

National Center for Multiscale Modeling of Biological Systems

Virtual Hands-on Workshop on Computational Biophysics

October 12-15, 2020

The workshop will cover a wide range of physical models and computational approaches for the simulation of biological systems at multiple scales, consisting of both theoretical and hands-on lectures.

Instructors:

Ivet Bahar, PhD
Emad Tajkhorshid, PhD
Pemra Doruker, PhD

Software:

*ProDy API, NAMD,
FFTK, VMD, DynOmics,
NMWiz, and DruGUI
plugins for VMD &
QwikMD*



Carnegie
Mellon
University



This workshop is supported by NIH-NIGMS P41GM103712. Please see mmbios.org for additional information.



Emad Tajkhorshid

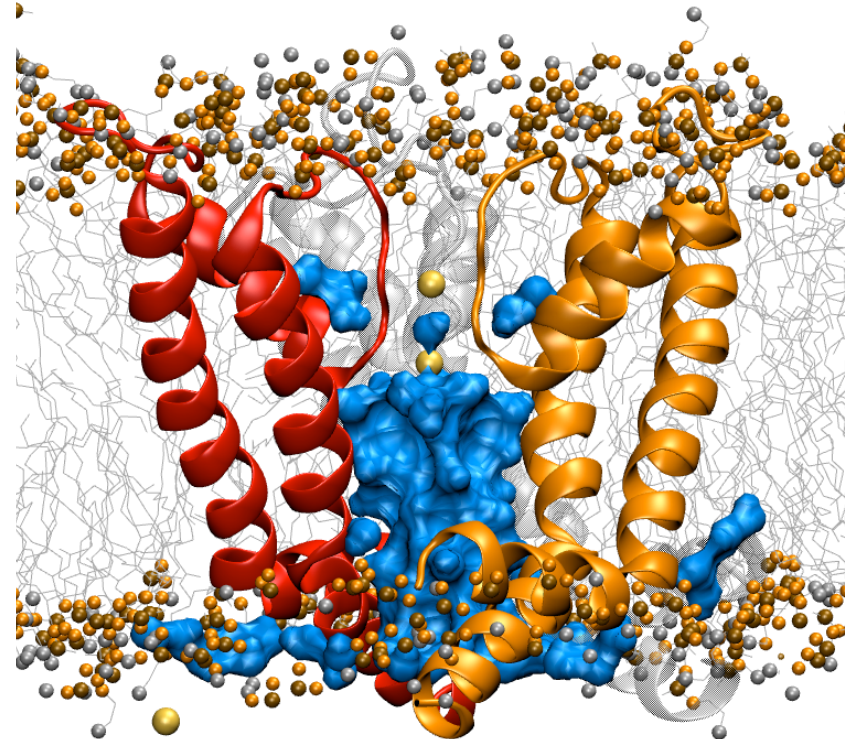
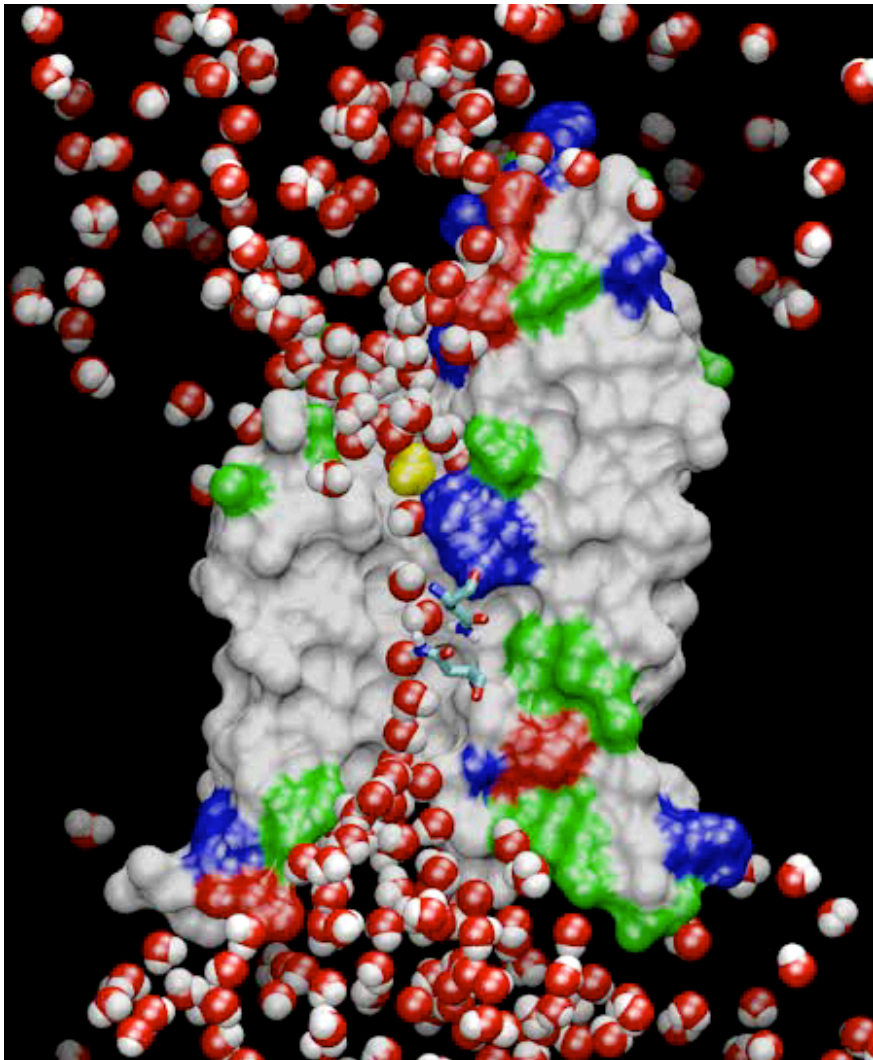
NIH Center for Macromolecular Modeling and Bioinformatics
Beckman Institute for Advanced Science and Technology
University of Illinois at Urbana-Champaign

Investigating Biological Membranes and Membrane Proteins Using Advanced Simulation Technologies



Emad Tajkhorshid

NIH Center for Macromolecular Modeling
and Bioinformatics, Beckman Institute,
University of Illinois at Urbana-Champaign

