Single molecule investigations of the pH-dependent interaction between nanoparticles and an α-hemolysin protein pore

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Topics

Short introduction: pH, pKa, aminoacids, intermolecular interactions

Interactions between nanoparticles (NP) and protein pore

Transport process at the single molecule level

Relevance of the study of the interaction between α–HL with NPs.

pH= -lg[H^+]

pKa

$$HA \rightleftharpoons H^+ + A^-$$

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

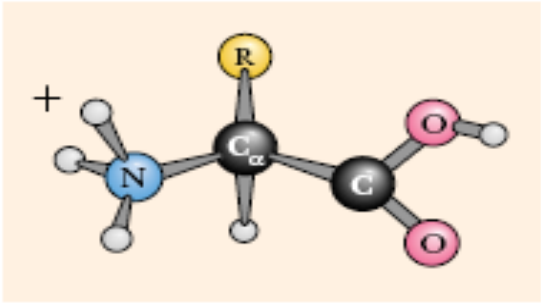
$$\lg [H^+] = \lg K_a + \lg \frac{[HA]}{[A^-]}$$

$$pH = pK_a - \lg \frac{[HA]}{[A^-]} \quad \textit{or} \quad pH = pK_a + \lg \frac{[A^-]}{[HA]}$$

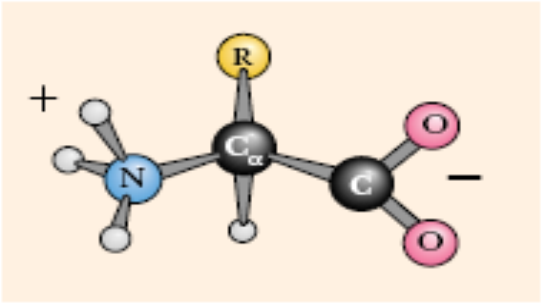
Henderson- Hasselbalch equation

Aminoacids

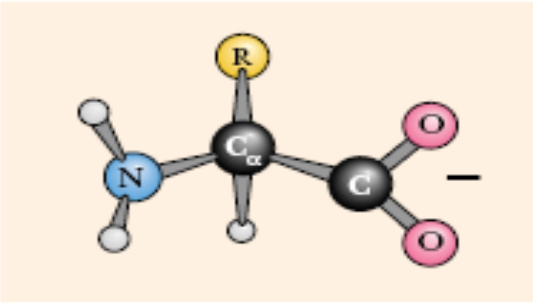
pH 1 Net charge +1

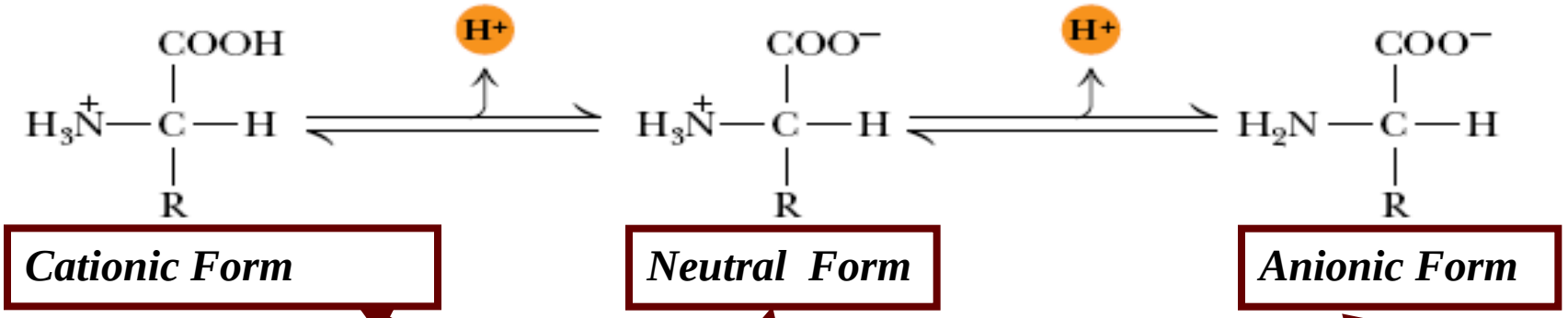


pH 7 Net charge 0



pH 13 Net charge –1

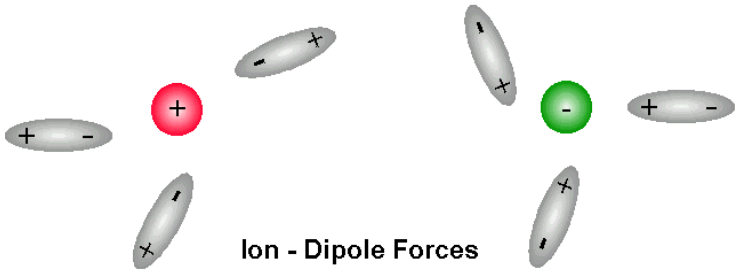




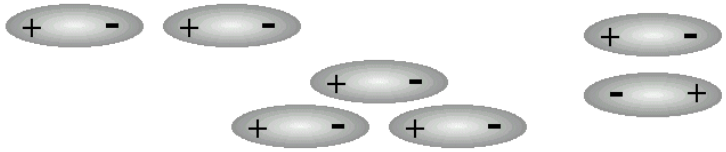
Cationic Form ***Neutral Form*** ***Anionic Form***

