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## Peptide foldamer-based self-assembled nanostructures containing cyclic beta-amino acids

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Peptide soft materials belong to an emerging branch of materials sciences due to their growing importance as responsive materials in diagnostics, therapeutics, and biomedical applications. The diversity provided by easily modifiable peptide sequences can be further increased by introducing nonnatural amino acids such as cyclic  $\beta$ -amino acids, leading to the formation of foldamers. Moreover, it is possible to combine peptide chains with other polymers, aromatic compounds, etc. to create hybrids with completely new properties and applications. In this review, we focus on the *cis/trans* enantiomers of three cyclic  $\beta$ -amino acids: 2-aminocyclobutane-1-carboxylic acid (ACBC), 2-aminocyclopentane-1-carboxylic acid (ACPC) and 2-aminocyclohexane-1-carboxylic acid (ACHC). The peptides discussed here either contain exclusively  $\beta$ -amino acids or are  $\alpha,\beta$ -peptides, and they undergo self-assembly by forming different interactions that lead to the creation of well-defined nanostructures.

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### Introduction

Peptide-based self-assembled structures possess many advantages over other organic and inorganic systems due to their chemical diversity, ability to adopt distinct secondary structures, biocompatibility, resemblance to proteins, large potential for modification, relatively easy and scalable synthesis, and ability to associate spontaneously.<sup>1,2</sup> The type, number, and sequence of amino acids can be modified to design peptides that can self-assemble to form nanotubes,<sup>3</sup> nanofibers,<sup>4</sup> nanoribbons,<sup>5</sup> nanospheres,<sup>6</sup> nanotapes<sup>7</sup> and nanorods<sup>8</sup> – all with controllable properties and a broad spectrum of applications in biotechnology,<sup>9</sup> bioengineering,<sup>10</sup> biomedicine<sup>11,12</sup> and more. Most of the nanostructures described in the literature consist of  $\alpha$ -amino acid residues with different charges, hydrophobicities, polarities, and sizes that allow the creation of various complex nanostructures with different characteristics.<sup>13,14</sup> Incorporation of unnatural amino acids such as  $\beta$ -amino acids into the sequence significantly increases the number of possible nanostructures.<sup>15</sup>

there are proteinogenic amino acids.<sup>16</sup> The most common and most important is H- $\beta$ hGly-OH, the so-called “ $\beta$ -alanine” (a component of the naturally occurring peptides anserine and carnosine<sup>17</sup>), as well as pantothenic acid (vitamin B<sub>5</sub>, a component of coenzyme A<sup>18</sup>). There are several advantages of using peptides with incorporated  $\beta$ -amino acids depending on their application.  $\beta$ -Amino acids are resistant to the enzymatic cleavage of peptides, and they seem to be extraordinarily stable in metabolic processes occurring in mammals, insects, and plants; additionally, they undergo microbial degradation very slowly. Many studies conducted thus far have demonstrated negligible biocidal or toxic properties of these peptides.<sup>16</sup>

The conformation of  $\alpha$ -amino acids can be represented by two torsion angles, denoted  $\varphi$  (phi) and  $\psi$  (psi). There is an extra carbon atom in the  $\beta$ -amino acid structure; hence, there is an additional torsion angle,  $\theta$  (theta) (Fig. 1). In cyclic  $\beta$ -residues, this  $\theta$  angle depends on  $\varphi$  and  $\psi$ , and its *gauche* conformation is inherently stable.<sup>19</sup> Changing the conformation of the additional torsional angle in  $\beta$ -peptides can even profoundly affect the folding of these molecules. 2-Aminocyclopentane-1-carboxylic acid (ACPC)-based peptides with the *cis* conformation at the  $\theta$  torsion angle have been shown to lead to an extended strand with a zigzag pattern rather than to the helix fold observed when  $\theta$  is in the *trans* conformation.<sup>20</sup> In amino acids with constrained conformations, C<sup>2</sup> and C<sup>3</sup> atoms are part of the ring, and cyclization at these atoms imposes an additional constraint on five- and six-membered rings because  $\theta$  is now constrained to  $\pm 60$  degrees due to sp<sup>3</sup> hybridization.<sup>21</sup> Consequently,  $\beta$ -amino acids have  $\varphi$  and  $\psi$  values that are arranged in a Ramanathan

### Review

#### $\beta$ -Amino acids as conformationally constrained building blocks

$\beta$ -Amino acids are considered “nonnatural” amino acids; however, there are many more  $\beta$ -amino acids in nature than

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Fig. 1 Torsion angles  $\phi$ ,  $\theta$ , and  $\psi$  of  $\alpha$ -,  $\beta$ - and cyclic  $\beta$ -amino acids.

diagram similar to that of a glycine residue,<sup>22</sup> and thus,  $\beta$ -amino acids tend to adopt the conformational space normally accessible only to glycine residues. This in turn opens a range of possible conformations available for foldamers.

The main advantage of  $\beta$ -peptides is their ability to adopt various types of helices,  $\beta$ -sheets, turns, and extended structures.<sup>23</sup> Their ability to form well-defined helical structures stabilized by hydrogen bonds is particularly valuable.  $\beta$ -Peptides demonstrate a higher folding propensity than  $\alpha$ -peptides of comparable length; for instance, properly designed  $\beta$ -peptides with only six residues adopt helical conformations in water,<sup>24</sup> while  $\alpha$ -peptides usually need to be two or three times longer to exhibit significant helicity.<sup>25</sup> Moreover, among a few types of hydrogen-bonded helices known for  $\alpha$ -peptides, the most common are only three:  $\alpha$ -,  $3_{10}$ - and  $\pi$ -helices, whereas  $\beta$ -peptides have been shown to form a variety of different helical H-bonded structures,<sup>26</sup> e.g., 10-helix,<sup>27</sup> 12-helix<sup>28</sup> and 14-helix.<sup>29</sup> If both  $\alpha$ - and  $\beta$ -residues are introduced into the sequence, the number of possible helical types is even higher.<sup>30,31</sup> Importantly, the type of secondary structure adopted can be predicted using a method called stereochemical patterning, which is based on the known tendency of individual units to prefer dihedral angles of a certain magnitude and sign.<sup>32</sup> Moreover,  $\beta$ -amino acids introduced into the  $\alpha$ -amino acid chain may induce changes in the peptide conformation, mainly by stabilizing the secondary structure,<sup>33</sup> which translates into biological functions<sup>34</sup> and a propensity for supramolecular self-assembly.<sup>35</sup> Additionally, it is possible to control the  $\alpha,\beta$ -helix handedness through small changes in the peptide termini.<sup>36</sup> It was also shown that changing the relative configurations of the  $\beta$ -peptide backbone may lead to the control of the secondary structure and the self-assembly pattern and, thus, the morphology of the obtained nanostructure.<sup>37</sup>

The basic and most versatile structures of cyclic  $\beta$ -amino acids are shown in Fig. 2.

