Nano-guided cell networks as conveyors of molecular communication

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Advances in nanotechnology have provided unprecedented physical means to sample molecular space. Living cells provide additional capability in that they identify molecules within complex environments and actuate function. We have merged cells with nanotechnology for an integrated molecular processing network. Here we show that an engineered cell consortium autonomously generates feedback to chemical cues. Moreover, abiotic components are readily assembled onto cells, enabling amplified and ‘binned’ responses. Specifically, engineered cell populations are triggered by a quorum sensing (QS) signal molecule, autoinducer-2, to express surface-displayed fusions consisting of a fluorescent marker and an affinity peptide. The latter provides means for attaching magnetic nanoparticles to fluorescently activated subpopulations for coalescence into colour-indexed output. The resultant nano-guided cell network assesses QS activity and conveys molecular information as a ‘bio-litmus’ in a manner read by simple optical means.

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It has become increasingly apparent that a wealth of molecular information exists, which, when appropriately accessed, can provide feedback on biological systems, their componentry and their function. Thus, there is a developing niche that transcends length scales to concurrently recognize molecular detail and at the same time provide understanding of the overall system. An emerging scheme is to develop nano- to microscaled tools that intimately engage with biological systems through monitoring and interacting at the molecular level, with synthetic biology being one such tool.

While synthetic biology is often viewed as an innovative means for ‘green’ product synthesis through the genetic rearrangement of cells, their biosynthetic capabilities and their regulatory networks can instead be tuned for executive functions. That is, cells can be rewired to survey molecular space as they have sophisticated capabilities to recognize, amplify and transduce chemical information. Further, they provide a means to connect biological systems with traditional microelectronic devices and in doing so present a potential interface between chemically based biomolecular processing and conventional vectors of information flow, such as electrons and photons.

Specifically, through engineered design, cell-based molecular processing can be further coupled to enable external abiotic responses. Cells, then, represent a versatile means for mediating the molecular ‘signatures’ common in complex environments, or in other words, they are conveyors of molecular communication.

Further, beyond clonal cell-based sensors, there is an emerging concept of population engineering to establish microorganisms in deliberate networks that enable enriched system identification through a combination of distinctive yet coexistent behaviours, including, perhaps, competitive or cooperative features. We posit the use of cell populations assembled in parallel where multiple microbes with distinct molecular recognition capabilities work congruently. An advantage is that populations, as opposed to few cells, can facilitate thorough sampling since the presence of many cells increases their spatial breadth and per-cell data contributions. Each cellular unit undergoes independent decision-making and contributes a datum to its entire constituency. The prevalence of data provided within the population, then, substantiates a collective output by the system based on the molecular landscape. As follows in a multipopulation system, molecular input thus influences the outcomes of each population, and elicits plural responses when the molecular input ranges overlap the ranges of the sensing populations, which can define classification boundaries.

Cell-mediated classification was posited in silico by Didovyk et al., where reporter libraries with randomized sensitivities to a molecular cue elicit concentration-dependent fluorescent patterns and these are elucidated by population screening. In the present construct, multiple populations enable multiplexed analysis, resulting, here, in a response gradation that is designed to index the molecular input ‘signature’. Consequently, the feedback information becomes transfigured beyond a dose-dependent cell-by-cell analysis. That is, the output is predicated by the comparison between the populations rather than accumulation of response within a total population.

With population engineering as a premise for enriched molecular information processing, we engineered cell species, each to achieve an appropriate output through genetic means. There is conceptual basis for incorporation into networks, such as through mobile surveillance and position-based information relay. Hence, it is conceivable that, in addition to autonomous molecular recognition and processing afforded by synthetic biology, the use of physical stimuli to enable cell response could confer similar networking properties.

For example, the complete information-processing ‘repertoire’ can be expanded beyond specific cell responses by the integration of external stimuli that serve to collate cell populations.
Specifically, we envision integration of nanomaterials that enable co-responses to molecular inputs, such that cell populations employ traditional reporting functions, that is, fluorescence marker expression, as well as responses that enable additional processing via the integration of stimuli-responsive abiotic materials (Fig. 1b).

In our example, cells are engineered to respond by permitting the attachment of magnetic nanoparticles (mNPs), such that each fluorescent cell becomes receptive to a magnetic field. Thus, the combination of cell-nanoparticle structures provides further dimensionality for the conveyance of molecular information (via magnetic stimulation). That is, without magnetic collation the fully distributed system would harbour diffuse responses; a magnetically stimulated system results in acute output due to a filtering and focusing effect (Fig. 1b)\textsuperscript{31,32}, allowing binned information to be readily, and fluoroescnetly, conveyed.

