Supplementary Information

Reduction-Chemistry-Assisted Nanopore Determination for Immunoglobulins Isotypes

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Experimental Section

Materials and Reagents

The wild-type alpha-hemolysin and other chemicals including NaCl, Trizma base and TCEP were purchased from Sigma-Aldrich (St. Louis, MO, USA). 1,2-Diphytanyol-sn-glycero-3-phosphocholine (DPhPc) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Immunoglobulins used in this study were the mouse Ig monoclonal antibodies purchased from Sigma-Aldrich (St. Louis, MO, USA). Streptavidin modified Mag-bead nanoparticles (4 mg/mL) and biotinylated IgG were obtained from New England BioLabs, Inc. (Ipswich, MA) and Fisher Scientific (Suwanee, GA), respectively. The materials for SDS-PAGE analysis were purchased from Bio-Rad (Hercules, CA). All reagents and materials are of analytical grade. All of the solutions were prepared in pure water (Milli-Q, 18.2 MΩ cm^{-1} resistivity), followed by being filtered using Amicon Ultra-0.5 centrifugal filter devices purchased from Millipore. The stock solutions of immunoglobulins were prepared at 5.0 mg/mL kept at -20°C before and after use, and the solution of immunoglobulins were prepared for IgG and IgM reduction at 33 μM and 10.5 μM, respectively.

Nanopore Ionic Current Measurements

Briefly, according to the Montal-Mueller method a planar lipid bilayer using 1, 2-diphytanoylphosphatidylcholine (Avanti Polar Lipids, Alabaster, AL) was formed on a 150 μm aperture of a Teflon film which separated the experimental chamber into two compartments (cis and trans). The potential was applied using Ag/AgCl electrodes. Unless otherwise noted, all the experiments were carried out at 25 ± 2 °C under symmetrical buffer solutions with both compartments filled with a 2.0 mL electrolyte solution comprising 1 M NaCl and 10 mM Tris · HCl (pH 7.5), where the Ig sample and the wild-type α-HL protein pore were both added to the *cis* compartment. The ionic current was recorded using a patch-clamp amplifier (Axopatch 200B, Molecular Devices, Sunnyvale, CA) under an applied potential bias of +160 mV, unless stated otherwise. The current recording was filtered with a four-pore low-pass Bessel filter at 5 kHz, and digitized with a 1440A converter (Molecular Device) at a sampling frequency of 25 kHz.

Chemical Reduction Reaction

The final IgG and IgM concentrations for chemical reduction were 33 μM and 10.5μM, respectively. TCEP concentrations varied at 33 μM, 66μM, 99μM, 165μM for IgG reduction and 50μM, 100μM, 200μM for IgM reduction, respectively. The reduction mixture was incubated for 120 min at 37 °C and kept at -20°C. The pH for IgG and IgM reduction is 4.5, 8.0 and 10.0 accordingly.

Serum Sample Analysis

The fetal bovine serum (100 μL) was first centrifugated and then pick up the supernatant for following use. We incubated the streptavidin modified Mag-bead nanoparticles (100 μL of 4 mg/mL) and IgG (1 mg/mL) in serum for 10 minutes . The supernatant was magnetically removed and followed by the buffer washing for at least three times. Briefly, Mag-beads-IgG was washed with 100 μL washing buffer (1.0 M NaCl, 10 mM Tris-HCl, pH 7.5). TCEP were consequently added for chemical reduction using the same procedure mentioned above, and magnetically remove the bottom suspensions and transferring the supernatant of reduction products (L chains remained, biotinylated H chains magnetically discarded) to the cis compartment for nanopore sensing (Supporting information, Figure S9). At least, three independent trials were performed.

Molecular Dynamic Simulation

Molecular dynamic (MD) simulation were performed using GROMACS 5.1.4 package. The amber99sb-ildn force field and TIP3P water model were employed. The model used in MD simulation consists of DPhPC lipid bilayers, α-hemolysin nanopore (PDB data: 7AHL), light-chain fragment of IgG (PDB data: 1IGT), water, Na^+ and Cl^- ions. All atoms were periodically arranged in x, y and z directions. The α-hemolysin located in the range of 8-18 nm in the z direction, and the light chain was placed about 5 Å above *cis* entrance of α-hemolysin nanopore. 1 M NaCl electrolyte was used for the simulation. An electric field with 180 mV amplitude was applied along z direction. Following the energy minimization process, NVT and NPT ensembles were carried out to maintain a constant temperature of 300K and a constant pressure of 1 bar, respectively.

