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Self-Assembling Peptide-Based Building Blocks in Medical Applications

Handan Acar[1,2], **Samanvaya Srivastava**[1,3], **Eun Ji Chung**[1,4], **Mathew R. Schnorenberg**[1,2,5], **John C. Barrett**[6], **James L. LaBelle**[2], and **Matthew Tirrell**[1,3,*]

[1]Institute for Molecular Engineering, University of Chicago, Chicago, IL 60637, USA

[2]Department of Pediatrics, Section of Hematology/Oncology, University of Chicago, Chicago, IL 60637, USA

[3]Institute for Molecular Engineering, Argonne National Laboratory, Argonne, IL 60439, USA

[4]Department of Biomedical Engineering, University of Southern California, Los Angeles, CA 90089, USA

[5]Medical Scientist Training Program, University of Chicago, Chicago, IL 60637, USA

[6]Biophysical Sciences Graduate Program, University of Chicago, Chicago, IL 60637, USA

Abstract

Peptides and peptide-conjugates, comprising natural and synthetic building blocks, are an increasingly popular class of biomaterials. Self-assembled nanostructures based on peptides and peptide-conjugates offer advantages such as precise selectivity and multifunctionality that can address challenges and limitations in the clinic. In this review article, we discuss recent developments in the design and self-assembly of various nanomaterials based on peptides and peptide-conjugates for medical applications, and categorize them into two themes based on the driving forces of molecular self-assembly. First, we present the self-assembled nanostructures driven by the supramolecular interactions between the peptides, with or without the presence of conjugates. The studies where nanoassembly is driven by the interactions between the conjugates of peptide-conjugates are then presented. Particular emphasis is given to in vivo studies focusing

[*]*Corresponding Author*: Matthew Tirrell, PhD: mtirrell@uchicago.edu, Institute for Molecular Engineering, University of Chicago, Chicago, IL 60637, USA.

Handan Acar, PhD: hacar@uchicago.edu, Institute for Molecular Engineering, University of Chicago, Chicago, IL 60637, USA

Samanvaya Srivastava, PhD: samsri@uchicago.edu, Institute for Molecular Engineering, University of Chicago, Chicago, IL 60637, USA

Eun Ji Chung, PhD: Institute for Molecular Engineering, University of Chicago, Chicago, IL 60637, USA

Mathew R. Schnorenberg: schnorenberg@uchicago.edu, Institute for Molecular Engineering, University of Chicago, Chicago, IL 60637, USA

John C. Barrett: jcbarrett@uchicago.edu, Institute for Molecular Engineering, University of Chicago, Chicago, IL 60637, USA

James L. LaBelle, MD, PhD: jlabelle@peds.bsd.uchicago.edu, Department of Pediatrics, Section of Hematology/Oncology, University of Chicago, Chicago, IL 60637, USA

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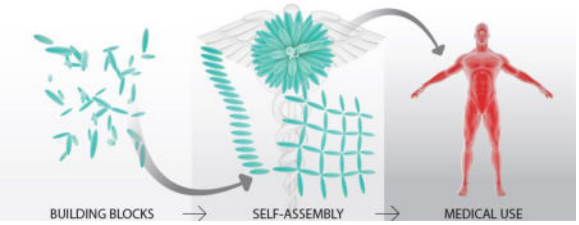
on therapeutics, diagnostics, immune modulation and regenerative medicine, and challenges and future perspective are presented.

Graphical abstract



Keywords

Peptide; Peptide-conjugates; Self-assembly; Medicine; Supramolecular

1. Introduction

Peptides in Medicine

Amino acids are like the letters of the alphabet. They are the building blocks of peptides and proteins in the way letters are the building blocks of words and sentences. In this sense, they convey information about structure and interactions. Peptides and proteins perform a wide range of functions within biological systems, including communication between cells. By tuning the amino acid sequence through the nucleic acid sequence of their genes, proteins fold in different conformations that alter their activities. For these reasons, peptides made of natural and synthetic building blocks are an increasingly popular class of biomaterials. Recent decades have witnessed a steep increase in the popularity of peptide-based targeting and therapeutic agents. Unlike small molecules (<550 Da) and biologics (>5000 Da), peptides offer a distinctive class of therapeutics with greater or equal specificity and potency as biologics but are more accessible for development akin to small molecules [1]. Because of this and recent advances in peptide production costs, efficiency, and use of non-natural amino acids, the market for therapeutic peptides is on the rise [1–3]. From 2009–2011 the US Food and Drug Administration (FDA) approved 76 new therapeutics, 58 molecular and 18 biologics (proteins, monoclonal antibodies, and enzymes) [4–7]. As of 2015, there were more than 60 FDA approved peptide therapeutics and this number is only expected to grow [3,8]. There are currently ~140 peptide-based drugs in clinical trials and more than 500 in preclinical development, reflecting predicted growth of a global market for peptide drugs from $14.1 billion (US dollars) in 2011 to $25.4 billion in 2018 [3]. The rise in demand for peptide drug development has spurred new mechanisms to develop feasible biological peptides beyond traditional methods.

Peptides used for pharmacological intervention have been traditionally derived from natural products isolated from plants, animals or humans (as is the case with hormone-based peptide agonists) [9,10]. However, biologically-potent peptides are increasingly mined from genetic or recombinant libraries as well as chemical and peptide screens [11]. Peptides have wide-

ranging applications in medicine because they can target proteins more selectively than small molecules, thus decreasing potential off-target side effects [12]. Their small size compared to proteins and antibodies allows peptides to better penetrate into tissues and solid masses such as tumors [12]. Peptides also have a lower immunogenicity profile than proteins and antibodies endowing them with greater potential for stable clinical therapeutic windows, predictable metabolism, and ability to repeat dosing. Peptides also offer several advantages over small molecules given that they are traditionally constructed in the likeness of the smallest functional part of a target protein. This fact endows them with greater efficacy, selectivity, and specificity [12–14].

Limitations of Peptide-Based Structures in Medical Applications

Despite recent successes, peptide-based therapeutics have been fraught with difficulties that limit their clinical translation including short circulation half-lives, poor chemical and physical stability in serum, bioavailability limitations via oral delivery, along with poor biodistribution, poor cellular penetration, high conformation flexibility limiting protein binding selectivity, and inability to home to diseased areas or target cell populations [12]. Innovative approaches have been proposed to tackle these issues. Not surprisingly, a major focus of translational chemical biology is to devise synthetic strategies to recreate the architecture of biologically active structures for both basic research and medicinal purposes [15–17]. For example, a considerable amount of effort has been directed towards preserving the peptide secondary structure, combating enzymatic degradation, and improving peptide half-lives [18]. One strategy, proposed by Verdine and coworkers, relies on hydrocarbon stapling the peptides by α,α-di-substituted non-natural amino acids bearing olefin tethers in optimal length and stereochemistry for ruthenium-catalyzed ring-closing metathesis (RCM) across one or two α-helical turns [15].

Hydrocarbon stapling was specifically developed to investigate and target α-helical interactions *in vitro* and *in vivo* [19,20]. Substitution/insertion of non-natural amino acids with olefin tethers at positions spanning either one (i,i+4) or two (i,i+7) turns of an α-helix followed by RCM crosslinks, or “staples”, effectively linking the non-natural amino acids to one another on one face of the helix. Stapled peptides can endow α-helical peptides with improved pharmacologic properties such as cellular penetration, protease resistance, and increased binding affinity. Although these peptide therapeutics can localize to the cytoplasm and nuclei of diseased cells, this and other methods to stabilize the natural secondary structure of peptide elements apart from the parent protein does not always guarantee cellular permeability, exact recapitulation of the natural secondary structure, non-interference at the protein binding interface, or cellular homing [21,22].

Self-Assembling Peptide-Based Structures

To address many of the limitations of peptide-based medicine, self-assembled nanostructures have emerged in recent years. The nanostructures protect the peptide against protease degradation and preserve the functionality of the individual peptides. Likewise, the sizes of the structures can be precisely controlled to provide optimal passive targeting abilities. At an optimal size (10–200 nm), self-assembled, peptide-based nanostructures can penetrate leaky tumor vasculature owing to the enhanced permeability and retention (EPR) effect [23,24].

Nanostructures enter cells through endocytosis and can increase the intracellular accumulation of the drug [25], do not affect healthy tissue, and are eventually removed from the body via renal clearance [26]. Furthermore, the peptide sequence can be designed for disease-specific enzymatic activity to control self-assembly [27] or disassembly [28], allowing for active targeting of various diseases.

Peptides can form various secondary structures, such as α-helices, β-strands, β -turns, and random coils, and they can self-assemble into a variety of structures including micelles, fibers, ribbons, tapes, and vesicles [29]. Typically driven by non-covalent supramolecular interactions (electrostatic, hydrogen bonding, hydrophilic and hydrophobic forces, van der Waals interactions, π-π stacking, etc.), well-controlled assembly is a combination of repulsive and attractive interactions between constituents of the system [30,31]. The repulsive forces are essential on the bio-functional sides of the peptide-based products to prevent the undesirable precipitation or steric hindrance through the structure [32].

