Peptoid-based hierarchically-structured biomimetic nanomaterials: Synthesis, characterization and applications

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ABSTRACT Peptoids (or poly-*N*-substituted glycines) are a promising class of bioinspired sequence-defined polymers due to their highly efficient synthesis, high chemical stability, enzyme hydrolysis resistance, and biocompatibility. By tuning the side chain chemistry of peptoids, it allows for precise control over sequences and achieving a large side-chain diversity. Due to these unique features, in the last several years, many amphiphilic peptoids were designed as highly tunable building blocks for the preparation of biomimetic nanomaterials with well-defined hierarchical structures and desired functionalities. Herein, we provide an overview of the recent achievements in this area by dividing them into the following three aspects. First, mica- and silica-templated peptoid self-assembly are summarized. The presence of inorganic substrates provides the guarantee of investigating their self-assembly mechanisms and interactions between peptoids and substrates using nanoscale characterization techniques, particularly *in situ* atomic force microscopy (AFM) and AFM-based dynamic force spectroscopy (AFM-DFS). Second, solution-phase self-assembly of peptoids into nanotubes and nanosheets is presented, as well as their self-repair properties. Third, the applications of peptoid-based nanomaterials are outlined, including the construction of catalytic nanomaterials as a template and cytosolic delivery as cargoes.

Keywords: peptoid, self-assembly, biomimetic nanomaterial, nanoscale characterization, assembly mechanism

INTRODUCTION

Bioinspired materials assembled from sequence-defined polymers are attracting increasing attention due to significant advantages over their natural counterparts: the ability to precisely tune their structures over a broad range of chemical and physical properties, increased sta-

bility, and improved processability [1–6]. Peptoids are non-natural mimetics of peptides and proteins composed of repeating *N*-substituted glycine monomer units, where the side chain is covalently attached to the nitrogen atom rather than the α -carbon (Fig. 1a) [1,2,7]. This subtle structural modification provides peptoids with unique structures and properties in contrast to peptides and proteins, such as high stability against protease digestion, enhanced chemical and thermal stability, and the lack of backbone hydrogen bond donors which simplifies the building of peptoid-peptoid and peptoid-surface interactions exclusively through side-chain chemistry. Because of such advantages, in the last several years, amphiphilic peptoids have been frequently designed and used as sequence-defined building blocks to assemble into biomimetic nanomaterials with various hierarchical structures both on the substrate surfaces and in the solution [1,2,7–18]. To access sequence-defined peptoids, a step-wise “sub-monomer” synthesis approach was developed by Zuckermann *et al.* [19] in 1992. This approach uses a two-step sub-monomer addition reaction to build peptoid side chains from primary amines, instead of amino acid precursors used in the solid-phase peptide synthesis (Fig. 1b). Because several hundreds of commercially available amines can be used for peptoid synthesis, this method provides the access to a nearly infinite diversity of peptoid sequences with precisely controlled side-chain chemistries, allowing a fine tuning of the structure and function of peptoid-based nanomaterials by incorporating protein-like high information content into the materials design [3,20,21].

The recent development of peptoid-based nanomaterials was involved with many state-of-the-art nanoscale characterization techniques. Although traditional meth-

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Figure 1 (a) The structures of peptides and peptoids, and peptoids bridge the gap between the natural polymers and synthetic polymers. (b) Solid-phase synthesis of sequence-defined peptoids through a repeating two-step submonomer synthesis method.

ods, such as X-ray diffraction (XRD) and dynamic light scattering (DLS), can be used to determine the crystallinity, size and surface charges of peptoid assemblies, they are limited in determining the assembly pathways and dynamics. Atomic force microscopy (AFM) is a beneficial tool with unprecedented spatial resolution and mechanical resolution to investigate the detailed structural parameters *in situ* under liquid conditions [22,23]. Besides, AFM-based dynamic force spectroscopy (AFM-DFS) allows characterizing the thermodynamic and kinetic parameters of the molecular assemblies [24,25]. A combination of *in situ* AFM imaging with DFS enables direct observation of the assembly process at the nanometer scale and deep understanding of the driving forces that control hierarchical self-assembly [26].

To date, several review articles on the synthesis, properties, and applications of peptoids have been published [2,27,28]. Herein, it is not our intention to give a comprehensive overview of the latest research progress in the field of peptoid-based nanomaterials, instead, we will give an overview of some recent achievements in the self-assembly of amphiphilic peptoids into hierarchically-structured materials, such as nanoribbons [10,13,16], nanomembranes [14,15] and nanotubes [8,9,12], highlighting our fundamental understandings of the peptoid assembly pathway and mechanisms using *in situ* AFM and AFM-DFS techniques. We believe some views and insights from this review will benefit materials scientists for the future design and development of peptoid-based biomimetic na-

nomaterials with desired structures and properties.