SDS-PAGE Analysis

Electrophoresis assay was carried out on Mini-PROTEAN Tetra Cell electrophoresis system (Bio-Rad, Hercules, CA). Vilber ST4-1100 (France) fluorescence image analysis system was used to take photos of the gel. SDS-PAGE gel 10% (wt/vol): The gel contains 1.5 M Tris-HCl (pH 8.8), 0.4% (wt/vol) SDS, acrylamide/bisacrylamide (30%/0.8%, wt/vol), water, 10% (wt/vol) APS and TEMED; Stacking gel contains 0.5 M Tris-HCl (pH 6.8), 0.4% (wt/vol) SDS, acrylamide/bisacrylamide (30%/0.8%, wt/vol), water, 10% (wt/vol) APS and TEMED. The gels should be freshly prepared. SDS loading buffer: 4× SDS loading buffer contains 100 mM Tris-Cl (pH 6.8), 10% (wt/vol) SDS, 0.2% (wt/vol) bromophenol blue, 30% (vol/vol) glycerol and 600 mM DTT. Store the SDS gel-loading buffer without DTT at room temperature. 1 M DTT can be stored at - 20 °C. SDS running buffer: SDS running buffer contains 25 mM Tris-Cl (pH 8.8), 192 mM glycine and 0.1% (wt/vol) SDS. This buffer can be stored at room temperature.

Data Analysis

Current signature events were manually selected for statistical analysis. Origin (Microcal, Northampton, MA) and Clampfit10.5 software (Molecular Device) were used for histograms construction, curve fitting and graph

presentation.

Supplementary Figures

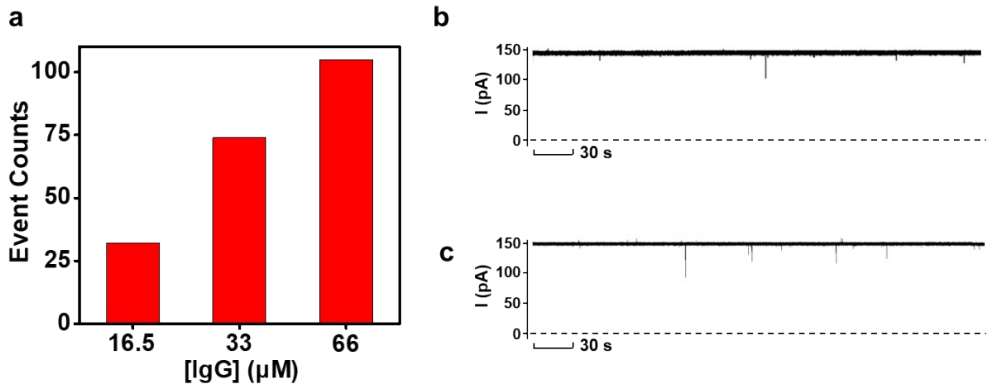

Figure S1. Determination of Immunoglobulin G (IgG) by α-hemolysin (αHL) nanopore. a Histograms shows the current signal increases as the IgG concentration increases. The final TCEP and IgG concentrations for chemical reduction were 1 mM, and 16.5μM, 33 μM, 66 μM, respectively that excess of TCEP over the amount of Ig was applied to the reduction reaction. Typical single channel recording trace segments with and without IgG in the α-HL. b Blank α-HL channel; c Pure IgG; α-HL and IgG were both added into *cis* chamber compartment. All experiments were performed at +160 mV in 1 M NaCl solution containing 10 mM Tris at pH 7.5. The concentration of IgG used for nanopore sensing was 33 nM.

