TOPICAL REVIEW

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Topical Review

Peptide-based vesicles and droplets: a review

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Abstract

Peptide assembly is an increasingly important field of study due to the versatility, tunability and vast design space of amino acid based biomolecular assemblies. Peptides can be precisely engineered to possess various useful properties such as the ability to form supramolecular assemblies, desired response to pH, or thermal stability. These peptide supramolecular assemblies have diverse morphologies including vesicles, nanotubes, nanorods and ribbons. Of specific interest is the domain of engineering peptides that aggregate into spherical nanostructures due to their encapsulation properties: the ability to hold, transport and release chemical payloads in a controllable manner. This is invaluable to the fields of nanomedicine and targeted drug delivery. In this review, the state of the art in the domain of peptide-based vesicles and nanospheres is summarized. Specifically, an overview of the assembly of peptides into nanovesicles and nanospheres is provided. Both aromatic as well as aliphatic side chain amino acids are discussed. The domain of aromatic side chained amino acid residues is largely dominated by phenylalanine based peptides and variants thereof. Tyrosine also demonstrates similar aggregation properties. Both experimentally and computationally driven approaches are discussed. The domain of aliphatic amino acid residues based vesicles and droplets is broader, and details multiple amino acid residues such as alanine, valine, lysine, glycine, proline, and aspartic acid. Finally, a discussion on potential future directions is provided.

Keywords: peptide, vesicle, droplet, nanosphere

(Some figures may appear in colour only in the online journal)

1. Introduction

Peptide assemblies are playing an increasingly important role in a wide range of applications including nanomedicine and drug delivery [1], organic electronics [2], neurodegenerative diseases [3], biosensing [4–7], and nanobiomaterials [8, 9]. Peptide assemblies adopt a diverse range of morphologies such as vesicles, droplets, nanotubes, or mono- and bi-layered lamellar sheets [10], with characteristics which enable precisely controlled morphological transitions via response to external stimuli including pH [11]. For many of these applications, vesicles, i.e. hollow (or solvent) cored or droplets, i.e. solid (peptide) cored spherical nanostructures are of great interest.

Vesicles are typically observed to be formed from lipids [12], whose chemical structure (namely, a hydrophilic head

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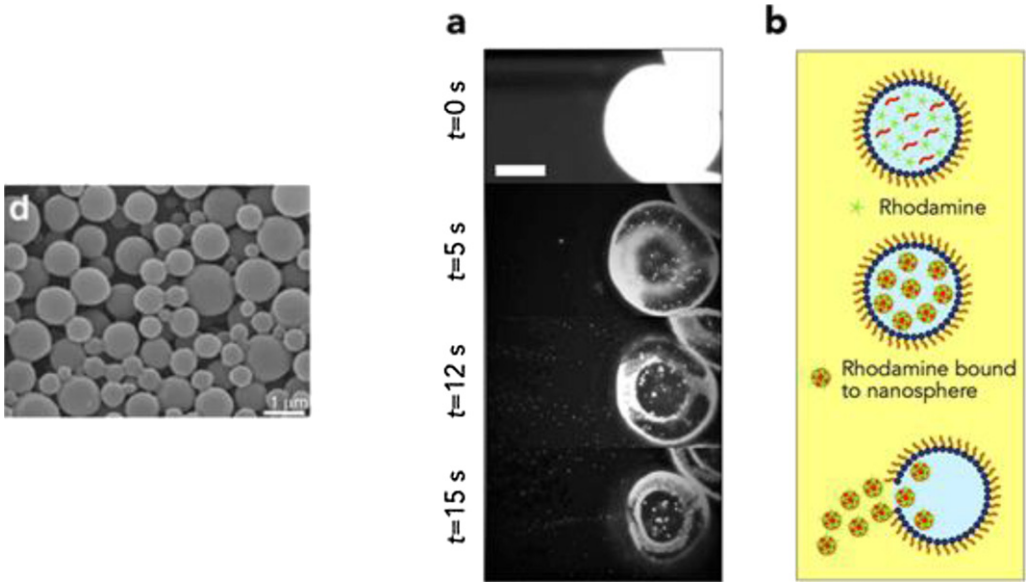

Figure 1. Boc-FF assembly (left), shows the formation of nanospheres [21]. Time lapse microscopy images (right) of Rhodamine nanoparticles being released from a Boc-FF nanovesicle [23].

group and one or two hydrophobic tails) endows them with an amphiphilic nature. This allows the lipids to organize themselves end-to-end in aggregates, forming bilayers that can fuse their free edges to yield vesicles [13]. Peptides have also been observed to assemble into hollow and solid core nanospherical structures. Due to the availability of high precision tuning of amino acid residue sequences in peptide synthesis, peptides can be engineered to have specific chemical traits, including amphiphilicity as well as molecular geometry of the molecule [14]. These chemical traits give the synthesized peptide sequences the ability to aggregate into ordered nanostructures with desired properties.

There are several excellent reviews that have handled many aspects of the aggregation of peptide sequences, the properties of the assemblies and potential applications. For example, Kundu *et al* [15], in their 2018 review, discuss spectroscopy and microscopy techniques to study vesicle structures, some of which are formed with phenylalanine. Another review [16] (Yan *et al* 2010) discusses the self-assembly and applications of diphenylalanine-based systems. Li *et al* [17] provide a review of the state of the art in applications using peptide-based nanomedicine. Du and Stenzel [1] provide a review on peptide-based drug carriers and discuss various methods of peptide drug delivery including drug carriers based on organic materials, peptide-polymer conjugates, encapsulation and surfactant based techniques. The present review focuses on vesicles and droplets that encompass solely peptide molecules, or if the formation of vesicles and droplets is driven by interactions between peptide sequences.

The review is organized as follows: the first section addresses current understanding of aromatic amino acid-based vesicles and droplets, and the second section addresses prior work on aliphatic amino acid-based vesicles and droplets. Due to the different emphases in the prior work related to the two sections, the subtopics in each section are handled somewhat differently. The section on aromatic amino acid residue-based vesicles and droplets consists of two subsections that deal with experimental and computational studies. Whereas the section on aliphatic amino acid-based aggregates is organized based on the various residues that are used in the studies discussed. Potential future directions are discussed at the end of the review. As an aside, several of the experimental studies use techniques such as drying, freeze-drying/vitrifying, and staining to clearly observe the formed nanostructures. This may have effects on the integrity of the structures formed and the results should therefore be interpreted with care.

2. Aromatic amino acid based droplets and vesicles

2.1. Experimental studies

Experiment driven approaches to study the assembly of peptide sequences are a critical part of the endeavor to understand these complex biomolecular systems. Gazit *et al* studied the aggregation of amyloid like peptides where multiple functional units of peptides were observed [18]. This study established the role of aromatic amino acids in aggregation, specifically the phenomenon of pi–pi stacking. Although the study pertained primarily to the investigation of amyloids and not droplets or vesicles, the study played a critical role toward the development in the understanding of peptide aggregation. The authors of the study found that diphenylalanine (FF) was the smallest unit that was required for aggregation to occur. The study involved a reductionist approach, i.e., iteratively shortening an aggregating peptide to find the smallest possible peptide fragment that led to amyloid formation [18]. This investigation was followed by other studies involving FF and derivatives thereof, including capped

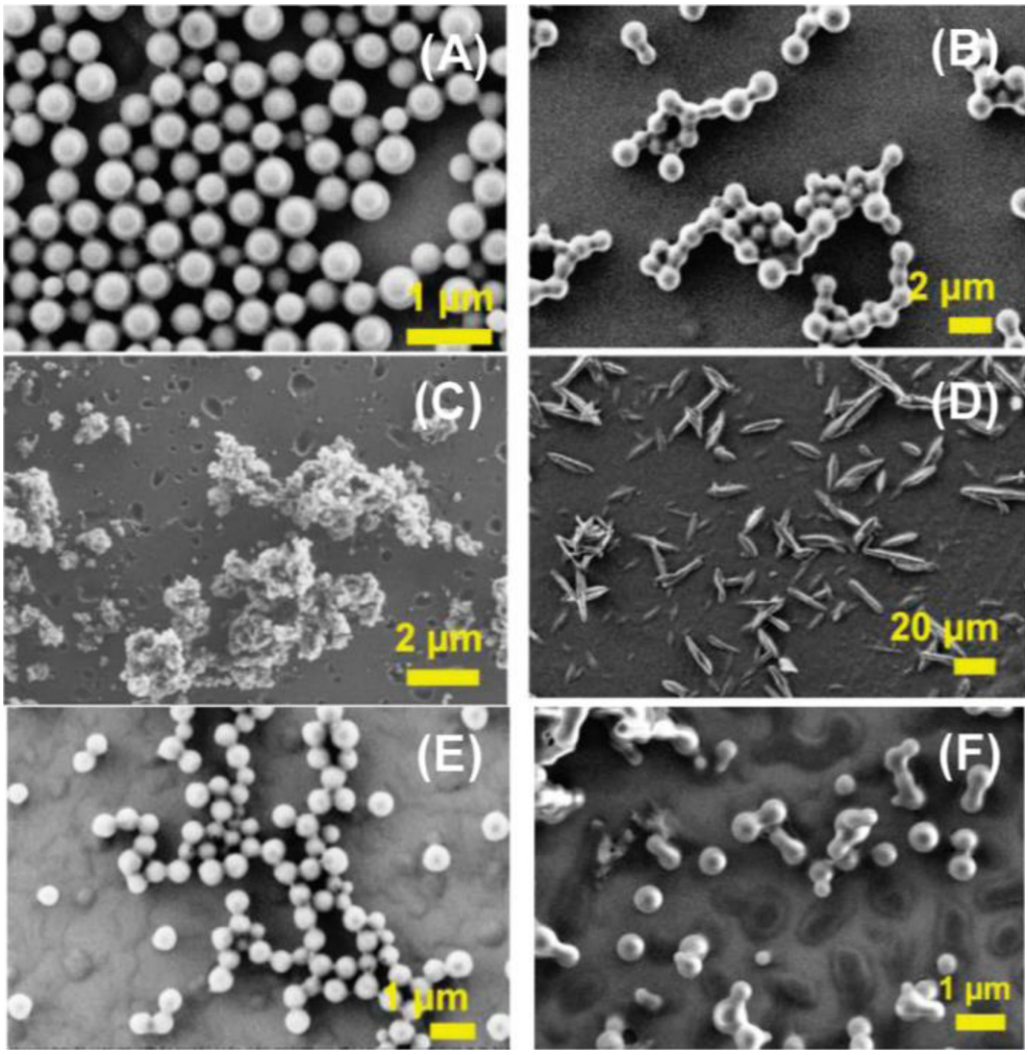

Figure 2. SEM images of nucleoside conjugated peptides forming vesicles and nanospherical structures [24].