The detection and interpretation of signalling molecules in our example is based on a microbial communication process known as quorum sensing (QS). The molecules, autoinducers (AIs), are secreted and perceived within a microbial community; once accumulated, the AI level indicates that the population size has reached a ‘quorum’\textsuperscript{33,34}. By surpassing a threshold concentration, the AI signalling coordinates population-wide phenotypic changes\textsuperscript{35}. We have designed a QS information processor that utilizes two cell populations to independently interrogate natural microbial communities and generate information about QS activity by accessing AI-2 (ref. 36). Each cell population becomes ‘activated’ in response to a characteristic AI-2 level by expressing a fluorescent marker and a streptavidin-binding peptide (SBP) on the outer membrane\textsuperscript{38}. SBP provides a means for collating data by binding mNPs that are introduced into the community. Using a post-processing magnetic sweep, the system as a whole interprets a molecular landscape and refines output into colour-categorized, or ‘binned,’’ states (no fluorescence, red, or red and green) through (1) parallel population processing and (2) acute focusing (Fig. 1c).

The use of engineered cells as data-acquiring units and selectively equipping each with functional nanomaterials to form a redistributable processing system merges two paradigms: decentralized, active probing at a molecular scale and self-organization of units through structured dependencies on stimuli\textsuperscript{42}. The population-based system overall contributes categorized feedback about a biological environment.

**Results**

**Surface expression of SBP and fluorescent protein fusions.** First, we established expression of a fusion protein consisting of a fluorescent marker (enhanced green fluorescent protein (eGFP) and variants) and SBP. Importantly, for SBP to function as a coupling agent between cells and mNPs, we used AIDAc (kindly shared by J. Larssen)\textsuperscript{40} to export the chimeric protein to Escherichia coli’s outer surface. Translocation to a cell’s surface utilizes a signal peptide (for inner membrane translocation) and AIDAc as an outer membrane autotransporter por\textsuperscript{38–41}, with the passenger protein linked to each. In Fig. 2a, we depict expression of three different constructs using Venus, eGFP and mCherry for optical transmission, and the AIDAc translocator domain for surface localization. These constructs are mapped in Supplementary Fig. 1. After induction with isopropyl β-D-1-thiogalactopyranoside (IPTG), controls were probed for surface expression of the SBP portion of the tagged fluorescent protein. Cells were incubated with fluorescently labelled streptavidin; the fluorophore of the streptavidin probe was orthogonal to the expressed fluorescent protein. The multiple fluorescence emissions were analysed by confocal microscopy without spectral overlap.

The fraction of cells (\(f_c\)) that exhibit colocalized fluorescent protein and the fluorescently-labeled streptavidin is reported in Fig. 2b, showing that SBP–Venus cells bound streptavidin at a slightly lower frequency than SBP–mCherry and SBP–eGFP, which exhibited statistically similar fractions (\(f_c = 0.7\)).

That is, microscopy results related to the colocalization analysis are depicted for pairings of Venus and blue-streptavidin (SA), eGFP and red-SA, and mCherry and green-SA (Fig. 2c). Strong signals were observed in both filter sets (the fluorescent protein (Column I) and the labelled streptavidin (Column II)). Overlaiding each image reveals colocalization, as indicated in Column III, where arrows point to examples of strong colocalization. In addition, Column IV plots fluorescence intensities across horizontal sections of the images, where cells that exhibit colocalized fluorescence are indicated by superimposed peaks. For + pSBP–Venus cells, those with both a blue and yellow signal are observed as pale blue-violet in the overlaid image. Cells with + pSBP–eGFP and + pSBP–mCherry and labelled streptavidin emit both green and red signals; their colocalization appears yellow. Controls shown in Supplementary Fig. 2, verify that fluorescent streptavidin (all colours) has specificity for only SBP-expressing cells over negative controls. Colocalization indicates that not only are both components of the fusion, SBP and the fluorescent protein, expressed, but that SBP is accessible to bind streptavidin on the cell’s surface. This is the first use of AIDAc for cell surface anchoring of fluorescent proteins, each having been functionalized with an affinity peptide.

