Lessons from applications of multi-scale techniques to analysis of the permeation and selectivity in KcsA ion channel

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Support

EPSRC Engineering and Physical Sciences Research Council

My expertise (a part)

Most probable (optimal) path in non-equilibrium systems
(Advances in Chemical Physics 119:469 - 524 2001)

Large Fluctuation and Energy-minimal Deterministic Control
(PhysRevLet 85(10):2100-3 2000, PhysRev E 67(5 Pt 1):051102 2003)

Non-Gaussian noise escape problem
(PhysRevB 89(8) 085419 2014)

Escape in excitable systems (systems without saddle point)
(PhysRevE 87(3) 032116 ·2013)

Bayesian inference of stochastic dynamics
(PhysRevE 77 061106 · 2008)

…

$$\dot{\mathbf{x}} = \mathbf{K}(\mathbf{x}, t) + \mathbf{Q}(\mathbf{x})\xi(t)$$

$\mathbf{K}(\mathbf{x}, t)$ Deterministic dissipative vector function

$\mathbf{Q}(\mathbf{x})$ Stochastic term

Outline

- **Molecular Dynamics (MD) simulations of ion channels, biased methods**
- **Permeation in KcsA, the knock-on mechanism and the height of the barrier**
- **Issues in free energy calculations**
- **Ion permeation in KcsA**

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- **Ion permeation in KcsA**

Study of ion channels

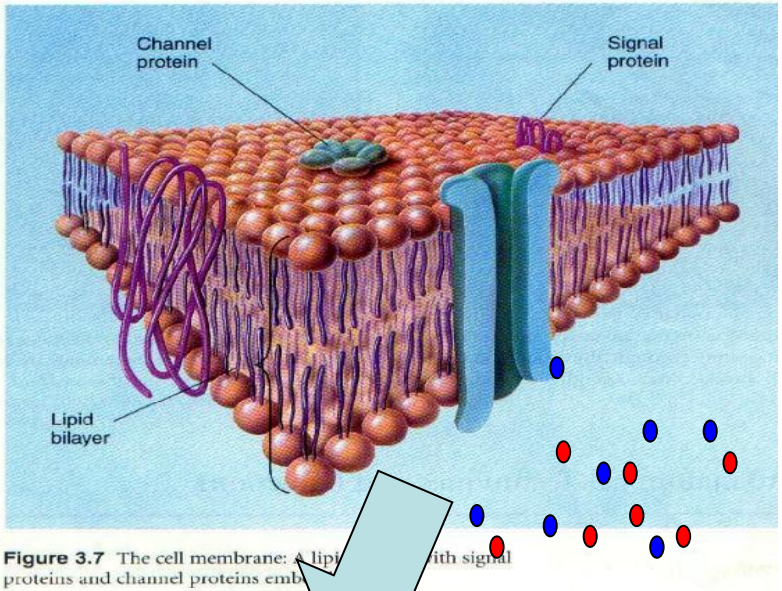
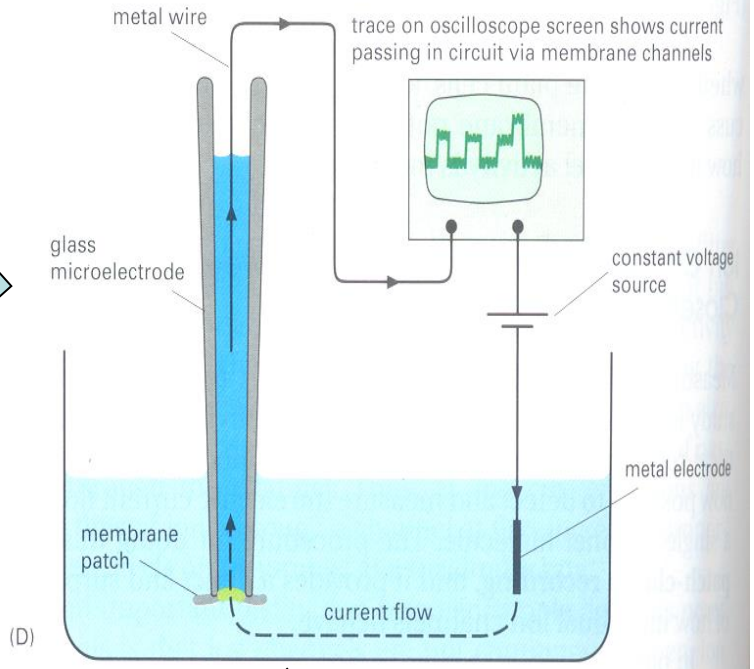
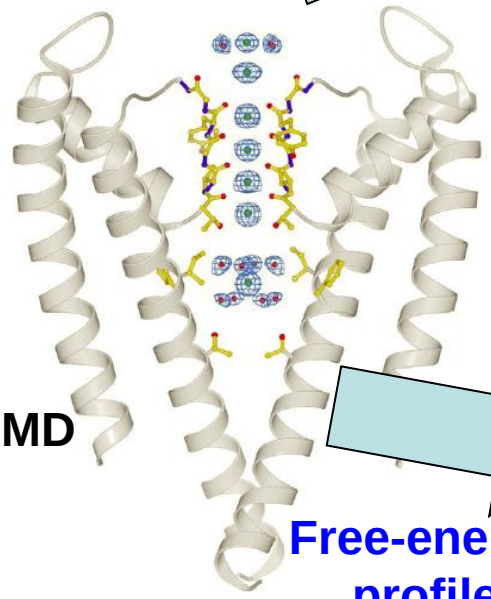


Figure 3.7 The cell membrane: A lipi… ith signal proteins and channel proteins emb…

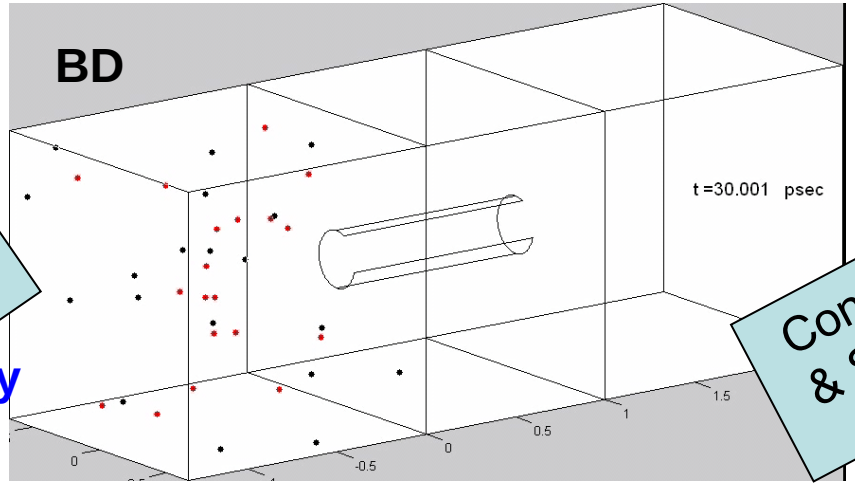


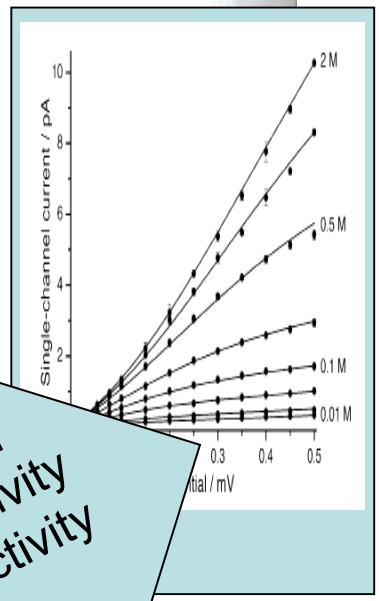
Protein structure



MD

Free-energy profile



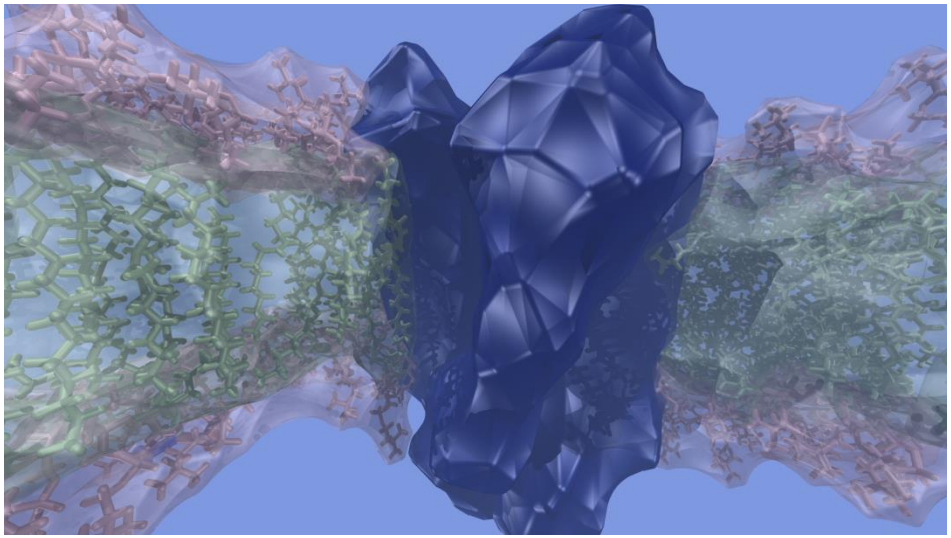


Conductivity & Selectivity

MD simulations of KcsA

Main steps in line with the tutorial: http://www.ks.uiuc.edu/Research/smd_imd/kcsa/

1. Building the full protein using the information available from the x-ray structure from MacKinnon group, 2.0 A resolution, Y. Zhou, J. H. Morais-Cabral, A. Kaufman, and R. MacKinnon, “Chemistry of ion coordination and hydration revealed by a K+ channel-Fab complex at 2.0 A resolution,” Nature, vol. 414, pp. 43–48, Nov. 2001.
2. Building a phospholipid bilayer;
3. Inserting the protein in the membrane;
4. Solvating of the entire system.
5. Relaxing the membrane to envelop the protein and to let it assume a natural conformation.



Software and

Computational resources

MD simulations of KcsA

Molecular Dynamics is the *solution* of the classical (Newtonian) equations of motion for a set of molecules.

Within the Born-Oppheneimer approximation, the Hamiltonian of a system can be expressed as a function of the nuclear coordinates $\mathbf{q}_i$ and momenta $\mathbf{p}_i$.