FF peptide sequences. For example, FF capped with a fluorenylmethoxycarbonyl group (Fmoc-FF) [19, 20], FF capped with tert-butyloxycarbonyl (Boc-FF) [19, 21], and tripeptides containing phenylalanine [22]. Levin *et al* demonstrated in 2014 that Boc-FF assembles via a multi-step process which involves numerous metastable states. The study carries out a detailed thermodynamic characterization of these phase transitions [21].

In another study, Levin *et al* explored the phenomenon of self-assembly mediated nanoparticle release from nanovesicles constituted of FF-Boc [23]. The authors of the study reported that these vesicles can be modulated for controlled release across oil/water interfaces, seen in figure 1.

Diphenylalanine has been observed to assemble into nanotubes or vesicles; the morphology of the self-assembled nanostructure is dependent upon the concentration. At low concentration of FF, vesicles are observed, whereas at higher concentrations nanotubes are reported [48]. Datta *et al* [24] studied the effect of modifying the FF motif with nucleosides on the morphology of the assemblies and demonstrated the formation of monodisperse nanovesicles (seen in figure 2). The authors also explored the stability of the vesicle under various external stimuli.

Polymorphism in phenylalanine-based peptides has been reported by an earlier study [22]. The study reported that carboxybenzyl protected FF (z-FF) can form assemblies with a wide range of morphologies including nanospheres. In addition, tripeptides consisting of phenylalanine (i.e. FFF) were to assemble just as efficiently, forming nanospheres and plate like structures.

Peptide nanovesicles have been studied for their potential as drug delivery vehicles [25]. A range of branched peptides varying between 15 and 23 amino acid residues were explored for their ability to form stable vesicles. These peptides were amphiphilic in nature and mimicked the structure of phospholipids. The assemblies resulted in nanospheres with diameters ranging from 50 nm to 200 nm as detected by dynamic light scattering (DLS), transmission electron microscopy (TEM) and scanning transmission electron microscopy (STEM) techniques. The study also reported that the linear variant of this peptide did not demonstrate any propensity to assemble (figure 3).

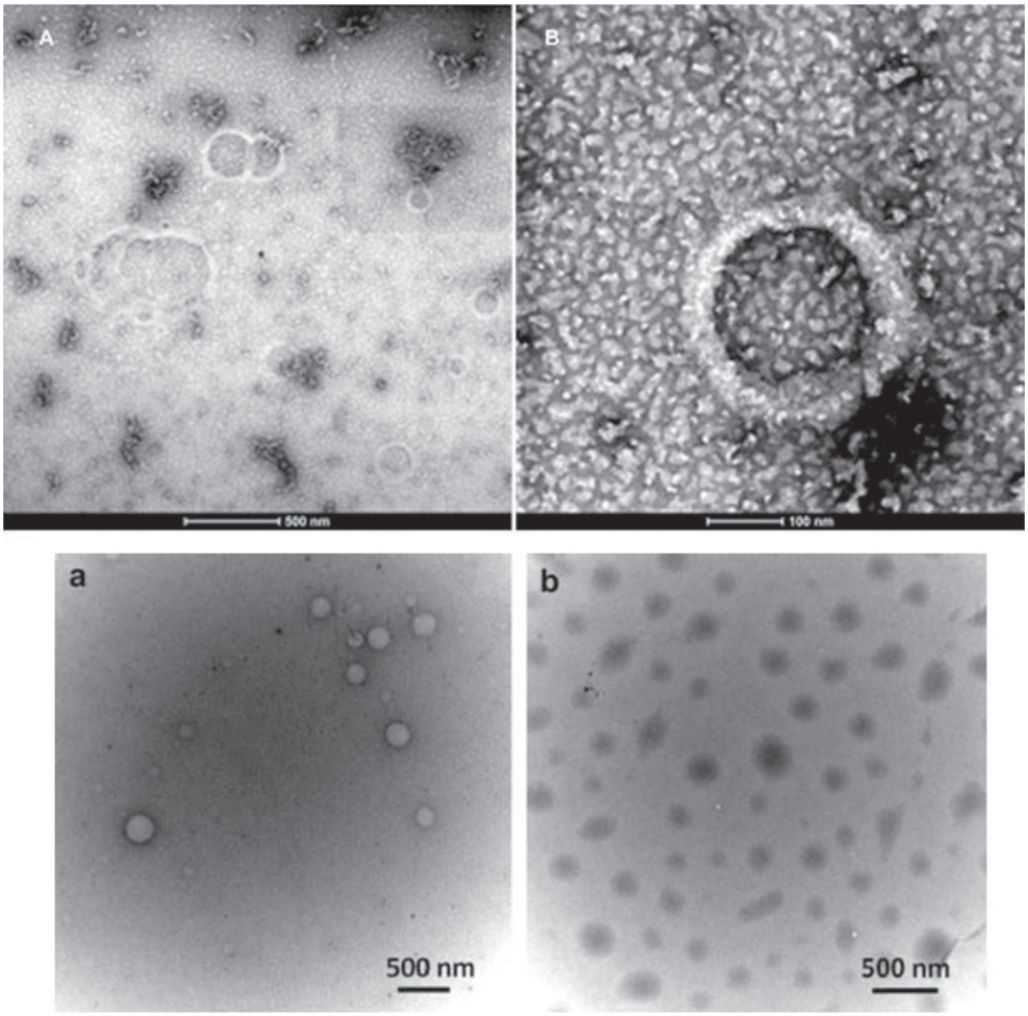

Figure 3. STEM micrograph of bis(h5)-K-K_4 and bis(h9)-K-K_4 vesicular assemblies [25] (top). TEM images of aggregates of (a) $K_{132}Y_{40}$ and (b) $K_{230}V_{66}$ [27] (bottom).

Diphenylalanine vesicles have been demonstrated to allow for bacterial membrane permeation, leading to inhibition of bacterial growth and proliferation [26]. This study is significant since it establishes a clear relationship between antimicrobial properties and self-assembly, and provides design principles for developing antimicrobial nanomaterials.

Aside from phenylalanine, tyrosine is another aromatic amino acid residue that has been investigated for its tendency to aggregate, albeit less frequently. In this context, lysine–tyrosine block copolypeptides have been studied and reported to form self-assembled micellar structures [27]. In this report, the authors also use the ratio of the radius of gyration to the hydrodynamic radius (Rg/Rh) to determine whether the structure is hollow or solid core. A ratio of 1 is considered indicative of vesicles by the authors, whereas a ratio of ∼0.77 is indicative of solid core spherical structures. The peptides were large (∼O(100) residues in length) and formed solid core aggregates with dimensions ranging from 100 nm to 350 nm. It is important to note that considering the large size of these molecules (200–300 amino acids long), these aggregates cannot be considered 'micellar'.

2.2. Computational studies

Computational approaches are a powerful tool for understanding aspects of molecular systems which cannot be accurately probed using experimental approaches. Computational approaches are particularly useful for probing systems at molecular or atomistic resolutions. These resolutions allow for the study of molecular level processes and mechanisms that drive the macroscale behavior of these systems. Among these systems, of particular interest are those that display the tendency to aggregate. Peptide motifs that encourage (or discourage) aggregation have been a topic of specific emphasis.

In this regard, aromatic amino acids have been shown to have a strong propensity to aggregate [28, 29]. Despite the studies reporting peptides forming amyloid fibrils and non-spherical assemblies, they highlight an important result: aromatic groups have a significant role in driving aggregation. The study by Gazit *et al* [18] is of specific importance: it signaled a paradigm shift in the approach to investigating aggregation of peptides. The core result of this study, as discussed before, was the identification of phenylalanine as the main

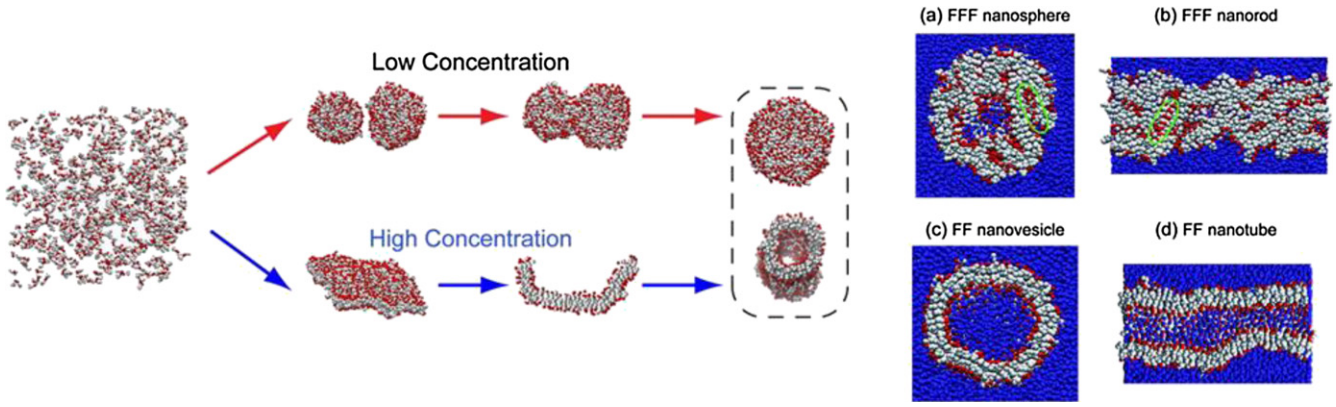

Figure 4. Assembly pathways of FF aggregation into vesicles and nanotubes (left) [36]. FF and FFF assemblies: (a) FFF nanosphere (b) FFF nanorod (c) FF vesicle (d) FF nanotube. (a) and (c) show the solid and hollow (water) cores of the FFF nanospheres and FF vesicles respectively [37] (right).

