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Heparin versus DNA: Chiral Preferences in Polyanion Binding to Self-Assembled Multivalent (SAMul) Nanostructures

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Supporting Information

ABSTRACT: This communication presents simple cationic self-assembling multivalent (SAMul) first generation dendrons based on L or D lysine, which form identical nanoscale assemblies in terms of dimensions and charge densities but toward which DNA and heparin exhibit different chiral binding preferences. However, higher generation dendrons with larger hydrophilic head groups are bound identically by these polyanions, irrespective of chirality. We propose that well-organized chiral ligands on the surface of self-assembled nanostructures can exhibit enantioselective polyanion binding. This demonstrates that small structural changes can be amplified by self-assembly and impact on nanoscale binding.

Polyanions are of vital importance in biological systems, where they dominate many environments (indeed, much of biology can be considered a "polyanion world"), and as such, selective recognition is crucial.[1] In electrostatic binding, it is frequently argued that selectivity is difficult, with charge density being the dominant factor which controls binding.[2] However, key recent studies have focused on the anisotropic and stoichiometric organization of charge, and its consequent ability to achieve more selective binding than might initially have been expected.[3]

Multivalent platforms are an ideal way of achieving interactions with nanoscale biological targets,[4] in order to manipulate biological processes or intervene as therapeutics in nanomedicine.[5] An approach to increase the synthetic accessibility of multivalent systems is to use a self-assembling multivalent (SAMul) approach, which only requires the synthesis of smaller "drug-like" monomer units that self-assemble to multiply up the ligand numbers presented to the target for binding.[6] The first example was reported by Whitesides and co-workers who assembled glycoclusters able to intervene in viral adhesion.[7] A number of other researchers have since built on this approach.[8] We adopted the SAMul approach to develop families of molecules targeted at DNA[9] and heparin,[10] both of which have therapeutic relevance.[11,12] As a result, we became interested in the factors that govern selectivity, but given that our SAMul systems simply display cationic ligands, we were unsure of the extent to which they could employ "precisely defined" interactions. As such, we wanted to determine whether the SAMul approach could achieve *any* polyanion discrimination.

Given the inherent chirality of DNA and heparin, we thought chiral SAMul systems might express some sort of binding preference. The binding of chiral ligands to DNA has been previously examined,[13] finding applications in areas such as enantiomeric purification[14] and asymmetric catalysis.[15] Individual DNA strands can also selectively bind helically complementary chiral peptide nucleic acids.[16] Similarly, heparin can discriminate chiral substrates, for example in capillary electrophoresis, where it has been used as a mobile phase additive.[17] However, work from Wang and Rabenstein suggested enantiomeric peptides interacted with heparin in an identical manner.[18] There have been studies in which heparin has been used to compete with DNA binding,[19] and very recently, CD was used to probe interactions between a synthetic ligand and these polyanions.[20] However, chiral polyanion discrimination remains rare, as does chiral recognition using micelles in cases where self-assembly is a prerequisite for effective multivalent binding across the surface.

We decided to probe self-assembling chiral cationic ligands for binding similar polyanions (Figure 1) and chose lysine as the chiral, cationic surface ligand owing to its biological ubiquity in polyanion-binding proteins.[21] Lysine has been shown to be among the most effective heparin binding groups.[22] We then modified our previously reported synthetic approach to SAMul ligands[10] to incorporate lysine, synthesizing compounds **1L** and **1D** (Figure 1), and control compound **1**, unmodified with a hydrophobic chain, which is incapable of self-assembly. Second

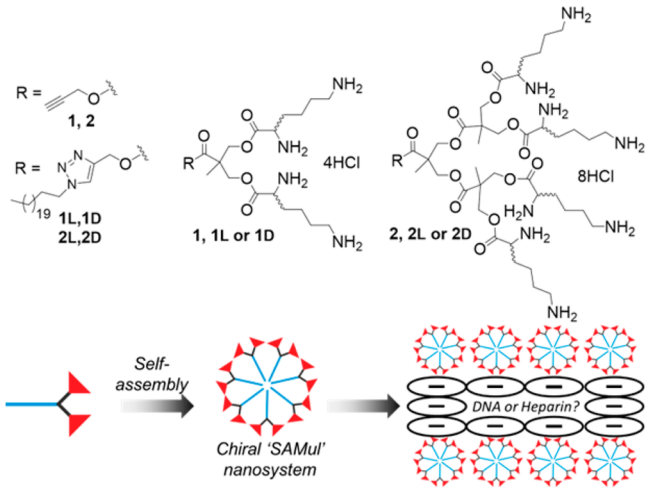

Figure 1. Monomer units **1L/D** and **2L/D** self-assemble to generate multivalent ligand arrays for polyanion binding.

