Spatiotemporal control and superselectivity in supramolecular polymers using multivalency


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Multivalency has an important but poorly understood role in molecular self-organization. We present the noncovalent synthesis of a multicomponent supramolecular polymer in which chemically distinct monomers spontaneously coassemble into a dynamic, functional structure. We show that a multivalent recruiter is able to bind selectively to one subset of monomers (receptors) and trigger their clustering along the self-assembled polymer, behavior that mimics raft formation in cell membranes. This phenomenon is reversible and affords spatiotemporal control over the monomer distribution inside the supramolecular polymer by superelective binding of single-strand DNA to positively charged receptors. Our findings reveal the pivotal role of multivalency in enabling structural order and nonlinear recognition in water-soluble supramolecular polymers, and it offers a design principle for functional, structurally defined supramolecular architectures.

One of the most fascinating features of living matter is the precise control over biological activity in space and time. The cell membrane provides a remarkable example of such high-fidelity spatiotemporal control in a complex biological setting, wherein thousands of different components, namely lipids and proteins, self-assemble into a 2D fluid mosaic (1). To perform the functions that the cell requires, lipids and proteins are heterogeneously distributed and specific biomolecules are segregated in active nanometer-sized domains often referred to as rafts (2). This distribution is highly dynamic, such that these platforms can be rapidly assembled and disassembled (3). The principles that underlie control over the molecular composition of the cellular microenvironment in space and time are the subject of great scientific debate as they are of crucial importance for cell functioning, signaling, growth, and division (4).

One of the main goals of supramolecular chemistry is the noncovalent synthesis of functional molecular architectures through weak and reversible interactions (5). In this framework, a key challenge is the design of molecular building blocks that are able to self-organize hierarchically and in a cooperative fashion (6), thus mimicking the dynamic and structural complexity of living systems as well as their functionality. Various modular multicomponent systems have been successfully developed (7), but the spatiotemporal control of the localization of distinct components within synthetic supramolecular assemblies has yet to be realized. Mastering the spatial distribution of assembled molecules in a noncovalent synthesis is as crucial for their functionality as regio-selectivity impacts the molecular properties of organic molecules synthesized in a classical covalent manner. An interesting supramolecular polymer, where multiple components coassemble cooperatively in water, is based on 1,3,5-benzenetricarboxamide derivatives (BTAs) (8). Of particular relevance for the present work, is the fact that reversible interactions between monomers confer a dynamic behavior to BTA supramolecular polymers, wherein monomers are constantly being exchanged with the bulk (8). Most of these studies were in organic solvent, but we recently describe how pegylated BTAs self-assemble in water through a combination of hydrogen bonding and hydrophobic effects to create long supramolecular polymers of ~0.1–10 μm in length (9).

In the biology and chemistry communities, there is intense interest in the role of multivalency as a tool to encode structure and function into soft materials. Several fascinating papers reported how nature exploits multivalency to exert control over spatial segregation and phase separation inside living cells (10). Segregation of proteins and sphingolipids in lipid raft domains (11) is a remarkable example of this phenomenon. Likewise, the chemistry community is keen to use the efficacy of multivalent interactions as a “chemical organization and action principle” (12). Interfacing chemistry with biology, two recent innovative studies demonstrate the use of synthetic multivalent binders to drive receptor clustering and raft formation on the cell membrane (13) (14). Collectively, these reports convincingly show how multivalency can be used to create well-defined structures and elicit a biological response. Moreover, a recent study has proposed an analytical model describing the nature of superselective binding as a consequence of multivalency (15). Can we now profit from the same organization principle to induce order into synthetic supramolecular structures?

Inspired by natural systems and the formation of rafts in the cell membrane in particular, we present a study that demonstrates control of the spatiotemporal distribution of assembled BTAs through dynamic and multivalent interactions in water. We find that both the dynamic nature of the molecular (dis) assembly and multivalency are key requirements to establish such control at the molecular level. Finally, we demonstrate the emergence of a strongly nonlinear effect, namely superselective recognition, as a direct consequence of the multivalent nature of synthetic supramolecular structures. The observation of these phenomena has important ramifications for the understanding and application of supramolecular materials in water. The control over the spatiotemporal distribution of distinct components in a supramolecular aggregate is an important step toward the design of switchable functional systems. Moreover, the adaptive behavior and the superselective binding, reported in this work, shows that BTA-based supramolecular polymers are versatile tools for molecular recognition in a broad range of applications including biomedical materials.


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Results and Discussion

Multivalent supramolecular polymers based on BTAs were prepared via coassembly of different types of pegylated BTAs (14) as displayed in Fig. 1 (see SI Text for details on the BTA synthesis). The three different building blocks reported in this work present the same hydrogen-bonding motif in the hydrophobic core but vary in the chemical composition of the peripheral side chains (Fig. 1A). Neutral BTA monomers have been coassembled together with cationic species, indicated as BTA$^{+\text{NH}_3}$, into multicomponent one-dimensional structures (9). BTA$^{+\text{Cy3}}$ and BTA$^{+\text{Cy5}}$ are asymmetric monomers bearing at the PEG termini two primary amines, positively charged at neutral pH, and a cyanine dye (Cy3 or Cy5, both bearing one positive charge) as a fluorescent label. In our design, the neutral monomers serve as a scaffold; i.e., they represent the synthetic analog of the liquid phase of the plasma membrane. The cationic monomers are incorporated in small amounts ranging from 0.1% to 8% and act as receptors capable of electrostatic binding to oppositely charged ligands. The coassembly process results in a supramolecular analog of random copolymers with a stochastic distribution of monomers along the polymer chain (Fig. 1B, Left). The ligand-mediated rearrangement, similar to an isomerization, represents a subsequent step in the noncovalent synthesis and results in nonrandom, segmented monomer sequences (Fig. 1B, Right). Here, we exploit single-stranded DNA (ssDNA) as the multivalent, polyanionic tool necessary to recruit the cationic BTA$^{+\text{Cy}}$ receptors and induce their clustering.

