Supramolecular Self-assembled Peptide-based Vaccines: Current State and Future Perspectives

1

Tuerdimaimaiti Abudula[1†], Khushbu Bhatt[2†], Loek Eggermont[3†], Nick O'Hare[3], Adnan Memic[1*], Sidi A. Bencherif[3,4,5,6*]

[1]Center of Nanotechnology, King Abdulaziz University, Jeddah, Saudi Arabia

[2]Department of Pharmaceutical Sciences, Northeastern University, Boston, USA

[3]Department of Chemical Engineering, Northeastern University, Boston, USA

[4]Department of Bioengineering, Northeastern University, Boston, USA

[5]Harvard John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA

[6]Sorbonne University, UTC CNRS UMR 7338, Biomechanics and Bioengineering (BMBI), University of Technology of Compiègne, Compiègne, France

* Correspondence:

Adnan Memic at amemic@kau.edu.sa

Sidi A. Bencherif at s.bencherif@northeastern.edu

[†]These authors contributed equally to this work.

Abstract

Despite the undeniable success of vaccination programs in preventing diseases, effective vaccines against several life-threatening infectious pathogens such as human immunodeficiency virus are still unavailable. Vaccines are designed to boost the body's natural ability to protect itself against foreign pathogens. To enhance vaccine-based immunotherapies to combat infections, cancer, and other conditions, biomaterials have been harnessed to improve vaccine safety and efficacy. Recently, peptides engineered to self-assemble into specific nanoarchitectures have shown great potential as advanced biomaterials for vaccine development. These supramolecular nanostructures (i.e., composed of many peptides) can be programmed to organize into various forms, including nanofibers, nanotubes, nanoribbons, and hydrogels. Additionally, they have been designed to be responsive upon exposure to various external stimuli, providing new innovations in the development of smart materials for vaccine delivery and immunostimulation. Specifically, self-assembled peptides can provide cell adhesion sites, epitope recognition, and antigen presentation, depending on their biochemical and structural characteristics. Furthermore, they have been tailored to form exquisite nanostructures that provide improved enzymatic stability and biocompatibility, in addition to the controlled release and targeted delivery of immunomodulatory factors (e.g., adjuvants). In this short review, we first describe the different types of self-assembled peptides and resulting nanostructures that have recently been investigated. Lastly, we discuss the recent progress and development trends of self-assembled peptide-based vaccines, their challenges, and clinical translatability, as well as their future perspectives.

1   Introduction

Vaccination has been considered as one of the crowning achievements of humankind, and gained remarkable triumph in treating many life-threatening and epidemic diseases, such as influenza, tuberculosis, and smallpox (Malonis et al., 2019; Piot et al., 2019). Vaccines can greatly reduce the burden of preventable infectious diseases by working with the body's natural defenses to safely develop immunity to disease (Piot et al., 2019; Parvizpour et al., 2020). For instance, to respond to the new and unprecedented coronavirus disease 2019 (COVID-19) crisis, vaccination is considered to be the best strategy to end this pandemic (Graham, 2020; Lurie et al., 2020). Conventional vaccination methods are based on pathogens (e.g., attenuated, inactivated, toxins, subunits) that can provoke immunity against antigens and provide long-lasting protection against the diseases (Chiang et al., 2010; Cockburn and Seder, 2018). Despite these advantages, this approach is suffering from a number of drawbacks, including low immunogenicity, high cost, manufacturing challenges, vaccine instability in the cold chain, and potential contaminations during manufacturing which could alter vaccine efficacy and cause a strong allergic shock (Skwarczynski and Toth, 2016).

Although great advances have been made in the development of vaccines and immunotherapies, there is an increasing demand for enhanced control over the immune responses induced against infectious diseases and cancer. Biomaterials can be leveraged for modulating the immune system and subsequently controlling immune responses (Bookstaver et al., 2018). For instance, recent immunization strategies have centered on biomaterial-based vaccines in which specific cellular components were used as antigens to stimulate the immune response against cancer (Bencherif et al., 2015; Malonis et al., 2019). Within these biomaterial-based vaccines, full-length proteins and peptides have been extensively studied as antigens, but they can also be used as structural biomaterials (Skwarczynski and Toth, 2016). Peptides are biomolecules that consist of sequences of 2-50 amino acids and that have a molecular structure that is generally much simpler than that of proteins (Malonis et al., 2019). Using peptides has become a widespread trend in vaccine development since they are easily processed and presented by antigen-presenting cells, leading to potent T cell–mediated immune responses (Purcell et al., 2007). Furthermore, current challenges associated with cell-based or protein-based vaccination, such as manufacturing complexity, biological contamination, off-target effects, and autoimmunity, can be prevented with peptide-based vaccines (Purcell et al., 2007; Skwarczynski and Toth, 2016). Additionally, peptides are capable of self-assembly into ordered supramolecular structures, making them excellent building blocks to form nanofibers, nanovesicles, nanotubes, nanomicelles, and hydrogels (Habibi et al., 2016; Qi et al., 2018). These peptide assemblies can have a multivalent character and present peptides in their native 3D conformation, which is essential for B-cell stimulation. Furthermore, they allow mixing of multiple components with precise stoichiometry (Wen and Collier, 2015). Ultimately, immunogenic, long-lasting, stable, and self-adjuvanted vaccines can be engineered by combining epitopes, antigens, and immunomodulatory moieties within the self-assembled peptide structures (Wen and Collier, 2015).

Besides being exploited as immunostimulatory materials in vaccine development, self-assembled supramolecular peptides are also excellent candidates to serve as carriers for the delivery of immunological factors (Sis and Webber, 2019; Xiao et al., 2019b). The macrostructural features of supramolecular peptide assemblies can be fine-tuned by altering the amino acid sequences. Additionally, bioactive segments can be introduced into the peptides during their self-assembly to design stimuli-responsive and cell- or organ-targeted vaccine delivery vehicles (Sis and Webber, 2019; Xiao et al., 2019a; Lampel, 2020). Moreover, combining immunogenic peptide epitopes with non-immunogenic peptides that form delivery carriers could enhance vaccine efficacy while reducing unwanted side effects (Eskandari et al., 2017).

119   In this short review, we first describe the various strategies employed to create self-assembled
120   supramolecular peptides with sophisticated hierarchical nanostructures. Next, their application in
121   subunit vaccine design and delivery for cellular and humoral immunity is highlighted. Finally, we
122   discuss challenges and clinical translatability of self-assembled peptide-based vaccines, as well as their
123   future perspectives.
124

125   **2   Design of supramolecular self-assembled peptides**

126   Molecular self-assembly is a bottom-up approach for achieving highly ordered and stable nanoscale
127   structures or patterns. This technique is based on the spontaneous assembly of small molecules or
128   nanosized building blocks under thermodynamic equilibrium conditions (Qi et al., 2018).
129   Intermolecular and intramolecular interactions, such as hydrogen bonding, amphiphilic interactions,
130   and aromatic stacking, have been used for supramolecular peptide assembly, allowing the construction
131   of complexes with stratified nanostructures (Lampel, 2020). Specifically, peptides can be designed to
132   exhibit distinctive secondary structures, such as β-sheets, β-hairpins, and α-helices, and these natural
133   motifs can be leveraged to drive a complex hierarchical architecture (Rad-Malekshahi et al., 2015). For
134   instance, β-sheets consisting of alternating hydrophobic and hydrophilic amino acids have been
135   extensively applied in driving self-assembly of peptides into extended fibrillar nanostructures (Moore
136   et al., 2018; Sis and Webber, 2019). Several groups have designed self-assembled nanofibrils from the
137   synthetic amphiphilic peptide RADA16 (RADARADARADARADA) through the β-sheet motifs
138   (**Figure 1A**), in which alternating regions of hydrophobic alanine and hydrophilic arginine/aspartate
139   residues can yield a stable β-sheet-rich structure (Cormier et al., 2013; Lu et al., 2019). Furthermore,
140   the antiparallel orientation of double-layered β-sheets, such as β-hairpins, have shown to generate
141   another secondary structure, which promotes the formation of peptide-based nanofibrous hydrogels
142   under physiological conditions (Worthington et al., 2017). Smith et al. developed a multiphase
143   transitioning, injectable hydrogel through the molecular self-assembly of a peptide-based β-hairpin.
144   Their anastomosis photocage 1 (APC1) peptide, which contains seven lysine residues, was folded into
145   a β-hairpin and rapidly self-assembled into a cross-linked fibrillar hydrogel (Smith et al., 2016).
146   Alternatively, α-helices could also be used to form a highly ordered structure through helical self-
147   assembly, which closely resembles the sophisticated hierarchical coiled coil-type structures of native
148   proteins, such as collagen (Lampel, 2020). Thomson et al. designed supercoiled coil α-helical barrels
149   by self-assembling 5-7 α-helices twisted around each other with cyclic or dihedral symmetry (**Figure
150   1B**). The peptides used in their study have a canonical hpphppp heptad repeat sequence, in which h
151   and p represent hydrophobic and polar amino acids, respectively (Thomson et al., 2014; Rhys et al.,
152   2018). Di-phenylalanine peptide (FF) is another interesting building block to trigger peptide self-
153   assembly into desirable nanostructures such as nanotubes, nanospheres, and nanoribbons (Habibi et
154   al., 2016). While self-assembly of the FF motif is usually achieved in the form of amyloid-like sheets
155   (Lampel, 2020), Bera et al. interestingly generated a helical architecture by simply adding one more
156   amino acid (proline) to the FF peptide, forming proline-phenylalanine-phenylalanine (PFF) (Bera et
157   al., 2019).
158

