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Bottom-up design of biomimetic assemblies

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Abstract

Nature has evolved the ability to assemble a variety of molecules into functional architectures that can specifically bind cellular ligands. Mimicking this strategy requires the design of a set of multifaceted molecules, where elements that direct assembly were conjugated to biologically specific components. The development of functional molecular building-blocks that assemble to form compartments for therapeutics addresses the desire to have controllable morphologies that interact with biological interfaces at nanometer length scales. The practical application of such 'bottom-up' assemblies requires the ability to predict the type of aggregated structure and to synthesize molecules in a highly controlled fashion. This bottom-up approach results in a molecular platform that mimics biological systems with potential for encapsulating and delivering drug molecules.

Keywords: Targeted drug delivery; Peptide assemblies; Block-co-polymer micelles; Bioconjugation

Contents

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1. Introduction

Biological molecules are particularly difficult to emulate with synthetic systems, because they possess the ability to interact in a highly specific fashion, and they do this on extremely small length scales. A design strategy, which does emulate nature, and is amenable to both characteristics is the use of self-assembling molecules, where the structure contains at least two distinct moieties, a biologically active portion and a fraction that directs assembly. This approach fits into a larger framework known as 'bottom-up' design, where complex functional structures are generated by assembling a group of molecularly interlocking parts [1]. The systems discussed in this review mimic biological architectures by using a modular set of small molecules that are capable of associating into a supramolecular structure. To accomplish this, the molecule must first be encoded with the appropriate biologically specific message or information, which can be via a peptide, nucleotide, sugar or specifically binding synthetic molecule. Second, a molecular region must be included to direct the assembly of the molecules into a controlled superstructure, such as micelles, vesicles, tubules, and other more complex structures. The assembly gives potency to the biological information it carries by several possible mechanisms. These include controlling spatial relationships among, and concentrating locally the biofunctional entities, as well as providing some opportunities for combinatorial selectivity. These assemblies have potential as vehicles for therapeutics by virtue of their inherent compartmentalization and targeting character.

There are significant challenges to realization of this potential. Though this article focuses on design, we do not really know very much about how to design for function. That is, we can design an increasing number of intriguing new structures, with ideas for function in mind, but structure function relationships in this type of assembly are very much needed to accelerate the effective design process. Beyond this fundamental gap in knowledge, many of these biomimetic aggregates are composed of a relatively new set of molecules such that the balance between curative efficacy and toxicity is poorly understood. Several unknown parameters for these nascent molecular assemblies can result in the restriction of use, primarily toxic side effects, but also the sterility, stability and scale-up of the complicated drug carrier systems [2]. This review focuses on the design aspects used to synthesize biomimetic self-assembling architectures, and these impediments to the realistic application will not be discussed in depth.

Nature frequently capitalizes on bottom-up strategies by coupling proteins into quaternary structures to create functional systems. Structural biologists are currently showing how nature maximizes utility while simultaneously minimizing the use of resources and economizing synthesis by assembling molecules in a variety of ways to give a spectrum of binding targets by mixing heteromeric assemblies together. Molecules that operate in this fashion include dimers such as antibodies, integrins, and leucine zippers, trimers such as collagen, and larger multimeric assemblies such as viral capsids. A recent survey of leucine zippers show that homodimers and heterodimers give quaternary structures such that several molecules are coupled together in a combinatorial fashion to give greater specificity and target diversity. The coupling reaction itself provides another mechanism of control, where the monomeric uncoupled form of the assembly does not possess the desired binding or function and is essentially 'turned off'. Cellular networks composed of these modular elements of a structurally similar class can selectively self-assemble revealing subtle functional correlations. For example, the coupling of

leucine zipper peptides involved in the circadian clock, where 'partnering selectivity' of homologous sequences on a protein array suggest connections between peptides in a regulatory network [3] (Fig. 1A) [4]. A more complex example is the viral capsid, where a variety of proteins come together to form a functional icosohedral cage. The bottom-up strategy is exemplified by the large capsid constructed from a small number of genes [5,6] (Fig. 1B) [7]. This type of modular aggregate would be ideal to mimic for drug delivery purposes, possessing specific binding properties coupled with encapsulation. Attempting to emulate this type of strategy with synthetic molecules will require a fundamental understanding of the dominant forces that drive assembly, innovative chemistry of molecules that assemble in a controlled manner, and practical application of self-assembling systems to succeed.

Several attempts have been made to imitate biological structure and function by using bottom-up strategies to design molecules that are capable of self-assembly. This class of molecules offers a modular system that can be tailored to interact with cellular elements. Many of the supramolecular assemblies described here could be developed to encapsulate, display or facilitate the delivery of drugs. We will review the rapidly growing toolbox of molecules that can access complex biological systems by emulating their characteristics on nanometer length scales.

2. Design

Designing multifunctional molecules that assemble into supramolecular biologically active structures can be a complicated endeavor. Nature's elegant ability to

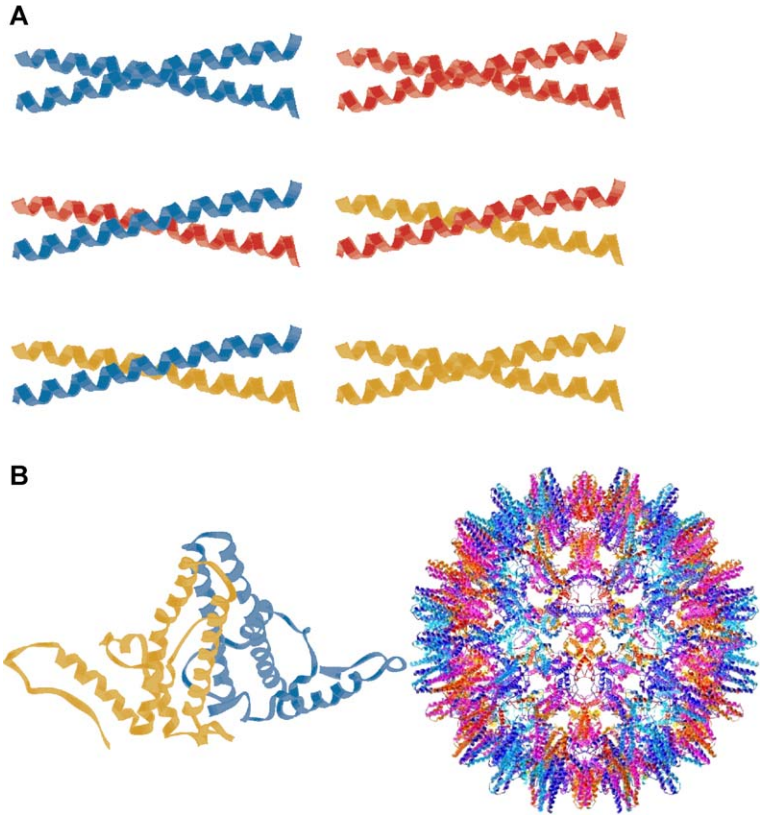


Fig. 1. (A) Leucine zippers maximize binding diversity by assembling several permutations of homo- and hetero- dimeric structures. (B) Viral Capsid of Hepatitis B, the homodimeric unit is shown (at left), and the complete capsid aggregate is shown (at right) composed of 120 dimers in a $T=4$ icosahedral symmetry [7].

fold and self-assemble molecules is not yet entirely understood or predictable. Moreover, it is difficult to duplicate in all its richness with synthetic molecules. Micelles, vesicles, lamella, bicontinuous structures, and fibrils are among the more common types of self-assemblies engendered by amphiphilic molecules; one can imagine that directing the formation of such assemblies is the most fundamental step for bottom-up constructs. A variety of physical parameters govern the assembly of supramolecular structures, predicting and controlling these aggregates a priori often becomes overwhelmingly complex. The synthesis of simple molecular building blocks can lead to intricate self-assembled morphologies beyond the original structural hypothesis [8–10]. The resulting morphology can subsequently be rationalized based on the physics of assembly. It is worthwhile therefore at the outset to describe some of the physical parameters and chemical principles that can be employed for the design of assembled structures, including intermolecular forces that dominate assembly mechanisms, thermodynamics of assembly, and surfactant number theory.

2.1. Intermolecular forces

Many of the ideas that are used to predict the assembly of molecules parallel those used to describe protein folding, where the intramolecular interactions that govern folding can be applied to intermolecular assembling systems. This collection of forces has been well established and is described in detail by in Creighton [11]. This review will focus specifically on three of these forces, hydrophobic interactions, hydrogen bonding and columbic forces [12]. These forces or a combination of them in a particular molecule have proven to be particularly prevalent in design efforts to control self-assembly.

2.1.1. Hydrophobic

Perhaps the most common driving for the rational design of self-assembling molecules is the hydrophobic interaction. Amphiphilic moleculaes, comprising a moiety that loves water and one that hates water, use the hydrophobic interaction to form a variety of aggregated morphologies. The portion that is hydrophobic acts as the principle driving force for assembly, but ΔG° actually can stems from several factors that make aggregation favorable (or unfavorable). Some of these terms are as follows:

$$\Delta G^\circ = \Delta G_{\text{hydrophobic}} + \Delta G_{\text{contact}} + \Delta G_{\text{Hydrophobic packing}} + \Delta G_{\text{Headgroup}}$$

The first term represents the transfer of tails from an aqueous environment into the aggregate (negative). For a saturated hydrocarbon this term can be roughly estimated by

$$\Delta G_{\text{hydrophobic}} = -(n_c - 1)3.0 - 9.6$$

The contact term refers to the alkyl groups that are assembling together and interacting with the solvent; this term is a function of the micelle area (positive). Hydrophobic packing results in the decrease in entropy associated with the loss of conformational freedom within the aggregate (positive). And, the headgroup term refers to the attractive or (typically) repulsive interactions of the head-groups, examples of this are electrostatic repulsion in charged head-groups or steric interactions of polymeric head-groups, understanding these interactions will help describe the general driving forces for aggregate formation [13,14].

Functional amphiphilic molecules have been the subject of heightened design efforts, where complex structures are required with biological functionalities. Designing functional amphiphiles is the subject of a review by Luk and Abbott [15]. They describe an assortment of headgroup interactions that are biologically relevant. Several groups have studied the function and structure of amphiphiles conjugated with peptides [16,10] or carbohydrates [17], also polymerizable elements in the hydrophilic head groups [18] and the hydrophobic cores [19] can be included for stability. Nature's use of amphiphiles is analogous to the applications pursued by chemists and engineers, where two parallel design goals are characteristic in both systems: the formation of organelles (compartmentalized environments) and the presentation of concentrated specific signals at the interface of those enclosures. Assembly can be controlled by subtle modulation of hydrophobic interactions and chemical structure, where a variety of assemblies including micelles, vesicles, sheets, bundles and tubes are observed [15].

