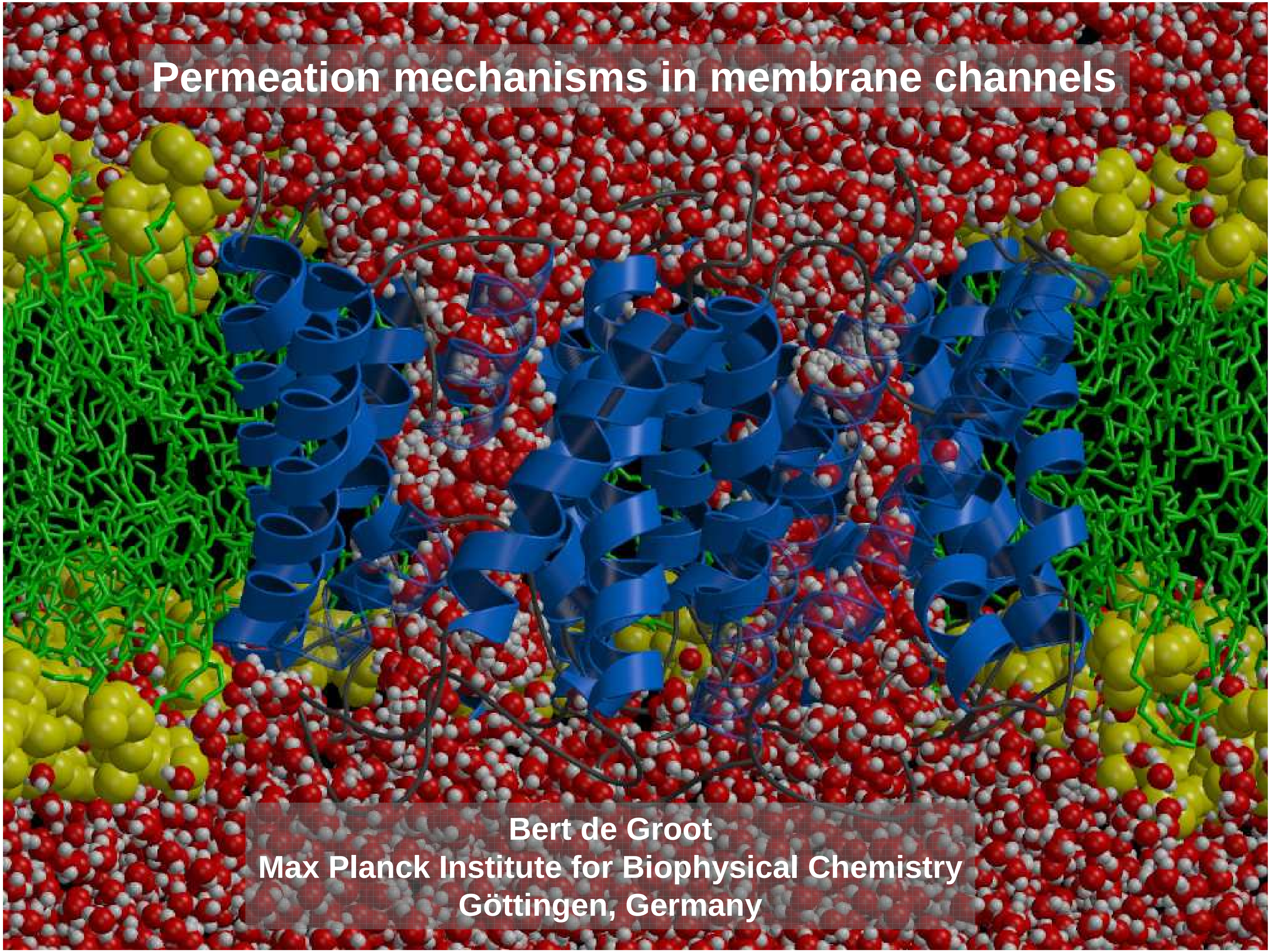
Permeation mechanisms in membrane channels



Bert de Groot
Max Planck Institute for Biophysical Chemistry
Göttingen, Germany

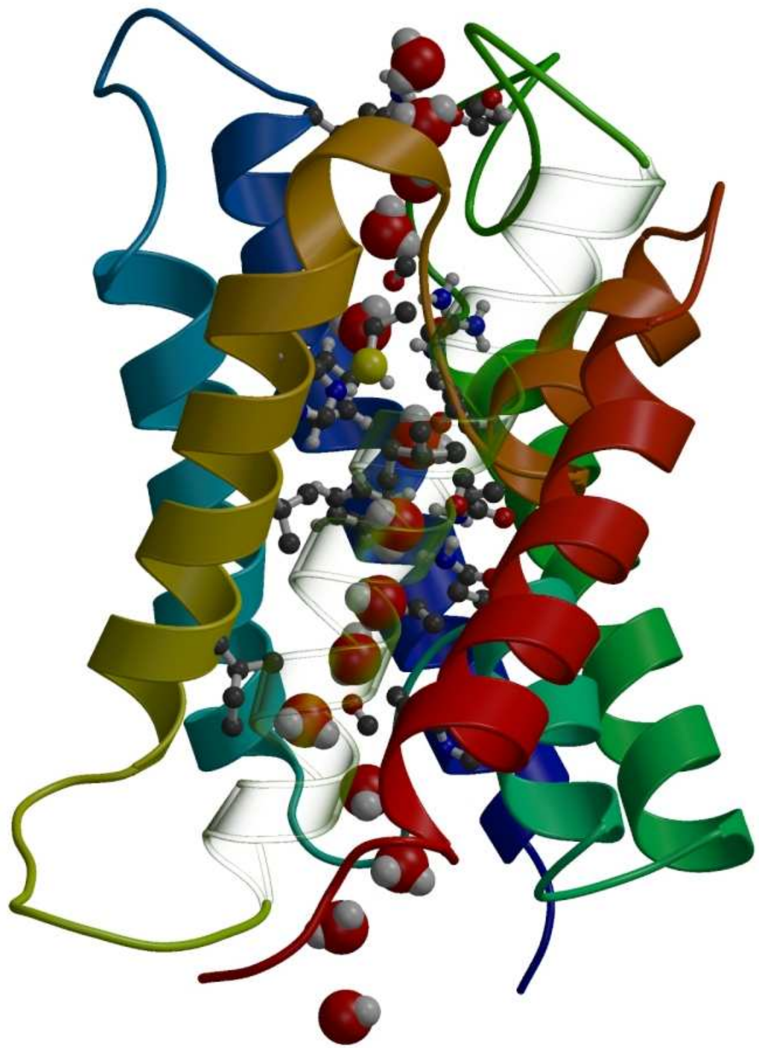
Aquaporin Water Channels

Aquaporins are highly selective, efficient water channels (10^9/s)

Question:

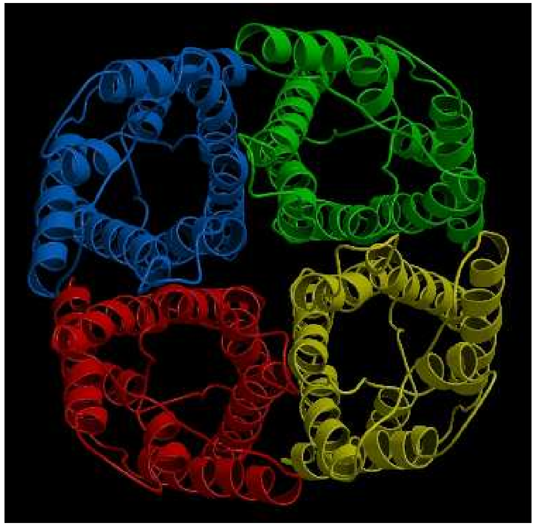
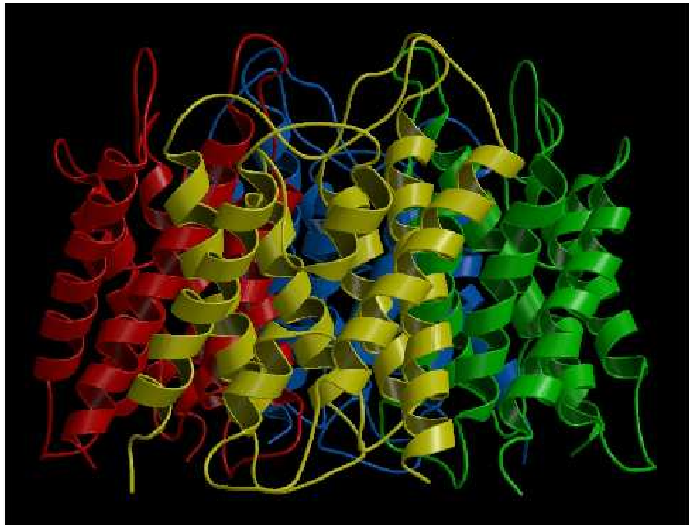
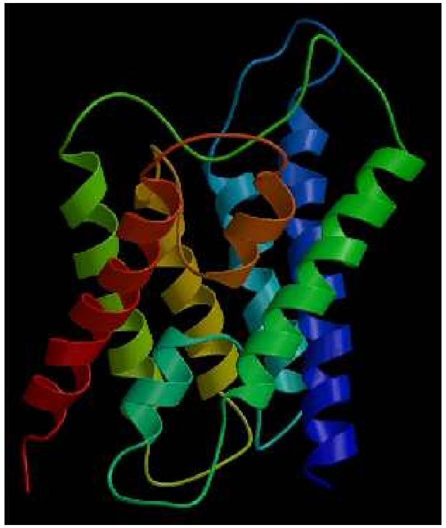
How can aquaporins be so selective and at the same time so efficient?

