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Bacteria clustering by polymers induces the expression of quorum-sensing-controlled phenotypes

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Bacteria deploy a range of chemistries to regulate their behaviour and respond to their environment. Quorum sensing is one method by which bacteria use chemical reactions to modulate pre-infection behaviour such as surface attachment. Polymers that can interfere with bacterial adhesion or the chemical reactions used for quorum sensing are therefore a potential means to control bacterial population responses. Here, we report how polymeric 'bacteria sequestrants', designed to bind to bacteria through electrostatic interactions and therefore inhibit bacterial adhesion to surfaces, induce the expression of quorum-sensing-controlled phenotypes as a consequence of cell clustering. A combination of polymer and analytical chemistry, biological assays and computational modelling has been used to characterize the feedback between bacteria clustering and quorum sensing signalling. We have also derived design principles and chemical strategies for controlling bacterial behaviour at the population level.

Non-lethal means of targeting bacteria[1,2], such as the stimulation of host immune systems[3,4], interference with cell adhesion[5,6] or bacterial communication[7,8], are emerging as attractive means to avoid resistance against antimicrobial therapies. In recent years, polymeric antimicrobials have increasingly been a focus of attention due to their ability to present multiple functionalities for detecting, binding and inactivating pathogens[9–11]. There are now examples of polymers that can prevent cell growth in multi-drug-resistant strains[11], or that can sequester specific bacteria[12–14], toxins[15,16] and/or cell-signal molecules[17–19].

Particularly promising are materials that can prevent bacteria binding to hosts[5,6], a prerequisite for most infections and particularly those related to invasive pathogens[19]. Two main strategies have been exploited, the first utilizing antifouling surfaces to directly inhibit bacterial adhesion[20–22], and the second displaying multiple ligands that bind competitively to the surface of the bacteria, thus inhibiting their attachment to host surface ligands[12–14]. Depending on the material design, one of the consequences of the second approach is the aggregation of bacteria into clusters, a microenvironment where diffusion of nutrients and signals can be significantly affected.

A number of publications have now described the significant effects of local concentration and spatial confinement, as well as molecule and bacteria diffusion, on bacterial cell–cell communication networks[23–28]. Bacterial communication, also known as quorum sensing[29,30], is an important regulator of bacterial behaviour, including swarming, aggregation, production of exoenzyme and toxins, as well as processes preceding infection such as surface colonization and biofilm formation[31–34]. Quorum sensing signalling in bacteria often involves complex feedback mechanisms and is regulated by gene circuits and multiple interconnected control mechanisms[29,35]. This feedback between cell clustering and quorum sensing signalling has stimulated intense debate regarding the nature of quorum sensing and whether it is always a population density response rather than a function of cell clustering and signal diffusion[36,37].

We recently reported preliminary data indicating that certain polymers can modulate the luminescence of *Vibrio harveyi*, a marine pathogen that responds to the quorum-sensing signal AI-2 by producing light. These materials were designed to cluster bacteria while simultaneously reducing the concentration of AI-2, a component of the quorum-sensing circuit of several bacteria[38]. Unlike conventional polymers that are able to bind only to the quorum-sensing signals, and therefore inhibit light production in a dose-dependent way, some of those polymers were able to induce luminescence in *V. harveyi* under specific experimental conditions, suggesting interdependence between bacteria clustering and the quorum-sensing response[39].

We report here how a polymeric 'bacteria sequestrant', which induces bacterial aggregation through electrostatic interactions and has no functionalities to interfere with the quorum-sensing signals, is able to induce quorum-sensing-related responses in a range of bacteria. These include not only the model microorganism *V. harveyi*, but also the human pathogens *Escherichia coli* and *Pseudomonas aeruginosa*. We used synthetic and analytical chemistry, biological assays and computational modelling to demonstrate that quorum-sensing-associated behaviour occurs as a direct consequence of bacteria clustering. Furthermore, the responses of *V. harveyi* as a model organism were simulated and compared against a representative 'quorum quencher', which should only bind to quorum-sensing signals, and a 'dual-action' polymer, with the ability to bind both the surface of bacteria and the

