"Non-standard amino acids and peptides: from self-assembly to nanomaterials"

Francesca Clerici, Emanuela Erba, Maria Luisa Gelmi, Sara Pellegrino*

Dipartimento di Scienze Farmaceutiche, Università degli Studi di Milano, Via Venezian 21, 20133 Milano, Italy

Abstract The exploitation of peptides in the development of smart nanomaterials is gaining increasing attention in the last few years. Amino acids are indeed able to drive the self-assembly and the self-organization at the molecular level. By using non-standard amino acids, it is possible to expand the scope of the possible applications, ranging from biomaterials, biosensors to drug delivery systems. In this digest, the recent advances in this field are presented.

Introduction

Amino acids are the building blocks of peptides and proteins. In million years of evolution, Nature has optimized their structures to absolve numerous functions in almost all the biological processes. They are indeed able to induce a high molecular complexity starting from relatively simple molecules, being at the molecular basis of the living world. Taking inspiration from Nature, scientists are now trying to develop smart peptide materials for a wide range of applications, such as biomolecular devices, biosensors, and hybrid catalysts. The modularity of amino acids and their ability at driving self-assembly and self-organization leads to a high versatility in functions. Amino acids can both function as single molecules and when inserted in peptides. Furthermore, by tailoring the functional groups on the side chains and at the N-and C-terminus it is possible to expand endlessly their molecular variability. Different kinds of materials could be thus developed, and, depending on the solvent and the environmental conditions, hydro- and organo-gel, nanoarchitectures, vesicles and micelles have been obtained. In the case of standard or coded amino acids, many papers, some reviews and books have recently been published.[1] In this digest, we aimed to explore the advances in using non-standard amino acids for the design of nanomaterials, highlighting in particular some new developments which have been reported since 2010. A comprehensive review of all obtained peptide nanostructures is beyond the scope of this manuscript. We focused our attention on the different amino acid features, starting from simple variation at N- and C-terminus of standard amino acids to unnatural α, β and γ-amino acids. We envisaged the use of non-coded amino acids to be of particular relevance, expanding the scope of the nanoarchitectures so obtained. In particular, the use of non-coded amino acids opens the doors to more stable materials that could be, for instance, exploited in biomedical applications such as drug delivery systems.

NON-STANDARD AMINO ACIDS:
- N- and/or C-terminus capping
- Side chains modification
- β-amino acids
- γ-amino acids

NANOMATERIALS:
- Hydro- and organo-gels
- Vesicles
- Nanotubes
- Nanorings
- Complex architectures (catenanes, collagens….)

Figure 1. Examples of non-standard amino acids and nanomaterials developed

Capped Natural Amino Acids

A simple and widely used strategy to modify the amino acid scaffold is the introduction of substituents at N- or C-terminus.[2] The most common functionalization is the attachment of an aromatic moiety, such as pyrene[3] and ferrocene[4], through amide bond formation. In these examples, π–π interactions drive the self-assembly of the constructs yielding, in most cases, hydrogelators. Naphthalene group has been used to functionalize dendrons composed of aspartic acid (Asp) and alanine (Ala), (**Nap-G1** and **Nap-G2**, Figure 2). [5]

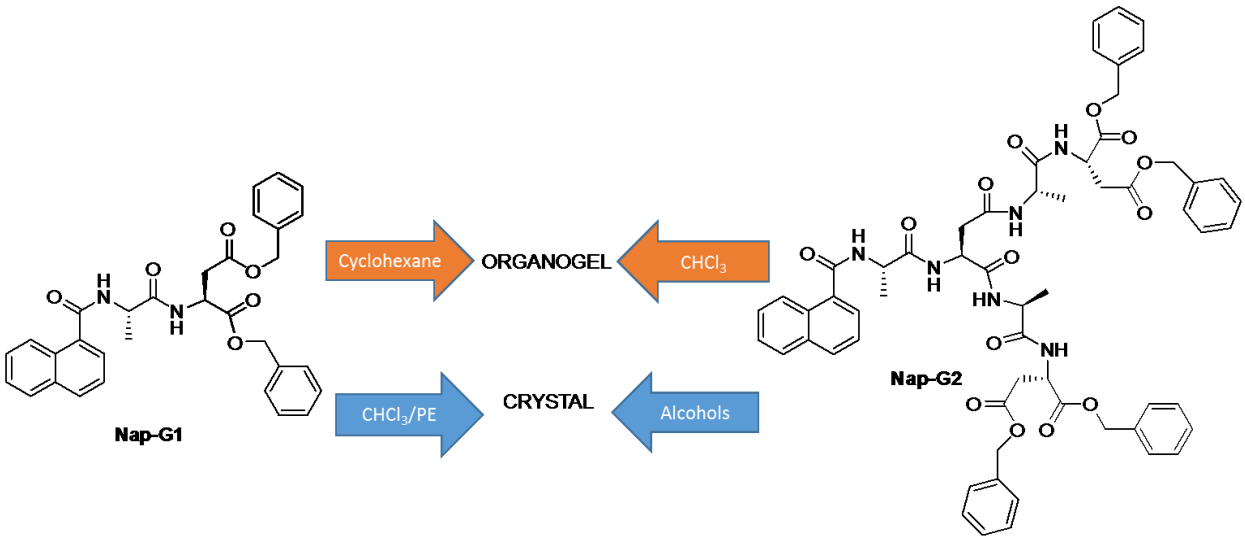


Figure 2. Naphtalene functionalized dendrons.

Nap-G1 in cyclohexane develops a gel, formed by a fibrous network with β-sheet architecture, but in mixed solvents (chloroform/petroleum ether 1:5, v/v) exhibits a spherulitic network (Figure 2). On

the other hand, **Nap-G2** acts as an efficient organogelator in chloroform but forms crystalline microbelts in relatively high polarity solvents, such as acetone and methanol (Figure 2). These polymorphic features are due to the bulky naphtyl group at the N terminus that drives the molecular architectures formation during the self-assembly in different solvents.

The derivatization of amino acids with arylenediimides has also been investigated. Particularly, naphthalenediimides (**NDI**s) and perylenediimides (**PDI**s) (Figure 3) give access to a wide variety of applications ranging from biomedicine to electronics, as reviewed in 2012.[6] For example, Ala forms spiral nanorings, while Phe nanospheres. Several papers related to their use in the field of molecularly engineered organization, hydrogelators, host-guest interactions, dynamic combinatorial chemistry, molecular recognition, photosystems and biomedical applications have been published more recently.[7]

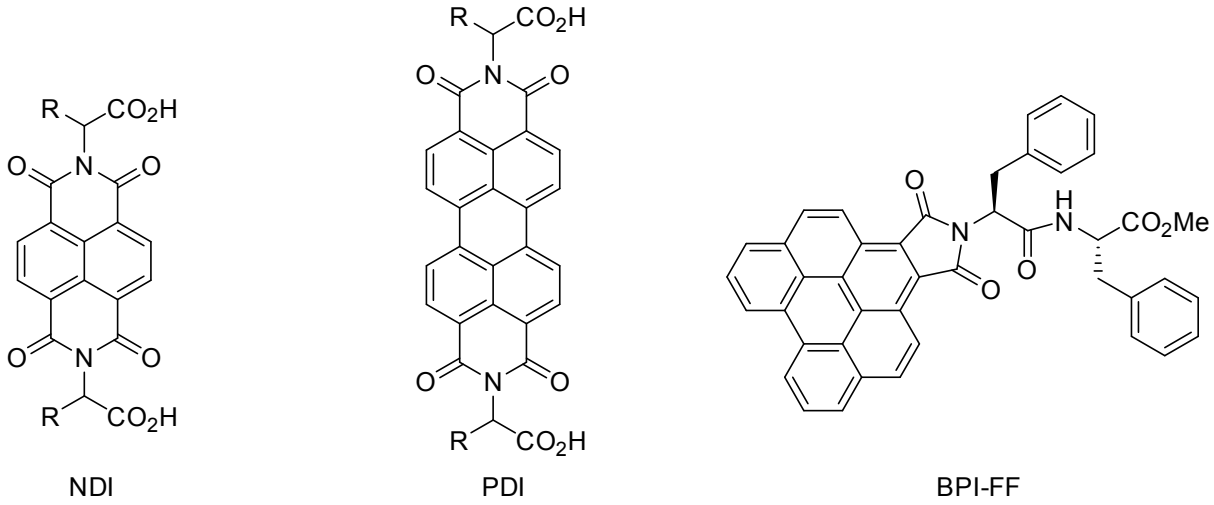


Figure 3. *N*-Arylenediimides derivatives of single amino acids (**NDI** and **PDI**) and of Phe-Phe dipeptide (**BPI-FF**

)

The aromatic dipeptide Phe-Phe, which is known to be the minimal self-assembling peptide sequence, has been functionalized with benzo[*ghi*]perylene monoimide (**BPI-FF**, Figure 3) showing optical behaviour and self-assembly in different organic solvents.[8]

Few examples dealing with C-terminus functionalization are reported. Stilbene derivatives of Phe-Phe dipeptides (**STL-FF**, Figure 4) self-assemble in various organic solvents to form an organogel. Interestingly, due to the *cis–trans* conformational isomerization of the double bond, the organogel is photoresponsive and a gel–sol transition occurs by irradiating the gel with UV light at 365 nm.[9]

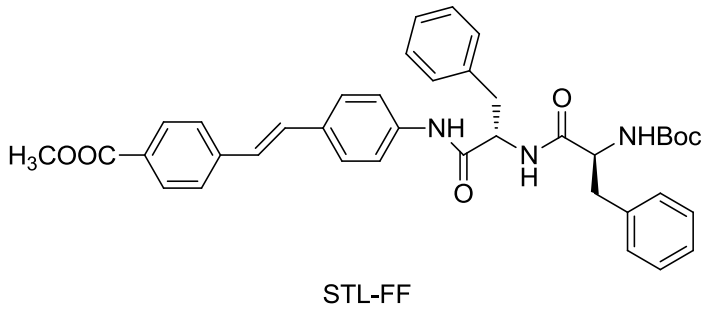


Figure 4. *C*-stilbene substituted Phe-Phe dipeptide.

An interesting class of *N*-substituted amino acids is obtained by the reduction of the corresponding Schiff bases to *N*-aryl derivatives (Figure 5).[10] These compounds have been used for the preparation of Coordination Polymers (CPs) or supramolecular assemblies of Cu^{II} under non-hydrothermal conditions. In particular, the presence or absence of a coordinated water molecule in the metal–ligand complex is responsible for the formation of supramolecular assemblies over CPs.