The characterization of the chemical composition (i.e., the monomer sequence) of multicomponent supramolecular polymers is complicated by the fact that their structure is dynamic and will change with a variation of the experimental conditions, such as monomer concentration, temperature, and the presence of ligands. In fact, the presence of spatial inhomogeneities in the dynamic environment of the plasma membrane remained controversial for decades, precisely because of the lack of suitable experimental techniques to probe such a complex system with sufficient temporal and spatial resolution. In recent years, progress has been made as various assays, mostly based on fluorescence, have been developed to probe the formation and breakup of solid-like domains within the otherwise liquid-like cell membrane (16). Similarly, the present study relies on sensitive fluorescence assays to probe the reversible assembly and disassembly of clusters of charged BTAs in a neutral BTA scaffold. For this purpose, we labeled BTA$^{+\text{Cy}}$ monomers with a pair of dyes well suited for Förster resonance energy transfer (FRET). It is one of the most powerful biophysical tools used to demonstrate clustering of receptors inside the plasma membrane (17). We selected the FRET pair Cy3/Cy5, with a Förster radius of 50 Å (18), to probe monomer–monomer proximity on the molecular scale.

Exploiting this fluorescent tool, we first evaluated the dynamics of the multicomponent supramolecular polymers as it is a prerequisite for spatiotemporal control over the monomer sequence. Obviously, BTA receptors can only cluster upon binding to a multivalent ligand if they are able to exchange position within the supramolecular chain. To investigate whether water-soluble supramolecular polymers are able to reconfigure on a molecular level, we separately assembled supramolecular polymers incorporating either Cy3 or Cy5 BTAs as shown in Fig. 2A. After equilibration, the two polymer solutions were mixed and the FRET ratio monitored in time. If the structures are unable to exchange monomers, no change in fluorescence should be observed after mixing. However, if the polymers are endowed with a dynamic nature, mixed fibers containing both Cy3 and Cy5 should be obtained resulting in an increase of FRET due to proximity.

Fig. 2B shows the results of the mixing experiments. As can be observed, FRET increases in time after mixing and reaches a plateau in ~3 h. This indicates that the monomer sequence is not frozen like in a covalent polymer but molecules can dynamically exchange position. This exchange is remarkably slow in comparison with what has been observed for supramolecular...
polymers in organic solvent, where the exchange occurs on a second timescale (19). This difference can be attributed to the stronger association in water owing to the hydrophobic effect, which results in an enhanced BTA aggregation that slows down the depolymerization of the aggregates. To gain insight into the rearrangement process, we measured the exchange kinetics at different concentrations (Fig. 2B) and temperatures (Fig. 2C). Concentration does not significantly influence the rate of the reorganization over one order of magnitude. In contrast, the exchange rate is significantly faster at higher temperatures. These results resemble observations on self-assembled polymeric micelles, in which monomer expulsion and insertion is the rate-determining step of system dynamics (20, 21). Moreover, monomer polymerization and depolymerization are reported to be the main exchange mechanism of supramolecular polymers in organic solvents (22). The limited effect of concentration on the exchange rate indicates that stack–stack interactions do not play a pivotal role while free monomers seem to be the mediators of the polymer chain rearrangement. The information obtained about the dynamic behavior and the timescales for monomer exchange are of great importance for the study of spatiotemporal control proposed in this work.

Having verified that it is possible to rearrange the monomer sequence within and between supramolecular polymers, we continued to study the change in BTA distribution upon ssDNA addition. ssDNA is particularly appealing as recruiter due to its multivalency (i.e., the number of charged groups) can be precisely tuned through solid-phase DNA synthesis. Fig. 3A shows the plot of FRET versus time upon DNA treatment.

The FRET signal displays a dramatic increase upon ssDNA addition, as a result of the interactions of the polyanion with the labeled charged receptors. The change in FRET is correlated to the significant reduction in the average distance between charged monomers that occurs when the random distribution is converted into a more segmented one. This finding supports the idea that the DNA is able to bind the charged monomers and, due to its multivalent character, force their clustering along the stack into 1D domains. Interestingly, when a large excess of a monovalent anionic competitor, phosphate ion, is added, the trend is completely reverted (Fig. 3A). This result has two major implications. First, it confirms that the binding is dominated by electrostatic interactions, as an excess of phosphate ions disrupts the clustering. It also reveals the necessity of multivalency for receptor clustering: the FRET ratio measured in the presence of monovalent phosphate ions indicates that the monomer distribution is comparable to the stochastic one.

The clustering kinetic is remarkably slow: more than 24 h are needed to reach a plateau in FRET efficiency. However, this time frame is in full agreement with the time needed for monomer exchange. Interestingly, BTAs retain their dynamic behavior even when bound to ssDNA, owing to the reversible nature of electrostatic interactions. Indeed, when ssDNA is added to the individual polymers with only Cy3 or Cy5 BTAs first, followed by mixing, monomers can still exchange between different clusters as shown in SI Text. This interesting feature further highlights the fundamental difference between the exertions of multiple weak interactions compared with the use of permanent covalent crosslinking. Noncovalent interactions afford control over the monomer distribution without irreversibly “freezing” the polymer structure.

To clarify the role of multivalency in monomer clustering, we performed a series of FRET experiments varying recruiter length and receptor density (Fig. 3B). As expected, the highest efficiencies are obtained for the combinations of high density and long DNA strands (top right corner of the 3D plot in Fig. 3B). However, a marked nonlinear dependence on both receptor density and DNA length is observed, as highlighted in Fig. 3C–E. At lower densities (from 0.1% to 0.5%), no clustering occurs regardless of the length of the DNA (Fig. 3C). At a critical threshold around 1% (Fig. 3D), an increase of FRET is observed, which becomes stronger upon an increase in the receptor density (Fig. 3E). Moreover, it is evident from Fig. 3E that the clustering has a nonlinear dependence on the length of the DNA; no effect is observed for short DNA strands and clustering starts to occur for strands longer than 12–15 DNA bases.

To fully understand the observed experimental results, we performed μVT lattice Monte Carlo (MC) simulations (23) of a coarse-grained model. DNA chains are represented as beads
on a lattice connected through bonds, with each bead representing a DNA base. We simulated only a single BTA columnar stack, by prealigning BTA molecules (represented as single lattice sites) along the z direction of the simulation box. When a bead from the DNA chain is in nearest-neighbor contact with a charged BTA molecule, the system lowers its energy by an amount equal to $\varepsilon$. In this work, $\varepsilon$ was varied between 0 and 10 kT, where $k$ is Boltzmann’s constant and $T$ is the absolute temperature. To simulate the dynamical behavior of the molecules in the polymer, the positions of charged and uncharged molecules are exchanged by means of standard Metropolis MC moves (23). A detailed description of the implementation of the simulations is reported in SI Text.

