Supplementary Information (SI) for "Signal discrimination by differential regulation of protein stability in quorum sensing"

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1. Derivation of the analytical expression of steady-state fidelity *f* (Eq. 5) from the quorum-sensing model (Ordinary Differential Equations 1-3 from the main text) using *Mathematica*®

Please see the file "derivation_of_f.nb".

2. Instability of LuxR alone does not endow bistability in the LuxR-positive feedback loop mediated by the quorum sensing signaling molecule

Recent analysis by Buchler et al. illustrated that nonlinear protein degradation coupled with a positive feedback loop (PFL) may enlarge the parameter regime wherein bistability occurs (1). It remains unclear whether the bistable region can be enhanced by the much higher instability of the LuxR-type monomer, as compared to its dimer, in a quorum-sensing mediated PFL. To address this issue, we use bifurcation theory to model a PFL mediated by quorum sensing (see Figure S5A). A dimensionless model of this system is as follows:

$$\frac{dR}{d\tau} = a_R \frac{R^{*n}}{1+R^{*n}} - b_R R - a_b AR + a_{R0} \quad (1)$$

$$\frac{dR^*}{d\tau} = a_b AR - R^* \quad (2)$$

where R, R^* and A represent the dimensionless concentrations of the transcription factor R, activated complex R^*, and the signal A, respectively. a_R is the synthetic rate constant of R. a_{R0} is the basal synthetic rate constant of R. b_R is the degradation rate constant of R. Note that R is much less stable than R^* (i.e, $b_R >> 1$). a_b is the binding rate constant of R with A. Considering physiologically feasible scenarios, the base values are chosen as a_b=1, a_R =1, a_{R0} =1, b_R=5.

In the model, translation of *luxR* mRNA is not explicitly described, and is lumped into a single synthesis process of the R-protein. Dimerization of R^* is assumed to be fast. The synthetic rate expression of R is a Hill function with coefficient n ($n \geq 1$) to represent that R^* forms a homodimer prior to binding to the *lux* promoter and inducing the expression of *luxR*.

Under the approximation of quasi-steady-state, Equations (1) and (2) can be reduced to a nonlinear algebraic equation:

$$f(R,\ A;\ b_R,\ a_R,\ a_{R0},\ a_b)\ =\ a_R \cdot \frac{(a_b A)^2 R^2}{1+(a_b A)^2 R^2} - b_R R - a_b AR + a_{R0} = 0 \qquad (3)$$

We aim to determine the region of the parameter space $\underline{\mathbf{P}}^* = (a_R,\ b_R)$ where a bistable response ("R versus A" curve) can occur. According to bifurcation theory (2), the loci of Hysteresis separate the parameter space into regions, each corresponding to monotonic or bistable responses. We obtain the analytical expression of the loci of Hysteresis as Equation (4):

$$a_R = \frac{4n}{(n-1)^2} a_{R0} \qquad (4)$$

This expression indicates two essential features of this PFL. First, bistability cannot be generated with cooperativity (n = 1). Second, in the $(a_R,\ b_R)$-plane the bistable region (with cooperativity, $n > 1$) is above a critical value of a_R and independent of the value of b_R (Figure S5B). If the LuxR synthesis rate is lower than this critical value, no matter how one increases the b_R value (*i.e.,* the decay rate or instability of the LuxR monomer), bistability cannot occur.

3. Increasing LuxR decay rate can endow bistability in a LuxR-LuxI-double positive feedback loop

If the R-protein and the signal A are both regulated in their synthesis by PFLs (Figure S5C), the instability of the R-protein will impact the ability of the system to generate bistability. A dimensionless model that describes this network architecture is as follows:

$$\frac{dR}{d\tau} = a_R \frac{R^{*n}}{1+R^{*n}} - b_R R - a_b AR + a_{R0} \qquad (5)$$

$$\frac{dA}{d\tau} = a_A \frac{R^{*n}}{1+R^{*n}} - b_A A - a_b AR + a_{A0} \qquad (6)$$

$$\frac{dR^*}{d\tau} = a_b AR - R^* \qquad (7)$$

where the notation is identical to that in Equations (1) and (2). In addition, a_A is the synthetic rate constant of the signal A. a_{A0} is the basal synthesis rate constant of A. b_A is the degradation rate constant of A. Here we consider the case that R^* does not cooperatively bind to the promoter, *i.e.,* the Hill coefficient $n = 1$.