Aminoacids charge at different pH

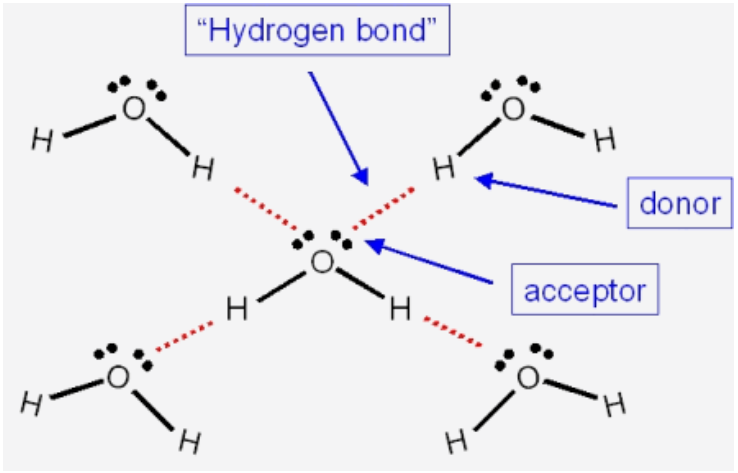
Intermolecular interactions

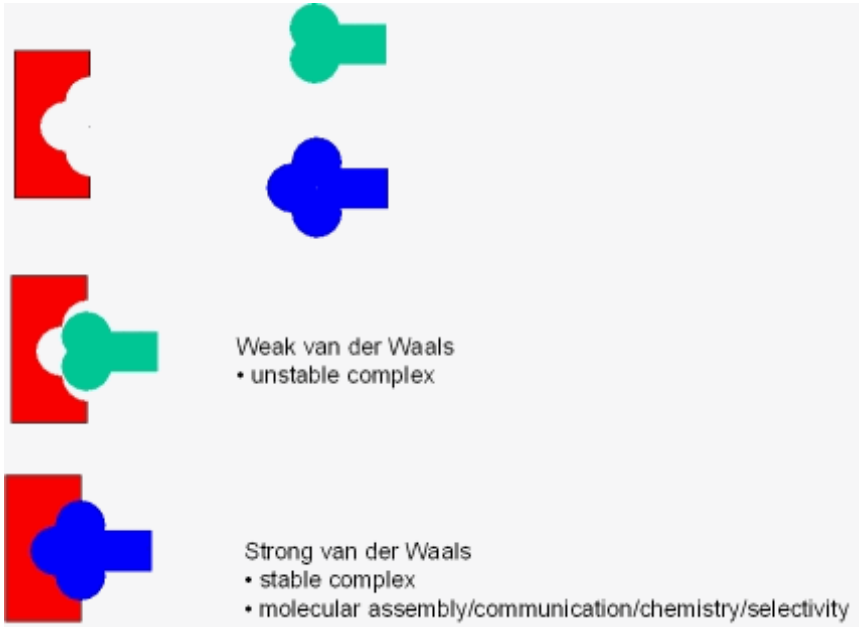


Ion - Dipole Forces

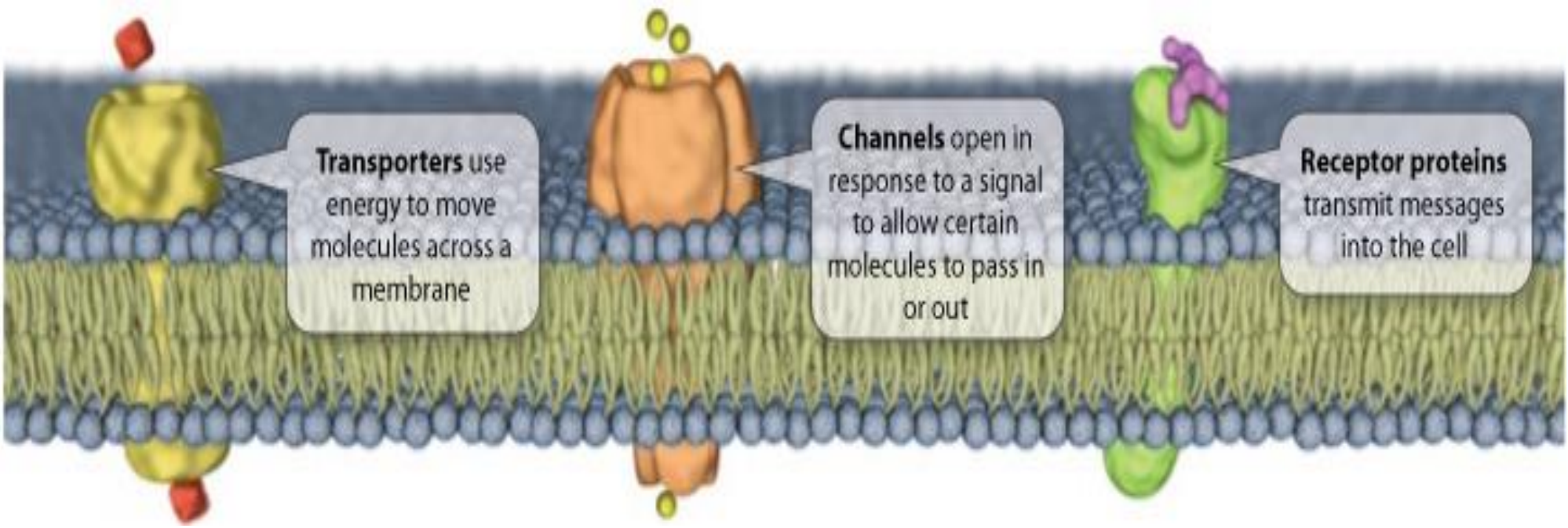


Attractive Dipole-Dipole Interactions





Ion Channels: gates in the cell wall



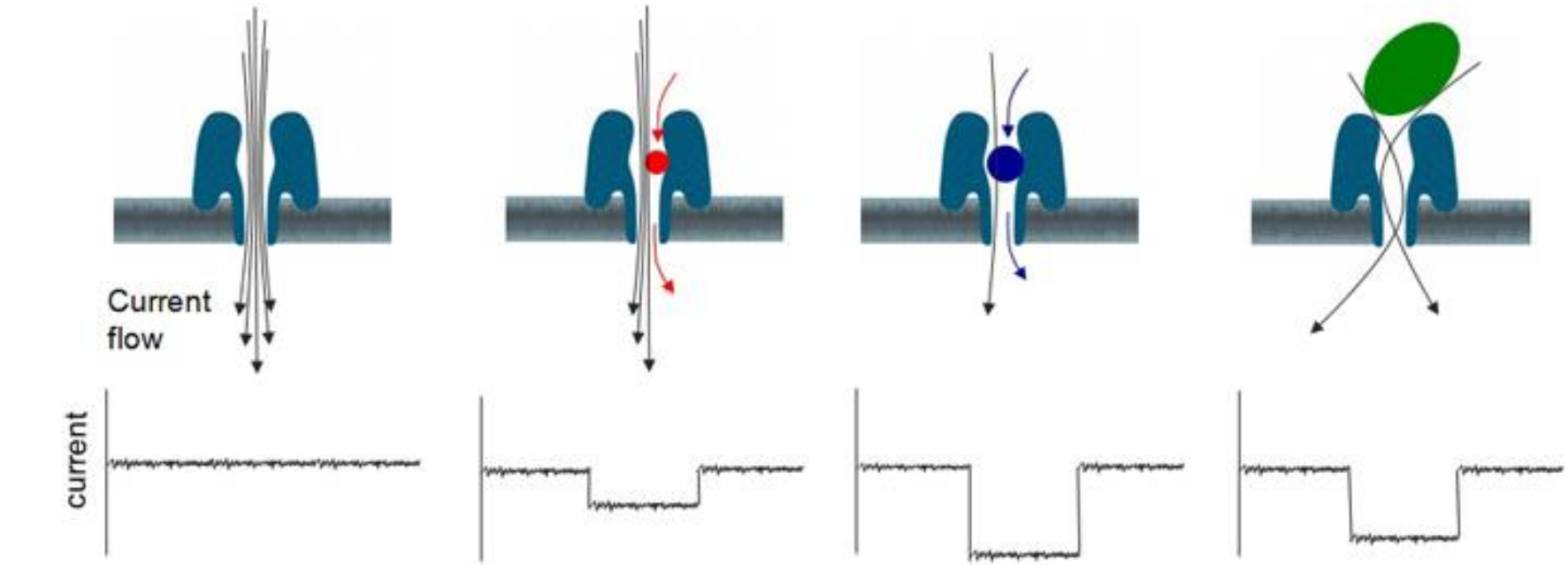
http://learn.genetics.utah.edu/content/begin/cells/membranes/

Why nanopore protein?

