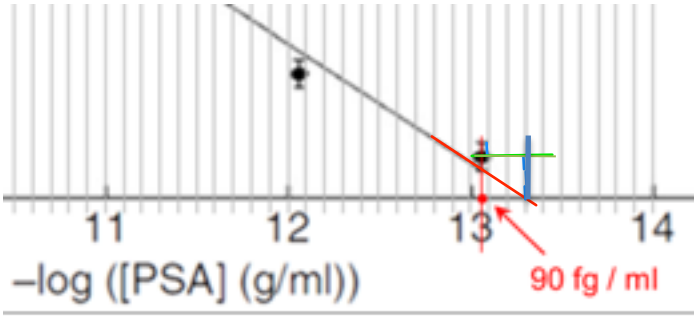
SOLUTION SESSION 8: BINDING KINETICS ON NANO-SIZED DEVICES

Exercise 1

The paper derives a limit of detection of 75 fg/ml (3X signal-to-noise ratio), which corresponds 13.12 on the logarithmic scale of the x-axis.

On the plot in Figure 1c, the interpolated value for zero signal (interpolated offset) corresponds to circa 13.3 (50 fg/ml). The numerical value of standard deviation (St.D.) and the value of the slope are not reported. However, we can estimate that 3X St.D. would correspond to a PSA concentration of 90 fg/ml (see the red drawing on the plot below), 13.05 in log scale, which is close to the value reported.



Exercise 2

<u>Paper (1):</u>

Given the value of the dissociation constant and of the dissociation rate we can calculate:

$$k_{on} = \frac{k_{off}}{K_D} = 2.4 \times 10^9 M^{-1} \cdot s^{-1}$$

$$\tau_{diss} = \frac{1}{k_{off}} = 115\ hours$$

Moreover, in the case of biotin /streptavidin we can also calculate the characteristic time of the association, considering a concentration of streptavidin of 250 nM, given in Figure 2B:

$$\tau_{ass} = \frac{1}{k_{off} + k_{on}C} = 1.7\ ms$$

The association rate is fast and dissociation is very long. We notice that the association times that can be observed on the plot of Figure 2b-2e is longer than the theoretical one. This could be due to mass-transport phenomena or small volumes. On the other side, dissociation is negligible in the 5-10 minutes range, which is in agreement with the dissociation time calculated.

Since no information is given about the density of biotin molecules in paper (1), the text of the exercise suggests to consider the that the surface density of biotin is the same than the density of PSA in paper (2) (although it will be probably much larger since the size of biotin is way smaller than the PSA antibody). Given $\Gamma_{biotin} = 0.2 \times 10^6\ \mu m^{-2}$, we can calculate:

$$\Gamma_{eq} = \frac{C\Gamma_{biotin}}{C + K_D} = 0.2 \times 10^6\ \mu m^{-2}$$

In theory, each biotin molecule binds a streptavidin molecule, resulting in a total number of streptavidin molecules on the nanowire equal to 126'000. We are therefore in the condition of saturation coverage.

[Diameter of nanowire: $d = 20 \times 10^{-9} m$

Length of nanowire: $l = 20 \times 10^{-6} m$

Area nanowire: $A = (d \cdot \pi) \cdot l = 1.26 \times 10^{-12} m$

Available area for binding: $A_{eff} = 0.5 \cdot A = 0.63 \times 10^{-12} m$

Number of probes (area of one probe 5 nm^2):

$N_{biotin} = \Gamma_{biotin} \cdot A_{eff} = 126000\ molecules$]

These calculations do not take into account the larger space occupied by the streptavidin compared to biotin. If biotin molecules are too packed, some of them will not be able to bind a streptavidin from the solution. Moreover, streptavidin consists of four subunits and has four binding sites. Thus, one streptavidin could bind multiple biotins on the surface.

<u>Paper (2):</u>

Assuming kinetic constants for PSA/PSA-Ab1 binding as follows (given in the text of the exercise):

Dissociation constant: $K_D = 1.1 \times 10^{-9} M$

Association rate: $k_{ON} = 4.1 \times 10^4 M^{-1} \cdot s^{-1}$

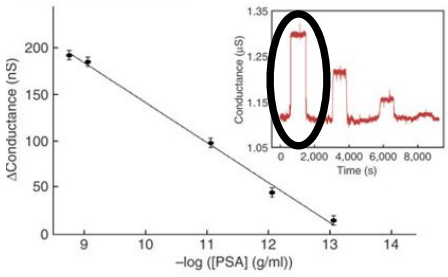
Dissociation rate: $k_{OFF} = 4.5 \times 10^{-5} s^{-1}$

The concentration of PSA is reported in Figure 1c as 0.9 ng/ml. Assuming PSA MW ≈ 26 KDa, the molar concentration is equal to 34 pM.

We can estimate the characteristic times for association and dissociation as

$$\tau_{ass} = \frac{1}{k_{off} + k_{on}C} = 6\ hours\ (k_{on} \gg k_{off}C)\ ;\ \ \tau_{diss} = \frac{1}{k_{off}} = 6\ hours$$

These time values define a much longer dynamics than the one that can be observed in the sensor response represented in the paper (see figure on the right), in which association and dissociation are in the order of minutes.



It is difficult to deduce why the Langmuir model does not provide an accurate prediction of the times of association and dissociation kinetics. We can think of different hypothesis:

1) The Langmuir kinetics model is valid even for a single molecule. However, it only applies to the case of thermodynamic equilibrium, which might not be valid in this experimental set, due to a low number of molecules.

2) The rapid response time might be caused by small size of the flow chamber which promotes the analyte transport to the sensor surface.

3) The authors also claimed that they observed experimentally a dependence of the speed of response on the frequency of the electrical measurement performed, the so-called "electrokinetic effect". The voltage applied to record the sensors conductance might generate an electric field that attracts the analyte molecules and increases their concentrations at the sensors surface.

Exercise 3

As a first remark, we notice that in both studies no specific passivation layer was applied to the surface to prevent unspecific binding of molecules onto the nanowire sensor. The complete absence of non-specific binding is therefore unlikely.

Paper (1):

Two different experiments are carried out to characterize the binding of non-specific molecules to the nanowire surface. The shift in conductance was recorded for the case of streptavidin injected onto the sensor bare surface (without probe molecules) and of saturated streptavidin (without available binding sites) on a biotin-modified nanowire sensor. We do observe in the two cases a small rise in conductivity when the sample is injected, however these signals due to unspecific binding are negligible, according to the authors. In both scenarios, some physisorption of molecules onto the sensor surface is expected (in particular, the binding in Fig. 2C is purely due to physisorption onto the bare nanowire surface). In the case of Fig. 2D, there might be additional signal since a few molecules of streptavidin, which were already bound to biotin in solution (the antigen was saturated with 4x excess biotin prior to injection), might still bind to the biotin on the sensors surface.

Paper (2):

To characterize pure non-specific binding, a solution of 10 μg/ml of BSA protein has been injected. In this case no substantial conductance change is recorded, even if the BSA concentration is very large if compared with the pg/ml range of measurement of PSA. Moreover, the delivery of a solution containing 0.9 pg/ml PSA and 10 μg/ml BSA showed a slight decrease in the output signal. This could be a result of completion and steric hindrance. However, no signal is observed when BSA is injected alone, which might be due to the fact that this low shift is not above the noise level of the transducing system. Nevertheless, the decrease in conductance due to the presence of BSA is minimal and the authors do not consider it as relevant interference to the PSA quantification.