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Supramolecular Chemistry

Self-Assembled Multivalency: Dynamic Ligand Arrays for High-Affinity Binding

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molecular recognition · multivalency · non-covalent interactions · self-assembly · supramolecular chemistry

Multivalency is a powerful strategy for achieving high-affinity molecular recognition in biological systems. Recently, attention has begun to focus on using self-assembly rather than covalent scaffold synthesis to organize multiple ligands. This approach has a number of advantages, including ease of synthesis/assembly, tunability of nanostructure morphology and ligands, potential to incorporate multiple active units, and the responsive nature of self-assembly. We suggest that self-assembled multivalency is a strategy of fundamental importance in the design of synthetic nanosystems to intervene in biological pathways and has potential applications in nanomedicine.

1. Introduction

The concept of multivalency, or polyvalency, the simultaneous interaction of multiple binding groups on one molecule with the complementary receptors on another, is often used by biology to achieve high-affinity binding in very competitive aqueous environments, and in recent years has been extensively exploited by supramolecular chemists.[1] Nanoscale molecules with multiple ligands, such as dendrimers, have been extensively studied for their ability to achieve multivalent recognition.[2] In multivalent interactions between host and guest, the binding of the second ligand can be considered to be favored as a consequence of being "intramolecular" in nature. In essence, the effective concentration (or effective molarity)[3] of the second ligand is enhanced by virtue of it being directly connected to the first. As such, rigid multivalent arrays can, if well-organized, benefit from greater ligand pre-organisation and less entropic cost of binding, but flexible multivalent systems can also have advantages, such as a greater ability to optimize the individual interactions in enthalpic terms, and the ability to provide screening from the surrounding competitive medium.[4] The binding of the multivalent ligand to a receptor is often compared with the binding of multiple individual (monovalent ligands). The average free energy of interaction between a ligand and receptor in a multivalent system can either be greater than, equal to or less than the free energy in the analogous monovalent interaction. These classes of multivalent interaction are referred to as positively cooperative (synergistic), non-cooperative (additive), or negatively cooperative (interfering), respectively. Positively cooperative multivalent binding is very rare.[5] Multivalency does not, however, necessarily require positive cooperativity—the key aspect is whether the multivalent system has higher affinity for its target than the monovalent analogue. Whitesides and co-workers defined a binding enhancement factor, a ratio of multivalent to monovalent binding, to capture this enhancement in overall affinity.[1a] Given the importance of multivalency in biological systems, and the ability of multivalent interactions to adhere synthetic nanoscale surfaces to one another, it has become a primary tool in the armoury of chemists working in the fields of biomolecular recognition and nanotechnology.

Self-assembly, the spontaneous association of molecules into well-defined structures held together by non-covalent interactions,[6] is elegantly demonstrated in Nature by the tobacco mosaic virus; composed of over 2000 identical amino acid based subunits spontaneously arranged in a helical formation around a single strand of RNA.[7] There are many advantages to employing self-assembly as a tool to generate nanoscale structures. Constructing large molecules held together by covalent bonds often requires a considerable number of synthetic steps whereas self-assembly often only requires smaller building blocks which aggregate spontaneously as a consequence of their carefully designed molecular structures. Interestingly, biological systems frequently use self-assembly as a strategy to organize molecular recognition events—in particular in membranes, where receptors or

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ligands can cluster together to spontaneously generate a multivalent region.[8]

There are numerous examples reporting molecules which are capable of self-assembly and that bind to large biological targets.[9] For example amphiphilic cationic lipids which aggregate in aqueous solution are ubiquitous in DNA binding and gene delivery,[10] such systems can be considered to mimic certain aspects of viruses in terms of their self-assembly and genetic cargo.[11] There are also a number of examples in which peptides have been incorporated into self-assembled materials in order to enhance their adhesion to biological targets and hence mimic extracellular matrices—such work from the groups of Stupp, Ulijn and others has potential exciting applications in the field of tissue engineering.[12] In the majority of examples, however, it is unclear whether self-assembly enhances the binding interaction (and if so by how much), or whether it is primarily being used as a materials formulation tool. In very recent years, it has been realized that spontaneous self-assembly is a powerful and general tool for generating synthetic multivalent nanoscale binding arrays with biomolecular applications.[13] There are a number of key advantages of taking a self-assembly approach to multivalency (Figure 1), including:

1) spontaneous assembly (easy to make),
2) well-defined low-molecular-weight building blocks (relatively straightforward for clinical approval),

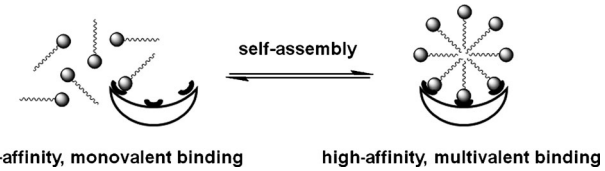

Figure 1. Self-assembly can enable high-affinity multivalent binding.

3) easily tunable ligands (can bind diverse partners),
4) tunable nanostructure morphologies (can modify binding),
5) ability to assemble different active components into a single nanostructure (leading to synergistic effects),
6) simple or triggered disassembly/degradation (can switch off multivalency and/or limit toxicity).