SURFACE-TEMPLATED PEPTOID SELF-ASSEMBLY

Inspired by self-assemblies in nature, proteins and peptides have been frequently used as building blocks to assemble functional materials at solid surfaces, such as materials for biocompatible implants and tissue regeneration. However, due to the complexity of protein and peptide folding, the prediction of protein and peptide assembly represents a formidable challenge, and thus the rational design and controlled assembly of proteins or peptides on surfaces remain underdeveloped. Peptoids, due to their lack of backbone hydrogen bonds, enable the tuning of peptoid-peptoid and peptoid-surface interactions exclusively through side-chain chemistry. The exploitation of peptoid side-chain interactions for the β -sheet-like assembly was first reported by Nam *et al.* [29] in 2010, where they used charged hydrophilic side chains that form electrostatic interactions and built complementary sequences by alternating them with the hydrophobic side chains. The assembly of these peptoids into nanosheets was made possible through an interface-assisted process of monolayer compression [29–31]. Zuckermann and colleagues [32,33] later used this design principle to develop self-complementary sequences that assembled into nanosheets. A similar strategy was also used to design 30-mer amphiphilic peptoids that assembled into homochiral superhelices [34]. Inspired by

these studies, Chen *et al.* [16,35] recently designed similar peptoid sequences with *N*-[2-(4-chlorophenyl)ethyl]glycine ($N_{4-Cl}pe$) hydrophobic sidechains and used Ca^{2+} -carboxylate interactions to build β -sheet-like inter-peptoid interactions for mica-templated peptoid self-assembly.

Mica-templated formation of hexagonally patterned networks of nanoribbons

Design of peptoid and self-assembly into nanoribbons

In specific, 12-mer peptoid (Pep-1) with alternating polar *N*-(2-carboxyethyl)glycines (Nce) and nonpolar $N_{4-Cl}pe$ side chains (Fig. 2a) were synthesized and self-assembled

into hexagonally patterned networks of nanoribbons on mica surfaces. When this peptoid was dissolved in Tris buffer at pH 8.0, the Nce groups became fully deprotonated and could coordinate with Ca^{2+} cations in terms of inter-peptoid and peptoid-mica interactions while the aromatic $N_{4-Cl}pe$ side chains offered hydrophobic interactions, thus driving the formation of hexagonally patterned nanoribbons on mica surfaces.

The mechanism of mica-templated nanoribbon formation

To investigate the detailed assembly pathways and mechanism of this mica-templated peptoid self-assembly system [16,35], *in situ* AFM imaging and AFM-DFS were

Figure 2 (a) Structure of Pep-1 and a proposed model showing the multilayered peptoid ribbons connected through Ca^{2+} -carboxylate coordination bonds and hydrophobic interactions (nitrogen, blue; oxygen, red; chloride, cyan; calcium cation, purple). (b) Time dependent *in situ* AFM images showing Pep-1- Ca^{2+} complexes assembly pathway on mica surface, in which Pep-1- Ca^{2+} nanoparticles were directly transformed into hexagonally patterned nanoribbons. The inset of each image is a 2D Fourier transform showing the six-fold symmetry. (c) Dynamic force spectra of Pep-1-modified AFM tips with bare mica or preassembled Pep-1. Fitting the mean rupture forces between Pep-1 and bare mica (top curve) or between Pep-1 and preassembled Pep-1 on mica (bottom curve) yields the single-molecule binding free energy. Reprinted with permission from Ref. [16], Copyright 2016, American Chemical Society.

used. Specifically, Pep-1- CaCl_2 solution was injected into the AFM fluid cell, and the obtained *in situ* AFM results showed that the Pep-1- Ca^{2+} solution initially presented as discrete nanoparticles on the mica surface. As time continued, these nanoparticles transformed into oriented, nanorod-like structures (Fig. 2b). The two-dimensional (2D) Fourier transform of the time-dependent *in situ* AFM images showed that all resulting nanoribbons were packed hexagonally after 186-minute incubation and formed a highly ordered network structure. Besides, only discrete nanoparticles of Pep-1- Ca^{2+} complexes were observed without introducing mica surfaces, indicating that the peptoid-mica interaction is critical for forming this ordered network structure. To better understand the roles of peptoid-mica and peptoid-peptoid interactions, AFM-DFS was used to acquire the force-distance curves and obtain the single-molecule binding free energy of peptoid-mica and peptoid-peptoid. The binding free energy for peptoid-mica [$\Delta G_{(\text{pep-mica})}$] was $-17.7 \pm 1.5 k_B T$ and

the peptoid-peptoid binding free energy [$\Delta G_{(\text{pep-pep})}$] was $-7.8 \pm 0.4 k_B T$ (Fig. 2c), showing that peptoid bound to mica surface much stronger than preassembled peptoid nanoribbons. These DFS results further demonstrated that the peptoid-mica interaction was sufficiently strong to drive the transformation of discrete nanoparticles to hexagonally patterned nanoribbons.

