Multivalent Cluster Nanomolecules for Inhibiting Protein-Protein Interactions

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Abstract

Multivalent protein-protein interactions serve central roles in many essential biological processes, ranging from cell signaling and adhesion to pathogen recognition. Uncovering the rules that govern these intricate interactions is important not only to basic biology and chemistry, but also to the applied sciences where researchers are interested in developing molecules to promote or inhibit these interactions. Here we report the synthesis and application of atomically precise inorganic cluster nanomolecules consisting of an inorganic core and a covalently linked densely-packed layer of saccharides. These hybrid agents are stable under biologically relevant conditions and exhibit multivalent binding capabilities, which enable us to study the complex interactions between glycosylated structures and a dendritic cell lectin receptor. Importantly, we find that subtle changes in the molecular structure lead to significant differences in the nanomolecule’s protein-binding properties. Furthermore, we demonstrate an example of using these hybrid nanomolecules to effectively inhibit protein-protein interactions in a human cell line. Ultimately, this work reveals an intricate interplay between the structural design of multivalent agents and their biological activities toward protein surfaces.
Multivalency is a prevalent phenomenon that facilitates many important biological processes in nature. Some of the most fascinating examples are found in our own immune system, where multivalency plays a crucial role in modulating several central functions of the immune cells, including cell signaling, cell-cell interaction, and pathogen recognition. A notable example of these intricate interactions takes place between glycoproteins and lectins, whose specificity and affinity toward each other are greatly amplified through multivalency. The important role multivalency plays in nature has fascinated both biologists and chemists alike, who are mutually interested in understanding the fundamental mechanisms behind these supramolecular recognition events as well as developing abiotic tools that are inspired by natural phenomena.

An important biological target for studying multivalency is a C-type lectin receptor called dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN). Predominately expressed on the surface of dendritic cells, it organizes into a homotetrameric structure that is critical for the multivalent recognition of pathogens. In particular, DC-SIGN is able to bind specific high-mannose glycoproteins and glycolipids on pathogens with high avidity, which activates a sequence of downstream responses including pathogen uptake and degradation as well as subsequent antigen processing and presentation. However, various pathogens such as HIV-1 have been observed to escape the intracellular degradation pathway following DC-SIGN-facilitated uptake. While the mechanism behind this unusual behavior is not well understood, it is clear that DC-SIGN plays an instrumental role in transmitting HIV-1 to the T cells and enhancing the infection in its early stages. Therefore, there is significant interest in 1) uncovering the rules that govern the multivalent interactions between DC-SIGN and high-mannose glycoconjugates and 2) inhibiting the DC-SIGN-dependent attachment and uptake of certain pathogens. One of the most promising approaches that can potentially tackle both
challenges is centered around building molecules that can mimic the dense multivalent display of carbohydrates on the pathogen surface.\textsuperscript{8,9,17–19}

Previously, several promising classes of glycomimetic ligands for DC-SIGN have been designed and synthesized, which include but are not limited to small molecules,\textsuperscript{20,21} peptides,\textsuperscript{22,23} linear and dendritic polymers,\textsuperscript{24–30} fullerenes,\textsuperscript{31,32} supramolecular assemblies,\textsuperscript{33–35} and hybrid nanoparticles.\textsuperscript{36–38} These constructs are capable of engaging DC-SIGN with high avidities ($K_D$ spanning nM–µM), which allowed several of these systems to inhibit viral entry and infection. In particular, rigid three-dimensional (3D) architectures such as thiol-capped gold nanoparticles (AuNPs) are attractive glycomimetic platforms due to the ease of generating tunable and well-defined multivalent agents. Nevertheless, due to the weak bonding interactions between gold and thiol-based ligands, the surface morphology of these systems is poorly defined and highly dynamic, especially under biologically relevant conditions.\textsuperscript{39–42} This ultimately hinders researchers’ ability to understand the precise structure-activity relationships of these systems with respect to biomolecular recognition and binding events.

