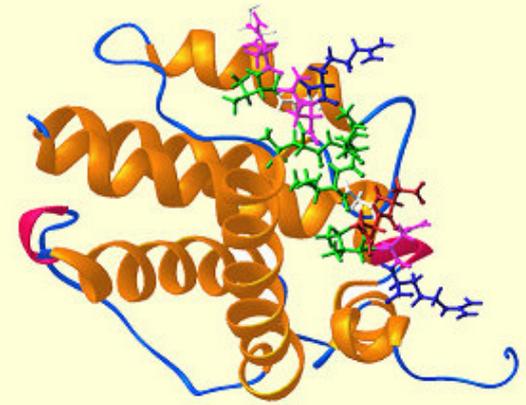


Nanoscopic interrogation of molecular interactions with protein nanopores

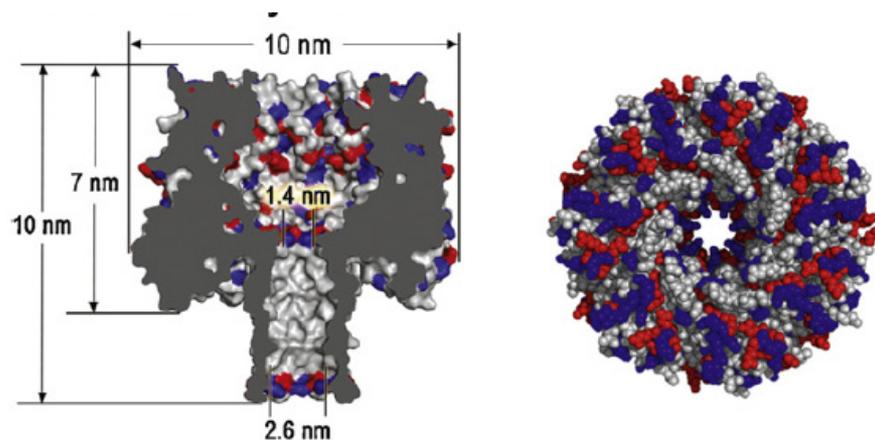
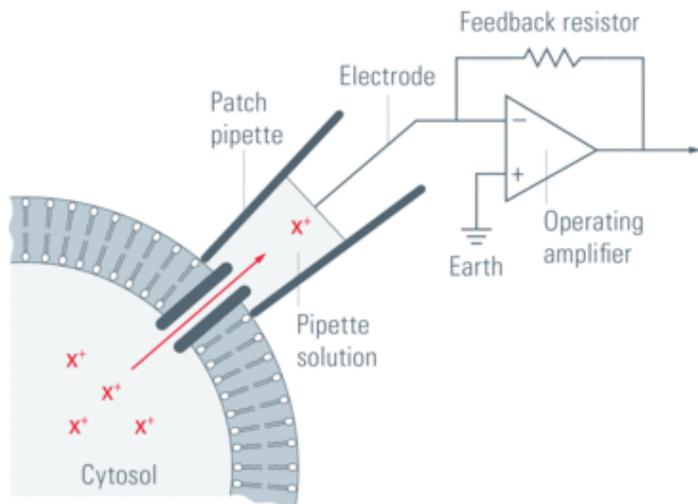
Tudor Luchian, Department of Physics, Laboratory of Molecular Biophysics
and Medical Physics, Alexandru I. Cuza University, Iasi, Romania
E-mail: luchian@uaic.ro

Nanoscopic interrogation of molecular interactions with protein nanopores



- Brief overview of the nanopore sensing technique**
- Molecular braking of single-molecule passage through a protein nanopore**
- Salt-dependent folding of model antimicrobial peptides**
- Bioanalytical applications of nanopore-based devices.
Application to metals sensing**

Nanopore-based, single-molecule sensing and analysis – general principle

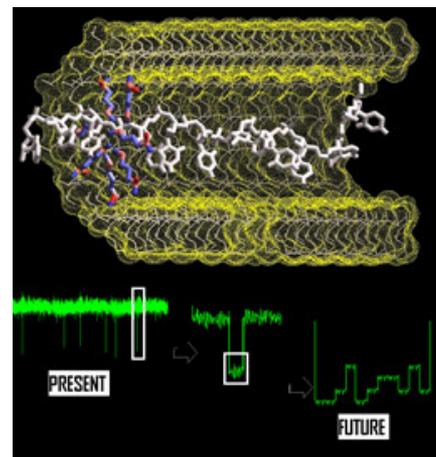


Song, L. Z. et al., *Science*, 1996, 274, 1859–1866

The Nobel Prize in Physiology or Medicine 1991 awarded jointly to Erwin Neher and Bert Sakmann "for their discoveries concerning the function of single ion channels in cells"



<http://www.nobelprize.org/>



Kasianowicz, J. J. et al., *Proc. Natl. Acad. Sci. U. S. A.* 1996, 93, 13770–13773

Bayley, H. et al., in *'Single Molecules and Nanotechnology'*, 2008, 251–277

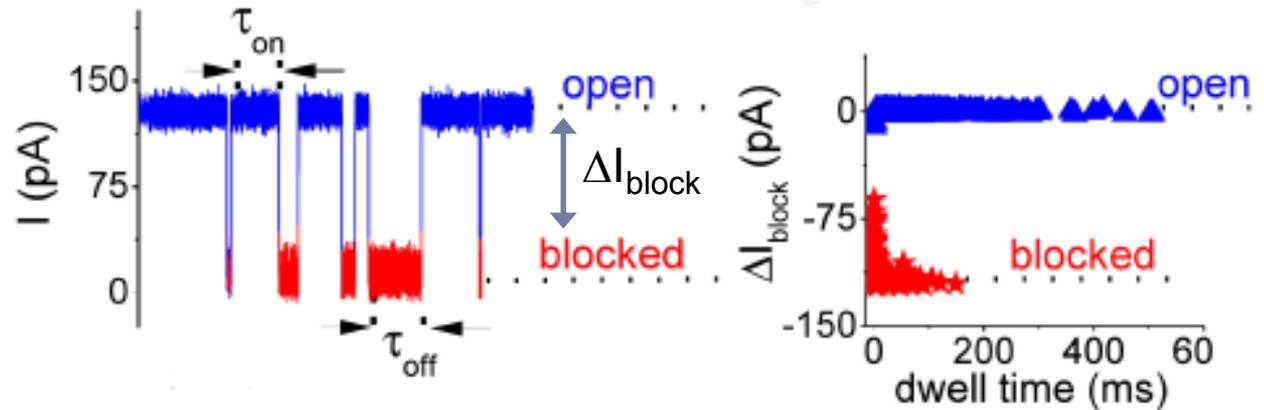
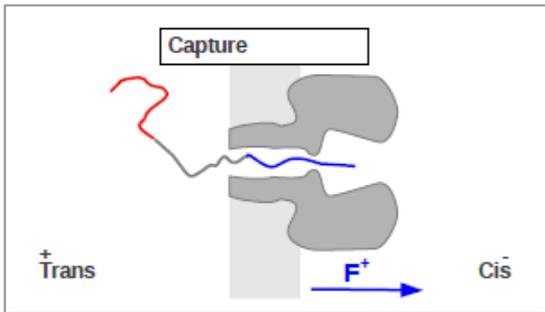
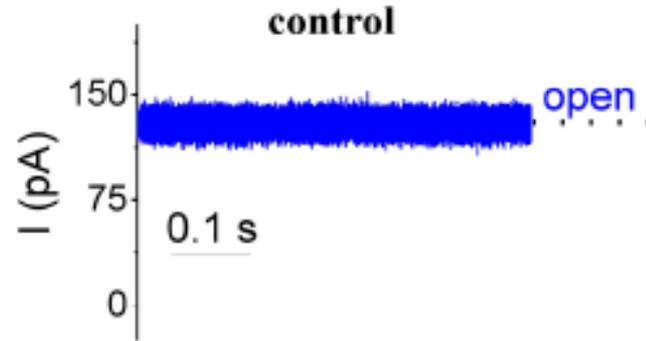
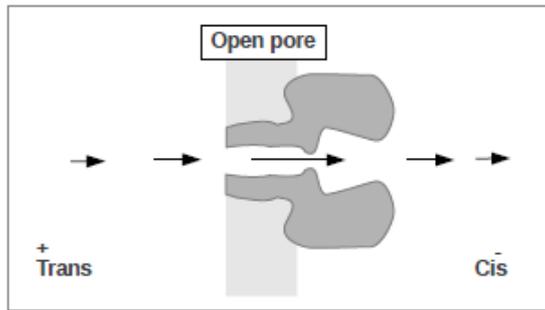
Majd, S. et al. *Curr. Opin. Biotechnol.* 2010, 21, 439–476

Wanunu, M. *Physics of Life Reviews* 2012, 9, 125–158

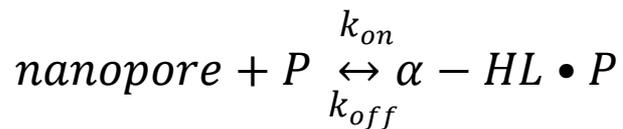
Oukhaled, A. et al. *ACS Chem. Biol.* 2012, 7, 1935–1949

Luchian, T. et al., *Angew. Chem. Int. Ed.* 2003, 42, 3766–3771

Nanopore-based, single-molecule sensing and analysis – general principle



Key facts: the association and translocation time, along with the amplitude of the current blockade depends on the charge, size and conformation of the molecule.



$$\delta = \frac{\Delta I_{block} (l_{pore} + 0.8d_{pore})^2}{\gamma \sigma \Delta V}$$

$$k_{on} = \frac{1}{[P] \hat{\tau}_{on}} \quad k_{off} = \frac{1}{\hat{\tau}_{off}}$$

Concise applications of the nanopore technology

- sensing and separation (DNA, RNA, peptides, proteins, metals)
- single-molecule physics and chemistry
- study of proteins folding-unfolding

Oxford NANOPORE Technologies

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Technology

- Introduction to nanopore sensing
- Electronics for nanopore sensing
- The MinION™ device: a miniaturised sensing system
- The PromethION™ system
- The GridION™ system
- Workflow versatility: no fixed run time
- Nanopore sensing: informatics
- Automatic optimisation of system performance
- Analyses and applications: DNA, RNA, proteins
- DNA: An introduction to nanopore sequencing
- DNA strand sequencing

DNA: An introduction to nanopore sequencing

Oxford Nanopore is developing 'strand sequencing', a method of DNA analysis that is compatible with the Company's GridION™ and MinION™ systems.

'Strand sequencing' is a technique that passes intact DNA polymers through a protein nanopore, sequencing in real time as the DNA translocates the pore. Oxford Nanopore intends to commercialise this technology independently.

The following movie is an introduction to nanopore DNA sequencing. For more information please visit the specific pages linked above.

DNA sequencing with nanopores

from Oxford Nanopore

GGTGTTCCTCTGTTGGTGGCGATATTCCTTTGAC

genomeweb

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Home » Business, Policy & Funding » Business News » Illumina Licenses Nanopore-based Sequencing Technology from UAB-UW

Illumina Licenses Nanopore-based Sequencing Technology from UAB-UW

Oct 15, 2013 | a GenomeWeb staff reporter

NEW YORK (GenomeWeb News) – The University of Alabama, Birmingham announced on Monday that it and the University of Washington have licensed to Illumina the rights to nanopore sequencing technology developed by a UAB microbiologist and a University of Washington physicist.

The deal gives Illumina exclusive worldwide rights to develop and market the technology developed by UAB's Michael Niederweis and UW's Jens Gundlach. The UAB Research Foundation and UW have filed patent applications covering the technology. Niederweis will serve as a consultant to Illumina as part of the deal.

Further terms of the agreement were not disclosed.

"Many companies and universities are looking at the potential of nanopore technology, but the technology developed by Niederweis and Gundlach is among the most promising," Christian Henry, senior vice president and general manager of Illumina's Genomic Solutions business, said in a statement.

