Lysozyme recognition with aptamer-modified cylindrical nanopores

M. Ali[1,2,#], S. Nasir[1,2], C. Trautmann[1,2], and W. Ensinger[2]

[1]GSI, Darmstadt, Germany; [2]TU Darmstadt, Germany.

Recently nanofluidic channels/pores have attracted a remarkable attention to miniaturize biosensing devices [1]. The working principle of these nanosized sensors is based on the modulation of ionic transport through the nanopore. The protein pores, *e.g.*, α-hemolysin, have been frequently employed for the sensing of a variety of biomolecules. But the fragile embedding lipid bilayers restrain their suitability for more practical purposes. To date, various routes have been investigated to fabricate synthetic robust analogues of biological ion channels. Amongst the various techniques, ion track technology permits control over the number of pores cm^{-2} (from single to multipore membranes), dimensions and shape of the nanopores. Moreover, the pore surface properties can be tuned on demand *via* exploiting the inherent chemical groups on the pore surface.

Ion current rectification is the main characteristics of pores with asymmetric geometries or pores having non-homogeneous fixed charge distributions on their inner pore surface. Here, we demonstrate that aptamer-protein bioconjugates can provide another useful strategy to incorporate a non-homogeneous fixed charge distribution in cylindrical nanopores leading to ion current rectification.

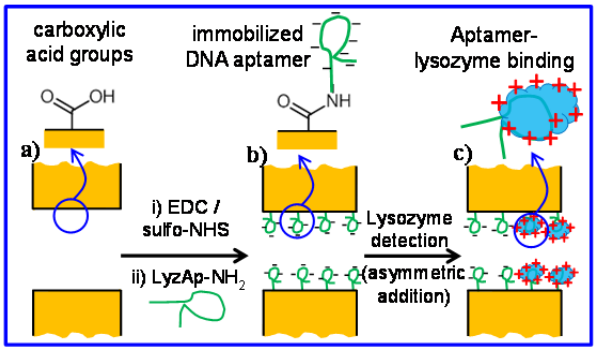

Figure 1: Scheme representing **a)** a cylindrical pore with surface carboxylic acid groups, **b)** the immobilization of DNA aptamer (LyzAp-NH_2) via carbodiimide coupling chemistry, and **c)** protein (lysozyme) conjugation.

In this study, single swift heavy ion irradiated polyethylene terephthalate (PET) membranes of 12 µm thickness were used to fabricate single cylindrical nanopores by symmetric track-etching method [2]. The amine-terminated single-stranded DNA aptamer, *i.e.*, 5′-NH_2-$(CH_2)_{12}$-ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3′ (LyzAp–NH_2) which has the ability to selectively bind with lysozyme (Lyz) protein was selected as model system. The LyzAp–NH_2 molecules were covalently immobilized on the pore walls via carbodiimide coupling chemistry (Fig. 1) [3].

After aptamer immobilization, the next step was to study the biomolecular recognition events inside the confined geometries. Figure 2a shows the *I–V* curves obtained when the modified pore was exposed asymmetrically to lysozyme (*p*I = 11.35) solutions of various concentrations. Due to its high isoelectric point, Lyz molecules were positively charged in our experimental conditions. Therefore, the binding of Lyz with aptamer chains resulted in the switching of surface charge polarity (Fig. 1c). Because of the opposite polarity of fixed surface charges on either half of the pore, the applied electric potential resulted in an asymmetric flow of ions from the two pore ends. This led to ion current rectification because of high resistance for the flow of ions at positive voltages ($V > 0$) compared to negative ones ($V < 0$).

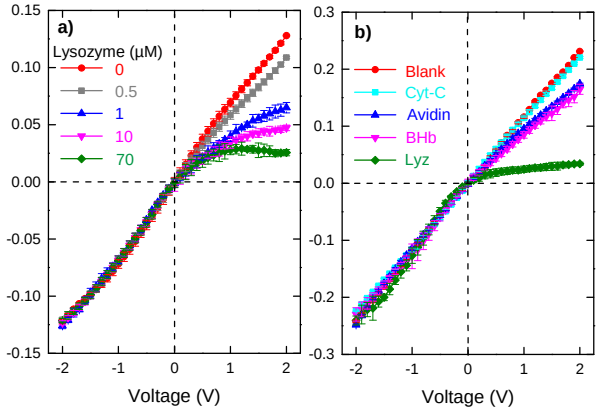

Figure 2: a) *I–V* curves of aptamer-modified single cylindrical nanopore (d ~20 ± 3 nm) when exposed to different protein concentration, asymmetrically. b) *I–V* curves of modified single cylindrical pore (d ~ 40 ± 3 nm) upon asymmetrically exposing to an electrolyte solution containing no protein, cytochrome C (180 µM), avidin (5 µM), bovine hemoglobin (5 µM) and lysozyme (70 µM), separately.

Moreover, we have also demonstrated sensor specificity against Cyt-C, avidin and BHb proteins. From the respective *I–V* curve (Fig. 2b) a slight reduction in positive currents was noticed due to the physical adsorption of large sized protein molecules on the pore opening. But for Cyt-C (almost similar to Lyz in size and polarity) we did not observe any change in *I–V* curve.

In summary, we have miniaturized a nanofluidic biosensing device based on synthetic nanopores modified with DNA-aptamer molecules. We believe that based on the modulation of nanopore transport properties such nanoporous systems can be further extended for the recognition of a variety of proteins and small organic molecules which exhibit an affinity towards a specific aptameric probe.

References

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M.Ali@gsi.de