Here we report the synthesis of a family of atomically precise glycosylated cluster nanomolecules featuring robust inorganic cluster scaffolds as nanoparticle core templates. Specifically, we developed conditions that allow the rapid functionalization of perfluoroaryl-based moieties covalently grafted onto a rigid dodecaborate core via “click”-like nucleophilic aromatic substitution (S$_{N}$Ar) chemistry, thus leading to fully covalent nanomolecules with a densely packed layer of saccharides.\textsuperscript{43,44} This chemistry mimics the operational simplicity with which thiol-capped AuNPs are synthesized, yet produces well-defined assemblies that are stable under biologically relevant conditions.\textsuperscript{44} Importantly, direct binding studies between these hybrid assemblies and DC-SIGN reveal the multivalency-enhanced avidity in addition to the carbohydrate specificity of the
lectin and the structural requirements for the multivalent ligands. Furthermore, competitive binding data suggest the mannose-coated nanomolecules can inhibit the protein-protein interactions between DC-SIGN and an HIV-1 envelope glycoprotein, gp120. Moreover, we found that the nanomolecules exhibit no apparent toxicity to a human lymphoblast-like cell line at 0.5–50 µM concentrations. This allowed us to perform cellular experiments, which revealed that the mannose-functionalized clusters are capable of preventing the cell uptake of gp120 by blocking cell-surface DC-SIGN. Therefore, we demonstrate that easily accessible, precisely engineered hybrid cluster-based nanomolecules can be utilized to not only study the rules governing multivalent recognition, but also inhibit protein-protein interactions in cells.

**Results and Discussion**

Given our success in installing a wide scope of thiols onto the perfluoroaryl-perfunctionalized clusters using S_N_Ar chemistry,^44^ we hypothesized that this strategy could be applied to generate a library of atomically precise nanomolecules featuring a variety of saccharides densely packed on the rigid 3D surface. Using the perfluoroaryl-perfunctionalized cluster 2 (Figure 1a) and 1-thio-β-D-mannose tetraacetate,^44^--^47^ we performed S_N_Ar reactions in the presence of base in dimethylformamide (DMF), stirring under a N_2 atmosphere. These test conjugation reactions revealed significant conversions, as determined by 19F NMR spectroscopy. Following efficient optimization facilitated by *in situ* 19F NMR spectroscopy, we found that employing an excess of the thiol and potassium phosphate (K_3PO_4) allowed the nearly quantitative (≥99%) substitution of 2 with the substrate within 48 hours. The product was briefly treated with sodium methoxide (NaOMe) to remove all the acetyl groups, then purified by a desalting centrifugal filter to yield the mannose-coated nanomolecule 2a (Table 1, entry 1) in 80% isolated yield (see the Supporting Information for experimental details). The purified 2a was subsequently subjected to
characterization via $^1$H, $^{11}$B, and $^{19}$F NMR spectroscopy and electrospray ionization-high resolution mass spectrometry (ESI-HRMS), which support the proposed structure and composition (see the Supporting Information for characterization data). Furthermore, we found that a similar strategy could be used to perfunctionalize 2 with 1-thio-$\beta$-D-galactose tetraacetate within 48 hours,47,48 giving rise to the purified nanomolecule 2b (Table 1, entry 2), after isolation in 84% yield (see the Supporting Information for experimental details and characterization data).

Additionally, we prepared previously reported glucose- and poly(ethylene glycol) (PEG)-coated structures 2c and 2d (Table 1, entries 3 and 4),44 and notably the isolated yield for 2c was
significantly improved (17% to 65%) through the new purification strategy (see the Supporting Information for experimental details). Overall, these results demonstrate that perfluoroaryl-thiol S₅Ar chemistry can be utilized to assemble a panel of well-defined, multivalent hybrid nanomolecules functionalized with various saccharides including mannose, galactose, and glucose. Moreover, both the glycosylated and PEGylated nanomolecules can be easily purified using desalting centrifugal filters, which streamlines access to the pure materials. Ultimately, these nanomolecules provide us with the ability to evaluate the biological activities of multivalent assemblies as a function of the molecular structure precisely displayed in 3D space.

