Supplementary Information

Differentiation of Selectively Labeled Peptides using Solid-State Nanopores

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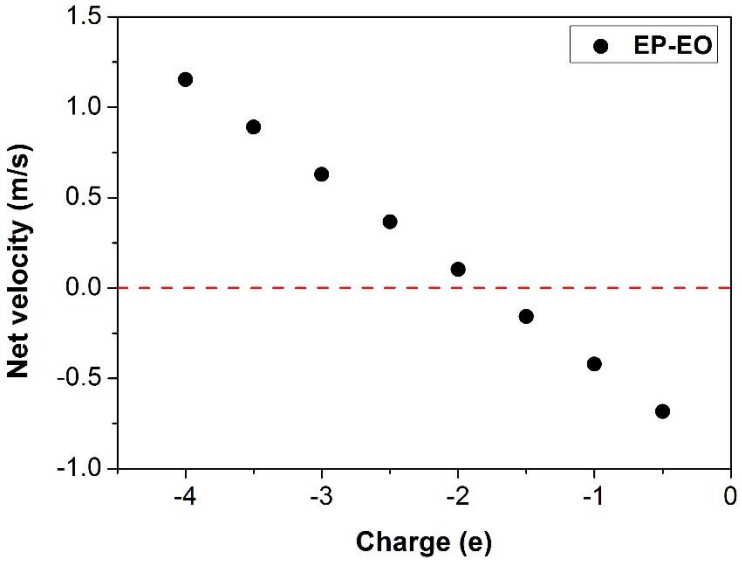
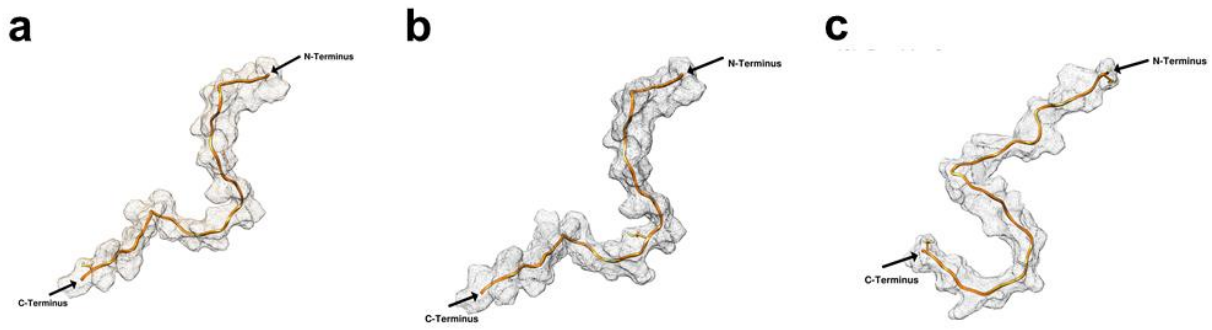


