Water Mediated Proton Conduction: from Nanotubes to Cytochrome c Oxidase

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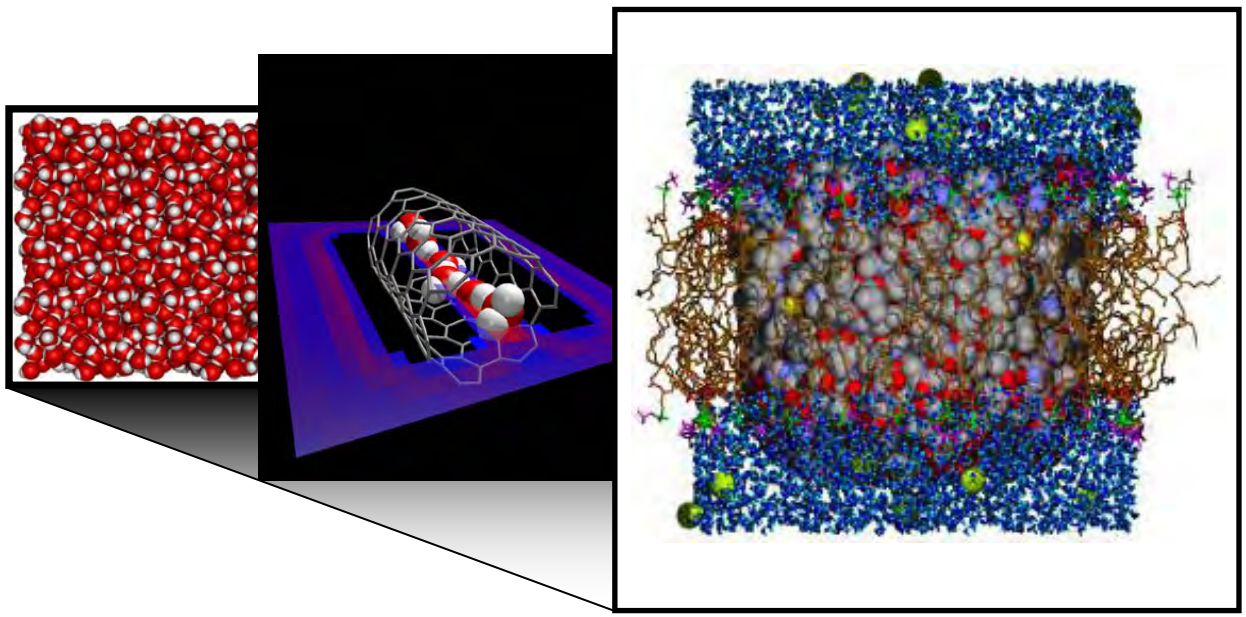

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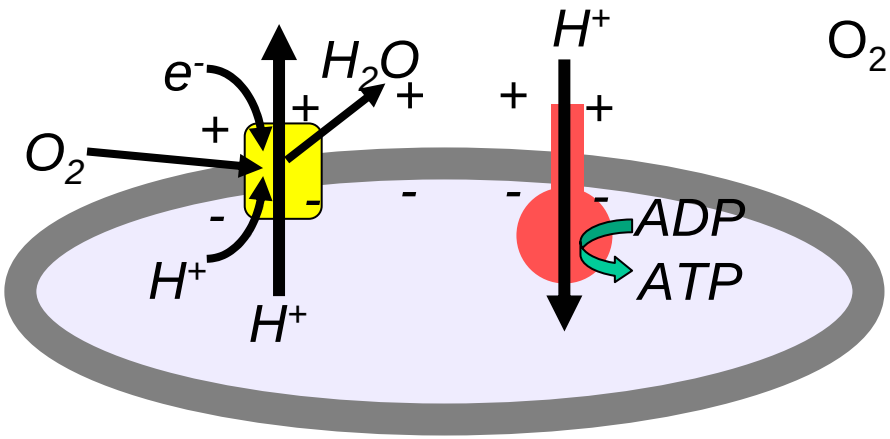
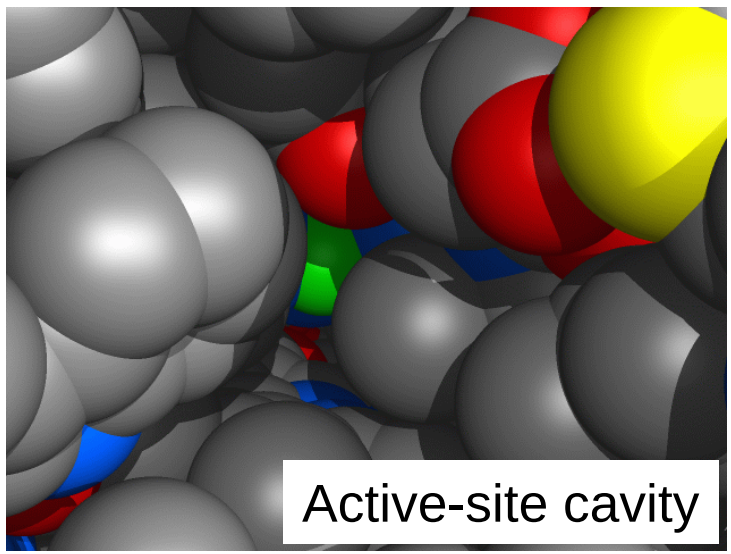
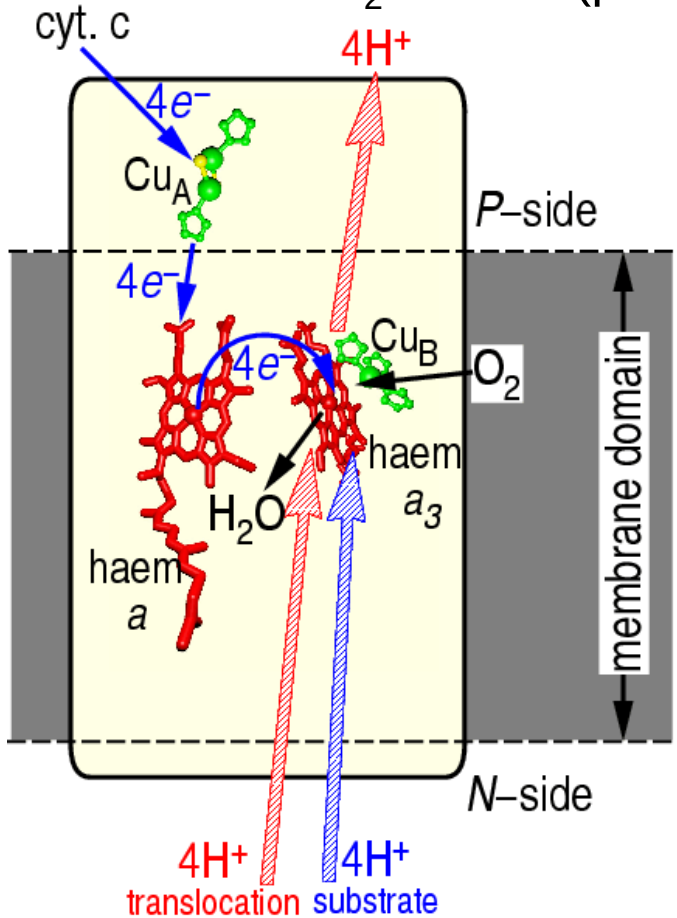
(Department of Health and Human Services)

Biological “Fuel Cell”: Proton Pump Cytochrome c Oxidase



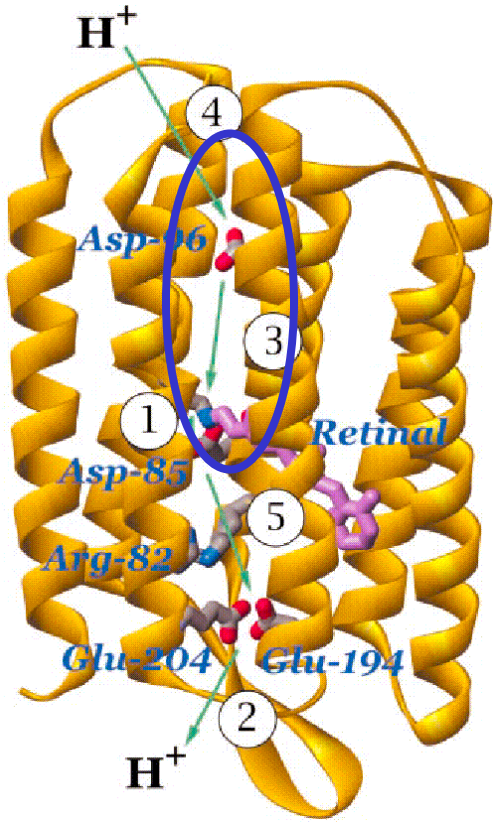


$O_2 + 8H^+ + 4e^- \rightarrow 2H_2O + 4H^+(\text{pumped})$



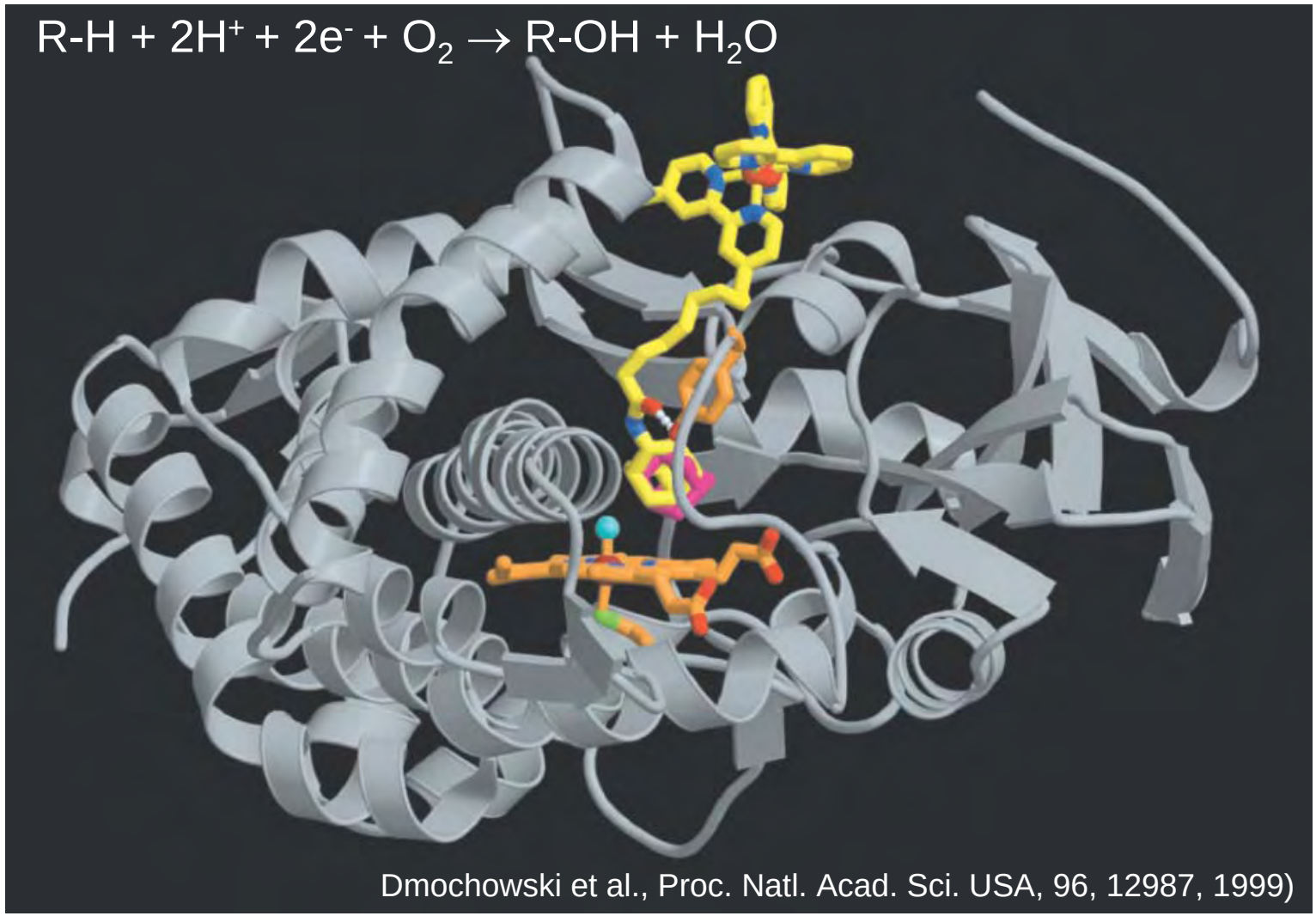
Bacteriorhodopsin – “Bio-Solar Cell”

- Energy of absorbed photons used to pump protons across membrane
- Hydrophobic proton access channel on cytoplasmic side



(Luecke, Richter and Lanyi, *Science* **289**, 1934, 1998)

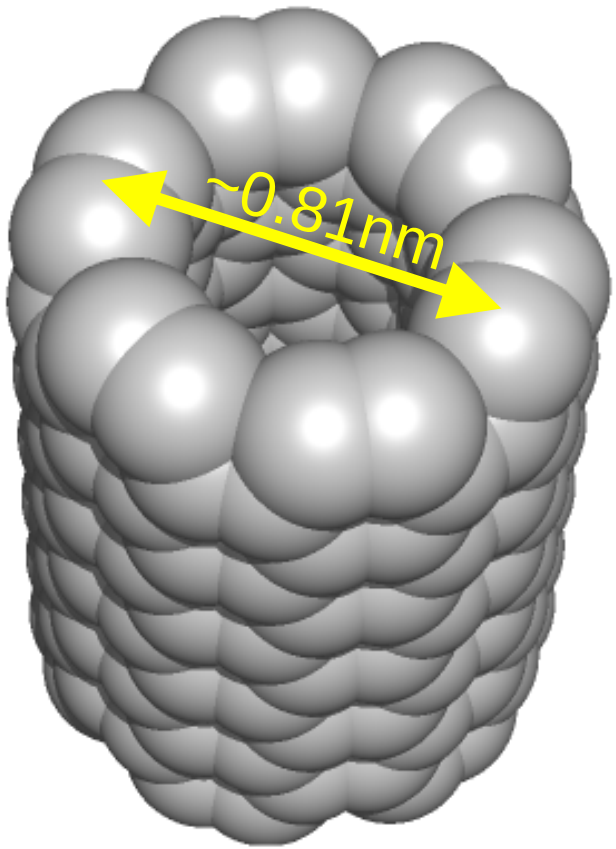
Cytochrome P450: “Bio-Chemistry”



Objectives: Role of Water in Biological Proton Transfer

1. Does water fill hydrophobic (i.e., “water repelling”) proton access channels?
2. What are the thermodynamic, dynamic, and structural properties of such molecularly confined water molecules?
3. Can water-filled nonpolar channels provide efficient mediators for proton transfer?
4. Role of protein and solvent dynamics in biological proton transfer

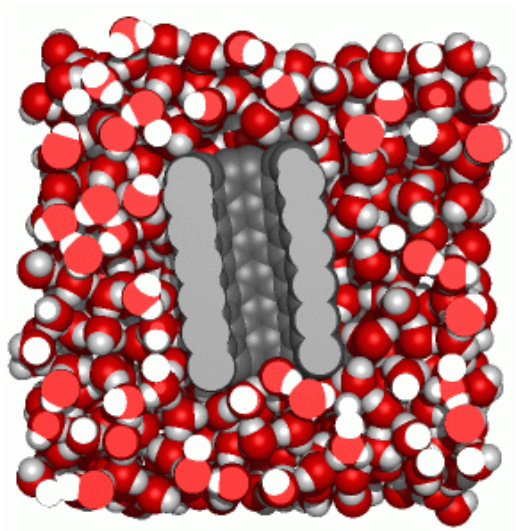
1. Does water fill nonpolar channels? Carbon Nanotube as Simplest Nonpolar Molecular Channel

