Supporting Information: Information processing and signal integration in bacterial quorum sensing

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I. TWO-STATE MODEL FOR RECEPTORS

We model receptors using a simple two-state model in which receptors exist in two states: a low kinase activity state we call "off" and a high kinase activity state, we call "on" (Swem et al., 2008; Keymer et al., 2006). Ligands, in our case autoinducers, act by binding to the receptor protein and changing the free energies and therefore the thermal occupancies of the two activity states. There are a total of four free-energy states with corresponding free energies: (i) on without ligand-bound $E^{\rm on}$, (ii) on with ligand-bound $E^{\rm on} - \log\left([\mathrm{L}]/K^{\rm on}\right)$, (iii) off without ligand-bound $E^{\rm off}$, and (iv) off with ligand bound $E^{\rm off} - \log\left([\mathrm{L}]/K^{\rm off}\right)$. In the absence of ligands, the receptors favor the on state but ligand causes switching to the off state. This implies that $K^{\rm on} \gg K^{\rm off}$ and that $E^{\rm on} < E^{\rm off}$. At equilibrium, the probability that a receptor is in the on state is a function of the difference in free energies between the "on" state and the "off" state:

$$f = \epsilon + \log\left(\frac{1+[\mathrm{L}]/K^{\rm off}}{1+[\mathrm{L}]/K^{\rm on}}\right) \tag{SI-1}$$

with $\epsilon = E^{\rm on} - E^{\rm off}$ where all energies are expressed in units of the thermal energy $k_B T$. In particular, one has

$$p_{\rm on} = \frac{1}{1+e^{f}}. \tag{SI-2}$$

For $K^{\rm on}$ much larger than the typical ligand concentration and ϵ large and negative as in the quorum-sensing network (Swem et al., 2008), the probability that a receptor is on becomes

$$\begin{aligned} p_{\rm on} &= \frac{1}{1+e^{\epsilon}\left(\frac{1+[\mathrm{L}]/K^{\rm off}}{1+[\mathrm{L}]/K^{\rm on}}\right)} \\ &\approx \frac{1}{1+\frac{[\mathrm{L}]}{K^{\rm off}e^{-\epsilon}}}. \end{aligned} \tag{SI-3}$$

Defining a half-maximal inhibition constant $K_I = K^{\text{off}} e^{-\epsilon}$, one has the simple non-cooperative Hill function,

$$p_{\text{on}} \approx \frac{1}{1 + \frac{[L]}{K_I}} \tag{SI-4}$$

We denote the probabilities that LuxN and LuxPQ are in their on states by X and Y, respectively. and we denote the kinase activities in the on states of the two receptors by k_X and k_Y, respectively. Furthermore, for notational simplicity and consistent with experiment, we assume that the kinase activity in the off state for both receptors is negligible. We also assume, based on experimental evidence, that the receptors have state-independent phosphatase activities, which we denote p_X and p_Y. The phosphorelay can be modeled using simple differential equations of the form

$$\begin{aligned}
\frac{d[\text{LuxU-P}]}{dt} &= (k_x X + k_y Y)([\text{LuxU}]_{\text{T}} - [\text{LuxU-P}]) - p'[\text{LuxU-P}] \\
&- k_+[\text{LuxU-P}]([\text{LuxO}]_{\text{T}} - [\text{LuxO-P}]) + k_-[\text{LuxO-P}]([\text{LuxU}]_{\text{T}} - [\text{LuxU-P}]) \\
\frac{d[\text{LuxO-P}]}{dt} &= k_-[\text{LuxU-P}]([\text{LuxO}]_{\text{T}} - [\text{LuxO-P}]) - k_+[\text{LuxO-P}]([\text{LuxU}]_{\text{T}} - [\text{LuxU-P}])
\end{aligned} \tag{SI-5}$$

.

where $p' = p_X + p_Y$, and $[\text{LuxU}]_{\text{T}}$ and .$[\text{LuxO}]_{\text{T}}$ are the total concentrations of LuxU and LuxO molecules, respectively. At steady state, we can set the left hand side of these equations to zero yielding,

$$\frac{[\text{LuxU-P}]}{[\text{LuxU}]_{\text{T}}} = \frac{k_X X + k_Y Y}{k_x X + k_y Y + p'}. \tag{SI-6}$$

A very similar expression can be derived for the fraction of phosphorylated LuxO, which we denote Z in the main text, by setting the left hand side of the bottom equation in (**??**) equal to zero and plugging in (**??**). This yields

$$\frac{[\text{LuxO-P}]}{[\text{LuxO}]_{\text{T}}} = \frac{k_X X + k_Y Y}{k_x X + k_y Y + p} \tag{SI-7}$$

with $p = \frac{k_-}{k_+} p'$.

