Supramolecular Polyvalency

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A Supramolecular Polymer as a Self-Assembling Polyvalent Scaffold**

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Adequate recognition and strong affinities in biological systems are mainly the result of polyvalent interactions.[1] The polyvalent recognition elements are typically supramolecular assemblies in a self-reorganizing biological environment, such as the cell membrane. Induction or inhibition of these types of interactions provides entry into targeting diseases and infections related to them.[1] Small-molecule inhibitors usually do not feature the affinity required for effective competition. Therefore, synthetic polyvalent inhibitors have been generated, with a large molecular diversity in their scaffolds.[2] Nevertheless, the question of how to design and synthesize a macromolecule that optimally mimics and matches the arrangement of its multiple targets[3] in the membrane and adapts itself to the dynamics of these targets, is essentially still unsolved. Ligand placing, polymer folding, and active adjustment of ligand positioning are topics that need to be addressed for the generation of polyvalent systems that respond to the dynamic rearrangement of the biological interaction partners. Synthetic supramolecular systems are self-assembling and dynamic scaffolds that might feature these properties. Vesicular architectures[4] and pseudopolyrotaxanes[5] have been shown to be promising supramolecular architectures in this respect, with applications in, for example, biomedical engineering[4] and bacterial detection.[5,6] There is, however, a need for new self-assembling synthetic systems, with diverse topology, composition, and assembly dynamics. Herein we show for the first time that synthetic supramolecular polymers[7] are ideal polyvalent scaffolds to target polyvalent biological systems. The design, synthesis, and biological evaluation of a biocompatible, auto-fluorescent, polyvalent columnar supramolecular polymer are presented. The discotic monomers reversibly assemble into a columnar polymer at low concentrations in water and can be decorated with ligands to target carbohydrate–lectin polyvalent interactions.[8] The ligand density in the polymer can be controlled by reversible exchange of monomers. This supramolecular polymer features strong binding to bacteria, is easily detected by fluorescence microscopy, and the control over its ligand density allows easy optimization of bacterial clustering (Figure 1).

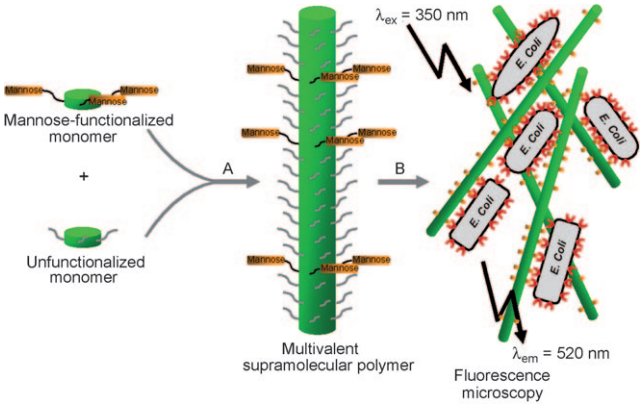

Figure 1. Schematic representation of the formation of polyvalent columnar supramolecular polymers. A) ratio of monomers controls the composition of the supramolecular polymer, B) binding of bacteria, which results in clustering and detection of the bacteria.

Disc-shaped molecule **1** (Scheme 1) assembles into columnar supramolecular polymers,[9] at low concentrations in water[10] and other polar media.[11] The decoration of the supramolecular polymer with a shell of solubilizing ethylene glycol chains ensures water-solubility and prevents unspecific interactions with biological matter. Upon polymerization, these molecules become highly fluorescent. Based on **1**, compound **2** was designed featuring three selectively introduced azide functionalities at the periphery of the molecule, envisaged to provide a flexible platform for modifications with biological ligands (Scheme 1).[12] Compound **2** was synthesized in a multistep approach in a convergent fashion (see Supporting Information for synthesis). Propargyl-α-D-mannopyranoside was selected as the ligand for the interaction with bacterial lectins and prepared in three steps using the tin tetrachloride catalyzed glycosidation procedure.[13] For the threefold modification of **2** with this mannose derivative, standard conditions of 2 mol% $CuSO_4$, 5 mol% of sodium ascorbate, and 15 equivalents of 1-propargyl-mannose per azide residue in a water/*t*BuOH mixture were applied.[14] In contrast to many previous reports on the copper-catalyzed azide–alkyne cycloaddition for sugars,[15] the modification of **2** to **3** by this reaction was relatively slow. Approximately 30% of the azide functionalities reacted rapidly within the first hours, but the complete functionalization of all groups required longer times (up to four weeks). Apparently the polymerization of **2**/**3** under the reaction conditions in water

