Multi-scale Ion Channel Simulations

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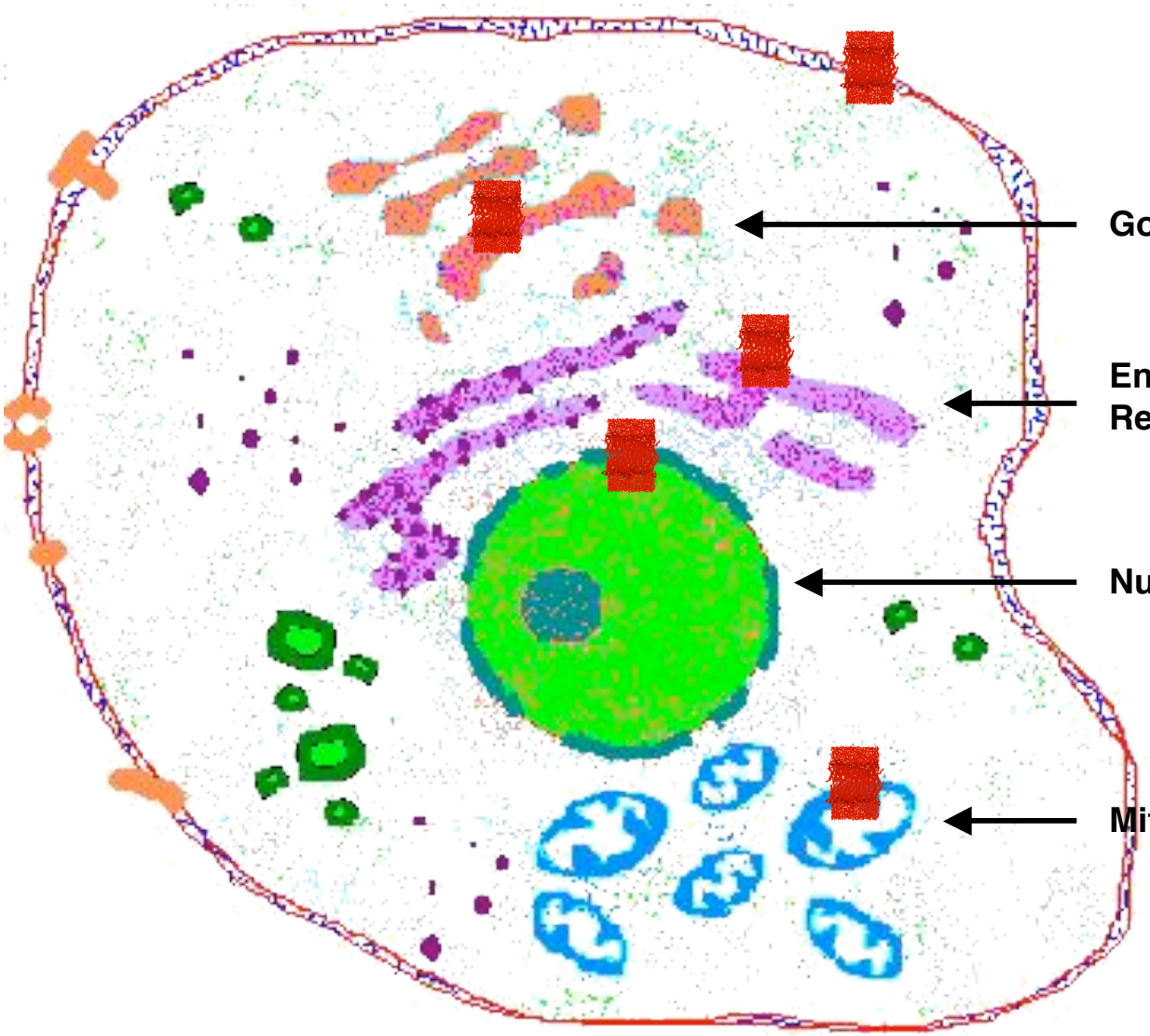
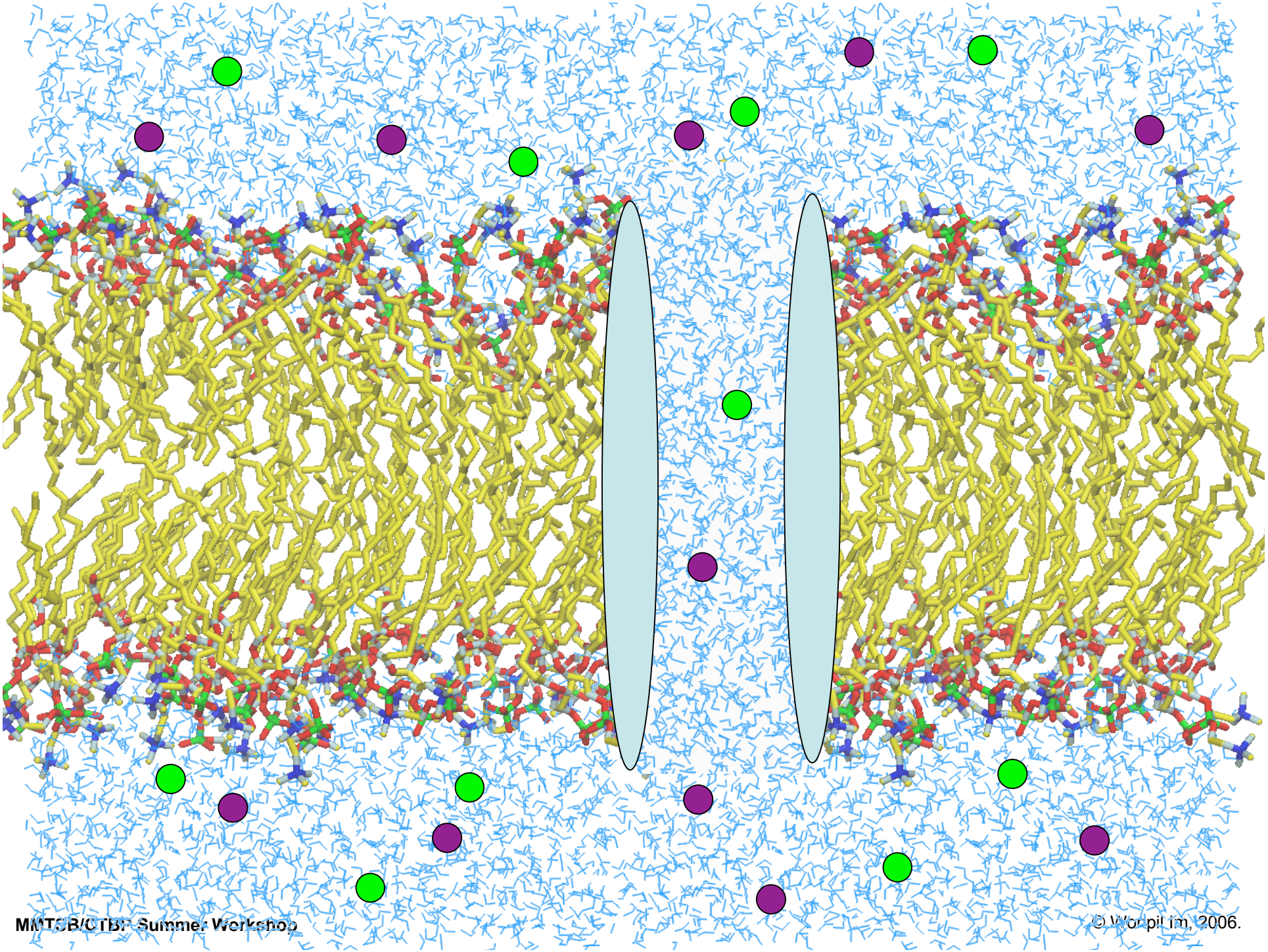
MMTSB/CTBP
2006 Summer Workshop



http://www.usd.edu/~bgoodman/ReviewFrames.htm



Permeation Models:
Molecular Dynamics

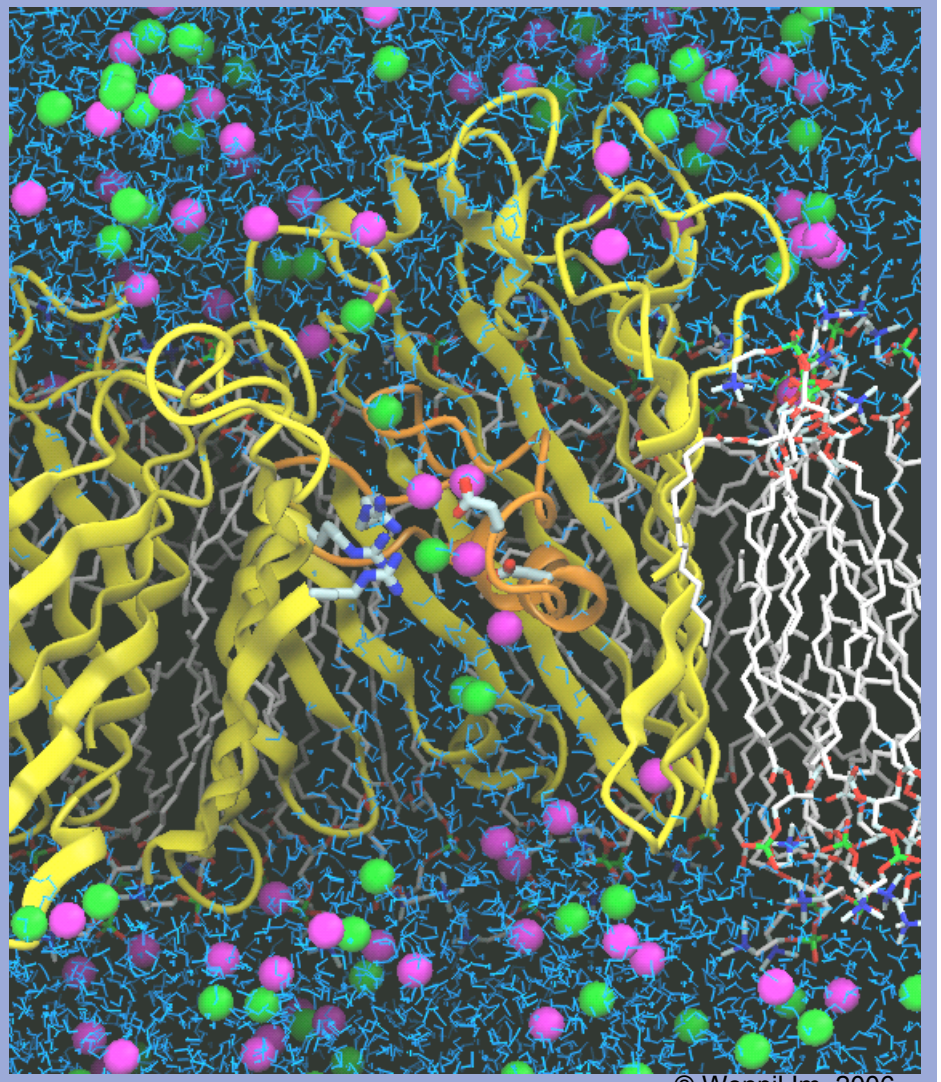
- Newton's classical equations of motion *F=MA*
- Potential energy function

$$U(r_1,r_2,..,r_N) = \sum_{\text{bonds, i}} \tfrac{1}{2} k_i^b \cdot (r_i - r_i^0)^2 + \sum_{\text{angles, i}} \tfrac{1}{2} k_i^\theta \cdot (\theta_i - \theta_i^0)^2 +$$

$$\sum_{\text{torsions, i}} k_i^\phi \cdot [1 + \cos(n_i \phi_i - \delta_i)] +$$

$$\tfrac{1}{2} \sum_{\text{nonbond pairs, (i,j)}} \sum \left\{ \varepsilon_{\min}^{ij} \left[\left(\frac{r_{\min}^{ij}}{r_{ij}} \right)^{12} - 2 \left(\frac{r_{\min}^{ij}}{r_{ij}} \right)^{6} \right] + \frac{q_i q_j}{\varepsilon r_{ij}} \right\}$$

- 1 nS => 1 ion / 1.6 ns at 100 mV



Output

- Potential of mean force
- Ion diffusion constants
- Short-range ion-ion interactions
- Ion exclusion radius

Permeation Models:
Brownian Dynamics

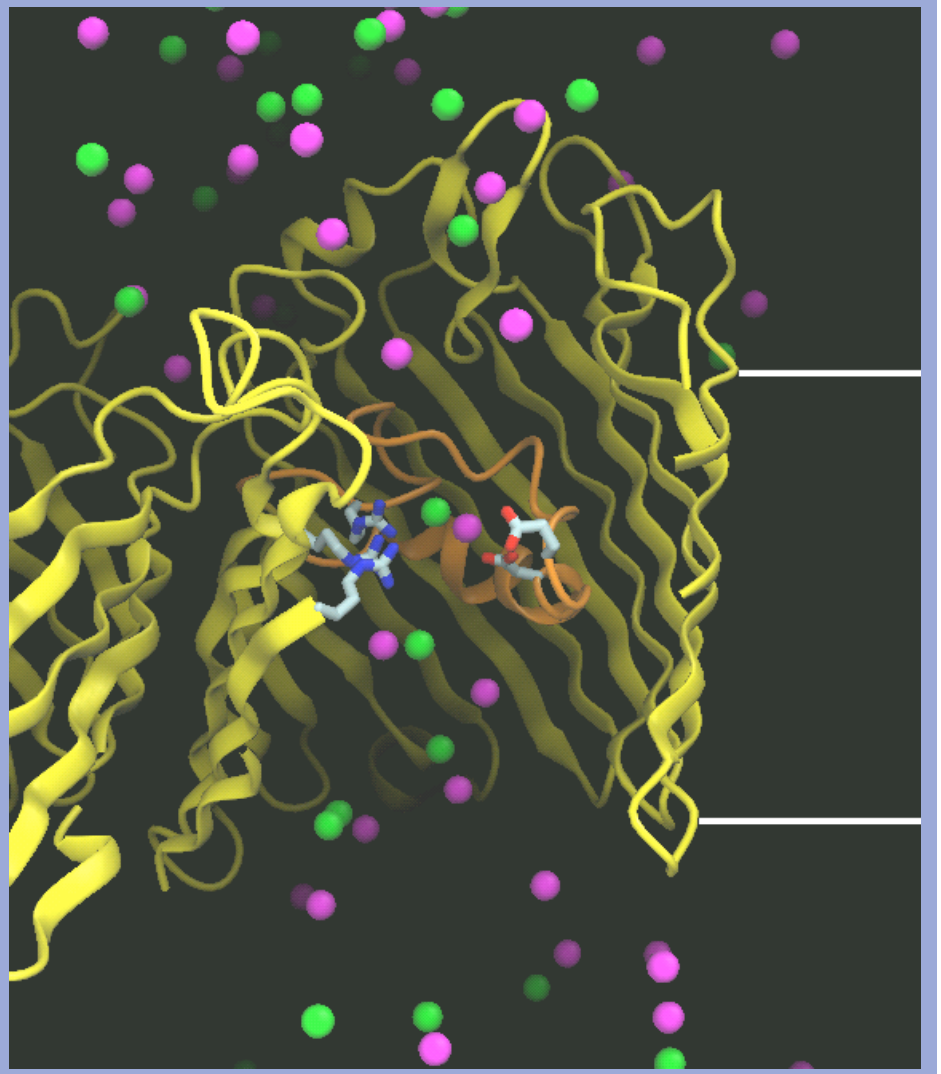
- Brownian equations of motion

$$\dot{\mathbf{r}}_i(t) = \frac{D_i(\mathbf{r})}{k_B T}\langle \mathbf{F}_i \rangle + \frac{\partial D_i(\mathbf{r})}{\partial \mathbf{r}_i} + \xi_i(t)$$

- Multi-ion PMF (continuum electrostatics)
- Rigid channel proteins
- Ion-ion correlations

Output

- **I-V curve**
- **Channel conductance**
- **Reversal potential**



Permeation Models:
Poisson-Boltzmann & Poisson-Nernst-Planck

- Mean-field theories
- PNP electrodiffusion equations

Nernst-Planck (NP) equation :

$$\mathbf{J}_\alpha(\mathbf{r}) = -D_\alpha(\mathbf{r})\left(\nabla C_\alpha(\mathbf{r}) + \frac{q_\alpha}{k_B T} C_\alpha(\mathbf{r})\nabla\phi(\mathbf{r})\right)$$

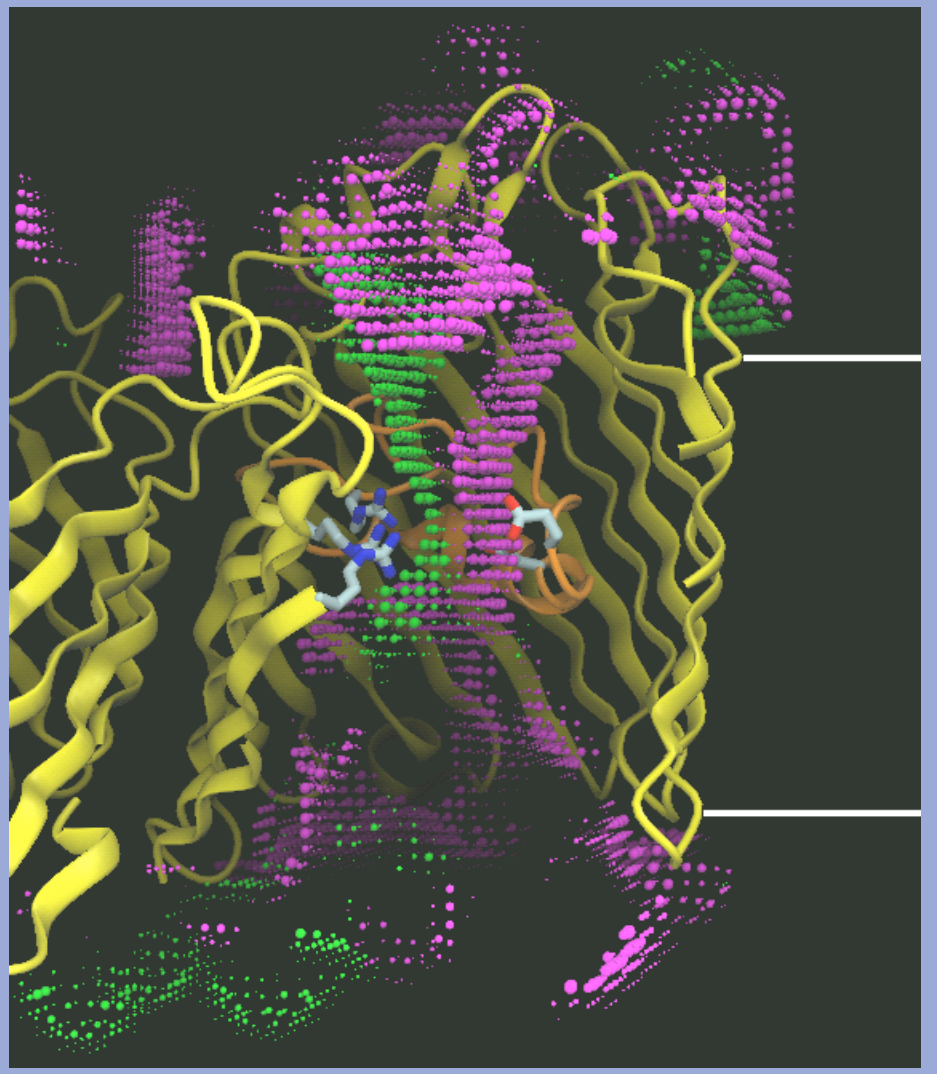
Poisson equation :

$$\nabla \bullet \left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right] = -4\pi\left(\rho_p(\mathbf{r}) + \sum_\alpha q_\alpha C_\alpha(\mathbf{r})\right)$$

- Continuum electrostatics
- Rigid channel proteins

Output

- **I-V curve**
- **Channel conductance**
- **Reversal potential**



Permeation Models

MD

- Newton's classical equations of motion *F=MA*
- Potential energy function

Output
- Potential of mean force
- Ion diffusion constants
- Short-range ion-ion interactions
- Ion exclusion radius

BD

- Brownian (or Langevin) equations of motion
- Multi-ion PMF (continuum electrostatics)
- Rigid channel proteins
- Ion-ion correlations

Output
- I-V curve
- Channel conductance
- Reversal potential

PB & PNP

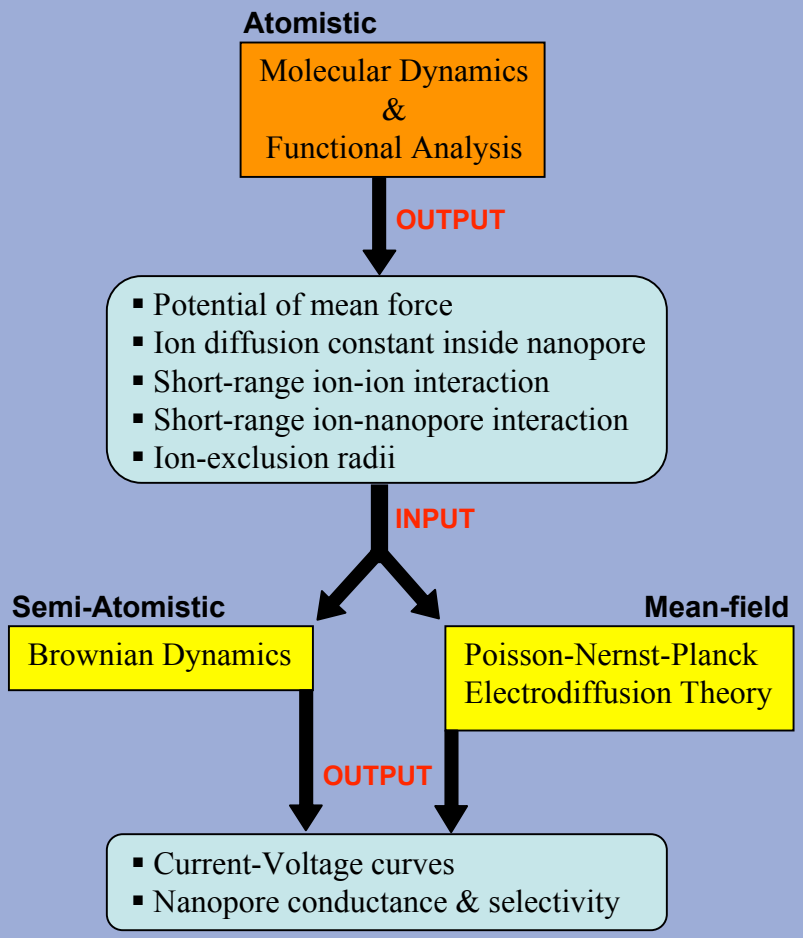
- Mean-field theories
- PNP electrodiffusion equations
- Continuum electrostatics
- Rigid channel proteins

