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Enhanced Single Molecule Mass Spectrometry Via Charged Metallic Clusters

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ABSTRACT:

Water-soluble metallic clusters have been used for a number of important applications. One of the most stable clusters is $Au_{25}(SG)_{18}$, which are negatively charged in solution and highly monodisperse making them ideal for characterization and analysis applications. We present here a new application where these clusters are shown to increase the mean residence time of polyethylene glycol (PEG) molecules within an alpha hemolysin (αHL) nanopore. The effect appears over a range of PEG sizes and ionic strengths. This increases the resolution of the peaks in the single molecule mass spectrometry (SMMS) current blockade distribution and suggests a means for reducing the ionic strength of the nanopore solute in the SMMS protocol.

INTRODUCTION:

Nanopore detectors are used to measure the physical and chemical properties of single molecules in solution.[1] These detectors consist of a nanoscale hole in a membrane that partitions an electrolyte solution. An applied transmembrane potential drives ionic current through the pore and individual molecules, commensurate in size with the pore, enter the hole and significantly reduce the flow of ions for an extended period of time. This gives rise to measurable current blockades.

Here we describe a new method to increase the residence times of PEG molecules within an αHL pore. Single $Au_{25}(SG)_{18}$ metallic clusters enter the *cis* side of an αHL nanopore under an applied electric field. The clusters remain in the pore for extended periods, but only partially block the flow of ions (~25% reduction) while oppositely charged analyte molecules enter into the narrower *trans* side of the pore. The interaction between the oppositely charged analyte and cluster within the pore leads to increased residence times, which improve the efficacy of nanopore-based SMMS analysis in two ways, by improving the quality of the SMMS current blockade distributions and lowering the KCl concentration threshold for detecting PEG molecules.

EXPERIMENTAL METHOD:

All experiments were performed using αHL nanopores inserted into unsupported painted lipid bilayer membranes. A patch clamp method was used to isolate individual pores to allow for relatively high bandwidth detection (B =10 kHz).[2] We preloaded the gold clusters into the patch pipette electrode to ensure CIS side cluster loading (see figure 1A).

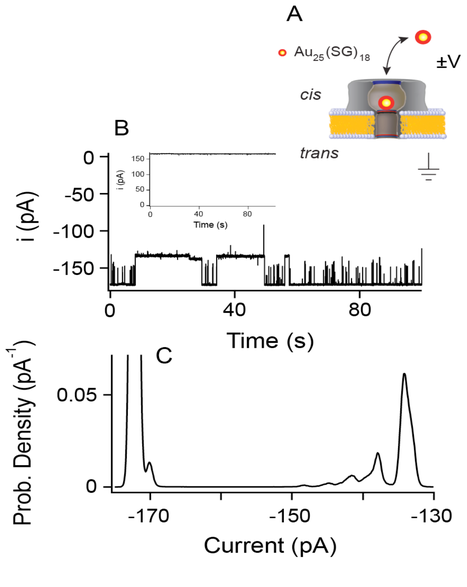

Figure 1: Nanopore detection of gold clusters verifies cluster charge and yields multiple blockade states. (**A**) Clusters randomnly enter and exit the pore from the *cis* side under an applied negative transmembrane potential. (**B**) The open state current with no gold present in i_{open}(-50mV)=(-172.3±0.6)pA. (**B, inset**) Under a positive applied potential, the open state current is i_{open}(-50mV)=(-172.3±0.6)pA and no blockades are seen. (**C**) An all-points histogram of the negative voltage current trace demonstrates the quantized nature of the current blockades. The large peak corresponds to the open pore current and smaller peaks between -150 pA and -130 pA, the gold cluster blockade states.

INCREASED RESIDENCE TIME:

The residence times of the clusters are sufficiently long (ca. several minutes) in some cases so that the clusters appear to be trapped within the nanopore volume. These trapped clusters act as traps that should hold positively charged analyte for extended periods of time.

To demonstrate this, we performed nanopore residence time measurements with gold cluster and polyethylene glycol (PEG) mixtures under various electrolyte and applied transmembrane potentials. These experiments showed that with clusters inside the pore the PEG residence time increases significantly. This leads to more easily resolvable peaks in the current blockade distribution.

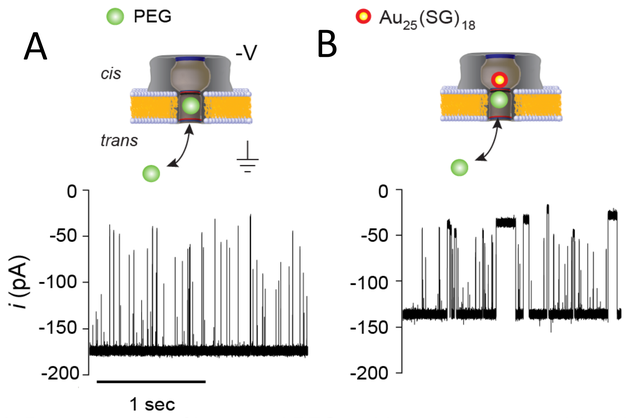

Figure 2: Demonstration of increased PEG residence time inside αHL with a single $Au_{25}(SG)_{18}$ cluster. A) The empty pore exhibits an open state current of i_{open}(-50mV)=(-174.0±2.7)pA. PEG molecules enter the pore and yield short lived current blockades. (B) When a cluster enters the *cis* side of the pore the current is reduced to $i_{open,gold}$(-50mV)=(-136.5.0±2.6)pA. With the negatively charged gold cluster held in the pore, the slightly cationic PEG molecule[17] induces longer lived current blockades.

