Supramolecular Biofunctional Materials

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Abstract:

This review discusses supramolecular biofunctional materials, a novel class of biomaterials formed by small molecules that are held together via noncovalent interactions. The complexity of biology and relevant biomedical problems not only inspire, but also demand effective molecular design for functional materials. Supramolecular biofunctional materials offer (almost) unlimited possibilities and opportunities to address challenging biomedical problems. Rational molecular design of supramolecular biofunctional materials exploit powerful and versatile noncovalent interactions, which offer many advantages, such as responsiveness, reversibility, tunability, biomimicry, modularity, predictability, and, most importantly, adaptiveness. In this review, besides elaborating on the merits of supramolecular biofunctional materials (mainly in the form of hydrogels and/or nanoscale assemblies) resulting from noncovalent interactions, we also discuss the advantages of small peptides as a prevalent molecular platform to generate a wide range of supramolecular biofunctional materials for the applications in drug delivery, tissue engineering, immunology, cancer therapy, fluorescent imaging, and stem cell regulation. This review aims to provide a brief synopsis of recent achievements at the intersection of supramolecular chemistry and biomedical science in hope of contributing to the multidisciplinary research on supramolecular biofunctional materials for a wide range of applications. We envision that supramolecular biofunctional materials will contribute to the development of new therapies that will ultimately lead to a paradigm shift for developing next generation biomaterials for medicine.

Keywords: supramolecular, hydrogels, noncovalent interaction, biofunctional, materials, applications

1. Introduction

In this review, supramolecular biofunctional materials refer to the biomaterials that are held together mainly by noncovalent intermolecular interactions among small molecules. Being formed via molecular self-assembly (usually in water) and possessing ordered superstructures, supramolecular biofunctional materials have found increased numbers of biomedical applications.[1] Moreover, when molecular self-assembly integrates with cellular events (e.g., enzymatic reactions, ligand-receptor binding, or electron transfer), it provides a bioinspired, yet fundamentally new path to generate molecular processes in cell milieu for simultaneously interacting with multiple protein targets,[2] cells, and even organs. Because of their potentials, over the last two decades, supramolecular biofunctional materials and related molecular processes have received intensive research attentions. For example, Stupp et al. used the pH-controlled self-assembly of peptide amphiphiles (PAs) to develop a group of supramolecular fibrous scaffolds with promising biological applications, like directing formation of hydroxyapatite *in vitro* to mimic biomineralization[3] or inducing rapid differentiation of progenitor cells into neurons while discouraging the development of astrocytes in cell assays.[4] Using supramolecular hydrogels as a platform for incorporating different artificial receptors, Hamachi and coworkers developed a class of semi-wet sensor chips, which recognized a variety of chemicals such as cations, saccharides, anionic fluorescent dyes, phosphate derivatives, polyamines, and polyols.[5-10] They also reported a smart multicomponent system consisting of self-sorted supramolecular nanofibers.[11] Van Esch et al. demonstrated that the self-assembly of molecular building blocks driven by a chemical fuel led to the transient formation of an active material, in which reaction kinetics and fuel levels determined the properties such as lifetime, stiffness, and self-regeneration capability of the material.[12] Ulijn et al. expanded the development of supramolecular materials based on Fmoc-capped small molecules and used computational tools to screen the self-assembling propensity of more than 8,000 possible tripeptides in water.[13] Taking advantages of the chemical structures and the self-assembling ability of peptides, Cui et al. developed a novel class of drug amphiphiles (one-component nanomedicine) by covalently linking one or more anticancer drugs to a rationally chosen/designed peptide backbone through a biodegradable linker to achieve precise control of

1 drug loading and physicochemical properties of nanomedicines.[14-17] Yang et al. developed a promising
2 vaccine adjuvant made of self-assembling peptide hydrogels. Compared with the clinically used alum
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adjuvant, the L- and D-peptide hydrogels increased the IgG production against the proteins (e.g.,
ovalbumin (OVA)), [18] and such D-peptide hydrogels are being explored as an adjuvant for HIV vaccine
based on DNA.[19] Parallel to these studies, a new and versatile progress, firstly reported in 2004, is to
integrate enzymatic reactions and self-assembly (i.e., enzyme-instructed self-assembly (EISA)) to build
up supramolecular biofunctional materials for the applications in biomedicine such as cancer therapy,[2,
20-24] infectious diseases,[25] and drug delivery[26, 27]. Moreover, besides the exploration of the self-
assembling drug molecules,[28, 29] the development of D-peptides, which usually resist endogenous
proteases and act as novel biofunctional materials, has generated fruitful results and opened a new
direction for the development of biomaterials.[21, 30-33] All these research activities have generated
considerable exciting and promising results, which warrant a brief review of supramolecular biofunctional
materials.

We arrange this review in the following way: we first compare the supramolecular hydrogels with
traditional polymeric hydrogels to highlight the merits of supramolecular interactions (i.e., noncovalent
interactions) and orders of molecular arrangement in the context of hydrogels or nanostructures. After
introducing the basic building blocks of supramolecular hydrogels (amino acids, nucleic acid
architectures, and saccharides) by discussing their unique properties, we focus on the potential biological
and medical applications of these supramolecular biomaterials and relevant molecular processes, followed
by the perspectives and outlooks of this field. In this review, we intend to provide a snapshot of recent
achievements at the intersection of supramolecular chemistry and biomedical science in hope of
contributing to the multidisciplinary research on the development of supramolecular biofunctional
materials for a wide range of applications. Due to the fast development of the field, the choice of
examples for this review inevitably is arbitrary and incomplete. For more comprehensive discussions,
interested readers can refer to other authoritative review and research articles.[34-45]

2. Supramolecular Hydrogels as Biofunctional Materials.

2.1. Polymeric Hydrogels and Supramolecular Hydrogels

Sharing certain characteristics with traditional polymeric biomaterials, the most explored and prominent type of supramolecular biofunctional materials are supramolecular hydrogels.[41] Thus, it is appropriate to understand supramolecular biofunctional materials by comparing conventional polymeric hydrogels[46] to supramolecular hydrogels.[1] As illustrated in Figure 1A, conventional polymeric hydrogels, composed of 3D elastic networks formed by macromolecules (either synthetic or biological polymers), are able to hold or immobilize substantial amount of water through surface tension and capillary forces. Depending on the nature of crosslinking, polymeric hydrogels can be further classified to type I (covalent crosslinking) and type II (noncovalent crosslinking).[47] Composed of polymer chains interconnected to each other by covalent bond, type I polymeric hydrogels are generally permanent and irreversible, but may still have certain dynamic freedom. Being crosslinked by covalent bonds, this type of hydrogels is brittle and unable to re-build (i.e., self-healing) after the network is broken. Since the formation of type I polymeric hydrogels usually involves chemical reactions that require metal catalysts, initiators, or UV, the processes for generating the hydrogels are hardly biocompatible. Having limited dynamic freedom, type I hydrogels usually are insensitive to external stimuli. All these disadvantages largely limit the applications of type I polymeric hydrogels in biomedicine. In contrast, type II polymeric hydrogels, in which the crosslinking relies on transient noncovalent interactions between the polymer chains (referred as “gellants”), usually are weaker and susceptible to external disturbs/stimuli. Nevertheless, this dynamically transient nature can be an advantage as it makes the formation of the polymeric hydrogels reversible (or “self-healing”).[48] Compared to type I polymeric hydrogels, type II polymeric hydrogels find applications more readily in biological research due their two desirable characteristics—“self-healing” and responsiveness derived from the transient nature of noncovalent interactions. Since 1955 when Czech chemists Drahoslav Lím and Otto Wichterle developed the first polymeric hydrogel (poly-2-hydroxyethyl methacrylate) for potential biomedical applications,[49]

polymeric hydrogels have independently defined a remarkable research area and constituted a large group of biomaterials. Having been used in a variety of biological disciplines (e.g., superabsorbent materials,[50] drug storage and delivery in biomedicine,[51] scaffolds to reconstitute artificial extra-cellular matrix,[52, 53] contact lenses,[49] and tissue engineering,[34, 54, 55]), these polymeric hydrogels are still expanding the scope of their applications.

In the past two decades, the successful development of polymeric hydrogels and low-molecular-weight organogelators have stimulated the emergence and thriving of small molecular hydrogelators (being named to correspond to gellants in polymeric hydrogels). The self-assembly of hydrogelators in water results in supramolecular “chains”, nanofibers, or other nanoscale assemblies as the 3D networks of the hydrogels to immobilize water. The resulting hydrogels thus are named as supramolecular hydrogels or molecular hydrogels.[1, 41] Resulted from self-assembly of small building molecules (e.g., peptide derivatives) via noncovalent interactions, the supramolecular “chain”, nanofibers, or other nanoscale assemblies usually possess orders that, however, are transient and reversible. Unlike the crosslinks in polymeric hydrogels, the crosslinks among nanofibers of supramolecular hydrogels are noncovalent (Figure 1B). Thus, the supramolecular hydrogels are often thermally reversible or easily respond to certain stimuli. However, the major limitation of supramolecular hydrogels is their mechanical strength.

We compare type I and type II polymeric hydrogels with supramolecular hydrogels on several important characteristics that are important for biological and biomedical applications in Table 1.

Table 1. Comparison between two different types of hydrogels – polymeric hydrogels and supramolecular hydrogels.

	polymeric hydrogels		supramolecular hydrogels
	type I (covalent crosslink)	type II (noncovalent crosslink)	
mechanical strength	extremely strong	relatively strong	weak
tunability	poor	good	excellent
responsiveness to biological stimuli	poor	medium to excellent	excellent
biocompatibility	not good	medium to excellent	excellent
mimicry of extracellular matrices (e.g., signal presentation, network topology)	poor	excellent	good to excellent
reversibility	brittle and irreversible	less reversible	reversible

degradation/clearance	poor	medium to excellent	excellent
incorporation of multiple biological functionalities	poor	excellent	excellent

Figure 1. Schematic illustration of (A) covalent crosslinking of functional polymer chains/precursors to form covalent polymeric hydrogels (type I) and noncovalent crosslinking of functional polymer chains/precursors to form polymeric hydrogels (type II) and (B) small molecules to form supramolecular nanofibers via noncovalent interactions and self-assembly, which further entangle with each other to form the network of supramolecular hydrogels.

2.2. Merits of Supramolecular Biofunctional Materials

Due to their noncovalent nature, supramolecular hydrogels, as a valuable addition to polymeric hydrogels, contribute a large portion of functional biomaterials, and are being extensively explored today. Molecular self-assembly is a process in which molecules adopt a defined arrangement spontaneously and is ubiquitous in nature. As a key concept in supramolecular chemistry, self-assembly is primarily based on noncovalent interactions (e.g., van der Waals force, hydrogen bonding, electrostatic interaction, and hydrophobic interactions) between molecules. Among these noncovalent interactions, hydrophobic interactions, as the result of a nonpolar group in water and acting over long range, are uniquely important for the self-assembly of hydrogelators in water. The self-assembly of hydrogelators to form nanofibers as

networks/matrix of supramolecular hydrogels, which shares many characteristics of certain biological processes in nature (e.g., the dynamics of cytoskeletons such as actin and tubulin), is a fundamental molecular process, typically noncovalent, three-dimensional, structural, dynamic, adaptive, responsive, and organized. This kind of molecular process can easily interact with, interfere, or even mimic the cellular events in various biological systems, thus promising various fundamentally new biological and biomedical applications (e.g., mimicking the function of glycoproteins[56, 57]). The extensive noncovalent nature of molecular self-assembly renders supramolecular biofunctional materials with several unique merits as highlighted below.