This Minireview will focus on recent key selected examples which demonstrate the power of solution-phase self-assembly to produce systems with significantly enhanced multivalent binding compared to their monovalent non-assembled counterparts.

2. Protein Binding

2.1. Carbohydrate Ligands for Lectin Binding

The wide variety of structural variation available in carbohydrates allows them to control cell signaling and many cell–cell interaction processes, however, the difficulty of binding neutral sugars in a hydrogen-bonding medium like water means that biology makes extensive use of sugar clustering in order to maximize binding affinity.[14] As a consequence, a large amount of attention in multivalent interactions has been directed towards exploiting carbohydrate–protein interactions.[15] More recently, many groups have begun to utilize self-assembly as a simple means to generate multivalent carbohydrate ligands.

In a very early example, Whitesides and co-workers conjugated sialic acid (SA) to a lipid chain and incorporated it into a phosphatidylcholine and cholesterol-based liposome (Figure 2).[16] The resulting SA-coated liposomes were found to bind to the protein hemagglutinin up to 10^4 times better than the equivalent monovalent system—a very significant

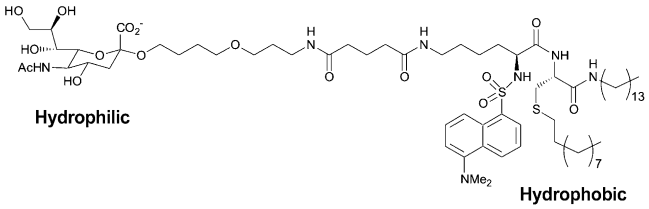

Figure 2. Structure of lipid-modified sialic acid derivative developed by Whitesides and co-workers and self-assembled in liposomes to achieve multivalent recognition of hemagglutinin.[16]

binding enhancement factor indicating effective multivalency was achieved by self-assembly of the sialic acid ligands within the liposomal nanostructure. A similar approach using lipid-functionalized sialic acid was also exploited by Kanie, Wong and co-workers, who are interested in the application of such self-assembled multivalent systems as inhibitors of viral adhesion to cell surfaces.[17]

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Anna Barnard studied Chemistry at Durham University, and is currently a PhD student in the group of Professor David K. Smith at the University of York. Her research interests include developing self-assembling dendritic ligands for gene delivery and heparin binding. In 2011, she was awarded the Bronze medal for Chemistry at "SET for Britain" at the Houses of Parliament and the Kathleen Mary Stott Prize at the University of York.

Thoma and co-workers synthesized a series of novel carbohydrate-functionalized dendrons which were found to assemble into nanoscale particles as a consequence of π-stacking and solvophobic effects in the highly aromatic core.[18] The ability of these dendrons to inhibit immunoglobulin binding was then investigated in vitro for the inhibition of anti-αGal antibodies which are responsible for the hyperacute rejection of pig organs transplanted into primates. The dendrons capable of forming large nanoparticles were the most effective inhibitors. Evidence for the involvement of self-assembled multivalency as opposed to simple covalent dendritic multivalency came from the observation that a dendron without the potential to self-assemble was inactive. The most potent system was tested in an animal model system and shown to completely eliminate anti-αGal antibody-mediated hemolysis.

In an eye-catching recent example, Brunsveld and co-workers employed a supramolecular polymer as a self-assembling scaffold for polyvalent binding (Figure 3).[19] They designed a mannose-functionalized discotic compound which assembles into supramolecular polymers in aqueous solution as a consequence of hydrophobic interactions between the aromatic cores.[20] The mannose-functionalized polymer remained bound to bacteria through carbohydrate–lectin interactions even in the presence of a 10^6-fold excess of monovalent mannose, clearly demonstrating the advantages of self-assembled multivalency in this case, and the density of the mannose groups on the self-assembled structure controlled the degree of bacterial aggregation. The supramolecular polymers were also found to be very effective inhibitors of concanavalin A (Con A) binding to yeast cells—around eight times stronger than the monovalent reference compound.

Lee and co-workers designed a series of mannose-functionalized amphiphiles which can self-assemble into a variety of architectures depending on the molecular structure—demonstrating one of the key advantages of self-assembly in being able to fine-tune the nanoscale morphology.[21] The ability of these systems to inhibit Con A-promoted erythrocyte agglutination was between 800- and 1800-fold higher than monovalent mannose, and the nanostructures were able to bind to the surfaces of *E. coli* bacteria with high affinity. They synthesized a series of molecular structures which, dependent on the relative size balance of the hydrophobic and hydrophilic blocks, assembled in a predictable way into either vesicles, spherical, or cylindrical micelles (Figure 4).[22] This allowed the effect of aggregate size and shape

