
Computational Biophysics:

Treating biological processes with mathematical rigorosity



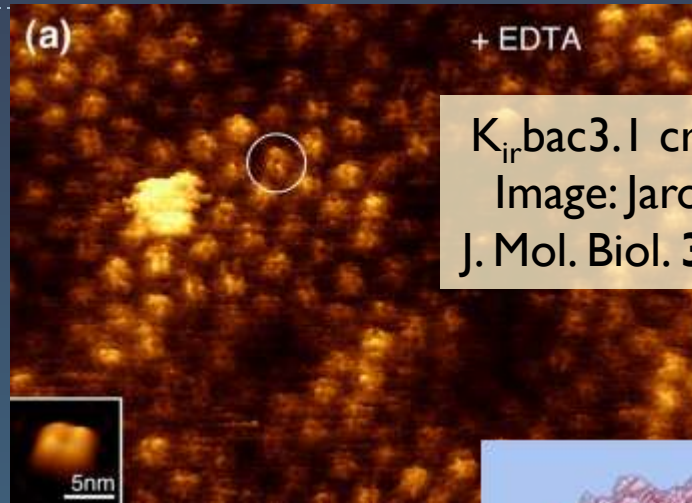
What does biophysics mean?

- ▶ A cross between biology, chemistry, physics and a bit of mathematics, developed in mid 20th century
 - ▶ Molecules behave according to laws of physics and chemistry
 - ▶ Therefore, processes must involve interactions/reactions of these molecule
- ▶ This subject includes the various techniques in physics like X-ray crystallography and NMR-resonance
 - ▶ Applied into biological problems
- ▶ I wish to show you the various applications that are possible, as carried out by our group



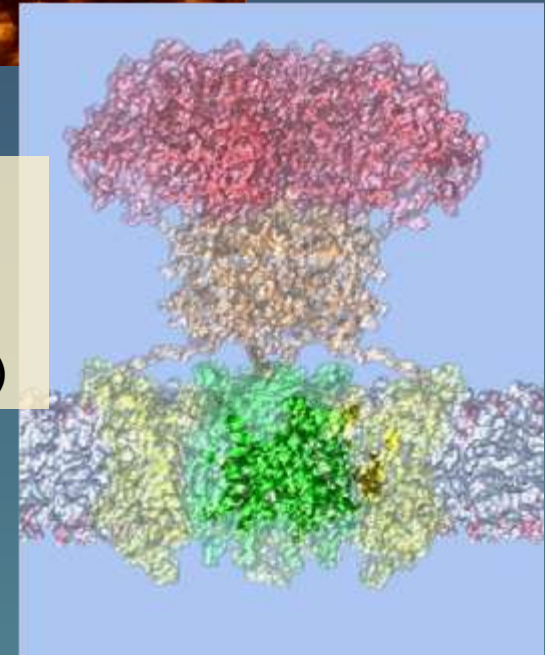
What this Biophysics group does

- ▶ We study molecular processes with computational models
 - ▶ Construct systems of particles *mimicking* the real environment
 - ▶ Using some or all of the atoms
- ▶ Simulated systems obey statistical mechanics
 - ▶ Easy to manipulate
 - ▶ Can measure properties relevant to the real system



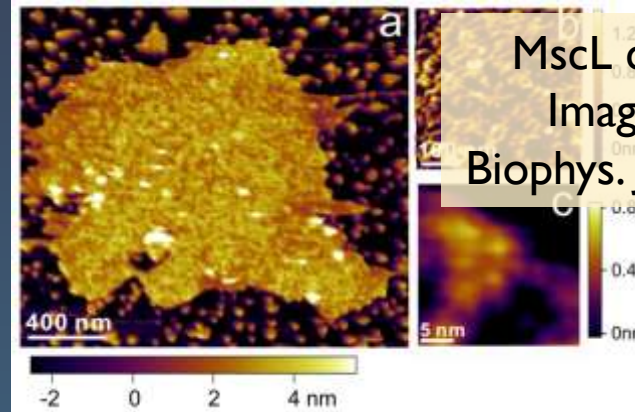
$K_{ir}3.1$ crystals by AFM.
Image: Jaroslowski *et al.*,
J. Mol. Biol. **374**:500 (2007)

$K_v1.2$ in membrane.
Simulation system
based on X-ray
structure. (2R9R.pdb)

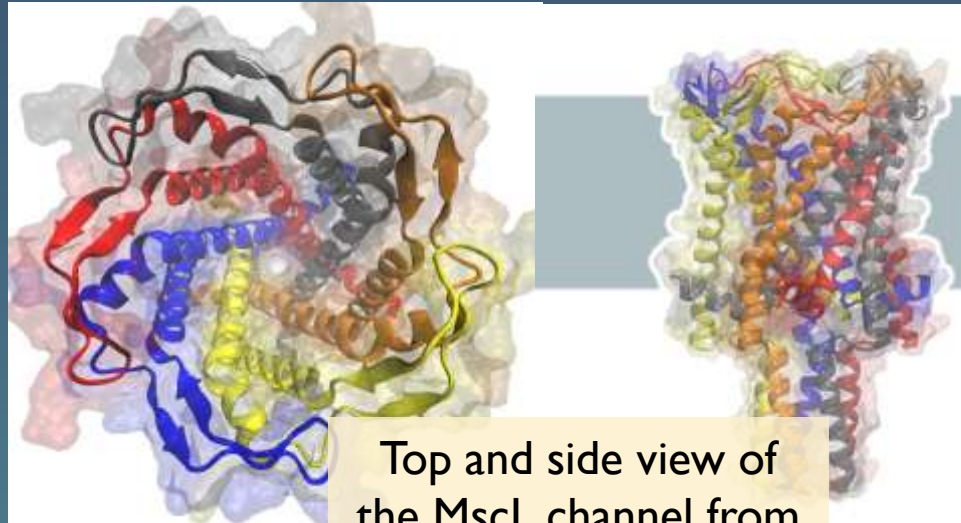


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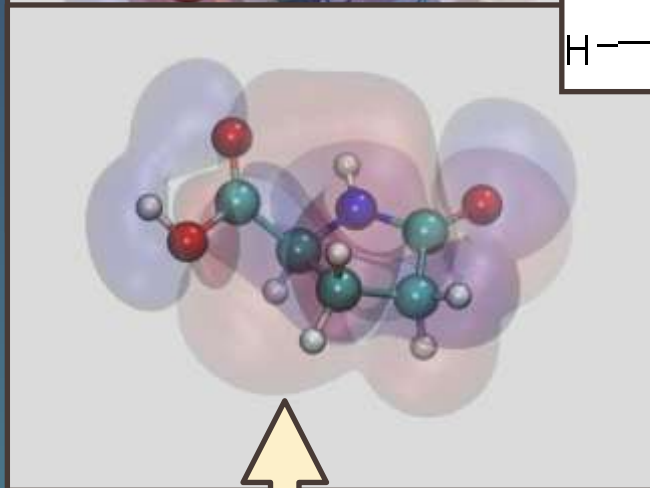
MscL clusters by AFM.
Image: Grage *et al.*,
Biophys. J. **100**:1252 (2011)



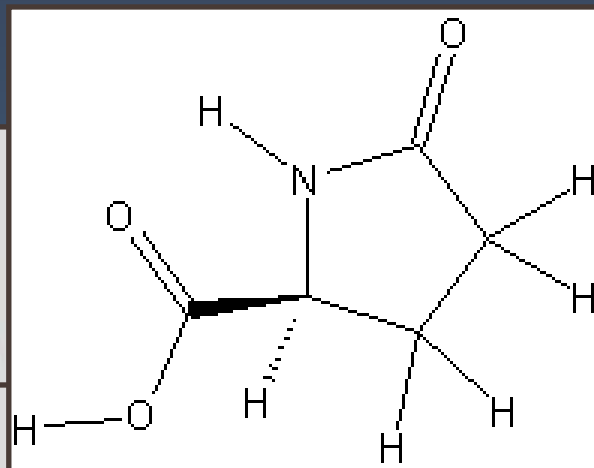
Top and side view of
the MscL channel from
X-ray structure.
(2OAR.pdb)