UAB and UW's technology is based on attaching DNA polymerases to DNA strands, which move through nanopores. One problem with nanopore-based sequencing has been that DNA strands move too quickly through nanopores for electric signatures to be captured. UAB said. But in the technology.

RNA-Seq, STANDARDIZED

Breaking News

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- NIH to Fund Coordination Center for Alzheimer's Research
- Q&A: Clinical Microbiology Lab Director Nathan Ledeboer on MDx Trends
- PositveID to Issue \$4M in Senior Notes to Investor
- Genomics Ltd. Raises \$12.6M in Private Financing
- Cancer Research UK, Illumina Ink Sequencing Tech Supply Deal for Lung Cancer Trial

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TECHNOLOGY

Technology References

Beyond 1000

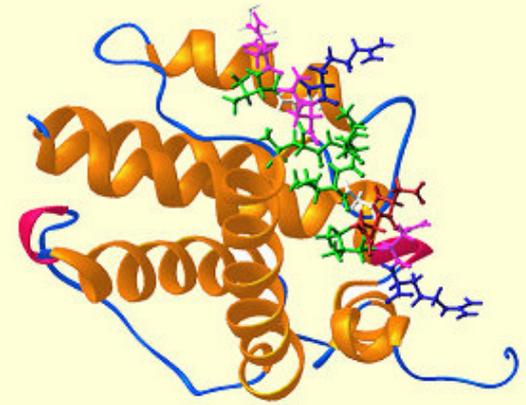
As the genomics community pushes toward the \$1,000 genome, Genia is looking beyond, to the \$100 genome, bringing the benefits of genome sequencing out of the lab and into the everyday world.

Biological Nanopores: Structure-Based Sequencing of Single DNA Molecules

Genia's technology reduces the price of sequencing and increases speed, accuracy, and sensitivity by moving away from complex sample preparation and optical detection. The heart of Genia's technology is the **biological nanopore**, a protein pore embedded in a lipid bilayer membrane. Our planar electronic sensor technology enables highly efficient nanopore-membrane assembly and accuracy of current readings, overcoming many of the limitations faced by earlier nanopore sequencing efforts.

Genia's NanoTag sequencing approach, developed in collaboration with Columbia and Harvard University, uses a DNA replication enzyme to sequence a template strand with single base precision as base-specific engineered tags cleaved by the enzyme are captured by the nanopore. As the cleaved tags travel through the pore, they attenuate the current flow across the membrane in a sequence-dependent manner (Figure 1).

1. Molecular braking of single-molecule passage through a protein nanopore



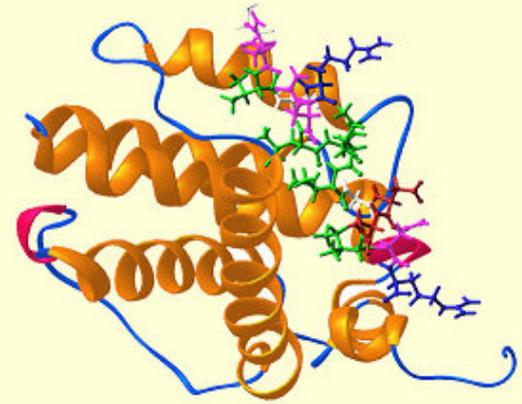
- Usually the translocation process is too fast and precludes the precise characterization of the transiently present moieties inside the nanopore.

$$x = 2.5 \text{ nm}, D = 0.5 \times 10^{-9} \text{ m}^2\text{s}^{-1}$$
$$\langle t \rangle \sim 6.25 \text{ ns}$$

- Using pH-tuned single-molecule electrophysiology we demonstrate how peptide passage through the α -hemolysin protein can be sufficiently slowed down to observe intermediate single-peptide sub-states along the pore.



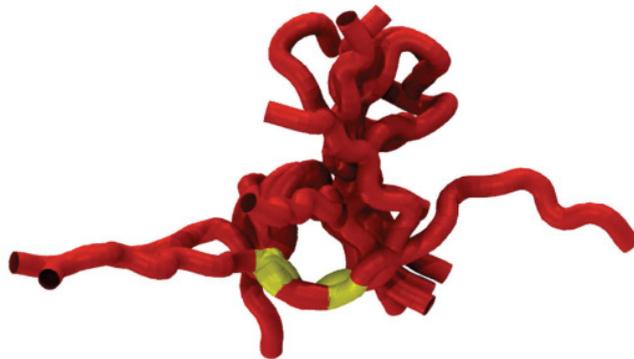
Molecular braking of single-molecule passage through a protein nanopore



CAMA3; net charge ~ + 8 at neutral and acidic pH

KWKLFKKIGIGKFLQSAKKF-NH₂

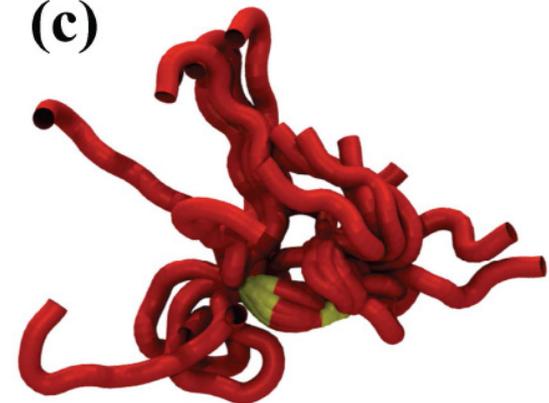
(a)



(b)

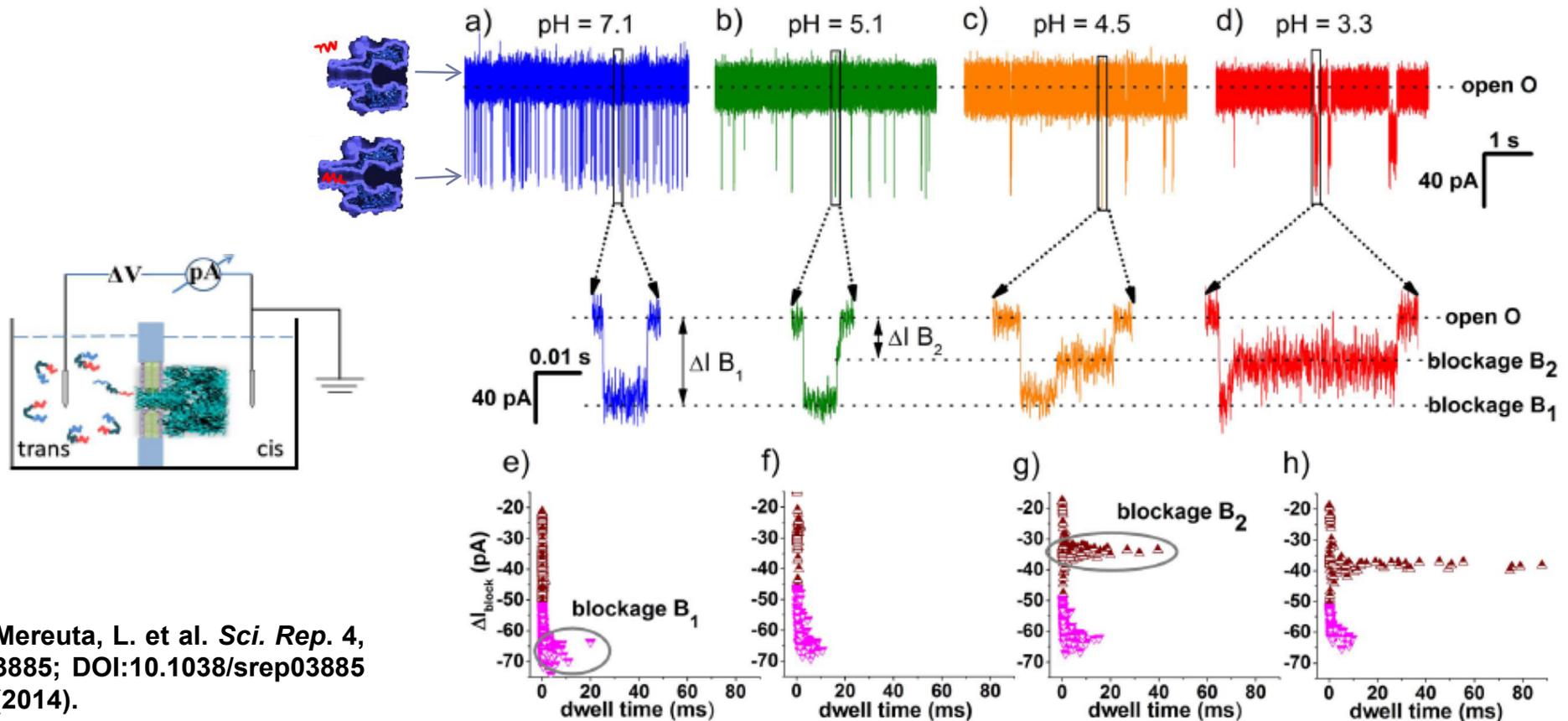
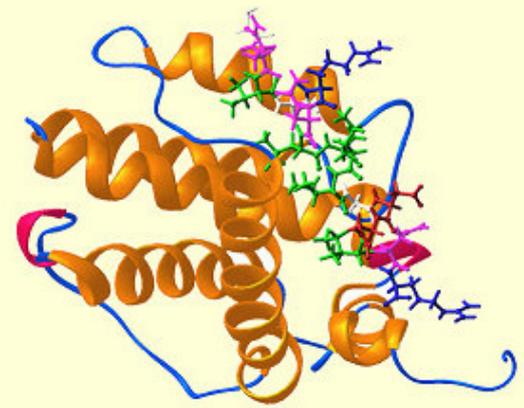


(c)



Peptide in (a) bulk, (b) α -HL' lumen and (c) α -HL's vestibule

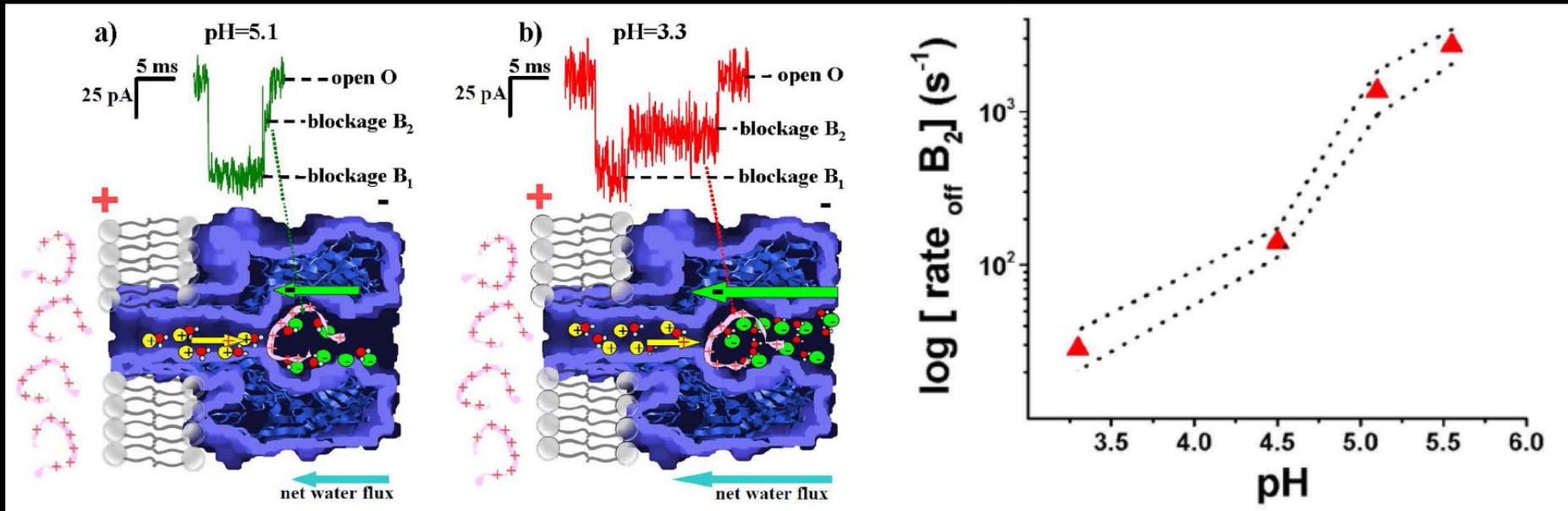
Molecular braking of single-molecule passage through a protein nanopore