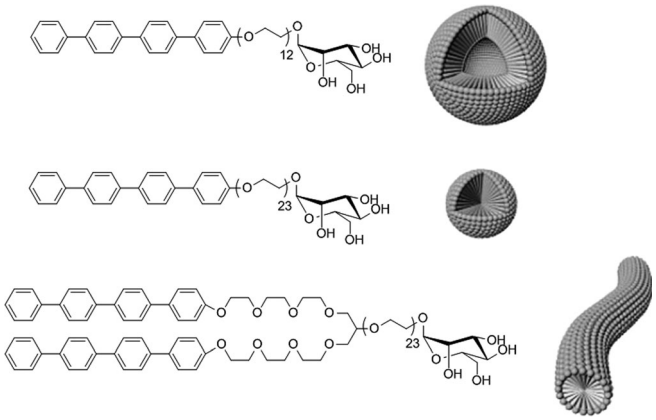

Figure 4. Structure of mannose-functionalized amphiphiles and their corresponding aggregate structure which can inhibit Con A-promoted erythrocyte agglutination.[21]

on the biological activity to be determined. The amphiphiles which formed the spherical micelles were found to be the most potent inhibitors indicating that in this case, higher surface curvature results in more effective inhibition.[21b]

Ravoo and co-workers have decorated self-assembling amphiphilic cyclodextrin derivatives with sugar residues.[23] These systems exhibit strong affinity for either Con A when functionalized with maltose, or peanut agglutinin when

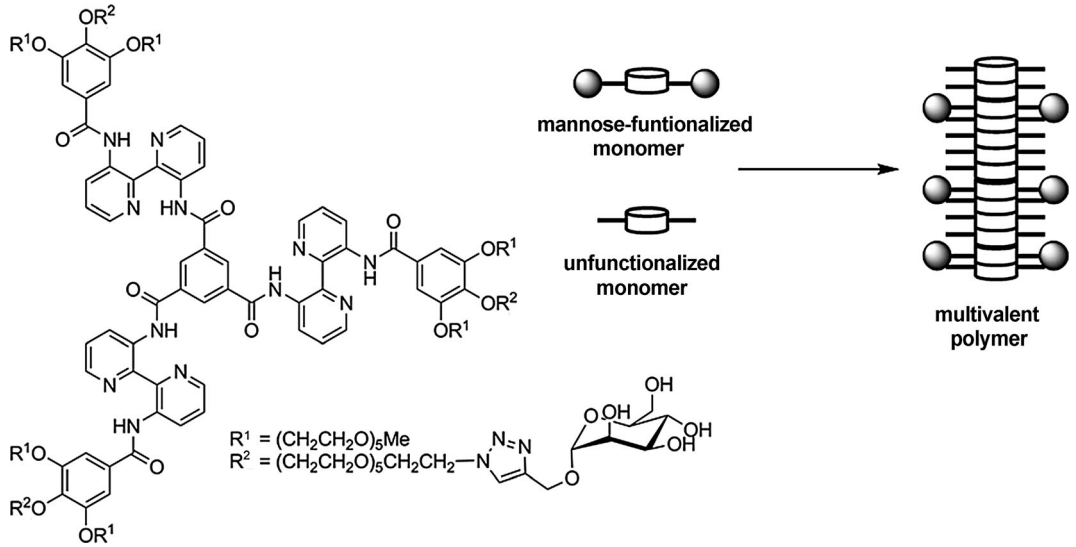

Figure 3. Structure of a mannose-functionalized discotic compound which assembles into a columnar polymer and then exhibits multivalent binding to lectins on bacterial surfaces.[19]

functionalized with lactose. When the carbohydrate ligands are not self-assembled they do not display measurable affinity for their corresponding protein target.

Stoddart and co-workers have also utilized self-assembly to organize cyclodextrins—however, they have used host–guest encapsulation within the cyclodextrin cavity as the means of assembly.[24] By choosing a polyviologen as a guest, cyclodextrins with additional pendant sugars can then thread themselves along the polymer chain to yield a self-assembled pseudorotaxane (Figure 5). Seventeen cyclodextrins were

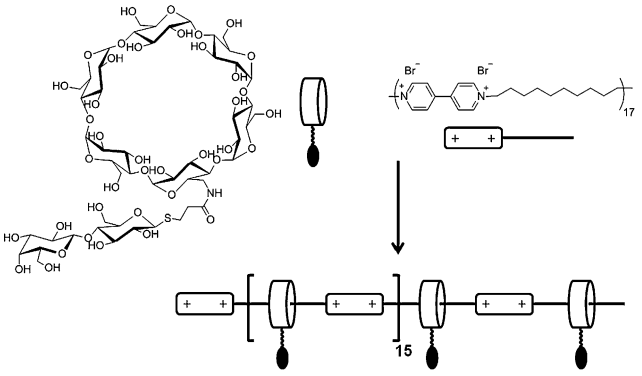

Figure 5. Structure of lactoside–cyclodextrin and polyviologen which assembles into a pseudopolyrotaxane.[24]

able to thread onto the polyviologen and were capable of binding to galectin-1 (a typical member of the family of galactoside-binding lectins, which mediate cell adhesion, signaling, and death) almost eight times more effectively than non-assembled lactose–cyclodextrin and ten times better than simple lactose.