- *water permeation*
- *proton exclusion*
- *permeation of other solutes?*



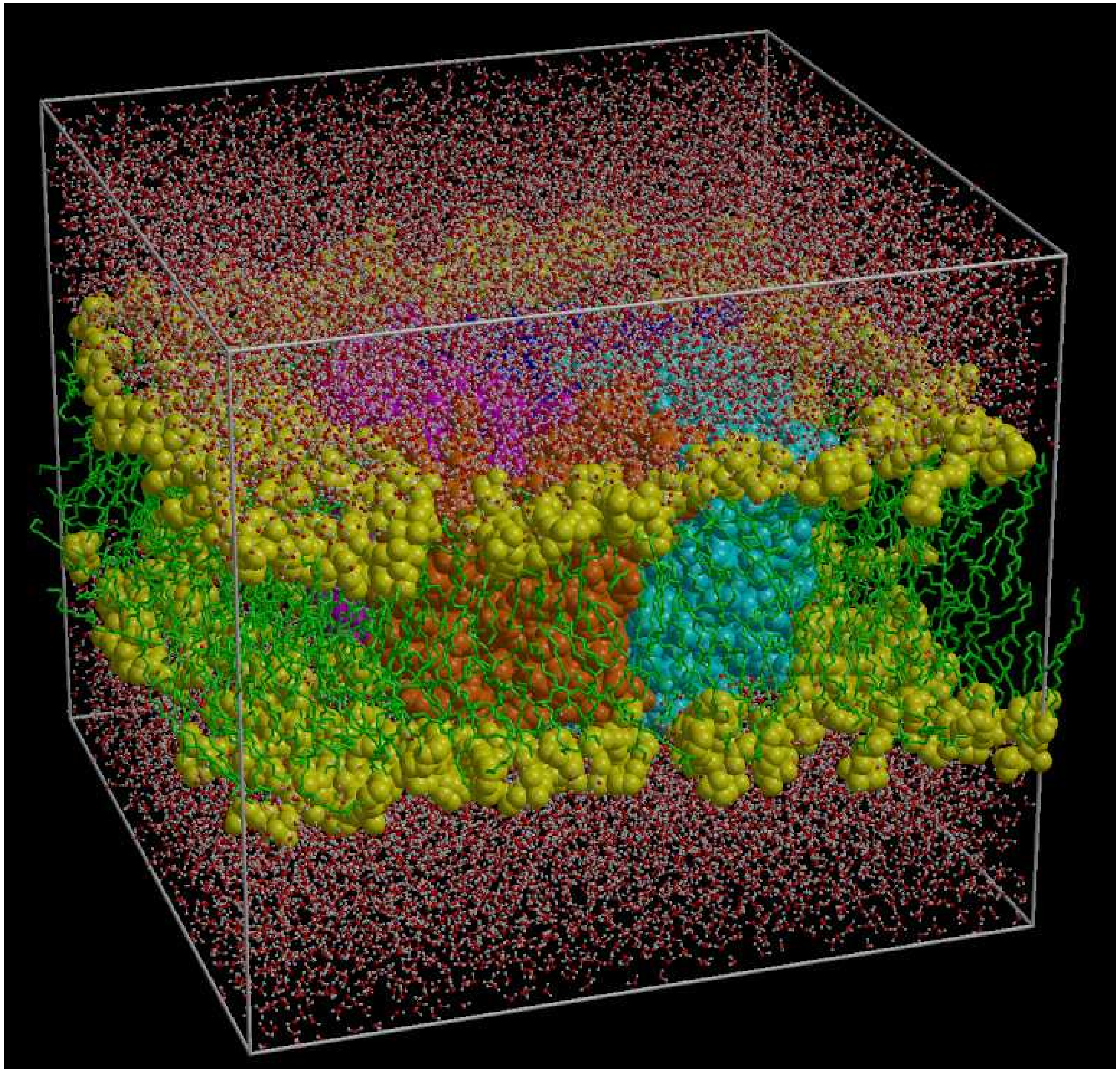
B.L. de Groot, A. Engel, H. Grubmüller, *FEBS Lett.* **504** (2001) 206

Aquaporin Structure

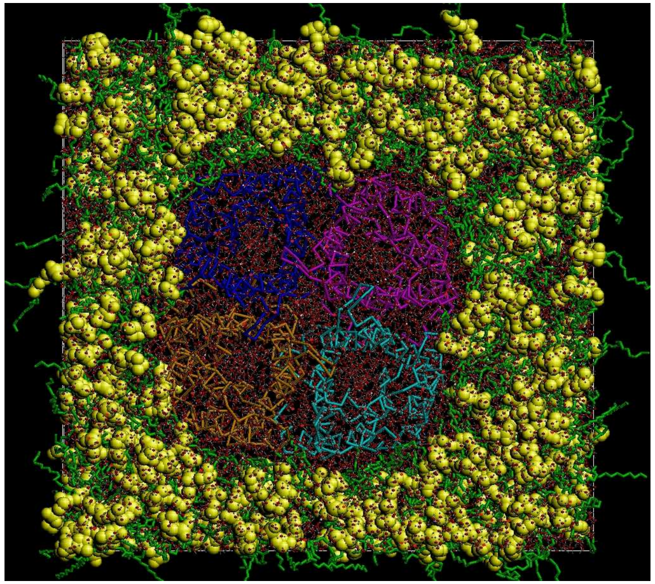
  

- *tetrameric channel with each monomer providing a pore*
- *6 transmembrane spanning helices*
- *loops B and E (with NPA) fold back into the protein*
- *central constriction region about 3 Å wide*

MD simulations of water permeation

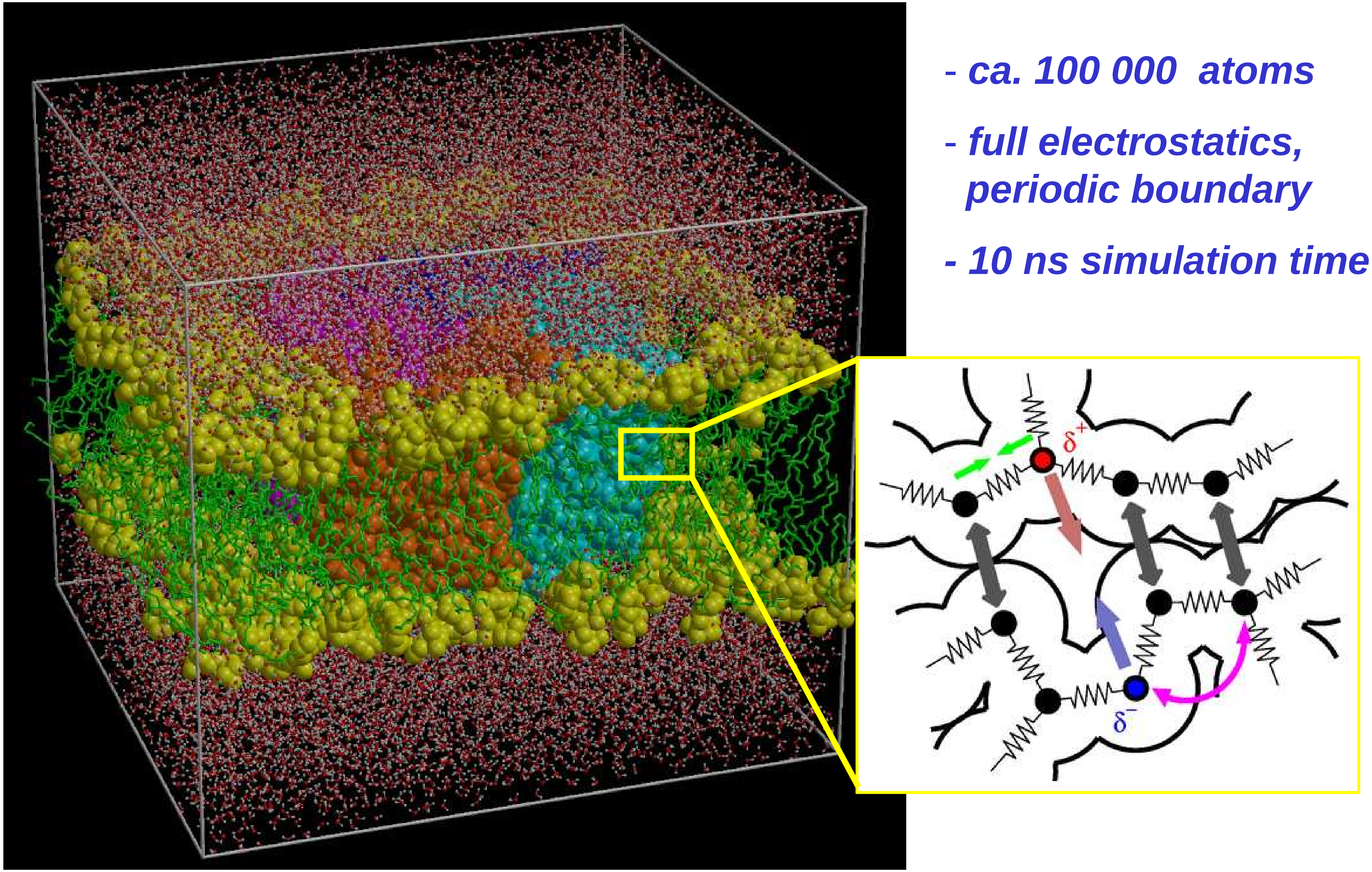


- *ca. 100 000 atoms*
- *full electrostatics, periodic boundary*
- *10 ns simulation time*



B.L. de Groot, H. Grubmüller, *Science* **294**, 2353 (2001)

