Solutions to the test exam

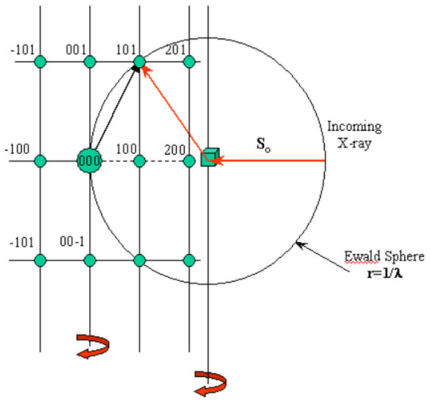
Question 1.

a. Patterson map – a plot of Patterson function, a simplified electron density function with omitted phase information which provides a map of the interatomic vectors (relative atomic positions), the height of its maxima being proportional to the number of electrons of the atoms implied.

b. Heavy atoms (with more electrons) create strong signals on the Patterson map which can be interpreted to get their absolute positions and obtain the phases of the diffracted beams and thus calculate the electron density function and thus solve the crystal structure.

Question 2.

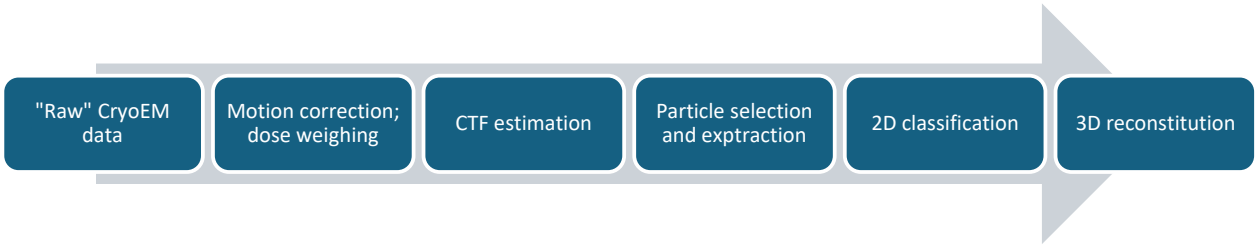
Ewald sphere – a sphere with radius 1/λ in the center of which we imagine the analyzed crystal. If a reciprocal lattice point of the crystal falls on the surface of the sphere the Bragg condition is satisfied, and reflection will occur.



Question 3.

After the CryoEM data is obtained – which are 2D images of the analyzed particles (molecules) – these data should be processed in certain ways before 3D reconstruction.

First the images are motion corrected and passed through dose weighing filters. Next, contrast transfer function is estimated for the images. Then individual particle images are selected and extracted, after which they undergo 2D classification. Finally, from these 2D images – which represent hundreds of projections of the sample from many different angles – the 3D object can be reconstituted via a reverse Fourier transfer.



Question 4.

First, the MSD (mean square displacement) of the molecule from its extracted trajectory is calculated. Then the diffusion coefficient can be calculated from the MSD values plotted as a

function of time. For simple diffusion, there is a linear relationship between MSD and time, and the diffusion coefficient can be found using the following relationship: $D = \frac{MSD}{4t}$ (when measured in 2D).

Question 5.

a) 1 – directed motion

2 – Brownian motion

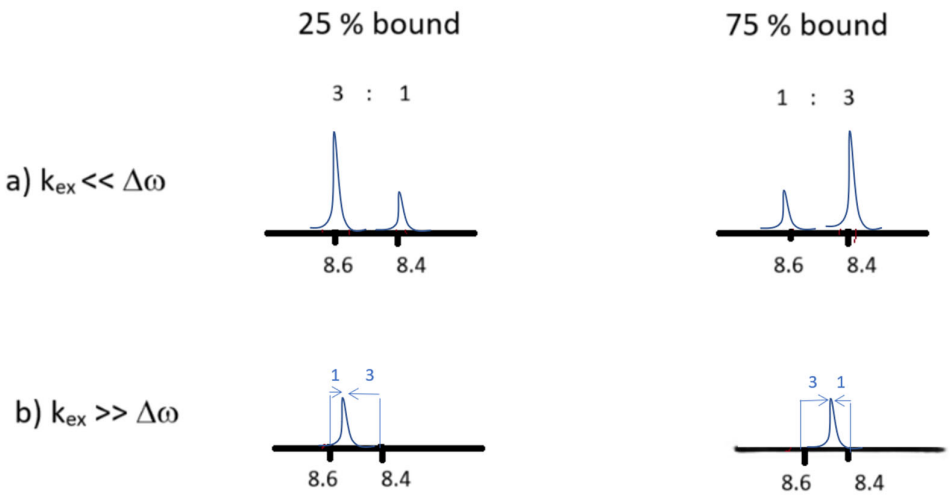
3 – Anomalous diffusion

b) 1 – Myosin: It moves along actin filaments, and its motion is the combination of simple diffusion and active translocation.

2 – GFP, normal Brownian diffusion. MSD is linear.

3 – Acetylcholine receptor: The diffusion is anomalous as the protein is bound to the membrane and corralled by the internal cytoskeleton.

Question 6.



Question 7.

a) $A_{open} \Leftrightarrow B_{closed}$

$\Delta G = -RT\ln([A]/[B]) = -8.314 * 298 * \ln(5/95) = 7295$ J or 7.3 kJ

b) Using the Arrhenius equation:

$k_{cl} = k_0 * e^{-\Delta\Delta G\neq/RT}$

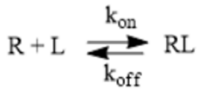
$50 = 10^8 * e^{-\Delta\Delta G^{\neq}/(8.314* 298)}$

$\Delta\Delta G^{\neq} = 35946.2$ J or 35.9 kJ

c) Error in the question: **we should calculate kop**

here: Keq = kcl/kop = 5/95 = 50 s-1/ kop -> kop = 950 s-1

Question 8.



