Supporting Information

A biomimetic zinc activated ion channel

Ye Tian,[a] Xu Hou,[c] Liping Wen,[a] Wei Guo,[b] Yanlin Song,*[a] Hongzhe Sun,[d] Yugang Wang,*[b] Lei Jiang,*[a] and Daoben Zhu[a]

[a] Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, P. R. China. Fax: (+86)10-8262-7566; Tel: (+86)10-8262-1396; E-mail: jianglei@iccas.ac.cn, ylsong@iccas.ac.cn
[b] State Key Laboratory of Nuclear Physics and Technology, Peking University, Beijing 100871, P. R. China. E-mail: ygwang@pku.edu.cn
[c] National Center for Nanoscience and Technology, Beijing 100190, P. R. China.
[d] Department of Chemistry and Biochemistry and Open Laboratory of Chemical Biology, University of Hong Kong, Pokfulam, Hong Kong, China.

Materials

Poly(ethylene terephthalate) (PET, 12μm thick) membranes were irradiated with single heavy ion (Au) of energy 11.4 MeV/nucleon at UNILAC linear accelerator (GSI, Darmatadt, Germany). The zinc finger, Pro-Tyr-Ala-Cys-Pro-Val-Glu-Ser-Cys-Asp-Arg-Arg-Phe-Arg-Ser-Asp-Glu-Leu-Thr-Arg-His-Ile-Arg-Ile-His-Thr-Gly-Gln-Lys, which is derived from finger 1 of ZIF268, [1] and the control peptide, Pro-Tyr-Ala-Gly-Pro-Val-Glu-Ser-Gly-Asp-Arg-Arg-Phe-Ser-Arg-Ser-Asp-Glu-Leu-Thr-Arg-Gly-Ile-Arg-Ile-Gly-Thr-Gly-Gln-Lys were synthesized by GL Biochem Ltd. (GLS, China), 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide (EDC), N-hydroxysulfosuccinimide (NHSS), 1,4-dithiothreitol (DTT), zinc chloride ($ZnCl_2$),

calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$) were purchased from Sigma-Aldrich. 1,1,1-tris(hydroxymethyl)methylamine (Tris), hydrochloride acid (HCl, 37%), sodium hydroxide (NaOH), potassium chloride (KCl), potassium dihydrogen phosphate (KH_2PO_4), potassium hydrogen phosphate (K_2HPO_4) and formic acid (HCOOH) were purchased from Sinopharm Chemical Reagent Beijing Co. , Ltd. (SCRC, China). All solutions were prepared in MilliQ water (18.2 MΩ).

Nanochannel fabrication

The PET membrane was exposed to the UV light for 1h, [2] then the single nanochannel was prepared by chemical etching method which was developed by others. [3] Briefly, the PET membrane was embedded between the two chambers of a conductivity cell at room temperature (about 25 ^{o}C), one chamber was filled with etching solution (9 M NaOH), while stopping solution (1 M KCl + 1 M HCOOH) was added in the other. Then a voltage of 1 V was applied across the membrane, conical nanochannel with desired diameter could be obtained by monitoring the transmembrane current. The membrane was soaked in MilliQ water (18.2 MΩ) to remove residual salts. The diameter of the large opening of the conical nanochannel which was called base (D) was determined by scanning electron microscopy (SEM) in parallel etching experiments, the diameter of the small opening which was called tip (d_{tip}) was estimated by the following relation:

$$d_{tip} = \frac{4LI}{\pi k(c)UD}$$

Where L is the length of the pore; I and U is the measured current and the applied voltage, respectively; d_{tip} is the tip diameter, D is the base diameter; $k(c)$ is the

special conductivity of the electrolyte. For 1M KCl solution at 25 ^{o}C, *k(c)* is 0.11173 $\Omega^{-1}cm^{-1}$. In this work, the base diameters were several hundred nanometers and the tip diameters were from 7 nm to 45 nm.