MD simulations of water permeation



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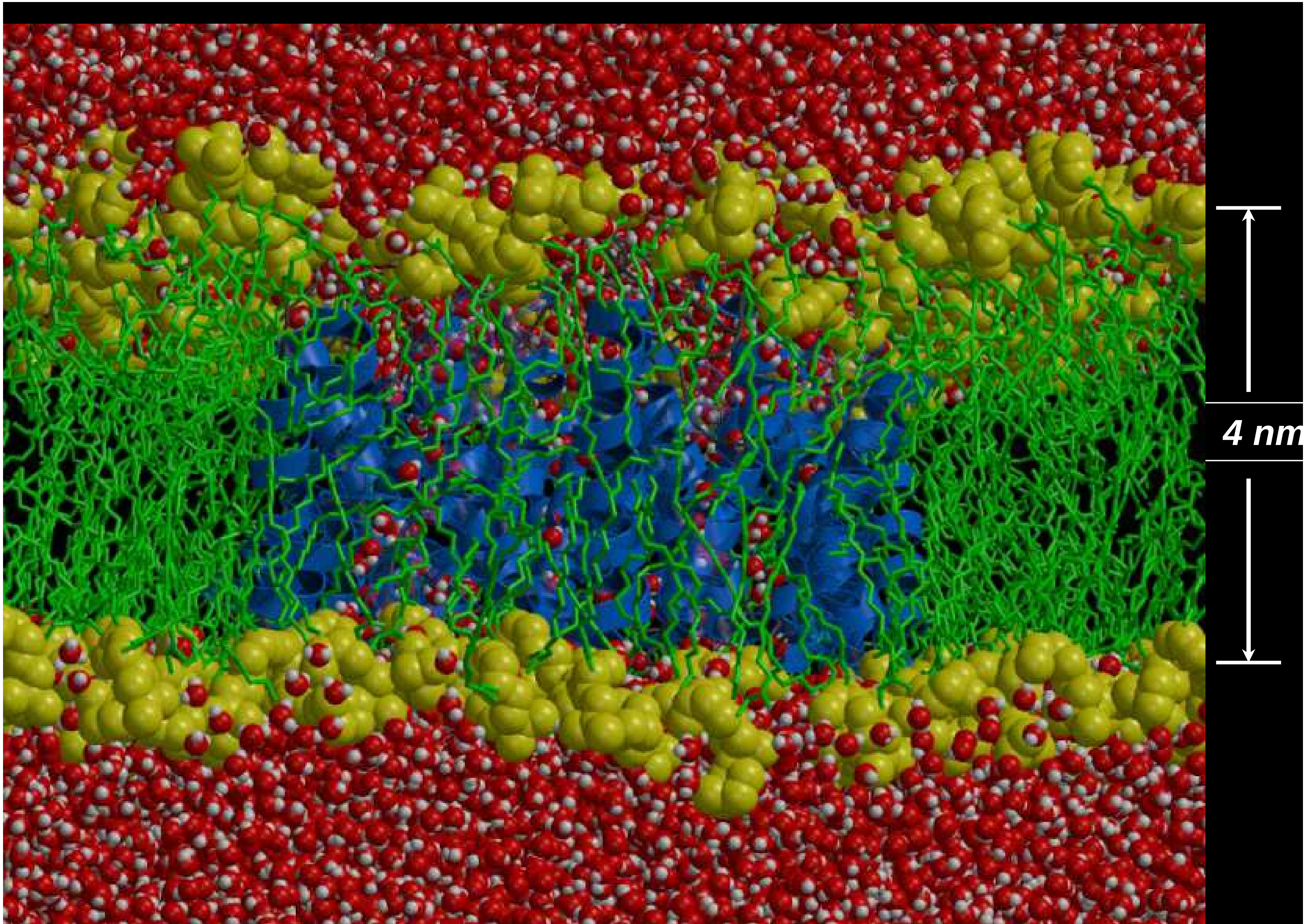
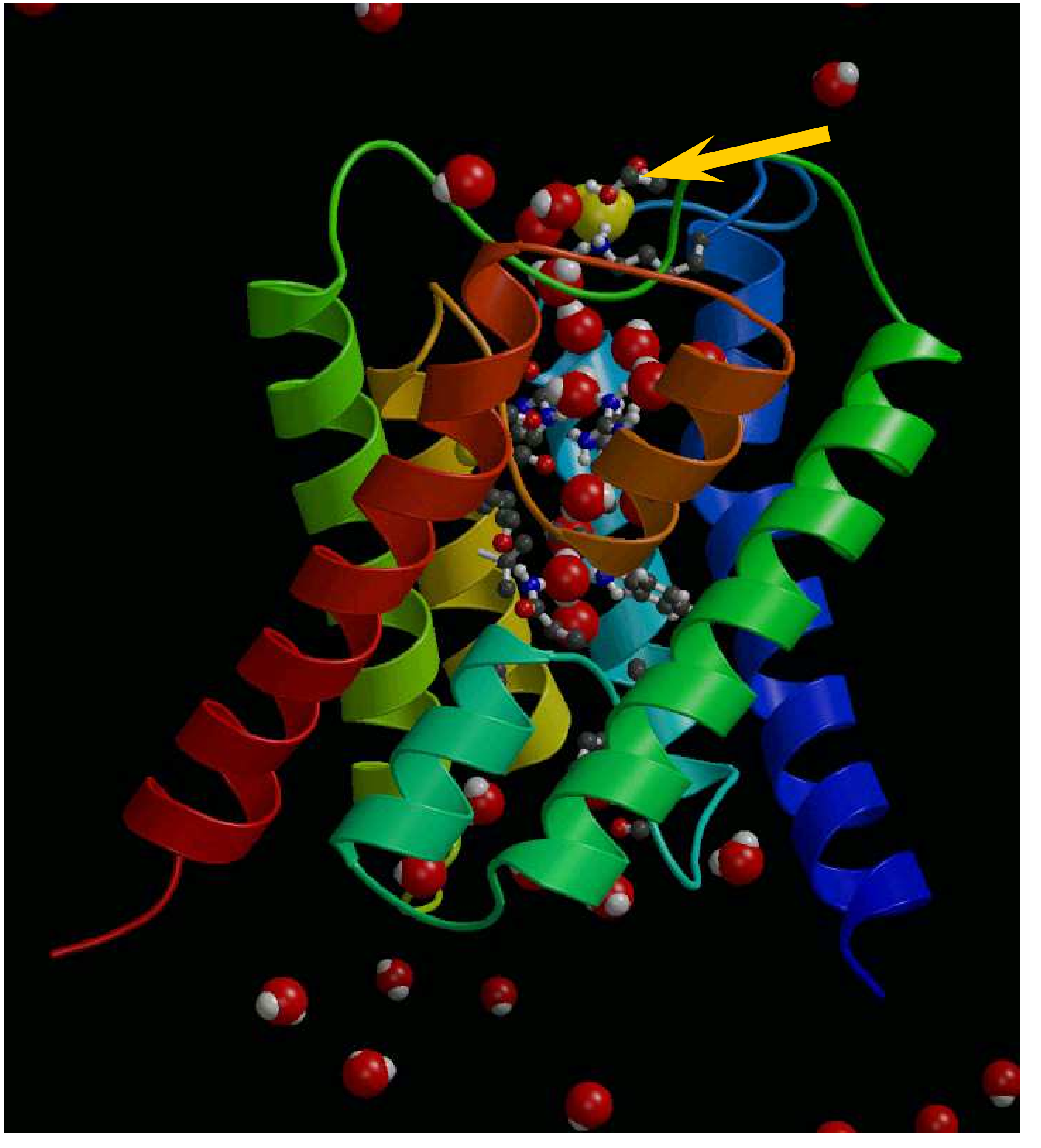


Molecular dynamics simulation, 1s $\hat{=}$ 2 ·10 $^{-11}$s

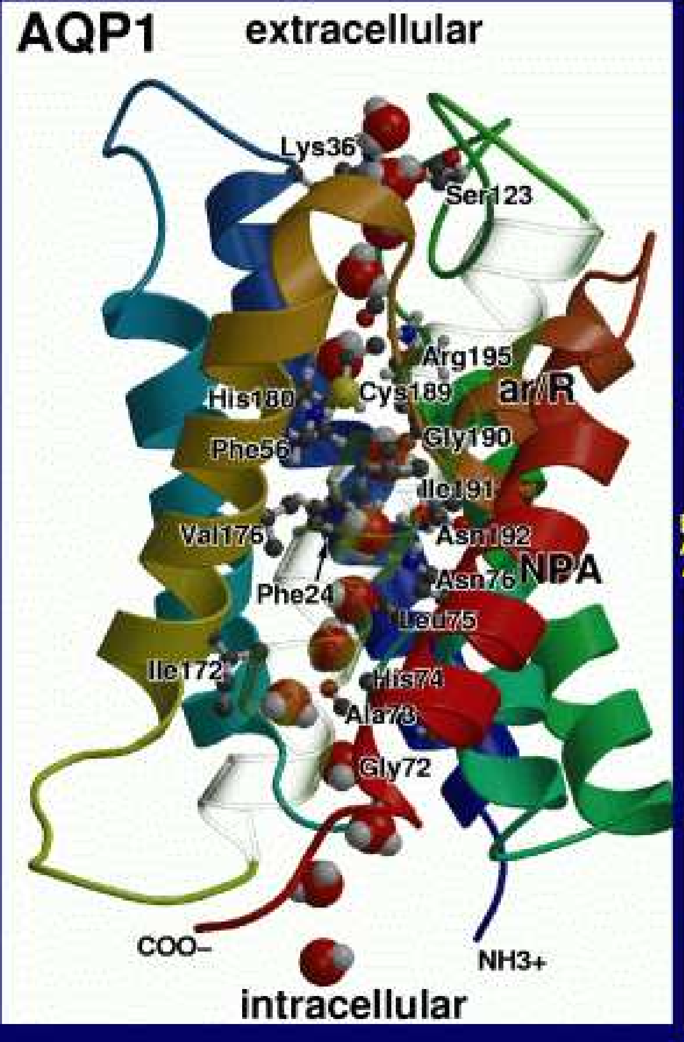
Water Permeation proceeds in steps

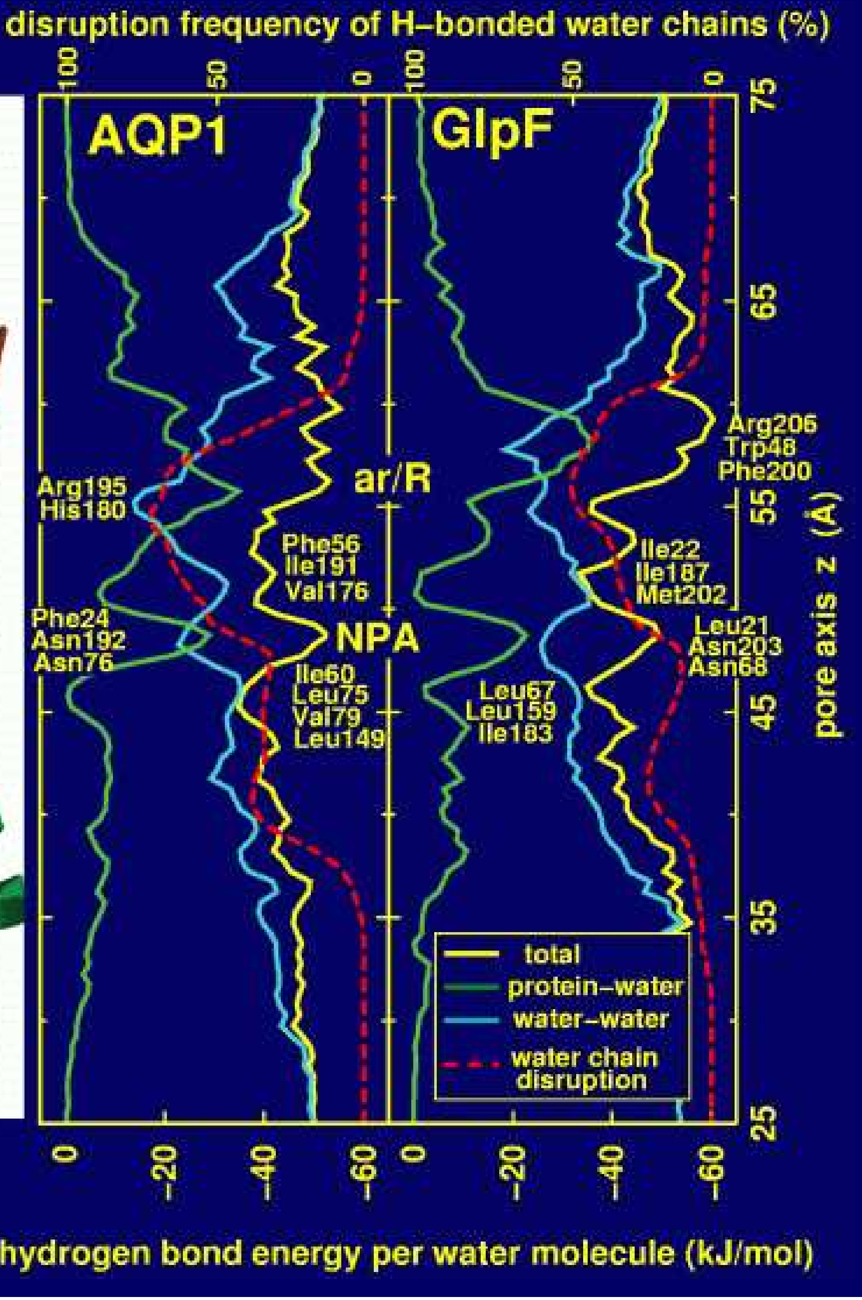
one out of 16 full spontaneous permeation events (2 ns)