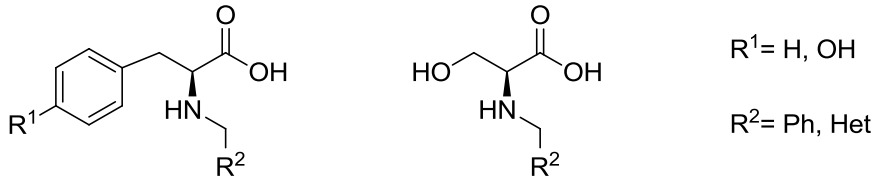


Figure 5. *N*-Aryl derivatives

Novel supramolecular soft gel materials were prepared exploiting the triazole isosteric substitution of peptide amide bond. Disubstituted 1,2,3-triazoles are indeed known as powerful non-classical isosters of amides as they can mimic either a *trans*- or a *cis*- configuration of the amide bond depending on the substitution pattern of the triazole (1,4- or 1,5-, respectively). This replacement has been widely used in medicinal chemistry for the rational design of new drugs. Interestingly, the isosteric replacement paradigm has been now transferred from medicinal chemistry to soft materials. Taking inspiration from *N*-stearoyl-*L*-glutamic acid (**C18-Glu**,) which is known to self-assemble in many solvents at suitable concentrations, the analogue **click-Glu** can be easily synthesized *via* click chemistry by the copper(I)-catalyzed azide–alkyne cycloaddition.[11] Isosteric gelators **C18-Glu** and **click-Glu** give a variety of physical hydrogels/organogels with different thermal, mechanical, morphological and diffusional properties (Figure 6). In particular, **click-Glu** revealed better gelator performance in polar protic solvents, whereas **C18-Glu** exhibited improved properties in non-polar ones. These systems have been successfully applied for fine-tuning the release of the antibiotic vancomycin.

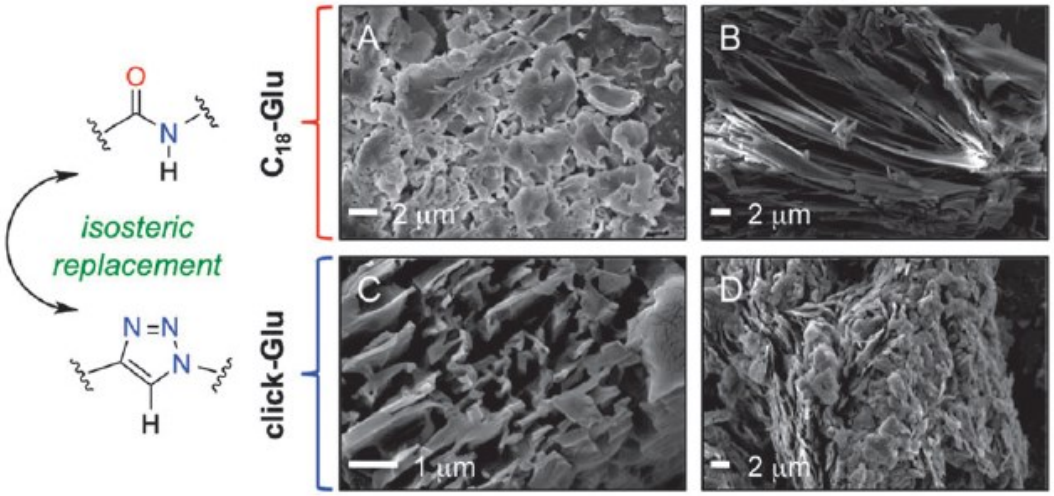


Figure 6. FESEM images of xerogels prepared by freeze drying the corresponding gels. (A) C_{18}-Glu in H_2O (25 g L^{-1}); (B) C_{18}-Glu in $CHCl_3$ (100 g L^{-1}); (C) click-Glu in H_2O (25 g L^{-1}); (D) click-Glu in $CHCl_3$ (100 g L^{-1}). (From Bachl, J. et al Chem. Commun., 2015, 51, 5294—5297)

Non standard α-amino acids

A large number of non-standard α-amino acid, *i. e.* amino acids in which the side chain is modified with respect to natural ones, have been synthesized in the last few years.[12] Nevertheless, their application in nanomaterials preparation, both as single molecules and when inserted in peptides, has still not been completely exploited even if there has been an increase in their use which can be seen from literature.

Linear α-amino acids

Non-coded linear amino acids are able to self-assemble on different type of metal nanoparticles (gold, silver, platinum…). As an example, α-methyl *L*-cysteine (**αMe-*L*-Cys**, Figure 7) has been used for decorating gold and silver nanoparticles (NPs).[13] The gold NPs show higher aqueous stability against aggregation in comparison with the nanoparticles capped with unmodified *L*-cysteine. Such prevention of coalescence is due to the conformational restriction imposed by the α-methyl group, which affects the organization of the amino acid with respect to both the gold surface and the neighboring molecules. On silver nanoparticles, significantly lower adsorbed molecules of **αMe-*L*-Cys** than for cysteine itself, was observed. Apparently, the small variation in structure causes a substantial change in optical activity of the systems. This unexpected behavior could be due to steric hindrance of an additional methyl group that does not allow 3D supramolecular structures to be formed on nanoparticles surface.

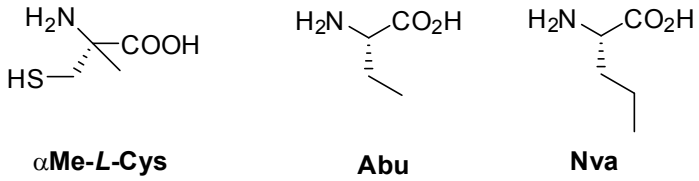


Figure 7. Examples of linear non standard α amino acids

Dipeptides containing hydrophobic side chains are an exceptional source of microporous organic materials, although there are few examples of compounds composed of non standard amino acids. Gorbitz and coworkers reported several new dipeptides containing non-proteinogenic *L*-2-aminobutanoic acid (**Abu**, Figure 7) and/or *L*-2-aminopentanoic acid (**Nva**, Figure 7).[14] These dipeptides crystallize in permanently porous architectures characterized by parallel and independent chiral channels with distinct diameters and helicities. Interestingly, through the fine-tuning of the channel cross-section by systematically changing the amino acid side chains, the empty space can be exploited for accommodating guests.

Dehydrodipeptides, *i. e.* dipeptides containing dehydroamino acids, such as dehydrophenylalanine (**ΔPhe**, Figure 8), dehydroaminobutyric acid **(ΔAbu**, Figure 8), and dehydroalanine (**ΔAla**, Figure 8) have been used for preparing protease resistant hydrogelators functionalized with the nonsteroidal anti-inflammatory drug naproxen.[15] These hydrogels consist of networks of micro/nanosized fibers formed by peptide self-assembly through stacking interactions of naproxene aromatic groups. Furthermore, the planar geometry of dehydroamino acids and the presence of substituents at the β carbon (**ΔPhe** and **ΔAbu**), impart rigidity to the dipeptides, stabilizing the conformation and inducing protease stability.

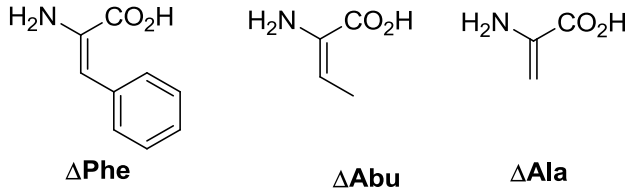


Figure 8. Examples of linear dehydro α-amino acids

A very interesting application takes advantage of the incorporation of photoswitching molecules into molecular building blocks. This creates the possibility of obtaining photoresponsive materials in which the self-assembled architecture or self-assembling process can be controlled by the external light stimuli. Among the photoswitching molecules, azobenzene has been used most widely by virtue of the large photoinduced changes in its molecular geometry and physical properties. Several α-amino

acids bearing side-chain azobenzene moieties, such as **azo-Phe** and other derivatives (*e.g.* **azo-Dbg, A4** and **bis-A4**) have been used for this purpose (Figures 9 and 10).[16] It has been demonstrated by density functional theory calculations that **Azo-Phe** adopts different conformations depending on the *cis/trans* conformation of the azo-function.[16c] In the case of *cis* isomer, water tends to stabilize the helical backbone arrangement. This conformation is sterically forbidden for the *trans* one that thus adopts a β conformation. **Azo-Phe** has been introduced in low-molecular-weight (LMW) peptides that were used for the preparation of multistimuli-responsive supramolecular gels. Interestingly, the azobenzene residue can be used as a versatile regulator to reduce the critical gelation concentration and enhance both the thermal stability and mechanical strength of the gels.