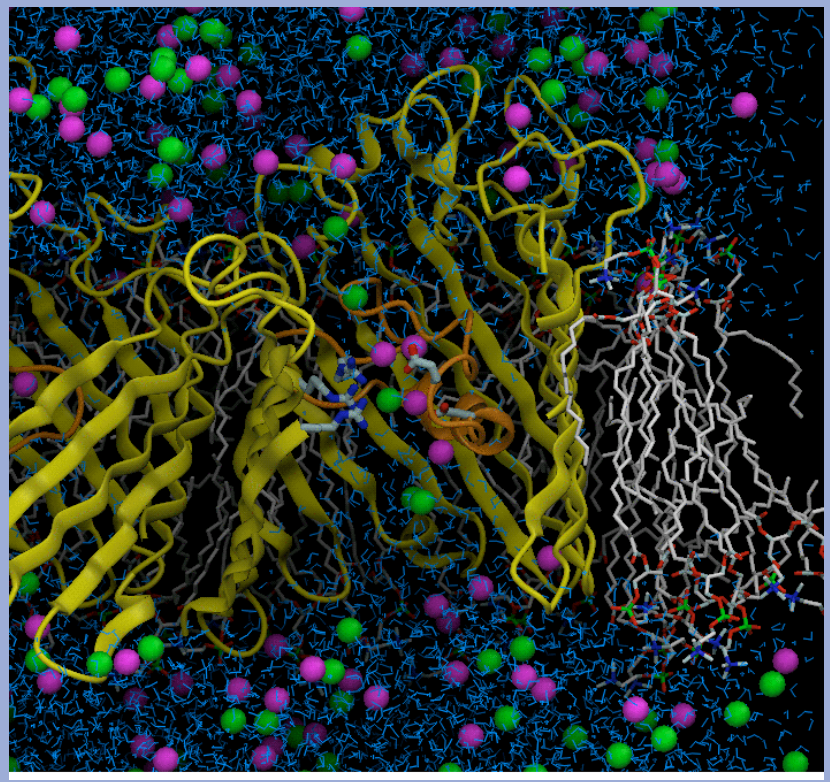
Output
- I-V curve
- Channel conductance
- Reversal potential

more details ⟷ less details

Roux et al. (2004) *Q. Rev. Biophys.*

The Hierarchical Approach

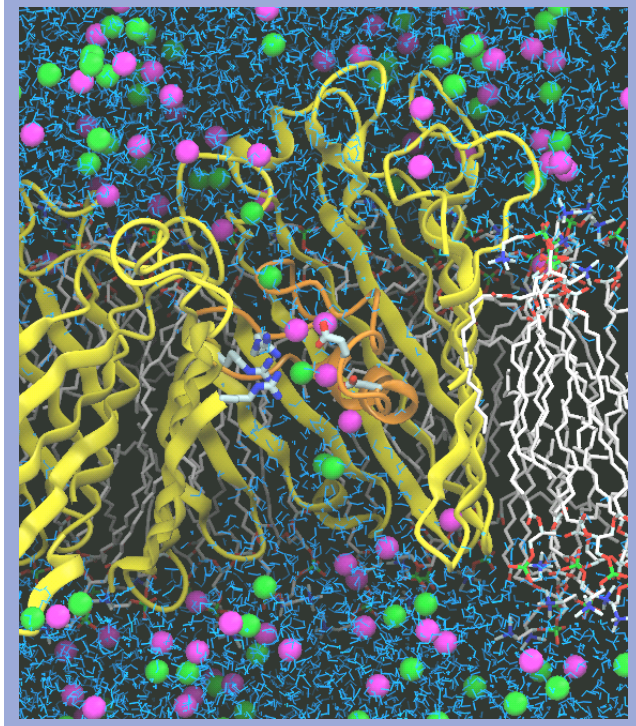


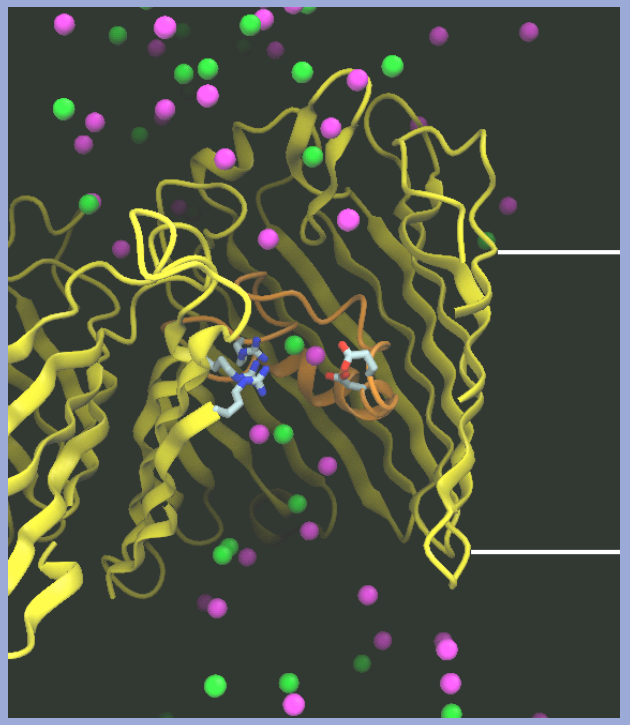


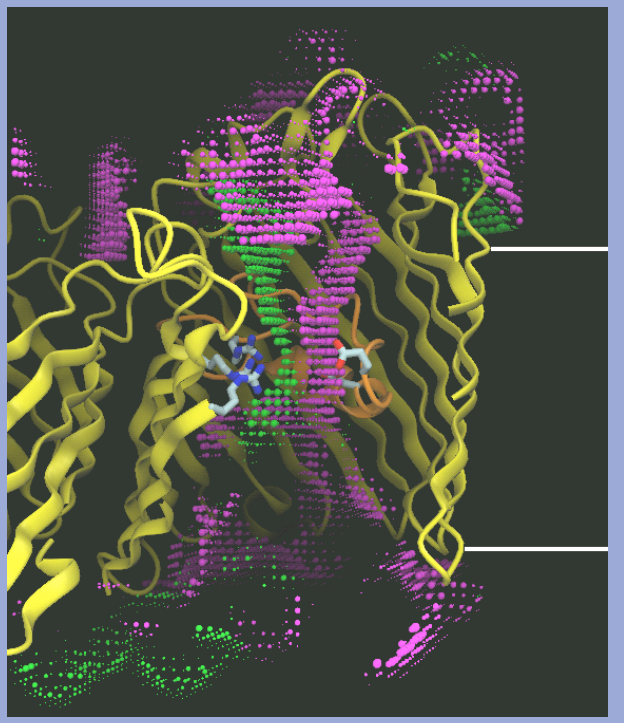
The Hierarchical Approach

- Ion diffusion constants
- Short-range ion-ion interactions
- Ion exclusion radius

MD → BD → PB & PNP





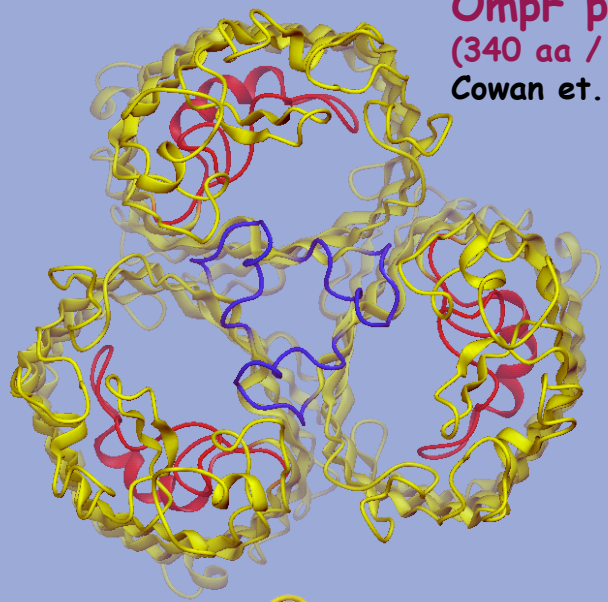


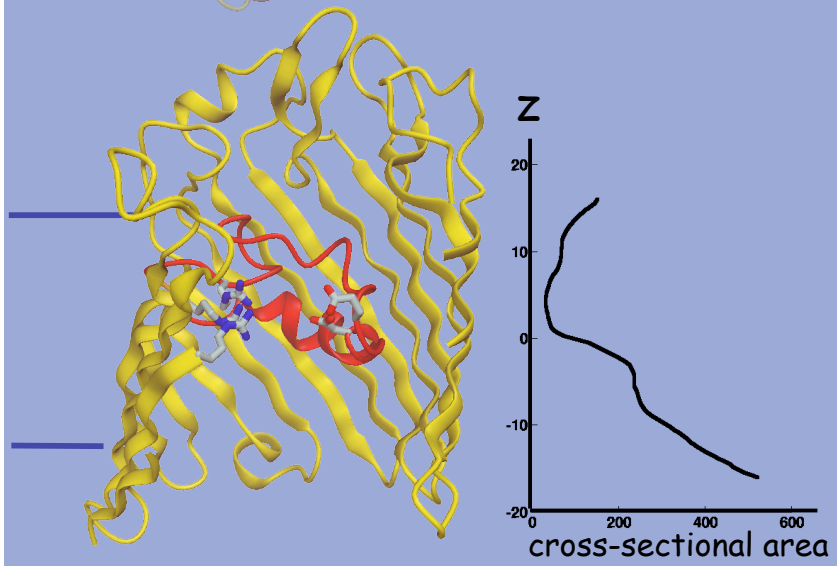
1. Validity of approximate approaches such as BD, PB, and PNP
2. Validity of the hierarchical approach
   [microscopic information (inputs) from MD simulations]

Im & Roux (2002) *J. Mol. Biol.*

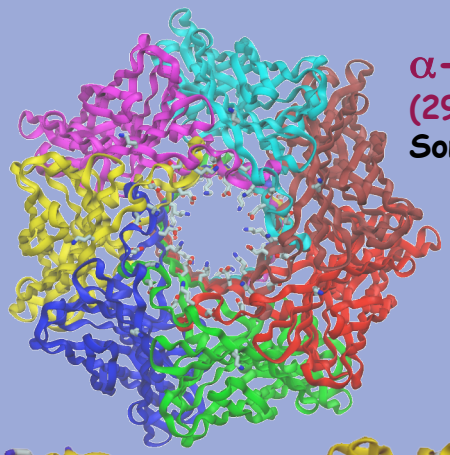
Model Systems

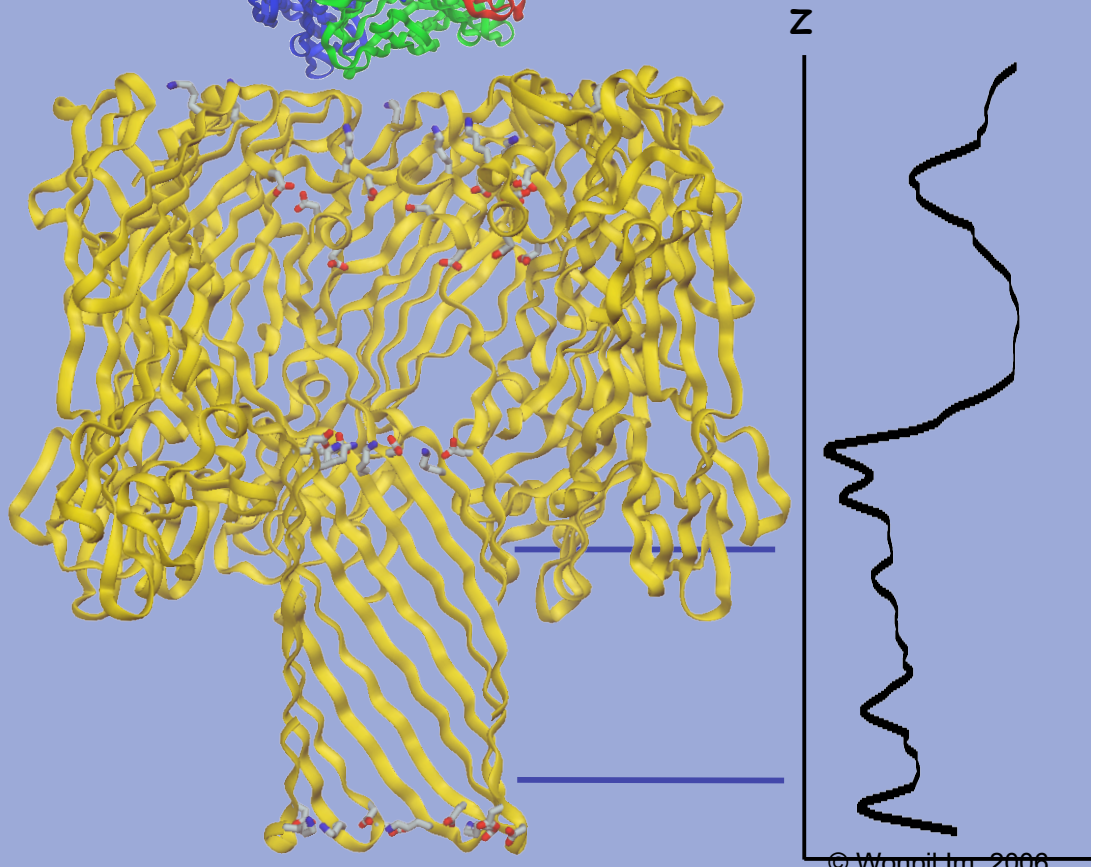
OmpF porin
(340 aa / monomer)
Cowan et. al. (1992) *Nature*





α-Hemolysin (α-HL)
(293 aa / monomer)
Song et. al. (1996) *Science*





When Reconstituted into Planar Membranes

- **Distinct large single-channel conductance in 1M KCl**

  OmpF: **1.25** nS at 160 mV and **1.13** nS at -160 mV

  α-HL : **1.01** nS at 40 mV and **0.78** nS at -40 mV

  1 nS => 1 ion / 1.6 ns at 100 mV or 1000 ions / 1.6 μs

- **Ion selectivity**

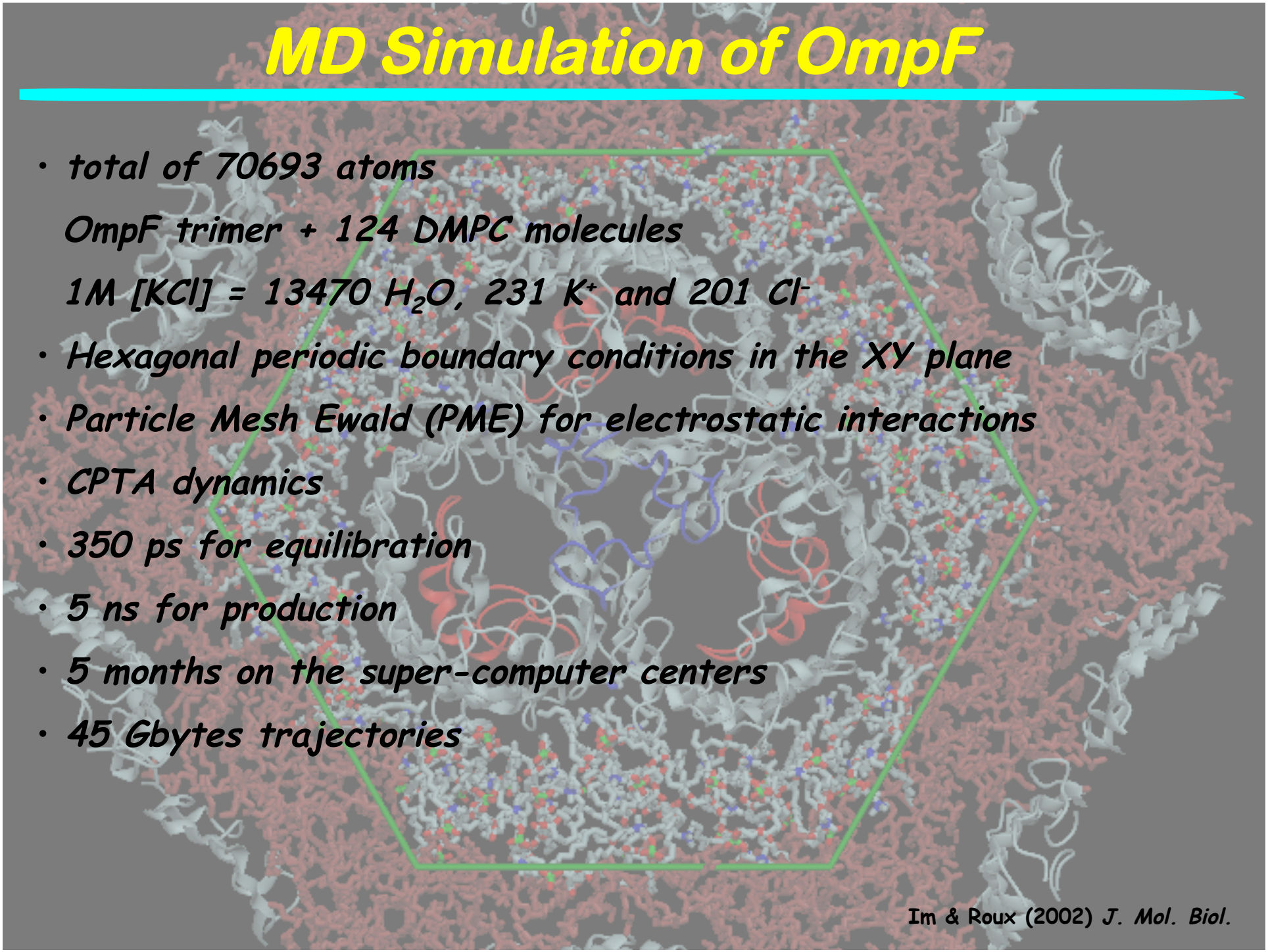
  OmpF: V_{rev} = **24.3** mV (0.1/1.0 M [KCl]) <---> P_K/P_{Cl} = **3.3**

  α-HL : V_{rev} = **9.2** mV (1.0/0.2 M [KCl]) <---> P_K/P_{Cl} = **0.55**

- **Voltage gating behavior**

Miles et al. (2002) *Protein Sci.*
Gu et al. (2001) *Prot. Natl. Acad. Sci. USA*
Gu & Bayley (2000) *Biophys. J.*
Schirmer et al. (1999) *J. Mol. Biol.*
Saint, personal communication