### Table 1. Glycosylation and PEGylation of Clusters 2 and 3

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>L</th>
<th>R</th>
<th>Time (h)</th>
<th>In situ yield(^a) (%)</th>
<th>Isolated yield(^b) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2a</td>
<td>none</td>
<td>(\text{mannose} ) (\text{OH} ) (\text{CH}_3 ) (\text{OH} ) (\text{OH} ) (\text{OH} ) (\text{OH} ) (\text{OH} ) (\text{OH} ) (\text{OH} ) (\text{OH} ) (\text{OH} ) (\text{OH} )</td>
<td>48</td>
<td>(\geq 99)</td>
<td>80</td>
</tr>
<tr>
<td>2</td>
<td>2b</td>
<td>none</td>
<td>(\text{galactose} ) (\text{OH} ) (\text{CH}_3 ) (\text{OH} )</td>
<td>48</td>
<td>(\geq 99)</td>
<td>84</td>
</tr>
<tr>
<td>3</td>
<td>2c(^*)</td>
<td>none</td>
<td>(\text{glucose} ) (\text{OH} ) (\text{CH}_3 ) (\text{OH} )</td>
<td>24</td>
<td>(\geq 99)</td>
<td>65</td>
</tr>
<tr>
<td>4</td>
<td>2d(^*)</td>
<td>none</td>
<td>(\text{mannose} ) (\text{OH} ) (\text{CH}_3 ) (\text{OH} )</td>
<td>24</td>
<td>(\geq 99)</td>
<td>76</td>
</tr>
<tr>
<td>5</td>
<td>3a</td>
<td>(\text{P} ) (\text{F} )</td>
<td>(\text{mannose} ) (\text{OH} ) (\text{CH}_3 ) (\text{OH} )</td>
<td>0.3</td>
<td>(\geq 99)</td>
<td>83</td>
</tr>
<tr>
<td>6</td>
<td>3b</td>
<td>(\text{P} ) (\text{F} )</td>
<td>(\text{galactose} ) (\text{OH} ) (\text{CH}_3 ) (\text{OH} )</td>
<td>0.3</td>
<td>(\geq 99)</td>
<td>67</td>
</tr>
<tr>
<td>7</td>
<td>3c</td>
<td>(\text{P} ) (\text{F} )</td>
<td>(\text{glucose} ) (\text{OH} ) (\text{CH}_3 ) (\text{OH} )</td>
<td>0.3</td>
<td>(\geq 99)</td>
<td>77</td>
</tr>
<tr>
<td>8</td>
<td>3d</td>
<td>(\text{P} ) (\text{F} )</td>
<td>(\text{mannose} ) (\text{OH} ) (\text{CH}_3 ) (\text{OH} )</td>
<td>1.5</td>
<td>(\geq 99)</td>
<td>84</td>
</tr>
</tbody>
</table>

\(^a\)Yield determined by \(^{19}\)F NMR spectroscopy; \(^b\)Isolated yield after purification; \(^*\)Previously reported compounds. r.t., room temperature.
With the successful synthesis of glycosylated nanomolecules 2a–c, we sought to build a new generation of multivalent architectures that share the precision and rigidity of the first-generation assemblies, but feature a rationally designed linker that will modularly extend the cluster scaffold. We envisioned that the new class of larger-sized glycosylated nanomolecules featuring a distinct multivalent display of saccharides, when studied alongside 2a–c, will allow us to further investigate the complex relationship between molecular structure and activity in the multivalent constructs. Keeping the downstream biological applications in mind, we set out to find a rigid linker that could ideally lead to water-soluble glycosylated nanomolecules. After testing multiple linker designs, we found a sulfone-bridged biphenyl derivative (Figure 1a) to be the most suitable candidate. The rationale behind choosing this linker was two-fold – not only could the polar sulfone group promote the overall water solubility of the nanomolecule (our attempt to use a biphenyl motif resulted in a poorly water-soluble glycosylated cluster), but also a similar molecule, decafluoro-biphenylsulfone, was recently found to exhibit remarkably fast $S_N$Ar reactivity toward cysteine residues on peptides under aqueous conditions. Therefore, we hypothesized that perfunctionalization of 1 (Figure 1a) with the sulfone-bridged linker could enhance the $S_N$Ar reaction kinetics and impart aqueous compatibility to the cluster conjugation, resulting in a water-soluble glycosylated species. The target benzyl bromide linker containing a terminal $SO_2C_6F_5$ functional group was synthesized in three steps (see the Supporting Information for experimental details and characterization data). Using a microwave-assisted synthesis method, we observed nearly quantitative conversion of 1 to the perfunctionalized cluster within 30 minutes, based on $^{11}$B NMR spectroscopy and ESI-HRMS. The cluster species was isolated from the residual organic-based starting materials via silica gel chromatography in 94% yield. After subjecting the compound to a sodium ion exchange column, 3 (Figure 1a) was isolated as a light salmon-colored...
solid (see the Supporting Information for experimental details). $^1$H, $^{11}$B, and $^{19}$F NMR spectroscopy (Figure 1c) and ESI-HRMS results of 3 are consistent with the proposed structure and composition of the dodeca-functionalized B$_{12}$-based cluster (see the Supporting Information for characterization data).