A typical snapshot of such simulations is shown in Fig. 4 A and shows that DNA binds selectively to the charged monomers forcing their clustering along the BTA chain. To compare the computational results with the FRET measurements reported, we analyzed the simulations and gathered information about clustering and selectivity (Fig. 4 B–D). As FRET between red and green dyes is effective only within a cutoff distance of a few nanometers, we tracked in the simulations the number of nearest-neighbor red–green pairs as an indication of the FRET signal strength (Fig. 4B). Note that taking contributions beyond nearest neighbors into account was shown to have an insignificant effect on the observed results (SI Text). We find that, for DNA–BTA$^{3+}$ energy of interaction $\varepsilon = 4.50$ kT, the simulation results (Fig. 4B) are remarkably similar to the experimental results presented in Fig. 3, whereas a variety of qualitatively different behaviors are found for other values of $\varepsilon$ (SI Text). For low fractions of charged BTA (<1%), no clustering is observed and the number of green–red dye pairs is not dependent on DNA length. In contrast, for higher receptor densities (>1%), we observed a distinctive nonlinear behavior: no signal is observed below a critical DNA length (~12-mer), whereas a rapid increase of clustering is observed above that threshold.

The excellent agreement between the experiments and simulations is remarkable given the simplicity and coarse-grained nature of our model, suggesting that the principles behind multivalency and recruitment are general and system independent. Indeed, the nonlinearity of our results can be understood in the light of multivalency and superselectivity. We previously reported an analytical model in which is demonstrated that, under specific conditions, multivalent particles are capable of superselective binding to receptors, i.e., that the fraction of bound particles varies nonlinearly with the receptor density (15). Although this model was originally developed for the case in which the receptors are “fixed” to the surface (i.e., nondynamic behavior), we have extended it for the case in which the receptors are mobile (SI Text). In this case, the model predicts that the mobility of the receptors allows for an even sharper dependence of the number of bound particles on receptor density, increasing the superselective behavior of the system at the appropriate conditions (SI Text). In fact, our MC simulations show that the number of bound ssDNA molecules depends sharply on the receptor (i.e., BTA$^{3+}$) concentration. Hence, for a given DNA length, there should be a critical charge density in the polymer above which DNA binds significantly to the supramolecular polymer and receptors can be clustered. At low receptor concentration, few chains are bound and little clustering occurs. However, as the percentage of receptors increases and we approach the threshold concentration, the number of bound chains starts to increase dramatically resulting in receptor clustering. Because for dilute solutions this transition becomes more abrupt—in a
nonlinear way—more clustering occurs for longer chains. Thus, at the conditions studied we can expect that for chains with lengths above the 12-mer the threshold receptor concentration occurs slightly above 1% of charged BTA, whereas for shorter chains the threshold concentration is higher than the concentrations studied (i.e., 8%) and therefore is not observed.

As discussed in ref. 15, the threshold concentration corresponds to the receptor concentration at which the quantity $\alpha$ reaches a maximum value. The quantity $\alpha$ is defined as follows:

$$\alpha \equiv \frac{d \ln N_b}{d \ln n_R}$$  \[1\]

where $N_b$ is the number of DNA chains bound to the supramolecular polymer and $n_R$ is the charge density. Because locally $N_b \sim n_R^\gamma$, the value of $\alpha$ indicates how fast the number of bound particles changes with receptor concentration. Hence, at the point where $\alpha$ is maximum, the number of bound particles changes the fastest with $n_R$, therefore indicating the onset of DNA binding and clustering. To test this hypothesis, we computed $\alpha$ from the simulations for various DNA lengths (Fig. 4C).

DNA chains with lengths of 4- and 10-mers do not reach a peak in $\alpha$ for $n_R < 8\%$, indicating that shorter chains do not reach the threshold concentration and cannot induce significant clustering. Conversely, longer chains (e.g., 20-mers) present a peak in the value of $\alpha$ around 1–2% consistent with the experimental results. Thus, our simulations strongly suggest that, in the experiments, our supramolecular system is behaving superselectively. Hence, the phenomenon of superselectivity, which has only been described for biological systems, can explain the behavior of fully synthetic architecture as well.

To further understand the change in receptor distribution along the polymer, we turned to a quantification of BTA$^{3+}$ clustering. We divided the supramolecular polymer into arbitrary “macrolattices” of a large enough size (20 beads in this work) and we computed the frequency of a given number of charged monomers within these macrolattices. Fig. 4D displays such distributions for a 20-mer chain, with 8% charged monomers, for different values of $\varepsilon$. When there is no attractive energy (i.e., $\varepsilon = 0$), the charged beads distribute randomly and the distribution approaches a binomial distribution. However, as the attractive interaction becomes stronger, the distribution becomes more bimodal, indicating the existence of regions of the polymer with many charged monomers (i.e., clusters) and regions of no charged monomers, while keeping the total charge fixed. The appearance of clustering under conditions of multivalency, high values of $\varepsilon$, and low bulk concentration of DNA, is consistent with the prediction of the simple analytical model presented in SI Text, where we have extended the multivalency model proposed in ref. 15 to take the mobility of the receptors into account.

The computational analysis provides important insights into the roles of multivalency and dynamics in our supramolecular system. From a kinetic point of view, a critical number of initial binding interactions is required to stably anchor DNA onto the BTA fiber after which recruitment of more charged monomers can occur. The initial number of anchoring points depends both on DNA multivalency and receptor density, which is consistent with the observed nonlinear dependence on these two quantities. According to the model proposed by Hlavacek and coworkers for multiple receptor–ligand interaction (24), the number of initial binding sites determines the time that the DNA is bound to the BTA stack before detachment. If the residence time is comparable to the timescale of monomers rearrangement, which is about 2–3 h (Fig. 2), monomer exchange can provide additional receptors for DNA binding. The resultant increase in the number of binding sites further strengthens the DNA–polymer interaction...
and augments the residence time of the DNA onto the BTA stack. This synergy creates a positive-feedback loop in which multivalency and dynamics, if synchronized, act in a cooperative fashion to induce order in self-assembled systems.

**Conclusions**

In conclusion, we present a supramolecular system in which multiple components coassemble in a dynamic fashion. We investigated the role of multivalency and dynamics and show how they drive clustering of molecules within a synthetic self-assembled structure. These phenomena have two main implications. First, these findings demonstrate how a multivalent binder can induce order into a supramolecular polymer, allowing control over the spatiotemporal distribution of the monomers. This is of great interest to design well-defined functional supramolecular systems through noncovalent synthesis. Second, we have shown how the dynamic behavior of noncovalent architectures makes them adaptive, and affords superselective recognition of specific biomolecules. This responsive behavior is of great interest for biological recognition and makes functional supramolecular polymers a promising scaffold for several applications in the biological environment.