159   Additionally, non-peptidic compounds such as alkyls, amino acids, and metal ions have been used to
160   form self-assembled peptide complexes with a wide variety of structures and functionalities (Sis and
161   Webber, 2019). Alkyls, for instance, have been used as a hydrophobic tail to produce peptide
162   amphiphiles (PAs) that undergo self-assembly via hydrophobic shielding (Sis and Webber, 2019). As
163   a result, the nanostructure and other properties of self-assembled PAs can be controlled by switching
164   their hydrophobic components (Qi et al., 2018). Choi et al. proved that the nanostructure of self-
165   assembled amphiphilic Janus peptide dendrimers (JPDs) can be induced via a simple chemical

166 bifurcation. Various JPDs were synthesized by varying the length of their hydrophilic and hydrophobic
167 regions, and their self-assembly resulted in the formation of spherical/cylindrical micelles or bi-layered
168 vesicles (Choi et al., 2019). PAs can also be made by conjugating peptides with functional polymers
169 (Habibi et al., 2016). Chin et al. designed a muscle-inspired anisotropic actuator in the form of a hollow
170 fibrous peptide-based hydrogel, in which VVVAAAEEE peptides were functionalized with a lysine-
171 coupled bromoisobutyryl moiety to enable the grafting of thermo-responsive polymers via atom
172 transfer radical polymerization (Chin et al., 2018). Recent studies also revealed that the nanostructure
173 of self-assembled peptides can be reshaped in a controllable manner by incorporating metal ions (Sis
174 and Webber, 2019). Knight et al. studied the effect of different metal ions on the morphological
175 transformation of self-assembled PAs consisting of hexahistidine as a hydrophilic head and
176 oligostyrene as a hydrophobic tail (**Figure 1C**). Interestingly, the presence of manganese(II) promoted
177 the formation of multilamellar vesicles, while nickel(II) and cadmium(II) engendered micelle
178 nanoparticles. In contrast, zinc(II), copper(II), and cobalt(II) led to agglomerated micelles (Knight et
179 al., 2018).
180
181 Finally, there has been a growing interest in designing smart nanomaterials that initiate the assembly
182 of molecular building blocks upon application of external stimuli such as enzymes, pH, heat, and light.
183 This strategy can provide additional spatiotemporal control over the formation and structure of
184 supramolecular peptides (Lampel, 2020). For instance, enzyme-instructed self-assembly (EISA) of
185 peptides has recently been applied to allow assembly in complex biological systems (Wang et al.,
186 2019). Li et al. synthesized several dipeptidic precursors, consisting of FF with various stereoisomers,
187 in which N- and C-terminal peptide sequences are linked to 2-(naphthalen-2-yl)acetic acid and 2-(4-
188 (2-aminoethoxy)-4-oxobutanamido)ethane-1-sulfonic acid, respectively. Molecular self-assembly
189 (i.e., EISA) of these peptides is induced by carboxylesterase, which is present inside the cells and in
190 the extracellular space of tissues. Using this approach, intracellular and intercellular peptide self-
191 assembly could be achieved at different rates (Li et al., 2018). Additionally, some stimuli-responsive
192 peptides, such as elastin-like polypeptides (ELPs), exhibit reversible transition behavior upon exposure
193 to the specific stimuli (Saha et al., 2020). Dreher et al. prepared several ELP block copolymers (with
194 varying molecular weights and block ratios) in a linear AB diblock architecture by homogeneously
195 fusing an N-terminal hydrophilic ELP block to a C-terminal hydrophobic ELP block. They observed
196 that copolymers with suitable diblock ratios are highly water-soluble at normal body temperature (37
197 ᵒC) and can self-assemble into spherical micelles at the tumor temperature (42 ᵒC). When the
198 temperature was lowered again, the copolymers showed the inverse transition behavior (Dreher et al.,
199 2008). Hassouneh et al. developed a theoretical model to explain the mechanism for this special
200 reversible transition behavior of the copolymers(Hassouneh et al., 2015).
201

202 **3   Application of self-assembled peptides in vaccine design and delivery**

203 A wide variety of vaccines have been designed to stimulate the immune system to combat pathogens
204 (e.g., bacteria, viruses) or tumors. In particular, vaccines aim to induce an adaptive immune response
205 that leads to immunological memory, as illustrated in **Figure 1D**. There are two subdivisions of the
206 adaptive immune system: the cell-mediated immune response, which is executed by cytotoxic T
207 lymphocytes (CTLs) that can kill infected or cancerous cells, and the humoral immune response, which
208 is mediated by activated B cells that produce antibodies to neutralize extracellular pathogens (Molnar
209 and Gair, 2015). Peptide-based vaccines generally require three major components—an antigen, an
210 adjuvant, and a delivery vehicle—to generate efficient adaptive immune responses. When peptides are
211 used as antigen, the conformation of the specific regions recognized by the immune system, known as
212 epitopes, is particularly important for inducing humoral immunity. Specifically, B cells need to bind

213   epitopes in their native conformation to allow antibody binding. B-cell epitopes usually have α-helical,
214   loop, and β-strand conformations that are generally integrated into vaccines as longer peptides to allow
215   them to adopt their native conformation (Skwarczynski and Toth, 2016; Malonis et al., 2019). When
216   designing vaccines, self-assembly of peptides can be utilized to ensure correct folding of antigenic
217   epitopes. In vaccine applications where antibody affinity and titer are essential, self-assembling
218   peptides that also have inherent CD4+ T-cell epitopes, such as those included in the Coil29
219   (QARILEADAEILRAYARILEAHAEILRAD) peptide, can be incorporated to induce strong follicular
220   helper T-cell engagement that further promotes B-cell responses (Wu et al., 2020). Similar to CD4+
221   T-cell stimulation, the peptide sequence is more critical than the epitope conformation itself to induce
222   CTL-mediated cellular immunity. Therefore, shorter peptides can be used to induce T-cell responses,
223   as CD4+ T cells recognize 12-16 amino acid long peptides presented by MHC II on antigen-presenting
224   cells (APCs), while CD8+ T cells bind to slightly shorter 8-10 amino acid long peptides displayed by
225   MHC I (Skwarczynski and Toth, 2016; Malonis et al., 2019). Unlike attenuated pathogen-based
226   vaccines, peptide-based vaccines generally incorporate adjuvants to boost the overall immune response
227   to antigens and mimic the natural "danger signals" which follow infections. The choice of adjuvants
228   depends on several factors such as immunogenicity and toxicity. Several studies have established that
229   the adjuvanticity of self-assembled peptides is mediated via antigen presentation in an ordered and
230   repetitive array that resembles pathogen-associated molecular patterns (PAMPs), resulting in strong
231   immune responses mediated through Toll-like receptor (TLR)-inflammasome signaling pathways via
232   TLR2 and TLR4 activation (Azmi et al., 2014; Negahdaripour et al., 2017; Tandon et al., 2018; Zottig
233   et al., 2020). Additionally, self-assembling peptides may act as adjuvants themselves by forming an
234   antigen depot, directing vaccines to APCs, and ultimately enhancing immune-cell priming (Grenfell et
235   al., 2015; Acar et al., 2017; Negahdaripour et al., 2017).