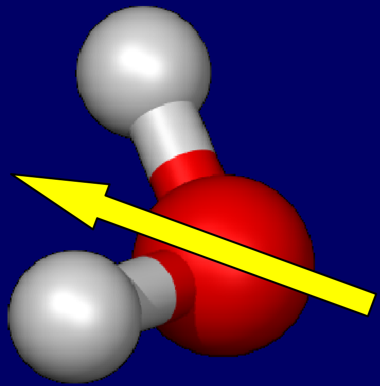
(outside the channel, only few water molecules are shown)

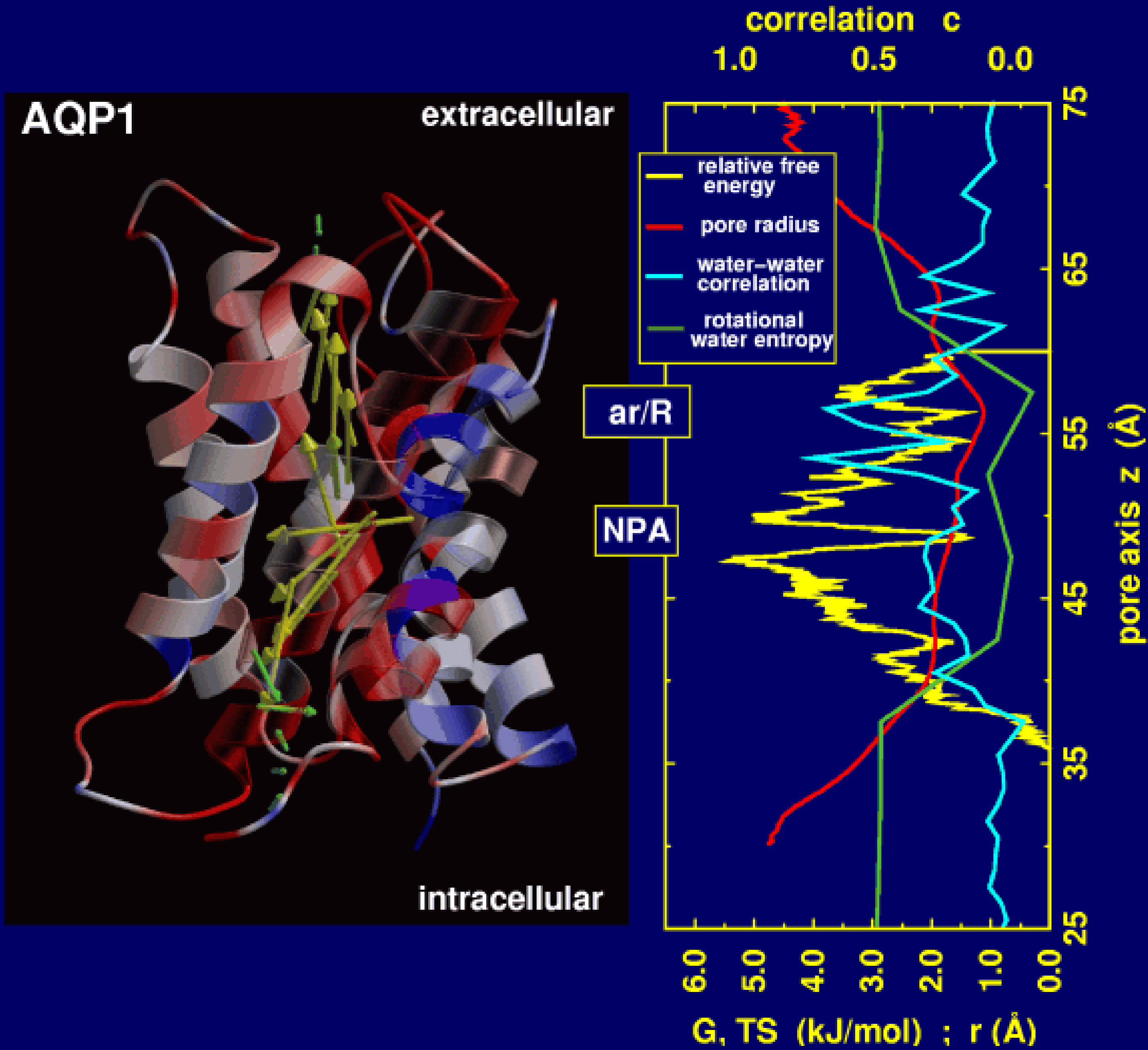
Water pathway and hydrogen bonding





Choreography of water molecules in Aquaporin-1

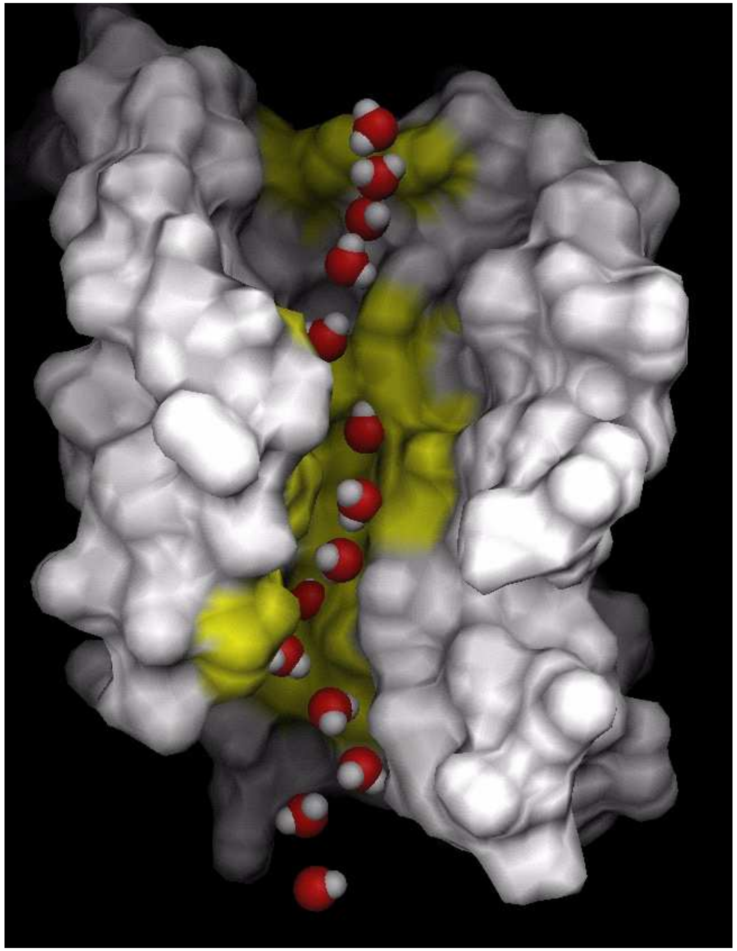




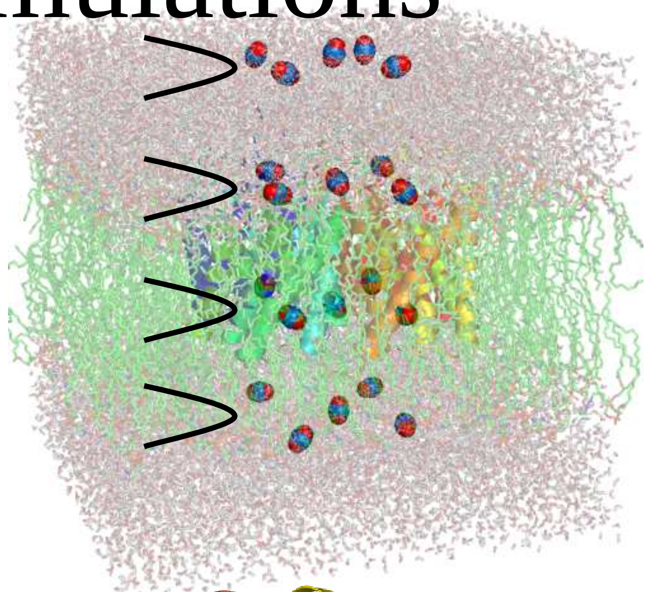
Other functions than water permeation?

Permeation beyond water and glycerol?

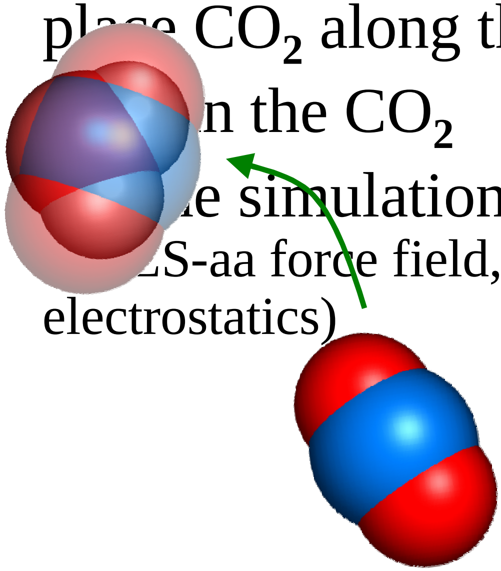
- CO_2
- O_2
- NH_3
- urea
- (glycerol)



Umbrella sampling simulations

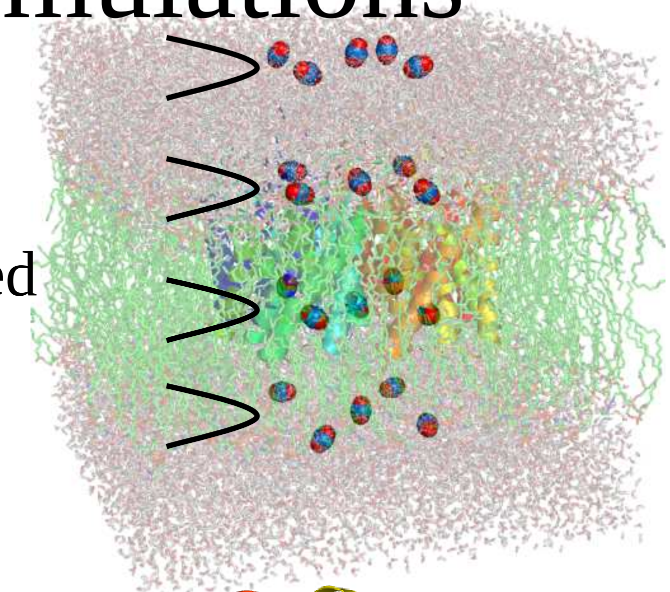


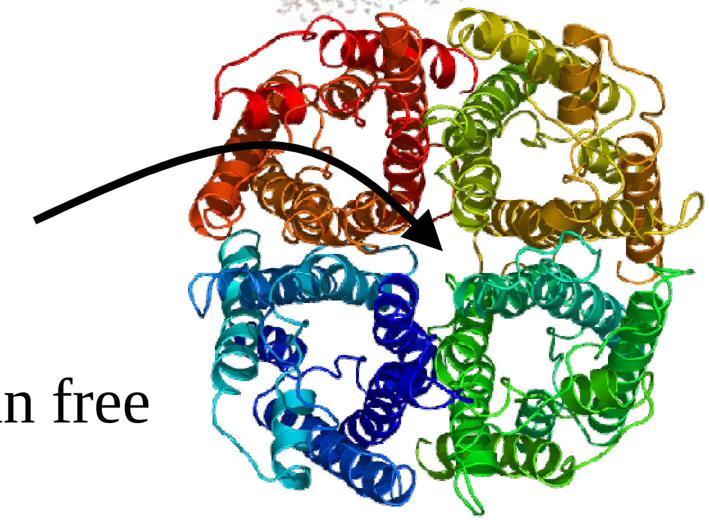
- start with a equilibrium molecular dynamics simulation
- select frames from the trajectory
- replace water molecules by CO_2 at desired positions along the channels
- place CO_2 along the ***central cavity***
- ...n the CO_2
- ...e simulation
  (...S-aa force field, 300K, NPT, PME electrostatics)



