Title: Observation of Single Protein and DNA Macromolecule Collisions on Ultramicroelectrodes

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Supplementary Materials:

Figures S1 – S4, including materials and methodology and additional experimental data.

Materials and Methodology:

Chemicals and Reagents:
Water used in each experiment was Milli-Q water (Massachusetts, USA). Phosphate buffer was purchased from Fisher scientific. Ferrocyanide was purchased from Fisher Scientific and used without further purification and used for all collision experiments. Ferrocenedimethanol was purchased and used without further purification and was used to test the electrodes after laser pulling and focused ion beam milling. Catalase from bovine liver and glucose oxidase from aspergillus niger were purchased form Sigma Aldrich. Horseradish peroxidase was purchased from Thermo Scientific. All enzymes were purchased as a lyophilized powder. The 22 nm polystyrene beads were purchased from Thermo Scientific as a standard with a certificate of calibration and traceability as NIST certified spheres. The certified mean diameter was 22 nm plus or minus 2 nm, which agrees well with electrochemical data. All chemicals were used as received. Mouse monoclonal anti-gB neutralizing antibody (Clone MAb97.3) was provided by Dr. Michael Mach (University of Erlangen, Germany). Plasmid DNA was provided by the laboratory of Dr. Jason Upton at the University of Texas at Austin.

Instrumentation:
Electrochemical experiments were performed using a CHI model 920C potentiostat (CH Instruments, Austin, TX). The ultramicroelectrodes were prepared using a previously reported procedure using a laser-pulling instrument (Sutter Instruments)[i] followed by milling with a focused ion beam (FEI Strata DB235 dual beam SEM/FIB). In short, a 20 um Pt wire was placed in a glass capillary, which was then pulled with a laser puller to produce a sharp tip of nanometer dimensions. The size of the electrode was determined by SEM imaging and electrochemistry. The three-electrode cell was placed in a faraday cage and grounded to a pipe. A Ag/AgCl (1M KCl) wire was used (BASi, West Lafayette, Indiana) as the reference electrode, and a Pt wire was used as the auxiliary electrode.

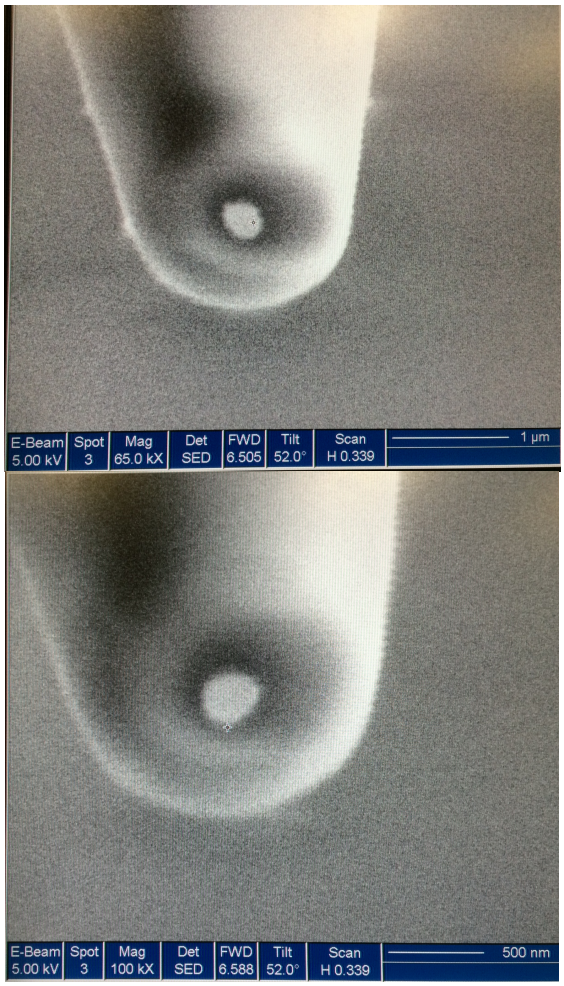


Figure S1. SEM Images of Electrodes used in these experiments after FIB milling.