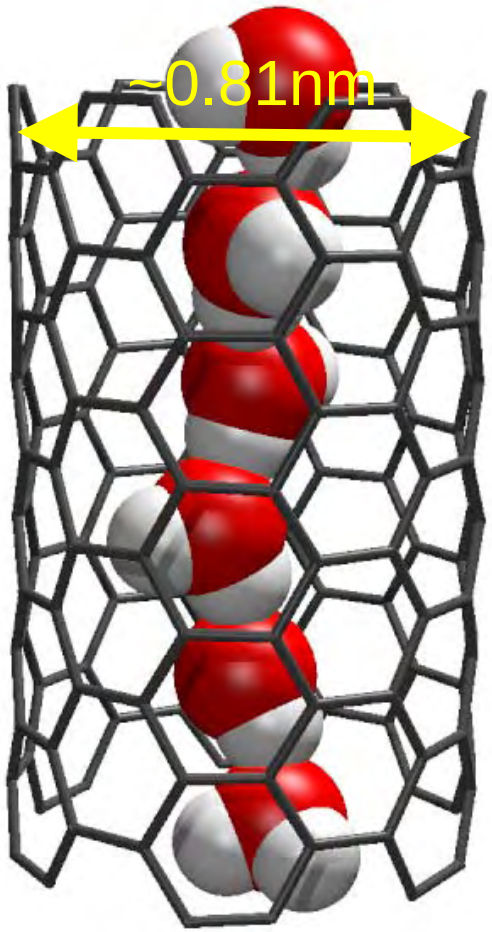


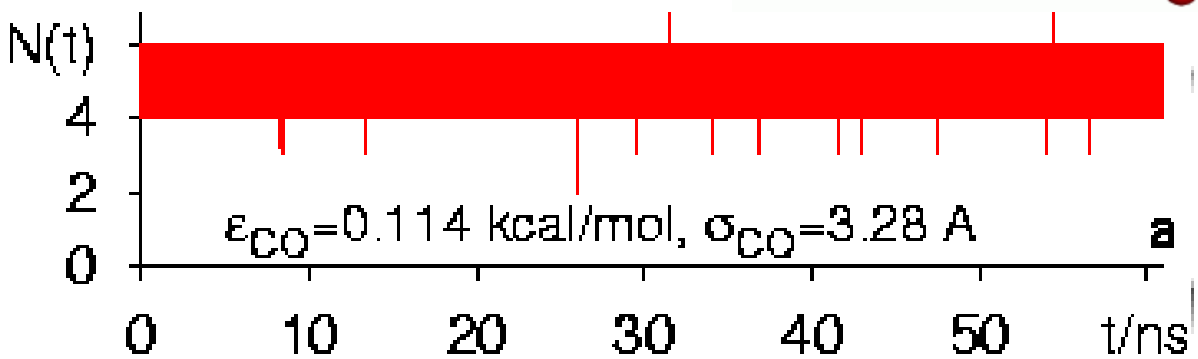
- Fullerene-type cylindrical molecules
- sp^2 carbons in `honeycomb' lattice
- Open or closed ends
- Single or multi-wall structure
- Diameters of ~1 nm and larger
- Chemically functionalizable

Molecular Dynamics Simulations of Carbon Nanotube in Water (Hummer, Rasaiah & Noworyta, *Nature* **414**, 188, 2001)

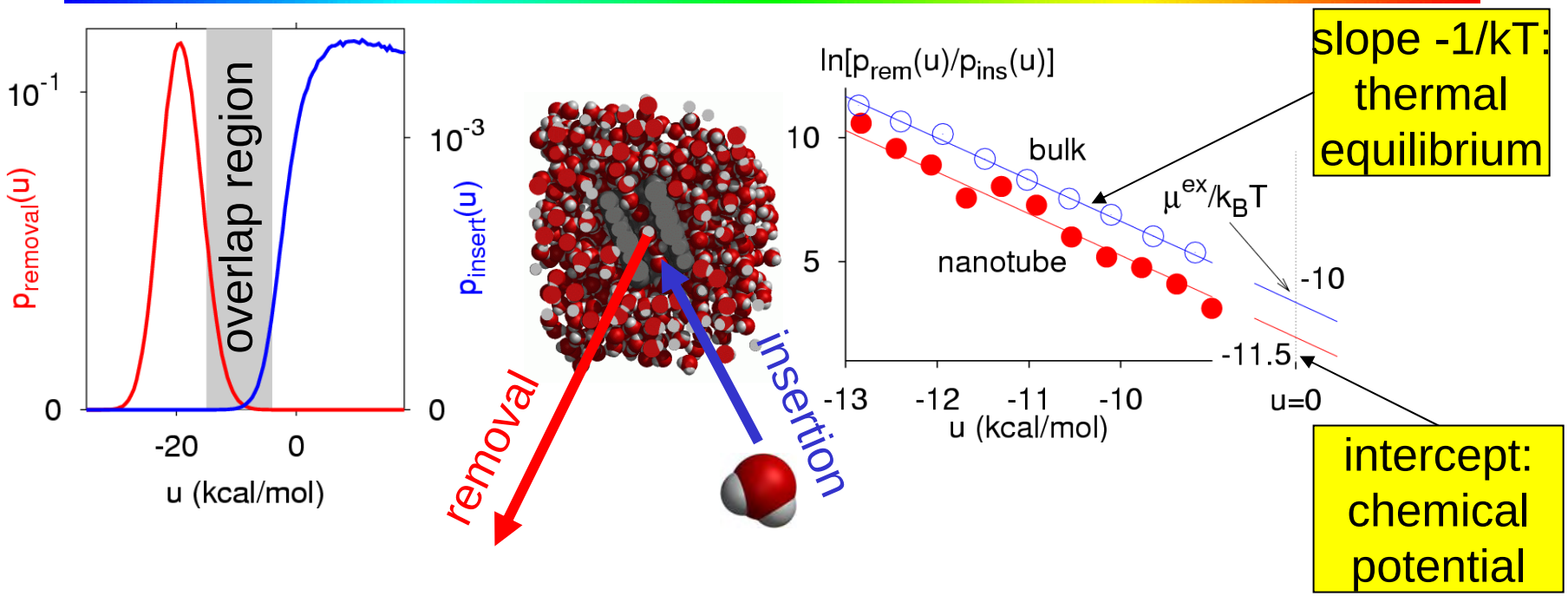
- Nanotube fills within picoseconds and remains filled for 66 nanoseconds
- Loss of hydrogen-bond energy?
- Loss of entropy because of 1D order?







Thermal and Chemical Equilibrium between Water in Nanotube and Bulk Phase



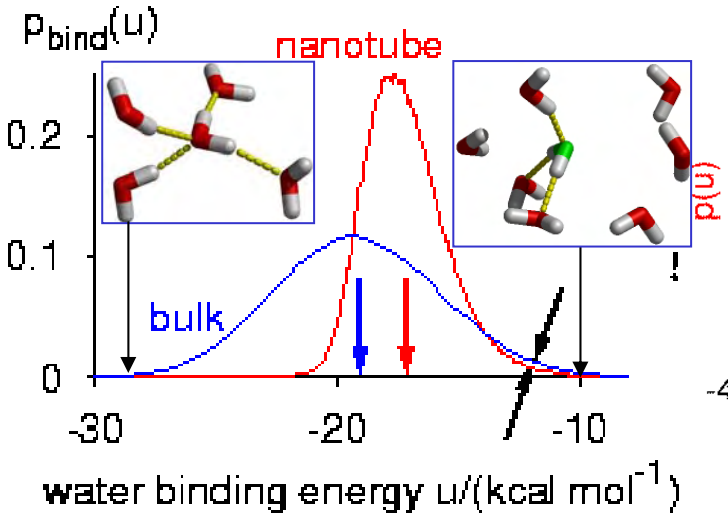
- Excess chemical potentials from histogram analysis
  - μ^{ex}_{w} = -6.05 ± 0.02 kcal/mol (bulk TIP3P water)
  - μ^{ex}_{nt} = -6.87 ± 0.07 kcal/mol (nanotube)
  - $-kT \ln(\langle N \rangle/\rho\Delta V)$ = -0.87 kcal/mol ~ $\mu^{ex}_{nt} - \mu^{ex}_{w}$

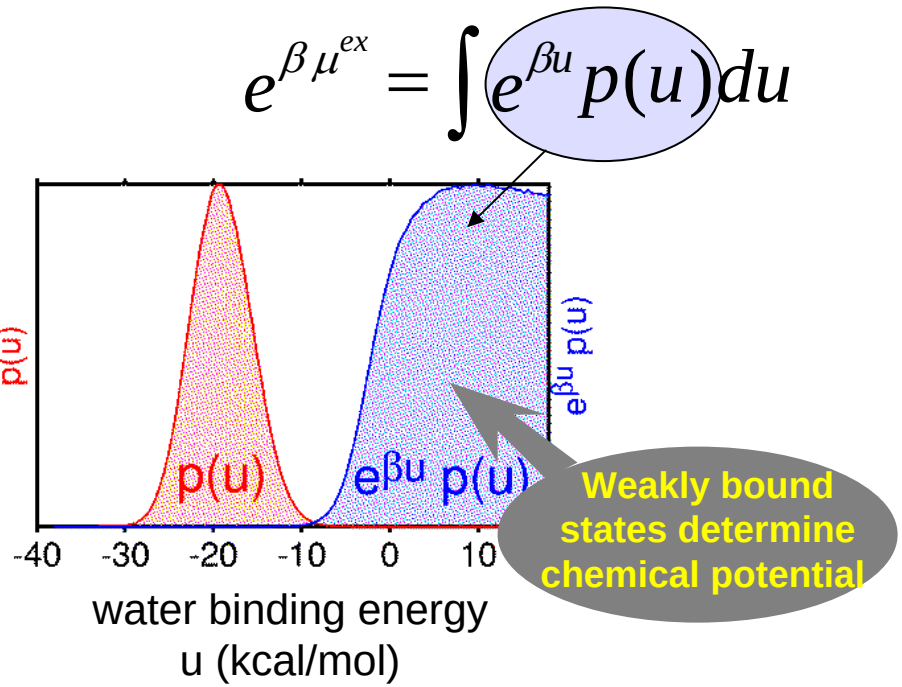
} chemical equilibrium

Thermodynamics from Binding Energies: High-Energy Tail Determines Vapor Pressure

- Channel shields from fluctuations
  - Degenerate ground state: low population of high energy states

$$e^{\beta \mu^{ex}} = \int e^{\beta u} p(u) du$$





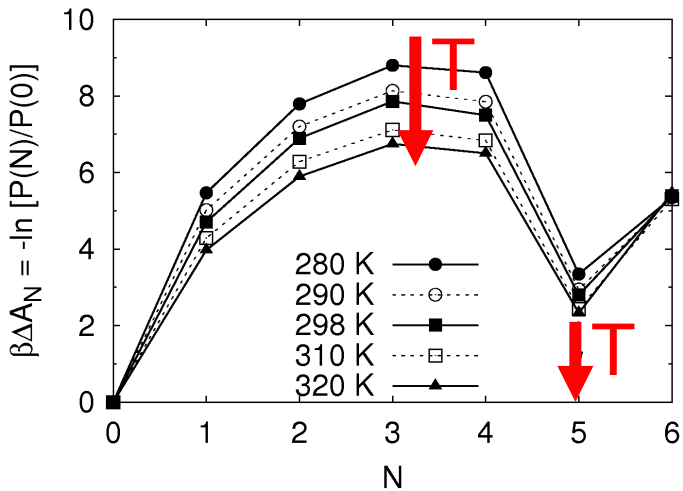
Thermodynamics of Filling from Grand-Canonical Partition Function for Infinite Periodic Tube

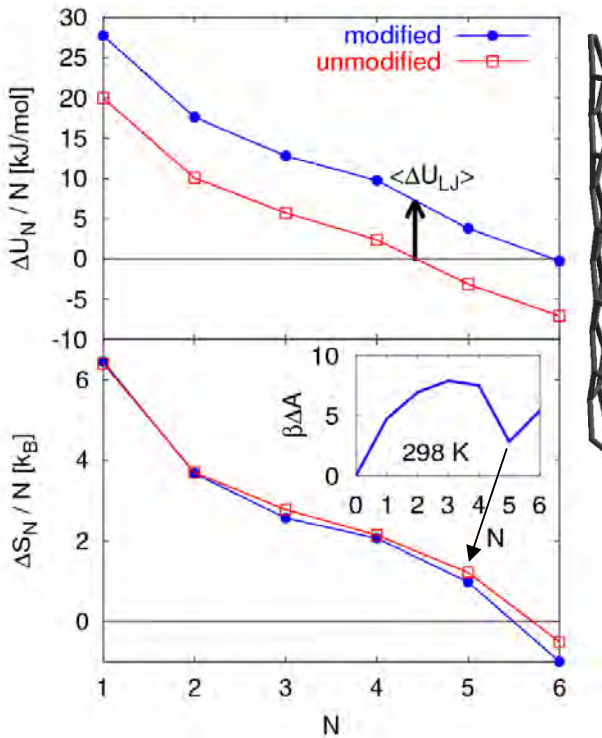
(Vaitheeswaran, Rasaiah, and Hummer, J. Chem. Phys. **121**, 7955, 2004)

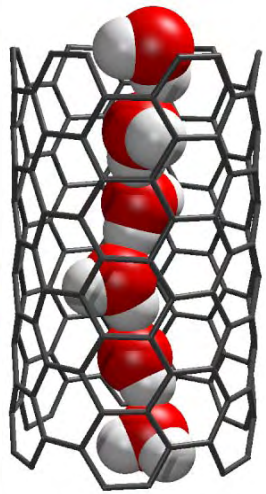
- Grand-canonical partition function calculated term-by-term using histogram method

$$\frac{P(N+1)}{P(N)} = \frac{\rho V}{N+1}\left\langle \exp[-\beta(U_{N+1}-U_N)]\right\rangle_N$$

