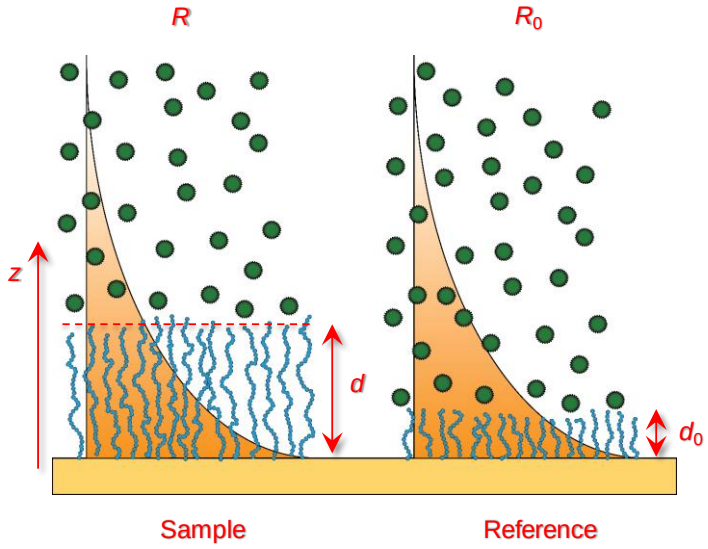
Home assignment 2018

A few years ago a research group in Switzerland recently came up with a new kind of application for SPR, namely to determine the thickness of thin hydrated films by injecting "non-interacting probes". The idea is quite creative: They use the refractive index change by molecules in the liquid bulk instead of those caused by molecules bound to the surface. As long as the "probe" molecule does not adsorb to the surface the method can be used to obtain a height which represents the thickness d of the film on the surface below which the probe cannot penetrate. The response R from the probes is compared with the response R_0 when the probes are injected in a reference channel which contains an inert coating with a known thickness d_0.



(A)

You have already encountered the standard way to quantify the SPR response R by an exponentially decaying field as a mathematical weight to the refractive index changes:

$$R = \frac{S_0}{\delta}\int_0^\infty \Delta n \times \exp\left(-\frac{z}{\delta}\right) \mathrm{d}z$$

Use this formula to derive an expression for the thickness d determined by the non-interacting probes method. Assume the reference surface has the same bulk sensitivity S_0 and decay length δ. (The refractive index change from the injected probes is also the same for the reference surface.)

If the refractive index change occurs above the film ($z > d$) the response becomes:

$$R = \frac{S_0}{\delta}\Delta n \int_d^\infty \exp\left(-\frac{z}{\delta}\right) \mathrm{d}z = \frac{S_0}{\delta}\Delta n \left\{-\delta \exp\left(-\frac{z}{\delta}\right)\right\}_{z=d}^{z=\infty} = S_0 \Delta n \exp\left(-\frac{d}{\delta}\right)$$

The same thing for the reference channel:

$$R_0 = S_0 \Delta n \exp\left(-\frac{d_0}{\delta}\right)$$

Now take the ratio:

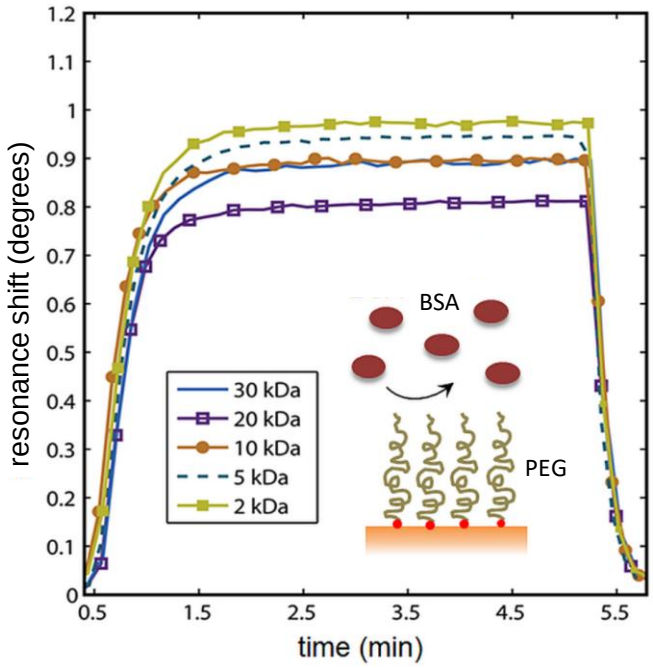
$$\frac{R}{R_0} = \exp\left(-\frac{d-d_0}{\delta}\right)$$

Thus the thickness is:

$$d = \delta \log\left(\frac{R_0}{R}\right) + d_0$$

(B)

The figure shows the SPR response when bovine serum albumin (BSA) is injected to surfaces coated with poly(ethylene glycol) (PEG) of different molecular weight. The proteins cannot enter the polymer film. The decay length is δ = 184 nm. Calculate the thickness of the 20 kD PEG layer by using a known thickness of 6 nm for the 2 kD PEG.