MD Simulation of OmpF

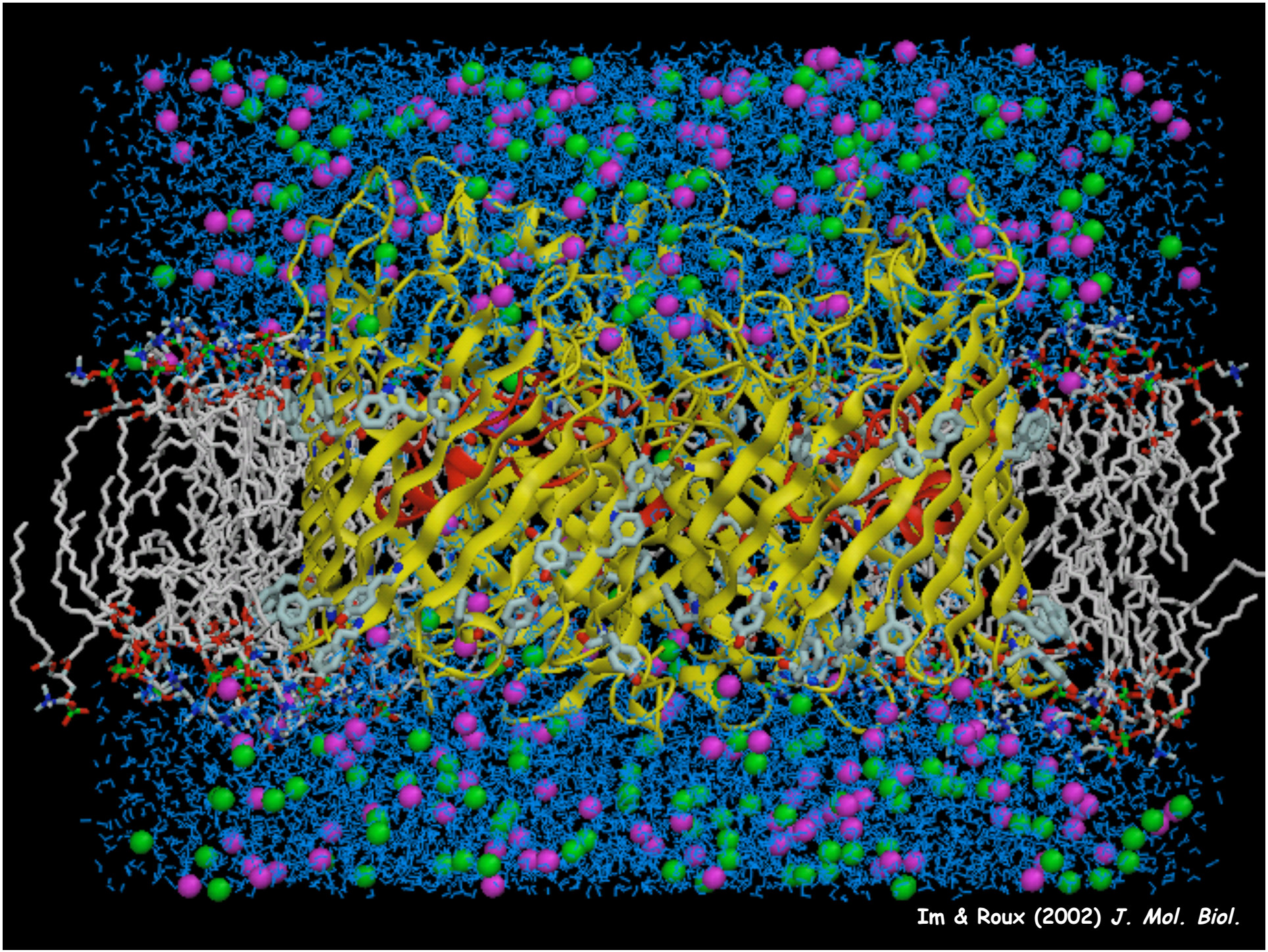


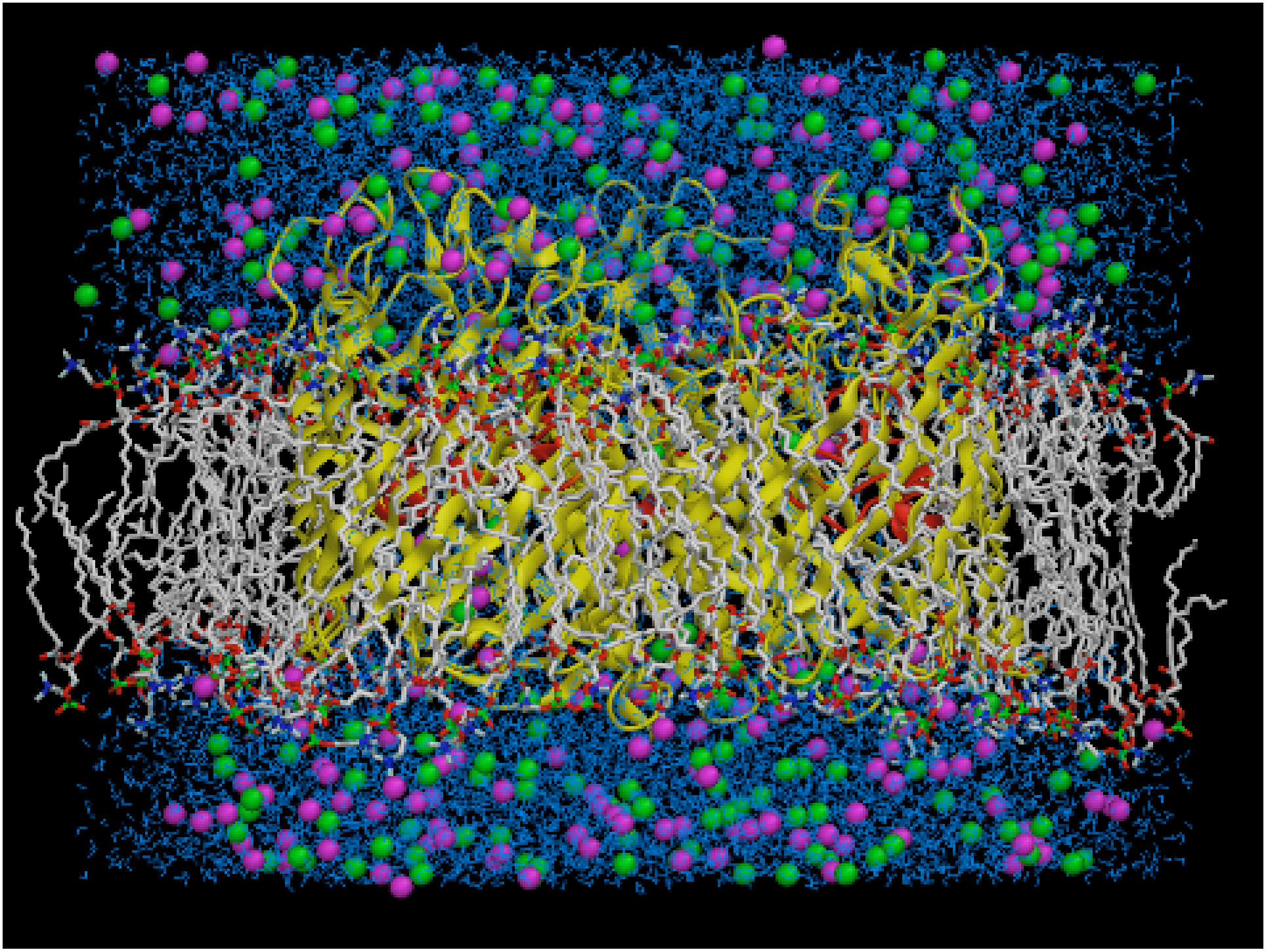
- total of 70693 atoms

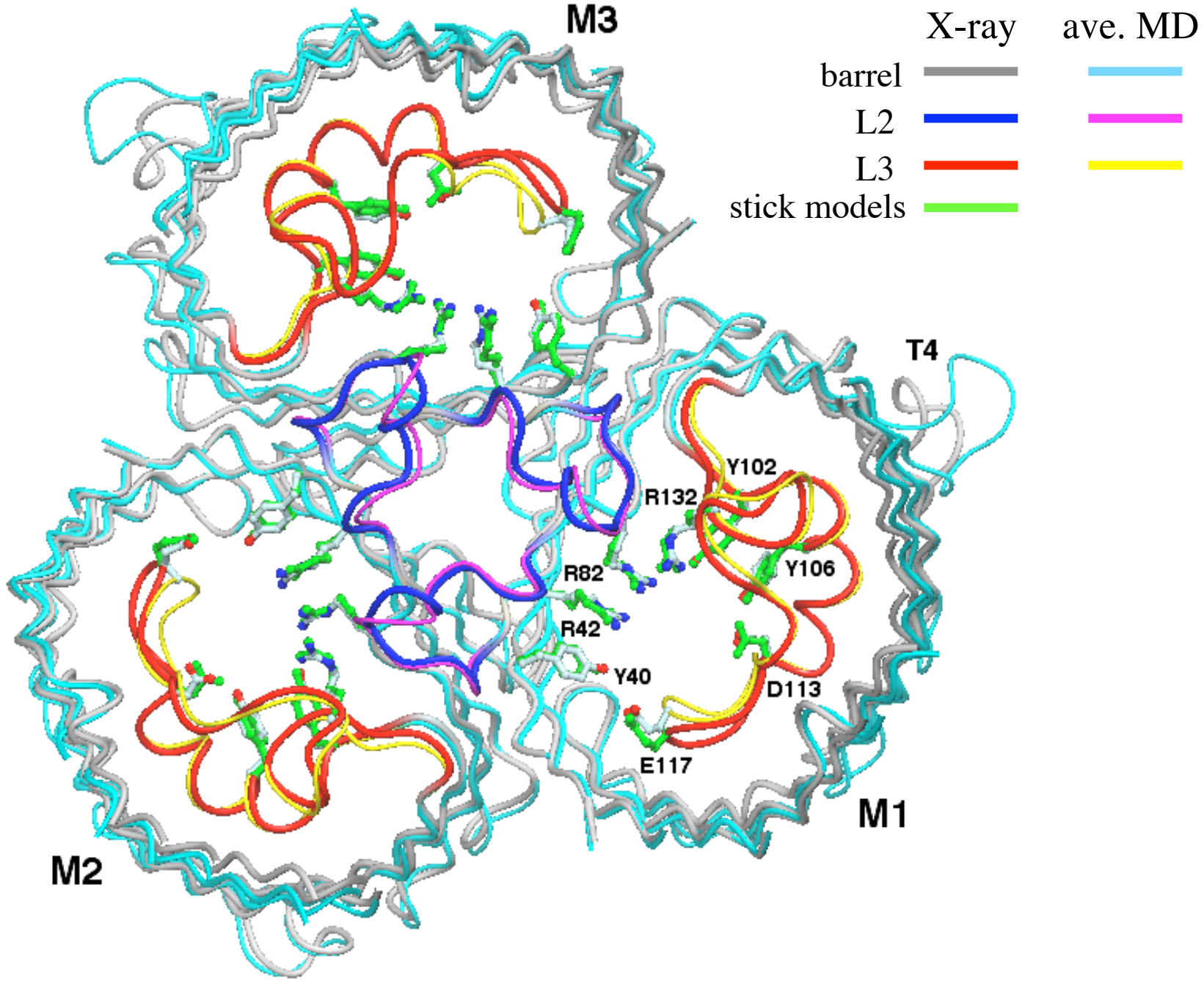
  OmpF trimer + 124 DMPC molecules

  1M [KCl] = 13470 H_2O, 231 K^+ and 201 Cl^-
- Hexagonal periodic boundary conditions in the XY plane
- Particle Mesh Ewald (PME) for electrostatic interactions
- CPTA dynamics
- 350 ps for equilibration
- 5 ns for production
- 5 months on the super-computer centers
- 45 Gbytes trajectories

Im & Roux (2002) *J. Mol. Biol.*


Im & Roux (2002) J. Mol. Biol.

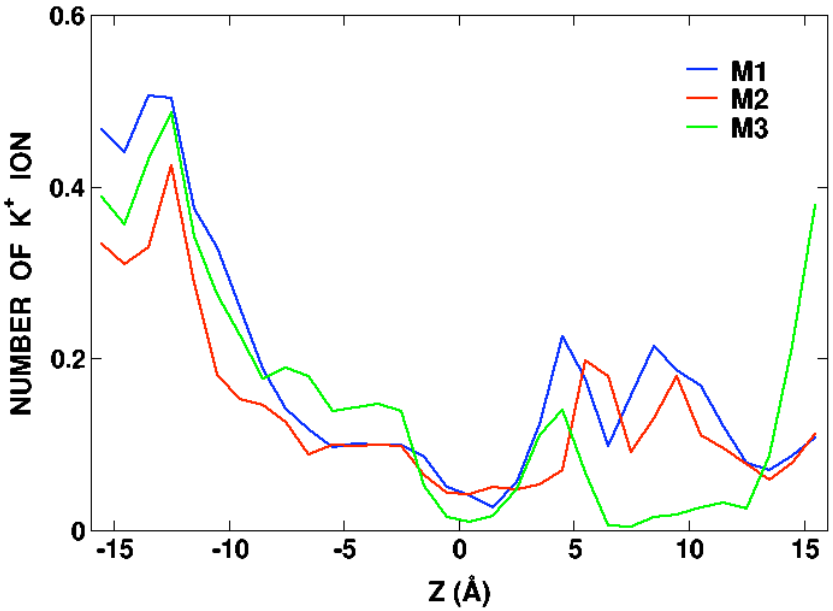


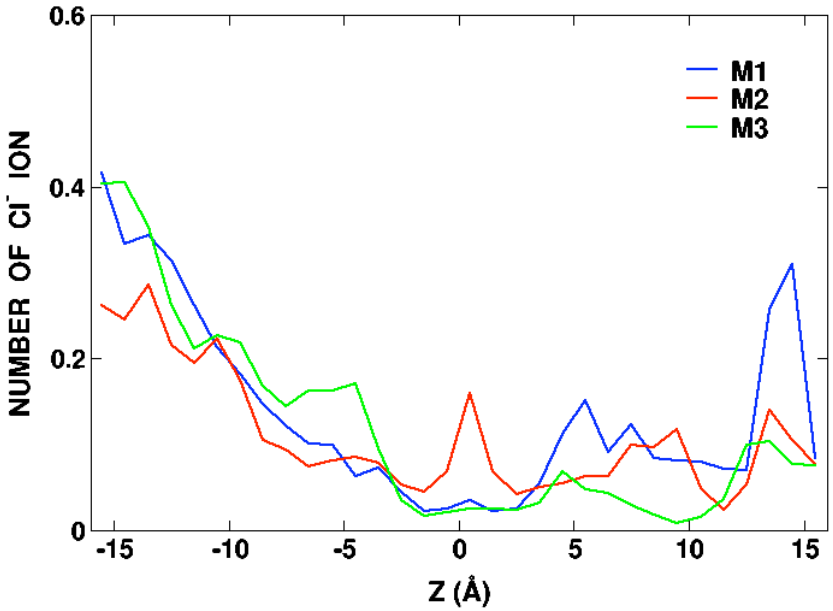


In a Large Aqueous OmpF Pore,

- what is the average number of ions inside the pore?
- is there a specific ion pathway along the pore?
- does ion solvation change inside the pore?
- is ion pairing important in ion permeation?
- does ion diffusion constant change inside pore?

Ion Distribution along OmpF pore





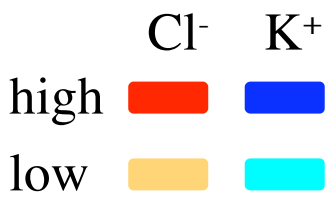
simulation captures the preference of OmpF porin for cations

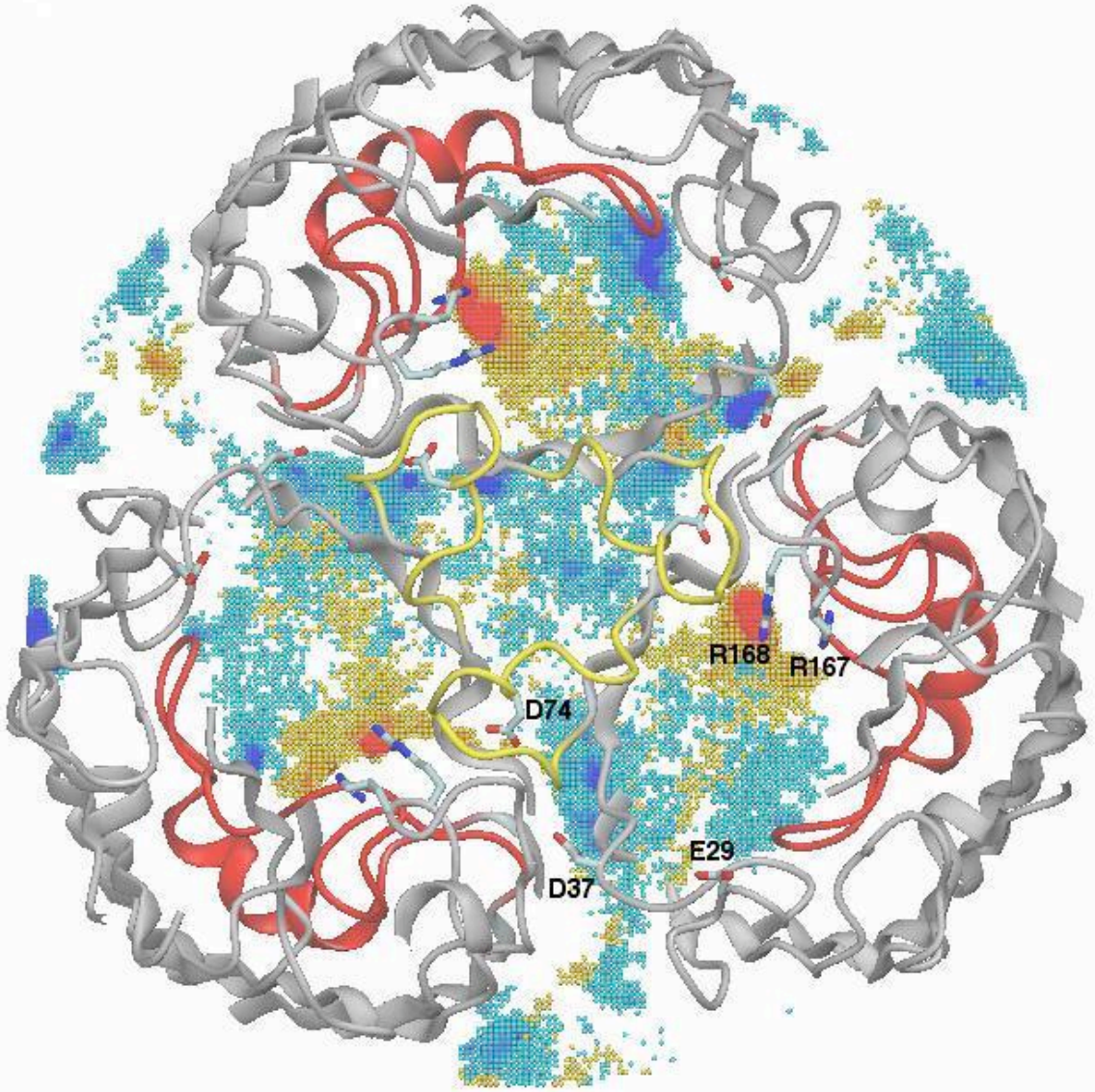
average number of K^+ in each pore is 5.8 (M1), 4.5 (M2), and 4.9 (M3)

average number of Cl^- in each pore is 4.4 (M1), 3.6 (M2), and 3.8 (M3)

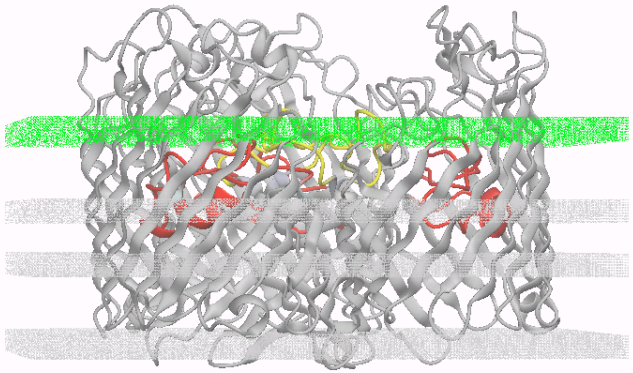
(5 ns is not sufficient to obtain very well converged average distribution)