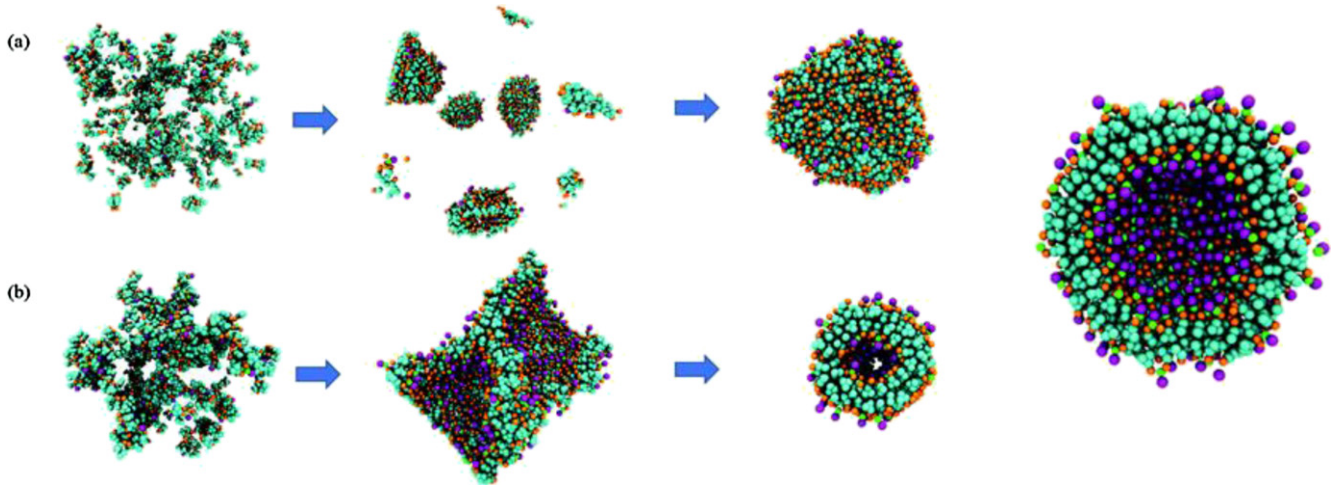

Figure 5. Left: assembly pathways of FF–FNF co assemblies forming (a) vesicles and (b) nanotubes at different concentrations. Right: exploded view of FF–FNF co-assembled vesicle simulated using the Martini cosrae-grained model [39].

assembly-driving amino acid due to the pi–pi stacking of the aromatic side chains.

Following Gazit and co-workers' demonstration of aggregation in phenylalanine based peptides, there have been several computational studies that focused on aggregation of peptides due to the hydrophobic effect of the aromatic groups. Selected studies from among these have been discussed below. These studies have included both atomistic as well as coarse grained approaches. Atomistic approaches provide a high resolution and allow for the study of properties at the sub-molecular and intermolecular levels, whereas coarse grained approaches trade away this resolution for computational efficiency, providing the ability to study significantly larger spatiotemporal scales.

2.3. Atomistic approaches

Molecular simulations with complete atomistic detail have been a mainstay in computational biomolecular research. They provide high resolution and allow for the study of molecular mechanisms at an extremely fine-grained detail, including properties such as the individual bonds and angles in molecules. In the context of peptides and their assembly, these studies provide insight into the mechanisms involving sub-residues that drive aggregation and other properties of interest.

A hybrid computational/experimental study has examined the mechanisms underlying the aggregation of FF assemblies [30]. German *et al* studied aggregation of phenylalanine using atomistic molecular dynamics simulations and reported that phenylalanine forms aggregates in groups of four molecules [31]. Uyaver *et al* performed atomistic simulations of a wide range of aromatic peptides including phenylalanine, tyrosine and tryptophan-based peptide sequences, and reported the formation of fibril like structures. The study reported that increasing the presence of tryptophan pushes the system toward forming disordered aggregates [32]. Other studies have employed multiscale approaches [33]. One investigation [34] combined coarse grained Monte Carlo simulations with atomistic molecular dynamics simulations to investigate the mechanics underlying the aggregation of a tyrosine containing peptide sequence, *GNNQQNY*. The study reported an interesting assembly pathway: first the formation of stable beta

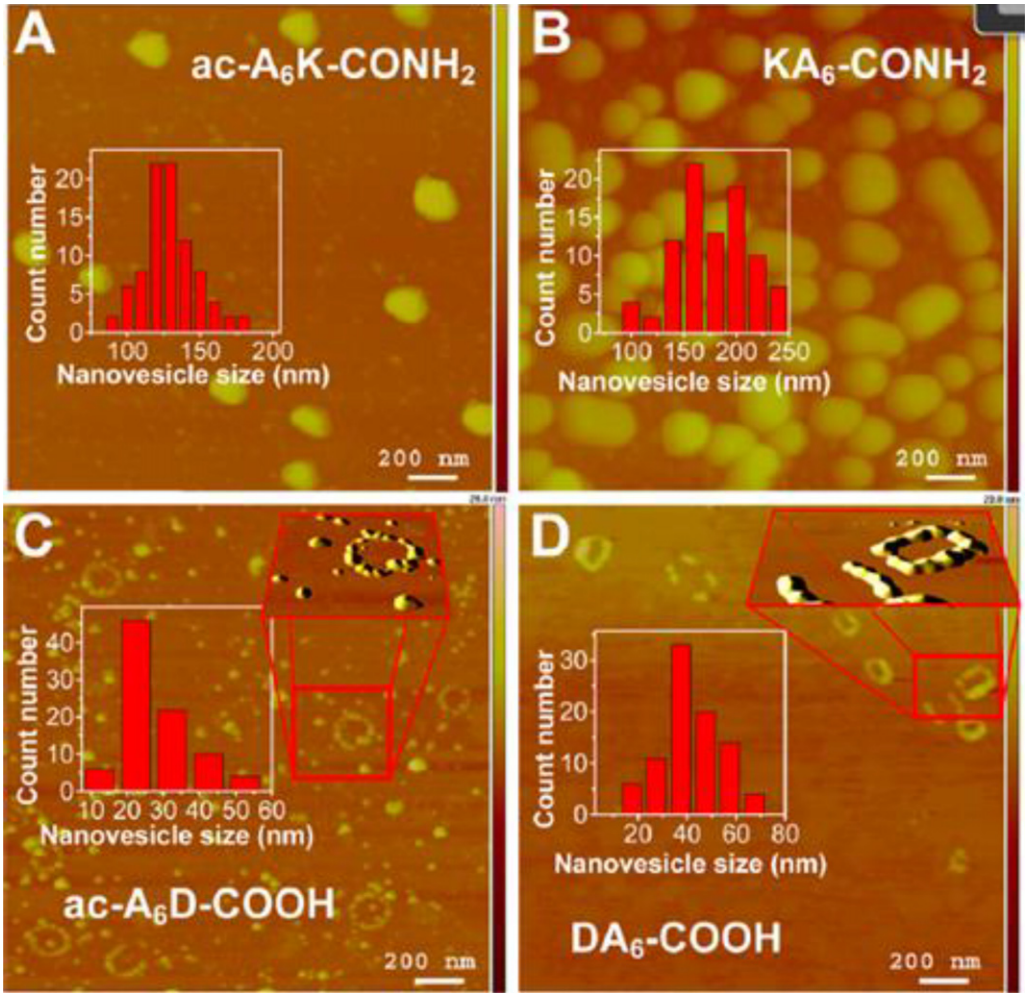

Figure 6. AFM images of the resultant self-assembled vesicle nanostructures that formed for each of the four lipid-like peptides on mica and dried with nitrogen gas. Histograms detailing the size distribution of the formed vesicles are included with their respective images. The nanovesicles containing aspartic acid as the charged amino side chain (C) and (D) appeared to be assembled into connected chains of vesicles. This was revealed to be caused by the drying conditions, in which quick drying of the sample (∼5 min) caused the nanostructures to slightly disassemble and interact with one another [50].

sheet dimers, followed by the formation of a 'polar zipper' motif with charged groups aligned in an antiparallel orientation. Further, atomistic approaches have focused on amyloid and amyloid-like peptides, mainly due to the tremendous interest in these molecules in the domain of neurodegenerative diseases. Since this review focuses on peptide droplets and vesicles, these applications are not within the scope of the present review.