Because peptoid assembly is determined by various driving forces (e.g., hydrophobic interactions, cations), a modification of self-assembling peptoids with different heads, or tails can significantly influence peptoid assembly pathways. For example, when a hydrophobic tail was attached at the end of Pep-1 to synthesize Pep-2 (Fig. 3a), *in situ* AFM was used to directly monitor the whole assembly process of Pep-1 and Pep-2, from the initial state mixing both sequences with the CaCl_2 solution to the formation of the patterned nanoribbon networks. *In situ* AFM results showed that the peptoid assembly pathways are sequence dependent [35], and the attachment of hy-

Figure 3 (a) Structures of Pep-1 and Pep-2. (b) *In situ* AFM imaging of Pep-2 (left) and Pep-1 (right) assembly process to form porous networks. (c) Proposed model for Pep-2 assembly process at early (left) and late (right) stages showing the effect of hydrophobic conjugate on the propensity for peptoid aggregation. (d, e) Kinetics of crystal formation. Nuclei number density *versus* time for Pep-2 (d) and Pep-1 (e). Reprinted with permission from Ref. [35], Copyright 2017, Nature Publishing Group.

drophobic tail to the end of Pep-1 resulted in the switching of the assembly pathway from a single-step to a two-step process. Specifically, Pep-1 exhibited a simple single-step assembly pathway, and only 3-nm-high particles were observed (Fig. 3b and c). Pep-2 showed an interesting two-step crystallization pathway which began with 5-Å-high clusters. These clusters were then converted into 3-nm-high nuclei particles before ultimately forming a three-dimensional porous network. The kinetics of peptoid assembly were also determined in different concentrations of peptoids and Ca^{2+} . For Pep-1, the formation rates decreased with time in accordance with a simple Langmuir dependence and were dependent on both peptoid and Ca^{2+} concentrations (Fig. 3d). Within contrast to Pep-1, at relatively high Pep-2 concentrations, the formation rate of 3-nm-high particles transitioned from a low to high value after a time τ that decreased with the increase of both peptoid concentration and Ca^{2+} concentration. However, at low Pep-2 concentrations, no such transition was observed, and the formation rates remained low (Fig. 3e). After the transition, the formation rate increased with Pep-2 concentration but was independent of Ca^{2+} concentrations. Pep1-Pep1 and Pep1-mica binding free energies under different Ca^{2+} concentrations were determined by AFM-DFS. These AFM-DFS results showed the opposite dependence of Ca^{2+} concentration: an increase of Ca^{2+} concentration led to a decreased binding of Pep-1 to the surface, providing a reasonable explanation for peptoid crystallization kinetics on Ca^{2+} concentration. These findings based on *in situ* AFM and AFM-DFS shed new light on crystallization mechanisms and have implications for the design of self-assembled peptoid materials.

SiO₂-templated self-assembly of cylindrical micelles

Developing synthetic systems for supramolecular self-assembly of hierarchical structures to achieve hierarchy by functionalizing precise atomic locations and incorporating complex sequence information into current synthetic platforms is limited. In a recent work, a significant challenge in the self-assembly of peptoids with hierarchical structures was reported by using one previously reported lipid-like peptoid with a site-specific functionalization.

Design of peptoid and self-assembly of cylindrical micelles

Recently, Jin *et al.* [14,15,36] reported that the lipid-like peptoid (Nce)₆(N_{4-Cl}pe)₆ (Pep-3) self-assembled into highly stable and self-repairable membrane-mimetic 2D nanomaterials. The incorporation of β -cyclodextrin (CD)

headgroup into the hydrophilic domain brought the opportunity to access various self-assembled structures [37]. Specifically, we investigated the Pep-3-CD (Fig. 4a) assembly on a few different substrates (such as quartz with uncharged surface and mica with a negatively charged surface) at pH values in the range of 2.1 to 7.5 and Ca^{2+} concentrations in the range of 5 to 50 mmol L⁻¹. Pep-3-CD self-assembled into various hierarchical structures from 1D worm-like or cylindrical micelles to 2D membranes to 3D intertwined ribbons dependent on the substrate and solution conditions [10].

