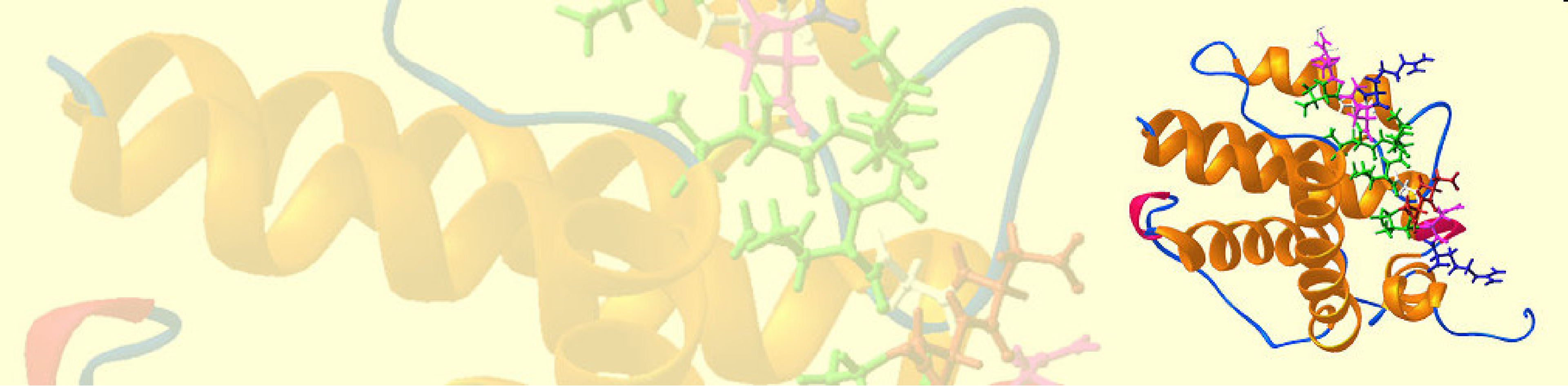
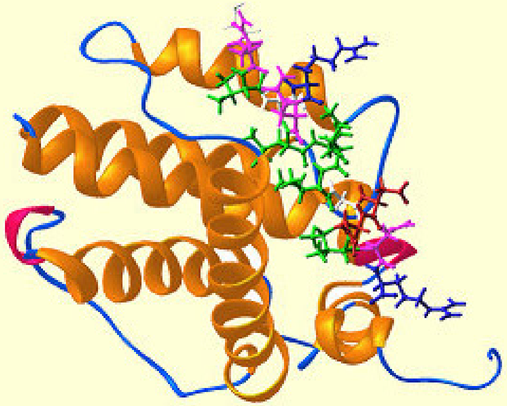


Nanoscopic interrogation of molecular interactions with protein nanopores

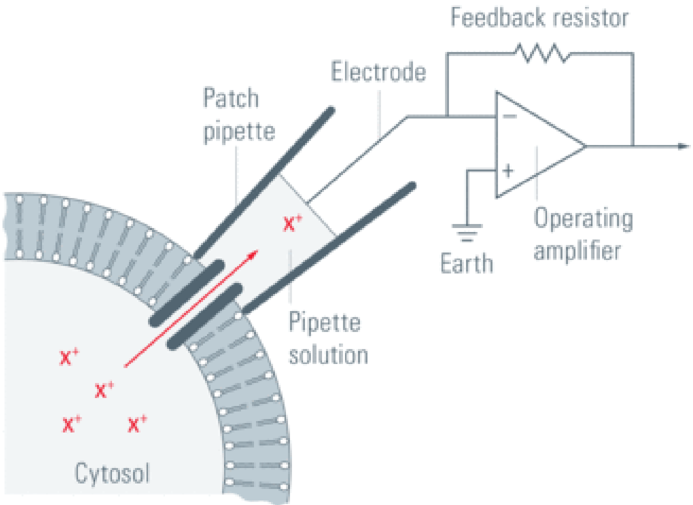
Tudor Luchian, Department of Physics, Laboratory of Molecular Biophysics and Medical Physics, Alexandru I. Cuza University, Iasi, Romania
E-mail: luchian@uaic.ro

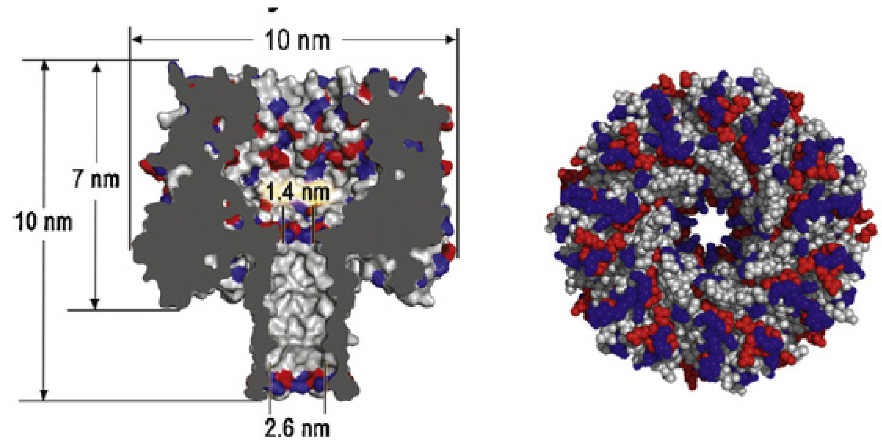
Nanoscopic interrogation of molecular interactions with protein nanopores



- 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

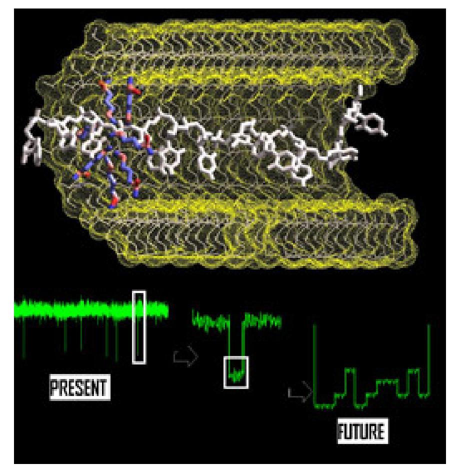
Nanopore-based, single-molecule sensing and analysis – general principle

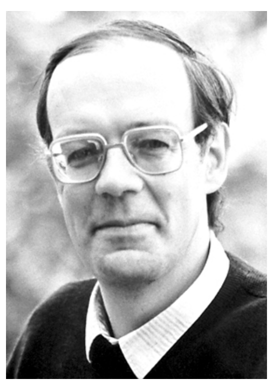




Song, L. Z. et al., *Science,* 1996*,* 274, 1859–1866

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"*

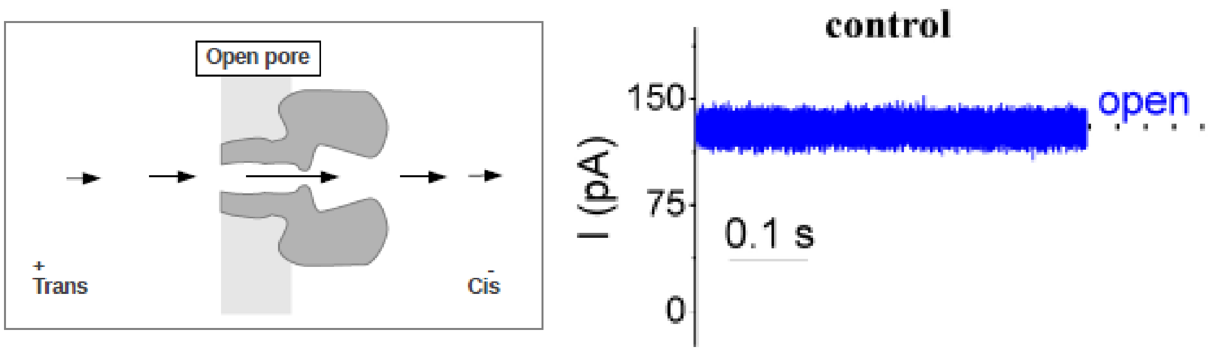


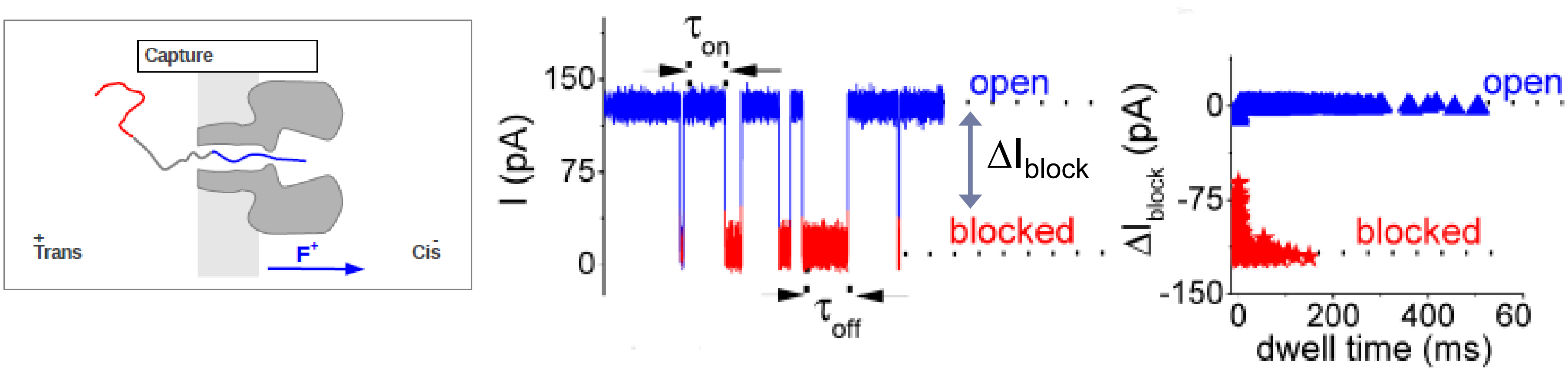


http://www.nobelprize.org/

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. Biotech*nol. 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. E*d. 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.

