

Label-free determination of protein–surface interaction kinetics by ionic conductance inside a nanochannel

Nicolas F. Y. Durand* and Philippe Renaud

Received 28th June 2008, Accepted 2nd October 2008

First published as an Advance Article on the web 4th November 2008

DOI: 10.1039/b811006a

We have developed a label-free conductometric platform for the rapid measurement of kinetics parameters for the adsorption and desorption of proteins on surfaces. Adsorbed Bovine Serum Albumin (BSA) has been detected electrically with response times in the minute range, and kinetic models were elaborated and compared to the measurements. The device presents similar characteristics to Surface Plasmon Resonance (SPR) immunosensors, but takes advantage of a simpler, low-cost electronic measurement unit.

Introduction

Measurement of the kinetics of adsorption of proteins on surfaces and equilibrium adsorption isotherms have been reported using different techniques, including Optical Waveguide Lightmode Spectroscopy (OWLS),^{1,2} ellipsometry,³ Total Internal Reflectance Fluorescence (TIRF)^{4–8} or Surface Plasmon Resonance (SPR).^{9–11} Kinetic effects are often complex, dependant of salt concentration, protein charge or dipole moment, and surface charge or hydrophobicity.⁵

Micro- and nanofluidics^{12,13} for immunology applications have received much interest over the last 10 years, mainly because of the reduction in detection time due to the large surface to volume ratio and to the reduced sample volume. Diffusive transport at that length scale implies short diffusion times and increases the speed of the analysis.

Recently Karnik *et al.*¹⁴ and Schoch *et al.*¹⁵ have reported possibilities of electrical detection of chemical binding inside nanochannels. They have demonstrated that molecules entering a confined area change the volume conductance, which can be easily measured. Such electrical biosensors on a chip are holding promises due to the low-cost and the simplicity of the detection system.

In this study, we supply theoretical models for the effect of proteins adsorption on conductance in a nanoslit, and based on simple adsorption experiments, we describe the different regimes governed by the solution characteristics (ionic strength, pH, charge and concentration of molecules, etc.). This study is the preliminary step for understanding future immunological detection based on coupling nanofluidic devices with electrical measurements.

As presented in figure 1, the device used in the experiment consists of 2 microchannels linked together by a short but wide nanoslit (height $h = 50$ nm, length $l = 3$ μ m, width $w = 2.5$ mm). The width of the nanoslit is large in order to decrease the measured impedance and allow measurement when the solution has a low ionic concentration.

External positive and negative pressures allow driving the flow in each 500nm deep microchannel. Platinum electrodes placed on each side near the nanoslit allow the measurement of the nanoslit impedance.

Theory

Adsorption kinetics

The Langmuir isotherm model¹⁶ is an adsorption kinetics theory accounting for reversible adsorption. The exponential model for the capture rate at constant concentration, which is governed by the rate of adsorption K_{on} and desorption K_{off} , is usually used to describe the behavior of molecules adsorption:

$$\Gamma(t) = \Gamma_0(1 - e^{-t/\tau}) \quad (1)$$

where the surface concentration $\Gamma(t)$ is a function of time t , and the effective surface coverage Γ_0 is a function of the full coverage surface concentration Γ_{max} :

$$\Gamma_0 = \Gamma_{max} \frac{K_{on} \cdot c_0}{K_{on} \cdot c_0 + K_{off}} \quad (2)$$

where c_0 is the protein concentration. The time constant τ to get the proteins adsorbed, is given by

$$\tau = \frac{1}{K_{on} \cdot c_0 + K_{off}} \quad (3)$$

After the injection of the proteins, when the equilibrium is reached, the effective surface coverage Γ_0 is reduced compared to the full coverage Γ_{max} due to the adsorption and desorption of proteins, as described by eqn 2. The ratio of the rate of adsorption over the rate of desorption K_{on}/K_{off} determines the equilibrium constant K , which is constant for a given type of protein.

Effect of the presence of proteins on the nanoslit conductance

In the absence of adsorbed biomolecules on the nanoslit walls, the nanoslit conductance is governed by the surface charges at low ionic concentration, resulting to an offset conductance as observed by Stein.¹⁷ At high ionic concentration, the nanoslit conductance is governed by the channel geometry.