For Cartesian coordinates:

$$\dot{\mathbf{r}}_i = \mathbf{p}_i / m_i$$
$$\dot{\mathbf{p}}_i = -\nabla \mathcal{V}_i(\mathbf{r}_i)$$

Empirical CHARMM Force Field, **VMD, NMAD**

$$V(r) = \sum_{bonds} K_b(b - b_0)^2 + \sum_{angles} K_\theta(\theta - \theta_0) + \sum_{dihedrals} K_\phi[1 + cos(n\phi - \phi_0)] + \sum_{impropers} k_\psi(\psi - \psi_0)^2$$
$$+ \sum_{i,j} 4\epsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] + \sum_{Urey-Bradley} K_u(u - u_0)^2 + \sum_{i,j} \frac{q_i q_j}{\epsilon_D r_{ij}}$$

From MD to Experiments via BD

Both permeation (conductivity) and selectivity can be discussed via the **Free Energy or the Potential of Mean Force (PMF)** , thermodynamics description

The main idea: If there is PMF $F(z)$, then time scale of diffusion through the channel can be estimated via Kramers rate. The coordinate z describes the transition.
(T. W. Allen, PNAS, vol. 108, no 44, 17963-17968, 2011)

$$rate = \frac{D\sqrt{K_b K_w}}{2\pi k_b T}\exp(-\Delta F / k_b T)$$

Unknown:

D – diffusion constant;

$F(z)$ – PMF, ΔF is a barrier;

T is assumed to be as in the bulk

PMF via special approach as

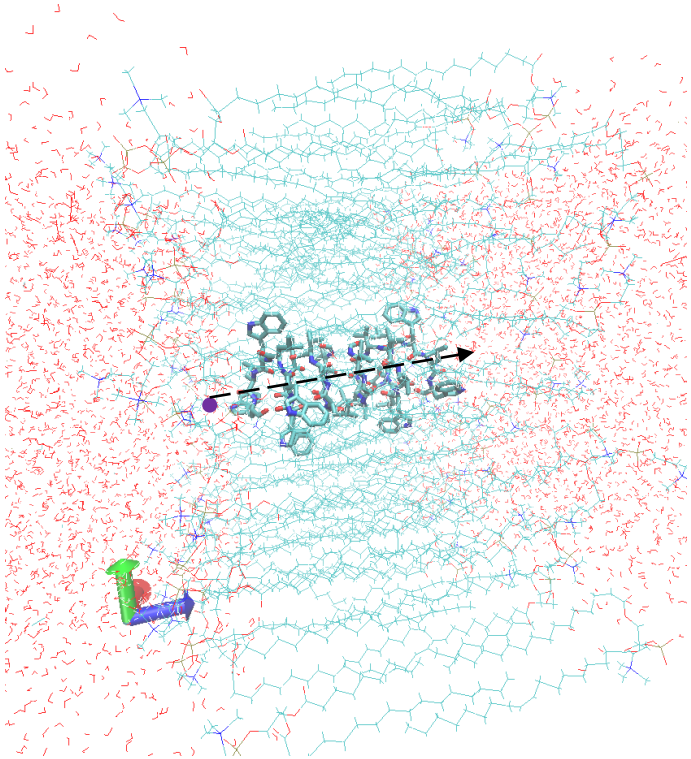
Umbrella Sampling (US)

$$F(z) = -\frac{1}{k_b T}\ln P(z) - Bias(z)$$

P(z) is distribution from MD trajectories
Bias(z) – is an external biased potential

or others (MetaDynamics)

From MD to Experiments via BD



All-Atom Molecular Dynamics (MD)

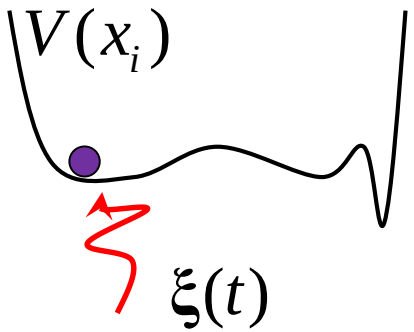
Hamiltonian Equation:

$$H = \sum_{k=1}^{N} \frac{\mathbf{p}_k \cdot \mathbf{p}_k}{2m_k} + U(\mathbf{r}_1, \mathbf{r}_2, \ldots \mathbf{r}_N)$$

$$\frac{d\mathbf{r}_i}{dt} = \frac{\partial H}{\partial \mathbf{p}_i} \equiv \frac{\mathbf{p}_i}{m_i}, \quad \frac{d\mathbf{p}_i}{dt} = -\frac{\partial H}{\partial \mathbf{r}_i} \equiv -\nabla_{\mathbf{r}_i} U$$

$$N \sim 10^6 - 10^7$$

One-Atom Brownian Dynamics (BD) **Overdamped Langevin Equation:**



$$\dot{\mathbf{r}}_i(t) = -\frac{1}{m_i \gamma(\mathbf{r}_i)} \frac{\partial V(\mathbf{r}_i)}{\partial \mathbf{r}_i} + \sqrt{\frac{2k_B T}{m_i \gamma(\mathbf{r}_i)}} \boldsymbol{\xi}(t)$$

$\gamma(\mathbf{r}_i)$ damping coefficient

$\boldsymbol{\xi}(t)$ white Gaussian noise

Potential of mean force (PMF), Free Energy

Biased simulations

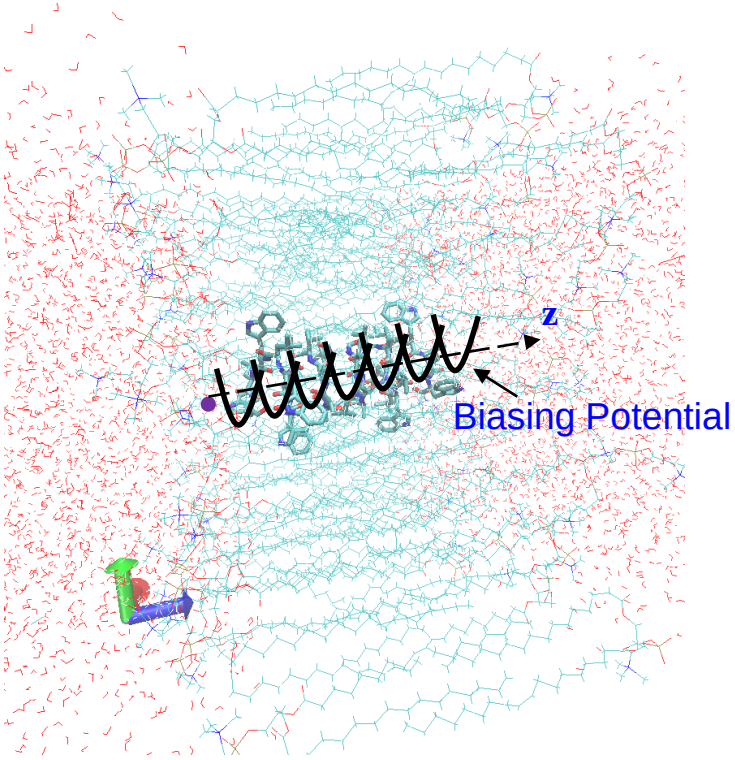
$$H_b(\mathbf{r},\mathbf{p}) = H_0(\mathbf{r},\mathbf{p}) + U(\mathbf{z}) \quad \Rightarrow \quad V(\mathbf{z})$$

Biased Hamiltonian

Initial Hamiltonian

Biasing Potential

Resulting PMF



Assumptions:

"… the underlying system's dynamics **must be**

Markovian and

Ergodic

for any bias…"

MetaDynamics

- reconstruct of free energy surfaces;
- force the system to escape from minima;
- based on a small number of collective variables (CVs) able to describe the relevant transitions; CVs correspond to transition coordinates $\mathbf{z}$
- a history-dependent potential is created as a sum of Gaussians centred along the trajectory of the CVs.
- In the simpler case of standard MetaDynamics and one CV, the biasing potential is given by

$$V(S(\mathbf{z}),t) = w \sum_{t=\tau,2\tau,\ldots} \exp\left[-\frac{(S(\mathbf{z}) - s(t))^2}{2\delta s^2} \right]$$

where
$S(\mathbf{z})$ is the chosen CV function of the coordinates $\mathbf{z}$ and
$s(t)$ its value at the time t;
w and δ are the Gaussian height and width;
τ is the deposition interval time.

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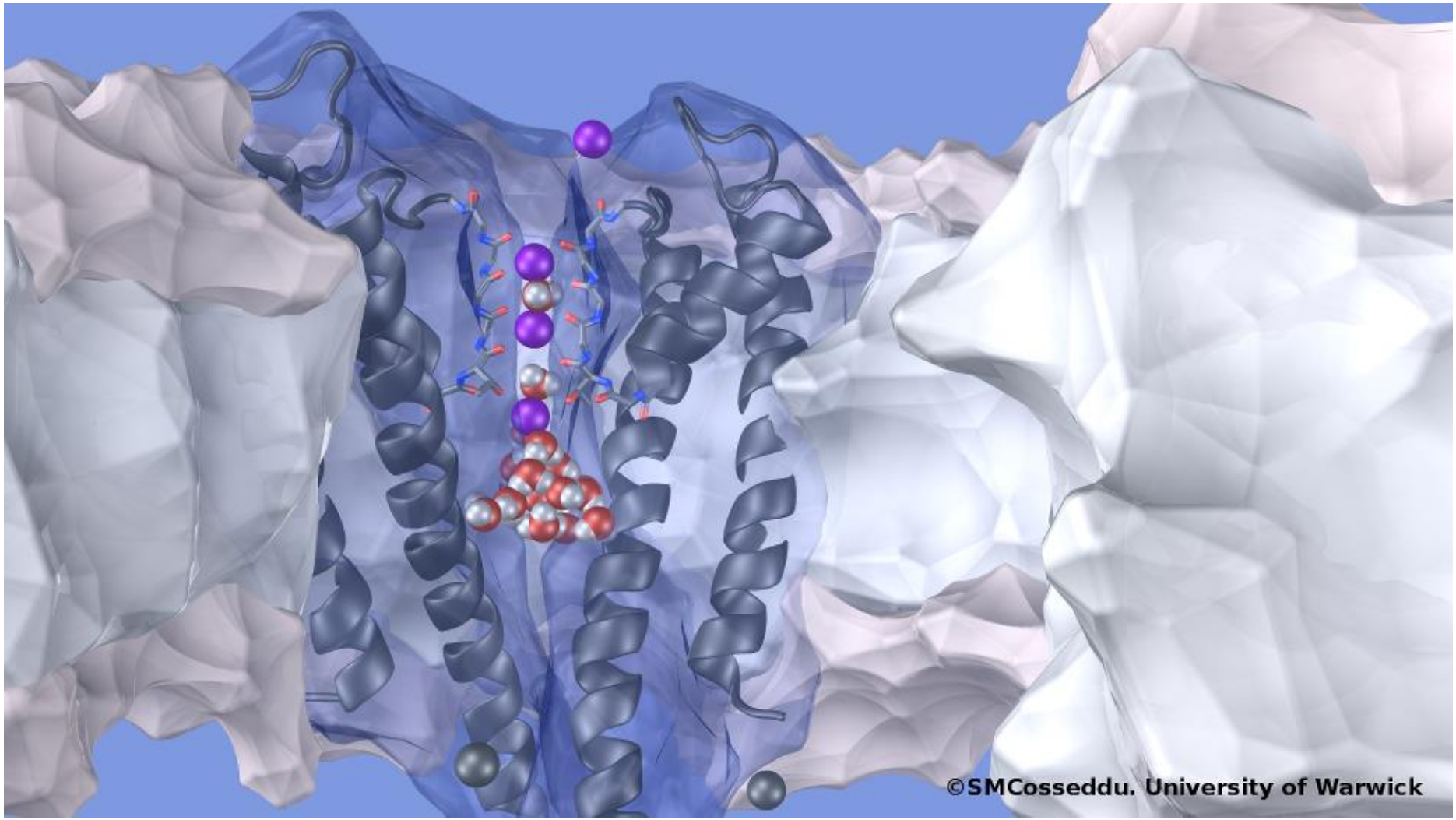
- Molecular Dynamics (MD) simulations of ion channels, biased methods

