Transport through (biological) nanopores

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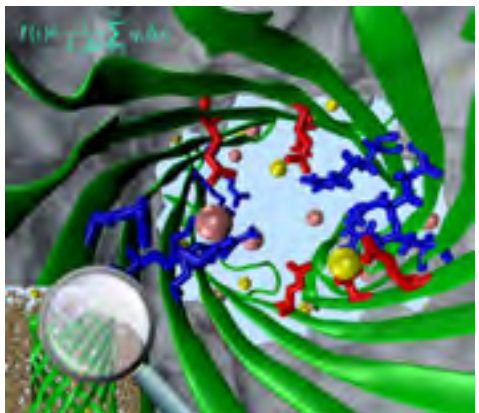
Jacobs University Bremen



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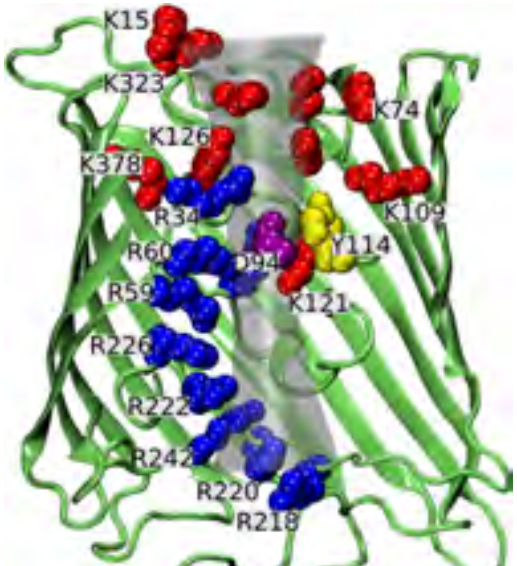
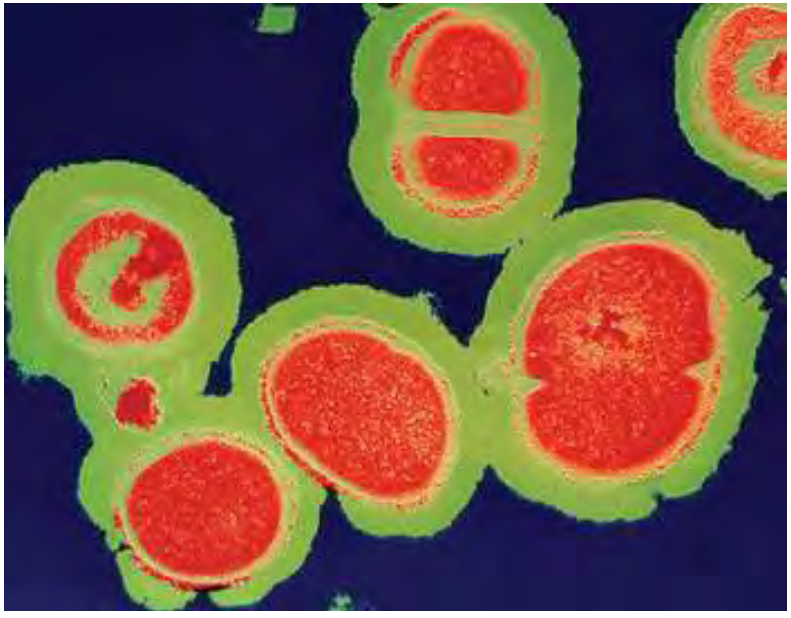
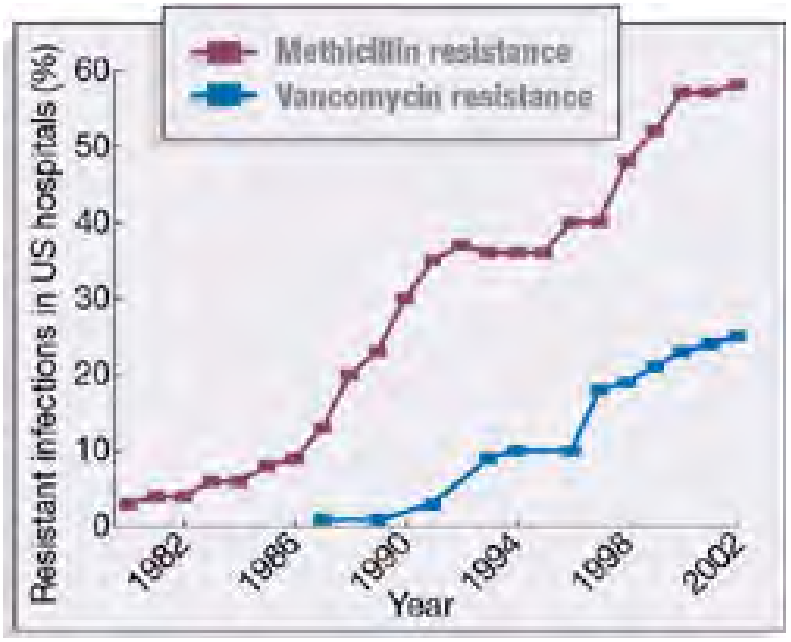


Outline

1. Motivation for studying ion transport
2. Ion transport through membrane proteins
3. Larger ions through OmpF
4. Phosphate-selective porin OprP
5. Excitation energy transfer in light-harvesting complexes

The problem: Antibiotic Resistance

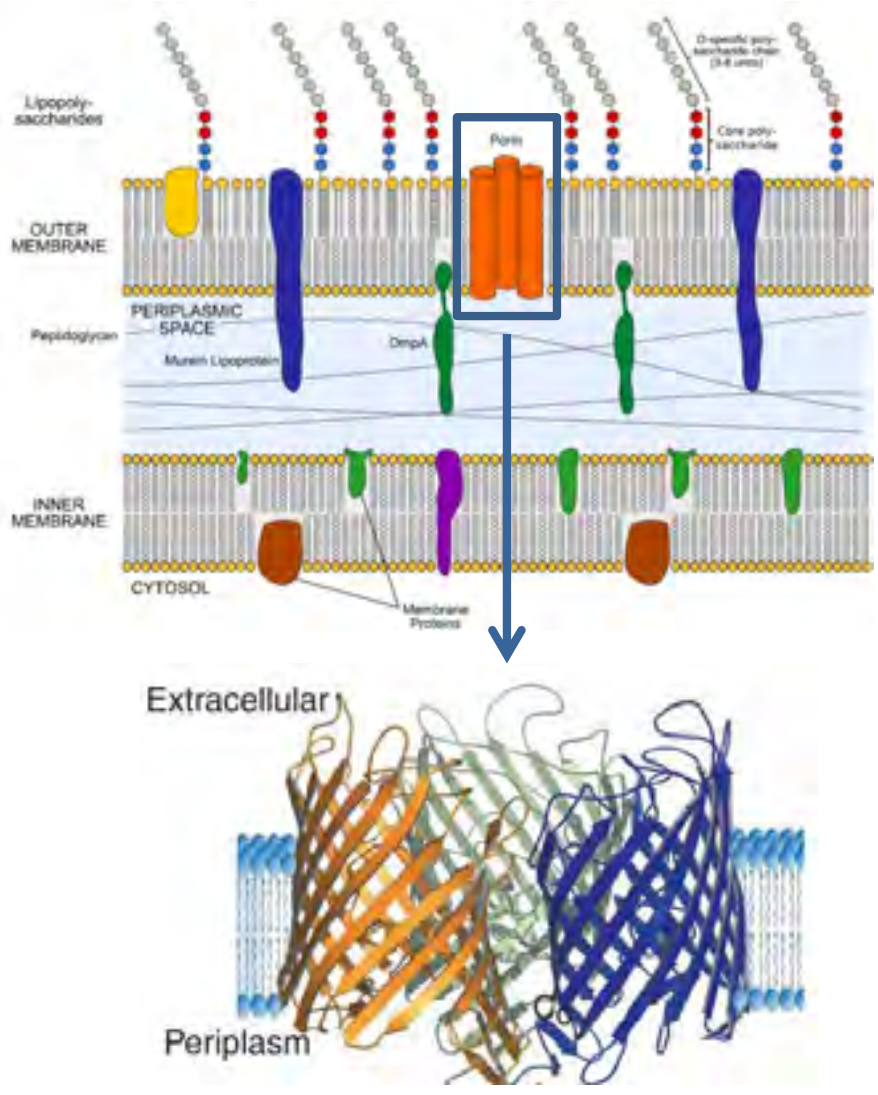




- Bacteria are acquiring antibiotic resistance at a rate that outpaces the ability of the scientific world to provide the medical world with new antibiotics.

Porins

- Mainly proteins present in the outer membrane of Gram-negative bacteria
- β-barrel structure
- Allow permeation of small solutes across bacterial outer membrane
- Play a crucial role in permeation of antibiotics through outer membrane of Gram-negative bacteria (Nestorovich, et al. *PNAS,* 99(2002), 9789)
- Non-specific porins: OmpF, OmpC
- Specific porins: phosphate selective OprP, carbohydrate specific LamB and ScrY
- Provide a testbed system to understand ion transport processes



http://en.wikipedia.org/wiki/Gram-negative_bacteria

Example: The outer membrane protein F (OmpF)

- general diffusion channel for small molecules incl. water and also ions
- also main pathway for β-lactam antibiotics
- experiments of translocation of antibiotics through OmpF, for example, Winterhalter group at Jacobs University
- earlier molecular dynamics simulations model only equilibrium properties, here non-equilibrium molecular dynamics

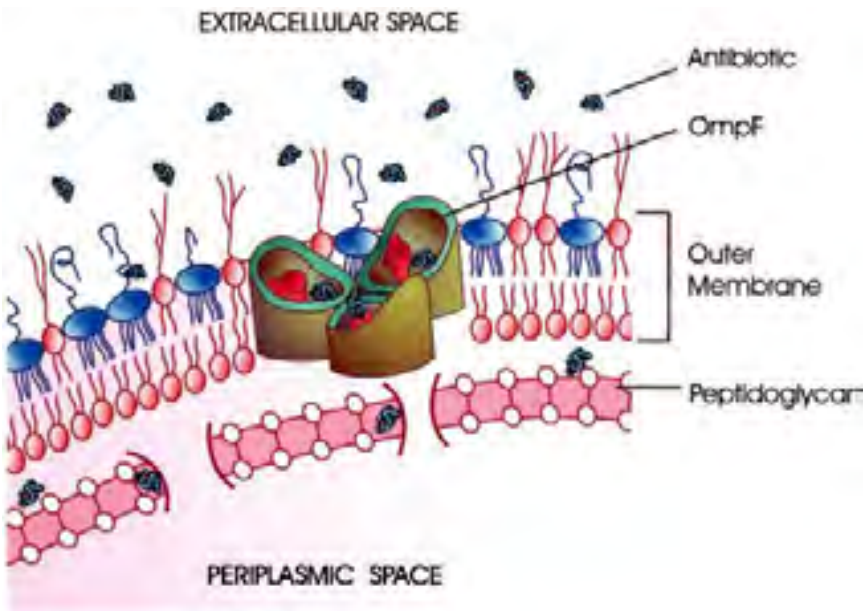
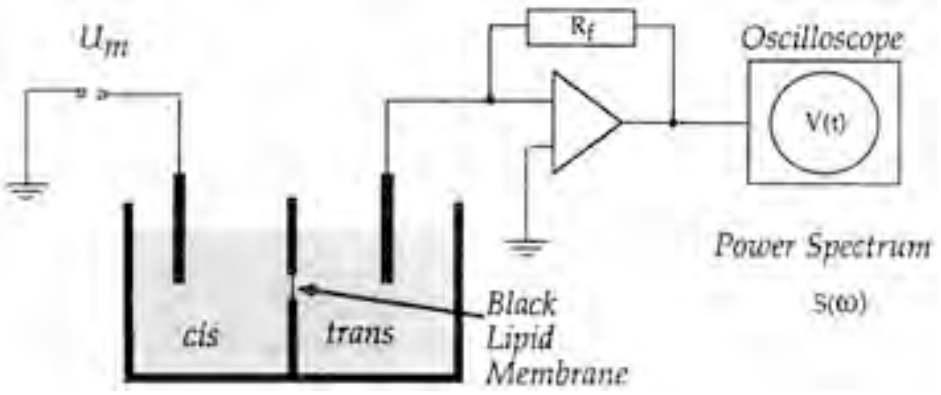
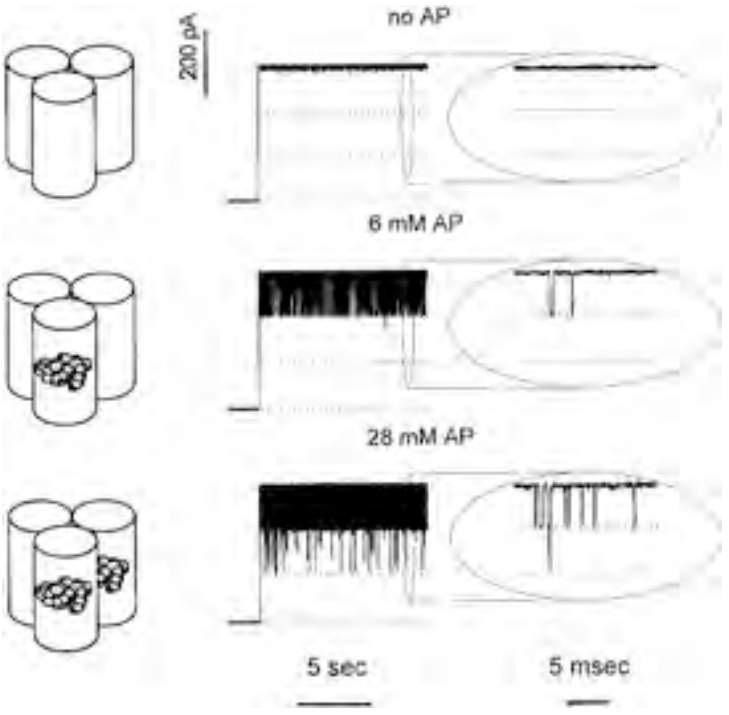


figure taken from Nestorovich et al., PNAS **99**, 9789 (2002)

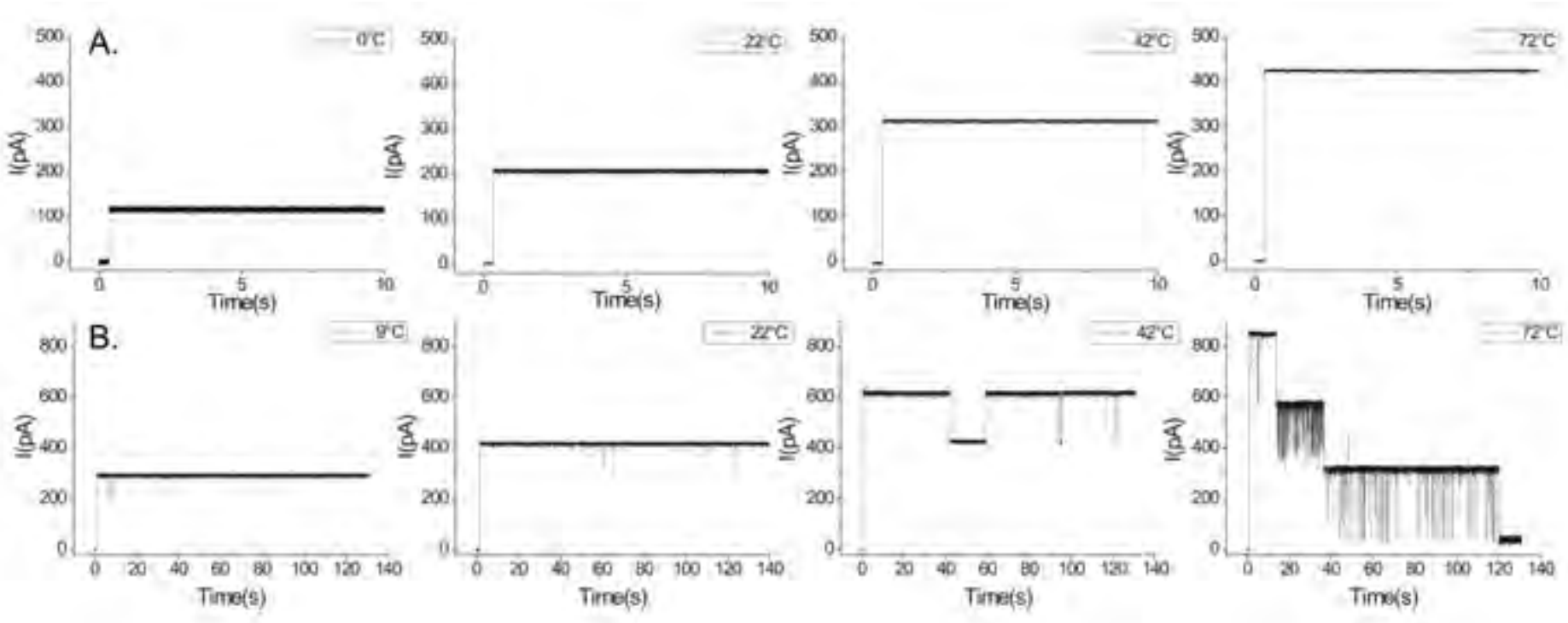
Electrophysiology I





- Split simulation problem into two parts
  - Simulation of ion current
  - Blocking through antibiotics