Peptides immobilization

The peptides were immobilized on the PET surface and inner pore by a direct amidation method. [4] The PET films were first exposed to an aqueous solution of EDC (15 mg/ml) and NHSS (3 mg/ml) for 1 h, then reacted with a solution of 2 μM peptide and 10 mM DTT in deoxygenated phosphate buffer (pH 7.5) for 4 h. The films were rinsed with extensive deoxygenated MilliQ water (18.2 MΩ) and soaked in deoxygenated phosphate buffer (pH 7.5) for 1 h to remove the unreacted peptides and other reagents. The films were washed with extensive deoxygenated phosphate buffer (pH 7.5) further before current-voltage measurement.

Zinc activated procedure

The $ZnCl_2$ solution was added into the solution of each chamber with a concentration from 10 μM to 100 μM for 4 h for different diameters. Then the films were soaked in and washed with tris-HCl buffer solution (pH 7.5) for three times before current-voltage measurements. For comparison, 100 μM $CaCl_2$ and $MgCl_2$ solution were added into the chambers for 12 h with the same method as described above.

Ion current measurement

The film was placed between the two chambers of the conductance cell, [2] each chamber was filled with 1.7 mL 0.1 M KCl in 5 mM deoxygenated tris-HCl buffer

solution (pH 7.5), Ag/AgCl electrodes were used to apply a transmembrane potential across the film. The ion current was measured by a Keithley 6487 picoammeter (Keithley Instruments, Cleveland, OH). The transmembrane potential used in this work was a scanning voltage varied from -1 V to +1 V on the base side while the tip side of the nanochannel was connected to the ground electrode.

Circular dichroism characterization

Circular dichroism (CD) spectra were collected on a JASCO J-810 CD spectrometer at a peptide concentration of 28 μM in a 1 mm path-length cuvette. The spectra were obtained in 5 mM phosphate buffer (pH 7.5) at 20 ^{o}C with or without 100 μM $ZnCl_2$, $CaCl_2$, $MgCl_2$, respectively. Buffers were deoxygenated with N_2 prior to dissolution of peptides.

X-ray photoelectron spectra characterization

X-ray photoelectron spectra (XPS) data were obtained with an ESCALab220i-XL electron spectrometer from VG Scientific using 300W Al Kα radiation. All peaks were referenced to C 1s (CHx) at 284.8 eV in the deconvoluted high resolution C 1s spectra, and the analysis software used was provided by the manufacturer.

Schematic description of the conformational change of the zinc finger:

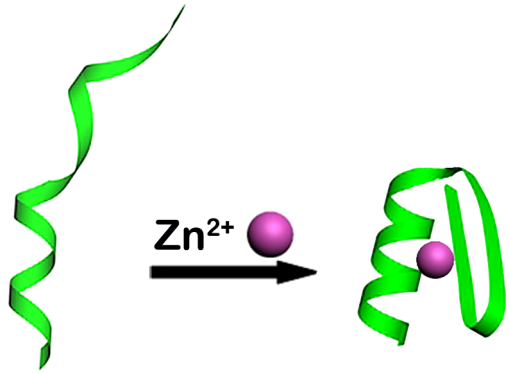

Fig. S1 Schematic description of the conformational change of the zinc finger. In the absence of Zn^{2+}, the zinc finger shows an unfolding conformation, and folds to a finger-like conformation after Zn^{2+} binding.

CD and XPS characterizations of zinc finger:

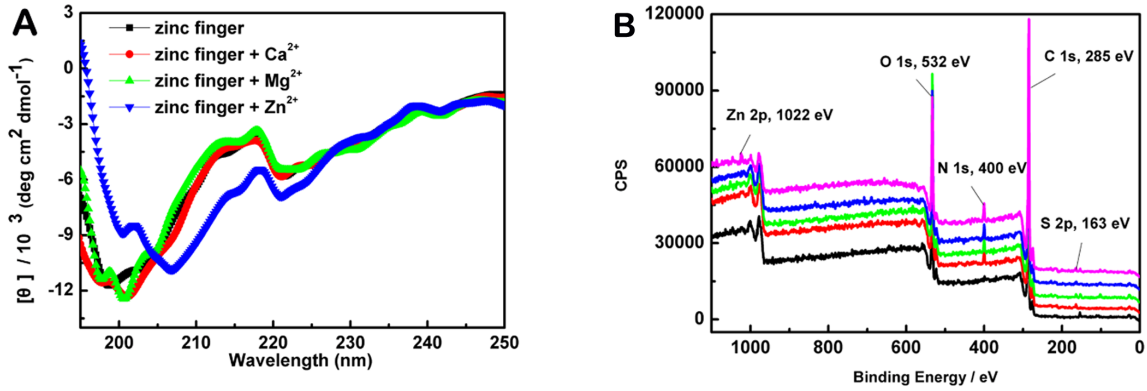

Fig. S2 (A) CD spectra of zinc finger (PYACPVESCDRRSFRSDELTRHIRIHTGQK) in the presence of different ions. The concentrations of peptide and ions are 28 μM and 100 μM, respectively. The differences between the blue line and the black line indicated the Zn^{2+} induced folding of the zinc finger, and the other lines showed slightly differences which corresponded to the almost unchanged conformation of the peptides. [5] (B) XPS spectra of the zinc finger immobilized PET films. The different colour lines corresponding to the naked PET film (black), after zinc finger immobilization (red), and following by interacting with Ca^{2+} (green), Mg^{2+} (blue) and

Zn^{2+} (magenta), respectively. The sulfur element corresponds to the cysteine residues, the nitrogen element exists in all the residues of the zinc finger, and the existence of zinc element in the magenta line indicates that the retention of the zinc binding properties of the zinc finger after immobilizing on PET film, the absence of Ca^{2+} and Mg^{2+} demonstrates that they could not binding to the zinc finger.

Table S1 The analysis of element content of the zinc finger immobilized PET films, 1~5 correspond to the naked PET film (1), after zinc finger immobilization (2), and following by interacting with Ca^{2+} (3), Mg^{2+} (4) and Zn^{2+} (5), respectively.

Sample / Element	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
C	**74.74**	**72.52**	**74.13**	**70.81**	**79.02**
O	**25.26**	**23.72**	**21.76**	**25.23**	**16.22**
N	—	**2.97**	**3.37**	**3.19**	**3.64**
S	—	**0.79**	**0.74**	**0.77**	**0.81**
Zn	—	—	—	—	**0.31**

CD and XPS characterizations the control peptide:

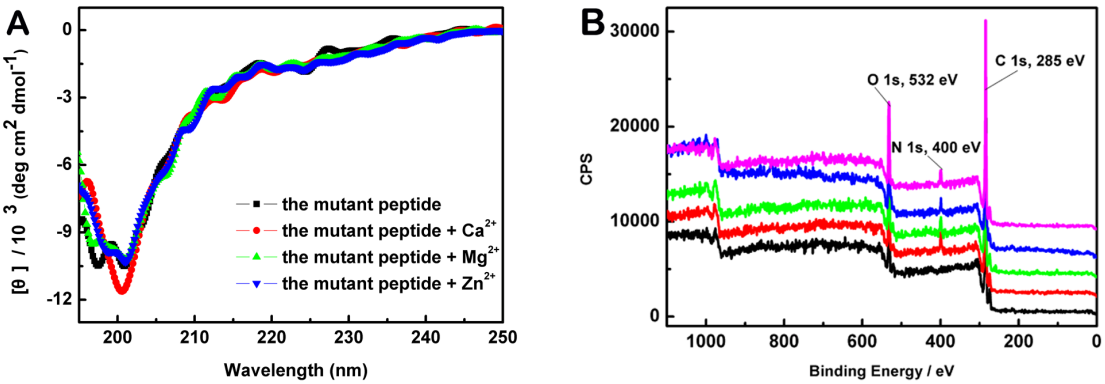

Fig. S3 (A) CD spectra of the control peptide (PYAGPVESGDRRFSRSDELTRGIRIGTG- QK) in the presence of different ions. The concentrations of peptide and ions are 28 μM and 100 μM, respectively. These lines showed slightly differences corresponded to the almost unchanged conformation of the control peptide. (B) XPS spectra of the control peptide immobilized PET films. The different colour lines corresponding to the naked PET film (black), after the control peptide immobilization (red), and following by interacting with Ca^{2+} (green), Mg^{2+} (blue) and Zn^{2+} (magenta), respectively. The nitrogen element confirms the successful immobilization of the control peptide, and from the other lines, we can conclude that the control peptide could not bind with these three kinds of ions.