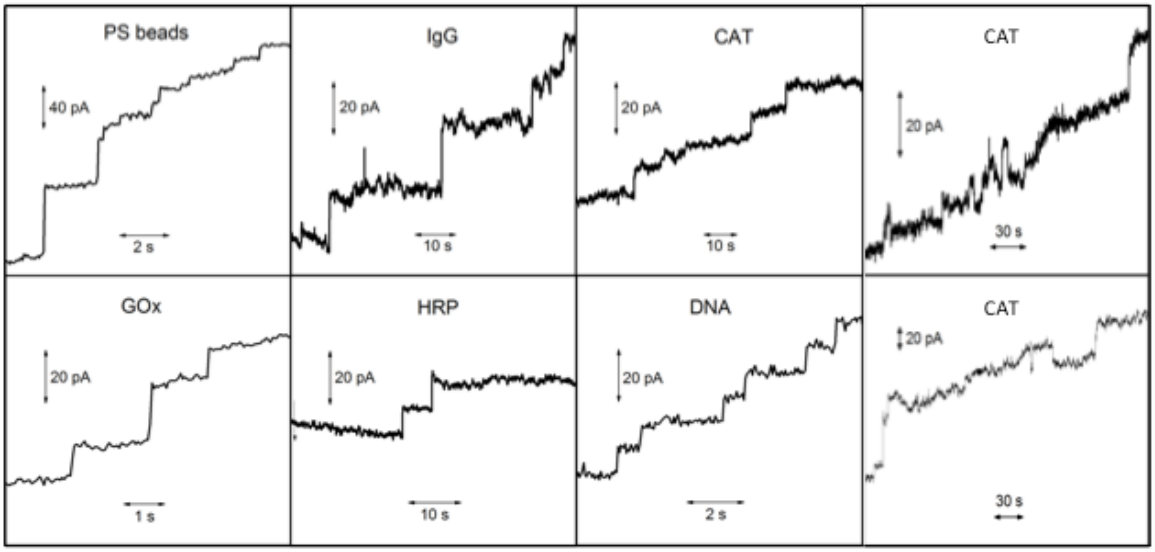


Figure S2. i-t curves for various proteins and polystyrene nanospheres. The last two panels, however, show interesting behavior seen with larger proteins, such as catalase, which manifests itself also in the IgG antibody data.

Addition of Acid to Catalase solution

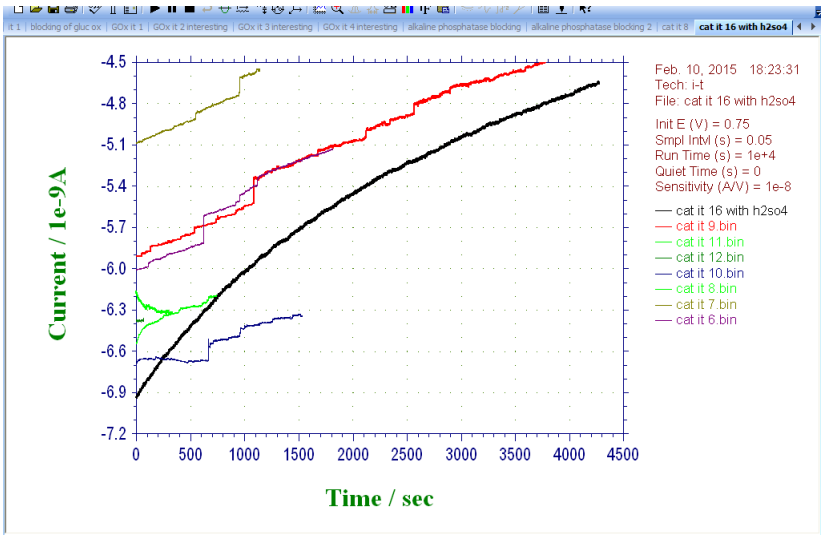


Figure S3. Addition of acid (black curve, pH ~ 0) to the catalase solution (other colored curves) showed no blocking events, which indicates the denatured protein's inability to block the electrode.