Somewhat analogous to cyclodextrins, cucurbiturils have also been used as building blocks to organize self-assembled saccharide arrays. For example, Kim and co-workers utilized an amphiphilic cucurbit[6]uril, capable of self-assembling into vesicles.[25] By taking advantage of the strong affinity of the molecular cavity for polyamines they were able to use a mannose–spermine conjugate to decorate the vesicle surface with mannose ligands. The binding of the vesicle to Con A was three orders of magnitude greater than the free mannose–spermine ligand. In elegant work, Scherman and co-workers also employed cucurbiturils in a self-assembled lectin-binding system which could be switched off with a simple chemical trigger which caused disassembly.[26] A cucurbit[8]uril was threaded onto a polymer chain and a mannose–viologen guest was then coordinated to each cucurbit[8]uril through hydrophobic interactions. The self-assembled three-component system bound effectively to Con A but on addition of sodium dithionite, which reduced viologen to a radical cation, complex disassembly occurred and the high-affinity lectin binding was switched off.

Carbon nanotubes (CNTs) have been used by Bertozzi and co-workers as structural scaffolds for molecular self-assembly and multivalent sugar expression. A C_{18} aliphatic chain-functionalized polymer decorated with α-*N*-galactosamine residues was assembled onto the surface of a CNT through hydrophobic interactions (Figure 6). The functionalized nanotubes were able to interact specifically with cell surface lectins.[27]

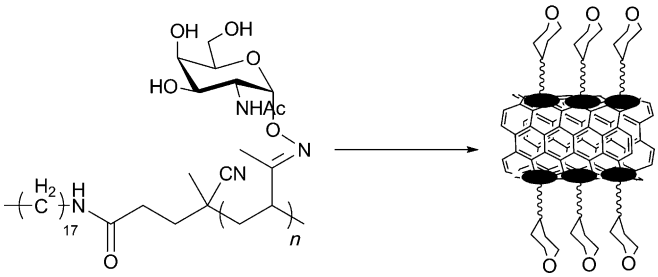

Figure 6. Structure of C_{18}-terminated galactosamine-conjugated polymer capable of assembling onto a CNT surface and then binding cell surface lectins with enhanced affinity.[27]

A number of groups have utilized gold nanoparticles as a surface to assemble thiolated carbohydrates.[28] For example, Lin and co-workers coated gold nanoparticles with mannose producing particles with approximately 200 mannose residues appended.[28a] A 2000-fold excess of monovalent mannose was required to competitively outperform the nanoparticles in binding to *E. coli* bacteria, clearly demonstrating the benefits of the self-assembled multivalent array. More recently, Yan and co-workers employed a fluorescence-based competition assay in order to provide more quantitative analysis of the binding affinity of these glyconanoparticles with lectin.[28f] Nanoparticles functionalized with between one and three mannose ligands exhibited binding affinities for Con A between 0.013 and 0.43 nM, several orders of magnitude stronger binding than the corresponding free ligands which have μM affinity. In some ways, however, gold nanoparticles, although often referred to as self-assembled,[29] can still really be considered as synthetic objects, as they require some purification after assembly, and their formation is not truly reversible—as such the remainder of this article will not discuss gold nanoparticles in terms of self-assembled multivalency.

2.2. RGD Peptides for Integrin Binding

Integrins are heterodimeric, transmembrane proteins which have important roles in biology in both cell signaling and adhesion making them of particular interest in tissue engineering applications.[30] Moreover, some integrins are over-expressed on the surface of cancer cells making them an interesting target for anti-cancer treatments.[31] Arg–Gly–Asp (RGD) peptides have been shown to target integrin binding.[32] As such, there has been considerable interest in the development of RGD-functionalized ligands for integrin binding. Integrin itself only has a single RGD-binding site, and as such, the only benefits of multivalent ligand binding to free integrin would be an effective ligand concentration effect. However, in biological systems, integrins are found in cell membranes and are known to cluster in order to achieve focal adhesion—as such multivalency can offer additional benefits to binding in such circumstances, and a number of

reports of RGD-functionalized dendrimers binding integrin, with some increase in binding affinity have been published.[33]

In a number of cases RGD-containing lipopeptides have been shown to have enhanced binding affinity for integrins when inserted into hydrophobic vesicles.[34] There have also been studies into amphiphilic RGDs which self-assemble in their own right into RGD-functionalized nanostructures.[35] However, far fewer examples exist where it has been quantitatively demonstrated that the assembly process leads directly to enhanced integrin binding.

Work from our own group has aimed to compare, contrast, and quantify dendritic and self-assembled strategies to RGD multivalency.[36] We found that a simple amphiphile C12-RGD (Figure 7), capable of assembly into micellar structures, bound integrin significantly more effectively ($EC_{50} = 200\ \mu M$)