**Cell hybridization via mNPs.** Given that expression of a fluorescent protein tagged with SBP enabled external binding of streptavidin, we employed this interaction for fastening streptavidin-functionallized materials directly to the cell surface. We chose streptavidin-conjugated mNPs, 100 nm in diameter (an order of magnitude smaller than a cell), for binding to a cell surface (Fig. 3a) to impart the abiotic magnetic properties. Scanning electron microscopy (SEM) was used to observe surface interaction between cell surface-expressing SBP and streptavidin-functionalized mNPs. Supplementary Fig. 3a,b shows electron micrographs of E. coli cells (dimensions 1.5–2 μm in length) and the mNPs (~100 nm in diameter). The SEM image in Fig. 3b, shows a magnetically isolated SBP-expressing cell with streptavidin-mNPs. The sample was prepared by mixing SBP-expressing cells with streptavidin-mNPs, then collecting or ‘focusing’ into a magnetized pellet via magnetic field, then separating from unbound cells in the supernatant. The cells were then washed and resuspended. In Fig. 3b, clusters of surface-bound mNPs are observed. In addition, the elemental composition was analysed with energy-dispersive X-ray spectroscopy, shown in Fig. 3c by an element map superimposed with carbon (red) and iron (green). While the cell appears to be of a uniform carbon composition, the particles localized at the cell surface (highlighted with arrows) were found having a strong iron composition; thus, elemental analysis confirmed particle identity as iron oxide mNPs. Additional characterization of magnetic functionality, including detailed SEM and fluorescent microscopic analysis prior to and after application of magnetic fields, is described in the Supplementary Information (Supplementary Fig. 3).

In sum, the well-known affinity interaction between streptavidin and the peptide SBP is harnessed to endow cells with non-natural abiotic properties. Here coupling a functionalized nanomaterial to the surface-displayed peptide physically extends the fusion protein and also adds physical (magnetic) functionality to the cell.

**Linking expression to AI-2 recognition.** The expression system for pSBP–Venus was then put under AI-2 control so that the
protein is expressed in the presence of AI-2 instead of IPTG. That is, we coupled the native QS signal transduction circuitry to the reporter cassette. To ensure ample expression (as the native operon is fairly weak), we placed expression of T7 RNA polymerase under control of the natural QS circuitry. Phosphorylated AI-2 activates the system through derepression of the regulator LsrR, naturally upregulating AI-2 import and phosphorylation, and, by design, the T7 RNA polymerase on a...
sensor plasmid. When sbp–Venus is included downstream of a T7 promoter region on a second plasmid, expression is then triggered by AI-2 uptake (Supplementary Fig. 4a). Then, we used two host sensor strains engineered to provide varied AI-2 sensitivity (denoted responders ‘A’ and ‘B’). In ‘A’, luxS, genes required for internally phosphorylated AI-2 degradation are deleted. Also, both strains lack the terminal AI-2 synthase, luxS, so they cannot produce AI-2 and, instead, must ‘receive’ AI-2 from an external source (Supplementary Fig. 4a). The phenotypic difference between A and B is the threshold level of AI-2 that activates the genetic response. Fully constructed, these cells are designed to take up and process AI-2 to generate fluorescence output (that co-functions with streptavidin binding).

We next evaluated the kinetics of surface-fusion protein expression and effects on cell growth. The AI-2-induced expression for AIDAc-linked and SBP-tagged fluorescent proteins did not alter growth kinetics for either cell type (Supplementary Fig. 4b,c). Expression efficacy was also evaluated via immunoblot assay of the outer membrane, probing for AI-2-induced surface display. After induction with 20 μM AI-2, extracts from cell types A and B were size-separated and blotted using alkaline phosphatase-conjugated streptavidin to probe for the SBP-tagged protein fusion (Supplementary Fig. 5). The 88 kDa AIDAc–Venus–SBP protein was only found in the membrane-containing pellet fraction (Fig. 4a). Analogously, protein orientation was assessed by immunolabeling the fluorescent protein. Cell type B transformed with pSBP–eGFP was induced with 20 μM AI-2 overnight; cell surfaces were then probed for eGFP using a mouse anti-GFP primary antibody and red-labelled secondary anti-mouse IgG. Simultaneously, cells were observed using phase contrast and fluorescence confocal microscopy. We noted a punctate pattern for eGFP, which was in one-to-one correspondence with red immunostaining of the surface-expressed protein. The positive staining of eGFP-expressing cells for red fluorescence, contrasted by the absence of negative control immunostaining indicated surface exposure of the fusion (Supplementary Fig. 6). Confocal microscopy confirmed precise colocalization of the eGFP and red-labelled antibodies within the confines of individual cells (Fig. 4b). Therefore, efficient transport of this functionality to the membrane under AI-2 induction was demonstrated in each host.

