Supporting Information

Gating protein transport in solid state nanopores by single molecule recognition

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Experimental

Nanofabrication: The different steps in the nanofabrication were performed as described in earlier work.1-2

Cleaning: Prior to experiments all samples were cleaned with basic piranha for 25 min. After rinsing with Milli-Q water the samples were sonicated in ethanol (99.7%) for 5 min. The samples were then dried using nitrogen and immediately transferred to the polymer grafting solution or mounted in a flow-cell.

Chemicals: Phosphate buffered saline (PBS) tablets were dissolved in milli-Q water. Avidin (NeutrAvidin, ThermoFisher) and bovine serum albumin (Sigma) were stored frozen at 1 g/L in water. The PEG antibody E11 was kindly provided by Dr. Steve Roffler at the Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan. The grafting of thiol terminated PEG (Laysan Bio) was performed as described earlier.3 Alkanethiol-EG6 (OEG) was purchased from Prochimia.

Electron microscopy: Images were acquired using a Zeiss Supra 60 VP.

AFM: High speed AFM (RIBM, Japan) was performed with a homebuilt scanner that achieved a maximum scan speed of 200 ms per frame. AC10 cantilevers (Olympus) were used for all measurements. The nominal spring constant was 0.1 N/m and the resonance frequency in liquid was ~400 kHz. Amorphous carbon tips were fabricated on top of the silicon lever 10° tilted from the direction perpendicular to the sample surface using a scanning electron microscope. AFM data post-processing was performed as described previously.4 The relative height change was calculated by comparing the height of the planar region to the pore region.2 The real-time movie of the pore opening available as Supporting Information for this paper starts just after the injection of the AB.
SPR: The angular reflectivity spectrum was obtained using a SPR Navi instrument (BioNavis, Finland) with ordinary Au sensor slides. The grafting density on planar Au was determined by fitting Fresnel models to data as described previously.\(^3\)

Fluorescence: All fluorescence measurements were conducted using a Zeiss Axio Imager Z2 upright microscope equipped with an Axiocam506 camera. Membrane transport was measured using a Plan-Apochromat 63× water immersion objective (NA = 1.0) immersed in a 300 µL droplet of PBS buffer placed on top of the membrane. After the first frame was captured 0.8 µM fluorophore (BSA labelled with Alexa 488, ThermoFisher) was injected in the flow chamber (chamber volume ~300 µL, below the membrane) using a peristaltic pump (1.5 mL of fluorophore was injected in ~80 s). Images were acquired every 20 s with a 100 ms exposure time. The fluorescence was analyzed by defining a 25×25 µm\(^2\) square starting 25 µm from the membrane edge. The initial frame (background) of each image stack was subtracted and then all values were normalized to the fluorescence in the center of the membrane at 10 minutes.

**Quartz crystal microbalance (QCM) results**

QCM was used to test the amount of antibody adsorbing to glass, which is the material exposed in the cavities under the nanopores in gold. Measurements were performed using quartz crystal microbalance with dissipation monitoring (Q-sense E4, Biolin Scientific), using sensor crystals coated with borosilicate (Biolin Scientific). Fig. S1 shows the response upon addition of the antibody in PBS buffer. The antibody binding results in a very small signal (<2 Hz) compared to the monolayer formation of average sized proteins, e.g. avidin, under identical conditions (40 Hz).\(^2\) Since the IgG AB is a large protein it can be concluded that it binds very little to glass, at least in PBS buffer at the concentration used.
**Height measurement using non-interacting molecules**

To determine the effect of the AB on the protein exclusion height of the PEG brush we conducted measurements using non-interacting molecules. The heights in Fig. 1 of the main text were calculated in the same manner as described previously, except that normalization to the TIR angle was used as described below. For the heights at lower AB concentrations, mixtures of AB and BSA were used and compensated for as follows: First, repeated injections of BSA (10 g/L) were performed over the formed brush. Next, AB injections were performed to be able to subtract the effect of AB binding from the BSA and antibody mixture. Finally, to see the effect of the antibody on the height, different concentrations of AB was added to the BSA solution and injected. All injections were normalized to the change in refractive index, determined by the shift in the TIR angle to make the injections independent of variations in the concentration of BSA.

