

1 Crowder-induced forces

The goal here is to consider a process in which two objects bind together, and how the presence of “crowders” changes the free energy of binding. The example we will be using will be a transcription factor binding to a site on DNA. We model the transcription factor as a sphere of radius r_t and the DNA as a cylinder of radius r_d . As for the crowders, they are spheres of radius r_c , and they are mobile.

In the beginning, we will consider only the consequences of hard-core (excluded volume) interactions. Later, we will add the effects of other potentials acting between different molecular species.

2 Definition of $\Delta F_{\text{crowd}}(\phi)$

In a dilute solution, the process of interest looks like Fig. 2. With this process is associated some free energy change ΔF_{dilute} . Considering what goes on when a transcription factor binds to a particular site on DNA, it is clear that calculating ΔF_{dilute} would be a very complicated business; we would need a very detailed understanding of this process in atomistic detail. We are *not* going to be calculating ΔF_{dilute} . Rather, our goal is to understand how the total binding free energy changes when crowding is added. In general, we quantify the concentration of crowders in terms of their volume fraction ϕ :

$$\phi \equiv \frac{N_{\text{crowd}} V_{\text{crowd}}}{V_{\text{box}}}, \quad (1)$$

where we are considering our system to be enclosed in a box of volume V_{box} and containing N_{crowd} crowders. Our binding process is shown in Fig. 3, but now with crowders. The free energy of binding will differ from that in the dilute case. We define the crowding contribution to the binding free energy as this difference:

$$\Delta F_{\text{crowd}}(\phi) \equiv \Delta F(\phi) - \Delta F_{\text{dilute}} \quad (2)$$

Note that so far we have made no assumptions or approximations, merely defined what it is that we wish to calculate, namely $\Delta F_{\text{crowd}}(\phi)$.

2.1 Key assumption

2.2 Calculation of ΔF_{crowd} to first order in ϕ

Clearly $\Delta F_{\text{crowd}}(\phi)$ vanishes as ϕ goes to zero. What we want now is the behavior of ΔF_{crowd} to linear order in ϕ . That is, how does the binding free energy change as we add an infinitesimal volume fraction of crowders?

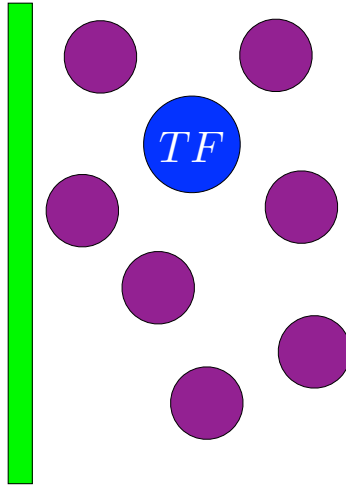


Figure 1: Schematic depiction of DNA, transcription factor (TF), and crowders.

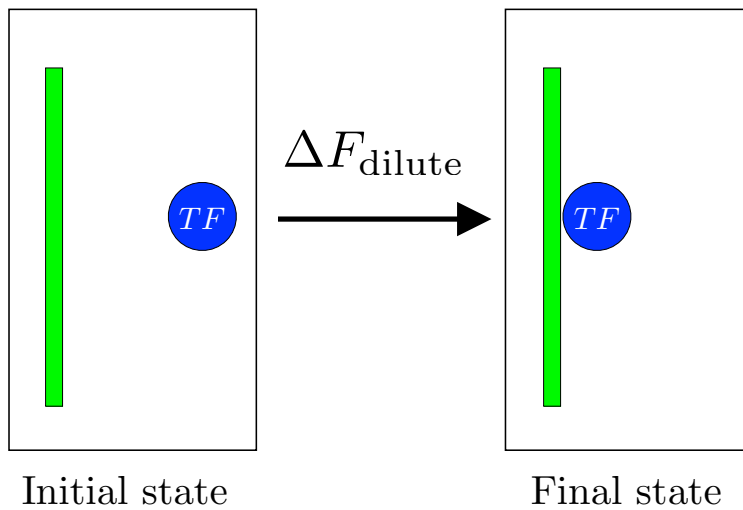


Figure 2: The initial and final states of the binding process. In the initial state, you should think of the two objects as being very far apart.

