Molecular Mechanism of Proton Transport in Membrane Proteins

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Why study mechanisms of ion transport?

Biological importance

- Ionic gradients are essential to proper biological function
  → nervous system, metabolism, ATP synthesis…

- Membranes block ion flow

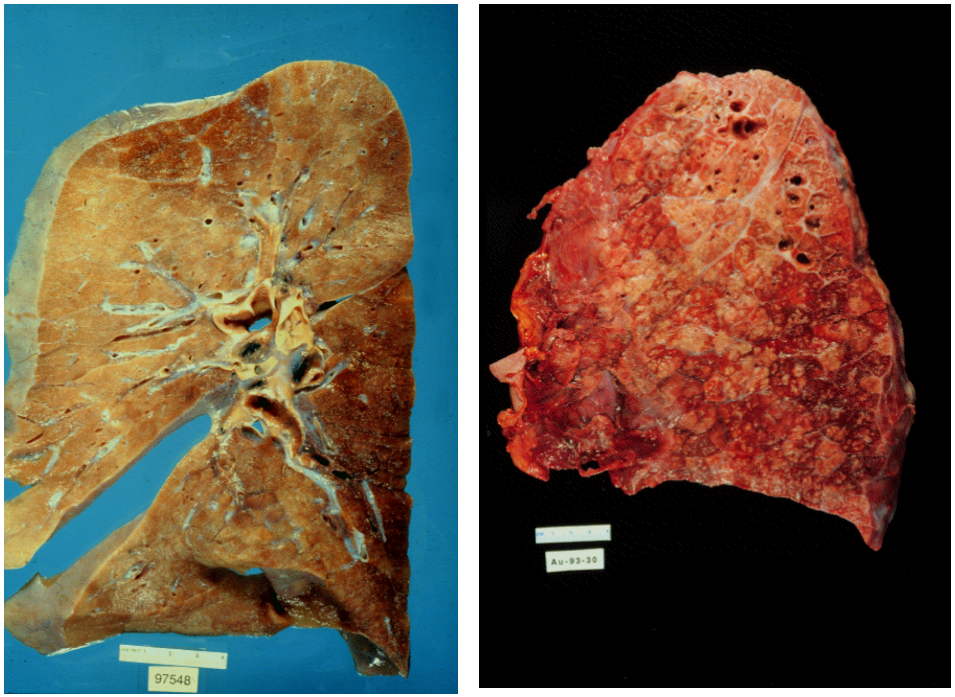
- Specific transport is mediated by membrane proteins
  = channels, transporters, pumps

→ ion permeation (potassium, chloride, sodium…)
→ ion exclusion (aquaporins = water specific)
→ ion pumps

Medical relevance

Malfunction of channels is linked to disease:

– Cystic fibrosis ← Chloride channel

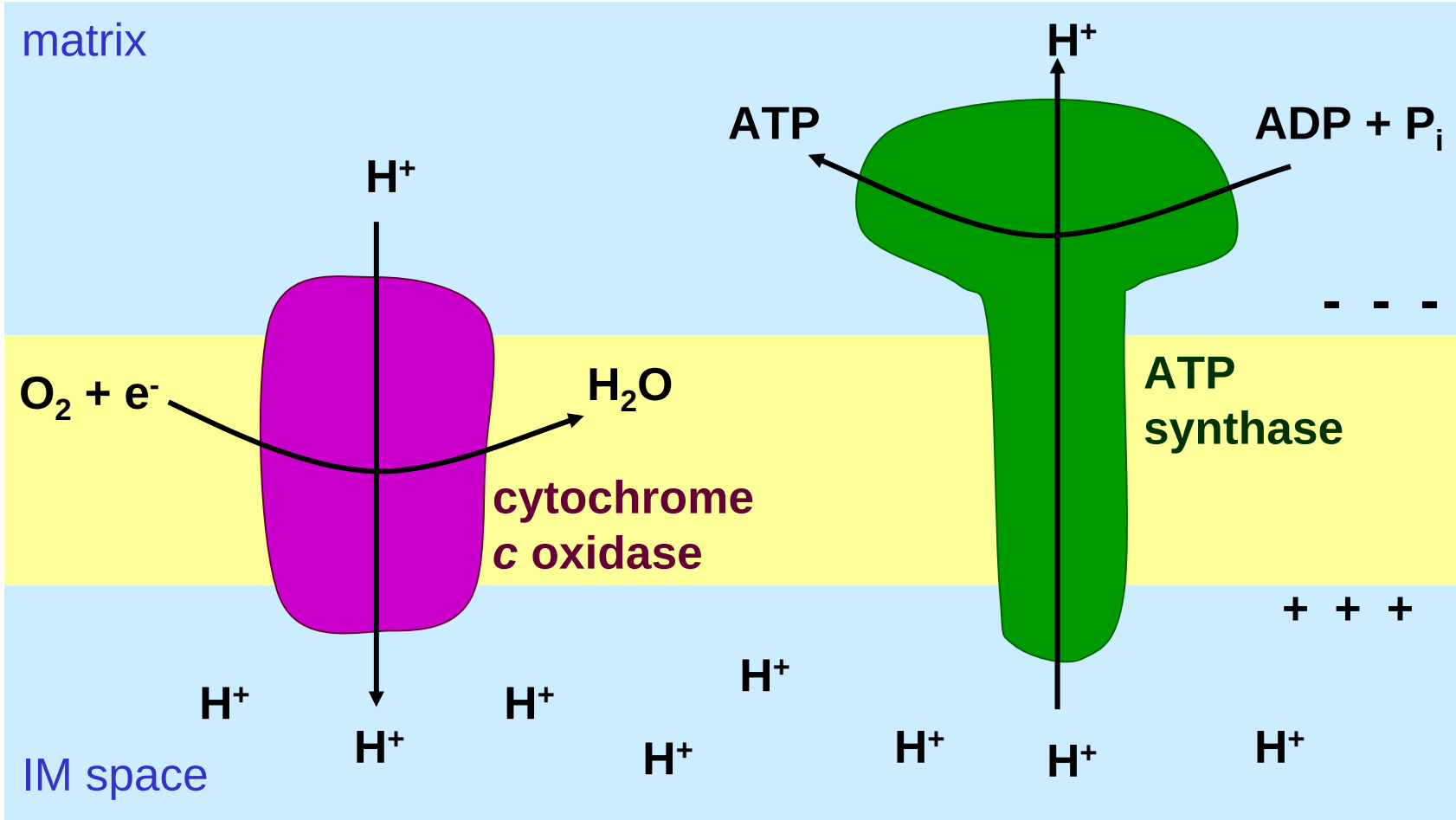


– Aquaporins → cataract, diabetes, …

– But there's no life without proton pumping!

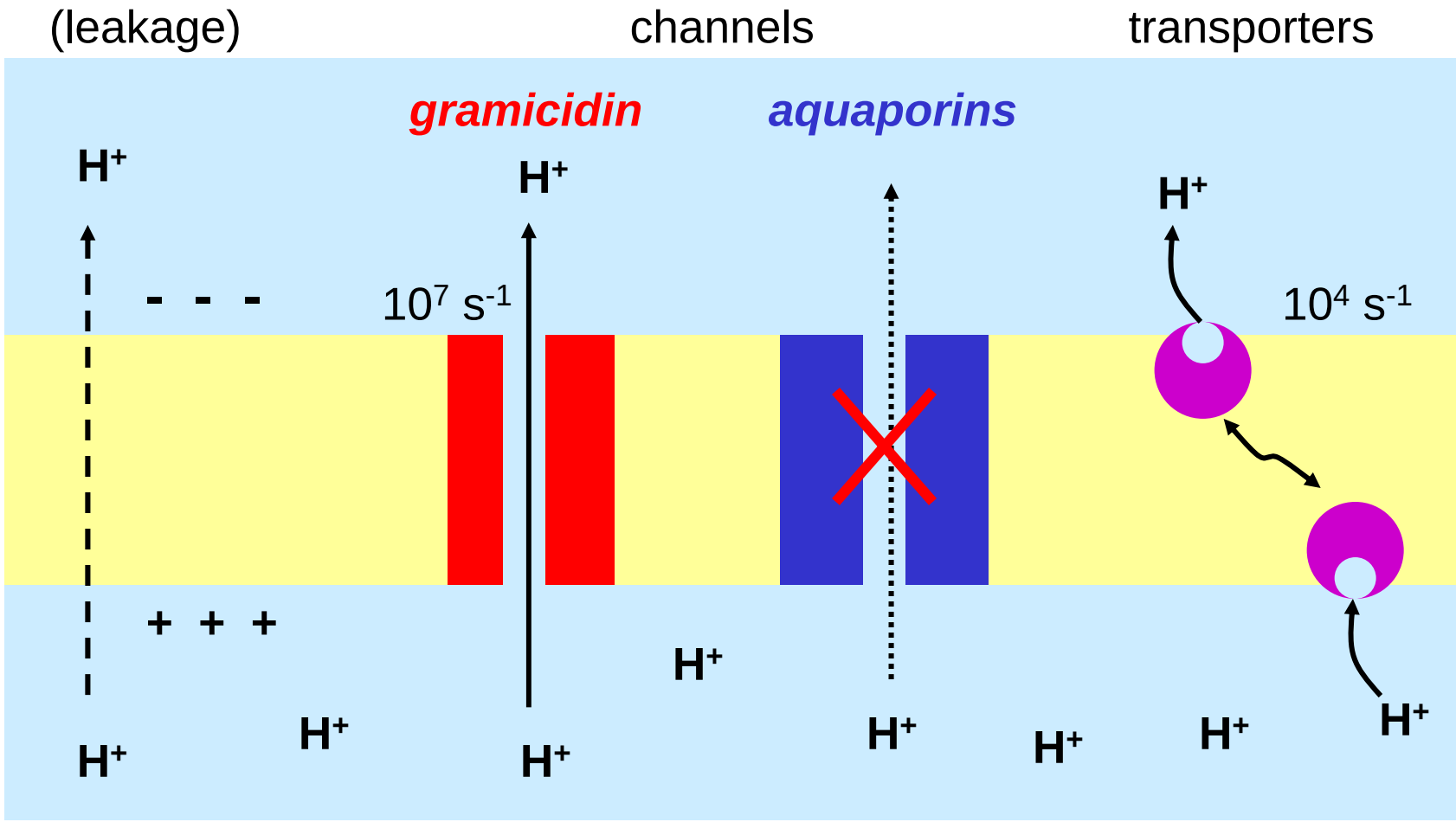
H^+ transport across biomembranes

Chemiosmotic coupling

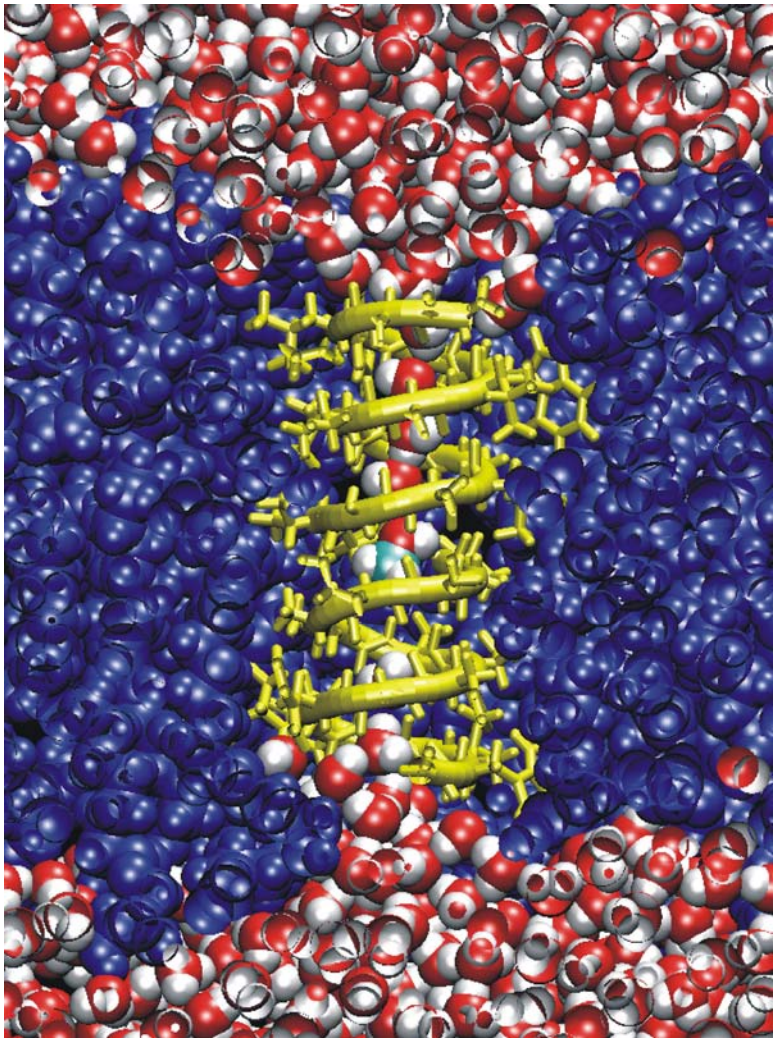


H^+ pumping is required for ATP synthesis

H^+ transport across biomembranes



Passive H^+ transport destroys proton-motive force



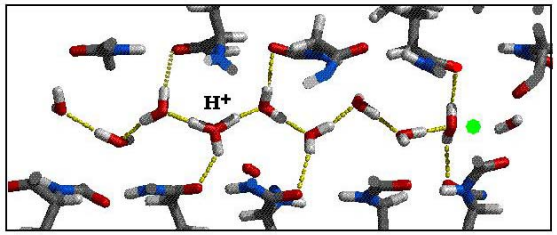
Physical basis of permeation

Channels = narrow, water-filled pores

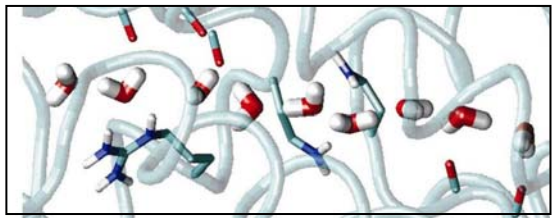
Selective to the passage of certain ions and/or small molecules

Allow ions to cross the dielectric barrier of the membrane

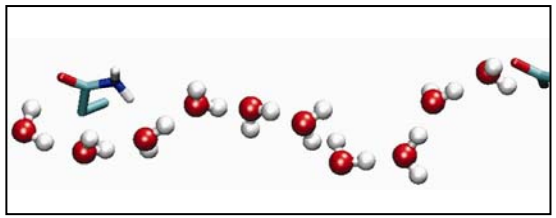
Proton transport and blockage



1. Relay of H^+ translocation in gramicidin



2. Exclusion of protons from aquaporins



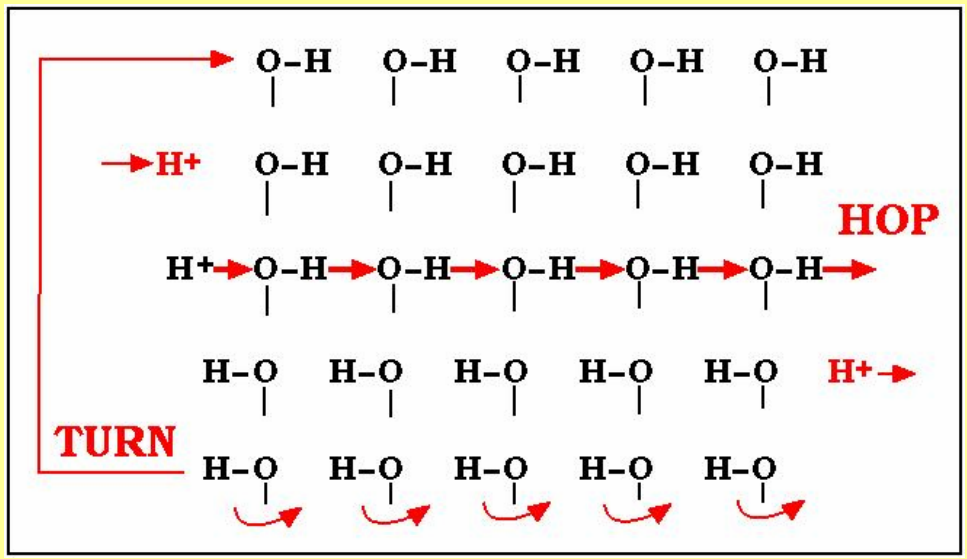
3. Uptake of protons in cytohrome *c* oxidase

Biological Proton Translocation

Opposed by the dielectric barrier

H^+ reactivity → special transport properties

The Grotthuss mechanism



Annales de Chimie 58 : 54 - 74 (1806)

MÉMOIRE

Sur la décomposition de l'eau et des corps qu'elle tient en dissolution à l'aide de l'électricité galvanique,

PAR C. J. T. DE GROTTHUSS (1).