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generation target molecules were synthesized in a related convergent manner. Full experimental methods and characterization can be found in the Supporting Information (SI). Chiralities of **1L**/**1D** and **2L**/**2D** were examined by circular dichroism (CD) spectroscopy and shown to be equal and opposite.

The ability of **1L** and **1D** to self-assemble in water was examined using a Nile Red encapsulation assay,[23] and critical micelle concentrations (CMCs) of 29 ± 9 and 27 ± 13 μM, respectively, were derived (see SI), essentially identical. For **2L** and **2D** these values were 25 ± 8 and 20 ± 6 μM, respectively. Control compounds **1** and **2** did not self-assemble at concentrations up to 1 mM. The morphologies of **1L** and **1D** self-assemblies were characterized by transmission electron microscopy (TEM). Both **1L** and **1D** appeared to form spherical micellar assemblies with approximate diameters of 8 nm (Figure 2). Compounds **2L** and **2D** also formed micelles ca. 9−11 nm in

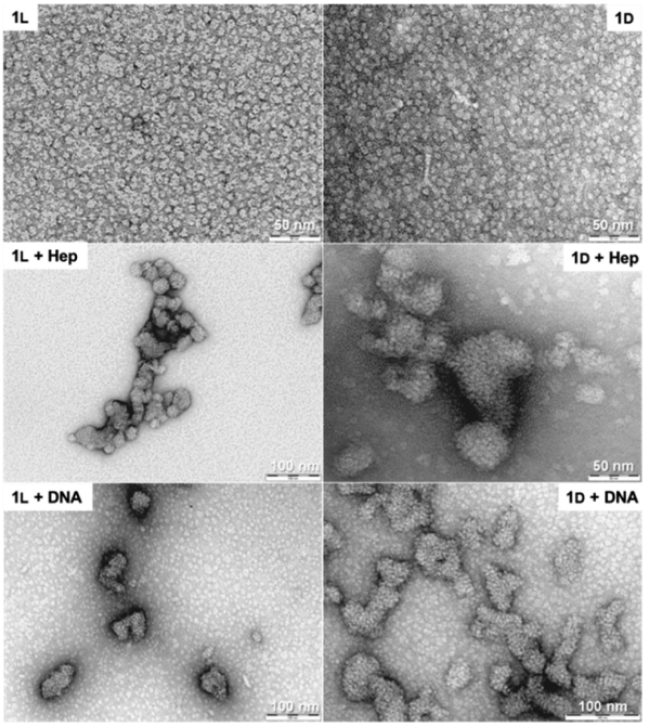

Figure 2. TEM images of **1L** (left) and **1D** (right) in the absence (top) and presence of heparin (middle) and DNA (bottom).

diameter; however, accurate sizing was complicated by the tendency of the sample to deteriorate under the electron beam (see SI). In the presence of heparin, in each case the micelles became structured in a pseudocrystalline array and had apparent diameters of ca. 7 nm. Conceptually related structured arrays have previously been reported by ourselves,[10] and also by Kostiainen and co-workers;[24] the spherical polycations can be considered to be packing with the linear polyanions in a nanoscale ionic-lattice-like arrangement.

Dynamic light scattering (DLS) measurements were used to estimate the solution-phase dimensions (Table 1). In 10 mM Tris HCl buffer, **1L** and **1D** formed aggregates ca. 7.7 nm in diameter, in good agreement with TEM. On the addition of 150 mM NaCl, the aggregates enlarged, with diameters of ca. 8−9 nm. This is due to a combination of salt-mediated charge screening at the micellar surface and an enhanced hydrophobic

Table 1. DLS Data for 1L and 1D under Different Electrolytic Conditions

	diameter (nm)		
compd	Tris	Tris + NaCl	zeta potential
1L	(7.6 ± 0.3)	(9.0 ± 0.3)	(+33.0 ± 1.4)
1D	(7.8 ± 0.2)	(8.2 ± 0.3)	(+26.3 ± 8.4)
2L	N/A	(6.0 ± 0.2)	(+29.0 ± 1.3)
2D	N/A	(6.4 ± 0.3)	(+26.4 ± 6.9)

effect.[10b] DLS suggested that in solution, the self-assemblies formed by **2L** and **2D**, have diameters of about 6 nm, suggesting the larger hydrophilic head groups limit the growth of larger nanostructures, owing to their volume requirement.