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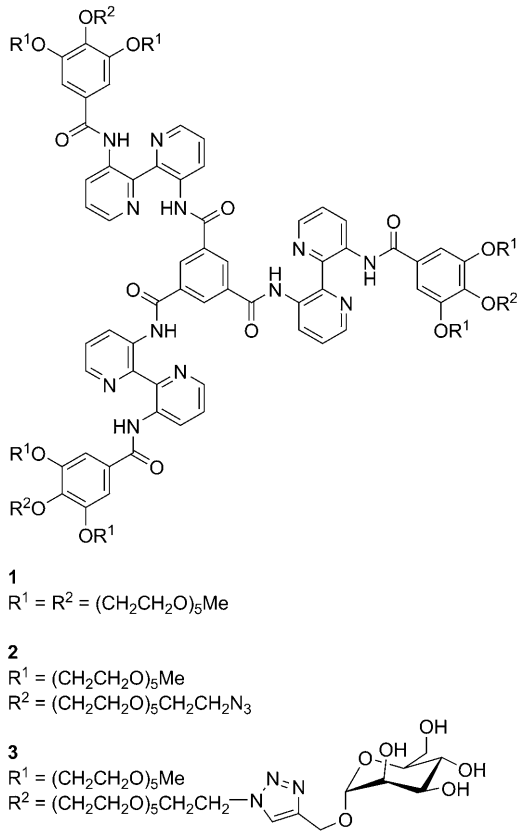

1
$R^1 = R^2 = (CH_2CH_2O)_5Me$

2
$R^1 = (CH_2CH_2O)_5Me$
$R^2 = (CH_2CH_2O)_5CH_2CH_2N_3$

3
$R^1 = (CH_2CH_2O)_5Me$
$R^2 = (CH_2CH_2O)_5CH_2CH_2$—N

Scheme 1. Discotic compounds: **1** with inert glycol side chains, **2** with azide functionalities for attachment of ligands, **3** mannose functionalized.

results in molecular crowding (see below) of the reactive functional groups.[16] The dynamic rearrangement of the discotic monomers nevertheless allows full conversion of the free azide groups over time, probably at the chain-ends of the supramolecular polymer. The pure product **3** could be isolated after size-exclusion chromatography, separating the excess of mannose, in 80% yield.

All discotic compounds dissolve readily in water and form supramolecular polymers (K_{ass} **1** $= 10^8\,M^{-1}$).[10] The binding of polyvalent supramolecular polymers built up out of **1** or **3** and mixtures thereof to bacteria was investigated by microscopy studies with the *E. coli* strain BL 21α. The bacteria were cultivated in LB (lysogeny broth) media, washed, resuspended and incubated with either nonfunctionalized discotic **1**, mannosylated discotic **3**, or with water, for 1 h at room temperature.[6] The total concentration of discotic monomers **1** and **3** was kept dilute at 10^{-7} M. After washing and mounting on glass slides, binding to the bacteria was evaluated with a fluorescent microscope (Figure 2), taking advantage of the strong auto-fluorescence of the discotic monomers when present as supramolecular polymer.[10,11] Only when the bacteria were incubated with mannose-modified discotic **3** could a strong fluorescence of the bacterial aggregates be observed, colocalizing with the brightfield image. Both the control experiments with nonfunctionalized scaffold **1** and with water did not show fluorescence. These results show that the supramolecular polymers of **3** bind to the bacteria and

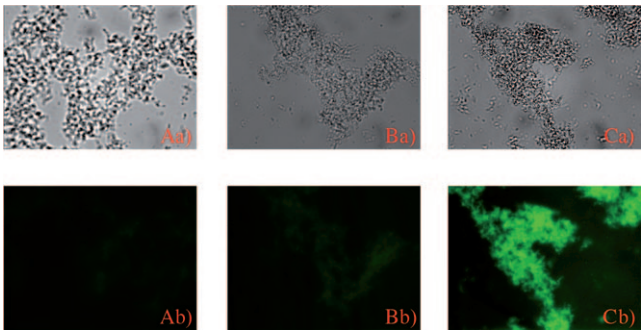

Figure 2. Microscopy pictures in a) brightfield and b) fluorescence ($\lambda_{ex} = 360$ nm, $\lambda_{em} = 490$ nm) mode on *E. coli* incubated with A) water, B) inert discotic **1**, C) mannose discotic **3**.

demonstrate the absence of unspecific binding of supramolecular polymers of **1**.

The specificity of the interaction of **3** with the mannose-binding FimH receptors on the bacterial surface was evaluated through binding studies on two *E. coli* strains, ORN178 and ORN208.[17] These strains differ in their mannose-binding-properties owing to the over-expression or suppression of the FimH receptor, respectively. Mixtures of **3** and ORN178 featured a strong fluorescence response where the bacteria were located, whereas no binding of **3** to ORN208 could be detected (Figure 2 in the Supporting Information). The supramolecular polymers thus bind to the bacteria through the mannose–FimH receptor interaction selectively and do not induce unspecific binding.

The polyvalent effect of the supramolecular polymers was evaluated on dilute bacteria samples, with the bacteria present in non-aggregated state. When these samples were incubated with **3** the bacteria clustered and a colocalized fluorescence of the bacteria could be observed. Control experiments with either **1** or water showed dispersed bacteria only (Figure 3). Apparently the supramolecular polymers are

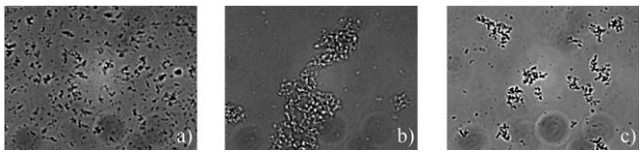

Figure 3. Microscopy pictures in brightfield mode on dilute bacterial samples incubated with a) 100% **1**, b) 99/1 **1**/**3**, and c) 100% **3**.

long enough to function as polyvalent cross-linking scaffolds, similar to conventional polymers.[6] The supramolecular polymers are furthermore most probably stabilized by the polyvalent interaction with the bacteria, which increases the degree of polymerization.