- Positive entropy of filled state





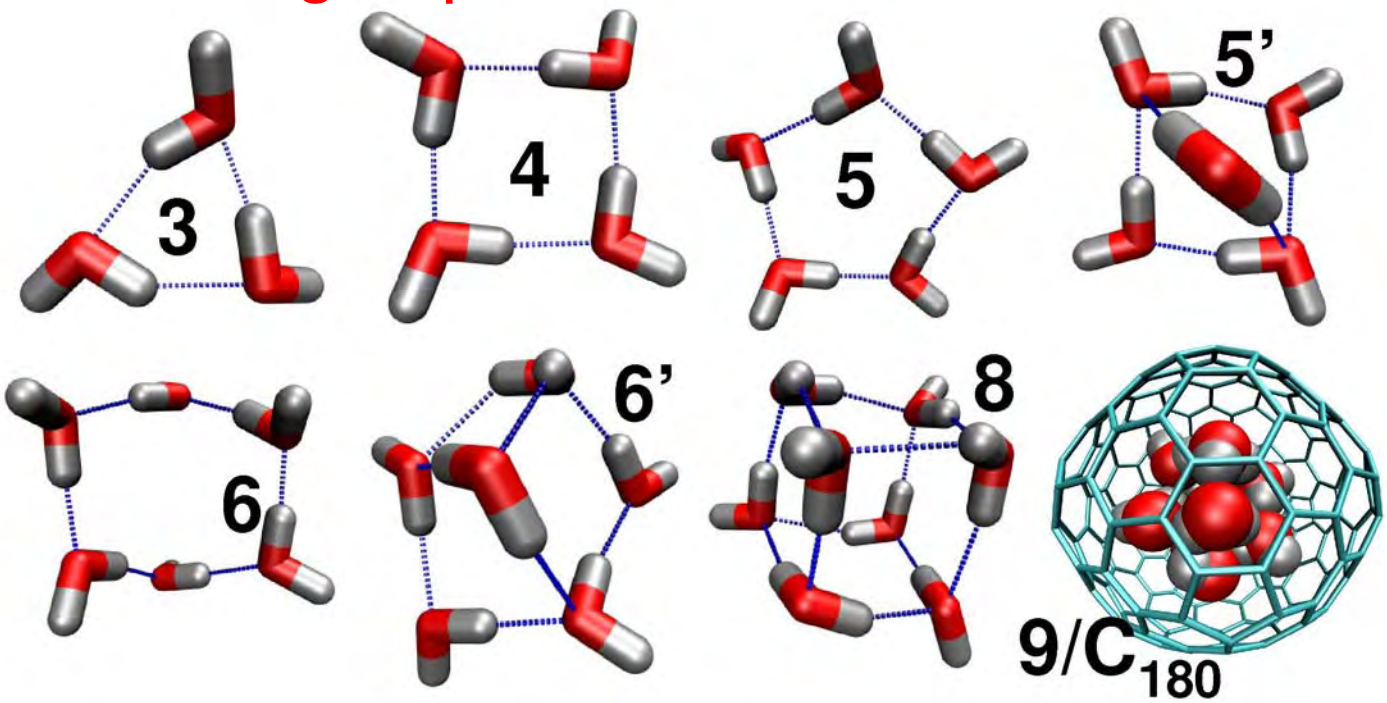


Dangling OH bond compensates entropy loss of 1D ordering

Water in Nonpolar Cavities

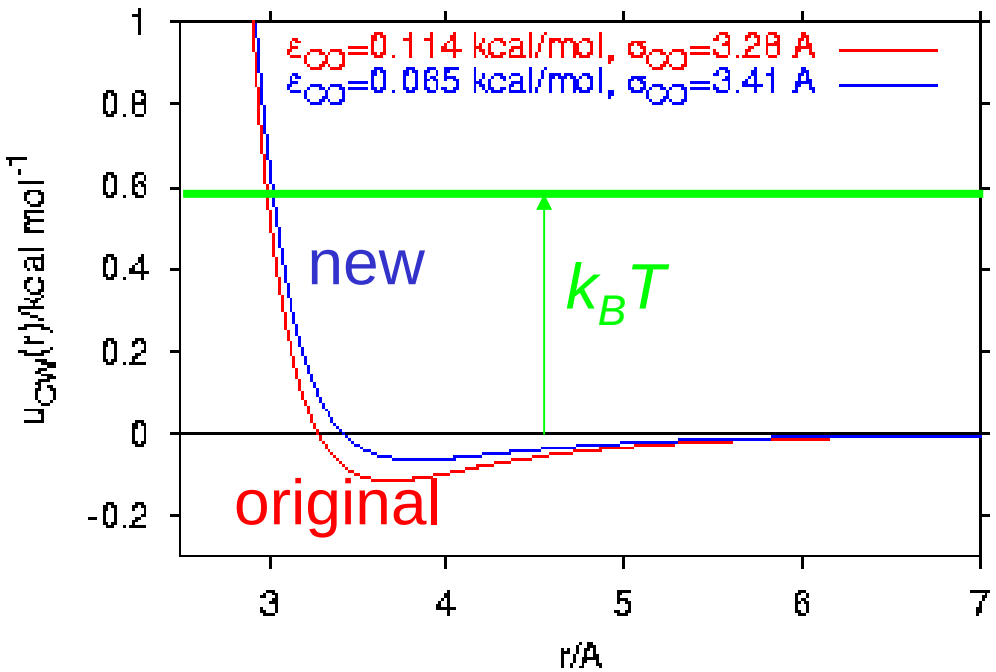
(Vaitheeswaran, Yin, Rasaiah, and Hummer, *Proc. Natl. Acad. Sci. USA* **101**, 17002, 2004)

- Water clusters in weakly polar spherical cavities can be thermodynamically stable and resemble gas-phase clusters

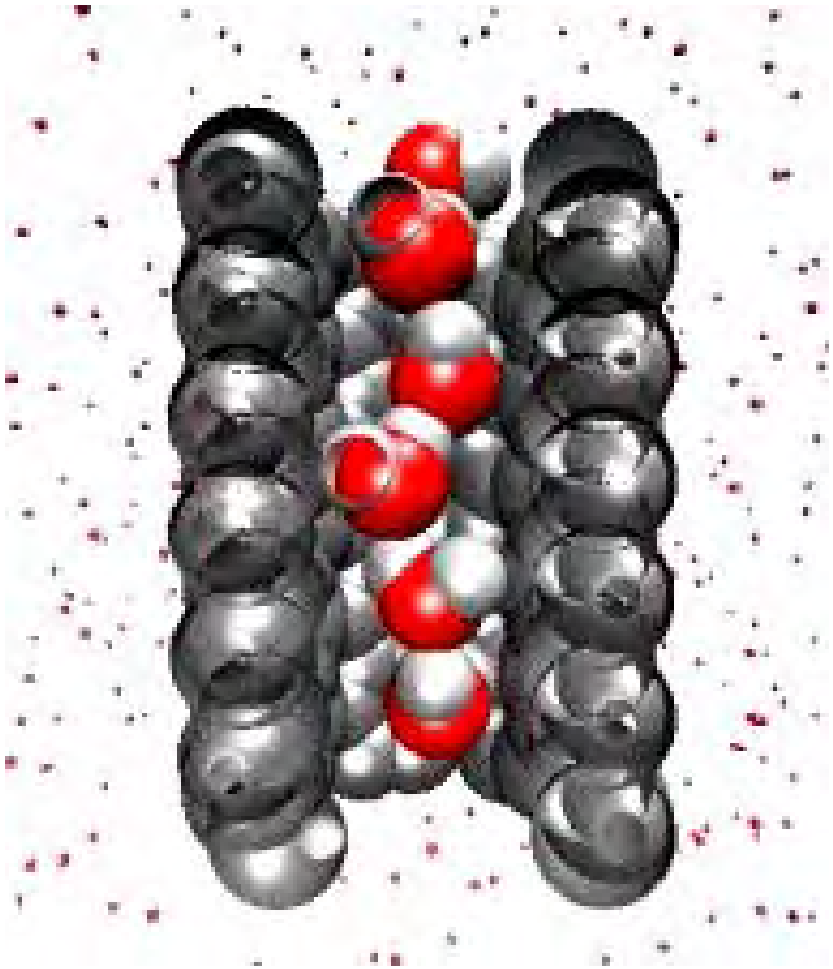


Effects of Interaction Potentials and Solvent Conditions: Modified Carbon-Water Attractions

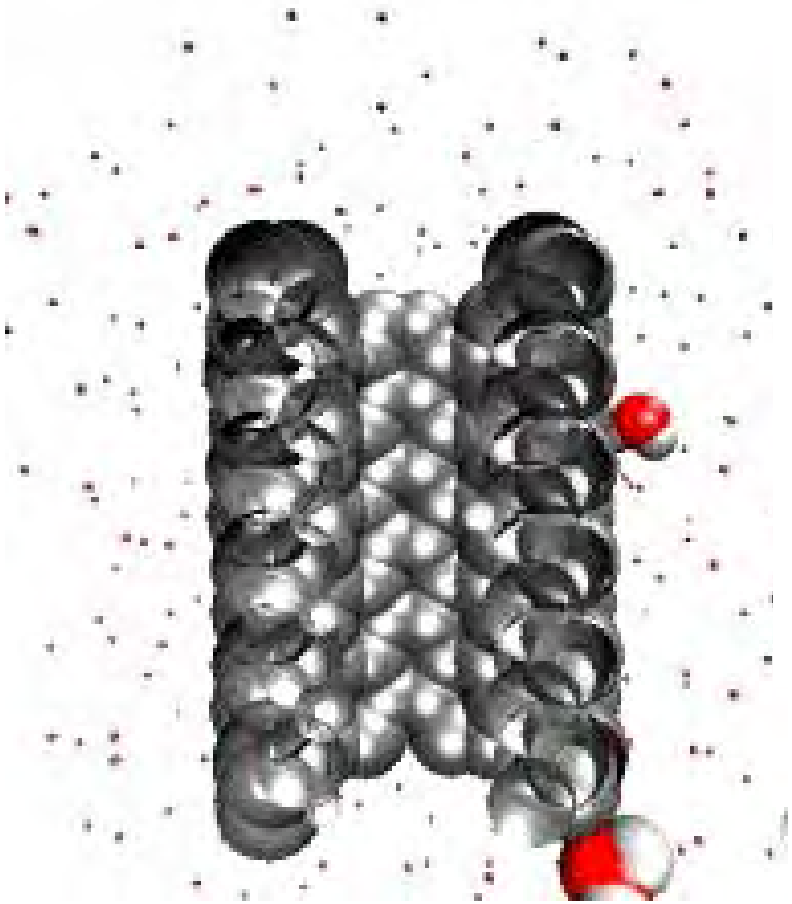
- Modified carbon parameters
  - ε_{CO} = 0.065 (0.114) kcal/mol
  - σ_{CO} = 3.41 (3.28) A

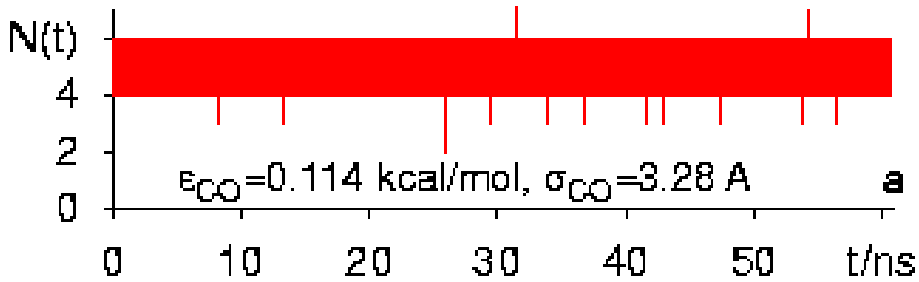


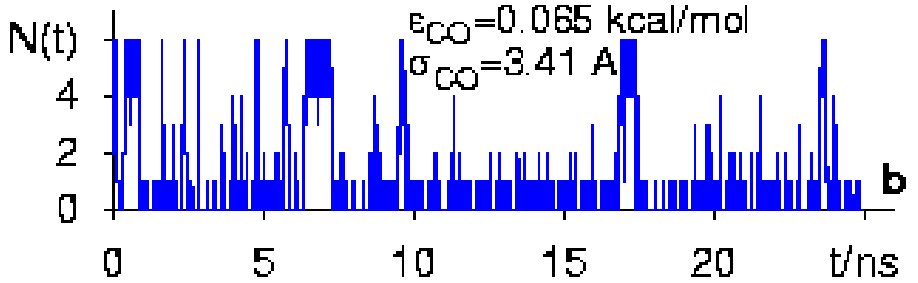
Emptying Transition



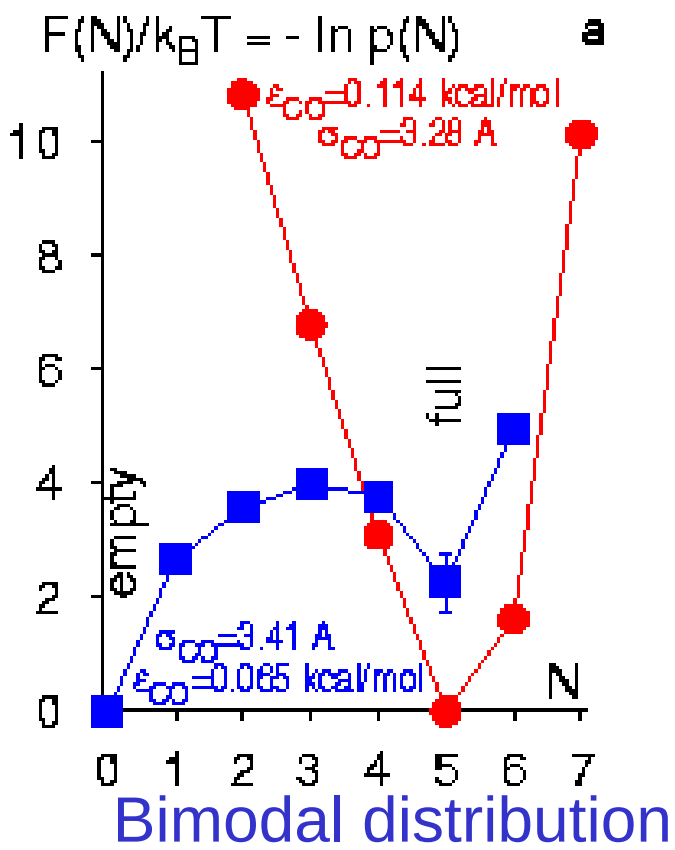
Filling/Emptying Transitions





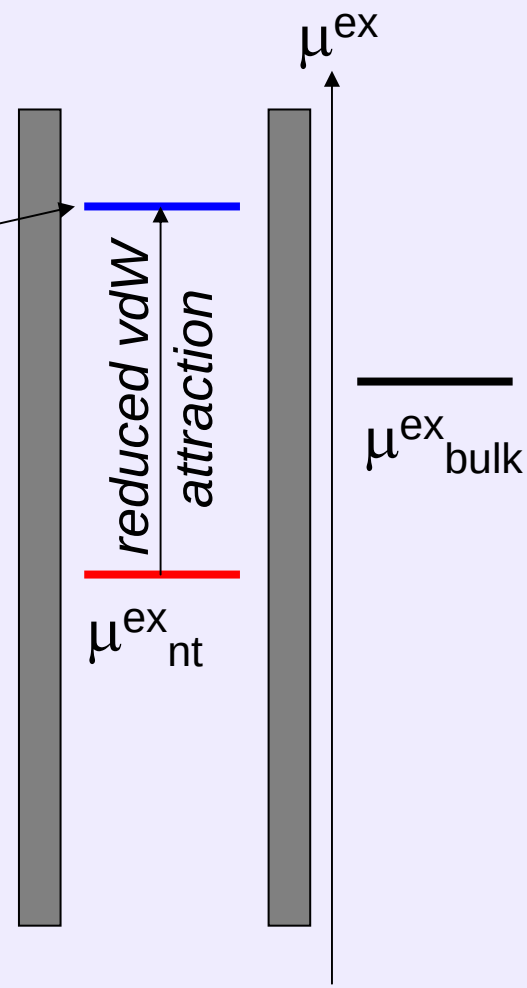


Change in Potential Parameters/Solvent Conditions Results in Bimodal Occupancy Distribution

