Dynamics of biomolecular processes – Exercises & questions

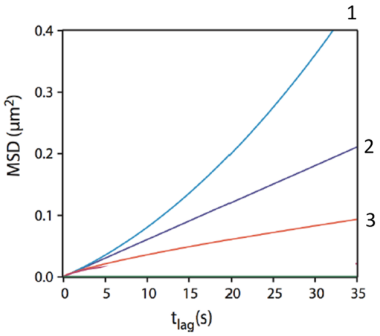
1. Explain in keywords:
a. What is a Patterson Map and what is it used for?
b. Why are heavy metals used to obtain an interpretable Patterson Map for a protein?

2. Which geometrical construction is used to determine which lattice points of the reciprocal lattice are resulting in a detectable reflection? Explain your answer with a sketch.

3. Explain, using keywords, how a 3D structure is obtained from cryoEM data (use sketches if appropriate).

4. The diffusion of fluorescently labelled molecules within a membrane can be investigated by single-molecule imaging. Explain how the diffusion constants for the individual molecules are extracted from the single-molecule trajectories obtained by a single-molecule imaging experiment.

5. You label 3 proteins with a fluorescent marker. The 3 proteins are: GFP, myosin and the acetylcholine receptor (a membrane protein). The motions of the 3 proteins are tracked in the cell. Their displacement over time is shown in the plot below.



a. What is the name of the type of motion shown for the 3 tracks, and what is the underlying process leading to his behavior?
b. Which trace (1, 2 or 3) corresponds most likely to which protein?

6. A protein **P** binds a ligand **L** in a dynamic reaction $P + L \underset{}{\overset{k_{ex}}{\rightleftharpoons}} PL$

Binding of the ligand results in a chemical shift change for a specific proton within the binding pocket of protein **P**. The chemical shift for this proton is 8.4 ppm in the bound state, and 8.6 ppm in the free state. Sketch a hypothetical 1D NMR spectrum for this specific proton for two cases: Either **25%** or **75%** of the protein are bound to the ligand and:

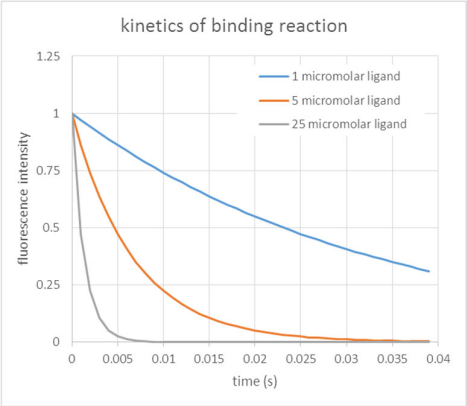
a) and the binding reaction is **much slower** than the NMR timescale (i.e. $k_{ex} << \Delta\omega$)
b) Assume that the binding reaction is **much faster** NMR timescale (i.e. $k_{ex} >> \Delta\omega$)

7. At 25° C, an enzyme exists in two states, an open state (**A**) and a closed state (**B**). In equilibrium, the enzyme is 95% of the time in **A** (open) and 5% of the time in **B** (closed).

a. What is the energy difference $\Delta G°$ between the closed and open states?
b. The closing reaction proceeds with a rate constant $k_{cl} = 50\ s^{-1}$. Assuming a pre-exponential factor of $k_0 = 10^8\ s^{-1}$, what is the barrier height for the closing reaction ($\Delta\Delta G°^{\ddagger}$)?
c. Calculate the closing rate constant for this protein, again at 25° C.

8. You measure the association reaction of a receptor (at 1 nM concentration) with its ligand using a change in tyrosine fluorescence and obtain the data to the right.

Calculate the bimolecular association rate constant.

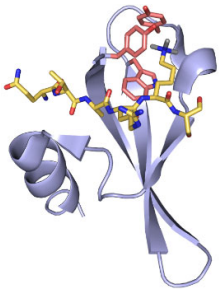


9. Transcription factors have been observed to bind their DNA targets very rapidly, via a process called facilitated diffusion. A transcription factor with a 3D diffusion coefficient $D_{3D} = 8 \cdot 10^{-8}$ $cm^2 s^{-1}$ binds a target DNA site with radius $1 \cdot 10^{-7}$ cm within the human genome (total DNA length 200 cm) in a human cell nucleus (volume $9.05 \cdot 10^{-10}$ cm^3).

a. Describe the concept of facilitated diffusion. Use a sketch.
b. The experimentally determined ideal sliding length for this transcription factor is 7 nm. What is the 1D diffusion coefficient D_{1D} for this transcription factor when sliding on the DNA?

10. Microscopy using visible light has a resolution limit of ~ 300 nm. Describe a method how the position of a protein can still be determined with an accuracy of ~ 1 nm using light microscopy.

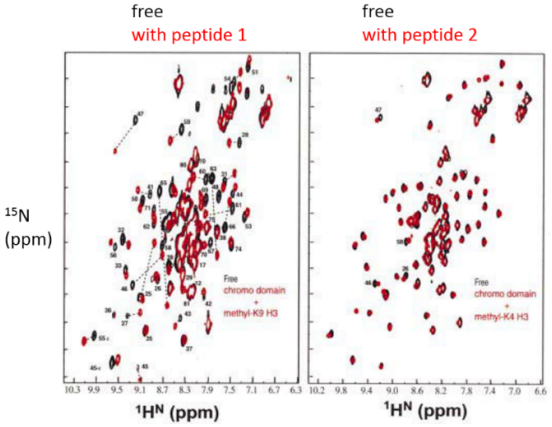
11. Consider this protein structure (PDB code: 1KNE) of the HP1 chromodomain (2002 Science) bound to a histone peptide at 2.4 Å. Shown in blue is the protein backbone (cartoon representation), yellow is the histone peptide (stick representation) and crucial amino acid residues in the protein are given in red (sticks representation).



a. What are the major secondary structure elements in the protein, how many elements can you discriminate?
b. The proteins binds to a tri-methylated lysine residue. What are the amino acids mainly responsible for this interaction?
c. At 2.4 Å resolution, which features of the structure are well defined, and where might still remain some uncertainty?

12. A HSQC spectrum of the chromodomain of HP1 (see question 3) was measured alone (black spectra) and in the presence of three different peptides.

- The first spectrum represents an overlay of the free chromodomain and the chromodomain in the presence of a peptide (**peptide 1**).



- The second spectrum represents the free chromodomain overlaid with the chromodomain in the presence of **peptide 2**.

- When exposed to **peptide 3** (not shown), 30% of HSQC peaks vanish.

- Explain what is observed for all three peptides, and rank the peptides according to their binding affinity to the protein.

13. Why is cryoEM data usually recorded using a slight underfocus? Explain.

14. You determine the folding kinetics of a protein which folds in a two-state process by stopped-flow measurements using a denaturant (guanidinium hydrochloride). In water, the protein folds rapidly, with an observed rate constant of 10^3 s^{-1}. At 3 M GmdCl the relaxation kinetics are slowest with 10 s^{-1}.

- Sketch the dependency between the logarithm of the **observed relaxation rate constant** (λ) and the **denaturant concentration** [D] for this protein.

- You now add a ligand that stabilizes the folded state of the protein (by lowering its free energy), but otherwise does not change the energy landscape. Sketch the resulting (λ) vs [D] plot for the folding kinetics.

- In a different sample, you add a second ligand, that both stabilizes the native state as well as the transition state. Sketch the resulting (λ) vs [D] plot for the folding kinetics.