We can compare these expressions to experiments in (Long et al., 2009) by noting that from (**??**)

$$X \approx \frac{1}{1 + \frac{[\text{AI}-1]}{K_I^{\text{AI}-1}}} \tag{SI-8}$$

and

$$Y \approx \frac{1}{1 + \frac{[\text{AI}-2]}{K_I^{\text{AI}-2}}}. \tag{SI-9}$$

II. FORMULAS FOR PRIORS USED IN MAIN TEXT

The lack of knowledge about the ecology of *V. harveyi* makes it difficult to quantitatively define a prior for input signals. Therefore, as discussed in the main text, we performed our calculations for several different choices of priors and verified that our conclusion are essentially independent of our choice of prior. We present results for three different choices of priors: a flat prior, a symmetric bimodal prior, and a non-symmetric bimodal prior. As discussed in the main text, we take as our inputs X and Y the probabilities that LuxN and LuxPQ, respectively, are in their kinase-active states. The advantage of this formulation is that input signals are bounded to be between 0 and 1. Explicitly, the priors we used are given by the expressions:

Flat prior:

$$q(X,Y) = 1/N \tag{SI-10}$$

with N a normalizing constant equal to 1.

Symmetric bimodal prior:

$$q(X,Y) = \frac{1}{N_s}\left(e^{-\frac{(X-\bar{X}_1)^2}{\sigma^2}} + e^{-\frac{(X-\bar{X}_2)^2}{\sigma^2}}\right)\left(e^{-\frac{(Y-\bar{Y}_1)^2}{\sigma^2}} + e^{-\frac{(Y-\bar{Y}_2)^2}{\sigma^2}}\right) \tag{SI-11}$$

with $\bar{X}_1 = \bar{Y}_1 = 0.25$, $\bar{X}_2 = \bar{Y}_2 = 0.75$, $\sigma = 0.2$, and N_s a normalizing constant to ensure the integral of $q(X,Y)$ is one.

Nonsymmetric bimodal prior:

$$q(X,Y) = \frac{1}{N_{ns}}\left(e^{-\frac{(X-\bar{X}_1)^2}{\sigma^2}} + e^{-\frac{(X-\bar{X}_2)^2}{\sigma^2}}\right)\left(Ae^{-\frac{(Y-\bar{Y}_1)^2}{\sigma^2}} + e^{-\frac{(Y-\bar{Y}_2)^2}{\sigma^2}}\right), \tag{SI-12}$$

with all parameters as above in Eq. (**??**) plus the asymmetry parameter $A = 5$, and the normalizing constant N_{ns} chosen so that the integral over the distribution is 1.

In the last section of the main text, we restrict our input space so that $X \geq Y$. For this calculation, we use priors on the lower-half triangle of the form $q_{\text{half}}(X,Y) = q(X,Y)\theta(X - Y)/N_h$ where $q(X,Y)$ is as above, $\theta(X)$ is the Heaviside function, and N_h is a normalizing constant that ensures the integral over $q_{\text{half}}(X,Y)$ is 1.

III. MUTUAL INFORMATION VIA SADDLE-POINT

A. Justification for saddle-point approximation

In the low-noise regime, we can derive approximate expressions for mutual information using a saddle-point approximation. As in all saddle point approximations, we exploit a large parameter. In our case, the large parameter is the signal-to-noise ratio. We interpret the mean value $f(X,Y)$ as the signal and $\sigma(X,Y)$ as the noise around the signal. When the noise is small, or equivalently the signal-to-noise ratio is high, we know that $\frac{f(X,Y)}{\sigma(X,Y}} \gg 1$. Thus we can write $\frac{f(X,Y)}{\sigma(X,Y}} = \lambda S(X,Y)$, where $\lambda \gg 1$ is a constant of order the signal to noise ratio and $S(X,Y)$ is a function of order 1. In the calculation below, λ serves as the implicit large parameter. This implies that the saddle-point approximation is valid as long as signal-to-noise is much larger than 1.