To quantify their relative heparin binding abilities, the compounds were tested using our recently reported Mallard Blue (MalB) competition assay in which displacement of MalB from heparin by addition of the nanoscale binder, as monitored by UV−vis spectroscopy, indicates the extent of binding.[25] The DNA binding abilities were also examined using a competition assay involving ethidium bromide (EthBr) as the indicator dye, the fluorescence of which is quenched on displacement.[26] Titration curves are shown in Figure 3. All assays had to be performed on freshly prepared samples, owing to the relatively fast degradation of the compounds.[9c,27] The analysis of the data from this kind of assay is challenging for several reasons. Polyanions such as heparin and DNA have multiple binding sites along the polymer chain. The first assemblies that bind to the biopolymers therefore do so with significantly different affinity to the last assemblies; as more cationic assemblies bind to the polyanion, charge−charge repulsions between them mean the binding becomes less effective. Furthermore, heparin and DNA are polydisperse in terms of their constitution. This problem is particularly acute for heparin, which consists of a number of different saccharides, all of which have different patterns of anionic functionalization.[28] Hence, there are significantly variable binding sites along the polymer. As such, it is difficult, if not meaningless, to extract a simple "binding constant" or thermodynamic parameters from these assays. For simple comparison of relative binding performances, we calculated charge excess values (CE_{50}) at 50% dye displacement (Table 2). This reflects how much cationic charge (relative to polyanion charge) is required to displace 50% of the indicator dye from its complex with the polyanion (a lower number indicates a greater efficiency and "better" binding). The CE_{50} value reflects the binding when ca. half of the biopolymer has been bound by SAMul nanostructures.

Nonassembling molecules **1** and **2** were unable to displace the indicator dyes from their complexes. Control compound **2** was slightly better than **1**, perhaps as a consequence of it having double the number of ligands. As such, in the absence of self-assembly the individual lysine-based ligands cannot bind these polyanions to any significant degree. In contrast, both **1L** and **1D** were able to displace the indicator dyes in both assays; it is therefore evident that the SAMul effect is responsible for binding in each case.

The data further suggest that **1D** (1.13 ± 0.19) uses its charges more efficiently than **1L** (1.94 ± 0.38) when binding heparin, while the opposite is true when binding DNA, i.e., **1L** (1.99 ± 0.54) is more effective than **1D** (3.51 ± 0.37). These differences are well beyond the error range and were repeated across multiple runs. As such, they can be considered significant. The SAMul nanostructures formed by **1L** and **1D** have identical sizes

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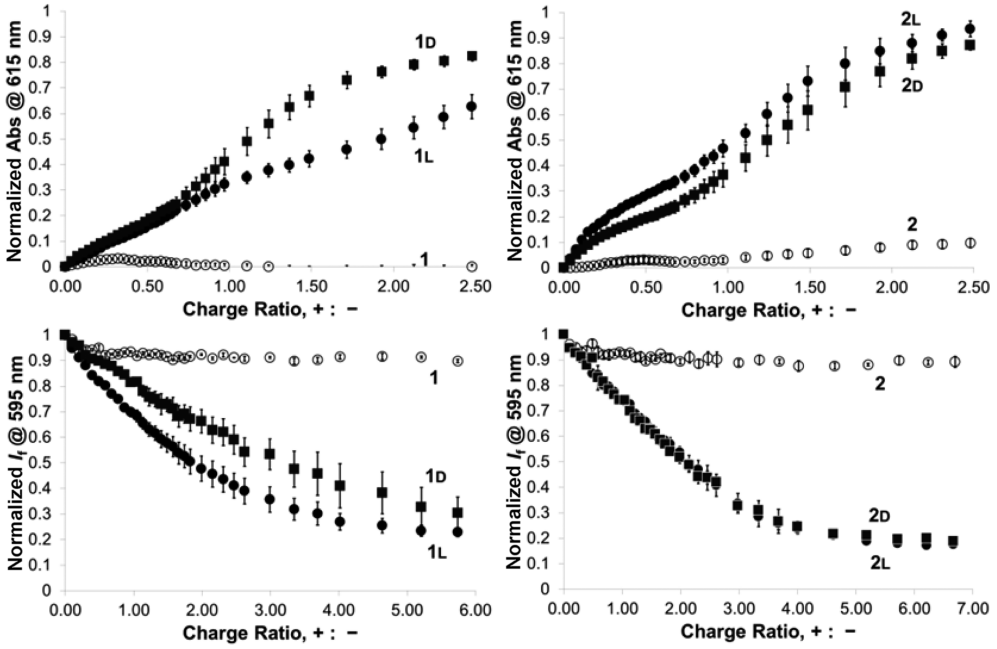

Figure 3. Characterization of the binders using the MalB displacement assay (heparin binding ability, top) and EthBr displacement assay (DNA binding ability, bottom).

