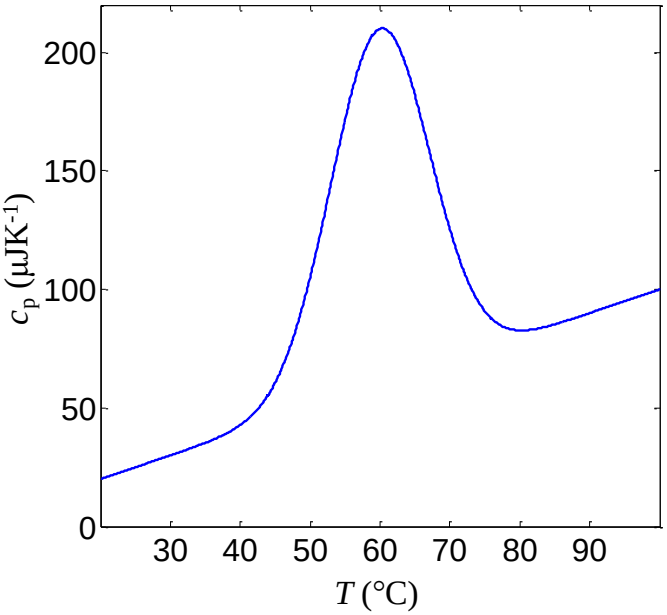
2015

A calorimeter slowly sweeps the temperature of a 1 mL solution containing a 80 kDa protein at 1 g/L which denatures reversibly. The data is shown in the figure (reference chamber subtracted).



The definition of heat capacity at constant pressure is:

$$c_p = \left.\frac{\partial H}{\partial T}\right|_P$$

(A) Estimate the enthalpy of denaturation per mole.

There are 1.25×10^{-8} moles in the chamber. The area under the peak (subtracting the baseline) is approximately 3 mJ. The denaturation enthalpy is then 240 kJ/mol. (Large deviations are fine here if you explain how you did the estimate.)

(B) Estimate the probability that a protein molecule of this type is denatured at 50 °C. (The estimate can be very rough but you must explain how you got the value.)

The scanning is slow so one can assume the system is in equilibrium at each temperature. The area under the peak represents the progress of denaturation. At 50 °C we have only a small fraction of the total integral (baseline subtracted), about 15%. This is the probability of denatured state.

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Commercial surface plasmon resonance instruments, like those from Biacore (GE Healthcare), contain thin microfluidic channels that efficiently deliver molecules to the sensor surface and experiments are performed under steady liquid flow. Why does one go through so much trouble

(time, money…) to create this kind of microfluidic systems? (Excitation of surface plasmons is clearly possible even without microfluidics.) Give two reasons!

The two reasons are reduced material consumption and preventing mass transport from influencing the binding kinetics for more simple and accurate determinations of rate constants.

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(A) The Langmuir model of binding kinetics is given by:

$$\Gamma(t) = \frac{k_{on} C_0 \Gamma_{max}}{k_{on} C_0 + k_{off}} \left[1 - \exp\left(-\left[k_{on} C_0 + k_{off}\right] t\right)\right]$$

A target with concentration 1 nM in solution starts binding to its receptor on a surface with k_{on} = 4×10^2 $s^{-1}mol^{-1}m^3$ and k_{off} = 2×10^{-4} s^{-1}. According to the Langmuir model, what fraction of the receptors have a target captured after 10 min?

The fraction of receptors with a target captured is simply Γ/Γ_{max}. You can insert the values into the equation as long as you convert the units accurately. For instance, C_0 should be in mol/m^3 if you use the given values of k_{on} and k_{off} as is. The time should be inserted in seconds. This gives Γ/Γ_{max} = 0.2015…

(B)

What is the probability that a receptor have a target bound to it at any point in time after equilibrium has been established?

At equilibrium $t \to \infty$ per definition so the second factor becomes unity, which gives the equilibrium condition. The sought probability is again simply Γ/Γ_{max}. $K_D = k_{off}/k_{on} = 5\times10^{-10}$ M and C_0 = 1 nM gives Γ/Γ_{max} = 2/3.

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Formulate schematically the basics of electron transfer on redox enzyme electrodes (provide a sketch). Explain the advantage of mediated electron transfer in electrochemical biosensors. Formulate principle of operation of glucose biosensor and provide an example of new technical solutions. (For instance, explain what SPCEs technology is.)

Immobilized enzyme catalyses the oxidation of glucose. The enzyme is regenerated by electron transfer from the mediator in solution, like ferrocyanide. The mediator can then easily react at the electrode to form a steady current. The enzyme does not have to be just at the surface. SPCEs are screen printed carbon electrodes, cheap electrochemical detection chips based on carbon.