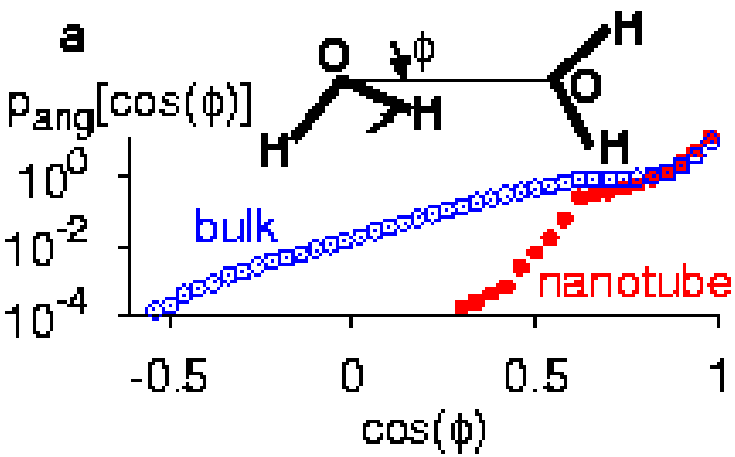


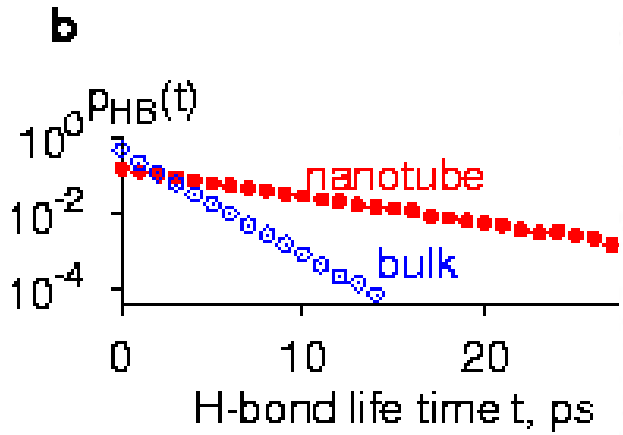
Bimodal distribution

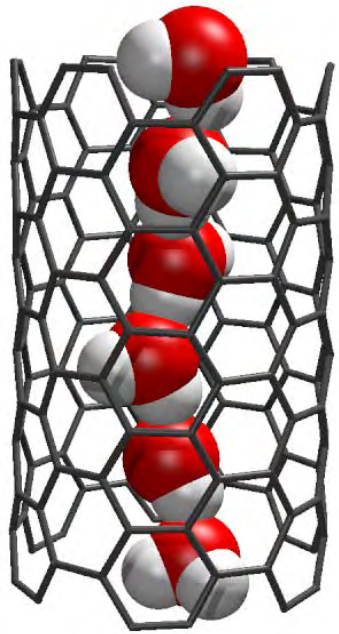
Average occupancy $N \sim \exp(-\beta \Delta \mu^{ex})$ corresponds to 'unstable' fragmented chain
→ fluctuations between filled and empty states ('coexistence')



2. Properties of 1D Confined Water: Long-Lived and Strongly Oriented Hydrogen Bonds in Narrow Channel



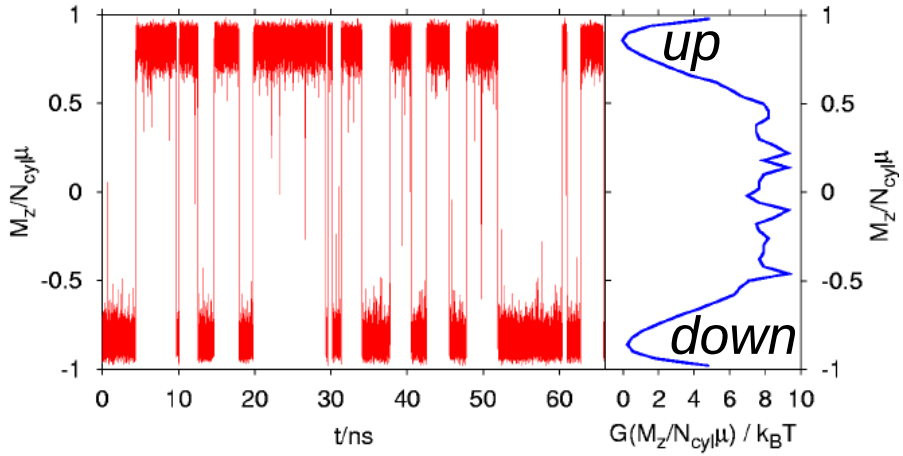


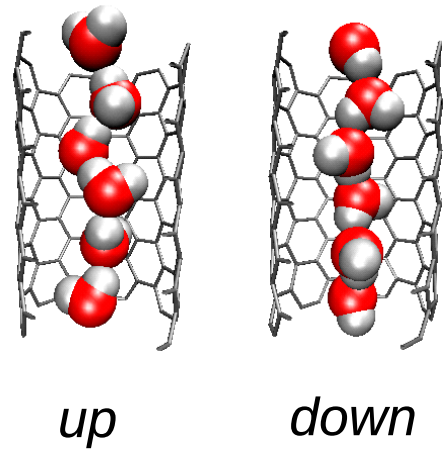


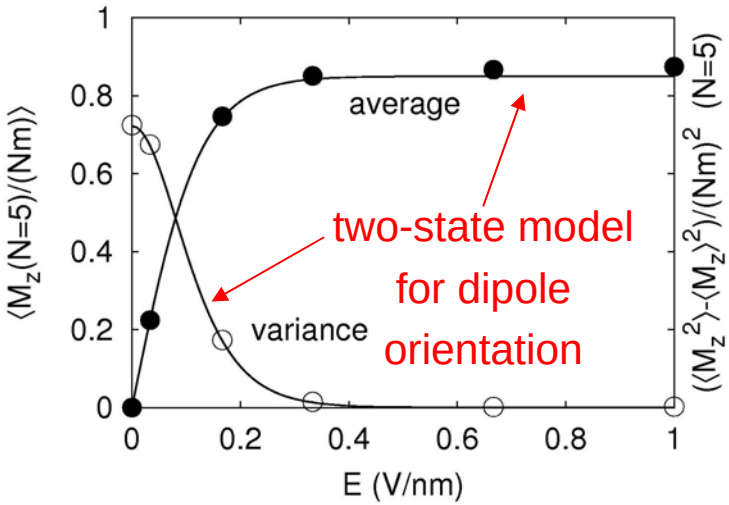
- H-bond angles > 30^{o}
  - Water: 37%
  - Nanotube: <15%
- H-bond Lifetime
  - Water: 1ps
  - Nanotube: 5ps

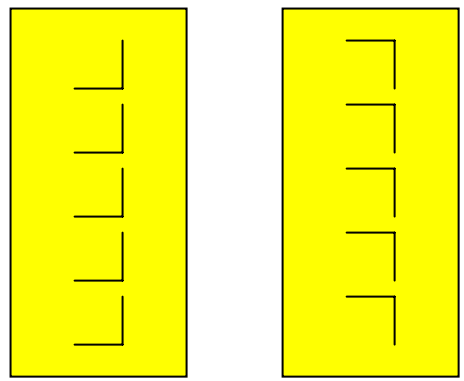
Collective Dipolar Orientation of Water Chain Governed by Local Electric Field

(Vaitheeswaran, Rasaiah, and Hummer, J. Chem. Phys. **121**, 7955, 2004;
Best and Hummer, Proc. Natl. Acad. Sci. USA, in press, 2005)



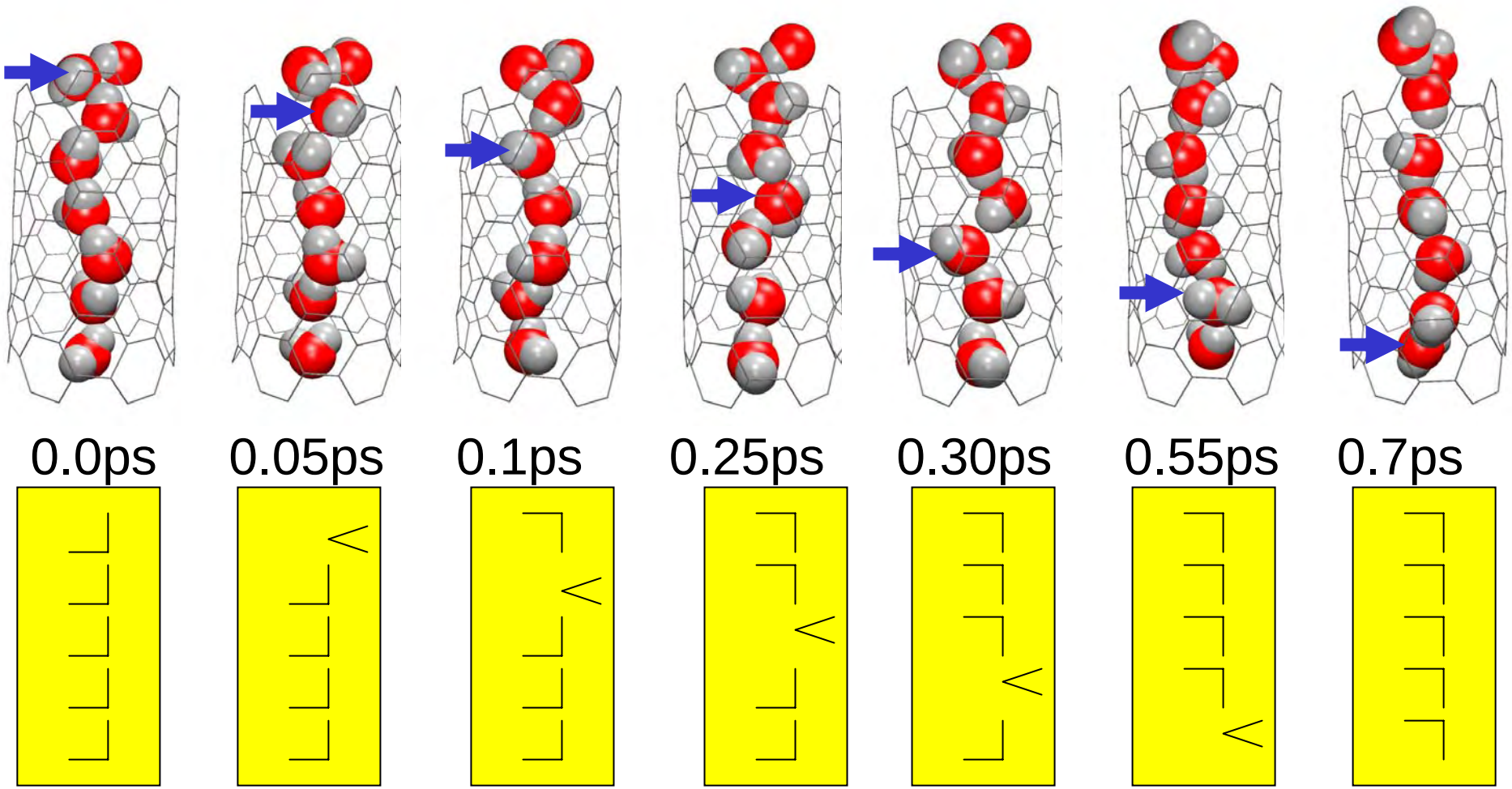






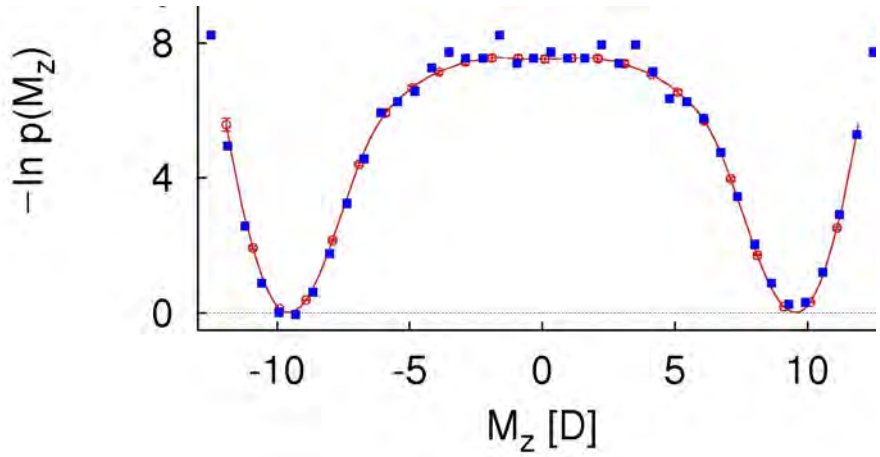
Propagating Hydrogen Bond Defect Reorientates Chain

(Best and Hummer, Proc. Natl. Acad. Sci. USA, in press, 2005)



Rate of Dipolar Reorientation is Slow

- Defect motion is diffusive
  - Typically ~7 recrossings of M_z=0 dividing surface
  - Langevin damping frequency of ~200 ps^{-1}, with an average transition path duration of ~2 ps

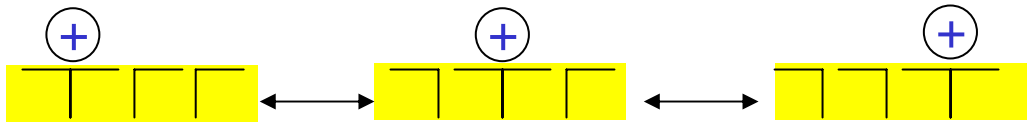


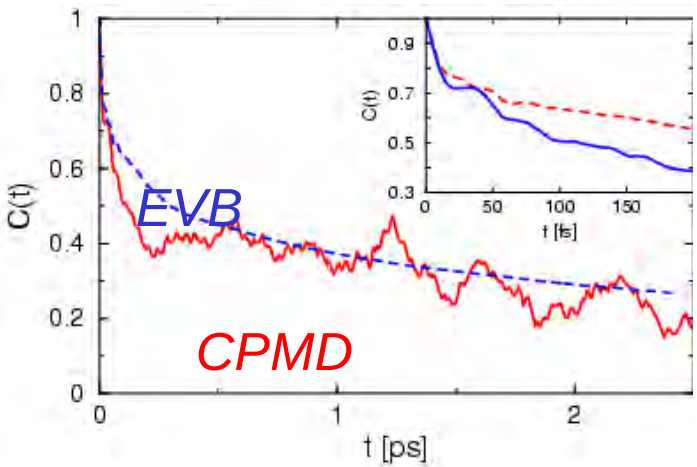
- Rate of dipole reorientation is slow
  - k ~ 1/(2 ns) for solvated tube filled with ~5 H_2O
  - k > 1/(20 ns) for tube in low-dielectric environment
  - Free energy barrier of ~8 k_BT dominated by electrostatics

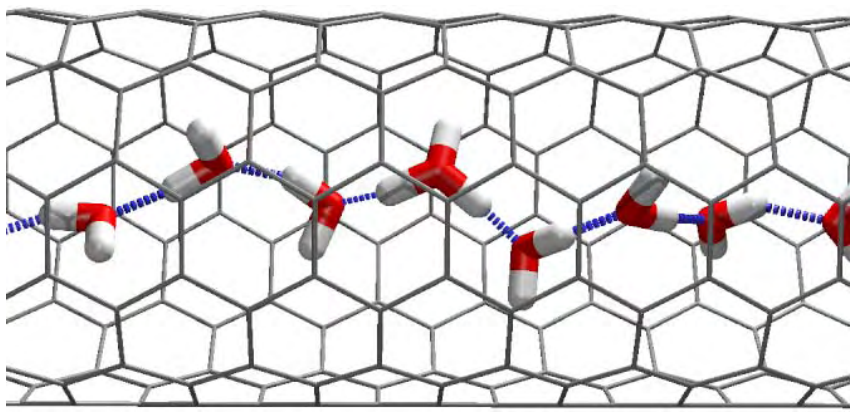
3. Proton Transport along 1D Water Wires

(Dellago, Naor and Hummer, *Phys. Rev. Lett*. **90**, 105902, 2003)

- Molecular dynamics simulations of water and excess proton in nanotube
  - Car-Parrinello dynamics (DFT/BLYP)
  - Empirical-valence-bond model (Warshel and Weiss, *J. Am. Chem. Soc.* **102**, 6218, 1980; Schmitt and Voth, *J. Phys. Chem. B* **102**, 5547, 1999)
  - Grotthuss H^+ relay (de Grotthuss, C. J. T. *Annal*. *Chim*. **58**, 54, 1806):