We used the blocking collision methodology to try and analyze the quality of a molecular sample by looking at the difference between a 3-year-old sample of lyophilized HRP (stored in freezer) and a new sample of HRP, which was also stored in the freezer.

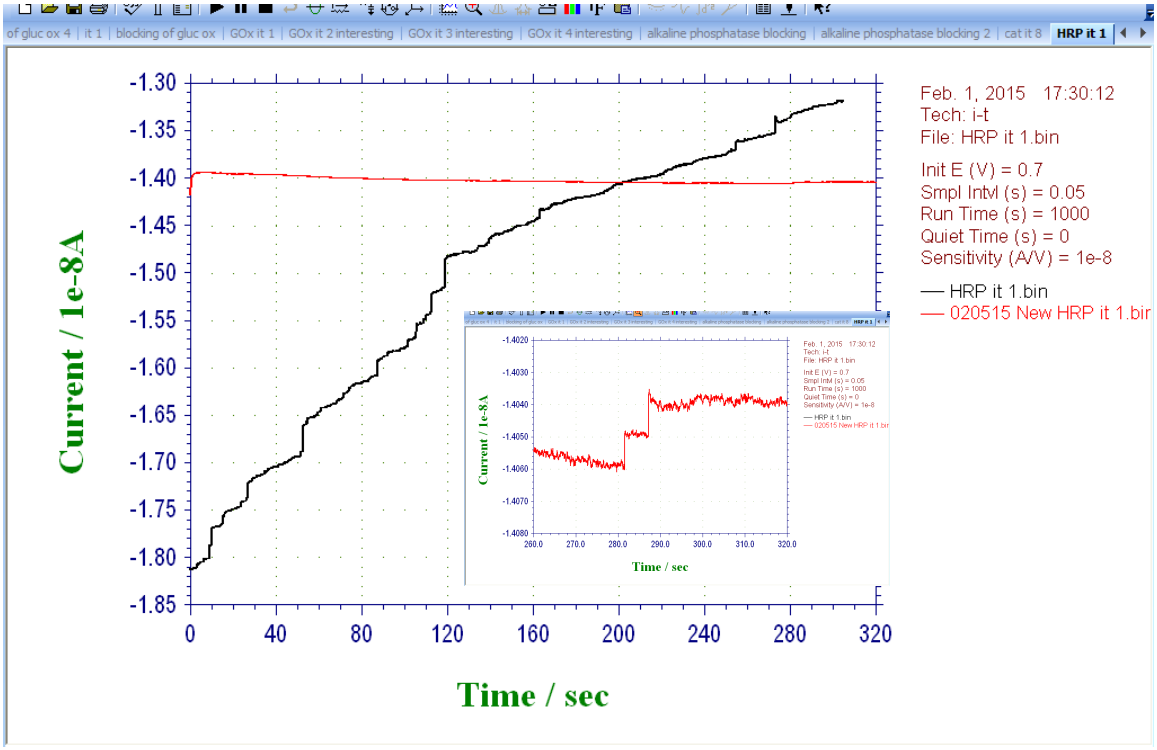


Figure S4. Difference between old and new HRP samples.

From the i-t behavior in Figure S4, it is evident that the there are much larger current step heights in the older solution compared to the new solution, which may indicate a high extent of aggregation of the older proteins.