Modelling real molecules

QM

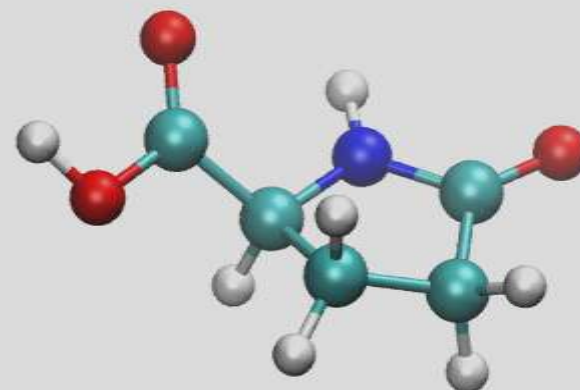


Calculate electron
wave-functions
directly



MM

Springs governing
bonds, angles, and
dihedrals



Modelling real molecules

Quantum Mechanics

- ▶ Models behaviours of electron in bonding and atomic orbitals
- ▶ Iteratively solves Schrodinger's equations
- ▶ Time-consuming:
 - ▶ picoseconds
- ▶ QM methods select kinds of electronic behaviour to include in description

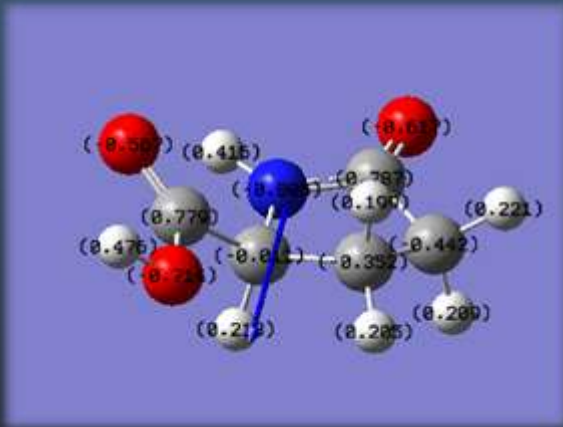
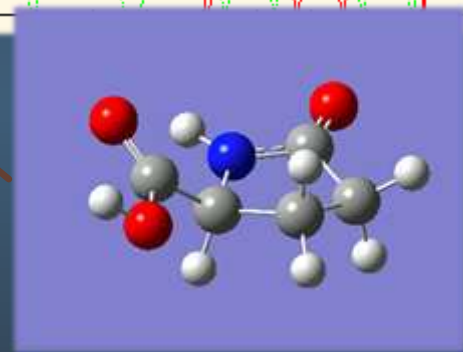
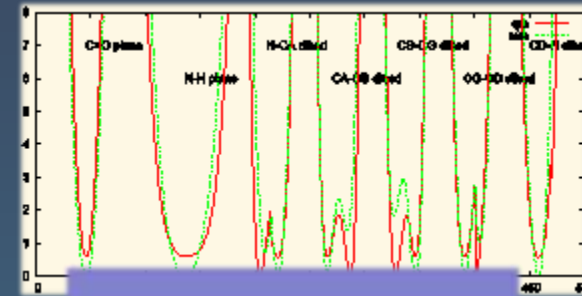
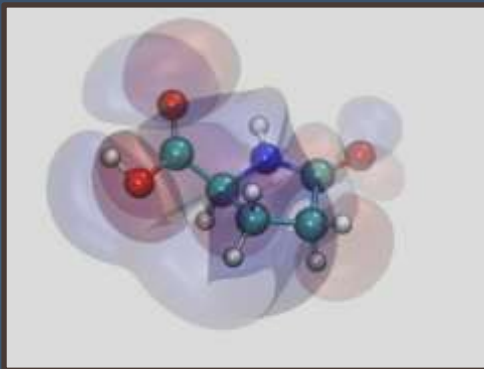
Molecular Mechanics

- ▶ Models electronic interactions implicitly with harmonic potentials ('springs')
- ▶ Iteratively solves Newtonian equations
- ▶ Faster, more approximate:
 - ▶ Nanoseconds, microseconds
- ▶ MM methods must design force-fields to approximate the real behaviour.



Modelling real molecules

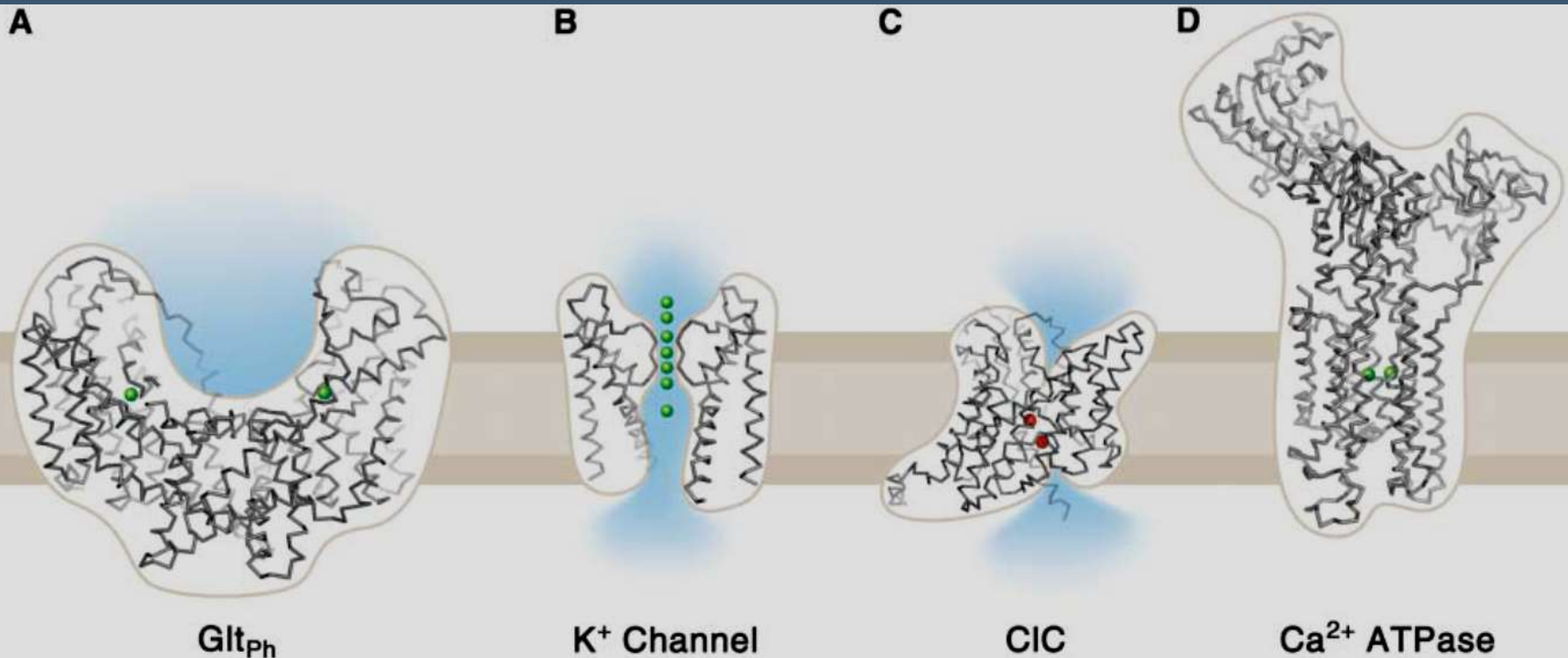
NB: **molecular mechanics** must use information from **quantum mechanics** and **experiment** to setup a correct description



What this particular biophysics group does:

We study:

- Various protein channels of known structure that resides in the cell membrane
- Their function, location and regulation