- **Permeation in KcsA, the knock-on mechanism and the height of the barrier**

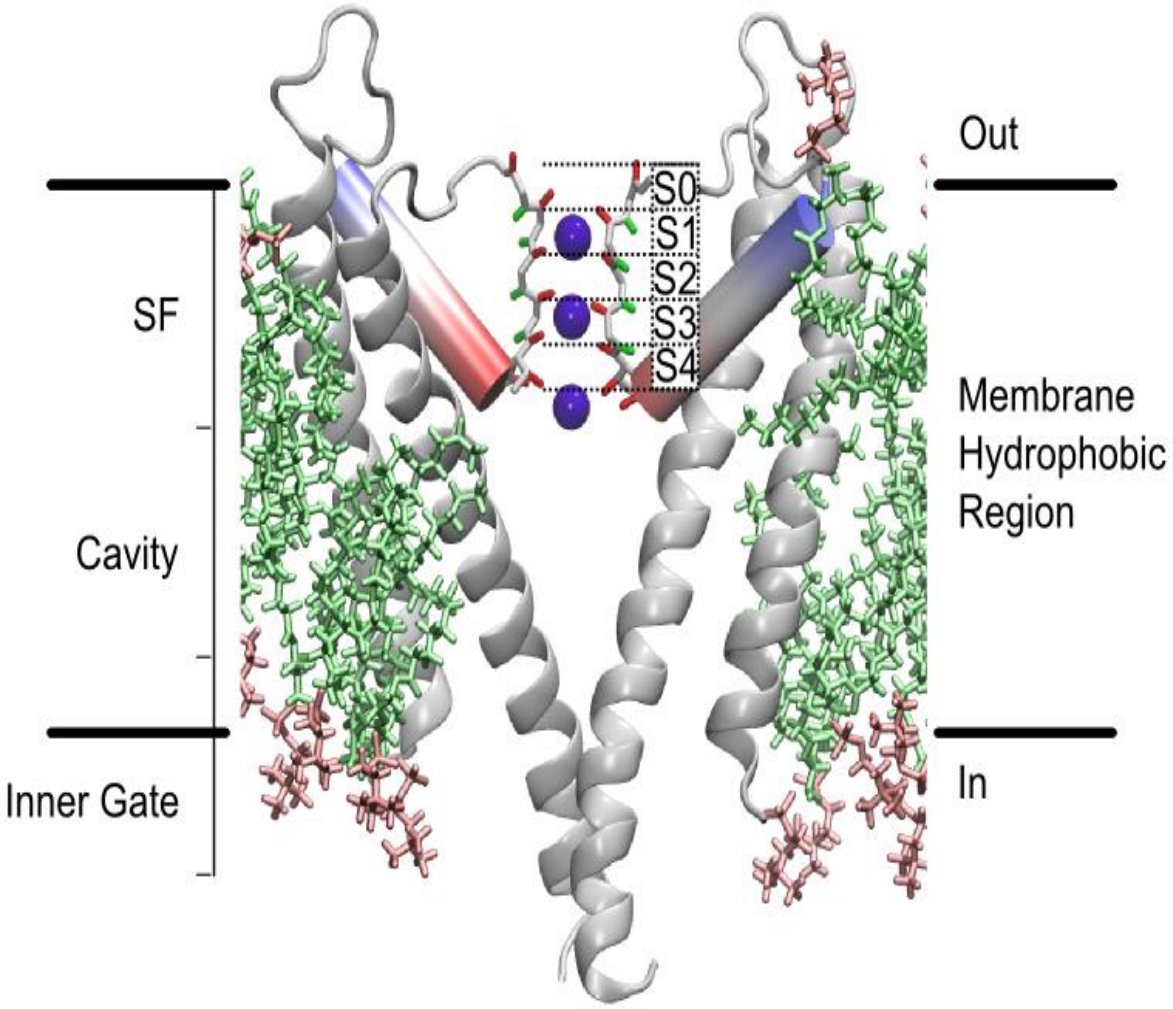
- Issues in free energy calculations

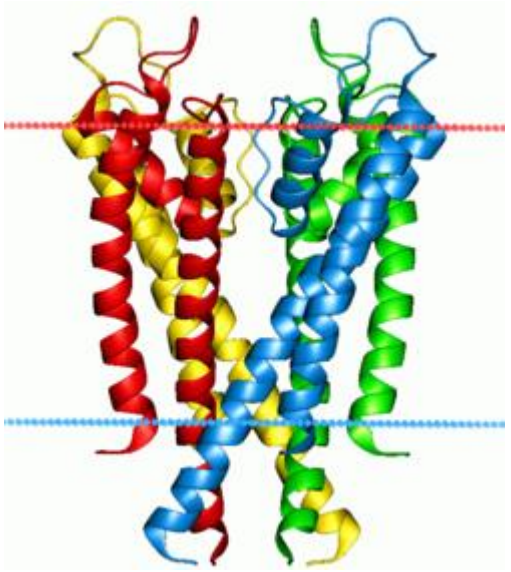
- Ion permeation in KcsA

Potassium channel KcsA



Potassium channel KcsA





KcsA is a tetramer composed of four identical subunits

Main features:

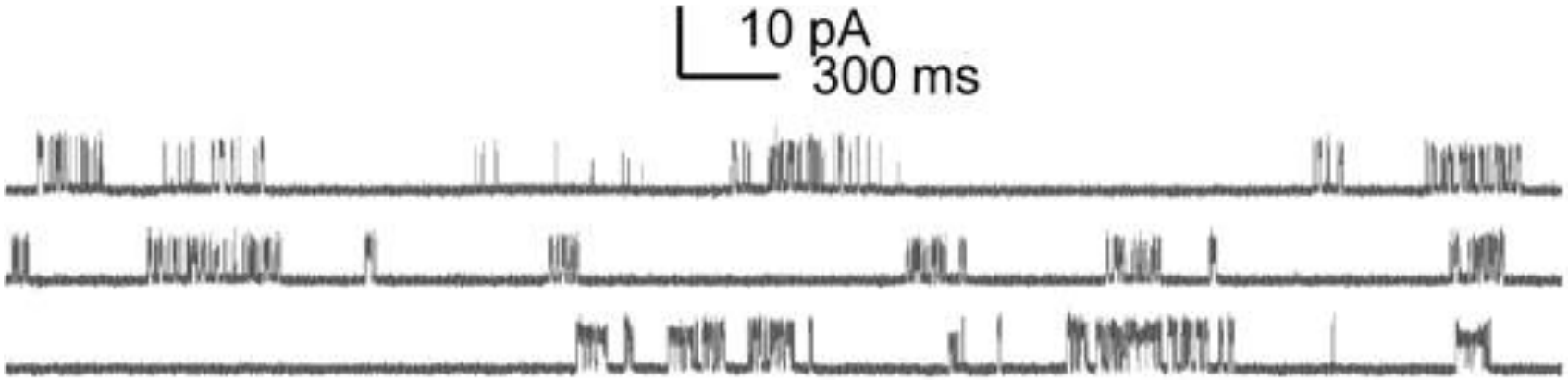
Permeation

Selectivity

Gating

Experimental data, KscA

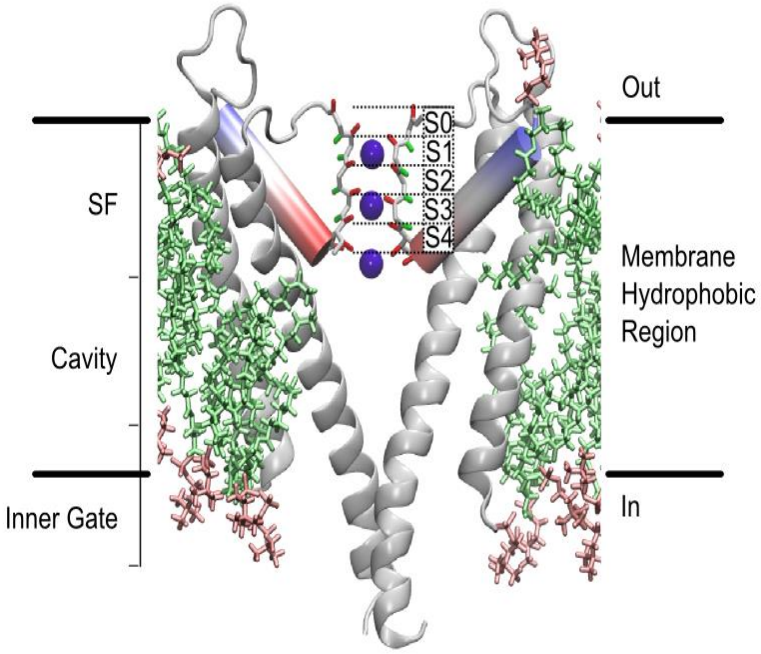
A typical single-channel Recording:

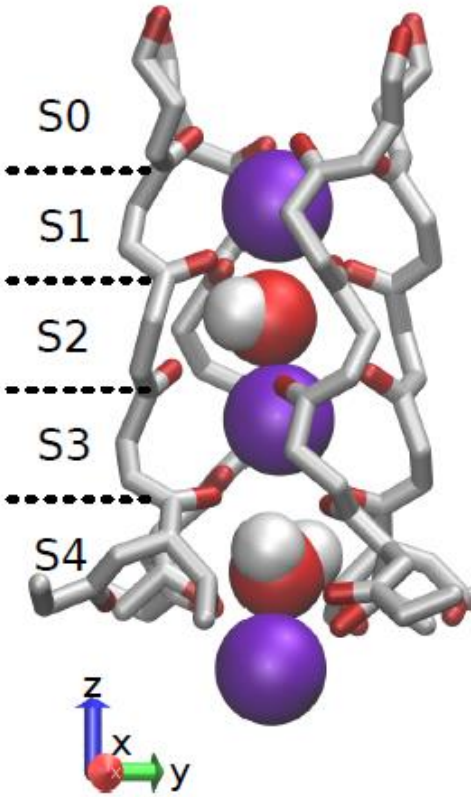


from Cordero-Morales et al., Nat. Struct. & Mol. Biol. 13 (2006).

Main features: **Permeation and Gating**

Permeation in KcsA





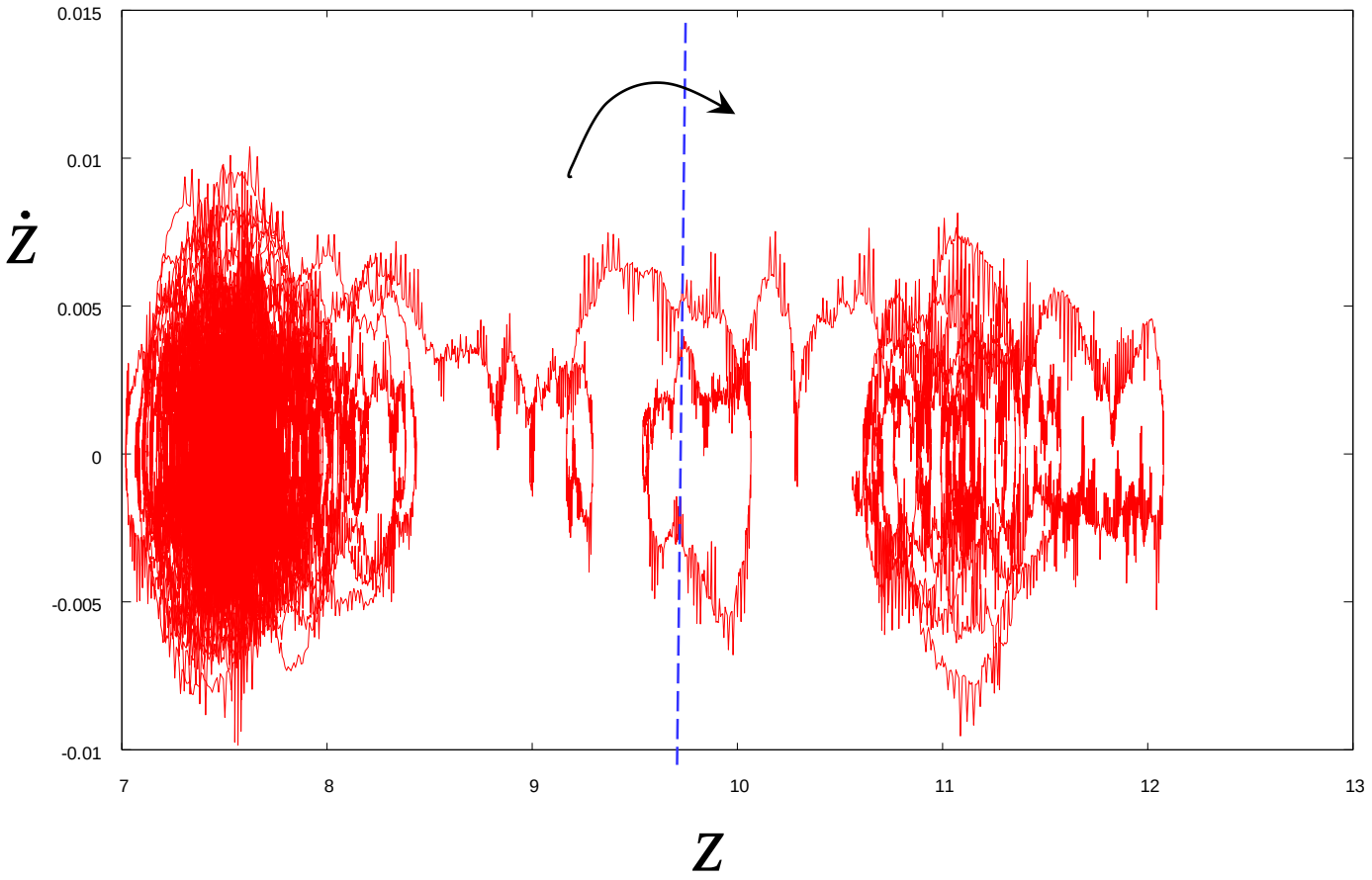
Binding sites S0, S1, S2, S3, S4 are observed experimentally and in MD simulations.