Peptide-Conjugates and Synthesis Strategies

Peptide-conjugates are expanding the ability to manipulate forces that drive self-assembly toward a desired direction [33,34] and already proving far more effective than naturally occurring peptides in medical applications [18]. For instance, conjugation of hydrophobic moieties to peptides, creating peptide amphiphiles (PAs), can initiate self-assembly driven by the aggregating tendencies of the hydrophobic non-polar groups, dictating the local structure. PAs provide an alternative method of intracellular delivery and stabilization of bioactive peptides. As reviewed in the conjugation-driven self-assembly section of this review, PAs consist of a biofunctional peptide headgroup linked to a hydrophobic alkyl lipid-like tail to create molecules with distinct hydrophobic and hydrophilic ends, akin to natural lipids [16]. PAs self-assemble into a variety of nanoscale structures, including rod-like and spherical micelles, based upon charges in the peptide portion of the molecule and the nature of the hydrophobic tail. The hydrophobic tails of PAs promote cellular membrane anchoring and internalization and because of their dynamic structure, individual monomers can escape and insert their tails into other hydrophobic compartments [35,36]. Advantages of micelle-based peptide delivery systems are (i) delivery of a high concentration of peptide to its target (60–90% of the introduced concentration) [37], (ii) stabilization of peptide secondary structure (e.g. α-helices) [38,39], (iii) protection from proteolytic degradation [40,41], (iv) modular building potential to target malignant cells, and (v) the ability to load with multiple different amphiphiles directed at non-redundant protein-protein interaction (PPI) targets. Importantly, these compounds can also be targeted to cells using extracellular targeting ligands, receptor-specific peptides, or antibodies. Concurrently, the directional forces, such as hydrogen-bonding and π- π stacking, can regulate the larger structure, and lead to formation of core-shell one-dimensional fibril formation. Furthermore, the conformation of the fibril can be tuned by addition of attractive or repulsive electrostatic interaction promoter amino acid [42,43], chemical functionalization [44], or addition of a bulky group [45]. Larger molecules, such as polyethylene glycol (PEG) have also been shown to be useful in therapeutics to enhance their water solubility, reduce immunogenicity, increase *in vivo* half-life, and aid the self-assembly of peptides and PAs [46–49]. Natural (alginate, chitosan, hyaluronic acid, gelatin, heparin etc.) or synthetic (polyethylene, polyethylene glycol,

polyesters etc.) polymers can be efficiently conjugated to bioactive peptides with highly efficient coupling approaches [50,51]. The supramolecular interactions between synthetic polymers and specific conjugations for peptide modifications can be engineered for the purpose of controlled self-assembly [52]. Thus, self-assembled peptide nanostructures are extending the utility and use of peptides into many fields, including drug delivery and tissue engineering [53].

At this point, it is important to briefly discuss the various chemical synthesis approaches that have been followed for conjugation.

The chemoselective strategies for the conjugation of natural and synthetic polymers on peptides highly vary and are reported [54–59]. The conjugation of the non-peptidic group can be performed during solid phase peptide synthesis on resin. The orthogonal groups of the amino acids provide specific regions for conjugation within a peptide sequence, or the option for conjugation onto the N-terminus [60].

The strategies for conjugation on peptides have largely focused on the modification of cysteine and lysine side chains with orthogonal protecting groups. [61]. Formation of disulfide bonds is one of the most common modification on the cysteine side chain and for most antibody-drug conjugations [61]. Another common approach regarding side modifications on cysteins is maleimide chemistry [54,55,62].

Site-specific modifications of complex functional peptides are challenging yet imperative to simultaneously retain functionality and introduce modularity. A recent approach called "**π**-clamping" tailored biomolecules with the help of a natural small peptide sequence, and is providing to be a very exciting strategy that expands the ability of bio-conjugation chemistry [63,64]. Another approach is bioorthogonal ligand tethering (BOLT), in which a genetically encoded, unnatural amino acid on a protein is bioorthogonally reactive with an inhibitor conjugate to enable reversible regulation of protein activity in mammalian cells [65]. The unnatural amino acids are commonly used probes for selective conjugation, and their use can be extended to several pairs of fluorophores with Förster resonance energy transfer in physiological conditions [66]. These site-selective approaches to design peptide-based agents provide higher control on the structure of the end product, which also is highly dependent on the nature of the conjugate. The examples of conjugates to form self-assembled hydrogel materials are highly versatile [52,67]. These strategies can be enriched with chemical ligation in solution [68]. Comprehensive reviews on this topic have been described from other groups [56–59] . In this review, we survey the recent progress in the use of peptide and peptide-conjugates for self-assembled nanostructures in medicine (Figure 1). We focus primarily on studies of peptide and peptide-conjugate building blocks, which are self-assembled after synthesis, and their applications in therapeutics, diagnostics, immune modulation and regenerative medicine during the last five years. The integration of biofunctional peptides into self-assembled structures after the self-assembly process is beyond the scope of this review, as are inorganic nanoparticle conjugates. Many valuable reviews related with peptide-based therapeutics and perspectives about their future directions can be found elsewhere [1–3,69,70].

In the following section, structures formed via self-assembly driven by the interactions between peptides are reviewed, followed by discussions on self-assembly of structures driven by the interactions between the conjugates. Lastly, we end with a discussion on the challenges and the future of peptide-based nanostructures in medicine.

2. Peptide-Peptide Interaction Driven Self-Assembly

Recent technologies have associated many protein-protein interactions (PPIs) with various human diseases [71,72]. Amino acids, because of their various physical and chemical properties, are reliable building blocks for material scientists. With sufficient knowledge of the properties of amino acids, one can alter interactions to design molecular building blocks to self-assemble into various architectures, such as spherical, one-, two-, or three-dimensional, or even tetrahedral architectures by using orthogonal dimerizing segments of peptides [73]. In this section, we provide examples from self-assembled peptide-based structures, in which the self-assembly occurs through the interactions between amino acids in the sequence. While in some cases peptide sequences form bio-functional materials, in other cases they were used as a complementary part responsible for self-assembly and do not serve any biological function.

2.1. Diagnostic Probes Based on Peptide-Peptide Interaction Driven Self-Assemblies

Magnetic resonance imaging (MRI) has evolved into a highly-reliable non-invasive imaging methodology that relies on altering the relaxation time of water molecules. It allows for imaging of opaque deep tissue, provides excellent cellular scale resolution, and is used extensively in both clinical and medical research. Higher concentrations of the contrast agents, which alter the relaxation times of the water, are required to improve the sensitivity and the signal-to-noise ratio of the images. While employing macromolecules that contain multiple contrast agent moieties would be beneficial in this regard, they have been shown to exhibit higher cytotoxicity. A self-assembling contrast agent is thus better suited – sensitivity can be increased in the region of interest while preserving the surrounding tissue, the self-assembled structures lead to longer retention by the cells, and small molecule components are more susceptible to cellular uptake prior to self-assembly – leading to highly efficient and superior contrast agents [74]. Peptide-based molecules have been employed in such context to design gadolinium (Gd) containing molecules that condense into amphiphilic dimers post intra-cellular disulfide reduction, and they subsequently self-assemble into Gd-containing nanoparticles [75]. Further specificity of self-assembly was imparted by introducing susceptibility to furin, a protease overexpressed in tumors, into the Gd-containing peptides. *In vivo* experiments on mice with subcutaneously xenografted tumors reported preferential assembly of these molecules into nanostructures in the tumors, thus providing an excellent contrast for MRI imaging as compared to non-cleavable Gd-containing peptides [76].

An alternate MRI technique relies on nuclear magnetic spectroscopy of ^{19}F nuclide, with generally higher selectivity but lower sensitivity than ^{1}H MRI. Inspired by the self-assembling ^{19}F MRI probes that disassemble in presence of target proteins to produce strong signals [77], Yaun *et al.* designed self-assembling particles that assemble via the reduction-

condensation reaction as described earlier [75,76]. They then disassemble in presence of Legumain (Lgmn), an asparaginyl endopeptidase associated with various physiological events, such as protein catabolism and renal homeostasis, and also with diseases including the inhibition of osteoclast formation and bone resorption, atherosclerosis, stroke, and cancer [78]. *In vivo* investigations in zebrafish showed exceptional selectivity of the self-assembling and Lgmn-mediated disassembling probe, thus opening exciting avenues for future research in ^{19}F MRI techniques [78].

Self-assembled peptide structures are also finding applications as targeted fluorescent imaging *in vivo*. Typically, self-assembly of fluorophore containing small molecules into nanostructures brings the fluorophores closer and leads to fluorescence resonance energy transfer (FRET) effect to quench the fluorescence signal. Enhanced quenching has been employed by researchers to detect the activity of enzymes (ex. caspase 3 [79], alkaline phosphatase [80], etc.). However, self-assembly can also leads to a reduction of the charge transfer between the fluorophore and the polar solvent, and thus an increment of fluorescence [81]. Various studies have exploited enzymatic hydrogelation – self-assembly of nanofibers upon enzymatic reaction with a hydrogelator precursor –to induce peptidic self-assembly inside the cells. Gao *et al.* employed one such precursor, a combination of 4-nitro-2,1,3-benzoxadiazole (NBD), and a phosphorous ester on the tyrosine residue of a small peptide, to illustrate the growth of nanofibers inside the cell. The nanofibers also facilitated the confirmation of the endoplasmic reticulum as the location of the enzymatic activity thorough *in vitro* spatiotemporal fluorescence measurements [82]. Self-assembly of peptide nanofibers is typically driven by aromatic–aromatic interactions and produces slightly hydrophobic regions inside the fibers, thus leading to enhanced fluorescence of the probes. Fluorescence imaging can therefore be employed to understand the interactions of peptides with various enzymes; D-peptides, which typically resist most enzymes, were shown to be susceptible to enzymatic dephosphorylation by alkaline phosphatase [83]. However, the self-assembling tendencies and the distribution of the self-assembled fibers in the cellular environment can be heavily influenced by the nature of the fluorophore, and can also induce cytotoxicity in some cases. Thus, extreme care must be exercised in designing the fluorophore precursors to achieve the desired imaging objectives [84].

A recent illustration of enzymatic hydrogelation utilizing caspase-3/7 cleavage and thiol reduction to drive a first-order bioorthogonal cyclization reaction was reported by Rao and coworkers [85]. This cyclization instigated self-assembly of the small peptide, leading to aggregation and enhanced fluorescence of the fluorophore, as shown in Figure 2a. The bioorthogonality of the self-assembly process was shown to occur selectively in apoptotic tumor cells (Figure 2b), and the drug treated tumors were selectively identified compared to untreated tumors in human tumor xenograft mouse models of chemotherapy (Figure 2c), thus demonstrating a facile, non-invasive super-resolution fluorescence microscopy technique to demonstrate the efficacy of chemotherapeutic treatments [85].