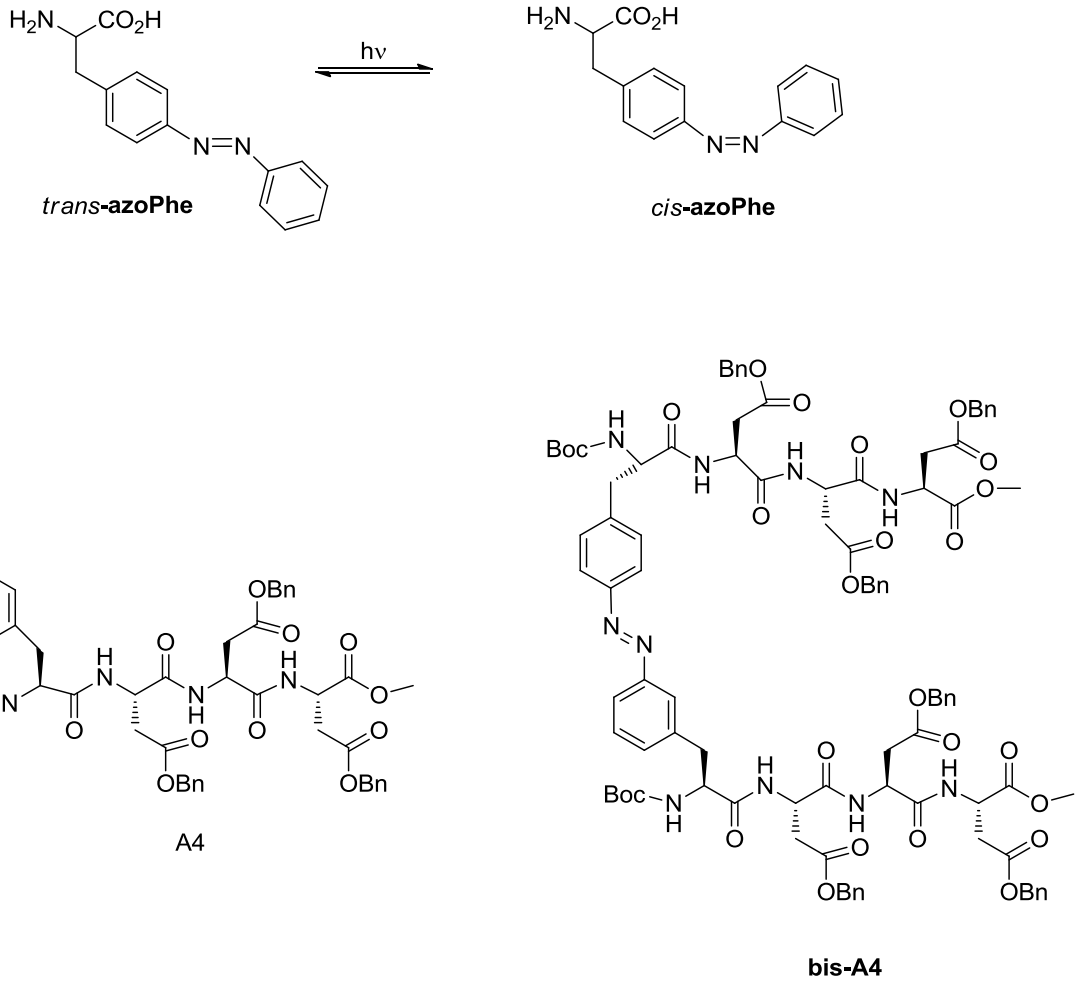


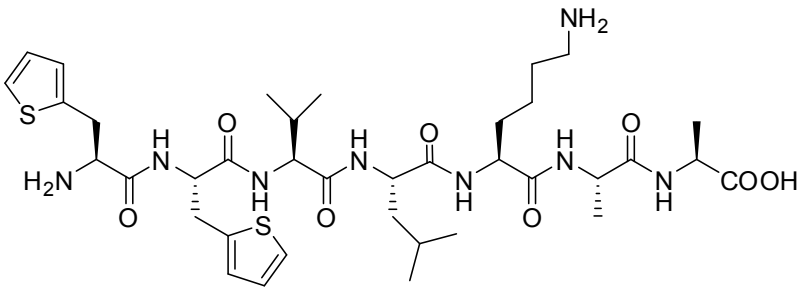
Figure 9. Examples of **azo-Phe** peptides

***p*Azo-Dbg**, an α,α-disubstituted aminoacid bearing two azobenzene moieties covalently linked to the glycine α-carbon atom through a methylene group (Figure 10), has been used to create interesting supramolecular systems.

Figure 10. ***p*AzoDbg** α-amino acid

As an example, ***p*Azo-Dbg** has been used for substituting a phenylalanine moiety in Phe-Phe dipeptide retaining its self-assembly behavior. The presence of ***p*azo-Dbg** α-amino acid is particularly profitable, because: 1) the two side-chain azobenzene moieties give rise to aromatic stacking interactions strong enough to drive the self-assembly process; 2) these interactions can be disrupted by light, thus rendering the supramolecular structure photoresponsive. The nanoarchitectures indeed undergo multiple, reversible isomerizations in a variety of solvents upon irradiation with Vis light (450 nm) or UV light (350 nm). Furthermore, they are able to load metal nanoparticles (Au, Ag and Pt nanoparticles) retaining the reversible photoswitching properties. [17]

The non-coded amino acid thienylalanine (**Thi**) has been used in the development of polypeptides with conductivity properties, arising from the creation of extended conjugated electronic systems.[18] The (**2-Thi**)(**2-Thi**)VLKAA sequence, in which the phenylalanine residues (F) are replaced by **2-Thi** units, was designed and synthesized taking inspiration from AAKLVFF amyloid motif (Figure 11). β-2-Thienylalanine (**2-Thi**) residue is expected to confer interesting electronic properties due to charge delocalization and π-stacking. The peptide is shown to form β-sheet-rich amyloid fibrils with a twisted morphology, in both water and methanol solutions at sufficiently high concentration. The molecular dynamics simulations on these systems revealed well-defined folded structures (turn-like) in dilute aqueous solution, driven by self-assembly of the hydrophobic aromatic units, with charged lysine groups exposed to water.



(2-Thi)(2-Thi)VLKAA

Figure 11. Chemical formula of (**2-Thi**)(**2-Thi**)VLKAA peptide.

Cyclic α-amino acids

There are a few examples of nanostrucures involving cyclic α-amino acids, most of them exploiting 4-substituted prolines. The presence of ring constraints is indeed extremely efficient in stabilizing specific secondary structure of the peptides and thus in driving the self-assembly.

Dipeptides containing 4-F or 4-OH prolines, and cyclic α-substituted and/or α,α-disubstituted amino acids self-assemble into octameric [2]-catenanes (Figure 12).[19] Using dynamic combinatorial chemistry, mixtures of dipeptide monomers were combined to probe how the structural elements affect the equilibrium stability of self-assembled [2]-catenanes versus competing noncatenated structures. The catenanes are stabilized by a combination of intra- and inter-macrocyclic hydrogen bonds, multiple aromatic interactions, and CH-π interactions. The core structure has been evidenced as being especially important, as well as β-turn conformation that is the critical feature predicting stability.

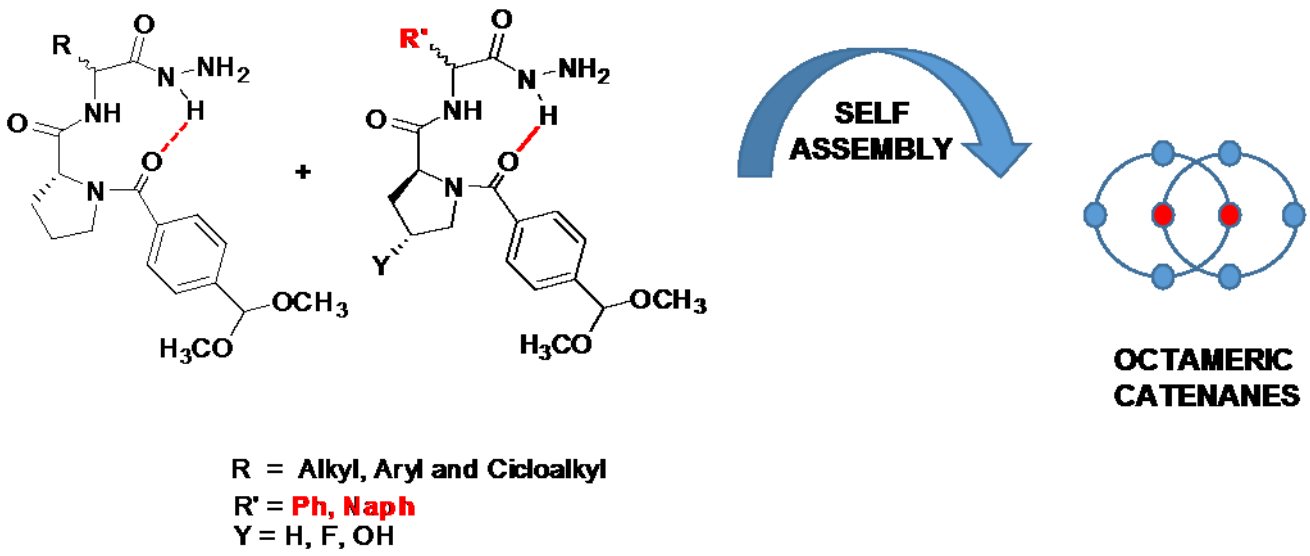


Figure 12. Self-assembly of mixed Pro dipeptides to octameric catenanes

Unnatural 4-azido proline has been used for the preparation of different classes of hybrid nanomaterials.[20] The presence of azido group, indeed, allows Pro- functionalization with different chemical entities, through click reaction.

Wennemers et al. exploited 4-azido proline for the development of collagen derivatives, introducing in the triple helix various functionalities such as carbohydrates (Figure 13)[20a]. They observed that the conformational stability of the functionalized collagens depends on the position of the substituents. Sterically demanding substituents should be attached to (4R)-configured amidoprolines in the Xaa position or to (4S)-configured amidoprolines in the Yaa position.

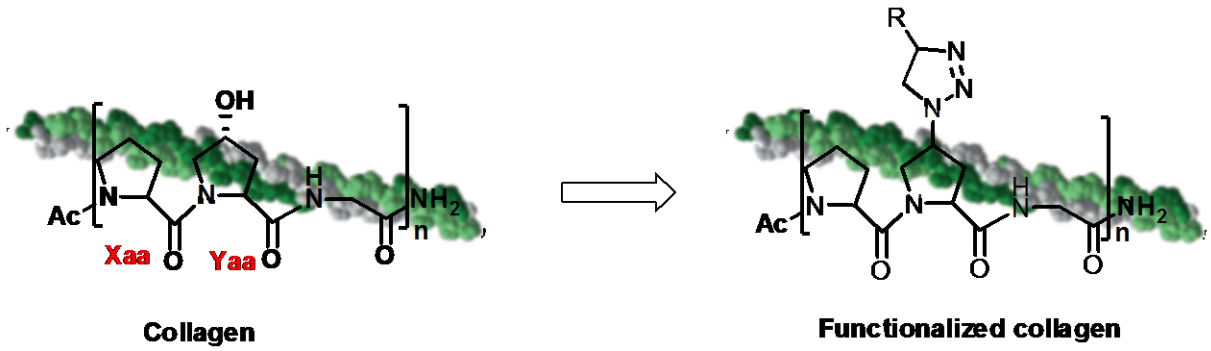


Figure 13. Functionalized collagen derivatives

The same research group reported the click conjugation between azido functionalized oligoprolines and sterically demanding perylenemonoimides (**PMIs**).[20b] The so obtained hybrid materials form worm-like hierarchical supramolecular self-assemblies, via bundles of rigid fibers to nanosheets and nanotubes (Figure 14). The spatial orientation between π systems directly triggers the self-assembly, allowing a fine tuning of supramolecular aggregation.