2.1.2. Electrostatics

The energy associated with an electrostatic interaction, E, between charged atom A and B is takes the following form:

$$\Delta E = \frac{Z_a Z_b \varepsilon^2}{D r_{ab}},$$

where Z is the number of charges, ε is the charge of an electron, r is the distance between atoms and D is the dielectric constant [11], which varies with salt concentration such that $D \sim \exp(c_i^{1/2})$ [14]. Electrostatics provides a powerful handle on the dynamics of self-assembly, because it simultaneously provides an attractive and repulsive force at relatively long ranges, which can be controlled by adjusting the salt concentration.

Schnarr and Kennan [20] provide an example of designing assemblies that use electrostatic interaction to create a coiled-coil heterotrimeric assembly. The molecular assembly combines the use of hydrophobic interactions to drive assembly and electrostatic forces to uniquely match together the building blocks. Manipulation of charge-charge interaction controls the specificity of assembly, providing a design that is selective for trimeric α-helical coiled-coils as building blocks. Typical coiled-coil motifs are septet-repeats of amino acids that contain a hydrophobic 'streak' to promote assembly, the amino acids are designated abcdefg, where *a* and *d* are typically hydrophobic [21]. The core interactions of the trimer were controlled at positions *a*/*d* by coupling one cyclohexylalanine with two alanines, preventing undesired arrangements by steric interactions. Hydrophilic residues (e/g) promoted stability by coupling favorably electrostatic interactions, namely including lysine–glutamic acid pairs to locally enforce the association of a particular heterotrimeric arrangement. This study shows that specificity can be designed into the assembly process by using electrostatics. These types of biologically specific assemblies can be used to present therapeutics in a geometrically controlled-polyvalent fashion at the interface. The combination of hydrophobic forces and electrostatics gives 'independent control' of complex assembly by manipulating the sequence of three peptides to locally control the position of electrostatic interactions [20].

2.1.3. Hydrogen bonding

Hydrogen bonding is often considered a subset of electrostatic interactions [13], because it originates between a hydrogen atom with a partial positive charge and an electronegative atom with a partial negative charge. The hydrogen atom's partial positive charge is due to a covalent bond to another electronegative atom. Quantifying this interaction is difficult as the interaction is dependent on the chemical constituents and the angle of the three atoms involved [11].

The β-type aggregation of cyclic D,L-peptides is an example of designing assemblies that are energetically favorable because of hydrogen-bonding effects. This type of assembly was first studied in 1971 with gramicidin A, a transmembrane channel with 15 amino acids containing alternating L,D-chirality. Urry suggested a β-type structure for gramicidin A, where hydrogen bonding occurs in a head-to-head and tail-to-tail arrangement, which is corroborated by spectroscopic and conformational analysis [22]. The theoretical treatment of Urry's spectroscopy experiments validates the existence of a pi(L,D) helix for enantiomerically regular sequences [23]. Fig. 2 shows a cyclic analogue of the gramicidin A peptide, where the N-terminus and C-terminus are covalently connected with an amide bond, and the amino acids alternate in chirality. Harterink et al. uses this molecular pattern to synthesize a building block for a tubular aggregate, also shown in Fig. 2, based on the formation of anti-parallel β-sheet hydrogen bonding and three other physical design principles. First, ring strain of the cyclic peptide should be approximately eight amino acids as shown by molecular modeling, because short sequences would lead to a strained amide backbone, and larger rings have too much flexibility. Second, the register of the stack are designed such that the rings will align with homochiral residues as closest neighbors, preventing cross strained steric interactions between the side chain and backbone of a hetero chiral alignment. And third, manipulations of the side chain-side chain interactions using glutamine that participates in intra- and inter-hydrogen bonding for molecular assembly. The careful construction of these molecules resulted in extremely stable stacked assemblies (solvent, temperature, pH) as verified by FTIR and electron diffraction [24].

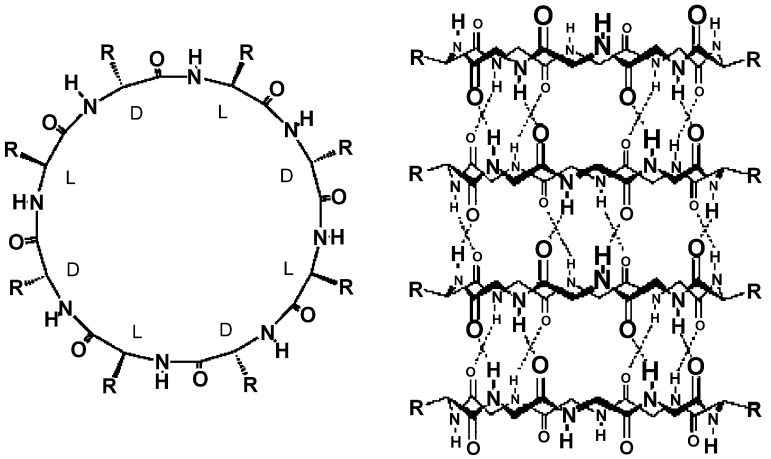


Fig. 2. Molecular structure of a cyclic D,L-peptide (left), and stacks of ring-like peptides self-assembling into tube-like structure (right) [86].

In the end, self-assembling systems must be designed for particular applications based on the class of molecules used and the forces that dominate their assembly. Several of these systems are discussed in section four of this review, where applications can be derived from self-assembly. For example, the D,L-cyclic peptide stacks mentioned above can be functionalized to partition into the plasma membrane, resulting in increased membrane permeability [25]. Realistic predictions of self-assembled structure should take into account a combination of forces that describe self-assembly as a complex milieu with several local energy minima, much like protein folding.

2.2. Thermodynamics of self-assembly

Thermodynamics provides another perspective on predicting the self-assembly of molecules. Obtaining thermodynamic parameters for aggregate formation will help to control (or at least rationalize) self-assembled structures [14,26].

A simple treatment of the assembling systems combines all these interactions, where forces can be evaluated using the chemical potential, μ, the generic driving force for aggregation:

$$\mu_N = \mu_N^o + \frac{kT}{N}\log\left(\frac{X_N}{N}\right) \quad \text{and}$$

$$X_N = N\left[X_1 \exp\left(\frac{\mu_1^o - \mu_1^o}{kT}\right)\right]^N$$

where μ_N^o is the standard chemical potential, X_N is the concentration of aggregates, and N is the size of each aggregate. The primary impetus for aggregation is $\mu_N \ll \mu_1$. It should be noted that the chemical potential is a function of a variety of physical parameters including temperature, ionic strength, and pH.

For amphiphilic systems, the minimization of hydrophobic interaction energy drives aggregation to form at a critical concentration of amphiphiles known as the critical micelle concentration (CMC). At this concentration,

$$\Delta G^\circ = \mu^o(\text{micelle}) - \mu^o(\text{water}) = \text{RTlnCMC}$$

To obtain the CMC (thus the ΔG°) we can use an equilibrium model,

$$NS \Leftrightarrow S_N \quad \text{and} \quad K_N = [S_N]/[S]^N$$

where S is the monomer concentration, S_N is the concentration of aggregates of N amphiphiles, and K_N is the equilibrium constant. The CMC can be considered the measure of total amphiphile concentration, $[S]_T$, where molecules entering the system begin to preferentially enter aggregated structures.

$$[S]_T = N[S_N] + [S] = NK_N[S]^N + [S] \quad \text{and}$$

$$\left.\frac{\partial\{N[S_N]\}}{\partial[S]_T}\right|_{CMC} = \left.\frac{\partial[S]}{\partial[S]_T}\right|_{CMC} = 0.5$$

The migration of amphiphiles to self-assemblies with increasing total concentration is shown in Fig. 3 [14]. These two equations can be solved to give a CMC:

$$\{[S]_{\mathrm{CMC}}\}^{N-1} = (N^2 K_N)^{-1}$$

and

$$\mathrm{CMC} = [S]_{\mathrm{CMC}}(1+N^{-1}) = (N^2 K_N)^{-1/(N-1)}(1+N^{-1})$$

Assuming that $N>>1$ such that $\ln N/N \to 0$, we can obtain a ΔG°.

$$\Delta G^\circ = RT\left[\frac{-1}{N-1}(2\ln N + \ln K_N) + \ln\left(1+\frac{1}{N}\right)\right]$$
$$= -RT\ln K_N$$

The free energy can related back to the collection of forces that drive assembly, some of which are described in Section 2.1. A typical argument using free energy considerations for the assembly of block copolymers is given by Kataoka et al. [27], describing the balance of forces that govern the formation of micelles from block copolymers as governed by three principle characteristics: a hydrophobically driven decreased free energy at the interface, a decrease in entropy due to the elongation of the core segments, and an increased steric repulsion of segments at the interface. This last term causes the area of the head group to increase as a function of the molecular weight of the hydrophilic polymer head group, taking these types of geometric considerations into account is the subject of surfactant number theory.

2.3. Surfactant number theory

A commonly used method to evaluate and predict the type of self-assembled structures is to consider the dimensionless surfactant number [26],

$$N_s = \frac{v}{a_o l}$$

where v is the volume of the tail-groups, a_o is the head-group area and l is the fully extended hydrocarbon tail. Using the surfactant number, the morphology of the aggregate can be predicted or controlled (see Fig. 4) [26]. Amphiphilic systems can be examined using these theoretical approximations in an attempt to control, predict or simply justify microstructure. One relatively simple route to control the surfactant number is to manipulate the tail group. Either changing the tail length (l_c) or the number of tails, therefore volume, (v) will result in a proportional change in the surfactant number. Modifications in the degree of saturation in the tail will affect the geometry of the hydrophobic regions (accounting for this is slightly more difficult). Also, manipulating the head-group is possible by covalently attaching charged or polymeric functionalities, causing an increase in a_o. Another

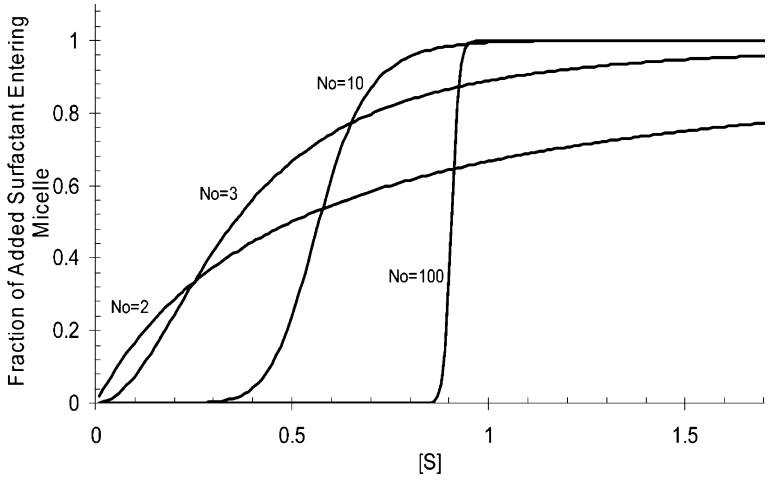


Fig. 3. Fraction of molecules entering the aggregate of size N_o [14].

