Interactions Between Peptide Assemblies and Proteins for Medicine

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Abstract: Peptide assembly is attractive not only to develop biotechnological tools and smart nanomaterials, but also to treat pathologies in new ways. This Review focuses on recent progress made in the exciting area pertaining the interaction between peptide assemblies and proteins. Earlier works aimed to identify proteins able to bind peptide assemblies for therapeutics’ delivery and biocompatible scaffolds. Recent advancements cover more applications that go beyond tissue regeneration and biomaterials. On one hand, self-assembling peptides interacting with proteins can inhibit pathological amyloid fibrillation, or boost the immune response to vaccines. On the other, they have been exploited to promote protein crystallization, also for therapeutics’ delivery. As research advances in this exciting area, it opens the way towards a qualitative leap in the clinical translation of supramolecular medicinal chemistry to creatively tackle unmet challenges of modern disease treatment and prevention.

Keywords: peptide · self-assembly · proteins · biomaterials · vaccines

1. Introduction

The ability of proteins to interact with short peptides has been used not only to trigger peptide fibril formation, but also to promote selective in vitro and in vivo protein-peptide interactions. In this manner, composite materials find applications in the biomedical field, encompassing tissue engineering, protein crystallization, protein delivery, cancer therapy, bioimaging, vaccines, etc.[1]

A popular class of proteins that is widely studied comprises those with enzymatic activity. In particular, the use of enzymes to tailor peptide self-assembly is a widely reviewed topic that will thus be covered here only pertaining applications in cellula.[2] Enzymes can act as convenient endogenous triggers for diagnostic tools that exploit aggregation-induced-emission, possibly even coupled with drug release towards theranostics.[3] Alternatively, self-assembling peptides can be convenient enzyme mimics as recently reviewed elsewhere,[4] with hydrolyses being amongst the most studied targets,[5] although their effective design is not always trivial[6] and undesired side reactions may lead to catalyst modification.[7]

This Review focuses on recent progress on the mastering of interactions between peptide assemblies and proteins. We believe that the recent advances at the exciting interface between nanotechnology, medicinal and supramolecular chemistry, is opening the door towards the clinical translation of therapeutic concepts that were once unimaginable to address the unmet medical challenges of our time.

2. In Vitro and In Vivo Studies for Therapy

2.1 Identification of Proteins Interacting with Peptide Assemblies

Early works were proofs of concept that short-peptide fibrils were able to interact with certain proteins in a non-specific manner. Based on this premise, Hamachi et al. developed a peptide hydrogel used as medium to detect and measure enzymatic activity.[8] Xu et al. devised a strategy to discover which proteins from HeLa cell lysates had higher capacity to

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interact with peptide fibrils. To fix the interacting biomolecules, a photo-leucine was incorporated in the peptide backbone. After lyase incubation, application of UV light allowed protein-peptide cross-linking, thus favoring the purification and recognition of the protein-peptide complex by simply washing, boiling, centrifuging, and running an electrophoresis gel for separation. Results proved that peptide fibrils interacted with actins, tubulins, and other proteins, probably, chaperonins. The same group also proved that the morphology of the peptide assemblies had a strong impact on their interaction with proteins. This effect might be due to the different surface-to-volume ratio of the aggregate, or to a different hydrophobic/hydrophilic balance, or to chemical groups on the surface of the supramolecular assembly.

The interactions between peptides and proteins having an impact on the morphology of the resulting aggregates were also observed by Ulijn et al. They proved that different protein clusters had a direct effect on peptide self-assembly modifying the structural and mechanical properties of the resulting gels and even the chirality of the protein-peptide cooperative co-assemblies.

2.2 Pathological Amyloids Interacting with Peptide Assemblies

A study by Mirave and Escalet et al. shed light over the cooperative growth of peptide-protein assemblies, using a family of short motifs presenting a different backbone distribution of phenylalanine and aspartic acid residues and the amyloid peptide fragment Aβ(1–40) involved in Alzheimer’s disease. Interestingly, specific combinations of amino acids showed the ability to interact with Aβ(1–40), thus giving rise to long fibrils formed by the cooperative co-assembly of the short peptides and the amyloid fragment. Conversely, heterochiral tripeptides that could self-organize into nanoparticles were designed by Marchesan’s group to inhibit Aβ(1–42) fibrillation by exploiting the amyloid-derived diphenylalanine as bait for binding, and proline as β-breaker (Figure 1). Despite the presence of a D-amino acid, the most effective tripeptide inhibitor displayed good biocompatibility in vitro. This data supported earlier findings on similar amyloid-derived short motifs, whose cytotoxicity was alleviated by the heterochiral design that impeded the hierarchical formation of toxic microfibrils. Table 1 summarizes the short-peptide sequences used to inhibit Aβ fibrillation.