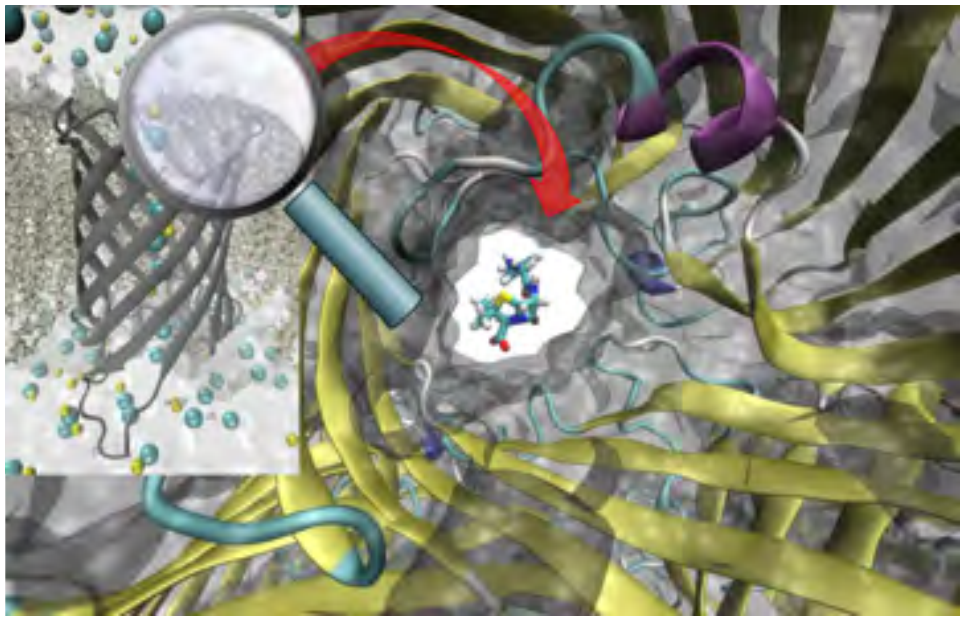
Electrophysiology II



- upper panel: 50 mV transmembrane potential
- lower panel: 100 mV transmembrane potential

Combating Antibiotic Resistance: New Drugs 4 Bad Bugs: TRANSLOCATION

- Project of the “Innovative Medicine Initiative”
- academic lead: Prof. Mathias Winterhalter, Jacobs University
- total budget 29.3 MEuro (incl. in-kind and other contributions)
- includes AstraZeneca, GlaxoSmithKline, Sanofi, Basiliea, ...



efpia

imi
Innovative Medicines Initiative

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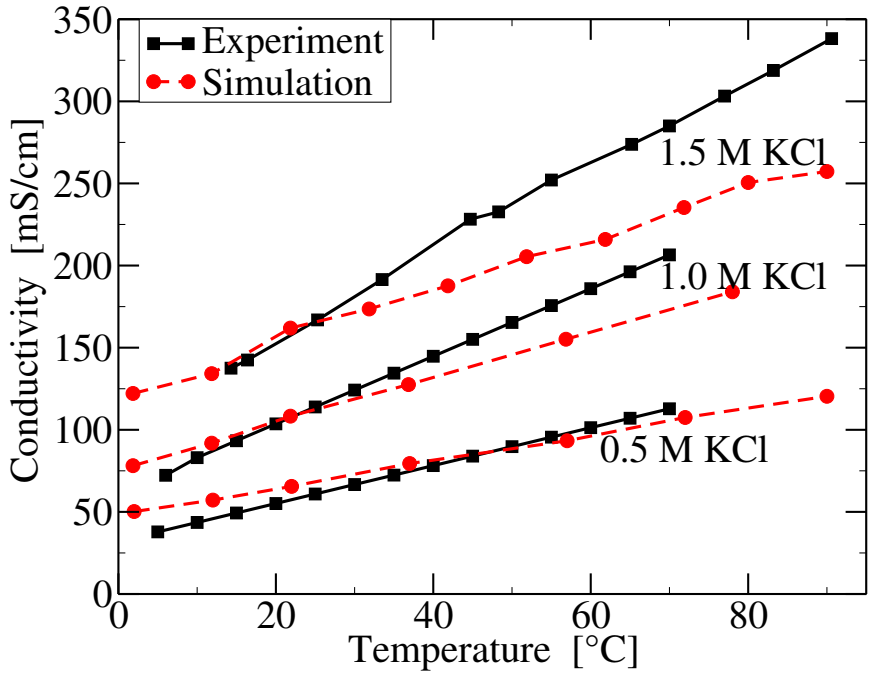
Conductivity of KCl in bulk water

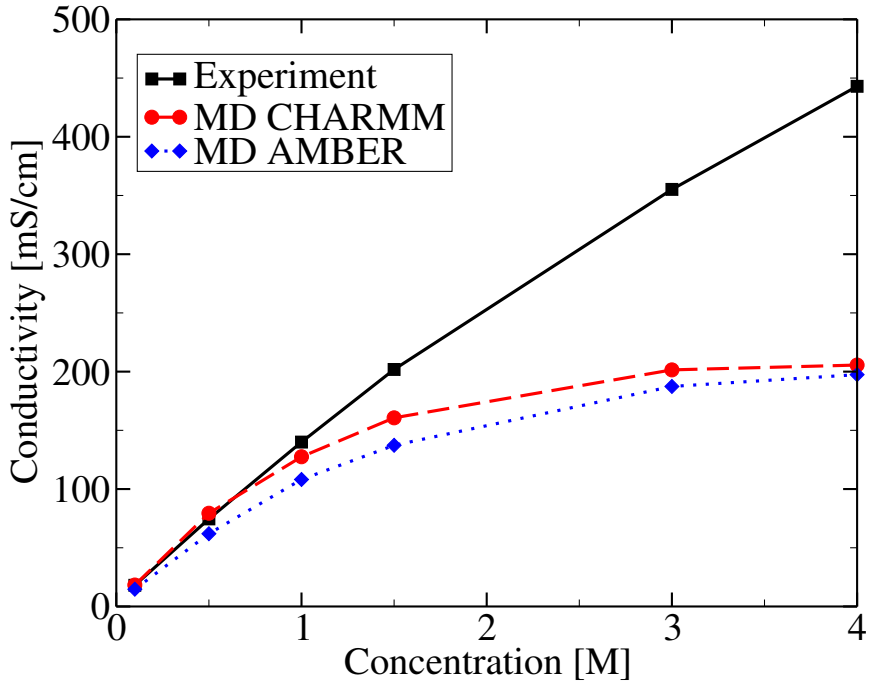
- simulation in cube of water with side length 4.2 nm
- 1 molar KCl solution: 45 K and 45 Cl ions in about 2500 water molecules



- conductivity measurements in group of Mathias Winterhalter, Jacobs University Bremen
- homogenoues electic field: $\vec{\nabla} V = \vec{E}$, $V = EL_z$
- tinfoil boundary conditions

Conductivity of KCl in bulk water

- simulation in cube of water with side length 4.2 nm
- room temperature



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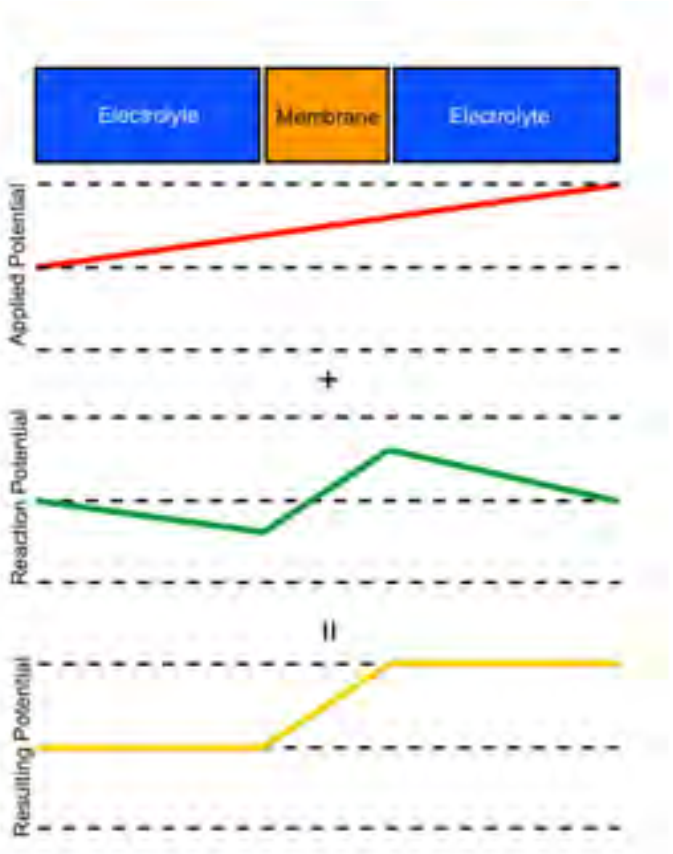
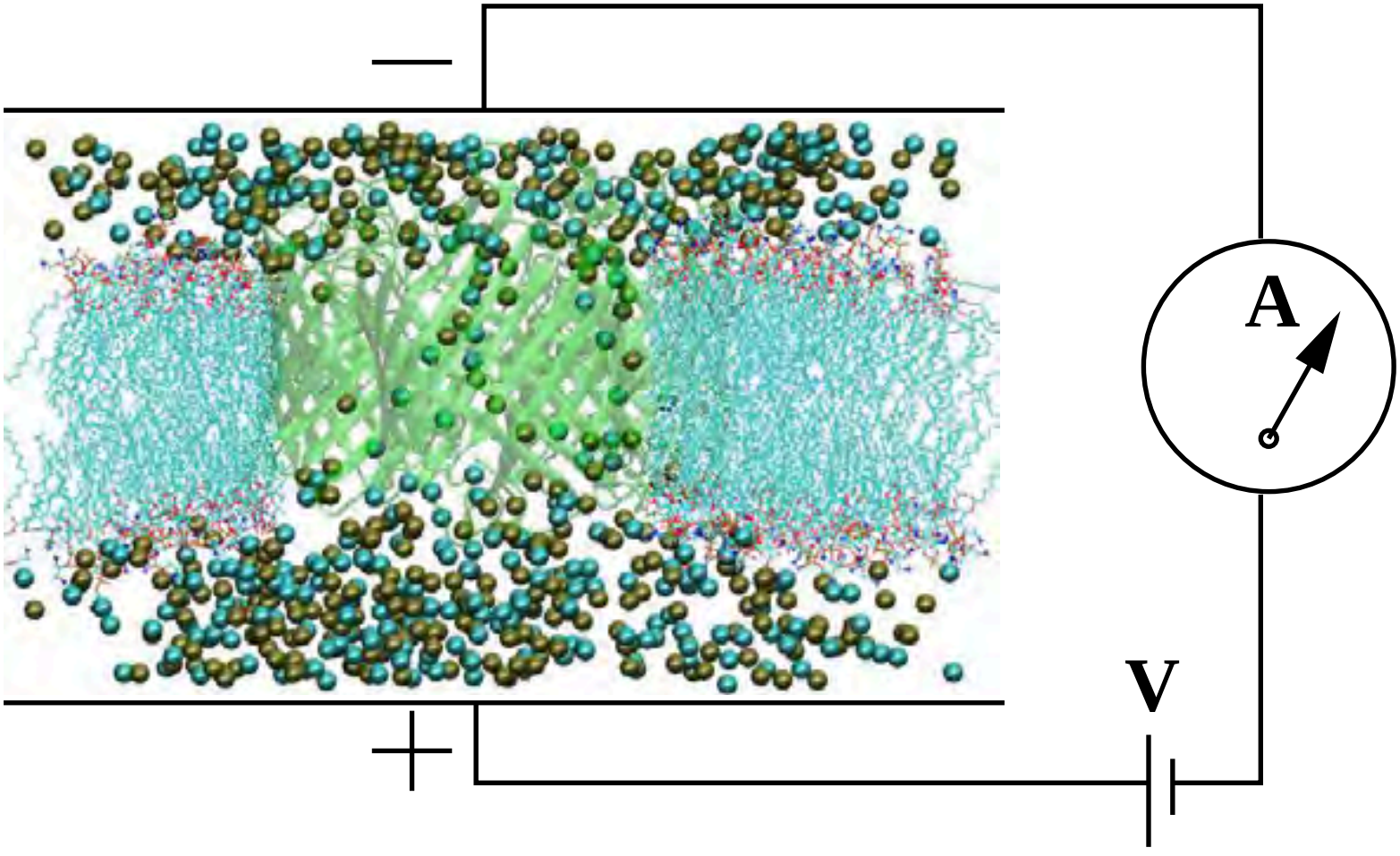
Potential in membrane systems



- water molecules and ions are free move around and will rearrange
- ions and water molecules generate reaction potential
- figure shows applied, reaction, and resulting potential

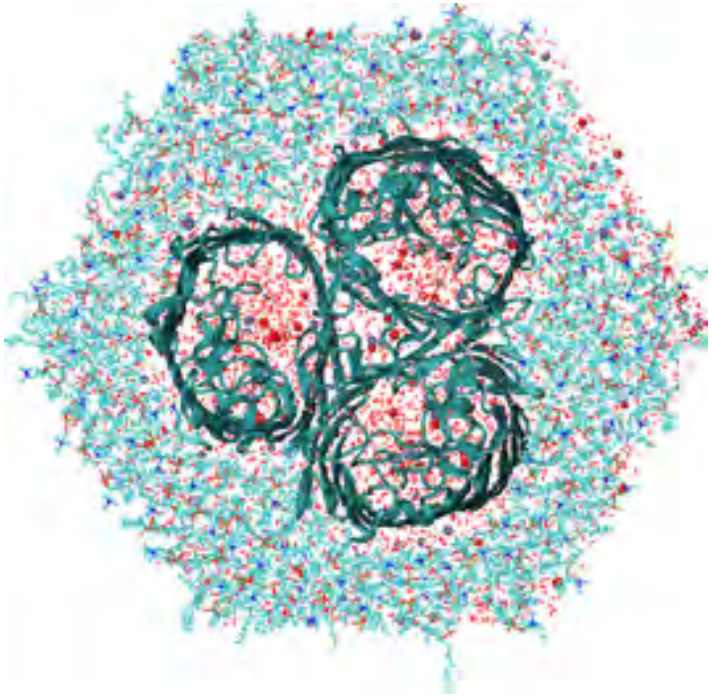
Conductance of KCl through an OmpF trimer

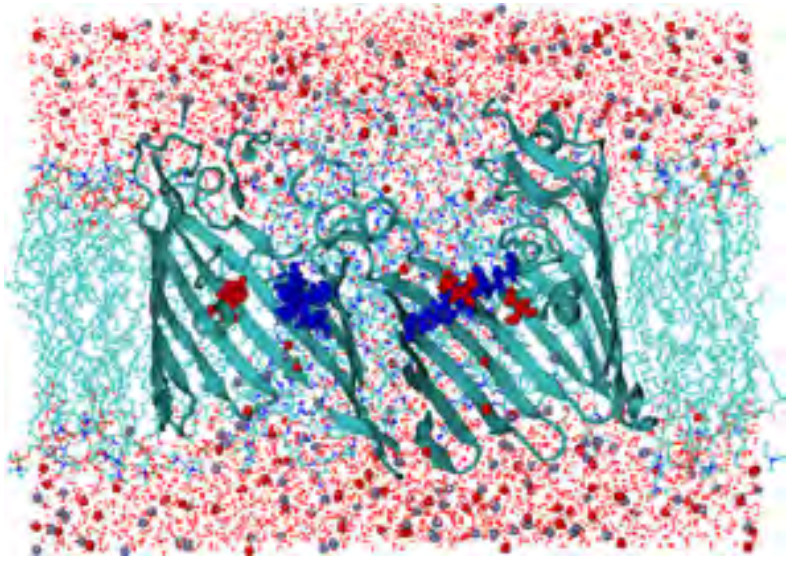
- 1 molar KCl solution with a voltage of ± 1 V across the membrane



- resulting bias voltage $V = -EL_z$

Setup for OmpF Simulations

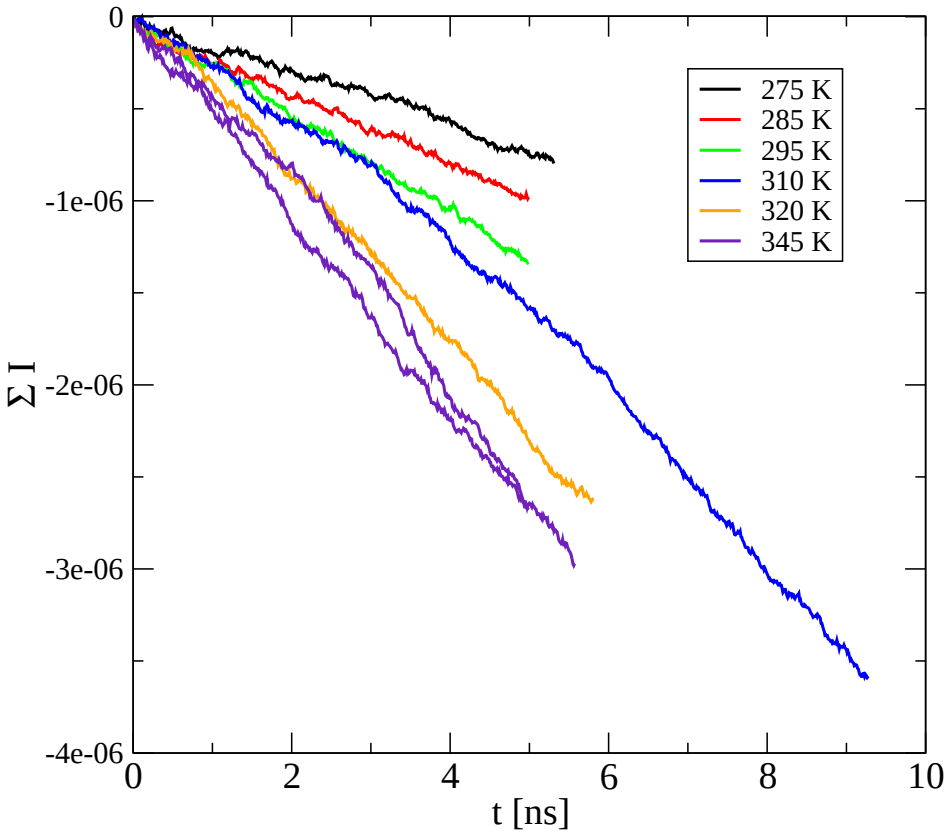




- of about 80.000 atoms about half belong to water
- trimer from protein 2OMF inserted into POPE membrane
- CHARMM27 force fields using NAMD2
- electric field applied in z-direction with tin foil BC