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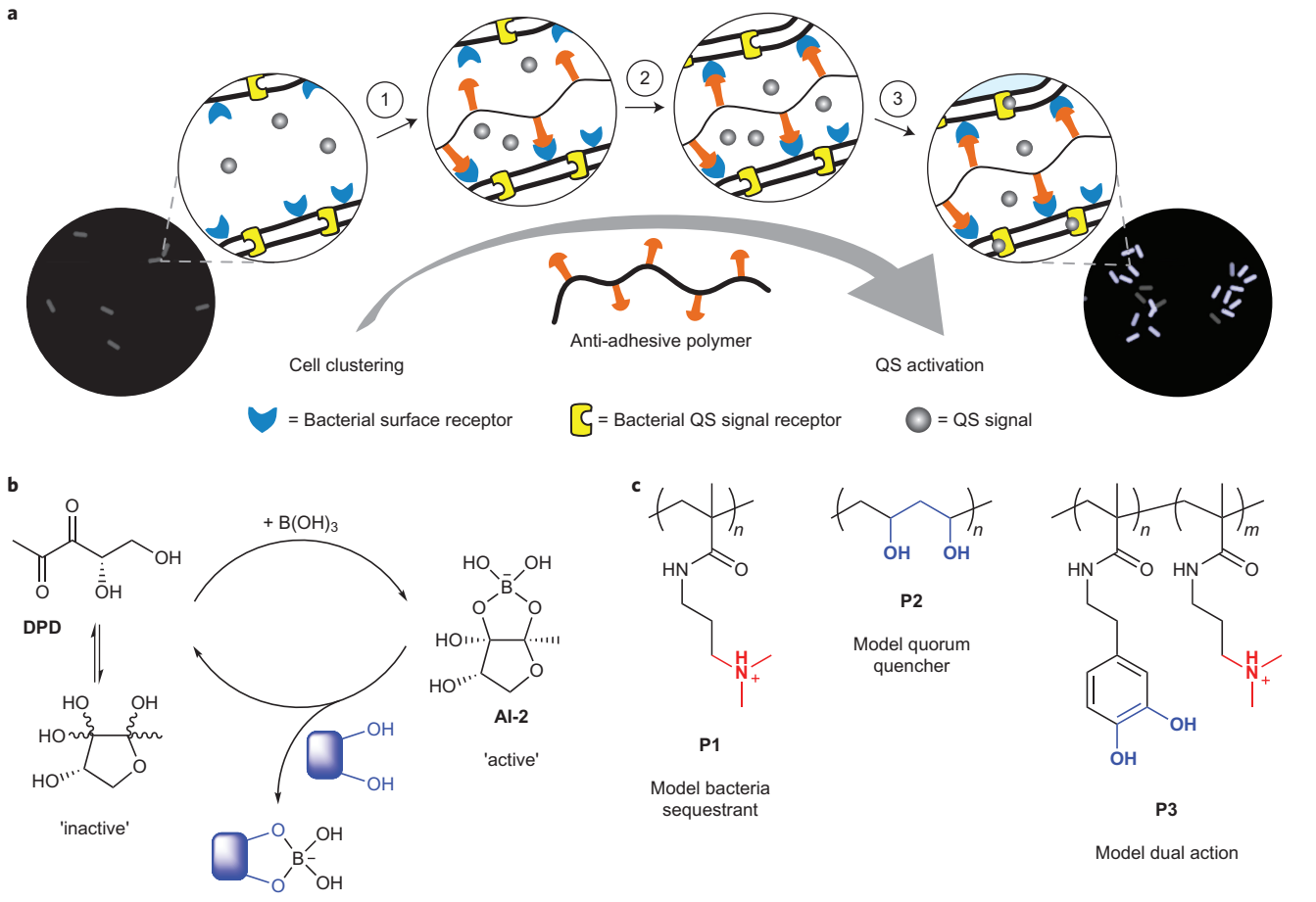

Figure 1 | Quorum sensing induction in the AI-2 network. a, Schematic representation of quorum sensing (QS) activation by bacteria sequestrants that promote bacteria clustering. Step 1: polymer binds to the surface of the bacteria via multivalent interactions. Step 2: bacteria are crosslinked as polymer interacts with different bacteria. Step 3: signal diffusion is limited, maintaining a high concentration within the cell cluster. **b**, Key components of the AI-2 network, including 4,5-dihydroxy-2,3-pentanedione (**DPD**) and the active species formed in the presence of $B(OH)_3$ in the media, and the mechanism of AI-2 quenching by competitive binding with diols. **c**, Structure of the polymers used in this work: poly(*N*-[3-(dimethylamino)propyl] methacrylamide) (**P1**), poly(vinyl alcohol) (**P2**) and poly(*N*-dopamine methacrylamide-co-*N*-[3-(dimethylamino)propyl] methacrylamide) (**P3**).

signal molecules. The results give important insight into the unexpected consequences of feedback between bacteria clustering and quorum-sensing signalling. Furthermore, the data suggest entirely new chemical design principles not only for novel anti-adhesive materials, but also for inducing consequences of quorum-sensing responses that are beneficial, such as antibiotic production[40,41].

Results

The starting hypothesis was that polymeric materials with the ability to aggregate bacteria into clusters would be able to induce the expression of quorum-sensing-controlled phenotypes (Fig. 1a)[39]. We thus derived a model that predicted, from a phenomenological point of view, induction of a feedback loop into quorum-sensing signalling by bacteria clustering, interrelating polymer (P) concentration, bacterial (B) aggregation and quorum-sensing signals (S). Three classes of polymers were therefore defined in order to predict all the potential interactions between polymers, bacteria and signals: (1) bacteria sequestrants, which should only bind to bacteria, inducing cell clustering? (2) quorum quenchers, which would only be able to bind the signals? and (3) dual-action polymers, with the ability to bind both signals and bacteria. The predicted clustering and quorum-sensing responses were validated against experimental data using *V. harveyi* and its AI-2 network (Fig. 1b) as a model.

Poly(*N*-[3-(dimethylamino)propyl] methacrylamide) (**P1**), a cationic polymer that should bind to the surface of bacteria through electrostatic interactions, was synthesized as a representative bacteria sequestrant. Controlled radical polymerizations (RAFT) were used to tune the molar mass and the materials were characterized by NMR and gel permeation chromatography (GPC). The behaviour of bacteria in the presence of **P1** was determined and compared to model polymers of the other classes. Because AI-2 in *V. harveyi* is a borate ester, and its concentration in solution can be reduced by competitive binding to the boric acid precursor with polymeric diols (Fig. 1b), commercially available poly(vinyl alcohol) (**P2**), and poly(*N*-dopamine methacrylamide-co-*N*-[3-(dimethylamino)-propyl] methacrylamide) (**P3**)[39] were chosen as representative quorum quenchers and dual-action polymers, respectively (Fig. 1c).