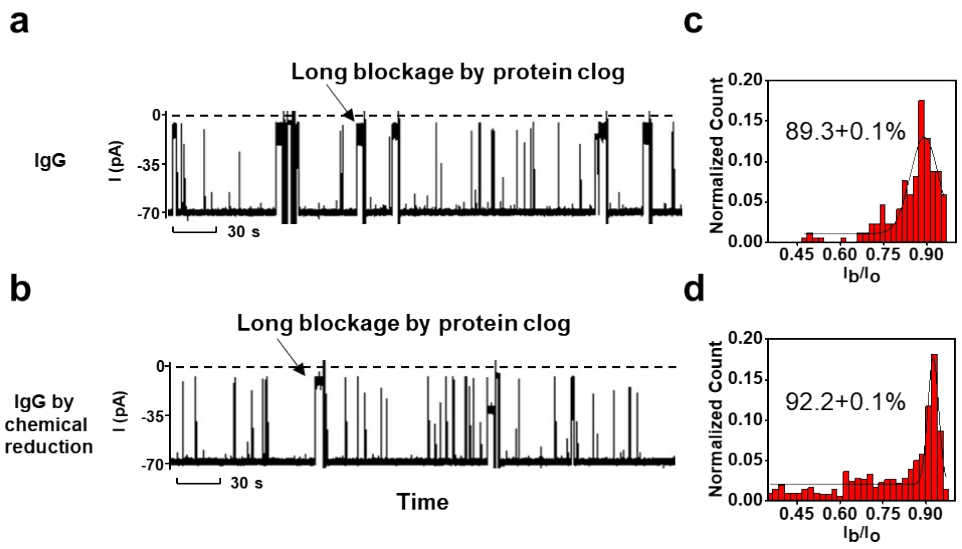

Figure S2. Typical single channel recording trace segments with and without IgG in the α-HL. a IgG only; b IgG after chemical-reduction treatment; IgG samples were added into *trans* chamber compartment. c and d: Corresponding plots of I_b/I_0 as a function of normalized event counts. All experiments were performed at -160 mV in 1 M NaCl solution containing 10 mM Tris at pH 7.5. The concentration of IgG and TCEP in chemical reduction was 33 uM and 99 uM, respectively. The concentration of IgG used for nanopore sensing was 33 nM.

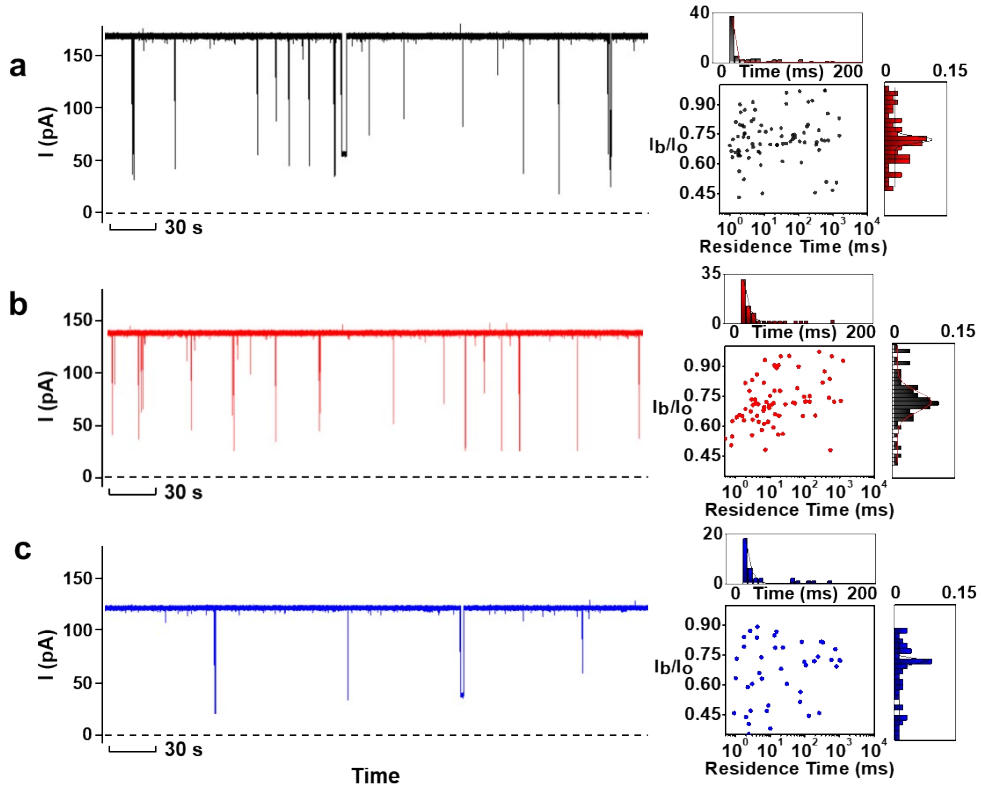

Figure S3. Determination of IgG by αHL nanopore with various applied potential bias. Typical single channel recording trace segments in the α-HL and corresponding scatter plots, histograms of I_b/I_0 (72.12±0.9%, 72.14±0.6% and 71.79±0.8%, respectively) and residence time as a function of signature event counts. a 180 mV; b 160 mV; c 140 mV; All experiments were performed in 1 M NaCl solution containing 10 mM Tris at pH 7.5. The concentration of IgG and TCEP used for chemical-reduction was 33 uM and 99 uM, respectively. The concentration of IgG used for nanopore sensing was 33 nM.

