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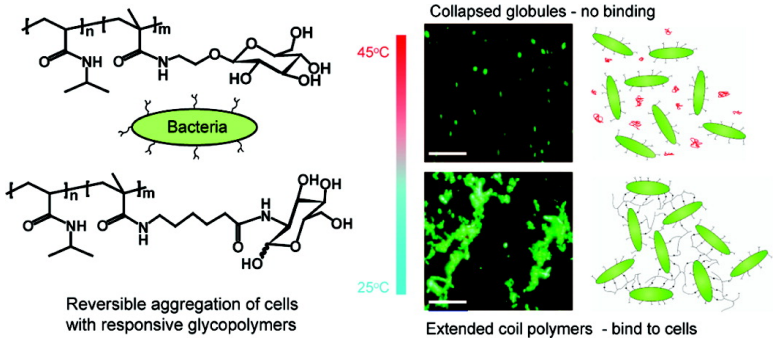


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# Control of Bacterial Aggregation by Thermoresponsive Glycopolymers

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The ability to control the interactions of bacteria with receptors and surfaces is fundamental to pathogen detection, anti-infection strategies and, ultimately, to public health.[1] Many bacteria have evolved specific adhesion structures such as fimbriae that bind to host cell glycoconjugates, thus exploiting the recognition and signaling pathways of sugar-bearing biopolymers that constitute the "glycocode".[2] Oligosaccharides have been used to block bacteria−cell interactions as a means to combat infection, while artificial glycopolymers that agglutinate bacteria through multiple cooperative polymer−cell interactions have been described for detection and deactivation of pathogens.[3] However, for practical use, reversibility in the material−cell-binding behavior is needed, i.e., that interactions with bacteria can be switched on for capturing the organism and switched off for removal of cells prior to the next assay. In addition, reversible cell binding could be used as a means for controlling biofilm formation. Here we describe the reversible aggregation of a specific bacterial strain controlled by thermoresponsive glycopolymers as the first step toward robust and reusable cell-sensing materials. Furthermore, we show that polymer activity in bacterial agglutination is achievable with rather simple sugar functionality, employing multiple glucose residues able to control cell aggregation through a combination of the cluster glycoside effect and polymer conformation.

The polymers were designed to contain pendant sugar residues that could be exposed or hidden via an externally controlled coil-to-globule phase transition.[4] We used poly(*N*-isopropylacrylamide) as the central component, owing to its well-known lower critical solution temperature (LCST) of 32 °C in water, and incorporated glucose functionality into the chain such that the LCST occurred above 37 °C. Two sets of thermoresponsive polymers were synthesized, and glucose residues were attached via either the anomeric carbon (**P1**) or the 2-amino position of glucosamine (**P2**). For both polymers, sharp phase transitions (LCST **P1** = 41 °C, **P2** = 44 °C) were apparent for relative ratios of sugar:thermoresponsive segments of <0.22 mol % (Figure 1).

To demonstrate the reversible display of the sugar moieties around the LCST we investigated both multiple aggregative interactions with lectins and individual glucose-binding reactions with small-molecule ligands. As expected, both glycopolymers bound with high specificity to concanavalin A at low temperatures (25 °C) as detected by UV turbidity assays, i.e. when the polymers were in the chain-extended conformations and fully solvated below their LCST (ESI). To probe the mechanism underlying the changed accessibility of the sugars below and above LCST we employed a three-component system comprising alizarin red S dye (AR), phenylboronic acid (PBA), and **P1** or **P2**. AR is inherently nonfluorescent but fluoresces strongly when bound to PBA in alkaline conditions. The covalent, but reversible (in response to pH[5] and temperature[6]), binding of PBA with the catechol diol

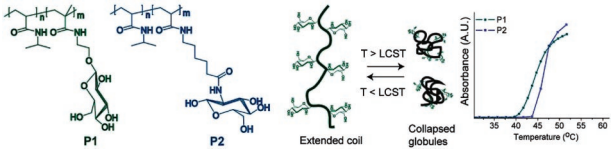


***Figure 1.*** Structure of thermoresponsive glycopolymers and phase behavior in PBS (pH 7.4) solution.

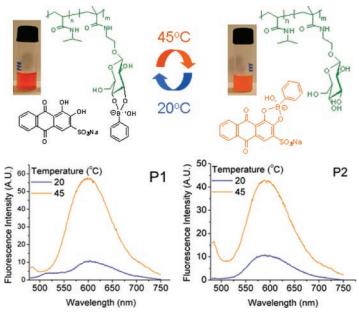


***Figure 2.*** (Top) Visual inspection of diol binding and release at temperatures below (left) and above LCST (right) for polymer **P1**. (Bottom) Corresponding fluorescence spectra using AR and PBA in glycine buffer (0.1 M, pH 9.3).

groups induces emission at 578 nm. The introduction of glucose, which has high affinity to PBA, in the polymer results in competition for diol-binding sites on PBA between AR and polymer-bound glucose. Binding of glucose to PBA was thus monitored by variations in the AR−PBA complex fluorescence intensity as the concentration of glucose from the glycopolymer changed. The polymer-bound glucose reacted with PBA when the polymer was in a soluble phase ($T$ < LCST) as shown by reduction in AR−PBA complex fluorescence intensity. By contrast, an increased fluorescence intensity of the AR−PBA complex was observed at $T$ > LCST, when the polymer was in a globular state and the glucose residues were not available for competitive binding with AR for PBA. The effect was apparent to the naked eye, with the color change of a vial containing all three components (AR, PBA, and **P1** or **P2**) from burgundy to orange at low and high temperatures, respectively (Figure 2).