2.4. Coarse grained/multiscale approaches

Atomistic approaches provide insight into molecular systems due to their very high resolution, but at the expense of computational efficiency. This results in the atomistic approaches being unsuitable for examining larger spatiotemporal scales which are necessary for studying morphological properties of peptide aggregates. All atom studies of peptides are usually limited to a small number of peptides and cannot scale further without becoming extremely expensive in terms of computational resources. Coarse grained simulations address this challenge by reducing the number of degrees of freedom in the system, allowing for the study of biomolecular systems at considerably larger spatiotemporal scales at which phenomena such as aggregation dynamics or phase separation may be investigated.

The power of coarse-grained approaches in exploring vastly larger spatiotemporal scales than atomistic approaches enables the study of aggregation of peptides and the morphology of the aggregates. In this regard, Ulijn *et al* [35] performed an extensive coarse-grained study of the entire library of the 8000 possible tripeptides and ranked them according to an aggregation propensity score. The authors reported that hydrophobicity alone is insufficient to predict aggregation in tripeptide sequences: one must also consider the sequence itself, with positively charged residue sidechains (if sidechains are present) preferably occupying the N-terminus, and negatively charged residue side chains occupying the C-terminus. The authors of the study propose that this is because of charge effects at the termini creating strong salt bridges across the peptides and promoting aggregation.

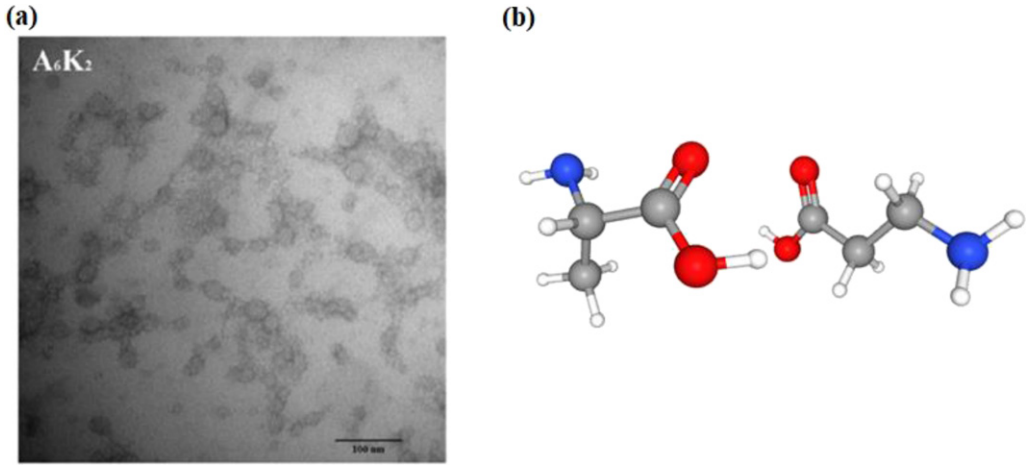

Figure 7. (a) TEM image (negatively stained with uranyl acetate) of the self-assembled nanovesicles formed by A_6K_2 at a concentration of 1.0 mg ml^{-1} in water. The nanovesicles ranged from 30–40 nm in diameter [59]. (b) A visual comparison of alanine (left) and β-alanine (right). Unlike alanine, β-alanine lacks a chiral center due to the location of its amino group. In β-alanine, the amino group is at the β-position from the carboxylate group. Images generated via https://pubchem.ncbi.nlm.nih.gov/.

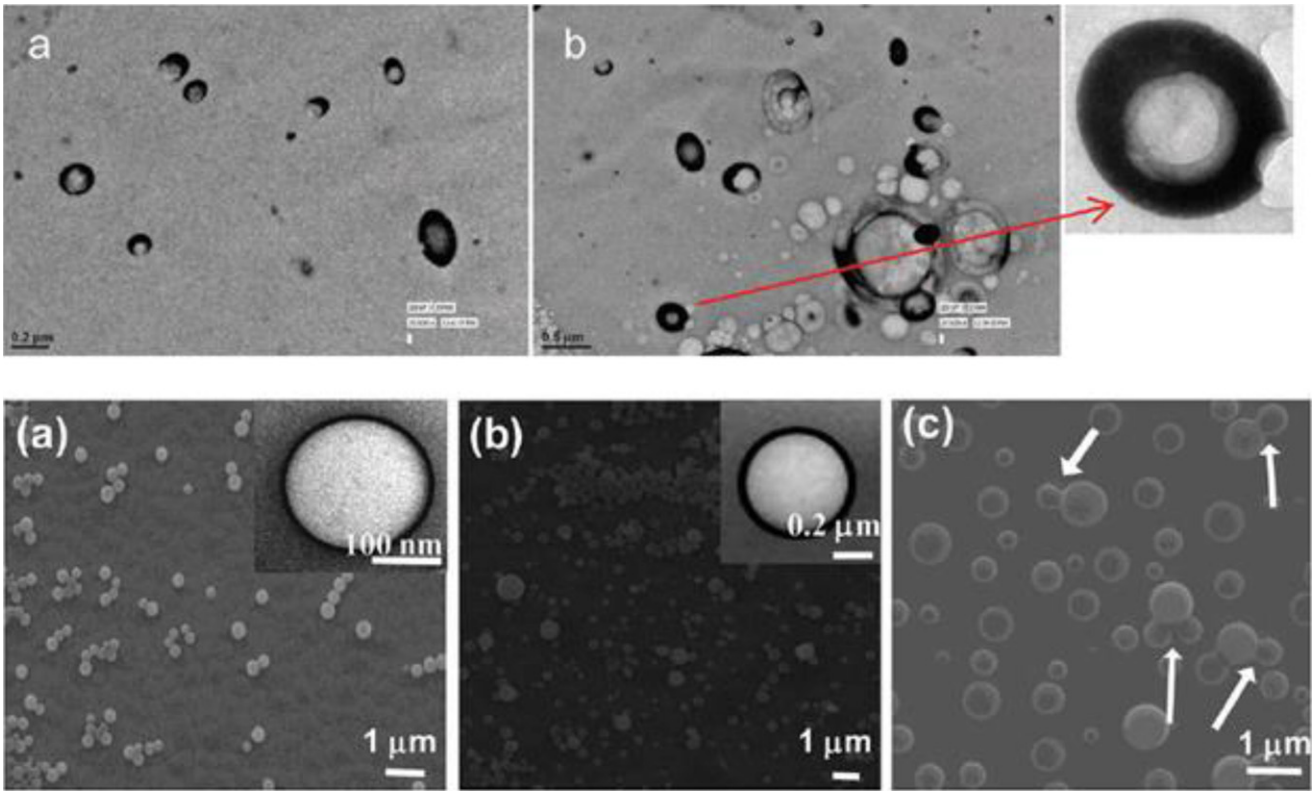

Figure 8. TEM images of the self-assembled $(\beta\text{-Ala})_4$ nanovesicles. These nanovesicles ranged in size from 100 nm–250 nm. (a) Shows the nanovesicles at a scale of 200 nm while (b) shows the nanovesicles at a scale of 500 nm with a close-up of one of the nanovesicles [51] (top). FE-SEM images of Boc-β-Ala–N, N^0-dicyclohexylurea in two concentrations: (a) at 1 mM and (b) at 10 mM. TEM images of vesicles from each of the two concentrations are included as respective inserts to demonstrate the hollowness of the assembled nanostructures. (c) Is an enlarged image of (b) which demonstrates the spherical morphology of the assembles present at 10 mM [52] (bottom).

The concentration dependence of the morphology of nanostructures such as lamellae, vesicles and nanotubes encompassing FF dipeptides has been studied through the use of coarse grained models in conjunction with molecular dynamics simulations [36]. This study reported that low concentrations (∼50 mg ml^{-1}) increase the tendency of the system to yield hollow vesicle like structures, and nanotubes and lamellae at higher concentrations. Further, the study reported distinct assembly pathways for the formation of vesicles and other morphologies, as seen in figure 4.

Tripeptides consisting of phenylalanine (FFF) form nanodroplets and nanorods, as demonstrated by Guo *et al* [38]

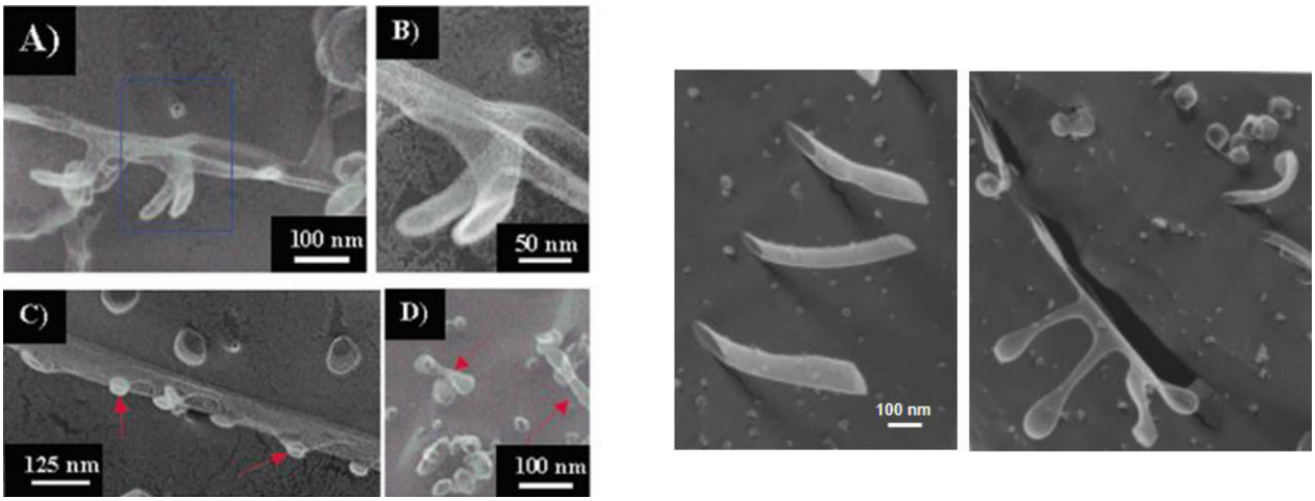

Figure 9. (Left) TEM images of the dynamic self-assembly of G_6D_2. (a) Show branches coming off of the main stem of the assembly. (b) Is an enlargement of the blue box in (a). (c) Shows a nanotube with openings which may be the starting point for the growth of the branches. A few nanovesicles are visible in the image. (d) Shows nanovesicles which are potentially undergoing fission with one another [54]. (Right) transmission electron microscope images of V_6D aggregates [48].