Microsystems Laboratory, STI-LMIS, EPFL, CH-1015 Lausanne, Switzerland

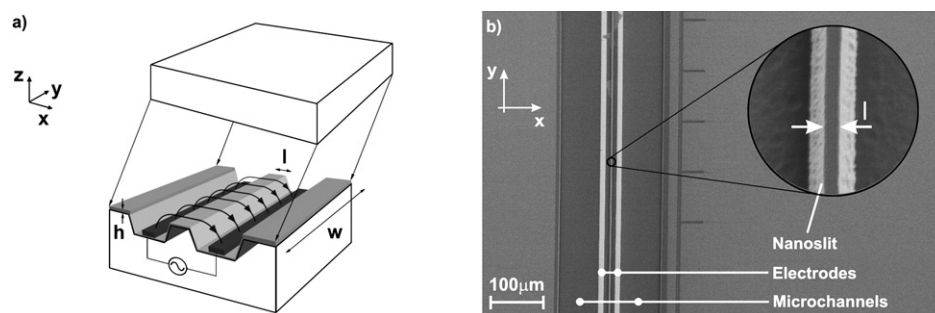


Fig. 1 (a) 3D schematic of the nanofluidic biosensor (cross-section, not to scale) constituted of 2 microchannels linked together by a short but wide nanoslit ($h = 50$ nm, $l = 3$ μ m, $w = 2.5$ mm). A 50 nm aSi layer defines the height of the nanoslit and a platinum electrode is deposited in each microchannel. A driving voltage is applied on the electrodes, generating an electric field through the nanoslit (represented by lines with arrows). (b) SEM image of the top view of the microchannels and the electrodes. The inset picture presents the topography of the nanoslit.

The nanoslit conductance G in steady-state conditions of a bare nanoslit before protein adsorption, when filled with a 1:1 electrolyte solution, has been proposed by Schoch *et al.*¹⁸ and is given by

$$G = \underbrace{(\mu_+ + \mu_-)c_i \cdot N_A \cdot e \frac{w \cdot h}{l}}_{G_V} + \underbrace{2\mu_{\text{opposite}} \cdot |\sigma_0| \frac{w}{l}}_{G_S} \quad (4)$$

where μ is the ionic mobility (subscripts denote cation/anion and “opposite” indicates counterions of opposite charge to the surface charge), σ_0 the surface charge density in the diffuse layer (assuming that the species in the inner Helmholtz plane are immobile), N_A the Avogadro constant, e the electron charge and c_i the electrolyte ionic concentration. The conductance G in the nanoslit is obtained through the addition of the bulk conductance G_V and the conductance G_S due to excess counterions equilibrating surface charges.

In our model, we assume that the protein concentration along the nanoslit is constant and that the presence of biomolecules adsorbed to the channel surfaces can change the nanoslit conductance mainly for 2 reasons: i) the decrease of the effective volume of the nanochannel due to the hindrance of the proteins, and ii) the change of the surface charges because of the charged adsorbed biomolecules and thus of the counterions concentration.

We assume constant properties of proteins and no lateral interactions between adsorbed molecules.

The immobilized proteins contribute to the change of conductance related to the channel geometry. As the volume of the proteins decreases the nanoslit section, a resulting decrease in nanoslit conductance occurs:

$$\frac{\Delta G_V}{G_V} = -\frac{\Delta V(I)}{V_0} = -\frac{2V_p}{h} \cdot \Gamma \quad (5)$$

where $\Delta G_V/G_V$ is the normalized conductance change of the nanoslit relatively to G_V , ΔV is the decrease of volume of the nanoslit, V_0 the initial volume of the channel and Γ is the surface concentration of bound molecules.

The adsorbed proteins also change the nanoslit conductance G_S because of the change of the effective surface charge density σ_S . In our model, we assume that the initial surface charges are progressively replaced by every protein adsorbing on the surface. Each protein also brings new counterions to the surface:

$$\sigma_S = \left(1 - \frac{\Gamma}{\Gamma_{\text{max}}}\right) \sigma_0 + n_p \cdot e \cdot \Gamma \quad (6)$$

where n_p is the charge of one protein, depending on pH.