CHAPITRE PREMIER.

Action de l'électricité galvanique sur certains corps dissous dans l'eau.

§. PREMIER.

SANS m'arrêter à la discussion d'une foule d'hypothèses imaginées pour expliquer la décomposition de l'eau par l'appareil électromoteur, j'exposerai une théorie générale de la décomposition des liquides par l'électricité galvanique, qui me paroît réduire les effets de celle-ci à une explication simple

(1) Ce Mémoire a été imprimé à Rome en 1805. Nous avons pensé qu'on le trouveroit ici avec plaisir, et l'auteur lui-même a désiré qu'on le réimprimât.

et satisfaisante. J'ai été conduit à cette théorie par les observations suivantes.

§. II.

Lorsqu'on fait passer à travers une dissolution métallique saturée, un courant d'électricité galvanique, dont l'intensité se trouve proportionnée à l'intervalle occupé par le liquide, et compris entre les extrémités des deux fils conducteurs, on observe des phénomènes intéressans, même pour celui qui ne se mêle pas d'en approfondir la cause. A l'extrémité du fil en contact avec le disque de zinc, l'oxigène se dégage, pendant qu'à l'extrémité du fil en contact avec le disque de cuivre, les molécules du métal en dissolution se trouvent revivifiées, en prenant un arrangement symmétrique qui s'étend dans la direction du courant galvanique.

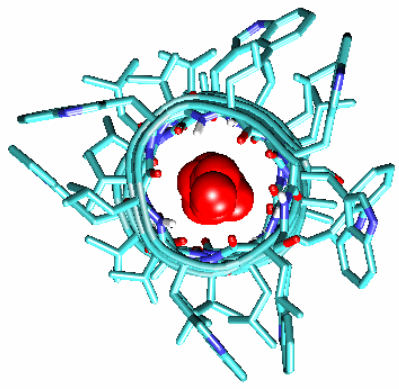
§. III.

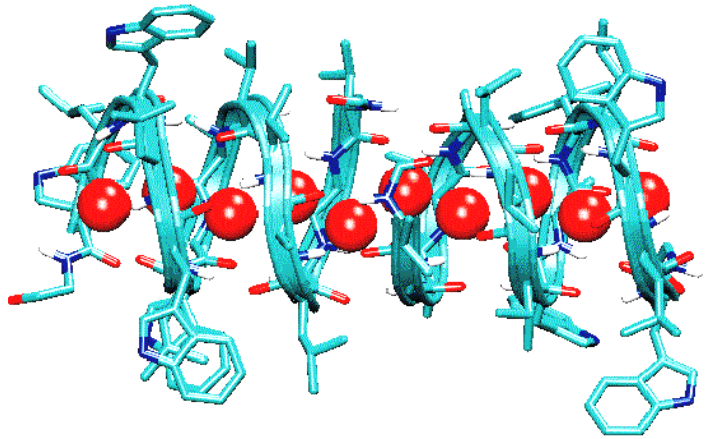
Cet arrangement n'est qu'une cristallisation imparfaite des molécules métalliques, parfaitement semblable à celle qu'on connoît sous le nom d'*arborisation*, et qui a lieu en précipitant les métaux en dissolution par d'autres métaux. Les anciens ajou-

Theodor Grotthuss

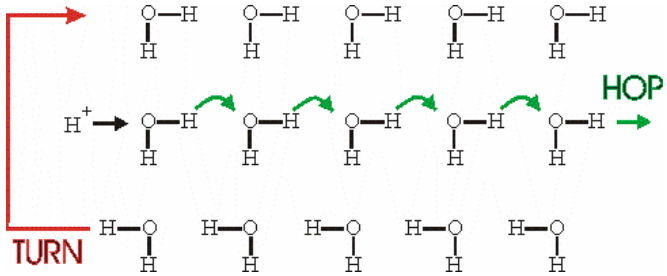
(1785-1822)

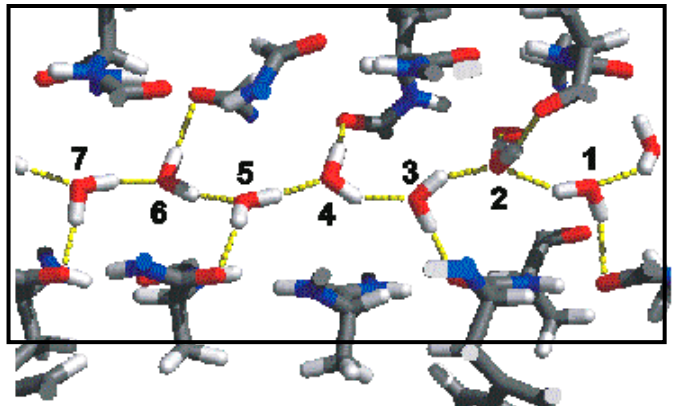
The Grotthuss mechanism owes its name to a paper published in 1806





1. Proton relay in the gramicidin channel

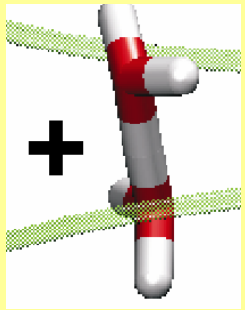


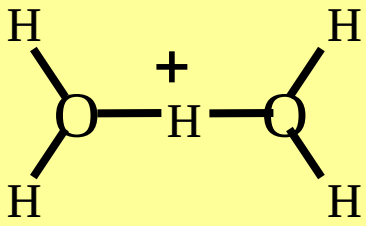


Pomès & Roux, *Biophys. J.* 1996, 2002

Proton solvation and hydrogen bonds

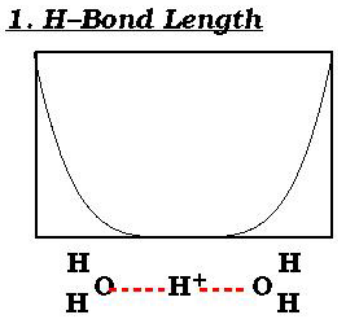
- H^+ is very **reactive** ➔ it doesn't exist by itself in biological systems
- The hydrated proton exists primarily in two forms:

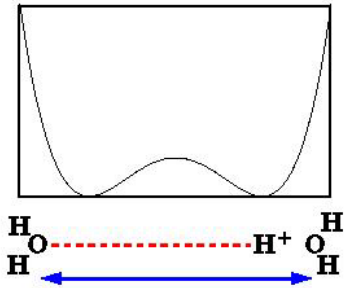


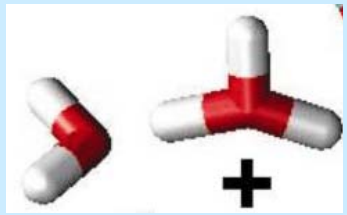


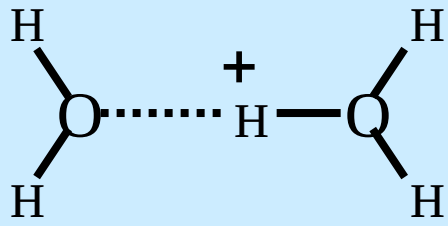
0.24 nm

Zundel ion









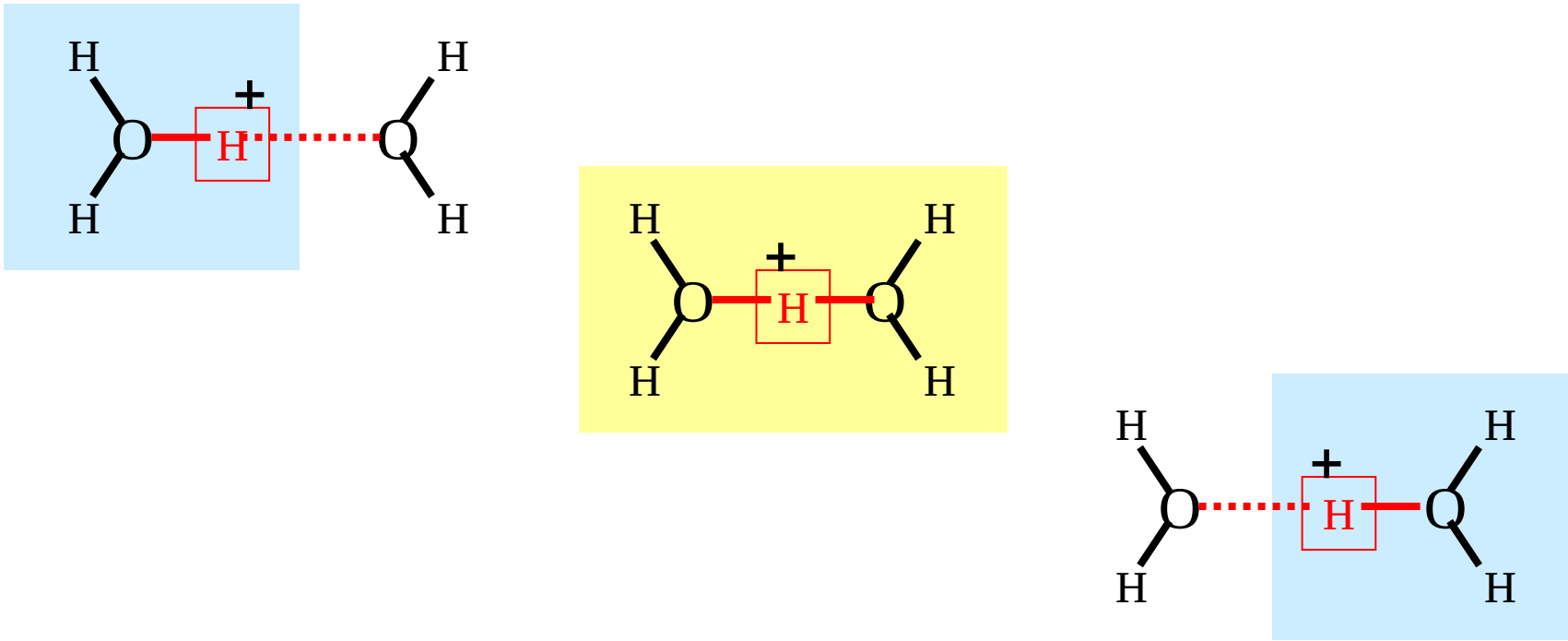
0.28 nm

hydronium

- These two species differ in the length of the hydrogen bonds

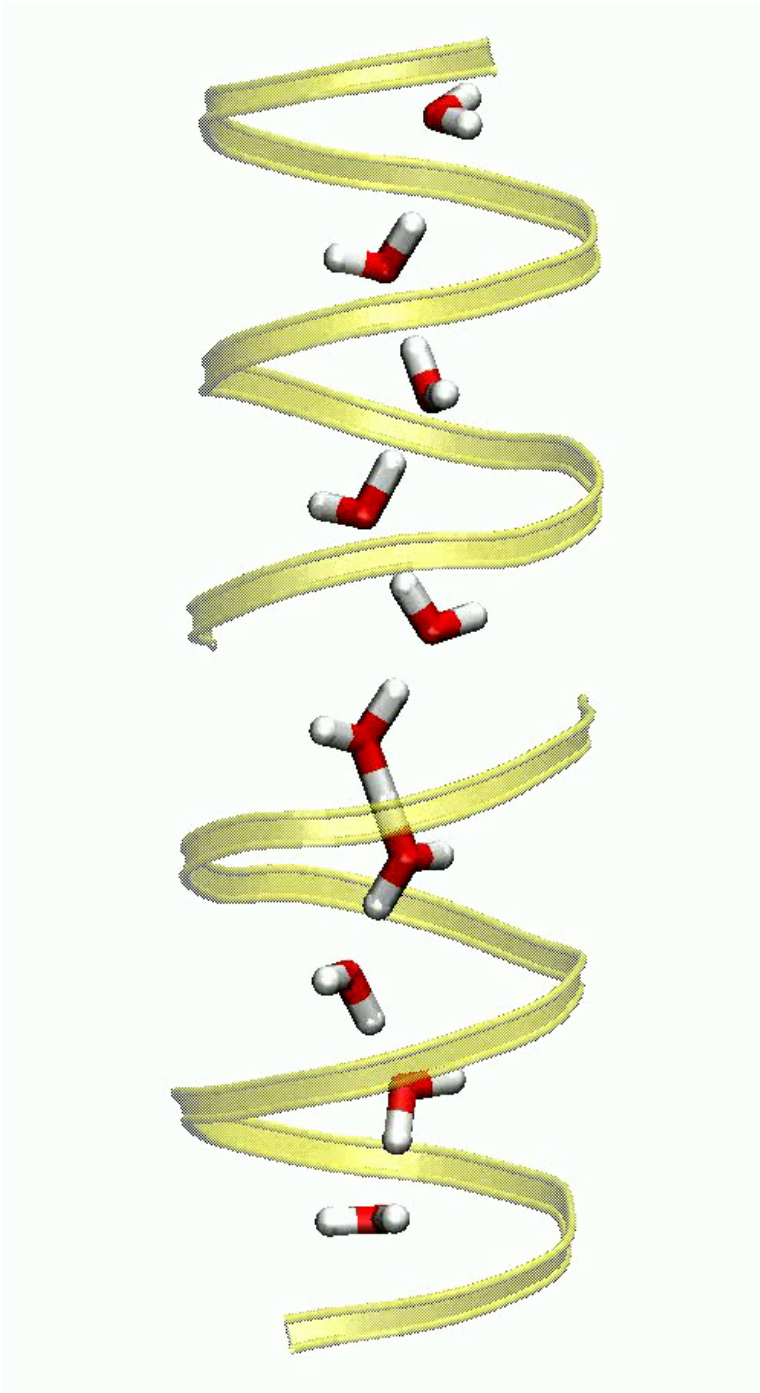
The Grotthuss mechanism: proton exchange

