Recent advances in short peptide self-assembly

DOI:

[10.1016/j.cocis.2019.08.003](https://doi.org/10.1016/j.cocis.2019.08.003)

Document Version

Accepted author manuscript

[Link to publication record in Manchester Research Explorer](#)

Citation for published version (APA):

Hu, X., Liao, M., Gong, H., Zhang, L., Cox, H., Waigh, T. A., & Lu, J. R. (2020). Recent advances in short peptide self-assembly: from rational design to novel applications. *Current Opinion in Colloid and Interface Science*, 45, 1-13. <https://doi.org/10.1016/j.cocis.2019.08.003>

Published in:

Current Opinion in Colloid and Interface Science

Citing this paper

Please note that where the full-text provided on Manchester Research Explorer is the Author Accepted Manuscript or Proof version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version.

General rights

Copyright and moral rights for the publications made accessible in the Research Explorer are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Takedown policy

If you believe that this document breaches copyright please refer to the University of Manchester's Takedown Procedures [<http://man.ac.uk/04Y6Bo>] or contact uml.scholarlycommunications@manchester.ac.uk providing relevant details, so we can investigate your claim.

Current Opinion in Colloid & Interface Science 2020, 45:1–13.

Recent Advances in Short Peptide Self-assembly: from Rational Design to Novel Applications

*Xuzhi Hu¹, Mingrui Liao¹, Haoning Gong¹, Lin Zhang¹, Henry Cox¹, Thomas A. Waigh^{1,2},
Jian R. Lu^{1*}*

¹Biological Physics Group, School of Physics and Astronomy, University of Manchester, Oxford Road, Manchester, M13 9PL, UK.

²Photon Science Institute, University of Manchester, Oxford Rd., Manchester, M13 9PL, UK.

* To whom all correspondence should be made. E-mail: j.lu@manchester.ac.uk. Tel: (+44)-161-200-3926.

Keywords: Short peptides, self-assembly, non-covalent interactions, molecular design, nanostructures, characterisation techniques, STROM, SANS, hydrogels.

Abstract

Due to their structural simplicity and robust self-assembled nanostructures, short peptides prove to be an ideal system to explore the physical processes of self-assembly, hydrogels, semi-flexible polymers, quenched disorder and reptation. Rational design in peptide sequences facilitates cost-effective manufacturing, but the huge number of possible peptides has imposed obstacles for their characterisation to establish functional connections to the primary, secondary and tertiary structures. This review aims to cover recent advances in the self-assembly of designed short peptides, with a focus on physical driving forces, design rules, characterisation methods and exemplar applications. Super-resolution microscopy in combination with modern image analysis have been applied to quantify the structure and dynamics of peptide hydrogels whilst SANS and ssNMR continue to provide valuable information on structures over complementary lengths. Short peptides are attractive in biomedicine and nanotechnology, e.g., as antimicrobials, anticancer agents, vehicles for controlled drug release, peptide bioelectronics and responsive cell culture materials.

1. Introduction

Peptides self-assemble into a diverse range of nanostructures. The field is of key interest in biomaterials science because it plays a fundamental role in fabricating hierarchical architectures that present good biocompatibility and functionalities that can be widely exploited in biotechnology and biomedicine [1]. Over the past few decades, extensive studies have been devoted to understanding peptide self-assembly, with a focus on the rational design of peptides composed of sequences shorter than 20 amino acids [2-6]. Amino acids have a broad range of physical properties, such as polarity, hydrophobicity and charge, so short peptide sequences reduce the level of complexity in structural design and synthesis costs, making it easier to establish the relationship between primary sequences, secondary and tertiary structures, and their functionalities.

Based on their chemical structures, short peptides can be classified as linear [6], cyclic [4] or branched [7]. The rich variety of amino acid side-chains means that the interactions can be diverse even within short peptides, although short peptides do present much more tractable systems to approach the problems of protein folding and aggregation. In aqueous solutions, short peptides adopt molecular conformations that minimise their total free energy. This often

means that their side-chains must be projected away from the backbone to optimise their interactions. Depending on the exact molecular sequence, secondary structures such as α -helices or β -sheets may subsequently form. The early stages of structural templating between peptide molecules are driven by intermolecular forces. The main intra- and inter-molecular forces include hydrogen bonding, hydrophobic interactions, π - π stacking, electrostatic interactions and metal ion coordination [8]. These forces often work cooperatively in the dynamic process of self-assembly. As a result, linear peptides and their derivatives often self-assemble into nanostructures, such as nanofibers, nanoribbons, nanotubes or vesicles [5]; cyclic peptides normally stack into nanotubes [9] and branched peptides often form micelles or vesicles [10].

These early stage nanostructures often template a hierarchy of structures on larger length scales and the final sizes of these self-assemblies vary greatly, from a few nanometers to hundreds of microns. These nanostructures can be influenced by environmental perturbations including solution pH, solvent polarity, ionic strength, temperature, irradiation with visible or UV light, metal ion complexation and enzymatic reactions [11]. The resultant structures often percolate the sample volume creating hydrogels that can be functionalized for specific applications, e.g., made temperature sensitive [12].

To capture the effects of different environmental conditions, different techniques must be employed to characterise primary, secondary, tertiary and hydrogel structures of the peptides [13]. These characterisation techniques include transmission electron microscopy (TEM), atomic force microscopy (AFM), super-resolution fluorescence microscopy (specifically stochastic optical reconstruction microscopy, STORM), small-angle neutron scattering (SANS), circular dichroism (CD), Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR). Due to the delicate and complex nature of the self-assembly processes these techniques are chosen, since, with the exception of TEM, they can function with aqueous solutions at room temperature with minimally invasive sample preparation.

In spite of their different sizes and shapes, short peptide nanostructures tend to have amphiphilic nanodomains and they have been extensively explored as nanocarriers for drug and gene delivery [14]. The large surface area and tuneable surface properties of peptide hydrogels also make them ideal as scaffolds for cell culturing and tissue/organ regeneration [15, 16]. Cyclic peptides tend to form nanotubes with a hollow central cavity. These

nanotubes have been extensively explored as drug carriers [17] or as model ion channels inserted into lipid bilayers [18].

We will discuss advances in short peptide self-assembly, with an initial focus on the physical forces that drive short peptide self-assembly and some rational design rules. Then we will outline some techniques employed to characterise the self-assembly processes of designed short peptides, followed by semi-flexible models for peptide hydrogels, the development of new industrial applications (particularly with functional hydrogels) and the future of the field.

2. Physical driving forces and environmental effects that determine short peptide self-assembly

In equilibrium in aqueous solution, a peptide molecule adopts a conformation that minimises its total free energy. The main forces involved in the intramolecular interactions that determine how side-chains of the constituent amino acids project into the solution include hydrogen-bonding (inter and intramolecular), π - π , cation- π , electrostatic, hydrophobic and van der Waals (vdW) interactions [8]. Peptide self-assembly refers to the association of two or more molecules mediated by these multiple physical interactions. These non-covalent interactions are relatively insignificant for isolated peptides, since they are generally weaker than covalent interactions. However, forces such as hydrogen bonding, cation- π and anion- π interactions can be reasonably long-ranged and when they act co-operatively can have energies equivalent to a weak covalent bond [19]. They thus govern the structural conformations of peptide aggregates and determine the thermodynamically stable structures. **Figure 1** shows representative non-covalent interactions and their cooperativity.