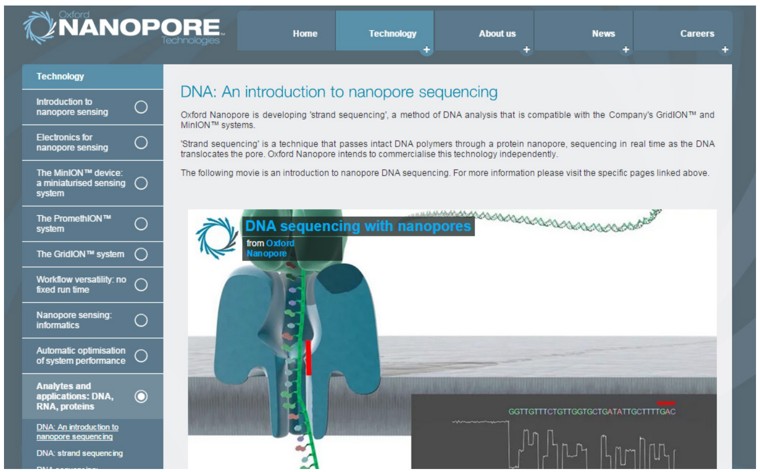
$$nanopore + P \underset{k_{off}}{\overset{k_{on}}{\leftrightarrow}} \alpha - HL \bullet P$$

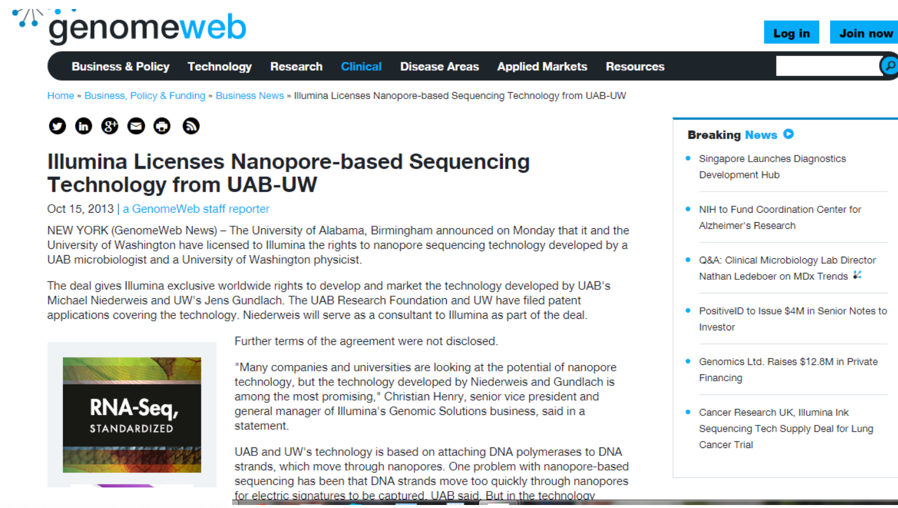
$$\delta = \frac{\Delta I_{block}\left(l_{pore} + 0.8 d_{pore}\right)^2}{\gamma \sigma \Delta V}$$

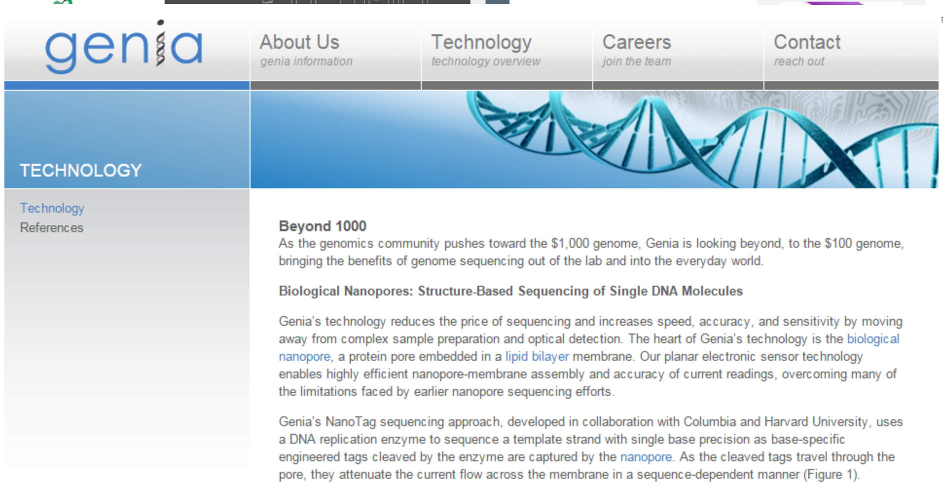
$$k_{on} = \frac{1}{[P]\hat{\tau}_{on}} \quad k_{off} = \frac{1}{\hat{\tau}_{off}}$$

Concise applications of the nanopore technology

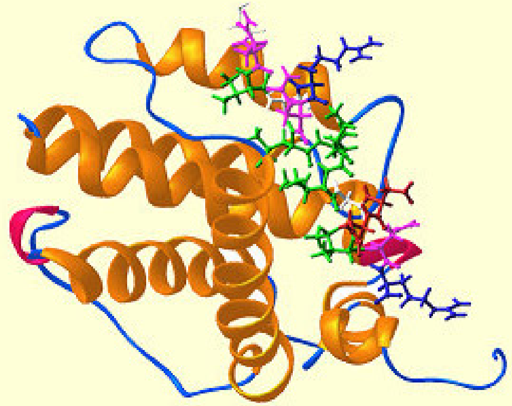
- sensing and separation (DNA, RNA, peptides, proteins, metals)
- single-molecule physics and chemistry
- study of proteins folding-unfolding







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

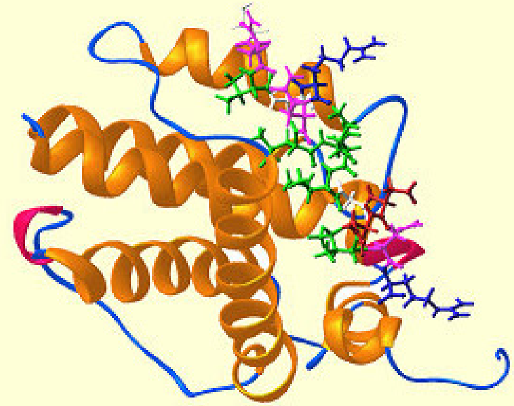


- ❑ Usually the translocation process is too fast and precludes the precise characterization of the transiently present moieties inside the nanopore.

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

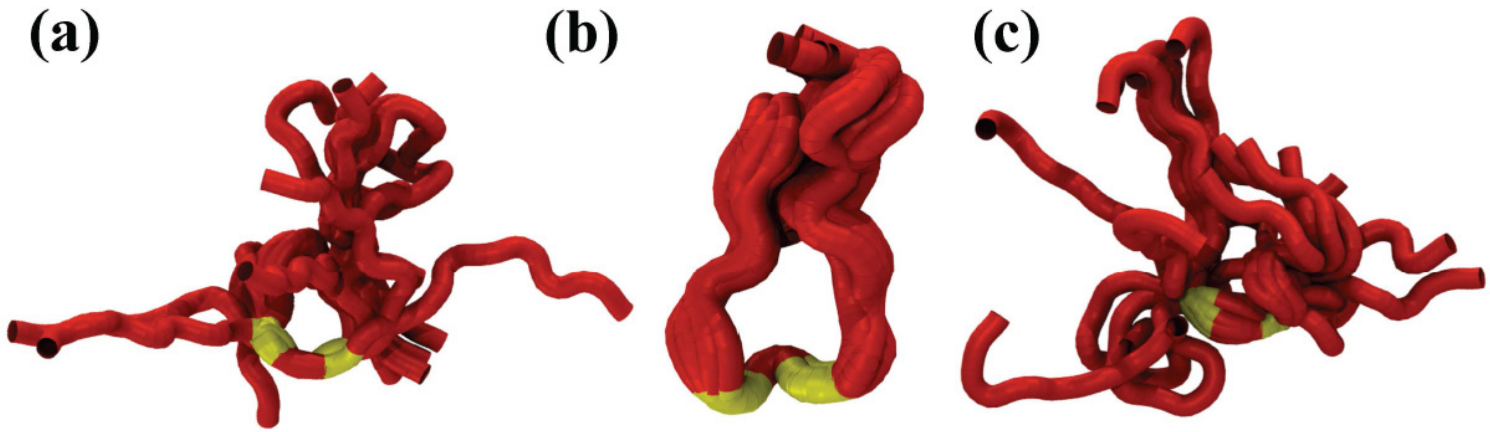
- ❑ Using pH-tuned single-molecule electrophysiology we demonstrate how peptide passage through the α-hemolysin protein can be sufficiently slowed down to observe intermediate single-peptide sub-states along the pore.

Molecular braking of single-molecule passage through a protein nanopore



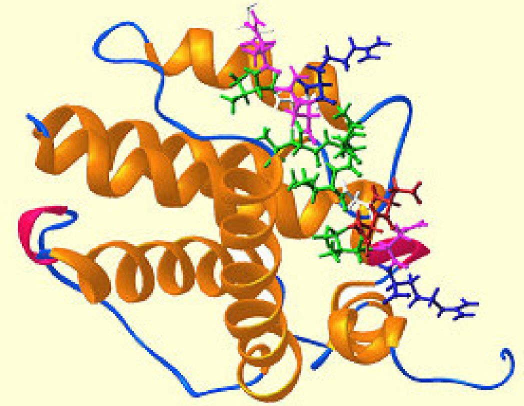
CAMA3; net charge ~ + 8 at neutral and acidic pH