The viability of *V. harveyi* in the presence of these polymers was assessed by monitoring cell growth during luminescence experiments. For the relevant duration of the experiment (0–8 h), before solvent evaporation in the well plates becomes significant, no differences were observed in the optical density of the cultures in the absence and presence of increasing amounts of each polymer (Supplementary Figs 9b–14b). In addition, the viability of *V. harveyi* in the presence of **P1**, a polymer, which might be expected to exhibit toxicity due to a higher content of tertiary amines[42,43], was also investigated using nuclear staining and fluorescent microscopy. When compared against cultures in the

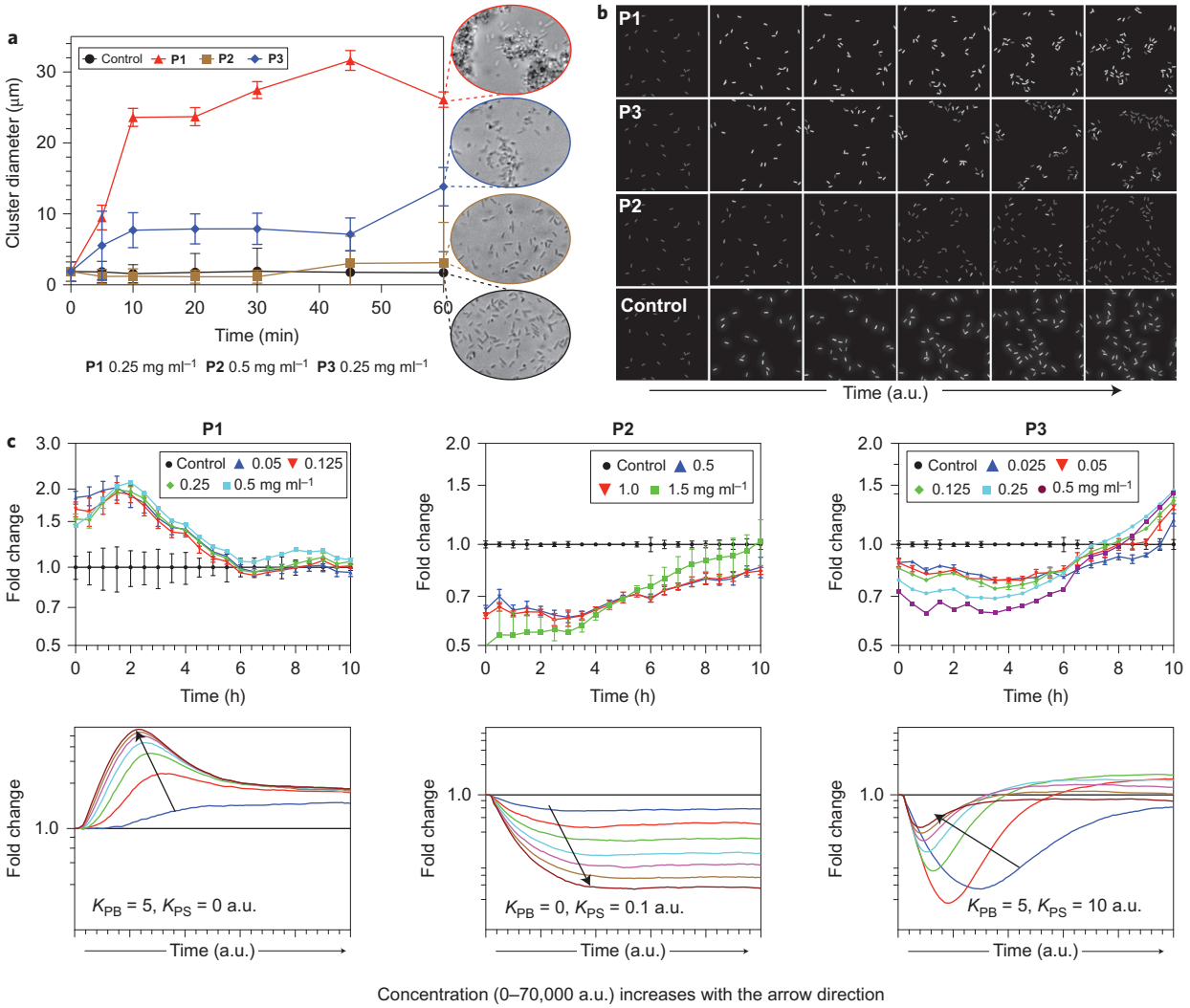

Figure 2 | Effect of polymers on *V. harveyi* MM32 behaviour. a,**b**, Aggregation of bacteria in the presence of polymers, measured in a Coulter Counter and by optical microscopy (**a**), were in good agreement with those predicted by the computational stochastic simulation, observed from simultaneous screenshots of simulated bacteria cultures in the absence and presence of polymers (**b**). Mean value and polydispersity index are reported. **c**, Quorum-sensing signalling in the presence of polymers, measured by luminescence (top row), was in good agreement with that predicted by the computational model (bottom row). Bacteria sequestrants (**P1**) enhanced luminescence (fold change ≥ 1) throughout the duration of the experiment (left). Quorum quenchers (**P2**) reduced luminescence (fold change ≤ 1) during the same timeframe (middle). For dual-action polymers (**P3**), both induction and quenching were observed (right). Mean value and standard deviation are reported. Two-way Anova analysis of experimental results indicates that significant differences in fold change are observed as polymer concentration increases, for the relevant duration of the experiment (0–8 h). See Supplementary Figs 2, 7 and 9–11 for further details.

absence of polymer (positive control) and cultures in the presence of methanol (negative control), the ratio between viable (green) and non-viable (red) bacteria in the presence of **P1** was similar to that of the positive control (untreated bacteria) and significantly different from the negative control (Supplementary Fig. 27). This indicates that **P1** was not altering quorum sensing through a direct toxic response.