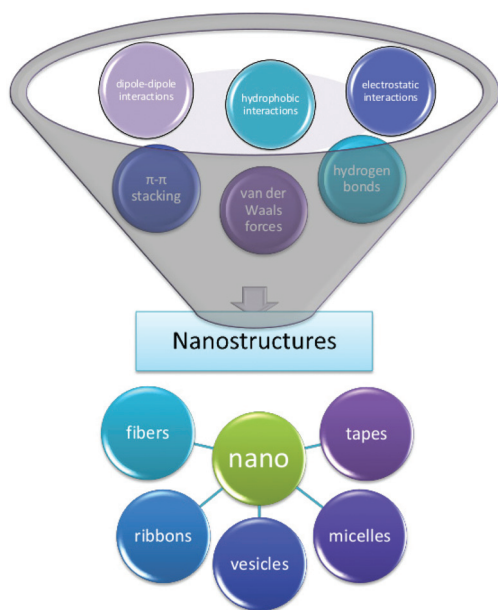


Fig. 2 *Cis* and *trans* enantiomers of 2-aminocyclobutane-1-carboxylic acid (ACBC), 2-aminocyclopentane-1-carboxylic acid (ACPC) and 2-aminocyclohexane-1-carboxylic acid (ACHC) residues.

### Self-assembly process in nanostructure formation

The term self-assembly is used to describe a wide variety of processes in which molecules associate into larger species.<sup>38</sup> This spontaneous organization of small units into higher-order structures is possible due to the collective balance of noncovalent interactions, mainly electrostatic interactions, hydrogen bonding, and hydrophobic interactions, with a contribution from  $\pi$ - $\pi$  stacking and other dispersive forces.<sup>39,40</sup> While these interactions are rather weak compared to covalent bonds, they account for relatively stable higher-order self-assembled structures, thanks to the additive effect of these secondary forces; *i.e.*, self-assembled structures are thermodynamically more stable since their Gibbs free energy values are lower than the energy of the individual components.<sup>41</sup> The formation of peptide aggregates may or may not be reversible; they may be amorphous or highly structured aggregates, and they may assemble both in solution and on surfaces after adsorption.<sup>42</sup> The precise mechanisms of self-aggregation of foldameric peptides remain insufficiently understood, despite many efforts already made in different fields of peptide research, *e.g.*, research on beta-amyloid aggregation.<sup>43</sup> The elucidation of the self-assembly mechanism is not trivial, mainly due to the complexity of the interactions between molecules building nanostructures. Numerous hydrogen bond patterns play an important role in supporting various elements of the secondary structure, among which  $\alpha$ -helices and  $\beta$ -strands have been mainly observed in peptide materials.<sup>44</sup> For example, peptides forming  $\beta$ -sheets exhibit hydrogen bond interactions along the backbone but can also be found in arrays with ionic interactions, hydrophobic interactions, van der Waals interactions, and water-mediated hydrogen bonds.<sup>45</sup> In particular, the “bottom up” approach mechanisms require a deep understanding; therefore, rational methods of nanostructure formation should be developed considering the structure and the ability of the individual molecular building blocks to self-aggregate (Fig. 3).<sup>46</sup>





**Fig. 3** Self-assembly of peptides into higher-order nanostructures (nanofibers, nanoribbons, nanovesicles, etc.) is possible due to the collective balance of noncovalent interactions (hydrogen bonding, hydrophobic interactions, electrostatic interactions, etc.).

### Peptide foldamer-based self-assembled nanostructures containing cyclic beta-amino acids

Peptide foldamers are oligomers containing nonnatural amino acids that fold into well-defined three-dimensional structures.<sup>47</sup> If they are correctly designed, they can undergo a self-assembly process within minutes and without external stimuli, forming distinct nanostructures.<sup>46</sup> Examples of the different nanostructures formed by particular building blocks containing cyclic  $\beta$ -amino acids along with reported interactions/mechanisms leading to their self-association are described in Table 1.

$\beta$ -Peptides containing only a few residues of cyclic  $\beta$ -amino acids are known to form well-defined secondary structures that are stable in solution and possess a high propensity to form different nanostructures. Hetenyi *et al.* showed that *trans*-2-aminocyclohexane-1-carboxylic acid (*trans*-ACHC) homo-oligomers can form different types of helices depending on the number of residues in the sequence.<sup>69</sup> For instance, (*trans*-ACHC)<sub>4</sub> adopts a 10-helix motif, whereas (*trans*-ACHC)<sub>5</sub> and (*trans*-ACHC)<sub>6</sub> form 14-helices. All these peptides can form helical bundles in solution. Rua and coworkers combined the rigidity and amphiphilic properties of another tetrapeptide, namely,  $\beta$ -strands mimicking *cis*-2-aminocyclobutane-1-carboxylic acid (*cis*-ACBC)<sub>4</sub>, and obtained ribbon-like fibrils that formed a gel in some media.<sup>49</sup> Vertically amphiphilic self-stabilizing helix units containing 3–4 residues of *trans*-ACHC were also reported, in which the hydrophobic surface of the helix was large enough to facilitate solvent-driven association into a continuous helix-bundle motif.<sup>55</sup> The cog-wheel-like mutual fit of the helix side chains further enhances the stabi-