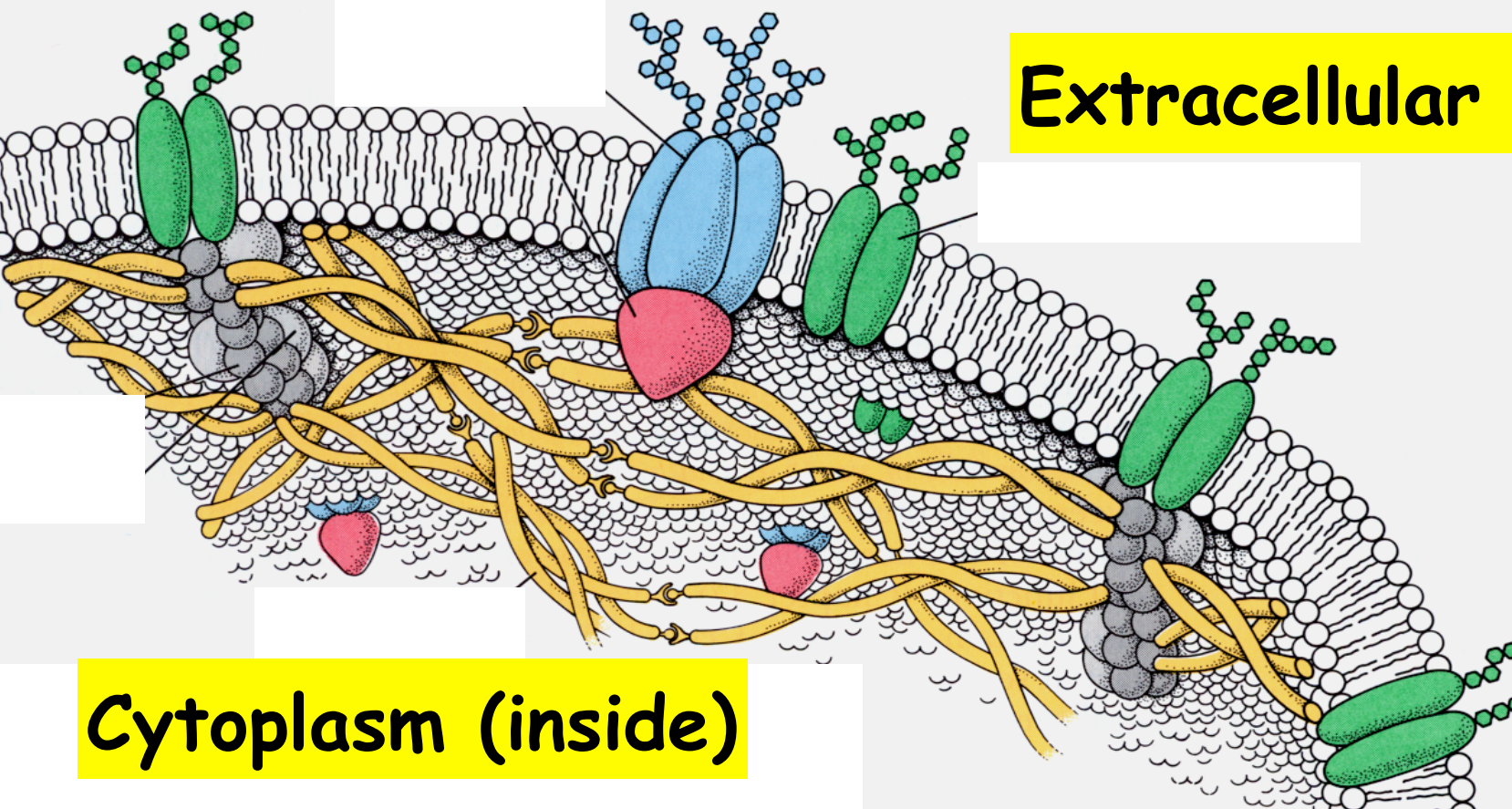


Overcoming Timescale limitation

Sampling

- Living cells also need to exchange materials and information with the outside world

... however, in a highly selective manner.

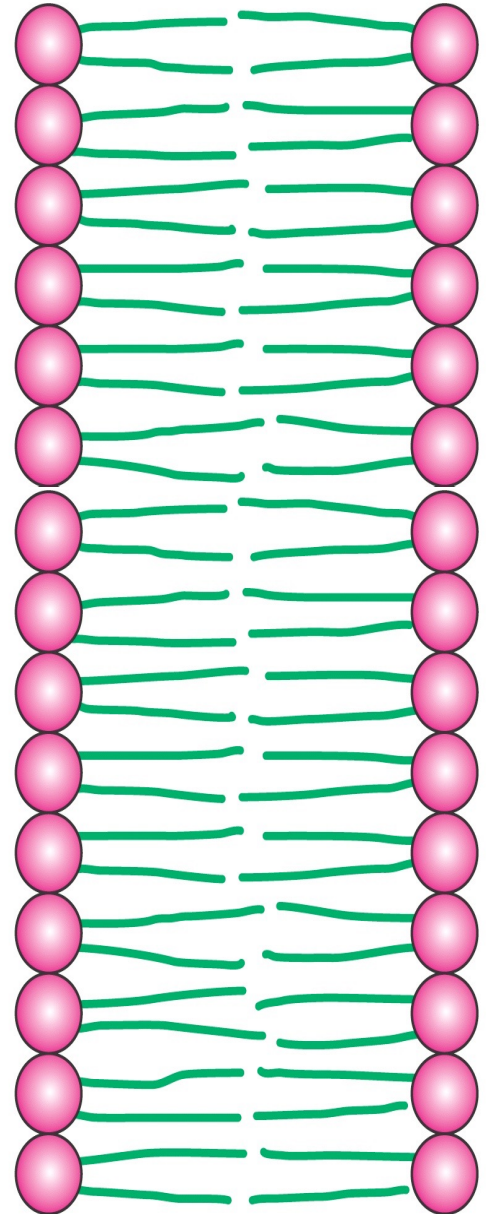


Extracellular (outside)

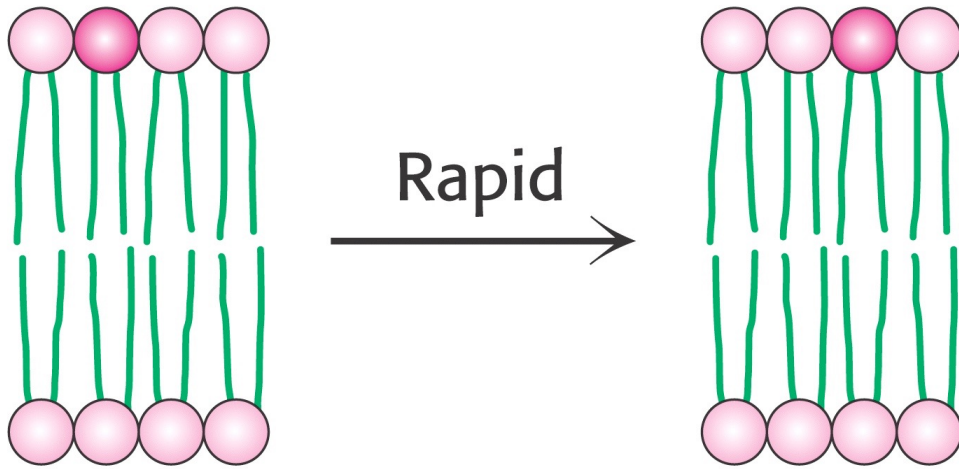
Cytoplasm (inside)

Phospholipid Bilayers Are Excellent Materials For Cell Membranes

- Hydrophobic interaction is the driving force
- Self-assembly in water
- Tendency to close on themselves
- Self-sealing (a hole is unfavorable)
- Extensive: up to millimeters



Lipid Diffusion in a Membrane



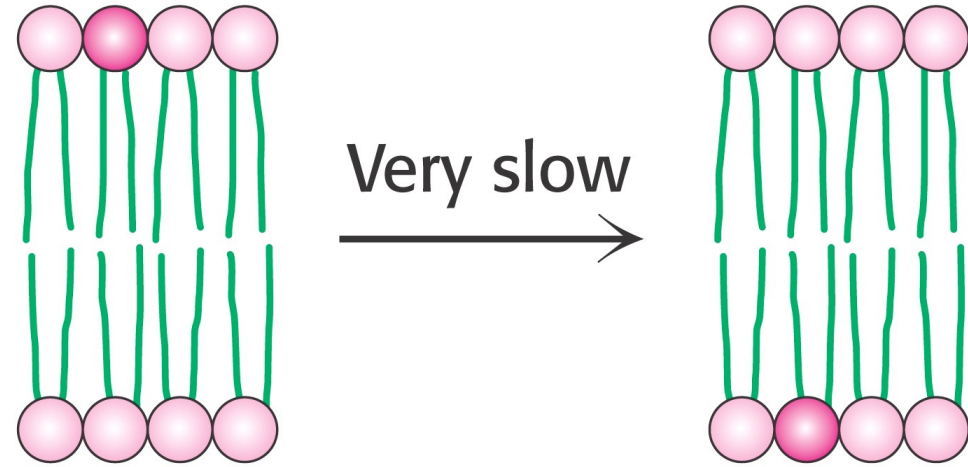
Lateral diffusion

$$D_{\text{lip}} = 10^{-8} \text{ cm}^2.\text{s}^{-1}$$

(50 Å in $\sim 5 \times 10^{-6} \text{ s}$)

$$D_{\text{wat}} = 2.5 \times 10^{-5} \text{ cm}^2.\text{s}^{-1}$$

Modeling mixed lipid bilayers!



Transverse diffusion
(flip-flop)

Once in several hours!