Accumulated current through OmpF trimer

- 1 molar KCl solution with a voltage of ± 1 V across the membrane

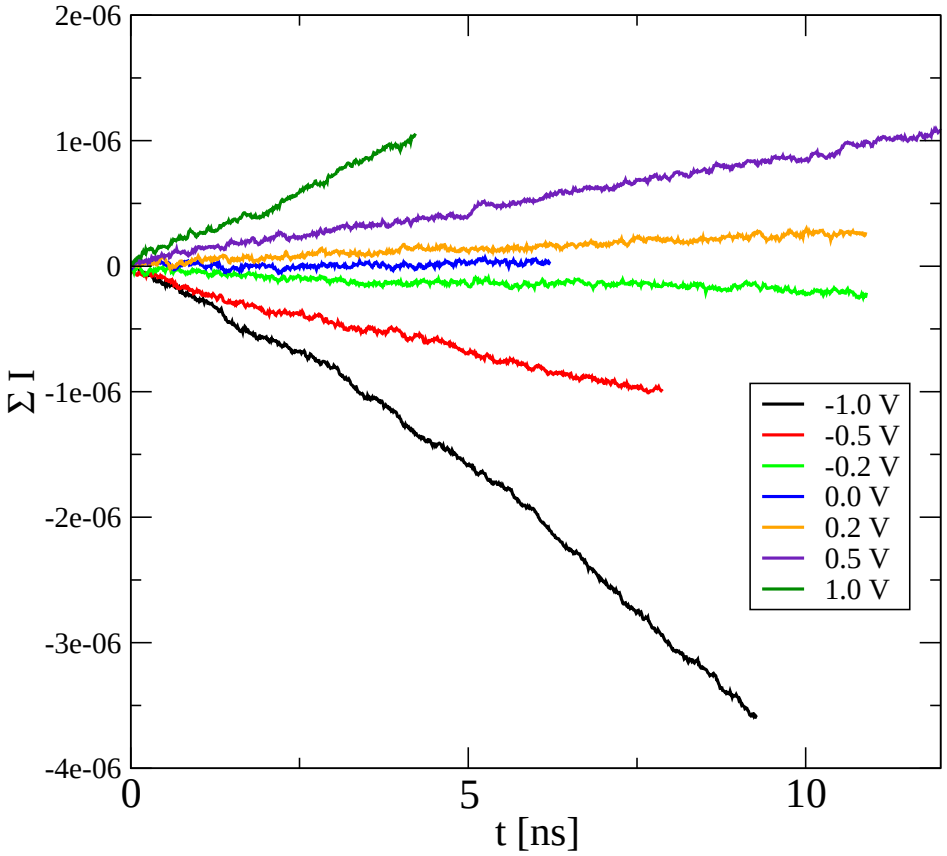


- accumulated current $I(t) \times \Delta t$ summed up during the simulation as

$$I(t) = \frac{1}{\Delta t \times L_z} \sum_{i=1}^{N} q_i \left[z_i(t + \Delta t) - z_i(t) \right]$$

Accumulated current through OmpF trimer

- 1 molar KCl solution with a different voltages



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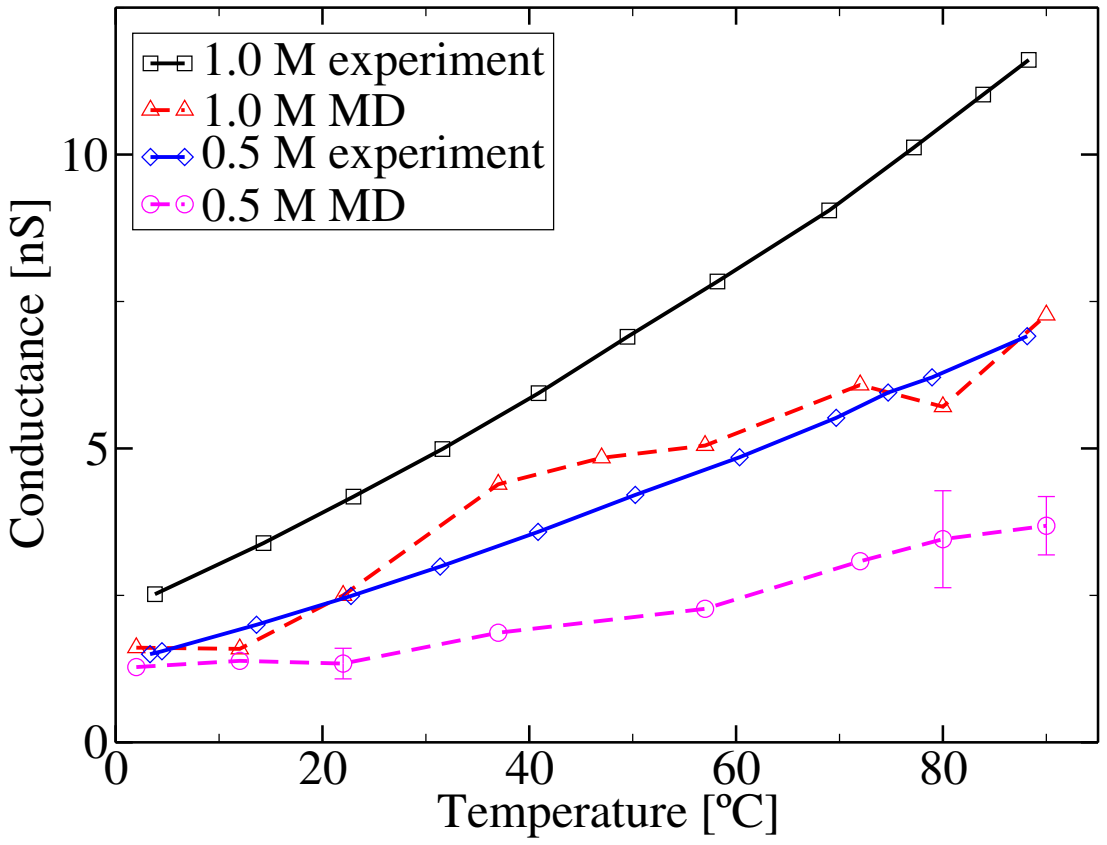
Conductance of KCl through an OmpF trimer

- 1 molar KCl solution with a voltage of ± 1 V across the membrane
- measurements at 150 mV



C. Chimerel, L. Movileanu, S. Pezeshki, M. Winterhalter, U. Kleinekathöfer, Eur. Biophys. J. **38**, 121 (2008)

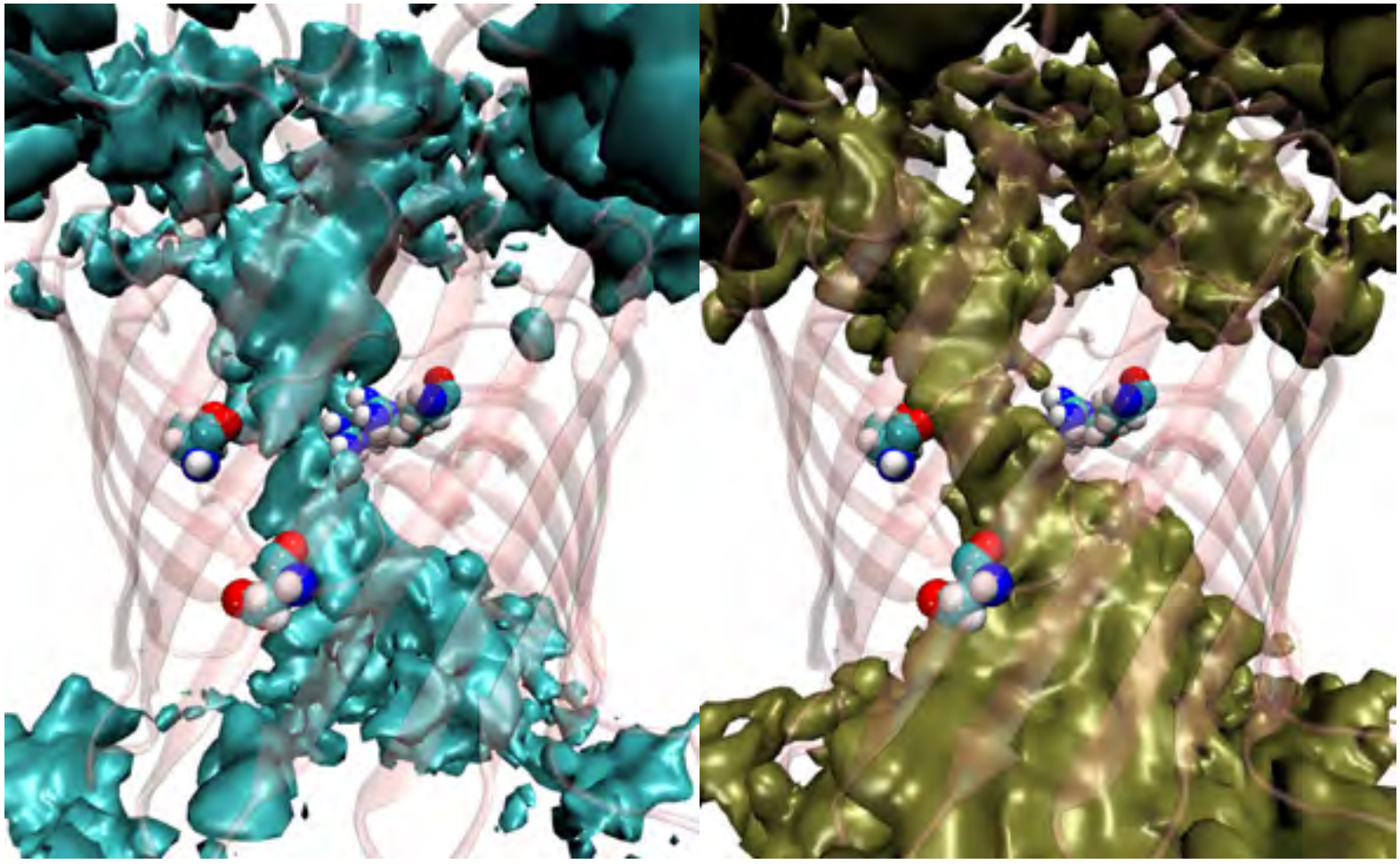
Mutation studies

- Wild type constriction zone:
  E117, D113, R42, R82, R132, $\Rightarrow$ 2 acids and 3 bases
- Mutations with neutralized bases, acids and all amino acids in the constriction zone

Acronym	mutated residues	G_{MD} [nS]	G_{exp} [nS]	I_{K^+} / I_{Cl^-}	S_{exp}	S_{est}
wt (DERRR)	-	3.0	4.0	1.2	3.5$\pm$ 0.2	3.5
NQAAA	neg. & pos. neutralized	3.4		3.6		10.5
NQRRR	negative neutralized	1.9	1.8	0.7	2.9$\pm$ 0.2	2.0
DEAAA	positive neutralized	4.7	4.2	4.5	14.1$\pm$ 1.4	13.1
RRRRR+EE	neg. to pos. & pos. to neg.	1.2		0.1		0.3
DEERE	pos. to neg.	4.1		5.6		16.3

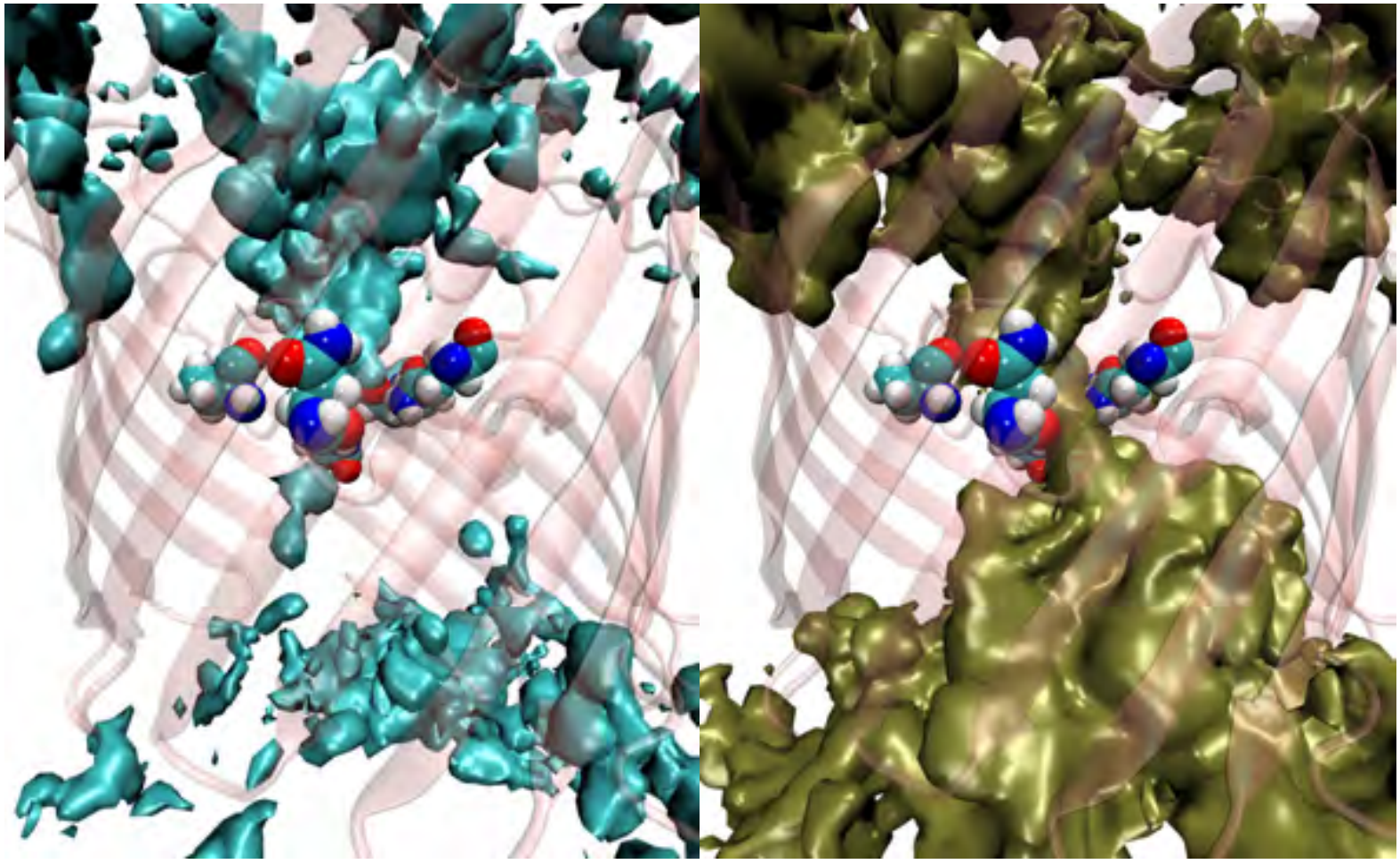
S. Pezeshki, C. Chimerel, A. Bessenov, M. Winterhalter, U. Kleinekathöfer, Biophys. J. **97**, 1898 (2009).