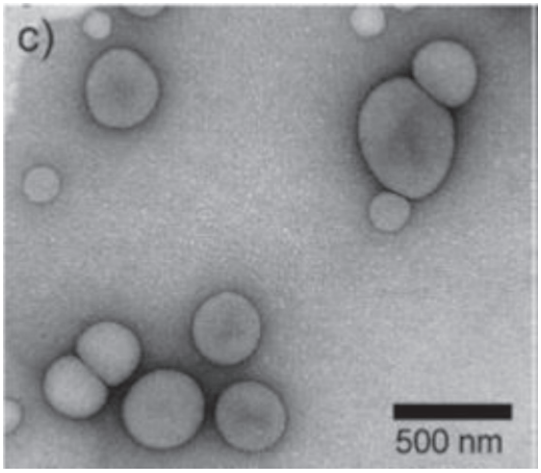

Figure 10. TEM image (negatively stained with uranyl acetate) of self-assembled spherical $P_{10}R_3$ nanostructures in water [56].

using a similar coarse-grained approach. The study demonstrated the fundamental differences between FF nanostructures and FFF nanostructures; the FFF assemblies are not hollow, i.e. they do not possess water cores, whereas FF assemblies do. Figure 4 highlights this difference in subfigures (a) and (c), right side.

A vast structural diversity can be achieved in aggregates by adopting a co-assembly approach using FF dipeptides and FFF tripeptides [37]. The equilibrium morphologies include nanodroplets, rods, tubes and even toroidal structures. The morphology of the supramolecular assemblies could be controlled by varying the ratio of the two peptides in the system. This implies that co-assembly can be a powerful tool to achieve a rich polymorphism in the assembly molecular parameter space.

Co-assembled peptides need not be limited to a single amino acid [39, 40]. These studies reported a rich polymorphism in the morphologies of the aggregates formed in mixed systems of FF dipeptides and FNF tripeptides. The morphologies included vesicles, nanotubes and lamellae depending on the relative concentrations of the two components as well as the total concentration. At low concentrations of total peptides (∼0.15–0.20 peptides/ cu. nm) the system largely yielded hollow nanospheres and the relative ratio of the two peptide sequences had little effect. Whereas a higher total concentration exaggerated the effect of the presence of the tripeptide and caused the system to generate more curved structures such as vesicles and nanotubes when the tendency of the pure FF systems was to yield flat lamellae. Distinct assembly pathways were observed in the formation of vesicles at low concentrations than other nanostructures formed at higher concentrations, this is seen in figure 5.

Dignon *et al* [41] established a novel coarse grained force field based simulation method that helped capture changes in the phase diagram due to specific features in peptide molecules arising from certain mutations. Dignon *et al* [42] used a coarse-grained molecular dynamics approach to establish the dependence of aggregation properties on peptide/protein sequence and demonstrated the phenomenon of phase separation in mixed aggregate systems. They showed that intrinsically disordered regions in protein sequences are a major driving force for the aggregation. Shuai *et al* [43] performed coarse grained simulations of self-assembled P_5VP_5 symmetrical nanospheres and their bactericidal properties. The nanospheres showed excellent thermal stability which was validated experimentally.

3. Aliphatic amino acid based vesicles and droplets

3.1. Experimental studies

Zhang *et al* [44] demonstrated self-assembling amphiphilic peptides to organize into ordered supramolecular assemblies. The structures formed were observed to span a range of

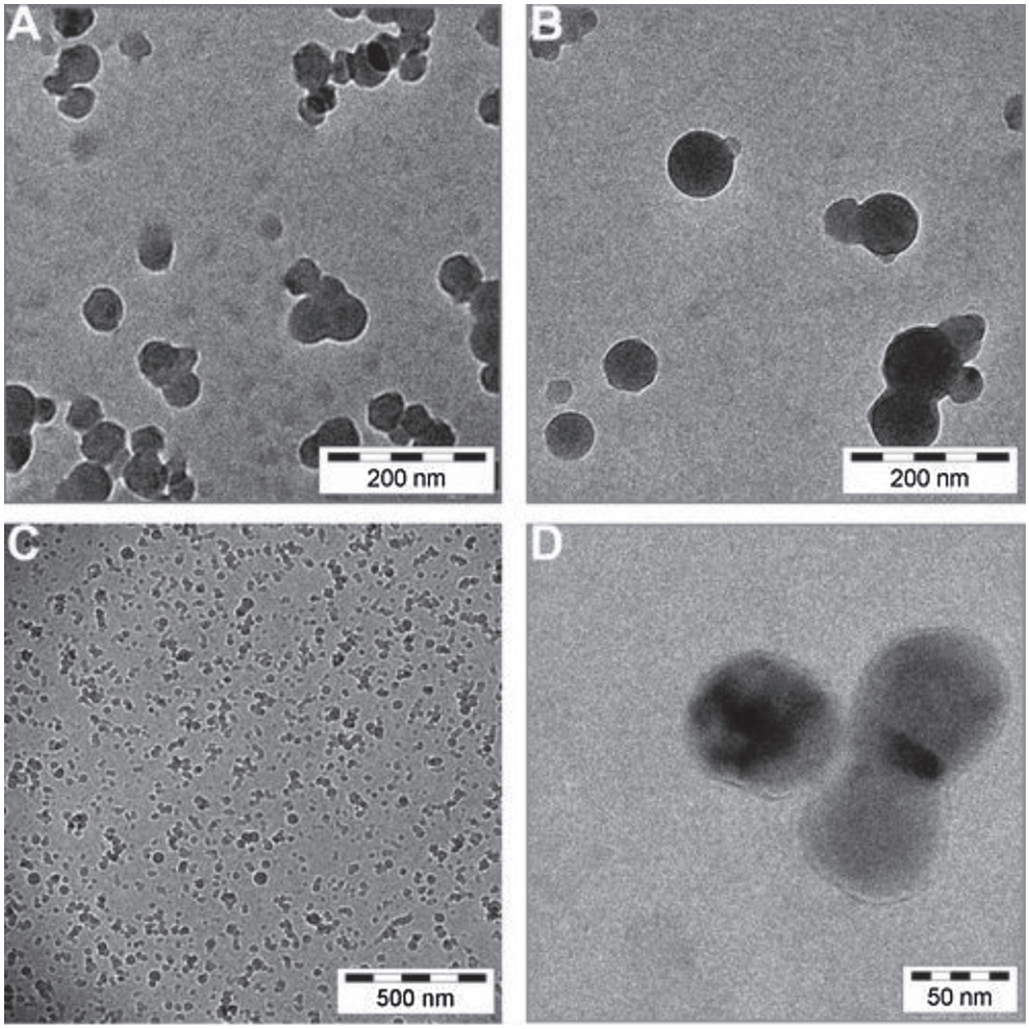

Figure 11. TEM images of the self-assembled peptide nanostructures. (A) SA2, (B) SA7, and (D) SA5 peptides assembled to yield spherical nanostructures. (C) shows a zoomed out image [57].

morphologies which included nanotubes, rods, lamellar bilayers, vesicles, droplets and nanotapes. Several factors were attributed to influence the formation of the resultant nanostructures, including amino acid-specific and environmental conditions.

The aggregation of amphiphiles into assemblies is determined by three key factors: the hydrophobic effect, hydrogen bonding, and electrostatic repulsion. Aliphatic amino acids comprise an amphiphile's hydrophobic regions while charged amino acids make up the hydrophilic regions. These distinct sections interact differently in the presence of water, with the charged amino acids preferentially interacting with water. Assemblies are further stabilized through hydrogen bonds between the peptides, while repulsion between similarly charged hydrophilic regions hinders the aggregation [45]. The formation of assemblies is an outcome of the competition between these three factors. The impact of each factor is dependent upon the aliphatic and the charged amino acids making up the peptide amphiphile. The morphology of the assembly is also influenced by the geometry of its polar (charged) head groups. For example, amphiphiles with large polar head groups but with comparatively smaller hydrophobic, aliphatic tails will form spherical micelles in water [46].

For the purposes of this review, a peptide is deemed to be aliphatic if the amino acid is non-polar, hydrophobic, and non-aromatic; therefore, this section of the review will focus on alanine, glycine, isoleucine, leucine, proline, and valine. Glycine is considered as aliphatic as it assumes a hydrophobic role during the formation of the presented assemblies.