For a standard BSA injection the ratio between the SPR signal and TIR angle is defined as:

$$\Delta R_{BSA} = \frac{\Delta BSA_{SPR}}{\Delta BSA_{TIR}}$$

In the case of the mixture the ratio was calculated by subtracting the effect of the antibody itself (average shift of 3 injections for each concentration) to obtain the bulk signal:
\[ \Delta R_{AB} = \frac{\Delta M_{\text{SPR}} - \langle \Delta A_{\text{SPR}} \rangle}{\Delta M_{\text{TIR}} - \langle \Delta A_{\text{TIR}} \rangle} \]

To calculate the absolute height the injections were compared to injections made over a gold surface functionalized with a short self-assembled monolayer of OEG. The ratio for BSA injections over the OEG layer, \( \Delta R_{\text{ref}} \) was determined to be 1.293 ± 0.007 at 785 nm and 1.577 ± 0.013 at 670 nm.

The height was then calculated by the following formula:

\[ d_{\text{PEG}} = \log \left( \frac{\Delta R_{\text{ref}}}{\Delta R_{\text{sample}}} \right) \times \frac{\delta}{2} + d_{\text{OEG}} \]

Where \( \Delta R_{\text{ref}} \) is the normalized shift for BSA injections on the OEG surface, \( \Delta R_{\text{sample}} \) the BSA shift measured for PEG with/without antibody present, \( \delta \) is the effective surface plasmon decay length at the wavelength used (254 nm for 670 nm laser, 364 nm for 785 nm laser), and \( d_{\text{OEG}} \) the thickness of the OEG layer used as a reference surface for the injections (~2 nm).

**Figure S2.** Example of measured SPR and TIR shifts when injecting BSA, AB or a mixture of the two.

**Membrane pores**
The same membranes with plasmonic nanopores as in previous work were used. An example is shown in Fig. S3. The fabrication method is the same as for nanowells, only that the membrane is used as a support instead of SiO$_2$. Thus the final pores have a shape identical to the nanowells except that there is an opening at the other end.\textsuperscript{1} The Si$_3$N$_4$ membrane is 50 nm thick and square shaped with a side length $>100 \mu$m.

![Figure S3](image.jpg)

**Figure S3.** Example electron microscopy image of a membrane with plasmonic nanopores.

**Kinetic model for AB binding to PEG**

The kinetic models proposed can qualitatively replicate the observed binding measured using SPR. When varying the factor $n$ representing the greater surface area occupied by strong antibodies it can be shown that a value $>2$ is needed to obtain similar kinetics as measured. Both models can replicate the decrease in signal shown for the higher concentrations used. While the heterogenous model shows a greater decrease when weak antibodies are replaced with stronger binding ones, the sequential model shows a slower association phase, even slower than the measured data. To perfectly replicate both the fast association and the decrease in signal proved difficult with either model, which could reflect that both sequential and parallel binding to the strong state may occur. Regardless, the model confirms the presence of two binding states where one occupies $n$ times more PEG (with $n \approx 3$) and shows that a quasi-equilibrium can be established for lower concentrations (a few
µg/mL) and short exposure times (tens of min). Additional examples of solutions to the association and dissociation kinetics are shown in Fig. S4. Simulated binding curves for longer association times are shown in Fig. S5.

By UV spectroscopy (Nanodrop, ThermoFisher) we measured the protein content in the AB solution to be 42% of the mass-based concentration. All concentrations ($C_0$) are specified as those obtained as weight over volume, but in the calculations the concentration was multiplied by a factor of 0.42 to account for this.

Note that in order to translate the mass coverage into an SPR shift, calculations were performed as described previously. This is a generally established methodology for SPR quantification, but a small degree of uncertainty remains in the values for protein refractivity, field extension etc. However, any error will only influence the $\Gamma_{\text{max}}$ parameter, not the rate constants.

![Figure S4](image)

**Figure S4.** Example solutions to the differential equations with rate constants set to $k_{\text{on}} = 1.25 \times 10^3 \text{M}^{-1}\text{s}^{-1}$, $k_{\text{off}} = 7 \times 10^4 \text{s}^{-1}$, $k_{\text{par}} = 3 \times 10^2 \text{M}^{-1}\text{s}^{-1}$, $k_{\text{seq}} = 1.5 \times 10^8 \text{cm}^2\text{s}^{-1}$ and $\Gamma_{\text{max}} = 3 \times 10^{12}$ cm$^2$. The parallel model is shown in solid lines and the sequential in dashed lines. Note that the simulated injection of ABs is longer in the left graph.
**Figure S5.** Example solutions for longer association phases plotted as fractions of AB bound by strong or weak interactions. The equilibrium state is that all AB become strongly bound but it takes a long time to establish.
References (also cited in main text)

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