What this Biophysics group does

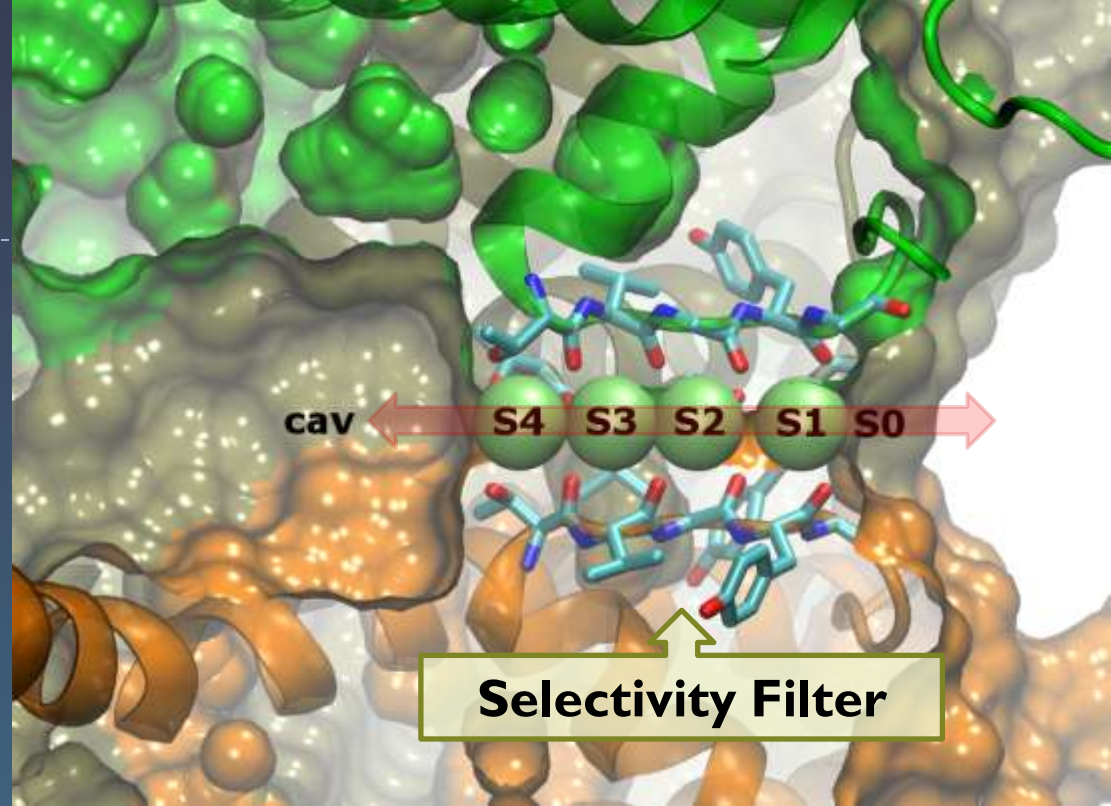
- ▶ Ligand flow through channels
 - ▶ Potassium ion permeation (K^+ -channels)
- ▶ Ligand binding to channels, and channel-like receptors
 - ▶ Block of K^+ -channels by small proteins, *i.e.* peptide toxins
 - ▶ Binding of the glutamate receptor
- ▶ Polarisation properties of ions in water
 - ▶ One of the major limitations in molecular mechanics



K⁺ permeation

Biophys J. (2011)

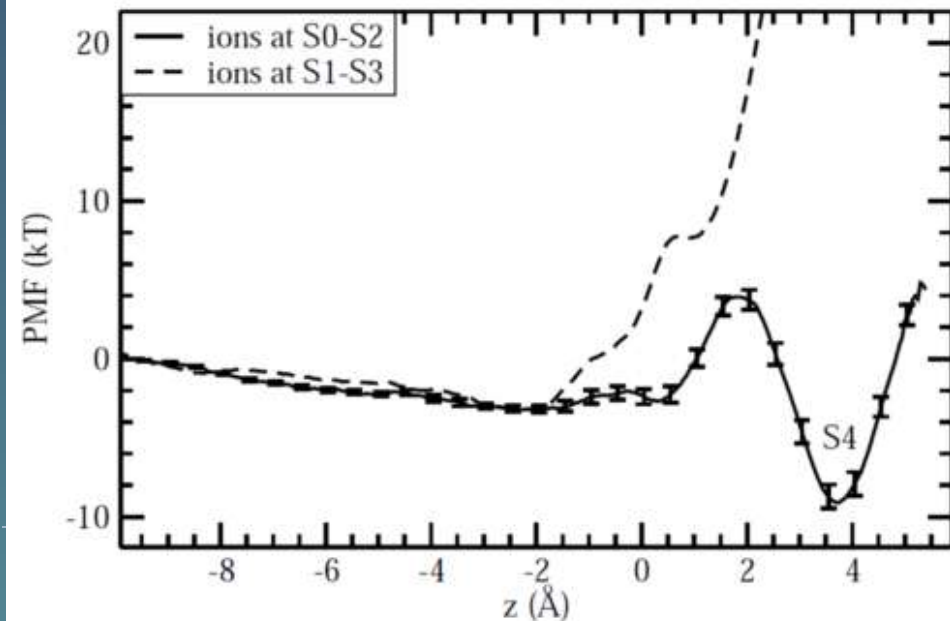
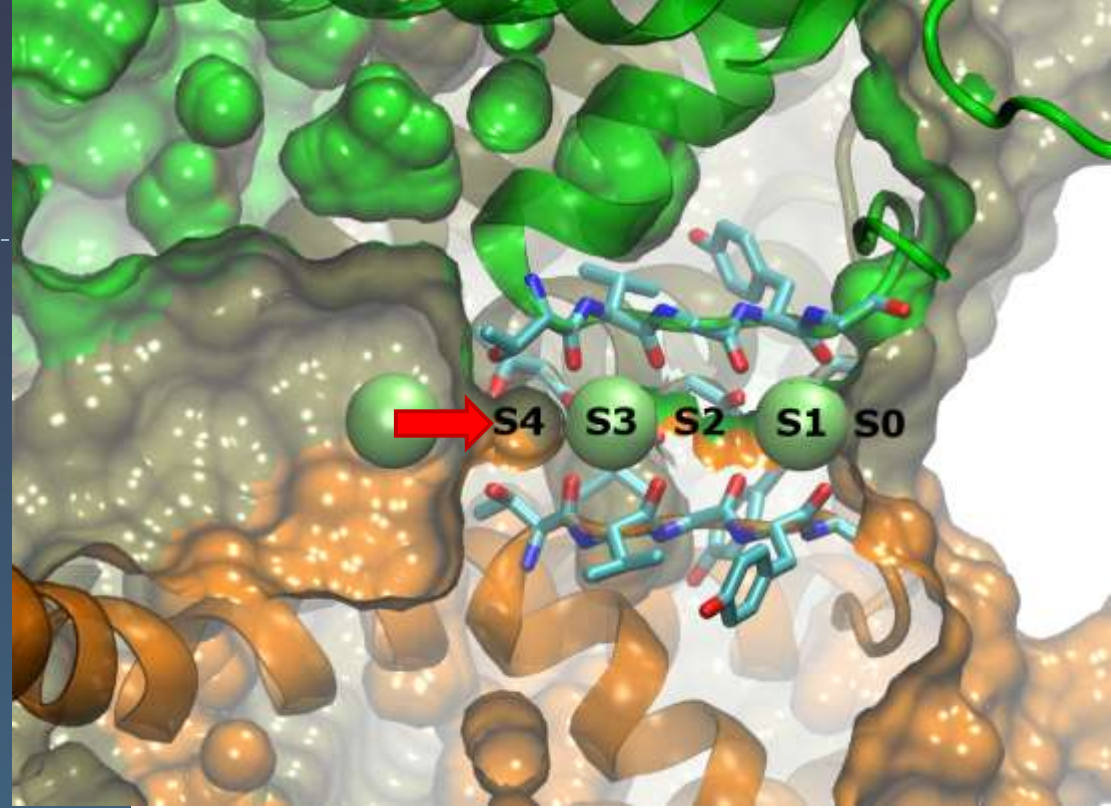
- ▶ Ion balance requires selective control of various electrolytes
 - ▶ Channels are distributed throughout the body to regulate this
 - ▶ Used in neuronal and cardiac signals
- ▶ In potassium channels, K⁺ ion is favoured by the pore structure
 - ▶ Coordination by oxygens replace the function of water
 - ▶ Conduction involve concerted movement of these ions



K⁺ permeation

Biophys J. (2011)