237   *Self-assembled peptide-based vaccines for cellular immunity*
238   Supramolecular self-assembling peptides can form excellent structures to induce cytotoxic immune
239   responses, which is particularly important for cancer immunotherapy. For this purpose, peptide
240   assemblies can function as a platform for safe and controlled delivery of antigens, adjuvants, immune
241   cells and/or drugs (**Table 1**). For example, Wang et al. utilized a tumor-penetrating peptide Fmoc-
242   KCRGDK-based hydrogel formulation to encapsulate a BRD4 inhibitor, a photothermal agent
243   (indocyanine green), and autologous tumor cells. Upon laser irradiation, the personalized cancer
244   vaccine released tumor-associated antigens. This process promoted DC maturation, T-cell infiltration,
245   the formation of memory immune cells to prevent tumor relapse, and inhibited distant tumors (Wang
246   et al., 2018b). Interestingly, Xu et al. observed that the configuration and the number of lysine residues
247   in the peptide are critical to enhance CTL response. They described a supramolecular NF-κB-activating
248   nano-adjuvant hydrogel, synthesized by pH-triggered self-assembly of Ada-GFFYGKKK-NH2
249   peptide, for cancer immunotherapy. According to their findings, nano-adjuvants containing D-
250   configured peptides and 3 lysine residues encapsulated antigens more efficiently through charge-
251   charge interaction than 2 lysine residues, and as a result, generated more robust adaptive and innate
252   immune responses than peptides with L-configuration (Xu et al., 2019). Yang et al. showed that a
253   nanofibrous RADA16 peptide-based hydrogel scaffold encapsulating bone-marrow derived DCs,
254   model antigen ovalbumin (OVA), and anti-PD-1 antibody recruited and stimulated endogenous and
255   exogenous DCs. This process increased DC migration to the lymph nodes, driving a more robust
256   antigen-specific immune response against EG7-OVA tumors (Yang et al., 2018). Furthermore, Wang
257   et al. developed glutathione-responsive nanocomposites by co-assembling a positively charged cell-
258   penetrating CWWRCRCRC peptide with a negatively charged protein such as ovalbumin (OVA) via

259 electrostatic interactions. After being internalized by APCs, intracellular glutathione degraded the
260 disulfide bonds of the peptide, inducing rapid release of the antigen into the cytoplasm, which was then
261 cross-presented to induce potent CD8+ T-cell responses. When compared to free OVA, the peptide-
262 based nanocomposites improved antigen uptake by DCs, promoted DC activation and maturation, and
263 enhanced cellular as well as humoral immune responses (Wang et al., 2018a). Overall, the use of self-
264 assembled peptides as a delivery vehicle provides multiple benefits, including efficient cell and antigen
265 loading, minimal loss of active components, as well as controlled and targeted release in the tumor
266 microenvironment (Zhang, 2017; Lee et al., 2019).

267

268 Apart from acting as a delivery vehicle, self-assembling peptides can also serve as an antigen source
269 themselves to induce cellular immunity. For example, Black et al. developed cancer vaccines consisting
270 of self-assembling tumor antigen peptides with enhanced immunogenicity. Conjugating a synthetic
271 lipid tail with two palmitic residues to an OVA-derived peptide containing a CTL epitope initiated the
272 self-assembly of PAs into cylindrical micelles, resulting in multivalent epitope presentation. These
273 micelles were internalized by APCs, likely due to fusion of their hydrophobic tails with the cell
274 membrane, leading to CTL activation in absence of additional adjuvants. Additionally, these
275 cylindrical micelles act as antigen depots, thereby protecting the peptides from degradation and
276 prolonging antigen exposure to the APCs. Improved in vivo protection was observed against OVA-
277 expressing tumor cells after immunization with the diC16-OVA micelles compared to free OVA
278 peptide formulated with incomplete Freund's adjuvant (Black et al., 2012). Xing et al. developed
279 injectable, peptide-based supramolecular hydrogels by co-assembling poly-L-lysine (PLL) and FF
280 dipeptide via electrostatic coupling. Fmoc-FF/PLL-SH hydrogels have a nanofibrous structure with α-
281 helical conformation which resembles natural fimbrial antigens, thereby acting as an adjuvant. When
282 injected around a tumor, the hydrogels activated T-cell responses and efficiently suppressed tumor
283 growth without the addition of other adjuvants or antigens. In this case, the tumor cells themselves act
284 as the source of antigen to stimulate the immune response (Xing et al., 2017). Self-assembly can also
285 be used to incorporate longer peptides containing multiple epitopes to ensure optimal APC stimulation,
286 increase the magnitude of CTL response, and simultaneously promote helper T-cell activity (Lynn et
287 al., 2020). Overall, peptide-based self-assembly can induce robust cellular immune responses by
288 serving as a durable source of antigen that allows easy uptake and processing by APCs, providing
289 multiple epitopes, and conferring self-adjuvanticity to the vaccine.

290

291 *Self-assembled peptide-based vaccines for humoral immunity*
292 Robust humoral immune responses are essential to treat infectious diseases. For this purpose, self-
293 assembly designs have included approaches such as flanking the antigen with a self-assembling peptide
294 sequence to display the secondary structure of the antigen, presenting antigens in a highly ordered and
295 repetitive form, and optimizing the distance between repeated epitopes for optimal B-cell receptor
296 (BCR) engagement (Raman et al., 2006; Black et al., 2010; Babapoor et al., 2011; Trent et al., 2015;
297 Skwarczynski et al., 2020). Self-assembling supramolecular peptides can serve as a delivery scaffold
298 to present antigenic peptides, and they can simultaneously possess self-adjuvanting characteristics to
299 induce B-cell responses. For instance, Grenfell et al. exploited RADA4 peptide-based hydrogels that
300 self-assembled into hydrated nanofibers after injection into the tissue to form a gel matrix depot, to

deliver a recombinant antigen for the hepatitis B virus. This system elicited enhanced adjuvant-free humoral and cellular responses when compared to the antigen delivered with aluminum hydroxide (alum) and complete Freund's adjuvant. The authors credited the slow release of antigen from the depot to have improved the activation of APCs and prolonged immunostimulation (Grenfell et al., 2015). In a different study, Tian et al. utilized a supramolecular hydrogel-based nanovector comprised of Nap-GFFY-NMe (naphthylacetic acid-modified tetrapeptide GFFY with C-terminal methyl amide group), using an EISA approach to encapsulate a DNA sequence encoding gp145, an envelope glycoprotein of the human immunodeficiency virus (HIV). Alkaline phosphatase triggered self-assembly of the peptide, leading to formation of nanofibrous hydrogels. The strong cellular and humoral immune responses were attributed to the ability of left-handed nanofibers to effectively condense DNA and prevent it from degradation, thus enhancing DNA transfection and gene expression in cells (Tian et al., 2014).

Self-assembling peptides can also be the antigen source to induce humoral immunity. For instance, Kaba et al. utilized a self-assembling peptide-based nanoparticle platform to present repeated immunodominant B-cell circumsporozoite peptide epitope $(DPPPPNPN)_2D$ of the malarial parasite. The polypeptide consisted of two oligomerization domains—a de novo trimeric coiled-coil domain and a pentameric coiled-coil domain—which were fused together with flexible diglycine residues. The self-assembled nanoparticles, containing multiple coiled-coil domains, displayed the B-cell peptide epitope in a highly ordered and repetitive array, thereby triggering robust helper T cell–dependent and long-lasting antibody responses with higher avidity and titer (Kaba et al., 2009). Pimentel et al. utilized a similar polypeptide-based self-assembling nanoparticle platform to display the C-terminal heptad repeat region (HRC) of the SARS-CoV S-protein in its native α-helical trimeric coiled-coil conformation. These nanoparticles not only maintained this conformational integrity, but also provided repetitive presentation of the B-cell epitope and displayed icosahedral symmetry that resembled viral protein capsids. The SARS-CoV vaccine evoked conformation-specific neutralizing antibodies against the B-cell epitope without additional adjuvants (Pimentel et al., 2009). A potent peptide-based vaccine against *Streptococcus pneumoniae* was proposed by Dorosti et al., who incorporated CTL epitopes such as pneumococcal surface protein A (PspA) and choline-binding protein A (CbpA), helper T-cell epitopes such as pneumococcal histidine triad protein D (PhtD) and a lipoprotein from pneumococcal iron ABC transporter (PiuA) and universal helper T-cell epitopes like diphtheria toxoids on a coiled-coil self-assembling interface. The designed vaccine was predicted to exhibit stronger immunogenic responses compared to an analogous epitope-based vaccine (Dorosti et al., 2019). In addition, Rudra et al. developed a peptide-based vaccine to prevent malaria by combining self-assembling β-sheet peptides with epitopes of protozoan parasite *P. falciparum* (Rudra et al., 2012). In another example, Trent et al. designed a self-adjuvant peptide-based vaccine for group A streptococcus (GAS). When the J8 antigenic peptide of the GAS-M protein is taken out of its native protein environment, its α-helical structure is lost, and as a result, it adopts a random conformation in the solution. The addition of two C16 alkyl chains at the N terminus of the J8 peptide triggered its self-assembly into cylindrical micelles, and reinforced α-helicity of the antigen, which subsequently induced a strong B-cell response in mice. Since the hierarchical micellar structure kept thousands of peptides in close proximity, it conferred potent adjuvanticity while maintaining the native antigen conformation at the injection site.