L is in excess, thus the reaction can be assumed to be pseudo-first order. Then, in terms of fluorescence, we can write:

$$F(t) = F_0 \cdot e^{-(k_{on\ app.}+k_{off})t} \quad (1)$$

$$k_{on\ app.} = k_{on}[L] \quad (2)$$

$$k_{obs} = k_{on}[L] + k_{off} \quad (3)$$

We want to find k_{on}. From the plots:

1) At $[L]_0$ = 1 uM:

t = 0.01 s → F = 0.75

t = 0.025 s → F = 0.47

From eq. (1):

$$\frac{F(t_1)}{F(t_2)} = e^{k_{obs}\frac{t_2}{t_1}}$$

k_{obs} *(at $[L]_0$ = 1 uM)* = 0.1869

2) At $[L]_0$ = 5 uM:

t = 0.005 s → F = 0.48

t = 0.015 s → F = 0.11

k_{obs} *(at $[L]_0$ =51 uM)* = 0.4911

3) From eq. 3:

$0.1869 = 1*k_{on} + k_{off}$

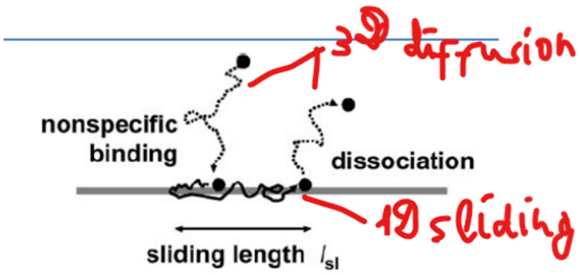
$0.4911 = 5 * k_{on} + k_{off}$

From this system of equations:

$k_{on} = 0.076\ uM^{-1}s^{-1}$.

Question 9.

a) In the case of TF–DNA system: facilitated diffusion is a combination of TF 3D diffusion (random motion) with its 1D sliding along the DNA.



b) l^*_{sl} = 7 nm, $D_{3D} = 8 \cdot 10^{-8}$ cm^{2}s^{-1}, V = $9.05 \cdot 10^{-10}$ cm^3, L = 200 cm

then use

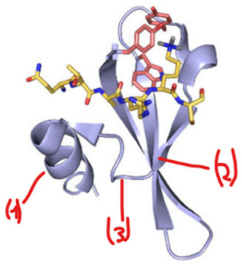
$$l^*_{sl} \sim \sqrt{\frac{D_{1D}}{D_{3D}} \frac{V}{4\pi L}}$$ → $D_{1D} = 1.09 \cdot 10^{-7}$ cm^{2}s^{-1}

Question 10.

To determine the proteins' positions with accuracy of ~1 nm one should be able to detect fluorescently labelled proteins at single molecule level (the signals from individual molecules should not overlap). A sparce subset of fluorophores should be seen in the microscope at a time. Photoswitchable fluorophores can be used for this purpose. Then the observed fluorophore PSF (point spread function) can be fitted with a 2D-Gaussian function – the centre of the PSF can be determined – and the position of the protein reconstructed with high precision. Importantly, to get high resolution enough photons should be collected from each fluorophore.

Question 11.

a) (1) α-helix; (2) anti-parallel β-sheet; (3) disordered region.



b) 2 Tyr residues; 1 Trp residue.

c) Protein backbone, tertiary/secondary structure are resolved well.

Some side chains of amino acids and flexible regions may remain some uncertainty.

Question 12.

^{1}H ^{15}N HSQC is a 2D NMR experiment that correlates proton chemical shifts with nitrogen-15 shifts. It allows for the assignment of amide resonances to specific residues of the protein.

For peptide 1: the HSQC spectrum almost didn't change in comparison to the spectrum of the protein alone. It means that the peptide doesn't interact with the protein.

For peptide 2: certain signals shifted significantly – peptide binds to the protein.

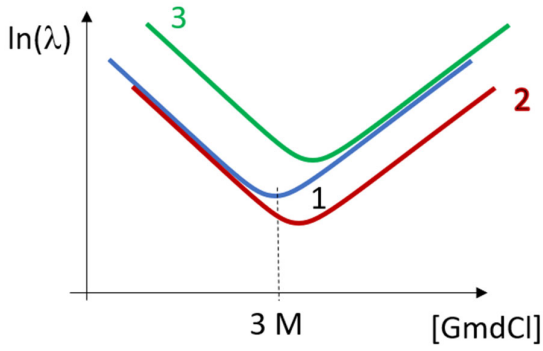
For peptide 3: disappearance of 30 % of the peaks means that binding–unbinding equilibrium is fast, with an exchange rate constant in the intermediate regime. The peptide binds to the protein but the binding is not strong (k_{off} is rather high).

Thus: peptide 2 > peptide 3 >peptide 1.

Question 13.

Slight underfocus in CryoEM is used to improve image contrast. By applying underfocus, the phase if the scattered electrons from the sample shifts, this causes interference with the unscattered beam, creating visible contrast (termed phase contrast).

Question 14.



Curve 1: [D] = 0 => lg(λ) = 3; [D] = 3 => lg(λ) = 1

Curve 2: folded state stabilized – ku is getting slower, so overall curve shifts to the right

Curve 3: folded and transition state stabilized – the folding kinetics (kf) is faster; curve shifts upwards / to the right.