BIOLOGICAL ION CHANNELS AS NANOSCALE DEVICES

Approaches to Simulation: Continuum and Particle Methods

T.A. van der Straaten

Beckman Institute for Advanced Science and Technology
University of Illinois at Urbana-Champaign

Department of Molecular Biophysics and Physiology
Rush Medical College, Chicago

OUTLINE

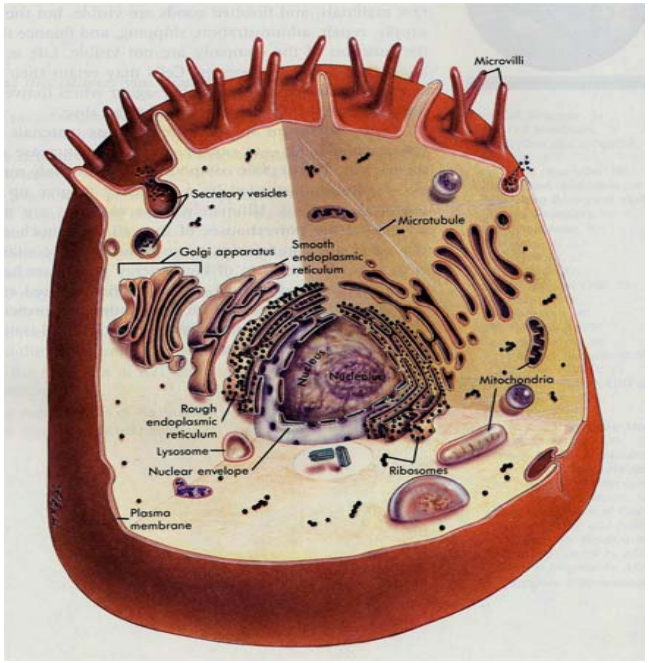
- Background on Protein chemistry, Ion Channels etc
- Simulation Methods
- Continuum (Drift-Diffusion) simulations
  - gramicidin
  - porin
- Monte-Carlo simulations – gramicidin

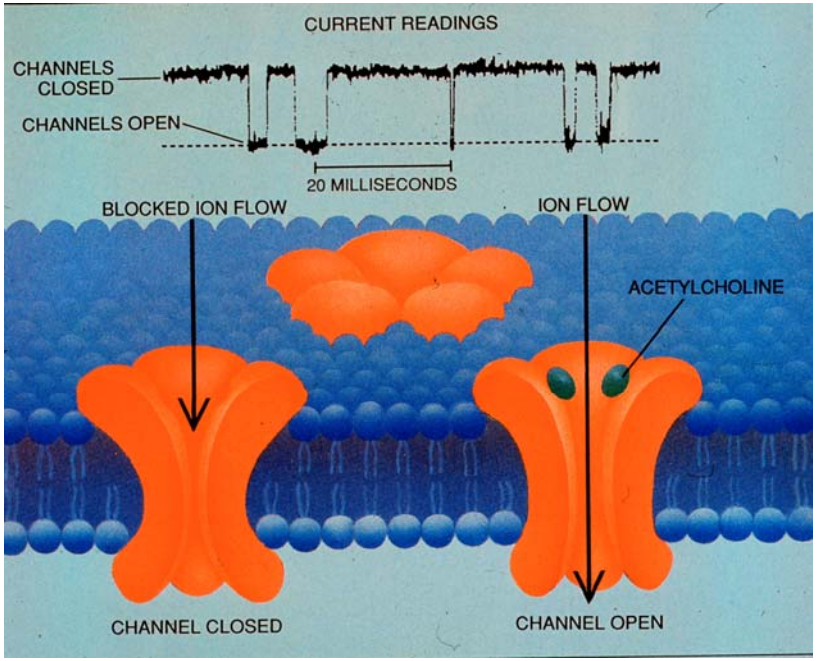
BIOLOGICAL ION CHANNELS

Proteins that form **nanoscopic** aqueous tunnels in cell membrane

Physiological Role – **regulate ion flow** and composition inside cell control, **electrical signaling** in the nervous system, muscle contraction, **drug** delivery

Disease – malfunctioning channels





BIOCHEMISTRY OF ION CHANNELS

Amino acids – building blocks of proteins

Side-chain distinguishes the amino acid

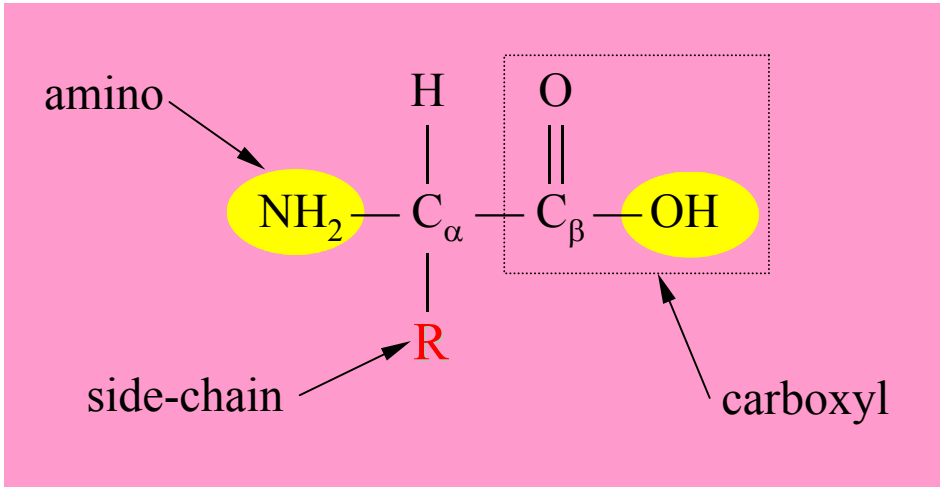
Some of the side-chains are ionizable – **proteins are highly charged.** Strong and steeply varying charge density is critical to the I-V characteristics of the open channel.

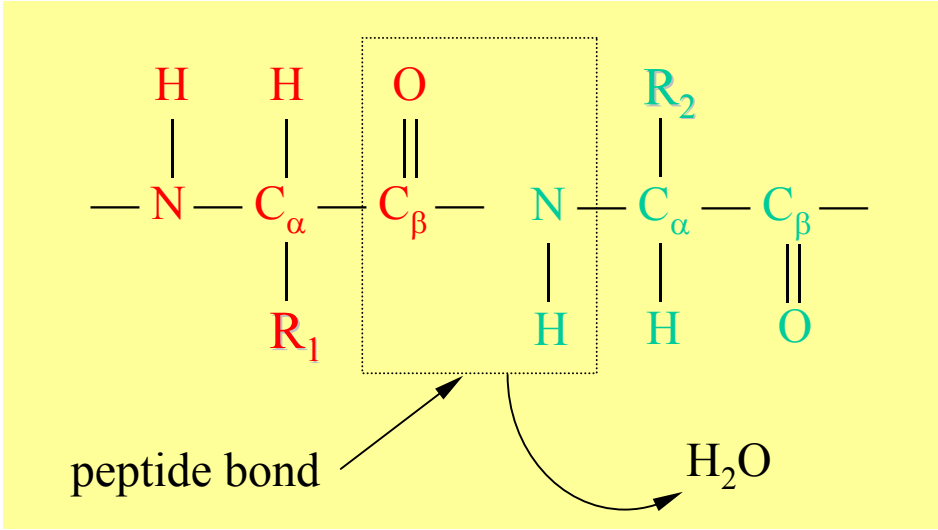
neutral pH: $NH_2 \rightarrow NH_3^+$

$COOH \rightarrow COO^-$

Amino acids are linked together by **peptide bonds**

Polypeptide chains **fold** to form proteins





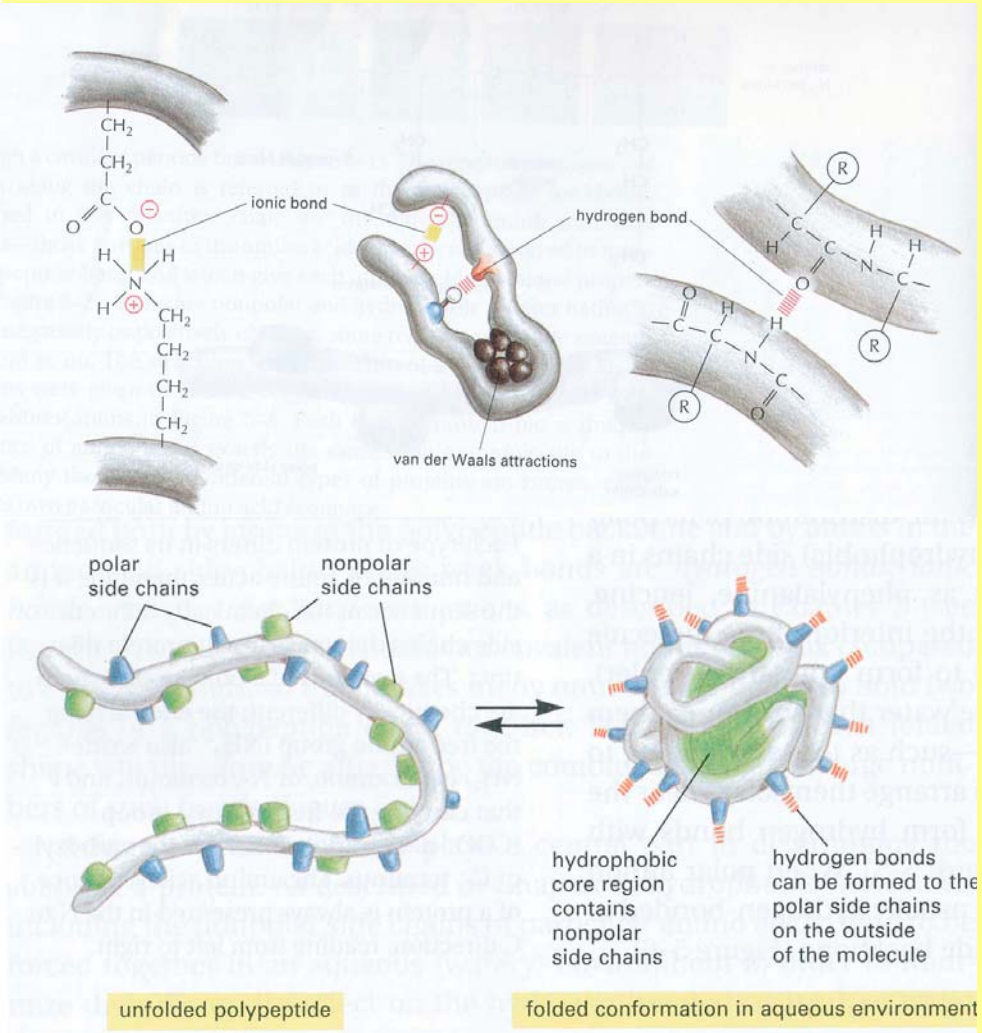
AMINO ACID			SIDE CHAIN
Aspartic acid	Asp	D	negative
Glutamic acid	Glu	E	negative
Arginine	Arg	R	positive
Lysine	Lys	K	positive
Histidine	His	H	positive
Asparagine	Asn	N	uncharged polar
Glutamine	Gln	Q	uncharged polar
Serine	Ser	S	uncharged polar
Threonine	Thr	T	uncharged polar
Tyrosine	Tyr	Y	uncharged polar

POLAR AMINO ACIDS

AMINO ACID			SIDE CHAIN
Alanine	Ala	A	nonpolar
Glycine	Gly	G	nonpolar
Valine	Val	V	nonpolar
Leucine	Leu	L	nonpolar
Isoleucine	Ile	I	nonpolar
Proline	Pro	P	nonpolar
Phenylalanine	Phe	F	nonpolar
Methionine	Met	M	nonpolar
Tryptophan	Trp	W	nonpolar
Cysteine	Cys	C	nonpolar

NONPOLAR AMINO ACIDS

PROTEIN FOLDING



Ionic bonds
Hydrogren bonds } **electrostatic**
van der Waals attraction

3D structure is determined by order of amino acids in sequence and energy considerations - folded structure is that which minimizes free energy

Conformational changes can occur when protein interacts with other molecules - crucial to function of protein

GRAMICIDIN

Small simple channel forming molecule
Each monomer is made up of 15 amino acids (~ 500 atoms) folded into a helical structure.
Expressed by certain bacteria perhaps to kill other microorganisms by collapsing the ion gradients that are required for their survival. Useful as antibiotic

ompF PORIN

Large trimeric channel, sits in the outer membrane of *e. coli*
Each monomer is made up of 340 amino acids (~90 000 atoms)

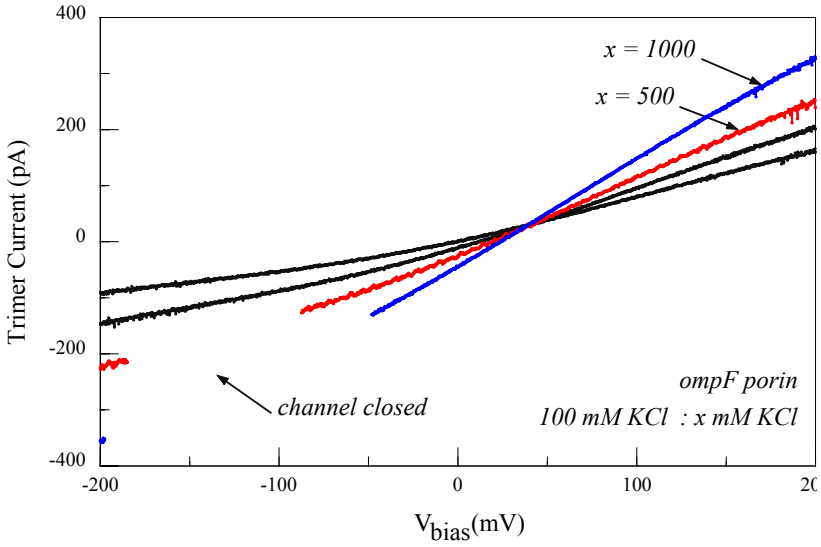
RASMOL www.umass.edu/microbio/rasmol/

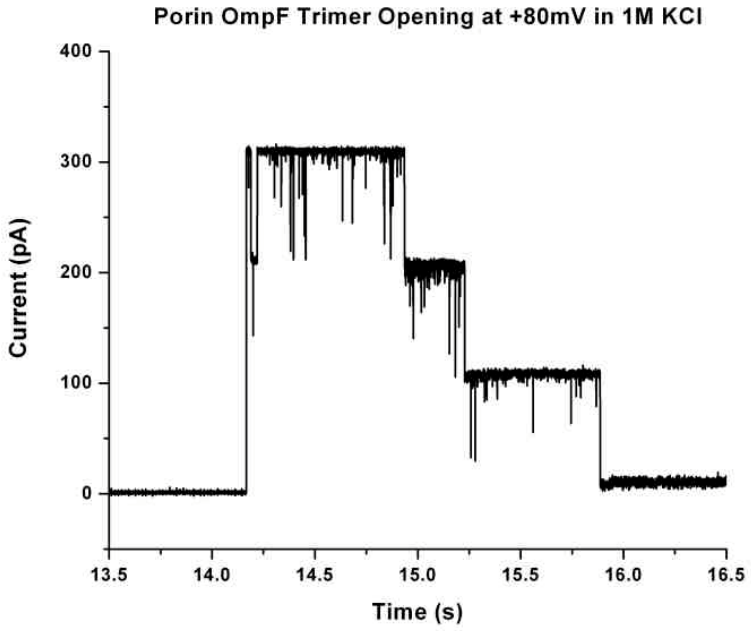
PROTEIN DATABANK www.rcsb.org/pdb/

GATING / SWITCHING

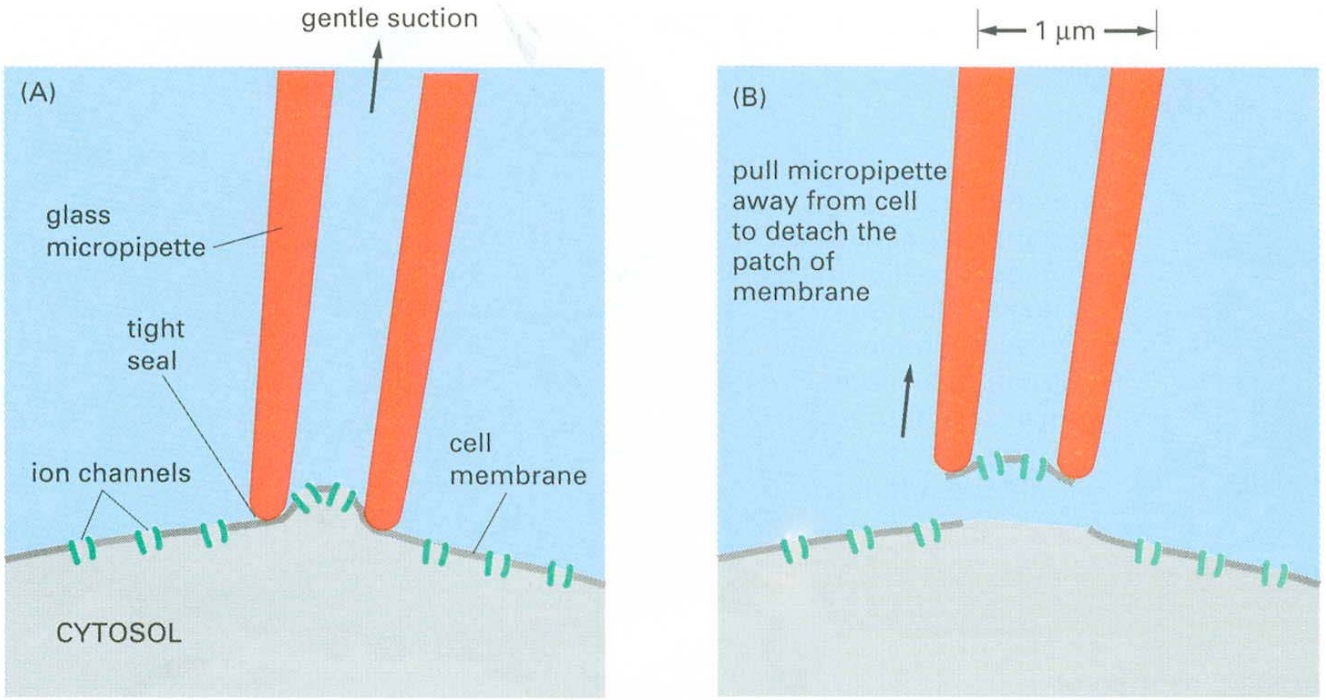
Many channels exhibit **switching** properties similar to electronic devices.