343   In addition, the micellar structure increased the local antigen concentration available to immune cells
344   in comparison to free peptide, which quickly diffused away from the injection site (Trent et al., 2015).
345   Taken together, these studies have demonstrated that self-assembled peptides can efficiently induce
346   humoral immune responses through the display of secondary structures, thereby maintaining the
347   conformational integrity of epitopes. Furthermore, these self-assembly platforms offer a highly ordered
348   and multivalent display of relevant antigens, resulting in efficient stimulation and proliferation of B
349   cells, and their subsequent differentiation into antibody-secreting plasma cells. Inclusion of helper T-
350   cell epitopes further enhances vaccine efficacy by imparting long-lasting B-cell immunity.
351

4   Challenges and future perspectives

353   Despite the great potential of self-assembled supramolecular peptides for vaccine design and
354   engineering, many challenges still persist. Compared to conventional vaccines containing live-
355   attenuated or inactivated pathogens (viruses, bacteria, etc.), most peptide-based vaccines induce a weak
356   immune response (Malonis et al., 2019). The use of adjuvants can overcome this obstacle, and deepen
357   our understanding of their mechanism of action and their safety (Skwarczynski and Toth, 2016).
358   Additionally, the stability and efficacy of peptide-based vaccines in complex biological environments
359   remain challenging. For instance, their rapid enzymatic degradation and structural integrity in the body
360   is a major limiting factor (Kim et al., 2019). Self-assembled peptides must exhibit improved stability
361   when interfacing with biological barriers (e.g., pH, enzymes) that are encountered upon administration
362   in tissues (Eskandari et al., 2017). Rational systematic design of the supramolecular peptide structure
363   to include covalent cross-linking—or via improved intermolecular interactions such as hydrogen
364   bonding, π-π stacking, and hydrophobic interactions—may augment their resilience and mechanical
365   stability (Khalily et al., 2015; Li et al., 2019). The development of supramolecular self-assembled
366   peptide-based vaccines is hindered by our limited understanding of the interface between self-
367   assembled nanostructures and immune cells. In-depth investigation of the interactions between the self-
368   assembled structures and the receptors on human immune cells, their uptake by APCs, as well as their
369   effect on DC maturation, B-cell and T-cell priming, and cytokine profiling, is needed (Zhao et al.,
370   2017). Challenges exist in translating the ease in design and small-scale synthesis of supramolecular
371   peptides to an industrial scale. Furthermore, advanced engineering of supramolecular assembly (e.g.,
372   cell-penetrating peptides) may target intracellular vaccine delivery more precisely, ultimately
373   enhancing antigen uptake, promoting endosomal escape and cross-presentation by APCs, which are
374   critical steps in inducing a robust cellular immune response (Yang et al., 2019). Existing technologies
375   such as machine learning, bioinformatics, and computational modeling can potentially be leveraged
376   for the macromolecular engineering of immunostimulatory peptide assemblies with improved vaccine
377   immunogenicity, efficacy, and safety (Kim et al., 2019). Moving forward, biologically inspired
378   supramolecular peptides, an excellent and sparsely explored class of materials, could be further
379   exploited in designing the next generation of vaccines that are effective, safe, affordable, and accessible
380   to everyone. Despite their importance and great potential, self-assembled peptide-based vaccines do
381   require further investigation and validation prior to regulatory approval and clinical use.
382
383
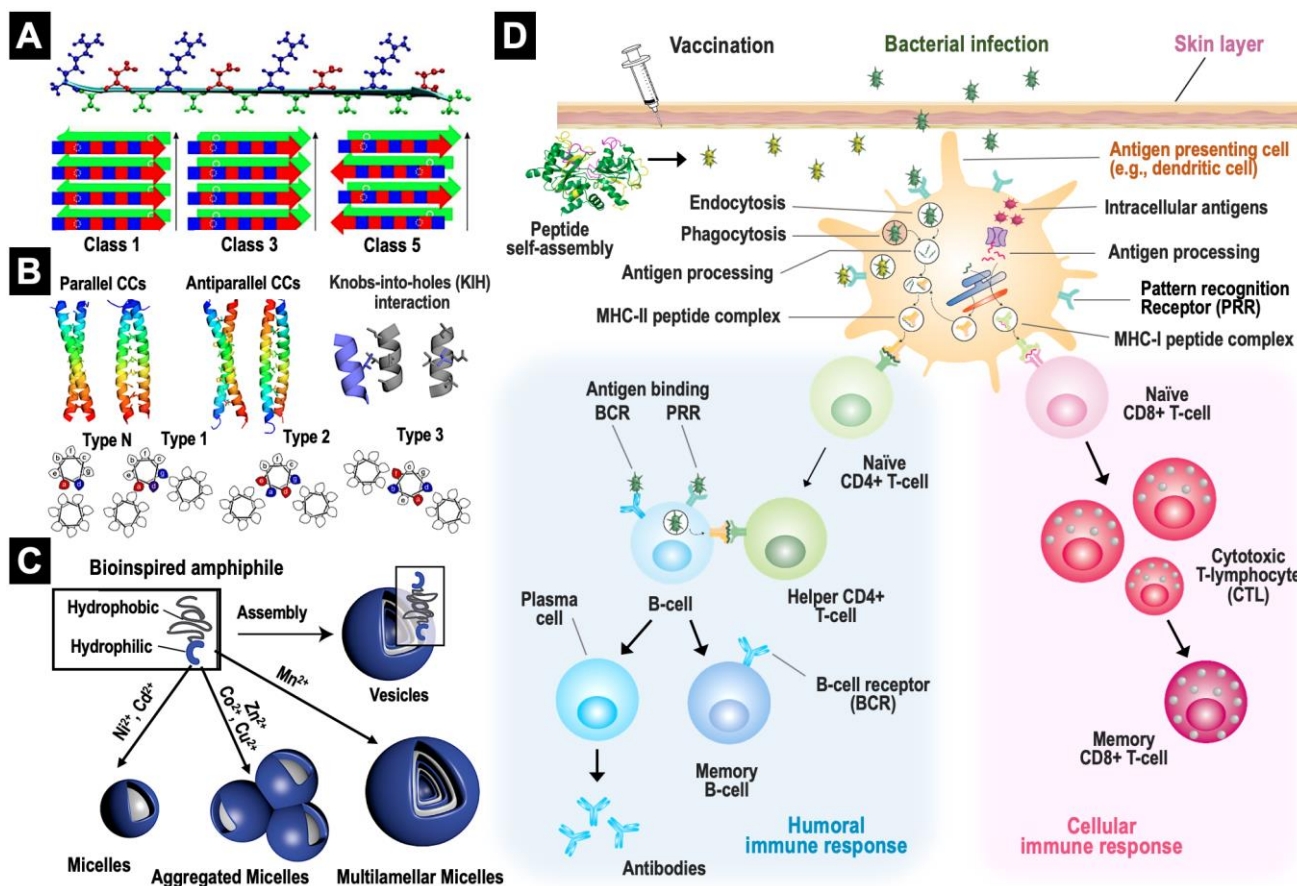384
385
386
387
388

389 **5   Figures**

390
391
392 **Figure 1. Strategies to induce supramolecular self-assembly of peptides and peptide-based**
393 **vaccines.** (**A**) Formation of β-strands from self-assembling peptide RADA16-I (top) and their
394 arrangement into nanofibers with different symmetry classes arranged into stacks of two β-sheets
395 (bottom). β-sheets are formed by alternating segments of hydrophobic alanine (green) and hydrophilic
396 arginine/aspartate (blue/red) regions (reproduced with permission from Cormier et al., 2013). (**B**)
397 Symmetry (top) and sequence (bottom) of coiled-coils (CCs) made of peptides containing a heptad
398 repeat of hydrophobic (h) and polar (p) residues in a hpphppp pattern. Helical wheels for classical
399 Type-N, and Type-1, Type-2, and Type-3 interfaces are depicted. All are viewed along the α-helices
400 from the N to C termini, labels are for the canonical a–g nomenclature and the teardrop shapes indicate
401 the direction of Cα–Cβ bonds (reproduced with permission from Rhys et al., 2018). (**C**) Effect of
402 different divalent transition metal ions on the morphological transformation of self-assembled PAs,
403 consisting of hexahistidine as a hydrophilic head and oligostyrene as a hydrophobic tail (reproduced
404 from Knight et al., 2017). (**D**) Schematic illustration of immune stimulation by self-assembled peptide-
405 based vaccines. Peptides undergo self-assembly upon intra-muscular or subcutaneous vaccination,
406 after which they can induce humoral and/or cellular immune responses to protect individuals against
407 pathogens. Humoral immune response arises once B cells encounter the antigen and are subsequently
408 activated in the secondary lymphoid organs. Mechanistically, B-cell activation occurs upon binding of
409 antigens to the B-cell receptor (BCR), resulting in the internalization of antigens via receptor-mediated
410 endocytosis. These antigens are then processed, and antigenic peptide-loaded MHC II is displayed on
411 the cell surface, allowing CD4+ helper T-cell binding. Once activated, B cells undergo proliferation,
412 immunoglobulin (Ig) class-switching, and differentiation into antigen-specific antibody-secreting
413 plasma cells and memory B cells. To induce CTL-mediated cellular immune responses, antigens need

