Biotechnical Physics TIF040 exam questions

2014

(A) Explain briefly the detection principle in a surface-based biosensor or "surface sensitive" technique! Give at least two examples of such techniques (name is sufficient).

Any type of molecular binding to the surface generates a detectable signal (optical, electrical etc.) such as in a surface plasmon resonance or quartz crystal microbalance instrument.

(B) Explain one major advantage and one major disadvantage that are generic for surface-based biosensors.

Major advantages are that any type of biomolecular interaction can be studied and operation in real-time. Major disadvantage is nonspecific binding which gives false positive signals when the sensor is used to detect analytes from complex samples. The fact that one molecule must be immobilized on the surface can also be considered a generic disadvantage.

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(A) Surface Plasmon Resonance and Isothermal Titration Calorimetry are two important tools for studying biomolecular interactions in drug development. Explain briefly the physical principles behind these methods.

In SPR, surface plasmons, i.e. electromagnetic waves that propagate along an interface, are excited with light. The resonance wavelength or (angle) changes when the refractive index changes at the surface, as it does when molecules bind. ITC measures the heat needed to maintain the same temperature of a reaction chamber (containing the interacting molecules) as a reference chamber (same solvent but no molecules).

(B) Give at least two advantages for each method when compared to the other, i.e. explain what benefits one technique offers that the other cannot provide.

SPR monitors binding kinetics (k_{on} and k_{off}) and requires much smaller amounts of the molecules. Multiplexing is also possible by SPR imaging. ITC provides a direct measure of reaction enthalpy and by varying temperature the standard free energy can be determined (relates equilibrium constant with Gibbs). Also, in ITC the molecules can interact freely in solution (instead of on a surface).

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Dynamic Light Scattering and Fluorescence Correlation Spectroscopy are both based on analyzing an autocorrelation function. However, the fluctuations in the intensity that generates this function arise from completely different mechanisms. For each of the two methods, give a brief explanation of why the intensity data exhibits fluctuations related to diffusivity!

DLS is based on coherent light scattering: The objects scatter light and the waves can interfere with each other in local maxima or minima. When the objects have moved a distance comparable to the wavelength of the probing light the intensity will fluctuate strongly. Fluorescence light is incoherent so in FCS, the fluctuations are instead based on molecules diffusing in and out of a

very small excitation volume. The concentration needs to be small enough so that there are only a few molecules that can emit light at a given time.

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In an experiment one wants to study single viruses (~50 nm in diameter) that adsorb on a planar surface using a light beam focused to a 1 μm^2 probing area. The surface and the solution containing the viruses (water at room temperature) can be considered as very large. There are 10^{10} viruses per mL in the solution, which is introduced instantly. How long time should one expect that it will take before one virus has landed on the illuminated spot?

The Ilkovic model for diffusion limited binding can be used. The virus have a diffusion constant $D = 8.64\times10^{-12}$ m^2s^{-1} (Einstein-Stokes). If there should be one virus on 1 μm2, we have $\Gamma = 10^{12}$ m^{-2} (coverage in terms of number of entities). Further, the bulk concentration is $C = 10^{16}$ m^{-3} (again in number of entities). Using these values we can solve Ilkovic directly and get t = 15 min.

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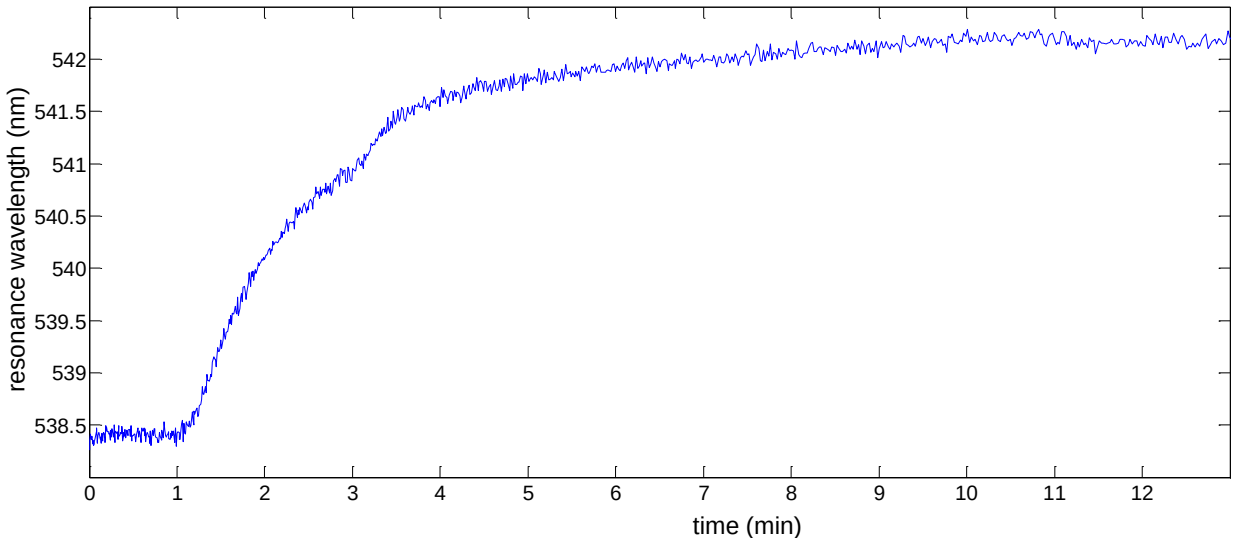
The binding curve below comes from avidin binding to biotin on gold nanoparticles as measured by extinction spectroscopy. (It was generated by one of the groups during the lab exercise.) Estimate the sensor detection limit in terms of surface coverage (avidin has M = 60 kD and is approximately spherical with 5 nm diameter).