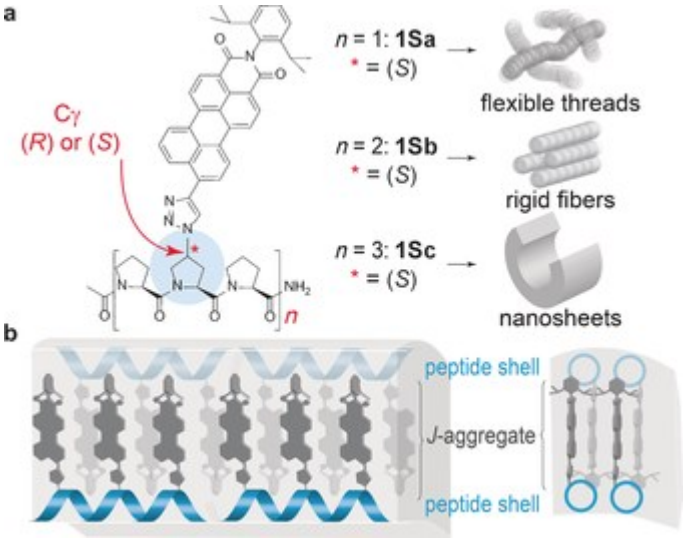


Figure 14. Hybrid collagens from 4-azido proline a) General structure of oligoproline-PMI conjugates. b) Model of the supramolecular organization, front view (left) and side view (right). (From Lewandowska, U. et al. Chem. Eur.J. 2016, 22, 3804)

Thermoresponsive polyproline dendrons have been obtained by grafting azido-polyproline to oligo(ethylene glycol) (OEG) polymers (Figure 15).[20c] The arrangement of the dendrons along the polyproline backbone influences both the helical propensity and the thermally-induced phase transitions. Polyprolines carrying one OEG in every three proline moieties adopt PPI conformation in aqueous conditions; however, polyprolines with reduced number of OEGs, adopt a stable PPII conformation.

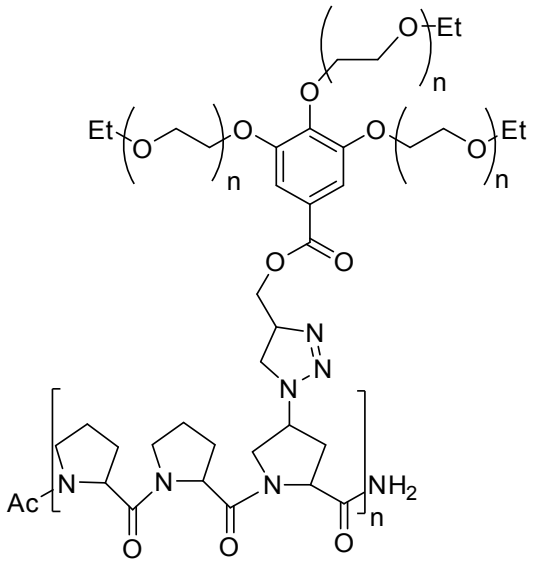


Figure 15. Polyproline dendrons

1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acids (**TIC**, Figure 16) have been used for preparing new simple hydrogelators allowing the *in situ* formation of Pt and Ir nanocrystals.[21] The Pt nanocrystals were successfully used as a catalyst for hydrogenation of the nitro group.

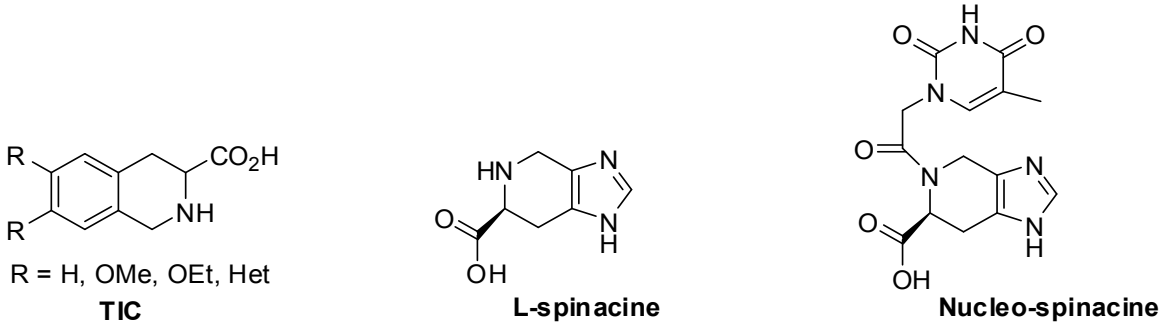


Figure 16. Examples of cyclic non-standard α-amino acids

Another example of application of a cyclic amino acid was reported by Pedone and coworkers.[22] In this work, authors describe the synthesis of a novel nucleoamino acid based on a *L*-spinacine residue bearing the DNA nucleobase thimine anchored to its N-α (Figure 16). The investigation of the self-assembly properties of the novel nucleoamino acid, lead to important information on the assembly of supramolecular networks based on the peptidyl nucleoside analog. From UV and LS studies it has been possible to demonstrate that these structures change as a result of the interaction with metal ions (Cu^{2+}). Furthermore, by experiments with fresh human serum, the enzymatic stability of the novel peptidyl nucleoside analogue was also demonstrated.

In 2012, Gatto and co-workers developed a novel method to build peptide self-assembled monolayers (SAMs) on gold nanoparticles by exploiting exclusively helix---helix macrodipole interactions.[23] A

peptide was prepared containing Cα,α-disubstituted amino acids such as α-aminoisobutyric acid (**Aib**), 4-aminopiperidine-4-carboxylic acid (**Api**, Figure 17) and Cα-methyl norvaline (***L*-(αMe)Nva**) residues, and bearing a 1-pyrenyl (Pyr) unit in the proximity of the N-terminus. This peptide generates a stable supramolecular nanostructure where the pyrenylpeptide is incorporated into the SSA4WA palisade. More recently, **Oxo-Azn**, a α,α-disubstituted constrained glutamine analogue containing the azepino ring (Figure 17), has been designed to decorate gold nanoparticles through a covalent linkage.[24] Interestingly, **Oxo-Azn** was also able to stabilize 3_{10} helix structure in ultra-short peptide.

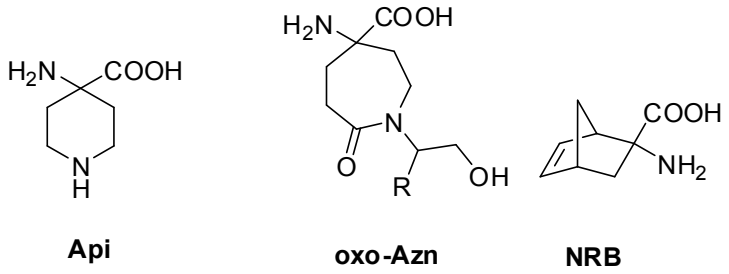


Figure 17. Examples of cyclic α,α disubstituted amino acids

Non-proteinogenic norbornene amino acid (**NRB**, Figures 17 and 18) has been inserted in short peptides containing alanine and Aib that are able to form supramolecular assembly of spherical shapes in water. These nanoaggregates are stable in fetal bovine serum.[25]

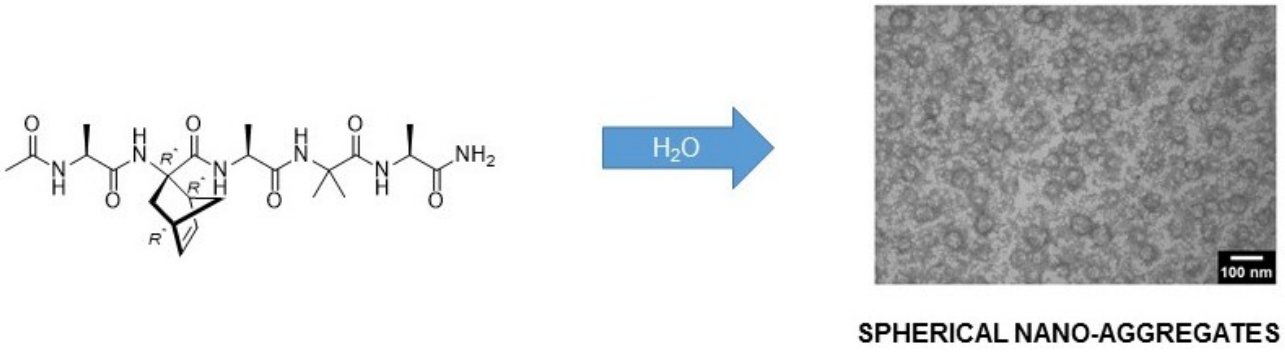


Figure 18. Self-assembly of NRB containing peptides

Non-coded cyclic amino acids have been used for the preparation of spherical nanostructures as drug delivery systems. As an example, a study has been made on the influence on peptide self-assembly of the amino acid residue at position 7 of *Degarelix*, a gonadotropin-releasing hormone (GnRH) peptide antagonist (Figure 19).[26] Peptides containing a Cα,α-tetrasubstituted amino acids (such as **Acc3**, **Acc5**, **Acc6**, Figure 19) are able to form stable vesicles and act as long active compounds.

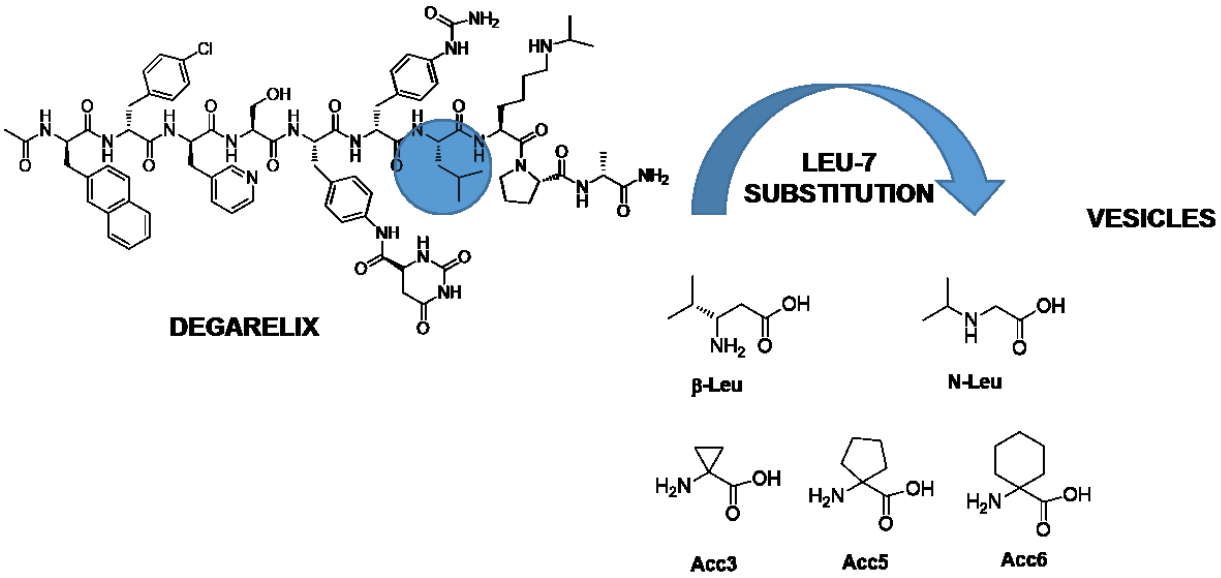


Figure 19. Degarelix analogues

β-Amino acids

β-Amino acids are commonly considered as unnatural amino acids, although some representatives of this class occur in nature as secondary metabolites or as components of complex natural products. With respect to α-amino acids, β-amino acids are characterized by an additional carbon atom between the carboxylic and amine functionalities. In the presence of substituents, the two obtained regioisomers are called β^2 and β^3-amino acids (Figure 20).