Establishing molecular ranges for cell interrogation. Importantly, the engineered cells each provide a characteristic response to the level of AI-2. Recently, we showed that AI-2 level influences the quorum size of responding engineered populations but does not alter the expression level within each quorum. Here we evaluated our engineered AI-2 responders, again for quorum size (or in other words, percentage of AI-2-responsive cells in the population), this time varying the compositions of molecular input and the configuration of responders (Fig. 5a).

First, we added AI-2, synthesized in vitro, to each of the two responder populations (Fig. 5b). We also added conditioned medium (CM), the spent medium from an AI-2 producer culture containing metabolic byproducts, as well as AI-2 (refs 36,49; Fig. 5c). We also mixed the responder populations and added AI-2 to gauge responses in complex cultures (Fig. 5d).

Specifically, in Fig. 5b, A and B populations were incubated at mid-exponential phase with in vitro-synthesized AI-2 (refs 50,51) at concentrations: 0, 2, 10, 28 and 75 μM. After 12 h, samples were observed for fluorescence by confocal microscopy and then quantified by fluorescence-activated cell sorting (FACS; Supplementary Fig. 4c). We found that SBP–Venus expression for responder A cells occurred at the lowest tested level (2 μM AI-2), where 56% of the population expressed SBP–Venus and this fraction increased with AI-2 reaching a maximum of 90% at 28 μM. For type B, a more gradual trend was found; only ~1% was fluorescent from 0–2 μM, and this increased from 9 to 46% as AI-2 was increased to 28 μM. Finally, the highest fraction of fluorescing cells was found at the highest concentration tested, 75 μM.

We next isolated CM, which contains a dynamic composition of unfiltered metabolites and media components, from W3110 E. coli cultures at intervals during their exponential growth, throughout which AI-2 accumulates (AI-2 levels for the samples are indicated in Supplementary Fig. 7). CM aliquots were mixed with either A or B cells and cultured in triplicate for 12 h. Through FACS analysis it was found, again, that a larger subpopulation of A expressed Venus compared with population B at any concentration (Fig. 5c). Statistically relevant expression from B was not apparent until incubated with CM from cultures at an optical density (OD) of 0.23. In all cases, population A recognized AI-2 presence, including from media isolated at a W3110 OD of 0.05, the minimum cell density tested in this study.
The sensitivities of both strains to AI-2-mediated induction corroborate previous literature. These trends demonstrate that strains engineered for altered sensitivity to molecular cues provide discrimination of concentration level. That is, the identical plasmid expression system was transformed into different hosts, providing robust and distinct levels of expression.

Having developed cell types A and B with differential ability to detect AI-2, we next altered the reporters so that each cell type expressed a unique SBP-fluorescence fusion for colour-coded designation. Cell type A was engineered with pSBP-mCherry and type B with pSBP-eGFP, resulting in red and green fluorescence, respectively. These populations were mixed together in equal proportion at mid-exponential phase, introduced to a range of AI-2 concentrations, and incubated overnight. Populations A and B exhibited equal growth rates when cultured alone and together. When measured in cocultures. Phenomenologically, as expected, an initial accumulation of red type A responders was found. Then, at higher AI-2 levels, we found an emergence of a green subpopulation (type B). Above 28 μM, there was no longer an apparent differential response that would otherwise enable discrimination of AI-2 concentration; based on the consistency with modelled behaviour, coculturing contributed to dampen the response as the maximum percentage of responding cells in cocultures is 50% instead of 100%. However, the overall fluorescence output is enriched by the combination of multiple populations since the ranges of sensitivity overlap and effectively expand that of the master population (Supplementary Fig. 8d). Specifically, because the fluorescence of B is described by a larger constant for each population independently and in cocultures.