2.2.Therapeutics based on Peptide-Peptide Interaction Driven Self-Assemblies

Amino acids with different charges let the engineer have control over the location of the forces within a sequence at the molecular level. Although, cationic amino acids cannot self-

assemble due to the repletion of their high charge, they are known for their cell penetrating abilities. One of the most popular examples of cell-penetrating peptides (CPPs), transactivator of transcription (TAT), has been widely used for anti-cancer drug conjugates to increase cellular uptake and intracellular retention in cervical cancer cells [86,87].

Self-assembly of TAT was achieved via DNA conjugation. Lande *et al.* showed that TAT-DNA complexes can lead to the chronic activation of plasmacytoid dendritic cells (pDCs), which causes systemic lupus erythematosus (SLE), a severe and incurable autoimmune disease [88]. As described thoroughly in earlier studies, on human autoimmune diseases, the pDCs sense and respond to self-DNA [89]. In 2011, Lande *et al.* suggested that the reason for activation of pDCs by TAT-DNA complexes is its ability to break the innate tolerance to self-DNA, and to activate the toll-like receptor 9 on pDCs [90]. However, in a recent following study, the authors investigated the mechanism by which the self-assembled structure of the TAT-DNA complex leads to SLE, using X-ray scattering, computer simulations, microscopy and experimental analysis [91]. The parallel conformation of dsDNA ligands in liquid-crystalline TAT-DNA complexes can form a parallel grill-like arrangement of DNA with Toll-like receptor 9 on the pDCs. This leads to multivalent electrostatic interactions and amplifies binding of DNA to the receptor, and therefore creates an immune response. Furthermore, their findings suggested that this kind of amplified interaction can be driven by a large repertoire of antimicrobial peptide sequences and other cationic molecules.

Cell membrane penetration with non-cationic amino acids is also possible through self-assembly on the cell membrane. Self-assembly of cyclic peptide (*cyclo*[Gln-(D-Leu-Trp)$_4$-D-Leu], CP) forms artificial transmembrane nanochannels on the cell membrane for the permeation of anti-cancer drugs, which are smaller than 1 nm. The *in vivo* study with these nanochannels in a grafted solid tumor model in mice showed that tumor growth was greatly inhibited by the combination of anti-cancer drugs with the cyclic peptide nanochannels [92].

The ability to trigger self-assembly to construct or degrade the therapeutic structure is an important tool to achieve successful therapy on the targeted area, while reducing the side effects on healthy tissue. Webber's recent review is focused on supramolecular biomaterials, specifically tunable and reversible non-covalent interaction in medical application [93]. Designing a cancer specific factor to trigger the self-assembly of peptide building blocks is a promising cancer-targeting therapy strategy. Self-assembly of engineered building blocks of amino acids around the cancer cell membrane can lead to the death of the cancer cells [94]. Cancer related pericellular enzyme has been widely used to trigger N-terminal naphthyl group capped tri- [95,96] and tetra- [95] peptide blocks, which are made of D-amino acids with one or two phospho-tyrosine residues. When these special peptides are induced to the medium, alkaline phosphatase, an overexpressed enzyme by cancer cells, catalyzes the phosphorylation of the peptides to form hydrogelator just on the pericellular space of the cancer cells. This selectively forms a hydrogel to block critical cellular activities of the cancer cell and enhance apoptosis. The remarkable chemical complementarity and structural compatibility of the self-assembled D- and L- amino acids make them beneficial materials and structures in medical applications. A recent review based on D-amino acids and their applications in cancer therapy can be found [27,97,98].

The same strategy can be applied to peptide-conjugate building blocks with another cancer related enzyme, matrix metalloproteinase-7 [99]. Although self-assembly happens through the amphiphilic region, the peptide sequence plays the most important role to repel the amphiphiles before being triggered by the enzyme. Since the enzyme is specific to cancer cells, the cytotoxicity of the peptide amphiphile precursor was low for normal cells. Indeed, cancer cells and healthy cells were co-cultured and the selectivity of the precursor to cancer cells was shown. Enzyme cleaves the peptide-amphiphile on the pericellular space of the cancer cell before internalization. The internalized peptide-amphiphiles self-assemble into one-dimensional fibrils, which create a vital stress and cause cell-death (Figure 3).

Carefully designed amino acid sequences can lead to the arrangement of supramolecular interactions on the molecular building block. Alternating charged and hydrophobic residues of the 16 amino acid EAK16-II (n-AEAEARARAEAEARAR-c) facilitate self-assembly into highly stable platforms to carry specifically hydrophobic anticancer drugs, such as ellipticine (EPT) [100–102]. The self-assembled EAK16-II was shown to stabilize EPT through electrostatic interactions in aqueous solution. It shows resistance to enzymatic digestion, good biocompatability, and enhanced circulation time relative to the dull (free?) EPT in the circulation [103]. The self-assembled EAK16-II and EPT complex enter cells through a caveolae-dependent endocytosis mechanism, and enhances the anticancer efficacy of EPT *in vivo* [70]. Recently, lysine in the EAK16-II sequence was substituted with arginine because of its higher binding efficiency to EPT and its ability to penetrate the cell. The sequence of amino acids in EAR was studied to understand the effect on the size and morphology of the peptide self-assembly and the peptide-EPT complex, as well as their delivery efficacy, in order to optimize this self-assembling peptide-based platform for future clinical applications [104]. Not only alternating, but also localized hydrophobic and charged amino acids form amphiphilic peptides without conjugation of any hydrophobic tail [105–107]. An aspartic acid or lysine hydrophilic head and a hydrophobic tail composed of six alanines (i.e., ac-A_6 K-$CONH_2$, KA_6 – $CONH_2$, ac-A_6 D-COOH, and DA_6 -COOH) were designed to form lipid-like peptides, which form vesicles in physiological conditions [108]. Encapsulation of hydrophilic and hydrophobic compounds in these vesicles showed sustained release through the peptide bilayer. The permeability enhancing properties of these lipid-like peptides were studied *in vitro* and *ex vivo* through the intestinal barrier [109].

In addition to the previous sequence, the amphiphilic peptide sequence was obtained with C-terminus attachment of two hydrophobic adhesive sequences, FLIVIGSII and FLIVI, in a parallel array into branched structures with predetermined amino acids, resulting in self-assembly towards solvent-filled bilayered vesicles with 50–200 nm range [110] (Figure 4). The *in vitro* studies showed the cellular uptake of vesicles, their accumulation within the perinuclear space membrane, and their potential for encapsulating larger molecules such as hydrophilic drugs, peptides, proteins and large plasmids [110].

Having knowledge of self-assembling short amino acid sequences is important to design peptide structures [111]. Computational and experimental analyses on short amino acid sequences were studied to uncover the design rules for self-assembling sequences [34]. The key motif of self-assembly, like amyloid-beta (Aβ) peptide, LVFFA, has been studied to understand the complex fibrillation process. Aβ peptides self-assemble into a β-sheet

ordered fibril structure, which is a known cause of Alzheimer's disease [112]. Protein aggregation and amyloid formation has become the subject of rapidly increasing interdisciplinary research activities, in order to understand and control the triggering factors of aggregation. The native form of Aβ is unfolded but can aggregate into fibrillar structures under various conditions and environmental manipulations, such as solvents [113], inorganic elements [114], or nanoparticles [115]. The key motif of Aβ peptides induces cellular membrane disruption, which has made it a potential target for pharmacological inventions recently [116–118].The kinetic and thermodynamic parameters of the dominant pathway of cellular disruption have been studied to be able to enhance pharmacological applications [119].

For the conjugation-driven self-assembly of peptide-conjugate structures, the side chain interactions of the peptides are important feature that determines the architecture of nanostructures. These interactions can determine the shape, conformation and even packing of building blocks at the molecular level. The hydrophobic conjugates of peptides typically form one-dimensional fibrils and tuning the resulting structure is possible by pre-determining the side chain interactions [120]. The location of hydrophobic and hydrophilic sequences, even small sequences such as tetrapeptides, can influence the self-assembly to form helical, twisted or cylindrical nanofibers.

2.3. Immune Modulators Based on Peptide-Peptide Interaction Driven Self-Assemblies

Considerable effort has been put toward developing subunit vaccines to overcome the limitations of vaccines based on killed or attenuated pathogens. Subunit vaccines theoretically offer greater control over the immune response by providing only the minimum required antigen to raise a protective immune response. Unfortunately, subunits suffer from poor immunogenicity due to inefficient antigen presentation and uptake. Ironically, in order to improve the subunit immune response, some control over the immune response must be surrendered to adjuvants which cause inflammation and lead to a complex combination of downstream effects. To re-claim control of subunit vaccines, platforms have been engineered to contain highly defined components, including generally low quantities or no supplemental adjuvants at all. Supramolecular assemblies of both peptide-peptide and peptide-conjugate interactions are major players in this new field of immunoengineering. These assemblies have several advantages over other antigen carrier systems such as biodegradability and ease of production. And their immunological properties arise from many controllable features, including size, shape, multivalency, and molecular content of different functional components.