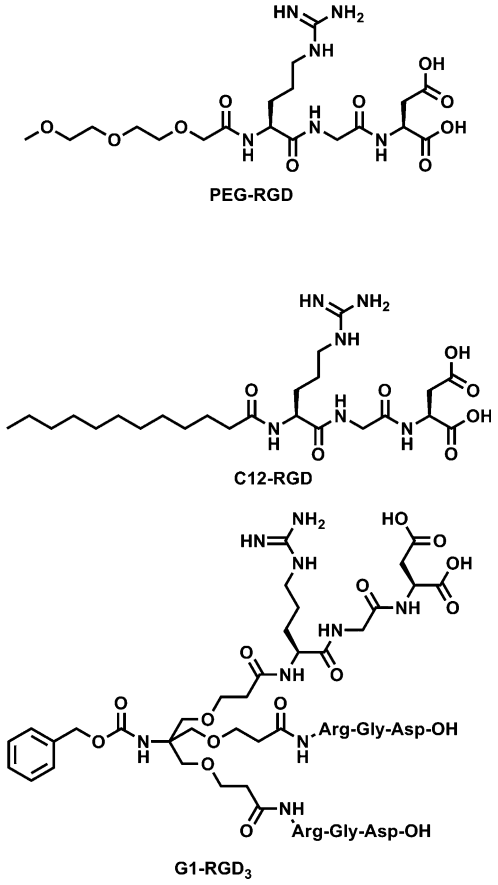

Figure 7. Structure of self-assembling (C12-RGD) and dendritic arrays (G1-RGD) and a negative control (PEG-RGD). The lipid-functionalized C12-RGD is as effective an integrin binder as covalent multivalent G1-RGD.[36]

than a related polyethylene glycol(PEG)-functionalized system which does not self-assemble ($EC_{50} > 1\ mM$). Notably, the self-assembly strategy was comparable to, if not more effective than, a dendritic multivalent array ($EC_{50} = 125\ \mu M$, per RGD unit $= 375\ \mu M$). This clearly demonstrated in quantitative terms that self-assembly is an effective strategy for ligand organization and can compete with a covalent approach.

In elegant work Chilkoti and co-workers have used thermal triggering to induce the assembly of elastin-like polypeptides which exhibit an inverse phase transition at a specific temperature.[37] They employed an RGD-functionalized diblock copolymer of two peptides with different transition temperatures. When the temperature was increased from 23 to 40 °C the hydrophobic block became insoluble and a micelle-like structure formed in solution with the RGD-functionalized hydrophilic block located on the periphery. Using this system they were able to selectively switch from a monovalent low-affinity system, which showed no evidence of cell uptake by both flow cytometry and confocal microscopy, to a multivalent one with enhanced integrin mediated cell uptake in around 50 % of cells. This clearly illustrates how self-assembled multivalency offers the advantage of being highly responsive.

2.3. Alternative Ligands for Protein Binding

Alternative ligands have also been self-assembled in order to achieve the enhanced multivalent binding of different protein targets. Cucurbiturils have been used as ligands capable of binding to the hydrophobic/aromatic side chain of tryptophan. Urbach and co-workers assembled cucurbit[8]uril onto a scaffold functionalized with either one, two or three viologens, which bind to one, two or three cucurbiturils, respectively.[38] The trivalent system was able to bind peptides displaying multiple tryptophan residues with an affinity up to 280-fold greater than the monovalent complex.

Hamilton and co-workers utilized a spontaneously assembling pentameric DNA scaffold with phosphocholine head groups.[39] The assembled pentaplex was found to bind to human C-reactive protein three orders of magnitude more effectively than the corresponding single-stranded phosphocholine. By changing the linker length and self-assembly conditions, the binding affinity could be significantly altered, and as much as 1000-fold increase in binding strength was observed in one case.[40] In general, pentameric assemblies were found to perform significantly better than the corresponding tetrameric system and linker lengths of less than 32 Å were found to hinder the binding event as the receptors become too tightly packed to achieve saturation, illustrating the vital importance of optimizing the geometry of the self-assembled nanostructures.

Merkx and co-workers functionalized a PEGylated phospholipid with CNA35, a collagen-binding domain present in an adhesion protein, resulting in the formation of CNA-functionalized micelles.[41] High-affinity binding of these micelles to collagen was only observed above the critical micelle concentration of 5 μM, demonstrating that effective self-assembly was a pre-requisite for collagen binding. Interestingly, it was noted that collagen binding did not damage the self-assembled micelles, indeed it significantly enhanced the micellar stability. The same group also developed a choline-functionalized fifth generation dendrimer capable of binding multiple copies of a fusion protein composed of C-LytA bound to CNA35 (Figure 8).[42] The binding affinity of the dendrimer decorated with wild-type

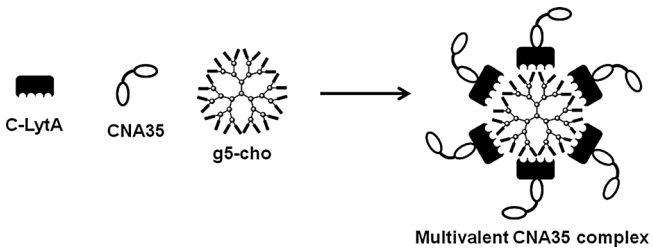

Figure 8. Structure of the multivalent collagen-binding system comprising a 5th generation choline dendrimer (g5-cho) and self-assembled surface-bound C-LytA/CNA35 fusion proteins.[42]

self-assembled fusion protein showed similar collagen binding affinity to the monovalent equivalent. However, when using the mutant weakly-binding CNA35-Y175K protein, the binding affinity showed an eight-fold enhancement in the presence of the G5 dendrimer compared to in its absence—as the dendrimer promotes self-assembly of a multivalent array of mutant fusion proteins. This demonstrates how multivalency can, in particular, benefit weak (sub-optimal) binding events, such as that shown by the mutant protein in this case.

