Electronic Supplementary Material (ESI) for Chemical Science.
This journal is © The Royal Society of Chemistry 2017

Supplementary Information

Selective Single Molecule Nanopore Sensing of Proteins Using DNA Aptamer-functionalised Gold Nanoparticles

Xiaoyan Lin, Aleksandar P. Ivanov,* and Joshua B. Edel*

Department of Chemistry, Imperial College London, South Kensington, London SW7 2AZ, United Kingdom

Materials and Methods

Fabrication of nanopipettes. Quartz capillaries (Intracel Ltd, UK), with an outer diameter of 1.0 mm and an inner diameter of 0.5 mm including inner filament, were cleaned by the plasma cleaner. The capillaries were then pulled by a laser-based pipette puller (Sutter Instrument, P-2000) through a two-line protocol: (1) HEAT, 520; FIL, 3; VEL, 35; DEL, 185; PUL, 45; (2) HEAT, 900; FIL, 0; VEL, 15; DEL, 128; PUL, 200. It should be noted that the above parameters are instrument specific and were optimized to yield 20 nm openings at the tip of the nanopipette.

Reagent and solution. 5 nm gold nanoparticles (G-5-20) were purchased from Stratech Scientific Limited. Lysozyme (L6876), trypsin (T4799), cytochrome C (C2037) and tween 20 were obtained from Sigma Aldrich. TCEP solution and DNA aptamers were ordered from Thermo Scientific. PEG - thiol (MW 1000, MPEG-SH-1000-1g) was from Laysan Bio Inc. The translocation buffer was made up of 10 mM Tris, 1 mM EDTA, 100 mM KCl, pH 7.4. The reaction buffer for aptamer and lysozyme contained 20 mM Tris, 2 mM EDTA, 100 mM NaCl, 5 mM $MgCl_2$, pH 7.4. The AuNP-LBAs were incubated with the proteins in the reaction buffer for 40 min before the translocation studies.

Attachment of aptamers to the surface of AuNPs. The disulphide modification on the aptamer oligonucleotides was cleaved by the addition of 10 mM TCEP and incubated at room temperature for 0.5 h. 100 nM freshly cleaved aptamers were added to 50 nM AuNPs and allowed to incubated at room temperature for 1.5 h with continuous mixing, after which 0.01% tween 20 and 200 nM PEG-thiol were added.[1] The sample was centrifuged at 12000 rpm for 30 min and the supernatant was removed. The PEG-thiol modified AuNPs were prepared in the same way but without the addition of DNA aptamers.

Transmission Electron Microscopy (TEM). 20 μL of AuNPs samples were loaded on the grids (Agar Scientific) and characterised by JOEL 2000FX TEM. The diameter was calculated by averaging 200 particles from the TEM images using Image J software (developed at the National Institutes of Health).

UV-Vis Spectroscopy Measurement. UV-Vis measurement was conducted to investigate the concentration and stability of the AuNPs samples. All UV-Vis spectra were recorded using Nanodrop 2000c (Thermo Scientific), scanning from 200 nm to 850 nm. The extinction coefficient (ε) for 5 nm AuNPs is 8.56×10^6 L/ (mol·cm).[2]

Fluorescence Measurement. Fluorescence measurements were carried out on a Horiba Jobin Yvon Spectrophotometer (Horiba, Germany). The excitation wavelength was set at 495 nm, with a 2 nm bandwidth and 0.2 s integration time. The emission spectra were obtained by scanning the emission from 510 to 800 nm in steps of 1 nm/s. Fluorophore-labelled DNA (FAM-DNA) was used to determine the number of DNA per AuNP.[3] Fluorescent intensity of different concentrations of FAM-DNA was measured to give a standard curve. After the attachment, the DNAs on the surface of AuNPs were cleaved with TCEP by incubating at room temperature for 5 h. Centrifugation was conducted to separate the free DNAs from AuNPs, after which the fluorescent intensity of DNAs was measured. The number of DNA per AuNP was calculated by dividing the concentration of DNA to the concentration of AuNP.

Ion Current Measurement. The ion currents of translocation were measured with AxoPatch 200B patch clamp amplifier (Molecular Devices, US). Electrodes (Ag/AgCl) were inserted into the external bath (patch electrode) and the nanopipette (ground electrode), respectively. The ion currents were filtered with a 10 kHz Bessel filter and recorded by the software pClamp 10 (Molecular Devices). For the sub-complex molecular information study, the high bandwidth amplifier VC100 (Chimera Instruments) was used to record the current signal (sampling at 1MHz) and the data was filtered with a 30 kHz-100kHz digital Bessel filter. The data was analysed using a custom-written MATLAB code.