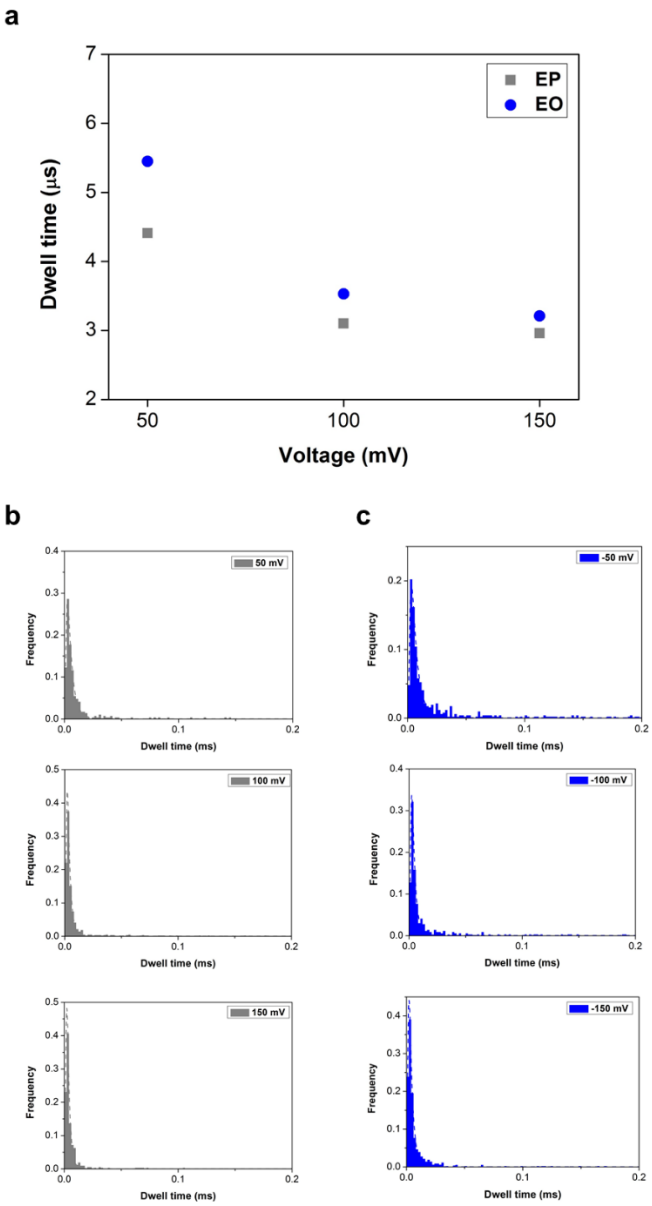
Supplementary Figure 1. Calculated velocities of Pep A in EO and EP directions

The electrophoretic velocity, v_{EP} and electroosmotic velocity, v_{EO} of the Pep A in ionic solution can be calculated as follows. According to Stokes-Einstein relation, $v_{EP} = \mu E = \frac{qD}{k_B T} E = \frac{qE}{6\pi\eta r}$, where μ is electrophoretic mobility, E is the applied electric field, q is the charge of the peptide, D is diffusion coefficient, k_B is the Boltzmann constant, T is the absolute temperature, η is viscosity of the electrolyte solution, and r is the radius of the peptide. Since the Pep A used in the experiment did not have a spherical shape, it was assumed r = $\sqrt[3]{\left(\frac{3}{4\pi}\right) V_{peptide}}$, where $V_{peptide}$ is the volume of the Pep A. Based on the calculation of amino acids sequences, (Supplementary Figure 2) $V_{peptide}$ is ~5.29 nm^3 and r = ~1.08 nm. Using E = 200 mV/3 nm, η = 10^{-3} Pa·s, the theoretical v_{EP} was calculated according to the charge of the peptide. At a high ionic strength of 1 M, the Debye length is very thin, ~0.3 nm, and the velocity of the electroosmotic flow, v_{EO} can be expressed as $v_{EO} = -\frac{\varepsilon_0 \varepsilon_r \zeta_{pore} E}{\eta}$, where ε_0 is vacuum permittivity and ε_r is relative permittivity, and ζ_{pore} is the zeta potential of the pore wall. Since the net velocity $v_{net} = v_{EP} - v_{EO}$ of the Pep A, having a net charge of -0.973 e, is less than 0, the EO direction translocation is more dominant.