The ability of the polymers to cluster bacteria and their effect over quorum-sensing networks was investigated in cultures of two strains of *V. harveyi* (MM32 and BB170). Initial experiments were carried out with *V. harveyi* MM32, which responds to exogenous AI-2 but does not produce the quorum-sensing signal precursor 4,5-dihydroxy-2,3-pentanedione (**DPD**). Concurrent with the experimental assays, polymer–bacteria interactions were simulated based on a simple affinity model. Cell aggregation experiments (Fig. 2a) showed a good match with the computationally predicted results (Fig. 2b), with **P1** inducing rapid bacterial clustering, **P2** producing no apparent difference compared to bacterial suspensions alone, and **P3** forming aggregates with bacteria at a similar rate to **P1**. Computationally predicted results were simulated ten times using different randomizations in order to obtain statistically reliable results. Effects were consistent within the ten simulations. Initial conditions (cell positioning, random seeds, affinities and polymer concentrations) for simulations with different polymers were identical (Fig. 2b, Supplementary Figs 28–30).

We then considered the effects of clustering on the quorum-sensing response as reported by luminescence. Taking into consideration feasible diffusion rates and affinities for the interactions between bacteria, signals and polymers, we predicted changes in luminescence with the addition of bacteria sequestrants (**P1**), quorum quenchers (**P2**) and dual-action polymers (**P3**). As is apparent from Fig. 2c, when compared to a control in the absence

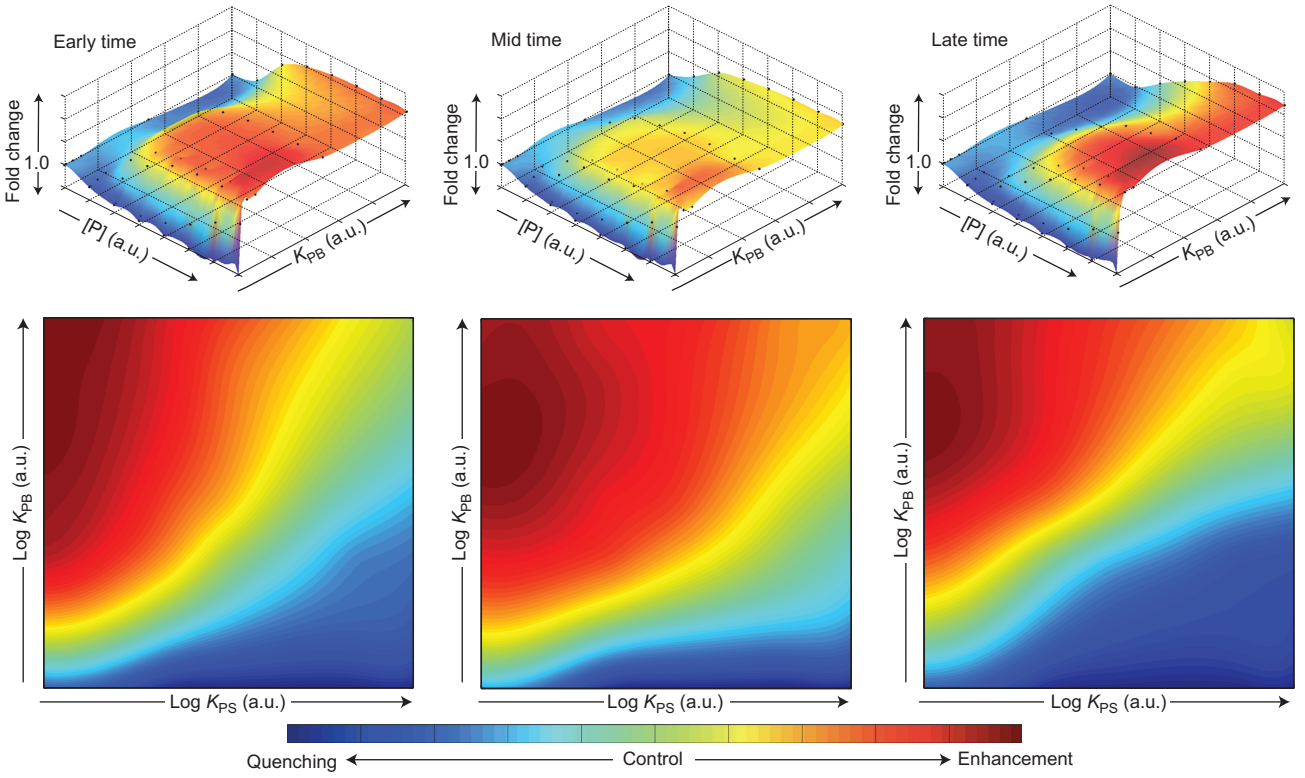

Figure 3 | Effect of relative binding affinities on light production. The effect of polymer affinity for signals (K_{PS}) and bacteria (K_{PB}) on light production was predicted by the model. In the presence of weak polymer–bacteria interactions, dual-action polymers (**P3**) quench light production, regardless of the polymer concentration [P] and time at which light production is evaluated (top). As K_{PB} increases, the overall outcome of polymer interference changes, and enhancement of light production is expected at higher polymer concentrations. In addition, overall light production depends on the relative intensities (K_{PB} and K_{PS}) of both affinities as well as the time at which light production is evaluated (bottom). To obtain dual-action polymers that consistently quench light production, polymers with low K_{PB} are required. Initial conditions (cell positioning, random seeds) for the simulations are identical. Times of 5,000, 15,000 and 30,000 a.u. were selected as representative early, mid and late times, respectively, for the simulations. Top: $K_{PS} = 0.1$ a.u. was selected as a representative value. See Supplementary Figs 31–39 for further details.

