Supporting Information

Resistive-pulse nanopore sensing of ligand exchange at the single nanocluster limit for peptide detection

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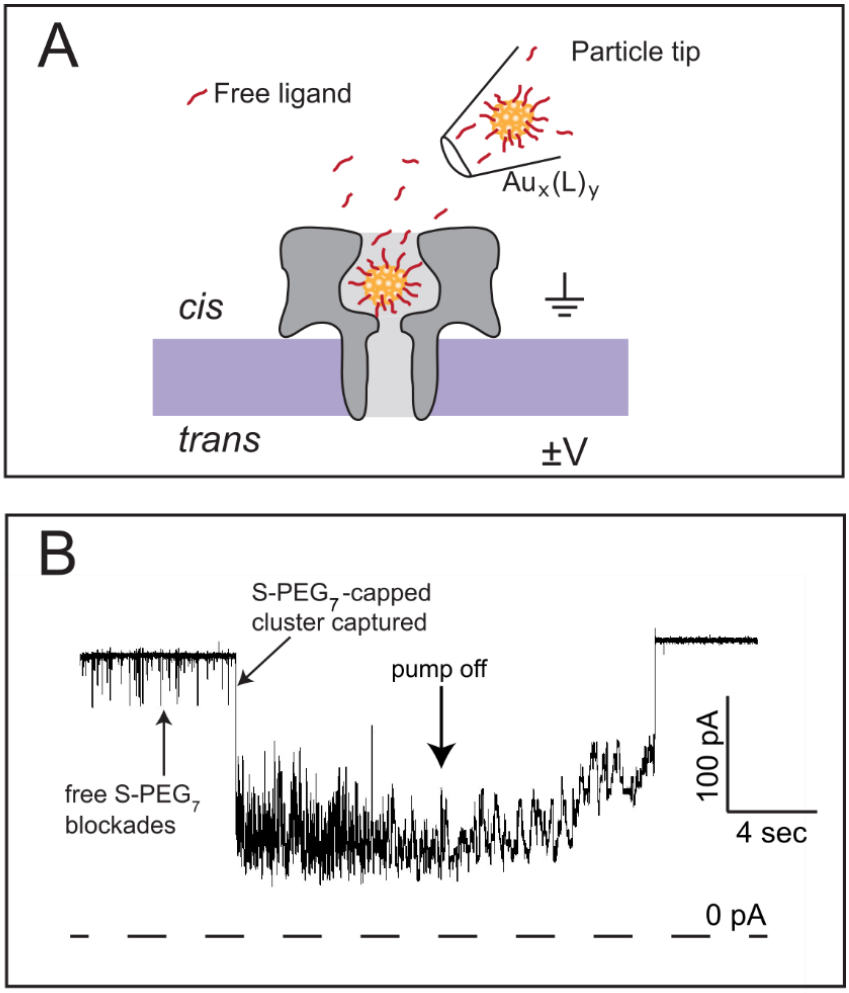

Figure S1. (A) Single-tip homogenous exchange occurs when a cluster is exposed to free ligands from the particle-containing tip. (B) Typical current trace shows the capture of a S-PEG$_7$-capped gold cluster. Approximately 10 s after capture the ejection is turned off and excess S-PEG$_7$ diffuses away from the pore. The short downward spikes in the current before the cluster capture result from individual S-PEG$_7$ molecules striking the pore. We also note the current increases shortly after the backing pressure on the PEG tip is turned off. This may result from PEG ligands detaching from the cluster, which has been reported previously.[1] This one-tip methodology was used to collect the data shown in Fig. 2A of the main article.