3. DNA Binding

Through applications in gene therapy, DNA binding has enormous potential in medicine to provide treatments for genetic conditions such as cystic fibrosis or as therapeutics in the treatment of cancer.[43] In order to successfully deliver genetic material into cells it is necessary to use a transport vector.[44] Both self-assembly and multivalency have proved highly effective strategies for designing DNA binding ligands.[10,11,45] However, fewer examples exist where self-assembly has itself been used as a strategy to generate multivalent arrays of DNA-binding ligands.

Cheng and Gabrielson modified spermine, a well-known, naturally occurring, DNA-binding ligand, with two hydrophobic oleyl groups (Figure 9a).[46] On its own, spermine has relatively weak binding and is incapable of gene delivery, but when conjugated to a lipophilic tail the resulting assembly into larger multivalent nanoscale aggregates drastically increases the affinity and transfection efficiency of the compound. Functionalization of cell-targeting groups (folate) with similar oleyl groups allowed them to be co-assembled with the transfection vector. In this way, additional/orthogonal functionality can also be simply incorporated into the vector by using self-assembly methods.

Diederich and co-workers have demonstrated that the self-assembly properties of a family of branched amphiphiles had a profound effect on both DNA-binding affinity and transfection efficiency (Figure 9b).[47] In particular, the hydrophobic–hydrophilic balance was important in mediating the mode of self-assembly, and the morphology of the resulting aggregates. The most effective vector, **2**, was found to assemble into more flexible multilamellar vesicles, whereas **1** and **3** could only form micelles. This difference in morphology helped prevent sensitivity to serum, allowing high levels of gene delivery to be maintained.

In our own group, we have used oligoamine ligands such as spermine, on the surface of hydrophobically modified dendrons to generate very well-defined self-assembled multi-

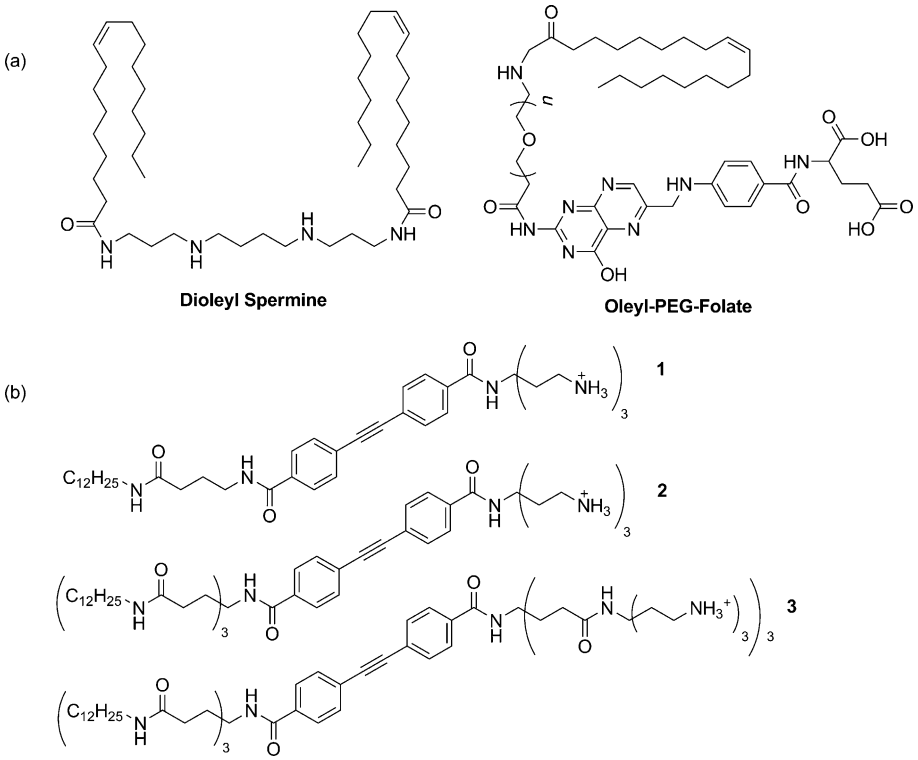

Figure 9. a) Structure of DNA-binding dioleyl spermine and cell-targeting oleyl-PEG-folate.[46] b) Structures of branched amphiphiles **1**–**3** designed for gene transfection and optimized on the basis of self-assembled morphologies.[47]

