Dual-Activity Polymers

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Synthetic Polymers for Simultaneous Bacterial Sequestration and Quorum Sense Interference**

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Materials that interfere with bacteria–host interactions are an attractive approach for controlling infectious diseases (Figure 1a, route 1).[1] Synthetic polymers are especially useful in this context, because their affinity with cell surface receptors can be tailored through multivalent ligand display, while at the same time avoiding selection pressure and resistance invoked in bacteria by antibiotics. However, many bacterial species employ additional strategies for environmental adaptation and host invasion, thus confounding therapies that depend on cell sequestration alone. Amongst these mechanisms are communication systems such as quorum sensing (QS), which allows bacteria to synchronize transcriptional changes at the population level (Figure 1a, route 2).[2–5] Inhibition of QS is itself a potential further method of controlling bacterial infection,[6] but targeting QS signals in isolation can still allow bacterial infection by alternative pathways. Materials that could interfere with both communication mechanisms and cell adhesion/aggregation at the same time would allow greater flexibility in anti-infective strategies. Herein, we report polymers that combine potent activity in binding QS autoinducers with effective adhesion at bacterial surfaces (Figure 1).

We designed polymers to attach to *Vibrio* species (such as the human pathogen *V. cholerae*), while at the same time binding specific autoinducers for QS. For this, we used the marine bacterium *V. harveyi*, as QS in this species not only controls virulence but also bioluminescence, thereby facilitating readout of the effects of polymers on the QS network. *V. harveyi* uses two types of autoinducers (AIs), acyl homoserine lactones (AI-1) and the AI-2 network, a "universal" pathway that functions in both Gram-positive and Gram-negative bacteria.[7] The key compound in the AI-2-mediated QS pathway is 4,5-dihydroxy-2,3-pentanedione (DPD), which can exist in several forms. In *V. harveyi* and other marine species, DPD reacts with boric acid to yield the active furanosyl borate ester (**1**), one of the few known biomolecules incorporating boron (Figure 1b).[8,9] We reasoned that if

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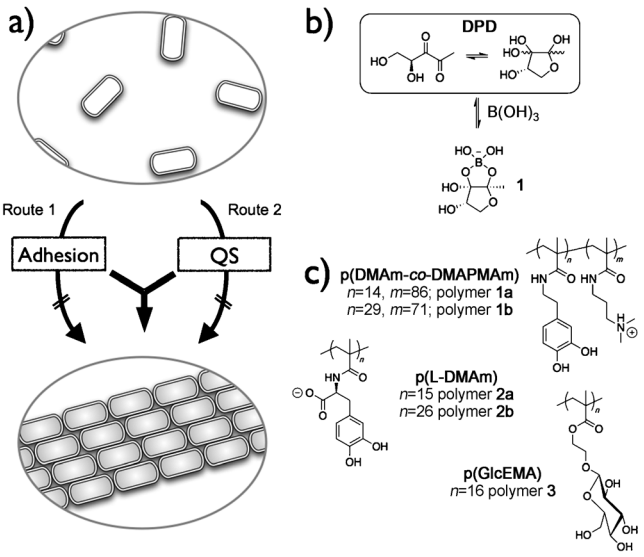

Figure 1. a) Polymer-mediated interference in bacterial aggregation and signaling. b) Structures of the key components in the autoinducer AI-2 network. c) Structures of polymers. DPD = 4,5-dihydroxy-2,3-pentanedione, DMAm = *N*-dopamine methacrylamide, DMAPMAm = *N*-[3-(dimethylamino)propyl]methacrylamide, p(GlcEMA) = poly(β-D-glucosyloxyethyl methacrylate).

polymers were designed to bind bacteria while at the same time sequestering borate, the levels of "active" AI-2 (**1**) would be reduced and QS, as reported by bioluminescence, should be suppressed.