"open/close" or "on/off" states – response to environment





PATCH-CLAMP MEASUREMENTS

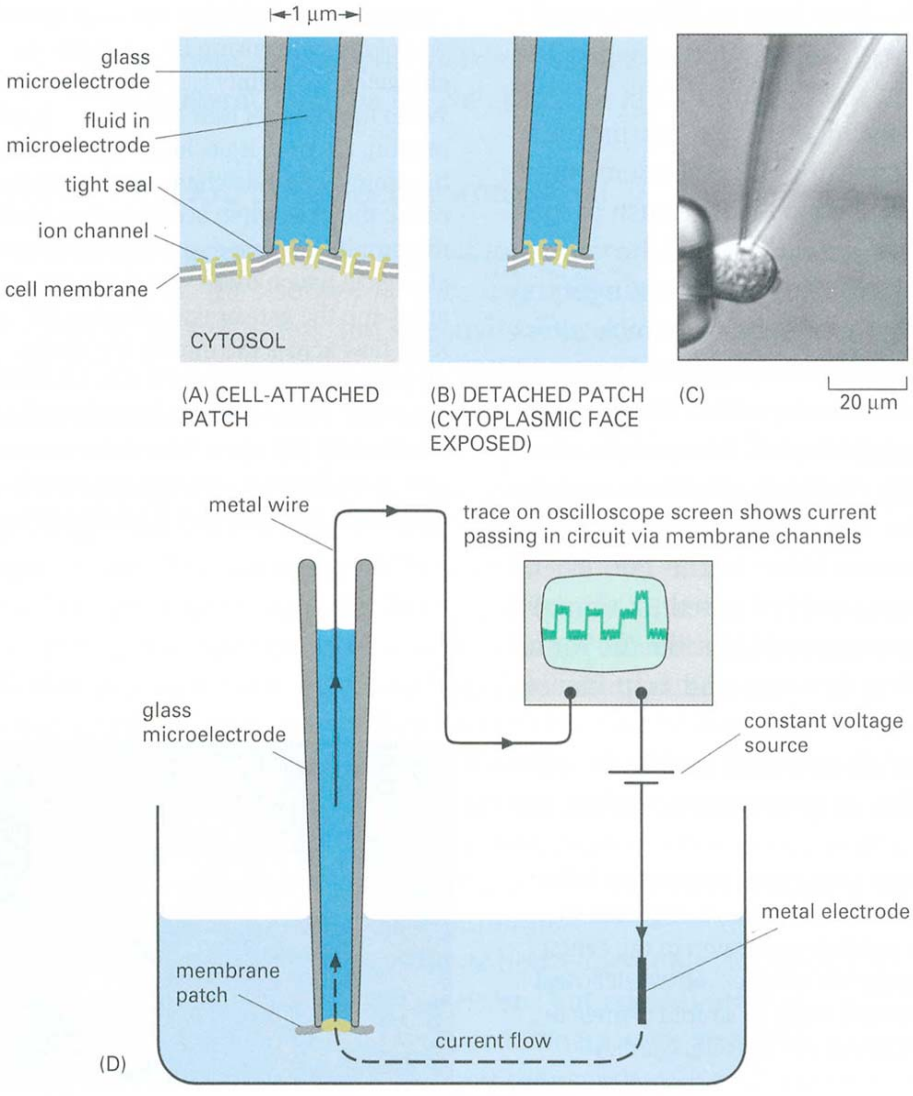


Allows single channel currents to be recorded

Channel is either fully open or fully closed

Open channel conductance is constant - aggregate current reflects the total number of channels open at any given time

PATCH-CLAMP MEASUREMENTS



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GATING MECHANISMS

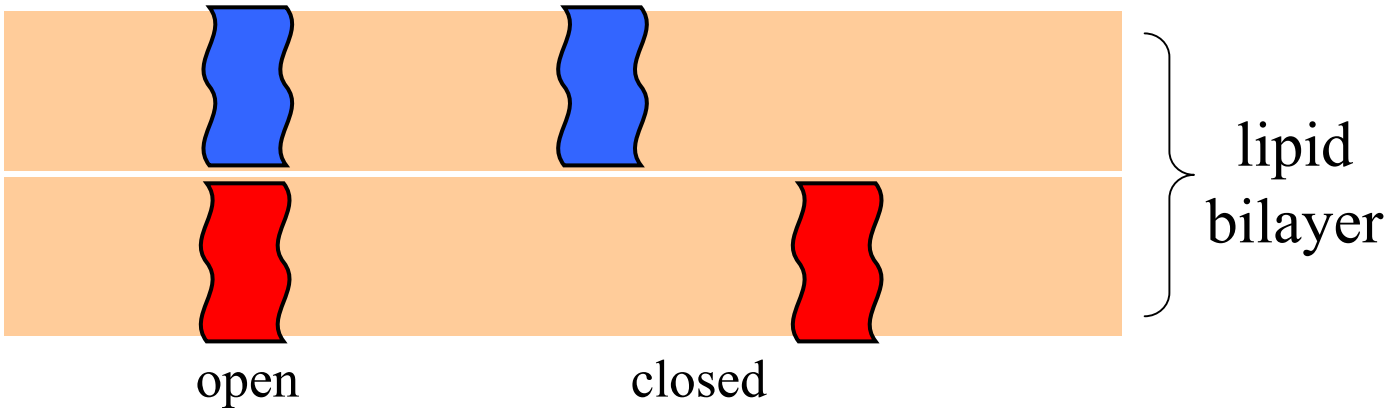
Voltage Gating – the open probability is a function of voltage – e.g., Na^+, K^+, and Ca^{2+} channels are essential for conducting a nerve pulse down an axon and to another nerve cell (or neuron).

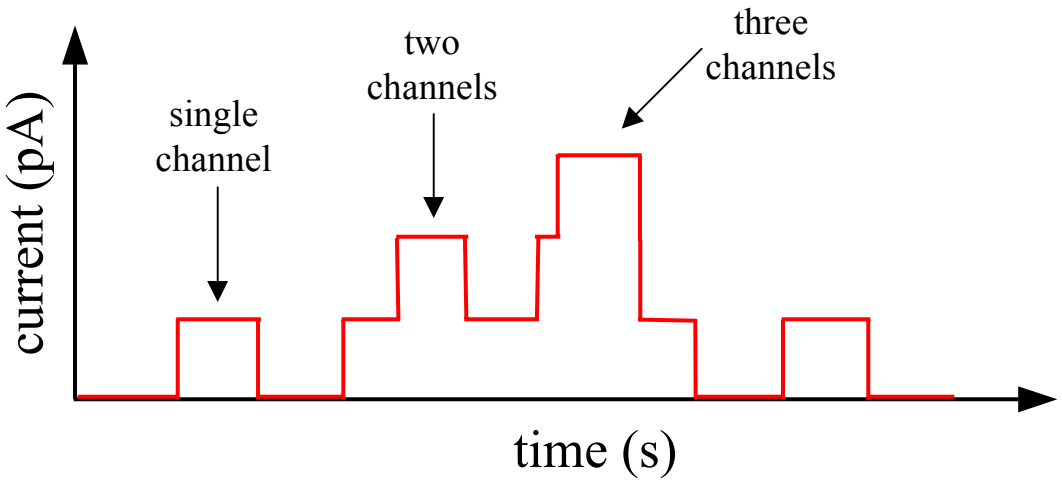
pH Gating –open probability is a function of pH

Ligand Gating – small molecule binding to the channel affects its open probability. Important in **chemical synaptic transmission** (the most common way of transfering a signal from one neuron to another). These channels are gated by **neurotransmitters**, molecules that actually carry the signal between two nerve cells.

Mechanical Gating – channel is directly gated by a mechanical trigger –e.g., cation channel in the hair cell of the inner ear, which is directly gated by a mechanical vibration caused by sound. Not well studied because of technical difficulties

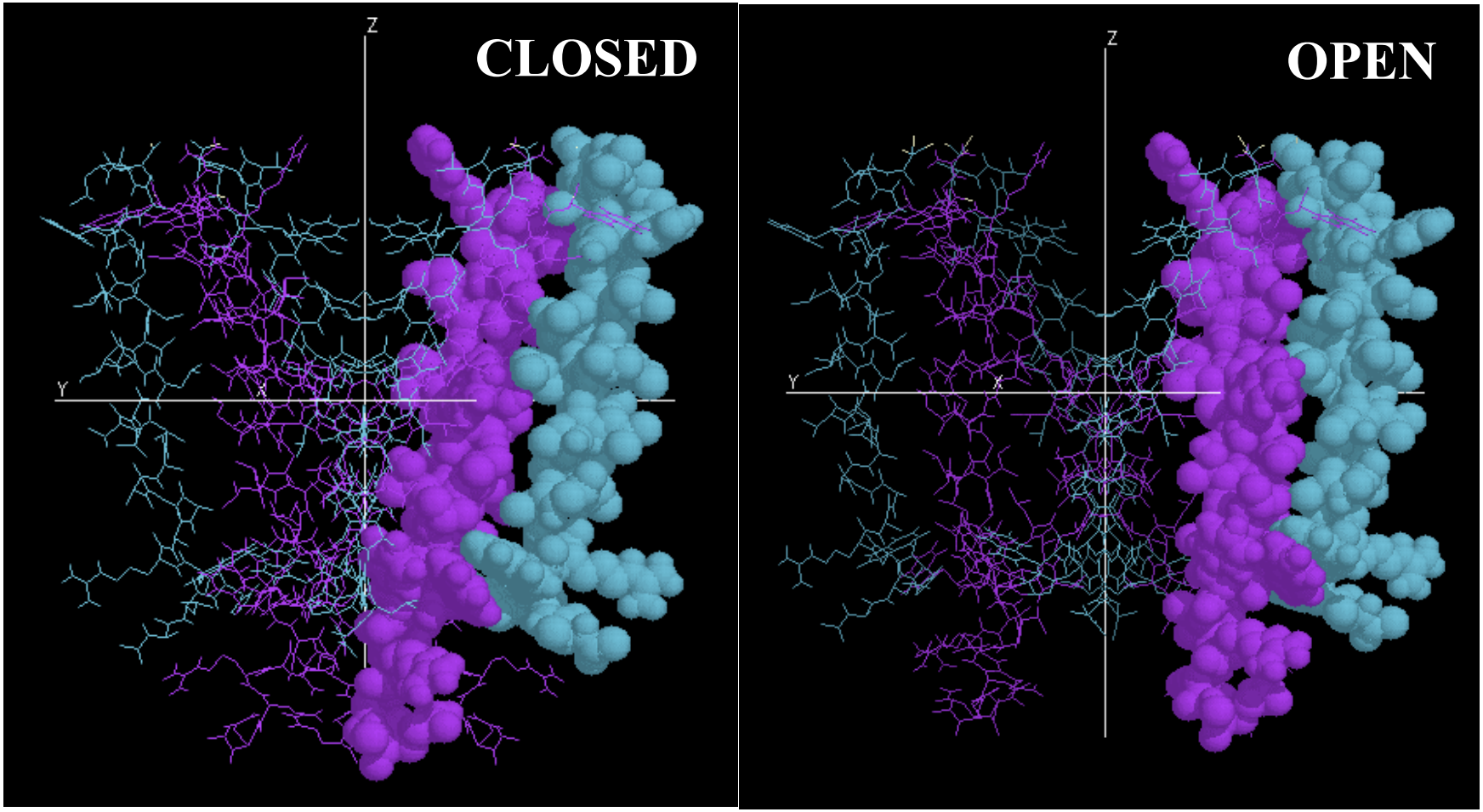
GRAMICIDIN FORMS DIMERS



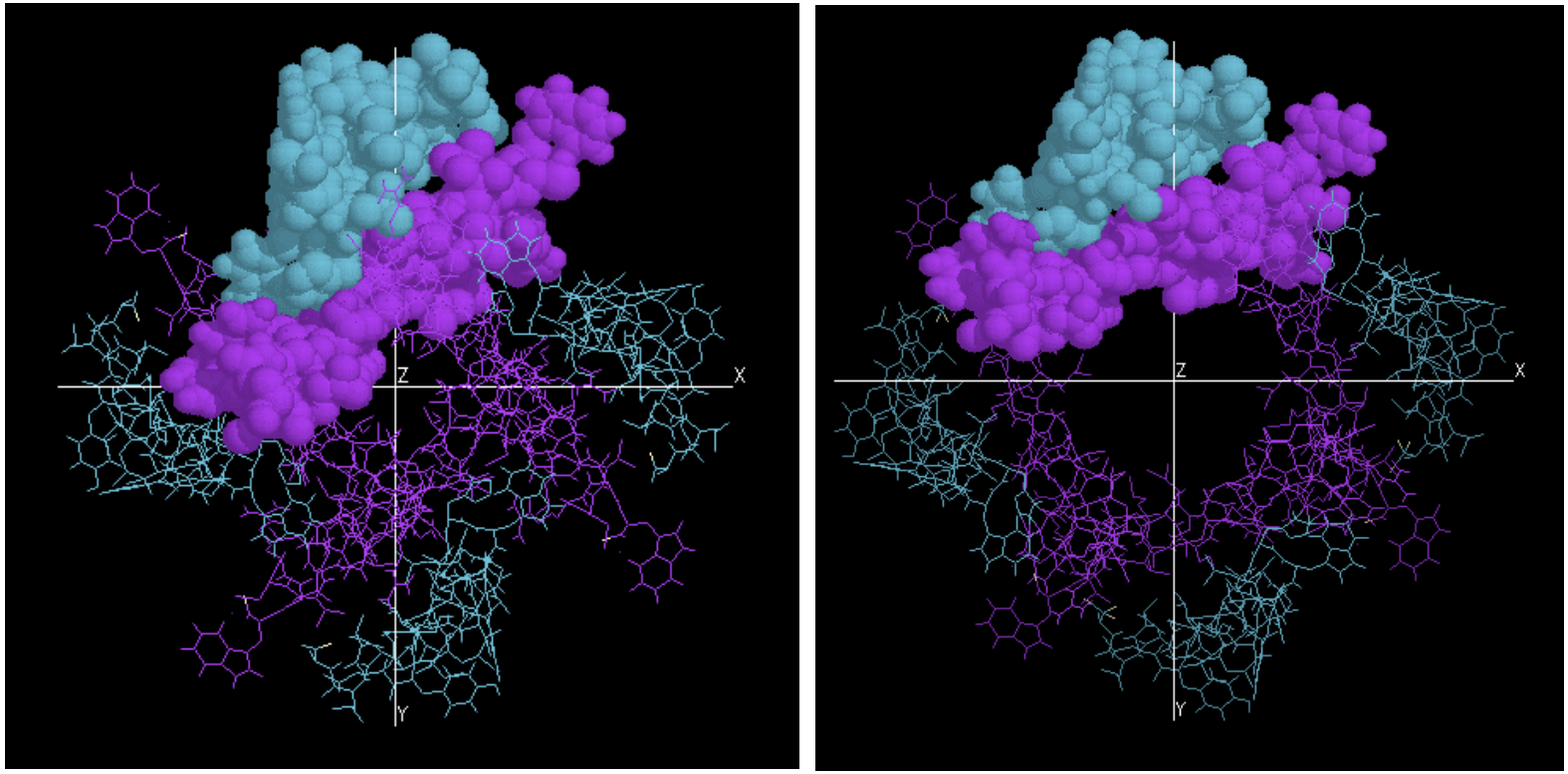


KcsA CHANNEL - a natural pH sensor

pH induced conformational changes causes KcsA channel to switch between conducting and non-conducting states



CLOSED **OPEN**

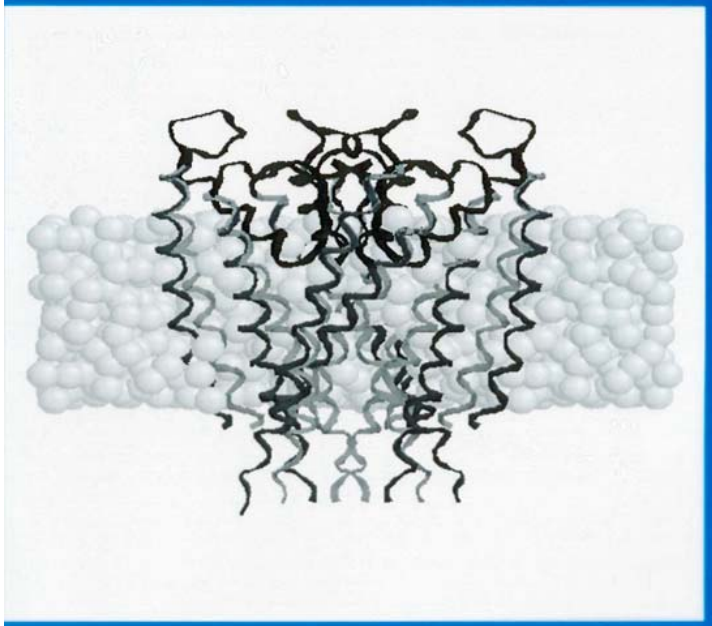


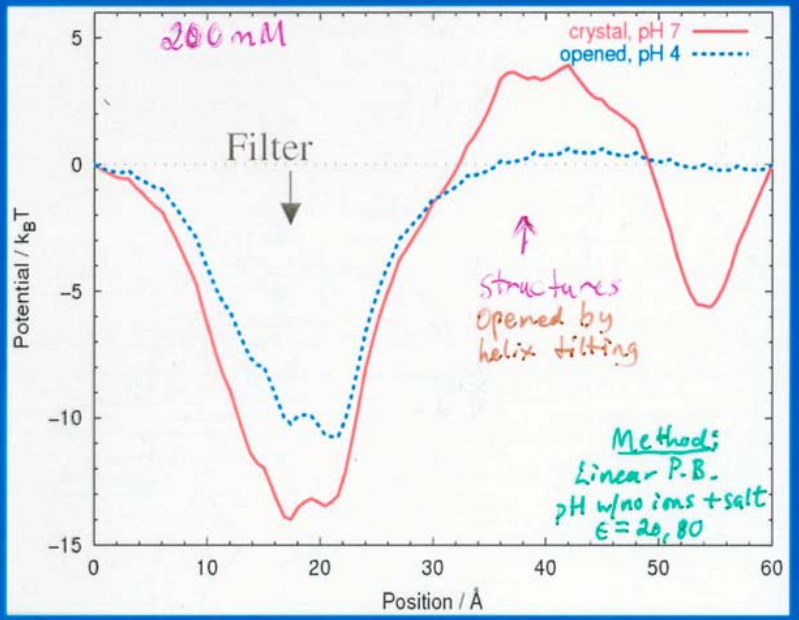
Changes in pH → protein charge → electrostatic fields → conformational changes → lowering energy barrier to permeation

(Jay Mashl, computational biology)

Permeation features of an open KcsA Model

Electrostatic ion potential energy





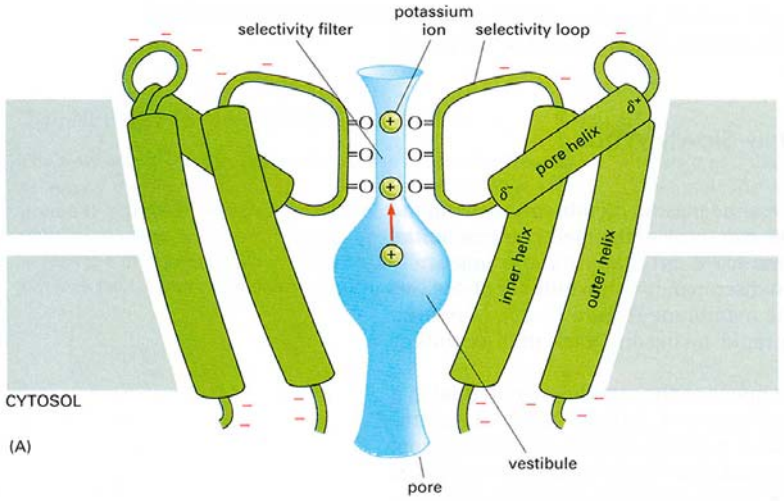
- Energy surface results from charge distribution / pKa's
- Channel geometry creates dielectric barrier to ion entry

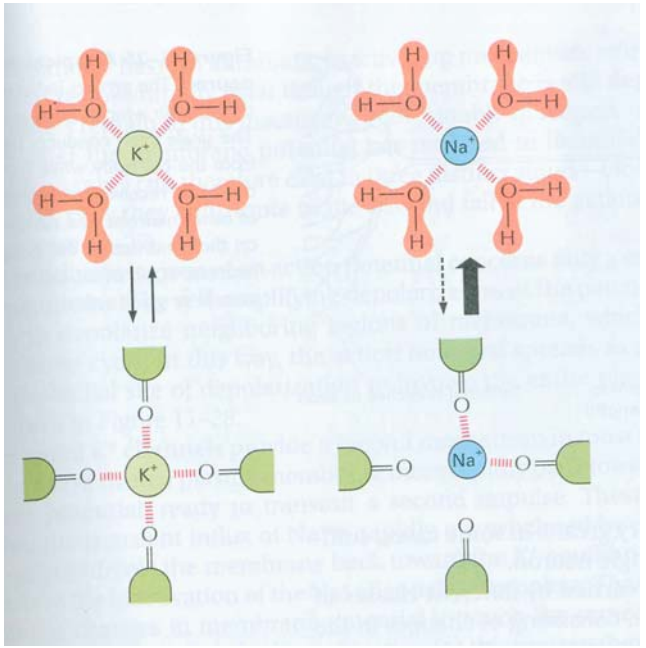
Ref. *Biophys. J.* (2001) 81:2473-2483.