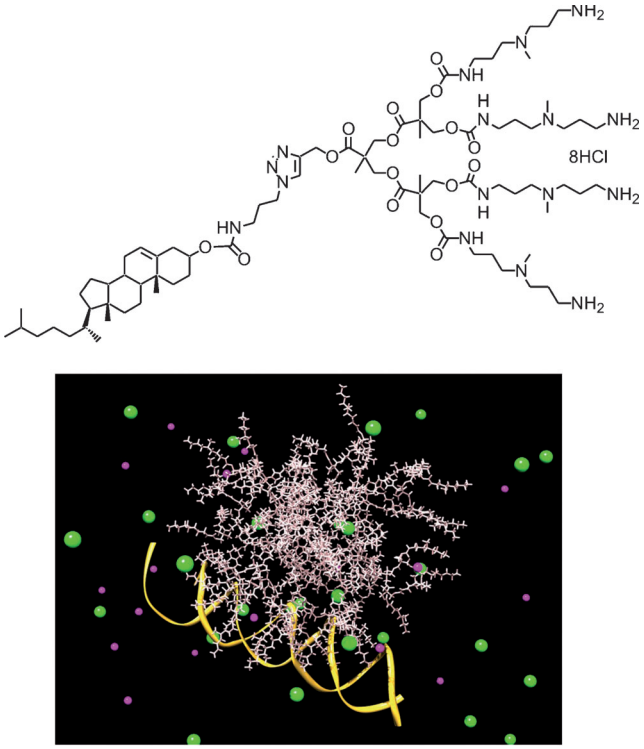

Figure 10. Structure of cholesterol-modified self-assembling dendron and multiscale modeling of self-assembled nanoscale micelle binding to DNA.[49b]

valent systems (Figure 10).[48] Using multiscale modeling, we were able to show that the mode of self-assembly plays a vital role. The choice of hydrophobic unit controls the surface charge density of the resulting nanostructure, which can be directly correlated with the DNA binding affinity.[48c] Furthermore, the size of the hydrophobic unit controls the morphology of the aggregates, which directly impacts upon gene transfection ability.[48b] In recent studies, we have employed degradable ester-based dendrons,[49] and demonstrated that on hydrolytic degradation in water at pH 7.4, the ligands become disconnected from the hydrophobic unit, the nanostructures disassemble, and are no longer capable of binding DNA—the individual dendrons have lost all their self-assembled multivalency, and do not have sufficient affinity. This responsive nature of self-assembled multivalency is one of its key advantages over static multivalent arrays. Once bound to DNA, hydrolytic dendron degradation, which is mediated by intramolecular catalysis from the surface ligands, is inhibited.[49b] Efforts to employ this switch-off self-assembled multivalency mechanism in cellular delivery are ongoing.

Ravoo and co-workers have recently employed monovalent spermine ligands functionalized with an azobenzene derivative in a self-assembling system (Figure 11).[50] The azobenzene group can undergo a reversible photo-induced isomerization from the *trans* to the *cis* isomer. The *trans* isomer was able to complex with a vesicle-like cyclodextrin assembly, yielding an aggregate capable of high-affinity multivalent DNA binding. However, by converting the azobenzene to its corresponding *cis* isomer the modified

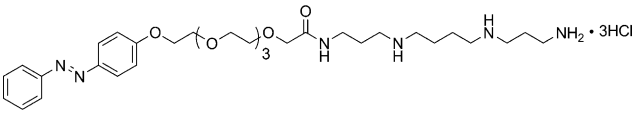

Figure 11. Structure of *trans*-azobenzene spermine conjugate which exhibits light switchable self-assembly and multivalency behavior.[50]

spermine was no longer able to complex with the cyclodextrin vesicle, causing dissassembly from the nanostructure, and a transition to the low-affinity monovalent binding system. This elegant system was found to be fully reversible over a number of cycles between low- and high-affinity states and demonstrates the potential of self-assembled multivalent systems to be responsive to a wide range of different stimuli.

4. Other Targets

In addition to proteins and DNA, the concept of self-assembled multivalency can be applied to a wide range of different targets—both chemical and biological. For example, Williams, Hunter and co-workers developed a cholesterol-functionalized dansylamine capable of binding to copper(II) (Figure 12). The dansylamine alone had very low Cu^{2+} affinity

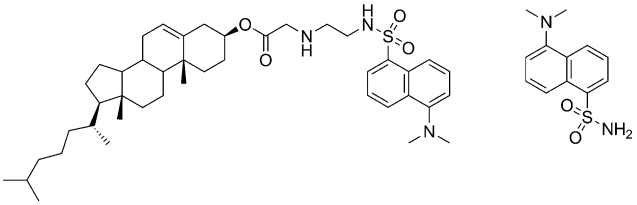

Figure 12. Structure of dansylamine-functionalized cholesterol which shows enhanced affinity for Cu^{II} on self-assembly and the monovalent dansylamine ligand.[47]

but when assembled into unimolecular micelles as a consequence of the hydrophobicity of the cholesterol group, the binding affinity increased by two orders of magnitude. When the cholesterol-modified ligand was assembled within a lipid bilayer the binding stoichiometry between ligand and metal ion was significantly altered.[51] This demonstrates that the self-assembled multivalency strategy is not necessarily limited to large biological binding partners. In related work, Webb and co-workers inserted metal-bound ligands into vesicles, and only if ligand clustering occurred within the membrane could the vesicle show adhesion to a different conjugate-ligand-coated vesicle.[52] This control of nanoscale adhesion via self-assembly-induced clustering mimics some aspects of cell adhesion processes.