of polymers, **P1** induced an increase in light production in *V. harveyi* MM32 cultures throughout the duration of the experiment (fold change in luminescence ≥ 1), despite not being targeted to quorum sensing and lacking the functionalities to interfere with the signals. **P2** was able to reduce luminescence during the same time (fold change ≤ 1). As expected, **P3** showed a dual mode of behaviour, with the ability either to enhance or reduce light production depending on the specific polymer concentration and time (bacteria density). For **P1**, the absolute change in luminescence was higher at later stages of the experiments, when cell numbers were higher (Supplementary Fig. 8), but the relative difference (fold change) in luminescence was higher at earlier stages. Relative variations in luminescence at early stages of bacterial growth can appear exaggerated in cases of low initial values of luminescence, because the timescale for aggregation is considerably smaller than that for light production. Therefore, the effects of cell clustering were most apparent at an early time in the experiment, and became less pronounced as bacterial growth matched the density and viscosity within clusters. Thus, at early time periods, it was expected that slow diffusion of signals from the cell clusters enabled bacteria to sense a higher concentration of quorum-sensing signals more rapidly. Indeed, during the key timescales of the experiment (that is, 0–8 h, after which cell numbers increased markedly), the effect of polymer on quorum-sensing-controlled luminescence matched well that predicted by the theoretical model.

To evaluate further the feedback between the ability of polymers to induce aggregation and quorum-sensing-controlled light production, several simulations were performed in which the affinities towards bacteria (K_{PB}) and signals (K_{PS}) of **P3** were systematically varied. Affinities were investigated over a three order of magnitude range, and different combinations of K_{PB} and K_{PS} were simulated (see Supplementary Figs 31–39 for further details). As can be seen in Fig. 3, the potential of a polymer to inhibit or enhance light production was highly dependent on polymer concentration, experiment time and the polymer affinities for signals and bacteria. Variations in any of these parameters were predicted to lead to, and indeed showed, marked changes in quorum-sensing signalling, manifest in the light production. For instance, polymers with a high affinity for bacteria enhanced light production, regardless of their concentration and the time of the analysis, even if they showed a high affinity towards the signals.

The effects bacterial density and growth rate have on the activity of dual-action polymers were also investigated. Polymer affinities for bacteria and signals were fixed and simulations with different initial densities of bacteria B_0 or different growth rates, as expressed by bacteria doubling time T, were performed (see Supplementary Figs 40–42 for further details). When the initial density of bacteria B_0 was reduced by an order of magnitude (Supplementary Fig. 41), **P3** was able to induce quorum-sensing signalling throughout the duration of the simulation and regardless of the concentration of polymers, as opposed to the dual action exhibited when the starting number of bacteria was higher (Supplementary Fig. 40). Similarly, the ability of **P3** to induce or inhibit light production was significantly affected in the presence of bacteria growing at different rates (compare Supplementary Figs 40 and 42).