B. Approximate probability distributions

Often, the mean transfer functions of biological signaling systems are monotonic in the inputs. This is true for the *V. harveyi* quorum-sensing circuit. In this case, it is useful to reparameterize the space of input signals in order to perform calculations. In particular, we will utilize two different coordinate systems given by the coordinate transforms: $(X,Y) \rightarrow (f = f(X,Y), \theta = Y)$ and $(X,Y) \rightarrow (f = f(X,Y), \theta = X)$. For these two different coordinate transforms, by definition, we have, respectively,

$$q(f,\theta) = |\frac{\partial f}{\partial Y}|^{-1} q(X,Y) \tag{SI-13}$$

and

$$q(f,\theta) = |\frac{\partial f}{\partial X}|^{-1} q(X,Y) \tag{SI-14}$$

where, for simplicity, we denote all distributions by the same symbol q whether they are a function of X and Y or f and θ. By definition one has,

$$\begin{aligned} p(Z) &= \int df d\theta \; p(Z|(f,\theta)) q(f,\theta) \\ &= \int df d\theta \; \frac{1}{\sqrt{2\pi\sigma^2(f,\theta)}} e^{-\frac{(Z-f)^2}{2\sigma^2(f,\theta)}} q(f,\theta) \\ &\approx \int d\theta \; q(Z,\theta) \end{aligned} \tag{SI-15}$$

where, to obtain the last line, we performed the saddle-point approximation. Furthermore,we define the probability distributions

$$q(\theta) = \int df\ q(f,\theta) \tag{SI-16}$$

and

$$p(Z,f,\theta) = p(Z|f,\theta)q(f,\theta) = \frac{1}{\sqrt{2\pi\sigma^2(f,\theta)}} e^{-\frac{(Z-f)^2}{2\sigma^2(f,\theta)}} q(f,\theta). \tag{SI-17}$$

A final distribution of interest to us is $p(Z,\theta)$ given by

$$\begin{aligned} p(Z,\theta) &= \int df\ p(Z,f,\theta) \\ &= \int df\ \frac{1}{\sqrt{2\pi\sigma^2(f,\theta)}} e^{-\frac{(Z-f)^2}{2\sigma^2(f,\theta)}} q(f,\theta) \\ &\approx q(f,\theta). \end{aligned} \tag{SI-18}$$

Again, to obtain the last line, we have utilized the saddle-point approximation.

C. Calculation of relevant Shannon entropies

To calculate the mutual informations, we need several entropies:

$$\begin{aligned} H(Z) &= -\int dZ\ p(Z)\log_2 p(Z) = -\int dZ d\theta\ q(Z,\theta)\log_2\left[\int d\theta' q(Z,\theta')\right] \\ H(\theta) &= -\int d\theta\ q(\theta)\log_2 q(\theta) = -\int d\theta df\ q(f,\theta)\log_2\left[\int df' q(f',\theta)\right] \\ H(Z,\theta) &= -\int d\theta dZ\, q(Z,\theta)\log_2 q(Z,\theta). \end{aligned} \tag{SI-19}$$

A final entropy of interest to us is the entropy $H(z,r,\theta)$. Once again, we use the saddle-point approximation to obtain this entropy. Namely, one has

$$\begin{aligned} H(Z,f,\theta) &= -\int dZ df d\theta\ \frac{1}{\sqrt{2\pi\sigma^2(f,\theta)}} e^{-\frac{(Z-f)^2}{2\sigma^2(f,\theta)}} q(f,\theta)\log_2\left[\frac{1}{\sqrt{2\pi\sigma^2(f,\theta)}} e^{-\frac{(Z-f)^2}{2\sigma^2(f,\theta)}} q(f,\theta)\right] \\ &\approx -\int df d\theta q(f,\theta)\left[\log_2 q(f,\theta) + \log_2\frac{1}{\sqrt{2\pi}e\sigma(f,\theta)}\right] \\ &= H(f,\theta) - \langle\log_2\frac{1}{\sqrt{2\pi}e\sigma(f,\theta)}\rangle_{q(f,\theta)}, \end{aligned} \tag{SI-20}$$

where, in the second line, we utilized the saddle-point approximation, and the second term in the last line is the expectation value of the logarithm of the standard deviation of the noise.

