

Short peptide based self-assembled nanostructures: implications in drug delivery and tissue engineering

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Self-assembly of biomolecules facilitates the creation of a diverse range of hierarchical nanostructures from a wide range of polymeric and non-polymeric materials. Peptides and specifically short peptides are very attractive in this respect due to their unmatched biocompatibility, ease of synthesis, functionality as well as tunable bioactivity along with the availability of rich chemistry for fine-tuning the structure and function of peptides according to environmental conditions. Self-assembled peptide based nanostructures such as tubes, filaments, fibrils, hydrogels, vesicles, and monolayers have been studied by many research groups and found application as three-dimensional cell growing scaffolds, dental implants, neural tissue engineering scaffolds and as carriers for drugs, proteins and genes, and nucleotides. Nanostructures are also being developed from designed or modified amino acids to have enhanced cellular as well as *in vivo* stability. These modified nanostructures showed enhanced drug delivery properties both under *in vivo* and *in vitro* conditions.

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Introduction

Self-assembly is defined as the autonomous organization of components into ordered patterns or structures.¹ Living cells consist of many self-assembling systems that work in synchronization to achieve a defined goal. Self-assembly, as a process contributes significantly in biology such as maintaining cell integrity, performing important cellular functions as well as inducing abnormalities that cause diseases.¹ Thus, understanding life necessitates a better understanding of self-assembly. Concepts of self-assembly have also been used in many disciplines for constructing useful materials. Molecular self-assembly is in fact a very practical way of making ensembles of nanostructures. The ubiquitous existence of self-assembly processes in living systems along with the prevalence of various non-covalent interactions (van der Waals, electrostatic, and hydrophobic metal–ligand, π – π stacking interactions, hydrogen and coordination bonds) in biology, have resulted in rapid development of self-assembling biomaterials as a promising research area.^{2–4} Self-assembly provides the flexibility of developing novel materials with tailored morphologies and desired functions through single-molecule design and engineering. This results in controlling the bulk properties of the resultant material by modulating individual monomeric building blocks. Thus, by modulating structural changes in constituent molecules it becomes possible to dictate the behavior of the end product.

In recent past, many self-assembling nanomaterials have been generated from various organic polymers such as carbohydrates, nucleic acids, proteins *etc.* either to gain a better understanding of the phenomenon or to use them for applications ranging from molecular devices to delivery systems or scaffolds.^{1,2}

For example, different polysaccharides due to their many merits have been investigated as templates for the synthesis of nanomaterials. Polysaccharides are of natural origin; they are biodegradable, non-toxic and safe. They have abundant natural resources and have a low cost of purification. Polysaccharides such as alginate, pectin, dextran, chitosan, hyaluronic acid *etc.* have been studied for constructing nanostructures. Polysaccharide nanostructures are prepared by methods such as covalent crosslinking, ionic crosslinking, polyelectrolyte complex formation, and by the self-assembly of polysaccharides modified with hydrophobic groups. However, cross-linking agents such as glutaraldehyde could be a limiting factor in the potential biomedical application of these nanostructures prepared by the cross-linking method. Polysaccharides like chitosan, dextran either in a native or modified form or in association with other polymers form nanoparticles that can encapsulate various drugs, genes, proteins, and peptides.⁵ Chitosan has been investigated for making a variety of nanocarriers,^{6,7} and chitosan nanocarriers have been shown to encapsulate drugs like retinol,⁸ utilized for DNA delivery.⁹ Also, calcium-alginate nanoparticles have been shown to encapsulate anti-tuberculosis drugs like isoniazid, pyrazinamide and rifampicin with high efficiency and loading resulting in increased bioavailability of these drugs.¹⁰

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Furthermore, biomolecules like DNA, RNA and proteins are endowed with many desirable properties which make them suitable templates for constructing self-assembled nanostructures. DNA nanostructures with either two or three dimensions and with varied shapes like, icosahedral, tubular, tetrahedral, and Y-shaped geometries are being constructed and have been shown to deliver cargos like, drugs (anticancer, various antibiotics), oligo-nucleotides, dyes, inorganic nanoparticles, proteins *etc.* to different cells or to display groups that induce immunostimulation.¹¹ The superiority of DNA nanoarchitectures as compared to other self-assembling systems lies in the flexibility of fine tuning the geometry and size of DNA nanoarchitectures very accurately because of the well-known self-recognition properties of DNA and knowledge of the exact structure of the double helix on the atomic level. This allows the ability of controlling spatial distribution of cargoes and ligands on DNA nanostructures.

Multifunctional DNA nanostructures simultaneously decorated with targeting agents, drug payloads as well as imaging agents are also being investigated for theranostic applications.¹² Hybrid nanoparticles like DNA-gold nanoparticles have additional features like magnetic properties, plasmonic effects or the ability of fluorescence quenching that could find application in imaging, detection and as transfection agents and gene regulation materials.^{13–15} DNA-block copolymer micelles (DBC)s have also been shown to have applications in biomaterial purification,^{16,17} templated synthesis¹⁸ and in nanoelectronics.¹⁹ For *in vitro* and *in vivo* experiments usually larger quantities of biomaterials are needed. However due to cost issues, it is important that while designing DNA nanostructures the number of strands per DNA nanostructure should be kept at the minimum and this is one of the major limitations of DNA nanostructures.

Like DNA, RNA molecules by virtue of their inter and intra molecular interactions can self-assemble to form nanostructures by both template or non-template mediated assembly. RNA self-assembly plays a significant role in nanofabrication by virtue of its ability to form 3D nanostructures, to produce reversible self-assembly and self-repair. RNA nanoparticles have shown application in pathogen detection, drug/gene delivery, and various other therapeutic applications.²⁰ For instance, motor pRNA of the bacteriophage phi29 formed nanostructures of various shapes like twins, tetramers, rods, triangles, and 3D arrays of micron size *via* interaction of programmed helical regions and loops. These structures were resistant to a wide range of temperatures, salt concentrations, and pH and have shown potential to deliver oligonucleotides like ribozyme or siRNA to kill cancer cells both *in vitro* and *in vivo* or Hepatitis B virus.^{21,22}

Like DNA, RNA and carbohydrates, proteins due to the existence of varied structures, shapes and chemical properties and availability of a large database can act as good templates for molecular self-assembly and many protein based nanostructures are being investigated.^{23–25}

Though all these systems have their own demerits and limitations, their relative instability within living systems and other deleterious effects can hinder the potential applications of these systems for safe human use.

The idea of generating peptide based biomaterials essentially came from the observation that many soluble cellular proteins could self-assemble to form well-ordered tubular deposits in neurodegenerative diseases such as Alzheimer's, Parkinson's and in prion related diseases.²⁶ Though, disastrous to the normal physiology,²⁶ these protein assemblies formed the basis of developing novel nanobiomaterials.²⁷ These observations have led to many efforts focusing on studying self-assembling peptides and several peptide based nanostructures have been described/developed.^{28–30}

There are many advantages associated with using peptides as building blocks to make ensembles of nanostructures. These include the availability of detailed structural and functional information for many peptides and proteins, easy and rapid chemical synthesis, tunability to various environmental conditions (like pH, temperature, and ionic strength), and adoption of well defined helical or β -hairpin/sheet secondary structures that can energetically favor self-association and assembly.³¹ Peptides however generally are labile to enzymatic degradation, but if being developed as delivery agents, there are different strategies to circumvent this problem. For example, by introducing non-coded residues in the peptide design, the peptide based nanostructures can be made more resistant to enzymatic degradation.^{32,33}

Nanostructures generated by peptide self-assembly

Peptides, based on their design, can assemble into different kinds of supramolecular architectures (Fig. 1) such as nanotubes^{34,35,36} and monolayers with a nanoscale order and as vesicular structure.^{28–30} Nanostructures have been developed from different types of peptides including cyclic peptides, amphiphilic peptides, peptides containing β -sheeted motifs, helical-structures or β -turns.

Cyclic peptide based nanostructures

Ghadiri *et al.* for the first time developed peptide nanotubes based on cyclic polypeptides with an even number of alternating

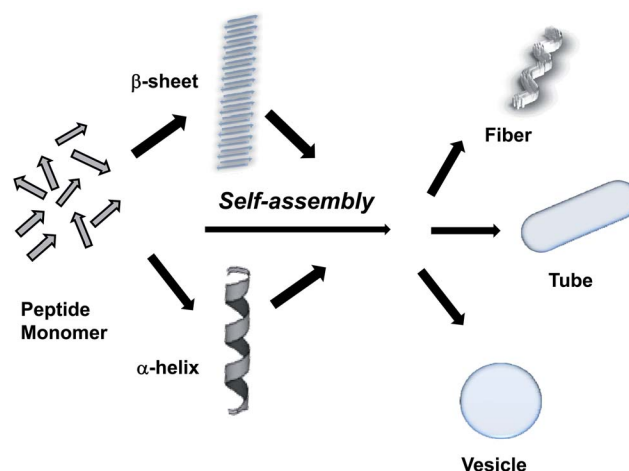


Fig. 1 Self-assembly of peptides into different types of nanostructures.

D- and L-amino acids. These peptides assembled by virtue of inter-molecular hydrogen bonding and formed self-assembled nanotubes with the internal diameter ranging from 7–8 Å (Fig. 2).^{37,38} Because of the alternating D and L-amino acid sequences, the peptide side chains in these assemblies lie on the outward direction creating a hollow tubular structure. The diameters of these nanotubes could be varied depending on the cyclic peptide ring size. Lanreotide growth hormone inhibitor, a natural cyclic peptide has also been shown to self-assemble to form ordered nanotubes by virtue of aromatic and hydrophobic interactions.³⁹ These nanotubes showed potential for a wide range of applications as antimicrobial agents, biosensors, catalysts *etc.*^{40,41}

Nanostructures generated from beta-sheeted or alpha-helical peptides

A large number of peptides have been shown to self-assemble by forming beta-sheeted secondary structures.⁴² Beta-sheeted peptides have been used to synthesize a variety of supramolecular architectures such as ribbons,^{43,44} nanotubes,^{36,45,46} monolayers with nanoscale order,^{47,48} and nanotubes with delocalized electronic states.⁴⁹ An example of a pH responsive self-assembling beta sheeted peptide is P11-4 (QQRFEWEFEQQ). This peptide is pH sensitive due to the ionizable glutamate and arginine side chains. At concentrations below $<10 \text{ mg ml}^{-1}$ and at neutral pH, the peptide is soluble but adopts a hydrogel state at low pH (pI: 4.2). This occurs due the formation of anti-parallel β -sheet tapes, which then stack together to form fibrils. At its critical gelling concentration of 10 mg ml^{-1} , the peptide also forms hydrogel like structures in culture medium or in the presence of 140 mM salt.⁴³ Similarly, MAX1, a 20 residue long peptide, with two β -strands with alternating valine and lysine residues connected through a type II β -turn, when kept under basic conditions, first folds into a β -hairpin like structure which then self-assembles into a hydrogel network.⁵⁰

Powers *et al.*, also investigated the self-assembly of designed β -hairpin peptides and found that these peptides can self-assemble at the air/water interface to form various nanostructures.⁵¹ Other designed peptides like MAX-8, the modified version of MAX-1 with lysine at the 15th position being replaced by glutamic acid and MAX-3 with 7th, 12th and 16th valine

residues of MAX-1 being replaced by threonine residues, have been shown to undergo triggered folding in response to pH and/or temperature changes, to adopt a β -hairpin conformation that undergoes self-assembly to form various nanostructures.^{52,53} Large polypeptides with alternating segments of polar and non-polar residues like the 16 residue peptides RADA-16 I and II have been shown to form β -sheeted structures that self-assembled into pH responsive hydrogels.⁵⁴ RADA16 (RADARADARADARADA) is also marketed under the name of PuraMatrix™ (3DM, Inc., Cambridge, MA, USA). This 16 amino acid peptide readily self-assembles into a nanofiber network. Gel formation can also be triggered by an increase in ionic strength or by a change in pH. In another approach, linear peptides were assembled into nanotubes by utilization of highly directional metal–ligand interactions.⁵⁵ The ability of these peptides to form polymeric β -sheets can be modulated by pH and salts in a manner that is dictated by the number and positioning of charged amino acid residues.