We have recently developed multivalent self-assembling cationic systems to target the binding of polyanionic heparin.[53] This important anticoagulant drug plays an important role during surgery. When surgery is complete, a heparin reversal agent, protamine, has to be employed, but unfortunately protamine causes a significant number of negative side-effects. We reasoned that self-assembled nanostructures

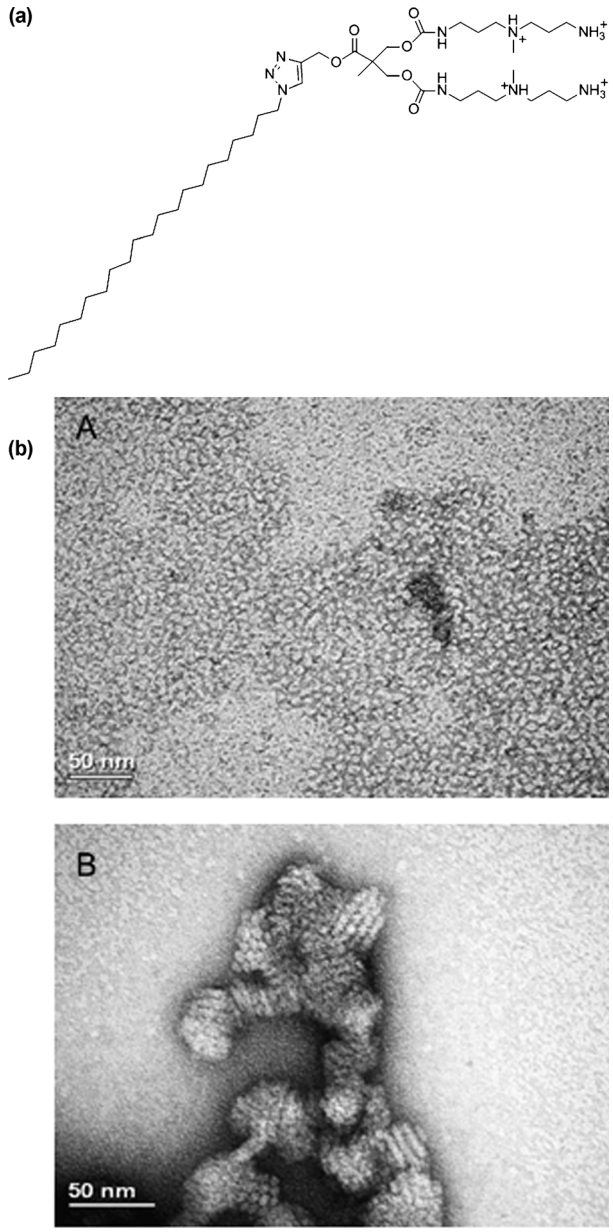

Figure 13. a) Structure of self-assembling heparin-binding ligand and b) TEM images of self-assembling ligand taken A) without heparin and B) in the presence of heparin.[53]

might mimic protamine (a covalently bound protein) in terms of size and charge, but have better clinical outcomes. We reported that our self-assembling system bound heparin with similar affinity to that of protamine, and visualized the heparin–nanostructure binding using electron microscopy methods (Figure 13). This technique demonstrated that the micelles remain intact in the presence of heparin and indeed, appeared to be organized on the surface of it. Heparin binding did not appear to induce any loss in stability or morphological change in the nanoscale assemblies. We have since been able to demonstrate that equivalent non-assembling compounds have limited heparin binding affinity, and that self-assembly plays a key role in allowing high-affinity recognition under biologically relevant conditions.[54]

5. Summary and Outlook

This Minireview has described selected key examples in which self-assembly has been used as an effective and synthetically straightforward tool to achieve high-affinity binding through multivalency. This concept can be applied to a wide variety of binding interactions and can be used to mimic a large number of different biological processes and intervene in biological pathways. In particular, such self-assembled systems are ideal for interfacing with large biological surfaces, such as those of proteins, nucleic acids, viruses, and bacterial and human cells. As such, this approach may see applications in inhibition of key protein–protein interactions, gene therapy, prevention of infection, tissue engineering, etc. It is predicted that self-assembled multivalency will therefore be of fundamental importance in the emergent field of nanomedicine.

The rapid expansion of supramolecular chemistry in recent years, and its increasing focus on the nanoscale, has enabled the generation of self-assembled nanosized objects with highly tuneable sizes and shapes—features which can directly impact on the nature of the multivalency expressed. Furthermore, self-assembled systems are highly dynamic and responsive, and this opens the possibility of generating systems which can turn their multivalency on and off in response to a wide range of different triggers—both chemical and physical. Such systems will be of great value in achieving smart recognition or controlled delivery. For example it should be possible to generate systems which can show high-affinity multivalent binding to a target (e.g. a therapeutic protein or nucleic acid) and then, under triggered conditions, disassemble and effectively switch off the binding—achieving controlled release of the therapeutic agent.

Importantly, self-assembled multivalent systems are constructed from well-defined, relatively low-molecular-weight building blocks, and this makes them ideal for medical application, as their behavior at the molecular level can be clearly defined and understood—a clear advantage in terms of the regulatory process.

In summary, the use of self-assembly to create dynamic multivalency is a powerful strategy, with some significant advantages over the use of static multivalent arrays, it mimics processes which occur naturally within cell membranes, and has a wide range of potential applications, both in nanomaterials science and nanomedicine.

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