**Methods**

Chemicals were purchased from Sigma-Aldrich and used without further purifications. Cy3-NHS and Cy5-NHS esters were purchased from Lumiprobe. DNA oligos with different length were obtained from Eurofins. Dialysis membranes were obtained from Spectrum Laboratories. 1H-NMR and 13C-NMR spectra were recorded on a Varian Mercury Vx 400 MHz NMR spectrometer. Matrix-assisted laser desorption/ionization mass spectra were obtained on a PerSeptive Biosystems Voyager DE-PRO spectrometer. Thermogravimetric analysis was performed on a Perkin-Elmer TGA 7 instrument. ATR sampling was performed on a Perkin-Elmer Spectrum Two FT-IR spectrometer, equipped with a Perkin-Elmer Universal ATR Sampler Accessory. The synthetic scheme for the synthesis of cationic BTA3+ is reported in Fig. S1. The detailed procedures for the synthesis are reported in SI Text. The synthesis of neutral pegylated BTAs was previously reported (9). For BTA polymers assembly procedure stock solution of BTA (10 mM) and labeled BTA3+ (1 mM) in MeOH were prepared. The organic solvent solutions were mixed at the desired ratio to control the molar ratio of BTA3+ on BTA, injected in filtered Milli-Q water (total concentration BTA, 50 μM) and equilibrated for 24 h before experiment. Samples at different concentrations were obtained by serial dilution with Milli-Q water of the 50 μM stock. For mixing kinetic experiments, solution containing either 2% of BTA-Cy3 or 2% of BTA-Cy5 were mixed 1:1 in a 500-μL glass cuvette and immediately measured at the concentration of 25 μM was added to the BTA solutions to reach a final concentration of 500 nM.

To achieve FRET ratio, samples were excited at 540 nm (Cy3 excitation) and fluorescence emission measured at 570 nm (Cy3 emission) and 670 nm (Cy5 emission). Temperature was kept at 20 °C with the in-built peltier of the fluorimeter. The array of FRET measurements reported in Fig. 3b was obtained by means of a plate reader. Samples with different BTA3+ densities were prepared (total volume, 50 μL) and incubated with different DNA strands for 48 h in a 96-well plate at 20 °C. Three samples were independently prepared for every BTA3+-BTA combination. After 48 h, fluorescence emission at 570 and 670 nm was measured for every sample and the FRET ratio ± SD (n = 3) calculated.

The details of the MC simulation and the analytical model for the binding of multivalent recruiting to dynamic receptors are reported in SI Text.

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4. Leslie M (2011) Mysteries of the cell. Do lipid rafts exist?
Supporting Information

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SI Text

SI Methods

Chemicals were purchased from Sigma-Aldrich and used without further purifications. Cy3-N-hydroxy succinimide (NHS) and Cy5-NHS esters were purchased from Lumiprobe. DNA oligos with different length were obtained from Eurofins. Dialysis membranes were obtained from Spectrum Laboratories. Flash chromatography was performed on a Biotage flash chromatography system using 200–425 mesh silica gel (type 60A, grade 633). Water was purified on an EMD Millipore Milli-Q Integral Water Purification System. 1H-NMR and 13C-NMR spectra were recorded on a Varian Mercury Vx 400 MHz (100 MHz for 13C) or a Varian Mercury Plus 200 MHz (50 MHz for 13C) NMR spectrometer. Chemical shifts are given in parts per million (ppm) values relative to residual solvent or tetramethylsilane (TMS). Splitting patterns are labeled as s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; and b stands for broad. Matrix-assisted laser desorption/ionization mass spectra were obtained on a PerSeptive Biosystems Voyager DE-PRO spectrometer or a Bruker autoflex speed spectrometer using α-cyano-4-hydroxycinnamic acid (CHCA) and 2-(2′-E)-3-(4-tert-butylyphenyl)-2-methylprop-2-ylidenemalononitrile (DCTB) as matrices. Infrared spectra were recorded on a Perkin-Elmer Spectrum One 1600 FT-IR spectrometer or a Perkin-Elmer Spectrum Two FT-IR spectrometer, equipped with a Perkin-Elmer Universal ATR Sampler Accessory.

Synthetic Procedures

The synthetic scheme for the synthesis of cationic BTA2+ is reported in Fig. S1. The detailed procedures for the synthesis are reported in the following. The synthesis of neutral pegylated 1,3,5-benzenetetracarboxamide derivatives (BTAs) was previously reported (1).

Tetraethylene glycol monotosylate (1). A round-bottom flask (1 L) was charged with tetraethylene glycol (0.51 mol, 99.4 g) and THF (100 mL). The mixture was placed in an ice bath and NaOH (0.078 mmol, 3.10 g) was dissolved in water (18 mL) and added carefully. The mixture was stirred for 15 min and p-toluene-sulfonyl chloride (0.052 mmol, 3.10 g) was dissolved in water (10 mL). The organic layer was added in 2 h using an addition funnel. The mixture was stirred for 2 h. Water (300 mL) was added and the THF was removed in vacuo. The remaining aqueous mixture was extracted three times with dichloromethane (200 mL). The organic fractions were combined and washed three times with water (200 mL). The organic layer was washed with brine (200 mL) and dried with sodium sulfate. The obtained material was dissolved in acetonitrile (100 mL), was added in 2 h using an addition funnel. The mixture was stirred for 2 h. Water (300 mL) was added and the THF was removed in vacuo. The remaining aqueous mixture was extracted three times with dichloromethane (200 mL). The organic fractions were combined and washed three times with water (200 mL). The organic layer was washed with brine (200 mL) and dried with sodium sulfate. The obtained material was dissolved in acetonitrile (100 mL), and concentrated in vacuo, and this was repeated with toluene (100 mL). Yield = 6.7 g, 98%. 1H NMR (400 MHz, CDCl3, δ): 3.81–3.57 [m, 14H, O-(CH2)2-O], 3.40 (t, J = 4.9 Hz, 2H, N3-CH2), 2.49 (t, J = 6.0 Hz, 1H, CH2-OH). 13C NMR (100 MHz, CDCl3, δ): 72.47, 70.72, 70.69, 70.62, 70.37, 70.06, 61.78, 50.68.