Nanomedicine Cancer treatment
Delivery of macromolecules into cells
Development of antimicrobial drugs

Sensing Detection of small ions and organic molecules
Probing the translocation and structure of polymers, polynucleotides and polypeptides
Monitoring protein-binding interactions, enzyme activity, chemical reactions

Nanoelectronics Nanofluidic diodes
Development of bio-inspired batteries



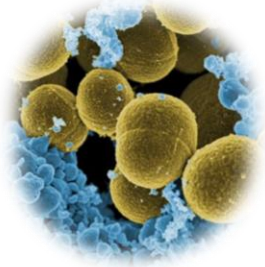
www.nanoporetech.com

- The ability to detect individual molecules using a single nanopore has been used to quantify analytes and distinguish between different ones.

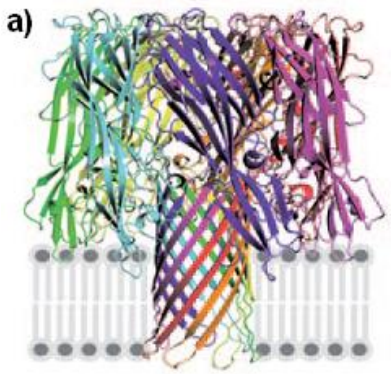
- The chance to detect the non-covalent block of the wild-type α-HL protein pores by small organic molecules, which may prove crucial in medicine, environmental monitoring and domestic defense

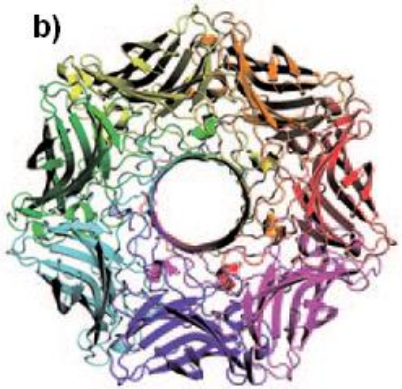
- A particular emphasis of protein engineering has been placed on the identification and characterization at the single-molecule level of single-stranded genomic DNA or RNA. Knowing that the interaction of peptides with protein pores is of fundamental importance in biology, a litany of studies have been devoted to examining the partitioning of peptides into the α-HL protein pore

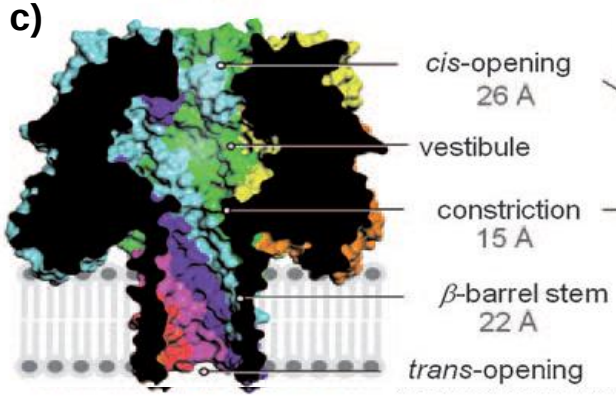
Structure of the α-HL and interaction between α-HL and NP