SELECTIVITY

Many channels selectively transmit or block a particular ion species

• Gramicidin passes only small monovalent positive ions (e.g., H^+, Li^+, Na^+, K^+) – electrostatic and steric barriers
• Porin ompF channel shows a mild preference for cations
• Potassium channel selects K^+ over Na^+ by a factor of 10^4, even though these ions are similar in size – dehydration of Na^+ presents an energy barrier





ENGINEERING APPLICATIONS

Currently only ~ 50 known channel structures
~ 30% of all the genes in the human genome code for ion channels

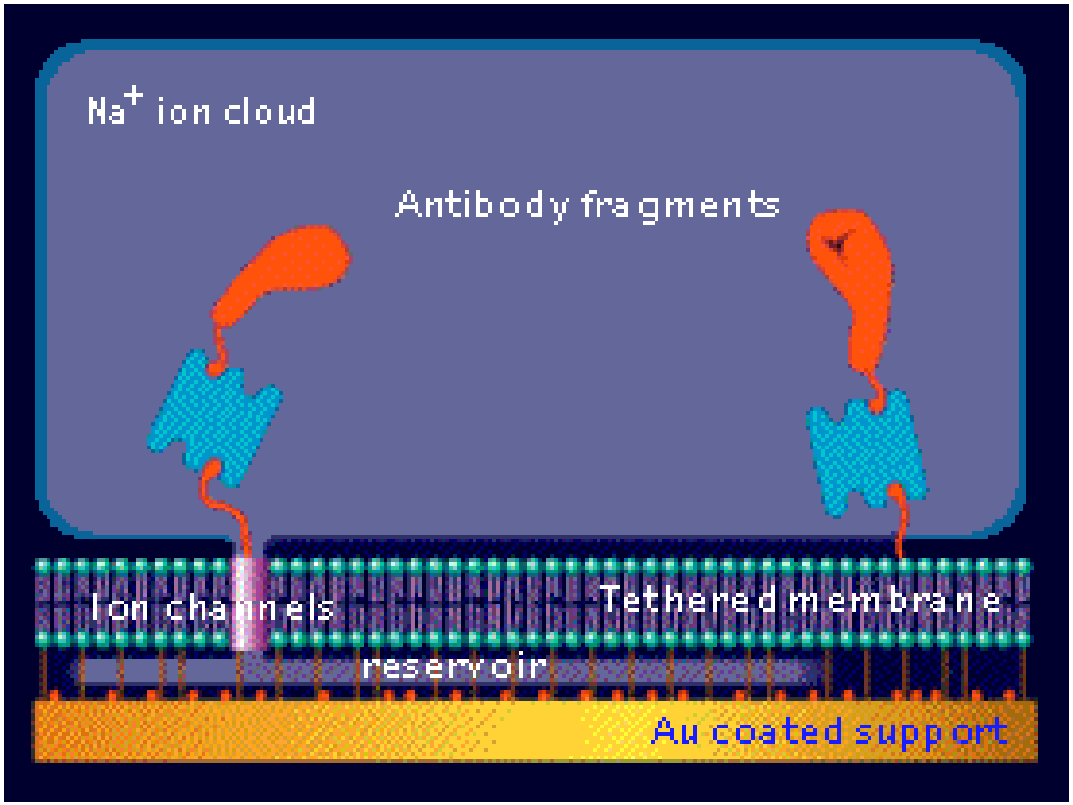
Device feature sizes are shrinking, how much further can we go?

Channels are naturally occurring device elements

- **self-assembled**
- **perfectly reproducible**
- have many specific built-in **features** and **functions**
- can be **mutated**

Design channels with specific conductances, selectivities and functions.
Bio-devices – circuit elements, biosensors

AMBRI® BIOSENSOR



- Chemical to be detected binds to the antibodies
- Prevents the formation of current-conducting dimers
- Marked reduction in aggregate current

www.ambri.com.au

ION CHANNELS AS NANODEVICES

solid state		ion channels
Crystal bandstructure	⟺	Water solution.
Carriers are quasi-particles with a small effective mass	⟺	Carriers are ions with relatively large mass and finite volume
Conduction channel is delimited by depletion layers or potential energy steps at heterojunctions	⟺	Conduction channel is delimited by a highly charged protein membrane
Ultra-scaled structures suffer from fluctuations of doping and sizes	⟺	Channel structures are always perfect replicas. Stable mutations can be used to modify channel behavior.
Energetic carriers may cause structural damage	⟺	Ion are thermalized by interaction with water.

COMPUTATIONAL ISSUES

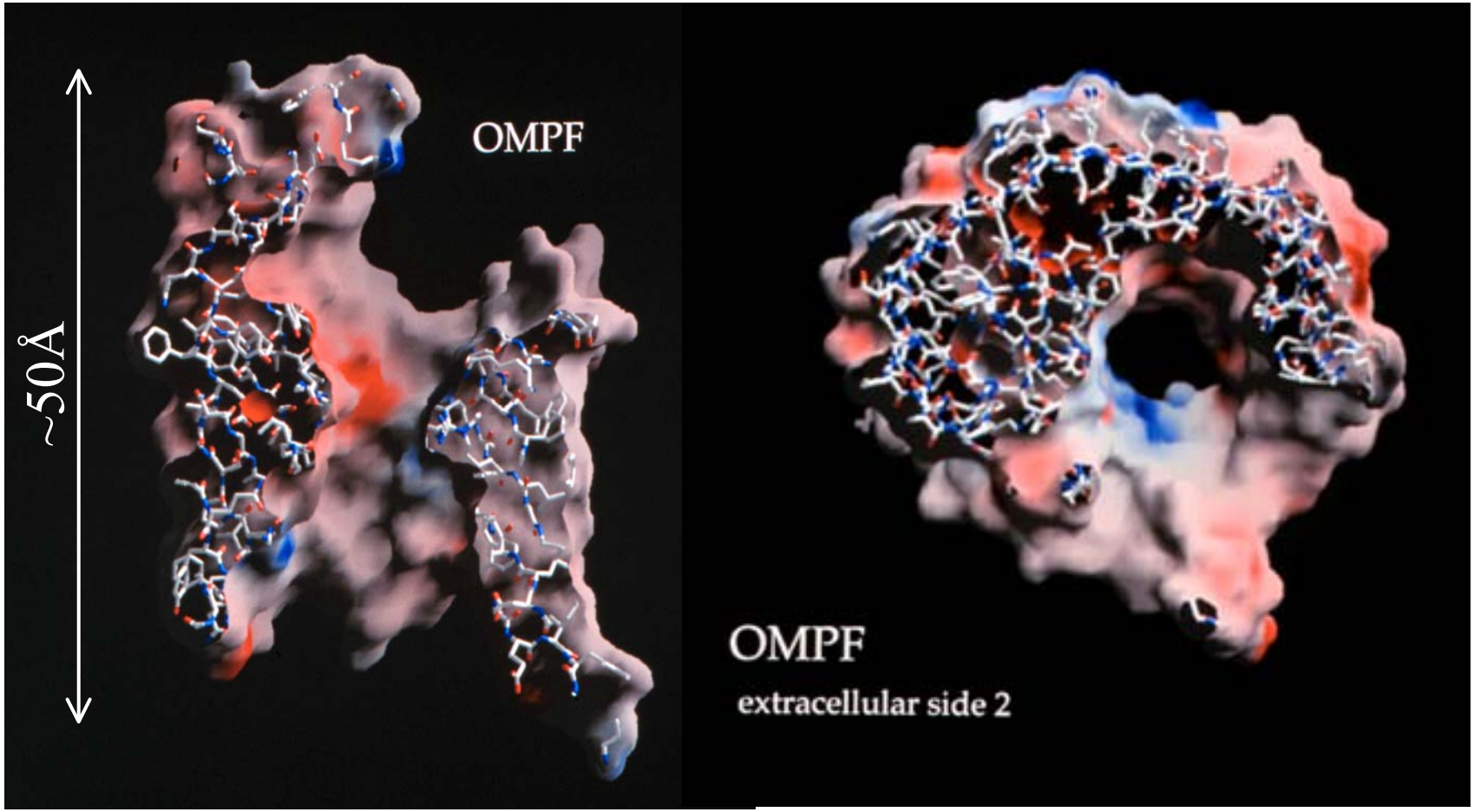
Permeation takes place on timescales of the order of milliseconds
Scattering rates are high (10^{13} - 10^{14} Hz)

System length scales ~ 100 Å
Feature length scales ~ 1 Å (steep gradients)
Complicated, temporally fluctuating structures, difficult to mesh

Representation of trace ionic concentrations

Dielectric constant - protein? lipid? inside channel?
Ion-water scattering rates? Other types of interaction with water?
Water behavior at the nanoscale?

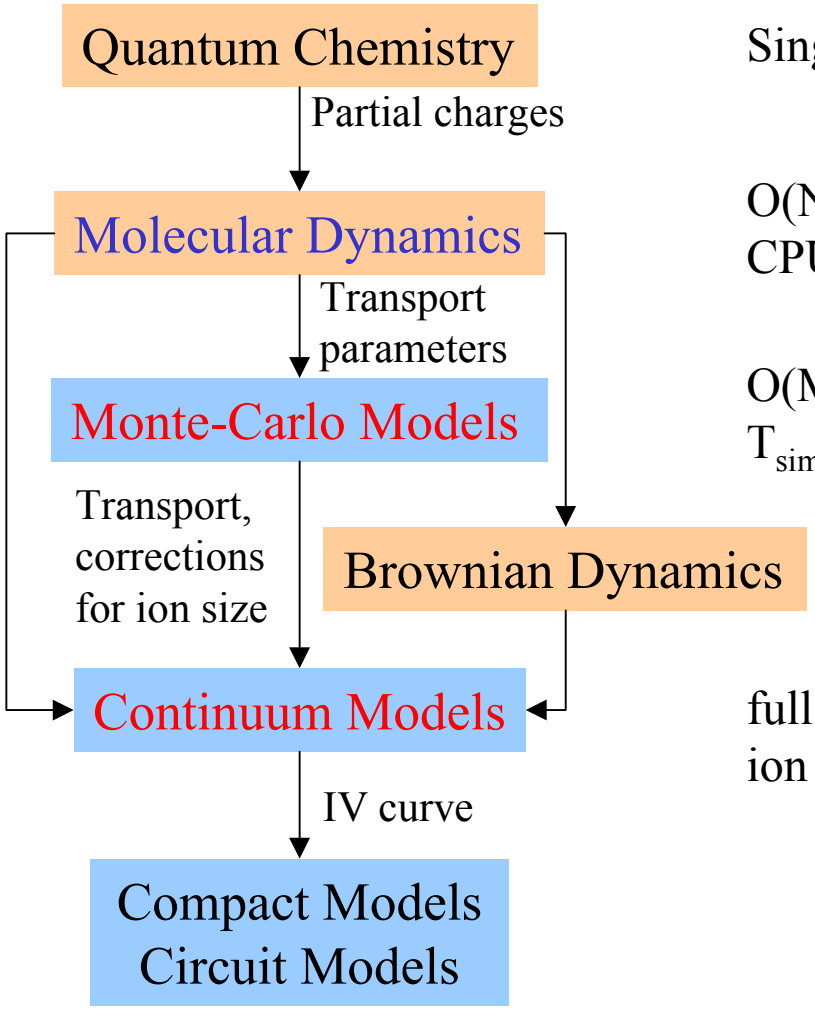
IRREGULAR PROTEIN GEOMETRY



topology and charge varies on scale-length of $\lesssim$ 1Å

molecular "surface" rendered with GRASP

SIMULATION HIERARCHY



Single amino acid (10 to 20 atoms), no dynamics

$O(N^2)$ $N \sim 10^5$ $\Delta t \sim 1fs$ $T_{sim} \sim 1\text{-}10ns$
CPU weeks-months

$O(M)$ $M \sim 10^4$ $N \sim 100$ $\Delta t \sim 10fs$
$T_{sim} \sim 100ns$ CPU days-weeks

Langevin Eq, overdamped limit, $\Delta t \sim 100fs$
$T_{sim} \sim 0.1\text{-}1\mu s$ CPU days, problems with $\varepsilon(r)$

full IV curve CPU mins-hours, overestimates ion densities, requires transport parameters

BECKMAN INSTITUTE

- combine **computational electronics** and **computational biochemistry** tools to simulate the behavior of ionic channels as devices.
- study how ion channels could be incorporated in traditional electronic systems performing functions, and learn how to simulate **wet/dry interfaces**.
- **understand** how specialized functions are accomplished by natural channels, and investigate how to **embed** similar function in **synthetic** channels.
- **exploit self-assembly feature** of molecular structure to arrive at new device architectures.

DRIFT-DIFFUSION

Poisson's Equation $\nabla\cdot(\varepsilon\nabla\psi) = -(\rho_{fixed} + \rho_+ + \rho_-)$

Drift-diffusion Equation $j_\pm = -(D_\pm / kT)\rho_\pm \nabla\phi_\pm$

Electrochemical potential $\phi_\pm = q\psi \pm kT \ln \rho_\pm + \phi_\pm^{ex}$

Continuity Equation

$$\nabla \bullet j_{\pm} + \frac{\partial \rho_{\pm}}{\partial t} = S_{\pm}$$

Boundary Conditions

$$\psi_{right} - \psi_{left} = V_{bias}$$

$$\rho_{\pm,right} = C_{right}$$

$$\rho_{\pm,left} = C_{left}$$

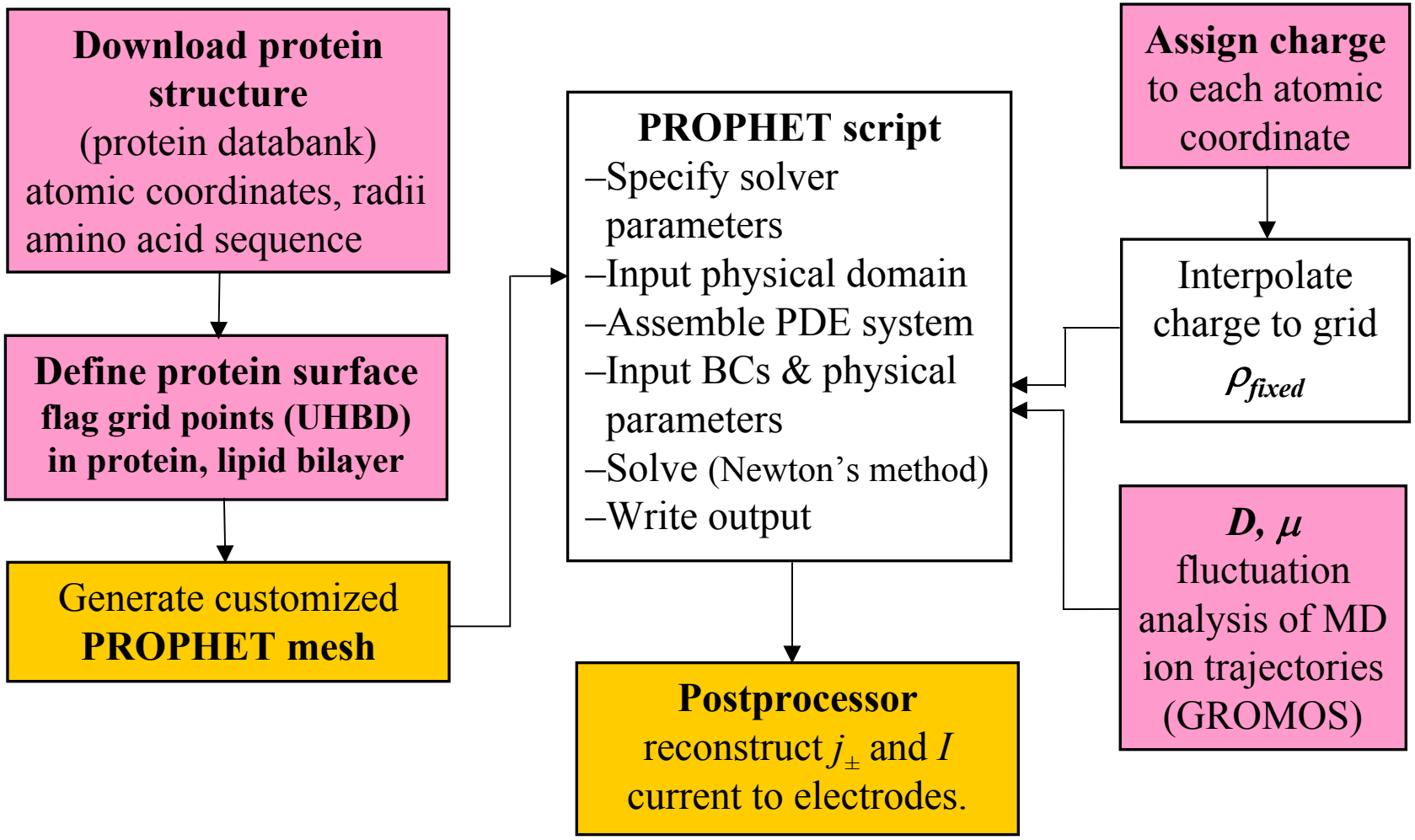
PROPHET SIMULATOR

- **PROPHET** – a rapid prototyping computational environment developed at Lucent Technologies (Conor Rafferty) for solving **multi-dimensional systems of PDEs.**
- **Dial-an-Operator** scripting language allows user to construct a system of equations at an abstract level, using standard 'building blocks' (e.g., Laplacian operator).
- **Database hierarchy** for numerical and physical parameters with 'inheritance' features – data can be created or modified 'on the fly'.
- Physical models can be developed independently of the details of the discretization library, solvers, grid structure, etc.
- Handles **arbitrary multi-dimensional geometries** and physical systems.

Application of PROPHET to Ion Channels

- The **Stanford** group (R.W. Dutton, Z.Yu, D.Yergeau) are developing the new generation of PROPHET with extensions to general device simulations.
- A three-way collaboration (**UIUC, Stanford, Rush**) is underway to apply PROPHET to simulate ion permeation through protein 'nano-channels' embedded in cell membranes, using a continuum transport model
- Detailed protein geometry and fixed charge distribution are constructed using a variety of existing molecular biology and chemistry software tools (**UHBD, GROMOS**). This structural information is then translated into PROPHET native mesh format using a customized mesh generator.