Though β -sheeted peptide motifs are being widely scrutinized as templates for forming self-assembled nanoscale materials,^{50,56} the design of α -helical nanoscale materials has seen only limited advancement. As models of fibrillar structures based on α -helical coiled coil motifs are prevalent in the body such as in the cytoskeleton and extracellular matrix,⁵⁷ α -helical synthetic peptides have been attractive targets for *de novo* design of fibrillar nanostructures. For instance, filamentous nanostructures were generated from coiled-coil peptide motifs 25–50 residues long. These peptides were based on heptad repeats $(\text{abcdefg})_n$, with positions a and d being occupied by hydrophobic residues and polar residues anywhere else in the sequence. Hydrophobic residues formed an inter-helical hydrophobic core, providing a stabilizing interface between the helices. Charged residues at positions “e” and “g” participate in electrostatic interactions contributing to coiled-coil stability. These peptides form 2–5 helices that wrap around each other in a superhelical fashion to form nanoscale fibers.^{58–60} Woolfson and co-workers designed peptides (29–36 residue long) based on helical coiled-coils,^{61,62} that self-assembled into nanofibers. Their peptides were also based on a heptad sequence repeat, abcdefg, with hydrophobic residues isoleucine and leucine at the ‘a’ and ‘d’ sites, respectively. The hydrophobic residues promoted helix oligomerisation through hydrophobic collapse. To promote staggered assembly of peptides and fibril formation, lysines were incorporated at the ends of the peptides with central glutamates to allow ionic interactions resulting in a fibrous assembly.^{63,64} Hartgerink and co-workers have also used heptad repeats producing helical coiled-coils that form nanofibres in a concentration-dependent manner. They used pH and ionic strength as triggers for self-assembly with incorporation of isoleucine and leucine residues at positions ‘a’ and ‘d’ of the heptad, and glutamate at positions ‘e’ and ‘g’ providing an acidic region. Hence, at low pH ionic repulsion was eliminated and carboxylic acid side chains hydrogen bonded with each other to form assembly.⁶⁴

Hydrogels are three dimensional (3D) networks with the capacity to imbibe and retain water. They have been shown to possess a wide range of biomedical applications.^{65,66} Hydrogels

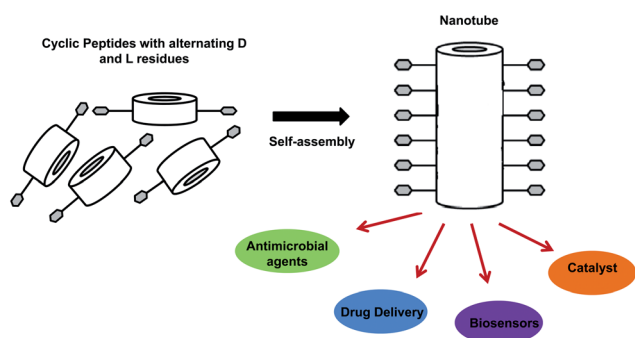


Fig. 2 Self-assembly of cyclic peptides with alternating D and L residues and their potential applications.

have also been developed from self-assembly of helical polypeptides based on 'aba' triblock motifs that are composed of a central random coil block flanked by two coiled-coil forming sequences. The self-assembly occurred as a balance between the oligomerization of the helical ends and swelling of the central water-soluble random coil segment.^{67–69} Interestingly, it was observed that hydrogel formation and their physical properties could be fine-tuned by changing the structure and length of the coiled-coil building blocks. Consequently, stimuli responsiveness (to temperature and/or pH, salt) could be introduced in the self-assembled hydrogels by modulating the amino acid sequence of the coiled-coil domains. Denaturation of coiled-coil domains by guanidine hydrochloride (GdnHCl) resulted in disassembly of the hydrogels and removal of GdnHCl by dialysis caused coiled-coil refolding and hydrogel reassembly.⁶⁸

Various research groups have designed leucine zippers (a condition where the interacting surface between the helices contained leucine) for constructing nanostructures with different properties.^{70,71} O'Shea *et al.* were the first to propose a peptide design in which one strand with acidic residues and the other with basic residues yielded a parallel heterodimer to form a model called 'Peptide Velcro'.⁷⁰ In this design, a single asparagine residue in the sequence forms a hydrogen bond with a corresponding asparagine residue in the other subunit contributing to the directional specificity of the helix orientation and oligomerization state. Thus, it appears that simple sequences can be designed to have a very high preference to pair with each other to form useful structures. These 'Peptide Velcro's can be utilized to bring two molecules together, which may be useful in sensing applications.

Self-assembly of amphiphilic peptides

Zhang and co-workers demonstrated that 7–8 residue long surfactant-like peptides, characterized by well-defined hydrophilic and hydrophobic residues that could self-assemble in water to form well-ordered nanotubes and nanovesicles.⁷² The hydrophilic head groups of these peptides were composed of aspartic acids and their tails were composed of hydrophobic amino acids such as alanine, valine, or leucine. Like other surfactants, self-association by hydrophobic interactions was the major governing force for the self-assembly of these peptides.⁷²

Amphiphilic peptides generally consist of a hydrophilic peptide headgroup and a hydrophobic alkyl tail where the tail participates in aligning the head group to form various secondary, super-secondary and tertiary conformations.^{73–75} These peptides self-assembled to form a variety of morphological structures with nanodimensions such as micelles, vesicles or tubules.^{36,44,54,72} Tsonchev *et al.* demonstrated that the self-assembly of amphiphilic peptides was driven by both hydrophobic as well as electrostatic interactions.⁷⁶

Bolaamphiphiles are also a class of amphiphilic molecules consisting of two hydrophilic groups flanked by a hydrocarbon chain.⁷⁷ Their peptide segment undergo β -sheet hydrogen bonding to form various supramolecular nanostructures such as fibers, rods, tubes, ribbons, spheres *etc.*^{78–81} Bolaamphiphilic

nanotubes have been shown to have several interesting applications such as use in viral assays,^{82,83} protein sensing⁸⁴ *etc.*

All of the above described strategies of making assembled structures have utilized large polypeptide sequences with the use of either purely L-amino acids, D-amino acids or both. However, the associated expense and complexity of synthesis of large linear peptides, cyclic and dendritic structures have strongly limited the applicability of such peptides for practical purposes. Moreover, their proteolytic instability has also been a major concern for their application under biological conditions or in *in vivo* situations.

Self-assembly of short peptides into different nanostructures

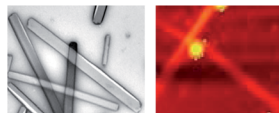
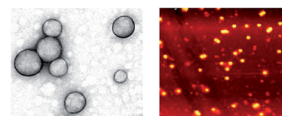
Lately, it was demonstrated that very small peptides could also self-assemble into various nanomorphologies, thereby minimising the difficulty and cost of their synthesis and simultaneously enhancing their stability (Fig. 3). These short peptide fragments were mostly discovered in a quest to determine the minimal required sequence for amyloid formation. An example for this is, NFGAIL (hIAPP22–27), a hexapeptide fragment of the islet amyloid polypeptide (IAPP) that forms well-ordered amyloid fibrils similar to those formed by the full length polypeptide.⁸⁵ FGAIL (hIAPP23–27) a pentapeptide fragment of the IAPP polypeptide, also formed a fibrillar structure.⁸⁵ Similarly, Westermarck *et al.*, discovered AILSS to be the strongly amyloidogenic region of IAPP and Rechtes *et al.* discovered NFGSVQ to be the minimally active amyloidogenic peptide fragments of the Aortic Medial Amyloid.^{86,87}

KLVFF, a pentapeptide fragment of the amyloid β -peptide A β -42, also self-assembled in phosphate buffered saline (PBS) to form a hydrogel.⁸⁸ It was hypothesized that shielding of the electrostatic charges of the peptide by salt ions favored β -sheet formation that self-associated to form a nanofibrillar gel network. Later, DFNKF, a pentapeptide fragment of human calcitonin, was also found to form well-ordered amyloid fibrils similar to those formed by the full length polypeptide.⁸⁹ In a quest to find out the minimal requisite peptide sequences of the protein TAR–DNA binding protein (TDP 43) that are involved in

Self-assembly of short peptides (≤ 5 amino acid) into nanostructures

Vesicle forming sequences

CFF, EAF, KAF
RAF, MAF
Phg-Phg, Acp-TE, di-para-nitro-F



Tube forming sequences

FF, (D)-F-(D)-F, NH₂-FF-NH₂, Boc-FF
FΔF, LL, LF, di-pentafluoro-F, NFGSV
FL, IL, WG, βAA, δAvaF, Ac-FF

Fibril forming sequences

NFGAI, FGAIL
NFLVH
DFNK, GAIL, GFIL, KLVFF, DFNKF
NFGSV, Boc-AUV-Ome, Boc-AUI-Ome
FΔF, Cbz-FF, IF, Fmoc-F, Fmoc-FF

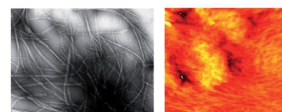


Fig. 3 Sequences of short peptides (≤ 5 amino acids long) that can self-assemble into nanostructures.

aggregation and plaque formation in amyotrophic lateral sclerosis and frontotemporal lobar degeneration, Akash *et al.* discovered 10–13 residue peptides (like EDLIKGISV, MNFGAFSINPAMM) that formed well ordered fibrillar structures similar to those formed by a full length protein.⁹⁰ All these studies revealed that almost all of the amyloid forming peptides have minimally active shorter peptide fragments which carry the capacity to form amyloid fibrils similar to those formed by large native polypeptides. These studies also suggested that aromatic residues in general play a critical role in amyloid fibril formation.⁹¹

Hauser and co-workers created a unique class of natural tri- to heptapeptides made of simple, non-aromatic amino acids that self-assembled in water to form hydrogels.⁹² The amphiphilic peptide motif consisted of a tail of aliphatic nonpolar amino acids (N terminus) with decreasing hydrophobicity and a hydrophilic head group of acidic, neutral, or basic nonaromatic polar amino acids (C terminus). This assembly involved a conformational transition of the structurally unorganized monomers into metastable α -helical intermediates that terminated in cross- β structures. The peptides had a characteristic sequence motif consisting of an aliphatic amino acid tail of decreasing hydrophobicity capped by a polar head, which endowed them with amphiphilicity. A decrease in hydrophobicity from the N-terminus (acetylated to suppress charge effects) to the C-terminus strongly improved ease of self-assembly, stability and strength of nanostructures. It was observed that the length of the hydrophobic tail and the polarity of the head group were integral elements that supported facile hydrogel formation. Hexamers typically formed gels more readily than pentamers, tetramers, and trimers. Stronger gels were derived from head groups with acidic residues (D and E), followed by neutral (S and T) and basic (K) polar, non-aromatic amino acids.

Hauser and co-workers also carried out morphological evaluation of diverse nanostructures formed by varying the amino acid sequence and concentration of a class of small self-assembling peptides. They modified these peptides by replacing the aliphatic amino acid at the C-terminus with different aromatic amino acids. The best gelling hexamer LIVAGD and the smallest trimer peptide IVD, were modified by replacing the aspartic acid residue at the C-terminus with an aromatic amino acid (F, W and Y) residue and tracked for the effect of the introduced aromatic residues on the self-assembly and morphology of resulting nanostructures. Where, aliphatic peptides formed long, helical fibers that entangled into hydrogel meshes, the modified peptides contrastingly formed short, straight fibers with a flat morphology. No helical fibers were observed for the modified peptides. Such a study dealing with the assembly of small peptides derived from simple aliphatic amino acids is significant and may be relevant and helpful in understanding chemical evolution leading to the origin of life on Earth. These aliphatic peptide based self-assembled nanostructures were proposed to have a variety of potential applications in bioengineering and nanotechnology.⁹³

Johansson and his group showed that even tetrapeptides are capable of forming amyloid fibrils. They hypothesized that hydrophobic interactions alone are not sufficient enough for

the self-assembly of these peptides⁹⁴ and there are other factors which favour peptide self-assembly into fibers.

Working with smaller peptides, Maji *et al.* demonstrated that tripeptides like Boc-AUV-OMe, Boc-AUI-OMe and Boc-AGV-OMe, where U is α -amino isobutyric acid, Boc is *tert*-butoxycarbonyl and Me is methyl, self-associated to form supermolecular β -sheet structures which further assembled into amyloid-like fibrils.⁹⁵ In another piece of work Banerjee and co-workers demonstrated pH-responsive nanostructural transition of a tripeptide, TUA, from nanotubes to nanovesicles.⁹⁶

Self-assembly of dipeptides and their modified versions into distinct nanostructures

Moving ahead in this direction researchers tried to explore the self-assembly of peptides as small as dipeptides (Fig. 4). The first report for dipeptide self-assembly came from Gazit's group who investigated the nature of self-assembly of the dipeptide, FF, a core motif of the amyloid β (A β) polypeptide segment. This dipeptide self-assembled into highly ordered nanotubes/microtubes,^{46,97–99} nanowires¹⁰⁰ and nanoforests.¹⁰¹ Interestingly, FF nanotubes were found to be thermally stable, a unique property desired to be present in any biologically inspired material.¹⁰² Gazit *et al.* further used FF to construct arrays of nanotubes that can act as high surface-area electrodes for storing energy and making microfluidic chips.⁹⁷ A newer type of assembly method based on vapor deposition was used for high scale production of FF nanotubes. This method was not only novel but also provided the handle to fine-tune the length and density of nanotubes by controlling monomer supply from the gas phase. This further demonstrated that the mode and nature of assembly of a given structure can be fine-tuned based on the method and conditions provided.⁹⁷ FF based self-assembled nanotubes and nanowires have been investigated further for other mechanical applications such as construction of nanodevices, nanobiosensors and low resistance conducting nanowires.^{34,103} Ihee and his group demonstrated a fascinating morphological transformation between diphenylalanine based nanowires and nanotubes. Nanotubes were obtained by dissolving the peptide in water by sonication followed by heating,

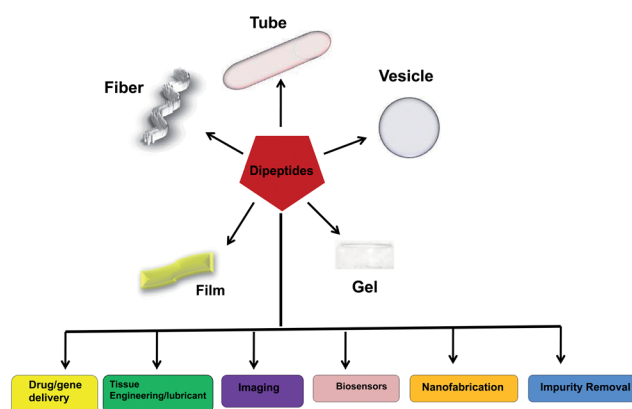


Fig. 4 Formation and potential biomedical applications of dipeptide based nanostructures.

whereas, nanowires were obtained in water at high ionic strength. These two morphologies are inter-convertible.¹⁰⁴

Görbitz *et al.* showed that hydrophobic dipeptides like LL, LF, FL, and IL self-assembled into nanotubes by forming head-to-tail ($\text{NH}^{3+}\cdots\text{OOC}$) hydrogen bonds.^{105–109} Water-filled nanotubes of the dipeptide WG also demonstrated the ability of negative thermal expansion.^{110,111} These workers also showed that dipeptides VA, LS and FF can form nanoporous structures.^{105,108,109,112,113} Ripmeester's work further demonstrated that dipeptide-based nanoporous materials could adsorb inert gases, such as xenon.^{114–116}

Gazit and co-workers further demonstrated that addition of a thiol group in FF changed its assembly from tubular to spherical structures.¹¹⁷ They further demonstrated the assembly of other aromatic homodipeptides into nano-spheres, nanoplates, nano-fibrils and hydrogels.¹¹⁸ These peptide nanostructures can be used as a casting mold for the fabrication of metallic nano-wires and coaxial nano-cables,^{46,119} and can have biomedical applications in biosensing, tissue engineering, molecular imaging *etc.*^{120–124}