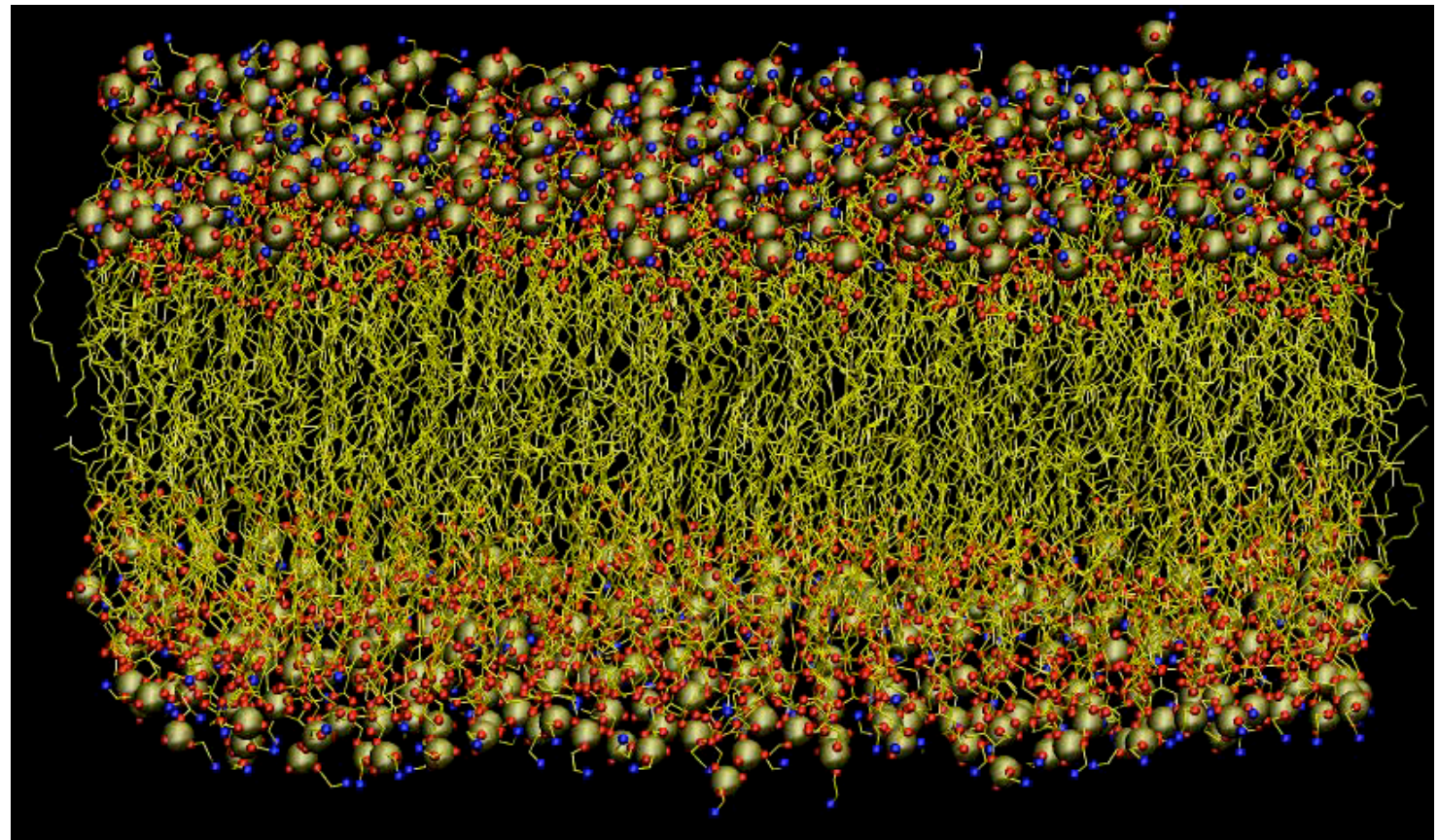
($\sim 50 \text{ Å}$ in $\sim 10^4 \text{ s}$)

*~ 9 orders of magnitude slower
ensuring bilayer asymmetry*

Technical difficulties in Simulations of Biological Membranes

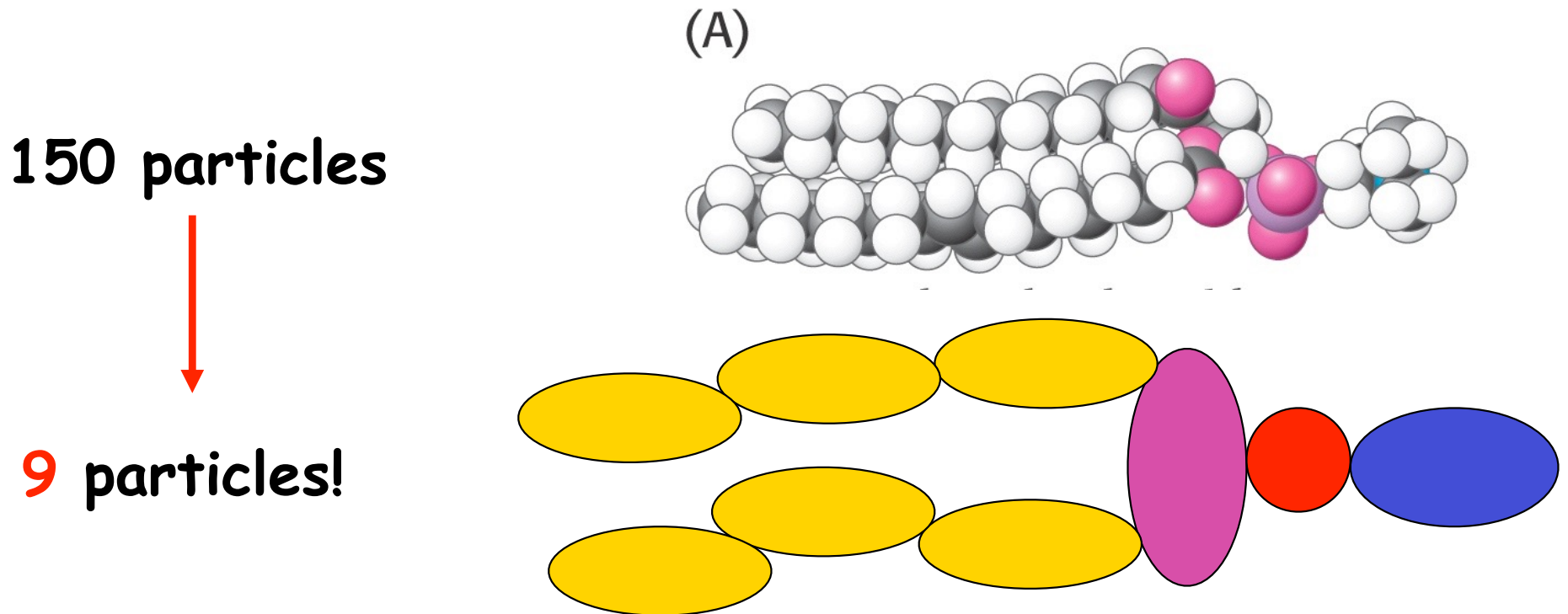
- Time scale
- Heterogeneity of biological membranes ☹️

60 x 60 Å
Pure POPE
5 ns
~100,000
atoms

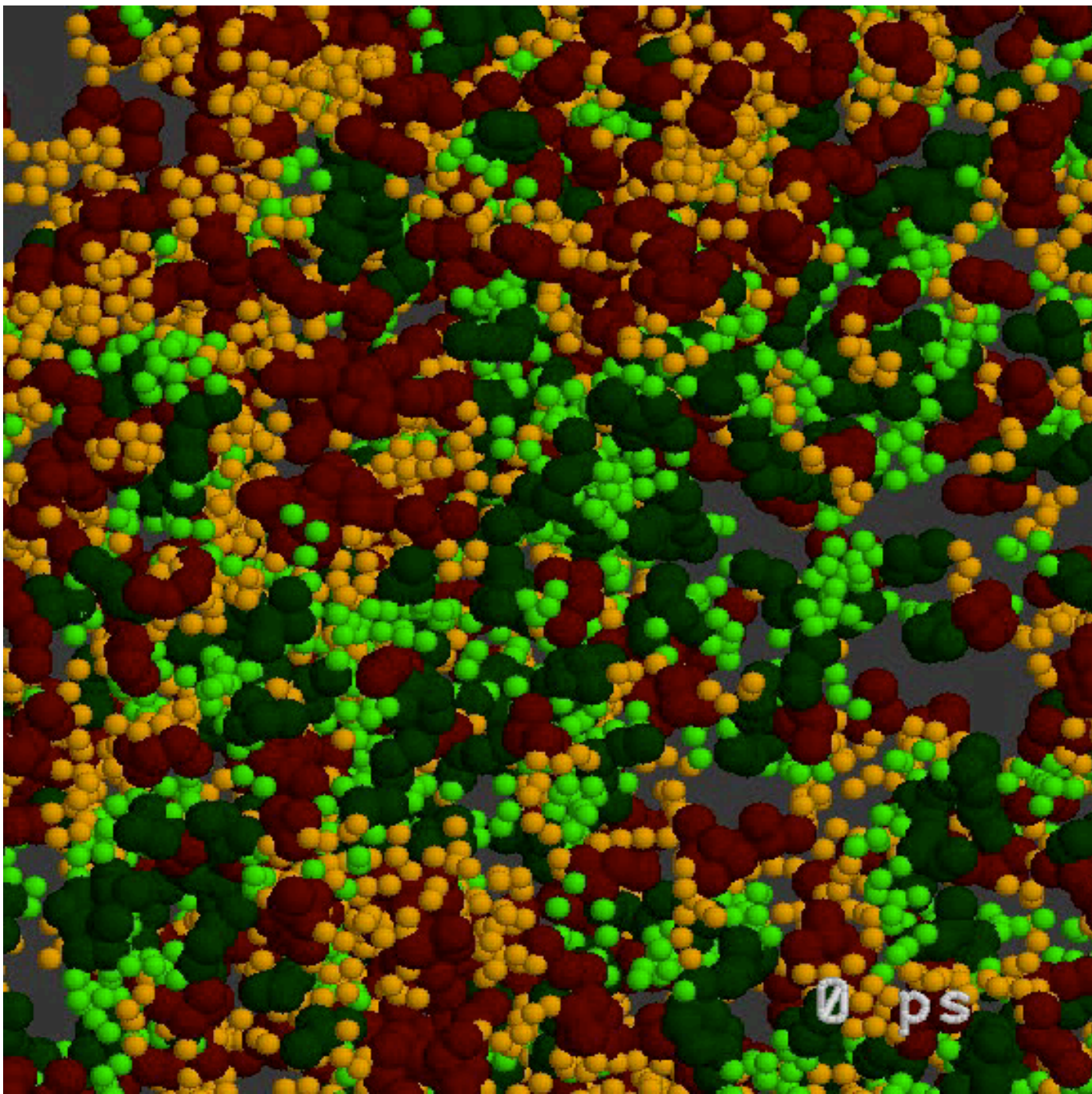


Battling the Timescale - Case I

Coarse-grained modeling of lipids



Also, increasing the time step by orders of magnitude.