Self-assembling β-sheet peptide fibers are an example of a high-aspect ratio platform used for vaccine development. The Q11 peptide (QQKFQFQFEQQ) is able to form fibril structures with functional epitopes or proteins conjugated to them without disrupting the self-assembling capacity [121–123]. The peptide fibers themselves, even when delivered with complete Freund's adjuvant (CFA), did not raise a detectable immune response. However, when ovalbumin 323–339 ($OVA_{323–339}$, an allergenic and antigenic epitope of the ovalbumin protein) peptide was attached, antibody levels of IgG and IgG subclasses against OVA were comparable to those induced by the peptide delivered with CFA [124,125]. Even

without adjuvants, the humoral immune response raised by the nanofiber vaccine is shown to be robust with a response up to 40 weeks long against $(Asn\text{-}Ala\text{-}Asn\text{-}Pro)_3$ -$(NANP)_3$ - malaria peptide [125]. The modularity of the platform is exemplified by its ability to raise an immune response against two different epitopes without decreasing the immune response to either epitope [125].

The immune response was also shown to be tunable in an experiment where a fiber was formed by co-assembly of a B cell (E214-Q11) and T cell (PADRE-Q11) epitope [126]. By varying the concentrations of each B and T cell epitope, the optimal T helper cell and antibody responses were selected. Co-assembly of the B and T cell epitopes in the same nanofiber was required to raise an immune response, as separate injections of PADRE-Q11 and E214-Q11 were unable to raise antibody responses. This confirms the earlier finding that Q11 fiber vaccines were shown to be T-cell dependent and strongly associated with the self-assembling structure [124]. The cytotoxicity of the nanofiber vaccines was also evaluated [127]. Chen *et al.* proved that the OVA-Q11 nanofibers did not induce any swelling or local inflammation at the site of injection when compared to OVA formulated with alum adjuvant. Additionally, the OVA-Q11 peptide vaccine did not cause any cell death in a range of concentrations, while alum formulations produced dose dependent death.

Low aspect ratio self-assembling peptide platforms have also joined in vaccine design. Generally, these systems form nanoparticles that resemble virus like particles. One self-assembling polypeptide nanoparticle was composed of linear peptide monomers containing two coiled-coil oligomerization domains (pentameric and trimeric domains) [128,129]. Antigen epitopes were added to the N and C terminals flanking these self-assembling domains, and displayed on the nanoparticle surface when fully assembled. In one experiment, three epitopes were added to the monomer (a B cell epitope, a T helper epitope and a cytotoxic T cell epitope) and were shown to be able to raise long lasting humoral and cellular immunity against malaria for up to one year [128].

2.4. Peptide-Peptide Interaction Driven Self-Assemblies in Regenerative Medicine

The extracellular matrix (ECM) serves many purposes in the human body. It directs the growth of the organs during the growth of the embryo, and provides for them mechanical support later in life. More importantly, it guides new tissue formation by affecting the cells that are in its contact by providing a medium to grow and providing physical and chemical signals for growth, physical architecture, and sequestration of growth factors, respectively [130,131]. Any biomaterial being developed as a scaffold for tissue growth and regenerative medicine needs to imitate the complex three dimensional structure as well as physical and chemical functionality of the ECM of the target organ.

Peptide-based structures are attractive in tissue engineering and regenerative medicine owing to advantages they offer, including biocompatibility and biodegradability, as discussed previously. Self-assembling structures based on ionically complementary peptides (ICP) are particularly attractive for these applications as these peptides predominantly assume a beta-sheet structure that then can further self-assemble spontaneously into fibrils and hydrogels [132]. Also known as peptide *lego*, their beta sheet structures possess hydrophobic and hydrophilic moieties on either side. In addition, they also have alternating positive and

negative charges, which renders specificity of interactions and arrangements to the beta-sheets, as shown in the schematic in Figure 5a. Thus, these ICPs exploit electrostatic interactions in addition to the usual hydrogen bonding and van der Waals interactions to direct their self-assembly. In addition, functional and bioactive motifs have been successfully assimilated into the ICP backbones without interfering with their fibrillating, self-assembling, and gelation tendencies, thus allowing for introduction of growth factors and other nutrients, and mimicking the microenvironment in the ECM around the target organ.

The most commonly employed ICPs are known as RAD16-I ($COCH_3$-RADARADARADARADA-$CONH_2$). These form fibrous hydrogels [133] readily upon dissolution in water while retaining substantial amounts of water as well as high porosities. They allow for easy integration with the host tissue for *in vivo* applications along with facile functionalization with growth factors and other biological motifs. Various researchers have investigated the efficiency of modified RADA16 based gels for supporting tissue growth ranging from neural regeneration, angiogenesis and cardiac tissue regeneration, homeostasis and mucosal and skin regeneration to cartilage regeneration and bone repair and regeneration, and they have typically reported superior performance compared to passive gel scaffoldings. We shall refer the reader to a few recent reviews that have thoroughly surveyed the progress in this area for further information [53,131,134–138], and rather focus on a couple of studies that have demonstrated the use of RADA16 based gels for dual purposes of myocardial protection and enhanced angiogenesis [139] and hemostasis and accelerated osteosis [140], respectively.

Kim *et al.* [139] injected mice with myocardial infarction with RADA16 based self-assembling gel with conjugated angiogenic (FGF-2) and arteriogenic (PDGF-BB) factors to promote recruitment of endothelial cells and vascular smooth muscle cells for enhancing vascularization and preventing cardiac fibrosis. Mice that received the dual factor containing gels exhibited the best recovery when compared to those that received no medication, gels with only one of the growth factors, and both the growth factors without the gels. Thus, this study was instrumental in showing the versatility of the RAD16 platform for combinatorial therapy. More recently, Wu *et al.* [140] employed unmodified RADA16-I gels for achieving rapid homeostasis and promoting osteosis in New Zealand rabbit ilium bone defect model. As shown in Figure 5b and 5c, RADA16 based gels were able to quickly arrest blood flow, and the bone was shown to heal significantly faster than untreated bone or bone defect filled with bone wax. The non-toxic and biodegradable gel formed a seamless interface with the native tissue and allowed for osteoblast penetration and settlement while providing the supporting matrix, thus enabling bone regeneration.

3. Conjugate-Conjugate Interaction Driven Self-Assembly

Supramolecular self-assembly of peptide conjugates utilizes the conjugate domain to drive self-assembly of a supramolecular nanoparticle. Here, we focused on the interactions between the conjugates of the peptide-conjugates. These interactions are not the only driving force in most of the structures. In many studies, clear distinction of the forces and the source of self-assembly is not easily defined . Although, the interactions between the peptide

regions are contributing to the self-assembly, we focused on the studies in which the conjugate portion ultimately determines the conformation of the end product and the overall process.

In conjugate interactions driven self-assembled peptide-based structures, peptide can be incorporated before or after self-assembly and the most popular applications for peptides are active targeting and cell penetration, but they have also been used for modulating cell signaling, small molecule drug loading, and environmentally stimulated drug release. PA micelles are a notable example in which the peptide is conjugated to a hydrophobic domain prior to self-assembly such that a single population of PAs can self-assemble into a micellar nanoparticle.

3.1. Diagnostic Tools Based on Conjugate-Conjugate Interaction Driven Self-Assemblies

Nanoparticles with peptide conjugates are often self-assembled through the hydrophobic interactions provided by the conjugate component of the molecule. For diagnostic applications used in preclinical studies in recent years, particles include those that consist of peptide amphiphiles and synthetic amphiphilic polymers and include fluorescence, MRI and positron electron tomography (PET) capabilities. The majority of studies focus on cardiovascular and cancer detection. Although other supramolecular nanoparticles such as liposomes, viral capsids, and nanoemulsions incorporate peptide conjugates, peptides are often added after self-assembly of the particle, and we refer the reader to other recent articles and reviews [141–144].

The majority of nanoparticles that consist of PAs used in the context of diagnostics have conjugated a biologically active, and targeting peptide “headgroup” to a hydrophobic, alkyl “tail” through a bulky PEG spacer in between (i.e. DSPE-PEG2000). The primary driving force behind PA aggregation is the need for the hydrocarbon tails to be shielded from the aqueous environment, making up the core of the micelle and allowing for bioactive peptides to be presented to the environment [46]. The PEG spacer not only allows for enhanced blood circulations times, but allows monomers to obtain a large headgroup to a relatively short and stiff tail (packing parameter, $P < 1/3$). This forms a cone-type geometry which favors aggregates with a high degree of curvature, i.e. spherical micelles.

DSPE-PEG2000 PAs assemble into spherical micelles with an aggregation number of 90, a CMC of 1 μM, and a hydrodynamic diameter of 8–20 nm [145,146]. The modularity of this system allows for the formation of multifunctional micelles with controlled size and shape through simple mixing of different PEG-lipid monomers [38,147]. Therefore, targeting, diagnostic, and theranostic micelles derived from PAs have been reported with this approach [143,147–151]. A potential drawback of PEG lipids is reduced peptide folding due to the increased conformational freedom afforded by the flexible PEG chain; however, the small size allows for intravenous injection and circulating in narrow blood vessels without obstructing blood flow, and recent reports show no toxicity and clearance through both the reticulo-endothelial system (RES) and the renal system [150]. By seven days, the majority of PAs are cleared out of the body, which is an important consideration for diagnostic applications, especially for patients needing additional scans.

Fluorescently-labeled PAs with the fibrin-binding peptide, cysteine-arginine-glutamic acid-lysine-alanine (CREKA), have been used to for both cancer and atherosclerosis diagnostics [147,148]. For cancer applications, Chung *et al.* took advantage of the fibrin deposition that is characteristics to tumor vasculature and constructed spherical, Cy7-labeled, DSPE-PEG2000 micelles to target glioblastoma multiforme, an aggressive and malignant form of brain tumors, known to carry a 5-year survival rate of less than 5% [148]. Upon intravenous administration to GL261 glioma bearing mice, non-targeting micelles passively accumulated at the brain tumor site via the EPR effect, and Cy7-CREKA-micelles displayed enhanced tumor homing via active targeting as early as 1 hour after administration (Figure 6), with no signs of cytotoxicity and tissue damage in diseased organs. In addition to fluorescence, DSPE-PEG2000-CREKA molecules have also been mixed with 18:0 PE DTPA(Gd) to form micelles that can be utilized as molecular MRI contrast agents for tumor-targeting [151].