The normalized conductance change of the nanoslit relative to G_S can be written as follows:

$$\frac{\Delta G_S}{G_S} = \frac{|\sigma_S| - |\sigma_0|}{|\sigma_0|} \quad (7)$$

The total normalized conductance change $\Delta G/G$ is obtained through the combination of eqns 4, 5 and 7:

$$\frac{\Delta G}{G} = \frac{G_V}{G} \cdot \frac{\Delta G_V}{G_V} + \frac{G_S}{G} \cdot \frac{\Delta G_S}{G_S} \quad (8)$$

With high ionic strength solutions, conductance changes in the nanoslit are dominated by the channel geometry and the first term of eqn 8 is dominant. However, at low salt concentration, conductance becomes increasingly dominated by surface charges and the second term of eqn 8 becomes dominant. This last term gives a positive conductance change upon protein adsorption and is characterized by the protein charge.

A strong dependence on the pH is therefore expected since protein charge n_p will change with pH. The protein charge is positive below the isoelectric point (pI) and is negative at pH above pI.

Electrical model

The electrical model of the system we used, in which electrodes are placed in the 2 microchannels linked by a nanoslit, is presented in figure 2a. The equivalent electrical circuit is composed by resistive elements and parasitic capacitances. R_{nS} and $R_{\mu C}$ correspond respectively to the electrical resistance of the nanoslit and microchannels. The value of the resistances of the microchannels, $R_{\mu C}$, is much lower than the resistance of the nanoslit.

The capacitances C_{EDL} result from the interface between the electrodes and the ionic solution. C_{stray} is the parasitic capacitance between the two electrodes through the glass.

C_{stray} can be measured when the chip does not contain solution and was measured to be $C_{\text{stray}} = 1.2 \pm 0.2$ pF.

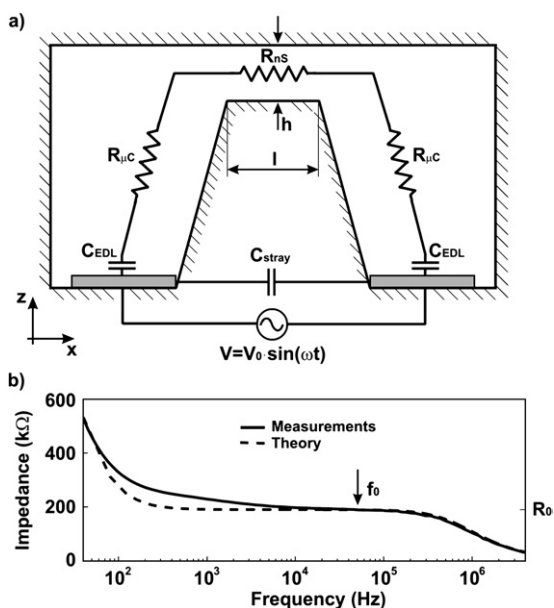


Fig. 2 (a) Equivalent electrical model showing the electrical resistance of the channels, the capacitance due to the electrical double layer and the stray one. (b) Impedance measurement as a function of the frequency for a chip filled with a solution composed of 10% PBS and 90% deionized water. f_0 corresponds to the most resistive case (when the phase is close to zero).

To avoid electrochemical reactions at the embedded electrodes interface, a low AC voltage (100 mV) was chosen. The working frequency f_0 was tuned for the system to provide a resistive behavior, and a phase close to zero. Figure 2b shows the impedance measurement for a large range of frequencies when the device is filled with 10% PBS and 90% deionized water.

Experimental section

Device microfabrication

Microfabrication processes were used to produce the devices. Microchannels were wet-etched on a Pyrex wafer and 150 nm thick platinum electrodes were deposited and structured using standard lift-off techniques. A 50 nm layer of amorphous silicon was sputtered and structured by plasma etching. The thickness of this layer defines the height of the nanoslit. A second Pyrex wafer, in which access holes were drilled by sandblasting, was anodically bonded onto the first wafer. Afterwards, the wafers were diced into individual chips and UV photosensitive glue was used to isolate the electric connections from the fluidic part. This process allows observing the microchannels by the use of an optical microscope, which allows potential correlations between electrical and fluorescent measurements. Figure 1b shows a SEM picture of a manufactured nanoslit.