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José A. Gavira obtained his PhD at University of Granada (2000), First Prize within the PhD-Chemistry program. Postdoctoral fellow at the University of Huntsville (UAH) in Alabama (USA) and the Marshal Space Flight Centre (NASA), in 2003, joined back the Laboratory of Crystallographic Studies (LEC) of the Spanish National Research Council (CSIC) to lead the protein crystallography research line where holds a permanent position (2007). Since 2021 he is the head of the LEC.

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Silvia Marchesan did her PhD at The University of Edinburgh (2008), and postdoc research at the University of Helsinki, Monash University, and CSIRO. She then moved to Italy to set-up in 2015 the Superstructures Lab (www.marchesanlab.com). In 2018, she became associate professor, got habilitated as full professor, and was selected by Nature as Rising Star in the natural sciences. Since 2021 she is visiting the University of Cambridge (UK).
2.3 Peptide Assemblies as Depots for Protein Inhibitors

Several therapeutics act as protein inhibitors. Their initial design often takes inspiration from the natural peptides that normally interact with the target protein, for instance as substrates or natural inhibitors. However, peptides have been traditionally considered an impractical choice for therapeutics, in light of their limited stability against enzymatic degradation and poor pharmacokinetics, although new approaches are enabling clinical use also orally.\(^{29}\)

In this regard, self-association of bioactive peptides has been envisioned as an interesting strategy to provide a depot for their long-term release to overcome such limitations. Roches and co-workers have successfully applied this approach to inhibit HIV-1 integrase (Figure 2), whereby the target enzyme can shift the equilibrium from the fibril to the active monomer.\(^ {39}\) The same concept can be further extended to other morphologies and active principles. As an example, spherical particles of self-assembling peptides could be loaded with small molecules to deliver them within cells.\(^ {33}\) Systems of this kind can be further implemented towards theranostic applications, by coupling drug release with a displacement-based fluorescence response, to track both release rate and localization of the drug.\(^ {33}\) Alternatively, peptide-based hydrogels can provide effective vehicles for the delivery of antimicrobial peptides, such as polymyxin B over several days, as recently demonstrated by Azevedo and co-workers.\(^ {24}\)

2.4 Protein and Peptide Assemblies for Biomaterials

Interactions between short-peptide hydrogels and proteins have also been explored for the delivery of therapeutic...
proteins. Proteins are complex molecules to stabilize, due to their large size and three-dimensional structure. Protein delivery vehicles must allow high protein loadings, and guarantee their stability and activity in vivo. Peptide hydrogels have many advantages for protein delivery since they have a high water content, they are biocompatible, and they form under mild and physiological conditions, allowing for gelation in the presence of proteins. Additionally, many of them can be administered by injection, thus with minimal invasiveness. Interactions between peptide fibrils and proteins can also stabilize proteins and modify their release rate. Furthermore, the reticular hydrogel structure can prevent the incorporation of degradative enzymes, thus increasing protein in vivo stability and effectiveness.\[25\]

Self-assembling peptides are ideally suited to mimic proteins and deliver functional biomaterials.\[26\] Zhang et al. pioneered the use of self-assembling peptides as vehicles for proteins. In a preliminary work, they tested the peptide Ac-(RADA)\(_n\)-CONH\(_2\) (RADA16-I) to study the influence of peptide concentration and hydrodynamic radii of different model proteins on their delivery. Four proteins, namely bovine serum albumin (BSA), lysozyme, immunoglobulin G (IgG), and soybean trypsin inhibitor were tested. The major factors controlling protein diffusion were indeed protein size and peptide concentration.\[27\] The same group studied the influence of the electrostatic interactions between peptides and proteins in their release, which was inhibited when using oppositely charged components.\[28\] Since then, many shorter amphiphilic sequences have been reported to self-assemble and gel,\[29\] also with bioactive motifs to interact with integrins and induce cell adhesion,\[30\] and the use of modern techniques such as high-resolution cryo-TEM are proving key for the structural elucidation of the nanoassemblies.\[31\]