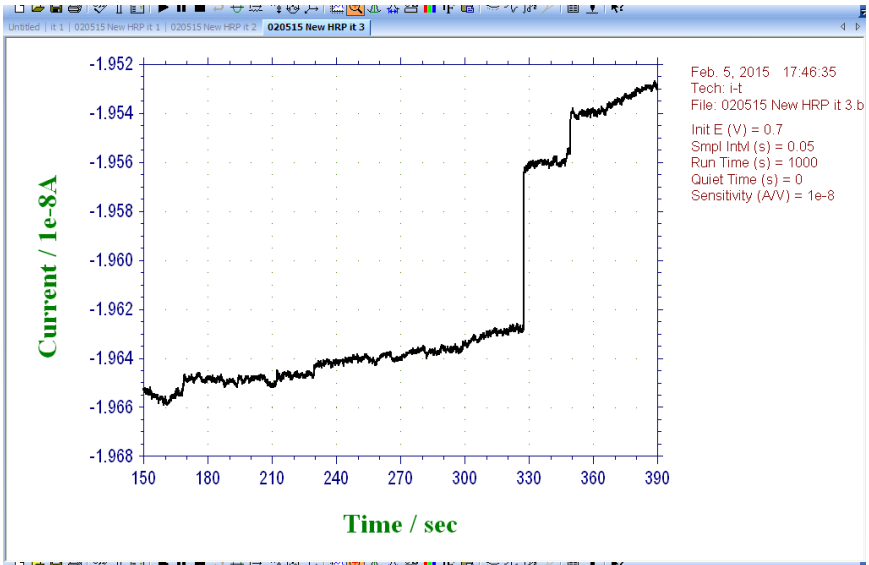
Calculation of size of pDNA:

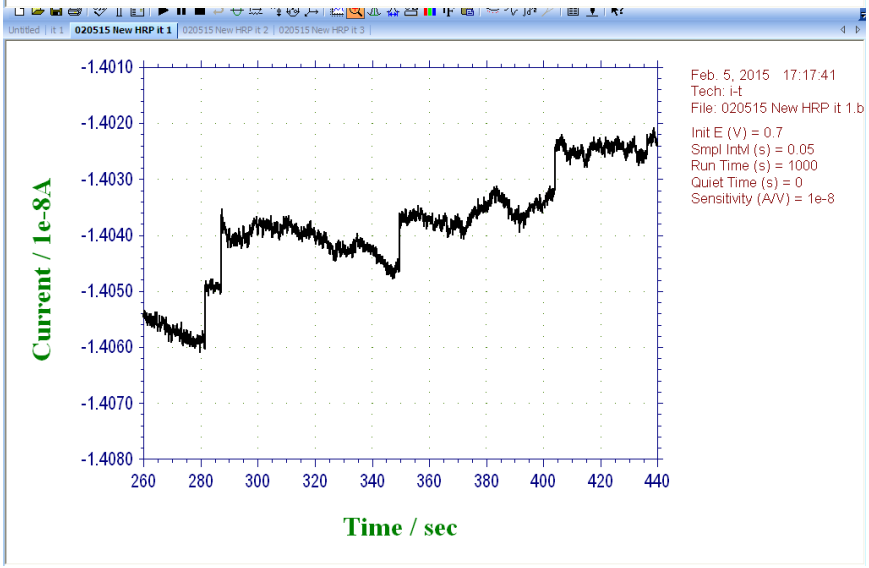
Composition of pDNA:

- pDNA is Bacterial DNA grown in E-Coli
- Composition for one strand:
  - Adenine: 1,140
  - Guanine: 1,246
  - Cytosine: 1,285
  - Thymine: 1,062
- 4733 bases at 3.4 angstroms apiece is 1610 nm circumference = 250 nm radius