Schematic representation of pH-augmented electroosmotic (ELO) braking

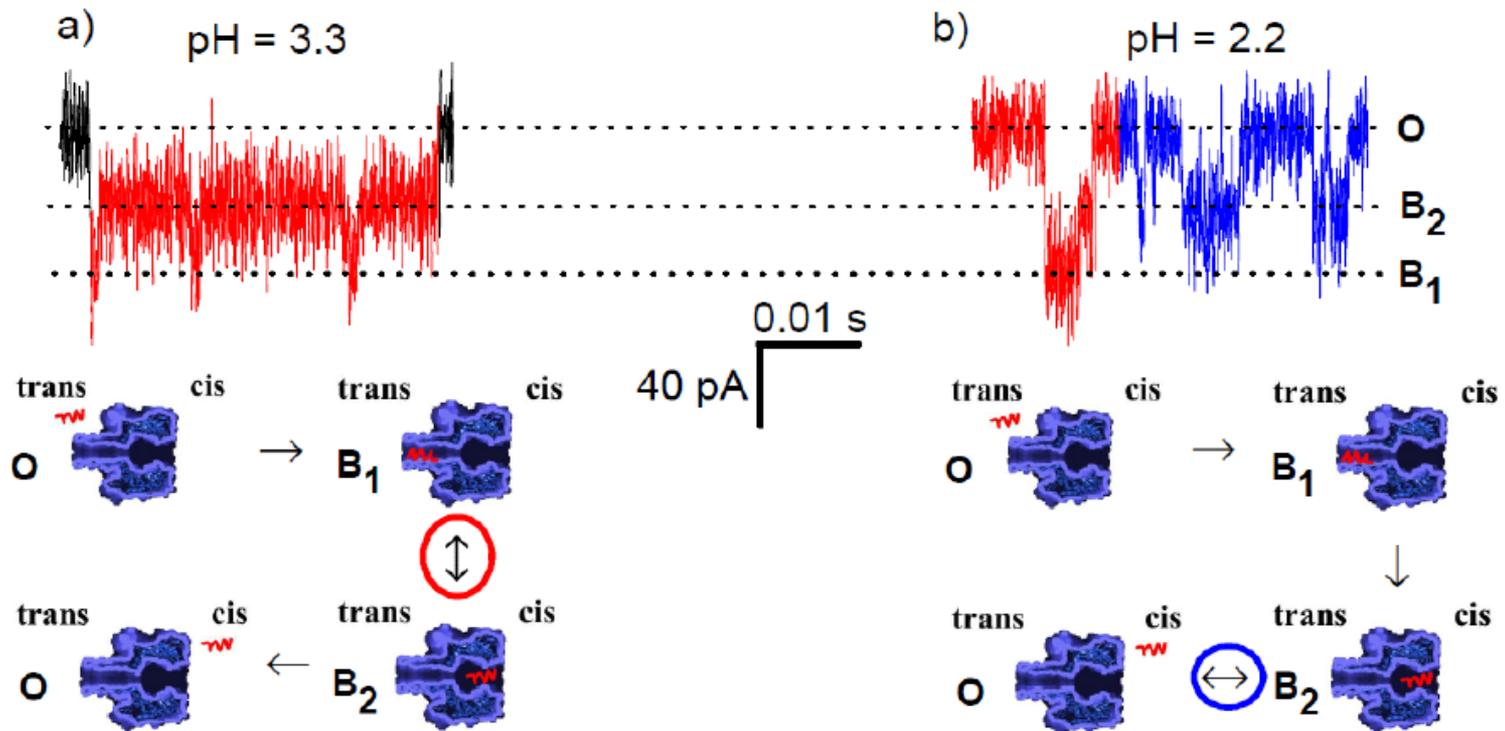
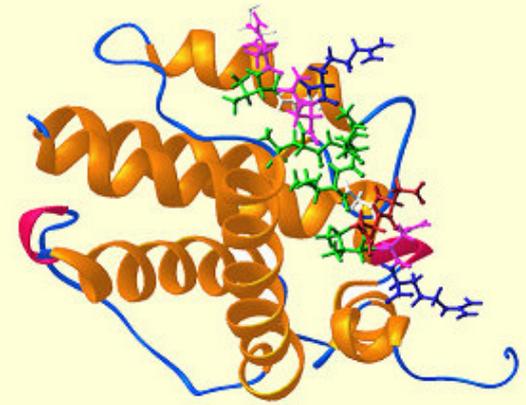
$P_{K^+}/P_{Cl^-} = 0.86$; pH 7.1

$P_{K^+}/P_{Cl^-} = 0.44$; pH 4.4



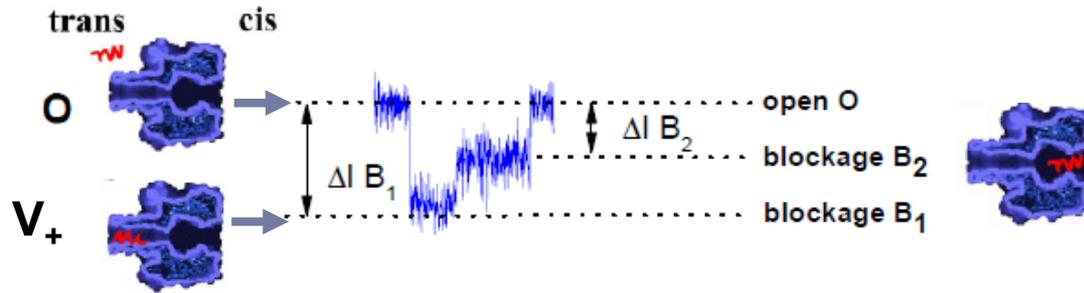
$$v_{drift} = v_{electrophoretic} - v_{electroosmotic} = \mu \frac{\Delta V}{l_{pore}} - \frac{(P_{Cl^-} - P_{K^+})}{(P_{Cl^-} + P_{K^+})} N_h I / (|e^-| S_{pore} [H_2O])$$

Controlling the balance between the ELF and ELO forces helps trapping the translocating peptides

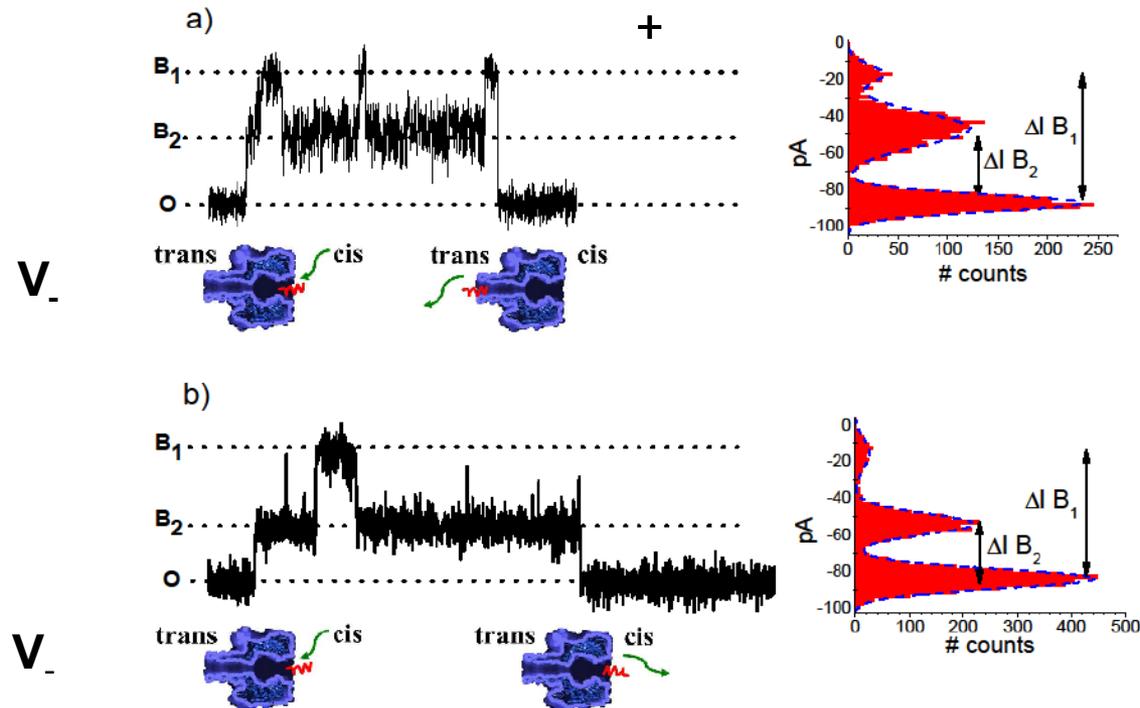


Peptide braking unravels the directionality of peptide motion across the protein nanopore

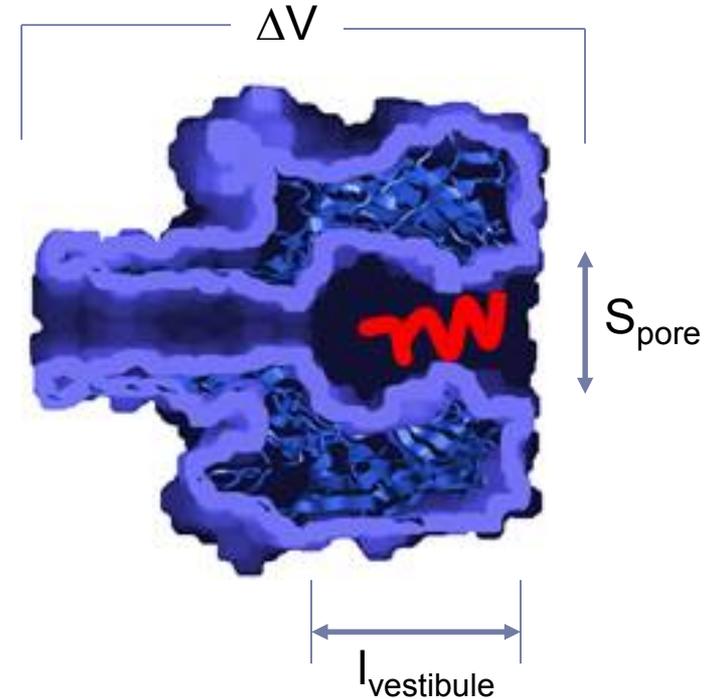
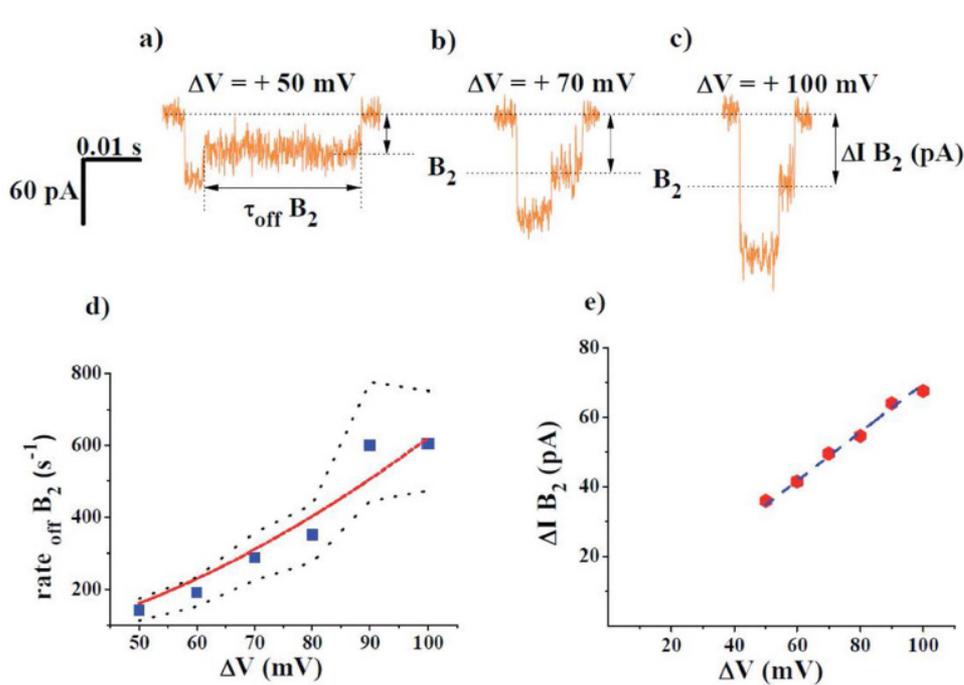
Peptide added from trans (pH = 4.5):



