Computational Model of Gating in Proteins: Kinetic Monte Carlo Reaction Path Following

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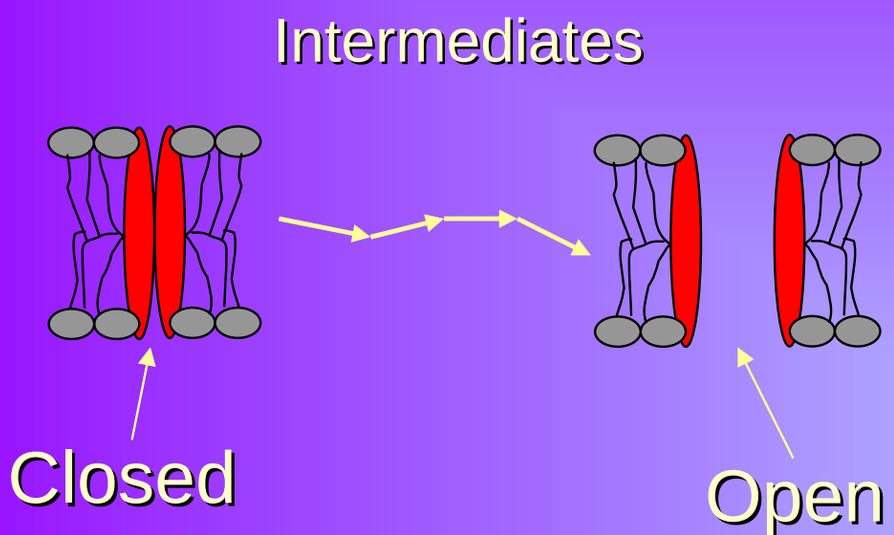
Protein Gating Problem

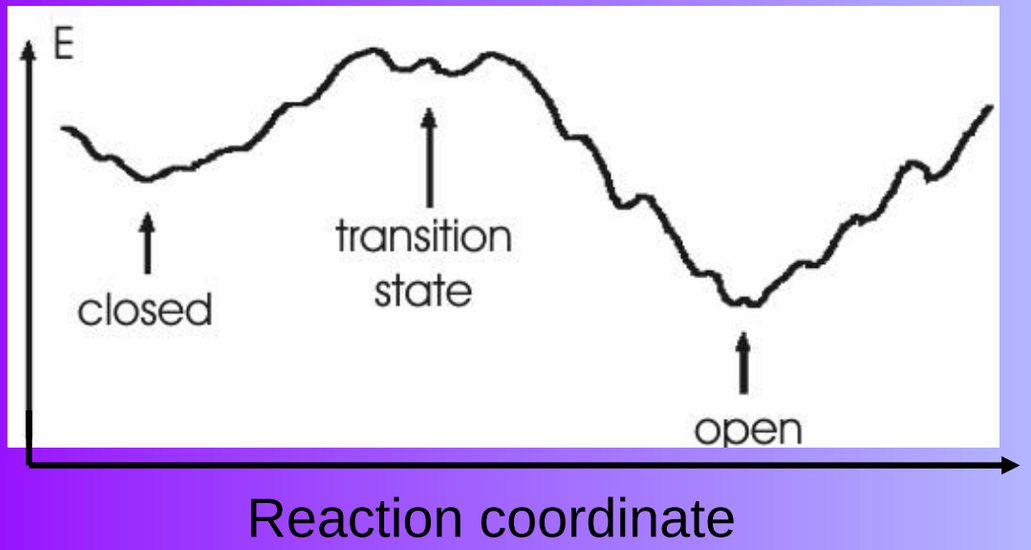


long-lived closed or open states

transition is short duration event

protein changes its conformation evolving along a gating pathway

for large proteins the reaction coordinate can not be defined by a single degree of freedom



during gating the reaction coordinate involves coupling of many of degrees of freedom

protein surrounding takes effect on a reaction coordinate

How to construct a reaction coordinate?

Steered Molecular Dynamics

simulates gating using applied external force

Examples:

- placing a balloon (vdW sphere) inside the KcsA channel and gradually inflating it to generate an open-state KcsA model (Sansom's group, BJ 2002:83(4) 1867)
- constant ''radial'' forces applied to CA atoms in the MscL channel along a vector normal from the channel axis to gate the MscL protein open (Schulten's group, BJ 2003:85(4) 2087)

Drawbacks:

- arbitrary choice of reaction coordinate
- too short timescales (nano-seconds) for gating transitions
- high pulling speeds (6–9 orders of magnitude too rapid)
- non-equilibrium process with strong energy dissipation

Principal Component Analysis

- **phase space reduction method for proteins**
- **extracts essential degrees of freedom from MD trajectory**

Example:

"dimer-of-dimers like motion" was proposed as a possible gating mechanism in the Kir channels based on extraction of principal components from 10-ns MD trajectories (Sansom's group, BJ 2005:88(5) 3310)

However …

there is an analytic proof that principle component analysis does not reveal any reliable information on time scales which are not actually sampled; long time dynamics (slow modes) can not be determined reliably (Schulten's group, J. Phys. Chem. 1996:100(7) 2567)

Purpose:

Predict the protein transition state structures at the amino acid level along gating pathways

methods that will focus sampling on a relatively brief transition event between long-lived stable states

1. method to determine a reaction coordinate separating the relevant degrees of freedom from orthogonal variables which might be regarded as random noise
2. method to evolve a complex protein system along a reaction coordinate
3. method to calculate the free-energy profile along a reaction coordinate

finally, other special purpose standard techniques can be used to evaluate the transition rates and kinetics

kinetic Monte Carlo Reaction Path Following

- uses Metropolis Monte Carlo (MMC) technique
- applies unidirectional constraint on evolution of the system along a predefined degree of freedom (reaction coordinate)
- lowest-energy downhill or Boltzmann-weighted uphill configurations along the transition path are only accepted
- moves are thermally activated (constrained MMC method)
- trial moves are sampled as in a kinetic MC (kMC) method, but move steps are not fixed

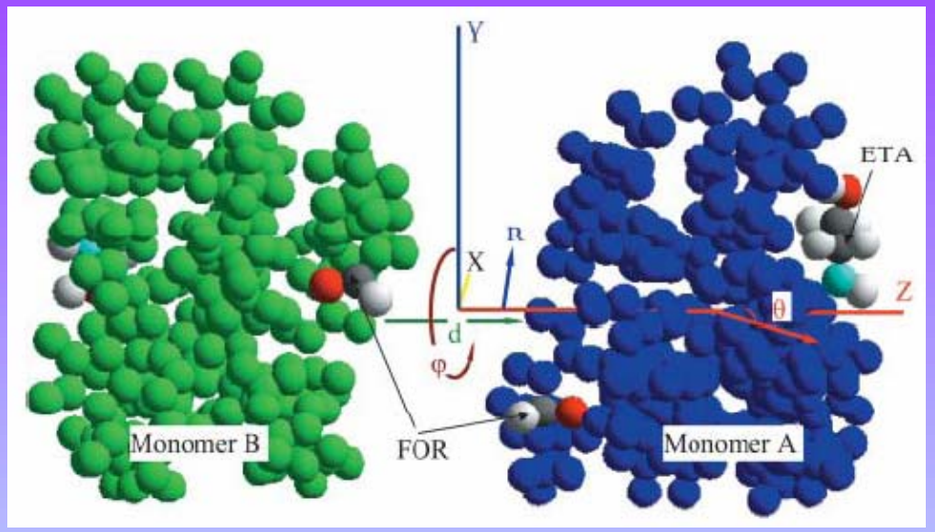
kMCRPF:

- evolves the system along a reaction coordinate by small kMC moves
- determines protein transition state conformations along the reaction pathway

*Miloshevsky & Jordan (2005) J. Chem. Phys. **122**:214901*

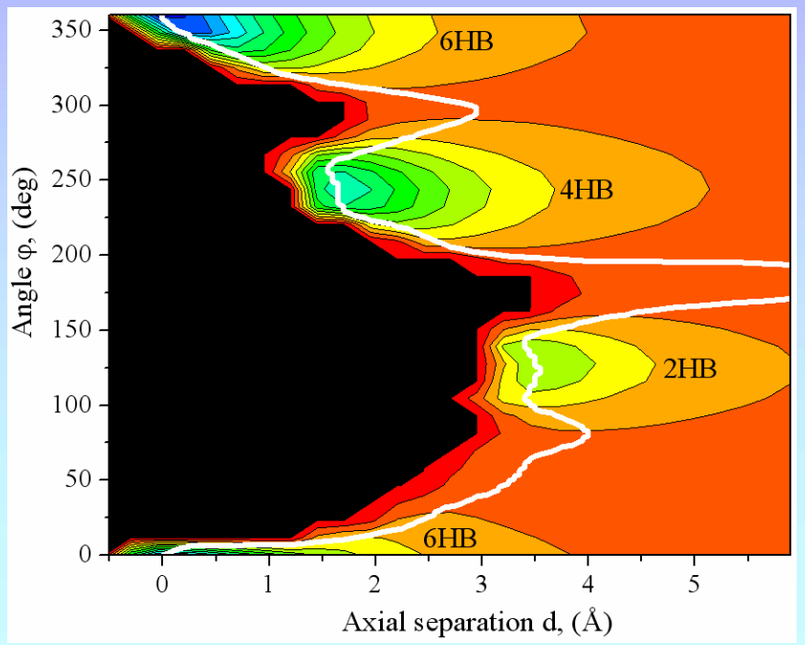
GRAMICIDIN GATING

Molecular Details Of Gramicidin Gating



- gating in gramicidin is transition between multiple open and closed states through coupled monomer rotation and lateral displacement

