Submitted to small

Small

Label-free Attomolar Detection of Proteins Using Integrated Nanoelectronic and Electrokinetic Devices

*Jian-Ru Gong**

Supporting Information

A roughly calculation, regarding the geometry of the sample cell and the flow regime operative in the experiment, was made to estimate the feasibility of the protein detection limit.

1. Antibody binding sites on NW surface

First, derive an approximate value for Γ_{lim} (represents the density of binding sites on the NW surface). Assuming that the protein comprising the surface immobilized binding site is roughly globular with a value for the radius, $r_0 \sim 2\times10^{-9}$ m, for close packing of spheres the site density (Γ_{lim})[1] is simply $1/2\sqrt{3}\, N_A r_0^2$ (N_A: Avogadro constant) M m^{-2} giving $\Gamma_{lim} \sim 1.125\times10^{-7}$ M m^{-2}.

$\Gamma_{lim} = 1.125\times10^{-7}$ M $m^{-2} \times 6.02 \times 10^{23}$ $M^{-1} \approx 7\times10^{16}$ m^{-2}

Then, calculate the average surface area of NW ($\mathbf{S}_{NW}$), length of NW (L) is *ca.* 3 μm, radius of NW (r), 20 nm.

$\mathbf{S}_{NW} = 2\pi rL = 2 \times 3.14 \times 20 \times 10^{-9}$ m $\times 3 \times 10^{-6}$ m $\approx 3.8 \times 10^{-13}$ m^2

Last, get antibody binding sites on NW surface (**N**)

$\mathbf{N} = \Gamma_{lim} \times \mathbf{S}_{NW} = 7 \times 10^{16}$ $m^{-2} \times 3.8 \times 10^{-13}$ $m^2 \approx 2.7 \times 10^4$

2. Feasible detection limit

From equilibrium considerations, the following expression in Equation (1) forms a useful basis to assess the likely performance of immunosensors, although its application to the description of antigen binding at surfaces will be subject to the well-known limitations of the Langmuir treatment.

$$1/\theta_{eq} = 1 + K/[A]_{eq} \quad (1)$$

Where, θ_{eq} is equilibrium surface coverage, as a function of normalized concentration, [A]/K. In our case, the equilibrium dissociation constant of PSA antibody and antigen, K, is *ca.* 0.2 nM;[2] PSA detection limit without AC electrokinetic application, $[A]_{eq}$ is 100 fM; Based on equation (1), $\theta_{eq} \sim 1/2000$. At this low fractional surface coverage, that is, *ca.* 14 ($\mathbf{N}\theta_{eq} = 2.7 \times 10^4 / 2000 = 14$) antibody binding sites on NW surface, 100 fM detection limit should be feasible.

From kinetic considerations, two cases will serve to illustrate the important features of the surface binding reaction. The first is linear diffusion, and the second is the "Nernst diffusion layer" treatment of convective systems.

In the case of without AC excitation, the protein-NW collision/binding frequency (J) is estimated in Equation (2).

$$J = \mathrm{k}\,[\mathrm{PSA}] \quad (2)$$

Assuming the binding is diffusion limited, based on Fick's law, the number of molecules entering collision cross-section (cylinder shape) per unit time (rate)

$J = 2\pi r\, L\Phi_B = 2\pi r\, L\, D_B\, d[B]_r/dr$

Surface flux $\Phi_r(r) = -D_B \nabla C_B = -D_B\, d[B]_r/dr$

$$\int_{[B]_{R^*}}^{[B]} d[B]_r = \int_{R^*}^{d} \frac{J}{2\pi r L D_B} dr$$

$$[\mathrm{B}]-[B]_{R^*} = [\mathrm{B}] = \frac{J}{2\pi L D_B} \ln[r]_{R^*}^{d}$$

L, the length of NW; D_B, the diffusion coefficient, for most of proteins for similar size to PSA, ~10^{-10} m^2 s^{-1}; R^*, the radius of cross-section of interaction (wire+receptor); d, the distance where protein concentration equals to bulk, *i.e.*, wire+receptor+protein

$$k = \frac{J}{[B]} = \frac{2\pi L D_B}{\ln \frac{d}{R^*}}$$

So, for d = 30 nm, R^* = 25 nm, L = 3 μm, $D_B = 10^{-10}$ m^2 s^{-1}, the encountering rate k = 6.3×10^{12} (M^{-1} s^{-1})

Through the comparison of the PSA-NW encounter rate and signal rising time in Table 1, it is feasible to have a number of binding events occurring at the lowest concentration (100 fM) without AC excitation in our experiment.

Table 1. PSA-NW encounter rate and event without electrokinetic application

[PSA] [pM]	PSA-NW encounter rate [s^{-1}][a]	Time for one event of PSA-NW encounter [s][a]	Signal rising time[s][b]	Possible encounter event during rising [b]
1	6.3	0.16	115	725
0.5	3.15	0.32	220	693
0.1	0.63	1.6	550	347

[a] Calculation result; [b] Experimental result.