- The exchange between the two forms of hydrated H^+ drives transport:

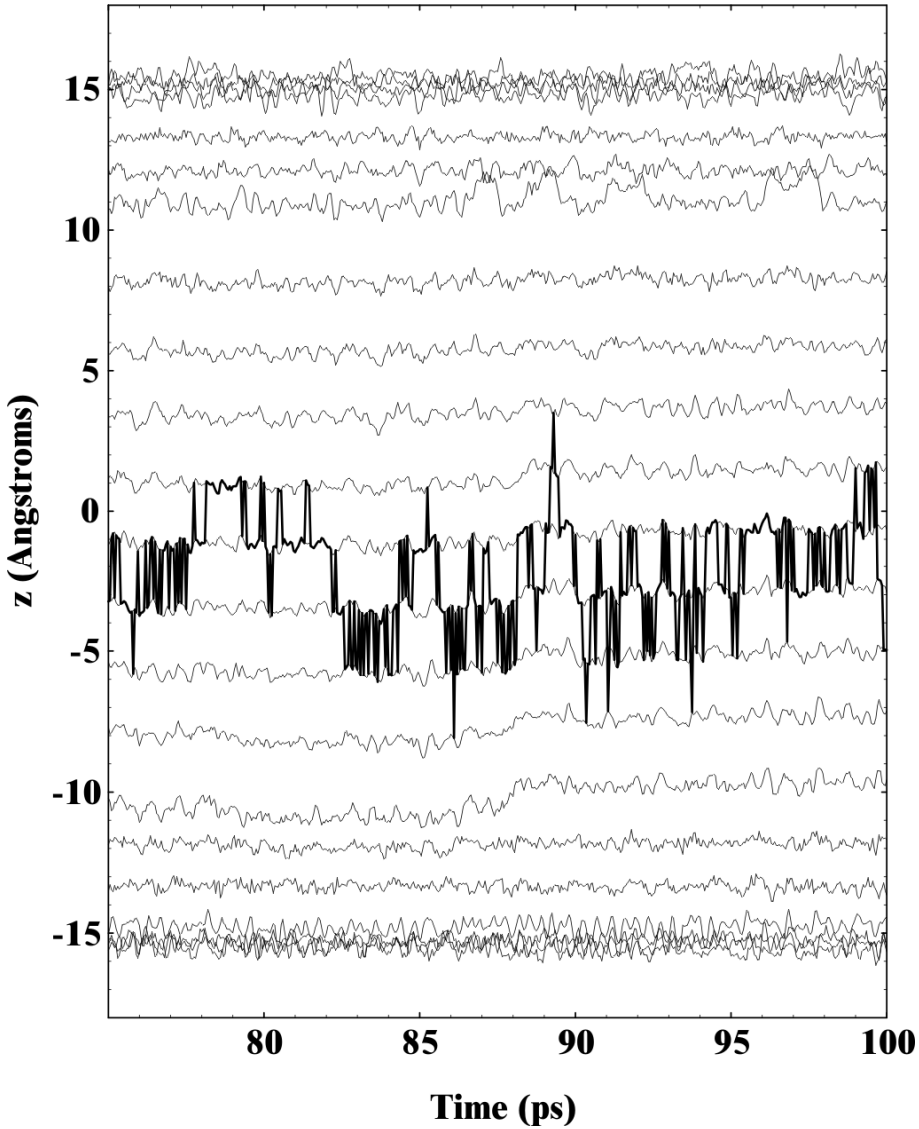


- This relay process hinges on fluctuations of small amplitude (~1 Angstrom)
  - on ps timescale

  → translocation of H^+ across large distances (10's of Angstroms)
  - in 10^{-9} second or even faster.



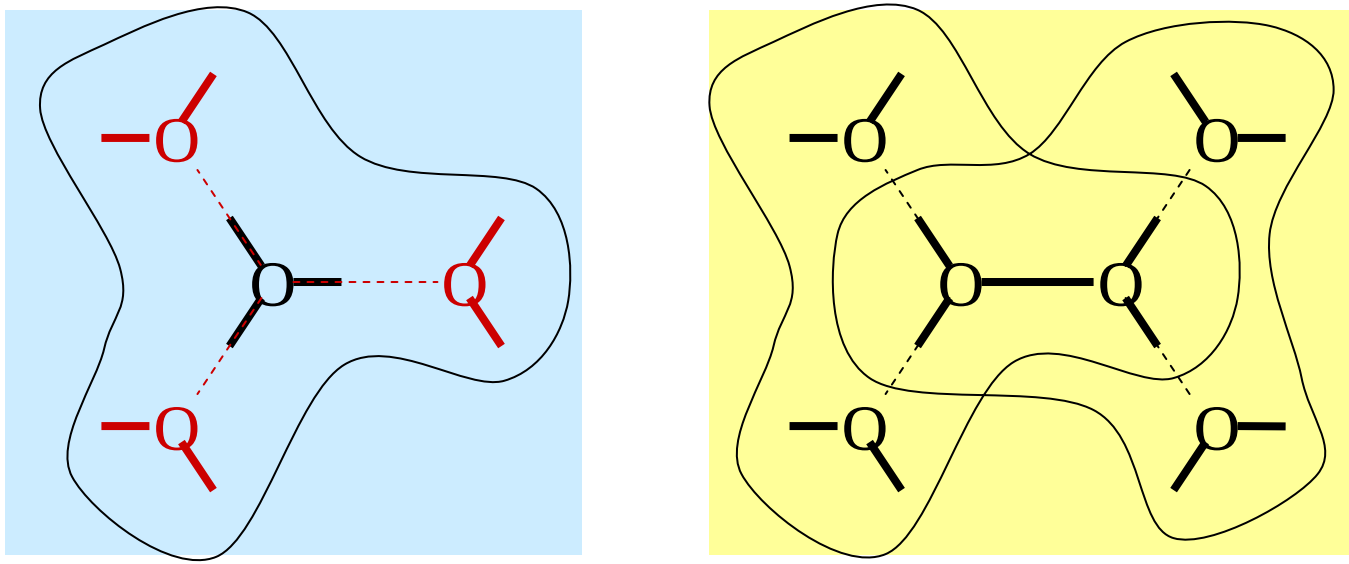
The exchange between OH_3^+ and $O_2H_5^+$ is the elementary step of proton relay



What is the role of the channel in the mechanism?

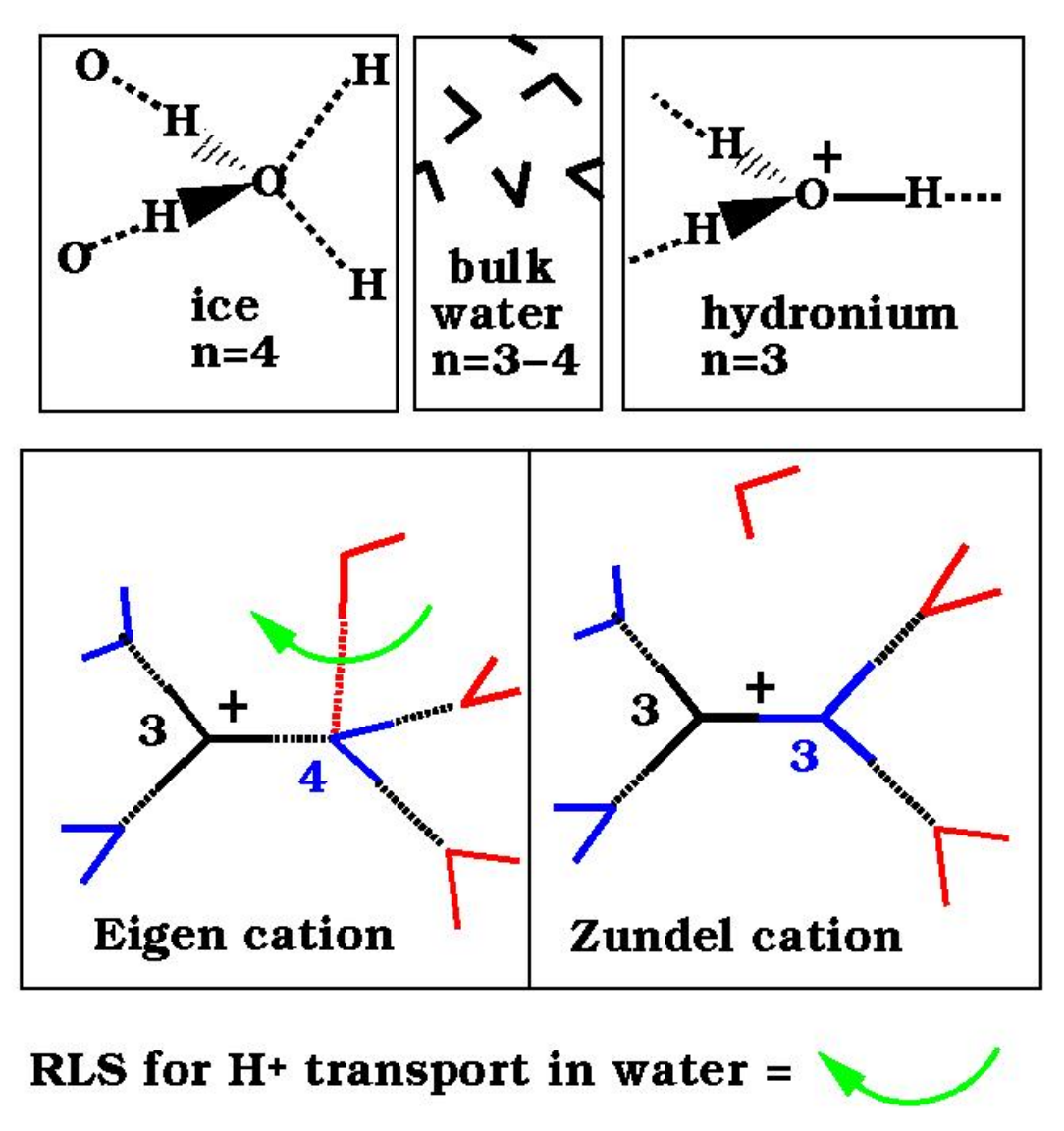
Proton solvation and hydrogen-bonded networks

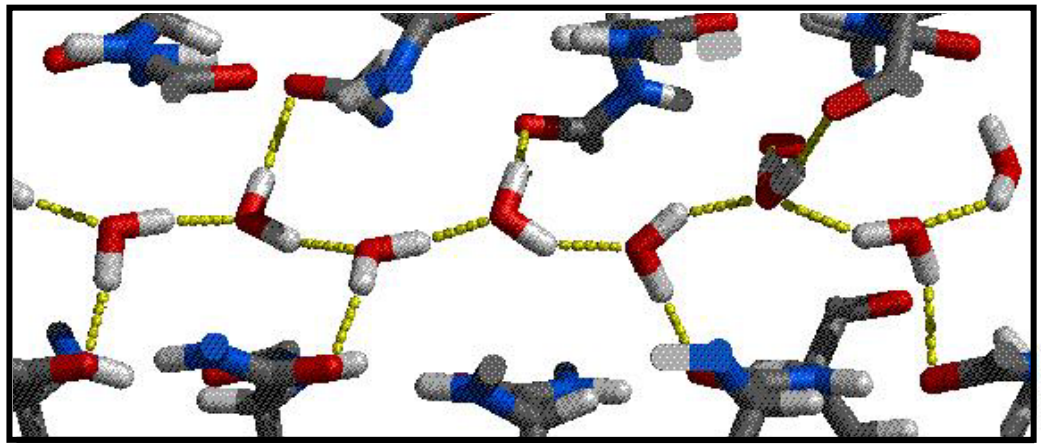
- Both forms of the hydrated proton are stabilised by **hydrogen-bonding donation to 3 neighbors**



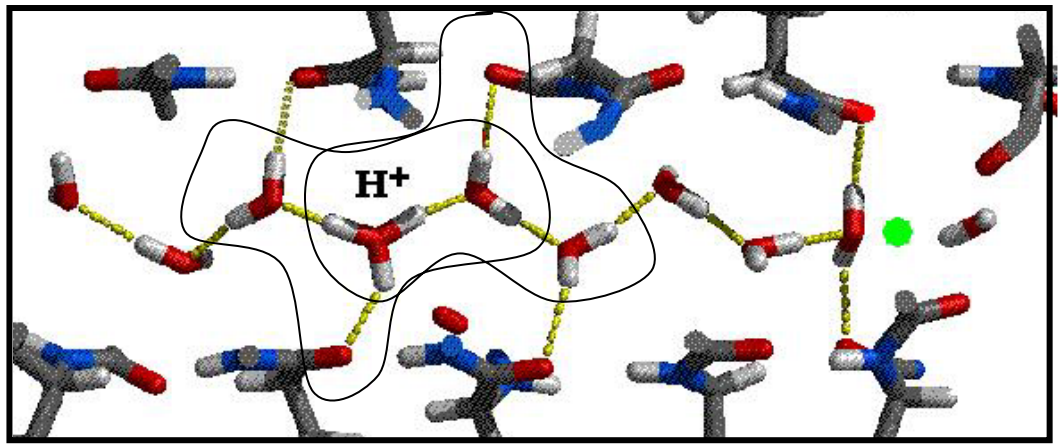
- In water, hydrogen bonds are constantly formed and broken
- In gramicidin, the channel backbone provides ideal coordination

Mechanism of proton transport in water



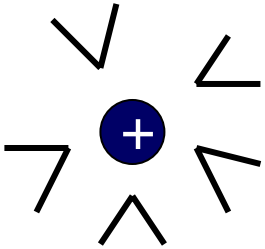


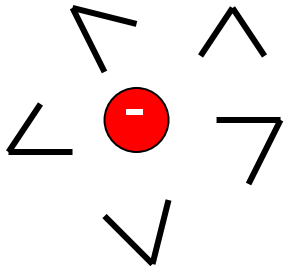
Gramicidin offers a local environment well suited to rapid proton transport (solvation and mobility)