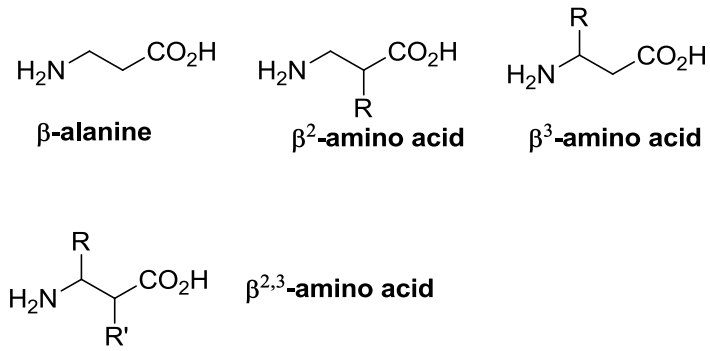


Figure 20. Nomenclature of β-amino acids

Peptides containing β-amino acids are stable to proteolytic degradation and present a great chemical variability that leads to the formation of different and complex secondary structures. These molecular architectures are often able to self-assemble and have been exploited in different nanomaterial

applications. In particular, their metabolic stability is particularly profitable for drug delivery systems. Reviews dealing on the different geometries deriving from the self-assembly of β-peptides have been recently published.[27]

Linear β*-amino acids*

The β-alanine itself has been found able to generate different nanostructures depending on the solvent, temperature, and surface functionality of the materials on which it is assembled. As an example, *N,N*-dicyclohexylurea-β-alanine adduct forms nanovescicles in methanolic solutions. These nanovescicles have been used to encapsulate metotrexate, a potent anticancer drug, suggesting their potential use as a drug carrier. [28]

β-Alanine homotetramers (H_2N–βAla–βAla–βAla–βAla–$CONH_2$) form nanovesicles in an aqueous medium. These vesicles are able to encapsulate L-DOPA, and to release it by lowering the pH to 6.2. The driving force in nanovesicles formation is a conformational switch in the helical structure of the peptides during the self-assembly. TEM and SEM micrographs show that the self-assembled peptides form spherical particles with diameters in the range of 100 nm to 250 nm and possess a central core.[29]

A large number of papers deal with the self-assembly of β^3-amino acids, while, to the best of our knowledge, no examples are reported on β^2-amino acids. β^3-Dipeptides containing β-phenylalanine are able to form different nanostructures depending on environment conditions (pH, temperature, solvent). With respect to α-Phe-Phe nanoarchitectures, β-phenylalanine dipeptides possess thermal and proteolytic stability and can be used in biomedical applications.[30] They have also been used for decorating carbon nanotubes that self-assemble forming regular dendritic-like morphologies.[31]

Aguilar works show that ultrashort β^3-peptides, containing different side chains, spontaneously self-assemble into fibers from solution in a unique head-to-tail fashion. It is possible to obtain large structures that can be seen with the naked eye and handled easily.[32] Side chain polarity does not affect their ability to undergo head-to-tail self-assembly into helical nanorods. They thus represent a robust self-assembling platform that can support polar functional groups in fibrous nanomaterials. As applications, helical N-acetylated β-tripeptides can form hydrogel that can be decorated with cell adhesion signals IKVAV and RGD obtaining bioscaffolds that support the growth of cells.[33] Helical bundles composed of β^3- and α/ β^3-peptides have been extensively studied by Gellman and by several other research groups (see also below in cyclic paragraph). It has been demonstrated that the packing of β-3_{14} helix into coiled-coils of varying stoichiometries is a function of amino acid sequence. These helical bundles have been used in several applications, such as metal ion binding,[34] catalysis[35] and cation nanochannels mimics.[36]

Few examples of nanostructures formed by linear disubstituted $\beta^{2,3}$-amino acids are reported. Dipeptides containing a fluorine substituted $\beta^{2,3}$-diarylamino acid and *L*-alanine (Figure 21) self-assemble into proteolytic stable nanotubes, that are able to load small molecules and enter the cells locating in the cytoplasmic region.[37]

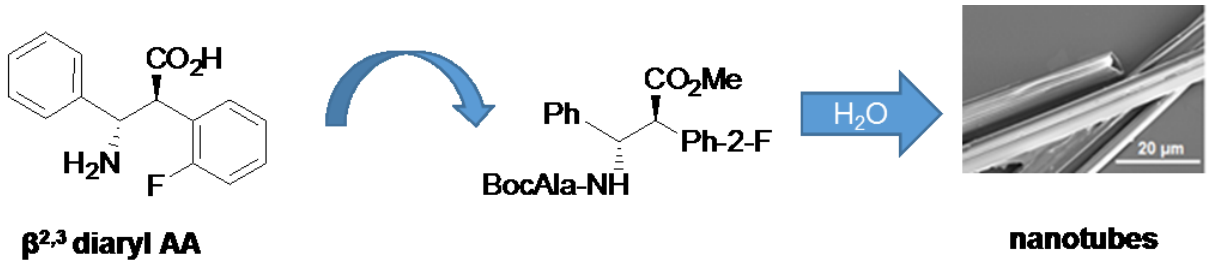


Figure 21. Dipeptide nanotubes containing $\beta^{2,3}$-diarylamino acid

11-Helical α,$\beta^{2,3}$-hybrid peptides give access to hexagonal or rod-like microcrystalline structures depending on the chemical environment and on the sequential use of organic solvents during the self assembly.[38]

Cyclic β-amino acids

$\beta^{2,3}$-Cyclic β-amino acids have been widely used for the preparation of both β- and α,β-peptides. As observed for linear amino acids, they are able to induce the formation of molecular architectures that self-assemble in different geometries.

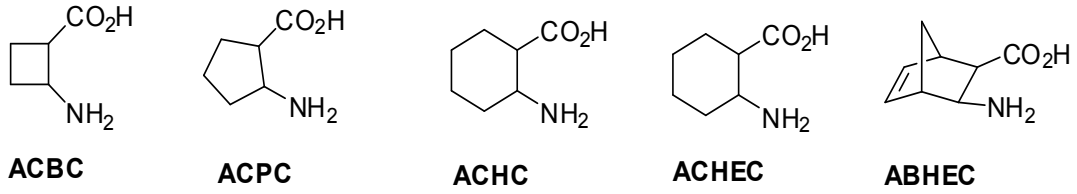


Figure 22. Examples of cyclic β-amino acids

Ortuño and co-workers deeply studied systems containing cyclobutane β-aminoacids (**ACBC**, Figure 22), and investigated the effect of *cis/trans* stereochemistry on molecular organization. Small anionic amphiphiles, containing a lipophilic chain anchored to the amino function of **ACBC**, form spherical micelles, with a morphology depending on the stereochemistry. In diluted solutions, the *cis* geometry stabilizes the headgroup solvation and the anionic-charge, through intramolecular hydrogen-bonding and charge-dipole interactions. Furthermore, the relative configuration of **ACBC** influences the chiral recognition ability of the spherical micelles for bilirubin enantiomers.[39] β-Dipeptides containing two *trans*-**ACBC**, or one *trans* and one *cis* fragment, assemble into nanoscale helical aggregates that form solid-like networks. The gel–sol transition temperature of this gelator is around 270 K in toluene at a

concentration of about 15 mM.[40] *cis*-**ACBC** dipeptides functionalized with the π-electron-rich tetrathiafulvalene (TTF) moiety form fibers able to conduct electricity.[41] By increasing the number of *cis*-**ACBC**, the formation of six-membered hydrogen-bonded rings is induced and a strand conformation is observed in solution. These oligomers self-assemble into nano-sized fibres.[42]

Seung-Lee and co-workers devised the new term "foldectures", indicating any 3D molecular architecture that is derived from the self-association of foldamers in solution. They showed that helical β-peptide foldamers self-assemble into 3D molecular architecture when they are composed of *trans*-(*R,R*)-2-aminocyclopentanecarboxylic acid (*trans* (*R,R*)-**ACPC**, Figure 22) and C-protected with a benzyl group. In particular, they observed that four individual left-handed helical monomers constitute a right-handed superhelix similar to the supercoiled structure of collagen.[43] The 7-mer sequence, that in organic solution possesses a 12-helical structure, self-assembles into a homogeneous windmill-shaped morphology in an aqueous environment.[44] The hexamer gives rise to nanoarchitectures with a molar tooth shape in aqueous solution.[43] Starting from the shorter *trans*-**ACPC** tetramer, microtubes with a rectangular cross-section can be observed. What is relevant is that the tetramer lacks full helical propensity in solution. The self-assembly is indeed solvent evaporation induced.[45] Recently, the authors discovered that these foldectures, as free carboxylic acids, uniformly align with respect to an applied static magnetic field, and possess instantaneous orientational motion in a dynamic magnetic field (Figure 23). These motions resemble the magnetotactic behaviour of magnetosomes in magnetotactic bacteria.[46]