- stable 6HB, 4HB and 2HB states correspond to open pores



- intermediates correspond to closed pores

- conductance pathway is interrupted and reformed during gating

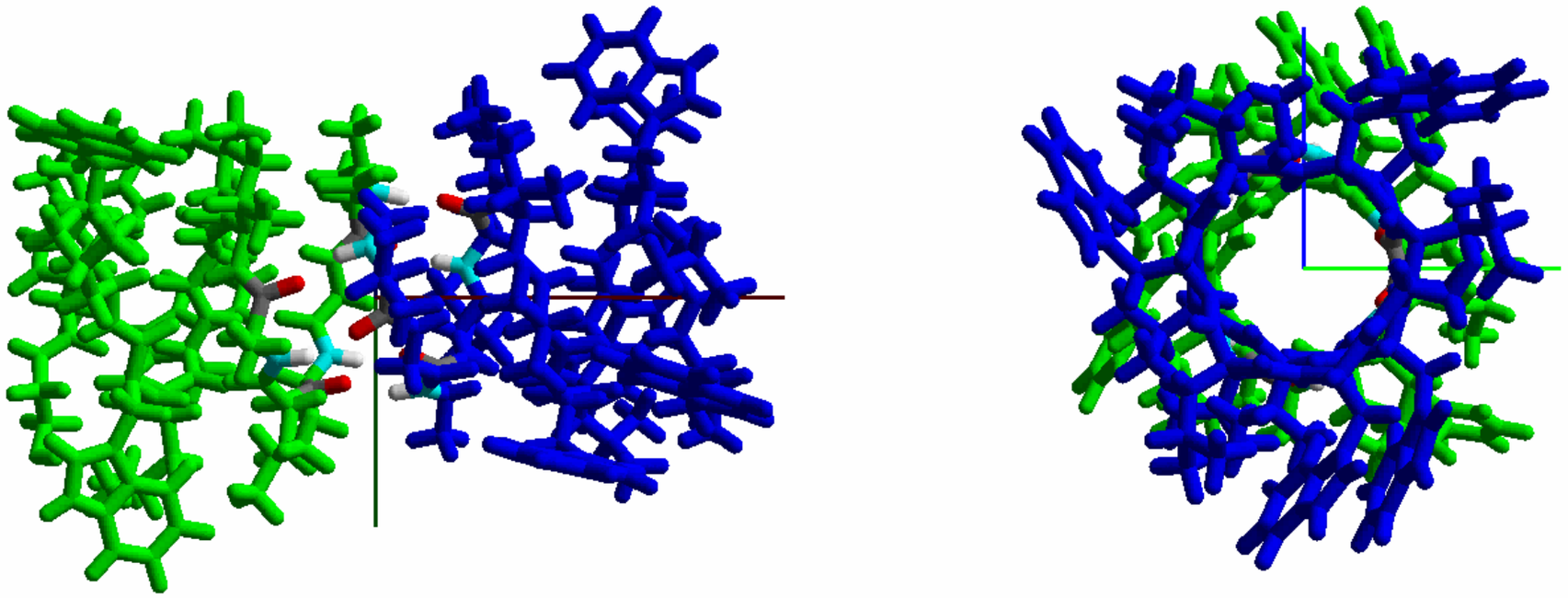
- fluctuations (flickers) in the open states

- gramicidin dimer undergoes rotational and translational diffusion in the membrane

Miloshevsky & Jordan (2004) Biophys. J. 86:92

GRAMICIDIN GATING

Molecular Details Of Gramicidin Gating



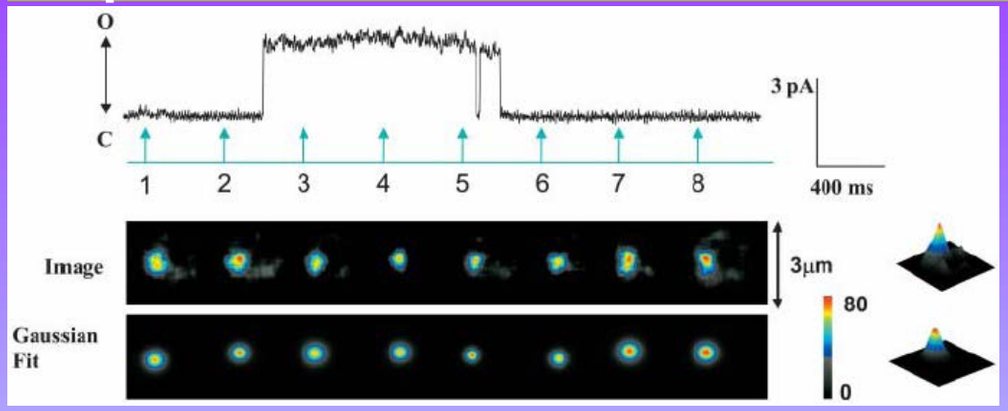
1) large hydrophobic mismatch greatly aids the formation of 4HB and 2HB states in a thick membrane