lizing hydrophobic surface, and head-to-head and head-to-tail contacts of the helices enable the formation of a membrane. More information about the interaction forces between amphiphilic molecules in aggregates and how they are affected by solution conditions can be found elsewhere.<sup>70</sup> Other interesting examples of  $\beta$ -peptides were described by Torres *et al.*<sup>49</sup> They reported the synthesis of *cis*-ACBC derivatives and their incorporation into  $\beta$ -peptides consisting of 2–8 residues bearing different *N*-protecting groups. The obtained oligomers adopted a  $\beta$ -strand-type conformation induced by the formation of intrasidic six-membered hydrogen-bonded rings to form *cis*-fused [4.2.0]octane structural units that show the high rigidity of these  $\beta$ -peptides. The synthesized peptides self-associated to form nanofibers and, under certain conditions, gels. The authors elucidated a model in which a parallel molecular arrangement is preferred and the conformation is similar to that observed in a solution in which both hydrogen bonding and hydrophobic interactions are responsible for assembly. As demonstrated by Martinek and coworkers, 4–6 residues of *cis*-ACPC can self-assemble to form ribbons 2–3 nm high, which corresponds to the distance between the N and C termini of a single (*cis*-ACPC)<sub>*n*</sub> strand.<sup>55</sup> It was concluded that since the width of the ribbon increased with the size of the oligomer and the polarity of the solvent, the ribbons must be organized by hydrophobic forces between the side chains in the corresponding dimension, thus leading to a continuous pleated-sheet sandwich tertiary structural motif. The supramolecular structure was organized by interstrand hydrogen bonds formed by peptide moieties parallel to the long fibril axis. Mandity and coworkers showed that not only the  $\alpha$ -helix but also the  $\beta$ -peptidic H10/12 helix can tolerate side chains containing alicyclic rings.<sup>65</sup> Conformational polymorphism was observed for the alternating *cis*-ACHC hexamer, in which chemical exchange occurred between the major left-handed H10/12 helix and a minor folded conformation. For this peptide, vesicles with diameters of  $\sim$ 100 nm formed as a result of hydrophobic self-assembly.

Most of the published research concerns nanostructures in which the building element is the *trans* enantiomer rather than the *cis* enantiomer (Table 1). This is most likely related to the difficulties in the synthesis and the purchase cost of the *ci* enantiomer *s*. Several authors have accepted the challenge of comparing the conformations and properties of peptides, including those of each enantiomer. For example, Gorrea *et al.* described studies on two  $\beta$ -dipeptides, one with two *trans*-ACBC fragments and another with one *trans* residue and one *cis* residue.<sup>53</sup> Studies on noncovalent interactions responsible for the self-assembly, morphology and size of the obtained nanostructure showed head-to-head (a dipeptide with two *trans* residues) or head-to-tail (a dipeptide with *trans* and *cis* ACBC residues) molecular arrangements present in the studied peptides. Helical structures corresponding to hydrogen-bonded single chains that interact with each other in an anti-parallel way to form bundles were obtained. Hydrogen bonds formed between the two NH groups of one molecule and the CO<sup>3</sup> and CO<sup>9</sup> of the other molecule. These hydrogen bonds

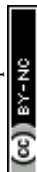


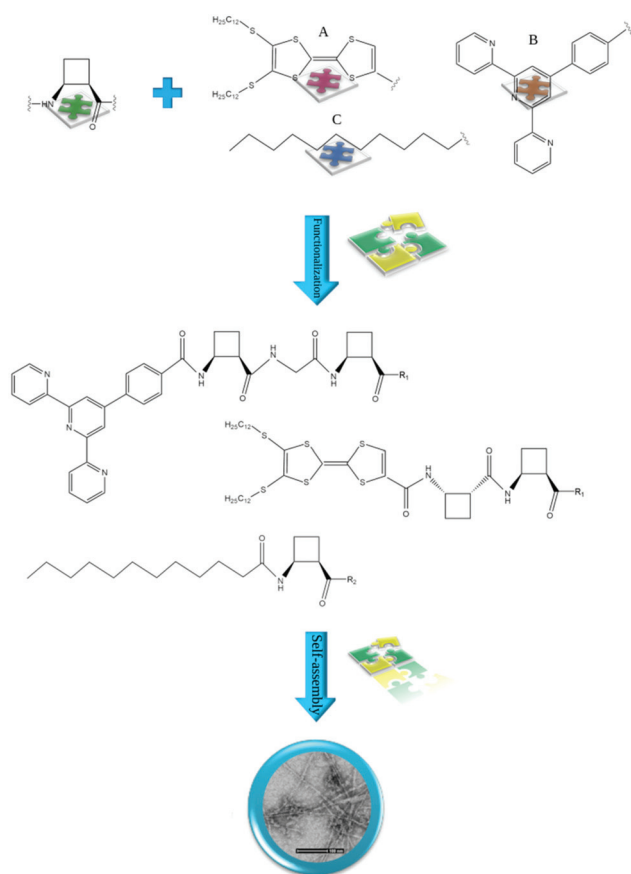
**Table 1** Examples of the different types of nanostructures formed by cyclic  $\beta$ -amino acid constituents along with a description of the interactions/mechanisms leading to self-association