The efficacy of the delivery system has been also evaluated by measuring the biological activity of the therapeutic proteins once released in the media. Different growth factors have been delivered and their activity has been evaluated in vitro and in vivo. The administration and slow release of IGF-1 (insulin-like growth factor 1) together with cardiacomyocytes in a rat's infarcted heart showed an improved myocardium recovery that could not be ascribed only to the administration of cells.\[32\]

Schneider and Pochan used β-hairpin peptide hydrogels, such as, MAX1 (VKKVKVK-V\(_n\)-PPT-KVKVKVKVNIH2) and MAX8 (VKKVKVK-V\(_n\)-PPT-KVEVVKVKVINH2). The influence of peptide concentration and charge was evaluated with lysozyme, IgG, lactoferrin, α-lactalbumin, myoglobin and BSA.\[33\] The ability to control the release rate by electrostatic interactions between peptides and proteins was extended to media in which the ionic strength was relevant, such as physiological conditions, using highly charged self-assembling peptides.\[34\]

Aromatic short peptides containing a naphthyl group (Nap=2-naphthylacetic acid) have also been explored as protein vehicles. Yang et al. developed differently charged peptides of NapGGYY(X)ySSE (X stands for K, E, or S) that can form supramolecular fibrils upon glutathione reduction. Confocal laser scanning microscopy was used to monitor the intracellular distribution of the enhanced green fluorescent protein. Data showed that the co-assemblies were essential to internalize the protein, with the K-derivative being better for intracellular administration.\[35\]

The co-assembly of short-peptides and proteins has been explored with the intention of creating an injectable hydrogel for application in tissue engineering. Li et al. created a novel hydrogel by the co-assembly of Nap-FF and silk fibroin from Bombyx mori. The inclusion of the vascular endothelial growth factor showed that the subcutaneous injection of the hydrogel was able to generate new blood capillaries in vivo.\[36\]

Nap-peptides have also been used to promote periodontal bone regeneration. The simultaneous co-assembly of Nap-FF-OH with stromal cell derived factor-1 and bone morphogenetic protein-2 showed the regeneration and reconstruction of periodontal bone tissues in vitro and in vivo. After 8-week treatment of bone defect areas using this hydrogel, a bone regeneration rate of 56.7% was achieved in models rats.\[37\]

Peptide amphiphiles (PAs) formed by conjugating a peptide to a lipid tail were developed mainly by Stupp et al. and were used for the delivery of therapeutic proteins too.\[38\] In particular, the delivery of growth factors was evaluated in vivo. This group has designed a PA that can be co-assembled with heparin, so that heparin arranged on the surface of peptide fibrils, being able to interact more efficiently with angiogenic growth factors. The ability to promote angiogenesis was evaluated in vivo using a rat corneal assay. The heparin-peptide nanostructures combined with growth factors promoted neovascularization.\[39\] The same heparin system has used for the in vivo delivery of growth factors, leading to an increase of blood vessel density in the mouse omentum that enhanced islet engraftment. Diabetic mice recovered glycemia levels when treated with islet cells and heparin-peptide containing growth factors, at a higher rate (78%) than control animals treated without growth factors (30%) or with growth factors alone (20%).\[40\] The authors have also used the same system to deliver an angiogenic growth factor inside islet cells. The administration of the combination of PA and growth factors improved cell viability and function in vitro.\[41\]

Interestingly, Mata et al. showed that the co-assembly of an elastin-like protein with amphiphilic peptides formed robust membranes that could evolve into tubular structures. These sophisticated morphologies proved to act as bioactive scaffolds in tissue engineering.\[42\] The exploitation of biological organization into tissues is indeed a promising strategy to advance biofabrication of multiple biomolecules into tissues for regeneration purposes.\[43\] In particular, in a collaborative effort with the group of Adler-Abramovich, Mata and co-workers showed that self-assembling short peptides can stabilize intrinsically disordered elastin-like polypeptides (ELP) into scaffolds (Figure 3) that allow pre-osteoblast cell adhesion and proliferation, and pH-dependent cargo delivery too.\[44\]
2.5 Peptide Assemblies to Promote Protein Crystallization

A different approach to take advantage of short-peptide fibril-protein interactions has been explored by the groups of Gavira and Alvarez de Cienfuegos. They studied the influence of short-peptide hydrogels as media to grow protein crystals and to create novel protein-composite crystals. Protein crystallization in hydrogel media is a technique that has been known for more than a century. It has been demonstrated that crystals formed in hydrogels tend to be of higher quality and of bigger size, relative to those grown in solution, and both aspects are useful to obtain X-ray diffraction (XRD). Hydrogels are advantageous for protein crystallogenesis, because they avoid sedimentation, reduce convection currents, and, in some cases, promote nucleation. Traditional gels used for protein crystallization have been agarose, silica, or polyethylene glycol (PEG). Although these gels proved useful for protein crystallization, the possibility to tune their chemical composition, morphology, or chirality is either not possible or very limited, thus reducing the application of gels in this field.