D. Expressions for the individual inputs

We calculated the information $I(Z,\theta)$. By definition,

$$I(Z,\theta) = H(Z) + H(\theta) - H(Z,\theta). \tag{SI-21}$$

We can use the formulas for these entropies from above to obtain the expression

$$I(Z,\theta) = \int dZ d\theta\, q(Z,\theta) \log_2 \frac{q(Z,\theta)}{\left[\int d\theta' q(Z,\theta')\right] \times \left[\int dZ' q(Z',\theta)\right]}. \tag{SI-22}$$

From this formula, we can calculate the information theoretic quantities of interest to us, $I(Z,X)$ and $I(Z,Y)$ by utilizing the two different coordinate transforms discussed above: $(X,Y) \rightarrow (r = f(X,Y), \theta = X)$ and $(X,Y) \rightarrow (r = f(X,Y), \theta = Y)$. From these transforms, we know that $I(Z,\theta)$ is simply $I(Z,X)$ or $I(Z,Y)$ respectively. Note that these expressions are independent of $\sigma(f,\theta)$ and thus do not depend on the noise in the system.

E. Expression for the total information

We now calculate the total mutual information $I(Z,(f,\theta))$ between the output Z and the individual inputs X and Y. This mutual information can be expressed in terms of the entropies as

$$I(Z,(f,\theta)) = H(Z) + H(f,\theta) - H(Z,f,\theta). \tag{SI-23}$$

Use of (**??**) yields the following simple expression,

$$I(Z,(r,\theta)) = \langle \log_2 \frac{1}{\sqrt{2\pi} e \sigma(r,\theta)} \rangle_{q(r,\theta)} + H(Z), \tag{SI-24}$$

where $H(Z)$ is given in (**??**). Since information is invariant under coordinate transforms one has $I(Z,(X,Y)) = I(Z,(r,\theta))$. This expression is analogous to that found for the case of circuit with one input and one output (Tkacik et al., 2008). This follows intuitively because $I(Z,(X,Y))$ is insensitive to the identity of the individual signals X and Y and thus the circuit effectively has a single input (X,Y) and a single output Z.

IV. CALCULATING TOTAL INFORMATION TRANSMISSION FROM EXPERIMENTAL DATA

We calculated total information transmission in the *Vibrio harveyi* quorum-sensing circuit using data from Long et al. (2009) for a variety of priors. In particular, we calculated the

mutual information between the GFP output signal and the inputs, $I(\mathrm{GFP},(X,Y))$ and found that it is of order 1.5 bits for most reasonable priors.

Here we outline our basic procedure. In Long et al. (2009), single-cell measurements were performed for a ten by ten grid of values in the $X-Y$ plane. We calculated the mean GFP level, $f(X,Y)$ as well as the variance of the GFP, $\sigma(X,Y)$, from the data for each of these points. We susbsequently used these data to infer $f(X,Y)$ and $\sigma(X,Y)$ for all values of X and Y between 0 and 1 using quadratic interpolation. Next, we calculated the noisy transfer function $P(\mathrm{GFP}|X,Y)$ using Eq. 1 in the main text:

$$P(Z|X,Y)=\frac{1}{\sqrt{2\pi\sigma^2(X,Y)}}\exp\left(-\frac{(Z-f(X,Y))^2}{2\sigma^2(X,Y)}\right) \tag{SI-25}$$

From the transfer function (**??**), we constructed the distributions $p(Z,X,Y)$ and $p(Z)$ for various priors using the formulas

$$p(Z,X,Y)=p(Z|X,Y)q(X,Y). \tag{SI-26}$$

and

$$p(Z)=\int dXdYp(Z,X,Y). \tag{SI-27}$$

We then used these formulas and the definition of total information to obtain

$$I(Z,(X,Y))=\int dZdXdYp(Z,X,Y)\log_2\left(\frac{p(Z,X,Y)}{p(Z)q(X,Y)}\right). \tag{SI-28}$$

We found that for nearly all priors $I(Z,(X,Y))$ was between 1.2 and 1.7 bits.