3.2. Alanine

Alanine, known for its role in the glucose–alanine cycle [47], is the least hydrophobic amino acid of the aliphatics studied [48] by Zhang *et al*. The small hydrocarbon side chain on alanine, endowing it with low hydrophobicity, promotes the self-assembly of the peptides encompassing it. These weak interactions result in the large size range of assemblies containing alanine, as they tend to evolve over time due to their instability [49]. However, alanine-containing amphiphilic vesicles have recently been synthesized for potential use in drug delivery [50]. Four different amphiphiles (ac-A_6K-$CONH_2$, KA_6-$CONH_2$, ac-A_6D-COOH, and DA_6-COOH) self-assembled

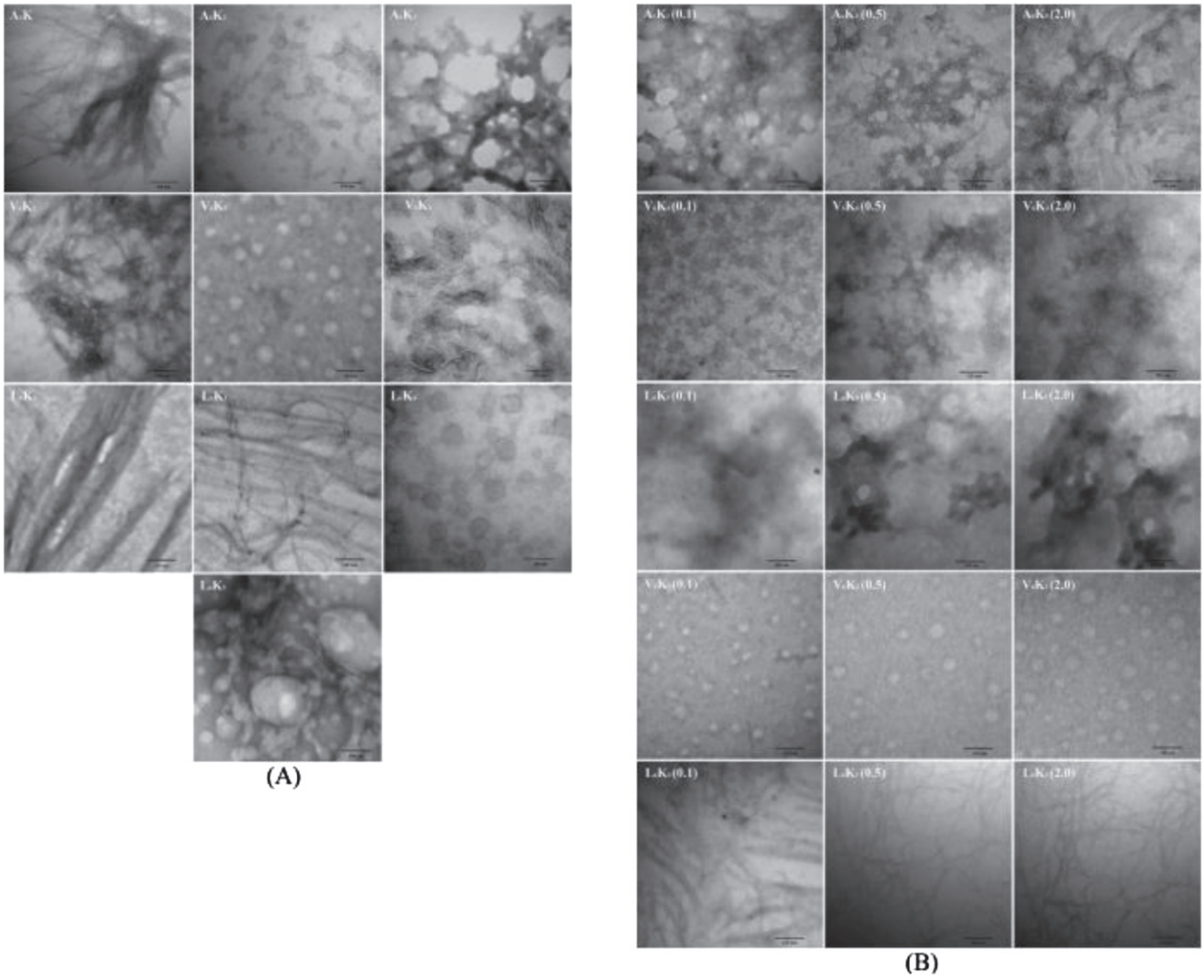

Figure 12. (A) TEM images of self-assembled nanostructures formed by A_6K, A_6K_2, A_6K_3, V_6K_2, V_6K_3, V_6K_4, L_6K_2, L_6K_3, L_6K_4, and L_6K_5 at a concentration of 1.0 mg ml^{-1} in water and (B) A_6K_3, V_6K_4, L_6K_5, V_6K_3, and L_6K_3 under different concentrations (0.1, 0.5, and 2.0 mg ml^{-1}) as reported by Meng *et al* [59]. It must be noted that this paper reports air drying, which may affect the integrity of the nanostructures.

above their critical micelle concentration to form vesicles of varying sizes (figure 6). Atomic force microscopy (AFM) imaging was used to validate the existence of nanovesicles in the system while DLS was used to determine the characteristics of each type of vesicle. Vesicles with lysine as the charged group formed larger assemblies than those with aspartic acid due to the difference in size of the charged amino acid side chains. Aspartic acid has a smaller side chain than lysine; therefore, the peptides containing aspartic acid were able to self-assemble into more closely packed structures than those encompassing lysine residues.

The influence of alanine, in comparison to other aliphatics, along with the increasing size of the hydrophilic region of the peptide molecule on morphology of the peptide assemblies has been reported by an earlier study [59]. In this study, six alanine residues were chemically grafted to an increasing number of lysine residues (resulting in peptide sequences A_6K, A_6K_2, and A_6K_3) and the resultant self-assembled nanostructures were compared against those created with valine and leucine. TEM was used to visualize each of the nine systems studied, while DLS confirmed the existence of the proposed nanostructures in solution. Vesicle formation occurred with A_6K_2, V_6K_3, and L_6K_4; an image of the A_6K_2 system is seen in figure 7(a). The hydrophobic regions of these three peptides varied in length while the three peptides sequences each self-assembled to yield vesicles. This result indicated that the composition of the hydrophobic region influenced the packing of the peptides such that the same self-assembled nanostructure was achieved under equilibrium.

3.3. *β-alanine*

β-alanine, a form of alanine in which the amino group of alanine is at the β-position from the carboxylate group, has been reported to form nanovesicles in solution. A comparison between alanine and β-alanine can be found in figure 7(b), with alanine on the left and β-alanine on the right.

H_2N–βAla–βAla–βAla–βAla–$CONH_2$ [(β-Ala)$_4$] has been reported to self-assemble into nanovesicles [51]. TEM

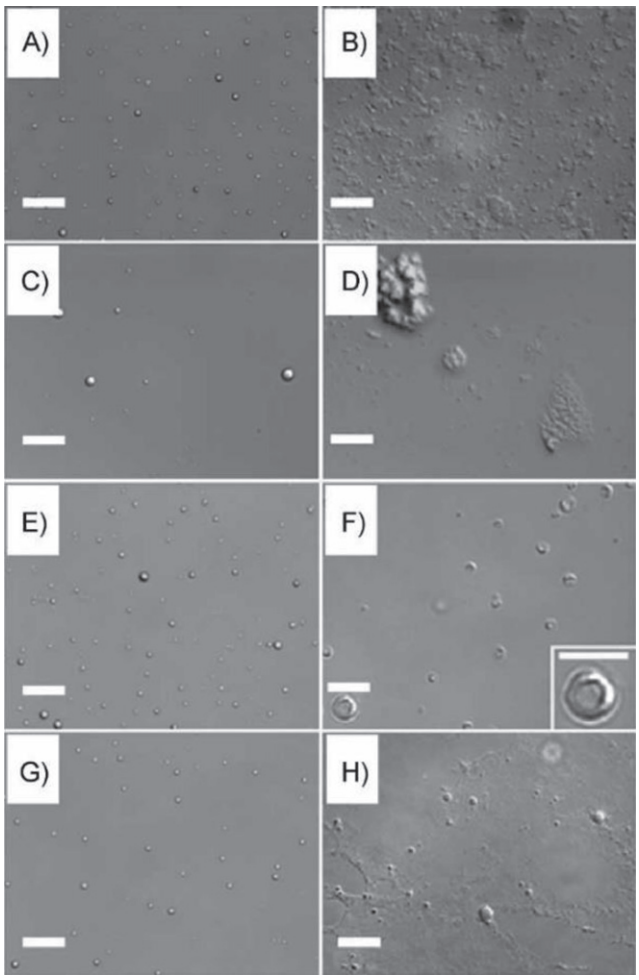

Figure 13. TEM images of vesicles formed by (A) $K_{60}DOPA_{20}$ (B) $K_{60}L_{20}$ (C) $K_{60}DOPA_{20}$ (D) $K_{60}L_{20}$ (E) $K_{60}DOPA_{20}$ and (F) $K_{60}L_{20}$ (G) $K_{60}DOPA_{20}$ and (H) $K_{60}L_{20}$ as reported by Holowka *et al* [60]. The drying technique reported consists of membrane extrusion and air drying/drying with filter paper, potentially affecting structural integrity and must be interpreted with care.

images shows that the tetrapeptide assembles into nanovesicles with dimensions ranging from 100 nm to 250 nm (figure 8). These vesicles were tested in the delivery L-Dopa, an anti-Parkinson's drug which needs a carrier to help mediate its transition across the blood-brain barrier. The results suggest that the drug is released slowly over a period of 24 h, making these vesicles potential drug-delivery systems.

Boc-β-Ala–N, N^0-dicyclohexylurea can form several different nanostructures depending upon its external environment [52]. The two caps of the peptide were chosen for their influence on their assembly, availability and biological use. Field emission scanning electron microscopy (FE-SEM) was used to study the system under changes in concentration and temperature. Changes in concentration from 1 mM to 10 mM resulted in an increase in the vesicle size (figure 8), as the formation of larger vesicles results in a decrease in the free energy of the system. Changes in temperature from 50 °C to 100 °C, 150 °C, and 170 °C resulted in a transition from vesicles to fibrils to plate-like structures. The morphology of these structures is believed to change due to the hydrophobic effect switching from entropy-driven at room temperature to enthalpy driven at higher temperatures, which changes how the side chains of the capping groups interact [52].