2) direct dissociation due to the membrane elastic force pulling the monomers apart was observed only from 4HB and 2HB states, but not from 6HB state

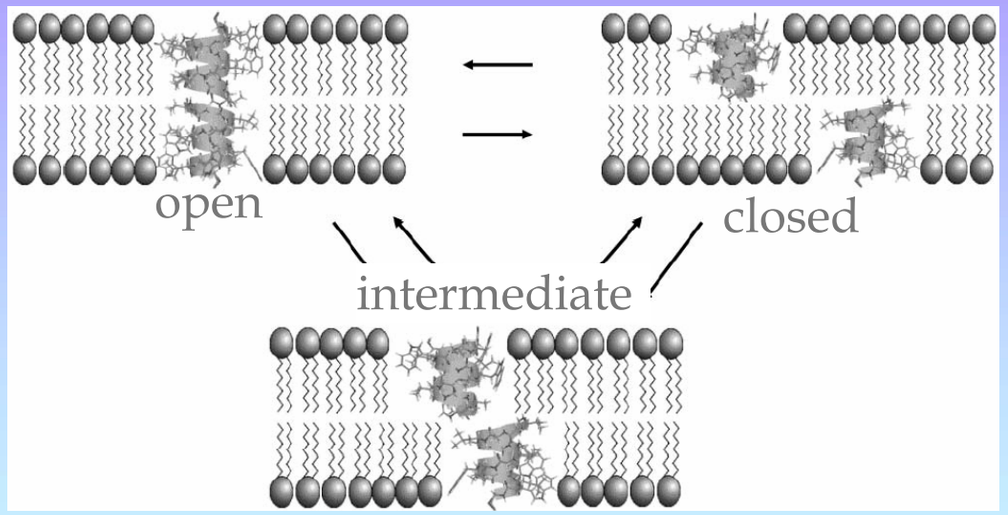
Miloshevsky & Jordan (2004) Biophys. J. 86:92

GRAMICIDIN GATING

Experimental Evidence Of Conformational Changes



Patch-clamp Fluorescence Microscopy - single-molecule fluorescence spectroscopy & single-channel current recordings



- multiple conformational states
- intermediate conformers result from the six hydrogen bond fluctuations
- variation in channel conductance with no significant change in inter-monomer separation
- inhomogeneous and spatially confined dynamics and kinetics
- channel can be in closed states even when the monomers are still in an intermediate dimerized state

Harms et al. (2004) Appl. Phys. Lett. 84:1792

Harms et al. (2003) Biophys. J. 85:1826

Reaction Path-finding Strategy

1. equilibrate the protein in a particular stable conformation
2. construct a harmonic approximation of the global potential well around this conformation
3. use normal mode analysis to identify low-energy modes that describe collective domain motions of protein
4. associate a lowest-energy normal mode vector (indicates direction and amplitude) with a reaction coordinate
5. use kMCRPF to progress the collective motion along a reaction coordinate by a random amount of amplitude
6. after a trial move is accepted by a MMC criterion, equilibrate the molecular system
7. use this new protein transition state conformation as a starting point and go to step 3

Methods & Implementations

✓ Fast Multipole Methods (FMM) to treat long-range electro-static effects; computation cost scales as O(N log N) or O(N)

✓ FMM incorporated in Boundary Element Solver to treat efficiently the effects of the reaction field

✓ preferential sampling methods to focus computation on collective protein domain motions and their neighborhood

✓ rigid protein group decomposition method partitioning protein chains into rigid blocks of one or more residues to calculate efficiently low-frequency normal modes

✓ efficient implementation of numerical methods to solve the eigenvalue problem with the Hessian matrix

SUMMARY

- kMCRPF method samples gating transitions along the predefined degree of freedom (reaction coordinate) rapidly and correctly; open or closed initial protein conformation is only required

- kMCRPF predictions of the gating process in gramicidin channels are in good agreement with experimental data

- kMCRPF method supplemented with path-finding technique will simultaneously define a reaction coordinate, progress along it, and determine the transition state structures and the free energy profiles in large proteins

Acknowledgements

Prof. Peter Jordan

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