V. FEEDBACK ON RECEPTOR NUMBER

Bacteria can manipulate receptor kinase rates using feedbacks on receptor numbers. In general, the maximal kinase activity of a pathway depends on two separate quantities: (1) the total number of receptors, and (2) the kinase activity of a single receptor. Explicitly, the maximal kinase rates of the X (AI-1) and Y (AI-2) pathways of *V. harveyi* obey $k_X=k_X^0N_X$ and $k_Y=k_Y^0N_Y$, with N_X and N_Y the number of receptors in the X and Y pathways, respectively, and k_X^0 and k_Y^0 the maximal kinase activities of single receptors. Consequently, bacteria can modulate the maximal kinase activity of a pathway by changing the number of receptors using a feedback.

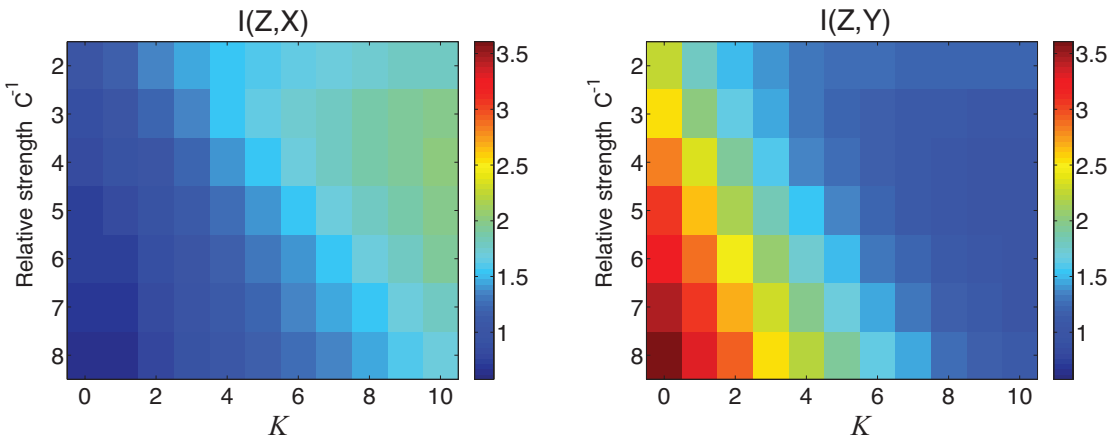

FIG. SI-1: $I(Z,X)$ and $I(Z,Y)$ for a positive-feedback architecture as a function of K and feedback strength, $C^{-1} = (\delta C)$.

A. Positive Feedback on Receptors

In the low-noise limit, the mutual informations $I(Z,X)$ and $I(Z,Y)$ only depend on three combinations of parameters, the ratios of the maximal kinase activities in the presence and absence of feedback, and the half-maximal value of the feedback K (data not shown). Thus, we can consider the equivalent transfer function

$$Z \approx X + Y(C + \frac{\delta C Z}{K + Z}) \tag{SI-29}$$

with $C = k_Y^0 N_{Y0}/k_X$, $\delta C = k_Y^0 \delta N_Y/k_X$. We have calculated the mutual informations $I(Z,X)$ and $I(Z,Y)$ for this transfer function using our low-noise expressions for a flat prior with inputs limited to the domain $X \geq Y$ and the results are plotted in Fig. **??** for various choices of K between 0 and 10. In order to reduce parameters we have considered the case where $C^{-1} = \delta C = 2, 3, \ldots, 8$. Notice that by an appropriate choice of K, cells can learn as much, or even more, about both signals as in the absence of the feedback. This finding shows that by using a positive feedback on receptor number N_Y, bacteria can preferentially pay attention to AI-2 (Y) at low cell densities and AI-1 (X) at high cell densities while simultaneously learning about both input signals.

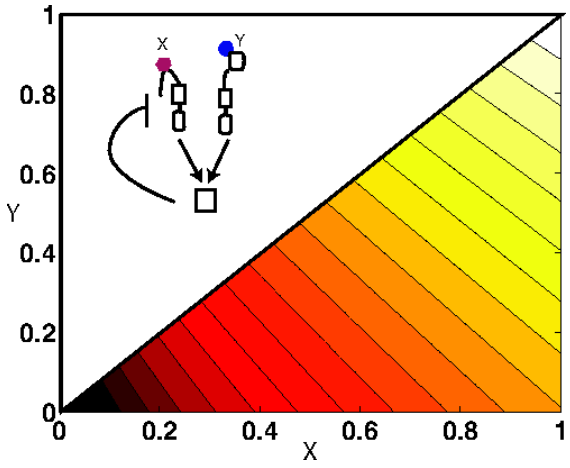

FIG. SI-2: Graphical representation of input-output relations in the presence of a negative feedback on receptor number restricted to the domain $X > Y$. Equally spaced, onstant-output Z contours for a signaling circuit with negative feedback on receptor number (see inset). Parameters are $K = 0.7$ and $D = d$.