We found, in Fig. 5d, the general trends in response to an increasing AI-2 level were as predicted by modelled response curves (Supplementary Table 4), which were also well-correlated to Fig. 5b data (Supplementary Fig. 8a,b). That is, the saturation constants that describe dependence on AI-2 were unchanged when measured in cocultures. Phenomenologically, as expected, an initial accumulation of red type A responders was found. Then, at higher AI-2 levels, we found an emergence of a green subpopulation (type B). Above 28 μM, there was no longer an apparent differential response that would otherwise enable discrimination of AI-2 concentration; based on the consistency with modelled behaviour, coculturing contributed to dampen the response as the maximum percentage of responding cells in cocultures is 50% instead of 100%. However, the overall fluorescence output is enriched by the combination of multiple populations since the ranges of sensitivity overlap and effectively expand that of the master population (Supplementary Fig. 8d).

Specifically, because the fluorescence of B is described by a larger saturation constant, its fluorescence continually increases at higher AI-2 concentrations, while the fluorescence of A remains unchanged. Thus, coculturing between A and B enables resolvable output that is lower than the detection limit of B (due to A) yet surpasses the upper limit at which A saturates by the inclusion of isolated culturing. We evaluated the Monod-type saturation constant for each population independently and in cocultures.

Figure 5 | Single and multi-population cell responses to autoinducer-2. (a) Fluorescence output is linked to small molecule input, derived from purified or crude sources. Fluorescence from Responders A and B was analysed after exposure to autoinducer-2 (AI-2) in mono and mixed culture environments. (b) Venus expression from in vitro-synthesized AI-2 added to monocultures of A and B. (c) Venus expression from conditioned media (CM) added to monocultures of A and B. CM was isolated from WT W3110 E. coli cultures sampled at indicated OD. Data are averages from triplicate cultures with s.d. indicated. (d) Red and green fluorescence responses to AI-2 during co-incubation of Responders A (pSBP-mCherry, +) and B (pSBP-eGFP, -). Representative fluorescence images show colocalization of red and green cells. Scale bar, 10 μm. The average cell count per responder cell is plotted against AI-2 concentration, as determined by image analysis in quadruplicate. All data are plotted as averages of at least triplicate samples with s.d.
Figure 6 | Binning molecular information through cell-based parallel processing and magnetically focusing fluorescence into collective consensus output. (a) A and B cell types were co-incubated with AI-2 levels ranging from 0 to 55 μM AI-2 (left axis), then imaged after magnetic nanoparticle coupling and magnetic collation. Fluorescence results (centred directly over the magnet) are shown from high to low input (top left to bottom right). (b) Quantification of red and green fluorescence cell densities per AI-2 level. (c) The process of accessing molecular information begins by distributing Responders A and B within the environment of an AI-2 producer, P. A and B independently express fluorophore fusions and are linked with magnetic nanoparticles on processing autoinducer-2. Magnetic focusing translocates fluorescing responders. Image analysis of the magnetically collated cell aggregate reveals classified fluorescence output, representing the AI-2 composition of the interrogated environment. (d) Bright field (left) and fluorescence (right, red and green filters) images positioned over the edge of a magnet, as indicated by the inset. The sample in the bottom image pair was isolated from an environment of low AI-2 accumulation. The sample in the top image pair was isolated from a high AI-2 environment. (e) Quantification of visual space occupied by collated cells (eGFP and mCherry expressers) while distributed (- magnet) and magnetically focused (+ magnet). Scale bars, 50 μm.

Consensus feedback through multidimensional processing. We hypothesized that the value of cell-based sensing would be enhanced if the cell output could be collated in an unbiased manner that in turn were easily ‘read’ using optical means. We engaged magnetic processing, which represents an abiotic processing step that enhances the signal by focusing the collective response. Hence, cells were equipped with streptavidin-conjugated mNPs (Fig. 3). The ability of a magnetic field to refine fluorescence output through filtering and focusing is described in the Supplementary Information (Supplementary Fig. 11). Thus, in our combinatorial approach, fluorescence feedback about molecular information within a microbial community entails biotic processing through constituencies of two independent cell types in conjunction with magnetic post-processing that is enabled by guidance at the nanoscale (Fig. 6c). Moreover, since the fluorescence feedback data is provided through two constituencies, consensus from each independently provides an aggregate output; in our example, the output becomes relayed as a distinctive ‘binned’ category due to finite colour-combinations generated from constituencies A and B (Fig. 6c).