Ion densities: Wild type Ompf



- iso-density surfaces
- Cl density (left) and K density (right)
- averaged over trajectory

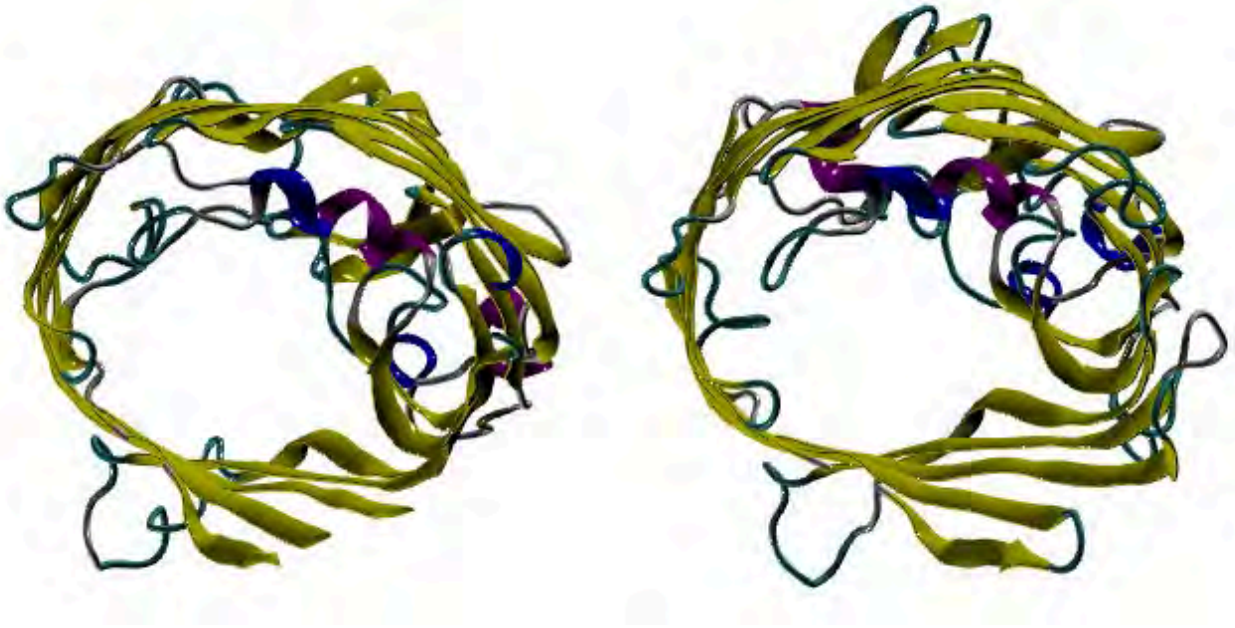
Ion densities: R42Q, R82Q, R132Q, E117Q, D113N



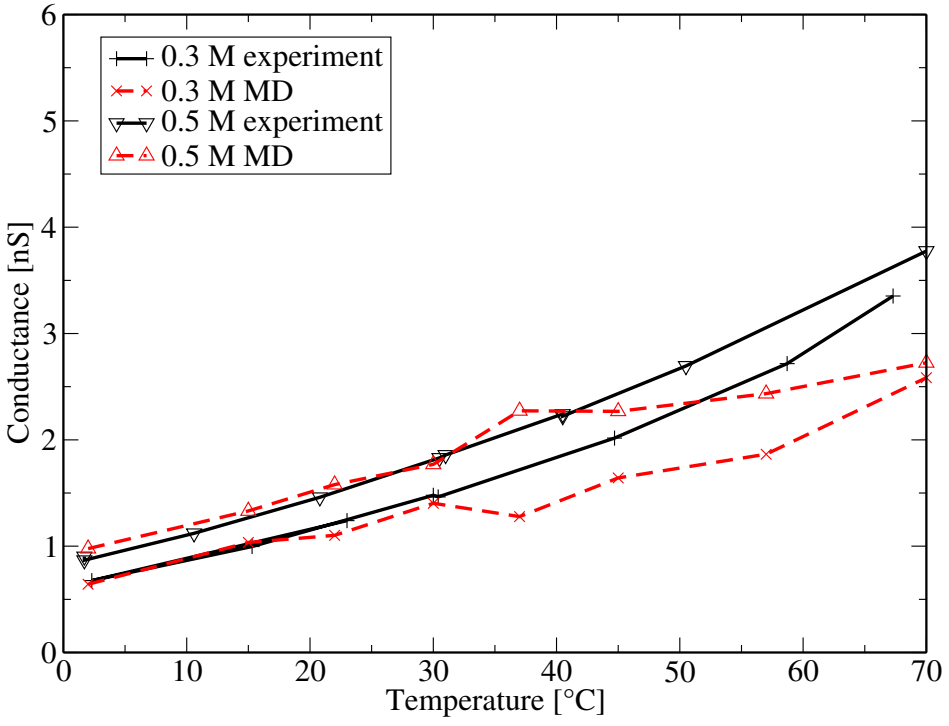
- iso-density surfaces
- Cl density (left) and K density (right)
- averaged over trajectory

OmpF versus OmpC

- sequences 60 % identical, 74 % of pore lining residues conserved
- OmpF dimension: elliptic channel (37 Å, 30 Å) with 50 Å length
- OmpC dimension: elliptic channel (34 Å, 28 Å) with 45 Å length
- OmpC slightly more cation selective than OmpF
- to distinguish OmpC from OmpF: electrostatics important, atomic details more important than pore size



Conductance of KCl through an OmpC trimer



I. Biro, S. Pezeshki, H. Weingart, M. Winterhalter, U. Kleinekathöfer, Biophys. J. **98**, 1830 (2010).

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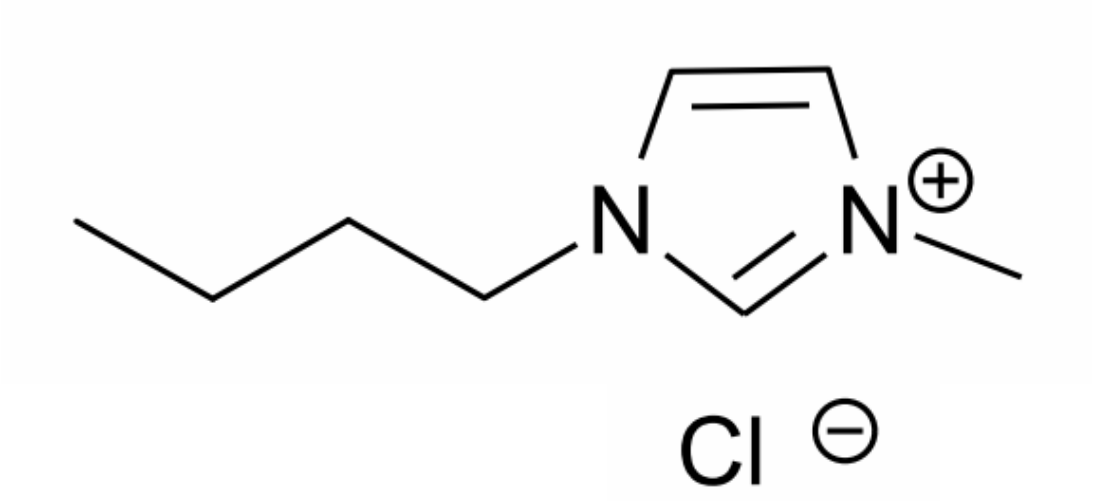
Ionic Liquid

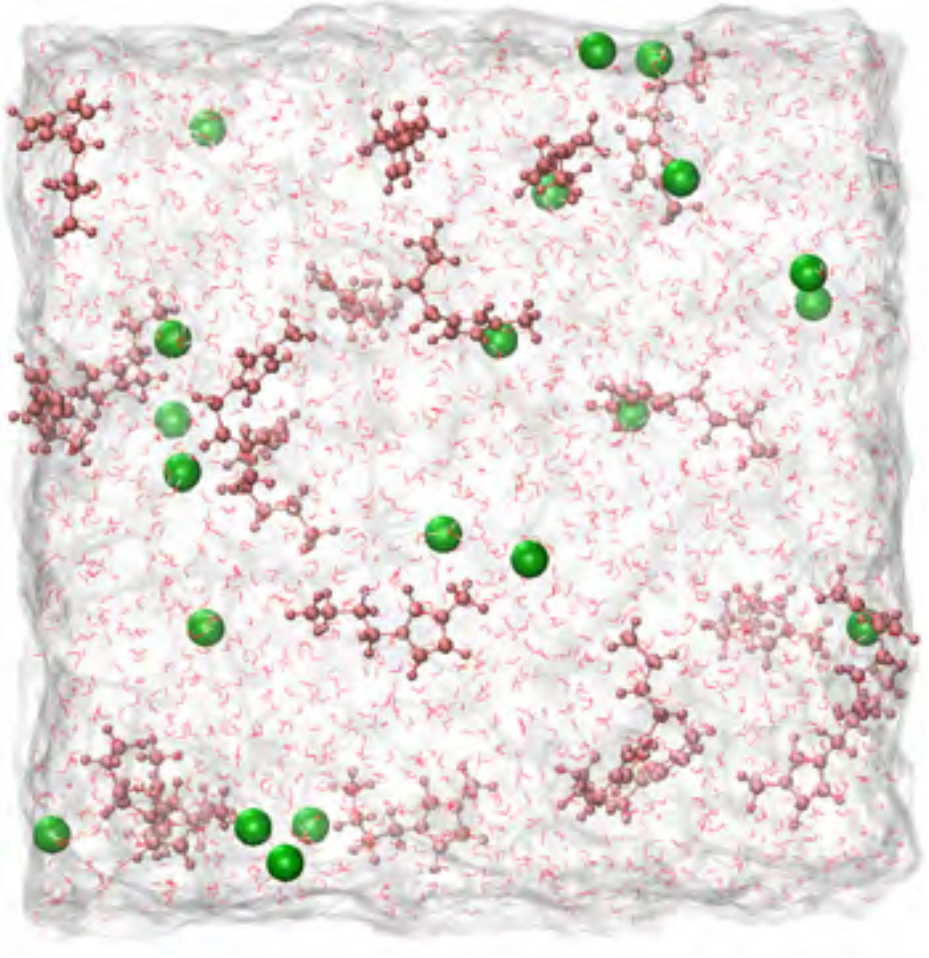


BMIM-Cl

- Bulky and asymmetric cation
- Weakly coordinating ions
- Interaction with solvent molecules and bio-molecules determined by **van der Waals interactions** and **H-bond donor/acceptor interactions** (if any) in addition to **electrostatic interactions**

Bulk Conductivity

- To benchmark the agreement between theory and experiment



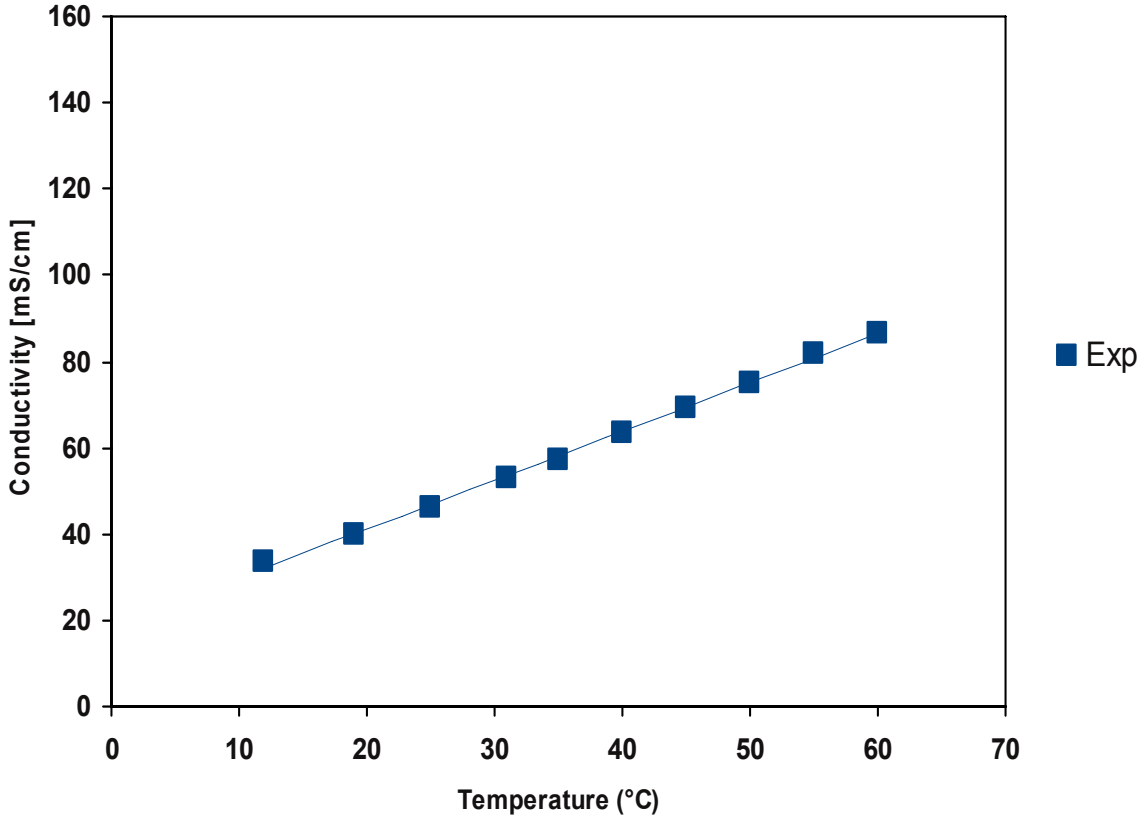
All atom molecular dynamics simulation with

- ~7000 atoms
- Different water models: TIP3P, TIP4P, TIP4P-Ew, TIP4P-2005
- $BMIM^{+}$ and Cl^{-} ions
- NAMD software package
- CHARMM force field and BMIM parameters
- Periodic boundary conditions
- Applying homogeneous electric field

Bulk Conductivity: Temperature dependence

$$I(t) = \frac{1}{\Delta t L_z} \sum_{i=1}^{N} q_i [z_i(t + \Delta t) - z_i(t)]$$

Water Models	Self Diffusion 10^{-5} cm^2/s
Experimental	2.27



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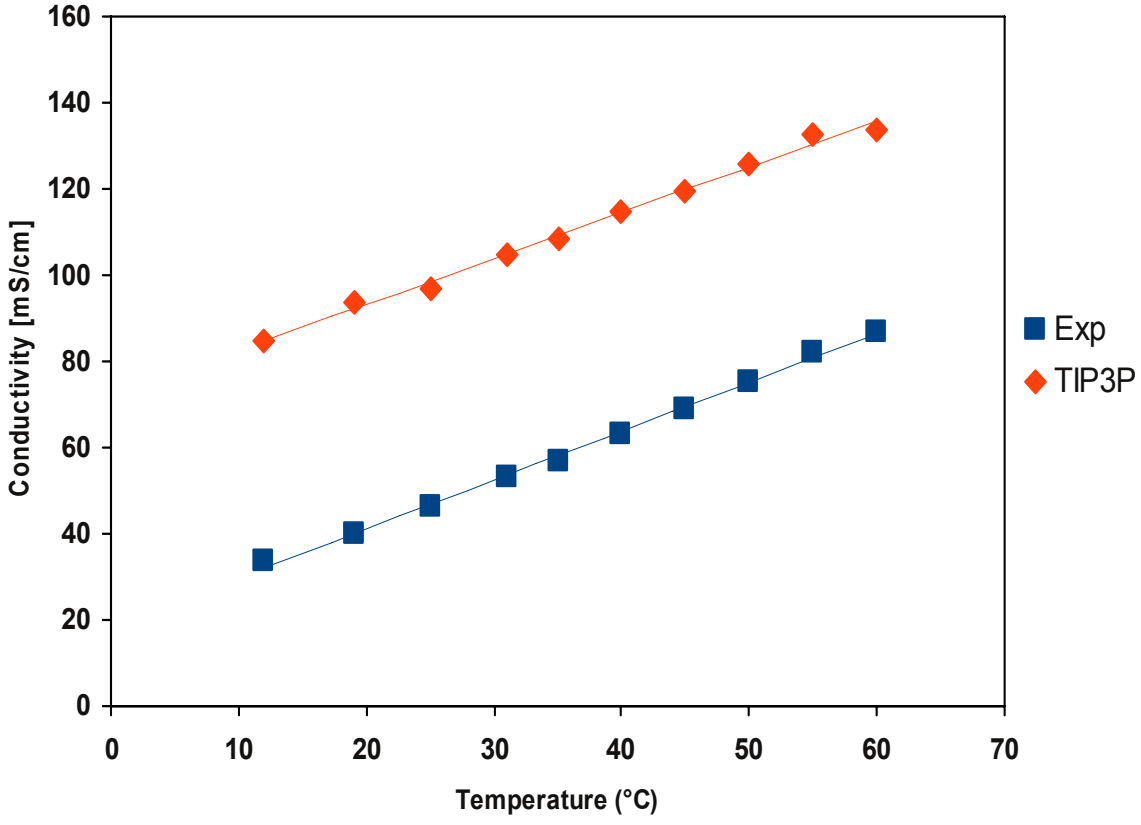

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TIP3P	5.19

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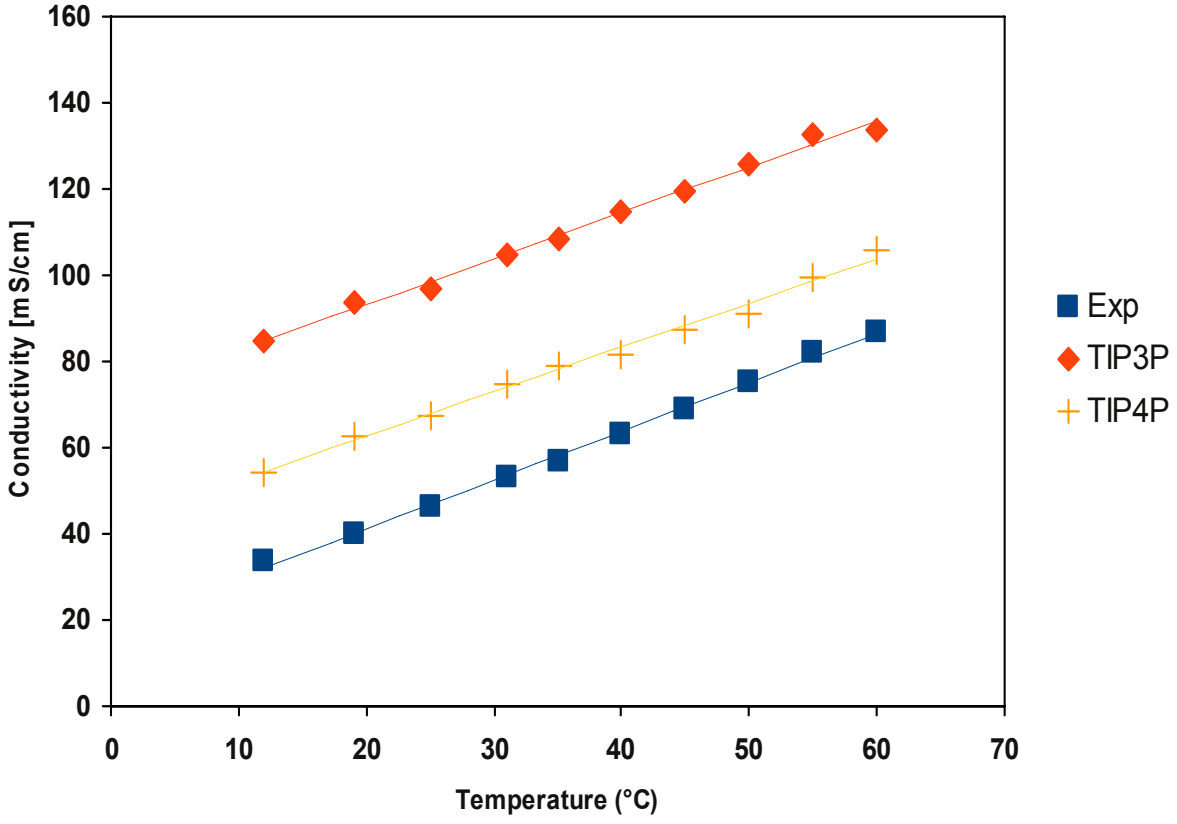