Peptide added from cis (pH = 4.5)



Insights into molecular behavior of a peptide inside the pore, subjected to confinement effects: (i) the diffusion coefficient



$D = 1.5 \cdot 10^{-12} \text{ m}^2 \text{ s}^{-1}$ (drift-diffusion model)

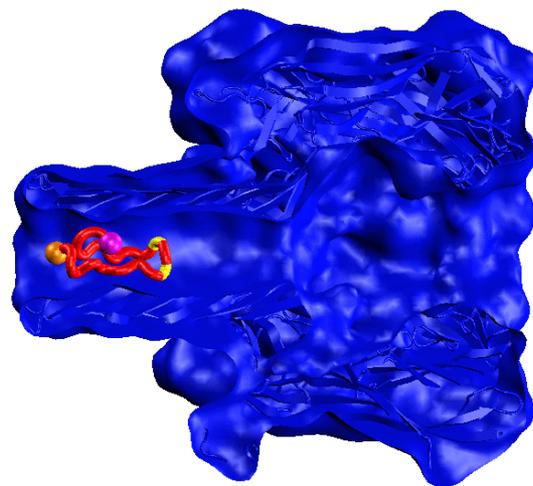
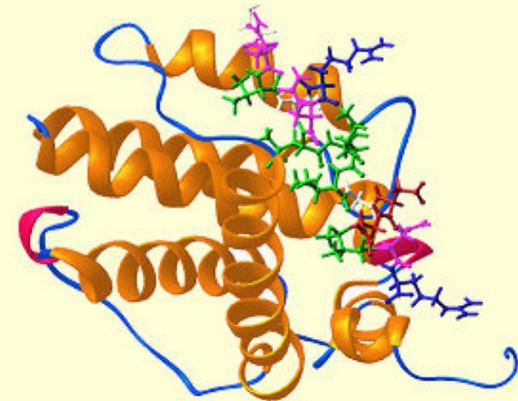
Diffusion of peptide with comparable size *in water*: $D = 5 \cdot 10^{-10} \text{ m}^2 \text{ s}^{-1} \div 10^{-9} \text{ m}^2 \text{ s}^{-1}$.

A similarly sized peptide in a buffer slightly more viscous than water, was measured to have $D = 2.4 \cdot 10^{-12} \text{ m}^2 \text{ s}^{-1}$.

$$\langle t_{\text{off}} \rangle = \frac{1}{\text{rate}_{\text{off}} B_2} = \int_0^{\infty} \frac{t(v_{\text{drift}} t + l_{\text{vestibule}}) e^{-\left[\frac{(l_{\text{vestibule}} - v_{\text{drift}} t)^2}{4Dt} \right]}}{2t\sqrt{4D\pi t}} dt$$

$$v_{\text{drift}} = \mu \frac{\Delta V}{l_{\text{pore}}} - \frac{N_{\text{h}} I \left(\frac{P_{\text{Cl}^-}}{P_{\text{K}^+}} - 1 \right)}{|e^-| \left(\frac{P_{\text{Cl}^-}}{P_{\text{K}^+}} + 1 \right)} \frac{1}{S_{\text{pore}} [\text{H}_2\text{O}]}$$

Molecular simulation of the peptide transit across the α -HL pore

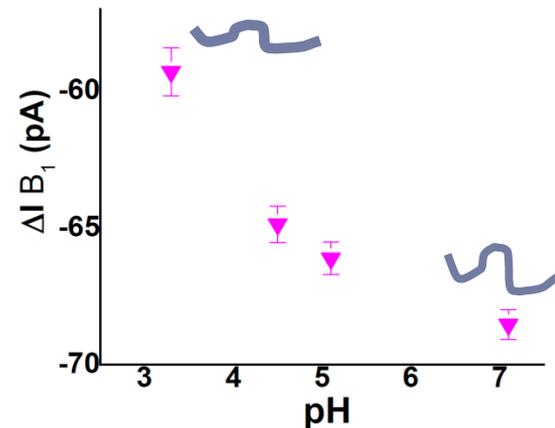
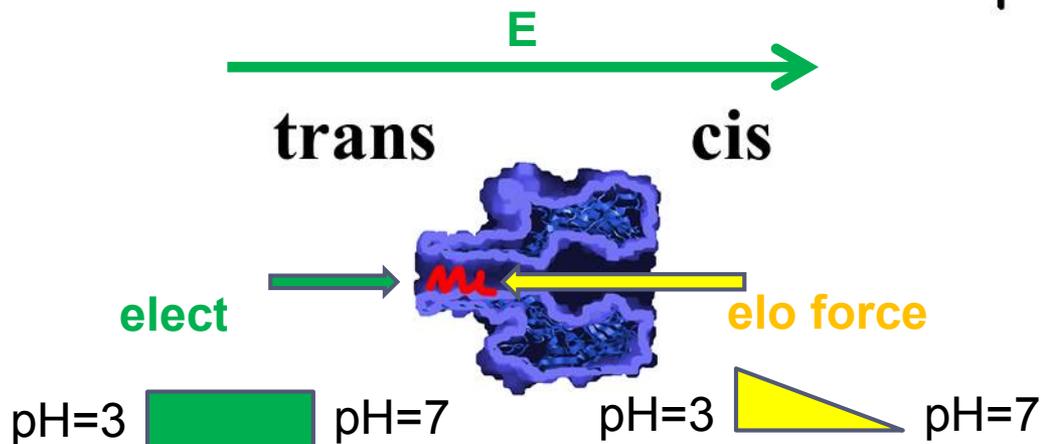
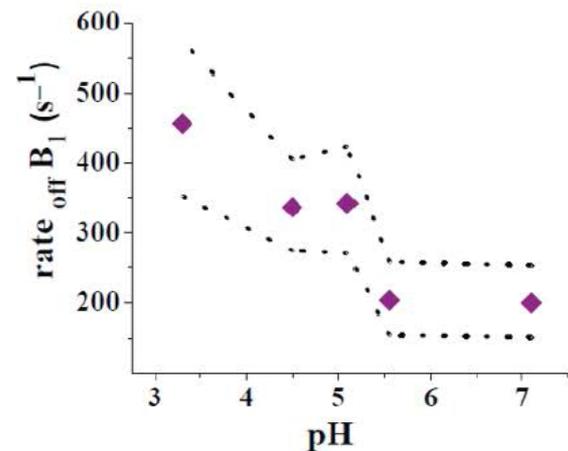
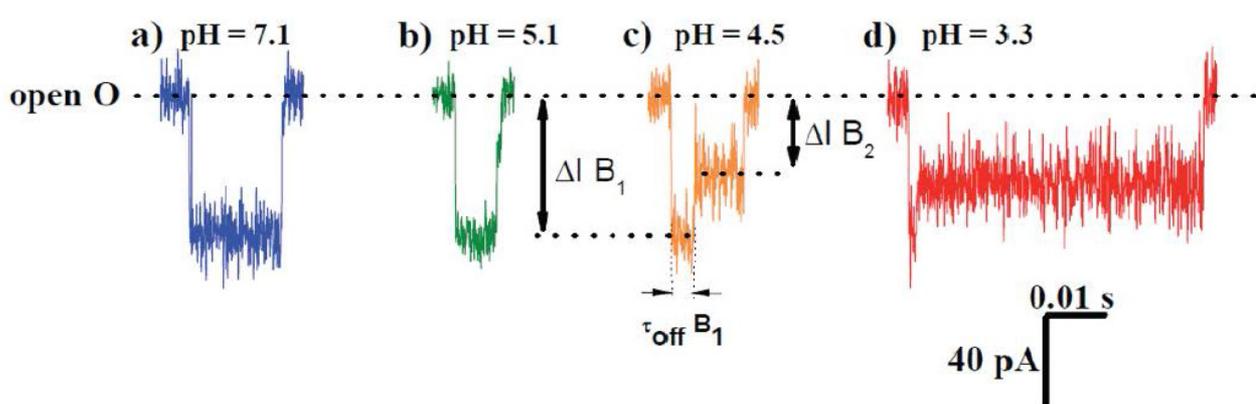


Ioan Andricioaei's Lab, University of California, Irvine, USA

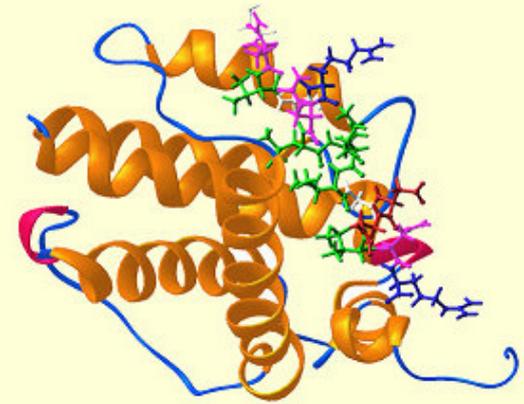
Mereuta, L. et al. *Sci. Rep.* 4, 3885; DOI:10.1038/srep03885 (2014).

Mereuta, L. et al., *ACS Applied Materials & Interfaces*, 2014 6(15):13242-56.

Insights into molecular behavior of a peptide inside the pore, subjected to confinement effects: (ii) peptide unfolding under ELF and ELO forces



2. Salt-dependent folding of model antimicrobial peptides

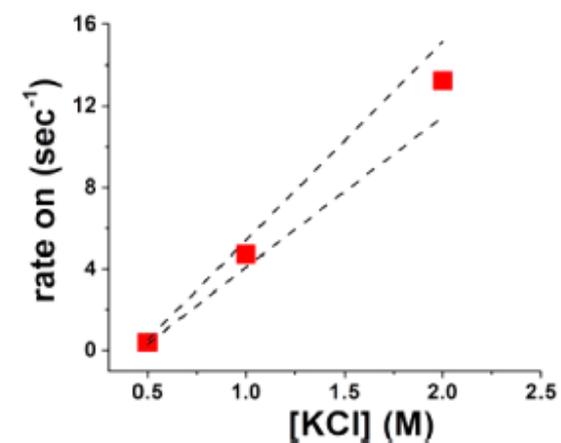
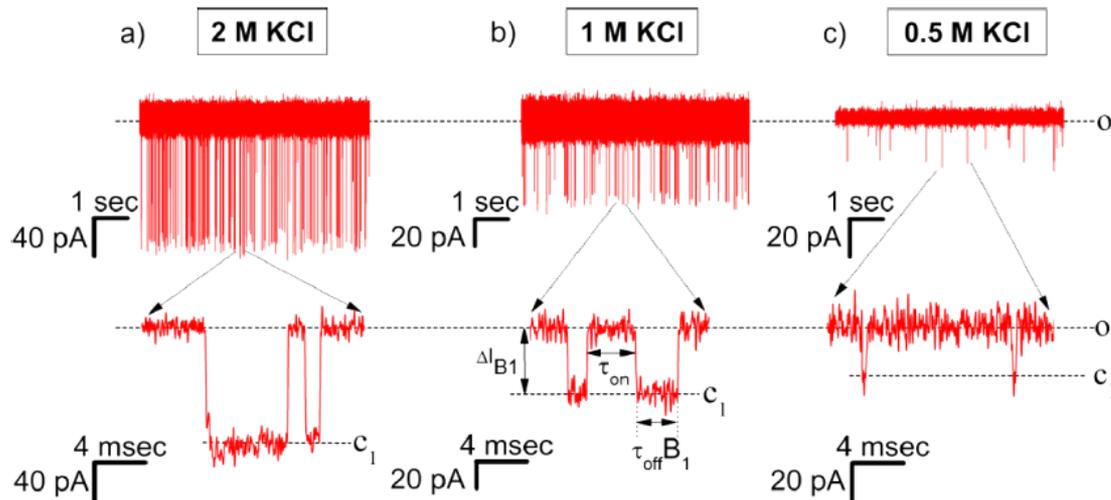