Again, type A transmits red output (SBP–mCherry +) and type B transmits green (SBP–eGFP +). These were first co-incubated with titred concentrations of AI-2, to obtain results similar to those of Fig. 5d. By coupling mNPs to the responsive parallel populations, we tested for aggregate two-colour output to provide informative feedback within a set of outcomes ranging from no colour, red-only to red + green. After overnight co-incubation and a magnetic sweep with streptavidin-mNPs, fluorescence results are shown in Fig. 6a, where the recovered cells are displayed above a magnet’s center in order from highest to lowest AI-2 level (top left to bottom right). The processing output generated by the range of conditions was quantitatively assessed for contributions from A and B responders. The spatial density of each fluorophore, or the area occupied by fluorescent responders as a percentage of total visible area, was quantified and plotted in Fig. 6b. Here the trend of increasing fluorescence with AI-2 is followed by both A and B cell types; however, red A cells accumulate at a higher rate than green B cells. This relationship
between A and B processing is not only consistent with their previous characterizations (Fig. 5) but indicates that the aggregate output is unbiased regardless of assembly with mNPs and magnetic-stimulated redistribution (Supplementary Information, Supplementary Fig. 14a).

Next, A and B cells were added together to probe the QS environment of *Listeria innocua*, an AI-2-producing cell type that is genetically and ecologically similar to the pathogenic strain *L. monocytogenes*. The environment was biased towards low and high cell density conditions by altering nutrient levels to develop contrasting scenarios of AI-2 level. Preliminary characterization in the Supplementary Information indicated that *L. innocua* proliferation is unperturbed by the presence of *E. coli* responders (Supplementary Fig. 12) and that type A cells detect AI-2 at low *Listeria* densities limited by sparse nutrients; then with rich nutrient availability, cell proliferation permits a higher AI-2 level that can be detected by type B (Supplementary Fig. 13). Replicating these conditions, we expected red fluorescence to be observed at low culture density and for green fluorescence to be reported when high (Fig. 6c). Two conditions were tested: *L. innocua* was proportioned to responder cells at 20:1 in dilute media to establish a low culture density condition or, alternatively, a ratio of 200:1 in rich media for a high culture density condition. After overnight co-incubation and a magnetic sweep (applied directly to the triple strain cultures) with streptavidin-mNPs, the recovered cells are displayed above a magnet’s edge (shown in Fig. 6d). Acute focusing of the fluorescence signals, contributed by each subset population of the processor (A and B), is visually apparent. The magnetic field had a physical effect of repositioning the ‘on’ subsets to be tightly confined within the magnetic field.

The processing output generated by the contrasting culture conditions was again assessed for the respective contributions of A and B, and for changes in spatial signal density due to the magnetic sweep (Fig. 6e). The analysis was based on images provided in Supplementary Fig. 14b. Data in Fig. 6e indicate that

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**Figure 7 | Extension of nano-guided cell networks for hypothetical regulatory structures.** (a) Rows 1 and 3 depict 10 hypothetical genetic responses to molecular inputs for pairs of fluorescence-reporting cell populations (red, R and green, G). Rows 2 and 4 depict genetic responses as phase-plane plots yielding distinct patterns. This establishes a visual field, showing the extent of any population-population bias (illustrated in example case 1). (b) Left panel: a two-population pairing (shown in case 10) defines visual output that inherently bins into three quadrants: Q1, negligible colour; Q2, red bias due to majority red cell output; and Q4, combined red and green output. Right panel: data from Figs 5d and 6b are plotted analogously, where each data point represents an autoinducer-2 input (labelled, μM). As expected, red and green outputs were binned into Q1, Q2 and Q4 as indicated by coloured outlines.
red type A cells are prevalent regardless of culture condition (except negative controls). However, compared with the low AI-2 condition, the abundance of green cells is 100-fold higher in the high AI-2 condition. In addition, the ratio of green to red was consistent prior to and after magnetic concentration, substantiating observations in the distributed system. Further, data show that magnetic refining increased per-area fluorescence 100-fold or 10-fold in low and high cell culture studies, respectively.

Based on the thresholds established for responder populations A and B, we found colour-coded binning corresponded to AI-2 level, where ‘red-only’ represented less AI-2 than ‘red + green’ (Fig. 5d). Thus, we found a binned output was established via this multidimensional molecular information-processing system and that this matched the expectations. Red feedback (from responder A) indicated dilute AI-2 accumulation occurred in the low density culture. In the dense cultures, high AI-2 accumulation turned on both A and B for combined red and green feedback.