by: J. Siewert-Jan Marrink and Alan E. Mark, University of Groningen, The Netherlands

Battling the Timescale - Case II

Steered Molecular Dynamics is a non-equilibrium method by nature

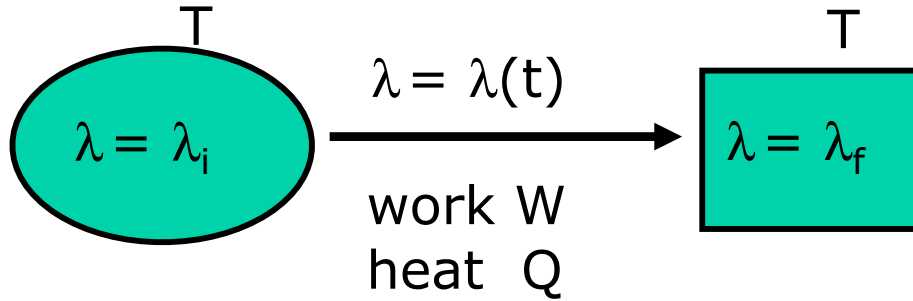
- A wide variety of events that are inaccessible to conventional molecular dynamics simulations can be probed.
- The system will be driven, however, away from equilibrium, resulting in problems in describing the energy landscape associated with the event of interest.

Second law of thermodynamics

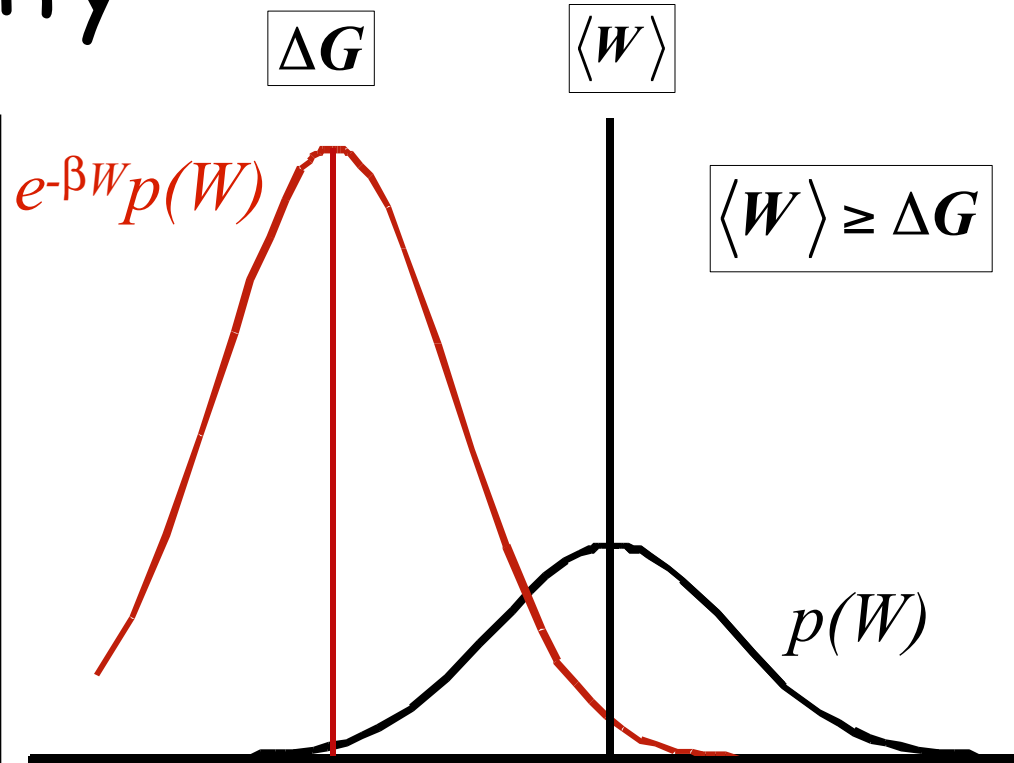
$$\longrightarrow W \geq \Delta G$$

Jarzynski's Equality

Transition between two equilibrium states



$$\Delta G = G_f - G_i$$



C. Jarzynski, *Phys. Rev. Lett.*, **78**, 2690 (1997)

C. Jarzynski, *Phys. Rev. E*, **56**, 5018 (1997)

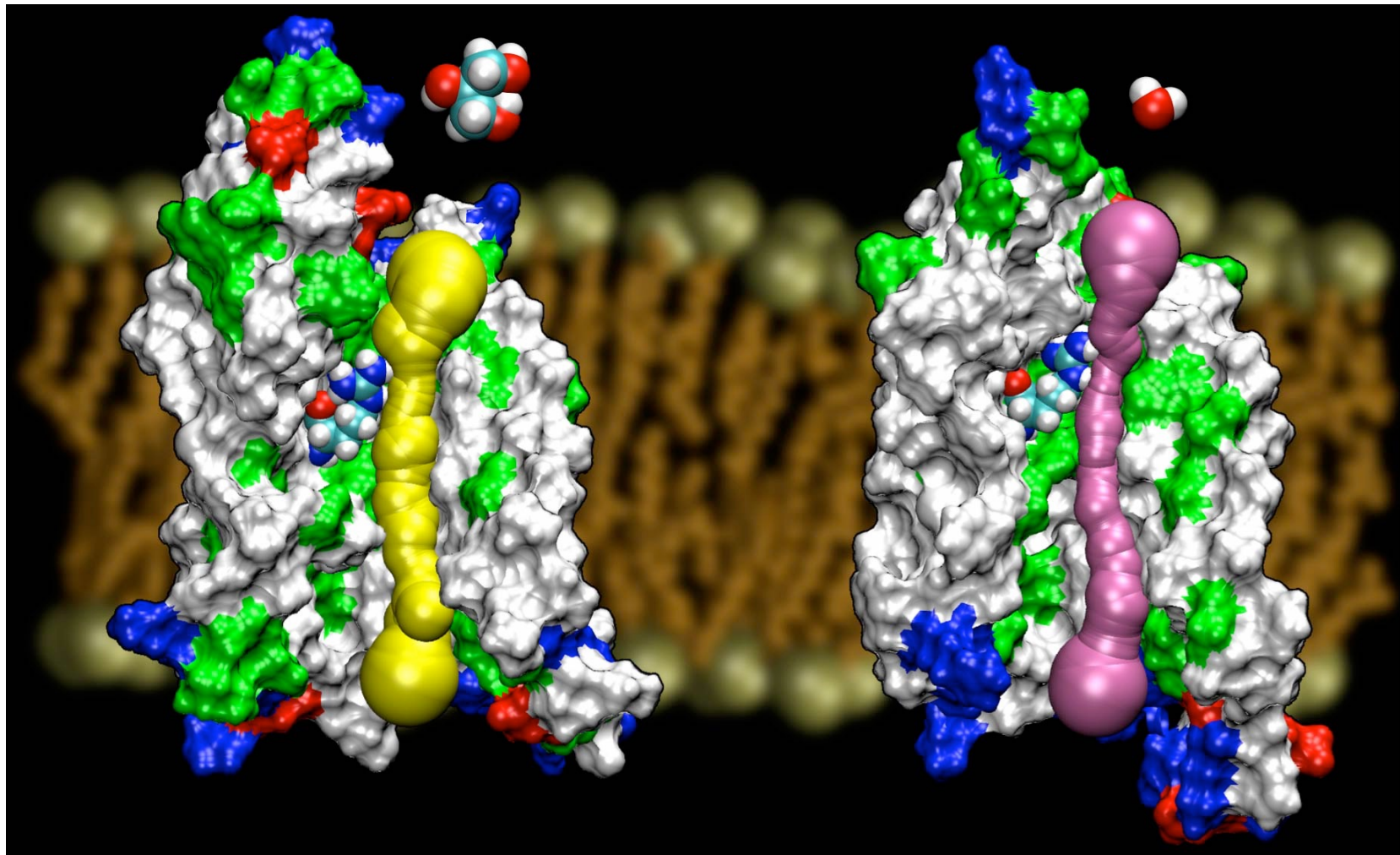
$$\langle e^{-\beta W} \rangle = e^{-\beta \Delta G}$$

$$\beta = 1/k_B T$$

In principle, it is possible to obtain free energy surfaces from repeated **non-equilibrium** experiments.

