

Very good! 

# Microbial Signaling Systems: Methods and Applications

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## Introduction

Intercellular communication and regulation is an essential feature of both multicellular systems and aggregates of unicellular organisms. Familiar examples such as growth factors, reproductive hormones, and other endocrine signals in the human body present attractive targets for synthetic signal-response or signal-modulation systems, while simpler interactions among unicellular organisms – such as bacterial quorum sensing or induction of slime-mold plasmodium formation – offer opportunities for the construction of biological “circuits.”

Weiss and Knight (2000) describe an engineered microbial signaling system in which two distinct cell populations serve as signal “senders” and “receivers” in a unidirectional signal transduction process. This system represents a first step toward the construction of synthetic signaling systems using biological substrates.

## Exploitation of Bacterial Quorum Sensing

Weiss and Knight exploit the genetic control features found in the Lux operon of the bacterium *Vibrio fischeri*, which uses quorum sensing to induce density-dependent luminescence in its population (Engebrecht et al., 1983). Briefly, *V. fischeri* and related luminescent bacteria produce a species-specific “autoinducer” molecule capable of permeating cell membranes and thereby diffusing out of the originating cell and into its neighbors. This creates an autoinducer concentration gradient that varies with distance from the originating cell. When a population is small and cell density is low, overall autoinducer concentration will be low, and no signaling will occur. However, increasing cell density yields increasing extracellular concentration of autoinducer, and above a certain concentration threshold, transcription of the Lux genes will be activated. The overall effect is a non-linear density-dependent activation of light-producing activity in bacterial populations.

Although the natural system does not segregate senders and receivers into separate populations, this can be achieved using clever genetic engineering. Weiss and Knight constructed one recombinant *E. coli* population, termed the senders, in which the production of autoinducer was placed under the genetic control of tetracycline-responsive elements. The receiver population, grown in the same medium as the senders, contained the gene for green fluorescent protein (GFP, a popular reporter gene in microbial work) under the control of the autoinducer-responsive elements of the Lux operon. In the presence of non-toxic levels of anhydrotetracycline (a less-toxic tetracycline variant), the sender cells produce high levels of autoinducer, which activates GFP expression in the receiver cells upon reaching the thresh-

old concentration. Incident UV light is required to induce fluorescence and to detect the success of the signal transduction.

## Microbial Digital Circuits

Although this signal transduction mechanism is not truly “all or none” and thus not truly digital, it is as close an approximation as can be realistically expected in biological systems. Expression of even tightly regulated genes is likely to occur at some low basal rate in the absence of activating signals, and the maximal rate at which a cell expresses an inducible gene under activating conditions will vary with cell size, available nutrients, rate of cell division, and a variety of other difficult-to-control biological factors. The use of a large population of cells in circuit constructs can serve to mitigate this variation by linking signal transduction to aggregate cellular behavior, which minimizes the effect of “outlier” cells and more closely approximates a digital system.

Genetically engineered circuits more elaborate than those of Weiss and Knight could potentially be constructed by coupling alternative effectors to the Lux genes in the receiver population. For example, a positive-feedback effect could be arranged by placing genes for the production and export of anhydrotetracycline (or an alternative originating signal, if the senders are appropriately modified) under the control of the Lux genes in the receiver. Alternatively, the receiver could secrete a different species' autoinducer, and a third cell population could be added with an engineered response to the secondary autoinducer. A series of links in the chain could be constructed in this way, limited primarily by diffusion of

signaling molecules and the challenge of maintaining multiple distinct cell populations in a single nutritive medium.

Nonlinear signal transduction mechanisms could also plausibly be used for more complicated constructions such as logic gates in a summative manner similar to that observed in neurons. It is easy to envision – although perhaps not so easy to implement – a scheme in which two distinct sender populations produce different inducer molecules in response to independent stimuli, and a single receiver population has an effector activity (GFP expression, or possibly a secondary signaling molecule) that is only expressed when *both* of the sender populations are active. This would amount to an AND gate. Similar constructions using known excitatory or inhibitory genetic elements (for example, promoters extracted from the well-characterized Lac operon) could be used for other Boolean operations.