- ❑ Folding events of β -hairpin peptides are triggered by the presence of salt, as a direct consequence of the electrostatic screening between charged amino acids within the peptide.
- ❑ Knowing that:
 - ❑ *the hairpin-like conformations constitute an important trigger for the toxicity onset of peptides and proteins associated with human disorders*
 - ❑ *given the impact of histidine residues in establishing peptides activity*

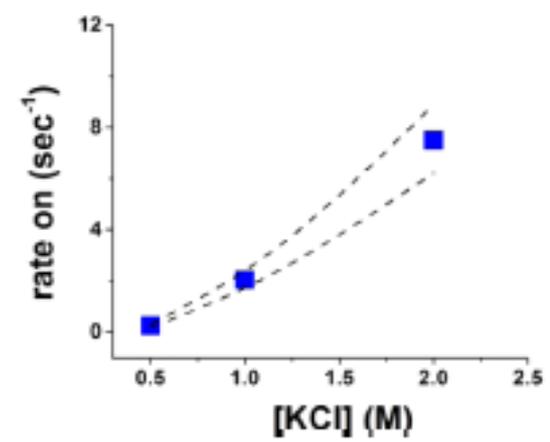
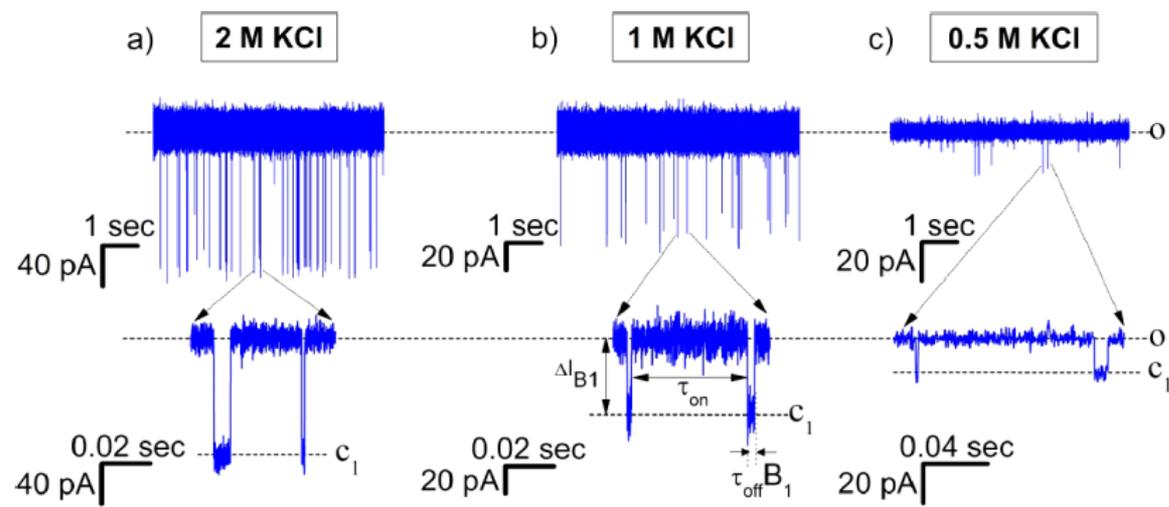
We used the nanopore-based, ‘single-molecule’ analysis technique to investigate the salt dependence of folding state of histidine-containing, β -hairpin-like peptides.

Salt-dependent folding of model antimicrobial peptides

KWKLKKHIGIGKHFLSAKKF-NH₂ + 8 e⁻ (CAMA 3)

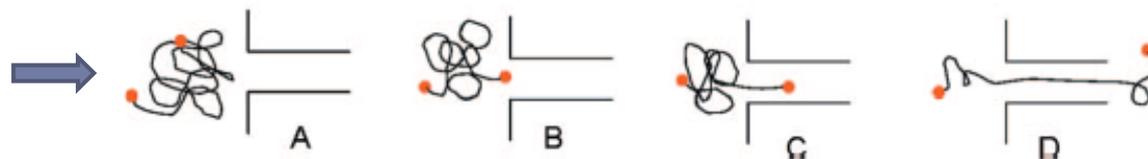


KWKLFKKIGIGKHFLSAKKF-NH₂ + 8 e⁻ (CAMA 1)



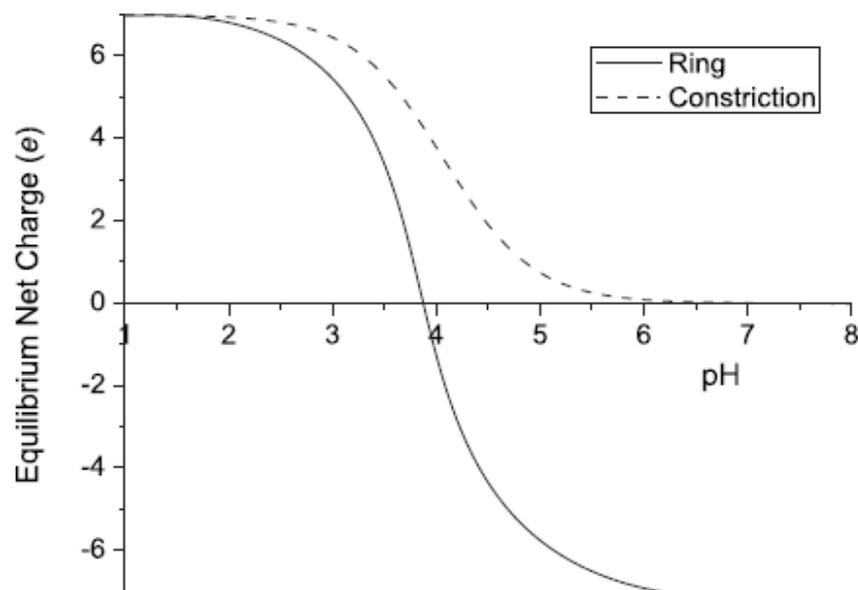
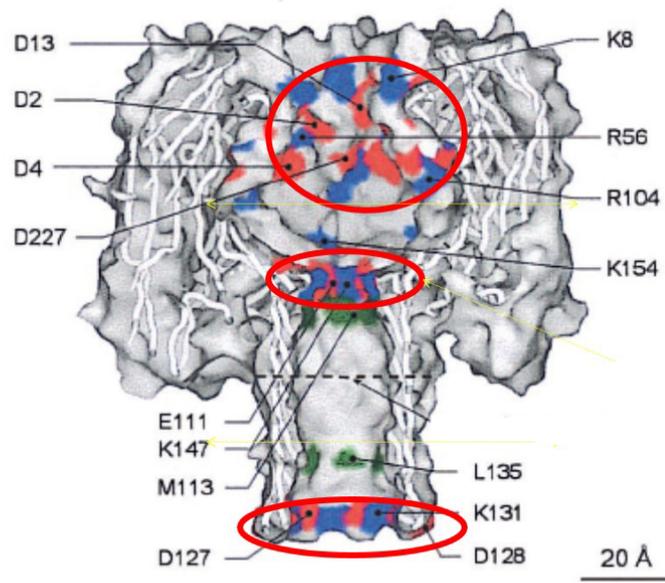
Salt-dependent folding of model antimicrobial peptides

The essential steps of peptide partitioning inside α -HL



Muthukumar, M., *The Journal of Chemical Physics* **132**, 195101, 2010

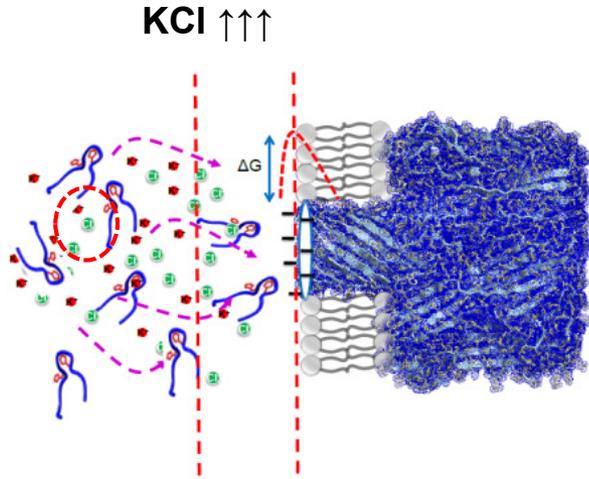
The electrostatic field around distinct α -HL domains changes with pH and ion strength



Gu, L-Q. et al., *PNAS*, **97**, 3959–396, 2000

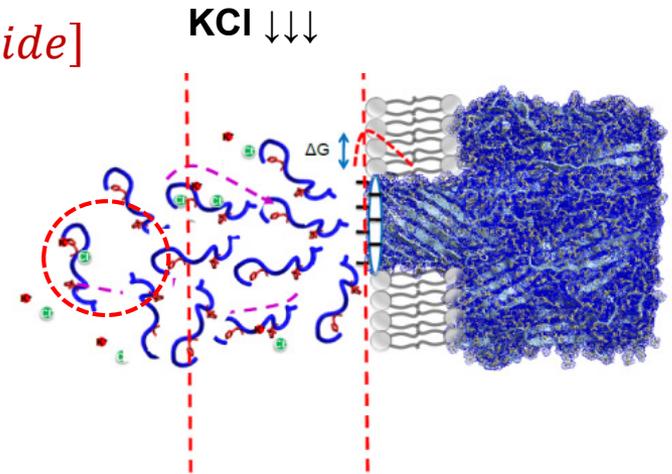
Wong, C.T.A. and Muthukumar, M.,
The Journal of Chemical Physics **133**, 045101, 2010

Salt-dependent folding of model antimicrobial peptides



$$rate_{on} = k_{on}[peptide]$$

$$k_{on} \approx e^{-\frac{\Delta G}{RT}}$$

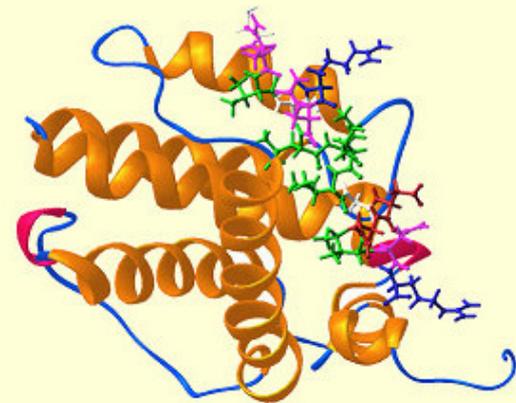


$$\frac{rate_{on;CAMA3}}{rate_{on;CAMA1}} = \frac{e^{-\left(\frac{\Delta H_{CAMA3}^* - T\Delta S_{CAMA3}^*}{RT}\right)}}{e^{-\left(\frac{\Delta H_{CAMA1}^* - T\Delta S_{CAMA1}^*}{RT}\right)}} = e^{\left(\frac{\Delta\Delta S_{CAMA3-CAMA1}^*}{R}\right)}$$

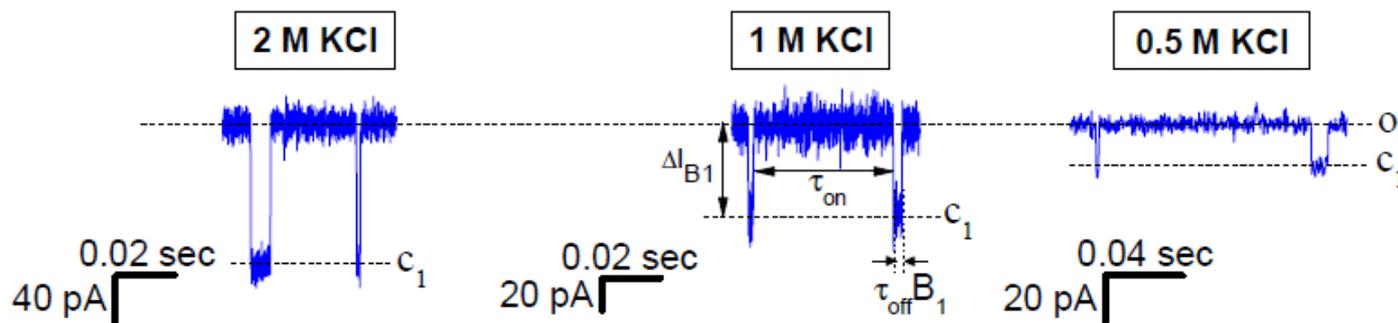
$$\Delta\Delta S_{CAMA3-CAMA1}^* = R \ln \left(\frac{rate_{on;CAMA3}}{rate_{on;CAMA1}} \right) = 4.7 \text{ J K}^{-1} \text{ M}^{-1}$$

- The approach provides quantitative insights into the entropic barrier differences of rather similar peptides to partitioning within confined nano-volumes
- This can be used to probe shifts in the relative populations of distinct folding states of peptides that occur in response to precise altering of inter-residues electrostatic, coordinative or aromatic interactions, via single-point mutagenesis.