Table 2. CE_{50} Data for Binders Interacting with Heparin and DNA Obtained by Displacement Assays with MalB and EthBr, Respectively

ligand	heparin	DNA
1	no binding	no binding
1L	1.94 ± 0.38	1.99 ± 0.54
1D	1.13 ± 0.19	3.51 ± 0.37
2	no binding	no binding
2L	1.07 ± 0.20	2.15 ± 0.31
2D	1.28 ± 0.26	2.03 ± 0.25

and charge densities, and their only difference is chirality, suggesting that the spatial arrangement of the cationic charges is important when binding these polyanions and indicating some degree of chiral recognition.

Considering the titration curves for **1L** and **1D** more fully, it is worth noting that for heparin binding, when only small amounts of SAMul nanostructures are present, there is little difference in affinity between **1L** and **1D**, as reflected by the initially overlapping binding profiles in the titration curve in Figure 3. This would suggest that the "primary" binding sites on heparin from which MalB can be displaced show no preferential binding between enantiomers and bind based on charge density. However, as more of the SAMul system is added, and about one-third of the MalB dye has been displaced, the differences between **1L** and **1D** become significant and the titration curves diverge (as also reflected in the CE_{50} value at 50% displacement of dye). We therefore suggest that for effective binding to these "secondary" binding sites on heparin, more than charge density matters, and chiral organization is significant. We have reported previously that the stoichiometry of binder−heparin complexes is important in determining the degree of binding;[29] however, this is the first time enantiodiscrimination has been observed. For DNA binding, however, significant differences between **1L** and **1D** exist throughout the titration. The distribution of phosphate anions down the DNA backbone is regular in this polymer (unlike the high variability of anionic sites in heparin). As such, we suggest that there is less differentiation between "primary" and "secondary" binding sites on DNA, and a consistent enantiopreference is expressed throughout the titration as a result.

Compounds **2L** and **2D** displace 50% of MalB at charge efficiencies of 1.07 ± 0.20 and 1.28 ± 0.26; no significant difference between enantiomers (Table 2). Indeed, it is clear that this is the case across the whole titration (Figure 3). It therefore appears that despite the same chiral ligands being present in the second generation binders, this chiral information is less apparent to heparin. Similar trends were observed for DNA binding; compounds **2L** and **2D** bind DNA with almost identical efficiencies and identical titration curves, indicating chirality has no influence. We suggest that increased crowding at the surface of the dendritic structure on the smaller self-assemblies formed by **2L** and **2D**, as a consequence of the larger, more sterically hindered hydrophilic ligands, masks the differences in chiral expression.

It should be noted that all self-assembling ligands achieved effective multivalent polyanion binding at concentrations below their CMC values. The presence of polyanions such as DNA can lower the effective CMC; indeed, such effects are well-known for ionic surfactants.[30] Such a phenomenon would suggest that multivalency-enhanced self-assembly is operating here, in addition to self-assembly enhanced multivalency; i.e., these two mechanisms reinforce one another. Taken overall, these data suggest that, in some cases, these two biological polyanions exhibit chiral preferences when presented with enantiomeric versions of the same SAMul binding unit; noteworthy as it contradicts observations that suggested such interactions primarily depend on charge density.[2,31]

In summary, SAMul is a useful approach for achieving some selectivity in polyanion binding. Although the effects are quite small, this is a very challenging problem, and this is a rare example of enantioselective binding in molecular recognition between nanoscale surfaces with different biomolecules exhibiting different preferences. The appropriate organization of the multivalent ligand array through self-assembly is important in

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transcribing the binding selectivity onto the nanoscale surface. Innate differences between the molecular recognition potential of heparin and DNA may have biological significance in the polyanion rich biological environment, and we suggest these results are important in understanding binding in biorelevant conditions.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b04344.

Synthetic methods, characterization data, assay methods, CMC and anion binding data, compound degradability, and TEM images (PDF)

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Notes

The authors declare no competing financial interest.

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