To evaluate the influence of ligand density, supramolecular polymers consisting of mixtures of **1** and **3** were generated and similarly evaluated at dilute bacteria concentrations. These supramolecular copolymers induced bacterial aggregation at all mixture compositions evaluated (Figure 3 and Supporting Information Figure 3). A functional-mono-

mer percentage as low as 1% of **3** still resulted in the formation of bacterial aggregates. Evidently, only a limited number of mannose functionalities at the periphery of the supramolecular polymer are required to induce clustering of the bacteria around the columns. Furthermore, the supramolecular polymers are sufficiently long to bridge the distances between the few functional monomers **3** to induce bacterial clustering (Figure 1). Interestingly, the aggregates observed for the supramolecular polymers containing predominantly nonfunctionalized discotic **1** were typically larger than those induced by polymers consisting of **3** only. This result indicates that optimal binding to bacteria occurs when only a limited number of mannose-functionalized monomers is present, surrounded by nonfunctionalized monomers. "Overcrowding" of the supramolecular polymer with ligands, thus results in a sub-optimal binding. The supramolecular nature of the polymer allows for easy adjustments and optimization of this monomer composition.

A competition experiment between **3** and mannose on binding to bacteria was performed to examine the strength of the polyvalent binding of the supramolecular polymer. To allow the most effective competition by the mannose, the bacteria were first incubated with different concentrations of mannose for ten minutes, after which discotic **3** was added

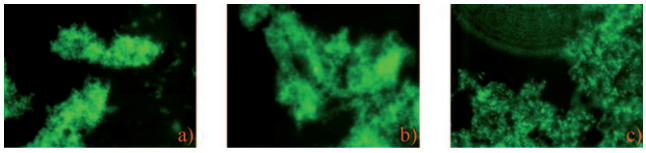

Figure 4. Microscopy pictures in fluorescence mode ($\lambda_{ex}=360$ nm, $\lambda_{em}=490$ nm) of bacteria incubated with **3** (10^{-7} M) and mannose as a competitor. a) pure **3** (10^{-7} M); b) **3** (10^{-7} M) and mannose (3×10^{-4} M); c) **3** (10^{-7} M) and mannose (3×10^{-1} M).

(Figure 4 and Supporting Information Figure 4). The bacteria remained fluorescent over the whole mannose concentration regime studied (up to a 10^6 fold excess of mannose). Apparently the supramolecular polymers feature highly efficient polyvalent binding to the bacteria in this assay.

To quantify the polyvalent binding of the supramolecular polymers of **3**, an enzyme linked lectin assay (ELLA) was performed.[18] Mannose-coated polyvalent structures are known to competitively inhibit Concanavalin A (Con A) binding to the yeast cell surface receptor mannan. Con A is a tetramer at neutral pH value, containing four spatially well separated binding sites (6.5 nm) for oligosaccharides.[19] Experiments using horseradish peroxidase labeled Con A (HRP-Con A) as the lectin and yeast mannan as the surface-fixed-ligand were carried out in 96-well plates. After pre-incubation with different concentrations of **3**, or methyl α-D-mannopyranoside as the reference compound, binding of HRP-Con A to mannan was measured photospectrometrically (see Supporting Information). The IC_{50} value for the methyl glycoside was around 3000 μM in our assay and the IC_{50} value for **3** around 120 μM (360 μM, valency corrected). The relative valency-corrected binding of **3** was 8.3 times stronger than the reference compound (24.9 per molecule **3**), showing the polyvalent inhibition of the supramolecular polymers. Typically, small trivalent ligands do not show significant valency-corrected enhancement effects to higher valency in specific lectin–ligand assays, such as this ELLA assay, as they cannot span the distances between the binding sites on the Con A.[20] For these small scaffolds, higher valencies improve the total potency, but do not improve valency-corrected potency, which results merely from the "cluster glycoside effect".[18,21] The supramolecular polymers formed by **3** allow effective polyvalent binding up to high valencies and show high potency per ligand. Apparently, because of the polymeric nature of **3**, the compounds are capable of spanning the distance between the mannose binding sites on Con A.

With the mannose–lectin interaction as an example, we have shown that columnar supramolecular polymers are effective polyvalent scaffolds for binding and detecting bacteria. The simple generation of polymers with different monomer compositions showed that decreasing the amount of mannose component actually enhanced bacterial aggregation, by reducing steric crowding. The self-assembly into polymers enhances the potency of the monomers significantly more than is to be expected on the basis of the cluster glycoside effect alone. Supramolecular polymers are ideal systems to generate polyvalent architectures for binding and modulation of biological interactions. The reversible self-assembly of monomers into polymers provides control over ligand density, polymeric architecture, and environmental response to the biological interaction partner, not accessible with covalent polymeric systems.

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