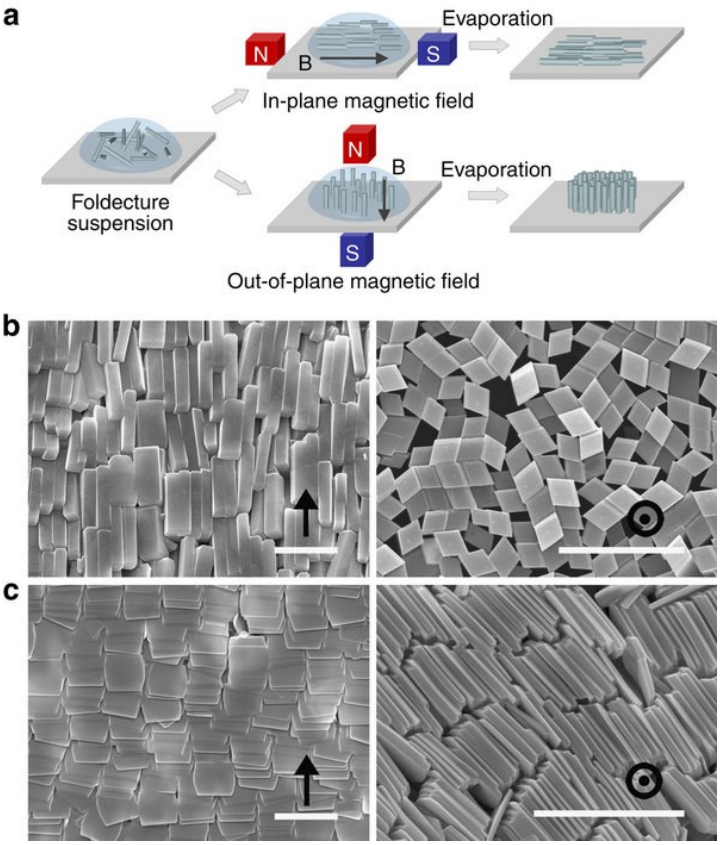


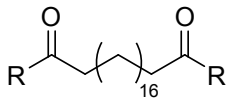
Figure 23. Alignment of *trans*-**ACPC** foldectures by static magnetic field. (a) Schematic diagram of experimental process for alignment of foldectures under static magnetic field. (b) SEM images of rhombic rod foldectures (F1) deposited on Si substrates under (left) in-plane magnetic field and (right) out-of-plane magnetic field. (c) SEM images of rectangular plate foldectures (F2) deposited on Si substrates under (left) in-plane magnetic field and (right) out-of-plane magnetic field. Arrows and circles indicate direction of magnetic field. Scale bars, 5 μm. (From Kwon S. et al. Nature Comm., 2015,DOI: 10.1038/ncomms9747)

The self-assembly of α/β-peptide foldamers containing **ACPC** and Aib was also studied. These foldectures possess a 11 helix structure in solution and give rise to parallelogram plate shapes in the solid state [47] and to hollow truncated trigonal bipyramid shapes in water.[48]

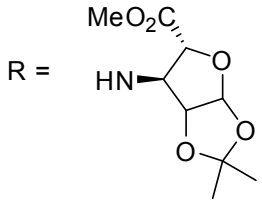
β-Peptides containing *trans*-2-aminocyclohexanecarboxylic acid (*trans*-**ACHC**, Figure 22) in solution form H_{14} helices that self-assemble into helix bundles, vesicle-forming membranes, and lyotropic liquid crystals.[49] On the other hand, *cis*-**ACHC** peptides possess $H_{10/12}$ helix conformation that in water self-assemble with the formation of vescicles with an average diameter of 100 nm. A similar behavior is observed for β-peptides containing *cis*-**ACHEC** (Figure 22) and *exo*-**ABHEC** (Figure 22).[50] Dipeptides containing *cis*-**ACHC** together with either Aib or *L*-Phe form organogel through the self- assembly of monomers possessing turn-type β-sheet arrangement. This gel has been

used for oil spill recovery from a biphasic mixture of oil and water.[51] Synthetic polymers containing **ACHC** and cationic $\beta^{2,3}$ substituted amino acid have been developed by Gellman. These nylon-3 polymers (poly-β-peptides) display significant and selective toxicity toward the most common fungal pathogen among humans, Candida albicans.[52]

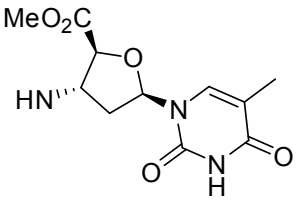
Cyclic β-amino acids containing the furanosyl ring (Figure 23) have been used for the synthesis of bolamphiphiles, molecules having two hydrophilic groups at both ends of a sufficiently long hydrophobic hydrocarbon chain. These compounds self-assemble in different structures depending on the hydrophilicity/hydrophobicity of the heads.[53]



Bolamphiphiles



sugar β-AA



AZT β-AA

Figure 23. Bolamphiphiles containing cyclic β-amino acids

γ-Amino acids

Recently, γ-amino acids have gained increasing attention considering them as molecular building blocks for nanomaterials. Although the introduction of two additional carbon atoms on the backbone reduces the number of potential hydrogen bonds, γ-peptides adopt various stable conformations, such as helices, sheets and turn.[54] In particular, γ-peptides helices are surprisingly stable and have been observed for ultra-short sequences. The conformation stability is increased by introducing substituents on the backbone chain (γ^4–,$\gamma^{4,2}$–, $\gamma^{4,4}$-amino acids, Figure 24)

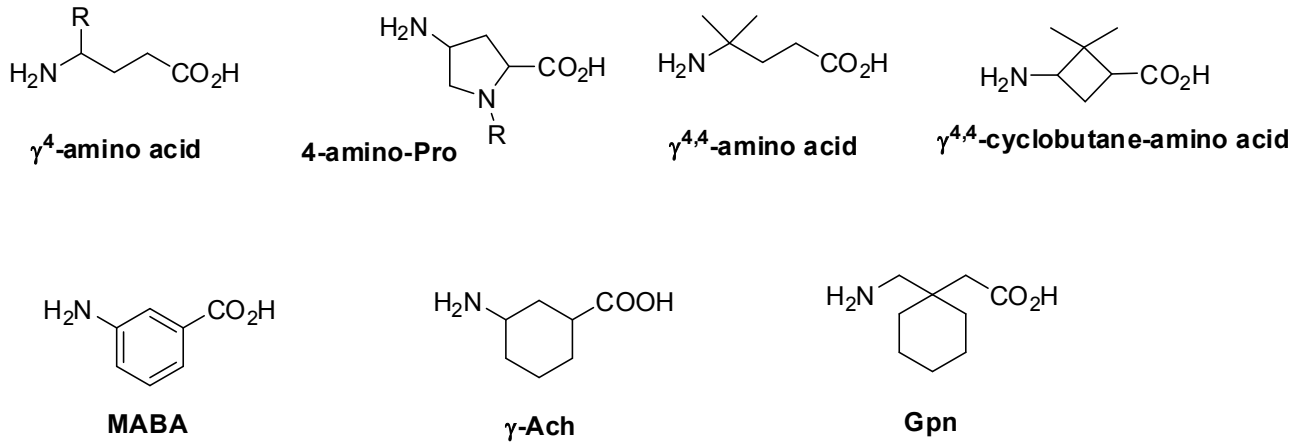


Figure 24. Examples of γ-amino acids.

Oligomers containing γ-cyclobutane amino acid and 4-amino Proline (Figure 22) have been studied by Ortuno et al.[55] These peptides have high tendency to aggregation providing stable vesicles of nanometric size.

Helical peptides containing *m*-aminobenzoic acid (**MABA**, Figure 24) form a supramolecular sheet structure in polar protic solvent, caused by a conformational switch of the helical strand to a more open (extended) structure. However, in solvent like chloroform the helical structure helps to accommodate the second molecule in the intertwining processes and thus increases the stability of the supramolecular double helix.[56] Dipeptides containing **MABA** in combination with several α-amino acids, form pH-sensitive nanostructures through aromatic π–π interactions. At acidic pH (pH 4.2–6.0) nanowires have been observed, while increasing the pH of the solution, only nanovesicles have been formed.[57] As far as γ-peptides composed of gem-dimethyl $\gamma^{4,4}$-amino acids (Figure 24) are concerned, they adopt extended polar sheet type structure and spontaneously self-assemble into nanofibrillar superstructures. Interestingly they display unprecedented thermoreversible gelation in various solvents.[58] In addition to the homo-oligomers, the mixed sequences consisting of α- and homologated β- and γ-amino acids have been studied. The reason being the variety of structures that can be generated by changing the order and position of amino acids. Gopi and coworkers studied the self-assembling properties of α,γ-hybrid peptides composed of alternating α- and γ-amino acids.[59] All α,γ 4-hybrid peptides show 12-helical conformations in single crystals. Based on the nature of the γ-amino acid side chains, they displayed remarkable divergent supramolecular assemblies such as ribbons, fibers, rods and tubes. The heptapeptides with alternating α- and γ-Phe residues showed remarkable elongated nanotubes which were explored as a template for biomineralization of silver ions to silver nanowires. Other hybrid peptides, in particular γ,α-hybrid peptides were studied by Das

et al.[60] Gabapentin (**Gpn**, Figure 24) containing hybrid peptides with sequence Boc-**Gpn**-Aib-Xaa-Aib-OMe (where Xaa = phenylalanine, leucine or tyrosine) show a C12/C10 hydrogen-bonded double turn conformation. The circular dichroism studies show distinct CD patterns for the peptides in an aqueous methanol medium which can be attributed to the third residue side-chain effect. Both scanning and transmission electron microscopy observations demonstrate solvent dependent self-assembled morphological features.