The mechanism of SiO₂-templated formation of cylindrical micelles

In situ AFM was used to monitor the pathways and kinetics of Pep-3-CD assembly. The Pep-3-CD assembly on SiO₂ surface exhibited a worm-like micelle structure without a particular orientation. This is mainly due to the introduction of CD group in the peptoid hydrophilic domain, resulting in increased electrostatic repulsion between the hydrophilic blocks. A proposed model shows that Pep-3-CD monomers are orderly packed with the CD headgroups pointing outward and the hydrophobic N_{4-Cl}pe tails organizing in the core (Fig. 4a). This model was confirmed by the Pep-3-CD assemblies at different pH conditions. While Pep-3-CD assembled into cylindrical micelles on quartz surfaces at pH values between 2.1 and 2.8, Pep-3-CD monomers remained in small particles at higher pH because the Nce residues were deprotonated and the electrostatic repulsion was increased. At lower pH, the Pep-3-CD solubility significantly decreased, resulting in the formation of large aggregates. We tracked the formation process of Pep-3-CD cylindrical micelles on SiO₂, and *in situ* AFM results showed a two-step self-assembly process, starting with 3–4 nm spheroidal precursors and then aggregated and transformed into larger worm-like micelles 6–7 nm in size. Finally, these micelles continued to grow until forming the final cylindrical micelles (Fig. 4b and d). A remarkable fact is the Pep-3-CD assembly process on mica is quite different from that on the SiO₂ surface. *In situ* AFM study of Pep-3-CD assembly on mica showed that Pep-3-CD firstly formed highly convoluted worm-like micelles, then the coverage of Pep-3-CD micelles continued to increase and eventually completely covered the surface to form 2D films with a thickness of ~4.5 nm which is similar to the thickness of 2D membrane (Fig. 4c and d). When Ca^{2+} was introduced, the 3D intertwined ribbon was formed on top of the 2D film, demonstrating that Ca^{2+} served as a bridge to facilitate the interactions between CD head-

Figure 4 (a) Structures of Pep-3-CD. (b) *In situ* AFM time series showing two-step assembly of Pep-3-CD cylindrical micelles and (f) kinetics of spheroidal precursors (black) transformed into cylindrical micelles (blue) on quartz. (c) *In situ* AFM time series images showing Pep-3-CD assembly into 2D films on mica at pH 2.8. (d) AFM images showing Pep-3-CD self-assembled into 1D cylindrical micelles on quartz surface (i), 2D films on mica surface (ii) and 3D intertwined ribbons with 5 mmol L⁻¹ CaCl₂ on mica surface (iii). (e) Illustration of the mechanisms of Pep-3-CD self-assembly into cylindrical micelles, 2D films and 3D intertwined ribbons. Reprinted with permission from Ref. [10], Copyright 2019, Wiley.

groups and carboxylate groups and drive the assembly of Pep-3-CD cylindrical micelles into the final 3D intertwined ribbons (Fig. 4d). *In situ* AFM results further revealed the assembly kinetics of Pep-3-CD film formation on mica. The rate of coverage is dependent on the peptoid concentration, solution pH and Ca²⁺ concentration. Higher peptoid concentration levels increased the rate of coverage, while an increase of solution pH slowed the kinetics of assembly. The introduction of low Ca²⁺ concentration facilitated the assembly, but a high concentration of Ca²⁺ had strong binding to the Pep-CD monomer, which delayed the formation of the worm-like micelle precursors and reduced the rate of coverage. These *in-situ* AFM results described a picture of Pep-3-CD self-assembly into cylindrical micelles, 2D films and 3D intertwined ribbons (Fig. 4e) and provided insight of mechanism and kinetics of peptoid self-assembly into hierarchical structures.

SELF-ASSEMBLY OF PEPTOIDS INTO CRYSTALLINE NANOMATERIALS IN SOLUTION

Peptoid-based crystalline nanomembrane

Owing to their unique structures and intrinsic physical

and chemical properties, 2D nanomaterials have recently attracted intense interest as promising materials in a broad range of applications in photovoltaics, electronics, chemical sensing, catalysis, water filtration and also in biomedical areas [7,37–39]. Molecular self-assembly offers a unique ‘bottom-up’ approach to build up well-defined functional 2D nanostructures [36]. Jin *et al.* [14,15] recently reported the first example of self-assembled highly stable and self-repairing membrane-mimetic 2D nanomaterials by designing lipid-like sequence-defined peptoids with aromatic hydrophobic sidechains.