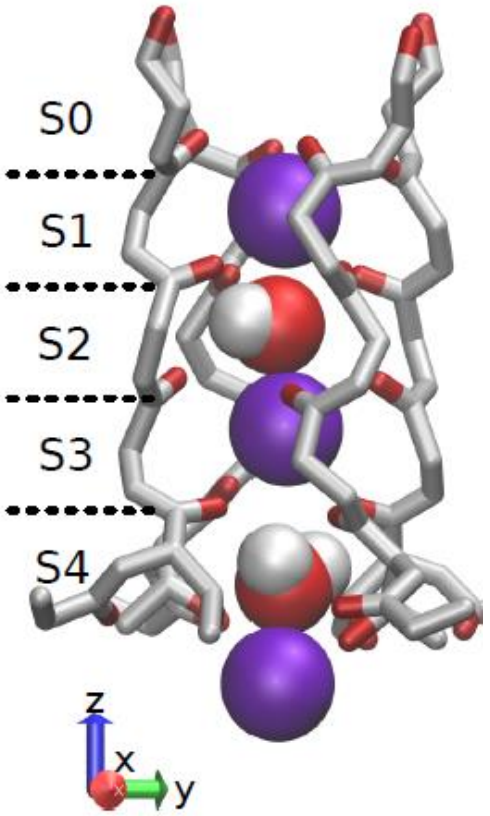
A permeation event corresponds to transition of ion(s) from one site to another.

Two-ions in the selectivity filter is a conductive configuration.

Ions activation dynamics in MD unbiased simulations

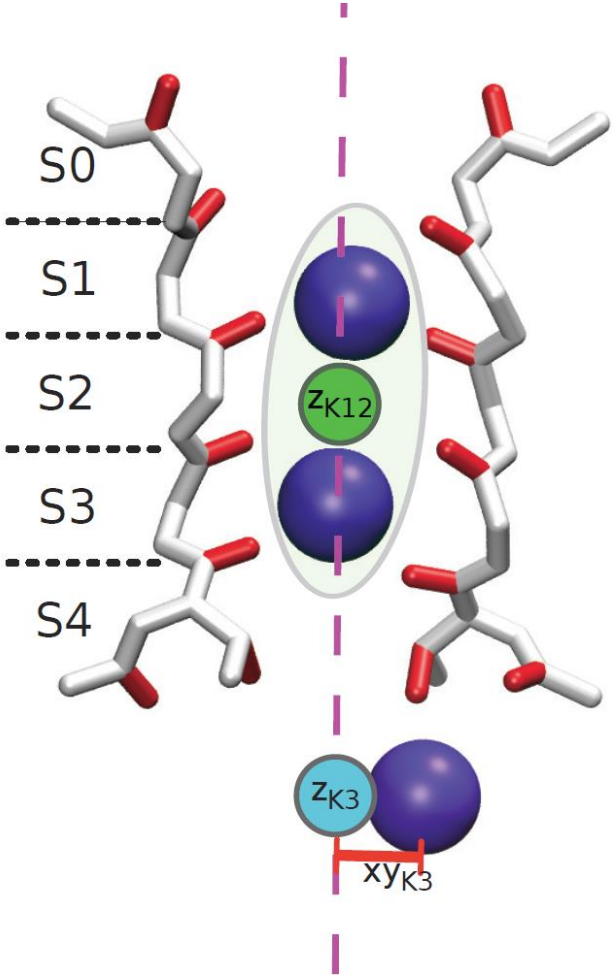
Transition from site to site





Selectivity filter

Collective variables for biased calculations



Collective variables z_{K12} and z_{K3} describe ions' permeation.

z_{K12}: the position of centre of mass of ion K1 and ion K2 along channel axis.

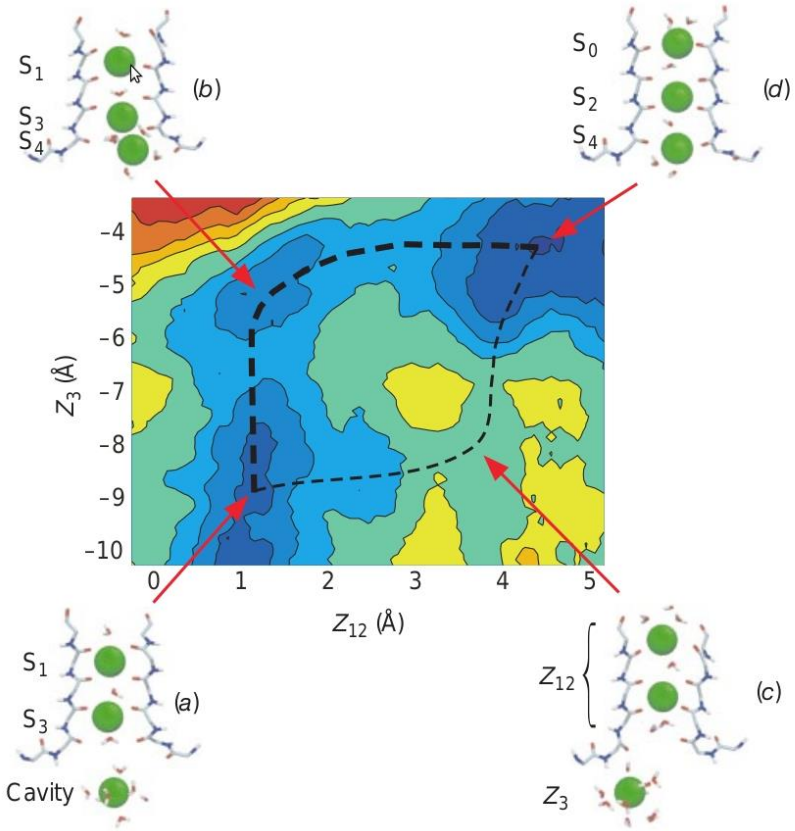
z_{K3}: the position of ion K3 along channel axis.

xy_{K3}: radial distance of ion K3 with respect of channel axis

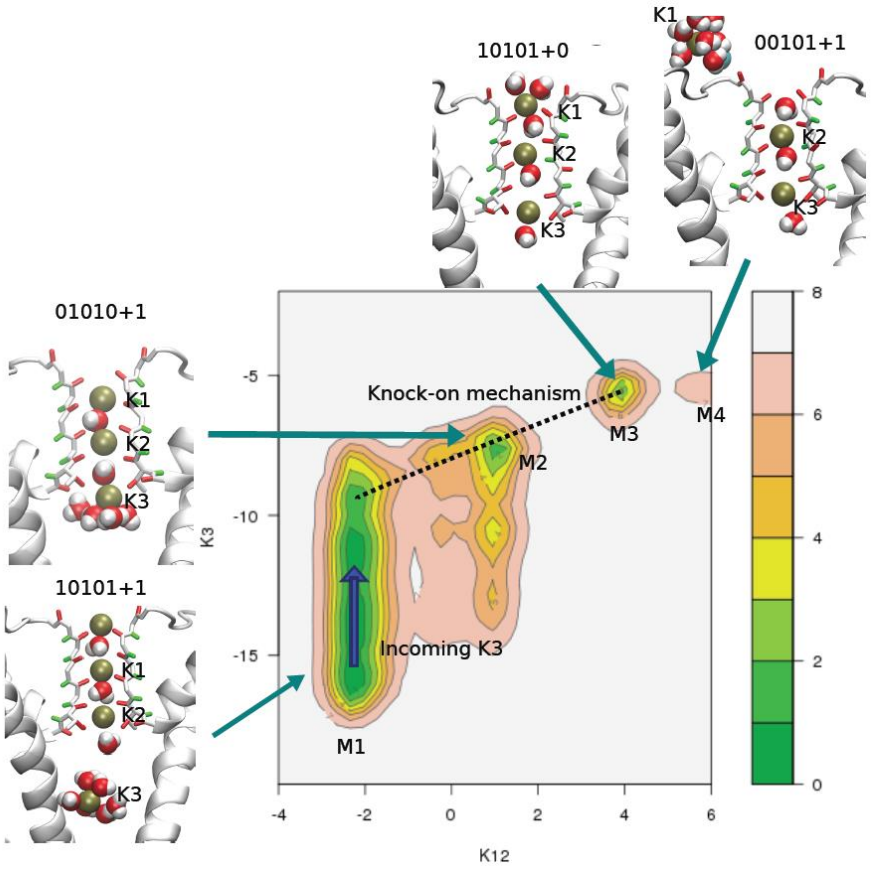
The classic theory for conduction is the **knock-on mechanism**, firstly proposed by Hodgkin and Keynes (1955): the driving factor is an incoming ion that 'knocks on' ions already bound within the selectivity filter. **Knock-on assumes zero barrier.**

Difference in PMF

2-dimensional Umbrella Sampling
Berneche and Roux, Nature 2001



Our calculations, MetaDynamics

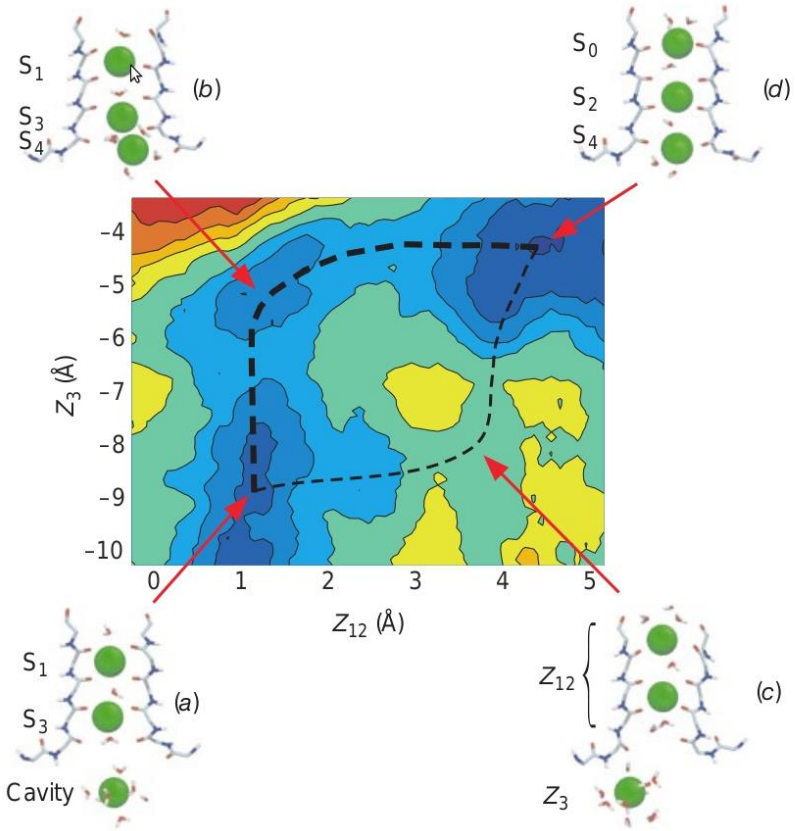


Barrier 6-7 kcal/mol

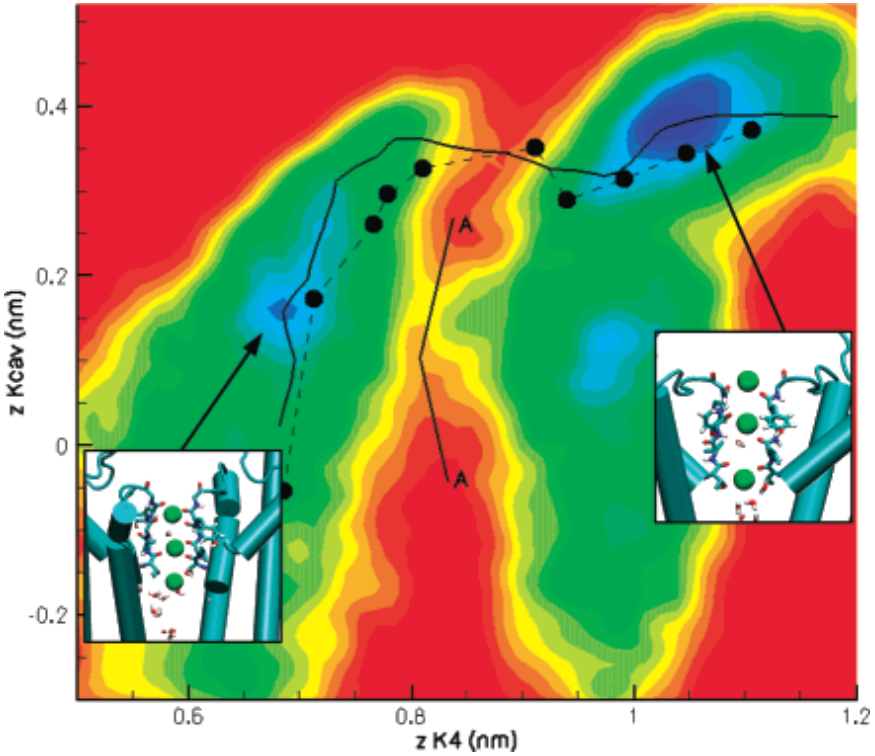
Barrier 2-3 kcal/mol, order of noise intensity

