L07-02785R-Electrical Characterization of Protein Molecules in a Solid-State Nanopore

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SUPPLEMENTARY MATERIALS.

Chemiluminescent ELISA assay. A sensitive chemiluminescence enzyme-linked immunoabsorbance assay (ELISA) was used to confirm that BSA molecules were passed through a nanopore from the *cis* to the *trans* chamber during nanopore experiments. About ~100 µl solution from the *trans* chamber was collected after a BSA translocation experiment. An Immunoenzymetric assay kit (Cygnus Technologies, Catalog #F030) was used to quantify the amount of translocated BSA with an optimized assay protocol. Initially, 100 µl of diluted BSA standards (0, 1.0, 5.0, 10, 50 and 100 pg), along with the trans chamber sample, were mixed with 100 µl HRP (Horse Radish Peroxidase) conjugate sheep anti-BSA antibody and incubated overnight in a test tube. The 200 µl mixtures were subsequently separated into two identical sets of 100 µl each and transferred to anti-BSA antibody coated microtiters and incubated for 1 hour to capture the BSA-HRP-antibody complex. The liquid was then aspirated and the residual was blotted with absorbent paper. The sample wells were then washed 6 times and a chemiluminescent substrate for HRP (Luminigen PS-atto, Lumigen) was added. Reaction of the substrate with HRP generates a high-intensity and sustained luminescent signal with a stable peak intensity achieved within seconds and maintained for hours. The sustained luminescent signal provided a distinct advantage when the measurement was made using a luminometer set to integration mode. Two sets of samples were measured and five independent measurements were performed for each set. For our work, we acquired the luminescence signal using two different methods: a TD20/20 luminometer (Turner Biosystems) and an Alpha Innotec FluorChem 8900 imaging system. The sensitivity and the acquisition time for the luminometer were established so that the integrated luminescence intensity (relative Luminescence Units, RLU) was within the mid-scale for the most concentrated sample (50 pg/100 µl). The luminescence intensity of all samples was determined (five luminescence measurements per sample) for the standards and the test samples. This chemiluminescent assay has the sensitivity to detect as few as 10,000 BSA molecules (www.lumigen.com).

The measured luminescence from the 5 BSA standards: 0, 0.5, 2.5, 5, 25 and 50 pg per 100 µl, as well as the sample collected from the trans chamber is shown in Fig. 3. One set of the standards and the *trans* chamber sample were also analyzed using a CCD imaging system set (10 min acquisition time) to confirm the data. The 0 pg standard (well 0) shows the background signal (Fig. S1), and the luminescence decreases proportionally as the concentration of BSA decreases from 50 pg (well 1) to 0.5 (well 5). The luminescence images are recorded as halo circles around the wall of each well, demonstrating that the luminescence is originated from the enzymatic activity of the HRP on the HRP-labeled antibody that is attached to the wall. The luminescence intensity is estimated within the range of 0.5 to 2.5 pg, in agreement with the data obtained with the luminometer.

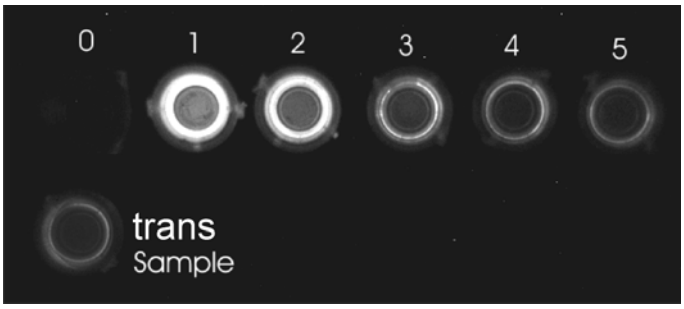

FIG. S1. Luminescence measurements for BSA standards: (0)-0pg BSA, (1)-50 pg BSA, (2)-25 pg BSA, (3)-5 pg BSA, (4)-2.5 pg BSA, (5)-0.5 pg BSA, and the *trans* chamber sample.