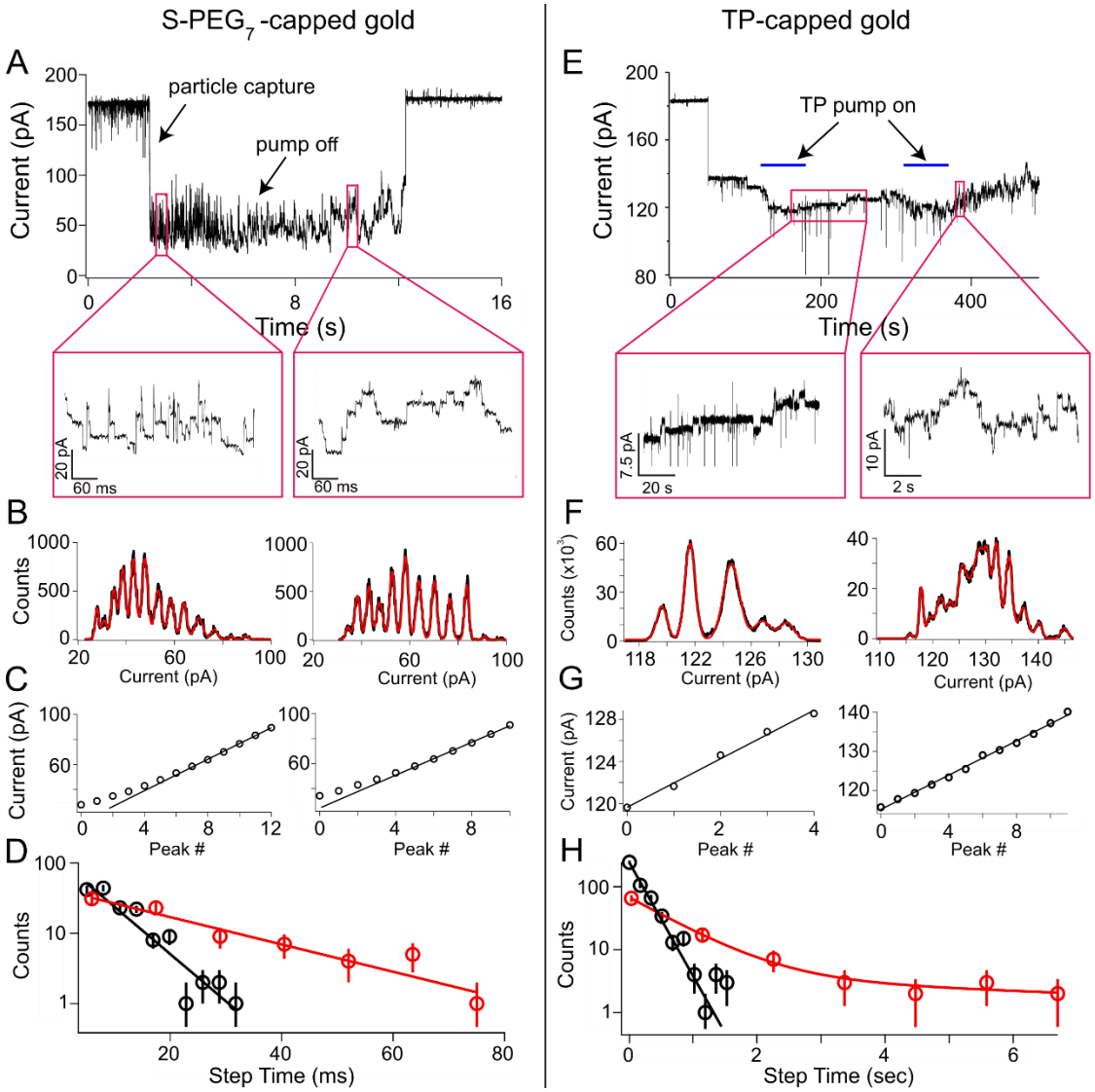

Figure S2. Homogenous exchange for (left) S-PEG$_7$ and (right) TP-capped gold clusters. (A, E) Reproduced from Fig. 2 of the main article. (B) All-points histograms of the current traces (left) before and (right) after the ligands are removed with a corresponding Gaussian-mixture model fit (red). (C) The peak positions extracted from the Gaussian fits show a linear dependence for currents above 60 pA showing evenly spaced current states with spacing (6.3 ± 0.1) pA and (6.6 ± 0.1) pA before and after the pump is off respectively. (D) Step time distributions before the pump is off (black) and after the pump is off (red) are exponentially distributed with mean step times of τ_{before} = (6.5 ± 0.6) ms and τ_{after} = (22.3 ± 3.1) ms. (F-H) Similar analysis for TP-capped clusters exposed to free TP ligands. (G) The peak spacing after the first TP-ejection was (2.3 ± 0.1) pA and after the second TP-ejection was (2.2 ± 0.1) pA. (H) The step time distributions show double exponential dependence after the first ejection with time constants (0.75 ± 0.06) s and (11.4 ± 10) s and a single exponential dependence after the second ejection (240 ± 10) ms. Data was collected in 3M KCl at pH 8 under a 70 mV applied transmembrane potential. Ligand concentrations in the S-PEG$_7$ and TP tips were 22 μM and 165 μM respectively.