Cross-sectional Ion Distribution



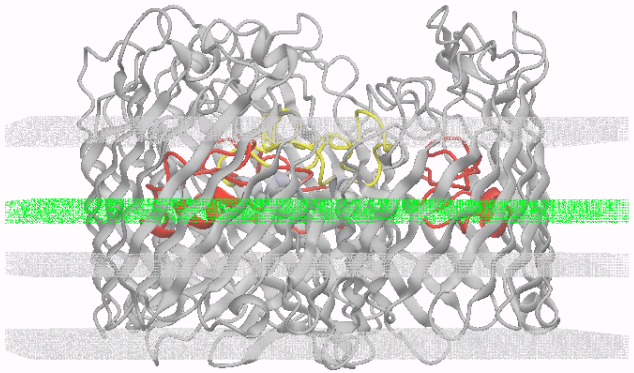


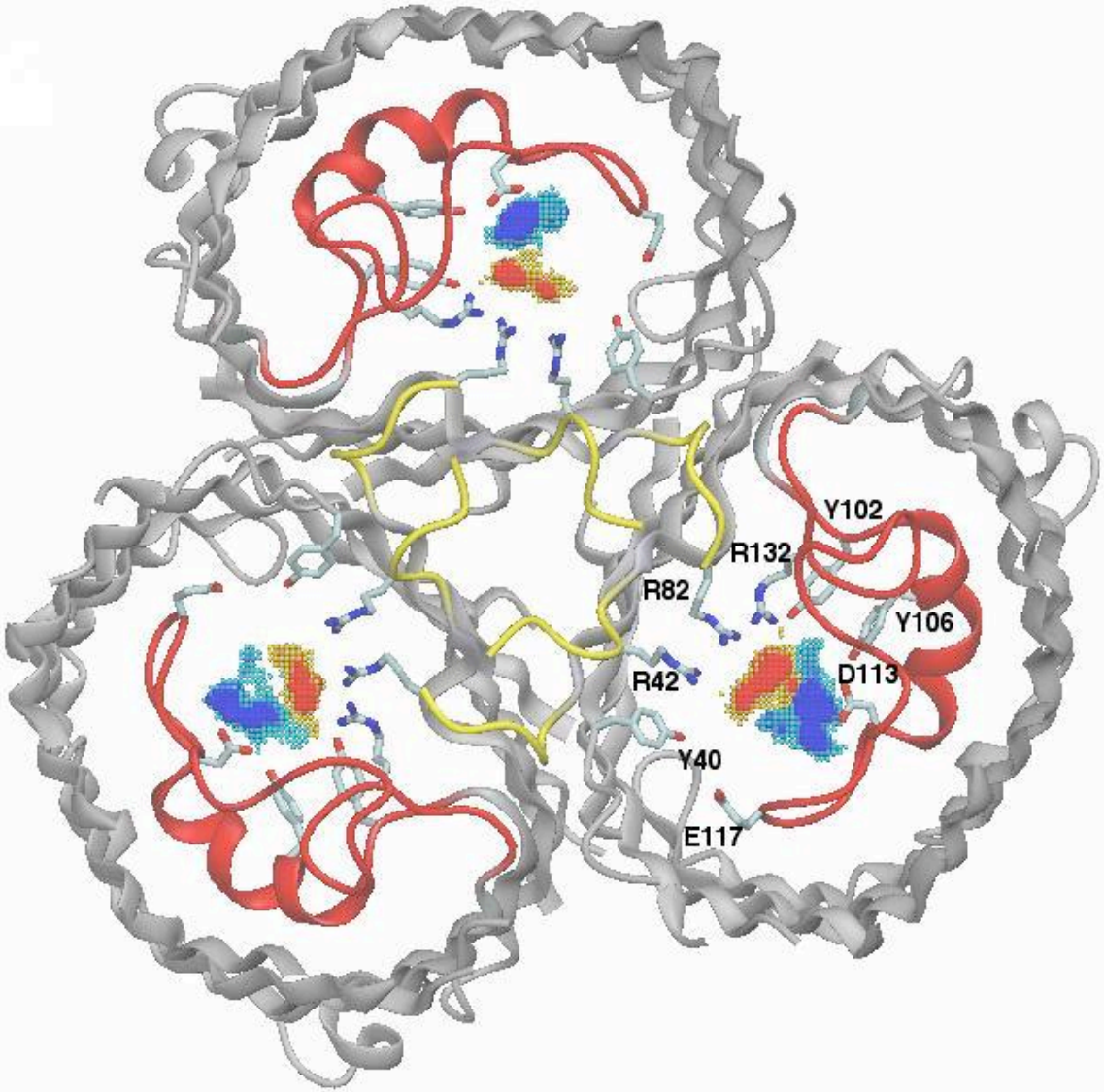
Extracellular vestibule



Cross-sectional Ion Distribution

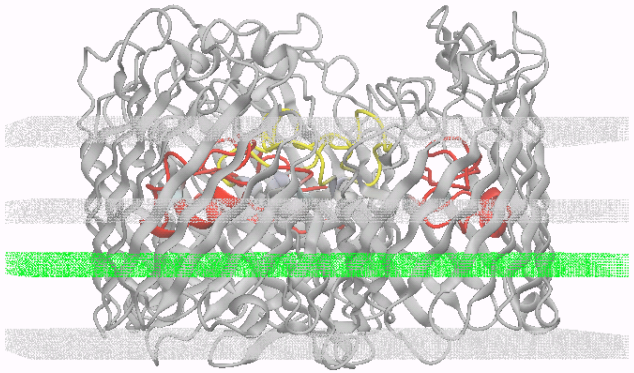
Constriction zone

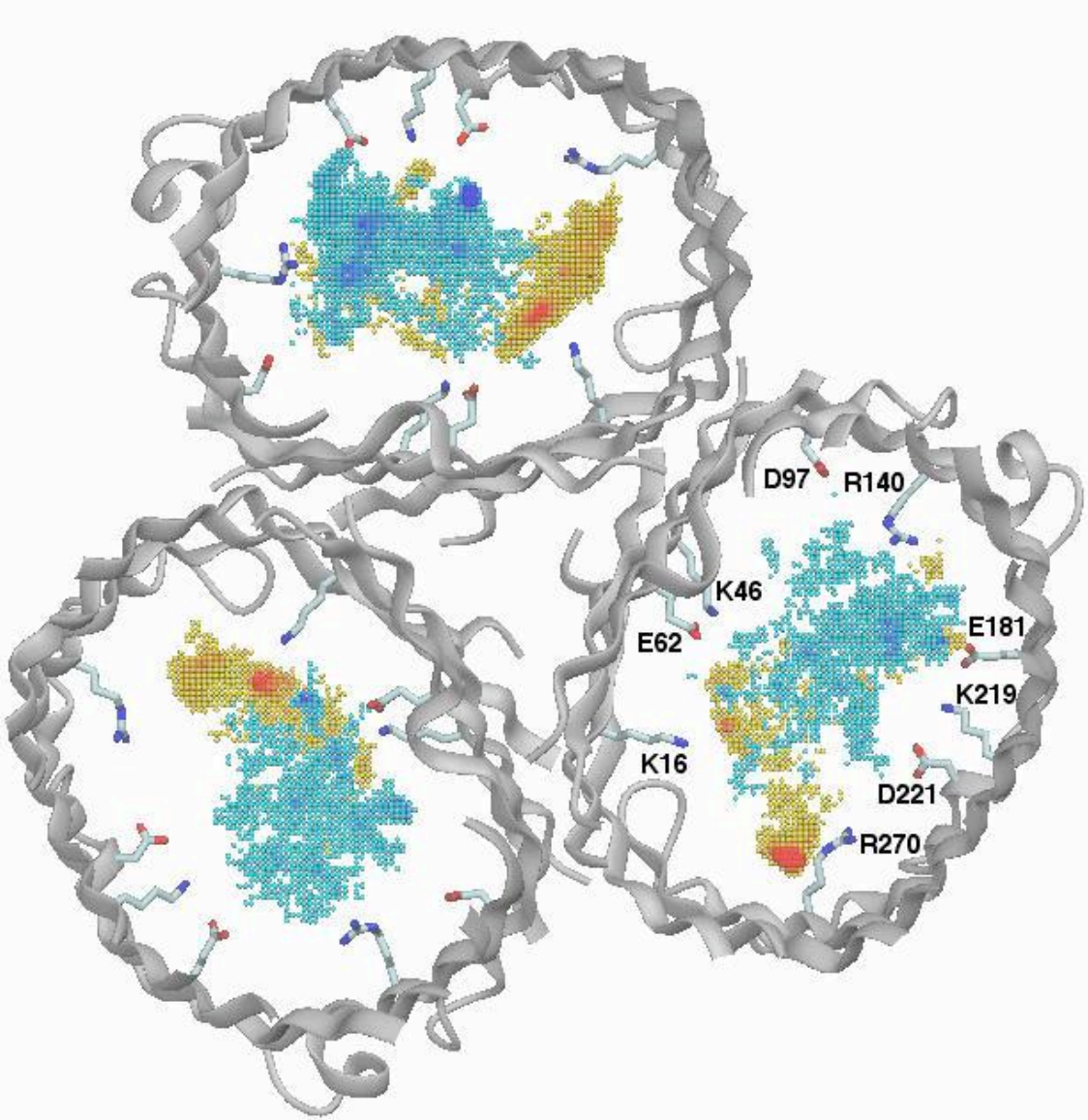




Cross-sectional Ion Distribution

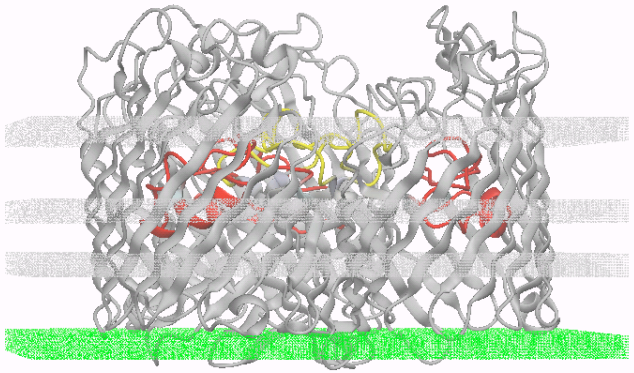
Pore periplasmic side

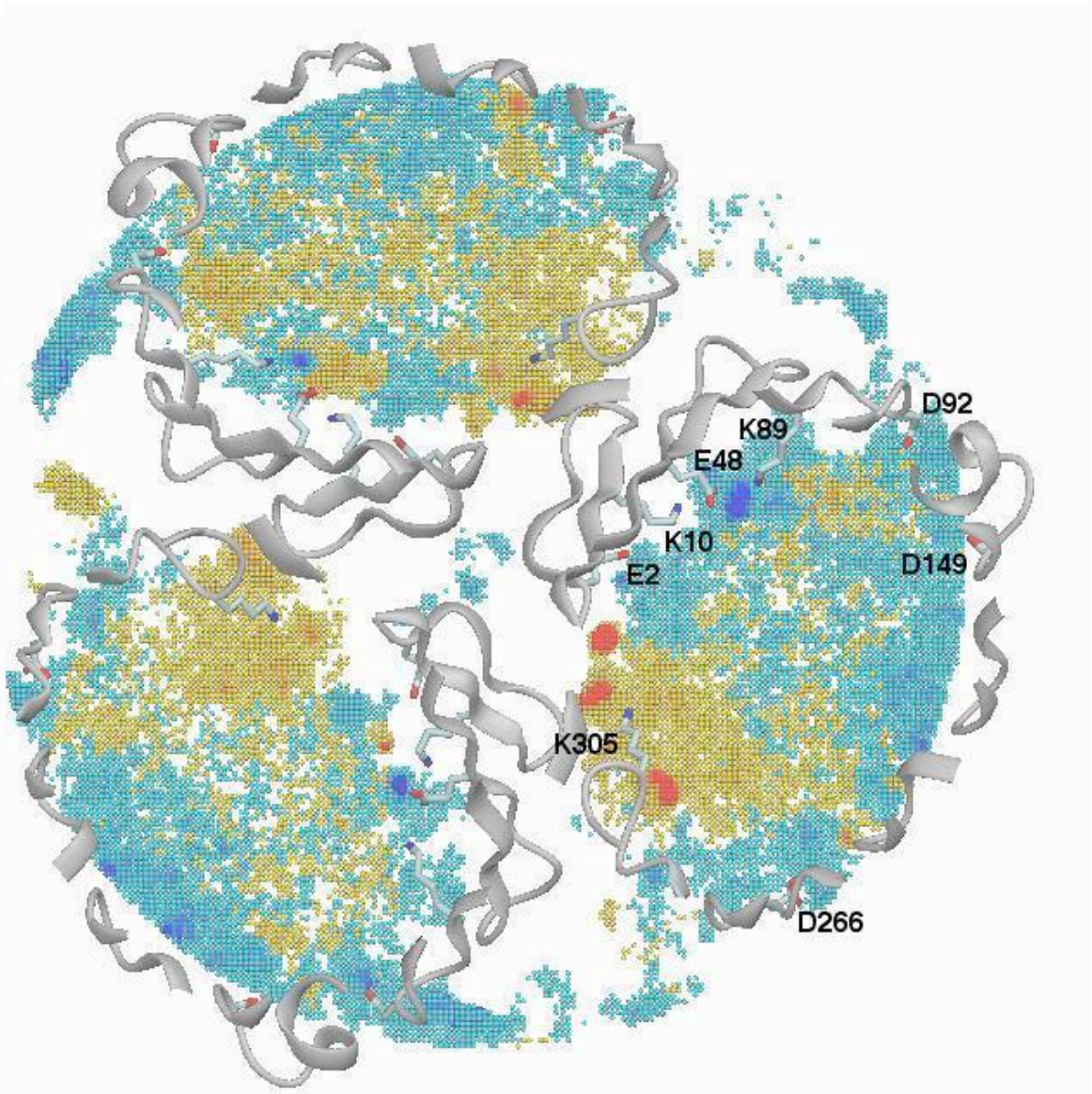




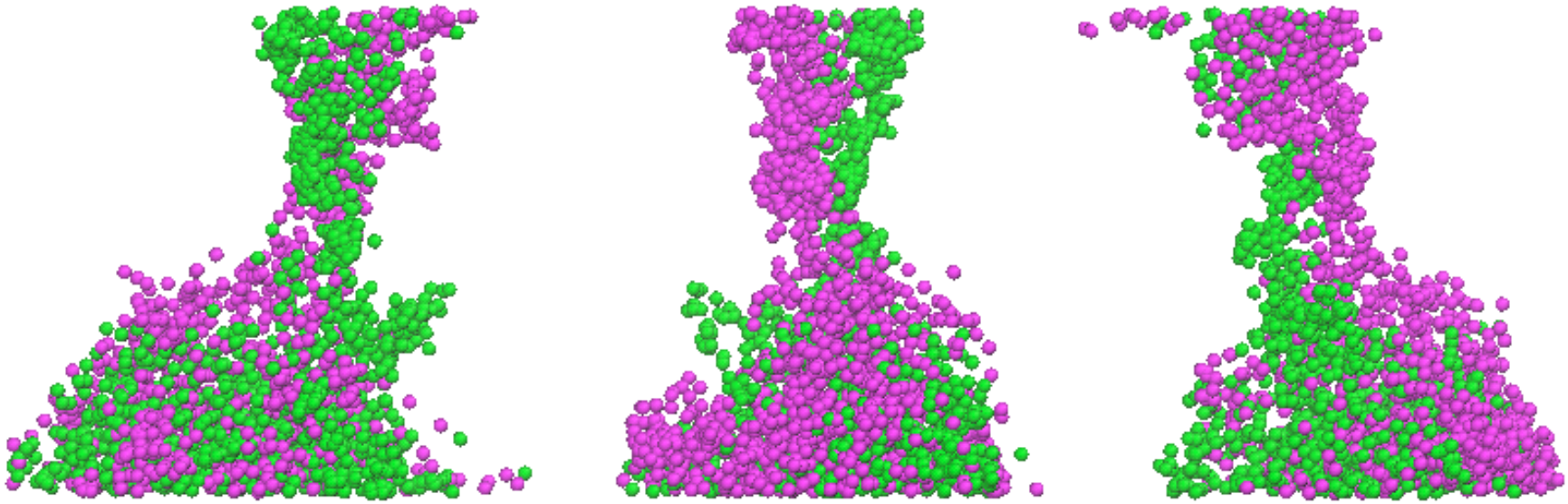
Cross-sectional Ion Distribution

Periplasmic side



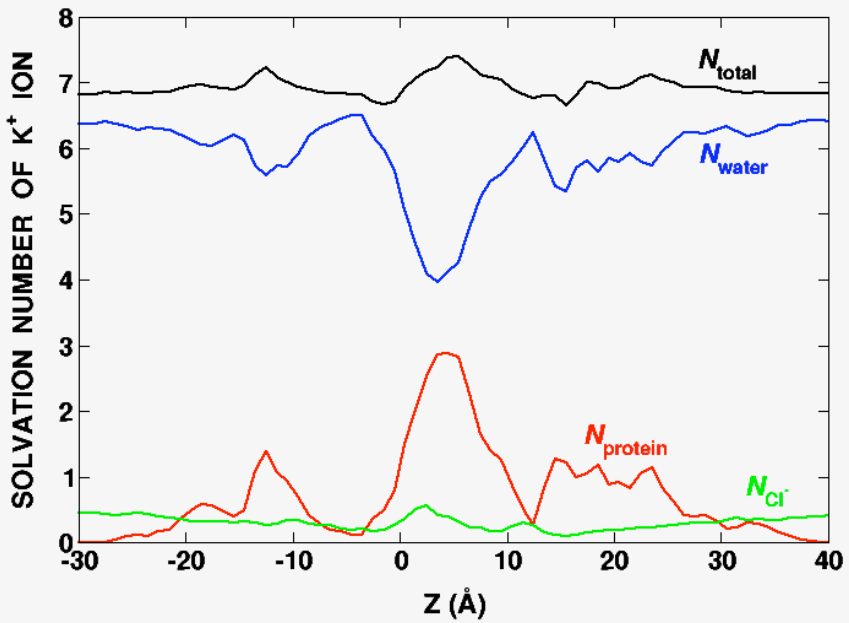


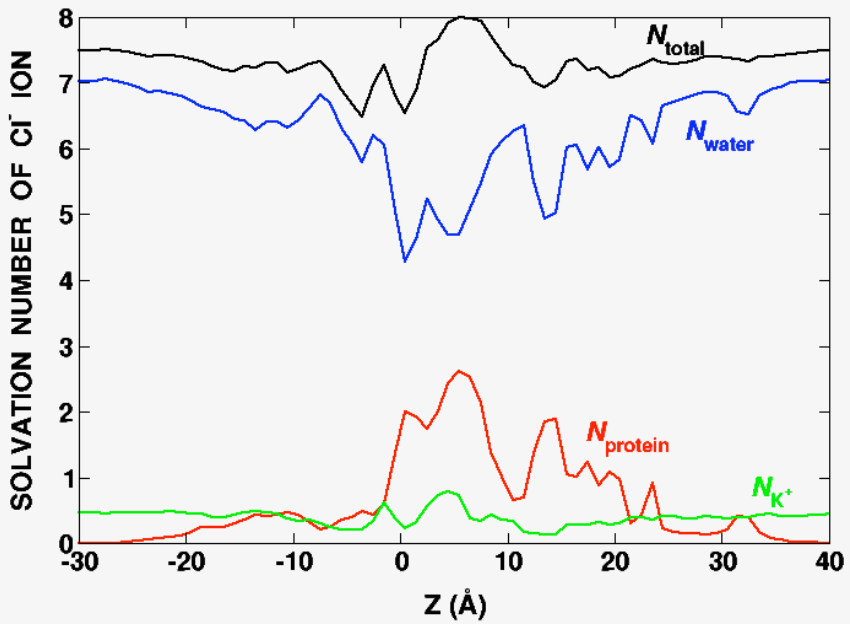
On average, K^+ and Cl^- follow two well-separated left-handed screw-like paths spanning nearly over 40 Å along the axis of the pore crossing at the constriction zone



Three views rotated by 120°

Ion Solvation along OmpF Pore

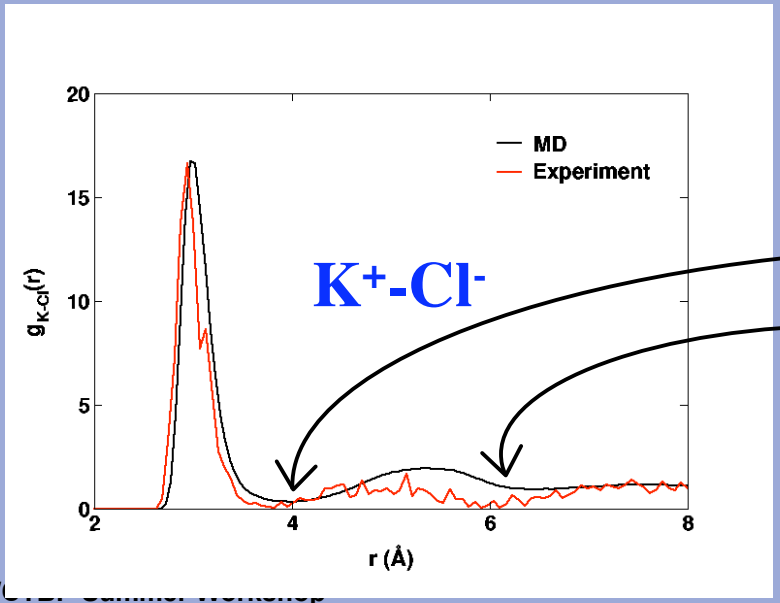


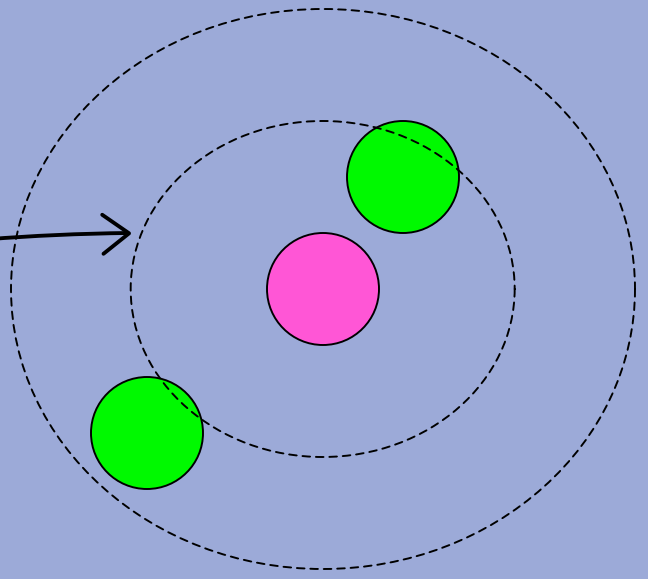


- the total solvation number is similar to that in bulk solution throughout the pore
- the total contributions from water and the protein are complementary
- at least 4 water molecules around both ions even in the constriction zone
- the contributions from the protein are asymmetric

Ion Pairing

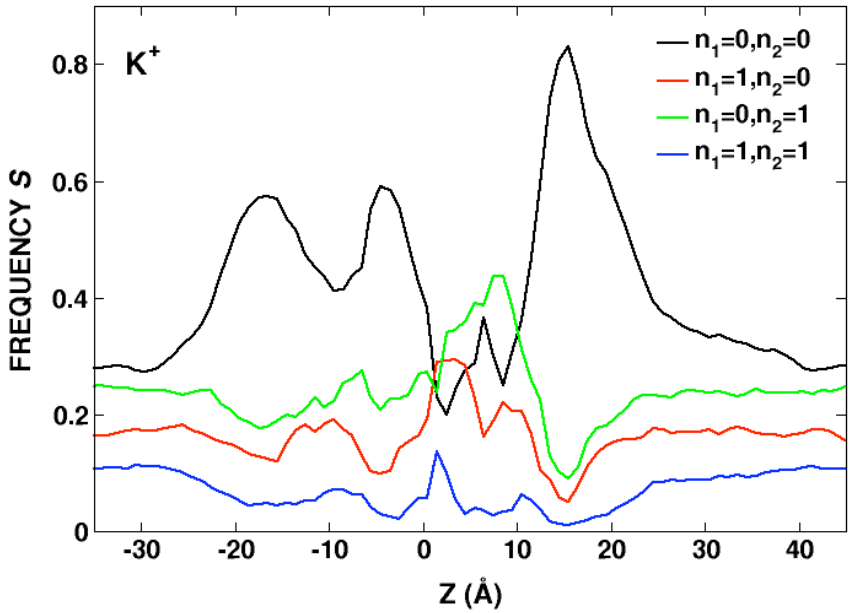
- $S_i(n_1=0,n_2=0)$ represents the frequency that an ion *i* has zero counterion neighbor in the first shell and zero in the second shell
- $S_i(n_1=1,n_2=0)$ represents the frequency that an ion *i* has one counterion neighbor in the first shell and zero in the second shell
- $S_i(n_1=1,n_2=1)$ represents the frequency that an ion *i* has one counterion neighbor in the first shell and one in the second shell

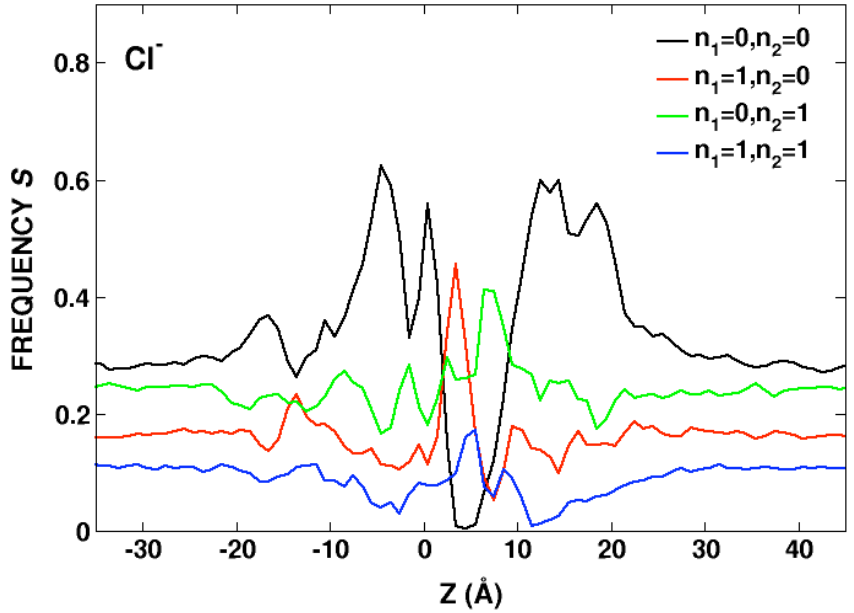




K+
Cl-

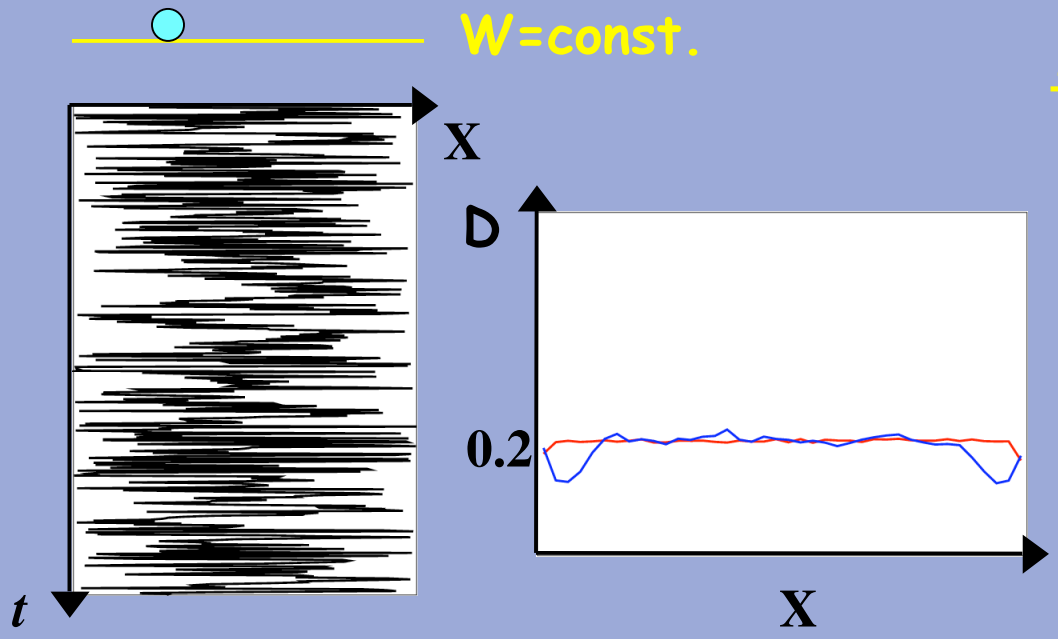
Ion Pairing along OmpF Pore

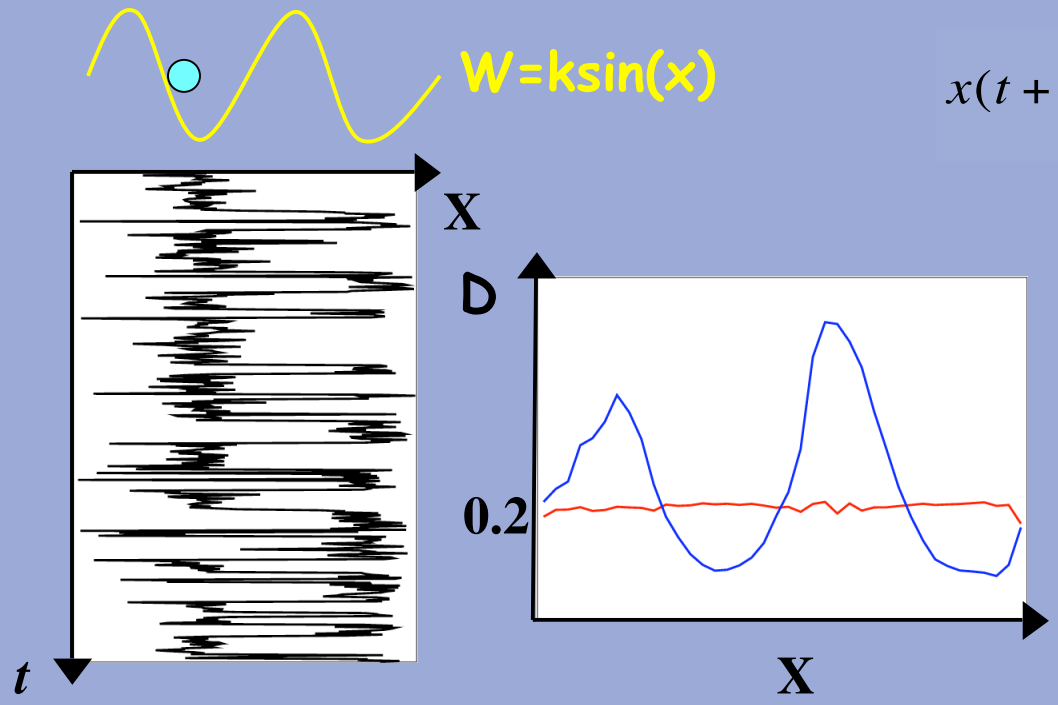




- ion pairing is reduced in the membrane-solvent interface and inside the pore
- ion pairing is **NOT** reduced in the constriction zone
- a K^+ ion alone can pass the constriction zone
- a Cl^- ion cannot go through the constriction zone without the presence of K^+ ions

Diffusion Constant





$$D = \lim_{t\to\infty} \frac{\left\langle \left[x(t) - x(0) \right]^2 \right\rangle}{2t}$$