Bacteria strongly bind cationic surfaces[10] and glycopolymers,[11] but less is known about the ability of polymers to quench AI-2 in *Vibrio sp.*[12] Representative diols were investigated as potential AI-2 scavengers via reversible ester formation with boric and boronic acids, with special attention to carbohydrate derivatives. Titration studies at pH 7.4 using the Alizarin Red S (AR-S) assay (Figure S2 in the Supporting Information)[13,14] established binding affinities for boron–diol species, which were in accord with prior reports, that is, linear diols < cyclic diols (sugars) < aromatic diols or catechols.[14,15] Although DPD has a similar structure to carbohydrates, its affinity was much higher than that for the other saccharides analyzed, and only the catechols gave comparable affinities (Figure S2 in the Supporting Information).

These observations were confirmed by using mass spectrometry and ^{11}B NMR spectroscopy. When a solution of DPD in a borate/phosphate buffer at pH 7.4 was treated with 2 equivalents of the catechols, complete suppression of the signals at $\delta = 4.8$ and 6.2 ppm, corresponding to the AI-2 (**1**),[16] occurred, while new signals at $\delta = 6.9$ and 7.9 ppm, assigned to the dopamine and L-3,4-dihydroxyphenylalanine (L-DOPA) complexes, respectively, were observed (Figure 2a and b).

Dopamine was found to be the best candidate for polymeric QS control, showing a similar affinity for boron compared to DPD and the ability to decrease the concentration of AI-2 (**1**) in solution. To test for effects on both bacterial adhesion and QS interference, *N*-dopamine methacrylamide (DMAm)[17] was copolymerized with *N*-[3-(dimethylamino)propyl]methacrylamide (DMAPMAm) to form a cationic polymer (**1a**,**b**, Figure 1c). The amine functionality was designed to confer water solubility at neutral pH and bacterial adhesion, as well as coordinate to boronic acids.[18] Since dopamine and L-DOPA showed similar affinities for borate, we prepared and polymerized L-DOPA methacrylamide (L-DMAm), thereby generating two novel anionic water-soluble homopolymers (**2a**,**b**, Figure 1c), which we reasoned would still bind AI-2 strongly but attach less readily to bacterial surfaces because of charge–charge repulsion. Despite a lower affinity for boronic acid, we also prepared carbohydrate-based poly(β-D-glucosyloxyethyl methacrylate) (p(GlcEMA), **3**), shown in prior assays to be a highly active bacterial sequestration agent.[19,20]

Bacterial attachment assays indicated that, as expected, the cationic polymers sequestered the negatively charged *V. harveyi*, with the formation of dense "mats" of polymer–cell aggregates (Figure 3b–d and the Supporting Information). In addition, since *V. harveyi* has been shown to bind to glucose,[21] the fact that polymer **3** induced bacterial aggregation was also anticipated (Figure 3d and the Supporting Information). The negatively charged polymers also formed dense polymer–cell aggregates (Figure 3c and the Supporting Information), a less predictable outcome we attribute to the inherent adhesive properties of L-DOPA-derived cate-