The introduction of short-peptide hydrogels in protein crystallogenesis opened the possibility to study in more detail how peptide-protein interactions affect the resulting crystal properties. A first study was conducted to evaluate the influence of peptide fibril chirality in protein crystal formation. To this end, the authors developed two enantiomeric hydrogels based on dimers of cysteine from L- and D-amino acids, respectively. Studies performed with these hydrogels showed that enantiomeric hydrogels had an impact on the quality and on the polymorphism of the protein crystals. When crystals grew in a hydrogel, they occluded the gel material in their interior, giving rise to composite crystals. These composite crystals can have different physicochemical properties based on the composition of the hydrogel. For example, ultrastable protein crystals have been obtained growing lysozyme in silica gel.

Gavira and Alvarez de Cienfuegos proved that lysozyme crystals grown in Fmoc-dipeptide hydrogels presented different ratios of dissolution in water once formed. Surprisingly, lysozyme crystals grown in fluoresceinmethylxocarbonyl-dialanine (Fmoc–AA) presented a rate of dissolution that was extremely slow, relative to crystals grown in other peptide hydrogels, highlighting again the important role of non-specific protein-peptide interactions to modulate the final outcome.

The effects of short peptide fibrils in reducing the rate of dissolution of the composite lysozyme crystals were later explored using insulin as therapeutically essential protein. In this case, the authors were able to obtain homogeneous batches of very small and narrow size distribution of insulin composite crystals, which were ideal for subcutaneous injections. Insulin crystals grown in Fmoc–AA showed a slower dissolution rate and higher thermal stability and, more importantly, these properties were maintained in vivo after subcutaneous injections in rats (Figure 4).
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The structural diversity of peptides was also explored to obtain hydrogels containing specific functional groups that, once occluded in the crystals, could provide them with novel properties or functions. It is known that repetitive cycles of synchrotron irradiation on protein crystals end up in losing the structural information. This is due to protein degradation being mediated by the formation of reactive radical species, which arise from the internal water that is occluded in the crystals.

This phenomenon is known as radiation damage, and to minimize this effect, XRD is performed at 100 K which is not an ideal situation, since the three-dimensional structure of a protein can be different, relative to room temperature. Different scavengers have been used to minimize this effect and enable XRD at room temperature, although most of them did not have a significant impact. To mitigate this effect, short peptide hydrogels containing cysteine (Flmoc-CF) were used. Lysozyme crystals grown in this hydrogel were more resistant to the radiation damage generated after nine cycles of synchrotron irradiation at room temperature, than crystals grown in agarose. The protection was observed in the more sensitive groups of the protein, i.e., cysteine disulfide bonds, and methionine residues.

It is known that aromatic short peptides have a great capacity to interact with highly hydrophobic molecules and nanostructures, such as carbon nanotubes (CNTs), giving rise to composite or hybrid hydrogels. Taking advantage of this property, Askenasy, Gaviria and Alvarez de Cienfuegos et al. tried to obtain composite protein crystals containing in their interior CNTs with the intention to obtain electronic conductive protein crystals. First, they prepared hybrid hydrogels of Flmoc-FF containing increasing concentrations of CNTs that were well dispersed over the whole volume of the hydrogels, and then, they succeeded in growing lysozyme crystals in these hydrogels. The resulting composite crystals occluded CNTs all over their internal structure. This specific distribution of CNTs in the interior of the protein crystals created conductive channels through the whole crystals, thus rendering them electric conductors.

The combination of assembling peptides, proteins, and CNTs bears great innovation potential not only in bioelectronics, but also sensing and bioimaging.

2.6 Vaccine Development

The development of vaccines based on attenuated pathogens presents important drawbacks, which include the cost of production and manufacture, batch-to-batch variability in the composition, and thermal instability that requires costly cold chains. Therefore, a considerable effort has been devoted to the development of subunit or purified-antigen vaccines. This type of approach comes at an expense, as these types of vaccines are less immunogenic, thus requiring the use of adjuvants to elicit an adequate immune response. However, these may display some disadvantages too, as some of them can cause an undesired inflammatory response.