The response from BSA injected over 2 kD PEG is 0.98 and 0.81 for 20 kD PEG. Using the formula from before gives d = 184 × log[0.98/0.81] + 6 = 41 nm.

Home assignments 2017

1

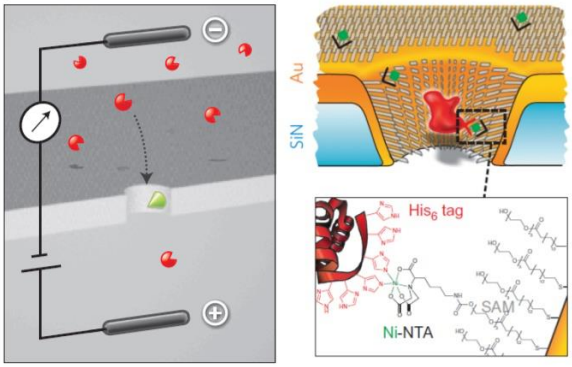
Virus capsids are shells of self-assembled proteins. Assume capsids that are approximately spherical with radius 30 nm are adsorbing irreversibly to a large planar surface from a large stagnant liquid. Each capsid consists of 120 proteins with molecular weight 40 kg/mol. The concentration of capsids is 10 µg/mL. How long time does it take to fill up the surface so that no more binding occurs?

Hint: When spheres bind to a surface in a random manner, the maximum areal coverage is 54.7%.

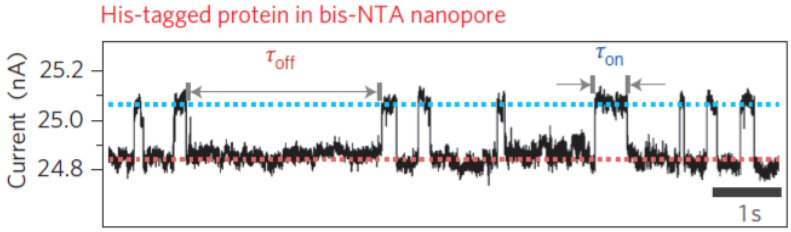
Assume water at room temperature. Get diffusion constant from Einstein-Stokes: $D = k_B T/[6\pi\eta R) = 7.3\times10^{-12}$ m^2/s. Each capsid has a weight of 4800 kg/mol, so the molar concentration is $C_0 = 2.1\times10^{-6}$ mol/m^3. The cross-section area of the virus is πR^2 and 0.547 divided by this number is equal to Γ when the surface is filled (also divide with Avogadro's number), which gives $\Gamma = 3.2\times10^{-10}$ mol/m^2. Now use Ilkovic to solve for the time $t = [\Gamma/[2C_0]]^2\times\pi/D = 42$ min.

2

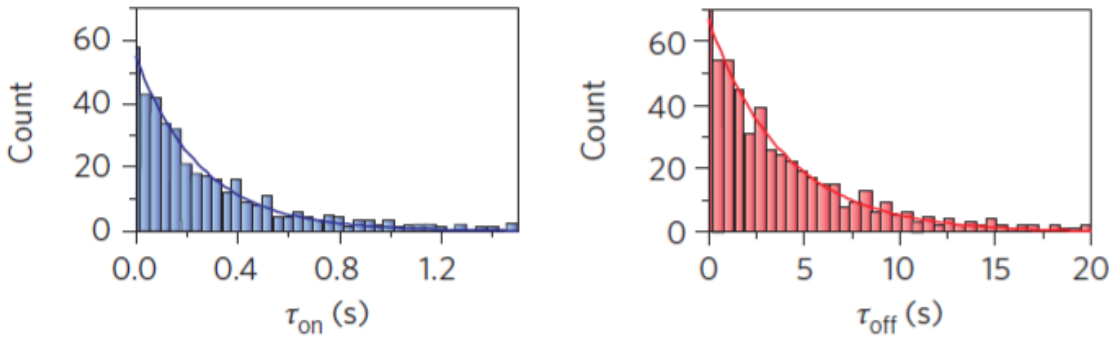
By measuring the ion current through single nanopores it is possible to make an extremely small sensor. When receptors are immobilized inside the pore it is possible that only a single receptor contributes to the sensor response:



Reversible binding and dissociation events can then be monitored and the sensor has two states. The following is an example of what the data can look like:



The events can be summarized in histograms. The following data is for 800 nM protein concentration in solution:



Estimate the dissociation constant of the interaction! (Figures from Wei et al. *Nature Nanotechnology* 2012, 7 (4), 257-263.)

We need k_{on} and k_{off}. When a molecule is bound the distribution of times until dissociation should follow an exponential decay just like in an ensemble average measurement. The right curve has decreased by a factor $1/e$ at ~4 s and thus $k_{off} = 1/4\ s^{-1}$. For the association the principle is the same, i.e. exponential decay of probability distribution for time until the event occurs, but we need to include the concentration. The curve has decreased by a factor $1/e$ at ~0.3 s and thus $k_{on} = 1/[0.3\times800\times10^{-9}] \approx 4.16\times10^{6}\ s^{-1}M^{-1}$. This gives $K_D = k_{off}/k_{on} = 60$ nM.