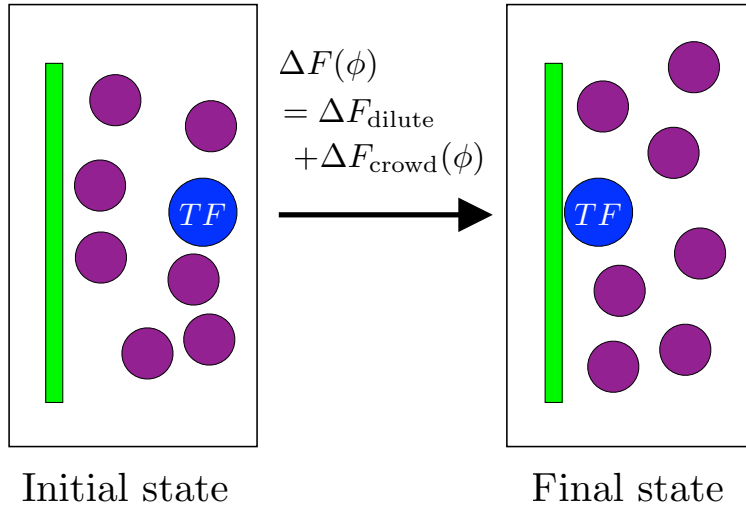


Figure 3: The binding process in a crowded environment. Definition of $\Delta F_{\text{crowd}}(\phi)$. By ϕ we mean the volume fraction of crowders.

Of course, if we keep the box size V_{box} fixed, it makes no sense to talk about infinitesimal volume fractions of crowders, since there is no such thing as a fraction of a crowder. Instead, we let the box size go to infinity, and we calculate the contribution to the binding free energy when there is a *single crowder*, in which case

$$\phi = \frac{1 \cdot V_{\text{crowd}}}{V_{\text{box}}} \quad (3)$$

becomes infinitesimal as $V_{\text{box}} \rightarrow \infty$. This reformulation of the question is convenient, because the problem turns out to be tractable for a single crowder. The binding process is depicted in Fig. 4 for the case where there is a single crowder. The figure shows the region of excluded volume for the DNA and the TF (or for the DNA-TF complex) in gray. These are the regions where it is impossible to put the *center of mass* of a crowder. The excluded volume region of the TF alone is a sphere of radius $r_t + r_c$ centered on the TF. The excluded volume region of the DNA is a cylinder of radius $r_d + r_c$. Very importantly, in the bound state there is an overlap between these two region, so that the overall excluded volume decreases during binding.

Now we can calculate free energies. What we will really be doing is calculating the partition functions of the crowder itself. Let's start with the partition function for the initial state. We can place the crowder anywhere except in the excluded volume regions around the TF and the DNA. Therefore

$$Z_{\text{initial}} = \int_{\text{box}} \exp[-\beta U(\vec{r})] d^3 \vec{r} = V_{\text{box}} - V_{\text{DNA}}^{\text{excl}} - V_{\text{TF}}^{\text{excl}} \quad (4)$$