For vascular applications, the CREKA targeting peptide, an anithrombin peptide called hirulog, and fluorescence molecules were conjugated to the DSPE-PEG2000 hydrophobic tail to form a multifunctional nanoparticle for theranostic applications for atherosclerosis [147]. Novel methods of diagnosing atherosclerotic plaques are particularly needed because of their asymptomatic yet deadly nature; while current clinical imaging options are limited in their ability to detect rupture instability, cardiovascular diseases remain to be the single leading cause of deaths globally [152]. In an effort to provide a novel molecular imaging tool, fluorescently-labeled spherical PAs have also incorporated targeting peptides to monocytes [149], endothelial cells [153], and collagen [154] to detect vulnerable, diseased, and injured blood vessels. Additional studies conducted in large animals will be the next step to determine the potential of PAs as a potential molecular imaging tool for the clinic.

Other spherical nanoparticles based on amphiphilic peptide conjugates have been tested *in vivo* for diagnostic applications in vascular injury and cancer [155–158]. Luehmann *et al.* [155] developed CCR5 receptor-targeting, poly(methyl methacrylate)-core/PEG-shell amphiphilic comb-like nanoparticles with ^{64}Cu for PET imaging in atherosclerotic mice. CCR5 is an important chemokine receptor that is upregulated in subsets of monocytes and mediates plaque progression in atherosclerosis. These nanoparticles were found to be significantly uptaken at lesion sites and competitive PET receptor blocking studies confirmed CCR5 receptor-specificity of particles with low nonspecific nanoparticle uptake. In addition to comb-like nanoparticles, Miki and colleagues [156] report on tumor targeting by nanoparticles consisting of amphiphilic copolymers prepared through ring-opening metathesis polymerization (ROMP). The ROMP-based polymer brushes of poly(methacrylate)(PMA) was grafted with PEG, near-infrared fluorescent (NIRF) dyes, and cyclic RGD peptides and in a tumor mouse model, demonstrated high selectivity.

3.2. Therapeutics Based on Conjugate-Conjugate Interaction Driven Self-Assemblies

In addition to diagnostic applications, therapeutic PAs have been recently developed. For extensive information on other types of nanoparticles (e.g. liposomes or polymeric nanoparticles), we direct the reader to recent review articles and primary literature examples [159–166].

Numerous examples of targeting peptides have been incorporated into PAs to deliver therapeutics to specific cell populations. RGD peptides are a popular example that preferentially bind $\alpha_V\beta_3$ integrin overexpressed by some tumors [167]. Saraf *et al.* used RGD PA self-assembly to make micelles that preferentially bound melanoma cells and were internalized *in vitro*. They then loaded a hydrophobic small-molecule anti-cancer drug called paclitaxel into the hydrophobic core, and the targeted micelles preferentially killed melanoma tumors in an *in vivo* mouse model.

In addition to loading small molecule drugs into the core of a micelle, a peptide-conjugate's peptide domain can itself be therapeutic. Recently, Zha *et al.* coupled a peptide domain from an anti-angiogenic protein, maspin, to an alkyl tail to form supramolecular nanostructures [168]. The maspin peptide domain interacts with endothelial cells to upregulate their adhesion, down-regulate their migration, and thereby inhibit angiogenesis during tumor growth [169–171]. They found that their maspin-mimetic nanorods inhibited angiogenesis *in vivo* and at far lower doses than when peptide was administered alone [168].

In addition to aiding in the delivery of a therapeutic, a supramolecular nanoparticle's structure can itself be harnessed as a therapeutic. Morgan *et al.* showed that circulating nanofibers can be actively targeted to accumulate at the site of blood vessel disruption to control hemorrhage and minimize blood loss [172]. They conjugated a peptide targeted against tissue factor to a beta-sheet forming peptide domain and an alkyl tail to form stabilized nanofibers. The nanofibers normally circulated without binding anything and were naturally cleared from the body.

However, upon blood vessel disruption by injury, the nanofibers encountered and bound tissue factor in the intravascular space, where they accumulated to stop blood flow from the wound. The peptide domain of a peptide conjugate can also be used to control the stability of a micelle in the circulation and thereby control pharmacokinetics. Dong *et al.* recently coupled a peptide domain, designed to form a 3-helix coiled coil, to a hydrophobic tail to form 15 nm spherical micelles that circulated in the bloodstream for at least 48 hours with minimal cargo leakage [173]. In the context of actively-targeted micelles, this degree of stability could be important to ensure the micelles reach their targets before releasing their payloads.

3.3. Immune Modulators Based on Conjugate-Conjugate Interaction Driven Self-Assemblies

In vivo applications of self-assembled peptide-conjugates as vaccine nanoparticles, driven by interactions of their conjugate domains, are growing in importance. There are also many vaccine nanoparticle applications in which peptides are conjugated to nanoparticles after self-assembly. For more on those examples and for general information on nanoparticle vaccines, we refer the reader to recent reviews [174,175]. Here, we briefly mention liposomes in which peptide conjugates were incorporated during conjugate-driven self-assembly and then discuss in more detail recent PA micelle vaccines.

While many vaccines successfully induce humoral immunity, there are still many vaccine preventable infections, chronic diseases, and emerging diseases that remain [176].

Liposomal and PA vaccines are two emerging strategies to elicit previously elusive immune responses by simply conjugating a peptide antigen to a hydrophobic “tail” domain, which then drives self-assembly.

Peptides are often included in or on the surface of liposomes to introduce them to the adaptive immune system and induce a response. Beyond incorporating antigen into liposomal vaccines, non-antigenic peptide-conjugates have been used to enhance liposome delivery and antigen presentation. For example, cell penetrating peptides (CPPs) consisting of eight arginine residues (R8) were conjugated to a lipid tail and incorporated into liposomes during self-assembly to deliver liposomes into cells and enhance antigen trafficking and presentation [177–183]. For more detailed information and recent research on liposomal vaccines, we refer the reader to recent reviews and studies [174,184,185].

PA micelle vaccines are a prime example in which peptides are conjugated before self-assembly. As described in section 3.1, PAs are formed by conjugating peptides to an alkyl tail, with or without a spacer in between. Self-assembly of PAs is then driven by hydrophobic interactions between the tail domains to form spherical micelles, cylindrical micelles, or liposomes, depending on the packing parameter of the peptide-conjugate as mentioned above PAs create a unique set of vaccine nanoparticles that can self-adjuvant to elicit either humoral or cell-mediated immunity by simply conjugating an antigen to a self-assembling tail domain. They have also been shown to elicit immune responses that have otherwise remained elusive from clinical development. For example, *streptococcus pyogenes* (group A streptococcus, or GAS) is an infectious bacterial disease that has evaded clinical vaccine development despite many platforms being in clinical and preclinical studies [186]. In 2015, Trent *et al.* coupled a GAS B-cell antigen (J8) to a diC_{16} tail, which drove self-assembly of cylindrical micelles, enhanced antigen alpha-helicity, and elicited strong IgG1 antibody titers [187]. Notably, no molecular adjuvant (e.g. IFA) or CD4+ T-helper epitope (e.g. KLIP) was necessary; the self-assembled nanostructures were sufficient to induce humoral immunity *in vivo*, while the peptide co-delivered with an unloaded micelle was not. Similar self-adjuvant results for PA vaccines have been shown against Herpes simplex virus (HSV) *in vitro* [188].

While most clinical vaccine development focuses on humoral immunity, antigen-specific CD8+ T-cell mediated immunity remains elusive [189,190]. CD8+ T cells use their T cell receptors (TCRs) to “read” the intracellular antigens processed presented on all cells via major histocompatibility complex (MHC) class I molecules on the cell surface. When the CD8+ T cells encounter a cell presenting a peptide antigen recognized as “non-self,” the T cell kills the diseased cell. This is important, for example, in anti-cancer or viral immune responses in which antibodies would not be able to access intracellular disease antigens. Interestingly in 2012, Black *et al.* conjugated a model cytotoxic T-cell epitope to a diC_{16} tail to form PA micelles, which were sufficient to induce an antigen-specific CD8+ T cell response *in vivo* and impart protective immunity against tumors bearing that antigen (Figure 7) [191].

The hydrophobic conjugate domain used to drive self-assembly of vaccine nanoparticles has also been shown to bind albumin and target the peptide to the draining lymph nodes [192].

Liu *et al.* in 2014 reported that for a PA cancer vaccine, increasing the length of a PEG space between the antigenic peptide and tail (i) reduced insertion of the lipid tail into cell membranes, (ii) enhanced accumulation of PAs in draining lymph nodes, presumably by hydrophobic tails binding albumin, and (iii) induced strong, cell-mediated immunity when co-delivered with a nanoparticle adjuvant made from CpG amphiphiles [192].

While these studies are promising for the field of vaccine research, more research is needed to understand the unique mechanisms by which vaccine nanoparticles elicit immune responses, including the peptides' antigenicity and the nanoparticle's self-adjuvanting properties.

3.4. Conjugate-Conjugate Interaction Driven Self-Assemblies in Regenerative Medicine

The majority of supramolecular nanoassemblies derived from peptide conjugates designed for regenerative medicine use cylindrical PAs [193–195]. Cylindrical micelles require a packing parameter of ≤ ½ and typically incorporate a short alkyl chain and a short peptide sequence composed of hydrophobic amino acids that have a strong propensity to form β-sheets and intermolecular hydrogen bonding, a necessity for one-dimensional self-assembly info nanostructures [196,197]. Moreover, charged amino acids can be included to enhance solubility in water and allow for salt or pH-responsiveness to form networks of one-dimensional nanostructures into scaffolds for regenerative medicine. Lastly, bioactive peptides for epitope-specific biological interactions can also be incorporated; for example, cell adhesion molecules are often incorporated to mimic native extracellular matrices (Figure 8). In recent years, the majority of *in vivo* applications using cylindrical PA-based scaffolds include regeneration for, bone [198], nerves [199–202], and reproductive organs [203].