Avidin binds irreversibly to biotin, so when equilibrium is reached one can expect to have a completely filled surface, i.e. a monolayer of proteins such that no more can fit. This represents an areal coverage of ~50%. If we do the calculation on, for instance, 1 cm^2 we have (at saturation) a number of molecules equal to $0.5/[\pi[2.5\times10^{-7}]^2] = 2.55\times10^{12}$ molecules. This corresponds to a mass of 254 ng (multiply with $60\times10^3/N_A$). Since one can assume that the plasmonic signal is proportional to surface coverage, the detection limit is acquired by dividing this value with the signal to noise, which is about 20 (around 3.7 nm signal, almost 0.2 nm noise level). The detection limit is then approximately 13 $ngcm^{-2}$.

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Note: The important thing here is to understand how to do the estimate. If you use a slightly different value for the noise (it is a bit hard to see exactly) you still get full points.

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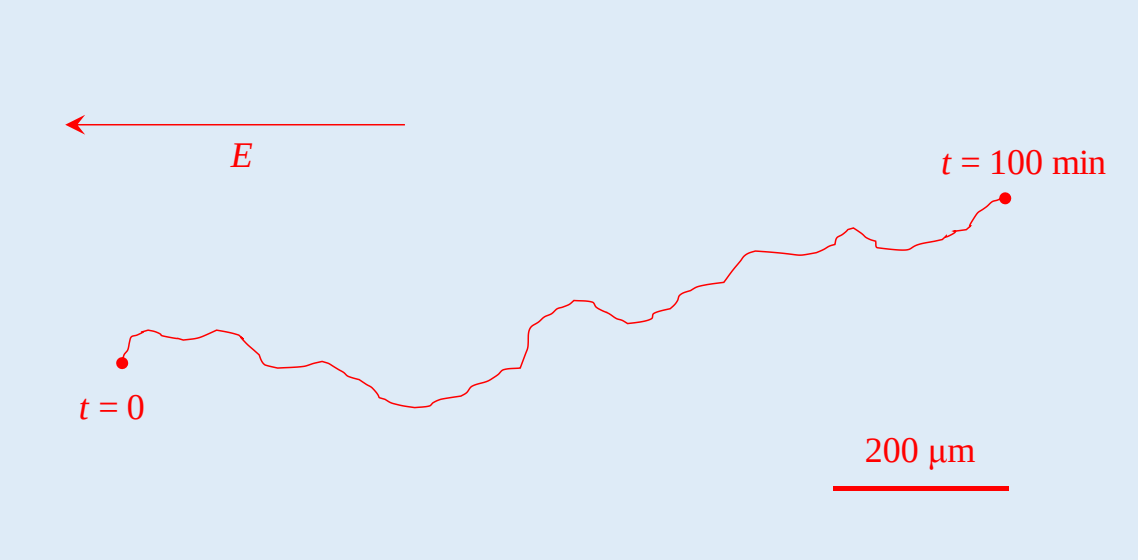
(A) Gold nanoparticles charged by carboxylic acid ($-COO^-$) groups undergo electrophoresis in 10 mM $CaCl_2$ at room temperature. The motion path (can be considered as 2D) of individual particles is detected by light scattering. A representative path is drawn in the figure below. The field strength is E = 4.0 V/m. Assuming there are no adsorbed ions, estimate the surface coverage of (deprotonated) carboxylic acid groups on the particle.



The particle has moved about 1 mm along the direction of the field (the movement perpendicular to the electrophoresis path should not be included) during 100 min. The mobility is per definition $\mu = 10^{-3}/[100\times60]/4 = 4.17\times10^{-8}$ $m^2V^{-1}s^{-1}$. Schmoluchowski approximation can be used to get a zeta potential of ζ = 59 mV. This is a low potential so to get an approximation we can assume only a diffuse layer (no adsorbed ions) and that ζ is also the surface potential. The general Grahame equation is then used to get the surface charge density. (Note that there is no need to explicitly calculate the Debye length and that concentration must be inserted as number of molecules per m^3.) This should give 0.37 nm^{-2} after converting the resulting σ (comes out in Cm^{-2}) by dividing with the elementary charge (one charge on each chemical group).

(B) Estimate the size of the particle and its total charge.

The particle has diffused approximately 200 µm perpendicular to the direction of the field during 100 min. In this dimension there is no electrophoretic mobility so it can be assumed to represent pure Brownian motion in one dimension. The diffusion constant can then be determined to $D = 3.33\times10^{-12}$ m^2s^{-1} and Einstein-Stokes gives R = 65 nm. (So the Schmoluchowski model is valid.) The total charge is surface area multiplied by σ from before. This should give 284 pC.

(C) The surface of the particles also contains a receptor that can capture a target in the solution. How could data from traces such as the one in the figure be used to detect this binding? (Not plasmonic sensing, i.e. measuring the spectrum of the scattered light.)

If the particle captures a molecule, it will effectively grow in size and should diffuse slower.