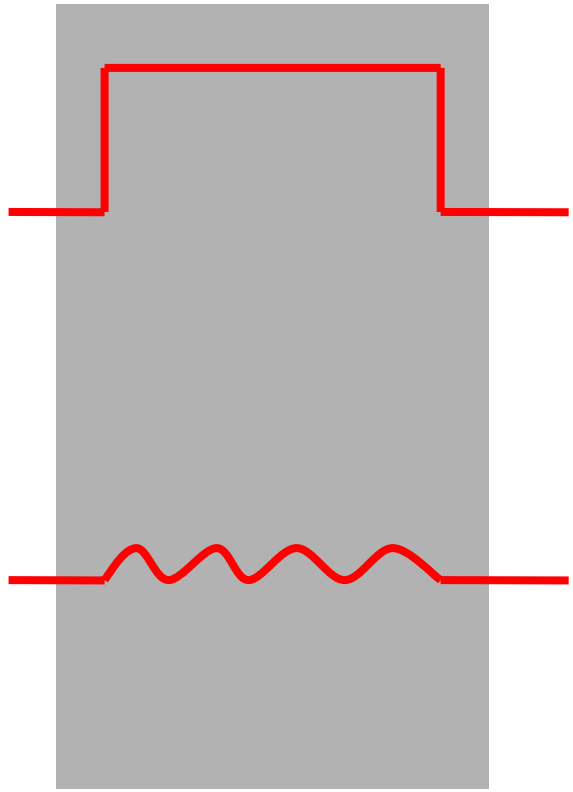


Role of membrane channels in ionic solvation and mobility

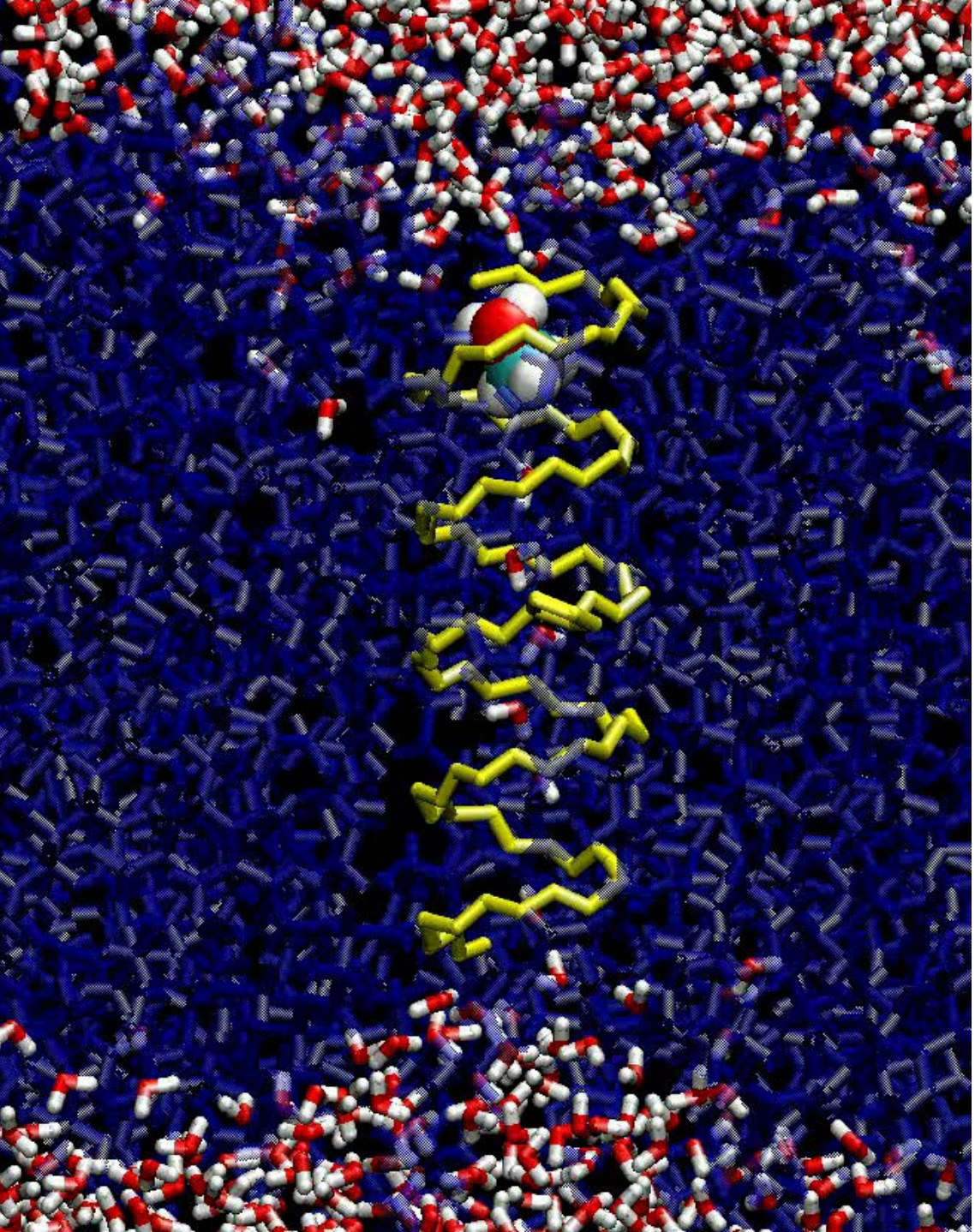
- "ideal" channel:
  - Chemical potential ~ that in water







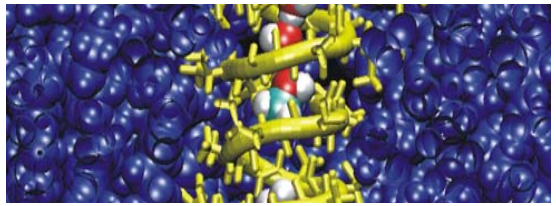
  - Low barriers
- Multiple "binding sites"

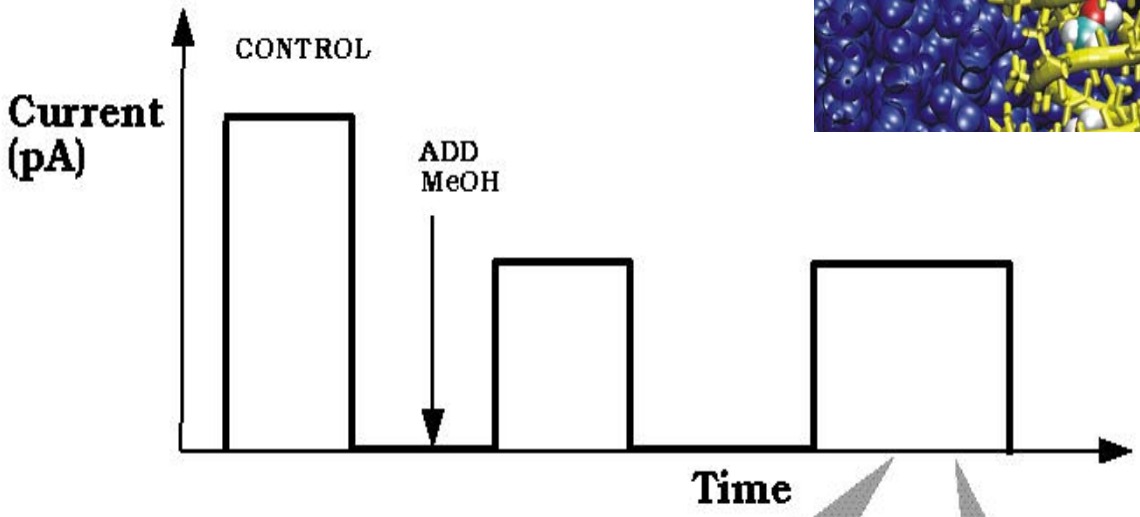


Origin of the attenuation of H^+ conductance by methanol?

Diffusion of methanol in gramicidin

Attenuation of proton permeation by methanol

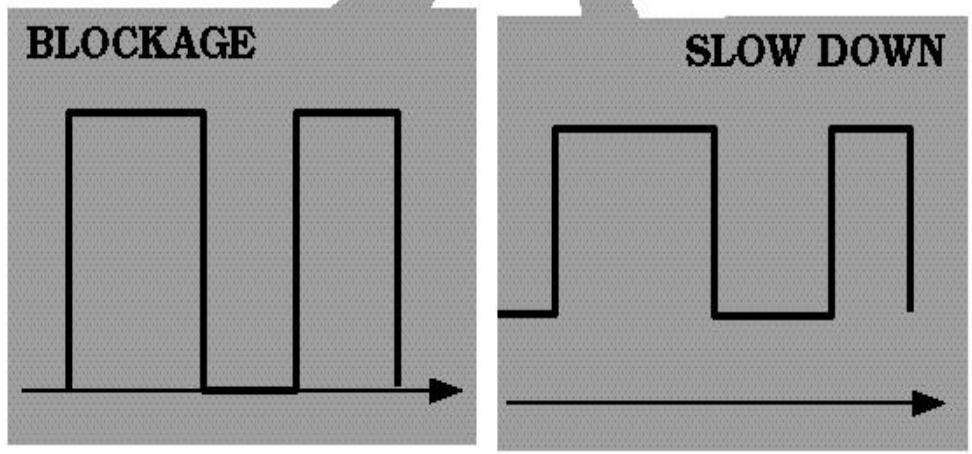




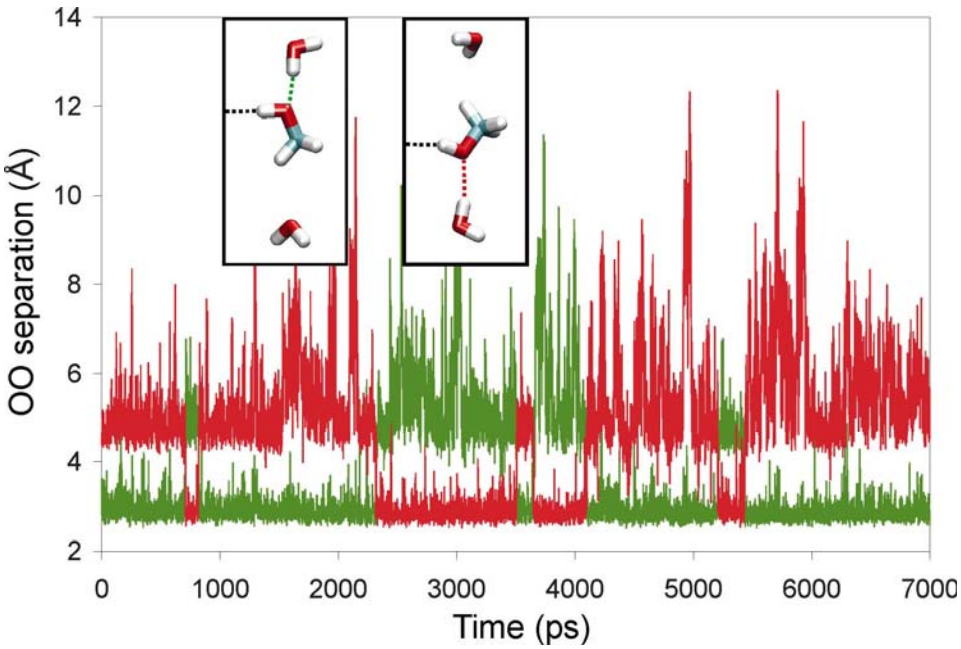
Not observed w/
other ROH

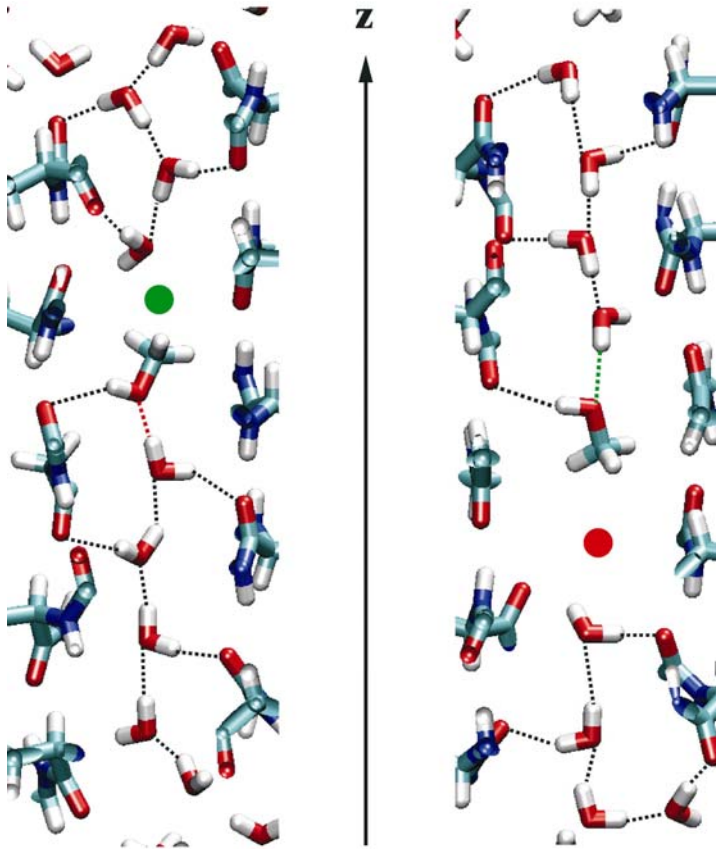
Hypothesis :
MeOH gets in
the single file

Then what?