Hydrogels with PA nanofibers with binding affinity to bone morphogenic protein-2 (BMP-2) was tested for promoting osteogenesis in a spinal fusion model [198]. Interestingly, the gels promoted osteogenesis with 10 to 100 times lower BMP-2 dose than those use clinically in collagen scaffolds, the standard of care today. In a rat posterolateral lumbar inter-transverse spinal fusion model, PA nanofiber gels exhibited enhanced spinal fusion rates verified by computed tomography (CT). Interestingly, the authors found nanofibers without the addition of exogenous BMP-2 resulted in enhanced fusion rates, suggesting nanofibers have the ability to recruit the endogenous growth factors. Overall, the authors conclude that this bioactive nanofiber system has promise for bone grafting procedures without undesirable side effects of high doses of BMP-2.

Regarding applications in the nervous system, recent attention has been focused on bioengineering nerve constructs for peripheral nerve injuries [199–202]. Li *et al.* designed PAs with RGDS and IKVAV, peptides for cell attachment and proliferation, in order to develop a scaffold that can support and guide axonal regeneration without morbidity and functional loss, a contrast to current standard of care [202]. The PA solution loaded onto PLGA conduits was passed through a 40 μm mesh screen to align nanofibers in response to shear flow, in order to promote directional axonal regeneration. The scaffolds supported Schwann cell growth *in vitro*, and in a critical sized, sciatic nerve defect in rats, PLGA conduits filled with aligned PAs demonstrated motor and sensory recovery, with increased axonal and Schwann cell regeneration within the nerve gap after 12 weeks via histology. In

addition, the ability of PA nanofiber hydrogels to regenerative collagen in response to cavernous nerve injury within prostatectomy and diabetic patients have also been reported, providing another promising use for PA use in regenerating tissues [203].

4. Conclusions

The future of peptide-based therapeutic clinical translation will rely on addressing four current major therapeutic hurdles, namely protein target specificity, stability of secondary structure, explicit cellular targeting and high-concentration delivery into cells. Many current forms of peptide-based therapeutics in pre-clinical or clinical development address one or two of these challenges but alone fall short of addressing them all. Self-assembling peptide-based nanoassemblies are currently being used to address all of these four hurdles and have vast medical implications for a variety of applications including drug delivery, diagnostics, vaccination, and tissue engineering. In the age of ‘personalized medicine,’ peptide-based self-assemblies also offer exciting new opportunities for explicit cellular homing and intracellular targeting of disease causing intracellular PPIs.

PPIs control essential cellular processes from the level of the plasma membrane to the inner workings of the nucleus. The biological activity of these interactions would ideally be targeted for therapeutic intervention due to explicit fidelity of contact points between binding partners. The biological potential of such peptides reflects millennia of evolution and endow biofunctional peptides explicit specificity and low off-target effects. Because of this, the breadth of potential targets vastly outnumbers other classes of biologic and small molecule therapeutics. However, many PPIs have been challenging to therapeutically target because of their often large, geographically complex, dynamic, and relatively flat surfaces. Although biologics like antibodies are able to effectively target such surfaces, these therapeutics lack the ability to efficiently target PPIs within cells and their large size and non-specific uptake in the liver and reticuloendothelial systems results in poor bioavailability and, for example, poor solid organ or tumor penetration. To target these interactions clinically, there has been a recent reliance on small molecules. However, many diseased PPIs are ‘off limits’ to these compounds because their binding relies on complex networks of protein-protein or protein-nucleic acid interactions, as is the case with transcription factors, deeming such high-reward targets “undruggable”. The future of peptide-based therapeutics will extend from those targeting extracellular membrane domains, receptor activating and blocking peptides, and hormonally-based therapeutics to vaccine-related and cytoplasmic and nuclear PPI engaging peptides. As crystal structures of protein binding partners and advanced screening approaches for biologic peptides continue to mature so too will opportunities for mechanistic studies and therapeutic benefit.

The impact of new production technologies will be critical to the future of self-assembling peptide use, innovation, and clinical development. Novel drug delivery systems and peptide therapeutics with increasingly high specificity and low toxicity can now be manufactured and tested together in real time for relatively low cost and increasingly higher efficiency. Self-assembling peptide-based nanoassemblies that remain stable in circulation or the gastrointestinal tract represent the future of targeted drug delivery and hold the promise of opening previously inaccessible avenues for therapeutic translation and where opportunities

to make tractable improvements in patients' health are on the verge of greater success. In the coming years, collaboration between chemists, engineers, biologists, and physicians will be increasingly vital to the development of these promising therapies for effective clinical translation.

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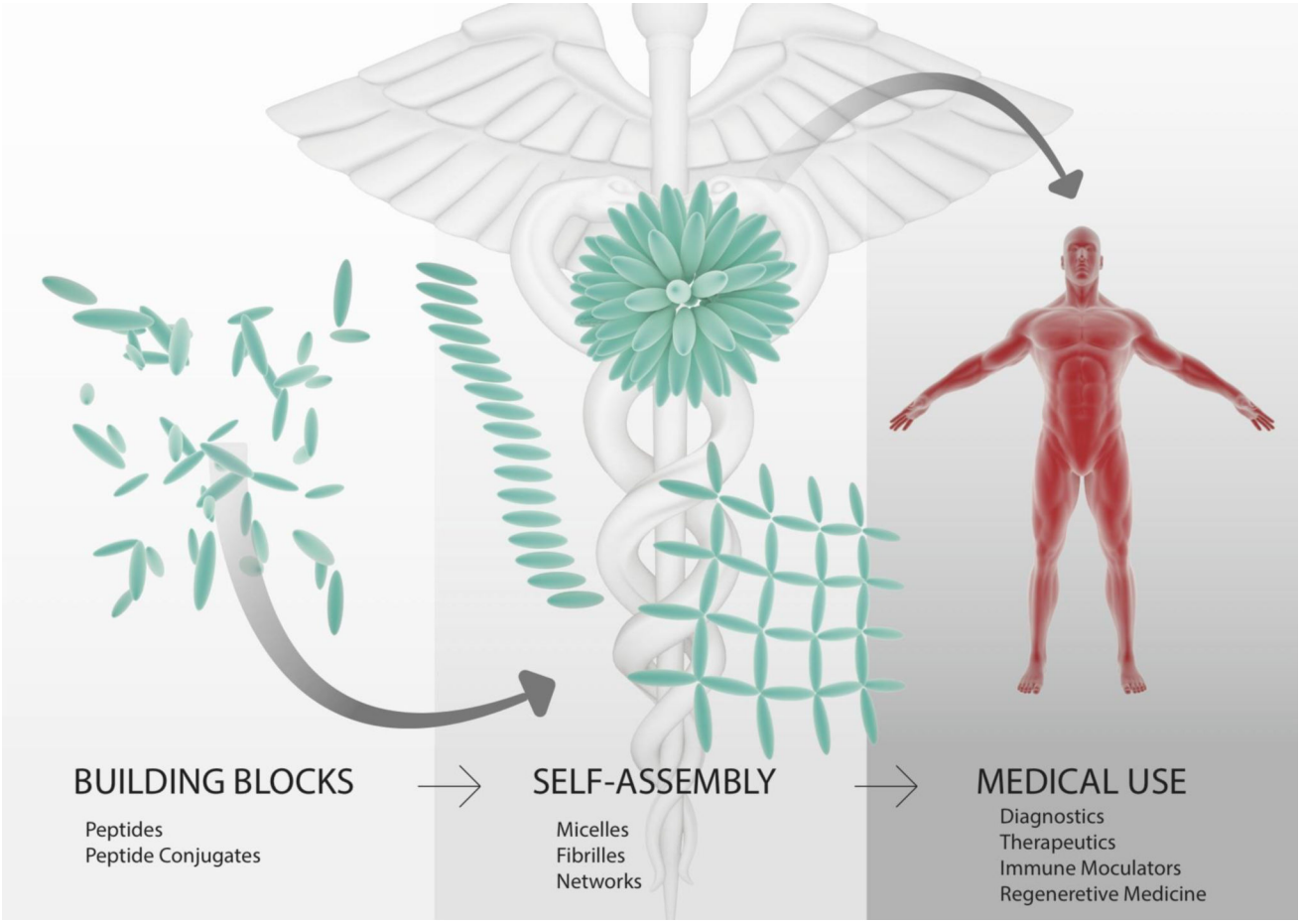

Figure 1. Self-assembling peptide-based structures

This review is focused on peptides and peptide-conjugates as building blocks for self-assembly of various structures for medical applications.