$\beta$ -Amino acid	Building blocks	Type of nanostructure	Interactions/mechanism	Ref.	
<i>cis</i> -ACBC	(ACBC) <sub>4</sub> ( $\beta$ -strands)	Ribbon-like fibrils; gels	Interactions between amphiphilic molecules; hydrogen bonds	48	
	(ACBC) <sub>2-8</sub> ( $\beta$ -strands)	Fibers; gels	Hydrogen bonds; hydrophobic interactions	49	
	TTF-functionalized (ACBC) <sub>2</sub>	Tape-like fibers	Head-to-head interactions; complex twisted bilayers	50	
	hcptpy-functionalized (ACBC) <sub>4</sub>	Helical aggregates; fibers; xerogels	$\pi$ - $\pi$ stacking; hydrogen bonds	51	
<i>trans</i> -ACBC	Lipophilic chain anchored to ACBC ( <i>trans</i> -ACBC)( <i>cis</i> -ACBC)	Fibers; spherical micelles; liquid crystals	Hydrogen bonds; charge-dipole interactions	52	
	1,2-difunctionalized ACBC	Helical aggregate; solid-like networks; xerogels	Head-to-tail molecular arrangement; hydrogen bonds	53	
	hcptpy-functionalized (ACBC) <sub>2</sub>	Vesicles; fibers (bilayer tubes)	Hydrogen bonds	54	
	Lipophilic chain anchored to ACBC (ACBC) <sub>2</sub>	Helical aggregates; fibers; xerogels	$\pi$ - $\pi$ stacking; hydrogen bonds	51	
<i>cis</i> -ACPC	1,2-difunctionalized ACBC	Fibers; spherical micelles; liquid crystals	Hydrogen bonds	52	
	hcptpy-functionalized (ACBC) <sub>2</sub>	Helical aggregates; solid-like networks; xerogels	Head-to-head molecular arrangement; hydrogen bonds	53	
	1,2-difunctionalized ACBC	Irregular aggregates; fibers (bilayer tubes)	Hydrogen bonds	54	
	(ACPC) <sub>4-6</sub>	Nonpolar strand; ribbonlike fibrils; fibrils of pleated-sheet sandwiches	Hydrophobic forces; hydrogen bonds	55	
	<i>trans</i> -ACPC	$\alpha,\beta$ -Peptides (helices)	Helical bundles	Hydrophobic forces; polarization/ionic interactions	56
		<i>N</i> -Boc-( <i>trans</i> -( <i>S,S</i> )-ACPC) <sub>7</sub> -OBn (12-helix)	Windmill-shaped supramolecular architectures	Hydrophobic forces; head-to-tail intermolecular hydrogen bonds	57
		BocNH-( <i>trans</i> -( <i>R,R</i> )-ACPC) <sub>6</sub> -OBn (12-helix)	Square plate; molar tooth	Hydrophobic forces; head-to-tail intermolecular hydrogen bonds	58
		BocNH-(ACPC) <sub>6</sub> -OH (12-helix)	Rhombic plate; rhombic rod	Hydrophobic forces; head-to-tail intermolecular hydrogen bonds	59
		BocNH-(Aib-ACPC) <sub>3</sub> -Aib-OBn (11-helix)	Tripod shape; trigonal bipyramids	Hydrophobic forces; head-to-tail intermolecular hydrogen bonds	60
		BocNH-(Aib-ACPC) <sub>3</sub> -OBn (11-helix)	Microrods; parallelogram plates	Hydrophobic forces; head-to-tail intermolecular hydrogen bonds	61
Boc-(ACPC) <sub>8</sub> -OBn (12-helix)		Rectangular plate	Hydrophobic forces; head-to-tail intermolecular hydrogen bonds	62	
Boc-(ACPC) <sub>4</sub> -OBn (12-helix)		Tubular foldectures	Hydrophobic forces; head-to-tail intermolecular hydrogen bonds	63	
<i>cis</i> -ACHC	BocNH-(ACPC) <sub>6</sub> -Leu <sub>2</sub> -OBn (12-helix)	Hexagonal plate	Hydrophobic forces; hydrogen bonds	64	
	$\alpha,\beta$ -Peptides (helices)	Fibrils	Cyclopentyl zipper	46	
	H <sub>2</sub> N-(ACHC) <sub>4</sub> or <sub>6</sub> (10/12-helix)	Vesicles	Hydrophobic forces	65	
	Boc-ACHC-Aib-Phe-OMe (turn-type $\beta$ -sheet)	Fibers; organogels	Hydrogen bonds; $\pi$ - $\pi$ stacking interactions	66	
	(ACHC-ACHC- $\beta$ -Lys) <sub>n</sub> (14-helix)	Globular aggregates; nanofibers; LC phase	Cyclohexyl zipper; interactions between amphiphilic molecules	67	
	$\beta$ -Peptides (14-helix)	Helix bundles; vesicle-forming membranes; lyotropic liquid crystals	Cyclohexyl zipper; interactions between amphiphilic helix units	68	
<i>trans</i> -ACHC	(ACHC) <sub>3-4</sub> (helix)	Spherical objects; helix-bundle membranes	Interactions between amphiphilic helix units; head-to-head and head-to-tail contacts; hydrophobic forces	55	
	(ACHC) <sub>4</sub> (10-helix) (ACHC) <sub>5-6</sub> (14-helix)	Helical bundles	Hydrophobic forces	69	

propagate along the axis of the aggregates, inducing a helical aggregation mode. The work of Pi-Boleda and coworkers aimed to investigate the influence of the stereochemistry and regiochemistry of peptides on their ability to serve as surfactants and undergo self-organization.<sup>54</sup> The authors showed that the interplay between stereoisomerism (*cis*- versus *trans*-1,2 disubstituted cyclobutane) and regiochemistry (N-centered versus C-centered amide bonds), enhanced by the rigidity of the cyclobutane ring, had a significant impact on the physicochemical properties of the obtained peptides. Regiochemistry has been found to govern the morphology of supramolecular aggregates (*i.e.*, long fibers versus plates or spherical assemblies), while the critical micelle concentration (cmc value) is

governed mainly by stereochemistry. A similar issue was investigated by Sorrenti and coworkers, who studied the effect of *cis/trans* stereochemistry on the molecular organization and recognition properties of small diastereomeric anionic amphiphiles based on a rigid cyclobutane  $\beta$ -amino acid scaffold.<sup>52</sup> Peptides containing lipophilic chains anchored to the amino group of ACBC moieties self-assembled to form fibers, spherical micelles, and liquid crystals with a morphology that depends on the stereochemistry (Fig. 4). The self-association process was mediated by the formation of hydrogen bonds and cooperative hydrogen bond networks between secondary amide groups in the heads of neighboring molecules. The authors concluded that the relative stereochemistry mainly





**Fig. 4** Examples of *cis*-ACBC residues functionalized with: (A) TTF,<sup>50</sup> (B) hcptpy,<sup>51</sup> and (C) lipophilic chain<sup>52</sup> self-assembling to form fibrils/fibers.  $R_1 = \text{OMe}$  and  $R_2 = \text{ONa}$ .

affects the solvation of the headgroup and anionic charge stabilization in a dilute solution, resulting in better stabilization of the *cis* diastereoisomer due to intramolecular hydrogen bonds and/or charge-dipole interactions. Furthermore, the relative configuration of ACBC residues affects the chiral recognition ability of spherical micelles for bilirubin enantiomers.

Introducing cyclic  $\beta$ -amino acids into peptide sequences can prompt hydrophobically induced aggregation or can help to direct association in the desired direction.<sup>71</sup> The plausible advantages of the substitution of  $\alpha$ -residues by  $\beta$ -residues depend on the application and whether a single modification or an entire  $\beta$ -peptide is considered. Szeftczyk *et al.* proposed a hierarchical approach for the construction of nanofibrils based on  $\alpha,\beta$ -peptide foldamers, in which the incorporation of a helix-promoting *trans*-ACPC residue at the outer positions of the model coiled-coil peptide led to its increased conformational stability and allowed for additional interactions between coiled-coil-like structures to direct the self-assembly process toward the formation of well-defined nanofibrils.<sup>46</sup> In their publications, Pomerantz and coworkers described globally amphiphilic helices undergoing a hydrophobically driven association process by interdigitating ACHC residues at the interface between neighboring  $\beta$ -peptides, creating a “cyclo-