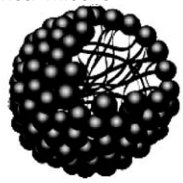
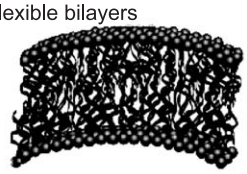
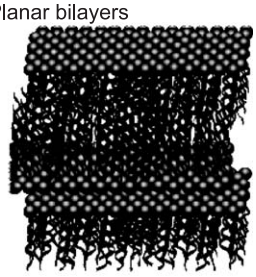
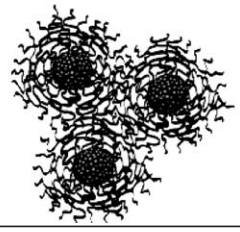
Critical Packing parameter ($v/a_o l_c$)	Critical packing shape	Structure Formed
<1/3	Cone 	Spherical micelle 
1/3 – 1/2	Truncated cone 	Cylindrical micelle 
1/2 - 1	Truncated cone 	Flexible bilayers 
~1	Cylinder 	Planar bilayers 
>1	Inverted truncated cone 	Inverted micelles 

Fig. 4. Aggregate morphology as predicted by the dimensionless surfactant number, adapted from [26].

method to 'tune' a_o is through the use of ions (salts or pH) that can screen charge effects.

Gelbart and Ben-Shaul present a phenomenological theory as an alternative approach to examine the assembly of surfactant systems. This approach focuses its analysis on the curvature dependence of surface energies, resulting in the correlation of self-assembly to phase changes [28]. Polymers and oligomers offer another means to generate self-organized bio mimetic materials, typically where the substituent monomers are functionalized to direct assembly and interact with natural ligands. For polymers, Matsen and Bates

describe the prediction of self-assemblies from block copolymers as a competition between interfacial tension and an entropic penalty that occurs when polymer coils are stretched. This interpretation of forces that govern self-assembly results in parameters that are analogous to those considered in surfactant number theory, such that self-consistent field theory predicts transition of polymeric amphiphiles to go from lamellar, perforated lamellar, gyroid, diamond, cylindrical, spherical to disordered assemblies (L-PL-G-D-C-S-disordered) with increasing <H>, area averaged mean curvature. Fig. 5 shows the changing phases [29].

Understanding these principles is crucial to the rational design of molecules that are biomimetic and capable of self-assembly. A perspective of the physical parameters that governs why molecules assemble, and under what condition they will disassemble will give insights into the application of these structures to drug delivery. This perspective is actively applied in the following section, which describes the chemistry associated with synthesizing biocompatible assemblies whose interface can be conjugated with specific binding molecules.

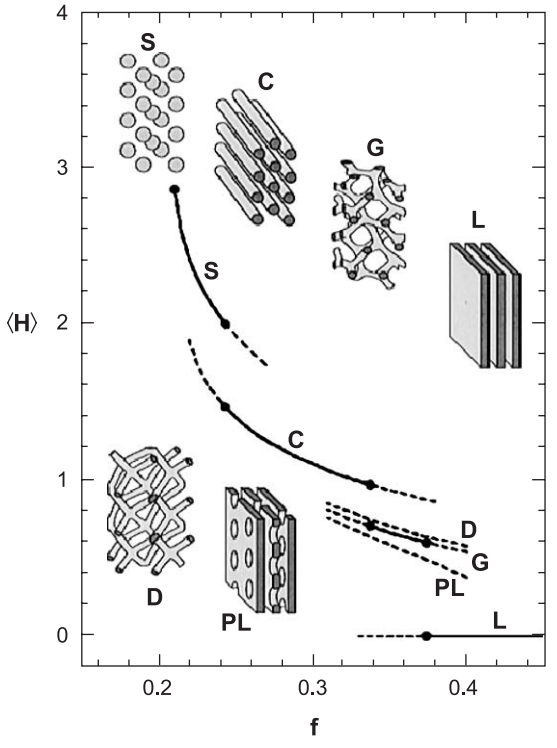


Fig. 5. Matsen and Bates, phase diagram showing the changing assembled morphology with change in <H>, area averaged mean curvature as a function of the volume fraction, f, of a particular block [29].

3. Chemistry and assembly

Nature uses 20 monomeric units (amino acids) to create molecules that possess an elaborate diversity of structure and function, from catalysts to fluorophores. The cornerstone of this monumental variety is sequence control. Natural systems are the archetype for precisely controlled synthesis. In order to mimic natures ability to self assemble molecules with highly specific function, experimental chemists must develop chemistry that is equally regular in sequence. This sort of highly control synthesis is the subject of several reviews [30,31]. These reviews stress the interdisciplinary nature of the field, where biologists, chemists, physicists, and engineers contribute complimentary skill sets to create more effective novel molecules.

There is a growing supply of innovative chemistry that provides the building blocks for biomimetic assemblies. The synthesis of these molecules can be divided into several classes of molecules from short oligomeric synthetic molecules, peptides, and sugars to large macromolecular assemblies that can be subsequently crosslinked to form drug carriers with controlled release properties. Peptides can be synthesized on a solid resin or by genetically engineering bacteria to give precise monodisperse sequences, which include unusual architectures (branching and rings) or unnatural amino acids. Polymer chemistry allows us to create assemblies that have much greater length scales and are crosslinkable to be stable over longer time periods. The initial generations of biomedical polymers has been to synthesized with biodegradable and biocompatible properties, but now chemists are devising ways to transform these inert macromolecules into specifically interacting biomimetic assemblies [32,33].

3.1. Solid phase peptide chemistry

The use of peptide sequences derived from the biologically active fragment of their protein complements is a simple way to mimic binding sites on a

cellular interface. Conjugating these sequences to drug delivery vehicles provides a mechanism to target particular cell types in a biomimetic manner. Alternatively, the peptides themselves can be capable of self-assembly into supramolecular architectures that contain some type of targeting element to direct delivery. Several (surprisingly) short sequences have been studied that can be correlated to the binding of cellular membranes: GRGDSP [34], REDV and LDV [35] in fibronectin YIGSR and IKVAV in laminin [36] and DGEA in collagen I [37]. The conjugation of these sequences to self-assemblies requires rigorous purity and sequence control of synthetic products to elicit specific biological response.

Merrifield developed the methodology for extremely precise synthesis on a solid matrix where an active Ribonucease A enzyme composed of 124 amino acids could be generated [38]. This methodology known as solid phase peptide synthesis simultaneously offers the requisite sequence fidelity by adding amino acids one-by-one to a solid support with a high yield for each independent step (>99%), see Fig. 6 [39]. Peptide synthesis has advanced significantly since the synthesis of Ribonuclease A such that long peptide sequences are produced in an automated fashion with minimal error. Merrifield originally used the Boc group as the protecting group as it was the most common for solution synthesis at the time, but it was found that repeated treatments with trifluoroacetic acid to remove the Boc group in the deprotection step is can be harmful to the growing peptide. Therefore peptide chemists currently use a base-labile protecting group, Fmoc, which is reviewed in detail by Fields and Noble [40]. This synthetic scheme provides facile design and control over sequence and can be varied to give novel architectures and constituent molecules that self-assemble, including non-linear sequences[41], alkylated sequences [42], and atypical amino acids [43].

Solid phase synthesis of other biologically relevant molecules, such as nucleotides and saccharides has also become more common. Oligonucleotides can be protected with allyl and (allyloxy)carbonyl groups, preventing side reactions between internucleotide bonds and nucleoside bases [44]. Recently, Plante et al developed a scheme for the automated synthesis of oligosaccharides, where the individual hydroxyl groups are selectively protected. Using a solid phase support and glycosyl phosphates, they were able to synthesize a branched hexasaccharide [45]. These highly controlled synthetic methodologies allow for the construction of molecules that are designed to self-assemble. Solid phase chemistry in general provides the capability to program into the molecular architecture signals that direct the morphology of the aggregate while using specific biological oligomers.

3.2. Non-biological oligomers

Mimicking protein structure and function with designed synthetic molecules can be a challenge; a tactic for the creation biomimetic self-assemblies is the use of ‘template-assembled’ molecules. This strategy circumvents designing a protein-mimic de novo by using well-known molecular assemblies that are simple and strongly energetically favorable with minimal folding requirements [46]. This can be accomplished by using typical peptide folding motifs like four helix bundles or synthetic molecules that are designed to have a strongly folded structure.

Synthetic self-assemblies carry the same design parameters described for peptide based assemblies, but the non-biological nature of this class of molecule gives more freedom to examine a variety of chemistries, resulting in changes in folding and assembly. The Moore and Wolynes group set out to design and synthesize a class of non-biological molecules that can fold and assemble into supramolecular structures. These assemblies should mimic proteins with synthetic molecules and respond to the environment in a predictable and controllable fashion. The set of intermolecular forces associated with the assembly of supramolecular structures is analogous to those forces involved in the folding of proteins (one might even say mimetic of these forces). Fig. 7 shows the phenyl acetylene molecule and its fold [47].