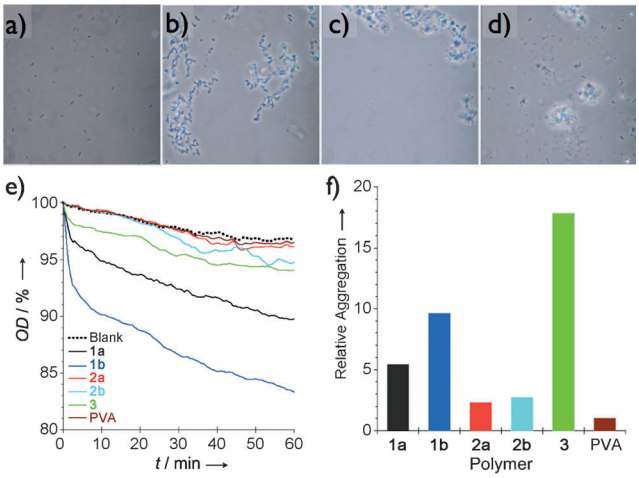

Figure 3. a–d) Aggregation of *V. harveyi* MM32; a) no polymer added and in the presence of b) **1b**, c) **2a**, and d) **3** (1000× magnification). e, f) Relative cell aggregation in PBS for all polymers (polymer concentrations: 0.25 mg mL^{-1} for **1** and **2**, 0.05 mg mL^{-1} for **3**, and 0.50 mg mL^{-1} for PVA). OD = optical density.

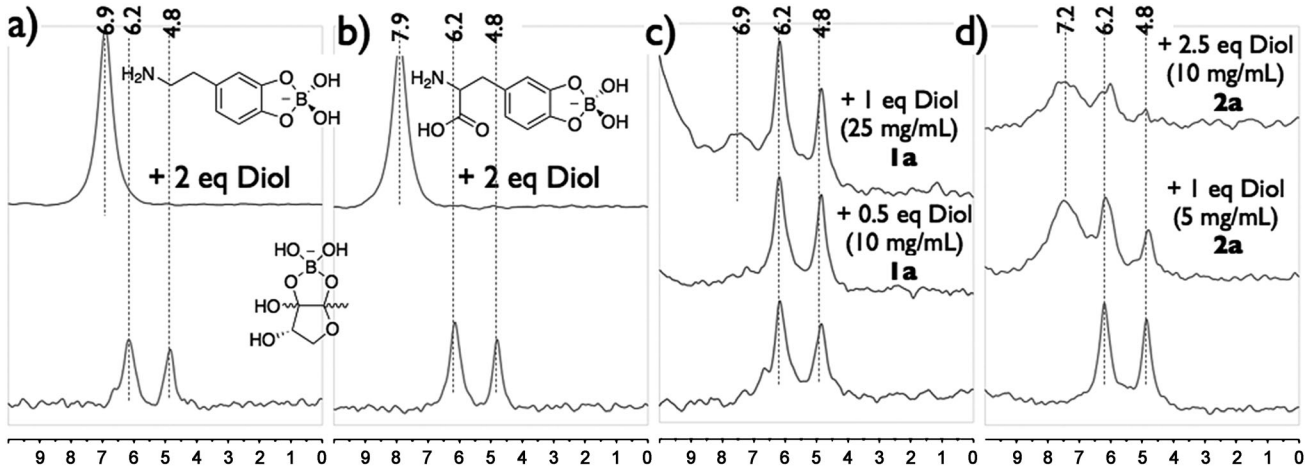

Figure 2. Sequestration of AI-2 (**1**) by a) dopamine, b) L-DOPA, c) polymer **1a**, and d) polymer **2a** as shown by ^{11}B NMR spectroscopy.