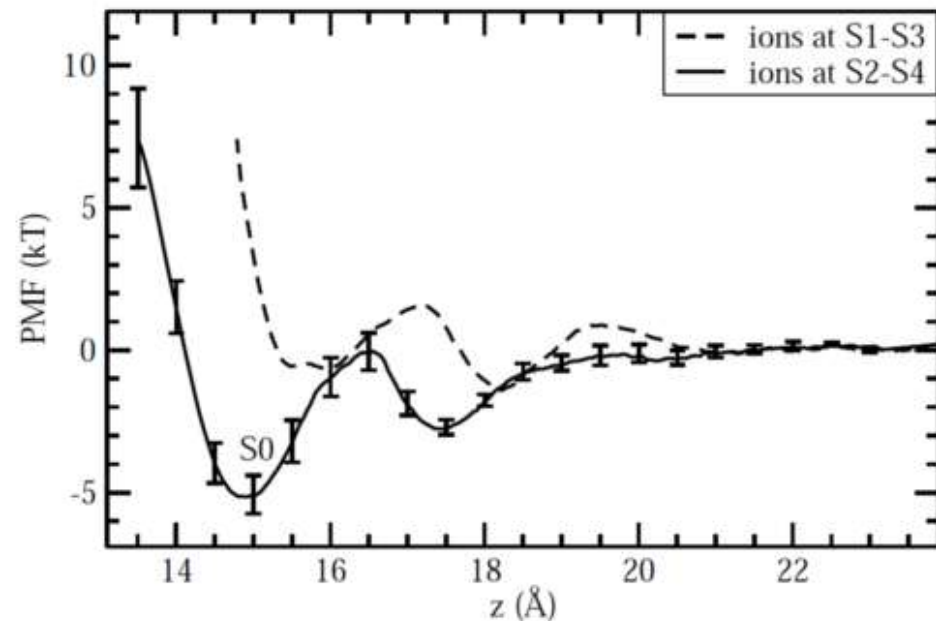
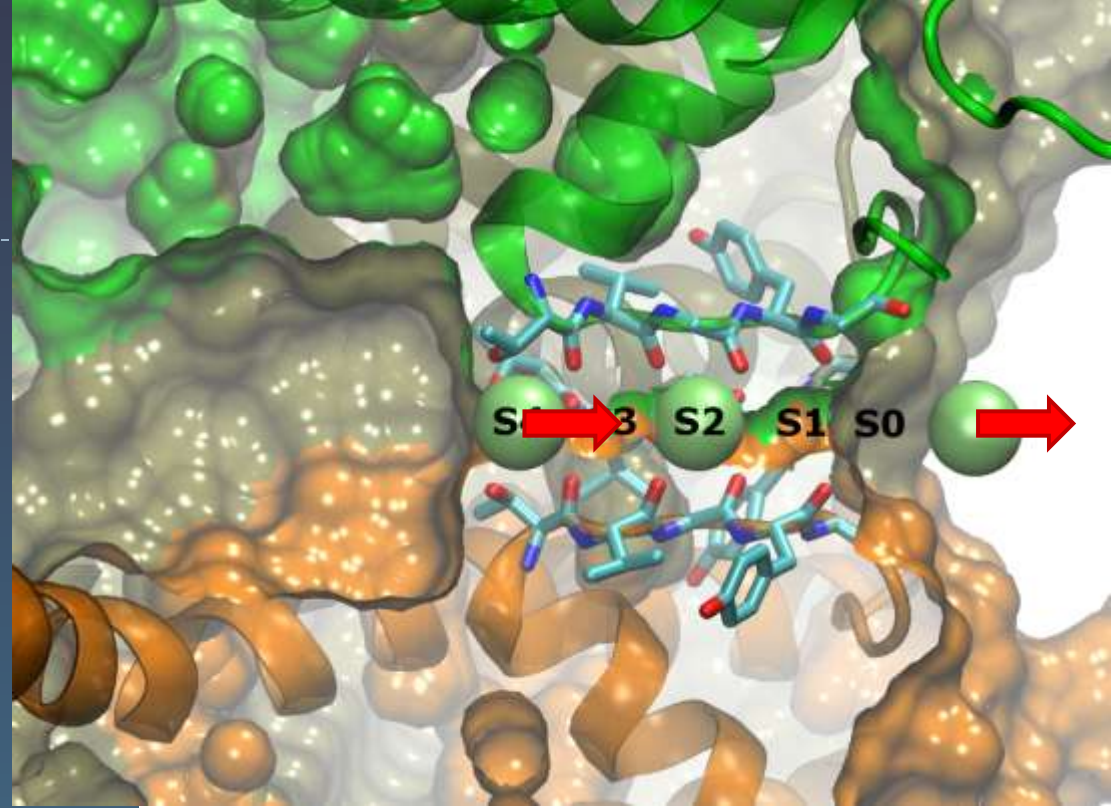
- ▶ Two ions normally reside in the filter
- ▶ Approach of additional ions
 - ▶ Let's say, from left
- ▶ Creates a three-ion state



K⁺ permeation

Biophys J. (2011)

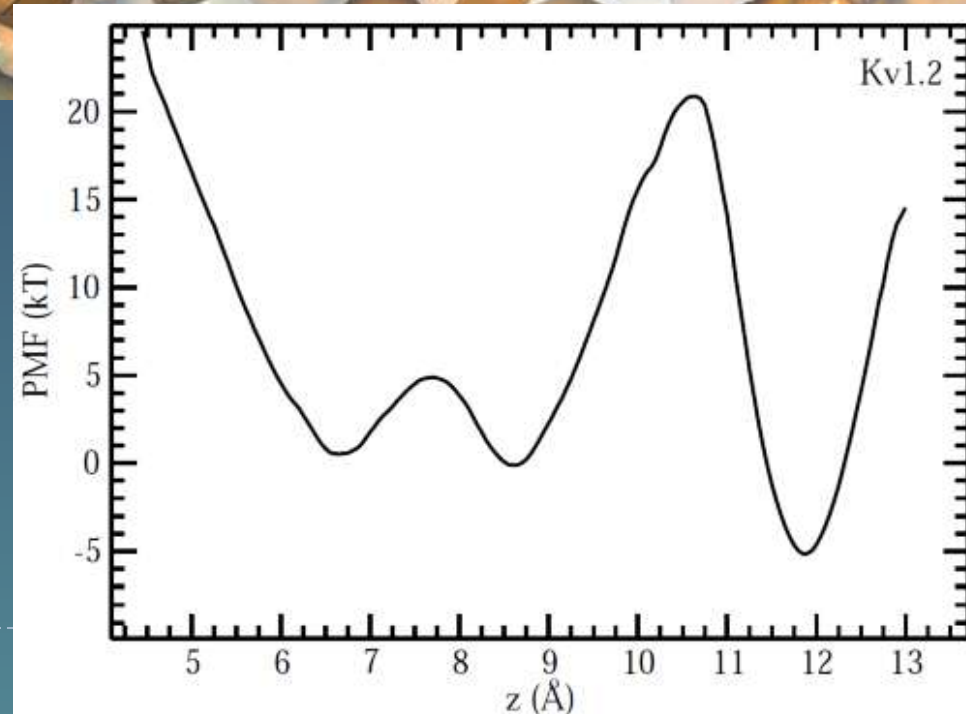
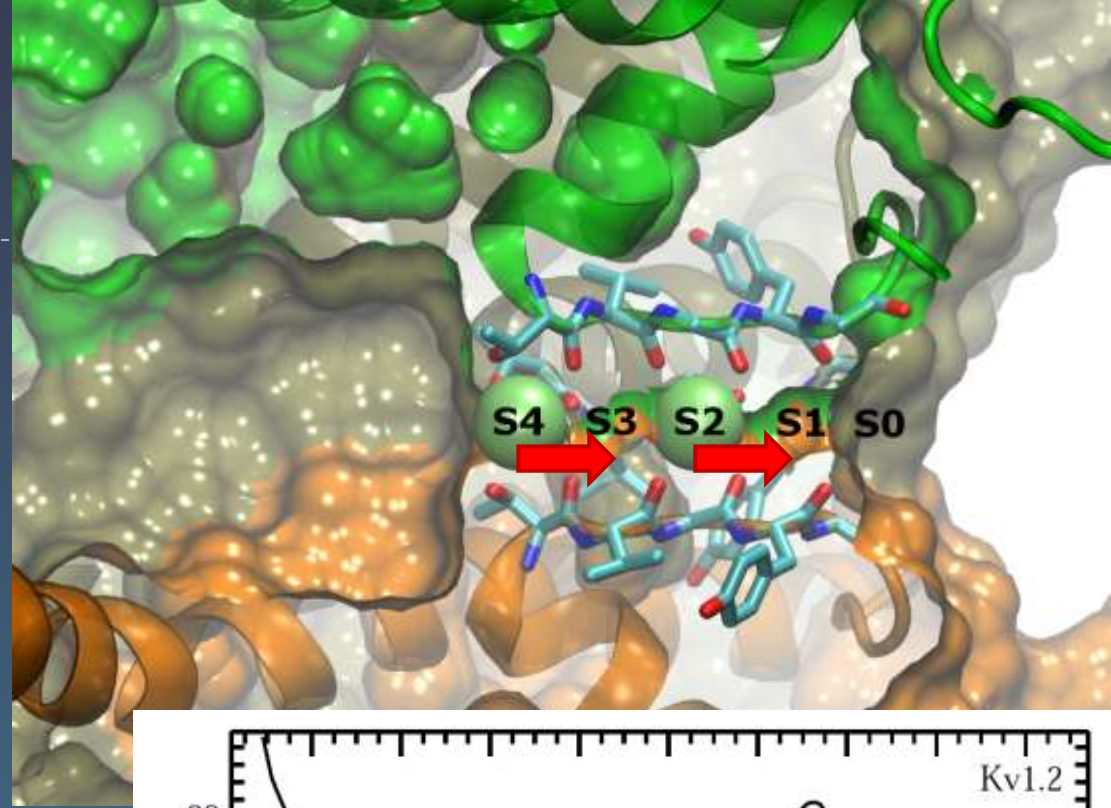
- ▶ Cav/S3/S1 -> S4/S2/S0
- ▶ The S0 ion can readily exit
- ▶ Back to two ions



