

Solutions to problem set 6

Problem 1

DNA overstretching transition.

- a) Van Marmeren *et al.* argue that DNA melts during the overstretching transition, i.e. that the double-stranded (ds) DNA is converted to two single strands (ss = single-stranded). Quoting from the paper (last paragraph of “Conclusion”):

In conclusion, we have unveiled that, independent of the details of strand attachment, DNA overstretching unambiguously comprises a gradual conversion of dsDNA to ssDNA.

- b) There are several lines of evidence for DNA melting during overstretching presented in the paper:

1) From fluorescence imaging of DNA stretched in optical tweezers, they show that YOYO binding (linearly) decreases when the DNA is overstretched (Figure 2). YOYO is an *intercalator* that is known to bind to B-form DNA and not to ssDNA. The authors note themselves that this experiment is not conclusive, though, since it is not known whether YOYO binds to S-DNA or not.

2) Using the same assay, they observe that fluorescently labeled mitochondrial single-stranded binding protein (mtSSB) starts binding upon overstretching the DNA and that the amount bound corresponds to the amount of DNA overstretched (Figure 3).

3) Finally, they perform two-color experiments where they label the (B-form) dsDNA with intercalating dyes (YOYO or POPO) and the single-stranded parts with fluorescently labeled mtSSB or RPA (another single-stranded DNA binding protein) (Figure 4).

- c) The experiments reported by van Marmeren *et al.* convincingly indicate that the overstretching transition can involve DNA melting. However, they do not rule out that S-DNA formation can also occur. First, as the authors note at least partially themselves, the measurements with intercalating dyes are inconclusive in this regard, since it is not known whether or to what extent intercalating dyes bind S-DNA. If intercalators bind strongly only to B-form DNA, then loss of fluorescence only means that overstretched DNA is no longer B-form, but it could still be either melting or S-form. Even the experiments with the single-stranded binding proteins do not rule out S-DNA formation upon overstretching. Strictly speaking, it is not

known whether they bind S-form DNA or not; this seems unlikely, though, from what we know structurally. More importantly, even if we assume (similar to the authors' implicit assumptions) that the single-stranded binding proteins only bind single-stranded DNA, it is important to realize that adding a binding partner for ssDNA will shift the thermodynamic equilibrium in favor of single-stranded DNA. So whatever equilibrium between melting and S-DNA formation occurs upon overstretching, the equilibrium will be shifted towards more melting in the presence of the single-stranded binding proteins.

It turns out that subsequent publications showed that both S-DNA formation and melting occur upon DNA overstretching and that the balance sensitively depends on solution conditions (salt concentration, temperature, etc.), GC content and pulling speed. See e.g. Bosaeus, *et al. PNAS* 2012 (<http://www.pnas.org/content/109/38/15179.full.pdf>), Zhang, *et al. PNAS* 2012 (<http://www.pnas.org/content/109/21/8103.full.pdf>), King *et al. PNAS* 2013 (<http://www.pnas.org/content/110/10/3859.full.pdf>)

Problem 2

FJC, revisited.

a) Radius of gyration

$$\begin{aligned}
R_g^2 &= \frac{1}{N} \sum_{i=1}^N \langle (\vec{r}_i - r_{mean}^{\vec{}})^2 \rangle \\
&= \frac{1}{N} \sum_{i=1}^N \langle (\vec{r}_i - \frac{1}{N} \sum_{j=1}^N \vec{r}_j)^2 \rangle \\
&= \frac{1}{N} \sum_{i=1}^N (\langle r_i^2 \rangle - 2 \frac{1}{N} \sum_{j=1}^N \langle \vec{r}_i \vec{r}_j \rangle + \frac{1}{N^2} \langle (\sum_{j=1}^N \vec{r}_j)^2 \rangle) \\
&= \frac{1}{N} \sum_{i=1}^N \frac{1}{N} \sum_{j=1}^N \langle r_i^2 \rangle - 2 \frac{1}{N^2} \sum_{i=1}^N \sum_{j=1}^N \langle \vec{r}_i \vec{r}_j \rangle + \frac{1}{N^2} \frac{1}{N} \sum_{i=1}^N \langle (\sum_{j=1}^N \vec{r}_j) (\sum_{k=1}^N \vec{r}_k) \rangle \\
&= \frac{1}{N^2} \sum_{i,j=1}^N \langle r_i^2 \rangle - \frac{2}{N^2} \sum_{i,j=1}^N \langle \vec{r}_i \vec{r}_j \rangle + \frac{1}{N^2} \sum_{i,j=1}^N \langle \vec{r}_i \vec{r}_j \rangle \\
&= \frac{1}{N^2} \sum_{i,j=1}^N (\langle r_i^2 \rangle - \langle \vec{r}_i \vec{r}_j \rangle) \\
&= \frac{1}{2N^2} (\sum_{i,j=1}^N \langle r_i^2 - \vec{r}_i \vec{r}_j \rangle) + \sum_{i,j=1}^N \langle r_j^2 - \vec{r}_i \vec{r}_j \rangle \\
&= \frac{1}{2N^2} \sum_{i,j=1}^N \langle r_i^2 - 2\vec{r}_i \vec{r}_j + r_j^2 \rangle \\
&= \frac{1}{2N^2} \sum_{i,j=1}^N \langle (\vec{r}_i - \vec{r}_j)^2 \rangle
\end{aligned}$$

b) Radius of gyration for FJC

$$\begin{aligned}
R_g^2 &= \frac{1}{2N^2} \sum_{i=1}^N \sum_{j=1}^N |i - j| b^2 \\
&= \frac{1}{2N^2} \int_0^N di \int_0^N dj |i - j| b^2 \\
&= \frac{1}{N^2} \int_0^N di \int_0^i dj |i - j| b^2 \\
&= \frac{1}{6} N b^2
\end{aligned}$$

Problem 3

3D Gaussian chain. Use the formula for $P(\vec{R}; N)$:

$$P(\vec{R}; N) = \left(\frac{3}{2\pi \cdot Nb^2} \right)^{3/2} \exp\left(-\frac{3R^2}{2 \cdot Nb^2} \right) \quad (1)$$

a) Boltzmann relation: $S(\vec{R}) = k_B \ln(P(\vec{R}; N))$. Now we want to go from the unperturbed end-to-end length $\sqrt{Nb^2}$ to a new value $\sqrt{R^2}$. The change in entropy is

$$\Delta S = S(R^2) - S(Nb^2) = k_B \left(\frac{3}{2} - \frac{3}{2} \frac{R^2}{Nb^2} \right) = \frac{3}{2} k_B \left(1 - \frac{R^2}{Nb^2} \right) \quad (2)$$

b) The Gaussian chain has only entropy as a contribution to its free energy. Thus $\Delta G = -T\Delta S$. In addition, we have that the force is given by $F = -\partial\Delta G/\partial R$, therefore we find

$$F = \frac{3k_B T}{Nb^2} R \quad (3)$$

c) The spring constant is simply the pre-factor in front of the R in the last equation, i.e. $\frac{3k_B T}{Nb^2}$. This agrees with the result that we obtained in the lecture from looking at the low force limit of the FJC force-extension relationship.