Azidotetraethylene glycol-12-bromododecyl ether (3). A round-bottom flask (250 mL) was charged with azidotetraethylene glycol (2) (38.8 mmol, 8.5 g) and THF (160 mL) was added. The stirred solution was placed in an ice bath and sodium hydride (60% in mineral oil, 2.1 g, 52.5 mmol) was added in portions. After 1 h, the ice was washed and 1,12-dibromododecane (0.26 mol, 87 g) was added. The mixture was stirred at room temperature overnight. The mixture was filtered, and the filtrate was concentrated in vacuo. The obtained material was dissolved in hot methanol. Upon cooling, a large portion of the 1,12-dibromododecane crystallized out and was removed by filtration. The filtrate was concentrated, yielding an oil that was purified by dry column vacuum chromatography (column diameter, 6 cm; height, 6 cm) (elucent heptane/ethylic acetate, 100/0–50/50). Yield = 7.4 g, 41%. 1H NMR (400 MHz, CDCl3, δ): 3.76–3.53 [m, 14H, O-(CH2)2-O], 3.51–3.33 (m, 6H, CH2-CH2-CH2-O, CH2-CH2-Br, NH2-CH2), 1.93–1.79 (m, 2H, CH2-CH2-Br), 1.62–1.51 (m, 2H, CH2-CH2-CH2-O), 1.47–1.37 (m, 2H, CH2-CH2-CH2-O). 1.37–1.16 (m, 14H, aliphatic). 13C NMR (100 MHz, CDCl3, δ): 71.53, 70.70, 70.68, 70.63, 70.62, 70.59, 70.04, 70.02, 50.68, 34.05, 32.82, 29.62, 29.55, 29.52, 29.50, 29.46, 28.75, 28.16, 26.07.

Azidotetraethylene glycol-12-phthalimidododecyl ether (4). A round-bottom flask (250 mL) was charged with azidotetraethylene glycol-12-bromododecyl ether (3) (13.6 mmol, 6.34 g) and methyl isobutyl ketone (MIBK) (80 mL). To the mixture, 18-crown-6 (1.9 mmol, 0.50 g) and potassium phthalimide (40.5 mmol, 7.5 g) were added, and the mixture was stirred at reflux overnight. The reaction mixture was allowed to cool to room temperature and, subsequently, filtrated. The filtrate was concentrated in vacuo and purified by column chromatography (elucent heptanes/ethylacetate, 100/0–0/100). Yield = 6.4 g, 88%. 1H NMR (400 MHz, CDCl3, δ): 7.84 (dd, J = 5.4, 3.0 Hz, 2H, phthalimide), 7.70 (dd, J = 5.4, 3.1 Hz, 2H, phthalimide), 7.34–7.51 [m, 16H, O-(CH2)2-O, CH2-CH2-CH2-N], 3.44 (t, J = 6.8 Hz, 2H, CH2-CH2-CH2-O), 3.38 (t, J = 5 Hz, 2H, N3-CH2), 1.74–1.62 (m, 3H, CH2-CH2-CH2-O), 1.59–1.46 (m, 2H, CH2-CH2-CH2-O), 1.38–1.18 (m, 16H, aliphatic). 13C NMR (100 MHz, CDCl3, δ): 168.46, 133.81, 132.19, 131.36, 131.73, 71.53, 70.70, 70.68, 70.63, 70.62, 70.59, 70.04, 70.02, 50.68, 34.05, 32.82, 29.62, 29.55, 29.52, 29.50, 29.46, 28.75, 28.16, 26.07.

Azidotetraethylene glycol-12-aminododecyl ether (5). A round-bottom flask (250 mL) was charged with azidotetraethylene glycol-12-phthalimidododecyl ether (4) (12.0 mmol, 6.4 g) and ethanol (160 mL). To the stirring solution, hydrazine monohydrate (6.0 mL) was added, and the mixture was stirred at reflux overnight. The solvent was removed in vacuo, yielding a white solid. The obtained material was dissolved in dichloromethane (400 mL) and extracted with sodium hydroxide (1 M, 250 mL). The aqueous layer was extracted with dichloromethane (120 mL). The organic fractions were combined, extracted with brine (200 mL), dried with sodium sulfate, filtrated, and concentrated in vacuo. The aqueous mixture was extracted two times with chloroform (200 mL). The organic fractions were combined, washed with brine (100 mL), and dried with sodium sulfate. The obtained material was dissolved in acetonitrile (100 mL), and concentrated in vacuo, and this was repeated with toluene (100 mL). Yield = 6.7 g, 98%. 1H NMR (400 MHz, CDCl3, δ): 3.81–3.57 [m, 14H, O-(CH2)2-O], 3.40 (t, J = 4.9 Hz, 2H, N3-CH2), 2.49 (t, J = 6.0 Hz, 1H, CH2-OH). 13C NMR (100 MHz, CDCl3, δ): 72.47, 70.72, 70.69, 70.62, 70.37, 70.06, 61.78, 50.68.
vacuo. Yield = 4.7 g, 97%. 1H NMR (400 MHz, CDCl 3): 3.76–3.53 [m, 14H, O-(CH2)2-O], 3.44 (t, J = 6.8 Hz, 2H, CH2CH2CH2O), 3.39 (t, J = 4.9 Hz, 2H, NH2CH2), 2.68 (t, J = 7.0 Hz, 2H, CH2CH2NH2), 1.64–1.50 (m, 2H, CH2CH2CH2O), 1.49–1.37 (m, 2H, CH2CH2CH2O), 1.37–1.12 (m, 16H, aliphatic). 13C NMR (100 MHz, CDCl 3): 71.52, 70.68, 70.66, 70.62, 70.51, 70.58, 70.05, 70.01, 50.67, 42.28, 33.88, 29.65–29.45, 26.88, 26.07. FT-IR (ATR) ν (cm -1): 3.578, 2.923, 2.853, 2.100, 1.595, 1.465, 1.349, 1.286, 1.250, 1.109, 1.039, 992, 851, 722, 646, 556, 506. Liquid chromatography-MS (electrospray ionization): Rt = 7.47 min, calculated M+ = 402.32 g/mol, observed m/z = 403.50 [MH+].

N′,N′,N′,N′-tris(1-azido-3,6,9,12-tetraoxatetradecan-24-yl)benzene-1,3,5-tricarboxamide (6). A round-bottom flask (250 mL) was charged with azidotetraethyleneglycol-12-aminoxydodecylether (5) (1.0 mmol, 0.438 g) and CHCl3 (10 mL) and kept at 0 °C by means of an ice bath. To this stirring solution, 160 mg (1.5 eq) of triethylamine and 90 mg (0.3 eq) of 1,3,5-benzenetricarbonyl trichloride were added. The resulting solution was stirred at 0 °C for 30 min and at room temperature overnight. The product was purified by column chromatography (eluent CHCl3/methanol, 100/1–2/10). Yield = 335 mg, 82%. 1H NMR (400 MHz, CDCl3, δ): 3.75–3.60 [m, 42H, O-(CH2)2-O], 3.41 (t, 6H, CH2CH2CH2O), 2.84 (t, 6H, NCH2CH2), 1.62–1.12 (m, 60H, aliphatic), 3.42 (t, 6H, NH2CH2), 3.24 (t, 6H, CH3CH2NH2), 1.62–1.12 (m, 60H, aliphatic), 8.36 (s, 3H, Ar-H), 6.73 (s, 3H, CO-NH).

