



MAX-PLANCK-INSTITUT
FÜR POLYMERFORSCHUNG

JOHANNES GUTENBERG
UNIVERSITÄT MAINZ



Controlling Order and Dynamics in Peptide Assemblies by Molecular Design and Light

Dissertation
zur Erlangung des Grades

„Doktor der Naturwissenschaften“ (Dr. rer. nat.)

im Promotionsfach Chemie
am Fachbereich Chemie, Pharmazie,
Geographie und Geowissenschaften

der Johannes Gutenberg-Universität Mainz

Julian Link

Geboren in Meiningen

Mainz, 2026

CC-BY 4.0

1. Gutachter/in Prof. Dr. Tanja Weil
2. Gutachter/in Prof. Dr. Pol Besenius

Datum der mündlichen Prüfung: 16.06.2026

[illegible]

Abbreviations

AAP	arylazopyrazol	GSH	glutathione
ADP	adenosine diphosphate	GSSG	oxidized glutathione
AFM	atomic force microscopy	HEPES	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
Ala, A	alanine	HPLC	high performance liquid chromatography
Arg, R	arginine	Ile, I	isoleucine
ATP	adenosine triphosphate	LC	liquid chromatography
Boc	tert-butoxycarbonyl	LCMS	liquid chromatography coupled mass spectrometry
CAC	critical aggregation concentration	Lys, K	lysine
CD	circular dichroism	MALDI	matrix -assisted laser desorption/ionization
COSY	correlated spectroscopy	MS	mass spectrometry
CPP	cell-penetrating peptide	NMR	nuclear magnetic resonance
Cy5	cyanine5	Nvoc	nitroveratryloxycarbonyl
DCM	dichloromethane	PBS	phosphate buffered saline
DIPEA	diisopropylethylamine	Phe, F	phenylalanine
DLS	dynamic light scattering	PPG	photoprotecting group
DMF	dimethylformamide	PSS	photostationary state
DMS	dimethyl sulfone	PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
DMSO	dimethyl sulfoxide	RNA	ribonucleic acid
DNA	deoxyribonucleic acid	ROS	reactive oxygen species
ESI	electrospray ionization	Ser, S	serine
F-Actin	filamentous actin	SPPS	solid-phase peptide synthesis
FLIM	fluorescence lifetime imaging	TAT	transactivator of transcription
Fmoc	fluorenylmethoxycarbonyl	TEM	transmission electron microscopy
G-actin	monomeric globular actin	TFA	trifluoroacetic acid
Gln, Q	glutamine	TFE	trifluoroethanol
Gly, G	glycine	TIPS	triisopropylsilane

Table of Contents

Acknowledgements	I
Abbreviations	II
Table of Contents	III
Abstract	1
Abstract	1
Kurzfassung	2
1. Introduction.....	3
1.1 Principles of Self-Assembly.....	4
1.1.1 Thermodynamic Interactions	4
1.1.2 Kinetic Considerations.....	7
1.1.3 Supramolecular Polymerization Mechanisms	9
1.2 Peptide Self-Assembly	10
1.2.1 Peptide Assemblies in Nature	11
1.2.2 Synthetic Peptide Assemblies	13
1.2.3 Intracellular Self-Assembly	21
1.3 Photoresponsiveness and Photopharmacology.....	24
1.3.1 Photocages	26
1.3.2 Photodimerization.....	27
1.3.3 Photoswitches	28
1.3.4 Photoreversible Self-Assembly.....	32
2. Motivation	35
3. Decoding the Role of Spacer Length and Flexibility in Molecular Peptide Self-Assembly	39
4. Light-Triggered Disassembly of Peptide Nanostructures.....	107
5. Photoswitchable Peptides as Molecular Tools to Encode Structural Order and Disorder in Intracellular Assemblies.....	133
6. Summary and Outlook	185

7. Bibliography.....	189
Appendix	201
Curriculum Vitae	201
Scientific Publications	202

Abstract

Abstract

The transition between order and disorder is a central element governing self-assembly in both natural and synthetic systems. However, achieving control over assembly pathways, reversibility and the rational design of functional structural order remain challenging, particularly under far-from-equilibrium and biologically relevant conditions. Although numerous peptide-based self-assembling systems have been reported, rational design principles linking molecular structure to dynamic supramolecular behavior remain insufficiently explored.

In this thesis, minimalistic peptide sequences are employed to investigate how subtle molecular design parameters govern supramolecular order and disorder in a stimulus-responsive manner. Systematic analysis of linker flexibility is used to identify its relevance for effective intermolecular interactions and the formation of ordered assemblies. Subsequently, light is introduced as a non-invasive external stimulus offering high spatiotemporal precision to actively regulate order-disorder transitions. Both irreversible and reversible photoresponsive strategies are explored to probe assembly pathways and non-equilibrium behavior.

Building on these concepts, photoreversible peptide systems are developed that enable repeated switching between ordered and disordered states through photoisomerization. Using isopeptide chemistry, the peptide scaffold is further extended to facilitate cellular uptake, while glutathione-responsive motifs allow these systems to exhibit intracellular activity in such biological environments. This approach enables correlations between supramolecular organization and cellular response to be examined. Collectively, the results demonstrate that molecular-level design enables active control over peptide self-assembly pathways beyond static equilibrium structures.

This work establishes design principles for adaptive supramolecular peptide systems and contributes to bridging supramolecular physical chemistry with dynamic behavior in complex, life-inspired environments.

Kurzfassung

Der Übergang zwischen Ordnung und Unordnung stellt ein zentrales Element dar, das die Selbstassemblierung sowohl in natürlichen als auch in synthetischen Systemen bestimmt. Die gezielte Kontrolle von Assemblierungswegen, Reversibilität und das rationale Design funktioneller struktureller Ordnung bleibt jedoch eine große Herausforderung, insbesondere unter fern-vom-Gleichgewicht liegenden und biologisch relevanten Bedingungen. Obwohl zahlreiche peptidbasierte selbstassemblierende Systeme beschrieben wurden, sind rationale Designprinzipien, die die molekulare Struktur mit dem dynamischen supramolekularen Verhalten verknüpfen, bislang nur unzureichend erforscht.

In dieser Arbeit werden minimalistische Peptidsequenzen eingesetzt, um zu untersuchen, wie subtile molekulare Designparameter die supramolekulare Ordnung und Unordnung in stimulus-responsiver Weise steuern. Eine systematische Analyse der Flexibilität des Linkersegments dient dazu, deren Bedeutung für effektive intermolekulare Wechselwirkungen und die Ausbildung geordneter Assemblierungen zu identifizieren. In einem weiteren Schritt wird Licht als nicht-invasiver externer Stimulus mit hoher räumlich-zeitlicher Präzision eingeführt, um Übergänge zwischen Ordnung und Unordnung aktiv zu regulieren. Sowohl irreversible als auch reversible photoresponsive Strategien werden untersucht, um Assemblierungswege und Nicht-Gleichgewichtsverhalten zu analysieren.

Aufbauend auf diesen Konzepten werden photoreversible Peptidsysteme entwickelt, die ein wiederholtes Umschalten zwischen geordneten und ungeordneten Zuständen durch Photoisomerisierung ermöglichen. Mithilfe von Isopeptid-Chemie wird das Peptidgerüst weiter modifiziert, um eine zelluläre Aufnahme zu erleichtern, während Glutathion-responsive Motive es diesen Systemen erlauben, in biologischen Umgebungen intrazelluläre Aktivität zu entfalten. Dieser Ansatz ermöglicht es, Zusammenhänge zwischen supramolekularer Organisation und zellulärer Antwort zu untersuchen. Insgesamt zeigen die Ergebnisse, dass molekulares Design eine aktive Kontrolle von Peptid-basierten Selbstassemblierungswegen über statische Gleichgewichtsstrukturen hinaus erlaubt.

Diese Arbeit etabliert Designprinzipien für adaptive supramolekulare Peptidsysteme und trägt dazu bei, die supramolekulare physikalische Chemie mit dynamischem Verhalten in komplexen, lebensinspirierten Umgebungen zu verbinden.

1. Introduction

The ability of molecules to recognize and associate with one another underlies the organization of matter in both biological and synthetic systems. Through a multitude of weak and reversible interactions, molecular components can spontaneously and autonomously associate into higher-order structures, giving rise to complexity and function. Understanding how such organization emerges from molecular recognition is a central objective of the field of supramolecular chemistry. In 1987, the Nobel Prize in chemistry was awarded to Cram, Lehn and Pedersen “for their development and use of molecules with structure-specific interactions of high selectivity” which established supramolecular chemistry as a well-accepted chemical discipline.¹

“A prerequisite for all life processes is that molecules recognize each other and bind to each other in order to be able to react.”²

–Prof. Salo Gronowitz, Nobel Prize Ceremony 1987

Self-assembly describes the process of spontaneous organization of smaller molecular entities into higher-ordered structures through non-covalent interactions.^{3,4} Because of their intrinsic dynamics, self-assembled systems can reorganize, respond, and adapt. This inherent dynamicity arises from the reversible and environment-dependent nature of the intermolecular interactions, which distinguishes self-assembly from covalent synthesis and underlies the formation of complex architectures across multiple length scales. The importance of self-assembly becomes clear when investigating the very processes that determine life itself. Molecular recognition drives the dynamic formation of organized yet adaptable structures that build up cells, tissues and organs. In a synthetic context, self-assembly is a powerful strategy to construct nanoscopic to macroscale functional materials from simple, accessible monomeric building blocks. Elucidating the principles that govern self-assembly is therefore essential for both understanding natural systems and enabling the rational design of supramolecular materials.

Among the various molecular building blocks explored for supramolecular assembly, peptides have emerged as particularly versatile candidates. Their sequence-defined structure enables precise control over intermolecular interactions while maintaining the dynamic and reversible character essential for self-assembly. Combined with their inherent biocompatibility, this structural programmability supports applications ranging from materials science to the life sciences, making peptides attractive platforms for exploring functional self-assembly.

The following sections therefore briefly introduce the fundamental principles of molecular self-assembly, highlight peptides as building blocks in natural and synthetic contexts, and outline design principles for achieving structure and function in such biomaterials.

1.1 Principles of Self-Assembly

The self-assembly of monomers into ordered structures arises from the collective and cooperative action of multiple non-covalent interactions.⁵ Together, these interactions result in a net decrease in free energy, providing the thermodynamic driving force that favors assembly over the monomeric state. However, the structures that form are not determined by thermodynamics alone, but they are also strongly influenced by the kinetic pathways through which the system evolves toward its energy minimum. Dynamic association and dissociation processes are therefore central to structure formation, as they enable continuous structural rearrangement and pathway dependent organization. Through the interplay of kinetic and thermodynamic factors, a single monomeric building block can thus give rise to the stepwise organization into hierarchically structured assemblies.

This chapter introduces the individual intermolecular forces underlying self-assembly, discusses the balance between thermodynamic driving forces and kinetic pathways, and concludes with an examination of supramolecular polymerization mechanisms that lead to hierarchical structure formation.

1.1.1 Thermodynamic Interactions

Firstly, five main classes of non-covalent interactions that contribute to self-assembly are described. Individually, these interactions are weak compared to intramolecular forces such as covalent bonds (10^0 – 10^1 kJ/mol for non-covalent interactions and 10^2 – 10^3 kJ/mol for covalent interactions^{6–8}), however, their collective action can result in strong overall binding while retaining a degree of dynamic behavior that is inaccessible to covalent chemistry.

Hydrogen Bonding

Hydrogen bonds are a distinctive form of dipole-dipole-interactions between an electropositive hydrogen-atom, covalently bound to an electronegative donor atom, and a second electronegative acceptor atom.⁹ While often described as a special form of dipole–dipole interaction, hydrogen bonds exhibit partial covalent character due to contributions from charge transfer, orbital overlap, and electron delocalization. Because of this orbital involvement, hydrogen bonding is highly directional, with interaction strength strongly dependent on the donor–hydrogen–acceptor angle. This directionality underlies the formation of well-defined secondary structures in polypeptides, where hydrogen bonds form between amide groups along the peptide backbone. In peptide-based systems, hydrogen bonding frequently represents a dominant driving force for self-assembly and plays a key role in directing the formation of higher-ordered supramolecular architectures.^{10,11}

π - π -Interactions

π - π -interactions are attractive non-covalent forces that arise between π -systems of different molecules.¹² They are moderately directional and rather weak individually (2–10 kJ/mol) but can act additively to drive the self-assembly of short amphiphiles or other aromatic-containing building blocks. These interactions can promote directional growth and are strongly modulated by the surrounding solvent.¹³ While the detailed physical origin of π - π interactions remains an active area of research¹⁴, their functional relevance in stabilizing supramolecular assemblies is well established.

Van der Waals Interactions

Van der Waals interactions describe a plethora of dispersion forces that arise from transient fluctuations in electron density. These interactions are non-directional, short-range additive effects in nature and have an approximated strength of a few kilojoules per mole (≈ 5 kJ/mol). They are particularly relevant in systems containing aliphatic segments but also play a supporting role in the self-assembly of more polar building blocks, including peptides.¹¹

Table 1: Typical binding free energies for important noncovalent interactions.⁷ It should be noted that the interaction strengths given are context-dependent and vary with molecular environment and experimental conditions; the values shown therefore represent approximate ranges rather than universal constants.

Type	Typical $-\Delta G$ [kJ/mol]	Notes
Electrostatic	5–8	Strongly reduced in aqueous environment. Way higher in gas phase (200–500 kJ/mol).
Hydrogen Bonding	2–10	Directional. Influenced by pH and ions. Significant in peptides.
π - π -stacking	2–30	Influenced by solvent. Controversially discussed.
Hydrophobic	1–3 per $-\text{CH}_2-$	Rather entropic than enthalpic.
Van-der-Waals	0–10	Ubiquitous. Additive but influenced by each other.

Hydrophobic Interactions

Hydrophobic interactions arise from the unfavorable interaction of apolar molecular surfaces with polar solvents such as water.¹⁵ Consequently, hydrophobic segments of amphiphilic molecules tend to associate in an aqueous environment, minimizing their exposure to the solvent. This effect is predominantly entropic in origin, resulting from the release of structured water molecules upon aggregation rather than from direct attractive forces between hydrophobic groups. Hydrophobic interactions therefore play a crucial role in the design of amphiphilic building blocks, where they can strongly promote self-assembly processes. However, if the hydrophobic driving force becomes too dominant, the balance shifts from controlled self-assembly to nonspecific aggregation and eventual precipitation. In contrast to more directional interactions such as hydrogen bonding or π – π stacking, hydrophobic interactions are less ordered and typically contribute binding free energies of a few kilojoules per mole (1–3 kJ/mol per methylene unit).^{11,16}

Electrostatic Interactions

Electrostatic interactions are based on coulomb interactions between opposite charges leading to the formation of ion-pairs. In isolation, such interactions are among the strongest non-covalent forces (≈ 500 kJ/mol)¹⁷, however their effective contribution to self-assembly is strongly attenuated in aqueous environments due to solvation effects. As a result, the interaction strength in water is reduced to only a few kilojoules per mole per ion pair (ca. 5 kJ/mol per ion-pair)^{18,19} and becomes highly dependent on pH and ionic strength. Consequently, electrostatic interactions often play a secondary role in the initial stages of structure formation in aqueous systems, while becoming increasingly important at later stages, for example by stabilizing folded conformations through the formation of salt bridges.

The fundamental forces described above drive self-assembly processes but must be considered in a holistic and synergistic manner, as it is not a single interaction but their collective interplay that determines the stability and structure of a supramolecular system. In the rational design of small monomeric building blocks, molecular dynamics simulations can be employed to map these interactions and predict structural motifs that may enable self-assembly.²⁰

While such computational approaches significantly enhance the efficiency of screening and design, they cannot replace the synthesis and experimental characterization of the actual compounds, as successful self-assembly is not guaranteed. This limitation arises in part because self-assembly is governed not only by thermodynamic driving forces but also by kinetic factors, which can strongly influence pathway selection and the resulting morphology. The following section therefore addresses the balance between thermodynamic control and kinetic trapping in supramolecular self-assembly.

1.1.2 Kinetic Considerations

While the thermodynamic parameters and forces determine whether self-assembly is energetically favorable for the system, the kinetic factors determine the reaction rates as well as the specific pathways and intermediates of assembly. In supramolecular systems, self-assembly rarely occurs via a single-step process but instead follows complex, multistep pathways that involve nucleation events, transient intermediates, and energy barriers. As a result, the structures that emerge may reflect kinetic accessibility rather than thermodynamic preference. Establishing the fundamental kinetic principles underlying self-assembly is therefore essential for understanding and controlling supramolecular organization. To visualize this, the free energy can be plotted as a function of the assembly coordinate. The resulting profile describes the energy landscape of self-assembly and typically exhibits multiple local minima (compare Figure 1) separated by energy barriers. Each minimum corresponds to a distinct spatial organization of the molecular building blocks, potentially leading to different structural states. These structural variations, in turn, determine the resulting physical, chemical, and biological properties, including mechanical strength, molecular recognition, transport behavior, and stimuli-responsiveness. Thus, function emerges as a direct consequence of structural organization across multiple length scales.²¹

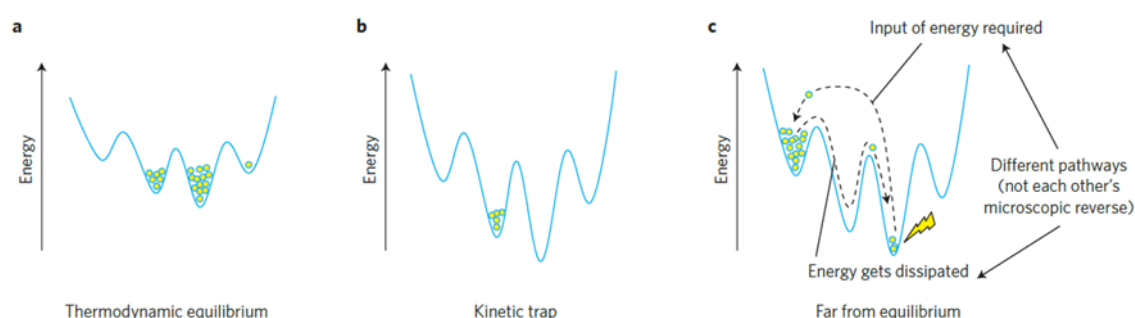


Figure 1: Energy diagrams of assembly processes. (a) Thermodynamic equilibrium assemblies, where the final state is governed by the Boltzmann distribution. (b) Kinetically trapped assemblies, where the final state is set by the synthetic pathway. (c) Far-from-equilibrium assemblies, where the final state arises from the balance of continuous synthesis, degradation, and energy dissipation. Taken with permission from²². Copyright 2015 Springer Nature.

Considering the interplay of energy landscapes and kinetic barriers, three thermodynamic regimes can be identified: equilibrium assemblies, kinetically trapped assemblies and far-from-equilibrium assemblies. Equilibrium assemblies, governed by the Boltzmann distribution, represent structure formation under thermodynamic control, in which reversible interactions allow continuous molecular exchange while maintaining a free-energy-minimized, time-invariant macroscopic state (Figure 1a). Synthetic systems largely fall into this category. In contrast, kinetically trapped assemblies stay in local minima, persisting due to high energy barriers that hinder relaxation to the global minimum (Figure 1b). These states are metastable and need time or external energy to be converted into a more stable global minimum.²³ Lastly,

the far-from-equilibrium state describes systems constantly needing energy input to function. Their functionality depends on a steady supply of energy, otherwise their collapse upon energy withdrawal leads to relaxation into equilibrium or kinetic trap states. This non-equilibrium behavior is central to biological systems, where continuous activity requires kinetic control rather than thermodynamic equilibrium.^{3,24} Thermodynamically stable equilibrium assemblies derive their macroscopic properties from the molecular structures of their monomers and the resulting supramolecular interactions. Despite the apparent simplicity of this class, highly complicated architectures have been realized, ranging from intricate host-guest-systems²⁵ and sophisticated 3D frameworks^{26,27} to supramolecular polymers with finely tunable mechanical properties.²³ Such advances have been enabled by emerging designing techniques such as dynamic combinatorial chemistry, which allow access to highly complex yet thermodynamically controlled systems.²⁸ For a given system, multiple individual kinetically trapped states may exist, each associated with different structural and therefore functional outcomes. Control over the parameters leading to these states can therefore enable a multitude of functions from a single system. Classical examples of kinetically trapped assemblies include phospholipid bilayer vesicles, which form rapidly but transiently convert into thermodynamically more favored crystalline phases.²⁹ In the class of kinetically trapped states, the pathway complexity of supramolecular assemblies becomes particularly evident. Variations in external parameters, including concentration,³⁰ temperature,³¹ or solvent composition,³² can direct the system toward markedly different structures, such as inverted helicity³³ or fibers with varying lengths and thicknesses³⁴. Far-from-equilibrium assemblies are distinguished from the aforementioned regimes by the requirement of continuous energy-input to maintain stability. This energy influx can either drive the system into a specific local minimum or sustain it in transient, dissipative state.³⁵ While various energy sources, such as chemical or electrical, can be employed to fuel such systems,³⁶ recent approaches highlight the benefits of using light energy due to its spatiotemporal precision, non-invasive nature and inherent reversibility. Accordingly, many reported examples rely on photoswitches as energy-harvesting motifs since they can reversibly alter their structural output upon irradiation with light of different wavelengths.^{37,38} Among the most prominent examples of light-driven far-from-equilibrium systems are molecular motors and machines.^{39,40} Inspired by these findings, photoacids and photoinduced electron transfer processes have been employed to activate pH- or redox-sensitive building blocks, enabling supramolecular polymers with reversible assembly behaviour^{41,42} or dynamic morphological alterations.^{43,44} In these examples, light enables access to reaction pathways that are distinct from thermally driven processes, thereby introducing directionality and temporal control into the assembly process. As a result, dissipative behavior can be sustained in a clean and spatiotemporally controlled manner. Nevertheless, a key challenge remains to achieve true out-of-equilibrium systems that fully circumvent kinetically trapped high energy states.³⁵

1.1.3 Supramolecular Polymerization Mechanisms

By combining the underlying thermodynamic driving forces with kinetic control parameters, three principal mechanisms of supramolecular polymerization can be identified. Isodesmic polymerization (Figure 2a) is characterized by the stepwise association of monomers without a distinct nucleation event. The binding constant remains identical at each stage of growth, from initial dimer formation to extended aggregates. As polymer growth proceeds gradually and with limited kinetical control, the resulting assemblies typically exhibit broad size distributions. This behavior closely parallels step-growth polymerization in conventional covalent polymer chemistry.²³ In contrast, in ring-chain supramolecular polymerization (Figure 2b), self-assembly is governed by a continuous competition between cyclization and chain elongation, dictated by monomer design and flexibility. The building blocks are typically ditopic monomers with a flexible linker segment tethering the functional groups, establishing an equilibrium between closed rings and open chains, and rendering these systems predominantly thermodynamically controlled. Polymerization is therefore strongly concentration dependent, with chain growth becoming increasingly favored at higher concentrations. Owing to the entropic cost of ring opening relative to chain growth, no strict nucleation barrier is present. Consequently, such systems often display characteristic, concentration-dependent length distributions and are comparable to the covalent ring-chain polymerization.²³

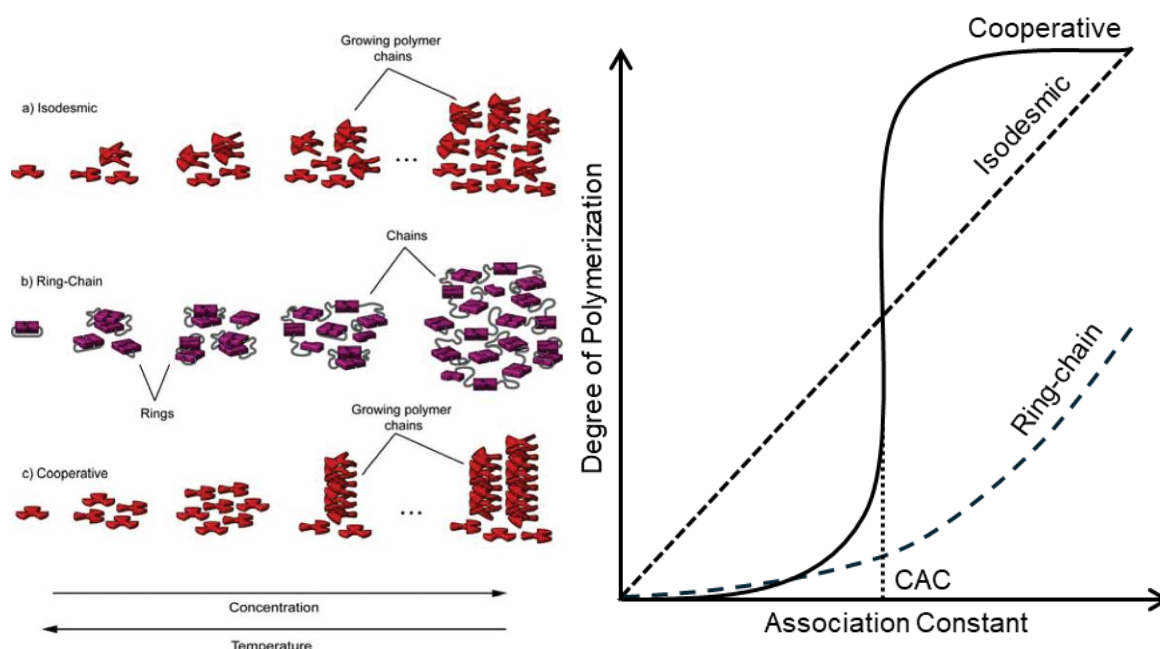


Figure 2: Schematic representation of the three distinct mechanisms of supramolecular polymerization: a) isodesmic polymerization. b) ring-chain mediated polymerization. c) cooperative polymerization. Taken with permission from²³. Copyright 2009 American Chemical Society. Right side: Corresponding plot of polymerization degree vs association constant, recreated with information from^{23,45,46}.

The last mechanism is cooperative supramolecular polymerization (Figure 2c), which is prevalent in biological examples, including peptide assembly and the formation of multi-subunit protein complexes. It proceeds via a two-step nucleation-elongation process that gives rise to cooperative or anti-cooperative growth behavior.⁴⁷ Initially, monomers associate through an isodesmic mechanism with a fixed association constant until a critical nucleation size is reached. Beyond this point, elongation occurs with a different association constant in comparison to the initial phase, reflecting enhanced or diminished binding due to (anti-) cooperative effects. The requirement of a nucleation event introduces a characteristic lag phase in the self-assembly process, as elongation cannot proceed in the absence of nuclei. Supplying the system with preformed nuclei can dramatically accelerate the assembly kinetics. Nucleus formation requires a specific threshold monomer concentration, the critical aggregation concentration. The resulting growth is non-linear with a sharp assembly transition. Because this critical value is highly sensitive to external parameters such as temperature, solvent composition and ionic strength, cooperative supramolecular polymerization displays a strong environmental responsiveness.⁴⁸ This intrinsic threshold behavior enables improved control over self-assembly, resulting in narrower length distribution in comparison to the purely isodesmic systems. Therefore, this mechanism is particularly attractive for the construction of well-defined supramolecular polymers with controlled lengths and functions.²³

1.2 Peptide Self-Assembly

Supramolecular self-assembly has been realized using a wide variety of molecular scaffolds, ranging from small organic molecules and synthetic polymers to nucleic acids, proteins, and hybrid systems. Low-molecular-weight gelators and amphiphilic block copolymers offer access to soft materials with tunable mechanical properties, while DNA-based architectures enable highly programmable assembly through sequence-encoded recognition. Protein-derived systems provide unparalleled structural complexity but often require elaborate folding pathways and stringent conditions. Each of these scaffold classes offers distinct advantages, yet they also impose limitations in terms of synthetic accessibility, adaptability, or control over dynamic behavior.

Within this diverse landscape, peptides occupy a unique intermediate position, combining the synthetic simplicity of small molecules^{49,50} with the structural richness and functional relevance of biological macromolecules. Peptides consist of amino acids linked by covalent amide bonds and allow precise control over molecular sequence, straightforward incorporation of functional motifs, and access to all major classes of non-covalent interactions discussed in **Chapter 1.1.1**, thereby providing a versatile platform for the rational, bottom-up design of well-defined nanostructures.^{51–53} Using the proteinogenic amino acids, peptide-based systems are

inherently biocompatible and readily adaptable to aqueous and biological environments, making them particularly attractive for studying dynamic and stimulus-responsive supramolecular organization.⁵⁴ For these reasons, the following chapter focuses on peptide self-assembly as a model platform for investigating how molecular design governs supramolecular structure, dynamics, and function.⁵⁵

1.2.1 Peptide Assemblies in Nature

In nature, structure formation through self-assembly is ubiquitous across a wide range of length scales. From spiderwebs extending over millimeters to meters, to fibrin networks that enable wound closure on the micrometer-to-millimeter scale, and down to the cytoskeleton operating from nanometers to micrometers, living organisms exploit self-assembly to generate sophisticated materials and structures from simple building blocks.^{56,57} At the molecular level, these building blocks are primarily the 20 canonical amino acids, which differ in the chemical nature of their side chain and thus encode different physicochemical properties to the peptide. Polar, apolar, basic and acidic functionalities combine within a peptide sequence, and the overall behavior of the peptide emerges from the collective behavior between the side-chain interactions and the strong, directional hydrogen-bonding of the backbone.⁵⁸

The linear sequence of amino acids, commonly represented by a one-letter code, defines the **primary structure** of a peptide or protein. Depending on its length and amino-acid composition, this sequence can locally fold into recurring conformations that are primarily stabilized by hydrogen-bonding along the peptide backbone. These locally ordered conformations constitute the **secondary structure**. The most prevalent secondary structure motifs are α -helices and β -sheets, although β -turns and Ω -loops are also known.^{59,60} Additional interactions between side chains lead to the folding of one or more secondary structure elements into a defined three-dimensional arrangement, known as the **tertiary structure**. The highest level of structural organization is the **quaternary structure**, in which multiple protein or peptide subunits assemble into a functional complex. In cells, this hierarchical folding and assembly process is tightly regulated and assisted by molecular chaperones, which prevent misfolding and ensure the formation of functional proteins.^{61,62}

The importance of correct protein folding becomes particularly evident when considering the origin of neurodegenerative diseases such as Alzheimer's, Parkinson's or Huntington's disease. In these disorders, the mechanisms of protein-quality control in cells fail, allowing specific proteins to adopt aberrant conformations that promote pathological self-assembly into toxic oligomers and aggregates. The accumulation of these misfolded species disrupts cellular homeostasis and ultimately leads to neuronal dysfunction and cell death.^{51,63,64}

These examples demonstrate that protein folding is not a static process, but a dynamically regulated one requiring continuous cellular control. This requirement for active maintenance of structural order however is not limited to individual proteins. At larger length scales within the cell, many functional assemblies are inherently dynamic and rely on continuous energy consumption to sustain their organization. Prominent examples are cytoskeletal systems such as actin filaments and microtubules, which are assembled from folded protein subunits but exist only as long as chemical energy is supplied. These assemblies represent a distinct class of biological structures that operate far from thermodynamic equilibrium, continuously balancing assembly and disassembly through ATP- or GTP-driven processes.⁶¹

Actin filaments and microtubules are composed of actin monomers and tubulin dimers, respectively, and undergo continuous dynamic assembly and disassembly driven by nucleotide hydrolysis. Monomeric actin (G-actin) binds ATP, which promotes its self-assembly into filamentous actin (F-actin). Subsequent hydrolysis of ATP to adenosine diphosphate (ADP) destabilizes the polymeric form, favoring depolymerization (Figure 3c). Owing to the intrinsic polarity of actin filaments, elongation preferentially occurs at the barbed end, while depolymerization dominates at the more polar pointed end. This asymmetry gives rise to treadmilling, a dynamic steady state in which monomers appear to move along the filament while its overall length remains constant.^{58,61}

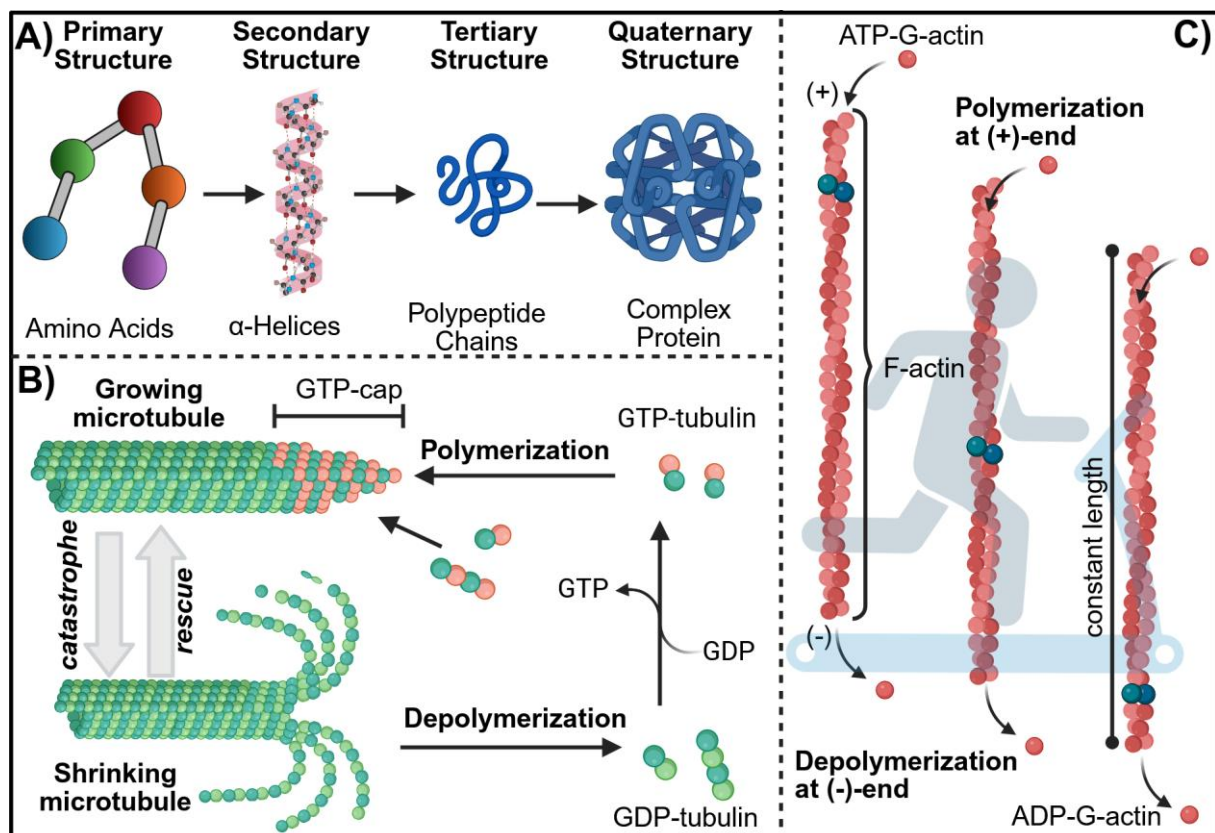


Figure 3: Overview of natural occurring self-assemblies. A) Peptide structure from primary to quaternary structure (adapted from⁵⁸, Created with Biorender.com.), B) (De-)Polymerization of microtubule (taken from Biorender.com) C) Actin Treadmilling (adapted from⁶⁵, Created with Biorender.com).

A similar directional growth is observed for microtubules, which assemble from α/β -tubulin heterodimers through GTP binding and hydrolysis at the β -tubulin subunit. The resulting protofilaments are intrinsically polar, exposing β -tubulin at the plus (+) end and α -tubulin at the minus (–) end, with polymerization predominantly occurring at the + end. Thirteen such protofilaments associate laterally to form the hollow cylindrical microtubule structure. In contrast to actin filaments, both elongation and depolymerization of microtubules occur majorly at the + end of the filaments (Figure 3b). This dynamic behavior is tightly regulated by tubulin conformation, which depends on whether GTP or GDP is bound. GTP-bound tubulin adopts a straight conformation that favors incorporation into the growing filament, whereas GTP hydrolysis to GDP induces a curved conformation that destabilizes the lattice. The presence of a GTP-cap at the + end therefore constitutes the key physical control parameter of microtubule stability: as long as the rate of GTP-bound tubulin addition exceeds the GTP hydrolysis, the structure is stabilized and remains to grow. When the hydrolysis rate surpasses the incorporation of new monomers, the GTP cap is lost, triggering depolymerization in a process known as catastrophe.^{58,61}

This process of rapid growth and hydrolysis is highly dynamic and influenced by a multitude of auxiliary proteins that support either of the processes. With the help of these proteins, as well as with the regulation of the GTP concentration, the cell can organize the structure formation with high precision.⁶¹

1.2.2 Synthetic Peptide Assemblies

Inspired by these highly dynamic and regulated biological assemblies, increasing efforts have been devoted to replicating such principles synthetically. While these biological systems illustrate how complex and dynamic structures can be generated and maintained, their intrinsic complexity poses significant challenge for systematic investigation and rational design. Proteins comprise long sequences with multiple interacting domains, post-translational modifications, and tightly regulated folding pathways, making it difficult to isolate the contribution of individual structural elements. To translate the principles of biological self-assembly into synthetic systems, it is therefore necessary to reduce this complexity in a controlled and deliberate manner. Short peptide sequences offer an attractive compromise, retaining essential interaction motifs found in proteins while providing improved synthetic accessibility and modularity. By reducing protein architectures down to minimal peptide building blocks, key design parameters governing self-assembly, such as sequence composition, interaction strength, and environmental responsiveness, can be probed. This reductionist approach enables the development of simplified model systems that capture

fundamental aspects of biological assembly while remaining experimentally tractable.

Utilizing these concepts, systems can adopt a plethora of nanoarchitectures, such as nanofibers, ribbons, tapes and bands, as well as higher-ordered networks and hydrogels.^{66,67}

These various morphologies are particularly attractive for applications in biomaterials, tissue engineering, drug delivery, and responsive soft matter, since their mechanical properties, stability and transport and diffusion characteristics enable tailor-made properties for their respective applications.⁶⁸

Synthetic peptide self-assembling systems can be broadly divided into two conceptual categories: biomimetic and non-natural. The first category is directly inspired by naturally occurring peptides and proteins, leveraging insights into how primary amino-acid sequences encode defined secondary structure. By analyzing sequence-structure relationships, design rules have been established for peptides that form β -sheets, α -helices or coiled-coil motifs. The second category extends beyond natural peptide architectures by introducing non-peptidic modification to the peptide scaffold, most commonly hydrophobic moieties such as alkyl chains or aromatic π -blocks, which promote and support self-assembly.⁶⁸

β -Sheets

β -sheet-forming peptides are well known for their pronounced self-assembly propensity, as exemplified by their abundance in amyloid plaques.^{51,63} A common structural motif of β -sheets is the alternation of hydrophilic and hydrophobic residues, which gives rise to amphiphilic sheets with distinct polar and apolar faces. Upon dimerization of two sheets, the hydrophobic surfaces can be buried within the interior, while the hydrophilic faces remain exposed to the surrounding medium.⁶⁹ A variety of different supramolecular nanostructures, such as nanoparticles, nanofibrils, nanotubes or nanosheets, can be realized from β -sheet forming peptides, underscoring the importance of controlling the assembly process to achieve uniform and reproducible architectures.⁷⁰ For a given sequence, such control can be exerted through parameters including peptide concentration, ionic strength or temperature.^{71,72} In general, higher peptide concentrations favor the formation of higher-order structures, until a critical threshold is reached beyond which solubility is exceeded and amorphous precipitation occurs. Systematic studies on hydrophobic residues have further shown that increasing the hydrophobicity of the utilized amino acid residues enhances self-assembly and lowers the critical aggregation concentration. Furthermore, sequence length also plays a crucial role: very short peptides often require additional hydrophobic modifications to assemble efficiently, whereas longer sequences may exhibit higher aggregation thresholds due to an increased entropic penalty competing with favorable enthalpic contributions.⁷³ Since electrostatic interactions often contribute significantly to the self-assembly process of β -sheets, the salt concentration can directly affect intermolecular forces and assembly outcomes, especially at

low peptide concentrations.⁷⁴ Notably, β -sheet derived hydrogels often display the ability to recover their macroscopic properties after mechanical stress by external energy sources like ultra sonification.⁷⁵ This self-healing behavior renders them a promising candidate for responsive and adaptive biomaterials.⁶⁸

α -Helices and Coiled-Coils

Another naturally inspired class of self-assembling peptide systems is based on α -helices forming coiled-coil assemblies. In these structures, two or more α -helical strands wind around one another to create fibrous architectures, stabilized by tailored interactions between repeating heptad motifs that bury hydrophobic residues within the interior of the superhelix.^{76,77} Such coiled-coil assemblies are highly specific and programmable, as the structural role of each amino acid position within the heptad repeat is well understood and can be precisely engineered.⁷⁸ When heterodimeric coiled-coil strands are designed with staggered hydrophobic interfaces, unpaired helical segments, commonly labeled as “sticky ends”, remain available for further coiled-coil interactions, enabling directional propagation of the assembly along the long axis of the coiled-coil.^{79,80} Overall, α -helical self-assembling peptide systems offer a high degree of structural control due to the well-established design rules governing coiled-coil formation. This precision, however, typically comes at the cost of longer and more complex sequence designs compared to β -sheet peptide assemblies.⁶⁸

Peptide Amphiphiles

In contrast to the category of natural self-assembling peptides, non-natural peptide amphiphiles are typically minimalistic constructs designed to achieve a comparable diversity of structures and functions with reduced molecular complexity. These systems are generally easier to synthesize and modify, making them well suited for systematically probing how subtle molecular variations influence self-assembly. To ensure sufficient self-assembly propensity, peptide amphiphiles are functionalized with a hydrophobic segment, either an alkyl chain or an aromatic π -block, which reinforces hydrophobic interactions (Figure 4a).^{68,81} In aqueous environments, alkyl modified peptides commonly assemble into high aspect ratio nanorods featuring a hydrophobic core surrounded by radially oriented peptide segments that often adopt β -sheet secondary structures.^{82,83} This architecture closely parallels the behavior of covalent block-copolymers, highlighting the conceptual similarities between supramolecular peptide amphiphiles and polymeric amphiphilic systems.⁸⁴

Studies on the minimalistic diphenylalanine (FF) dipeptide, commonly found in the core sequences of amyloid peptides, have highlighted the crucial interplay between backbone hydrogen bonding and aromatic π - π -interactions in driving supramolecular order.^{85,86} Building on these insights, subsequent work extended this concept to *N*-terminally modified short

peptides bearing a variety of aromatic moieties that promote π - π -stacking, including the carbobenzyloxy, naphthalene or fluorenylmethoxycarbonyl (Fmoc) groups.^{54,68,87}

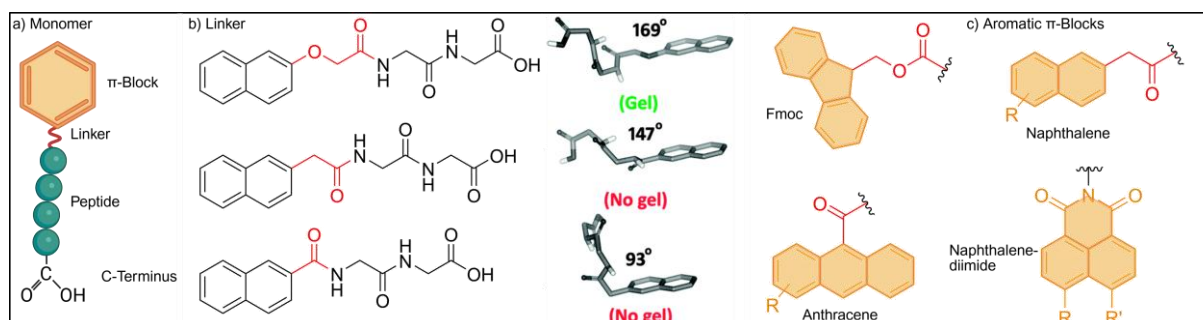


Figure 4: An overview of aromatic peptide amphiphile self-assembly; (a) a simplified aromatic peptide amphiphile; (b) The influence of the linker and its geometry on the gelation properties of naphthalene-containing peptides; (c) examples of assembly-promoting aromatic π -blocks. Created with Biorender.com. Partly adapted with permission.⁵⁴ Copyright 2014 Royal Society of Chemistry.

N-terminal Modifications

Among aromatic capping groups, the Fmoc moiety holds both historic and practical significance. It was first reported as part of a thermoreversible hydrogelator formed from the Fmoc-LD peptide,⁸⁸ and remains the most widely used protecting group in Fmoc-based solid-phase peptide synthesis.⁵⁰ As a consequence, most amino acids are readily available commercially with Fmoc-protected *N*-termini.^{89,90} Beyond its synthetic utility, the Fmoc group has been shown to promote self-assembly in a wide range of peptide-based systems more effectively than simple hydrophobic protecting groups, such as *tert*-butoxycarbonyl (Boc), due to its ability to engage in strong π - π -interactions.^{91–94} This effect has been demonstrated in numerous dipeptide and amino acid based systems, where Fmoc-capped monomers outperformed their Boc-protected analogues as supramolecular gelators.⁹⁵ Since *N*-terminal capping is the defining design element that distinguishes aromatic peptide amphiphiles from other classes of self-assembling peptides, an extensive library of potential building blocks is available (Figure 4c). For successful self-assembly in aqueous environments, the hydrophobicity of the peptide sequence must be carefully balanced with that of the π -block. For example, the VYGGG pentapeptide becomes too hydrophobic to undergo hydrogelation when coupled to a pyrene unit, whereas similar systems bearing less hydrophobic Fmoc or pyrene caps do form hydrogels.⁹⁶ Despite such empirical insights, reliably predicting the optimal combination of peptide sequence and *N*-terminal modification for hydrogelation remains challenging.⁹⁷ An additional strategy to fine-tune self-assembly involves modulating the electronic nature of the aromatic π -system through ring substitution. Such modifications directly influence the strength of π - π -interactions while simultaneously altering overall hydrophobicity.¹² In this context, highly electron-withdrawing and hydrophobic fluorine substituents in a pentafluorophenyl moiety have been shown to promote the formation of

hydrogels, whereas their non-fluorinated analogues failed to assemble.⁹⁸ In these systems, self-assembly is proposed to be stabilized by quadrupole-quadrupole interactions between aromatic amino acid side chain functionalities and the *N*-terminal capping-group.⁵⁴

Beyond structural control, the aromatic caps can also introduce additional functionality into peptide amphiphiles. While peptides themselves are inherently non-fluorescent, many aromatic systems possess intrinsic fluorescence, enabling direct visualization and tracking of self-assembly processes by confocal fluorescence microscopy and thereby extending the characterization toolbox beyond transmission electron microscopy (TEM).⁹⁹ Moreover, the use of spectroscopically active π -systems enable even advanced techniques such as phasor-based fluorescence lifetime imaging microscopy (Phasor FLIM), which maps fluorescence lifetimes in a fit-free manner therefore allowing real-time analysis of self-assembly kinetics.¹⁰⁰

The Linker Segment

Another key parameter governing the self-assembly propensity of aromatic peptide amphiphiles is the flexibility of the linker segment connecting the aromatic π -block to the peptide backbone. In general, a certain degree of flexibility is required to allow constructive π - π -stacking and efficient self-assembly, whereas overly rigid linkers can impede optimal aromatic-backbone alignment. For example, naphthoxy-capped peptides, which exhibit increased rotational freedom due to the presence of a bridging oxygen atom, have been shown to promote gelation more effectively than analogues in which the naphthyl substituent is directly attached to another sp^2 -hybridized carbon (Figure 4b).¹⁰¹ These experimental results have been partially rationalized by molecular modelling studies that evaluate the preferred linker conformations and corresponding bond angles. Systems exhibiting increased curvature in their energy-minimized state tend to assemble less efficiently, while gel-forming compounds generally favor more linear average geometries in the linker segment that support effective aromatic stacking.¹⁰² In addition to conformational freedom, some linker motifs can actively contribute stabilizing intermolecular interactions. For instance, the carbamate moiety present in the Fmoc protecting group displays a higher rotational energy barrier than the otherwise comparable ethylcarbonyl linker.¹⁰³ The length of the spacer segment also influences the resulting supramolecular structures. A systematic study varying the number of methylene units in the linker has demonstrated pronounced effects on chiroptical properties, as evidenced by circular dichroism (CD)-spectroscopy, demonstrating that the spacer length can directly modulate the overall chirality of the assemblies.¹⁰³ Despite its clear importance, the linker segments remains comparatively underexplored relative to other design parameters, highlighting it as a promising focus for future investigations in supramolecular peptide chemistry.

The Peptide Sequence

In aqueous environments, the influence of permanent dipoles within peptide sequences on the self-assembly energy landscape is largely attenuated by hydrogen-bonding with the solvent and electrostatic interactions with dissolved electrolytes. As a result, hydrophobic interactions typically dominate the assembly process in such systems. This is reflected in the prevalence of aromatic amino acids reported in literature on aromatic peptide amphiphiles.^{104–107} However, direct comparison between reported systems is often complicated by the wide variety of experimental protocols employed across studies. Furthermore, a noticeable bias toward phenylalanine-containing sequences can be identified, stemming from seminal work by the Gazit group^{86,108,109} and the central role of FF-motifs in amyloid formation.^{110–112}

Beyond backbone and terminal modifications, side-chain functionalities offers additional possibilities for the rational design of amphiphilic peptide materials. For instance, cysteine-containing peptides can be reversibly crosslinked via covalent disulfide bridges, enabling redox-responsive modulation of macroscopic properties.^{34,113} Expanding the monomer toolbox beyond the 20 canonical amino acids further increases design flexibility, allowing for novel or altered functionalities, such as side-chain attached fluorophores,¹¹⁴ modulation of hydrophobicity through protection of charged residues,¹¹⁵ or incorporation of novel metal containing amino acids.^{116,117} Together, these strategies broaden the structural and chemical space accessible to self-assembling peptides.

C-terminal Modifications

The C-terminus of short amphiphilic peptides is typically left unsubstituted, as the carboxyl group plays a crucial role in establishing an appropriate balance between ionized and neutral species, which is essential for solubility and, consequently, for self-assembly.¹¹⁸ Many such systems exhibit pronounced pH-dependent behavior that can be directly attributed to the protonation and deprotonation of the C-terminal carboxylate under various environmental conditions. Notably, experimentally determined pK_a values in the assembled state are often shifted to higher values compared to those of the free acid, reflecting changes in the local dielectric environment and cooperative electrostatic coupling between neighboring carboxylic acids upon aggregation.¹¹⁹ Chemical modifications of the C-terminus markedly alters this behavior. Functionalization generally reduces the system's pH-sensitivity while extending its stability over a broader pH range.¹²⁰ However, such modifications can be detrimental to self-assembly. Esterification may lead to solubility limitations^{121,122}, whereas amidation often suppresses assembly altogether.¹²³ These findings underscore the impact of even simple C-terminal modifications on solubility, responsiveness and self-assembly propensity of short-amphiphilic peptides.

Co-Assembly

Integrating all desired functionalities into a single self-assembling molecule can become challenging. Co-assembly strategies, in which multiple individual monomers jointly assemble into one common nanostructure, therefore represent a powerful alternative to introduce new functional groups. Similar to covalent copolymerization, supramolecular co-assembly can be classified as cooperative, orthogonal (or self-sorted), random or destructive (Figure 5).¹²⁴ Cooperative co-assembly describes an alternating arrangement of different monomers into a single supramolecular architecture yielding multifunctional nanostructures with a high degree of intrinsic order. Such systems can enable electronic exchange between donor and acceptor components, facilitating supramolecular charge-transfer processes relevant to optoelectronic applications.^{125,126} Cooperative co-assembly typically requires monomers with closely related structures to ensure selective and alternating incorporation, closely paralleling the concept of alternating covalent copolymers. Such structural compatibility can be achieved by matching interaction motifs so that heterotypic A-B interactions are energetically favored over homotypic A-A or B-B interactions. In peptide systems, this can be accomplished through complementary β -sheet regions, aromatic interactions, electrostatic pairing, or hydrophobic patterning.¹²⁷

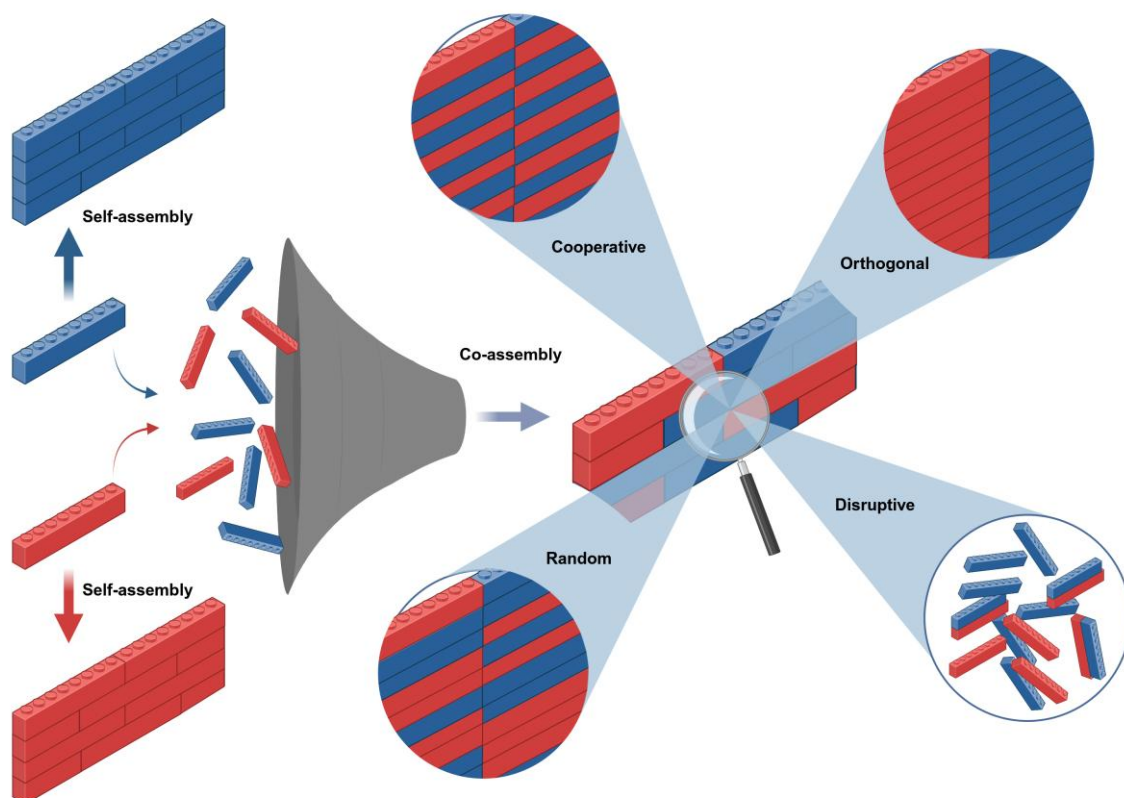


Figure 5: Peptide supramolecular co-polymers. Schematic illustration of how two peptide building blocks (blue and red bricks) can self-assemble into an ordered architecture (blue or red wall); mixing them results in a complex co-assembled architecture (wall comprises both blue and red bricks) via four possible supramolecular co-polymer arrangements such as cooperative, orthogonal (or self-sorting), random and disruptive co-assembly.¹²⁴ Created with Biorender.com. Adapted with permission¹²⁴. Copyright 2018 Royal Society of Chemistry.

In contrast, orthogonal or self-sorted co-assemblies consist of components that assemble independently of one another, resulting in phase-separated hybrid materials. This behavior is conceptionally analogous to block copolymers and may be advantageous for applications requiring spatial separation of functionalities, such as in photovoltaic systems.¹²⁸

Most amphiphilic peptide co-assembly systems, however, fall between these two extremes and are best described as random supramolecular polymerizations, comparable to random covalent copolymers. In such assemblies, the different peptide building blocks are arranged without strict order, and their distribution is governed by relative concentration, kinetics, and diffusion. Lastly, destructive co-assemblies can occur when the attachment of one peptide disrupts or terminates the assembly of another, effectively acting as a supramolecular chain stopper. This mechanism offers a unique handle for precisely tuning size, morphology, and physical properties of the resulting material.¹²⁹

Beyond the bonding motifs and thermodynamic interactions between the different monomers, another fine-tuning option exists in the respective monomer ratio. Assemblies can show crucially different behavior at different ratios, also exceeding the performances of the individual single-component assemblies.^{129–131} For example, stoichiometric mixtures of Fmoc-FF and Fmoc-GG form hydrogels with a storage modulus of 111 kPa in comparison to the storage modulus of 3.2 kPa for Fmoc-FF alone¹³². Similarly, coassemblies of Fmoc-FF and Fmoc-RGD exhibit a pronounced maximum in mechanical stiffness at an Fmoc-RGD content of 20%, reaching storage moduli of approximately 10000 kPa, whereas Fmoc-FF alone displays a storage modulus of only 1.9 kPa.¹³³ Other co-assembly strategies utilize more extreme ratios where only small amounts of co-monomers are added to insert a designated functional group. Examples for this are the co-assembly of dye-modified peptides within the structure of a non-fluorescent peptide amphiphile. This approach enables imaging of the structures via confocal fluorescence microscopy and thus tracking of the self-assembly even in biological setups.^{134,135} Such stoichiometry-dependent behavior closely mirrors biological assembly principles. In living cells, polymeric scaffolds dynamically recruit otherwise non-assembling monomers into pre-formed structures, thereby enabling adaptive modulation of mechanical properties, assembly kinetics, and surface chemistry in response to changing demands. Such recruitment processes are governed by multivalency and stoichiometry, leading to a high dependence on the relative ratios of the participating components.^{136,137} Self-assembling peptide amphiphiles provide a valuable model system for investigating these principles under well-defined conditions. Insightful examples are offered by short peptides that form β -sheet rich nanofibers composed of two distinct monomers, each of which is incapable of self-assembly on its own. Only when combined in distinct stoichiometric ratios that they specifically co-assemble into nanofibers.¹³⁸ This behavior highlights the dominance of specific peptide-peptide interactions over nonspecific hydrophobic interactions in directing assembly. These insights provide a

conceptual bridge between synthetic supramolecular materials and biological organization and lay the foundation for the following subchapter, which focuses on synthetic strategies for intracellular self-assembly, where stoichiometry, recruitment, and environmental responsiveness are key design parameters.

1.2.3 Intracellular Self-Assembly

Understanding the dynamics of biological structures and translating these insights into design principles for synthetic self-assembly in biological environments is a central objective of supramolecular chemical biology. This approach holds promise for both fundamental research and therapeutic applications. Synthetic, non-natural building blocks can often be distinguished more readily from native cellular components, facilitating the analysis of self-assembly processes within living cells. Moreover, the use of nanostructures rather than small molecule drugs offers several advantages.⁵⁶ The formation of supramolecular architectures at the target site is associated with an increased local concentration of the drug, thereby reducing the likelihood of drug resistance.¹³⁹ In addition, the assembly process itself can generate mechanical stress and disrupt endogenous cellular structures, ultimately promoting apoptotic pathways.^{100,134,135,140} Achieving controlled self-assembly inside cells requires the rational design of biocompatible building blocks that allow for self-assembly inside of the crowded cellular environment with high spatiotemporal control. Crucially, these components must be equipped with efficient and selective mechanisms for cellular entry, that minimize nonspecific toxicity and enable self-assembly specifically at the desired target site. In this context, the incorporation of cell-penetrating peptides (CPPs) has proven particularly effective. When attached to molecular cargo either covalently or non-covalently, CPPs facilitate their combined uptake via direct membrane translocation or active endocytic pathways.¹⁴¹ A prominent example is the trans-activator of transcription (TAT) peptide derived from the human immunodeficiency virus, whose arginine-rich, positively charged sequence interacts strongly with negatively charged cell-surface components, thereby promoting efficient endocytic uptake.¹⁴²

After successful cellular uptake, the typically highly hydrophilic transporter sequence must be decoupled from the self-assembling amphiphile to enable intracellular assembly. Control over this process can be achieved by combining bioresponsive trigger groups with deliberate modifications of the peptide backbone. One such strategy involves the introduction of isopeptide linkages, which generate a kinked backbone geometry that suppresses self-assembly (Figure 6).¹⁴³ For example, the side-chain hydroxyl group of a serine residue can be esterified with the carboxyl group of a subsequent amino acid, yielding a sterically hindered isopeptide structure (compare Figure 6).^{144,145} To stabilize this metastable construct and prevent thermodynamically favored linearization via an *O,N*-acyl shift, the respective amine

functionality of the serine must be either protected with an appropriate caging group or maintained in a fully protonated state at strongly acidic pH ($\text{pH} \leq 2$). Here, the local pH directly influences the rearrangement kinetics, as both the nucleophilicity of the amine and the reactivity of the side chain ester are pH dependent. Under acidic conditions, protonation activates the ester group but simultaneously suppresses the nucleophilicity of the *N*-terminus. At near neutral pH, partial amine deprotonation enables efficient intramolecular nucleophilic attack, typically resulting in the highest reaction rates.^{146,147} At more basic pH values, although amine nucleophilicity is further enhanced, competing base-catalyzed ester hydrolysis becomes significant and can reduce the efficiency of the desired rearrangement reaction.¹⁴⁸ Incorporation of specific bioresponsive caging groups at this position enables precisely tailored stimulus-responsiveness. Removal of the caging group liberates the *N*-terminus, allows backbone linearization and ultimately triggers self-assembly of the released monomers inside the cell.

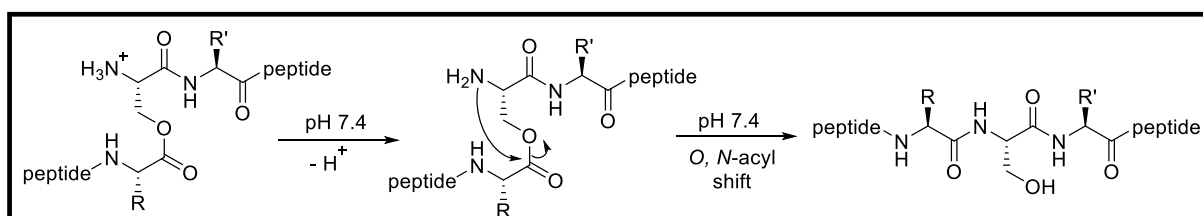


Figure 6: *O, N*-Acyl Shift of *isopeptides*. Intramolecular rearrangement from the kinked backbone of the *isopeptide* to the linear backbone of the native peptide.¹⁴⁴

Among these bioresponsive triggers are intracellular stimuli, such as pH ^{149,150}, redox environment^{151,152} or enzymes^{153,154} that can be exploited to generate tissue specificity since the tumor microenvironment typically exhibits a lower pH ¹⁵⁵, and higher concentrations of enzymes and redox-active compounds such as reactive oxygen species¹⁵⁶ (ROS) or glutathione¹⁵⁷ (GSH). Moreover, external stimuli like light can be used to improve the spatiotemporal control of the assembly and instill reversibility.^{158,159}

Introducing pH responsiveness provides a powerful strategy for intracellular self-assemblies, particularly in systems that enter cells via endocytic pathways, which are accompanied by a progressive decrease in pH to values of ≤ 5 . These endogenous pH gradients can then be exploited as internal triggers to transform an inert cargo into a self-assembling molecule. For instance, dynamic covalent bonds between salicylhydroxamates and phenyl boronic acids are stable at neutral pH but cleave under mildly acidic conditions ($\text{pH } 5.5\text{--}6.5$).¹⁶⁰ Incorporation of a self-immolative phenyl boronic acid that also acts as a sensor for reactive oxygen species further enhances selectivity, yielding a system that readily undergoes cleavage and backbone linearization only when both pH and ROS levels exceed defined thresholds (Figure 7 right).¹³⁵ Beyond pH and ROS responsiveness, the reducing intracellular environment, particularly pronounced in cancer cells, can also be harnessed to impart functionality and specificity.

Glutathione, the most abundant cellular reducing agent, has been widely explored in the design of GSH-responsive drug-delivery systems.¹⁶¹ These platforms typically rely on redox-sensitive disulfide linkers that are cleaved upon exposure to intracellular GSH upon cell uptake, releasing the active cargo at the target site. As an example, the pro-assembling Fmoc-ISA isotriptide was caged with a GSH-responsive self-immolative disulfide linker connecting it to the cell-penetrating TAT-peptide. Upon intracellular reduction of the linker inside the cytosol, the liberated peptide rearranged into a linear, self-assembling motif, disrupted the cytoskeleton, and ultimately induced apoptotic processes (Figure 7 left). Inclusion of a fluorescent component via a co-assembly approach allowed for real-time tracking of this intracellular self-assembly process by confocal fluorescence microscopy in both 2D and 3D cell culture models.¹³⁴

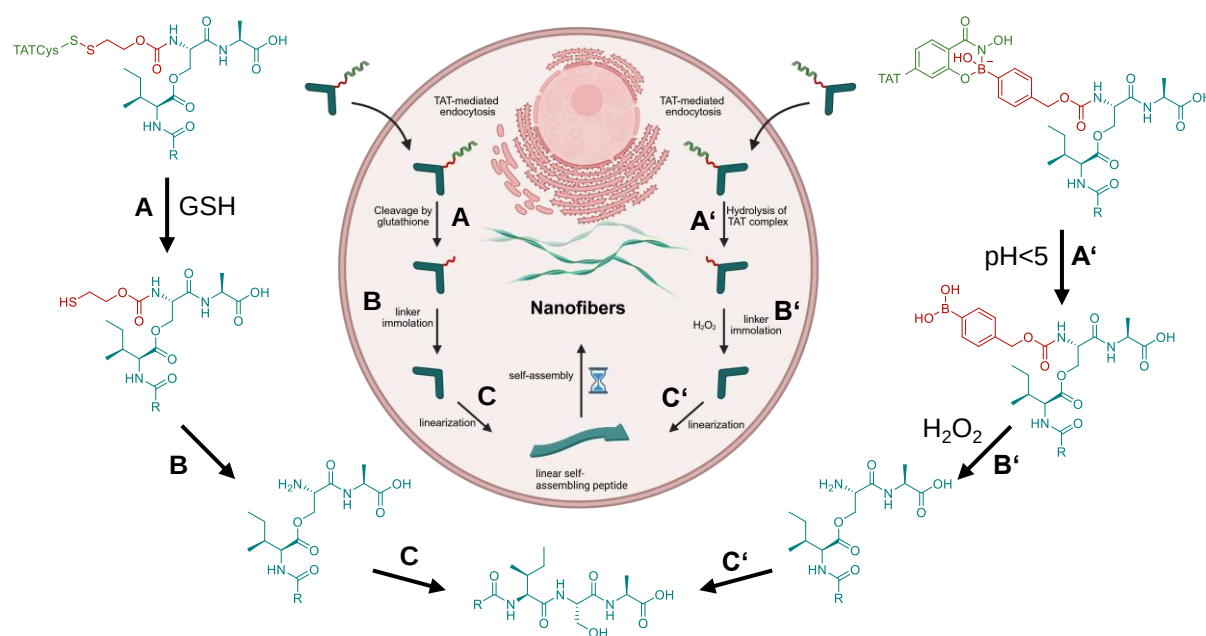


Figure 7: Schematic representation of glutathione (GSH)-induced (left pathway) or pH and reactive oxygen species induced (right pathway) peptide assembly inside living cells. The peptides enter the cell via transactivator of transcription (TAT)-mediated endocytosis and are subjected to GSH or pH dependent cleavage, followed by a multistep conversion into linear self-assembling peptides that assemble into nanofibers. Reproduced from^{134,135} under the terms of CC BY 4.0. Created with Biorender.com.

Tailoring trigger groups to intrinsic biological or chemical cues enables spatially controlled activation of self-assembly within specific cellular regions or compartments. In peptide-based systems, exploiting naturally occurring enzymes, such as metalloproteinases,^{162,163} alkaline phosphatase^{87,164} or furin⁹⁹, offers an elegant and highly selective strategy.^{165,166} Even subtle polarity changes induced by enzymatic dephosphorylation have been shown to be sufficient to initiate self-assembly.¹⁰¹ In other approaches, site-specific proteolytic cleavage by the furin enzyme at the RVRK recognition motif liberates a previously masked proassembling sequence, thereby generating the active, self-assembling monomer.⁹⁹

The inherent complexity and dynamic nature of chemical and biological networks often

necessitates external control elements that enable regulation without interfering with the system itself. While intracellular cues such as pH gradients, redox potentials, or enzymatic activity are powerful tools to generate targeting or probe intracellular environments, their intrinsic coupling to cellular homeostasis often leads to lacking spatiotemporal resolution and renders them difficult to modulate independently without inducing off-target effects. Moreover, such endogenous triggers are typically irreversible or diffusely distributed, limiting their suitability for repeated, localized, and externally programmable control.

In this context, light emerges as a uniquely attractive stimulus, as it can be applied remotely in a non-invasive way with exceptional spatiotemporal precision, tunable intensity, wavelength selectivity, and reversibility. Importantly, light enables external regulation of molecular and supramolecular processes without direct chemical interference with the surrounding environment, thereby minimizing interference with endogenous cellular pathways.¹⁶⁷ These features make light particularly well suited for probing and controlling dynamic supramolecular systems, especially in complex biological settings. Given the central relevance of light-controlled processes to the concepts and applications discussed in the further chapters, the subsequent chapter provides a detailed discussion of photopharmacology, photoresponsiveness and photoreversibility as key strategies for achieving spatiotemporally resolved control over supramolecular organization.

1.3 Photoresponsiveness and Photopharmacology

Light is one of the most fundamental regulatory signals in biology, with visual perception serving as a prominent example. In phototransduction, the initiating step is the isomerization of 11-*cis*-retinal to all-*trans*-retinal, which subsequently activates Rhodopsin *via* a cascade of intermediate steps.⁵⁸ Such processes enable organisms to sense and respond to their environment with extraordinary temporal precision, and illustrate how light can be translated into biochemical and structural responses, thereby affording precise spatiotemporal control over biological function. Beyond these life-science examples, the unique properties of light also make it highly attractive for application-oriented approaches. Incorporation of photoresponsive units into peptides enables remote control over parameters such as intensity, wavelength, duration, and spatial localization, providing external regulation of both structure and function with high spatiotemporal resolution. Furthermore, light represents a non-invasive stimulus with low biological toxicity and can be applied repeatedly without chemical waste generation.¹⁶⁸ Together, these advantageous characteristics render photostimulation particularly well suited for applications in living systems.¹⁶⁹

As such, the field of photopharmacology has emerged as a strategy to mitigate off-target activity and thereby reduce adverse side-effects.¹⁷⁰ By relying on small molecules rather than the larger protein constructs employed in optogenetics, photopharmacological approaches remain compatible with classical pharmacology and drug discovery strategies.^{171,172} Because pharmacokinetic and pharmacodynamic properties are intrinsically linked to the molecular structure, photoinduced structural changes can be rationally designed and modulated toward enhanced therapeutic efficacy.¹⁷³ Despite the many advantages associated with light as an external stimulus, several limitations must be considered. Since the active building blocks must first reach the target site, the achievable spatial resolution is ultimately diffusion limited. In addition, applications in tissues and *in vivo* studies are limited by the penetration depth of light, which depends strongly on wavelength: longer wavelengths penetrate more deeply, whereas shorter wavelengths restrict activation primarily to superficial tissues.^{174,175}

While the advantages of light as an external stimulus are well established, its relevance for supramolecular chemistry arises from how photonic energy can be translated into changes in molecular structure, interactions, and energetics. In self-assembling systems, supramolecular organization is governed by a delicate balance of non-covalent forces and kinetic barriers, such that even subtle molecular perturbations can shift assembly pathways or alter the stability of existing architectures. Light provides a unique tool to introduce such perturbations in a controlled and reversible manner by selectively modifying molecular geometry, polarity, or connectivity without changing the surrounding chemical environment. As a result, light can be used not only to trigger assembly or disassembly, but also to bias pathways, modulate order–disorder transitions, and access non-equilibrium states in supramolecular systems.

In general, three major molecular strategies have been reported for light-directed self-assembly of peptides in chemical and biological systems: photocleavable, photodimerizable and photoswitchable units.¹⁶⁸ The first strategy involves caging of a functional group with a photoprotecting group (PPG), yielding a construct that undergoes irreversible activation upon irradiation. The second relies on intermolecular photodimerization of unsaturated motifs, forming dimeric adducts with altered structural properties. The third approach is based on photoisomerization, in which light induces reversible photoswitching between distinct isomeric states and, consequently between different structural and functional configurations. While photocleavage represents a strictly unidirectional process, both photodimerization and photoswitching can, in principle, be implemented in a reversible manner.

1.3.1 Photocages

Photocleavable systems enable, albeit irreversible, control over self-assembly by inducing one-way chemical transformations that often proceed with high quantum yields and pronounced structural changes. PPGs can be installed at the peptide termini, within side chains, or directly into the peptide backbone, and their cleavage wavelengths can be readily tuned through substitution of the aromatic scaffold.¹⁷⁶ Since those aromatic π -systems are capable of contributing to self-assembly processes by hydrophobic and π - π -interactions, they can further substitute aromatic groups within peptide scaffolds. This substitution thus confer polarity changes to the assembly precursor should they be removed upon irradiation. Therefore, upon irradiation, photocleavage can either liberate a latent self-assembling motif or disrupt an existing one, thereby triggering assembly or disassembly.¹⁷⁷ A key limitation of this strategy, beyond its inherent unidirectionality, is the release of chemical byproducts that can accumulate within the system and lead to adverse side effects.^{176,178} Consequently, the rational design of PPGs requires careful consideration of the potential effects of the cleavage products on the self-assembly or the respective environments. Further requirements for good photocages are a distinct and narrow absorption band, a high quantum yield, and minimal intra- and intermolecular energy transfer. The development of PPGs exhibiting bathochromically shifted absorption maxima, has further expanded their applicability, enabling external control of biological processes using non-toxic and deep-tissue-penetrating NIR light.¹⁷⁹

A widely used class of photoprotective groups comprises *o*-nitrobenzyl-based photocages.¹⁸⁰ These systems combine straightforward synthesis with efficient photorelease upon UV irradiation. However, their reliance on relatively high-energy UV light limits their applicability in biological contexts due to restricted tissue penetration and increased phototoxicity.¹⁸¹ The photorelease mechanism of *o*-nitrobenzyl photocages involves intramolecular hydrogen transfer from the *o*-alkyl substituent to the nitro group, generating transient radical transitional states that subsequently relax through bond cleavage.¹⁷⁶ A prominent example within this class is the nitroveratryloxycarbonyl (Nvoc) group, which is typically cleaved using light at ~360 nm. After photoinduced cleavage, a redox-active aromatic nitroso species is released, which can promote oxidative dimerization of thiols to form disulfides.¹⁸² Combining the nvoc photocage with a masked thiol on a cysteine containing peptide yields a peptide system that can undergo photoinduced rearrangement cascade, ultimately leading to autooxidative disulfide formation, thereby giving rise to complex, pathway-dependent self-assembly behavior, illustrating both the versatility and mechanistic complexity associated with *o*-nitrobenzyl-based photocaging strategies.³⁴

To minimize pronounced cytotoxicity in biological environments, effective systems require activation with low-energy visible light or near-infrared light and the formation of biologically benign byproducts upon uncaging. Coumarin derivatives constitute a class of photocages that

fulfil these criteria (Figure 8 left). Depending on their substitution pattern, their absorption maxima can be tuned across a broad range (ca. 300–500 nm).¹⁸³ Substitution of the carbonyl oxygen in the coumarin core with a malononitrile group increases polarizability, resulting in a bathochromic shift toward visible green light ($\approx$ 505 nm). In the resulting peptide conjugate, a self-assembling peptide sequence is linked to a hydrophilic pentaarginine motif via the modified coumarin. The inherent strong hydrophilicity of the pentaarginine segment initially prevents self-assembly. Upon irradiation, the individual building blocks are released, leading to peptide self-assembly with high spatiotemporal precision.¹⁵⁹

Another class of intricate photocages solving the reliance on UV light for the decaging process by shifting the absorbance into the far visible/ near-infrared light is based on 4,4-difluoro-4-bora-3a,4a-diaza-s-indacenes, more commonly labeled as BODIPY.^{184,185} The bathochromic shift of activation in combination with promising photophysical properties, including large molar absorption coefficients, robustness to photofatigue, high fluorescence quantum yields and tunable spectral properties by multiple modification sites, renders them well-suited for biomedical applications.^{186,187} Despite these excellent premises, the utilization of BODIPY-based photocages in triggered self-assembly of peptides has yet to be explored, with literature rather exploiting the emissive,^{188,189} or photosensitizing¹⁹⁰ properties of the dye.

1.3.2 Photodimerization

Photodimerization is a light-induced addition reaction in which two identical molecules form a covalent dimer upon irradiation.¹⁹¹ A variety of aromatic motifs are known to dimerize when exposed to light of appropriate wavelength. These processes typically proceed via [2+2]- or [4+4]-cycloadditions, which are thermally forbidden under the Woodward-Hoffmann rules¹⁹² but become photochemically allowed, as excitation of an electron alters orbital symmetry and enables cycloaddition.^{191,193} Representative motifs include coumarins and cinnamates for [2+2]-cycloadditions, and anthracenes for [4+4]-cycloadditions (Figure 8 right). Because access to an excited state is crucial for this mechanism, photodimerization is highly sensitive to redox-active species such as oxygen or thiols, which can quench excited states. As such, photodimerization is typically inefficient in the reductive environment of cells.^{176,194} Moreover, effective orbital overlap requires the reacting chromophores to be in close molecular proximity and in a suitable suprafacial conformation, making the reaction strongly dependent on local concentration and specific molecular orientation. Consequently, preorganization within supramolecular assembled structures can potentially impart selectivity and efficiency to photodimerization reactions.¹⁹⁵ Depending on the applied system, photodimerization can

induce both assembly and disassembly, however it is most used to stabilize supramolecular assemblies through light-induced crosslinking.