2.2.1. Responsiveness

Due to the dynamic nature of noncovalent interactions, the biofunctional materials based on supramolecular hydrogels exhibit stimuli responsiveness to various physical, chemical, or biological stimuli, like temperature,[58] ultrasound,[59, 60] light,[61] magnetic field,[62] pH,[63-65] ionic strength,[66, 67] redox agent,[68] ligand-receptor interactions, [69-72], enzymes (Figure 2A),[25, 73-80] and many other reaction agents and catalysts.[81-83] Utilizing this property, one is able to not only control the self-assembly/disassembly process as *in situ* response to external stimuli for desired biomedical applications, but also to generate chemical, physical, and biological sensors for the detection of various external stimuli.

Figure 2. (A) Gel-sol or sol-gel transformation is responsive to different physical, chemical, biological stimuli. (B) Illustration of the enzyme-instructed self-assembly (EISA) of small molecules to form a supramolecular hydrogel. (Adapted from © 2004 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim with permission.[84])

One of the most common ways to control the reversible self-assembly is temperature, and a large amount of supramolecular hydrogels undergo a sol-gel or gel-sol transition upon the change of temperature. With the change of temperature (usually increasing and then decreasing), the noncovalent interactions between molecules likely undergo rearrangement to favor more ordered molecular assemblies that are thermodynamically more stable. Ultrasound, an efficient approach to promote dissolution or dispersion of molecules (usually) in a solvent, is also widely used for preparing supramolecular hydrogels by disrupting, redistributing, and rebuilding the stacking of small molecules, making the hydrogels more homogenous.[59, 60] Light-controlled self-assembly requires the presence of light sensitive motifs, such as tetrazole,[61, 85] merocyanine,[86] α -carboxy-2-nitrobenzyl (CNB),[87] and azobenzene.[88] Although light is a physical stimulus, chemical reactions and structural changes, in most cases, also occur to initiate the light-triggered sol-gel or gel-sol transition, and the resulting conformation of the molecules typically favor more compact stacking when a hydrogel forms. An interesting but much less used physical trigger is magnetic field,[62, 89, 90] which is contactless, homogeneously effective over the whole sample and can produce structured materials with different sizes. However, this technique heavily relies on the (dia)magnetic susceptibility of the sample and the strength of the applied magnetic field.[62] The adjustment of pH serves as the most common and convenient chemical process for preparing supramolecular hydrogels. The protonation or deprotonation mediated by changing pH directly influences not only the intensity and strength of hydrogen bonding between hydrogelators, but also the conformations of the hydrogelators to promote the rearrangement of the molecular stacking.[63-65] Adjusting ionic strength is another effective way to induce hydrogelation and to modulate hydrogel properties, including mechanical, structural, and transport properties of hydrogels.[91-93] Many kinds of chemical reactions (e.g., redox reactions,[68, 94, 95] click chemistry,[81, 85] Michael addition,[82] and ligations[83]) have recently found increasing applications to generate supramolecular hydrogels. Generally, the non-self-assembling reactants turn into hydrogelators to self-assemble. Another important and increasingly explored hydrogelation pathway is ligand-receptor interactions.[66, 67] However, the ligand-receptor pairs between small molecules in nature are rather rare.[69, 70] One important kind of

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4 ligand-receptor interactions is metal–ligand interactions, as a form of noncovalent interaction or
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6 alternative weak driving force commonly leading to gelation.[71, 72] Recently, many studies have
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8 exploited enzyme-responsive motifs and enzymatic reactions to control molecular self-assembly since
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10 enzymatic reactions govern most of the molecular assembly or signal event in biology.[76-78, 96] In
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12 general, enzymes initiate the self-assembly by simply converting a non-self-assembling precursor into a
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14 self-assembling molecule via bond cleavage or formation. Such a self-assembly of small molecules
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16 usually results in the formation of supramolecular nanoscale assemblies (e.g., nanofibers or nanoparticles)
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18 in water, and above a threshold concentration, hydrogelation (Figure 2B).[84] This seemingly simple
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20 approach, in fact, holds great promises for the development of supramolecular biofunctional materials.
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24 Since many physical, chemical, and biological stimuli are able to trigger molecular self-assembly,
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26 self-assembly/aggregation/hydrogelation, as an observable phenomenon, can reciprocally report the
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28 presence of these external triggers for developing chemo- or biosensors as economical, rapid, and high-
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30 throughput diagnostics. For example, a simple approach is to develop the assays based on the
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32 hydrogelation of small molecules for quick detecting the presence of enzymes (e.g., phosphatase), as well
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34 as enzyme inhibitors.[97] As the substrate of phosphatase, a precursor transforms into a hydrogelator
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36 upon enzyme catalysis, which induces the formation of a hydrogel. When inhibitors bind with the active
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38 site of the enzyme and block the conversion of the precursor, no hydrogel forms. Such an easily
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40 observable change can serve as an inexpensive and facile visual assay where the colorimetry or
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42 fluorescence approach fails. In a work using the similar concept demonstrated by Hamachi et al.,[98]
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44 Yang and coworkers developed a phosphorylated short peptide with adamantane-capping group, which
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46 self-assembled to form nanospheres after the treatment of phosphatase. Upon the formation of
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48 nanospheres, ^{19}F -NMR signal of the fluoride containing backbone faded away, but would reappear after
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50 the treatment of tyrosinase, which led to the disassembly of the nanospheres into unassembled small
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52 molecules. Apparently, the dephosphorylated molecules are less soluble, thus self-assemble. After being
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54 oxidized, the molecules change structure to disfavor self-assembly. ^{19}F -NMR signal broadens and
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56 attenuates upon the formation of large aggregates because ^{19}F has a large chemical shift anisotropy while
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the transverse relaxation of ^{19}F -NMR signal is sensitive to the apparent molecular weight due to the CSA relaxation mechanism.[99] Jong and Kim designed and prepared a novel stimuli-responsive supramolecular hydrogel based on the host-guest interaction between gamma-cyclodextrin and an azo dye. Because the azo dye, containing an 8-hydroxyquinolinem, also is a ligand for metal ions, different metal ions lead to different binding patterns, which are responsible for different morphological transitions of the supramolecular assembly in the hydrogels.[100]

2.2.2. Reversibility

Due to the transient nature of noncovalent interactions, supramolecular networks are capable of restoring their mechanical properties after deformations and destructions. In other words, the transient character confers reversibility to supramolecular biofunctional materials, which is essential not only for the spatiotemporal control of functions, but also for extending the lifetime and improving the safety and performance of materials, particularly, for biomedical applications. For example, MAX1 and MAX8, two peptides developed by Schneider et al., were highly responsive to mechanical shear—when exposed to a shear stress, the hydrogel formed by either MAX1 or MAX8 shear-thinned and flowed with very low viscosity, while the hydrogel immediately restored its original rigidity upon the removal of the stress (Figure 3).[101] In this regard, this inherent property of supramolecular hydrogels allows the hydrogel (incorporated with drugs) to be delivered via syringe with precision to target sites. Homogenous cellular distribution and cell viability are unaffected by the shear thinning process and the hydrogel reconstructs and stays fixed at the point of introduction, suggesting that these kind of hydrogels may be useful for the delivery of cells to targeted disease sites for tissue regeneration. For example, Banerjee and coworkers developed a cell-compatible synthetic tripeptide-based hydrogel with similar mechanical properties for entrapment and sustained release of an antibiotic vancomycin and vitamin B12 at physiological pH.[102] Moreover, due to their transient nature, the formed supramolecular hydrogels ultimately dissociate after certain period, which minimizes long-term toxicity.

Figure 3. (A) Self-assembly, shear-thinning, and self-healing mechanism allowing rapid formation of hydrogels that can be delivered by syringe. (B) Peptide sequences of MAX8 and MAX1. (Adapted from © 2007 National Academy of Sciences with permission.[101])

2.2.3. Tunability

In order to tune the mechanical properties of covalent polymeric hydrogels, material scientists typically modulate the density or distance between crosslinking points according to traditional network theory of polymeric gels.[103] This approach has inspired the modulation of the mechanical properties of supramolecular hydrogels by simply varying the concentrations of self-assembling units or the density of molecular recognition motifs. Since hydrophobic interaction, one of the most critical interactions within supramolecular hydrogels, is much weaker than electrostatic interactions, the combination of aromatic-aromatic and electrostatic interactions, becomes an effective approach to tailor the elasticity of supramolecular hydrogels.[104] For example, simply using calcium ions to increase interfiber cross-linking was able to enhance the elasticity of the hydrogels based on aromatic-aromatic interactions over several orders of magnitude, even by a subtle increase of calcium concentration.[91] In addition, molecular recognition is also able to enhance the elasticity of the supramolecular hydrogels. For instance, the addition of the ligands (i.e., vancomycin) into mechanically weak hydrogels of a derivative of receptors (pyrene-D-Ala-D-Ala) leads to up to a 10⁶-fold increase of storage modulus (Figure 4A).[105]

Figure 4. (A) Addition of vancomycin to the supramolecular hydrogel of self-assembled pyrene-D-Ala-D-Ala increases the storage modulus of the hydrogel of pyrene-D-Ala-D-Ala by about 10^6 -fold. (B) The precursor with three phenylalanines formed a birefringent hydrogel upon enzymatic transformation while the precursor with only two phenylalanines formed a non-birefringent hydrogel. (Adapted from © 2004, 2014 American Chemical Society with permission.[105, 106])

Altering the molecular structure of the building blocks for supramolecular materials directly leads to changes in macroscopic properties, such as optical properties, cell compatibility, and biostability. For example, the increase of the intermolecular aromatic-aromatic interaction of hydrogelators during enzymatic hydrogelation resulted in spontaneous formation of an anisotropic hydrogel (Figure 4B).[106]

The design and preparation of two pairs of precursors/hydrogelators that differed only in the number of phenylalanine residues has demonstrated that the precursor with three phenylalanines formed a birefringent hydrogel upon enzymatic transformation while the precursor with only two phenylalanines formed a non-birefringent hydrogel. Also, it is possible to build systems that exhibit different levels of cell compatibility to achieve selective targeting,[20, 21] or enhanced biostability by utilizing non-natural amino acids, such as D-amino acids.[21, 27, 30-33] For example, based on that placental alkaline phosphatase represents a generic difference between cancer and normal cell, a small D-peptide containing a tyrosine phosphate as an enzymatic trigger selectively inhibited cancer cells while sparing normal cells.[107]

2.2.4. Biocompatibility and Biomimicry

Biological systems are extremely complicated, and there remains a great demand to develop synthetic materials that are capable of mimicking the structural and functional complexity of biological materials in nature. Since the networks of supramolecular hydrogels largely rely on noncovalent interactions among small molecules (M.W. < 2,000 g/mol), this kind of hydrogels are typically biocompatible, and in most cases biodegradable. This feature makes supramolecular hydrogel an excellent platform for cell culture and tissue engineering, for example, mimicking extracellular matrix that supports cell survival, assisting cell adhesion and division, or even promoting cell proliferation and differentiation. Ulijn and coworkers reported the 2D and 3D cell culture using hydrogels formed by Fmoc-dipeptides/tripeptides, Fmoc-FF (Figure 5A) and Fmoc-RGD.[108, 109] The fibrous structures of the networks of the hydrogels were stable under cell-culture conditions and had similar dimensions to the fibrous components of the extracellular matrix, thus capable of supporting cell culture of chondrocytes in two and three dimensions. Several other groups also showed that it was feasible to use supramolecular hydrogels as cell compatible biofunctional materials.[110-112] A notable example is the hydrogelator containing D-glucosamine, which resulted in a hydrogel possessing the potential to mimic glycoproteins and was able to promote the wound healing and even to reduce the formation of scars.[56]

Figure 5. (A) The short peptide of diphenylalanine linked to fluorenylmethoxycarbonyl (Fmoc) spontaneously form fibrous hydrogels under physiological conditions, for 3D cell culture. (B) Molecular graphics illustration of an IKVAV-containing peptide amphiphile molecule and its self-

assembly into nanofibers, as shown in SEM image. (C) Schematic presentation of proposed core-shell nanostructures in a hydrogel formed by the co-assembly of Fmoc-diphenylalanine and Fmoc-serine and the hydrogel is able to control the differentiations of stem cells cultured in it. (Adapted from © 2006 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim,⁹⁸ 2004 American Association for the Advancement of Science,[4] 2016 Elsevier Inc. with permission.[113])

By controlling the display of bioactive epitopes (e.g., RGD and IKVAV) on supramolecular biomaterials, scientists are able to tailor the functional biomimicry of biological signal in nature. For example, molecular hydrogels that can mimic the structure or function of collagen in nature (the most abundant component of extracellular matrix) could be useful in the preparation of artificial cell scaffolds or in replicating the role of collagen in biomineralization.[3, 114-118] Stupp et al. showed that self-assembly of peptide amphiphiles (PA) with the neurite-promoting laminin epitope IKVAV encapsulated neural progenitor cells to induce rapid differentiation of the cells into neurons (Figure 5B).[4] Ulijn and co-workers recently tried to control the stem cell differentiations in supramolecular hydrogels by modulating the stiffness of these hydrogels. They cultured perivascular stem cells on supramolecular peptide hydrogels of three different stiffness and observed neuronal, chondrogenic, and osteogenic differentiation, respectively (Figure 5C).[113] One novel approach is to use the molecular self-assembly of the conjugate of nucleobase, amino acids, and saccharide to mimic complex glycans for promoting the proliferation of murine embryonic stem cells and the development of mouse zygotes into blastocysts.[57] This approach is noteworthy because it greatly expands the diversity of self-assembling building blocks for developing supramolecular biofunctional materials.