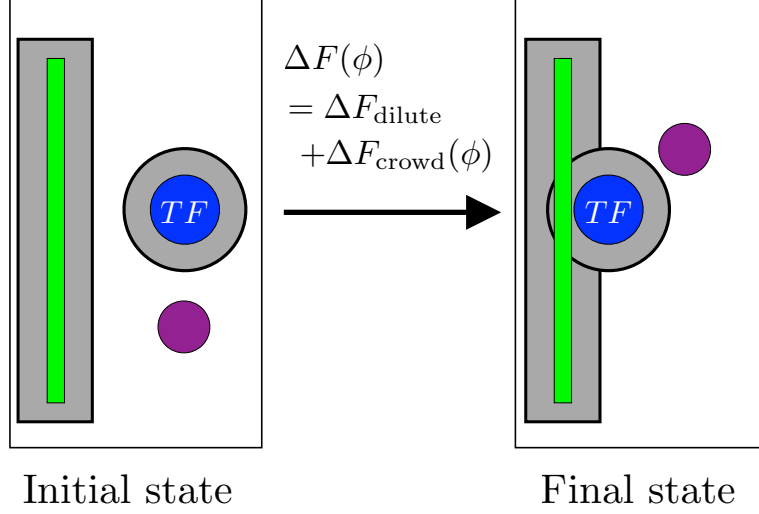


Figure 4: The binding process, with only one crowder. Excluded volume regions are shown in gray.

For the final state, the total excluded volume is not the sum of the excluded volumes of the DNA and the TF, since this would double-count the overlap region. Rather,

$$\begin{aligned}
 Z_{\text{final}} &= \int_{\text{box}} \exp[-\beta U(\vec{r})] d^3\vec{r} = V_{\text{box}} - (V_{\text{DNA}}^{\text{excl}} + V_{\text{TF}}^{\text{excl}} - V_{\text{overlap}}^{\text{excl}}) \\
 &= V_{\text{box}} - V_{\text{DNA}}^{\text{excl}} - V_{\text{TF}}^{\text{excl}} + V_{\text{overlap}}^{\text{excl}}
 \end{aligned} \tag{5}$$

The free energy difference between initial and final states is

$$\Delta F_{\text{crowd}} = -k_B T \ln Z_{\text{final}} + k_B T \ln Z_{\text{initial}} \tag{6}$$

It is convenient to work with the minus the free energy difference:

$$\begin{aligned}
 -\beta \Delta F_{\text{crowd}} &= \ln \left(\frac{Z_{\text{final}}}{Z_{\text{initial}}} \right) \\
 &= \ln \left(\frac{V_{\text{box}} - V_{\text{DNA}}^{\text{excl}} - V_{\text{TF}}^{\text{excl}} + V_{\text{overlap}}^{\text{excl}}}{V_{\text{box}} - V_{\text{DNA}}^{\text{excl}} - V_{\text{TF}}^{\text{excl}}} \right)
 \end{aligned} \tag{7}$$

We will divide through by V_{box} since we are letting this quantity become very large.

$$\begin{aligned}
-\beta\Delta F_{\text{crowd}} &= \ln\left(\frac{1 - V_{\text{DNA}}^{\text{excl}}/V_{\text{box}} - V_{\text{TF}}^{\text{excl}}/V_{\text{box}} + V_{\text{overlap}}^{\text{excl}}/V_{\text{box}}}{1 - V_{\text{DNA}}^{\text{excl}}/V_{\text{box}} - V_{\text{TF}}^{\text{excl}}/V_{\text{box}}}\right) \\
&= \ln\left(1 - V_{\text{DNA}}^{\text{excl}}/V_{\text{box}} - V_{\text{TF}}^{\text{excl}}/V_{\text{box}} + V_{\text{overlap}}^{\text{excl}}/V_{\text{box}}\right) \\
&\quad - \ln\left(1 - V_{\text{DNA}}^{\text{excl}}/V_{\text{box}} - V_{\text{TF}}^{\text{excl}}/V_{\text{box}}\right)
\end{aligned} \tag{8}$$

The ratios of volumes are all very small as $V_{\text{box}} \rightarrow \infty$, so we are justified in Taylor expanding

$$\begin{aligned}
-\beta\Delta F_{\text{crowd}} &\approx -V_{\text{DNA}}^{\text{excl}}/V_{\text{box}} - V_{\text{TF}}^{\text{excl}}/V_{\text{box}} + V_{\text{overlap}}^{\text{excl}}/V_{\text{box}} \\
&\quad + V_{\text{DNA}}^{\text{excl}}/V_{\text{box}} + V_{\text{TF}}^{\text{excl}}/V_{\text{box}} \\
&= V_{\text{overlap}}^{\text{excl}}/V_{\text{box}}
\end{aligned} \tag{9}$$