N′,N′,N′,N′-tris(1-amino-3,6,9,12-tetraoxatetradecan-24-yl)benzene-1,3,5-tricarboxamide (7). A round-bottom flask (10 mL) was charged with N′,N′,N′,N′-tris(1-azido-3,6,9,12-tetraoxatetradecan-24-yl)benzene-1,3,5-tricarboxamide (6) (180 mg) and methanol (5 mL), and N2 (g) was led through the stirred solution for 10 min. Subsequently, Pd/C (catalytic amount) was added and a balloon filled with H2 (g) was connected. The reaction mixture was vigorously stirred under H2 (g) atmosphere overnight at room temperature. The reaction mixture was filtered over celite and concentrated in vacuo, yielding 7 as a yellow wax. Yield = 144 mg, 85%. 1H NMR (400 MHz, CDCl3, δ): 3.75–3.60 [m, 42H, O-(CH2)2-O], 3.41 (t, 6H, CH2CH2CH2O), 2.84 (t, 6H, NCH2CH2), 1.62–1.12 (m, 60H, aliphatic), 8.37 (s, 3H, Ar-H), 6.73 (s, 3H, CO-NH). 13C NMR (100 MHz, CDCl3, δ): 165.80, 135.21, 128.12, 128.12, 73.30, 71.52, 70.60, 70.58, 70.54, 70.25, 70.02, 41.71, 40.33, 29.56, 29.52, 29.49, 29.44, 29.38, 29.2, 26.94, 26.02. MALDI: calculated M+ = 1,284.98 g/mol, observed m/z = 1,286.00 [MH+].

General Procedure for BTA + Labeling. To perform FRET experiments, cationic BTA were labeled either with Cy3 or Cy5 dyes. The conjugation has been achieved by means of commercially available activated NHS esters of the dyes. Briefly, N′,N′,N′,N′-tris(1-amino-3,6,9,12-tetraoxatetradecan-24-yl)benzene-1,3,5-tricarboxamide (7) (5 mg) was dissolved in 1 mL of DMSO and triethylamine (5 eq) and Cy dye (0.8 eq) added. The solution was stirred overnight at room temperature and then diluted with 5 mL of water. Purification was achieved by water dialysis (molecular weight cutoff = 1,000 Da) to remove the uncured dye. Dye conjugation was verified by means of UV-visible spectroscopy and MALDI mass spectroscopy.

Monte Carlo Simulations

We performed μVT lattice Monte Carlo (MC) simulations, in which DNA chains are inserted and removed from the system to establish equilibrium with a reservoir fixed at a bulk concentration of 8.13 × 10−7 chains per lattice site. As an approximation, DNA chains are represented as beads on a lattice connected through bonds, with each bead representing a DNA base. We use a simple cubic lattice in which bonds are allowed between the edges of each site as well as between diagonals sites, yielding to a total of 26 neighbors per lattice site. If we assume that the length of each lattice site corresponds to ~0.3 nm, we obtain that the bulk concentration of chains is roughly 500 nM in agreement with experimental conditions.

In addition to DNA chains, the system must contain BTA molecules clustered in columnar arrangements. In the interest of simplicity, we represent only a single one of these columnar clusters, by prealigning BTA molecules (represented as single lattice sites) along the z direction. Because of the periodic boundary conditions, the cluster becomes effectively of infinite length. Because BTA molecules are not allowed to move in the x–y plane but can only exchange position with other (aligned) BTA molecules, the cluster preserves its original shape during the simulation. Also, to this level of approximation, BTA molecules interact with each other only through excluded volume interactions (i.e., two molecules cannot be in the same lattice site at the same time).

There are three types of BTA molecules: neutral (gray), charged (red), and charged (green). Although all of the BTA molecules exhibit excluded volume interactions with the DNA chains, only the charged BTA molecules experience a nearest-neighbor attraction toward DNA chain beads. In particular, when a bead of a chain is in nearest-neighbor contact with a charged BTA molecule, the system gains an interaction energy equal to −ε. In this work, ε was varied between 0 and 10 kT, with k, Boltzmann’s constant, and T, the absolute temperature. An interaction energy of ε = 4.50 kT best represented the experimental conditions. Because BTA molecules are stacked along the z direction, in principle each BTA molecule has four nearest-neighbor sites with which to have potential attractive interactions.

However, because the real system has only three charged sites and the Kuhn length of ssDNA is longer than a single base, we expect that the experimental system will not have a tendency to wrap around the columnar cluster. Therefore, to decrease the tendency of the simulated chains to wrap around the BTA molecules, but preserving the symmetry of the lattice, we further assume that each BTA molecule can only interact attractively with two of the possible four nearest-neighbor sites. Furthermore, we assume that these attractive sites oppose each other in the x–y plane and are stacked on top of each other along the z direction. We expect, however, that the precise choice of geometry and number of attractive sites will have little effect on the very general trends observed during these simulations. This is confirmed by the agreement observed between the experiments and the very general analytical model described below.

Simulations were typically performed on systems with a dimension of 20 × 20 × 8,000 lattice sites. The percentage of charged BTA molecules was varied between 0.25% and 8%, and the length of the DNA chains was varied between 1 and 24 beads. Usually, simulations were run for more than 109 MC steps, with each step selected from a pool of insertion/deletion, chain rearrangement, or BTA swap moves, performed in the standard way (2).