System response patterns defined by parallel populations. Our example demonstrates the concept of an amorphous processing system that utilizes several biotic and abiotic components for multidimensional information processing. Interestingly, a binning effect was enabled: our system yields an index of colour-categorized feedback that characterizes the sampled environment. In Fig. 7, we present a means to extend our approach to multidimensional systems, those with more than one molecule-of-interest and at different concentrations. That is, by appropriate design of the cell responders, we can further enrich the methodology, its depth and breadth of applicability. We depict 10 hypothetical pairs of responses (with defining equations located in Supplementary Table 5)—those that can be driven by appropriately engineering cells to portend altered genetic responses. For example, rows 1 and 3 provide genetic outcomes as a function of analyte (AI-2) concentration. The hypothetical depictions are feasible as ‘designer’ signal transduction and a function of analyte (AI-2) concentration. For example, rows 1 and 3 provide genetic outcomes as appropriately engineering cells to portend altered genetic responses. For example, rows 1 and 3 provide genetic outcomes as appropriately engineering cells to portend altered genetic responses.

Discussion

While cell-based sensors work well in well-defined assay conditions, extension to complex environments remains a challenge. They grow, they move, they perturb their environments, they report in a time and concentration-dependent manner, small numbers of sensor cells may require signal amplification and so on. Also, increasingly, bacterial cells are engineered for user specified ‘executive’ functions in complex environments. Their performance depends on their ability to filter out extraneous noise while surveying the molecular landscape, and providing informed actuation.

Our system interrogates the molecular space by focusing on bacterial QS and a widely distributed signal molecule, AI-2. In addition to genetic attributes of the AI-2-responding sensor cells, AI-2 is a chemorepellent for E. coli, and hence E. coli engineered to sense and respond to AI-2 will naturally move towards its sources, enabling full sampling of the prevailing state. Each strain evaluates AI-2 with a distinct sensitivity. When ‘activated’ in response to a characteristic level, the cells simultaneously expressed a fluorescent marker and a SBP on the outer membrane via AID AAC translocation. SBP provides a means for cell hybridization through its strong affinity to streptavidin, and here, aids in binding mNPs. This enables the non-genetically coded property of cell translocation within a magnetic field through physically stimulated focusing and binning.

By making use of a diversity of biotic and abiotic features, our multidimensional system of ‘responder’ populations exemplifies several key metrics that promote executive performance in such environments: active molecule capture, post-capture refining of the detection output and finally the utilization of multiple feedback thresholds. Here, cells facilitate AI-2 recognition autonomously and actively because, as a distributed network they reside planktonically, chemotaxing to and continually processing signals over time. When AI-2 is detected, a processor cell’s cognate machinery responds by upregulation of the native QS operon, leading to rapid signal uptake and thereby creating an active-capture signal-processing mechanism. To maximize information acquisition and account for a potentially heterogeneous molecular landscape, cells serve as molecular sampling units among a distributed population, which leads to data fed back as a consensus of fluorescent ‘datapoints’. Then, distributed data collection can be selectively reversed via the incorporated abiotic feature: mNPs, fastened externally on the cell through affinity-guided self-assembly. As such, responding cells obtained this extendable feature, thereby becomes sensitized to repositioning within a magnetic field.

The layered nature of the processor here, from the subcellular to multicellular scale, permits a series of selective steps: it commences with the AI-2-triggered expression cascade which releases a tight repressor, surface localization of both the fluorescent protein and SBP tag, and finally nanoparticle binding for recovery. In addition, multiple layers of amplification result in orthogonal fluorescence feedback. The AI-2 detection event leads to whole-cell fluorescence through expression of many protein copies. Then their physical collection further amplifies the signal, yielding a macroscopic composite of many individual cell units. When utilized as a network of multiple constituencies, responder cell types A and B contribute individual recognition results (off, red or green) to a single consensus output. Finally, due to their overlapping thresholds for recognition of the same molecule, in this case, AI-2, parallel processing by A and B performance. Rather, we suggest such a strategy may be used to guide the dynamics of population architecture for actuation of by-design response patterns at a systems level.
Ampicillin was used at 100 μg/mL during the exponential phase, at which point experiments for triggered expression were performed from an overnight culture and grown at 37 °C. In many respects, the elucidation of layered information processing via information theory enables the integration of synthetic biology and nanomaterials design. We suggest this approach provides a rich opportunity to direct many formats of multi-population response through genetic tuning and systems-level engineering. Further development of cellular networks and incorporation of alternate abiotic attributes can expand the depth and breadth of molecular communication for user specified action.