Crystalline dipeptides like AV, VA, IV and VI have been shown by Sozzani and his co-workers to self-assemble into nanoporous materials with the capacity to adsorb, separate, and store various gases such as methane, carbon dioxide, and hydrogen.¹²⁵ Ventura and his co-workers reported that a dipeptide, IF, at 1.5 wt% and at pH 5.8 self-associated into a transparent, thermo-reversible gel composed of a network of nanofibrous structures in water.¹²⁶

Modified dipeptides have also been explored as templates for making biologically functional self-assembled nano or microstructures owing to their enhanced proteolytic stability.^{127,128} Two modified dipeptides [β -AA; δ -Ava-F] containing an N-terminally positioned ω -amino acid residue [β -alanine β -A/ δ -amino valeric acid (δ Ava)] self-associated to form nanotubes in the solid state as well as in an aqueous solution. TEM images of these two dipeptides revealed the formation of uniform and well ordered hollow nanotubular structures with varying dimensions.¹²⁹ Interestingly, the nanotubular structures formed by these peptides in the solid state and in solution differed significantly demonstrating their differential assembly behavior and packing arrangements in these two states. These nanotubes were found to be stable over a wide range of pH values and temperature.¹²⁹ The above studies indicated that water molecules by virtue of their intermolecular hydrogen bonding capacity, always play a pivotal role in the formation and stabilization of the nanotubular assemblies.^{129,130} In another study three water-soluble short peptides each having a common motif, a hybrid of β , α -amino acid residues (β -A-Xaa, Xaa = V/I/F), were found to self-assemble to form hollow nanotubes. These nanotubes could tolerate heat up to 80 °C, a wide range of pH (2–10), and were resistant to proteolytic degradation. These dipeptide-based robust crystalline nanotubes have been used as suitable templates for fabricating dipeptide-stabilized gold nanoparticles on their outer surfaces.¹³¹

Dipeptide-based nanoporous materials obtained from two water-soluble synthetic dipeptides namely β -A-Phg (Phg: phenylglycine) and Phg- β -A have shown the capacity to adsorb N_2

gas. Interestingly, these nanoporous materials obtained from dipeptides were degradable by the soil bacterial consortium suggesting their ecofriendly nature.¹³² They were not only very different from the existing type of nanoporous materials generated from zeolites, metal organic frameworks (MOFs) and others, but also had the advantage of biodegradability and eco-friendliness generally lacking in other organic and polymeric systems.¹³²

Fmoc-FF formed hydrogels with a nanofibrillar morphology in an aqueous solution with physical properties superior to those of hydrogels formed by longer polypeptides,¹²⁸ whereas, an uncharged peptide analogue, Ac-FF-NH₂, self-assembled into tubular structures.¹³³ Gazit *et al.* further explored other amine and carboxyl modified diphenylalanine peptide analogues and revealed that these dipeptides formed ordered tubular structures at the nanometric scale.¹³³ Ulijn and co-workers demonstrated that Fmoc dipeptides, derived from a combination of four different amino acids, namely glycine, alanine, leucine and phenylalanine, formed hydrogels whose structural and physical properties varied depending on the nature of the amino acids present in the peptide building blocks.¹³⁴ Peptides containing aromatic moieties like Fmoc,^{128,135–137} beta or D-amino acid residues,^{138,139} and pyrene^{140,141} have been proven to be good templates for making nanofibrillar hydrogel networks by the virtue of π - π stacking and hydrophobic interactions. The dipeptide amphiphile Fmoc-LG has been shown to self-assemble into thin surface supported hydrogel gel films and gap-spanning hydrogel membranes whose thicknesses can be closely controlled from tens of nanometers to millimeters. The films and membranes were stable once formed and could be reversibly dried and collapsed, then reswollen to regain the gel structure.¹⁴²

Dipeptides formed by the substitution of phenyl groups with naphthyl groups such as di-D-1-Nal and di-D-2-Nal also formed ordered fibrillar nanostructures. Di-D-1-Nal (Nal: naphthalene) formed fibers with 10 nm diameter. Di-D-2-Nal (Nal: naphthalene) peptides assembled into wider tubular structures with a diameter of about 50 nm and were more bundled than the fibrils formed by the di-D-1-Nal peptide. Interestingly, these naphthylalanine-based peptide structures showed single or low-number of walls as compared to multi-walled structures of the diphenylalanine tubes.¹¹⁸ Self-assembly of diphenylalanine peptides with nitro and phenyl groups has also been investigated. Di-*para*-nitro-Phe at a concentration of 5 mg ml⁻¹ formed spherical nanostructures with different diameters embedded in fibrillar nanostructures. The di-4-phenyl-Phe homo-dipeptide self-assembled into square plates with various dimensions. These square plates appeared to be very thin and symmetrical with clear borders.¹¹⁸ It was also observed that the aromatic dipeptide, diphenylglycine, self-assembled into well ordered closed-caged nanospheres.¹⁴³

Similar to FF, the dipeptide, (D)-F-(D)-F made up of two D-amino acids, self-assembled in water to form nanotubular structures with diameters ranging from 2 nm to 100 nm. Interestingly, both vesicles and nanotubes were found upon dilution of the solution with an appropriate volume of water suggesting that the concentrations of peptide can play a key role

in determining the formation of nanotubes alone or formation of a mixture of nanotubes and nanovesicles.¹⁴⁴ Junbai Li and his co-workers also showed nanotube to nanovesicle conversion of dipeptides. They demonstrated that a cationic dipeptide [FF-(NH₂)-HCl] could form self-assembled nanotubes at physiological pH, which in turn spontaneously converted into spherical vesicle-like structures after dilution.^{145,146}

Yet in another interesting example, the peptide Acp-YE (Acp, ϵ -amino caproic acid) demonstrated a concentration dependent nanovesicle to nanotube transformation. At a concentration of 6.9 mg ml⁻¹, Acp-YE formed vesicles and nanotubular structures were obtained at a peptide concentration of 2.3 mg ml⁻¹, whereas an intermediate concentration of 3.4 mg ml⁻¹ of the peptide led to the formation of an array of fused vesicular structures that fused to form nanotubular structures upon dilution. Thus peptide concentration played a significant role in modulating the peptide assembly from nanovesicles to nanotubes or fused vesicular structures.¹⁴⁷

Our own work has been focussed on the development and biomedical applications of dipeptides containing an unnatural amino acid α,β -dehydrophenylalanine (Δ Phe; Δ F) in the peptide backbone. We found that the dipeptide F Δ F, similar to FF assembled into distinct tubular structures with a mean diameter of 27–30 nm in water (Fig. 5), which were stable over a broad range of pH conditions and also in the presence of proteases.³² FTIR and CD studies demonstrated that the dipeptides adopted a beta-turn like structure in the tubular state.³² High stability of the self-assembled tubes over a broad range of pH conditions and to a highly nonspecific proteolytic enzyme, proteinase K degradation, makes these tubes interesting candidates for future applications in drug delivery.

Two amphiphilic Δ F containing dipeptides, E Δ F and K Δ F, self-assembled into anionic and cationic vesicular structures respectively.¹⁴⁸ E Δ F assembled into pleomorphic spherical structures ranging from 50–200 nm with a mean diameter of $\sim$ 110 nm, whereas, vesicles formed by K Δ F appeared mostly spherical with a mean diameter of $\sim$ 370 nm and a relatively narrow size range (250–450 nm). They were also stable to proteinase K. Light scattering studies of these particles demonstrated that E Δ F formed particles with a mean size of 370 ± 30 nm and K Δ F formed vesicles with a mean hydrodynamic diameter of 400 ± 160 nm.

We further extended these studies with remaining dipeptides with C-terminal Δ F but with varying N-terminal residues,

where the N-terminal residue was any one of the 20 naturally occurring amino acids. Out of these, the ones with aromatic N-terminal amino acid formed nanotubes, whereas those with charged N-terminal residues formed vesicles. The dipeptides with hydrophobic N-terminal residues also formed vesicles (unpublished work) (Fig. 5). It was further observed that Δ F dipeptides with hydrophobic groups at their N-termini formed visible assemblies whereas those with hydrophilic N-termini formed assembled structures invisible to the naked eye. Such an observation is interesting and points towards flexibility in peptide design which can be exploited to modulate the overall nanostructure morphology. We also found some dipeptides like M Δ F, I Δ F and L Δ F that self-assembled in a mixture of methanol and water (50 : 50, v/v) to form various nanostructures can also load hydrophobic drugs. In a 50 : 50 mixture of methanol and water, L Δ F formed large visible aggregates with a hydrodynamic diameter in the micrometer range, whereas I Δ F formed nanostructures with a mean diameter of approximately 600 nm. The mean hydrodynamic diameter of M Δ F nanoparticles was found to be approximately 160 nm. TEM showed that in a 50 : 50 mixture of methanol and water, all three dipeptides exhibited different assembly behaviour and formed nanostructures with varied morphologies. I Δ F formed micelle-like structures with an average diameter of 20 nm. L Δ F, with an equal molecular mass but differing in the position of a side chain methyl group of leucine, assembled into fibrillar structures, with diameters in the nanometer range. M Δ F formed very regular vesicular structures with a mean diameter of $\sim$ 40 nm.¹⁴⁹

Another interesting study showed that the dipeptide F Δ F, at a higher concentration and under appropriate assembling conditions could form stable hydrogels having dimensions in the nanometer range. The dipeptide gel was colorless and translucent in appearance. The gel was elastic in nature with higher storage modulus ($G' \sim 209$ kPa) than loss modulus ($G'' \sim 19.7$ kPa) and also had higher mechanical strength (evident from stable G' and G'' values at changing frequency). Electron micrographs further showed that the gel matrix was composed of a highly dense network of fibers, which provided it with high mechanical strength and solvent retention properties. The gel also showed responsiveness towards various environmental conditions such as salt concentration, temperature and pH, a desirable feature of hydrogels used in drug delivery.³³



Fig. 5 TEM images of Δ F dipeptide nanostructures (nanotubes and nanovesicles).



Fig. 6 Tumour targeted delivery by folic acid derivatized ΔF dipeptide nanoparticles (DNPs).

Self-assembly behavior of single amino acids or modified single amino acids

Single amino acids with various chemical modifications have also been investigated for their potential to make self-assembled structures. Ryan *et al.* studied self-assembly of modified single amino acids and showed that Fmoc-F formed self-assembled hydrogels. They demonstrated that side chain functionalization of Fmoc-F can have a significant effect on its self-assembly and hydrogelation behaviour.¹⁵⁰ Fluorinated derivatives of Fmoc-F, such as penta-fluorophenylalanine (5-Fl-Phe) and tri-fluorophenylalanine (3-Fl-Phe), when dissolved in water demonstrated spontaneous assembly into fibrils which later on formed a hydrogel network. Ryan *et al.* further investigated the effect of end group functionalization on the self-assembly and hydrogelation pattern of Fmoc-F derivatives by converting the C-terminal carboxylic acid moieties of Fmoc-5-Fl-F-OH and Fmoc-3-Fl-F-OH into amide and methyl ester groups. Their results depicted that though, C-terminal amide derivatives showed faster assembly than the parent carboxylic acids, the resultant hydrogels were weak and unstable to shear stress because of the lower water solubility of the amide functionality. On the other hand C-terminal esters owing to their high hydrophobicity, self-assembled into only a fibrous structure. From these results it became clear that the monomer/solvent interactions are in general very complex and they influence the self-assembly and hydrogelation pattern to a great extent. Overall, variation of either the fluorinated aromatic side chain or N-terminal functionalization influenced the hydrogelation pattern of these molecules. This also signified that fluorine and π - π interactions as the primary determinants for molecular recognition and self-assembly. A better understanding of these interactions would facilitate the development of optimal amino

acid based low molecular weight (lmw) hydrogelators.¹⁵⁰ Fmoc protected tyrosine (Fmoc-Y) also self-assembled into hydrogels by controlled enzyme-triggered dephosphorylation of Fmoc-phosphotyrosine.¹⁵¹ Such enzymatic hydrogelation of small molecules has also been investigated for various applications.^{152,153} In a very recent and interesting study, Gazit and co-workers demonstrated self-assembly of just a single amino acid phenylalanine into ordered fibrils at a pathological concentration found in the mental disorder phenylketonuria. The fibrils had an amyloid-like morphology and exhibited a well-ordered electron diffraction pattern. These assemblies exhibited cytotoxicity towards PC12 cells that was neutralized by the antibodies generated against the fibrils. These fibrils were shown to be present in the hippocampus mice model and in parietal cortex brain tissue from individuals with phenylketonuria.¹⁵⁴

Apart from experimental investigations a lot of work has also been done on the theoretical modelling and computer simulations of self-assembling systems. Schatz, Ratner, and group used bead and packing models to study the self-assembly of peptide amphiphiles (PAs).^{155–157} In their study of cone-shaped amphiphiles, Tsonchev *et al.* found that electrostatic interactions between charged residues of peptides induced a void volume in the hydrophobic tail region which guided the cluster to a cylindrical rather than a spherical shape.¹⁵⁷ McCullagh and co-workers using molecular modelling demonstrated that fiber formation was dependent on the choice of peptide residues.¹⁵⁶ Lee *et al.* studied the relaxation of self-assembled structures of 144 PA molecules, consisting of a hydrophobic alkyl chain attached to the N-terminus of the sequence SLSLAAAEIKVAV, into cylindrical nanofibers using atomistic molecular dynamics simulations in explicit water with physiological ion concentration. Self-assembly of the molecules is initiated in a cylindrical configuration based on prior experimental and theoretical

investigations, and the resulting cylindrical configuration was found to be stable during 40 nanosecond simulations. It was observed that water and sodium ions can penetrate into the peptidic part of the fiber but not between the alkyl chains. The electrostatic interactions between the PAs and the sodium counterions and the van der Waals interactions between the PAs were found to be the most important interactions stabilizing the nanofiber architecture.¹⁵⁸ Lee *et al.*, also used a coarse-grained molecular dynamic simulation based on the MARTINI force-field to study the self-assembly process of 140 IKVAV epitope bearing PAs for 16 μ s and found that PAs first formed spherical micelles (during first 0–0.05 μ s), which then formed a three-dimensional network with neighboring micelles *via* van der Waals interactions, then the tails of different micelles merge and formed fibers.¹⁵⁹ Velichko *et al.* developed a simplified coarse-grained model to study the influence of hydrogen-bond formation on the self-assembly of PAs and found that fibrous assemblies were formed by both hydrophobic interactions and the network of hydrogen bonds.¹⁶⁰ It has been suggested that the formation of β -sheets parallel to the axis of the fiber is the driving force for the formation of cylindrical fibers rather than spherical micelles.^{160–163}