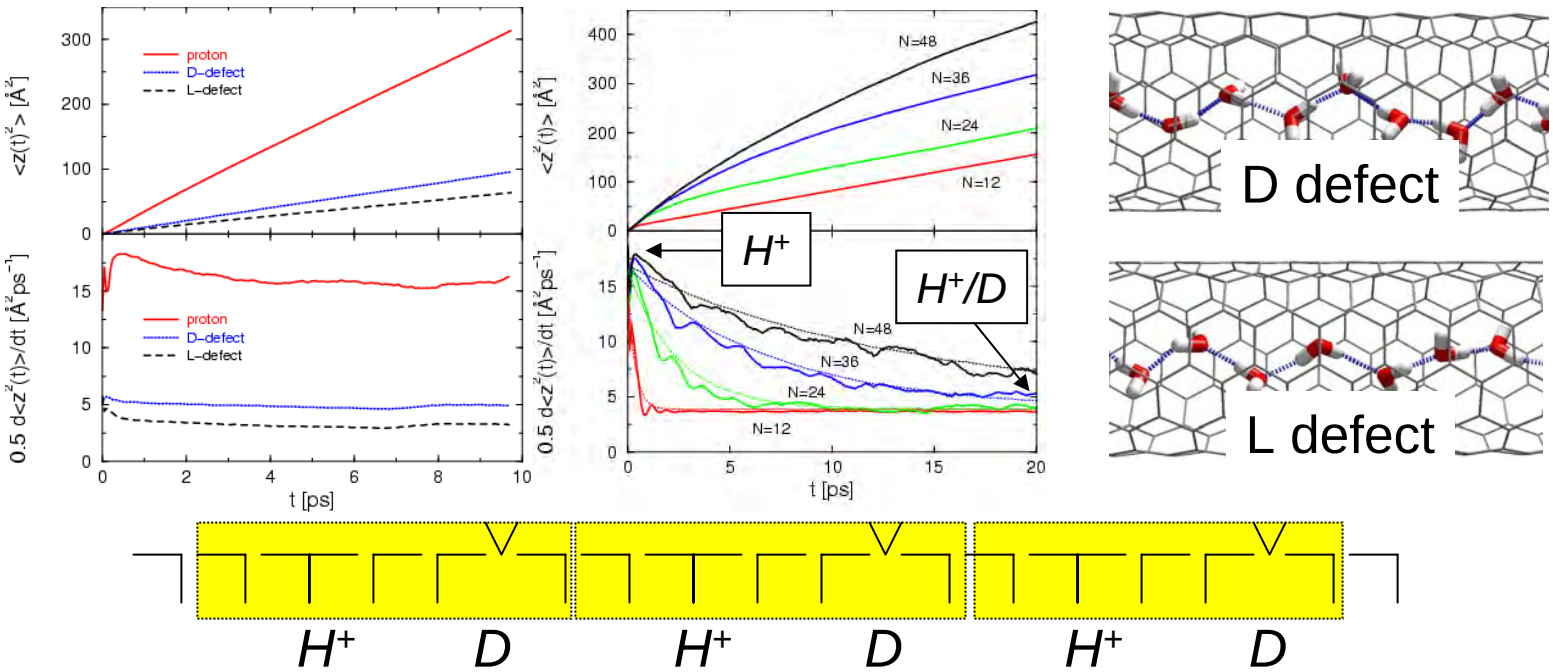






1D Proton Transport Coupled to Defect Motion

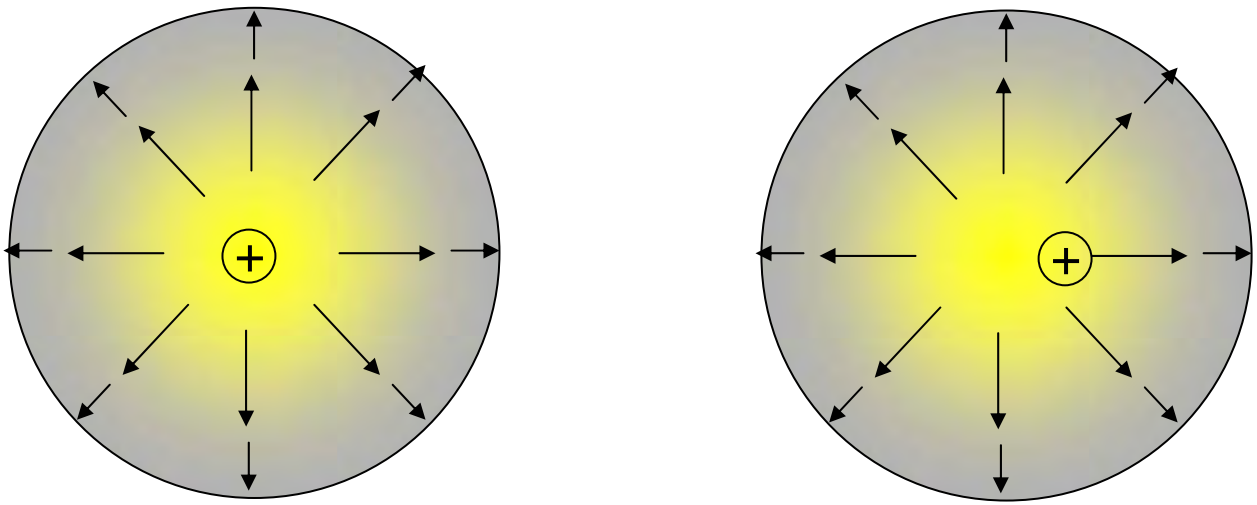
- Strong 1/r electrostatic coupling between H^+ and H-bond (D) defect in periodic tube (both defects carry positive effective charge):
  - 10-fold reduction of apparent diffusion constant



1D vs 3D Proton Conduction

- Proton diffusion approximately 40 times faster than in bulk water: $D(H^+) \approx 170 \times 10^{-5}\ cm^2 s^{-1}$
- 1D: local

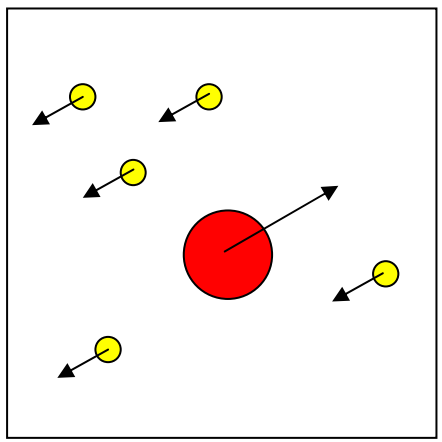
- 3D: non-local



System-size dependence of diffusion coefficients

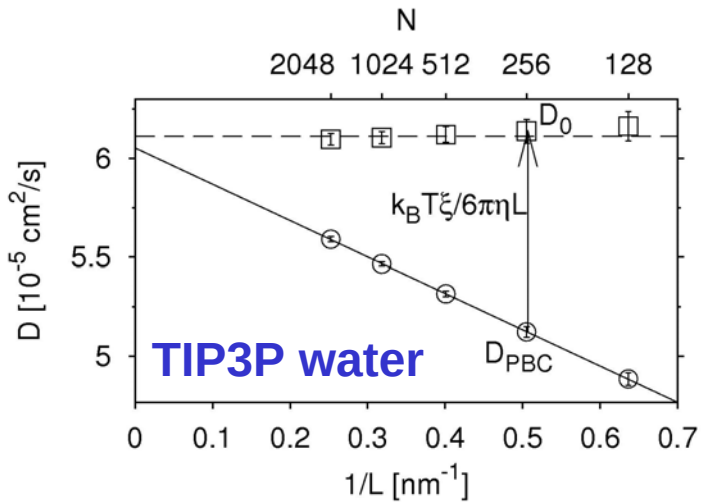
(Yeh and Hummer, *Biophys. J.* **86**, 681, 2004; *J. Phys. Chem. B* **108**, 15873, 2004;
Dünweg & Kremer, *J. Chem. Phys.* **99**, 6983, 1993)

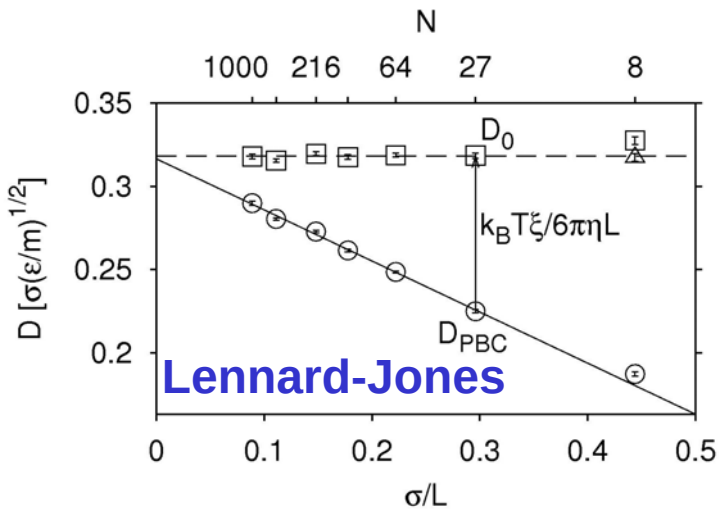
- Conservation of momentum imposes zero net force



- Hydrodynamic theory

$$D_{\mathrm{app}}(L) = D_0 - \frac{k_{\mathrm{B}} T \xi_{\mathrm{EW}}}{6\pi\eta L}$$

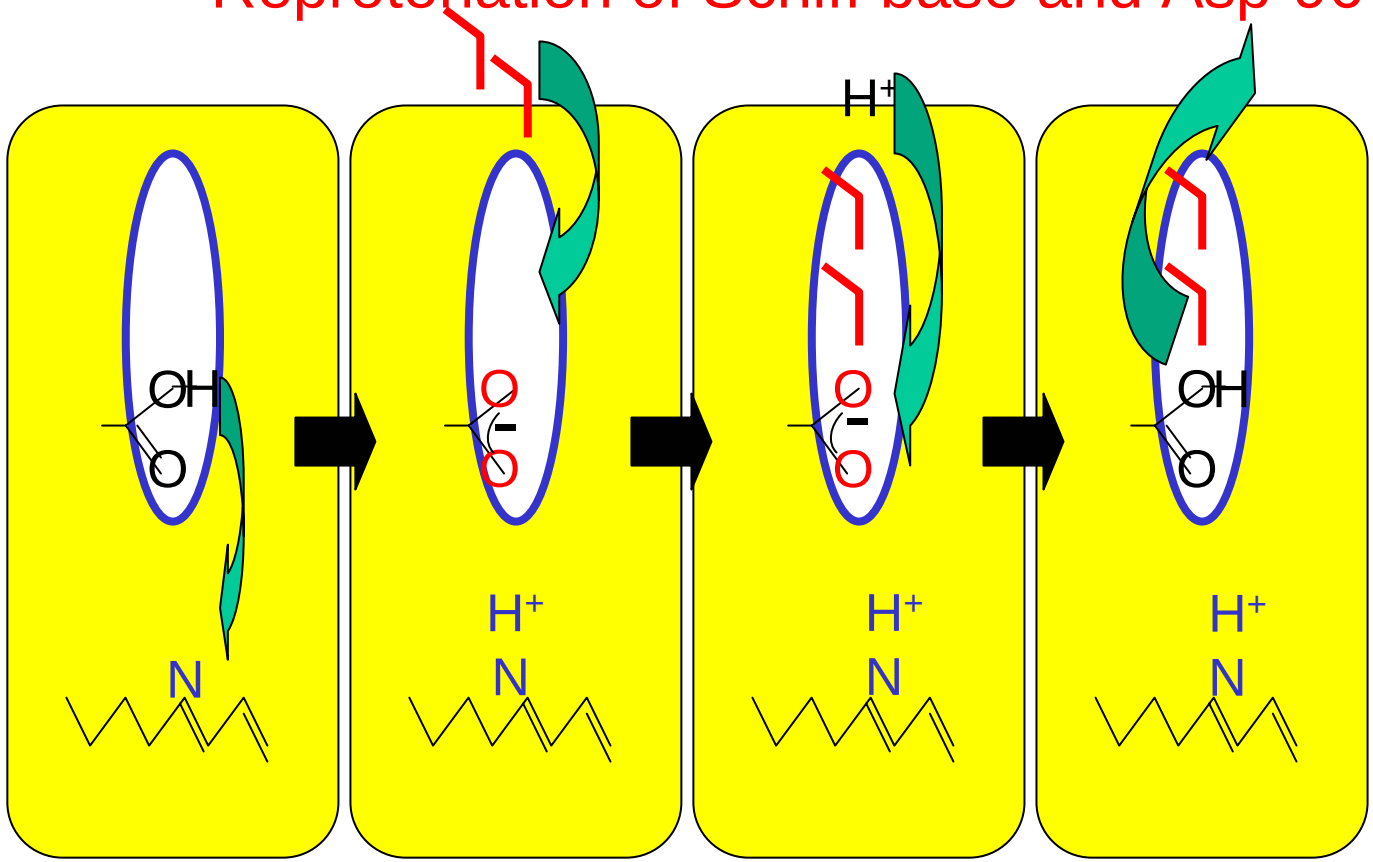


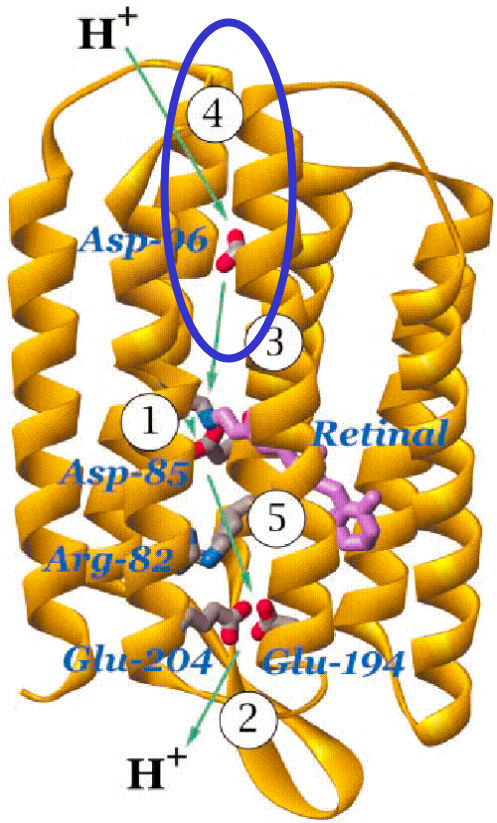


3. Water Mediated Proton Transfer in Light-Driven Proton-Pump Bacteriorhodopsin

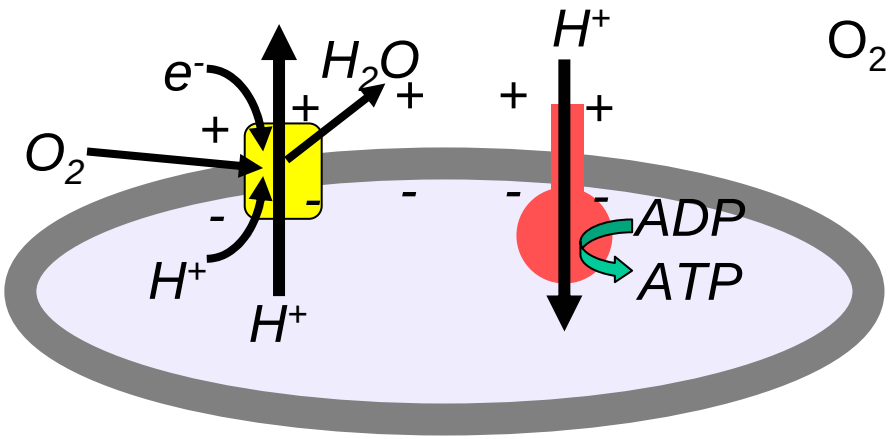
(Hummer, Rasaiah, Noworyta, ICCN Proceedings, 2002)