2.2.5 Modularity and Multiple Functions

Supramolecular hydrogelators have flexible molecular scaffolds that are able to incorporate a large variety of functional entities (e.g., drugs) to make multifaceted or multifunctional biomaterials. Particularly, these hydrogelators can conjugate with many clinically used drugs for producing hydrogels as “self-delivery” drugs, thus eliminating the step of drug encapsulation and sometimes offering enhanced performance.[28, 29]

Figure 6. (A) Upon the action of an enzyme, the phosphorylated precursor turns into a hydrogelator, which self-assembles to form nanofibers and affords a supramolecular hydrogel of the taxol derivative for controllable release of drugs. (B) Schematic illustration of the designed and synthesized drug amphiphiles (DAs). (Adapted from © 2009, 2012 American Chemical Society with permission.[14, 31])

In most cases, the modification of self-assembling molecules with drugs, hardly disrupts their self-assembly properties. For example, with rational molecular design, the integration of enzymatic reaction of self-assembly and drug molecules (e.g., taxol) provided a powerful method to create molecular hydrogels of clinically used therapeutics without compromising bioactivities of the drugs. Specifically, the conjugation of taxol (which binds to β -tubulin subunit of microtubules to result in cell apoptosis) to a self-assembling precursor with tyrosine phosphate as an enzymatic trigger resulted in a new molecule that was able to form a hydrogel through EISA. This hydrogel was capable of slowly releasing anticancer drugs and at the same time prevented the quick clearance of this drugs, thus achieving enhanced retention time[26] and high efficacy in animal model (Figure 6A).[31] Realizing that the noncovalently encapsulated drugs may still diffuse away quickly, Cui et al. directly incorporated the hydrophobic anticancer drug, camptothecin (CPT), into hydrophilic peptide backbones by a biodegradable linker for a high and quantitative drug loading. The loading amount of CPT determined the morphologies of the resulting nanostructures, which could be either nanofibers or nanotubes. The formation of nanostructures provided protection for the CPT drug from the external environment, thus achieving controlled release of CPT. These drug nanostructures could release the bioactive form of CPT and showed enhanced *in vitro* efficacy against a number of cancer cell lines (Figure 6B).[14] Such strategies promise to generate

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4 nanostructures made of other types of anticancer drugs, thus presenting new opportunities for the
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6 development of self-delivering drugs[28] as cancer therapeutics. In addition to the incorporation of drugs,
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8 the incorporation of a fluorophore (NBD) to generate a fluorescent hydrogelator has allowed the first
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10 demonstration of self-assembly of small molecules inside cells. The imaging contrast conferred by the
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12 nanofibers of the hydrogelators allowed the evaluation of intracellular self-assembly, the dynamics, and
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14 the localization of the nanofibers formed by the hydrogelators in live cells.[119]
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17 18 **2.2.6. Predictability and Ordered Molecular Arrangement**

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20 **Being ubiquitous in nature, noncovalent interactions** drive protein folding, protein-protein/nucleic
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22 acid/ligand interactions, cytoskeleton dynamics, DNA-double-helix formations, RNA structure formation,
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24 antibody-antigen, receptor/ligand/hormone, drug-target interactions, and enzyme-substrate interactions.
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26 Most cases mentioned above are complicated, so simplified models are always helpful. Supramolecular
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28 hydrogelation of small molecules is such a model, though still far away from actual biological
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30 counterparts, offering a useful system to study the self-assembly of biomacromolecules in biological
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32 systems since these smaller molecules are easy to **synthesize/engineer** and more suitable for high-
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34 resolution structural techniques such as X-ray, NMR, EM, and **fluorescent imaging**. For example, Lynn et
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36 al. used the self-assembly of small peptides to build up a couple of nucleated conformational conversion
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38 models of amyloid assembly in hope to elucidate the role of A β in Alzheimer's disease (Figure 7A).[120-
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44 Sophisticated biological systems are an unlimited source of inspiration to scientists.[123] For example,
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46 that most proteins exist in dimeric forms has led to the generation of a pair of new nucleopeptides, with
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48 the nucleobases conjugating to the short peptide sequences selected from the interface of a heterodimer of
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50 proteins (Figure 7B). Driven by hydrogen bonds, this pair of nucleopeptides self-assembled to form
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52 nanofibers upon mixing in water, and the resulting hydrogel exhibited excellent proteolytic resistance
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54 against proteinase K.[124]
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Figure 7. (A) Model for the progressive transitions observed for Aβ (16–22). (B) Molecular structures of the nucleopeptides containing the epitopes from stargazin. Hydrogels form upon mixing. (Adapted from © 2014 American Chemical Society, 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim with permission.[120, 124])

Unlike polymeric hydrogels, supramolecular hydrogels have more ordered structures, which usually lead to well-defined configurations and conformations. Based on the increased amount of structural data, it becomes increasingly easy to design self-assembling molecules with controllable nanostructures for predictable biological functions and desired biomedical applications.

3. Supramolecular Hydrogels Made of the Basic Biological Building Blocks

3.1 Amino Acids--the Most Prevalent Building Blocks for Supramolecular Biofunctional Materials

In fact, life relies on noncovalent interactions that maintain the 3D-structures of biomacromolecules (e.g., protein, nucleic acids, and polysaccharides) and control specific binding/recognition events in biological systems. This fundamental fact offers material scientists blue prints for using basic biological building blocks, typically amino acids or peptides, to build up self-assembling molecules for generating supramolecular biofunctional materials. Compared with other small organic molecules, such as urea derivatives, used for the early development of supramolecular hydrogels,[41] the use of the unified

building blocks of life[125] are more suitable and general for biomedical applications. Of course, the design of such kind of supramolecular biofunctional materials requires an understanding of the nature's underlying principles of noncovalent interactions resulted from these essential building blocks of life. The following section use the supramolecular hydrogels made of peptides or peptide derivatives to illustrate the advantages, versatility, and opportunity of the functional biomaterials derived from amino acids, the simple but essential building blocks of life.

3.1.1 A Large Space for Molecular Design

As the building blocks of proteins, amino acids are ideal for generating supramolecular biofunctional materials, including hydrogels. First, according to permutation and combination rules, the 22 natural amino acids, together with non-natural ones (40+, including D-version enantiomers), provide an enormous molecular space for generating self-assembling peptides, considering that even a dipeptide, for example, diphenylalanine, can self-assemble to form well-ordered, tubular, and elongated assemblies (Figure 8A).[126] Second, even a subtle difference may have a profound impact on the capabilities to self-assemble as well as the consequent physical and biological properties. For example, dipeptide Ile-Phe and Val-Phe, differing in only one methyl group, exhibited dramatically different self-assembling behaviors – the former one was able to form a hydrogel consisting of persistent nanofibers while the latter one didn't even self-assemble (Figure 8B).[127] Third, the carboxylic acid (-COOH), amine (-NH₂), and thiol (-SH) on the peptide terminals and side chains allow facile incorporation of a variety of small molecular entities into the self-assembling backbones to generate even more diverse self-assembling molecules.[84, 94, 128-132]

Figure 8. (A) Self-assembly of Phe-Phe dipeptide to form well-ordered and elongated peptide nanotubes (B) Ile-Phe forms a gel with nanofibers as the network whereas Val-Phe does not aggregate in solution. (Adapted from © 2003 American Association for the Advancement of Science,[126] 2007 Elsevier Inc. with permission.[127])

Based on their affinity to water and the hydrophobicity (or hydrophilicity) of their side chains, 22 amino acids roughly fall into two categories: hydrophobic amino acids (e.g., phenylalanine, tyrosine, valine, leucine, and methionine) and hydrophilic amino acids (e.g., aspartic acid, glutamic acid, arginine, and lysine). The side chains of hydrophobic amino acids usually contain aromatic or alkyl group and hydrophilic amino acids typically have -COOH or -NH₂ on their side chains. Recently, Frederix and Ulijn aimed to generate designing rules for peptidic hydrogelators by computationally assessing the ability of more than 8,000 tripeptides to self-assemble in water. They have generated considerable results regarding the properties of specific amino acid and their combinations, and identified a limited number of hydrogelators from over 8000 candidates.[13] This research is a valuable effort towards *a priori* design of a hydrogelator consisting of amino acids.

3.1.2. Easy Synthesis

The development of solid phase peptide synthesis (SPPS) and the commercialization of peptide synthesizers, largely reduces the burden of peptide synthesis and allow large scale preparations of peptides to be quick and even automatic.[133] Although SPPS typically has an upper limit of approximately 50 amino acids,[134] it is feasible to synthesize a longer sequence by conjugating two oligopeptides using native chemical ligations.[135] Moreover, the reactive terminals (-NH₂, -COOH, and -SH) of peptides readily provide available sites for incorporating one or more functional motifs into the molecules, affording conjugates with further tailored functions. Meanwhile, the easiness for synthesizing peptide derivatives stimulates the development of synthetic biomacromolecules mimics, such as glycans, collagens, in the situation where it is difficult to study the structure, assembly, and biochemistry of natural biomacromolecules. For example, the synthetic challenges (e.g., branched structures, anomeric linkages, and selective bond formations) in glycobiology and glycochemistry hinder the development of

glycobiomaterials for biomedicine. A promising approach emerged from the self-assembling peptides was to use the self-assembly of the conjugate of small glycoside and amino acids for mimicking the functions of glycoproteins/proteoglycans.[57, 136, 137]

3.1.3. Chirality for Structural Diversity

All natural amino acids, except glycine (G), are chiral and exist exclusively in the form of L-configuration. L-Amino acids form L-peptides in nature, which, in most cases, are compatible to cells and thus promise a range of biomedical applications, like cell culture, tissue engineering, and drug delivery. D-peptides, consisting of D-amino acids, usually resist endogenous proteases and are presumably insensitive to most enzymes, readily finding applications in a variety of areas, including cancer therapy and immunomodulation. For example, the conjugation of a naphthyl group with D-Phe-D-Phe afforded a self-assembling molecule, which formed a hydrogel and resisted proteolytic hydrolysis to offer long-term biostability.[27] The nanofibers formed by this D-peptide derivative efficiently inhibited the glioblastoma cells but exhibited little toxicity toward a neuronal cell line.[138] Moreover, acting as *de novo* amyloid oligomers, the nanofibers also effectively inhibited the tumor progression both in vitro and in vivo (Figure 9A).[139] Mechanistic study revealed that the nanofibers selectively accumulated in cancer cells via macropinocytosis and induced apoptosis by impeding the dynamics of cytoskeletal filaments via promiscuous interactions with cytoskeletal proteins.[129] In a different but related study, after the *in situ* enzymatic dephosphorylation of a precursor of such kind of D-peptides, the product formed the pericellular nanofibers that selectively inhibited cancer cells.[107]