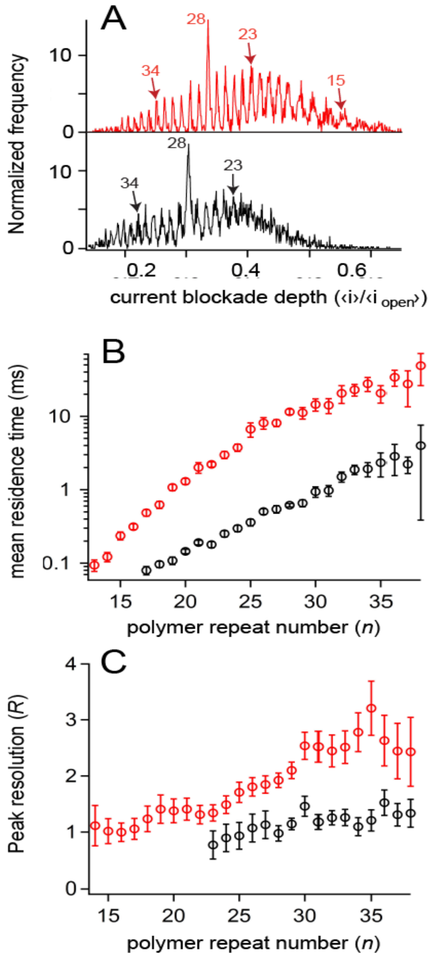

Figure 3: Current blockade, mean residence time and peak resolution distributions for PEG molecules with (red) and without (black) gold trapped clusters. (A) The distribution of normalized PEG current blockades yields peaks corresponding to different sized PEG molecules.[3,4] The large peak in both distributions corresponds to the n=28 monodisperse PEG that calibrates the polymer repeat number for all peaks. (B) Gold-clusters bound to the pore increase the mean PEG residence time by roughly an order of magnitude. This trend is observed across the entire range of PEG sizes. (C) The increased residence time yields narrower peaks in the current blockade distribution across the entire range of PEG sizes. The error bars on all figures correspond to 1 S.D.

DECREASED IONIC STRENGTH FOR NANOPORE ANALYSIS:

The mean residence time of PEG in the aHL nanopore depends on the ionic strength of the electrolyte solution. Detecting PEG without the aid of clusters requires working in high ionic strength conditions, which limits the applicability of nanopore detection. The cluster modified PEG residence times can be used to reduce the KCl concentration threshold to approximately 1.5 mol/L at 10 kHz detection bandwidth (see figure 4).

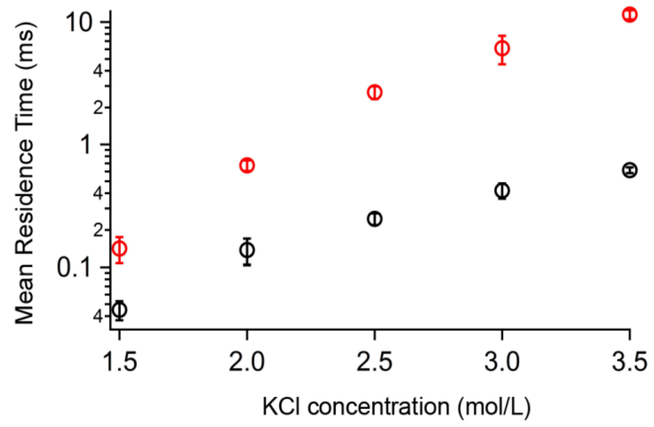

Figure 4: Mean residence times over a large range of KCl concentrations with (red circles) and without (black triangles) gold clusters present in the pore. 1.5 mol/L, 2.0 mol/L and 2.5 mol/L data used monodisperse PEG micture. The 3.0 mol/L and 3.5 mol/L data used in polydisperse PEG mixture. The error bars show 1 S.D. from the mean.

CONCLUSION:

The goal of this work is to establish a new method for increasing the mean residence time of PEG molecules in αHL pores. This increases the range and resolution of PEG with nanopore detectors and provides a means for working in solutions that more closely resemble physiological conditions. Negatively charged clusters enter the *cis* side of the pore and interact with a cationic PEG molecule that enters from the *trans* side of the pore. This interaction increases the PEG residence time in the pore by over an order of magnitude across a range of PEG sizes and ionic strengths.

REFERENCES:

1. Kasianowicz, J. J.; Robertson, J. W. F.; Chan, E. R.; Reiner, J. E.; Stanford, V. M. *Annu. Rev. Anal. Chem.* **2008**, *1*, 737-766.
2. Reiner, J. E.; Robertson, J. W. F.; Burden, D. L.; Burden, L. K.; Balijepalli, A.; Kasianowicz, J. J. *J. Am. Chem. Soc.* **2013**, *135*, 3087-3094.
3. Balijepalli, A.; Robertson, J. W. F.; Reiner, J. E.; Kasianowicz, J. J.; Pastor, R. W. *J. Am. Chem. Soc.* **2013**, *135*, 7064-7072.
4. Reiner, J. E.; Kasianowicz, J. J.; Nablo, B. J.; Robertson, J. W. F. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 12080-12085.
5. Robertson, J. W. F.; Rodrigues, C. G.; Stanford, V. M.; Rubinson, K. A.; Krasilnikov, O. V. ; Kasianowicz, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 8207-8211.