Umbrella sampling simulations

- start with a equilibrium molecular dynamics simulation
- select frames from the trajectory
- replace water molecules by CO_2 at desired positions along the channels
- place CO_2 along the ***central cavity***
- restrain the CO_2
- run the simulation
  (OPLS-aa force field, 300K, NPT, PME electrostatics)



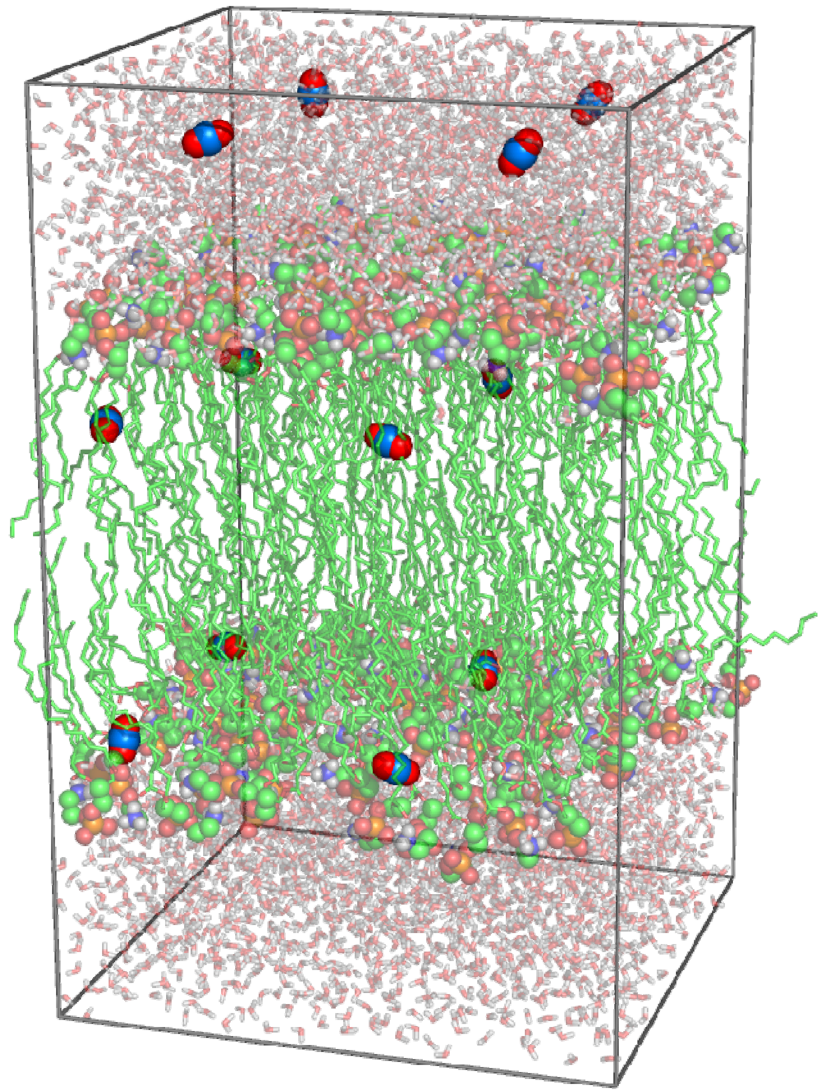


- extract histograms
- perform WHAM procedure to obtain free energy profile

Membrane

Physiological relevance of CO_2 permeation through AQP1?

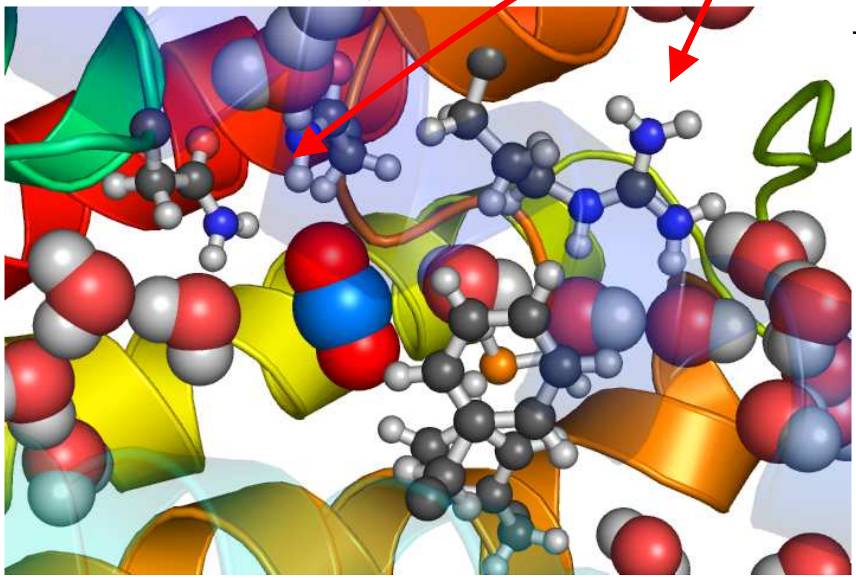
- comparison with membrane
- pure POPE membrane as a model bilayer

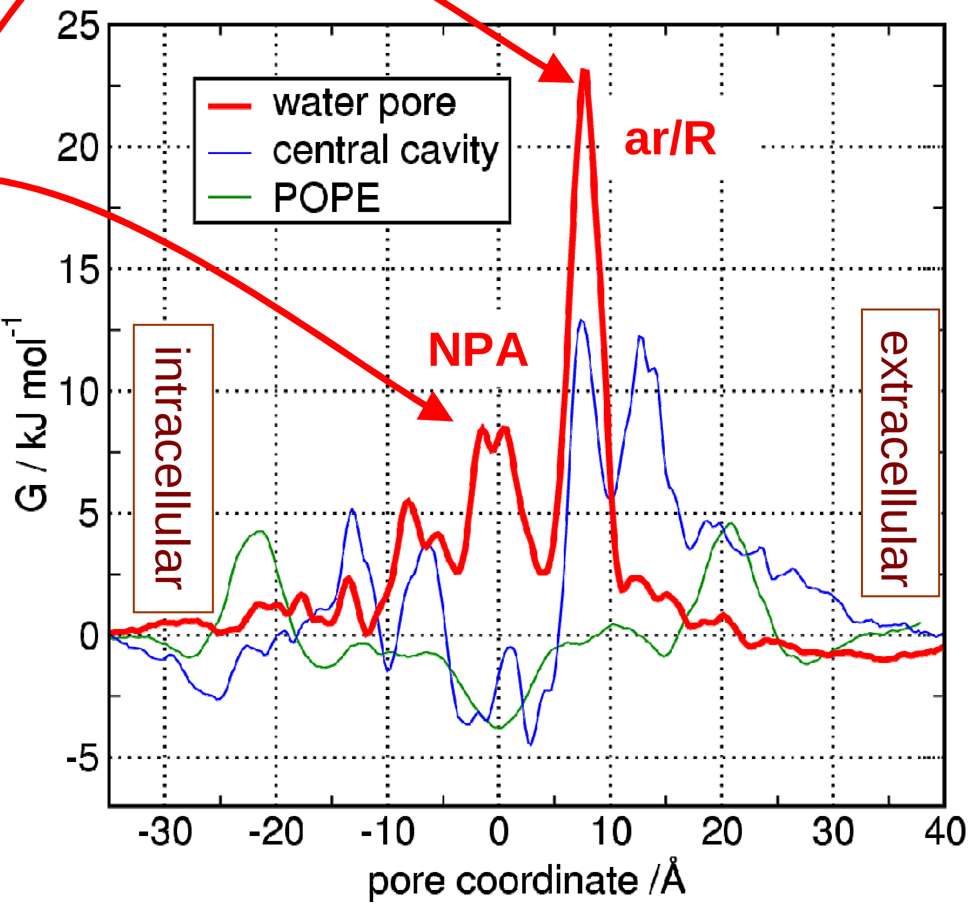


Potentials of mean force

main barriers:

- **water pore**
  - ar/R, 23 kJ/mol





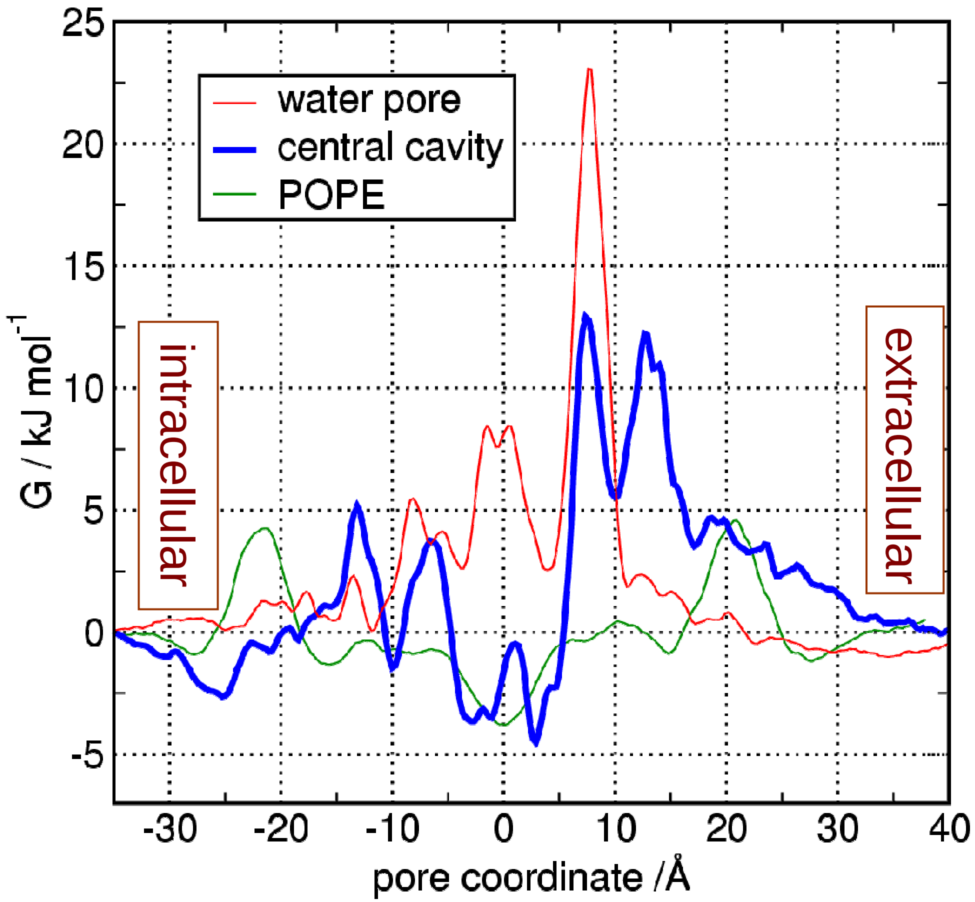
J. S. Hub and B. L. de Groot, *Biophys. J.*, **91**, 842-848 (2006)