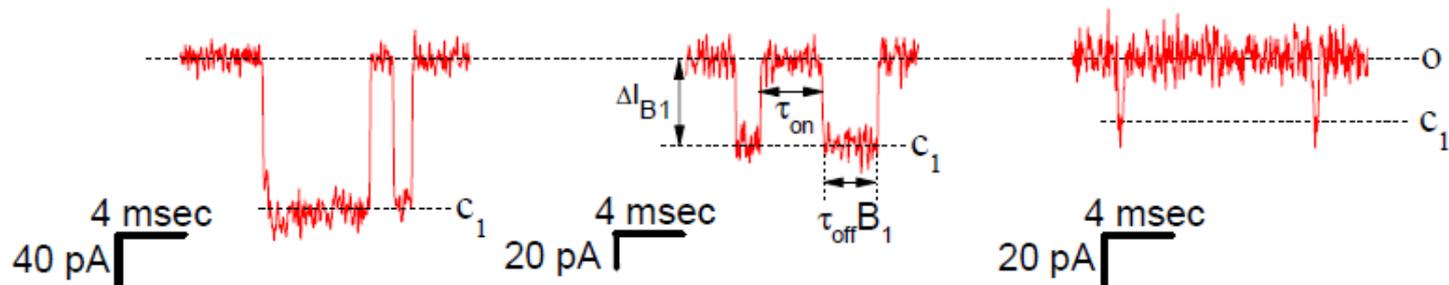
Salt-dependent folding of model antimicrobial peptides



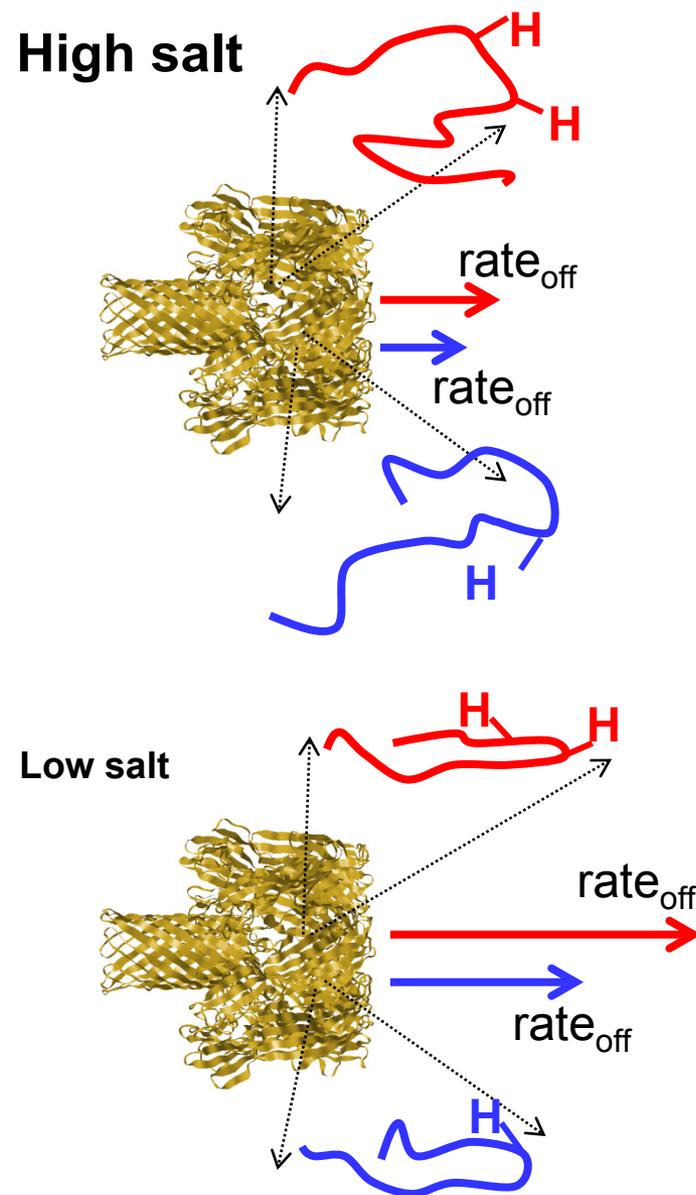
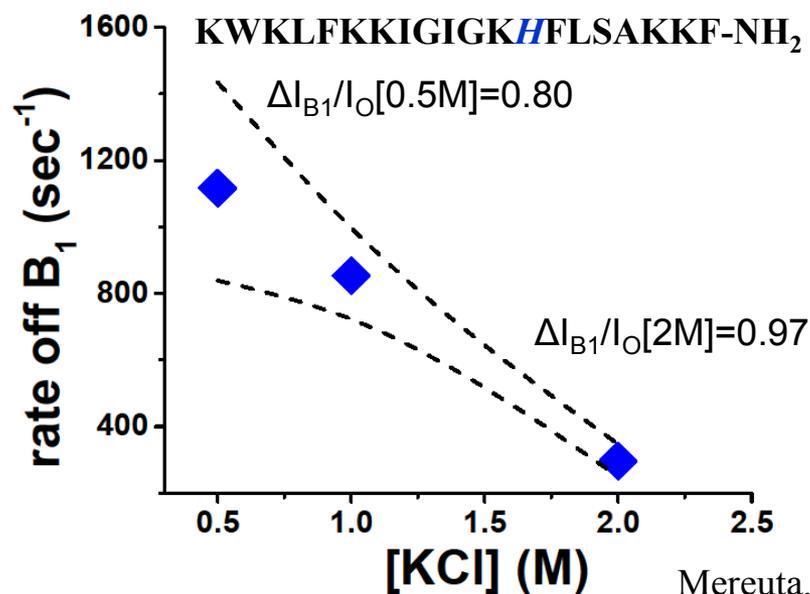
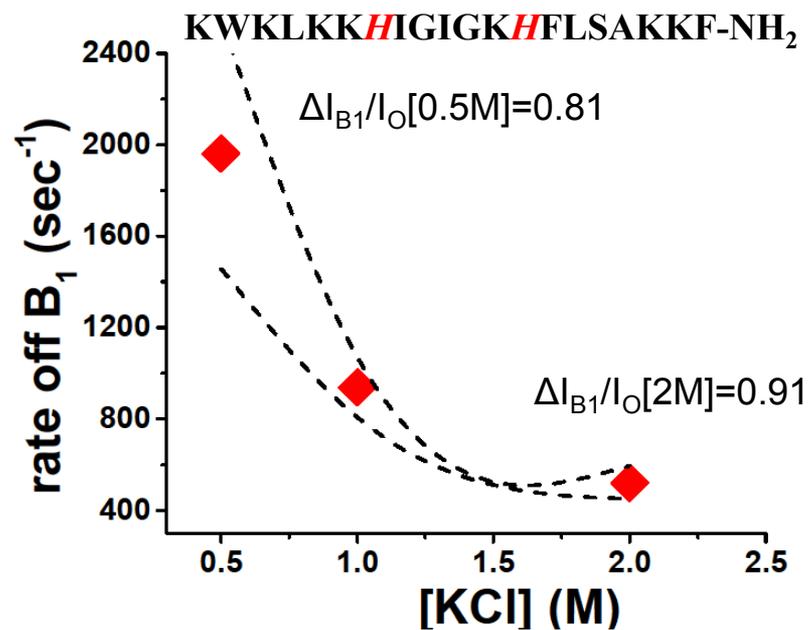
KWKLFKKIGIGK**H**FLSAKKF-NH₂



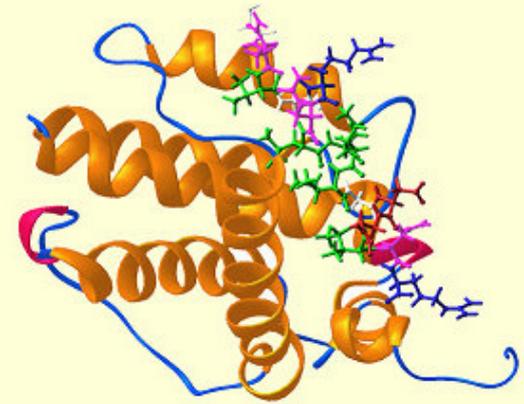
KWKLKK**H**IGIGK**H**FLSAKKF-NH₂



Salt-dependent folding of model antimicrobial peptides



3. Bioanalytical applications of nanopore-based devices. Application to metals sensing.



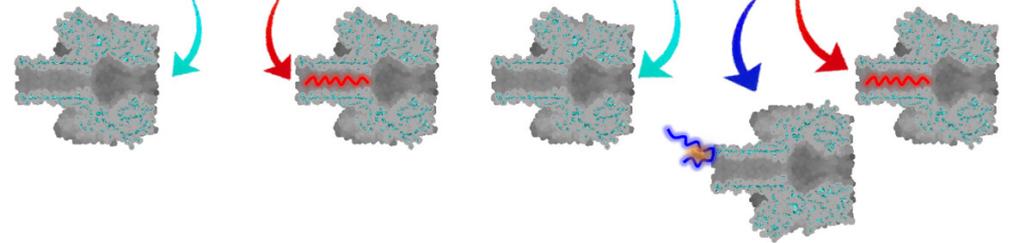
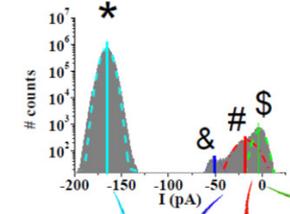
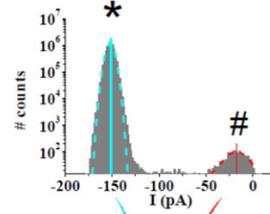
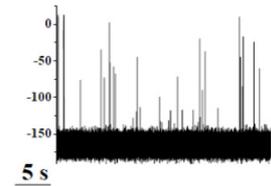
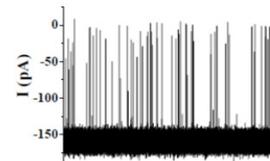
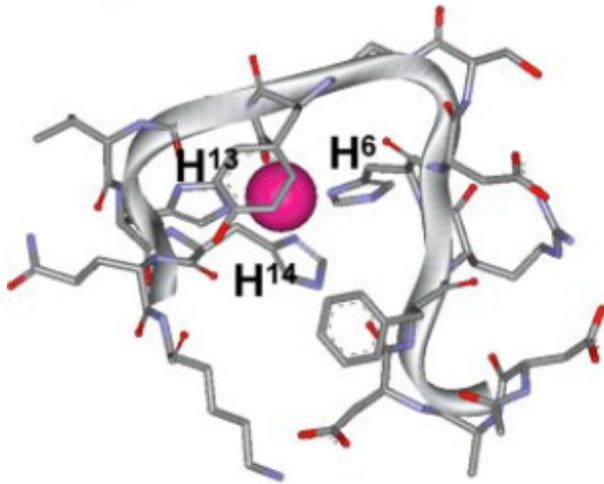
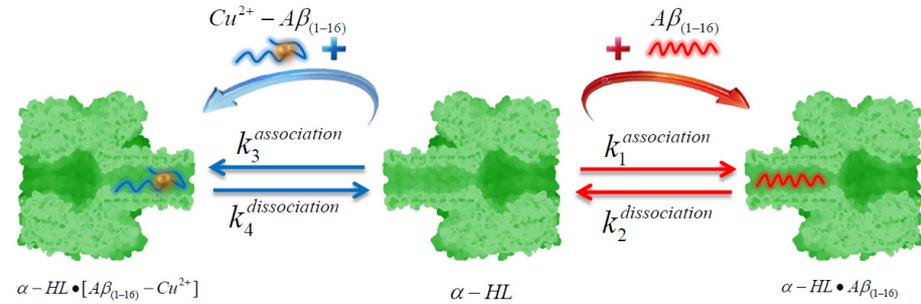
- ❑ Detection of transition metal ions has become significant because low contaminations of heavy and transition metal ions in waters were extremely toxic to humans and other living organisms.
- ❑ Although zinc ions are essential for human health, excess of zinc ions in human body was reported to be responsible for several diseases including Alzheimer's disease.
- ❑ A *nanopore-based, single-molecule approach* to various ions sensing would provide benefits with respect to *sensitivity, selectivity, portability, low cost, ease of use, and rapid response*, in real-world environmental or biological samples, which contain ions in complex mixtures.