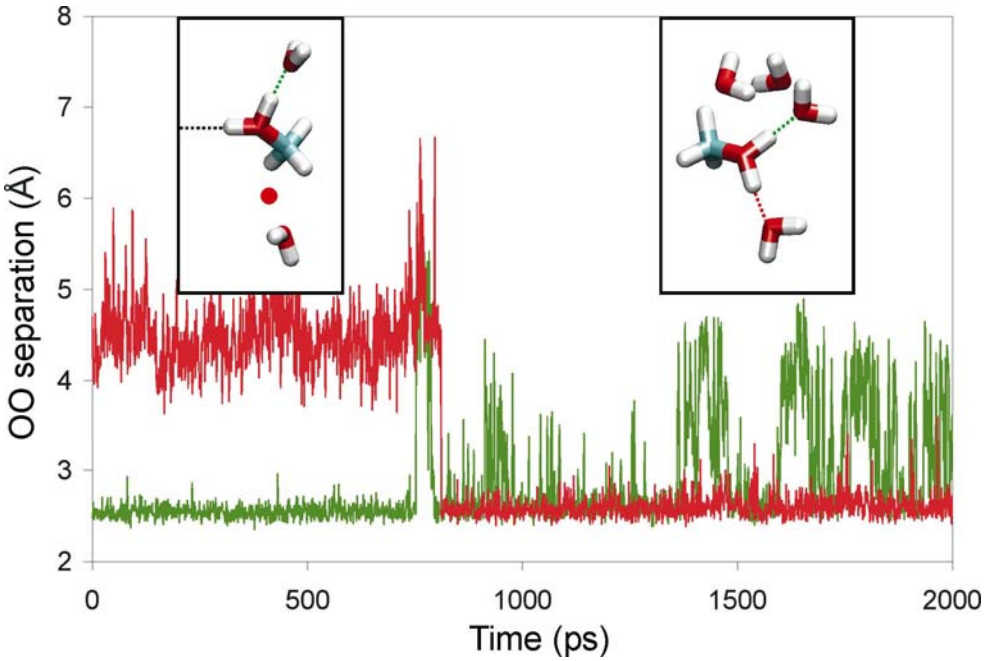


- Methanol fits in the pore of gramicidin
- It blocks proton relay because it **never forms a continuous chain**





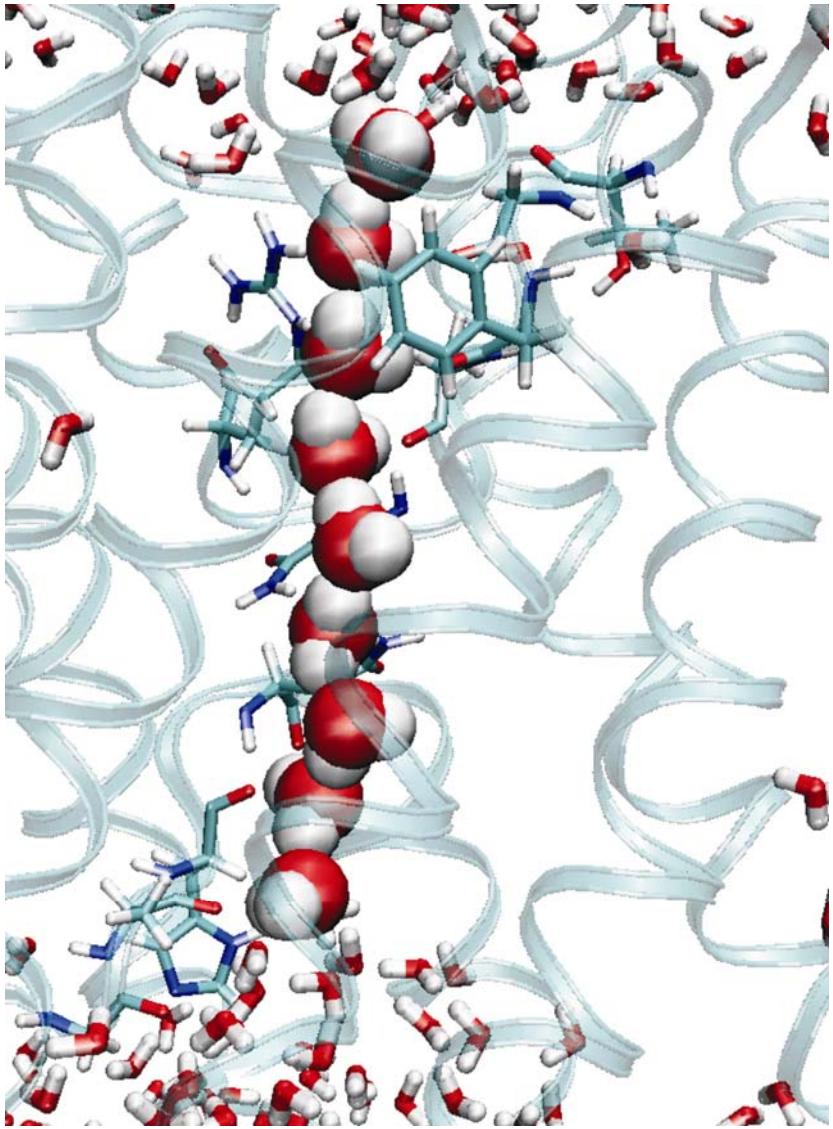
$MeOH_2^+$ does NOT form a continuous chain
+ does NOT tumble when inside the channel



<u>Conclusion</u>: blockage of H^+ relay when MeOH is in

Pomès & Yu, *Front. Biosc.* 2003

2. Proton exclusion from aquaporins



Water-selective channels

10^9 H_2O s^{-1}

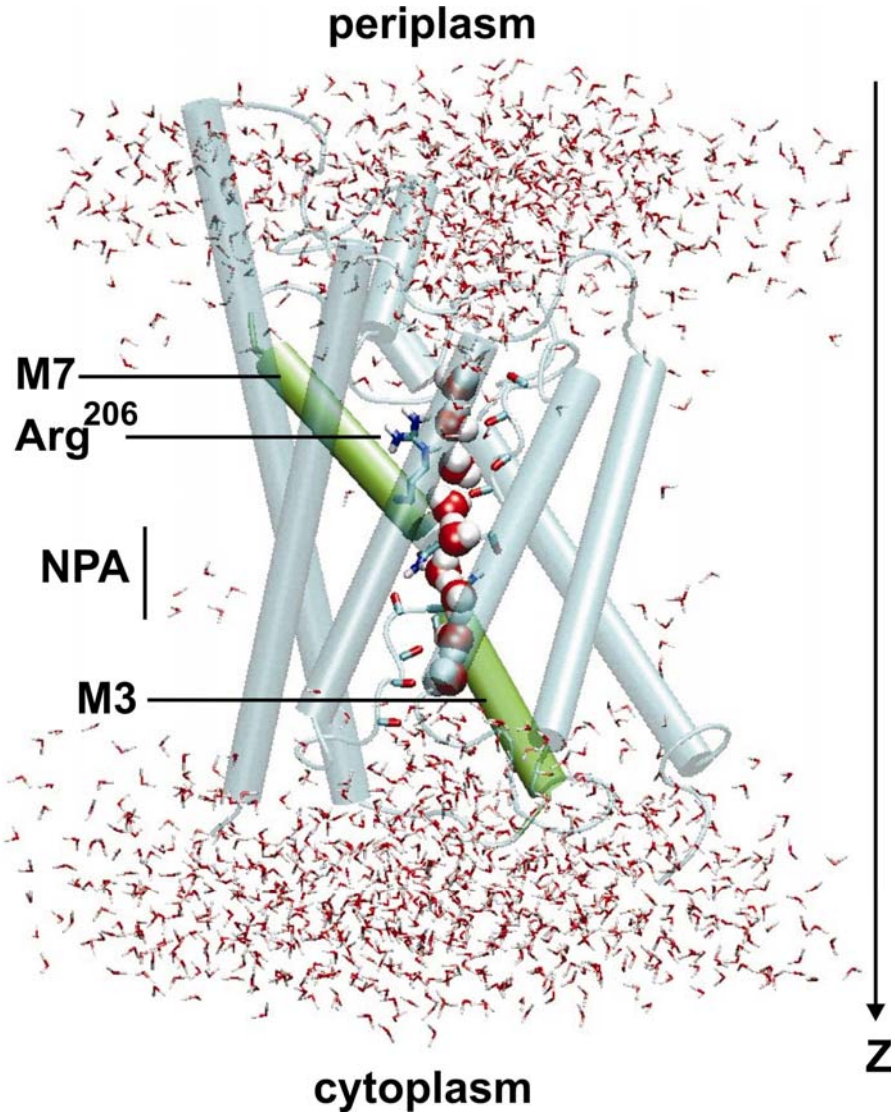
Water diffusion is coupled
to their reorientation

(DeGroot & Grubmuller, *Science* 2001)
(Tajkhorshid et al., *Science* 2002)

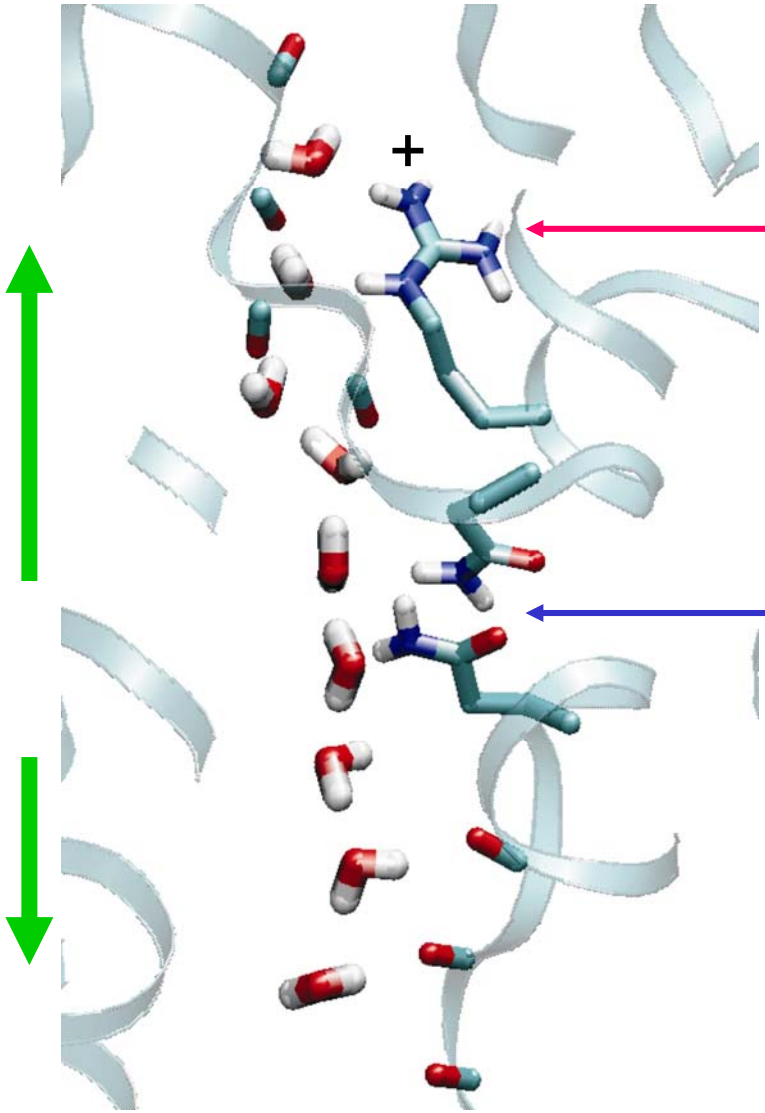
Exclude ions

Hydrogen-bonded water chain

Physical basis
of proton blockage?


periplasm
M7
Arg206
NPA
M3
Z
cytoplasm

Proton exclusion from water channels

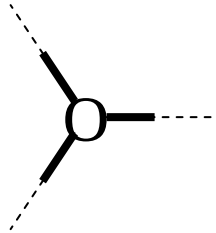


Some observations:

Arg206 could prevent approach of +
← charge-charge repulsion

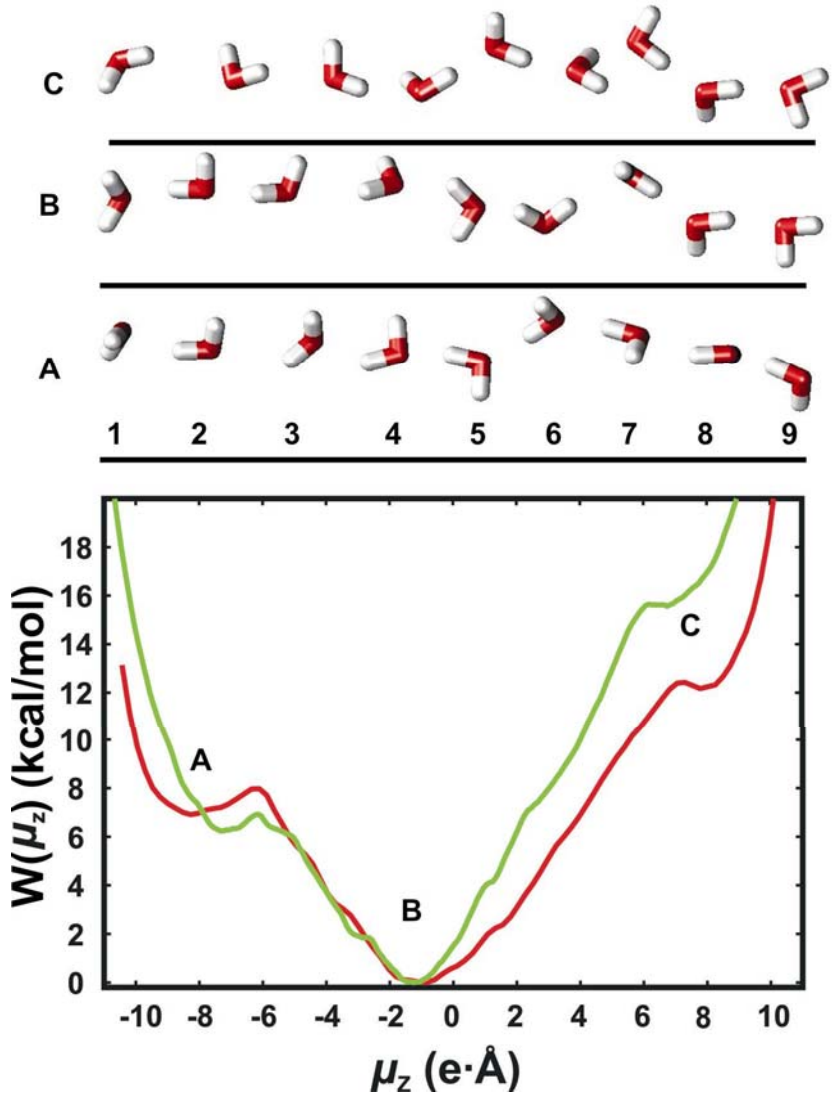
Adverse polarisation of water molecules incompatible with intrusion of proton

Local interactions with N68, N203 incompatible with solvation of hydrated proton

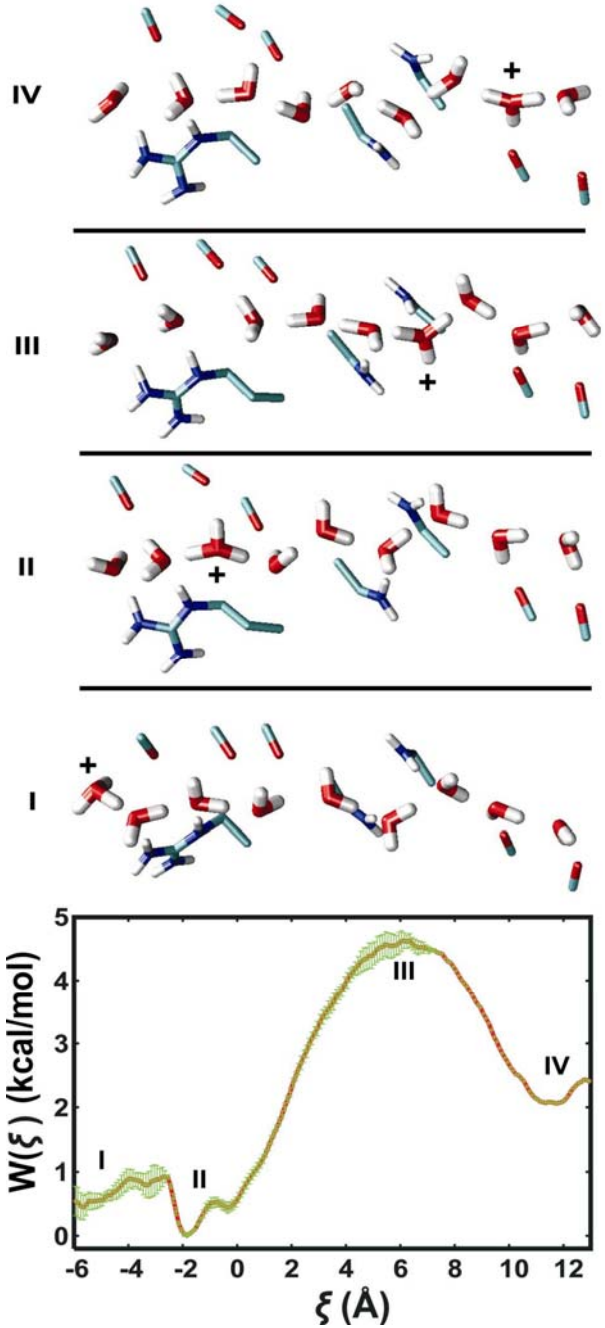


Free energy profile for water reorientation

Strong preference
for bipolar organization
opposes the turn step
of structural diffusion