K⁺ permeation

Biophys J. (2011)

- ▶ The two remaining ions resets
 - ▶ S4/S2 → S3/S1
 - ▶ Ready for next cycle
- ▶ This cycle goes both ways, depending on external environment
 - ▶ Electric potential
 - ▶ Ion concentration

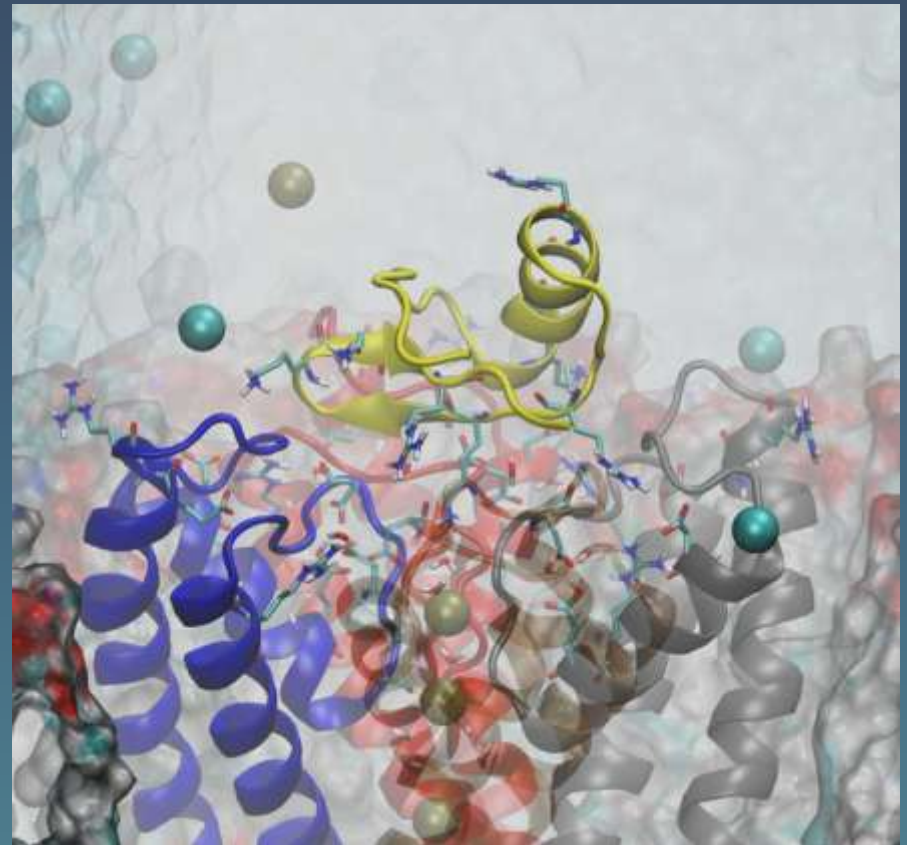


Ligand binding simulations

Motivation

- ▶ Some ion channels serve neural functions
 - ▶ Blocked by venom of paralyzing predators
- ▶ This block is 'selective'
 - ▶ Its action on non-relevant ion channels are much weaker
- ▶ Find structure-function relationships and investigate the potential of new toxins

Case Study: KcsA-ChTX



Calculations and connection to experiment

Gibbs free energy of binding

- ▶ Statistical ensembles give equilibrium constants

- ▶
$$K_{eq} = \iiint e^{-\frac{W(\vec{r})}{kT}}$$

- ▶
$$\Delta G_{bind} = -kT \log(K_{eq}C^0)$$

- ▶ Also a measure of free energy
 - ▶ Gibbs means ‘constant pressure and temperature’

Ligand affinity

- ▶ Consider binding of a ligand to protein, in a simple reaction:



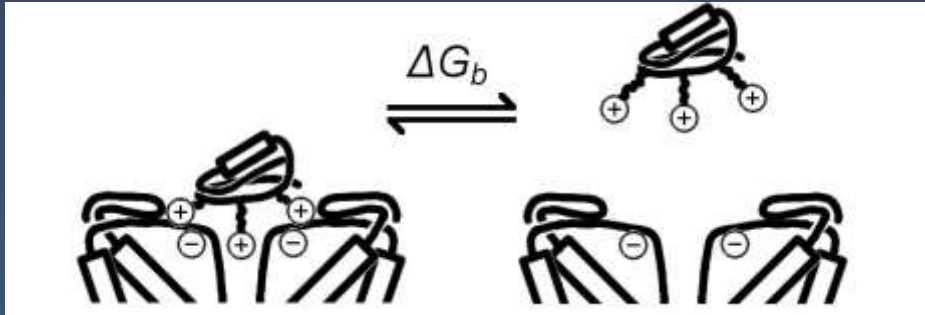
- ▶
$$K_{eq} = \frac{[LP]}{[L][P]}$$

- ▶ Standard measure of ‘experimental affinity’ is:

- ▶
$$K_D = 1/K_{eq}$$



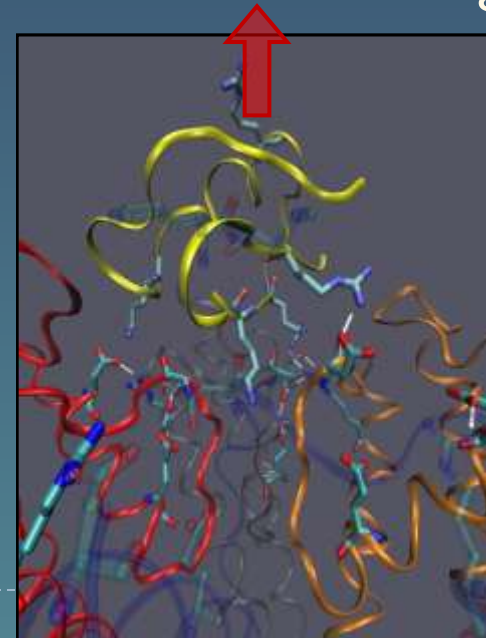
Free energy calculation



Simulation procedure

- ▶ Replicate the (un)binding process
- ▶ Measure average forces on the system
- ▶ Convert to free energy

