2013

Fill out the table below correctly by adding ”yes” or ”no” in each empty cell.

(not empty)	Nanoparticle Plasmon	Surface Plasmon
The plasmon couples only to a few defined resonance frequencies of light.		
The plasmon can be explained by Maxwell’s equations and classical physics.		
The plasmon propagates and carries a momentum.		
The plasmon energy is (at least to some extent) absorbed by the metal.		

Nanoparticle plasmon: Yes, yes, no, yes. Surface plasmon: No, yes, yes, yes.

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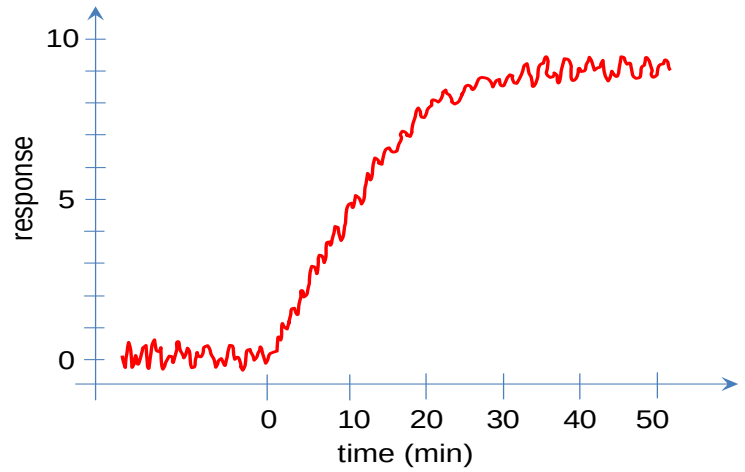
(A) An infinite stagnant water solution at room temperature contains proteins at a concentration of 1 nM that are approximately spherical with a molecular weight of 10 kD. The proteins bind to a surface. Assume the first 10 min of the binding process represent diffusion controlled binding. How many molecules have landed on 1 μm^2 after this time? Assume the density of a protein is around 1.4 g/cm^3.

Calculate the volume of the proteins by dividing the molecular weight with volume and assume a spherical shape (R = 1.41 nm). Calculate a diffusion constant for the proteins with Einstein-Stokes ($D = 1.53\times10^{-10}$ m^{2}s^{-1}). Use Ilkovic equation to get surface coverage. Note that the concentration unit used in the Ilkovic equation will define the unit for surface coverage so converting the molar concentration into number of molecules per volume is suitable. C_0 = 1 nM = $10^{-9}\times10^3\times N_A$ molecules per m^3. For t = 10 min this should give Γ = 206 molecules on 1 μm^2.

(B) The binding corresponding to the system in (A) is monitored by a refractometric plasmonic biosensor as shown below. The sensor response is proportional to the surface coverage Γ. What is Γ at equilibrium (e.g. in ng/cm^2)?



Using the information from the previous question, we have Γ after 10 min. Equilibrium corresponds to the final value which is about two times higher. Multiply with molecular weight to get mass coverage (0.68 ngcm^{-2}).

(C) The protein in (A) and (B) is captured by receptors at the surface that have a dissociation constant K_D = 2 nM. What is the maximum possible surface coverage (C_0 very high) if one receptor can bind one protein?

Langmuir equilibrium can be used directly. Since $K_D = 2C_0$, Γ_{max} must be 3 times higher than the value of Γ from (B). (Even if there was diffusion limited binding initially, the equilibrium still follows the Langmuir condition.) So Γ_{max} = 2.04 ngcm^{-2}.

(D) Estimate the detection limit in terms of concentration in solution (under the assumption that equilibrium can be established within a reasonable time for any concentration).

Noise level is ~10% of the signal. Assuming the response is proportional to Γ the minimal surface coverage that can be detected is ~0.07 ngcm^{-2}. Using the Langmuir equilibrium condition again with Γ_{max} from (C) gives C_0 = 69 pM at the detection limit.

(E) How small (in terms of area) must you make the sensor to make it possible to detect the binding (or release) of the proteins one after the other? Assume that the noise level remains unchanged even if the sensor is made smaller.

One molecule should correspond to a surface coverage representing the detection limit (0.07 ngcm^{-2}). The mass is $10^4/N_A$. The area is then 0.024 μm^2.

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A biomolecule switches between two states. You can readily observe the fraction of molecules occupying each state. How would you design an experiment that yields information for determining the standard enthalpy $\Delta H°$ and entropy $\Delta S°$ for the transition?

Change the temperature in the system and record the fraction of molecules in each state for different T. Use the relation between equilibrium constant and free energy. The best way to

determine the standard enthalpy and entropy is through an Arrhenius plot. Note that calorimetry is NOT suitable here since it measures phase transitions, enzymatic reactions and interactions between biomolecules.

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(A) Describe briefly the main physical mechanisms behind the various techniques listed below. In addition, mention for each technique one advantage and one disadvantage in comparison with at least one of the other techniques.

1 Atomic Force Microscopy (AFM)

2 Optical tweezers (or laser tweezers)

3 Total Internal Reflection Fluorescence microscopy (TIRFM)

4 Quartz crystal microbalance with dissipation monitoring (QCM-D)

AFM: A very sharp tip attached to a cantilever is in contact with the surface. Light is reflected on the cantilever. When it deflects due to surface morphology this is detected in the position of the reflected light beam. A piezoelectric stage scans the surface with the tip. Can be used in liquid.

Optical tweezers are based on the gradient and scattering forces in a focused light beam. Both these forces make a particle trapped in the focal spot. Particles can then be moved around.

TIRFM: TIR means total internal reflection, only an evanescent field of light is present at the surface. Adding fluorescence means that this evanescent field of light is used to excite fluorophores only very close to the surface. Adding microscopy means that the fluorophores at the surface are imaged in a microscope.

QCM-D is a variant of QCM, which is based on a piezoelectric quartz crystal. By having metal electrodes on each side the crystal can be mechanically oscillating by applying an AC voltage. At certain resonance frequencies, determined by crystal thickness, the amplitude is enhanced. This resonance frequency changes when something attaches to the crystal. With dissipation monitoring, also the lifetime or damping rate of the oscillation is measured by disconnecting the voltage after excitation.

Some examples of pros and cons: AFM has extremely good resolution but images relatively slowly and is difficult to use on soft matter. Optical tweezers is the only technique that can move objects around in 3D, but the focused light beam causes a lot of heating. TIRFM gives fast imaging but the resolution is limited compared to AFM. QCM-D can be used to quantify the amount of material on the surface, unlike the other techniques, but gives no imaging.

(B) Integrins are transmembrane proteins that mediate the interactions between a cell and its surroundings and are, for example, important for attachment of cells to an artificial implant. It is therefore of interest to study these proteins and their interactions with solid surfaces. Explain what type of information you could get from each experimental technique in (A). If a technique is likely impossible to use for the task, explain why.

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Some examples: AFM can probably give information about the shape of an integrin adsorbed on the surface because of its high resolution. It could tell something about the structure. Optical tweezers are likely not suitable since it is hard to entrap single proteins and also because they could denature due to high temperature. In any case it does not give any information about the protein on the surface. TIRFM can be used to image the integrins but requires that they are labelled. It could even be used on live cells interacting with the surface. QCM-D could be used to give some estimate of the amount of integrin adsorbed on different surfaces and maybe information about its rigidity.