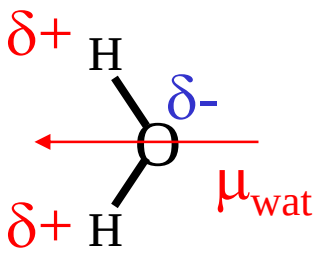


Free energy profile for H^+ transfer

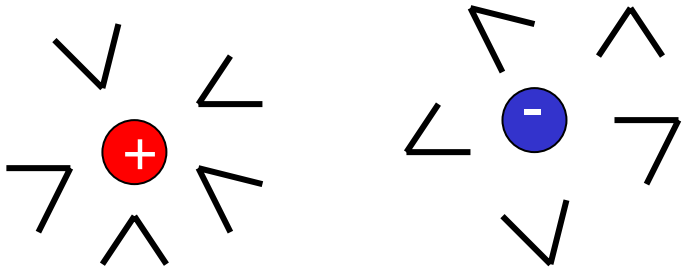


Ionic solvation: charge-dipole interactions

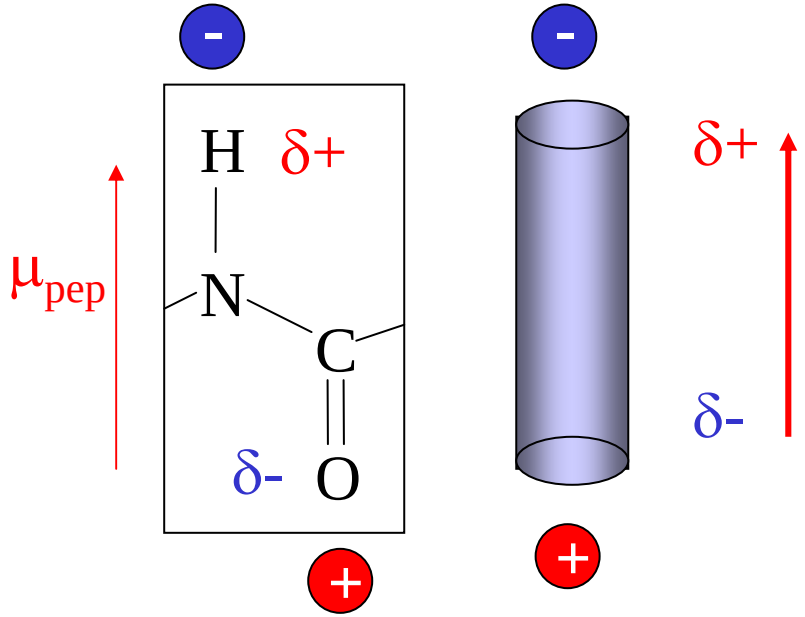
• O is electronegative, H is electropositive.



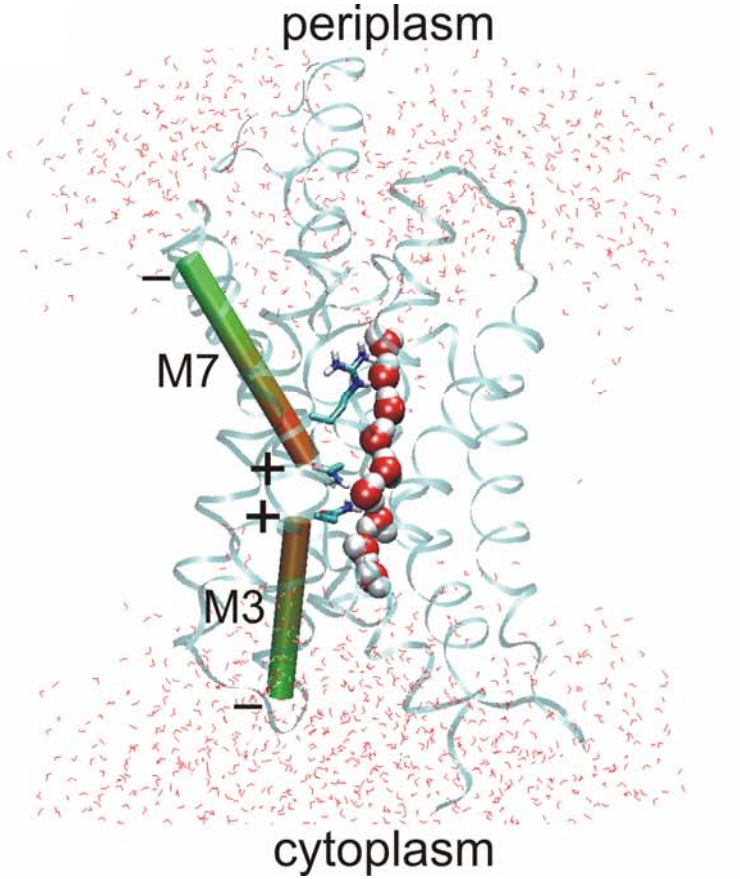
• The dipole moment of water molecules (charge separation) stabilises ions:

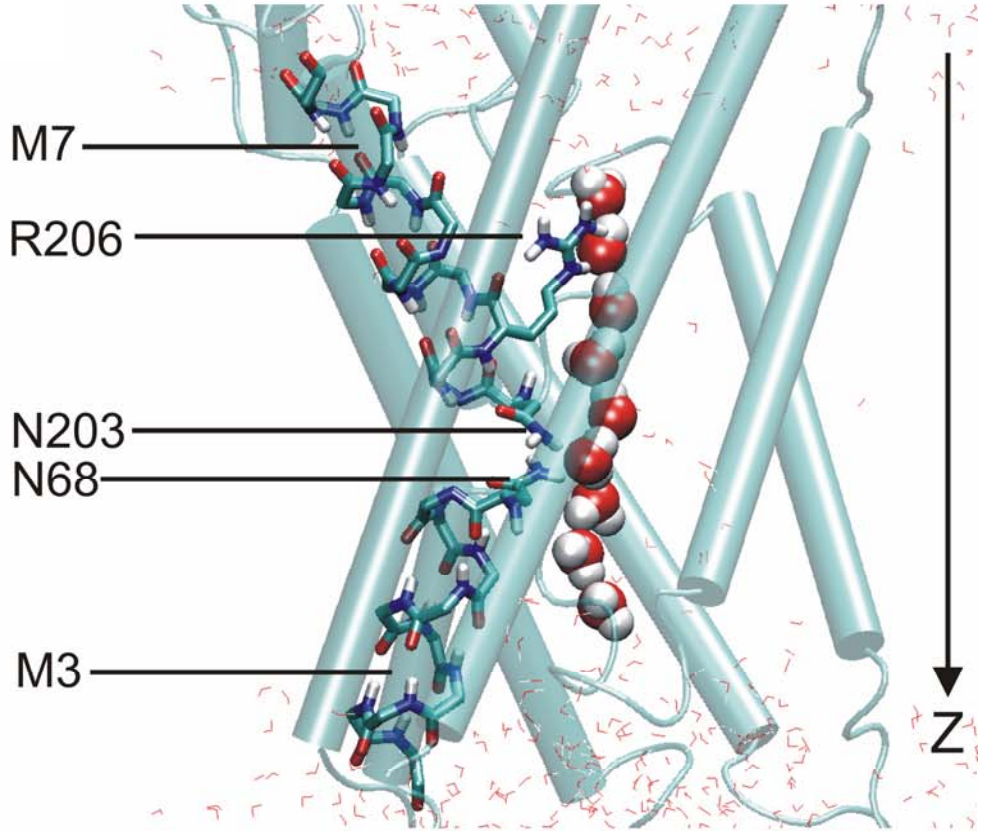


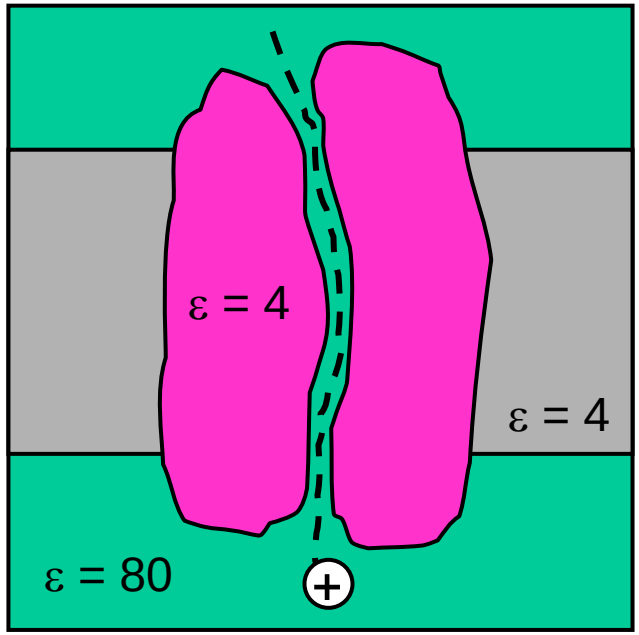
- Peptide bonds also have a dipole
- The dipole moment of peptide bonds and α helices stabilises ions

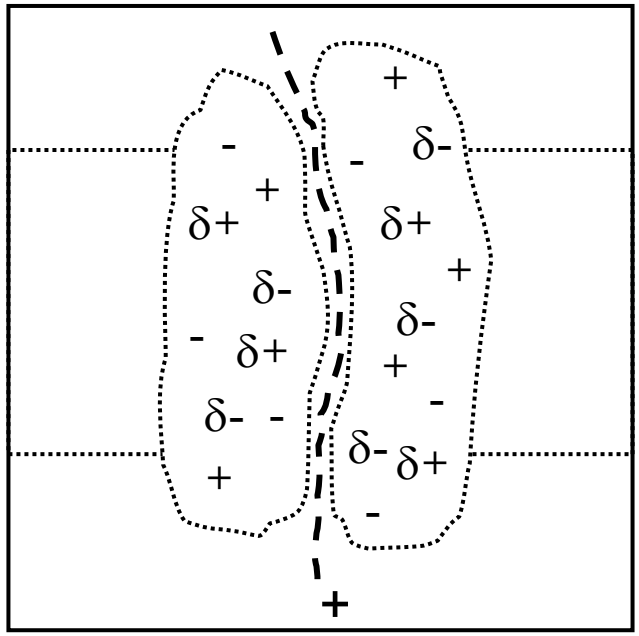


Adverse charge-dipole interactions give rise to proton exclusion







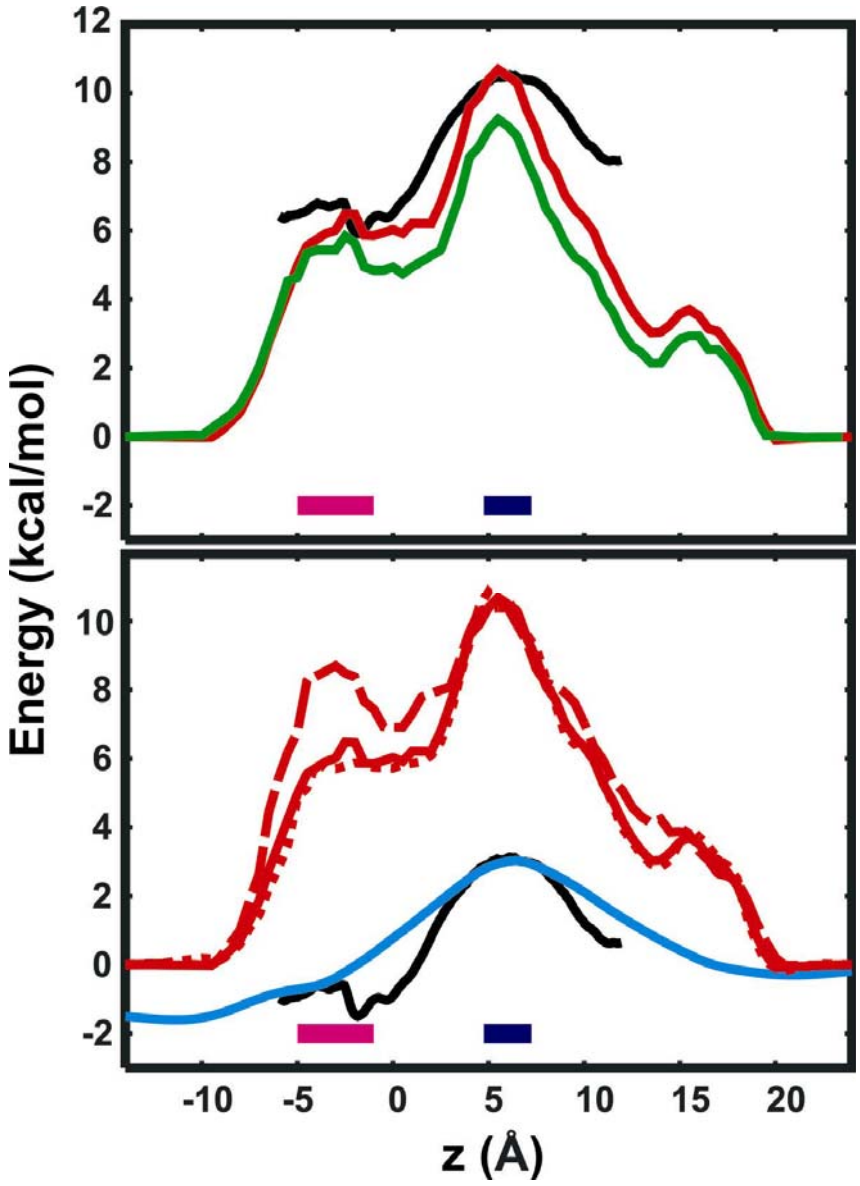


Continuum electrostatic calculations

Total electrostatic energy =

reaction field
← dielectric boundaries

static field
← charge distribution



Electrostatic origin
of the free energy barrier
opposing proton translocation

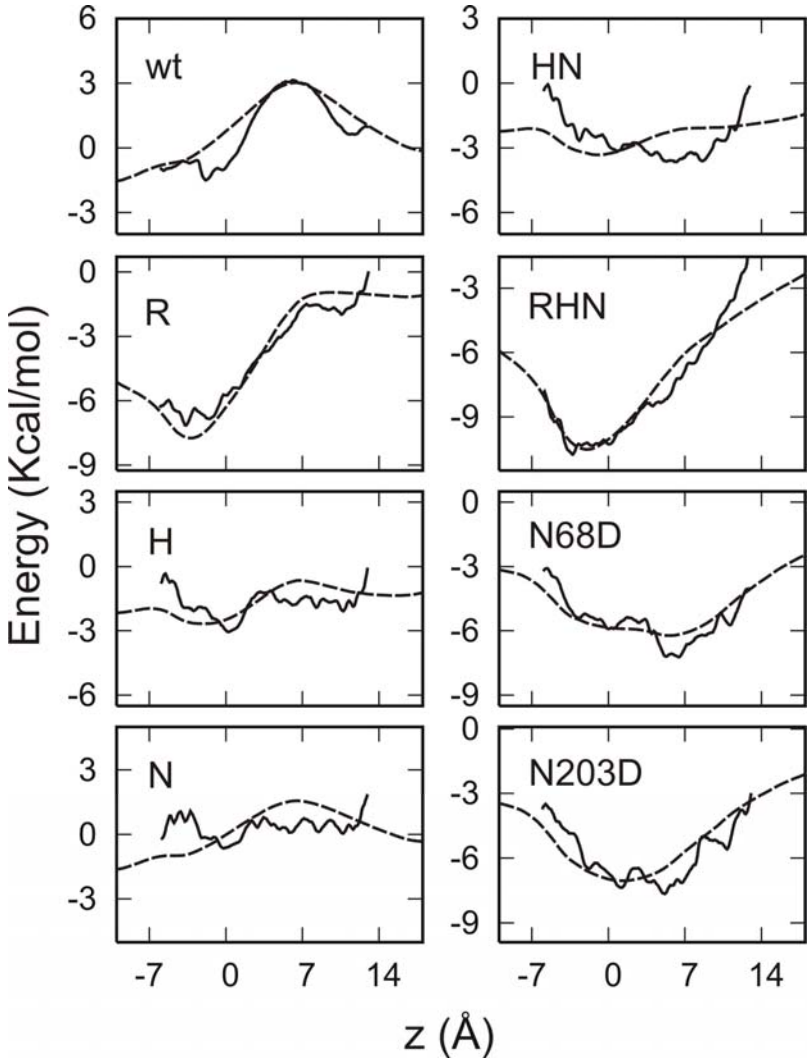
Hop PMF
Full ESP = charge + dielectric
No membrane dielectric