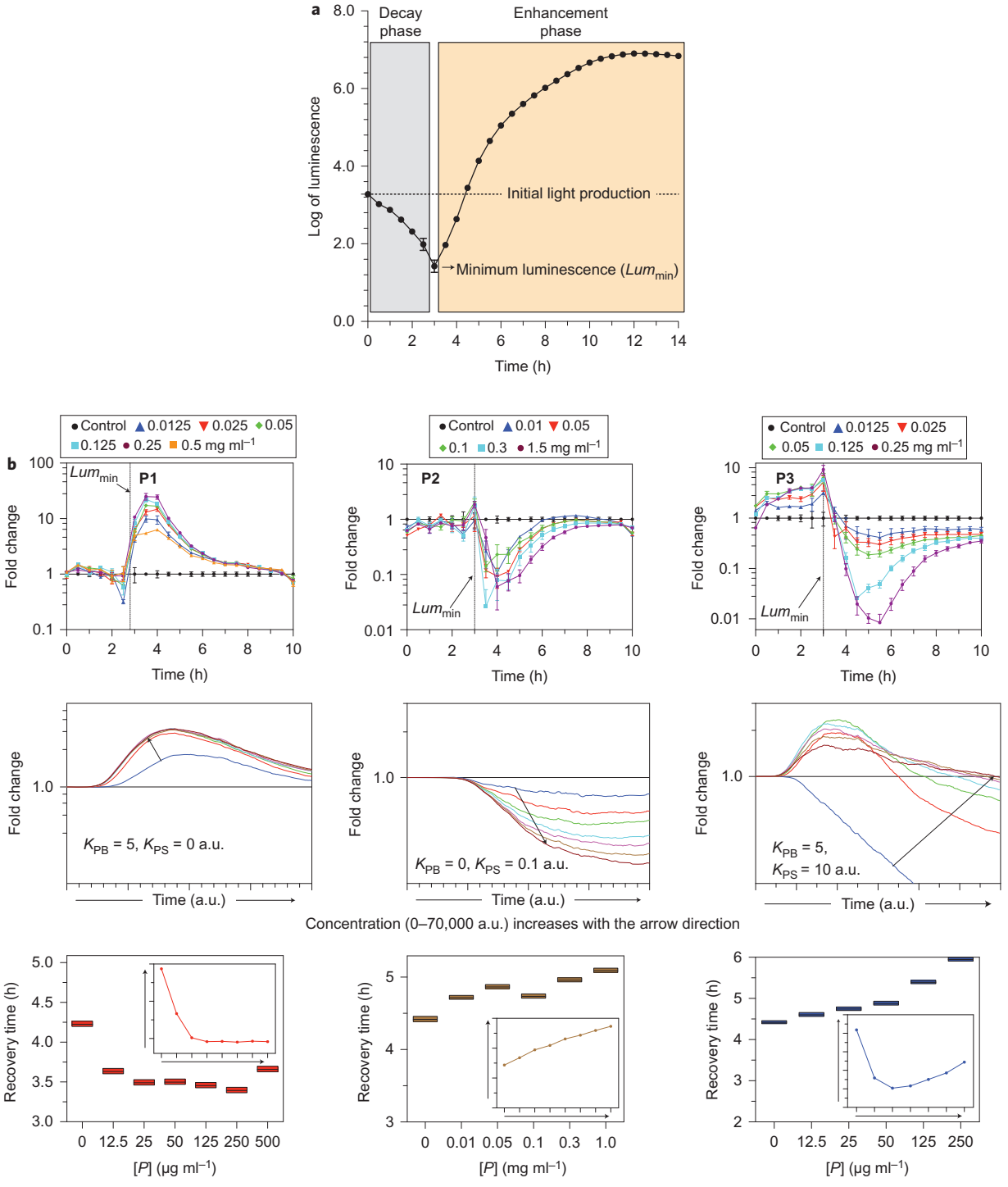

Figure 4 | Effect of polymers on *V. harveyi* BB170 luminescence. a, Luminescence in *V. harveyi* BB170 shows two phases: a decay phase where luminescence is reduced and an enhancement phase, after the minimum concentration of AI-2 for luminescence induction is reached. **b**, Polymer interference with quorum-sensing signalling, as measured by luminescence (top row), was in good agreement with that modelled (middle row). Bacteria sequestrants (**P1**) enhanced luminescence (fold change ≥ 1) (left); quorum quenchers (**P2**) reduced light production (fold change ≤ 1) (middle); and for dual-action polymers (**P3**) both induction and quenching of luminescence were observed (right). Additionally, differences in the time necessary to recover the initial intensity of luminescence could be observed (bottom row). **P1** induced earlier light production and **P2** delayed the recovery time. In this case, **P3** behaved as a quorum quencher and delayed the onset of luminescence. This behaviour was well predicted by the model (bottom row, insets). Mean value and standard deviation are reported. Two-way Anova analysis of experimental results indicates that significant differences in fold change are observed, as polymer concentration increases, for the relevant duration of the experiment (0–8 h). See Supplementary Figs 12–17 for further details.

The interaction of polymers and bacteria was also investigated using the *V. harveyi* BB170 strain, which is capable of producing **DPD**. In this case, light production in the absence of polymers shows two phases (Fig. 4a). In the first phase, luminescence decreased as the bacteria responded to the lower concentrations of **DPD** in the sample media as compared to the pre-culture

medium, before their production of endogenous **DPD**. When a threshold of **DPD** concentration was achieved, a new phase was attained wherein light production increased as a function of **DPD** concentration.

Despite the differences in light production profile for both strains, the effect that all three classes of polymers had on BB170 quorum-sensing signalling (Fig. 4b, top and middle), as measured by luminescence, was very similar to that described for MM32 (Fig. 2c, top). Throughout the duration of the experiments, bacteria sequestrants (**P1**) were able to induce light production, quorum quenchers (**P2**) reduced the overall production of light, and dual-action polymers (**P3**) showed both induction and quenching of luminescence. The effect was weaker during the decay phase (time lower than the time required for Lum_{min}), as the concentration of AI-2 will be well below the detection threshold for most of this period.

The effect of polymers on BB170 quorum-sensing signalling was also reflected in the duration of the decay phase and the time taken for bacteria to sense a concentration of AI-2 above the threshold. During this decay phase, light production was reduced because luciferase production was switched off while bacteria re-adapted to the low concentration of AI-2 after dilution. As the population of bacteria increased, the amount of AI-2 in solution increased accordingly, so that quorum-sensing signalling could recover. This effect was easily monitored from the light production plots by measuring the time taken by the bacterial suspension to recover a significant level of light intensity, for instance, the initial value of light production (Fig. 4a).

Interestingly, by confining cells into clusters, bacteria sequestrants (**P1**) were able to induce an earlier activation of quorum-sensing signalling as a consequence of the local higher concentration of AI-2. Conversely, quorum quenchers (**P2**) were able to delay the time needed to do so, as the concentration of AI-2 in solution was reduced. For dual-action polymers (**P3**), a combination of both effects was expected. In the reported example, **P3** showed an overall quenching effect, increasing the time needed for the recovery of the initial luminescence (Fig. 4b, bottom).

To investigate the generality of this effect and the potential for directing quorum-sensing-controlled phenotypes in relevant human pathogens, further experiments were performed with *E. coli* and *P. aeruginosa*. *Escherichia coli lux*-based acylhomoserine lactone (HSL) biosensors, JM109 pSB1075 (ref. 44), and JM109 pSB536 (ref. 45), which produce light in response to *N*-(3-oxododecanoyl)-L-homoserine lactone (3O-C12-HSL)[46] and *N*-butyryl-L-homoserine lactone (C4-HSL)[47], respectively, were selected as representative *E. coli* strains. *Pseudomonas aeruginosa* PA01 *pqsA* CTX-*pqsA*::*lux*[48], which produces light in response to 2-heptyl-3-hydroxy-4(1H)-quinolone, usually termed the *Pseudomonas* quinolone signal (**PQS**)[49], was selected as a representative *P. aeruginosa* strain.