Remembering that for our one-crowder system, $\phi = V_{\text{crowd}}/V_{\text{box}}$, we can rewrite this as

$$-\beta\Delta F_{\text{crowd}} = \frac{V_{\text{overlap}}^{\text{excl}}}{V_{\text{crowd}}} \cdot \phi + O(\phi^2) \tag{10}$$

2.3 Numerical values

Now we can use numerical values: $r_c = 3$ nm, $r_t = 4$ nm and $r_d = 1$ nm. Then $V_{\text{crowd}} = 36\pi \text{ nm}^3 = 113.1 \text{ nm}^3$. The volume of the overlap between the excluded volume regions, $V_{\text{overlap}}^{\text{excl}}$, is not easy to calculate analytically (the volume of the intersection of a cylinder with an off-center sphere). We can do it numerically, however, with the result that $V_{\text{overlap}}^{\text{excl}} = 363.1 \text{ nm}^3$. With these numerical values, Eq. 10 gives the estimate

$$-\beta\Delta F_{\text{crowd}}(\phi) \approx 3.2 \phi \tag{11}$$

Figure 5 compares this approximation with Monte Carlo simulation results, and shows that the approximation is actually quite good.

2.4 Adding crowder-TF attractive potential

The good news is that the ‘‘single crowder’’ problem is still tractable when there is an attractive potential between the TF and the crowder. It is a little bit more complicated, and we will need some notation to talk about the various regions and their intersections and so on. We denote the ‘‘difference’’ of two regions V_1 and V_2 by $V_1 \setminus V_2$. That is the set of points within V_1 but not within V_2 . The intersection of two regions is denoted $V_1 \cap V_2$ and their union $V_1 \cup V_2$. We will commit the abuse of notation of using the same symbol (for example, V_{box}) for both a region and the volume of that region. In the following it may help to consult Fig. 4 often.

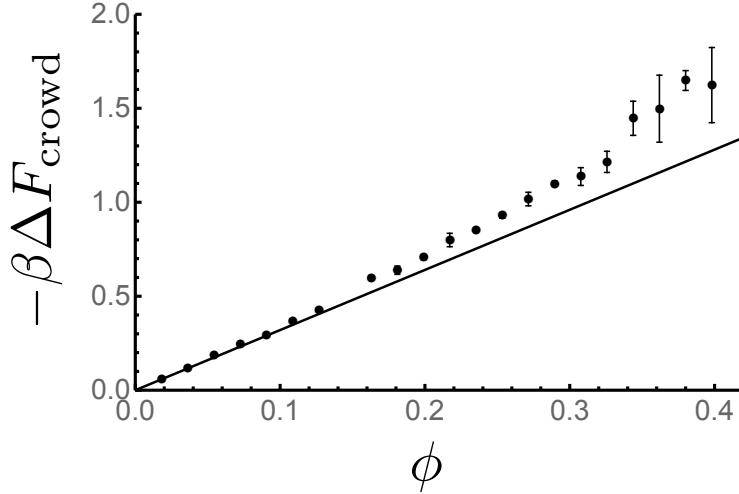


Figure 5: Solid line: linear approximation for $-\beta\Delta F_{\text{crowd}}(\phi)$ using numerical values. Symbols: MC simulation results.

Suppose, then, that there is an attractive potential $U(r)$ between the TF and the crowders, where r is the distance between their centers of mass. We assume that for large r the potential decays reasonably fast. The partition function for the initial state is

$$Z_{\text{initial}} = (V_{\text{box}} - V_{\text{DNA}}^{\text{excl}} - V_{\text{TF}}^{\text{excl}}) + \int_{V_{\text{box}} \setminus V_{\text{TF}}^{\text{excl}}} [\exp(-\beta U(r)) - 1] d^3\vec{r} \quad (12)$$

In the integral, the origin is taken to be the center of the TF. This equation needs some explanation. The first three terms (in parentheses) are the result in the absence of any potential. However, to ignore the potential is a mistake, which we correct by adding the integral of $[\exp(-\beta U(r)) - \exp(0)]$ over the region where the potential exists – that is, the region outside the excluded volume region of the TF. If you are careful, you will note that we are assuming that the potential $U(r)$ surrounding the TF vanishes in the vicinity of the DNA, since they are taken to be very far apart.