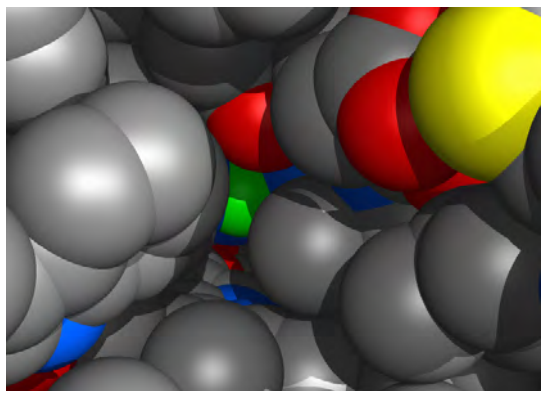
- Transient water networks in trapped intermediates (e.g., Schobert, Brown, Lanyi, J. Mol. Biol. 330, 553, 2003)
- Reprotonation of Schiff base and Asp-96 from solvent





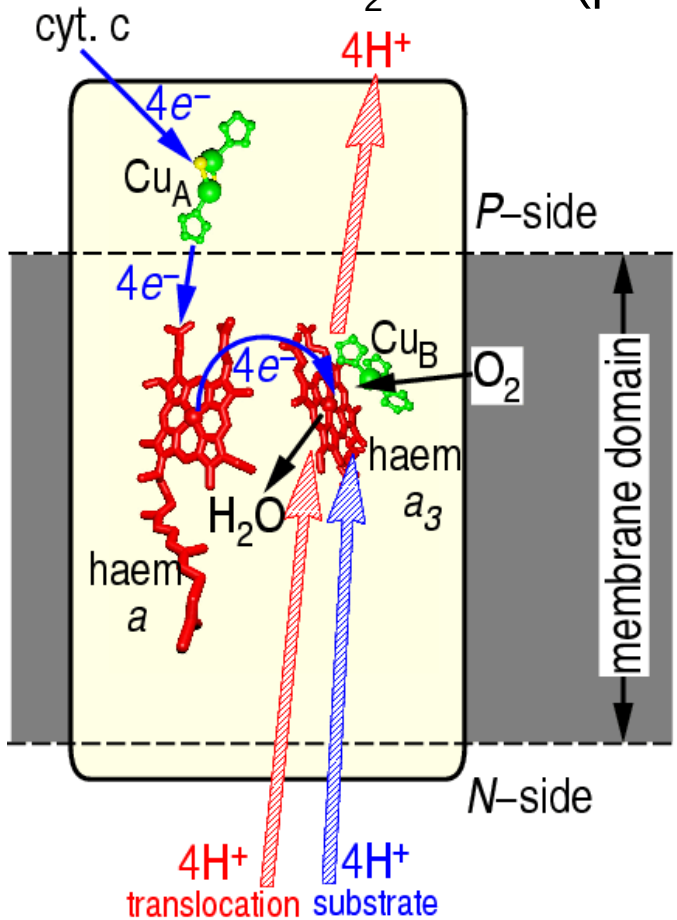
Cytochrome c Oxidase





Functional role of water in nonpolar active-site cavity?

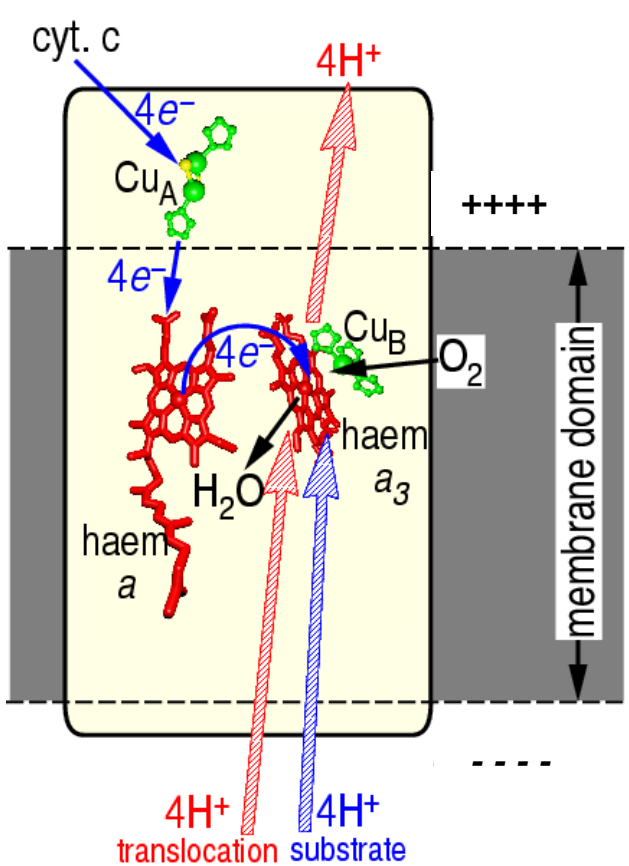
$O_2 + 8H^+ + 4e^- \rightarrow 2H_2O + 4H^+(\text{pumped})$

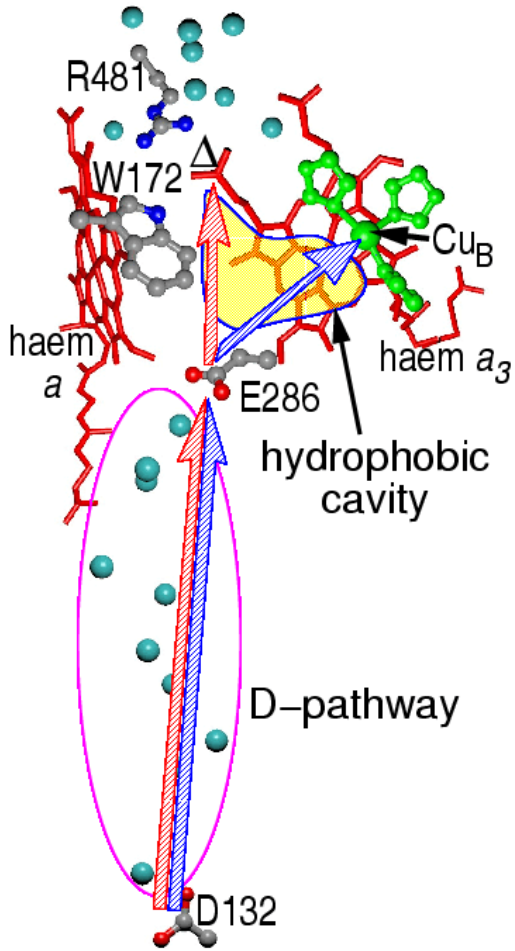


Proton Pumping in Cytochrome C Oxidase

(Wikström, Verkhovsky and Hummer, *Biochim. Biophys. Acta-Bioenerget.* **45238**, 1, 2003)

- Gating of *'chemistry'* and *'pumping'*
  - Pumped protons (for energy conservation) and chemical protons (for water production) use same pathway
  - Hydrophobic cavity between hemes is possible fork in pathway

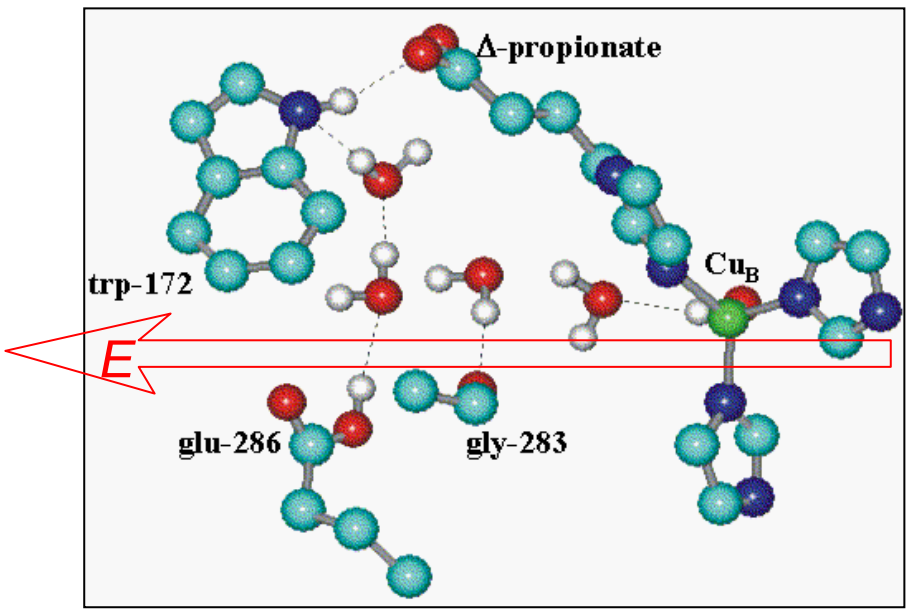




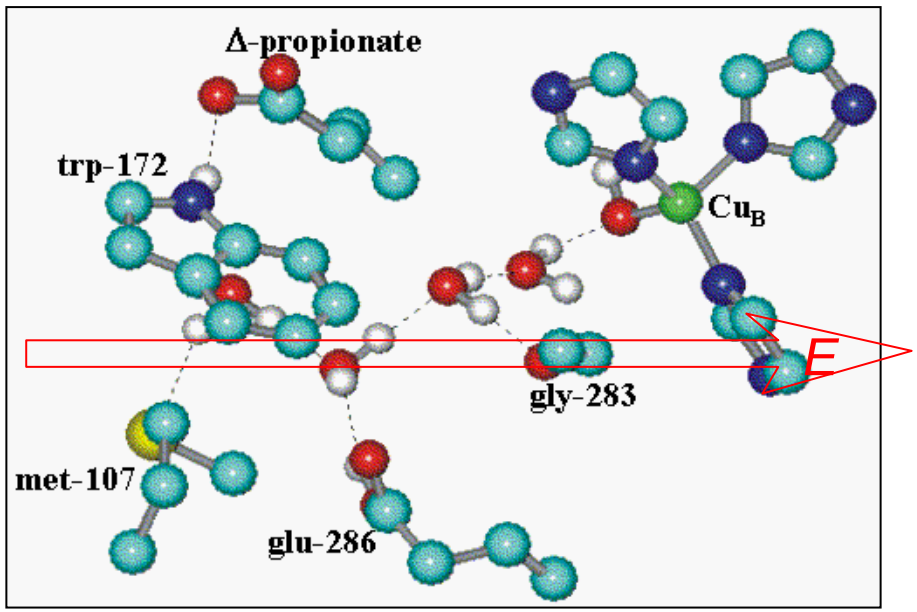
Heme-a/Heme-a_3/Cu_B Charge Distribution Switches Water-Chain Orientation

- Reorientation within ~1 ps of electron transfer in molecular dynamics simulations

- Heme-a reduced
- Heme-a_3/Cu_B oxidized



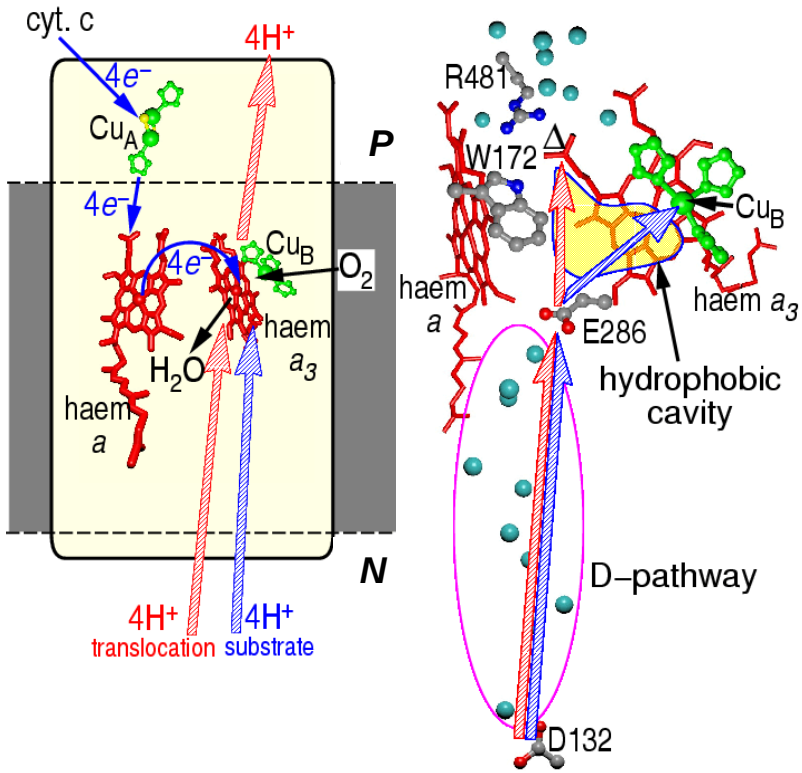
- Heme-a oxidized
- Heme-a_3/Cu_B reduced



Proton Pumping in Cytochrome C Oxidase

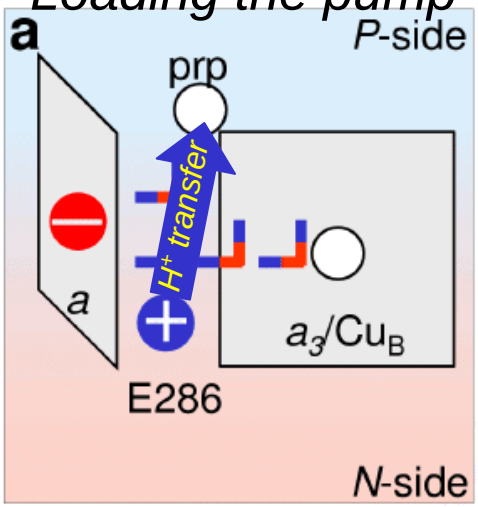
(Wikström, Verkhovsky and Hummer, *Biochim. Biophys. Acta-Bioenerget.* **45238**, 1, 2003)

- Gating of *'chemistry'* and *'pumping'*
  - Water in cavity orientated by electric field between hemes a and a_3/Cu_B
  - Switching between H^+ paths by water orientation (*'H^+ diode'*)
- Coupling of O_2 reduction to pumping
  - H^+ transfer from E286 to heme-a_3 propionate provides gate (*'solvent fluctuation'*) for electron transfer from heme a to heme-a_3/Cu_B
  - 'Pumped' H^+ trapped by electron transfer and reprotonation of E286