The complexity of this nonlinear system prevents an analytic expression of the loci of Hysteresis. We therefore use numerical analysis employing the XPPAUT software. Biologically feasible parameter values are used: a_b=0.5, a_R=1, a_{R0}=0.2, a_A=6, a_{A0}=0.2, b_R=2, and b_A=1.

Bifurcation analysis, as illustrated in Figure S5D, shows that increasing the decay rate constant of the *R*-protein (b_R) can give rise to bistability.

4. Detailed explanation for content in Table 1

LuxR – Urbanowski et al (3) claim that LuxR requires its cognate signaling molecule (3OC6HSL) for active LuxR to be obtained. In particular, 3OC6HSL is required in the nascent stage of the LuxR polypeptide synthesis in order for it to fold into its stable tertiary conformation. Figure 2 from Collins, 2006 indicates that, of those tested, LuxR responds only minimally to signals other than its cognate signal (4).

LuxR G2A-H – Using directed evolution, Collins et al (2005) (5) and Collins et al (2006) (4) illustrate the plasticity of the binding affinity of LuxR, in terms of the ability to tune this binding affinity towards promiscuity, away from specificity to its cognate signal, and subsequently back towards specificity to an AHL molecule other than its cognate signal. Collins et al, 2006 also indicates that the presence of 3OC6HSL results in significant accumulation of LuxR and LuxR-G2E and the presence of C10HSL results in significant accumulation of LuxR-G2E and LuxR-G2E-R67M, whereas none of these proteins accumulates to a significant concentration in the absence of both signals. These results suggest that the AHL molecules serve as “folding switches” that result in significant stabilization of the LuxR-type proteins to which they bind.

TraR – Zhu and Winans (1999) shows that soluble TraR is not observed in the absence of its cognate signal (3OC8HSL)(6). Further analysis indicates that the half life of TraR increases from 3.5 minutes without the cognate signal to ~92 minutes (~30fold) with the signal present (7).

LasR – LasR was found to be nonfunctional when expressed in the absence of its cognate signal (3OC12HSL) (8). LasR is shown to bind the signal so tightly that it cannot be removed even following prolonged dialysis. Also, Bottomley et al, 2007 shows that LasR is only soluble in the presence of its cognate signal (9).

SdiA – Michael et al (2001) shows that SdiA responds to multiple AHL signals with different structures (10). Yao et al (2006) also shows that multiple AHL signals serve as "folding switches" for SdiA. However, in contrast to TraR, Yao et al (2006) shows that SdiA in inclusion bodies could be refolded upon the addition of C8HSL (11). This result indicates that the signals that bind to SdiA need not be present during the SdiA synthesis in order to bind to and induce folding in SdiA.

CepR – Weingart et al (2005) shows that CepR, similar to TraR, requires its cognate AHL to accumulate in the soluble fraction of cell lysate (12).

RhlR –Ventre et al (2003) shows that RhlR exists as a dimer in the absence of any AHL molecule. The addition of C4HSL, the cognate signal for RhlR, does not result in a change in the oligomerization state of RhlR (13) but rather switches its activity from repression to activation (14). The exposure of RhlR to the LasR cognate signal (3OC12HSL) results in the decoupling of the RhlR dimer into its monomeric subunits (13).

ExpR – It has been shown that ExpR serves as an activator of pectic enzyme expression (15, 16). Without its cognate AHL, ExpR forms a dimer and can repress its own expression. With its signal, however, the repression is relieved and ExpR activates the expression of target genes.

CarR – When expressed in *E. coli* in the absence of AHL, ~20% CarR is found in the soluble fraction. The fraction of CarR in the insoluble fraction can be refolded in the absence of its cognate AHL signal (17). CarR tends to multimerize in the absence of its cognate AHL. It can activate its target function independent of its cognate AHL upon even slight overexpression (18). CarR has been found to bind to multiple AHL signals as shown in Welch et al (2007).