PROTEIN STRUCTURE → IV CURVE



```
dbase prefix=library/math/systems/default_numerical_parameters
dbase create name=method sval=iterative
dbase create name=maxNewton ival=50
dbase create name=accel sval=bicg
dbase create name=precon sval=ilu
dbase create name=maxfil ival=1
dbase create name=dynfil ival=0
dbase create name=NewtonMaxUpd rval=1.0e11

dbase prefix=""
dbase create name=/options/ignoreFPE ival=0
dbase create name=library/math/memory.size ival=770

dbase createlist name=library/physics/channel
dbase create name=library/physics/channel/max.process.temperature rval=1500
dbase createlist name=library/physics/protein
dbase create name=library/physics/protein/max.process.temperature rval=1500
dbase createlist name=library/physics/lipid
dbase create name=library/physics/lipid/max.process.temperature rval=1500

load pas=/home/amiga/trudy/prophet_examples/porin_NLP.pas

boundary name=left
+ xmin=-48.0 xmax=48.0
+ ymin=-48.0 ymax=48.0
+ zmin=-48.0 zmax=-48.0

boundary name=right
+ xmin=-48.0 xmax=48.0
+ ymin=-48.0 ymax=48.0
+ zmin=48.0 zmax=48.0
```

specify solver parameters

input domain

define electrodes

assemble PDE system

```
system name=pnp
+ sysvars=K,Cl,phi
+ nterm=10
#
# Solve transport in channel only
#
+ term0=box_div.sg_ddTherm(phi,K,tl,K_mobility|K)@{channel}
+ term1=box_div.sg_ddTherm(phi,Cl,tl,Cl_mobility|Cl)@{channel}
#
# Dirichlet B.C. for carrier densities at left & right boundaries
#
+ term2=dirichlet.default_dirichlet(0|K)@{channel/left,channel/right}
+ term3=dirichlet.default_dirichlet(0|Cl)@{channel/left,channel/right}
#
# Solve Poisson everywhere
#
+ term4=-1*box_div.lapflux(phi|phi)@{channel,protein,lipid}
+ term5=nodal.copy(K|phi)@{channel}
+ term6=-1*nodal.copy(Cl|phi)@{channel}
+ term7=nodal.copy(rho_fixed|phi)@{protein,lipid}
#
# Dirichlet B.C. for phi at left & right boundaries
#
+ term8=dirichlet.default_dirichlet(0|phi)@{channel/left,channel/right}
#
# constrain phi to be continuous across channel/protein/lipid interfaces
#
+ term9=constraint.continuity(phi|phi)@{channel/protein,protein/lipid,channel/lipid}
```

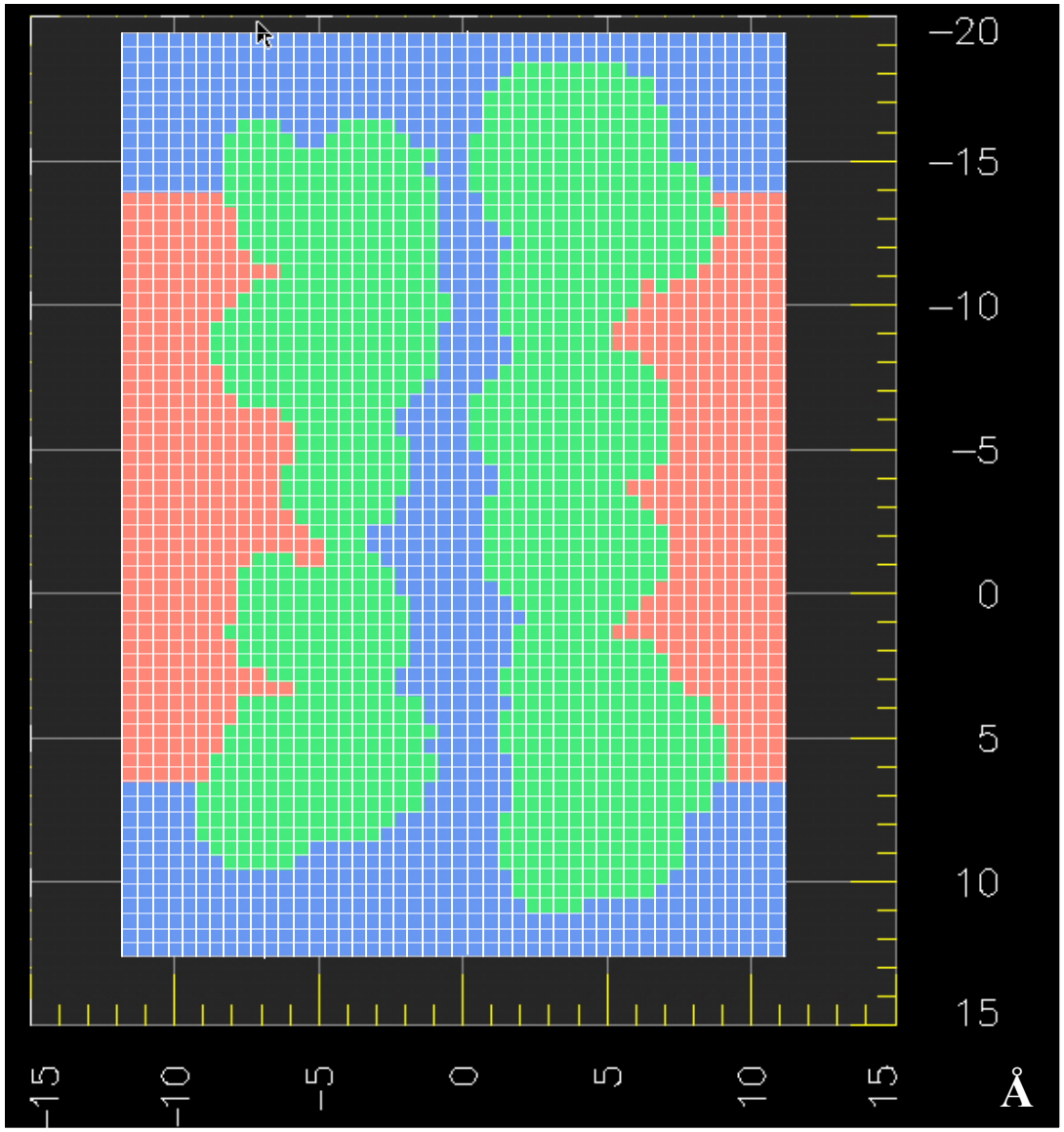
physical parameters & boundary conditions

```
# define the K in channel #

dbase createlist name=library/physics/channel/K
dbase prefix=library/physics/channel/K
dbase create name=background rval=1.0e-5
dbase create name=Cstar rval=1                # ?
dbase create name=esign rval=1                # ?
dbase create name=scale rval=1.0e-5           # ?
dbase create name=dirichlet.left rval=0.602214e6  # 100 mM
dbase create name=dirichlet.right rval=0.602214e6 # 100 mM
dbase prefix=""

... similarly for Cl ...

# define the phi in channel #

dbase createlist name=library/physics/channel/phi
dbase prefix=library/physics/channel/phi
dbase create name=scale rval=1.0e-7
dbase create name=background rval=0.0
dbase create name=Cstar rval=1
dbase create name=Dix rval= 0.4425e10         # Dix = eps/q eps = 80*eps0
dbase create name=dirichlet.left rval=0.0     # zero bias
dbase create name=dirichlet.right rval=0.0
dbase prefix=""

... similarly for phi in protein and lipid ...
```

```
field set=rho_fixed profile3d="porin_charge"
field set=K_mobility profile3d="K_mobility"
field set=Cl_mobility profile3d="Cl_mobility"
field set=tl val=300

# dump out flux data
field set=flux solvar=K type=edge
field set=flux solvar=Cl type=edge

# print out the Newton convergence behavior
dbase create name=/options/loops ival=1

solve system=pnp
save pas=porin.0mV.pas

dbase prefix=library/physics/channel/phi
dbase modify name=dirichlet.left rval=0.0
dbase modify name=dirichlet.right rval=0.01    # bias 10 mV
dbase prefix=""
solve system=pnp
save pas=porin.10mV.pas
```

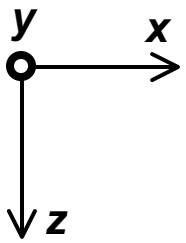
physical parameters

solve system & write output

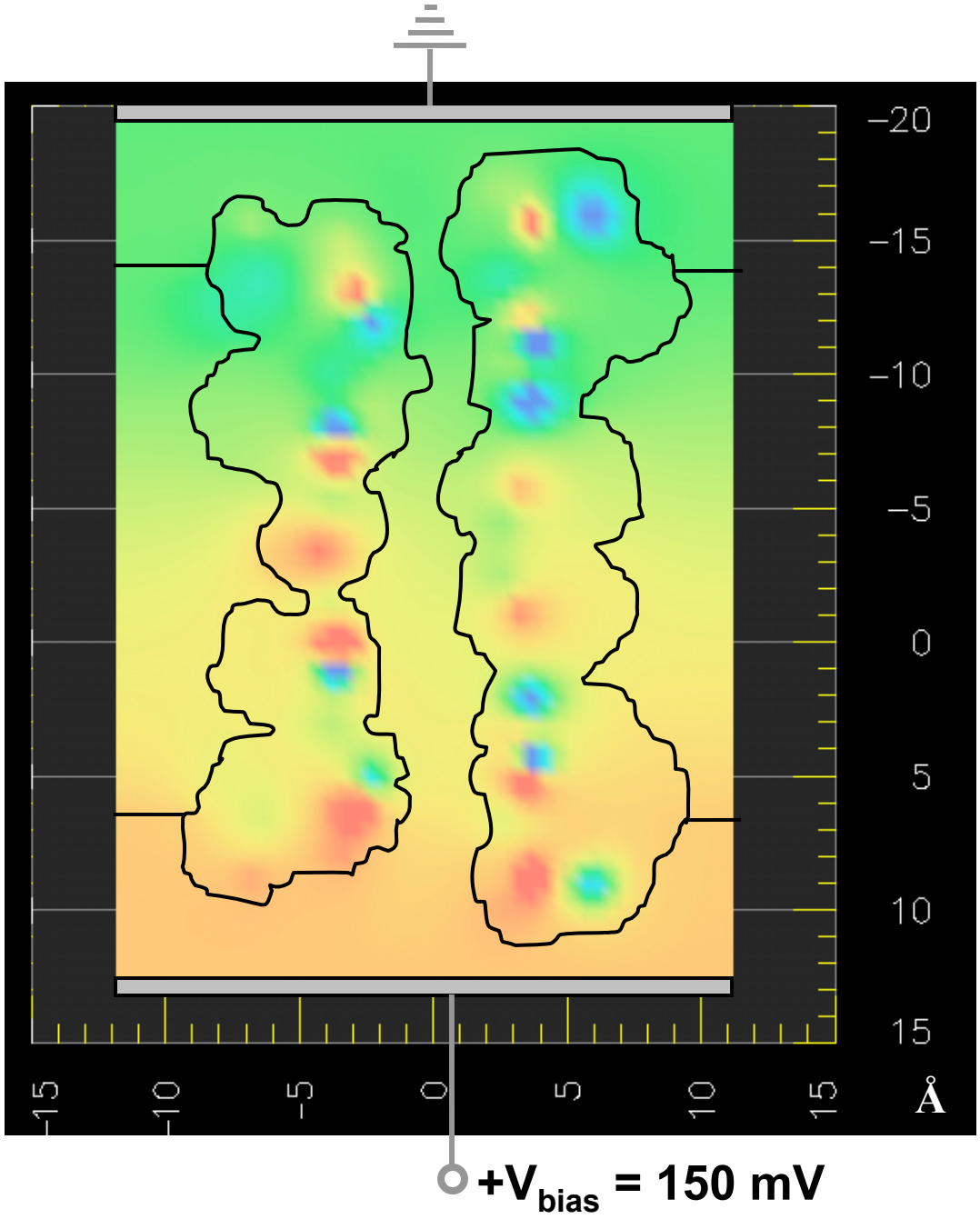


GRAMICIDIN

Lipid	$\varepsilon_r = 2$
Protein	$\varepsilon_r = 20$
Solution bath (NaCl)	$\varepsilon_r = 80$



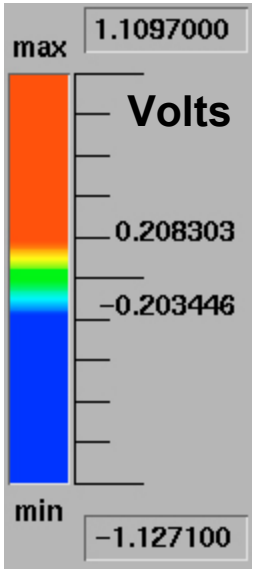
Slice through the channel of the 3D mesh used in PROPHET. (48x48x64 grid points, 0.5Å mesh spacing)

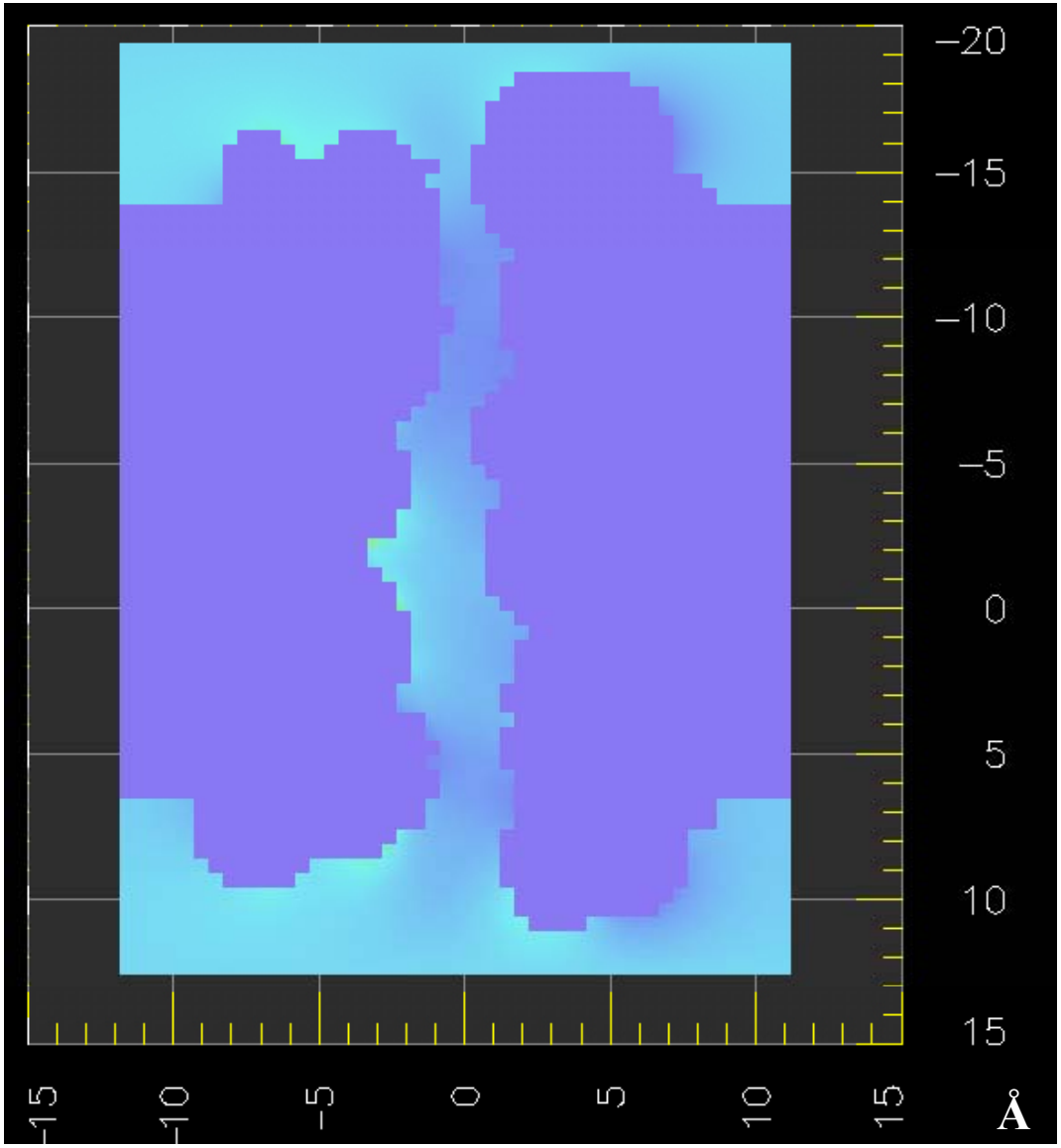


GRAMICIDIN
1.0 molar NaCl solution

Potential distribution, on a slice throught the channel.

Large positive and negative potential spikes in the protein region are due to the fixed charges.