414 to be processed and presented by antigen-presenting cells (APCs), such as dendritic cells and
415 macrophages. APCs take up the antigen by pinocytosis, phagocytosis, and receptor-mediated
416 phagocytosis, cleave the antigen into smaller peptide fragments, and present these peptides on MHC.
417 In the lymph nodes, extracellular antigenic peptides are presented on MHC II to CD4+ T cells, whereas
418 intracellular proteins, including viral and tumor-associated antigens, are presented on MHC I to CD8+
419 T cells. A subset of APCs can also present extracellular antigenic peptides on MHC I in a process
420 known as cross-presentation. Once activated, naïve CD8+ T cells proliferate into CTLs to mediate
421 antigen-specific cytotoxicity. A fraction of CD8+ T cells become memory CD8+ T cells to ensure long-
422 term protection. Upon activation, naïve CD4+ T cells differentiate into helper T cells which secrete
423 cytokines to support CTL expansion and survival along with B-cell proliferation and differentiation.
424 Memory B and T cells persist in the body for a long time and mount an immediate and efficient antigen-
425 specific immune response upon reinfection with the same pathogen.
426

427 **6 Tables**

428
429 **Table 1:** Summary of supramolecular peptide-based vaccines for cancer and infectious diseases

Peptide	Active ingredient	Features	Applications	References
RADA16	Anti-PD-1 + DCs + OVA peptide	β-sheet-rich nanofibrous hydrogel	Delivery system for DC-based vaccine in EG7-OVA tumor model	(Yang et al., 2018)
$K_2(SL)_6K_2$	STING agonist	Nanofibrous hydrogel with sustainable release properties	Delivery system in MOC_2-E_6E_7 tumor model	(Leach et al., 2018)
Fmoc-KCRGDK	BRD4 inhibitor + indocyanine green + autologous tumor cells	Micellar hydrogel with tumor penetrating properties	Delivery system for postoperative cancer immunotherapy in 4T1 tumor model	(Wang et al., 2018b)
Ac-I3SLKG-NH2	G(IIKK)3I-NH2	MMP-2 mediated enzyme responsive fibrillar hydrogels, sustained and targeted release properties	Delivery system for MMP-2 overexpressing HeLa tumor model	(Chen et al., 2017)
$OVA_{253-266}$ peptide	$OVA_{253-266}$ peptide conjugated with dialkyl lipid tail and 2 palmitic chains	Cylindrical micelles displaying epitopes at multiple valences with self-adjuvanting properties	Delivery system plus peptide tumor antigen in EG7-OVA tumor model	(Black et al., 2012)
$OVA_{254-267}$-HBc (Hepatitis B core protein)	$OVA_{254-267}$ peptide	Nanocage with controlled properties and high-density epitope display	Delivery system plus tumor antigen plus adjuvant for B16-OVA-Luc tumor model	(Shan et al., 2019)
Peptide-MHC/ANXA5	Peptide-MHC (pMHC)	Liposome	Antigen for B16-OVA tumor model	(Mao et al., 2018)
Ada-GFFYGKKK-NH2	OVA peptide	Nanofibrous hydrogel with NF-κB activating properties	Nano-adjuvant for B16-OVA cancer immunotherapy	(Xu et al., 2019)

Nap-GFFpY-OMe	OVA peptide	Nanofibrous hydrogel formed by phosphatase enzyme	Vaccine adjuvant for EG7-OVA tumor model	(Wang et al., 2016)
Q11 (QQKFQFQFEQQ)	Mucin 1 (MUC1) glycopeptide	Nanofibers, β-turn structure with self-adjuvanting properties	Delivery system plus adjuvant for MCF-7 tumor model	(Huang et al., 2012)
Ac-AAVVLLLW-COOH	OVA$_{250-264}$ + HPV16 E7$_{43-57}$	Nanostructure	TC-1 tumor model	(Rad-Malekshahi et al., 2017)
Cholesterol-aK-Cha-VAaWTLKAa-LEEKKGNYVVTDH	EGFRvIII + PADRE epitopes	Lipopeptide micelles with self-adjuvanting properties	Cellular and humoral immune response in B16-EGFRvIII tumor model	(Chen et al., 2018)
Coil29 (QARILEADAEILR AYARILEAHAEILR AD)	EGFRvIII, PADRE, SIINFEKL, PEPvIII	α-helical coiled-coiled peptide fiber	Induction of CD4+ T-cell, and CD8+ T-cell and B-cell response in mice	(Wu et al., 2017)
DEAP-DPPA-1	PD-L1 antagonist (^{D}PPA-1) + peptide substrate of MMP-2 + indoleamine-dioxygenase inhibitor (NLG919)	Nanoparticle responding to dual stimuli for targeted delivery and controlled release	B16-F10 tumor model	(Cheng et al., 2018)
GE11 (EGFR ligand)	Acetylcholinesterase gene + Doxorubicin	Self-assembling peptide nanovesicle	Drug and gene delivery system targeted towards EGFR expressing cancer	(Liang et al., 2016)
S4-8Q (QAEPDRAHYNIVT FCCKCD conjugated to a 4-arm star polymer)	8Q (HPV-16 E7 epitope)	Dendrimers with self-adjuvanting properties	TC-1 tumor model	(Liu et al., 2013); (Liu et al., 2015)
Nap-GFFY-NMe	DNA encoding gp145	Nanofibrous hydrogel	Strong cellular and humoral immune response for HIV treatment and prevention	(Tian et al., 2014)
EAK16-II	SL9 (HIV specific CTL epitope) + TL13 (CD4+ T-cell epitope) + R848 (TLR7/8 agonists) + DCs	β-sheet-rich nanofibers	Delivery system for HIV-1 vaccine	(Ding et al., 2016)
p41	p41 peptide (analogue derived from HCV protein NS5A)	α-helical nanocomplexes	HIV and HCV co-inhibition	(Zhang et al., 2013)
P6HRC1	HRC1 (B-cell epitope from S-protein)	Coiled-coil polypeptide nanoparticles	Coronavirus mediated SARS (severe acute respiratory syndrome) infection	(Pimentel et al., 2009)
KFE8	EIII (West Nile Virus envelope protein domain)	β-sheet-rich nanofibrous hydrogel with self-adjuvanting properties	West Nile virus vaccine	(Friedrich et al., 2016)
Pentamer and trimer	PspA and CbpA (CTL	5-stranded + 3-stranded	*Streptococcus*	(Dorosti et

sequence	epitopes) + PhtD and PiuA (helper T-cell epitopes) + DTD (universal T-helper)	coiled-coils nanoparticle scaffold with self-adjuvanting properties	*pneumoniae* vaccine	al., 2019)
J8 (B-cell epitope: (SREAKKQVEKAL)	J8 conjugated with dialkyl hydrophobic moiety (diC16)	Cylindrical micelles with self-adjuvanting properties	Group A *Streptococcus pyogenes* vaccine	(Trent et al., 2015)
MAX1	Inherently antibacterial	β-hairpin-rich hydrogel	Broad spectrum bacterial resistance to gram-positive and gram-negative bacteria	(Salick et al., 2007)
Fmoc-F_2	Silver nanoparticles	Macroscopic hydrogels	Anti-bacterial wound dressing	(Paladini et al., 2013)
Phage-VS-LK	Sap2 (VS) + Hsp90 (LK) peptides	Nanofiber	*Candida albicans* detection via ELISA and vaccine	(Wang et al., 2018b)
Cholesterol-G3R6TAT	Inherently antimicrobial	Nanoparticles	*Cryptococcus neoformans*-induced mengitis	(Wang et al., 2010)
Q11	NANP$_3$ (circumsporozoite protein epitope)	β-sheet-rich nanofibers	*P. falciparum* vaccine for malaria	(Rudra et al., 2012)

430

7 Conflict of Interest

Author Khushbu Bhatt is currently a student at Northeastern University and employed by Moderna Inc. However, Moderna Inc. was not involved in the writing of this review article or the decision to submit it for publication. The remaining authors declare that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

436

8 Author Contributions

The manuscript was written and edited with contribution from all authors. All authors have given approval to the final version of the manuscript.

440

9 Funding

446

10 Acknowledgments

450

11   References

Acar, H., Srivastava, S., Chung, E.J., Schnorenberg, M.R., Barrett, J.C., LaBelle, J.L., et al. (2017). Self-assembling peptide-based building blocks in medical applications. *Adv. Drug Del. Rev.* 110**,** 65-79. doi: 10.1016/j.addr.2016.08.006.

Azmi, F., Ahmad Fuaad, A.A.H., Skwarczynski, M., and Toth, I. (2014). Recent progress in adjuvant discovery for peptide-based subunit vaccines. *Hum. Vaccin. Immunother.* 10(3)**,** 778-796. doi: 10.4161/hv.27332.

Babapoor, S., Neef, T., Mittelholzer, C., Girshick, T., Garmendia, A., Shang, H., et al. (2011). A novel vaccine using nanoparticle platform to present immunogenic M2e against avian influenza infection. *Influenza Res. Treat.* 2011**,** 1-12. doi: 10.1155/2011/126794.