Successful signal transduction in these systems will be critically dependent on the ability of diffusion to get signaling molecules to their target cells in appropriate concentrations. This places restrictions on the types of molecules that can serve as inducers; they must be able to diffuse through an aqueous medium, but must also be detectable by downstream cells, either by diffusing through the cell membrane or by binding to external receptors. Molecules that enter their target cells must be sufficiently soluble in both water and lipid membranes to diffuse through both. Although bacteria are larger, more complicated, and less predictable systems than DNA alone, building circuits from bacterial populations rather than DNA molecules as previously outlined and described (e.g., Seeman and Carbone, 2002; Mao et al., 2000) allows additional layers of redundancy that could protect the system from error. Implementations

or cells must have channels for these molecules.

of DNA computation that rely on near-perfect base-pairing accuracy are highly vulnerable to catastrophic error that may yield “false positive” results or even halt the progress of the computation; because the microbial circuits rely on the aggregate behavior of millions of cells, the system is partially buffered against such large-scale errors.

## Biomedical and Environmental Applications

Synthetic intercellular signaling systems may also find potential applications interacting with the natural world. However, because the conditions under which such systems would operate are potentially highly variable or ill-defined, it may be some time before these applications come to fruition despite their promise.

Many human diseases can be traced to inappropriate or misdirected intercellular signaling. However, artificially detecting, modulating, and controlling such signals within the body is difficult. One can imagine deliberately infecting patients with benign strains of bacteria engineered to respond to the presence (or absence) of a particular signaling molecule. Difficulties inherent in this approach include the fact that, while the body is a natural host of bacterial flora in the gut, most signaling occurs in areas where the presence of bacteria is not well tolerated, such as the bloodstream. Engineering human cells to undertake the same duties is likely to present major experimental challenges, since eukaryotic cells are more difficult to genetically modify. Introduction of novel genetic material on plasmid vectors is less effective in eukaryotic cells than in bacteria, and viral vectors can be difficult to work with because toxic or damaging portions of the viral genome must be removed. While eukaryotic

expression vectors can be effective in transient transfection, stably transfected cells would be much more useful for signaling applications.

Bacterial populations engineered to respond to a particular signal may also find applications in environmental management. Bacteria capable of degrading hydrocarbons have been characterized and are commonly found to migrate naturally to oil or petrochemical spill sites (Xu and Obbard, 2003), leading to the early-stage development of bioremediation products that introduce these bacteria to a spill site artificially (e.g., U.S. Microbics' Remediline products). However, some applications would benefit from an dependable "automated" solution that does not rely on natural microbial populations, but also does not require human intervention to become active. Bacterial populations could exist naturally in a quiescent state, either as spores or feeding off non-petrochemical nutrients, until an influx of petrochemicals or other degradable pollution occurs. Activation of the sender population by pollutants induces the secretion of inducer, which (assuming an environment where diffusion occurs at a reasonable rate) activates the receiver population to degrade the pollutants.

A first glance would suggest that the multi-population system offers few advantages over a single population competent in both detection and degradation. The advantage of the multi-population signal transduction system is twofold: first, separation of function allows independent fine-tuning of sender and receiver properties without concern that optimizing degradative activity would interfere with sensing or vice versa. Second, signal transduction also provides an opportunity for signal amplification, since a single sender cell can contribute to the activation of many receivers. Highly optimized systems could also couple the growth

rate of the receiver directly to the concentration of inducer, allowing receivers to respond to induction not only by activating degradation pathways but also by rapidly producing more receivers.

## Conclusions

Genetic engineering of bacterial populations to create artificial intercellular signaling systems offers a wide variety of potential applications, both in ongoing research into biological computation and in practical scenarios such as disease treatment and environmental management. Although optimal conditions vary depending on the type of application – for example, computational work would require very stringent conditions for receiver activation, while bioremediation would work even with occasional spurious activation – microbial signaling systems allow a great deal of flexibility. While work in this area is in its infancy and signaling systems are still both fragile and brittle, the general robustness of biological systems suggests that many current practical difficulties can be overcome.

## References

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