Bioanalytical applications of nanopore-based devices.

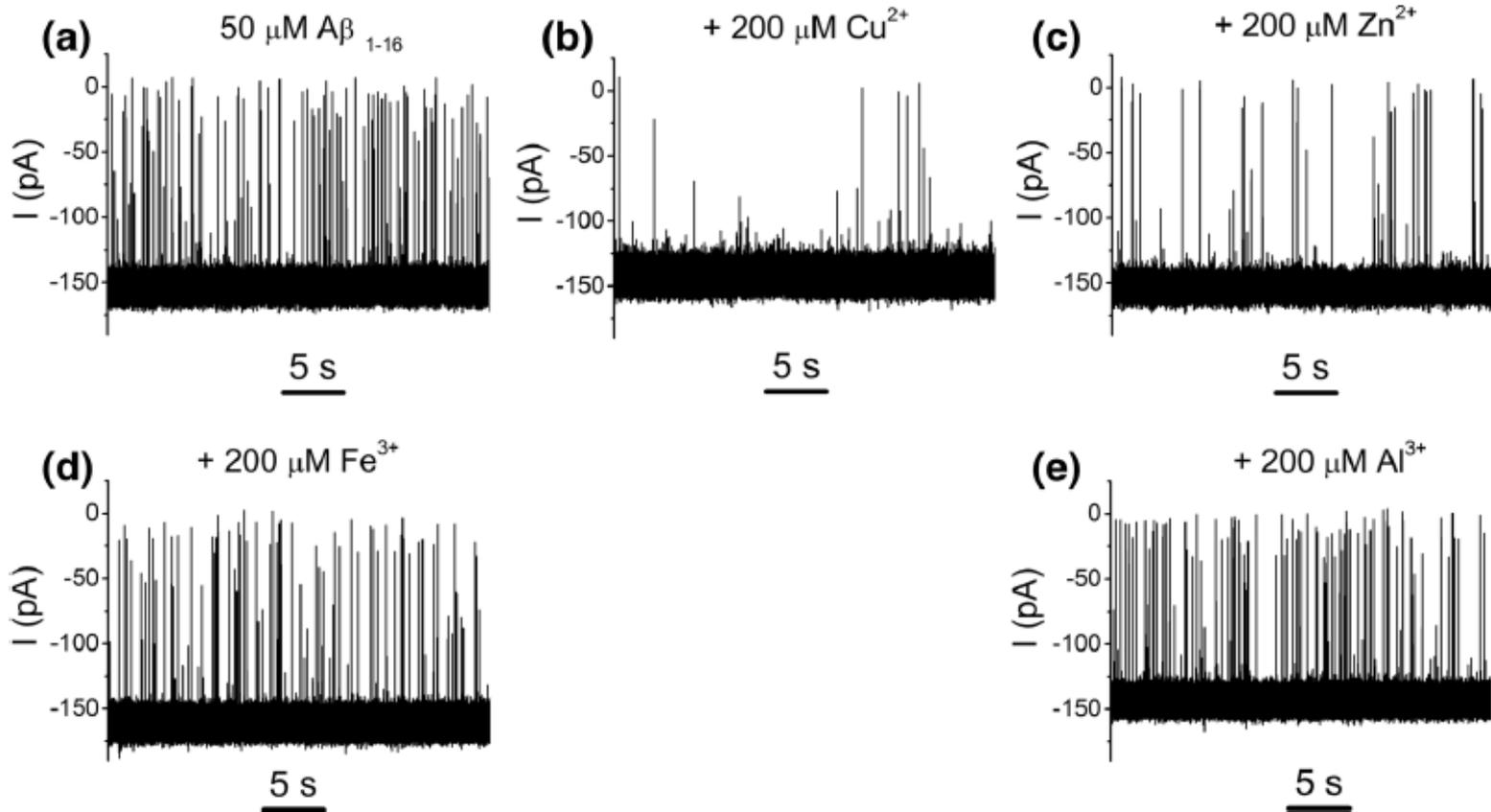
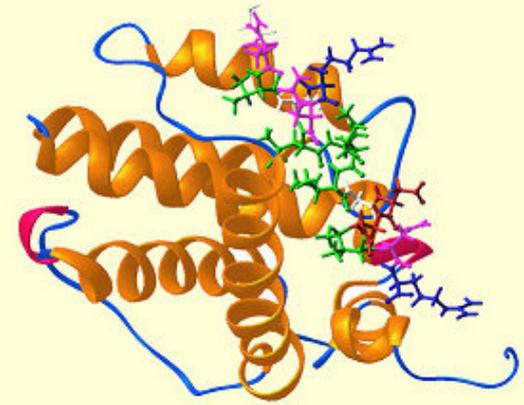
Application to metals sensing.

A β (1-16): intrinsically disordered peptide

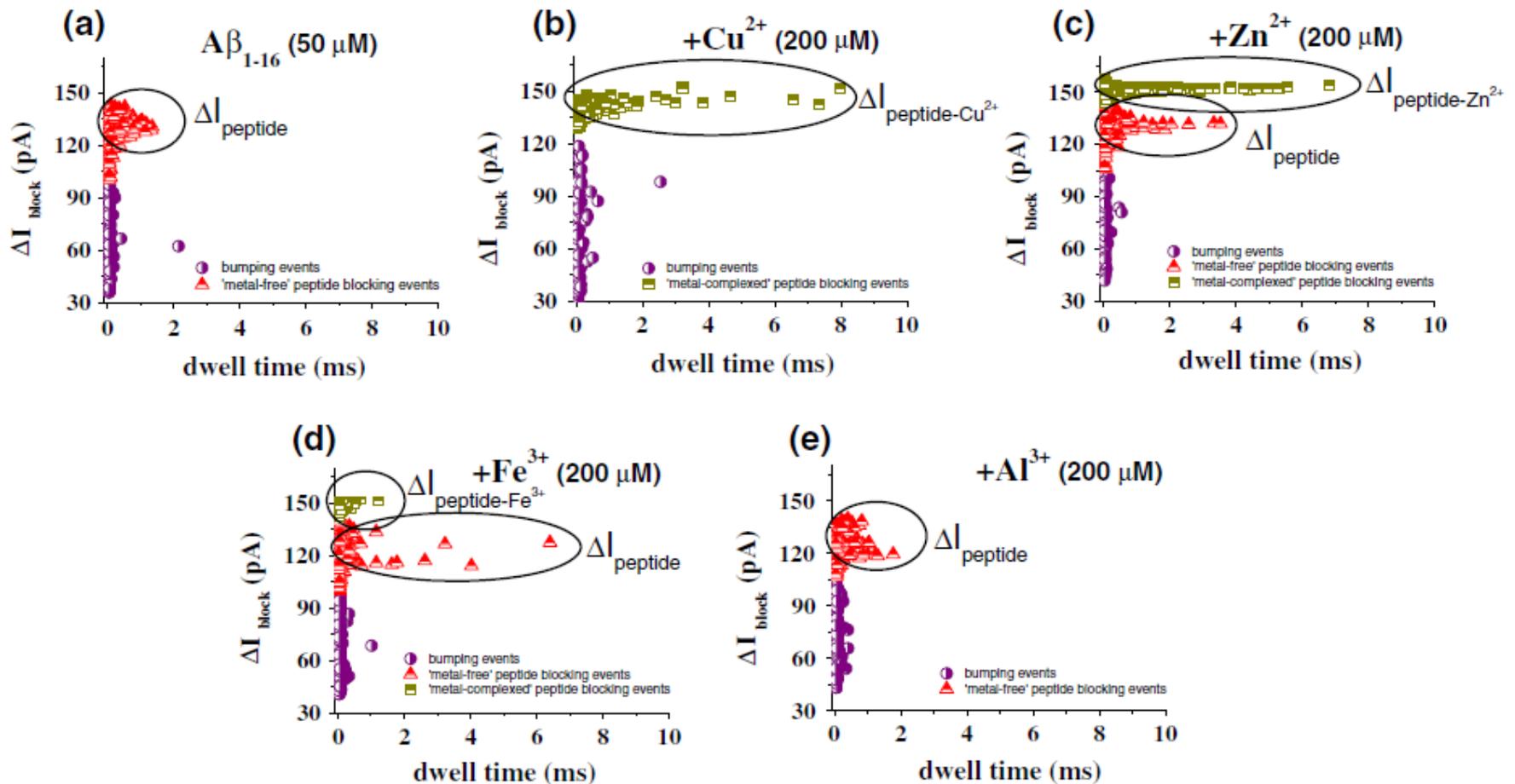
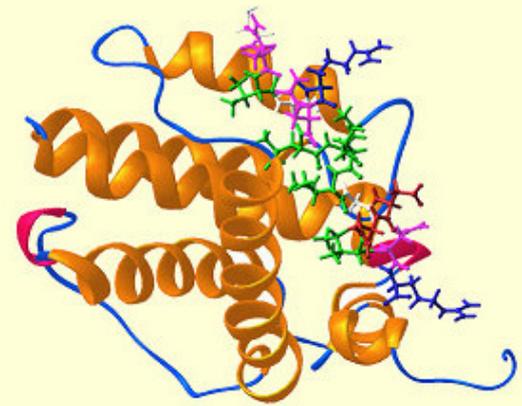
Asp-Ala-Glu-Phe-Arg-His⁶-Asp-Ser-Gly-Tyr-Glu-Val-His¹³-His¹⁴-Glu-Lys