An alternative approach has been more recently explored to provide new solutions to these problems, and it relies on self-assembling peptides to reduce, or even eliminate, the use of adjuvants. The chemical composition of these synthetic derivatives can be finely controlled, and designed to tune the immune response as needed. The basic principle consists in attaching a self-assembling peptide to one or several epitopes, to generate a supramolecular aggregate displaying multiple copies of antigens. The immunogenic capacity is also modulated by the final structure of the supramolecular assemblies, with peptide nanofibers, peptide amphiphiles, and nanoparticles succeeding to elicit an adequate immune response.

Collier et al. were the first to demonstrate that the covalent attachment of an epitope to a self-assembling peptide Q11 (i.e., QQKQFQQEQQ) did not impede fibrillation and could lead to a strong immune response without the use of external adjuvants. The same Q11 peptide has been derivatized with other antigens of different size, such as, B-cell and T-cell epitopes, alun (i.e., QQKQFQQEQQ) did not impede fibrillation and could lead to a strong immune response without the use of external adjuvants. The same Q11 peptide has been derivatized with other antigens of different size, such as, B-cell and T-cell epitopes, alun (i.e., QQKQFQQEQQ) did not impede fibrillation and could lead to a strong immune response without the use of external adjuvants. The same Q11 peptide has been derivatized with other antigens of different size, such as, B-cell and T-cell epitopes, alun (i.e., QQKQFQQEQQ) did not impede fibrillation and could lead to a strong immune response without the use of external adjuvants.

Peptide amphiphiles have also been explored in this area. The group of Tirrell has shown that these peptide amphiphiles can stabilize the secondary structure of some epitopes and elicit a strong immune response. The antigenic peptide is conjugated to a dialkyl lipid tail forming cylindrical micelles in phosphate-buffered saline. This system was able to elicit a strong T-cell response when it displayed a model cytotoxic T-cell epitope on the surface of the micelle. In vivo evaluation of this system reduced the growth of tumors and increased survival in mice. The same strategy has been used to develop a vaccine against group A Streptococcus. Micelles presenting a relevant B-cell epitope produced strong IgG and IgM responses.

Supramolecular peptide fibrils have also been used to condense DNA to develop a vaccine against HIV. It has been shown that the supramolecular fibrillar structure is essential to elicit a strong cellular and humoral response. Yang et al. have developed a series of Nap-tetrapeptides as promising vaccine adjuvants (Figure 5). The co-assembly of these peptides with antigenic proteins, such as ovalbumin, has been designed to form hydrogels by enzymatic catalysis or by a heating-cooling process. Other injectable peptide hydrogels were tested as H1N1 influenza vaccine adjuvants.

2.7 Self-Assembly of Peptides in Cell Milieu

With the advances in controlling peptide self-assembly under physiological conditions, recently, researchers have focused on promoting the self-assembly of peptides inside cells or on their surfaces, triggered by their enzymatic machinery. By doing
this, supramolecular peptide fibrils can interact with multiple cellular proteins providing a new approach to modulate cell behavior. In particular, this strategy was used to induce cancer cell death and for imaging enzymatic activity in cells. The group of Xu has been pioneering this field. They developed a synthetic strategy to create gelator precursors that could self-assemble inside cells by the action of an endogenous enzyme. This strategy, called EISA (enzyme-instructed self-assembly) was first demonstrated using HeLa cells. For this purpose, they developed a Nap-FF functionalized with a cleavable butyric diacid. Once the peptide was inside the cells, an esterase cleaved the butyric residue liberating the Nap-FF gelator that was able to form fibrils and a hydrogel inside the cells, provoking their death. This work was the first example in which the design and self-assembly of small molecules can modulate cell fate. In a following work, the authors studied in more detail the mechanism of cell death induced by peptide Nap-FF. This peptide was able to alter the dynamics of microtubules, causing apoptosis of T98G glioblastoma cells. Interestingly, the same peptide did not show toxicity for PC12 neuronal cells, demonstrating that the tolerability of peptide nanofibrils inside cells is cell-dependent. Peptide hydrophobicity was also essential for cytotoxicity.

Later, the same group explored the possibility to trigger peptide self-assembly in the pericellular space, that is, on cell surfaces by the action of secreted alkaline phosphatases (ALP). Importantly, due to the overexpression of surface ALP in cancer cells, a nanomet/hydrogel was formed selectively around them. This peptide film blocked cellular mass exchange inducing apoptosis.