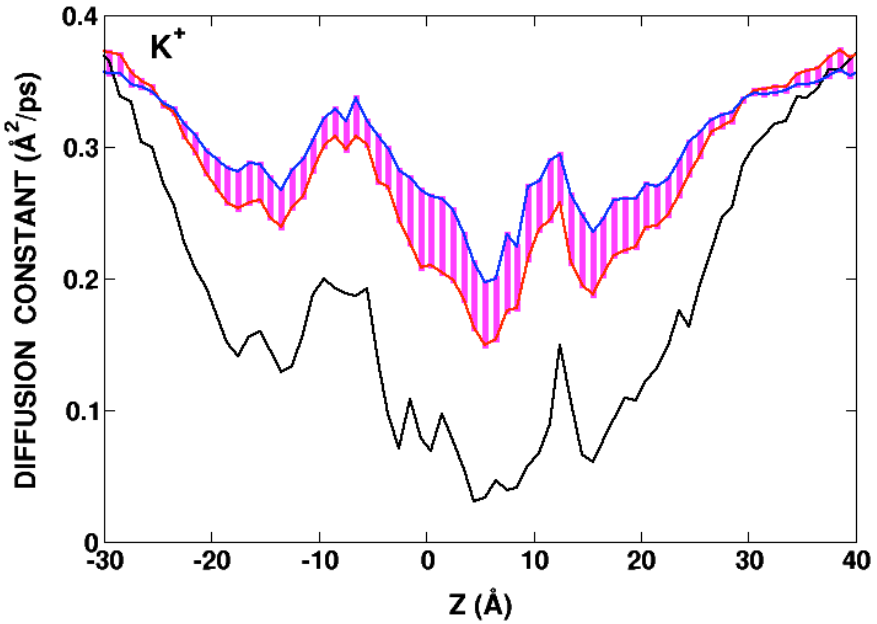
0.2 Å²/ps 10 fs

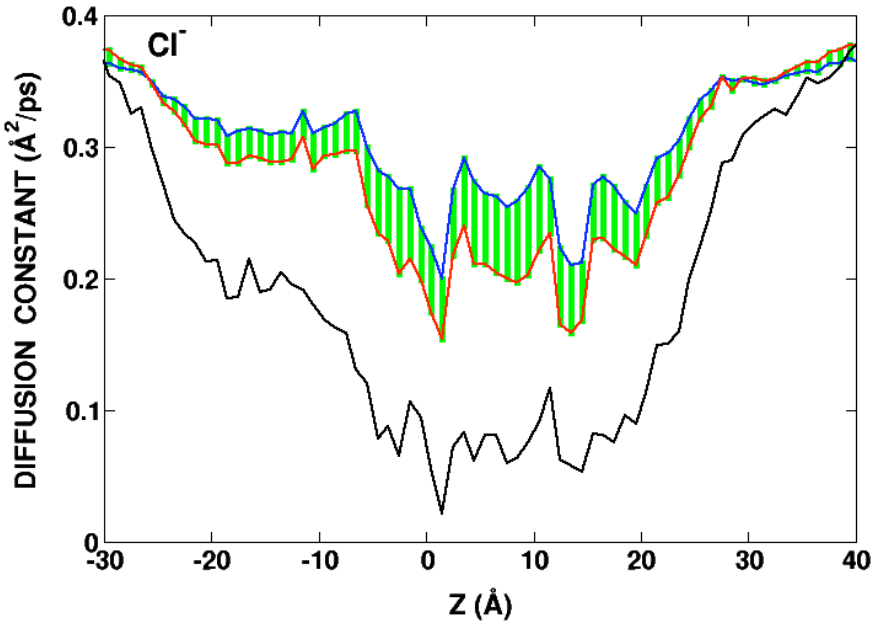
$$x(t+\Delta t) = x(t) - \underbrace{\frac{D}{k_B T}\frac{dW}{dx}\Delta t}_{<\Delta x(t)>} + \sqrt{2D\Delta t}\,R(t)$$

$$D = \frac{\left\langle \left[\Delta x(t) - \left\langle \Delta x(t) \right\rangle \right]^2 \right\rangle}{2\tau},$$

$$\Delta x(t) = x(t+\tau) - x(t)$$

Diffusion Constant along OmpF Pore





$$D = \lim_{t\to\infty} \frac{\left\langle [z(t) - z(0)]^2 \right\rangle}{2t}$$

$$D = \frac{\left\langle [\Delta z(t) - \langle \Delta z(t) \rangle]^2 \right\rangle}{2\tau}, \quad \Delta z(t) = z(t+\tau) - z(t)$$

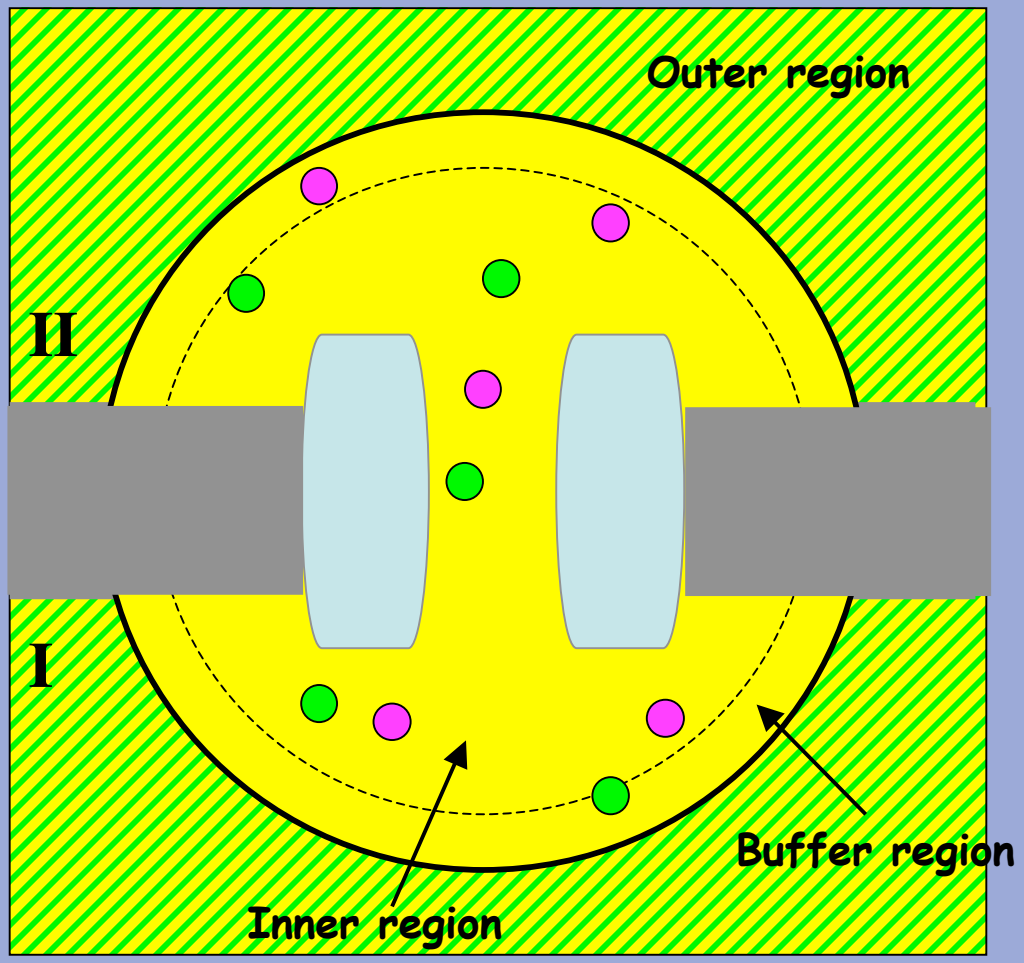
- inside the channel ion mobility is reduced to about 50% of the bulk value
- the diffusion profile can be used for BD and PNP calculations

Conclusions from MD Simulations

- more K^+ ions are found than Cl^- inside the pore in the ion density profile
- K^+ and Cl^- ions follow their own anti-clockwise screw-like ion pathways
- the contributions from water and the protein are complementary to keep the total solvation number of both ions similar to that in bulk solution
- ion pairing is quite reduced in the membrane-solvent interface and inside the pore except in the narrow constriction zone
- a Cl^- ion cannot go through the constriction zone without the presence of K^+
- inside the channel ion mobility is reduced to about 50% of the bulk value

Semi-continuum Approach: Brownian Dynamics (GCMC/BD)

Im et. al. (2000) *Biophys. J.*



- **BD : Inner region + Buffer region**

$$\dot{\mathbf{r}}_i(t) = \frac{D_i(\mathbf{r})}{k_B T}\langle \mathbf{F}_i \rangle + \frac{\partial D_i(\mathbf{r})}{\partial \mathbf{r}_i} + \xi_i(t)$$

$$\langle \mathbf{F}_i \rangle = -\frac{\partial W(\mathbf{r})}{\partial \mathbf{r}_i}$$

- **GCMC : Buffer region**

particle creation

$$P_{\text{creat}} = \frac{\left(\bar{n}_\alpha/(n_\alpha+1)\right) n_\alpha \exp\left[-(\Delta W - \bar{\mu}_\alpha)\right]}{1+\left(\bar{n}_\alpha/(n_\alpha+1)\right) n_\alpha \exp\left[-(\Delta W - \bar{\mu}_\alpha)\right]}$$

particle destruction

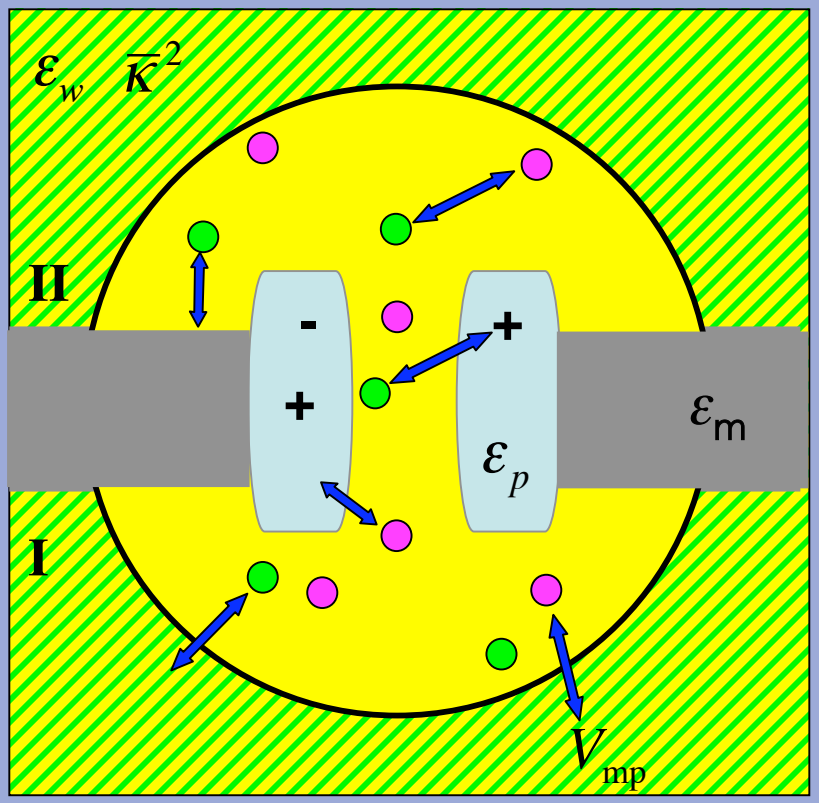
$$P_{\text{destr}} = \frac{1}{1+\left(\bar{n}_\alpha/(n_\alpha+1)\right) n_\alpha \exp\left[-(\Delta W - \bar{\mu}_\alpha)\right]}$$

chemical potential $\bar{\mu}_\alpha(\text{I}) = \Delta\mu_\alpha + q_\alpha V_{\text{mp}}$

$\bar{\mu}_\alpha(\text{II}) = \Delta\mu_\alpha$

GCMC/BD program freely available at
http://thallium.bsd.uchicago.edu/RouxLab/gcmc.html

Multi-ion Potential of Mean Force



$$W(\mathbf{R}_1,\mathbf{R}_2,\cdots) =$$

$$\sum_{\alpha\gamma}\sum_{ij} u_{\alpha\gamma}(\mathbf{r}_\alpha^i - \mathbf{r}_\gamma^j)$$ ion-ion interactions

$$+$$

$$\sum_{\alpha,i} U_{\text{core}}(\mathbf{r}_\alpha^{\mathbf{i}})$$ core repulsive potential

$$+$$

$$\Delta W_{\text{sf}}(\mathbf{R}_1,\mathbf{R}_2,\cdots)$$ static external field

$$+$$

$$\Delta W_{\text{rf}}(\mathbf{R}_1,\mathbf{R}_2,\cdots)$$ reaction field

Im et. al. (2000) *Biophys. J.*
Im & Roux (2001) *J. Chem. Phys.*

Multi-ion Potential of Mean Force

$$W(\mathrm{R}_1,\mathrm{R}_2,\cdots) =$$

$$\underbrace{\sum_{\alpha\gamma}\sum_{ij} u_{\alpha\gamma}(\mathbf{r}_\alpha^i - \mathbf{r}_\gamma^j)}_{\text{ion-ion interactions}} + \underbrace{\sum_{\alpha,i} U_{\text{core}}(\mathbf{r}_\alpha^i)}_{\text{core repulsive potential}} + \underbrace{\Delta W_{\text{sf}}(\mathbf{R}_1,\mathbf{R}_2,\cdots)}_{\text{static external field}} + \underbrace{\Delta W_{\text{rf}}(\mathbf{R}_1,\mathbf{R}_2,\cdots)}_{\text{reaction field}}$$

$$u_{\alpha\gamma}(r) = 4\varepsilon_{\alpha\gamma}\left[\left(\frac{\sigma_{\alpha\gamma}}{r}\right)^{12} - \left(\frac{\sigma_{\alpha\gamma}}{r}\right)^{6}\right] + \frac{q_\alpha q_\gamma}{\varepsilon_{\text{bulk}} r} + w_{\text{sr}}(r)$$

3d-grid map

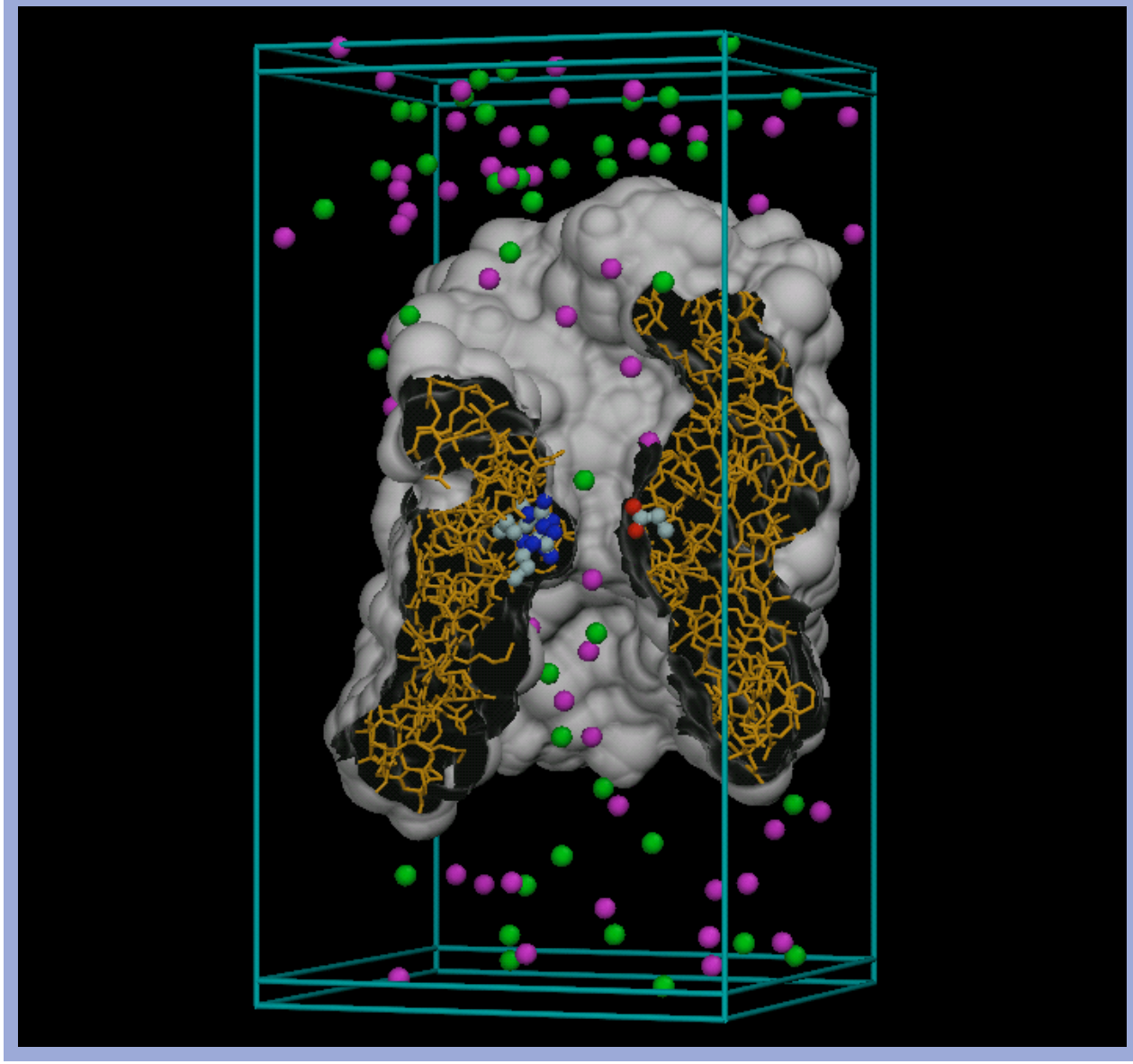
PB equations

multipolar basis-set expansion method

Roux (1999) *Biophys. J.*
Im et. al. (2000) *Biophys. J.*
Im et. al. (2001) *J. Chem. Phys.*
Im & Roux (2001) *J. Chem. Phys.*

BD System of OmpF Porin

ion movements in continuum representation



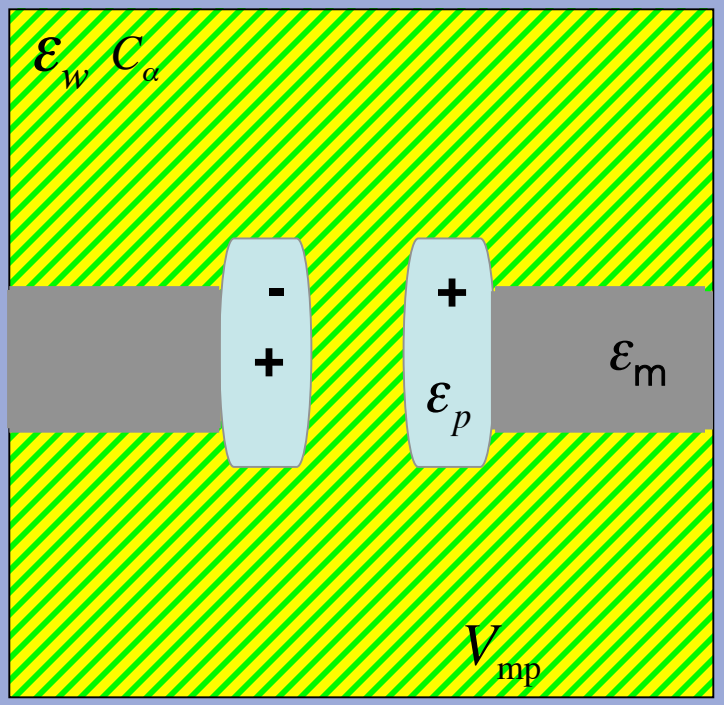
GCMC/BD simulation:
OmpF porin

15 ps/frame
150 mV
1 M [KCl]

K^+
Cl^-

Im et. al. (2000) *Biophys. J.*
Im et. al. (2001) *J. Chem. Phys.*
Im & Roux (2001) *J. Chem. Phys.*
Im & Roux (2002) *J. Mol. Biol.*

Continuum Approach: PNP Electrodiffusion Theory



Nernst-Planck (NP) equation :

$$\mathbf{J}_\alpha(\mathbf{r}) = -D_\alpha(\mathbf{r})\left(\nabla C_\alpha(\mathbf{r}) + \frac{q_\alpha}{k_B T} C_\alpha(\mathbf{r})\nabla\phi(\mathbf{r})\right)$$

Poisson equation :

$$\nabla\bullet\left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right] = -4\pi\left(\rho_p(\mathbf{r}) + \sum_\alpha q_\alpha C_\alpha(\mathbf{r})\right)$$

under equilibrium conditions, i.e., $J_\alpha(r)=0$

non-linear PB Equation :

$$\nabla\cdot\left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right] = -4\pi\left[\rho_{prot}(\mathbf{r}) + \sum_\alpha q_\alpha C_\alpha^{bulk}\exp\left(-q_\alpha\phi(\mathbf{r})/k_B T\right)\right]$$

3d-PNP program freely available at
http://thallium.bsd.uchicago.edu/RouxLab/pbpnp.html

PNP Electrodiffusion Theory in 3D

$$\mathrm{J}_\alpha(\mathrm{r}) = -D_\alpha(\mathrm{r})\exp\left(-q_\alpha\phi(\mathrm{r})/k_\mathrm{B}T\right)\nabla\left[C_\alpha(\mathrm{r})\exp\left(q_\alpha\phi(\mathrm{r})/k_\mathrm{B}T\right)\right]$$

under steady-state condition $\left(\nabla \bullet \mathbf{J}_\alpha(\mathbf{r}) = 0\right)$

$$\nabla \bullet \left[D_\alpha(\mathrm{r})\exp\left(-q_\alpha\phi(\mathrm{r})/k_\mathrm{B}T\right)\nabla C_\alpha(\mathrm{r})\exp\left(q_\alpha\phi(\mathrm{r})/k_\mathrm{B}T\right)\right] = 0$$

$$\nabla \bullet \left[\varepsilon^*(\mathbf{r})\nabla\phi^*(\mathbf{r})\right] = 0$$

This is just like the Poisson equation which can be routinely solved using finite-difference methods

$$\nabla \bullet \left[\varepsilon(\mathbf{r})\nabla\phi(\mathbf{r})\right] = -4\pi\left(\rho_\mathrm{p}(\mathbf{r}) + \sum_\alpha q_\alpha C_\alpha(\mathbf{r})\right)$$

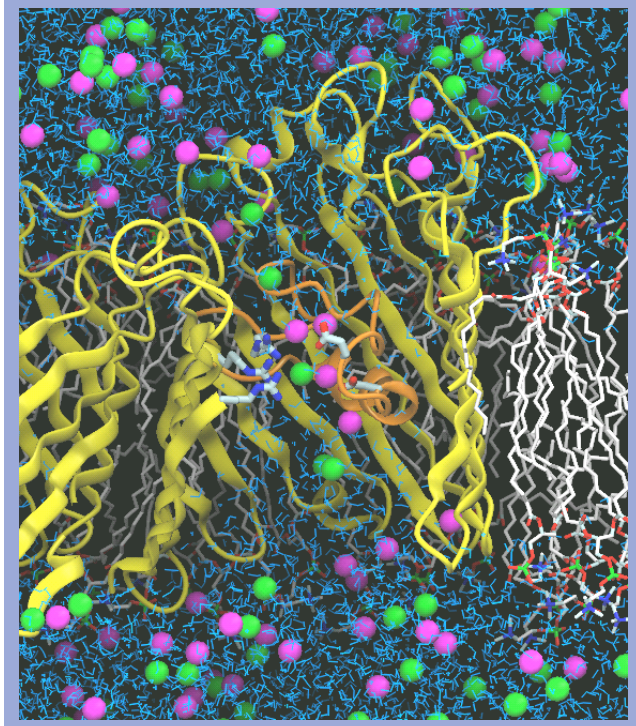
$$I_\alpha(z) = q_\alpha \int dx \int dy\ \mathbf{z} \bullet \mathbf{J}_\alpha(x,y,z)$$ Ion current

