SESSION 8: BINDING KINETICS ON NANO-SIZED DEVICES

The exercises are based on the following papers

(1) Cui, Y., Wei, Q., Park, H., & Lieber, C. M. (2001). Nanowire nanosensors for highly sensitive and selective detection of biological and chemical species. *Science (New York, N.Y.)*, *293*(5533), 1289–1292. http://doi.org/10.1126/science.1062711

(2) Zheng, G., Patolsky, F., Cui, Y., Wang, W. U., & Lieber, C. M. (2005). Multiplexed electrical detection of cancer markers with nanowire sensor arrays. *Nature Biotechnology*, *23*(10), 1294–1301. http://doi.org/10.1038/nbt1138

Exercise 1

Discussion on the value of the limit of the detection derived from the measurements.

In paper (2), the authors claim to be able to detect very low concentrations. They employ arrayed silicon-nanowire field-effect transistors to detect cancer markers down to femtomolar concentrations. They report in the text a limit of detection of 75 fg/ml for prostate specific antigen (PSA). Is it possible to deduce this number from Figure 1C (here below)?

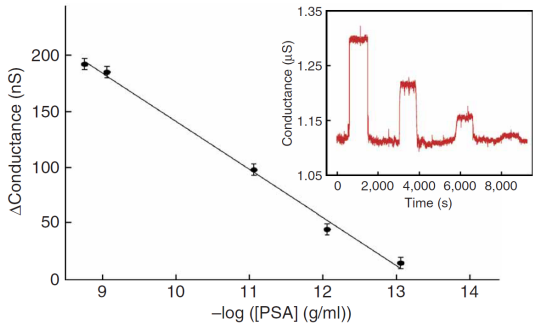

Figure 1C - paper (2) Change in conductance versus concentration of PSA for a p-type silicon nanowire modified with PSA-Ab1 receptor. Inset: Conductance-versus-time data recorded after alternate delivery of PSA and pure buffer solutions; the PSA concentrations were 0.9 ng/ml, 9 pg/ml, 0.9 pg/ml and 90 fg/ml, respectively. The buffer solutions used in all measurements were 1 mM phosphate (potassium salt) containing 2 mM KCl, pH 7.4.

Exercise 2

Considerations on the binding kinetics.

The kinetics of the observed phenomena are represented Fig. 1c and 1d (paper 2)) and in Fig 2 (in paper 1). Both works consist of conductivity measurements on silicon nanowires modified with molecular ligands. In paper (1) the sensor surface is modified with biotin probes which bind streptavidin as antigen. In paper (2), anti-PSA antibodies are immobilized on the surface of the NWs and a PSA detection is performed under static conditions.

The kinetics of association and dissociation differ substantially between the two experiments. In particular, the dissociation rate is extremely low in (1) and very quick in (2), the latter showing a fully reversible binding upon the simple injection of buffer solution in between successive injections of samples at different concentrations.

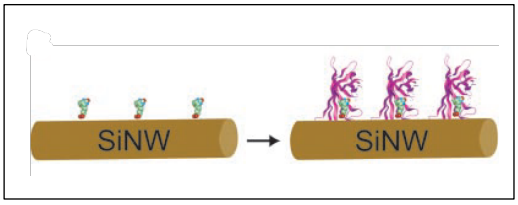

Figure 2A – paper (1) Schematic illustrating a biotin-modified SiNW (left) and subsequent binding of streptavidin to the SiNW surface (right).

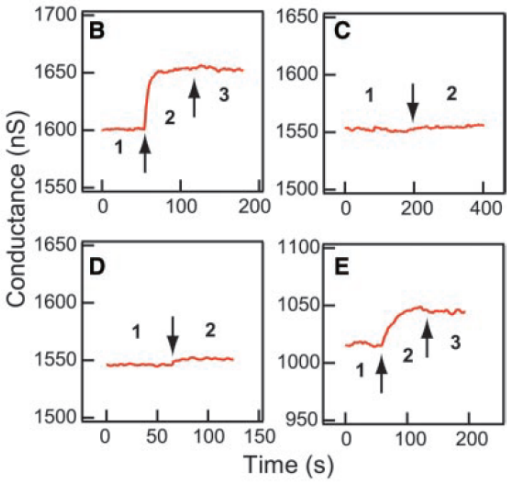

Figure 2 – paper (1) (B) Plot of conductance versus time for a biotin-modified SiNW, where region 1 corresponds to buffer solution, region 2 corresponds to the addition of 250 nM streptavidin, and region 3 corresponds to pure buffer solution. (C) Conductance versus time for an unmodified SiNW; regions 1 and 2 are the same as in (B). (D) Conductance versus time for a biotin-modified SiNW, where region 1 corresponds to buffer solution and region 2 to the addition of a 250 nM streptavidin solution that was preincubated with 4 equivalents d-biotin. (E) Conductance versus time for a biotin-modified SiNW, where region 1 corresponds to buffer solution, region 2 corresponds to the addition of 25 pM streptavidin, and region 3 corresponds to pure buffer solution. Arrows mark the points when solutions were changed.

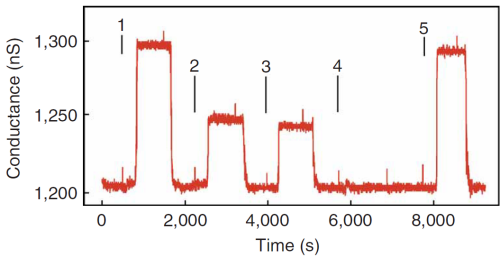

Figure 2D - paper (2) Conductance-versus-time data recorded for a PSA-Ab1-modified p-type silicon nanowire after alternate delivery of the following protein and pure buffer solutions: (1) 9 pg/ml PSA, (2) 0.9 pg/ml PSA, (3) 0.9 pg/ml PSA and 10 mg/ml BSA, (4) 10 mg/ml BSA and (5) 9 pg/ml PSA. *(BSA, bovine serum albumin, is a protein which is not supposed to specifically bind the anti-PSA probes).*

The nanowire can be modeled by a cylinder of diameter $d = 20\ nm$ and length $l = 20\ \mu m$, of which half of the surface is covered by probe molecules. The area of one probe is 5 nm^2.

Derive the theoretical kinetics (characteristic association and dissociation times) for the two cases and compare it with the experiments. How do they compare? (to this end, make sure to calculate the percentage of binding analytes at equilibrium for the two cases and their number)

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

Assume the following kinetic constants for biotin-streptavidin binding:

Dissociation constant: $K_D = 1 \times 10^{-15} M$

Dissociation rate: $k_{OFF} = 2.4 \times 10^{-6} s^{-1}$

Assume the molecular weight of biotin to be 150 KDa and the concentration of the injected sample equal to C = 250 nM.

Assume the density of biotin probe to be $\Gamma_{biotin} = 0.2 \times 10^6\ \mu m^{-2}$

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

Assume the following kinetic constants for PSA/PSA-Ab1 binding:

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

Assume the molecular weight of PSA to be 26 kDa and the concentration of the injected sample equal to C = 0.9 pg/mL

The density of anti-PSA probe is $\Gamma_{anti-PSA} = 0.2 \times 10^{6}\ \mu m^{-2}$, as reported in the paper.

Exercise 3

Non-specific binding.

Consider and comment the non-specific binding observed in papers (1) and (2), with reference to Figure 2C and Figure 2D of paper (1), and Figure 1D of paper (2). The figures are shown above.