Bencherif, S.A., Sands, R.W., Ali, O.A., Li, W.A., Lewin, S.A., Braschler, T.M., et al. (2015). Injectable cryogel-based whole-cell cancer vaccines. *Nat. Commun.* 6(1)**,** 1-13. doi: 10.1038/ncomms8556.

Bera, S., Mondal, S., Xue, B., Shimon, L.J., Cao, Y., and Gazit, E. (2019). Rigid helical-like assemblies from a self-aggregating tripeptide. *Nat. Mater.* 18(5)**,** 503-509. doi: 10.1038/s41563-019-0343-2.

Black, M., Trent, A., Kostenko, Y., Lee, J.S., Olive, C., and Tirrell, M. (2012). Self-assembled peptide amphiphile micelles containing a cytotoxic T-Cell epitope promote a protective immune response in vivo. *Adv. Mater.* 24(28)**,** 3845-3849. doi: 10.1002/adma.201200209.

Black, M., Trent, A., Tirrell, M., and Olive, C. (2010). Advances in the design and delivery of peptide subunit vaccines with a focus on toll-like receptor agonists. *Expert Rev. Vaccines* 9(2)**,** 157-173. doi: 10.1586/erv.09.160.

Chen, C., Zhang, Y., Hou, Z., Cui, X., Zhao, Y., and Xu, H. (2017). Rational Design of Short Peptide-Based Hydrogels with MMP-2 Responsiveness for Controlled Anticancer Peptide Delivery. *Biomacromolecules* 18(11)**,** 3563-3571. doi: 10.1021/acs.biomac.7b00911.

Chen, Y., Yuan, F., Jiang, X., Lv, Q., Luo, N., Gong, C., et al. (2018). Discovery of a self-assembling and self-adjuvant lipopeptide as a saccharide-free peptide vaccine targeting EGFRvIII positive cutaneous melanoma. *Biomater. Sci.* 6(5)**,** 1120-1128. doi: 10.1039/c8bm00017d.

Cheng, K., Ding, Y., Zhao, Y., Ye, S., Zhao, X., Zhang, Y., et al. (2018). Sequentially Responsive Therapeutic Peptide Assembling Nanoparticles for Dual-Targeted Cancer Immunotherapy. *Nano Lett.* 18(5)**,** 3250-3258. doi: 10.1021/acs.nanolett.8b01071.

Chiang, C.L.-L., Benencia, F., and Coukos, G. (2010). Whole tumor antigen vaccines. *Semin. Immunol.* 22(3)**,** 132-143. doi: https://doi.org/10.1016/j.smim.2010.02.004.

Chin, S.M., Synatschke, C.V., Liu, S., Nap, R.J., Sather, N.A., Wang, Q., et al. (2018). Covalent-supramolecular hybrid polymers as muscle-inspired anisotropic actuators. *Nat. Commun.* 9(1)**,** 1-11. doi: 10.1038/s41467-018-04800-w.

486 Choi, S.j., Kwon, S.h., and Lim, Y.b. (2019). 3D2 Self-Assembling Janus Peptide Dendrimers with
487 Tailorable Supermultivalency. *Adv. Funct. Mater.* 29(9)**,** 1808020. doi: 10.1002/adfm.201808020.

488 Cockburn, I.A., and Seder, R.A. (2018). Malaria prevention: from immunological concepts to effective
489 vaccines and protective antibodies. *Nat. Immunol.* 19(11)**,** 1199-1211. doi: 10.1038/s41590-018-0228-
490 6.

491 Cormier, A.R., Pang, X., Zimmerman, M.I., Zhou, H.-X., and Paravastu, A.K. (2013). Molecular
492 structure of RADA16-I designer self-assembling peptide nanofibers. *ACS nano* 7(9)**,** 7562-7572. doi:
493 10.1021/nn401562f.

494 Ding, Y., Liu, J., Lu, S., Igweze, J., Xu, W., Kuang, D., et al. (2016). Self-assembling peptide for co-
495 delivery of HIV-1 CD8+ T cells epitope and Toll-like receptor 7/8 agonists R848 to induce maturation
496 of monocyte derived dendritic cell and augment polyfunctional cytotoxic T lymphocyte (CTL)
497 response. *J. Control. Release* 236**,** 22-30. doi: 10.1016/j.jconrel.2016.06.019.

498 Dorosti, H., Eslami, M., Nezafat, N., Fadaei, F., and Ghasemi, Y. (2019). Designing self-assembled
499 peptide nanovaccine against Streptococcus pneumoniae: An in silico strategy. *Mol. Cell. Probes* 48**,**
500 101446. doi: 10.1016/j.mcp.2019.101446.

501 Dreher, M.R., Simnick, A.J., Fischer, K., Smith, R.J., Patel, A., Schmidt, M., et al. (2008). Temperature
502 triggered self-assembly of polypeptides into multivalent spherical micelles. *J. Am. Chem. Soc.* 130(2)**,**
503 687-694. doi: 10.1021/ja0764862.

504 Eskandari, S., Guerin, T., Toth, I., and Stephenson, R.J. (2017). Recent advances in self-assembled
505 peptides: Implications for targeted drug delivery and vaccine engineering. *Adv. Drug Del. Rev.* 110**,**
506 169-187. doi: 10.1016/j.addr.2016.06.013.

507 Friedrich, B.M., Beasley, D.W.C., and Rudra, J.S. (2016). Supramolecular peptide hydrogel
508 adjuvanted subunit vaccine elicits protective antibody responses against West Nile virus. *Vaccine*
509 34(46)**,** 5479-5482. doi: 10.1016/j.vaccine.2016.09.044.

510 Graham, B.S. (2020). Rapid COVID-19 vaccine development. *Science* 368(6494)**,** 945-946. doi:
511 10.1126/science.abb8923.

512 Grenfell, R.F., Shollenberger, L.M., Samli, E.F., and Harn, D.A. (2015). Vaccine self-assembling
513 immune matrix is a new delivery platform that enhances immune responses to recombinant HBsAg in
514 mice. *Clin. Vaccine Immunol.* 22(3)**,** 336-343. doi: 10.1128/CVI.00714-14.

515 Habibi, N., Kamaly, N., Memic, A., and Shafiee, H. (2016). Self-assembled peptide-based
516 nanostructures: Smart nanomaterials toward targeted drug delivery. *Nano Today* 11(1)**,** 41-60. doi:
517 10.1016/j.nantod.2016.02.004.

518 Hassouneh, W., Zhulina, E.B., Chilkoti, A., and Rubinstein, M. (2015). Elastin-like polypeptide
519 diblock copolymers self-assemble into weak micelles. *Macromolecules* 48(12)**,** 4183-4195. doi:
520 10.1021/acs.macromol.5b00431.

521 Huang, Z.H., Shi, L., Ma, J.W., Sun, Z.Y., Cai, H., Chen, Y.X., et al. (2012). A totally synthetic, self-
522 assembling, adjuvant-free MUC1 glycopeptide vaccine for cancer therapy. *J. Am. Chem. Soc.* 134(21)**,**
523 8730-8733. doi: 10.1021/ja211725s.

524 Kaba, S.A., Brando, C., Guo, Q., Mittelholzer, C., Raman, S., Tropel, D., et al. (2009). A
525 nonadjuvanted polypeptide nanoparticle vaccine confers long-lasting protection against rodent malaria.
526 *J. Immunol.* 183(11)**,** 7268-7277. doi: 10.4049/jimmunol.0901957.

527 Khalily, M.A., Goktas, M., and Guler, M.O. (2015). Tuning viscoelastic properties of supramolecular
528 peptide gels via dynamic covalent crosslinking. *Org. Biomol. Chem.* 13(7)**,** 1983-1987. doi:
529 10.1039/C4OB02217C.

530 Kim, J., Narayana, A., Patel, S., and Sahay, G. (2019). Advances in intracellular delivery through
531 supramolecular self-assembly of oligonucleotides and peptides. *Theranostics* 9(11)**,** 3191. doi:
532 10.7150/thno.33921.

533 Knight, A.S., Larsson, J., Ren, J.M., Bou Zerdan, R., Seguin, S., Vrahas, R., et al. (2018). Control of
534 amphiphile self-assembly via bioinspired metal ion coordination. *J. Am. Chem. Soc.* 140(4)**,** 1409-
535 1414. doi: 10.1021/jacs.7b11005.

536 Lampel, A. (2020). Biology-Inspired Supramolecular Peptide Systems. *Chem* 6(6)**,** 1222-1236. doi:
537 10.1016/j.chempr.2020.03.005.

538 Leach, D.G., Dharmaraj, N., Piotrowski, S.L., Lopez-Silva, T.L., Lei, Y.L., Sikora, A.G., et al. (2018).
539 STINGel: Controlled release of a cyclic dinucleotide for enhanced cancer immunotherapy.
540 *Biomaterials* 163**,** 67-75. doi: 10.1016/j.biomaterials.2018.01.035.