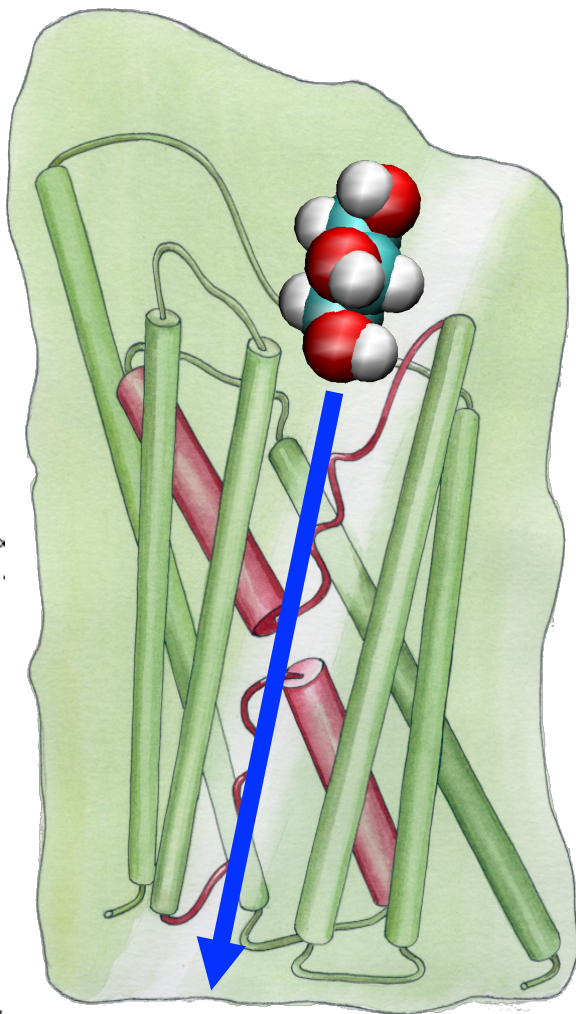
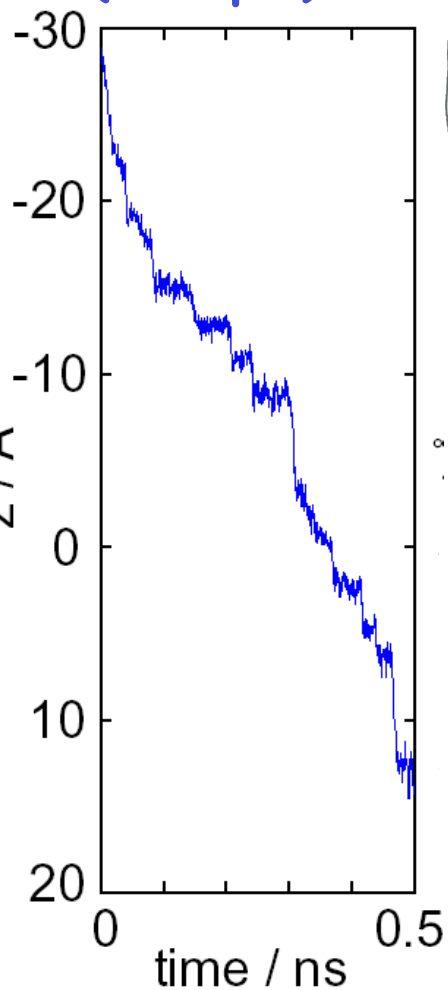
AqpZ vs. GlpF

- Both from *E. coli*
- AqpZ is a pure water channel
- GlpF is a glycerol channel
- We have high resolution structures for both channels