Table S2 The analysis of element content of the control peptide immobilized PET films, 1~5 correspond to the naked PET film (1), after the control peptide immobilization (2), and following by interacting with Ca^{2+} (3), Mg^{2+} (4) and Zn^{2+} (5), respectively.

Element \ Sample	1	2	3	4	5
	(%)	(%)	(%)	(%)	(%)
C	75.16	79.17	80.00	77.08	76.08
O	24.84	15.84	15.53	18.72	19.36
N	—	4.98	4.47	4.18	4.56

Current-voltage (*I-V*) characteristics:

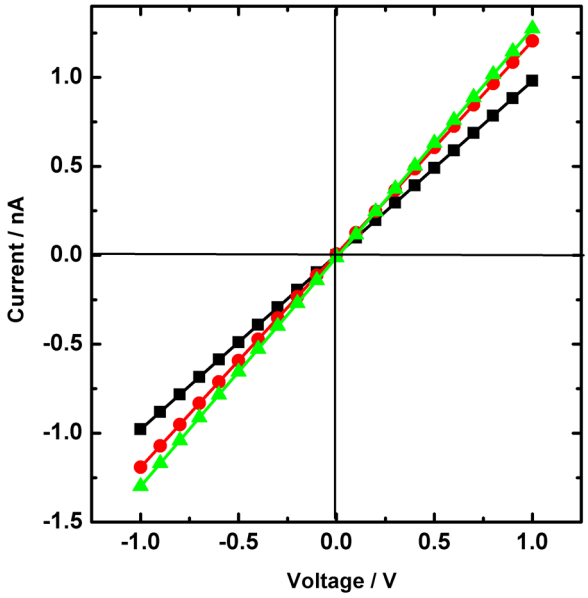

Fig. S4 *I-V* characteristics of zinc fingers immobilized nanochannel in 0.1 M KCl with 0 μM (■, black) 10 μM (●, red) and 100 μM (▲, green) Zn^{2+}. Before zinc fingers immobilization, the diameters of the tip and base are about 28 nm and 650 nm, respectively.

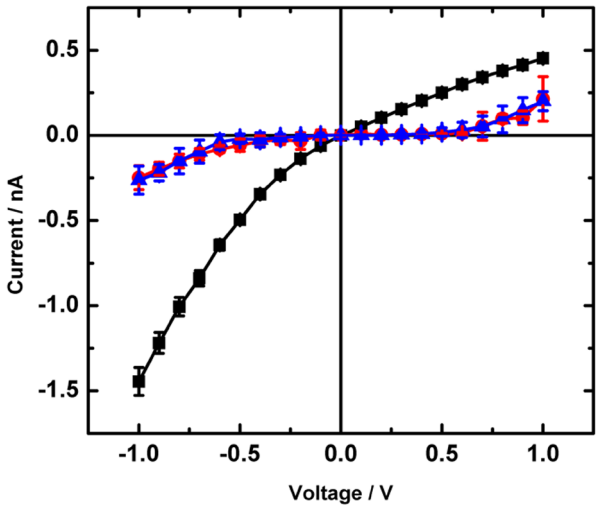

Fig. S5 *I-V* characteristics of a single polymeric nanochannel in 0.1 M KCl before (■, black) and after (●, red) zinc finger immobilization and followed by interacting with 100 μM Zn^{2+} (▲, blue). Each data point is the average of five replicate measurements. Before zinc fingers immobilization, the diameters of the tip and base are about 7 nm and 470 nm, respectively.