541 Lee, S., Trinh, T.H., Yoo, M., Shin, J., Lee, H., Kim, J., et al. (2019). Self-assembling peptides and
542 their application in the treatment of diseases. *Int. J. Mol. Sci.* 20(23)**,** 5850. doi: 10.3390/ijms20235850.

543 Li, J., Bullara, D., Du, X., He, H., Sofou, S., Kevrekidis, I.G., et al. (2018). Kinetic analysis of
544 nanostructures formed by enzyme-instructed intracellular assemblies against cancer cells. *ACS nano*
545 12(4)**,** 3804-3815. doi: 10.1021/acsnano.8b01016.

546 Li, J., Xing, R., Bai, S., and Yan, X. (2019). Recent advances of self-assembling peptide-based
547 hydrogels for biomedical applications. *Soft Matter* 15(8)**,** 1704-1715. doi: 10.1039/C8SM02573H.

548 Liang, X., Shi, B., Wang, K., Fan, M., Jiao, D., Ao, J., et al. (2016). Development of self-assembling
549 peptide nanovesicle with bilayers for enhanced EGFR-targeted drug and gene delivery. *Biomaterials*
550 82**,** 194-207. doi: 10.1016/j.biomaterials.2015.12.015.

551 Liu, T.Y., Hussein, W.M., Giddam, A.K., Jia, Z., Reiman, J.M., Zaman, M., et al. (2015). Polyacrylate-
552 based delivery system for self-adjuvanting anticancer peptide vaccine. *J. Med. Chem.* 58(2)**,** 888-896.
553 doi: 10.1021/jm501514h.

554 Liu, T.Y., Hussein, W.M., Jia, Z., Ziora, Z.M., McMillan, N.A., Monteiro, M.J., et al. (2013). Self-
555 adjuvanting polymer-peptide conjugates as therapeutic vaccine candidates against cervical cancer.
556 *Biomacromolecules* 14(8)**,** 2798-2806. doi: 10.1021/bm400626w.

557 Lu, L., Armstrong, E.A., Yager, J.Y., and Unsworth, L.D. (2019). Sustained Release of Dexamethasone
558 from Sulfobutyl Ether β-cyclodextrin Modified Self-Assembling Peptide Nanoscaffolds in a Perinatal
559 Rat Model of Hypoxia–Ischemia. *Adv. Healthc. Mater.* 8(11)**,** 1900083. doi:
560 10.1002/adhm.201900083.

561 Lurie, N., Saville, M., Hatchett, R., and Halton, J. (2020). Developing Covid-19 vaccines at pandemic
562 speed. *N. Engl. J. Med.* 382(21)**,** 1969-1973. doi: 10.1056/NEJMp2005630.

563 Lynn, G.M., Sedlik, C., Baharom, F., Zhu, Y., Ramirez-Valdez, R.A., Coble, V.L., et al. (2020).
564 Peptide–TLR-7/8a conjugate vaccines chemically programmed for nanoparticle self-assembly enhance
565 CD8 T-cell immunity to tumor antigens. *Nat. Biotechnol.* 38(3)**,** 320-332. doi: 10.1038/s41587-019-
566 0390-x.

567 Malonis, R.J., Lai, J.R., and Vergnolle, O. (2019). Peptide-based vaccines: current progress and future
568 challenges. *Chem. Rev.* 120(6)**,** 3210-3229. doi: 10.1021/acs.chemrev.9b00472.

569 Mao, C.P., Peng, S., Yang, A., He, L., Tsai, Y.C., Hung, C.F., et al. (2018). Programmed self-assembly
570 of peptide-major histocompatibility complex for antigen-specific immune modulation. *Proc. Natl.*
571 *Acad. Sci. U. S. A.* 115(17)**,** E4032-E4040. doi: 10.1073/pnas.1718434115.

572 Molnar, C., and Gair, J. (2015). 23.2. Adaptive Immune Response. *Concepts of Biology-1st Canadian*
573 *Edition*.

574 Moore, A.N., Silva, T.L.L., Carrejo, N.C., Marmolejo, C.A.O., Li, I.-C., and Hartgerink, J.D. (2018).
575 Nanofibrous peptide hydrogel elicits angiogenesis and neurogenesis without drugs, proteins, or cells.
576 *Biomaterials* 161**,** 154-163. doi: 10.1016/j.biomaterials.2018.01.033.

577 Negahdaripour, M., Golkar, N., Hajighahramani, N., Kianpour, S., Nezafat, N., and Ghasemi, Y.
578 (2017). Harnessing self-assembled peptide nanoparticles in epitope vaccine design. *Biotechnol. Adv.*
579 35(5)**,** 575-596. doi: 10.1016/j.biotechadv.2017.05.002.

580 Paladini, F., Meikle, S.T., Cooper, I.R., Lacey, J., Perugini, V., and Santin, M. (2013). Silver-doped
581 self-assembling di-phenylalanine hydrogels as wound dressing biomaterials. *J. Mater. Sci. Mater. Med.*
582 24(10)**,** 2461-2472. doi: 10.1007/s10856-013-4986-2.

583 Parvizpour, S., Pourseif, M.M., Razmara, J., Rafi, M.A., and Omidi, Y. (2020). Epitope-based vaccine
584 design: a comprehensive overview of bioinformatics approaches. *Drug Discov. Today* 25(6)**,** 1034-
585 1042. doi: 10.1016/j.drudis.2020.03.006.

586 Pimentel, T.A., Yan, Z., Jeffers, S.A., Holmes, K.V., Hodges, R.S., and Burkhard, P. (2009). Peptide
587 nanoparticles as novel immunogens: design and analysis of a prototypic severe acute respiratory
588 syndrome vaccine. *Chem. Biol. Drug Des.* 73(1)**,** 53-61. doi: 10.1111/j.1747-0285.2008.00746.x.

589 Piot, P., Larson, H.J., O'Brien, K.L., N'kengasong, J., Ng, E., Sow, S., et al. (2019). Immunization:
590 vital progress, unfinished agenda. *Nature* 575(7781)**,** 119-129. doi: 10.1038/s41586-019-1656-7.

591 Purcell, A.W., McCluskey, J., and Rossjohn, J. (2007). More than one reason to rethink the use of
592 peptides in vaccine design. *Nat. Rev. Drug Discov.* 6(5)**,** 404-414. doi: 10.1038/nrd2224.

593   Qi, G.B., Gao, Y.J., Wang, L., and Wang, H. (2018). Self-Assembled Peptide-Based Nanomaterials
594   for Biomedical Imaging and Therapy. *Adv. Mater.* 30(22)**,** 1703444. doi: 10.1002/adma.201703444.

595   Rad-Malekshahi, M., Fransen, M.F., Krawczyk, M., Mansourian, M., Bourajjaj, M., Chen, J., et al.
596   (2017). Self-Assembling Peptide Epitopes as Novel Platform for Anticancer Vaccination. *Mol. Pharm.*
597   14(5)**,** 1482-1493. doi: 10.1021/acs.molpharmaceut.6b01003.

598   Rad-Malekshahi, M., Visscher, K.M., Rodrigues, J.o.P., De Vries, R., Hennink, W.E., Baldus, M., et
599   al. (2015). The supramolecular organization of a peptide-based nanocarrier at high molecular detail. *J.*
600   *Am. Chem. Soc.* 137(24)**,** 7775-7784. doi: 10.1021/jacs.5b02919.

601   Raman, S., Machaidze, G., Lustig, A., Aebi, U., and Burkhard, P. (2006). Structure-based design of
602   peptides that self-assemble into regular polyhedral nanoparticles. *Nanomed. Nanotechnol. Biol. Med.*
603   2(2)**,** 95-102. doi: 10.1016/j.nano.2006.04.007.

604   Rhys, G.G., Wood, C.W., Lang, E.J., Mulholland, A.J., Brady, R.L., Thomson, A.R., et al. (2018).
605   Maintaining and breaking symmetry in homomeric coiled-coil assemblies. *Nat. Commun.* 9(1)**,** 1-12.
606   doi: 10.1038/s41467-018-06391-y.

607   Rudra, J.S., Mishra, S., Chong, A.S., Mitchell, R.A., Nardin, E.H., Nussenzweig, V., et al. (2012). Self-
608   assembled peptide nanofibers raising durable antibody responses against a malaria epitope.
609   *Biomaterials* 33(27)**,** 6476-6484. doi: 10.1016/j.biomaterials.2012.05.041.

610   Saha, S., Banskota, S., Roberts, S., Kirmani, N., and Chilkoti, A. (2020). Engineering the Architecture
611   of Elastin-Like Polypeptides: From Unimers to Hierarchical Self-Assembly. *Adv. Ther* 3(3)**,** 1900164.
612   doi: 10.1002/adtp.201900164.

613   Salick, D.A., Kretsinger, J.K., Pochan, D.J., and Schneider, J.P. (2007). Inherent antibacterial activity
614   of a peptide-based beta-hairpin hydrogel. *J. Am. Chem. Soc.* 129(47)**,** 14793-14799. doi:
615   10.1021/ja076300z.