Potentials of mean force

main barriers:

- **water pore**
  - ar/R, 23 kJ/mol
  - NPA, 8 kJ/mol

- **central cavity**
  - entrance into empty cavity, 13kJ/mol
  - between 4 Asp50, 13kJ/mol



J. S. Hub and B. L. de Groot, *Biophys. J.*, **91**, 842-848 (2006)

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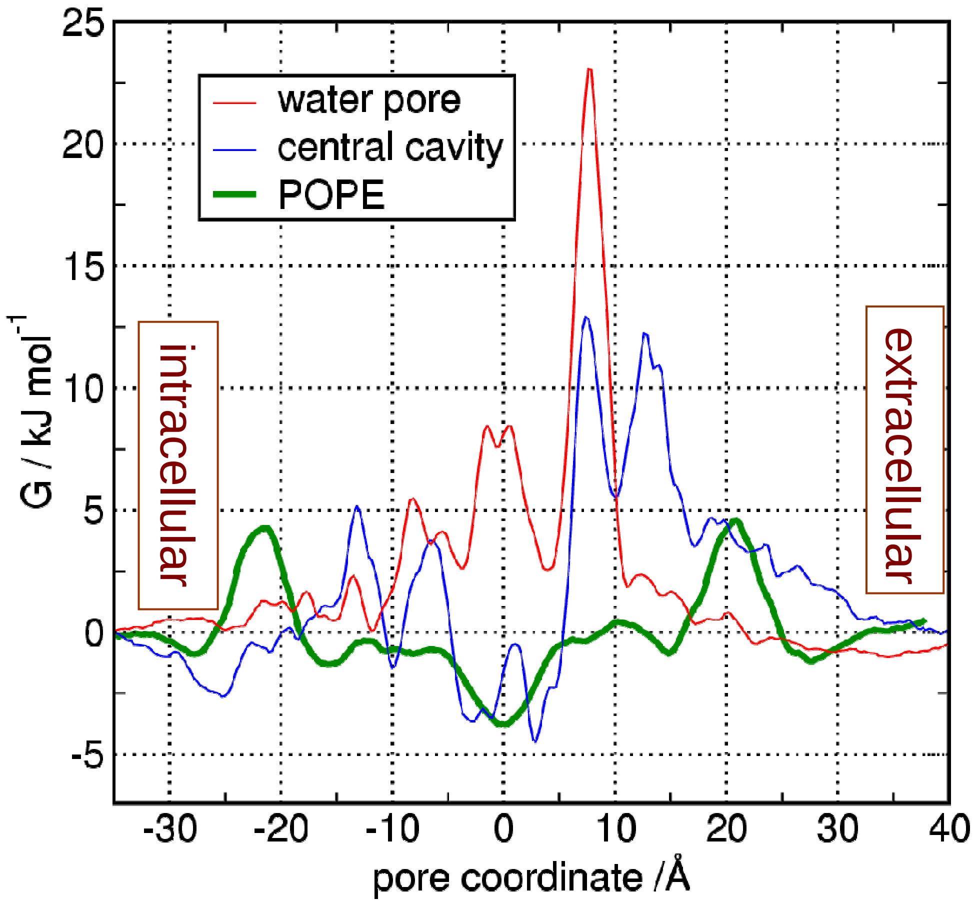
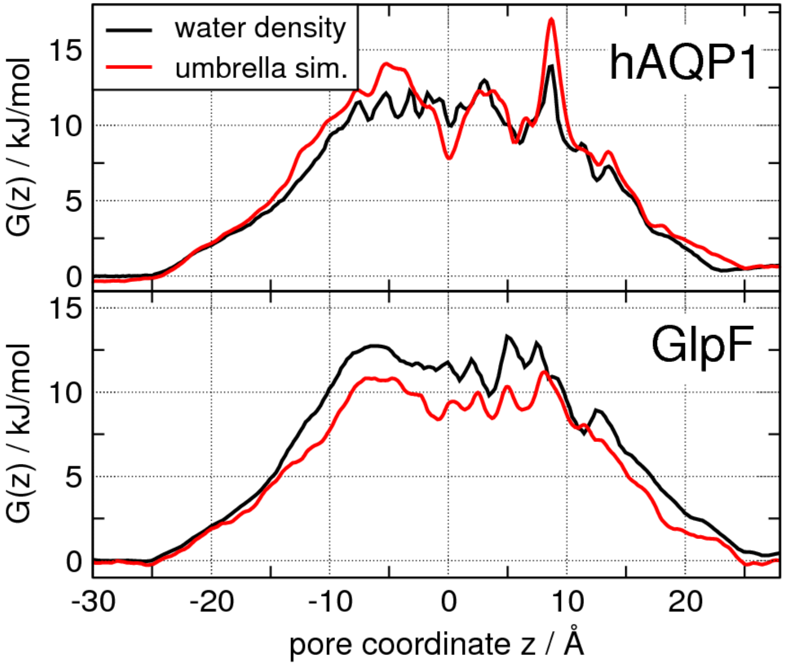
- **POPE bilayer**



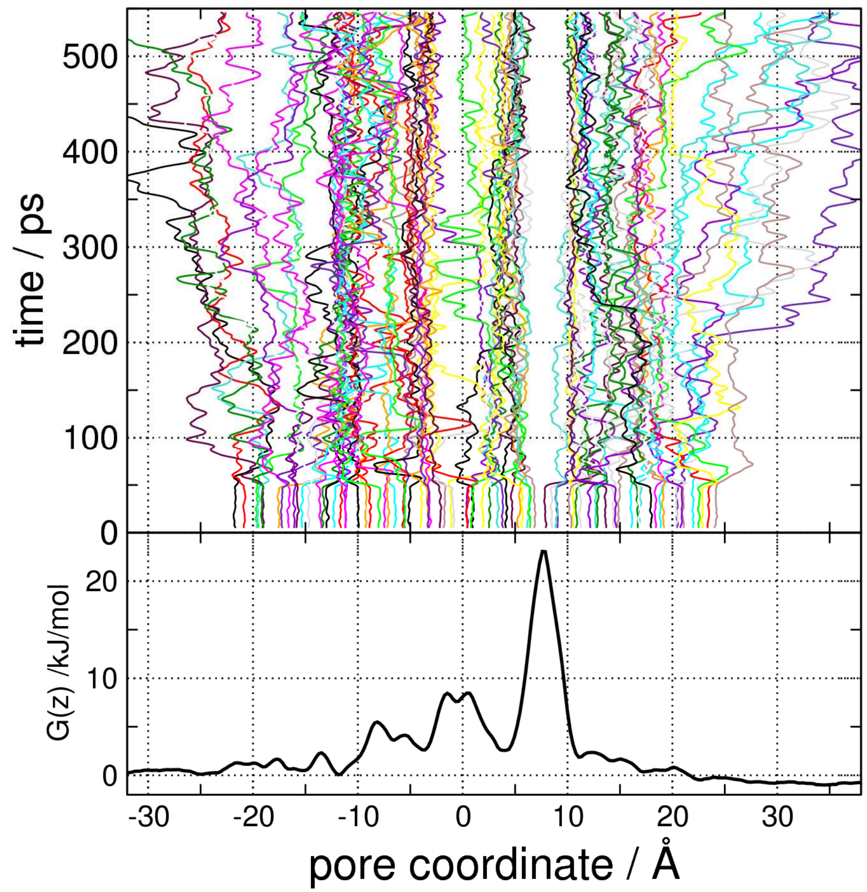
J. S. Hub and B. L. de Groot, *Biophys. J.*, **91**, 842-848 (2006)