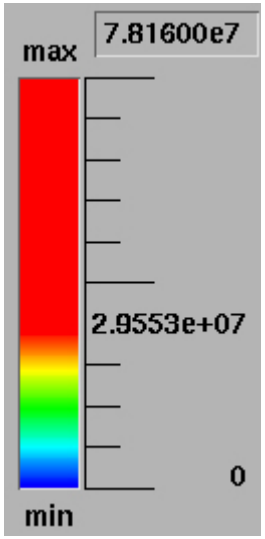
GRAMICIDIN
3-D PROPHET SIMULATION

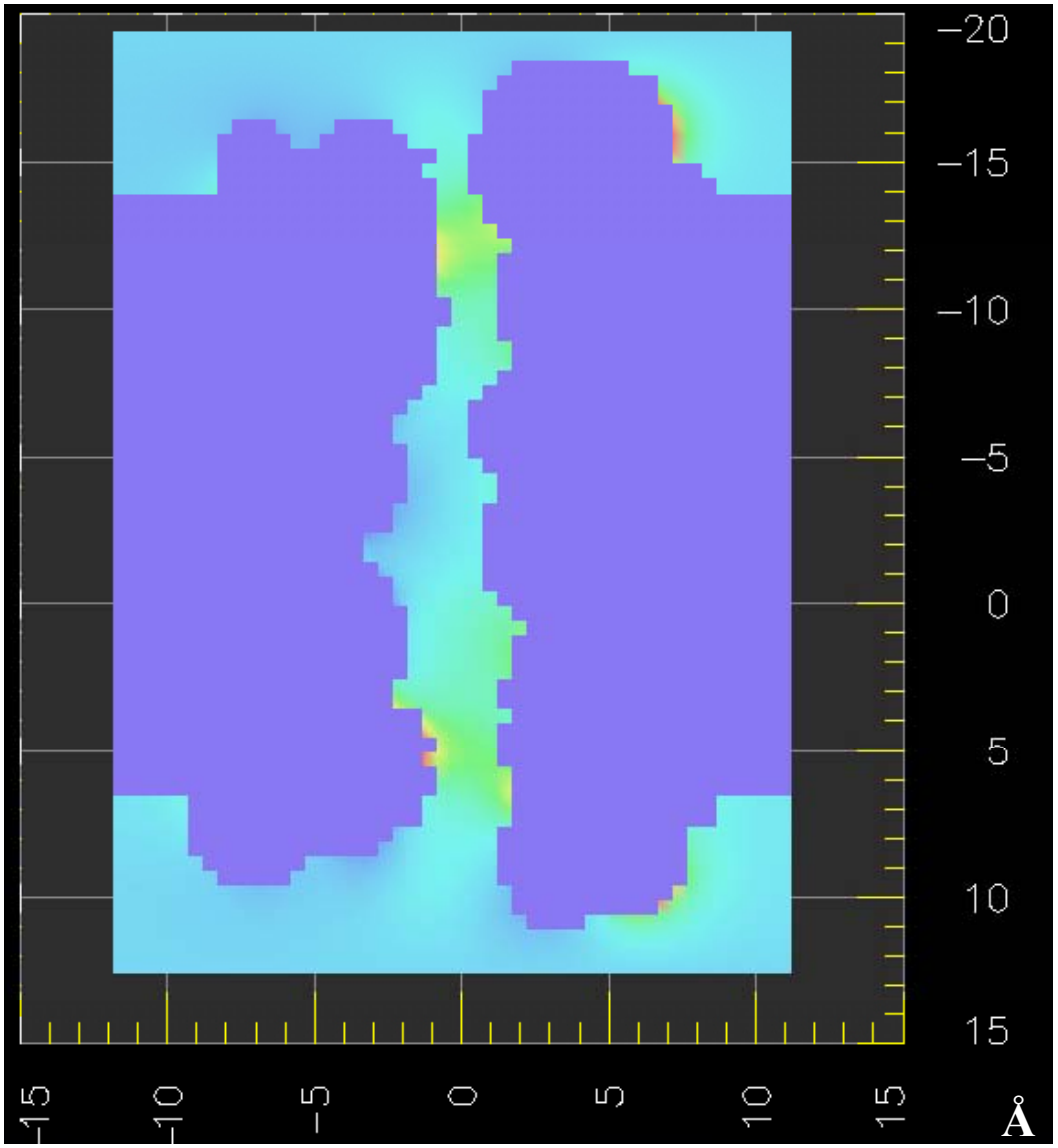
Cl^- Anion distribution

BIAS = 150 mV

Bath solution = 1.0 molar NaCl

$6.02 \times 10^6 = 1$ molar





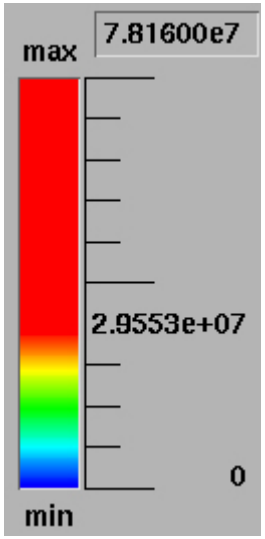
GRAMICIDIN
3-D PROPHET SIMULATION

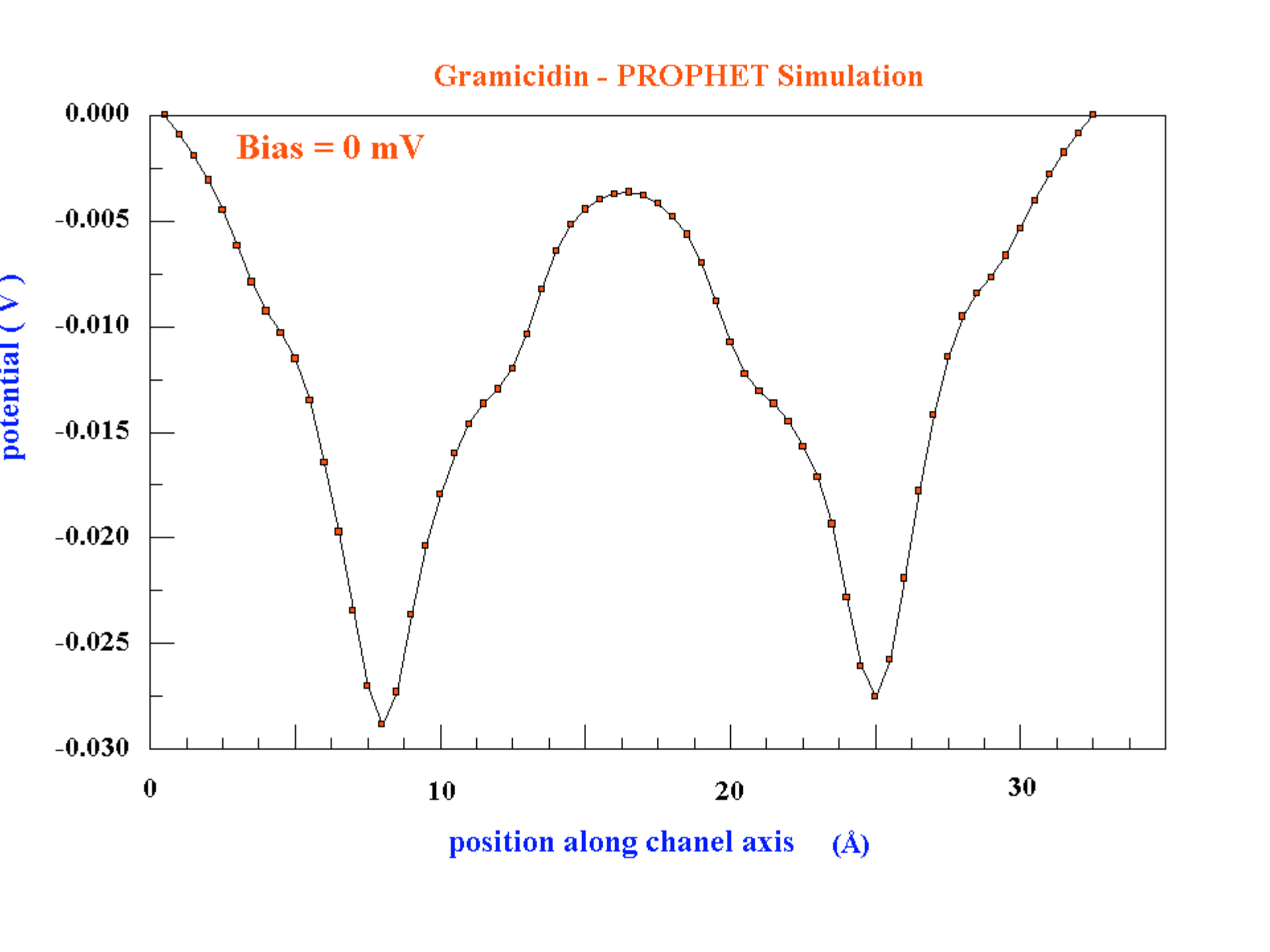
Na^+ Cation distribution

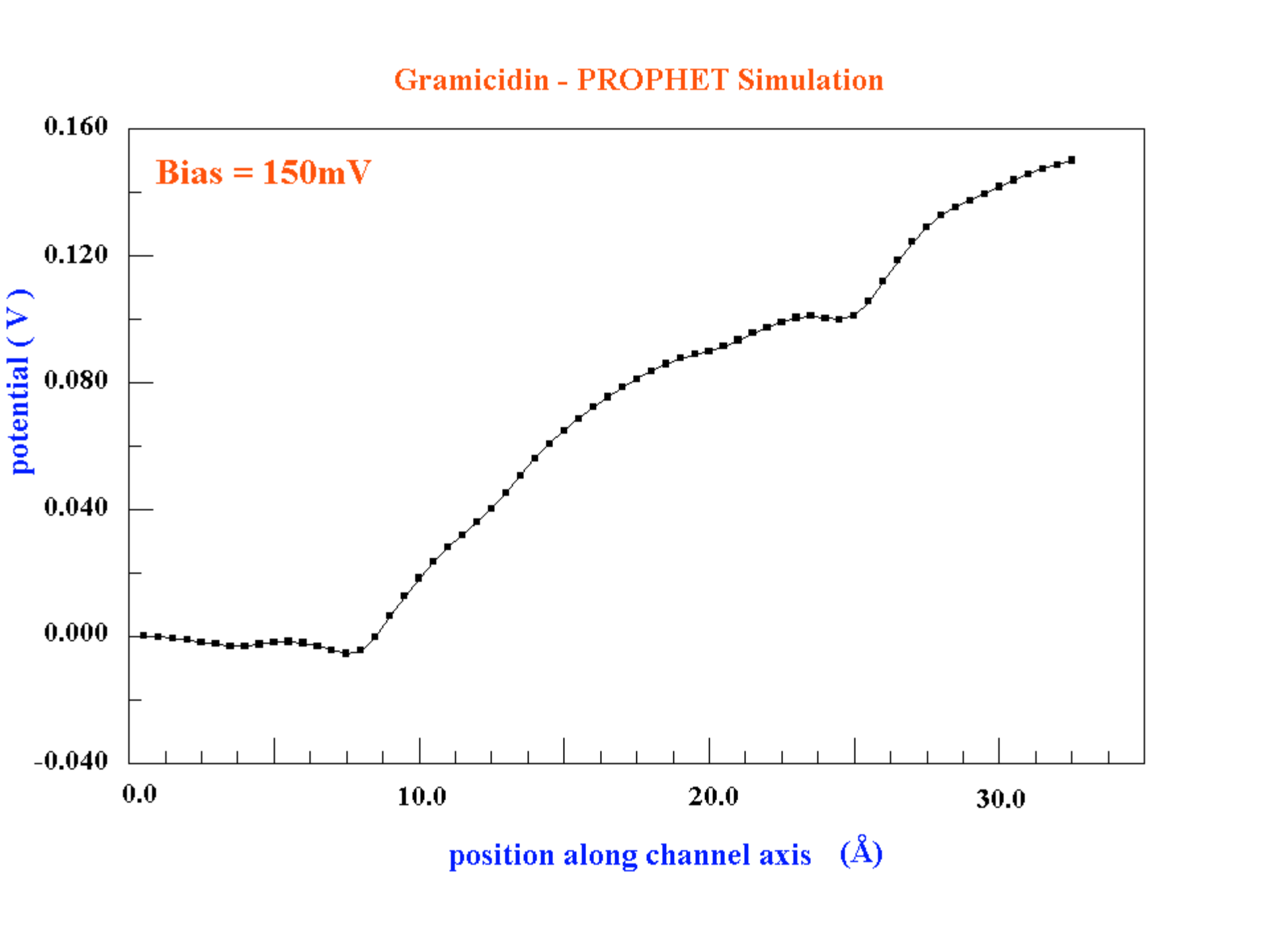
BIAS = 150 mV

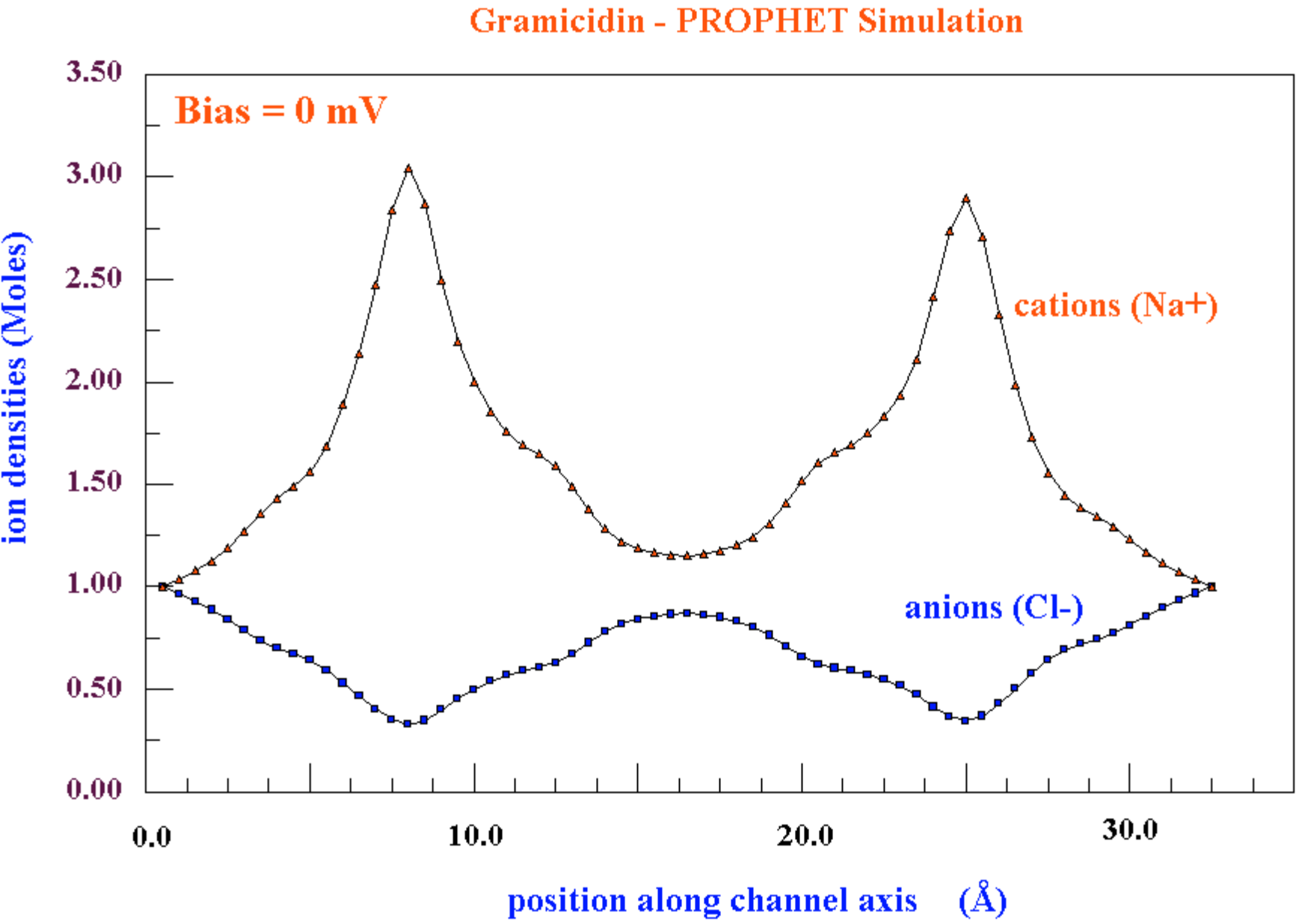
Bath solution = 1.0 molar NaCl

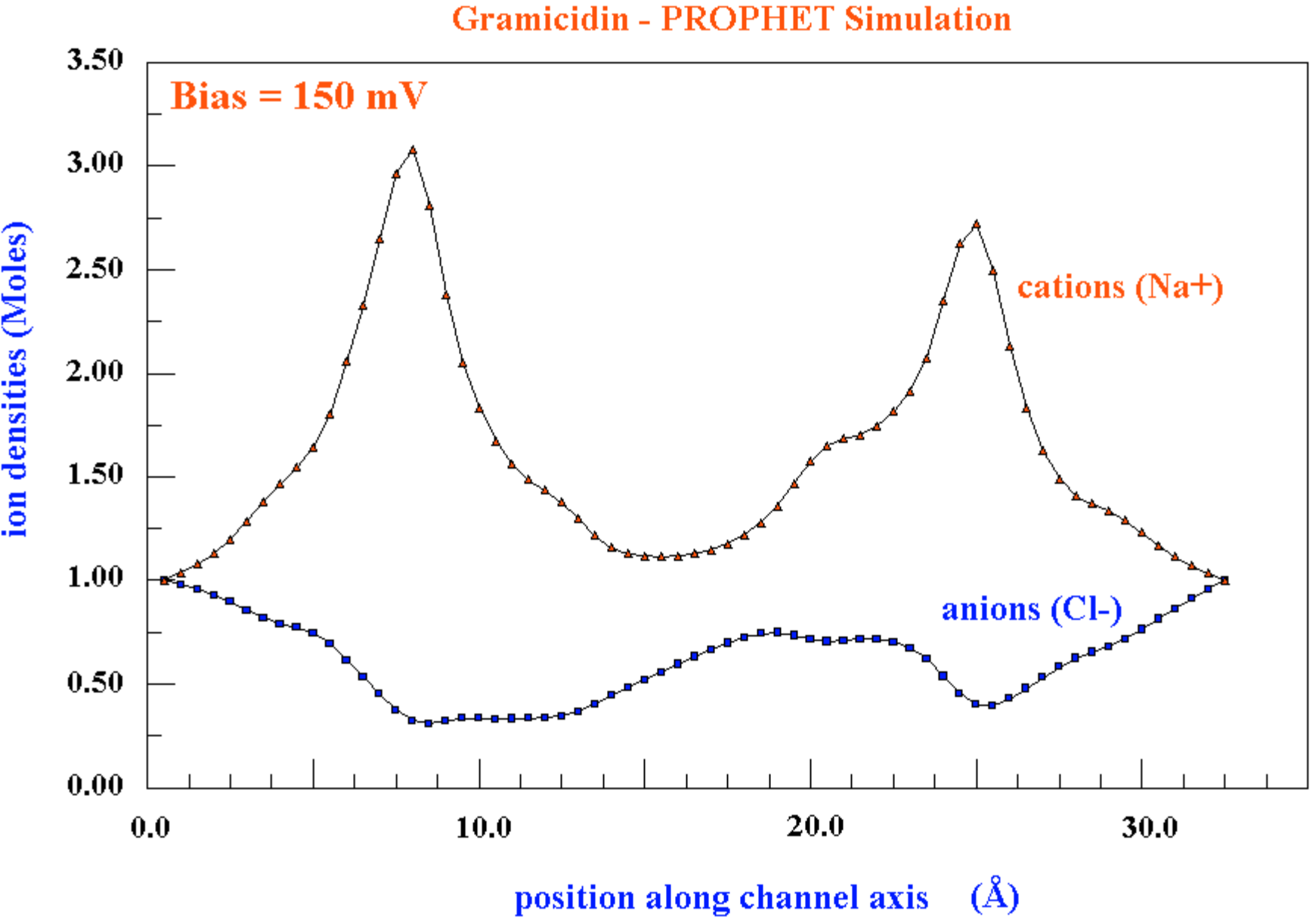
$6.02 \times 10^6 = 1$ molar



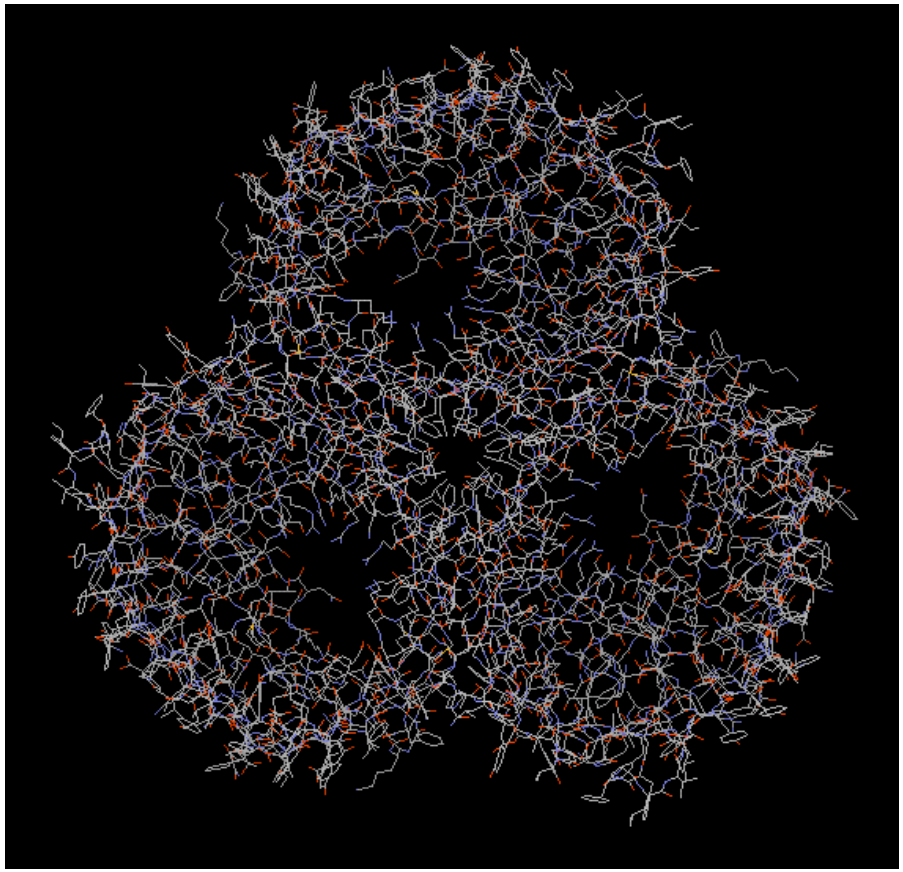

Gramicidin - PROPHET Simulation
Bias = 0 mV
0.000
-0.005
-0.010
-0.015
-0.020
-0.025
-0.030
potential (V)
0
10
20
30
position along chanel axis (Å)


Gramicidin - PROPHET Simulation
Bias = 150mV
0.160
0.120
0.080
0.040
0.000
-0.040
potential (V)
0.0
10.0
20.0
30.0
position along channel axis (Å)


Gramicidin - PROPHET Simulation
Bias = 0 mV
cations (Na+)
anions (Cl-)
ion densities (Moles)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
0.0
10.0
20.0
30.0
position along channel axis (Å)