Design of peptoid and self-assembly of nanomembrane

Specifically, Jin *et al.* [14] designed amphiphilic lipid-like peptoids (Pep-3) consisting of one block with six polar Nce residues, and another with six nonpolar N_{4-Cipe} residues (Fig. 5a). The self-assembly of Pep-3 was induced by an evaporation-induced crystallization process, and *ex situ* AFM data showed that gel-like materials formed after the crystallization process, which contained a large amount of 2D nanosheets with straight edges (Fig. 5a) and a thickness comparable to the cell membranes. XRD data showed that these peptoid-based nanomembranes were highly crystalline. These peptoid membranes were highly

Figure 5 (a) Structure of Pep-3 and the scheme showing its assembly into membrane-mimetic 2D nanomaterials, and an AFM image of Pep-3 nanomembranes with straight edges. (b) TEM images showing the elongated nanoribbon intermediates before the nanomembrane formation. (c) Schematic illustration of the AFM mechanical manipulation and peptoid membrane repair process. (d) *In situ* AFM images showing the self-repair process of peptoid membranes on mica surface. Reprinted with permission from Ref. [15], Copyright 2016, Wiley.

stable and exhibited a salt-induced thickness change.

Peptoid nanomembrane formation mechanism

Time-dependent transmission electron microscopy (TEM) and XRD characterizations were utilized to investigate the nanomembrane formation process, these results showed that the amphiphilic peptoids started as amorphous spherical particles in solution. These particles were then transformed into elongated nanoribbons before they were finally converted into highly crystalline nanomembranes (Fig. 5b). Based on AFM, TEM, and XRD results, a model showing the packing of peptoids to form 2D nanomembrane was proposed, in which we reasoned that peptoid membranes were stabilized by the enhanced hydrophobic interactions, such as π - π interaction as a result of ordered packing of hydrophobic N₄-ClP side chains.

Self-repair of peptoid nanomembrane

Peptoid nanomembranes exhibit a variety of unique properties similar to cell membranes, such as the ability to self-repair. To demonstrate that, a widely-used AFM lithography-based mechanical scratch method [40] was used to create defects along both x - and y -directions of the peptoid nanomembrane (Fig. 5c). After introducing the pre-assembled peptoid solution, *in situ* AFM was then used to monitor the repairing process in real time. AFM results showed that the defects were completely repaired

after 40 min, and the repair rate along the x -direction was faster than that along the y -direction (Fig. 5d). To better understand such a self-repairing property, three different substrates, atomically flat mica which is hydrophilic and negatively charged, mica modified by aminopropyl silatrane (APS) which is positively charged, and highly ordered pyrolytic graphite which is hydrophobic, were used. *In situ* AFM-based repairing results showed that the peptoid nanomembranes self-repaired on both mica and APS-mica surfaces, and even without the surface support. The detailed kinetic data generated from *in situ* AFM studies showed that the self-repair is a two-stage anisotropic process, in which the repair during the first stage is an order of magnitude slower than that in the second stage. Moreover, when a peptoid with the same hydrophobic block and different hydrophilic tails was introduced to the defect-containing nanomembranes, the repair was also observed and the added peptoids formed a new pattern in the nanoscale defect area, further demonstrating the self-repair ability to create nanoscale patterns of functional groups within peptoid-based nanomembranes. *In situ* AFM results further showed that the peptoid concentration or pH of the repairing solution has a significant impact on the membrane repairing rates. As indicated by these repairing results, AFM was demonstrated as a suitable tool to create nanoscale patterns for the nanomembranes, and it offered a direct observation of self-repair process and nanoscale patterning of

functional groups in real time, providing us with new insights into the understanding of membrane self-assembly and self-repair mechanisms.

Peptoid-based crystalline nanotube

Hollow tubular nanostructures offer a variety of applications in chemistry, biochemistry and materials science as specific molecular reaction vessels [41], screening activities of membrane channels and the nanoscale fluidic transport systems [42]. However, highly stable and programmable organic nanotubes (ONT) are very rare [43,44]. Recently, by changing the hydrophobic domains of self-assembling peptoids, we designed and synthesized a new class of highly tunable and dynamic stiff nanotubes formed through a unique rolling-up and closure of the nanosheet mechanism [12].

Design of peptoid and self-assembly of nanotubes

When the hydrophobic block ($N_4\text{-Clpe}$)₆ residues of membrane-forming peptoid were replaced with five to seven residues of *N*-[(4-bromophenyl)methyl]glycines (Nbpm)₆ (Pep-4, Fig. 6a), the resulting peptoids self-assembled into single-walled peptoid nanotubes (SW-PNTs) under a similar evaporation-induced crystallization process. AFM, TEM and XRD studies showed that the nanotubes were highly crystalline and stable, and they survived the exposure to high temperature (60°C) and organic solvents (CH₃CN and EtOH).