To evaluate the activity of the endogenous tyrosine phosphates in promoting cancer cell death, different gelator precursors containing one or two phosphorylated tyrosines were evaluated. The mono-phosphorylated precursors were able to gel faster and exhibited a higher degree of inhibition on cancer cells, than the derivatives having two phosphorylases. This result highlights the role of peptide self-assembly kinetics in inhibiting cancer cells. The kinetics of self-assembly have been explored to selectively inhibit one type of cancer cells over another, based on the different concentration of the same enzyme. Saso-2 and HepG2 cells express the alkaline phosphatase (TNA), but Saso-2 cells show higher level of TNA on the cell membrane. Liang et al. have developed a gelator precursor that is able to self-assemble into two different morphologies based on the different cellular location. Outside the cells, the peptide is able to self-assemble into fibrils by the action of ALP. Inside the cells after the action of the ALP, this peptide forms a self-assembling dimer, upon the action of glutathione. Considering that some cancer cells have higher levels of both ALP and glutathione, this peptide can selectively kill cancer cells by the action of two cellular mechanisms.

The concept of EISA has been explored with other types of enzymes overexpressed in cancer cells. Marumaya et al. have shown that matrix metalloproteinase-7 (MMP-7) is able to promote the intracellular self-assembly of a peptide amphiphile. The precursor is innocuous to normal cells, but once assembled has shown cytotoxicity against five different cancer cell lines.

Chen et al. have also used this strategy for cancer theranostics. In vivo co-assembly of indocyanine green, a molecule that has a unique near infrared absorbance/emission property and safety profile, with phosphorylated NapFKK, by the action of ALP gave rise to composite fibrils inside cancer cells. Indocyanine green behaved as a fluorescence/photoacoustic imaging probe showing improved photothermal conversion efficiency.

Besides using the self-assembly of peptides in living cells to promote their death, this strategy was used for imaging the enzymatic activity of cells in vivo too. For this purpose it is necessary that the self-aggregating peptides contain a type of fluorophore able to luminesce in the aggregate state as type AIE (aggregation-induced emission). Thanks to their biocompatibility and biodegradability together with the ability to self-assemble under different conditions or stimuli, fluorescent peptide fibrils or hydrogels have great potential for bio-imaging applications.

Xu et al. have used this strategy to image extracellular ALP on live cells (Figure 6). A phosphorylated 4-nitro-2,1,3-benzoazadizole (NBD)-conjugated D-peptide was used as a probe. When this peptide was dephosphorylated by ALP, it self-assembled into non-diffusive nanofibrils that luminesced locally. This probe was used to demonstrate the different ALP enzymatic activity of HeLa versus stromal cells (HS-5), and...
also between two pairs of drug-sensitive (A2780 and A2780cis) and drug-resistant (MES-SA and MES-SA/dx5) cancer cell lines and a cell line (e.g., MCF-7) under hormonal stimulus. [84]

3. Summary and Outlook

Self-assembling short peptides are uniquely positioned to take advantage of both worlds of synthetic molecules and proteins. On one hand, they can be easily made at low cost on a large scale to enable their convenient commercialization, and they display the robustness and stability of small molecules. On the other, they can self-assemble into supramolecular structures that mimic protein functions, and are enzymatically processable and biodegradable. They are thus ideally positioned to interact with endogenous proteins to realize new paradigms in medicine.

Over the last decades, great advances have been made in their use to treat challenging pathologies, and even to prevent them through vaccine development. In particular, the gain of selectivity towards organisms (e.g., to treat infections) and cell types (e.g., cancerous cells) has opened the way to make a qualitative leap in cancer therapy. Kinetic studies and the combination of different functions, such as bioactive delivery and targeted imaging, is particularly promising in theranostics, whereby diagnosis can be performed along treatment.

Exciting opportunities lie ahead in the further advancement of proof-of-concept studies on organelle selectivity to finely control cells’ fate in vivo and minimize adverse effects. Fundamental investigations to extract general design rules will be key to extend their wider applicability. We are witnessing the unveiling of fine mechanistic and thermodynamic details over the influence of supramolecular order imparted by self-assembling peptides over disordered proteins, or proteins’ packing into high-order structures and crystals. This research area is opening the way to gain a deeper understanding to mimic, and even correct when needed, biomolecular organization over several timescales into functional 3D structures, as it occurs in nature. In conclusion, the field is now gaining maturity to make a qualitative leap in medicine and provide innovative solutions to unsolved challenges for the wider benefit of society.

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