Electrical measurement

The conductance of the nanoslit was measured using the precision LCR meter HP 4284A (Agilent Technologies, Inc., Englewood, CO) with a sinusoidal voltage of 100 mV peak-to-peak.

Acquisition was controlled by a Matlab program, allowing recording measurements at a given frequency.

Materials

Bovine Serum Albumin (Sigma-Aldrich, St-Louis, MO) was used due to its well-known capacity to adsorb to non-pretreated surfaces. BSA is a globular protein composed of 610 amino acids, has a molecular mass of 66 kDa and an isoelectric point around $pH = 4.9$.¹⁹

For the preparation of solutions, Phosphate Buffered Saline (Sigma-Aldrich, St-Louis, MO) containing 137 mM sodium chloride, 10 mM phosphate buffer and 2.7 mM potassium chloride, was diluted with deionized water (18 MΩ cm) and adjusted to the wanted pH with addition of HCl or KOH. For all experiments, no pre-treatments were performed on the chip; solutions were directly injected in microchannels and flow across the nanoslit. All solutions were prepared and degassed just before use, avoiding problems with air bubbles (only 5% of experiments were aborted due to air bubbles). The solution containing the proteins is dispensed in the inlet by a syringe. The liquids from other ports are driven by air aspiration using an air-pressure regulator (Bellofram Corp., Newell, WV) from 0 to −800 mbar. In order to avoid a lateral flow through the nanoslit during the measurement, the proteins of interest were injected in the first microchannel with the same flow rate as the rinsing solution in the second one. Diffusion is considered as the only phenomenon occurring in the nanochannel.

Results and discussion

Experiments were carried out to evaluate the conductance changes of the nanoslit due to the adsorption and desorption of BSA on its walls: first the microchannels and the nanoslit were filled with a clean PBS solution by establishing a flow between the inlet and the outlet ports. When the PBS solution fills the whole device, the measurement of the impedance is started.

After being inserted in one microchannel, BSA diluted in PBS diffuses in the nanoslit, resulting in a change of the impedance of the nanoslit as measured in figure 3. The impedance reaches an asymptotic value when BSA molecules have reached the maximum effective coverage.

Conductance changes due to BSA adsorption

Figure 3 shows an adsorption experiment where a 600 μM BSA solution was injected in the micro/nano fluidic system containing a) high ionic concentrated PBS solution (137 mM NaCl) and b) the same solution diluted 100x in deionized water (1.37 mM NaCl) in order to reduce the ionic concentration.

At high ionic strength, the conductance of the nanoslit is decreasing with time due to the dominance of first term in eqn 4. The curve shows a typical behavior of adsorption kinetics at surfaces.

At low ionic strength, the conductance of the nanoslit is increasing due to the dominance of surface charges effects, described by the second term of eqn 4. The kinetic behavior is however different from the expected adsorption kinetic curve. There is a systemic overshoot followed by a slower decay to the equilibrium value $\Delta G/G$. Similar behaviour was also