616   Shan, W., Zheng, H., Fu, G., Liu, C., Li, Z., Ye, Y., et al. (2019). Bioengineered Nanocage from HBc
617   Protein for Combination Cancer Immunotherapy. *Nano Lett.* 19(3)**,** 1719-1727. doi:
618   10.1021/acs.nanolett.8b04722.

619   Sis, M.J., and Webber, M.J. (2019). Drug Delivery with Designed Peptide Assemblies. *Trends*
620   *Pharmacol. Sci.* 40(10)**,** 747-762. doi: 10.1016/j.tips.2019.08.003.

621   Skwarczynski, M., and Toth, I. (2016). Peptide-based synthetic vaccines. *Chem. Sci.* 7(2)**,** 842-854.
622   doi: 10.1039/C5SC03892H

623   Skwarczynski, M., Zhao, G., Boer, J.C., Ozberk, V., Azuar, A., Cruz, J.G., et al. (2020). Poly (amino
624   acids) as a potent self-adjuvanting delivery system for peptide-based nanovaccines. *Sci. Adv.* 6(5)**,**
625   eaax2285. doi: 10.1126/sciadv.aax2285.

626   Smith, D.J., Brat, G.A., Medina, S.H., Tong, D., Huang, Y., Grahammer, J., et al. (2016). A multiphase
627   transitioning peptide hydrogel for suturing ultrasmall vessels. *Nat. Nanotechnol.* 11(1)**,** 95-102. doi:
628   10.1038/nnano.2015.238.

629 Tandon, A., Pathak, M., Harioudh, M.K., Ahmad, S., Sayeed, M., Afshan, T., et al. (2018). A TLR4-
630 derived non-cytotoxic, self-assembling peptide functions as a vaccine adjuvant in mice. *J. Biol. Chem.*
631 293(51)**,** 19874-19885. doi: 10.1074/jbc.RA118.002768.

632 Thomson, A.R., Wood, C.W., Burton, A.J., Bartlett, G.J., Sessions, R.B., Brady, R.L., et al. (2014).
633 Computational design of water-soluble α-helical barrels. *Science* 346(6208)**,** 485-488. doi:
634 10.1126/science.1257452.

635 Tian, Y., Wang, H., Liu, Y., Mao, L., Chen, W., Zhu, Z., et al. (2014). A peptide-based nanofibrous
636 hydrogel as a promising DNA nanovector for optimizing the efficacy of HIV vaccine. *Nano Lett.* 14(3)**,**
637 1439-1445. doi: 10.1021/nl404560v.

638 Trent, A., Ulery, B.D., Black, M.J., Barrett, J.C., Liang, S., Kostenko, Y., et al. (2015). Peptide
639 amphiphile micelles self-adjuvant group A streptococcal vaccination. *AAPS J.* 17(2)**,** 380-388. doi:
640 10.1208/s12248-014-9707-3.

641 Wang, H., Feng, Z., and Xu, B. (2019). Assemblies of Peptides in a Complex Environment and their
642 Applications. *Angew. Chem. Int. Ed.* 58(31)**,** 10423-10432. doi: 10.1002/anie.201814552.

643 Wang, H., Luo, Z., Wang, Y., He, T., Yang, C., Ren, C., et al. (2016). Enzyme-Catalyzed Formation
644 of Supramolecular Hydrogels as Promising Vaccine Adjuvants. *Adv. Funct. Mater.* 26(11)**,** 1822-1829.
645 doi: 10.1002/adfm.201505188.

646 Wang, H., Xu, K., Liu, L., Tan, J.P., Chen, Y., Li, Y., et al. (2010). The efficacy of self-assembled
647 cationic antimicrobial peptide nanoparticles against Cryptococcus neoformans for the treatment of
648 meningitis. *Biomaterials* 31(10)**,** 2874-2881. doi: 10.1016/j.biomaterials.2009.12.042.

649 Wang, K., Yang, Y., Xue, W., and Liu, Z. (2018a). Cell penetrating peptide-based redox-sensitive
650 vaccine delivery system for subcutaneous vaccination. *Mol. Pharm.* 15(3)**,** 975-984. doi:
651 10.1021/acs.molpharmaceut.7b00905.

652 Wang, T., Wang, D., Yu, H., Feng, B., Zhou, F., Zhang, H., et al. (2018b). A cancer vaccine-mediated
653 postoperative immunotherapy for recurrent and metastatic tumors. *Nat. Commun.* 9(1)**,** 1-12. doi:
654 10.1038/s41467-018-03915-4.

655 Wen, Y., and Collier, J.H. (2015). Supramolecular peptide vaccines: tuning adaptive immunity. *Curr.*
656 *Opin. Immunol.* 35**,** 73-79. doi: 10.1016/j.coi.2015.06.007.

657 Worthington, P., Langhans, S., and Pochan, D. (2017). β-Hairpin peptide hydrogels for package
658 delivery. *Adv. Drug Del. Rev.* 110**,** 127-136. doi: 10.1016/j.addr.2017.02.002.

659 Wu, Y., Kelly, S.H., Sanchez-Perez, L., Sampson, J., and Collier, J.H. (2020). Comparative study of
660 α-helical and β-sheet self-assembled peptide nanofiber vaccine platforms: Influence of integrated T-
661 cell epitopes. *Biomater. Sci.* 8 3522-3535. doi: 10.1039/D0BM00521E.

662 Wu, Y., Norberg, P.K., Reap, E.A., Congdon, K.L., Fries, C.N., Kelly, S.H., et al. (2017). A
663 Supramolecular Vaccine Platform Based on α-Helical Peptide Nanofibers. *ACS Biomater. Sci. Eng.*
664 3(12)**,** 3128-3132. doi: 10.1021/acsbiomaterials.7b00561.

665 Xiao, T., Qi, L., Zhong, W., Lin, C., Wang, R., and Wang, L. (2019a). Stimuli-responsive nanocarriers
666 constructed from pillar [n] arene-based supra-amphiphiles. *Mater. Chem. Front* 3(10), 1973-1993. doi:
667 10.1039/C9QM00428A.

668 Xiao, T., Zhong, W., Xu, L., Sun, X.-Q., Hu, X.-Y., and Wang, L. (2019b). Supramolecular vesicles
669 based on pillar [n] arenes: design, construction, and applications. *Org. Biomol. Chem.* 17(6), 1336-
670 1350. doi: 10.1039/C8OB03095B.

671 Xing, R., Li, S., Zhang, N., Shen, G., Möhwald, H., and Yan, X. (2017). Self-assembled injectable
672 peptide hydrogels capable of triggering antitumor immune response. *Biomacromolecules* 18(11), 3514-
673 3523. doi: 10.1021/acs.biomac.7b00787.

674 Xu, Y., Wang, Y., Yang, Q., Liu, Z., Xiao, Z., Le, Z., et al. (2019). A versatile supramolecular
675 nanoadjuvant that activates NF-κB for cancer immunotherapy. *Theranostics* 9(11), 3388. doi:
676 10.7150/thno.34031.

677 Yang, J., Luo, Y., Shibu, M.A., Toth, I., and Skwarczynskia, M. (2019). Cell-penetrating peptides:
678 Efficient vectors for vaccine delivery. *Curr. Drug Del.* 16(5), 430-443. doi:
679 10.2174/1567201816666190123120915.

680 Yang, P., Song, H., Qin, Y., Huang, P., Zhang, C., Kong, D., et al. (2018). Engineering dendritic-cell-
681 based vaccines and PD-1 blockade in self-assembled peptide nanofibrous hydrogel to amplify
682 antitumor T-cell immunity. *Nano Lett.* 18(7), 4377-4385. doi: 10.1021/acs.nanolett.8b01406.

683 Zhang, J., Mulvenon, A., Makarov, E., Wagoner, J., Knibbe, J., Kim, J.O., et al. (2013). Antiviral
684 peptide nanocomplexes as a potential therapeutic modality for HIV/HCV co-infection. *Biomaterials*
685 34(15), 3846-3857. doi: 10.1016/j.biomaterials.2013.01.026.

686 Zhang, S. (2017). Discovery and design of self-assembling peptides. *Interface Focus* 7(6), 20170028.
687 doi: 10.1098/rsfs.2017.0028.

688 Zhao, G., Chandrudu, S., Skwarczynski, M., and Toth, I. (2017). The application of self-assembled
689 nanostructures in peptide-based subunit vaccine development. *Eur. Polym. J.* 93, 670-681. doi:
690 10.1016/j.eurpolymj.2017.02.014.

691 Zottig, X., Côté-Cyr, M., Arpin, D., Archambault, D., and Bourgault, S. (2020). Protein
692 Supramolecular Structures: From Self-Assembly to Nanovaccine Design. *Nanomaterials* 10(5), 1008.
693 doi: 10.3390/nano10051008.

694