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Binding Bacteria to Highly Branched Poly(*N*-isopropyl acrylamide) Modified with Vancomycin Induces the Coil-to-Globule Transition

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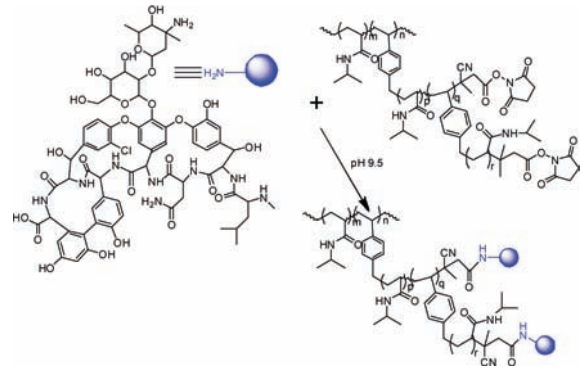
It is well-known that at temperatures below the lower critical solution temperature (LCST), stimulus-responsive polymers in water are solvated in an open-coil form.[1−3] However, when the temperature is raised to the LCST [32 °C for linear poly(*N*-isopropylacrylamide) (PNIPAM)], the polymer becomes desolvated and switches to a more compact conformation. This coil-to-globule transition is the basis of emergent sensing devices, drug delivery systems, and various new tools in biotechnology.[4] In view of current difficulties in clinical management of infections,[5−7] developing responses of devices to biological entities is a highly desirable goal.[8] However, few examples of coil-to-globule transitions in response to binding have been reported.[9]

Recently, work on linear PNIPAM targeted at bacteria by Pasparakis and co-workers[10,11] showed that linear PNIPAM functionalized along the main chain with sugars that interact with bacteria did not bind or aggregate *Escherichia coli* above the LCST because the sugar groups were buried within the globule. The linear polymer was bound to the bacteria only in the open-coil state, and an external thermal stimulus was required to drive the polymer through the LCST. This concept, that PNIPAM shields functionality above the LCST, is well-established. On the other hand, Ding et al.[12] showed that the stimulus-responsive polymer poly(*N*,*N*-diethylacrylamide), when attached to streptavidin, blocked the binding of biotinylated bovine serum albumin (BSA) below the LCST, although binding did occur when the polymer was in the globular state. Also, Liu et al.[13] recently showed that binding of BSA to anti-BSA conjugated to PNIPAM occurred only above the LCST. From these results, it appears to us that polymer architecture can have a significant effect on the ability of binding sites to interact with their targets: that is, in the case of the work of Pasparakis et al.,[11] a receptor on a bacterial cell is prevented from binding to its ligand when a linear polymer is in the globular state, whereas in the case of the work of Ding et al.[12] and Liu et al.,[13] the globular form allows easier access to a binding site.

In branched polymers, the collapse of the polymer from the open chain to the globule should not prevent the binding groups at the chain ends from interacting with bacteria. In this study, we prepared highly branched polymers with vancomycin end groups (HB-PNIPAM-van) capable of binding to Gram-positive bacteria, and we examined their ability to interact with Gram-positive and Gram-negative bacteria from 4 to 37 °C.

HB-PNIPAM with carboxylic acid end groups (HB-PNIPAM-COOH) was prepared by our previously disclosed method.[14] Vancomycin was attached to the chain ends at pH 9.5 by amidation of the end groups activated as *N*-hydroxysuccinimides, as shown

Scheme 1. Conjugation of Vancomycin to HB-NIPAM



in Scheme 1. Approximately 10% of the end groups were modified with vancomycin. To facilitate visualization, the polymer was also labeled by addition of aminoanthracene to a fraction (1.1 mol %) of the chain ends prior to modification with the binding groups. HB-PNIPAM-COOH passed through a cloud point at 42 °C, and the polymer precipitated from solution to form mainly a coagulated mass. HB-PNIPAM-van showed a cloud point at 55 °C, but the polymer remained suspended as a colloidal dispersion. HB-PNIPAM-van and *Staphylococcus aureus* bacteria or polymers alone in phosphate buffered saline (PBS) as controls were incubated in U-shaped polystyrene wells at 37 °C for 1 h (Figure 1a).

In wells containing polymer alone, there was no visible change after incubation. In wells containing *S. aureus* alone, the cells rolled

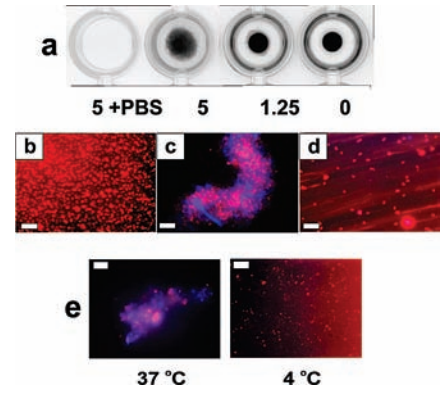


Figure 1. Bacteria in contact with polymers: (a) HB-PNIPAM-van in decreasing concentrations (mg cm^{-3}) incubated with *S. aureus* or polymer in PBS (5 + PBS). (b−d) Fluorescence images (bacteria labeled with Dylight-649, polymer labeled with anthracene): (b) *S. aureus*; (c) *S. aureus* + HB-NIPAM-van; (d) *P. aeruginosa* incubated with HB-PNIPAM-van. (e) Fluorescence images of HB-PNIPAM-van + *S. aureus* at 37 and 4 °C. Scale bars = 10 μm.