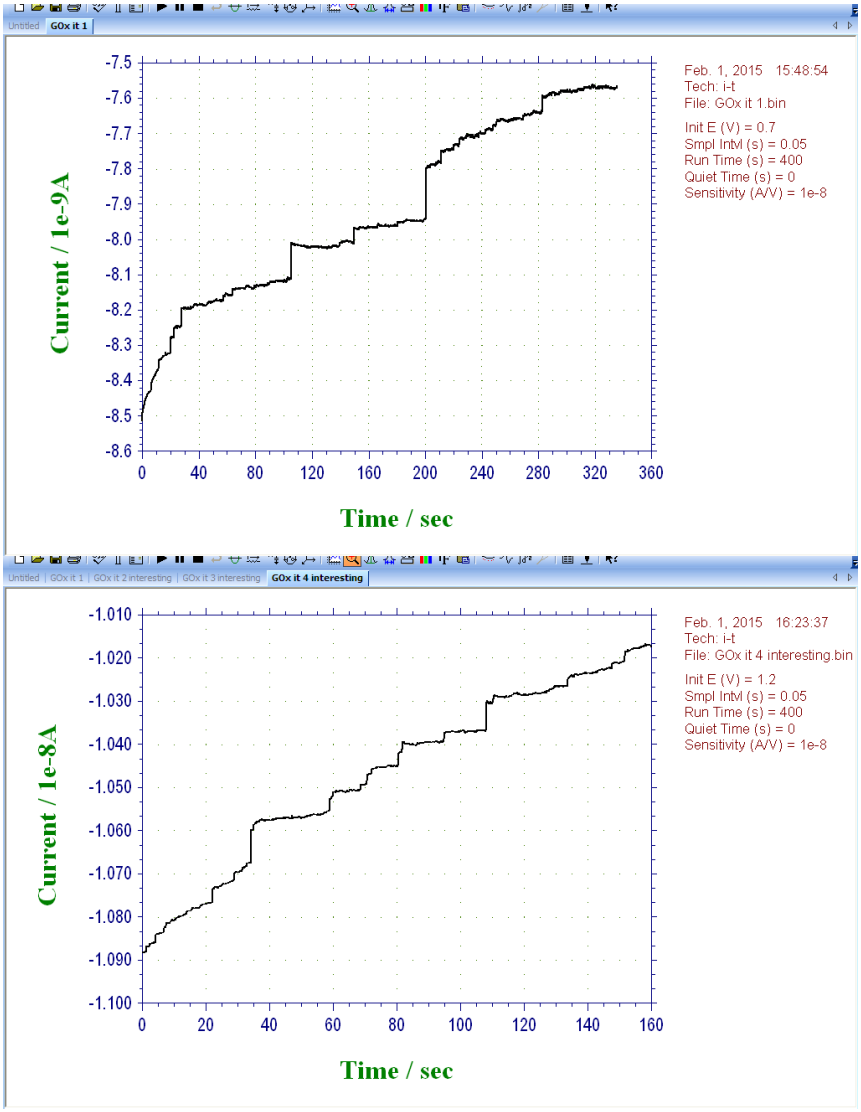
More experimental examples of blocking by type:

Examples of Horseradish Peroxidase:

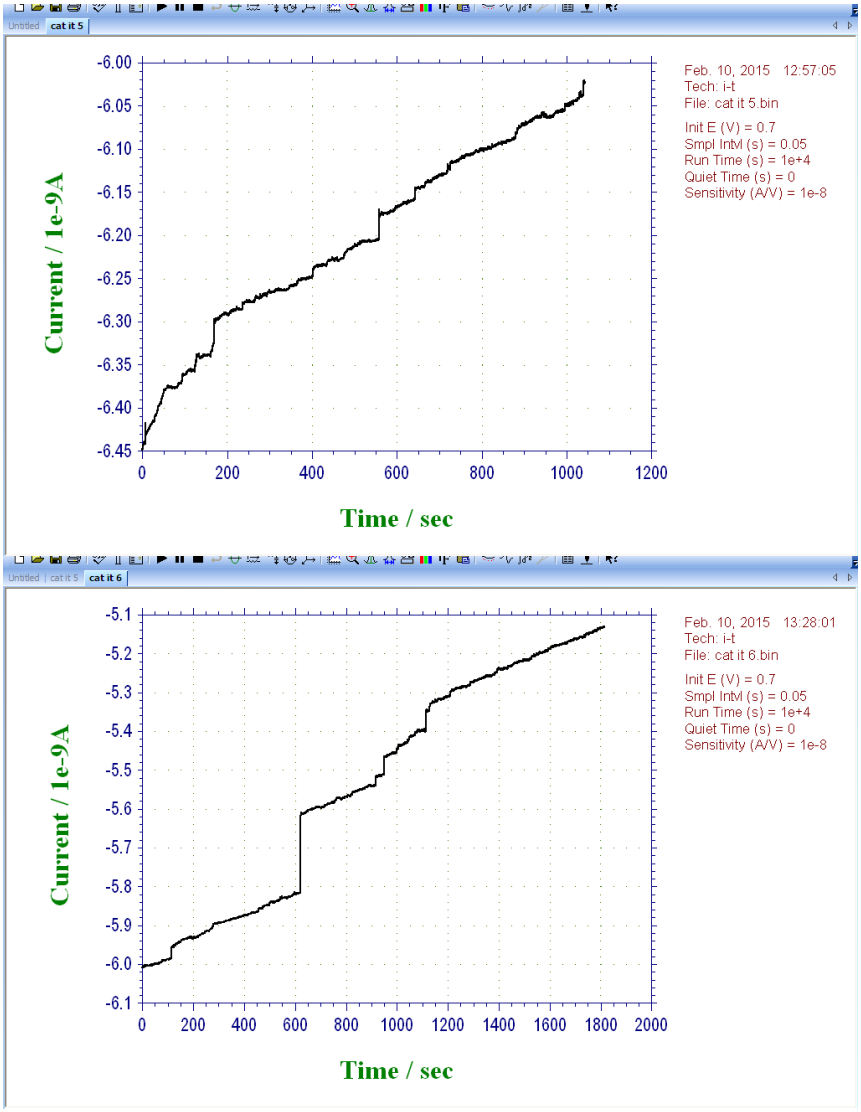


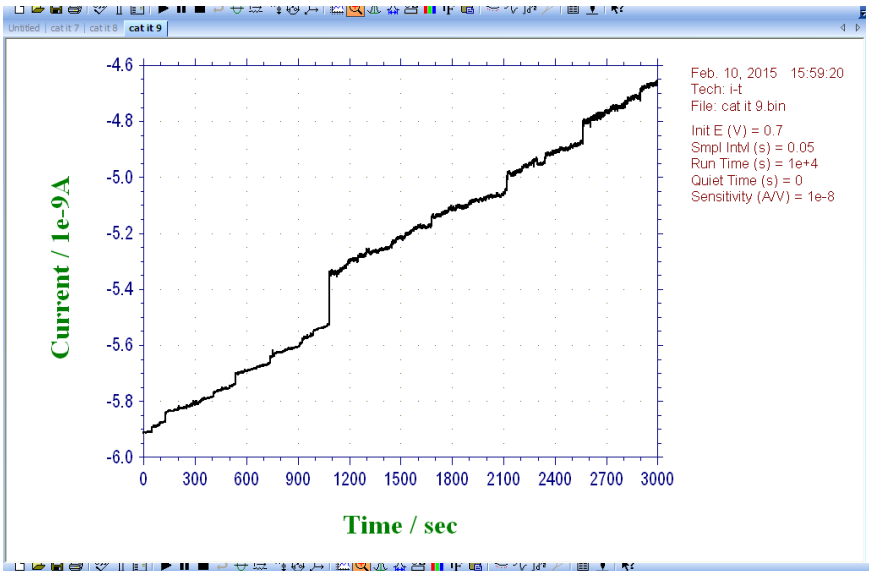


Examples of Glucose Oxidase:



Examples of Catalase:

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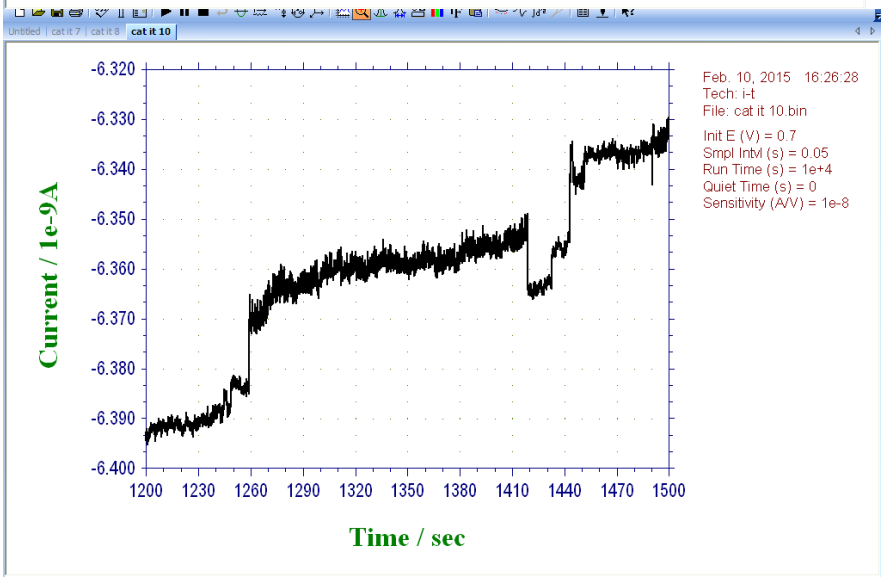
Time / sec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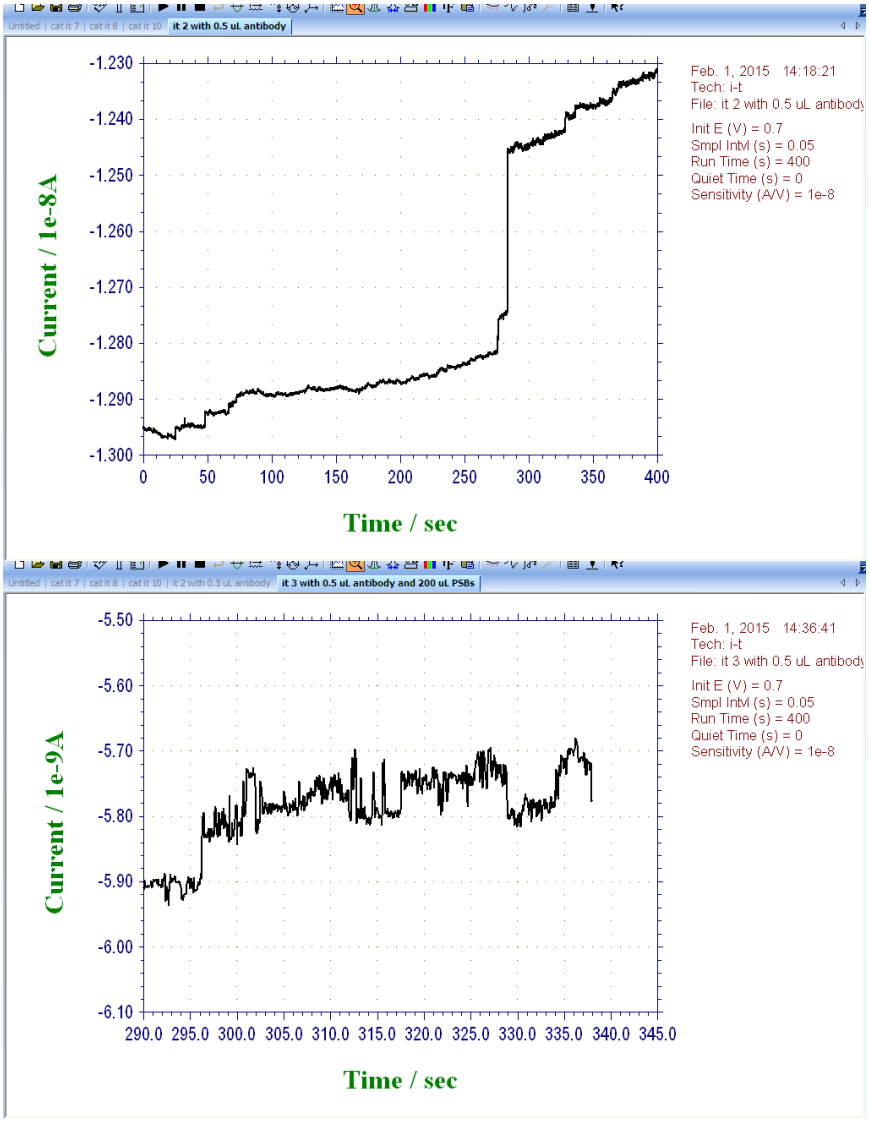