In order to understand the A β fibrilization mechanism as well as structural properties of FF nanostructures, simulation studies were carried out using both all atom^{164,165} and coarse-grained peptide models.^{166,167} Tamamis *et al.* studied the association of FF peptides using an implicit water model, and found transient formation of ring-like structures which are reminiscent of the nanotubular structure.¹⁶⁵ However, these studies used only a few peptide molecules (96 FF chains) or short simulation time and only resulted in disordered aggregates. Energy minimization¹⁶⁸ simulations and molecular dynamics¹⁶⁴ simulation studies of FF showed the formation of cylindrical structures which are reminiscent of tubular structures.¹⁶⁹ Guo *et al.*¹⁷⁰ used a coarse-grained peptide model to study FF nanostructures and their concentration dependence. They suggested that the FF peptide behaved like a surfactant that first formed vesicles and then these vesicles fused to form nanotubes. Their molecular dynamic trajectories showed the formation of ordered spherical, vesicular or tubular nanostructures and a formation of either vesicles or tubes was concentration dependent. At low concentrations fusion of vesicles or the fusion of vesicles with a bilayer occurred, whereas at high concentrations, first a bilayer was formed, that bent and closed to form tubes.¹⁷⁰

Recently, Jeon *et al.* explored the initial stages of FF assembly by carrying out molecular dynamic simulations on zwitter ionic and capped FF nanotubes. They showed that electrostatic interactions between peptides led to the formation of ordered dimers and trimers, whereas hydrophobic interactions between side chains were involved in deciding the structures of larger oligomers.¹⁷¹ Also FF peptides with charged termini because of electrostatic steering first formed dimer or trimer ladders that further facilitated hydrophobic association of side chains and formed more ordered and compact structures as compared to those of uncharged FF peptides that associated by hydrophobic interactions.¹⁷¹ Simulations of the crystal structure of FF suggested that the strongest interactions occurred between

side chains and the charged termini formed salt bridges.¹⁷¹ All these studies along with experimental investigations shed light on the potential phenomena behind peptide assembly and significantly improved understanding of these systems.

Potential applications of peptide based self-assembled nanostructures in drug-delivery

Different types of biocompatible, inorganic nanomaterials have been developed for the drug delivery purpose.¹⁷² However, many of them contain potentially toxic elements^{173–176} and have been proven to be less promising for human use. For instance, positively charged lipid-based nanoparticles are known to trigger strong immune responses. Liposomes present technological limitations such as poor reproducibility and stability, and low drug entrapment efficiency and poor control of drug leaching. Polymer nanosystems may be potentially useful alternatives, but their surface functionalization for improving drug-targeting is usually complicated and rather ineffective. Besides, most nanostructures based on naturally occurring polymers have problems of eliciting unwanted immune response and also present lot to lot variability which makes it difficult to predict their behavior in living systems.

Designed peptide based nanoparticles, due to their biocompatibility, ease of synthesis and functionality, in principle can be excellent candidates for drug and gene delivery. Encapsulation by self-assembled peptide nanostructures holds particular promise in the delivery of biological molecules, including DNA, water insoluble drugs and tagged molecules for imaging.

Drug delivery using nanostructures generated from long peptides

Tanaka *et al.* developed TV-XIIa, an 11-residual peptaibol derived carrier peptide for the delivery of antisense oligonucleotides. TV-XIIa was derivatised with a 10-mer of lysine at the C-terminus to make the designed carrier peptide, Ac-U-N-I-I-U-P-L-L-U-P-I-K-K-K-K-K-K-K-K-K-OH (U: α -aminoisobutyric acid), which electrostatically interacted with oligodeoxynucleotides (ODNs) and formed a complex with ODNs, capable of crossing membranes of NIH3T3 cells to accumulate in the cytoplasm and the nucleus.¹⁷⁷

A novel class of core-shell like self-assembled nanoparticles developed from an amphiphilic peptide, cholesterol-G3R6YGRKKRRQRRR, abbreviated as CG3R6TAT, where TAT is the transcriptional activator protein of the human immunodeficiency virus type-1, by Yang *et al.* demonstrated powerful antimicrobial activities against a variety of microbes (bacteria, yeasts and fungi).¹⁷⁸ The nanoparticles possessed a broad spectrum of antimicrobial activities and were active against a variety of Gram-positive as well as drug-resistant Gram-positive bacteria, fungi and yeast with low minimal inhibitory concentration (MIC) values and very low haemolysis. More interestingly, the peptide nanoparticles could cross the blood brain barrier (BBB) in a *Staphylococcus aureus*-induced meningitis rabbit model and minimised bacterial growth in brain without causing any significant toxicity to the major organs. These

properties indicate that nanoparticles can be developed as efficient antimicrobial agents in treating brain infections. Yang *et al.* further demonstrated that the efficacy of these peptide nanoparticles could be extended to other infectious diseases such as methicillin-resistant *Staphylococcus aureus* associated infections, *Candida albicans*-caused brain infections, and *Stachybotrys chartarum* infections. Taken together, these nanoparticles were found to be promising antimicrobial agents that can be used to treat brain infections and other infectious diseases.¹⁷⁸ The cyclic peptide nanotubes developed by Ghadiri *et al.*, also served as nanocontainers for effective drug delivery¹⁷⁹ and also as anti-microbial agents.^{180,181}

Peptide-Based-Nanoparticle Devices (PBNDs), which are described as short amphipathic peptides with a capacity to form stable nanoparticles with proteins and/or nucleic acids have also been explored for their potential as drug and gene delivery vehicles. Divita *et al.* developed a PBND named MPG, a 27-residue-long primary amphipathic peptide (acetyl-GALFLGFLGAAGSTMGAWSQPKKKRKVcysteamide), containing three distinct domains: an N-terminal hydrophobic motif (GALFLGFLGAAGSTMGA) derived from the fusion sequence of the HIV-1 gp 41 (glycoprotein 41) for interaction with the lipid moiety of the cell membrane, a hydrophilic domain (KKKKRKV) derived from the nuclear localization sequence of simian virus 40 large T-antigen for promoting interactions with nucleic acids and intracellular trafficking of the cargo, and a linker domain (WSQP), which improves the flexibility and integrity of the hydrophobic and the hydrophilic domains. This PBND showed siRNA condensing properties by virtue of a basic peptide domain and membrane destabilizing properties due to the presence of a hydrophobic peptide sequence, formed stable nanoparticles with siRNA and entered the cell independent of the endosomal pathway to efficiently deliver siRNA into a variety of cell lines^{182,183} Other PBND-family peptides named CADY and PEP also acted as gene carriers that entered a variety of challenging cells independent of the endosomal pathway for efficient delivery.¹⁸⁴

Nanocarriers generated from chondroitin sulfate A (CSA) coated and PEGylated poly-L-lysine-based dendrimers have also been developed for controlled and sustained delivery of an antimalarial drug chloroquine phosphate (CQ). Entrapment in the peptidic carrier led to a significant reduction in the cytotoxicity as well as hemolytic activity of the drug. Also a significant reduction in levels of ring and trophozoite stages of *Plasmodium falciparum* were found after being treated with drug loaded CSA coated dendrimers as compared to the free drug.¹⁸⁵

Yang and co-workers developed self-assembled micelles generated from synthetic oligopeptide amphiphiles (*i.e.* A₁₂H₅K₁₀ and A₁₂H₅K₁₅) that acted as efficient gene delivery vehicles.¹⁸⁶ In another study they developed nanostructures from an oligopeptide amphiphile, Ac-(AF)₆-H₅-K₁₅ (FA32), and evaluated them as carriers for co-delivery of the anticancer drug doxorubicin (Dox) with a luciferase reporter and p53 genes. Co-delivery of the drug and genes using FA32 micelles demonstrated a synergistic cytotoxic effect between the p53 gene and Dox with an increase in the p53 mRNA expression level as well as end point cytotoxicity towards HepG2 cells.¹⁸⁷

Drug delivery using nanostructures generated from short peptides

Peptide-based vectors owing to their easy functionality and better design strategies can be developed as efficient gene delivery vehicles. Zhang and his group developed a series of surfactant peptides like LLLLLLKK, which are composed of a hydrophobic tail attached to a polar head group at the C- or N-terminus. This peptide self-assembled into nanovesicles and nanotubes that acted as DNA delivery vehicles.^{188,189} In DNA solution, the positively charged peptides self-assembled into a tube that encapsulated the negatively charged DNA. This 'minivan' was then able to deliver DNA to growing cells, at least in some cases. These systems can further be developed as smarter materials by tagging the minivan surface with a cell specific marker.¹⁹⁰ Using β -sheeted peptide-based nanoribbons as scaffolds, filament-shaped artificial viruses for gene and drug delivery have been developed.¹⁹¹

Stimuli-responsive peptide nanostructures are particularly attractive as drug delivery vehicles since these can have stimuli triggered drug release at target sites. For example, stimuli responsive peptide nanovesicles can be loaded with drugs and other important biomolecules to release them in response to environmental systems like pH, temperature and others.¹⁴⁷ Tri-peptide derivatives conjugated with olsalazine, an anti-inflammatory prodrug, self-assembled in water to form prodrug-containing supramolecular hydrogels.¹⁹² It was also shown that the controlled release of an anti-inflammatory agent 5-amino-salicylic acid could be achieved from the gel by the disruption of the supramolecular hydrogel caused by the reduction of the azo group. This method can be generalised for developing new nanobiomaterials for site specific drug delivery.¹⁹² Banerjee *et al.*'s group developed nanovesicles from dipeptides containing glutamic acid residues at the C terminus. Though these vesicles were stable over a wide range of pH, they showed responsiveness towards the presence of calcium ions. These vesicles encapsulated the anticancer drug Dox, fluorescent dyes, various biologically active molecules and were capable of releasing them in response to calcium ions. They have also been shown to act as delivery agents for biologically active molecules, such as cyclic adenosine monophosphate within the cells, while preserving their biological activity.¹⁹³ Another fascinating example of peptide nanostructure for drug delivery was the one generated from the oligopeptide Acp-YE (Acp, ϵ -amino caproic acid), which showed nanotube to vesicle transition and was responsive to calcium ions in the solution as well as to pH change.¹⁴⁷ These nanovesicles could entrap Dox and release it in a triggered manner in the presence of calcium ions.

Peptide based self-assembled hydrogels in drug delivery

Hydrogels are an important class of biological materials with a wide range of applications in drug delivery and tissue engineering. Peptide hydrogels/nanogels are particularly important biomaterials as they do not use harmful chemicals (*e.g.*, toxic cross-linkers *etc.*) to initiate gelation, they are non-toxic, non-immunogenic, biodegradable and degrade into natural amino acids. It has been shown that coiled-coil polypeptides based on,

aba tri-block polymers self-associated to form hydrogels with potential application in sustained protein delivery.⁶⁹

Hydrogels prepared from MAX1 and MAX8 encapsulated and released model biomacromolecules in a controllable fashion. Fluorescence recovery after photobleaching (FRAP) measurements and bulk release studies of a series of FITC-labeled dextrans within gel networks of differing peptide weight percents, demonstrated that probe diffusion inside the hydrogel network depended on the probe size, the peptide sequence, and the mesh size of the gel. Further, the electrostatic interactions between a given macromolecule and the gel network also influenced their release pattern. This study suggested that the release of macromolecules with varied characteristics such as size and charges from these self-assembling β -hairpin peptide hydrogels could be controlled and fine-tuned by simply modifying the amino acid sequences and the peptide weight percent of the gel.¹⁹⁴

Hydrogels generated from oligopeptides, GAIL and GFIL, demonstrated thermo and pH responsive behaviour. At their minimum gelling concentration, GAIL and GFIL gels were shown to entrap 8.62×10^{-3} (M) and 3.79×10^{-3} (M) of Dox respectively. They also showed controlled drug release behaviour with almost 85% and 90% of the drug molecules getting released from the gel matrix after 45 h respectively.¹⁹⁵

Self-assembled hydrogels generated from Fmoc-diphenylalanine encapsulated various enzyme bioreceptors (*e.g.*, glucose oxidase or horseradish peroxidase) and fluorescent reporters [*e.g.*, CdTe and CdSe quantum dots (QDs)]. Enzyme loaded hydrogels were smartly used for biosensing purpose and for the detection of analytes such as glucose and toxic phenolic compounds by using a photoluminescence quenching of the hybridized QDs. These results suggest that the peptide hydrogels can act as intelligent optical biosensing platforms by virtue of their simple fabrication method (by self-assembly), efficient analyte diffusion, and high encapsulation efficiencies for fluorescent reporters and bioreceptors.¹⁹⁶

Dipeptide hydrogels derived from beta-amino acids by Banerjee and co-workers have been shown to encapsulate vitamins like B₂ and B₁₂ and sustain their release for 3 days at physiological pH (7.46) and temperature (37 °C). This depicts the potential of these gel-based biomaterials for sustained release of drugs and other important biomolecules.¹⁹⁷