hexyl zipper”.<sup>67,68</sup> It has been revealed that hydrophobically driven assembly processes are promoted not only by the overall lipophilicity of a molecule but also by specific arrangements of lipophilic elements on the molecular surface and specific associations of lipophilic elements in intermolecular contacts. Unpaired ACHC residues that elongated into nanofibers were the predominant self-assembled structures leading to the lyotropic LC phase. Additionally,  $\beta^3 \rightarrow$  cyclic replacements that showed generally good accommodation in helix-bundle quaternary structures were also investigated.<sup>56</sup> The authors investigated  $\alpha/\beta$ -peptide foldamers with a regular  $\alpha$ -residue/ $\alpha$ -residue/ $\beta$ -residue ( $\alpha\alpha\beta$ ) backbone motif, which exhibited a four-helix bundle quaternary structure in the crystalline state. The folded structures were stabilized by the close packing of hydrophobic side chains in the core, with additional interactions between the polar residues. Kwon and coworkers described several peptides containing highly hydrophobic helices arising from the presence of *trans*-ACPC residues displayed on the lateral exterior of the helix and N- and C-termini protection by the Boc group and the benzyl group, respectively.<sup>57–60,62,63</sup> The described peptides were able to self-assemble, creating nanostructures of various shapes, *i.e.*, wind-mill-shaped structures, square plate (in water) and molar tooth (in surfactant) structures, microrods (in water), and parallelogram plates (in surfactant), depending on the sequence modifications and the presence of a surfactant in the solution. The authors concluded that the helices self-assembled in an aqueous solution *via* lateral hydrophobic interactions between the helical faces. Head-to-tail intermolecular hydrogen bonds and highly ordered anisotropic molecular packing motifs present in the foldamer building blocks were responsible for their unique shapes.

Peptides containing  $\beta$ -amino acids can also be functionalized with various molecules to obtain nanostructures with interesting properties. Torres *et al.* described homochiral dipeptides composed of *cis*-ACBC residues that were functionalized with a  $\pi$ -electron-rich tetrathiafulvalene (TTF) moiety (Fig. 4).<sup>50</sup> Microscopic analysis revealed the formation of fibers in which the molecules were packed into dimeric tapes with the peptide head groups at the center. There are complex twisted bilayers whose deformation is caused partially by the chiral nature of the molecular components. The films of the obtained material showed electrical conductivity. Porcar-Tost and coworkers described dipeptides and tetrapeptides built with either *cis*- or *trans*-ACBC residues functionalized with a terpyridine derivative, namely, 4'-(4-carboxy)phenylterpyridine (hcptpy) (Fig. 4).<sup>51</sup> The obtained conjugates self-associated as a result of the  $\pi$ - $\pi$  interactions provided by the aromatic moieties that cooperate with intermolecular hydrogen bonds between NH and CO in the amide groups. The morphology of the created nanostructures, *i.e.*, helical aggregates, fibers or xerogel, is strongly dependent on the solvent. Moreover, ruthenium(II) complexes derived from these ligands showed electrochemical behavior and activity as catalysts; as organogelators, these conjugates were able to gel various solvents. The mechanistic aspects of the supramolecular ordering of



$\beta$ -amino acid-based peptides in several organic solvents and fuel oils were investigated by Konda and coworkers.<sup>66</sup> The studied peptide Boc-*cis*ACHC-Aib-Phe-OMe was able to self-assemble into a bundle of nanofibers and entrap various organic solvents and oils, governing the formation of self-supporting gels. The self-assembly process was mediated by hydrogen-bonding and  $\pi$ - $\pi$  stacking interactions.

In summary, both *cis* and *trans* enantiomers of 2-aminocyclobutane-1-carboxylic acid (ACBC), 2-aminocyclopentane-1-carboxylic acid (ACPC) and 2-aminocyclohexane-1-carboxylic acid (ACHC) proved to possess propensities for the formation of diverse self-assembled nanostructures, *e.g.*, spherical micelles, fibers and gels. In the reported research, the self-assembly process was mediated mainly by hydrogen bond formation, hydrophobic interactions, head-to-head and head-to-tail molecular arrangement, and/or interactions between amphiphilic molecules. In some cases, the spontaneous arrangement of the building blocks was shown to be a hierarchical process leading to the formation of nanostructures with different organization levels, *e.g.*, globular aggregates  $\rightarrow$  nanofibers  $\rightarrow$  liquid crystals. Moreover, it was proven that the morphology of the nanostructures can be controlled, for instance, by the appropriate modification of the peptide sequence or addition of a surfactant. The above findings, together with the continuous development of high-resolution methods (*e.g.*, XRD, SAXS/WAXS, and cryo-TEM) for the characterization of foldameric nanostructures and intense research on another group of self-aggregating peptides – amyloids – may in the near future lead to a full mechanistic description of the self-association process of peptides and produce a breakthrough in the wide application of soft materials.

## Conclusions

In conclusion, the presence of  $\beta$ -residues increases the diversity in peptide sequences, which is translated to the functions and applications of the obtained peptides. Cyclic  $\beta$ -amino acids help to form peptide foldamers with well-defined 3D structures. The self-assembly process, which is mediated through various types of interactions and enhanced by hydrophobic interactions, provides numerous nanostructures that can result in interesting properties and can be used in many applications.  $\beta$ -Peptide modularity for tailoring properties is indisputable, but there are still ongoing efforts to clarify the relationship among the  $\beta$ -peptide sequence, its 3D structure, and the self-assembly process leading to the nanostructure formation. In particular, more studies are essential to elucidate rational methods for peptide design that can explain the fundamental interactions that enhance their self-association and help to drive the self-assembly process in the desired direction.

## Abbreviations

ACBC 2-Aminocyclobutane-1-carboxylic acid

ACPC	2-Aminocyclopentane-1-carboxylic acid
ACHC	2-Aminocyclohexane-1-carboxylic acid
Aib	$\alpha$ -Amino isobutyric acid
Boc	<i>tert</i> -Butyloxycarbonyl
cmc	Critical micelle concentration
hcptpy	4'-(4-Carboxy)phenylterpyridine
Phe	Phenylalanine
SAXS	Small-angle X-ray scattering
TEM	Transmission electron microscopy
TTF	Tetrathiafulvalene
WAXS	Wide-angle X-ray scattering
XRD	X-ray diffraction

## Conflicts of interest

The authors declare no potential conflicts of interest.