Water-Gated Proton Pump

Loading the pump



H+ transfer

E286 to propionate

electron transfer

water reorientation

E286 H+ uptake from N- side

substrate H+ transfe

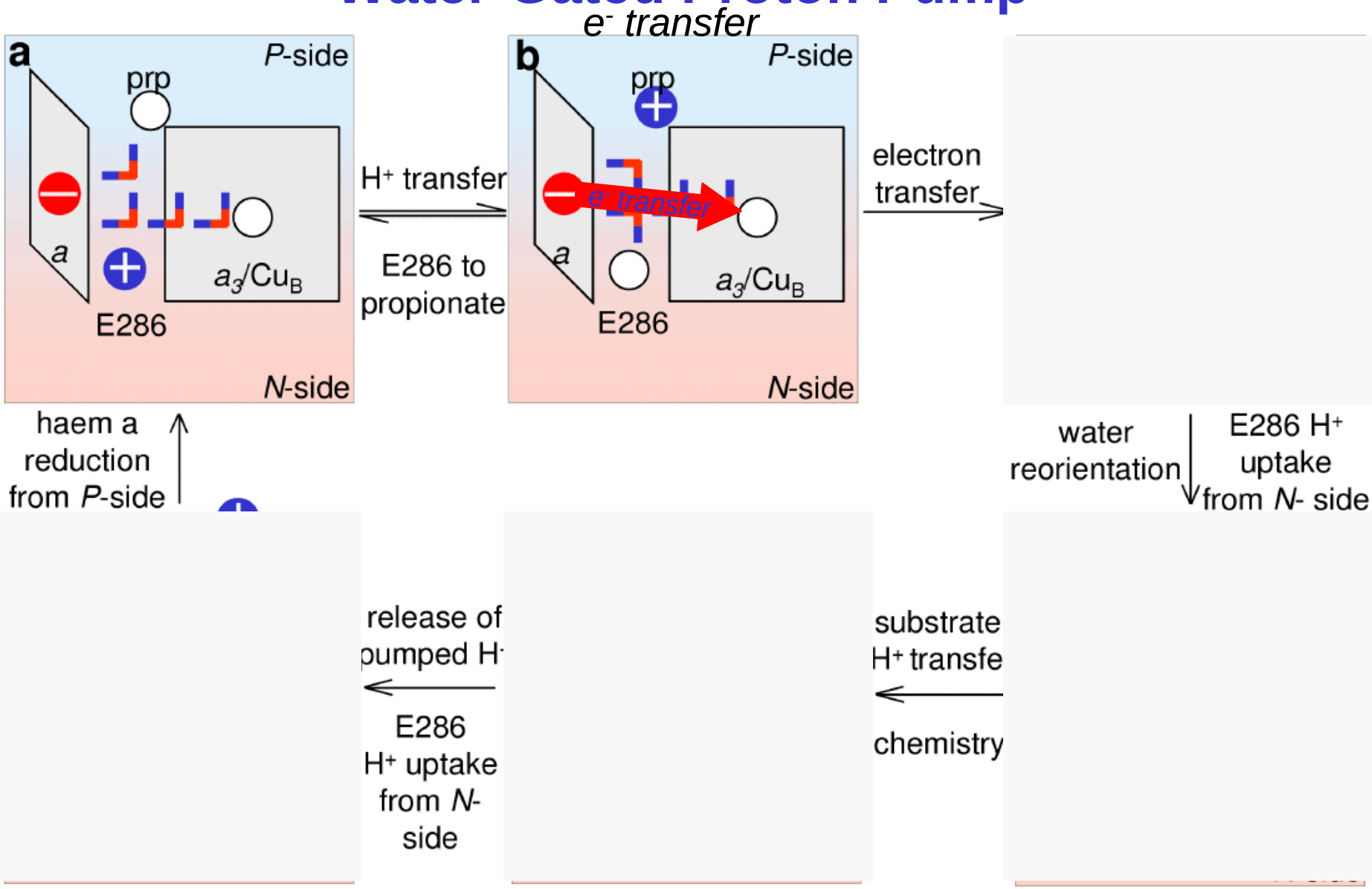
chemistry

release of pumped H+

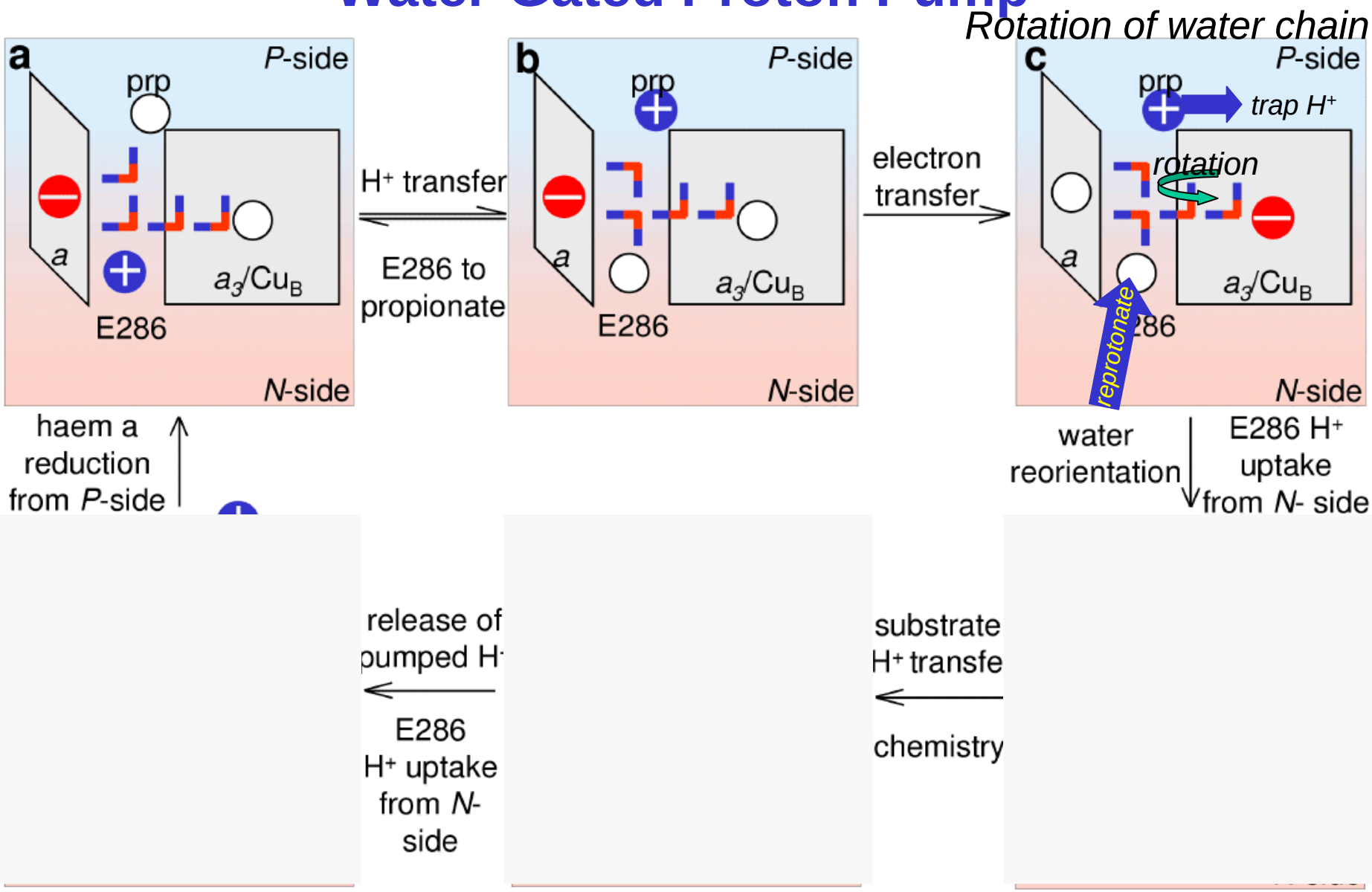
E286 H+ uptake from N-side

haem a reduction from P-side

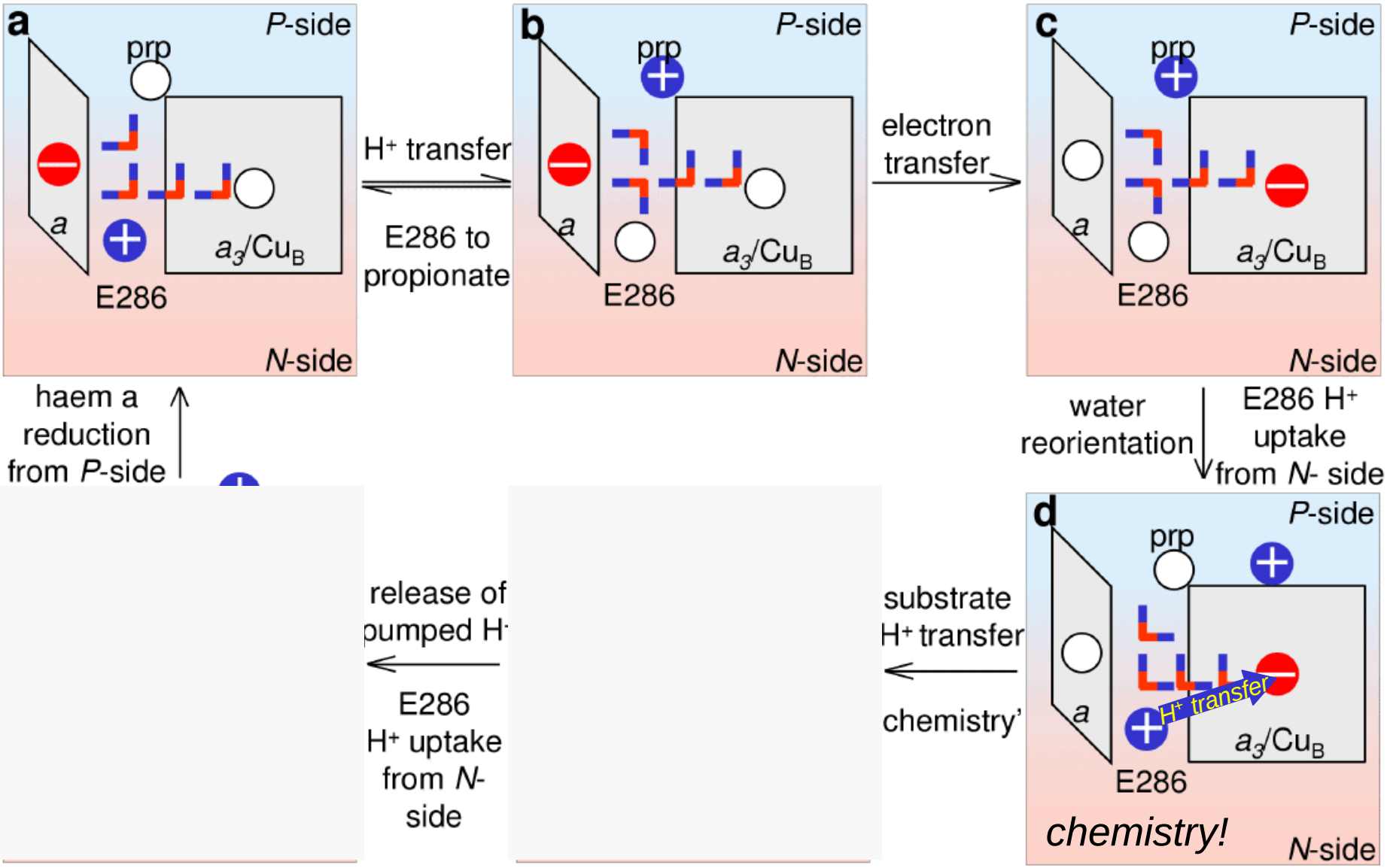
Water-Gated Proton Pump



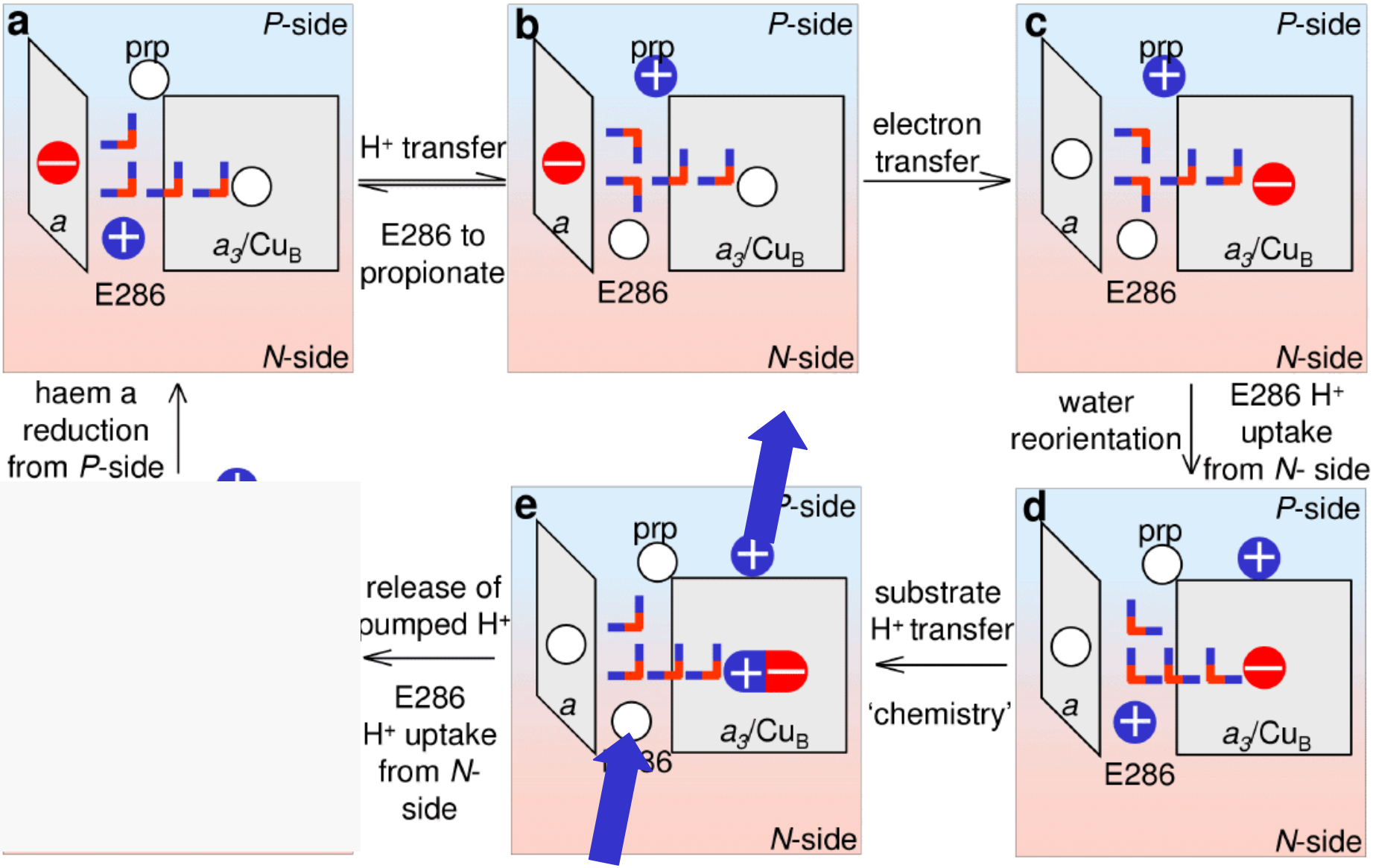
Water-Gated Proton Pump



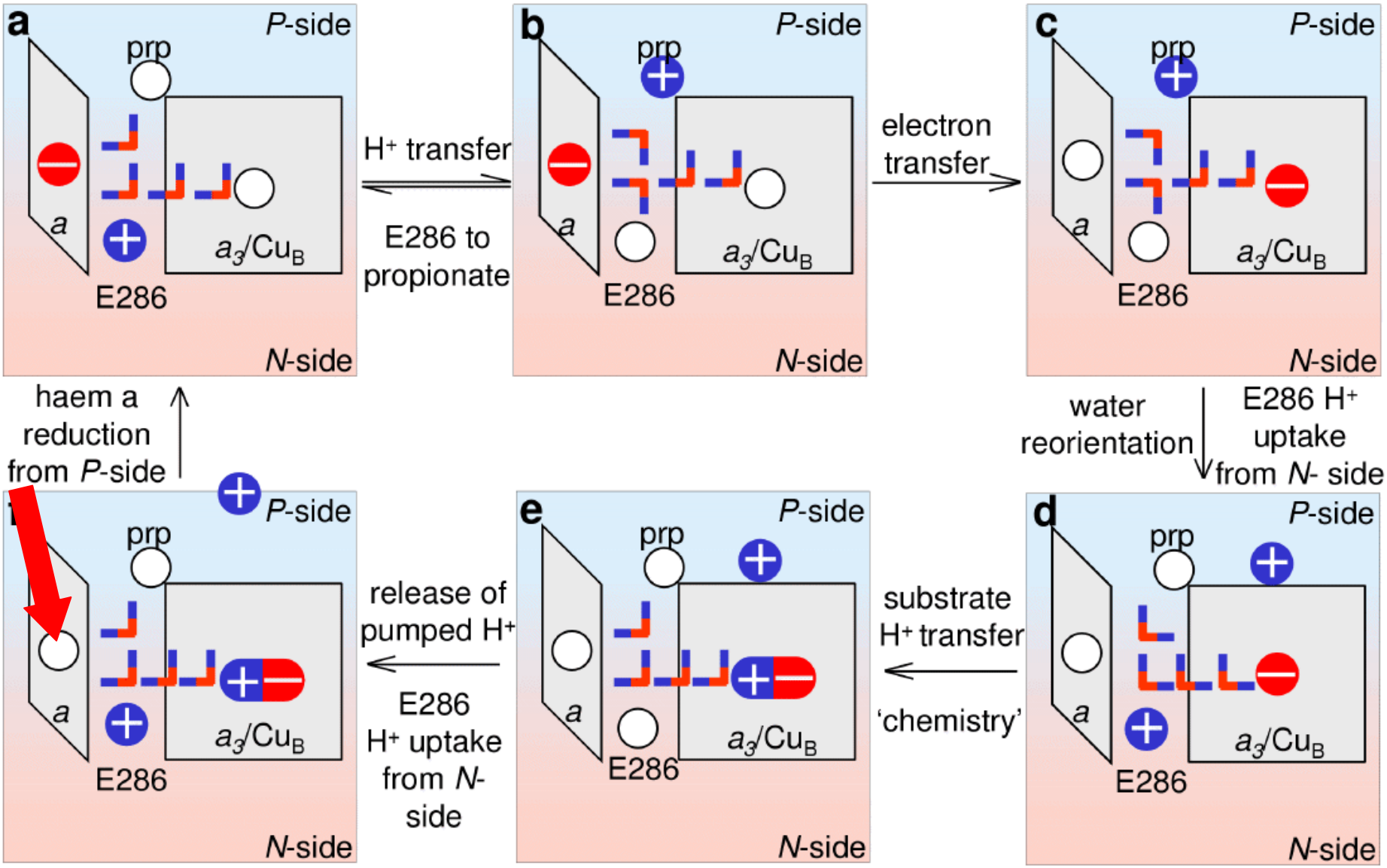
Water-Gated Proton Pump



Water-Gated Proton Pump

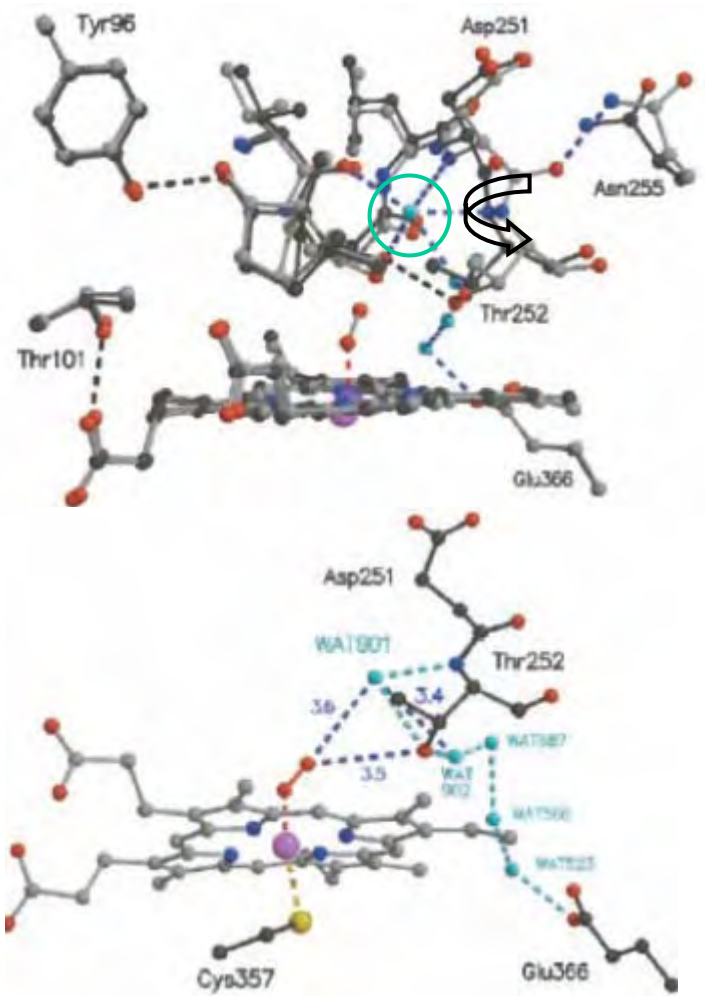


Water-Gated Proton Pump



4. Role of Protein Dynamics in Biological Proton Transfer: Water Penetration and Side Chain Isomerization in Cytochrome P450cam

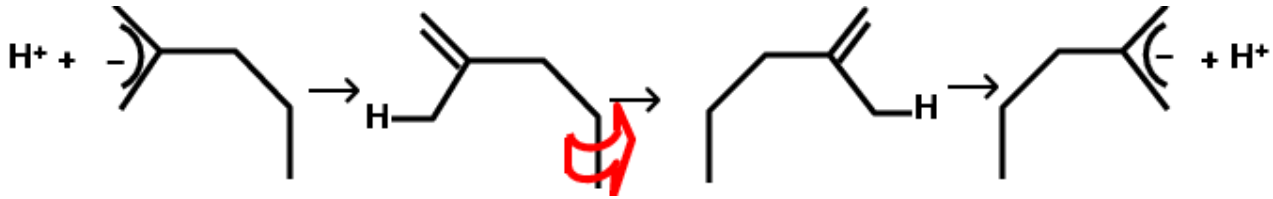
- Cryo-trapped intermediate with dioxygen bound
  - Exposure of amide group to previously empty non-polar cavity induces water filling (Schlichting et al, *Science* **287**, 1615, 2000)



Network Model of Proton Transfer in Proteins

(Taraphder and Hummer, *J. Am. Chem. Soc*. **125**, 3931, 2003)

- H^+ transfer via H bonds and shuttles of ionizable side-chains: $H^+ + AH\ldots OH\ldots B^- \rightarrow HA\ldots HO\ldots HB$

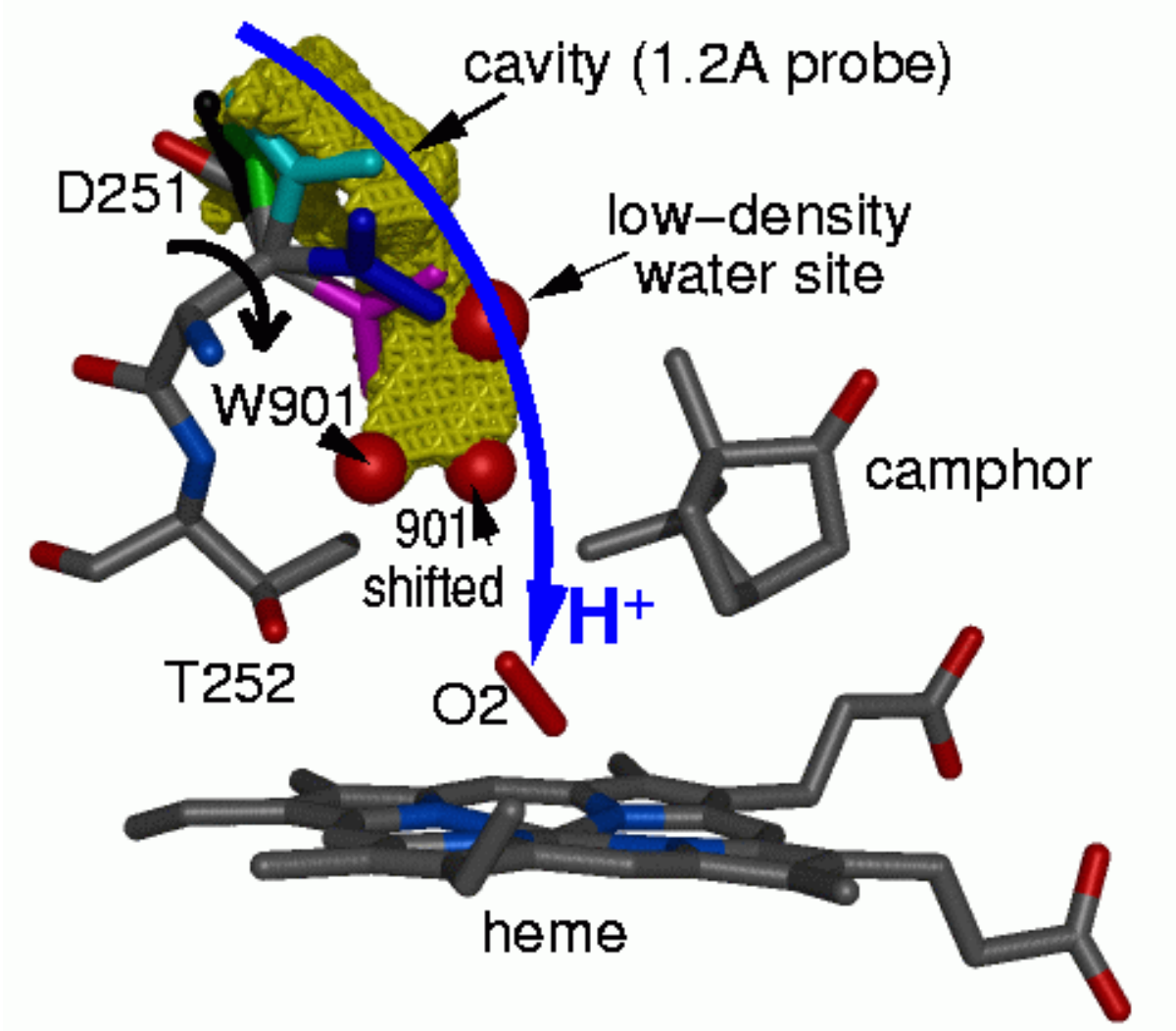


- Recursive network analysis of H^+ relay groups
  - Sample fluctuations in side-chain orientations and hydration to collect possible H^+ paths
  - Rank paths by using “steric” action

$$p(i \rightarrow j) = \exp(-\beta \Delta E_{ij}) / \sum_k \exp(-\beta \Delta E_{ik})$$

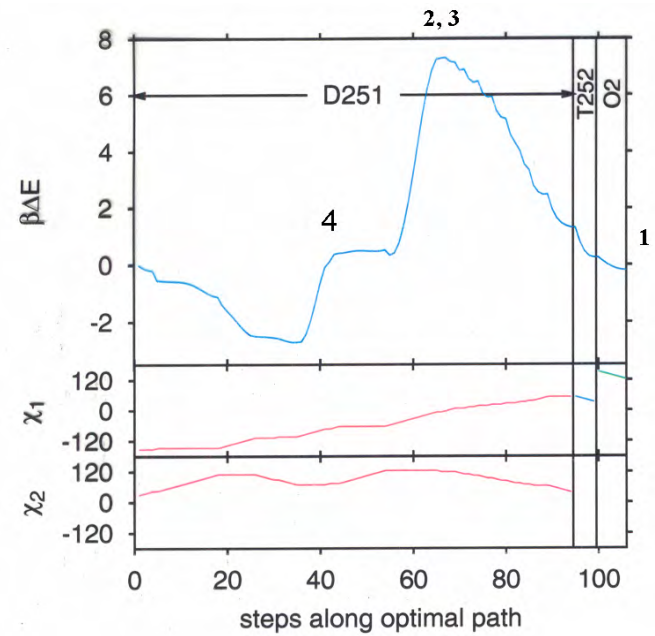
$$p(i_1 \rightarrow i_2 \rightarrow \ldots . i_n) = \prod p(i_k \rightarrow i_{k+1}) \equiv \exp(-S)$$

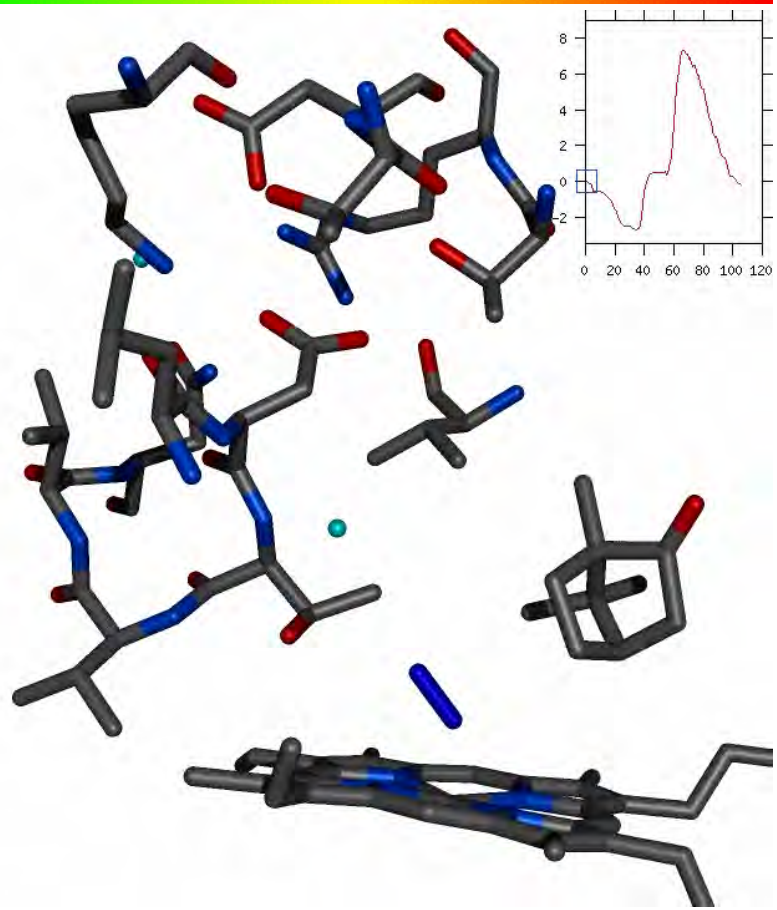