Aggregation of these strains in the presence of **P1** was very fast, and a dose-dependent increase in optical density of the cultures could be observed as soon as **P1** was added (Supplementary Figs 19–26, time 0). In addition, the growth of both *E. coli* strains in the presence of **P1** was significantly compromised, notably in the case of pS536 reporter. This lack of growth had an impact on luminescence production for these strains. As **P1** concentration increased, the production of light decreased, in agreement with the decreased viability of *E. coli* in the presence of **P1**. With higher polymer concentrations, recovery of light production was observed as a consequence of light induction being triggered by clustering (Supplementary Figs 20 and 22). *P. aeruginosa* showed better viability in the presence of **P1**, and induction of light production in the presence of bacteria sequestrant **P1** was clearly observed, particularly at higher polymer concentrations (Supplementary Figs 23–26).

Discussion

The initial finding that polymers intended to suppress quorum sensing could in fact enhance cell signalling (as reported by light production)[39] was unexpected and suggested that bacteria confinement into clusters could be responsible for quorum-sensing induction, thus producing an effect opposite to that desired.

Spatial confinement is inherent to quorum sensing as cell density helps regulate bacteria behaviour. Significantly, recent publications[23,24,28] have suggested that a confined individual bacterium can show quorum-sensing-type behaviour. This behaviour has been computationally anticipated[50] in studies, suggesting that programmable compartmentalization is a Turing-complete mechanism, and can thus be a potentially useful tool for the control of population responses in synthetic biology. In addition, as noted in the case of *Pseudomonas* species, quorum-sensing signal gradients have a context-dependent action, with limited effects on biofilm growth in liquid culture but pronounced and significant effects on confined cell communities attached to surfaces, that is, when bacteria and signals are in close proximity[27].

Therefore, to understand how quorum sensing could be activated by bacteria sequestrants, we derived a phenomenological synthetic biology model to simulate and predict quorum-sensing-controlled luminescence as a function of binding affinities towards bacteria and signals, in two different mutants of *V. harveyi*. We used the MM32 strain, which responds to, but cannot produce, its quorum-sensing signal (**DPD**, the AI-2 precursor), and the BB170 strain, which is capable of synthesizing **DPD** and thus introduces natural variability and nonlinearity into the system.

We synthesized a model bacteria sequestrant, **P1**, intended to bind to the surface of bacteria through electrostatic interactions. The ability of **P1** to aggregate bacteria into clusters was confirmed by measuring cluster size and via optical microscopy. In addition, **P1** induced light production throughout the incubation assay, despite not being targeted to quorum sensing and having no specific functionalities to interfere with the signals. Taking into account the ability of the polymers to cluster bacteria, the predicted relative affinity of the monomer units within the polymers for the bacteria[39], the behaviour of *V. harveyi* in the presence of **P1** was well predicted by the computational model.

In contrast, the behaviour of *V. harveyi* in the presence of **P2**, a model quorum quencher, was markedly different to that in the presence of **P1**. In addition, a combination of the two responses could be observed when using **P3**. We termed **P3** a dual-action polymer as it incorporated both the cationic groups that bind to the surface of bacteria, inducing cluster formation, and diols capable of binding the boronic acid needed to activate AI-2. The numbers of monomer units, that is, the components in each repeating section of the polymers able to 'bind' signals or cells, were broadly similar across **P1** to **P3** (degrees of polymerization, 100–400), and no significant differences in response were observed when **P1** with different molecular weight was used (Supplementary Figs 6 and 18). Nevertheless, from a phenomenological point of view, there was good correlation between simulated and experimental quorum-sensing responses in the presence of these polymers.

The goal of the model was not only to understand the feedback between aggregation and light production, but also to derive design principles for quorum-sensing control. We therefore performed a series of simulations where the relative affinities of **P3** towards bacteria and signal were systematically varied. As can be seen in Fig. 3, to design efficient polymeric materials that can cluster bacteria, dual-action polymers have to be considered where the balance between the affinity towards the bacteria (K_{PB}) and the affinity towards the signal (K_{PB}) prevents induction of quorum-sensing-controlled phenotypes (green to blue colour in the graphs). In a similar way, the model predicted, and experiments showed, that quorum quenchers designed to reduce the expression of quorum-sensing-controlled behaviour should also exhibit a very low affinity for bacterial surfaces in order to retain their intended effects on bacterial populations. Most notably, the models and experiments

showed that small changes in initial bacterial density and growth rates could tip the balance to strongly opposing effects, so either luminescence enhancement or quenching could be seen for the same polymer under very similar conditions. This variability in conditions is likely to be most apparent in therapeutic applications of polymers, where the numbers and growth for pathogens will differ significantly across patients, or the degree of infection.

The model developed herein was designed to be 'agnostic' to the nature of the bacteria, as well as the type of response triggered by quorum sensing. In principle, therefore, any bacteria behaviour under quorum-sensing control, such as the production of exoenzymes and toxins, or biofilm formation[31,32,34], could be triggered if cell clustering is induced by bacteria sequestrants.

We therefore investigated whether the reported enhancement of light production by a bacteria sequestrant could also be detected using different bacteria and different signalling molecules. Experiments under the same conditions optimized for *V. harveyi* were performed using *E. coli* and *P. aeruginosa* luminescence reporters for HSLs and PQS, respectively. These signals are significantly different to AI-2 in terms of their chemical functionality, and the quorum-sensing response of the microorganisms is not synchronized in the way that *V. harveyi* responds to AI-2. Despite these differences, and the lower viability of *E. coli* strains in the presence of **P1**, the ability of this polymer to induce light production as a consequence of aggregation was also observed, establishing the generality both of the quorum sensing/polymer/bacteria feedback model and the mechanism of activity of the dual-action polymers.

Conclusions

In conclusion, we have shown how polymeric bacteria sequestrants, with high affinity for bacterial surfaces, have the ability to interfere with non-targeted signalling pathways such as quorum sensing in a range of prokaryotes. We have defined a theoretical and practical framework for understanding bacterial responses to quorum-sensing interference in the presence of polymers with the ability to bind bacteria and/or signalling molecules, which should aid the development of novel non-antibiotic anti-infectives.

