Single-cell Study of Bacterial Signaling in Quorum Sensing

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***Short Abstract* — Quorum sensing is a process that bacteria communicate with one another by signaling molecules called autoinducers. To investigate quorum-sensing response of single cell and uncover the mystery of integration of multiple autoinducers, we constructed mutant strains with fluorescent protein reporters and applied quantitative microscopy. We quantified cell-to-cell variation in response to various levels of homogeneous autoinducer concentrations and found that cells can tell more than just low and high cell densities. We obtained dose response features of two autoinducer sensing pathways when they present alone or together. Our results suggest that two autoinducers are integrated additively and there is no discrimination of the two distinct autoinducers.**

***Keywords* — single cell, bacterial signaling, quorum sensing.**

BACTERIAL cells communicate with one another through a process called quorum sensing. During this process, cells produce, release and detect signaling molecules called autoinducers. Since autoinducer accumulation directly depends on cell density, detection of autoinducers enables bacteria to sense their population cell density. Bacteria change their gene expressions coordinately in response to cell density and therefore change their cellular behaviors such as bioluminescence, virulence factor secretion, and biofilm formation [1]. Although quorum sensing enables bacteria to behave collectively as multi-cellular organisms, the events inside single cells dictate the behavior of the entire population. Despite decades of accumulated effort on studying quorum sensing, all experiments were carried on at bulk-scale dealing with large populations of cells. Nothing has been known about quorum sensing response of individual cells.

Quorum sensing systems in most bacterial species consist of more than one type of autoinducers [1]. Typically, different autoinducer-sensing pathways converge and all autoinducers share the same response regulator. It is a long lasting mystery that how the multiple autoinducers are integrated and whether they can be discriminated.

To investigate quorum sensing at single-cell level and unveil the mystery of autoinducer integration, we focused on a model system – *Vibrio harveyi* – whose quorum sensing mechanism is well understood and very typical [1]. It produces and detects three types of autoinducers: AI-1, CAI-1, and AI-2, which are suggested for intra-species, intra-vibrios, and inter-species communications respectively. The three autoinducers are detected by their cognate receptors: LuxN, CqsS, and LuxPQ. All three receptors transfer information of autoinducer binding to the phosphorylation of their common response regulator LuxO by phosphorelay.

To investigate single-cell behavior and explore the accuracy of cell-density detection, we fused GFP to a promoter activated by LuxO-P and used it as the output reporter for the system. We also introduced mCherry driven by a constitutive promoter as internal control. To discover the mechanism of multiple autoinducer integration and find out whether cells can distinguish different autoinducers, we need to dissect each autoinducer sensing pathway alone and also their combination. We constructed three sensor mutant strains: LuxN+ with only LuxN receptor left, LuxPQ+ with only LuxPQ receptor left, and LuxN+ LuxPQ+ with two of the three receptors left. Synthases of autoinducers are knocked out and autoinducers can only be added exogenously. Thus we can control autoinducer concentration precisely and use it as input signal of the system.

We applied quantitative microscopy to measure cell-to-cell variation in response to various levels of homogeneous autoinducer concentrations. We found that GFP distribution of a population of cells is always monomodal. There is no bistability in the response. Despite variation within populations exists, cells can still tell more than three autoinducer concentration ranges, which means they can detect not only low and high cell densities but also some intermediate cell-density states. Data from the dose response experiments of the sensor mutants suggest that cells cannot really distinguish AI-1 versus AI-2. Analysis further indicates that cells integrate AI-1 and AI-2 in a simple additive way. Additivity imposes strong constraints on the biochemical reactions occurred in the system, from which some relative relations of parameters can be inferred.

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