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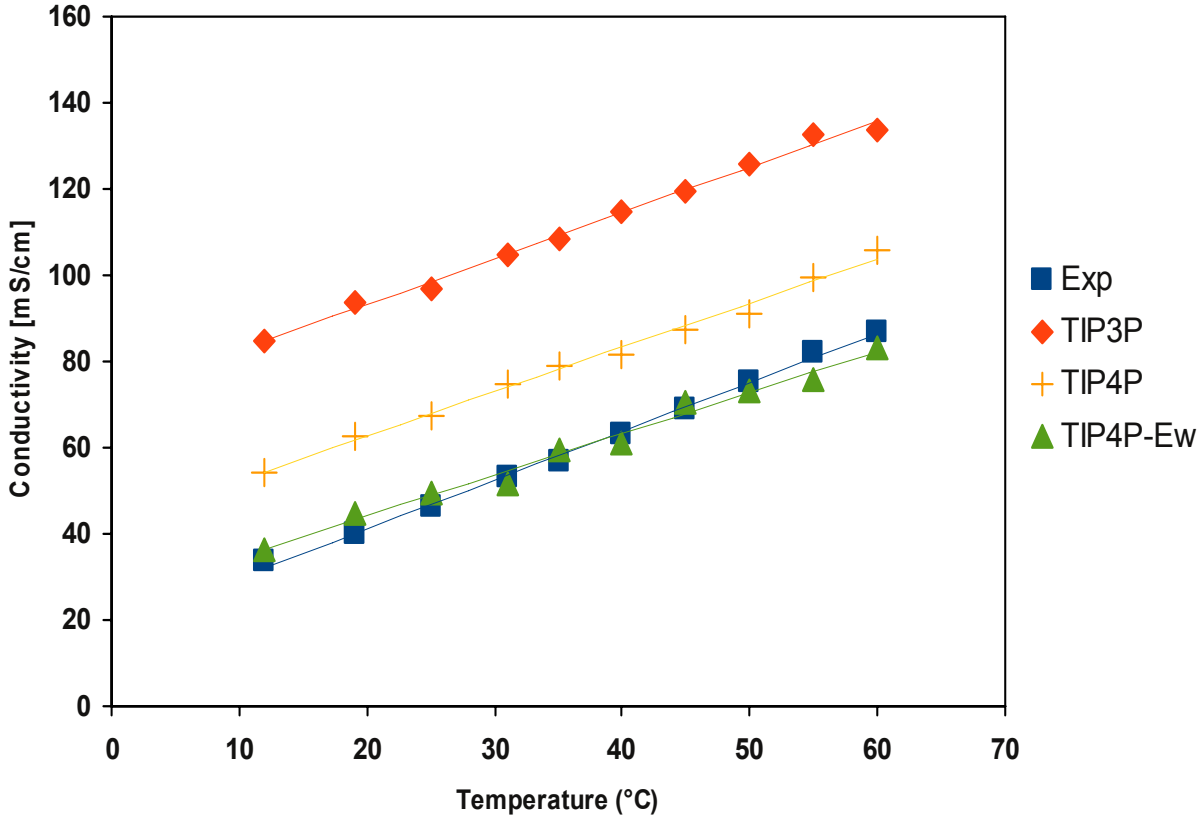

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TIP4P-Ew	2.4

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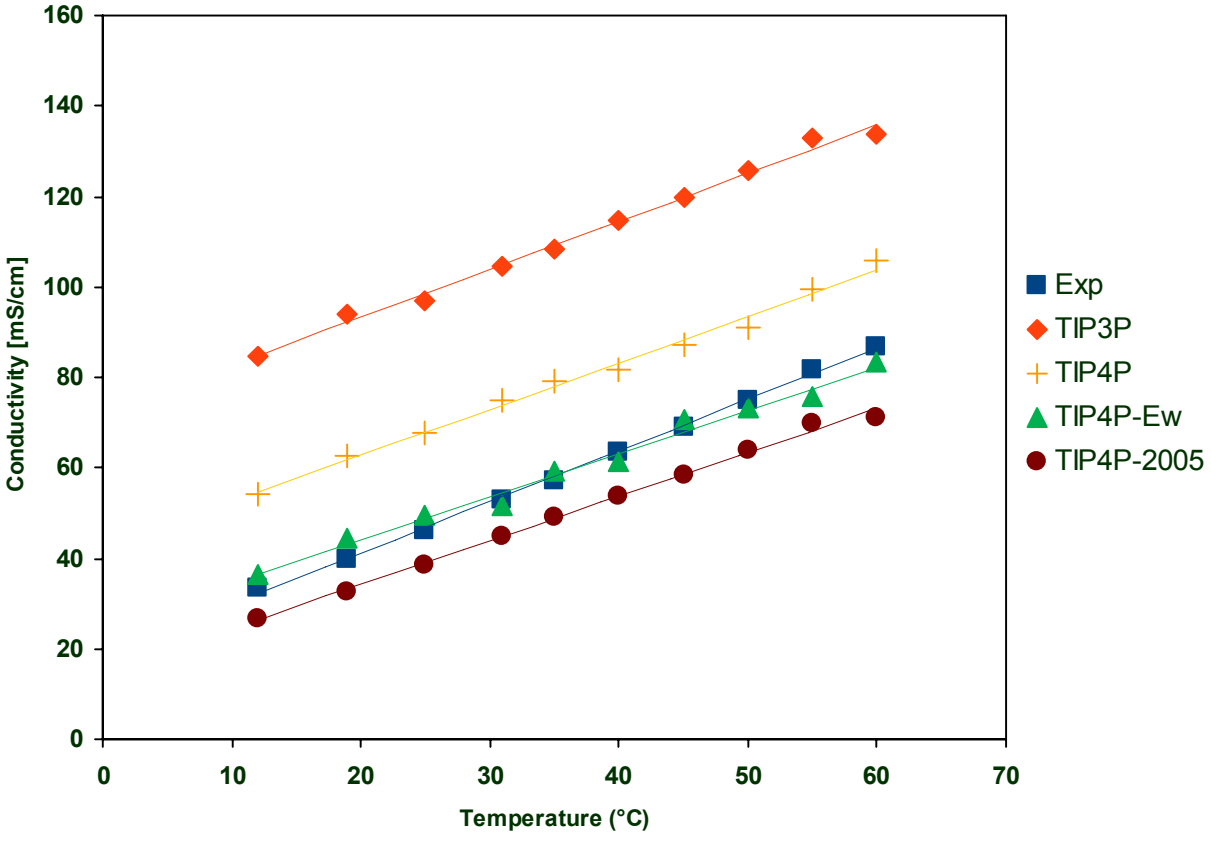

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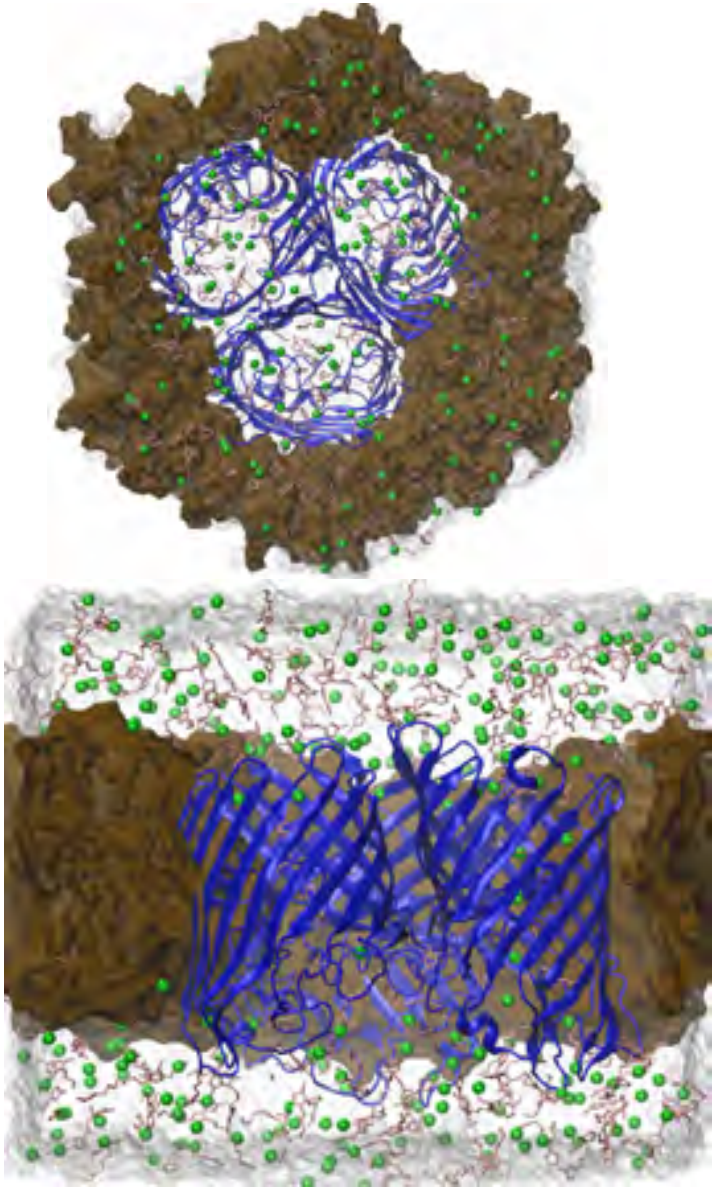
TIP4P-2005	2.08

System Setup



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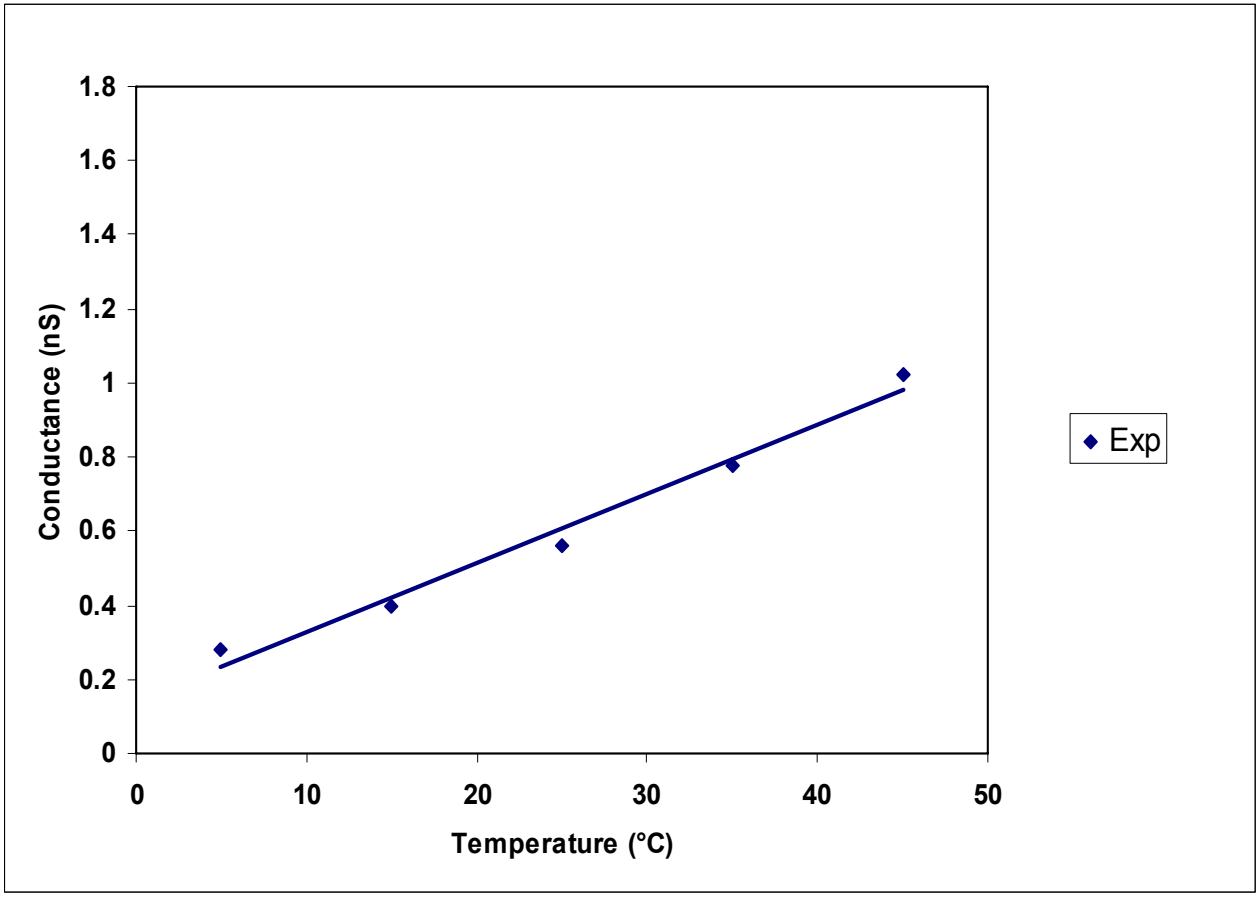
Pore conductance: Temperature dependence



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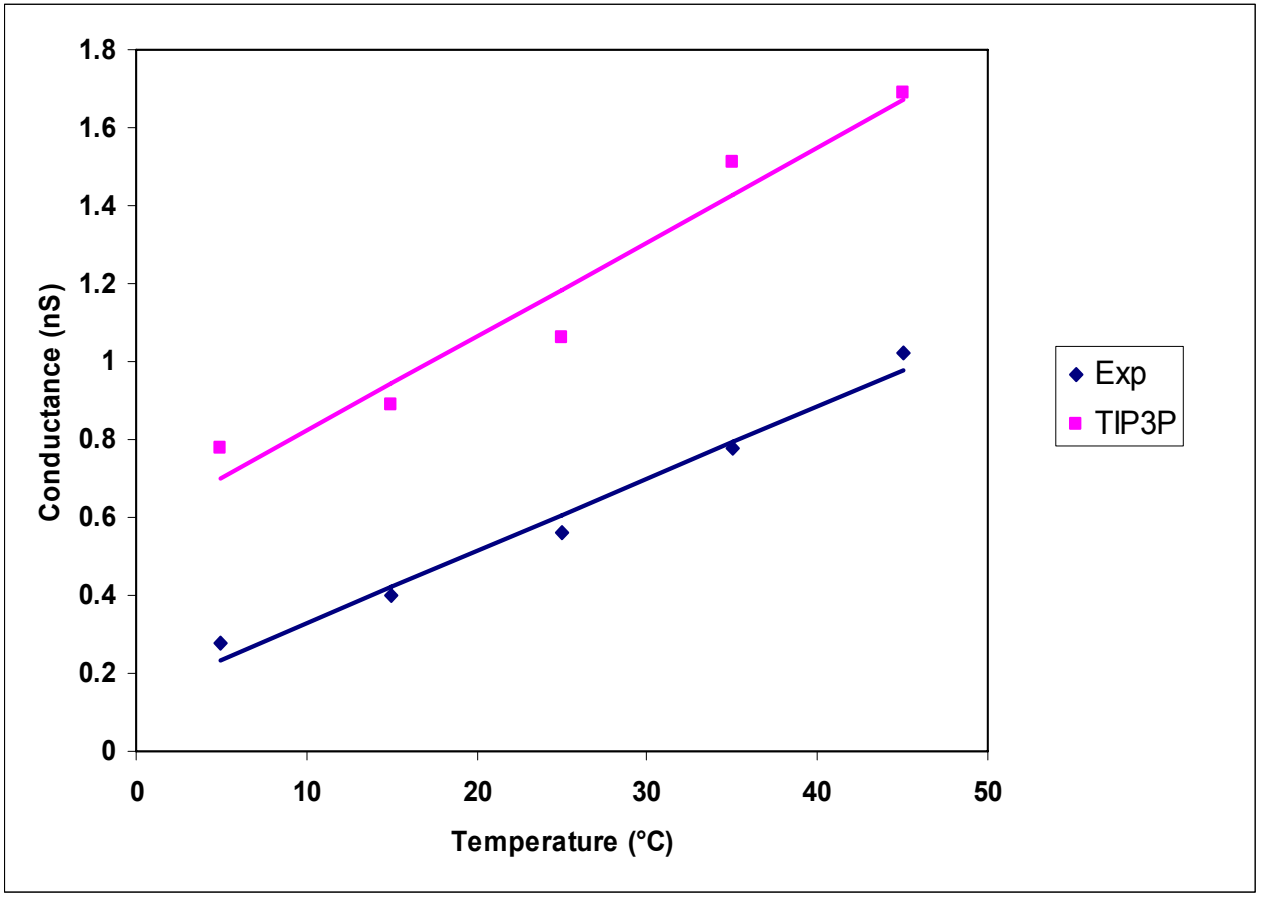