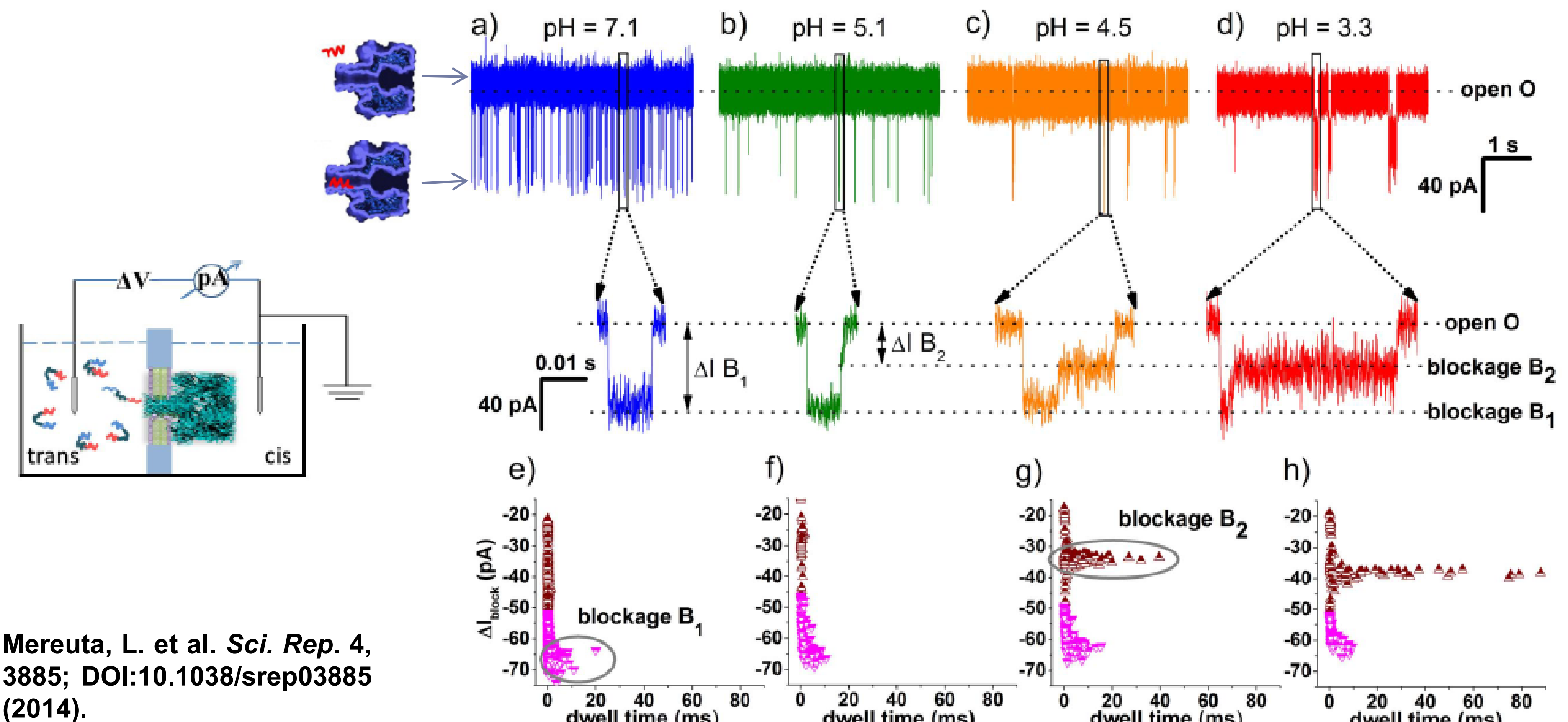
KWKLFKKI<u>GIG</u>KFLQSAKKF-NH2



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

Molecular braking of single-molecule passage through a protein nanopore

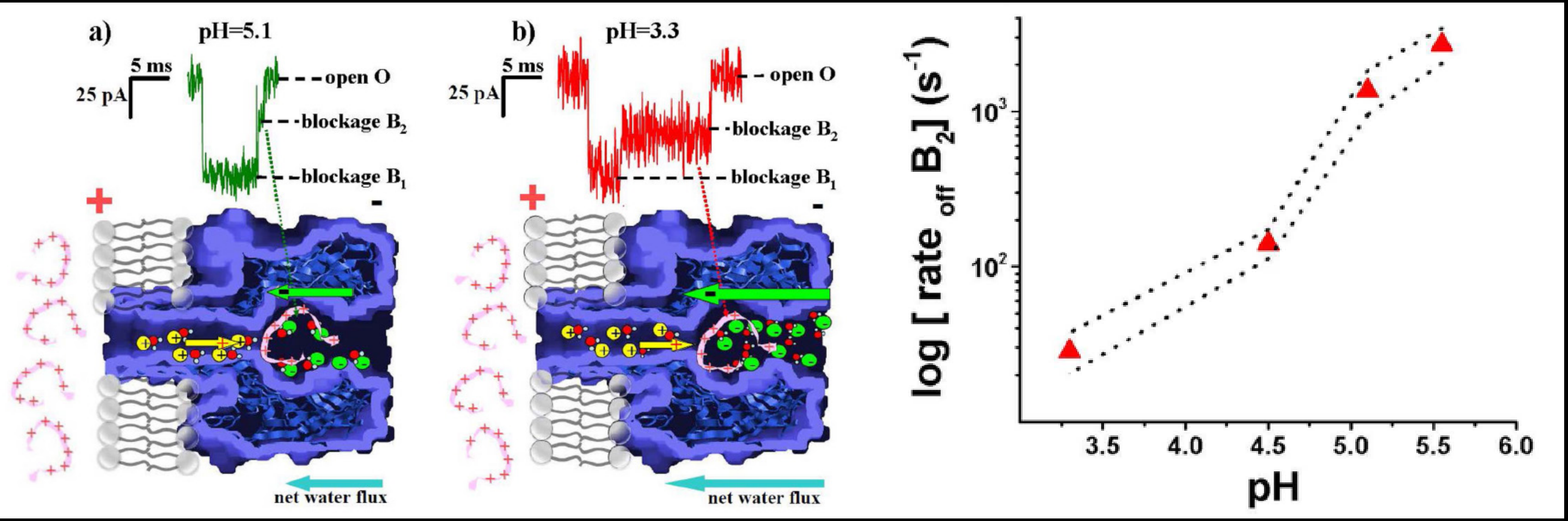




Mereuta, L. et al. *Sci. Rep.* 4, 3885; DOI:10.1038/srep03885 (2014).

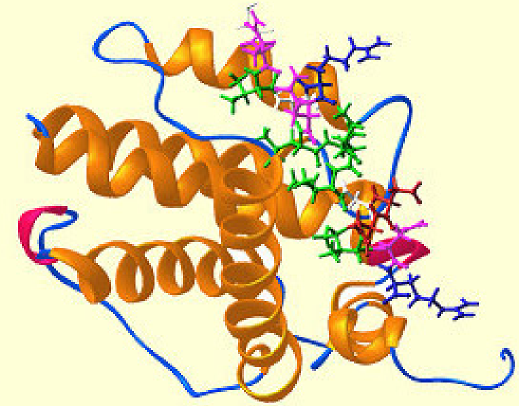
Schematic representation of pH-augmented electroosmotic (ELO) braking

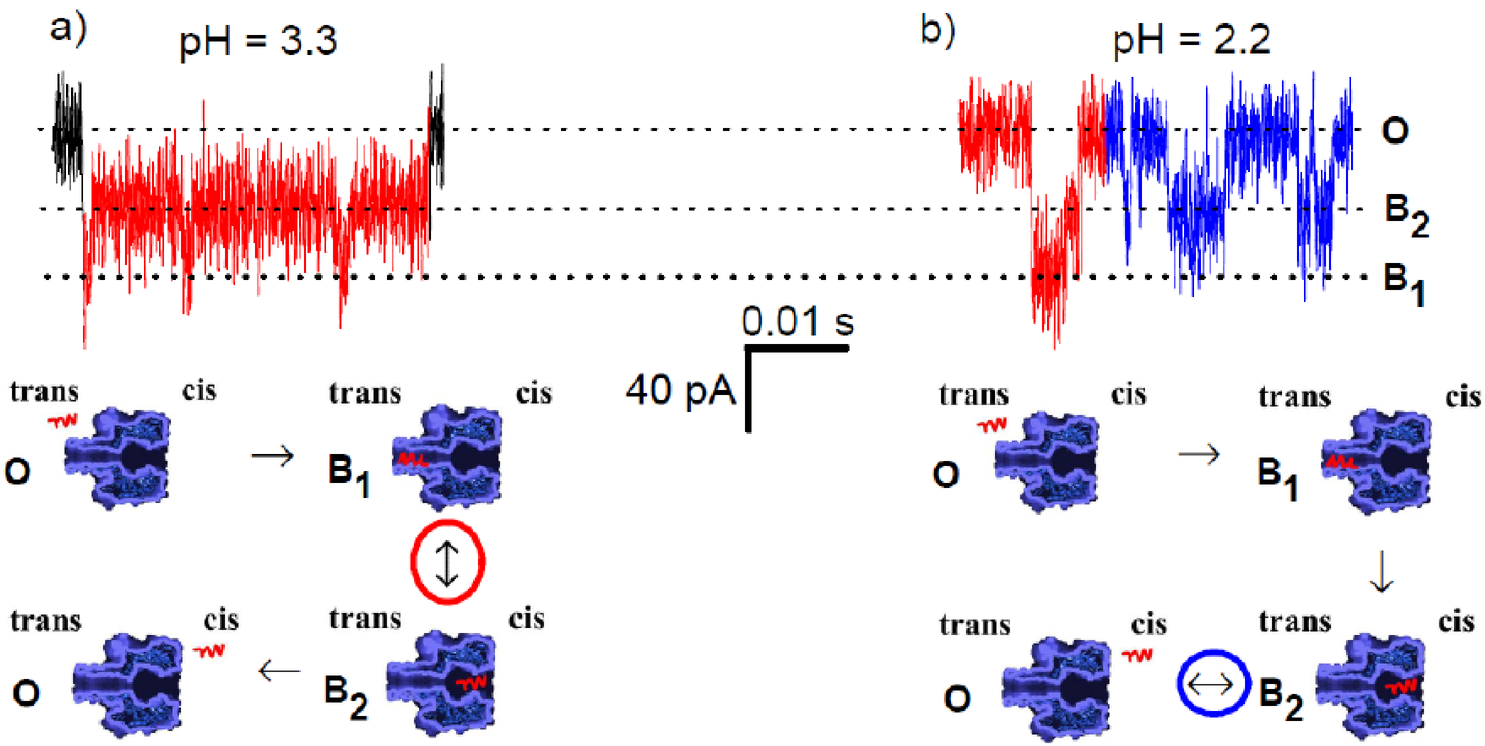
$P_{K^+}/P_{Cl^-} = 0.86$; pH 7.1
$P_{K^+}/P_{Cl^-} = 0.44$; pH 4.4



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

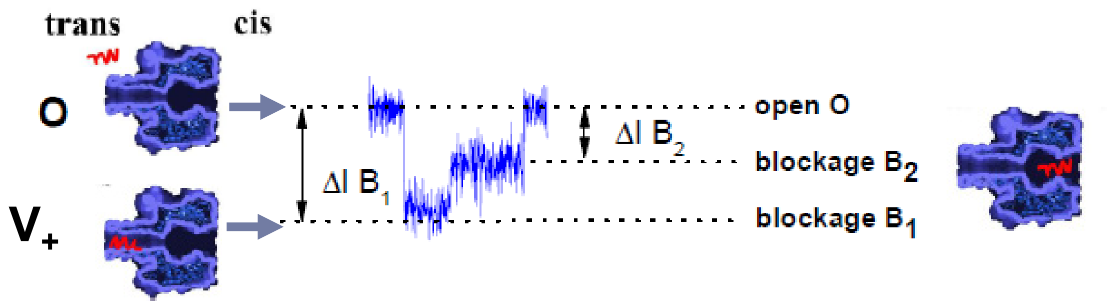
Controlling the balance between the ELF and ELO forces helps trapping the translocating peptides



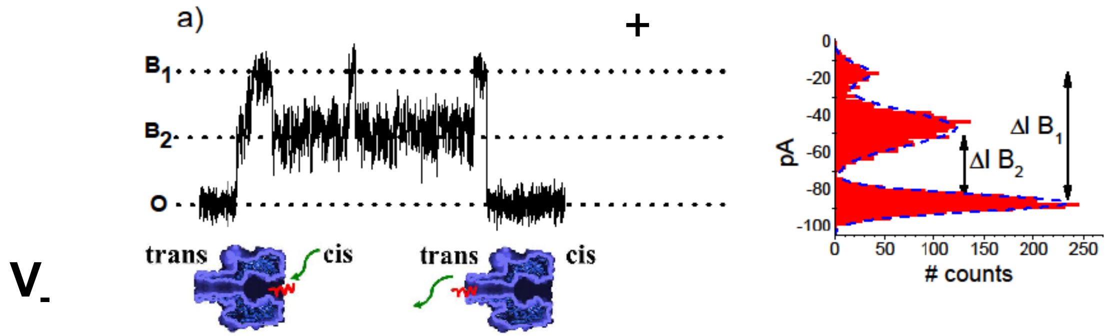


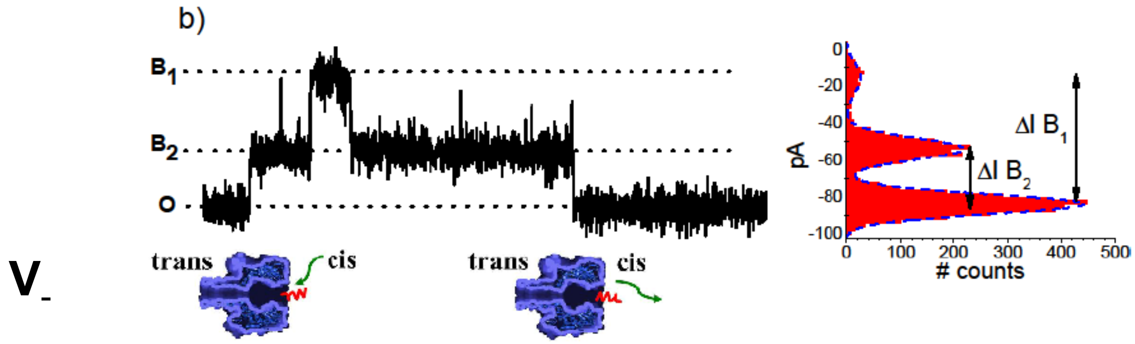
Peptide braking unravels the directionality of peptide motion across the protein nanopore

Peptide added from trans (pH = 4.5):