Drug delivery using Δ F dipeptide nanostructures

Since the last decade, our group has been working on the design and synthesis of Δ F dipeptide based nanostructures for drug delivery applications. Dipeptide nanovesicles formed by amphipathic dipeptides K Δ F and E Δ F could encapsulate bioactive molecules such as vitamin B₁₂, amodiaquin (antimalarial), ampicillin (antibiotic), mitoxantrone (anticancer), polypeptide insulin, synthetic anti-microbial peptides *etc.* The ability of these vesicles to entrap proteins of various sizes such as recombinant malaria vaccine candidates namely, merozoite surface protein-119 (Pf MSP-119; 11.2 kDa), merozoite surface protein-3N (Pf MSP-3N; 25 kDa), and *Plasmodium falciparum* Histidine Rich Protein-2 (Pf HRP-II; 32.9 kDa), in addition to

chicken egg lysozyme (16.2 kDa), bovine serum albumin (BSA; 66.4 kDa), and anti-mouse goat IgG (150 kDa) were also investigated. Results showed that all proteins interacted with the nanovesicles to varying degrees. TEM imaging carried out to determine the position of proteins on the dipeptide vesicles showed that protein Pf HRP-II was encapsulated in both the vesicles, whereas Pf MSP-119 localized preferentially on the surface of the vesicles. However, TEM studies also suggested that some other proteins like BSA, lysozyme, Pf MSP-3N, and IgG destabilized the nanovesicles. The dipeptide vesicles were taken up by mammalian cells and were not cytotoxic.¹⁴⁸

Other Δ F dipeptides like R Δ F, L Δ F formed vesicular and micellar nanostructures. R Δ F also formed self-assembled nanostructures with a mean hydrodynamic diameter of approximately 250 ± 50 nm. TEM showed that the dipeptide formed vesicular nanostructures with mean size ranging between 30 and 50 nm. L Δ F with a hydrophobic N-terminus, assembled into micellar structures with a hydrodynamic diameter of ~ 250 nm.^{198,199} Nanostructures formed by the F Δ F, R Δ F, L Δ F and E Δ F entrapped drugs like riboflavin, niacin, amodiaquin, mitoxantrone, ampicillin with varying entrapment efficiency. They also showed non-cytotoxicity towards a variety of mammalian cells. R Δ F nanostructures could be labeled with the radioisotope Technetium-99 (Tc99) with stannous tartarate as the reducing agent. Biodistribution studies carried out using Tc-labeled R Δ F nanostructures, showed accumulation in the kidney and bladder along with some radioactivity in the heart and blood vessels 1 hour post-injection. Interestingly, the biodistribution profile revealed that R Δ F nanostructures can evade uptake by reticulo endothelial system (RES) organs such as the liver (no significant accumulation) to remain in blood circulation for a long time.¹⁹⁸

Delivery of hydrophobic drugs has always been a challenge. Thus in order to improve the loading of hydrophobic drugs, we tried to develop novel self-assembled dipeptide nanostructures in an aqueous/organic mixture. We chose curcumin as the model drug and tried to load it in Δ F dipeptide nanoparticles. Among all the dehydrodipeptides tested, M Δ F demonstrated the maximum curcumin loading efficiency and could release the drug in a sustained manner. Loading of curcumin in M Δ F nanoparticles, increased its solubility and improved cellular availability. Curcumin-M Δ F nanoparticles showed an enhanced cytotoxic effect in different cancerous cell lines such as human cervical cancer cell line (HeLa), human breast cancer cell line (MCF-7) and human hepatocarcinoma cell line (HuH-7), as compared to native curcumin. These nanostructures also enhanced curcumin's *in vivo* efficacy towards inhibiting tumor growth in Balb/c mice bearing a B₁₆F₁₀ melanoma tumor.¹⁴⁹ Such dipeptide nanoparticles are also expected to improve the delivery of other potent hydrophobic drug molecules with poor cellular uptake, bioavailability, and efficacy.

Dipeptides E Δ F, K Δ F, R Δ F and D Δ F, with charged amino acids at N-termini, were synthesized and investigated for their assembly behavior. Out of the four, R Δ F formed vesicular nanoparticles that could be easily derivatized with folic acid. Folic acid derivatized nanoparticles showed enhanced cellular-uptake in various cancer cells like human breast adenocarcinoma (MDA-MB-231) and HeLa that over-expressed folic acid

receptors. Folic acid derivatized RΔF nanoparticles also exhibited high Dox encapsulation efficiency and Dox loaded nanoparticles demonstrated enhanced cytotoxicity towards cancer cells over-expressing folic acid receptors (Fig. 6). Bio-distribution and tumor distribution studies carried out using Tc99 labeled RΔF and folic acid-RΔF nanoparticles in Ehrlich ascitic tumor bearing Balbc mice, exhibited enhanced tumor targeting and accumulation as compared to underivatized nanoparticles. In comparison with underivatized nanoparticles or native drug, Dox loaded folic acid-RΔF nanoparticles showed enhanced tumor regression in breast tumor bearing nude mice as well as ascitic tumor bearing Balbc mice.¹⁹⁹

Recently, we have also shown the potential of FΔF nanotubes for intravitreal delivery of pazopanib, a multi-targeted tyrosine kinase inhibitor with efficacy for treating various cancers as well as ocular disease like choroidal neovascularisation in wet age related macular degeneration. The drug could be loaded in the nanotubes by both pre and post-loading methods. The pre-loading method was found to be more efficient in loading the drug in nanotubes (25% w/w pazopanib loading and ~55% loading efficiency) compared to the post-loading (8% w/w pazopanib loading and ~17% loading efficiency). Plain and peptide loaded nanotubes were non-cytotoxic to retinal pigment epithelial cells. The tubes sustained *in vitro* release of pazopanib for 35 days. They also demonstrated a sustainable eye delivery for a period of 15 days following intravitreal injection using a 33 gauge needle.²⁰⁰

We also investigated gene delivery potential of nanoparticles synthesized from cationic ΔF dipeptides RΔF and KΔF and found that the cationic dipeptides condensed plasmid DNA into discrete vesicular nanostructures with RΔF forming more regular and ordered vesicular nanostructures.²⁰¹ Dipeptide nanoparticles were non-cytotoxic and showed enhanced cellular uptake. They protected DNAs condensed inside them from enzymatic degradation and could successfully deliver these DNAs to different types of cancer cells such as HeLa and HuH 7 (Fig. 7). GFP encoding plasmid DNA loaded dipeptide NPs showed positive gene transfection as well as gene expression in HuH 7 cells. By virtue of their simple dipeptide origin, ease of synthesis, high enzymatic stability as well biocompatibility, these nanostructures can be foreseen to be developed as vehicles for effective gene therapy.

Thus, our work showed that the ΔF dipeptide nanostructures can act as potential candidates for drug delivery applications. The most important point here is that these dipeptide nanostructures are biocompatible with no adverse side effects both *in vitro* and *in vivo*. Due to the presence of ΔF residue in the peptide design, these dipeptide nanostructures are expected to show enhanced stability to enzymatic degradation^{202,203} and thus would have high *in vivo* half-life.

Similarly, the FΔF hydrogel also showed drug loading and release behavior (Fig. 8). The peptide gel could effectively entrap vitamins like ascorbic acid, riboflavin, and vitamin B12, antibiotics like ampicillin and chloramphenicol, antimalarial drugs such as amodiaquin, anticancer drugs like fludarabine and mitoxantrone and anti-tuberculosis drugs like L-cycloserine and isoniazid. The polypeptide insulin was also entrapped in the



Fig. 7 Cationic dipeptide–DNA nanoparticles for cellular delivery.

dipeptide gel. All the entrapped molecules in the hydrogel, exhibited sustained release behavior from the gel matrix with their diffusion coefficient (D) values ranging between $D = 2.9 \times 10^{-11}$ to 5.6×10^{-10} . The release rates of drugs from the gel matrix (D values) were also found to be dependent on the nature of drugs like their molecular weight, $C \log P$ values and net charge. The gel further demonstrated stimuli responsive behavior and released entrapped molecules in response to changes in environmental pH or salt-concentration.³³ The ability of the dipeptide hydrogel to encapsulate a diverse kind of bioactive molecules within its matrix and their “depot-like” sustained release behavior, as well as increased proteolytic stability, makes the described dipeptide gel a promising candidate for potential drug delivery applications.

Potential application of self-assembling peptides in tissue engineering

Successful tissue regeneration requires that cells should be provided with an environment appropriate for regeneration, induction, and development of neo-tissue. The behavior pattern of cells changes when they are constrained to two dimensional (2D) culture in Petri dishes, after being isolated from their complex native tissue environment. Hence, it is necessary that cells should be grown in a three dimensional (3D) matrix to maintain their natural phenotypic shape and behavior patterns. Scaffolds required for the 3D growth of cells to generate specific organs should ideally be biodegradable with nontoxic degradation products easily eliminated by the host. One important aim in the design of synthetic tissue scaffolds is to mimic the structure and function of the extracellular matrix (ECM) for providing the cells a microenvironment similar to that encountered *in vivo*. The ECM organizes cells into tissue, affects overall cellular architecture, provides pathways for migratory cells, participates in signal transduction pathways and strengthens tissues. The architecture of the natural ECM components has been an inspiration for researchers to synthesize materials with a similar structure for biomedical applications such as hydrogels, for cell-based therapeutics^{204,205} and soft tissue engineering scaffolds.²⁰⁶



Fig. 8 Schematic diagram showing drug entrapment and release in a peptide (FΔF) hydrogel.

The present day cell-based therapeutic hydrogels are either derived from natural biopolymers (such as collagen, hyaluronate, fibrin, alginate, agarose, and chitosan) or from synthetic polymers (such as poly(acrylic acid), poly(ethylene oxide), poly(vinyl alcohol), and polyphosphazene).²⁰⁶ The natural polymers suffer from limitations such as the presence of variable properties based on their source of origin or presence of viral contaminants, whereas, synthetic polymers usually lack functional sites for cellular interactions and may show adverse side-effects.

Self-assembling peptides generally show favorable properties concerning biocompatibility, immunogenicity and biodegradability, producing non-toxic waste products. In recent past several self-assembled peptide based hydrogels have been explored as scaffolds for 3D cell growth and tissue engineering purpose.²⁰⁷ Self-assembling peptide nanofiber scaffolds with amino terminal with sequences derived from collagen IV or laminin demonstrated enhanced adhesion and spreading of human aortic endothelial cells.²⁰⁸ In order to mimic the ECM structure and porosity, peptide nanofibers were further combined with epithelial growth factors. The modified nanofibers showed an improved rate of wound healing on a human skin equivalent tissue model.²⁰⁹ Such modified scaffolds have also showed enhanced growth properties in mouse neural stem cells²¹⁰ and pre-osteoblasts.²¹¹ Zhang *et al.* developed a unique type of peptide based scaffolds which could be injected into the myocardium, creating an intra-myocardial microenvironment for mouse endothelial cells that promoted vascular cell recruitment.²¹²

Hartgerink *et al.* developed various peptide amphiphiles which could form different types of nanofibers.²¹³ Peptide amphiphile molecules were derivatized with biologically active

peptides containing RGD for promoting cell adhesion and phosphorylated serine residues for promoting hydroxyapatite mineralization. Linear and cyclic RGDS²¹⁴ as well as various other bioactive signals such as biotin²¹⁵ were also presented on the N-terminal of peptide amphiphile nanofibers to promote better cell growth and differentiation. Peptide nanofibers were further assembled to form branched peptide amphiphiles, leading to greater accessibility of binding sites. These nanofibers were developed as bioactive scaffolds with improved epitope recognition for enhanced cell adhesion^{214,216} for tissue engineering applications,^{217,218} and magnetic resonance imaging.²¹⁹ Sargeant *et al.* fabricated hybrid bone implants by self-assembling peptide amphiphile nanofibers within porous titanium.²²⁰ These implants demonstrated cell encapsulation, vascularization and mineralization of calcium phosphate with a calcium : phosphate ratio similar to that of hydroxyapatite.

Self-assembling nanofibers have also been shown to act as scaffolds for neural progenitor cells,²²¹ dental stem cells²²² as well as have been used for cell entrapment²²³ and stimulation of angiogenesis.²²⁴ RGD containing β -sheet forming peptide sequences with capacity to self-assemble into nanoribbons, have been shown to encapsulate hydrophobic molecules as well as cells through integrin receptors. Hydrogel scaffolds produced by the peptide Ac-(KL₃DL)₃-CONH₂ have been shown to support the growth and differentiation of chondrocytes along with stimulating the synthesis and accumulation of the extracellular matrix.²²⁵ Self-assembling peptides were also being developed as scaffolds for cartilage repair and promotion of nerve cell growth.²²⁶ A peptide amphiphile, C16-G3A4-IKVAV, shown to promote the re-growth of nerve cells in rats was made by including a neurite-promoting laminin epitope tag IKVAV.²²⁷

The β -hairpin hydrogel developed from the peptide MAX 1, promoted growth and proliferation of fibroblast cells.²²⁸ Another study demonstrated hydrogel formation from an N-terminally protected peptide sequence naphthalene-FFGRGD.²²⁹ This was used for surface coating on poly(3-caprolactone) (PCL) films, and to promote cell attachment and growth.

