

S1 Text. RNAP fugacity

When calculating the fold-change we have thus far implicitly assumed that the RNAP fugacity does not change upon addition of transcription factors. This is not necessarily the case. To illustrate this, we will explicitly calculate the fugacity of RNAP in the presence (λ_P) and absence (λ_P^0) of transcription factors. We write

$$P = N\theta_P(\lambda_P, \lambda_R) + N_{\text{ns}}\theta_P^{\text{ns}}(\lambda_P, \lambda_R), \quad (\text{S.1})$$

In the case of simple repression, the average occupation numbers can be found as

$$\left. \begin{aligned} \theta_P(\lambda_P, \lambda_R) &= \frac{\lambda_P x_P}{1 + \lambda_P x_P + \lambda_R x_R} \simeq \frac{\lambda_P x_P}{1 + \lambda_R x_R}, \\ \theta_P^{\text{ns}}(\lambda_P, \lambda_R) &= \frac{\lambda_P}{1 + \lambda_P} \simeq \lambda_P. \end{aligned} \right\} \quad (\text{S.2})$$

Isolating λ_P from eq. (S.1), we obtain

$$\left. \begin{aligned} \lambda_P &= \frac{P}{\frac{N}{1 + \lambda_R x_R} x_P + N_{\text{ns}}}, & \left(\simeq \frac{P}{N_{\text{ns}}} \right) \\ \lambda_P^0 &= \frac{P}{N x_P + N_{\text{ns}}}. & \left(\simeq \frac{P}{N_{\text{ns}}} \right) \end{aligned} \right\} \quad (\text{S.3})$$

We write down the fraction λ_P/λ_P^0 as a series expansion.

$$\frac{\lambda_P}{\lambda_P^0} \simeq 1 + \frac{N x_P}{N_{\text{ns}}} \theta_R - \left(\frac{N x_P}{N_{\text{ns}}} \right)^2 \frac{\theta_R^2}{\lambda_R x_R} + \dots \quad (\text{S.4})$$

Since $\theta_R \leq 1$, we see that λ_P/λ_P^0 becomes unity as long as $N x_P/N_{\text{ns}} \ll 1$. Typically, the number of non-specific sites is overwhelmingly large. In *E. coli*, N_{ns} is of the order 5×10^6 and $\epsilon_P \sim -2.9 k_B T$. This means that decoupling is justified for even large gene copy numbers, provided that $N \ll 3 \times 10^5$. Similarly, in activation architectures, we can write down a similar argument to show that the decoupling remains valid there. The RNAP fugacity in the case of simple activation becomes

$$\lambda_P = \frac{P}{N \frac{(1 + \lambda_A x_A x_{AP})}{1 + \lambda_A x_A} x_P + N_{\text{ns}}}, \quad (\text{activation}) \quad (\text{S.5})$$

which leads to the following series expansion

$$\frac{\lambda_P}{\lambda_P^0} = \left\{ \begin{aligned} &1 + \frac{N x_P}{N_{\text{ns}}} \theta_A (1 - x_{AP}) \\ &- \left(\frac{N x_P}{N_{\text{ns}}} \right)^2 \theta_A (1 - x_{AP}) \frac{1 + \lambda_A x_A x_{AP}}{1 + \lambda_A x_A} + \dots \end{aligned} \right. \quad (\text{S.6})$$

This means that decoupling the RNAP fugacity is justified when $N x_P \ll N_{\text{ns}}$ and $N x_P \theta_A (1 - x_{AP}) \ll N_{\text{ns}}$. Since usually the number of non-specific sites in the genome of a cell is overwhelmingly large, the approximation is nearly always justified. In *E. coli*, this is the case when $N \ll 2 \times 10^3$.