Peptoid nanotube formation mechanism

In order to obtain a better view of the intermediate state

of PNT formation, the crystallization rate was slowed down by reducing the initial peptoid concentration from 5.0 to 0.5 mmol L⁻¹. Time-dependent TEM results showed an assembly process beginning with homogeneous nanoparticles with a diameter of 40 nm. As the solvent evaporated, these particles crystallized to form nanoribbons. After 24 h, the nanoribbons rolled up from both edges and closed up to completely form nanotubes in 72 h (Fig. 6b). Based on that, we reasoned that the PNTs were formed through a rolling-up, folding and closure of nanoribbon mechanism through hydrophobic interactions between the aromatic residues. We further demonstrated the tuning of PNT diameter and wall thickness by changing the number of Nbpm groups. Although *ex-situ* TEM offers a clear picture of the PNT formation process, the detailed evolution of these intermediates remains unclear. We are currently working on using *in situ* AFM to investigate the self-assembly pathways and mechanisms of the PNT formation.

Dynamic feature of peptoid-based nanotubes

Because liquid-cell AFM enables the direct monitoring of the changes and dynamics of soft matters in a liquid environment, and tube-forming peptoids exhibit pH-dependent deprotonation and protonation, liquid-cell AFM was also used to monitor the dynamic behaviors of PNTs in response to solution pH [12]. Specifically, when the solution pH changed from 8.0 to 3.6, and then to 8.0 again, AFM results showed that the PNT height decreased from 24.3 to 13.1 nm as solution pH decreased, and then returned to the original height of 24.3 nm when the so-

Figure 6 (a) Structure of Pep-4 and a proposed model showing the assembly of Pep-4 into SW-PNTs. (b) Time-dependent TEM images show the assembly process of Pep-PNTs. The Pep-4 assembled into a mixture of nanospheres, then converted into nanosheets with partially rolled up edges, and these sheets further fold and closure to form nanotubes after 72 h. (c) Liquid cell AFM data showing the pH-triggered PNT height changes. (d) AFM-based measurement of the PNT mechanical property, in which the PNTs underwent an intense deformation after an indentation induced by the AFM tip. Reprinted with permission from Ref. [12], Copyright 2018, Nature Publishing Group.

lution pH was increased back to 8.0 (Fig. 6c). Such pH-responsive dynamic behavior was fully reversible. When the solution pH was above 3.3, the carboxyl group started to deprotonate. Therefore, the electrostatic repulsion was believed to play a key role in the pH-dependent dynamic structural changes of the PNTs.

AFM-based measurement of PNT mechanic property

The outstanding mechanical property of nanotubes is crucial for their applications. AFM-based nanoindentation is an effective tool to measure the mechanical parameter of nanomaterials. Through the analysis of tip indentation into the sample δ and the cantilever deflection Δd , which can be converted to force F ($F=k \times \Delta d$, k is the spring constant of AFM cantilever), the mechanical properties of the nanomaterial sample (such as Young's modulus) then can be obtained by force-distance curves. Peak force quantitative nanomechanics (PFQNM) is a new AFM technique for measuring Young's modulus of materials [45] at each contact point in real-time with high spatial resolution and surface sensitivity [46]. This technique was recently used to directly probe the PNT's mechanical property in liquid conditions (Fig. 6d). When the applied force increased to 37 nN, no damages of the tubular structure were observed for the PNTs assembled from peptoids with six and seven Nbpm groups, and these PNTs returned to their original shapes once the applied forces were removed. In contrast, PNTs assembled from peptoids with five Nbpm groups collapsed when the applied force was over 30 nN. These AFM-based nanoindentation results showed that PNTs exhibited Young's modulus in the range of 13 to 17 GPa,

which is comparable to the stiffest of peptide nanotubes in previous reports [47].

APPLICATIONS OF NANOMATERIALS BASED ON PEPTOID ASSEMBLY

Self-assembly plays a crucial role in constructing various nanostructures that have potential applications in nanoscience and nanotechnology [48–51]. Self-assembling peptoids provide various building blocks for the fabrication of functional materials with well-ordered nanostructures. Different nanostructures were designed for specific applications. For example, hexagonally patterned nanoribbons offer unique advantages for biomimetic coating [16], nanomembranes could be used for tissue engineering [14] and nanotubes are suitable for biosensor fabrication [12].