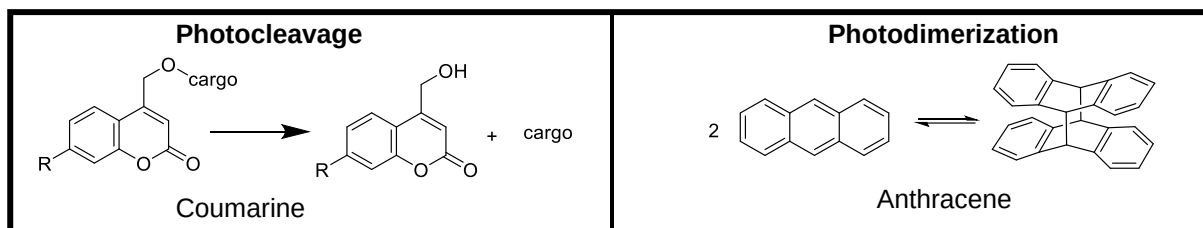


Figure 8: Two classes of photoresponsive motifs used for the controlled assembly of peptides.

A representative example of photo-crosslinking is provided by the enhancement of the mechanical properties of hydrogel formed from a lysine dipeptide functionalized with 7-(diethylamino)-3-coumarincarboxylic acid, where irradiation at 365 nm induces coumarin dimerization and significantly increases stiffness.¹⁹⁶ In the context of nanotubes, photodimerization of anthracene motifs implemented into cyclic peptides scaffolds has been exploited to trigger light-induced bending of peptide nanotubes.¹⁹⁷ The importance of the molecular scaffold, rather than solely the dimerizing chromophore, is highlighted by further studies involving anthracene-modified peptides. Hydrogels formed from anthracene functionalized phenylalanine or tyrosine undergo disassembly upon long-wavelength UV irradiation, as evidenced by clear gel-to-sol-transition. Detailed analysis by COSY NMR spectroscopy revealed the formation of at least three different dimerization products, indicating that inefficient stacking disrupts key π - π -interactions responsible for maintaining the supramolecular network.¹⁹⁸

1.3.3 Photoswitches

Molecular photoswitches are chromophores that undergo reversible isomerization between two states with distinct absorption properties upon irradiation with light of an appropriate wavelength. Typically, one isomer is thermodynamically more stable and can be reached either photochemically or via thermal relaxation from the metastable other state.^{199,200} This interconversion is commonly accompanied by changes in molecular geometry, polarity or π -conjugation, which in turn modulate intermolecular interactions relevant for self-assembly. A key advantage of photoswitches over the aforementioned monodirectional approaches is their reversibility, which enables repeated cycling between states and access to adaptive or far-from-equilibrium behavior, rendering them particularly suited for dynamic and dissipative assemblies.²⁰¹ Moreover, since photoswitching does not rely on bond cleavage, it generally avoids accumulation of chemical byproducts at the target site, reducing the risk of undesired

side effects. In addition, the extent and location of switching can be controlled through irradiation wavelength, intensity, and spatial distribution of the light, enabling graded modulation of material properties. Importantly for bioapplications, many contemporary photoswitch scaffolds can be tuned toward red-shifted visible light switching, improving tissue penetration and compatibility with biological environments.^{202,203} When designing an application-oriented photoswitch system, several photophysical and practical parameters must be considered. First, photoswitching never yields quantitative conversion. Instead, irradiation generates a mixture of isomers whose steady-state distribution (isomerization at equilibrium) under continuous irradiation is termed as the photostationary state (PSS).^{191,203} Thus, a given wavelength may produce, for example, an 80:20 *cis/trans* mixture, and this coexistence can strongly influence assembly behavior. Second, the structural perturbation induced by photoswitching may be too small to overcome strong peptide-peptide interactions in a peptide-photoswitch conjugate, resulting in weak responsiveness at the supramolecular level. Third, repeated cycling can lead to photobleaching effects, diminishing performance over multiple switching cycles.²⁰⁴ In an ideal case, each isomer would selectively absorb at a wavelength where the other isomer is effectively transparent, and both photoisomerization processes would proceed with high quantum yields. Under such conditions, near-quantitative enrichment of a single isomer could be achieved (PSS $\approx$ 100%). While this represents a theoretical limit, PSS values exceeding 95% are accessible in selected systems, typically in combination with favorable absorption profiles and photoisomerization quantum yields in the range of 0.2–0.5.²⁰⁰ Notably, complete switching is often not required to elicit biological effects, as biological responses can be highly nonlinear with respect to the fraction of the active species.²⁰⁵ In nature, only a limited set of photoswitching motifs, predominantly *cis/trans*-isomerizations, are known. In contrast, synthetic chemistry has produced a broad repertoire of photoswitch scaffolds with tunable properties.^{169,172} In the following, key classes of photoswitches are outlined and discussed with respect to their suitability for controlling self-assembly in supramolecular systems. both in vitro and in living cells.

Azobenzenes

Azobenzenes represent the classical and most widely studied class of photoswitchable scaffolds. First reported in the 19th century,²⁰⁶ they have since become central building blocks in molecular switching and have been extensively explored in peptide-based systems.^{207,208} Their appeal stems from generally favorable photochemical properties, straightforward synthesis and a comparatively pronounced geometric change upon irradiation.²⁰⁰ In particular, photoisomerization from the thermodynamically stable, near planar *trans*-isomer to the metastable, bent *cis*-isomer is typically rapid and, for many derivatives, can be cycled with strong resistance to photobleaching.^{203,207} Despite these advantages, conventional

azobenzenes show several limitations relevant to biological and aqueous self-assembly environments. Efficient switching often requires UV or near-UV light, and the partial spectral overlap of the absorption bands can restrict achievable PSSs (commonly 80–95%).²⁰⁰ In addition, the thermal half-lives of the *cis*-isomer are frequently modest, which may complicate applications requiring longer-lived metastable states. Finally, unmodified azobenzenes are often susceptible to reduction under biological relevant conditions, making them less suitable for intracellular applications without further structural optimization.²⁰⁷ Examples for the use of azobenzenes in peptide self-assembly include the incorporation in the peptide backbone as well as in the side chain residues, leading to changes from cross- β , native like fibrils into amorphous aggregates.²⁰⁹

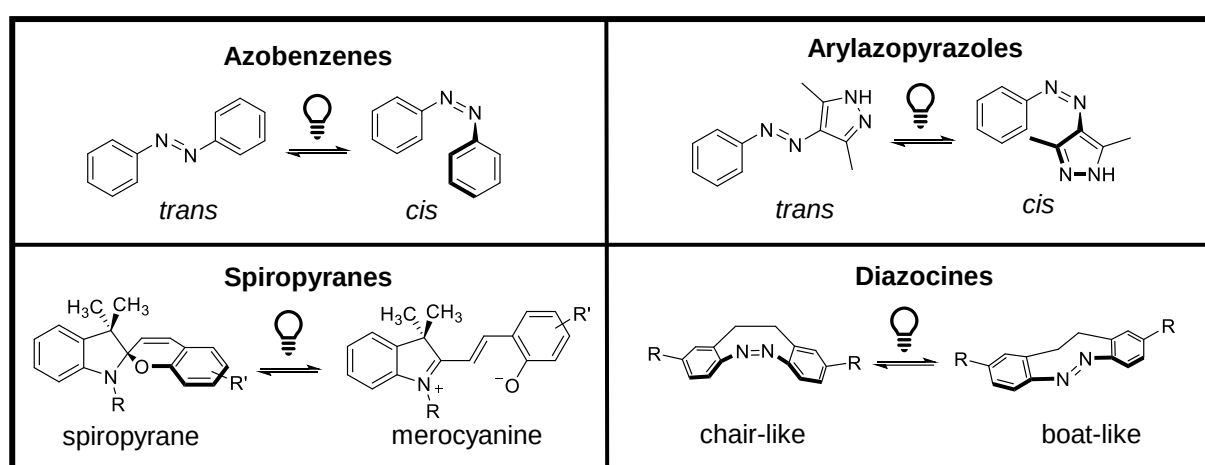


Figure 9: Structures of different photoswitchable scaffolds.

Spiropyranes

Spiropyranes exhibit one of the most pronounced changes in molecular geometry and polarity among photoswitches, as the closed, neutral spiropyran undergoes light-induced ring opening to the planar, zwitterionic merocyanine form. Since this transformation is accompanied by charge separation and a substantial increase in conjugation, dramatic effects on optical properties, dipole moment and solubility are observed.²¹⁰ The spiropyran-merocyanine interconversion is, in principle, reversible, with thermal or visible-light induced back-isomerization restoring the closed form. As such, the incorporation of a spiropyran motif in the backbone of an amphiphilic peptide-alkyl conjugate has been shown to lead to photoresponsive transition between cylindrical fibers and spherical micelles.²¹¹ However, the formation of a highly reactive open-chain merocyanine during the photoisomerization renders spiropyranes susceptible to photofatigue, particularly under the high-energy UV light often required for efficient switching.^{212,213} In addition, the merocyanine form is highly sensitive to environmental factors such as pH, solvent polarity and the presence of oxygen or metal ions and typically display rapid thermal relaxation.²¹⁴ These factors complicate predictable switching

behavior and ultimately limit the suitability of spiropyrans for applications in biological environments.^{212,215}

Diazocines

Diazocines constitute a class of photoswitches that can be regarded as cyclic or bridged azobenzene analogues in which the azo unit is conformationally constrained. As a result of this geometric restriction, diazocines typically exhibit an inverted thermodynamic stability pattern compared to their acyclic counterparts, with the boat-shaped *cis*-isomer being more stable than the chair-like *trans*-isomer. This intrinsic geometric frustration is accompanied by large structural changes upon photoisomerization, making this class of photoswitches particularly interesting for applications requiring large conformational modulation.²¹⁶ A key advantage of diazocines is their responsiveness to visible light, as both forward and backward switching can often be achieved using wavelengths in the visible or near-visible region.²¹⁷ Moreover, many diazocines display high quantum yields and near complete photostationary states. These promising photophysical properties, however, come at the cost of synthetic complexity, as the constrained architecture requires more elaborate synthetic routes than those of unbridged azobenzenes. Moreover, back-isomerization to the metastable *trans* form can be inefficient, and thermal half-lives can be relatively short, ranging from 4–74 h.²¹⁸ Owing to these limitations, diazocines have so far been explored only sparingly in the context of peptide self-assembly.²¹⁹

Arylazopyrazoles

Arylazopyrazols (AAPs) are a class of azoheteroarene photoswitches in which the azogroup bridges a phenyl ring and a five-membered pyrazole heterocycle. Compared to classical azobenzenes, AAPs represent a relatively young but rapidly emerging photoswitch scaffold that is readily accessible with a broad scope of substitution patterns from simple aniline precursors.²²⁰ In general, AAPs exhibit outstanding photophysical properties, including high photostationary state compositions (typically $\geq 90\%$) and exceptionally long thermal half-lives of the metastable *cis* isomer, often surpassing those of their comparable parent azobenzenes.^{220,221} Here, the five-membered pyrazole ring allows access to a *cis*-isomer conformation impossible for six-membered rings and with half-lives of up to 1000 days. Steric tuning of this metastable state by substitution on the heterocycle enhances the $n\text{-}\pi^*$ absorption, resulting in a separation of the absorption profiles of the individual isomers not achievable with azobenzenes.^{220–222} In addition, the incorporation of the heteroaromatic pyrazole ring enhances aqueous solubility and confers increased resistance toward reductive environments, addressing two major limitations of conventional azobenzenes.²²³ These features enable reliable and sustained photoswitching under physiological conditions and

make AAPs particularly well suited for applications in chemical biology and photopharmacology.²²⁴ Ravoo and coworkers have reported the synthesis of carboxylic acid bearing derivatives and highlight their photoreversible behavior with cyclodextrin-functionalized vesicles and gold nanoparticles. In this host-guest approach, excellent light-induced aggregation and dispersion was achieved in water.²²⁴

1.3.4 Photoreversible Self-Assembly

In the previous chapter, the molecular design principles and photophysical properties of photoswitches relevant to peptide-based materials were discussed in detail. Building on this foundation, the present chapter shifts the focus from the photoswitch itself to its functional integration into supramolecular systems. Rather than reiterating photoswitch chemistry, emphasis is placed on how reversible photoisomerization can be translated into reversible control over peptide self-assembly, enabling dynamic modulation of supramolecular order and material properties.

Photoreversible self-assembly refers to a process where assembly and disassembly can be repeatedly and reversibly controlled by light, without permanent chemical changes to the building blocks and without the formation of chemical waste products. It enables pronounced spatiotemporal control over material properties over multiple cycles.^{169,217}

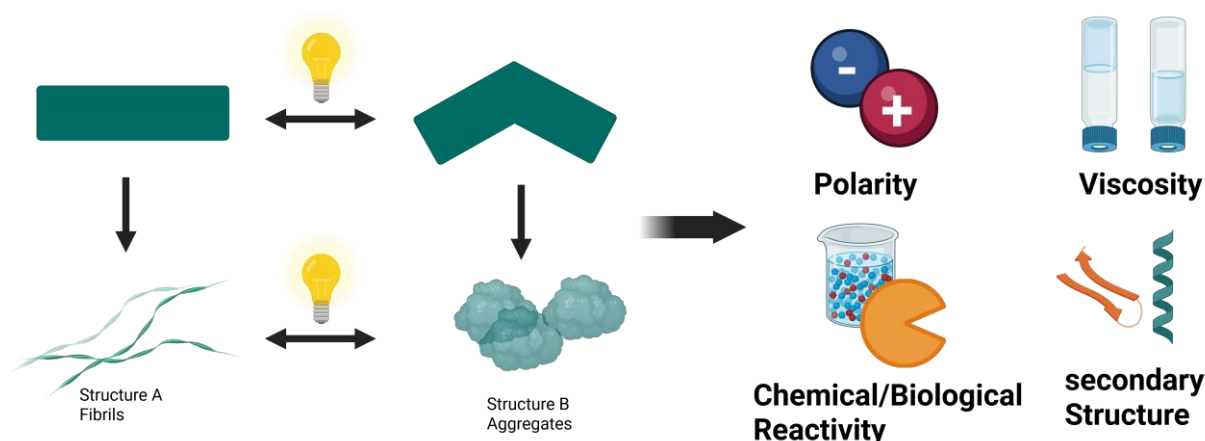


Figure 10: Photoswitching causes changes in molecular orientation leading to different macroscopic structure formation. The structures differ in parameters, such as polarity, bulk viscosity, chemical or biological reactivity and secondary structure. Created with Biorender.com.

To render a self-assembling peptide system photoreversible, both the supramolecular assembly kinetics and the properties of the incorporated photoswitch must be carefully balanced. In contrast to irreversible light-activated systems, both photoinduced states must be sufficiently populated to influence the self-assembly outcome, precluding kinetically trapped states and requiring compatibility with repeated cycling. A key design parameter is therefore

the relationship between the photostationary state and the cooperativity of the assembly process. In cooperative supramolecular polymerization, structure formation or disassembly occurs only once a critical aggregation concentration of monomer is exceeded.²³ Operating at concentrations close to this threshold enables relatively small changes in effective monomer concentration to induce pronounced nanostructure modulation. However, this concentration regime is inherently delicate, since incomplete PSS, kinetically trapping, or photofatigue may limit the reversibility of the process and attenuate corresponding macroscopic responses.^{3,169} Equally important is the interplay between the thermal half-lives of the photoisomeric states and the timescale of supramolecular reorganization. To achieve effective modulation, the lifetime of each isomer must match or exceed the time required for assembly or disassembly with shorter half-lives preventing equilibration, resulting in incomplete or transient states.²⁰¹ Optimal performance therefore demands precise matching of intrinsic switching kinetics with supramolecular dynamics. Beyond molecular switching, photostability at the supramolecular level must also be considered. Repeated irradiation can lead to accumulated photodamage, side reactions or the formation of supramolecular defects, which gradually impair reversibility and alter assembly pathways. Such local defects may impose steric constraints, modify light-penetration behavior, or initiate secondary chemical reactions, ultimately decoupling molecular photoisomerization from the macroscopic material response.^{225,226} Finally, achieving photoreversible self-assembly in biological environments introduces additional challenges, including tissue-dependent light-absorption,²²⁷ competing interactions with biomolecules, and reduced chemical stability in reductive intracellular conditions.^{169,170} Collectively, these considerations highlight the intrinsic difficulty of balancing supramolecular physical chemistry and photophysics, and underscore the need for integrated design strategies when developing photoreversible self-assembling systems.

In general, both photoswitchable scaffolds and photodimerization approaches can be employed to realize photoreversible self-assemble. In peptide-based systems, even subtle conformational changes can strongly influence intermolecular forces and thereby determine self-assembly efficiency. Consequently, alterations in polarity and geometry within a preformed structure may induce disassembly by weakening directional thermodynamic contributions such as hydrogen bonding, or π - π -interactions. Reversing the photochemical stimulus restores the original molecular state and can re-establish the conditions required for self-assembly.

A second mode of photoreversible control relies on the reversible crosslinking of building blocks, which can lead to network formation, increased rigidity, or gelation, while back-irradiation regenerates the original, unlinked state. In photodimerization-based systems, this behavior arises from the dynamic formation and cleavage of inter-unit covalent connections without fundamentally disrupting the underlying supramolecular framework. Such reversible dimerization processes are often based on [2+2]-cycloadditions of coumarin groups,

exemplified by the light-induced reversible self-assembly of gold nanoparticles over four irradiation cycles.²²⁸ The stringent requirement for close molecular proximity in photodimerization reactions can also be exploited in host-guest-systems, where confinement within a host cavity enables fine-tuning of dimerization kinetics. This concept has been demonstrated using anthracene derivatives as guest molecules encapsulated within a flexible coordination cage.²²⁹ Photoswitchable scaffolds can likewise be incorporated into such host-guest-systems to achieve reversible crosslinking, resulting in pronounced sol-gel transitions in hydrogel materials. In such systems, the photoswitch scaffold typically acts as a guest that fits selectively into the cavity of a host receptor. Photoisomerization then alters its geometry and binding affinity, thereby triggering disassembly. Common host motifs include crown-ethers²³⁰ and cyclodextrins, yielding hydrogels whose stiffness can be externally modulated by light. These materials were demonstrated to have excellent self-healing ability, as softened gels can merge after mechanical damage and subsequently be re-stiffened upon irradiation.^{231,232}

Beyond binary on-off assembly-disassembly behavior, photoreversible self-assembly can also enable reversible transitions between distinct supramolecular morphologies. Light-induced changes in molecular shape, polarity and interaction anisotropy may modulate assembly between fibers, sheets, micelles or aggregates without complete disassembly. Here, self-assembly is not disrupted entirely, but reprogrammed toward alternative packing arrangement, introducing a form of pathway complexity by enabling reversible access to multiple functional states from the same building blocks. Exploiting the pronounced changes in molecular geometry and dipole moment induced by photoisomerization, a single molecular building block can give rise to a variety of supramolecular architectures depending on the irradiation conditions. In such systems, shorter irradiation times have been shown to promote the formation of wormlike micelles, lamellae structures, and bigger vesicles, whereas prolonged irradiation favored the generation of small micelles.²³³ Moving toward more application-oriented systems, photoinduced and reversible transition between vesicles and nanoparticles has been successfully employed to explore switch-controlled drug release.²³⁴ Beyond these microscale effects, photoisomerization has also been exploited at the macroscopic level, where light-induced structural transitions between nanohelical assemblies and irregular aggregates resulted in dynamic photoactuating behavior driven by external irradiation.²³⁵

2. Motivation

Self-assembly in nature gives rise to highly ordered architectures formed from minimal building blocks. While the second law of thermodynamics dictates that isolated systems evolve toward states of maximal entropy, local order formation is fully compatible with this principle in open systems that exchange energy and matter with their environment. Living systems maintain such organized states far from thermodynamic equilibrium by continuously dissipating energy. In his seminal work "*What Is Life?*", Erwin Schrödinger described this capability as feeding on "negative entropy"²³⁶, highlighting how biological systems sustain and regulate order under dynamic, non-equilibrium conditions. While originally formulated to rationalize biological organization, this principle poses a broader physical question: how ordered structures can emerge, persist, and adapt in systems governed by reversible interactions and energy flux. In supramolecular chemistry, self-assembly provides a synthetic foundation to explore this challenge, where molecular building blocks organize into higher-order architectures through non-covalent, reversible interactions. However, unlike biological systems, synthetic supramolecular systems are largely designed to converge toward a single equilibrium state, limiting their adaptability and functional diversity.

Supramolecular peptide self-assembly offers access to adaptive and functional nanomaterials, yet precise control over assembly pathways, reversibility, and structural order remains challenging, particularly under non-equilibrium and biologically relevant conditions. Despite extensive reports of peptide-based assemblies, rational design principles that directly link molecular structure to dynamic supramolecular behavior are still insufficiently explored.

The objective of this thesis was therefore to identify molecular design principles that enable controlled, reversible, and stimulus-responsive self-assembly of peptide-based supramolecular systems. Particular emphasis was placed on understanding how even subtle structural modifications, such as linker flexibility, aromatic interactions and the incorporation of photoresponsive elements, translate into distinct transitions between ordered and disordered states.

To address these objectives, minimalistic peptide systems are designed to systematically probe the influence of individual molecular parameters on self-assembly. First, the influence of linker flexibility between the peptide backbone and the self-assembly-promoting π -block is examined as a key, yet often overlooked, design parameter for generating ordered supramolecular architectures. In amphiphilic peptide conjugates, the spacer does not merely connect two functional units, but can also dictate how efficiently aromatic π - π -interactions, backbone hydrogen bonding, and hydrophobic collapse cooperate during nucleation and growth. In particular, increased flexibility is expected to enable the aromatic motif to adopt orientations supporting ordered packing without imposing strain on the peptide backbone. This

should enhance cooperative coupling between π -stacking and peptide hydrogen bonding, reduce packing frustration and thereby lower kinetic barriers to assembly. As a result, more flexible linkers are anticipated to promote stronger assembly propensity and potentially increase long-range order. Conversely, overly rigid or geometrically mismatched linkers may decouple these interactions, favoring the formation of disordered aggregates. To address these hypotheses, a systematic series of short, pro-assembling peptides bearing *N*-terminal aromatic modifications is developed. While maintaining a constant combination of peptide sequence and attached π -block, the peptide conjugates differ only in the length and flexibility of the spacer. This minimalistic design allows spacer effects to be isolated from changes in overall composition and aromatic content. By correlating spacer properties with assembly readouts, such as nanostructure morphology and concentration-dependent assembly behavior, structure-assembly relationships are assessed to determine how linker flexibility and length govern the emergence of supramolecular order or disorder.

Second, external stimuli are introduced to actively regulate transitions between order and disorder. Among the available external input signals, light offers exceptional spatiotemporal precision while remaining non-invasive and, when employed with photoswitches, avoiding the generation of chemical by-products. One such strategy to achieve light-induced regulation is the photodimerization of aromatic moieties, which is accompanied by pronounced changes in molecular geometry and planarity and can thereby strongly alter intermolecular interactions that govern self-assembly. Incorporation of such photoactive anthracene units into peptide sequences enables access to distinct supramolecular architectures whose stability can be modulated by irradiation. To introduce an additional level of control, pH-responsiveness is embedded within otherwise comparable peptide sequences, allowing environmental sensing of the supramolecular organization. This design promotes preorganization of the photoactive units within the assemblies, which can then be exploited to enhance the rate of photodimerization reactions through spatial proximity. This photodimerization is expected to disrupt effective aromatic interactions and introduce unfavorable stacking geometries, thereby inducing a transition from ordered nanostructures to disordered states. While this anthracene-based photodimerization approach enables efficient light-induced disassembly, it is inherently unidirectional. Introducing dynamic control into supramolecular systems therefore represents a critical next step, as external energy input enables switching between distinct photostationary states, providing deeper insight into the self-assembly pathways and the kinetics governing structural transitions.

To overcome this unidirectionality of the photodimerization approach, a peptide system is designed to enable reversible switching between supramolecular order and disorder. Incorporation of a photoswitchable moiety at the *N*-terminus of a short pro-assembling peptide sequence yields a photoresponsive conjugate capable of undergoing reversible structural

modulation upon irradiation. Arylazopyrazols were selected as representative photoswitch scaffolds owing to their favorable photophysical properties, including long thermal half-lives, high resistance to photofatigue, and well-defined photostationary states. Photoisomerization of the switch is expected to induce pronounced changes in molecular geometry and aromatic interactions, thereby enabling reversible transitions between ordered and disordered supramolecular states. Such reversible control over distinct states enables repeated interrogation of assembly and disassembly pathways.

To translate this concept to biological environments, several prerequisites must be met. The assembly precursor must (i) enter cells in a monomeric, non-assembled state and (ii) be transformed intracellularly into the assembling motif. A common strategy to achieve this relies on masking the assembling unit with a hydrophilic unit that is cleaved inside the cell. However, multi-step activation cascades offer additional advantages: they allow the integration of multiple bioresponsive triggers into the design of the precursor, thereby providing tighter control over the active monomer generation. Incorporating a photoresponsive unit as the self-assembly promoting π -block further complements these endogenous cues with an orthogonal external control element.

To address these requirements, the initially linear peptide design can be converted into a kinked isopeptide that acts as a non-assembling precursor. Such isopeptides contain an ester linkage at a serine side chain, which introduces a conformational kink and disrupts premature assembly. Crucially, this architecture also enables the installation of a bioresponsive protecting group at the amino group of the esterified serine. Upon intracellular uncaging, the isopeptide undergoes rearrangement, restoring the native linear backbone and thereby regenerating the self-assembling sequence.

A particularly useful intracellular trigger is glutathione, a highly abundant reducing agent in mammalian cells. Attaching a glutathione-cleavable disulfide linker to the serine amino group enables reductive cleavage inside the cytosol and simultaneously provides a handle for conjugation to a cell-penetrating peptide to promote uptake. After endocytosis and glutathione-mediated disulfide reduction, the caging group undergoes self-immolation, releasing the free amino group and ultimately yielding the linearized self-assembling building block. Coupling this activation strategy to photoswitch-controlled assembly facilitates efficient cellular delivery while preserving precise control over supramolecular organization, thereby allowing the effects of intracellular order versus disorder to be probed using a single molecular framework. By correlating aggregation propensity with cell viability, this approach aims to elucidate how intracellular supramolecular assemblies influence cellular processes, including pathways associated with apoptosis and cell death.

Collectively, this work aims to contribute to the development of adaptive supramolecular

peptide systems that operate beyond static equilibrium structures and approach the dynamic complexity of biological assemblies.

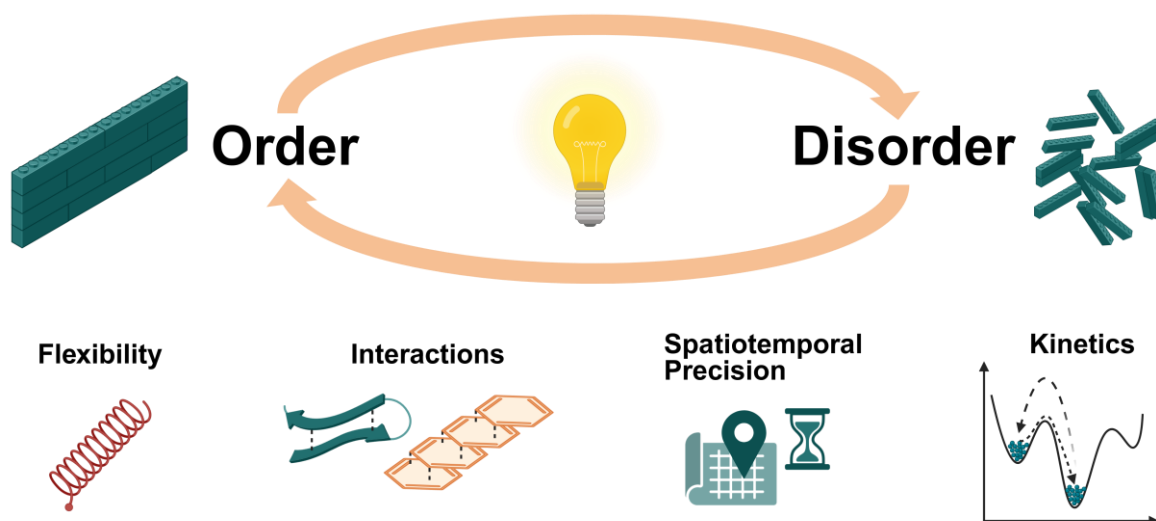


Figure 11: Scope of this thesis: Identifying fundamental design principles of self-assembling peptides (chemical design) with the control over their thermodynamics and kinetics (energy landscape) to achieve reversible spatio-temporal control of their assemblies and order-disorder transitions. Created with Biorender.com.

3. Decoding the Role of Spacer Length and Flexibility in Molecular Peptide Self-Assembly

Authors

Julian Link[‡], Albin Lahu[‡], Manfred Wagner, David Y. W. Ng^{*}, Tanja Weil^{*}

[‡] Authors contributed equally to this work

^{*} Corresponding Author

The work presented in this chapter was submitted to Beilstein Journal of Organic Chemistry.

Date of Submission: 02.03.2026

Scientific Context and Research Aim

The rational design of peptide-based nanomaterials relies on the control of non-covalent interactions governing the self-assembly. While the influence of the peptide sequence and the aromatic moiety on the self-assembly of short amphiphilic peptides has been extensively studied, the role of the linker segment connecting these two functional domains remains comparatively underexplored. This represents a substantial gap in the fundamental understanding of supramolecular structure-property relationships. Specifically, the influence of spacer flexibility on the cooperativity of π - π stacking and hydrogen bonding and, consequently on the fidelity of the resulting nanostructures remains unclear. The primary aim of this project was therefore to systematically investigate the influence of linker flexibility on the self-assembly behavior of short amphiphilic peptides. By addressing this question, the study tries to establish design rules that link molecular geometry to macroscopic outcomes.

Experimental Approach and Methodology

To address this challenge, a systematic series of ICA peptides was designed, each N-terminally modified with a naphthalene-imide fluorophore. The peptide sequence and aromatic unit were kept constant while only varying the length and flexibility of the spacer segment. This minimalist approach allowed for the isolated analysis of linker effects without other variables. The assembled structures were analyzed using complementary spectroscopic and microscopic methods. Secondary structure formation was investigated via circular dichroism (CD) spectroscopy, while morphology was visualized using transmission electron microscopy

(TEM) and atomic force microscopy (AFM). To scrutinize the thermodynamic stability and assembly propensity, NMR spectroscopy was employed using organic cosolvent titration methods. Furthermore, the photophysical properties of the naphthalene-imide unit were exploited to analyze the supramolecular environment.

Personal Contribution

I was deeply involved in all stages of this project, from conceptualization to finalizing the manuscript. Specifically, I contributed to the conceptual design of the molecular library and the experimental workflows. I synthesized all peptide conjugates and associated reference molecules, ensuring high purity for all subsequent analyses. My primary responsibility was the structural and photophysical characterization of the systems. I performed and analyzed the CD spectroscopy data to determine secondary structure content and conducted TEM imaging to assess nanoscale morphologies. Furthermore, I performed the photophysical investigations, performing UV/Vis- and fluorescence spectroscopy to correlate assembly states with optical properties of the naphthalene-imide segment. I conducted the NMR spectroscopy experiments, including the cosolvent titration series, to quantify self-assembly propensity and determine critical concentration regimes. Finally, I took a leading role in the interpretation of the data and the writing and editing of the final manuscript.

Collaboration and Supervision

This project was conducted in close collaboration with Albin Lahu, with whom I share the first authorship. We jointly conceptualized the project and shared responsibilities in peptide synthesis, CD spectroscopy, and TEM analysis. Albin Lahu additionally led the aggregation assays using HPLC and Proteostat methods. Support in NMR data analysis and experimental setup was provided by Manfred Wagner. The overall research concept and results were discussed and assessed with David Y. W. Ng and Tanja Weil, who also provided critical feedback and supported the editing of the manuscript. Funding for this project was acquired by Tanja Weil that enabled this research.

Outcome and Significance

The work successfully demonstrated that linker flexibility is a critical design parameter capable of tuning supramolecular order. The findings revealed that increased spacer flexibility promotes cooperative interactions and ordered structure formation, whereas rigid linkers can lead to structural heterogeneity. These insights provide a foundational framework for the rational design of peptide-based materials with tailored functional properties.

Decoding the Role of Spacer Length and Flexibility in Peptide Self-Assembly

Julian Link^{†1}, Albin Lahu^{†1}, Manfred Wagner¹, David Y. W. Ng^{*1}, Tanja Weil^{*1}.

¹Max Planck Institute for Polymer Research, D-55128 Mainz, Germany.

Email: ng@mpip-mainz.mpg.de, weil@mpip-mainz.mpg.de.

* Corresponding authors.

† These authors contributed equally.

Abstract

Spacer length is a key molecular parameter governing the self-assembly of short peptides. Here, we investigate isoleucine-cysteine-alanine (ICA) tripeptides containing carbon spacers of 6, 3, or 0 atoms linking the peptide backbone to a hydrophobic naphthalene (Nap) π -block. Using complementary spectroscopic and microscopic techniques, we show that spacer length controls the balance between conformational flexibility and directional non-covalent interactions, thereby dictating assembly pathways and material properties. The results establish a correlation between spacer length and assembly propensity, with the longest spacer (C_6) consistently promoting aggregation more effectively than the intermediate analogue (C_3), whereas peptides containing the rigid C_0 -spacer fail to develop ordered nanostructures. These findings identify spacer length as a powerful design parameter for tuning peptide self-assembly across multiple length scales.

Keywords

Peptide Amphiphiles; Structure-Property Relation; Self-Assembly; Spacer Length; Nanotechnology

Introduction

Short self-assembling peptides are an eminent class of materials used in supramolecular chemistry due to their advantageous properties such as facile synthesis, programmable molecular information^[1–4], structural diversity^[5,6], biocompatibility^[7–9] and responsiveness to environmental stimuli.^[10–12] These peptide monomers can be synthesized in high throughput through solid-phase peptide synthesis (SPPS) and have shown a great structural versatility ranging from the formation of different secondary structures, such as α -helices^[13], β -sheets^[14,15] and β -turns^[16], to the assembly into distinct supramolecular morphologies, for instance nanosheets^[17,18], droplets^[19], nanotubes^[20,21], nanofibrils^[22,23] and coiled coils.^[24,25] The information of the secondary structures and supramolecular morphologies are encoded within the sequence, similar to the primary structure of proteins, therefore enabling on-demand production of hierarchical nanostructures. Besides the sequence, the implementation of hydrophobic elements, such as aromatic π -blocks, steroids or alkyl chains, have been successfully applied to enable and modulate the self-assembling properties of short peptides through directional and cooperative non-covalent forces.^[26,27] Coupling these to the *N*-terminus of the peptide sequence leads to the formation of peptide amphiphiles with a general architecture of a hydrophobic tail attached to a hydrophilic peptide domain.^[28,29] Such peptide amphiphiles have emerged as powerful building blocks to engineer highly-ordered nanostructures in biomedicine^[30], nanoscience^[31], and cosmetics.^[32]

While extensive efforts have focused on varying peptide sequences^[33] and hydrophobic motifs^[34], the role of the linker (or spacer) between these domains has received comparatively little attention. Previous studies indicate that the flexibility and rigidity of the spacer segment strongly influence self-assembly, gelation and bulk mechanical properties of hydrogels.^[35] In contrast, studying peptide amphiphiles at lower concentrations, where ordered nanostructures form without macroscopic gelation, important in elucidating the molecular principles of self-assembly.

To address this gap, we designed a series of peptide amphiphiles with systematically varied spacer lengths. The peptide scaffold consists of the pro-assembling tripeptide **Ile-Cys-Ala** (ICA), which forms fibrous networks upon functionalization with aromatic groups at the *N*-terminus.^[36] To enable both, π – π interactions and fluorescence-based characterization, we chose a naphthalene imide fluorophore as the hydrophobic segment (Nap). The fluorophore was attached either directly to the peptide *N*-terminus (C_0 -spacer), via β -alanine (C_3 -spacer) or 6-aminohexanoic acid (C_6 -spacer), yielding a systematic set of peptide-fluorophore conjugates (Scheme 1). This design allows us to dissect the relationship between spacer length and hierarchical self-assembly. The peptides have been analyzed with respect to their assembly properties using assembly conversion determinations, fluorescence microscopy, transmission electron microscopy (TEM), atomic force microscopy (AFM), circular dichroism

(CD) spectroscopy and nuclear magnetic resonance (NMR) spectroscopy. The results of this work emphasize the importance of π -block flexibility on supramolecular assembly as improved assembly characteristics can be observed with increasing spacer-lengths. Therefore, we hypothesize that the spacer-length influences assembling properties of amphiphilic peptides directly through the rotational freedom of the π -block, thus enabling or limiting the possible intermolecular interactions. These results help understanding the fundamental role of spacers within peptide amphiphiles and improve the rational design of future peptide assemblies.

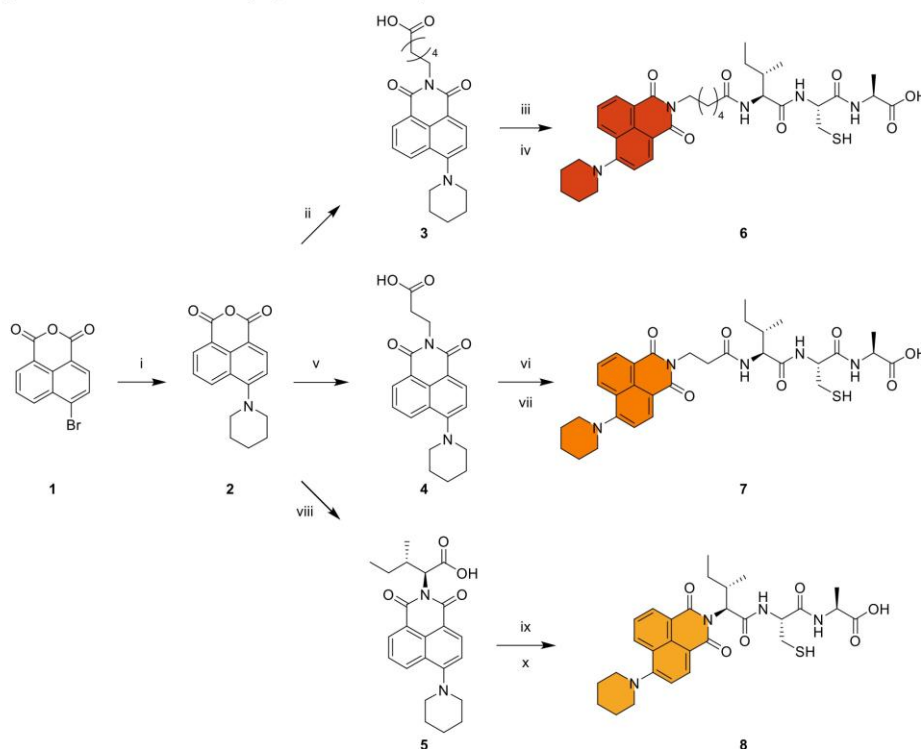
Results and Discussion

Synthesis

The naphthalene imide spacers were synthesized in two steps starting from 4-bromo-1,8-naphthalic anhydride **1** (Scheme 1 and S1). First, the bromo-substituent in compound **1** was replaced by piperidine in a metal-free Buchwald-Hartwig amination reaction to obtain 6-(piperidin-1-yl)-1*H*,3*H*-benzo[de]isochromene-1,3-dione **2** with good yield (79 %, Figures S1-S6) after purification through recrystallization from ethanol.^[37] Compound **2** was used as the precursor for the synthesis of the C₆-/C₃- and C₀-spacer (**3**, **4** and **5**) building blocks. To synthesize the C_x-spacers, the anhydride was transformed into the imides by refluxing the corresponding amines with the anhydride in the presence of a catalytic amount of 4-(dimethylamino)pyridine (4-DMAP). Through reaction of **2** with 6-amino-hexanoic acid, 6-(1,3-dioxo-6-(piperidin-1-yl)-1*H*-benzo[de]isoquinolin-2(3*H*)-yl) hexanoic acid (C₆-Nap) **3** was obtained. 1*H*-Benz[de]isoquinoline-2(3*H*)-propanoic acid (C₃-Nap) **4** was synthesized through reaction of **2** with β -alanine and (2*S*,3*S*)-2-(1,3-dioxo-6-(piperidin-1-yl)-1*H*-benzo[de]isoquinolin-2(3*H*)-yl)-3-methylpentanoic acid (C₀-Nap) **5** was obtained from compound **2** and L-isoleucine. The products were washed with 1 M HCl and brine and did not require further purification. The naphthalene imides **3**, **4** and **5** were obtained with good yields (87–99 %) and in high purities (90–95 %) as characterized by liquid chromatography mass spectrometry (LC-MS) and NMR spectroscopy (Figures S7-S24).

The target tripeptide (ICA) was synthesized in a microwave assisted peptide synthesizer using the fluorenylmethyloxycarbonyl (Fmoc) solid-phase peptide synthesis strategy^[38], synthesizing the peptide from the C- to N-terminus according to the general procedure described in the experimental section. To synthesize the C₆- and C₃-spacer variants, C₆-Nap-ICA **6** and C₃-Nap-ICA **7**, the free amine was functionalized with the naphthalene imides **3** and **4** respectively, using ethyl cyanohydroxyiminoacetate (OxymaPure) and *N,N'*-diisopropylcarbodiimide (DIC) in dry dimethylformamide (DMF). After the coupling, the peptides were cleaved from the resin under acidic conditions (95 % TFA) in the presence of TIPS (2.5 %) and H₂O (2.5 %) acting as reducing agents and carbocation scavengers to suppress side reactions.^[39] Furthermore, for the peptide synthesis chlorotriyl (*Clt*)-resin was chosen instead of the commonly used *Wang*-

resin, to minimize S-alkylation of the thiol side-chains.^[40] C₀-Nap-ICA **8** was obtained through coupling of C₀-Nap **5** to Cys-Ala-Clt-resin using benzotriazol-1-yloxytriethylphosphonium hexafluorophosphate (PyBOP®) and *N,N*-diisopropylethylamine (*N,N*-DIPEA). All peptides were purified via high performance liquid chromatography (HPLC) and subsequently characterized using LC-MS and NMR spectroscopy revealing high purities (> 95 %) with overall yields between 8–20 % (Figures S25-S33).



Scheme 1: Synthetic scheme for the naphthalene-peptide conjugates C₆-Nap-ICA **6**, C₃-Nap-ICA **7** and C₀-Nap-ICA **8**. i) Piperidine, 2-methoxyethanol, 125 °C, overnight; ii) 6-aminohexanoic acid, ethanol, reflux, overnight; iii) ICA-Clt-resin, Oxyma, DIC, DMF, room temp., overnight; iv) TFA (95 %), TIPS (2.5 %), H₂O (2.5 %), room temp., 2 h; v) 3-aminopropanoic acid, ethanol, reflux, overnight; vi) ICA-Clt-resin, Oxyma, DIC, DMF, rt, overnight; vii) TFA (95 %), TIPS (2.5 %), H₂O (2.5 %), room temp., 2 h; viii) L-isoleucine, ethanol, reflux, overnight, ix) CA-Clt-resin, PyBOP®, *N,N*-DIPEA, DMF, room temp., 2 h; x) TFA (95 %), TIPS (2.5 %), H₂O (2.5 %), room temp., 2 h.

Supramolecular Self-Assembly

Next, we analyzed the naphthalene-containing peptides **6**, **7** and **8** regarding their self-assembling properties. Initially, the peptides were dissolved in 2,2,2-trifluoroethanol (TFE) to form a stock solution (10 mM) and added to phosphate-buffered saline (PBS, 50 mM, pH 7.4) to obtain 100 μM final peptide and 1 % final TFE concentrations.

By diluting the peptides in neutral aqueous buffer conditions, the hydrophobic intermolecular interactions of the peptide molecules are promoted, thereby inducing self-assembly into supramolecular structures. Here, the π - π -interactions of the naphthalene-moieties, which are

expected to be directly influenced by the flexibility of the spacer-length, become the driving force for the self-assembly of the peptide monomers.

The assembly conversion of peptide monomers to supramolecular structures was analyzed using an analytical HPLC-based study (Figure 1A).^[41] Using high concentration stock solutions in TFE (10 mM) followed by dilution into PBS buffer (50 mM, pH 7.4), final peptide concentrations of 100 μ M with 1 % TFE were obtained. The diluted solutions were incubated for 24 h at room temperature to allow for self-assembly. Identical preparations in methanol (1 % TFE) were used as non-assembled, molecular references. After incubation, the samples were filtered (0.2 μ m) to remove any aggregates or assembled supramolecular structures in solution and Tyramine (200 μ M in MeOH, Standard) was added to the filtrate as an internal standard. In methanol, where no assembly occurs, the retention of the analyzed peptides occurs between 16 and 18 min (Figure S34). In contrast, lower peptide monomer signals were detected in the PBS-incubated samples as self-assembly removes the peptide monomers from the bulk solution. The assembly conversion rates were calculated based on the integrated absorbance intensities at 214 nm and are presented in Figure 1A as bar plots. Interestingly, high assembly conversion of the molecular state to the assembled state can only be observed for C₆-Nap-ICA **6**, (86 ± 3 %). At the tested concentration, C₃-Nap-ICA **7** assembles at (41 ± 1 %), whereas C₀-Nap-ICA **8** only assembles minimally, having a low assembly conversion rate of (14 ± 6 %) and therefore indicating that intermolecular interactions are weaker for the peptide monomers with short spacer-lengths. The obtained results were confirmed by Proteostat® Aggregation Assay (Figure 1B). Proteostat® fluorescence increases upon binding to hydrophobic environments present in supramolecular peptide assemblies, particularly β -sheets.^[42] Compared to the PBS buffer (50 mM, pH 7.4, 1 % TFE) controls, an increase in fluorescence can be observed with increasing spacer length, with highest fluorescence detected for C₆-Nap-ICA **6**.

Subsequent analysis of the peptide structures using fluorescence microscopy with a green fluorescence filter ($\lambda_{\text{ex}} = 470/40$ nm, $\lambda_{\text{em}} = 525/50$ nm) further confirmed the absence of self-assembled structures for C₀-Nap-ICA **8** (Figure 1D and S35). For C₃- (**7**) and C₆-Nap-ICA **6**, the formation of supramolecular structures was observed. Determination of the aggregate sizes revealed large aggregates for C₆-Nap-ICA **6** at (20 ± 12) μ m compared to small aggregates for C₃-Nap-ICA **7** at (4 ± 2) μ m size (Figure 1C,D).

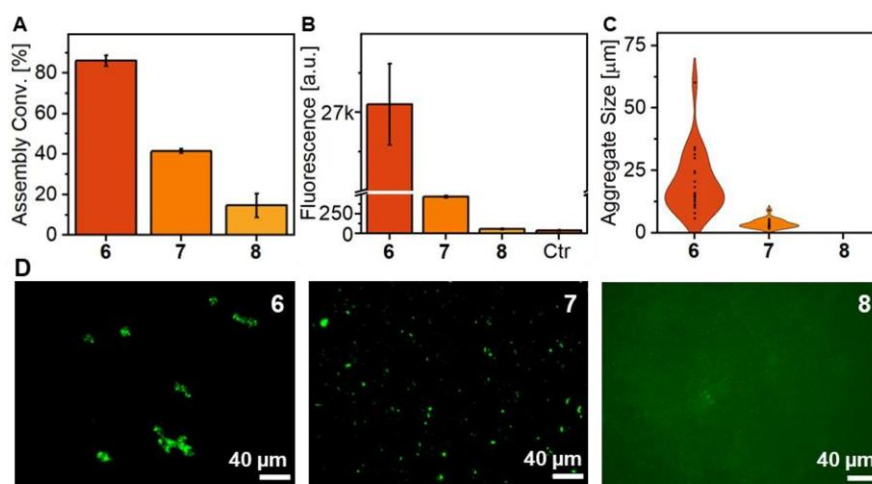


Figure 1: **A** Bar plot representing the assembly conversion of C₆-Nap-ICA **6**, C₃-Nap-ICA **7** and C₀-Nap-ICA **8** (all 100 μM) in PBS (50 mM, pH 7.4, 1 % TFE) determined through analytical HPLC. **B** Bar plot showing the fluorescence intensity of Proteostat® at $\lambda_{em} = 600$ nm ($\lambda_{ex} = 500$ nm) after incubation with C₆-Nap-ICA **6**, C₃-Nap-ICA **7** or C₀-Nap-ICA **8** (all 100 μM) in PBS (50 mM, pH 7.4, 1 % TFE) for 24 h at room temperature. Control shows the fluorescence of Proteostat® in PBS (50 mM, pH 7.4, 1 % TFE). **C** Violin plot depicting the distribution of assembly sizes for C₆-Nap-ICA **6**, C₃-Nap-ICA **7** and C₀-Nap-ICA **8** (all at 100 μM) in PBS (50 mM, pH 7.4, 1 % TFE) after 24 h incubation at room temperature. The sizes were determined using Keyence Microscopy, $n = 21$. **D** Fluorescence microscopy images of C₆-Nap-ICA **6**, C₃-Nap-ICA **7** or C₀-Nap-ICA **8** (all 100 μM) in PBS (50 mM, pH 7.4, 1 % TFE), scale bar = 40 μm.

Concentration-dependent TEM studies were performed to gain deeper insight into the correlation of spacer-length and the formation of nanostructures (Figure 2B, Figure S36). For this purpose, three peptide concentrations were tested, namely 50, 100 and 200 μM in PBS (50 mM, pH 7.4, 1 % TFE). Direct attachment of the fluorophore to the peptide backbone (**8**) did not result in ordered structures at any tested concentration: no assemblies were observed at 50 μM, while only small disordered aggregates were formed at 100 and 200 μM. In contrast, both the C₃- and C₆-spacer variants formed well-defined nanofibers. The C₃-variant **7** exhibited fibers only at 100 and 200 μM, whereas the C₆-variant **6** displayed fibers even at 50 μM (Figure S36). To further analyze these fibrillar morphologies, dry-state AFM measurements with tapping mode at 250 kHz were performed (Figure S37). The peptides were assembled in MilliQ-H₂O (1 % TFE) with ordered, fibrillar structures observed for C₆-Nap-ICA **6** with height profiles ranging from 2.4–6.9 nm. C₃-Nap-ICA **7** also displayed fibrillar structures, however their vertical distance was observed to be much lower (0.7–0.8 nm). In contrast, **8** showed no supramolecular structures (Figure S37). Control TEM-experiments in MilliQ-H₂O (1 % TFE) revealed fibrillar structures for peptides **6** and **7** and no supramolecular structures for **8** (Figure S38). Therefore, the AFM-results confirm the results of observed supramolecular structures in TEM and indicate a strong correlation of spacer-length and supramolecular assembly as well as morphology.

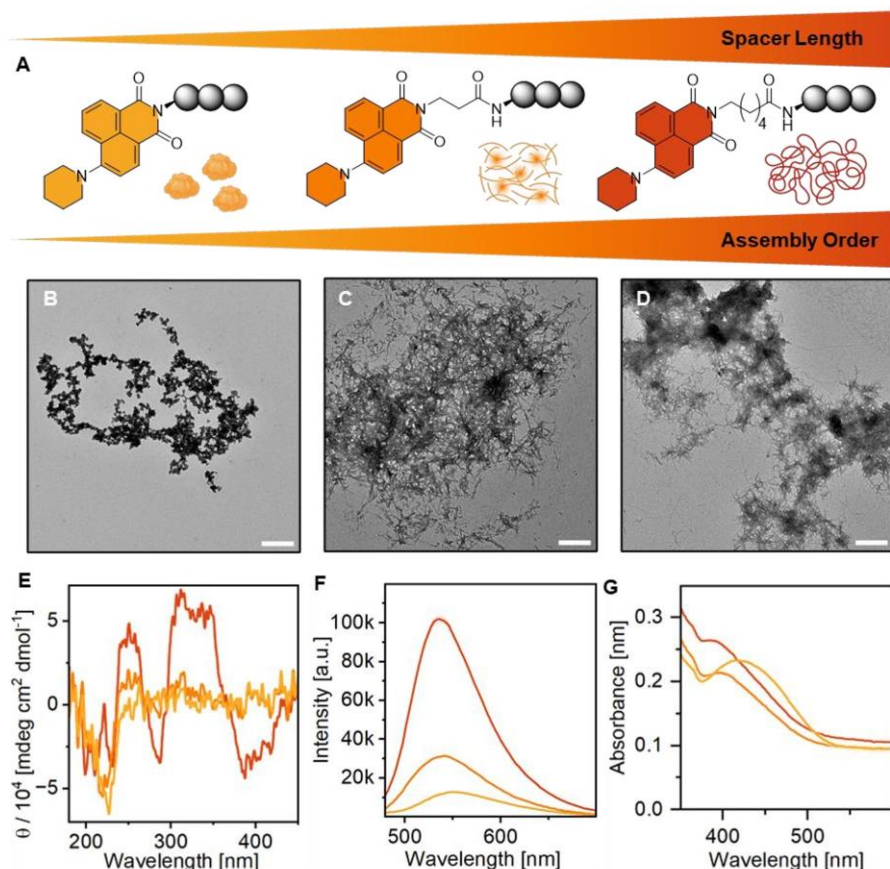


Figure 2: **A** Scheme depicting the self-assembly process in aqueous media. **B** TEM micrograph of C₀-Nap-ICA **8** (100 μM) in PBS (50 mM, pH 7.4, 1 % TFE) after 24 h incubation at room temperature. **C** TEM micrograph of C₃-Nap-ICA **7** (100 μM) in PBS (50 mM, pH 7.4, 1 % TFE) after 24 h incubation at room temperature. **D** TEM micrograph of C₆-Nap-ICA **6** (100 μM) in PBS (50 mM, pH 7.4, 1 % TFE) after 24 h incubation at room temperature. The structures were stained with uranyl (IV) acetate (4 %), scale bar = 0.5 μm. **E** Circular Dichroism spectra of C₆-Nap-ICA **6**, C₃-Nap-ICA **7** and C₀-Nap-ICA **8** (all 100 μM) in PBS (50 mM, pH 7.4, 1 % TFE). The molar ellipticity is plotted against the wavelength. **F** Fluorescence emission and **G** Absorbance of C₆-Nap-ICA **6**, C₃-Nap-ICA **7** and C₀-Nap-ICA **8** (all 100 μM) in PBS (50 mM, pH 7.4, 1 % TFE) at λ_{em} = 480–700 nm (λ_{ex} = 405 nm). The intensities are plotted against the wavelength.

Next, CD spectroscopy was used to analyze the secondary structures of the self-assembling peptides. The CD spectra of the three different conjugates revealed major differences in secondary structure (Figure 2D). The C₀-spacer variant **8** exhibits minima at 200, 217 and 225 nm. The minimum at 200 nm reflects disordered regions consistent with random coil formation, while the minima at 217 nm and 225 nm correspond to n→π* transitions of the peptide backbone in β-sheet secondary structures, with an additional aromatic contribution of the naphthalene imide units.^[43–46]

The C₃-spacer variant **7** displays a small positive maximum at 198 nm and similar minima at 217 and 225 nm, but lacks the minimum at 200 nm, suggesting increased order and enhanced

β -sheet content. In the near-UV region, maxima at 280 and 390 nm and a minimum at 330 nm indicate strong aromatic π - π^* contributions.^[47] Finally, the C₆-spacer variant **6** displays a far-UV CD spectrum more similar to the C₀-spacer **8**, with minima at 200, 215 and 230 nm, consistent with a mixture of β -sheet contents, disordered regions and aromatic contributions. However, in the near-UV, it shows pronounced differences. With intense maxima at 250 nm and a broad feature at 300–350 nm as well as corresponding minima at 280 and 390 nm, the spectrum displays the characteristic bisignate dichroic peaks arising from aromatic exciton coupling.^[43] Altogether, the CD spectroscopy results indicate improved aromatic π -block interactions with increasing spacer-lengths. These improvements in interactions are expected to cause the observed differences in self-assembly and therefore supramolecular morphologies.

During self-assembly, the attached fluorophores are brought into close proximity, enabling electronic coupling between transition dipoles that can result in characteristic spectral shifts.^[48] This behavior is commonly rationalized within the framework of H- and J-aggregates. H-type aggregates typically exhibit a hypsochromic (blue) shift of the absorption maximum, whereas J-type aggregates display a bathochromic (red) shift.^[49,50] To evaluate the influence of spacer length on the spectroscopic properties of the otherwise identical fluorophores, the three peptide conjugates were investigated at 100 μ M under non-assembling conditions (100 % TFE) and assembling conditions (1 % TFE, 99 % PBS). In the dissolved state (100 % TFE, Figure S39), all three peptide conjugates exhibited similar absorbance maxima at (430 $\pm$ 2) nm. Upon switching to aqueous assembling conditions, pronounced hypsochromic shifts were observed for C₆-Nap-ICA (**6**) and C₃-Nap-ICA (**7**), with absorption maxima at 392 nm (**6**) and 396 nm (**7**), respectively. In contrast, the C₀-variant (**8**), which does not form ordered nanostructures, showed only a minor shift to 426 nm. The substantial blue shifts for **6** and **7** are consistent with excitonic H-type coupling arising from π - π -stacking interactions within ordered assemblies, as also suggested by the CD data. The magnitude of these shifts suggests the formation of electronically coupled face-to-face chromophore stacks, whereas **8** remains largely monomeric or in a non-ordered state. Analysis of the emission spectra reveals subtle differences already in the dissolved state: the C₀-spacer **8** emits at 540 nm, while the C₃- (**7**) and C₆-spacer (**6**) both emit at 546 nm, indicating a slightly different microenvironment of the fluorophore in **8** even under non-assembling conditions. Upon transitioning to the aqueous buffer system, **6** and **7** exhibit mild blue shifts of their emission maximum, whereas **8** shows a red shift. Additionally, the fluorescence intensities follow the trend C₆ > C₃ > C₀. While ideal H-aggregates are often associated with fluorescence quenching due to exciton splitting and forbidden lowest-energy transitions, the increased emission intensity observed for **6** and **7** suggests deviation from the idealized limit. The enhanced fluorescence likely reflects suppression of non-radiative decay pathways upon self-assembly, potentially due to

conformational restriction of the fluorophore, including reduced flexibility of the piperidine unit. In contrast, the red shift observed for **8** under aqueous conditions may arise from changes in solvent polarity surrounding the fluorophore.

Overall, the pronounced hypsochromic absorption shifts for C₆-Nap-ICA (**6**) and C₃-Nap-ICA (**7**) in assembling conditions support excitonic H-type coupling within ordered supramolecular assemblies. These findings are consistent with TEM, AFM and Proteostat® aggregation assay results, which demonstrates more pronounced and ordered nanostructure formation for C₆-Nap-ICA (**6**) and C₃-Nap-ICA (**7**). The obtained results from all aforementioned analysis tools are summarized in the following Table 1.

Table 1: Summary of the assembly characteristics of C₆-Nap-ICA **6**, C₃-Nap-ICA **7** and C₀-Nap-ICA **8** at 100 μ M in PBS (50 mM, pH 7.4, 1 % TFE).

Peptide	Assembly Conversion ^a [%]	Aggregate Sizes ^b [μ m]	Morphology ^c	Secondary Structure ^d [nm]	Absorbance / Emission Maximum ^e [nm]
6 (C ₆)	86 $\pm$ 3	20 $\pm$ 12	Fibrils	β -sheet	392 / 537
7 (C ₃)	41 $\pm$ 1	4 $\pm$ 2	Fibrils	β -sheet	396 / 543
8 (C ₀)	14 $\pm$ 6	—	Amorphous	random coil	426 / 550

^aDetermined using assembly conversion assay at $\Delta = 214$ nm on analytical HPLC. ^bDetermined using fluorescence microscopy with a green fluorescence filter ($\lambda_{\text{ex}} = 470/40$ nm, $\lambda_{\text{em}} = 525/50$ nm), $n = 21$;

^cDetermined via TEM; ^dDetermined using CD-spectroscopy. ^eDetermined through absorbance and fluorescence ($\lambda_{\text{ex}} = 405$ nm) scans on a Tecan Spark 20M multi-plate reader in the aggregated state.

¹H-NMR spectroscopy provides valuable insight into the self-assembly behavior of molecules by monitoring spectral changes upon increasing the fraction of an organic co-solvent. A gradual, linear change in signal intensity or chemical shift with increasing co-solvent content is indicative of simple dissolution and bulk solvent effects. In contrast, a pronounced transition from broad, poorly resolved resonances arising from large, slowly tumbling aggregated species to sharp, well-resolved signals characteristic of smaller, faster-tumbling species points to a cooperative self-assembly process.^[51–54] To probe this behavior, the three peptide conjugates were dissolved in mixtures of d₃-trifluoroethanol (d₃-TFE) and PBS buffered D₂O with increasing d₃-TFE content (1/99, 10/90, 20/80, 30/70, 40/60, 50/50, and 100/0, v/v) and analyzed by ¹H-NMR spectroscopy (compare Figure 3).

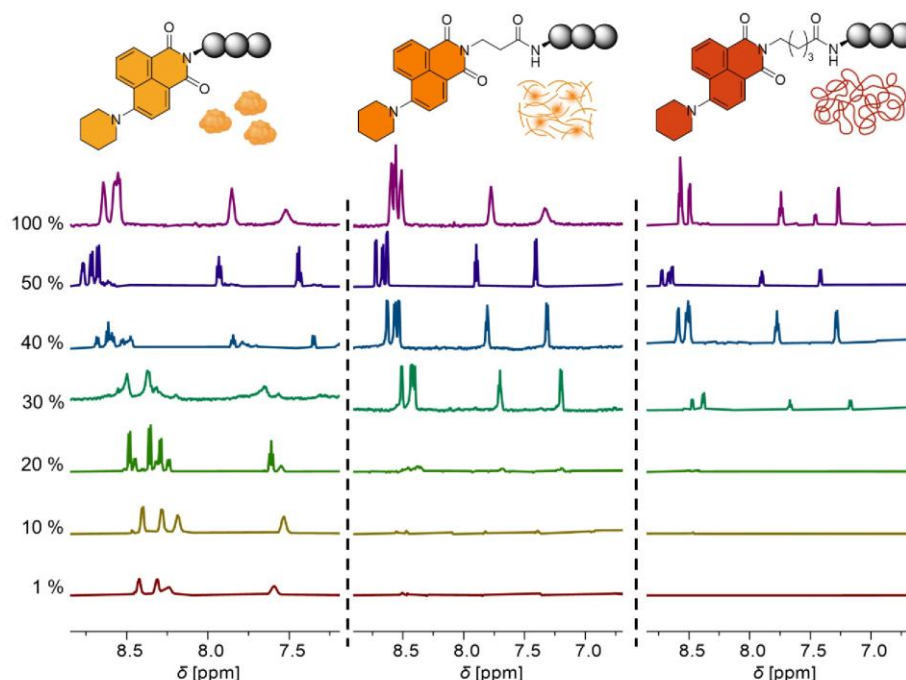


Figure 3: ^1H -NMR spectroscopy data (700 MHz, 298 K, mixtures of d_3 -TFE and D_2O) of the C_0 -spacer **8** (left), the C_3 -Spacer **7** (middle) and the C_6 -spacer **6** (right) with increasing d_3 -TFE content. Spectra were normalized to internal standard of dimethyl sulfone. Here, the aromatic region of the naphthalene signals is shown for comparison between the analytes. Full spectra are available in the supporting information (Figures S40-S60).

At 1 % d_3 -TFE content, the C_0 -spacer exhibits detectable resonances, even though line broadening effects are noticeable. This observation is consistent with a largely monomeric state in aqueous medium with a small population of aggregates contributing to slower molecular tumbling and thus line broadening. In contrast, the peptide conjugates with longer spacer lengths show no observable NMR signals at this co-solvent composition due to the formation of substantially larger aggregates that undergo rapid transverse relaxation. The pronounced aggregation of these conjugates is further evidenced by inspection of the corresponding NMR tubes, where clear solid-liquid phase separation is observed (compare Figure S61).

Upon increasing the d_3 -TFE content to 20 %, the resonances of the C_0 -spacer **8** conjugate sharpen and well-defined multiplicities become visible as the aggregates are dissolved into monomeric states. At this composition, the first signals appear for the C_3 -spacer **7**, whereas no resonances are yet observed for the C_6 -spacer peptide **6**. The requirement of a higher d_3 -TFE fraction for visible NMR signals indicates higher intrinsic aggregation propensity for the C_6 -spacer in comparison to the C_3 -spacer. Further increasing the d_3 -TFE content to 30 % results in the emergence of sharp, well-resolved signals for both peptides **6** and **7**. This pronounced transition suggests that the aggregates present in aqueous media are disrupted

once a critical level of organic co-solvent is reached. Additional increases in d_3 -TFE content to 50 % do not produce further qualitative changes in the spectra, indicating that the systems remain in a predominantly solvated, molecular regime. In summary, this ^1H -NMR spectroscopy study demonstrates that the self-assembly propensity of the peptide conjugates increases with spacer length. These results highlight the importance of flexibility within the linker segment for enabling productive intermolecular interactions that promote the formation of ordered self-assembled structures.

Conclusion

In this work, we investigated how the spacer segment connecting a short self-assembling peptide core to an *N*-terminal π -block modification influences the resulting self-assembly behavior. Although previous studies have extensively varied both peptide sequences and assembly-promoting π -blocks, the role of the spacer region has received comparatively little attention. By analyzing a systematic series of structurally related peptide conjugates with increasing spacer lengths, we demonstrate that flexibility within this segment is essential for enabling cooperative intermolecular interactions and efficient self-assembly.

Peptide conjugates bearing the most rigid spacer (C_0) failed to form ordered nanostructures, whereas constructs with longer aliphatic spacers (C_3 and C_6) assembled readily into well-defined supramolecular architectures. Moreover, spectroscopic and biochemical analyses reveal a clear correlation between spacer length and assembly propensity. Circular dichroism spectroscopy, NMR spectroscopy, and critical aggregation concentration assays consistently show that the C_6 -spacer promotes self-assembly more effectively than the C_3 -spacer. These results establish the spacer segment as a decisive structural element in the design of short amphiphilic peptides.

The present study focuses on assemblies primarily driven by hydrophobic interactions of the π -block and supported by peptide-peptide interactions. In addition, the investigated spacers were limited to relatively short, hydrophobic aliphatic chains. Future work could expand these design principles by exploring longer peptide sequences as well as a broader range of spacer lengths and compositions, including more polar and synthetic linkers such as PEG-based segments. Such studies will further refine the understanding of spacer-controlled peptide self-assembly and provide additional strategies for the rational development of responsive peptide materials.

Experimental

Microwave-assisted Peptide Synthesis

Peptides were synthesized using the Fmoc-SPPS strategy^[38], synthesizing the peptide from C- to *N*-terminus in a microwave-assisted peptide synthesizer on a Fmoc-L-Ala *C/t*-resin at

scales of 0.10 or 0.25 mmol. Fmoc-L-Ala *Clt*-resin (0.10/0.25 mmol) was swollen in DMF (5 mL) at room temperature for one hour before transferring it into the peptide synthesizer. DMF was removed via a draining process and the resin was swollen with DMF (20 mL) for 20 s. DMF was removed again through the draining process. Prior to every coupling, the Fmoc-protecting group was cleaved by two deprotection steps using a solution of 20 % piperidine (DMF, 3 mL) for two and five minutes at 75 °C. The solvent was drained and the resin was washed three times with DMF. The amino acids (0.2 M in DMF), DIC (0.5 M in DMF) and Oxyrna Pure (1.0 M in DMF) were added to the resin and the solution was heated to 70 °C for 30 s followed by a heating cycle to 90 °C for 120 s. The reaction mixture was drained, the Fmoc-group was removed as described before and washed three times with DMF. The following coupling steps were performed in the same way.

Assembly Conversion Rate Determination

The monomeric peptides were dissolved in TFE at high concentrations (10 mM) before dilution to different concentrations using PBS (50 mM, pH 7.4) and methanol. The final peptide concentrations were 100 μ M in PBS (50 mM, pH 7.4, 1 % TFE) and 100 μ M in MeOH (1 % TFE). The samples were incubated at room temperature for 24 h. Then, the samples were filtered through a syringe filter (0.2 μ m) and diluted with an internal standard, Tyramine (200 μ M in MeOH, 1:1, v/v).

Conversion rate measurements were performed on an *Atlantis T3* column (4.6 $\times$ 100 mm, 5 μ m) at a flowrate of 1 mL/min. All measurement steps were performed using gradients of ACN and MilliQ-H₂O, each acidified with 0.1 % TFA. The gradient started at 0 % ACN content and was linearly increased to 100 % over 24 min. Absorbance was recorded at 214 and 254 nm wavelengths. The software's *LabSolutions* by Shimadzu and *Origin Pro* by OriginLab® were used to process the generated HPLC spectra.

Assembly Characterization by NMR

To investigate the self-assembly propensity of the three peptide conjugates, the compounds were dissolved in mixtures of d₃-TFE and PBS-buffered D₂O with increasing d₃-TFE content (1/99, 10/90, 20/80, 30/70, 40/60, 50/50, and 100/0, v/v) and analyzed by ¹H-NMR spectroscopy. The aqueous component of the solvent mixtures was prepared by lyophilizing 40 mL of commercial PBS to dryness and redissolving the residue in the same volume of D₂O. Dimethyl sulfone (DMS, 6.96 mg) was added as an internal reference.

Each peptide conjugate was initially dissolved in d₃-TFE, followed by the addition of the DMS-containing PBS/D₂O buffer (250 μ L) and additional PBS/D₂O buffer without DMS to achieve the desired solvent compositions. The exact volumes used are summarized in Table S1.

Supporting Information

The data that support the findings of this study are available in the Supporting Information of this article.

Supporting Information File 1:

File Name: Supporting Information Spacer Length

File Format: pdf.

Title: Decoding the Role of Spacer Length and Flexibility in Peptide Self-Assembly

Acknowledgements

The authors acknowledge the members of MS core facility for the support with mass spectrometry measurements in this project. Furthermore, the authors thank Helma Burg (Scanning Probe Core Facility of the MPIP) for supporting our work with topography measurements. The authors gratefully acknowledge financial support from the Max Planck Society, the Max Planck-Bristol Centre for Minimal Biology as well as the Deutsche Forschungsgemeinschaft (DFG), project: SFB 1551 – R04 (Projektnummer: 464588647).

Author Contributions

T.W. conceived the project, J.L., A.L. and D.Y.W.N. were critically involved. J.L. and A.L. conducted TEM, Proteostat, UV-Vis experiments, synthesis and characterization. J.L. performed CD and NMR experiments. A.L. performed fluorescence microscopy and analytical HPLC experiments. M.W. contributed to the NMR studies. H.B. performed AFM measurements. J.L. and A.L. wrote the manuscript which was corrected by D.Y.W.N. and T.W.. T.W. supervised the overall project.

References

- [1] Y. Zhao, J. Wang, L. Deng, P. Zhou, S. Wang, Y. Wang, H. Xu, J. R. Lu, "Tuning the Self-Assembly of Short Peptides via Sequence Variations" *Langmuir* **2013**, *29*, 13457–13464.
- [2] X. Hu, M. Liao, H. Gong, L. Zhang, H. Cox, T. A. Waigh, J. R. Lu, "Recent advances in short peptide self-assembly: from rational design to novel applications" *Current Opinion in Colloid & Interface Science* **2020**, *45*, 1–13.
- [3] F. Sheehan, D. Sementa, A. Jain, M. Kumar, M. Tayarani-Najjaran, D. Kroiss, R. V. Ulijn, "Peptide-Based Supramolecular Systems Chemistry" *Chem. Rev.* **2021**, *121*, 13869–13914.
- [4] S. Chagri, D. Y. W. Ng, T. Weil, "Designing bioresponsive nanomaterials for intracellular self-assembly" *Nat Rev Chem* **2022**, *6*, 320–338.

- [5] M. H. Sangji, H. Sai, S. M. Chin, S. R. Lee, I. R. Sasselli, L. C. Palmer, S. I. Stupp, "Supramolecular Interactions and Morphology of Self-Assembling Peptide Amphiphile Nanostructures" *Nano Lett.* **2021**, *21*, 6146–6155.
- [6] N. J. Sinha, M. G. Langenstein, D. J. Pochan, C. J. Kloxin, J. G. Saven, "Peptide Design and Self-assembly into Targeted Nanostructure and Functional Materials" *Chem. Rev.* **2021**, *121*, 13915–13935.
- [7] M. Amit, S. Yuran, E. Gazit, M. Reches, N. Ashkenasy, "Tailor-Made Functional Peptide Self-Assembling Nanostructures" *Advanced Materials* **2018**, *30*, 1707083.
- [8] J. Chen, X. Zou, "Self-assemble peptide biomaterials and their biomedical applications" *Bioactive Materials* **2019**, *4*, 120–131.
- [9] F. Koch, K. Ekat, D. Kilian, T. Hettich, O. Germershaus, H. Lang, K. Peters, B. Kreikemeyer, "A Versatile Biocompatible Antibiotic Delivery System Based on Self-Assembling Peptides with Antimicrobial and Regenerative Potential" *Advanced Healthcare Materials* **2019**, *8*, 1900167.
- [10] S. Sun, H.-W. Liang, H. Wang, Q. Zou, "Light-Triggered Self-Assembly of Peptide Nanoparticles into Nanofibers in Living Cells through Molecular Conformation Changes and H-Bond Interactions" *ACS Nano* **2022**, *16*, 18978–18989.
- [11] Z. Li, Y. Zhu, J. B. Matson, "pH-Responsive Self-Assembling Peptide-Based Biomaterials: Designs and Applications" *ACS Appl. Bio Mater.* **2022**, *5*, 4635–4651.
- [12] S. Chagri, J. Fetzer, P. Roth, A. Lahu, N. Alleva, J. Zhang, M. Wagner, S. Si, I. Lieberwirth, K. Landfester, D. Y. W. Ng, T. Weil, "Programming Nanostructure Formation Through Furin-Triggered Isopeptide Conversion and Peptide Self-Assembly" *Macromolecular Bioscience* **2026**, *26*, e00427.
- [13] Z. Yang, G. Liang, B. Xu, "Supramolecular hydrogels based on β -amino acid derivatives" *Chem. Commun.* **2006**, 738–740.
- [14] A. Dutt, E. C. Spencer, J. A. K. Howard, A. Pramanik, "Studies of Amyloid-Like Fibrillogenesis through β -Sheet-Mediated Self-Assembly of Short Synthetic Peptides" *Chemistry & Biodiversity* **2010**, *7*, 363–375.
- [15] S. Kim, J. H. Kim, J. S. Lee, C. B. Park, "Beta-Sheet-Forming, Self-Assembled Peptide Nanomaterials towards Optical, Energy, and Healthcare Applications" *Small* **2015**, *11*, 3623–3640.
- [16] S. Deike, S. Rothmund, B. Voigt, S. Samantray, B. Strodel, W. H. Binder, " β -Turn mimetic synthetic peptides as amyloid- β aggregation inhibitors" *Bioorganic Chemistry* **2020**, *101*, 104012.
- [17] I. W. Hamley, J. Hutchinson, S. Kirkham, V. Castelletto, A. Kaur, M. Reza, J. Ruokolainen, "Nanosheet Formation by an Anionic Surfactant-like Peptide and Modulation of Self-Assembly through Ionic Complexation" *Langmuir* **2016**, *32*, 10387–10393.

- [18] R. Meyer, J. Link, L. Gunkel, A. Lahu, H. Demirezen, T. Weil, D. Y. W. Ng, "Light-Triggered Disassembly of Peptide Nanostructures" *ChemBioChem* **2025**, 26, e202500414.
- [19] J. Sastre, A. Thatte, A. M. Bergmann, M. Stasi, M. Tena-Solsona, C. A. Weber, J. Boekhoven, "Size control and oscillations of active droplets in synthetic cells" *Nat Commun* **2025**, 16, 2003.
- [20] Y. Zhao, J. Wang, L. Deng, P. Zhou, S. Wang, Y. Wang, H. Xu, J. R. Lu, "Tuning the Self-Assembly of Short Peptides via Sequence Variations" *Langmuir* **2013**, 29, 13457–13464.
- [21] Y. Zhao, W. Yang, D. Wang, J. Wang, Z. Li, X. Hu, S. King, S. Rogers, J. R. Lu, H. Xu, "Controlling the Diameters of Nanotubes Self-Assembled from Designed Peptide Bolaphiles" *Small* **2018**, 14, 1703216.
- [22] Y. Ren, Z. Zhou, I. Harley, Ö. Aydin, L. Driehaus-Ortiz, A. Kaltbeitzel, J. Xing, K. Maxeiner, I. Lieberwirth, K. Landfester, D. Y. W. Ng, T. Weil, "Intracellular assembly of supramolecular peptide nanostructures controlled by visible light" *Nat. Synth* **2025**, 4, 673–683.
- [23] J. Link, L. Burg, S. Chagri, H.-C. Nguyen, D. Y. W. Ng, B. J. Ravoo, T. Weil, "Photoswitchable Peptides as Molecular Tools to Encode Structural Order and Disorder in Intracellular Assemblies" *Angewandte Chemie International Edition* **2026**, 65, e14781.
- [24] F. Thomas, N. C. Burgess, A. R. Thomson, D. N. Woolfson, "Controlling the Assembly of Coiled–Coil Peptide Nanotubes" *Angewandte Chemie International Edition* **2016**, 55, 987–991.
- [25] J. L. Beesley, D. N. Woolfson, "The *de novo* design of α -helical peptides for supramolecular self-assembly" *Current Opinion in Biotechnology* **2019**, 58, 175–182.
- [26] M. J. Sis, Z. Ye, K. La Costa, M. J. Webber, "Energy Landscapes of Supramolecular Peptide–Drug Conjugates Directed by Linker Selection and Drug Topology" *ACS Nano* **2022**, 16, 9546–9558.
- [27] D. Zhang, L. Feng, X. Zhu, H. Li, H. Liu, B. Sun, "Aromatic short peptides: from self-assembled architectures to fluorescent sensing applications in food safety" *Trends in Food Science & Technology* **2025**, 166, 105361.
- [28] S. Fleming, R. V. Ulijn, "Design of nanostructures based on aromatic peptide amphiphiles" *Chem. Soc. Rev.* **2014**, 43, 8150–8177.
- [29] C. J. C. Edwards-Gayle, I. W. Hamley, "Self-assembly of bioactive peptides, peptide conjugates, and peptide mimetic materials" *Organic & Biomolecular Chemistry* **2017**, 15, 5867–5876.
- [30] H. Xing, A. Rodger, J. Comer, A. S. Picco, C. Huck-Iriart, E. L. Ezell, M. Conda-Sheridan, "Urea-Modified Self-Assembling Peptide Amphiphiles That Form Well-Defined Nanostructures and Hydrogels for Biomedical Applications" *ACS Appl. Bio Mater.* **2022**, 5, 4599–4610.