Zhang et al. describes a synthesis and purification scheme that allows one to control accurately the formation of short phenyl acetylene sequences. These ‘non-biological’ molecular sequences possess the ability to self-organize intramolecularly into helices [47] and intermolecularly into columnar aggregates [48]. The chemistry provides the flexibility to synthesize molecular building blocks with tailored assembly and binding properties. The monomeric units are trimethylsilyl derivatives that can be (cross)-coupled to-

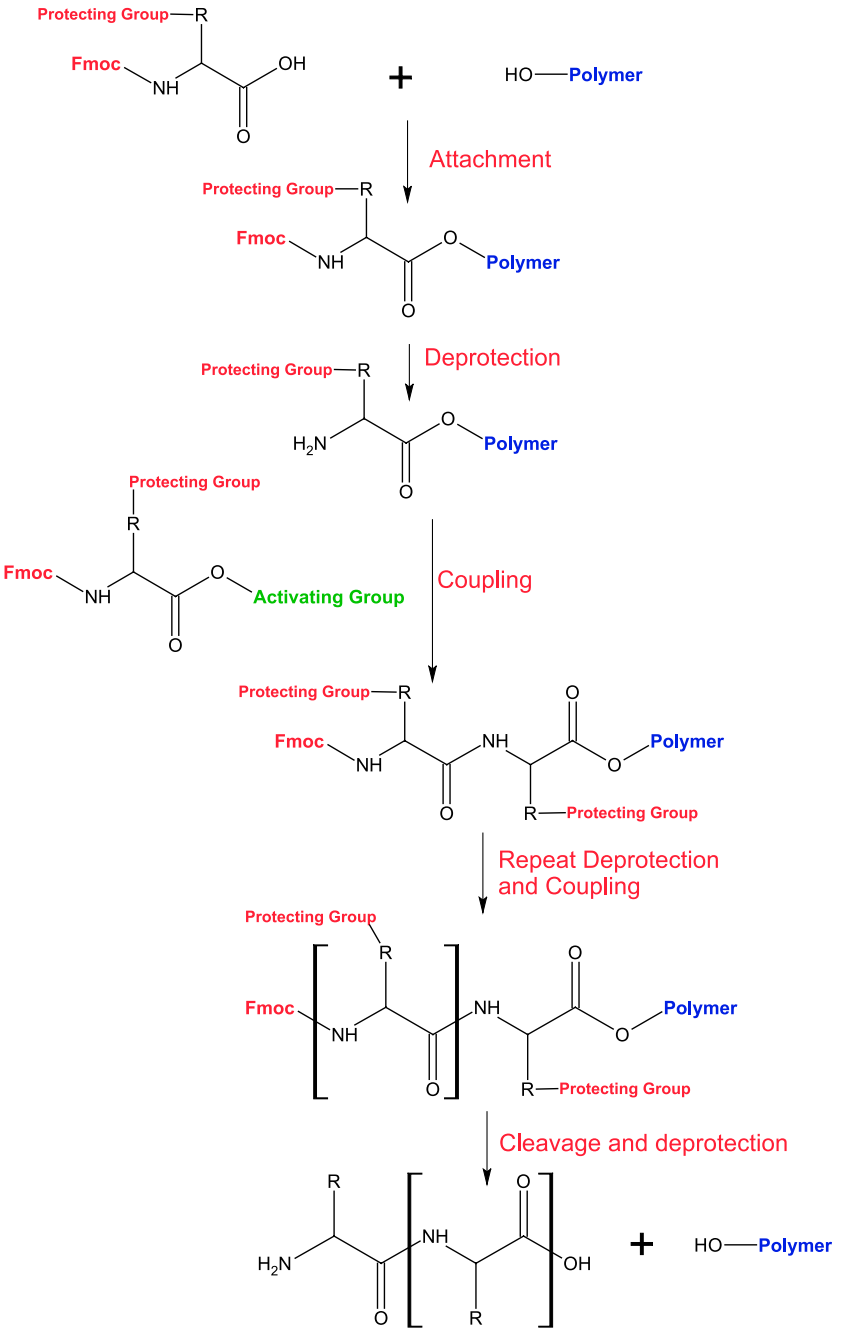


Fig. 6. Solid phase peptide synthesis.

gether using a palladium catalyst to form oligomers with 2^n units, where n is the number of reaction cycles. Other sequence lengths can be formed by combining the cycles (ex. reacting a tetramer with a dimer). Cyclization is then accomplished by using the Pd catalyst in an oxygen free environment under semi-

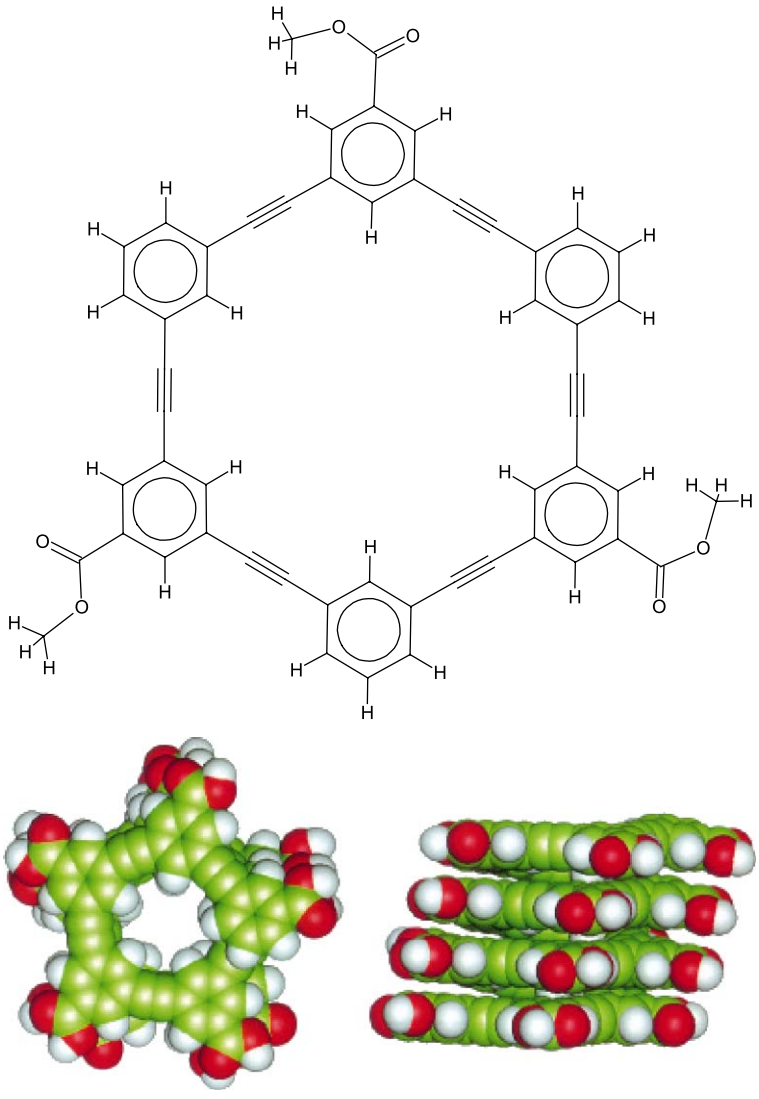


Fig. 7. Shows the polymerization scheme to form the phenylacetylene oligomers and cyclic structures [47].

dilute conditions after the phenyl acetylene oligomer is at its desired length [9].

These oligomers can fold and unfold (much like a peptide) with changes in the solvent and temperature. Also, the $\Delta G=(G_{\text{helix}}-G_{\text{coil}})$ for the formation helices can be calculated giving a critical oligomeric length for the formation of secondary structure, therefore assembly. Knowing these thermodynamic parameters allows for the design of molecules that fold and unfold in response to their environment [47]. Using this chemistry Shetty et al. describes the assembly of these macrocycles into stacks, where cyclic phenylacetylene hexamers can form offset face-to-face rods, see Fig. 7. NMR experiments suggest an aromatic stacking of oligomers into a barrel-like architecture that are interlocked by a pi stacking interaction [48]. This non-biological construct creates a binding cavity that can be chemically modified to bind biological ligands or drug molecules. One can image a system based on these synthetic assemblies that associate and dissociate in an environment dependent fashion where the exterior is functionalized with biologically specific

ligands and the interior is designed to carry particular drug molecules.

Several non-biological molecules that are analogous to peptides have been synthesized; these molecules have been shown to bind cellular ligands and assemble similarly to proteins. Oligocarbamates (see Fig. 8a) have ethylene backbones with a carbamate linker between residues. The α-carbon contains a side chain similar to peptides, but the β-carbon is unsubstituted. Synthesis of this molecule is accomplished on a solid phase resin from N-protected *p*-nitrophenyl carbonate monomers, where the methodology is analogous to solid phase peptide peptide synthesis. The chemical composition of these mimics results in a greater hydrophobilicity than their peptide complements, which may

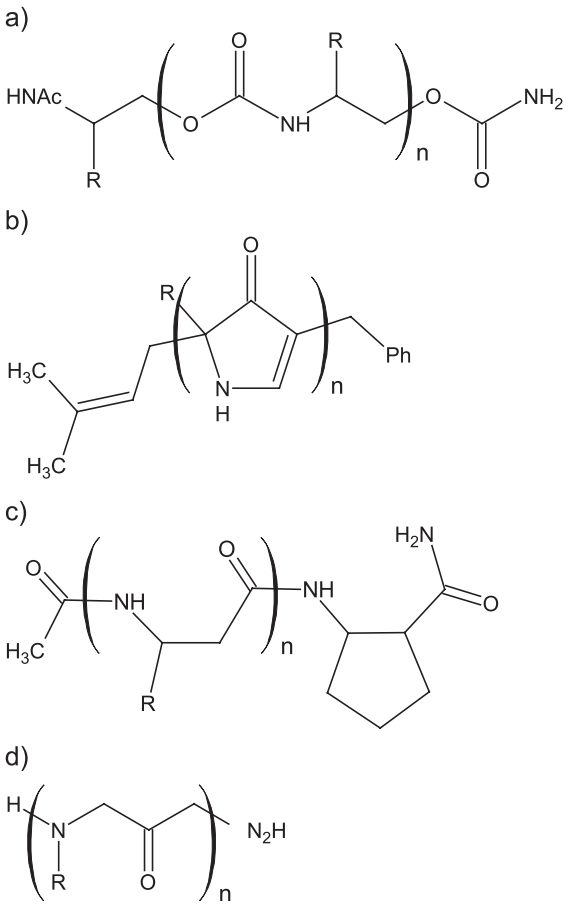


Fig. 8. Non-biological oligomers: (a) oligocarbamates, (b) oligopyrrolinones, (c) β-peptides, and (d) peptoids.

be used in the design of supramolecular assemblies [49]. Oligopyrrolinone (Fig. 8b) molecules with 'peptidomimetic' side chains can be synthesized via solution phase [50] or solid phase chemistry [51]. These oligomeric structures exhibit β-sheet analogous intermolecular assemblies as well as β-strand and β-turn-like intramolecular structure [52]. This class of molecule is a particularly interesting self-assembling peptide mimetic because it has been shown to be a potent HIV-1 protease inhibitor [53]. The synthesis of β-peptides (Fig. 8c) can also be accomplished via solid phase synthesis of Fmoc protected pyrrolidine based β-amino acids [54]. β-Peptides are also capable of folding, where the non-biological oligomer has been dubbed a 'foldamer' because of its ability to form sheet structures [55] and robust helical structures. Moreover, these helical conformations can be rationally altered to fold into a 12-helix or a 14-helix by adjusting the hydrogen bonding of the helix [56]. The additional flexibility in the β-peptide backbone provides the ability to design atypical conformations that can subsequently self-assemble to encapsulate therapeutics. Peptoid oligomers are comprised of N substituted glycine-like amino acids (Fig. 8d). These non-biological molecules are synthesized on a solid resin with Fmoc protected peptoid monomers. In contrast to previously described peptide mimics, the peptoid oligomer has no chirality or hydrogen bond donors on its backbone. Instead the side chain plays the dominant role in the formation of stable helical structures that fold and unfold cooperatively [57].