Size selectivity at R206

Static field = effect of charge distribution of the channel

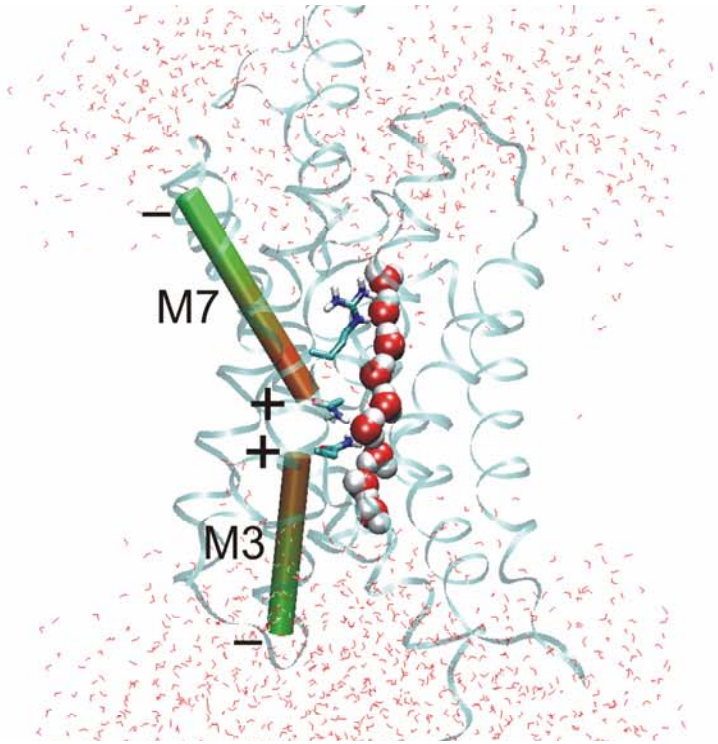
Chakrabarti et al., *Structure* 2004

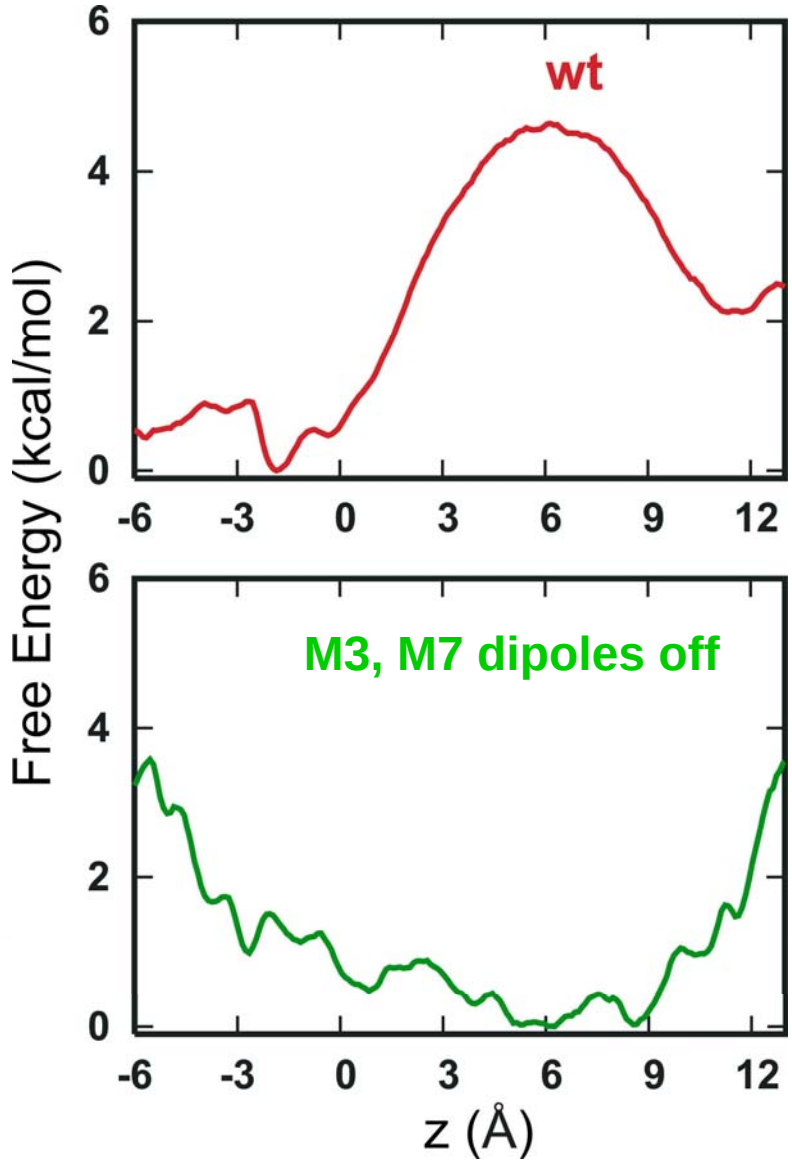
PMF vs PB in aquaporin channel variants



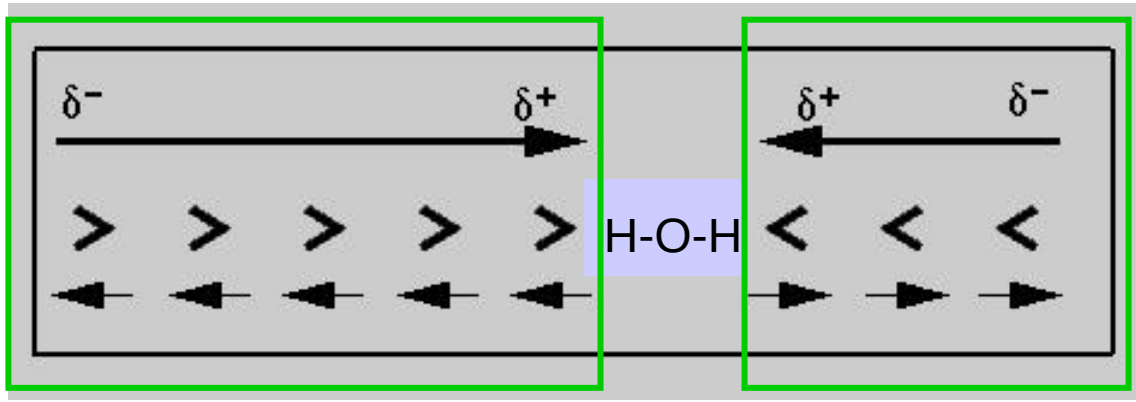
H^+ PMF is essentially determined by the distribution of charged and polar groups

Structural determinants of H^+ blockage: charge-dipole interactions

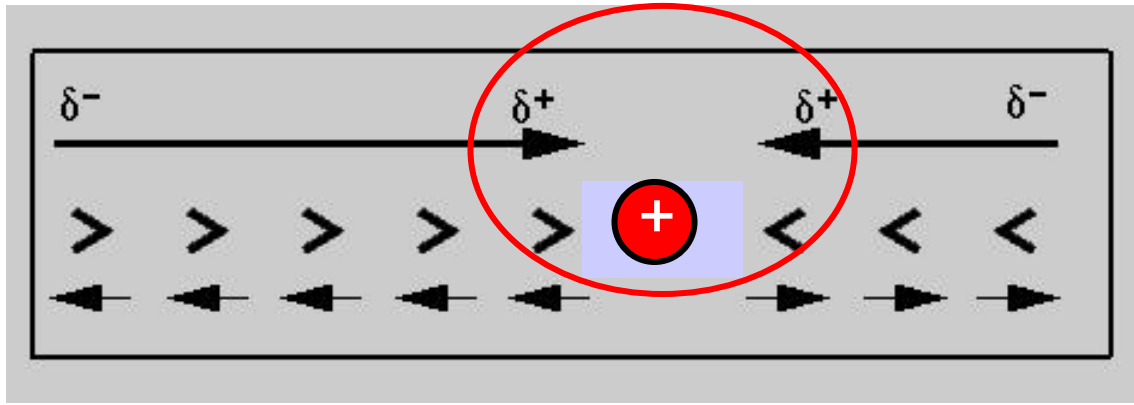




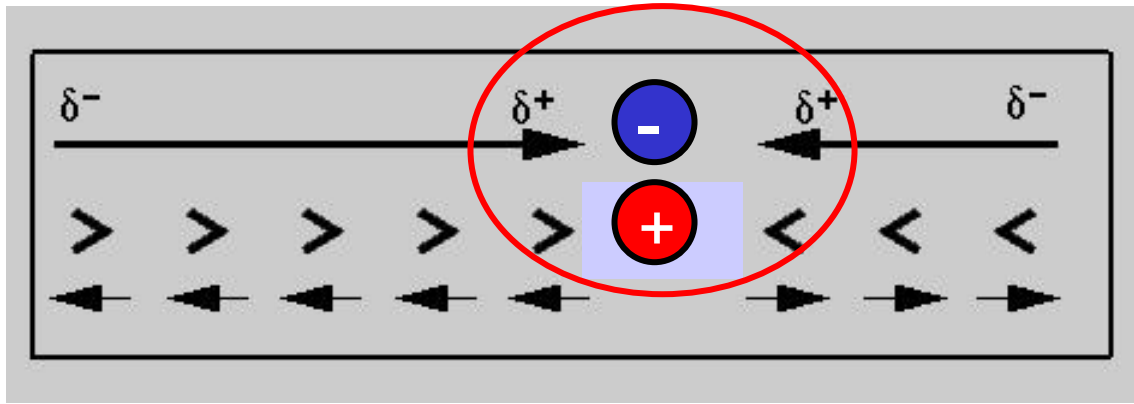
Mechanism of proton exclusion in aquaporins



Favored



Forbidden

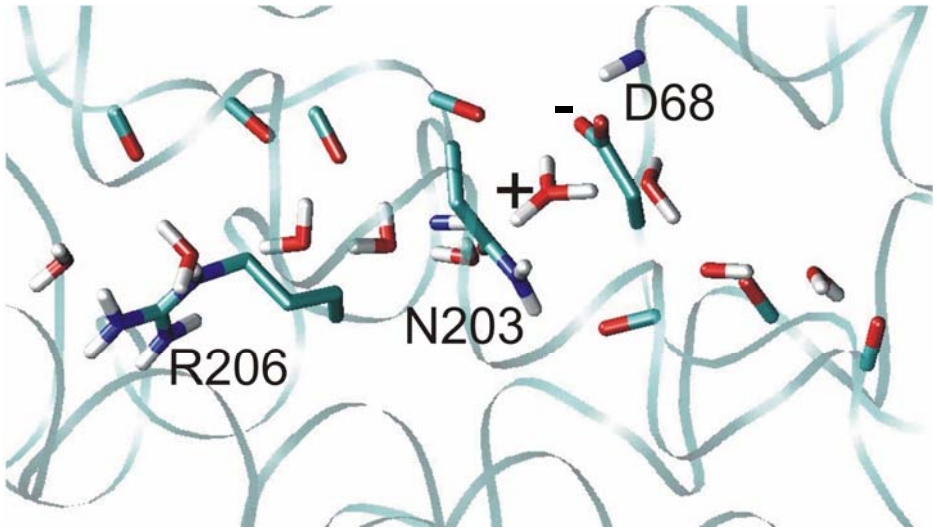


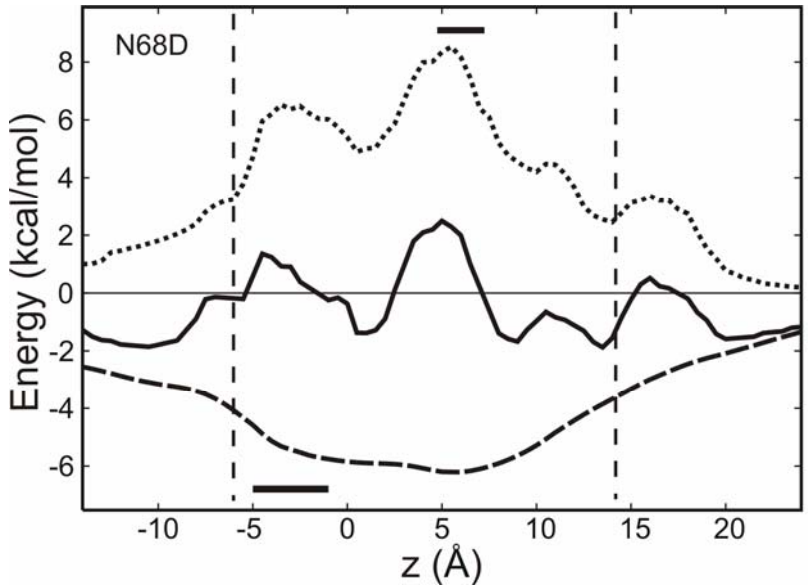
??

Compromising proton impermeability of aquaporins?