An intricate balance of the multiple non-covalent forces plays a significant role in the interaction of peptide secondary structures and can cause further aggregation through a cascade of dynamic structure transitions with short peptides. Xing et al [20] incorporated a charge-induced secondary structural transition into an Fmoc-FF model system. As a result, the self-assembled structure of the neutral Fmoc-FF changed from a typical fibrous network to longer helical fibrils after the Fmoc-FF monomer was deprotonated. The molecular configurations and intramolecular interactions experienced huge changes during this transformation due to the additional electrostatic repulsion which affected hydrogen bonding, π - π stacking and hydrophobic interactions. The secondary structural conversion of short peptides, especially the transformation between β -sheet and α -helical structures, thus underpins the subsequent self-assembly of the modified dipeptide.

Even some single amino acids can associate into α -helix structures under suitable solution pH and salt conditions, e.g., phenylalanine can form oligomers, metaclusters and even fibrillar aggregates [21]. The presence of counterions may then disrupt the stability of the phenylalanine metaclusters which rearrange themselves into more compact clusters. This results in the same structure as in the case of neutral pH. Notably, the phenylalanine monomers arrange themselves to create a hydrophilic core composed of zwitterionic termini with exposed hydrophobic aromatic side-chains on the outside. This is totally different from the ordered molecular packing formed by larger Fmoc-FF peptides due to similar π - π stacking and hydrophobic interactions [21]. The electrostatic interactions are due to the zwitterionic termini of phenylalanine monomers (a “zig-zag” interaction mode [22]) which play an important role in phenylalanine aggregation.

The main forms of non-covalent interactions that control the assembly processes of short peptides are now discussed (**Figure 1**).

Figure 1: The main non-covalent interactions that control the cooperative self-assembly of short peptides include hydrogen bonds, cation- π bonds, lone-pair- π bonds, anion- π bonds, halogen bonds and π - π bonds. Reprinted from [23] with permission from American Chemical Society.

Hydrogen bonding. This is an important driving force for the fabrication of peptide architectures, especially in the stabilisation of the peptide's secondary structure, its folding and subsequent interactions. For example, Kim et al [24] have shown that with diphenylalanine (FF) the peptide concentration tunes the ratio of hydrogen bonding between each of the FF monomers and water molecules (intermolecular hydrogen bonds). Ions can also reduce the number of free water molecules around each FF monomer, presumably weakening hydrogen bonding between the peptides and the water molecules. Additionally, elevated temperature and sonication may break intermolecular hydrogen bonds, but increase the hydrophobic effect. The hydrogen bond strength modulated by these kinetic factors enables structural transitions between nanowires and nanotubes [25]. More importantly, the selectivity and high directionality of hydrogen bonds can convert short peptides into diverse 1-dimensional (1-D), 2-D and 3-D nanostructures [26]. Whilst the main hydrogen bonds are formed between the peptide backbones, the side-chains of several amino acids carry hydroxyl, carboxylic, amine, amide and imidazole groups, which also participate in hydrogen bonding, altering the direction of structural templating. Because some of these polar groups can dissociate in a pH-dependent manner, the exact self-assembling cascades for a given short peptide may be adjusted by altering solution pH as well as ion type and ionic strength [27].

Hydrophobic interactions. In spite of its few directional constraints, hydrophobicity is a well-established driving force in surfactants and thus surfactant-like peptide systems. For example, molecular dynamics (MD) simulations of Stupp et al [28] showed how the self-assembly process of amphiphilic peptides is tuned by the relative strength of hydrogen bonding compared with the hydrophobic interaction. They found that a dominant hydrophobic interaction leads to micellar structures, while dominant hydrogen bonding leads to the aggregation of 1D β -sheets, resulting in very different aggregate shapes. Individual amphiphilic molecules cannot interact favourably with water molecules in bulk solution, but as their concentration increases, the hydrophobic parts of these molecules tend to aggregate to minimize their surface area in contact with water, which leaves their hydrophilic parts exposed to water [29]. Such hydrophobic interactions are inherently entropic effects rather than enthalpic. Hydrophobic forces in peptide amphiphiles can be adjusted by selecting an appropriate acyl or alkyl tail group. In dipeptides, such as Fmoc-FF, hydrophobic interactions can be enhanced with an increase of temperature. The aromatic residues of peptide building blocks may play an important role through either hydrophobic interactions or π - π interactions:

the organization mode of the aromatic residues in the hydrophobic interaction is commonly disordered, whilst in π - π interactions it is well-ordered.

π - π and cation- π interactions. π - π stacking can drive self-assembly from π -conjugated peptides using amino acids such as phenylalanine or tyrosine [30]. Importantly, the π - π stacking can induce directional peptide growth. The interaction is often very robust in water because of the limited solubility of the molecules containing the aromatic groups. The π - π stacking can also be a distinct driving force in organic solvents such as toluene. Moreover, the introduction of a cationic residue at the N-terminus and an aromatic residue at the C-terminus of a collagen-related peptide can generate a “head-to-tail” assembly behaviour. Such cation- π interactions can promote the self-assembly of collagen triple helices into higher-order structures and serves as an effective force similar to π - π interactions in peptide self-assembly.

Electrostatic interaction. Screened Coulombic interactions between opposite charges in solution can lead to the formation of ion pairing. The strength of an ionic bond is dependent on the solvent (particularly its dielectric) and the presence of mobile ions. Ionic bonds are generally stronger and of longer range than hydrogen bonding [31], although ionic interactions at long distances can be weaker than hydrogen bonds at much smaller distances (both are also affected by salt in non-trivial ways). Unlike the roles of hydrogen bonding and hydrophobic interactions, electrostatic interactions often provide structural specificity for charged peptides. The importance of various electrostatic interactions in the process of the formation of peptide nanostructures has been reviewed by Rehm et al [32]. Previously, Voyer et al [33] described the self-assembly of a crown ether functionalized peptide via the mediation of electrostatic interactions. In the absence of specific metal ions, the peptide adopts a β -sheet type structure which is not affected by sodium or potassium cations. However, Cs^+ can form a sandwich complex with the crown ether and thus leads to macrocyclisation. This mechanistic action changes the peptide from a β -sheet conformation to a β -turn conformation. The effect of screened electrostatic interactions on the aggregation of peptides is a subtle field. The most relevant references are still the calculations of critical micelle concentrations for charged surfactants [34] and the assembly of larger protein systems [35].

van der Waals (vdW) interaction. vdW is of comparable strength to hydrogen bonding, but it is non-selective, non-directional and non-additive [31]. The vdW forces, such as the

interactions between aliphatic tails in peptide amphiphiles, represent the main contribution to the non-covalent interactions and are ubiquitous in peptide self-assembly. There are however only a few examples that employ vdW interaction as a dominant force for the control of peptide nanostructuring [36, 37]. The self-assembly of oligopeptides (FKFEFKFEFKFE) occurred when solution conditions reduced intermolecular electrical double-layer repulsion below the threshold of vdW attraction, e.g., when FKFEFKFEFKFE forming gels were neutralized with NaOH.

Synergistic effects of non-covalent interactions. Rather than using one single type of interaction, the combination of different types of non-covalent interactions is one of the most efficient approaches to achieve stable self-assembly for the fabrication of nanostructures. Recently, Ji et al [38] achieved a secondary structural transformation of amyloid-like dipeptides through metal ion-modulation (including different types and concentrations of metal ions) from an amyloid-like β -sheet into a superhelix or random coil due to a mechanism involving multiple interactions including metal coordination, hydrogen bonding and π - π stacking. Similarly, aromatic tripeptide amphiphiles (Fmoc and pyrene peptides) are able to stabilise emulsions by the formation of nanofibrous networks at the oil/water interface through a combination of π -stacking and hydrogen bonding interactions [39].