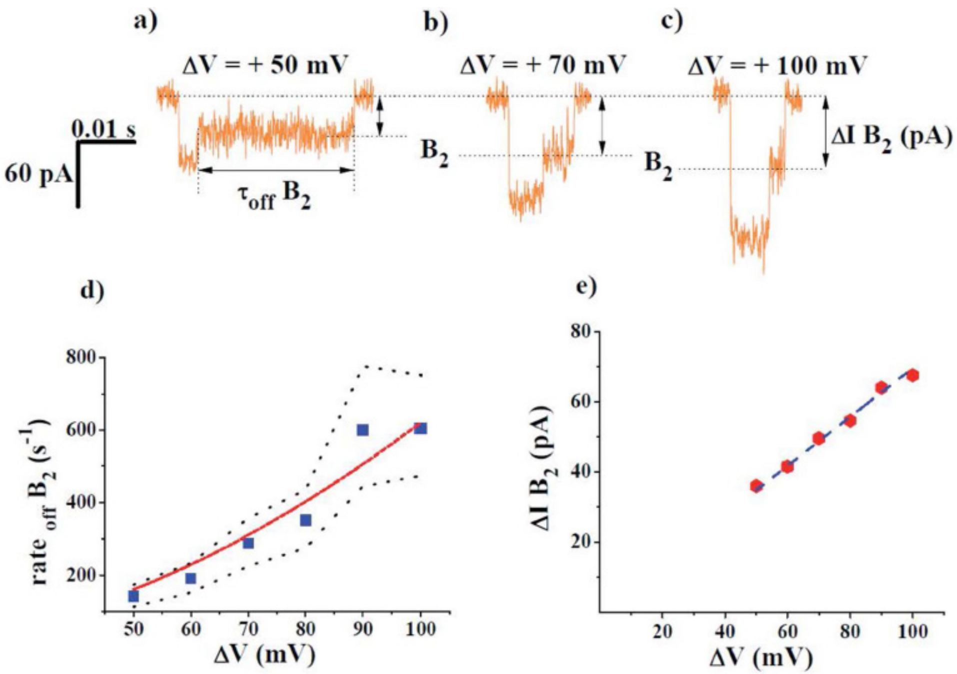


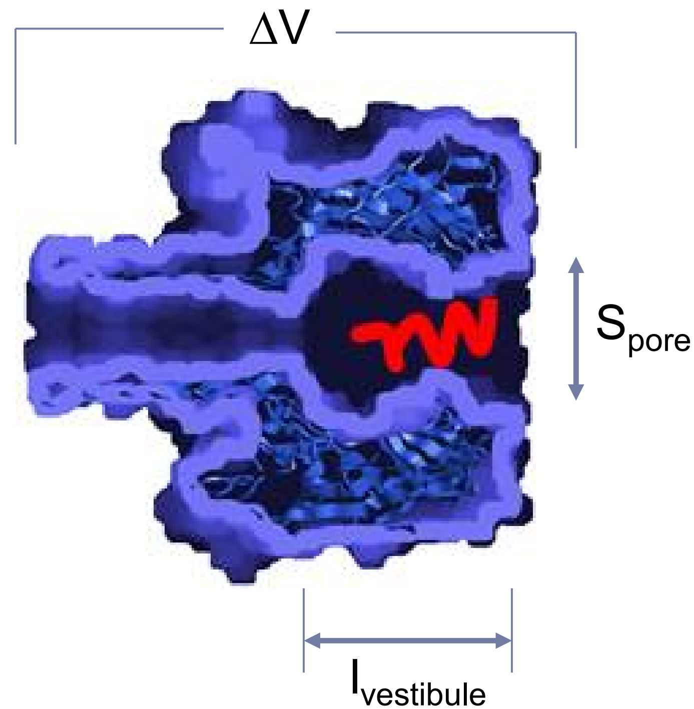
Peptide added from cis (pH = 4.5)





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





D = 1.5*10^{-12} m^{2}s^{-1} (drift-diffusion model)

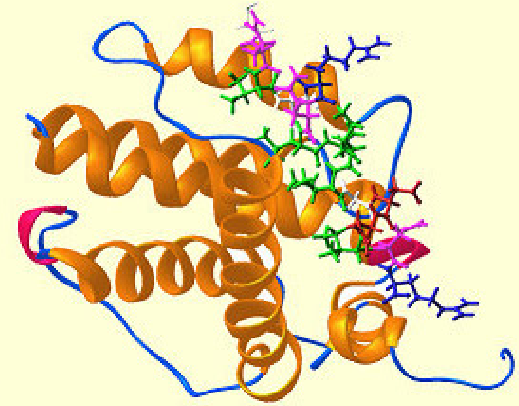
$$\langle t_{off} \rangle = \frac{1}{\mathrm{rate}_{off} B_2} = \int_0^{\infty} \frac{t(v_{drift}t + l_{vestibule})e^{\left[-\frac{(l_{vestibule} - v_{drift}t)^2}{4Dt}\right]}}{2t\sqrt{4D\pi t}}\, dt$$

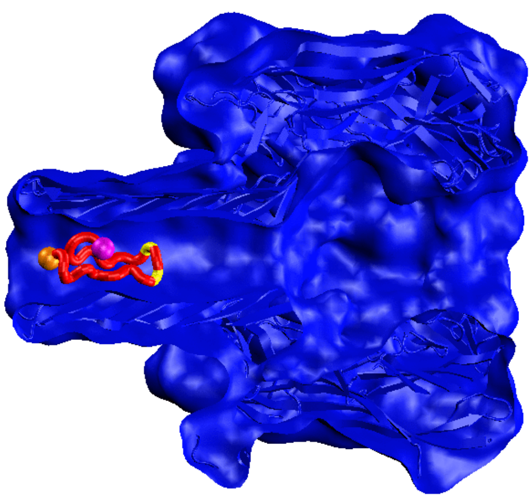
$$v_{drift} = \mu \frac{\Delta V}{l_{pore}} - \frac{N_h I}{|e^-|} \frac{\left(\frac{P_{Cl-}}{P_{K+}} - 1\right)}{\left(\frac{P_{Cl-}}{P_{K+}} + 1\right)} \frac{1}{S_{pore}[H_2O]}$$

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

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

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



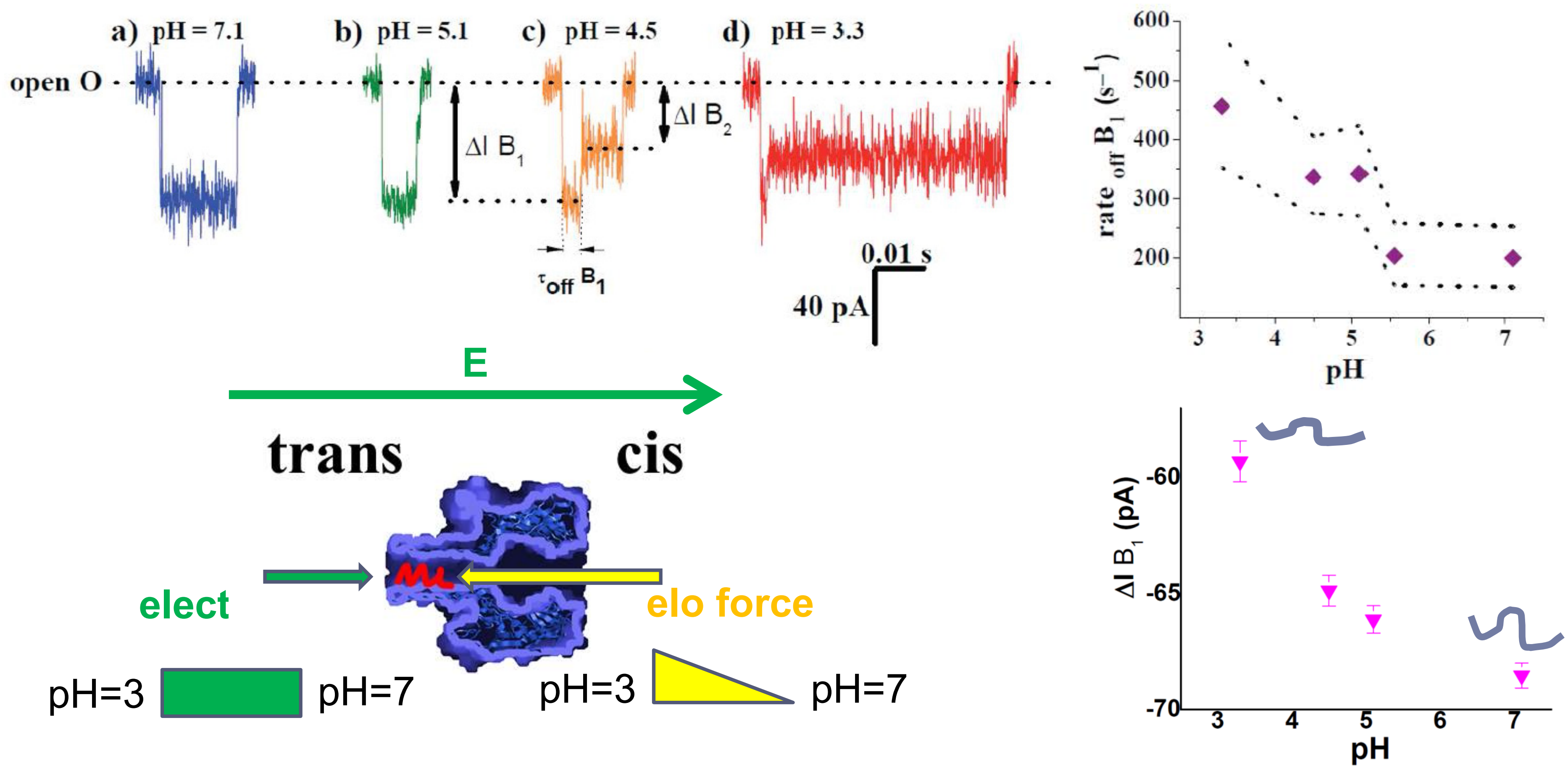


Ioan Andricioaei's Lab, University of California, Irvine, USA

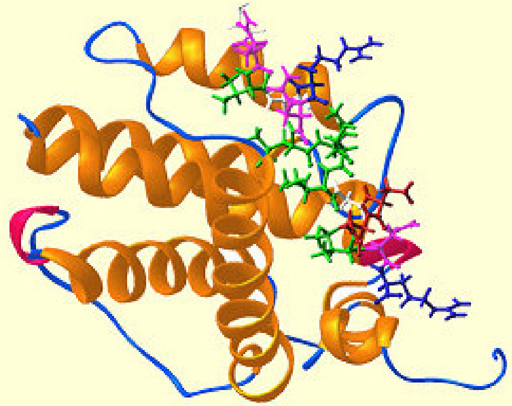
Mereuta, L. et al. *Sci. Rep*. 4, 3885; DOI:10.1038/srep03885 (2014).

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



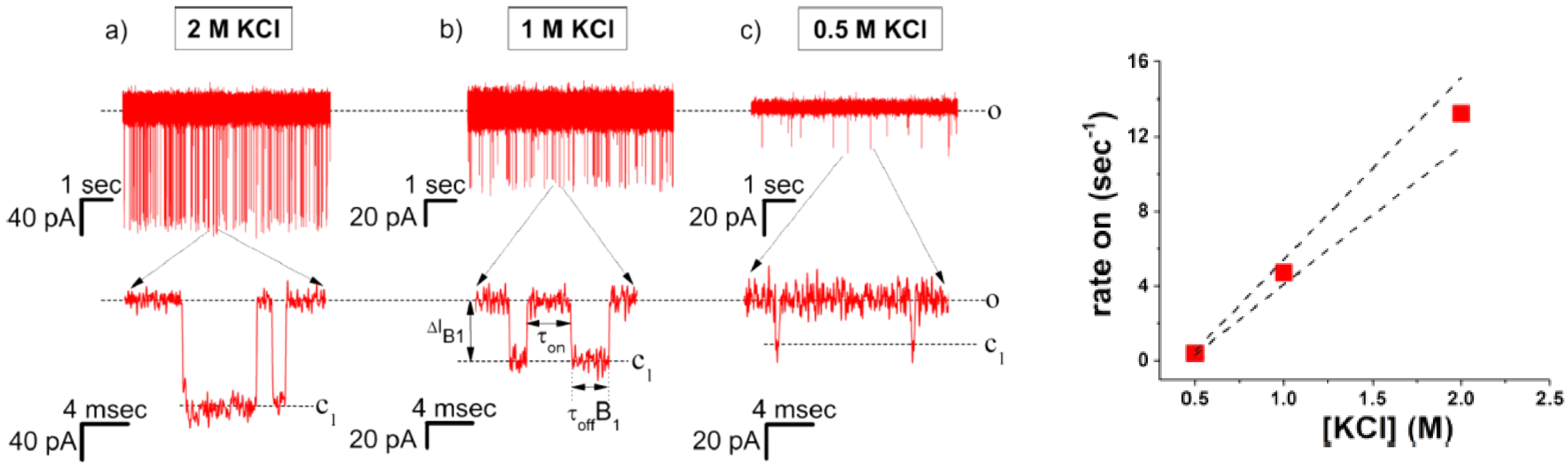
- Folding events of β-hairpin peptides are triggered by the presence of salt, as a direct consequence of the electrostatic screening between charged amino acids within the peptide.