Proton shuttle through concerted water penetration and side chain motion



Energetics of Asp-251 Shuttle in P450cam

- Exhaustive search of side-chain conformers
- Sterically optimal path for Asp-251 isomerization





Conclusions: Water in Nonpolar Channels

- Water can favorably occupy nonpolar channels, despite loss of hydrogen bonds
- Channels protect from fluctuations
  - Narrow distribution of energies
  - Unbound states rarely populated
  - Increased life time of hydrogen bonds with rare defects
  - Long life-time of water-chain orientation

Conclusions: Drying

- Water occupancy in nanotube channel extremely sensitive to attractive interactions
  - Difficult to predict whether filling of narrow channels occurs under ambient conditions, but small perturbations to near-ambient conditions (polarity, *T*, *p*, osmolality, etc.) can tune filling
- Sharp two-state transitions between empty and filled states
  - Intermediate states are rarely populated because of fragmented hydrogen bonds

Proton Transfer in Proteins

- Proton wires ‘on demand’
  - Increase in local polarity can trigger water’ influx to establish protonic connectivity
- High ‘delivery speed’
  - High mobility of a *single* proton along ordered water chain inside hydrophobic pore (40x bulk!)
- Unidirectional wires (‘diode’)
  - Hydrogen-bond orientation controlled by electrostatics
- High ‘fidelity’
  - Only single proton delivered without reorientation

Acknowledgments

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  - J. C. Rasaiah (U. Maine)
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  - C. Dellago (U. Vienna)
- Proton transport in cytochrome P450
  - S. Taraphder (NIH, IIT Kharagpur)
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