To test whether cluster 3 exhibits enhanced S$_{\text{NAr}}$ reactivity toward thiols, we exposed 3 dissolved in DMF to a stoichiometric amount of an unprotected thiolated saccharide, 1-thio-$\beta$-D-glucose sodium salt, and observed by $^{19}$F NMR spectroscopy a nearly quantitative ($\geq$99%) conversion to 3c (Table 1, entry 7) within 20 minutes (Figure 1b, c). The purified water-soluble 3c was obtained via a desalting centrifugal filter, and was subjected to analysis via $^1$H, $^{11}$B, and $^{19}$F NMR spectroscopy and ESI-HRMS, which support the proposed structure and composition (see the Supporting Information for experimental details and characterization data). Notably, due to the rapid kinetics, this reaction did not require a N$_2$ atmosphere in order to proceed to completion, therefore all subsequent conjugation reactions of 3 were performed under ambient conditions. Parallel experiments monitoring the S$_{\text{NAr}}$ reaction conversion over time of 2 and 3 by $^{19}$F NMR spectroscopy revealed the significantly improved conversion rates of 3 over 2 (Figure 1b), which is consistent with our hypothesis. We then proceeded to test whether 3 tolerates water in the conjugation reaction by subjecting 3 to a stoichiometric amount of 1-thio-$\beta$-D-glucose sodium salt in 1:1 DMF:water and 1:1 acetonitrile (MeCN):water mixtures, and in both cases observed nearly quantitative ($\geq$99%) conversion to 3c within 15 minutes (Figure 1c) (see the Supporting Information for experimental details). These remarkably fast reaction kinetics in mixed aqueous/organic media are consistent with the observations by Kalhor-Monfared et al. and furthermore may be facilitated by the enhanced solubility of 1-thio-$\beta$-D-glucose sodium salt in water.$^{49}$ Overall, these studies demonstrate that by employing rational linker design, the S$_{\text{NAr}}$
reaction characteristics including kinetics and aqueous compatibility can be dramatically
enhanced, allowing for the rapid assembly of atomically precise, densely glycosylated
nanomolecules.

Based on the successful glycosylation of 2 to yield functionalized nanomolecules 2a–c, we
hypothesized that 3 could likewise be glycosylated by mannose and galactose in addition to
-glucose (vide supra). Treatment of 3 with the sodium salts of 1-thio-α-D-mannose and 1-thio-β-
D-galactose in 1:1 DMF:water mixtures resulted in nearly quantitative (≥99%) conversions within
15 minutes to 3a and 3b (Table 1, entries 5 and 6), respectively. Following purification, 3a and 3b
were subjected to characterization via 1H, 11B, and 19F NMR spectroscopy and ESI-HRMS, which
support the proposed structures and compositions (see the Supporting Information for
experimental details and characterization data). Furthermore, we were able to fully PEGylate 3
within 90 minutes, giving rise to purified 3d (Table 1, entry 8) after isolation in 84% yield (see the
Supporting Information for experimental details and characterization data). These experiments
demonstrate that cluster 3 can rapidly lead to a library of multivalent hybrid entities featuring
diverse functional groups, which allows us to study how the specific surface chemistry affects the
protein-binding properties. Ultimately, the family of precisely engineered multivalent
nanomolecules (2a–3 and 3a–d, vide supra) creates a framework which can potentially enable us
to study the fundamental rules that govern multivalent biological recognition events.