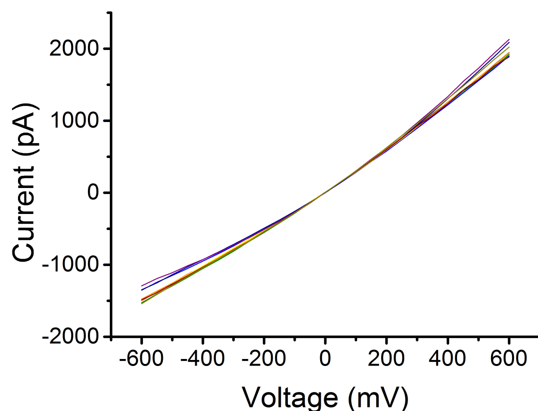

Figure S1. Current – voltage curves of 10 representative nanopipettes using 100 mM KCl, exhibiting current rectification behaviour with an average rectification ratio $I_{500\ mV}/ I_{-500\ mV}$ of 1.4 ± 0.1. The nanopipettes showed a resistance of 354 ± 18 MΩ as measured in (− 0.1 V, 0.1 V) regime. The nanopipettes used in this work had resistance variation within 5%.

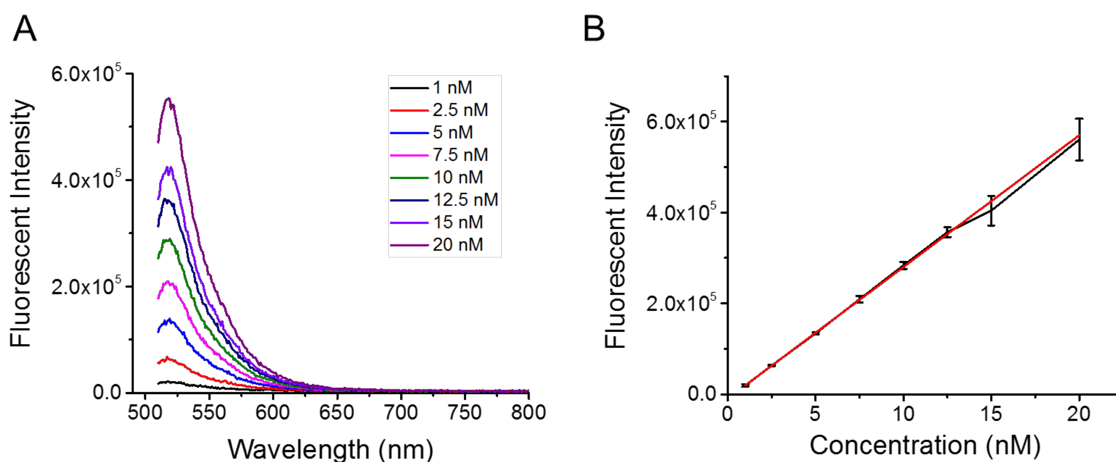

Figure S2 A. Fluorescent spectra of different concnetrations of FAM-DNA. **B.** The fluorescent intensity of FAM-DNA as a function of concentration, serving as the standard curve to determine the concentration of FAM-DNA. The FAM-DNAs were cleaved from the surface of AuNPs by TCEP and separated from the AuNPs by centrifugation. The concentration of the FAM-DNA was determined from the fluorescence measurement and the number of DNA per AuNP was calculated by dividing the concentration of DNA to the concentration of AuNP.

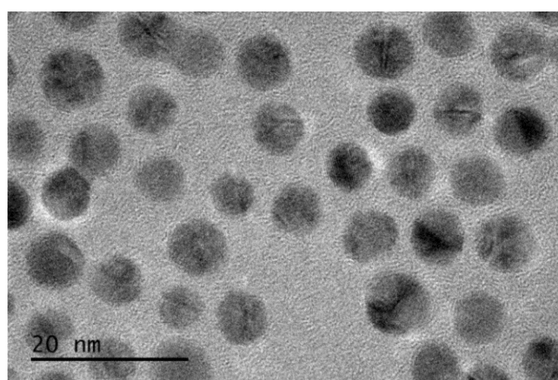

Figure S3. TEM image of the aptamer-modified AuNPs (AuNP-LBAs), hilighting the good uniformity and dispersion of the AuNPs after the functionalisation of LBA.

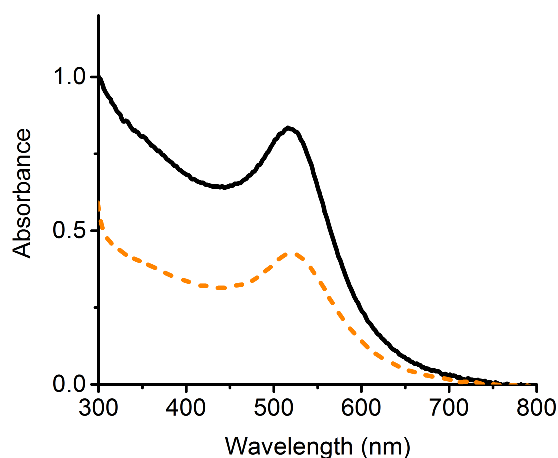

Figure S4. UV-Vis spectra of AuNP-LBAs (solid, black) and AuNP-LBA/lysozyme complexes (dashed, orange), confirming no aggregation after the binding between AuNP-LBA and lysozyme.