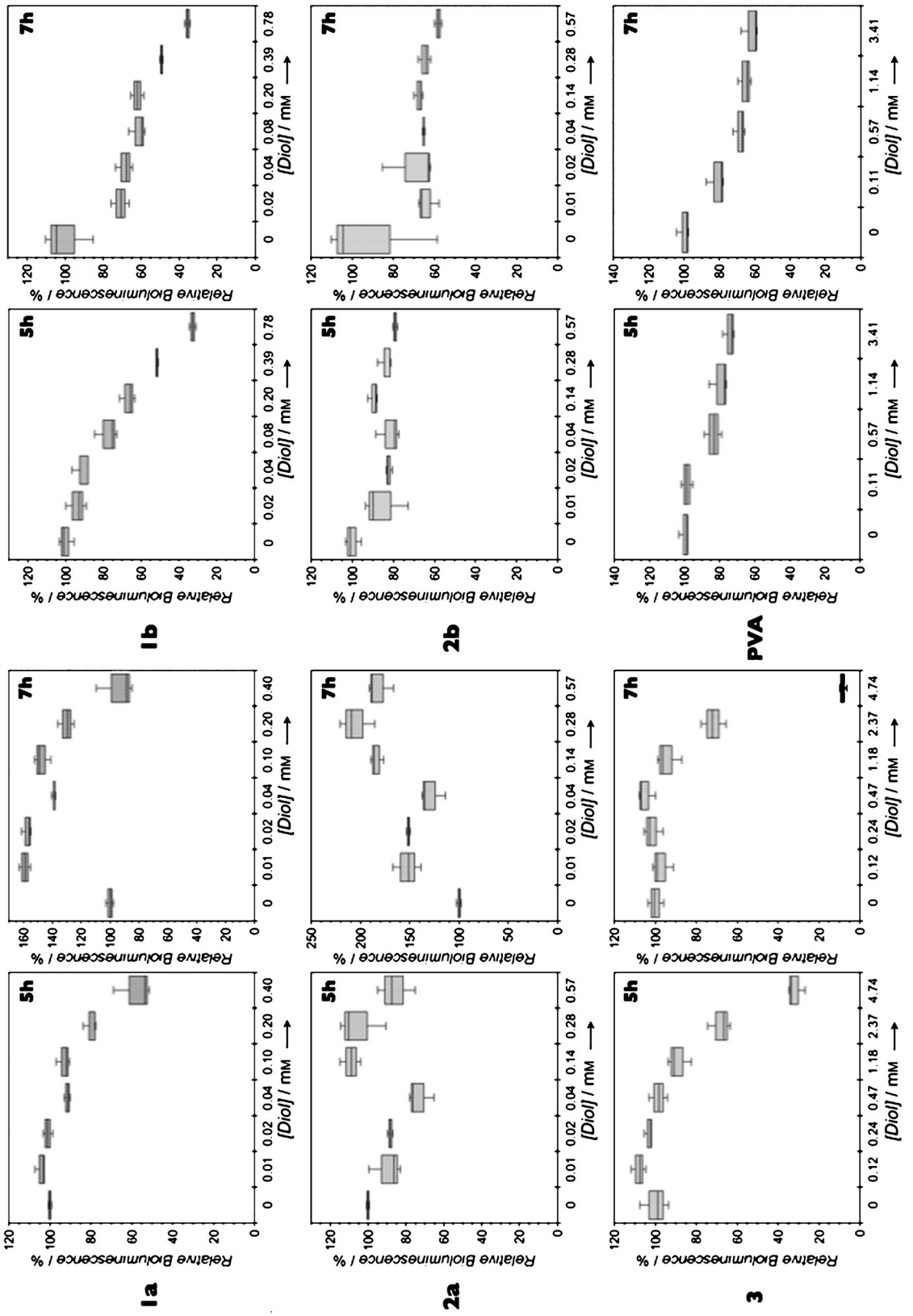

Figure 4. Effects of diol concentration on relative bioluminescence at 5 and 7 h time points for *V. harveyi* MM32 in the absence and presence of cationic polymers **1a** and **1b**, anionic polymers **2a** and **2b**, glycopolymer **3**, and control polymer PVA.

chols.[17,22] Aggregation experiments using phosphate-buffered saline (PBS) and Assay Broth (AB) medium (Figure 3e and f and the Supporting Information) confirmed this behavior for all the tested polymers and, importantly, showed that the strength of the binding affinity could be tuned as a function of the monomer composition. The abilities of polymers **2a** and **2b** to bind bacteria were not a result of nonspecific association of polymeric alcohols with *V. harveyi*, as shown by experiments with poly(vinyl alcohol) (PVA) as a control.[23]

All polymers quenched significantly the fluorescence signal corresponding to the AR-S/boronate complex, and in most cases affinities were higher than those calculated for the small-molecule analogues (Figure S2 in the Supporting Information). Interestingly, when we analyzed p(GlcEMA) (**3**), a 50-fold increase in affinity was observed when compared to methyl-β-D-glucopyranoside,[24] which suggests that, at least in the case of the carbohydrates, multivalency could play a significant role in the binding with boronic acids, and therefore in QS control. In addition, like their monomeric units, polymers **1** and **2** were able to reduce the concentration of AI-2 in solution, as shown by ^{11}B NMR spectroscopy (Figure 2c and d).