Following the assembly and isolation of the glycosylated and PEGylated clusters, we proceeded
to uncover the binding characteristics of the various nanomolecules toward an important dendritic
cell receptor, DC-SIGN. Among the existing techniques that can experimentally elucidate the
binding affinities between complex molecules and biomolecular targets, the surface plasmon
resonance (SPR) technology represents a “gold standard” used by researchers in both academic
and biotechnology communities.\textsuperscript{51,52} Given the ability of the SPR technology to perform real-time, label-free detection of biomolecular interactions with high sensitivity,\textsuperscript{52} we decided to use it for studying the binding interactions between the multivalent cluster nanomolecules and DC-SIGN. In the first set of SPR-based direct binding experiments, the tetrameric DC-SIGN extracellular domain (ECD) was immobilized on a commercial sensor chip \textit{via} standard amide coupling, and the mannose-functionalized nanomolecules 2\textit{a} and 3\textit{a} were injected over the protein surface for real-time visualization of their respective binding interactions with DC-SIGN (see the Supporting Information for experimental details). The resulting sensorgrams (Figure 2\textit{a}, \textit{d}) reflect changes in the refractive index as molecules interact with the lectin surface, and reveal the dose-dependent binding response of 2\textit{a} and 3\textit{a}, respectively, toward DC-SIGN. By fitting the Langmuir 1:1 binding model to the binding curves of the mannose-coated clusters, we estimated $K_D$ values of 0.11 $\mu$M for 2\textit{a} and 5.0 $\mu$M for 3\textit{a}. Compared to D-mannose (low mM affinity),\textsuperscript{11} these multivalent systems exhibit avidities three to four orders of magnitude higher for DC-SIGN through the cluster glycoside effect.\textsuperscript{3} To further understand the dynamics of the multivalent interactions, we performed computational studies using a tetrameric model derived from an X-ray structure of DC-SIGN (see the Supporting Information for experimental details).\textsuperscript{12,53} Molecular dynamics (MD) simulations of the interactions between the DC-SIGN model and 2\textit{a}/3\textit{a} over 40 ns were conducted, and snapshots were taken at the end of both simulations (Figure 2\textit{b}–\textit{c}/\textit{e}–\textit{f}, respectively; see the Supporting Information for experimental details and movies). The MD movies and snapshots suggest that consistent with previous reports using monosaccharides and oligosaccharides,\textsuperscript{11,12} the equatorial 3-OH and 4-OH groups on the cluster-linked mannose residues engage in $Ca^{2+}$-mediated binding in the carbohydrate recognition sites. Furthermore, 2\textit{a} was observed to stay longer than 3\textit{a} near the binding site of the protein model (Figure S16), which agrees with the lower $K_D$ value of
determined from the SPR experiments. A possible explanation for the observed difference in avidity is the flexibility of the linker – while the extended linker in 3a is still rigid, it allows more flexibility compared to the benzylic linker in 2a. Although a more flexible linker can relax the requirements for the precise positioning of ligands on a multivalent scaffold, it can also lower the overall affinity for a target protein.\textsuperscript{5}