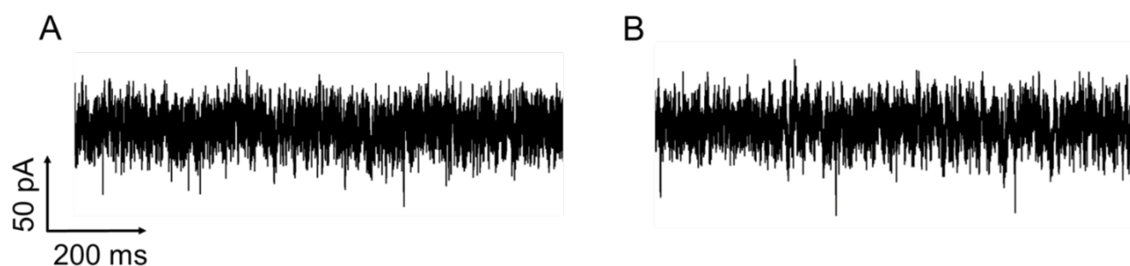

Figure S5. Current – time traces of LBA + lysozyme (**A**) and AuNPs + lysozyme (**B**) at 600 mV. Both showed small current amplitude, further comfirming the events with large current amplitude were brought by the AuNP-LBA/lysozyme complexes.

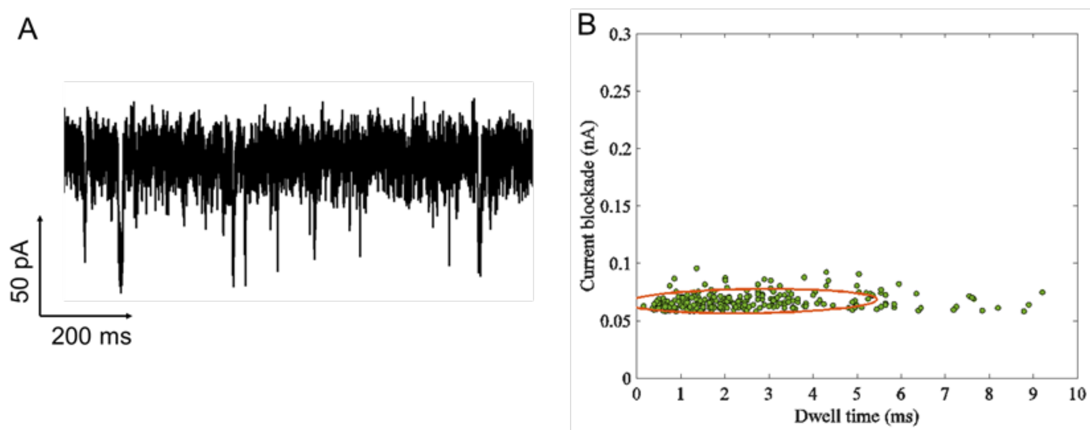

Figure S6. (A) Current-time trace of lysozyme binding with AuNPs functionalised with average 2 aptamers, with some events exhibiting long dwell time; **(B)** Scatter plot of the current blockade versus dwell time for the translocation events of lysozyme binding with AuNPs functionalised with average 2 aptamers, showing broader distribution compared with AuNPs functionalised with one aptamer. Translocations were only observed with larger-sized nanopipettes, which can be explained by the larger size of the AuNPs binding with more aptamers and lysozymes that cannot go through the 20 nm nanopipettes.

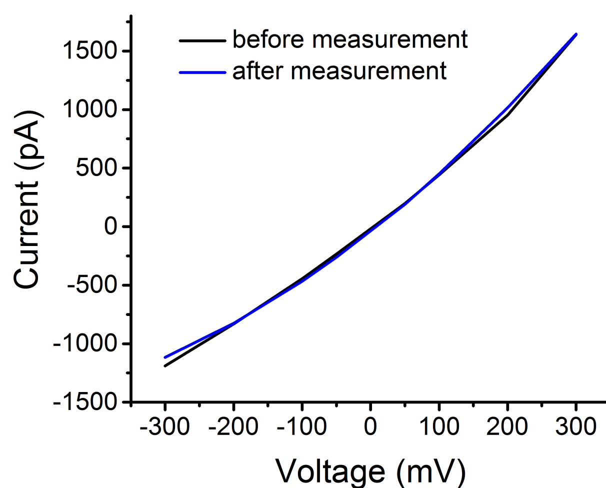

Figure S7. Current – voltage curves of the nanopipette before and after the translocation experiment of the AuNP-LBA/lysozyme complexes. There was little variation, indicating minimal of any analyte adsorption to the nanopore surface.

References

(1) Li, J. X.; Zhu, B. Q.; Yao, X. J.; Zhang, Y. C.; Zhu, Z.; Tu, S.; Jia, S. S.; Liu, R. D.; Kang, H. Z.; Yang, C. J. *Acs. Appl. Mater. Inter.* **2014**, *6*, 16800.

(2) Liu, X. O.; Atwater, M.; Wang, J. H.; Huo, Q. *Colloids Surf., B* **2007**, *58*, 3.

(3) Hurst, S. J.; Lytton-Jean, A. K.; Mirkin, C. A. *Anal. Chem.* **2006**, *78*, 8313.