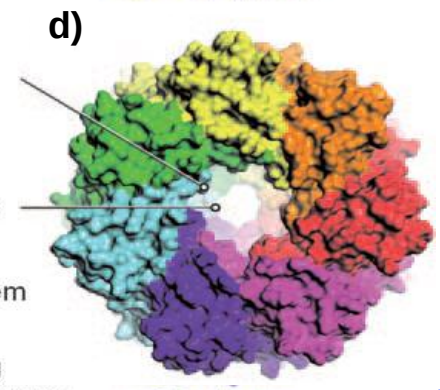


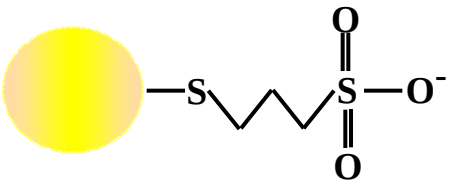
Staphylococcus aureus

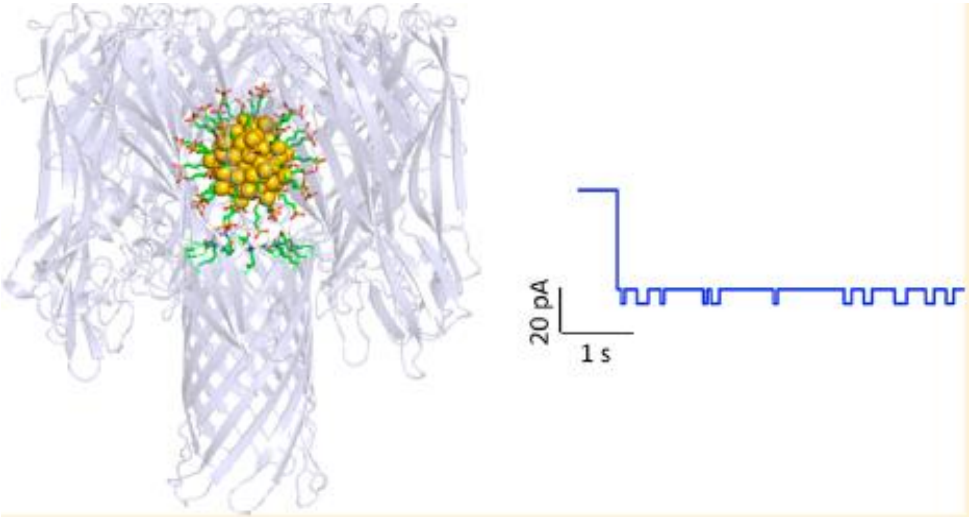








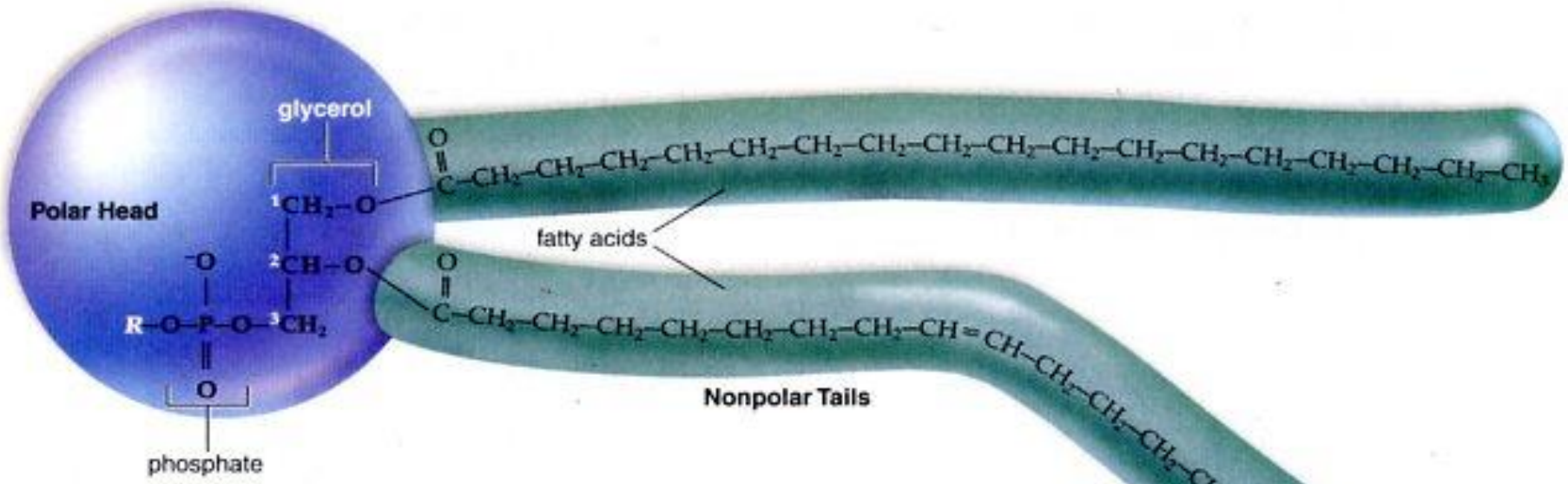




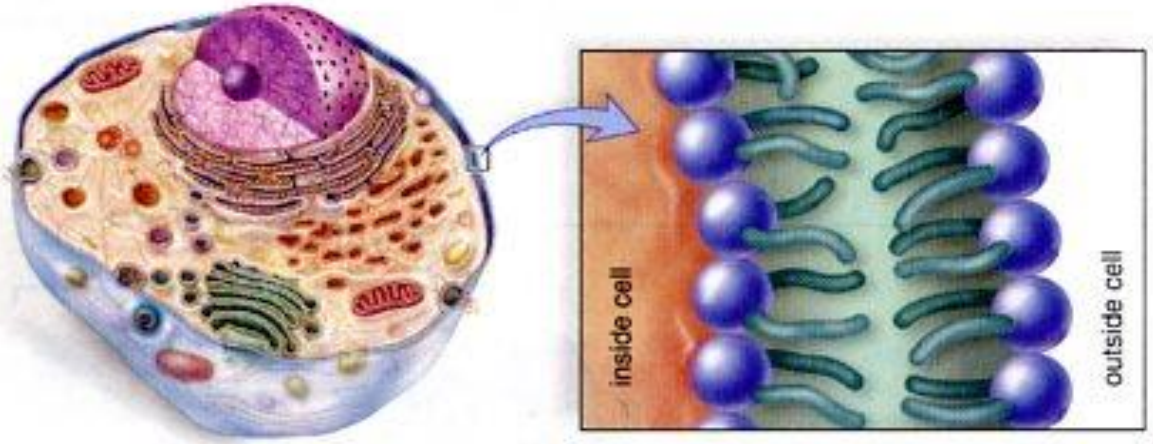
L. Ma and S.L. Cockroft, 2010, *ChemBioChem* 11, 25-34

E. Campos, A. Asandei, C.E. McVey, J.C. Dias, A. S. F. Oliveira, C. M. Soares, T. Luchian, Y. Astier, 2012, *Langmuir* 28, 15643−15650

Lipid Structure



a. Phospholipid structure

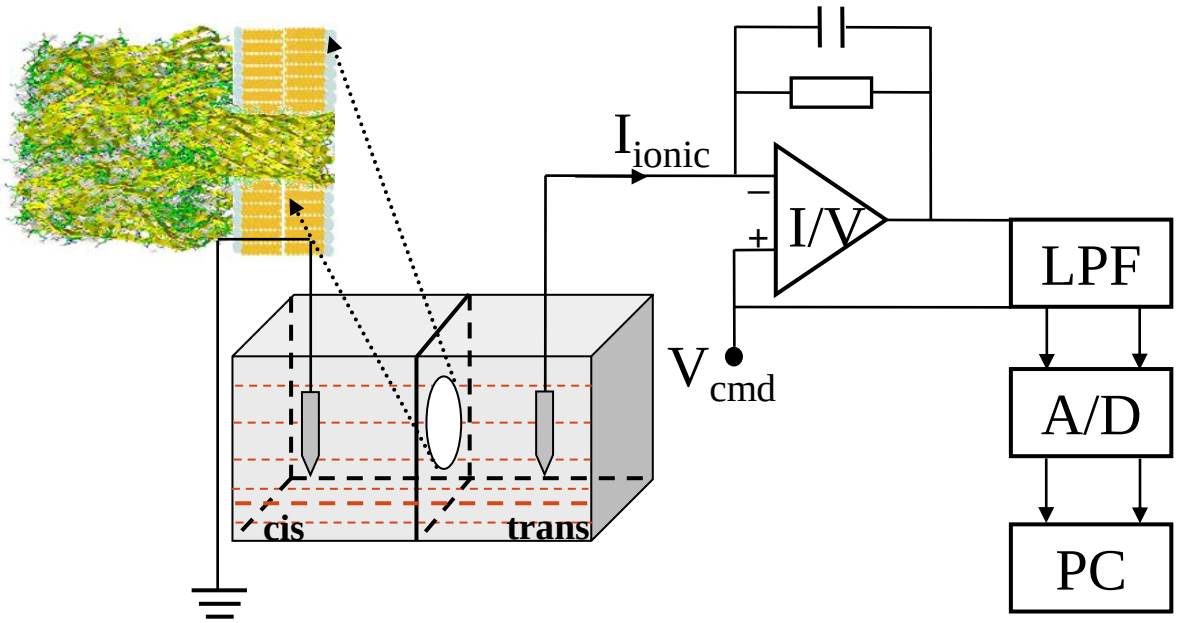


b. Plasma membrane of a cell

Phospholipids form membranes.