**Methods**

**Engineered strains.** All plasmids and strains used in this study are listed in Supplementary Table 1. The vectors designed for this study, pSBP–Venus, pSBP–mCherry and pSBP–eGFP, were derived from pAIDA-I, which was generously donated by Larsson and previously used for covalent surface display of fusions up to 110 kDa in size. The plasmids pSBP–Venus, pSBP–mCherry and pSBP–eGFP were constructed as described in the Supplementary Information using primers listed in Supplementary Table 2 and the gene sequences of Supplementary Table 3 as templates. The resulting plasmid constructs are mapped in Supplementary Fig. 1. Plasmids were transformed into chemically competent BL21 (DE3) E. coli (Life Technologies) for testing T7-regulated expression of the surface display fusion SBP–fluorescent protein-AIDAc. Next, the plasmids were introduced by electroporation into the electrically competent strains CT104 (+ pCT6) and MDA12 (+ pCT6). Strains were made competent by standard procedures.

**Protein expression and labelling.** Chemically competent BL21 (DE3) cells (Life Technologies) were transformed with pSBP–Venus, pSBP–eGFP or pSBP–mCherry. Cultures were grown to mid-exponential phase, then induced with 500 μM isopropyl-β-D-1-thiogalactopyranoside (IPTG, purchased from Sigma-Aldrich, USA). The induced cells were incubated at 37 °C, 250 r.p.m. shaking, for 6 h. Alexafluor488- and Alexafluor594-labelled streptavidin (Life Technologies, #S-11223) and Alexafluor594-labelled streptavidin (Life Technologies, #S-11223 and S-11227) were prepared to a working concentration of 20 μg/mL. In 10 mM PBS, Dylight405-labelled streptavidin was prepared from a ThermoScientific labeling kit and diluted to a working concentration of 500 μg/mL. Culture aliquots were washed once in PBS, centrifuged (4,000 g, 5 min), resuspended in the fluorescent streptavidin solution and labelled for 1 h at room temperature. Finally, cells were again washed in PBS and resuspended for imaging. Imaging parameters for each fluorophore were consistent for each composite. To avoid immunolabel surface expression, cells were washed in PBS and incubated with anti-GFP monoclonal mouse IgG (Rockland Immunochemicals, #600-301-215), diluted 1:100 in PBS for 1 h at room temperature. Samples were washed twice in PBS, then incubated with Alexafluor594 (red) anti-mouse polyclonal goat IgG (Molecular Probes, #A-11032) as a secondary antibody, diluted 1:200 in PBS, again for 1 h at room temperature.

**Cell culture.** E. coli cultures were prepared by 1% reincoculation into LB medium from an overnight culture and grown at 37 °C, 250 r.p.m. shaking, until mid exponential phase, at which point experiments for triggered expression were initiated. Antibiotics were added according to the plasmids contained by the strain. Ampicillin was used at 100 μg/mL for pCT6 transformants and kanamycin at 50 μg/mL for pSBP transformants. CM were isolated from W3110 E. coli by pelleting cells and resuspended in 10 mM PBS and incubated with anti-GFP monoclonal mouse IgG (Rockland Immunochemicals, #600-301-215), diluted 1:100 in PBS for 1 h at room temperature. Samples were washed twice in PBS, then incubated with Alexafluor594 (red) anti-mouse polyclonal goat IgG (Molecular Probes, #A-11032) as a secondary antibody, diluted 1:200 in PBS, again for 1 h at room temperature.

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were applied directly to overnight cultures at 2% of the culture volume. After 20 minutes, the cells and mNPs in a 96-well plate at 4 °C for 20 minutes. A 2.2 × 1.6 cm (μM) was added to the coverslip. A 0.8 ml of suspended mNPs and cells bound with mNPs was added to the opposite side of the coverslip, directly on top of the magnet, set for 2 minutes, after which another coverslip sealed the sample for imaging at × 200 magnification. Characterization of streptavidin-coated mNPs for SBP-surface-expressing cells is provided in the Supplementary Information.

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Author contributions
Design and cloning of genetic constructs were done by H.-C.W. and J.L.T. Experimental data were obtained by J.L.T. Magnetic characterization was carried out by N.B.B. and J.L.T., W.E.B. supervised the project. J.L.T., W.E.B., G.F.P. and H.-C.W. contributed to the writing of the manuscript. All authors contributed to the intellectual content.

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