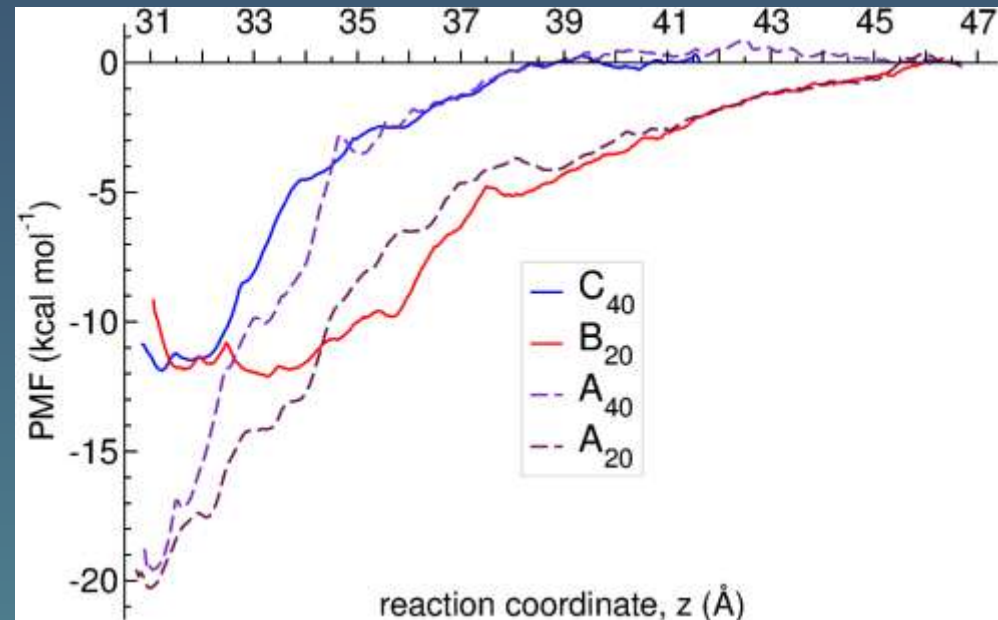
- ▶ The reaction $L + P \leftrightarrow LP$ involves a change in free energy
- ▶ We can calculate this free energy and give a prediction of the experimental affinity



Free energy calculation

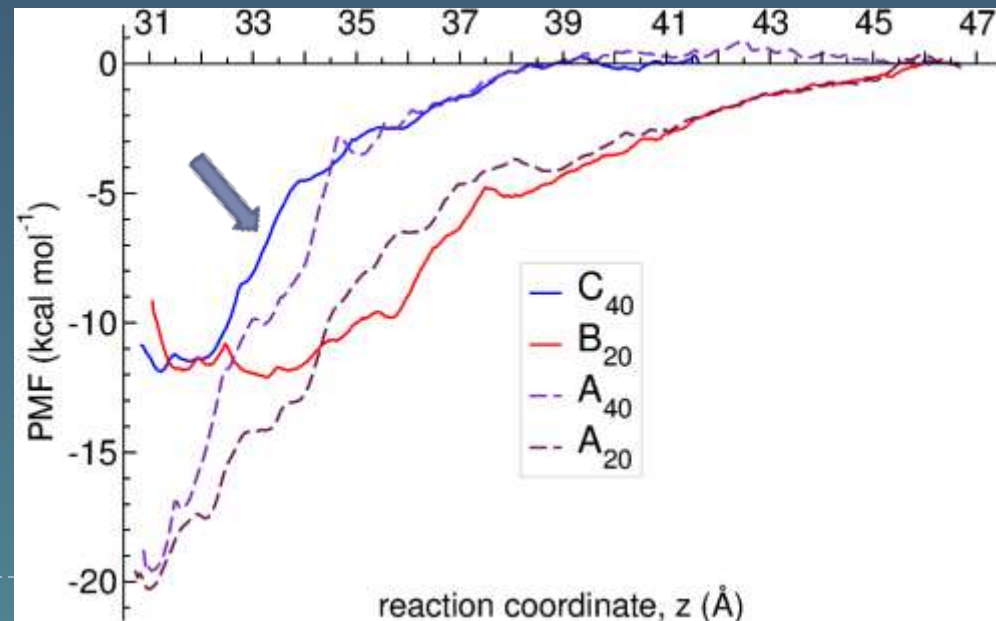
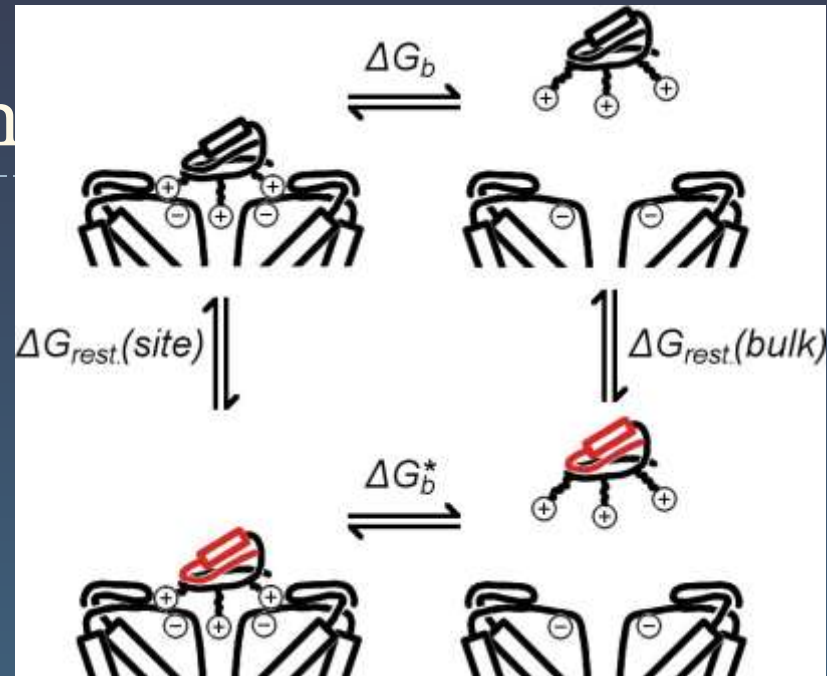
- ▶ The unbinding process of an entire peptide cannot be easily simplified
 - ▶ Naïve manipulations can unfold the protein and introduce errors into the calculation
- ▶ These are compensated by protecting the peptide during the process

Attempts	FE of binding (kcal/mol)
A ₂₀	-17.1 ± 0.9
A ₄₀	-16.8 ± 2.3
B ₂₀	-8.7 ± 1.7
C ₄₀	-7.6 ± 1.1
Expt.	-8.3