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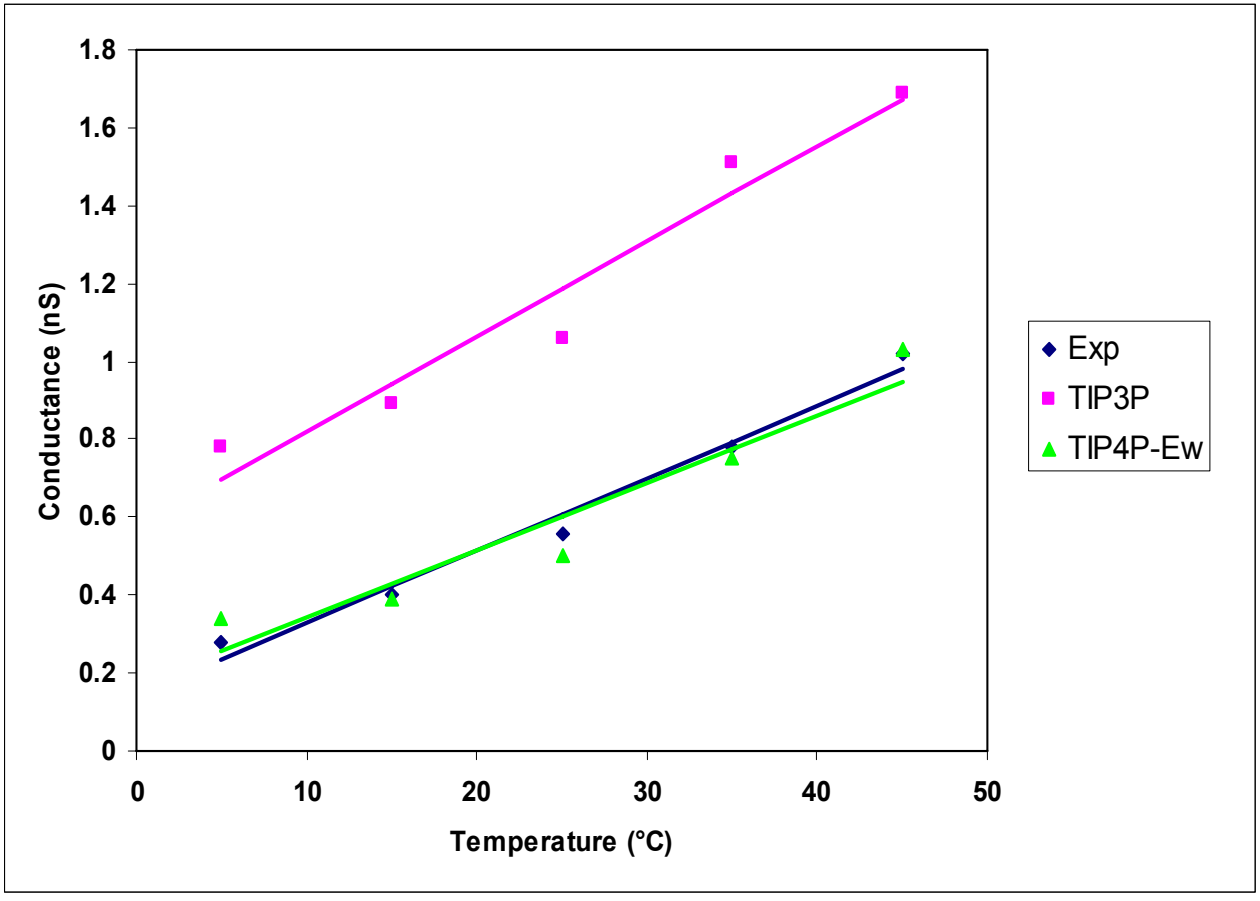

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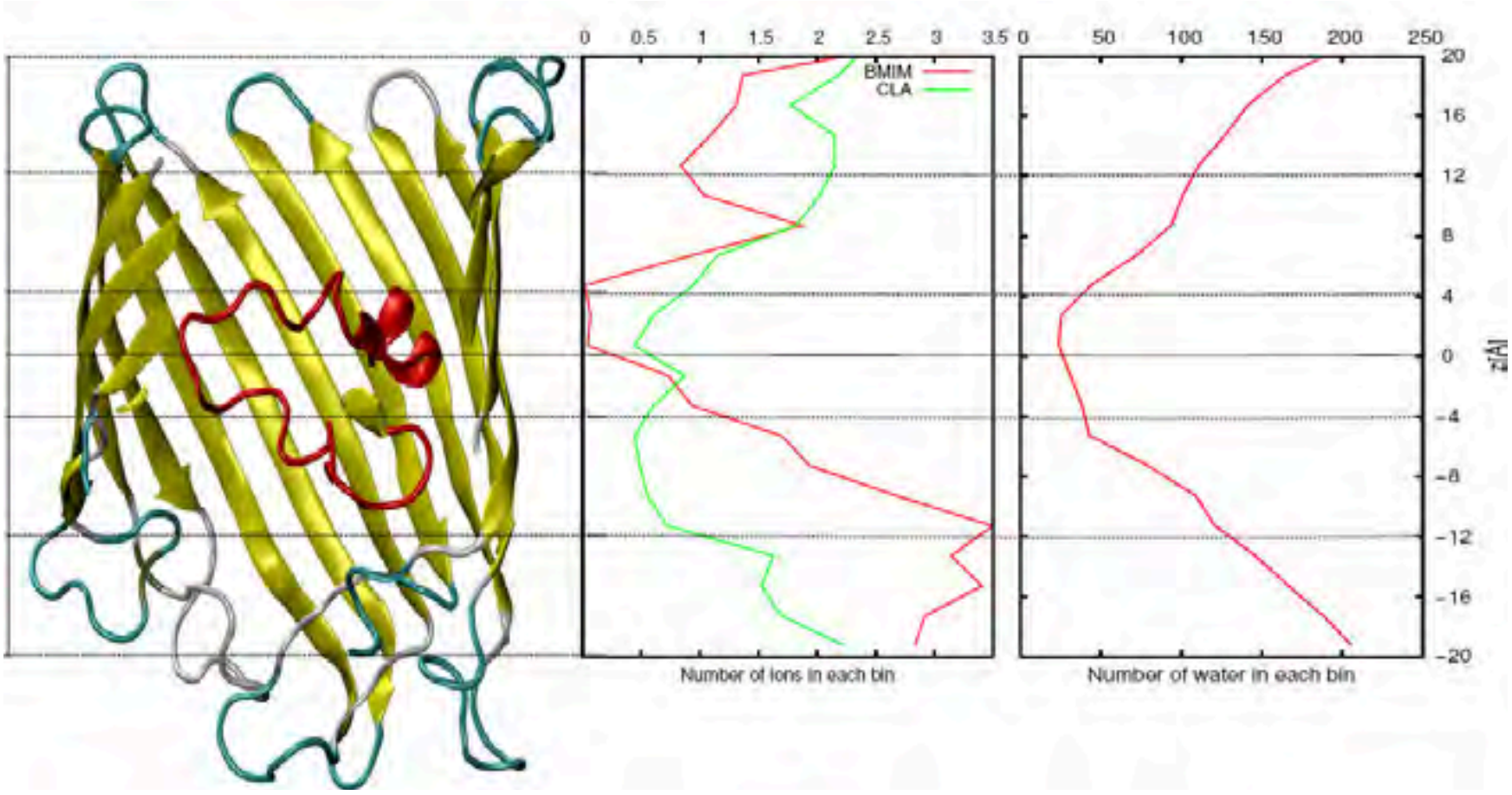
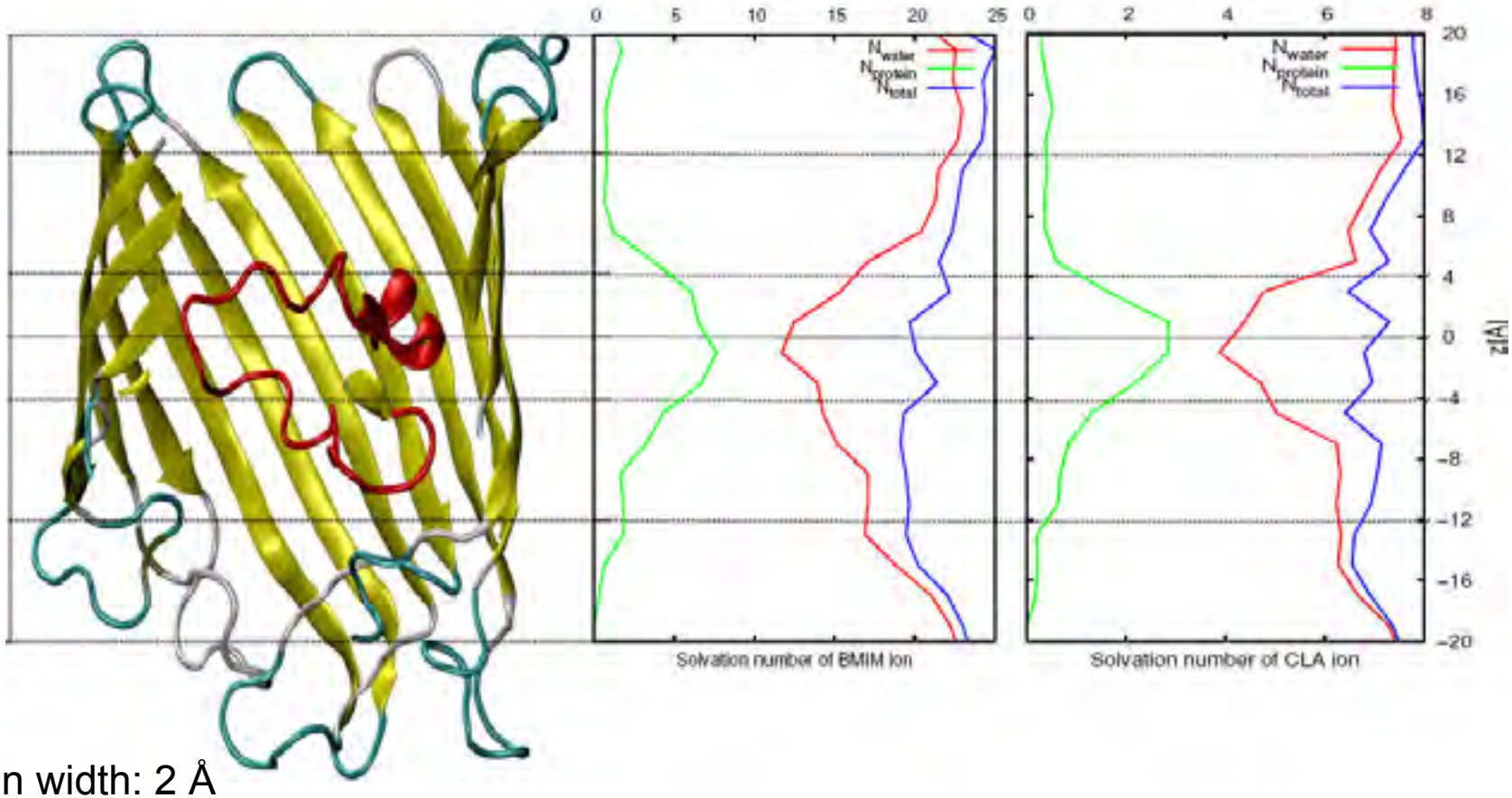
Water and Ions inside OmpF



- Number of water and ions in a particular bin
- Bin width: 2 Å

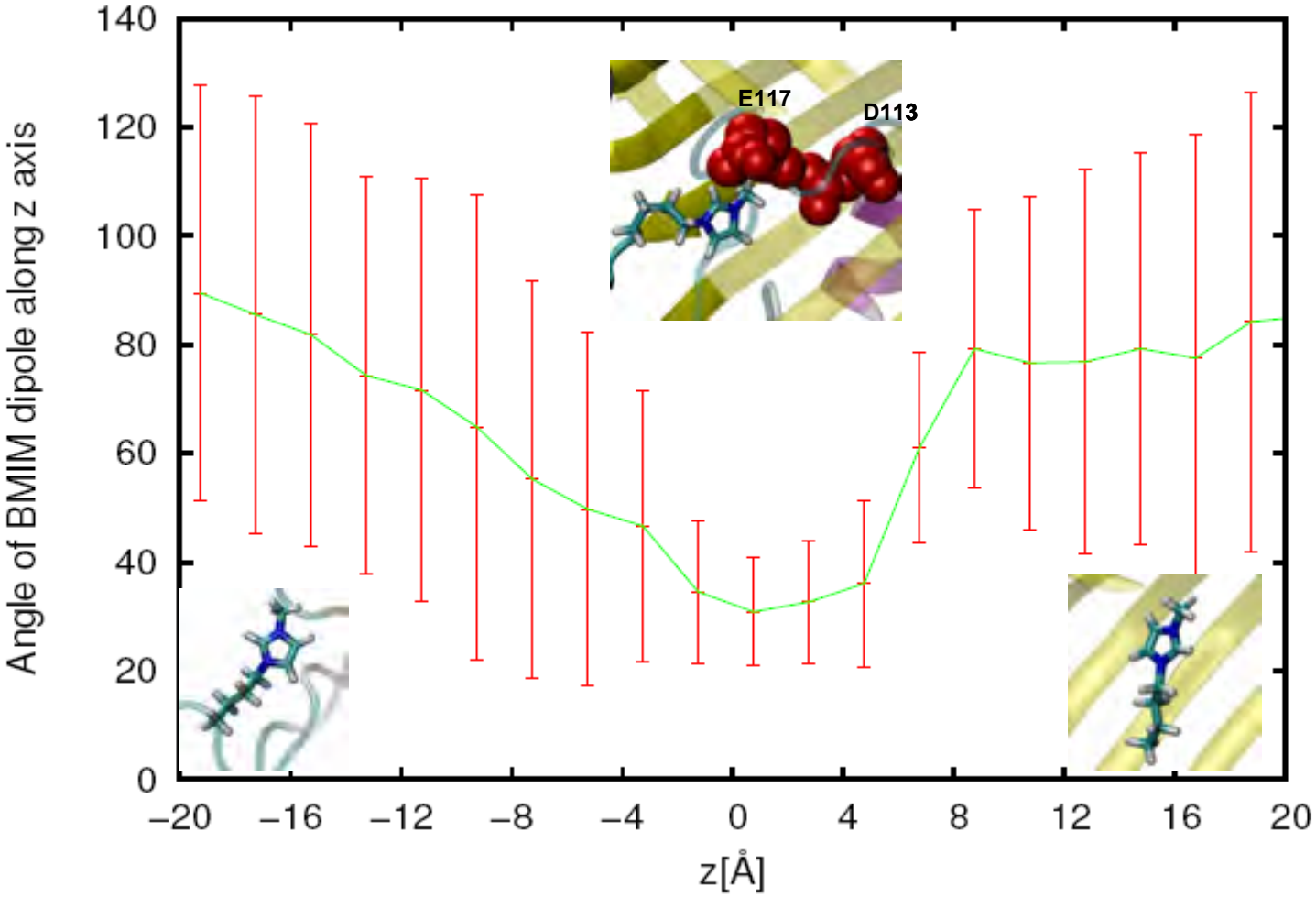
Ion Solvation

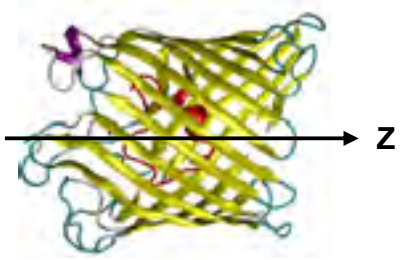


- Bin width: 2 Å

Ion type	Water Solvation	Protein Contacts
BMIM	Number of water within 4.1 Å from center of charge of BMIM	Number of oxygen atoms of protein within 4.1 Å from center of charge of BMIM
Cl	Number of water within 3.2 Å of Cl	Number of nitrogen atoms of protein within 3.2 Å of Cl

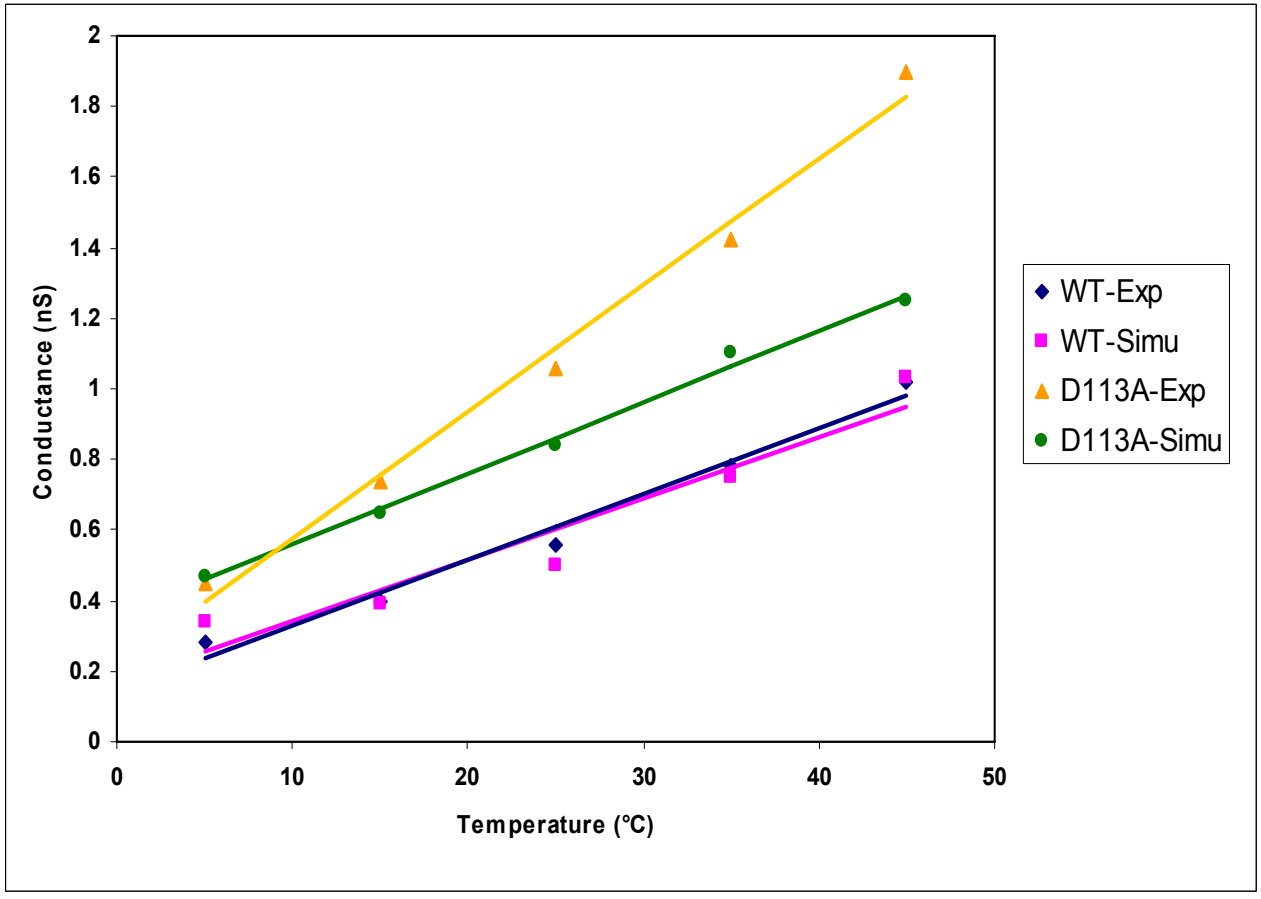
Orientation of BMIM





- Angle of BMIM dipole with respect to Z axis in a particular bin
- Bin width: 2 Å
- More consistent orientation of BMIM in constriction zone

