Supporting Information

Copper Sensing with a Prion Protein Modified Nanopipette

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Materials and Methods

Reagents

The 4-octarepeat prion peptide (PrP 23-28, 57-91: Ac-KKRPKPWGQ(PHGGGWGQ)$_4$-COOH) was synthesized by standard Fmoc solid-phase peptide synthesis using a Liberty1 microwave peptide-synthesizer (CEM; Mathews, NC). Amino acids were purchased from AAPPTec (Louisville, KY). 0.25 mmoles of H-Rink amide ChemMatrix resin (Sigma-Aldrich Co; St. Louis, MO) was used to begin the synthesis. Fmoc deprotection was performed using 20% Piperidine in DMF. Fmoc amino acids were double-coupled with 0.625 mM diisopropylcarbodiimide (DIC) and 1.25mM 1-hydroxybenzotriazole hydrate (HOBt) and then capped with 10% acetic anhydride in DMF. The peptide was cleaved from the resin in 90% TFA, 5% triisopropylsilane, and 5% 1,2-ethanedithiol. The crude product was then purified by C18 reverse-phase high-performance liquid chromatography. The purified peptide was confirmed by mass spectrometry using a ZMD 4000 (Waters; Milford, MA). Copper sulfate, Calcium chloride, Zinc Phosphate, 3-(N-morpholino)propanesulfonic acid hemisodium salt were purchased from Sigma Aldrich (Saint Louis, MO). Aqueous reagents were prepared using ultrapure water with >18MΩ cm^{-1} resistance.

Nanopipette fabrication

Nanopipettes are fabricated from quartz capillaries with filaments, with an outer diameter of 1.0 mm and an inner diameter of 0.70mm (QF100-70-5; Sutter Instrument, Novato, CA).

The capillary was drawn into a nanopipette using a P-2000 laser puller (Sutter Instrument, Novato, CA) preprogrammed to fabricate nanopipettes with an inner diameter of ~50 nm. Parameters used were: Heat 620, Fil 4, Vel 60, Del 150, and Pul 190. The settings are variable depending on the puller and were adjusted as needed to provide nanopipettes showing the desired conductance.

Measurement setup

All measurements were performed in a two electrode setup since the current flowing through the nanopipette is too small to polarize a reference electrode. The nanopipette, acting as the working electrode, was backfilled with 0.1M KCl, 10 mM MOPS (pH 7), and a Ag/AgCl electrode was inserted. Another Ag/AgCl ground electrode was placed in bulk solution, 0.1M KCl 10 mM MOPS (pH 7), acting as auxiliary/reference electrode. Both electrodes were connected to a MultiClamp 700B (Molecular Devices, Sunnyvale, CA) amplifier with a DigiData 1322A digitizer (Molecular Devices, Sunnyvale, CA), and a PC equipped with pClamp 10 software (Molecular Devices, Sunnyvale, CA). The system remained unstirred for the duration of the measurement, which was conducted at room temperature.

4-Octarepeat physisorption

We prepared aqueous solutions of the 4-octarepeat and quantified their concentration by spectrophotometry. While applying a sinusoidal voltage (500mV, 5Hz) to the nanopipette, we added 20 µL of the 4-octarepeat aliquot (~1 mg/ml) in the reservoir. The addition instantaneously caused a decrease in the measured ionic current indicating the physisorption of the 4-octarepeat on the negatively charged nanopipette surface. Nanopipette functionalization was determined to be stable if the resulting change in ionic current was maintained during immersion in a fresh buffer solution.