3.4. Glycine

Glycine is considered to be the simplest amino acid due to the composition of its side chain being comprised of only one hydrogen. However, this hydrogen (as opposed to the carbon located in other amino acid side chains) allows for glycine to induce greater flexibility when incorporated into a peptide sequence [53].

It has been shown that varying the length of the hydrophobic regions of an amphiphile when glycine is the aliphatic amino acid changes the morphology of the resulting self-assembled nanostructures [54]. The peptides were studied at neutral pH and were only capped at their C-terminus, resulting in a −3 charge per peptide in the system. The hydrophilic region was composed of two aspartic acids and the hydrophobic region encompassed between 4 and 10 glycine residues. TEM images show that primarily vesicles were formed from G_6D_2 peptides, while nanotubes and meshes were observed for G_4D_2, G_8D_2, and $G_{10}D_2$. However, further study of the G_6D_2 system showed that the assemblies were dynamic, with the coexistence of nanotubes and vesicles. Figure 9 highlights this.

Glycine has also been a prominent component of synthetic cell membranes [55], which are peptide vesicles that have been used to study and replicate cellular systems. The cellular membrane which encapsulates the system is composed of elastin-like peptides which contain a large proportion of glycine for flexibility. The final peptide sequence which makes up the membrane contains a hydrophilic region mainly composed of GEGVP pentapeptides and a hydrophobic region containing mainly GFGVP pentapeptides. The vesicles formed allowed for simple RNA transcription as well as growth when the membrane peptide was available in the environment.

3.5. Proline

Proline, like glycine, is a unique amino acid in that its side chain varies from the other amino acids. Proline is the only amino acid where its side chain connects to its backbone twice. This provides peptide sequences encompassing proline additional rigidity of the backbone.

A rod-coil amphiphile containing proline and Tat cell-penetrating peptide has been shown to assemble into vesicles [56]. $P_{10}R_3$ was first synthesized in attempts to characterize the assembly. The self-assembled nanostructures were characterized by TEM and were determined to be vesicles (and not micelles) based upon their dimensions (figure 14). After success with $P_{10}R_3$, P_{10}Tat was synthesized. The size of the vesicles encompassing P_{10}Tat increased; from 74 nm with $P_{10}R_3$ to 115 nm with P_{10}Tat. The ability of the vesicles to deliver drugs was then tested by encapsulating rhodamine B dye. Intracellular delivery using HeLa cells was confirmed with confocal laser scanning microscopy. It was determined that the chemical characteristics of the proline residues provided rigidity and phase separation between the hydrophobic and hydrophilic

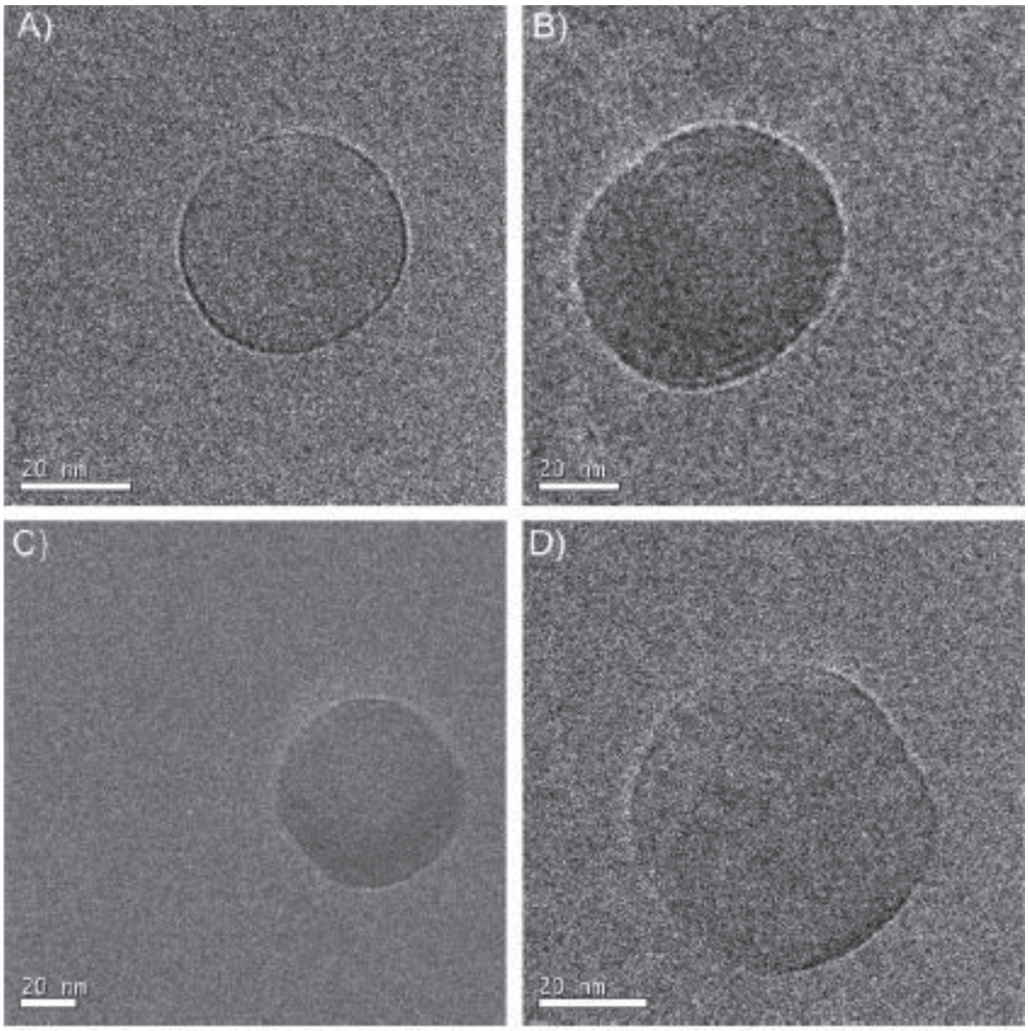

Figure 14. Elastin like copolymers (ELP's) forming stable vesicles (Cryo-TEM): (A) ELP-64/60, (B) ELP-96/90, (C) ELP-64/90, and (D) ELP-96/60 [61].

sections of the peptide, yielding vesicles sufficiently rigid to actively diffuse across a cell membrane (figure 10).

Amphiphilic peptides encompassing aliphatic amino acid residues have been demonstrated to display aggregation propensities and self-assemble into several nanostructures including nanovesicles, nanotubes and nanofibers [48]. The V_6D peptide sequence has six valine residues at the N-terminus and one aspartic acid residue at the C-terminus. This peptide sequence was observed to form a vesicle in aqueous solution (see figure 9) [48]. Both the nanotubes and nanovesicles were detected by DLS. These results suggest that the self-assembled nanostructures might be tuned by changing the sequence of the monomers or the environment (via pH). The TEM images of the supramolecular assemblies formed from V_6D (see figure 9) were captured by flash freezing in liquid propane (-180 °C) and surface-coating with a thin layer of platinum and carbon.

Recombinant amphiphilic oligopeptides [57] encompassing aliphatic amino acid residues assembled into spherical nanostructures. In this study, the N terminus acetylated (to prevent undesired charged distribution) peptides were observed using fluorescence spectroscopy, electron microscopy, dynamic and static light scattering. Two peptide sequences from those studied, Ac-Ala-Ala-Val-Val-Leu-Leu-Leu-Trp-Glu2-COOH (SA2) and Ac-Ala-Ala-Val-Val-Leu-Leu-Leu-Trp-Glu7-COOH (SA7) self-assembled in neutral pH aqueous solution (figure 11) [57]. These two peptides have glutamic acid residues as the hydrophilic domain and a relatively large interfacial area because of the side chain size and electrostatic repulsion. The critical aggregate concentration for SA2 and SA5 are respectively 4.9×10^{-7} M and 1.6×10^{-5} M. The TEM images were captured using the quick-freeze technique. DLS was used to determine the radii of the vesicles (63 nm and 59 nm respectively for peptide sequences SA2 and SA7). Static light scattering determined the radius of gyration of the assembled nanostructures to be 66 nm and 54 nm respectively for SA2 and SA7. The assembly of the SA2 peptide sequence was also examined using coarse grained molecular dynamics simulations [58]. Within the nanocarrier, the peptide sequence was determined to organize into an antiparallel, beta sheet. The simulation was performed for a duration of 54 μs at 300 K. All atom force fields (namely, AMBER ff99 SB-ILDN and

Table 1. Selected peptide sequences/peptide based molecules reviewed and the morphology of the assemblies reported. See figure 15 for further discussion of the implications of this table.

Peptide	Structure	Structure type (from figure 15)
FF motif	Vesicle	(c)
FFF motif	Droplet	(c)/(f) Hybrid due to free rotation of central F residue
P_5–V–P_5	Droplet	(g)
L_6K_4/A_6K/A_6D	Vesicle	L_6K_4 (a), A_6K/A_6D (c)
Beta alanine-Boc	Vesicle	(c)
G_6D_2	Vesicle	(c)
Long chain block copolypeptides $E_{60}L_{20}$/$K_{20}L_{20}$	Vesicle	$E_{60}L_{20}$ (d) /$K_{20}L_{20}$ (b)
R_3V_6	Droplet	(e)

GROMOS54a7) were used to show the stable beta sheet secondary structure.