When 10 aM PSA is delivered into the channel,

$$10 \times 10^{-18}\ \text{M} \times 6.02 \times 10^{23}\ \text{M}^{-1} = 6.02 \times 10^{3}\ \text{mL}^{-1}$$

at the delivery rate of 0.3 mL h^{-1},

$$0.3\ \text{mL h}^{-1} \times 6.02 \times 10^{3}\ \text{mL}^{-1} = 30\ \text{min}^{-1}$$

the number of molecule passing through the channel during the binding time of 600 s is,

$$30\ \text{min}^{-1} \times 600\ \text{s} = 300$$

Considering other possible negative effect, such as molecular adsorption in the channel, we conservatively assume that only 10% molecules could bind onto the NW after electrokinetic application, even so, still could get 30 molecules bound on the NW. Therefore, with the enrichment factor of 10^4, femtomolar protein concentration at the nanowire surface could be reached with the 10 aM injected concentration. Based on the calculation about

diffusion model for femtomolar concentration without AC excitation, the detection limit of 10 aM with AC excitation is feasible.

References

[1] M. J. Eddowes, *Biosensors* **1987/88**, *3*, 1.

[2] T. Soukka, J. Paukkunen, H. Harma, S. Lonnberg, H. Lindroos, T. Lovgren, *Clin. Chem.* **2001**, *47*, 1269.

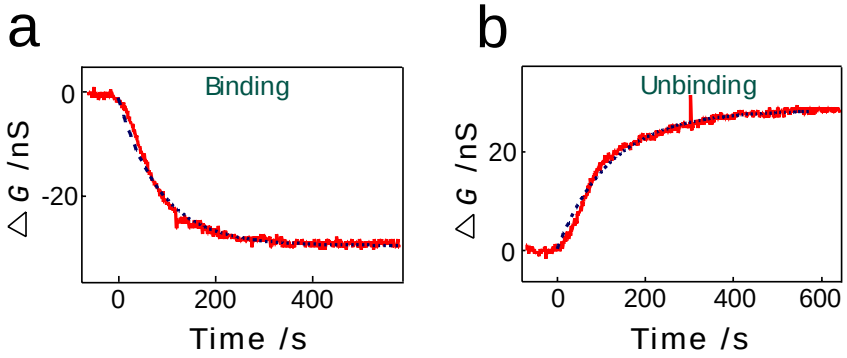

Figure 1. Kinetic analysis of curve fitting for CTB binding and unbinding with an expanded x-scale. (a) Binding and (b) unbinding curve (blue dotted lines) of 1.8 fM CTB with AC excitation. Single exponential fitting is used to fit the curve.

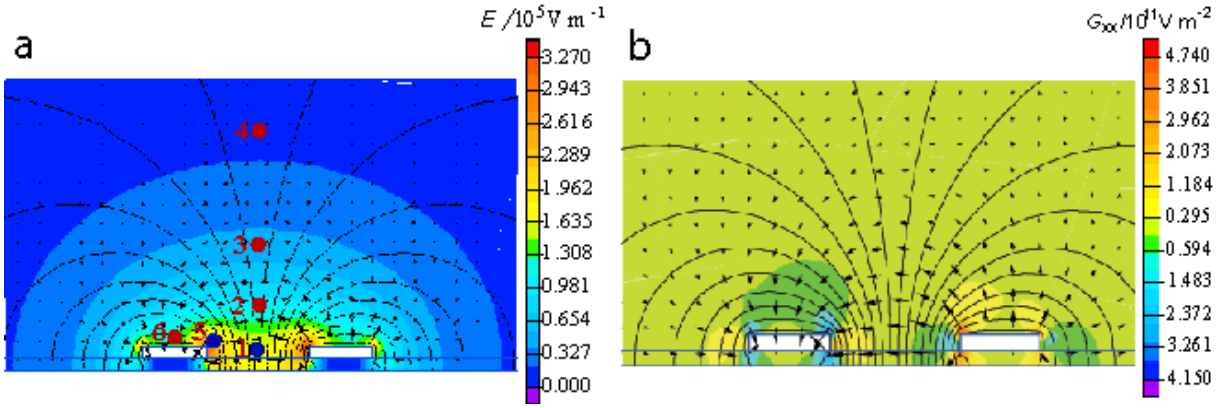

Figure 2. (a) Field strength and (b) field gradient near the electrokinetic electrodes from two-dimensional electrostatic simulations (Quickfield, Tera Analysis, Denmark). Positions of the electric field measurement in (a): 1) X = 0, Y = 0 (in the middle of the electrode pair gap on the substrate; 2) X = 0, Y = 2.5 μm; 3) X = 0, Y = 5 μm; 4) X = 0, Y = 10 μm; 5) electrode edge; 6) electrode center. The order of field strength at the specific position: 5 > 1 > 6 > 2 > 3 > 4.