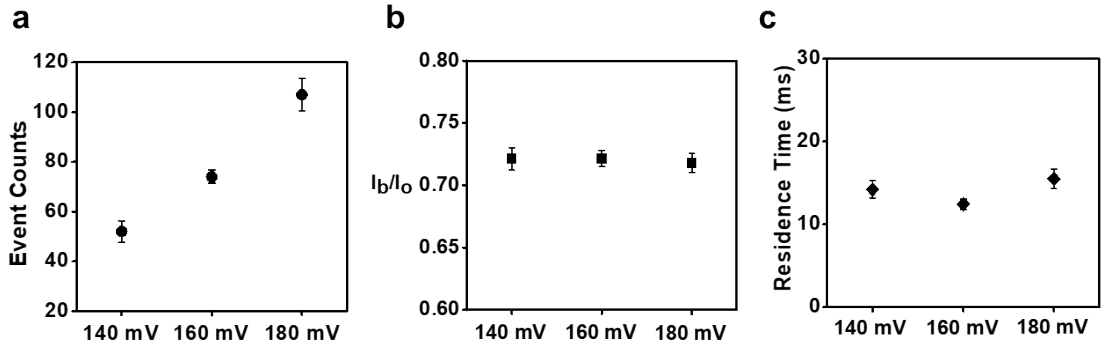

Figure S4. The voltage-dependent experiments on the performance of IgG determination with wild-type αHL nanopore. Plots of (a) Event counts and (b) Current blockage of I_b/I_0; and (c) Mean residence time. The experiments were performed in a solution comprising 1 M NaCl and 10 mM Tris•HCl (pH7.5). The concentration of IgG and TCEP used for chemical reduction was 33 uM and 99 uM, respectively. The concentration of IgG used for nanopore sensing was 33 nM.

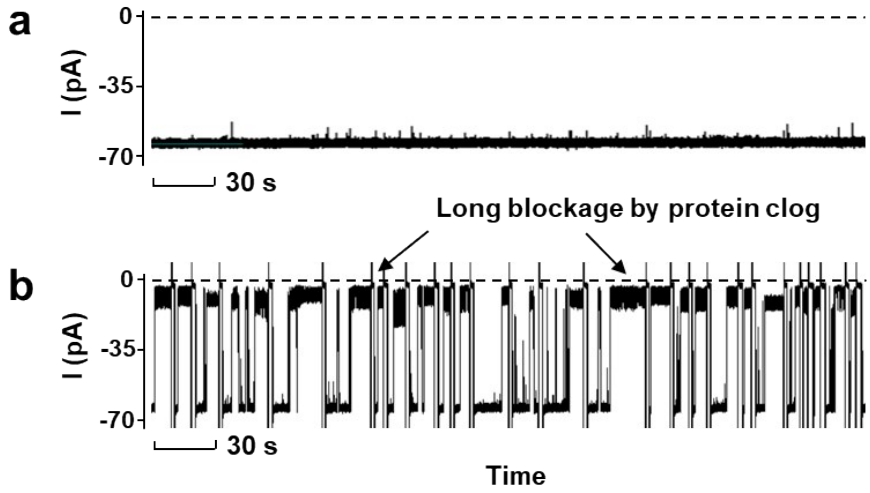

Figure S5. Determination of Immunoglobulin M (IgM) by αHL nanopore. Typical single channel recording trace segments with and without IgM in the α-HL. a Blank α-HL channel; b IgM only; α-HL and IgM were both added into ***trans*** chamber compartment. All experiments were performed at -120 mV in 1 M NaCl solution containing 10 mM Tris at pH 7.5. The concentration of IgM used was 330 nM.

