Supporting Text

A Simple Interpretation of Entropic Pulling from the Analytical Solution of a Simplified Polymer Model. The entropic effect due to the reduction of the number of polypeptide conformations upon Hsp70 binding is not easily pictured without some physics background. By introducing a simplified modeling of the polypeptide as a Gaussian chain, it is possible to write an explicit analytical solution of the problem, and to provide a simpler, more intuitive picture of the process.

According to the Gaussian chain model (1), the distribution function of the end-to-end vector $\vec{r}$ of a polymer of *n+1* monomers (hence of length *n*) is given by a Gaussian function,

$$G(\vec{r},n+1)=\left(\frac{3}{2\pi nb^2}\right)^{3/2}\exp\left[-\frac{3|\vec{r}|^2}{2nb^2}\right]$$

where *b* is the length of a Kuhn segment. Anchoring one of the end-points to the pore exit, and limiting the available volume by forbidding the half-space occupied by the membrane (that is, confining the polypeptide to the mitochondrial matrix), changes that distribution of the end-point to

$$G_{membrane}(\vec{r},n+1)=\left(\frac{3}{2\pi nb^2}\right)^{3/2}\frac{6z\varepsilon}{nb^2}\exp\left[-\frac{3|\vec{r}|^2}{2nb^2}\right]$$

which is a standard result of polymer physics, and where *z* is the distance of the free polypeptide end-point from the pore exit along the direction perpendicular to the membrane, and ε a small arbitrary constant (corresponding to the distance from the membrane, along the *z* direction, of the membrane anchored end-point that, for mathematical reasons, cannot be strictly at the membrane). If *N(n+1)* is the number of conformations available to a polymer of *n+1* monomers in free space, the number of polymer conformations constrained by the presence of the membrane is

$$N_{membrane}(n+1) = N(n+1)\int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} dy \int_{0}^{\infty} dz\, G_{membrane}(x,y,z,n+1)$$

where *x* and *y* span a coordinate system parallel to the membrane. Binding of mtHsp70 around the (*n+1*)th monomer reduces the number of polymer conformations because the end-point that is not anchored to the membrane cannot access values of the coordinate $z<R_{70}$ and the number of conformations becomes

$$N_{membrane,70}(n+1) = N(n+1)\int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} dy \int_{R_{70}}^{\infty} dz\, G_{membrane}(x,y,z,n+1) =$$
$$= \exp\left(\frac{3R_{70}^2}{2b^2}\right) N_{membrane}(n+1)$$

The free energy of a Gaussian chain is completely determined by its entropy *S*, which is the logarithm of the number of accessible conformations, by the formula $F=-TS=-k_B T \ln N(n+1)$, where k_B is the Boltzmann's constant. The free energy difference induced by chaperone binding is thus

$$\Delta F_{70}(n) = -k_B T\left[\ln N_{membrane,70}(n+1) - \ln N_{membrane}(n+1)\right] =$$
$$= -k_B T \ln \frac{N_{membrane,70}(n+1)}{N_{membrane}(n+1)} = \frac{3R_{70}^2}{2b^2}\frac{k_B T}{n}$$

where the last equality highlights the two most important functional dependences of $\Delta F_{70}(n)$, namely the direct proportionality to the thermal energy, a typical signature of entropic effects, and the inverse proportionality to the number of imported residues. This functional behavior is also shown in Fig.2*A*, and the curves from our mode detailed models (see *Materials and Methods*) can indeed be fitted with such a formula, although with model-specific constants.

The Gaussian chain approximation allows a perhaps more intuitive interpretation of our results. If we integrate the free-end probability distribution $G_{membrane}(\vec{r},n+1)$ only over *x* and *y*, and multiply by the total number of conformations of an unconstrained polymer of *n+1* monomers, we obtain the number of conformations such that the end-point is at distance *z* from the membrane

$$N_{membrane}(z,n+1) = N(n+1)\int_{-\infty}^{\infty} dx \int_{-\infty}^{\infty} dy\, G_{membrane}(x,y,z,n+1)$$

which gives

$$N_{membrane}(z,n+1) = \left(\frac{3}{2\pi n b^2}\right)^{1/2} \frac{6z\varepsilon}{nb^2} \exp\left(-\frac{3z^2}{2nb^2}\right) N(n+1)$$

The free energy profile of the polymer as a function of z is therefore $F(z,n+1)=-k_BT$ $\ln[N_{membrane}(z,n+1)]$ and takes the functional form

$$F(z,n+1) = -k_BT\left(\ln z - \frac{3z^2}{2nb^2}\right) + const$$

which is shown in Fig.5 (the constant only shifts the absolute value of the energy, which is not relevant since only energy differences have to be considered).

The graphical representation of the free energy of the system as a function of z allows for a simple interpretation. By identifying the free end-point of the polypeptide with a chaperone binding site, Fig. 5 can be interpreted as if the Hsp70 binding site effectively behaved as an un-tethered particle confined close to the membrane by a potential $F(z,n+1)$. Binding of Hsp70 to the free end-point reduces the available volume close to the membrane because the end-point cannot approach the membrane closer than a chaperone radius (shaded region in Fig. 5), whereas the quadratic constraint away from the membrane does not change. Effectively, the volume accessible to the end-point has been reduced. If we identify the binding site as a simple particle, it should obey the laws of gases that state that reducing, at constant temperature, the volume accessible to a gas increases its pressure. Thus, the binding site with locked Hsp70 confined close to the membrane exerts a pressure, thus a force, trying to increase its accessible volume. Since the confining potential on the membrane side is given by chaperone-membrane volume exclusion, which we assume as infinitely strong, the accessible volume can be increased only by weakening the constraining potential away from the membrane. This potential is $3k_BT\, z^2/2b^2n$ and the only way to make it softer is to increase n. In Fig. 5 we show two different curves corresponding to two different values of n (solid line, smaller n; dashed line, larger n).

By using a more realistic description of the polypeptide, with realistic values of the persistence length, and a realistic modelling of the chaperone can provide a better estimate of the free energies involved in the process, at the price of losing the ability to handle the equations analytically. Nonetheless, the intuitive interpretation offered here still applies.

1. de Gennes, P.-G. (1979) *Scaling Concepts in Polymer Physics* (Cornell University Press, Ithaca and London).