To increase the life time of the peptides, proteins, or antibodies for maintaining their bioactivities, Merrifield has pioneered the development of the analogues of the native peptide sequences using D-amino acids, namely retro-inverso peptides.[140] The retro-inverso peptides, consisting of D-amino acids in the reversed sequence, share a similar side-chain arrangement of the corresponding L-peptides, although their terminal carboxyl and amino groups point in opposing directions. If the binding of a small peptide to a target is independent to the secondary structure of the peptide, its retro-inverso peptide is likely to have a similar binding affinity with the target (Figure 9B).[141]

Besides D-peptides, L- and D-mixed peptides also exhibit tailored protease resistance. For example, replacing any one of the three L-amino acids in a hydrophobic tripeptidic precursor or its hydrogelator (Nap-Phe-Phe-pTyr/Nap-Phe-Phe-pTyr) with a D-amino acid significantly increased its proteolytic resistance[32]. Moreover, doping L-peptides with D-amino acids (or *vice versa*) provides a platform to control the intramolecular folding and the subsequent self-assembly of the monomeric peptides. This approach thus allows the design of materials that are responsive to this intramolecular folding event. One representative classes of peptides that use a D-amino acid to control molecular folding is the β -hairpin peptides (e.g., MAX1 and MAX8), pioneered by Schneider and Pochan et al. [34, 87, 142-145] As a general design, a β -hairpin contained two prolines in the middle (one is L- and the other is D-version) of the peptide and was able to respond to a range of stimuli (e.g., pH, temperature, ionic strength, or shear forces) to self-assemble in water and to afford hydrogels that were rich in β -sheet.

Figure 9. (A) Nap-Phe-Phe self-assembles in water to form nanofibers that inhibit tumor in vitro and in vivo by disrupting cytoskeletons. (B) An example of L-peptide and its retro-inverso isomer. (Adapted from © 2013, 2014 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 1994 National Academy of Sciences with permission.[27, 139, 141])

3.1.4. Peptides—Perfect Self-Assembling Backbones

As a kind of biomaterials, supramolecular hydrogels are noncovalently crosslinked nanofiber networks with high water content. Their formation requires a balance between the forces driving the association of nanofibers and those mediating solvation of the network. Considering that proteins have

evolved to fold and to function in aqueous environments and that many proteins self-assemble into larger structures, they would seem to be ideal candidates for developing hydrogelators or precursors. In fact, nature uses proteins to make hydrogels (e.g., collagens for cornea). Moreover, the segments of protein—peptides—are suitable for self-assembly. The amide structures in the peptide backbone provide both hydrogen bonding donors and acceptors to facilitate molecular stacking, which is essential in the process of self-assembly and hydrogelation. A common conformation in the supramolecular nanofibers of small peptidic hydrogels is β -sheet. For example, Zhang et al., while investigating the sequence of Ac-(AEAEAKAK)₂-NH₂ (EAK16) from yeast proteins, observed the gelation of a self-complementary peptide which consisted of opposite charged residues and hydrophobic residues. The pattern of alternating alanine (A) and glutamic acid (E) or lysine (K) residues promoted the formation of β -sheets in which the hydrophobic and charged side-chains were pointing towards opposite directions of the peptide backbone. Hydrogen bonding between peptides led to the formation of intermolecular β -sheets containing a hydrophobic alanine face and a charged Glu/Lys face.[146] They also designed several other peptides altering the sequences containing RAD motif, RADA16-I, (RADARADARADARADADA), and RAD16-II, (RARADADARARADADADA). These peptides have motif RAD that is similar to the ubiquitous integrin receptor binding site RGD. These peptides, similar to EAK16, formed well-ordered nanofibers by adopting a β -sheet conformation.[147] Compared to β -sheets, α -helical structures have played a less prominent role in the development of supramolecular hydrogels. There are few examples of the hydrogelation of α -helical peptides, which require more sophisticated design.[148] For example, Woolfson reported the first example of rationally designed and fully characterized self-assembling peptide hydrogels containing more than 99% water, based on linear peptides with purely α -helical structures. Two helices, formed by 28mers, wrapped around one another to form a dimer, with complete 3.5 residues per turn mediated by multiple hydrogen bonds.[149] Recently, it was reported that certain bacterial amyloids are α -helices,[150] implying that it should be feasible to use α -helices for generating supramolecular hydrogels.

3.1.5. Terminal or Side Chain Modifications to Expand Applications

One of the attractive aspects of peptides is that their C- and N-terminals, as well as side chain with -NH₂, -COOH, -SH, or -OH groups, are readily available for further functionalization. Such a conjugation accommodates a broad range of non-peptidic structural moieties without compromising the excellent self-assembling abilities provided by the peptides. The modification mainly falls into two categories—one is to give rise to an additional directional driving force for self-assembly of the peptides, and the other is to functionalize the peptides without hampering its self-assembling abilities. The former one generates numerous excellent supramolecular hydrogelators or precursors, such as peptide amphiphiles bearing an alkyl chain[3, 151-153] or peptides modified with aromatic groups.[27, 154-156] The latter one readily finds applications in a wide range of biological and biomedical areas, which would be discussed in more details later (see section 4). For aromatic rings like benzene, there is a partial negative charge on both faces of the benzene ring while there is a partial positive charge around the aromatic ring. In this case, a face-to-face stacking of π system is largely disfavored, and an edge-to-face stacking, where regions of positive charge directly interact with negative charged regions, dominates. Being similar to electrostatic interaction, such aromatic-aromatic interaction is strong and directional.[157]

Figure 10. Chemical structures of some hydrogelators.

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4 Stupp and co-workers have pioneered the development of peptide amphiphiles (PA) that self-
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6 assemble to form hydrogels for biological applications. Most of the peptide amphiphiles[158-162] contain
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8 a polar head (typically hydrophilic peptides) and a hydrophobic tail (typically alkyl chain) and self-
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10 assemble to form nanostructures like fibers, micelles, ribbons, tubes, and even sheets in water, which also
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12 afford supramolecular hydrogels upon pH changes or the addition of salts (Figure 10A). [163, 164] To
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14 augment the self-assembly ability of peptidic hydrogelators, aromatic groups, as the hydrophobic
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16 segments, are another prevalent choice to decrease the solubility of the peptides in water.[165-171]
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18 Shortly after the report of pyrene conjugated vancomycin to form a hydrogel with the dramatically
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20 enhanced antibacterial activity, fluorenylmethyloxycarbonyl motif (i.e., Fmoc, the commonly used
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22 protecting group of amine in amino acids) is found to turn simple dipeptides (e.g., Gly-Gly or Ala-Ala)
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24 into self-assembling molecules to form nanofibers as networking matrix of hydrogels.[172, 173]
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26 Although the Fmoc-dipeptide was reported to form a gel in 1995,[174] the observation of the self-
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28 assembled nanofibers of Fmoc-dipeptides was not reported until 2003 (Figure 10B).[175] Shortly after
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30 that, Ulijn et al. reported the development of Fmoc-diphenylalanine, one of the extensive investigated
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32 small molecular hydrogelators, which formed hydrogel either by adjusting the pH of its aqueous solution,
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34 or mixing with other hydrogelators.[63, 176-178] Being considered to be more biocompatible than Fmoc,
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36 2-(naphthalene-2-yl) (Nap-) has emerged as the aromatic group to conjugate with Phe-Phe at either C- or
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38 N-terminals for generating hydrogelators (Figure 10C). The Nap- group on the terminal provided the
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40 hydrophobic force to enhance self-assembly of these two hydrogelators in the aqueous environment.[90,
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42 179-182] The simplicity of this approach has result in the subsequent design and development of a large
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44 group of self-assembling, peptidic hydrogelators capped by Nap motif.[22, 106, 183-185]
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52 The existence of naturally occurring nucleopeptides has stimulated the development of self-
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54 assembling molecules by connecting nucleobases to small peptides in order to achieve increased
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56 hydrogen bonding interactions.[186-191] For example, the conjugation of nucleobases (e.g., thymine,
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58 adenine, cytosine, and guanine) to simple dipeptides, such as Phe-Phe, created a group of hydrogelators or
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precursors. Upon proper initiation (e.g., pH or enzyme), these nucleopeptides self-assembled in water to afford a new type of supramolecular hydrogels that not only were biocompatible but also exhibited significant resistance to proteases (Figure 10D).[192-196] The incorporation of sugar into peptide backbones usually increases the solubility of the peptides, which consequently decreases the self-assembling tendency of the molecule. However, the supramolecular hydrogels formed by the resulting molecules likely are biocompatible because they resemble natural glycopeptides and should be susceptible to enzymatic degradation.[197-200] A man-made substrate of β -galactosidase was the first example of such simple glycopeptides (i.e., simpler than the conjugate of pyrene and vancomycin[201]), which became a hydrogelator to self-assemble to form ordered nanostructure upon enzymatic deglycosylation (Figure 10E).[197] Another notable hydrogelator was an L-rhamnose containing glycopeptide that self-assembled to form nanofibers. The corresponding hydrogel was able to suppress the antibody response to phycoerythrin in mice, contrasting to the reported immunostimulatory properties of monomeric L-rhamnose.[202]

Nature, which exploits saccharides, peptides, and nucleobases as fundamental building blocks for the creation of biomacromolecules, has inspired the creation of a new class molecular conjugates consisting of saccharides, amino acids, and nucleobases (Figure 10F). [57, 137, 193, 203-206] These conjugates, surprisingly, acting as hydrogelators, self-assembled in water to form ordered nanostructures and supramolecular hydrogels that possessed multiple functions.

3.2. Other Building Blocks — Nucleic Acid Motifs and Saccharides

The combination of nucleic acid motifs (e.g., nucleoside, nucleotides, and nucleobase) with supramolecular chemistry principles provides another powerful approach to create supramolecular hydrogels/assemblies that exhibit tailored physical, chemical, biological properties and functions. For example, the pH-sensitive guanosine hydrazide, as an excellent hydrogelator, forms a stable supramolecular hydrogel based on a guanine quartet induced by metal cations (Figure 11A).[207] Besides, 2'-deoxyadenosine, having an octyl-lipid tail connected by a urea linker, gels water under ultrasonic

irradiation.[208] Barthélémy and coworkers have pioneered the research on glycosyl-nucleoside-lipid (GNL) amphiphiles.[209, 210] In 2005, Barthélémy reported a neutral amphiphile derived from uridine with two oleyl chain and one glucose on its sugar ring,[211] and demonstrated that such a glycol-nucleo-amphiphile was able to bind efficiently to the nucleic acid double helix structure. Barthélémy et al. also reported the use of GNL as a small hydrogelator for the delivery of oligonucleotides into cells. The conjugation of an oleyl chain and a glucose to a nucleoside via double “click chemistry” yielded a hydrogelator, which efficiently formed a hydrogel to encapsulate nucleic acids (Figure 11B).[212] The resulting nucleic acid loaded GNL hydrogel significantly increased the cellular uptake of nucleic acids even in presence of serum, without showing obvious toxicity. Fluorination of the lipid chain of such GNL amphiphile afforded a new family of glycosyl-nucleoside-fluorinate (GNF) compounds, which were able to form hydrogels by varying the temperature (Figure 11C).[213] Most recently, self-assembling molecules based on nucleic acid motif are finding more applications in biology and biomedicine.[214-216]