- [31] A. Dasgupta, D. Das, "Designer Peptide Amphiphiles: Self-Assembly to Applications" *Langmuir* **2019**, 35, 10704–10724.
- [32] X. Zhao, F. Pan, H. Xu, M. Yaseen, H. Shan, C. A. E. Hauser, S. Zhang, J. R. Lu, "Molecular self-assembly and applications of designer peptide amphiphiles" *Chemical Society Reviews* **2010**, 39, 3480–3498.
- [33] K. Kaygisiz, L. Rauch-Wirth, A. Dutta, X. Yu, Y. Nagata, T. Bereau, J. Münch, C. V. Synatschke, T. Weil, "Data-mining unveils structure–property–activity correlation of viral infectivity enhancing self-assembling peptides" *Nat Commun* **2023**, 14, 5121.
- [34] Y.-L. Tsai, P. Cavallo, Q. Lu, J. Yu, C. P. Ender, J. Link, K. Amann-Winkel, K. Endres, C. V. Synatschke, T. John, "Design of the Hydrophobic Core of Self-Assembling Peptide Fibrils for Enhanced Neural Regeneration" *Small Science* **2025**, 5, 2500224.
- [35] O. J. Ibukun, M. Gumtya, S. Singh, A. Shit, D. Haldar, "Effect of the spacer on the structure and self-assembly of FF peptide mimetics" *Soft Matter* **2023**, 19, 3215–3221.
- [36] P. Roth, R. Meyer, I. Harley, K. Landfester, I. Lieberwirth, M. Wagner, D. Y. W. Ng, T. Weil, "Supramolecular assembly guided by photolytic redox cycling" *Nat. Synth* **2023**, 2, 980–988.
- [37] A. T. Peters, M. J. Bide, "Amino derivatives of 1,8-naphthalic anhydride and derived dyes for synthetic-polymer fibres" *Dyes and Pigments* **1985**, 6, 349–375.
- [38] E. Atherton, H. Fox, D. Harkiss, C. J. Logan, R. C. Sheppard, B. J. Williams, "A mild procedure for solid phase peptide synthesis: use of fluorenylmethoxycarbonylamino-acids" *J. Chem. Soc., Chem. Commun.* **1978**, 537–539.
- [39] D. A. Pearson, M. Blanchette, M. L. Baker, C. A. Guindon, "Trialkylsilanes as scavengers for the trifluoroacetic acid deblocking of protecting groups in peptide synthesis" *Tetrahedron Letters* **1989**, 30, 2739–2742.
- [40] Y. Fujiwara, K. Akaji, Y. Kiso, "Racemization-Free Synthesis of C-Terminal Cysteine-Peptide Using 2-Chlorotriyl Resin" *Chemical & Pharmaceutical Bulletin* **1994**, 42, 724–726.
- [41] A. Lahu, S.-L. Wu, M. Schuler, F. Mazzotta, A. Ramadani, E. Koca, I. Lieberwirth, K. Landfester, T. John, D. Y. W. Ng, T. Weil, "Co-Assemblies Regulate the Catalytic Activity of Peptide Fibrils" *Angewandte Chemie International Edition* **2026**, 65, e11165.
- [42] S. Navarro, S. Ventura, "Fluorescent dye ProteoStat to detect and discriminate intracellular amyloid-like aggregates in Escherichia coli" *Biotechnology Journal* **2014**, 9, 1259–1266.
- [43] M. C. Manning, M. Illangasekare, R. W. Woody, "Circular dichroism studies of distorted α -helices, twisted β -sheets, and β -turns" *Biophysical Chemistry* **1988**, 31, 77–86.
- [44] K. Wang, J. D. Keasling, S. J. Muller, "Effects of the sequence and size of non-polar residues on self-assembly of amphiphilic peptides" *International Journal of Biological Macromolecules* **2005**, 36, 232–240.

- [45] C. J. Bowerman, W. Liyanage, A. J. Federation, B. L. Nilsson, "Tuning β -Sheet Peptide Self-Assembly and Hydrogelation Behavior by Modification of Sequence Hydrophobicity and Aromaticity" *Biomacromolecules* **2011**, *12*, 2735–2745.
- [46] E. L. Bakota, O. Sensoy, B. Ozgur, M. Sayar, J. D. Hartgerink, "Self-Assembling Multidomain Peptide Fibers with Aromatic Cores" *Biomacromolecules* **2013**, *14*, 1370–1378.
- [47] S. K. M. Nalluri, R. V. Ulijn, "Discovery of energy transfer nanostructures using gelation-driven dynamic combinatorial libraries" *Chem. Sci.* **2013**, *4*, 3699–3705.
- [48] N. J. Hestand, F. C. Spano, "Expanded Theory of H- and J-Molecular Aggregates: The Effects of Vibronic Coupling and Intermolecular Charge Transfer" *Chem. Rev.* **2018**, *118*, 7069–7163.
- [49] F. Würthner, T. E. Kaiser, C. R. Saha-Möller, "J-Aggregates: From Serendipitous Discovery to Supramolecular Engineering of Functional Dye Materials" *Angewandte Chemie International Edition* **2011**, *50*, 3376–3410.
- [50] S. Ma, S. Du, G. Pan, S. Dai, B. Xu, W. Tian, "Organic molecular aggregates: From aggregation structure to emission property" *Aggregate* **2021**, *2*, e96.
- [51] S. Yao, G. J. Howlett, R. S. Norton, "Peptide self-association in aqueous trifluoroethanol monitored by pulsed field gradient NMR diffusion measurements" *J Biomol NMR* **2000**, *16*, 109–119.
- [52] X. Chang, D. Keller, S. I. O'Donoghue, J. J. Led, "NMR studies of the aggregation of glucagon-like peptide-1: formation of a symmetric helical dimer" *FEBS Letters* **2002**, *515*, 165–170.
- [53] M. Fioroni, M. D. Diaz, K. Burger, S. Berger, "Solvation Phenomena of a Tetrapeptide in Water/Trifluoroethanol and Water/Ethanol Mixtures: A Diffusion NMR, Intermolecular NOE, and Molecular Dynamics Study" *J. Am. Chem. Soc.* **2002**, *124*, 7737–7744.
- [54] M. Wallace, J. A. Iggo, D. J. Adams, "Using solution state NMR spectroscopy to probe NMR invisible gelators" *Soft Matter* **2015**, *11*, 7739–7747.

Supporting Information

Decoding the Role of Spacer Length and Flexibility in Peptide Self-Assembly

Julian Link^{†1}, Albin Lahu^{†1}, Manfred Wagner¹, David Y. W. Ng^{*1}, Tanja Weil^{*1}.

^{†1}Max Planck Institute for Polymer Research, D-55128 Mainz, Germany.

E-Mail: david.ng@mpip-mainz.mpg.de, weil@mpip-mainz.mpg.de.

Table of Contents

1	Materials and Instruments.....	4
1.1	Materials	4
1.2	Instruments	4
1.2.1	Microwave Peptide Synthesizer	4
1.2.2	Thin-Layer Chromatography (TLC).....	4
1.2.3	High-Performance Liquid Chromatography (HPLC).....	4
1.2.4	Nuclear Magnetic Resonance Spectroscopy (NMR).....	5
1.2.5	Liquid Chromatography-Mass Spectrometry (LC-MS)	5
1.2.6	Matrix-Assisted Laser Desorption/Ionization – Time of flight (MALDI-TOF)	5
1.2.7	Self-Assembly	5
1.2.8	Transmission Electron Microscopy (TEM)	6
1.2.9	Atomic Force Microscopy (AFM)	6
1.2.10	Circular Dichroism (CD) Spectroscopy.....	6
1.2.11	Assembly Conversion Rate Determination.....	6
1.2.12	Fluorescence Microscopy	7
1.2.13	General Procedure for Automated Peptide Synthesis	7
1.2.14	Purification of Peptides	7
2	Synthesis.....	8
2.1	Synthesis of Small Molecules	8
2.1.1	Synthesis of 4-Piperidinyl-1,8-naphthalic anhydride	8
2.1.2	Synthesis of 6-(1,3-dioxo-6-(piperidin-1-yl)-1 <i>H</i> -benzo[de]isoquinolin-2(3 <i>H</i>)-yl)hexanoic acid (C ₆ -Nap).....	12
2.1.3	Synthesis of 1 <i>H</i> -Benz[de]isoquinoline-2(3 <i>H</i>)-propanoic acid (C ₃ -Nap)	16
2.1.3	Synthesis of (2 <i>S</i> ,3 <i>S</i>)-2-(1,3-dioxo-6-(piperidin-1-yl)-1 <i>H</i> -benzo[de]isoquinolin-2(3 <i>H</i>)-yl)-3-methylpentanoic acid (Nap-Ile).	20
2.2	Synthesis of Peptides.....	24
2.2.1	Synthesis of C ₆ -Nap-ICA	24
2.2.2	Synthesis of C ₃ -Nap-ICA	26
2.2.3	Synthesis of C ₀ -Nap-ICA	29

Supporting Information

3	Self-Assembly.....	31
3.1	Assembly Conversion Rate	31
3.2	Fluorescence, Transmission Electron and Atomic Force Microscopy	32
3.3	UV/Vis- & Fluorescence Emission Spectroscopy.....	34
4	NMR study with increasing TFE content	34
	References.....	48

1 Materials and Instruments

1.1 Materials

Reagents and solvents were purchased from commercial vendors and used without further purification. For the synthesis of peptides, peptide synthesis grade reagents were used. High performance liquid chromatography (HPLC) was performed using acetonitrile (ACN) in HPLC grade. MilliQ-H₂O for HPLC and reactions was obtained from a Millipore purification system by *Merck Millipore*.

1.2 Instruments

1.2.1 Microwave Peptide Synthesizer

A *LibertyBlue* automated microwave synthesizer from *CEM Corporation* was used to perform solid-phase peptide synthesis (SPPS). The system was purged with dimethylformamide (DMF) prior and during usage. Piperidine was used for deprotection of the peptide protecting groups and the used amino acids were dissolved in DMF. *N,N*-Diisopropylcarbodiimide (DIC) and ethyl cyanohydroxyiminoacetate (Oxyma Pure) were employed as coupling agents and dissolved in DMF as well.

1.2.2 Thin-Layer Chromatography (TLC)

The analysis of compounds by means of thin layer chromatography (TLC) was performed using *Alugram Sil G/UV₂₅₄* plates, by *Macherey-Nagel*. The analytes were detected by extinction of ultraviolet (UV) absorption at a wavelength of 254 nm and 365 nm or by staining of the TLC-plate using potassium permanganate solution.

1.2.3 High-Performance Liquid Chromatography (HPLC)

Peptides were purified by preparative HPLC using a setup from *Shimadzu*. For purification, a *Phenomenex Gemini 5 μm NX-C18 110 Å 150 × 30 mm* column was used at a flowrate of 25 mL/min. All measurements and purification steps were performed using gradients of ACN and MilliQ-H₂O, each acidified with 0.1 % trifluoroacetic acid (TFA). The absorbance was recorded at wavelengths of 214 and 254 nm. For processing of the HPLC spectra, the software's *LabSolutions* by *Shimadzu* and *PowerPoint* by *Microsoft Corporation* were used. Analytical measurements were performed on an *Atlantis T3* column (4.6 × 100 mm, 5 μm) at a flowrate of 1 mL/min. All measurements and purification steps were performed using gradients of ACN and MilliQ-H₂O, each acidified with 0.1 % TFA. Absorbance was recorded at 214 and 254 nm wavelengths. The software's *LabSolutions* by *Shimadzu* and *PowerPoint* by *Microsoft Corporation* were used to process the generated HPLC spectra.

1.2.4 Nuclear Magnetic Resonance Spectroscopy (NMR)

^1H - and ^{13}C -NMR-measurements were conducted in *AVANCE 400*- and *AVANCE 700*-spectrometers by *Bruker* at 278 K in deuterated solvents. All deuterated solvents were used without further purification and purchased from *Sigma Aldrich*. The internal standards were set depending on the solvent used.

CHCl_3 : ^1H : $\delta = 7.26$ ppm, ^{13}C : $\delta = 77.16$ ppm; $\text{DMSO-}d_6$: ^1H : $\delta = 2.50$ ppm, ^{13}C : $\delta = 39.52$ ppm; CF_3CHDOD : ^1H : $\delta = 3.88$ ppm, ^{13}C : $\delta = 61.50, 126.30$ ppm.

The acquired data was processed using the software *MestReNova 14.2.0* by *Mestrelab Research*. The chemical shifts δ were indicated in parts per million (ppm). Multiplets were abbreviated as following: s = singlet, d = doublet, t = triplet, q = quartet, p = quintet, m = multiplet.

1.2.5 Liquid Chromatography-Mass Spectrometry (LC-MS)

Compounds were analyzed by HPLC-ESI-MS on a LC-MS 2020 from *Shimadzu* using a Kinetex 2.6 μm EVO C_{18} 100 $\text{\AA}$ LC 50 $\times$ 2.1 mm column. MilliQ- H_2O , acidified with 0.1 % formic acid, and ACN, acidified with 0.1 % formic acid, were used as solvents for all measurements. The solvent gradient started with 5 % ACN and 95 % MilliQ- H_2O . This solvent ratio was kept constant for 2 min, then the ACN content was linearly increased to 95 % in 14 min. Generated data was processed with *LabSolutions* by *Shimadzu* and *Origin Pro* by *OriginLab*[®].

1.2.6 Matrix-Assisted Laser Desorption/Ionization – Time of flight (MALDI-TOF)

For further characterization of peptides, MALDI-ToF spectra were recorded on a *rapifleX* MALDI-ToF/ToF by *Bruker*. The samples were dissolved in ACN, MeOH and/or MilliQ- H_2O and mixed with a saturated solution of the matrix, α -cyano-4-hydroxycinnamic acid (CHCA), in $\text{H}_2\text{O}/\text{ACN}$ (1:1, v/v, 0.1 % TFA) prior to the measurement. The software's *mMass* and *Origin Pro* by *OriginLab*[®] were used to process the collected data.

1.2.7 Self-Assembly

The monomeric peptides were dissolved in TFE at high concentrations (20 mM) before dilution using phosphate-buffered saline (PBS) buffer (50 mM, pH 7.4) with final TFE concentrations of 1 %. The samples were incubated at room temperature for 24 h.

1.2.8 Transmission Electron Microscopy (TEM)

TEM images were recorded on a *JEOL 1400* transmission electron microscope at a voltage of 120 kV. The images were visualized using the software *ImageJ*. Unless stated otherwise, all samples were prepared in PBS (50 mM, pH 7.4, 1 % DMSO) and incubated for 24 h at room temperature. The samples were applied to Formvar/carbon-film coated copper grids (300 mesh) by *Plano GmbH*. The TEM grids were prepared by covering the grid with a 3 μ L droplet of the sample solution and incubation for 5 minutes. The excess solution was removed and the grids were stained with 7 μ L uranyl (IV) acetate solution (4 %) for 2.5 min. The TEM grids were washed three times with MilliQ-H₂O and dried before being used for microscopy.

1.2.9 Atomic Force Microscopy (AFM)

Atomic force microscopy (AFM) (*Park NX20* and *Bruker Dimension ICON*) was employed to characterize the morphology of the system. Briefly, samples at different time intervals were diluted to 100 μ M and air-dried prior casting on freshly cleaved silicon wafer. Imaging was performed under tapping mode using a cantilever with a resonance frequency of 285 kHz and a spring constant of 42 N·m⁻¹. The resulting AFM images were further processed and analyzed with *Gwyddion 2.63*.

1.2.10 Circular Dichroism (CD) Spectroscopy

CD spectra were recorded on a *JASCO J-1500* spectrometer in a 1 mm High Precision Cell by *Hellma Analytics*. The collected data was processed using *Spectra Analysis* by *JASCO* and *Origin Pro* by *OriginLab*®. Samples were prepared in 50 mM PBS buffer (pH 7.4) with peptide concentrations of 100 μ M and 1 % 2,2,2-trifluoroethanol (TFE). The samples were incubated for 24 h at room temperature and the spectra were recorded at wavelengths from 185 nm to 300 nm with a bandwidth of 1 nm, data pitch of 0.2 nm and scanning speed at 5 nm/min, while using 300 μ L of the prepared sample solution. The samples were measured three times and the collected spectra were averaged. Background measurements were performed using PBS buffer (50 mM, pH 7.4, 1 % TFE, 300 μ L) and subtracted from the peptide spectra.

1.2.11 Assembly Conversion Rate Determination

The monomeric peptides were dissolved in TFE at high concentrations (10 mM) before dilution to different concentrations using PBS (50 mM, pH 7.4) and methanol. The final peptide concentrations were 100 μ M in PBS (50 mM, pH 7.4, 1 % TFE) and 100 μ M in MeOH (1 % TFE). The samples were incubated at room temperature for 24 h. Then, the samples were filtered through a syringe filter (0.2 μ m) and diluted with an internal standard, Tyramine (200 μ M in MeOH, 1:1, v/v).

Conversion rate measurements were performed on an *Atlantis T3* column (4.6 × 100 mm, 5 µm) at a flowrate of 1 mL/min. All measurement steps were performed using gradients of ACN and MilliQ-H₂O, each acidified with 0.1 % TFA. The gradient started at 0 % ACN content and was linearly increased to 100 % over 24 min. Absorbance was recorded at 214 and 254 nm wavelengths. The software's *LabSolutions* by Shimadzu and *Origin Pro* by OriginLab® were used to process the generated HPLC spectra.

1.2.12 Fluorescence Microscopy

Fluorescence images of the peptides were taken using a Keyence BZ-X810 fluorescence microscope with a 40x LD PH lens. The fluorescence of the naphthalene moiety was analyzed using a GFP filter with the following wavelength settings: $\lambda_{\text{ex}} = 470/40$ nm, $\lambda_{\text{em}} = 525/50$ nm.

1.2.13 General Procedure for Automated Peptide Synthesis

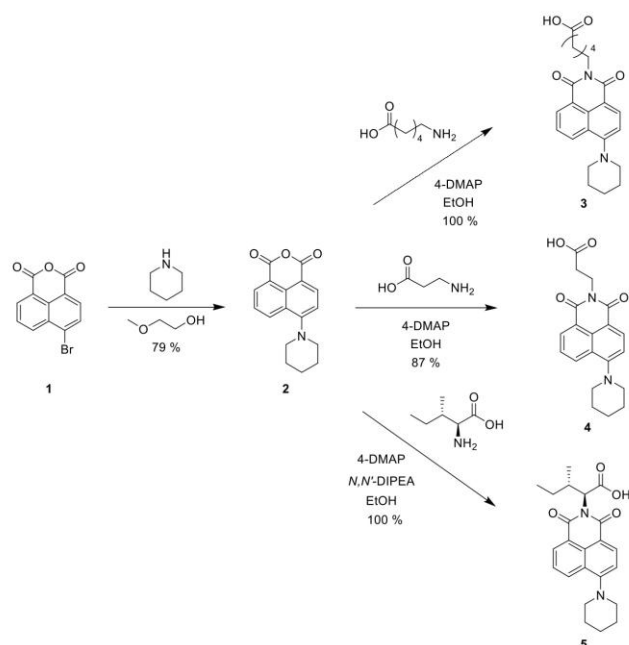
Peptides were synthesized using the Fmoc-SPPS strategy^[1], synthesizing the peptide from C- to N-terminus in a microwave-assisted peptide synthesizer on a Fmoc-L-Ala *Clt*-resin at scales of 0.10 or 0.25 mmol. Fmoc-L-Ala *Clt*-resin (0.10/0.25 mmol) was swollen in DMF (5 mL) at room temperature for one hour before transferring it into the peptide synthesizer. DMF was removed via a draining process and the resin was swollen with DMF (20 mL) for 20 s. DMF was removed again through the draining process. Prior to every coupling, the Fmoc-protecting group was cleaved by two deprotection steps using a solution of 20 % piperidine (DMF, 3 mL) for two and five minutes at 75 °C. The solvent was drained and the resin was washed three times with DMF. The amino acids (0.2 M in DMF), DIC (0.5 M in DMF) and Oxyma Pure (1.0 M in DMF) were added to the resin and the solution was heated to 70 °C for 30 s followed by a heating cycle to 90 °C for 120 s. The reaction mixture was drained, the Fmoc-group was removed as described before and washed three times with DMF. The following coupling steps were performed in the same way.

1.2.14 Purification of Peptides

Crude peptide products were purified via HPLC. The crudes were dissolved in 20 mL of ACN:MilliQ-H₂O (9:1, v/v, 5 % TFA) and filtered through a syringe filter (0.2 µm). For purification, a *Phenomenex Gemini* 5 µm *NX-C18* 110 Å 150 × 30 mm column was used at a flowrate of 25 mL/min. The gradient started at 0 % ACN (0.1 % TFA) and was linearly increased to 100 % ACN (0.1 % TFA) through the span of 20 min. Fractions were collected and detected by measurements of the absorbance at wavelengths of 214 and 254 nm. The compounds were then identified using LC-MS and MALDI-Tof. The product fractions were collected in a round-bottom flask (RBF) and narrowed down to 40 mL under reduced pressure. The residual solvents were removed via lyophilization overnight.

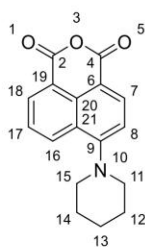
2 Synthesis

2.1 Synthesis of Small Molecules



Scheme S1: Two-step synthesis of the naphthalene- π -blocks C₆-Nap **3**, C₃-Nap **4** and C₀-Nap **5** starting from 4-bromo-1,8-naphthalic anhydride **1**.

2.1.1 Synthesis of 4-Piperidinyl-1,8-naphthalic anhydride



The synthesis was performed according to a general procedure by Li.^[2] 4-Bromo-1,8-naphthalenedicarboxylic anhydride **1** (1.00 g; 3.61 mmol; 1.0 eq.) was dissolved in 2-methoxyethanol (10 mL) and stirred for 10 min at room temperature. Then, piperidine (750 μ L; 7.22 mmol; 2.0 eq.) was added and the reaction mixture was stirred overnight under reflux conditions. The reaction mixture was cooled to room temperature. The suspension was further cooled in an ice-bath and filtered. The orange solid was washed three times with small amounts

Supporting Information

of cold EtOH and the orange crude was recrystallized from EtOH. 4-Piperidinyl-1,8-naphthalic anhydride **2** (800 mg; 2.85 mmol; 79 %) was obtained as orange crystals and used without further purification.

LC-MS: (pos.): calculated for $[M + H]^+$: $m/z = 282.3$; measured: $m/z = 282.1$.

R_f: 0.58 (DCM/MeOH = 9:1, v/v).

¹H-NMR, COSY (400 MHz, CDCl₃, 298 K): δ [ppm] = 8.58 (dd, 1H, $J = 7.3$ Hz, 1.2 Hz, H_{16}); 8.50 (d, 1H, $J = 8.2$ Hz, H_{18}); 8.43 (dd, 1H, $J = 8.5$ Hz, 1.2 Hz, H_7); 7.71 (dd, 1H, $J = 8.5$ Hz, 7.3 Hz, H_{17}); 7.19 (d, 1H, $J = 8.2$ Hz, H_8); 3.33–3.25 (m, 4H, $H_{11,15}$); 1.90 (p, 4H, $J = 5.8$ Hz, $H_{12,14}$); 1.75 (tq, 2H, $J = 7.9$ Hz, 4.1 Hz, H_{13}).

¹³C-NMR, HSQC, HMBC (101 MHz, CDCl₃, 298 K): δ [ppm] = 161.7, 160.9, 158.6, 135.2, 133.3, 132.3, 125.8, 115.1, 54.5, 26.3, 24.4.

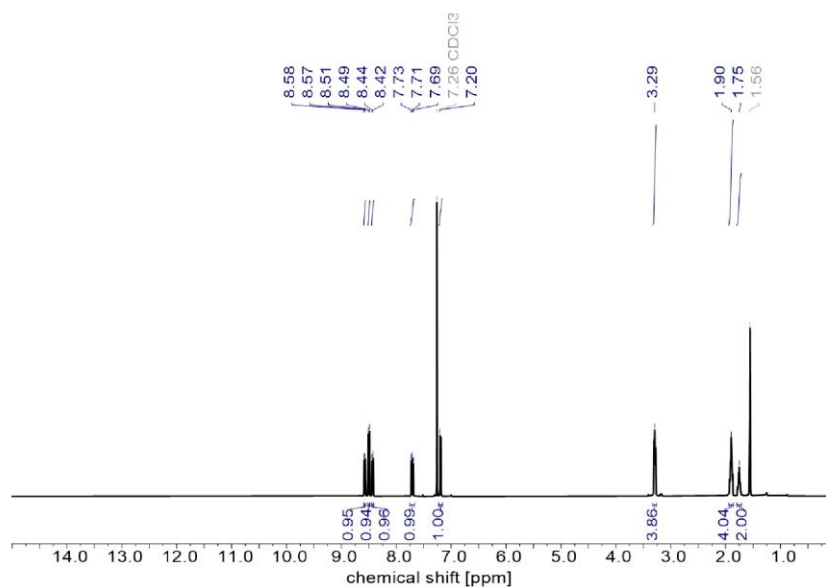


Figure S1: ¹H-NMR spectrum (CDCl₃, 400 MHz, 298 K) of 4-Piperidinyl-1,8-naphthalic anhydride **2**.

Supporting Information

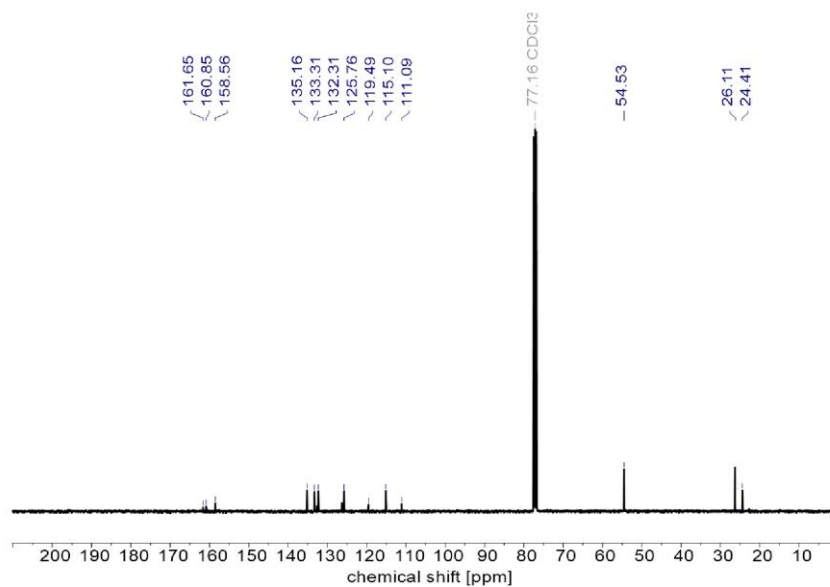


Figure S2: ^{13}C -NMR spectrum (CDCl_3 , 101 MHz, 298 K) of 4-Piperidiny-1,8-naphthalic anhydride **2**.

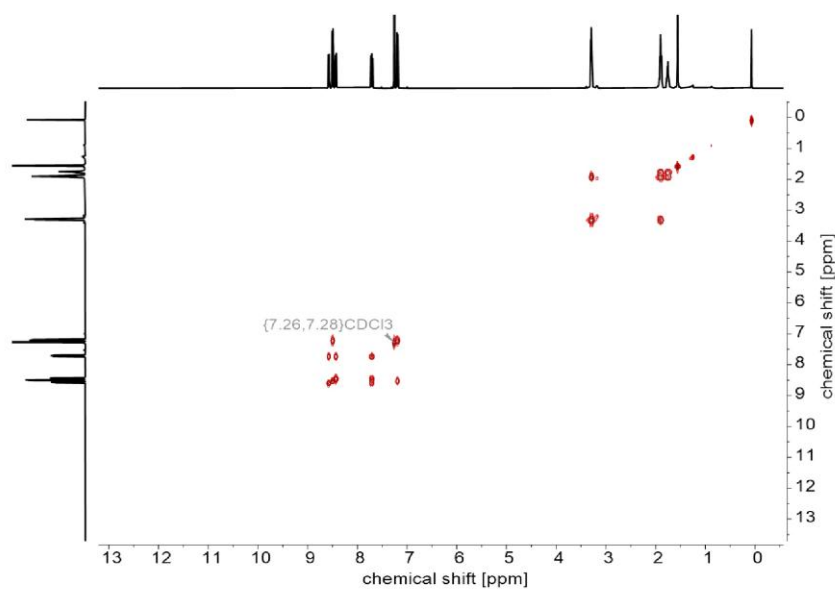


Figure S3: ^1H - ^1H -COSY-NMR spectrum (CDCl_3 , 400 MHz, 298 K) of 4-Piperidiny-1,8-naphthalic anhydride **2**.

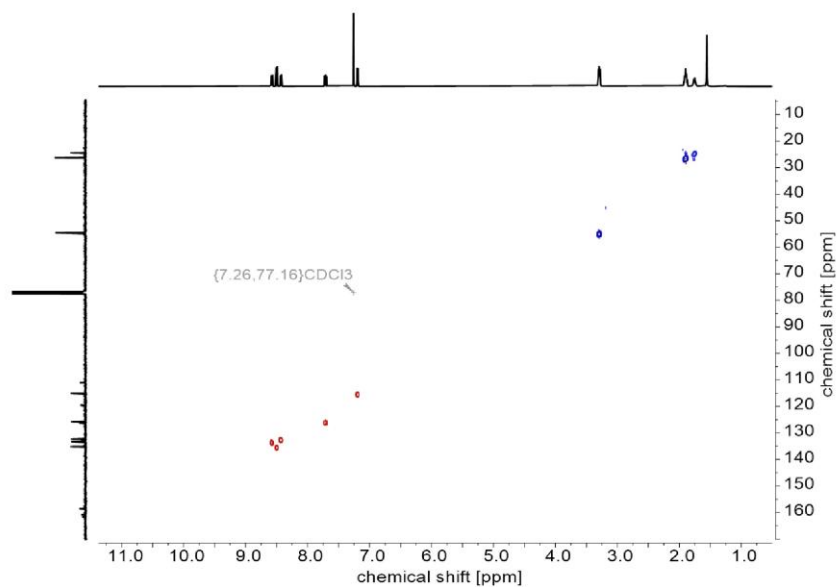


Figure S4: ^1H - ^{13}C -HSQC-NMR spectrum (CDCl_3 , 101 MHz, 298 K) of 4-Piperidiny-1,8-naphthalic anhydride **2**.

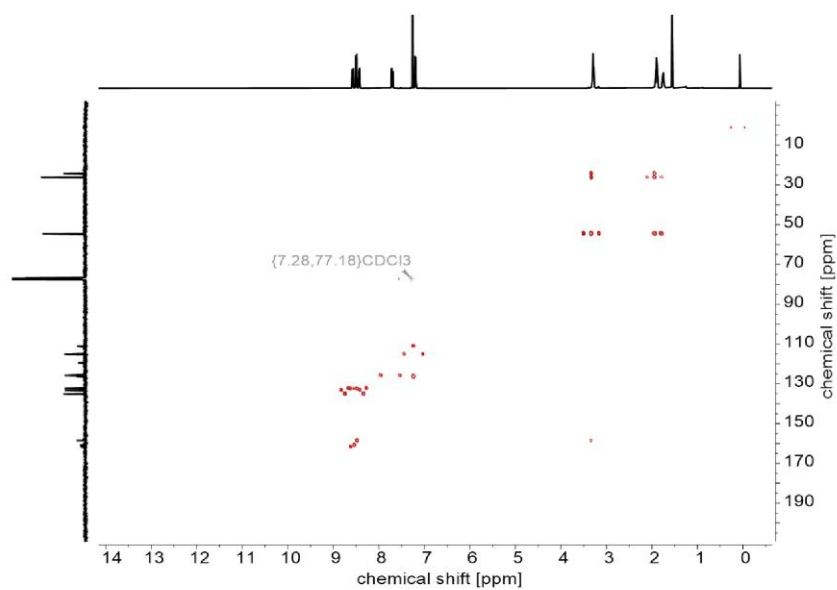


Figure S5: ^1H - ^{13}C -HMBC-NMR spectrum (CDCl_3 , 101 MHz, 298 K) of 4-Piperidiny-1,8-naphthalic anhydride **2**.

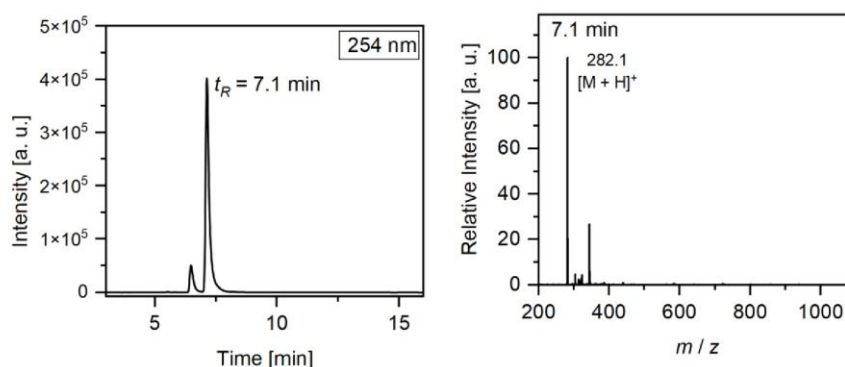
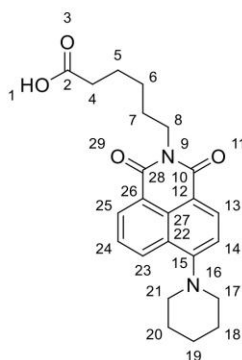


Figure S6: LC elugram (left, detection at 214 nm) and corresponding mass data (right) of 4-Piperidinyl-1,8-naphthalic anhydride **2**.

2.1.2 Synthesis of 6-(1,3-dioxo-6-(piperidin-1-yl)-1*H*-benzo[de]isoquinolin-2(3*H*)-yl) hexanoic acid (**C₆-Nap**)



4-Piperidinyl-1,8-naphthalic anhydride **2** (100 mg; 0.36 mmol; 1.0 eq.), 6-amino-hexanoic acid (55 mg; 0.43 mmol; 1.2 eq.) and 4-DMAP (5 mg; 0.04 mmol; 0.1 eq.) were covered with EtOH (10 mL). The reaction mixture was stirred under reflux conditions for 24 h. Then, the mixture was diluted with DCM (10 mL). The solution was washed with 1 M HCl (3 x 5 mL) and brine (1 x 10 mL). The organic layer was dried over MgSO₄, filtered and the solvents were removed under reduced pressure. **C₆-Nap 3** (155 mg; 0.36 mmol; 100 %) was obtained as a bright orange solid.

LC-MS: (pos.): calculated for [M + H]⁺: m/z = 395.5; measured: m/z = 395.2; calculated for [2 M + Na]⁺: m/z = 811.9; measured: m/z = 811.5.

R_f: 0.15 (DCM/MeOH = 9:1, v/v; 1 % AcOH).

Supporting Information

¹H-NMR, COSY (400 MHz, CDCl₃, 298 K): δ [ppm] = 8.57 (dd, 1H, J = 7.2 Hz, 1.2 Hz, H_{23}); 8.49 (d, 1H, J = 8.1 Hz, H_{25}); 8.39 (dd, 1H, J = 8.5 Hz, 1.3 Hz, H_{13}); 7.67 (dd, 1H, J = 8.5 Hz, 7.3 Hz, H_{24}); 7.17 (d, 1H, J = 8.2 Hz, H_{14}); 4.21–4.13 (m, 2H, H_8); 3.33–3.19 (m, 4H, $H_{17, 21}$); 2.38 (t, 2H, J = 7.5 Hz, H_4); 1.88 (p, 4H, J = 5.8 Hz, $H_{18, 20}$); 1.82–1.66 (m, 6H, $H_{5, 7, 19}$); 1.48 (tt, 2H, J = 9.9 Hz, 6.4 Hz, H_6).

¹³C-NMR, HSQC, HMBC (101 MHz, CDCl₃, 298 K): δ [ppm] = 157.5, 132.9, 131.2, 130.8, 126.4, 125.5, 123.3, 116.1, 114.9, 54.7, 53.6, 40.1, 33.7, 27.9, 26.7, 26.4, 24.6, 24.5.

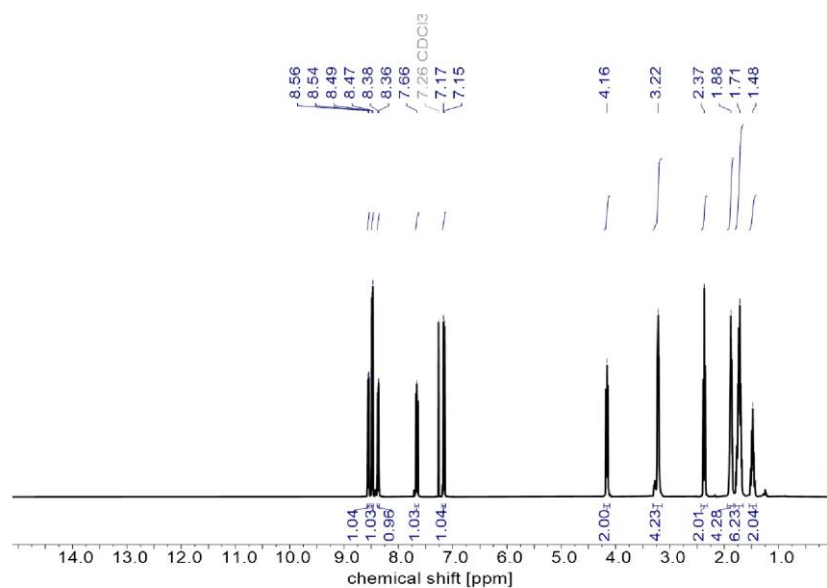


Figure S7: ¹H-NMR spectrum (CDCl₃, 400 MHz, 298 K) of C₆-Nap 3.

Supporting Information

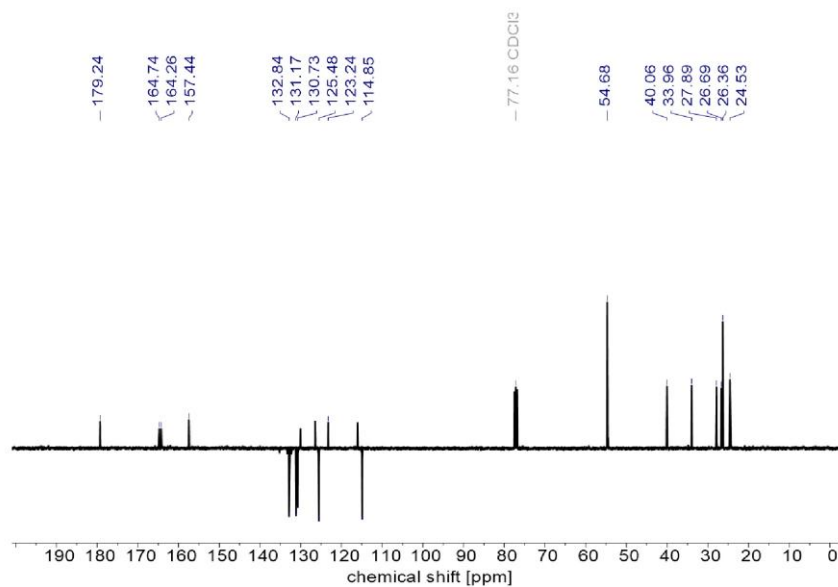


Figure S8: ^{13}C -NMR spectrum (CDCl_3 , 101 MHz, 298 K) of $\text{C}_6\text{-Nap 3}$.

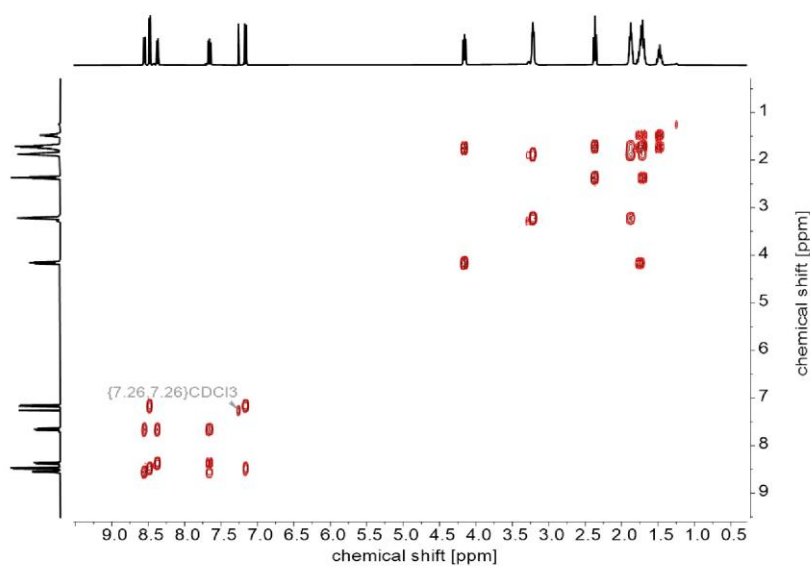


Figure S9: ^1H - ^1H -COSY-NMR spectrum (CDCl_3 , 400 MHz, 298 K) of $\text{C}_6\text{-Nap 3}$.

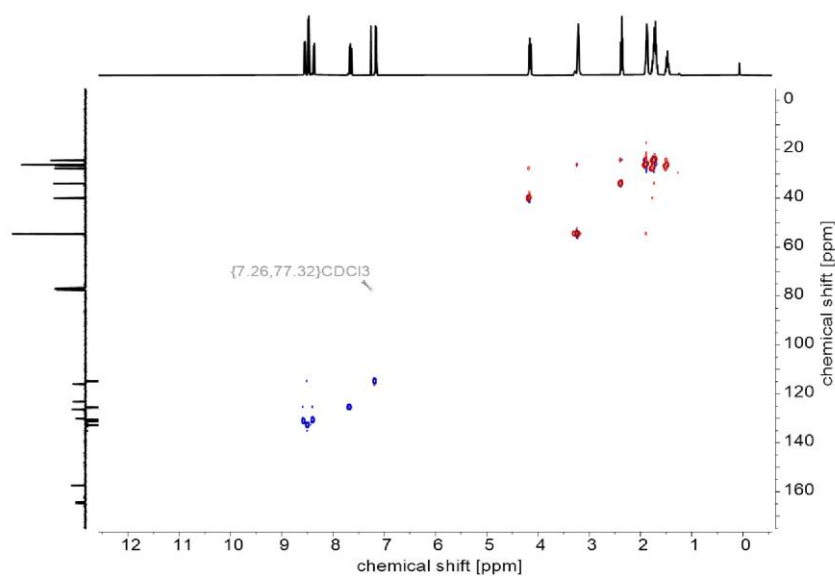


Figure S10: ^1H - ^{13}C -HSQC-NMR spectrum (CDCl_3 , 101 MHz, 298 K) of **C₆-Nap 3**.

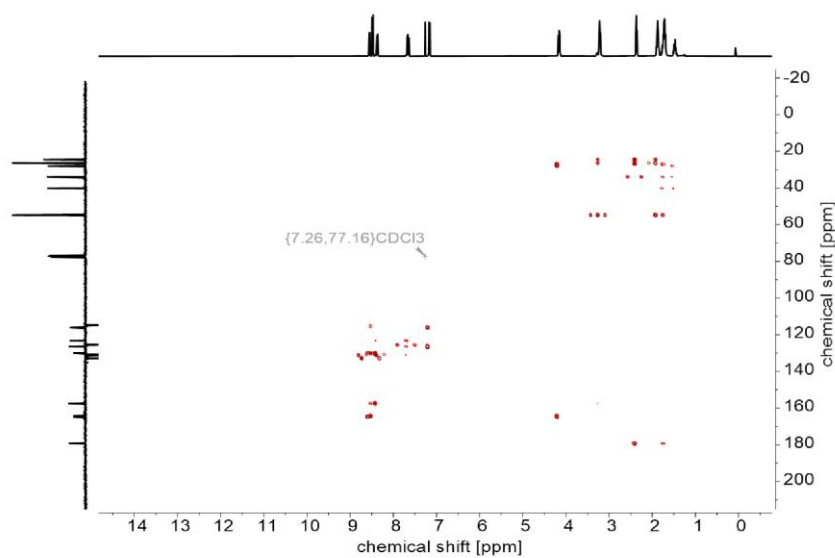


Figure S11: ^1H - ^{13}C -HMBC-NMR spectrum (CDCl_3 , 101 MHz, 298 K) of **C₆-Nap 3**.

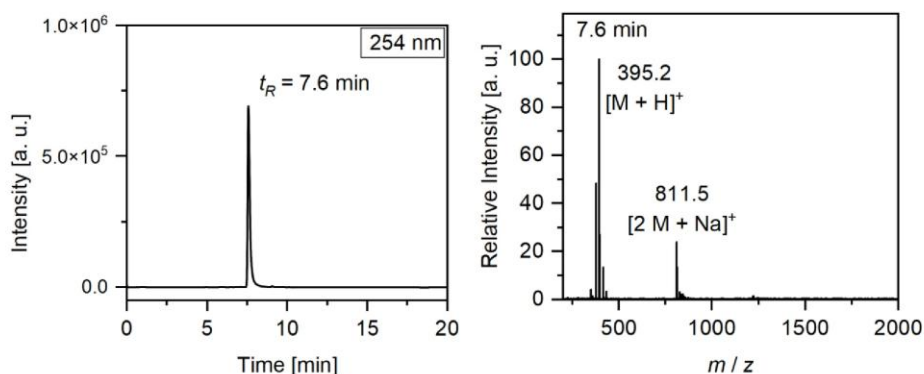
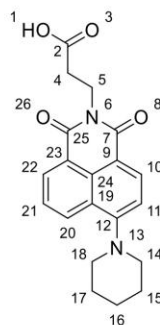


Figure S12: LC elugram (left, detection at 254 nm) and corresponding mass data (right) of **C₆-Nap 3**.

2.1.3 Synthesis of 1*H*-Benz[de]isoquinoline-2(3*H*)-propanoic acid (**C₃-Nap**)



4-Piperidiny-1,8-naphthalic anhydride **2** (100 mg; 0.36 mmol; 1.0 eq.), β -alanine (32 mg; 0.36 mmol; 1.0 eq.) and 4-DMAP (5 mg; 0.04 mmol; 0.1 eq.) were covered with EtOH (10 mL). The reaction mixture was stirred under reflux conditions for 24 h. The mixture was diluted with DCM (10 mL) and the solution was washed with 1 M HCl (3 x 5 mL) and brine (1 x 10 mL). The organic layer was dried over MgSO_4 , filtered and the solvents were removed under reduced pressure. **C₃-Nap 4** (110 mg; 0.31 mmol; 87 %) was obtained as an orange solid.

LC-MS: (pos.): calculated for $[\text{M} + \text{H}]^+$: $m/z = 353.4$; measured: $m/z = 353.2$; calculated for $[2 \text{ M} + \text{Na}]^+$: $m/z = 727.8$; measured: $m/z = 727.4$.

R_f: 0.72 (DCM/MeOH = 7:3, v/v; 1 % AcOH).

¹H-NMR, COSY (400 MHz, CDCl_3 , 298 K): δ [ppm] = 8.58 (dd, 1H, $J = 7.3$ Hz, 1.2 Hz, H_{20}); 8.50 (d, 1H, $J = 8.1$ Hz, H_{22}); 8.40 (dd, 1H, $J = 8.4$ Hz, 1.2 Hz, H_{10}); 7.68 (dd, 1H, $J = 8.4$ Hz, 7.3 Hz, H_{21}); 7.18 (d, 1H, $J = 8.1$ Hz, H_{11}); 4.50 (dd, 2H, $J = 7.9$ Hz, 6.9 Hz, H_5); 3.24 (t, 4H, $J = 5.3$ Hz, $H_{14, 18}$); 2.83 (dd, 2H, $J = 7.9$ Hz, 7.0 Hz, H_4); 1.89 (p, 4H, $J = 5.6$ Hz, $H_{15, 17}$); 1.73 (t, 2H, $J = 5.8$ Hz, H_{16}).

^{13}C -NMR, HSQC, HMBC (101 MHz, CDCl_3 , 298 K): δ [ppm] = 175.0, 164.7, 164.2, 157.8, 133.1, 131.4, 131.1, 130.2, 126.4, 125.5, 123.0, 115.7, 114.9, 54.7, 35.8, 32.6, 26.4, 24.5.

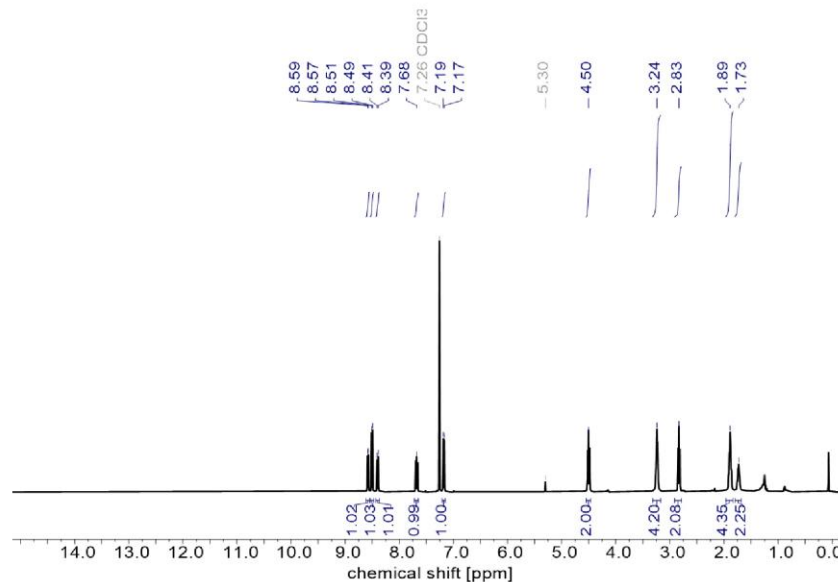


Figure S13: ^1H -NMR spectrum (CDCl_3 , 400 MHz, 298 K) of **C₃-Nap 4**.

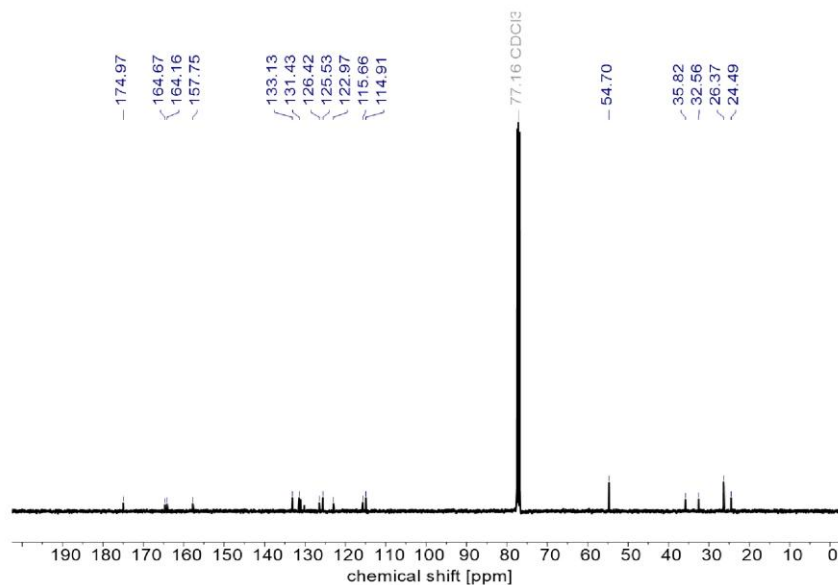


Figure S14: ^{13}C -NMR spectrum (CDCl_3 , 101 MHz, 298 K) of **C₃-Nap 4**.

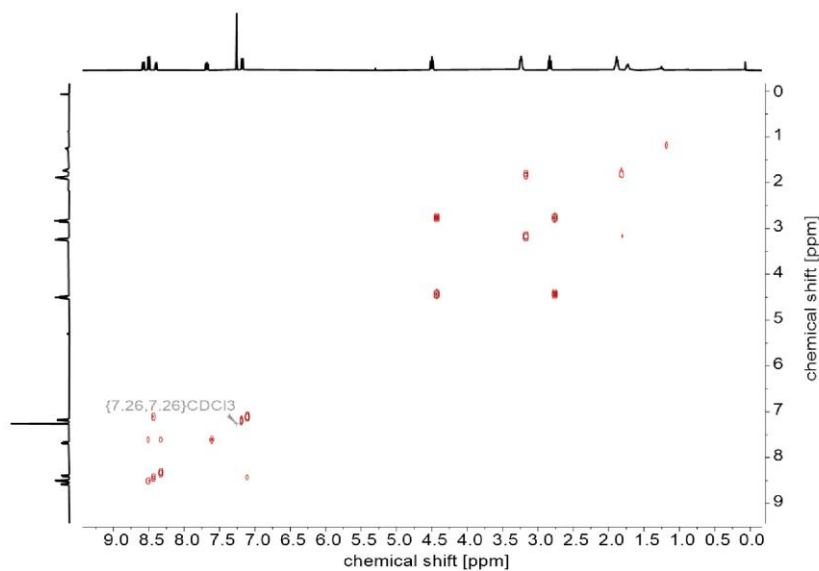


Figure S15: ^1H - ^1H -COSY-NMR spectrum (CDCl_3 , 400 MHz, 298 K) of $\text{C}_3\text{-Nap 4}$.

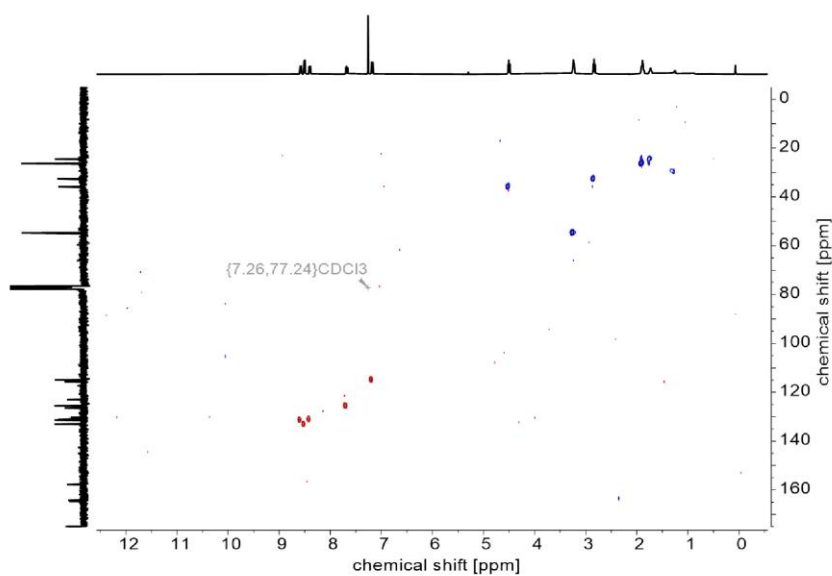


Figure S16: ^1H - ^{13}C -HSQC-NMR spectrum (CDCl_3 , 101 MHz, 298 K) of $\text{C}_3\text{-Nap 4}$.

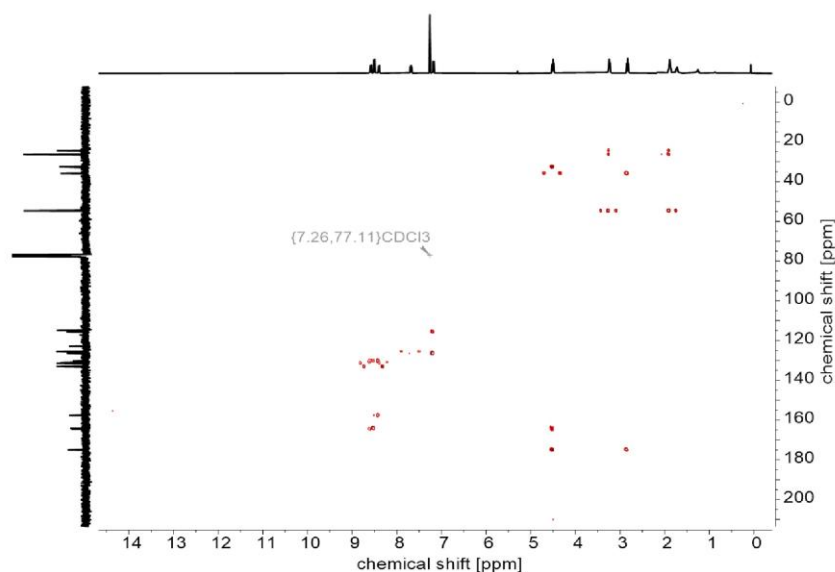


Figure S17: ^1H - ^{13}C -HMBC-NMR spectrum (CDCl_3 , 101 MHz, 298 K) of $\text{C}_3\text{-Nap 4}$.

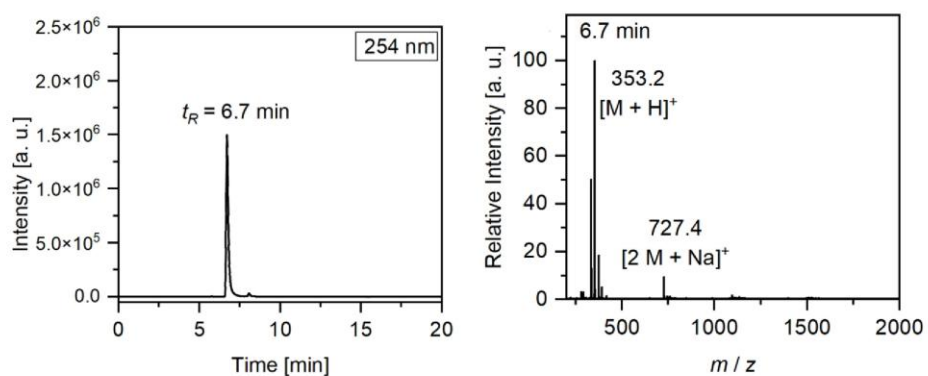
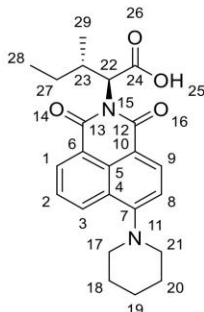


Figure S18: LC elugram (left, detection at 254 nm) and corresponding mass data (right) of $\text{C}_3\text{-Nap 4}$.

2.1.3 Synthesis of (2*S*,3*S*)-2-(1,3-dioxo-6-(piperidin-1-yl)-1*H*-benzo[de]isoquinolin-2(3*H*)-yl)-3-methylpentanoic acid (Nap-Ile).



4-Piperidinyl-1,8-naphthalic anhydride **2** (200 mg; 0.71 mmol; 1.0 eq.), L-isoleucine (115 mg; 0.85 mmol; 1.2 eq.) and 4-DMAP (9 mg; 0.07 mmol; 0.1 eq.) were covered with EtOH (10 mL). *N,N'*-DIPEA (150 μ L; 0.85 mmol; 1.2 eq.) was added in one portion. The reaction mixture was stirred under reflux conditions for 24 h and diluted with DCM (10 mL). The solution was washed with 1 M HCl (3 x 5 mL) and brine (1 x 10 mL). The organic layer was dried over MgSO_4 , filtered and the solvents were removed under reduced pressure. Nap-Ile **5** (280 mg; 0.71 mmol; 100 %) was obtained as an orange solid.

LC-MS: (pos.): calculated for $[\text{M} + \text{H}]^+$: $m/z = 395.5$; measured: $m/z = 395.2$; calculated for $[2 \text{M} + \text{Na}]^+$: $m/z = 811.9$; measured: $m/z = 811.5$.

R_f: 0.75 (EtAc/^cHex = 1:2, v/v; 1 % TFA).

¹H-NMR, COSY (400 MHz, CDCl_3 , 298 K): δ [ppm] = 8.57 (dd, 1H, $J = 7.3$ Hz, 1.2 Hz, H_3); 8.50 (d, 1H, $J = 8.1$ Hz, H_1); 8.40 (dd, 1H, $J = 8.4$ Hz, 1.2 Hz, H_9); 7.68 (dd, 1H, $J = 8.4$ Hz, 7.3 Hz, H_2); 7.18 (d, 1H, $J = 8.1$ Hz, H_8); 5.47 (d, 1H, $J = 9.3$ Hz, H_{22}); 3.24 (t, 4H, $J = 5.3$ Hz, $H_{17, 21}$); 2.62 (tdd, 1H, $J = 10.1$ Hz, 6.6 Hz, 3.7 Hz, H_{23}); 1.88 (p, 4H, $J = 5.6$ Hz, $H_{18, 20}$); 1.73 (q, 2H, $J = 6.2$ Hz, H_{19}); 1.34–1.26 (m, 1H, H_{27}); 1.24 (d, 3H, $J = 6.5$ Hz, H_{29}); 1.09–0.91 (m, 1H, $H_{27''}$); 0.80 (t, 3H, $J = 7.4$ Hz, H_{28}).

¹³C-NMR, HSQC, HMBC (101 MHz, CDCl_3 , 298 K): δ [ppm] = 174.3, 164.6, 164.1, 157.9, 133.6, 131.9, 131.3, 130.4, 126.4, 125.6, 122.7, 115.2, 114.9, 58.2, 54.7, 33.5, 31.1, 26.4, 25.2, 24.5, 18.0, 11.2.

Supporting Information

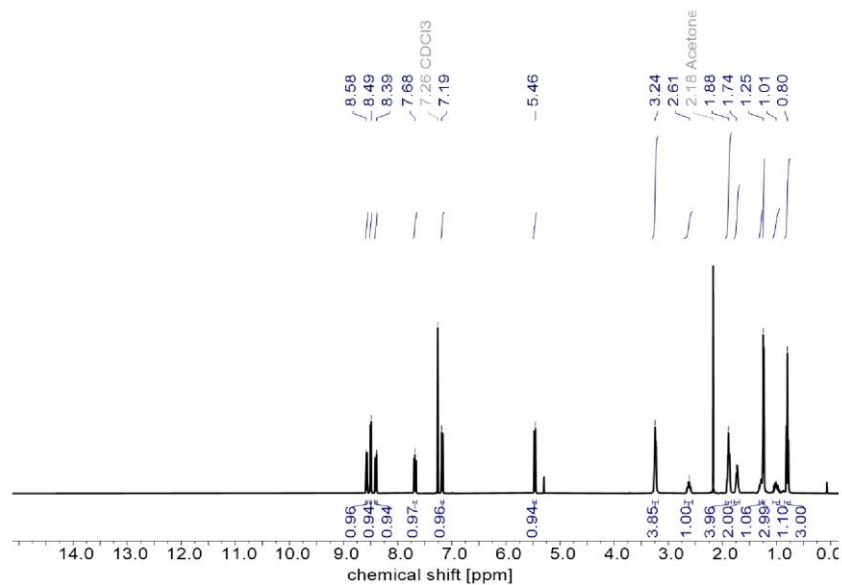


Figure S19: ¹H-NMR spectrum (CDCl₃, 400 MHz, 298 K) of C₀-Nap **5**.

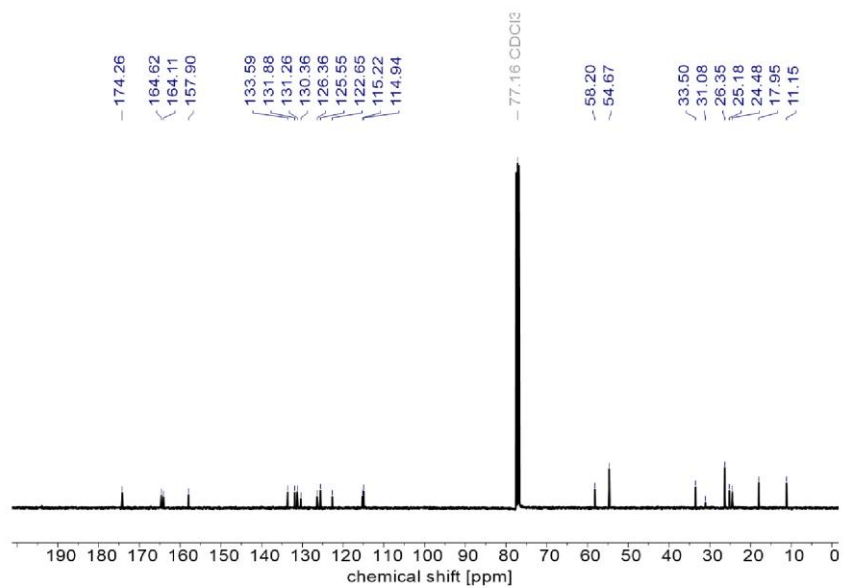


Figure S20: ¹³C-NMR spectrum (CDCl₃, 101 MHz, 298 K) of C₀-Nap **5**.

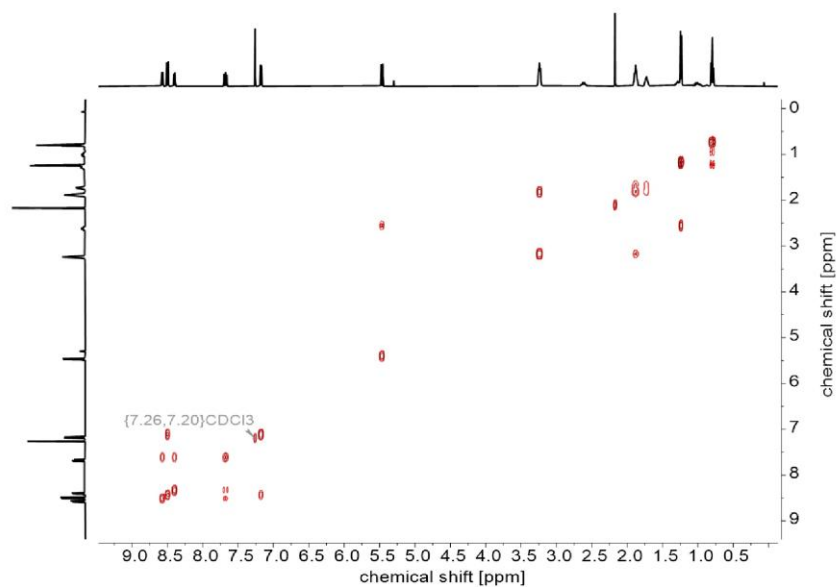


Figure S21: ^1H - ^1H -COSY-NMR spectrum (CDCl_3 , 400 MHz, 298 K) of C_0 -Nap **5**.

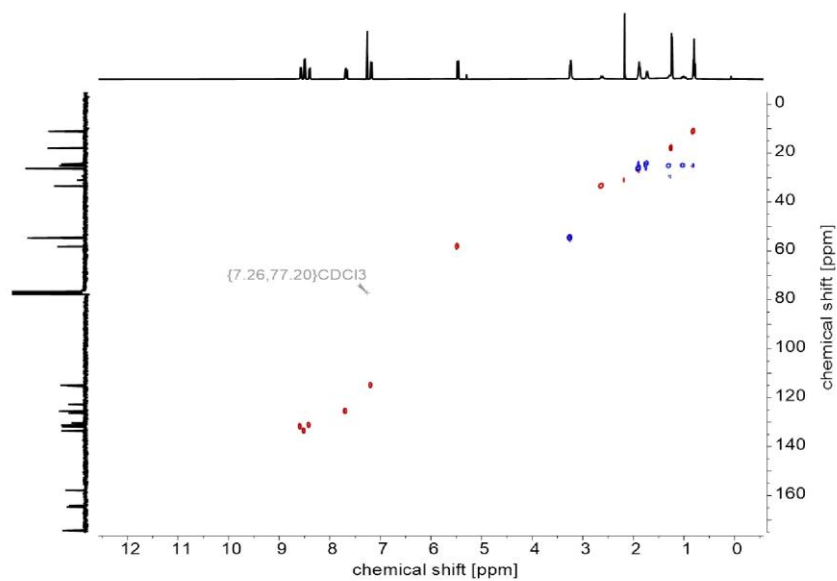


Figure S22: ^1H - ^{13}C -HSQC-NMR spectrum (CDCl_3 , 101 MHz, 298 K) of C_0 -Nap **5**.

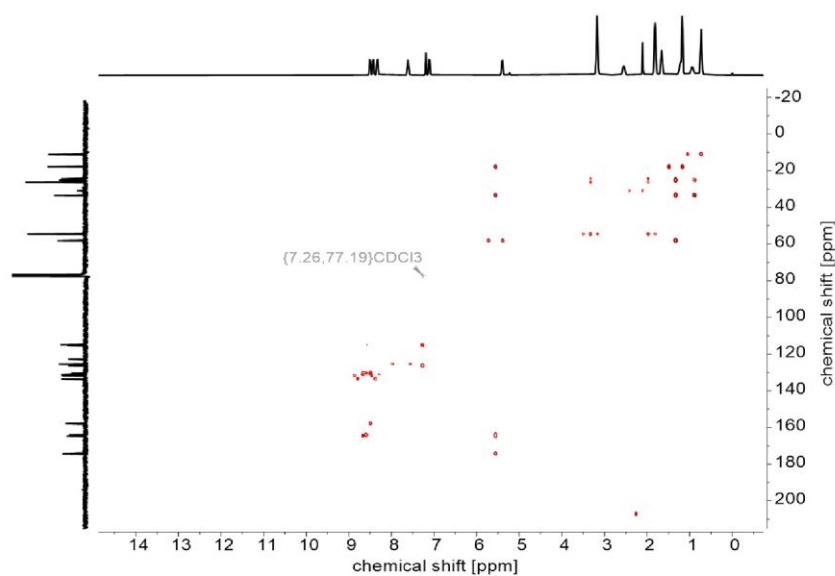


Figure S23: ^1H - ^{13}C -HMBC-NMR spectrum (CDCl_3 , 101 MHz, 298 K) of $\text{C}_0\text{-Nap 5}$.

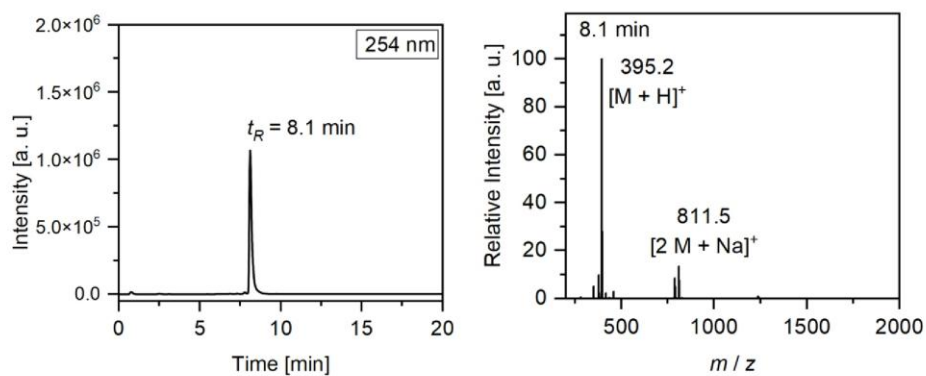
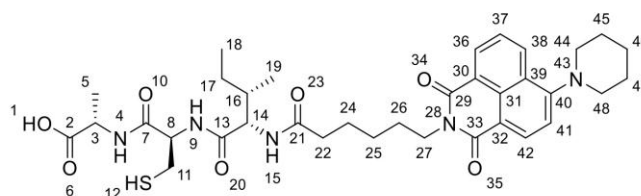


Figure S24: LC elugram (left, detection at 254 nm) and corresponding mass data (right) of $\text{C}_0\text{-Nap 5}$.

2.2 Synthesis of Peptides

2.2.1 Synthesis of C₆-Nap-ICA



C₆-Nap-ICA **6** was synthesized using SPPS at a scale of 0.10 mmol on Fmoc-L-Ala *Clt*-resin according to the general procedure for peptide synthesis. After deprotection of the final Fmoc-protecting group, the resin was washed with DMF (3 x 5 mL). C₆-Nap **3** (50 mg; 0.12 mmol; 1.2 eq.), PyBOP® (155 mg; 0.30 mmol; 3.0 eq.) and *N,N'*-DIPEA (100 μ L, 0.60 mmol; 6.0 eq.) were added to the resin and covered with DMF (5 mL). The reaction mixture was mixed overnight at room temperature. The mixture was filtered and washed with DMF (1 x 5 mL) and DCM (2 x 5 mL). The resin was treated with a cleavage cocktail consisting of 95 % TFA, 2.5 % TIPS and 2.5 % H₂O for two hours at room temperature. The mixture was filtered and the resin was washed with DCM (3 x 5 mL). The solvents were removed under reduced pressure. The crude was purified using HPLC according to the general procedure. C₆-Nap-ICA **6** (12 mg; 0.02 mmol; 20 %) was obtained as a yellow solid.

LC-MS: (pos.): calculated for [M + H]⁺: m/z = 682.9; measured: m/z = 682.4; calculated for [M + Na]⁺: m/z = 704.9; measured: m/z = 704.4; calculated for [2 M + Na]⁺: m/z = 1386.7; measured: m/z = 1385.9.

¹H-NMR, COSY (400 MHz, DMSO-d₆, 298 K): δ [ppm] = 12.61 (s, 1H, *H*₁); 8.47 (dd, 1H, *J* = 7.3 Hz, 1.2 Hz, *H*₃₈); 8.43–8.36 (m, 2H, *H*_{36, 42}); 8.15 (d, 1H, *J* = 7.1 Hz, *H*₉); 7.98 (d, 1H, *J* = 7.9 Hz, *H*₄); 7.87 (d, 1H, *J* = 8.4 Hz, *H*₁₅); 7.81 (dd, 1H, *J* = 8.5 Hz, 7.3 Hz, *H*₃₇); 7.31 (d, 1H, *J* = 8.2 Hz, *H*₄₁); 4.43 (td, 1H, *J* = 7.7 Hz, 5.1 Hz, *H*₈); 4.16 (dt, 2H, *J* = 13.8 Hz, 7.5 Hz, *H*_{3, 14}); 4.01 (t, 2H, *J* = 7.4 Hz, *H*₂₇); 3.20 (t, 4H, *J* = 5.3 Hz, *H*_{44, 48}); 2.84–2.63 (m, 2H, *H*₁₁); 2.26 (dd, 1H, *J* = 9.0 Hz, 7.9 Hz, *H*₁₆); 2.41 (qd, 1H, *J* = 14.3 Hz, 7.1 Hz, *H*₂₂); 1.82 (p, 4H, *J* = 5.6 Hz, *H*_{45, 47}); 1.75–1.45 (m, 8H, *H*_{24, 25, 26, 46}); 1.03–0.88 (m, 3H, *H*₁₈); 0.81–0.72 (m, 6H, *H*_{18, 19}).

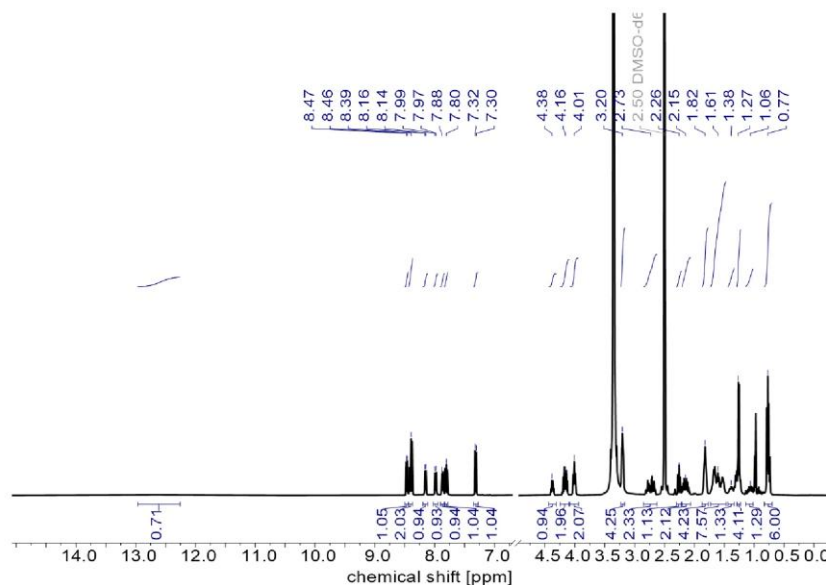


Figure S25: ¹H-NMR spectrum (DMSO-d₆, 400 MHz, 298 K) of C₆-Nap-ICA **6**.