Pore conductance: Mutant (D113A)



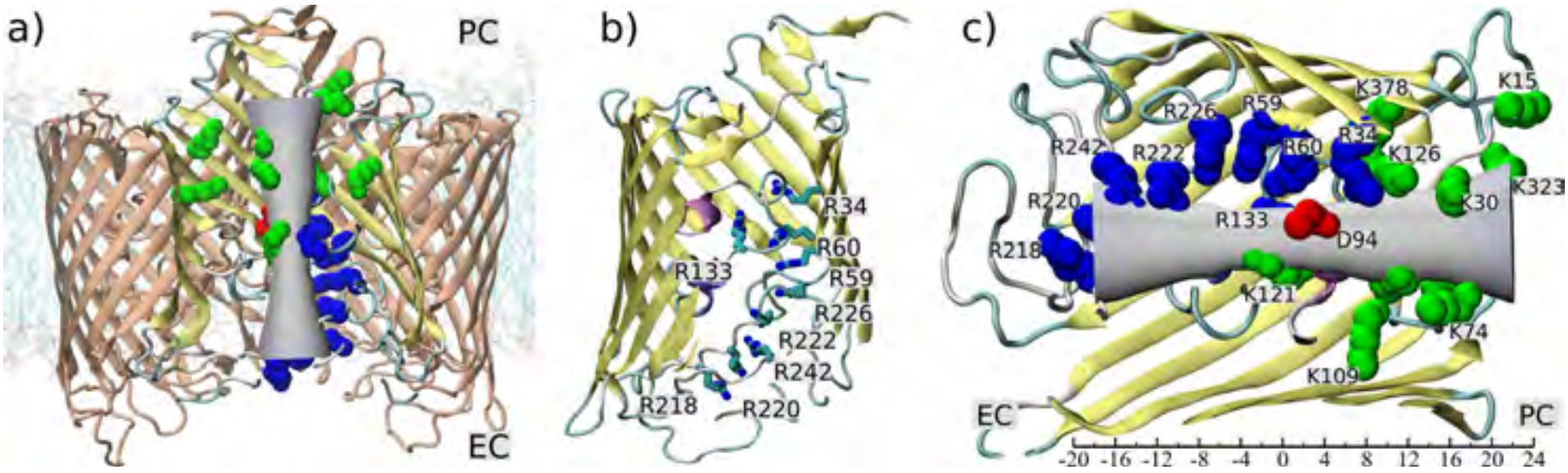
	Ratio (Cl/BMIM)
WT	3 to 5
D113A	5 to 8

- D113A mutant has higher conductance value than WT
- Mutant is more anion selective
- Indicates the influence of **negative charge** of **Asp** in determining ion conductance and ion selectivity

Outline

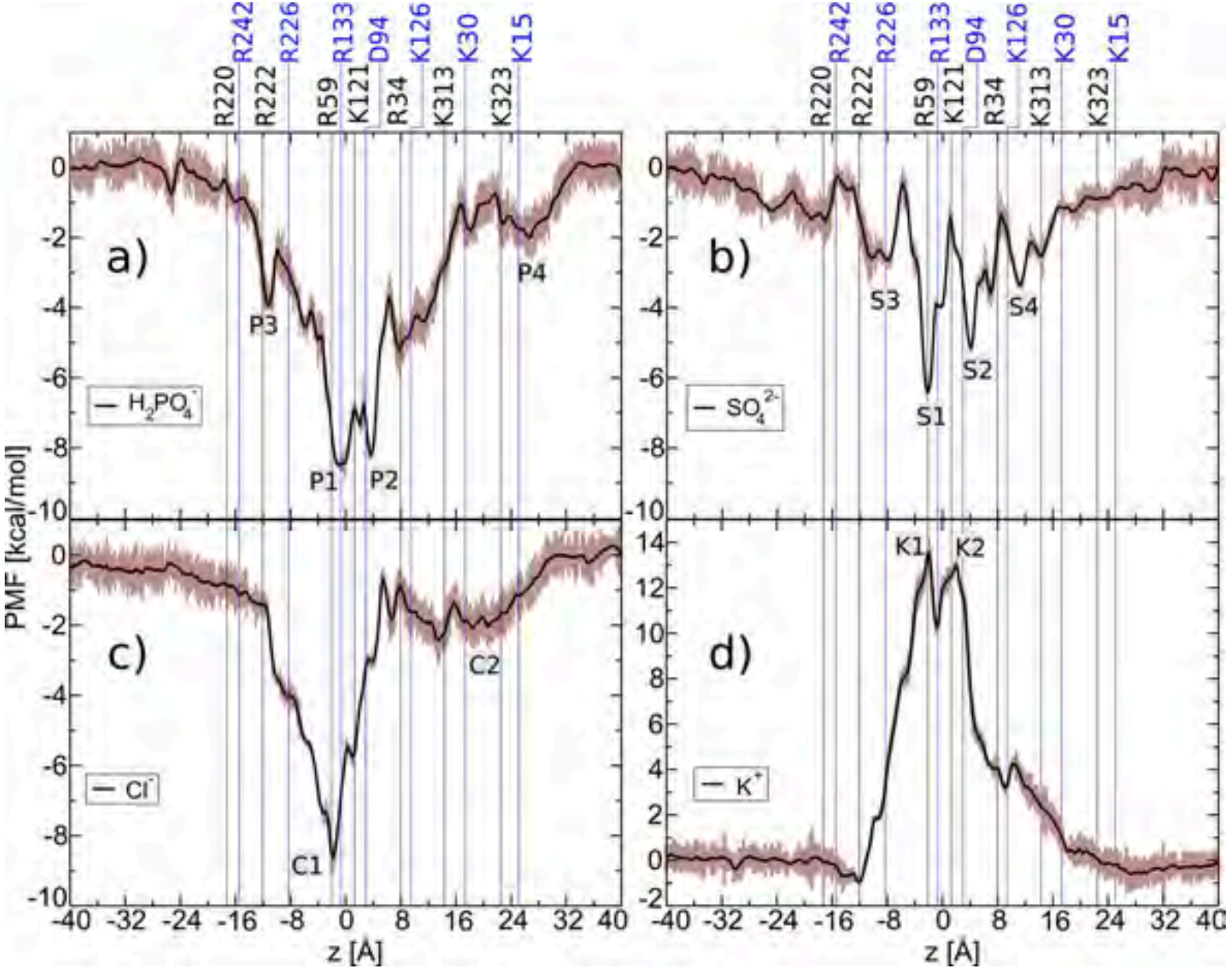
1. Motivation for studying ion transport
2. Ion transport through membrane proteins
3. Larger ions through OmpF
4. Phosphate-selective porin OprP
5. Excitation energy transfer in light-harvesting complexes

Phosphate-selective porin OprP

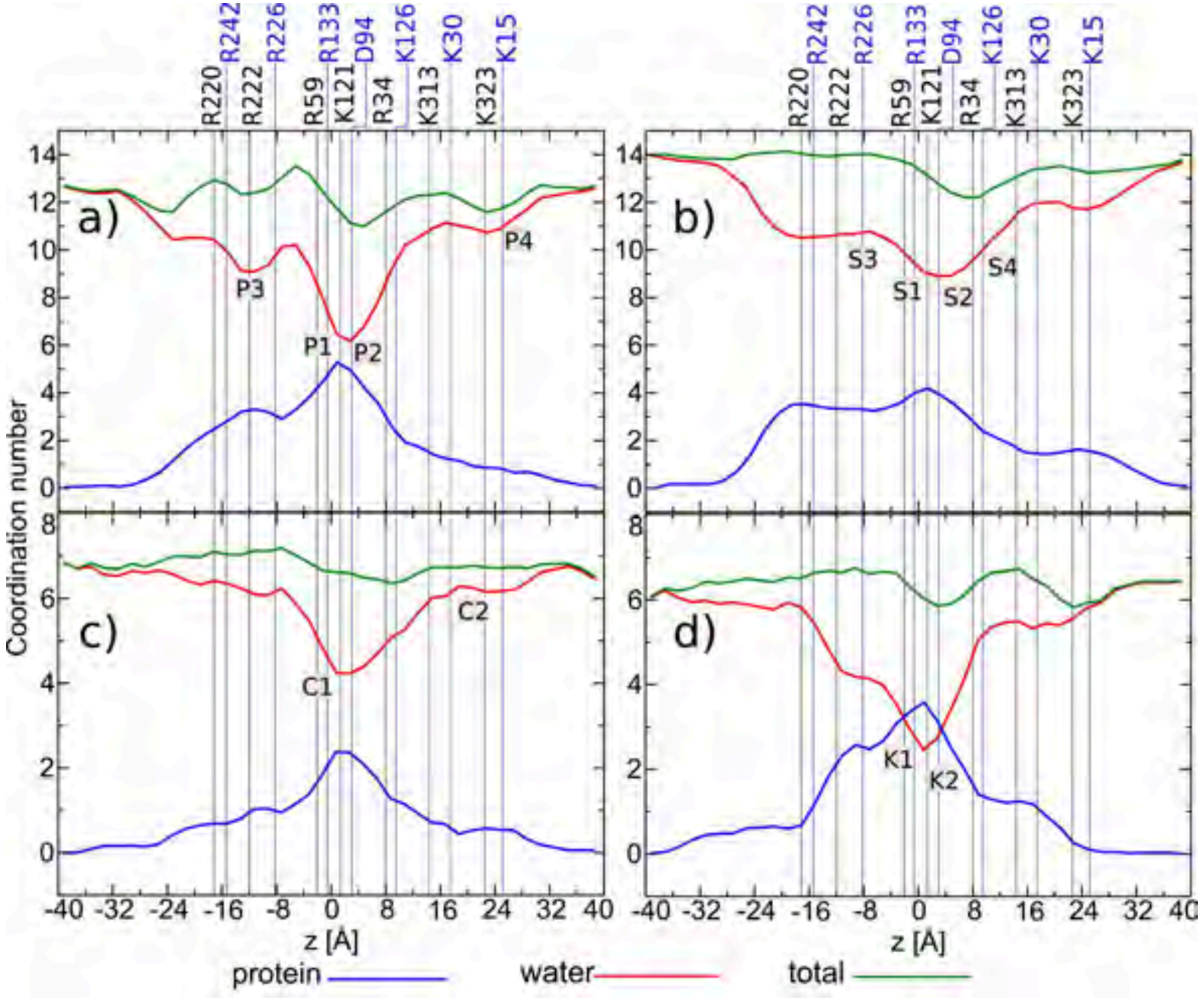


Modi, Benz, Hancock, Kleinekathöfer, J. Phys. Chem. Lett. **2**, 3639 (2012).
Modi, ... , Benz, Hancock, Kleinekathöfer, Biochemistry 52, 5522 (2013).

Free energy surfaces



Protein and Water Contacts



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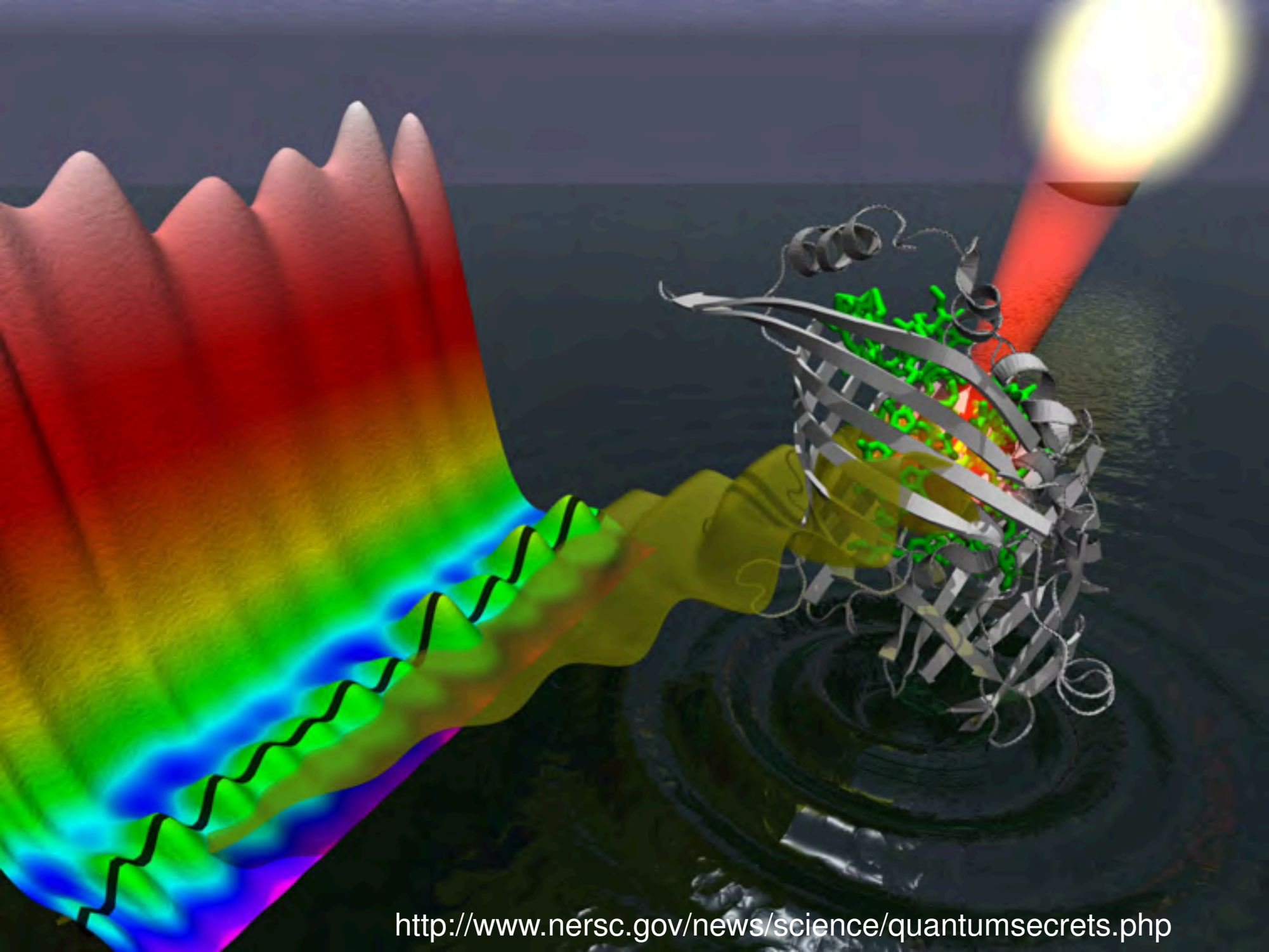
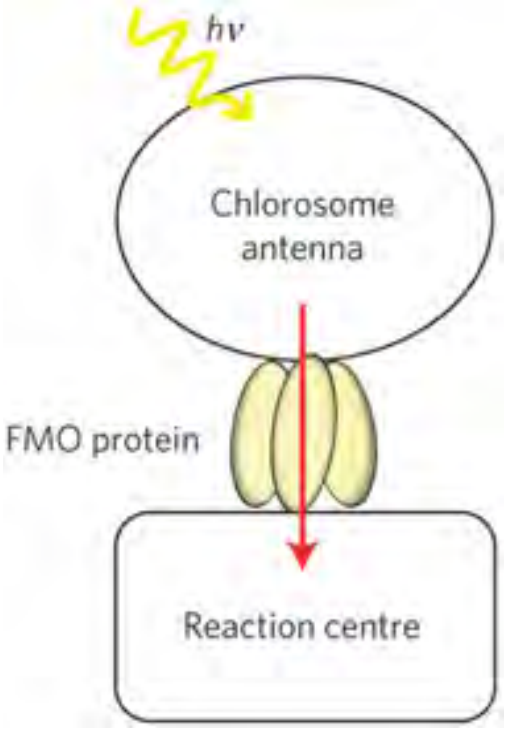
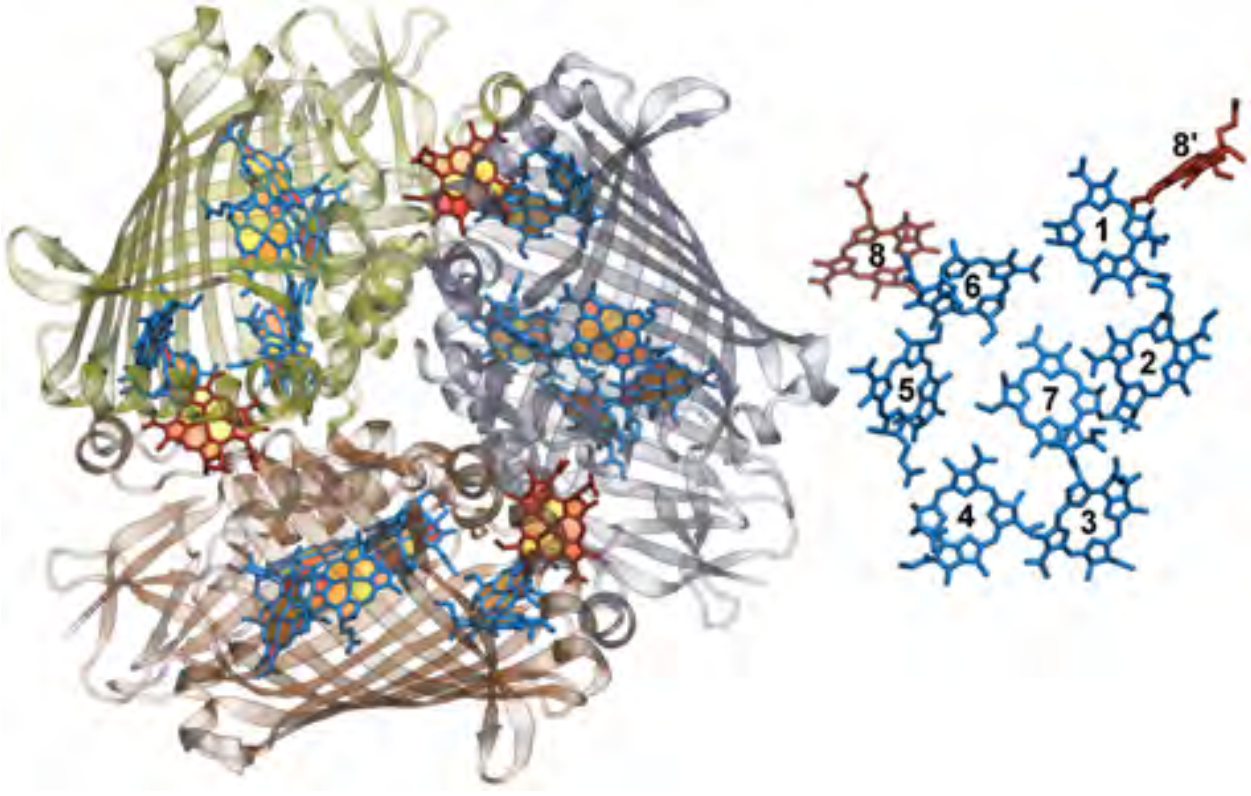