Difference in PMF

2-dimensional Umbrella Sampling
Berneche and Roux, Nature 2001



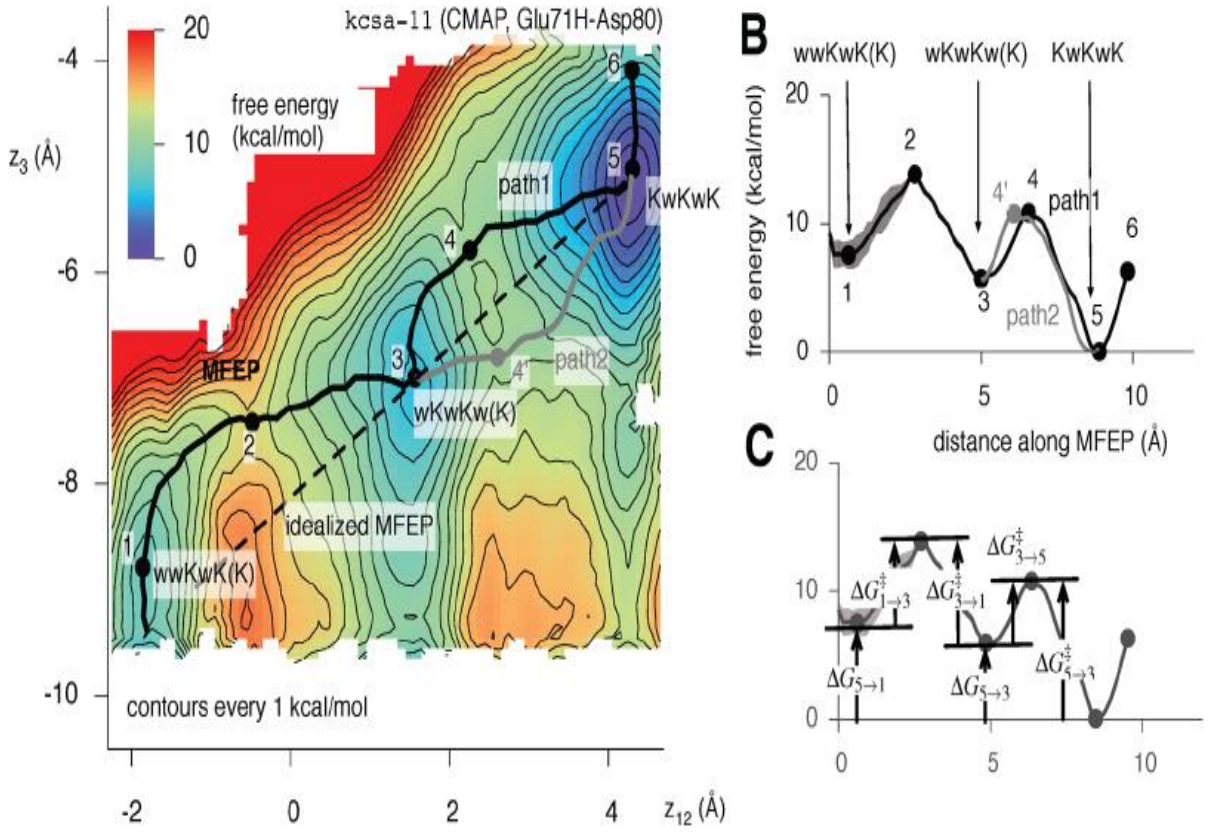
Piccinini etal, J. Chem. Theory Comput. 2008, MetaDynamics



Barrier 9-10 kcal/mol

Barrier 2-3 kcal/mol, order of noise intensity

Ion permeation and PMF. State of the art



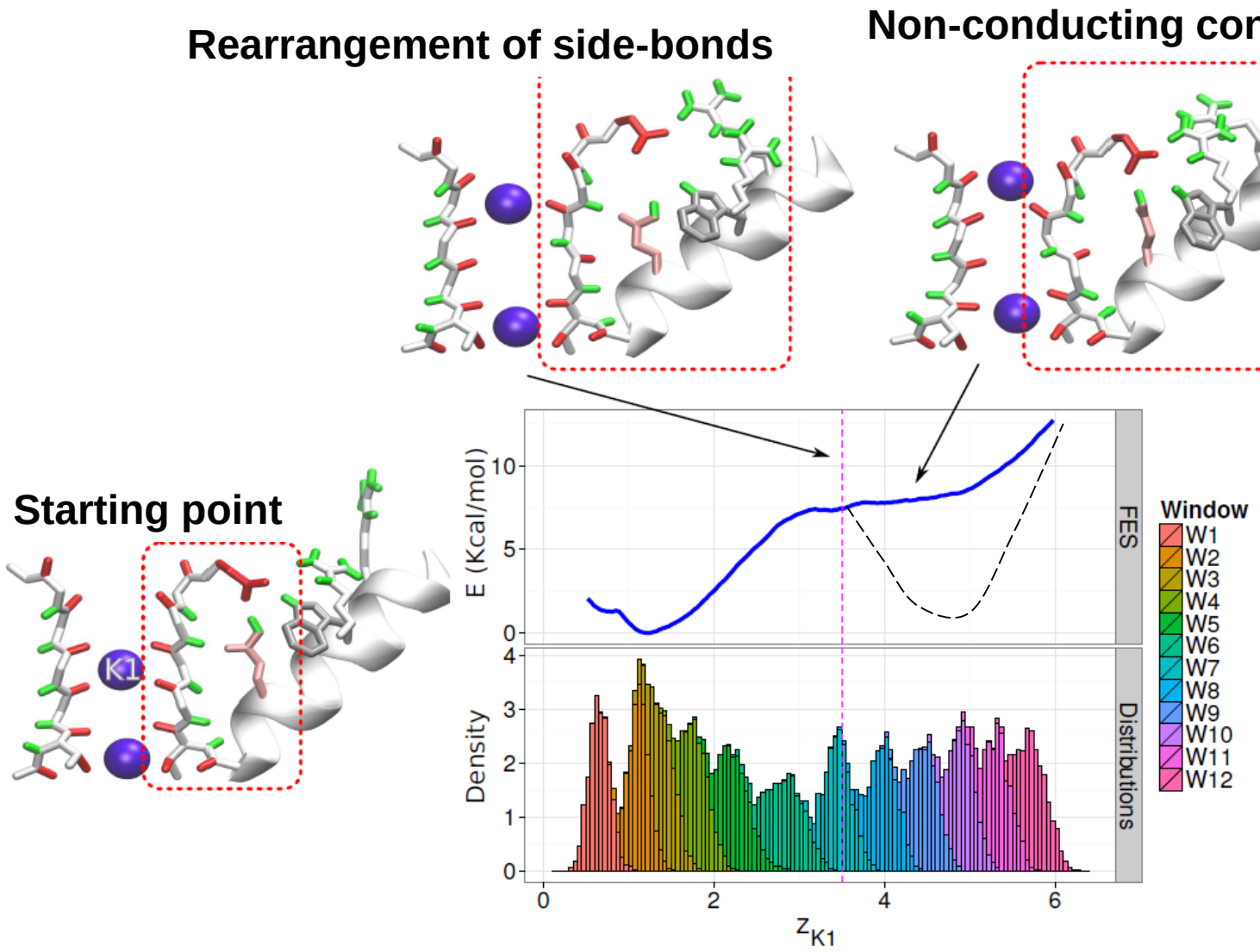
From
P.W. Fowler et al
J Chem Theory Comput
2013, 9, 5176-5189.

Abstract. "… the heights of the kinetic barriers for potassium ions to move through the selectivity filter are, in nearly all cases, *too high to predict conductances in line with experiment*. This implies it is **not** currently feasible to predict the conductance of potassium ion channels, but other simpler channels may be more tractable."

Outline

- Molecular Dynamics (MD) simulations of ion channels, biased methods
- Permeation in KcsA, the knock-on mechanism and the height of the barrier
- **Issues in free energy calculations**
- Ion permeation in KcsA

Example: 1-dimensional PMF. Umbrella sampling

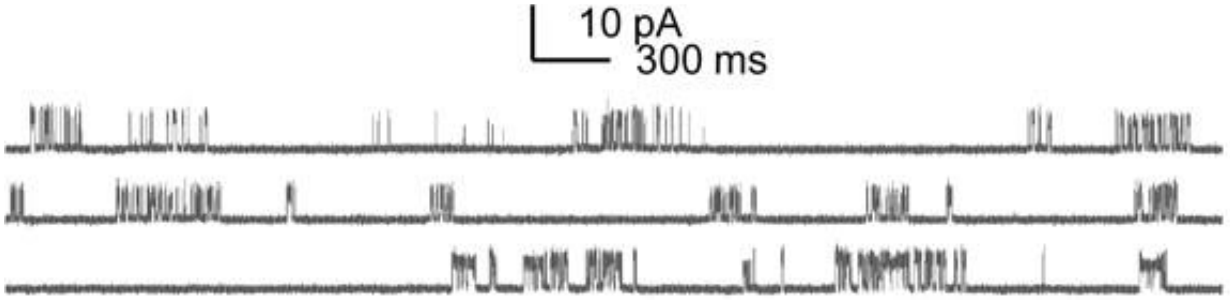


Similar picture is observed for MetaDynamics

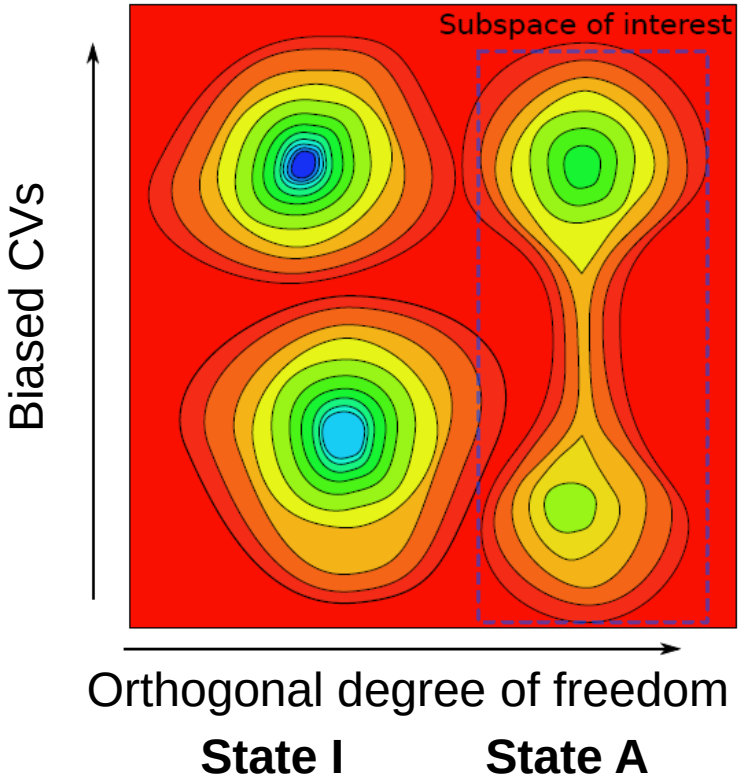
A single ion transition between sites S2 – S1.
Both sites are metastable states for an ion.

Permeation and inactivation as orthogonal degree of freedom

KcsA channel can easily switch into an inactive state in experiment.



An applied bias switch the channel into an inactive state in simulation.