B. Negative Feedback on Receptors

We have also considered a negative feedback on receptors in the X-pathway (see Figure **??**. Once again the transfer function $Z = f_{\rm fb}(X, Y)$, describing the output signal (the fraction of phosphorylated output regulators), as a function of the inputs X and Y (the probability that the corresponding receptors are in their on states) is obtained by solving for the steady state of a set of differential equations, in this case

$$\begin{aligned} \frac{dZ}{dt} &= (k_X X + k_Y Y)(1 - Z) - pZ \\ &= (k_X^0 N_X X + k_Y Y)(1 - Z) - pZ, \\ \tau \frac{dN_X}{dt} &= \frac{\bar{K} N_{X0}}{\bar{K} + Z} - N_X, \end{aligned} \tag{SI-30}$$

where N_{X0} is the number of receptors at $X = Y = 0$ and $\bar{K}$ sets the scale for the negative feedback. We obtain the steady-state solution by setting the left hand sides of the above equations to zero and recalling that $p \gg k_X, k_Y$. This analysis yields (where for simplicity we also denote the steady-state output by Z)

$$Z \approx \frac{k_X^0 N_{X0} \bar{K}}{p(\bar{K} + Z)} X + \frac{k_Y}{p} Y. \tag{SI-31}$$

This equation can be solved for Z to obtain the transfer function in the presence of feedback, $f_{\rm fb}(X, Y)$. The mutual information is invariant under a constant rescaling $Z \to \frac{p}{k_Y} Z$. Thus,

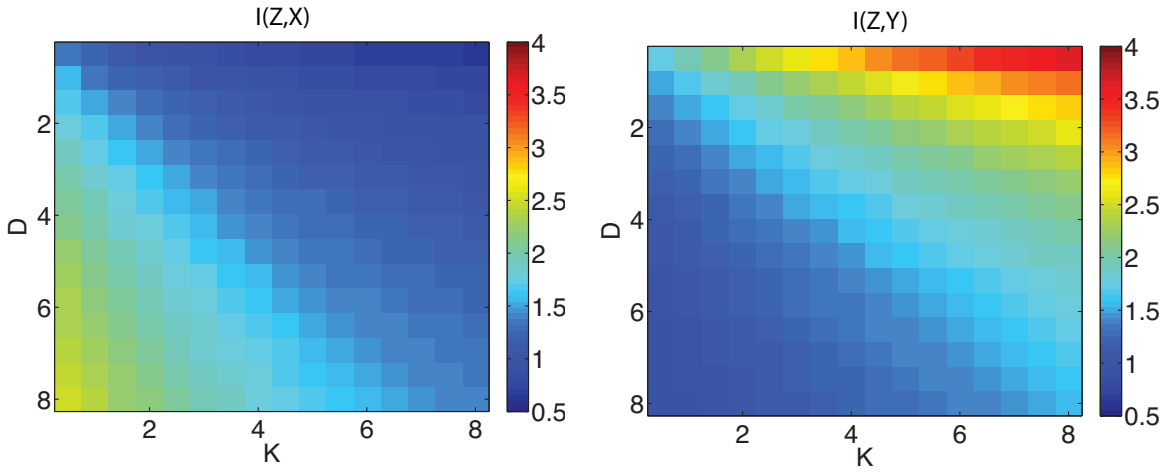

FIG. SI-3: $I(Z,X)$ and $I(Z,Y)$ for a negative feedback architecture (Eq. **??**) as a function of K and D.

to reduce the number of parameters we have calculated the mutual informations $I(Z,X)$ and $I(Z,Y)$ for a family of functions (for the rescaled Z) of the form

$$Z \approx \frac{D}{K+Z}X + Y, \tag{SI-32}$$

with $D = \bar{K}k_X^0 N_{X0} p/k_Y^2$ and $K = p\bar{K}/k_Y$, and the results are shown in Fig. **??**. In the plots we have used a flat prior with inputs restricted to the domain $X \geq Y$.

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