Similarly self-assembled fibers generated from the RADA16 peptide were used in a wide range of biomaterial applications, including cartilage tissue repair,²²⁶ osteoblast proliferation and differentiation,²¹¹ bone regeneration²³⁰ and axon regeneration.²³¹ Cardiomyocytes or non-differentiated stem cells loaded fibril-forming peptide gels when injected into damaged heart tissues led to the improvement of transplanted cell survival and wound healing after a myocardial infarction.^{212,232}

Scaffolds derived from peptide-amphiphiles have been shown to promote the attachment of primary human bladder cells, demonstrating the potential biological application of PAs for augmenting the biocompatibility of polymeric materials conventionally used for tissue engineering.²¹⁸

Another wonderful application of PA-based nanofibers includes the adhesion and migration of neural cells *in vitro*.²²⁷ Remarkably, these PA-based nanofibers exhibited extremely promising results in an animal model based on a spinal cord injury.²³³ Another amphiphilic peptide construct, containing a heparin-binding site, exhibited very exciting results in promoting angiogenesis.²³⁴ These types of peptides were further modified with biotin²¹⁵ and a Gd³⁺ metal-chelating moiety suitable for detection by magnetic resonance imaging (MRI).²¹⁹

Vescovi and group reported about short assembling peptides containing the bone marrow homing peptide 1 (BMHP 1) functional motif (PFSSTKT) (BMHP1-SAPs). These peptides assembled to form nano or microstructures of varied shape like tubular fibers, twisted ribbons, tubes and sheets. Interestingly, despite having a heterogeneous nanostructure morphology and varied scaffold stiffness, all the BMHP1-SAPs exhibited β -sheets and β -turns like the secondary structure. A few of these 10-mer peptides promoted adhesion, differentiation, and proliferation of human neural stem cells. These SAPs could be derivatized with the cell adhesion promoting motif like RGDGG, for promoting enhanced cell attachment. *In vivo* experiments carried out using these SAPs exhibited negligible side reactions towards host nervous tissue.²¹⁰ Hydrogels prepared from the P11-4 peptide were also found to have tissue engineering applications such as enamel remineralization,²³⁵ injectable scaffolds²³⁶ and joint lubricants.²³⁷

Zhou *et al.* reported a peptide based bioactive hydrogel using molecular self-assembly of a mixture of two aromatic short peptide derivatives: Fmoc-FF (fluorenylmethoxycarbonyl diphenylalanine) and Fmoc-RGD (arginine-glycine-aspartate). This biomimetic nanofibrous hydrogel acted as a 3D-scaffold for anchorage-dependent cells.¹³⁴

An array of FF nanofibers grown on gold microelectrodes led to the development combined cell culture and biosensing platforms. Peptide nanowires were modified with conductive polymers for enabling detection of dopamine at physiological concentrations as well as to grow different cell lines such as PC12 and HeLa.²³⁸

Our group has also reported 3D growth of mammalian cells [HeLa and fibroblast (L929)] on a chemically functionalized F Δ F hydrogel. The peptide hydrogel was functionalized with the cell adhesion motif "RGD" containing pentapeptide to facilitate cell growth and proliferation. This functionalized hydrogel provided a wonderful support for 3D cell growth for more than two weeks with maintaining cell viability and spreading. This study provides an excellent example of a simple peptide based-hydrogel to attain increased cell growth promoting properties, with high enzymatic stability.²³⁹ This gel-based soft material acts as a convenient template for 3D cell growth with probable use in tissue engineering and cell biology.

All these results encourage the use of peptide-based biomaterials in regenerative medicine and pave the way for the development of novel self-assembling peptide sequences that may be useful for materials science and regenerative medicine applications. In addition to the above mentioned applications peptide based self-assembled structures can have many more applications such as materials for surface engineering, bio-sensing devices and many more to be discovered in near future.²⁹

Conclusion

Self-assembly is a marvelous strategy to make an ensemble of nanostructures. Small peptide-based self-assembled nanostructures, due to their inherent biocompatibility, easy tunability, simple and cost-effective synthesis could offer a myriad of potential uses in biomedical applications. Peptides could spontaneously self-assemble into tubular or fibrillar or vesicular nanostructures. The nanostructures have been shown to entrap a wide range of bioactive molecules with a controlled release pattern. Peptide based hydrogels with a nanofibrillar morphology have been shown to support 3D growth of various cell types along with promoting cell differentiation in many cases. Thus small peptides have tremendous potential to be developed as intelligent biomedical scaffolds with many biomedical applications. Thus peptide self-assembly is emerging as a new area of research and has spurred intensive interest in the fabrication of nanoscale devices and demand for miniaturization in both academia and industries necessitate progress in this area.

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References

- 1 G. M. Whitesides and B. Grzybowski, *Science*, 2002, **295**, 2418–2421.
- 2 S. Zhang, *Nat. Biotechnol.*, 2003, **21**, 1171–1178.
- 3 S. Stupp, *Nano Lett.*, 2010, **10**, 4783–4786.

- 4 G. M. Whitesides, J. P. Mathias and C. T. Seto, *Science*, 1991, **254**, 1312–1319.
- 5 Z. Liu, Y. Jiao, Y. Wang, C. Zhou and Z. Zhang, *Adv. Drug Delivery Rev.*, 2008, **60**, 1650–1662.
- 6 K. A. Janes, P. Calvo and M. J. Alonso, *Adv. Drug Delivery Rev.*, 2001, **47**, 83–97.
- 7 M. Prabakaran and J. F. Mano, *Drug Delivery*, 2005, **12**, 41–57.
- 8 D. G. Kim, Y. I. Jeong, C. Choi, S. H. Roh, S. K. Kang, M. K. Jang and J. W. Nah, *Int. J. Pharm.*, 2006, **319**, 130–138.
- 9 Z. R. Cui and R. J. Mumper, *J. Controlled Release*, 2001, **75**, 409–419.
- 10 A. Zahoor, S. Sharma and G. K. Khuller, *Int. J. Antimicrob. Agents*, 2005, **26**, 298–303.
- 11 J. W. de Vries, F. Zhang and A. Herrmann, *J. Controlled Release*, 2013, **172**, 467–483.
- 12 D. Kim, Y. Y. Jeong and S. Jon, *ACS Nano*, 2010, **4**, 3689–3696.
- 13 J. I. Cutler, E. Auyeung and C. A. Mirkin, *J. Am. Chem. Soc.*, 2012, **134**, 1376–1391.
- 14 N. L. Rosi, *Science*, 2006, **312**, 1027–1030.
- 15 J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc.*, 1998, **120**, 1959–1964.
- 16 M. D. Costioli, I. Fisch, F. Garret-Flaudy, F. Hilbrig and R. Freitag, *Biotechnol. Bioeng.*, 2003, **81**, 535–545.
- 17 M. Safak, F. E. Alemdaroglu, Y. Li, E. Ergen and A. Herrmann, *Adv. Mater.*, 2007, **19**, 1499–1505.
- 18 F. E. Alemdaroglu, K. Ding, R. Berger and A. Herrmann, *Angew. Chem., Int. Ed.*, 2006, **45**, 4206–4210.
- 19 M. Kwak, J. Gao, D. K. Prusty, A. J. Musser, V. A. Markov, N. Tombros, M. C. A. Stuart, W. R. Browne, E. J. Boekema, G. ten Brinke, H. T. Jonkman, B. J. van Wees, M. A. Loi and A. Herrmann, *Angew. Chem., Int. Ed.*, 2011, **50**, 3206–3210.
- 20 P. Guo, *J. Nanosci. Nanotechnol.*, 2005, **5**, 1964–1982.
- 21 S. Guo, N. Tschammer, S. Mohammed and P. Guo, *Hum. Gene Ther.*, 2005, **16**, 1097–1109.
- 22 A. Khaled, S. Guo, F. Li and P. Guo, *Nano Lett.*, 2005, **5**, 1797–1808.
- 23 E. N. Marsh and W. F. DeGrado, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 5150–5154.
- 24 H. Gradišar and R. Jerala, *J. Nanobiotechnol.*, 2014, **12**, 1–9.
- 25 C.-J. Tsai, J. Zheng, D. Zanuy, N. Haspel, H. Wolfson, C. Alema and R. Nussinov, *Proteins: Struct., Funct., Bioinf.*, 2007, **68**, 1–12.
- 26 E. Gazit, *Drugs Future*, 2004, **29**, 613–619.
- 27 I. W. Hamley, *Angew. Chem., Int. Ed.*, 2007, **46**, 8128–8147.
- 28 E. Gazit, *Chem. Soc. Rev.*, 2007, **36**, 1263–1269.
- 29 A. Lakshmanan, S. Zhang and C. A. Hauser, *Trends Biotechnol.*, 2012, **30**, 155–165.
- 30 K. Rajagopal and J. P. Schneider, *Curr. Opin. Struct. Biol.*, 2004, **14**, 480–486.
- 31 T. J. Deming, *Adv. Drug Delivery Rev.*, 2002, **54**, 1145–1155.
- 32 M. Gupta, A. Bagaria, A. Mishra, P. Mathur, A. Basu, S. Ramakumar and V. S. Chauhan, *Adv. Mater.*, 2007, **19**, 858.
- 33 J. J. Panda, A. Mishra, A. Basu and V. S. Chauhan, *Biomacromolecules*, 2008, **9**, 2244–2250.
- 34 S. Scanlon and A. Aggeli, *Nano Today*, 2008, **3**, 22–30.
- 35 D. Ranganathan, M. P. Samant and I. L. Karle, *J. Am. Chem. Soc.*, 2001, **123**, 5619–5624.
- 36 S. Santoso, W. Hwang, H. Hartman and S. Zhang, *Nano Lett.*, 2002, **2**, 687–691.
- 37 M. R. Ghadiri, J. R. Granja, R. A. Milligan, D. E. McRee and N. Khazanovich, *Nature*, 1993, **366**, 324–327.
- 38 J. D. Hartgerink, J. R. Granja, R. A. Milligan and M. R. Ghadiri, *J. Am. Chem. Soc.*, 1996, **118**, 43–50.
- 39 C. Valery, M. Paternostre, B. Robert, T. Gulik-Krzywicki, T. Narayanan, J. C. Dedieu, G. Keller, M. L. Torres, R. Cherif-Cheikh, P. Calvo and F. Artzner, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 10258–10262.
- 40 S. Fernandez-Lopez, H. S. Kim, E. C. Choi, M. Delgado, J. R. Granja, A. Khasanov, K. Kraehenbuehl, G. Long, D. A. Weinberger, K. M. Wilcoxon and M. R. Ghadiri, *Nature*, 2001, **412**, 452–455.
- 41 R. J. Brea, C. Reiriz and J. R. Granja, *Chem. Soc. Rev.*, 2010, **39**, 1448–1456.
- 42 A. Aggeli, I. A. Nyrkova, M. Bell, R. Harding, L. Carrick, T. C. McLeish, A. N. Semenov and N. Boden, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 11857–11862.
- 43 A. Aggeli, M. Bell, L. M. Carrick, C. W. Fishwick, R. Harding, P. J. Mawer, S. E. Radford, A. E. Strong and N. Boden, *J. Am. Chem. Soc.*, 2003, **125**, 9619–9628.
- 44 D. M. Marini, W. Hwang, D. A. Lauffenburger, S. Zhang and R. D. Kamm, *Nano Lett.*, 2002, **2**, 295–299.
- 45 K. Lu, J. Jacob, P. Thiagarajan, V. P. Conticello and D. G. Lynn, *J. Am. Chem. Soc.*, 2003, **125**, 6391–6393.
- 46 M. Reches and E. Gazit, *Science*, 2003, **300**, 625–627.
- 47 S. Colfer, J. W. Kelly and E. T. Powers, *Langmuir*, 2003, **19**, 1312–1318.
- 48 G. Xu, W. Wang, J. T. Groves and M. H. Hecht, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 3652–3657.
- 49 N. Ashkenasy, W. S. Horne and M. R. Ghadiri, *Small*, 2006, **2**, 99–102.
- 50 J. P. Schneider, D. J. Pochan, B. Ozbas, K. Rajagopal, L. Pakstis and J. Kretsinger, *J. Am. Chem. Soc.*, 2002, **124**, 15030–15037.
- 51 E. T. Powers, S. I. Yang, C. M. Lieber and J. W. Kelly, *Angew. Chem., Int. Ed. Engl.*, 2002, **41**, 127–130.
- 52 L. Haines-Butterick, K. Rajagopal, M. Branco, D. Salick, R. Rughani, M. Pilarz, M. S. Lamm, D. J. Pochan and J. P. Schneider, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 7791–7796.
- 53 D. J. Pochan, J. P. Schneider, J. Kretsinger, B. Ozbas, K. Rajagopal and L. Haines, *J. Am. Chem. Soc.*, 2003, **125**, 11802–11803.
- 54 T. C. Holmes, S. de Lacalle, X. Su, G. Liu, A. Rich and S. Zhang, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 6728–6733.
- 55 M. V. Tsurkan and M. Y. Ogawa, *Chem. Commun.*, 2004, 2092–2093.
- 56 A. Aggeli, M. Bell, N. Boden, J. N. Keen, P. F. Knowles, T. C. McLeish, M. Pitkeathly and S. E. Radford, *Nature*, 1997, **386**, 259–262.