Free energy calculation

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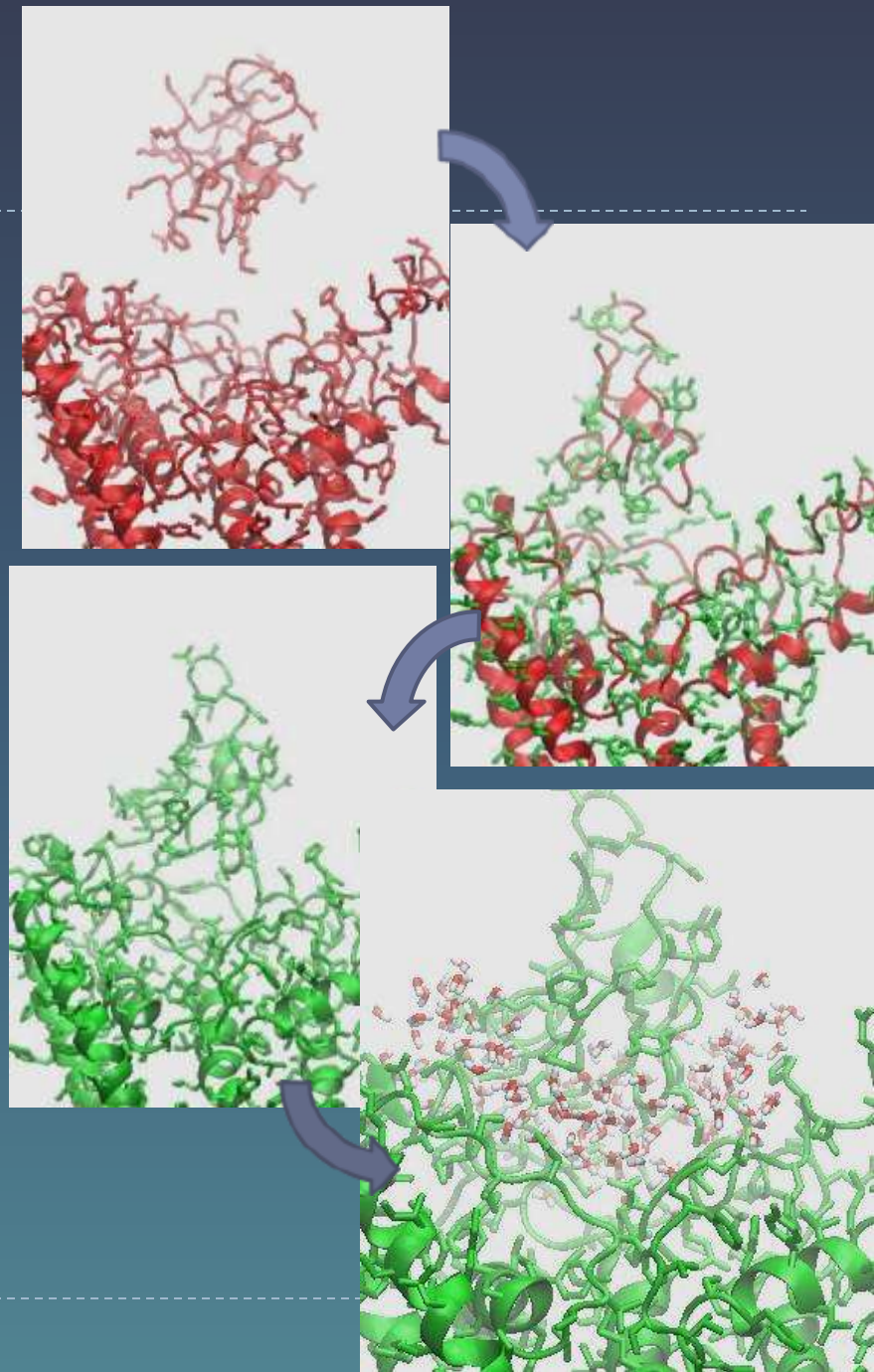
Predicting the binding site of a ligand

- ▶ Of course, we cheated in the previous example.
 - ▶ We already have a correct bound complex ready to go
 - ▶ We merely measured the unbinding reaction, which is just the reverse of the original binding interaction
 - ▶ What if we didn't know the exact location?
- ▶ The binding process itself takes μs or ms
 - ▶ Not feasible to copy by simulations alone
- ▶ Find solution directly by 'docking'
 - ▶ Take random samples of possible binding sites/orientations and refine them
 - ▶ Evaluate each to find a likely real candidate

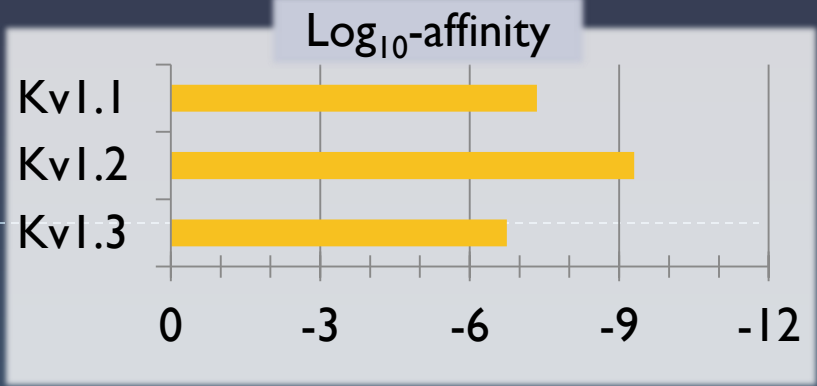


Docking two proteins

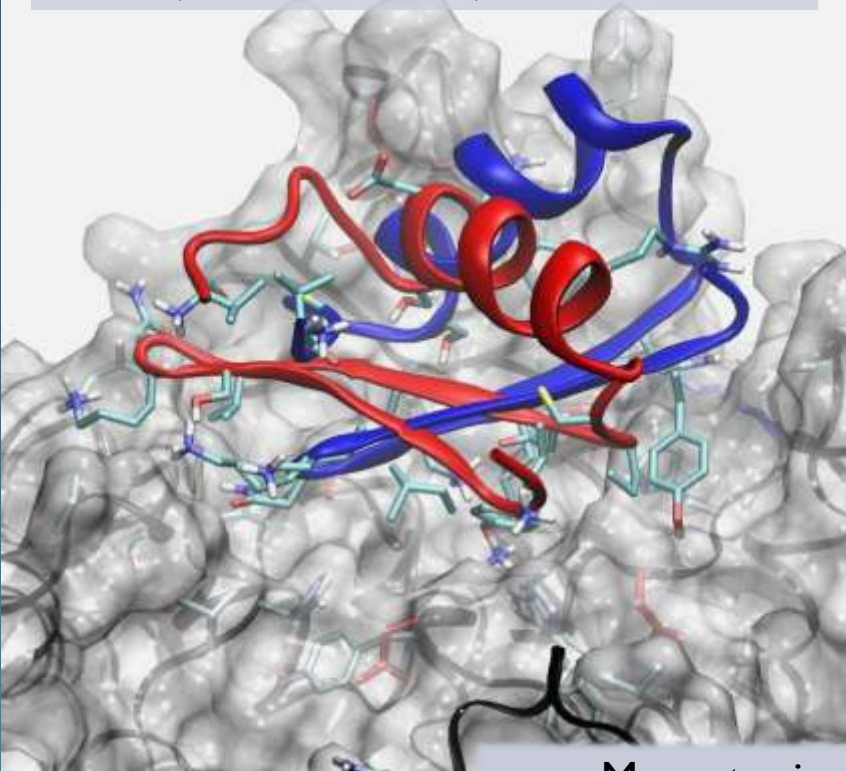
- ▶ Begin by making rough search of possible binding orientations
- ▶ Refine hopeful candidates and trim unwanted ones
 - ▶ Rigid body
 - ▶ Add flexibility
 - ▶ Add water environment
- ▶ Principle: low to high accuracy searches