- 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***

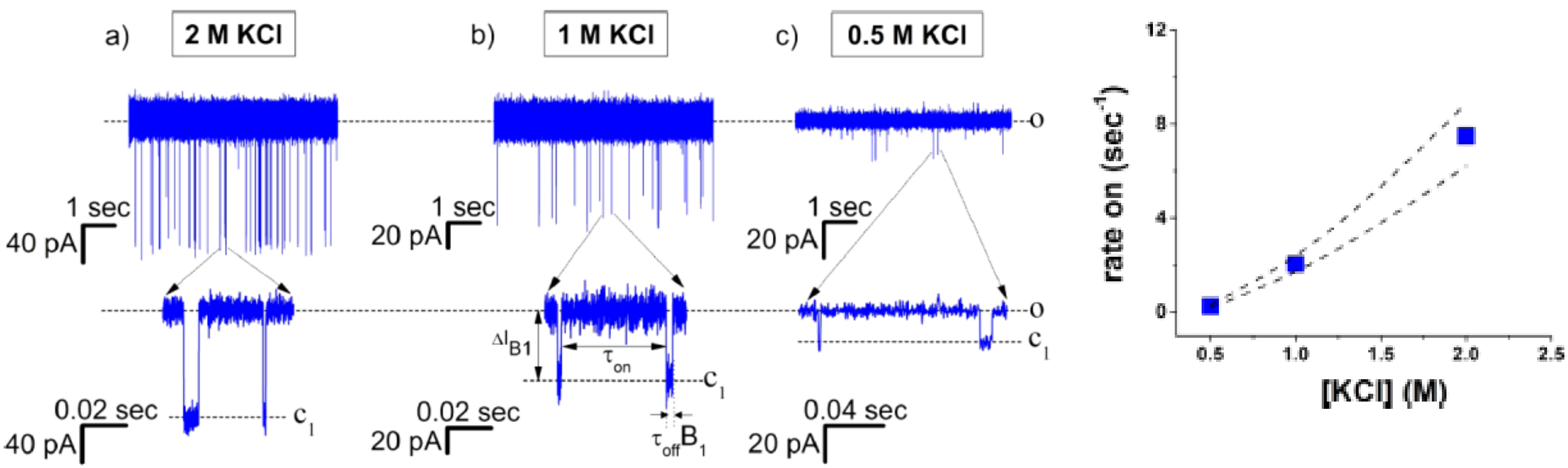
We used the nanopore-based, 'single-molecule' analysis technique to investigate the salt dependence of folding state of histidine-containing, β-hairpin-like peptides.

Salt-dependent folding of model antimicrobial peptides

KWKLKK*H*IGIGK*H*FLSAKKF-NH_2 + 8 e^- (CAMA 3)



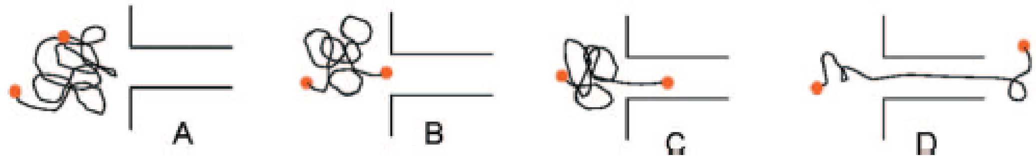
KWKLFKKIGIGK*H*FLSAKKF-NH_2 + 8 e^- (CAMA 1)



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

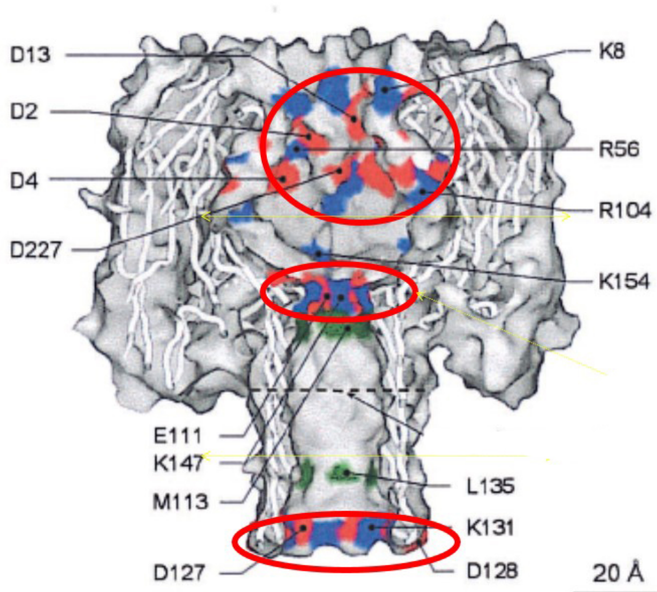
Salt-dependent folding of model antimicrobial peptides

The essential steps of peptide partitioning inside α-HL

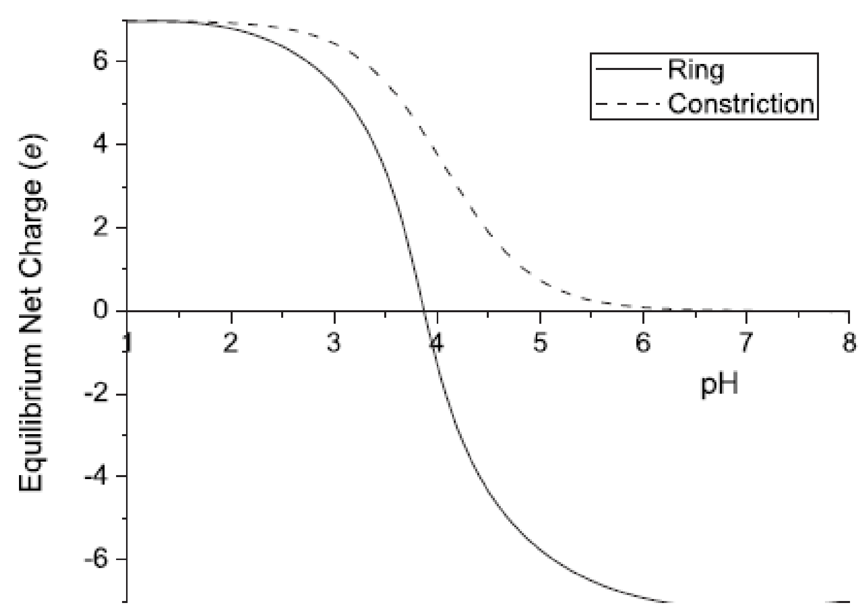


Muthukumar, M., *The Journal of Chemical Physics* **132**, 195101, 2010

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

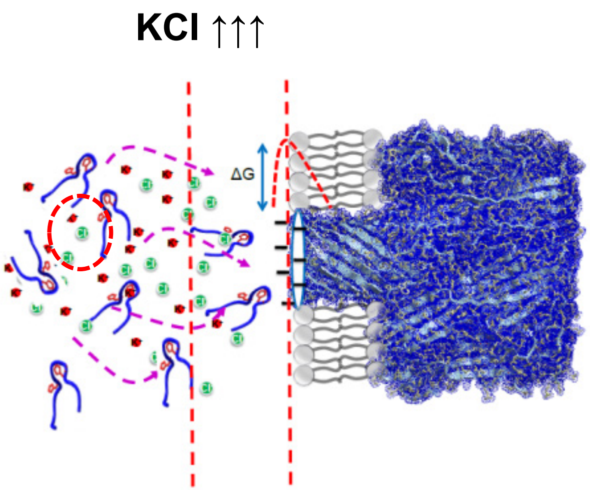


Gu, L-Q. et al., *PNAS*, **97**, 3959–396, 2000



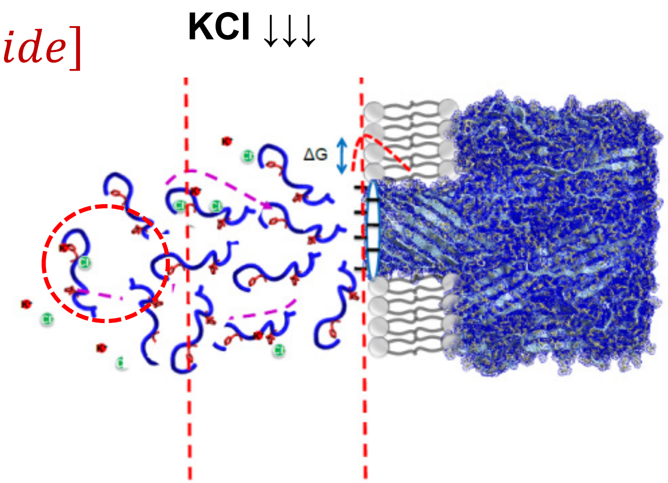
Wong, C.T.A. and Muthukumar, M.,
The Journal of Chemical Physics **133**, 045101, 2010

Salt-dependent folding of model antimicrobial peptides



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

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

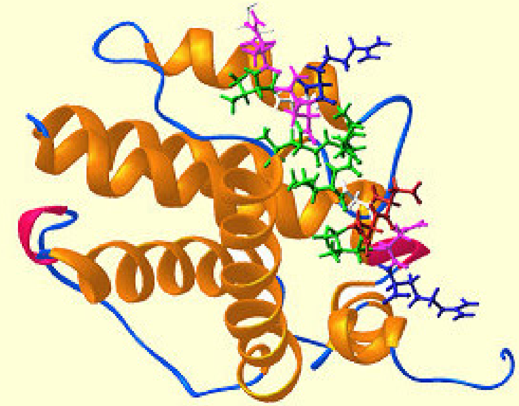


$$\frac{rate_{on;CAMA\,3}}{rate_{on;CAMA1}} = \frac{e^{-\left(\frac{\Delta H^*_{CAMA\,3} - T\Delta S^*_{CAMA\,3}}{RT}\right)}}{e^{-\left(\frac{\Delta H^*_{CAMA\,1} - T\Delta S^*_{CAMA\,1}}{RT}\right)}} = e^{\left(\frac{\Delta\Delta S^*_{CAMA\,3-CAMA\,1}}{R}\right)}$$

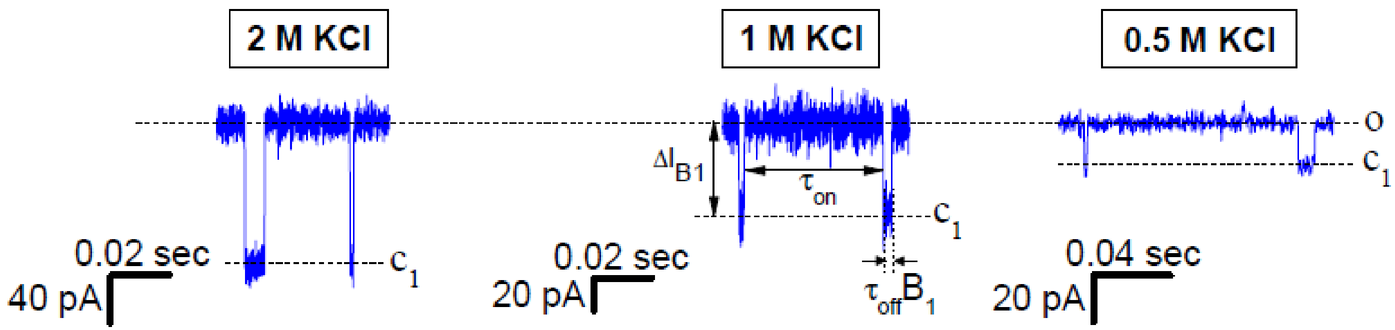
$$\Delta\Delta S^*_{CAMA\,3-CAMA\,1} = Rln\left(\frac{rate_{on;CAMA\,3}}{rate_{on;CAMA1}}\right) = 4.7 \text{ J K}^{-1}\text{ M}^{-1}$$

- 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.