As templates to control the formation and assembly of catalytic materials

Metal nanoparticles have high catalytic activities due to their large surface-to-volume ratio. One major disadvantage is that the metal nanoparticles readily aggregate and thus lose their catalytic activity. Self-assembled peptoid structures were recently used as scaffolds to control the formation of catalytic active Pd nanomaterials [52] (Fig. 7a). When Pep-1 was conjugated with one 6-aminohexanoyl (Aha) at the N-terminus to synthesize Pep_{pd-2}, this peptoid formed crystalline 2D nanomembranes at pH 2.1 and less crystalline nanofibers at pH 5.6 [52]. To use these nanomembranes and nanofibers as scaffolds for controlling the formation of Pd nanomaterials, the stock solution of peptoid nanomem-

Figure 7 (a) The scheme showing the synthesis of Pd⁰ nanomaterials templated by peptoid assemblies with two different morphologies. (b) Catalytic analysis of the resulting Pd nanomaterials showing that fiber-templated Pd⁰ nanomaterials exhibited significant enhancement for olefin hydrogenation over membrane-templated Pd⁰ materials. Reprinted with permission from Ref. [52], Copyright 2018, Royal Society of Chemistry.

branes or nanofibers was incubated with potassium tetrachloropalladate (II) (K_2PdCl_4) which was reduced by sodium borohydride ($NaBH_4$) to form the Pd nanomaterials. The catalytic activities of the prepared Pd nanomaterials were evaluated for the olefin hydrogenation reaction. These catalysis results showed that Pd nanomaterials templated by Pep_{Pd-2} nanofibers exhibited higher catalytic activity than the membrane-templated counterparts (Fig. 7b), demonstrating that the morphology of peptoid assembly can significantly influence the properties of templated catalytic nanomaterials.

As cargoes for efficient cytosolic delivery

Efficient delivery of macromolecule drugs (e.g., nucleic acids, peptides, and proteins) is essential for many genome therapeutic applications. Although non-viral strate-

gies including lipid- [53], nanoparticle- [54] and peptide-based methods [55] have shown the improved cellular uptake of therapeutic macromolecule drugs, these approaches are usually problematic, such as high cytotoxicity and low endosomal release efficiency [56]. To address these challenges, we recently designed and synthesized crystalline nanoflower-like nanoparticles assembled from fluorinated peptoids ($Pep-5$) [17] (Fig. 8a). The cytosolic delivery efficiency of these fluorinated peptoid crystals (FPC) was investigated upon their cellular uptake, toxicity, and cargo release pathway. The cytosolic delivery results showed that these FPCs enabled a nearly 80% of cytosol delivery rate (Fig. 8b), and a rapid escape from early endosomes without causing pores (Fig. 8d) and minimal cytotoxicity (Fig. 8c). Furthermore, we demonstrated the application of these FPCs as efficient nano-

Figure 8 (a) Structure of $Pep-5$ and the scheme showing self-assembly of fluorinated peptoids into crystalline nanoflowers. (b) Cell incubated with FPC (100 nmol L^{-1}) for 1 h. (c) Cell viability of H1299 cells after incubating with different concentrations of FPC. (d) Characterization of the localization of the FPCs in the cell. Reprinted with permission from Ref. [17], Copyright 2018, Wiley.

carriers to deliver model macromolecule drugs, such as ssDNA and mRNA. These FPCs led to a successful intracellular gene expression with a transfection rate as high as around 71% (Fig. 8d, right image).

As functional nanomaterials for other applications

To demonstrate the high tunability of peptoid-based nanomaterials, a variety of functional groups were attached at the N-terminus of either membrane- or tube-forming peptoids. AFM results showed these modifications, including conjugation of self-assembling peptoids with *N*-(1-pyrenemethyl)glycines (Npyr) or CD groups, did not disturb the formation of intact nanomembranes (Fig. 9a, left image) [14] and nanotubes (Fig. 9a, right image) [12]. The high tunability of these peptoid-based nanomaterials enabled various applications. For example, When Arg-Gly-Asp (RGD) peptides were introduced as functional groups at the N-terminus of tube-forming peptoids, the self-assembled RGD-containing PNTs exhibited an enhanced cellular adhesion and an increased cellular uptake (Fig. 9b). The second example is to precisely engineer PNTs for water decontamination [12]. Due to the large surface area and high porosity of the peptoid nanotubes, functionalized PNTs assembled from CD-conjugated peptoids were able to remove azo-containing dye contaminants from contaminated water, as shown by UV-Vis analysis, demonstrating the potential use of peptoid-based nanomaterials for water purification (Fig. 9c) [12]. Based on the hierarchical self-assembly of Pep-3-CD, the