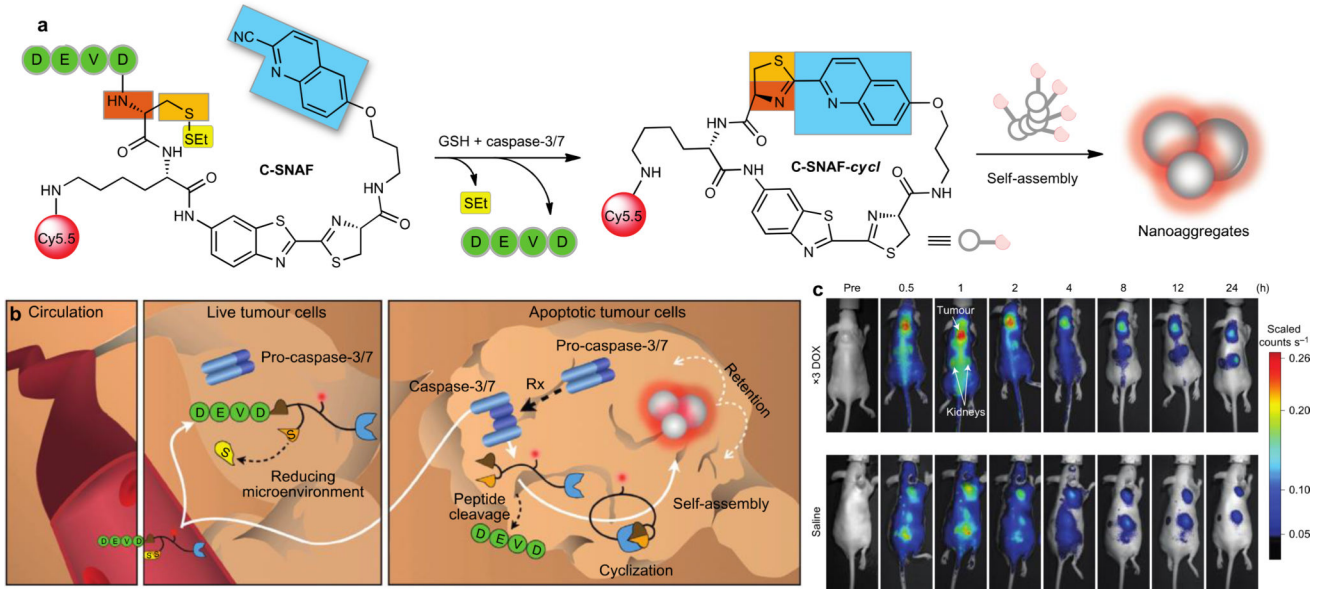

Figure 2. Enzyme-responsive self-assembly of tumor-targeting fluorescence probes

(**A**) *In vivo* imaging of caspase-3/7 activity in human tumor xenograft mouse models through the bioorthogonal intramolecular cyclization reaction and subsequent nanoaggregation via self-assembly. (**b**) The bioorthogonal intramolecular cyclization reaction occurs only in the presence of caspase-3, which is only found in apoptotic tumor cells. (**c**) Non-invasive fluorescence imaging of ×3 doxorubicin (DOX)-treated (top) and saline-treated (bottom) tumor-bearing mice. Enhanced fluorescence clearly indicates the effectiveness of the DOX treatment. White arrows indicate the anatomical locations of the tumor and the kidneys. Adapted by permission from Macmillan Publishers Ltd: [Bioorthogonal cyclization mediated in situ self-assembly of small-molecule probes for imaging caspase activity *in vivo*] [85] copyright (2016).

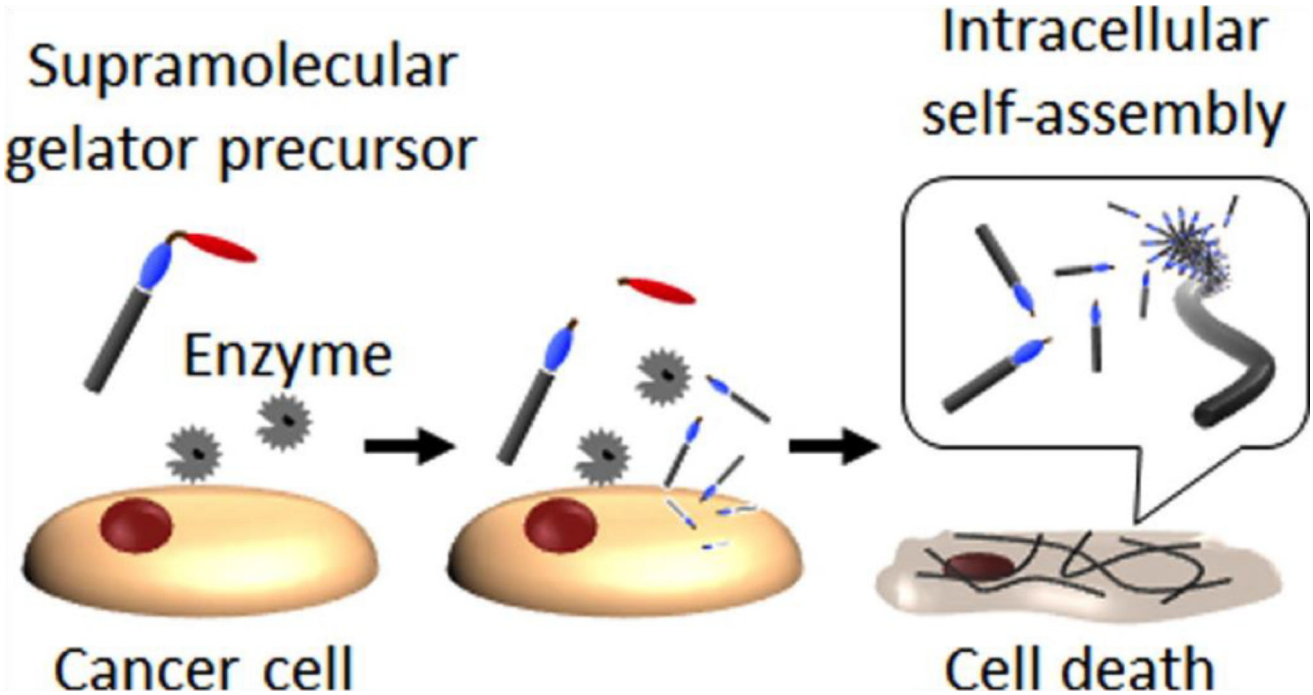

Figure 3. Enzyme-driven self-assembly of peptide fibrils causes related cell death

The peptide part repels the precursor building blocks to prevent the gelation in normal tissue. A cancer-related enzyme, matrix metalloproteinase-7, cleaves the supramolecular gelator precursor, and the amphiphilic region self-assembles into one-dimensional fibrillar structures and causes cell death. Adapted with permission from [99]. Copyright (2016) American Chemical Society.

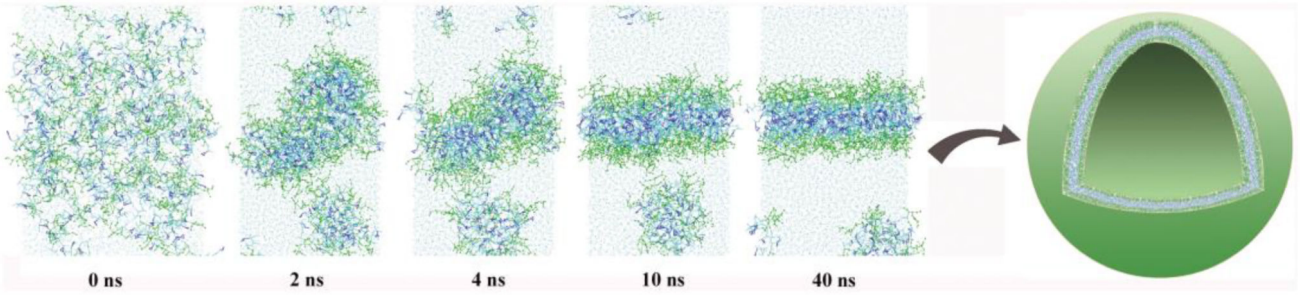

Figure 4. Self-assembling bilayered vesicles

Self-assembly kinetics of branched peptides with hydrophobic adhesive sequences FLIVIGSII and FLIVI into solvent-filled bilayered vesicles with a size range of 50–200 nm. Adapted with permission from Gudlur et al [110].

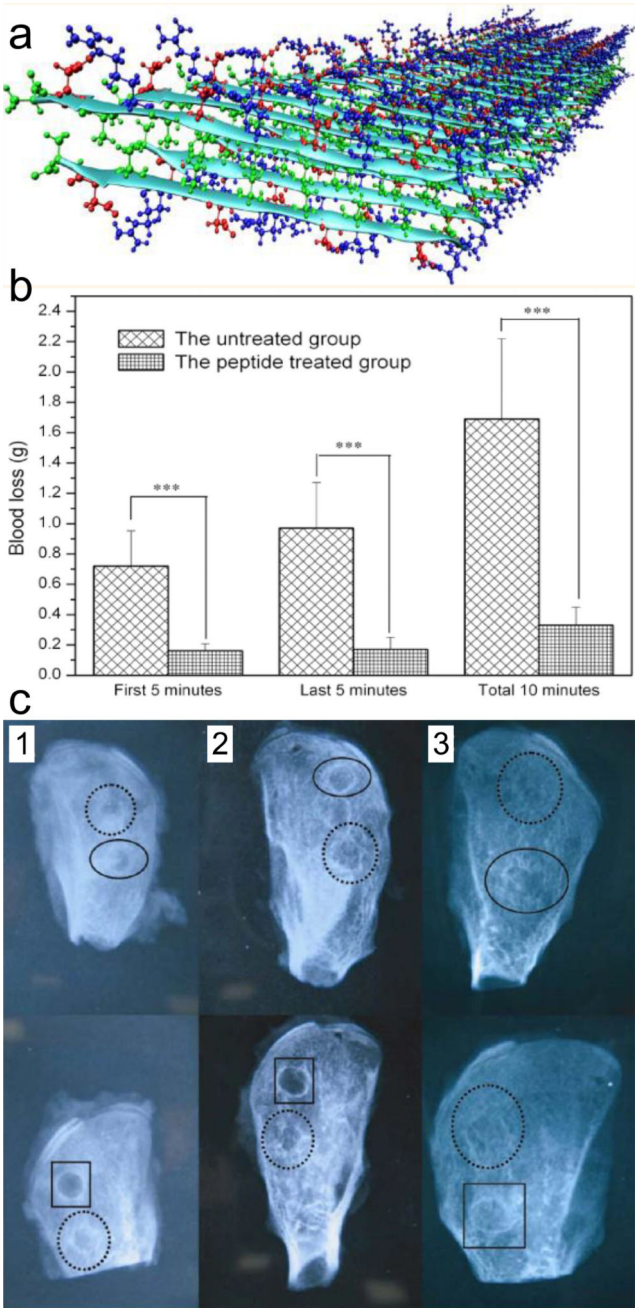

Figure 5. Sheet formation and applications of *lego* peptides

a. A molecular model for RADA16-I nanofibers, with ribbons denoting peptide backbones, and green, blue and red atoms denoting alanine, arginine, and aspartate residues, respectively. Adapted with permission from [133]. Copyright (2016) American Chemical Society. **b**. Blood loss and **c**. representative radiographs of the ilium bone defects of New Zealand rabbits at different times. In c., 1, 2 and 3 correspond to 4, 8 and 12 weeks, respectively post treatment. RADA16- I (dotted circle) or bone wax (rectangle) was used for treatment, and are compared against the untreated specimen (ellipse). Adapted with permission from [140]. Copyright (2016) American Chemical Society.