Analytical Model Adsorption of Multivalent Agents to a Surface with Mobile Recruiters

As explained in ref. 3, when we have a substrate with receptors in contact with a bulk solution of multivalent agents that have κ ligands that bind those receptors, and we assume that we can divide the surface in lattice sites such that when a multivalent agent binds no other agents can bind on the same lattice site and that each agent can only bind to the nR receptors within its lattice site, we can express the partition function Ω of the system as follows:
where $\beta = 1/kT$, $z$ is the activity of multivalent agents in solution, $N_{\text{max}}$ is the number of lattice sites on the substrate, and $q$ is the single-site bound-state partition function. The activity $z$ is defined as $z = \exp(\beta \mu)$, with $\beta \mu = \mu^\text{ex} + \ln(\rho_0)$, with $\rho$ the concentration of the multivalent in bulk solution and $\rho_0$ the standard concentration (usually 1 mol/L). Because $\mu^\text{ex}$, the excess chemical potential tends to zero for dilute solutions, $z$ can be approximated as $z = \rho/\rho_0$. Finally, the single-site bound-state partition function $q$, which is a function of the number of ligands $k$, the number of receptors in a lattice site $n_R$, the free energy of binding $\beta f_B$, and the geometry of the system, is defined as the ratio of single-particle partition functions in the bound and unbound state, at the reference concentration.

$$q = \frac{Q^{\text{b-bound}}}{Q^{\text{b-unbound}}} = \exp(\beta \mu)$$.  

where $Q^{\text{b-unbound}}$ is the partition function of a single unbound particle in solution at concentration $\rho_0$ and $Q^{\text{b-bound}}$ is the partition function of a single bound particle. Because the ratio of partition functions at is equal to the ratio of probabilities of being bound/unbound, we find that at the reference concentration:

$$q = \frac{P^{\text{b-bound}}}{P^{\text{b-unbound}}} = \frac{\sum_{i=1}^{n_{\text{max}}} P(\lambda)}{\sum_{i=1}^{n_{\text{max}}} P(\lambda)}$$

where $P(\lambda)$ is the probability of being bound with $\lambda$, and $\lambda_{\text{max}}$ is the maximum number of bonds that an agent can form within its lattice site [i.e., $\lambda_{\text{max}} = \min(k,n_R)$].

Once the function $q$ is obtained, the average number of bound particle is easily obtained from the following:

$$\theta(\kappa,n_R,\beta f_B,z) \equiv \frac{N}{n_{\text{max}}} = \frac{1}{n_{\text{max}}} \frac{\partial \ln Z}{\partial \mu} = \frac{z \times q(\kappa,n_R,\beta f_B)}{1 + z \times q(\kappa,n_R,\beta f_B)}$$.  

Now, in this treatment, we have assumed that each lattice site has exactly $n_R$ receptors. However, in many practical applications, the receptors are mobile and can be “recruited” and “clustered” by the multivalent agents. To achieve this, we need a formalism in which the number of receptors on each lattice site is allowed to fluctuate while the total number of receptors on the surface remains fixed. Strictly speaking, for a finite number of lattice sites this can be achieved by summing over all possible permutations of receptor rearrangements among the lattice sites such that the total number of receptors remained fixed. In general, this constrained sum is difficult to accomplish. However, as the surface in consideration becomes larger, the correlation between the number of receptors in two different lattice sites disappears and each lattice site becomes independent, with the rest of the lattice sites acting as a reservoir that keeps the average number of receptors $<n_R>$ constant. In this limit, we can write the grand canonical partition function $\Xi$ in which the number of receptors (as well as the number of bound colloids) is allowed to fluctuate. Thus, we can write the following:

$$\Xi = \sum_{n_R=0}^{\infty} q^{\rho \mu_{\text{P}}}(1 + z \times q(\kappa,n_R,\beta f_B)) / n_R!$$

where $\mu_R$, the receptor chemical potential must be adjusted such that the average number of receptors $<n_R>$ matches the desired value. To achieve this, we obtain the average number of receptors from the following:

$$<n_R> = \frac{d \ln \Xi}{d \mu} = \sum_{n_R=0}^{\infty} \frac{\partial \Xi}{\partial \mu} n_R = \frac{z \times q(\kappa,n_R,\beta f_B)}{1 + z \times q(\kappa,n_R,\beta f_B)}$$.  

where we have defined in the last equality the normalized probability $P(n_R)$ for a lattice site to have $n_R$ receptors as follows:

$$P(n_R) = \frac{\exp(\beta \mu_{\text{P}})(1 + z \times q(\kappa,n_R,\beta f_B))}{n_R!}$$

Similarly, the average number of bound colloids can be found from:

$$<\theta(\kappa,\mu_R,\beta f_B,z)> \equiv \frac{<n_R>}{N_{\text{max}}} = \frac{1}{N_{\text{max}}} \frac{\partial \ln \Xi}{\partial \mu} = \frac{z \times q(\kappa,n_R,\beta f_B)}{1 + z \times q(\kappa,n_R,\beta f_B)}$$

As discussed in ref. 3, the precise form of $q$ will be particular to the system being studied. However, a useful limit is obtained when we assume that all of the $k$ ligands are within reach of the $n_R$ receptors within the lattice; in this case, we have the following:

$$q(\kappa,n_R,\beta f_B) = \sum_{\lambda=1}^{\min(k,n_R)} e^{-\beta f_B \times \lambda} \frac{\lambda^k n_R!}{(\kappa - \lambda)!}$$

Although this situation corresponds to a somewhat optimistic case, it provides a reasonable basis to understand the multivalent effects. Now that we have a formalism calculate the number of bound multivalent particles to a substrate in the case of mobile receptors, we can proceed to compare the behavior of the parameter $\alpha$ defined as follows:

$$\alpha = \frac{d \ln <\theta>}{d \ln <n_R>}$$

To compare the selectivity of a system where receptors are mobile with the ones in which the receptors are distributed randomly (Poisson process) but are fixed. The derivative in Eq. S11 must be carried out numerically, and it is important to note that for each value of $<n_R>$ we must find (implicitly) from Eq. S6 the appropriate value of $\mu_R$. The results are shown in Fig. S6 for a system with $k = 10$, $\beta f_B = 0$, and $z = 10^{-5}$. We observe that the system with mobile receptors exhibits a higher value of $\alpha$ at
the peak indicating a superior sensitivity to the receptor concentration although in a somewhat narrower range of \( n_R \).

Finally, we can compare the probabilities \( P(n_R) \) of finding a lattice site with \( n_R \) receptors at different values of \( \beta f_B \) while keeping constant \( \langle n_R \rangle \geq 1, z = 1e-5, \) and \( \kappa = 5 \) in Fig. S7. Note that because \( \langle n_R \rangle \) is a function of both \( \beta f_B \) and \( \mu_R \), we need to adjust the value of \( \mu_R \) for each \( \beta f_B \) to keep \( \langle n_R \rangle \) constant.

If the \( \beta f_B \) is large and positive, the particles do not bind and the receptors distribute randomly on the surface and \( P(n_R) \) approaches a Poisson distribution. As \( \beta f_B \) becomes more negative, the fraction of empty lattices \( P(n_R = 0) \) increases while a peak starts to develop around \( n_R = 5 \) consistent with clustering or recruiting of receptors by the multivalent agents. Notice that this bimodal distribution persists even in the limit of infinitely strong bonds. Interestingly, at these conditions the system is prevented from adsorbing more particles as all of the receptors are already bound. Hence, at these conditions the surface thermodynamically self-limits the number of bound particles even as the binding strength goes to infinity.