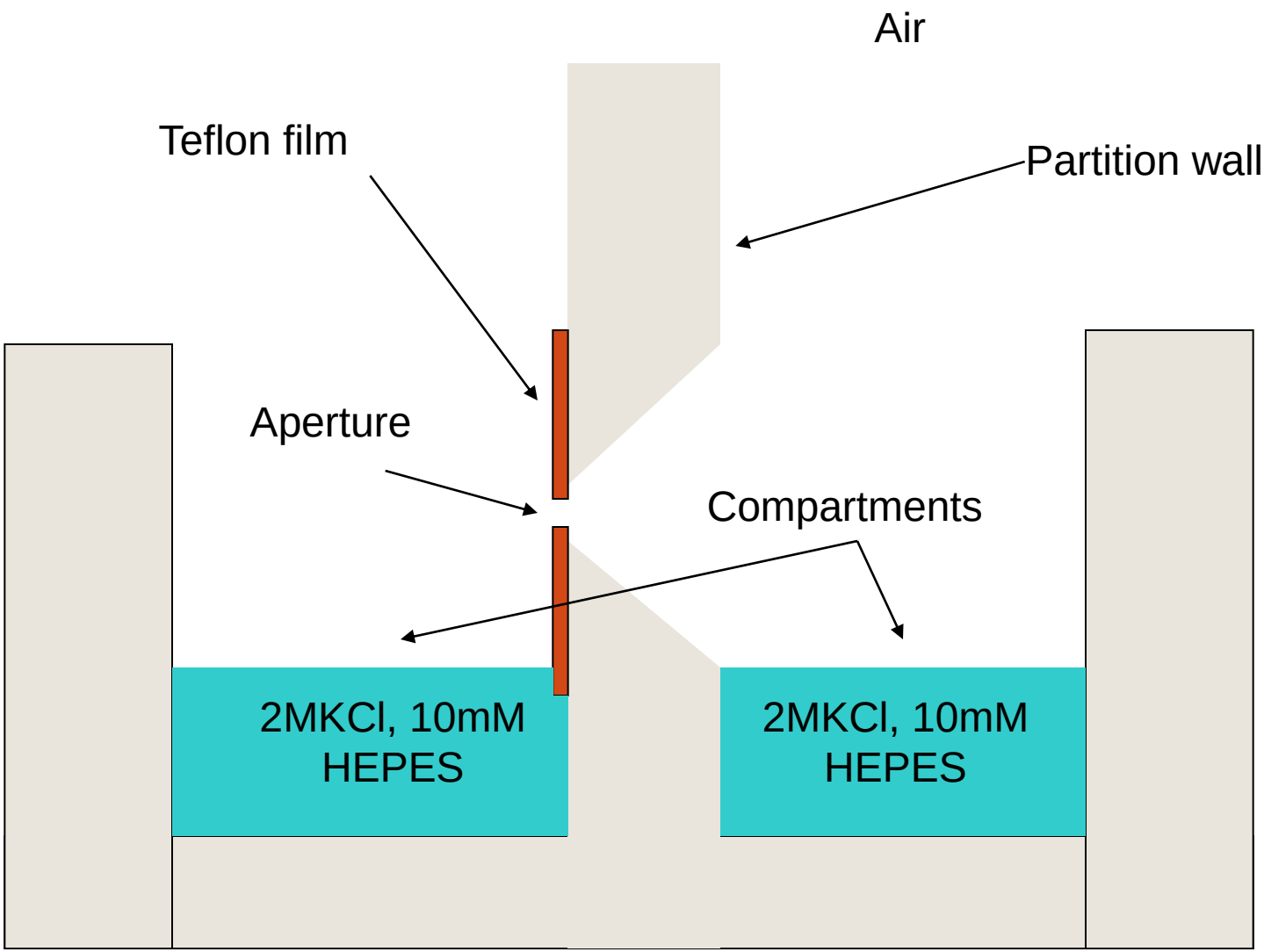
a. Phospholipids are constructed like fats, except that in place of the third fatty acid, they have a polar phosphate group. The hydrophilic (polar) head is soluble in water, whereas the two hydrophobic (nonpolar) tails are not. A tail has a kink wherever there is an unsaturated bond. **b.** Because of their structure, phospholipids form a bilayer that serves as the major component of a cell's plasma membrane. The fluidity of the plasma membrane is due to kinks in the phospholipids' tails.

Experimental set-up



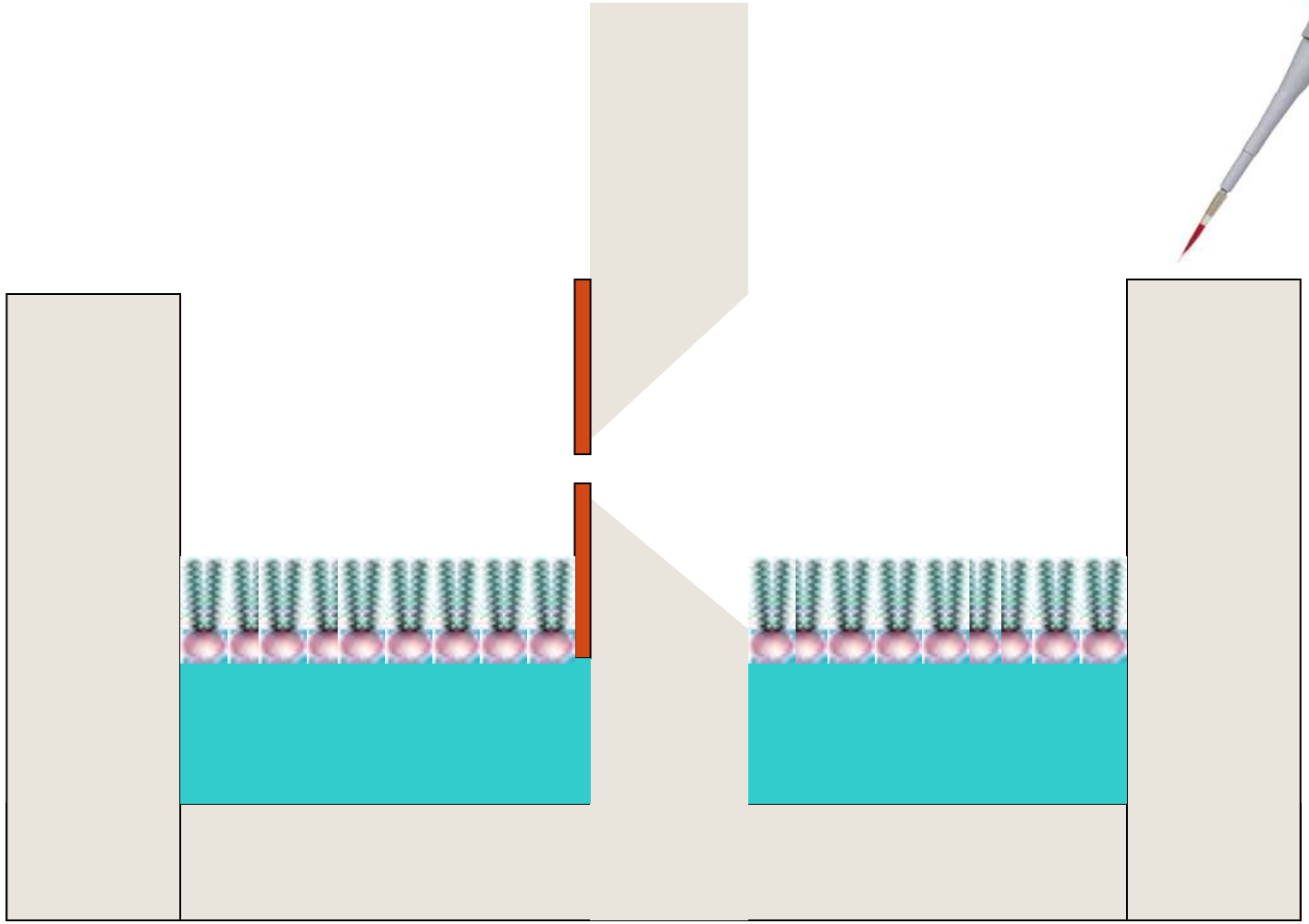
Experimental set-up for undertaking single-molecule electrical measurements of the interaction between α–HL pore with NPs at various pH