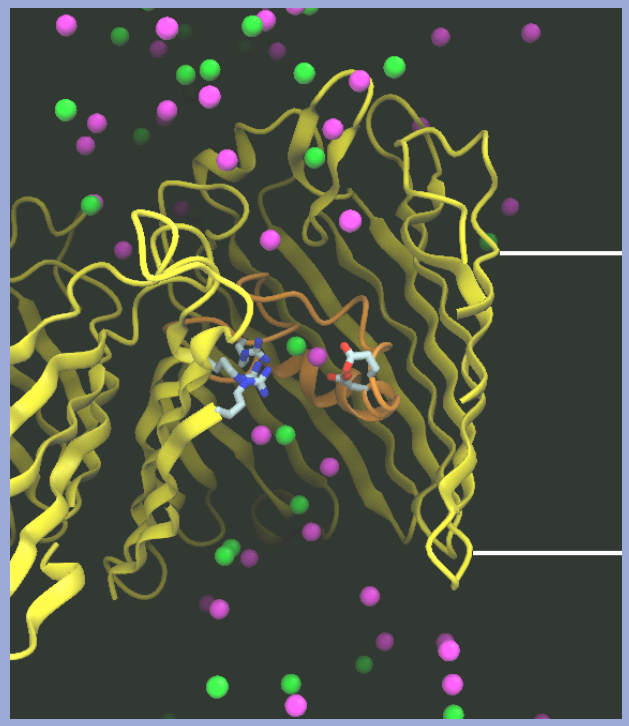
Kurnikova et. al. (1999) *Biophys. J.*

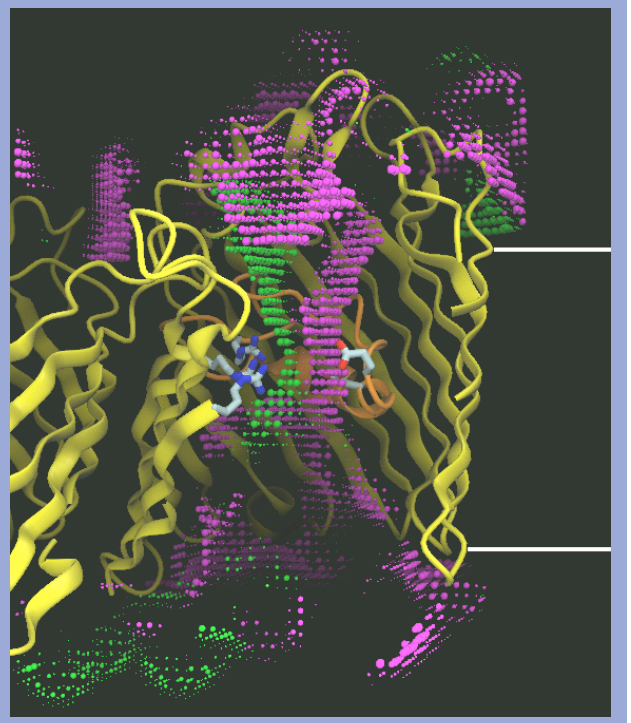
Permeation Models: OmpF Porin

- Ion diffusion constants
- Short-range ion-ion interactions
- Ion exclusion radius

MD → BD → PB & PNP



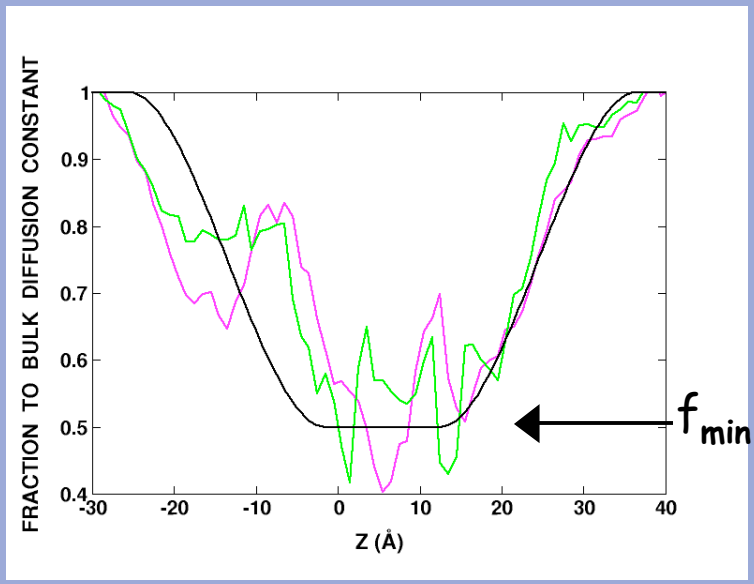




Im & Roux (2002) *J. Mol. Biol.*

Microscopic Information from MD

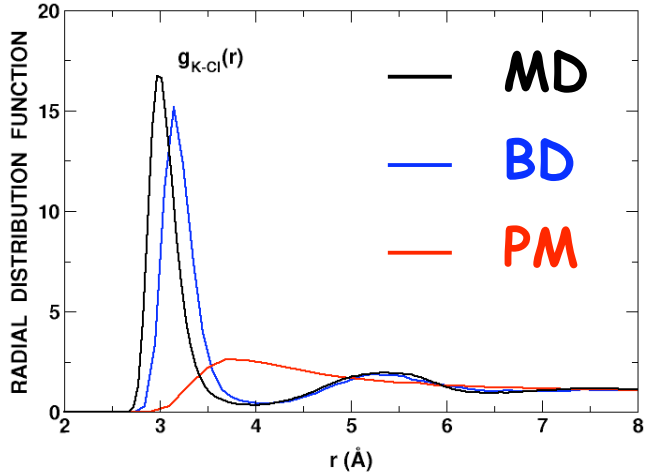
1. Profile of ion diffusion constant

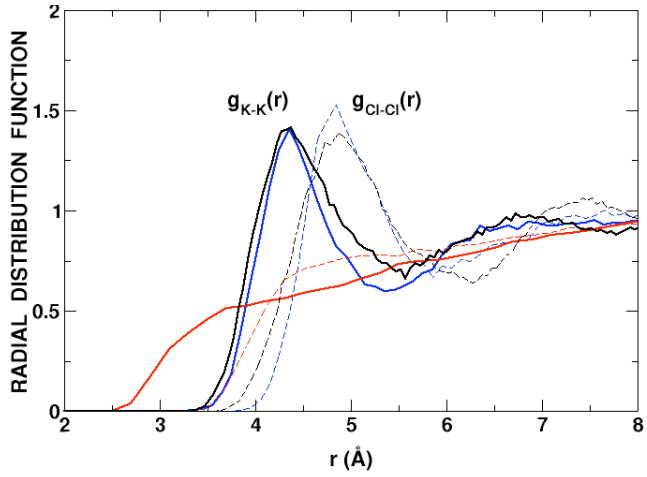


D_K=0.196 Å^2/ps D_{Cl}=0.203 Å^2/ps

2. Short-range ion-ion interactions

$$w_{sr}(r) = c_0 \exp\left[\frac{c_1 - r}{c_2}\right]\cos\left[c_3(c_1 - r)\pi\right] + c_4\left(\frac{c_1}{r}\right)^6$$

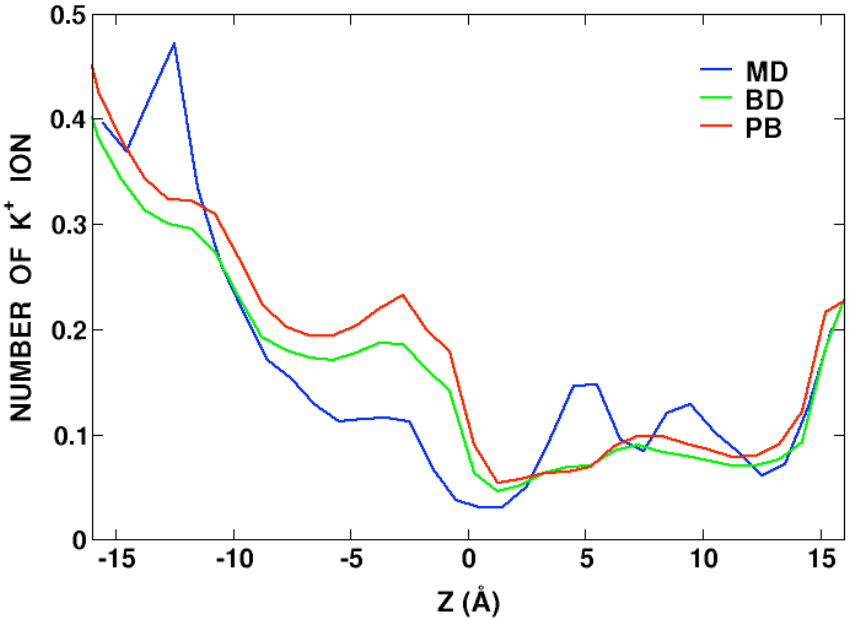


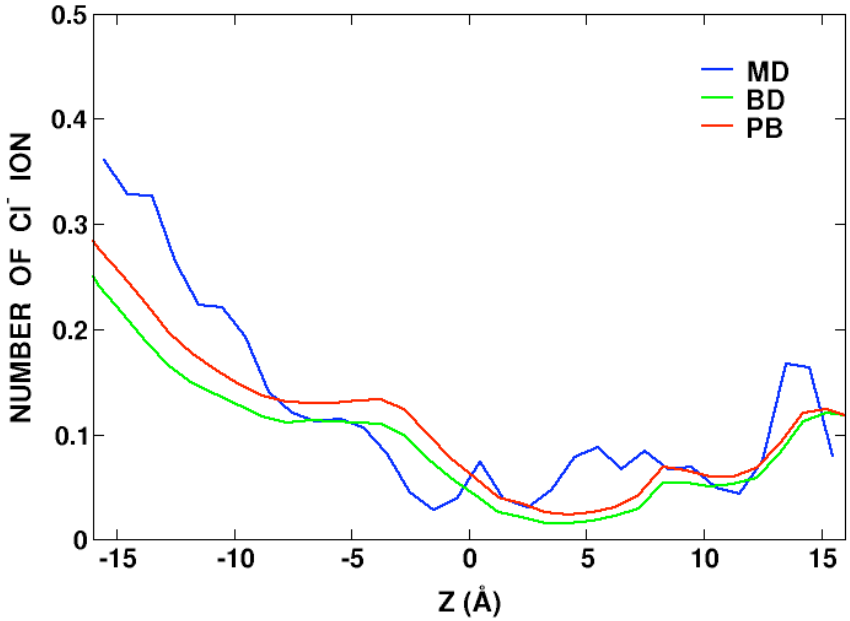


3. Ion-exclusion radius

0.75 Å for all nitrogen atoms
0.93 Å for all oxygen atoms
1.00 Å for all other heavy atoms

Ion Distribution along OmpF pore



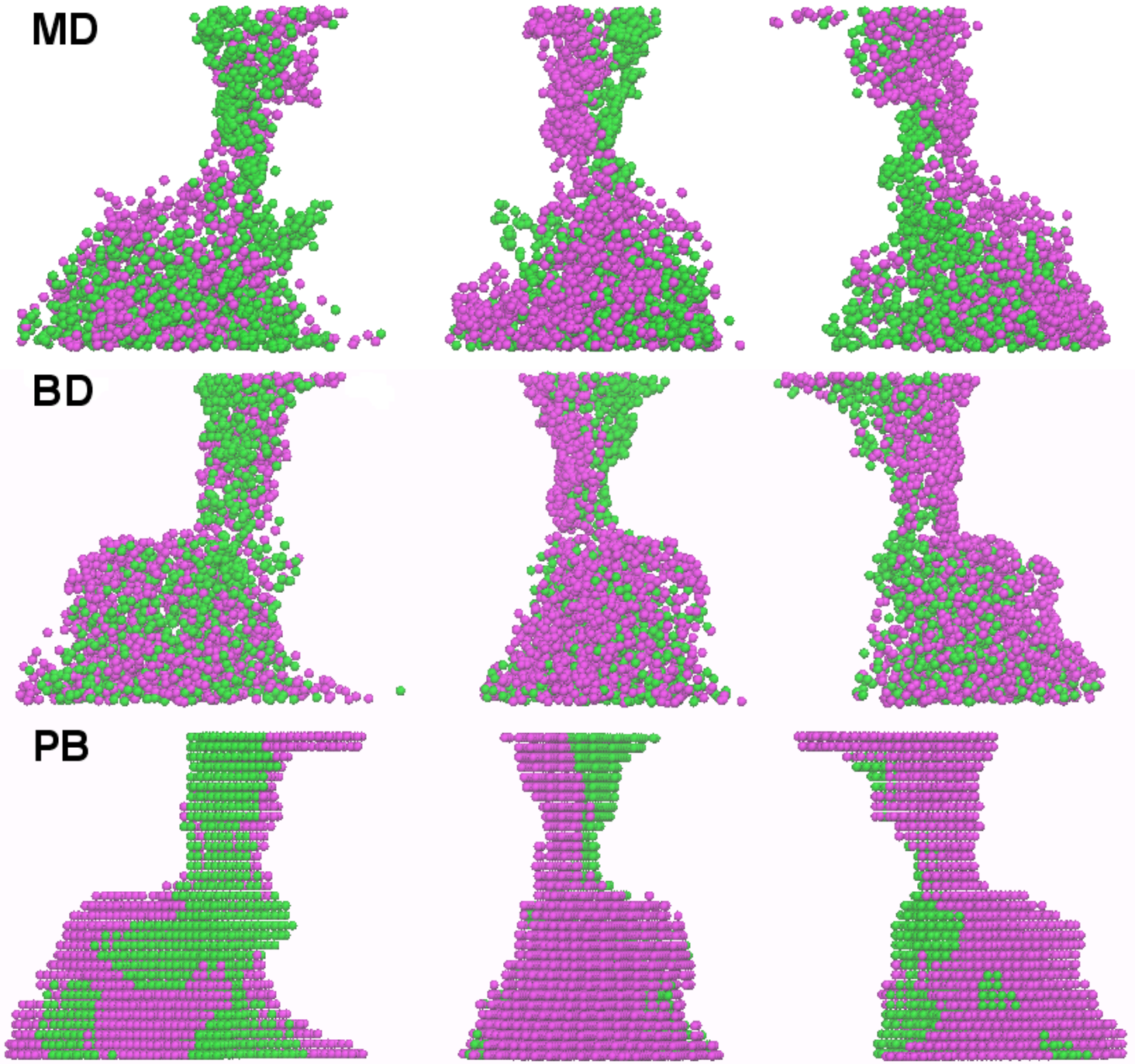


All computational approaches show the preference of OmpF for cations

average number of K^+ in each pore is 5.1 (MD), 4.9 (BD), and 5.6 (PB)

average number of Cl^- in each pore is 3.9 (MD), 2.8 (BD), and 3.4 (PB)

The agreement is quite reasonable between MD and BD (between BD and PB)



In Equilibrium Situations of 1M KCl Symmetric Solution

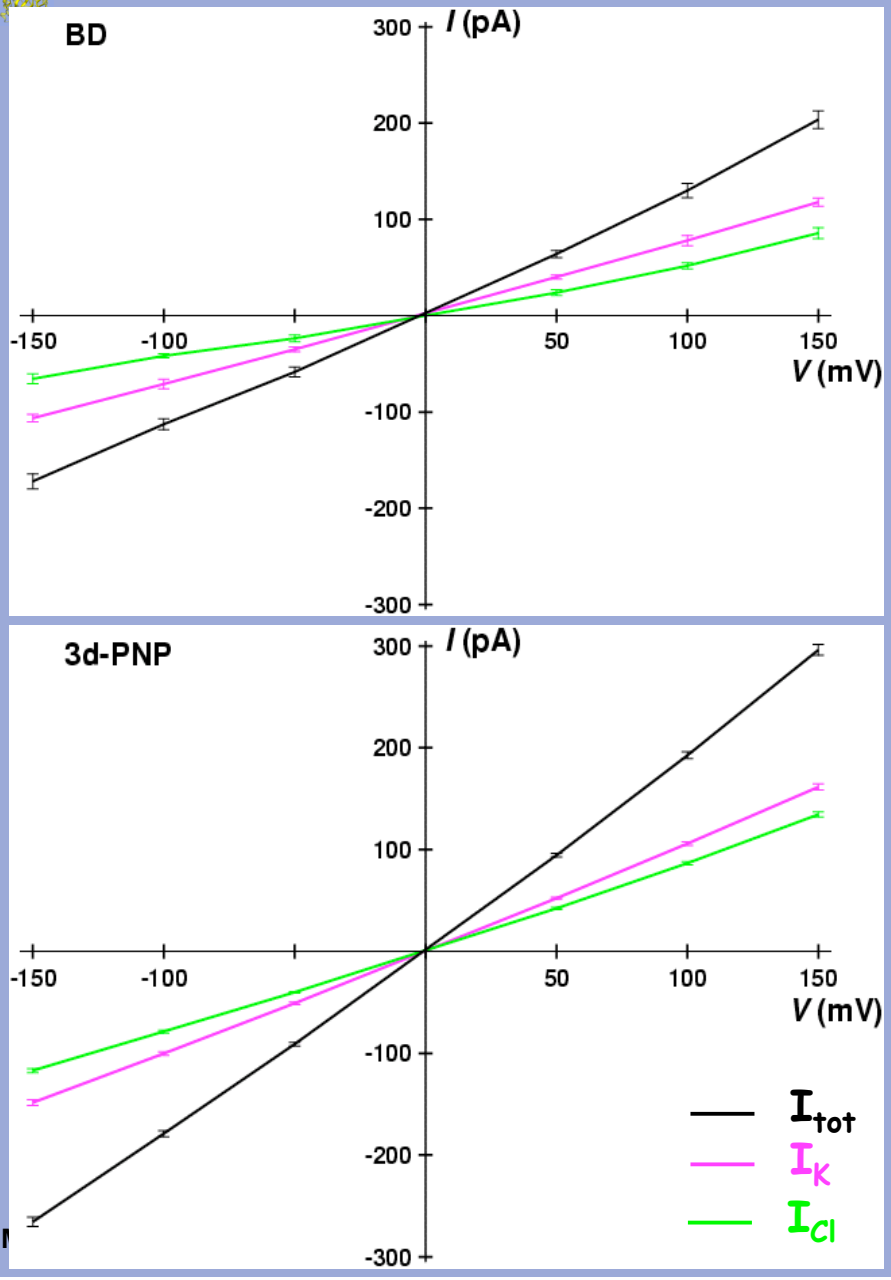
- BD and PB reproduce reasonably well the average ion distribution as well as the distinct ion pathways inside the pore observed in MD

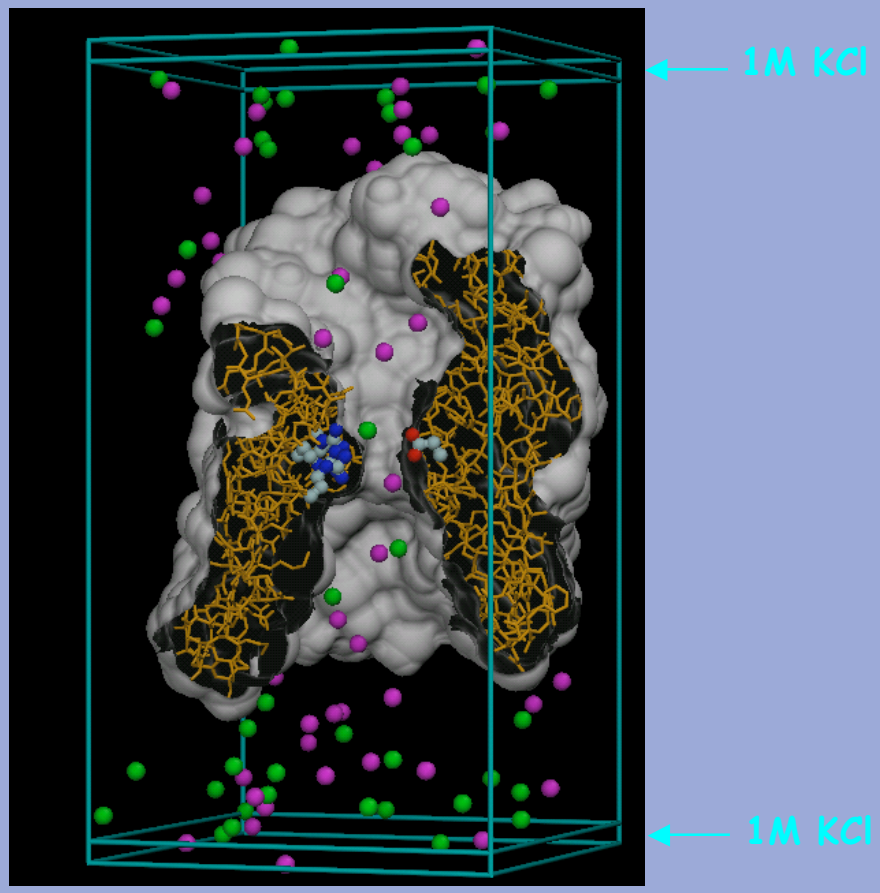
MD
- explicit all-atoms

BD
- continuum dielectric description of water
- rigidity of the protein

PB
- continuum dielectric description of water
- rigidity of the protein
- mean-field Approximation

I-V Curves in Symmetric 1M KCl



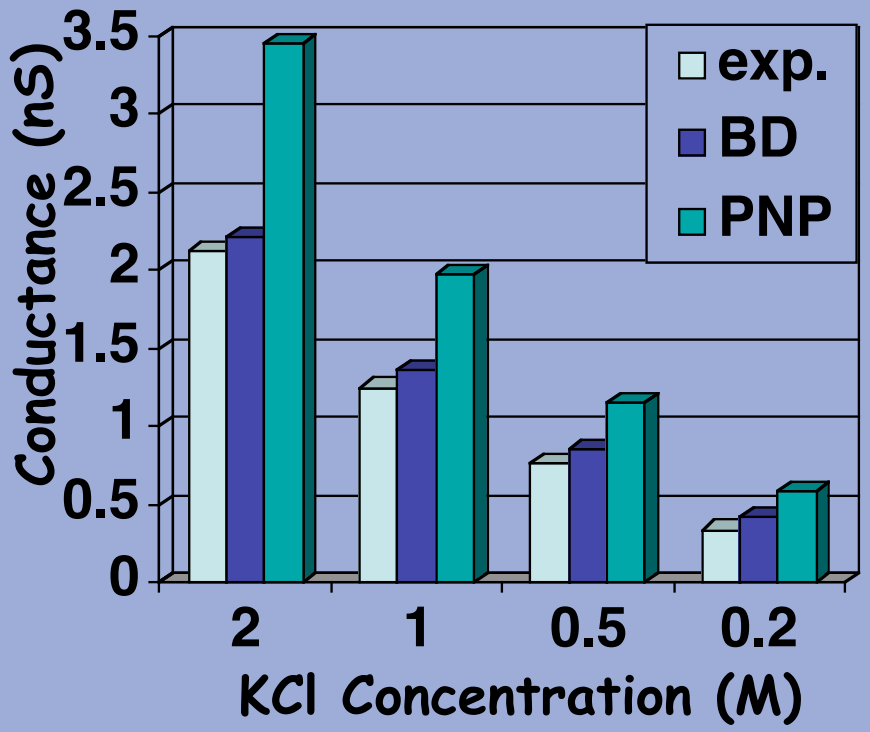


- I_K > I_{Cl} (cation selectivity)
- Asymmetry in I-V relation
- Current (PNP) > Current (BD)