Cyclopeptides

Several classes of cyclic oligopeptides self-assemble into peptide nanotubes (SPNs). SNPs are widely studied for their potential applications both in biology and materials science. By varying the number of amino acids in each ring, it is possible to control the diameter of the nanotube. On the other hand, the properties of nanotube outer surface can be easily modified by varying the amino acids side chain. In particular, it is crucial that the cyclopeptide adopts a flat conformation in which not only the amino acids side chains of the peptide rings have a pseudo-equatorial outward-pointing orientation but also the carbonyl and amino groups of the peptide bonds are oriented perpendicular to the ring.[61]

Cyclic homo-γ-tetrapeptides based on *cis*-3-aminocyclohexanecarboxylic acid (**γ-Ach**, Figures 24 and 25) residues, self-assemble to nanotubes.[62] Cyclic octapeptides composed of α-amino acids alternated with *cis*-**γ-Ach** (Figure 23a), self-assemble as drumlike dimers through β-sheet-like, backbone-to backbone hydrogen bonding.[63]

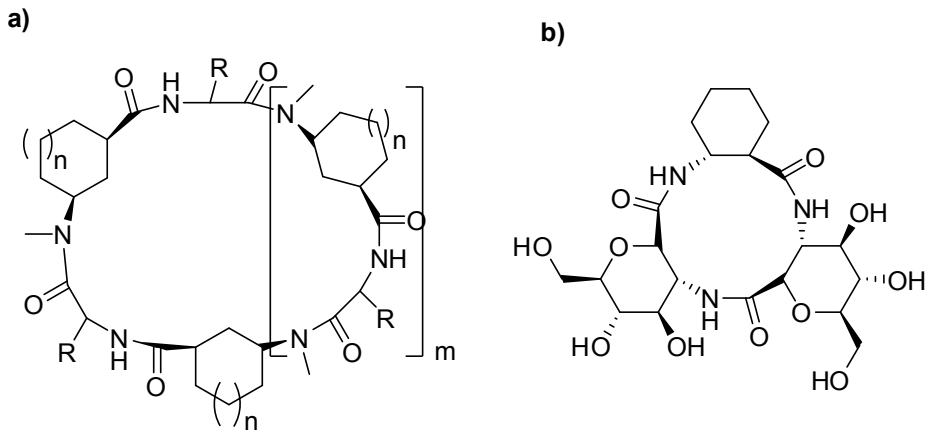


Figure 25. Cyclic peptides containing **γ-Ach**

Several other recent examples of cylic peptides incorporating sugar-like scaffolds have been reported. Granja and coworkers reported on peptide nanotubes composed exclusively of cyclic γ-amino acids with a saccharide-like outer surface.[64] Amphiphilic cyclic peptide composed of two *β*-glucosamino

acids and one *trans*-2-aminocyclohexylcarboxylic acid (Figure 25b) self-assembled into rodlike crystals or nanofibers depending on preparative conditions.[65]

The synthesis and the structural investigation on a cyclic tetrapeptide containing alternating a C-linked sugar β-amino acid (**(*S*)-β-Caa**) and *R*-aminoxy acid 2 (***R*-Ama**) was reported by Sharma (Figure 26).[66] At higher concentration this cyclic peptide forms nanorod aggregates as evidenced from TEM and AFM analysis.

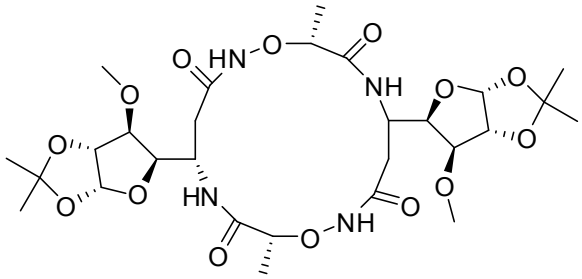


Figure 26. Cyclic peptides containing **(*S*)-β-Caa** and ***R*-Ama**

Replacement of amide bond by triazole isoster leads to cycles with very interesting properties which have been deeply studied by Chattopadhyay and coworkers.[67] For instance, two types of novel heterocyclic backbone modifed macrocyclic peptides have been obtained by incorporating an α- or β−amino acid and *cis*-β−furanoid (1,4)-linked triazole amino acid (Figure 27). The macrocyclic peptide **A**, featuring α-amino acid, is able to maintain pseudo cyclo-peptide conformation with predictable self-assembly pattern, while **B**, based on β-amino acid, forms only a conformationally homogeneous cyclic peptide that does not undergo self-assembly.

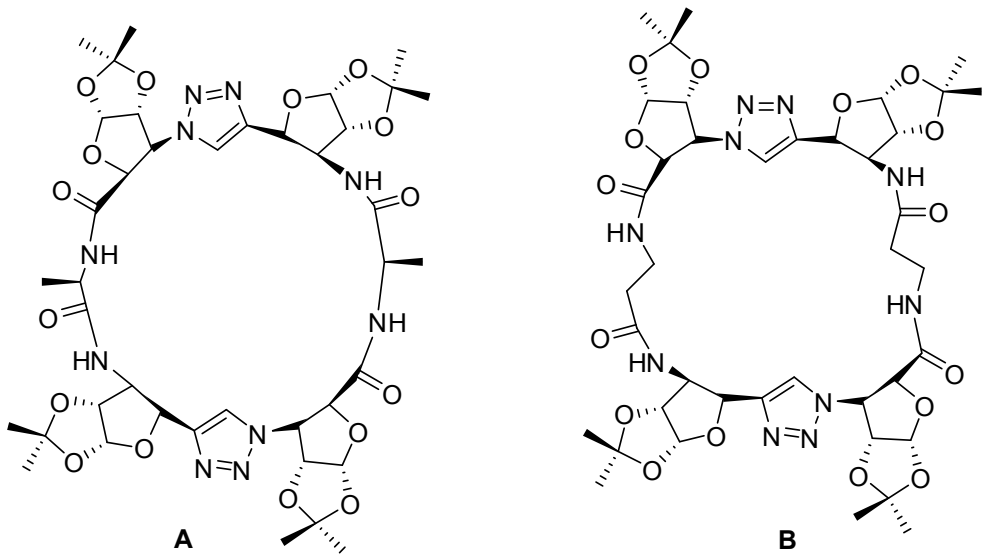


Figure 27. Cyclic peptides containing isosteric triazole amide replacement

Conclusions

Many synthetic efforts are devoted to obtaining new and different non-standard amino acids, claiming their importance in the development of new smart nanomaterials. In principle, the functional variability on the amino acid scaffold could be endlessly introduced, leading to an infinite number of molecular combinations and architectures. While a huge number of synthetic methodologies have been developed, the application of non-standard amino acids in nanomaterials preparation has yet to be completely exploited.

References

1. (a) Mondal, S.; Gazit, E. ChemNanoMat 2016, 2, 323 –332; (b) Alemàn, C.; Bianco, A.; Venanzi, M. Peptide Materials, John Wiley and Sons: U. K., 2013. (c) Mandal, D.; Shirazib, A. N.; Parang, K. Org. Biomol. Chem. 2014, 12, 3544. (d) Dasgupta, A.; Mondala, J. H.; Das, D. RSC Adv. 2013, 3, 9117. (e) Panda, J. J.; Chauhan, V. S. Polym. Chem. 2014, 5, 4418. (f) Lakshmanan, A.; Zhang, S.; Hauser, C. A. E. Trends in Biotechnology 2012, 30, 155; (g) Boyle, A. L.; Woolfson, D. N. Supramolecular Chemistry: From Molecules to Nanomaterials, John Wiley and Sons: U. K., 2012.

2. Fleming, S.; Ulijn, R. V. Chem. Soc. Rev. 2014, 43, 8150.

3. Nanda, J.; Biswas, A.; Adhikari, B.; Banerjee, A. Angew. Chem. Int. Ed. 2013, 52, 5041.

4. Sun, Z.; Li, Z.; He, Y.; Shen, R.; Deng, L.; Yang, M.; Liang, Y.; Zhang, Y. J. Am. Chem. Soc. 2013, 135, 13379.

5. Kuang, G-C.; Teng, M-J.; Jia, X-R.; Chen, E-Q.; Wei, Y. Chem. Asian J. 2011, 6, 1163.

6. Avinash, M. B.; Govindaraju, T. Adv. Mater. 2012, 24, 3905.

7. A) For a recent review on naphthalene diimides: M. Al Kobaisi, S. V. Bhosale, K. Latham, A. M. Raynor, S. V. Bhosale Chem. Rev. 2016, DOI: 10.1021/acs.chemrev.6b00160; b) Fan, Y.; Cheng, L.; Liu, C.; Xie, Y.; Liu, W.; Li, Y.; Li, X.; Li, Y.; Fan, X. RSC Adv. 2014, 52245; c) S. Bai, S. Debnath, N. Javid, P. W. J. M. Frederix, S. Fleming, C. Pappas, R. V. Ulijn Langmuir, 2014, 30, 7576–7584; d) S. Manchineella,V. Prathyusha, U. Deva Priyakumar, T. Govindaraju Chem. Eur. J. 2013, 19, 16615 – 16624 and ref. cited therein.

8. Manna, M. K.; Rasale, D. B.; Das, A.K. RSC Adv. 2015, 90158.

9. Maiti, D. K.; Banerjee, A. Chem. Asian J. 2013, 8.

10. Kumar, N.; Khullar, S.; Mandal, S. K. Dalton Trans. 2015, 44, 5672.

11. Bachl, J.; Mayr, J.; Sayago, F. J.; Cativiela, C.; Diaz Diaz, D. Chem. Commun. 2015, 51, 5294.

12. (a) Stevenazzi, A.; Marchini, M.; Sandrone, G.; Vergani, B.; Lattanzio, M. Bioorg. Med. Chem. Lett. 2014, 24, 5349. (b) Fanelli, R.; Ben Haj Salah, K.; Inguimbert, N.; Didiejean, C., Martinez J., Cavelier F. Org. Lett. 2015, 17, 449. (c) Pellegrino, S.; Contini, A.; Clerici, F.; Gori, A.; Nava, D.; Gelmi, M. L. Chem. Eur. J. 2012, 18, 8705. (d) Ruffoni, A.; Casoni, A.; Pellegrino, S.; Gelmi, M. L.; Soave, R.; Clerici, F. Tetrahedron, 2012, 68, 1951.

13. Koktan, J.; Sedlackova, H.; Osante, I.; Cativiela, C.; Diaz Diaz, D.; Rezanka, P. Coll. and Surf. A: Physicochem. Eng. Aspects 2015, 470, 142 and ref. cited therein.

14. Yadav, V. N.; Comotti, A.; Sozzani, P.; Bracco, S.; Bonge-Hansen, T.; Hennum, M.; Gçrbitz, C. H. Angew. Chem. 2015, 127, 15910.

15. Vilaça, H.; Hortelão, A. C. L.; Castanheira, E. M. S.; Queiroz, M. R. P.; Hilliou, L.; Hamley, I. W.; Martins, J. A.; Ferreira, P. M. T. Biomacromol. 2015, 16, 3562.