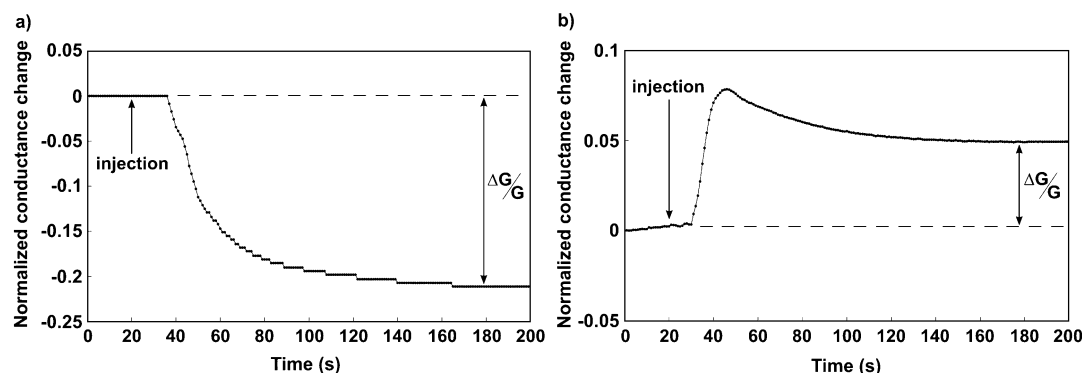


Fig. 3 Nanoslit conductance response of BSA adsorbed at Pyrex surfaces when channels are filled with (a) PBS solution and (b) 1%PBS diluted in deionized water, both corrected to pH = 6 by addition of HCl. The 600 μM BSA solution was injected at $t = 20$ s and adsorbed on the nanoslit's walls. The value $\Delta G/G$ is measured when the normalized conductance change reaches an asymptote. The delay observed between injection and start of change is due to the time to reach the nanoslit.

observed by Malmsten *et al.*^{3,20} with ellipsometry and by Wertz *et al.*⁶ with TIRF measurements. They explain this phenomenon by the occurrence of competitive spreading, in which initially adsorbed proteins undergo interfacial spreading, thereby occupying a larger interfacial area, and causing a fraction of the protein molecules to desorb. This is a possible explanation for the overshoot, assuming that high ionic strength solutions alter protein desorption due to competitive spreading.

Adsorption kinetics at high ionic strength

The adsorption experiment was reproduced with different initial values of the BSA concentration c_0 (from 600 nM to 600 μM) in high ionic strength conditions. The surface conductance G_S is negligible at high ionic concentration and the kinetics curve can be fitted using eqn 5.

Figure 4 shows the temporal evolution of the normalized conductance change during the adsorption of the proteins on the Pyrex nanoslit walls for various initial concentrations of BSA injected in the microchannel. When reducing the protein concentration, the time constant of the adsorption phenomenon increases strongly, in accordance with eqn 3, and the saturated conductance changes value decreases.

A simplified geometrical model of a spherical molecule with a radius $r_p = 4$ nm is used to calculate V_p . The assumption of full surface coverage Γ_{max} is made with the same model, postulating a compact hexagonal packing on surface:

$$\Gamma_{max} = \frac{1}{2\sqrt{3} \cdot r_p^2} \quad (9)$$

With this model, eqn 2 and 5 become

$$\Gamma_0 = \frac{1}{2\sqrt{3} \cdot r_p^2} \frac{K_{on} \cdot c_0}{K_{on} \cdot c_0 + K_{off}} \quad (10)$$

$$\frac{\Delta G}{G} = -\frac{8\pi \cdot r_p^3}{3h} \cdot \Gamma_0 \quad (11)$$

The equilibrium coverage ratio of the surface can be expressed by

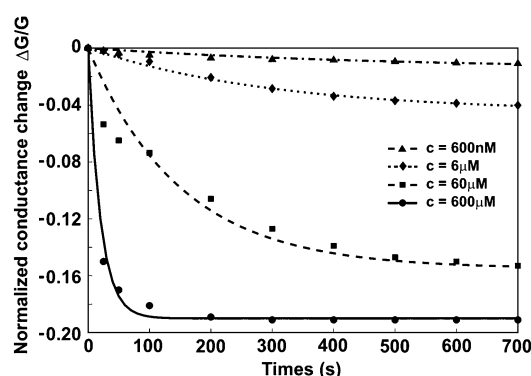


Fig. 4 Evolution of the normalized conductance change with time for various initial concentrations of BSA. The time constant of the adsorption phenomena decreases with increasing BSA concentration. The experiments are realized in PBS solution with pH of 4.9 corresponding to the isoelectric point of BSA. Points represent measurements and lines are exponential fits from which experimental adsorption kinetics values $\Delta G/G$ and τ are extracted (second column, table 1).

$$\frac{\Gamma_0}{\Gamma_{max}} = -\frac{3\sqrt{3}}{4\pi} \cdot \frac{h}{r_p} \cdot \frac{\Delta G}{G} \quad (12)$$

The maximum surface coverage $\Gamma_{max} = 1.8 \times 10^{16}$ mol/m² obtained with eqn 9 is close to the measured value in similar conditions by Duncan *et al.*¹⁹ of 2.6×10^{16} mol/m².