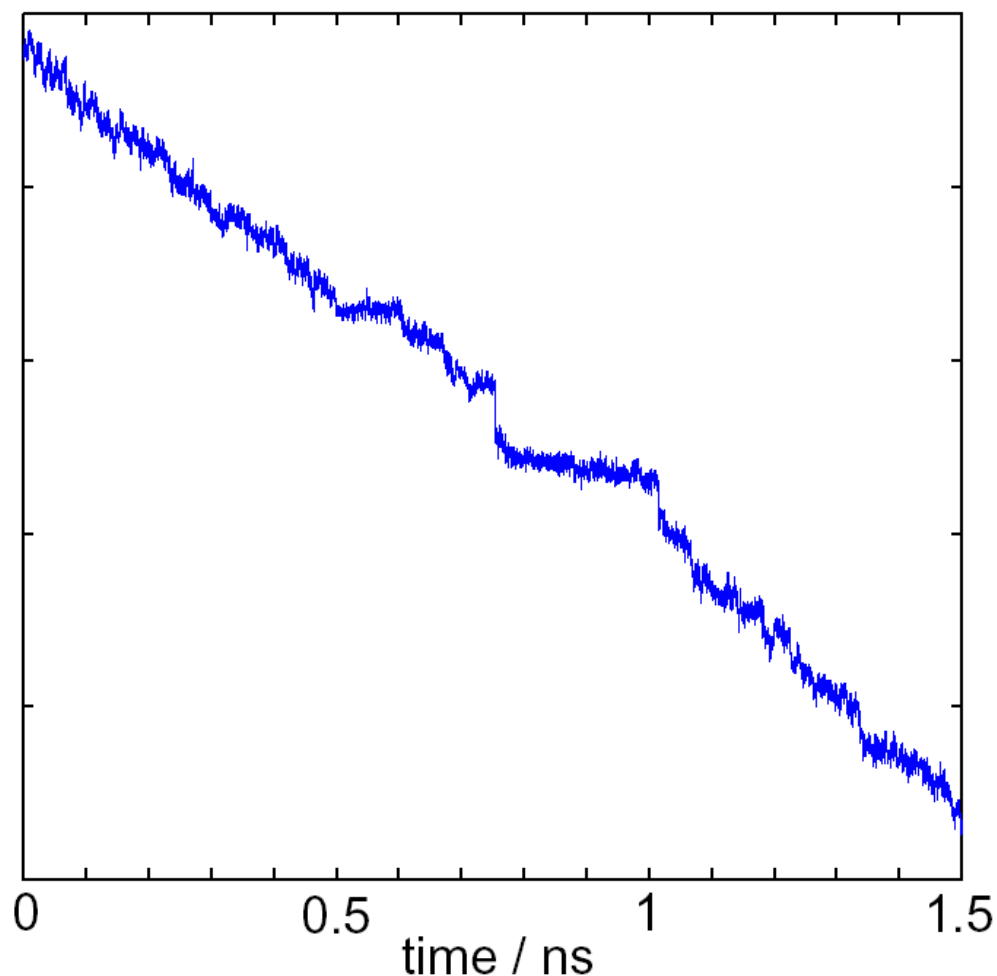


Steered Molecular Dynamics

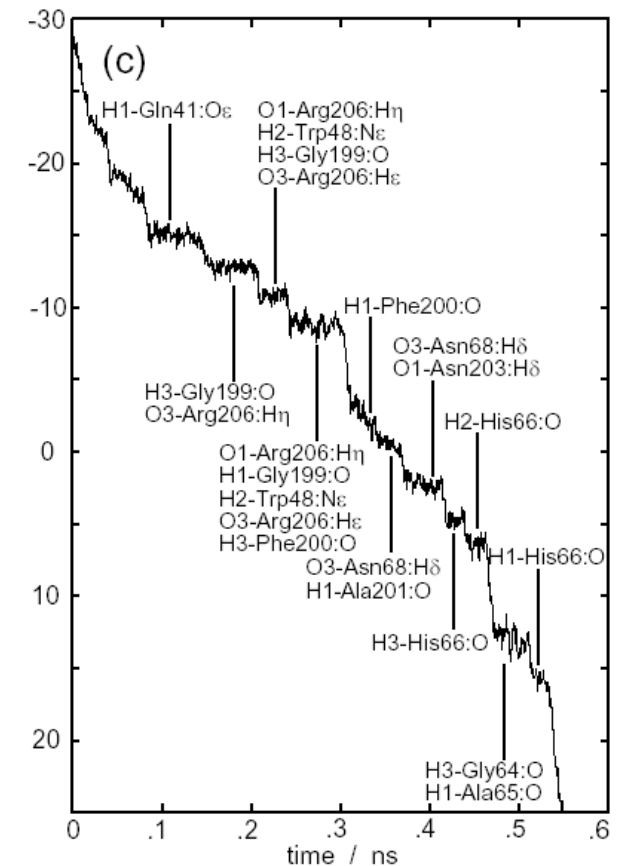
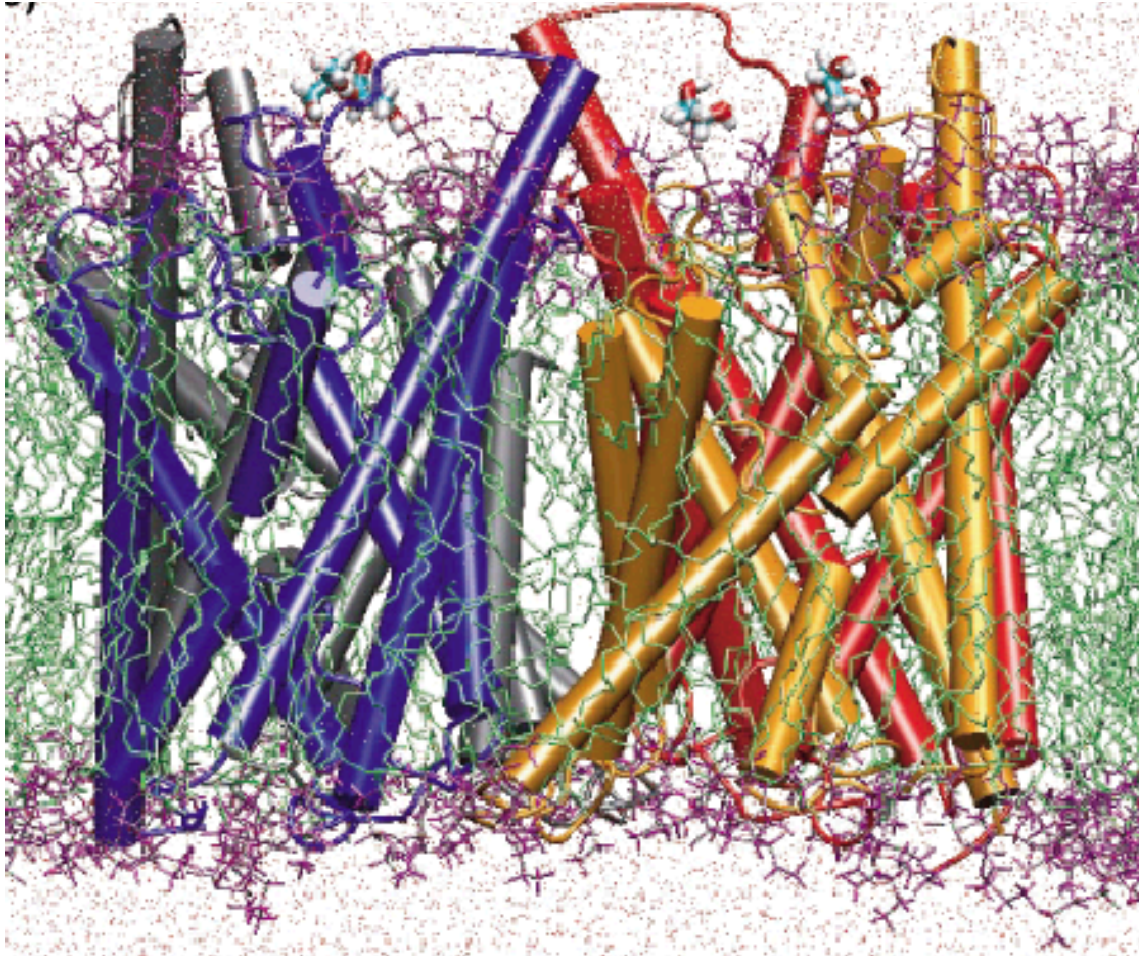
constant force
(250 pN)



constant velocity
(30 $\text{\AA}/\text{ns}$)