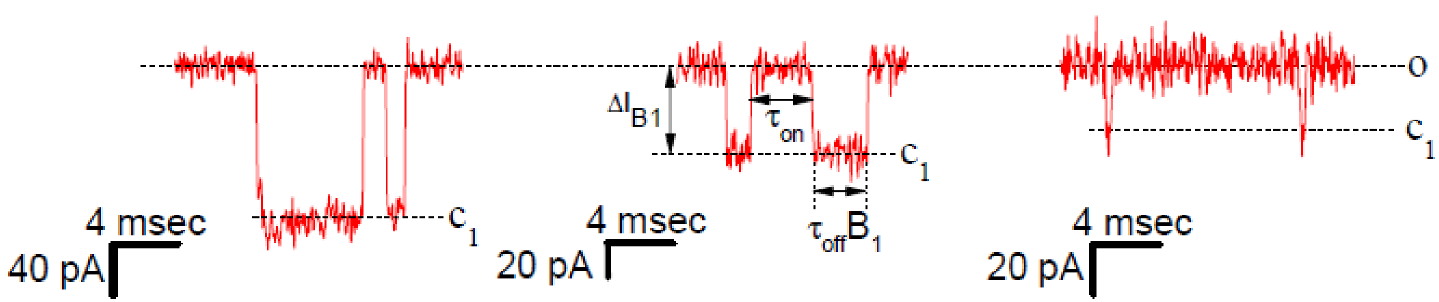
Salt-dependent folding of model antimicrobial peptides



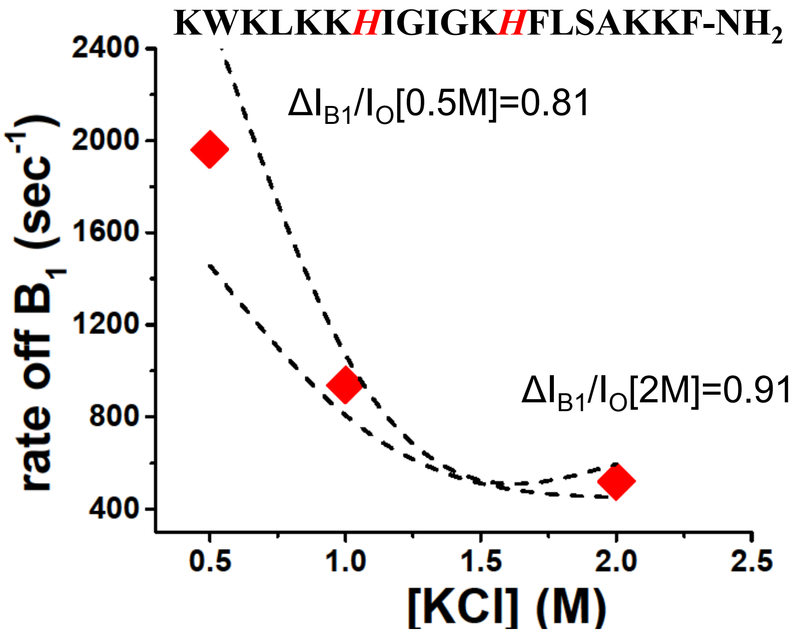
KWKLFKKIGIGK***H***FLSAKKF-NH_2

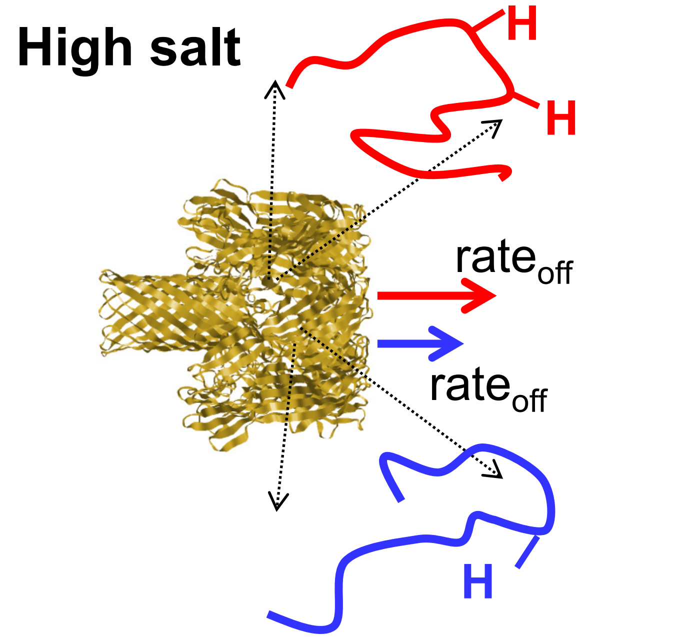


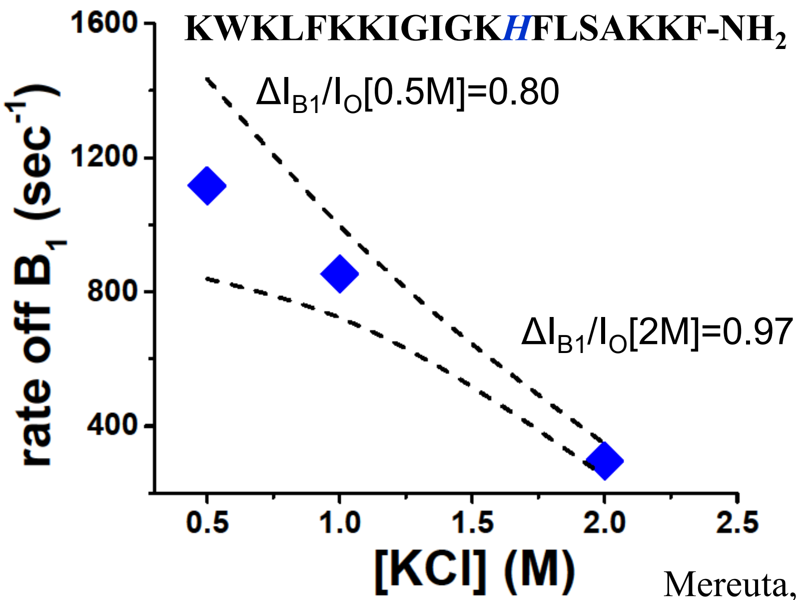
KWKLKK***H***IGIGK***H***FLSAKKF-NH_2

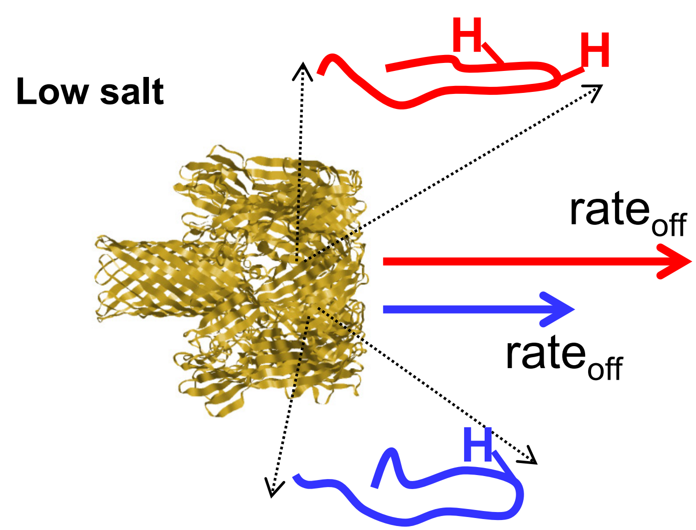


Salt-dependent folding of model antimicrobial peptides



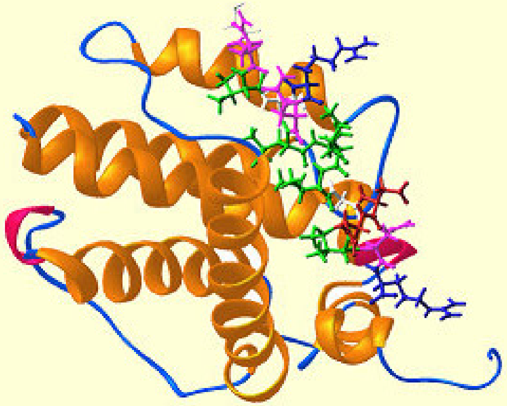






Mereuta, L. et al., *ACS Applied Materials & Interfaces*, 2014 6(15):13242-56.

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.

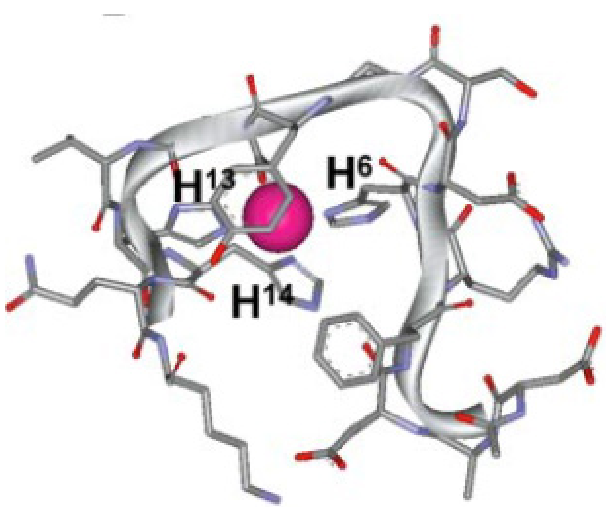
- Although zinc ions are essential for human health, excess of zinc ions in human body was reported to be responsible for several diseases including Alzheimer's disease.