The values of measured time constants and normalized conductance changes are presented in table 1 and compared to the calculated ones. Calculations are best fittings of experimental data, using $K_{on} = 100 \text{ M}^{-1} \text{ s}^{-1}$, $K_{off} = 0.002 \text{ s}^{-1}$ and $r_p = 4$ nm. We obtain remarkably good fits considering the simplicity of the model. This allows direct determination of the rate of adsorption K_{on} and desorption K_{off} of label-free proteins on surfaces interactions.

Effect of pH and ionic strength variations

The adsorption experiment was repeated for various values of pH, and for various ionic concentrations of PBS solution. Figure 5 shows the normalized conductance change of the

Table 1 Comparison between measured and calculated conductance changes due to BSA adsorption in a nanochannel. The experimental values $\Delta G/G$ and τ are extracted from the data shown in figure 4 with an exponential fit. The calculated values have been obtained using eqn 11, 3 and 10 with the following values: $K_{on} = 100 \text{ M}^{-1} \text{ s}^{-1}$, $K_{off} = 0.002 \text{ s}^{-1}$, $r_p = 4 \text{ nm}$ and $h = 50 \text{ nm}$. The relative surface coverage is calculated with the same parameters and displayed in the last column

c_0 (μM)	Experimental data, extracted from figure 4		Calculated data with eqn 3, 10 and 11		
	$\Delta G/G$	τ (s)	$\Delta G/G$	τ (s)	Γ_0 (mol/m^2)
600	-0.19 ± 0.02	20 ± 5	-0.19	16	1.75×10^{16}
60	-0.16 ± 0.01	150 ± 30	-0.15	125	1.35×10^{16}
6	-0.04 ± 0.005	300 ± 80	-0.04	385	4.15×10^{15}
0.6	-0.01 ± 0.005	500 ± 120	-0.01	485	5.41×10^{14}

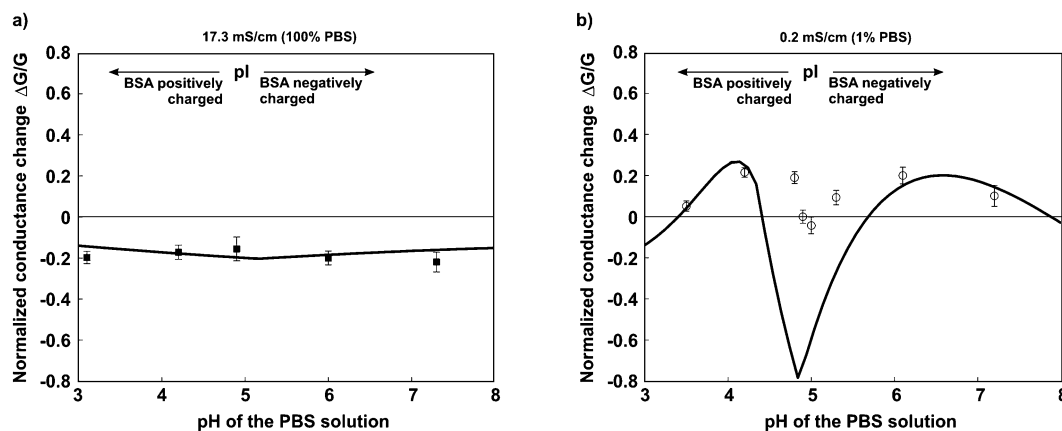


Fig. 5 Normalized conductance change of the nanoslit as a function of the pH of the PBS solution when injecting 600 μM BSA. (a) High ionic concentration solution is mainly dominated by nanoslit volume change and (b) low ionic concentration by charge effects. At high ionic concentrations the conductance difference due to proteins charges and proteins adsorption is less significant than for low ionic strength. Points are the measurements and lines are plotted using eqn 8, with n_p from Böhme *et al.*,²¹ $\Gamma(\text{pH})/\Gamma(\text{pI})$ from Clark *et al.*,²³ $\Gamma(\text{pI})_{1\% \text{ PBS}}/\Gamma(\text{pI})_{100\% \text{ PBS}} = 1.6$, $r_p = 4 \text{ nm}$, $\mu_{\pm} = 6.5 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$, $K_{on} = 100 \text{ M}^{-1} \text{ s}^{-1}$, $K_{off} = 0.002 \text{ s}^{-1}$, $\sigma_0 = -1.4 \times 10^{-2} \text{ C}/\text{m}^2$.