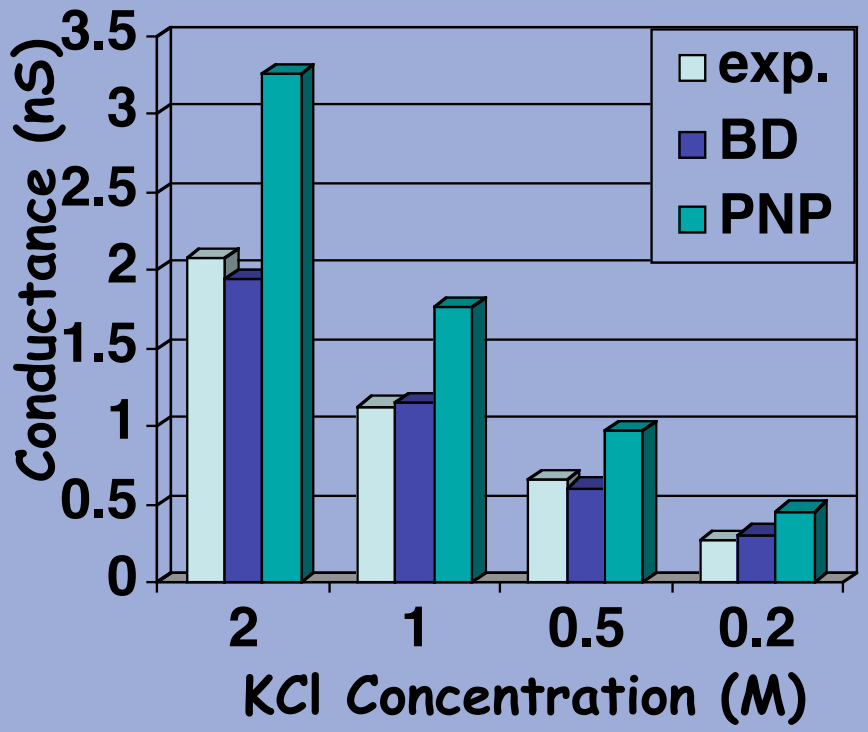
Comparisons with Experiments:

Single Channel Conductance

Vmp=+150 mV



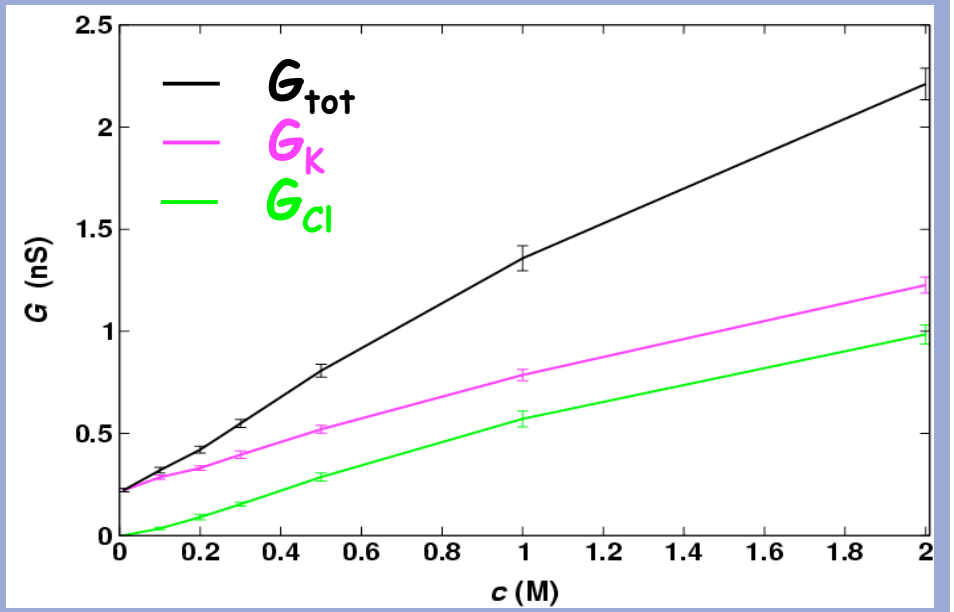
Vmp=-150 mV

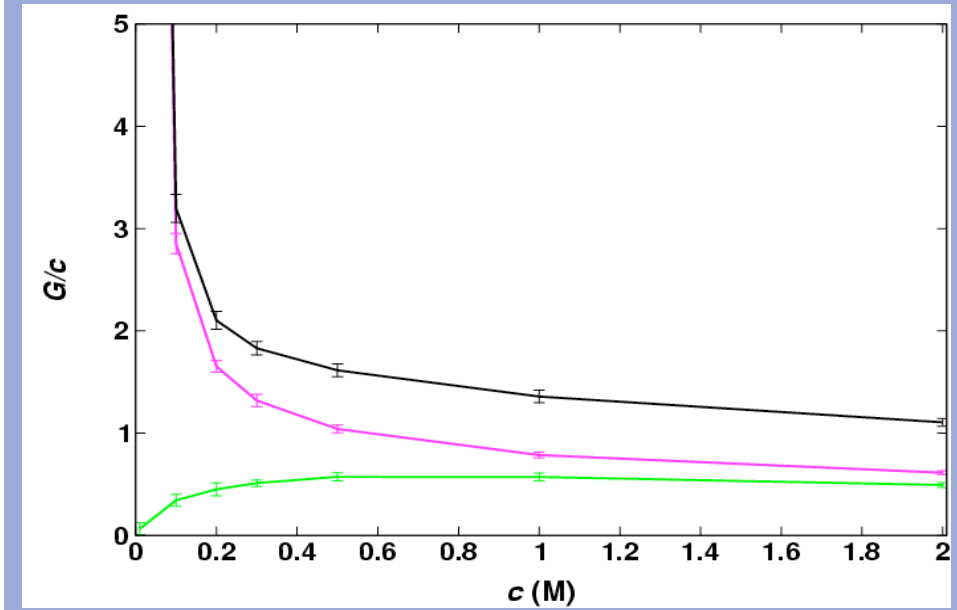


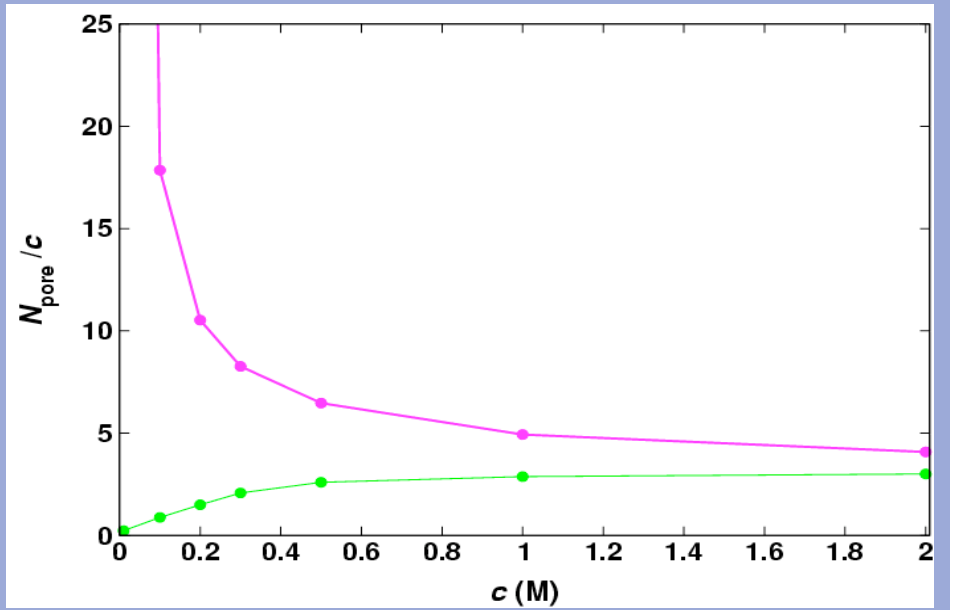
Experimental data from Nathalie Saint (Unpublished)

Conductance-Concentration Relation

from BD simulations in
0.01, 0.1, 0.2, 0.5, 1, 2M KCl
symmetric solutions with +150 mV

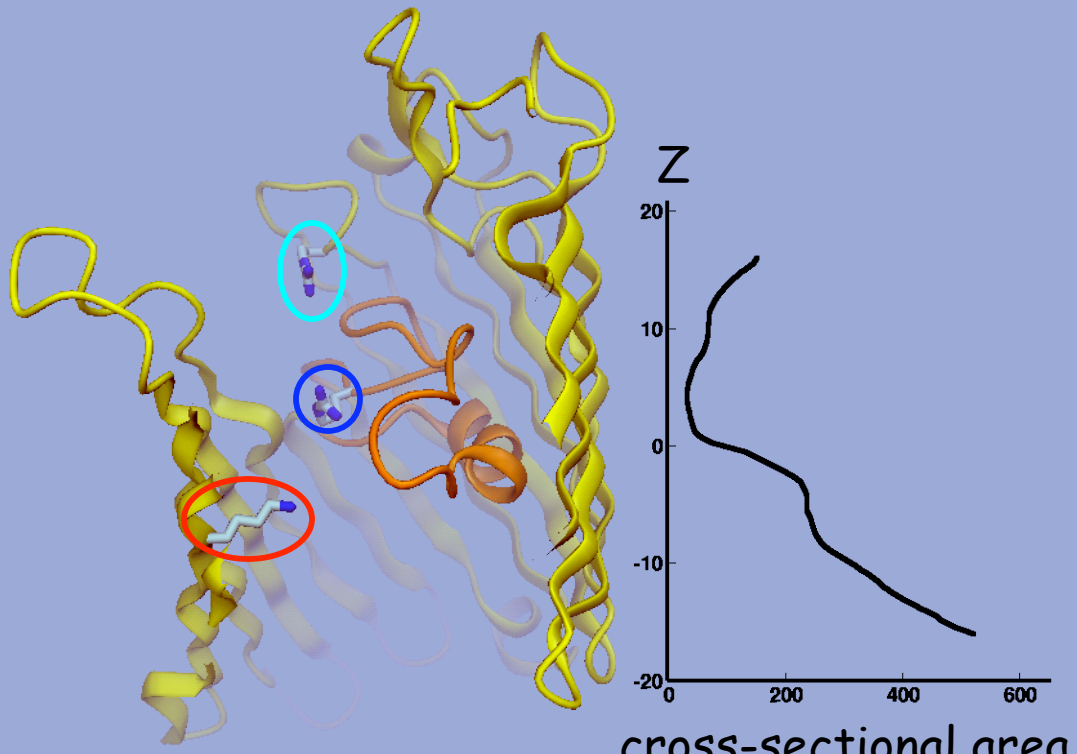






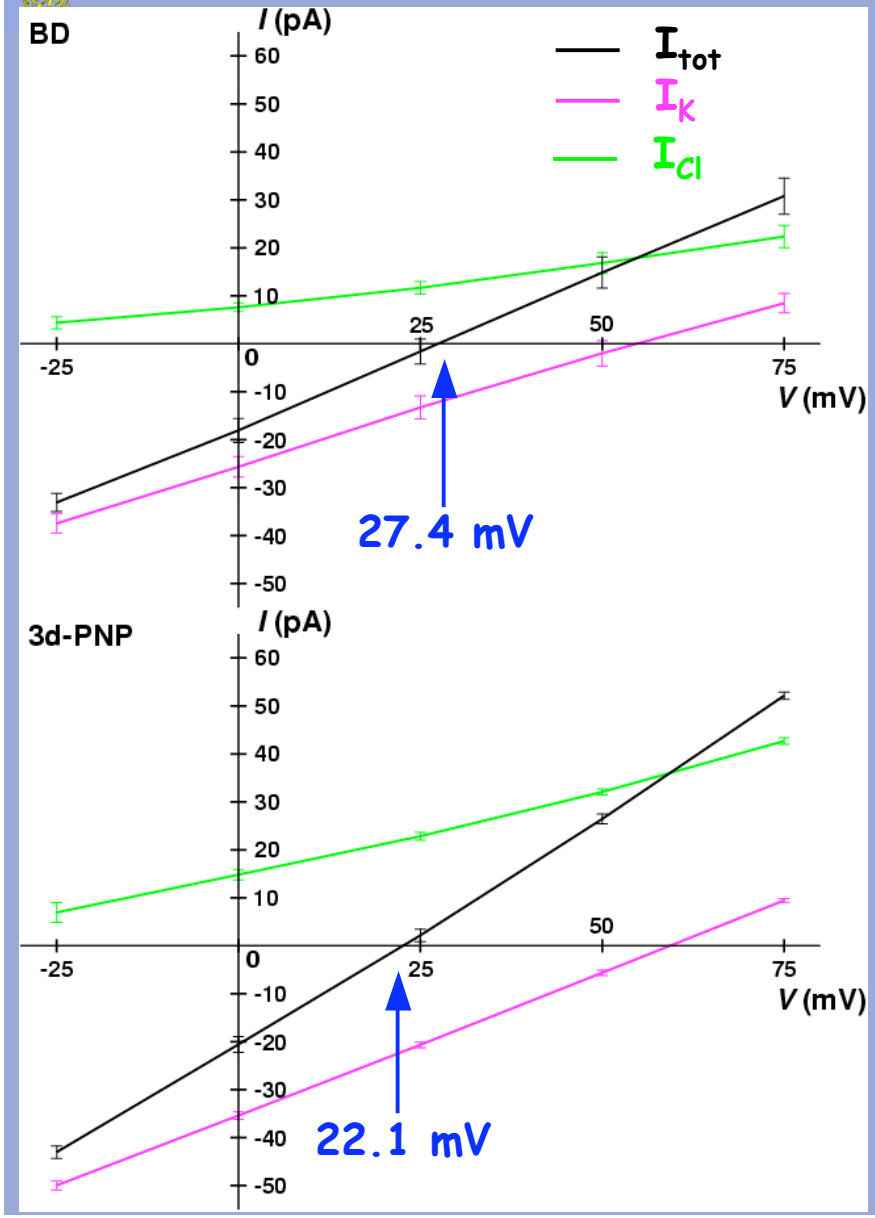
Asymmetry in Channel Conductance

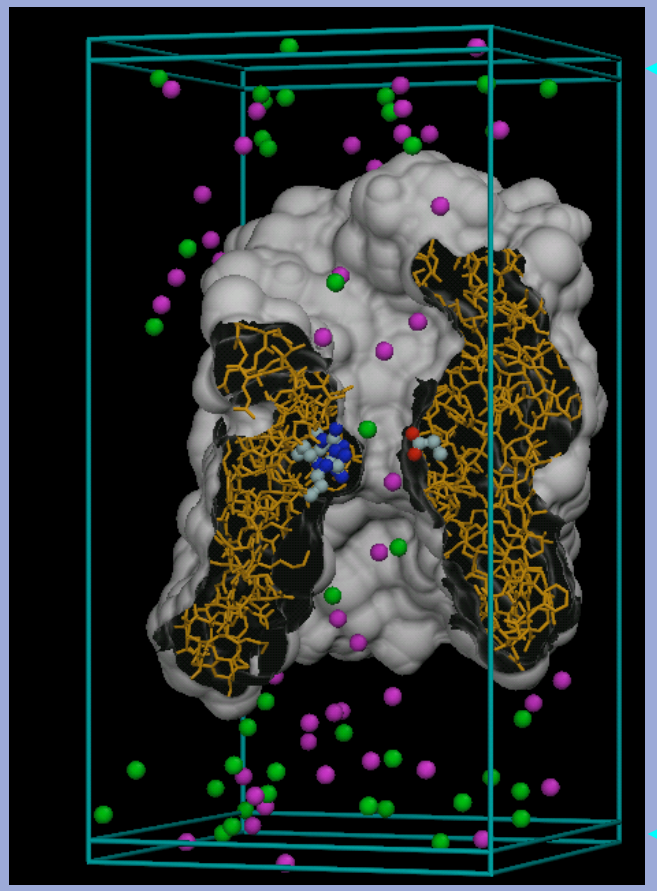
protein	Vmp = +150 mV			Vmp = -150 mV			G_+/G_-
	$I_K(+)$	$I_{Cl}(-)$	G_+	$I_K(-)$	$I_{Cl}(+)$	G_-	
OmpF, uncharged	54.4	54.8	0.73	-56.3	-55.8	0.75	0.97
OmpF	117.9	85.7	1.36	-106.4	-65.5	1.15	1.18
R168A	117.4	62.1	1.20	-124.6	-61.8	1.24	0.96
R132A	145.1	56.6	1.34	-122.5	-39.8	1.08	1.24
K16A	170.5	68.7	1.59	-110.9	-33.6	0.96	1.66



Comparisons with Experiments:

Reversal Potential in 0.1:1M KCl Solution





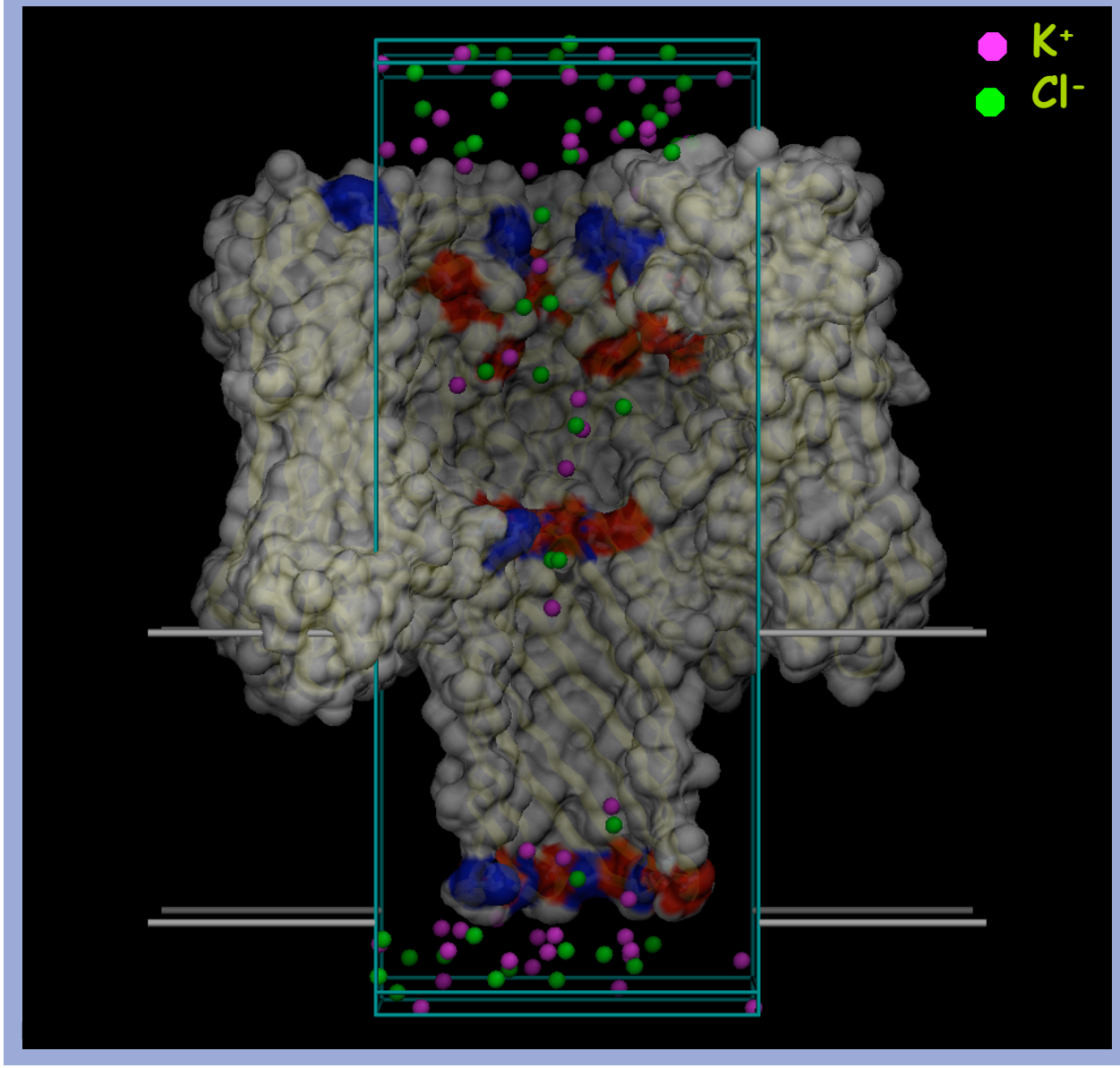
exp. 24.3 mV (Schirmer, 1999)

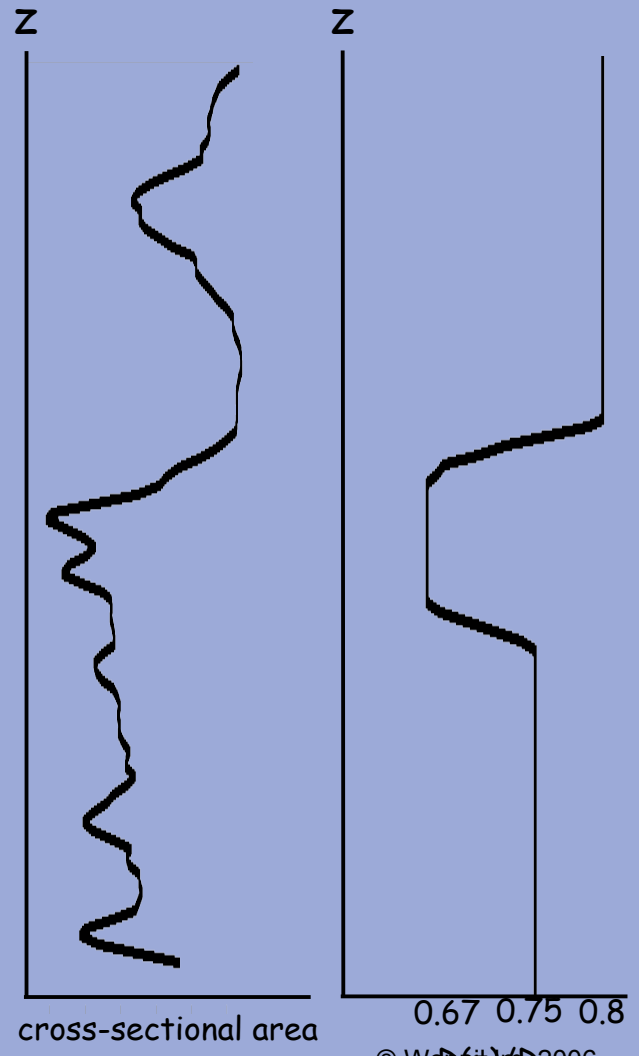
In Non-equilibrium Situations of Symmetric and Asymmetric Solutions