- 57 K. Beck and B. Brodsky, *J. Struct. Biol.*, 1998, **122**, 17–29.
- 58 R. Fairman and K. S. Akerfeldt, *Curr. Opin. Struct. Biol.*, 2005, **15**, 453–463.
- 59 S. A. Potekhin, T. N. Melnik, V. Popov, N. F. Lanina, A. A. Vazina, P. Rigler, A. S. Verdini, G. Corradin and A. V. Kajava, *Chem. Biol.*, 2001, **8**, 1025–1032.
- 60 D. E. Wagner, C. L. Phillips, W. M. Ali, G. E. Nybakken, E. D. Crawford, A. D. Schwab, W. F. Smith and R. Fairman, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 12656–12661.
- 61 E. Moutevelis and D. N. Woolfson, *J. Mol. Biol.*, 2009, **385**, 726–732.
- 62 D. Papapostolou, A. M. Smith, E. D. Atkins, S. J. Oliver, M. G. Ryadnov, L. C. Serpell and D. N. Woolfson, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 10853–10858.
- 63 C. Gribbon, K. J. Channon, W. Zhang, E. F. Banwell, E. H. Bromley, J. B. Chaudhuri, R. O. Oreffo and D. N. Woolfson, *Biochemistry*, 2008, **47**, 10365–10371.
- 64 H. Dong, S. E. Paramonov and J. D. Hartgerink, *J. Am. Chem. Soc.*, 2008, **130**, 13691–13695.
- 65 A. S. Hoffman, *Adv. Drug Delivery Rev.*, 2002, **54**, 3–12.
- 66 P. Gupta, K. Vermani and S. Garg, *Drug Discovery Today*, 2002, **7**, 569–579.
- 67 W. A. Petka, J. L. Harden, K. P. McGrath, D. Wirtz and D. A. Tirrell, *Science*, 1998, **281**, 389–392.
- 68 C. Xu, V. Breedveld and J. Kopecek, *Biomacromolecules*, 2005, **6**, 1739–1749.
- 69 C. Xu and J. Kopecek, *Pharm. Res.*, 2008, **25**, 674–682.
- 70 E. K. O'Shea, K. J. Lumb and P. S. Kim, *Curr. Biol.*, 1993, **3**, 658–667.
- 71 M. G. Ryadnov, B. Ceyhan, C. M. Niemeyer and D. N. Woolfson, *J. Am. Chem. Soc.*, 2003, **125**, 9388–9394.
- 72 S. Vauthey, S. Santoso, H. Gong, N. Watson and S. Zhang, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 5355–5360.
- 73 T. Gore, Y. Dori, Y. Talmon, M. Tirrell and H. Bianco-Peled, *Langmuir*, 2001, **17**, 5352–5360.
- 74 R. S. Tu and M. Tirrell, *Adv. Drug Delivery Rev.*, 2004, **56**, 1537–1563.
- 75 J. Schneider, P. Berndt, K. Haverstick, S. Kumar, S. Chiruvolu and M. Tirrell, *J. Am. Chem. Soc.*, 1998, **120**, 3508–3509.
- 76 S. Tsonchev, K. L. Niece, G. C. Schatz, M. A. Ratner and S. I. Stupp, *J. Phys. Chem. B*, 2008, **112**, 441–447.
- 77 R. M. Fuoss and D. Edelson, *J. Am. Chem. Soc.*, 1951, **73**, 269–273.
- 78 R. C. Claussen, B. M. Rabatic and S. I. Stupp, *J. Am. Chem. Soc.*, 2003, **125**, 12680–12681.
- 79 M. Kogiso, Y. Okada, T. Hanada, K. Yase and T. Shimizu, *Biochim. Biophys. Acta*, 2000, **1475**, 346–352.
- 80 F. Qiu, Y. Chen, C. Tang, Q. Zhou, C. Wang, Y. K. Shi and X. Zhao, *Macromol. Biosci.*, 2008, **8**, 1053–1059.
- 81 M. Z. Menzenski and I. A. Banerjee, *New J. Chem.*, 2007, **31**, 1674–1680.
- 82 R. I. MacCuspie, I. A. Banerjee, C. Pejoux, S. Gummalla, H. S. Mostowski, P. R. Krause and H. Matsui, *Soft Matter*, 2008, **4**, 833–839.
- 83 R. de la Rica, E. Mendoza, L. M. Lechuga and H. Matsui, *Angew. Chem., Int. Ed. Engl.*, 2008, **47**, 9752–9755.
- 84 N. Kameta, M. Masuda, G. Mizuno, N. Morii and T. Shimizu, *Small*, 2008, **4**, 561–565.
- 85 K. Tenidis, M. Waldner, J. Bernhagen, W. Fischle, M. Bergmann, M. Weber, M. L. Merkle, W. Voelter, H. Brunner and A. Kapurniotu, *J. Mol. Biol.*, 2000, **295**, 1055–1071.
- 86 P. Westermark, U. Engstrom, K. H. Johnson, G. T. Westermark and C. Betsholtz, *Proc. Natl. Acad. Sci. U. S. A.*, 1990, **87**, 5036–5040.
- 87 M. Reches and E. Gazit, *Amyloid*, 2004, **11**, 81–89.
- 88 M. J. Krysmann, V. Castelletto, A. Kellarakis, I. W. Hamley, R. A. Hule and D. J. Pochan, *Biochemistry*, 2008, **47**, 4597–4605.
- 89 M. Reches, Y. Porat and E. Gazit, *J. Biol. Chem.*, 2002, **277**, 35475–35480.
- 90 A. Saini and V. S. Chauhan, *ChemBioChem*, 2001, **12**, 2495–2501.
- 91 M. Kamihira, A. Naito, S. Tuzi, A. Y. Nosaka and H. Saito, *Protein Sci.*, 2000, **9**, 867–877.
- 92 C. A. Hauser, R. Deng, A. Mishra, Y. Loo, U. Khoe, F. Zhuang, D. W. Cheong, A. Accardo, M. B. Sullivan, C. Riekel, J. Y. Ying and U. A. Hauser, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 1361–1366.
- 93 A. Lakshmanan and C. A. Hauser, *Int. J. Mol. Sci.*, 2011, **12**, 5736–5746.
- 94 L. Tjernberg, W. Hosia, N. Bark, J. Thyberg and J. Johansson, *J. Biol. Chem.*, 2002, **277**, 43243–43246.
- 95 S. K. Maji, D. Haldar, M. G. B. Drew, A. Banerjee, A. K. Das and A. Banerjee, *Tetrahedron*, 2004, **60**, 3251–3259.
- 96 P. P. Bose, A. K. Das, R. P. Hegde, N. Shamala and A. Banerjee, *Chem. Mater.*, 2007, **19**, 6150–6157.
- 97 L. Adler-Abramovich, D. Aronov, P. Beker, M. Yevnin, S. Stempler, L. Buzhansky, G. Rosenman and E. Gazit, *Nat. Nanotechnol.*, 2009, **4**, 849–854.
- 98 M. Wang, L. Du, X. Wu, S. Xiong and P. K. Chu, *ACS Nano*, 2011, **5**, 4448–4454.
- 99 P. Zhu, X. Yan, Y. Su, Y. Yang and J. Li, *Chemistry*, 2010, **16**, 3176–3183.
- 100 J. Ryu and C. B. Park, *Adv. Mater.*, 2008, **20**, 3754–3758.
- 101 M. Reches and E. Gazit, *Nat. Nanotechnol.*, 2006, **1**, 195–200.
- 102 L. Adler-Abramovich, M. Reches, V. L. Sedman, S. Allen, S. J. Tendler and E. Gazit, *Langmuir*, 2006, **22**, 1313–1320.
- 103 N. Hendler, N. Sidelman, M. Reches, E. Gazit, Y. Rosenberg and S. Richter, *Adv. Mater.*, 2007, **19**, 1485–1488.
- 104 J. Kim, T. H. Han, Y. I. Kim, J. S. Park, J. Choi, D. G. Churchill, S. O. Kim and H. Ihee, *Adv. Mater.*, 2010, **22**, 583–587.
- 105 C. H. Görbitz, *New J. Chem.*, 2003, **27**, 1789–1793.
- 106 C. H. Görbitz, *Acta Crystallogr., Sect. E: Struct. Rep. Online*, 2004, **60**, o626–o628.
- 107 C. H. Gorbitz, *Acta Crystallogr., Sect. C: Cryst. Struct. Commun.*, 2006, **62**, o328–330.
- 108 C. H. Gorbitz, *Chemistry*, 2001, **7**, 5153–5159.
- 109 C. H. Gorbitz, *Acta Crystallogr., Sect. B: Struct. Sci.*, 2002, **58**, 849–854.

- 110 H. Birkedal, D. Schwarzenbach and P. Pattison, *Angew. Chem., Int. Ed. Engl.*, 2002, **41**, 754–756.
- 111 Y. Pan, H. Birkedal, P. Pattison, D. Brown and G. Chapuis, *J. Phys. Chem. B*, 2004, **108**, 6458–6466.
- 112 C. H. Görbitz, M. Nilsen, K. Szeto and L. W. Tangen, *Chem. Commun.*, 2005, 4288–4290.
- 113 C. H. Görbitz, *Chemistry*, 2007, **13**, 1022–1031.
- 114 D. V. Soldatov, I. L. Moudrakovski and J. A. Ripmeester, *Angew. Chem., Int. Ed. Engl.*, 2004, **43**, 6308–6311.
- 115 I. Moudrakovski, D. V. Soldatov, J. A. Ripmeester, D. N. Sears and C. J. Jameson, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 17924–17929.
- 116 R. Anedda, D. V. Soldatov, I. L. Moudrakovski, M. Casu and J. A. Ripmeester, *Chem. Mater.*, 2008, **20**, 2908–2920.
- 117 M. Reches and E. Gazit, *Nano Lett.*, 2004, **4**, 581–585.
- 118 M. Reches and E. Gazit, *Phys. Biol.*, 2006, **3**, S10–S19.
- 119 O. Carny, D. E. Shalev and E. Gazit, *Nano Lett.*, 2006, **6**, 1594–1597.
- 120 M. Yemini, M. Reches, J. Rishpon and E. Gazit, *Nano Lett.*, 2005, **5**, 183–186.
- 121 M. Yemini, M. Reches, E. Gazit and J. Rishpon, *Anal. Chem.*, 2005, **77**, 5155–5159.
- 122 N. Amdursky, I. Koren, E. Gazit and G. Rosenman, *J. Nanosci. Nanotechnol.*, 2012, **11**, 9282–9286.
- 123 N. Amdursky, M. Molotskii, D. Aronov, L. Adler-Abramovich, E. Gazit and G. Rosenman, *Nano Lett.*, 2009, **9**, 3111–3115.
- 124 L. Adler-Abramovich, M. Badihi-Mossberg, E. Gazit and J. Rishpon, *Small*, 2010, **6**, 825–831.
- 125 A. Comotti, S. Bracco, G. Distefano and P. Sozzani, *Chem. Commun.*, 2009, 284–286.
- 126 N. S. de Groot, T. Parella, F. X. Aviles, J. Vendrell and S. Ventura, *Biophys. J.*, 2007, **92**, 1732–1741.
- 127 R. Orbach, L. Adler-Abramovich, S. Zigerson, I. Mironi-Harpaz, D. Seliktar and E. Gazit, *Biomacromolecules*, 2009, **10**, 2646–2651.
- 128 A. Mahler, M. Reches, M. Rechter, S. Cohen and E. Gazit, *Adv. Mater.*, 2006, **18**, 1365–1370.
- 129 S. Guha, M. G. B. Drew and A. Banerjee, *Chem. Mater.*, 2008, **20**, 2282–2290.
- 130 S. Guha, M. G. Drew and A. Banerjee, *Small*, 2008, **4**, 1993–2005.
- 131 S. Guha and A. Banerjee, *Adv. Funct. Mater.*, 2009, **19**, 1949–1961.
- 132 S. Guha, T. Chakraborty and A. Banerjee, *Green Chem.*, 2009, **11**, 1139–1145.
- 133 M. Reches and E. Gazit, *Isr. J. Chem.*, 2005, **45**, 363–371.
- 134 M. Zhou, A. M. Smith, A. K. Das, N. W. Hodson, R. F. Collins, R. V. Ulijn and J. E. Gough, *Biomaterials*, 2009, **30**, 2523–2530.
- 135 T. Liebmans, S. Rydholm, V. Akpe and H. Brismar, *BMC Biotechnol.*, 2007, **7**, 88.
- 136 V. Jayawarna, M. Ali, T. A. Jowitt, A. F. Miller, A. Saiani, J. E. Gough and R. V. Ulijn, *Adv. Mater.*, 2006, **18**, 611–614.
- 137 V. Jayawarna, A. Smith, J. E. Gough and R. V. Ulijn, *Biochem. Soc. Trans.*, 2007, **35**, 535–537.
- 138 Z. Yang, G. Liang and B. Xu, *Chem. Commun.*, 2006, 738–740.
- 139 G. Liang, Z. Yang, R. Zhang, L. Li, Y. Fan, Y. Kuang, Y. Gao, T. Wang, W. W. Lu and B. Xu, *Langmuir*, 2009, **25**, 8419–8422.
- 140 B. Xing, C. W. Yu, K. H. Chow, P. L. Ho, D. Fu and B. Xu, *J. Am. Chem. Soc.*, 2002, **124**, 14846–14847.
- 141 Z. Yang, H. Gu, Y. Zhang, L. Wang and B. Xu, *Chem. Commun.*, 2004, 208–209.
- 142 E. K. Johnson, D. J. Adams and P. J. Cameron, *J. Am. Chem. Soc.*, **132**, 5130–5136.
- 143 S. Gilead and E. Gazit, *Supramol. Chem.*, 2005, **17**, 87.
- 144 Y. Song, S. R. Challa, C. J. Medforth, Y. Qiu, R. K. Watt, D. Pena, J. E. Miller, F. van Swol and J. A. Shelnutt, *Chem. Commun.*, 2004, 1044–1045.
- 145 X. Yan, Q. He, K. Wang, L. Duan, Y. Cui and J. Li, *Angew. Chem., Int. Ed. Engl.*, 2007, **46**, 2431–2434.
- 146 X. Yan, Y. Cui, Q. He, K. Wang, J. Li, W. Mu, B. Wang and Z. C. Ou-Yang, *Chemistry*, 2008, **14**, 5974–5980.
- 147 J. Naskar and A. Banerjee, *Chem. – Asian J.*, 2009, **4**, 1817–1823.
- 148 A. Mishra, J. J. Panda, A. Basu and V. S. Chauhan, *Langmuir*, 2008, **24**, 4571–4576.
- 149 S. Alam, J. J. Panda and V. S. Chauhan, *Int. J. Nanomed.*, 2012, **7**, 4207–4221.
- 150 D. M. Ryan, T. M. Doran, S. B. Anderson and B. L. Nilsson, *Langmuir*, 2011, **27**, 4029–4039.
- 151 Z. Yang, H. Gu, D. Fu, P. Gao, J. K. Lam and B. Xu, *Adv. Mater.*, 2004, **16**, 1440–1444.
- 152 Z. Yang and B. Xu, *Chem. Commun.*, 2004, 2424–2425.
- 153 Z. Yang, G. Liang and B. Xu, *Acc. Chem. Res.*, 2008, **41**, 315–326.
- 154 L. Adler-Abramovich, L. Vaks, O. Carny, D. Trudler, A. Magno, A. Caffisch, D. Frenkel and E. Gazit, *Nat. Chem. Biol.*, 2012, **8**, 701–706.
- 155 S. Tsonchev, G. C. Schatz and M. A. Ratner, *Nano Lett.*, 2003, **3**, 623–626.
- 156 M. McCullagh, T. Prytkova, S. Tonzani, N. D. Winter and G. C. Schatz, *J. Phys. Chem. B*, 2008, **112**, 10388–10398.
- 157 S. Tsonchev, A. Troisi, G. C. Schatz and M. A. Ratner, *J. Phys. Chem. B*, 2004, **108**, 15278.
- 158 O.-S. Lee, S. I. Stupp and G. C. Schatz, *J. Am. Chem. Soc.*, 2011, **133**, 3677–3683.
- 159 O.-S. Lee, V. Cho and G. C. Schatz, *Nano Lett.*, 2012, **12**, 4907–4913.
- 160 Y. S. Velichko, S. I. Stupp and M. O. de la Cruz, *J. Phys. Chem. B*, 2008, **112**, 2326.
- 161 E. T. Pashuck, H. G. Cui and S. I. Stupp, *J. Am. Chem. Soc.*, 2010, **132**, 6041.
- 162 J. C. Stendahl, M. S. Rao, M. O. Guler and S. I. Stupp, *Adv. Funct. Mater.*, 2006, **16**, 499.
- 163 H. Z. Jiang, M. O. Guler and S. I. Stupp, *Soft Matter*, 2007, **3**, 454.
- 164 D. Flock, G. Rossetti, I. Daidone, A. Amadei and A. Di Nola, *Proteins*, 2006, **65**, 914–921.