3.3. Polymer chemistry

Several reviews have been written that describe the use of polymeric molecules that can self-assemble into aggregates, which is analogous to the assembly of smaller amphiphiles, where macromolecules aggregate to form micelles [58] and rod-like micelles [59], driven by the differential solubility of the blocks [60]. These molecules can be subsequently conjugated with biological molecules to engender a specific binding character [61,60,31]. This class of biomimetic is perhaps the most quickly growing functional assembly, because of their implications for drug delivery. Analogies between bioconjugated block copolymer assemblies and viral architectures are commonly drawn: both possess self-assembled shells, similar

length scales, and cell specific targeting [62]. Drug molecules can be transported within stable polymeric assemblies to particular cell types, while their cargo can be slowly released in a controlled fashion, but the design and synthesis of a molecular building block that can be realistically applied to deliver drugs must take several factors into account: biocompatibility and toxicity, micellar stability, residence time in vivo, self-assembled morphology, drug loading and release [63,64].

There is a vast array of chemistry from which to construct these 'pilot-molecules' resulting in biocompatible and biodegradable polymers labile for conjugation to elements that are selective for target cells [27]. The chemistry described here reviews the synthesis polymeric assemblies that use molecules derived from biology or have some moiety that is biomimetic. One strategy is to synthesize the block-co-polymer (block-co-polypeptides) using amino-acid monomers, giving protein like secondary structure with longer length scales than typical proteins or peptides. Another strategy is to bioconjugate the water soluble interface of block-co-polymers with molecules that interact specifically with biological ligands.

Deming describes the synthesis of block-copolypeptides from α-aminoacid-*N*-carboxyanhydride monomers with an organonickel initiator. This synthetic scheme allows the formation of amino acid sequences far greater than solid phase peptide synthesis, resulting in molecular weights that are >100,000 g/mol. The polymerization mechanism is a living polymerization that gives a monomodal distribution with a low polydispersity index (1.05–1.15) [65,66]. These well-defined block copolypeptides formed via NCA monomers are capable of self-assembly and are inherently biologically compatible. The macromolecular structure and their potential application for drug delivery are discussed later.

The ability to conjugate polymers with sugars, peptides and proteins allows the formation of biomimetic assemblies. The formation of a biomimetic interface requires the polymer to have an asymmetric end-functionalization and, concurrently, a non-toxic linker to attach the biological molecule. Using synthetic molecules gives a chemist versatility in the preparation supramolecular structures, while several new chemistries allow for control over molecular weights and the selection of molecules that can respond to their environment. Hybridized assembling building blocks call for the careful selection of at least three parts (a tripartite structure): the polymer, a linker and a bioactive molecule. The rational selection of these three components is the subject of a review by Duncan [61], which describes several interesting strategies that effectively conjugate polymers with biologically active molecules.

The first element of this tripartite structure, the polymer, must be synthesized in a manner that is well controlled and biocompatible. For this purpose living polymerization has become a popular chemistry for those who are interested in constructing the biopolymers because there is a high degree of control over molecular weight, sequence (ability to form blocks), and end functionalization. Groups motivated to develop synthetic macromolecules that can emulate the structural complexity of natural systems require this level of control [12,67].

The chemistry for one such bioconjugated structure, which has been termed shell-crosslinked knedel like nanoparticles, SCK, has evolved based on a combination of interesting chemistry [68]. The first step involves the formation of a block-copolymer with a low polydispersity via anionic 'living' polymerization (other work accomplishes this with free radical 'living' polymerization [69]). Subsequently, the blocks are allowed to self-assemble at concentrations where spherical micelles are preferred, above the CMC, but below the concentration where intermicellar forces cause the formation of rod-like micelles [70]. Finally, the shell of these assemblies is crosslinked to various degrees using condensation [68] or free radical [71] chemistry. The resulting assembly has a hydrogel-like shell and polymer micelle architecture.

The next step in the evolution of these building blocks is to conjugate them with a biological functionality such that they are targeted towards particular cell types. Using block co-polymers to assemble complex nanostructured materials the Wooley group has developed a chemical methodology to functionalize block co-polymers with peptides, specifically the protein transduction domain (PTD) peptide from a HIV-1 TAT protein involved in cell binding. These bioconjugates can be realized by combining two reaction strategies that give highly controlled products (low polydispersity): solid phase peptide synthesis [40] and living polymerization [69]. The conjugation process

starts with forming a shell-crosslinked polymer micelle as described above where the hydrophilic block is functionalized with a carboxylic acid end group. These SCK particle is then attach to the N-terminus of the peptide sequence on a solid phase resin and subsequently cleaved from the resin [72]. A second method for bioconjugation carries out the entire synthesis on the solid resin, where a peptide is generated one amino acid at a time via condensation chemistry (Section 3.2), and the block-co-polymer synthesis occurs from the solid support via living free radical polymerization. This is accomplished by functionalizing the peptide N-terminus with an alkoxyamine to act as an initiator for polymerization from the solid support. The entire PTD functionalized block copolymer can then be cleaved from the resin, and the fidelity of these molecules are verified by NMR, showing the presence of peptide covalently linked to polymer [73]. Fig. 9 shows the synthesis scheme for the bioconjugated polymer that can function as a building block in micellar assemblies for drug delivery.

A family of chemistry exists around a particular molecule that has become ubiquitous for biomaterials synthesis. Poly(ethyleneglycol) (PEG) is perhaps the most commonly incorporated polymer for biological interfaces. This molecule is a flexible water soluble shell-forming segment that is has been shown to be biologically compatible. The inclusion of the PEG group creates a steric repulsion at the corona of the nanosphere that gives the assembly a ‘stealth’ character, such that proteins cannot adsorb to the interface. It has been shown that biomaterials functionalized with PEG become non-thrombogenic, preventing the aggregation of blood factors that cause vascular obstruction. The resulting inertness dramatically increases the circulation time of self-assemblies in vivo by avoiding the reticuloendothelial system (RES), which acts as the host defense system to remove drug delivery vehicles [74]. Additionally, increasing the residence time capitalizes on a passive targeting phenomenon known as the ‘enhanced permeability and retention effect’ (EPR), where the tumor vasculature is hyperpermeability to circulating macromolecules and the accumulation of assemblies is enhanced by poor tumor drainage [75].

There has been a call for the development of novel synthesis routes giving end-functionalized block-copolymers that include PEG as the water soluble moiety, resulting in an asymmetrically functionalized PEG-block with low polydispersity. The asymmetrically end-functionalized (heterobifunctional or heterotelechelic) PEG is synthesized via ring opening anionic polymerization of ethylene oxide with a 3,3-diethoxypropanolate initiator. The resulting polymer can then be end functionalized with a variety of components, in this case, aldehyde for protein conjugation and methacryloyl for grafting to other polymers [62]. Other end-functionalizations include hydroxyl, primary amino [76], acetal and mercapto [77] while the adjacent block polymer can include polylactides as biodegradable/non-toxic cores [78] and poly(ethylenimine) for DNA delivery in polyion complex micelles [77]. Several chemistries have been developed to copolymerize the PEG block with other polymer blocks and are reviewed by Otsuka et al. [58].

Taking advantage of the heterobifunctional PEG was accomplished by Yamamoto et al. [79], where

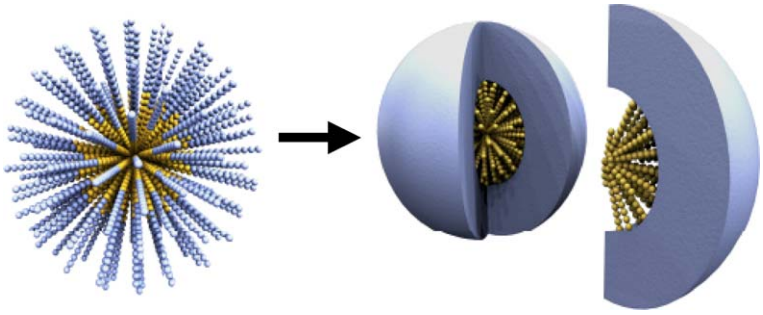


Fig. 9. Self-assembly and polymerization of a block copolymer yields a Shell-crosslinked knedel polymer assemblies, where the crosslinked shell can be decorated with biologically relevant ligands [68,71,72].

peptides were covalently linked to the aldehyde end group via schiff base formation and reductive amidation to control the surface charge. The resulting aggregate was characterized with dynamic light scattering and zeta-potential measurements to verify the formation of micelles and the correct peptidyl functionalization. Similar chemistry can be applied to attach sugar molecules to target glycoreceptors located on the plasma membrane. An example of this is the conjugation of PEG with glucose and galactose (specific for RCA-1), where the protected sugar is metalated and acts as the initiator for the PEG block and subsequently the PLA block [80]. Also, block-copolymers with poly(g-methylglutamate) cores and PEG shells can be synthesized that are conjugated to lactose, which is specific for RCA120 lectins [81].