Environmental effects. Kinetic structural control of short peptides is accomplished by multiple factors including pH, solvent, temperature and external stimuli. Slight changes in pH (between 6.0 and 6.5) can modulate the protonation of histidine residues; this results in the reversible disassembly of cylinder and sphere-forming peptide amphiphiles in acidic solutions [40]. Stupp et al [41] showed that hydrogen bonding, π - π , and hydrophobic interactions all tend to promote the aggregation of amphiphilic peptide molecules, whereas electrostatic repulsion causes their disassociation. Both the final properties and structures of assemblies reflect a delicate balance of the contributions from these interactions [42]. For instance, Stupp et al. [43] found that through changes in pH and concentration, nanobelts can transform into twisted ribbons due to the disruption of the balance of electrostatic repulsions and hydrogen bonding interactions. Sonication is also an effective method to make the kinetic control over the rearrangement of nanostructures by assisting peptide molecules to overcome the energy barriers of intermolecular non-covalent interactions. Ulijin et al. [44], found that tripeptides can form anisotropic gels that depend on the peptide sequence and solvent environment through directional ultrasonication. Solvent properties, such as polarity and

propensity for hydrogen bonding formation, will directly influence local interactions of peptide self-assembly at the molecular level. Dispersion, dipole-dipole and hydrogen bonding interactions are the three main components that determine the Hansen solubility parameters for a solvent [45]. Wang et al [46] reported that the incorporation of the counterion ferrocene with FF changes the conformation of the secondary structures from flat β -sheets into twisted β -sheets. Furthermore, through subtle modulations of counterion, temperature, and solvent, they could precisely control both the helical pitch and diameter, and the handedness of the self-assembled chiral nanostructures. The driving forces for forming twisted nanostructures are thus a balance of π - π stacking, hydrogen bonding and the chirality of ferrocene modified-FF molecules.

3. Simple design rules

There are some 20 polymerising amino acids that occur in eukaryotes which are either polar, apolar or charged, and the number of possible combinations for forming short peptides, such as those containing 3 amino acids, is high i.e. $\approx 20^3 \times 2 = 16,000$, where the factor of 2 is from the directionality of the peptide bond and the number is slightly overestimated due to symmetric sequences e.g. IKI would be double counted. In addition, it is possible to modify the topology of a short peptide so it is a linear chain or a circular ring with the first amino acid binding to the last one [4]. Furthermore, some amino acids have more than one active group, e.g. lysine has amine groups at both α and ϵ positions. Therefore, when this amino acid is bonded via either α or ϵ amine groups in the middle of a linear sequence of amino acids, it is possible to link with another side chain of a peptide sequence, resulting in a branched peptide [10]. Although molecular geometry plays an important role in the self-assembly of short peptides, for simplicity we focus on several simple design rules based on amino acid substitution, sequence variation and chirality.

3.1 Amino acid substitution

Because of the vastly different sizes and amphiphilic varieties of the 20 naturally occurring amino acids their different propensities for forming α -helices or β -sheets are widely exploited in peptide design. Both extensive experimental and computational studies have investigated the conformations of natural amino acids and dipeptides in aqueous solutions [47]. These studies are important for di- and tripeptides of practical relevance. By appropriate amino acid substitutions in a peptide, one or more non-covalent interactions can be altered. For instance, we showed that Ac-I₃K-NH₂ peptides self-assemble into long nanofibres due to the high hydrophobicity and strong propensity for β -sheet hydrogen bonding of isoleucine. Once the

three isoleucine amino acids are replaced by three leucine residues, only spherical nanostructures are observed for Ac-L₃K-NH₂ peptides [48]. In spite of similar hydrophobicity, I and L have different β -sheet propensities (-1.25 kcal/mol for I and -0.45 kcal/mol for L). This leads to a lack of β -sheet hydrogen bonding in Ac-L₃K-NH₂ and the absence of nanofibers.

3.2 Amino acid sequence variation

Intermolecular interactions can be simply altered when amino acid sequence is changed and the amino acid composition remains the same. Cui et al [49] have used the peptide C₁₆H₃₁O-VEVE and its analogues to show how sequence affects the resultant self-assembled structures. C₁₆H₃₁O-VEVE and C₁₆H₃₁O-EVEV with alternating hydrophilic and hydrophobic amino acid sequences form nanoribbons, while C₁₆H₃₁O-VVEE and C₁₆H₃₁O-EEVV self-assemble into nanofibers. This is because the alternating sequences lead to peptide molecular dimerization and eliminate the interfacial curvature between acyl tails and peptide fragments. As a result, the lateral association of the β -sheets is promoted and peptides form nanoribbons. With the block sequences, dimerization does not occur and only nanofibres are formed.

3.3 Amino acid chirality

All 20 eukaryotic amino acids, except glycine, are chiral and their chirality plays an important role in peptide aggregation. Amino acid chirality has been widely used to mediate the molecular structures of cyclic peptides. Ghadiri et al [50] demonstrated that circulating alternative D, L-amino acids can form cyclic peptides with a hollow centre and side-chains pointing outside the ring. The cyclic peptides can adopt β -sheet conformations and thus stack into nanotubes. Recently, Wang et al. designed ^LI₃^LK, ^DI₃^DK, ^LI₃^DK, ^DI₃^LK, ^{La}I₃^LK and ^{Da}I₃^DK peptides [51] to investigate the effects of amino acid chirality on the handedness of the self-assembled nanostructures. Unusually, the handedness of the twisted nanofibers is determined by the chirality of the C-terminal K amino acid for this type of short peptide.

4 Characterisation techniques

The dynamic reversible nature of the non-covalent interactions that contribute to the formation of short peptide nanostructures offers the ability to respond to external stimuli. However, these dynamic hierarchical self-assembly processes also make them difficult to characterise. Multiple characterisation techniques must be applied to gain information of different time and length scales. The most popular methods that are employed can be roughly classified as imaging, spectroscopy and scattering. Imaging techniques such as TEM, AFM

and optical microscopy can give valuable information about self-assembled morphologies and geometric parameters, such as size and diameter, typically in the range of 5 nm to 500 μm [52]. In contrast, spectroscopic methods including CD, NMR and FTIR can reveal the secondary structures, such as α -helix, β -sheet and random-coiled conformations. Although these structures can propagate along the major axial length well beyond 50 nm, the local structures that are detected between neighbouring molecules are usually <5 nm. Scattering techniques (e.g. SANS or light scattering) are widely used, but interpretation of results in reciprocal space can be demanding, although it is a large and well-developed field. Many of these techniques require specialised sample preparation and data analysis. Over the past few years, there have been many improvements facilitating the use of these imaging, spectroscopy and scattering tools. We consider in detail how STORM, SANS and ss-NMR can be integrated with more conventional techniques by referring to examples selected from our own work. **Figure 2** summarises these typical techniques in terms of the length scales and structures they can detect.

Figure 2: Typical length scales probed by different characterisation techniques combined with representative images of small peptide aggregates. STORM and AFM images are reprinted from [53], TEM images and SANS profiles are reprinted from [54] and CD, FTIR and NMR spectra were remodified from [55] with permissions from American Chemical Society, Wiley and Nature, respectively.