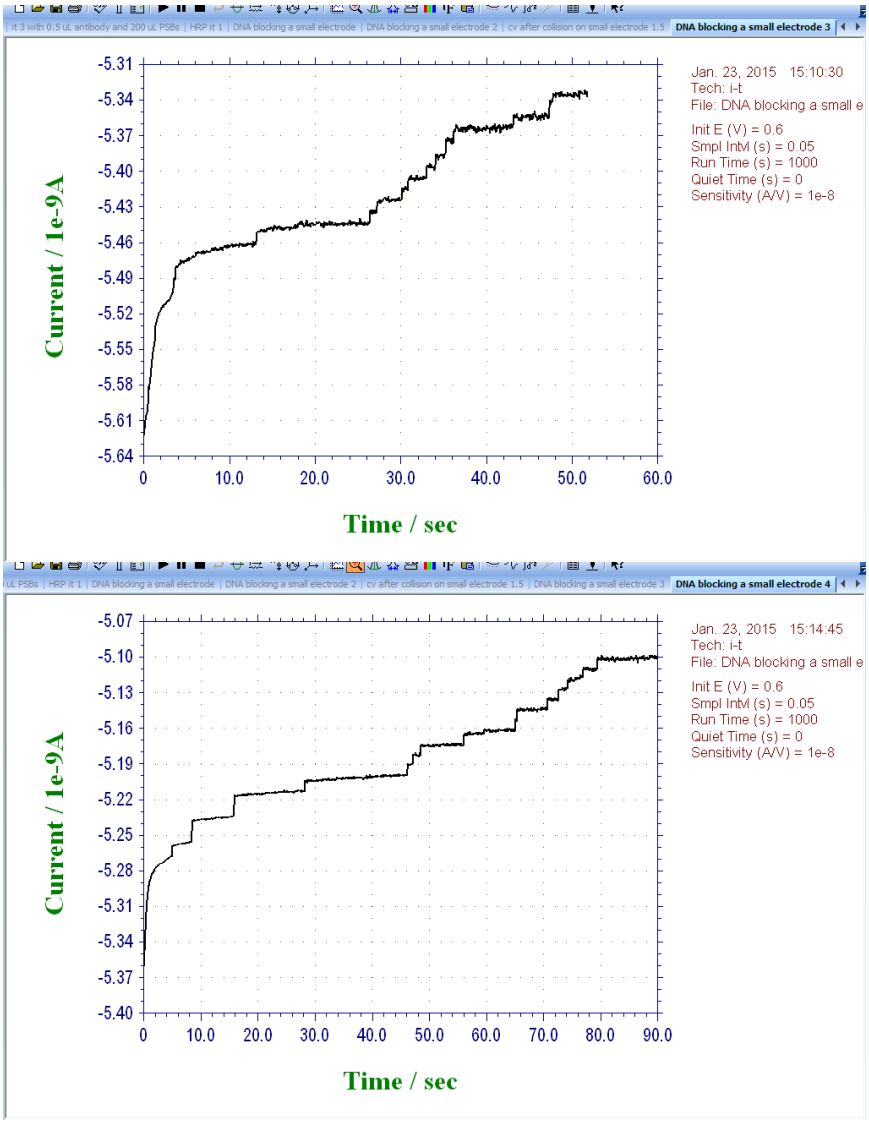
Time / sec

Examples of Mouse Monoclonal Antibody:



Examples of Plasmid DNA:



[i] Kim, J.; Izadyar, A.; Nioradze, N.; Amemiya, S.; J. Am. Chem. Soc., 2013, 135, 2321-2329.