http://www.nersc.gov/news/science/quantumsecrets.php

The Fenna-Matthews-Olson (FMO) Complex of Green Sulfur Bacteria

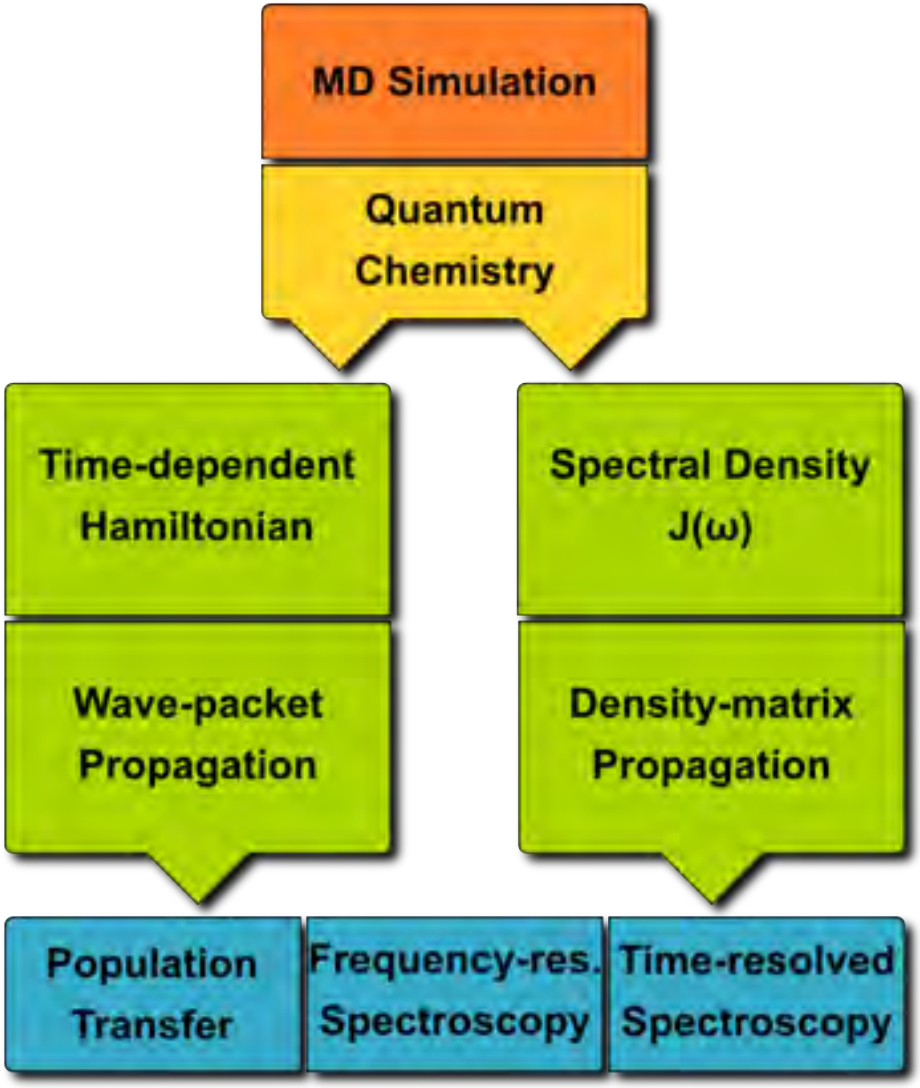




Sarova et al., *Nat. Phys.* **6** 462 (2010)

- Mediates excitation energy transfer from the chlorosome to the RC
- Trimeric structure, water soluble
- 8 BChls per monomer, embedded in protein scaffold

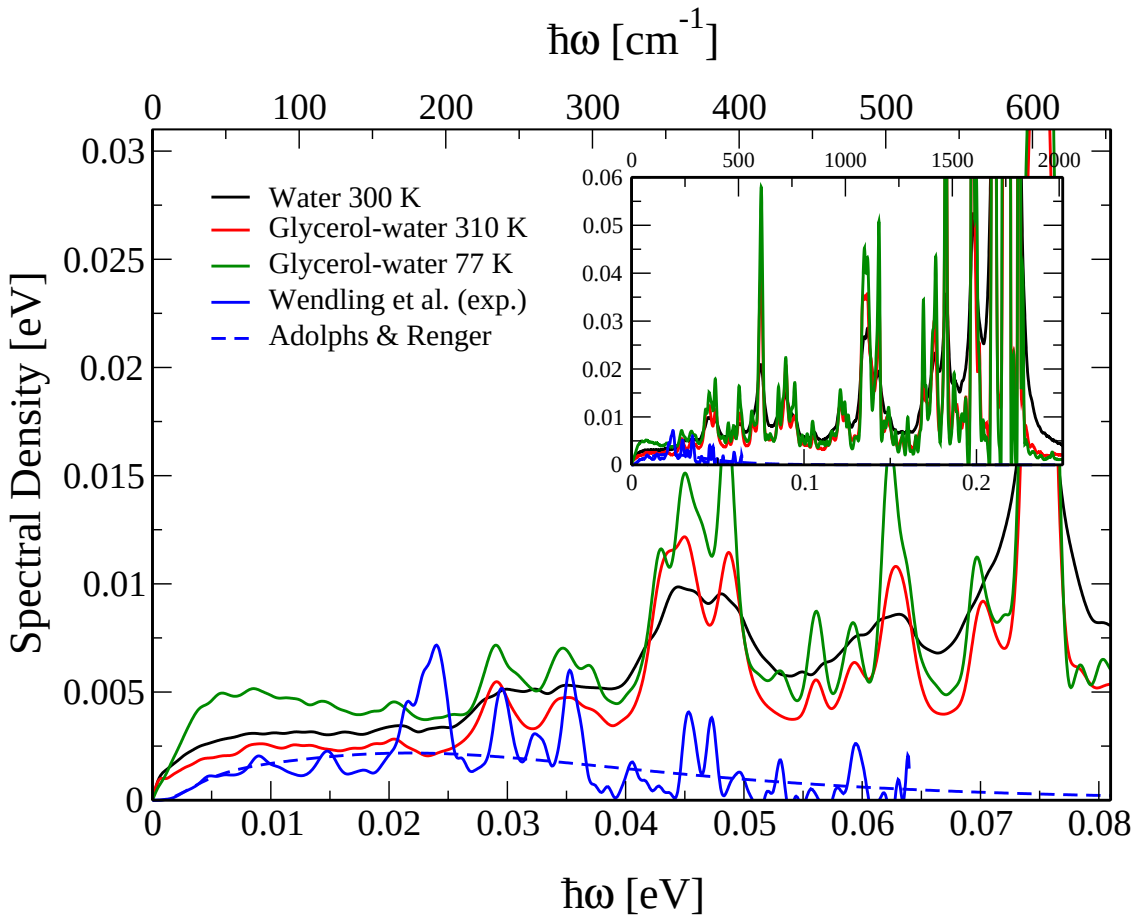
Simulation strategy of quantum effects in biomolecules



- **Molecular dynamics simulation**
  - Classical force-field based simulation of complete systems incl. water etc
  - Atomic details
  - No optical properties, energy transfer, etc.
- **Quantum chemistry**
  - Electronic structure calculations of individual pigments
  - Electronic couplings
  - Along MD trajectory
- **Dissipative Quantum Dynamics**

Spectral densities of the environment

$$J(\omega) = \frac{\hbar\omega}{\pi k_B T} \int_0^\infty dt \, \langle \Delta E(t) \Delta E(0) \rangle \, \cos \omega t$$



Olbrich, Strümpfer, Schulten, Kleinekathöfer, J. Phys. Chem. Lett. **2**, 1771 (2011)
Agthar, Strümpfer, Olbrich, Schulten, Kleinekathöfer, J. Phys. Chem. B **117**, 7157 (2013)

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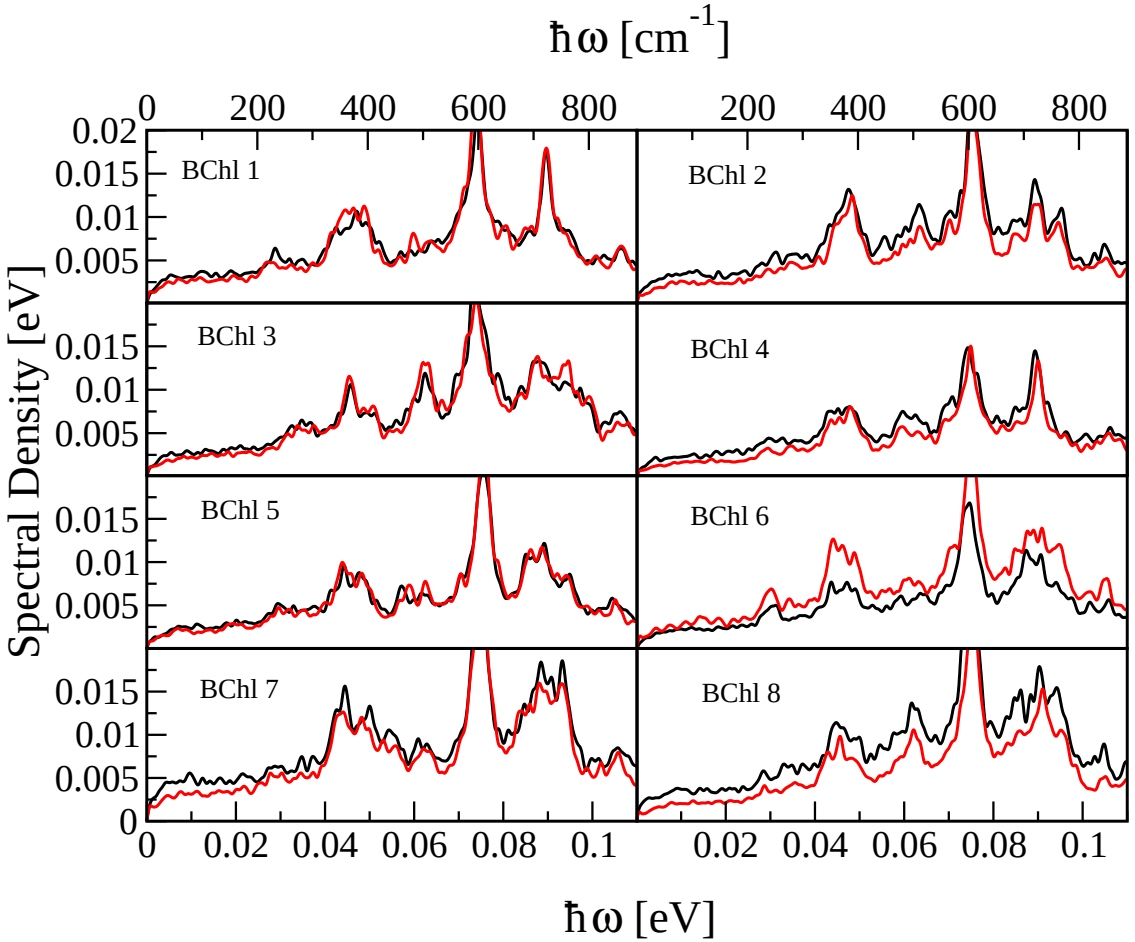

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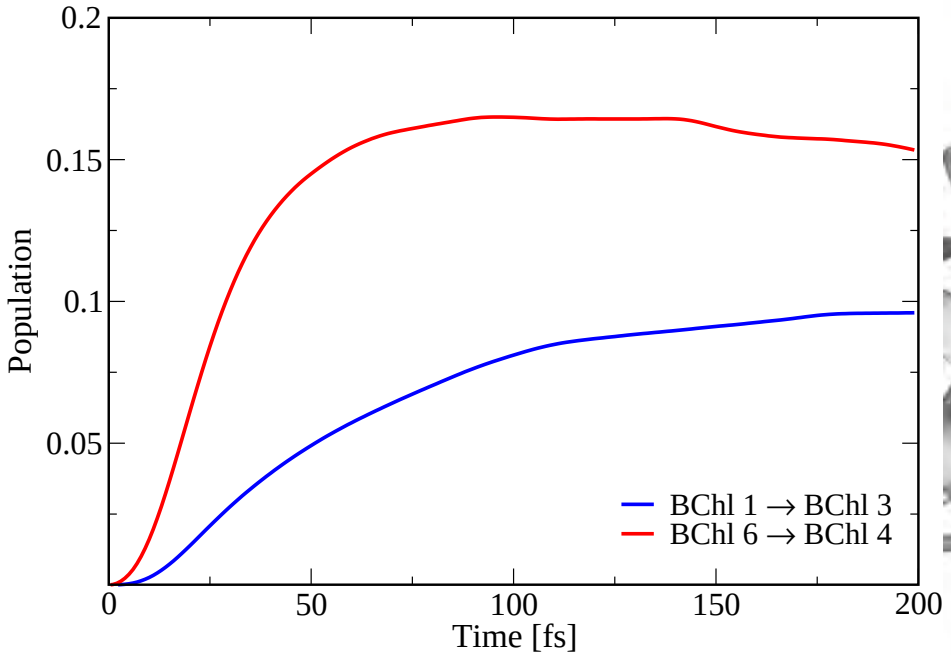
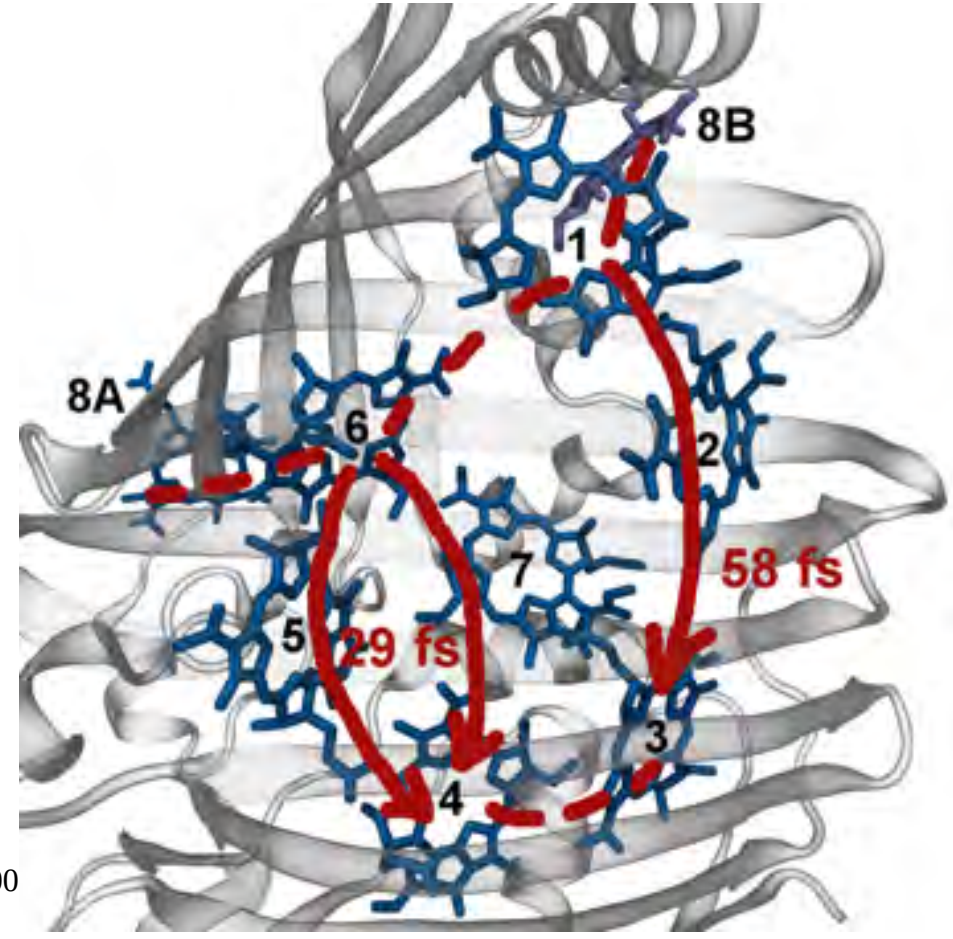
Examples for transfer between sites





- Fast transfer via intermediate sites
- Very slow transfer between the FMO monomers