STORM and single-molecule fluorescence microscopy. STORM (stochastic optical reconstruction microscopy) is a promising super-resolution fluorescence microscopy technique and we have demonstrated its use with short peptides over the last 2 years [56]. For a typical STORM experiment, self-assembling peptides are first labelled with fluorescent dyes. The dye molecules are then activated by lasers to induce a blinking state. This allows the position of each dye molecule to be established with very high precision and the super-resolution images are constructed by combining all of the localisations together. A distinct advantage of STORM is that it allows direct imaging of the assemblies in solution at room temperature with minimally invasive sample preparation. A combination of STORM with AFM allowed us to follow the dynamic self-assembling process of a short peptide I₃K [12, 53, 57]. The AFM image shown in **Figure 2** indicates that I₃K forms nanofibres with diameters of 5-20 nm. Although the diameters of this range are slightly beyond the STORM resolution (~20 nm), the single colour STORM images of I₃K conjugated to Cy3B fluorescent dye confirm the fibre shape (allowing the persistence length and mesh size to be calculated), but the fibril density is much lower than that observed from AFM imaging. This is because STORM images are recorded in bulk solution whilst the drying process applied before AFM scanning causes artificial condensation of layers of peptide nanofibrils onto the substrate surface. Another advantage of STORM is its ability to distinguish between peptides of the same variety by separately labelling the molecules with different dyes, known as multi-colour STORM. In the same work, we examined the long-term dynamic self-assembly process by mixing I₃K fibres labelled with Cy3B dye and Alexa Fluor 647 dye respectively. The two-colour STORM image shown in **Figure 2** indicates that the long nanofibers were formed without significant monomer exchange during the formation. This work demonstrates the feasibility of using STORM to follow dynamic events associated with different stages of self-assembly of short peptides by directly imaging the structural segments formed.

Using the same microscope as the STORM experiments, but with different data analysis and a slightly different optical set up it is possible to image the dynamics of single peptides (**Figure 3**). A rich range of information is then available on the dynamics of peptide fibres, which were analysed with a snakes image processing algorithm and then Fourier analysis, which provided evidence of prestressed fibres in the gel experiencing motion in reptation tubes (directly measured from the microscopy technique). These measurements were then extended to responsive short peptide gels that were mixed with PNIPAM (poly(N-

isopropylacrylamide) and temperature was observed to modulate the number of states of prestress.

Figure 3. A) A peptide fibril in a state of pre-stress inside a gel measured with single-molecule fluorescence microscopy. B) The reptation tube width as a function of distance along the fibre. Minima in the width as associated with sticky intra-chain cross-links. C) Probability distribution of tube widths for a single fibre has two populations: the smaller tube width is due to the cross-links (Gaussian) and the larger is due to the reptation dynamics (log-normal). The figure is reprinted from [57] with permission from American Chemical Society.

SANS. SANS is a non-destructive technique, which measures the intensity of a neutron beam scattered from a peptide solution. The scattering intensity profile contains information about the nanostructures formed from self-assembly of a short peptide. Structural features can be extracted by first calculating a theoretical scattering profile from an analytic model for simple geometrical shape(s) which is then fitted to the measured data. As the typical length scale for SANS is 0.3 - 100 nm, SANS is capable of unravelling the detailed structures of short peptide self-assemblies beyond the resolution of conventional imaging techniques [58]. Our recent work [54] has used both TEM and SANS to study the self-assemblies of 2 sets of hexapeptides, with Set I incorporating Ac-KI₄K-NH₂, Ac-KI₃NleK-NH₂, Ac-KI₃LK-NH₂ and Ac-KI₃TleK-NH₂ carrying different branching, but similar hydrophobicity, and Set II containing Ac-KI₃VK-NH₂, Ac-KI₂V₂K-NH₂, Ac-KIV₃K-NH₂ and Ac-KV₄K-NH₂ carrying similar branching, but different hydrophobicity. The TEM images indicated that whilst Ac-KI₃TleK-NH₂ and Ac-KV₄K-NH₂ have few ordered aggregates all the rest of the peptides self-assembled into tubular structures with different widths: 100-230 nm for Ac-KI₄K-NH₂, 38 ± 8 nm for Ac-KI₃VK-NH₂, and 5-15 nm for the remaining ones, with a typical set of images shown in Figure 2. As the detailed structural features, including inner radius and wall thickness, are beyond standard TEM resolution, we performed further SANS investigations.

The profile shown in Figure 2 for Ac-KI₄K-NH₂ was fitted to a lamellar model whilst those for the other peptides were fitted to a hollow cylindrical model. The wall thicknesses were measured as 2 nm for all self-assembled nanotubes, indicating that the wall was formed by a peptide monolayer. Apart from Ac-KI₄K-NH₂, inner radii were found to be 17 nm for Ac-KI₃VK-NH₂ and 2-4 nm for the rest, consistent with TEM observations. Thus, the combined TEM and SANS measurements revealed that variations in core side-chain branching and hydrophobicity can control peptide nanotube diameters. This work also showed that SANS is highly complementary to TEM and AFM imaging techniques in terms of unravelling detailed structural features, such as the inner diameter and wall thickness for self-assembled nanotubes.

Solid-state NMR (ss-NMR). NMR is commonly used to complement CD and FTIR in determining the molecular packing of self-assembled aggregates of short peptides. However, solution NMR measurements are often constrained by the mobility of peptide molecules, because molecular assembly usually broadens resonances from aggregates, reduces the intensity and shifts the frequency, making it difficult to interpret the spectra [13]. An alternative approach is either to focus on the small mobile molecules and ions dissolved in the solution [59] or to use ss-NMR to examine peptide self-assemblies (that are often formed from isotope labelled peptides such as ²H, ¹³C, ¹⁵N and ³¹P to allow specific labelling). We have previously examined the secondary structures self-assembled from a series of short peptides Ac-I₃XGK-NH₂ (X = Q, S, G, V) [55]. Our CD and FTIR results are shown in Figure 2. They indicate that the self-assembled aggregates adopted β -sheet conformations characterised by a positive CD maximum at ~200 nm, a negative CD minimum at ~220 nm and an FTIR band at ~1620 cm⁻¹. To determine how peptide molecules were packed within the β -sheet, one ¹³C- and one ¹⁵N-labelled Ile amino residue was incorporated into the sequence of I₃QGK, denoted as {¹⁵N}Ile1{¹³C}Ile3-Ac-I₃QGK-NH₂. The ¹H-¹³C cross polarisation spectrum by solution NMR confirmed a conformational homogeneity of the labelled peptide. The interatomic distance between neighbouring β -strands within a sheet was then determined by ¹³C{¹⁵N} rotational-echo double-resonance (REDOR) NMR. The REDOR curve together with MD simulations indicated two inter-strand ¹³C-¹⁵N distances of 7.4 and 8.1 Å, which implied a single residue shift in the anti-parallel packing occurred within the sheets as shown in **Figure 2**. This work clearly showed the complementary role of NMR, CD and FTIR in determining the precise molecular packing mode within β -sheet structures formed by peptide self-assembly.

5. Models for the scientific understanding of hydrogels

Hydrogels of semi-flexible polymers have been intensively studied due to their importance in biological systems, e.g., actins, microtubules, fibrins [35, 60]. Short peptides present a model system for pursuing the scientific understanding of hydrogels due to their low price, high purity, simplicity and robust long-lived self-assembled structures. For example, the diameters of short cationic peptide aggregates are often weakly dependent on peptide concentration. Using a combination of dynamic fluorescence imaging with static super-resolution fluorescence imaging, image analysis and machine learning (a self-consistent Bayesian mixture model) we were able to explore some new aspects of the physical properties of these short peptide hydrogels [53]. We found that self-assembled I₃K gels contain substantially more prestressed nanofibres than expected from Boltzmann statistics. Thus quenched disorder occurs in these gel systems and it is probably generic in a wide range of biopolymer gels, e.g., actins, microtubules, fibrins [60]. Furthermore, mixed systems of I₃K with PNIPAM that were temperature-sensitive gels, allowed the number of fibres in a state of prestress to be actively modulated [12]. The dynamic studies were also able to observe the reptation tubes of the fibres in the gels and observe the effects of light cross-linking on the internal fibre dynamics (**Figure 3**).