Polymeric self-assembled materials are not limited to diblock copolymers, triblocks have been designed and assembled to form supramolecular nano-structures as well. A 'rodcoil' tri-block that contains a rod-like component with pi-stacking attractive forces covalently attached to a coil-moiety that is sterically repulsive has been shown to form mushroom shaped nano-structures. Short styrene and isoprene blocks, the coil moiety, were synthesize using living anionic polymerization where the average degree of polymerization was nine. Subsequently, biphenyl and/or phenylene vinylene rod-like molecules were added by etherification [82]. The diversity that synthetic chemistry provides for designing materials that can self-assemble is limitless. Understanding the physics of assembly and available chemistry should allow the development of assemblies with an equally diverse set of morphologies for drug delivery vehicles.

Chemists are motivated to develop synthetic macromolecules that can emulate the structural complexity of natural systems, but many important parameters are still unexplored, particularly the conditions that define the rate of release. The assembled morphology, molecular weight, chemical composition all effect the kinetics of release [83]. These parameters can be addressed with chemistries that offer a diverse set of building blocks for the bottom-up construction of self-assembled molecular architectures, but there is an infinite parameter space for variations in biomimetic molecules, where synthetic chemists provide the toolbox for the rational design of drug delivery vehicles.

4. Applications

Murphy et al. states that the future development of biomaterials will be made at the submicron length scale, which can be accessed by the design of self assembling systems that mimic biological structures [84]. Several examples exemplify how design and chemistry are successfully employed to create a multifunctional molecule that assembles predictably and can be used to interact with specific biological ligands. Constructing a set of molecules that aggregates into functional biomimetic architectures that can subsequently be applied to drug delivery systems is the goal of the work discussed below.

4.1. Peptides

Solid-phase peptide chemistry (described above) has established a routine synthetic protocol to create highly controlled short peptides, typically <100 amino acids. This class of molecule is an ideal building block for bottom-up assembly, providing versatility of monomeric units (natural and unnatural) and control over molecular architecture. Additionally, using peptides (as opposed to fully synthetic oligomers), allows one to borrow structural motifs from nature, which have been evolved over millions of years.

4.1.1. Peptide-based self-assemblies

Simple peptides can be synthesized with an amphiphilic character, where hydrophobic peptides such as alanine and leucine repeats are used as the 'tail' and charged amino acids such as glutamic acid or lysine are used as the hydrophilic head group. These amphiphiles will self-assemble as dictated by the parameters such as the surfactant number, but are often more difficult to precisely predict than simple surfactants as they include the added complexity of an inherent chirality and torsional degrees of freedom dictated by the side groups.

Vauthey et al. have synthesized a series of peptides with tails composed of six repeated hydrophobic residues (valine, alanine or leucine) and head groups consisting of one or two hydrophilic amino acids (aspartic acid). It was suggested that the surfactant number can be used to help predict the type of assembly, but no attempt was made to calculate this for the assemblies presented. Also, a variety of mor-

phologies were observed for L_6D_2, including rod-like micelles, vesicles, and hollow tubes. These structures also appeared to be dynamic through the course of the experiment. This paper illustrates how solid phase peptide chemistry can provide a method that gives accurate molecular control and flexibility, but the design of particular assembly types can be challenging. Vauthey suggests that controlling the formation of these nanotubes will provide a platform for developing encapsulation tools for drug delivery or biologically compatible tissue scaffolds [85].

Another peptide self-assembly was described in Section 2.1.3, where the Ghadiri group uses solid phase peptide chemistry to form a self-assembled structure from d- and l- amino acids showing β-helix secondary structure and possessing membrane pore forming characteristics. These ring-like peptide assemblies mimic the Tobacco mosaic virus in their tubular form, which are formed from helical nucleocapsid structures that are capable of delivering RNA to specific cell types [6]. The synthesis involves forming cyclic peptides, in this case octapeptides of cyclo[-(L-Gln-D-Ala-L-Glu-D-Ala)$_2$-], to stack together to form a 'nano-tube'. The design of the particular D-,L-cyclopeptide combines hydrogen-bonding with electrostatics such that these molecules are allowed to interact only if the pH was low, because the glutamic acid residues would be electrostatically repulsive in an alkaline environment [41]. These assemblies are powerful tools in that they have simple controls over their self-assembly and surface functionalization. The circular (and therefore the cylindrical) diameter can be dictated by varying the number of peptides participating the peptide monomers. While simultaneously controlling the exterior character of the cylinder by varying the amino acid side chains [86].

Granja et al. [87] shows this bottom-up design of biomimetic transmembrane channels can be employed with cyclic peptides, where hydrophilic molecules are selectively transported across a lipid bilayer (Fig. 10). A van der Waals pore diameter of less than nine angstroms is required to transport a glucose molecule across the membrane, and the transport channel should have a hydrophobic character at the exterior to promote the partitioning of the nanotube into the bilayer. To accomplish both these functions a 10 residue peptide was synthesized, cyclo[Gln-(D-Leu-Trp)4-D-Leu]. The results show that these assemblies do transport glucose out of unilamellar lipid vesicles, but the eight residue analogous peptide with a 7.5 Å internal diameter is not capable of to exporting glucose. Biomimetic cylindrical assemblies that facilitate the transport of molecules across the lipid bilayer may be of interest for drug delivery application, as well as other functions that may be designed, where control over the size of the assembly and presentation of side chains are valuable tools.

Another purely peptide built self-assembly is based on leucine zipper assembled fibers, where coiled-coil heterodimers are designed to assemble together. The information that drives the formation of longer and longer assemblies is programmed into the sequence by including 'sticky-ends' to direct the formation of a continuous strand. These structures were designed such that a heterodimer with the proper register would be favored. This is accomplished by capitalizing on the septet repeat sequence (abcdefg), where positions

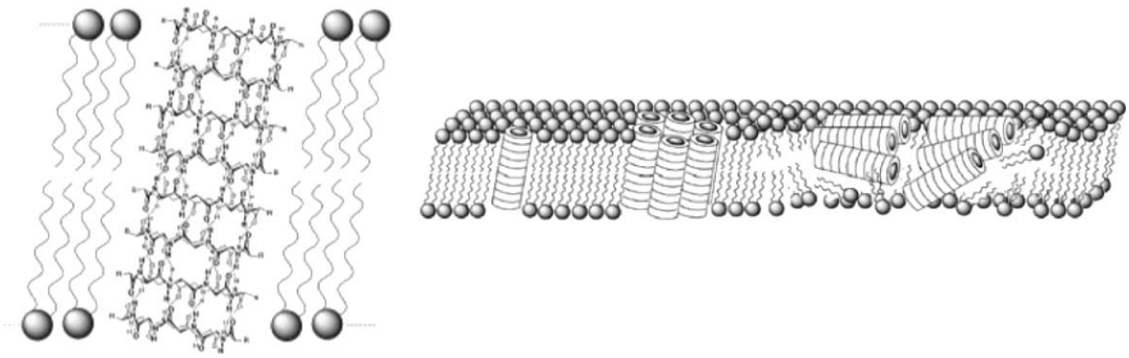


Fig. 10. Stack of cyclic D,L-peptides partitioning into lipid bilayer [25,86].

a and d are hydrophobic and positions e and g are polar and oppositely charged, this is similar to the matching that was introduced in Section 2.1.2. The sticky ends were generated by staggering the ends of the two 28-residue peptides, such that residues 1–14 of the first peptide preferentially interact with residue 15–28 of the second and vice-versa, see Fig. 11a. This design produced extremely long fibrillar structures that were thicker than anticipated (~ 43 nm), which was attributed to fiber–fiber interactions [88]. A variation on this strategy is to include fiber shaping peptides that are designed to produce kinking in the fibril formation. These fiber shaping peptides couple with the elongating fibers and create a discontinuity, by aligning the peptide fiber tail-to-tail or head-to-head, Fig. 11b. The fiber shaping peptides were shown to be capable of forming kinks and splitting along the leucine zipper backbone. The authors describe this type of assembly as having potential as biomaterials that can respond to cues by adding functional groups that can be controllably presented, scaffolds that are structurally similar to collagen or sensors for protein arrays and chips [89].

Another fibrous assembly is presented by Aggeli et al., where peptides are aligned in a latitudinal direction forming a stack of short anti-parallel beta-sheet structures to form fibrous 'nano-tape' [90]. The peptide was designed to minimize complexity, and it is suggested here that a minimum of six amino acids is required to have side chain interactions overcome the decrease in entropy on assembly of a β-sheet. An 11-residue peptide was eventually selected for three reasons (1) inter strand attraction, (2) exclusively

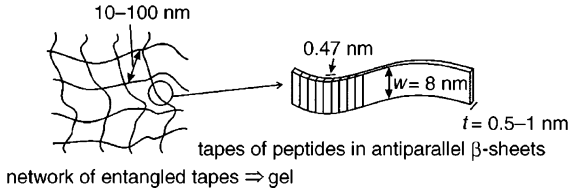


Fig. 12. From Aggeli, 1997. Formation of β-tape assembly. Gel networks of entangled rigid strands [90,91].

laterally bound assembly resulting in one dimensional elongation, and 3) solubility in water, Fig. 12 shows that assembled nano-tape. An array of intermolecular forces was included to engender the proper interstrand attraction, including hydrophobic, pi–pi interaction (Phe, Trp), and columbic (Arg, Glu). At low concentrations it was found that the designed peptide formed a gel with entangled networks (persistence length >13 nm), which is biocompatible and biodegradable. Electron micrographs of these entangled strands verified that the thickness of the tapes correlated to the length of the elongated peptide (~ 8 nm). The authors suggest that this type of peptide gel is mimetic of gelatin, actin, amylose and agarose. One can imagine forming an assembly of these peptides that gels and includes a functionality that is biologically specific. A small moiety for targeting in a peptide based gel that encapsulates a variety of hydrophilic or hydrophobic drugs, where the release kinetics are based on the persistence length, thus the degree of entanglement of the peptide tape [90]. Additionally, these assemblies can be designed such that the viscoelastic properties are controlled by changes in pH or shear [91].