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## References

- 1 D. M. Raymond and B. L. Nilsson, Multicomponent Peptide Assemblies, *Chem. Soc. Rev.*, 2018, **47**, 3659–3720.
- 2 E. Gazit, Self-Assembled Peptide Nanostructures: the Design of Molecular Building Blocks and their Technological Utilization, *Chem. Soc. Rev.*, 2007, **36**, 1263–1269.
- 3 Y. Zhao, X. Hu, L. Zhang, D. Wang, S. M. King, S. E. Rogers, J. Wang, J. R. Lu and H. Xu, Monolayer Wall Nanotubes Self-Assembled from Short Peptide Bolaamphiphiles, *J. Colloid Interface Sci.*, 2021, **583**, 553–562.
- 4 L. Lu, D. Morrison and L. D. Unsworth, Controllable Self-Patterning Behaviours of Flexible Self-Assembling Peptide Nanofibers, *Nanoscale*, 2020, **12**, 8133–8138.
- 5 A. Rani, I. Kavianinia, L. M. De Leon-Rodriguez, D. J. McGillivray, D. E. Williams and M. A. Brimble, Nanoribbon Self-Assembly and Hydrogel Formation from an NOctanoyl Octapeptide Derived from the Antiparallel  $\beta$ -Interface of a Protein Homotetramer, *Acta Biomater.*, 2020, **114**, 233–243.
- 6 Q. Zhang, P. Zhang, S. Jian, J. Li, F. Li, X. Sun, H. Li, Y. Zeng, Y. Zeng, S. Liang, P. Chen and Z. Liu, Drug-Bearing Peptide-Based Nanospheres for the Inhibition of Metastasis and Growth of Cancer, *Mol. Pharmaceutics*, 2020, **17**, 3165–3176.
- 7 A. Dehsorkhi and I. W. Hamley, Silica Templating of a Self-Assembling Peptide Amphiphile that Forms Nanotapes, *Soft Matter*, 2014, **10**, 1660–1664.



- 8 W. Wang and Y. Chau, Self-Assembled Peptidnanorods as Building Blocks of Fractal Patterns, *Soft Matter*, 2009, **5**, 4893–4898.
- 9 A. Levin, T. A. Hakala, L. Schnaider, G. J. L. Bernardes, E. Gazit and T. P. J. Knowles, Biomimetic Peptide Self-Assembly for Functional Materials, *Nat. Rev. Chem.*, 2020, **4**, 615–634.
- 10 Self-assembling peptides in biomedicine and bioengineering: Tissue engineering, regenerative medicine, drug delivery, and biotechnology in *Peptide Applications in Biomedicine, Biotechnology and Bioengineering*, ed. S. Koutsopoulos, Woodhead Publishing, 2018, pp. 387–408.
- 11 L. Sun, C. Zheng and T. J. Webster, Self-Assembled Peptide Nanomaterials for Biomedical Applications: Promises and Pitfalls, *Int. J. Nanomed.*, 2016, **12**, 73–86.
- 12 X. Zhang, C. Gong, O. U. Akakuru, Z. Su, A. Wu and G. Wei, The Design and Biomedical Applications of Self-Assembled Two-Dimensional Organic Biomaterials, *Chem. Soc. Rev.*, 2019, **48**, 5564–5595.
- 13 S. H. Yoo and H.-S. Lee, Foldectures: 3D Molecular Architectures from Self-Assembly of Peptide Foldamers, *Acc. Chem. Res.*, 2017, **50**, 832–841.
- 14 E. De Santis and M. G. Ryadnov, Peptide Self-Assembly for Nanomaterials: the Old New Kid on the Block, *Chem. Soc. Rev.*, 2015, **44**, 8288–8300.
- 15 S. Shankar, J. U. Rahim and R. Rai, Self-Assembly in Peptides Containing  $\beta$ - and  $\gamma$ -amino Acids, *Curr. Protein Pept. Sci.*, 2020, **21**, 584–597.
- 16 D. Seebach, A. K. Beck and D. J. Bierbaum, The World of Beta- and Gamma-Peptides Comprised of Homologated Proteinogenic Amino Acids and Other Components, *Chem. Biodivers.*, 2004, **1**, 1111–1239.
- 17 P. J. Quinn, A. A. Boldyrev and V. E. Formazuyk, Carnosine: Its Properties, Functions and Potential Therapeutic Applications, *Mol. Aspects Med.*, 1992, **13**, 379–444.
- 18 C. Spry, K. Kirk and K. J. Saliba, Coenzyme A Biosynthesis: an Antimicrobial Drug Target, *FEMS Microbiol. Rev.*, 2008, **32**, 56–106.
- 19 T. Beke, I. G. Csizmadia and A. Perczel, On the Flexibility of  $\beta$ -Peptides, *J. Comput. Chem.*, 2004, **25**, 285–307.
- 20 T. A. Martinek, G. K. Toth, E. Vass, M. Hollosi and F. Fulop, Cis-2-Aminocyclopentanecarboxylic Acid Oligomers Adopt a Sheetlike Structure: Switch from Helix to Nonpolar Strand, *Angew. Chem., Int. Ed.*, 2002, **41**, 1718–1721.
- 21 G. Ramanathan and A. Duley, Cyclic Beta-Amino Acids as Conformational Constraints in *Biomolecular Forms and Functions*, ed. M. B. N. Srinivasan, World Scientific Publishing Co. Pte. Ltd., Singapore, 2013, pp. 282–295.
- 22 G. N. Ramachandran, C. Ramakrishnan and V. Sasisekharan, Stereochemistry of Polypeptide Chain Configurations, *J. Mol. Biol.*, 1963, **7**, 95–99.
- 23 T. A. Martinek and F. Fulop, Peptidic Foldamers: Ramping up Diversity, *Chem. Soc. Rev.*, 2012, **41**, 687–702.
- 24 D. H. Appella, J. J. J. Barchi, S. R. Durell and S. H. Gellman, Formation of Short, Stable Helices in Aqueous Solution by  $\beta$ -Amino Acid Hexamers, *J. Am. Chem. Soc.*, 1999, **121**, 2309–2310.
- 25 A. Chakrabarty and R. L. Baldwin, Stability of  $\alpha$ -Helices, *Adv. Protein Chem.*, 1995, **46**, 141–176.
- 26 R. P. Cheng, S. H. Gellman and W. F. DeGrado,  $\beta$ -Peptides: from Structure to Function, *Chem. Rev.*, 2001, **101**, 3219–3232.
- 27 T. D. W. Claridge, J. M. Goodman, A. Moreno, D. Angus, S. F. Barker, C. Taillefumier, M. P. Watterson and G. W. J. Fleet, 10-Helical Conformations in Oxetane  $\beta$ -Amino Acid Hexamers, *Tetrahedron Lett.*, 2001, **42**, 4251–4255.
- 28 C. Fernandes, S. Faure, E. Pereira, V. Thery, V. Declerck, R. Guillot and D. J. Aitken, 12-Helix Folding of Cyclobutane beta-Amino Acid Oligomers, *Org. Lett.*, 2010, **12**, 3606–3609.
- 29 D. H. Appella, L. A. Christianson, I. L. Karle, D. R. Powell and S. H. Gellman,  $\beta$ -Peptide Foldamers: Robust Helix Formation in a New Family of  $\beta$ -Amino Acid Oligomers, *J. Am. Chem. Soc.*, 1996, **118**, 13071–13072.
- 30 L. K. A. Pilsl and O. Reiser,  $\alpha/\beta$ -Peptide Foldamers: State of the Art, *Amino Acids*, 2011, **41**, 709–718.
- 31 W. S. Horne and S. H. Gellman, Foldamers with Heterogeneous Backbones, *Acc. Chem. Res.*, 2008, **41**, 1399–1408.
- 32 I. M. Mandity, E. Weber, T. A. Martinek, G. Olajos, G. K. Toth, E. Vass and F. Fulop, Design of Peptidic Foldamer Helices: a Stereochemical Patterning Approach, *Angew. Chem., Int. Ed.*, 2009, **48**, 2171–2175.
- 33 J. L. Price, W. S. Horne and S. H. Gellman, Structural Consequences of  $\beta$ -Amino Acid Preorganization in a Self-Assembling  $\alpha/\beta$ -Peptide: Fundamental Studies of Foldameric Helix Bundles, *J. Am. Chem. Soc.*, 2010, **132**, 12378–12387.
- 34 C. Cabrele, T. A. Martinek, O. Reiser and L. Berlicki, Peptides Containing  $\beta$ -Amino Acid Patterns: Challenges and Successes in Medicinal Chemistry, *J. Med. Chem.*, 2014, **57**, 9718–9739.
- 35 K. Kulkarni, N. Habila, M. P. Del Borgo and M.-I. Aguilar, Novel Materials From the Supramolecular Self-Assembly of Short Helical  $\beta$ 3-Peptide Foldamers, *Front. Chem.*, 2019, **7**, 70.
- 36 M. Szeferczyk, E. Weglarz-Tomczak, P. Fortuna, A. Krzyszton, E. Rudzinska-Szostak and L. Berlicki, Controlling the Helix Handedness of  $\alpha\beta$ -Peptide Foldamers through Sequence Shifting, *Angew. Chem., Int. Ed.*, 2017, **56**, 2087–2091.
- 37 T. A. Martinek, I. M. Mandity, L. Fulop, G. K. Toth, E. Vass, M. Hollosi, E. Forro and F. Fulop, Effects of the Alternating Backbone Configuration on the Secondary Structure and Self-Assembly of  $\beta$ -Peptides, *J. Am. Chem. Soc.*, 2006, **128**, 13539–13544.
- 38 Y. Lin and C. Mao, Bio-Inspired Supramolecular Self-Assembly towards Soft Nanomaterials, *Front. Mater. Sci.*, 2011, **5**, 247–265.