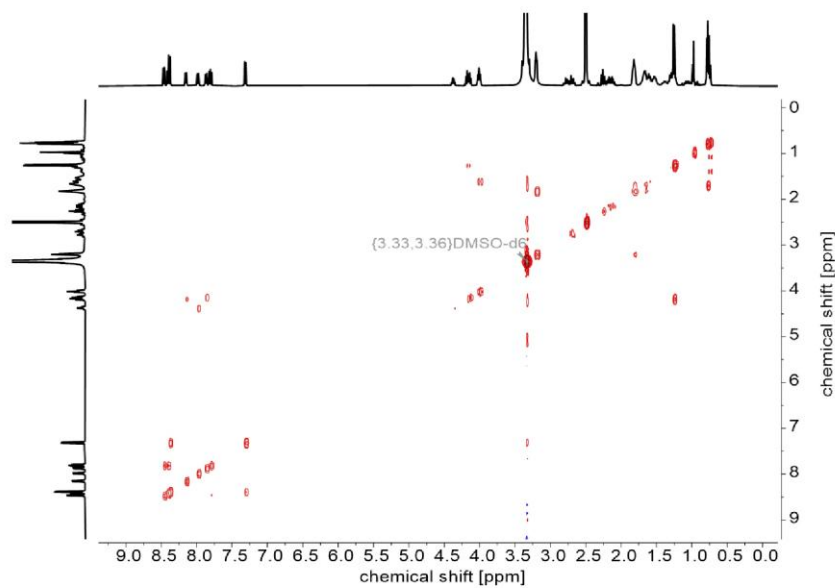


Figure S26: ¹H-¹H-COSY-NMR spectrum (DMSO-d₆, 400 MHz, 298 K) of C₆-Nap-ICA **6**.

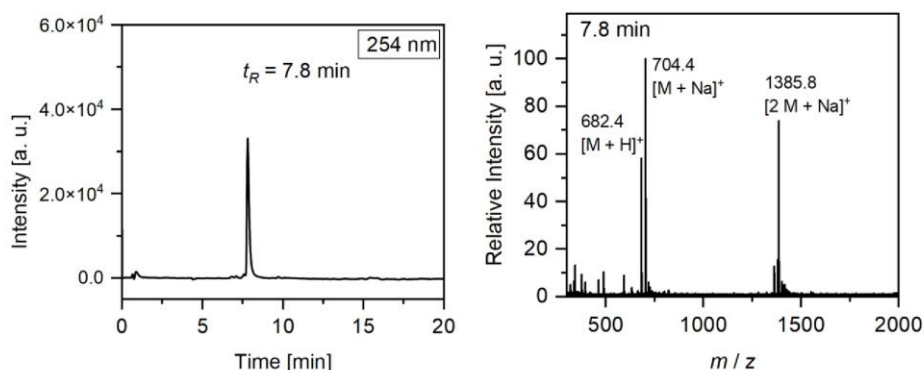
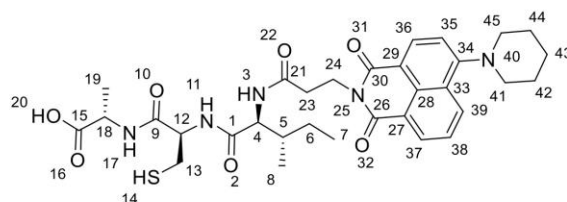


Figure S27: LC elugram (left, detection at 254 nm) and corresponding mass data (right) of **C₆-Nap-ICA 6**.

2.2.2 Synthesis of **C₃-Nap-ICA**



C₃-Nap-ICA 7 was synthesized using SPPS at a scale of 0.25 mmol on Fmoc-L-Ala *Clt*-resin according to the general procedure for automated peptide synthesis. After deprotection of the final Fmoc-protecting group, the resin was washed with DMF (3 x 5 mL). **C₃-Nap 4** (110 mg; 0.30 mmol; 1.2 eq.), PyBOP® (390 mg; 0.75 mmol; 3.0 eq.) and *N,N'*-DIPEA (260 μ L, 1.50 mmol; 6.0 eq.) were added to the resin and covered with DMF (5 mL). The reaction mixture was mixed overnight. The mixture was filtered and washed with DMF (1 x 5 mL) and DCM (3 x 5 mL). Then, the resin was treated with a cleavage cocktail consisting of 95 % TFA, 2.5 % TIPS and 2.5 % H₂O for two hours at room temperature. The mixture was filtered and the resin was washed with DCM (3 x 5 mL). The solvents were removed under reduced pressure. A yellow solid crude was obtained and purified via HPLC according to the general procedure. **C₃-Nap-ICA 7** (13 mg; 0.02 mmol; 8 %) was obtained as an orange solid.

LC-MS: (pos.): calculated for $[M + H]^+$: m/z = 640.8; measured: m/z = 640.3; calculated for $[M + Na]^+$: m/z = 662.8; measured: m/z = 662.3; calculated for $[2 M + H]^+$: m/z = 1280.5; measured: m/z = 1279.8; calculated for $[2 M + Na]^+$: m/z = 1302.5; measured: m/z = 1301.8.

¹H-NMR, COSY (700 MHz, DMSO-*d*₆, 298 K): δ [ppm] = 12.60 (s, 1H, *H*₂₀); 8.47 (dd, 1H, *J* = 7.3 Hz, 1.2 Hz, *H*₃₉); 8.42 (dd, 1H, *J* = 8.5 Hz, 1.2 Hz, *H*₃₇); 8.38 (d, 1H, *J* = 8.1 Hz, *H*₃₆); 8.13 (d, 1H, *J* = 7.1 Hz, *H*₁₁); 8.07 (d, 1H, *J* = 8.1 Hz, *H*₁₇); 8.04 (d, 1H, *J* = 8.0 Hz, *H*₃);

Supporting Information

7.81 (dd, 1H, $J = 8.4$ Hz, 7.2 Hz, H_{38}); 7.32 (d, 1H, $J = 8.1$ Hz, H_{35}); 4.39 (td, 1H, $J = 7.9$ Hz, 5.0 Hz, H_{12}); 4.27 (ddd, 1H, $J = 13.0$ Hz, 8.8 Hz, 6.6 Hz, H_4); 4.23–4.18 (m, 1H, H_{18}); 4.14 (dt, 2H, $J = 17.0$ Hz, 7.5 Hz, H_{24}); 3.21 (s, 4H, $H_{41,45}$); 2.85–2.79 (m, 1H, H_{13}); 2.75–2.68 (m, 1H, $H_{13''}$); 2.60–2.51 (m, 2H, H_{23}); 2.30 (t, 1H, $J = 8.5$ Hz, H_5); 1.82 (p, 4H, $J = 5.6$ Hz, $H_{42,44}$); 1.66 (s, 2H, H_{43}); 1.41–1.35 (m, 1H, H_6); 1.26 (d, 3H, $J = 7.3$ Hz, H_{19}); 1.07–0.99 (m, 1H, $H_{6'}$); 0.79 (d, 3H, $J = 6.9$ Hz, H_8); 0.73 (t, 3H, $J = 7.4$ Hz, H_7).

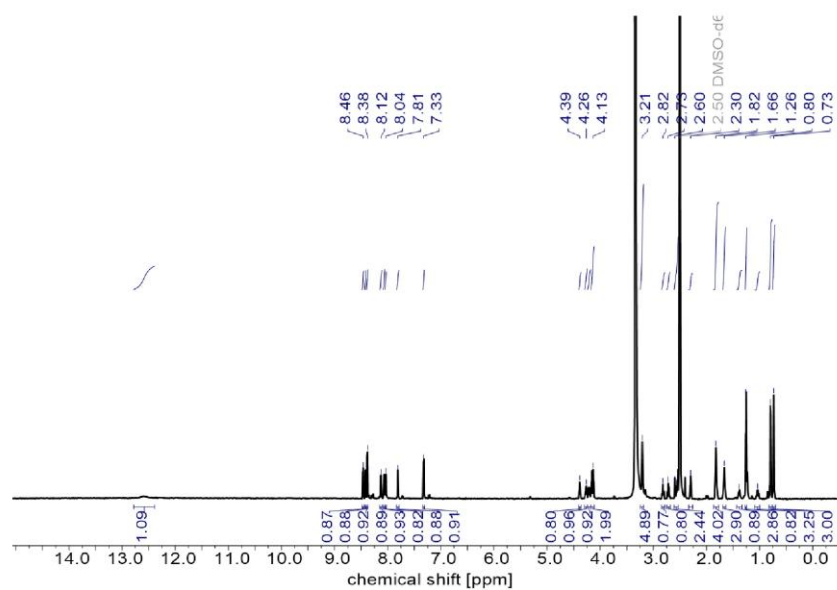
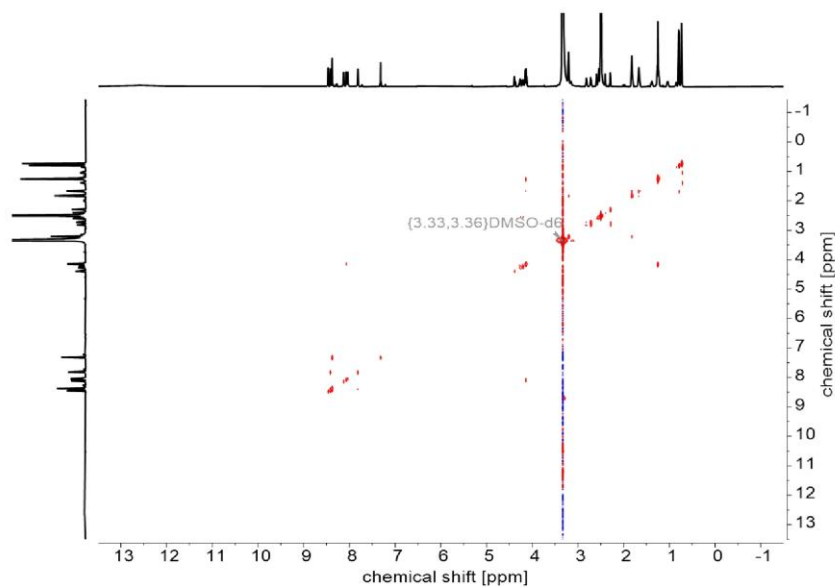
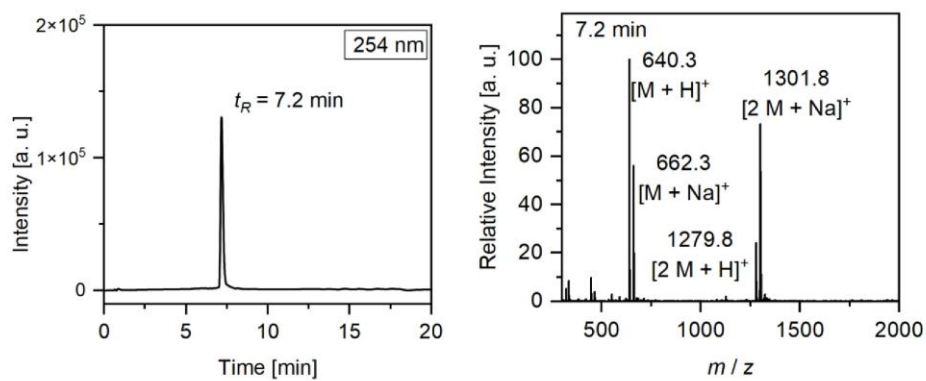
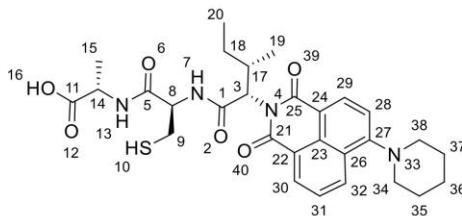


Figure S28: ^1H -NMR spectrum (DMSO-d_6 , 700 MHz, 298 K) of $\text{C}_3\text{-Nap-ICA 7}$.


 Figure S29: ^1H - ^1H -COSY-NMR spectrum (DMSO- d_6 , 700 MHz, 298 K) of C_3 -Nap-ICA 7.

 Figure S30: LC elugram (left, detection at 254 nm) and corresponding mass data (right) of C_3 -Nap-ICA 7.

2.2.3 Synthesis of C₀-Nap-ICA

The synthesis of C₀-Nap-ICA **8** was performed at a scale of 0.10 mmol. H-L-Ala *Clt*-resin (140 mg; 0.10 mmol; 1.0 eq.) was covered with DMF (5 mL) and mixed for one hour. Then, PyBOP® (210 mg; 0.40 mmol; 4.0 eq.), *N,N'*-DIPEA (104 μ L; 0.60 mmol; 6.0 eq.) and Fmoc-L-Cys(Trt)-OH (125 mg; 0.20 mmol; 2.0 eq.) were added and the mixture was mixed for 2 h. The resin was filtered and washed with DMF (3 x 5 mL). Then, the resin was treated twice with piperidine (20 % in DMF, 5 mL) for 10 min each. After filtration, the resin was washed with DMF (3 x 5 mL). PyBOP® (210 mg; 0.40 mmol; 4.0 eq.), *N,N'*-DIPEA (104 μ L; 0.60 mmol; 6.0 eq.) and Nap-Ile **6** (60 mg; 0.15 mmol; 1.5 eq.) were dissolved in DMF (5 mL) and added to the resin. The mixture was mixed for 2 h at room temperature. After filtration, the mixture was washed with DMF (3 x 5 mL) and DCM (3 x 5 mL). The resin was treated with a cleavage cocktail consisting of 95 % TFA, 2.5 % TIPS and 2.5 % H₂O for three hours. The mixture was filtered and washed with DCM (3 x 5 mL). The solvents were removed under reduced pressure. A yellow solid crude was obtained and purified via HPLC according to the general procedure. Nap-ICA **8** (8 mg; 0.01 mmol; 14 %) was obtained as a yellow solid.

LC-MS: (pos.): calculated for [M + H]⁺: m/z = 569.7; measured: m/z = 569.3; calculated for [2 M + H]⁺: m/z = 1138.4; measured: m/z = 1137.7.

¹H-NMR, COSY (700 MHz, DMSO-d₆, 298 K): δ [ppm] = 12.53 (s, 1H, H_{16}); 8.47 (d, 1H, J = 7.6 Hz, H_{32}); 8.44 (dd, 1H, J = 7.2 Hz, 1.2 Hz, H_{30}); 8.38 (d, 1H, J = 7.9 Hz, H_{29}); 8.19 (d, 1H, J = 7.1 Hz, H_7); 8.05 (d, 1H, J = 8.1 Hz, H_{13}); 7.83 (ddd, 1H, J = 14.0 Hz, 8.4 Hz, 7.2 Hz, H_{28}); 7.34 (dd, 1H, J = 14.6 Hz, 8.2 Hz, H_{31}); 5.15 (d, 1H, J = 9.2 Hz, H_3); 4.40 (qd, 1H, J = 8.6 Hz, 4.7 Hz, H_8); 4.15 (p, 1H, J = 7.3 Hz, H_{14}); 3.22 (t, 4H, J = 5.4 Hz, $H_{34, 38}$); 2.70–2.57 (m, 2H, $H_{9', 9''}$); 2.02–1.96 (m, 1H, H_{17}); 1.83 (p, 4H, J = 5.6 Hz, $H_{35, 37}$); 1.70–1.64 (m, 2H, H_{36}); 1.29 (d, 2H, J = 7.3 Hz, H_{18}); 1.11 (d, 3H, J = 6.4 Hz, H_{15}); 0.97–0.87 (m, 3H, H_{19}); 0.72 (t, 3H, J = 7.4 Hz, H_{20}).

Supporting Information

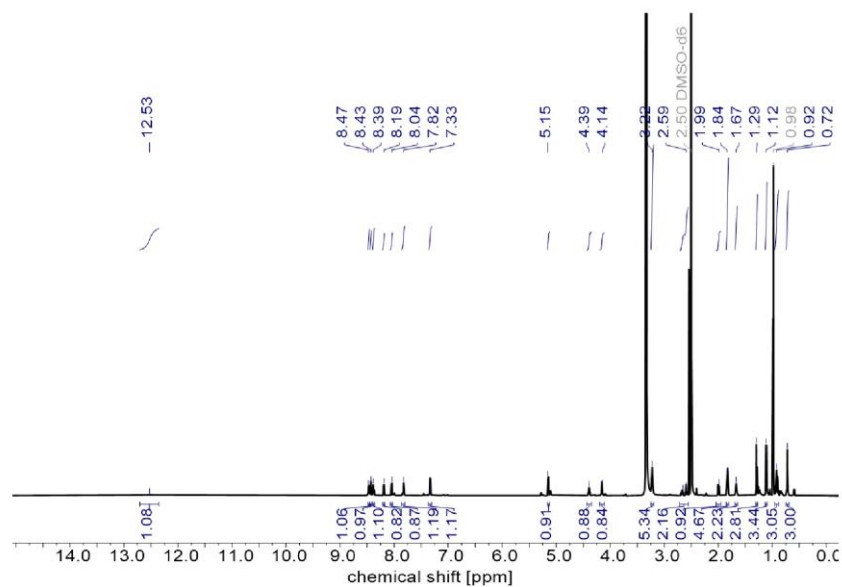


Figure S31: ^1H -NMR spectrum (DMSO- d_6 , 700 MHz, 298 K) of C₀-Nap-ICA **8**.

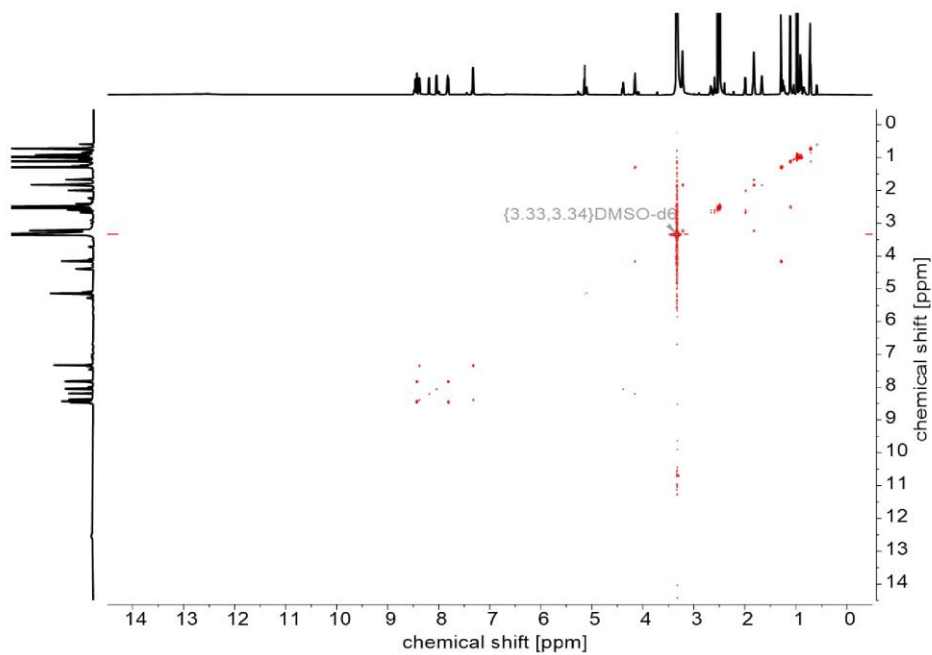


Figure S32: ^1H - ^1H -COSY-NMR spectrum (DMSO- d_6 , 700 MHz, 298 K) of C₀-Nap-ICA **8**.

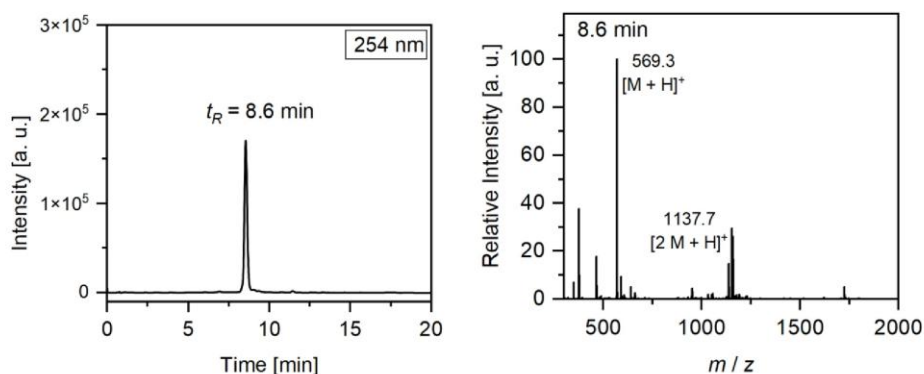


Figure S33: LC elugram (left, detection at 254 nm) and corresponding mass data (right) of Co-Nap-ICA 8.

3 Self-Assembly

3.1 Assembly Conversion Rate

The peptides were analyzed according to the general procedure for Conversion Rate determination. The elugrams were referenced to the standard and plotted using *Origin Pro* by *OriginLab*[®]. The Area under Curve (AUC) of the peptide signals in PBS (AUC_{PBS}) and in MeOH (AUC_{MeOH}) were calculated and used to determine the conversion rate (CR) using the following equation:

$$CR [\%] = \left(1 - \frac{AUC_{PBS}}{AUC_{MeOH}}\right) \cdot 100 \%$$

For peptides with full conversion rate, $CR > 95 \%$ was chosen due to the detection limit of the analytical HPLC.

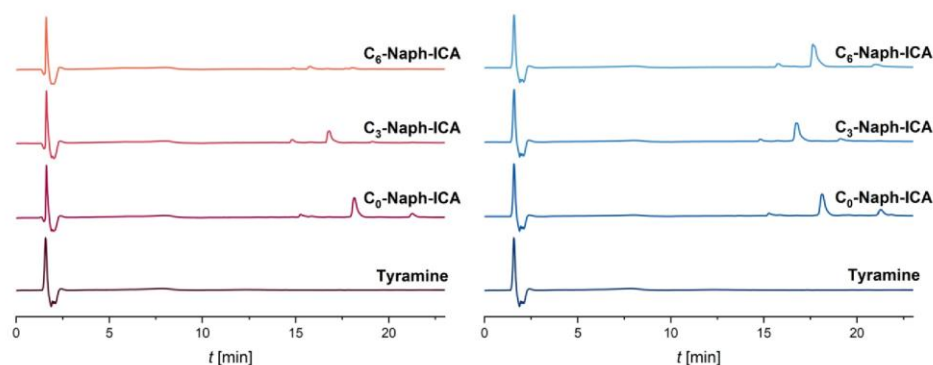


Figure S34: Elugrams of C_6 -Nap-ICA **6**, C_3 -Nap-ICA **7** and C_0 -Nap-ICA **8** (all 100 μ M) in PBS (50 mM, pH 7.4, 1 % TFE; *left*) and MeOH (1 % TFE; *right*) as well as the internal standard Tyramine (200 μ M in MeOH, **Standard**). The elugrams are shown as a plot of absorbance intensity at 214 nm against retention time t_R .

3.2 Fluorescence, Transmission Electron and Atomic Force Microscopy

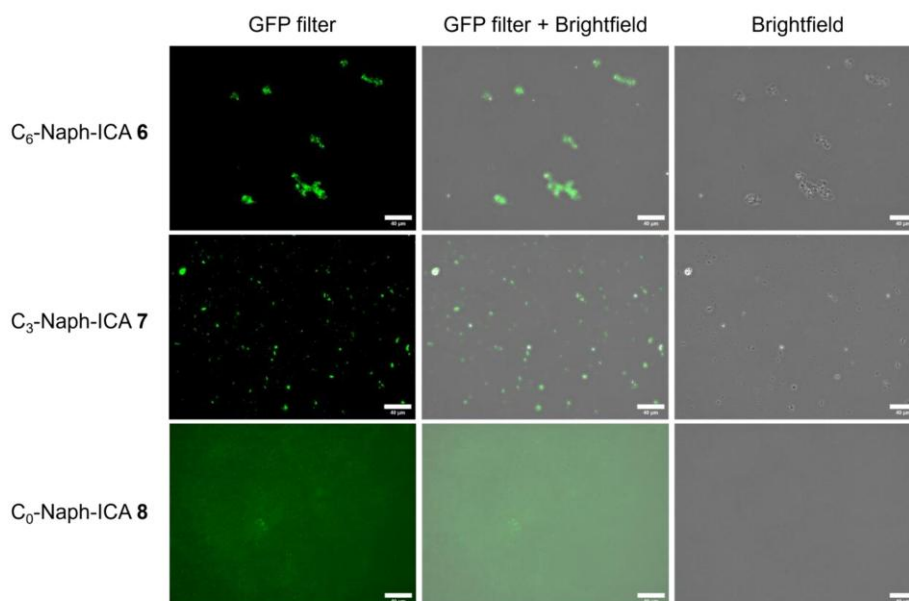


Figure S35: Fluorescence microscopy images of C_6 -Nap-ICA **6**, C_3 -Nap-ICA **7** or C_0 -Nap-ICA **8** (all 100 μ M) in PBS (50 mM, pH 7.4, 1 % TFE) including GFP-filter and brightfield, scale bar = 40 μ m.

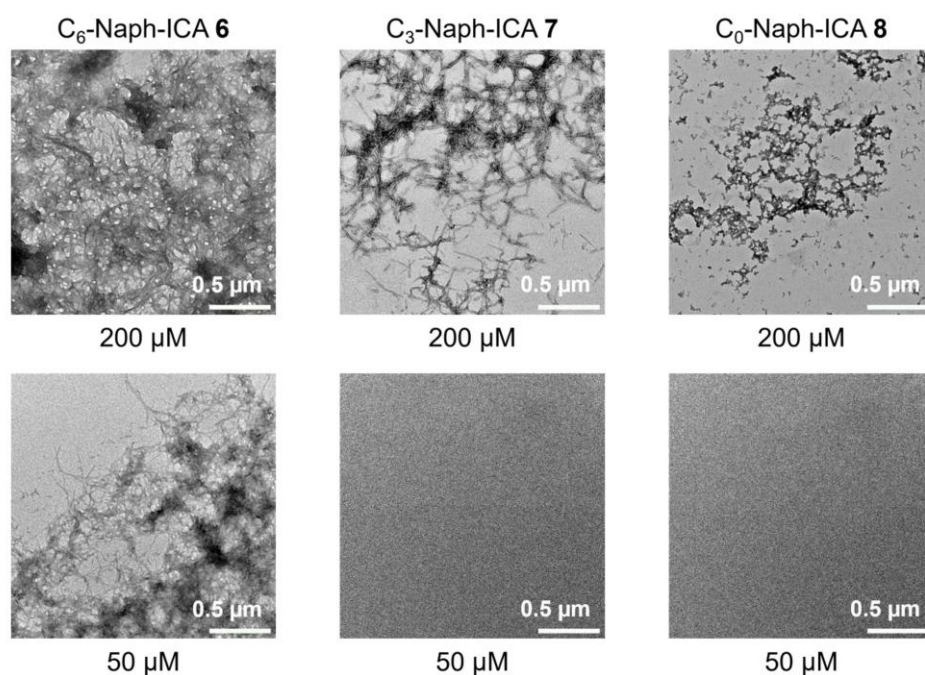


Figure S36: TEM micrographs of C₆-Nap-ICA 6, C₃-Nap-ICA 7 and C₀-Nap-ICA 8 (200/50 μM) in PBS (50 mM, pH 7.4, 1 % TFE) after 24 h incubation at room temperature. The structures were stained with uranyl(IV) acetate (4 %), scale bar = 0.5 μm.

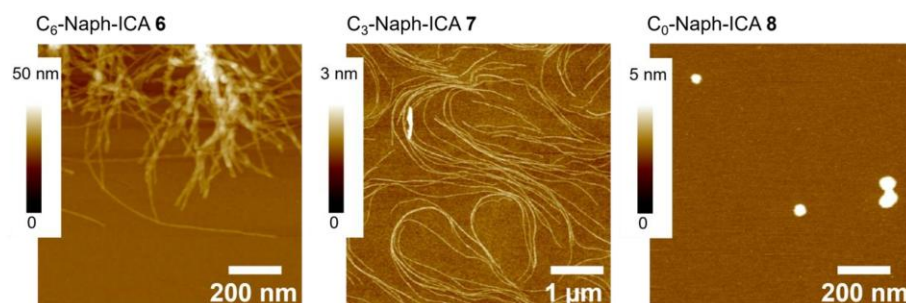


Figure S37: AFM images of supramolecular structures formed by C₆-Nap-ICA 6, C₃-Nap-ICA 7, C₀-Nap-ICA 8 samples at 100 μM in MilliQ-H₂O (1 % TFE) after 24 h incubation at room temperature.

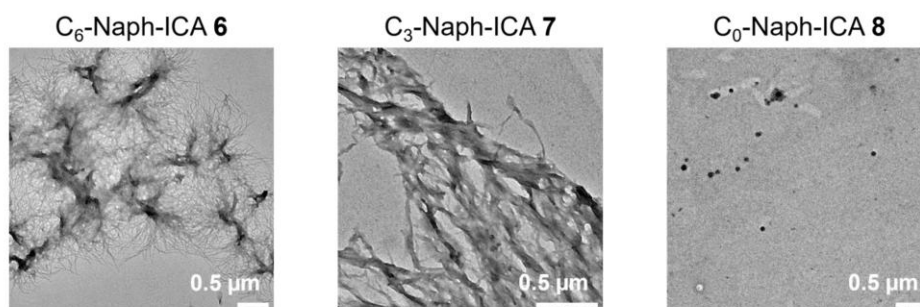


Figure S38: TEM micrographs of C₆-Nap-ICA **6**, C₃-Nap-ICA **7** and C₀-Nap-ICA **8** (100 μM) in MilliQ-H₂O (1 % TFE) after 24 h incubation at room temperature. The structures were stained with uranyl (IV) acetate (4 %), scale bar = 0.5 μm.

3.3 UV/Vis- & Fluorescence Emission Spectroscopy

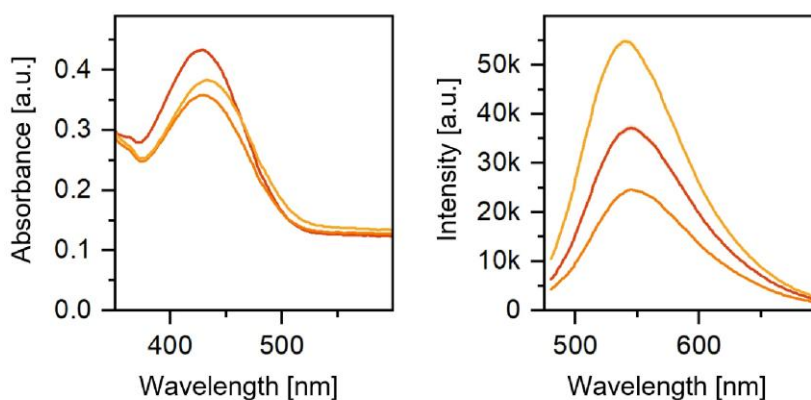


Figure S39: UV/Vis-absorbance spectroscopy (left) and fluorescence emission spectroscopy (right) of the three peptide conjugates C₆-Nap-ICA **6** (red), C₃-Nap-ICA **7** (light orange) and C₀-Nap-ICA **8** (yellow) at 100 μM in 100 % TFE.

4 NMR study with increasing TFE content

To investigate the self-assembly propensity of the three peptide conjugates, the compounds were dissolved in mixtures of d₃-trifluoroethanol and PBS-buffered D₂O with increasing d₃-TFE content (1/99, 10/90, 20/80, 30/70, 40/60, 50/50, and 100/0, v/v) and analyzed by ¹H-NMR spectroscopy.

Supporting Information

The aqueous component of the solvent mixtures was prepared by lyophilizing 40 mL of commercial PBS to dryness and redissolving the residue in the same volume of D₂O. Dimethyl sulfone (DMS, 6.96 mg) was added as an internal reference.

Each peptide conjugate was initially dissolved in d₃-TFE, followed by the addition of the DMS-containing PBS/D₂O buffer (250 μ L) and additional PBS/D₂O buffer without DMS to achieve the desired solvent compositions. The exact volumes used are summarized in Table S1.

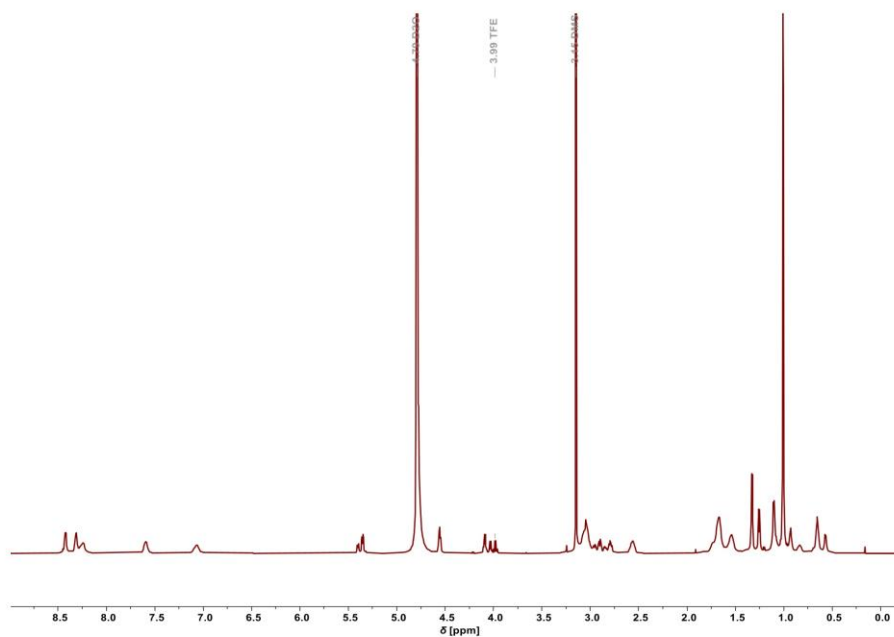


Figure S40: ¹H-NMR (700 MHz, 298 K, D₂O/d₃-TFE 99/1) spectrum of C₀-ICA-Nap **8**.

Supporting Information

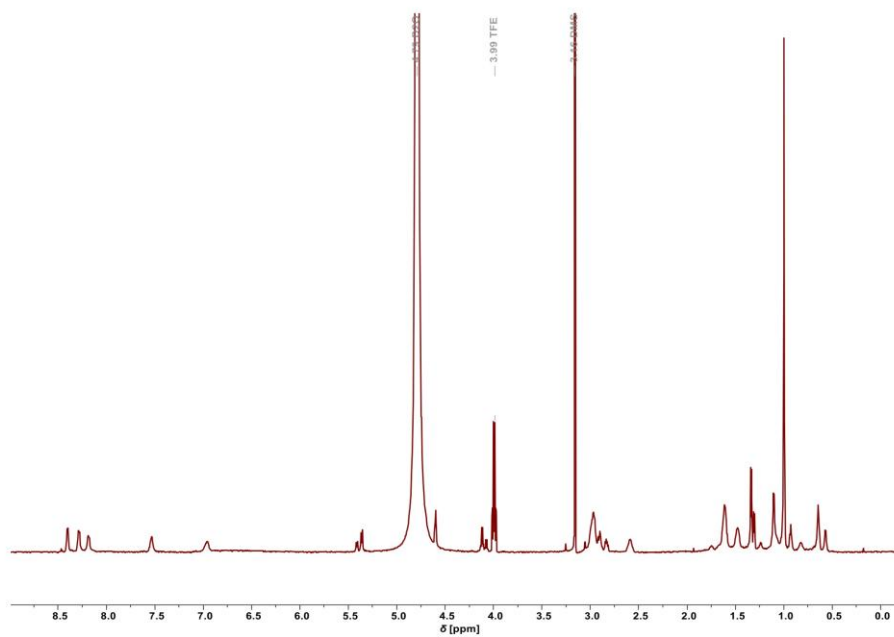


Figure S41: ¹H-NMR (700 MHz, 298 K, D₂O/d₃-TFE 90/10) spectrum of C₀-ICA-Nap **8**.

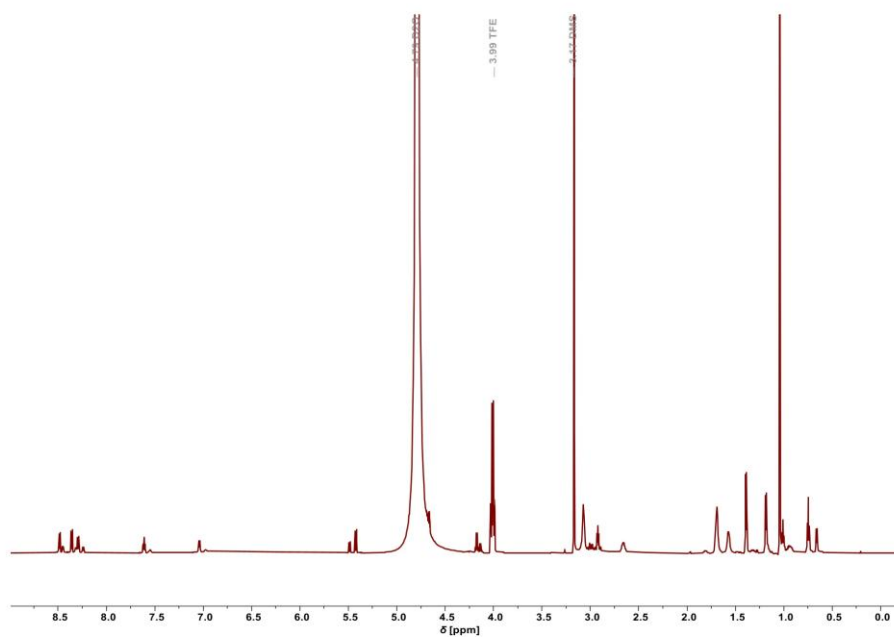


Figure S42: ¹H-NMR (700 MHz, 298 K, D₂O/d₃-TFE 80/20) spectrum of C₀-ICA-Nap **8**.

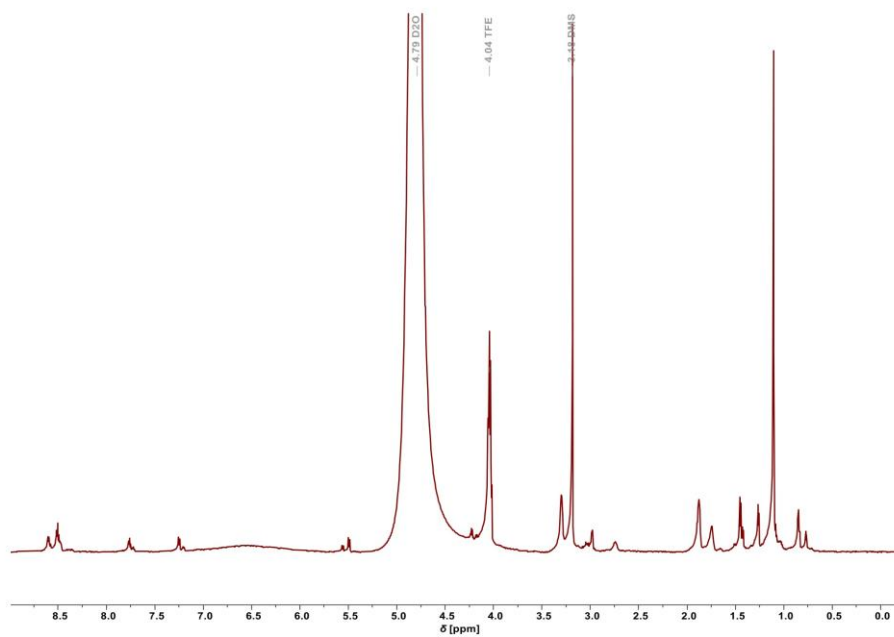


Figure S43: ¹H-NMR (700 MHz, 298 K, D₂O/d₃-TFE 70/30) spectrum of C₀-ICA-Nap 8.

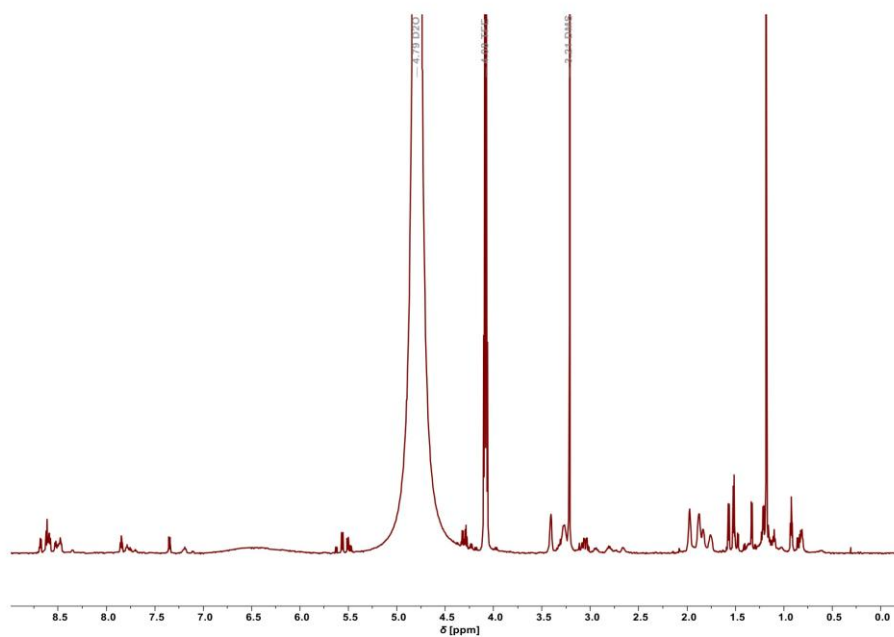


Figure S44: ¹H-NMR (700 MHz, 298 K, D₂O/d₃-TFE 60/40) spectrum of C₀-ICA-Nap 8.

Supporting Information

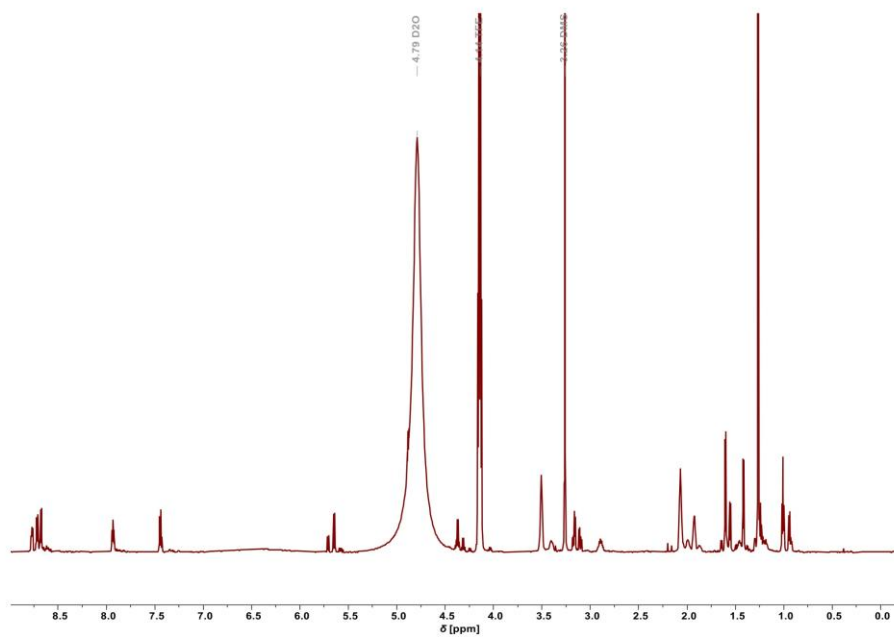


Figure S45: ¹H-NMR (700 MHz, 298 K, D₂O/d₃-TFE 50/50) spectrum of C₀-ICA-Nap **8**.

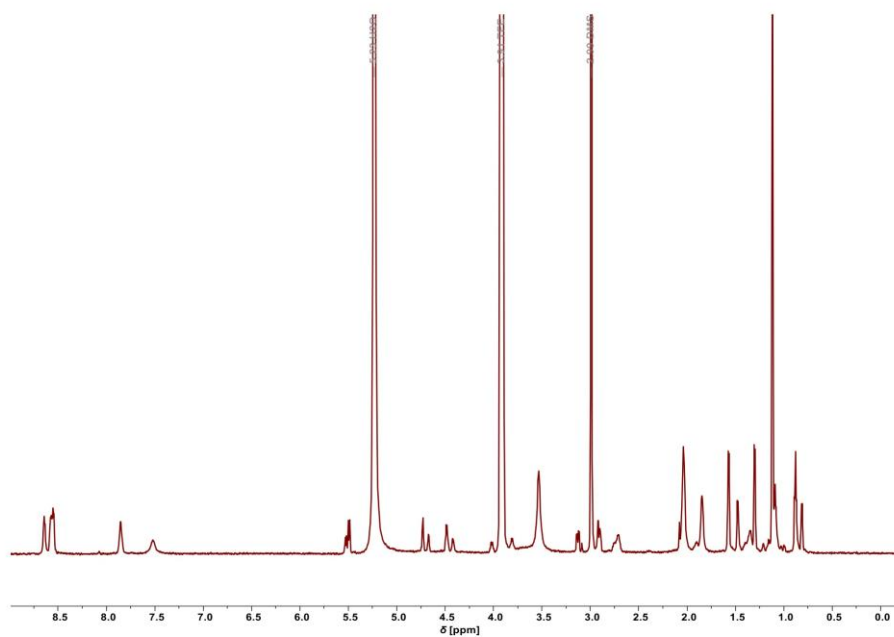


Figure S46: ¹H-NMR (700 MHz, 298 K, d₃-TFE) spectrum of C₀-ICA-Nap **8**.

Supporting Information

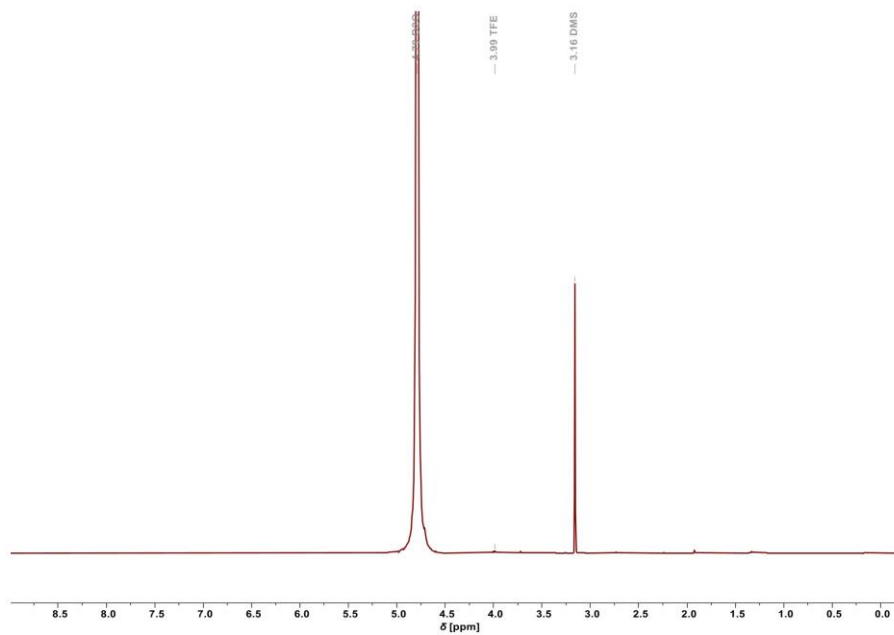


Figure S47: ^1H -NMR (700 MHz, 298 K, $\text{D}_2\text{O}/\text{d}_3\text{-TFE}$ 99/1) spectrum of $\text{C}_3\text{-ICA-Nap 7}$.

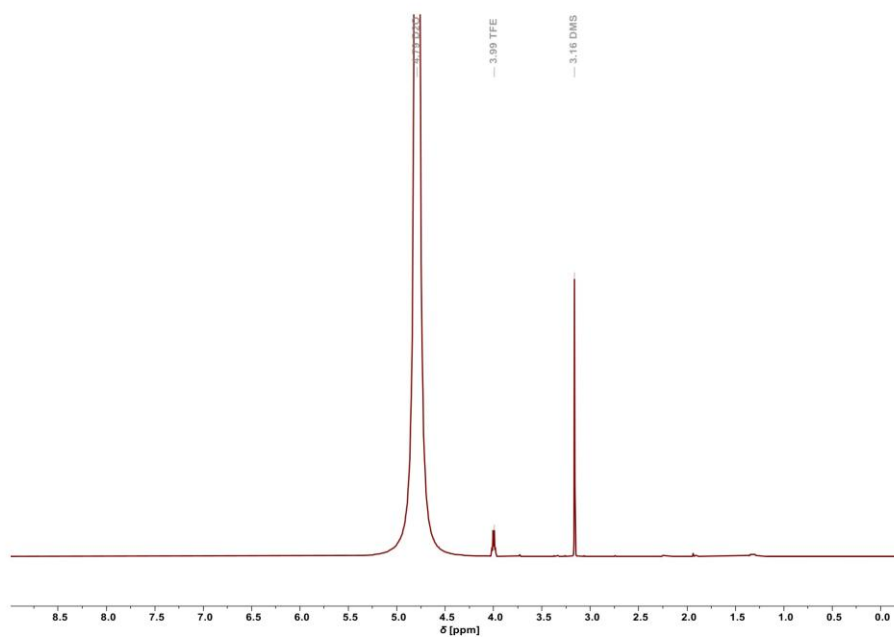


Figure S48: ^1H -NMR (700 MHz, 298 K, $\text{D}_2\text{O}/\text{d}_3\text{-TFE}$ 90/10) spectrum of $\text{C}_3\text{-ICA-Nap 7}$.

Supporting Information

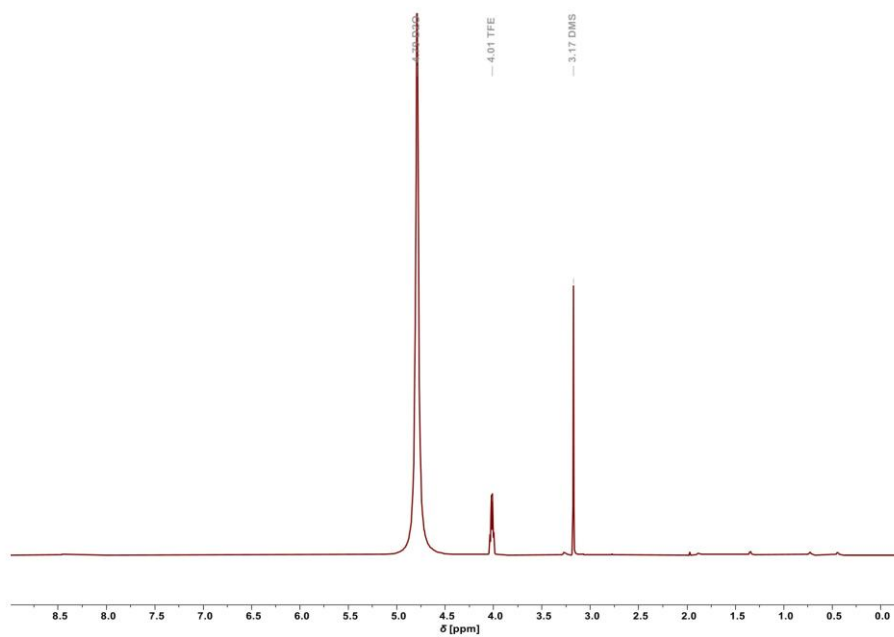


Figure S49: ^1H -NMR (700 MHz, 298 K, $\text{D}_2\text{O}/\text{d}_3\text{-TFE}$ 80/20) spectrum of $\text{C}_3\text{-ICA-Nap 7}$.

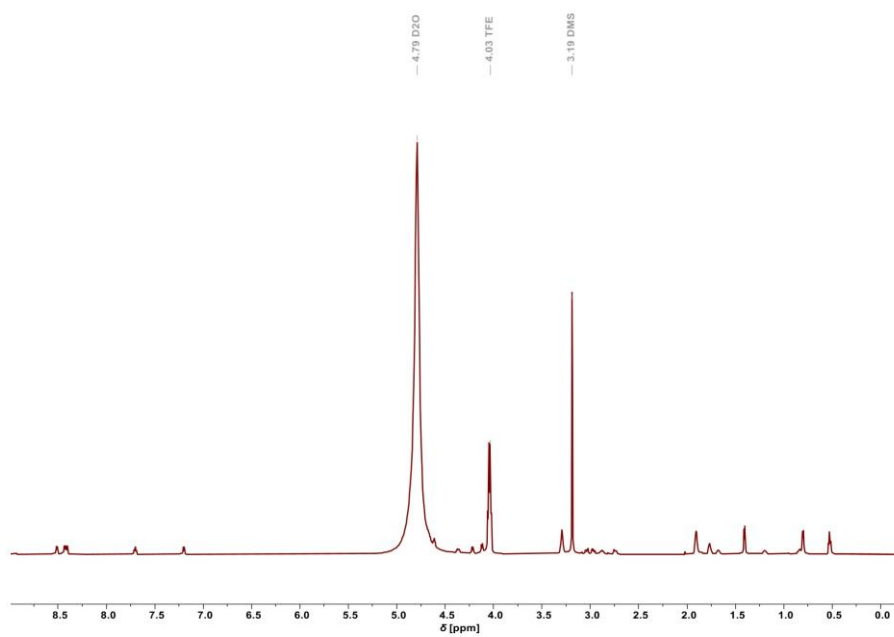


Figure S50: ^1H -NMR (700 MHz, 298 K, $\text{D}_2\text{O}/\text{d}_3\text{-TFE}$ 70/30) spectrum of $\text{C}_3\text{-ICA-Nap 7}$.

Supporting Information

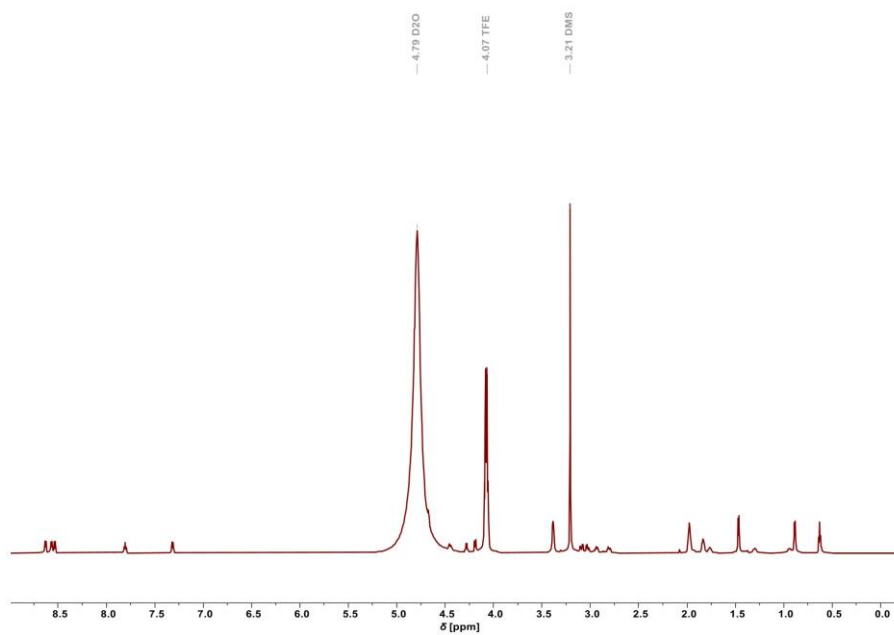


Figure S51: ¹H-NMR (700 MHz, 298 K, D₂O/d₃-TFE 60/40) spectrum of C₃-ICA-Nap 7.

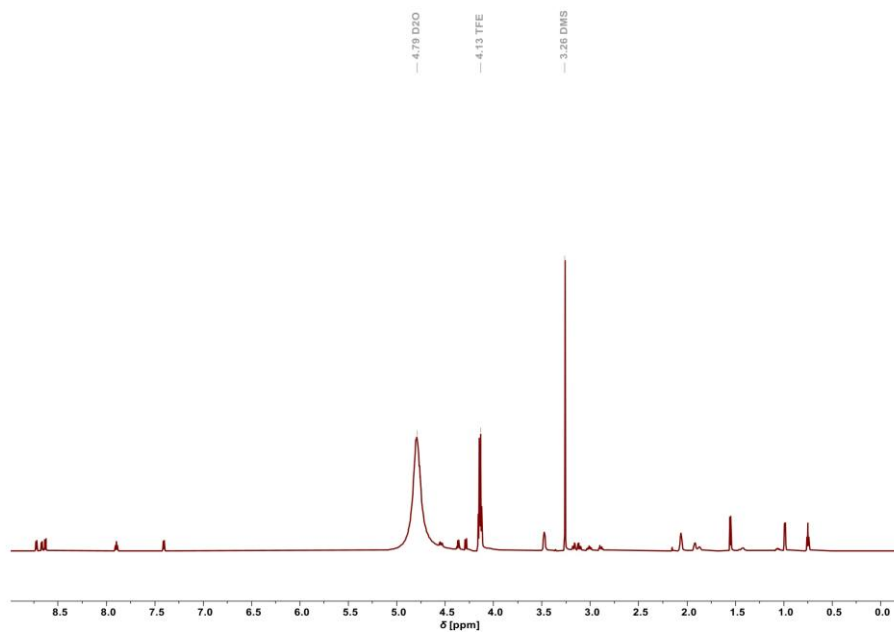


Figure S52: ¹H-NMR (700 MHz, 298 K, D₂O/d₃-TFE 50/50) spectrum of C₃-ICA-Nap 7.

Supporting Information

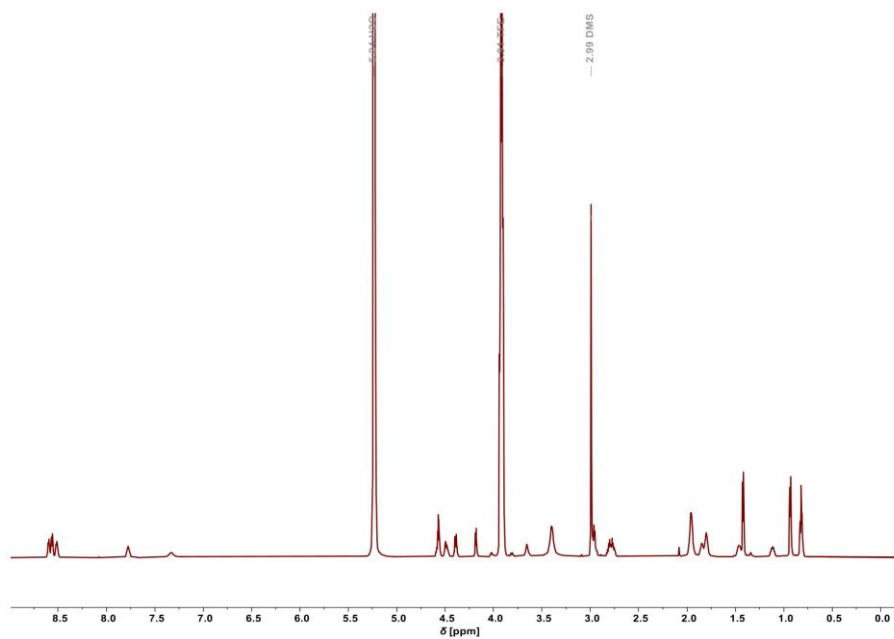


Figure S53: ^1H -NMR (700 MHz, 298 K, d_3 -TFE) spectrum of C_3 -ICA-Nap **7**.

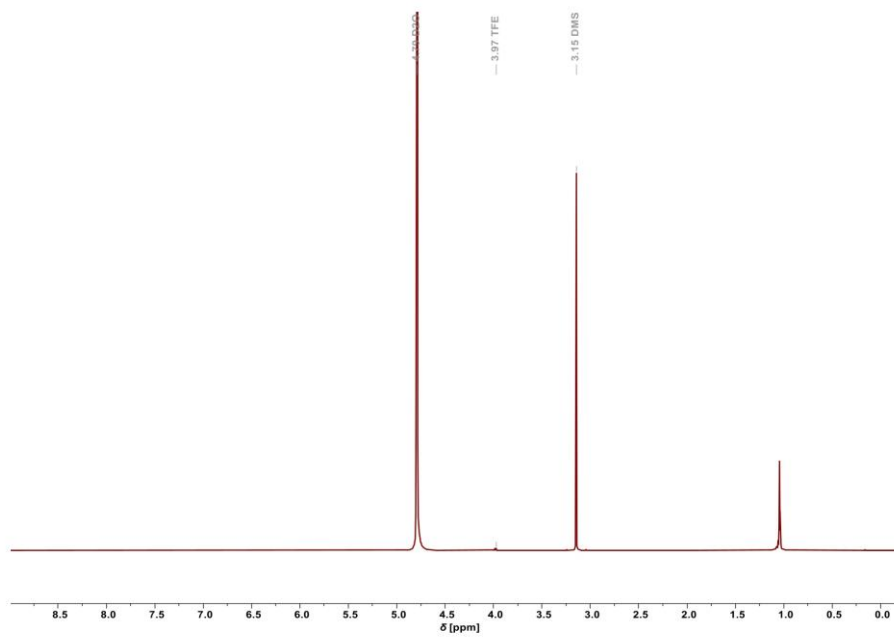


Figure S54: ^1H -NMR (700 MHz, 298 K, $\text{D}_2\text{O}/\text{d}_3$ -TFE 99/1) spectrum of C_6 -ICA-Nap **6**.

Supporting Information

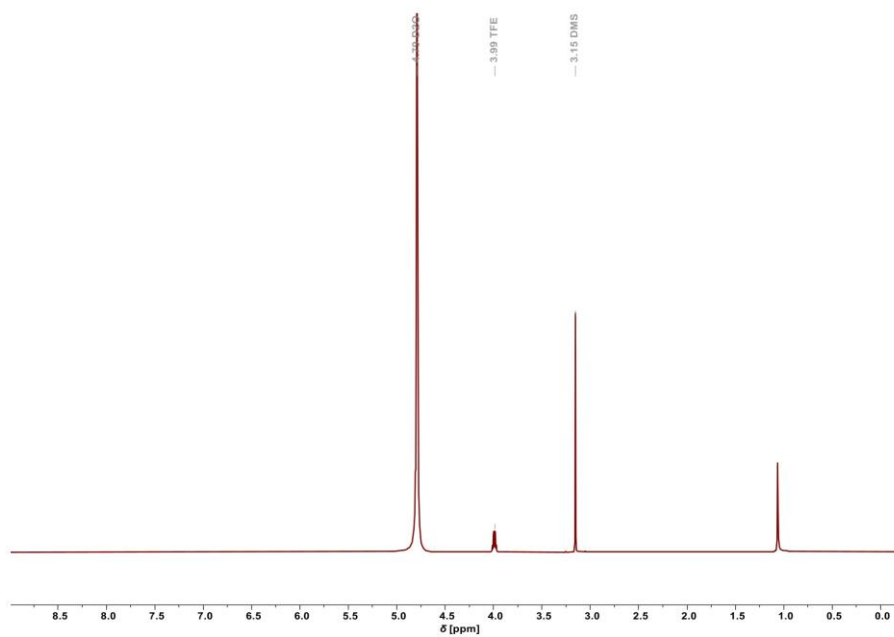


Figure S55: ¹H-NMR (700 MHz, 298 K, D₂O/d₃-TFE 90/10) spectrum of C₆-ICA-Nap 6.

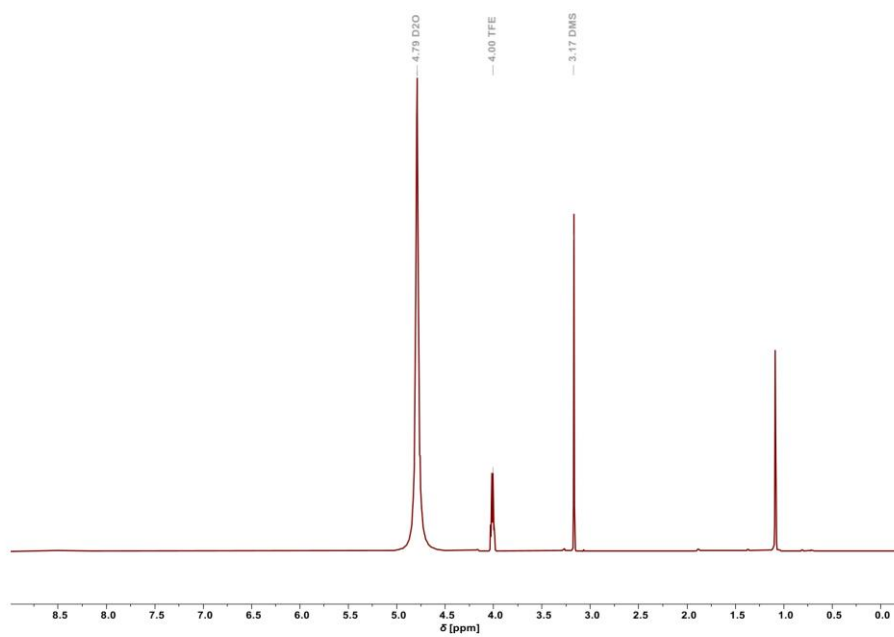


Figure S56: ¹H-NMR (700 MHz, 298 K, D₂O/d₃-TFE 80/20) spectrum of C₆-ICA-Nap 6.

Supporting Information

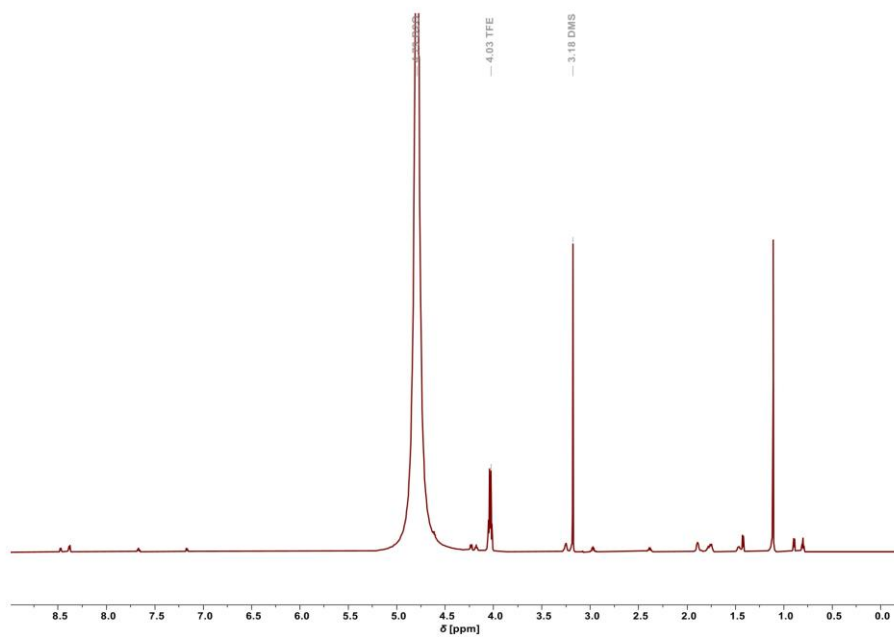


Figure S57: ^1H -NMR (700 MHz, 298 K, $\text{D}_2\text{O}/\text{d}_3\text{-TFE}$ 70/30) spectrum of **C₆-ICA-Nap 6**.

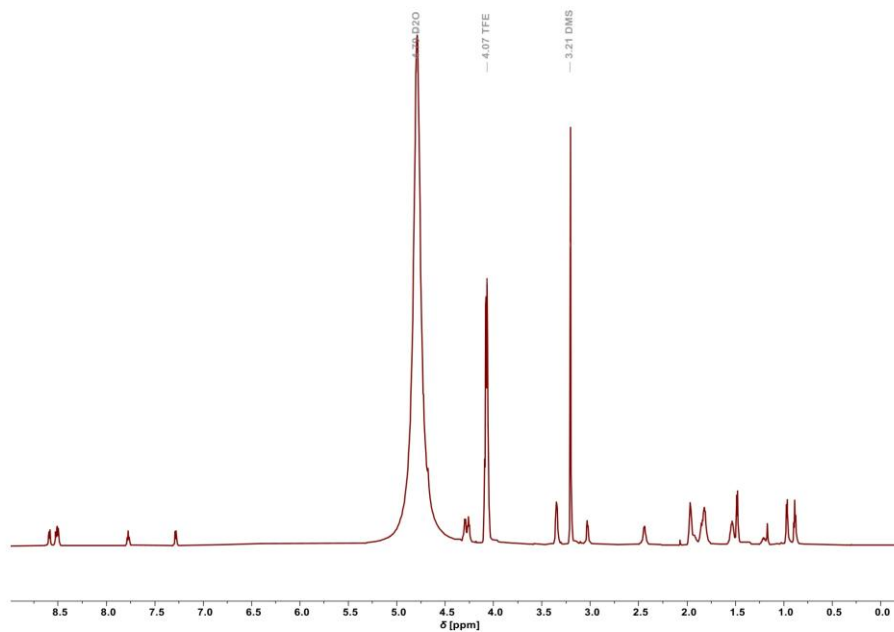


Figure S58: ^1H -NMR (700 MHz, 298 K, $\text{D}_2\text{O}/\text{d}_3\text{-TFE}$ 60/40) spectrum of **C₆-ICA-Nap 6**.

Supporting Information

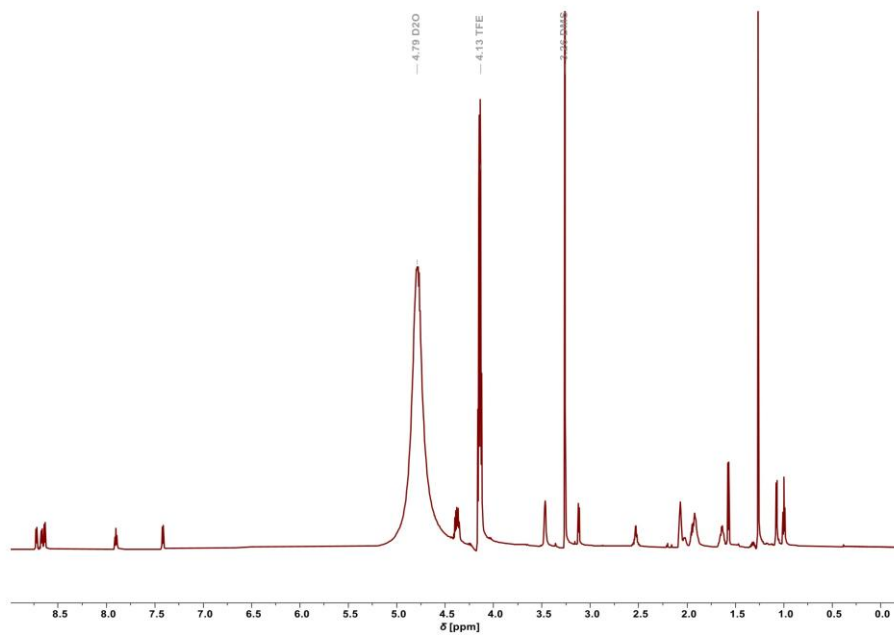


Figure S59: ¹H-NMR (700 MHz, 298 K, D₂O/d₃-TFE 50/50) spectrum of C₆-ICA-Nap **6**.

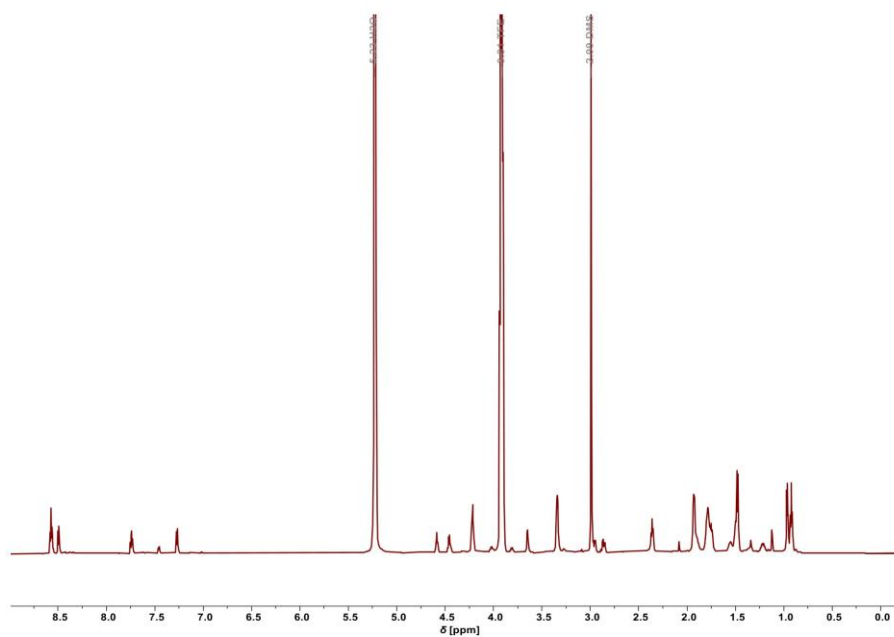


Figure S60: ¹H-NMR (700 MHz, 298 K, d₃-TFE) spectrum of C₆-ICA-Nap **6**.

Supporting Information

Table S1: Masses and amounts of peptides and masses of solvents for NMR experiment with increasing d₃-TFE contents

Compound and % TFE	<i>m</i> / ug	<i>n</i> / nmol	<i>m</i> _{TFE} / mg	<i>m</i> _{DMS-buffer} / mg	<i>m</i> _{noDMS-buffer} / mg
C ₀ -Nap-ICA 8 1%	497	0.874	6.3	275.3	273.3
C ₀ -Nap-ICA 8 10%	493	0.867	66.2	276.2	217.8
C ₀ -Nap-ICA 8 20%	500	0.879	132.5	275.9	162.5
C ₀ -Nap-ICA 8 30%	466	0.819	198.4	277.7	110.8
C ₀ -Nap-ICA 8 40%	512	0.900	267.3	277.6	53.4
C ₀ -Nap-ICA 8 50%	504	0.886	350.0	276.0	0
C ₀ -Nap-ICA 8 100%	508	0.893	702.3	0	0
C ₃ -Nap-ICA 7 1%	482	0.753	6.3	277.1	264.8
C ₃ -Nap-ICA 7 10%	475	0.742	65.9	276.1	217.1
C ₃ -Nap-ICA 7 20%	460	0.719	132.7	275.9	164.7
C ₃ -Nap-ICA 7 30%	466	0.728	198.3	276.1	110.2
C ₃ -Nap-ICA 7 40%	468	0.731	264.5	276.3	55.3
C ₃ -Nap-ICA 7 50%	488	0.763	329.8	277.7	0
C ₃ -Nap-ICA 7 100%	528	0.825	711.4	0	0
C ₆ -Nap-ICA 6 1%	514	0.754	6.1	275.1	272.1
C ₆ -Nap-ICA 6 10%	524	0.769	62.9	277.0	198.8
C ₆ -Nap-ICA 6 20%	534	0.784	133.0	277.7	163.0
C ₆ -Nap-ICA 6 30%	528	0.775	199.3	278.6	110.0
C ₆ -Nap-ICA 6 40%	480	0.705	264.8	278.0	53.4
C ₆ -Nap-ICA 6 50%	536	0.787	353.7	274.1	0
C ₆ -Nap-ICA 6 100%	507	0.744	708.9	0	0

Supporting Information

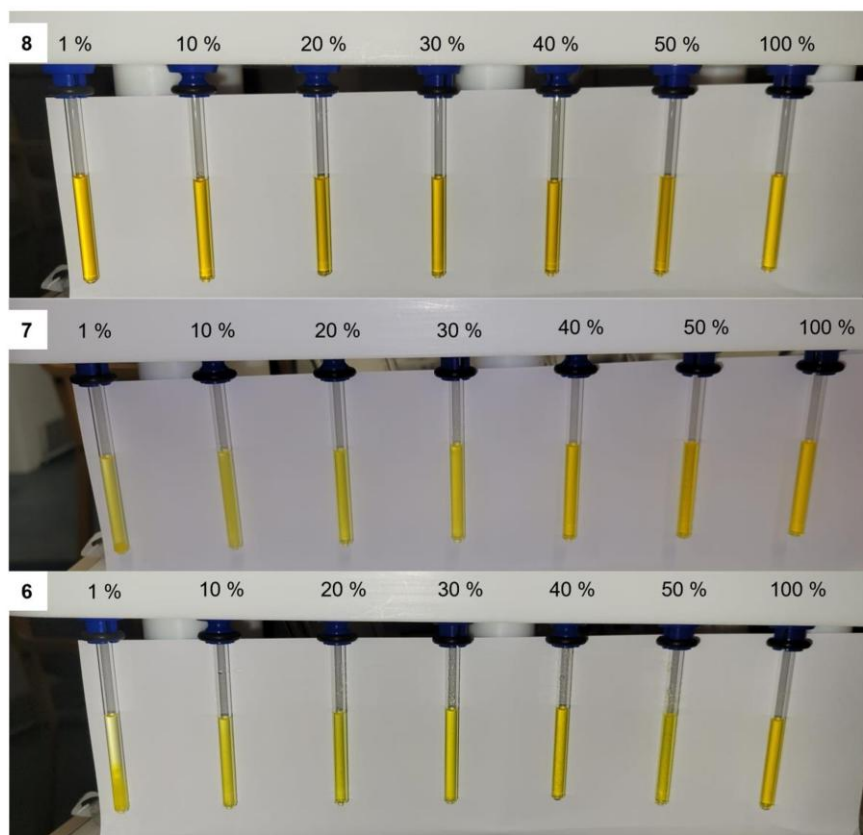


Figure S61: Photographs of the respective NMR tubes of the three peptide conjugates C₀-Nap-ICA **8** (top), C₃-Nap-ICA **7** (middle) and C₆-Nap-ICA **6** (bottom) with different d₃-TFE contents.

References

- [1] E. Atherton, H. Fox, D. Harkiss, C. J. Logan, R. C. Sheppard, B. J. Williams, "A mild procedure for solid phase peptide synthesis: use of fluorenylmethoxycarbonylamino-acids" *J. Chem. Soc., Chem. Commun.* **1978**, 537–539.
- [2] L. Li, Y. Wu, E. Li, C. Shen, H. Zhang, X. Xu, G. Wu, M. Cai, W.-H. Zhu, "Self-assembled naphthalimide derivatives as an efficient and low-cost electron extraction layer for n-i-p perovskite solar cells" *Chem. Commun.* **2019**, 55, 13239–13242.