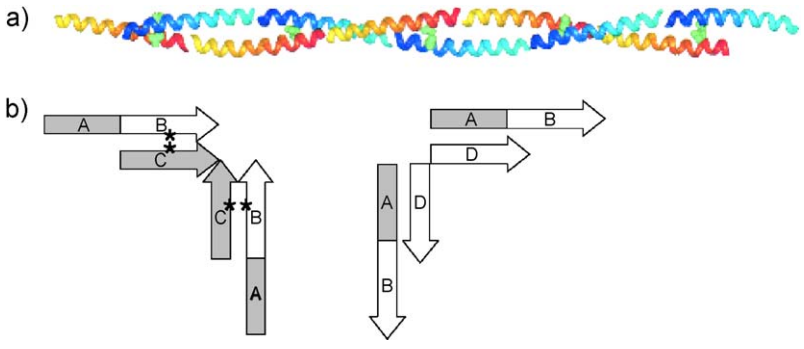


Fig. 11. (a) Design of a continuous leucine zipper fibril and (b) fiber shaping peptides that are able to 'kink' the assembly [89].

4.1.2. Peptide-amphiphiles

Nature uses post-translational modifications to covalently link non-amino acids to protein side chains. Resulting modifications, such as myristation and farnesylation allow proteins to partition into the plasma membrane. Synthetically mimicking this mechanism only requires an additional step in the solid phase synthetic procedure [42]; a scheme of this modification and the resulting molecule are shown in Fig. 13a.

The addition of alkyl tails to the N-terminus of the peptide results in a molecular conjugate structure that is referred to as a peptide amphiphile, which has been shown to self-assemble into micellar structures [10]. Interestingly, the formation of assemblies correlates with an enhancement in the secondary structure of the peptide head-group. The typical biological paradigm describes a system where specific function is derived from a unique assembled structure, but for peptide-amphiphiles self-assembly precedes secondary structure (and therefore function) to promote the formation of a protein-like aggregate.

The design goal of peptide alkylation is to non-covalently stabilize super-secondary, namely a triple helical structure, giving supramolecular architectures that mimic proteins. The target structure would be a trimeric assembly that can be verified using circular dichroism or NMR [92]. The protein that was chosen to emulate is the [IV-H1] peptide, GVKGDKGNPGWPGAP, which is derived from type IV collagen and has been shown to bind melanoma cells [93]. Also, included is a $[Gly\text{-}Pro\text{-}Hyp]_4$ sequence that exists to help nucleate the triple helical secondary structure [94,95]. Surfactant number theory can be invoked, where $Ns < 1/3$, as the peptide head groups are large, suggesting that the assemblies formed are micelles at several alkyl-tail lengths (Fig. 13b) [16].

Collagen-like peptides have been designed as described, and systematically functionalized with mono- and di-alkyl tails [96,97]. The synthetic lipidation of peptide molecules has been shown to control the assembly of peptides, giving 'well-defined' protein-like structures. The synthesis scheme of the molecules allows for the controlled variation of alkyl tail length such that one can correlate the thermal stability of protein-like molecular architectures with the propensity to self-assemble. CD and NMR results verify the changes in melting temperature with varying degrees of hydrophobicity, where the spectra of triple helices have a characteristic maximum at 225 nm, which degrades with increasing temperature as the peptide denatures allowing the estimation of the melting temperature. The $(GPP^*)4\text{-}IVH1\text{-}(GPP^*)_4$ peptide alone melts at 35.6 C, but the mono alkyl c6, c12 and c16 have melting temperatures of 42.2, 55.0 and 69.2, respectively. Analogous results were found with 1H NMR, where specific signals shift depending on their local orientation. This work hypothesizes that peptide amphiphiles provide a convenient template for developing stable molecular assemblies that possess specific biological activity, where the aggregate of amphiphiles resembles a viral envelope displaying peptides to target an encapsulated drug [97]. Other work suggests that the incorporation of alkylated collagen on liposomal assemblies promotes the targeting of the liver's Kupper cells [98], but this may be due to the natural removal mechanisms in vivo. Other work in vitro suggests that peptide amphiphiles that include the IVH1 sequence and dialkyl tails, which partition into favorably into liposomes, are capable of preferentially interaction with endothelial cell types.

Harterink et al. also uses peptide-amphiphiles in an attempt to engineer such a system to capture the complexities associated with hierarchical ordering of bone. These types of structures are particularly difficult to emulate because of the multiple length scales associated with the deposition of bone on soft tissue and the demand for particular crystal orientation of the inorganic phase, giving the proper mechanical properties.

A bottom-up strategy was employed to mimic the structural demands of bone growth, where the design of the peptide segment had to address engineering goals: robust in a cellular environment, bind to hydroxyapatite (HA), and, in parallel, bind to cells. These goals translated into a chimeric peptide with the following four elements: (1) four adjacent cystienes for crosslinking; (2) three glycine residues for flexibility and presentation; (3) a phosphoralated serine, which is known to bind to HA and (4) an RGD sequence, a common cell adhesion ligand from fibronectin.

Using a range of concentrations from 0.01 to 50 mg/ml, assemblies of these amphiphilic molecules offers a template for the deposition of HA in a highly ordered bottom-up manner. The authors also used the

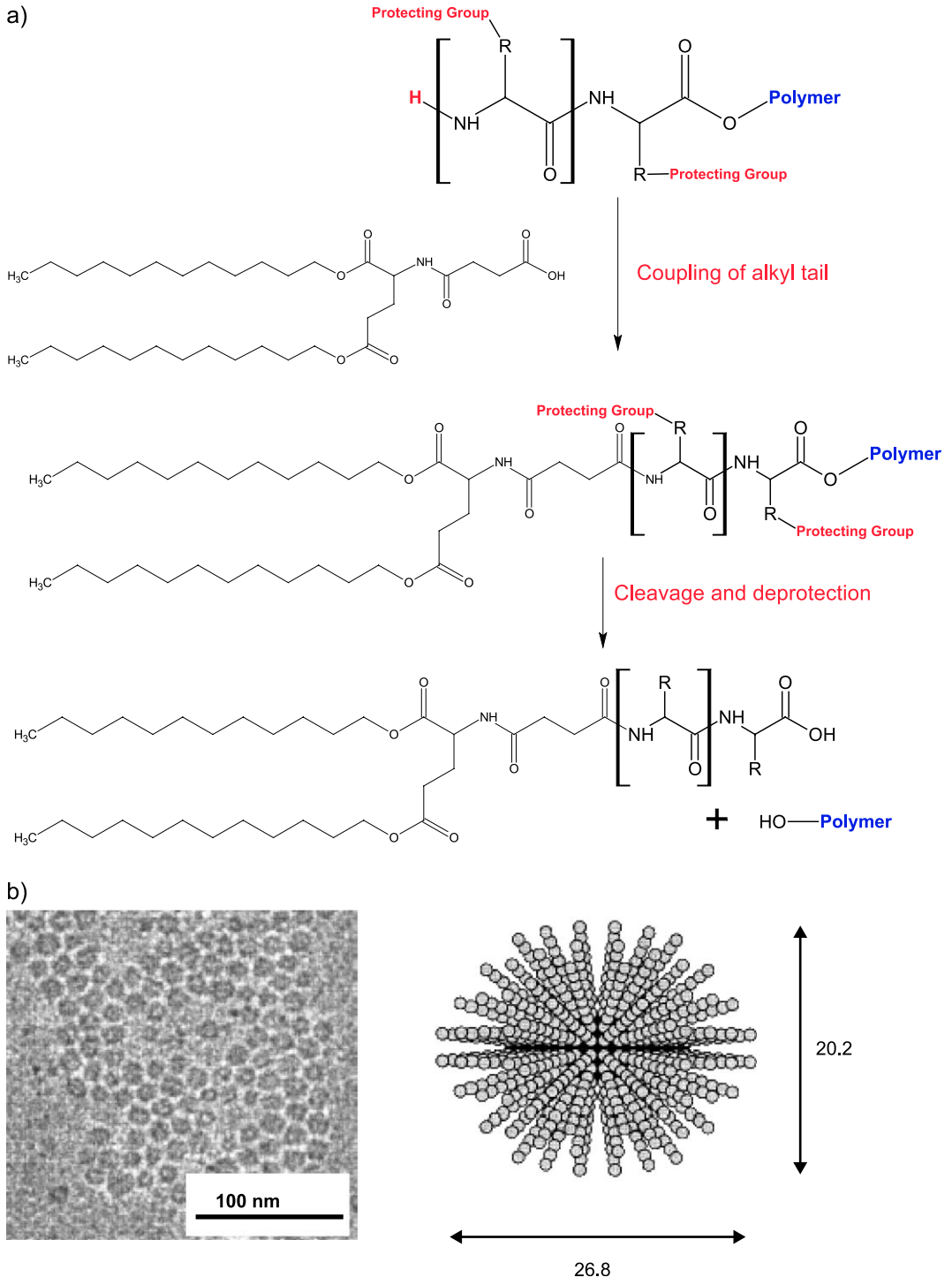


Fig. 13. Peptide amphiphile (a) synthesis and (b) assembly.

surfactant number to design and predict the formation of cylindrical micelles as the amphiphilic monomers were conical in shape. A 'second level of hierarchy' was seen with assembly, where both α -helix and β -sheet existed in the peptide, but secondary structure was not of primary importance to the biomimetic function in this case.

The resulting fibrous self-assembly was indeed observed to bind to HA in an oriented fashion along a crystallographic axis, whereas without the phosphoralated serine (only serine) the HA was amorphous. This example of bottom-up molecular scale engineering an amphiphilic molecule validates the strategy of creating a polyvalent assembly with a diversity of function [18]. It should be possible to incorporate a hydrophobic drug in an assembly that includes a peptide with several active sites such that the drug carrier could display a multiplicity of targets and functions.

Longer peptides and proteins can also be used to form self-assembled biomimetics, but synthetic constraints make genetically engineering these molecules a more practical method than solid phase peptide synthesis. Directly accessing biological machinery to make synthetically self-assembling proteins has two advantages: high fidelity of sequence and the use of preexisting evolved assemblies, which can be modified for drug delivery purposes.

Padilla et al. [99] uses genetic engineering to design polyhedra with hexagonal symmetry, where a fusion protein that is composed of an element that dimerizes and an element that trimerizes is coupled together, giving a nanohedral cage (Fig. 14). The design parameters call for oligomeric proteins with known three dimensional structures that are end functionalized with strongly α-helix forming sequences. These proteins can be genetically combined with a nine residue α-helical linker. Subsequently, molecular simulations can be employed to check for unwanted steric interactions. The fusion proteins are expressed in *E. coli* and purified in a variety of columns. Electron microscopy and light scattering are used to characterize the assembled structure where discrete particles with roughly spherical geometries are observed to be $\sim$ 7.5 nm, which is consistent with the proposed self-assembly. The protein-based cage-like assembly can be used to encapsulate and deliver drugs or genes, where subtle portions of the assembly's interface can be functionalized to target harmful cells. The resulting functionalization of the interface would be amplified because of the inherent polyvalence of the self-assembled nano-hedra. Yeates and Padilla also suggest that covalently linking drug molecules or therapeutic peptides could capitalize on the polyvalent character [100].