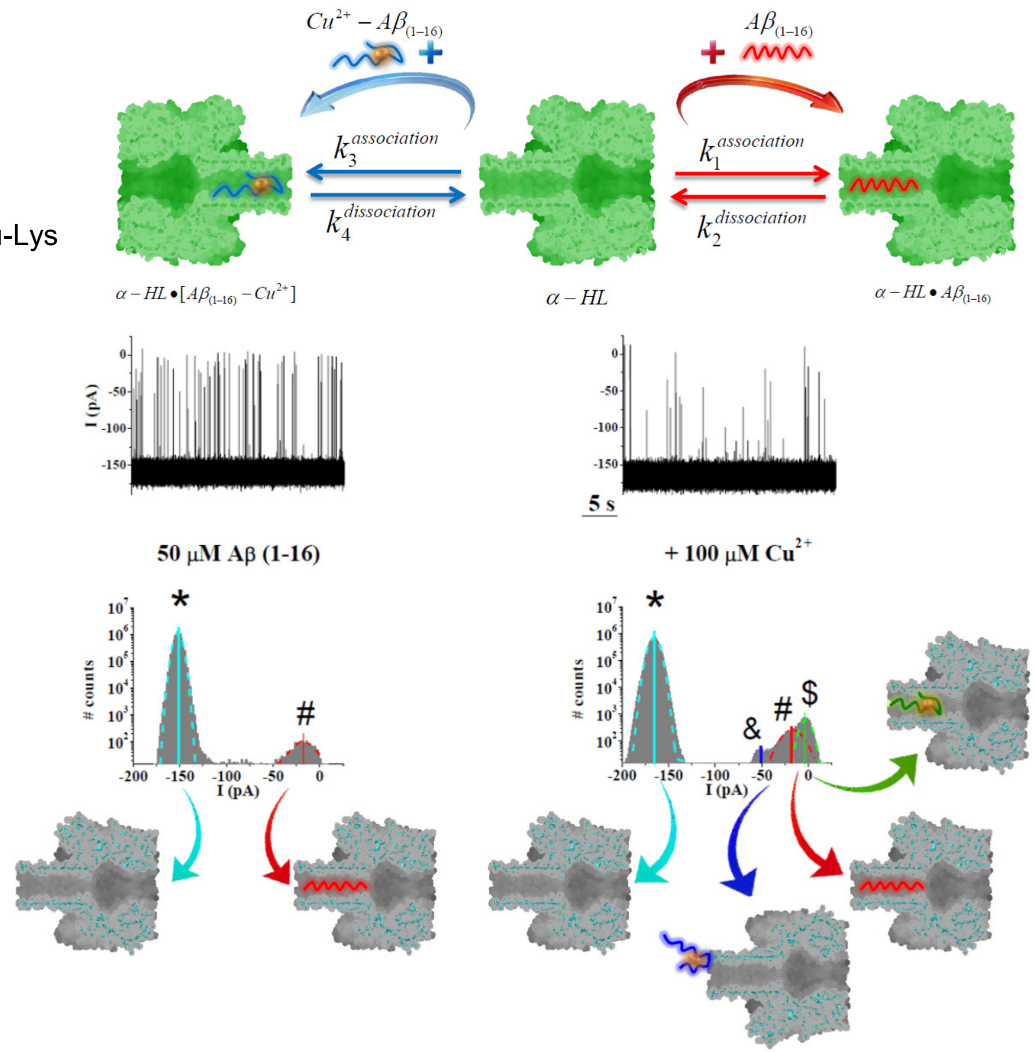
- *A nanopore-based, single-molecule approach* to various ions sensing would provide benefits with respect to *sensitivity, selectivity, portability, low cost, ease of use, and rapid response*, in real-world environmental or biological samples, which contain ions in complex mixtures.

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

Aβ (1-16): intrinsically disordered peptide

Asp-Ala-Glu-Phe-Arg-His[6]-Asp-Ser-Gly-Tyr-Glu-Val-His[13]-His[14]-Glu-Lys

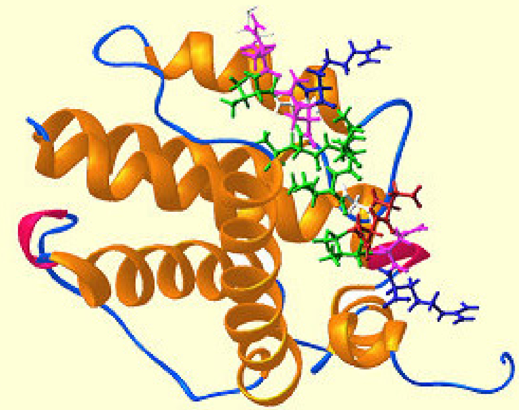


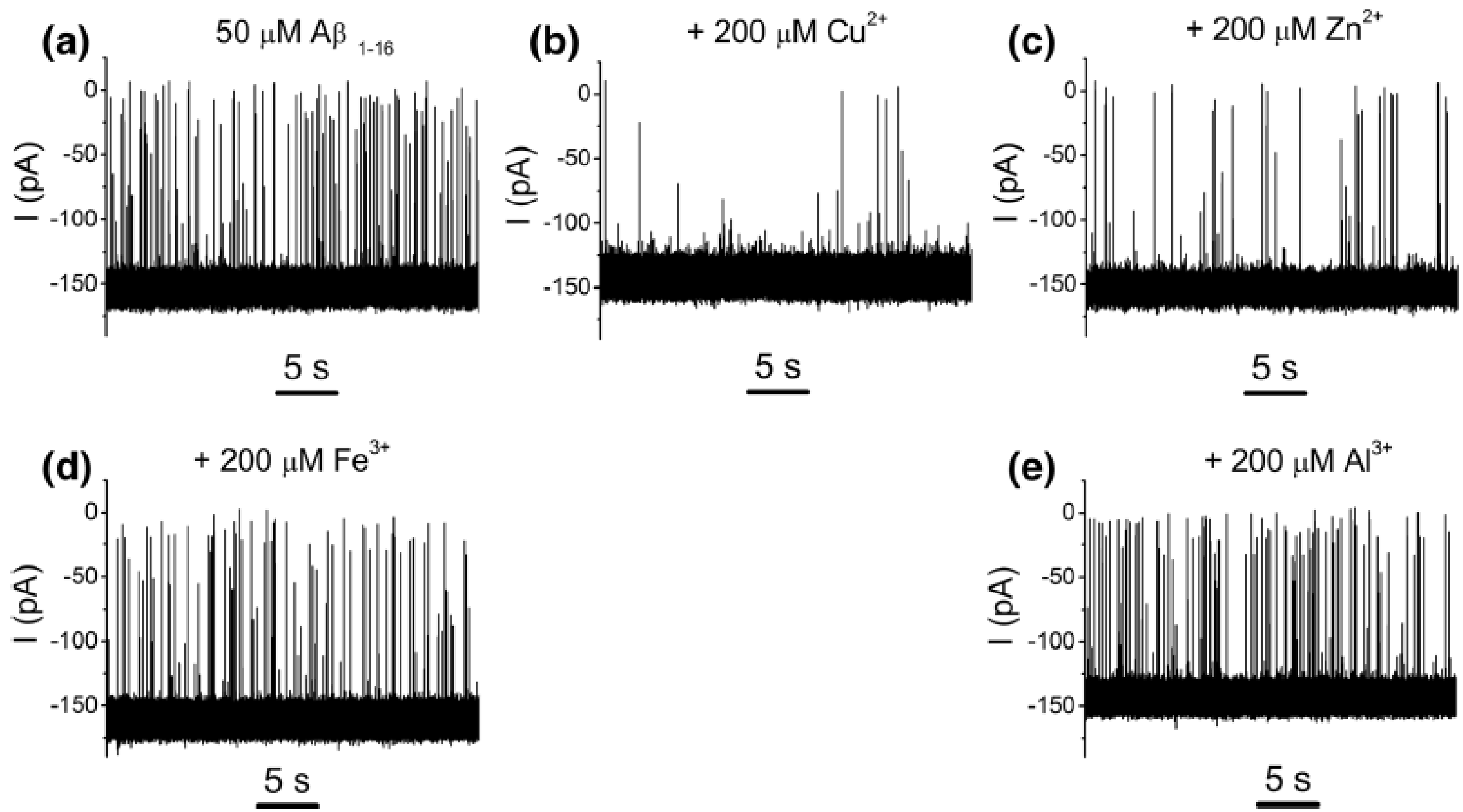


Peter Faller, P. et al., Acc. Chem. Res. 2014, 47, 2252−2259
Zirah, S. et al, *JBC*, 2006, 281, 2515-2161

Asandei A. et al., *Langmuir*, 2013, 29 (50), 15634-1564
Mereuta L. et al., *Langmuir*, 2012, DOI: 10.1021/la303782d

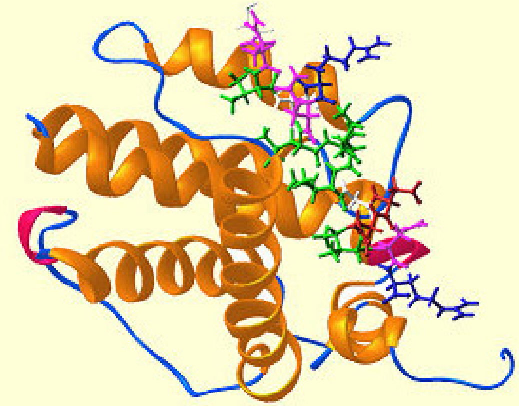
Bioanalytical applications of nanopore-based devices. Application to metals sensing.

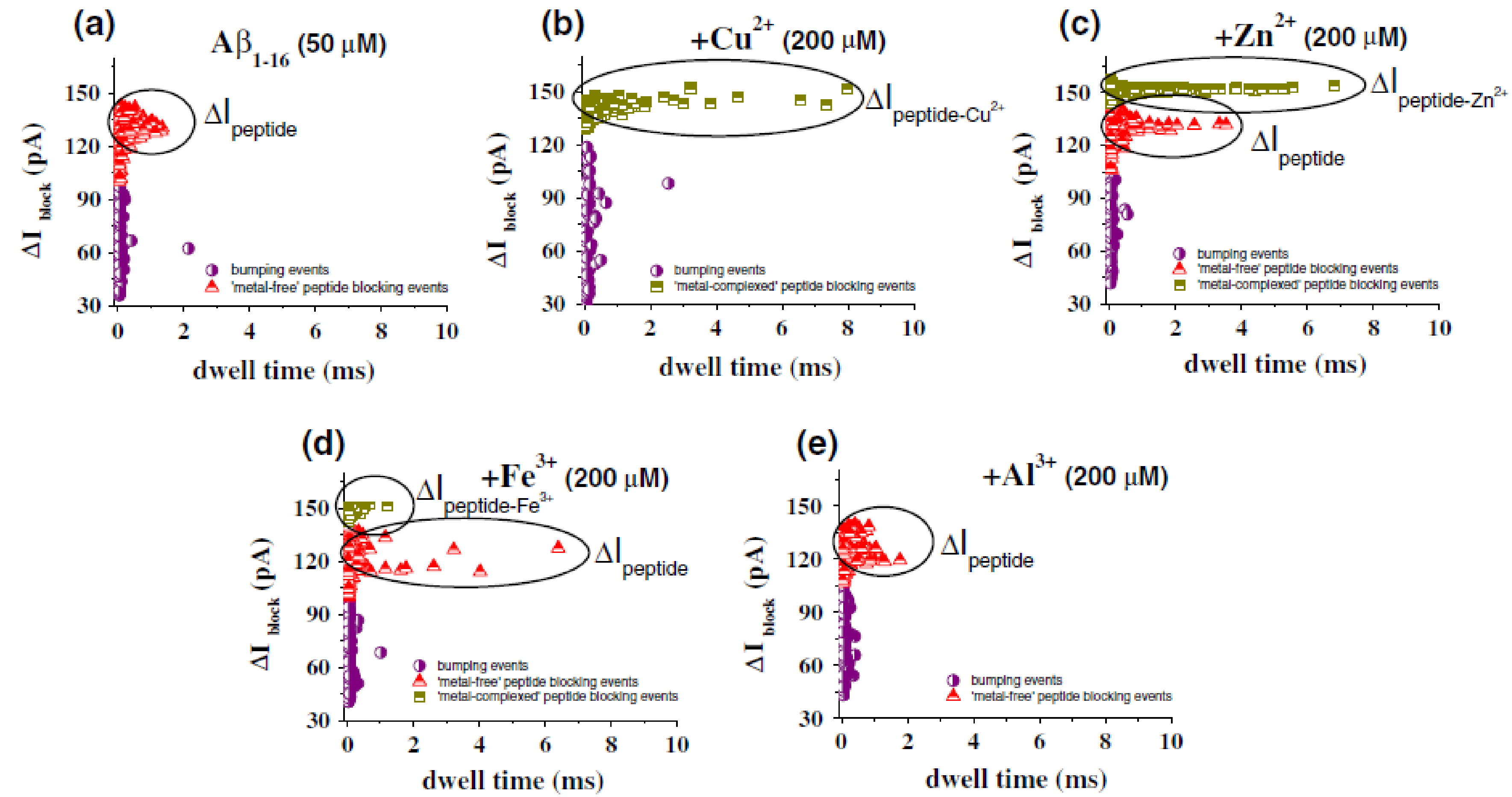




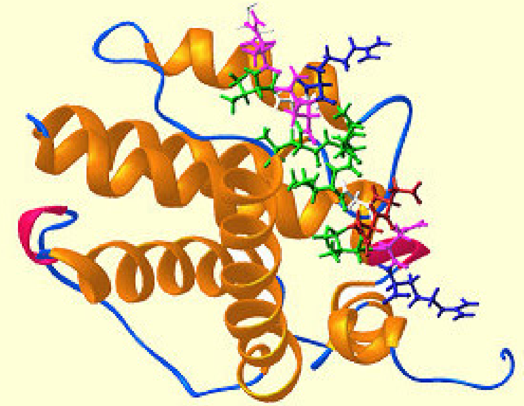
Asandei, A. et al., *J. Membr. Biol.*, 2014, Jun;247(6):523-30.