Control simulations

Verification from water density



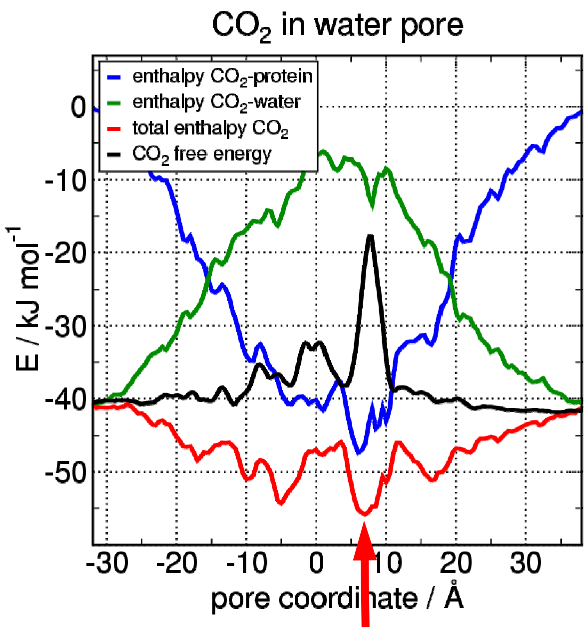
Comparison to free CO_2 simulations



Mechanisms of selectivity

Selectivity mechanism

solute/protein & solute/water interactions

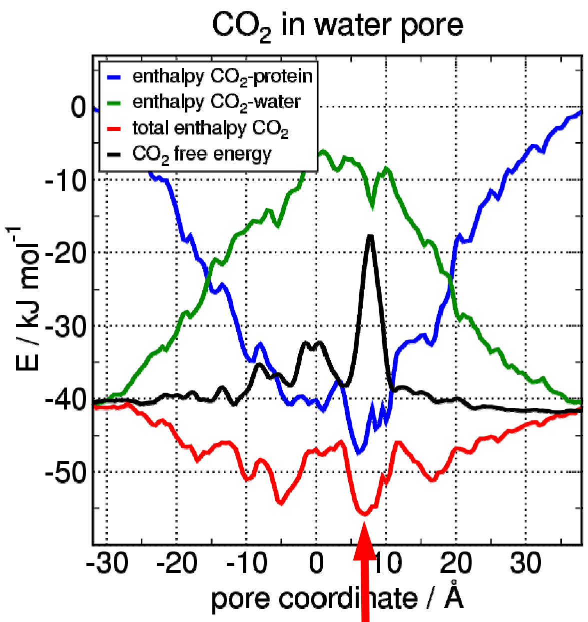


no enthalpic barrier

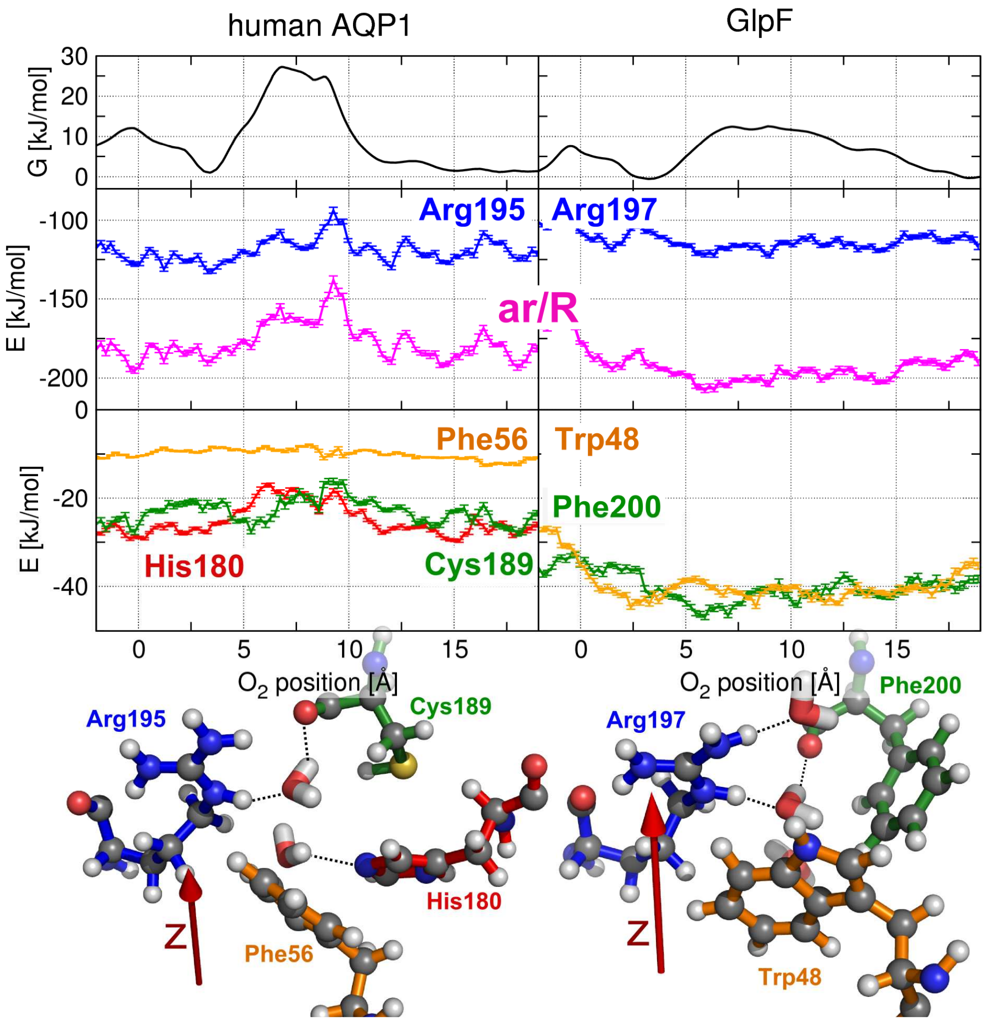
↳ indirect effects

Selectivity mechanism

solute/protein & solute/water interactions



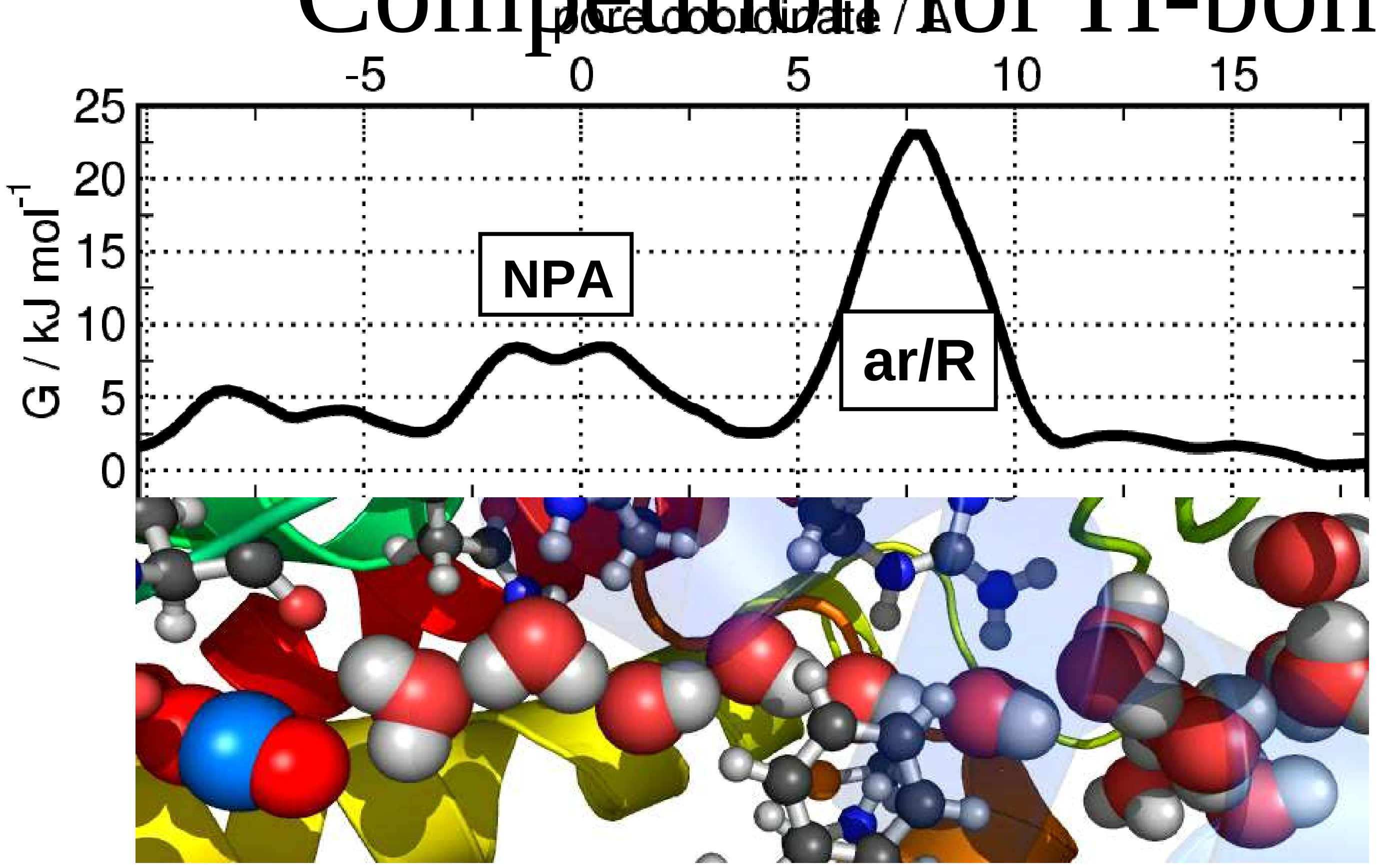
water/protein interactions as function of O_2 position



no *direct* enthalpic barrier

↳ indirect effects (unfavorable

Competition for H-bonds



barriers due to competition for H-bonds with

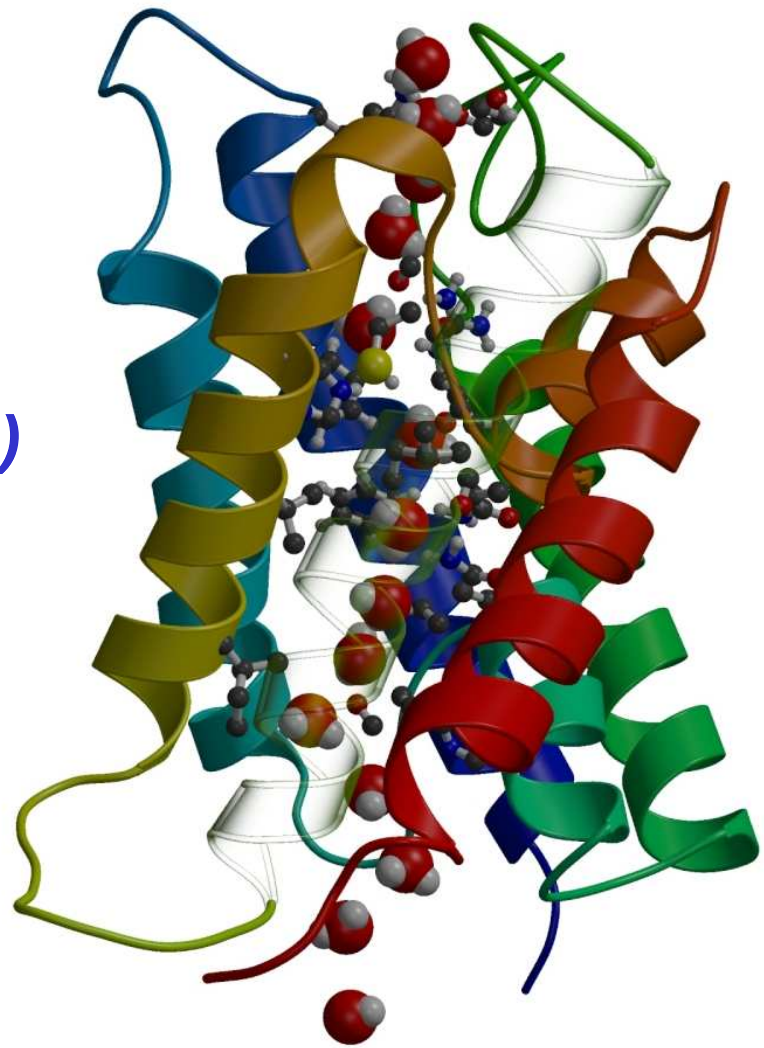
- Asn76 & Asn 192
- Arg195 !!

Inhibititors?