4. Light-Triggered Disassembly of Peptide Nanostructures

Authors

Raphael Meyer, **Julian Link**, Lucas Gunkel, Albin Lahu, Hakan Demirezen, Tanja Weil* & David Y. W. Ng*

* Corresponding Author

Published in ChemBioChem, 2025, Volume 26, Issue 16, e202500414

DOI: 10.1002/cbic.202500414

Date of Publication: 12th of September 2025

© 2025 The Author(s). ChemBioChem published by Wiley-VCH GmbH

This is an open access article under the terms of the Creative Commons Attribution License (available under <https://creativecommons.org/licenses/by/4.0/>), which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Scientific Context and Research Aim

While significant progress has been made in the design of self-assembling peptide materials, the controlled disassembly of these structures remains a considerable challenge. Most synthetic systems are designed to form stable architectures, yet the ability to trigger their dissolution on demand is crucial for applications in drug delivery and dynamic materials. Photochemical reactions offer a powerful tool for this purpose, allowing for spatiotemporal control without the addition of chemical triggers. This research project aimed to develop a light-responsive peptide system capable of undergoing controlled disassembly upon irradiation. Specifically, the study focused on incorporating anthracene units into short amphiphilic peptide sequences (GISVK and GISVKA_E) to exploit UV-induced [4+4] photodimerization. The primary objective was to understand how supramolecular preorganization influences the kinetics of the photochemical reaction and how environmental factors, such as pH, modulate the resulting nanostructure morphology.

Experimental Approach and Methodology

The project utilized a combination of synthetic chemistry and comprehensive physicochemical characterization. Peptide conjugates bearing *N*-terminal anthracene groups were synthesized and allowed to self-assemble into nanostructures. The structural evolution and disassembly processes were monitored using a multimodal approach. Secondary structure changes were tracked via Circular Dichroism (CD) and Fourier transform infrared (FTIR) spectroscopy, while the photochemical conversion of the anthracene units was quantified using UV-Vis and NMR spectroscopy. To visualize the macroscopic morphological changes, Transmission Electron Microscopy (TEM) was employed. Additionally, aggregation assays using Proteostat dye provided complementary data on the extent of assembly. The system was tested under varying pH conditions to evaluate the environmental sensitivity of the assembly and disassembly pathways.

Personal Contribution

Within this collaborative project, I took the primary responsibility for the electron microscopy work and the analysis of morphological transitions. I planned and performed all TEM measurements to characterize the macroscopic structure formation of Ant-GISVK and Ant-GISVKAE. A key aspect of my contribution was the systematic investigation of the pH effects on the assembly morphology. My analysis revealed that at neutral pH, Ant-GISVK assembled into freestanding, non-uniform 2D-sheets with irregular edges, whereas the extended sequence Ant-GISVKAE formed single twisted ribbons. I further discovered that at elevated pH, Ant-GISVK failed to form ordered nanostructures, yielding amorphous aggregates instead, while the morphology of Ant-GISVKAE remained robust. Following UV irradiation, I documented the complete disassembly of these structures into unstructured aggregates. These findings were critical for correlating the initial supramolecular order with the efficiency of the light-triggered disassembly process. I also contributed to the interpretation of the spectroscopic data and the drafting of the manuscript.

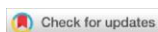
Collaborative Efforts and Supervision

This project was led by the first author Raphael Meyer, who was responsible for the conceptualization, peptide synthesis, and the majority of the spectroscopic characterization (UV-Vis, CD, NMR) as well as the irradiation experiments. We worked in close coordination to align the microscopic findings with the spectroscopic data. Lucas Gunkel contributed FTIR measurements and their interpretation. Additional TEM measurements and interpretation support were provided by Albin Lahu and Hakan Demirezen. The overall research concept and results were discussed regularly with Tanja Weil and David Y. W. Ng, who also provided critical

feedback on the manuscript. Tanja Weil was responsible for acquiring the funding that supported this work.

Outcome and Significance

The study successfully demonstrated that anthracene photodimerization can be used to trigger the disassembly of peptide nanostructures. A key scientific insight was that the photodimerization process was kinetically favored by the preorganization of the anthracene units within the supramolecular assemblies, highlighting the interplay between chemical reactivity and supramolecular order. Furthermore, the work established that morphology and stability could be tuned via peptide sequence extension and pH modulation. These findings contribute to the broader goal of designing dynamic materials where structural integrity can be externally controlled, paving the way for future applications in responsive biomaterials.



Light-Triggered Disassembly of Peptide Nanostructures

Raphael Meyer, Julian Link, Lucas Gunkel, Albin Lahu, Hakan Demirezen, Tanja Weil, and David Y. W. Ng*

While the assembly of supramolecular peptide nanostructures with diverse functions and morphologies has been extensively studied, the controlled disassembly of these architectures remains less understood. To address this, two short amphiphilic peptides incorporating anthracene as a light-responsive unit and lysine as a pH-sensitive residue are designed. These peptides self-assemble into nanosheets or nanoribbons with distinct secondary structures, which are further tunable by pH through modulation of peptide charge. Upon irradiation at 365 nm, the anthracene moieties undergo a bimolecular

[4 + 4] cycloaddition, disrupting the π - π stacking interactions by distorting the planarity of the aromatic units. This photoreaction leads to disassembly of the supramolecular architectures within 10 min. Unlike monomolecular reactions such as photocleavage, the kinetics of this bimolecular photodimerization are significantly influenced by the degree of preorganization within the assemblies. These findings underscore the critical interplay between supramolecular architecture and molecular photochemistry, enabling light-triggered, structure-dependent disassembly of diverse peptide nanostructures.

1. Introduction

Supramolecular peptide nanostructures have emerged as versatile biomaterials,^[1,2] addressing a broad range of applications in biomedicine^[3] and tissue engineering^[4] as they offer scaffolds that can be customized with diverse functionalities. Moreover, their synthesis routes are well-developed with the advent of modern biorthogonal chemistries, thus bringing forth the next generation of materials that exhibit various depths of adaptivity, mechanochemical, and viscoelastic properties.^[5–7] Despite these seminal advancements in the supramolecular assembly of peptide materials,^[8–13] their disassembly upon completion of their designated tasks, while equally critical, is much less explored.

One of the main reasons is that a large proportion of supramolecular peptide materials operates dominantly on β -sheet type interactions found in neurodegenerative amyloid plaques and pathogenic protein aggregates.^[14–16] The combined synergy between hydrogen bonding, π - π stacking, and hydrophobic forces results in the aggregate or material exhibiting high thermodynamic stability. Hence, disassembly strategies would need

to overcome a tremendous energy barrier to break apart the strong attractive forces holding them together. Using the concept of inducing molecular, geometric frustration to the aggregate by multiple strong local interactions, the development of small molecular β -sheet breakers^[17] or molecular tweezers^[18,19] has found preliminary successes. However, while these disassembling molecules are attractive as therapeutic solutions, they are cumbersome for material-based applications where a more integrative design is required for processing.

In this regard, supramolecular peptide scaffolds containing stimulus-responsive groups (pH,^[20] temperature,^[21] enzymes,^[22] redox reactions,^[23] or light^[24,25]) that chemically react, cleave, or isomerize have been exploited to disrupt intermolecular forces on demand. Among these, light offers a unique advantage in self-assembly processes as photons do not directly interfere with intermolecular forces, and the resultant photochemical reactions can be precisely controlled spatiotemporally.^[26] Therefore, light-responsive systems offer promising opportunities for biological applications, such as controlled drug release^[27] or harnessing light energy to generate heat or reactive oxygen species for use in photothermal or photodynamic therapy.^[28–30] In this regard, photo-switches (i.e., azobenzenes, spiropyranes) and photocleavable groups have been developed to control the aggregation threshold of a targeted self-assembling peptide.^[26] The resultant light-induced chemical change occurs within the self-assembling molecule (monomolecular), where structural isomerization or cleavage destabilizes the formed aggregate, favoring its dissolution. Using these motifs allow reactions to occur homogeneously, with every molecule exposed to light behaving in a similar fashion.^[31]

In contrast, bimolecular photocycloaddition reactions, such as the [4 + 4] photodimerization of anthracene, are dependent on how molecules are preorganized within the solid-state aggregate. Anthracene molecules that are stacked and sufficiently oriented to facilitate the cycloaddition will afford successful dimerization,

R. Meyer, J. Link, A. Lahu, H. Demirezen, T. Weil, D. Y. W. Ng
Department of the Synthesis of Macromolecule
Max Planck Institute for Polymer Research
Ackermannweg 10, 55128 Mainz, Germany
E-mail: david.ng@mpip-mainz.mpg.de

L. Gunkel
Department of Molecular Spectroscopy
Max Planck Institute for Polymer Research
Ackermannweg 10, 55128 Mainz, Germany

Supporting information for this article is available on the WWW under <https://doi.org/10.1002/cbic.202500414>

© 2025 The Author(s). ChemBioChem published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

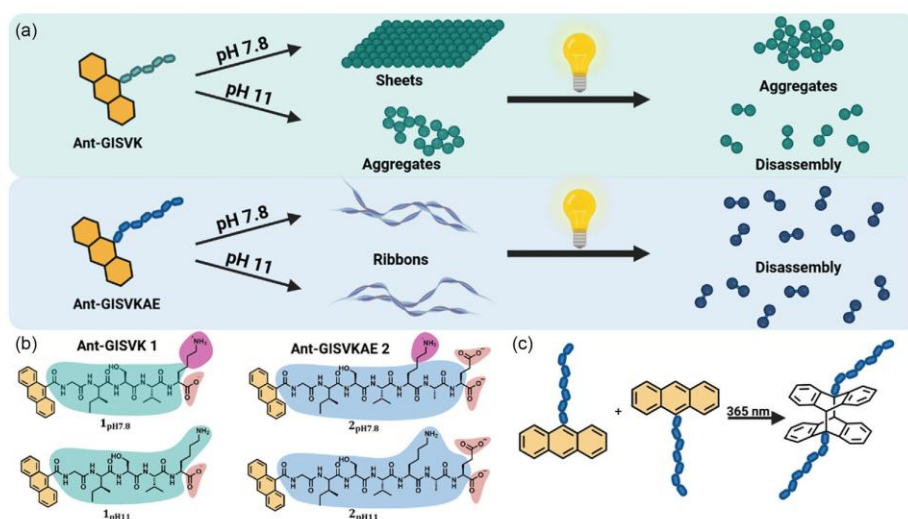


Figure 1. a) Schematic illustration of the assembly pathways of 1 and 2 at different pH and the disassembly pathway upon irradiation. b) Chemical structure of 1 and 2, respectively, at pH 7.8 or pH 11, showing the different charged states. c) [4 + 4] photodimerization of anthracene showing the bent configuration of the dimer.

leading to a distortion of the planar anthracene groups and disrupting the constructive π - π interactions. The geometric frustration generated as a consequence has recently been shown to induce morphological switching in the nanoscale, between fibers and vesicles, or the bending of nanotubes.^[32,33] Nonetheless, the extent of how 1D and 2D nanoscale structures succumb to the molecular deformation to allow structure-selective disassembly has not been explored.

In this study, we designed two anthracene-containing, short amphiphilic peptides, Anthracene-GISVK (Ant-GISVK) and Ant-GISVKAE, to assemble different pH-dependent β -sheet nanostructures, imparted by the lysine (K) and glutamic acid (E) residues (Figure 1a). At pH 7.8 and pH 11, the overall charge on the peptides can be modulated, which in turn dictates molecular preorganization within the assembly. Subsequently, the kinetics of the photodimerization of anthracene (Figure 1c) are monitored via UV-vis spectroscopy to correlate the reaction efficiency and the extent of structural disruption. The loss of secondary structure integrity and eventual disassembly are investigated via circular dichroism (CD) spectroscopy and transmission electron microscopy (TEM).

2. Results and Discussion

Ant-GISVK 1 and Ant-GISVKAE 2 (Figure 1b) were synthesized using Fmoc-solid phase peptide synthesis on Wang resin using *N,N'*-diisopropylcarbodiimide (DIC) and ethyl (2Z)-2-cyano-2-(hydroxyimino)acetate (OxymaPure) for the iterative peptide coupling steps and benzotriazol-1-yloxytripyrrolidino-phosphonium

hexafluoro-phosphate (PyBOP) and diisopropylethylamine (DIPEA) for the coupling of 9-anthracenecarboxylic acid to the peptide, respectively. Purification was achieved by HPLC, and the peptides subsequently were analyzed by nuclear magnetic resonance (NMR) spectroscopy and liquid chromatography-mass spectrometry (LCMS) (Figure S1–6, Supporting Information).

The peptides were then assembled in a solvent mixture of 5% DMSO in 95% NH_4HCO_3 buffer (10 mM) at pH 7.8 and pH 11 (adjusted with 1 M NaOH), respectively. Raising the pH to 11 results in the deprotonation of the side chain lysine ($\text{pK}_a = 10.4$),^[34] thereby, causing the overall charge of both peptides to become more negative compared to pH 7.8, where the lysine is positively charged (Figure 1b). Subsequently, we performed TEM of the peptides at both pH values and analyzed the influence of charge on the assembled nanostructure.

$1_{\text{pH}7.8}$ was observed to form freestanding, nonuniform 2D-sheets with irregular edges (Figure 2a) whereas $1_{\text{pH}11}$ did not assemble into ordered structures and formed only aggregates (Figure 2b). The increase in pH leads to a shift from an overall charge of 0 (pH 7.8), due to the zwitterion, to -1 (pH 11). This results in an enhanced electrostatic repulsion between the carboxylic acids of the C-terminus, leading to the destabilization and the formation of aggregates instead.^[35] In comparison to 1, 2 is extended by an alanine (A) and glutamic acid (E), resulting in an additional negative charge at pH 7.8 while preserving the alternating hydrophobic/hydrophilic pattern. The extended peptide sequence $2_{\text{pH}7.8}$ assembles into single twisted ribbons. While the increase in pH to 11 increases the overall charge by -1 , the same twisted ribbon morphology is retained for $2_{\text{pH}11}$ (Figure 2e,f).

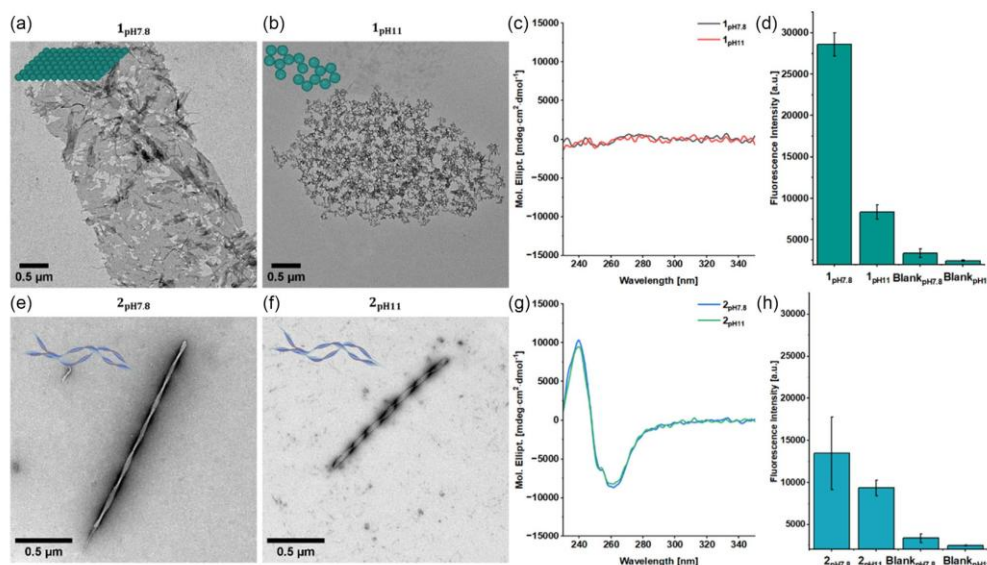


Figure 2. a) TEM image of $1_{\text{pH}7.8}$ revealing 2D-sheets. b) TEM image of $1_{\text{pH}11}$ revealing unordered aggregates. c) CD spectra of $1_{\text{pH}7.8}$ and $1_{\text{pH}11}$ indicating that there is no coupling of the anthracene chromophores. d) Proteostat assay showing increased levels of β -sheets for the sheets of $1_{\text{pH}7.8}$ compared to the aggregates of $1_{\text{pH}11}$. e) TEM image of $2_{\text{pH}7.8}$ revealing single twisted ribbons. f) TEM image of $2_{\text{pH}11}$ revealing single twisted ribbons. g) CD spectra of $2_{\text{pH}7.8}$ and $2_{\text{pH}11}$ indicating interactions of the anthracene according to the bisignate curve. h) Proteostat assay showing similar levels of β -sheets in the nanostructures of $2_{\text{pH}7.8}$ and $2_{\text{pH}11}$. For the Proteostat assay, excitation wavelength of 550 nm and an emission wavelength of 600 nm were used. Blanks are negative controls without sample to account for background fluorescence. All TEM scale bars are 0.5 μm . Data presented as mean $\pm$ SEM (n = 3).

Although both peptides demonstrate an analogous increase in negative charge at elevated pH levels, the position of the lysine within the sequence varies. Specifically, peptide 1 features lysine positioned at the C-terminus, exhibiting a pronounced alteration in morphology upon an increase in pH. Conversely, in peptide 2, the lysine is positioned at the sequence's center and, therefore, there is no change in the local chemical environment at the peptide terminus and no change in morphology at elevated pH. This observation indicates that the local chemical environment at the C-terminus, rather than the overall charge of the peptide, plays a pivotal role in determining its structural morphology.

The anthracene contribution toward the secondary structure was analyzed by CD spectroscopy from 230 nm onward as the presence of DMSO in the solvent composition shields the far UV region due to the absorbance of the S=O bond (<230 nm). As a result, signals corresponding to the peptide backbone's secondary structure, typically observed <220 nm, cannot be detected. $2_{\text{pH}7.8}$ and $2_{\text{pH}11}$ exhibit a bisignate curve with a negative cotton effect at 261 nm and a positive ellipticity at 240 nm, corresponding to the π - π^* transition of the anthracene. (Figure 2g). This observation suggests that the anthracenes are in close spatial proximity and possess a chiral orientation within the structure.^[36–38] Conversely, $1_{\text{pH}7.8}$ and $1_{\text{pH}11}$ exhibit no coupling of the anthracene chromophores, indicating that the interactions between the aromatic groups are not the dominant driving force for assembly (Figure 2c).

To address the missing structural information pertaining to the peptide backbone, Fourier-transform infrared spectroscopy (FTIR) and Proteostat assay were conducted. In addition, FTIR enables the visualization of the structural differences between the sheets of $1_{\text{pH}7.8}$ and the twisted ribbons of $2_{\text{pH}7.8}$. The spectrum of $1_{\text{pH}7.8}$ exhibits a peak at 1625 cm^{-1} , while $2_{\text{pH}7.8}$ displays peaks at 1610 and 1625 cm^{-1} (Figure S19, Supporting Information). These peaks can be attributed to intermolecular β -sheets, showing additional differences in secondary structure between $1_{\text{pH}7.8}$ and $2_{\text{pH}7.8}$.^[39,40] The β -sheet content and molecular order are subsequently quantified with the fluorogenic Proteostat assay.^[31,41] $1_{\text{pH}7.8}$ exhibited the highest fluorescence intensity, suggesting a high degree of order within the 2D, sheet-like structures. In contrast, $1_{\text{pH}11}$ which predominantly formed lower structural order aggregates exhibit reduced fluorescence intensity (Figure 2d). $2_{\text{pH}7.8}$ and $2_{\text{pH}11}$ showed comparable fluorescence intensities, which is in line with the CD and TEM results showing no difference in secondary structure or morphology upon increasing the pH (Figure 2h).

To establish the photochemistry of the anthracene units, we monitored the photodimerization reaction of peptides 1 and 2 using UV-vis spectroscopy, where the characteristic anthracene signal at 365 nm diminishes upon dimerization (Figure S9, Supporting Information). A quantitative framework for monitoring the dimerization is established by a calibration curve using

different concentrations of anthracene to correlate the change in absorption and the remaining anthracene concentration (Figure S7, 8, Supporting Information). Using the calibration plot as a reference, the reactivity of both peptides (1 and 2) in their molecular forms (100 μM) was analyzed in a solvent mixture of 1:1 DMSO/ NH_4HCO_3 buffer (10 mM) at pH 7.8 and pH 11 (adjusted with 1 M NaOH). Under these conditions, the monomers remain dissolved and no assembly takes place.

In the molecular state, the monomer concentration was found to be less than 10% of its initial value after 5 min of 365 nm irradiation for peptides 1 and 2 across both pH values (Figure 3a). Subsequently, the solvent composition was changed to 5:95 DMSO/ NH_4HCO_3 buffer (10 mM) at pH 7.8 and pH 11 to initiate assembly, and the irradiation kinetics was repeated under the new conditions (Figure 3b). $1_{\text{pH}7.8}$ exhibited slower kinetics compared to when it is dissolved in 50% DMSO, with substantial amount of monomer (16%) remaining even after 10 min irradiation. Conversely, $1_{\text{pH}11}$ and $2_{\text{pH}7.8}$ showed a lower remaining monomer concentration after 1 min and therefore a faster initial rate but still required 5 min to reach below 10%, similar to the irradiation in the nonassembled state. Peptide $2_{\text{pH}11}$ demonstrated the most accelerated kinetics in the assembled state compared to its solution state, with less than 10% monomer remaining already after 1 min of 365-nm irradiation (Figure 3b).

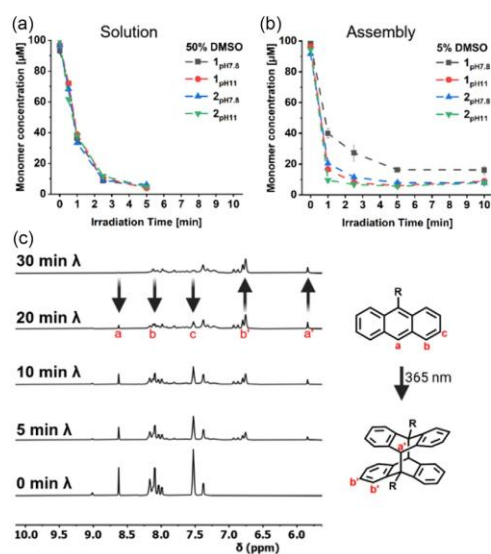


Figure 3. a) Consumption of the anthracene moiety of 1 and 2 in the nonassembled state with increasing irradiation time in 50/50 DMSO:Buffer (pH 7.8 or pH 11). b) Consumption of the anthracene moiety of 1 and 2 in the assembled state with increasing irradiation time in 5/95 DMSO:Buffer (pH 7.8 or pH 11). c) ^1H -NMR kinetic analysis of 1 at pH 7.8 at different irradiation times showing the decline in the anthracene signals while new signals of the dimer emerge. Data presented as mean $\pm$ SEM ($n = 3$).

The collective secondary structure information revealed the kinetic discrepancies observed during irradiation. $1_{\text{pH}7.8}$ demonstrates the highest degree of molecular order with no proximity coupling of the aromatic moieties, thereby preventing a substantial proportion of anthracene molecules from adopting the orientation needed for the dimerization reaction. For a successful photodimerization in the solid assembled state, the reaction centers need to be within $\approx 4.2 \text{ \AA}$,^[42] and the lateral slip between the corresponding π -orbitals to be less than $\approx 2 \text{ \AA}$.^[43] If the molecular order within the assembly does not satisfy these requirements, the photochemical reaction slows considerably and prevents the complete conversion of the anthracene peptides. In contrast, the preorganization in $2_{\text{pH}11}$ leads to an accelerated reaction, as the anthracenes are already in close spatial proximity within the assembly.

The [4 + 4] photodimerization was further confirmed by ^1H -NMR, which shows the emergence of a distinctive bridge proton signal at 5.8 ppm with the concomitant decrease of the respective anthracene signal at 8.6 ppm across all samples upon irradiation (Figure 3c). The bridge proton corresponds to the head-to-tail conformation of the dimer, which offers the least sterically hindered configuration, as evidenced by HMBC spectroscopy (Figure S14, Supporting Information). However, it was observed that only up to 60% conversion to the dimer was achieved, while the remaining monomer was found to be below 10% of the initial concentration (Figure S12, Supporting Information). While this result suggests the occurrence of side reactions upon irradiation, it is crucial to note that the overall concentration was 20-fold higher than the assembly conditions to achieve a sufficient signal-to-noise ratio. Therefore, these experiments were conducted in a solution of 80% DMSO- d_6 and 20% NH_4HCO_3 buffer (10 mM) at pH 7.8 and pH 11, a composition that was found to ensure the solubility of all compounds at higher concentration while maintaining control over pH. The side reactions were investigated by using 9-anthracenecarboxylic acid as a model compound and revealed the emergence of 9,10-anthraquinone upon irradiation besides the photodimer (Figure S15, Supporting Information). Additionally, it is important to note that the ratio between product and side reactions is dependent on the solvent composition (Figure S16, S17, Supporting Information). The difference in concentration and solvent composition can, in principle, alter the reactivity due to different mobility and electrostatic repulsion. The role of DMSO in this regard, compared to other organic co-solvents such as THF, not only facilitates initial solubilization but also plays a role in modulating assembly morphology and photoreactivity.

We hypothesize that the different dimerization kinetics and efficiency as a consequence of molecular preorganization within the supramolecular structure can lead to different nanoscale outcomes. Using TEM, each nanostructure ($1_{\text{pH}7.8}$, $1_{\text{pH}11}$, $2_{\text{pH}7.8}$, $2_{\text{pH}11}$) was subjected to 10 min irradiation, a duration that has been shown to ensure maximum consumption of anthracene units (Figure 3b). In general, irradiated samples are denoted with λ . TEM analysis of $1_{\text{pH}7.8}^\lambda$ revealed unordered aggregates, contrasting with the 2D-sheets observed for the nonirradiated $1_{\text{pH}7.8}$ (Figure 4a). The dimer, with its bent conformation of the phenyl

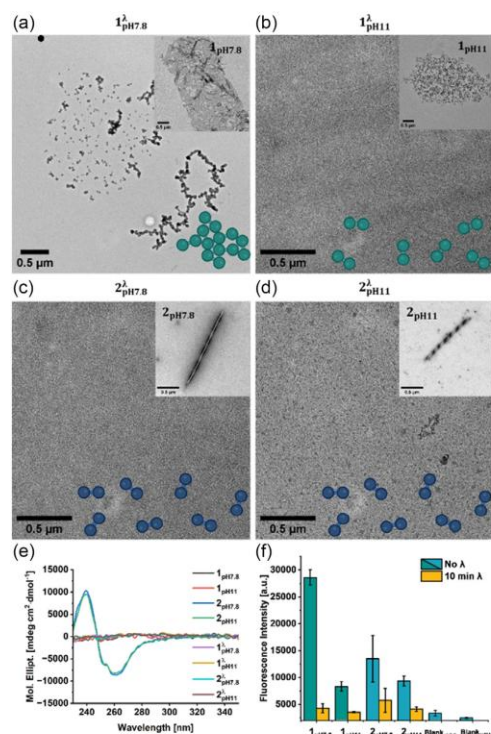


Figure 4. a) TEM image of $1_{\text{pH}7.8}^{\lambda}$ revealing unstructured aggregates. Inset: TEM image of $1_{\text{pH}7.8}^{\lambda}$ showing 2D-sheets. b) TEM image of $1_{\text{pH}11}^{\lambda}$ revealing the absence of nanostructures. Inset: TEM image of $1_{\text{pH}11}^{\lambda}$ showing unstructured aggregates. c) TEM image of $2_{\text{pH}7.8}^{\lambda}$ revealing the absence of nanostructures. Inset: TEM image of $2_{\text{pH}7.8}^{\lambda}$ showing twisted ribbons. d) TEM image of $2_{\text{pH}11}^{\lambda}$ revealing the absence of nanostructures. Inset: TEM image of $2_{\text{pH}11}^{\lambda}$ showing twisted ribbons. e) CD spectra of 1 and 2 at pH 7.8 and 11 before and after irradiation. The CD spectra of the irradiated peptides $1_{\text{pH}11}^{\lambda}$, $1_{\text{pH}7.8}^{\lambda}$, $2_{\text{pH}7.8}^{\lambda}$ and $2_{\text{pH}11}^{\lambda}$ show no signal overall. f) Proteostat assay of 1 and 2 in different pH with and without irradiation at 365 nm showing the disassembly upon irradiation. For the Proteostat assay, excitation wavelength of 550 nm and an emission wavelength of 600 nm were used. Blanks are negative controls without sample to account for background fluorescence. All TEM scale bars are 0.5 μm . Data presented as mean $\pm$ SEM ($n = 3$).

moieties, impedes the π - π stacking of the planar aromatic groups, thereby causing a structural change toward aggregates. However, the reduction in attractive forces, as well as the geometric frustration induced by the dimerization, proves insufficient to cause full disassembly. Irradiation exhibits similar structural destabilization compared to pH increase, as evidenced by the unstructured aggregates observed in both the irradiated $1_{\text{pH}7.8}^{\lambda}$ and the nonirradiated $1_{\text{pH}11}^{\lambda}$ at higher pH. The combination of pH and irradiation as destabilizing effects for $1_{\text{pH}11}^{\lambda}$, results in a complete disassembly of the nanostructure, due to the decrease of π - π stacking coupled with the increase in electrostatic repulsion (Figure 4b). The twisted ribbons of $2_{\text{pH}7.8}^{\lambda}$ exhibit a distinct response

to irradiation as evidenced by the absence of both assemblies and aggregates in $2_{\text{pH}7.8}^{\lambda}$ (Figure 4c). This emphasizes the necessity of balancing hydrophobic and hydrophilic moieties to facilitate the dissolution of assembling units, as opposed to the formation of aggregates due to the collapse of hydrophobic regions, as **2** features a greater number of hydrophilic charged groups such as glutamic acids in comparison to **1**. Furthermore, the observation of disassembly in $2_{\text{pH}11}^{\lambda}$ upon irradiation suggests that the morphology of the monomer and dimer assemblies of **2** is pH-independent (Figure 4d). However, this process is solvent dependent, as irradiation of the assemblies of **2** in 1/99 DMSO/ CHCl_3 does not lead to disassembly (Figure S18, Supporting Information). The effect of irradiation on the supramolecular structure was further confirmed by CD and Proteostat assay. The CD signals for $2_{\text{pH}7.8}^{\lambda}$ and $2_{\text{pH}11}^{\lambda}$ are removed, thereby confirming the disassembly of the structure. $1_{\text{pH}7.8}^{\lambda}$ and $1_{\text{pH}11}^{\lambda}$ continue to demonstrate an absence of CD signal (Figure 4e). Proteostat assay confirmed these observations as the fluorescence intensity decreased upon irradiation in all cases (Figure 4f).

3. Conclusion

In summary, we have demonstrated the assembly of short anthracene-containing peptides into different 1D and 2D morphologies by pH-dependent charge modulation. Each morphology expresses different levels of molecular order and preorganization, leading to distinct kinetic responses toward the [4 + 4] photodimerization of anthracene units on the self-assembling peptide. As a result, the extent of geometric frustration upon irradiation becomes selective toward nanostructures that promote proximity π - π interactions of the anthracene moieties. Our results highlight that bimolecular photochemistry is a highly attractive avenue to integrate supramolecular selectivity into complex responsive systems, while offering the unique advantages of light. We believe that the technology will provide a new dimension toward light-driven supramolecular materials and expand the toolbox for controlling disassembly processes.

4. Experimental Section

General Synthesis Procedure

The preloaded Wang resin was swollen in 5 mL DMF at room temperature for 1 h before transferring it into the peptide synthesizer. DMF was removed via a draining process, and the resin was swollen with 20 mL DMF for 20 s. Prior to each coupling step, as well as during the final deprotection, the N-terminal Fmoc group was removed using two deprotection steps: 20% piperidine in DMF was applied for 15 s at 75 $^{\circ}\text{C}$, followed by 50 s at 90 $^{\circ}\text{C}$. Fmoc-protected amino acids were coupled in a fivefold molar excess for 15 s and 110 s at 75 $^{\circ}\text{C}$ and 90 $^{\circ}\text{C}$, respectively, using a solution of DIC (0.25 M) and Oxyma (0.5 M) in DMF. The solution was drained, and the resin washed with DMF three times. Anthracene was then coupled to the peptide by adding 9-anthracene carboxylic acid (2 equiv.), PyBOP (2 equiv.) and DIPEA (4 equiv.) in DMF to the resin and shaking

overnight. The peptide was cleaved from the resin by adding 10 mL of cleavage cocktail (95% TFA, 2.5% TIPS, 2.5% MilliQ water) to the resin and shaking at room temperature for 2 h. The cleavage cocktail was drained, and the resin was washed with TFA. The TFA containing solutions were combined, and the liquids were removed under reduced pressure. The peptide was purified via HPLC with a YMC-Actus Triart C18 column (flow rate 20 mL min⁻¹). After lyophilization, the peptide was received as a colorless solid.

General TEM Procedure

Peptide 1 or 2 (100 µM) was dissolved in 5% DMSO and 95% NH₄HCO₃ buffer (10 mM) and incubated overnight at 25 °C while shaking at 300 rpm. The solutions for irradiation were then transferred to a glass vial equipped with a stirring bar and covered with a blanket of argon. They were then irradiated at 365 nm for 10 min while stirring at 300 rpm and again incubated overnight at 25 °C. Then TEM grids were prepared by pipetting 3 µL of irradiated or nonirradiated peptide solution onto a Formvar-coated copper grid and incubating for 5 min. After the incubation, the solutions were removed with filter paper, and the grids were stained with 7 µL 4% uranyl acetate solution for 2.5 min. The grids were washed three times with MilliQ water and dried before being measured.

Acknowledgements

The authors gratefully acknowledge financial support from the Max Planck Society, the Max Planck-Bristol Centre for Minimal Biology as well as the Deutsche Forschungsgemeinschaft (DFG)—SFB 1551 R04 (Projektnummer: 464588647). H.D. and T.W. would like to thank the support from the Carl Zeiss Stiftung (InteReg).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: disassembly · peptide amphiphile · photochemistry · photodimerization · supramolecular assembly

- [1] A. Levin, T. A. Hakala, L. Schneider, G. J. L. Bernardes, E. Gazit, T. P. J. Knowles, *Nat. Rev. Chem.* **2020**, *4*, 615.
- [2] H. Cui, M. J. Webber, S. I. Stupp, *Pept. Sci.* **2010**, *94*, 1.
- [3] J. Chen, X. Zou, *Bioact. Mater.* **2019**, *4*, 120.
- [4] T. Guan, J. Li, C. Chen, Y. Liu, *Adv. Sci.* **2022**, *9*, 1.
- [5] D. Schauenburg, T. Weil, *J. Am. Chem. Soc.* **2025**, *147*, 8049.
- [6] L. Dong, L. Li, H. Chen, Y. Cao, H. Lei, *Adv. Sci.* **2024**, *2403949*, 1.
- [7] J. T. Sharick, A. J. Atieh, K. J. Gooch, J. L. Leight, *J. Biomed. Mater. Res. - Part A* **2023**, *111*, 389.
- [8] S. Chagri, D. Y. W. Ng, T. Weil, *Nat. Rev. Chem.* **2022**, *6*, 320.
- [9] Y. Ren, Z. Zhou, K. Maxeiner, A. Kaltbeitzel, I. Harley, J. Xing, Y. Wu, M. Wagner, K. Landfester, I. Lieberwirth, T. Weil, D. Y. W. Ng, *J. Am. Chem. Soc.* **2024**, *146*, 11991.
- [10] S. C. Yuan, J. A. Lewis, H. Sai, S. J. Weigand, L. C. Palmer, S. I. Stupp, *J. Am. Chem. Soc.* **2022**, *144*, 16512.

- [11] H. Yuan, P. Han, Z. Tao, B. Xue, Y. Guo, D. Levy, W. Hu, Y. Wang, Y. Cao, E. Gazit, R. Yang, *ACS Appl. Mater. Interfaces* **2022**, *14*, 6538.
- [12] K. Dai, J. R. Fores, C. Wanzke, B. Winkelmann, A. M. Bergmann, O. Lieleg, J. Boekhoven, *J. Am. Chem. Soc.* **2020**, *142*, 14142.
- [13] J. Son, D. Kalafatovic, M. Kumar, B. Yoo, M. A. Cornejo, M. Contel, R. V. Ulijn, *ACS Nano* **2019**, *13*, 1555.
- [14] K. Hou, H. Pan, H. Shahpasand-Kroner, C. Hu, R. Abskharon, P. Seidler, M. Mekikittikul, M. Balbirnie, C. Lantz, M. R. Sawaya, J. L. Dolinsky, M. Jones, X. Zuo, J. A. Loo, S. Frautschy, G. Cole, D. S. Eisenberg, *Sci. Adv.* **2024**, *10*, 1.
- [15] E. Chuang, A. M. Hori, C. D. Hesketh, J. Shorter, *J. Cell Sci.* **2018**, *131*, 1.
- [16] X. Xing, C. Liu, A. Ali, B. Kang, P. Li, H. Ai, *ACS Chem. Neurosci.* **2020**, *11*, 45.
- [17] A. Frydman-Marom, R. Shaltiel-Karyo, S. Moshe, E. Gazit, *Amyloid* **2011**, *18*, 119.
- [18] E. Lump, L. M. Castellano, C. Meier, J. Seeliger, N. Erwin, B. Sperlich, C. M. Stürzel, S. Usmani, R. M. Hammond, J. Von Einem, G. Gerold, F. Kreppel, K. Bravo-Rodriguez, T. Pietschmann, V. M. Holmes, D. Palesch, O. Zirafi, D. Weissman, A. Sowislo, B. Wettig, C. Heid, F. Kirchhoff, T. Weil, F. G. Klärner, T. Schrader, G. Bitan, E. Sanchez-Garcia, R. Winter, J. Shorter, J. Munch, *Elife* **2015**, *4*, 1.
- [19] S. Sinha, D. H. J. Lopes, Z. Du, E. S. Pang, A. Shannugam, A. Lomakin, P. Talbiersky, A. Tennstaedt, K. McDaniel, R. Bakshi, P. Y. Kuo, M. Ehrmann, G. B. Benedek, J. A. Loo, F. G. Klärner, T. Schrader, C. Wang, G. Bitan, *J. Am. Chem. Soc.* **2011**, *133*, 16958.
- [20] X. Xu, Y. Li, H. Li, R. Liu, M. Sheng, B. He, Z. Gu, *Small* **2014**, *10*, 1133.
- [21] S. A. H. Jansen, E. Weyandt, T. Aoki, T. Akiyama, Y. Itoh, G. Vantomme, T. Aida, E. W. Meijer, *J. Am. Chem. Soc.* **2023**, *145*, 4231.
- [22] M. J. Webber, C. J. Newcomb, R. Bitton, S. I. Stupp, *Soft Matter* **2011**, *7*, 9665.
- [23] M. Pieszka, A. M. Sobota, J. Gaćanin, T. Weil, D. Y. W. Ng, *ChemBioChem* **2019**, *20*, 1376.
- [24] J. W. Fredy, A. Méndez-Ardoy, S. Kwangmettadam, D. Bochicchio, B. Matt, M. C. A. Stuart, J. Huskens, N. Katsonis, G. M. Pavan, T. Kudernac, *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 11850.
- [25] K. Kaygisiz, A. M. Ender, J. Gaćanin, L. A. Kaczmarek, D. A. Koutsouras, A. N. Nalakat, P. Winterwerber, F. J. Mayer, H. J. Räder, T. Marszalek, P. W. M. Blom, C. V. Synatschke, T. Weil, *Macromol. Biosci.* **2023**, *23*, 2200294.
- [26] K. Matsuura, H. Inaba, *Biophys. Rev.* **2023**, *4*, 041303.
- [27] Y. Ren, Z. Zhou, I. Harley, Ö. Aydin, L. Driehaus-Ortiz, A. Kaltbeitzel, J. Xing, K. Maxeiner, I. Lieberwirth, K. Landfester, D. Y. W. Ng, T. Weil, *Nat. Synth.* **2025**, *4*, 672.
- [28] R. Chang, L. Zhao, R. Xing, J. Li, X. Yan, *Chem. Soc. Rev.* **2023**, *52*, 2688.
- [29] Q. Zou, M. Abbas, L. Zhao, S. Li, G. Shen, X. Yan, *J. Am. Chem. Soc.* **2017**, *139*, 1921.
- [30] P. Zhu, X. Yan, Y. Su, Y. Yang, J. Li, *Chem. - A Eur. J.* **2010**, *16*, 3176.
- [31] P. Roth, R. Meyer, I. Harley, K. Landfester, I. Lieberwirth, M. Wagner, D. Y. W. Ng, T. Weil, *Nat. Synth.* **2023**, *2*, 980.
- [32] F. Novelli, M. Vilela, A. Pazó, M. Amorin, J. R. Granja, *Angew. Chemie—Int. Ed.* **2021**, *60*, 18838.
- [33] T. Xia, X. Cheng, X. Sun, G. Liang, *ACS Mater. Lett.* **2022**, *4*, 1962.
- [34] I. André, S. Linse, F. A. A. Mulder, *J. Am. Chem. Soc.* **2007**, *129*, 15805.
- [35] A. Ghosh, E. T. Dobson, C. J. Buettner, M. J. Nicholl, J. E. Goldberger, *Langmuir* **2014**, *30*, 15383.
- [36] S. G. Telfer, T. M. McLean, M. R. Waterland, *Dalt. Trans.* **2011**, *40*, 3097.
- [37] A. George, N. Jayaraman, *Carbohydr. Res.* **2023**, *533*, 108933.
- [38] M. Liu, L. Zhang, T. Wang, *Chem. Rev.* **2015**, *115*, 7304.
- [39] W. K. Surewicz, H. H. Mantsch, D. Chapman, *Biochemistry* **1993**, *32*, 389.
- [40] G. Zandomenighi, M. R. H. Krebs, M. G. McCammon, M. Fändrich, *Protein Sci.* **2004**, *13*, 3314.
- [41] J. Y. Li, C. M. Zhou, R. L. Jin, J. H. Song, K. C. Yang, S. L. Li, B. H. Tan, Y. C. Li, *J. Chem. Neuroanat.* **2024**, *138*, 102420.
- [42] C. J. Cook, W. Li, B. F. Lui, T. J. Gately, R. O. Al-Kaysi, L. J. Mueller, C. J. Bardeen, G. J. O. Beran, *Chem. Sci.* **2023**, *14*, 937.
- [43] I. Zouev, D.-K. Cao, T. V. Sreevidya, M. Telzhensky, M. Botoshansky, M. Kaftory, *CrystEngComm* **2011**, *13*, 4376.

Manuscript received: May 22, 2025

Revised manuscript received: July 22, 2025

Version of record online: August 14, 2025

Supporting Information

Light-Triggered Disassembly of Peptide Nanostructures

Raphael Meyer,^[a] Julian Link,^[a] Lucas Gunkel,^[b] Albin Lahu,^[a] Hakan Demirezen,^[a] David Y. W. Ng^{*[a]}
and Tanja Weil^{*[a]}

[a] R. Meyer, J.Link, A.Lahu, H.Demirezen, Dr. D.Y.W. Ng, Prof. T.Weil

Department of the Synthesis of Macromolecule

Max Planck Institute for Polymer Research

Ackermannweg 10, Mainz 55128, Germany

E-mail: david.ng@mpip-mainz.mpg.de, weil@mpip-mainz.mpg.de

[b] L. Gunkel

Department of Molecular Spectroscopy

Max Planck Institute for Polymer Research

Ackermannweg 10, Mainz 55128, Germany

Table of Contents

1	Materials and Instruments.....	3
1.1	Materials.....	3
1.2	Instruments.....	3
1.2.1	Microwave Peptide Synthesizer	3
1.2.2	High-Performance Liquid Chromatography (HPLC)	3
1.2.2	Nuclear Magnetic Resonance Spectroscopy (NMR)	3
1.2.3	1.2.4 Liquid Chromatography – Mass Spectrometry (LC-MS).....	3
1.2.4	Circular Dichroism Spectroscopy (CD).....	4
1.2.5	Fluorescence Spectroscopy	4
1.2.6	Transmission Electron Microscopy (TEM)	4
1.2.7	Irradiation setup	4
1.2.8	Fourier-transform Infrared spectroscopy	4
2	Synthesis.....	5
2.1	Ant-GISVK.....	5
2.2	Ant-GISVKAE	7
3	Characterization	10
3.1	Absorbance and Fluorescence spectroscopy	10
3.1.1	Calibration curves.....	10
3.1.2	Irradiation conversion in 50% DMSO	11
3.1.3	Irradiation conversion in 5% DMSO	11
3.1.4	Proteostat assay	12
3.2	Nuclear magnetic resonance	12
3.3	Transmission electron microscopy	15
3.3.1	Non-irradiated samples.....	15
3.3.2	Irradiated samples.....	15
3.4	Circular Dichroism.....	15
3.5	Fourier-transform Infrared spectroscopy.....	16

1 Materials and Instruments

1.1 Materials

All chemicals were purchased from commercial sources and were used without further purification. Solvents and reagents used for peptide synthesis were obtained in peptide grade. Purification with HPLC was performed with water obtained from a Millipore purification system and HPLC grade acetonitrile.

1.2 Instruments

1.2.1 Microwave Peptide Synthesizer

Peptides and peptide fractions were synthesized with a Liberty Blue Automated Microwave Peptide Synthesizer by CEM Corporation.

1.2.2 High-Performance Liquid Chromatography (HPLC)

The synthesized peptides were purified by preparative HPLC using a Shimadzu setup. A YMC-Actus Triart C18 column (150 x 20 mm, 5 μ m) with a flowrate of 20 mL/min or a Phenomenex Kinetex EVO C18 100 (150 x 30 mm, 5 μ m) with a flowrate of 25 mL/min was used for purification.

All purification steps were performed by using gradients of MilliQ water and acetonitrile, each acidified with 0.1% formic acid. Analytical measurements were performed by using gradients of MilliQ water and acetonitrile, each acidified with 0.1% trifluoroacetic acid. The HPLC data were processed with the software LabSolutions by Shimadzu and OriginPro 2024b by OriginLab.

1.2.2 Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra were recorded on a Bruker Avance III 700 MHz NMR spectrometer. For ^1H spectra, the chemical shifts are reported in parts per million (ppm) from high to low frequency using the residual solvent peak as the internal reference ($\text{DMSO-}d_6 = 2.50$ ($\text{CHD}_2\text{SOCD}_3$) ppm). All ^1H resonances are reported to the nearest 0.01 ppm. The multiplicity of ^1H signals are indicated as: s = singlet; d = doublet; t = triplet; q = quartet; p = pentet; m = multiplet; br = broad; or combinations of thereof. Coupling constants (J) are quoted in Hz and reported to the nearest 0.1 Hz. Where appropriate, averages of the signals from peaks displaying multiplicity were used to calculate the value of the coupling constant. The data was processed in MestReNova.

1.2.3 1.2.4 Liquid Chromatography – Mass Spectrometry (LC-MS)

Peptides were analysed by HPLC-ESI-MS on a LC-MS 2020 setup by Shimadzu using a Kinetex EVO C18 100 Å LC column (50 x 2.1 mm, 2.6 μ m). Acetonitrile (ACN) and MilliQ water acidified with 0.1% formic acid were used as solvents for all measurements. The solvent gradient

started with 5% ACN and 95% water, while the ACN content was linearly increased to 95% in 12 min. Data were processed in LabSolutions and OriginPro 2024b by OriginLab.

1.2.4 Circular Dichroism Spectroscopy (CD)

CD spectra of the peptides were recorded on a JASCO J-1500 spectrometer in a 0.1 cm High Precision Cell by HellmaAnalytics. The recorded data were processed in Spectra Analysis by JASCO and OriginPro 2024b by OriginLab.

1.2.5 Fluorescence Spectroscopy

To record the fluorescence intensity, a SPARK 20M microplate reader by Tecan Group Ltd. was used. The samples were measured in a Greiner 384 flat black well plate and data was processed in OriginPro 2024b by OriginLab. The absorbance was recorded on the same instrument with a Greiner UV-STAR® plate, 384 well, F-Bottom, clear.

1.2.6 Transmission Electron Microscopy (TEM)

TEM images of the peptide samples were recorded on a JEOL 1400 transmission electron microscope at a voltage of 120 kV. Formvar/carbon-film coated copper grids (300 mesh) by Plano GmbH were used to prepare the samples. The images were processed in Fiji ImageJ.

1.2.7 Irradiation setup

Irradiations were performed with a Thorlabs M365L3 lamp with a nominal wavelength of 365 nm, a bandwidth (FWHM) of 9 nm and a current of 1000 mA.

1.2.8 Fourier-transform Infrared spectroscopy

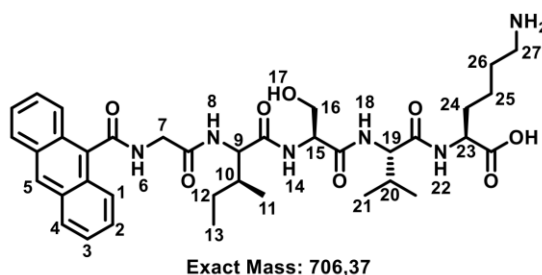
Infrared spectra were recorded in transmission geometry using a Bruker Vertex 70 IR spectrometer. The spectrometer has a resolution of 4 cm⁻¹ in a range from 400 cm⁻¹ to 4000 cm⁻¹.

2 Synthesis

2.1 Ant-GISVK

The Wang resin preloaded with Fmoc-Lys(Boc) (0.1 mmol) was swollen in 5 mL DMF at room temperature for 1 h before transferring it into the peptide synthesizer. DMF was removed via a draining process and the resin swollen with 20 mL DMF for 20 s. Prior to each coupling step, as well as during the final deprotection, the N-terminal Fmoc group was removed using two deprotection steps: 20% piperidine in DMF (3 mL) was applied for 15 seconds at 75 °C, followed by 50 seconds at 90 °C. Fmoc-protected amino acids (Fmoc-Val, Fmoc-Ser(tBu), Fmoc-Ile and Fmoc-Gly) were coupled in a 5-fold molar excess (5 eq. in 1.25 mL) for 15 seconds and 110 seconds at 75 °C and 90 °C, respectively, using a solution of DIC (0.25 M) and Oxyma (0.5 M) in 2.75 mL DMF. The solution was drained, and the resin washed with DMF three times. Anthracen was then coupled to the peptide by adding 9-anthracene carboxylic acid (44.45 mg, 2 equiv.), PyBOP (104 mg, 2 equiv.) and DIPEA (68 μ L, 4 equiv.) in 5 mL DMF to the resin and shaking overnight. The peptide was cleaved from the resin by adding 10 mL of cleavage cocktail (95% TFA, 2.5% TIPS, 2.5% MilliQ water) to the resin and shaking at room temperature for 2 h. The cleavage cocktail was drained, and the resin washed with TFA. The TFA containing solutions were combined and the liquids removed under reduced pressure.

The peptide was purified via HPLC with a YMC-Actus Triart C18 column (flowrate 20 mL/min). After lyophilisation, the product Ant-GISVK was received as a colourless solid (14.13 mg, 0.02 mmol, 20%).



$^1\text{H-NMR}$ (700 MHz, DMSO-d_6) δ [ppm] = 9.05 (t, J = 6.1 Hz, 2H, **6**), 8.65 (s, 1H, **5**), 8.25 (d, J = 36.1 Hz, 2H, **1**), 8.13–8.10 (m, 3H, **4,8**), 8.08 (d, J = 7.6 Hz, 1H, **14**), 8.03 (d, J = 8.2 Hz, 1H, **18**), 7.58–7.51 (m, 4H, **2,3**), 7.48 (d, J = 7.1 Hz, 1H, **22**), 4.51 (q, J = 6.6 Hz, 1H, **15**), 4.46 (dd, J = 9.0, 7.6 Hz, 1H, **9**), 4.13 (d, J = 49.6, 16.1, 6.0 Hz, 2H, **7**), 4.05 (dd, J = 8.2, 5.4 Hz, 1H, **19**), 3.83 (q, J = 6.2 Hz, 1H, **23**), 3.63 (qd, J = 10.7, 6.1 Hz, 2H, **16**), 2.71 (t, J = 9.6 Hz, 2H, **27**), 2.12 (m, dd, J = 13.0, 6.5 Hz, 1H, **20**), 1.79–1.73 (d, 1H, **10**), 1.59 (d, J = 8.9 Hz, 1H, **26**), 1.51 (dd, J = 14.4, 7.4 Hz, 4H, **12, 24, 26**), 1.36–1.19 (m, 2H, **25**), 0.910.82 (m, 12H, **11, 13, 21**).

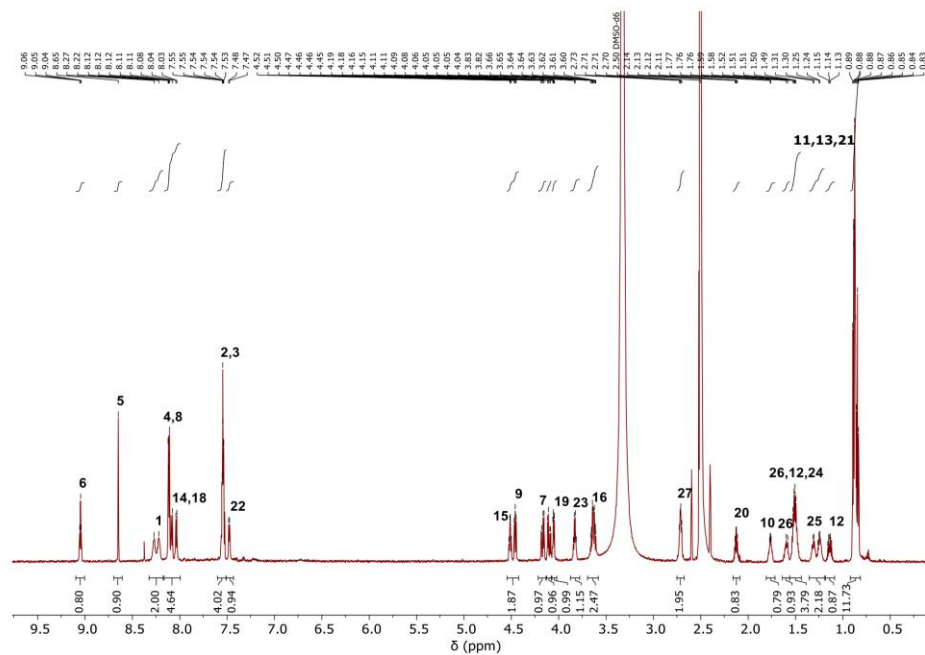


Figure 1: ^1H NMR spectrum (700 MHz, DMSO-d_6 , 298 K) of compound **1**

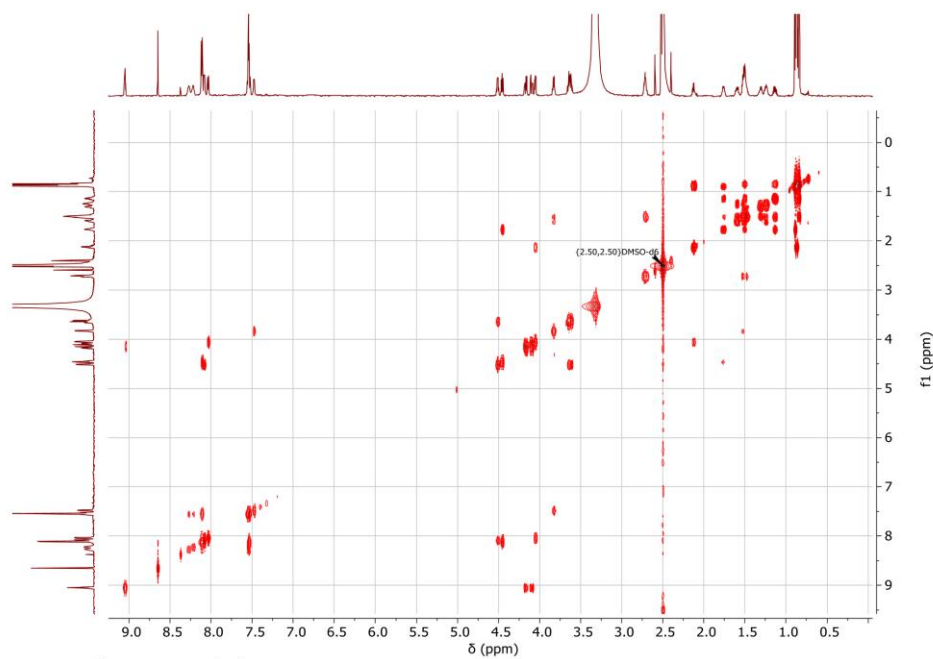


Figure 2: ^1H NMR and $^1\text{H}, ^1\text{H}$ COSY NMR spectra (700 MHz, DMSO-d_6 , 298 K) of compound **1**

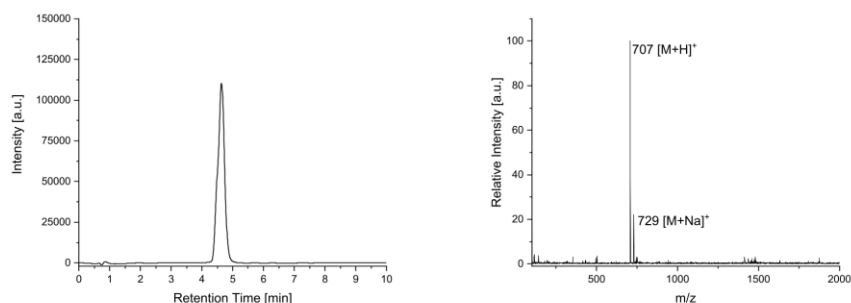
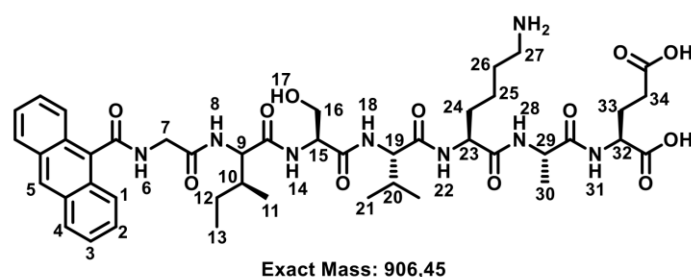


Figure 3: LC spectrum (left) and ESI MS (positive mode, right) of **1**. m/z calculated: $[M+H]^+ = 707$, found: $[M+H]^+ = 707$, $[M+Na]^+ = 729$.

2.2 Ant-GISVKAE

The Wang resin preloaded with Fmoc-Glu(OtBu) (0.25 mmol) was swollen in 5 mL DMF at room temperature for 1 h before transferring it into the peptide synthesizer. DMF was removed via a draining process and the resin swollen with 20 mL DMF for 20 s. Prior to each coupling step, as well as during the final deprotection, the N-terminal Fmoc group was removed using two deprotection steps: 20% piperidine in DMF (3 mL) was applied for 15 seconds at 75 °C, followed by 50 seconds at 90 °C. Fmoc-protected amino acids (Fmoc-Ala, Fmoc-Lys(Boc), Fmoc-Val, Fmoc-Ser(tBu), Fmoc-Ile and Fmoc-Gly) were coupled in a 5-fold molar excess (5 eq. in 1.25 mL) for 15 seconds and 110 seconds at 75 °C and 90 °C, respectively, using a solution of DIC (0.25 M) and Oxyma (0.5 M) in 2.75 mL DMF. The solution was drained, and the resin washed with DMF three times. Anthracene was then coupled to the peptide by adding 9-anthracene carboxylic acid (111 mg, 2 equiv.), PyBOP (260 mg, 2 equiv.) and DIPEA (170 μ L, 4 equiv.) in 5 mL DMF to the resin and shaking overnight. The peptide was cleaved from the resin by adding 10 mL of cleavage cocktail (95% TFA, 2.5% TIPS, 2.5% MilliQ water) to the resin and shaking at room temperature for 2 h. The cleavage cocktail was drained, and the resin washed with TFA. The TFA containing solutions were combined and the liquids removed under reduced pressure.

The peptide was purified via HPLC with a YMC-Actus Triart C18 column (flowrate 20 mL/min). After lyophilisation, the product Ant-GISVKAE was received as a colourless solid (50.6 mg, 0.06 mmol, 24%).



^1H NMR (700 MHz, DMSO) δ 9.02 (t, $J = 6.2$ Hz, 1H, **6**), 8.65 (s, 1H, **5**), 8.53 (d, $J = 7.6$ Hz, 1H, **28**), 8.27 (d, $J = 7.7$ Hz, 2H, **1**), 8.23 (s, 1H, **14**), 8.14 – 8.10 (m, 2H, **4**), 8.08 (d, $J = 9.0$ Hz, 1H, **8**), 8.04 (d, $J = 7.7$ Hz, 1H, **31**), 7.69 (d, $J = 8.8$ Hz, 1H, **18**), 7.54 (dd, $J = 7.1, 3.0$ Hz, 4H, **2, 3**), 7.33 (d, $J = 6.5$ Hz, 1H, **22**), 4.47 (t, $J = 8.0$ Hz, 1H, **9**), 4.41 (q, $J = 6.2$ Hz, 1H, **15**), 4.29 (q, $J = 7.6$ Hz, 1H, **32**), 4.24 – 4.05 (m, 4H, **7, 29, 19**), 3.81 (q, $J = 6.4$ Hz, 1H, **23**), 3.59 (d, $J = 6.1$ Hz, 3H, **16**), 2.83 – 2.66 (m, 2H, **34**), 2.26 – 2.06 (m, 2H, **27**), 1.96 (q, $J = 6.8$ Hz, 1H, **20**), 1.85 – 1.65 (m, 4H, **26', 24, 10**), 1.60 – 1.27 (m, 6H, **26', 33, 12', 25**), 1.22 (d, $J = 7.3$ Hz, 3H, **30**), 1.14 (dt, $J = 14.0, 7.7$ Hz, 1H, **12'**), 0.98 – 0.67 (m, 12H, **11, 13, 21**).

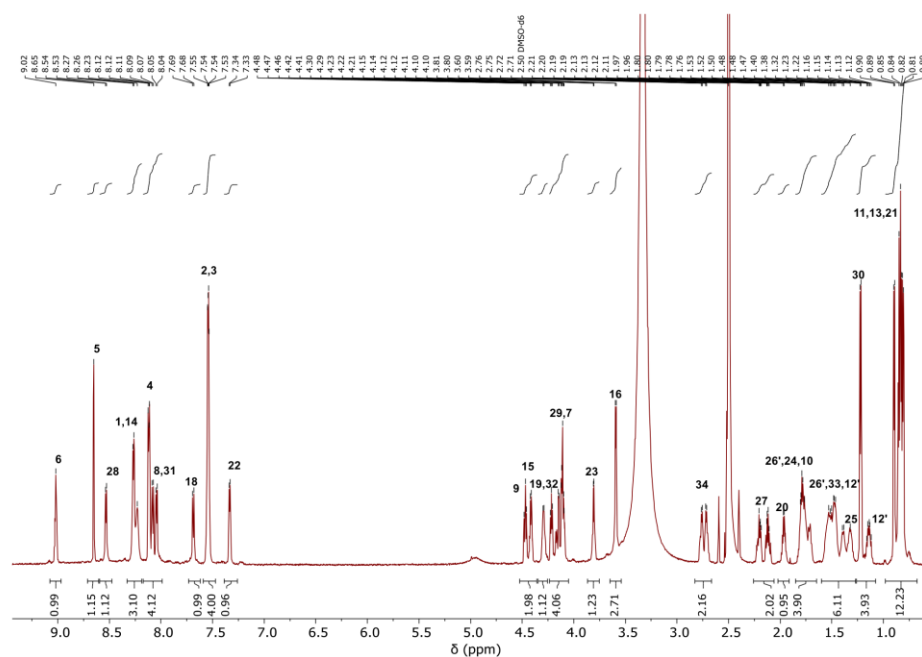


Figure 4: ^1H NMR spectrum (700 MHz, DMSO- d_6 , 298 K) of compound **2**



Figure 5: ^1H NMR and ^1H , ^1H COSY NMR spectra (700 MHz, DMSO- d_6 , 298 K) of compound **2**

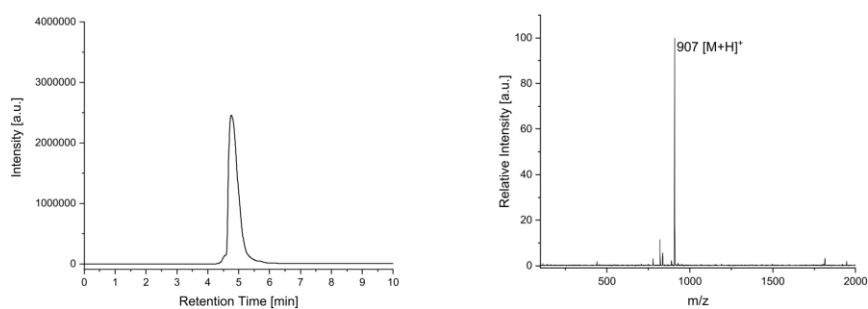


Figure 6: LC spectrum (left) and ESI MS (positive mode, right) of **2**. m/z calculated: $[\text{M}+\text{H}]^+ = 907$, found: $[\text{M}+\text{H}]^+ = 907$.

3 Characterization

3.1 Absorbance and Fluorescence spectroscopy

3.1.1 Calibration curves

1 was dissolved in 50% DMSO and 50% NH_4HCO_3 buffer (10 mM, pH 7.8) in the following concentrations: 100, 50, 25, 12.5, 6.25 and 0 μM . Then for each concentration three times 60 μL were transferred to a Greiner 384 UV-STAR® clear wellplate with flat bottom and the absorbance at 366 nm was measured. Absorbance was then plotted against the concentration and the points fitted linearly. The same procedure was conducted for **2**.

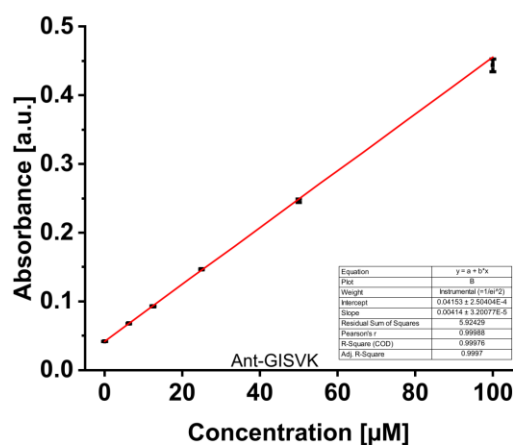


Figure 7: Linear fit of the absorbances of **1** at 366 nm with different concentrations in 50% DMSO and 50% NH_4HCO_3 buffer (10 mM, pH 7.8).

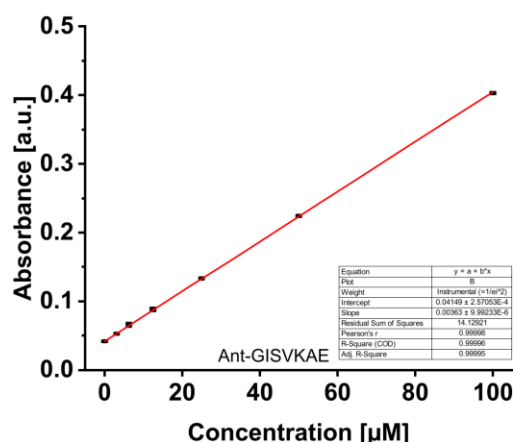


Figure 8: Linear fit of the absorbances of **2** at 366 nm with different concentrations in 50% DMSO and 50% NH_4HCO_3 buffer (10 mM, pH 7.8).

3.1.2 Irradiation conversion in 50% DMSO

1 (100 μM) was dissolved in 500 μL of 50% DMSO and 50% NH_4HCO_3 buffer (10 mM, pH 7.8) in a glass vial equipped with a stirring bar and covered with a blanket of argon. The solution was then irradiated at 365 nm for 0.5, 1, 2.5 and 5 min while stirring at 300 rpm. After each irradiation timepoint the absorbance at 366 nm was measured with three times 60 μL using a Tecan plate reader and Greiner 384 UV-STAR® clear wellplates with flat bottom. After each measurement the solution was filled back to the glass vial to keep the volume consistent. This procedure was repeated for **1** in 50% DMSO and 50% NH_4HCO_3 buffer (10 mM, pH 11 (adjusted with 1 M NaOH solution)) and for **2** at both pH values. The concentration of remaining **1** or **2** was then calculated with the absorbance values using the calibration curves of **3.1.1**.

3.1.3 Irradiation conversion in 5% DMSO

1 (100 μM) was dissolved in 150 μL of 5% DMSO and 95% NH_4HCO_3 buffer (10 mM, pH 7.8) and incubated overnight at 25°C while shaking at 300 rpm. The solution was then transferred to a glass vial equipped with a stirring bar and covered with a blanket of argon. The solution was then irradiated at 365 nm for 1 min while stirring at 300 rpm and then diluted with 135 μL DMSO to achieve a DMSO content of 50%. The absorbance at 366 nm was measured with three times 60 μL using a Tecan plate reader and Greiner 384 UV-STAR® clear wellplates with flat bottom. This process was repeated for irradiation times of 0, 2.5, 5 and 10 min of irradiation at 365 nm.

The procedure was conducted for **1** in 5% DMSO and 95% NH_4HCO_3 buffer (10 mM, pH 11) and for **2** at both pH values.

The concentration of remaining **1** or **2** was then calculated with the absorbance values using the calibration curves of **3.1.1** taking into account the dilution with DMSO.

3.1.4 Proteostat assay

1 (100 μ M) was dissolved in 5% DMSO and 95% NH_4HCO_3 buffer (10 mM, pH 7.8) and incubated overnight at 25°C while shaking at 300 rpm.

The Proteostat Protein Aggregation Assay Kit was purchased from *Enzo Life Sciences, Inc.* 0.52 μ L of the Proteostat stock solution was diluted with 98.48 μ L MilliQ water and 1 μ L assay buffer. 1 μ L of this solution was added to 9 μ L peptide solution in a Greiner 384 flat black wellplate three times for each sample. The solutions were incubated in the dark for 15 min while shaking at 500 rpm. The fluorescence intensity was measured at an excitation wavelength of 550 nm and emission wavelength of 600 nm and with a bandwidth of 20 nm. The procedure was conducted for **1** in 5% DMSO and 95% NH_4HCO_3 buffer (10 mM, pH 11) and for **2** at both pH values.

Samples for irradiation were transferred to a glass vial equipped with a stirring bar and covered with a blanket of argon. They were each irradiated at 365 nm for 10 min while stirring at 300 rpm and again incubated overnight at 25°C.

3.2 Nuclear magnetic resonance

1.4 mg of **1** was dissolved in 600 μ L DMSO- d_6 and 150 μ L NH_4HCO_3 buffer in H_2O (10 mM, pH 7.8). ^1H -NMR was then measured with water suppression. The sample was then covered with a blanket of argon and irradiated for 5 min at 365 nm while stirring at 300 rpm. ^1H -NMR with water suppression was again measured and this process repeated for a total of 10, 20 and 30 min of irradiation at 365 nm. The integral of the ^1H -singulett of the anthracene monomer at 8.63 ppm and of the anthracene-dimer at 5.84 ppm were compared to each other while using the peptide signal at 2.69 ppm as a standard.

The procedure was conducted for **1** in 80% DMSO- d_6 and 20% NH_4HCO_3 buffer in H_2O (10 mM, pH 11) and for **2** at both pH values.

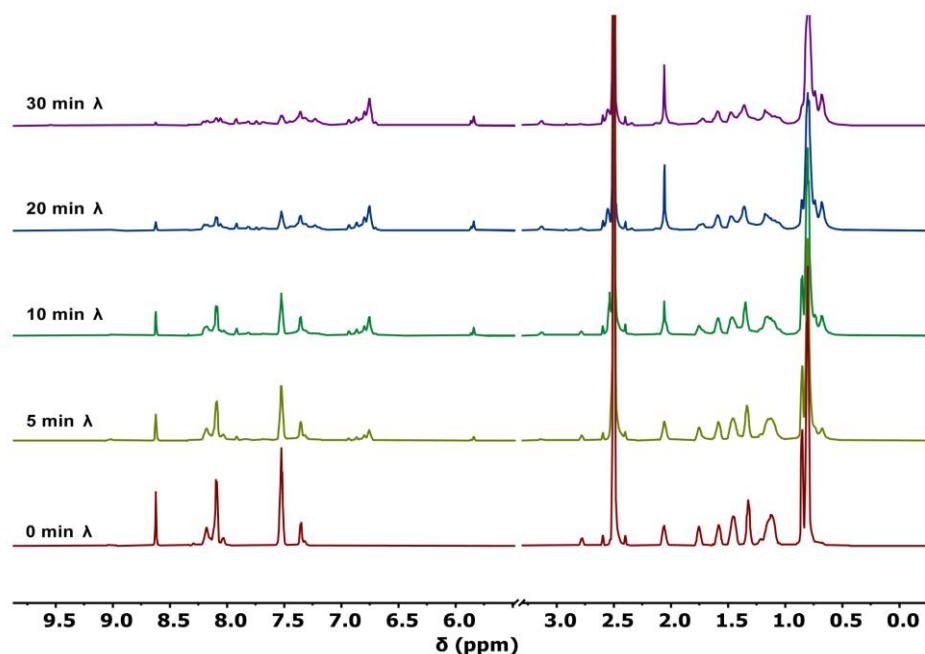


Figure 9: ^1H NMR kinetic (700 MHz, 298 K) of **1** at different irradiation times in 80% DMSO- d_6 and 20% NH_4HCO_3 buffer in H_2O (10 mM, pH 11).

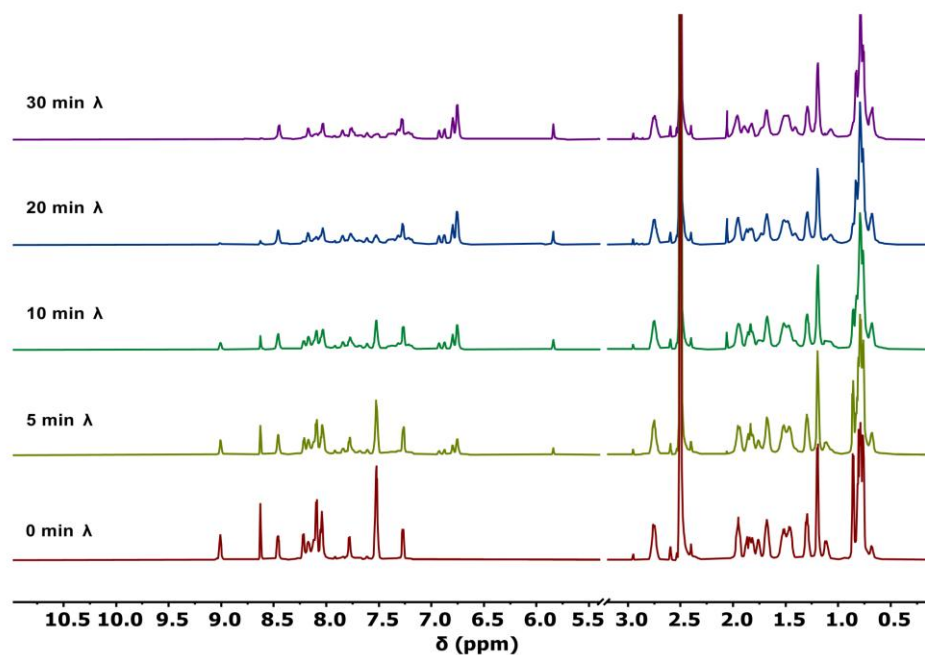


Figure 10: ^1H NMR kinetic (700 MHz, 298 K) of **2** at different irradiation times in 80% DMSO- d_6 and 20% NH_4HCO_3 buffer in H_2O (10 mM, pH 7.8).

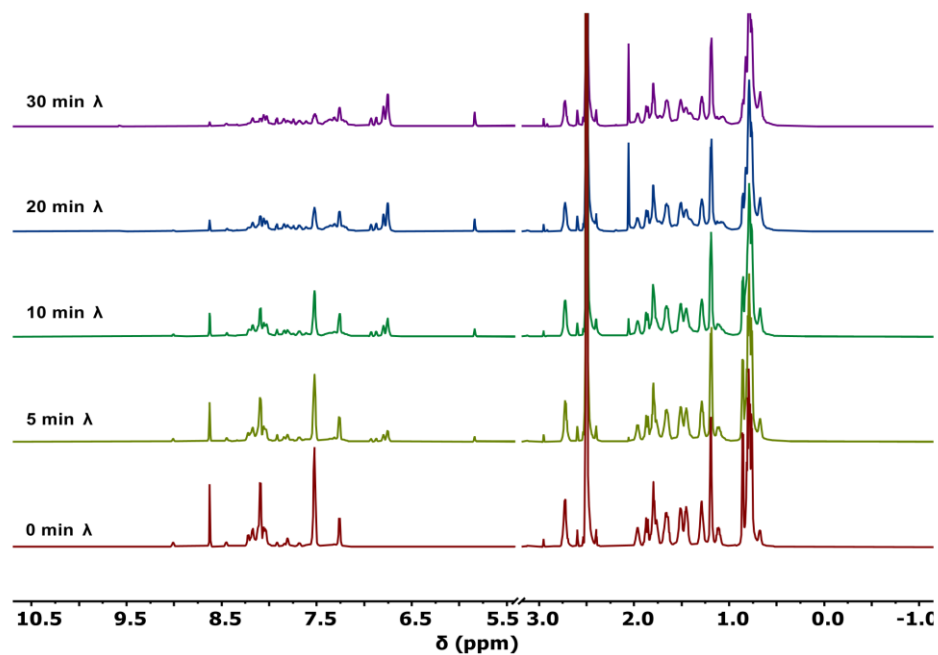


Figure 11: ^1H NMR kinetic (700 MHz, 298 K) of **2** at different irradiation times in 80% DMSO-d_6 and 20% NH_4HCO_3 buffer in H_2O (10 mM, pH 11).