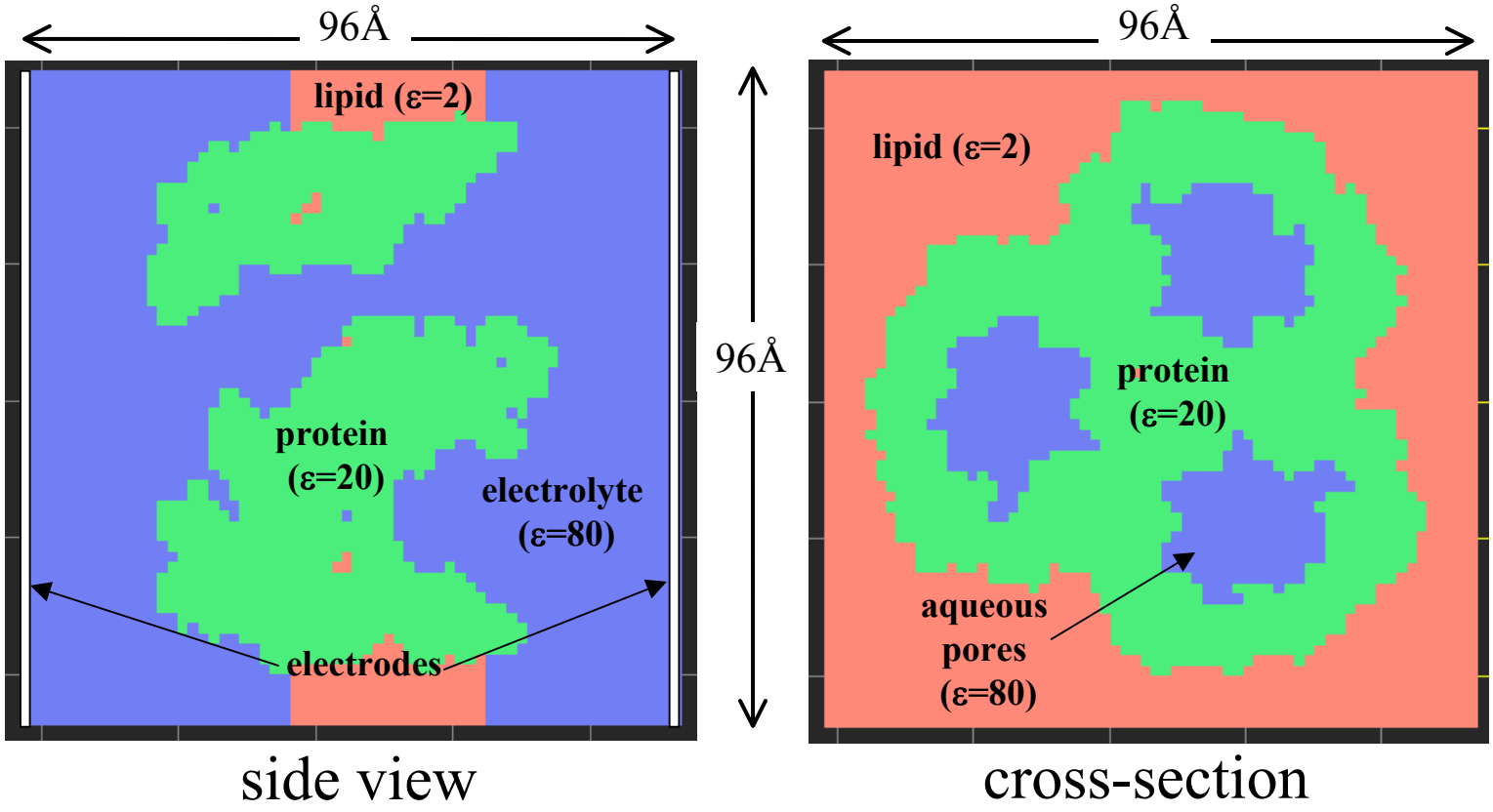

Gramicidin - PROPHET Simulation
Bias = 150 mV
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
ion densities (Moles)
cations (Na+)
anions (Cl-)
0.0
10.0
20.0
30.0
position along channel axis (Å)

ompF (OUTER MEMBRANE PORIN)

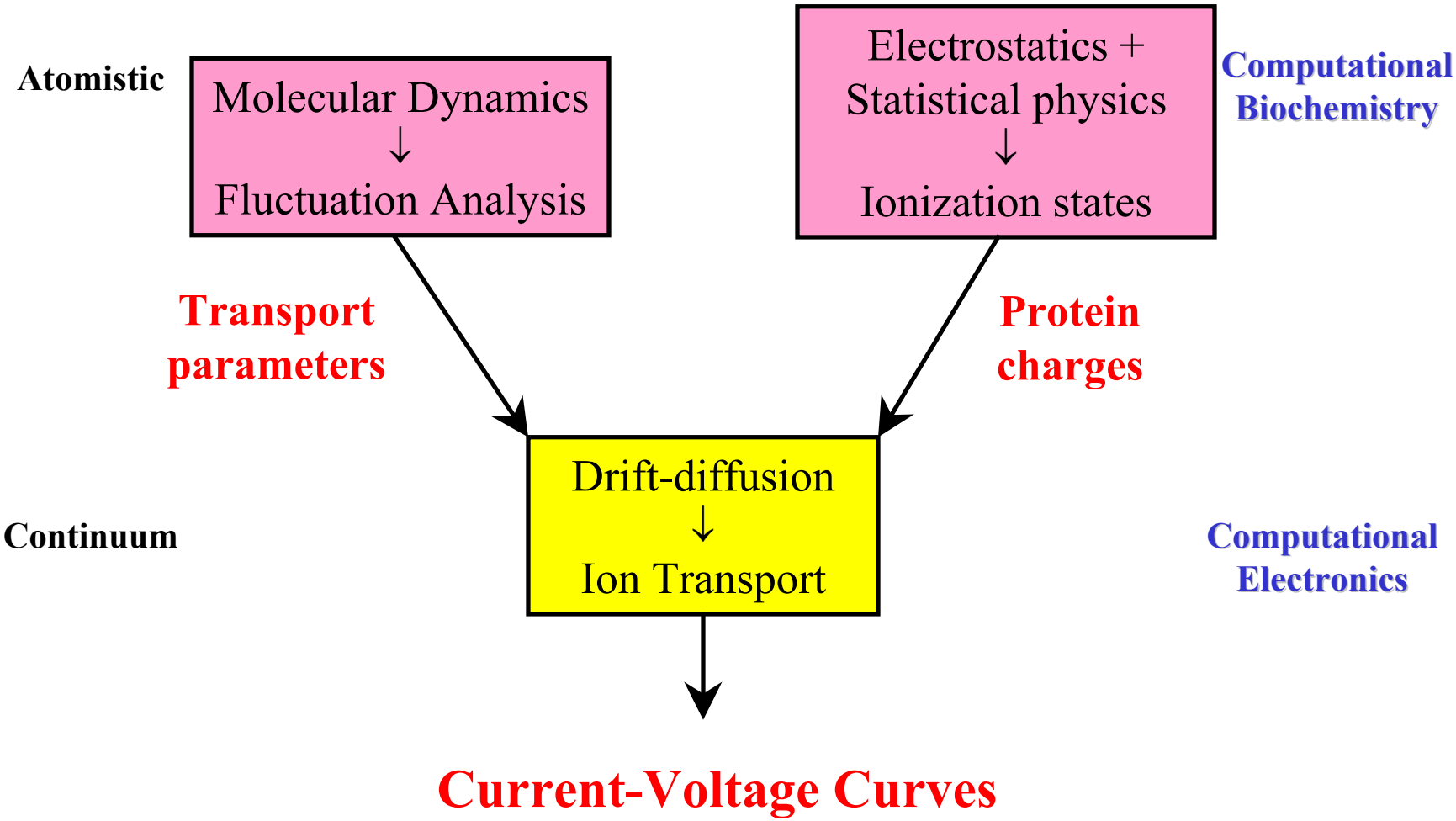


- **Trimeric** protein that resides in the outer membrane of *e-coli* bacterium.
- Well-known, **very stable** structure
- Net charge of ~ -30|*e*|
  Highly charged pore constriction.
  Moderate cation selectivity.
- Unknown gating mechanism
- Can be **mutated**.
  Ideal for experimental and simulation studies of ion permeation.
  Template for biodevices

PROPHET MESH - PORIN

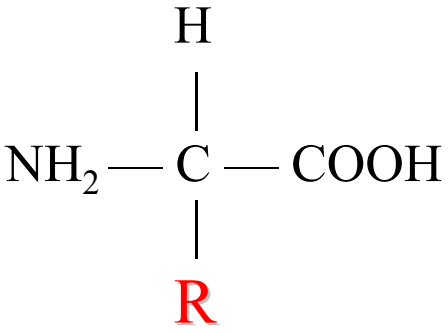


COMPUTATIONAL BIOCHEMISTRY



CALCULATING PROTEIN CHARGES

(Jakobsson group)



Amino acids …

Side chains are **ionizable** P_{ion}

P_{ion} depends on local electric field,

salt concentration and pH

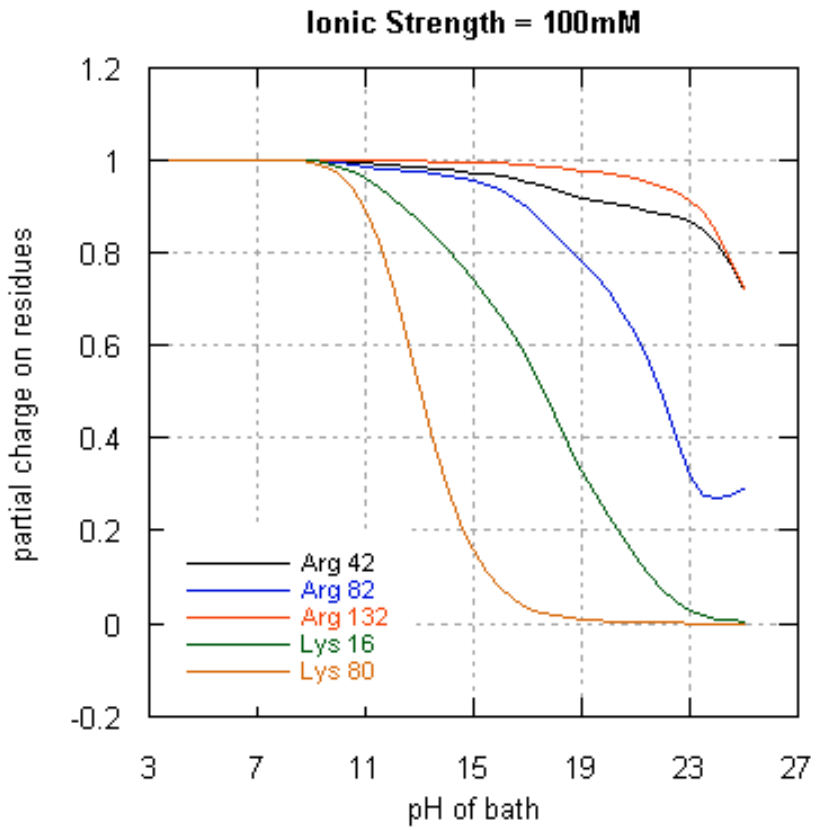
How to compute P_{ion} inside the folded protein?

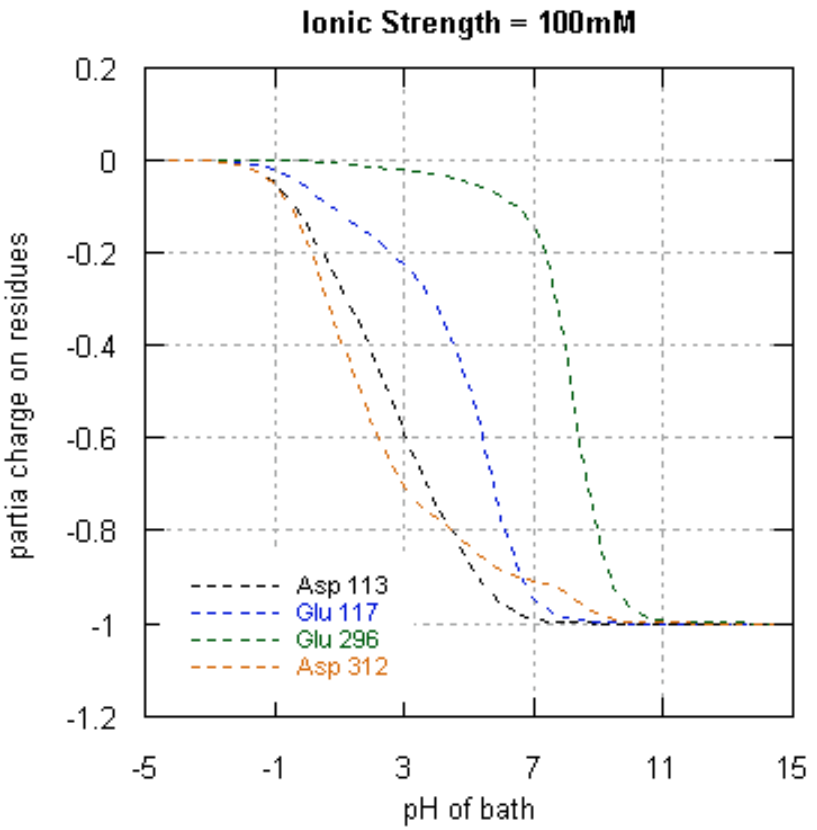
1. Δ(Free Energy): amino acid in $H_2O \rightarrow$ amino acid in protein

   Nonlinear Poisson (Poisson-Boltzmann) equation (UHBD)

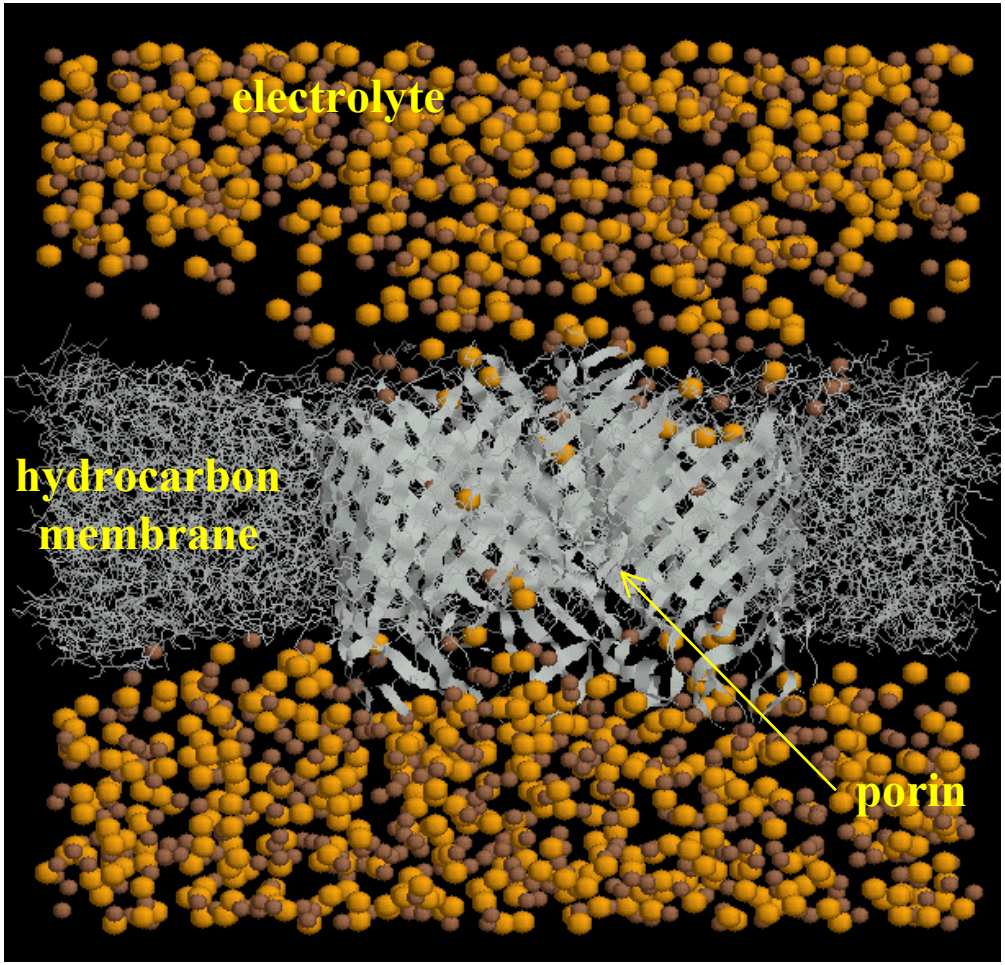
2. Effect of interaction of amino acids with each other

IONIZATION STATES FOR ompF PORIN





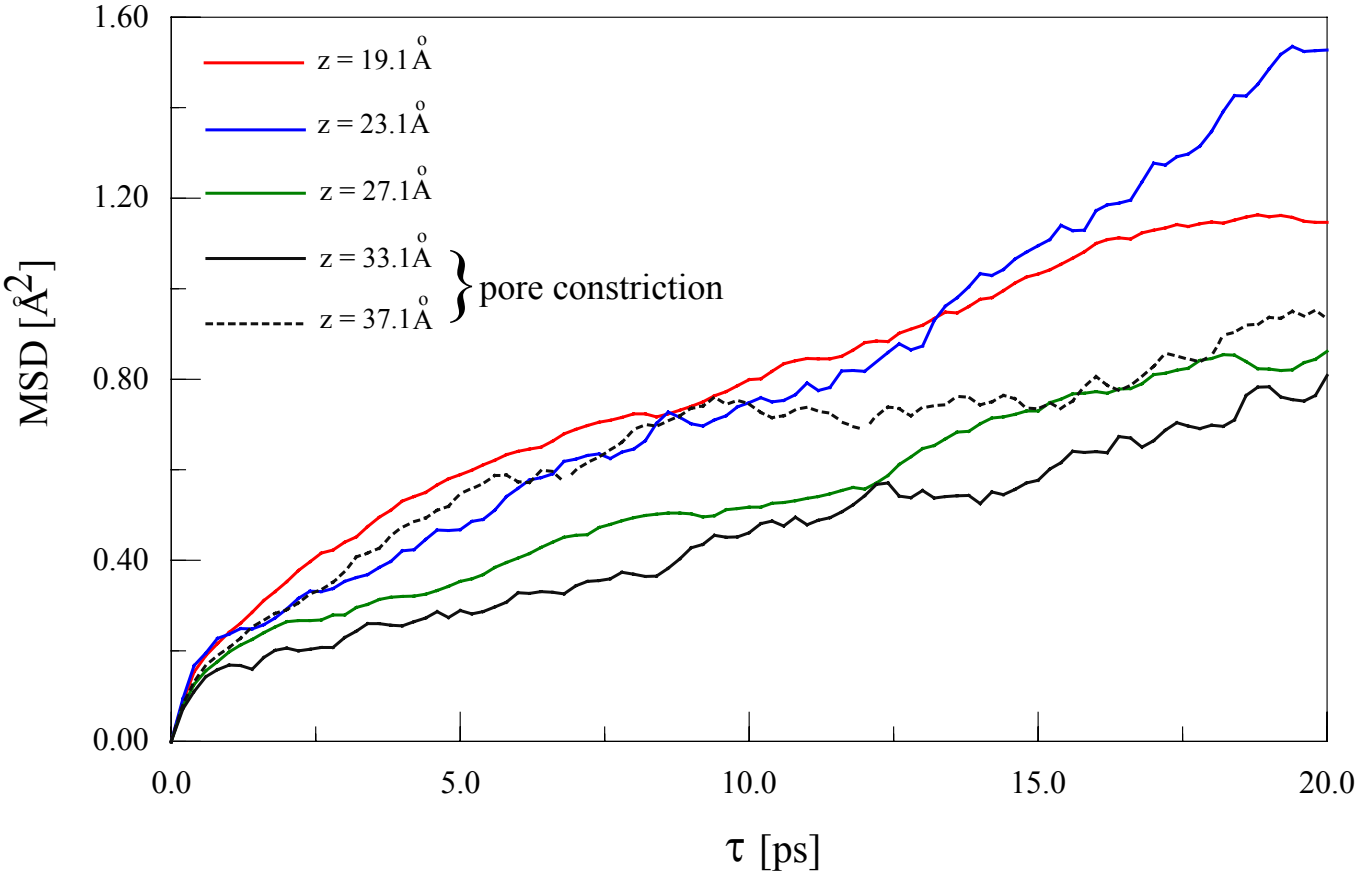
MOLECULAR DYNAMICS (Jakobsson group)