6. Applications

Research into the self-assembly of designed short peptides is driven by their potential in biomedical and biotechnological applications. A wide range of nanostructures formed by peptide bionanomaterials and related functional properties have been reported with potential applications in biomedical and biotechnological sectors. Nanofibrous architectures of supramolecular peptide hydrogels have been used as 3D scaffolds in cell culture and tissue regeneration [16, 61]. Nanotubular structures have been used as artificial membrane channels and biosensors [18, 62], whilst short peptide micelles are employed as gene or drug carriers [63].

6.1 Antimicrobial peptide nanofibers

Antimicrobial peptides (AMPs) kill bacteria via a rapid membrane disruptive mechanism with low multiple drug resistance (MDR) [64] [65]. Compared with many natural and biomimetic AMPs, designed AMPs can have their *de novo* sequences optimised, are easier to synthesise and scale up and more importantly, they do not tend to illicit unknown side effects. Furthermore, structural design and optimisation can make them selective against certain

pathogens, with little toxicity to the host mammalian cells. Most designed AMPs are cationic and amphiphilic. They select microorganisms by an initial electrostatic interaction, followed by subsequent hydrophobic interactions. Because the membrane-lytic processes are fast (on the time scale of minutes) and the killing is caused by leakage of internal materials due to structural disruption of the microbial membranes, the occurrence of resistance is less likely. In contrast, traditional antibiotics kill by slower genetic, metabolic or enzymatic interferences which are on the time scale of hours and days.

Recent studies [66-68] have revealed that the self-assembled nanostructures of short peptides can also have significant antimicrobial activity in spite of their relatively weak membrane disrupting power in the monomeric form. Schnaider et al [68] showed a higher bactericidal activity of the nanofibers self-assembled from FF dipeptides than the non-assembled GG and FF dipeptides. These results show that peptide self-assemblies with longer length and multiple cationic charges confer a greater ability to disrupt bacterial membranes. Their work has demonstrated an attractive strategy to develop cost effective AMP nanostructures against MDR infections.

6.2. Smart delivery platforms

Current drug delivery using self-assembling peptide systems is focused on “smart” processes. In these processes the bioactives (drug, protein, DNA or RNA) can be loaded and transported in peptide nanocarriers, and released in a controlled manner at the targeted sites [69]. Thus, the peptides need to either respond to specific biological environments, e.g., through enzymatic cleavage, or use other forms of drug incorporation to provide responsiveness or triggering. Recent work by He et al [10, 70] and Cao et al [71] showed smart drug release by the enzymatic cleavage of two peptide derivatives, Nap-FFK(^εG-FLAG)Y and Nap-FFGPLGLARKRK, respectively. In the former case, the peptide conjugated to a protein tag allows self-assembly into micelles that can trap small drug molecules. There is also a sequence of amino acids in the molecule that can be triggered by an enterokinase or enteropeptidase in mitochondria. After being taken up by eukaryotic cells, the micelles are turned into nanofibers through the action of the enzyme, thus releasing the cargos from the micelles at mitochondria. In the latter case, the peptide self-assembles into long nanofibers with hydrophobic inner cores which encapsulate a considerable amount of anticancer drugs. The enzyme-cleavable fragment of -GPLGLA- can be activated by certain enzymes at the tumour sites, leading to fibre degradation and thus release of the drug. In spite of different structural changes, both systems together showcased a new approach using enzymatic

cleavage to realise controlled drug release at the targeted sites. Moreover, recent studies from Yan et al [72-74] directly used hydrophobic photosensitizers as peptide carriers for a photodynamic therapy (PDT). The clear advantage lies in the flexible control of carrier sizes and thus drug loading by simple adjustment of the ratio of photosensitizer to peptide. In this case, the use of the carrier peptide material was substantially reduced and drug loading efficiency was significantly enhanced. **Figure 4** summarises representative progresses in recent smart drug delivery platforms using short peptide self-assembly.

Figure 4. (a): smart drug release by the enzymatic cleavage of Nap-FFGPLGLARKRK, reprinted from [71], with permission from American Chemical Society; (b): smart drug release by the enzymatic cleavage of Nap-FFK(^EG-FLAG)Y, reprinted from [70], with permission from American Chemical Society; (c): fabrication of photosensitive nanocarriers by amphiphilic dipeptide- or amino-acid-tuned self-assembly, reprinted from [72], with permission from Wiley.

6.3 Peptide bioelectronics

A big fundamental challenge in the field of peptide bioelectronics is whether the electricity is conducted by electrons or protons in peptides, e.g. electrons are transferred across conjugated bonds (as per conventional organic electronics) or the hopping of H^+ ions associated with acidic groups. The field is still wide open in this respect and has enormous implications for the design of peptides for bioelectronics [75]. Conventional inorganic semi-conductors have

poor biocompatibility, which limits their use in biological systems. A promising candidate for bioinspired semiconductors is self-assembled architectures formed by short peptides. Some peptide materials, especially the F- or W- based aromatic dipeptide assemblies, display semi-conductivity via π - π interactions [76]. The π -electrons and protons are generated from the interlocked aromatic groups and hydrogen bonds of peptide β -sheets, respectively, functioning as quantum dots. Tao et al [77] showed that a cyclic dipeptide (cyclo-GW) adopted mechanically robust and thermally stable monoclinic crystals, with an elastic modulus up to 24 GPa and a transition temperature up to 370 °C. These characteristics arise from its molecular packing which includes β -sheet hydrogen bonding and herringbone type aromatic interactions. The directional driving forces of self-assembling groups further conferred unique piezoelectric properties, so that the crystals are capable of producing a voltage of 1.2 V because of its high piezoelectric coefficient of 14.1 pC N⁻¹. This work demonstrated the feasibility of using peptide self-assemblies as microelectronic devices in biological systems.

7. Concluding remarks and future perspectives

Designed short peptides can self-assemble into diverse nanostructures that attract ever-increasing attention for applications in biomedicine and biotechnology. The self-assembly process of short peptides is mostly driven by non-covalent forces that depend upon their molecular structures. Their design has largely focused on their constituent units, i.e. natural amino acids. Due to the complex dynamic and structural features at different length scales, various characterisation techniques have been employed to follow the self-assembly processes of rationally designed short peptides and seek new applications for them.

Three powerful but not widely used methods were introduced, including STORM, SANS and ss-NMR which are highly complementary to conventional techniques such as AFM and TEM which are widely adopted in characterising the self-assemblies of short peptides. Next generation high resolution spectroscopy methods are becoming available, such as non-linear Raman [78], nitrogen vacancy single-molecule NMR [79], 2D IR and frequency comb IR [80]. It would be fascinating to study small peptide aggregation with these methodologies.

Progress has been made in exploring further physical properties of short peptide hydrogels. Strong evidence has been provided that states of prestress exist in the gels of short peptide fibres. However, quantitative models still require development and it would be informative to examine the impact of cross-link rigidity, geometry (e.g. coordination number and angle) and

life time on their existence (and cooperative effects such as floppy modes and states of self-stress) [81]. It would also be interesting to make more detailed studies of the non-linear rheology of peptide nanofibres, including shear banding, elastic turbulence and wall slip effects [82] which would be useful in understanding their performance in ink jet printing and processing applications.

