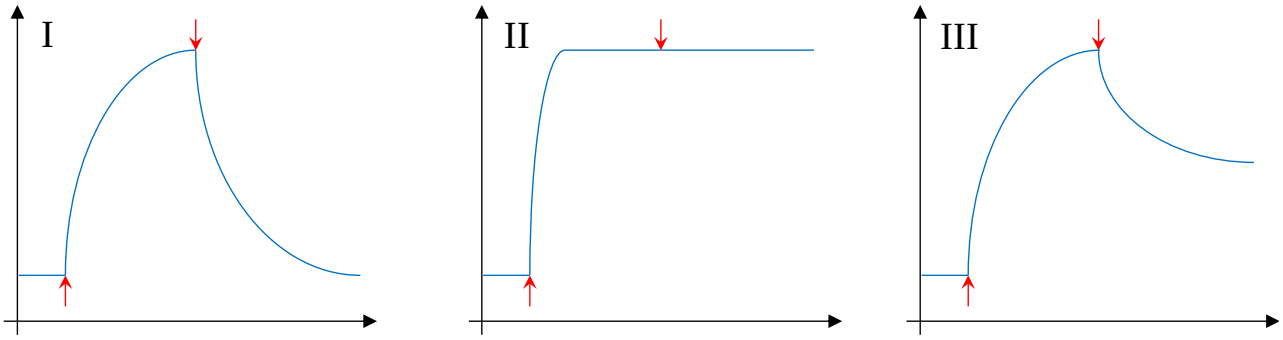
Question 1

The figures show different types of SPR sensorgrams (angle of reflectivity minimum against time) during binding of proteins to receptors on the surface. The arrow ↑ shows when the protein is introduced and the arrow ↓ shows when the liquid chamber is rinsed.



A (3p)

Which sensorgram shows an irreversible interaction?

Sensorgram II since upon rinsing there is no dissociation ($k_{off} = 0$).

B (3p)

In reality one often encounters curves like sensorgram III when one expects to see a curve like sensorgram I. Give one probable explanation why the dissociation in sensorgram III is incomplete?

The most likely explanation is that there is also some other interaction happening between the introduced protein and the surface and this binding is irreversible. The protein may be adsorbing to the underlying surface (highly likely). The protein may bind to the receptor in an alternate configuration which is irreversible (less common).

Note: One suggestion was that the released proteins would accumulate near the surface and rebind to some extent. This is very unlikely in practice unless the liquid region near the surface is extremely thin, but not impossible. I gave some points for this answer.

C (4p)

A set of experiments were performed with different liquid flow rate. Upon increasing the flow rate, the association part of the sensorgram went from looking like I to looking more like II. What does this say about the binding kinetics? Would it be possible to use the Langmuir model to determine rate constants from data?

If increasing the flow leads to increased binding the kinetics are at least to some extent influenced by mass transport. The Langmuir model is then not applicable since it assumes the concentration near the surface remains constant. (The association rate constant k_{on} is not changing, it is the model which is wrong.) If it should anyway be used it is more likely to be accurate for the case of faster binding.

Question 2

A 20 mM NaCl solution in water at room temperature contains lipid vesicles with a diameter of 100 nm consisting of the neutral lipid POPC and the lipid PS, which carries one negative charge. It can be assumed that the PS lipids orient themselves in the outer leaflet so that the head group faces the exterior.

A (4p)

A homogenous field of 300 V/m is applied, which makes the vesicles move with a velocity of 1.5 cm/h. Estimate the zeta potential by the Smoluchowsky model:

$$\mu = \frac{v}{E} = \frac{\varepsilon\varepsilon_0\zeta}{\eta}$$

Also, explain what implicit assumption you have made by using this model and why this seems reasonable in this case!

Using Smoluchokvski directly gives ζ = -19.6 mV. The major assumption is that the Debye length is smaller than the particle size. This can also be formulated as the planar surface approximation for describing the diffuse layer. It should be reasonable at this ionic strength (and is shown to be in the next question).

B (6p)

Determine the number of PS lipids on each vesicle. You may assume that there are no adsorbed ions and that the surface potential is equal to the zeta potential. The following relations may be used:

$$\kappa = \left[\frac{2C_0e^2}{\varepsilon\varepsilon_0 k_B T}\right]^{1/2}$$

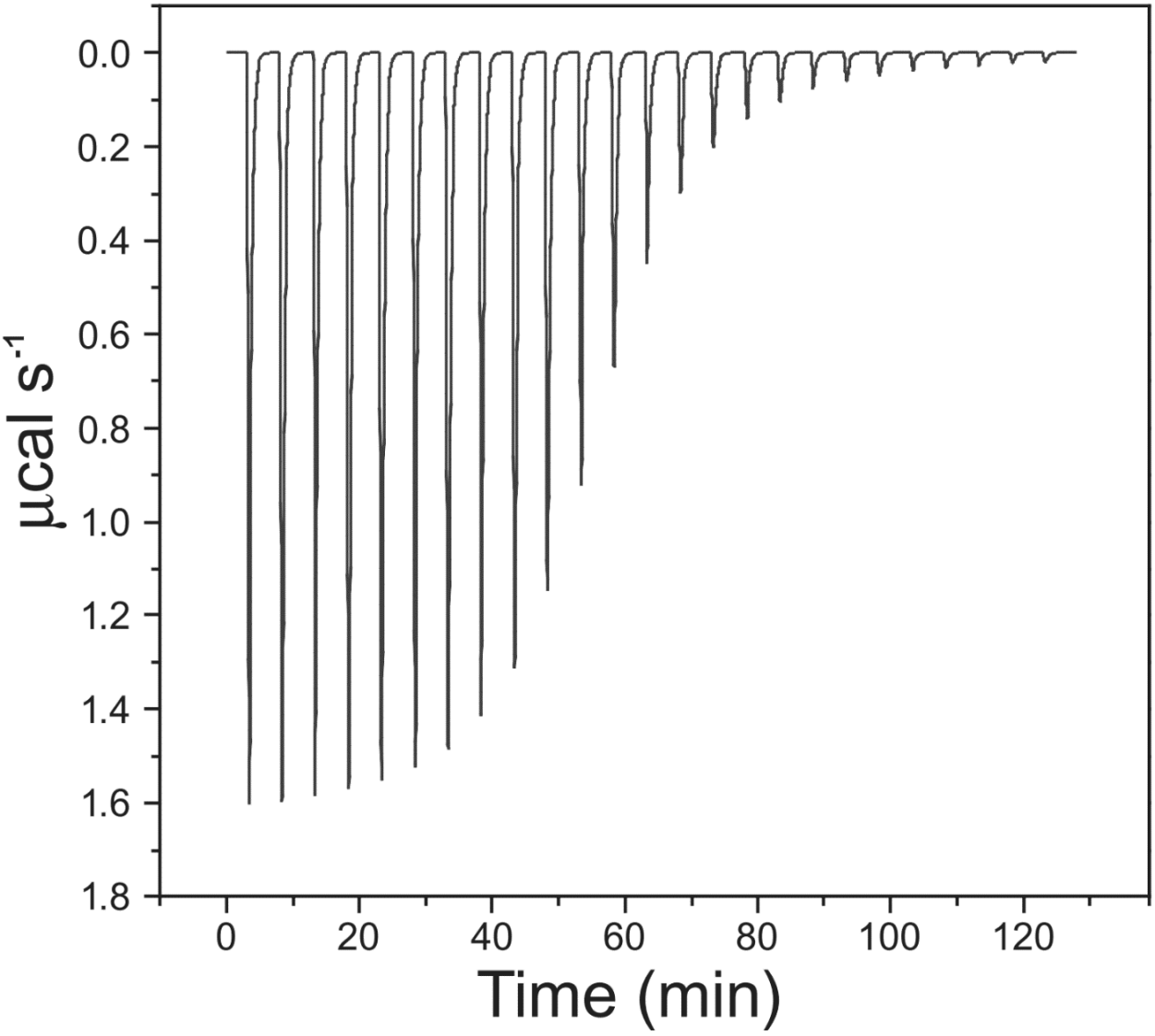
$$\sigma_0 = \varepsilon\varepsilon_0\psi_0\kappa$$

Using the formula for Debye length should give κ^{-1} = 2.16 nm. The potential is the simplified Grahame can be replaced with zeta potential. This gives σ = -0.0064 C/m^2. This translates into 0.04 PS lipids (one elementary charge) per nm^2. The area of the vesicle is $4\pi R^2$ = 3.14×10^4 nm^2. This gives 1260 PS lipids on each vesicle.

Question 3

A (6p)

The data shows the first injections in an isothermal titration calorimetry experiment (1 cal = 4.184 J) upon injections of 10 µL ligand solution at 3 mM concentration. Use the data to estimate the reaction enthalpy per mole. (Since it is hard to see the data in detail a rough estimate is sufficient as long as you explain how you do the analysis.)



Since the first two or maybe three injections are identical, it means all ligands introduced become bind to their targets. We should thus estimate the integrated heat from the first injection. The area can be approximated with a triangle of base 2 min and height 1.6 µcal, which gives 4×10^{-4} J. The injection contains $10\times10^{-6}\times3\times10^{-3} = 3\times10^{-8}$ mol. The binding enthalpy is then ~13 kJ/mol.

Note: For full points you must motivate why you use the first injection spike.

B (4p)

How should one design and analyse additional ITC experiments in order to estimate the standard entropy of binding? Hint, remember van't Hoff:

$$\log(K) = \frac{\Delta S^\circ}{k_B N_A} - \frac{\Delta H^\circ}{k_B N_A} \times \frac{1}{T}$$

The temperature is fixed in each titration. Perform several ITC experiments at different temperatures. Get the affinity constant for each case from the change in binding with each injection. Plot logarithm of equilibrium constant against 1/*T* to get entropy from the intersect with y-axis.

Question 4 (5p)

Describe how Förster resonance energy transfer can be used to investigate protein denaturation (unfolding). How does it work and what equipment is needed?

Two fluorophores are conjugated to the protein. The emission of one overlaps with the absorption of the other so that FRET is possible. In the folded state the protein is more compact so the distance between the fluorophores is short, giving a detectable FRET signal (acceptor emission). Upon unfolding the distance between the fluorophores will be larger on average so the FRET yield goes down. This can be detected by any kind of fluorescence setup, microscope or spectrometer. (One could also monitor changes in donor absorption.)

Question 5 (5p)

Describe the principle of operation (the signal transduction) in common electrochemical glucose sensors. Explain which molecular species are involved.

A redox enzyme immobilized on the surface of an electrode catalyzes the oxidation of glucose. A mediator then takes an electron from the enzyme so that it can continue to work. The mediator is then oxidized again at the electrode. The current generated from this process gives the glucose concentration in the sample. The mediator is mobile and makes electron transfer possible since the enzyme cannot be immobilized directly on the metal surface with preserved activity.