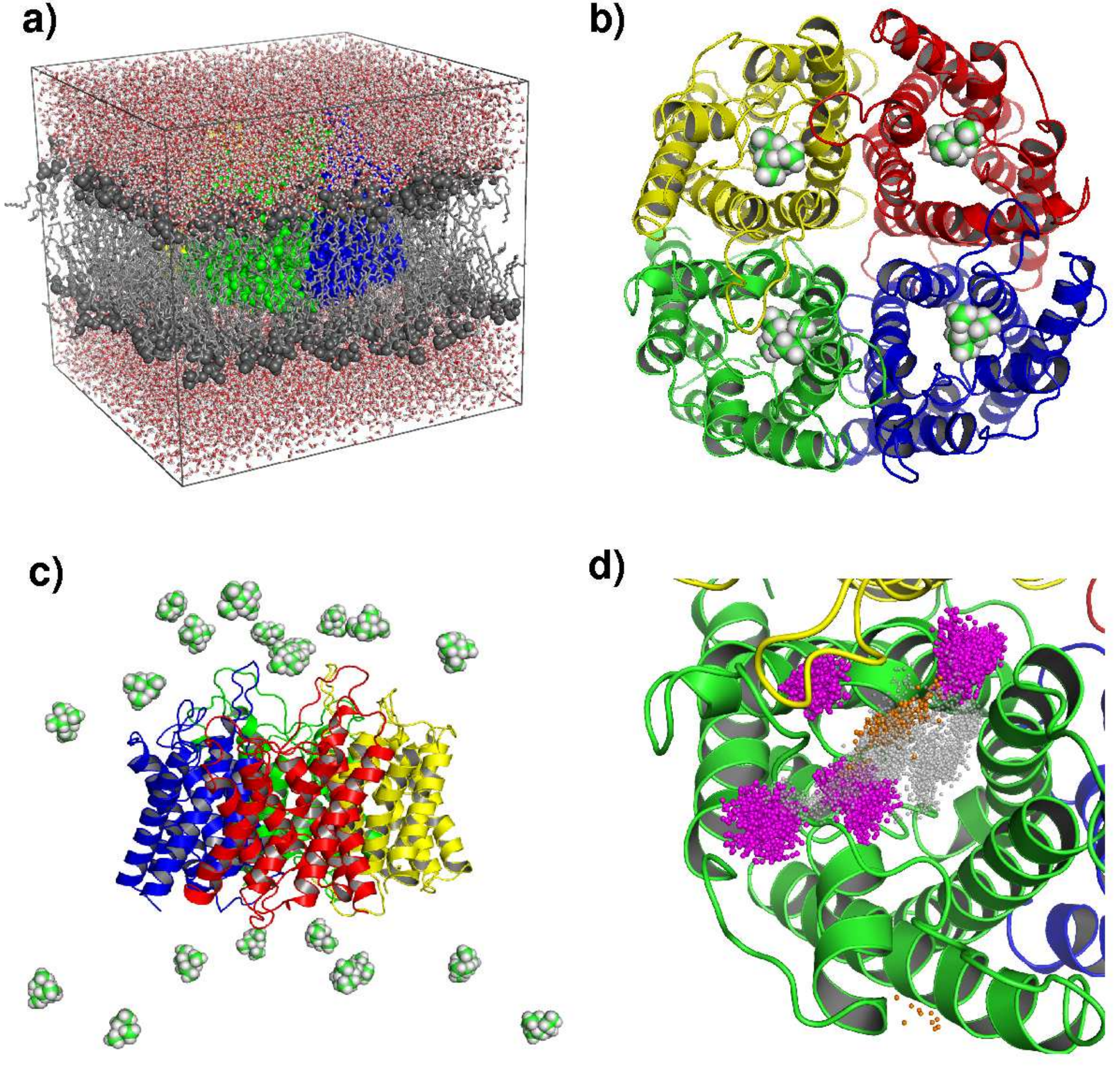
Need for Aquaporin inhibitors

Aquaporins involved in:

- ***glaucoma (AQP1)***
- ***diabetes insipidus (AQP2)***
- ***head trauma (AQP4)***
- ***cancer (AQP1)***

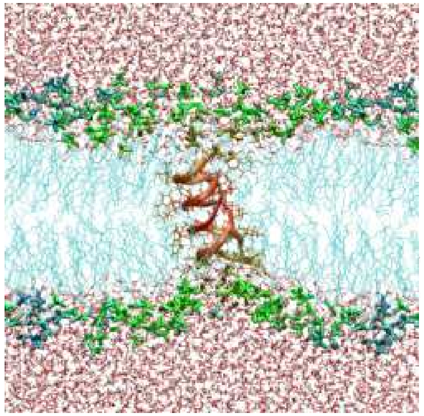


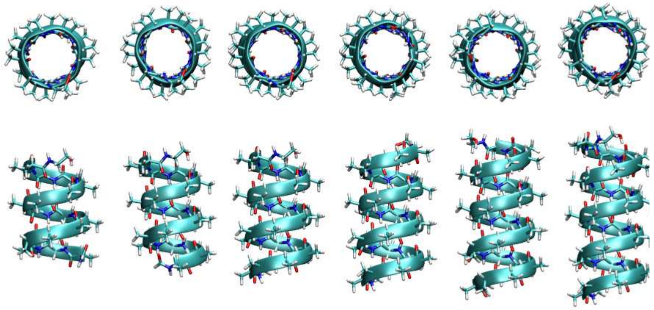
Aquaporin-1 inhibition by TEA?

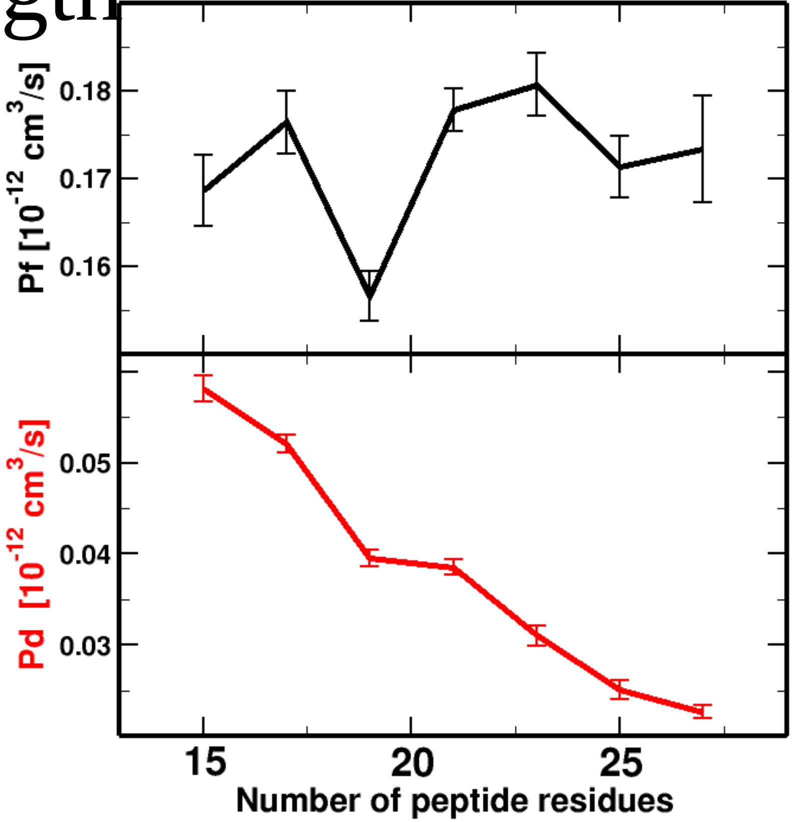


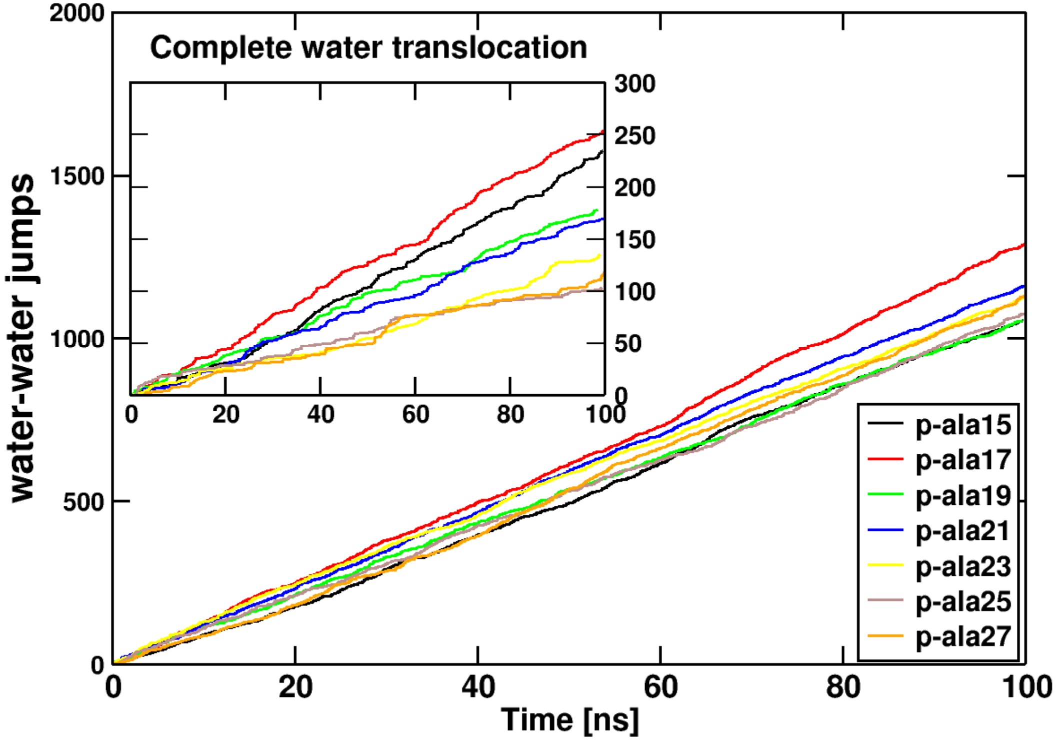
Model channels

Dependence of water permeability on pore length









-Osmotic permeability invariant with pore length

-Linear length dependence on diffusive permeability

G. Portella, P. Pohl, B. L. de Groot, Biophys. J. 92:3930 (2007)

Conclusions I

Aquaporins:

1. Efficient water permeation by hydrogen bond complementarity
2. Proton exclusion dominated by electrostatic effects
3. CO_2 permeation through AQP1 plays – if the tetrameric channel is blocked – a physiological role only in tissues with membrane barriers well above 20 kJ/mol.
4. Experimental suggestions:
   - Test for CO_2 permeation through AQP1 embedded in POPE or POPC
   - Do mutations in the ar/R region enhance CO2 permeability (e.g. His180Ala/Arg195Val)?

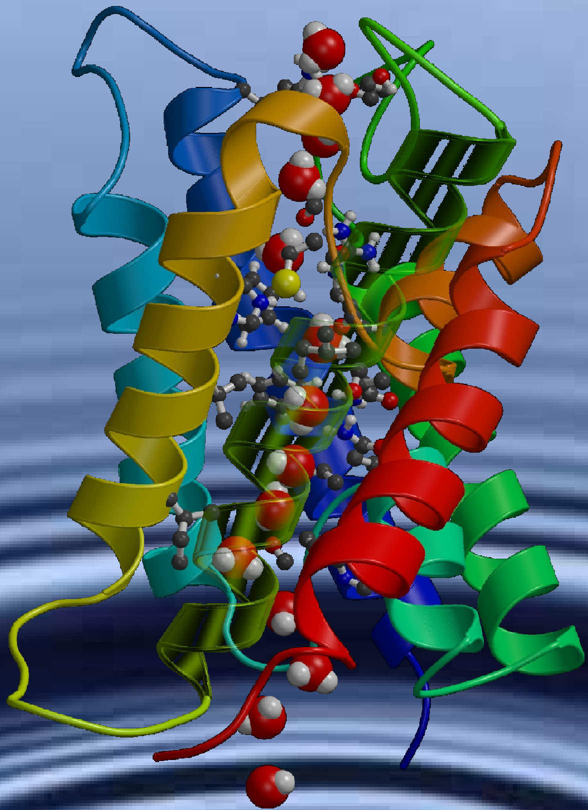
Conclusions II

5. Weak TEA inhibition of AQP1 confirmed

6. Water permeation channel length-independent

7. Ion permeation strongly channel length dependent
   → role of water entropy?
   → naturally ocurring channels have short constriction regions

Thanks:



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Helmut Grubmüller

Basel:
Andreas Engel

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Jochen Hub

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€: MPG, DFG, EU

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