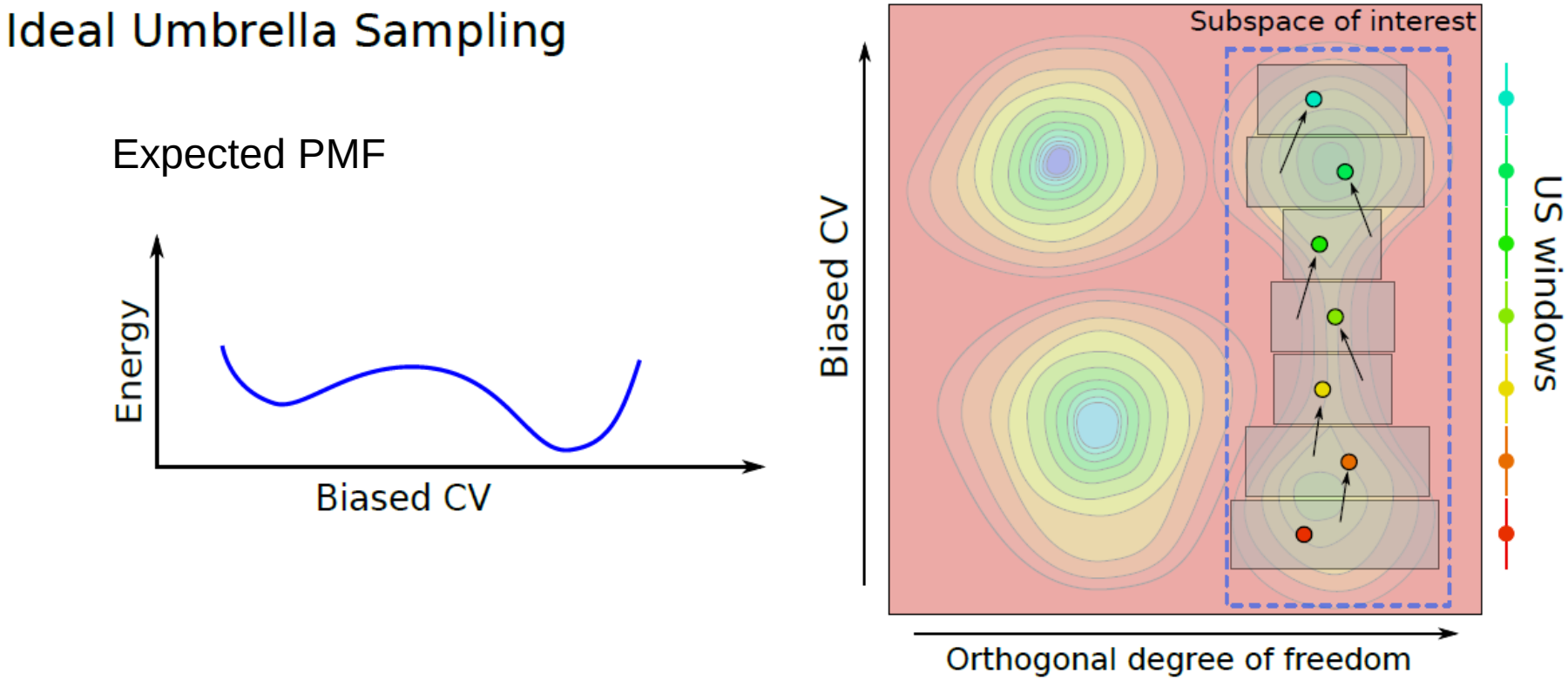


State A is a conducting state
State I is a non-conducting state

A bias leads to an exploration of an **irrelevant** state.

Let me stress that the transition to an inactive state (inactivation) has a **different** mechanism.

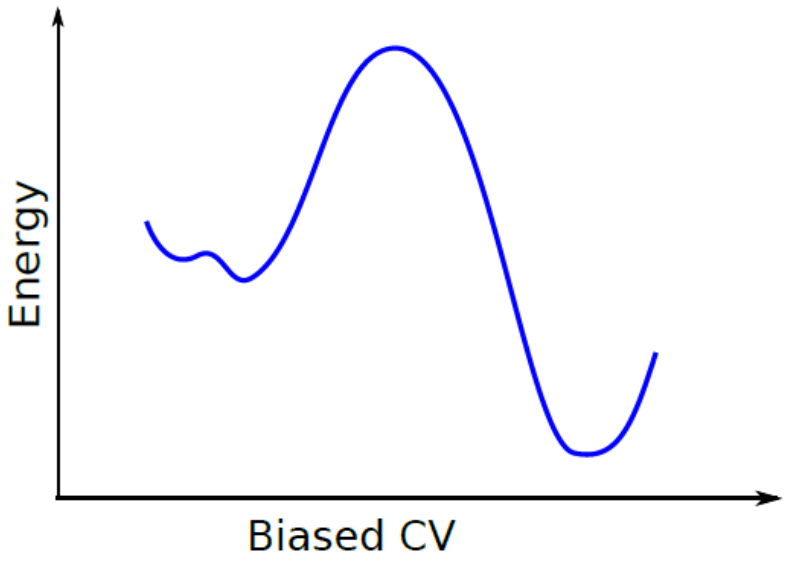
An ideal Umbrella sampling

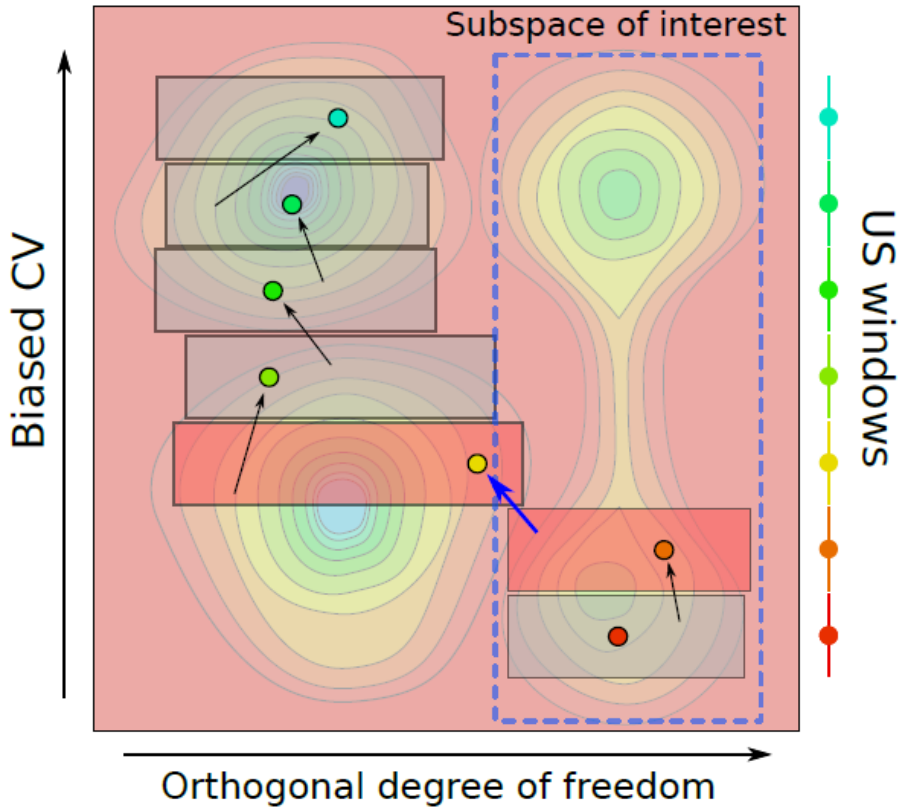


Usually we just hope to see such picture.