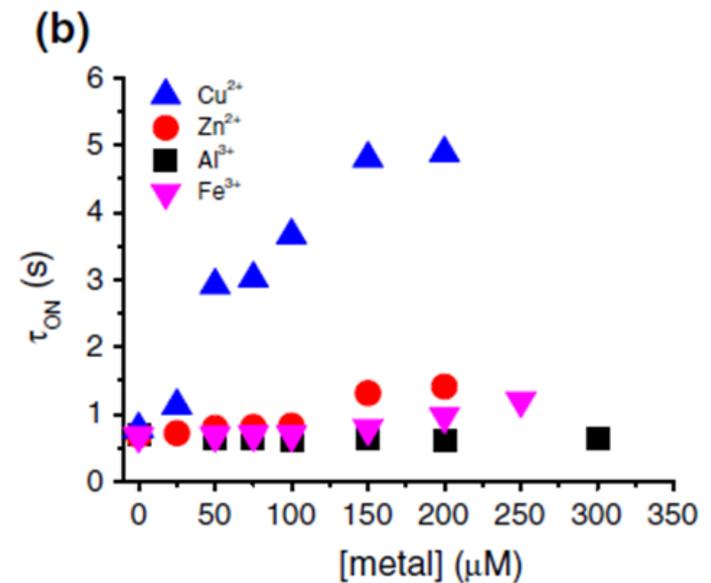
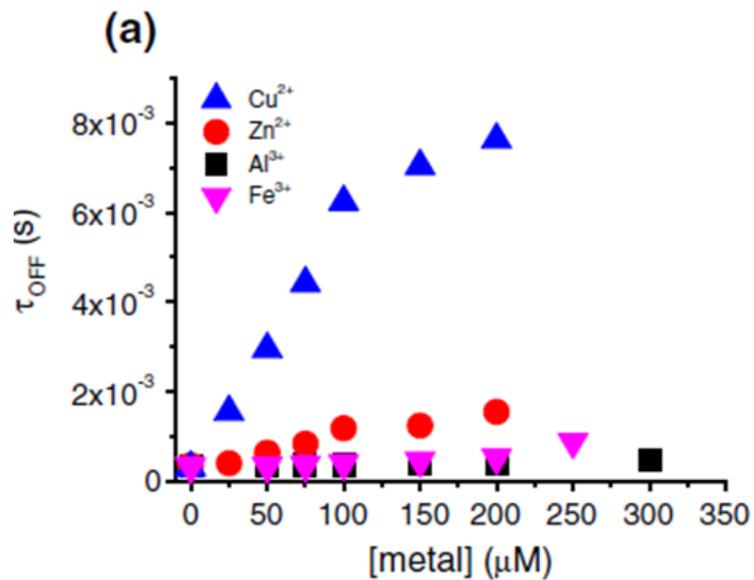
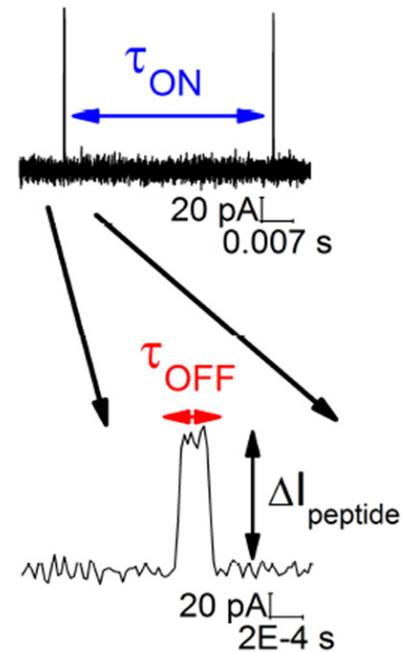
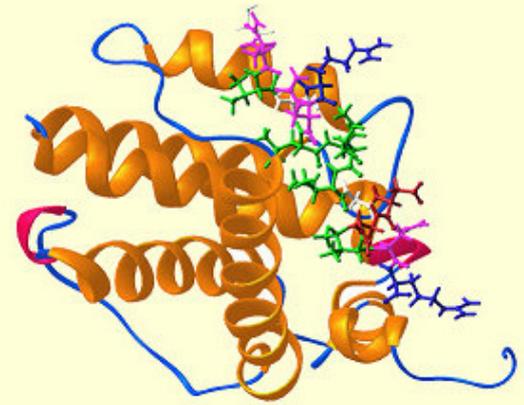
Bioanalytical applications of nanopore-based devices. Application to metals sensing.



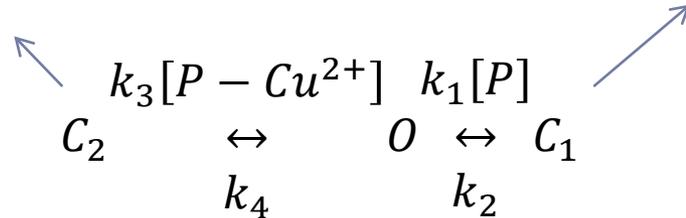
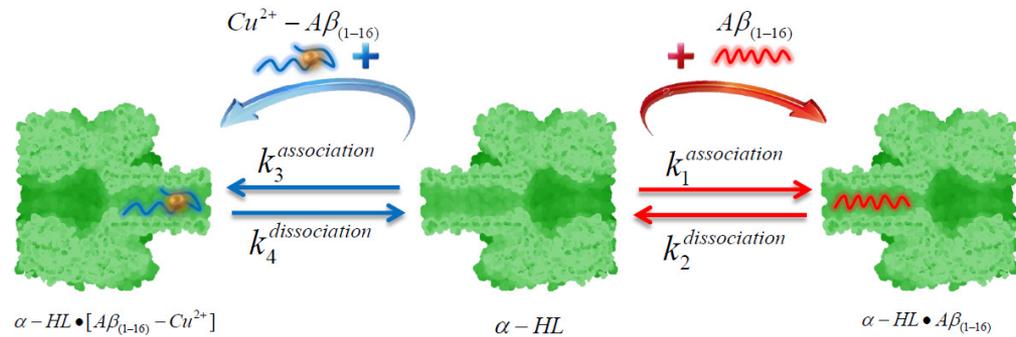
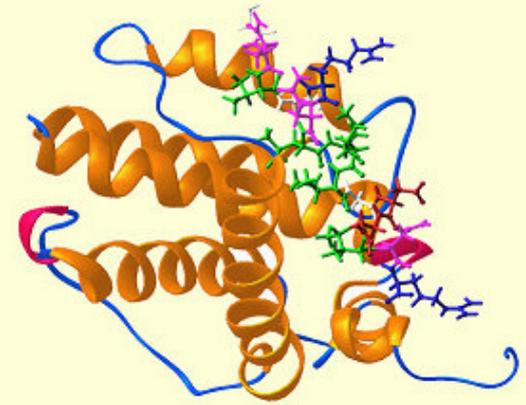
Bioanalytical applications of nanopore-based devices. Application to metals sensing.



Bioanalytical applications of nanopore-based devices. Application to metals sensing.



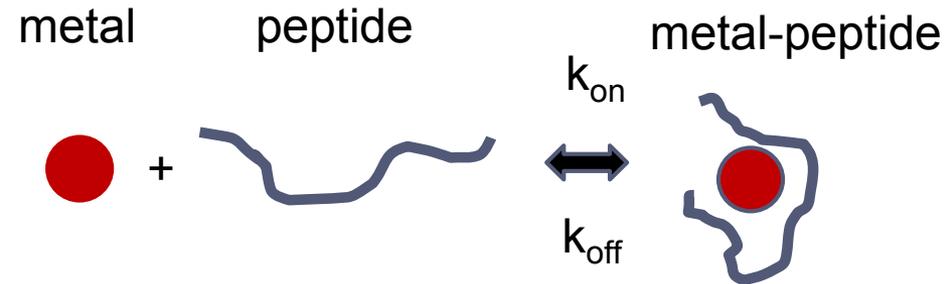
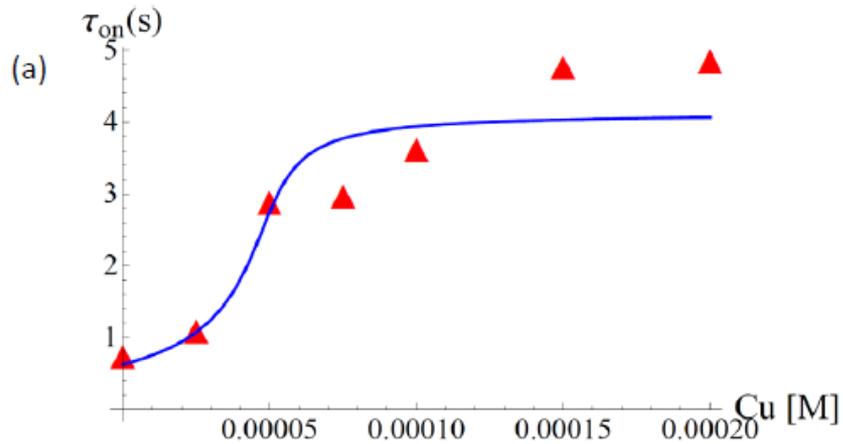
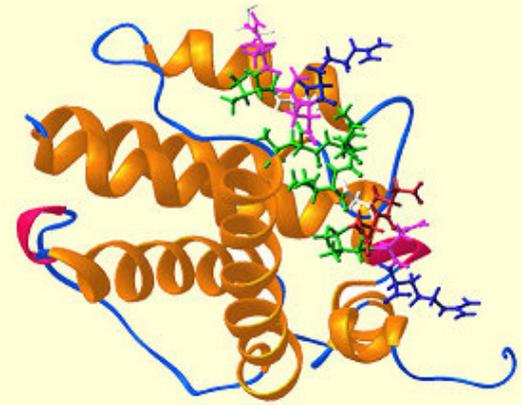
Bioanalytical applications of nanopore-based devices. Application to metals sensing.



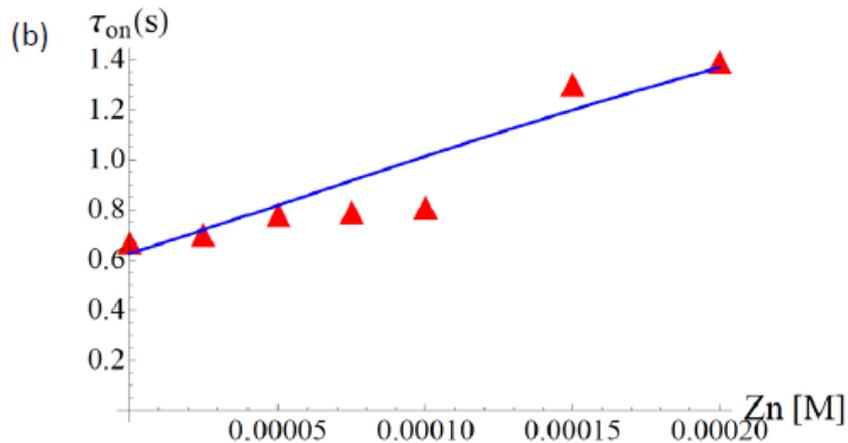
$$\tau_{ON} = \frac{1}{k_3[P - X]_{eq.} + k_1([P]_{eq.})} = \frac{1}{k_3[P - X]_{eq.} + k_1([P_0] - [P - X]_{eq.})}$$

$$[P - X]_{eq.} = \frac{([P_0] + [X_0] + K_d) - \sqrt{([P_0] + [X_0] + K_d)^2 - 4[P_0][X_0]}}{2}$$

Bioanalytical applications of nanopore-based devices. Application to metals sensing.



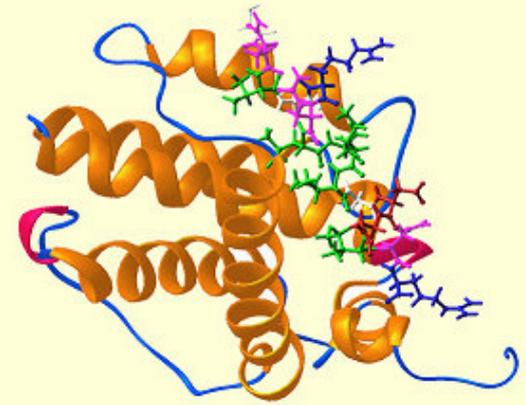
$$K_d = k_{off}/k_{on}$$



$$K_{d, Cu^{2+}} = 4.5 \times 10^{-7} \text{ M}$$

$$K_{d, Zn^{2+}} = 9.2 \times 10^{-5} \text{ M}$$

Acknowledgements



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Thermal unfolding of goulash meat