Having established binding of bacteria and boronic acids by polymers **1**–**3**, and their potential to reduce the concentration of AI-2 in solution, we investigated their effect on bacterial QS by means of bioluminescence. Unlike the expected dose-dependent suppression observed for conventional small-molecule AI-2 quenchers,[25,26] or polymers without the ability to bind bacteria,[12b] such as PVA (Figure 4 and the Supporting Information), the bioluminescence profiles reflected more complex behavior of *V. harveyi* in the presence of the dual-action polymers. Intriguingly, variations in time of onset, as well as magnitude, of light production were observed as the concentrations of different polymers in suspensions of bacteria varied (see the Supporting Information).

At the earlier time points (5 h, Figure 4) all the polymers exhibited dose-dependent suppression of light production except the lower-molar-mass anionic polymer **2a** (for which the effects were small). However, at intermediate time periods (7 h, Figure 4) increases in light production were observed in the presence of polymers **1a** and **2a** (and to a lesser extent **3**), with bioluminescence only suppressed at higher polymer concentrations. Over more extended times (see the Supporting Information) light production again was increased for low polymer concentrations and decreased at higher doses for all the polymers except PVA. These data suggest that the onset and duration of any quenching effects were strongly dependent on the cell density (cell growth cycle/time) as well as the affinity of the polymers towards the boron moiety and their ability to bind bacteria. Importantly, since QS itself partially depends on cell density[2] and clustering,[27,28] it is likely that those polymers able to aggregate bacteria as well as remove DPD were interfering in the cell signaling pathways by both a "cell-binding" and a "QS-quenching" mechanism. Since each of these processes could be in feedback, an apparent "oscillation" in light production can be inferred.

Although these experiments were performed independently for each of the polymers analyzed, the same dual behavior (bioluminescence inhibition versus enhancement) was observed in additional assays at lower polymer concentrations, as well as when polymers **1** and **2**, which show similar water solubility and boron affinity but different abilities in cell sequestration, were incubated using the same bacterial preculture in the presence of 0.4 mM borate to saturate AI-2 production (see the Supporting Information).

This suggests that in these systems reversible borate–diol binding reactions compete with irreversible bacteria–polymer clustering as well as bacteria–QS binding interactions. While the detailed mechanisms by which these interactions occur are the subject of ongoing studies, support for a feedback-competition model was obtained from experiments with PVA, which did not bind to bacteria but was able to sequester borate with low affinity, thus giving rise to simpler dose-dependent QS quenching (Figure 4 and the Supporting Information) but with no cell sequestration or cell-growth-dependent binding. By contrast, the dual-action polymers **1**–**3**, which have three different functional group types (cationic, saccharide, and catechol), each of which varied in total aggregation efficacy, were able to sequester *V. harveyi* through adhesion and interference with their QS network. Of crucial importance was that this range of polymers was able to maintain bacterial capture and suppression of QS signaling, but without damaging cell viability[29] (as judged by continuing growth in optical density measurements, see the Supporting Information). This is a key first step towards bacterial inactivation without invoking selection pressure.

In conclusion, we have prepared a series of polymers with dual function in binding and deactivating population responses in *V. harveyi*. The ability to sequester bacteria, while at the same time interfering with their QS response, is potentially a powerful route to novel diagnostics and antimicrobials. By tuning the affinities for cell–cell communication molecules and varying the potencies in cell aggregation through polymer design, a new class of signaling modulators and information carriers may emerge.

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