

Obtaining the looping J factor from titration data

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I. ON BINDING

We warm up by considering “simple” binding. Imagine that just the operator region of DNA is immobilized in a box of volume V containing solvent and a single LacR molecule.

We do statistical mechanics by enumerating states. Divide the states into two classes:

- a. The subset of states for which the LacR molecule is close to its perfect binding position and orientation. Specifically assume that we chose a fiducial point in the LacR and a triad inscribed on it. States “a” have this point lying within a volume δv of its binding position, and the triad lying within a group volume $\delta\omega$ of the preferred orientation.¹
- b. The complement of “a” (i.e. all other positions and orientations of LacR).

We implement “binding” by declaring that in the partition sum we will reward states in class “a” with an extra weight factor $8\pi^2 K(\delta v \delta\omega)^{-1}$. Here K is a constant with dimensions of [volume], that is, [concentration]⁻¹. Finally we take the limits $\delta v \rightarrow 0, \delta\omega \rightarrow 0$.

To see that that’s the right definition, let ψ be the indicator function for binding, i.e. $\psi = 1$ on “a” states and 0 otherwise. The partition function is

$$Z = \left[\frac{8\pi^2 K}{\delta v \delta\omega} \int_{\text{“a”}} d^3\mathbf{r} d^3\Omega \right] + \left[\int_{\text{“b”}} d^3\mathbf{r} d^3\Omega \right] = 8\pi^2 V + 8\pi^2 K$$

The probability of being bound is $\langle\psi\rangle = 8\pi^2 K/Z = K/(V + K)$.

We assumed that there was just one LacR in the box, so $[\text{LacR}] = V^{-1}$. So

$$P(\text{bound})/P(\text{unbound}) = \frac{K}{V + K} \frac{V + K}{V} = K[\text{LacR}]$$

Let’s compare to the usual picture. If the rate for operator to bind LacR is $k_+[\text{LacR}]$, and the ratio for bound state to dissociate is k_- , then in equilibrium we have $P(\text{bound})/P(\text{unbound}) = (k_+/k_-)[\text{LacR}]$, an expression equivalent to ours with the usual identification of K as the equilibrium constant.

If the operator is not isolated and immobilized, but instead is part of a long DNA and free in solution, everything above is unchanged. The many conformations of the long DNA are irrelevant.

¹ “Group volume” refers to volume on the group of rotation matrices; conventionally the volume of the whole group is the dimensionless number $8\pi^2$. Wu-Ki Tung, *Group theory in physics* p133.

II. ON LOOPING

See a similar analysis in (Vanzi *et al.*, 2006).

Now we suppose the operator O is part of a long DNA and free in solution, and there's a second operator O* with a second LacR* permanently bound to it. (For example O* may be stronger than O, so it's effectively always occupied in the concentration range of interest.) Again there's a single loose LacR in solution.

Now we have a competition: O can bind nothing, or the loose LacR, or the LacR* permanently bound to O*. We neglect the possibility of an alternate, looped state, with LacR in an "open" conformation. If there is such a state, it won't affect our calculation of the relative probability to be in the "closed loop" versus unlooped (see below). So we divide states into

- a. Looped
- b. Unlooped with the free LacR bound to O
- c. Unlooped with O unoccupied.

The partition function is now much more complicated than before, because the chain conformations enter nontrivially. But we can divide the problem. Suppose we study the chain with no LacR present at all. Suppose that the chain spends a certain fraction of its time in conformations where O* is within a volume δv of the preferred position for binding to LacR* (if LacR* had been present), and with orientation within a group volume $\delta\omega$ of the preferred orientation. Clearly this fraction will go to zero as δv or $\delta\omega$ become very small, so define the "looping J factor" as²

$$\tilde{J} = \lim_{\delta v \rightarrow 0, \delta\omega \rightarrow 0} (\text{fraction of time spent looped}) / \delta v \delta\omega$$

Again, this definition has nothing to do with LacR binding. It can readily be computed by MC simulation of chains. Note it naturally has the dimensions of concentration ($\delta\omega$ is dimensionless).

Again let $\mathbf{r}, \Omega$ be the position and orientation of the loose LacR. The partition function is then

$$Z = \left[\frac{8\pi^2 K}{\delta v \delta\omega} \delta v \delta\omega \right] + 8\pi^2 V \left[1 + \frac{8\pi^2 K}{\delta v \delta\omega} (\tilde{J} \delta v \delta\omega) \right] = 8\pi^2 (K + V + V 8\pi^2 K \tilde{J})$$

Let ψ be the indicator function for looping. Again by computing $\langle \psi \rangle / (1 - \langle \psi \rangle)$ we find

$$\frac{P(\text{unlooped})}{P(\text{looped})} = \frac{V + K}{V 8\pi^2 K \tilde{J}} = \frac{K^{-1} + [\text{LacR}]}{8\pi^2 \tilde{J}}$$

Note how the unit work out OK.

Lin measures the LHS as a function of [LacR], so for each DNA construct we find the slope and intercept to extract both K and $\tilde{J}$ (which we will predict from theory). As a check, K should always come out the same for each DNA construct.

² Better read this to see what they said about it: Flory, P. J.; Suter, U. W.; Mutter, M. Macrocyclization equilibria. 1. Theory. J. Am. Chem. Soc. 1976, 98, 5733-5739. See also (Czapla *et al.*, 2006).

A. Multiple looped states

Suppose there's an alternate conformation of LacR–DNA complex, also rigid, with a different “target” for binding; suppose it costs free energy ΔG to pop into it.

$$Z = 8\pi^2(K + V + V8\pi^2(K\tilde{J}_1 + K\tilde{J}_2e^{-\Delta G}))$$

We still have

$$\frac{P(\text{unloop})}{P(\text{loop } 1)} = \frac{K^{-1} + [\text{LacR}]}{8\pi^2\tilde{J}_1}$$

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