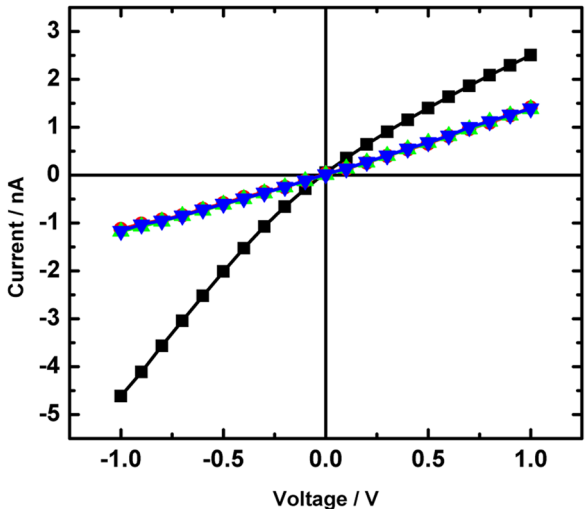

Fig. S6 *I-V* characteristics of a single nanochannel in 0.1 M KCl before (■, black) and after (●, red) zinc fingers immobilization and followed by interacting with 100 μM Ca^{2+} (▲, green) and 100 μM Mg^{2+} (▼, blue), respectively. Before zinc fingers immobilization, the diameters of the tip and base are about 27 nm and 620 nm,

respectively. Each data point is the average of five replicate measurements. Error bars are not shown in the *I-V* curves because they are approximately equivalent to the width of the lines in the plot.

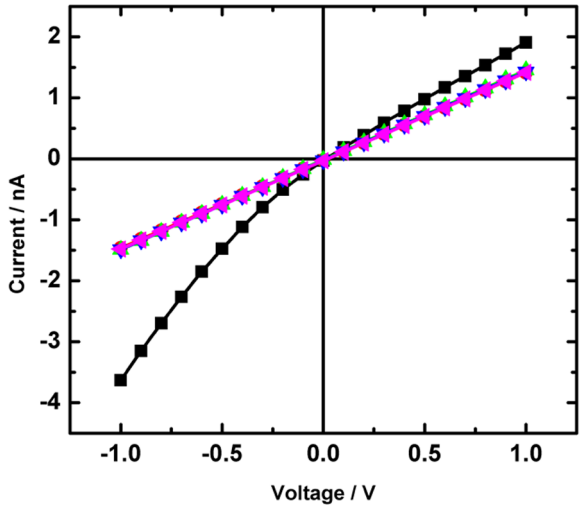

Fig. S7 *I-V* characteristics of a single nanochannel in 0.1 M KCl before (■, black) and after (●, red) the control peptides immobilization and followed by interacting with 100 μM Ca^{2+} (▲, green), 100 μM Mg^{2+} (▼, blue), and 100 μM Zn^{2+} (◀, magenta), respectively. Before the control peptides immobilization, the diameters of the tip and base are about 28 nm and 690 nm, respectively. Error bars are not shown in the *I-V* curves because they are approximately equivalent to the width of the lines in the plot.

References:

1. N. P. Pavletich and C. O. Pabo, *Science*, 1991, **252**, 809.

2. P. Apel, *Radiat. Measur.*, 2001, **34**, 559.

3. P. Y. Apel, Y. E. Korchev, Z. Siwy, R. Spohr and M. Yoshida, *Nucl. Instrum. Methods B*, 2001, **184**, 337.

4. G. T. Hermanson, *Bioconjugate techniques*, Academic Press: San Diego, California, 1996.

5. (a) G. Parraga, S. J. Horvath, A. Eisen, W. E. Taylor, L. Hood, E. T. Young and R. E. Klevit, *Science*, 1988, **241**, 1489; (b) A. Nomura and Y. Sugiura, *Inorg. Chem.*, 2002, **41**, 3693; (c) M. J. Lachenmann, J. E. Ladbury, N. B. Phillips, N. Narayana, X. Qian and M. A. Weiss, *J. Mol. Biol.*, 2002, **316**, 969.