nanoslit when 600 μM BSA proteins are injected in the system and have adsorbed on the nanoslit walls, as a function of the pH of the PBS solution. The points are representing the measurements and in order to plot eqn 8, various parameters have been measured or are taken from the literature: the effective charge of BSA as a function of pH has been taken from NMR measurements of Böhme *et al.*²¹ The initial surface density was measured to be $\sigma_0 = -1.4 \times 10^{-2} \text{ C}/\text{m}^2$ using the second term of eqn 4, which is very close with the value $\sigma_0 = -2.5 \times 10^{-2} \text{ C}/\text{m}^2$ reported by Schoch *et al.*¹⁸ The ions mobility $\mu_{Na} = 5.19 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ and $\mu_{Cl} = 7.91 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ were found in the literature²² and an average value $\mu_{\pm} = 6.5 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$ was used for all values of pH in order to avoid singularities.

The BSA surface coverage as a function of pH, $\Gamma(\text{pH})/\Gamma(\text{pI})$ has been taken from measurements of Clark *et al.*²³ They also reported that at high ionic strength, the surface coverage remains constant as a function of pH.

Finally, Shibata *et al.*⁷ and Duncan *et al.*¹⁹ highlighted an increase of the adsorption of proteins on surfaces when reducing the ionic strength of the solution. According to their measurements, we increase the surface coverage $\Gamma(\text{pH})$ by a factor 1.6 in our 1% PBS model relatively to the 100% PBS one.

Figure 5a highlights that for solutions of high ionic concentrations, the changes of conductivity $\Delta G/G$ are almost independent of the pH, and the conductance of the nanoslit is mostly

related to the apparent section of the nanoslit, and consequently to the number of proteins adsorbed on the nanoslit walls. The measurement of the normalized conductance change $\Delta G/G$ at high ionic concentrations can be used to detect the presence of biomolecules in the slit or the binding between species, as these phenomena are inducing changes of the apparent section of the nanoaperture. Schoch *et al.*,¹⁵ recently highlighted the possibility to detect binding between biotin and streptavidin in nanochannels. In future work, we will attach a primary antibody to the nanochannel walls, to quantify the binding with a specific antigen and compare it with other species. This may lead to immunological applications based on electrical detection of label-free antigens.

Figure 5b presents the nanoslit conductance changes for solutions of low ionic concentrations. We can distinguish 3 different regimes: i) close to the pI of the proteins, when BSA proteins are not charged and $|\sigma_S| < |\sigma_0|$, the surface conductance change $\Delta G_S/G_S$ is low and tends to -1 when all initial surface charges are covered by globally uncharged molecules (according to eqn 7). The model does not match the measurements when the pH is close to the pI because it is over-simplified and does not take into account the local inhomogeneity of the pH in the electrical double layer as reported by Van der Wouden *et al.*²⁴ and Bousse *et al.*²⁵ However the overall evolution of the model and experimental data are similar for values of the pH away from

pI. ii) When going away from the pI value, the global number of charge $|n_p|$ of the proteins increases, which also increase the conductance of the nanoslit. iii) Finally, when working with pH far away from pI, the surface coverage decreases too much and becomes the limiting factor, leading to a decrease of the conductance change.

Conclusions

We have demonstrated that protein adsorption kinetics can be measured in a nanoslit. When the ionic strength of the solution is high, the conductance change is dominated by volume effects due to the adsorbed or bound proteins and the conductance is independent of proteins charge. Applications in immunology and biomolecules detection requiring fast detection time could benefit from the development of a detection method based on these conditions. Additionally, the adsorption and desorption kinetics parameters for a given protein-interaction condition can be easily deduced from the experiment.