Experimental set-up

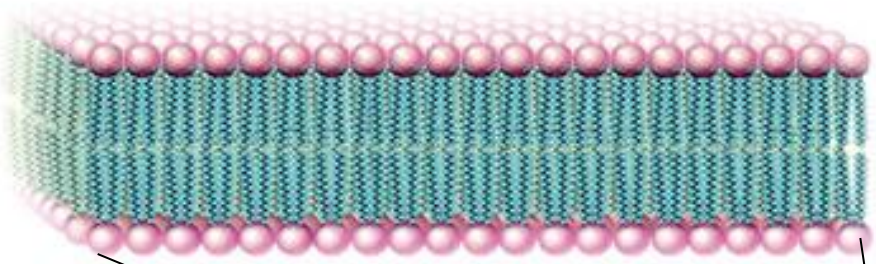


Membrane Support

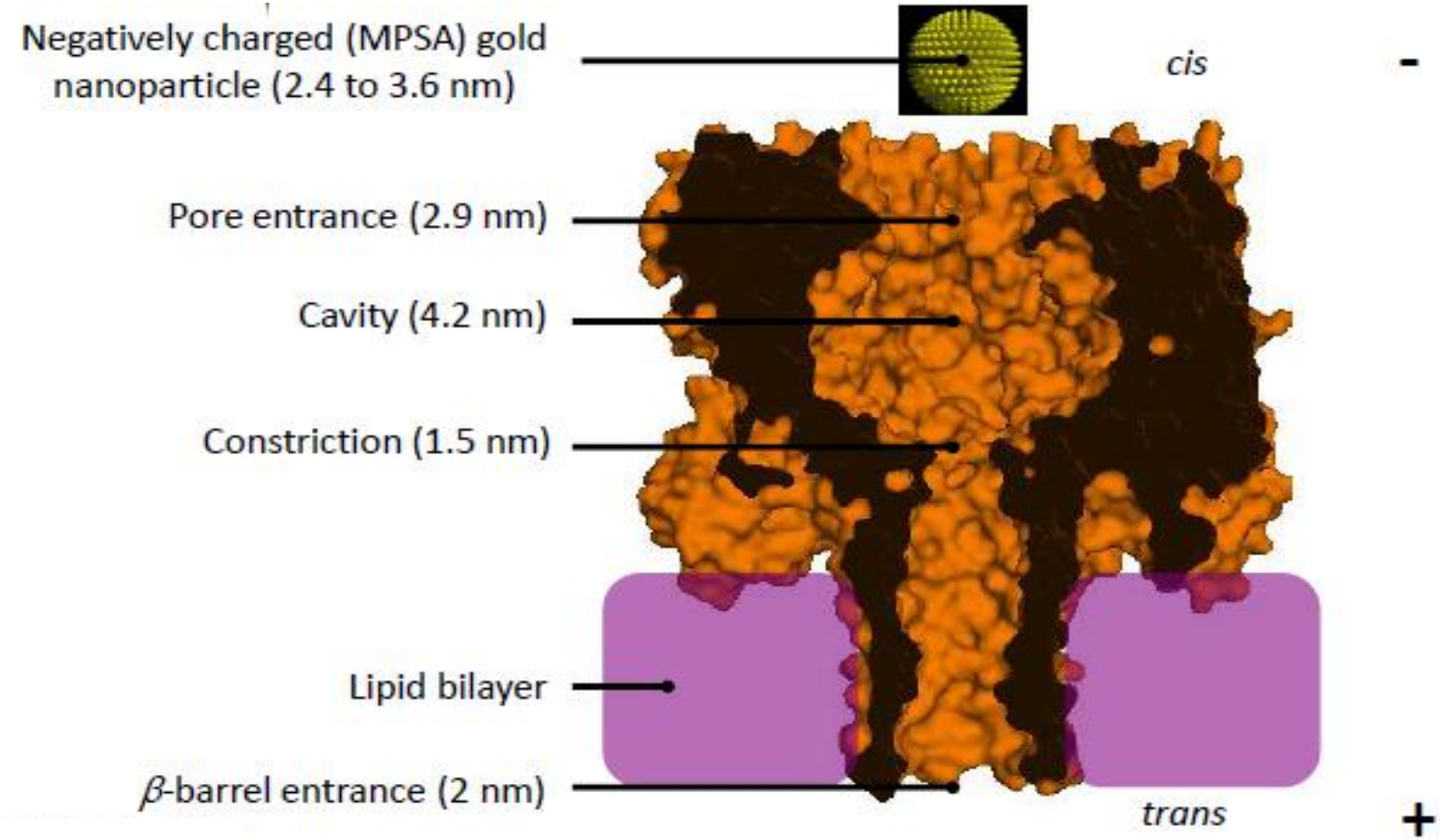
Experimental set-up



Experimental set-up



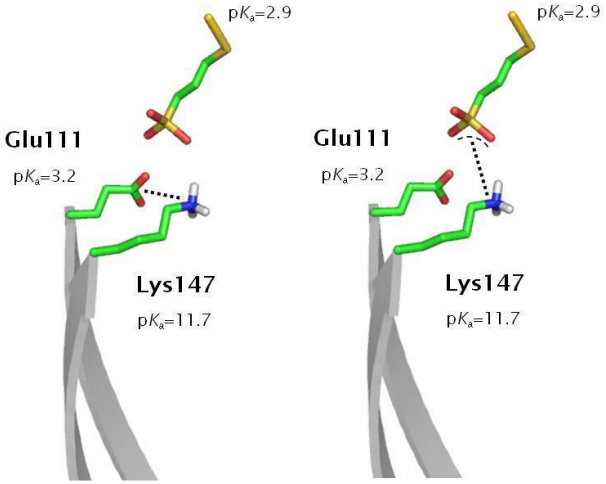
α-hemolysin (*Staphylococcus aureus*)