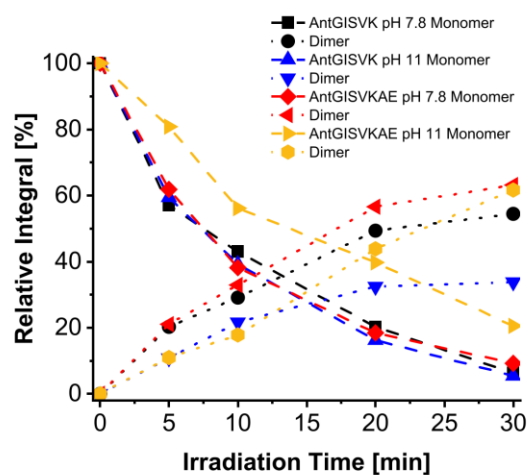


Figure 12: Relative integral of the anthracene monomer and dimer signals of **1** and **2** in 80% DMSO-d_6 and 20% NH_4HCO_3 buffer in H_2O (10 mM, pH 7.8 or pH 11) by ^1H NMR (700 MHz, 298 K).

3.3 Transmission electron microscopy

3.3.1 Non-irradiated samples

1 (100 μM) was dissolved in 5% DMSO and 95% NH_4HCO_3 buffer (10 mM, pH 7.8) and incubated overnight at 25°C while shaking at 300 rpm. Then TEM grids were prepared by pipetting 3 μL solution onto a Formvar-coated copper grid and incubated for 5 min. After the incubation, the solutions were removed with filter paper, and the grids were stained with 7 μL 4% uranyl acetate solution for 2.5 min. The grids were washed three times with MilliQ water and dried before being measured. The procedure was conducted for **1** in 5% DMSO and 95% NH_4HCO_3 buffer (10 mM, pH 11) and for **2** at both pH values.

3.3.2 Irradiated samples

1 (100 μM) was dissolved in 5% DMSO and 95% NH_4HCO_3 buffer (10 mM, pH 7.8) and incubated overnight at 25°C while shaking at 300 rpm. The solution was then transferred to a glass vial equipped with a stirring bar and covered with a blanket of argon. It was then irradiated at 365 nm for 10 min while stirring at 300 rpm and again incubated overnight at 25°C. Then TEM grids were prepared by pipetting 3 μL solution onto a Formvar-coated copper grid and incubated for 5 min. After the incubation, the solutions were removed with filter paper, and the grids were stained with 7 μL 4% uranyl acetate solution for 2.5 min. The grids were washed three times with MilliQ water and dried before being measured. The procedure was conducted for **1** in 5% DMSO and 95% NH_4HCO_3 buffer (10 mM, pH 11) and for **2** at both pH values.

3.4 Circular Dichroism

1 (100 μM) was dissolved in 5% DMSO and 95% NH_4HCO_3 buffer (10 mM, pH 7.8) and incubated overnight at 25°C while shaking at 300 rpm. CD spectra were recorded at wavelength from 300 to 185 nm with a bandwidth of 1 nm, data pitch of 0.2 nm and a scanning speed at 20 nm/min from 20°C. Spectra were measured three times. The procedure was conducted for **1** in 5% DMSO and 95% NH_4HCO_3 buffer (10 mM, pH 11) and for **2** at both pH values.

Samples for irradiation were transferred to a glass vial equipped with a stirring bar and covered with a blanket of argon. They were each irradiated at 365 nm for 10 min while stirring at 300 rpm and again incubated overnight at 25°C. The samples were measured as described above.

3.5 Fourier-transform Infrared spectroscopy

1 (500 μM) or **2** (500 μM) was dissolved in 5% DMSO and 95% NH_4HCO_3 buffer (10 mM, pH 7.8) and incubated overnight at 25°C while shaking at 300 rpm. Each sample was then measured in a cell between two CaF_2 windows separated by a 100 μm Teflon spacer.

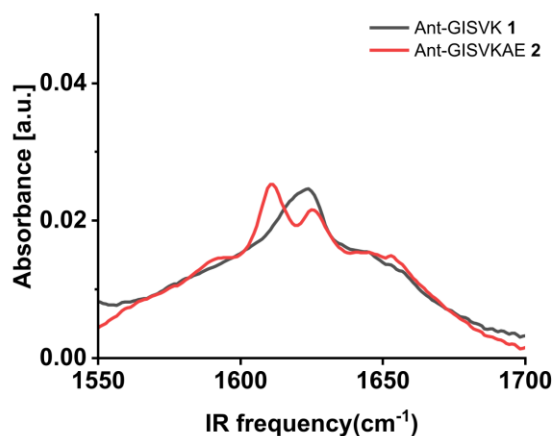


Figure 13: FTIR of **1**_{pH7.8} and **2**_{pH7.8} in 5% DMSO and 95% NH_4HCO_3 buffer (10 mM)

5. Photoswitchable Peptides as Molecular Tools to Encode Structural Order and Disorder in Intracellular Assemblies

Authors

Julian Link, Luca Burg, Sarah Chagri, Ha-Chi Nguyen, David Y. W. Ng*, Bart Jan Ravoo* & Tanja Weil*

* Corresponding Author

Published in *Angewandte Chemie International Edition*, 2026, Volume 65, Issue 3, e14781 & *Angewandte Chemie*, 2026, Volume 138, Issue 3, e14781

DOI: 10.1002/anie.202514781 (International Edition)

Doi: 10.1002/ange.202514781 (German Version)

Date of Publication: 4th of December 2025

© 2025 The Author(s). *Angewandte Chemie International Edition* published by Wiley-VCH GmbH & © 2025 Die Autoren. *Angewandte Chemie* veröffentlicht von Wiley-VCH GmbH

This is an open access article under the terms of the Creative Commons Attribution License (available under <https://creativecommons.org/licenses/by/4.0/>), which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Scientific Context and Research Aim

While the light-triggered disassembly demonstrated in the second project established a proof-of-concept for external control, the irreversible nature of the photochemical reaction limited the system's adaptability and potential for dynamic applications. To address this limitation, the third research project aimed to introduce full photoreversibility into the peptide assembly system. Furthermore, a key scientific gap remained in understanding how reversible supramolecular order-disorder transitions specifically impact biological environments. Most studies correlate static structures with biological function, leaving the dynamic interplay between switching states and cellular response largely unexplored. The primary aim of this work was to develop a photoswitchable peptide system capable of reversible cycling between ordered and disordered states and to investigate how these distinct supramolecular phases influence

cellular viability. By integrating a robust photoswitch with a bioresponsive delivery mechanism, the study sought to establish a direct link between photoreversible self-assembly and intracellular function.

Experimental Approach and Methodology

The molecular design centered on an arylazopyrazole (AAP) photoswitch integrated with the pro-assembling peptide ISA. This combination was chosen to enable efficient switching between a planar *trans*-isomer, capable of forming β -sheet-rich nanofibers, and a non-planar *cis*-isomer, which forms disordered aggregates. To facilitate biological application, the system was extended via isopeptide chemistry to include a glutathione (GSH)-responsive linker and a cell-penetrating TAT peptide. This design ensured intracellular activation and release of the assembly-competent monomer. The photophysical and supramolecular properties were characterized using UV-Vis and CD spectroscopy, TEM, and NMR spectroscopy. Reversibility was tested over multiple switching cycles. Biological evaluation involved cellular uptake studies visualized by confocal laser scanning microscopy (CLSM) and viability assays. Kinetic analysis of the intracellular release mechanism was performed using LC-MS.

Personal Contribution

As the first author of this project, I led the conceptualization and experimental execution from start to finish. I designed the molecular system and synthesized all peptide conjugates and associated reference molecules. I was responsible for the comprehensive structural characterization, performing circular dichroism (CD) spectroscopy to analyze secondary structure and transmission electron microscopy (TEM) to visualize macroscopic morphology. A significant part of my work involved assessing the robustness of the system; I conducted combined CD and TEM studies over 20 irradiation cycles to analyze supramolecular photofatigue and reversibility. I also determined the photostationary states of the linear peptide using NMR spectroscopy. For the biological component, I prepared all cell culture samples and managed the irradiation protocols required to trigger switching within the cellular context. Additionally, I performed the LC-MS kinetic analysis to verify the GSH-responsive release of the linear monomer from the isopeptide precursor for both isomeric forms. I analyzed the Proteostat aggregation data and took the lead in writing and editing the manuscript.

Collaborative Efforts and Supervision

This project benefited from specialized collaborations to complement my core work. Luca Burg contributed essential photophysical characterization, specifically determining the thermal half-life, photofatigue resistance, and switching kinetics of the photoswitch units. He also synthesized the initial arylazopyrazol unit. Sarah Chagri designed and performed the cell

uptake experiments, including the synthesis of the Cy5-containing peptide dye for visualization, while Ha-Chi Nguyen assisted with the execution of Confocal Laser Scanning Microscopy. The overall research concept and results were discussed with David Y. W. Ng, Bart Jan Ravoo and Tanja Weil. Bart Jan Ravoo and Tanja Weil were responsible for acquiring the funding that supported this research. All co-authors provided critical feedback and corrections on the manuscript.

Outcome and Significance

The project successfully demonstrated a photoreversible peptide assembly system capable of switching between ordered nanofibers and disordered aggregates over 20 cycles without significant fatigue. The AAP-ISA conjugate exhibited excellent photophysical properties, including long thermal half-lives and well-defined photostationary states. Crucially, the study established a direct correlation between supramolecular order and biological function: the ordered *trans*-assemblies exhibited significantly higher cytotoxicity compared to the disordered *cis*-states. By combining reversible photoswitching with GSH-responsive intracellular activation, this work provided a rare example of dynamic supramolecular control within living cells. These findings advance the field of photopharmacology and dynamic biomaterials by demonstrating that cellular responses can be modulated through reversible structural transitions rather than static chemical composition.



Peptides Hot Paper

How to cite: *Angew. Chem. Int. Ed.* **2026**, *65*, e14781
doi.org/10.1002/anie.202514781

Photoswitchable Peptides as Molecular Tools to Encode Structural Order and Disorder in Intracellular Assemblies

Julian Link, Luca Burg, Sarah Chagri, Ha-Chi Nguyen, David Y.W. Ng,* Bart Jan Ravoo,* and Tanja Weil*

Abstract: Understanding how self-assembled structure formation affects cells remains a central challenge in supramolecular chemistry. However, chemical tools that allow access to both ordered and disordered intracellular assemblies from a single molecular scaffold are rare due to design complexity. Here, we present a photoswitchable isotriptide incorporating an arylazopyrazole (AAP) unit, which undergoes intracellular cleavage to yield a self-assembling monomer. Upon photoisomerization, the planar *trans*-isomer forms β -sheet-rich nanofibers with strong aromatic interactions, while the non-planar *cis*-isomer assembles into disordered, random-coil aggregates lacking aromatic contribution. The structural dynamics of the assemblies are demonstrated by repeated photoswitching between the two states in buffered conditions. Notably, A549 cancer cell viability correlates with the isomer-dependent assembly behavior and critical aggregation concentrations (CACs): the *trans*-isomer, with higher aggregation propensity, exhibits greater cytotoxicity. This photoswitchable peptide system thus provides a powerful platform with fast, reversible and robust switching kinetics, long isomer half-lives, and high photostability to probe the intracellular consequences of supramolecular order and disorder using a single molecular scaffold.

Introduction

Artificial self-assembling peptides (SAPs) have emerged as a versatile class of programmable molecules capable of forming morphologically diverse nanostructures with various functions spanning from biological regulation to materials science.^[1–3] Their ability to mimic cytoskeletal elements such as actin filaments^[4,5] or mediate dynamic interactions akin to intrinsically disordered domains^[6,7] underscores their importance as bioinspired molecular tools to understand and modulate cellular functions. Synthetic modifications further extend the functionality of SAPs, allowing incorporation of stimuli-responsive elements, including pH-sensitive hydrazones,^[8,9] ROS-cleavable boronate esters,^[10–12] and photocleavable cages^[13,14] thereby enabling spatial and temporal control

over assembly behavior, structure formation, and cellular interactions.^[15]

Among these systems, β -amyloid-like motifs have gained prominence due to their well-defined self-assembly characteristics and growing utility beyond pathological models.^[16,17] Applications of SAPs now extend to piezoelectric nanodevices,^[18,19] nanoscale delivery vehicles,^[20–22] catalysis^[23,24] and responsive biomaterials.^[25,26] The biological impact of such artificial assemblies is increasingly recognized to depend on their diverse supramolecular architectures and their inherent assembly dynamics as well as other parameters such as intracellular distribution,^[27,28] biomolecular recognition,^[29,30] and signal transduction.^[31,32] However, chemical tools that use a single molecular scaffold to access both ordered and disordered intracellular assembly states remain scarce. Developing such systems is essential for disentangling how structural order governs cellular responses, ultimately guiding the rational design of self-assembling peptides for functional applications in biology and medicine.

The supramolecular organization of self-assembling monomers is dictated by a delicate interplay of non-covalent forces, including hydrogen bonding, van der Waals interactions, hydrophobic interactions, and π - π -stacking.^[33,34] These interactions are highly sensitive to molecular geometry, such that even minor structural variations can lead to pronounced differences in assembly behavior.^[35,36] Conventional strategies to modulate this behavior often rely on dynamic covalent chemistry or cleavable functional groups, which, while effective, can increase synthetic complexity and introduce potentially disruptive by-products.^[10,11] Non-assembling control peptides

[*] J. Link, Dr. S. Chagri, H.-C. Nguyen, Dr. D. Y. Ng, Prof. Dr. T. Weil
Max Planck Institute for Polymer Research, Ackermannweg 10 55128,
Mainz, Germany
E-mail: ng@mpip-mainz.mpg.de
weil@mpip-mainz.mpg.de

L. Burg, Prof. Dr. B. J. Ravoo
Center for Soft Nanoscience, Universität Münster,
Busso-Peuss-Strasse 10 48149, Münster, Germany
E-mail: b.j.ravoo@uni-muenster.de

Additional supporting information can be found online in the Supporting Information section

© 2025 The Author(s). Angewandte Chemie International Edition published by Wiley-VCH GmbH. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

used in these studies typically involve increasing the solubility by substituting hydrophobic amino acids for more hydrophilic ones. Although they function well as controls at the supramolecular level (assembling vs non-assembling), the molecular structural difference is often not considered or accounted for. In this regard, an emerging alternative exploits molecular isomerization to regulate phase separation and nanostructure formation without altering molecular composition becomes highly attractive. Among various chemical isomerization technologies, light-triggered isomerization offers a powerful, non-invasive approach to reversibly control molecular conformation with exceptional spatial and temporal precision.^[37,38]

When photochemistry is applied to modulate the biological activity of analytes by altering pharmacokinetic or pharmacodynamic properties, it is commonly referred to as photopharmacology.^[39–41] The most widely used tools in this field are photocleavable protecting groups, which enable the precise release of biological active molecules upon irradiation. Photoswitches complement this existing portfolio with their reversible switching capability, making them particularly well suited for probing dynamic processes in living systems.^[42] As such, photoswitches have been employed to control the activity of ion channels,^[43] enzymes^[44,45] or the cytoskeleton.^[46] A more recent approach to study aggregation processes relevant to Alzheimer's, Huntington's and Parkinson's disease involve the incorporation of unnatural amino acids bearing photoswitchable side chains into the backbone of a small tryptophan zipper peptide, enabling precise control over its secondary structure.^[47]

Several photoswitchable scaffolds, such as azobenzenes,^[48,49] spiropyrans,^[50,51] diazocines,^[38,52] and arylazopyrazoles (AAPs)^[38,53–55] have been developed for this purpose. Although spiropyrans undergo substantial polarity and geometry changes upon ring opening, their limited aqueous stability can hinder biological applications.^[56,57] Diazocines offer enhanced photostability and thermodynamic control but are more challenging to synthesize and often lack efficient bidirectional switching.^[58] In contrast, AAPs combine straightforward synthesis, efficient two-way switching, extended thermal half-lives, and excellent photostability.^[55,59,60] Their stability in aqueous and reducing conditions was demonstrated in cyclodextrin-based systems,^[54] heparin-conjugates,^[61] and opioid-receptor activation^[62] and makes them especially attractive for biological systems.^[63] Moreover, the potential of AAPs for photoresponsive supramolecular self-assembly in aqueous media has been demonstrated,^[64,65] however, their integration into live-cell environments for dynamic control of supramolecular behavior has yet to be fully explored.

Herein, we report a photoswitchable isotriptide incorporating an arylazopyrazole (AAP) unit that undergoes intracellular activation to yield photosensitive monomers with different aggregation dynamics and assembly morphology. This minimalistic construct enables reversible access to distinct supramolecular states, either ordered fibrillar nanostructures or disordered aggregates, through light-controlled isomerization. The assembly behavior is

governed by conformation-dependent steric effects, while the overall chemical constitution of the peptide remains unchanged between isomers. This design allows for a direct interrogation of how molecular geometry influences self-assembly behavior and its functional consequences for the interaction with biological structures inside living cells. By further integrating redox- and pH-responsive elements into the peptide-AAP framework, we establish a modular platform for an environment-sensitive assembly within complex cellular contexts. This work lays the foundation for spatiotemporally resolved studies of supramolecular organization and its role in cellular processes, advancing the application of dynamic molecular systems in living environments.

Results and Discussion

Design of the pH- and Glutathione-Responsive Photoswitchable AAP-ISA Tripeptide

The modified tripeptide AAP-ISA is designed as a minimalist model of a peptide photoswitch capable of forming distinct, isomer-dependent nanostructures driven by differences in steric demand of the aromatic head group. It features the pro-assembling peptide sequence Ile-Ser-Ala, which can self-assemble into nanofibers when a fluorenylmethoxycarbonyl (Fmoc) π -block is conjugated to its *N*-terminus.^[11] Replacement of the Fmoc-unit with an AAP photoswitch enables light-induced switching between a *trans*-isomer (**1_{trans}**) and a *cis*-form (**1_{cis}**). In the planar *trans*-form, efficient π - π interactions support self-assembly, whereas the steric kink of the *cis*-isomer disrupts stacking, thus preventing ordered self-assembly.

To enable cellular uptake, the linear peptide sequence has to be modified toward cell-penetrating capability. First, the structure of the peptide core is altered into a pro-assembling kinked isopeptide (Figure 2a, blue). A glutathione-responsive self-immolative linker is then attached to the *N*-terminus (Figure 2a, red). This linker is conjugated via a disulfide bond to a cysteine-extended cell-penetrating peptide (Figure 2a, green), specifically the *trans*-activator of transcription (TAT). Upon TAT-mediated uptake, the disulfide linker is cleaved by intracellular glutathione (GSH), yielding the kinked intermediate **3** (Figure 2b). Self-immolation of the linker is then triggered, resulting in the release of the isopeptide **iso-1**, as also demonstrated in previous studies.^[15,66] Lastly, the kinked peptide backbone undergoes an *O,N*-acyl shift, yielding the linearized monomer **1** (Figure 2b). This monomer subsequently self-assembles into either nanofibers (*trans*-isomer) or amorphous aggregates (*cis*-isomer, Figure 1). The kinetics of this transformation, both *cis*- and *trans*-isomer, in the presence of intracellular concentrations of GSH (10 mM)^[67] were monitored by liquid chromatography coupled with mass spectrometry (LC-MS) in a 1:1 mixture of NH_4HCO_3 buffer (50 mM, pH 7.4.) and methanol, ensuring the solubility of all components (Figure 2c). Immediate cleavage of the disulfide bond occurred followed by the formation of the isopeptide bearing the disulfide linker (**3**,

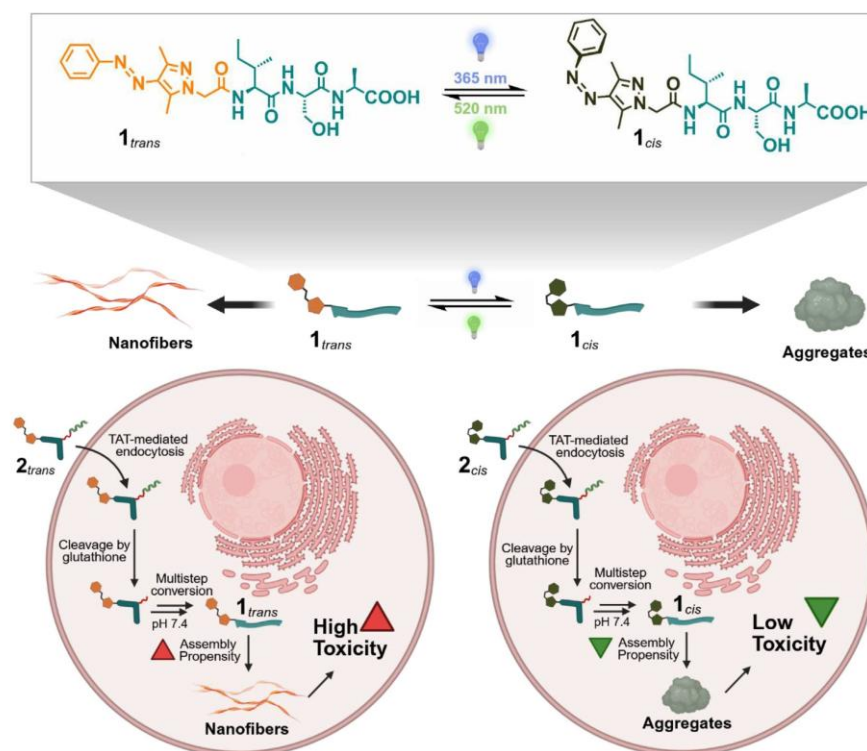


Figure 1. Schematic illustration of the photoswitchable peptide AAP-ISA (**1**) that self-assembles into a light-responsive phase separating material. The resulting nanostructure is dependent on the respective isomer of the photoswitch unit. Extending this peptide toward bioactivity leads to a compound that can enter the cell, be cleaved by glutathione and release **1** in a multistep conversion at physiological pH. The *trans*-isomer assembles into ordered nanofibers, whereas the *cis*-isomer forms disordered aggregates. Both isomers induce different cellular responses. Figure created with Biorender.com.

$t_R = 7.5$ min) and the corresponding GSH-conjugate (**3-GSH**, $t_R = 6.3$ min). Over 24 h, the peptide gradually converted into the free isopeptide (**iso-1**, $t_R = 5.5$ min) and subsequently into the linearized monomer **1** ($t_R = 6.5$ min) with both isomers achieving similar kinetic profiles (Figure 2d and Figures S31 + 32).

Molecular Characteristics and Photophysical Properties of the Photoswitchable Tripeptide **1**

The key characteristic of a photoswitch is its ability to reversibly transform between isomers upon irradiation. Previous studies on the AAP demonstrated that irradiation with UV light at 365 nm converts the native *trans*-isomer to the *cis*-isomer, while green light (520 nm) induces the reverse isomerization.^[53] This reversible *trans*-to-*cis* transition is marked by a decrease and a hypsochromic shift of the $\pi \rightarrow \pi^*$ band from 330 to 298 nm, along with an increase and bathochromic shift of the $n \rightarrow \pi^*$ band from 413 to 431 nm.

UV-vis spectroscopy (Figure 3b) revealed that the *trans*→*cis* isomerization was complete within 5 s, whereas the *cis*→*trans* transformation required approximately 120 s (Figure 3b). The thermal relaxation half-life in the dark at ambient conditions was determined to be 47.6 days, providing a stable time window for all subsequent experiments (Figure 3c). Remarkably, over the course of 10 switching cycles, the fidelity of **1_{trans}** is almost quantitatively preserved (>98%) (Figure 3d).

Switching ratios of **1** were quantified with ¹H-NMR-spectroscopy in DMSO-*d*₆, in which no self-assembly occurs (Figure 3e). The irradiation durations used in the NMR studies (15 s (365 nm), 240 s (520 nm)) were increased due to the higher concentrations (~3.8 mM) required for obtaining sufficient resolution compared to the other experiments (5 s (365 nm), 120 s (520 nm), 100–400 μM). The spectra were recorded immediately after the respective irradiation time intervals (Figure 3e, red→green). Even after 30 min of irradiation (520 nm), the ratios of **1_{trans}** versus **1_{cis}** did not change further (respective ratio of H3 protons 200.00:36.65),

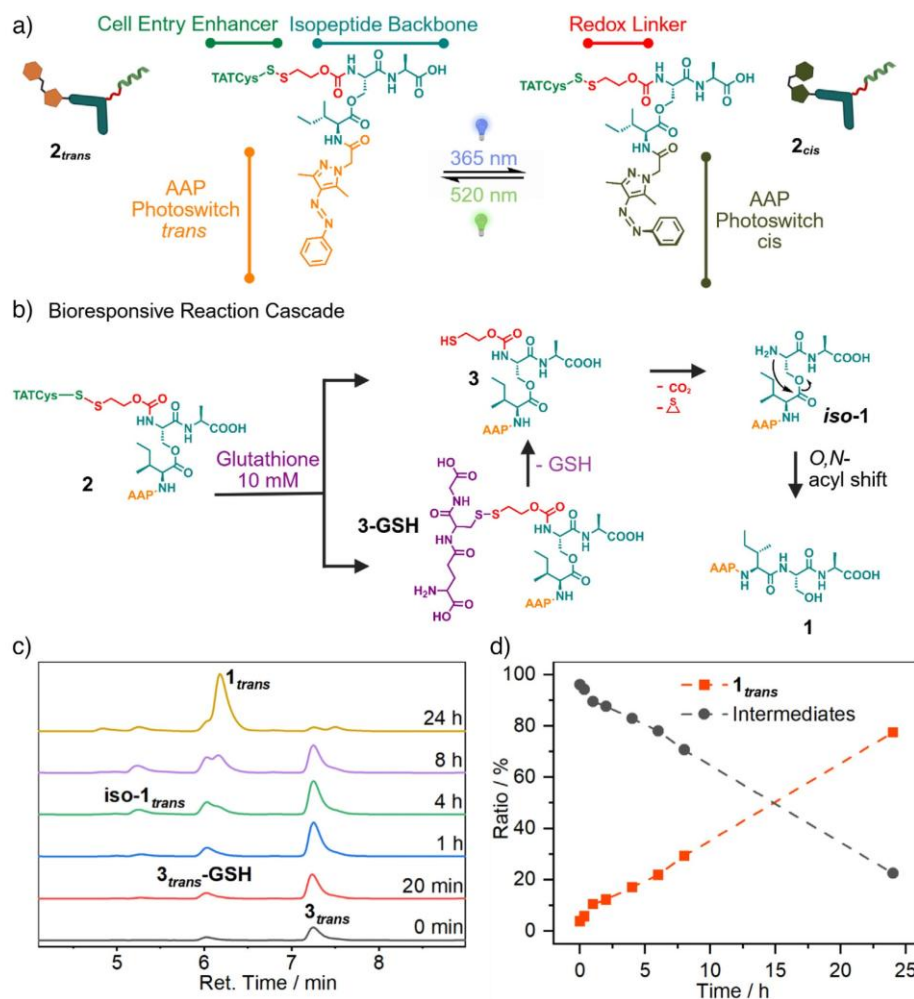


Figure 2. a) Chemical design of the triple-responsive isopeptide (2) composed of the cell-penetrating CysTAT peptide (green) attached to the self-assembly promoting iso-ISA peptide backbone (blue) via a redox sensitive disulfide linker (red) and the AAP photoswitch (orange/dark green). Upon irradiation with light at 365 nm the $trans$ - cis -isomerism (2_{trans} → 2_{cis}) and at 520 nm the cis - $trans$ -isomerism (2_{cis} → 2_{trans}) can be induced. b) Visualization of the chemical transformations within the bioresponsive reaction cascade. First, cleavage of the disulfide linker by glutathione, followed by self-immolation of the linker. Second, intramolecular rearrangement by O,N-acyl shift yielding the linearized AAP-ISA (1). c) LC-MS kinetic analysis of the glutathione-induced linearization of native 2_{trans} in NH₄HCO₃ buffer (50 mM, pH 7.4) and methanol (v/v 1:1) in the presence of intracellular concentrations of glutathione (10 mM) at room temperature. Corresponding masses can be found in the Supporting Information (Figure S31) d) Relative ratio of intermediates (iso-1, 3, 3-GSH) and final product 1 after the addition of glutathione-containing buffer, based on the peak integration at 300 nm. The data shown here is derived from the native $trans$ -isomer. The corresponding data for the cis -isomer can be found in the supporting information (Figure S32).

indicating completed conversion of 1_{cis} to 1_{trans} already after 240 s (Figure S30). Comparing the aromatic phenyl protons of 1, the signals of the $trans$ -state at 7.73, 7.51, and 7.43 ppm nearly vanished, while three new signals at lower chemical shift values at 7.01, 7.39, and 7.23 ppm emerged

corresponding to the respective phenyl protons of the cis -isomer. Additionally, the isoleucine and serine amide peaks shifted from 8.33 and 8.10 ppm to 8.13 and 8.08 ppm, respectively. Upon irradiation with green light (520 nm), the initial signals could be recovered, whereas the corresponding

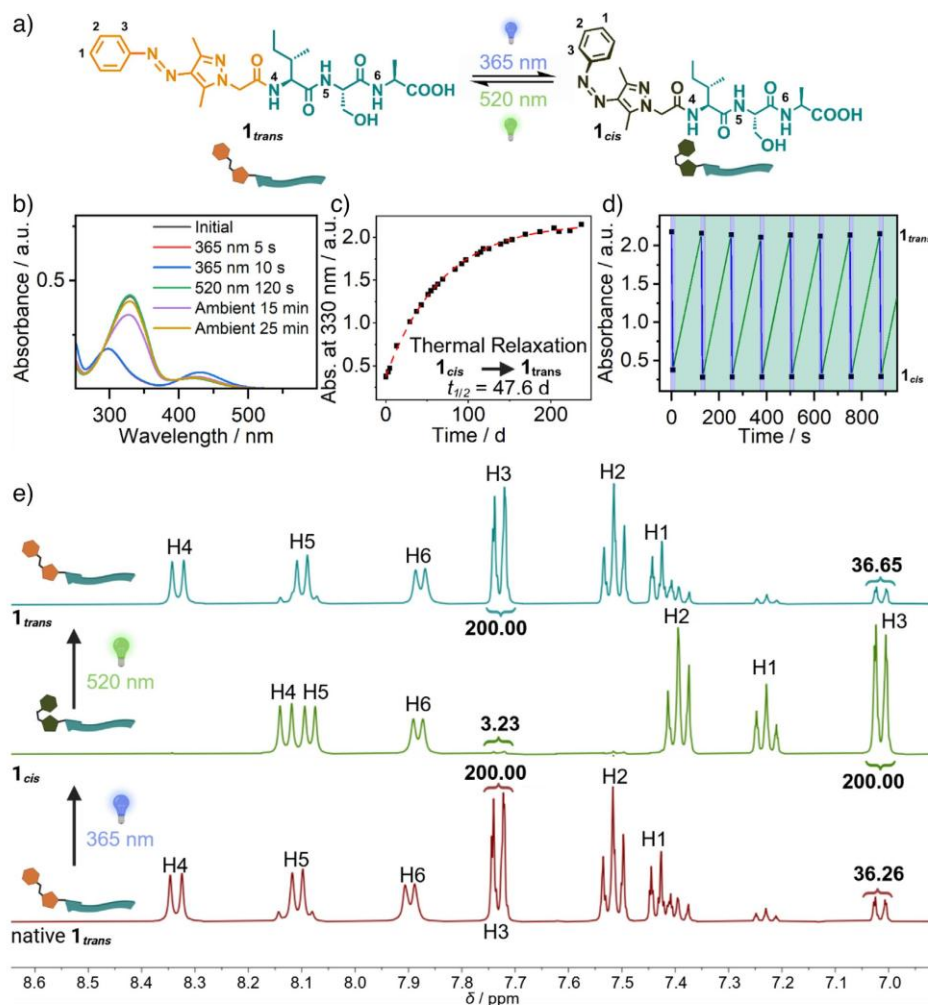


Figure 3. a) Chemical structures of AAP-ISA in *trans*- (1_{trans}) and *cis*-state (1_{cis}). b) UV-vis absorbance spectra of AAP-ISA (1) over different irradiation times at different wavelengths. c) Half-life time in the dark at ambient conditions for 1_{cis} . d) UV-vis absorbance of 1 over the course of 10 irradiation cycles at 365 nm for 5 s (indicated by blue box) and 520 nm for 120 s (green box). e) ^1H -NMR spectroscopy (DMSO- d_6 , 298K, 400 MHz, 2 mg mL^{-1}) analysis of AAP-ISA in native state (1_{trans} , red), after irradiation at 365 nm for 15 s (1_{cis} , green) and after irradiation at 520 nm for 240 s (1_{trans} , blue). Integrals were set to 200.00 for the respective H3-protons of the predominant isomer.

cis-signals disappeared (Figure 3e, green→blue). Based on peak integration in the native-state (Figure 3e), 85% of the molecules were in the *trans*-state (1_{trans}) and 15% were in the *cis*-configuration (1_{cis}). After irradiation with light at 365 nm, >98% of 1_{trans} transitioned to the *cis*-state (1_{cis}), with only <2% remaining in *trans*-form (1_{trans}). Subsequent irradiation with green light restored the native *trans*-state (87% *trans*-state, 13% *cis*-form). These results for absorbance, chemical shifts and switching efficiencies are in alignment

with the data set reported for the free AAP carboxylic acid.^[53]

Programming Supramolecular Order Versus Disorder of the Photoswitchable Tripeptide **1**

The peptide assemblies were analyzed using circular dichroism (CD) spectroscopy, transmission electron microscopy

(TEM), and a dynamic light scattering (DLS) aggregation assay as well as Proteostat aggregation assay. In our initial investigations, we examined the self-assembly behavior of the molecule in its *trans* and *cis* isomeric states independently, with the photoisomerization occurring prior to the assembly process. This approach allowed us to examine how each molecular state influences the structural organization and intermolecular interactions during self-assembly.

We prepared DMSO stock solutions of both isomers respectively, and diluted them to the effective conditions of self-assembly (99% buffer, 1% DMSO). TEM imaging in phosphate buffered saline (PBS) revealed that the *trans*-isomer formed twisted, tubular-like networks at concentrations ranging from 150 to 400 μM (Figure S22). These structures appear to arise from backbone alignment facilitated by π - π -interactions of the planar, hydrophobic *trans*-AAP photoswitch. Following the irradiation at 365 nm for 5 s, these well-defined architectures were entirely absent, suggesting that the twisted and more polar *cis*-AAP photo-switch hinders the self-assembly of the peptide. This behavior remained consistent across various buffer conditions, including hydroxyethylpiperazine ethane sulfonic acid (HEPES) and phosphate buffers supplemented with either chloride or fluoride salts, and in the absence of additional electrolytes (Figure S22).

Building upon these insights, we shifted our focus to dynamic systems where photoisomerization is induced after the assembly has taken place. This enables us to explore how structural transitions propagate through pre-formed assemblies, offering an understanding of the molecular responsiveness and potential reversibility within these supramolecular architectures. For this, the linear peptide in the native *trans*-form (**1_{trans}**) was dissolved in PBS at 200 μM and initial TEM images and CD spectra were recorded. At this concentration, the formation of fibrillar structures was observed for **1_{trans}**, while **1_{cis}** did not show structure formation. The sample was then irradiated for 5 s at 365 nm to initiate *trans*-*cis*-isomerism (**1_{trans}** $\rightarrow$ **1_{cis}**), followed by back-irradiation at 520 nm for 120 s to initiate the reverse *cis*-*trans*-isomerism (**1_{cis}** $\rightarrow$ **1_{trans}**). After the first cycle, a second 365 nm/520 nm irradiation cycle was performed to investigate the reversible behavior and any inherent structural adaptations of the supramolecular assemblies. Between each individual irradiation step, CD-spectroscopy was measured, and TEM samples were prepared (Figure 4) to analyze if the assembled structures of the *trans*-isomer could fully disassemble after the photoisomerization into the *cis*-monomer and reassemble upon the reverse switching. The experimental setup, including the irradiation conditions, is illustrated in Figure 4a. Data points are represented as colored symbols (orange = **1_{trans}**, green = **1_{cis}**) indicating the respective molar ellipticity at 325 nm as a function of total irradiation time.

The native *trans*-state before any irradiation (Figure 4, orange square) showed dense fibrillar network observed in TEM (Figure 4b), comparable to previously observed results (Figure S22). It was accompanied by CD-spectroscopy data indicating chiral secondary structure formation due to aromatic interactions and beta-sheet formation. The fiber-forming *trans*-isomer (**1_{trans}**) exhibited a maximum at 204 nm,

followed by a minimum at 217 nm, indicating n - π^* transitions associated with the presence of antiparallel β -sheets.^[68–72] A subsequent positive cotton effect observed at 233 nm (Figure 4, orange square) could be due to π - π stacking of the aromatic AAP photoswitch, a phenomenon also reported in peptides containing aromatic side chains.^[73,74] Additionally, the CD spectrum highlights a strong aromatic contribution from the photoswitch in the far-UV region (Figure 4, orange square). A bisignate dichroic peak with a minimum at 296 nm, followed by a maximum at 322 nm and a shoulder at ~ 360 nm, likely arises from π - π^* transitions of ordered AAP motifs. This negative-to-positive pattern indicates a positive exciton couplet and therefore a right-handed structure formation.^[71]

UV-irradiation of **1_{trans}** converted the *trans*- into the *cis*-isomer (Figure 4, green circle). In TEM, **1_{cis}** did not assemble into nanostructures (Figure 4, green circle) and the corresponding CD spectrum showed no signals indicating β -sheet structures or aromatic interactions (Figure 4, green circle). Instead, the *cis*-isomer showed a small CD minimum at 204 nm, suggesting a random coil conformation (Figure 4, green circle).^[64]

However, the reformation of **1_{trans}** by subsequent irradiation with green light restored these dense fibrillar networks with prominent β -sheet signatures, indicated by a maximum at 204 nm followed by a minimum at 217 nm, as well as aromatic contributions (minimum at 296 nm, maximum at 322 nm) completely (Figure 4, orange triangle). This process proved to be remarkably fast and fully reversible, with complete structural recovery observed after just 5 s (**1_{trans}** $\rightarrow$ **1_{cis}**) or 120 s (**1_{cis}** $\rightarrow$ **1_{trans}**) of irradiation. The system maintained its responsiveness and structural integrity over 10 cycles of irradiation (Figure S24), demonstrating its robust and dynamic supramolecular character. These observations are consistent with the photoresponsive self-assembly of AAP-containing hydrogelators described in previous reports.^[65,75]

Next, DLS and Proteostat assays were employed to determine the critical aggregation concentration (CAC) of the two isomers. For DLS, the mean count rate was measured at different concentrations and the CAC was identified by a steep increase in the count rate, as reported earlier.^[26] For Proteostat assay, the steep increase of absorbance of the dye indicated the CAC.^[69] For the *trans*-isomer, the CAC was found to be ~ 160 μM for both methods, while for the *cis*-isomer, it increased approximately two-fold, reaching ~ 360 μM (Figure S26). To assess whether CD and TEM data reveal significant differences at concentrations above the CAC of **1_{cis}**, the irradiation experiment shown in Figure 4 was repeated at 400 μM (Figure S25). The CD spectra showed no notable changes compared to those obtained at 200 μM . In TEM, the nanostructures formed by **1_{trans}** were conserved while **1_{cis}** exhibited the formation of amorphous aggregates. These observations are consistent with the CAC of **1_{cis}** (Figure S26) and highlight the impact of the photoswitch, regardless of whether the supramolecular assemblies form highly ordered structures (**1_{trans}**) or disordered aggregates (**1_{cis}**).

After analyzing the structure formation of the linear isotriptide **1** alone in detail, the structure formation upon

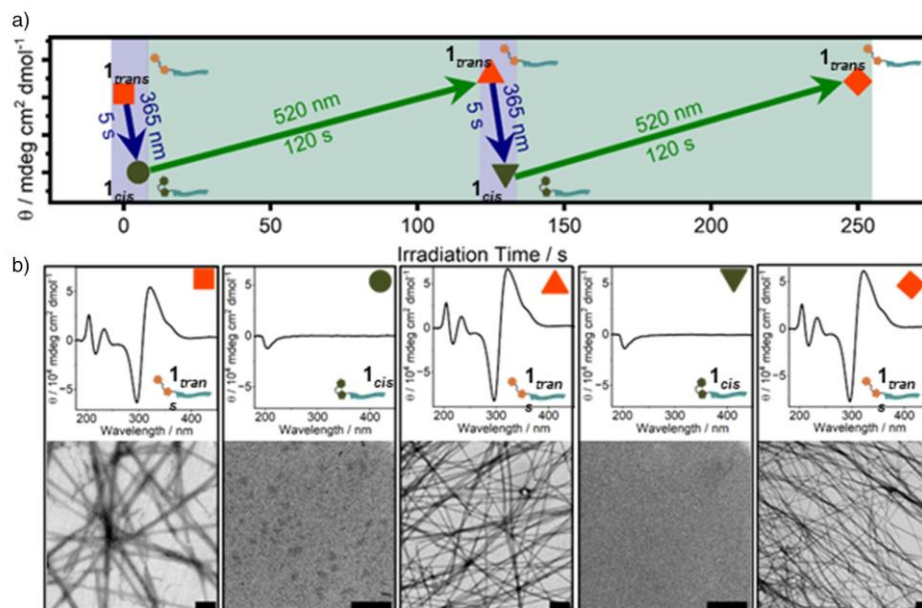


Figure 4. a) Molar ellipticity of **1** at 325 nm over the course of 2 irradiation cycles at 365 nm for 5 s (blue box) and 520 nm for 120 s (green box). In between the individual irradiation steps, corresponding TEM micrographs and CD-spectra at 200 μM in PBS were recorded b). Individual measurements are indicated by different symbol shape. Scale bar for TEM-micrographs = 500 nm.

GSH induced transformation from **2** into **1** was investigated. For this, **2** was incubated in PBS (200 μM) for 24 h and prepared for TEM analysis with and without irradiation for 5 s at 365 nm. The TAT-modified peptides (**2_{trans}** and **2_{cis}**) did not exhibit any defined nanostructure formation, regardless of photoisomerization (Figure S23I and J). However, after incubation with GSH (10 mM, 0–24 h), the *trans*-isomer gradually formed nanofibers, whereas the *cis*-isomer did not (Figure S23), indicating successful release of **1_{trans}** and **1_{cis}**.

Photoisomerism Determines Cellular Toxicity of the Isotriptide **2**

To enable fluorescence visualization of the assembly, co-assembly with Cyanine 5 (Cy5) modified Fmoc-ISA was performed (**Cy5-peptide**, Figure S27). Prior to cell experiments, we investigated the successful co-assembly in a 99/1 mixture of linear **1** and **Cy5-peptide** using both TEM and confocal microscopy at 200 μM in PBS (pH = 7.4) with 1% DMSO (Figure S28 + 29). TEM images revealed that the formed structures were visually indistinguishable from those of the pure AAP-ISA system, while confocal microscopy and fluorescence phase contrast microscopy showed fluorescent fibrous structures, supporting a successful co-assembly of the assembling monomer with the dye-containing **Cy5-peptide** (Figure S28 + 29).

Next, we investigated the cellular uptake of the TAT-bearing bioactive peptides and their subsequent GSH-induced transformation into the self-assembling linear peptide inside human lung adenocarcinoma cells (A549). Cells were treated with 100, 200, and 400 μM of the two different isomers of the TAT-bearing peptides in a 99/1 ratio (**2**/TAT-Cy5-peptide), respectively, incubated for 4 h, and analyzed using confocal laser scanning microscopy (CLSM). To better visualize cell morphology, cells were stained with MitoTracker Orange and NucBlue.

Confocal imaging confirmed cellular uptake for both isomers at various concentrations, as evidenced by strong Cy5 fluorescence (Figure 5d–f and Figure S33). No specific subcellular localization was detected, consistent with the ubiquitous cytosolic distribution of GSH.^[76] The cells treated with 400 μM of both **2_{trans}** (Figure 5h) and **2_{cis}** (Figure 5d) showed a strong Cy5 fluorescence indicating a high uptake of the isopeptides. Cells treated at these concentrations showed cell rounding and disruption of mitochondrial network, indicating mitochondrial stress and cell death. For incubation of cells at 100 μM , no dead cells were observed following treatment with the *cis*-isomer (Figure 5b), indicating no cytotoxicity at this concentration. In contrast, treatment with the *trans*-isomer at the same concentration resulted in weak toxic effects, like cell rounding and disruption of mitochondrial network (Figure 5g). At 200 μM , the difference between cells treated with the *trans*-isomer (Figure 5g) versus *cis*-isomer (Figure 5c)

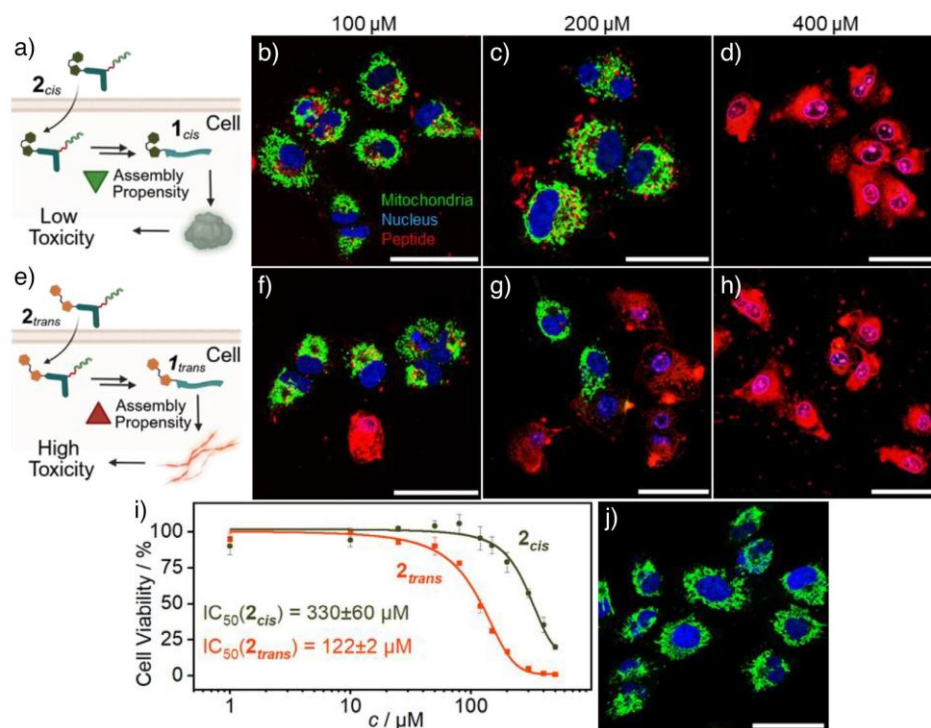


Figure 5. Schematic illustration of the internalization and assembly into nanostructures a) $2_{cis} \rightarrow 1_{cis}$, e) $2_{trans} \rightarrow 1_{trans}$. Confocal laser scanning micrographs of A549 cells treated for 4 h with 2_{cis} (100 b), 200 c) or 400 μM d) or with 2_{trans} (100 f), 200 g) or 400 μM h) or without any compound j) and prestained with Mitotracker Orange and NucBlue. For visualization, 1% of TAT-Cy5-peptide (compare Figure S27) was added and incubated with 2. Imaging under physiological condition (5% CO_2 , 90% humidity, and 37°C) using Leica STELLARIS 8 microscope, HC PL APO CS2 40x/1.25 GLYC. Imaging: 550 nm at 2.13% for MitoTracker Orange excitation, 405 nm at 2.23% for NucBlue excitation and 650 nm at 0.66% for sample excitation. Scale Bars: 50 μm . i) Cell viability of A549 cells incubated with 2_{cis} (green curve) or 2_{trans} (orange curve) for 4 h. The IC_{50} value of 2_{trans} is $122 \pm 2 \mu\text{M}$ and of 2_{cis} is $330 \pm 60 \mu\text{M}$. Data are presented as mean $\pm$ s.e.m, $n=4$.

became even more evident: Although cells treated with the *cis*-isomer remained largely viable, extensive cell death was observed for cells treated with the *trans*-isomer. These results highlight a marked difference in the cytotoxic profiles of the *cis*- and *trans*-isomers, with the *trans*-form exhibiting greater potency at lower concentrations.

To quantify these effects, we performed a CellTiter Glo Cell Viability Assay. A549 cells were treated with varying concentrations of both isomers, and the cell viability was assessed via an ATP dependent luminescence measurement after 4 h of incubation (Figure 5c). The *trans*-isomer exhibited an IC_{50} of $122 \pm 2 \mu\text{M}$, whereas the *cis*-isomer showed an IC_{50} of $330 \pm 60 \mu\text{M}$, representing a 2.7-fold increase in toxicity. These observations correlate with the differing assembly propensity of the *cis*- and *trans*-peptide isomer and their respective CACs (*trans*-isomer $\sim 160 \mu\text{M}$, *cis*-isomer $\sim 360 \mu\text{M}$, Figure S26), suggesting that higher aggregation propensity corresponds to higher cytotoxicity.

These results highlight that even subtle changes in molecular orientation can dramatically alter biological responses due to differences in assembly behavior between the isomers. Here, we designed a peptide system with an identical chemical composition, where the isomer less prone to self-assemble exhibits lower toxicity compared to the self-assembling isomer, allowing for the investigation of structure-bioactivity relationships based on no molecular compositional difference.

Conclusion

In this study, we developed a photosensitive self-assembling peptide capable of dynamically switching between a disordered and ordered assembled state in response to light. The peptide system displays exceptional photostability and half-life times, demands only short irradiation times, while offering precise spatiotemporal control over the self-assembly. Light-

induced switching between distinct β -sheet-rich nanofiber-forming structures and disordered random coil states was achieved rapidly and reversibly. Using isopeptide and redox-sensitive linker chemistry to control intracellular release in cells, this structural transition was further leveraged to modulate bioactivity, demonstrating that the structure-forming monomer exhibits significantly higher cytotoxicity than its aggregate-forming counterpart due to its greater propensity for assembly. These findings underscore how differences in superstructural order, without a change in molecular composition, can be controlled by photoisomerization and that cellular behavior toward synthetic structures formed in situ is largely affected by their respective assembly propensities.

Supporting Information

The authors have cited additional references within the Supporting Information.^[15,53]

Acknowledgements

The authors acknowledge funding by the Max Planck-Bristol Centre for Minimal Biology, the Max Planck Society and the University of Münster. This work was supported by the Max Planck Graduate Center (MPGC) with the Johannes Gutenberg University Mainz. The authors thank Leon Driebehaus-Ortiz for his contributions to the cell viability assays. The authors thank Albin Lahu and Raphael Meyer for their contribution to the GSH induced degradation kinetic by LCMS and Proteostat Aggregation Assay. The schematic illustrations in this manuscript were created with BioRender.com.

Open access funding enabled and organized by Projekt DEAL.

Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Keywords: Disassembly • Intracellular Self-Assembly • Light-responsive systems • Photoswitchable Peptides • Structural Order–disorder

- [1] S. Chagri, D. Y. W. Ng, T. Weil, *Nat. Rev. Chem.* **2022**, *6*, 320–338, <https://doi.org/10.1038/s41570-022-00373-x>.
- [2] A. Levin, T. A. Hakala, L. Schnaider, G. J. L. Bernardes, E. Gazit, T. P. J. Knowles, *Nat. Rev. Chem.* **2020**, *4*, 615–634, <https://doi.org/10.1038/s41570-020-0215-y>.
- [3] A. Reja, S. Pal, K. Mahato, B. Saha, M. Delle Piane, G. M. Pavan, D. Das, *J. Am. Chem. Soc.* **2023**, *145*, 21114–21121, <https://doi.org/10.1021/jacs.3c08158>.
- [4] A. M. Keane, I. P. Trayer, B. A. Levine, C. Zeugner, J. C. Ruegg, *Nature* **1990**, *344*, 265–268, <https://doi.org/10.1038/344265a0>.
- [5] R. J. Gübeli, D. Bertoldo, K. Shimada, C. B. Gerhold, V. Hurst, Y. Takahashi, K. Harada, G. K. Mothukuri, J. Wilbs, M. Harata, S. M. Gasser, C. Heinis, *ACS Chem. Biol.* **2021**, *16*, 820–828.
- [6] P. G. Argudo, J. J. Giner-Casares, *Nanoscale Adv.* **2021**, *3*, 1789–1812, <https://doi.org/10.1039/D0NA00941E>.
- [7] T. Ehm, H. Shinar, G. Jacoby, S. Meir, G. Koren, M. Segal Asher, J. Korpanty, M. P. Thompson, N. C. Gianneschi, M. M. Kozlov, S. Azoulay-Ginsburg, R. J. Amir, J. O. Rädler, R. Beck, *Biomacromolecules* **2023**, *24*, 98–108.
- [8] M. J. Webber, J. B. Matson, V. K. Tamboli, S. I. Stupp, *Biomaterials* **2012**, *33*, 6823–6832, <https://doi.org/10.1016/j.biomaterials.2012.06.003>.
- [9] S. Yu, M. J. Webber, *J. Mater. Chem. B* **2024**, *12*, 10757–10769, <https://doi.org/10.1039/D4TB01860E>.
- [10] Z. Zhou, K. Maxeiner, P. Moscarillo, S. Xiang, Y. Wu, U. Kaiser, K. Landfester, I. Lieberwirth, D. Y. W. Ng, T. Weil, *J. Am. Chem. Soc.* **2022**, *144*, 12219–12228.
- [11] M. Pieszka, S. Han, C. Volkmann, R. Graf, I. Lieberwirth, K. Landfester, D. Y. W. Ng, T. Weil, *J. Am. Chem. Soc.* **2020**, *142*, 15780–15789, <https://doi.org/10.1021/jacs.0c05261>.
- [12] Y. Ren, Z. Zhou, K. Maxeiner, A. Kaltbeitzel, I. Harley, J. Xing, Y. Wu, M. Wagner, K. Landfester, I. Lieberwirth, T. Weil, D. Y. W. Ng, *J. Am. Chem. Soc.* **2024**, *146*, 11991–11999, <https://doi.org/10.1021/jacs.4c01279>.
- [13] I. Elamri, C. Abdellaoui, J. K. Bains, K. F. Hohmann, S. L. Gande, E. Stirnal, J. Wachtveitl, H. Schwalbe, *J. Am. Chem. Soc.* **2021**, *143*, 10596–10603, <https://doi.org/10.1021/jacs.1c02817>.
- [14] Y. Ren, Z. Zhou, I. Harley, Ö. Aydin, L. Driebehaus-Ortiz, A. Kaltbeitzel, J. Xing, K. Maxeiner, I. Lieberwirth, K. Landfester, D. Y. W. Ng, T. Weil, *Nat. Synth.* **2025**, *4*, 673–683, <https://doi.org/10.1038/s44160-025-00751-5>.
- [15] S. Chagri, K. Maxeiner, M. J. S. A. Silva, L. Förch, J. Link, P. Roth, R. Meyer, J. Fetzner, A. Kaltbeitzel, I. Lieberwirth, K. Landfester, D. Y. W. Ng, T. Weil, *Adv. Sci.* **2025**, *12*, 2412606, <https://doi.org/10.1002/advs.202412606>.
- [16] Y. Zhang, H. Chen, R. Li, K. Sterling, W. Song, *Signal Transduct. Target. Ther.* **2023**, *8*, 1–26.
- [17] S. Sieste, T. Mack, E. Lump, M. Hayn, D. Schütz, A. Röcker, C. Meier, K. Kaygisiz, F. Kirchhoff, T. P. J. Knowles, F. S. Ruggeri, C. V. Synatschke, J. Münch, T. Weil, *Adv. Funct. Mater.* **2021**, *31*, 2009382, <https://doi.org/10.1002/adfm.202009382>.
- [18] S. Bera, S. Guerin, H. Yuan, J. O'Donnell, N. P. Reynolds, O. Maraba, W. Ji, L. J. W. Shimon, P. A. Cazade, S. A. M. Tofail, D. Thompson, R. Yang, E. Gazit, *Nat. Commun.* **2021**, *12*, 2634, <https://doi.org/10.1038/s41467-021-22895-6>.
- [19] H. Yuan, P. Han, K. Tao, S. Liu, E. Gazit, R. Yang, *Research* **2019**, *2019*, DOI <https://doi.org/10.34133/2019/9025939>.
- [20] Y. Zhao, T. Ji, Y. Zhao, Y. Ding, J. Wang, R. Zhao, J. Lang, H. Qin, X. Liu, J. Shi, N. Tao, Z. Qin, G. Nie, *Angew. Chem. Int. Ed.* **2016**, *55*, 1050–1055.
- [21] A. N. Shirazi, R. K. Tiwari, D. Oh, A. Banerjee, A. Yadav, K. Parang, *Mol. Pharmaceutics* **2013**, *10*, 2008–2020.
- [22] A. Altunbas, S. J. Lee, S. A. Rajasekaran, J. P. Schneider, D. J. Pochan, *Biomaterials* **2011**, *32*, 5906–5914, <https://doi.org/10.1016/j.biomaterials.2011.04.069>.
- [23] C. Zhang, R. Shafi, A. Lampel, D. MacPherson, C. G. Pappas, V. Narang, T. Wang, C. Maldarelli, R. V. Ulijn, *Angew. Chem. Int. Ed.* **2017**, *56*, 14511–14515, <https://doi.org/10.1002/anie.201708036>.
- [24] M. Samanta, N. Saad, D. Wu, N. S. A. Crone, K. Abramov-Harpaz, C. Regev, R. Cohen-Luria, A. L. Boyle, Y. Miller,

- A. Kros, G. Ashkenasy, *Angew. Chem. Int. Ed.* **2024**, *64*, e202413810, <https://doi.org/10.1002/anie.202413810>
- [25] A. M. Ender, K. Kaygisiz, H. J. Räder, F. J. Mayer, C. V. Synatschke, T. Weil, *ACS Biomater. Sci. Eng.* **2021**, *7*, 4798–4808, <https://doi.org/10.1021/acsbomaterials.1c00889>
- [26] K. Kaygisiz, A. M. Ender, J. Gačanin, L. A. Kaczmarek, D. A. Koutsouras, A. N. Nalakath, P. Winterwerber, F. J. Mayer, H. J. Räder, T. Marszałek, P. W. M. Blom, C. V. Synatschke, T. Weil, *Macromol. Biosci.* **2023**, *23*, 2200294, <https://doi.org/10.1002/mabi.202200294>
- [27] X. Liu, M. Li, J. Liu, Y. Song, B. Hu, C. Wu, A. A. Liu, H. Zhou, J. Long, L. Shi, Z. Yu, *J. Am. Chem. Soc.* **2022**, *144*, 9312–9323, <https://doi.org/10.1021/jacs.2c01025>
- [28] H. Wang, Z. Feng, Y. Wang, R. Zhou, Z. Yang, B. Xu, *J. Am. Chem. Soc.* **2016**, *138*, 16046–16055, <https://doi.org/10.1021/jacs.6b09783>
- [29] A. Tanaka, Y. Fukuoka, Y. Morimoto, T. Honjo, D. Koda, M. Goto, T. Maruyama, *J. Am. Chem. Soc.* **2015**, *137*, 770–775, <https://doi.org/10.1021/ja510156v>
- [30] Z. Chen, M. Chen, K. Zhou, J. Rao, *Angew. Chem. Int. Ed.* **2020**, *59*, 7864–7870
- [31] M. R. Ghadiri, J. R. Granja, L. K. Buehler, *Nature* **1994**, *369*, 301–304, <https://doi.org/10.1038/369301a0>
- [32] A. Roy, J. B. Dodd-O, A. S. Robang, D. He, O. West, Z. Siddiqui, E. D. Aguas, H. Goldberg, A. Griffith, C. Heffernan, Y. Hu, A. K. Paravastu, V. A. Kumar, *ACS Appl. Mater. Interfaces* **2024**, *16*, 364–375, <https://doi.org/10.1021/acsami.3c15660>
- [33] S. Fleming, R. V. Ulijn, *Chem. Soc. Rev.* **2014**, *43*, 8150–8177, <https://doi.org/10.1039/C4CS00247D>
- [34] C. J. Bowerman, B. L. Nilsson, *Biopolymers* **2012**, *98*, 169–184, <https://doi.org/10.1002/bip.22058>
- [35] K. Kaygisiz, L. Rauch-Wirth, A. Dutta, X. Yu, Y. Nagata, T. Bereau, J. Münch, C. V. Synatschke, T. Weil, *Nat. Commun.* **2023**, *14*, 5121
- [36] J. Song, X. Mo, X. Liu, B. Hu, Z. Zhang, Z. Yu, *Macromol. Rapid Commun.* **2023**, *44*, 1–9, <https://doi.org/10.1002/marc.202300308>
- [37] K. Matsuura, H. Inaba, *Biophys. Rev.* **2023**, *4*, 041303, <https://doi.org/10.1063/5.0179171>
- [38] A. Mukherjee, M. D. Seyfried, B. J. Ravoo, *Angew. Chem. Int. Ed.* **2023**, *62*, e202304437, <https://doi.org/10.1002/anie.202304437>
- [39] K. Hüll, J. Morstein, D. Trauner, *Chem. Rev.* **2018**, *118*, 10710–10747
- [40] M. M. Lerch, M. J. Hansen, G. M. van Dam, W. Szymanski, B. L. Feringa, *Angew. Chem. Int. Ed.* **2016**, *55*, 10978–10999
- [41] W. A. Velema, W. Szymanski, B. L. Feringa, *J. Am. Chem. Soc.* **2014**, *136*, 2178–2191, <https://doi.org/10.1021/ja413063e>
- [42] A. A. Beharry, L. Wong, V. Tropepe, G. A. Woolley, *Angew. Chem. Int. Ed.* **2011**, *50*, 1325–1327
- [43] P. Stawski, M. Sumser, D. Trauner, *Angew. Chem. Int. Ed.* **2012**, *51*, 5748–5751
- [44] M. J. Hansen, W. A. Velema, G. De Bruin, H. S. Overkleeft, W. Szymanski, B. L. Feringa, *ChemBioChem* **2015**, *16*, 2053–2057
- [45] L. Albert, J. Nagpal, W. Steinchen, L. Zhang, L. Werel, N. Djokovic, D. Ruzic, M. Hoffarth, J. Xu, J. Kaspareit, F. Abendroth, A. Royant, G. Bange, K. Nikolic, S. Ryu, Y. Dou, L. O. Essen, O. Vázquez, *ACS Cent. Sci.* **2022**, *8*, 57–66, <https://doi.org/10.1021/acscentsci.1c00434>
- [46] M. Borowiak, W. Nahaboo, M. Reyniers, K. Nekolla, P. Jalinot, J. Hasseroth, M. Rehberg, M. Delattre, S. Zahler, A. Vollmar, D. Trauner, O. Thorn-Seshold, *Cell* **2015**, *162*, 403–411
- [47] E. R. Cotroneo, T. C. Cheng, L. Kaltschnee, P. Maibach, M. Engelke, N. Schwegler, F. Thomas, C. M. Thiele, N. Simeth, *ChemRxiv Prepr.* **2025**, <https://doi.org/10.26434/chemrxiv-2025-jclbt>
- [48] A. Aemissegger, V. Kräutler, W. F. Van Gunsteren, D. Hilvert, *J. Am. Chem. Soc.* **2005**, *127*, 2929–2936, <https://doi.org/10.1021/ja0442567>
- [49] R. Siewertsen, H. Neumann, B. Buchheim-Stehn, R. Herges, C. Näther, F. Renth, F. Temps, *J. Am. Chem. Soc.* **2009**, *131*, 15594–15595, <https://doi.org/10.1021/ja906547d>
- [50] Y. Liang, S. Ogawa, H. Inaba, K. Matsuura, *Front. Mol. Biosci.* **2023**, *10*, 1–11
- [51] J. R. Nilsson, C. P. Carvalho, S. Li, J. P. Da Silva, J. Andréasson, U. Pischel, *ChemPhysChem* **2012**, *13*, 3691–3699, <https://doi.org/10.1002/cphc.201200468>
- [52] N. Preußke, W. Moormann, K. Bamberg, M. Lipfert, R. Herges, F. D. Sönnichsen, *Org. Biomol. Chem.* **2020**, *18*, 2650–2660
- [53] L. Stricker, E. C. Fritz, M. Peterlechner, N. L. Doltsinis, B. J. Ravoo, *J. Am. Chem. Soc.* **2016**, *138*, 4547–4554, <https://doi.org/10.1021/jacs.6b00484>
- [54] L. Stricker, M. Böckmann, T. M. Kirse, N. L. Doltsinis, B. J. Ravoo, *Chem. Eur. J.* **2018**, *24*, 8639–8647, <https://doi.org/10.1002/chem.201800587>
- [55] C. E. Weston, R. D. Richardson, P. R. Haycock, A. J. P. White, M. J. Fuchter, *J. Am. Chem. Soc.* **2014**, *136*, 11878–11881, <https://doi.org/10.1021/ja505444d>
- [56] Y. Shiraishi, T. Oshima, T. Hirai, *J. Phys. Chem. B* **2024**, *128*, 8797–8806, <https://doi.org/10.1021/acs.jpcc.4c03894>
- [57] A. Paschold, N. Starke, S. Rothenmund, W. H. Binder, *Org. Lett.* **2024**, 0–5
- [58] T. Tellkamp, J. Shen, Y. Okamoto, R. Herges, *European J. Org. Chem.* **2014**, *2014*, 5456–5461, <https://doi.org/10.1002/ejoc.201402541>
- [59] D. Prischich, A. M. J. Gomila, S. Milla-Navarro, G. Sangüesa, R. Diez-Alarcia, B. Preda, C. Matera, M. Batlle, L. Ramírez, E. Giral, J. Hernandez, E. Guasch, J. J. Meana, P. de la Villa, P. Gorostiza, *Angew. Chem. Int. Ed.* **2021**, *60*, 3625–3631
- [60] S. Ludwanowski, M. Ari, K. Parison, S. Kalthoum, P. Straub, N. Pompe, S. Weber, M. Walter, A. Walther, *Chem. Eur. J.* **2020**, *26*, 13203–13212
- [61] M. Stolarek, A. Pycior, P. Bonarek, M. Opydo, E. Kolaczowska, K. Kamiński, A. Mogielnicki, K. Szczubialka, *J. Med. Chem.* **2023**, *66*, 1778–1789, <https://doi.org/10.1021/acs.jmedchem.2c01616>
- [62] R. Lahmy, H. Hübner, M. F. Schmidt, D. Lachmann, P. Gmeiner, B. König, *Chem. Eur. J.* **2022**, *28*, e202201515
- [63] J. Volarić, W. Szymanski, N. A. Simeth, B. L. Feringa, *Chem. Soc. Rev.* **2021**, *50*, 12377–12449
- [64] E. L. Bakota, O. Sensoy, B. Özgür, M. Sayar, J. D. Hartgerink, *Biomacromolecules* **2013**, *14*, 1370–1378, <https://doi.org/10.1021/bm4000019>
- [65] C. W. Chu, L. Stricker, T. M. Kirse, M. Hayduk, B. J. Ravoo, *Chem. Eur. J.* **2019**, *25*, 6131–6140, <https://doi.org/10.1002/chem.201806042>
- [66] S. Chagri, A. Burgstaller, C. Schirra, J. Link, Z. Zhou, P. Roth, R. Meyer, J. Fetzer, Y. Ren, S. Si, F. Mazzotta, M. Wagner, I. Lieberwirth, K. Landfester, D. Y. W. Ng, O. Staufer, T. Weil, *ChemRxiv Prepr.* **2024**, <https://doi.org/10.26434/chemrxiv-2024-3lxgk>
- [67] J. M. Estrella, A. Ortega, E. Obrador, *Crit. Rev. Clin. Lab. Sci.* **2006**, *43*, 143–181, <https://doi.org/10.1080/10408360500523878>
- [68] A. Perczel, K. Park, G. D. Fasman, *Proteins Struct. Funct. Bioinform.* **1992**, *13*, 57–69, <https://doi.org/10.1002/prot.340130106>
- [69] P. Roth, R. Meyer, I. Harley, K. Landfester, I. Lieberwirth, M. Wagner, D. Y. W. Ng, T. Weil, *Nat. Synth.* **2023**, *2*, 980–988, <https://doi.org/10.1038/s44160-023-00343-1>
- [70] Q. Xing, J. Zhang, Y. Xie, Y. Wang, W. Qi, H. Rao, R. Su, Z. He, *ACS Nano* **2018**, *12*, 12305–12314, <https://doi.org/10.1021/acsnano.8b06173>

- [71] M. C. Manning, M. Illangasekare, R. W. Woody, *Biophys. Chem.* **1988**, *31*, 77–86, [https://doi.org/10.1016/0301-4622\(88\)80011-5](https://doi.org/10.1016/0301-4622(88)80011-5).
- [72] A. Micsonai, F. Wien, L. Kernya, Y. H. Lee, Y. Goto, M. Réfrégiers, J. Kardos, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E3095–E3103, <https://doi.org/10.1073/pnas.1500851112>.
- [73] K. Wang, J. D. Keasling, S. J. Muller, *Int. J. Biol. Macromol.* **2005**, *36*, 232–240, <https://doi.org/10.1016/j.ijbiomac.2005.06.006>.
- [74] C. J. Bowerman, W. Liyanage, A. J. Federation, B. L. Nilsson, *Biomacromolecules* **2011**, *12*, 2735–2745, <https://doi.org/10.1021/bm200510k>.
- [75] B. P. Nowak, B. J. Ravoo, *Soft Matter* **2020**, *16*, 7299–7304, <https://doi.org/10.1039/D0SM00835D>.
- [76] H. Lin, L. Wang, X. Jiang, J. Wang, *Curr. Opin. Chem. Biol.* **2024**, *81*, 102505, <https://doi.org/10.1016/j.cbpa.2024.102505>.

Manuscript received: July 07, 2025

Revised manuscript received: October 28, 2025

Manuscript accepted: October 29, 2025

Version of record online: December 05, 2025

Supporting Information

Julian Link^[a], Luca Burg^[b], Sarah Chagri^[a], Ha-Chi Nguyen^[a], David Y.W. Ng^{*,[a]}, Bart Jan Ravoo^{*,[b]}, Tanja Weil^{*,[a]}

[a] J. Link, Dr. S. Chagri, H.-C. Nguyen, Dr. D.Y.W. Ng, Prof. Dr. T. Weil
Max Planck Institute for Polymer Research
Ackermannweg 10, 55128 Mainz, Germany
weil@mpip-mainz.mpg.de, ng@mpip-mainz.mpg.de

[b] L. Burg, Prof. Dr. B.J. Ravoo
Center for Soft Nanoscience
Universität Münster
Busso-Peus-Strasse 10, 48149 Münster, Germany
b.j.ravoo@uni-muenster.de

Contents

1. General Information	3
1.1 Materials	3
1.2 Instruments	3
1.2.1 Nuclear Magnetic Resonance (NMR) Spectroscopy	3
1.2.2 Liquid Chromatography-Mass Spectrometry (LCMS)	3
1.2.3 Transmission Electron Microscopy (TEM)	3
1.2.4 Microwave Peptide Synthesizer	3
1.2.5 High-Performance Liquid Chromatography (HPLC)	4
1.2.6 CD-Spectroscopy	4
1.2.7 Fluorescence Spectroscopy	4
1.2.8 Irradiation Setup	4
1.2.9 Dynamic Light Scattering (DLS)	4
1.2.10 Matrix-Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry (MALDI-TOF-MS)	4
1.2.11 2D Cell Culture	5
1.2.12 Luminescence Detection of Cell Viability	5
1.2.13 Confocal Laser Scanning Microscopy (CLSM)	5
1.2.14 Fluorescence Phase Contrast Microscopy	6
1.2.15 UV/Vis Spectroscopy	6
2. Synthesis and Structural Characterization	7
2.1 Synthesis of Compounds 1 and 2	7
2.2 TEM Analysis of 1 in different buffers	24
2.3 TEM Analysis of incubation of 2 with GSH	25
2.4 Determination of CAC	29
2.5 Chemical Design of Coassembly Approach	30
2.6 Coassembly Analysis	31
2.7 ¹ H-NMR analysis of 1 with light switching	32
2.8 LCMS Kinetic	33
2.9 Confocal Laser Scanning microscopy of 2 at various concentrations	35
References	35

1. General Information

1.1 Materials

Reagents and solvents were purchased from commercial sources and were used without further purification. Peptide synthesis grade reagents were used for synthesizing the peptides. HPLC was performed using acetonitrile (CH₃CN) in HPLC grade and water obtained from a Millipore purification system. Flash column chromatography was carried out using Macherey-Nagel silica gel 0.04–0.063 mm.

1.2 Instruments

1.2.1 Nuclear Magnetic Resonance (NMR) Spectroscopy

NMR spectra of small molecules and peptides were recorded on a Bruker Avance III 700 MHz spectrometer and a Bruker Avance 400 MHz spectrometer. The solvent signal was used as a reference (deuterated chloroform CDCl₃ δ =7.26 ppm for ¹H, δ =77.16 ppm for ¹³C, DMSO-d₆ δ =2.50 ppm for ¹H and δ =39.52 ppm for ¹³C). All ¹H resonances are reported to the nearest 0.01 ppm. The multiplicity of ¹H signals are indicated as: s = singlet; d = doublet; t = triplet; q = quartet; p = pentet; m = multiplet; br = broad; or combinations of thereof. Coupling constants (J) are quoted in Hz and reported to the nearest 0.1 Hz. Where appropriate, averages of the signals from peaks displaying multiplicity were used to calculate the value of the coupling constant. Chemical shifts for protons of the compounds were assigned on the basis of COSY and HSQC correlations. The data was processed in MestReNova.

1.2.2 Liquid Chromatography-Mass Spectrometry (LCMS)

Compounds were analyzed by HPLC-ESI-MS on a LC-MS 2020 by Shimadzu using a Kinetex 2.6 μ m EVO C18 100 Å LC 50 $\times$ 2.1 mm column. MilliQ water acidified with 0.1% formic acid and CH₃CN were used as solvents for all measurements. The solvent gradient started with 5% CH₃CN and 95% water. This solvent ratio was kept constant for 2 min, then the CH₃CN content was linearly increased to 95% in 14 min. Data were processed in LabSolutions and Origin 2024b.

1.2.3 Transmission Electron Microscopy (TEM)

TEM images of the peptide samples were recorded on a JEOL 1400 transmission electron microscope at a voltage of 120 kV. Formvar/carbon-film coated copper grids (300 mesh) by Plano GmbH were used to prepare the samples. The images were processed in Fiji ImageJ.

1.2.4 Microwave Peptide Synthesizer

Peptides were synthesized in a Liberty Blue Automated Microwave Peptide Synthesizer by CEM Corporation.

1.2.5 High-Performance Liquid Chromatography (HPLC)

The synthesized peptides were purified by preparative HPLC using a Shimadzu setup. A YMC-Actus Triart C18 column (150 x 20 mm, 5 μ m) with a flowrate of 20 mL/min was used for purification. For analytical measurements an Atlantis T3 column (4.6 x 100 mm, 5 μ m) was used at a flowrate of 1 mL/min. All purification steps were performed by using gradients of MilliQ water and acetonitrile, each acidified with 0.1% formic acid. Analytical measurements were performed by using gradients of MilliQ water and acetonitrile, each acidified with 0.1% trifluoroacetic acid. Absorbance was recorded at 214, 254, and 400 nm wavelength. The HPLC data were processed with the software LabSolutions by Shimadzu and Origin 2024b by OriginLab.

1.2.6 CD-Spectroscopy

CD spectra of the peptides were recorded on a JASCO J-1500 spectrometer in a 0.1 cm High Precision Cell by HellmaAnalytics. The recorded data were processed in Spectra Analysis by JASCO and Origin 2024b by OriginLab.

1.2.7 Fluorescence Spectroscopy

To record the fluorescence intensity, a SPARK 20M microplate reader by Tecan Group Ltd. was used. The samples were measured in a Greiner 384 flat black well plate and data was processed in Origin 2024b by OriginLab.

1.2.8 Irradiation Setup

Irradiations were performed either with a Thorlabs M365L3 lamp with a nominal wavelength of 365 nm, a bandwidth (FWHM) of 9 nm and a current of 1000 mA or with a Avonec 3 W Power LED with a wavelength of 515-525 nm, a current of 750 mA and a voltage of 3.5-4.5 V.

1.2.9 Dynamic Light Scattering (DLS)

Single-angle DLS measurements were performed at 25 °C using a Malvern ZetaSizer Nano S purchased from Malvern Instruments Ltd. (Malvern, Great Britain) with a He/Ne Laser (λ = 633 nm) at a fixed scattering angle of 173°. All measurements were performed in triplicate. The obtained data was processed by cumulant fitting for D_h .

1.2.10 Matrix-Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry (MALDI-TOF-MS)

MALDI-TOF spectra were recorded on either a rapifleX MALDI-TOF/TOF from Bruker or MALDI Synapt G2-SI from Waters. Samples were mixed with a saturated solution of the matrix S7 α -cyano-4-hydroxycinnamic acid (CHCA) in NH_4HCO_3 buffer (20 mM)/ CH_3CN 1/1. Data processing was performed in mMass.

1.2.11 2D Cell Culture

A549 cells were cultured at 37 °C and 5% CO₂ in Dulbecco's Modified Eagle's Medium (DMEM, high glucose), supplemented with 10% fetal bovine serum (FBS). The cells were cultured in T75 culture flask and subcultivated two to three times per week.

1.2.12 Luminescence Detection of Cell Viability

A549 lung carcinoma cells were seeded into two 96-well half-area white plates (Greiner Bio-One, Cat# 675083) at a density of 2,500 cells per well in 50 µL of DMEM (Gibco, Cat# 11965092) supplemented with 10% FBS (Gibco, Cat# A5256801). Cells adhered overnight at 37°C in a humidified incubator with 5% CO₂.

Following incubation, the culture medium was carefully replaced with compound working solutions, prepared in DMEM with 10% FBS, including either **2_{trans}** or **2_{cis}** at concentrations ranging from 1 µM to 500 µM. Each solution contained 1% DMSO. Control wells received medium containing no compounds. Cells were exposed to the respective treatments for 4 hours.