The experiment could be repeated for several temperature cycles with consistent oscillation in fluorescence intensity for both polymers in respect to temperature, thus demonstrating the reversible binding character in a "switchable" manner. Thermoresponsive polymers without glucose residues and nonresponsive glycopolymers did not show temperature-dependent AR−PBA fluorescence intensity cycles (ESI).

Having established that the synthetic glycocode could be hidden and revealed by a temperature switch, we carried out bacterial binding assays with polymers **P1** and **P2** and a green fluorescent protein-tagged *Escherichia coli* strain (MG1655pGFP). This strain produces Type 1 fimbrae containing the *fim*H protein that possesses

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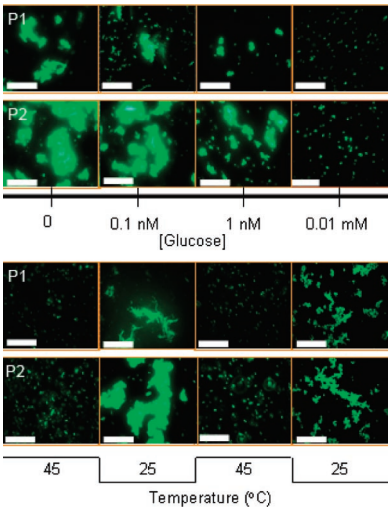


***Figure 3.*** Polymer−bacteria aggregation studies using *E. coli* MG1655pGFP (O.D. 0.8). (Top) Polymer (10 mg/mL)−glucose competition assay; gradual reduction of cluster size due to glucose increase. (Bottom) Control of bacterial aggregation formation by thermal oscillation across LCST in presence of **P1** and **P2** (white bars, 10 $\mu$m).

carbohydrate recognition sites (CRS) with high affinity for mannose ($K_d$ 2.3 $\mu$M) and glucose ($K_d$ 9.24 mM).[7] The interaction between cell-surface receptors and the multiple copies of sugar moieties on the polymers resulted in bacteria−polymer complex formation (Figure 3), and the mode of interaction was probed by competition assays with *fim*H ligands. Increasing amounts of added glucose showed a gradual decline in the size of the aggregates formed with respect to glucose concentration increase (Figure 3, upper panel). Total inhibition of bacterial cluster formation occurred when the concentration of added glucose in the polymer-bacterial suspension (300 $\mu$L) reached 0.01 mM, which correlated well with the numbers of glucose residues on the polymers (effective glucose concentration ~0.04 mM). By contrast, addition of sucrose, which exhibits lower affinity for *fim*H than glucose, did not inhibit formation of bacteria−polymer clusters.

The key experiment was to establish the reversibility of polymer−cell interactions via temperature-mediated ligand display. The strength of glucose binding to *fim*H[7] is lower than that to PBA[8] ($K_{eq}$, for the diol-boronate ester: 4.6 $M^{-1}$, pH 7.4), but crucially, the affinity constant for glucose−fimH[7] is lower than that of mannose−*fim*H, the principal biological target sugar for *E. coli* MG1655. We therefore hypothesized that the polyvalent interactions of the glucose−polymers would be strong enough to promote bacterial aggregation below LCST but sufficiently weak to facilitate reversibility upon application of a thermal stimulus, since the polymers showed reversible binding with PBA. Indeed, thermal cycling of bacteria−polymers suspensions revealed reversible bacterial aggregation (Figure 3, lower panel).

In cell suspensions at temperatures above LCST (45 °C) where the polymers were in a globular phase, bacteria were uniformly dispersed, but when left at room conditions below LCST, large aggregates were formed (ranging from 15 to 120 $\mu m^2$, ESI). Re-incubation of the suspensions at high temperatures for 5 min, followed by mild vortexing to break preformed clusters, and subsequent maintenance at 45 °C showed no aggregation. To close the thermal loop, cooling to room temperature promoted bacterial aggregation once more (Figure 3). This behavior was repeatable over three cycles. Larger bacterial aggregates were formed between **P2** and *E. coli* MG1655pGFP compared to those formed by **P1** and *E. coli*. This was most likely due to the higher molar mass of **P2** ($M_w \approx 20$ kDa) compared to **P1** ($M_w \approx 16$ kDa) and sugar content (~12 glucose units per chain for **P2** compared to 6 for **P1**). However, it is also probable that the different-length spacers and attachment positions of the glucose residues in copolymers **P1** and **P2** affected ligand−receptor accessibility and hence extent of cell interactions. Control experiments using a second *E. coli* strain, Top10, which does not produce Type 1 fimbriae, and **P1**, **P2** showed no aggregation behavior or thermal response, and the same experiments with precursor polymers lacking sugar residues and the MG1655pGFP strain also showed negligible bacterial aggregation under both temperature conditions (ESI).

In conclusion we have shown that it is possible to place bacterial aggregation under external control through a combination of thermoresponsive polymer chains, accessible functionality, and choice of sugar moiety. The advantages of using synthetic polymeric materials for bacterial interactions are numerous; the intrinsic "soft" nature of polymers confers compatibility with biological organelles, while the variety and flexibility of polymer architectures and structures through design suggests polymers can be highly effective materials for bacteria−matter interactions.[9] Future applications of these materials as mediators and controllers of cell−cell signaling are currently in progress.

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**Supporting Information Available:** Details of synthetic procedures, characterization, properties of glycopolymers, assay conditions, and quantification of cell binding. This material is available free of charge via the Internet at http://pubs.acs.org.

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