Figure 11. (A) Metal cations induce a guanine quartet to form a supramolecular hydrogel. (B) The conjugation of an oleyl chain and a glucose to a nucleoside via double “click chemistry” yields a hydrogelator. (C) Fluorination of the lipid chain of a GNL amphiphile affords a glycosyl-nucleoside-fluorinate (GNF) compound, which forms hydrogels upon changing temperature. (Adapted from © 2005 by the National Academy of Sciences of the USA with permission[207])

Polysaccharides, such as alginate, are a group of biomaterials that have found numerous biomedical applications. Most of them form hydrogels (i.e., noncovalent polymeric hydrogels) under mild conditions, which are particularly attractive in wound healing, drug delivery, and tissue engineering.[217] However, supramolecular hydrogels based on small saccharides remain less explored, and most of the saccharide-derived hydrogels are based on lipid conjugates. For example, Thorton et al. synthesized and characterized three series of methyl α -D-glucopyranoside derivatives (i.e., monosaccharide lipids (Figure 12A)). Most of them are excellent hydrogelators, which are able to form various self-assembled structures, including birefringent fibers and tubules.[218] Wang and coworkers also reported hydrogelators based on 4,6-O-benzylidene- α -D-methyl-glucopyranoside.[219] By clicking different saccharides as the polar heads onto a hydrophobic chain via copper(I)-catalyzed azide-alkyne [3 + 2] cycloaddition, Oriol et al. developed several glycolipid hydrogelators (Figure 12B), which, at room temperature, self-assembled to form fibrillar supramolecular structures as the matrices of stable hydrogels. Hamachi et al. reported a classical example of supramolecular hydrogels formed by saccharide derivative (Figure 12C). At high concentration, the resulting hydrogel exhibited sufficient stability and mechanical strength for different applications, such as holding various shapes, acting as gel capsules, serving as cell culture platforms, and sensing and targeting prostate cancer cells.[220] Zhu et al. developed a chemo-responsive hydrogel made of a saccharide-based hydrogelator (methyl-4,6-O-(4'-aldehydephenylidene)- α -D-glucopyranoside, Figure 12D). The hydrogel formed by this molecule not only showed thermal responsiveness, but also exhibited cysteine and pH responsiveness due to the presence of aldehyde and acetal groups.[221] Wang and coworkers conjugated different saccharides to a small peptide (Nap-Phe) as novel glycopeptide hydrogelators (Figure 12E). Such an approach integrated multiple types of building blocks into one single molecule, greatly expanding the candidate pool of supramolecular hydrogelators.[222]

Figure 12. Chemical structures of some saccharide-derived hydrogelators.

4. Applications of Supramolecular Biofunctional Materials

In the past two decades, the field of supramolecular chemistry has developed from merely seeking self-assembling molecules to the design of advanced materials with well-controlled biological activities. Such an advance provides scientists, engineers, and physicians with new options to address problems in biomedicine and biology. Here, we briefly discuss the applications/functions of these biofunctional materials into the following five areas: i) cell culture and tissue engineering; ii) drug delivery; iii) cancer therapy; iv) imaging; and vi) immunology. By highlighting the most recent and representative examples in each area, we discuss the specific features and properties that make supramolecular biofunctional materials the promising candidates for these applications.

4.1. Cell Culture and Tissue Engineering

As a class of biofunctional materials, supramolecular hydrogels hold up large amount of water and typically contain biological building blocks derived from nature. Due to their resemblance to extracellular environment, one of the potential applications of supramolecular hydrogels is to mimic extracellular

matrix for cell culture. Because cell culture is relatively easy to be assayed, considerable efforts on the research of supramolecular hydrogels have been made for 3D cell cultures.[112, 223-228] Hamachi and the co-workers reported the design of a hydrogelator containing zwitter-ionic amino acid and C=C double bond, which were both pH and UV sensitive. This hydrogelator was able to form a hydrogel as strong and multi-stimuli responsive as polymeric hydrogels, thus served as a unique molding template for fabricating various substrates, such as agarose containing proteins. They successfully mimicked a collagen gel by building up 2D/3D micro-channels and demonstrated its biocompatibility in the fabrication process.[229] Yang et al. developed a series of peptide derivatives with the sequences of Nap-GFFYX-ss-EERGD (X = E, K or S) so that glutathione (GHS) was able to break the disulfide bond to result in hydrogels.[230] Among the resulting hydrogels, only Egel (X = E) with storage modulus (G') of hundreds of pascals was suitable for 3D cell culture since the stiffness of the hydrogel had an influence on the cells adhesion, spreading, and proliferation. Besides stiffness, there was report that the change of chirality of nanofibrous structures greatly influenced cell adhesion and proliferation in the 3D extracellular matrix (Figure 13A)[231]—the nanofibers with left-handed helical structure promoted the cell adhesion and proliferation, whereas the nanofibers formed by its enantiomer showed the opposite effect. These results highlighted the crucial role of the stereochemistry of the nanofiber in cell adhesion and proliferation in 3D cell culture. Feng et al. reported the use of supramolecular self-assembly to create nanofibrous environments with the controllable wettability in both 2D and 3D environments.[232] They designed three 1,4-benzylidene-carboxamide-phenylalanine derived supramolecular hydrogelators, which formed stable hydrogels with different surface wetting properties. They found that the least wettable nanofibers gave the fast cell adhesion and proliferation, indicating that properties other than stiffness and chirality dictated the cell behaviors. Luo and coworkers reported the application of self-assembled D-peptide in 3D cell cultures.[233] In spite that the D-peptide hydrogel exhibited similar biocompatibility as the hydrogels of the corresponding L-peptides, D-peptides, have advantages over L-peptides, such as that D-peptides, being proteolytically resistant, are more stable in vivo.

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4 conjugates of nucleobase, amino acids, and saccharide (e.g., Ade-FRGD-Glucosamine) (Figure 13B).[57]
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6 Unexpectedly, the assemblies of Ade-FRGD-Glucosamine promoted the proliferation of mouse
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8 embryonic stem cells (mES), indicating that the self-assembly of the conjugate of nucleobase, amino
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10 acids, and saccharide might mimic glycoconjugates to control the cell behaviors.[57] Stupp and
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12 coworkers reported the design of a DNA nanotube, which enhanced the differentiation of neural stem
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14 cells into neurons (Figure 13C).[234] The DNA nanotubes are self-assembled from five strands, one of
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16 which was functionalized by the RGDS sequence. They demonstrated that the co-assembled bioactive
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18 DNA/peptide-DNA supramolecular nanostructure promoted the cell adhesion and differentiation. Stupp et
19
20 al. also reported the design of a peptide amphiphile (PA) that self-assembled into nanofibers, which could
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22 build up matrices and create self-supporting gels under cell culture conditions.[238] With phosphoserine
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24 residues in the peptide backbone, the peptide amphiphile promoted the hydroxyapatite formation in the
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26 culture medium. Experimental results suggested that human mesenchymal stem cell (hMSC) incorporated
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28 PA gels mineralized over time in the hMSC osteogenic medium supplemented with CaCl_2 , and the
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30 mineralized nanofibers promoted osteogenic differentiation of the hMSCs.
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A

PA1: C16-VVVAAAEEMQMKKVLDS

AIFC-PA6 (Control): C16-VVVAAAEEEKLMSQKMVD

SIS: small intestinal submucosa

B

Figure 14. (A) An anti-inflammatory peptide amphiphile, AIF-PA1, has found applications for bladder regeneration. (B) A peptide amphiphile, that self-assembles to form cylindrical nanostructures, serves as a therapeutic strategy to regenerate microcirculation and ischemic tissue in cardiovascular diseases. (Adapted from © 2014 Elsevier Ltd., 2010 National Academy of Sciences with permission.[239, 240])

In addition to cell culture, supramolecular biofunctional materials have also found increased applications in the field of tissue engineering. However, the development of tissue engineering using

supramolecular materials is a slow progress due to the complexity and limited knowledge of the dynamics of biological processes. Meanwhile, due to their simple compositions and weak mechanical strength, supramolecular materials only played supplementary roles when being used in complicated endogenous processes. Although challenges remain, researchers have made significant progresses in exploring the utilizations of supramolecular biofunctional materials in tissue engineering.[111, 241-250] Stupp et al. reported the design of mono-domain gels of $(C_{16})_2V_2A_2E_2-(NH_2)$, which contained aligned peptide amphiphile (PA) nanofibers, for delivering sonic hedgehog (SHH) protein to the cavernous nerve (CN).[251] This strategy imparted a unique advantage—directionality—for the delivery of proteins and the SHH to the CN via these aligned peptide amphiphile nanofibers. Indeed, this strategy promoted CN regeneration. The same group also reported a series of anti-inflammatory peptide amphiphiles (AIF-PAs) (e.g., AIF-PA1: C_{16} -VVVAAAEEMQMKKVLDS), for bladder regeneration (Figure 14A).[239] They observed significant regenerative advantages of the AIF-PAs such as potent angiogenic responses and limited tissue collagen accumulation. They demonstrated that AIF-PAs mitigated galvanic innate immune responses as well as providing a highly conducive regenerative environment. They also used heparin-binding peptide amphiphile (HBPA) and hyaluronic acid (HA) to form a self-assembled bioactive hierarchical membrane,[252] which had the potential capacity of binding and slowly releasing heparin-binding growth factors. Human mesenchymal stem cells (hMSCs) were able to attach to and remain viable on these membranes, which induced a significant and rapid enhancement of angiogenesis. By connecting K_3G , V_2A_2 , as well as C_{16} alkyl chain to the N terminus of the peptide sequence KLTWQELYQLKYKGI-NH₂ that mimicked VEGF, one of the most potent angiogenic signaling proteins, Stupp et al. generated a series of peptide amphiphiles for self-assembling to form cylindrical nanostructures (Figure 14B).[240] The polyvalent self-assembling nanofibers were efficacious in a hind-limb ischemia model of cardiovascular disease—a promising result for developing synthetic therapeutic strategy to regenerate microcirculation and ischemic tissue in cardiovascular diseases. Using the supramolecular platform, Stupp et al. also have explored supramolecular nanofibers in bone regeneration and repair, [253] bioengineered peripheral nerve repair,[254] the design of optimal neuronal scaffold

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4 biomaterials,[255] improvement of the regenerative efficacy of cell-based strategies in ischemic
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6 tissue,[256] and many others applications.[257, 258] These works have stimulated others to explore the
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8 supramolecular biofunctional materials in the field of tissue regeneration. For example, Wang et al.
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10 reported the application of the self-assembly of peptide RADA₁₆-IKVAV in brain tissue engineering.[259]
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12 The hydrogel formed by RADA₁₆-IKVAV enhanced survival of encapsulated neural stem cells, reduced
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14 the formation of glial astrocytes, and improved the regeneration and repair of injured brain. The same
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16 group also reported the design of two self-assembling peptides (RADA₁₆-GRGDS and RADA₁₆-YIGSR)
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18 as well as their application in liver tissue regeneration.[260] These two peptides were able to self-
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20 assemble into β -sheet structures and have shown satisfactory gelation behavior and excellent hemostasis
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22 efficacy in a rat liver model. Galler et al. reported a cell adhesive, enzyme-cleavable hydrogel, capable of
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24 encapsulating dental pulp stem cells together with the growth factors via heparin binding.[261] In vivo
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26 results revealed that the resulting supramolecular biomaterial helped to form a vascularized soft
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28 connective tissue similar to dental pulp.
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32 33 **4.2. Drug Delivery**