Small peptide gels tend to be fairly fragile (with moduli <kPa), so it will be important to investigate composite gels to improve their mechanical performance, e.g., for wound healing dressings where the peptides provide the antimicrobial component and the second component provides the strength. Double gels [83] and synergistic mixed gels [12] are both effective strategies to make peptide composite gels. For antimicrobial activity, STORM allows live bacteria to be imaged [84] and can successfully image peptides as they interact with bacterial membranes allowing their modes of action to be quantified.

Although natural and biomimetic AMPs have been investigated for their antimicrobial and anticancer behaviour, rationally designed AMPs bring distinct advantages including structural simplicity, shorter sequences, avoidance of side effects and low cytotoxicity. A highly promising area lies in exploring how nanostructures self-assembled from short cationic peptides can be used in antimicrobial and anticancer development. Because almost all designed small peptides can form amphiphilic nanodomains, they can be explored as nanocarriers for drug delivery, e.g., for hydrophobic drugs. However, unlike conventional drug delivery, short peptide drug delivery focuses on carrier design and controlled release that is responsive to the environment. Short peptide based nanomaterials have also shown piezoelectric properties with well-designed molecular packing combining herringbone type aromatic stacking and hydrogen bonding in β -sheet conformations, demonstrating the potential to work as peptide based microelectronic devices.

Another novel application of self-assembled short peptides is as efficient transmembrane transporters. Such endeavours require careful structural design of the short peptide with consideration of its selectivity, insertion into the target membrane, mediation of self-assembly and interaction with membrane lipids. Despite such daunting challenges, work in short peptide research has been largely driven by this type of application-led challenges and will remain so in the future. Advances in the technical capabilities of characterisation techniques will allow us to follow *in situ* self-assembling processes, e.g., the observations of short peptide self-assemblies and their responses to varied environments by using combined

measurements from SANS, ssNMR and STORM. In addition, computational modelling of the self-assembly of short peptides and their interactions with macromolecules, such as polymers or biomembranes, has evolved to make an important impact on the development of functional short peptides. The combination of simulations and experiments will help experimental design and data interpretation to develop a predictive capability, making the search for functional short peptides more efficient [12, 85]. With the knowledge, skills and technical capabilities accumulated so far, there are good reasons to believe that we are closer to expanded market successes for designed short peptide products over the next few years.

Acknowledgements

We thank the University of Manchester for support in the form of an overseas research studentship to X.H, M.L, H.G. and L.Z., an award from China Scholarship Council to M.L., L.Z. and H.G., research fund support from Syngenta to X.H., We thank ISIS Neutron Facility, STFC and Institut Laue Langevin, Grenoble for awards of neutron beam times. This work also benefited from the grant support from EPSRC (EP/F062966/1), Innovate UK (KTP009043) and a Marie Curie Fellowship ITN grant (grant number 608184) under SNAL (Small nano-objects for alteration of lipid-bilayers).

Papers of particular interest

* of special interest.

** of outstanding interest.

References

- [1] Zhao X, Pan F, Xu H, Yaseen M, Shan H, Hauser CA, et al. Molecular self-assembly and applications of designer peptide amphiphiles. *Chemical Society Reviews*. 2010;39:3480-98.
- *[2] Zhang S. Fabrication of novel biomaterials through molecular self-assembly. *Nature Biotechnology*. 2003;21:1171.
This is an early classic review on short peptide self-assembly.
- [3] Yan X, Zhu P, Li J. Self-assembly and application of diphenylalanine-based nanostructures. *Chemical Society Reviews*. 2010;39:1877-90.
- *[4] Chapman R, Danial M, Koh ML, Jolliffe KA, Perrier S. Design and properties of functional nanotubes from the self-assembly of cyclic peptide templates. *Chemical Society Reviews*. 2012;41:6023-41.
This is a classic review on design, structures and applications of cyclic peptide nanotubes.
- [5] Tao K, Levin A, Adler-Abramovich L, Gazit E. Fmoc-modified amino acids and short peptides: simple bio-inspired building blocks for the fabrication of functional materials. *Chemical Society Reviews*. 2016;45:3935-53.

*[6] Zhao Y, Yang W, Chen C, Wang J, Zhang L, Xu H. Rational design and self-assembly of short amphiphilic peptides and applications. *Current Opinion in Colloid & Interface Science*. 2018;35:112-23.

This is a recent review about rational designed amphiphilic peptide self-assembly and applications.

[7] Guler MO, Soukasene S, Hulvat JF, Stupp SI. Presentation and recognition of biotin on nanofibers formed by branched peptide amphiphiles. *Nano Letters*. 2005;5:249-52.

[8] Wang J, Liu K, Xing R, Yan X. Peptide self-assembly: thermodynamics and kinetics. *Chemical Society Reviews*. 2016;45:5589-604.

[9] Chapman R, Jolliffe KA, Perrier S. Multi-shell Soft Nanotubes from Cyclic Peptide Templates. *Advanced Materials*. 2013;25:1170-2.

[10] He H, Wang H, Zhou N, Yang D, Xu B. Branched peptides for enzymatic supramolecular hydrogelation. *Chemical Communications*. 2018;54:86-9.

[11] Zou R, Wang Q, Wu J, Wu J, Schmuck C, Tian H. Peptide self-assembly triggered by metal ions. *Chemical Society Reviews*. 2015;44:5200-19.

[12] Cox H, Cao M, Xu H, Waigh TA, Lu JR. Active modulation of states of pre-stress in self-assembled short peptide gels. *Biomacromolecules*. 2019;20:1719-30.

*[13] Draper ER, Adams DJ. How should multicomponent supramolecular gels be characterised? *Chemical Society Reviews*. 2018;47:3395-405.

A recent review summarises popular characterisation techniques for multicomponent supramolecular gels.

[14] Avila L, Aps L, Ploscaiu N, Sukthankar P, Guo R, Wilkinson K, et al. Gene delivery and immunomodulatory effects of plasmid DNA associated with branched amphiphilic peptide capsules. *Journal of Controlled Release*. 2016;241:15-24.

[15] Harrington DA, Cheng EY, Guler MO, Lee LK, Donovan JL, Claussen RC, et al. Branched peptide-amphiphiles as self-assembling coatings for tissue engineering scaffolds. *Journal of Biomedical Materials Research Part A: An Official Journal of The Society for Biomaterials, The Japanese Society for Biomaterials, and The Australian Society for Biomaterials and the Korean Society for Biomaterials*. 2006;78:157-67.

[16] Moore AN, Hartgerink JD. Self-assembling multidomain peptide nanofibers for delivery of bioactive molecules and tissue regeneration. *Accounts of Chemical Research*. 2017;50:714-22.

[17] Chen J, Zhang B, Xia F, Xie Y, Jiang S, Su R, et al. Transmembrane delivery of anticancer drugs through self-assembly of cyclic peptide nanotubes. *Nanoscale*. 2016;8:7127-36.

[18] Ghadiri MR, Granja JR, Buehler LK. Artificial transmembrane ion channels from self-assembling peptide nanotubes. *Nature*. 1994;369:301.

[19] Mahadevi AS, Sastry GN. Cooperativity in Noncovalent Interactions. *Chemical Reviews*. 2016;116:2775-825.

*[20] Xing R, Yuan C, Li S, Song J, Li J, Yan X. Charge-Induced Secondary Structure Transformation of Amyloid-Derived Dipeptide Assemblies from beta-Sheet to alpha-Helix. *Angewandte Chemie International Edition*. 2018;57:1537-42.

This recent work reports that incorporation of a charged-induced secondary structural transition into an Fmoc-FF system can alter the dipeptide assembly secondary structure.

[21] Do TD, Kincannon WM, Bowers MT. Phenylalanine oligomers and fibrils: the mechanism of assembly and the importance of tetramers and counterions. *Journal of the American Chemical Society*. 2015;137:10080-3.