These short oligomeric molecules provide a useful framework for molecules that have the capability to self-assembly into larger structures that can potentially act as drug carriers. Using peptides as a building block provides the added advantage of biocompatibility while providing a molecule that is amenable to the inclusion of targeting fragments. Longer polymeric molecules can also function as building blocks for self-assemblies of greater length scales. Additionally, these macromolecular assemblies can be designed to be extremely stable in biological environment and inherently have a capacity to carry a large volume of therapeutic molecules.

4.2. Polymeric assemblies

The largest molecular building block that will be discussed in this review will be block-co-polymers. This class of molecule follows similar physical rules that govern the assembly of much smaller amphiphiles with greater time and length scales. These macromolecular assemblies can be grouped into two classes. First, self-assembling block co-polypeptides, that have an inherent biological character, and second, block-copolymers that are given biomimetic character by conjugated the interface with specific ligands. Additionally, several groups have investigated the influence of cross-linking the assembly after its formation for more effect delivery characteristics. The generic goals for the development of the next generation of polymeric assemblies is to engender site and time selectivity with an architecture that is both biomimetic and has stealth like characteristics [72].

Nowak et al. [8] employs the previously mentioned block copolypeptides to form hydrogels at very low polymer weight percent. The polymers used have two control parameters, the amino-acid included in the block and the molecular weight of that block. As an example $(\text{lysine})_{160}$-β-$(\text{leucine})_{40}$ is shown to form a gel at 0.4 wt.%, and the leucine block can adopt an α-helical conformation. The formation of a network

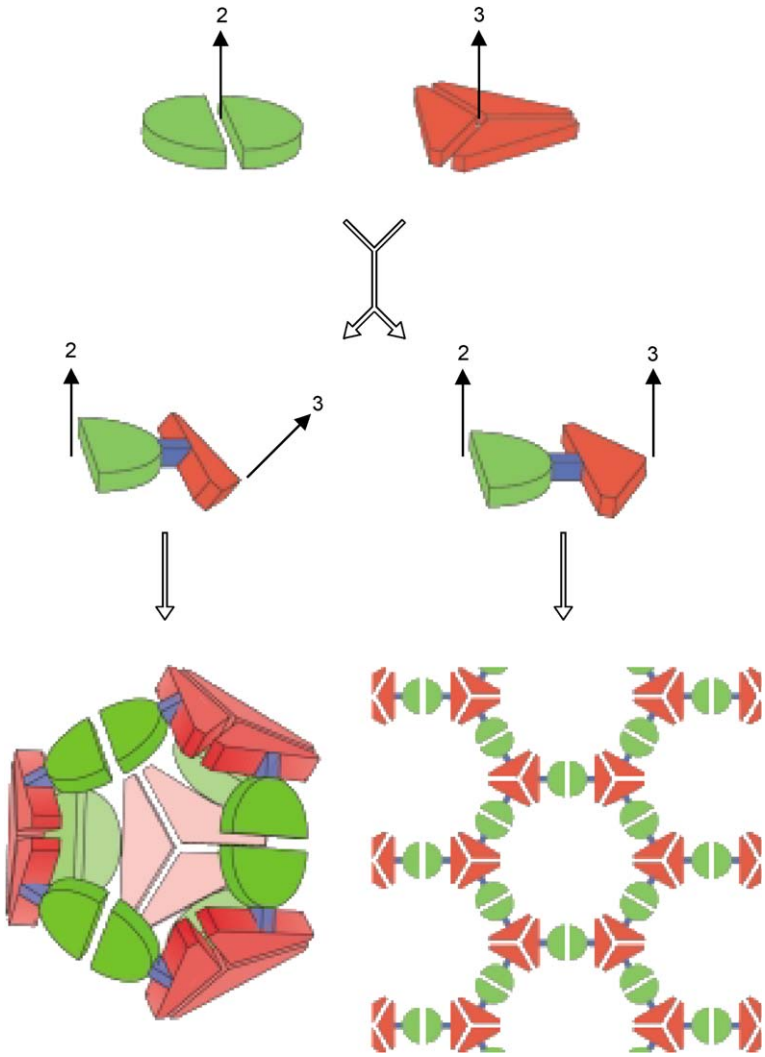


Fig. 14. Biologically synthesized nano-hedra [99].

actually depends on the secondary structure of the leucine block, evidenced by the lack of gel formation when a racemic, therefore unstructured, leucine block is used. Microscopy studies show that these gel structures have a homogenous pore structures due to the repulsion of the charged block. The large pore sizes coupled with the low weight fraction make this variety of polymeric self-assembly a potentially useful material for delivery of large hydrophilic drugs. One can also envision covalently functionalizing these assemblies with small biological ligands or even antibodies. Alternatively, one might attach therapeutics drugs directly to the gel forming polymeric self-assembly. Doxorubicin has been covalently linked to the hydrophobic block of a PEG-block-poly(aspartic acid) copolymer, resulting in increased drug accumulation at the tumor site and decreased non-specific distribution of the doxorubicin [27].

Rosler et al. suggests that there are three basic strategies to modify the structure of block copolymers to make them more effective drug delivery devices: crosslink the core, crosslink the shell or functionalized the surface with ligands that pilot or direct the assembly to specific sites [101]. The first type of

functionalization is exemplified in the giant worm-like micelles assembled from poly (ethyleneoxide)-poly(-butadiene) diblock copolymers, whose PB cores can be cross-linked freezing the aggregated morphology. Small angle neutron scattering and cryo-transmission electron microscopy are used image the formation of cylindrical assemblies and verify the unperturbed core-cross linked assemblies [59]. The formation of stabilized assembled molecules represents a useful strategy for building complex materials to encapsulate drug molecules, where shape, size and ordering can be designed into the aggregated structure by changing the parameters of the block copolymer.

The Wooley group has extensively researched the second strategy described by Rosler, functionalization of polymer assemblies by crosslinking the shell, producing shell-crosslinked knedel (SCK) nano-particles. These SCK building-blocks have been assembled from a variety of monomers including styrene, butadiene, e-capralactone, methyl methacrylate [101]. Again, the cross linking reinforces the assembly mechanically, altering the inherently fragile supramolecular assemblies to a more robust state. An added benefit of crosslinking SCK nano-particles is the hollow cage-like core coupled with a polymerized shell, resulting in a carrier with controllable permeability. The cross linked shell's physical characteristics are analogous to a permeable hydrogel coated nano-sphere. Variations in the choice of shell-monomer can result in added drug delivery characteristics, namely the use of positively charged monomers to condense DNA for gene delivery or use of monomers thermally responsive monomers that can load and release as a function of temperature change [60].

The third type of functionalization mentioned is bioconjugation, resulting in a polymeric assembly that has biomimetic characteristics. Liu et al. applies this chemistry to design a SCK surface to target specific cells. The nanocage is bioconjugated with a peptide from HIV-1 TAT protein (RRRQRRKKRGYGGGG), the four glycine repeats are attached closest to the polymer assembly to present the peptide flexibly away from the interface. The peptide functionalized polymer assembly is then used to selectively interact with CHO and HeLa cells. Fluorescence activated cell sorting showed that the bioconjugated assemblies promote binding to both cell types. The PTD nanocage bioconjugate represents the intersection of two strategies: controlled polymer assembly/crosslinking and bioconjugation for targeting, where the core of these polymeric assemblies can be used to package therapeutics from small molecules to large genes [72].

The chemistry and stealth benefits of using a poly (ethyleneglycol) water soluble block were described in a previous section. These molecules can be bioconjugated with either sugars or peptides, giving them a more biomimetic quality [58]. The authors of this work often refer to these assemblies as 'virus inspired vehicles.' It is shown that sugar–PEG–PLA core shell polymer is capable of binding lectin targets. Galactose and lactose conjugated assemblies were shown to be specific to RCA-1 lectin, and mannose sugars specifically bound to Con A lectin, where the lectins were immobilized on a column and an increased retention time was observed [102]. These polymer micelles can be further modified using heterobifunctional synthesis such that it forms a polyion that electrostatically interacts with DNA. This is accomplished by designing an α-aldehyde poly(ethyleneglycol)-block- poly(2-*N*,*N*-dimethylamino)ethyl methacrylate which can complex with DNA [103]. Work with heterobifunctional PEG chemistry has allowed the design of several biomimetic variations on multimolecular assemblies that can act as carriers for drugs.

Attempting to mimic the structure and function of viruses by using polymeric micelles is a goal that exemplifies the theme of this review. An 'ideal' drug carrier should emulate a system that is capable of residing in vivo for long periods of time, targeting particular cell types, compartmentalizing a large set of molecules, and releasing those molecules in the appropriate environment [63]. Nature accomplishes this phenomenal task with a small number of genes by capitalizing on bottom-up construction of self-assembled functional molecules.

5. Conclusions

Nature has evolved a variety of molecular assemblies that compartmentalize, transport and specifically bind cellular ligands. The imitation of these aggregated morphologies will require a fundamental understanding of the forces that direct assembly, the chemistry to synthesize complex building blocks with

biological function, and the application of the systems to biologically relevant problems. Developing bottom-up strategies that mimic cellular interfaces has several advantages that have been presented in this review, where the rational construction of biologically inspired analogues will be a valuable tool for drug delivery applications.

Creating a toolbox of biomimetic molecules that can self-assemble with biological precision on short length scales is a significant challenge. Several parallel efforts exist to develop hierarchical functional self-assemblies for other technologies, including materials with a photonic band-gap for optical properties and gigantic magnetoresistance for microelectronics [104]. The demands of these materials are similar to those described for drug design in that they require highly ordered structures that are spatially and chemically well controlled. Additionally, biomimetic self-assemblies require designs that carefully distill relevant biological signals, while simultaneously controlling nano-scale architecture [84]. This bottom-up approach provides a potential strategy to mimic biological systems, creating vehicles with features that make them very attractive for encapsulating and delivering drug molecules.

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