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Table S1: Base Parameter Set

Reaction	Definition	Parameter	Base value*	Justification
$*\xrightarrow{k_R} R$	Monomer synthesis	k_R	19.3 nM/min	Provides a saturating level of monomer
$R\xrightarrow{d_R} *$	Monomer degradation	d_R	0.2 min^{-1}	Set to the half-life of TraR (~3.5 mins) (7)
$R + A_i \xrightarrow{k_1} R_i$	Binding of cognate signal to monomer	k_1	0.096$(nM*min)^{-1}$	Based on K_D ≈10nM (7)
$R_i \xrightarrow{k_{1r}} R + A_i$	Unbinding of cognate signal from monomer	k_{1r}	1min^{-1}	Based on K_D ≈10nM (7)
$2R_i \xrightarrow{k_2} D_i$	Dimerization of cognate signal-monomer complex	k_2	0.096$(nM*min)^{-1}$	Based on K_D ≈10nM (7)
$D_i \xrightarrow{k_{2r}} 2R_i$	Dissociation of dimer	k_{2r}	1 min^{-1}	Based on K_D ≈10nM (7)
$R_i \xrightarrow{d_{R1}} *$ $D_i \xrightarrow{d_{R1}} *$	Complex and Dimer degradation	d_{R1}	0.023 min^{-1}	The measured dimer half-life is 92 mins (7). The given value takes into account dilution rate as the limiting factor (19).
	AHL signal concentration	A_i		Set to mimic the half-max induction concentration (f_a) or to provide a saturating concentration (f_s) of AHL signals
	Modulation of non-cognate signal degradation rate	α	1	Initially set to one to ensure symmetry
	Modulation of non-cognate signal dimer degradation rate	β	1	Initially set to one to ensure symmetry
	Modulation of non-cognate signal decoupling from monomer	γ	1	Initially set to one to ensure symmetry

*All values are based on a conversion of $9.64(10)^8$ m/M≈1 m/nM, which implicitly assumes a mean cellular volume of $1.6(10)^{-15}$ L/cell (20).

SI Figure Legend

Figure S1: Differential protein stabilization increases fidelity.

(A) The plot of f_a versus α where α_1=α_2=α and β=γ=1,10, and 50 demonstrates that, under otherwise symmetric parametric conditions, protein degradation plays a role in modulating the fidelity of the signal discrimination process based on the model analyzed herein. The parameter α is being modulated within the predefined range: $d_{R1}/d_R \le \alpha \le 1$. Less sensitivity of f_a to α is observed as β and γ increase.

(B) Equivalent to A, but for f_s.

Figure S2: Equivalent to figure 3A, but for f_s.

Figure S3: The minimal and maximal limits of fidelity in signal discrimination are also affected by the kinetic parameters that differentiate the signal binding and dimerization steps between the two pathways of Figure 1 (β and γ respectively). For f_a, β and γ set the baseline for the fidelity in terms of the monomer degradation rate, d_R. As d_R increases, for each value of β and γ, the dynamic range within which signal discrimination can take place shifts towards significantly higher fidelity.

Figure S4: Equivalent to S3, but for f_s. In this case, the baseline does not shift significantly for different values of β and γ; however, the maximal limit of f_s increases with d_R similarly to f_a.

Figure S5: Bifurcation analysis of the LuxR-positive feedback loop and LuxR-LuxI-double positive feedback loop mediated by quorum sensing.

(A) A positive feedback loop under the control of an AHL signal A. The transcription factor R (*i.e.*, LuxR), upon binding with the signal molecule A, forms an activated complex R^*, which is able to form a homodimer and bind to the promoter P_{luxI} (with a cooperative binding coefficient

(Hill coefficient) n that is larger than 1) to activate the gene expression of R itself, closing the autoregulatory PFL.

(B) Phase diagram in the parameter plane-$(a_R,\ b_R)$. The loci of Hysteresis separate the parameter space into two regions, each corresponding to a monotonic or bistable response (R *vs* A), as illustrated for different a_R values (insets). In the bistable switch ("*R versus A*" curve, upper inset), stable steady states are denoted by the solid line, unstable steady states by the dashed line.

(C) Quorum-sensing mediated double PFLs. Transcription factor R (*i.e.*, LuxR), upon binding with a signal molecule of A, forms an activated complex R^*, which is able to form a homodimer and bind to the promoter P_{luxI} (with a cooperative binding coefficient (Hill coefficient) n that is larger than 1) to activate the gene expression of R and I (and thus A) themselves, closing the autoregulatory PFLs.

(D) Phase diagram in the parameter plane-$(a_A,\ b_R)$. Insets illustrate the two types of bifurcation diagrams ("R versus a_R" curves) at different parameter regions separated by the loci of Hysteresis in the $(a_A,\ b_R)$-plane.