Extending the self-assembly of coiled-coil hybrids

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SUMMARY

Of the various biomolecular building blocks in use in nature, coiled-coil forming peptides are amongst those with the most potential as building blocks for the synthetic bottom-up self-assembly of nanostructures. There are many reasons for this: they have well defined size and surface functionality, the amino acid sequence can be designed to direct not just intra, but also highly specific intermolecular interactions, the binding energy of coiled coils is compatible with other self-assembly processes, and they can readily be conjugated with other molecular building blocks. The ease of working with and getting information about coiled coils has meant that they have been one of the most highly studied protein motifs. Coiled coils have been studied in isolation that form discrete coiled-coil complexes, and also that pack into larger assemblies. They have additionally been studied connected to water soluble blocks such that assemblies are constructed because of coiled-coil formation. However, one of the largest areas of potential has barely been delved into: native coiled coils are cogs from a great machine, and they clearly have the ability to function in and influence complex systems composed of multiple building blocks. The true potential of coiled-coil building blocks for synthetic chemists is only apparent when they are connected to other components, with each component contributing a function. Ideally a balance is struck between the different units such that all can play their role and novel functions emerge from their interactions. This thesis represents efforts at piecing together coiled coils with one or two other self-assembling components.

In Chapter 2 the coiled-coil unit that is to be used in the subsequent hybrids is investigated. The peptides, denoted E and K, are composed of three heptad repeats and form a parallel heterodimer in aqueous solution. The binding properties are determined using experimental and computational techniques, with the results from the two approaches supporting one another. The binding energy of the coiled-coil dimer was found to be ~ -11 kcal mol^{-1}. Using computational methods, details of binding are readily estimated. In the simulations the packing together of hydrophobic amino acid side chains in the coiled-coil core provided almost the entirety of the binding energy. On the contentious issue of whether salt-bridges exist and add to the binding energy, the simulations suggest that salt-bridges are not common, and that the charged side chains detract from the binding energy, but are necessary to impart specificity to the binding.

In Chapter 3 the first hybrid is constructed by coupling peptide E with a short polystyrene chain. The conjugation of E to the hydrophobic polystyrene provides a method to study orthogonal self-assembly. The self-assembly of PS_9-E is investigated on its own and together with peptide K and a hydrophilic hybrid K-PEG_{77}. Because E is water soluble it functions as the corona in the PS_9-E block copolymer, and tempers PS induced aggregation such that spherical micelles form. It is found that coiled-coil folding between E and K still occurs to a large extent when the peptides are conjugated with PS and/or

PEG, resulting in linear noncovalent di- and triblock copolymers. PS-E/K also forms spherical micelles, whereas PS-E/K-PEG undergoes hierarchical self-assembly into rod-like micelles. The coiled-coil linker in PS-E/K-PEG can be dissociated by raising the temperature. This reduction in the corona size causes a morphological change from rod-like to spherical micelles with a frozen core such that upon refolding of the coiled coil the micelles remain spherical. Thus, the coiled-coil forming block is utilized in three ways: one is for its hydrophilic character, it can act as a corona to control aggregation induced by the hydrophobic block; secondly, by forming a coiled coil the surface characteristics of the particles are modified and the range of hybrids that form organized aggregates can be extended; and finally the reversible nature of the coiled-coil connection infers additional environmental response to the nanostructures.

While Chapter 3 focused on the effect of the hydrophilic coiled-coil block on amphiphilic self-assembly, Chapter 4 extends the self-assembly of coiled-coil hybrids by varying the hydrophobic block. In order to create larger self-assembled structures, and to probe the limit of the hydrophobic block size, a series of poly(γ-benzyl L-glutamate)-E (PBLG-E) block copolymers were synthesized, and the self-assembly of the series in aqueous solution was investigated. PBLG was polymerized from E with average block length ranges from 36 monomers to 250 monomers. This approach lends itself to modular self-assembly, as both the hydrophobic block and the hydrophilic block/s can be varied. Up until 80 BLG monomers, peptide E was sufficient to induce well defined assembly of the amphiphile, while with longer PBLG blocks complexation of E with K or K-PEG was required for well-defined structural formation. Taking just 4 molecules from this small library – $PBLG_{36}$-E, $PBLG_{100}$-E, K, and K-PEG, polymersomes with a range of sizes, membrane thicknesses (ranging from 18 nm to at least 60 nm), and surface properties were accessible, as well as disk-like micelles.

The PBLG-E series represents the first of a new class of peptide: polypeptide-*b*-peptides, in which the flexibility of chain length of polypeptides is united with the flexibility of functionality of designed peptides. Peptide E was synthesized on solid support, and then PBLG was polymerized by ring-opening polymerization from the N-terminus of E while still on solid-support. The technique offers the advantage of very easy purification, as homopolymer, which is a scourge of NCA polymerizations, can be readily removed by washing the resin. Additionally, by using a relatively gentle cleavage mixture larger block copolymers are cleaved from the resin first, followed by those with shorter PBLG blocks, and finally peptide fragments, such that no further purification is necessary.