Meng *et al* [59] reported self-assembly of peptide sequences of the form X_6K_n where X is lysine, valine or alanine, and $n = 1$–6. Of these, at a 0.2 mM peptide concentration in water, V_6K_3 was observed to form vesicles with diameters ranging from 40 to 50 nm. Whereas, the peptide sequence L_6K_4 assembled to form vesicles of diameters ranging from 50 nm to 60 nm. The C-termini of the peptides were amidated and the N-termini were acetylated. The peptides were dissolved in water at neutral pH. DLS was used to measure the size distribution of the nanostructures. V_6K_3 and L_6K_4 had narrow size distribution respectively for 70 nm and 110 nm, 250 and 500 nm. Figure 12 shows the observed spherical assemblies along with other morphologies of the self-assembled nanostructures.

Long peptide block copolymers, such as $K_{60}L_{20}$ and $E_{20}L_{20}$[60], have also been observed to form vesicles. Holowka *et al* examined both pure as well as L-DOPA (l–3, 4-dihydroxyphenylalanine) substituted copolymers, in both crosslinked and non-crosslinked forms. The dimensions of the vesicles ranged between 0.8 μm and 5 μm. The vesicles were found to be stable under thermal and osmotic stress. Figure 13 shows the vesicles reported in this study.

Thermally responsive elastin-like polypeptides (ELPs) in a linear AB diblock architecture with an N-terminal peptide ligand were reported to self-assemble into spherical micelles when heated slightly above physiological temperature [61]. The peptides were observed to have two phase transition temperatures: intermediate temperature for an unimer-to-spherical micelle and higher temperature for micelle-to-bulk aggregate when the hydrophilic-to-hydrophobic block ratio was between 1:2 and 2:1. Figure 14 shows the nanovesicles obtained from ELPs examined in this study.

Peptide based micelles have shown promise for use as non-toxic gene carriers [62]. R_x–V_6 ($x = 1$–4) peptides which are composed of one to four arginine and six valine have two to five positive charges. These peptides assembled in aqueous solution to form micelles. These peptides were reported to have low cytotoxicity and moderate transfection efficiency.

4. Conclusions

Peptide assemblies are becoming increasingly prevalent in diverse applications related to biomedicine, energy and electronics. With twenty common amino acids, the design space for peptide sequences is enormous. The enormous variety of peptide sequences lends the potential to generate peptide assemblies with a diverse range of properties. These properties can be precisely controlled via the amino acid sequence and external conditions such as pH. One of the most commonly studied properties of peptide assemblies is their morphology as this characteristic is often closely tied with the intended function of the assembly. Peptide sequences have been observed to assemble into nanostructures such as vesicles, droplets, nanotubes and lamella [22, 48]. Of specific interest are peptide-based vesicles and droplets due to their ability to encapsulate, store and transport active agents [1]. This review has focused on the state of art in the area of peptide based vesicles and droplets.

Peptides encompassing aromatic amino acids such as phenylalanine have been observed to assemble into vesicles and nanospheres, or droplets. The assembly of phenylalanine based di- and tripeptides have been enabled by the pi–pi stacking between the aromatic rings. Dipeptides such as FF assemble into nanovesicles and nanotube, with equilibrium morphology dependent upon the peptide concentration [63]. Similarly, aromatic tripeptides have also been shown to assemble into nanovesicles and nanospheres. For example, assembly of FF-Boc yields nanovesicles [23] whereas FFF has been observed to assemble into nanospheres or droplets [22, 38]. Studies with peptides encompassing tyrosine residues have been found to assemble into micelles.

Experimental observations on the morphologies of assemblies associated with specific aromatic di- and tripeptide sequences have been supported by computational studies [22, 30, 37, 38] employing approaches suitable for different spatial and temporal resolutions. These tools include all atom or coarse-grained representation of the peptide sequences in conjunction with Monte Carlo or molecular dynamics simulations. Coarse grained molecular dynamics simulations have also been adopted to explore co-assembly [37, 39] of aromatic peptides to yield the morphology of the assembly as a function of the total and relative peptide concentration. These studies showed the formation of vesicles and droplets for specific concentrations of the two distinct peptide species [39].

Amphiphilic peptide sequences with aliphatic amino acid residues have also been observed to assemble into vesicles and droplets. Amphiphilic peptide sequences with alanine, glycine, isoleucine, leucine, proline, and valine [47–59] have

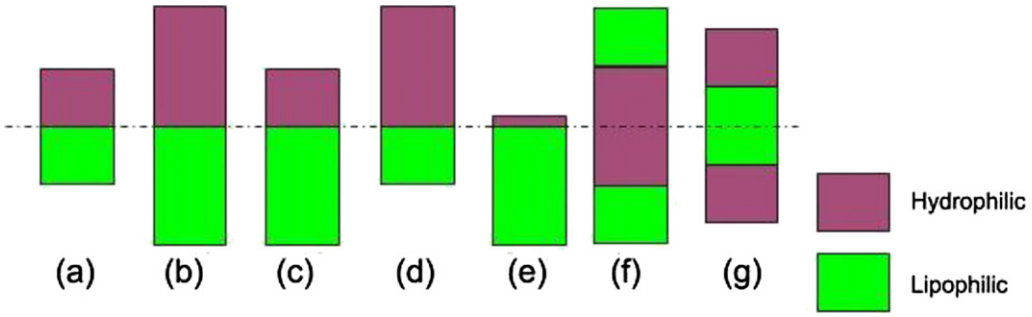

Figure 15. The schematic above shows amphiphilic molecules of various types. (a)–(d) Show cases where volumes of the hydro- and lipophilic sections are distinguishable (i.e. the molecules are asymmetric) and have comparable volumes. Types (a) and (b), while similar in volume ratios of the two zones, are represented as different cases since the differences in overall molecule size may have other steric effects that influence the assemblies. These molecules are generally expected to form hollow vesicles. (e) Shows a molecule where the hydrophilic section of the molecule is extremely small compared to the lipophilic section, these molecules are expected to form droplets. (f) and (g) Show symmetric molecules, i.e. the separation of the hydrophobic and lipophilic zones can no longer drive ordered aggregation. These are expected to form droplets as well. (a)–(e) have no more than one hydrophilic and lipophilic zone each, and they are clearly separated, forming what we term an 'amphiphilic dipole'.

been observed to assemble into vesicles or micelles. Whereas the aggregation of the amphiphilic peptides is dependent upon the hydrophobic effect, hydrogen bonding, and electrostatic repulsion, the equilibrium morphologies are determined by the peptide sequence. Most of the peptide sequences encompass polar amino acid residues which yield the assemblies to be sensitive to the pH of the environment [11, 23]. Amino acid residues that carry charged side chains are most responsive to pH changes, since they are most vulnerable to protonation or hydroxylation [64].

Computational studies on the assembly of peptide sequences with aliphatic amino acid residues is limited [58]. This limitation arises primarily due to the complexity of modeling the dynamically changing secondary structure of peptides along with the presence of hydrogen bonding. This difficulty can be addressed by further work in the development of suitable force field descriptions of peptide sequences. Progress in this area can significantly accelerate the sequence-structure-property predictions of peptide-based vesicles and droplets, thereby increasing their use in diverse disciplines.

The criteria for formation of vesicles or droplets, is often difficult to infer from experimental reports. This is largely due to the challenge of resolving the water cores of hollow assemblies through microscopy or similar techniques. This is reflected in the papers reviewed where they are frequently referred to as 'spherical nanostructures' (as opposed to vesicles or droplets). However, the ratio of the radius of gyration to the hydrodynamic radius (Rg/Rh) can be used to make distinctions between the two structures, as discussed in the report by Huang *et al* [27]. In addition, imaging techniques can be very insightful but also potentially be invasive, leading to compromising the structural stability of the supramolecular assemblies observed. With this in mind, a review of conceptual convergence between the various papers discussed is provided.

Table 1 lists the peptide sequences and classes reviewed. The sequences and motifs that were found to assemble into vesicles were the FF motif, asymmetrical block copolypeptides, and beta alanine-Boc. These peptides seem to share two common traits: the ability to maintain clear separation between the hydrophilic and hydrophobic sections, and comparable volumes between the hydrophilic and hydrophobic sections (see figure 15). The corresponding subfigure numbers from figure 15 representing the various molecular structures are also indicated in the table.

Peptides with the FF motif have clearly defined hydrophilic backbones and hydrophobic side chains as confirmed by computational studies. Asymmetrical block copolypeptides such as L_6K_4, A_6K, A_6K_2, A_6D and G_6D_2 demonstrate the ability to form hollow vesicles. As described by Israelachvili [46], the geometric packing factor plays a critical role in the formation of either hollow or solid supramolecular assemblies. Whereas Israelachvili defined the geometric packing factor in the context of lipids, analogous behavior can be expected in a wide range of amphiphiles. In all the above peptide sequences, the volumes of the hydrophilic and hydrophobic sections are comparable such that the packing factor ranges from 0.5 to 1.0. Therefore, we have two criteria we can point to in order to predict the formation of vesicles: (i) there must exist two clear, contiguous lipophilic/hydrophobic and hydrophilic regions of the molecule, forming an 'amphiphilic dipole' (analogous to an electric dipole but pertaining to amphiphilicity rather than charge), and (ii) the ratio of the exclusion volumes of these two regions must be comparable. However, in the peptide sequences that form solid structures, there is at least one of the two above criteria that is not satisfied. The FFF motif fails to maintain a separation between the hydrophobic and hydrophilic sections due to the free rotation of the amide bond, causing the middle F residue to rotate and point in the opposite direction to the flanking groups. The P_5VP_5 residue is symmetric, as shown in figures 15(f) and (g). The R_3V_6 has a much longer hydrophobic tail causing the formation of droplets, seen in figure 15(e). Further, the techniques reported by Huang *et al* [27] may be combined with the above concepts to make more accurate determinations of the supramolecular structural properties. This provides further momentum in the direction of developing a set of design principles that can be utilized to

accurately control morphologies of assembling peptide-based molecules.

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