After analyzing the binding interactions of mannose-coated cluster nanomolecules toward DC-SIGN, we hypothesized that the clusters grafted with other saccharides would exhibit different protein-binding behaviors. Therefore, we conducted another set of SPR-based direct binding studies with the glucose-coated nanomolecules (2c and 3c) (Figures S1 and S2), which yielded $K_D$
values of 0.18 and 30 µM, respectively. These similar but slightly higher $K_D$ values compared to
the mannose-coated analogs agree with results from previous reports using monosaccharides,$^{11,54}$
which suggest the equatorial 3- and 4-OH groups on glucose allow a similar binding interaction
with DC-SIGN. In contrast, the galactose-coated species (2b and 3b) were unable to engage DC-
SIGN with similar avidities (the estimated $K_D$ values were 0.87 and 96 µM, respectively; Figures
S3 and S4). This finding is also consistent with prior reports with monosaccharides and
glycopolymers,$^{11,24,54}$ since the axial 4-OH group on galactose prevents proper recognition by the
carbohydrate-binding sites on DC-SIGN. In contrast, the controls – PEGylated clusters (2d and
3d) and D-mannose – exhibit minimal to no binding to the protein surface when injected at the
highest mass concentrations with respect to 2a and 3a (Figure 2a, d). Overall, these experiments
reveal the dramatically enhanced binding avidities of the glycosylated cluster nanomolecules as a
result of multivalency and highlight a potentially intricate relationship between the scaffold
flexibility and the binding affinity. Nevertheless, in nature DC-SIGN is known to be a very flexible
transmembrane receptor that can reposition its carbohydrate recognition domains to adapt to the
ligands,$^{55}$ and this dynamic behavior is not fully captured by the immobilized protein setup in the
*in vitro* SPR and *in silico* MD experiments.

Therefore, we turned to SPR-based competitive binding assays in order to test 1) whether free
(vs immobilized) DC-SIGN exhibits different binding characteristics to the cluster nanomolecules
and 2) whether the mannose-coated species can inhibit the protein-protein interactions between
DC-SIGN and a sub-nM binder, HIV-1 gp120.$^{24,56}$ In these competition experiments, 100 nM DC-
SIGN and various concentrations of the nanomolecules were co-injected over the surface-
immobilized gp120, and the binding response of each injection was compared to that of each
preceding injection of DC-SIGN alone for an estimation of the % inhibition of the DC-SIGN–
gp120 interaction. As shown in Figure 3, 2a and 3a can both inhibit free DC-SIGN from attaching to gp120, with IC$_{50}$ values of 2.0 and 5.2 µM, respectively. These values are over three orders of magnitude lower than the reported IC$_{50}$ of monovalent D-mannose (6–9 mM),$^{54,57}$ indicating dramatically enhanced inhibition. Notably, compared with the IC$_{50}$ values from a similar SPR-based competition assay using a multivalent third-generation dendrimer (50 µM, 32 mannose residues),$^{27}$ these values are an order of magnitude lower. These results suggest that rigid inorganic cluster-based nanomolecules featuring significantly fewer (12) saccharides can serve as more potent inhibitors of this protein-protein interaction. Furthermore, in agreement with the direct binding data, the galactose-coated (2b, 3b) and PEGylated (2d, 3d) nanomolecules as well as D-mannose were less successful at inhibiting this interaction (Figures S5 and S6). Overall, these competition studies demonstrate for the first time the ability of multivalent glycosylated cluster nanomolecules to effectively compete against a sub-nM-binding viral glycoprotein for DC-SIGN. This suggests that a rigid cluster scaffold-based multivalent display of carbohydrates that mimics the natural highly glycosylated proteins on the surface of pathogens can be engineered to inhibit
the interactions between a cell-based lectin receptor and a viral glycoprotein. Moreover, the similarity in IC₅₀ values for 2a and 3a in contrast to their different Kᵤ values could be due to a combination of the free (vs immobilized) DC-SIGN better adapting to the more flexible nanomolecule 3a and the greater receptor surface coverage by the larger nanomolecule 3a.