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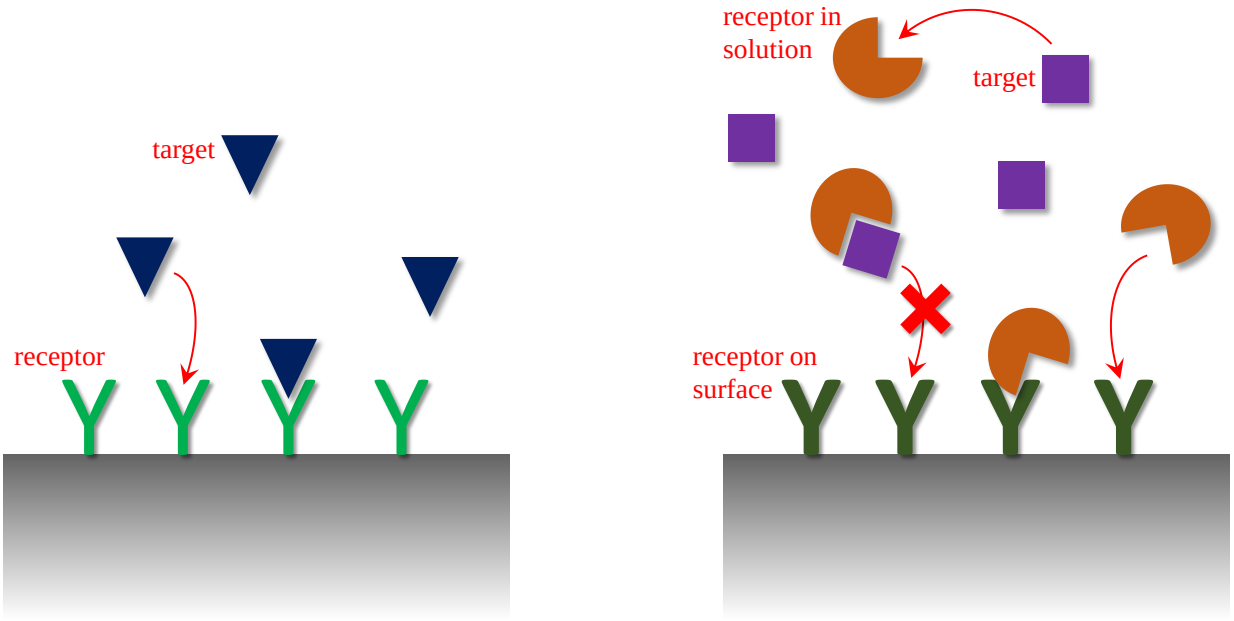
In a so-called *surface competitive assay* there are three molecules: The target, a receptor in solution and a second receptor on the surface. The receptor in solution binds to the target, while the receptor on the surface can capture the receptor in solution. The method can be used to indirectly detect binding of molecules such as drug candidates to their receptor. This occurs

when target binding interferes with the other interaction and leads to a reduced amount of binding to the surface. The assay can be used together with methods such as surface plasmon resonance or quartz crystal microbalance. Discuss advantages and disadvantages with this approach (right) in comparison with the "ordinary" approach to study biomolecular interaction, where there is just one receptor type on the surface and the target in solution (left)!



The advantages are that binding occurs in solution and that smaller molecules can be analysed since it is the big receptor that binds (or not) to the surface. The small molecule might not give a detectable signal. The main disadvantage is that there is no real-time monitoring of the interaction. Other potential disadvantages are that more material is needed and that it can be hard to find a suitable receptor pair that bind in the right way so that the target really interferes with their reaction.

Note: Almost nobody came up with the answer that the assay is useable for small molecules (typically drugs) that cannot be detected otherwise. (Maybe it should be emphasized more in the course that there are limits concerning detection with surface-based methods and that small molecules are hard to probe.)

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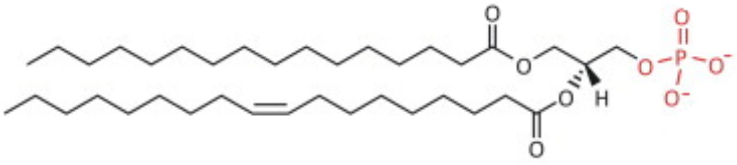
Phospholipids often have charged head groups which makes the lipid membrane a charged interface. One negatively charged lipid is PA (abbreviated) with structure:



The inverse Debye length in a monovalent salt solution is:

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$$\kappa = \left[\frac{2C_0 e^2}{\varepsilon \varepsilon_0 k_B T} \right]^{1/2}$$

The Grahame equation can then be simplified to:

$$\sigma_S \approx \varepsilon \varepsilon_0 \psi_S \kappa$$

(A) Lipid vesicles containing PA are present in 10 mM KCl (water, room temperature) at high pH. A zeta potential of 30 mV was calculated from their electrophoretic mobility. Use standard diffuse layer theory to estimate the packing density of PA (the number of PA per area) in the membrane! Important: To get all points you must explain two important assumptions you do in your estimation.

First calculate Debye length (3.1 nm) in this electrolyte with given formula (remember that the concentration must be inserted in number of molecules and not moles when using the formula as specified). Then use the simplified Grahame equation to get the charge density on the surface (in Coulombs per area). By dividing with two elementary charges you get the number of lipids per area since each carry two negative charges (~0.02 nm^{-2}). The most critical assumptions here is that by using these formula we assume a planar surface (which is not true for a vesicle) and that by using the zeta potential we assume the shear plane is just at the lipid heads. In reality there could be adsorbed or stuck ions which makes the conversion to number of lipids wrong since the Grahame equation is based on only a diffuse layer.

Notes: The zeta potential is given as an absolute number here. Of course, it is in principle a negative potential since the lipids make the vesicles negatively charged. This should be obvious from the design of this question but I guess I should have put a minus sign there to make it clear. Sorry about that if it confused anyone! As another point it is interesting that almost nobody got that the model is for a planar surface while the vesicle is spherical.

(B) Lipid bilayers can be used to cover the interior walls of nanochannels. The previously mentioned lipid vesicles (in 10 mM KCl) are used for this purpose in a channel where the walls are natively uncharged. After supported membrane formation, what will happen to the content inside the channel when a homogenous electric field is applied along it? Explain qualitatively!

The PA lipids will move to the anode along the walls since the membrane is fluid. There will also be an electroosmoic flow in the channel towards the cathode due to the net accumulation of K^+ in solution.

Notes: A lot of people wrote about “particles” moving inside the channel but without explaining what these particles are. The question relates only to the specified content: Lipid bilayer and ions. Also, it appears that almost nobody noticed that it is specified that there is a supported bilayer on the walls (so one can expect mobile lipids). I am not sure why…