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35 Recently, supramolecular hydrogels have emerged as an alternative to traditional polymeric hydrogels
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37 as promising carriers for therapeutic agents. The drugs or bioactive molecules can be either physically
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39 entrapped within the inert hydrogel matrix/scaffold or chemically conjugated to the self-assembling
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41 molecules. Thus, the applications of supramolecular hydrogels as drug delivery vehicles mainly fall into
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43 two categories: i) drugs encapsulated in hydrogels;[102, 262-277] ii) drugs conjugate covalently with the
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45 hydrogelators.[278-283]
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49 Examples of the first category are the hydrogels carrying controllable amount of bioactive small
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51 molecules or drugs via noncovalent interaction to maximize access, retention, and therapy. Yang et al.
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53 have been actively involved in the design and development of a variety of multi-responsive
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55 supramolecular hydrogels for drug delivery. For example, they developed an innovative hydrogel based
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57 on charge attractions, in which the nanofibers were formed by the co-assembly of negative charged self-
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59 assembling peptides (Nap-GFFYGRGD) and positive charged responsive self-assembling components
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(Myristoyl choline or Ada-GFFYKKK-NH₂). Myristoyl choline could respond to the enzyme acetylcholine esterase (ACE) and Ada motif could interact with methyl- β -CD (MCD) via ligand-receptor interaction. By controlling and modulating the enzymatic reactions or ligand-receptor interactions, they achieved sustainable release of drugs in biological systems.[284] Ulijn and co-workers reported a MMP-9 responsive peptide amphiphile, which self-assembled into micelles under physiological condition (Figure 15A).[285] In the presence of MMP-9, the self-assembled micelles transformed into nanofibers and released the encapsulated doxorubicin during the disassociation process. Thus, the micelles were able to serve as carriers for selectively delivering the anticancer drugs to tumor sites where MMP-9 was overexpressed. Pochan and Schneider demonstrated a curcumin delivery strategy for local delivery of curcumin to the targeting sites by encapsulating curcumin in a self-assembling peptide hydrogel.[286] The encapsulation of curcumin in the self-assembled hydrogel barely affected the biological activities of curcumin. Changing the concentration of the self-assembling peptide modulated the release rate and therapeutic efficacy of curcumin. Zhang and co-workers demonstrated the delivery of miRNA into live cells by a supramolecular hydrogel formed by Gly-Ala linked biphenyl-substituted tetrazole.[287] The hydrogel network delivered the encapsulated miRNA into the encapsulated cells, thus repressing the expression of target genes. Similarly, Kim et al. also used the molecular self-assembly derived from riboflavin to deliver VEGF-siRNA into human cells.[288] Liu et al. demonstrated the design of a metal ion triggered shrinkable supramolecular hydrogel, which showed excellent reversibility.[289] The unique shrinking features of the hydrogel were extremely useful in the controllable release of vitamin B₁, as well as many other bioactive molecules.

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donor.[292] The hydrogelator, which self-assembled to form hydrogels for local delivery of NO, was synthesized by conjugating a β -galactosidase-responsive caged NO molecule with a self-assembling short peptide (Nap-FFGGG). By adjusting the concentrations of β -galactosidase (for removing the capping group, sugar) loaded into the hydrogels, they were able to control the rate of NO release. The same group also reported the preparation of injectable hybrid hydrogels by mixing the hexameric proteins of 2T, 4T, and 6T with a peptide sequence NapGFFYGGGWRESAI.[293] The hexameric proteins of 2T, 4T, and 6T served as crosslinkers to interconnect the self-assembling nanofibers of NapGFFYGGGWRESAI, leading to the formation of hydrogels with tunable mechanical properties. Another drug delivery hydrogel, reported by Yang et al., is based on the conjugation of a peptide and two complementary anti-cancer drugs.[294] Upon the treatment of dithiothreitol (DTT), Dex-FFFK(Taxol/HCPT)E-ss-EE turned into Dex-FFFK(Taxol/HCPT)E, which self-assembled to form hydrogels for continuous co-delivery of anti-cancer drugs with enhanced retention time. Stupp et al. developed a peptide covalently containing hydrazide. This peptide self-assembled to form hydrogels for sustainable hydrazide release. Zhong and coworkers designed a synergistic dual-targeting hydrogel that was able to transport drugs for specifically targeting tumors.[295] Experimental results revealed the accumulation of hydrogel within the cancer cells *in vitro* as well as in breast tumor *in vivo*. They confirmed that the estrone and RGD peptide acted as the tumor-targeting moieties and contributed to the selective accumulation of the hydrogel in the breast tumor. The biocompatible and synergistic dual-targeting hydrogel efficiently assisted the release of the drug inside the cells.

4.3. Cancer Therapy

Although the understanding of cancer biology and the cancer therapies (e.g., traditional surgery, radiation and chemotherapy, hormone therapy, immunotherapy, stem cell transplant, and precision medicine) have progressed considerably in the last decade, cancer still remains one of the major challenges to public health.[296] Meanwhile, despite extensive efforts, pressing problems in the treatment of cancer are still emerging, such as drug resistance resulted from target mutations or redundant pathways. Thus, there is still an urgent need to develop innovative approaches for treating cancers. As discussed in

the previous section, supramolecular hydrogels have already shown promises as possible cancer drug delivery systems.[277, 295, 297-300] In this section we discuss the recent development in the use of supramolecular hydrogelators themselves as innovative anticancer therapeutics.

The investigation of the unusual cytotoxic profile of a versatile self-assembling motif, a diphenylalanine derivative (Nap-FF),[179] has found that the nanofibers of Nap-FF, referred as prion-like nanofibrils of small molecules (PriSM), were able to selectively inhibit glioblastoma cancer cells over neuronal cell in cell culture and effectively inhibit xenograft tumor in animal models (Figure 9A).[129, 138, 139] Mechanistic investigation revealed that the PriSM selectively inhibited different types of cancer cells, while remained innocuous to normal cells like bone marrow stromal cells (i.e., HS-5) and neuronal cells (i.e., PC12) at the concentration above critical concentration for self-assembly. After accumulating inside cancer cells via macropinocytosis, the self-assembly of the dipeptide derivatives impeded the dynamics of cytoskeletal filaments (e.g., microtubules, actins), inducing cell apoptosis. The modification of Nap-FF at its C-terminal with an ester bond resulted in a novel precursor, which was able to enter cells and self-assemble to form nanofibers upon hydrolysis catalyzed by carboxylesterase (CES). The resulting nanofibers killed most of human cancer cells (i.e., HeLa) while being nontoxic to mouse fibroblast cells (i.e., NIH3T3) due to significantly higher esterase level in HeLa cell than NIH3T3 cell.[301] The unexpected selectivity and new mechanism of molecular nanofibers inside cell against cancer have led to the utilization of intracellular EISA of D-peptide for a combination therapy that used traditional anticancer drugs, such as cisplatin.[23, 302] As shown in Figure 16A, the designed small D-peptide precursors, as a substrate of carboxylesterase (CES), turned into hydrogelators that self-assembled in water to form nanofibers upon the cleavage of ester bond catalyzed by CES. At certain concentration, the precursors were innocuous to cells, but they dramatically boosted the activity of cisplatin against drug-resistant ovarian cancer cells by 2- to 3-folds.[303]

Maruyama et al. also utilized enzymes to instruct the intracellular self-assembly of a peptide derivative to selectively inhibit cancer cells that express high level of metalloproteinase-7 (MMP-7) (Figure 16B).[304] The precursors developed by them became hydrogelators extracellularly in the

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4 presence of MMP-7, which self-assembled to form nanofibers after entering cells, inducing cell death.
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6 Notably, this small molecule exhibited remarkable cytotoxicity to several cancer cell lines while showed
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8 low cytotoxicity to normal cells due to the fact that cancer cells secrete excessive amount of MMP-7.
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10 Based on the report of the intracellular EISA of L-peptide derivative (Nap-FFK_pY),[119, 305, 306] Gao et
11
12 al. reported the use of Nap-FFK_pY to co-assemble with indocyanine green (ICG) for *in vivo* cancer
13
14 theranostics. The formation of ICG-doped nanofibers not only enhanced the tumor uptake of ICG (by 25-
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16 fold compared with free ICG), but also largely extended the retention time of ICG in tumor site, resulting
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18 in complete and precise tumor elimination by laser ablation (Figure 16C).[307]
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Figure 16. (A) Enzyme-instructed intracellular molecular self-assembly of a D-peptide derivative boosts activity of cisplatin against drug-resistant ovarian cancer cells (SKOV3). (B) MMP-7 mediated intracellular self-assembly of a peptide derivative selectively inhibits cancer cells. (C) An endogenous phosphatase-triggered co-assembly strategy to form tumor-specific indocyanine green (ICG)-doped nanofibers for cancer theranostics. (Adapted from © 2015 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2015 American Chemical Society with permission. [23, 304, 307])

Figure 17. (A) Enzyme-instructed pericellular self-assembly of a small D-peptide derivative selectively inhibits cancer cells over normal cells. (B) Regulating the rate of molecular self-assembly for selectively targeting cancer cells by tailoring the number of enzymatic triggers on a single molecule. (C) Enzymed mediated self-assembly of an aromatic carbohydrate amphiphile selectively inhibits cancer cells. (D) Ligand-receptor interaction (i.e., vancomycin and D-Ala-D-Ala) to catalyze the aggregation of small molecules via an auto catalysis manner, which induce cell necroptosis. (Adapted from © 2014, 2016

Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2016 Royal Society of Chemistry, 2015 American Chemical Society with permission. [20, 107, 308-310])

While the intracellular self-assembly of small molecules to form nanostructures for inhibiting cancer cells are having more instances, extracellular self-assembly of small molecule unexpectedly has shown the ability to form pericellular nanofibers for selective inhibiting cancer cells over normal ones (Figure 17A)[107]. This observation is the first example of using ectoenzyme to regulate the self-assembly of small molecules for controlling the fate of cells. Mechanism studies revealed that the selectivity came from that cancer cells overexpress certain phosphatase on their surface, as was confirmed by the fact that adding phosphatase inhibitor largely eliminated the cytotoxicity of the precursors. Notably, in the co-culture of cancer cells (i.e., HeLa) and normal cell (i.e., HS-5), which largely mimicked the tumor microenvironment, the ectophosphatase-instructed hydrogelation of the designed D-peptide derivative mainly occurred in the pericellular space of cancer cells (HeLa), inducing cancer cell death, while the majority of the normal cells (HS-5) remained alive (Figure 17 A).[107, 311] Mechanistic investigation indicated that the nanofibers, selectively formed around the cancer cells overexpressing phosphatases, acted as multifaceted inducers of apoptosis by interacting with extrinsic cell death ligands and receptors (Figure 17A).[311] In a related study, the minimal C-terminal modification (only a 2% change of the molecular weight) of that D-peptide significant enhanced the anticancer potency, decreasing the IC₉₀ against cancer cells (i.e., Saos-2) from 470 μ M to 36 μ M.[308] In fact, the enhancement of efficacy is remarkable because the anticancer potency of the assemblies of the D-peptide derivative is even higher than that of a clinical used drug (e.g., carboplatin) against the same cells. These promising results motivated a comprehensive molecular and cellular validation of EISA as a multiple step process for selectively killing cancer cells that overexpressed alkaline phosphatase (ALPs). The use of a series of D-tetrapeptides containing zero, one or two enzymatic triggers[21] demonstrated that the peptides without phosphate (i.e., enzymatic trigger) were innocuous to all the cell lines, while both the mono- and diphosphorylated peptides selectively inhibited the cancer cells but sparing normal cells. The observation that the mono- and diphosphorylated peptides exhibited different cytotoxicity against different cancer cell

lines, in fact, has led to the demonstration of selective inhibition of osteosarcoma cells over hepatocytes by regulating the rate of molecular self-assembly (Figure 17B).[20] Besides promising site selective drug delivery, this result opens a way to use the expression level (or activity) of enzymes to autonomously dose the drugs for selectively inhibiting cancer cells, which is certainly worth further exploration.