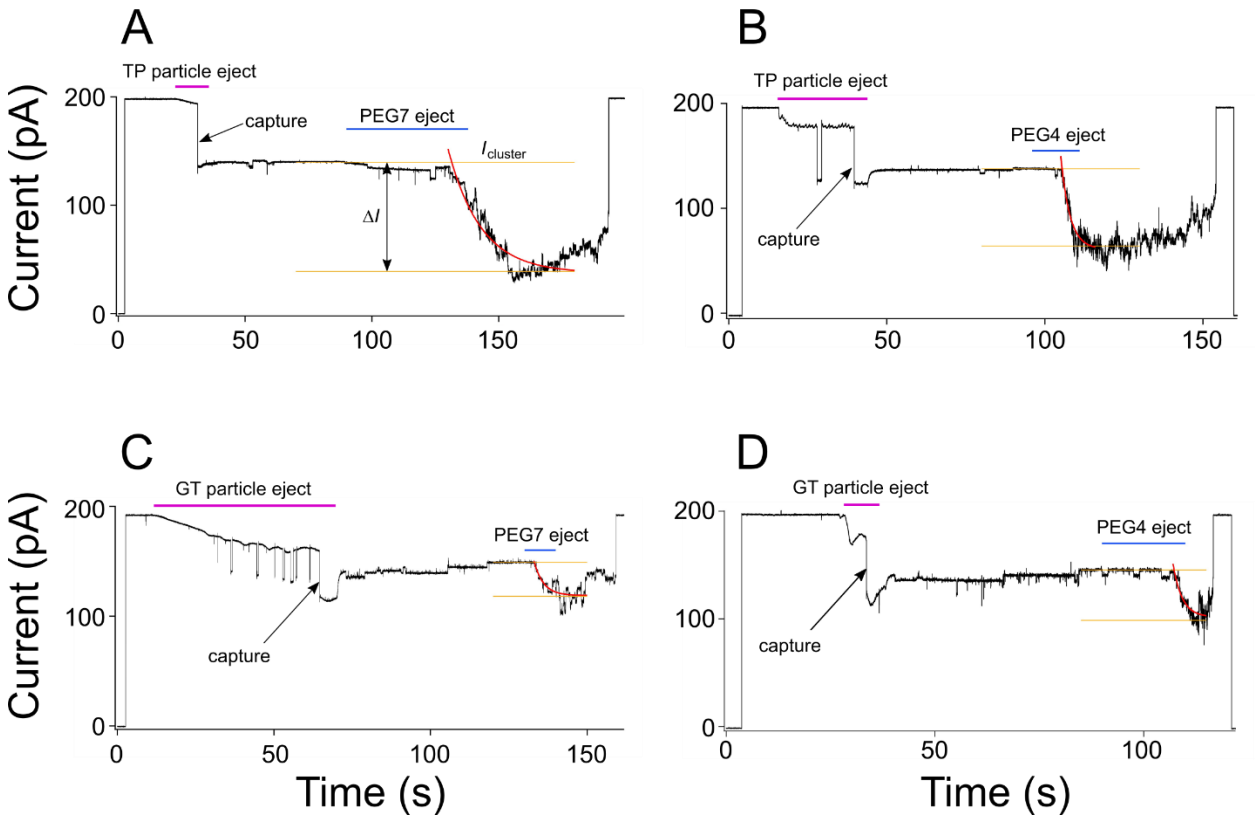

Figure S3. Sample traces of single-cluster heterogeneous exchange. (A,B) Nanopore-trapped TP-particles and (C,D) GT-particles exchange with S-PEG$_7$ and S-PEG$_4$. The pore begins in the open current state when particles are ejected in the vicinity of the pore (highlighted by the purple bar in each panel). Upon capture of a particle, the particle ejection is turned off and the current stabilizes for ca. one minute to a new value $I_{cluster}$. Following this, free ligands are ejected near and within the pore and this triggers the exchange process. The solid orange lines show the pre- and post-exchange current levels that are found from a two-term Gaussian mixture fit to the all points histogram of the current. The solid red curve in each panel is a least-squares fit to the current between the pre- and post-exchange current levels with a single exponential offset function $i(t) = A\exp((t-t_0)/\tau)$. These traces correspond to the following fitting parameters: TP-PEG$_7$: $\Delta I/I_{cluster} = 0.72$, $\tau = (13.3 \pm 0.1)$ s, TP-PEG$_4$: $\Delta I/I_{cluster} = 0.53$, $\tau = (2.95 \pm 0.05)$ s, GT-PEG$_7$: $\Delta I/I_{cluster} = 0.21$, $\tau = (3.12 \pm 0.05)$ s and GT-PEG$_4$: $\Delta I/I_{cluster} = 0.32$, $\tau = (2.23 \pm 0.05)$ s. All data shown here was collected in 3M KCl under a 70 mV applied transmembrane potential. All ligand tip concentrations were 540 µM except the TP-PEG$_7$ which was 200 µM.