- 39 L. Wang, C. Gong, X. Yuan and G. Wei, Controlling the Self-Assembly of Biomolecules into Functional Nanomaterials through Internal Interactions and External Stimulations: A Review, *Nanomaterials*, 2019, **9**, 285.
- 40 K. L. Wooley, J. S. Moore, C. Wu and Y. Yang, Novel Polymers: Molecular to Nanoscale Order in Three-Dimensions, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 11147–11148.
- 41 P. Kumaraswamy, S. Sethuraman, J. V. Yakhmi and U. M. Krishnan, Hierarchical Self-Assembled Peptide Nanoensembles, in *Handbook of Nanomaterials Properties*, ed. B. Bhushan, D. Luo, S. Schrickler, W. Sigmund and S. Zauscher, Springer, Berlin, Heidelberg, 2014.
- 42 K. L. Zapadka, F. J. Becher, A. L. Gomes dos Santos and S. E. Jackson, Factors Affecting the Physical Stability (Aggregation) of Peptide Therapeutics, *Interface Focus*, 2017, **7**, 20170030.
- 43 F. Chiti and C. M. Dobson, Protein Misfolding, Amyloid Formation, and, Human Disease: A Summary of Progress Over the Last Decade, *Annu. Rev. Biochem.*, 2017, **86**, 27–68.
- 44 E. De Santis and M. G. Ryadnov, Peptide Self-Assembly for Nanomaterials: The Old New Kid on the Block, *Chem. Soc. Rev.*, 2015, **44**, 8288–8300.
- 45 S. Zhang, Discovery and Design of Self-Assembling Peptides, *Interface Focus*, 2017, **7**, 20170028.
- 46 M. Szeftczyk, N. Szulc, M. Gasior-Glogowska, A. Modrak-Wojcik, A. Bzowska, W. Majstrzyk, M. Taube, M. Kozak, T. Gotszalk, E. Rudzinska-Szostak and L. Berlicki, Hierarchical Approach for the Rational Construction of Helix-Containing Nanofibrils Using  $\alpha,\beta$ -Peptides, *Nanoscale*, 2021, **13**, 4000–4015.
- 47 C. M. Goodman, S. Choi, S. Shandler and W. F. DeGrado, Foldamers as Versatile Frameworks for the Design and Evolution of Function, *Nat. Chem. Biol.*, 2007, **3**, 252–262.
- 48 F. Rua, S. Boussert, T. Parella, I. Diez-Perez, V. Branchadell, E. Giralt and R. M. Ortuno, Self-Assembly of a Cyclobutane Beta-Tetrapeptide to Form Nanosized Structures, *Org. Lett.*, 2007, **9**, 3643–3645.
- 49 E. Torres, E. Gorrea, K. K. Burusco, E. Da Silva, P. Nolis, F. Rua, S. Boussert, I. Diez-Perez, S. Dannenberg, S. Izquierdo, E. Giralt, C. Jaime, V. Branchadella and R. M. Ortuno, Folding and Self-Assembling with Beta-Oligomers Based on (1R,2S)-2-Aminocyclobutane-1-Carboxylic Acid, *Org. Biomol. Chem.*, 2010, **8**, 564–575.
- 50 E. Torres, J. Puigmarti-Luis, A. Perez del Pino, R. M. Ortuno and D. B. Amabilino, Use of Unnatural  $\beta$ -Peptides as a Self-Assembling Component in Functional Organic Fibres, *Org. Biomol. Chem.*, 2010, **8**, 1661–1665.
- 51 O. Porcar-Tost, B. Pi-Boleda, J. Garcia-Anton, O. Illa and R. M. Ortuno, Cyclobutane-Based Peptides/Terpyridine Conjugates: Their Use in Metal Catalysis and as Functional Organogelators, *Tetrahedron*, 2018, **74**, 7252–7260.
- 52 A. Sorrenti, O. Illa, R. Pons and R. M. Ortuno, Chiral Cyclobutane  $\beta$ -Amino Acid-Based Amphiphiles: Influence of *Cis/Trans* Stereochemistry on Solution Self-Aggregation and Recognition, *Langmuir*, 2015, **31**, 9608–9618.
- 53 E. Gorrea, P. Nolis, E. Torres, E. Da Silva, D. B. Amabilino, V. Branchadell and R. M. Ortuno, Self-Assembly of Chiral trans-Cyclobutane-Containing  $\beta$ -Dipeptides into Ordered Aggregates, *Chem. – Eur. J.*, 2011, **17**, 4588–4597.
- 54 B. Pi-Boleda, A. Sorrenti, M. Sans, O. Illa, R. Pons, V. Branchadell and R. M. Ortuno, Cyclobutane Scaffold in Bolaamphiphiles: Effect of Diastereoisomerism and Regiochemistry on Their Surface Activity Aggregate Structure, *Langmuir*, 2018, **34**, 11424–11432.
- 55 T. A. Martinek, A. Hetenyi, L. Fulop, I. M. Mandity, G. K. Toth, I. Dekany and F. Fulop, Secondary Structure Dependent Self-Assembly of  $\beta$ -Peptides into Nanosized Fibrils and Membranes, *Angew. Chem., Int. Ed.*, 2006, **45**, 2396–2400.
- 56 J. L. Price, W. S. Horne and S. H. Gellman, Structural Consequences of  $\beta$ -Amino Acid Preorganization in a Self-Assembling  $\alpha/\beta$ -Peptide: Fundamental Studies of Foldameric Helix Bundles, *J. Am. Chem. Soc.*, 2010, **132**, 12378–12387.
- 57 S. Kwon, A. Jeon, S. H. Yoo, I. S. Chung and H. S. Lee, Unprecedented Molecular Architectures by the Controlled Self-Assembly of a  $\beta$ -Peptide Foldamer, *Angew. Chem., Int. Ed.*, 2010, **49**, 8232–8236.
- 58 S. Kwon, H. S. Shin, J. Gong, J.-H. Eom, A. Jeon, S. H. Yoo, I. S. Chung, S. J. Cho and H.-S. Lee, Self-Assembled Peptide Architecture with a Tooth Shape: Folding into Shape, *J. Am. Chem. Soc.*, 2011, **133**, 17618–17621.
- 59 S. H. Yoo, T. Eom, S. Kwon, J. Gong, J. Kim, S. J. Cho, R. W. Driver, Y. Lee, H. Kim and H.-S. Lee, Foldecture as a Core Material with Anisotropic Surface Characteristics, *J. Am. Chem. Soc.*, 2015, **137**, 2159–2162.
- 60 J.-H. Eom, J. Gong, S. Kwon, A. Jeon, R. Jeong, R. W. Driver and H.-S. Lee, A Hollow Foldecture with Truncated Trigonal Bipyramid Shape from the Self-Assembly of an 11-Helical Foldamer, *Angew. Chem., Int. Ed.*, 2015, **54**, 13204–13207.
- 61 J.-H. Eom, J. Gong, R. Jeong, R. W. Driver and H.-S. Lee, Parallelogram Plate Shaped Foldecture from the Controlled Self-Assembly of  $\alpha/\beta$ -peptide Foldamer, *Solid State Sci.*, 2015, **48**, 39–43.
- 62 S. Kwon, B. J. Kim, H.-K. Lim, K. Kang, S. H. Yoo, J. Gong, E. Yoon, J. Lee, I. S. Choi, H. Kim and H.-S. Lee, Magnetotactic Molecular Architectures from Self-Assembly of  $\beta$ -Peptide Foldamers, *Nat. Commun.*, 2015, **6**, 8747.
- 63 J. Kim, S. Kwon, S. H. Kim, C. K. Lee, J. H. Lee, S. J. Cho, H.-S. Lee and H. Ihee, Microtubes with Rectangular Cross-Section by Self-Assembly of a Short  $\beta$ -Peptide Foldamer, *J. Am. Chem. Soc.*, 2012, **134**, 20573–20576.
- 64 E. Yoon, J. Gong, Y. Jung, W. Lee, R. W. Driver and H.-S. Lee, Unambiguous Characterization of Anisotropic Foldamer Packing in a Foldecture with an Elongated Hexagonal Plate Shape, *Chem. Commun.*, 2016, **52**, 5250–5253.
- 65 I. M. Mandity, L. Fulop, E. Vass, G. K. Toth, T. A. Martinek and F. Fulop, Building  $\beta$ -Peptide H10/12 Foldamer Helices with Six-Membered Cyclic Side-Chains: Fine-Tuning of Folding and Self-Assembly, *Org. Lett.*, 2010, **12**, 5584–5587.