When working with low ionic strength solutions the sensitivity to proteins charge was demonstrated and the behavior of the nanoslit conductance was described.

The detection time of our device is limited by the kinetics of the adsorption and desorption of molecules, as in the case with SPR immunosensors. However the technique presented in this paper has several advantages such as the simplicity of the measurements, the portability, low cost of production, suitability for fast multiplexing label-free detection of biomolecules on-chip and can become a powerful biophysical alternative for SPR devices in determining kinetic and equilibrium constants of molecular interactions.

Acknowledgements

This work was partially supported by Swiss National Foundation (FNS) grant no. 200021-111790. Cleanroom processing was performed at the EPFL Center of MicroNanoTechnology (CMI). The authors wish to thank Arnaud Bertsch and Jan Eijkel

for reviewing the manuscript and Joël Stadelmann for his help during the initial experiments.

References

- 1 C. Calonder, Y. Tie and P. R. Van Tassel, *P. Natl. Acad. Sci. USA*, 2001, **98**, 10664.
- 2 Y. Tie, C. Calonder and P. R. Van Tassel, *J. Colloid Interf. Sci.*, 2003, **268**, 1.
- 3 M. Malmsten, G. Siegel and W. G. Wood, *J. Colloid Interf. Sci.*, 2000, **224**, 338.
- 4 C. F. Wertz and M. M. Santore, *Langmuir*, 1999, **15**, 8884.
- 5 C. F. Wertz and M. M. Santore, *Langmuir*, 2001, **17**, 3006.
- 6 C. F. Wertz and M. M. Santore, *Langmuir*, 2002, **18**, 1190.
- 7 C. T. Shibata and A. M. Lenhoff, *J. Colloid Interf. Sci.*, 1992, **148**, 469.
- 8 C. T. Shibata and A. M. Lenhoff, *J. Colloid Interf. Sci.*, 1992, **148**, 485.
- 9 D. R. Shankaran, K. V. Gobi and N. Miura, *Sensor Actuat B-Chem*, 2007, **121**, 158.
- 10 R. L. Rich and D. G. Myszk, *Curr. Opin. Biotech.*, 2000, **11**, 54.
- 11 S. Sjölander and C. Urbaniczky, *Anal. Chem.*, 1991, **63**, 2338.
- 12 N. Lion, F. Reymond, H. H. Girault and J. S. Rossier, *Curr. Opin. Biotech.*, 2004, **15**, 31.
- 13 N. F. Y. Durand, A. Bertsch, M. Todorova and P. Renaud, *Appl. Phys. Lett.*, 2007, **91**, 203106.
- 14 R. Karnik, K. Castelino, R. Fan, P. Yang and A. Majumdar, *Nano Lett.*, 2005, **5**, 1638.
- 15 R. B. Schoch, L. F. Cheow and J. Han, *Nano Lett.*, 2007, **7**, 3895.
- 16 W. H. Reinmuth, *J. Phys. Chem.*, 1961, **65**, 473.
- 17 D. Stein, M. Kruithof and C. Dekker, *Phys. Rev. Lett.*, 2004, **93**, 035901.
- 18 R. B. Schoch and R. Philippe, *Appl. Phys. Lett.*, 2005, **86**, 253111.
- 19 M. R. Duncan, J. M. Lee and M. P. Warchol, *Int. J. Pharm.*, 1995, **120**, 179.
- 20 M. Malmsten, *Biopolymers at interfaces*; Marcel Dekker, 2003; Vol. 110.
- 21 U. Böhme and U. Scheler, *Chem. Phys. Lett.*, 2007, **435**, 342.
- 22 H. Morgan and N. G. Green, *AC Electrokinetics: colloids and nanoparticles*, 2003.
- 23 W. M. Clark, A. Bansal, M. Sontakke and Y. H. Ma, *J. Membrane Sci.*, 1991, **55**, 21.
- 24 E. J. Van Der Wouden, D. C. Hermes, J. G. E. Gardeniers and A. Van Den Berg, *Lab Chip*, 2006, **6**, 1300.
- 25 L. J. Bousse, S. Mostarshed and D. Hafeman, *Sensor Actuat B-Chem*, 1992, **B10**, 67.