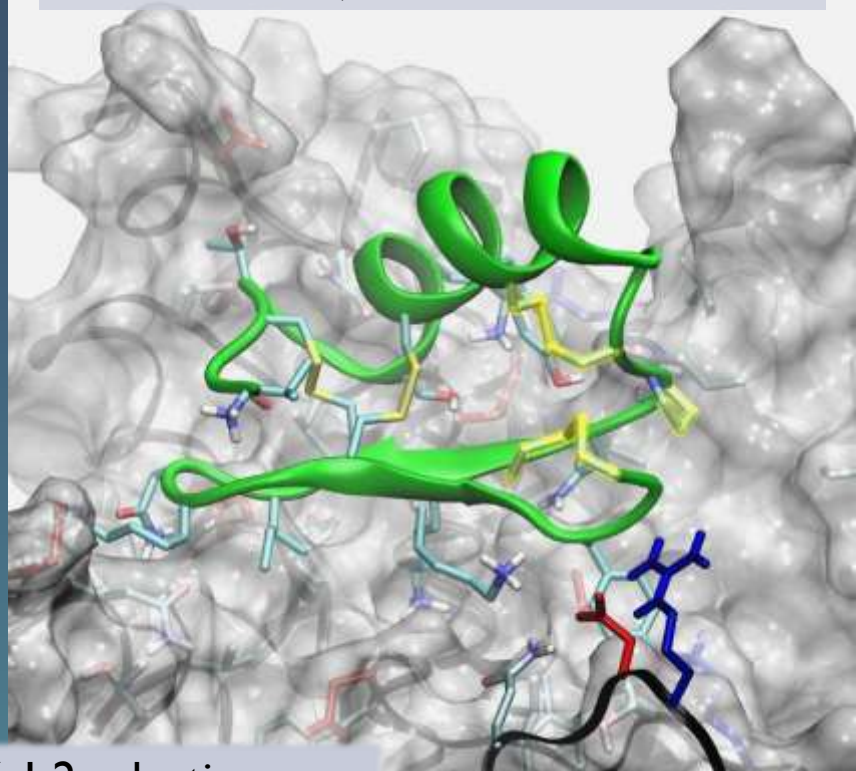
Example results



K_v1.1: 45 nM – K_v1.3: 180 nM



K_v1.2: 0.5 nM



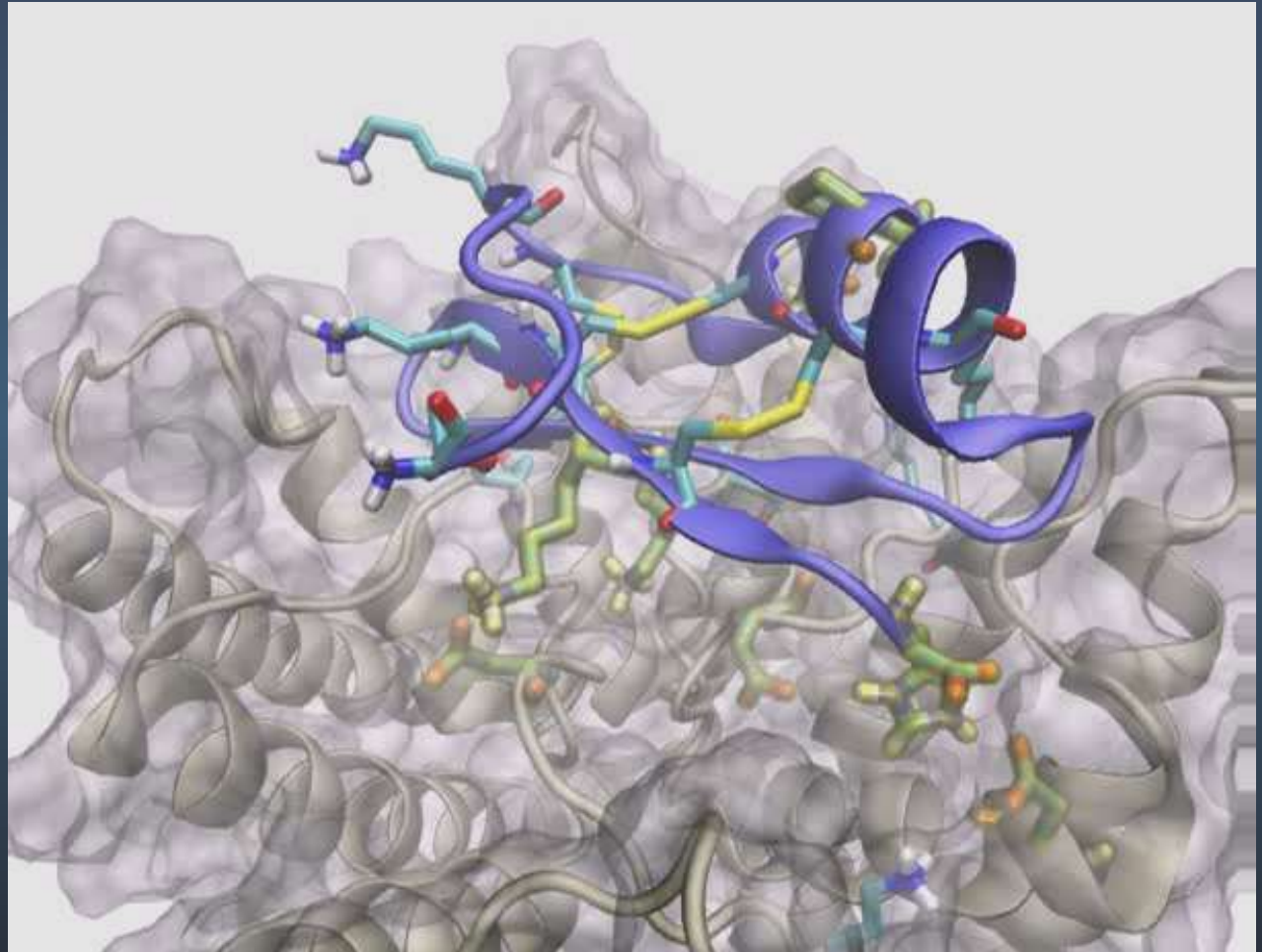
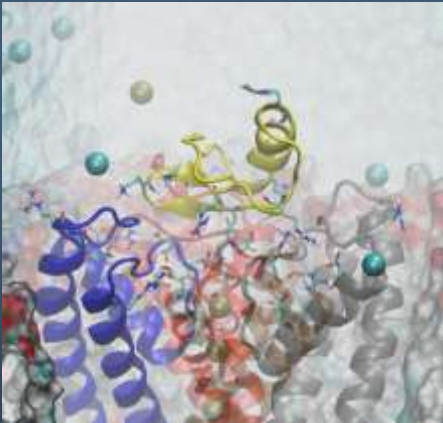
Maurotoxin: K_v1.2 selective



Margatoxin-Kv1.3 complex: demonstration

Many toxins bind in similar fashion.

Kcsa-ChTX



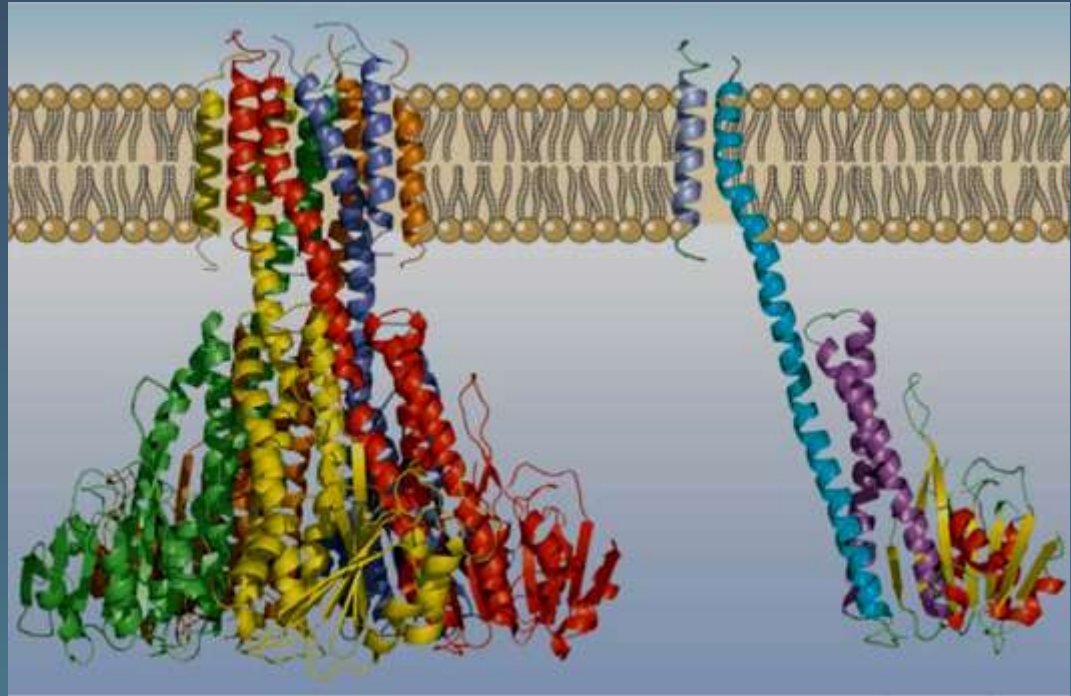
Summary

- ▶ Simulations take static coordinates of a system and find its dynamic behaviour
 - ▶ Mechanisms of ion-permeation, and toxin-binding
 - ▶ Energetic contributions
- ▶ These information contributes to the knowledge of molecular interactions
 - ▶ understanding of biological processes, and how they break down in mutations and disease
 - ▶ assists in designing new proteins



It's far from over

- ▶ There are numerous proteins that are either recently characterised or as yet unknown
- ▶ Computational simulations can explore the detailed mechanisms
 - ▶ ...given time



“CorA”, a magnesium channel. Image:
Moomaw and Maguire, *Physiology*, **23**:275 (2008)



Programs that you can use

- ▶ **NAMD**
 - ▶ Simulation work
 - ▶ www.ks.uiuc.edu/Research/namd/
- ▶ **VMD**
 - ▶ Visualisation
 - ▶ www.ks.uiuc.edu/Research/vmd/