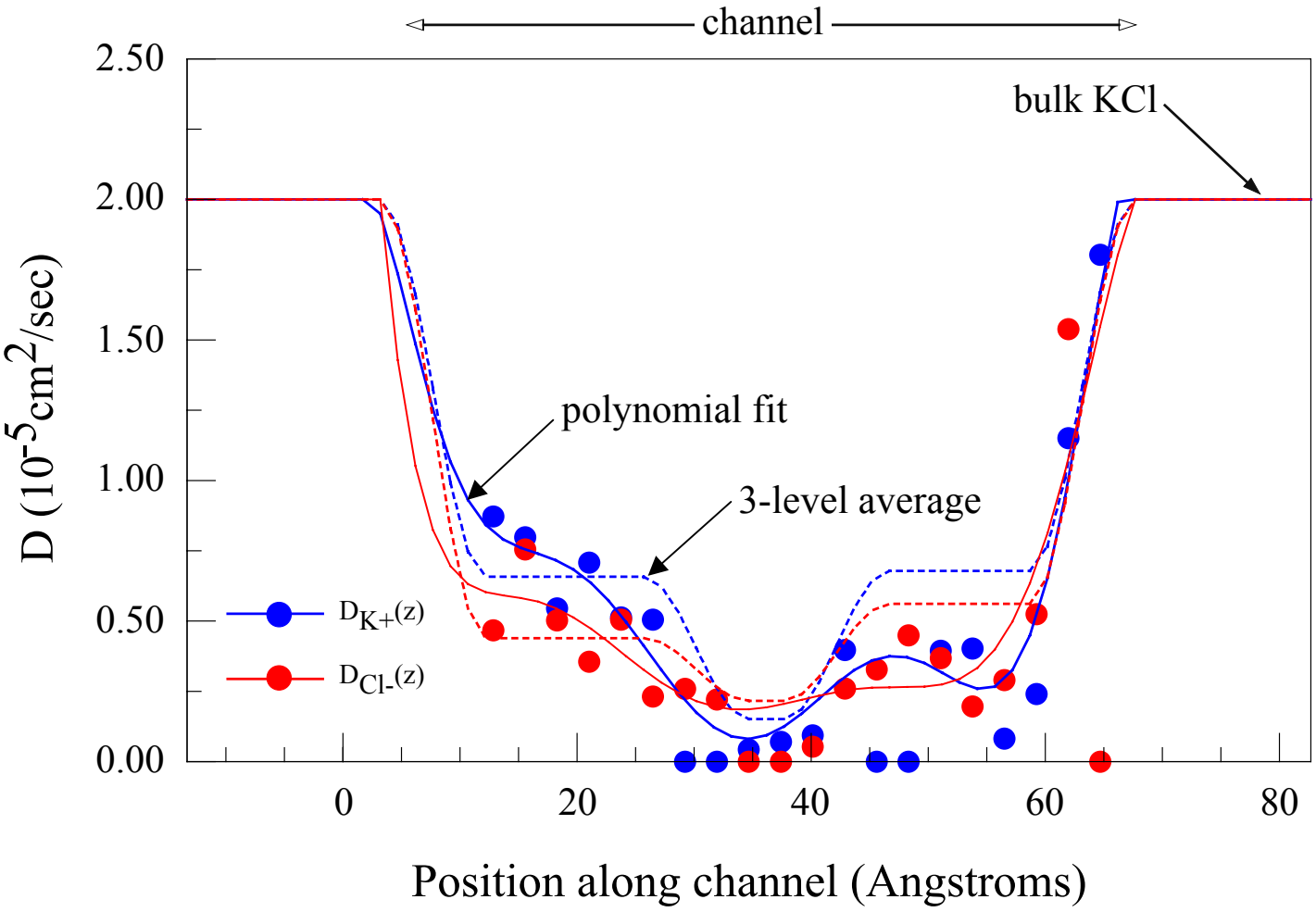
- System is represented by charged balls (atoms) connected by springs (bonds)
- System is initialized with a Maxwellian distribution and allowed to evolve according to Newtonian mechanics.
- Gromacs - efficient, scalable open source MD package

ESTIMATING DIFFUSIVITY FROM ION TRAJECTORIES

$$\mathrm{MSD}(\tau) = \left\langle (x(t+\tau) - x(t))^2 \right\rangle = 6D\tau$$

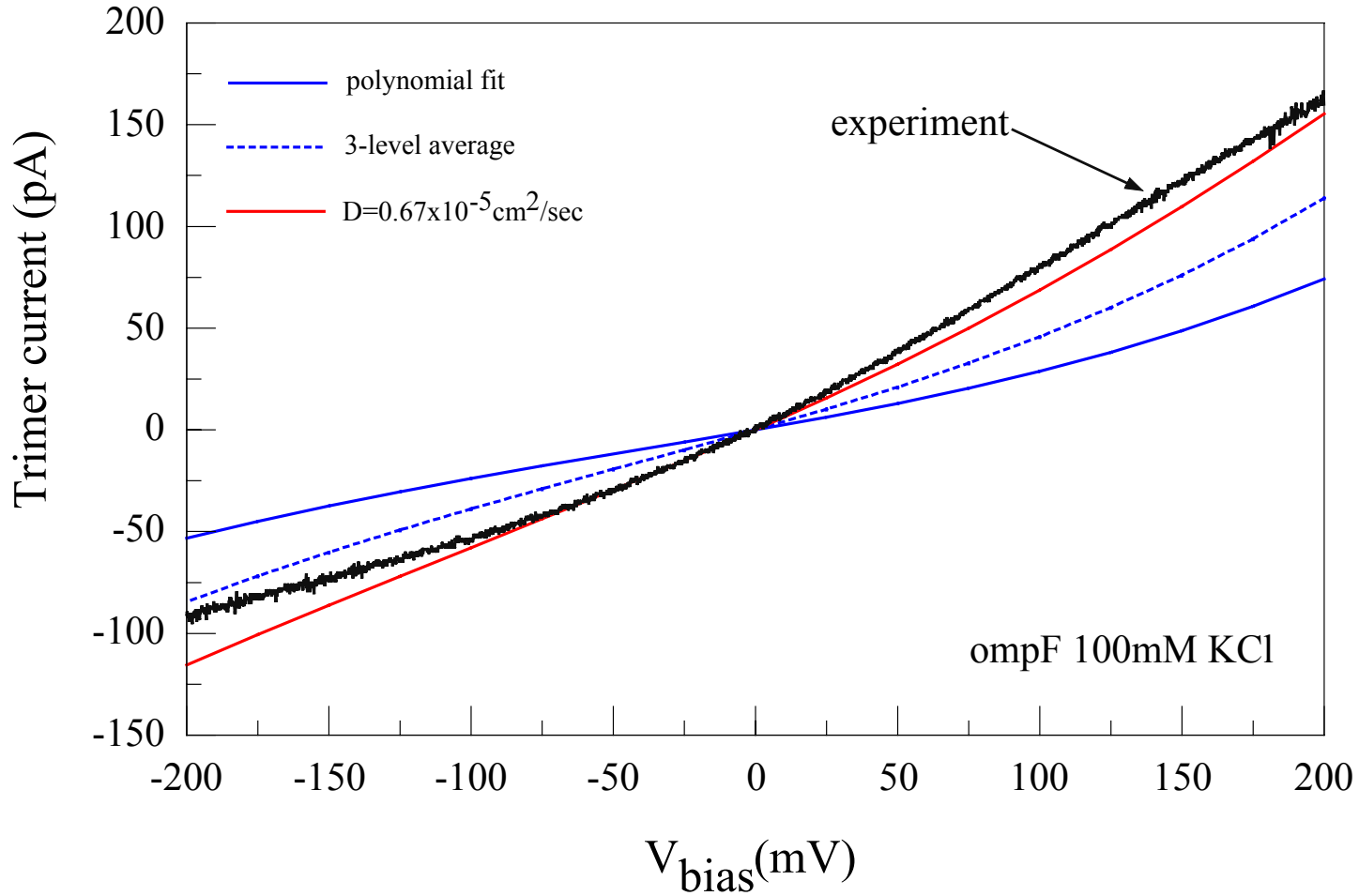


DIFFUSIVITY INSIDE CHANNEL

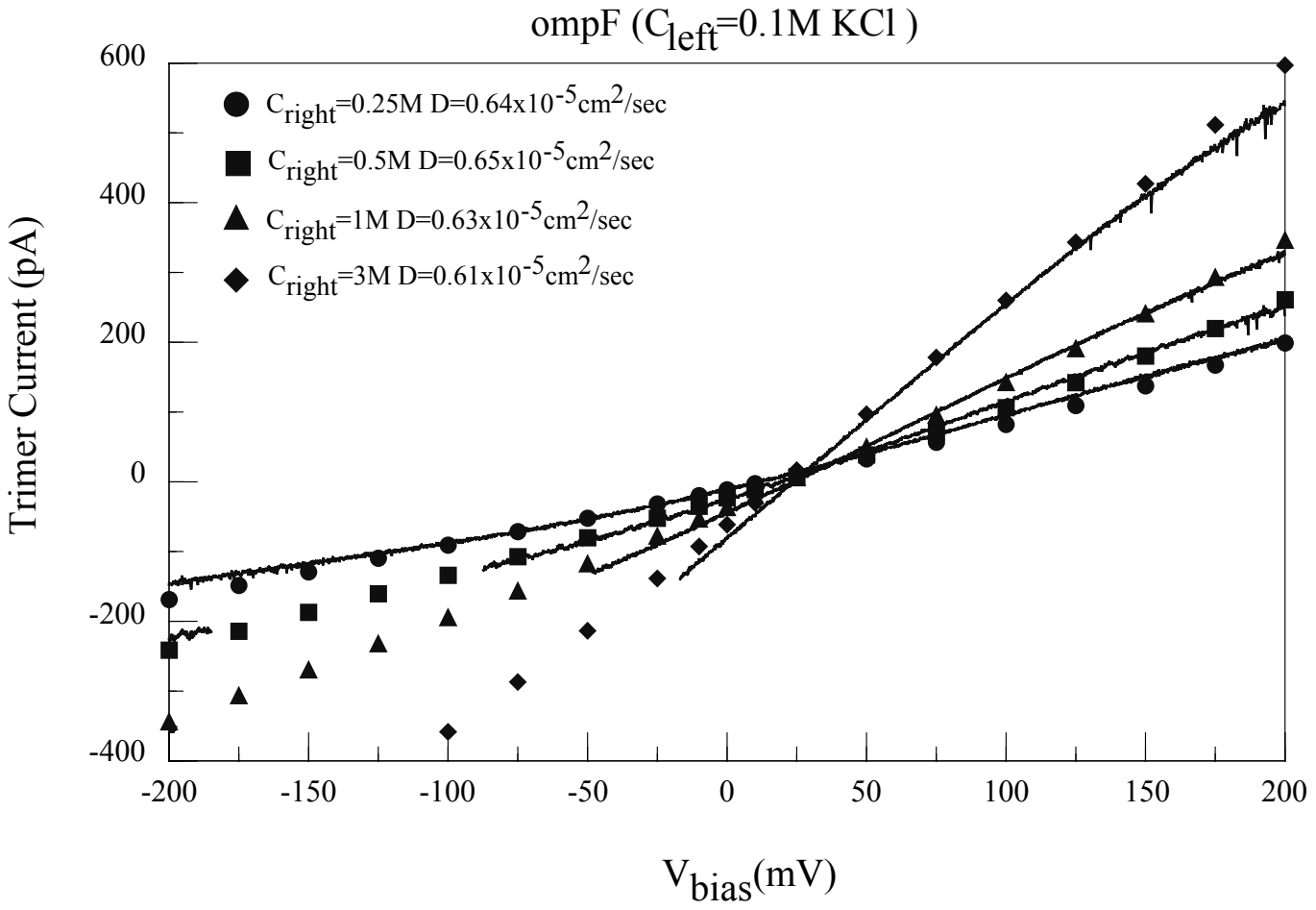


CURRENT-VOLTAGE CURVES

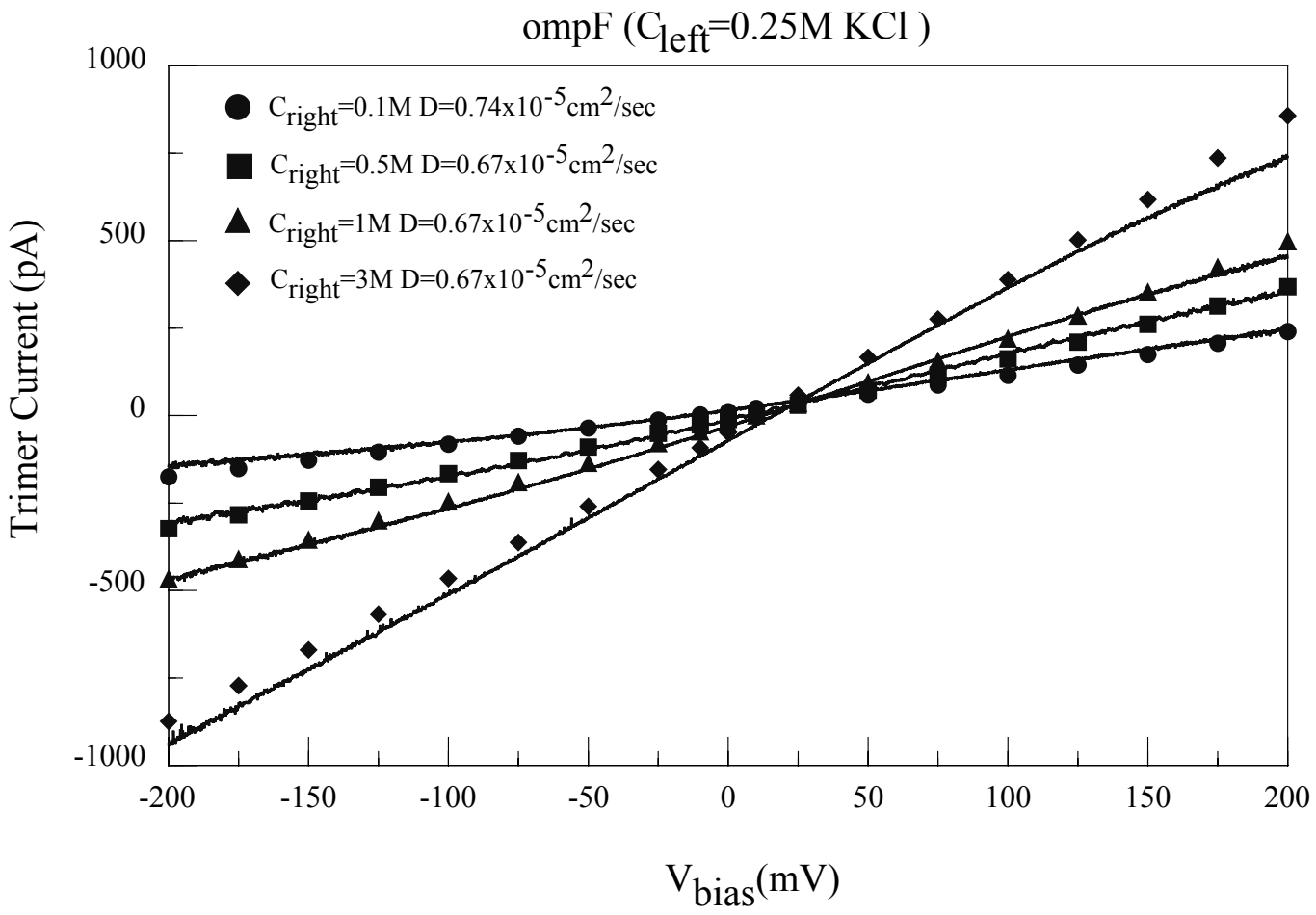
experiment (Rush) vs. simulation (UIUC)