Pitfalls of biased methods

Obtained PMF

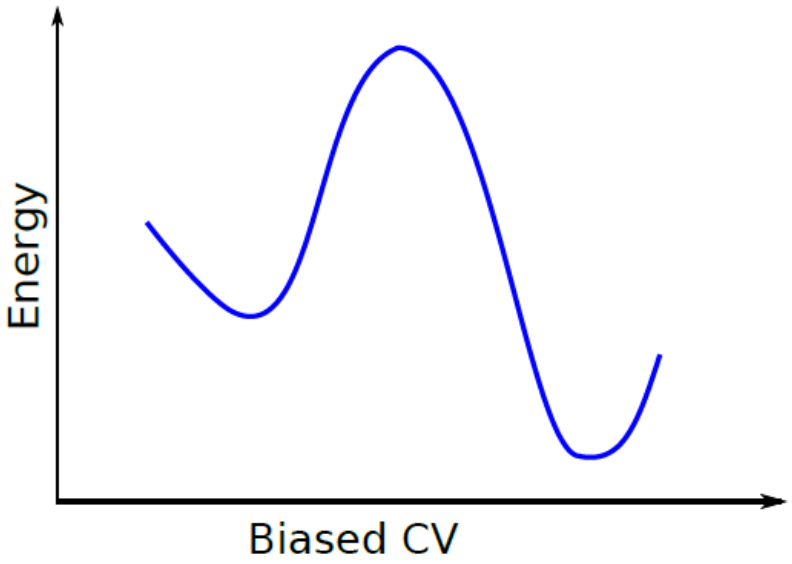


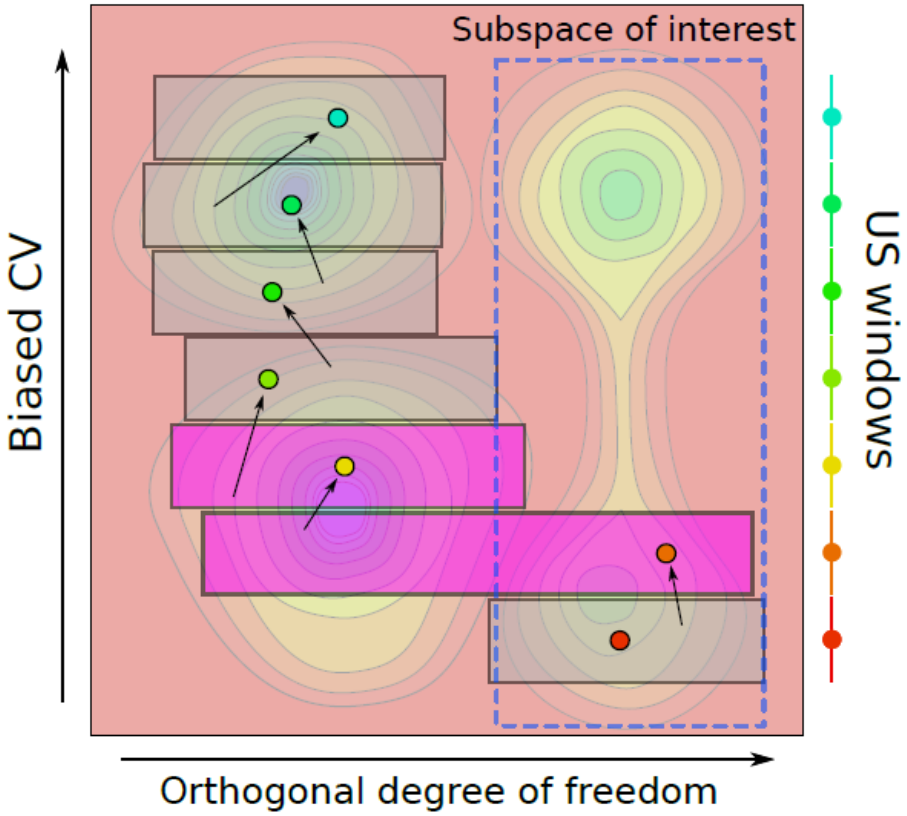


Issues in the initialisation

Pitfalls of biased methods

Obtained PMF

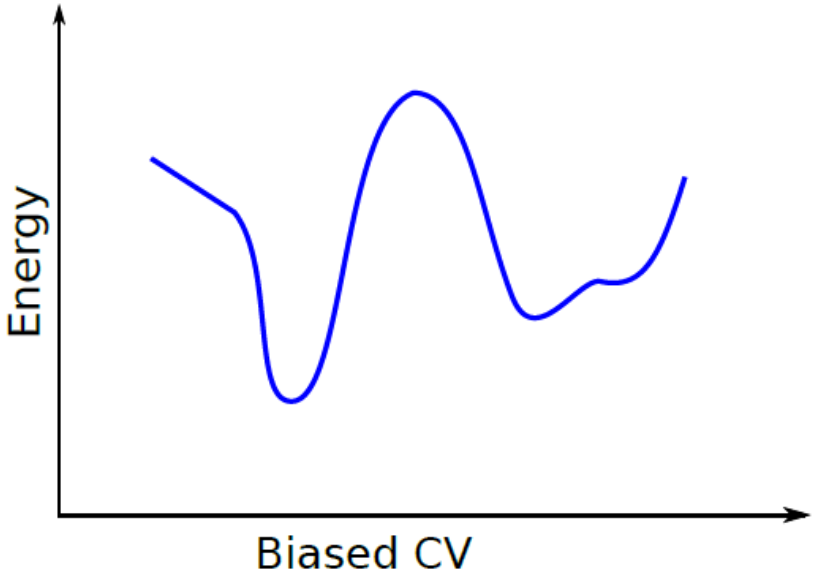


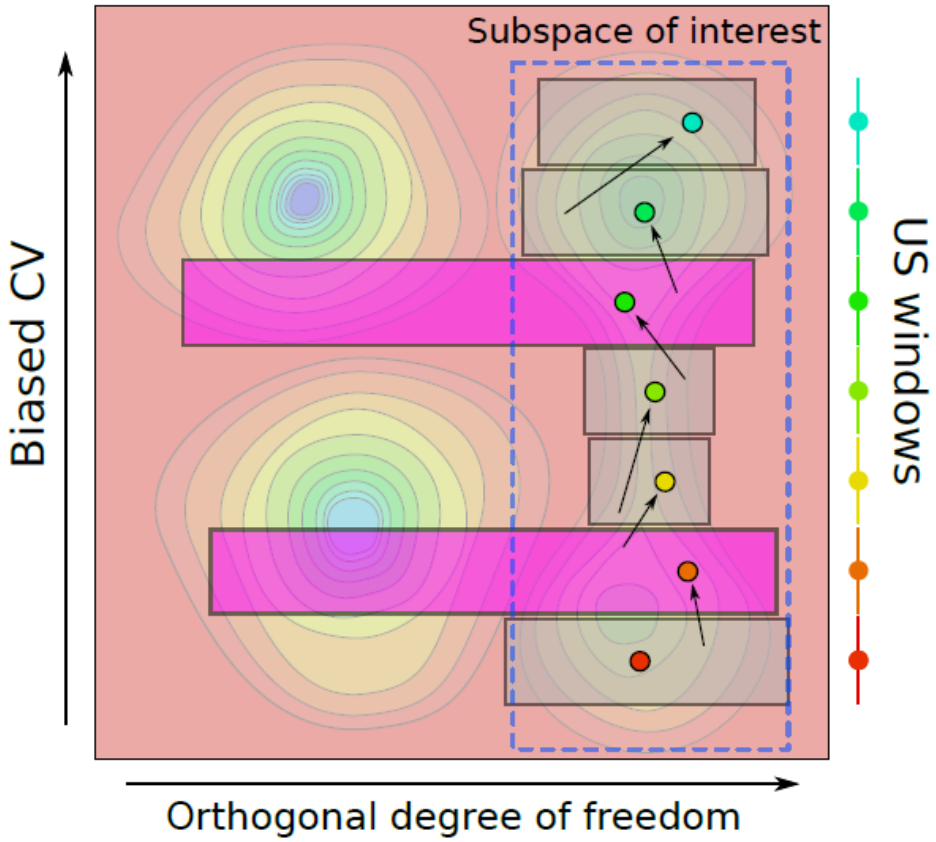


Trapping and sampling an irrelevant state

Pitfalls of biased methods

Obtained PMF





Improper sampling

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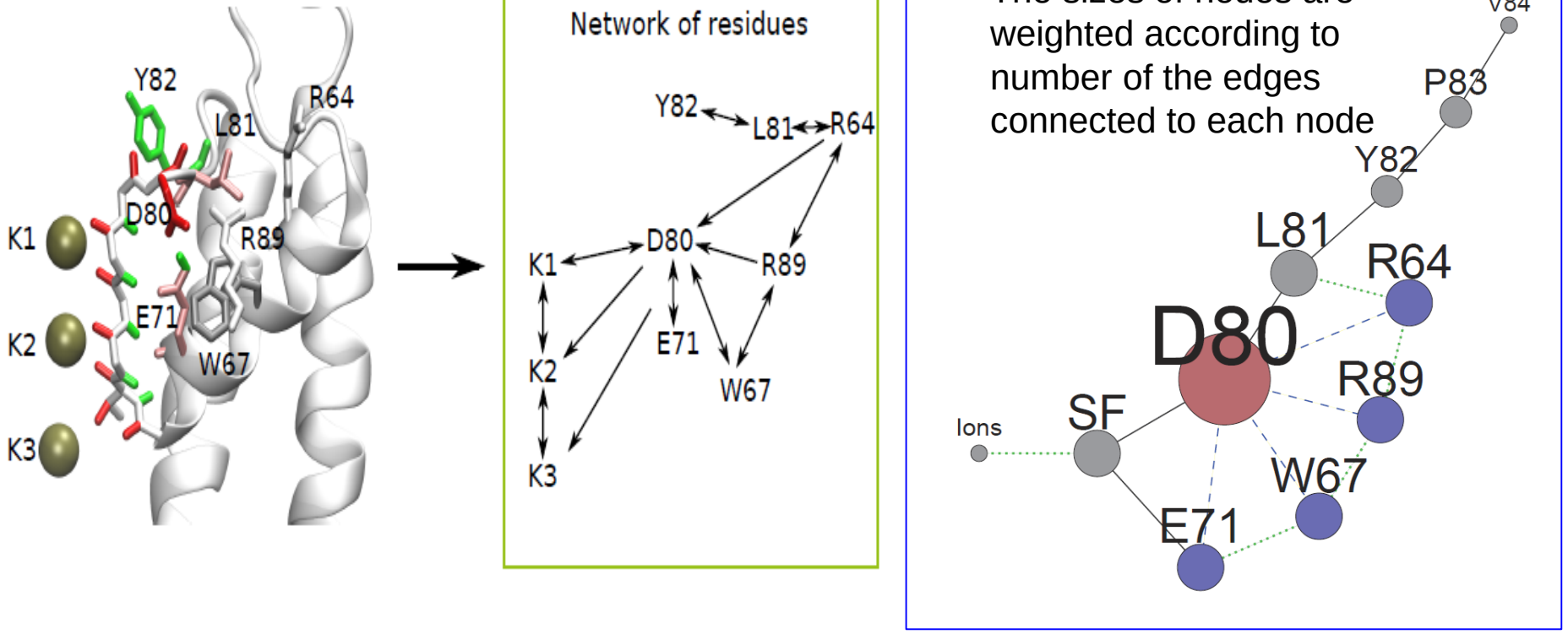
- Issues in free energy calculations
- **Ion permeation in KcsA**

Our solution

Understand quite well the whole system: dynamics of ions and side chains, study all properties conductivity, inactivation and selectivity together.

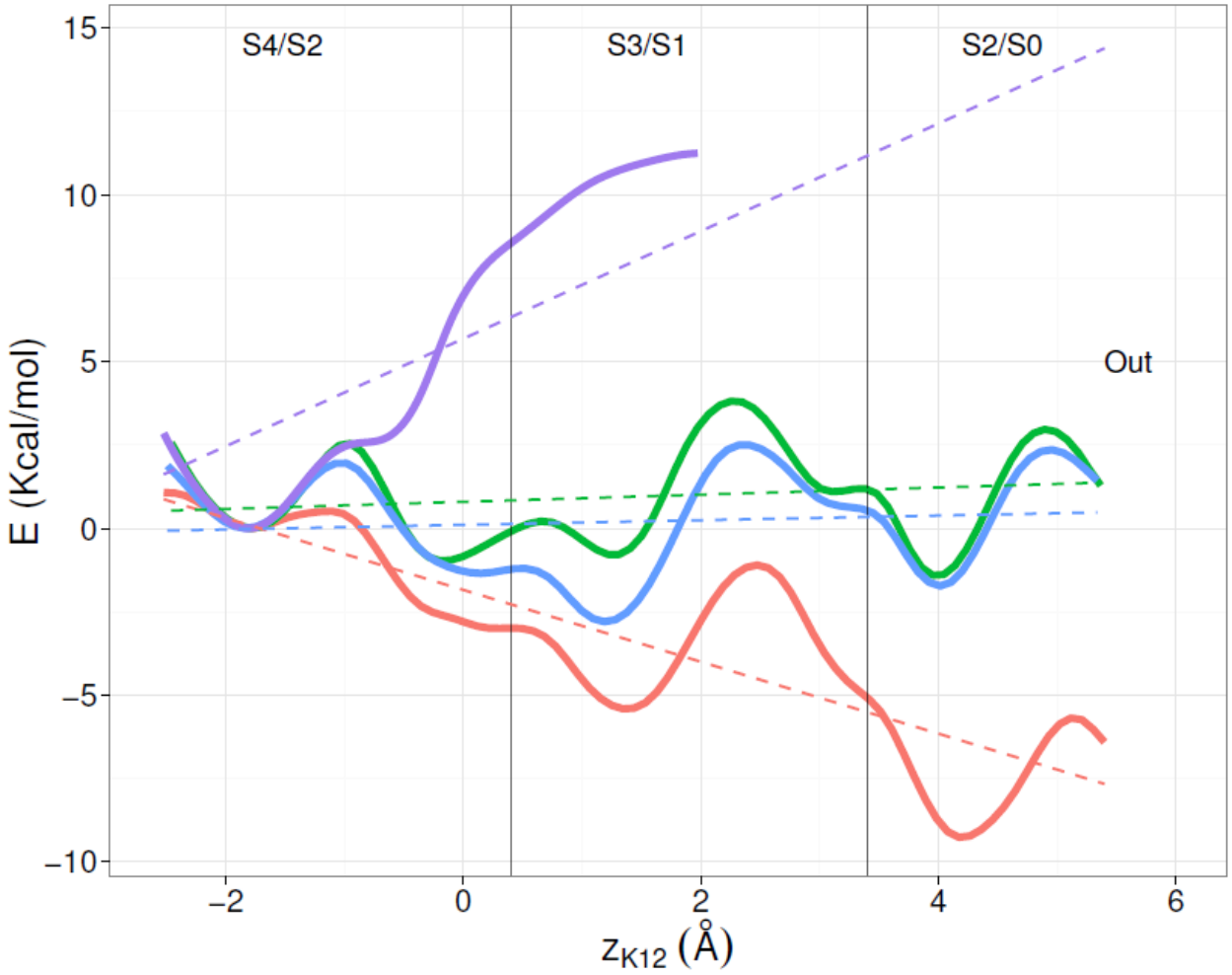
Keep the protein in **a conductive conformation** during biased MD simulation

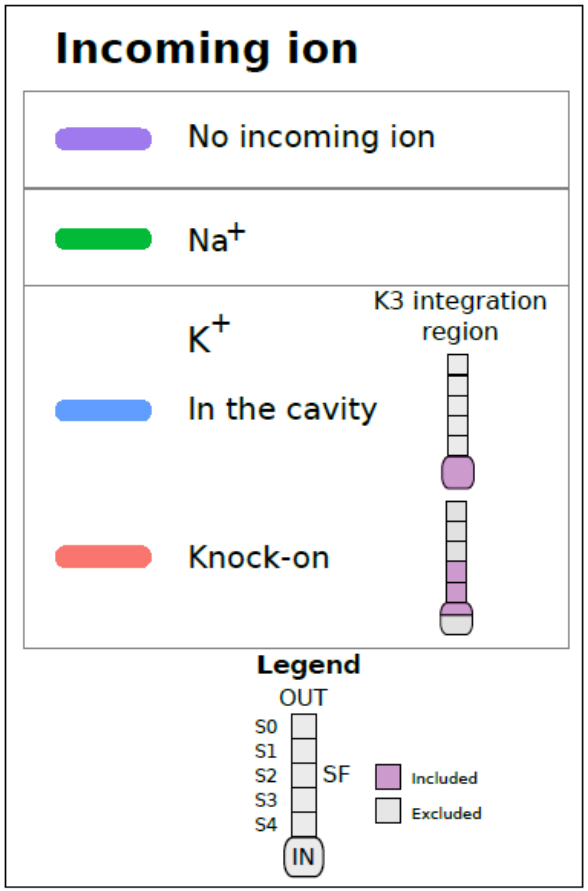


A formal approach(?)