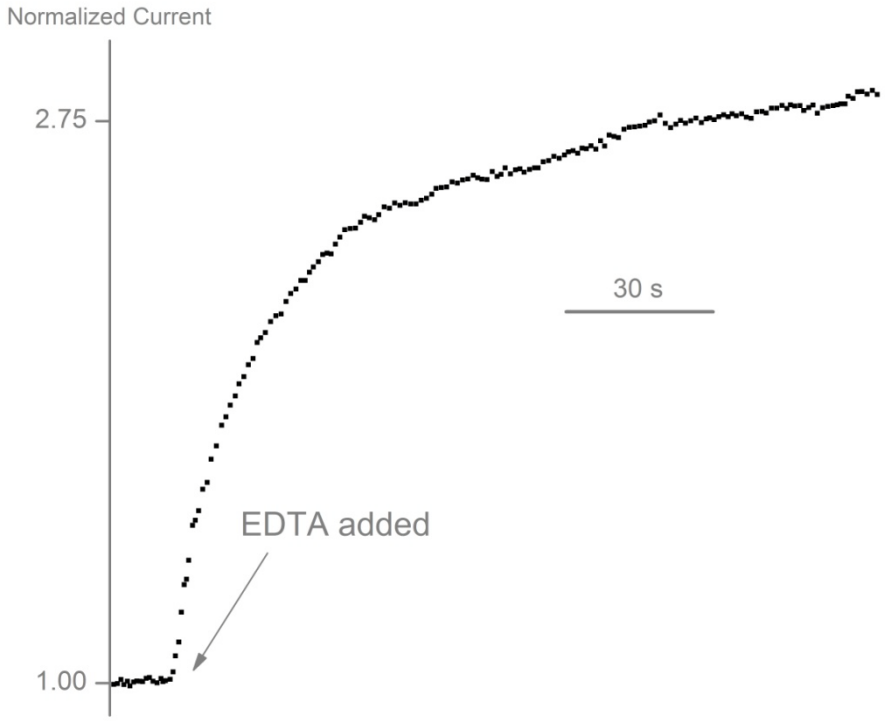

Fig.S1 Evolution of the normalized ionic current of a peptide-functionalized nanopipette sensor after chelation of Cu^{2+} and addition of 500 µM EDTA

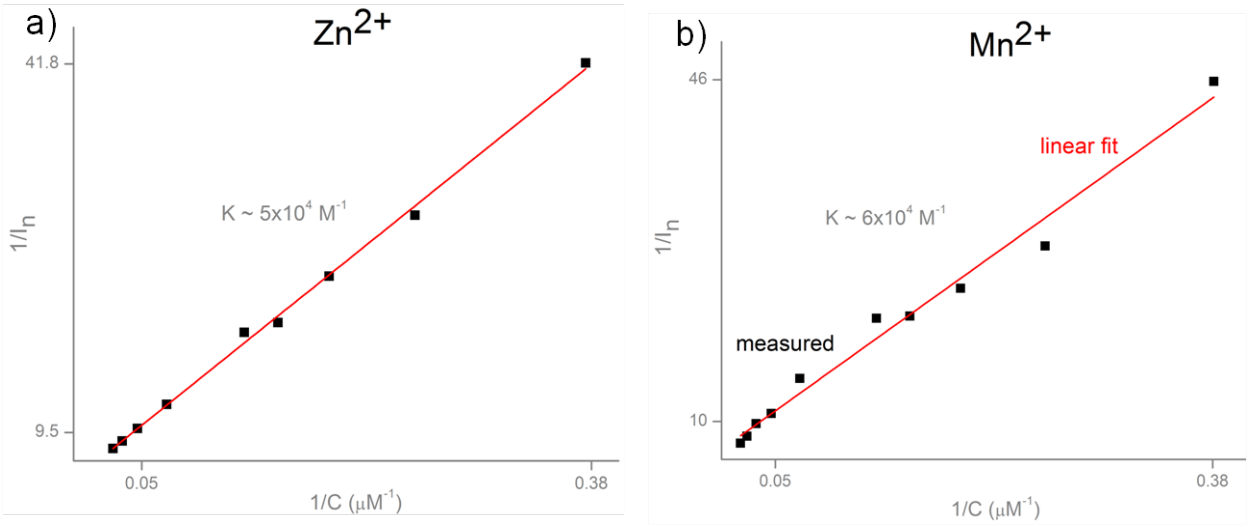

Fig.S2 Linear fit to the Langmuir isotherm of the dose response of Mn^{2+} (R^2=0.98) and Zn^{2+} (R^2=0.99)