Cell viability was assessed using the CellTiter-Glo Assay (Promega, Cat# G7571), which quantifies intracellular ATP as a reference for metabolically active cells. The assay relies on a thermostable luciferase that catalyzes the mono-oxygenation of luciferin to oxyluciferin in the presence of ATP, Mg²⁺, and O₂, producing a luminescent signal proportional to ATP levels.

50 µL of CellTiter-Glo reagent was added to each well. Plates were shaken gently for 2 minutes on an orbital shaker in the dark, followed by a period of incubation at room temperature without agitation, also shielded from light. Luminescence was recorded using a Tecan Spark 20M spectrophotometer. Data were processed in Microsoft Excel 2016 and graphs were generated in Origin 2024b.

1.2.13 Confocal Laser Scanning Microscopy (CLSM)

Cells were cultured in an ibidi µ-Slide 8 well at a density of 20,000 cells per well in DMEM and allowed to adhere overnight at 37°C and 5% CO₂. The medium was aspirated and samples were introduced to each well after dissolving them in DMEM at the respective concentrations. Incubation was performed at 37°C and 5% CO₂. Finally, cells were co-stained with MitoTracker Orange CMTMRos (Thermo Fisher Scientific).

Cells were imaged under physiological conditions using an incubator-equipped Leica Stellaris® 8 microscope (40x glycerol objective, HC PL APO CS2 40x/1.25 GLYC) with fast lifetime contrast (FALCON) module (Leica Microsystem GmbH). The incubator (okolab) is set and held constant at 37°C, 5% CO₂ and a relative humidity of 90% throughout all measurements.

Samples were excited using a 40 MHz pulsed white light laser tuned to 649 nm for intensity measurements. Emitted photons were detected using HyD[®] R detector with a filter window at 658-834 nm. Imaging was conducted with living cells with a scanning resolution of 1024 x 1024 pixels at 400 Hz for each image.

Experimental Preparation

A549 cells were seeded at a density of 20,000 cells per well in an 8-well chamber imaging slide. After adhering for 24 h, cells were treated with the sample for 4 h at 37°C. The samples were dissolved in DMSO at a concentration of 10 mM. Sample solutions were further diluted to an end concentration of 25, 50, 100 and 200 µM with DMEM and added to the cells (total DMSO content = 1.00 %). Cells were then co-stained with MitoTracker[®] Orange CMTMRos (Thermo Fisher Scientific) for 10 min.

1.2.14 Fluorescence Phase Contrast Microscopy

A 6-well plate was used to culture cells at a density of 80,000 cells per well in DMEM. After cells adhered overnight at 37 °C and 5% CO₂, the medium was replaced by samples after dissolving them in DMEM at the respective concentrations. Incubation was also performed at 37°C and 5% CO₂. Finally, cells were co-stained with MitoTracker Orange CMTMRos (Thermo Fisher Scientific).

Cells were imaged using a KEYENCE BZ-X810 microscope with a CCD-camera (Plan Fluorit 20x LD PH, NA 0.45 WD 8.8-7.5 mm Phase Contrast). Samples were illuminated using a 40 W LED light and using a Cy5 fluorescence filter cube (Ex: 620/60, Em: 700/75 nm).

Experimental Preparation

A549 cells were seeded at a density of 80,000 cells per well in an 6-well plate. After adhering for 24 h, cells were treated with the sample for 4 h at 37 °C. The samples were dissolved in DMSO at a concentration of 10 mM. Sample solutions were further diluted to an end concentration of 100 µM with DMEM and added to the cells (total DMSO content = 1.00 %). Cells were then co-stained with MitoTracker[®] Orange CMTMRos (Thermo Fisher Scientific) for 10 min.

1.2.15 UV/Vis Spectroscopy

UV/vis absorption spectra were recorded with a JASCO V-770 double-beam spectrophotometer at 20 °C using a 10 x 10 mm quartz cuvette by HellmaAnalytics. The recorded data were processed in Origin 2024b by OriginLab.

2. Synthesis and Structural Characterization

2.1 Synthesis of Compounds 1 and 2

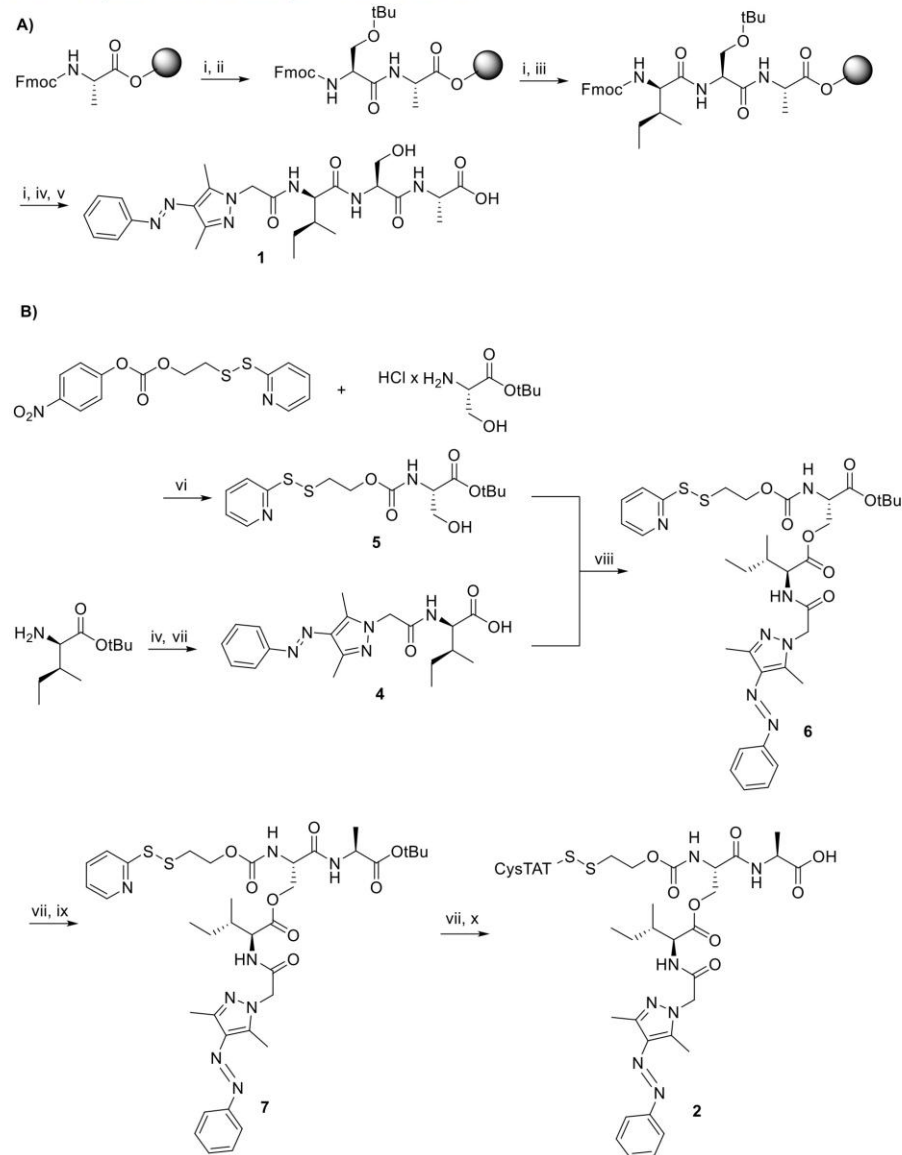
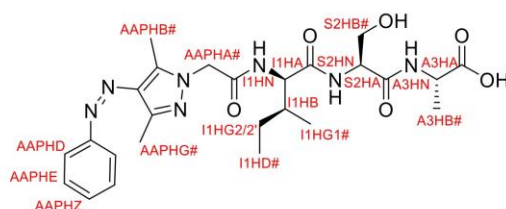


Figure S1: Synthesis of compounds **1** and **2**. **A)** Synthesis of **2**. (i) 20% Piperidine in DMF, 2x 10 min, RT. (ii) Oxyma, DIC, Fmoc-Ser(OTBu)-OH. (iii) Oxyma, DIC, Fmoc-Ile-OH. (iv) Oxyma, DIC, AAP. (v) TFA (95%), TIPS (2.5%), H₂O (2.5%). **B)** Synthesis of **1-TAT**. (vi) 2,2'-dipyridyldisulfide, MeOH, AcOH, 24h, RT. (vii) 4-nitrophenyl chloroformate, TEA, THF, 2 h, 0°C – RT. (viii) H₂N-Ser(OH)-OtBu, DIPEA, DCM, overnight, RT. (ix) TFA(50%) in DCM, 2 h, RT. (x) DIC, DMAP, DCM, overnight, RT. (xi) Fmoc-Ala-OH, PyBOP, DIPEA, overnight, RT. (xii) CysTAT, MeOH, overnight, RT.

The synthesis of the linear tripeptide **1** was carried out using Fmoc solid-phase peptide synthesis on an alanine-preloaded Wang resin using *N,N*-diisopropylcarbodiimide (DIC) and ethyl (2*Z*)-2-cyano-2-(hydroxyimino)acetate (OxymaPure) for the iterative coupling steps (Figure S1A). The photoswitch carboxylic acid was synthesized in a four-step reaction sequence starting from aniline as described in a previous report.^[50] It was then coupled to the ISA peptide on the solid support using DIC and OxymaPure, cleaved off from the resin with trifluoroacetic acid (TFA):triisopropyl silane (TIPS):H₂O (95:2.5:2.5) and purified by high-performance liquid chromatography (HPLC) to yield **1**.

The synthesis of the photoswitchable isotriptide **2** was carried out in solution (Figure S1B). The AAP photoswitch was attached to the *N*-terminus of isoleucine using DIC and OxymaPure, while a disulfide linker prepared from 2-mercaptoethanol was conjugated on the *N*-terminus of serine. Then, both fragments were combined via a Steglich esterification. The C-terminus was prolonged by alanine before the 2-mercaptopyridine substituent was replaced via a disulfide exchange reaction with Cys-TAT, yielding the bioactive molecule **2**, which was characterized by matrix-assisted laser desorption/ionization mass spectrometry (MALDI) and HPLC (Figure S20).



The Wang resin preloaded with Fmoc-Ala (0.5 mmol) was swollen in 5 mL DMF at room temperature for 1 h before transferring it into the peptide synthesizer. DMF was removed via a draining process and the resin swollen with 20 mL DMF for 20 s. After draining of the DMF, the Fmoc group was removed with 10 mL 20 % v/v piperidine in DMF and a heating cycle at 70°C for 25 s followed by another cycle at 90°C for 65 s. The solution was drained, and the resin washed with DMF three times. After the last washing step, Fmoc-Ser(tBu)-OH (0.2 M in DMF, 10 mL), DIC solution (0.5 M in DMF, 4 mL) and OxymaPure solution (1.0 M in DMF, 2 mL) was added to the resin and the solution heated to 70°C for 30 s followed by a heating cycle to 90°C for 120 s. The solution was drained, the Fmoc group removed as described before and washed with DMF three times. After the last washing step, Fmoc-Ile-OH (0.2 M in DMF, 10 mL), DIC solution (0.5 M in DMF, 4 mL) and OxymaPure solution (1.0 M in DMF, 2 mL) was added to the resin and the solution heated to 70°C for 30 s followed by a heating cycle to 90°C for 120 s. The solution was drained, and the resin washed with DMF three times.

The AAP photoswitch (171 mg, 0.66 mmol, 1.3 eq) was then coupled to the free *N*-terminus by addition of DIC (189 mg, 1.5 mmol, 3 eq) and Oxyma (426 mg, 3 mmol, 6 eq) in DMF (10 mL) by shaking at room temperature overnight. The resin was then washed with DMF (10 mL, 3x) and DCM (10 mL, 3x), drained and cleaved from the resin by adding 10 mL of cleavage cocktail (95% TFA, 2.5% TIPS, 2.5% MilliQ water) to the resin and shaking at room temperature for 2 h. The cleavage cocktail was drained and the liquids removed under reduced pressure. The peptide was purified via HPLC with a YMC-Actus Triart C18 column (flowrate 20 mL/min). After lyophilisation, the product Fmoc-ICA was received as a yellow solid (85 mg, 0.16 mmol, 32%).

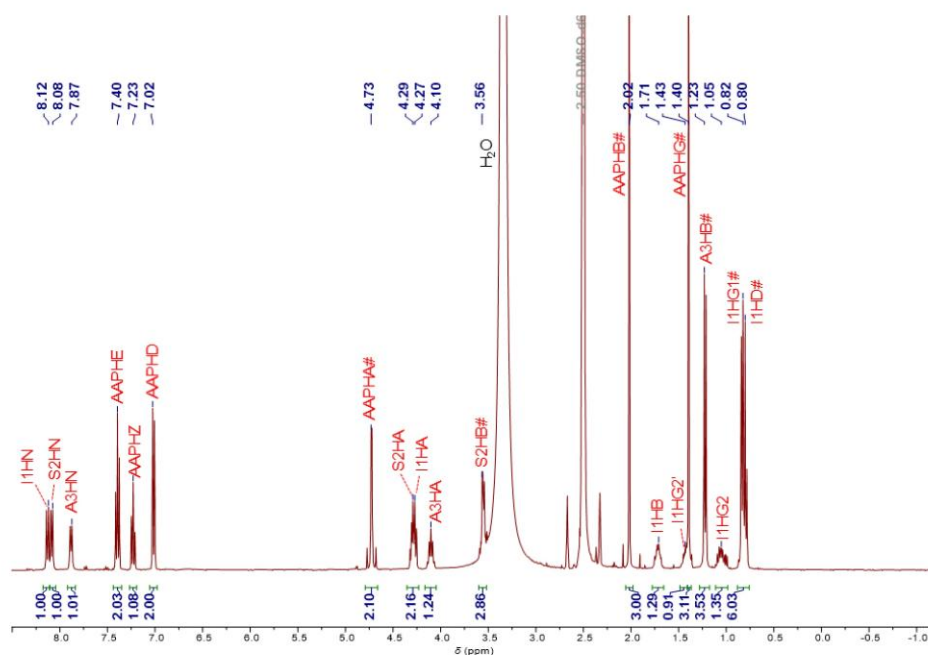


Figure S2: ¹H-NMR (400 MHz, DMSO-d₆, 298 K) of **1_{cis}**. Peak Assignments were made with comparison of subsequent 2D-NMR-spectra. ¹H NMR (400 MHz, DMSO-d₆) δ = 8.12 (d, 1H), 8.08 (d, 1H), 7.87 (d, 1H), 7.40 (t, 2H), 7.23 (tt, 1H), 7.02 (dd, 2H), 4.71 (q, 2H), 4.35 – 4.22 (m, 2H), 4.10 (p, 1H), 3.55 (dd, 2H), 2.02 (s, 3H), 1.78 – 1.65 (m, 1H), 1.49 – 1.41 (m, 1H), 1.40 (s, 3H), 1.22 (d, 3H), 1.11 – 0.97 (m, 1H), 0.89 – 0.73 (m, 6H).

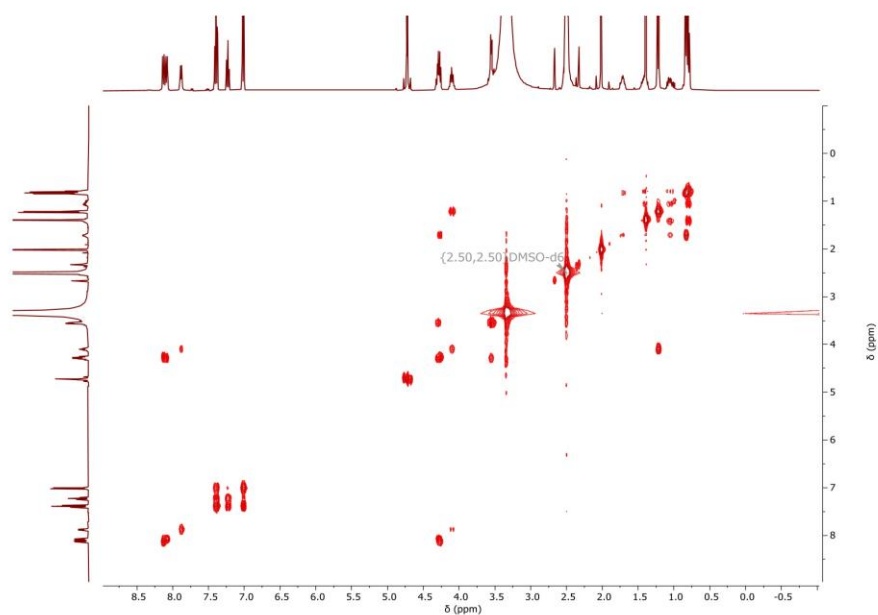


Figure S3: ^1H , ^1H -COSY NMR spectrum (400, 400 MHz, DMSO- d_6 , 298 K) of 1_{cis} . ^1H - ^1H NMR ((400, 400) MHz, DMSO- d_6) δ (8.14 4.27), (8.14 8.11), (8.09 4.27), (8.09 8.05), (7.89 7.87), (7.89 4.09), (7.41 7.01), (7.39 7.20), (7.39 7.38), (7.24 7.38), (7.24 7.01), (7.22 7.20), (7.02 7.38), (7.02 7.01), (4.73 4.73), (4.30 8.08), (4.29 3.54), (4.28 1.71), (4.27 4.27), (4.27 8.13), (4.10 1.21), (4.10 4.09), (4.10 7.88), (3.56 3.54), (3.55 4.27), (2.01 2.01), (1.72 4.27), (1.72 0.79), (1.71 1.04), (1.71 1.40), (1.71 1.71), (1.41 0.79), (1.41 1.04), (1.40 1.71), (1.39 1.40), (1.23 1.22), (1.23 4.09), (1.06 1.71), (1.05 0.79), (1.05 1.04), (1.04 1.40), (0.83 1.70), (0.82 0.79), (0.80 1.04), (0.79 0.79).

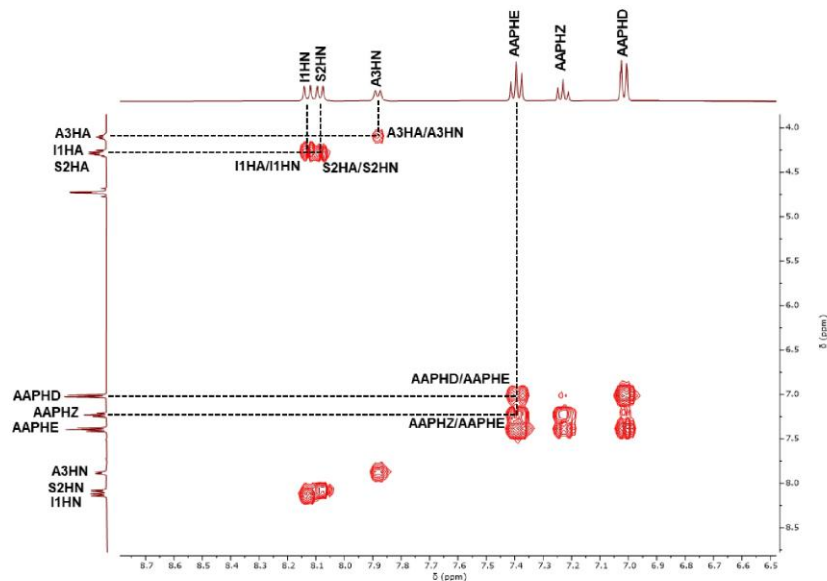


Figure S4: ^1H , ^1H -COSY NMR spectrum (400, 400 MHz, DMSO- d_6 , 298 K) of 1_{cis} . Zoom of fingerprint region relevant for interactions of the amide protons with the α -protons of the amino acids.

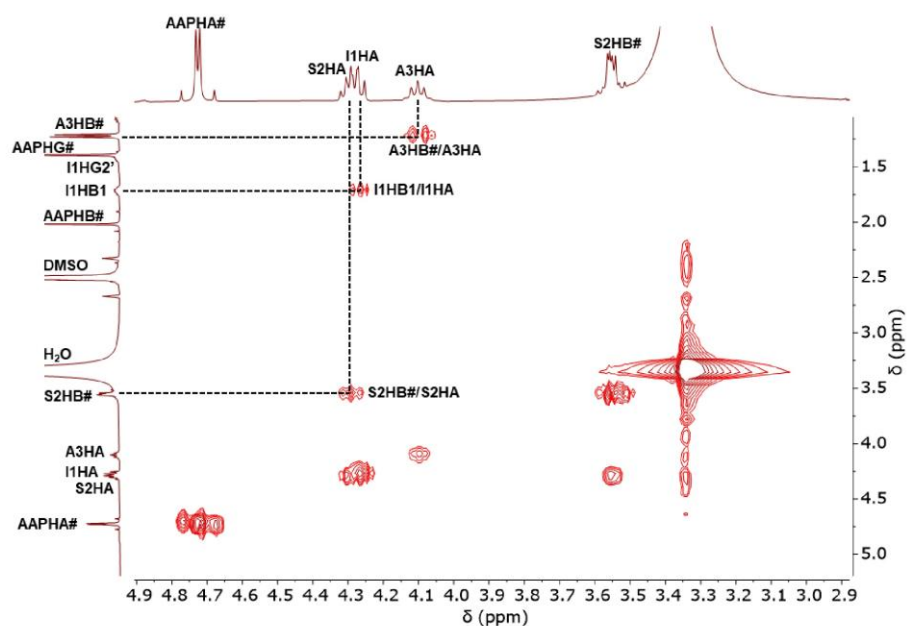


Figure S5: ^1H , ^1H -COSY NMR spectrum (400, 400 MHz, DMSO- d_6 , 298 K) of 1_{cis} . Zoom of region with the chemical shift of 5–3 ppm, 5–1 ppm.

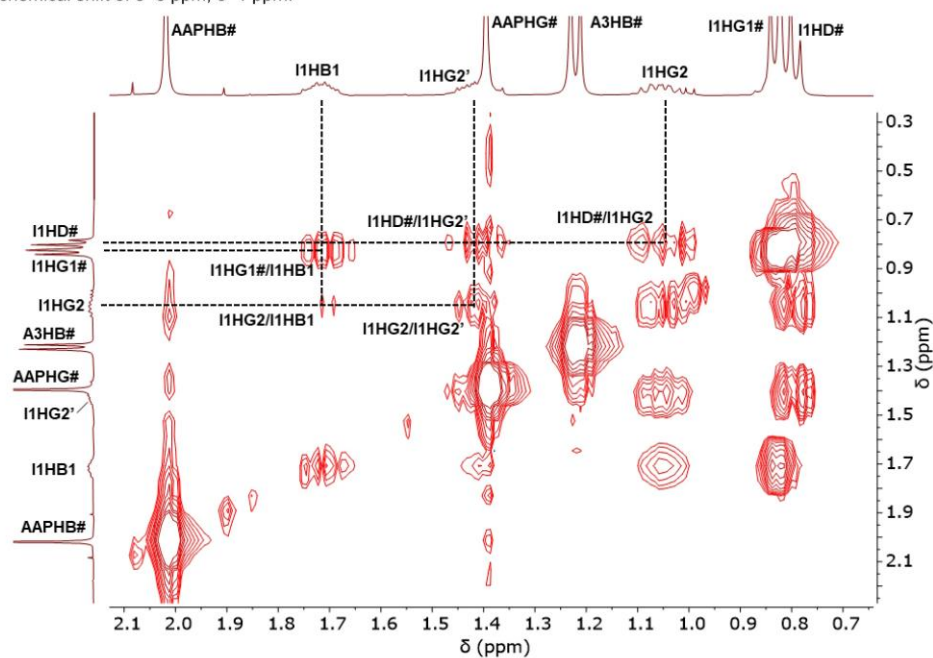


Figure S6: ^1H , ^1H -COSY NMR spectrum (400, 400 MHz, DMSO- d_6 , 298 K) of 1_{cis} . Zoom of region with the chemical shift of 2–0.5 ppm, 2–0 ppm.

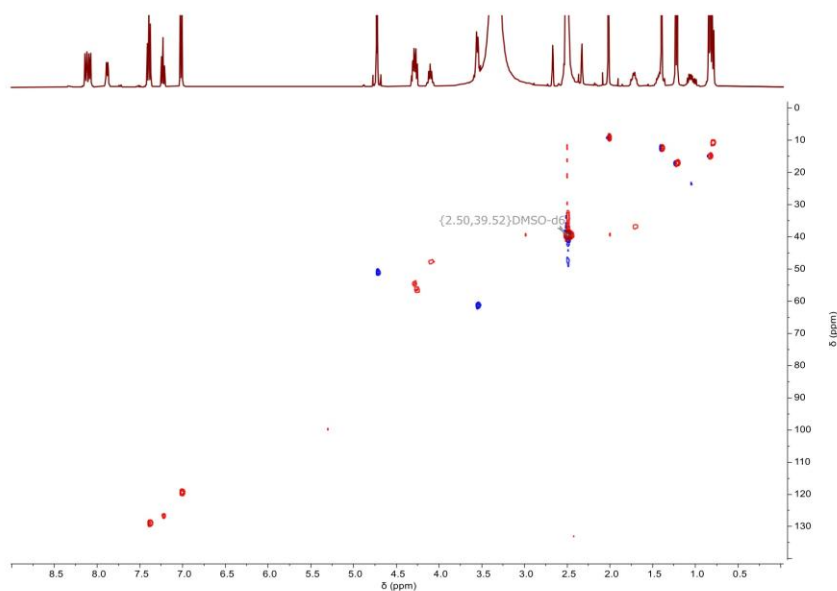


Figure S7: ^1H , ^{13}C -HSQC NMR spectrum (400, 101 MHz, DMSO-d_6 , 298 K) of **1_{cis}**. Red colour indicates CH/ CH_3 and blue colour indicates CH_2 . ^1H - ^{13}C NMR ((400, 101) MHz, DMSO-d_6) δ (7.40 129.35), (7.23 127.07), (7.01 119.68), (4.73 51.30), (4.29 54.99), (4.28 56.92), (4.10 47.96), (3.55 61.67), (2.02 9.46), (1.71 37.23), (1.44 23.87), (1.40 12.80), (1.22 17.48), (1.06 24.40), (0.82 15.26), (0.81 11.22).

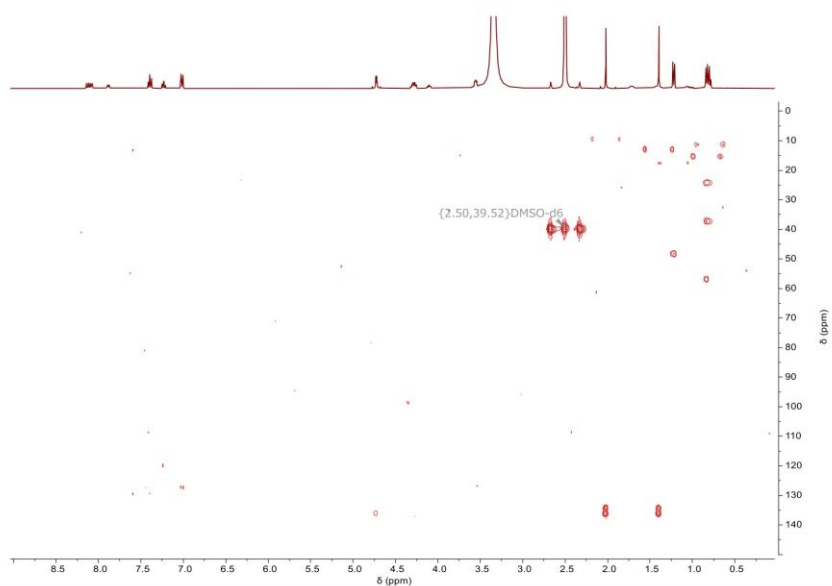


Figure S8: ^1H , ^{13}C -HMBC NMR spectrum (400, 101 MHz, DMSO-d_6 , 298 K) of **1_{cis}**. ^1H - ^{13}C NMR ((400, 101) MHz, DMSO-d_6) δ (7.24 119.92), (7.02 127.18), (4.72 136.09), (4.72 166.09), (2.17 9.52), (2.02 134.21), (2.02 136.09), (1.85 9.52), (1.55 12.80), (1.40 134.45), (1.40 136.09), (1.23 12.80), (1.22 174.04), (1.21 48.19), (1.05 17.49), (0.98 15.38), (0.95 11.16), (0.83 24.28), (0.83 37.18), (0.83 56.87), (0.66 15.38), (0.63 11.39).

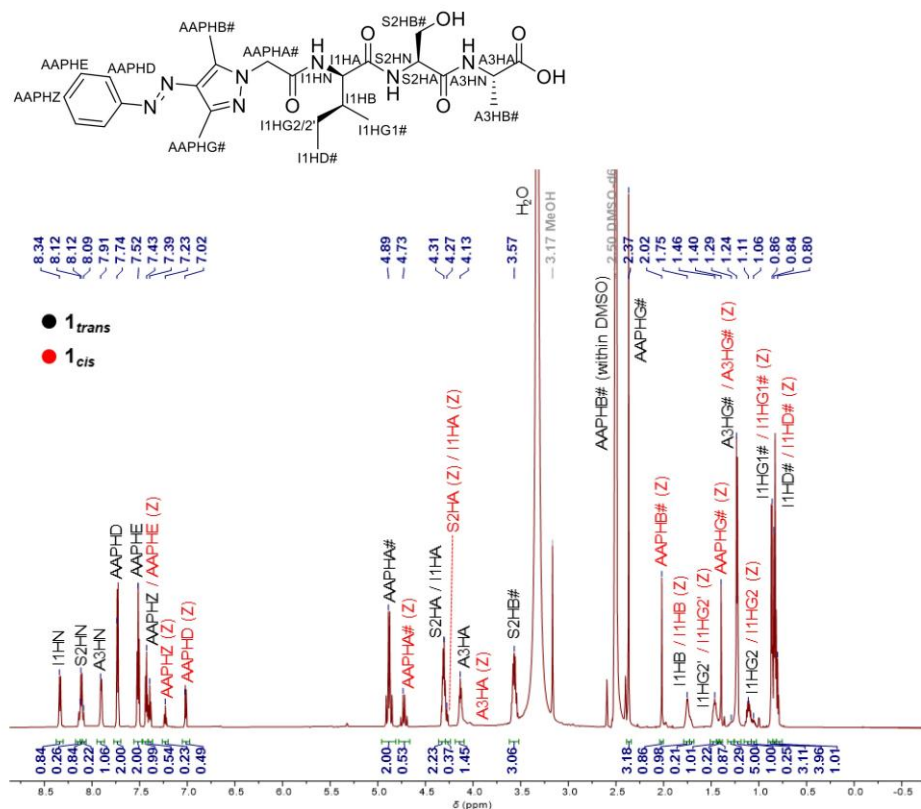


Figure S9: ¹H-NMR (700 MHz, DMSO-d₆, 298 K) of native **1**_{trans}. Peak Assignments were made with comparison of subsequent 2D-NMR-spectra. As described in the main text, the native state of compound **1** consists of approximately 85% **1**_{trans} and 15% **1**_{cis}. The resulting peaks of both isomers are assigned with different colours: black = **1**_{trans}, red = **1**_{cis}. ¹H NMR (700 MHz, DMSO-d₆) δ = 8.34 (d, 1H), 8.12 (d, 1H), 8.11 (d, 0H), 7.91 (d, 1H), 7.74 (d, 2H), 7.52 (t, 2H), 7.43 (t, 1H), 7.39 (t, 1H), 7.23 (t, 0H), 7.02 (d, 0H), 4.89 (q, 2H), 4.72 (q, 1H), 4.36 – 4.25 (m, 3H), 4.18 – 4.08 (m, 2H), 3.64 – 3.49 (m, 3H), 2.37 (s, 3H), 2.02 (s, 1H), 1.79 – 1.70 (m, 1H), 1.51 – 1.44 (m, 1H), 1.40 (s, 1H), 1.23 (d, 5H), 1.16 – 1.08 (m, 1H), 0.90 – 0.76 (m, 8H).

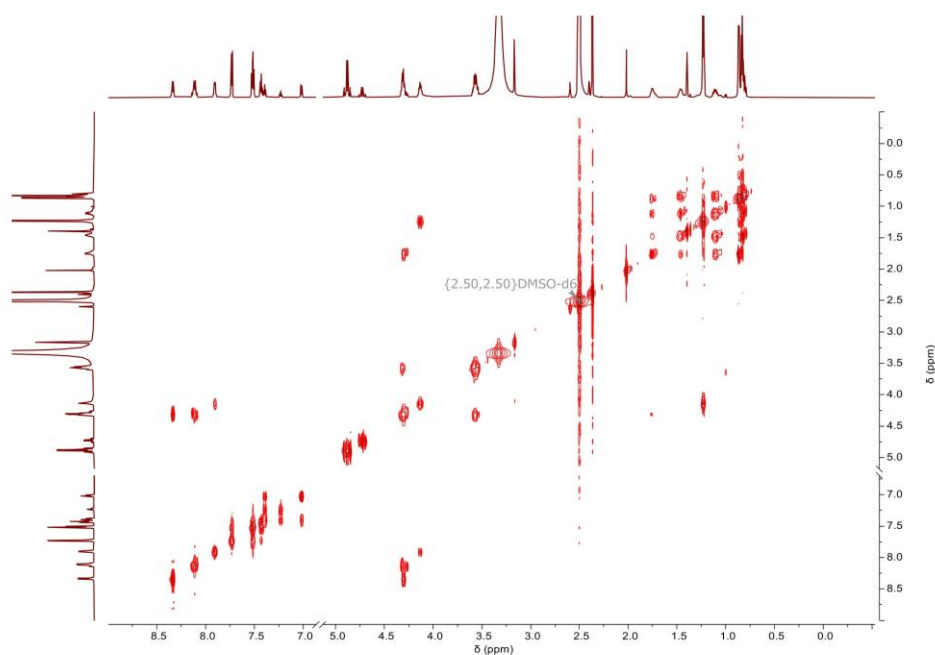


Figure S10: ^1H , ^1H -COSY NMR spectrum (700, 700 MHz, DMSO-d_6 , 298 K) of native **1_{trans}**. ^1H - ^1H NMR ((700, 700) MHz, DMSO-d_6) δ = (8.34 8.35), (8.34 4.31), (8.13 8.12), (8.12 4.31), (8.10 8.12), (8.10 4.31), (7.91 7.94), (7.91 4.13), (7.73 7.53), (7.72 7.76), (7.52 7.41), (7.51 7.71), (7.51 7.53), (7.44 7.71), (7.43 7.41), (7.40 7.00), (7.39 7.24), (7.39 7.41), (7.24 7.41), (7.23 7.24), (7.01 7.00), (7.01 7.41), (4.89 4.89), (4.72 4.72), (4.31 1.79), (4.31 8.12), (4.30 3.60), (4.30 4.31), (4.29 8.35), (4.14 1.26), (4.13 4.13), (4.13 7.94), (3.57 3.60), (3.56 4.31), (2.36 2.37), (2.02 2.02), (1.76 0.85), (1.76 1.49), (1.76 4.31), (1.75 1.14), (1.75 1.79), (1.46 0.85), (1.46 1.14), (1.46 1.49), (1.45 1.79), (1.23 4.13), (1.23 1.26), (1.10 0.85), (1.10 1.14), (1.10 1.49), (1.10 1.79), (0.87 0.85), (0.86 1.79), (0.84 1.49), (0.84 1.73), (0.83 0.85), (0.83 1.14).

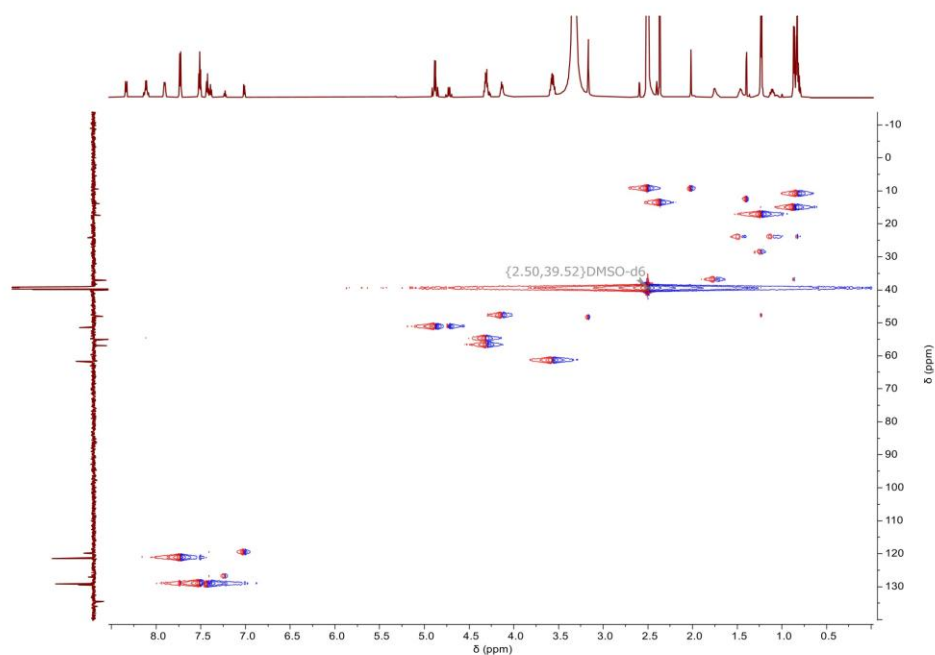


Figure S11: ^1H , ^{13}C -HSQC NMR spectrum (700, 176 MHz, DMSO-d_6 , 298 K) of native **1_{trans}**. ^1H - ^{13}C NMR ((700, 176) MHz, DMSO-d_6) δ = (7.74 121.11), (7.74 128.85), (7.52 128.85), (7.42 129.14), (7.24 126.80), (7.01 119.51), (4.90 51.05), (4.71 51.05), (4.33 54.70), (4.32 56.60), (4.12 47.70), (3.55 61.27), (2.50 9.16), (2.36 13.54), (2.01 9.31), (1.76 36.99), (1.46 23.88), (1.39 12.52), (1.23 28.57), (1.22 17.04), (1.09 23.90), (0.88 15.00), (0.85 10.77).

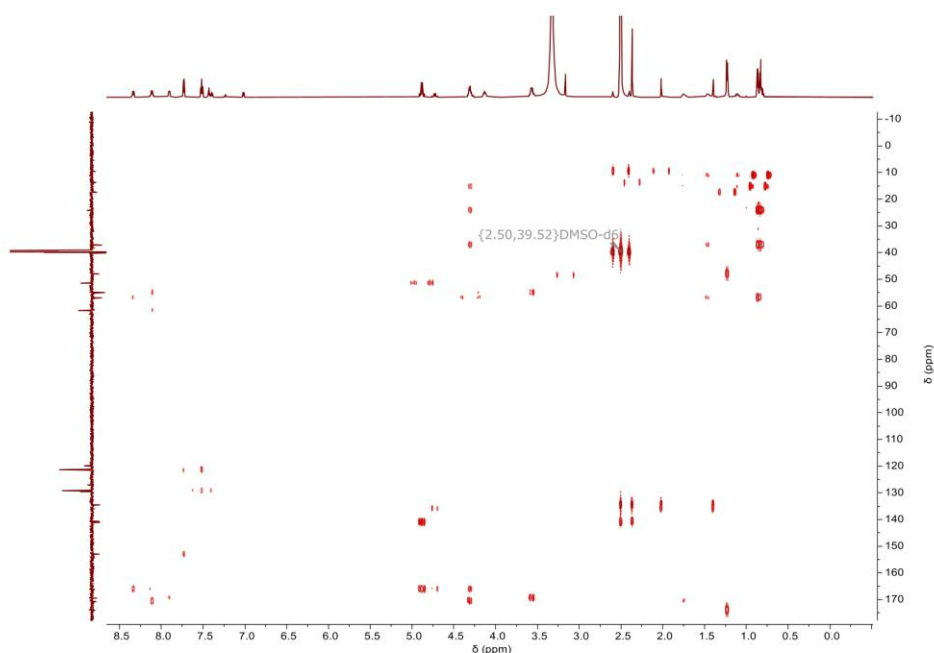


Figure S12: ^1H , ^{13}C -HMBC NMR spectrum (700, 176 MHz, DMSO-d_6 , 298 K) of native **1_{trans}**. ^1H - ^{13}C NMR ((700, 176) MHz, DMSO-d_6) δ = (8.34 56.74), (8.33 165.92), (8.11 170.59), (8.11 54.99), (7.90 169.42), (7.74 121.26), (7.73 152.78), (7.51 129.14), (7.41 128.85), (7.40 119.80), (4.89 140.82), (4.88 165.92), (4.88 50.62), (4.73 136.40), (4.73 165.98), (4.32 165.92), (4.31 36.89), (4.31 170.30), (4.31 55.77), (4.30 24.05), (4.29 15.29), (3.57 54.70), (3.57 169.13), (2.37 134.39), (2.36 140.52), (2.02 135.85), (1.76 170.59), (1.75 24.05), (1.74 10.91), (1.73 15.29), (1.47 10.91), (1.45 56.74), (1.45 37.18), (1.41 133.87), (1.23 173.80), (1.23 47.69), (1.23 16.94), (1.11 36.89), (1.11 10.91), (1.10 55.81), (0.87 36.89), (0.86 16.94), (0.86 24.05), (0.86 56.74), (0.84 24.05), (0.83 36.89), (0.83 10.38).

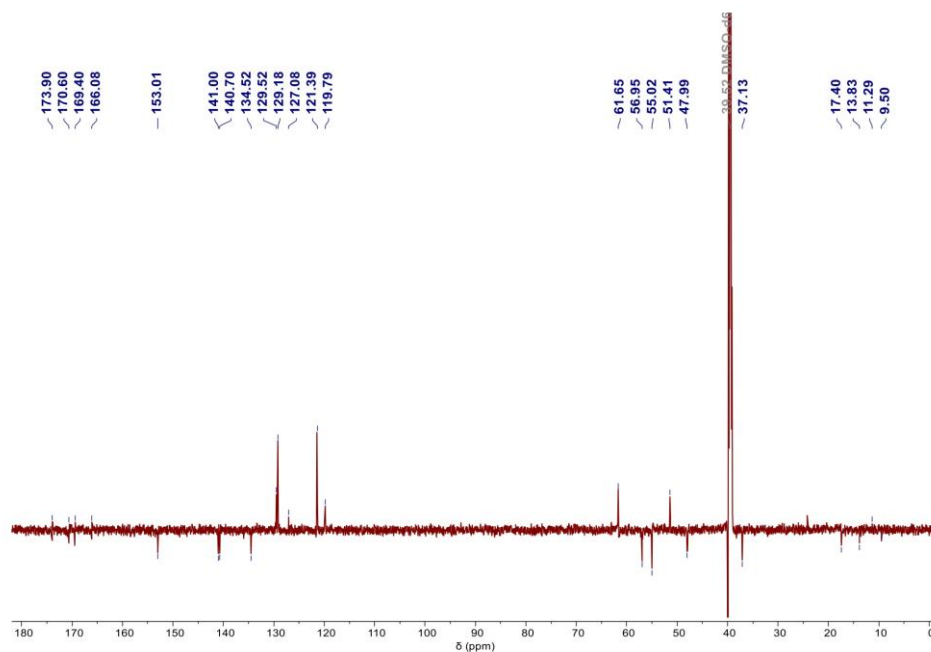
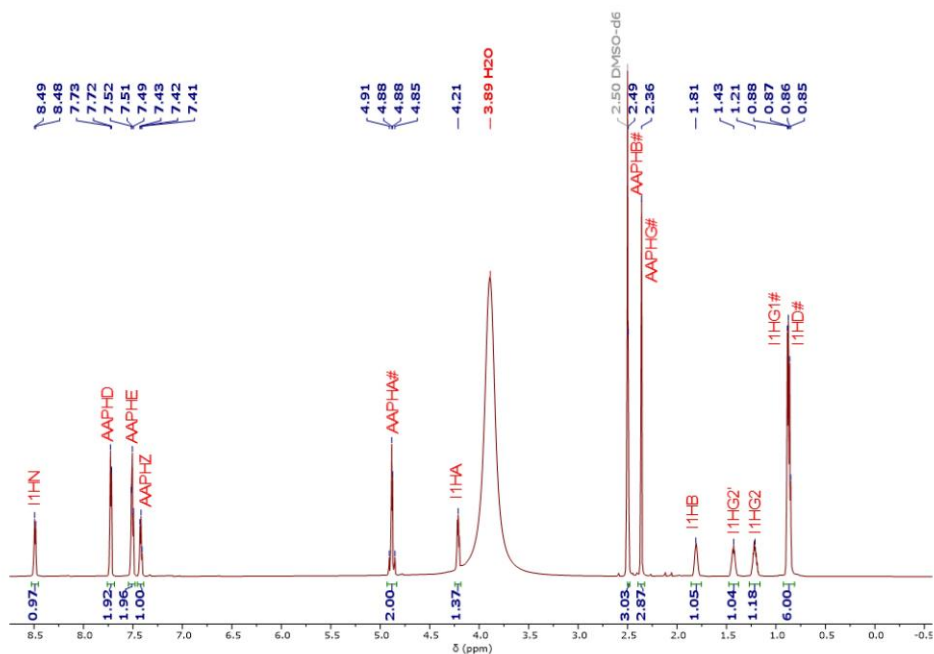


Figure S13: ^{13}C -NMR spectrum (176 MHz, DMSO-d_6 , 298 K) of native **1_{trans}**. ^{13}C NMR (176 MHz, DMSO-d_6) δ = 173.90, 170.60, 169.40, 166.08, 153.01, 141.00, 140.70, 134.52, 129.52, 129.18, 127.08, 121.39, 119.79, 61.65, 56.95, 55.02, 51.41, 47.99, 37.13, 17.40, 13.83, 11.29, 9.50.



¹H NMR (700 MHz, DMSO-*d*₆, 298 K) δ 8.49 (d, *J* = 8.1 Hz, 1H), 7.72 (d, *J* = 7.2 Hz, 2H), 7.51 (t, *J* = 7.4 Hz, 2H), 7.42 (t, *J* = 7.2 Hz, 1H), 4.88 (q, *J* = 5.9 Hz, 2H), 4.23–4.19 (m, 1H), 2.49 (s, 3H, in solvent peak), 2.36 (s, 3H), 1.84–1.77 (m, 1H), 1.47–1.40 (m, 1H), 1.25–1.18 (m, 1H), 0.90–0.84 (m, 6H).

¹³C NMR (176 MHz, DMSO-d₆, 298 K) δ 173.13, 166.87, 158.91, 158.70, 153.45, 141.59, 141.14, 134.99, 130.02, 129.67, 121.88, 116.53, 114.89, 56.93, 51.74, 40.43, 40.29, 40.17, 40.05, 39.93, 39.81, 39.69, 39.57, 37.01, 25.14, 16.02, 14.35, 11.80, 9.96.

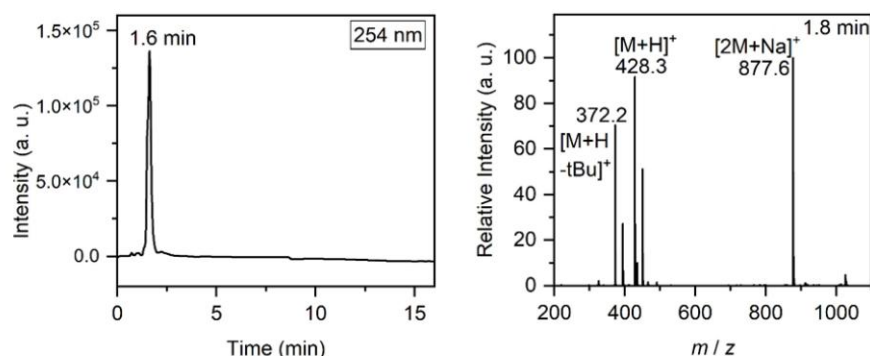
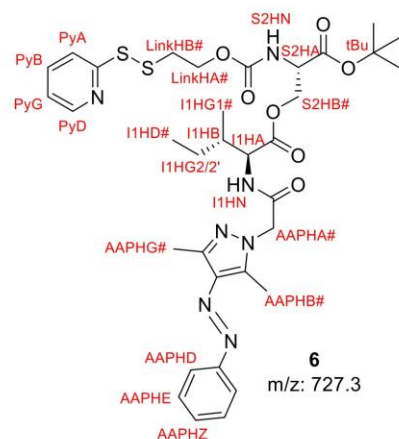


Figure S15: LC-MS data of AAP-Ile-tBu (**4**). Left: LC trace (254 nm) with $t_R = 1.6$ min. Right Convolved ESI-MS spectrum showing peaks of $[M+H]^+$ (calculated: 428.26), $[2M+Na]^+$ (calculated: 877.51) and $[M+H-tBu]^+$ (calculated: 372.20, protecting group removed in ESI-MS).



Compound **4** (31 mg, 1 eq) was dissolved in dry DCM (5 mL) and compound **5** (35 mg, 1.1 eq), DIC (25 μ L, 2 eq) and DMAP (10 mg, 1 eq) were added. The reaction mixture was stirred at room temperature overnight. The solvent was reduced in vacuo and the residue purified via HPLC yielding a yellow solid (25 mg, 34.3 μ mol, 43%).

¹H NMR (700 MHz, DMSO- d_6 , 298 K) δ 8.62 (d, $J = 8.8$ Hz, 1H), 8.52 (d, $J = 8.8$ Hz, 1H), 8.44 (d, $J = 4.7$ Hz, 1H), 7.81 (t, $J = 8.1$ Hz, 2H), 7.76 (d, $J = 7.8$ Hz, 1H), 7.73 (d, $J = 7.8$ Hz, 2H), 7.51 (t, $J = 7.7$ Hz, 2H), 7.42 (t, $J = 7.3$ Hz, 1H), 7.23 (t, 1H), 4.96–4.82 (m, 2H), 4.55 (dd, $J = 8.8$ Hz, 1H), 4.37–4.27 (m, 3H), 4.27–4.21 (m, 1H), 4.19 (t, 2H), 3.07 (t, $J = 6.3$ Hz, 2H), 2.36 (s, 3H), 1.93–1.83 (m, 1H), 1.39 (s, 10H), 1.35–1.23 (m, 1H), 1.20–1.08 (m, 1H), 0.84 (d, $J = 7.6$ Hz, 6H).

¹³C NMR (176 MHz, DMSO- d_6 , 298 K) δ 171.26, 166.73, 158.85, 155.81, 153.02, 149.65, 140.95, 140.60, 137.89, 134.58, 129.61, 129.25, 121.46, 121.32, 119.32, 81.55, 64.01, 61.92, 55.00, 53.42, 51.28, 40.02, 39.88, 39.76, 39.64, 39.52, 39.40, 39.28, 39.16, 37.26, 36.58, 27.58, 25.66, 24.76, 15.39, 14.68, 13.92, 11.59, 11.24, 9.53.

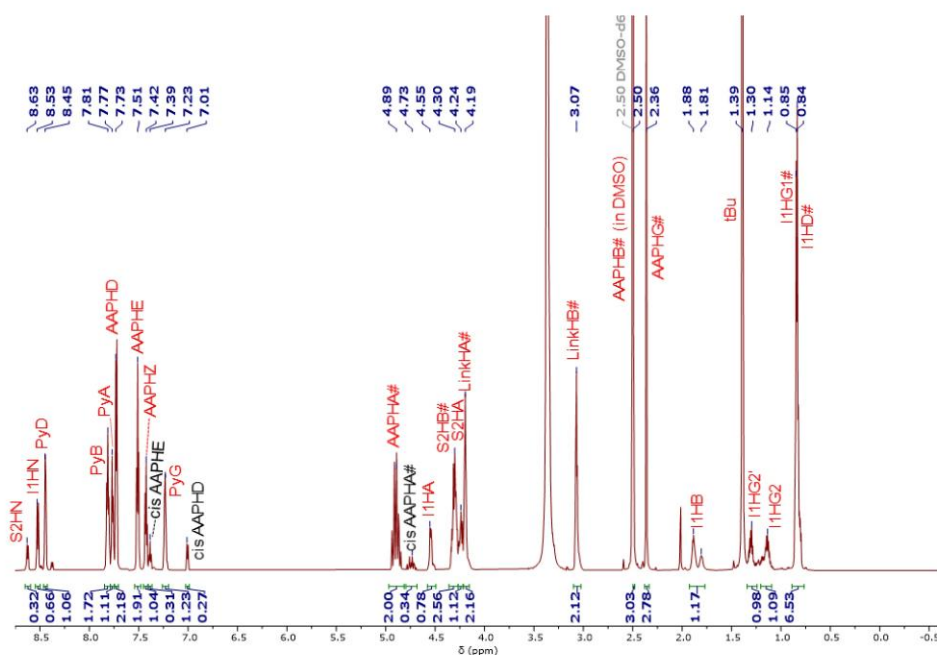


Figure S16: ^1H -NMR (700 MHz, DMSO-d_6 , 298 K) of iso-AAP-Ile-Ser-tBu (**6**).

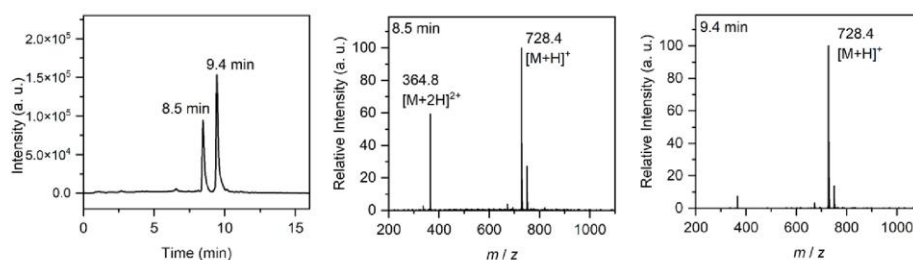
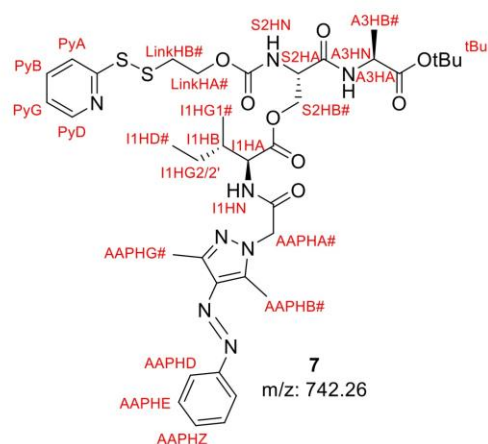


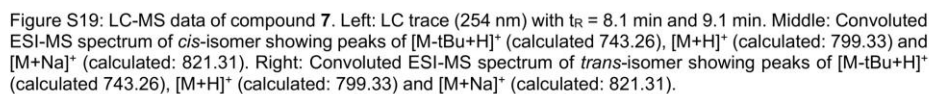
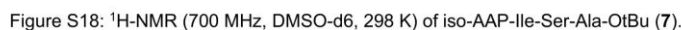
Figure S17: LC-MS data of **6**. Left: LC trace (254 nm) with $t_R = 8.5$ min and 9.4 min. Middle: Convolved ESI-MS spectrum of *cis*-isomer showing peaks of $[\text{M}+\text{H}]^+$ (calculated: 728.29) and $[\text{M}+2\text{H}]^{2+}$ (calculated: 364.65). Right: Convolved ESI-MS spectrum of *trans*-isomer showing peak of $[\text{M}+\text{H}]^+$ (calculated: 728.29).

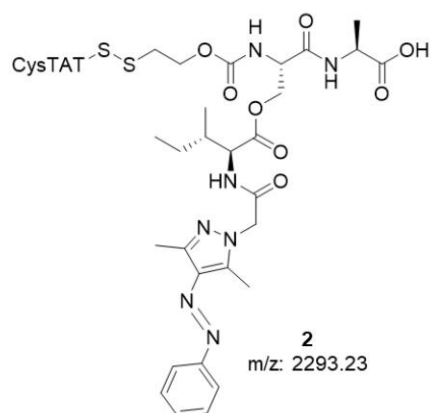


The tBu protecting group of compound **6** was removed from the C-terminus by stirring in TFA/DCM (1:1) for 2 h at room temperature. The solvents were removed in vacuo and, in a round-bottom flask, compound **6** (25 mg, 1 eq) was dissolved in dry DCM (5 mL) and DIPEA (40 μ L, 6 eq), PyBOP (45 mg, 2.3 eq) and H₂N-Ala-OtBu x HCl (13 mg, 1.9 eq) were added. The reaction mixture was stirred at room temperature overnight and the solvent removed in vacuo. The residue was purified by HPLC, stirred in TFA/DCM (1:1) for 2 h at room temperature and lyophilized, yielding a yellow solid (17 mg, 21.2 μ mol, 59%).

¹H NMR (700 MHz, DMSO-*d*₆, 298 K) δ 8.62 (d, *J* = 8.2 Hz, 1H), 8.53 – 8.47 (m, 1H), 8.44 (d, *J* = 5.6 Hz, 1H), 8.37 (d, *J* = 7.2 Hz, 1H), 7.81 (t, *J* = 7.2 Hz, 1H), 7.79 – 7.75 (m, 1H), 7.73 (d, *J* = 7.7 Hz, 2H), 7.51 (t, *J* = 7.6 Hz, 2H), 7.47 – 7.36 (m, 1H), 7.27 – 7.19 (m, 1H), 4.96 – 4.82 (m, 2H), 4.49 (dd, *J* = 8.7, 4.4 Hz, 1H), 4.43 – 4.35 (m, 1H), 4.35 – 4.28 (m, 1H), 4.25 – 4.16 (m, 3H), 4.16 – 4.04 (m, 2H), 3.06 (t, *J* = 6.1 Hz, 2H), 2.37 (s, 3H), 1.93 – 1.79 (m, 1H), 1.38 (s, 9H), 1.33 – 1.27 (m, 1H), 1.25 (d, *J* = 7.2 Hz, 3H), 1.18 – 1.08 (m, 1H), 0.91 – 0.74 (m, 6H).

¹³C NMR (176 MHz, DMSO-*d*₆) δ 171.90, 168.80, 167.19, 159.36, 156.13, 153.80, 150.07, 141.41, 138.32, 135.43, 129.95, 129.66, 121.87, 121.80, 120.15, 119.99, 81.03, 64.85, 62.36, 55.34, 53.86, 49.29, 37.66, 36.84, 33.34, 28.02, 26.05, 25.09, 17.77, 15.28, 14.34, 11.95, 11.70, 9.96.





In a round-bottom flask compound **7** (1.4 mg, 1.8 μ mol, 1 eq.) was dissolved in MeOH and CysTAT (5.0 mg, 3.0 μ mol, 1.6 eq.) was added. The reaction mixture was stirred at room temperature overnight. The solvent was removed in vacuo and the peptide purified using HPLC. Subsequently, TFA/DCM (1:1) was added and the solution was stirred for 2 h at room temperature. The solvents were removed in vacuo and the sample was lyophilized, yielding a yellow solid (3 mg, 1.3 μ mol, 72%).

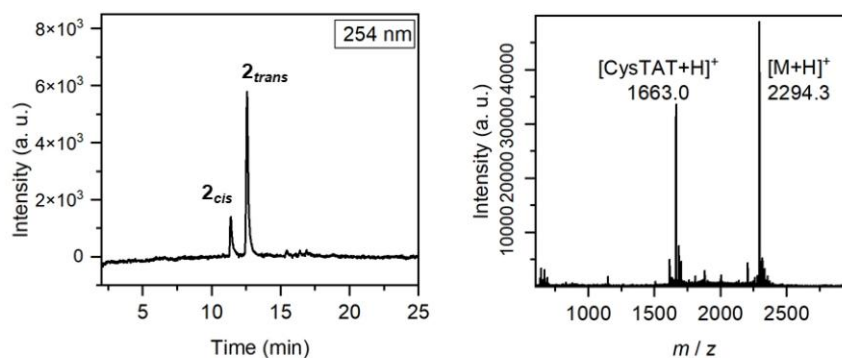


Figure S20: Left: HPLC trace (254 nm) of compound **2** with t_R = 11.4 min (**2_{cis}**) and 12.6 min (**2_{trans}**). Right: Convolved MALDI-MS spectrum of **2** showing peaks of [CysTAT+H]⁺ (calculated 1661.98, released due to strong ionization energy) and [M+H]⁺ (calculated: 2294.23).

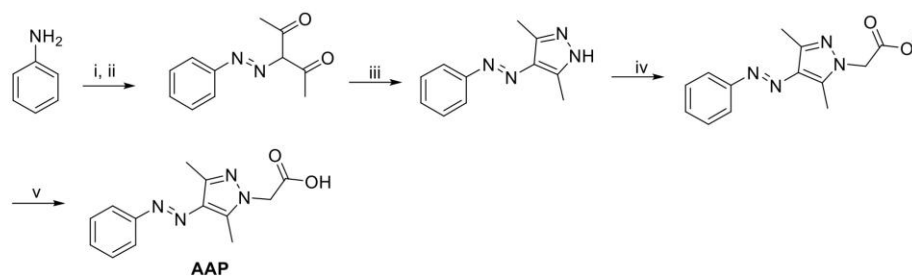


Figure S21: Synthesis of **AAP**¹. (i) HCl, NaNO₂, HOAc, 45 min, 0°C. (ii) pentane-2,4-dione, NaOAc, EtOH/H₂O, 1 h, RT. (iii) hydrazine, EtOH, 3 h, reflux. (iv) methyl 2-bromoacetate, K₂CO₃, ACN, 18 h, reflux. (v) LiOH, THF/H₂O, overnight, RT.

The AAP photoswitch was synthesized according to a previously reported procedure.¹

2.2 TEM Analysis of **1** in different buffers

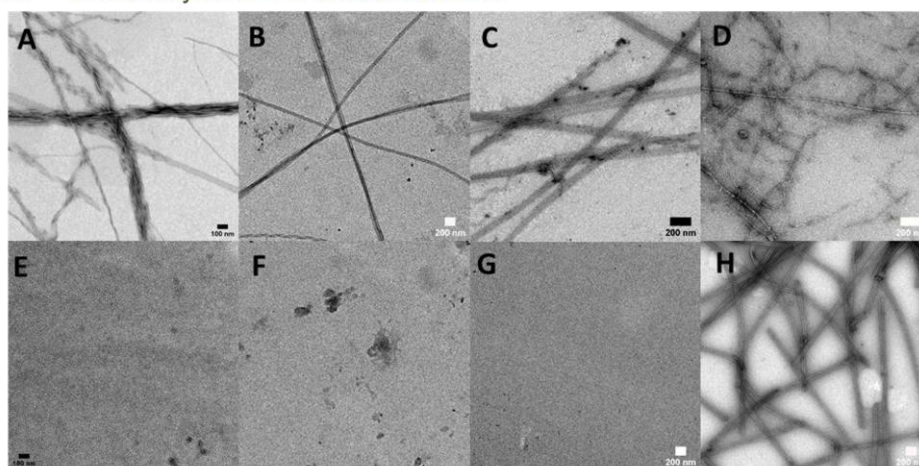


Figure S22: TEM images of **1**. A) **1**_{trans}, 200 μ M, HEPES buffer, 1% DMSO. B) **1**_{trans}, 200 μ M, PB buffer (10 mM Na₂HPO₄, 1.8 mM KH₂PO₄) with NaF (137 mM) and KF (2.7 mM). C) **1**_{trans}, 200 μ M, PBS, 1% DMSO. D) **1**_{trans}, 150 μ M, HEPES buffer, 10% DMSO. E) **1**_{cis}, 200 μ M, HEPES buffer, 1% DMSO, irradiated for 5 s at 365 nm. F) **1**_{cis}, 200 μ M, PB buffer (10 mM Na₂HPO₄, 1.8 mM KH₂PO₄) with NaF (137 mM) and KF (2.7 mM), irradiated for 5 s at 365 nm. G) **1**_{cis}, 200 μ M, PBS, 1% DMSO, irradiated for 5 s at 365 nm. H) **1**_{trans}, 400 μ M, PBS, 1% DMSO.

2.3 TEM Analysis of incubation of **2** with GSH

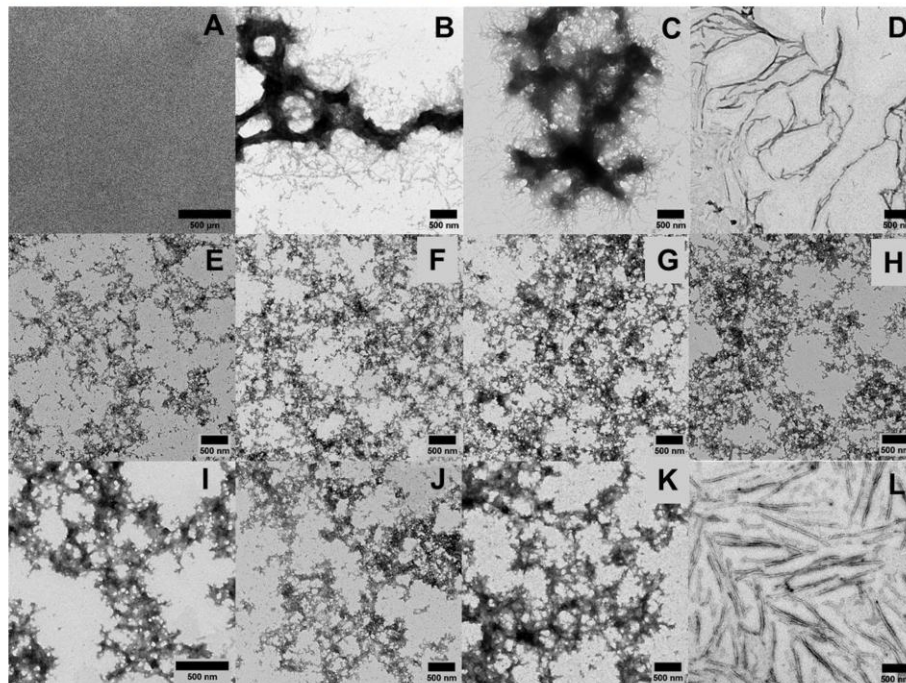
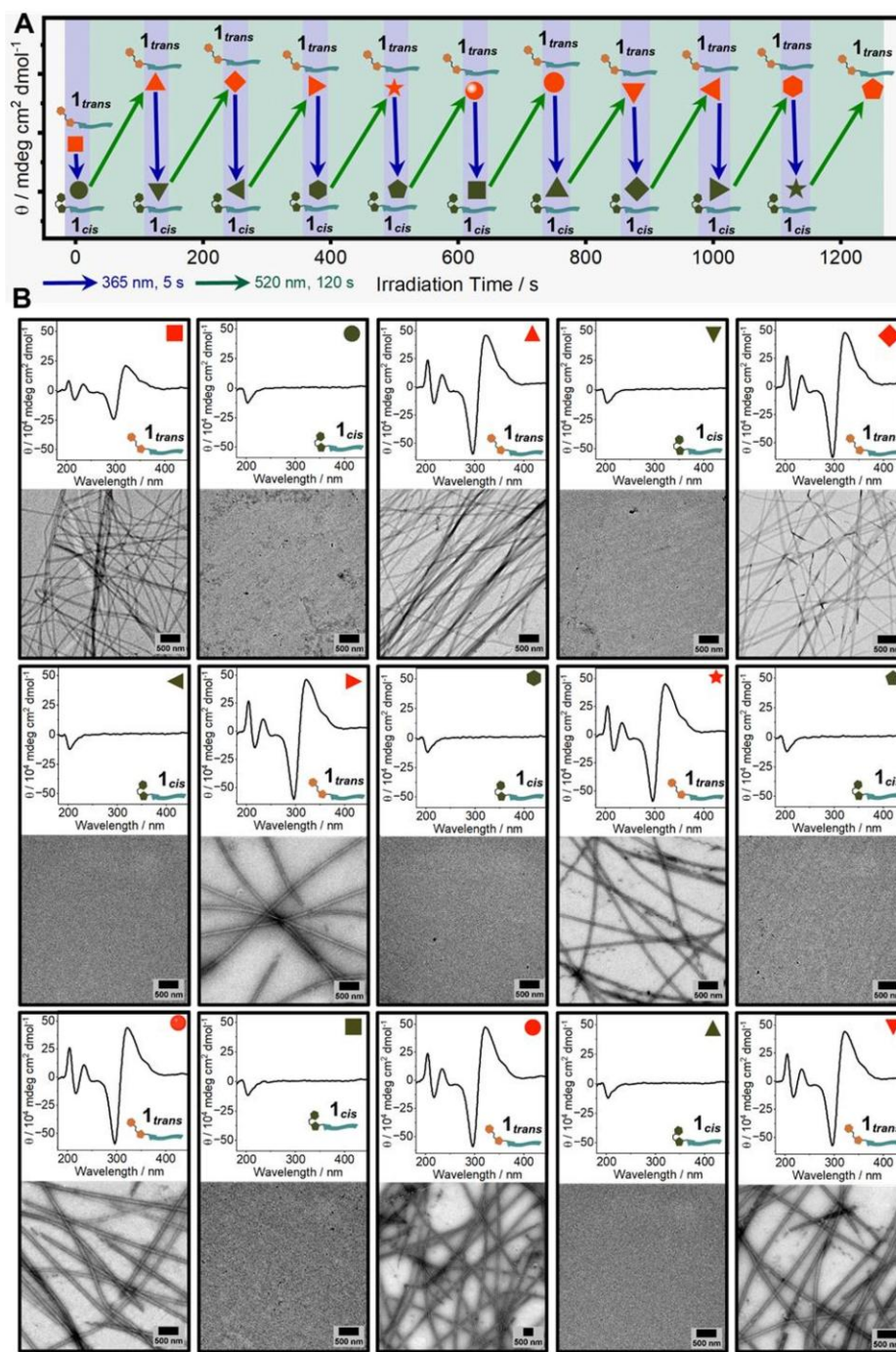


Figure S23: TEM images of **2** (200 μM, PBS) incubation with GSH (10 mM) over time. (A) **2**_{trans} after 0 h. (B) **2**_{trans} after 2 h. (C) **2**_{trans} after 4 h. (D) **2**_{trans} after 24 h. (E) **2**_{cis} after 0 h. (F) **2**_{cis} after 2 h. (G) **2**_{cis} after 4 h. (H) **2**_{cis} after 24 h. (I) **2**_{trans} after 24 h without GSH. (J) **2**_{cis} after 24 h without GSH. (K) Only GSH (10 mM) in PBS. (L) **2**_{trans} after 3 d.



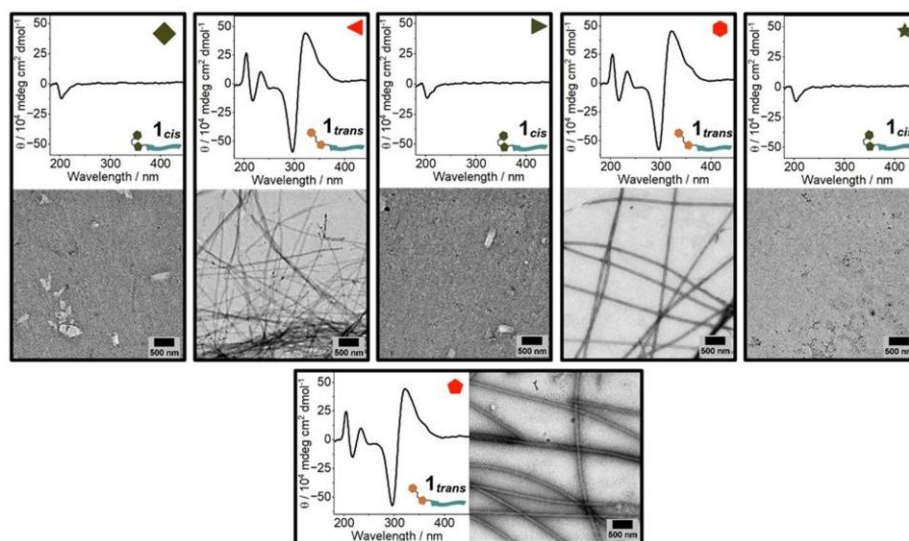


Figure S24: (A) Molar ellipticity of **1** at 325 nm over the course of 10 irradiation cycles at 365 nm for 5 s (blue box) and 520 nm for 120 s (green box). In between the individual irradiation steps, corresponding TEM micrographs and CD-spectra at 200 μM in PBS were recorded (B). Individual measurements are indicated by different symbol shape. Scale bar for TEM-micrographs = 500 nm.

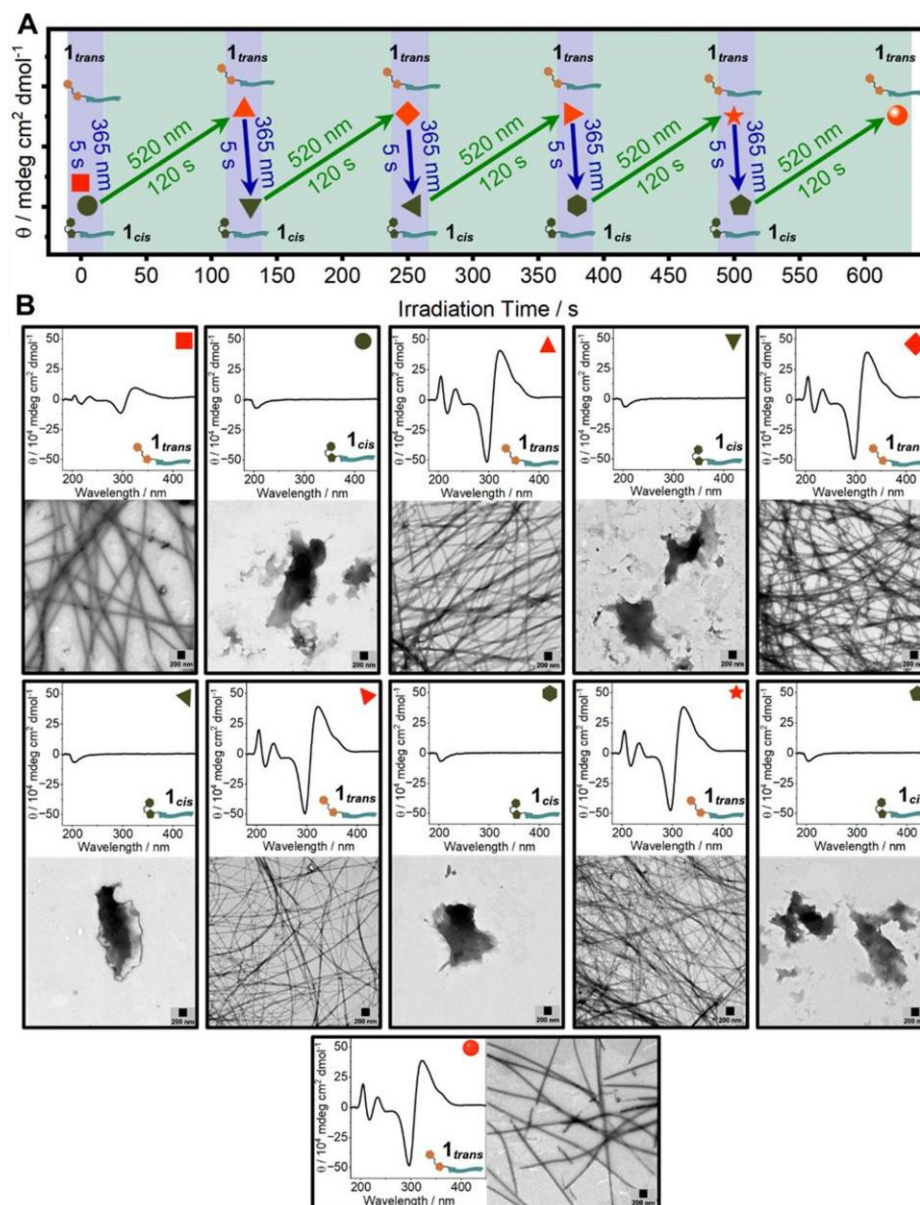


Figure S25: **(A)** Molar ellipticity of **1** at 325 nm over the course of 10 irradiation steps at 365 nm for 5 s (blue box) and 520 nm for 120 s (green box). Inbetween the irradiation steps, corresponding TEM micrographs and CD-spectra at 400 μM in PBS were recorded **(B)**. Individual measurements are indicated by different symbol shape. Scale Bars = 200 nm.

2.4 Determination of CAC

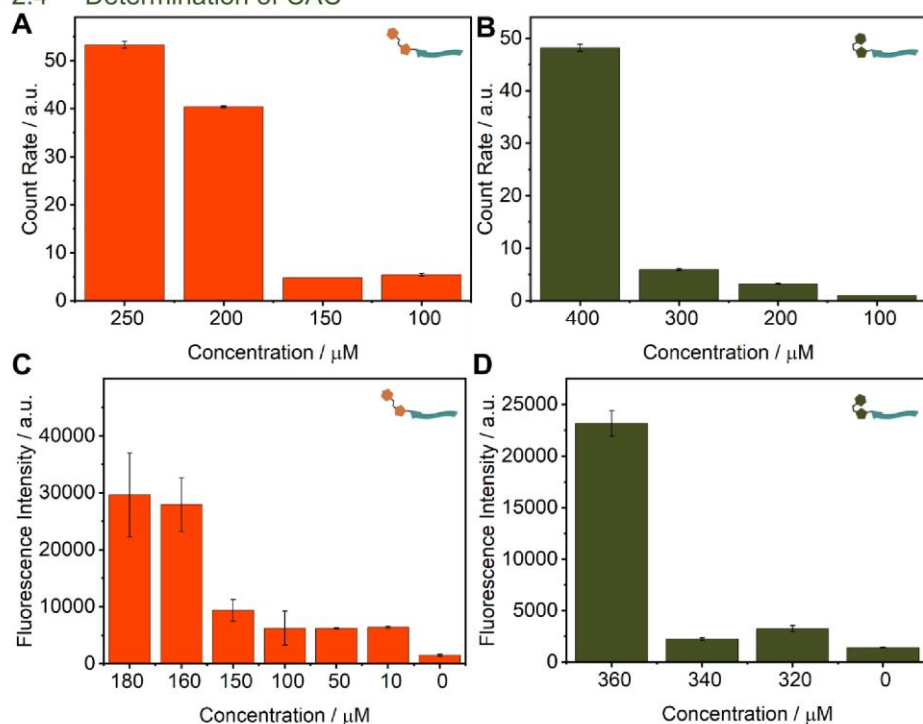


Figure S26: DLS count rate assay of 1_{trans} (A) and 1_{cis} (B) and Proteostat aggregation assay of 1_{trans} (C) and 1_{cis} (D). Measured in PBS with 1% DMSO. The data are presented as mean $\pm$ SEM.