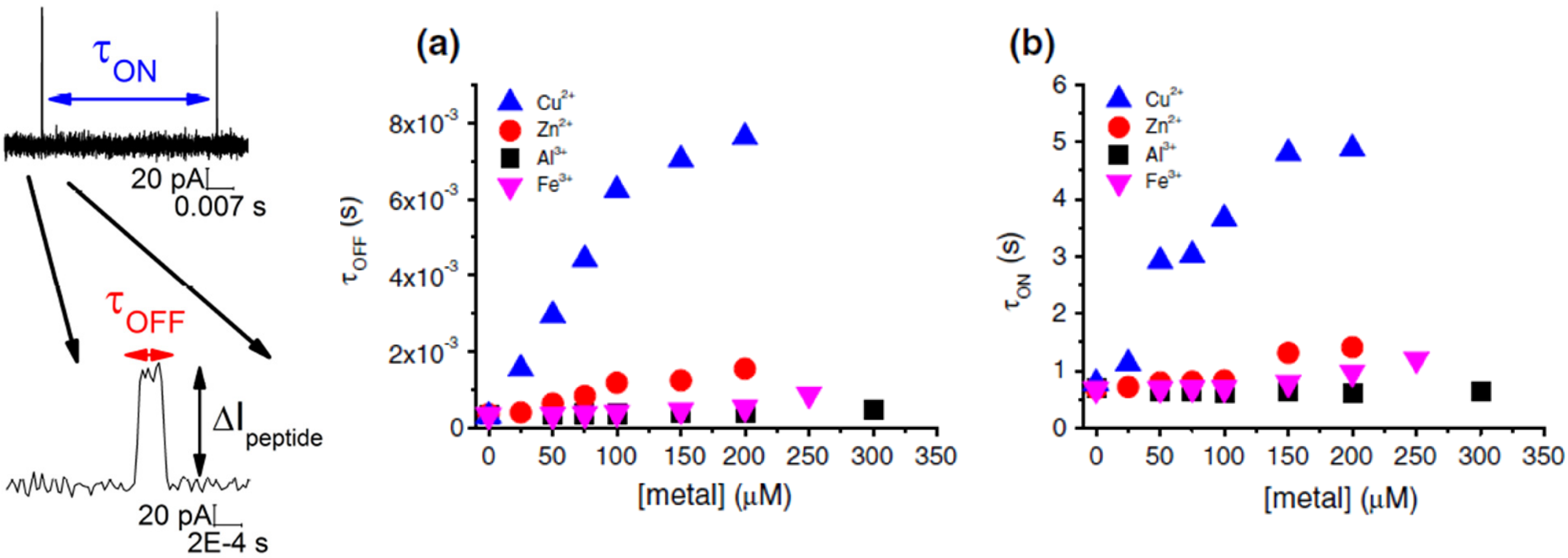
Bioanalytical applications of nanopore-based devices. Application to metals sensing.



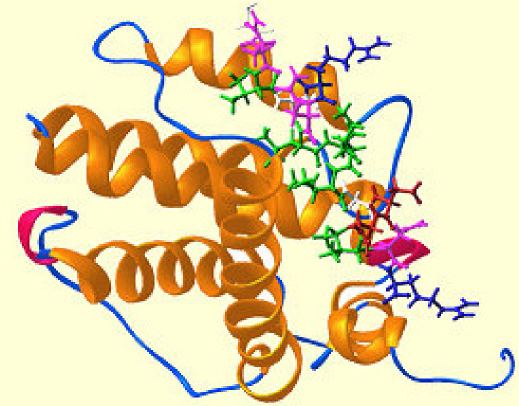


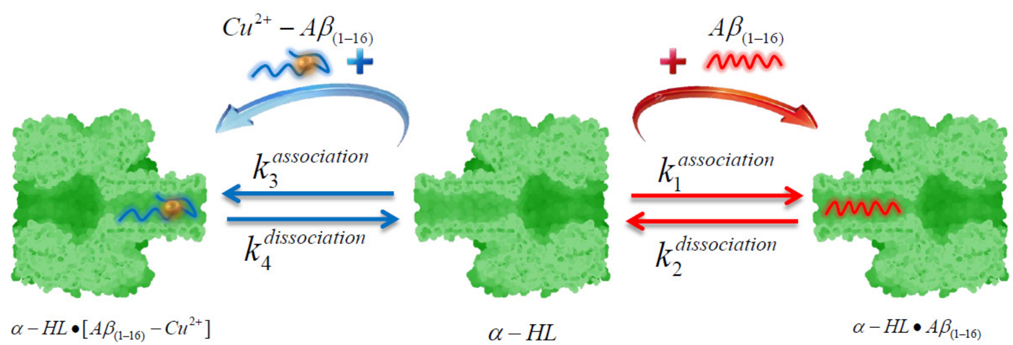
Bioanalytical applications of nanopore-based devices. Application to metals sensing.





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



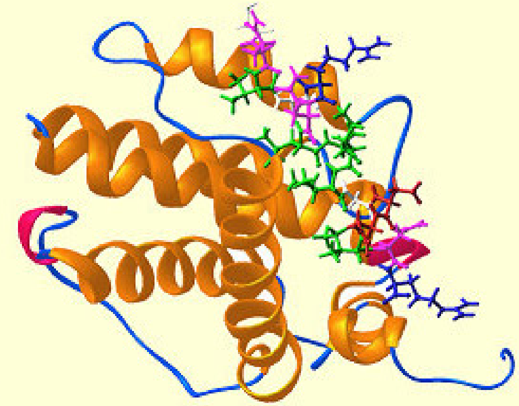


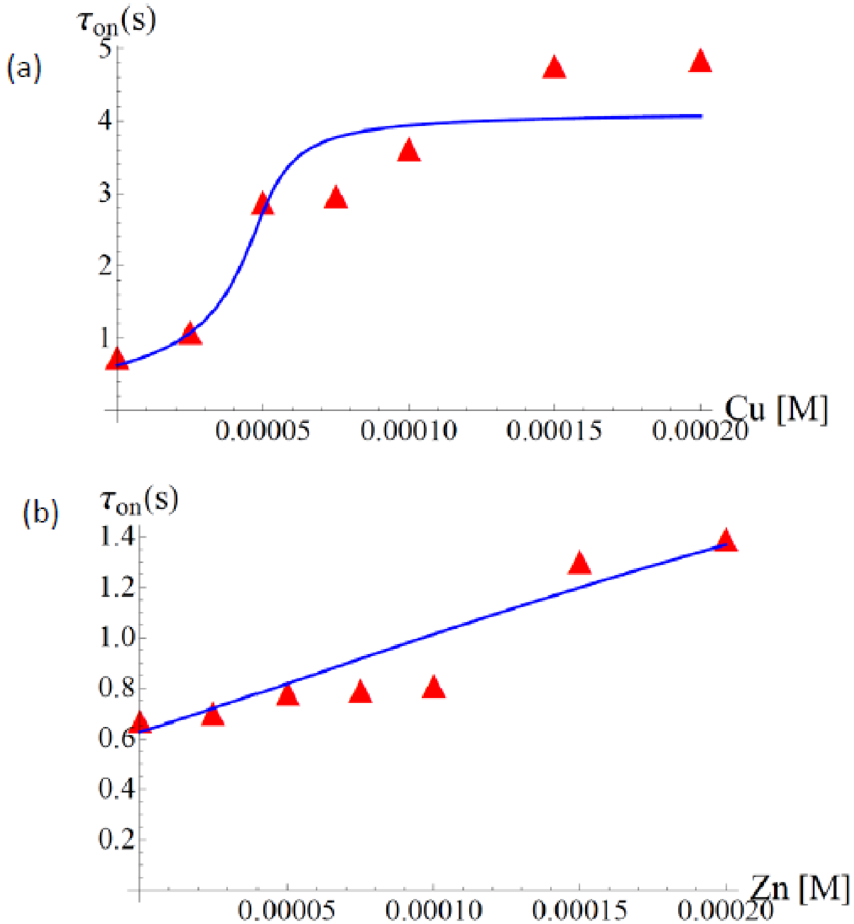
$$C_2 \underset{k_4}{\overset{k_3[P-Cu^{2+}]}{\leftrightarrow}} O \underset{k_2}{\overset{k_1[P]}{\leftrightarrow}} C_1$$

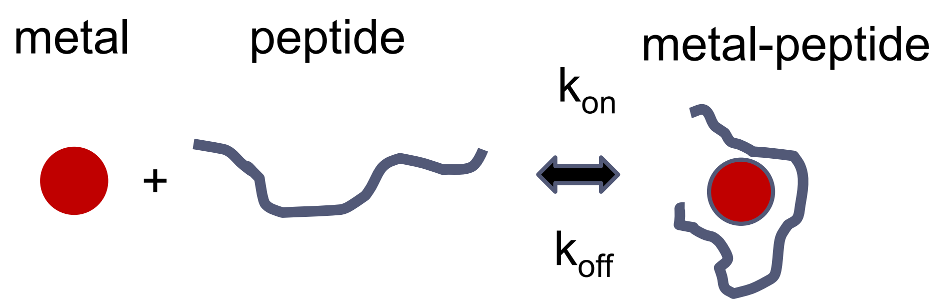
$$\overline{\tau_{ON}} = \frac{1}{k_3[P-X]_{eq.} + k_1([P]_{eq.})} = \frac{1}{k_3[P-X]_{eq.} + k_1([P_0]-[P-X]_{eq.})}$$

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

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







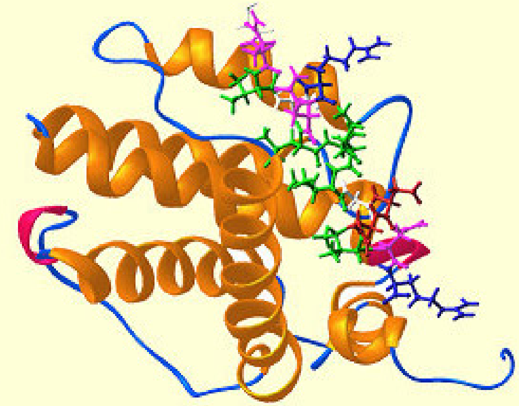
$$K_d = k_{off}/k_{on}$$

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

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

Asandei, A. et al., *J. Membr. Biol.*, 2014, Jun;247(6):523-30.

Acknowledgements



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Thermal unfolding of goulash meat