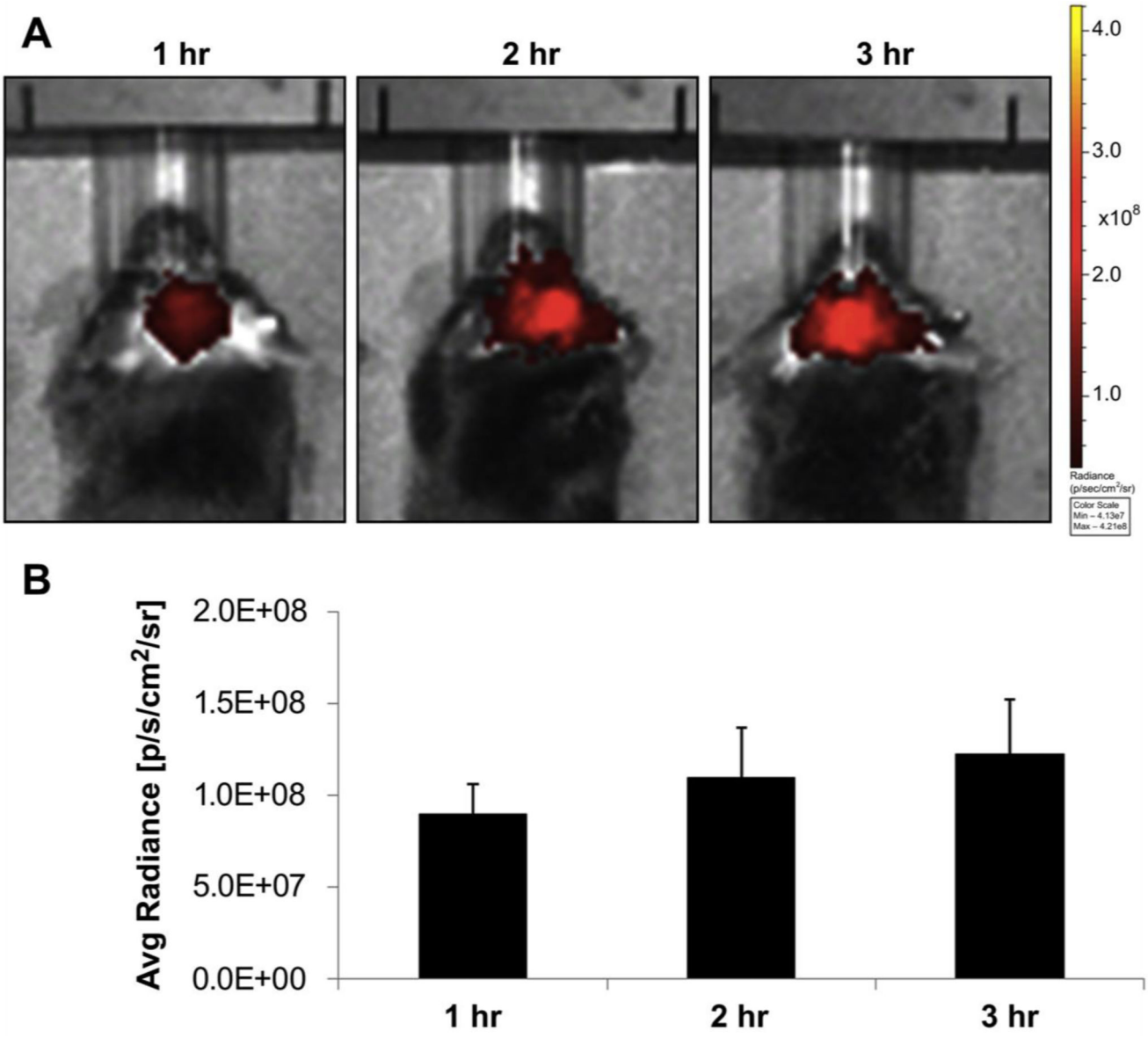

Figure 6. ***In vivo*** **images of Cy7-CREKA-micelles**

Cy7-CREKA micelles target gliomas as early as 1 hour post-injection intravenously. (**a**) *in vivo* images, (**b**) quantification of fluorescence for up to 3 hours. Adapted with permission from Adapted by permission from Macmillan Publishers Ltd: [Fibrin binding, peptide amphiphile micelles for targeting glioblastoma] [148] copyright (2016).

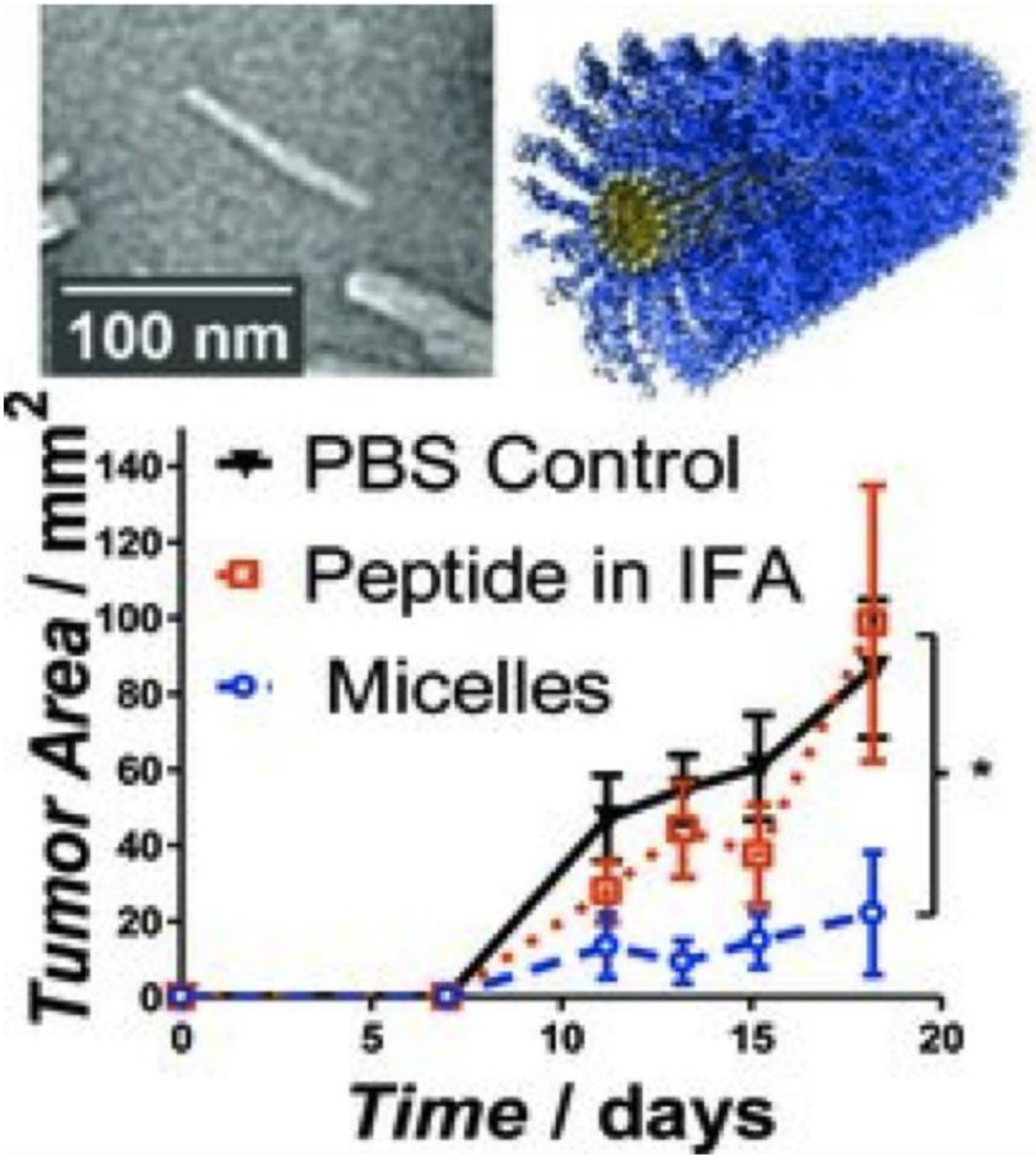

Figure 7. One-dimensional peptide vaccine formation

Antigenic peptide amphiphiles self-assemble into micelle vaccines, which are sufficient to induce antigen-specific cytotoxic T cell responses *in vivo* against tumors bearing the same antigen. Adapted by permission from Macmillan Publishers Ltd: [Self-Assembled Peptide Amphiphile Micelles Containing a Cytotoxic T-Cell Epitope Promote a Protective Immune Response *In Vivo*] [191] copyright (2016).

Figure 8. Molecular structure of cylindrical PAs with 4 rationally designed chemical regions

Adapted by permission from Macmillan Publishers Ltd: [Self-assembly of peptide amphiphiles: From molecules to nanostructures to biomaterials] [197] copyright (2016).