To understand under which circumstances we can find “self-limited” adsorption behavior, it is instructive to plot in Fig. S8 the average number of bound particles as a function of the activity \( z \) at a strong binding \( \beta f_B = -10 \) and \( \kappa = 5 \). In addition, we plot Fig. S9 the distributions \( P(n_R) \) for several values of \( z \). It can be seen that, when the bulk is extremely dilute such that \( z^* q(n_R = \kappa) \ll 1 \), particles do not bind to the surface and the distribution of receptors is essentially random. As the concentration increases (remember \( z = \rho / \rho_0 \) to a first approximation), particles start to bind on the surface primordially in sites with \( n_R = 5 \) as this is the maximum number of bonds our multivalent agents can form. As a consequence, particles start recruiting receptors and a bimodal distribution with a peak around \( n_R = 5 \) is observed for \( P(n_R) \) (Fig. 4A). When \( z^* q(n_R = \kappa) \sim 1 \), a significant number of particles is bound and recruiting is evident. As \( z \) keeps increasing, but still \( z^* q(n_R \leq n_R >) \ll 1 \), the multivalent agents recruit all of the receptors and no further particles can be absorbed. At these conditions, the system achieves maximum recruiting and the surface “self-limits” the number of adsorbed particles. This behavior continues until \( z^* q(n_R \leq n_R >) \sim 1 \) when the number of absorbed particles starts to increase again approaching \( \theta \to 1 \), by redistributing the surface receptors among the newly adsorbed particles. It is important to note however, that although at large \( z \) the state of saturated surface is thermodynamically stable, it is likely that in practice the system will become kinetically trapped as the rearrangement of bonds, requiring the breakage of strong bonds, is expected to be slow.

Fig. S2. Emission spectra of DNA-induced clustering. Emission spectra (excitation, 530 nm) of BTA+BTA\textsuperscript{3+} assemblies before (green line) and after (red line) addition of DNA (or water, in the center graph). Center and Right show no significant changes in the spectra in absence of ssDNA or neutral BTAs, indicating that all of the components are necessary for the noncovalent synthesis of segmented supramolecular polymers. Scattering is observed in the case of the absence of BTA (Right) indicating that labeled BTA\textsuperscript{3+} are poorly soluble when not associated to a BTA polymer.

Fig. S3. Dynamic exchange between BTA\textsuperscript{3+} clusters. Schematic representation of the experiment (Left). DNA was added to assemblies incorporating either BTA-Cy3 or BTA-Cy5 inducing clustering. After equilibration for 48 h, the two solutions were mixed, measuring the exchange of monomers between clusters. FRET kinetics (Right) is slower in the presence of ssDNA, probably due to a slower exchange kinetics due to the DNA/BTA interaction.

Fig. S4. Normalized number of green–red nearest-neighbor pairs in the \(\mu VT\) MC simulations as a function of the attractive energy \(\varepsilon\). For weak binding (\(\varepsilon = 4kT\)), DNA chains fail to bind the polymer, and little signal is observed. For the moderate binding (\(\varepsilon = 4.5kT\)), the behavior of the signal is similar to the one observed in experiments. However, when \(\varepsilon\) is further increased, we start to observe “saturation” in which the normalized signal for high receptor concentrations and long DNA chains becomes smaller than at low receptor concentration (\(\varepsilon = 4.75–6kT\)). Note, however, that not such saturation exists if we consider the absolute (i.e., nonnormalized) number of green–red pairs as these necessarily always increase with charge concentration. Finally, at very strong binding (\(\varepsilon = 8kT\)), all of the normalized curves are saturated and we observed a reversed behavior where little concentration charge has more signal than more concentration charge.
Fig. S5. Average fraction of red–green neighbors as a function of the DNA length. The qualitative behavior of the signal is independent of the number of neighbors considered. (A) Only nearest neighbors are considered. (B) Up to three neighbors are considered. (C) Up to five neighbors are considered.
Fig. S6. The value of $\alpha$ as a function of the receptor concentration $n_R$, in the case of fixed and mobile receptors. The parameters used are $x = 10$, $\beta f_B = 0$, and $z = 10^{-5}$. The mobile receptor system presents a more pronounced peak in $\alpha$, indicating a sharper dependency to receptor concentration albeit in a narrower range of $n_R$.

Fig. S7. Probability distributions of finding a lattice site with $n_R$ receptors, for a multivalent system with $\langle n_R \rangle \geq 1$, $z = 1e-5$, and $x = 5$. As the bond strength increases, the probability distributions deviate from random and become more bimodal, while preserving the mean receptor concentration.
Fig. S8. Fraction of bound particles as function of the activity \( z \) at strong binding \( \beta f_s = -10 \) and \( \kappa = 5 \). When the bulk concentration is extremely low (i.e., low \( z \)), the number of bound particles increases linearly with \( z \). At these conditions, each bound particle recruits as many receptors as possible (i.e., around \( \kappa \) receptors) and we say we are in the recruiting regime. As \( z \) and the number of bound particles increase, more and more receptors are recruited until at some point (\( z \sim 10^{-18} \) in the plot) no more receptors are available for further binding. From this point on, further increase in concentration has little effect in the number of bound particles (self-limiting regime). Finally, as \( z \) increases further, the translational entropy cost associated with recruiting receptors becomes larger than the cost of bringing nanoparticles from solution and particles start to “share” receptors, allowing the surface to be fully covered (saturation regime).
**Fig. S9.** Probability distributions of finding a lattice site with $n_R$ receptors, for a multivalent system with $\langle n_R \rangle \geq 1$, $\beta f_B = -10$, and $x = 5$ for different values of $z$. 

(A) For extremely dilute conditions, the receptors distribute randomly (see $z = 10^{-30}$). As the value of $z$ starts to increase, a peak around $n_R = 5$ starts to develop signaling clustering (recruiting regime; see $z = 10^{-20}$) until no receptors are left for further binding (self-limiting regime; see $z = 10^{-7}$). 

(B) When $z^* q(n_R \leq n_R >) \sim 1$, the self-limiting regime ends and further particles start to bind to the surface (saturation regime). In this case, the particles start to "share" receptors and clustering is diminished. As a consequence, the right peak of the bimodal probability distribution starts to shift to the left until it becomes unimodal again with a peak in $n_R \leq n_R >$.