hydrophobic core of these peptoid nanofibers was used as a “host” to accommodate donor molecules (hydrophobic NBD) and the CD cages on the surface of Pep-3-CD cylindrical micelles were used to capture acceptor molecules (Rhodamine B). The synthesized Förster resonance energy transfer (FRET)-active peptoid nanomaterials exhibited a high energy transfer efficiency in aqueous solution (Fig. 9d) [10]. Because functional groups can be precisely incorporated as peptoid side chains, peptoid-based self-assemblies offer a new platform for developing hierarchically-structured biomimetic nanomaterials for applications. Because functional groups can be precisely incorporated as peptoid side chains, peptoid self-assembly offers a new platform for developing hierarchically structured functional nanomaterials. Therefore, besides those applications mentioned above, we expect a wide range of applications for this type of nanomaterials will be reported in the near future.

SUMMARY AND OUTLOOK

In this review, we have summarized a few recent examples of sequence-defined peptoids that self-assembled into hierarchically-structured nanomaterials, including self-repairable nanomembranes that mimic cell membranes, highly tunable and dynamic single-walled stiff nanotubes formed through a unique “rolling-up and closure of nanosheet” mechanism, and networks of hexagonally patterned nanoribbons templated by mica surfaces. During these hierarchical self-assembly processes,

Figure 9 (a) Incorporating functional groups into self-assembling peptoids did not inhibit the formation of nanomembranes (left) and nanotubes (right). (b) Fluorescence image showing that RGD-containing peptoid nanotubes (PNTs) exhibit a high cellular uptake. (c) CD-containing PNTs were used as adsorbents for the removal of azo-dyes from water. (d) The FRET system based on Pep-3-CD cylindrical micelles, NBD donors and RB acceptors. Fluorescence emission results show a high FRET efficiency of this system in aqueous solution. Reprinted with permission from Refs [12,14], Copyright 2016 and 2018, Nature Publishing Group, and Ref. [10], Copyright 2019, Wiley.

nanoscale characterizations, such as *in situ* AFM and AFM-DFS, offered critical information for investigating the peptoid assembly pathways and mechanisms, and the properties (e.g., membrane self-repair and mechanical property of PNTs) of the self-assembled nanomaterials. These findings highlight the importance of nanoscale characterizations for understanding peptoid assembly mechanisms and for achieving predictive materials synthesis through controlled peptoid self-assembly. However, there are still many critical issues that need to be addressed for achieving predictable peptoid self-assembly. For example, despite many recent studies showing that peptoid self-assembly has mainly relied on hydrophobic interactions, we still do not fully understand the influence of the polar domain of diblock-like peptoids during their self-assembly. While *in situ* AFM and AFM-DFS offer important insights by monitoring the peptoid assembly pathways and by illustrating the driving forces that determine the peptoid self-assembly, achieving the assembly of peptoid-based nanomaterials with predictable morphologies still faces significant challenges. On the other hand, while the applications of peptoid-based nanomaterials still remain at the infancy stage, the unique features of these nanomaterials, such as highly programmable composition and biocompatibility, open up a wide range of potential applications in sensing, catalysis, tissue regeneration, antimicrobial, and drug delivery.

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Conflict of interest The authors declare that they have no conflict of interest.

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synthesis of bio-inspired functional materials that rival those found in biology.

基于类肽等级自组装结构仿生纳米材料: 合成, 表征和应用

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摘要 类肽(或N-取代甘氨酸)具有合成效率高、化学稳定性强、抗酶水解及生物相容性好等优点, 是一类潜在的可自定义序列的生物仿生聚合物. 通过调节类肽的侧链化学, 可以精确地控制类肽序列并实现侧链的多样性. 基于以上类肽分子独特的优势, 在过去的几年里, 研究者设计和合成了大量的双亲性类肽作为基本的结构单元, 通过自下而上的分子自组装作用构建了结构可控和具有特定功能的仿生纳米材料. 本文从以下三个方面综述了我们在类肽自组装领域取得的一些成果. 首先, 概述了以云母和硅片为无机基底辅助类肽自组装. 将原子力显微镜原位成像技术和单分子力谱技术相结合, 可实时观察类肽分子在表面的组装过程并直接测定类肽分子与基底间的相互作用, 揭示基底表面对类肽自组装的影响. 其次, 介绍了类肽分子在溶液中自组装成纳米管和具有自修复功能的纳米薄膜. 最后, 综述了基于类肽自组装纳米材料的应用, 包括以类肽自组装纳米材料为催化模板构建纳米仿生催化剂和作为细胞内输送物质的载体等.