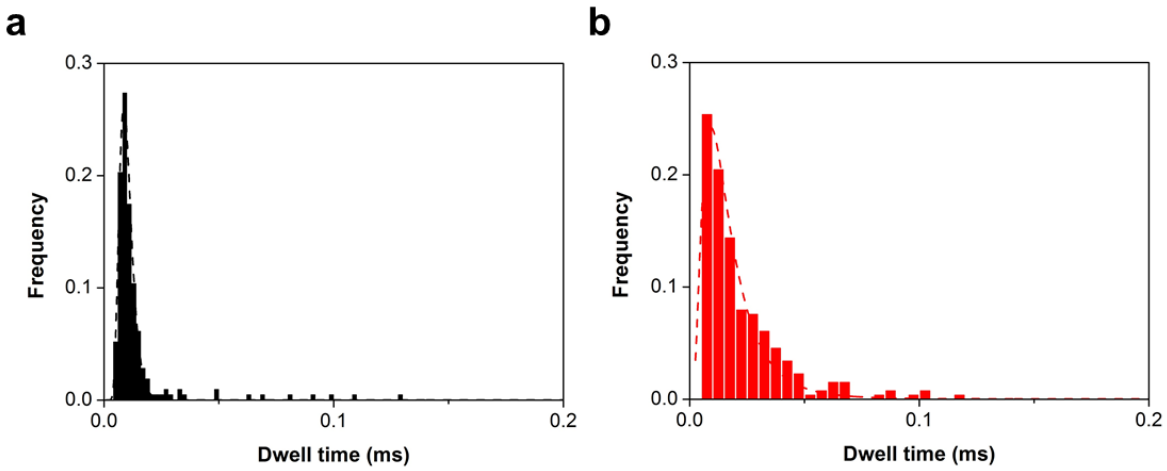


Supplementary Figure 2. Theoretical volume of the peptides used in the experiment. (a) Pep A, (b) Pep B, and (c) Pep C.

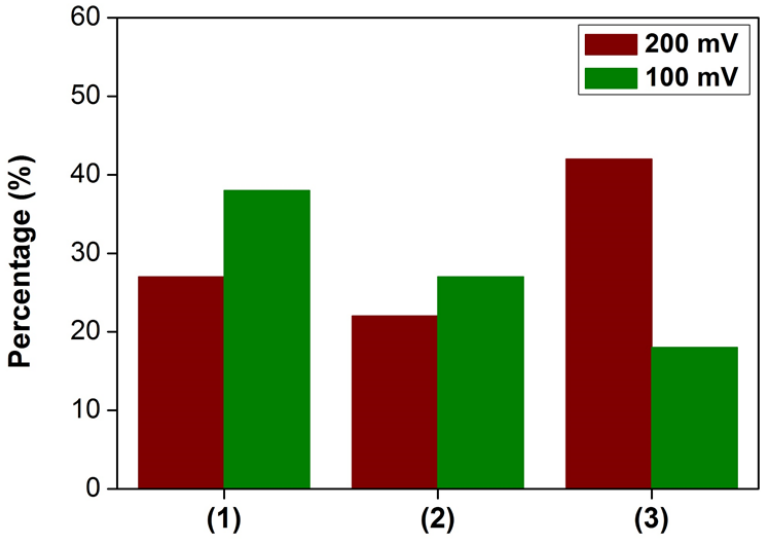
We performed simulations using the NAMD 2.9 package with the CHARMM 27 force field with the protein parameters incorporating the CMAP. The TIP3P water model was employed. Positions for Na^+ and Cl^- ions were generated with a condition of 5 Å between ions employing the AUTOIONIZE module of VMD to approximate the system to be in the physiological condition, 150 mM in NaCl. The particle mesh Ewald (PME) method was used for electrostatic interactions. The damping coefficient was 5 ps^{-1} for Langevin dynamics. The direct space cut off was 12 Å. We performed molecular dynamics simulation in the NPT ensemble (300 K, 1 atm) for 10 ns. Peptides were modelled using Model1er 9.19. The volume of the peptides is 5287 $Å^3$, 5278 $Å^3$, and 5541 $Å^3$ for Pep A, Pep B, and Pep C.



Supplementary Figure 3. Dwell time of the labeled Pep A. (a) Dwell time of the labeled Pep A in EO and EP direction translocations according to the applied voltages. Dwell time distributions for each voltage in (b) EP direction and (c) EO direction. In the EP translocation, the dwell time is 4.4, 3.1, and 2.9 μs at 50, 100, and 150 mV, respectively. In the EO translocation, the dwell time is 5.4, 3.5, and 3.2 μs at 50, 100, and 150 mV, respectively. The dwell time at 100 and 150 mV in both directions are close to the measurement limit for the signal distortion. (~3.3 μs at 200 kHz low-pass filter)



Supplementary Figure 4. Dwell time of single step events and peaks with specific conformations. (a) Dwell time of single step events occurred in the translocation of the labeled peptides. Dwell time of ~9 μs is obtained from the log-normal distribution fitting. (b) Dwell time of peak shape (1) - (3) occurred in the translocation of labeled peptides. Dwell time of ~15 μs is obtained from the log-normal distribution fitting.



Supplementary Figure 5. Peak shape occurrence ratio of the Pep B' at different applied voltages. (+100 and +200 mV)

Peak shape (3) decreased to less than half at 100 mV (~18 %) than at 200 mV (~42 %).