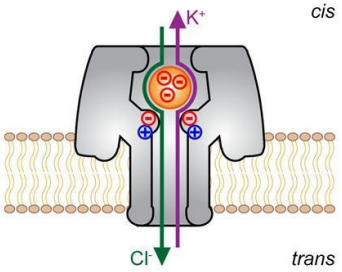
Astier et. al., 2009, Small, 1-6

Possible interaction between NP and constriction zone of the α–HL protein pore

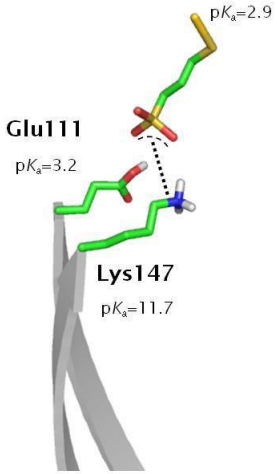
A



pH=8.0

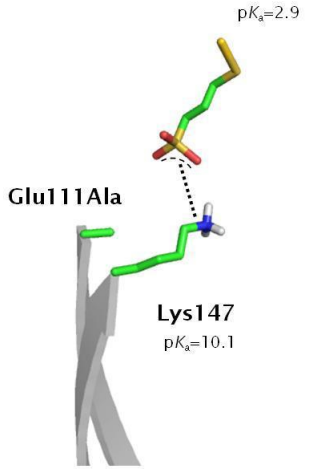


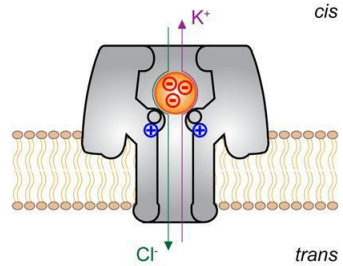
B



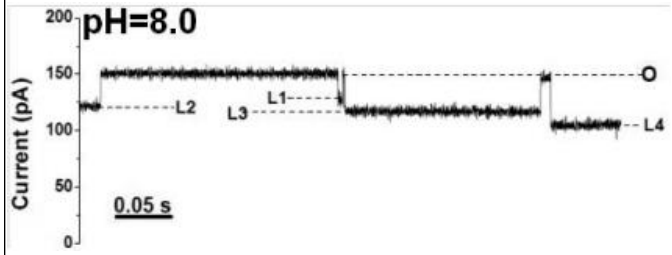
pH=2.8

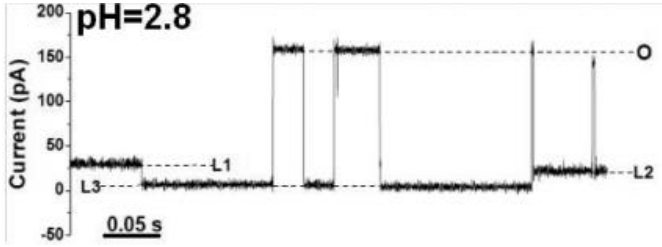
C

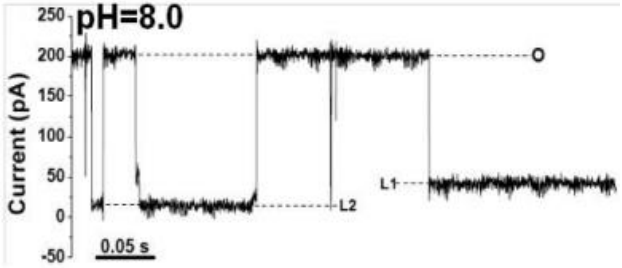


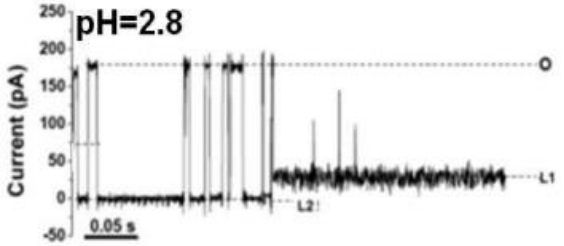


pH=8.0
pH=2.8

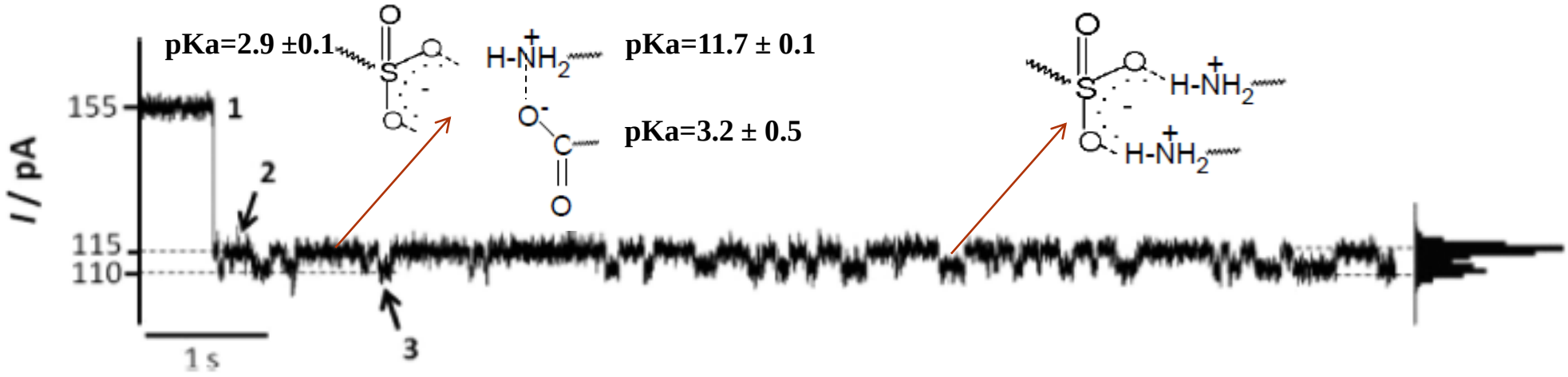






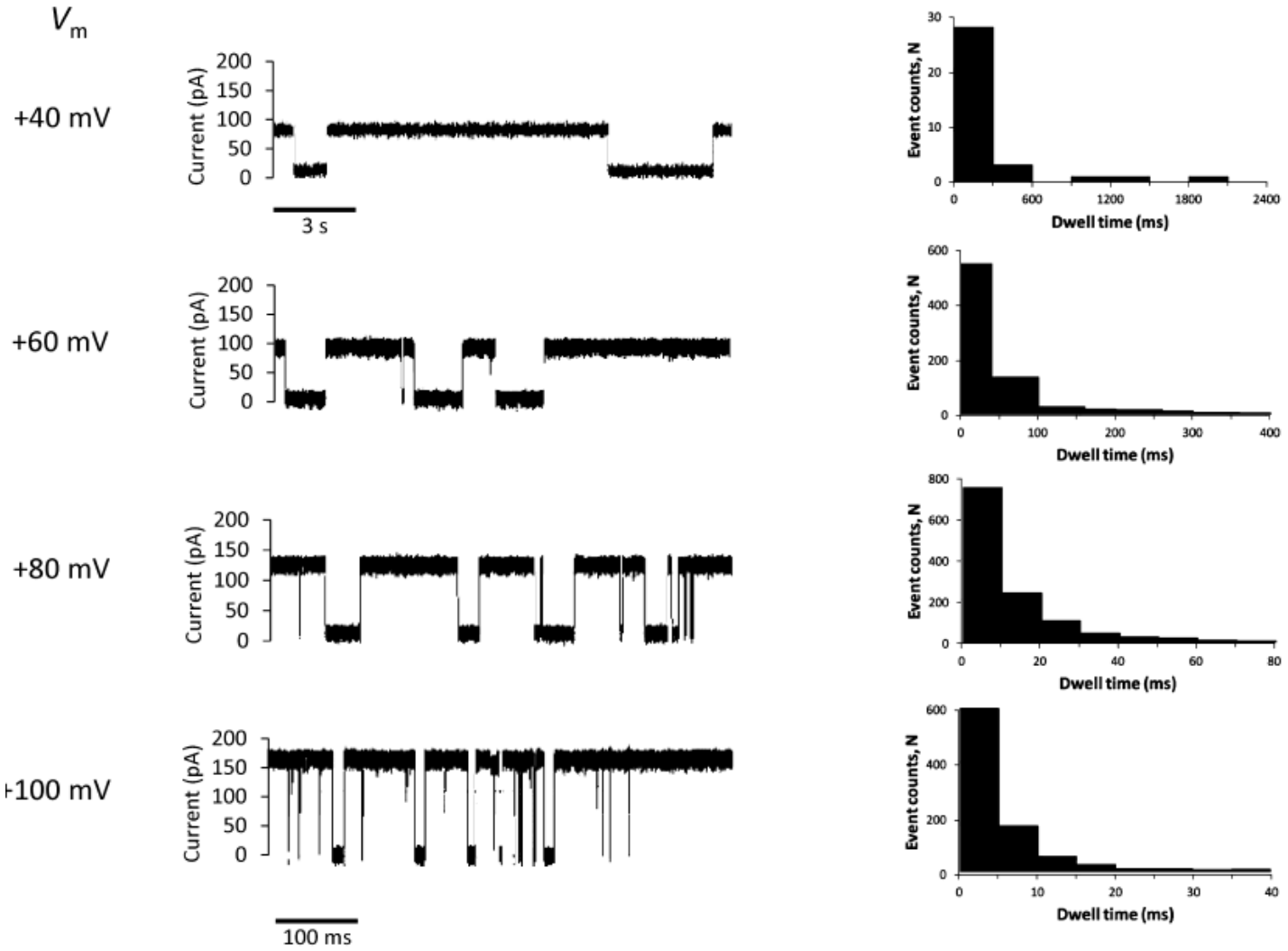


Transport process at the single molecule level



Representative capture event showing changes of current amplitude between defined amplitude levels, while the MPSA-NP is inside the single WT-αHL nanopore. (1) Empty pore current amplitude of 155 pA. (2) Nanopore current amplitude (115 pA) with a single MPSA-NP trapped inside, while Lys147 interacts with Glu111 through a salt bridge. (3) Nanopore current amplitude (110 pA) with a single MPSA-NP trapped inside, while Lys147 interacts electrostatically with MPSA-NP sulfonate ligands. A histogram of the amplitude changes (2 and 3) is plotted on the right. Recordings were made at +80 mV in 2 M KCl, 10 mM HEPES (pH 8.0), in the presence of 10 μg mL^{-1} MPSA-NP added on the cis side of the lipid bilayer.

E. Campos, A. Asandei, C.E. McVey, J.C. Dias, A. S. F. Oliveira, C. M. Soares, T. Luchian, Y. Astier, 2012, *Langmuir* 28, 15643−15650



Single-channel recordings of E111A nanopores after the addition of 10 μg mL^{-1} MPSA-NP to the cis compartment, at applied potentials of +40, +60, +80, and +100 mV. The right-handed charts are event histograms showing the dwell-time distribution for the most frequent block at each potential. Experiments were performed in 2 M KCl and 10 mM HEPES at pH 8.0.

E. Campos, A. Asandei, C.E. McVey, J.C. Dias, A. S. F. Oliveira, C. M. Soares, T. Luchian, Y. Astier, 2012, *Langmuir* 28, 15643−15650

	pH			
	2.8	6.9	8.0	9.9
WT-αHL	+8.3	−1.2	−3.9	−8.1
E111A	+11.2	+5.7[a]	+3.0	−3.0
MPSA-NP	−21.2	−44.0	−44.0	−44.0

Table 2. Summary of the MPSA-NP Capture Events in a Single WT-αHL and E111A Nanopore[a,b]

	+60 mV			+80 mV			+100 mV		
	% blockade	dwell time (s)	N	% blockade	dwell time (s)	N	% blockade	dwell time (s)	N