2.5 Chemical Design of Coassembly Approach

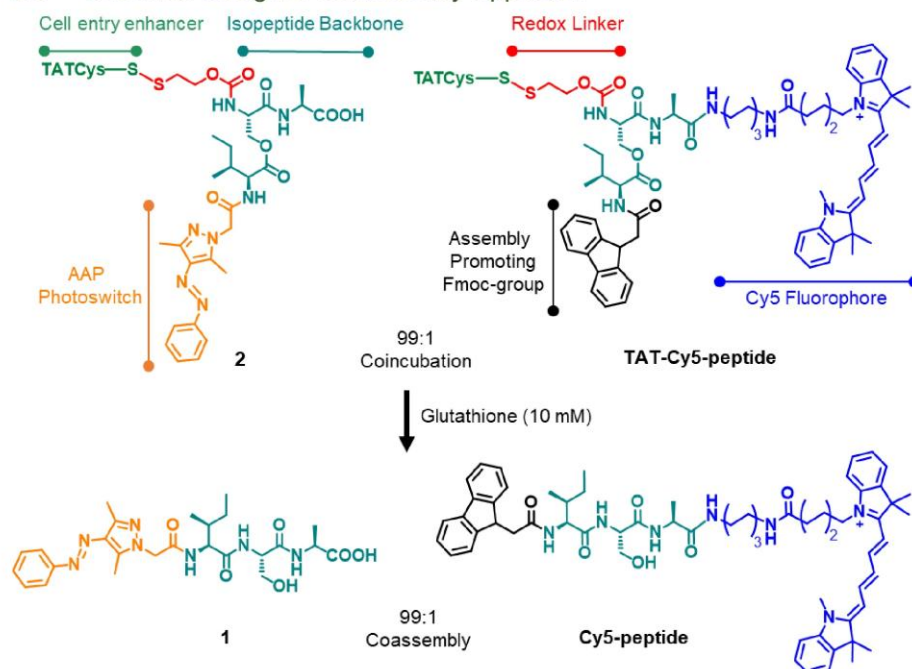


Figure S27: Chemical Design of the coassembly approach. Maintaining the isopeptide backbone, disulfide linker and cell-penetrating TAT-sequence, the AAP photoswitch was changed to an assembly promoting Fmoc-unit to circumvent energy transfer from Cy5, that was connected to the C-terminus of the peptide to enable fluorescence.² Cells were treated with the 99/1 coincubation of **2** with **TAT-Cy5-peptide**. Intracellular glutathione induces linker cleavage and subsequent rearrangement into **1** and **Cy5-peptide**, that coassemble into fluorescent nanostructures.

Procedure:

DMSO stock solutions of **2** and **TAT-Cy5-peptide** were prepared at 40 mM concentration. Then the stocks were combined at a ratio of 99/1 (**2**/**TAT-Cy5-peptide**) maintaining an overall peptide concentration of 40 mM. This stock was further diluted with DMSO to 20 mM or 10 mM respectively. Sample solutions were further diluted to an end concentration of 400, 200 or 100 μ M with DMEM and added to the cells (total DMSO content = 1.00 %).

2.6 Coassembly Analysis

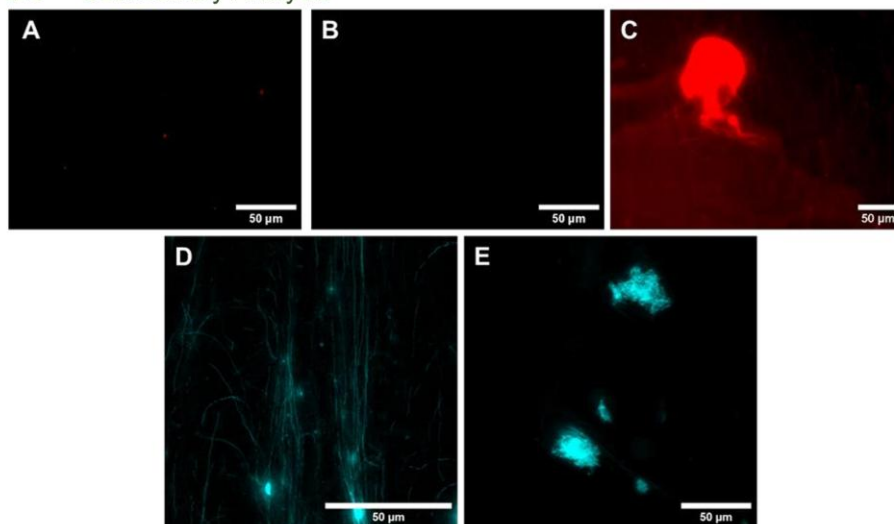


Figure S28: Wide-field fluorescence microscopy of the **Cy5-peptide** in the utilized effective concentration (2 μM) (A), of **1_{trans}** (200 μM) (B) and of the coassembly of **1_{trans}** with the **Cy5-peptide** (C, 99:1, 200 μM). Confocal laser scanning microscopy of the coassembly of **1_{trans}** with the **Cy5-peptide** (D, 99:1, 400 μM) and of **1_{cis}** with the **Cy5-peptide** (E, 99:1, 400 μM). Magnification of the coassembly of **1_{trans}** with the **Cy5-peptide** (F, 99:1, 200 μM).

Procedure:

DMSO stock solutions of **1_{trans}** and **Cy5-peptide** were prepared at 40 or 20 mM concentration. For the analysis of **1_{cis}**, the DMSO stock was irradiated for 5 s with 365 nm light. Then the stocks were combined at a ratio of 99/1 (**1/Cy5-peptide**) maintaining an overall peptide concentration of 40 or 20 mM. Sample solutions were further diluted to an end concentration 400 or 200 μM with PBS (total DMSO content = 1 %) and imaged by placing a drop (5 μL) on a glass slide and covered with a cover slip. For the control experiments, **Cy5-peptide** (A, 2 μM) or **1** (B, 200 μM) were prepared without the addition of the respective other component in the effectively used concentration and imaged likewise.

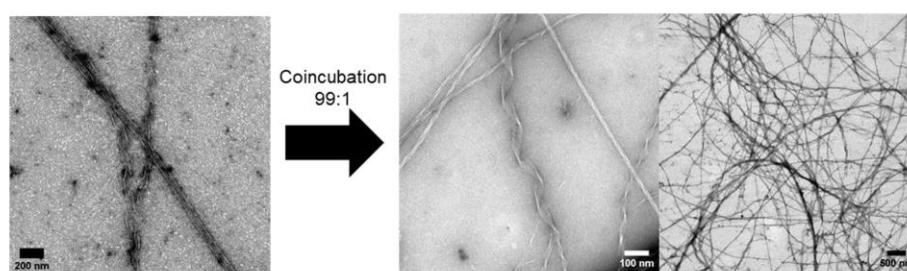


Figure S29: TEM micrographs of coassembly of **1_{trans}** with the corresponding linearized **Cy5-peptide**. Left side shows data for only **1_{trans}**. Right side shows corresponding data for the 99/1 coinubation with **Cy5 peptide**. Overall peptide concentration is 200 μM .

2.7 ^1H -NMR analysis of **1** with light switching

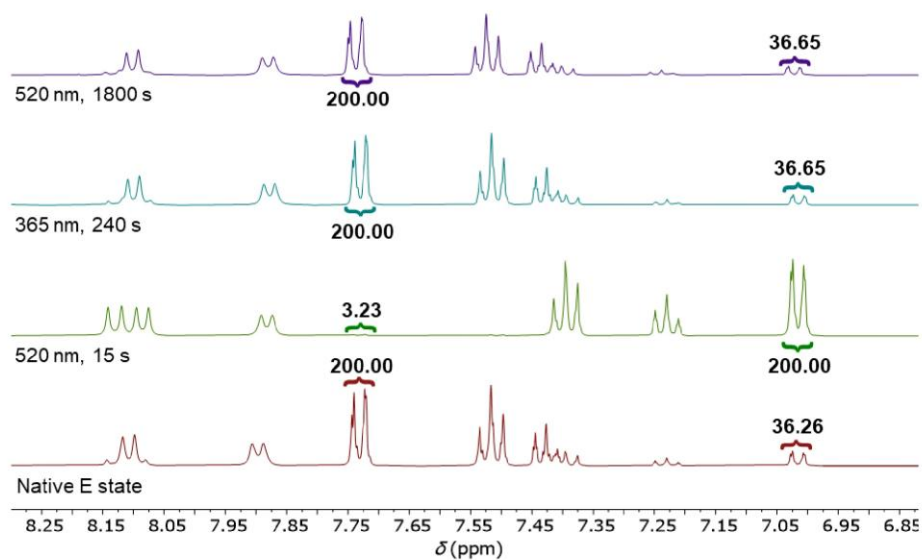


Figure S30: ^1H -NMR spectroscopy (DMSO- d_6 , 298K, 400 MHz, 2 mg/ml) analysis of **1** in native state (red), after irradiation at 365 nm for 15 s (green), after subsequent irradiation at 520 nm for 240 s (blue) and subsequent irradiation at 520 nm for 1800 s. Integrals were set to 200.00 for the respective H3-protons of the predominant isomer.

2.8 LCMS Kinetic

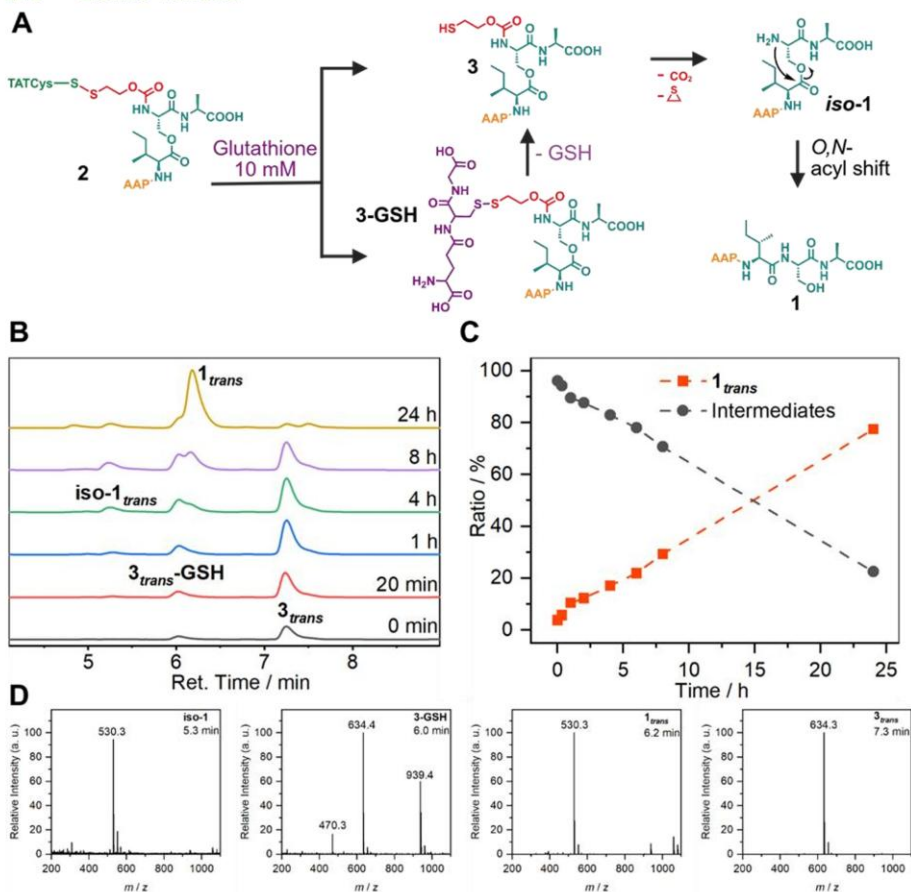


Figure S31: **(A)** Visualization of the chemical transformations within the bioresponsive reaction cascade. First, cleavage of the disulfide linker by glutathione, followed by self-immolation of the linker. Second, intramolecular rearrangement by *O,N*-acyl shift yielding the linearized AAP-ISA (**1**). **(B)** LC-MS kinetic analysis of the glutathione-induced linearization of **2**_{trans} in NH_4HCO_3 buffer (50 mM, pH 7.4) and methanol (v/v 1:1) in the presence of intracellular concentrations of glutathione (10 mM) at room temperature. **(C)** Relative ratio of intermediates (**iso-1**, **3**_{cis}, **3**_{trans}, **3-GSH**) and final product **1**_{trans} after the addition of glutathione-containing buffer, based on the peak integration at 300 nm. **(D)** Corresponding ESI MS spectra of the LCMS Kinetic.

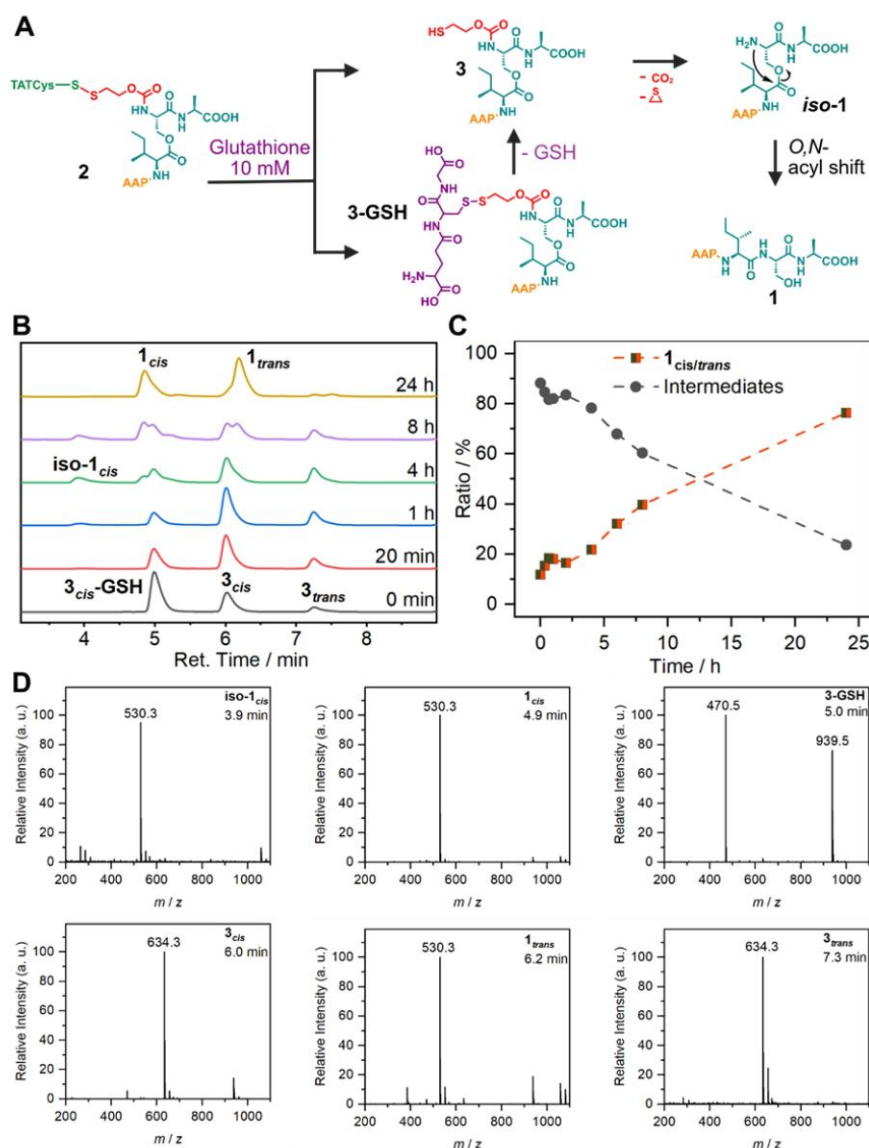


Figure S32: **(A)** Visualization of the chemical transformations within the bioresponsive reaction cascade. First, cleavage of the disulfide linker by glutathione, followed by self-immolation of the linker. Second, intramolecular rearrangement by O,N -acyl shift yielding the linearized AAP-ISA (**1**). **(B)** LC-MS kinetic analysis of the glutathione-induced linearization of **2_{cis}** in NH_4HCO_3 buffer (50 mM, pH 7.4) and methanol (v/v 1:1) in the presence of intracellular concentrations of glutathione (10 mM) at room temperature. **(C)** Relative ratio of intermediates (**iso-1**, **3_{cis}**, **3_{trans}**, **3-GSH**) and final products **1_{cis}** and **1_{trans}** after the addition of glutathione-containing buffer, based on the peak integration at 300 nm. **(D)** Corresponding ESI MS spectra of the LCMS Kinetic.

2.9 Confocal Laser Scanning microscopy of 2 at various concentrations

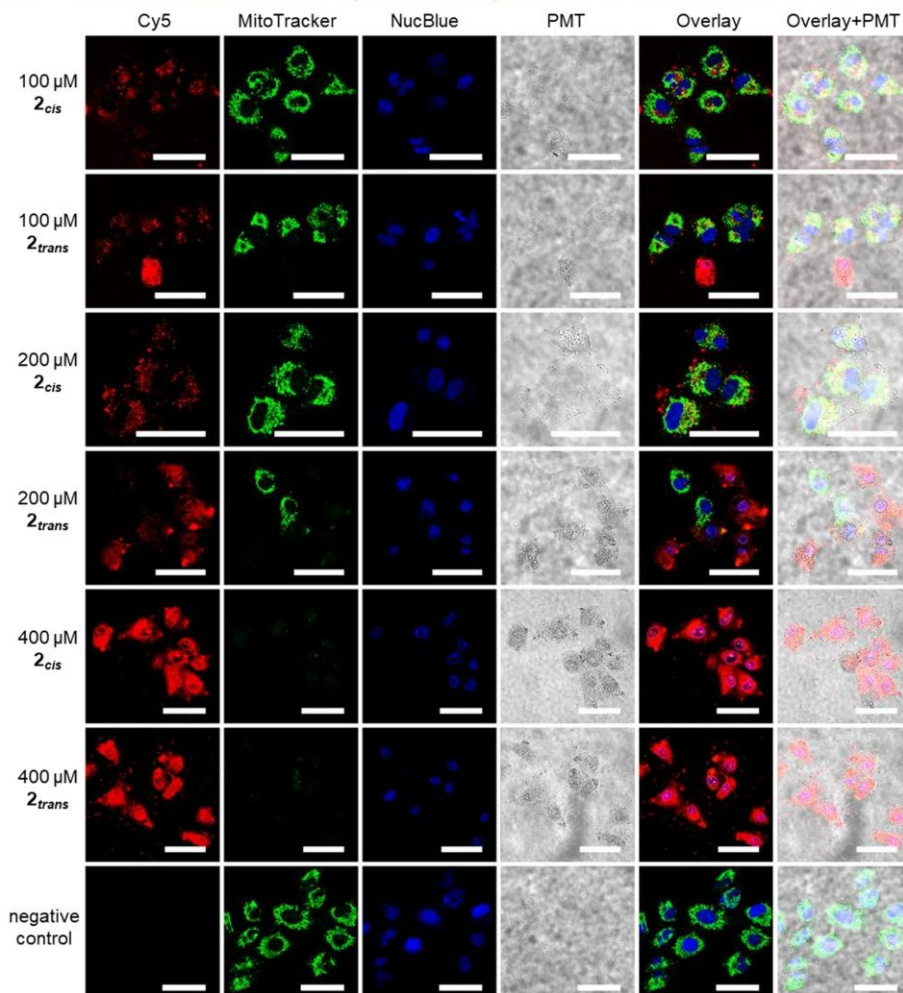


Figure S33: Confocal laser scanning micrographs of A549 cells treated for 4h with **2/Cy5-peptide** (99/1, 400, 200 or 100 μM) and prestained with Mitotracker Orange and NucBlue. Imaging under physiological condition (5% CO_2 , 90% humidity, 37°C) using Leica STELLARIS 8 microscope, HC PL APO CS2 40x/1.25 GLYC. Imaging: 550 nm at 2.13% for MitoTracker Orange excitation, 405 nm at 2.23% for NucBlue excitation and 650nm at 0.66% for sample excitation. Scale Bars: 50 μm .

References

- 1 L. Stricker, E. C. Fritz, M. Peterlechner, N. L. Doltsinis and B. J. Ravoo, *J. Am. Chem. Soc.*, 2016, **138**, 4547–4554.
- 2 S. Chagri, K. Maxeiner, M. J. S. A. Silva, L. Förch, J. Link, P. Roth, R. Meyer, J. Fetzer, A. Kaltbeizel, I. Lieberwirth, K. Landfester, D. Y. W. Ng and T. Weil, *Adv. Sci.*, 2025, 2412606.

6. Summary and Outlook

Living systems maintain a high degree of structural and functional order by operating far from thermodynamic equilibrium, continuously balancing forces that promote organization against those that drive disorder. Translating this principle into synthetic supramolecular systems remains a central challenge, as most assemblies are inherently biased toward static equilibrium states with limited adaptability.

The work presented in this thesis demonstrates that molecular-level design can be used to actively control supramolecular order, disorder, and reversibility in peptide-based systems, enabling access to dynamic assembly pathways that extend beyond static structures. By systematically linking molecular parameters, external stimuli, and environmental context to supramolecular behavior, this thesis establishes a framework for rationally designing adaptive peptide assemblies. The following paragraphs summarize the key findings of each project and discuss how they collectively address the overarching objective of controlling peptide self-assembly under non-equilibrium and biologically relevant conditions.

Chapter 3 investigates the role of linker flexibility in governing effective intermolecular interactions and, consequently, peptide self-assembly. A series of ICA tripeptides was *N*-terminally functionalized with a naphthalene-imide fluorophore using linker segments of varying lengths, comprising six, three or zero carbon atoms between the peptide backbone and the aromatic π -block. The longest spacer (C_6) consistently promoted aggregation more effectively, followed by the intermediate spacer (C_3), whereas the rigid, spacer-free system (C_0) failed to form ordered nanostructures. These results highlight the critical importance of spacer flexibility and constitution in enabling productive aromatic interactions and controlled self-assembly. While this study focused exclusively on methylene-based spacers, future work could expand this design principles to include more hydrophilic polyethylene glycol linkers or peptidic glycine spacers, as well as a broader range of aromatic π -blocks. In addition, the impact of linker-controlled packing parameters in biological environments remains unexplored. In this context, the cysteine side chain offers a versatile handle for the introduction of cleavable cell-penetrating peptide conjugates to facilitate cellular uptake. Combined with the intrinsic fluorescence of the π -block, this strategy would enable direct visualization and analysis of intracellular self-assembly using confocal laser scanning microscopy.

Chapter 4 investigates light-responsive self-assembly using the short amphiphilic peptides Anthracene-GISVK and Anthracene-GISVKAE. In their native states, the peptides assembled into distinct morphologies, namely 2D-sheets or twisted nanoribbons, which differ in their secondary structure signatures and packing interactions between the anthracene motifs. Notably, only the shorter Ant-GISVK displayed pronounced pH-sensitivity, undergoing a transition from sheets to disordered aggregates at elevated pH, whereas the nanoribbons

formed by Ant-GISVKAE peptide remained stable under the same conditions. Upon UV-irradiation, [4+4] photodimerization of the anthracene units is triggered. This reaction disrupts the planarity of the aromatic segments, weakens effective π - π -stacking, and consequently drives disassembly or conversion into disordered aggregates. Importantly, the dimerization shows clear morphology-dependent kinetics: preorganization within the assembled state modulates the reaction rate compared to the dissolved peptides, demonstrating how supramolecular order can actively shape the underlying chemistry and thereby control disassembly pathways. Overall, these results highlight the tight coupling between supramolecular organization, reaction kinetics, and macroscopic material response. To further enhance systems toward multi-step, pathway selective, and out-of-equilibrium assemblies, additional design elements that enable programmable reaction cascades are required. Crucially, the implementation of reversible photochemistry to allow recovery and repeated cycling between the observed morphologies is of interest.

These considerations inspired the design a photoresponsive peptide platform to probe how supramolecular organization influences cellular function. Accordingly, Chapter 5 introduces the photoswitchable peptide AAP-ISA. In its native *trans*-state, the peptide forms β -sheet rich nanofibers stabilized by pronounced aromatic interactions, whereas the non-planar *cis*-isomer assembles into disordered, random-coil aggregates. The peptide conjugates combine long thermal isomer half-lives with high photofatigue resistance, enabling robust cycling. The structural dynamics of the assembled architectures were demonstrated by repeated photoswitching between both isomeric states over 20 cycles. To translate this structural scaffold into a cellular setting, the system was extended via isopeptide chemistry to incorporate a cell-penetrating peptide through a GSH-responsive linker, allowing intracellular activation and release of the self-assembling monomer. Isomer-dependent conversion and release kinetics were first quantified *in vitro*, followed by uptake experiments and cell-viability measurements. Notably, A549 lung cancer cell viability correlated with the critical aggregation concentrations of the two isomers: the *trans*-isomer, with the higher aggregation propensity, exhibited markedly greater cytotoxicity. Overall, the combination of favorable photophysical properties with tunable bioactivity provides a powerful single-scaffold platform to interrogate the intracellular consequences of supramolecular order versus disorder.

A natural next step will be to exploit the robustness of this system to study switching within the cells in real-time, rather than pre-switching prior to incubation. Such experiments could address, for example, whether early apoptotic responses triggered by the fiber-forming *trans*-state can be halted or reversed by light-induced disassembly, or conversely whether cytotoxicity can be activated on demand by converting the less toxic *cis* isomers into the nanofiber-forming *trans* variant.

In summary, this thesis demonstrates how rational molecular design can enable control over supramolecular peptide self-assembly pathways, allowing reversible, adaptive and a stimulus-responsive behavior. It answers the question how even subtle molecular design parameters translate into controllable, adaptive supramolecular behavior in peptide-based systems.

While this thesis provides fundamental insights into peptide-based self-assembly, several key challenges remain. In particular, the development of synthetic systems that operate far-from-equilibrium is essential for understanding the dynamic behaviors characteristic of biological assemblies. Progress toward this goal will likely require more complex molecular designs that integrate several layers of stimulus responsiveness and environmental sensitivity, enabling multi-step pathways, pathway selection, and adaptive or dissipative behavior reminiscent of cytoskeletal elements. Embedding such responsiveness in a modular fashion further provides access to programmable supramolecular materials that implement molecular logic (e.g. logic gates) to couple intracellular cues with externally applied stimuli to spatiotemporally control self-assembly. Environment sensing is also expected to advance the mechanistic understanding of intracellular self-assembly, helping to rationalize why structures formed in cells can differ from those observed under simplified *in vitro* conditions. Closely related, the fundamentals of co-assembly remain a central topic, as they underpin recruitment processes and regulate the integration of multiple chemical distinct building blocks into a single nanoarchitecture. Finally, light provides a particularly powerful and orthogonal control input to address assemblies with high spatiotemporal precision, enable reversible cycling, and achieve on-demand activation and deactivation, features that are central to next-generation, biofunctional supramolecular systems.

Ultimately, this thesis contributes to the field by exploring fundamental design principles for adaptive peptide assemblies operating at the interface of supramolecular chemistry photochemistry, and chemical biology. By bridging molecular precision with macroscopic function, the work demonstrates that dynamic control over non-covalent interactions is a viable strategy for engineering biological response. The systems developed herein establish that supramolecular order itself can serve as a functional parameter, distinct from chemical composition, to modulate cellular outcomes. These findings lay the groundwork for a new generation of smart materials where structure, function, and environment are intricately linked through reversible chemical inputs, paving the way for future applications in dynamic biomaterials, photopharmacology, and synthetic biology.

7. Bibliography

1. Huang, F. & Anslyn, E. V. Introduction: Supramolecular Chemistry. *Chem. Rev.* **115**, 6999 (2015).
2. Gronowitz, S. Nobel Prize in Chemistry 1987 – Award Ceremony Speech. <https://www.nobelprize.org/prizes/chemistry/1987/ceremony-speech/> (1987).
3. Whitesides, G. M. & Grzybowski, B. Self-assembly at all scales. *Science*. **295**, 2418–2421 (2002).
4. Whitesides, G. M., Mathias, J. P. & Seto, C. T. Molecular Self-Assembly and Nanochemistry: A Chemical Strategy for the Synthesis of Nanostructures. *Science*. **254**, 1312–1319 (1991).
5. Wang, J., Liu, K., Xing, R. & Yan, X. Peptide self-assembly: Thermodynamics and kinetics. *Chem. Soc. Rev.* **45**, 5589–5604 (2016).
6. Gavezzotti, A. Comparing the strength of covalent bonds{,} intermolecular hydrogen bonds and other intermolecular interactions for organic molecules: X-ray diffraction data and quantum chemical calculations. *New J. Chem.* **40**, 6848–6853 (2016).
7. Biedermann, F. & Schneider, H.-J. Experimental Binding Energies in Supramolecular Complexes. *Chem. Rev.* **116**, 5216–5300 (2016).
8. Atkins, P. & De Paula, J. *Atkins' Physical Chemistry*. (Oxford University Press, 2006).
9. Arunan, E. *et al.* Definition of the hydrogen bond (IUPAC Recommendations 2011). *Pure Appl. Chem.* **83**, 1637–1641 (2011).
10. Weinhold, F. & Klein, R. A. Research and Practice What is a hydrogen bond? Resonance covalency in the supramolecular domain. *Chem. Educ. Res. Pract.* **15**, 276–285 (2014).
11. Israelachvili, J. N. Special Interactions: Hydrogen- Bonding and Hydrophobic and Hydrophilic Interactions. in *Intermolecular and Surface Forces* 151–167 (2011).
12. Hunter, C. A. & Sanders, J. K. M. The nature of .pi.-.pi. interactions. *J. Am. Chem. Soc.* **112**, 5525–5534 (1990).
13. Zhu, P., Yan, X., Su, Y., Yang, Y. & Li, J. Solvent-induced structural transition of self-assembled dipeptide: From organogels to microcrystals. *Chem. - A Eur. J.* **16**, 3176–3183 (2010).
14. Grimme, S. Do special noncovalent π - π stacking interactions really exist? *Angew. Chemie - Int. Ed.* **47**, 3430–3434 (2008).
15. Chandler, D. Interfaces and the driving force of hydrophobic assembly. *Nature* **437**, 640–647 (2005).
16. Tanford, C. The Hydrophobic Effect and the Organization of Living Matter. *Science*. **200**, 1012–1018 (1978).
17. Rehm, T. H. & Schmuck, C. Ion-pair induced self-assembly in aqueous solvents. *Chem. Soc. Rev.* **39**, 3597–3611 (2010).
18. Schneider, H. J. Linear free energy relationships and pairwise interactions in supramolecular chemistry. *Chem. Soc. Rev.* **23**, 227–234 (1994).
19. Schneider, H. J., Schiestel, T. & Zimmermann, P. The Incremental Approach to Noncovalent Interactions: Coulomb and van der Waals Effects in Organic Ion Pairs. *J. Am. Chem. Soc.* **114**, 7698–7703 (1992).
20. Tsonchev, S., Niece, K. L., Schatz, G. C., Ratner, M. A. & Stupp, S. I. Phase Diagram for Assembly of Biologically-Active Peptide Amphiphiles †. *J. Phys. Chem. B* **112**, 441–447 (2008).

21. Li, C. *et al.* Structure-activity collective properties underlying self-assembled superstructures. *Nano Today* **42**, 101354 (2022).
22. Mattia, E. & Otto, S. Supramolecular systems chemistry. *Nat. Nanotechnol.* **10**, 111–119 (2015).
23. Greef, T. F. A. De *et al.* Supramolecular Polymerization. *Chem. Rev.* **109**, 5687–5754 (2009).
24. Hou, X.-F. *et al.* Supramolecular dissipative self-assembly systems: Approaches and applications. *Responsive Mater.* **1**, e20230016 (2023).
25. Hamieh, S. *et al.* A “ Dial-A-Receptor ” Dynamic Combinatorial Library. *Angew. Chemie - Int. Ed.* **52**, 12368–12372 (2013).
26. Ponnuswamy, N., Cougnon, F. B. L., Clough, J. M., Pantos, G. D. & Sanders, J. K. M. Discovery of an Organic Trefoil Knot. *Science*. **338**, 783–785 (2012).
27. Chichak, K. S. *et al.* Molecular Borromean Rings. *Science*. **304**, 1308–1312 (2004).
28. Li, J., Nowak, P. & Otto, S. Dynamic Combinatorial Libraries : From Exploring Molecular Recognition to Systems Chemistry. *J. Am. Chem. Soc.* **135**, 9222–9239 (2013).
29. Israelachvili, J. N., Marčelja, S. & Horn, R. G. Physical principles of membrane organization. *Q. Rev. Biophys.* **13**, 121–200 (1980).
30. Sivagnanam, S. *et al.* Concentration-dependent fabrication of short-peptide-based different self-assembled nanostructures with various morphologies and intracellular delivery property. *Mater. Chem. Front.* **3**, 2110–2119 (2019).
31. Samdin, T. D., Wang, X., Fichman, G. & Schneider, J. P. Exploring the temperature dependence of β -hairpin peptide self-assembly. *Faraday Discuss.* **260**, 113–131 (2025).
32. Lin, Y. *et al.* Residue-Specific Solvation-Directed Thermodynamic and Kinetic Control over Peptide Self-Assembly with 1D/2D Structure Selection. *ACS Nano* **13**, 1900–1909 (2019).
33. Korevaar, P. A. *et al.* Pathway complexity in supramolecular polymerization. *Nature* **481**, 492–497 (2012).
34. Roth, P. *et al.* Supramolecular assembly guided by photolytic redox cycling. *Nat. Synth.* **2**, 980–988 (2023).
35. Sachini, B., Corra, S., Curcio, M., Groppi, J. & Cred, A. Defying chemical equilibrium with light. *Trends Chem.* **7**, 372–383 (2025).
36. Rieß, B., Grötsch, R. K. & Boekhoven, J. The Design of Dissipative Molecular Assemblies Driven by Chemical Reaction Cycles. *Chem* **6**, 552–578 (2020).
37. de Jong, J. J. D., Lucas, L. N., Kellogg, R. M., van Esch, J. H. & Feringa, B. L. Reversible Optical Transcription of Supramolecular Chirality into Molecular Chirality. *Science*. **304**, 278–281 (2004).
38. Jalani, K., Dhiman, S., Jain, A. & George, S. J. Temporal switching of an amphiphilic self-assembly by a chemical fuel-driven conformational response. *Chem. Sci.* **8**, 6030–6036 (2017).
39. Ragazzon, G., Baroncini, M., Silvi, S., Venturi, M. & Credi, A. Light-powered autonomous and directional molecular motion of a dissipative self-assembling system. *Nat. Nanotechnol.* **10**, 70–75 (2015).
40. Uhl, E., Mayer, P. & Dube, H. Active and Unidirectional Acceleration of Biaryl Rotation by a Molecular Motor. *Angew. Chemie Int. Ed.* **59**, 5730–5737 (2020).
41. Li, H. *et al.* Relative Unidirectional Translation in an Artificial Molecular Assembly Fueled by Light. *J. Am. Chem. Soc.* **135**, 18609–18620 (2013).

42. Yin, Z. *et al.* Dissipative Supramolecular Polymerization Powered by Light. *CCS Chem.* **1**, 335–342 (2019).
43. Cissé, N. & Kudernac, T. Light-Fuelled Self-Assembly of Cyclic Peptides into Supramolecular Tubules. *ChemSystemsChem* **2**, e2000012 (2020).
44. Chen, X.-M. *et al.* Light-activated photodeformable supramolecular dissipative self-assemblies. *Nat. Commun.* **13**, 3216 (2022).
45. Appel, W. P. J., Nieuwenhuizen, M. M. L. & Meijer, E. W. Multiple Hydrogen-Bonded Supramolecular Polymer. in *Supramolecular Polymer Chemistry* (ed. Harada, A.) 1–28 (WILEY-VCH, 2011).
46. Schubert, U. S., Newkome, G. R. & Winter, A. Supramolecular Polymers: General Consideration. in *Supramolecular Polymers and Assemblies* 1–28 (WILEY-VCH, 2021).
47. Zhao, D. & Moore, J. S. Nucleation–elongation: a mechanism for cooperative supramolecular polymerization. *Org. Biomol. Chem.* **1**, 3471–3491 (2003).
48. Smulders, M. M. J. *et al.* How to Distinguish Isodesmic from Cooperative Supramolecular Polymerisation. *Chem. – A Eur. J.* **16**, 362–367 (2010).
49. Merrifield, R. B. Solid Phase Peptide Synthesis. I. The Synthesis of a Tetrapeptide. *J. Am. Chem. Soc.* **85**, 2149–2154 (1963).
50. Chan, W. C. & White, P. D. *Fmoc Solid Phase Peptide Synthesis*. (Oxford University Press, 1999).
51. Dobson, C. M. Protein folding and misfolding. *Nature* **426**, 884–890 (2003).
52. Jumper, J. *et al.* Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589 (2021).
53. Berman, H. M. *et al.* The Protein Data Bank. *Nucleic Acids Res.* **28**, 235–242 (2000).
54. Fleming, S. & Ulijn, R. V. Design of nanostructures based on aromatic peptide amphiphiles. *Chem. Soc. Rev.* **43**, 8150–8177 (2014).
55. Li, T., Lu, X.-M., Zhang, M.-R., Hu, K. & Li, Z. Peptide-based nanomaterials: Self-assembly, properties and applications. *Bioact. Mater.* **11**, 268–282 (2022).
56. Chagri, S., Ng, D. Y. W. & Weil, T. Designing bioresponsive nanomaterials for intracellular self-assembly. *Nat. Rev. Chem.* **6**, 320–338 (2022).
57. Philp, D. & Stoddart, J. F. Self-Assembly in Natural and Unnatural Systems. *Angew. Chemie Int. Ed. English* **35**, 1154–1196 (1996).
58. Berg, J. M., Tymoczko, J. L., Gatto jr., G. J. & Stryer, L. *Stryer Biochemie*. (Springer US, 2018).
59. Pauling, L. & Corey, R. B. Configurations of Polypeptide Chains With Favored Orientations Around Single Bonds. *Proc. Natl. Acad. Sci.* **37**, 729–740 (1951).
60. Pauling, L., Corey, R. B. & Branson, H. R. The structure of proteins: Two hydrogen-bonded helical configurations of the polypeptide chain. *Proc. Natl. Acad. Sci.* **37**, 205–211 (1951).
61. Alberts, B. *et al.* *Molekularbiologie der Zelle*. (WILEY-VCH, 2017).
62. Dill, K. A. & MacCallum, J. L. The Protein-Folding Problem, 50 Years On. *Science*. **338**, 1042–1046 (2012).
63. Chiti, F. & Dobson, C. M. Protein Misfolding, Functional Amyloid, and Human Disease. *Annu. Rev. Biochem.* **75**, 333–366 (2006).

64. Knowles, T. P. J., Vendruscolo, M. & Dobson, C. M. The amyloid state and its association with protein misfolding diseases. *Nat. Rev. Mol. Cell Biol.* **15**, 384–396 (2014).
65. Osada, Y., Kawamura, R. & Sano, K.-I. Why Cytoskeletal Gel? in *Hydrogels of Cytoskeletal Proteins* 7–20 (Springer, 2016).
66. Zhang, S. Fabrication of novel biomaterials through molecular self-assembly. *Nat. Biotechnol.* **21**, 1171–1178 (2003).
67. Hamley, I. W. Self-assembly of amphiphilic peptides. *Soft Matter* **7**, 4122–4138 (2011).
68. Ulijn, R. V & Smith, A. M. Designing peptide based nanomaterials. *Chem. Soc. Rev.* **37**, 664–675 (2008).
69. Zhang, S., Holmes, T., Lockshin, C. & Rich, A. Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. *Proc. Natl. Acad. Sci.* **90**, 3334–3338 (1993).
70. Aggeli, A. *et al.* Hierarchical self-assembly of chiral rod-like molecules as a model for peptide β -sheet tapes, ribbons, fibrils, and fibers. *Proc. Natl. Acad. Sci.* **98**, 11857–11862 (2001).
71. Besenius, P. *et al.* Controlling the growth and shape of chiral supramolecular polymers in water. *Proc. Natl. Acad. Sci.* **107**, 17888–17893 (2010).
72. Aggeli, A. *et al.* Responsive gels formed by the spontaneous self-assembly of peptides into polymeric β -sheet tapes. *Nature* **386**, 259–262 (1997).
73. Caplan, M. R., Schwartzfarb, E. M., Zhang, S., Kamm, R. D. & Lauffenburger, D. A. Control of self-assembling oligopeptide matrix formation through systematic variation of amino acid sequence. *Biomaterials* **23**, 219–227 (2002).
74. Hong, Y., Pritzker, M. D., Legge, R. L. & Chen, P. Effect of NaCl and peptide concentration on the self-assembly of an ionic-complementary peptide EAK16-II. *Colloids Surfaces B Biointerfaces* **46**, 152–161 (2005).
75. Yokoi, H., Kinoshita, T. & Zhang, S. Dynamic reassembly of peptide RADA16 nanofiber scaffold. *Proc. Natl. Acad. Sci.* **102**, 8414–8419 (2005).
76. Lupas, A. N. & Gruber, M. The Structure of α -Helical Coiled Coils. in *Fibrous Proteins: Coiled-Coils, Collagen and Elastomers* vol. 70 37–38 (Academic Press, 2005).
77. Crick, F. H. C. Is α -Keratin a Coiled Coil? *Nature* **170**, 882–883 (1952).
78. Woolfson, D. N. The Design of Coiled-Coil Structures and Assemblies. in *Fibrous Proteins: Coiled-Coils, Collagen and Elastomers* vol. 70 79–112 (Academic Press, 2005).
79. Pandya, M. J. *et al.* Sticky-End Assembly of a Designed Peptide Fiber Provides Insight into Protein Fibrillogenesis. *Biochemistry* **39**, 8728–8734 (2000).
80. Woolfson, D. N. Understanding a protein fold: The physics, chemistry, and biology of α -helical coiled coils. *J. Biol. Chem.* **299**, 104579 (2023).
81. Hartgerink, J. D., Beniash, E. & Stupp, S. I. Self-Assembly and Mineralization of Peptide-Amphiphile Nanofibers. *Science*. **294**, 1684–1688 (2001).
82. Paramonov, S. E., Jun, H.-W. & Hartgerink, J. D. Self-Assembly of Peptide-Amphiphile Nanofibers: The Roles of Hydrogen Bonding and Amphiphilic Packing. *J. Am. Chem. Soc.* **128**, 7291–7298 (2006).
83. Pashuck, E. T., Cui, H. & Stupp, S. I. Tuning Supramolecular Rigidity of Peptide Fibers through Molecular Structure. *J. Am. Chem. Soc.* **132**, 6041–6046 (2010).
84. Stupp, S. I. & Palmer, L. C. Supramolecular Chemistry and Self-Assembly in Organic Materials Design. *Chem. Mater.*

- 26, 507–518 (2014).
85. Reches, M. & Gazit, E. Controlled patterning of aligned self-assembled peptide nanotubes. *Nat. Nanotechnol.* **1**, 195–200 (2006).
86. Reches, M. & Gazit, E. Casting Metal Nanowires Within Discrete Self-Assembled Peptide Nanotubes. *Science*. **300**, 625–627 (2003).
87. Yang, Z., Gu, H., Zhang, Y., Wang, L. & Xu, B. Small molecule hydrogels based on a class of antiinflammatory agents. *Chem. Commun.* 208–209 (2004) doi:10.1039/B310574A.
88. Vegners, R., Shestakova, I., Kalvinsh, I., Ezzell, R. M. & Janmey, P. A. Use of a gel-forming dipeptide derivative as a carrier for antigen presentation. *J. Pept. Sci.* **1**, 371–378 (1995).
89. Behrendt, R., White, P. & Offer, J. Advances in Fmoc solid-phase peptide synthesis. *J. Pept. Sci.* **22**, 4–27 (2016).
90. Merck KGaA. Aminosäuren, Harze und Reagenzien für die Peptidsynthese. <https://www.sigmaaldrich.com/DE/de/products/chemistry-and-biochemicals/amino-acids-resins-and-reagents-for-peptide-synthesis?country=DE&language=de&cmsRoute=products&cmsRoute=chemistry-and-biochemicals&cmsRoute=amino-acids-resins-and-reagents-for-peptide-> (2026).
91. Orbach, R. *et al.* Self-Assembled Fmoc-Peptides as a Platform for the Formation of Nanostructures and Hydrogels. *Biomacromolecules* **10**, 2646–2651 (2009).
92. Draper, E. R. & Adams, D. J. How should multicomponent supramolecular gels be characterised? *Chem. Soc. Rev.* **47**, 3395–3405 (2018).
93. Koshti, B. *et al.* Solvent-controlled self-assembly of Fmoc protected aliphatic amino acids. *Phys. Chem. Chem. Phys.* **25**, 11522–11529 (2023).
94. Adams, D. J. *et al.* A new method for maintaining homogeneity during liquid–hydrogel transitions using low molecular weight hydrogelators. *Soft Matter* **5**, 1856–1862 (2009).
95. Debnath, S., Shome, A., Das, D. & Das, P. K. Hydrogelation Through Self-Assembly of Fmoc-Peptide Functionalized Cationic Amphiphiles: Potent Antibacterial Agent. *J. Phys. Chem. B* **114**, 4407–4415 (2010).
96. Ma, M. *et al.* Aromatic–Aromatic Interactions Induce the Self-Assembly of Pentapeptidic Derivatives in Water To Form Nanofibers and Supramolecular Hydrogels. *J. Am. Chem. Soc.* **132**, 2719–2728 (2010).
97. Batra, R. *et al.* Machine learning overcomes human bias in the discovery of self-assembling peptides. *Nat. Chem.* **14**, 1427–1435 (2022).
98. Hsu, S.-M., Lin, Y.-C., Chang, J.-W., Liu, Y.-H. & Lin, H.-C. Intramolecular Interactions of a Phenyl/Perfluorophenyl Pair in the Formation of Supramolecular Nanofibers and Hydrogels. *Angew. Chemie Int. Ed.* **53**, 1921–1927 (2014).
99. Chagri, S. *et al.* Programming Nanostructure Formation Through Furin-Triggered Isopeptide Conversion and Peptide Self-Assembly. *Macromol. Biosci.* e00427 doi:10.1002/mabi.202500427.
100. Ren, Y. *et al.* Supramolecular Assembly in Live Cells Mapped by Real-Time Phasor-Fluorescence Lifetime Imaging. *J. Am. Chem. Soc.* **146**, 11991–11999 (2024).
101. Yang, Z., Liang, G., Guo, Z., Guo, Z. & Xu, B. Intracellular Hydrogelation of Small Molecules Inhibits Bacterial Growth. *Angew. Chemie Int. Ed.* **46**, 8216–8219 (2007).
102. Yang, Z., Liang, G., Ma, M., Gao, Y. & Xu, B. Conjugates of naphthalene and dipeptides produce molecular hydrogelators with high efficiency of hydrogelation and superhelical nanofibers. *J. Mater. Chem.* **17**, 850–854 (2007).

103. Fleming, S., Debnath, S., Frederix, P. W. J. M., Tuttle, T. & Ulijn, R. V. Aromatic peptide amphiphiles: significance of the Fmoc moiety. *Chem. Commun.* **49**, 10587–10589 (2013).
104. Frederix, P. W. J. M., Ulijn, R. V, Hunt, N. T. & Tuttle, T. Virtual Screening for Dipeptide Aggregation: Toward Predictive Tools for Peptide Self-Assembly. *J. Phys. Chem. Lett.* **2**, 2380–2384 (2011).
105. Smith, A. M. *et al.* Fmoc-Diphenylalanine Self Assembles to a Hydrogel via a Novel Architecture Based on π – π Interlocked β -Sheets. *Adv. Mater.* **20**, 37–41 (2008).
106. Sieste, S. *et al.* Supramolecular Peptide Nanofibrils with Optimized Sequences and Molecular Structures for Efficient Retroviral Transduction. *Adv. Funct. Mater.* **31**, 2009382 (2021).
107. Kaygisiz, K. *et al.* Data-mining unveils structure–property–activity correlation of viral infectivity enhancing self-assembling peptides. *Nat. Commun.* **14**, 5121 (2023).
108. Reches, M. & Gazit, E. Formation of Closed-Cage Nanostructures by Self-Assembly of Aromatic Dipeptides. *Nano Lett.* **4**, 581–585 (2004).
109. Kholkin, A., Amdursky, N., Bdikin, I., Gazit, E. & Rosenman, G. Strong Piezoelectricity in Bioinspired Peptide Nanotubes. *ACS Nano* **4**, 610–614 (2010).
110. Görbitz, C. H. The structure of nanotubes formed by diphenylalanine{,} the core recognition motif of Alzheimer{'}s β -amyloid polypeptide. *Chem. Commun.* 2332–2334 (2006) doi:10.1039/B603080G.
111. Krysmann, M. J. *et al.* Self-Assembly and Hydrogelation of an Amyloid Peptide Fragment. *Biochemistry* **47**, 4597–4605 (2008).
112. Gazit, E. A possible role for π -stacking in the self-assembly of amyloid fibrils. *FASEB J.* **16**, 77–83 (2002).
113. Sadownik, J. W. & Ulijn, R. V. Locking an oxidation-sensitive dynamic peptide system in the gel state. *Chem. Commun.* **46**, 3481–3483 (2010).
114. You, L. & Gokel, G. W. Fluorescent, Synthetic Amphiphilic Heptapeptide Anion Transporters: Evidence for Self-Assembly and Membrane Localization in Liposomes. *Chem. – A Eur. J.* **14**, 5861–5870 (2008).
115. Cheng, G., Castelletto, V., Moulton, C. M., Newby, G. E. & Hamley, I. W. Hydrogelation and Self-Assembly of Fmoc-Tripeptides: Unexpected Influence of Sequence on Self-Assembled Fibril Structure, and Hydrogel Modulus and Anisotropy. *Langmuir* **26**, 4990–4998 (2010).
116. Kraatz, H.-B., Luszyk, J. & Enright, G. D. Ferrocenoyl Amino Acids: A Synthetic and Structural Study. *Inorg. Chem.* **36**, 2400–2405 (1997).
117. Potter, N. *et al.* Tellurophene-Tagged Carfilzomib Enables Single-Cell Mass Cytometric Mapping of Proteasome Activity. *ACS Chem. Biol.* **20**, 2936–2942 (2025).
118. Raeburn, J. *et al.* Fmoc-diphenylalanine hydrogels: understanding the variability in reported mechanical properties. *Soft Matter* **8**, 1168–1174 (2012).
119. Rodriguez, A. L., Parish, C. L., Nisbet, D. R. & Williams, R. J. Tuning the amino acid sequence of minimalist peptides to present biological signals via charge neutralised self assembly. *Soft Matter* **9**, 3915–3919 (2013).
120. Gao, J. *et al.* Enzyme Promotes the Hydrogelation from a Hydrophobic Small Molecule. *J. Am. Chem. Soc.* **131**, 11286–11287 (2009).
121. Hughes, M. *et al.* Biocatalytic self-assembly of 2D peptide-based nanostructures. *Soft Matter* **7**, 10032–10038 (2011).

122. Hughes, M. *et al.* Sequence/structure relationships in aromatic dipeptide hydrogels formed under thermodynamic control by enzyme-assisted self-assembly. *Soft Matter* **8**, 5595–5602 (2012).
123. Ryan, D. M., Doran, T. M., Anderson, S. B. & Nilsson, B. L. Effect of C-Terminal Modification on the Self-Assembly and Hydrogelation of Fluorinated Fmoc-Phe Derivatives. *Langmuir* **27**, 4029–4039 (2011).
124. Makam, P. & Gazit, E. Minimalistic peptide supramolecular co-assembly: expanding the conformational space for nanotechnology. *Chem. Soc. Rev.* **47**, 3406–3420 (2018).
125. Liyanage, W., Vats, K., Rajbhandary, A., Benoit, D. S. W. & Nilsson, B. L. Multicomponent dipeptide hydrogels as extracellular matrix-mimetic scaffolds for cell culture applications. *Chem. Commun.* **51**, 11260–11263 (2015).
126. Pandeewar, M., Senanayak, S. P., Narayan, K. S. & Govindaraju, T. Multi-Stimuli-Responsive Charge-Transfer Hydrogel for Room-Temperature Organic Ferroelectric Thin-Film Devices. *J. Am. Chem. Soc.* **138**, 8259–8268 (2016).
127. Raymond, D. M. & Nilsson, B. L. Multicomponent peptide assemblies. *Chem. Soc. Rev.* **47**, 3659–3720 (2018).
128. Aida, T., Meijer, E. W. & Stupp, S. I. Functional Supramolecular Polymers. *Science*. **335**, 813–817 (2012).
129. Adler-Abramovich, L. *et al.* Controlling the Physical Dimensions of Peptide Nanotubes by Supramolecular Polymer Coassembly. *ACS Nano* **10**, 7436–7442 (2016).
130. Guo, C. *et al.* Expanding the Nanoarchitectural Diversity Through Aromatic Di- and Tri-Peptide Coassembly: Nanostructures and Molecular Mechanisms. *ACS Nano* **10**, 8316–8324 (2016).
131. Gila-Vilchez, C. *et al.* Insights into the co-assemblies formed by different aromatic short-peptide amphiphiles. *Polym. Chem.* **12**, 6832–6845 (2021).
132. Helen, W., de Leonardis, P., Ulijn, R. V, Gough, J. & Tirelli, N. Mechanosensitive peptide gelation: mode of agitation controls mechanical properties and nano-scale morphology. *Soft Matter* **7**, 1732–1740 (2011).
133. Zhou, M. *et al.* Self-assembled peptide-based hydrogels as scaffolds for anchorage-dependent cells. *Biomaterials* **30**, 2523–2530 (2009).
134. Chagri, S. *et al.* Intracellular Formation of Synthetic Peptide Nanostructures Causes Mitochondrial Disruption and Cell Death in Tumor Spheroids. *Adv. Sci.* 2412606 (2025) doi:<https://doi.org/10.1002/advs.202412606>.
135. Pieszka, M. *et al.* Controlled Supramolecular Assembly Inside Living Cells by Sequential Multistaged Chemical Reactions. *J. Am. Chem. Soc.* **142**, 15780–15789 (2020).
136. Alberts, B. The Cell as a Collection of Protein Machines: Preparing the Next Generation of Molecular Biologists. *Cell* **92**, 291–294 (1998).
137. Good, M. C., Zalatan, J. G. & Lim, W. A. Scaffold Proteins: Hubs for Controlling the Flow of Cellular Information. *Science*. **332**, 680–686 (2011).
138. Xiao, X. *et al.* De novo design of peptides that coassemble into β sheet–based nanofibrils. *Sci. Adv.* **7**, eabf7668 (2021).
139. Cheng, D. B. *et al.* Autocatalytic Morphology Transformation Platform for Targeted Drug Accumulation. *J. Am. Chem. Soc.* **141**, 4406–4411 (2019).
140. Zhou, Z. *et al.* In Situ Assembly of Platinum(II)-Metallopeptide Nanostructures Disrupts Energy Homeostasis and Cellular Metabolism. *J. Am. Chem. Soc.* **144**, 12219–12228 (2022).
141. Heitz, F., Morris, M. C. & Divita, G. Twenty years of cell-penetrating peptides: from molecular mechanisms to therapeutics. *Br. J. Pharmacol.* **157**, 195–206 (2009).

142. Frankel, A. D. & Pabo, C. O. Cellular uptake of the tat protein from human immunodeficiency virus. *Cell* **55**, 1189–1193 (1988).
143. Schnölzer, M. & Kent, S. B. H. Constructing Proteins by Dovetailing Unprotected Synthetic Peptides: Backbone-Engineered HIV Protease. *Science*. **256**, 221–225 (1992).
144. Sohma, Y. *et al.* Development of O-acyl isopeptide method. *Pept. Sci.* **88**, 253–262 (2007).
145. Coin, I. *et al.* Depsipeptide methodology for solid-phase peptide synthesis: Circumventing side reactions and development of an automated technique via depsidipeptide units. *J. Org. Chem.* **71**, 6171–6177 (2006).
146. Muir, T. W., Sondhi, D. & Cole, P. A. Expressed protein ligation: A general method for protein engineering. *Proc. Natl. Acad. Sci.* **95**, 6705–6710 (1998).
147. Dawson, P. E. & Kent, S. B. H. Synthesis of Native Proteins by Chemical Ligation*. *Annu. Rev. Biochem.* **69**, 923–960 (2000).
148. Skwarczynski, M. & Kiso, Y. Application of the O-N Intramolecular Acyl Migration Reaction in Medicinal Chemistry. *Curr. Med. Chem.* **14**, 2813–2823 (2007).
149. Dong, B. *et al.* Reversible Self-Assembly of Nanoprobes in Live Cells for Dynamic Intracellular pH Imaging. *ACS Nano* **13**, 1421–1432 (2019).
150. Waqas, M. *et al.* pH-Dependent In-Cell Self-Assembly of Peptide Inhibitors Increases the Anti-Prion Activity While Decreasing the Cytotoxicity. *Biomacromolecules* **18**, 943–950 (2017).
151. Guo, W.-W. *et al.* Intracellular Restructured Reduced Glutathione-Responsive Peptide Nanofibers for Synergetic Tumor Chemotherapy. *Biomacromolecules* **21**, 444–453 (2020).
152. Cheng, D.-B. *et al.* Endogenous Reactive Oxygen Species-Triggered Morphology Transformation for Enhanced Cooperative Interaction with Mitochondria. *J. Am. Chem. Soc.* **141**, 7235–7239 (2019).
153. Feng, Z. *et al.* Enzymatic Assemblies Disrupt the Membrane and Target Endoplasmic Reticulum for Selective Cancer Cell Death. *J. Am. Chem. Soc.* **140**, 9566–9573 (2018).
154. Wu, C., Zhang, R., Du, W., Cheng, L. & Liang, G. Alkaline Phosphatase-Triggered Self-Assembly of Near-Infrared Nanoparticles for the Enhanced Photoacoustic Imaging of Tumors. *Nano Lett.* **18**, 7749–7754 (2018).
155. Webb, B. A., Chimenti, M., Jacobson, M. P. & Barber, D. L. Dysregulated pH: a perfect storm for cancer progression. *Nat. Rev. Cancer* **11**, 671–677 (2011).
156. Trachootham, D., Alexandre, J. & Huang, P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat. Rev. Drug Discov.* **8**, 579–591 (2009).
157. Estrela, J. M., Ortega, A. & Obrador, E. Glutathione in cancer biology and therapy. *Crit. Rev. Clin. Lab. Sci.* **43**, 143–181 (2006).
158. Sun, S., Liang, H.-W., Wang, H. & Zou, Q. Light-Triggered Self-Assembly of Peptide Nanoparticles into Nanofibers in Living Cells through Molecular Conformation Changes and H-Bond Interactions. *ACS Nano* **16**, 18978–18989 (2022).
159. Ren, Y. *et al.* Intracellular assembly of supramolecular peptide nanostructures controlled by visible light. *Nat. Synth.* **4**, 673–683 (2025).
160. António, J. P. M., Russo, R., Carvalho, C. P., Cal, P. M. S. D. & Gois, P. M. P. Boronic acids as building blocks for the construction of therapeutically useful bioconjugates. *Chem. Soc. Rev.* **48**, 3513–3536 (2019).

161. Quinn, J. F., Whittaker, M. R. & Davis, T. P. Glutathione responsive polymers and their application in drug delivery systems. *Polym. Chem.* **8**, 97–126 (2017).
162. Zhang, M. *et al.* Matrix Metalloproteinase-Responsive Hydrogel with On-Demand Release of Phosphatidylserine Promotes Bone Regeneration Through Immunomodulation. *Adv. Sci.* **11**, 2306924 (2024).
163. Chien, M.-P. *et al.* Enzyme-Directed Assembly of Nanoparticles in Tumors Monitored by in Vivo Whole Animal Imaging and ex Vivo Super-Resolution Fluorescence Imaging. *J. Am. Chem. Soc.* **135**, 18710–18713 (2013).
164. Feng, Z., Wang, H. & Xu, B. Instructed Assembly of Peptides for Intracellular Enzyme Sequestration. *J. Am. Chem. Soc.* **140**, 16433–16437 (2018).
165. He, H. *et al.* Enzymatic Noncovalent Synthesis. *Chem. Rev.* **120**, 9994–10078 (2020).
166. Gao, J., Zhan, J. & Yang, Z. Enzyme-Instructed Self-Assembly (EISA) and Hydrogelation of Peptides. *Adv. Mater.* **32**, 1805798 (2020).
167. Hansen, M. J., Velema, W. A., Lerch, M. M., Szymanski, W. & Feringa, B. L. Wavelength-selective cleavage of photoprotecting groups: strategies and applications in dynamic systems. *This J. is Cite this Chem. Soc. Rev* **44**, 3358 (2015).
168. Matsuura, K. & Inaba, H. Photoresponsive peptide materials: Spatiotemporal control of self-assembly and biological functions. *Biophys. Rev.* **4**, 041303 (2023).
169. Szymański, W., Beierle, J. M., Kistemaker, H. A. V., Velema, W. A. & Feringa, B. L. Reversible Photocontrol of Biological Systems by the Incorporation of Molecular Photoswitches. *Chem. Rev.* **113**, 6114–6178 (2013).
170. Hüll, K., Morstein, J. & Trauner, D. In Vivo Photopharmacology. *Chem. Rev.* **118**, 10710–10747 (2018).
171. Deisseroth, K. Optogenetics. *Nat. Methods* **8**, 26–29 (2011).
172. Velema, W. A., Szymanski, W. & Feringa, B. L. Photopharmacology: Beyond proof of principle. *J. Am. Chem. Soc.* **136**, 2178–2191 (2014).
173. Lerch, M. M., Hansen, M. J., van Dam, G. M., Szymanski, W. & Feringa, B. L. Emerging Targets in Photopharmacology. *Angew. Chemie - Int. Ed.* **55**, 10978–10999 (2016).
174. Meinhardt, M., Krebs, R., Anders, A., Heinrich, U. & Tronnier, H. Wavelength-dependent penetration depths of ultraviolet radiation in human skin. *J. Biomed. Opt.* **13**, 44030 (2008).
175. Hang, Y., Boryczka, J. & Wu, N. Visible-light and near-infrared fluorescence and surface-enhanced Raman scattering point-of-care sensing and bio-imaging: a review. *Chem. Soc. Rev.* **51**, 329–375 (2022).
176. Klán, P. *et al.* Photoremovable Protecting Groups in Chemistry and Biology: Reaction Mechanisms and Efficacy. *Chem. Rev.* **113**, 119–191 (2013).
177. Ellis-Davies, G. C. R. Caged compounds: photorelease technology for control of cellular chemistry and physiology. *Nat. Methods* **4**, 619–628 (2007).
178. Bochet, C. G. Photolabile protecting groups and linkers. *J. Chem. Soc. { } Perkin Trans. 1* 125–142 (2002) doi:10.1039/B009522M.
179. Deiters, A. Principles and applications of the photochemical control of cellular processes. *ChemBioChem* **11**, 47–53 (2010).
180. Li, Y., Wang, M., Wang, F., Lu, S. & Chen, X. Recent progress in studies of photocages. *Smart Mol.* **1**, e20220003

- (2023).
181. Josa-Culleré, L. & Llebaria, A. In the Search for Photocages Cleavable with Visible Light: An Overview of Recent Advances and Chemical Strategies. *ChemPhotoChem* **5**, 296–314 (2021).
 182. Ellis, M. K., Hill, S. & Foster, P. M. D. Reactions of nitrosonitrobenzenes with biological thiols: Identification and reactivity of glutathion-S-yl conjugates. *Chem. Biol. Interact.* **82**, 151–163 (1992).
 183. Fournier, L. *et al.* Coumarinylmethyl Caging Groups with Redshifted Absorption. *Chem. – A Eur. J.* **19**, 17494–17507 (2013).
 184. Li, H., Wang, J., Jiao, L. & Hao, E. BODIPY-based photocages: rational design and their biomedical application. *Chem. Commun.* **60**, 5770–5789 (2024).
 185. Goswami, P. P. *et al.* BODIPY-Derived Photoremovable Protecting Groups Unmasked with Green Light. *J. Am. Chem. Soc.* **137**, 3783–3786 (2015).
 186. Boens, N., Leen, V. & Dehaen, W. Fluorescent indicators based on BODIPY. *Chem. Soc. Rev.* **41**, 1130–1172 (2012).
 187. Loudet, A. & Burgess, K. BODIPY Dyes and Their Derivatives: Syntheses and Spectroscopic Properties. *Chem. Rev.* **107**, 4891–4932 (2007).
 188. de Jong-Hoogland, D., Ulmschneider, J. & Ulmschneider, M. Computational investigation of the effect of BODIPY labelling on peptide-membrane interaction. *Sci. Rep.* **14**, 27726 (2024).
 189. Nikoloudakis, E. *et al.* Self-assembly of aliphatic dipeptides coupled with porphyrin and BODIPY chromophores. *Chem. Commun.* **55**, 14103–14106 (2019).
 190. Bassan, E., Gualandi, A., Cozzi, P. G. & Ceroni, P. Design of BODIPY dyes as triplet photosensitizers: electronic properties tailored for solar energy conversion, photoredox catalysis and photodynamic therapy. *Chem. Sci.* **12**, 6607–6628 (2021).
 191. Turro, N. J., Ramamurthy, V. & Scaiano, J. C. *Modern Molecular Photochemistry of Organic Molecules*. (University Science Books, 2010).
 192. Woodward, R. B. & Hoffmann, R. Stereochemistry of Electrocyclic Reactions. *J. Am. Chem. Soc.* **87**, 395–397 (1965).
 193. Woodward, R. B. & Hoffmann, R. The Conservation of Orbital Symmetry. *Angew. Chemie Int. Ed. English* **8**, 781–853 (1969).
 194. Foote, C. S. DEFINITION OF TYPE I and TYPE II PHOTSENSITIZED OXIDATION. *Photochem. Photobiol.* **54**, 659 (1991).
 195. Schmidt, G. . M. J. Photodimerization in the solid state. *Pure Appl. Chem.* **27**, 647–678 (1971).
 196. Kim, S. H., Sun, Y., Kaplan, J. A., Grinstaff, M. W. & Parquette, J. R. Photo-crosslinking of a self-assembled coumarin-dipeptide hydrogel. *New J. Chem.* **39**, 3225–3228 (2015).
 197. Novelli, F., Vilela, M., Pazó, A., Amorín, M. & Granja, J. R. Molecular Plumbing to Bend Self-Assembling Peptide Nanotubes. *Angew. Chemie Int. Ed.* **60**, 18838–18844 (2021).
 198. Chivers, P. R. A., Dookie, R. S., Gough, J. E. & Webb, S. J. Photo-dissociation of self-assembled (anthracene-2-carbonyl)amino acid hydrogels. *Chem. Commun.* **56**, 13792–13795 (2020).
 199. Crespi, S., Simeth, N. A. & König, B. Heteroaryl azo dyes as molecular photoswitches. *Nat. Rev. Chem.* **3**, 133–146 (2019).

200. Albert, L. & Vázquez, O. Photoswitchable peptides for spatiotemporal control of biological functions. *Chem. Commun.* **55**, 10192–10213 (2019).
201. van Rossum, S. A. P., Tena-Solsona, M., van Esch, J. H., Eelkema, R. & Boekhoven, J. Dissipative out-of-equilibrium assembly of man-made supramolecular materials. *Chem. Soc. Rev.* **46**, 5519–5535 (2017).
202. Weissleder, R. A clearer vision for in vivo imaging. *Nat. Biotechnol.* **19**, 316–317 (2001).
203. Bandara, H. M. D. & Burdette, S. C. Photoisomerization in different classes of azobenzene. *Chem. Soc. Rev.* **41**, 1809–1825 (2012).
204. Irie, M. Diarylethenes for Memories and Switches. *Chem. Rev.* **100**, 1685–1716 (2000).
205. Broichhagen, J., Frank, J. A. & Trauner, D. A Roadmap to Success in Photopharmacology. *Acc. Chem. Res.* **48**, 1947–1960 (2015).
206. Griess, P. Vorläufige Notiz über die Einwirkung von salpetriger Säure auf Amidinitro- und Aminitrophenylsäure. *Ann. Chem. Pharm.* **106**, 123–125 (1858).
207. Beharry, A. A. & Woolley, G. A. Azobenzene photoswitches for biomolecules. *Chem. Soc. Rev.* **40**, 4422–4437 (2011).
208. Woolley, G. A. Photocontrolling Peptide α Helices. *Acc. Chem. Res.* **38**, 486–493 (2005).
209. Doran, T. M., Anderson, E. A., Latchney, S. E., Opanashuk, L. A. & Nilsson, B. L. An Azobenzene Photoswitch Sheds Light on Turn Nucleation in Amyloid- β Self-Assembly. *ACS Chem. Neurosci.* **3**, 211–220 (2012).
210. Klajn, R. Spiropyran-based dynamic materials. *Chem. Soc. Rev.* **43**, 148–184 (2014).
211. He, J. *et al.* Photoswitchable supramolecular self-assembly of amphiphilic peptides enabled by backbone-integrated spiropyran. *Chem. Commun.* **61**, 16576–16579 (2025).
212. Berkovic, G., Krongauz, V. & Weiss, V. Spiropyrans and Spirooxazines for Memories and Switches. *Chem. Rev.* **100**, 1741–1754 (2000).
213. Kumar, S. *et al.* Eliminating Fatigue in Surface-Bound Spiropyrans. *J. Phys. Chem. C* **123**, 25908–25914 (2019).
214. Minkin, V. I. Photo-, Thermo-, Solvato-, and Electrochromic Spiroheterocyclic Compounds. *Chem. Rev.* **104**, 2751–2776 (2004).
215. Hirshberg, Y. & Fischer, E. Photochromism and reversible multiple internal transitions in some spiropyrans at low temperatures. Part I. *J. Chem. Soc.* 297–303 (1954) doi:10.1039/JR9540000297.
216. Siewertsen, R. *et al.* Highly Efficient Reversible Z–E Photoisomerization of a Bridged Azobenzene with Visible Light through Resolved S1($n\pi^*$) Absorption Bands. *J. Am. Chem. Soc.* **131**, 15594–15595 (2009).
217. Bléger, D. & Hecht, S. Visible-Light-Activated Molecular Switches. *Angew. Chemie Int. Ed.* **54**, 11338–11349 (2015).
218. Sell, H., Näther, C. & Herges, R. Amino-substituted diazocines as pincer-type photochromic switches. *Beilstein J. Org. Chem.* **9**, 1–7 (2013).
219. Tellkamp, T., Shen, J., Okamoto, Y. & Herges, R. Diazocines on molecular platforms. *European J. Org. Chem.* **2014**, 5456–5461 (2014).
220. Weston, C. E., Richardson, R. D., Haycock, P. R., White, A. J. P. & Fuchter, M. J. Arylazopyrazoles: Azoheteroarene photoswitches offering quantitative isomerization and long thermal half-lives. *J. Am. Chem. Soc.* **136**, 11878–11881 (2014).

221. Calbo, J. *et al.* Tuning Azoheteroarene Photoswitch Performance through Heteroaryl Design. *J. Am. Chem. Soc.* **139**, 1261–1274 (2017).
222. Mukherjee, A., Seyfried, M. D. & Ravoo, B. J. Azoheteroarene and Diazocine Molecular Photoswitches: Self-Assembly, Responsive Materials and Photopharmacology. *Angew. Chemie - Int. Ed.* **62**, e202304437 (2023).
223. Stricker, L., Böckmann, M., Kirse, T. M., Doltsinis, N. L. & Ravoo, B. J. Arylazopyrazole Photoswitches in Aqueous Solution: Substituent Effects, Photophysical Properties, and Host–Guest Chemistry. *Chem. - A Eur. J.* **24**, 8639–8647 (2018).
224. Stricker, L., Fritz, E. C., Peterlechner, M., Doltsinis, N. L. & Ravoo, B. J. Arylazopyrazoles as Light-Responsive Molecular Switches in Cyclodextrin-Based Supramolecular Systems. *J. Am. Chem. Soc.* **138**, 4547–4554 (2016).
225. Jangizehi, A., Schmid, F., Besenius, P., Kremer, K. & Seiffert, S. Defects and defect engineering in Soft Matter. *Soft Matter* **16**, 10809–10859 (2020).
226. Ikeda, T., Mamiya, J. & Yu, Y. Photomechanics of Liquid-Crystalline Elastomers and Other Polymers. *Angew. Chemie Int. Ed.* **46**, 506–528 (2007).
227. Weissleder, R. & Ntziachristos, V. Shedding light onto live molecular targets. *Nat. Med.* **9**, 123–128 (2003).
228. He, H., Feng, M., Chen, Q., Zhang, X. & Zhan, H. Light-Induced Reversible Self-Assembly of Gold Nanoparticles Surface-Immobilized with Coumarin Ligands. *Angew. Chemie Int. Ed.* **55**, 936–940 (2016).
229. Gemen, J. *et al.* Ternary host-guest complexes with rapid exchange kinetics and photoswitchable fluorescence. *Chem* **8**, 2362–2379 (2022).
230. Lohse, M. *et al.* Gating the photochromism of an azobenzene by strong host–guest interactions in a divalent pseudo[2]rotaxane. *Chem. Commun.* **51**, 9777–9780 (2015).
231. Davidson-Rozenfeld, G. *et al.* Light-responsive arylazopyrazole-based hydrogels: their applications as shape-memory materials[,] self-healing matrices and controlled drug release systems. *Polym. Chem.* **10**, 4106–4115 (2019).
232. Zhao, Y.-L. & Stoddart, J. F. Azobenzene-Based Light-Responsive Hydrogel System. *Langmuir* **25**, 8442–8446 (2009).
233. Lin, Y. *et al.* Creation of photo-modulated multi-state and multi-scale molecular assemblies via binary-state molecular switch. *Soft Matter* **6**, 902–908 (2010).
234. Zhang, H. *et al.* Reversible morphology transitions of supramolecular polymer self-assemblies for switch-controlled drug release. *Chem. Commun.* **51**, 15366–15369 (2015).
235. Meng, S., Chau, M.-H., Kajitani, T. & Leung, F. K.-C. Molecular Appendix Adjusted Architectural Propagations of Red-Light Controlled Macroscopic Photoactuating Performances. *Adv. Robot. Res.* **n/a**, e202500070.
236. Schrödinger, E. *What Is Life? The Physical Aspect of the Living Cell.* (Cambridge University Press, 1944).

Appendix

Curriculum Vitae

Name: Julian Link

[Redacted text block containing curriculum vitae details]

Scientific Publications

1. **J. Link**, L. Burg, S. Chagri, H.-C. Nguyen, D.Y.W. Ng*, B.J. Ravoo*, T. Weil*; Photoswitchable Peptides as Molecular Tools to Encode Structural Order and Disorder in Intracellular Assemblies; *Angew. Chem. Int. Ed.*, **2026**, 65, 3, e14781, <https://doi.org/10.1002/anie.202514781> & Photoschaltbare Peptide als molekulare Werkzeuge zur Kodierung von Strukturordnung und -Unordnung in intrazellulären Aggregaten *Angew. Chem.*, **2026**, 138, 3, e14781, doi:10.1002/ange.202514781
2. R. Meyer, **J. Link**, L. Gunkel, A. Lahu, H. Demirezen, T. Weil*, D.Y.W. Ng*; Light-Triggered Disassembly of Peptide Nanostructures; *ChemBioChem* **2025**, 26, 16, e202500414, doi:10.1002/cbic.202500414
3. Y.-L. Tsai, P. Cavallo, Q. Lu, J. Yu, C.P. Ender, **J. Link**, K. Amann-Winkel, K. Endres, C.V. Synatschke*, T. John*; Design of the Hydrophobic Core of Self-Assembling Peptide Fibrils for Enhanced Neural Regeneration; *Small Sci.* **2025**, 5, 10, 2500224, doi:10.1002/smss.202500224
4. S. Chagri†, K. Maxeiner†, M.J.S.A. Silva, L. Förch, **J. Link**, P. Roth, R. Meyer, J. Fetzter, A. Kaltbeitzel, I. Lieberwirth, K. Landfester, M. Wagner, D.Y.W. Ng*, T. Weil*; Intracellular Formation of Synthetic Peptide Nanostructures Causes Mitochondrial Disruption and Cell Death in Tumor Spheroids; *Adv. Sci.* **2025**, 12, 25 2412606, doi:10.1002/advs.202412606
5. M.T. Jeena, **J. Link**, J. Zhang, I. Harley, P. Turunen, R. Graf, M. Wagner, L.A. Baptista, H.R.A. Jonker, L. Cui, I. Lieberwirth, K. Landfester, J. Rao, D.Y.W. Ng*, T. Weil*; Chaperone-Derived Copper(I)-Binding Peptide Nanofibers Disrupt Copper Homeostasis in Cancer Cells; *Angew. Chem. Int. Ed.* **2024**, 63, 51, e202412477, doi:10.1002/anie.202412477 & Chaperon-Abgeleitete Kupfer(I)-Bindende Peptidnanofibrillen stören die Kupferhomöostase in Krebszellen *Angew. Chem.*, **2024**, 136, 51, e202412477, doi:10.1002/ange.202412477

† Marked authors contributed equally to this work

*Corresponding Author