Another report on EISA is the conjugate of cholesterol and phosphotyrosine to selectively inhibit drug-resistant ovarian cancer cells by simultaneously activating extrinsic (e.g., DR4 and DR5) and intrinsic (caspase 3 and caspase 8) cell death signaling.[312] Notably, the IC_{50} of this conjugates is about five times lower than the IC_{50} of cisplatin (a clinical drug) against platinum-resistant ovarian cancer cells (A2780cis), promising to be an innovative anticancer drug. The feasibility to utilize extracellular EISA for pericellular hydrogelation that inhibited cancer cell selectively was also confirmed by Pires and Ulijn, who reported an aromatic, phosphorylated derivative of carbohydrate for pericellular EISA (Figure 17C).[309] Treating osteosarcoma Saos-2 cell and prechondrocytes ATDC5 cell (expressing 15-20 times lower ALP on the cell surface than on Saos-2 surface) with the designed carbohydrate amphiphile resulted in different consequences—the amphiphiles were more inhibitory to Saos-2 cells than to ATDC5 cell. This concept is also valid for other self-assembly entities, such as nanoparticles.[313, 314] For example, the D-phosphotyrosine decorated magnetic nanoparticles selectively captured and killed cancer cells upon the dephosphorylation by alkaline phosphatase overexpressed on cancer cell surfaces.[313, 314] Specifically, in-situ dephosphorylation on the surface of cancer cells resulted in the aggregates of the nanoparticles to adhere to the cell surface. With the assistance of a magnet, cancer cells can be captured or sorted. Changing the chirality of phosphotyrosine abolished the capturing ability and inhibitory effect of the nanoparticles, indicating a facile approach that used chirality to control the reaction-diffusion of nanoparticles for selectively targeting cancer cells.[315]

Besides EISA, ligand-receptor interaction (e.g., vancomycin binding D-Ala-D-Ala) also was able to catalyze the self-assembly of small molecules in an autocatalysis manner, which resulted in cell death because the resulting aggregates adhered to the cell surface to induce necroptosis.[316-318] Mechanism

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4 investigation revealed that the cytotoxicity largely depended on the ligand-receptor interaction and
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6 aromatic-aromatic interactions (Figure 17D).[310]
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8 9 **4.4. Imaging**

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11 **Fluorescent imaging**, now a popular and important method in biological research, can provide new
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13 perspective and precise **details** on the structural organizations of cells, the sophisticated cellular events,
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15 and the dynamics of biomolecular assemblies **over** wide timescales. Since small self-assembling
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17 molecules (e.g., hydrogelators) have found increased applications in biology and biomedicine, it is
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19 important and necessary to gain a comprehensive understanding of their self-assembly behavior in
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21 biological environment, their interactions with organelles, and their dynamics in cellular milieu.
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23 Therefore, considerable efforts have focused on combining **fluorescent imaging** and supramolecular
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25 chemistry (e.g., molecular recognition or molecular self-assembly) because fluorescent imaging is a
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27 straightforward yet revealing approach to distinguish, depict, and record the behavior of supramolecular
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29 assemblies in cellular environment. In general, synthetic fluorophores have a small size, exhibit many
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31 colors spanning the whole spectrum of visible light, are readily available for chemical modification, and
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33 have advantages of brightness, photostability, and photoswitchability. Thus, they have the potential to
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35 substantially accelerate the broad applications of supramolecular biofunctional materials. The covalent
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37 incorporation of a suitable fluorophore to a self-assembling small molecule not only allows **monitoring** of
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39 the emergent properties of supramolecular assemblies, but also reveals the responses of biological
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41 systems (mainly cells) upon interaction of the supramolecular assemblies. In this section, we mainly focus
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43 on the applications of fluorescent hydrogelators for imaging in cellular milieu.
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Figure 18. (A) Imaging enzyme-triggered supramolecular self-assembly of a small molecule inside cells. (B) Imaging assembly-dependent spatial distribution of small molecules containing different fluorophores in a cellular environment. (C) EISA for spatiotemporal profiling of the activities of ALP on live cells. (D) The design of a probe with lower background fluorescence compared to conventional quenched probes by

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4 optimized molecular self-assembly. (Adapted from © 2012 2012 Macmillan Publishers Limited, part of
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6 Springer Nature, 2012 American Chemical Society, 2016 Elsevier Inc., 2015 Wiley-VCH Verlag GmbH
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8 & Co. KGaA, Weinheim with permission.[119, 306, 319, 320])
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11 Vancomycin (Van) is a powerful glycopeptide antibiotic against methicillin-resistant Gram-positive
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13 bacteria by specific binding to D-Ala-D-Ala sequence presented in bacteria cell wall. Utilizing this
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15 property and based on the self-assembly of vancomycin on bacteria surface,[201] Xing et al. developed a
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17 simple molecular strategy of divalent vancomycin-porphyrin for application in fluorescent imaging of
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19 vancomycin sensitive and resistant strains.[321] However, the imaging of self-assembly of small
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21 molecules inside cell requires more than just attaching a fluorophore, especially for the imaging of EISA
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23 in cellular milieu. The first report of the imaging of EISA of small molecules inside live cells was on the
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25 phosphatase-catalyzed self-assembly inside cells. The conjugate of a fluorophore to a self-assembling
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27 backbone with a phosphatase trigger (i.e., phosphotyrosine as a substrate of ALP) afforded a precursor
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29 (Nap-FFK(NBD)_pY) (Figure 18A) with good solubility so that the precursors dissolved well in
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31 physiological condition and diffused freely to make a homogeneous distribution of fluorophores. Upon
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33 the enzymatic dephosphorylation by ALP, the precursors turned into hydrogelators with decreased
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35 solubility to self-assemble to form nanofibers, which had fluorophores with much higher density thus to
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37 exhibit enhanced fluorescence. The principle for imaging molecular self-assembly inside live cells is that
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39 the resulting nanofibers are distinctly visible due to condensed and localized fluorophores after EISA
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41 (Figure 18A).[119] In another study about three fluorescent analogues consisting of the same L-peptide
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43 backbone, but with different fluorophores on the side chain of lysine, the fluorophores largely affected the
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45 EISA behavior of these molecules, thus further affecting the distribution of these small molecules in
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47 cellular environment (Figure 18B).[306] For example, the nanofibers of dansyl-bearing hydrogelators
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49 mainly adhered to the cell membrane and also exhibited significant cytotoxicity. The assemblies with
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51 DBD fluorophores were unable to enter cells and remained innocuous to cells. The molecules of
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53 rhodamine, being unable to self-assemble, showed homogenous fluorescence inside and outside of cells
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55 and barely affected the cells. These observations revealed that EISA could control the fate of cells via
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modulating the spatiotemporal distribution of the nanoscale assemblies of small molecules. To avoid the disadvantages of fluorescence labeling (e.g., alteration of intermolecular interactions, the need to confirm the innocence of the fluorophores, or the high cost of fluorophores), a doping method was used to assist the imaging of the self-assembly of small molecules without fluorescence inside mammalian cells. That was, the incorporation of tiny amount of dansyl-labeled hydrogelator into the self-assembly of the native molecules was able to determine the formation, localization, and progression of molecular assemblies generated from the non-fluorescent small molecular hydrogelators. The images taken with correlative light and electron microscopy (CLEM) confirmed the presence of ultra-structural assemblies of these precursors upon enzymatic catalysis in a cellular level.[305] The use of D-amino acids to replace the L-amino acids in the self-assembling molecule containing NBD fluorophore (mentioned above,[119] Nap-FFK(NBD)_pY in Figure 18A) yielded the enantiomer of the fluorescent precursor. Unexpectedly, the resulting D-enantiomer is still an excellent substrate of ALP. It, in the presence of the phosphatase, turned into a self-assembling molecule to form nanofibers.[30] Thus this D-peptide based precursors, having greatly enhanced biostability, was also able to serve as an effective molecular probe for imaging intracellular molecular self-assembly instructed by phosphatases.

Because NBD exhibited enhanced fluorescence upon the formation of nanofibers and D-peptides ensured proteolytic stability in cellular environment, a precursor containing two parts—NBD and a phosphorylated, self-assembling D-peptide backbone—acted as a fluorescent probe to profile the activities of ectophosphatases on live cells by EISA to form localized, fluorescent, biocompatible and non-diffusive nanofibers (Figure 18C).[319] This precursor revealed different ALP levels on different cell surfaces and even worked in the co-culture of cancer and normal cells. Being a supplementary approach to the existing methods, this activity probe for ectophosphatase would contribute to our understanding of reversible phosphorylation-dephosphorylation in extracellular domains.

Utilizing the environment-sensitive property of NBD motif, Yang et al. designed a NBD-capped molecular probe (i.e., NBD-FFYEEGGH) consisting of three parts: the NBD (providing environment-sensitive fluorescent signal), the FFYEE sequence (rendering the molecule better self-assembly capacity

and brighter fluorescence after self-assembly), and GGH motif (specifically binding to Cu^{2+} , making it sensitive to the present of Cu^{2+}). Based on that the specific binding to Cu^{2+} efficiently quenched the fluorescence of the formed nanofibers, Yang and co-workers confirmed the molecule as an excellent probe for the detection of Cu^{2+} *in vitro* and in cellular environment. Moreover, the incorporation of an enzyme cleavage site DEVD into this molecule afforded another probe NBD-FFFDEVDGGH, which not only was able to detect the presence of Cu^{2+} , but also was used for the detection of caspase-3 *in vitro* and in cells.[322]

Yang et al. found that the supramolecular self-assembly containing 4-fluoro phenylalanine ($^{\text{F}}\text{F}$) was able to greatly enhance the quenching effect of the quencher (i.e., dabcyI) in molecular probes by synergistic effects of FRET and ACQ. To optimize the quenching effect, they simply varied the number of $^{\text{F}}\text{F}$ being incorporated into the molecular backbone. Briefly, they conjugated a quencher dabcyI and a fluorophore (rhodamine or FITC) to the N-terminal and side chain of a peptide backbone ($\text{GF}_n\text{G}_{(3-n)}\text{DEVDGK}$) and also introduced aromatic $^{\text{F}}\text{F}$ on phenylalanine. The self-assembly of the resulting molecule largely enhanced the quenching effect of dabcyI to FITC, and this molecules was able to rapidly respond to caspase-3 to recover its fluorescence due to the presence of enzyme cleavage site DEVD (Figure 18D).[320] Ding and Yang et al. developed a highly sensitive colloidal fluorophore, as a turn-on bioprobe (TPE-GFFYK(DVEDEEAc)), through facile incorporation of a self-assembling peptide sequence GFFY between a fluorophore TPE and a caspase-3 cleavage site. This probe fluoresced more in the presence of caspase-3 since the removal of DVEDEEAc segment by enzyme promoted the self-assembly of the remaining TPE-GFFYK fragment. In this case this probe could be used for imaging caspase-3.[323] Yang et al. also designed and synthesized a far-red/near-infrared (FR/NIR) fluorescence light-up probe (DBT-2EEGWRESAI) by conjugating two peptides with same sequences (EEGWRESAI, tax-interacting protein-1 (TIP-1) specific binding ligands) to both sides of a fluorophore 4,7-di(thiophen-2-yl)-2,1,3-benzothiadiazole (DBT). DBT is an environment-sensitive dye—in aqueous solutions, the probe DBT-2EEGWRESAI is weakly fluorescent while it lighted up upon the specific binding with TIP-1 or polyprotein ULD-TIP-1 to form nanofiber network or spherical nano-complexes. The self-assembly-