				wild-type					
pH 2.8	81.91 ± 0.26	0.11 ± 0.02	114	82.81 ± 1.10	0.3 ± 0.1	75	86.28 ± 0.70	0.7 ± 0.1	21
	88.27 ± 0.62	0.5 ± 0.1	99	89.56 ± 1.54	0.8 ± 0.1	65	91.52 ± 0.38	1.9 ± 1.2	24
	95.87 ± 0.53	2.2 ± 1.2	59	96.49 ± 0.21	3.7 ± 1.5	43	96.46 ± 0.15	4.4 ± 2.1	26
	17.86 ± 0.52	0.1 ± 0.1	52	13.84 ± 0.79	0.004 ± 0.001	94	13.85 ± 0.26	0.005 ± 0.001	46
pH 8.0				17.30 ± 2.72	0.1 ± 0.1	34	17.30 ± 0.02	0.1 ± 0.1	42
				24.81 ± 2.29	8.6 ± 3.7	16	22.32 ± 0.75	3.8 ± 1.0	12
				30.98 ± 1.84	13.8 ± 5.5	12	26.22 ± 0.53	14.6 ± 5.4	12
							31.43 ± 0.72	17.8 ± 11.3	9
				E111A mutant					
pH 2.8	99.12 ± 1.83	0.29 ± 0.05	153	99.74 ± 0.48	0.09 ± 0.01	102	98.13 ± 5.02	0.06 ± 0.01	115
	88.81 ± 2.09	0.032 ± 0.004	42	88.79 ± 1.07	0.07 ± 0.01	86	79.32 ± 7.09	0.03 ± 0.01	38
pH 8.0	87.29 ± 2.11	0.037 ± 0.001	818	91.82 ± 3.88	0.012 ± 0.001	1423	90.39 ± 4.83	0.005 ± 0.0001	1084
	69.14 ± 0.92	0.008 ± 0.001	169	72.21 ± 5.33	0.006 ± 0.001	682	71.92 ± 8.86	0.003 ± 0.0002	384

Conclusions

Nanopores constitute - rapid and sensitive biosensors

- gold nanoparticles (NP) coated with 3-mercapto-1-propanesulfonate (MPSA) under 2.9 nm in diameter can be captured inside αHL nanopore cavity, decreasing the conductance of α-HL up to approximately 50%

- single-channel recordings carried out:

- at pH 6.9, 8.0, and 9.9 revealed several current blockade levels, whose values were around 20-30% and whose time duration (dwell time) ranging from 10 ms to 10 s.

- at pH 2.8, high current blockade around 80-90%, with dwell time around 1 s were observed. At pH 2.8, once Glu111 residues were mainly protonated, Lys147 residues were more likely to interact with NP's ligands.

- to confirm the role Lys147 on the interactions NP-protein, an engineered α-HL pore was designed in which Glu111 was substituted by alanine. Data obtained with α-HL-E111A mutant showed that, at pH 2.8 and 8.0, higher current blockades with dwell times from 10 to 100 ms were observed since Lys147 residues were able to interact with NP's ligands.

Our results suggest that Lys147 can be used to control opening and closing of αHL in the presence of MPSA modified gold NP. This approach can be applied in Biotechnology, namely, in controlling drug delivery to target cells.


Thank you!