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to the bottom of the wells, forming a "button"; this occurred at all temperatures. However, when HB-PNIPAM-van and *S. aureus* were incubated together, visible mats of insoluble assemblies of bacteria and polymer were formed. No aggregates were formed in wells containing HB-PNIPAM-van alone or with the Gram-negative bacterium *Pseudomonas aeruginosa.* Also, neither species interacted with HB-PNIPAM-COOH (see Supporting Figure 1). Mat formation was dependent on the concentration of polymer, with a decrease in size of visible complexes concurrent with a reduction in polymer concentration. Bacteria incubated in wells with free antibiotic did not form mats (not shown). To confirm that the mats were mixtures of polymer and bacteria, *S. aureus* was labeled with Dylight 649, a contrasting fluorochrome to anthracene. Microscopic evaluation showed aggregates forming only in wells containing HB-PNIPAM-van with *S. aureus* (Figure 1c). After 1 h of incubation at 37 °C, fluorescence microscopy revealed the aggregates to be Dylight-stained bacteria (red) intimately associated with insoluble polymer (blue) (Figure 1c). HB-PNIPAM-COOH (above its LCST) in the presence of *S. aureus* at 37 °C produced aggregates of polymer that were essentially free of bacteria (see Supporting Figure 2).

In the case of the wells containing polymer plus *P. aeruginosa* (Figure 1d) or *S. aureus* with PBS alone (Figure 1b), the bacteria remained dispersed throughout the solution. The polymer was applied below the LCST, and the dramatic aggregation effects displayed in Figure 1 suggested that the binding of the end groups was accompanied by a coil-to-globule transition. This would occur if the binding event produced a large perturbation of the extent of overall solvation, causing the LCST of the bound polymer to be lower than the application temperature (37 °C). In order to test this hypothesis, we observed the polymer−bacteria assemblies after cooling from 37 °C to 34, 30, 28, 24, and 4 °C (see Supporting Figure 3 and Figure 1e). As previously stated, an open mat was formed at 37 °C; it started to decrease in size after 3 h at 30 °C and at 28 °C, but most of the bacteria were released from the polymer at 24 °C and below. Figure 1e shows fluorescence micrographs of HB-PNIPAM-van + *S. aureus* at 37 and 4 °C. At 37 °C, the bacteria and polymer formed assemblies of intermingled colored aggregates from both labels (Dylight649 and anthracene). However, the anthracene-labeled polymer could not be detected at 4 °C, presumably because it was too disperse. Images taken at 34 °C were similar to those at 37 °C, but at 26 °C, the large aggregates had disintegrated and only a few small structures could be observed (see Supporting Figure 4). The data provide strong evidence for a modification of the LCST on binding to the bacteria: the LCSTs of the polymers are above 37 °C in the absence of bacteria, but binding reduces the LCST; thus, cooling to 26 or 4 °C takes the polymer through the new (bound) LCST, and the bacteria are released. Competitive binding experiments, in which free vancomycin was added both before and after formation of the assemblies, showed that the assemblies remained intact up to 100 μg mL^{-1} of vancomycin (the highest concentration studied). The data (see Supporting Figure 5) indicate that the aggregates form by the cooperative effects of both binding of chain ends and adhesion to the globular form of the polymer.

Energy transfer between chain-end anthracene groups following excitation at 380 nm resulted in increased emission at 520 nm from fluorescein on the bacteria and a decrease in the fluorescence lifetime of the anthracene excited state (Figure 2). Energy transfer was confirmed by measuring the fluorescence lifetime of the anthracene (donor), which decreased from 3.9 to 3.1 ns. The results indicate that the anthracene at the chain ends and the fluorescein at the surface of the bacteria were separated by an average distance of 4.2 nm. Particle size measurements on dispersions of HB-PNIPAM-van with *S. aureus* were obtained as a function of temperature. Figure 2b shows a

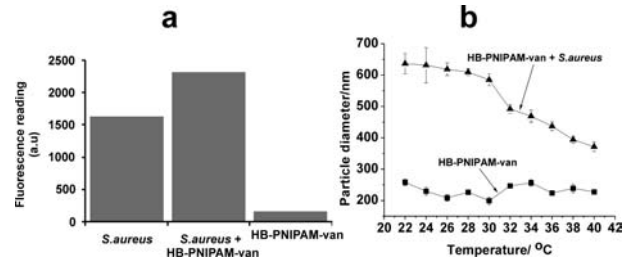

Figure 2. (a) Fluorescence intensity at 520 nm from fluorescein-labeled *S. aureus*, HB-NIPAM-van, and the bacteria combined with the polymer. (b) Particle sizes (PALS) of *S. aureus* and HB-NIPAM-van assemblies and HB-PNIPAM

discontinuity at ~30 °C, which we can assign to the new LCST of the polymer bound to the bacteria. The step-change increase in particle size occurs as the polymer swells into the coil conformation. On the other hand, no significant changes were observed as the polymer alone was heated. Thus, the data indicated that at 37 °C, the polymers within the polymer−bacteria assemblies were above the LCST in the globular conformation. Cooling to 4 °C progressed the polymer through the LCST.

These results are consistent with the idea that the chain ends of branched polymers do not penetrate into the polymer coil. A binding-induced transition can occur in both linear and branched polymers. In the linear polymers,[11] the collapse shields the interactions with the bacteria above the LCST. However, the chain ends in the highly branched polymers studied here remained available for binding above the LCST. Binding of the end groups to the bacteria decreased the LCST, and the polymer chain desolvated. These bound and desolvated polymers provided a substrate for the adhesion of *S. aureus*, which aggregated around the polymer to form clumps of bacteria mixed with polymer. Branched swollen structures are well-known as protein- and cell-repellant structures, and in these examples, cooling and swelling was sufficient to disrupt the binding of the end groups to the bacteria, which were released.

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Supporting Information Available: Full experimental details, images of HB-NIPAM-COOH with *S. aureus* and data at other temperatures, competition data, and complete ref 5. This material is available free of charge via the Internet at http://pubs.acs.org.

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