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4 induced FR/NIR fluorescence turn-on made DBT-2EEGWRESAI an excellent probe for detecting and
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6 visualizing specific protein/polyprotein-peptide interactions in both cell-free solutions and in live
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8 bacteria.[324]
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11 Besides supramolecular assemblies, molecular beacon[325] is another useful imaging tool based on
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13 nanoscale assemblies to reveal cellular and subcellular activities at molecular level by converting cellular
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15 events into optical signals. In 2013, Cui et al. reported a rational design of supramolecular spherical
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17 nanobeacons that served as a probe of cathepsin B.[326] Later in 2016, the same group developed another
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19 positively charged spherical nanobeacon for intracellular sensing of cathepsin B. They demonstrated that
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21 self-assembly of the well-designed molecules into filamentous nanobeacons significantly reduced their
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23 internalization by cancer cells, a useful property for probing extracellular protease activities.[327]
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27 In addition to fluorescent imaging, magnetic resonance imaging (MRI) is another useful approach to
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29 report real-time molecule distribution because of its superior spatiotemporal resolution and soft tissue
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31 contrast. There have been several attempts to incorporate MRI modalities into supramolecular assemblies
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33 for real-time imaging applications. For example, chemical conjugation of a MRI modality into a self-
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35 assembling peptide derivative yielded a dynamic material that changed shape and size in response to
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37 slight pH changes in solutions that mimicked serum. This morphological change was rapid and reversible
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39 and occurred under thermodynamic equilibrium, which was ideal for *in vivo* MRI imaging.[328] To track
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41 the fate of bioactive supramolecular nanostructures, Stupp and Meade designed a series of PA molecules
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43 based on the well-established β -sheet peptide (V_3A_3) conjugated to macrocyclic Gd(III) labels for MRI
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45 imaging. They confirmed the feasibility of such strategy as an effective tracking method for PA materials
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47 *in vivo* over the timescale of days.[329] Using the Gd-based compounds and materials, Cui et al. also
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49 developed a supramolecular strategy to convert an FDA-approved anticancer drug, Pemetrexed, to a
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51 molecular hydrogelator with inherent chemical exchange saturation transfer (CEST) MRI signals.[330]
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53 The CEST MRI signal enabled noninvasive monitoring of the *in vivo* distribution and drug release profile
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55 of Pemetrexed.
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59 60 4.5. Immunology 61 62 63 64 65

Immunology is an important branch of biological and medical sciences, where there has been recent interest in designing and developing novel therapies to modulate the immune system for treating cancers. Generally, supramolecular systems are able to modulate the responses of immune systems through either drug delivery or controlled presentation of immune signals (e.g., short peptide epitopes or antigens). Supramolecular hydrogels/assemblies, possessing artificial scaffolds that present multiple cell-interacting entities in spatially resolved network, are being explored as a new choice of adjuvant or delivery vehicles in immunotherapies.[18] Since we have discussed the supramolecular hydrogel for drug delivery in previous section, we will focus on presenting immunomodulating signals in this part. More specifically, the applications of supramolecular systems in immunology discussed here center on vaccine adjuvants and self-adjuvanting vaccines.

While safe and potent adjuvants are always in demand, supramolecular hydrogels made of peptide derivatives can serve as excellent candidates for adjuvants in immunotherapies and infectious diseases. For example, Collier et al. developed a self-assembling peptide OVA-Q11,[331] which contained three parts: the self-assembling transglutaminase substrate (Q-11), a spacer, and an epitope of OVA₃₂₃₋₃₃₉. [332] This peptide self-assembled to form long, unbranched nanofibers that displayed the epitope on their surfaces under physiological conditions and the resulting epitope-bearing nanofibers raised IgG1, IgG2a and IgG3 in levels similar to epitope peptide delivered in complete Freund's adjuvant (CFA). Importantly, it promoted IgM production even greater than the epitope peptide itself. Experimental data revealed the correlation between the enhancement of the immune responses and the self-assembly behaviors of the peptides, which were not immunogenic by themselves (Figure 19A).[333] By investigating the molecular determinants and immunological mechanisms leading to the significant immunogenicity of this self-assembling peptide, Collier and coworkers found that these responses were T-cell dependent because deleting amino acid sequences in T-cell recognizing region diminished the immunogenicity. Another self-assembling peptide OVA-KFE8, bearing the same epitope, also elicited strong antibody responses similar to OVA-Q11, indicating that the adjuvant action was not specific to certain self-assembling peptide sequences.[334] Besides varying the self-assembling peptides, Collier et al. developed another vaccine

adjuvant (NANP₃)-Q11 by incorporating another peptide epitope from plasmodium falciparum circumsporozoite protein into the self-assembling peptide. The resulting peptide raised long-lived T-cell-dependent antibody responses without frequent boosts. The co-assembling with a different epitope-bearing peptides had little influence on the antibody responses to either epitopes, suggesting the promises of developing multi-epitope vaccines.[335] The incorporation of p-nitrophenyl phosphonate (pNP) into the same peptide sequences Q11 afforded another self-assembling self-adjuvanting vaccine. The resulting peptide self-assembled into nanofibers and subsequently reacted with cutinase fusion proteins to afford protein-laden nanofibers, which had a control over the amounts of protein antigen and acted as self-adjuvanting vaccines in mice.[336] Similarly, by combining two native sequences form a spider silk elastic segment and a human muscle calcium channel segment, Sun et al. designed a self-assembling peptide h9e (FLIVIGSIIGPGGDGPGGD), which self-assembled to form a hydrogel either in acidic condition or in the presence of Ca²⁺. The hydrogel made by the addition of Ca²⁺ was self-recovering and was able to serve as an H1N1 influenza vaccine adjuvant.[337] By evaluating the immunological response of h9e as an adjuvant for PRRSV modified live virus (MLV) vaccines, Sun and coworkers demonstrated that the h9e hydrogel, as an adjuvant, improved vaccination-induced host protection against RRRSV infection.[338] Li and coworkers designed, synthesized, and evaluated several synthetic glycopeptides, as self-adjuvanting vaccine candidates with self-assembly domains (Q11). Briefly, they facilely incorporated different vaccine candidates, H1, H2, H3 and H4 into the self-assembling sequences Q11 and prepared nanofibers over 200 nm in length with the resulting molecules. Immunological evaluations revealed that the synthetic glycopeptide with Tn glycosylation in the PDTRP domain elicited significant immune responses. The induced antibody was able to recognize human MUC1-expressing tumor cell.[339] In another recent work, Jiang and Yang et al. reported that the EISA of a small peptide to form nanofibers could efficiently condense DNA, resulting in strong immune responses against HIV by promoting DNA transfection and enhancing gene expression in vitro (Figure 19B).[19] The same group also developed a supramolecular hydrogel formed by a self-assembling D-tetra-peptide, which was able to serve as a promising vaccine adjuvant to evoke both humoral and cellular immune responses.[340]

Modifying the same molecule with an enzymatic trigger and a C-terminal capping, Yang et al. demonstrated that the *in situ* formation of peptidic nanofibers facilitated the induction of multiple crucial immunities against HIV DNA vaccine, including polyfunctional T cell response, broad IgG subclasses response, and V1/V2 loop-specific antibody response, all of which could hardly be triggered by HIV DNA vaccine alone.[341]

Figure 19. (A) A self-assembling peptide (OVA-Q11) acts as an immune adjuvant. (B) A peptide-based nanofibrous hydrogel of G-NMe as a promising DNA nanovector for optimizing the efficacy of HIV vaccine. (C) De Novo chemoattractants form supramolecular hydrogels for immunomodulating neutrophils *in vivo*. (Adapted from © 2010 National Academy of Sciences, 2014 American Chemical Society with permission.[19, 333, 342])

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4 While molecular hydrogels or nanofibers are mostly used as self-adjuvanting vaccine or adjuvants,
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6 the incorporation of an unnatural amino acid into a well-established chemoattractant N-formyl-L-
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8 methionyl-L-leucyl-L-phenylalanine, fMLF, afforded a supramolecular hydrogelator that self-assembled
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10 to form *de novo* multifunctional nanofibers or hydrogels. The resulting hydrogels acted as
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12 chemoattractants that exhibited not only preserved cross-species chemoattracting activity to human and
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14 murine neutrophils, but also protease resistance (Figure 19C).[342]
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17 5. Perspective and Outlook

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19 Rather than focusing on replicating the exact molecular structure of biomacromolecules or natural
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21 products through tedious synthesis, supramolecular biofunctional materials, being resulted from self-
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23 assembly of simple molecular entities via noncovalent interactions, aim to mimic sophisticated functions
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25 in biological systems. Without the constraints imposed by complex structures, the functional mimicking is
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27 always much more effective and simpler than replicating. One important future direction of
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29 supramolecular chemistry is to explore self-assembly of small molecules in biological systems; the
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31 development of supramolecular biofunctional materials is a core activity of that mission. For example, by
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33 exploiting innovative methods and approaches, the development of supramolecular biofunctional
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35 materials will improve the spatiotemporal precision of supramolecular nanostructures for better
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37 mimicking the functional aspects of extracellular matrix in nature so that supramolecular biofunctional
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39 materials will be able to modulate the behaviors of biological entities in well-defined or highly
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41 controllable manners.
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47 By utilizing the biological building blocks for the construction of various biofunctional materials via
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49 self-assembly and noncovalent interactions, supramolecular chemistry represents the most molecular
50
51 example of the bioinspired researches, which use phenomena in biology to guide and to stimulate the
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53 interdisciplinary sciences and technologies of chemistry, physics, biology, and engineering.[123] Nature
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55 has displayed many strategies for creatures to survive and thrive. These strategies, in essence, are
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57 molecular phenomena over a wide length scale (from nanometers to meter), which provide unlimited
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59 inspirations for solving problems in everyday life and in biomedicine. The development of
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supramolecular biofunctional materials now and in the future heavily relies and will keep relying on biological systems as a source of inspiration—both in problems and solutions. Thus, the advancement of our understanding of biology will fuel the development of supramolecular biofunctional materials to meet new challenges in developing innovative strategies for biomedicine. On the other hand, as a mimicry or simplified model of real systems in biology, the progresses of the supramolecular biofunctional materials will deepen and broaden our understanding of biological systems. That is the reason why supramolecular biofunctional materials will remain a frontier in biomaterials and chemistry. Over the past three decades, supramolecular chemistry has provided guiding principles to understand intermolecular interactions, as well as for designing medicines based on those principles (e.g., molecular recognition). As the fundamental science of intermolecular interactions, supramolecular chemistry, in the coming years, will become a key strategy for creating biofunctional materials that promise unlimited opportunities and applications in biomedical and clinical practice.

Abbreviations

Abbreviation	Full name
EISA	enzyme-instructed self-assembly
CNB	α -carboxy-2-nitrobenzyl
CPT	camptothecin
NBD	4-nitro-2,1,3-benzoxadiazole
SPPS	solid phase peptide synthesis
G	glycine
EAK16	Ac-(AEAEAKAK) ₂ -NH ₂
A	alanine
E	glutamic acid
K	lysine
Nap	2-(naphthalene-2-yl)
Fmoc	fluorenylmethoxycarbonyl
GHS	glutathione
mES	mouse embryonic stem cell
PA	peptide amphiphile
SHH	sonic hedgehog
CN	cavernous nerve
AIF-PA	anti-inflammatory peptide amphiphile
HBPA	heparin-binding peptide amphiphile
hMSC	human mesenchymal stem cell
NSAID	nonsteroidal anti-inflammatory drugs
NO	nitric oxide
DTT	dithiothreitol
PriSM	prion-like nanofibrils of small molecules

CES	carboxylesterase
MMP-7	metalloproteinase-7
GNL	glycosyl-nucleoside-lipid
ICG	indocyanine green
ALP	alkaline phosphatase
Van	vancomycin
CLEM	correlative light and electron microscopy
¹⁹ F	4-fluoro phenylalanine
FR/NIR	far-red/near-infrared
TIP-I	tax-interacting protein-I
DBT	4,7-di(thiophen-2-yl)-2,1,3-benzothiadiazole
CFA	complete Freund's adjuvant
pNP	p-nitrophenyl phosphonate
MCD	methyl-β-CD
ACE	acetyl Cholin Esterase
MRI	magnetic resonance imaging
CEST	chemical exchange saturation transfer
GNF	glycosyl-nucleoside-fluorinate

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