The partition function for the final state is

$$Z_{\text{final}} = (V_{\text{box}} - V_{\text{DNA}}^{\text{excl}} - V_{\text{TF}}^{\text{excl}} + V_{\text{overlap}}) + \int_{V_{\text{box}} \setminus (V_{\text{DNA}}^{\text{excl}} \cup V_{\text{TF}}^{\text{excl}})} [\exp(-\beta U(r)) - 1] d^3\vec{r} \quad (13)$$

Again, the first line (in parentheses) is the answer without and potential and the second term corrects it by integrating $[\exp(-\beta U(r)) - \exp(0)]$ over the region where the potential

exists: again, outside of the excluded volume region of the TF, but *this time* also outside the excluded volume region of the DNA.

Now we use the same tricks as before. Pulling out a factor of V_{box} ,

$$\begin{aligned} \ln Z_{\text{initial}} &= \ln(V_{\text{box}}) \\ &+ \ln \left(1 - V_{\text{DNA}}^{\text{excl}}/V_{\text{box}} - V_{\text{TF}}^{\text{excl}}/V_{\text{box}} + \frac{1}{V_{\text{box}}} \int_{V_{\text{box}} \setminus V_{\text{TF}}^{\text{excl}}} [\exp(-\beta U(r)) - 1] d^3 \vec{r} \right) \\ &\approx \ln(V_{\text{box}}) - V_{\text{DNA}}^{\text{excl}}/V_{\text{box}} - V_{\text{TF}}^{\text{excl}}/V_{\text{box}} + \frac{1}{V_{\text{box}}} \int_{V_{\text{box}} \setminus V_{\text{TF}}^{\text{excl}}} [\exp(-\beta U(r)) - 1] d^3 \vec{r} \end{aligned}$$

and similarly

$$\begin{aligned} \ln Z_{\text{final}} &= \ln(V_{\text{box}}) \\ &+ \ln \left(1 - V_{\text{DNA}}^{\text{excl}}/V_{\text{box}} - V_{\text{TF}}^{\text{excl}}/V_{\text{box}} + V_{\text{overlap}}^{\text{excl}}/V_{\text{box}} + \frac{1}{V_{\text{box}}} \int_{V_{\text{box}} \setminus (V_{\text{DNA}}^{\text{excl}} \cup V_{\text{TF}}^{\text{excl}})} [\exp(-\beta U(r)) - 1] d^3 \vec{r} \right) \\ &\approx \ln(V_{\text{box}}) - V_{\text{DNA}}^{\text{excl}}/V_{\text{box}} - V_{\text{TF}}^{\text{excl}}/V_{\text{box}} + V_{\text{overlap}}^{\text{excl}}/V_{\text{box}} + \frac{1}{V_{\text{box}}} \int_{V_{\text{box}} \setminus (V_{\text{DNA}}^{\text{excl}} \cup V_{\text{TF}}^{\text{excl}})} [\exp(-\beta U(r)) - 1] d^3 \vec{r} \end{aligned}$$

And so

$$\begin{aligned} -\beta \Delta F_{\text{crowd}} &= \ln Z_{\text{final}} - \ln Z_{\text{initial}} \\ &\approx \frac{V_{\text{overlap}}^{\text{excl}}}{V_{\text{box}}} \\ &\quad + \frac{1}{V_{\text{box}}} \int_{V_{\text{box}} \setminus (V_{\text{DNA}}^{\text{excl}} \cup V_{\text{TF}}^{\text{excl}})} [\exp(-\beta U(r)) - 1] d^3 \vec{r} \\ &\quad - \frac{1}{V_{\text{box}}} \int_{V_{\text{box}} \setminus V_{\text{TF}}^{\text{excl}}} [\exp(-\beta U(r)) - 1] d^3 \vec{r} \\ &= \frac{V_{\text{overlap}}^{\text{excl}}}{V_{\text{box}}} - \frac{1}{V_{\text{box}}} \int_{V_{\text{DNA}}^{\text{excl}} \setminus V_{\text{TF}}^{\text{excl}}} [\exp(-\beta U(r)) - 1] d^3 \vec{r} \quad (14) \end{aligned}$$