[22] Liao M, Cheng G, Zhou J. Underwater Superoleophobicity of Pseudozwitterionic SAMs: Effects of Chain Length and Ionic Strength. *The Journal of Physical Chemistry C*. 2017;121:17390-401.

[23] Mahadevi AS, Sastry GN. Cooperativity in noncovalent interactions. *Chemical Reviews*. 2016;116:2775-825.

[24] Kim J, Han TH, Kim YI, Park JS, Choi J, Churchill DG, et al. Role of water in directing diphenylalanine assembly into nanotubes and nanowires. *Advanced Materials*. 2010;22:583-7.

- [25] Ziserman L, Lee H-Y, Raghavan SR, Mor A, Danino D. Unraveling the mechanism of nanotube formation by chiral self-assembly of amphiphiles. *Journal of the American Chemical Society*. 2011;133:2511-7.
- [26] Krone MG, Hua L, Soto P, Zhou R, Berne B, Shea J-E. Role of water in mediating the assembly of Alzheimer amyloid- β A β 16–22 protofilaments. *Journal of the American Chemical Society*. 2008;130:11066-72.
- [27] Aggeli A, Bell M, Carrick LM, Fishwick CW, Harding R, Mawer PJ, et al. pH as a trigger of peptide β -sheet self-assembly and reversible switching between nematic and isotropic phases. *Journal of the American Chemical Society*. 2003;125:9619-28.
- [28] Tsonchev S, Niece KL, Schatz GC, Ratner MA, Stupp SI. Phase Diagram for Assembly of Biologically-Active Peptide Amphiphiles. *The Journal of Physical Chemistry B*. 2008;112:441-7.
- [29] Israelachvili JN. *Intermolecular and surface forces*: Academic Press; 2011.
- [30] Zhu P, Yan X, Su Y, Yang Y, Li J. Solvent-Induced Structural Transition of Self-Assembled Dipeptide: From Organogels to Microcrystals. *Chemistry—A European Journal*. 2010;16:3176-83.
- [31] Faul CFJ, Antonietti M. Ionic Self-Assembly: Facile Synthesis of Supramolecular Materials. *Advanced Materials*. 2003;15:673-83.
- [32] Rehm TH, Schmuck C. Ion-pair induced self-assembly in aqueous solvents. *Chemical Society Reviews*. 2010;39:3597-611.
- [33] Voyer N, Roby J, Deschênes D, Bernier J. Design, synthesis, and supramolecular properties of bis-crown ether modified peptides. *Supramolecular Chemistry*. 1995;5:61-9.
- [34] Nagarajan R. Molecular packing parameter and surfactant self-assembly: the neglected role of the surfactant tail. *Langmuir*. 2002;18:31-8.
- [35] Michaels TC, Liu LX, Meisl G, Knowles TP. Physical principles of filamentous protein self-assembly kinetics. *Journal of Physics: Condensed Matter*. 2017;29:153002.
- [36] Caplan MR, Moore PN, Zhang S, Kamm RD, Lauffenburger DA. Self-assembly of a β -sheet protein governed by relief of electrostatic repulsion relative to van der Waals attraction. *Biomacromolecules*. 2000;1:627-31.
- [37] Tahara K, Lei S, Adisoejoso J, De Feyter S, Tobe Y. Supramolecular surface-confined architectures created by self-assembly of triangular phenylene–ethynylene macrocycles via van der Waals interaction. *Chemical Communications*. 2010;46:8507-25.
- [38] Ji W, Yuan C, Zilberzwige-Tal S, Xing R, Chakraborty P, Tao K, et al. Metal-Ion Modulated Structural Transformation of Amyloid-Like Dipeptide Supramolecular Self-Assembly. *ACS nano*. 2019.
- [39] Scott GG, McKnight PJ, Tuttle T, Ulijn RV. Tripeptide Emulsifiers. *Advanced Materials*. 2016;28:1381-6.
- [40] Moyer TJ, Finbloom JA, Chen F, Toft DJ, Cryns VL, Stupp SI. pH and amphiphilic structure direct supramolecular behavior in biofunctional assemblies. *Journal of the American Chemical Society*. 2014;136:14746-52.
- [41] Cui H, Webber MJ, Stupp SI. Self-assembly of peptide amphiphiles: From molecules to nanostructures to biomaterials. *Peptide Science*. 2010;94:1-18.
- [42] Wang J, Liu K, Xing R, Yan X. Peptide self-assembly: thermodynamics and kinetics. *Chemical Society Reviews*. 2016;45:5589-604.
- [43] Cui H, Muraoka T, Cheetham AG, Stupp SI. Self-assembly of giant peptide nanobelts. *Nano Letter*. 2009;9:945-51.
- [44] Pappas CG, Frederix PW, Mutasa T, Fleming S, Abul-Haija YM, Kelly SM, et al. Alignment of nanostructured tripeptide gels by directional ultrasonication. *Chemical Communications*. 2015;51:8465-8.
- [45] Wang J, Liu K, Yan L, Wang A, Bai S, Yan X. Trace Solvent as a Predominant Factor To Tune Dipeptide Self-Assembly. *ACS Nano*. 2016;10:2138-43.
- [46] Wang Y, Qi W, Huang R, Yang X, Wang M, Su R, et al. Rational Design of Chiral Nanostructures from Self-Assembly of a Ferrocene-Modified Dipeptide. *Journal of the American Chemical Society*. 2015;137:7869-80.

- *[47] Zhou P, Wang J, Wang M, Hou J, Lu JR, Xu H. Amino acid conformations control the morphological and chiral features of the self-assembled peptide nanostructures: Young investigators perspective. *Journal of Colloid and Interface Science*. 2019;548:244-54.
This recent review describes how amino acid conformation and chirality impact self-assembled peptide structures.
- [48] Han S, Cao S, Wang Y, Wang J, Xia D, Xu H, et al. Self-assembly of short peptide amphiphiles: the cooperative effect of hydrophobic interaction and hydrogen bonding. *Chemistry—A European Journal*. 2011;17:13095-102.
- [49] Cui H, Cheetham AG, Pashuck ET, Stupp SI. Amino acid sequence in constitutionally isomeric tetrapeptide amphiphiles dictates architecture of one-dimensional nanostructures. *Journal of the American Chemical Society*. 2014;136:12461-8.
- [50] Ghadiri MR, Granja JR, Milligan RA, McRee DE, Khazanovich N. Self-assembling organic nanotubes based on a cyclic peptide architecture. *Nature*. 1993;366:324.
- **[51] Wang M, Zhou P, Wang J, Zhao Y, Ma H, Lu JR, et al. Left or right: how does amino acid chirality affect the handedness of nanostructures self-assembled from short amphiphilic peptides? *Journal of the American Chemical Society*. 2017;139:4185-94.
This work reports the hardness evolution of short peptides from molecular chirality to self assembly handedness.
- [52] Mammadov R, Tekinay AB, Dana A, Guler MO. Microscopic characterization of peptide nanostructures. *Micron*. 2012;43:69-84.
- **[53] Cox H, Georgiades P, Xu H, Waigh TA, Lu JR. Self-assembly of mesoscopic peptide surfactant fibrils investigated by STORM super-resolution fluorescence microscopy. *Biomacromolecules*. 2017;18:3481-91.
This paper describes using STORM and AFM to observe the dynamic process for a short peptide I₃K self-assembly.
- *[54] Zhao Y, Yang W, Wang D, Wang J, Li Z, Hu X, et al. Controlling the diameters of nanotubes self-assembled from designed peptide bolaphiles. *Small*. 2018;14:1703216.
This paper describes combining TEM and SANS to investigate the wall thickness of different short peptide nanotubes.
- *[55] Wang M, Wang J, Zhou P, Deng J, Zhao Y, Sun Y, et al. Nanoribbons self-assembled from short peptides demonstrate the formation of polar zippers between β -sheets. *Nature Communications*. 2018;9:5118.
This work describes using ss-NMR and MD simulation to obtain molecular packing mode within peptide secondary structures.
- [56] Beun LH, Albertazzi L, Van Der Zwaag D, De Vries R, Cohen Stuart MA. Unidirectional living growth of self-assembled protein nanofibrils revealed by super-resolution microscopy. *ACS Nano*. 2016;10:4973-80.
- *[57] Cox H, Xu H, Waigh TA, Lu JR. Single-molecule study of peptide gel dynamics reveals states of prestress. *Langmuir*. 2018;34:14678-89.
This recent work describes using STORM and different data analysis and optical set-up to image the dynamic of single peptides.
- [58] Hu X, Gong H, Li Z, Ruane S, Liu H, Pambou E, et al. What happens when pesticides are solubilized in nonionic surfactant micelles. *Journal of Colloid and Interface Science*. 2019;541:175-82.
- [59] Wallace M, Iggo JA, Adams DJ. Probing the surface chemistry of self-assembled peptide hydrogels using solution-state NMR spectroscopy. *Soft Matter*. 2017;13:1716-27.
- [60] Broedersz CP, MacKintosh FC. Modeling semiflexible polymer networks. *Reviews of Modern Physics*. 2014;86:995.
- [61] Dou XQ, Feng CL. Amino acids and peptide-based supramolecular hydrogels for three-dimensional cell culture. *Advanced Materials*. 2017;29:1604062.
- [62] Motesharei K, Ghadiri MR. Diffusion-limited size-selective ion sensing based on SAM-supported peptide nanotubes. *Journal of the American Chemical Society*. 1997;119:11306-12.