- 165 P. Tamamis, L. Adler-Abramovich, M. Reches, K. Marshall, P. Sikorski, L. Serpell, E. Gazit and G. Archontis, *Biophys. J.*, 2009, **96**, 5020–5029.
- 166 A. Villa, N. F. A. van der Vegt and C. Peter, *Phys. Chem. Chem. Phys.*, 2009, **11**, 2068–2076.
- 167 A. Villa, C. Peter and N. F. A. van der Vegt, Self-Assembling Dipeptides: Conformational Sampling in Solvent-Free Coarse-Grained Simulation, *Phys. Chem. Chem. Phys.*, 2009, **11**, 2077–2086.
- 168 C.-J. Tsai, J. Zheng and R. Nussinov, *PLoS Comput. Biol.*, 2006, **2**, e42.
- 169 C. H. Gorbitz, *Chem. Commun.*, 2006, 2332.
- 170 C. Guo, Y. Luo, R. Zhou and G. Wei, *ACS Nano*, 2012, **6**, 3907–3918.
- 171 J. Jeon, C. E. Mills and M. S. Shell, *J. Phys. Chem. B*, 2013, **117**, 3935–3943.
- 172 D. Peer, J. M. Karp, S. Hong, O. C. Farokhzad, R. Margalit and R. Langer, *Nat. Nanotechnol.*, 2007, **2**, 751–760.
- 173 W. Wiwanitkit, A. Sereemasapun and R. Rojanathanes, *Fertil. Steril.*, 2009, **91**, e7–e8.
- 174 W. H. De Jong and P. J. A. Borm, *Int. J. Nanomed.*, 2008, **3**, 133–149.
- 175 D. B. Warheit, B. R. Laurence, K. L. Reed, D. H. Roach, G. A. Reynolds and T. R. Webb, *Toxicol. Sci.*, 2004, **77**, 117–125.
- 176 K. R. Vega-Villa, J. K. Takemoto, J. A. Yáñez, C. M. Remsburg, M. L. Forrest and N. M. Davies, *Adv. Drug Delivery Rev.*, 2008, **60**, 929–938.
- 177 S. I. Wada and R. Tanaka, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 2563–2566.
- 178 L. Liu, K. Xu, H. Wang, P. K. Tan, W. Fan, S. S. Venkatraman, L. Li and Y. Y. Yang, *Nat. Nanotechnol.*, 2009, **4**, 457–463.
- 179 M. R. Ghadiri, J. R. Granja and L. K. Buehler, *Nature*, 1994, **369**, 301–304.
- 180 V. Dartois, J. Sanchez-Quesada, E. Cabezas, E. Chi, C. Dubbelde, C. Dunn, J. Granja, C. Gritzen, D. Weinberger, M. R. Ghadiri and T. R. Parr, Jr, *Antimicrob. Agents Chemother.*, 2005, **49**, 3302–3310.
- 181 A. Montero, P. Gastaminza, M. Law, G. Cheng, F. V. Chisari and M. R. Ghadiri, *Chem. Biol.*, 2011, **18**, 1453–1462.
- 182 M. C. Morris, P. Vidal, L. Chaloin, F. Heitz and G. Divita, *Nucleic Acids Res.*, 1997, **25**.
- 183 F. Simeoni, M. C. Morris, F. Heitz and G. Divita, *Nucleic Acids Res.*, 2003, **31**, 2717–2724.
- 184 L. Crombez, M. C. Morris, S. Deshayes, F. Heitz and G. Divita, *Curr. Pharm. Des.*, 2008, **14**, 3656–3665.
- 185 D. Bhadra, S. Bhadra and N. K. Jain, *Pharm. Res.*, 2006, **23**, 623–633.
- 186 N. Wiradharma, M. Khan, Y. W. Tong, S. Wang and Y.-Y. Yang, *Adv. Funct. Mater.*, 2008, **18**, 943–951.
- 187 N. Wiradharma, Y. W. Tong and Y. Y. Yang, *Biomaterials*, 2009, **30**, 3100–3109.
- 188 G. von Maltzahn, S. Vauthey, S. Santoso and S. U. Zhang, *Langmuir*, 2003, **19**, 4332–4337.
- 189 X. Zhao and S. Zhang, *Trends Biotechnol.*, 2004, **22**, 470–476.
- 190 J. Gorman, *Sci. News*, 2003, **163**, 43–44.
- 191 Y. B. Lim, E. Lee, Y. R. Yoon, M. S. Lee and M. Lee, *Angew. Chem., Int. Ed. Engl.*, 2008, **47**, 4525–4528.
- 192 X. Li, J. Li, Y. Gao, Y. Kuang, J. Shi and B. Xu, *J. Am. Chem. Soc.*, 2010, **132**, 17707–17709.
- 193 J. Naskar, S. Roy, A. Joardar, S. Das and A. Banerjee, *Org. Biomol. Chem.*, 2011, **9**, 6610–6615.
- 194 M. C. Branco, D. J. Pochan, N. J. Wagner and J. P. Schneider, *Biomaterials*, 2009, **30**, 1339–1347.
- 195 J. Naskar, G. Palui and A. Banerjee, *J. Phys. Chem. B*, 2009, **113**, 11787–11792.
- 196 J. H. Kim, S. Y. Lim, D. H. Nam, J. Ryu, S. H. Ku and C. B. Park, *Biosens. Bioelectron.*, 2011, **26**, 1860.
- 197 J. Nanda and A. Banerjee, *Soft Matter*, 2012, **8**, 3380–3386.
- 198 J. J. Panda, A. Kaul, S. Alam, A. K. Babbar, A. K. Mishra and V. S. Chauhan, *Ther. Delivery*, 2011, **2**, 193–204.
- 199 J. J. Panda, A. Kaul, S. Kumar, S. Alam, A. K. Mishra, G. C. Kundu and V. S. Chauhan, *Nanomedicine*, 2013, 1927–1942.
- 200 J. J. Panda, S. Yandrapu, R. S. Kadam, V. S. Chauhan and U. B. Kompella, *J. Controlled Release*, 2013, **172**, 1151–1160.
- 201 J. J. Panda, A. Varshney and V. S. Chauhan, *J. Nanobiotechnol.*, 2013, **11**, 18.
- 202 D. Mendel, J. A. Ellman, Z. Chang, D. L. Veenstra, P. A. Kollman and P. G. Schultz, *Science*, 1992, **256**, 1798–1802.
- 203 M. L. English and C. H. Stammer, *Biochem. Biophys. Res. Commun.*, 1978, **83**, 1464.
- 204 A. C. Jen, M. C. Wake and A. G. Mikos, *Biotechnol. Bioeng.*, 1996, **50**, 357–364.
- 205 M. J. Lysaght and P. Aebischer, *Sci. Am.*, 1999, **280**, 76–82.
- 206 K. Y. Lee and D. J. Mooney, *Chem. Rev.*, 2001, **101**, 1869–1879.
- 207 S. Kyle, A. Aggeli, E. Ingham and M. J. McPherson, *Trends Biotechnol.*, 2009, **27**, 423–433.
- 208 E. Genove, C. Shen, S. Zhang and C. E. Semino, *Biomaterials*, 2005, **26**, 3341–3351.
- 209 A. Schneider, J. A. Garlick and C. Egles, *PLoS One*, 2008, **3**, e1410.
- 210 F. Gelain, D. Bottai, A. Vescovi and S. Zhang, *PLoS One*, 2006, **1**, e119.
- 211 A. Horii, X. Wang, F. Gelain and S. Zhang, *PLoS One*, 2007, **2**, e190.
- 212 M. E. Davis, J. P. Motion, D. A. Narmoneva, T. Takahashi, D. Hakuno, R. D. Kamm, S. Zhang and R. T. Lee, *Circulation*, 2005, **111**, 442–450.
- 213 J. D. Hartgerink, E. Beniash and S. I. Stupp, *Science*, 2001, **294**, 1684–1688.
- 214 M. O. Guler, L. Hsu, S. Soukasene, D. A. Harrington, J. F. Hulvat and S. I. Stupp, *Biomacromolecules*, 2006, **7**, 1855–1863.
- 215 M. O. Guler, S. Soukasene, J. F. Hulvat and S. I. Stupp, *Nano Lett.*, 2005, **5**, 249–252.
- 216 H. Storrie, M. O. Guler, S. N. Abu-Amara, T. Volberg, M. Rao, B. Geiger and S. I. Stupp, *Biomaterials*, 2007, **28**, 4608–4618.
- 217 Z. Huang, T. D. Sargeant, J. F. Hulvat, A. Mata, P. Bringas Jr, C. Y. Koh, S. I. Stupp and M. L. Snead, *J. Bone Miner. Res.*, 2008, **23**, 1995–2006.

- 218 D. A. Harrington, E. Y. Cheng, M. O. Guler, L. K. Lee, J. L. Donovan, R. C. Claussen and S. I. Stupp, *J. Biomed. Mater. Res., Part A*, 2006, **78**, 157–167.
- 219 S. R. Bull, M. O. Guler, R. E. Bras, T. J. Meade and S. I. Stupp, *Nano Lett.*, 2005, **5**, 1–4.
- 220 T. D. Sargeant, M. O. Guler, S. M. Oppenheimer, A. Mata, R. L. Satcher, D. C. Dunand and S. I. Stupp, *Biomaterials*, 2008, **29**, 161–171.
- 221 J. Guo, H. Su, Y. Zeng, Y. X. Liang, W. M. Wong, R. G. Ellis-Behnke, K. F. So and W. Wu, *Nanomedicine*, 2007, **3**, 311–321.
- 222 K. M. Galler, A. Cavender, V. Yuwono, H. Dong, S. Shi, G. Schmalz, J. D. Hartgerink and R. N. D'Souza, *Tissue Eng., Part A*, 2008, **14**, 2051–2058.
- 223 E. Beniash, J. D. Hartgerink, H. Storrie, J. C. Stendahl and S. I. Stupp, *Acta Biomater.*, 2005, **1**, 387–397.
- 224 K. Rajangam, H. A. Behanna, M. J. Hui, X. Han, J. F. Hulvat, J. W. Lomasney and S. I. Stupp, *Nano Lett.*, 2006, **6**, 2086–2090.
- 225 J. Kisiday, M. Jin, B. Kurz, H. Hung, C. Semino, S. Zhang and A. J. Grodzinsky, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 9996–10001.
- 226 T. C. Holmes, *Trends Biotechnol.*, 2002, **20**, 16–21.
- 227 G. A. Silva, C. Czeisler, K. L. Niece, E. Beniash, D. A. Harrington, J. A. Kessler and S. I. Stupp, *Science*, 2004, **303**, 1352–1355.
- 228 J. K. Kretsinger, L. A. Haines, B. Ozbas, D. J. Pochan and J. P. Schneider, *Biomaterials*, 2005, **26**, 5177–5186.
- 229 Z. Wang, H. Wang, W. Zheng, J. Zhang, Q. Zhao, S. Wang, Z. Yang and D. Kong, *Chem. Commun.*, 2011, **47**, 8901–8903.
- 230 H. Misawa, N. Kobayashi, A. Soto-Gutierrez, Y. Chen, A. Yoshida, J. D. Rivas-Carrillo, N. Navarro-Alvarez, K. Tanaka, A. Miki, J. Takei, T. Ueda, M. Tanaka, H. Endo, N. Tanaka and T. Ozaki, *Cell Transplant.*, 2006, **15**, 903–910.
- 231 R. G. Ellis-Behnke, Y. X. Liang, S. W. You, D. K. Tay, S. Zhang, K. F. So and G. E. Schneider, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 5054–5059.
- 232 M. E. Davis, P. C. Hsieh, T. Takahashi, Q. Song, S. Zhang, R. D. Kamm, A. J. Grodzinsky, P. Anversa and R. T. Lee, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 8155–8160.
- 233 V. M. Tysseling-Mattiace, V. Sahni, K. L. Niece, D. Birch, C. Czeisler, M. G. Fehlings, S. I. Stupp and J. A. Kessler, *J. Neurosci.*, 2008, **28**, 3814–3823.
- 234 R. F. Service, *Science*, 2005, **308**, 44.
- 235 J. Kirkham, A. Firth, D. Vernals, N. Boden, C. Robinson, R. C. Shore, S. J. Brookes and A. Aggeli, *J. Dent. Res.*, 2007, **86**, 426–430.
- 236 A. Firth, A. Aggeli, J. L. Burke, X. Yang and J. Kirkham, *Nanomedicine*, 2006, **12**, 189–199.
- 237 C. J. Bell, L. M. Carrick, J. Katta, Z. Jin, E. Ingham, A. Aggeli, N. Boden, T. A. Waigh and J. Fisher, *J. Biomed. Mater. Res., Part A*, 2006, **78**, 236–246.
- 238 L. Sasso, I. Vedarethinam, J. Emnéus, W. E. Svendsen and J. Castillo-León, *J. Nanosci. Nanotechnol.*, 2012, **12**, 3077–3083.
- 239 J. J. Panda, R. Dua, A. Mishra, B. Mittra and V. S. Chauhan, *ACS Appl. Mater. Interfaces*, 2010, **2**, 2839–2848.