Ion permeation in KcsA

Permeation in a conductive conformation. Results of well-tempered MetaDynamics (3D)





Lessons of KcsA channel

Advantage of MD technique

provide a bridge from the structure to functions

Pitfalls of MD technique

high degree of uncertainty in each step of the technique

"standard" approaches are not reliable for a formal implementation

require "experience" in the use of MD and understanding of studied systems

Further issues:

Non-Markovian effects and ergodicity

Assumptions:

"… the underlying system's dynamics **must be**

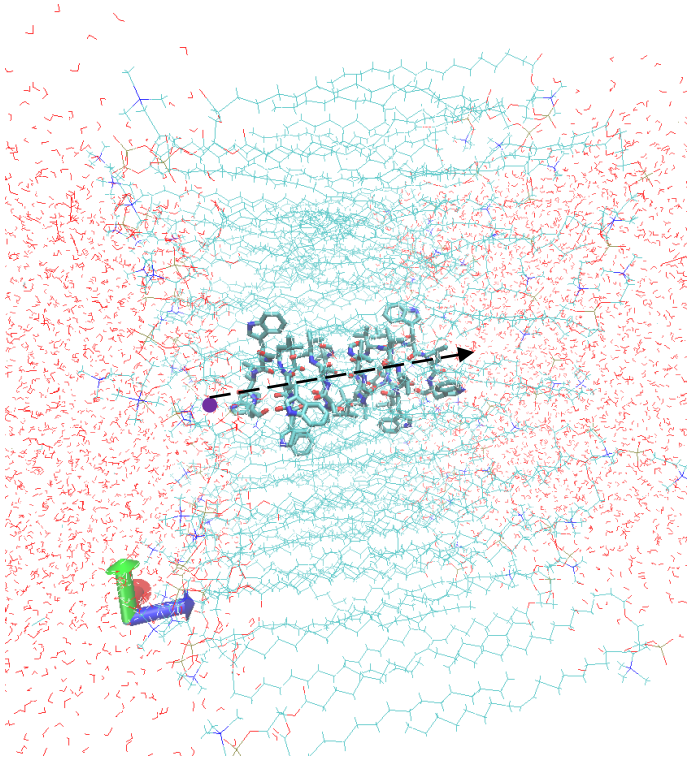
Markovian and **Ergodic**

for any bias…"

Easy to check!

(see The European Physical Journal Special Topics 222(10) ·2595-2605 2013; wrap.warwick.ac.uk/57511)

Potential of mean force (PMF) from MD simulations



All-Atom Molecular Dynamics (MD)

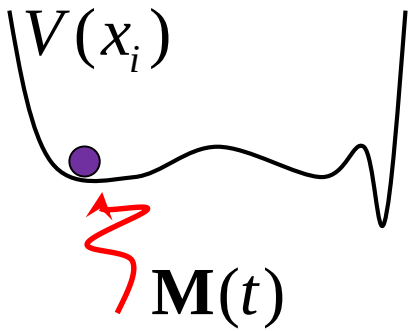
Hamiltonian Equation:

$$H = \sum_{k=1}^{N} \frac{\mathbf{p}_k \cdot \mathbf{p}_k}{2m_k} + U(\mathbf{r}_1, \mathbf{r}_2, \ldots \mathbf{r}_N)$$

$$\frac{d\mathbf{r}_i}{dt} = \frac{\partial H}{\partial \mathbf{p}_i} \equiv \frac{\mathbf{p}_i}{m_i}, \quad \frac{d\mathbf{p}_i}{dt} = -\frac{\partial H}{\partial \mathbf{r}_i} \equiv -\nabla_{\mathbf{r}_i} U$$

$$N \sim 10^6 - 10^7$$

One-Atom Brownian Dynamics (BD). **Generalized Langevin Equation:**



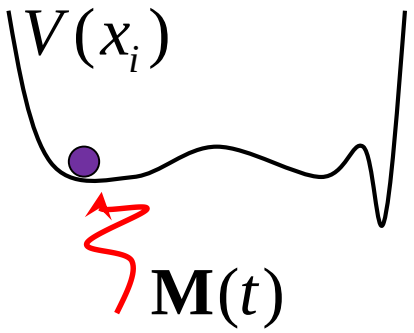
$$m_i \dot{\mathbf{v}}_i(t) = -\frac{\partial V(\mathbf{r}_i)}{\partial \mathbf{r}_i} - \int_0^t \mathbf{M}(t-\tau)\mathbf{v}_i(\tau)d\tau + \mathbf{R}(t)$$

$$\mathbf{M}(t) = \frac{1}{k_B T}\langle \mathbf{R}(0)\mathbf{R}(t) \rangle$$

Memory kernel

Markovian vs Non-Markovian Dynamics

One-Atom Brownian Dynamics (BD). **Generalized Langevin Equation:**



$$m_i \dot{\mathbf{v}}_i(t) = -\frac{\partial V(\mathbf{r}_i)}{\partial \mathbf{r}_i} - \int_0^t \mathbf{M}(t-\tau)\mathbf{v}_i(\tau)d\tau + \mathbf{R}(t)$$

$$\mathbf{M}(t) = \frac{1}{k_B T}\langle \mathbf{R}(0)\mathbf{R}(t)\rangle$$

Memory function

Typical assumption (See B. Roux, M. Karplus, J. of Chem. Phys., Vol 95, 4856, 1991)

Overdapmed Markovian diffusion:

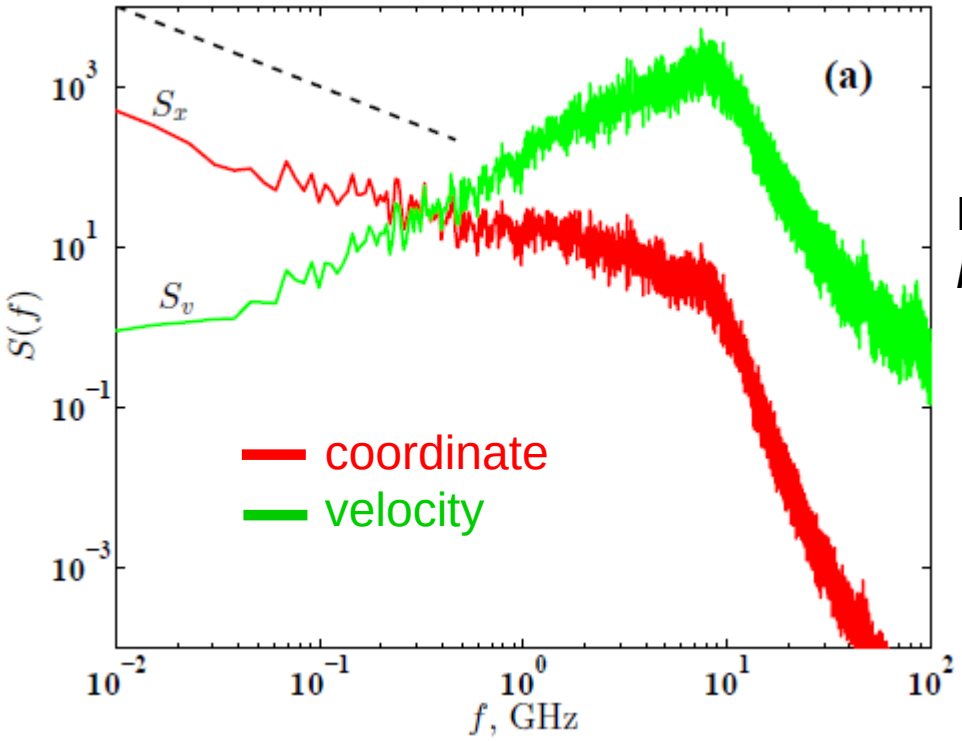
$$\dot{\mathbf{r}}_i(t) = -\frac{1}{m_i \gamma(\mathbf{r}_i)}\frac{\partial V(\mathbf{r}_i)}{\partial \mathbf{r}_i} + \sqrt{\frac{2k_B T}{m_i \gamma(\mathbf{r}_i)}}\boldsymbol{\xi}(t)$$

$\gamma(\mathbf{r}_i)$ damping coefficient

$\boldsymbol{\xi}(t)$ white Gaussian noise

Ion dynamics in the steady state, metastable state

Power spectrum



Ion demonstrates 1/f noise component:

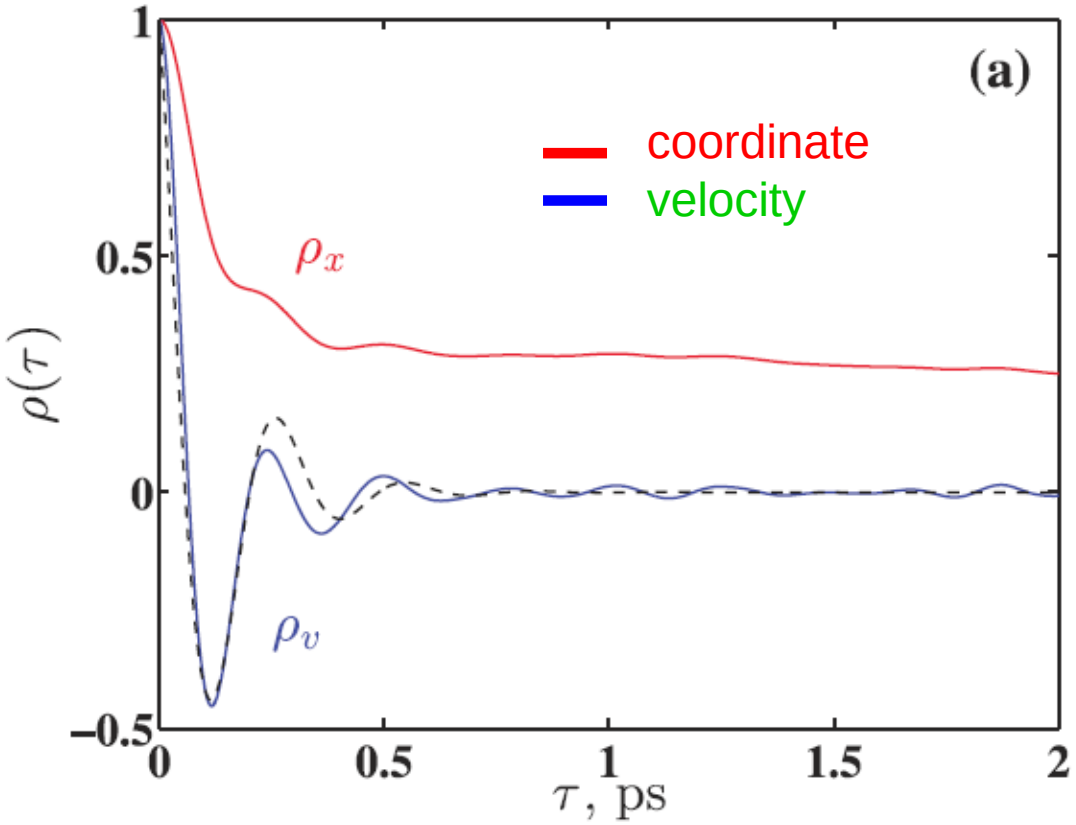
Fractional Kernel $M(t)$ and fractional noise $R(t)$ in the Generalized Langevin Equation

$$m_i\dot{\mathbf{v}}_i(t) = -\frac{\partial V(\mathbf{r}_i)}{\partial \mathbf{r}_i} - \int_0^t \mathbf{M}(t-\tau)\mathbf{v}_i(\tau)d\tau + \mathbf{R}(t),$$

1/f component indicates a strong influence of side-chains on ion states

Ion dynamics in the steady state, metastable state

Auto-correlation



Ion demonstrates the long-range (power-law) correlation

What is time-scale for averaging?

Summary of un-biased simulations

1/f component indicates a strong influence of side-chains on ion states

Ion dynamics is under-damped

Brownian dynamics model has the following form:

$$m_i \dot{\mathbf{v}}_i(t) = -\frac{\partial V(\mathbf{r}_i)}{\partial \mathbf{r}_i} - \int_0^t \mathbf{M}(t-\tau)\mathbf{v}_i(\tau)d\tau + \mathbf{R}(t)$$

$$\mathbf{M}(t) = \frac{1}{k_B T}\left\langle \mathbf{R}(0)\mathbf{R}(t)\right\rangle \propto t^{\gamma-1}$$

ESSENTIAL, check the underlying assumption