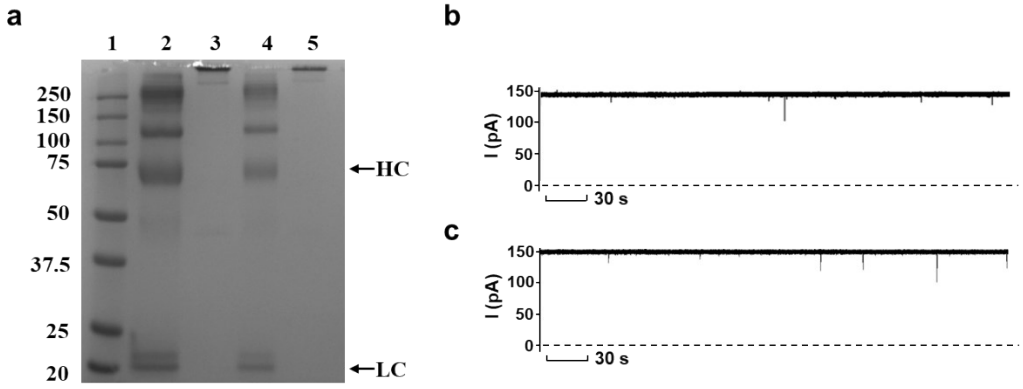

Figure S6. Determination of Immunoglobulin M (IgM). a PAGE analysis of IgM separated by reductant agents, Lane 1-5: molecular ladder, reduced IgM (10.5μM), pure IgM (10.5μM), reduced IgM (3 μM), and pure IgM (3 μM). Typical single channel recording trace segments with and without IgM in the α-HL. b Blank α-HL channel; c IgM only; α-HL and IgM were both added into ***cis*** chamber compartment. All experiments were performed at 160 mV in 1 M NaCl solution containing 10 mM Tris at pH 7.5. The concentration of IgM used in nanopore sensing was 330 nM.

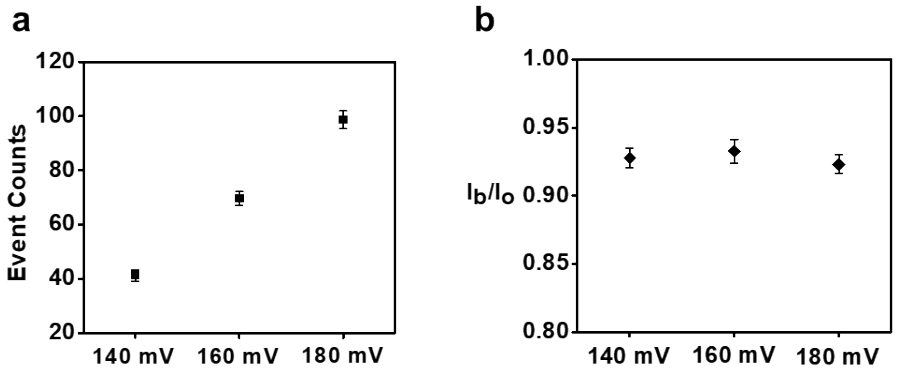

Figure S7. The voltage-dependent experiments on the performance of IgM determination with wild-type αHL nanopore. Plots of (a) Event counts and (b) Current blockage of I_b/I_0 (140 mV: 92.8±0.7%, 160 mV: 93.3±0.9% and 180 mV: 92.3±0.7%, respectively). The concentration of IgM and TCEP in chemical reduction was 10.5 uM and 200 uM, respectively. The experiments were performed in a solution comprising 1 M NaCl and 10 mM Tris•HCl pH7.5. The concentration of IgM used for nanopore sensing was 10.5 nM.