Many methods are required to produce polymer vesicles because for each method there is a limited range of vesicle properties that can be produced; not all block copolymers form vesicles with all methods; and additionally, in many instances some methods are not suitable due to the practical issue of availability of time or equipment, or requirements of the block copolymer itself or material to be incorporated. Chapters 5 and 6 investigate two new methods for producing vesicles from block copolymers. They take two methods that have been used for many decades to produce liposomes and that have particular advantages, and adapt them to make them suitable for block copolymers, which have different assembly characteristics than lipids. The detergent aided polymersome

preparation utilizes detergent molecules to molecularly disperse the block copolymer in aqueous solution, a role that is usually taken by organic solvents. The shielding effect of the detergent on the block copolymer is then reduced such that the intrinsic morphology of the block copolymer, i.e. polymersomes, emerges. This method has the advantage of not requiring organic solvent or a high energy input (e.g. sonication), hence will be of great utility when sensitive biomolecules are to be encapsulated in the polymersomes. The block copolymer used to demonstrate the technique, $PBLG_{36}$-E, has a much larger self-assembling energy than lipids do, and the method is explained with an emphasis on the adaptations necessary due to the properties specific to block copolymers. The other technique is termed water-addition solvent-evaporation, and is very rapid and simple. The block copolymer is dissolved in THF, the aqueous phase is added quickly, and the THF is evaporated. The parameters that drive aggregation and determine the sizes of the structures are very easily and quickly controlled: the solvent quality for the hydrophobic block is reduced to the desired level in less than two seconds, leading to microphase separation and structure formation; control of the interactions within the corona, which leads to precise tuning of the polymersome sizes, is also very straight forward, depending on the salt content in the water or the temperature at which the organic solvent is evaporated. Therefore, within a couple of minutes the polymersomes are formed, tuned, and stabilized, and retain their integrity for months. The same block copolymer, $PBLG_{50}$-K, could be made to assemble into polymersomes with a narrow size distribution, with the average size precisely adjusted between 200 nm and 2000 nm. The technique was demonstrated with charged peptidic block copolymers, non-covalent block copolymers, and traditional coil-coil block copolymers. This technique appears to be very practical in terms of time, equipment, the range of block copolymers for which it can be used, and the ability to tune the polymersome properties. Both techniques will open up possibilities in block copolymer vesicle research and applications.

Chapter 7 turns to a different type of hydrophobic block – lipids. The conjugation of coiled-coil peptides to lipids opens up new possibilities as the hybrids can be anchored into an orthogonal self-assembled assembly – lipid bilayers such as synthetic liposomes or cell membranes. The peptides E and K are utilized to create a minimal model for SNARE protein mediated membrane fusion. Hybrids are synthesized which mimic SNARE proteins – they contain the active, force producing coiled coils, a short flexible spacer, and a lipid tail to attach the hybrids onto liposomes. Not only do the hybrids mimic the structural components of SNARE proteins, but importantly they mimic the membrane fusion properties. Populations of liposomes are decorated with one each of the lipidated peptides and coiled-coil folding holds different liposomes in close proximity and induces the liposomes to fuse with lipid and content mixing and no leakage of the aqueous content. The fusion is not driven by membrane tension, and lipids with negative curvature restrict the fusion process. This is the first SNARE protein mediated membrane fusion model that meets all the key characteristics on native fusion, and is therefore a suitable analogue to study liposome fusion in vivo.

As the rest of this thesis hinted, the possible components, architectures, and properties of peptide-polymer block copolymers are multifarious. The conjugation of such disparate

blocks is not trivial; however the latest polymer-peptide hybrids exhibit excellent control over composition, structure, and functionality of the molecules. This is achieved through the careful combination of synthesis methods, tailored for particular classes of hybrids, as reviewed in Chapter 8.

PERSPECTIVES

Prior to using the coiled-coil dimer E/K to influence the self-assembly of coiled-coil hybrids, their properties as a unit were investigated. The experimental findings were backed up and elaborated upon by a molecular dynamics simulation. The next step to take would be to use the computational results to help design a coiled-coil unit that better suited a particular synthetic biology goal. At three heptad repeats the E/K unit is amongst the shortest heterodimeric coiled-coil unit that has been synthesized. Of course coiled-coil binding units of different lengths would have different effects on the systems into which they were incorporated. The design of peptides with three or more heptad repeats is routine, but heading in the other direction, at two heptad repeats only homodimers have been successfully designed and tested. Because the computational simulations give access to the binding contributions of each amino acid in a peptide it was reasoned that this may be able to be used as a tool to design a short heterodimeric coiled coil. In the simulations some residues were very destabilizing to the E/K dimer, particularly the negatively charged glutamic acid residues. These were exchanged for non-charged but polar amino acids. In this way a two heptad repeat peptide was produced that forms homo coiled coils, but as of yet the balance of destabilization of self-binding and heterodimer stability has not been achieved, and is an area of continuing research.

This thesis asked simple questions: can coiled coils function when covalently attached to large hydrophobic blocks? How large can the hydrophobic blocks be? Can coiled coils function when incorporated noncovalently with a supramolecular assembly? In answering these curiosities of fundamental science, coiled-coil hybrids were synthesized and their self-assembly investigated. As is often the case when new ground is explored, there were unexpected consequences, and some of the work from this thesis is already being developed for applications. PBLG-E and PBLG-K vesicles have a high potential as drug delivery devices as compounds can be loaded in the aqueous interior or in the hydrophobic membrane, and the nanocapsules themselves are very modular, with a large variation in membrane thickness and surface chemistry to choose from. These vesicles are currently being developed to deliver the influenza vaccine. The liposome fusion system is being adapted as a general drug delivery system, and is also being investigated for the delivery of fluorescent DNA markers to cells, to convey calcium carbonate to monolayers, to test *in situ* video atomic force microscopy, and to add a further orthogonal dimension by fusing liposomes which contain fiber-forming coiled-coil peptides.

This thesis uses one coiled-coil building block and a handful of other blocks to extend the self-assembly of coiled-coil units, assemblies, and systems; but naturally there are almost unlimited possibilities for functional systems involving coiled coils.