SMD Simulation of Glycerol Passage



Trajectory of glycerol pulled by **constant force**

Constructing the Potential of Mean Force

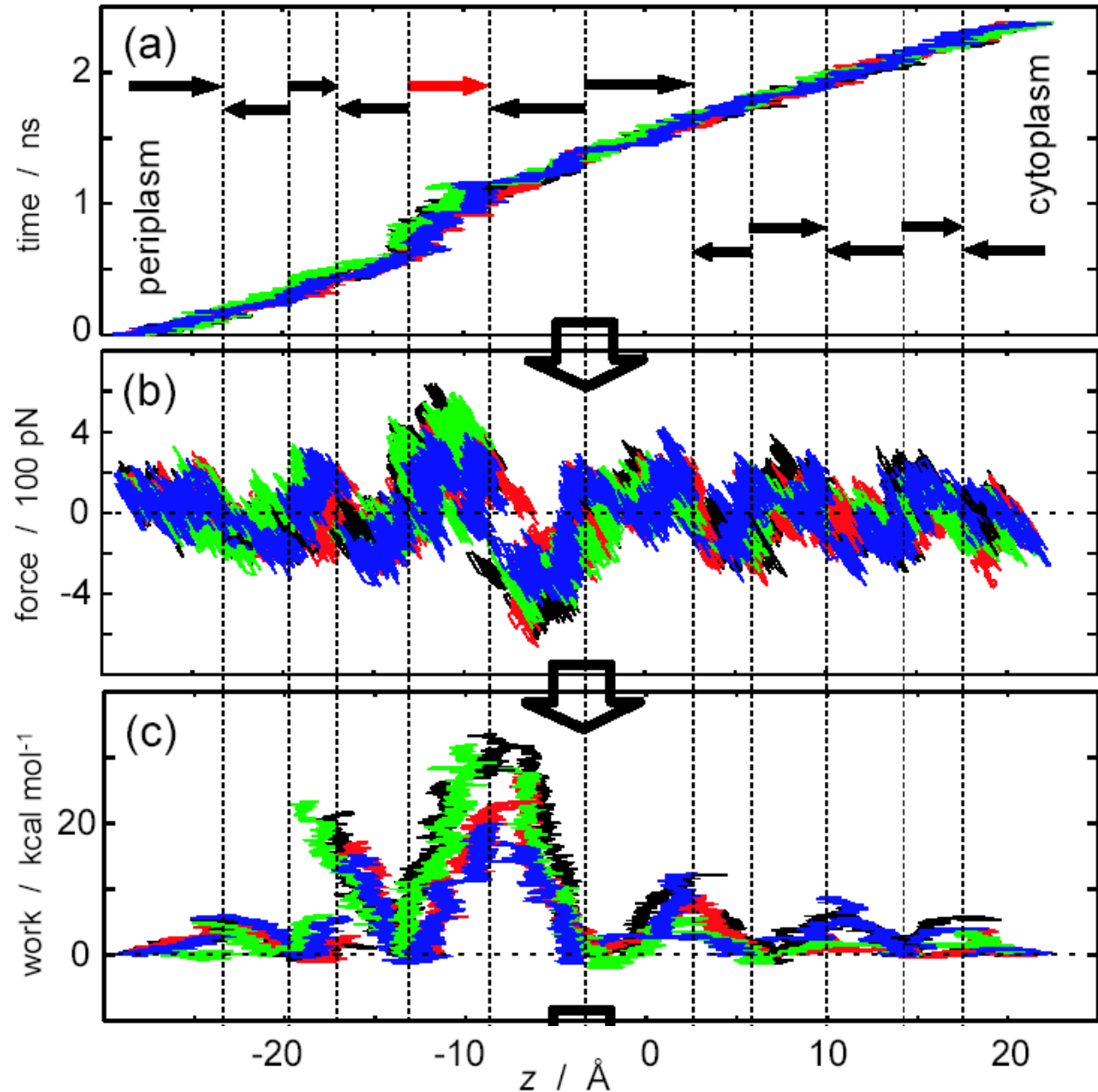
4 trajectories

$v = 0.03, 0.015 \text{ \AA/ps}$

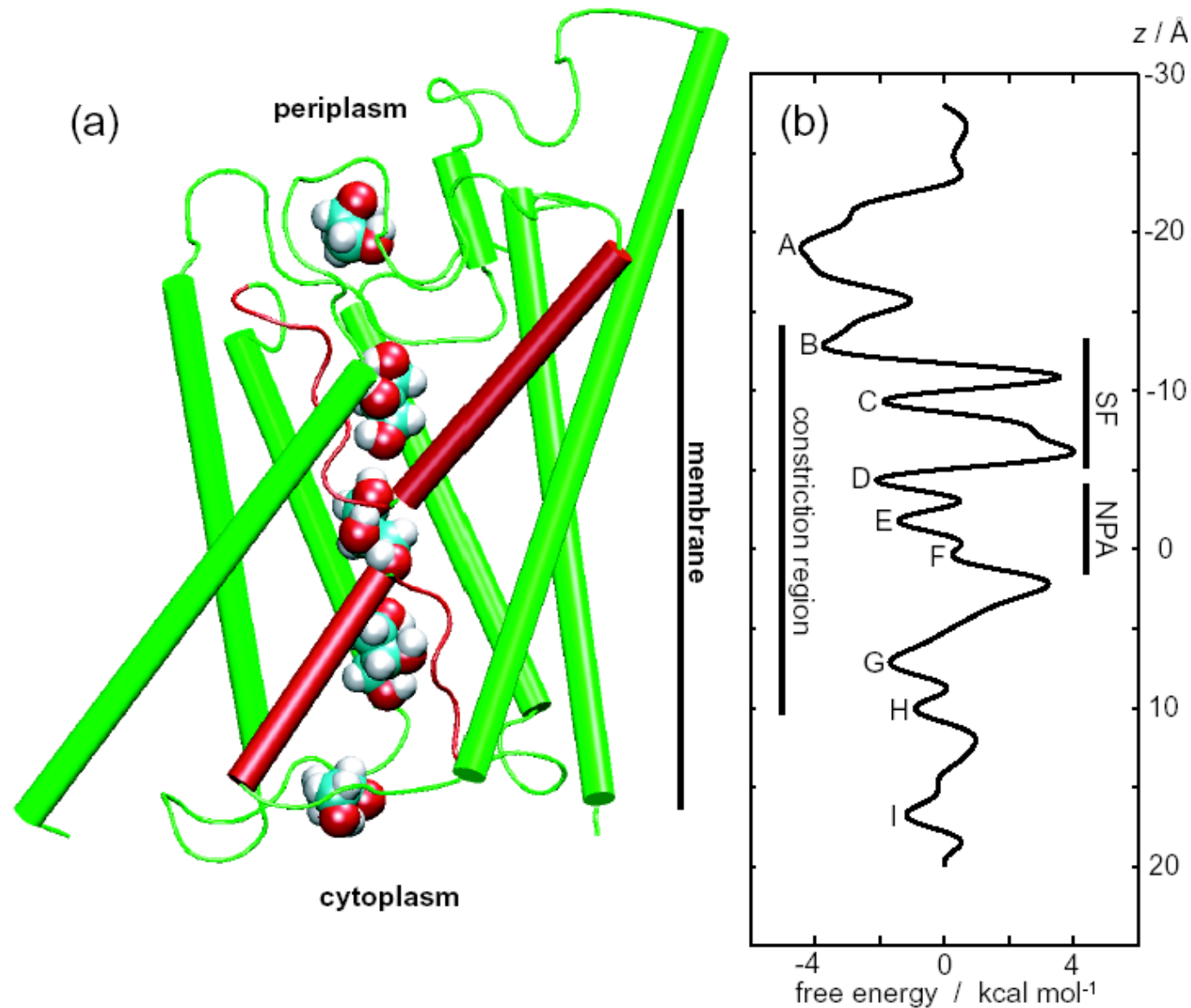
$k = 150 \text{ pN/\AA}$

$$f(t) = -k[z(t) - z_0 - vt]$$

$$W(t) = \int_0^t dt' v f(t')$$

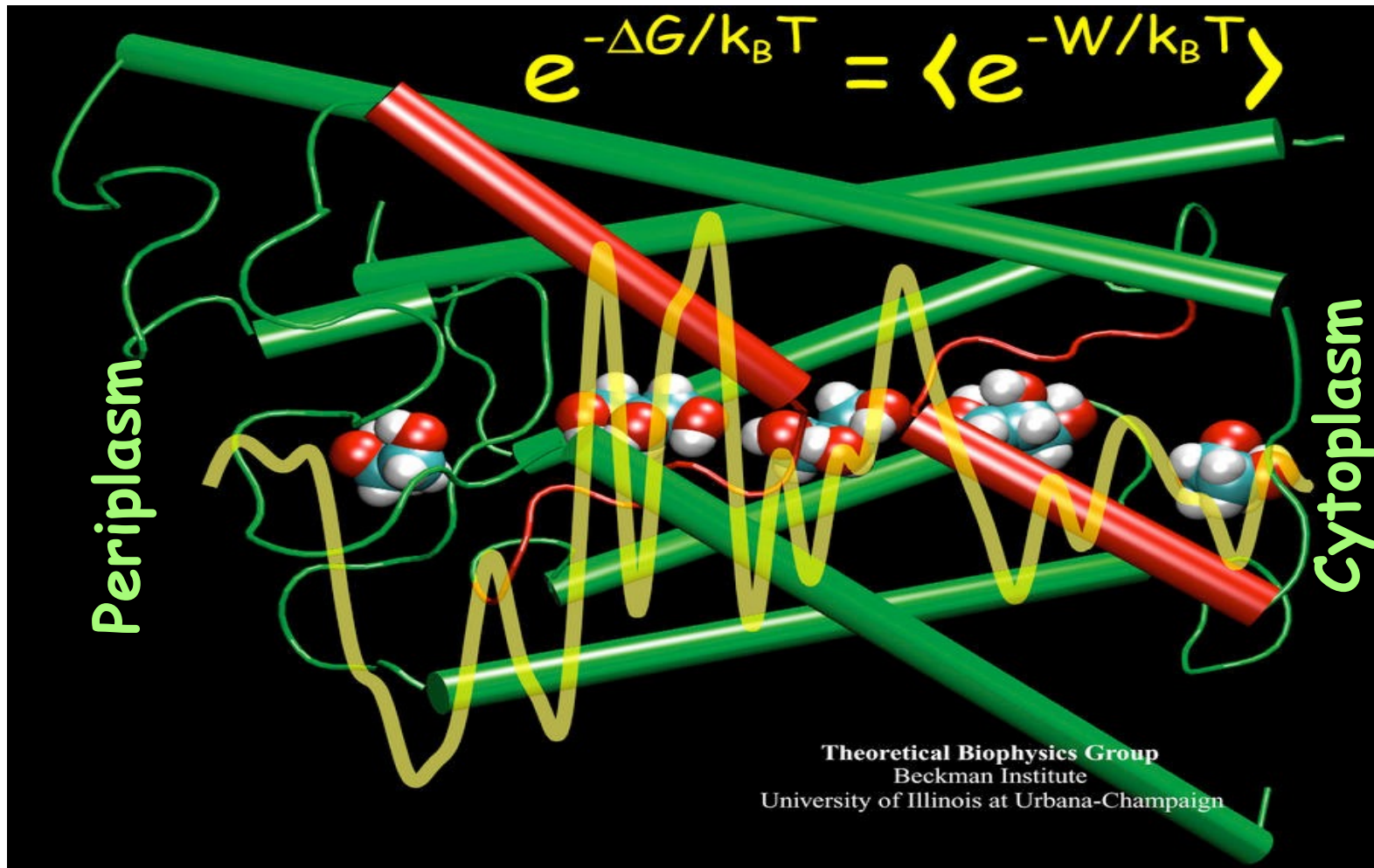


Features of the Potential of Mean Force



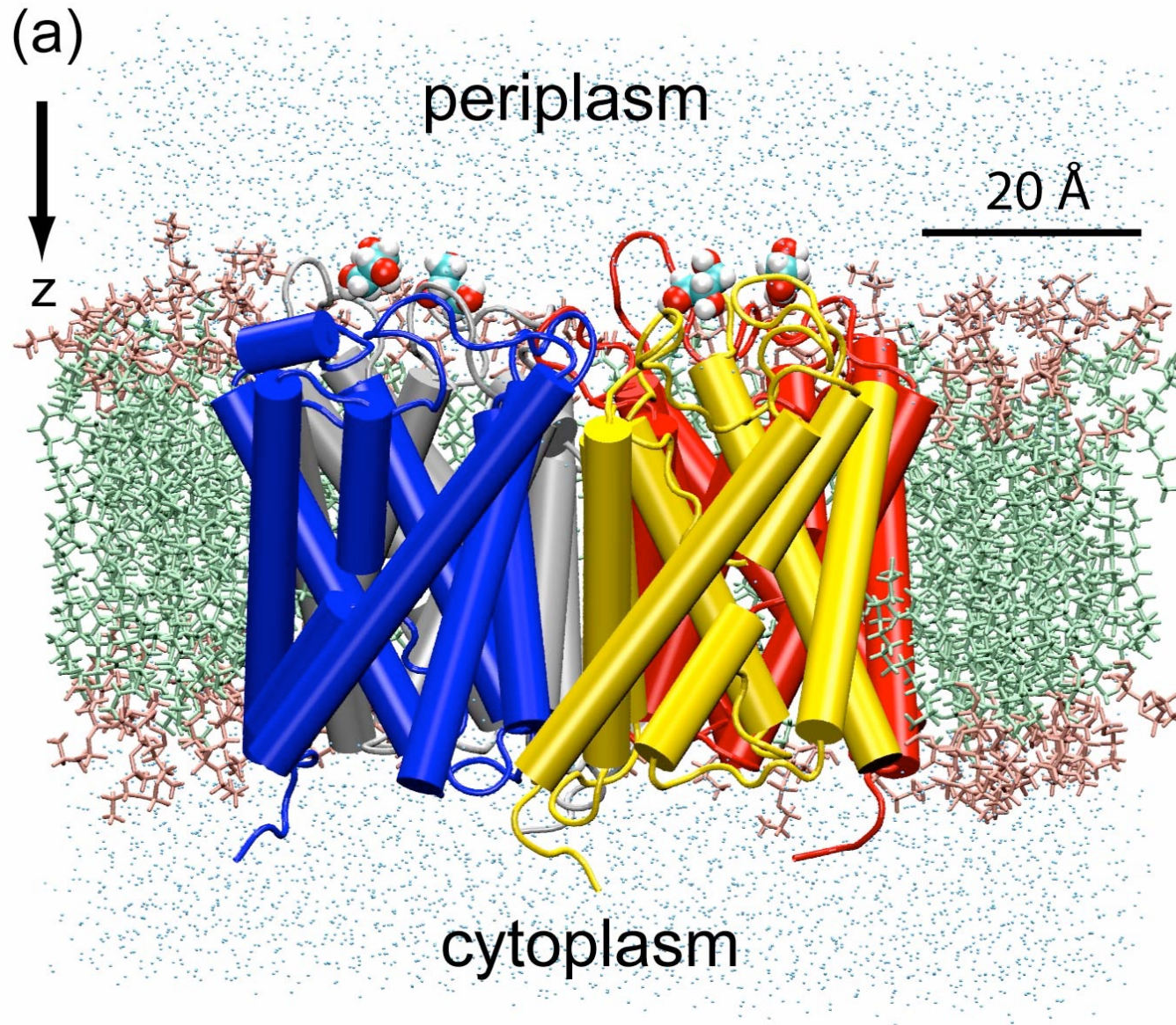
- Captures major features of the channel
- The largest barrier ≈ 7.3 kcal/mol; exp.: 9.6 ± 1.5 kcal/mol