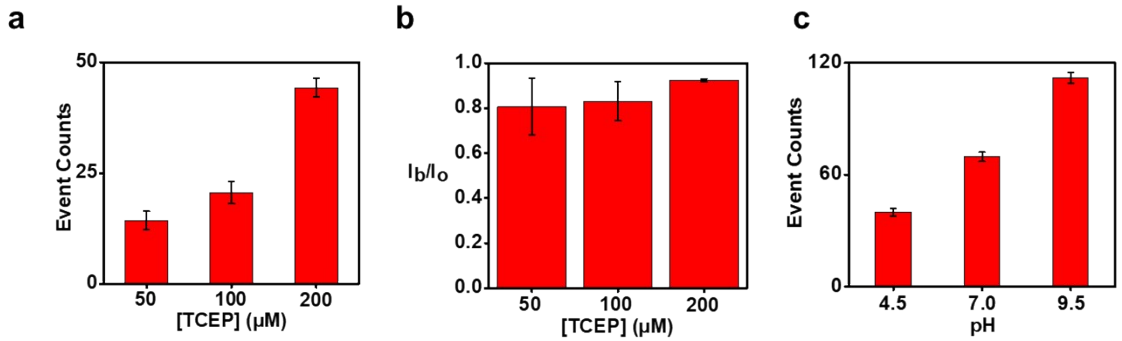

Figure S8. TCEP concentration-dependent and pH-dependent studies on IgM determination. TCEP concentration as a function of (a) Event counts and (b) Current blockage of $I_b/I_{0.}$ (80.7±12.6%, 83.0±8.7% and 92.3±0.7%, respectively). (c) pH-dependent as a function of event counts. The concentration of IgM and TCEP in chemical reduction was 10.5 uM and 50μM, 100μM, 200 uM, respectively. The experiments for nanopore sensing were performed in a solution comprising 1 M NaCl and 10 mM Tris•HCl. The concentration of IgM used for nanopore sensing was 10.5 nM.

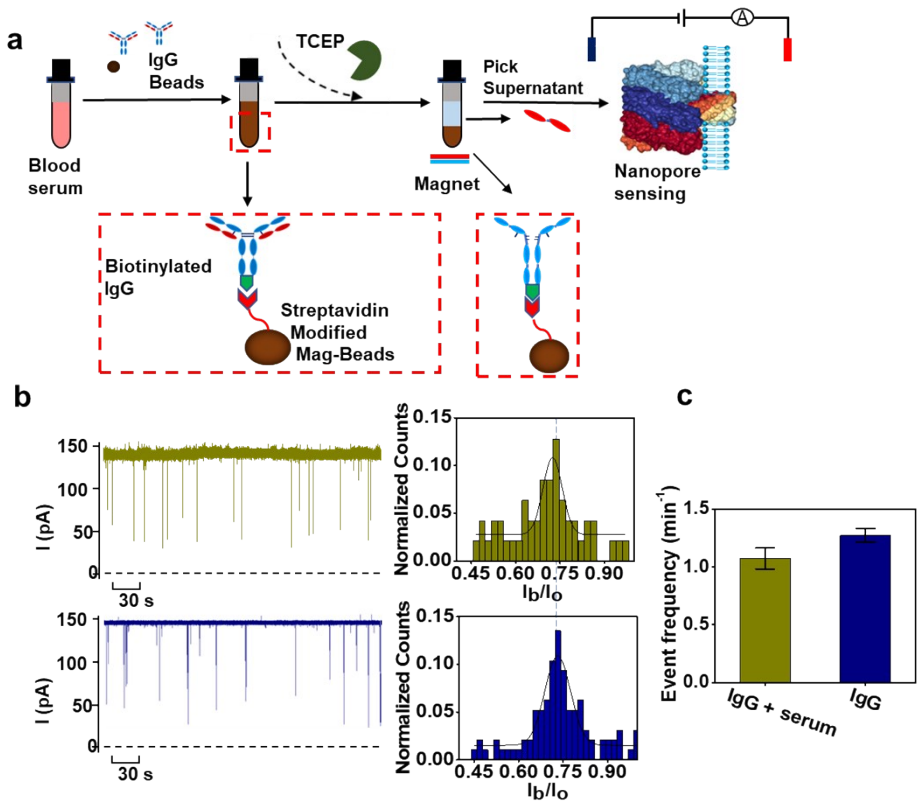

Figure S9. Nanopore sensor for selective immunoglobulins determination from blood serum sample. (a) Flow chart illustrates steps for detecting IgG in blood serum sample applying chemical-reduction method. (b) Corresponding current traces and histograms of detecting IgG with (upper panel) and without blood serum (lower panel), the Gaussian distribution showed constant current blockage of I_b/I_0 by polypeptide fragments. (c) Signature event frequency functioned as selectivity studies in the detection of immunoglobulins with blood serum. The fetal bovine serum (100 µL) was first centrifugated and then magnetic treated step by step to make sure the supernatant containing polypeptide fragments for following use. The final concentration for IgG sensing in fetal bovine serum is 33 nM. The experiments were performed in a solution comprising 1 M NaCl and 10 mM Tris•HCl (pH7.5).