Single point mutation introducing a negative charge at the NPA motif:

Asn68 → Asp



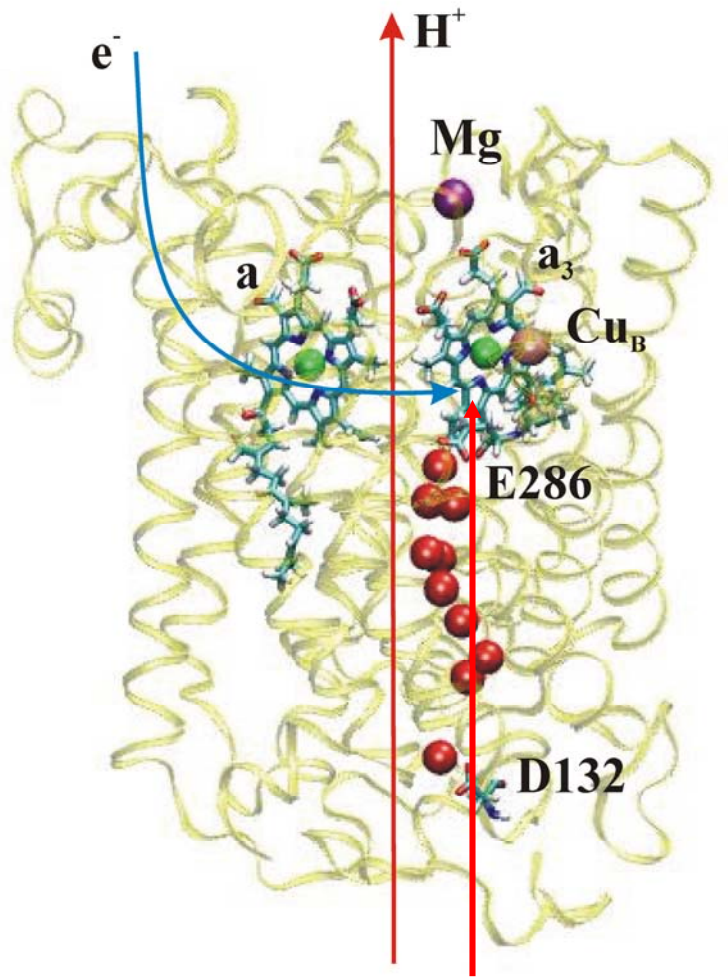


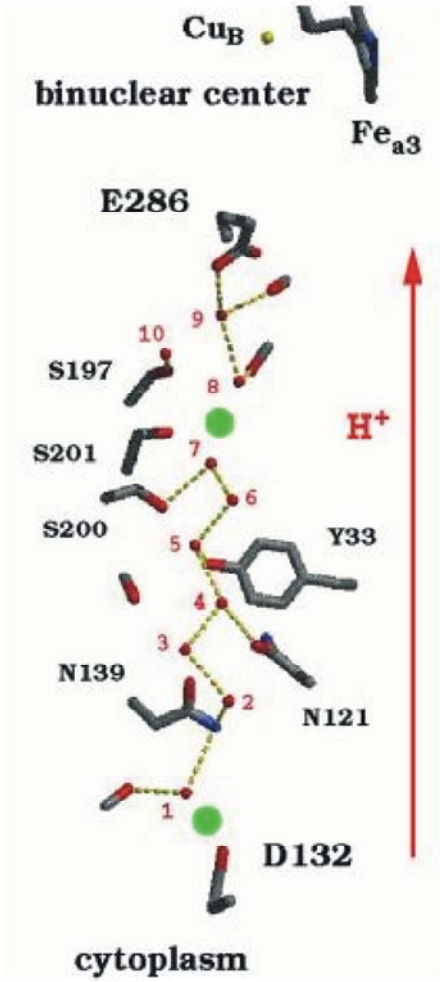
Predicted to leak protons…

Doesn't express!

Chakrabarti et al. J Mol Biol 2004

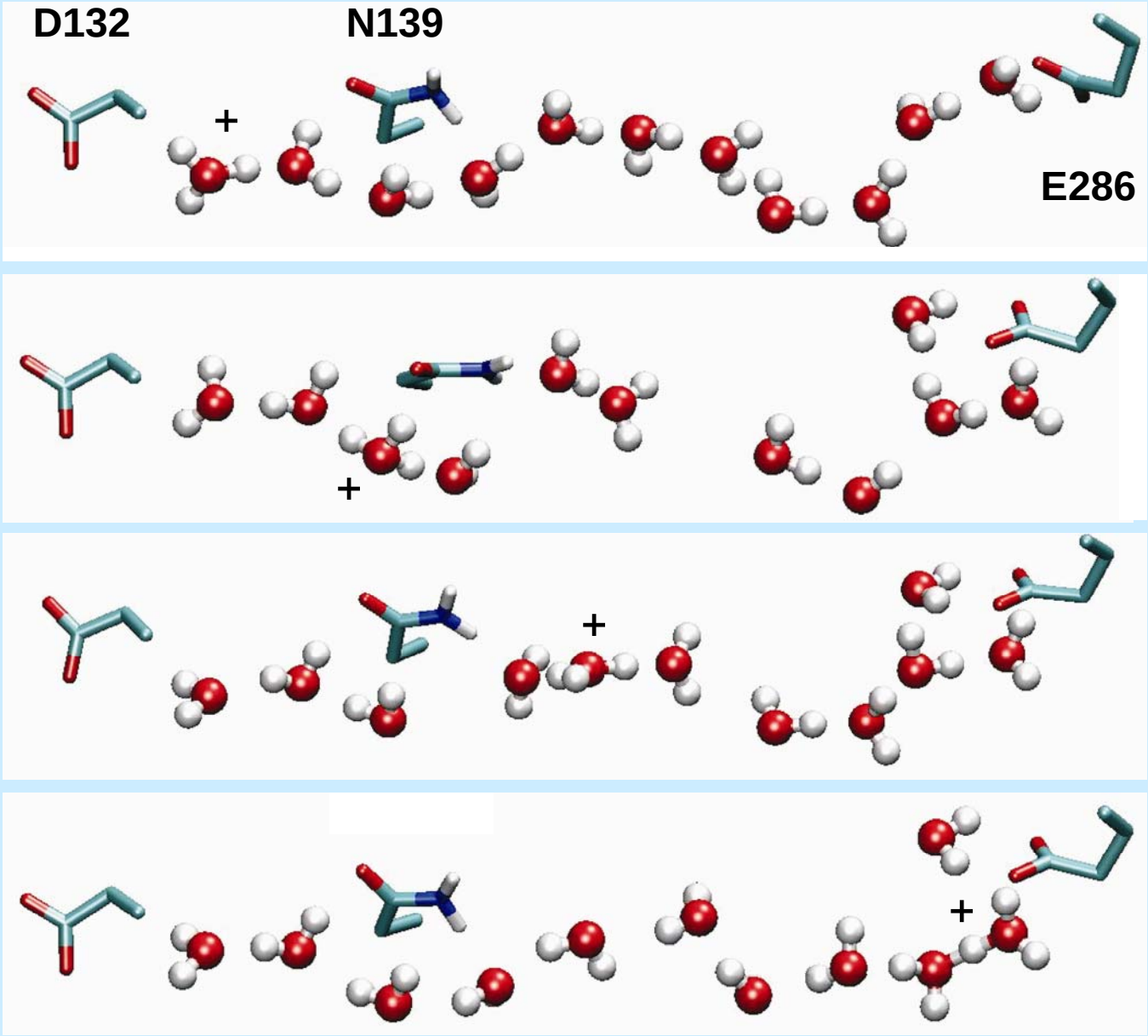
3. Proton pumping by cytochrome *c* oxidase



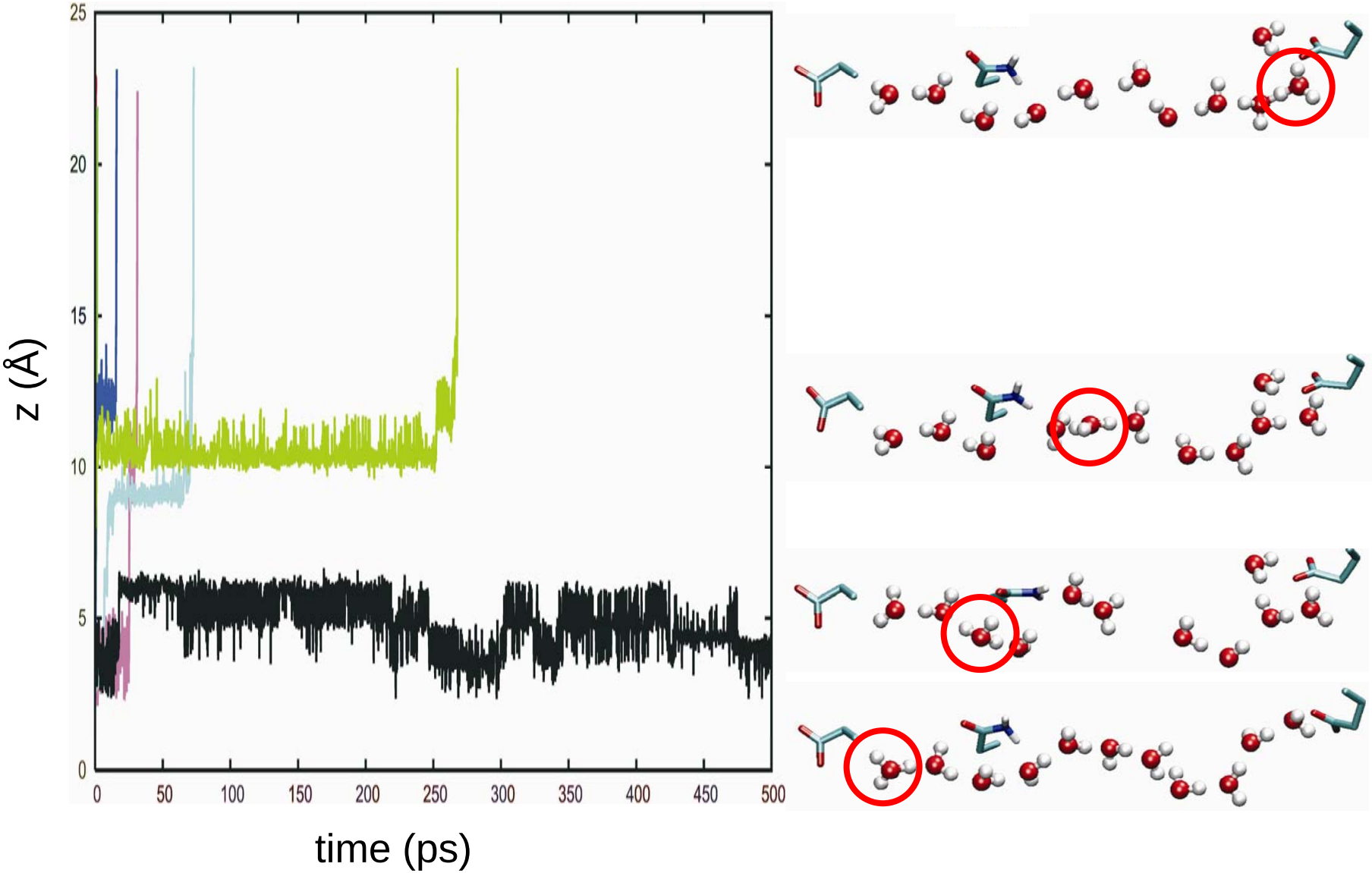


$O_2 + 4e^- + 4H^+ = 2H_2O$

A proton wire in the D channel



Water-mediated proton uptake



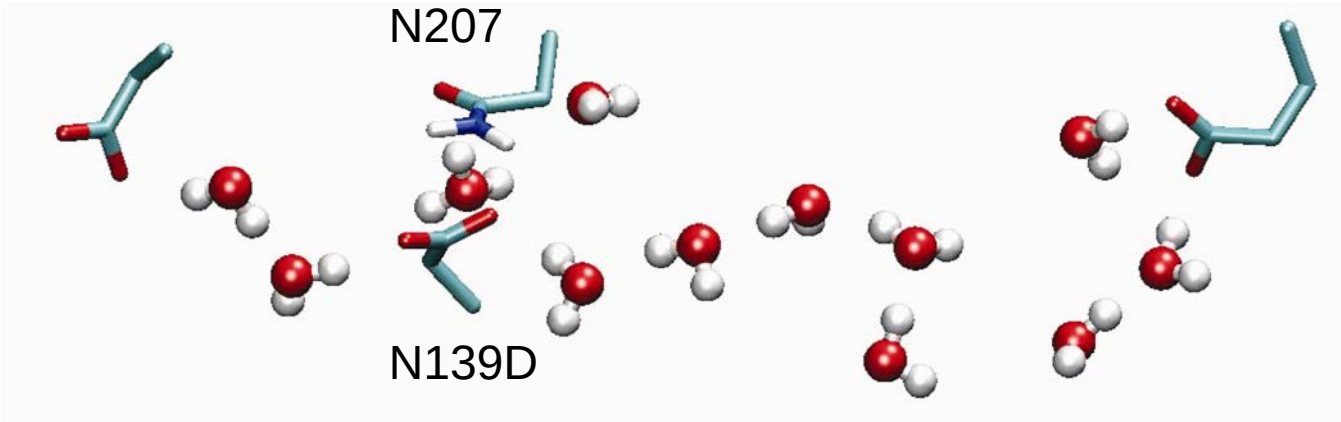
Kinetics of proton uptake in oxidase

The D channel is a proton sink: strongly non-equilibrium

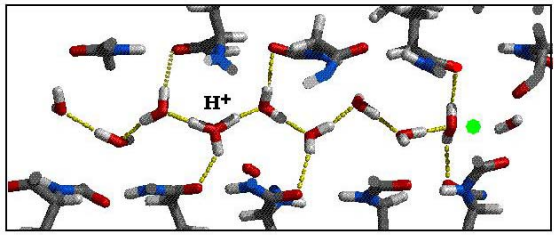
The kinetics of proton uptake is modulated by dynamic fluctuations of the water chain

The RLS corresponds to a bottleneck at residue 139

What is the origin of decoupling in N139D and N207D mutants?

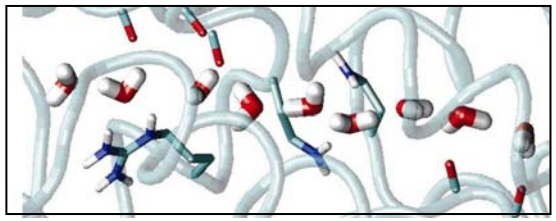


Conclusions



Detailed balance of microscopic forces

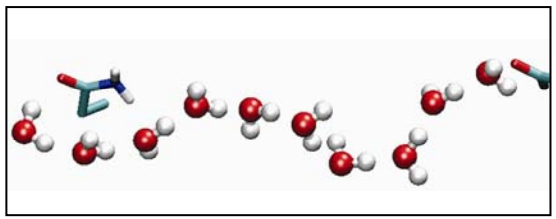
➔ physical basis of **proton transport** and **blockage** in membrane proteins



Atomistic simulations

→ events hard to observe experimentally

→ generate/refine testable mechanistic hypotheses



Next: mechanism of redox-coupled H^+ pumping in cytochrome *c* oxidase

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