Once more, we are talking about a single crowder so $\phi = V_{\text{crowd}}/V_{\text{box}}$ and we have

$$-\beta \Delta F_{\text{crowd}} = \phi \left[\frac{V_{\text{overlap}}^{\text{excl}}}{V_{\text{crowd}}} - \frac{1}{V_{\text{crowd}}} \int_{V_{\text{DNA}}^{\text{excl}} \setminus V_{\text{TF}}^{\text{excl}}} [\exp(-\beta U(r)) - 1] d^3 \vec{r} \right] + O(\phi^2) \quad (15)$$

In the integrals, the origin is at the center of the TF. The integration regions in these equations are shown in Fig. 6.

A few comments. First, when there is no potential, the integral vanishes and we recover the previous result of Eq. 10. Second, one sees that an attractive interaction between the TF and the crowders leads to a positive value of the integral. The effect of this is to decrease the strength of the effective attraction induced by the crowders. This makes sense: if the TF is very strongly attracted to the crowders, any direct contact with the DNA (although favored by the depletion interaction) also means less favorable TF-crowder contact.

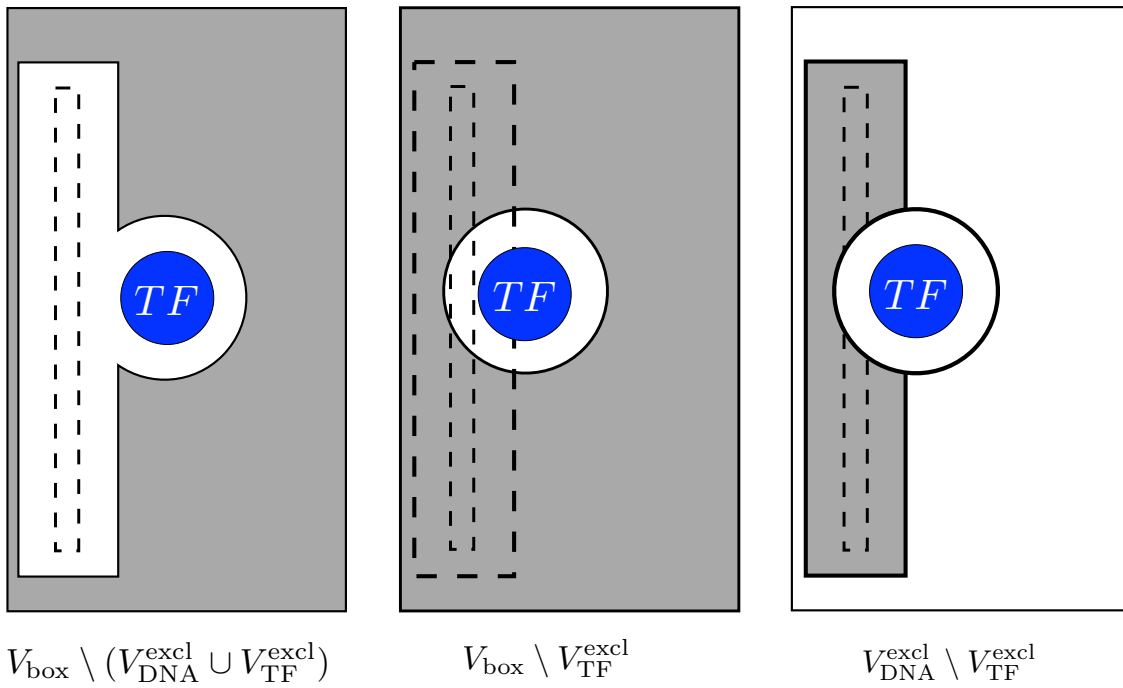


Figure 6: Schematic of several regions, including the integration region in Eq. 15 (right side).