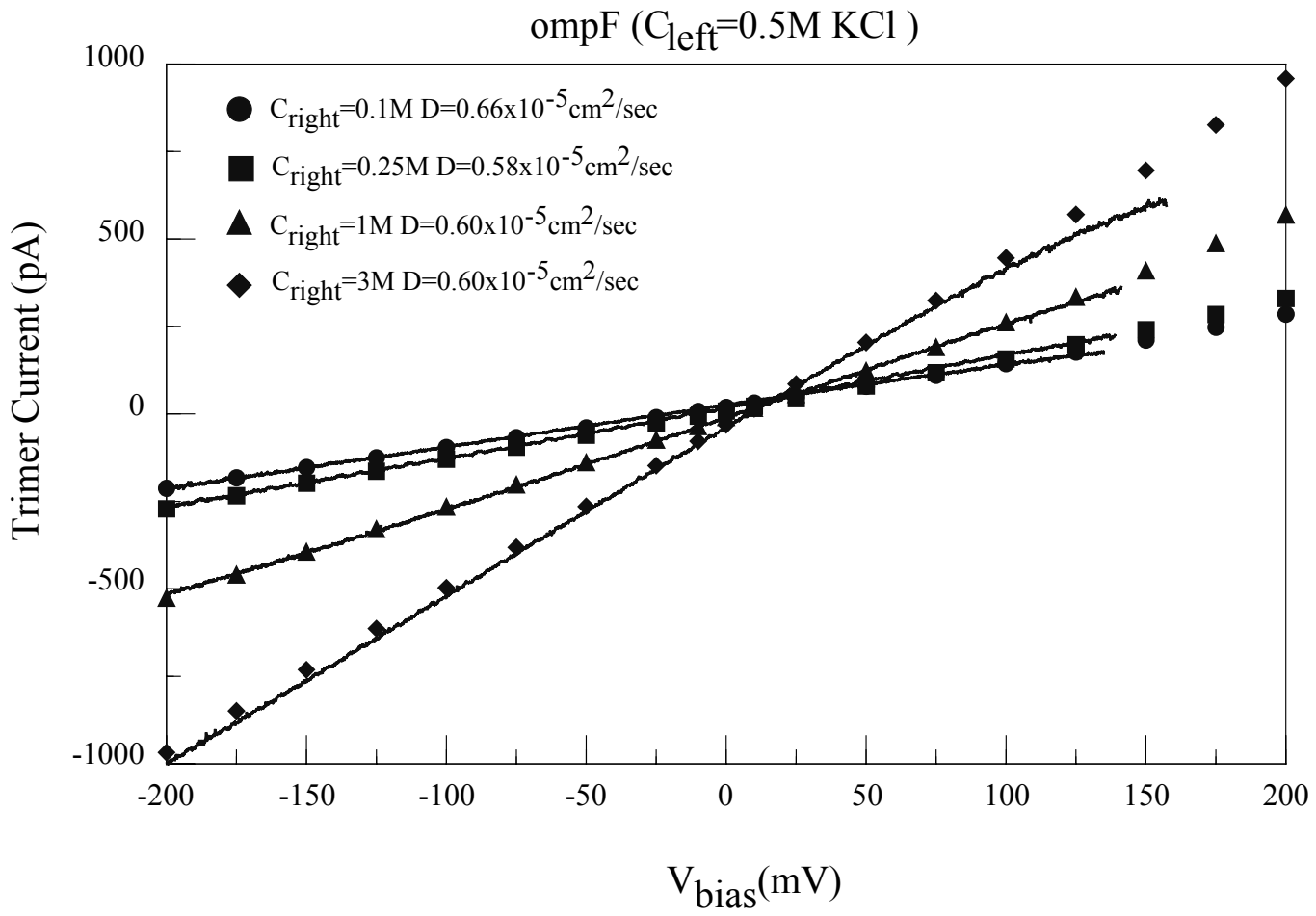
ASYMMETRIC BATH CONCENTRATIONS



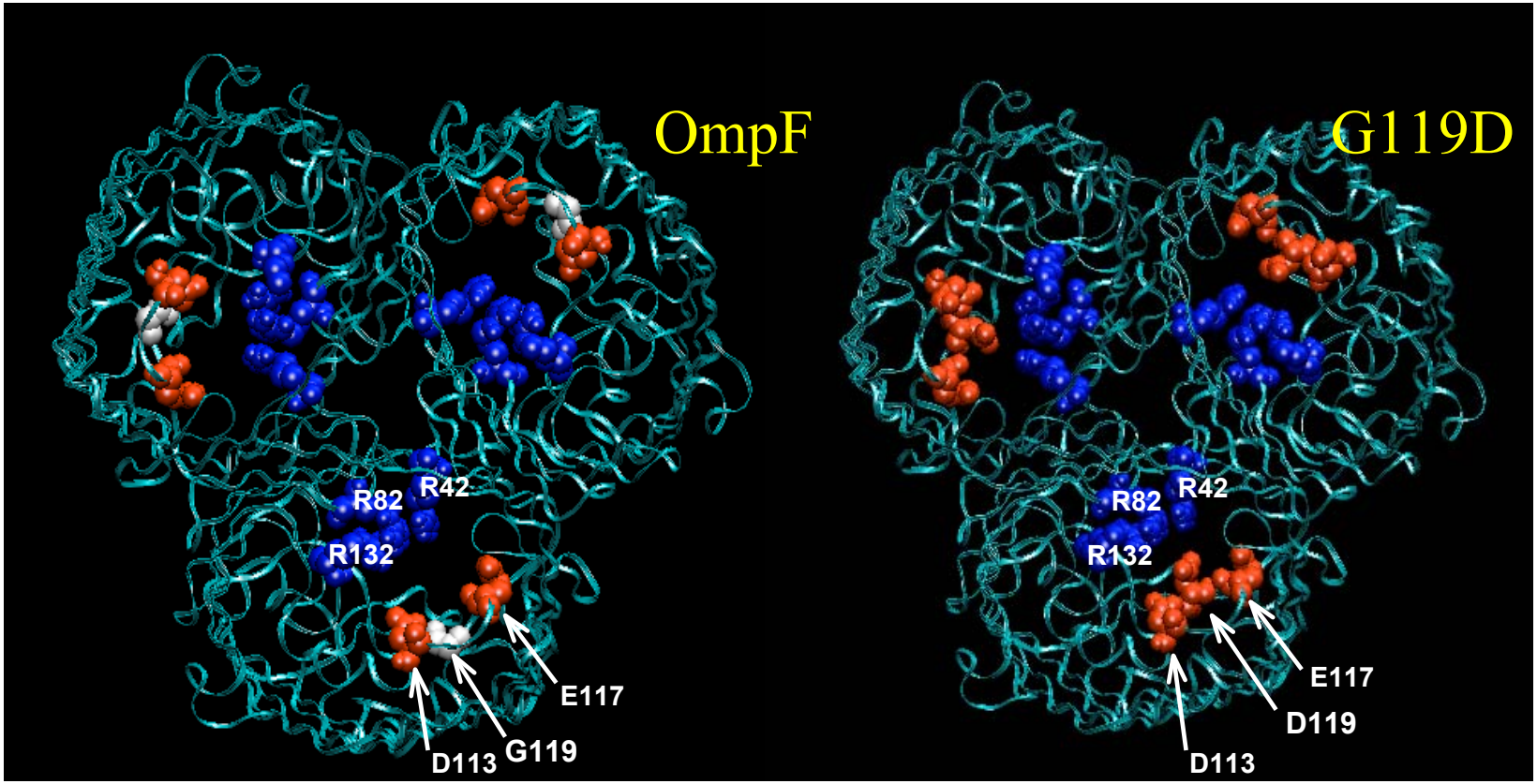
ASYMMETRIC BATH CONCENTRATIONS



ASYMMETRIC BATH CONCENTRATIONS



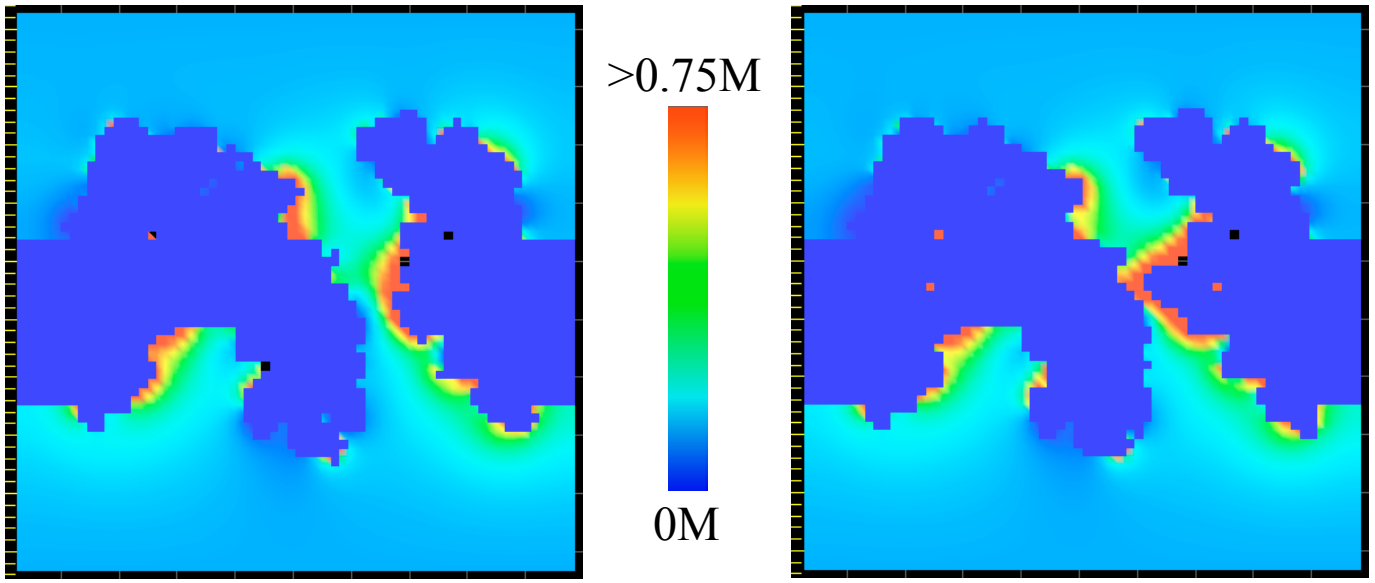
STUDYING MUTATIONS WITH PROPHET



OmpF → G119D: replace an uncharged glycine G119 (white), located in the pore constriction, with an aspartate D119 (red).

(visualization – VMD: http://www.ks.uiuc.edu/Research/vmd/)

EQUILIBRIUM K^+ DENSITY

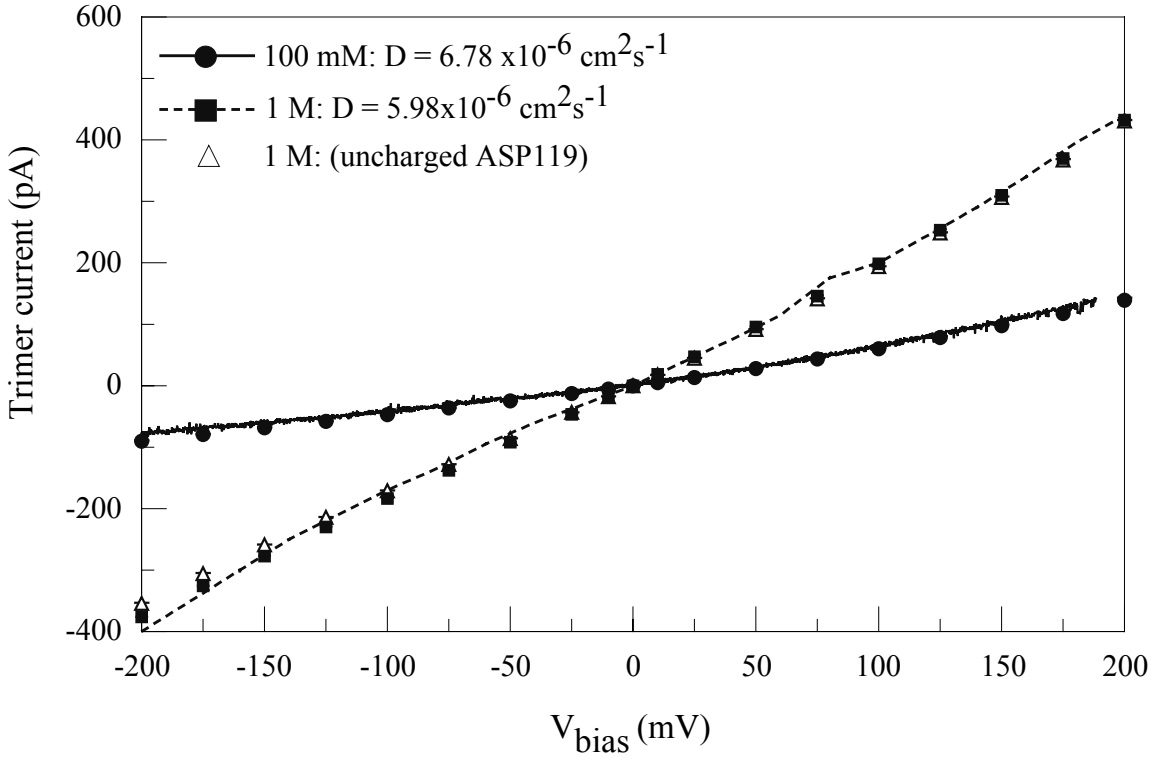


ompF

Mutation G119D

Effect of mutation: narrower channel, lower conductance (15-40% lower), higher cation selectivity

Separate geometric & electrostatic effects of the mutation by scaling down the charge distribution on the aspartate to zero - 'virtual' channel that is structurally identical to *G119D* but has the same charge as ompF.



Simulations at 1M reveal no change in the conductance.

Cation selectivity reduced

CONTINUUM SIMULATIONS – SUMMARY

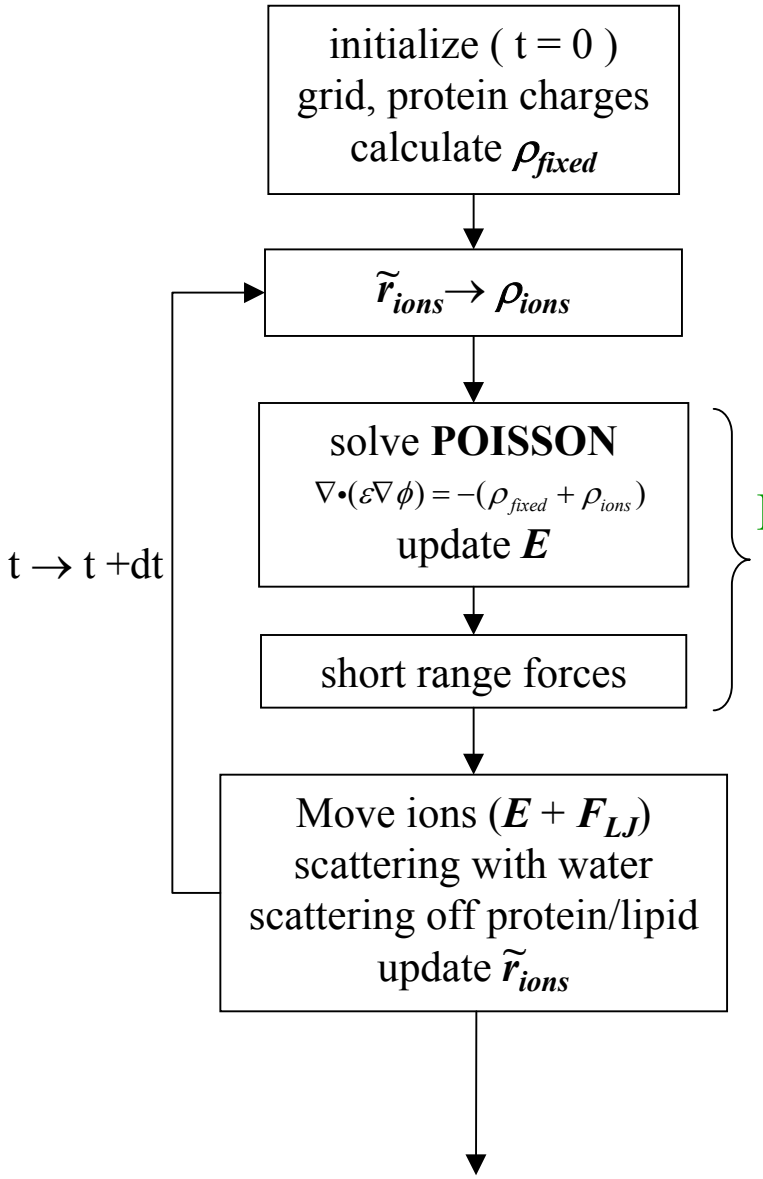
- Combine existing methods of computational biochemistry and computational electronics to study ion channels.
- Current-voltage characteristics computed for *ompF* in *KCl* agree reasonably well with experiments.
- Conductance sensitive to the diffusion coefficient profile, especially in the pore constriction
- Disagreement attributed to uncertainty in the diffusion coefficient and finite ion volume effects – work in progress

MONTE CARLO PARTICLE SIMULATION

• Particle trajectories are resolved in 3D. Electrostatic forces calculated self-consistently from Poisson equation (P^3M)

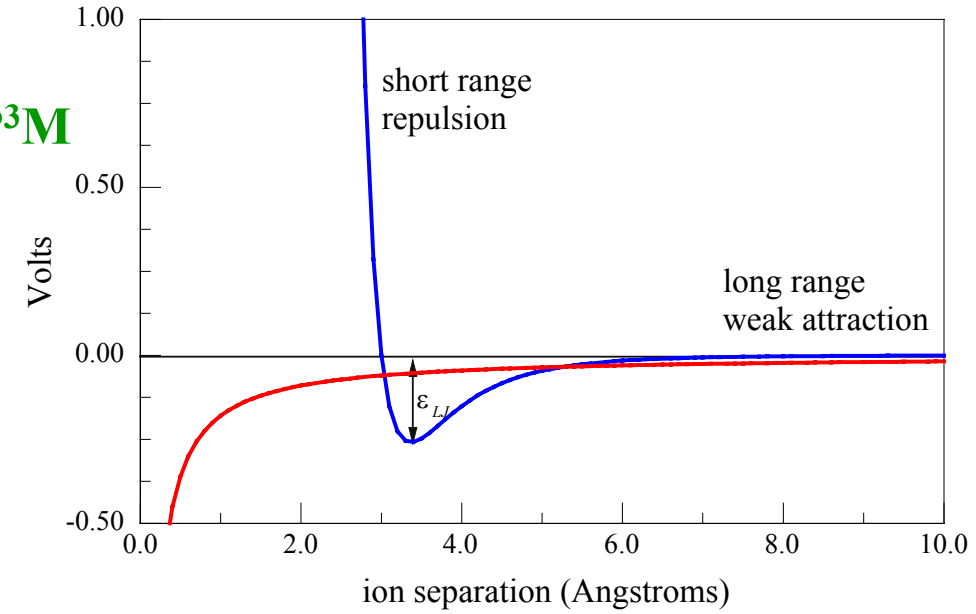
• Water is assumed to be a **continuum background** with a given permativvity ε. Interaction between ions and water is accounted for by a scattering rate. Flight-times between collisions are generated statistically from an average scattering rate. Scattering events are assumed to thermalize the ions.

• **Finite size of the ions** is accounted for by associating a radius and a Lennard-Jones potential to the ions.

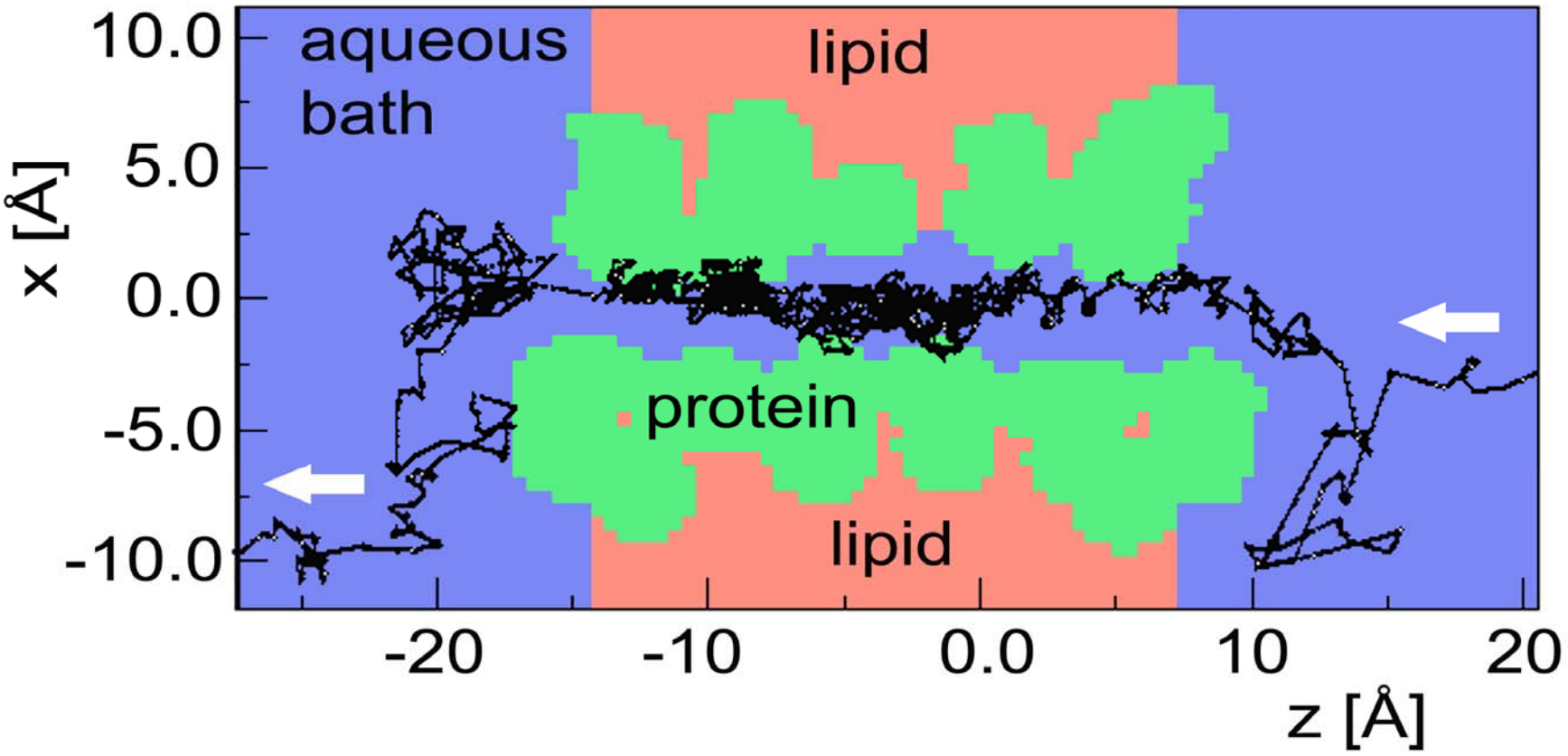


Lennard Jones 6-12 potential

$$\varphi_{LJ} = -4\varepsilon_{LJ}\left(\left(\frac{\sigma}{r}\right)^{6} - \left(\frac{\sigma}{r}\right)^{12}\right)$$



GRAMICIDIN CHANNEL



Na^+ trajectories that cross the channel are rare events

CHALLENGES

Poisson→ computational bottleneck
Small ensemble size (1M, 28000 Å^3 → ~ 40 ions) Coulomb (N^2 = 1600) is much faster than Poisson (N_{mesh}= 28000)
How to evaluate $F_{coulomb}$ when ions are separated by $\varepsilon(r)$?
Use a coarser mesh and include correction for short-range forces?
OK for baths – but close to protein need to have dielectric interface resolved properly. Coarse mesh also alters the channel cross-section and crossing probability.
→ 2 grids (fine grid for defining ion accessible volume, coarser grid for fields)
→ non-uniform, non-rectilinear mesh

Channel crossings are rare events (1pA → 6 ions crossing per μs)
Extended-ions (spheres) greatly reduces the probability of ion crossing channel – most CPU spend tracking ions that don't go anywhere near the channel mouth.
High ion-water scattering rate 10^{13} to 10^{14} Hz, $\Delta t < 10$fs so need about 10^8 Δt (1GHz processor, say $\Delta t \rightarrow$ 1s, 1 μs → 1yr)

Boundary conditions – what to do when ion hits electrode? Ion injection from electrodes? How far away from the protein do we need to put the electrodes?

Ions are not point charges

Ion-protein/lipid interaction modeled as hard-wall potential – ions within one ionic radius of protein/lipid surface are scattered randomly.

How is ionic charge distributed on the ion? How is this charge distribution mapped to grid? How to handle the short-range correction?

Test trajectories in *prescribed* field (PROPHET)
bias = 150 mV, 100 mM NaCl, 1μs simulation time

ions modelled as point charges: ~ 25 % current carried by Cl^-
ions modelled as "hard" spheres: < 4 % current carried by Cl^-
experimentally: Cl^- is zero

Simple 1-D PNP (drift-diffusion) solver for ionic-channel

On line at : http: // lipidraft.ncsa.uiuc.edu