To further investigate the ability of the mannose-functionalized cluster nanomolecules to inhibit the protein-protein interactions between DC-SIGN and gp120 in an experimental setup more reminiscent of natural systems, we moved to cell-based studies using a DC-SIGN-expressing human lymphoblast-like cell line (Raji DC-SIGN⁺ cells) and HIV-1 gp120 (Figure 4a).⁵⁸,⁵⁹ First, in order to gain a better understanding of the biocompatibility of the cluster nanomolecules, we conducted an MTS-based cell proliferation assay (see the Supporting Information for experimental details), and observed no apparent cytotoxic effects of the mannose-coated (2a, 3a) and PEGylated (2d, 3d) clusters toward Raji DC-SIGN⁺ cells at 0.5–50 µM concentrations (Figure 4b). This finding allowed the evaluation of the nanomolecules’ potential biological function in inhibiting the attachment of gp120 to cell-surface DC-SIGN. Fluorescein isothiocyanate-labeled gp120 (gp120-FITC) undergoes significant uptake by Raji DC-SIGN⁺ cells (Figure 4c), as observed by a confocal laser scanning microscopy-based assay (see the Supporting Information for experimental details and Figures S7–S15). This internalization is DC-SIGN-dependent since no gp120-FITC uptake was observed in a Raji cell line not expressing DC-SIGN (Figure 4c).⁶⁰,⁶¹ In order to test competitive inhibition, we introduced mixtures of gp120-FITC and mannose-coated clusters 2a/3a to Raji DC-SIGN⁺ cells, and observed reduced gp120-FITC uptake as a function of the cluster concentration (10 to 25 µM) (Figure 4c). Notably, at the same concentrations, 3a was more effective than 2a at preventing the binding and uptake of gp120-FITC. This result suggests that 3a can bind DC-SIGN in its natural transmembrane conformation better, which could be due
to its higher flexibility and larger size. Furthermore, these cell-based studies capture important information about the dynamic receptor-mediated antigen internalization process, thus enabling
us to assess both the nanomolecules’ binding to DC-SIGN and the inhibition of antigen uptake. Consistent with the presented SPR-based direct and competitive binding data, the control molecules – PEGylated clusters (2d and 3d) and D-mannose – were not able to bind to DC-SIGN and inhibit gp120 uptake at 25 µM (Figure 4c). Overall, the biological studies in cells reveal that biocompatible mannose-functionalized cluster nanomolecules are capable of competing against HIV-1 gp120 for cell-surface DC-SIGN and thereby preventing the receptor-mediated internalization of a viral envelope component.

Conclusions

We have demonstrated the rapid assembly of multivalent glycosylated inorganic cluster nanomolecules capable of inhibiting protein-protein interactions. Specifically, a dense layer of thiolated saccharides can be grafted on a rigid perfluoroaryl-perfunctionalized B_{12} cluster within 15 minutes in mixed aqueous/organic media using S_{N}Ar chemistry. The resulting fully covalent glycosylated assemblies can serve as multivalent binders with dramatically enhanced affinity compared to monovalent saccharides toward target lectins. We showed an example of using these hybrid agents for engendering ligand-specific, multivalent recognition with a biologically important dendritic cell receptor, DC-SIGN. Importantly, we demonstrated the ability of the cluster nanomolecules to inhibit protein-protein interactions between DC-SIGN and a sub-nM-binding HIV-1 envelope glycoprotein in a competitive binding study. We further found these clusters to be biocompatible in a human cell line and capable of preventing the internalization of gp120 by DC-SIGN-expressing cells. Notably, we uncovered an intricate interplay between the structural designs of multivalent binders and their biological activities. Ultimately, this work showcases a rare example of the application of tunable, stable inorganic cluster-based nanomolecules as
valuable tools for studying the rules that govern multivalent interactions and disrupting protein-protein interactions.\textsuperscript{63–65}

**Safety Statement**

No unexpected or unusually high safety hazards were encountered.

**Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website. All methods, synthetic procedures, characterization data, supplementary data, and MD simulation movies.

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**Author Contributions**

E.A.Q. and A.M.S. conceived the project and composed the manuscript. E.A.Q. designed and performed the experiments and analyzed the data. Y.H. and P.K. designed, conducted, and interpreted the computational studies. M.S.M. and H.D.M. contributed to the synthesis of \textit{2a} and \textit{2b}. All of the authors commented on the manuscript.

**Notes**

The authors declare no competing financial interest.
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