- [63] Wiradharma N, Tong YW, Yang Y-Y. Self-assembled oligopeptide nanostructures for co-delivery of drug and gene with synergistic therapeutic effect. *Biomaterials*. 2009;30:3100-9.
- [64] Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature*. 2002;415:389.
- *[65] Ciunac D, Gong H, Hu X, Lu JR. Membrane targeting cationic antimicrobial peptides. *Journal of Colloid and Interface Science*. 2019;537:163-85.
- This is a recent review on antimicrobial peptides from rational design to clinical trials.
- [66] Chen C, Hu J, Zhang S, Zhou P, Zhao X, Xu H, et al. Molecular mechanisms of antibacterial and antitumor actions of designed surfactant-like peptides. *Biomaterials*. 2012;33:592-603.
- [67] Li J, Chen Z, Zhou M, Jing J, Li W, Wang Y, et al. Polyoxometalate-Driven Self-Assembly of Short Peptides into Multivalent Nanofibers with Enhanced Antibacterial Activity. *Angewandte Chemie International Edition*. 2016;55:2592-5.
- [68] Schnaider L, Brahmachari S, Schmidt NW, Mensa B, Shaham-Niv S, Bychenko D, et al. Self-assembling dipeptide antibacterial nanostructures with membrane disrupting activity. *Nature Communications*. 2017;8:1365.
- [69] Habibi N, Kamaly N, Memic A, Shafiee H. Self-assembled peptide-based nanostructures: Smart nanomaterials toward targeted drug delivery. *Nano Today*. 2016;11:41-60.
- **[70] He H, Wang J, Wang H, Zhou N, Yang D, Green DR, et al. Enzymatic cleavage of branched peptides for targeting mitochondria. *Journal of the American Chemical Society*. 2018;140:1215-8.
- This pioneering work provides a new strategy for short peptides to target organelles and control drug release via enzyme cleavage.
- [71] Cao M, Lu S, Wang N, Xu H, Cox H, Li R, et al. Enzyme-Triggered Morphological Transition of Peptide Nanostructures for Tumor-Targeted Drug Delivery and Enhanced Cancer Therapy. *ACS Applied Materials & Interfaces*. 2019; 111:16357-66.
- [72] Liu K, Xing R, Zou Q, Ma G, Möhwald H, Yan X. Simple peptide-tuned self-assembly of photosensitizers towards anticancer photodynamic therapy. *Angewandte Chemie International Edition*. 2016;55:3036-9.
- [73] Li J, Wang A, Ren P, Yan X, Bai S. One-step co-assembly method to fabricate photosensitive peptide nanoparticles for two-photon photodynamic therapy. *Chemical Communications*. 2019.
- **[74] Xing R, Zou Q, Yuan C, Zhao L, Chang R, Yan X. Self-Assembling Endogenous Biliverdin as a Versatile Near-Infrared Photothermal Nanoagent for Cancer Theranostics. *Advanced Materials*. 2019:1900822.
- This work reports a new strategy of directly using hydrophobic photosensitizers as part of peptide carriers for a photodynamic therapy.
- [75] Amdursky N, Glowacki ED, Meredith P. Macroscale Biomolecular Electronics and Ionics. *Advanced Materials*. 2019;31:1802221.
- [76] Pinotsi D, Grisanti L, Mahou P, Gebauer R, Kaminski CF, Hassanali A, et al. Proton transfer and structure-specific fluorescence in hydrogen bond-rich protein structures. *Journal of the American Chemical Society*. 2016;138:3046-57.
- **[77] Tao K, Hu W, Xue B, Chovan D, Brown N, Shimon LJ, et al. Bioinspired Stable and Photoluminescent Assemblies for Power Generation. *Advanced Materials*. 2019;31:1807481.
- This pioneering work reports a short peptide cyclo-GW with benign piezoelectronic properties that can be used as micro electronic devices in biological systems.
- [78] Freudiger CW, Yang W, Holtom GR, Peyghambarian N, Xie XS, Kieu KQ. Stimulated Raman scattering microscopy with a robust fibre laser source. *Nature Photonics*. 2014;8:153.
- [79] Shi F, Zhang Q, Wang P, Sun H, Wang J, Rong X, et al. Single-protein spin resonance spectroscopy under ambient conditions. *Science*. 2015;347:1135-8.
- [80] Kowligy AS, Timmers H, Lind AJ, Elu U, Cruz FC, Schunemann PG, et al. Infrared electric field sampled frequency comb spectroscopy. *Science Advances*. 2019;5:eaaw8794.
- [81] Heussinger C, Frey E. Floppy modes and nonaffine deformations in random fiber networks. *Physical Review Letters*. 2006;97:105501.

[82] Malm A, Waigh T. Elastic turbulence in entangled semi-dilute DNA solutions measured with optical coherence tomography velocimetry. *Scientific Reports*. 2017;7:1186.

[83] Draper ER, Eden EG, McDonald TO, Adams DJ. Spatially resolved multicomponent gels. *Nature Chemistry*. 2015;7:848.

[84] Phanphak S, Georgiades P, Li R, King J, Roberts IS, Waigh TA. Super-resolution fluorescence microscopy study of the production of K1 capsules by *Escherichia coli*: evidence for the differential distribution of the capsule at the poles and the equator of the cell. *Langmuir*. 2019;35:5635-46.

*[85] Chen C, Starr CG, Troendle EP, Wiedman G, Wimley WC, Ulmschneider JP, et al. Simulation-guided rational de novo design of a small pore-forming antimicrobial peptide. *Journal of the American Chemical Society*. 2019;141:4839-48.

This work describes combining simulation and experiments to predict antimicrobial peptide behaviours on biomembranes.