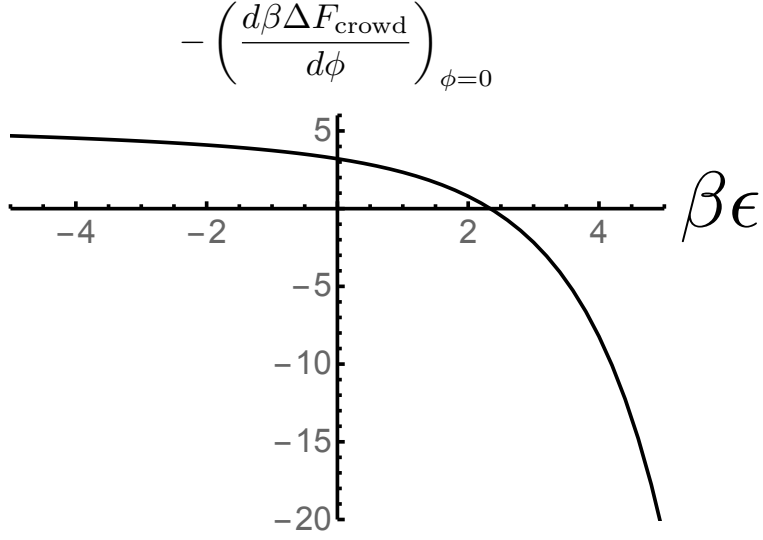


Figure 7: Coefficient of $-\beta\Delta F_{\text{crowd}}$ to first order in ϕ , including dependence on ϵ . Negative values of ϵ correspond to repulsion between TF and crowders; positive values correspond to attraction.

Numerical Values

To estimate the integral in Eq. 15 numerically, we need to specify the potential $U(r)$ acting between the TF and the crowders. We use

$$U(r) = -\epsilon \cdot \exp\left[-\frac{r - r_t - r_c}{\lambda}\right] \quad (16)$$

Note that a positive value of ϵ corresponds to an attractive potential. For the range of the interaction potential, we will use a value of $\lambda = 0.5$ nm. Now we can do the integral numerically. The result is shown in Fig. 7. One sees that, as expected, the coefficient decreases with ϵ , changing sign at $\epsilon \approx 2.3 k_B T$. It is interesting that attractive interactions can have a very strong effect, while repulsive interactions seem to have a small effect. The same information is shown in a different form in Fig. 8, which shows the binding free energy estimated, again, to first order in ϕ , but now with the exact ϵ dependence that came from treating the “one crowder” problem exactly. Note that as in Fig. 5 these should give the exact slope at small values of ϕ . An interesting question is: how do the actual curves deviate from these linear approximations? Do they always curve “up” as in Fig. 5? Do they always curve away from zero?

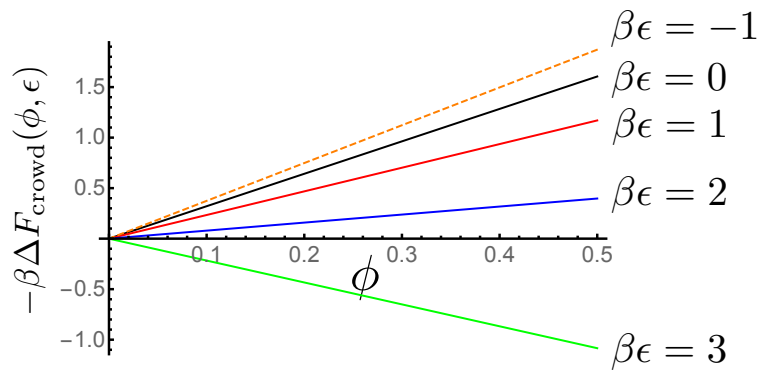


Figure 8: Approximation of $-\beta\Delta F_{\text{crowd}}$ to first order in ϕ , including the exact ϵ dependence.