16. Bachl, J.; Oehm, S.; Mayr, J.; Cativiela, C.; Marrero-Tellado, J. J. ; Diaz Diaz, D. Int. J. Mol. Sci. 2015, 16, 11766 and ref. cited therein; b) P. Fatas, J. Bachl, S. Oehm, A. I. Jimenez, C,s Cativiela, D. Diaz Diaz G. Chem. Eur. J. 2013, 19, 8861–8874; c) Revilla-Lopez, A. D. Laurent, E. A. Perpete, D. Jacquemin, J. Torras, X. Assfeld, C. Aleman J.Phys.Chem.B 2011, 115, 1232–1242

17. Mba, M.; Mazzier, D.; Silvestrini, S.; Toniolo, C.; Fatas, P.; Jiménez, A. I.; Cativiela, C.; Moretto, A. Chem. Eur. J. 2013, 15841 and ref. cited therein.

18. Hamley, I. W.; Brown, G. D.; Castelletto, V.; Cheng, G.; Venanzi, M.; Caruso, M.; Placidi, E.; Aleman, C.; Revilla-Lòpez, G.; Zanuy, D. J. Phys. Chem. B 2010, 114, 10674.

19. Chung, M.K.; Lee, S. J.; Waters, M. L.; Gagné, M. R. J. Am. Chem. Soc. 2012, 11430

20. (a) Siebler, C.; Erdmann, R. S.; Wennemers, H. CHIMIA Int. J. .Chem. 2013, .67, 891-895; (b) Lewandowska, U.; Zajaczkowski, W.; Pisula, W.; Ma, Y.; Li, C.; Mullen, K.; Wennemers H. Chem. Eur. J. 2016, 22, 3804; (c) Zhang, X.; Li, W.; Zhao, X.; Zhang, A. Macromol. Rapid Commun. 2013, 34, 1701−1707.

21. Kang, C.; Wang, L.; Bian, Z.; Guo, H.; Ma, X.; Qiu, X.; Gao, X. Chem. Comm. 2014, 50, 13979.

22. Roviello, G. N.; Mottola, A.; Musumeci, D.; Bucci, E. M.; Pedone, C. Amino Acids 2012, 43, 1465.

23. Gatto, E.; Porchetta, A.; Scarselli, M.; De Crescenzi, M.; Formaggio, F.; Toniolo, C.; Venanzi, M. Langmuir 2012, 28, 2817.

24. Pellegrino, S.; Bonetti, A.; Clerici, F.; Contini, A.; Moretto, A.; Soave, R.; Gelmi, M. L. J. Org. Chem. 2015, 80, 5507.

25. Ruffoni, A.; Cavanna, M. V.; Argentiere, S.; Locarno, S.; Pellegrino, S.; Gelmi, M. L.; Clerici, F. RSC Adv. 2016, 6, 90754 - 90759

26. Zhou, N.; Gao, X.; Lv, Y.; Cheng, J.; Zhou, W.; Liu, K. J. Pept. Sci. 2014, 868.

27. (a) Gopalan, R. D.; Del Borgo, M. P.; Mechler, A. I.; Perlmutter, P.; Aguilar, M.-I. Chem. & Biol. 22, 1417. (b) Wanga, P. S. P.; Schepartz, A. Chem. Comm. 2016, 52, 7420. (c) Hamley, I. W. Angew. Chem. Int. Ed. 2014, 53, 6866.

28. Kar, S.; Huang, B-H.; Wu, K-W.; Lee, C-R.; Tai, Y. Soft Matter 2014, 10, 8075.

29. Goel, R.; Gopal, S.; Gupta, A. J. Mater. Chem. B 2015, 3, 5849.

30. Parween, S.; Misra, A.; Ramakumarb, S.; Chauhan, V. S. J. Mater. Chem. B 2014, 2, 3096.

31. Dinesh, B.; Squillaci, M.A.; Ménard-Moyon, C.; Samorì, P.; Bianco, A. Nanoscale 2015, 7, 15873.

32. Seoudi, R. S.; Hinds, M. G.; Wilson, D. J. D.; Christopher G.; Adda, C. G.; Del Borgo, M. D.; Aguilar, M.-I.; Perlmutter, P.; Mechler, A. Nanotech. 2016, 27, 135606 and ref. cited therein.

33. Luder, K.; Kulkarni, K.; Wen Lee, H.; Widdop, R. E.; Del Borgo, M. P.; Aguilar M.-I. Chem. Comm. 2016, 52, 4549.

34. Wang, P. S. P.; Nguyen, J. B.; Schepartz, A. J. Am. Chem. Soc. 2014, 136, 14726.

35. Araghi, R. R.; Koksch, B. Chem. Comm. 2011, 47, 3544.

36. Otero, J. M.; van der Knaap, M.; Llamas-Saiz, A. L.; van Raaij, M. J.; Amorín, M.; Granja, J. R.; Filippov, D. V.; van der Marel, G. A.; Overkleeft, H. S.; Overhand, M. Cryst. Growth Des. 2013, 13, 4355.

37. Bonetti A.; Pellegrino, S.; Das, P.; Yuran, S.; Bucci, R.; Ferri, N.; Meneghetti, F.; Castellano, C.; Reches, M.; Gelmi, M. L. Org. Lett. 2015, 17, 4468

38. Balamurugan, D.; Muraleedharan, K. M. Soft Matter, 2012, 8, 11857.

39. Sorrenti, A.; Illa, O.; Pons, R.; Ortuño, R. M. Langmuir 2015, 31, 9608.

40. Gorrea, E.; Nolis, P.; Torres, E.; Da Silva, E.; Amabilino, D. B.; Branchadell, V.; Ortuño, R. M. Chem. Eur. J. 2011, 17, 4588.

41. Torres, E.; Puigmartí-Luis, J.; Pérez del Pino, A.; Ortuño, R. M.; Amabilino, D. B. Org. Biomol. Chem. 2010, 8, 1661.

42. Torres, E.; Gorrea, E.; Burusco, K. K.; Da Silva, E.; Nolis, P.; Rúa, F.; Boussert, S.; Díez-Pérez, I.; Dannenberg, S.; Izquierdo, S.; Giralt, E.; Jaime, C.; Branchadell, V.; Ortuño, R. M. Org. Biomol. Chem. 2010, 8, 564.

43. Kwon, S.; Shin, H. S.; Gong, J.; Eom, J.-H.; Jeon, A. ; Yoo, S. H. ; Chung, I. S.; Cho, S.J.; Lee, H.-S. J. Am. Chem. Soc. 2011, 133, 17618.

44. Kwon, S.; Jeon, A.; Yoo, S. H.; Chung, I. S.; Lee, H.-S. Angew. Chem. Int. Ed. 2010, 49, 8232.

45. Kim, J.; Kwon, S.; Kim, S. H.; Lee, C.-K.; Lee, J.-H.; June Cho, S. J.; Lee, H.-S.; Ihee, H. J. Am. Chem. Soc. 2012, 134, 20573.

46. Kwon, S.; Kim, B. J.; Lim, H.-K.; Kang, K.; Yoo, S. H.; Gong, J.; Yoon, E.; Lee, J.; Choi, I. S.; Kim, H.; Lee, H.-S. Nature Comm., 2015, DOI: 10.1038/ncomms9747.

47. Eom, J.-H.; Gong, J.; Jeong, R.; Driver, R. W.; Lee, H.-S. Solid State Sciences 48 (2015) 39.

48. Eom, J.-H.; Gong, J.; Kwon, S.; Jeon, A.; Jeong, R.; Driver, R. W.; Lee, H.-S. Angew. Chem. Int. Ed. 2015, 54, 13204.

49. Pomerantz, W. C.; Yuwono, V. M.; Drake, R.; Hartgerink, J. D.; Abbott, N. L.; Gellman, S. H. J. Am. Chem. Soc. 2011, 133, 13604.

50. Mándity, I. M.; Fülöp, L., Vass, E.; Tóth, G. K.; Martinek, T. A.; Fülöp, F. Org. let. 2010, 12, 5584.

51. Konda, M.; Maity, I. ; Rasale, D. B.; Das, A. K. ChemPlusChem. 2014, 79, 1482.

52. Liu, R.; Chen, X.; Hayouka, Z.; Chakraborty, S.; Falk, S. P.; Weisblum, B.; Masters, K. S.; Gellman, S. H. J. Am. Chem. Soc. 2013, 135, 5270.

53. Mainkar, P. S.; Sridhar, C.; Sudhakar, A.; Chandrasekhar, S. Helvetica Chimica Acta 96, 2013, 99

54. Bouillère, F.; Thétiot-Laurent, S.; Kouklovsky, C.; Alezra, V. Amino Acids, 2011, 41, 687-707.

55. Gutiérrez-Abad, R.; Carbajo, D.; Nolis, P.; Acosta-Silva, C; Cobos, J. A.; Illa, O.; Royo, M.; Ortuño, R. M. Amino Acids 2011, 41, 673.

56. Sarkar, R.; Debnath, M.; Maji, K.; Haldar, D. RSC Adv. 2015, 5, 76257.

57. Koley, P.; Pramanik, A. J. Mater Sci. 2014, 49, 2000.

58. Jadhav, S. V.; Gopi, H. N. Chem. Comm. 2013, 49, 9179.

59. Jadhav, S. V.; Misra, R.; Gopi, H. N. Chem. Eur. J. 2014, 20, 16523.

60. Konda, M.; Kauffmann, B.; Rasale, D. B.; Das, A. K. Org. Biomol. Chem. 2016, 14, 4089.

61. Brea, R. J.; Ririz, C.; Granja, J. R. Chem Soc Rev. 2010, 39, 1448.

62. Li, L.; Zhan, H.; Duan, P.; Liao, J.; Quan, J.; Hu, Y.; Chen, Z.; Zhu, J.; Liu, M.; Wu, Y.-D.; Deng, J. Adv. Func. Mat. 2012, 3051.

63. Brea, R. J.; Perez-Alvite, M. J.; Panciera, M.; Mosquera, M.; Castedo, L.; Granja, J. R. Chem. Asian J. 2011, 6, 110.

64. Guerra, A.; Brea, R. J.; Amorín, M.; Castedo, L.; Granja, J. R. Org. Biomol. Chem. 2012, 10, 8762.

65. Ishihara, Y.; Kimura, S. J. Pept. Sci. 2010, 16, 110.

66. Sharma, G. V. M.; Manohar, V.; Dutta, S. K.; Sridhar, B.; Ramesh, V.; Srinivas, R.; Kunwar, A. C. J. Org. Chem. 2010, 75, 1087,

67. Kulsi, G.; Ghorai, A.; Achari, B.; Chattopadhyay, P. RSC Adv. 2015, 5, 64675 and ref cited therein.