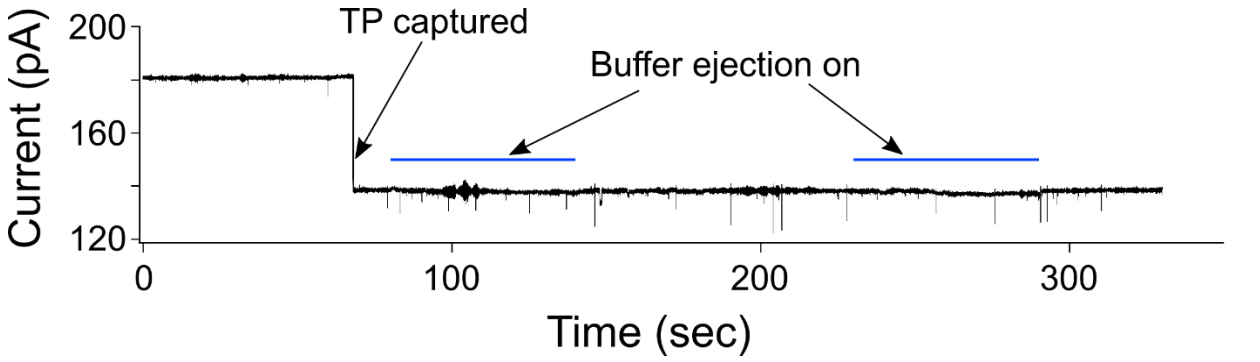

Figure S4. A blank control shows a TP-capped cluster exposed to an external buffer solution from *t* = 90s-130s and *t* = 230s-290s. No significant changes in current are observed. All data shown was collected in 3M KCl at pH 8.0 under a 70 mV applied transmembrane potential.

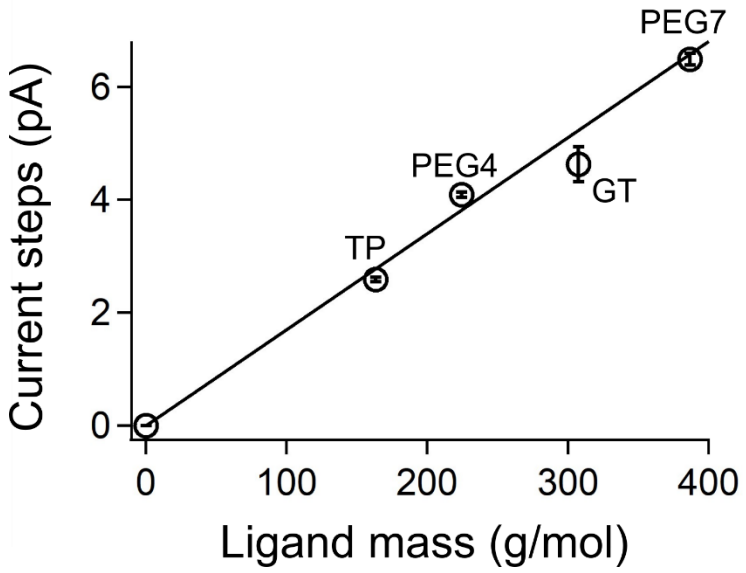

Figure S5. Ligand mass scales linearly with current steps. The steps were calculated from a minimum of five different particles prior to exchange experiments. The solid line is a least squares fit with slope (17 ± 1) fA/(g/mol). All data was taken in 3M KCl under a 70 mV applied transmembrane potential.

REFERENCES

(1) Schroedter, A.; Weller, H. Ligand Design and Bioconjugation of Colloidal Gold Nanoparticles. *Angew. Chem. Int. Ed.* **2002**, *41* (17), 3218–3221.