Given that many bacteria attach to host surfaces before colonization and invasion, our data suggest that materials designed to interfere with infection pathways should be designed so that they do not promote unwanted effects in cell signalling and quorum sensing. Significantly, the results show that materials that promote bacteria clustering induce unexpected responses in quorum-sensing-controlled phenotypes, and that these responses can be better modulated through control of the affinity towards both bacteria and signals. As a corollary, the combined model/experiment approach enables experimental data to be obtained regarding spatial effects on quorum sensing, which can be interrogated through computational models, which in turn can feed back into materials design. This combined chemistry/computation approach should enhance our understanding of quorum sensing in complex environments. In turn, the ability to utilize specific chemical design principles to control cell behaviour should facilitate the development of antimicrobials that avoid selection pressure and inform synthetic biology strategies wherein quorum sensing is used to induce the production of valuable metabolites.

Methods

Aggregation assay. A single colony of *V. harveyi* grown on Luria Bertani (LB) agar plates was used to inoculate 2 ml LB medium containing chloramphenicol (10 μg ml^{-1}), and kanamycin (50 μg ml^{-1}) in the case of BB170. The bacteria were grown with aeration at 30 °C overnight. Boron depleted assay broth (AB) medium was then inoculated with this preculture to give a bacterial suspension with an optical density (600 nm; OD_{600}) of 1.0. Aliquots of this culture were then mixed with known volumes of stock solutions of polymers in Dulbecco's phosphate buffer saline (DPBS). The values of polymer concentration reported for the aggregation experiments correspond to the polymer concentrations in these suspensions. To measure cluster size, these bacterial suspensions were added to a Coulter Counter flow cell filled with H_2O (<14 ml) to obtain an obscuration of 8–12%. Cluster size was then measured at different time intervals. For optical microscopy analysis, aliquots (10 μl) of the bacterial suspensions, in the absence and presence of polymers, were collected after 60 min, mounted on a glass slide with a coverslip on top, and examined with an optical microscope. See Supplementary Figs 1–8 for further details.

Microbiological assays. A single colony of *V. harveyi* grown on LB agar plates was used to inoculate 2 ml LB medium containing chloramphenicol (10 μg ml^{-1}), and kanamycin (50 μg ml^{-1}) in the case of BB170. The bacteria were grown with aeration at 30 °C overnight. Boron depleted AB medium was then inoculated with this preculture (5,000:1). For MM32, boric acid was added to a final concentration of 400 μM, and **DPD** was added to a final concentration of 22 μM. For BB170, boric acid was added to a final concentration of 22 μM. A volume of 180 μl of the inoculated medium was placed in each of the wells of a 96-well plate and combined with 20 μl of the samples to be analysed. Each compound was tested over at least three different concentrations. Light production and OD_{600} were recorded at 30 °C every 30 min for at least 10 h in a 96-well plate, after which time solvent evaporation became a significant issue. The experiments were carried out in triplicate and the plotted curves were derived from the mean value. The normalized luminescence was calculated by dividing the light output by the optical density at each time point.

Simulation methods. Because of the spatial and time scales of the system, a mesoscopic lattice-based model and an agent-based approach were used. Analysis of the results was carried out at the phenomenological level, that is, by capturing the characteristic effect of the three types of polymers. Modelled parameters were subsequently refined against measurable overall effects (that is, bacterial binding and luminescence production). The starting boundary conditions for the model were set so that any deviations from control experiments were caused by the polymers manipulating the immediate extracellular environment of the bacteria. Three types of object were considered in the model: bacteria (B), polymers (P) and signal molecules (S). The size of each B was fixed to occupy a square of 2 × 2 arbitrary lattice spaces. The sizes of S and P were considered to be negligibly small. One unit lattice space could thus contain a quarter of B and unlimited numbers of S and P. Two types of change were considered to take place in the system: chemical binding and diffusion of the objects. The reactions and diffusion of the different species were modelled using a Gillespie algorithm.

Three types of binding reaction were delineated in the model: binding between S and B, binding between B and P, and binding between P and S. Each interaction was considered to be reversible. In addition, there were delimiting conditions for the model: (1) B has separate binding sites for S and P; (2) each quadrant of B has a number of S binding sites, denoted as BS, and a number of P binding sites, BP. Similarly, each P has PS binding sites for S and two B binding sites, PB. Each S could thus only bind to one binding site (either B or P) and would not be available for other reactions once bound, until the reverse reaction occurred and the molecules and binding sites again became free. The same constraint was applied for P–B binding. We considered that S could only bind with P or B in the same lattice, and that bound S would move with P or B without further activation of the quorum-sensing network. An individual P was considered to bind with B inside the same lattice space. For S, once a single P was bound to a single B, they were considered to be fixed in a binding interaction over the timescale of the experiment. P bound to B were able to bind with other B in the neighbouring lattice. In such a case, the B–P–B complex moved as a single unit until associative interactions were lost. This mechanism represents key multiple binding interactions that could lead to bacterial clustering and aggregation. The values of binding affinities and diffusion rates are dependent on the concentrations of the different objects in the local environment (see Supplementary Sections 28–42 for further details).

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Author contributions

All authors conceived and designed the experiments, analysed the data and discussed the results. N.K. and C.A. secured funding, X.X., C.S., A.B., L.L. and F.F-T performed the experiments and L.L., F.F-T., N.K. and C.A. co-wrote the manuscript.

Additional information

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Competing financial interests

The authors declare no competing financial interests.