- the channel conductance calculated from BD is in excellent accord with the experimental data.
- the calculations conforms the asymmetry in the OmpF channel conductance
- the calculations also reproduce the experimentally well-known conductance-concentration relation
- the channel conductance of three OmpF mutants (R168A, R132A, and K16A) suggests that the asymmetric channel conductance arises mostly from the permanent charge distribution of the channel
- the calculated reversal potential in 0.1:1M KCl is 27.4 mV (BD) and 22.1 mV (PNP), in excellent accord with the experimental value of 24.3 mV
- All PNP calculations yield about 50% higher channel conductance than BD

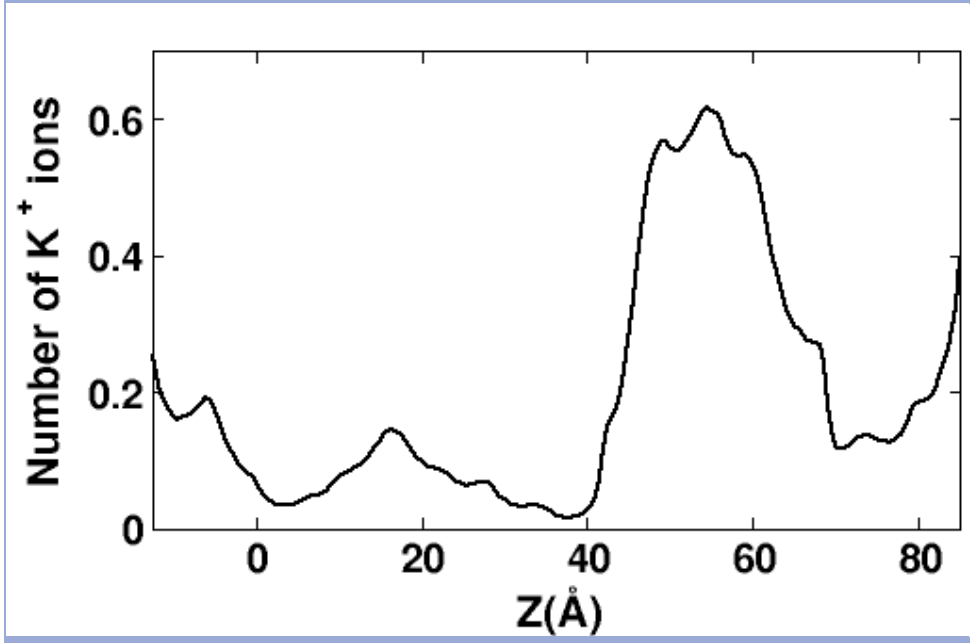
BD System of α-hemolysin (α-HL)

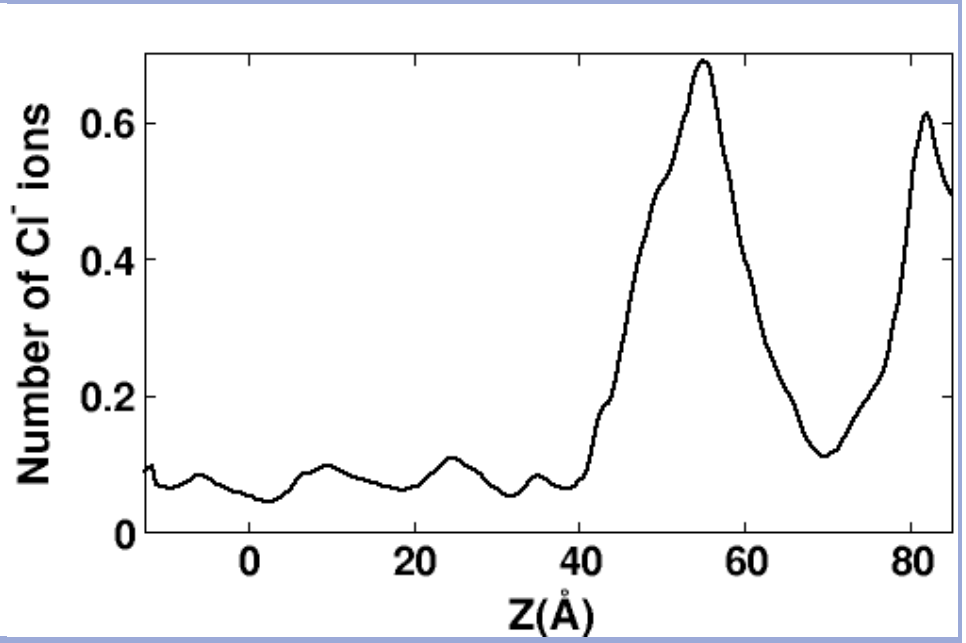
Noskov et. al. (2004) *Biophys. J.*





Ion Distribution along α-HL Pore

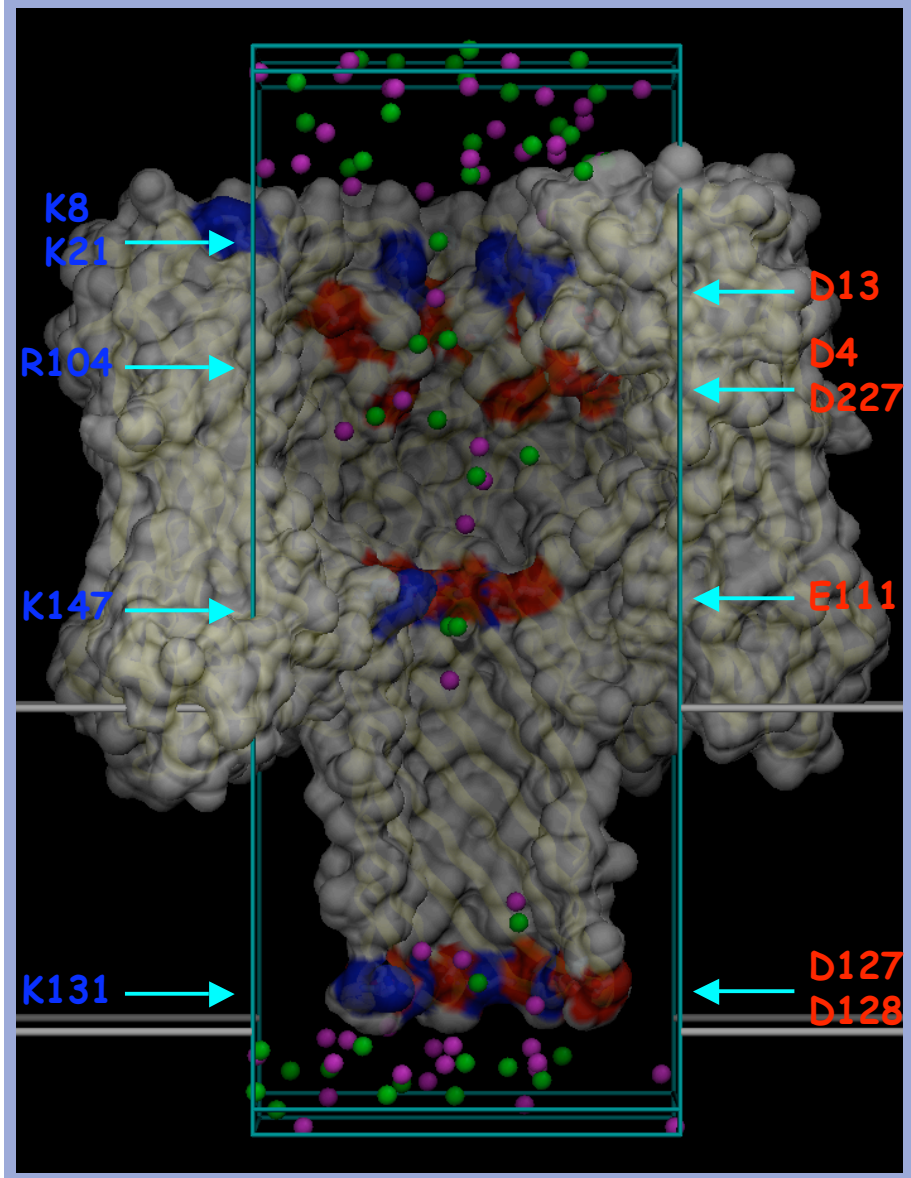




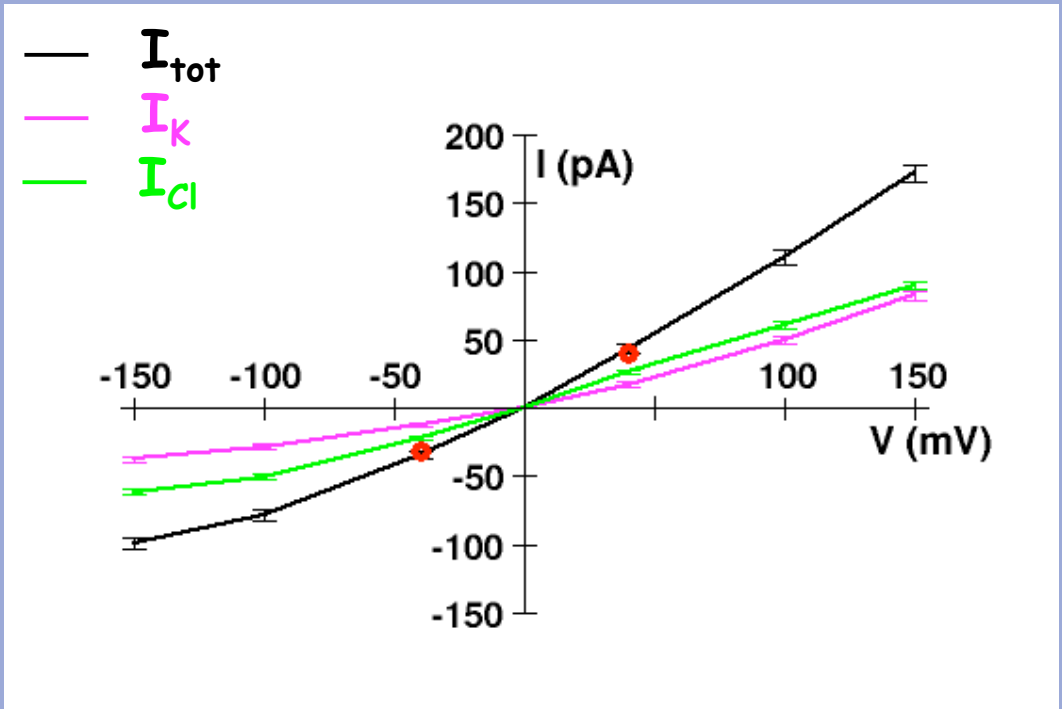
The multi-ion PMF captures the very weak-anion selectivity of α-HL

In 1M KCl, there are 22.9 K^+ and 23.2 Cl^- in α-HL pore.

Ion Distribution along α-HL Pore



I-V curves in Symmetric 1M KCl with Transmembrane Potential

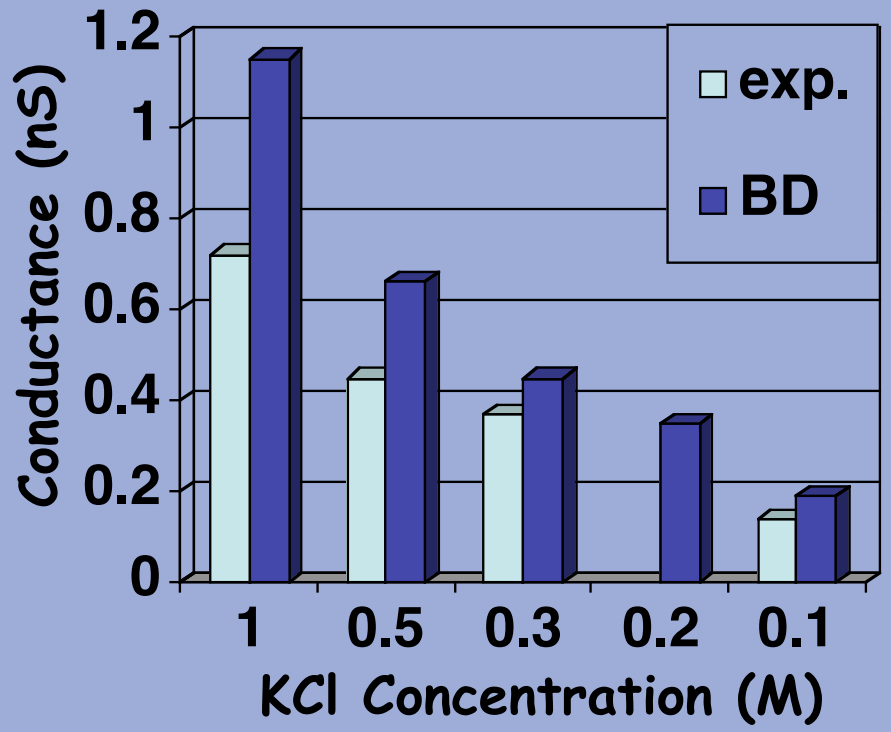


- $I_{Cl} > I_K$ (anion selectivity)
- Asymmetry in I-V relation

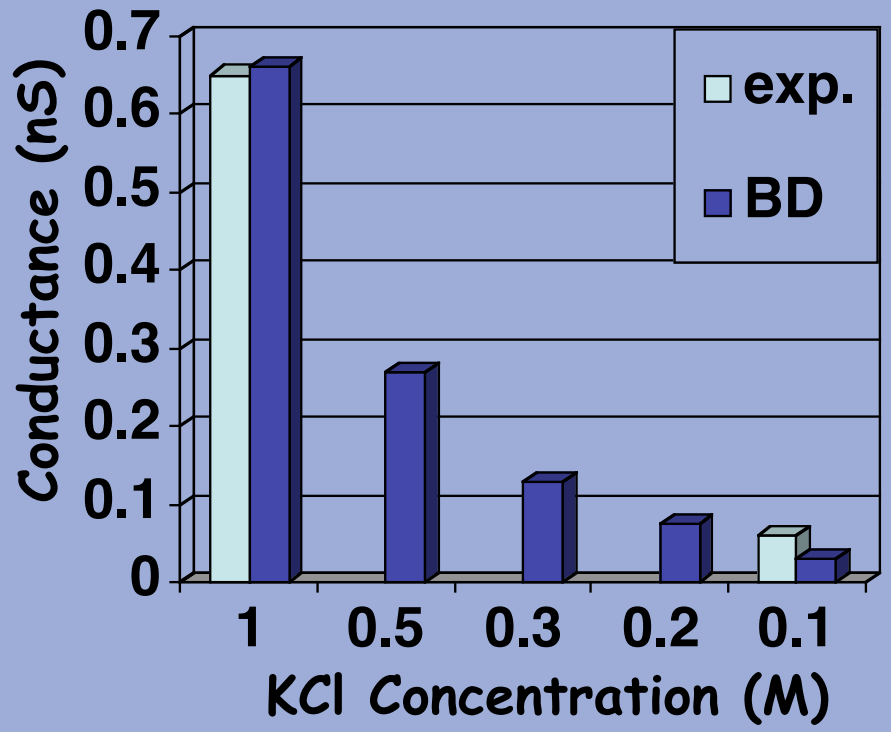
Comparisons with Experiments:

Single Channel Conductance

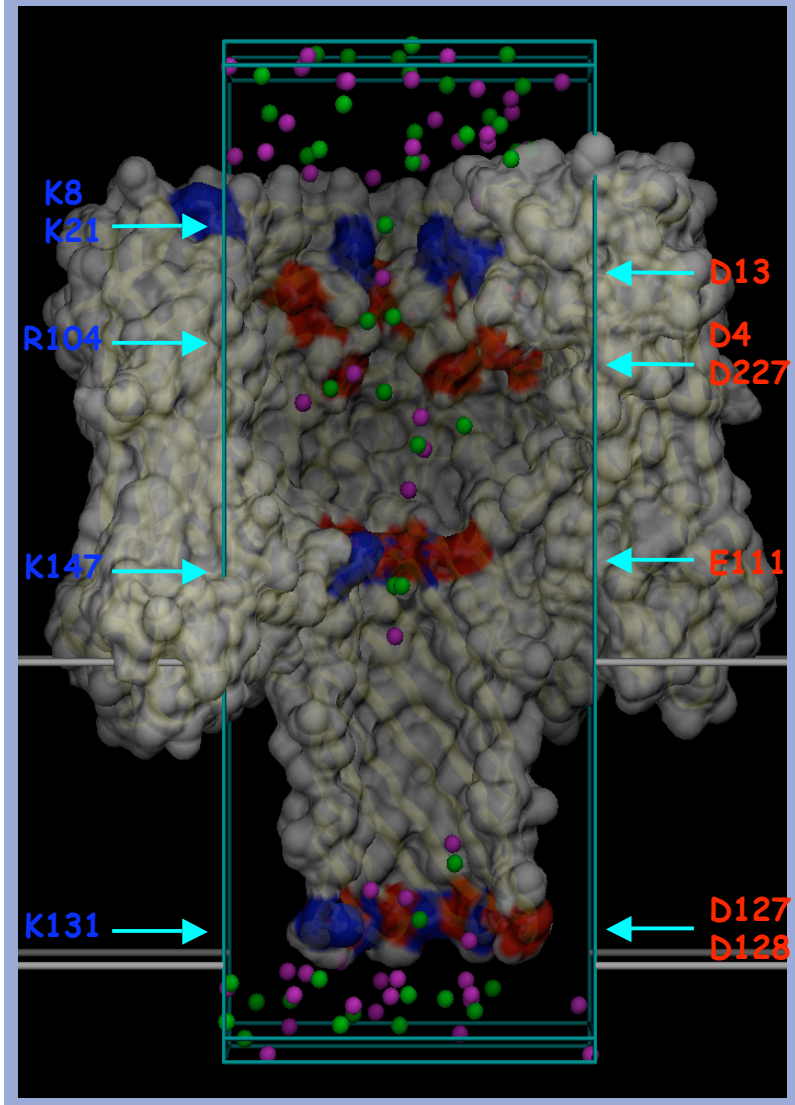
Vmp=+150 mV



Vmp=-150 mV



Asymmetry in Channel Conductance



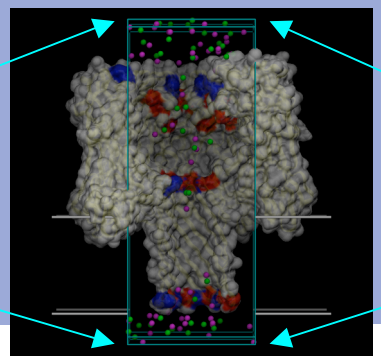
In 0.5M KCl

protein	Vmp = +150 mV			Vmp = -150 mV			G_+/G_-
	$I_K(+)$	$I_{Cl}(-)$	G_+	$I_K(-)$	$I_{Cl}(+)$	G_-	
Wild-type	48.0	51.0	0.66	-15.8	-24.7	0.27	2.44
E111	54.5	136.2	1.27	-10.7	-46.5	0.38	3.34
K147	83.3	18.7	0.68	-93.5	-33.1	0.84	0.81
E111-K147	89.8	40.8	0.87	-27.4	-39.7	0.44	1.98
D127	29.9	66.2	0.64	-37.4	-103.3	0.93	0.48
D128	43.0	77.4	0.80	-33.6	-85.2	0.79	0.99
K131	91.2	89.4	1.20	-28.9	-40.7	0.46	2.61
D127-D128-K131	35.0	91.6	0.84	-33.8	-94.3	0.85	0.99

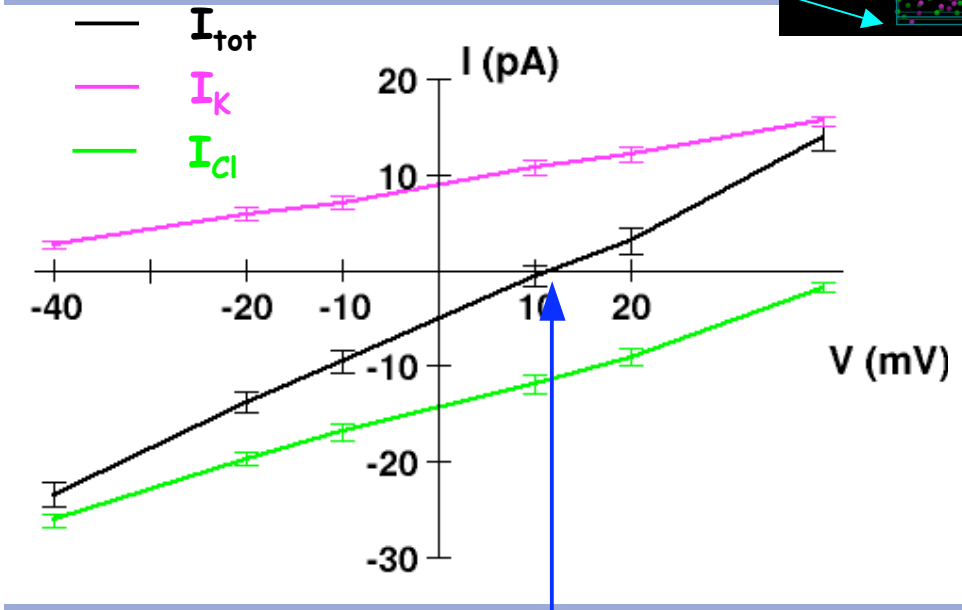
Comparisons with Experiments:

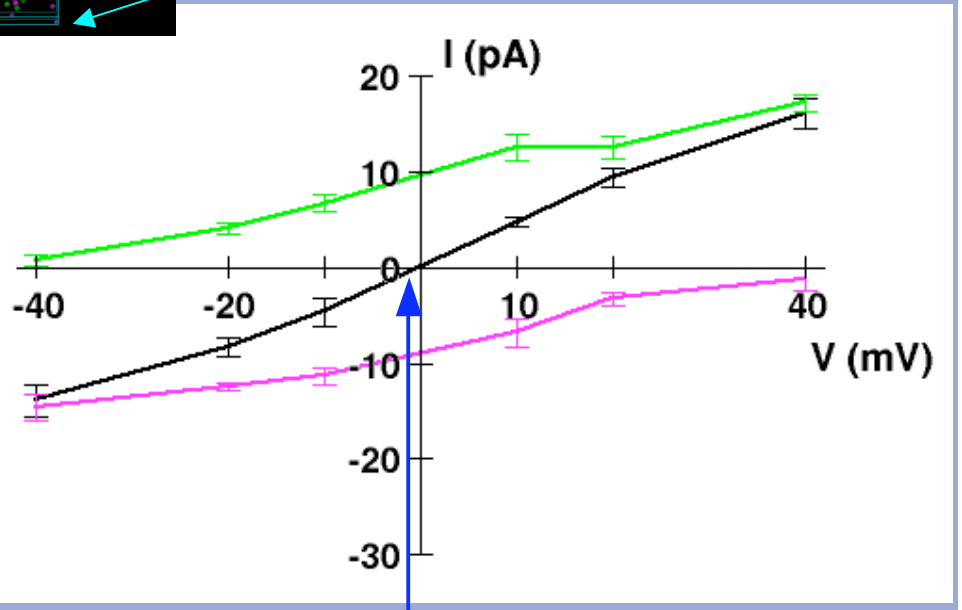
Reversal Potential in 0.2:1M KCl

In 1.0M:0.2M KCl



In 0.2M:1.0M KCl





11.2 mV

exp. 9.2 mV

-0.5 mV

-3.7 mV

Gu et al. (2001) *Prot. Natl. Acad. Sci. USA*

The Hierarchical Approach