- 66 M. Konda, I. Maity, D. B. Rasale and A. K. Das, A New Class of Phase-Selective Synthetic  $\beta$ -Amino Acid Based Peptide Gelator: From Mechanistic Aspects to Oil Spill Recovery, *ChemPlusChem*, 2014, **79**, 1482–1488.
- 67 W. C. Pomerantz, N. L. Abbott and S. H. Gellman, Lyotropic Liquid Crystals from Designed Helical  $\beta$ -Peptides, *J. Am. Chem. Soc.*, 2006, **128**, 8730–8731.
- 68 W. C. Pomerantz, V. M. Yuwono, C. L. Pizzey, J. D. Hartgerink, N. L. Abbott and S. H. Gellman, Nanofibers and Lyotropic Liquid Crystals from a Class of Self-Assembling  $\beta$ -Peptides, *Angew. Chem., Int. Ed.*, 2008, **47**, 1241–1244.
- 69 A. Hetenyi, I. M. Mandity, T. A. Martinek, G. K. Toth and F. Fulop, Chain-Length-Dependent Helical Motifs and Self-Association of  $\beta$ -Peptides with Constrained Side Chains, *J. Am. Chem. Soc.*, 2005, **127**, 547–553.
- 70 J. N. Israelachvili, 20 – Soft and Biological Structures, in *Intermolecular and Surface Forces*, ed. J. N. Israelachvili, Academic Press, 3rd edn, 2011, pp. 535–576.
- 71 R. D. Gopalan, M. P. Del Borgo, A. I. Mechler, P. Perlmutter and M. I. Aguila, Geometrically Precise Building Blocks: the Self-Assembly of  $\beta$ -Peptides, *Chem. Biol.*, 2015, **22**, 1417–1423.