Features of the Potential of Mean Force

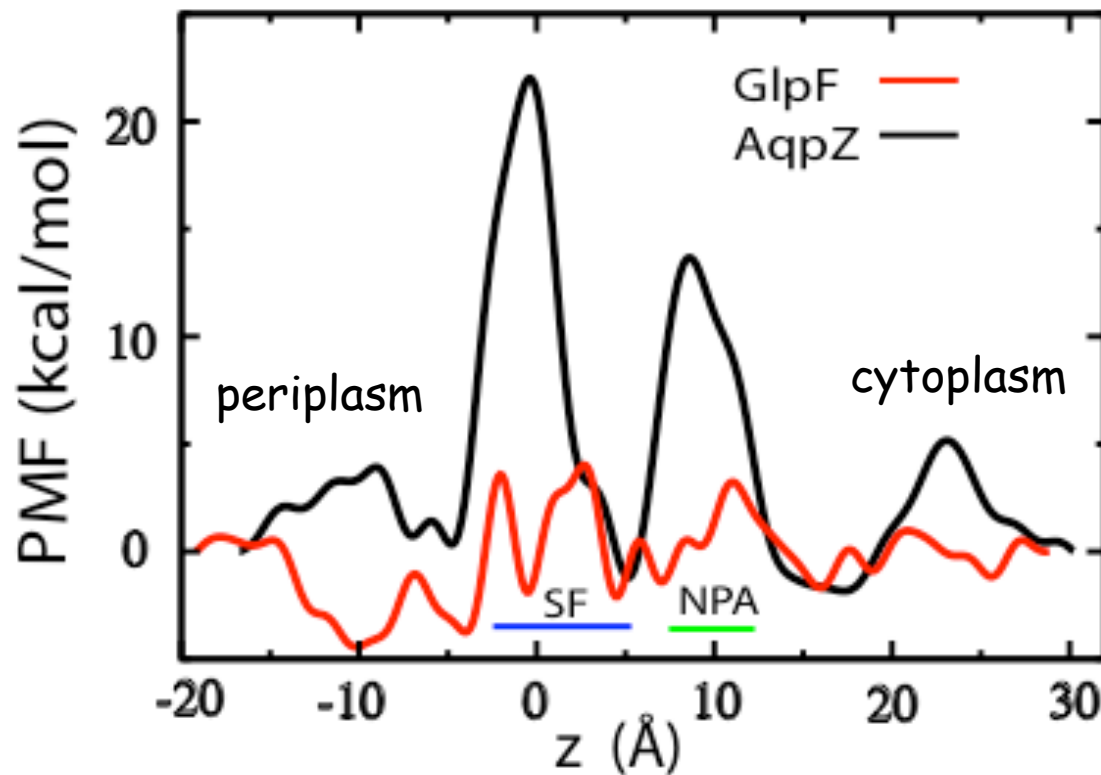


Asymmetric Profile in the Vestibules

Artificial induction of glycerol conduction through AqpZ

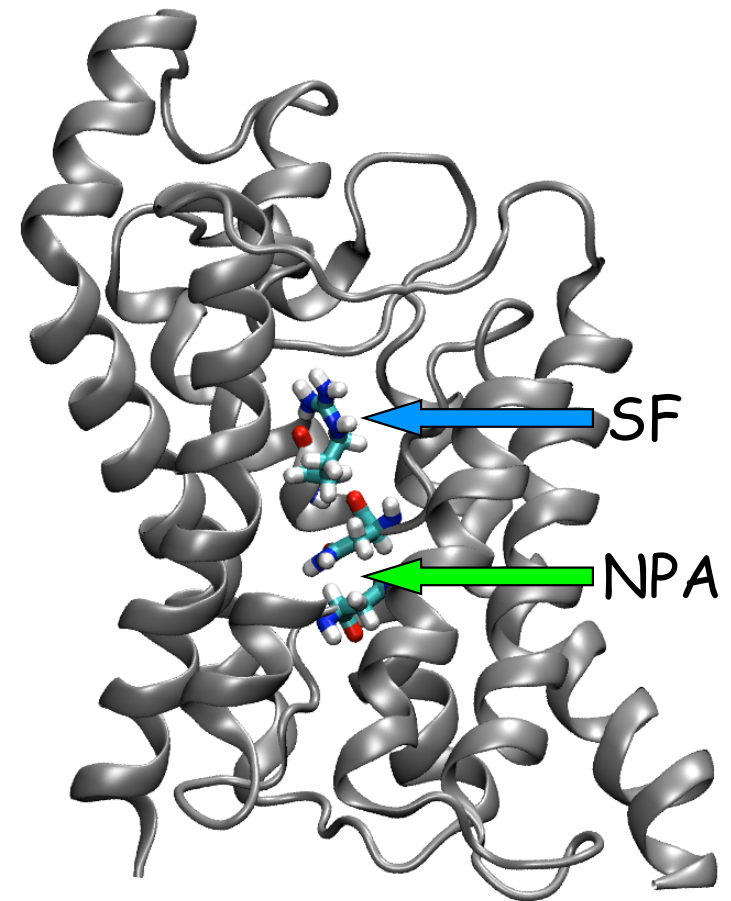


Three fold higher barriers

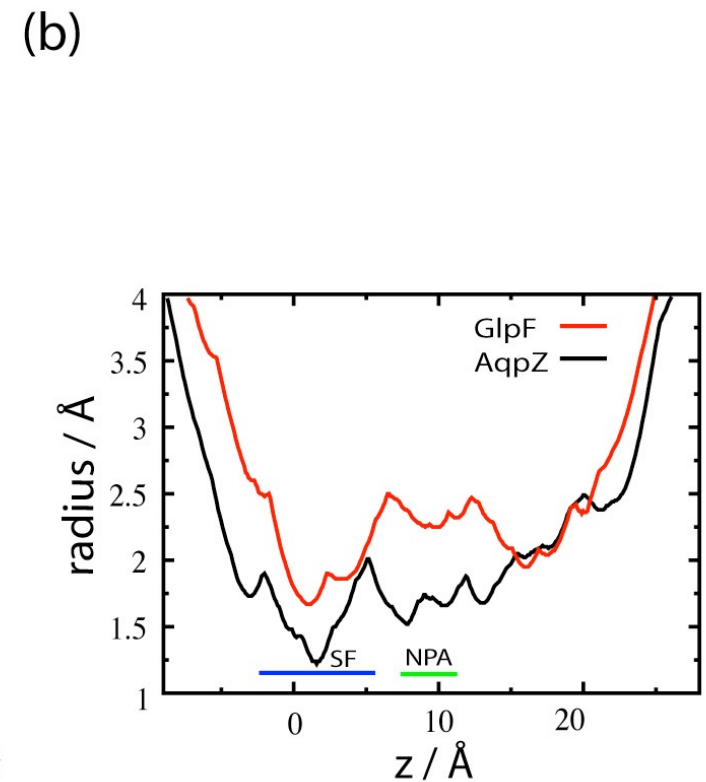
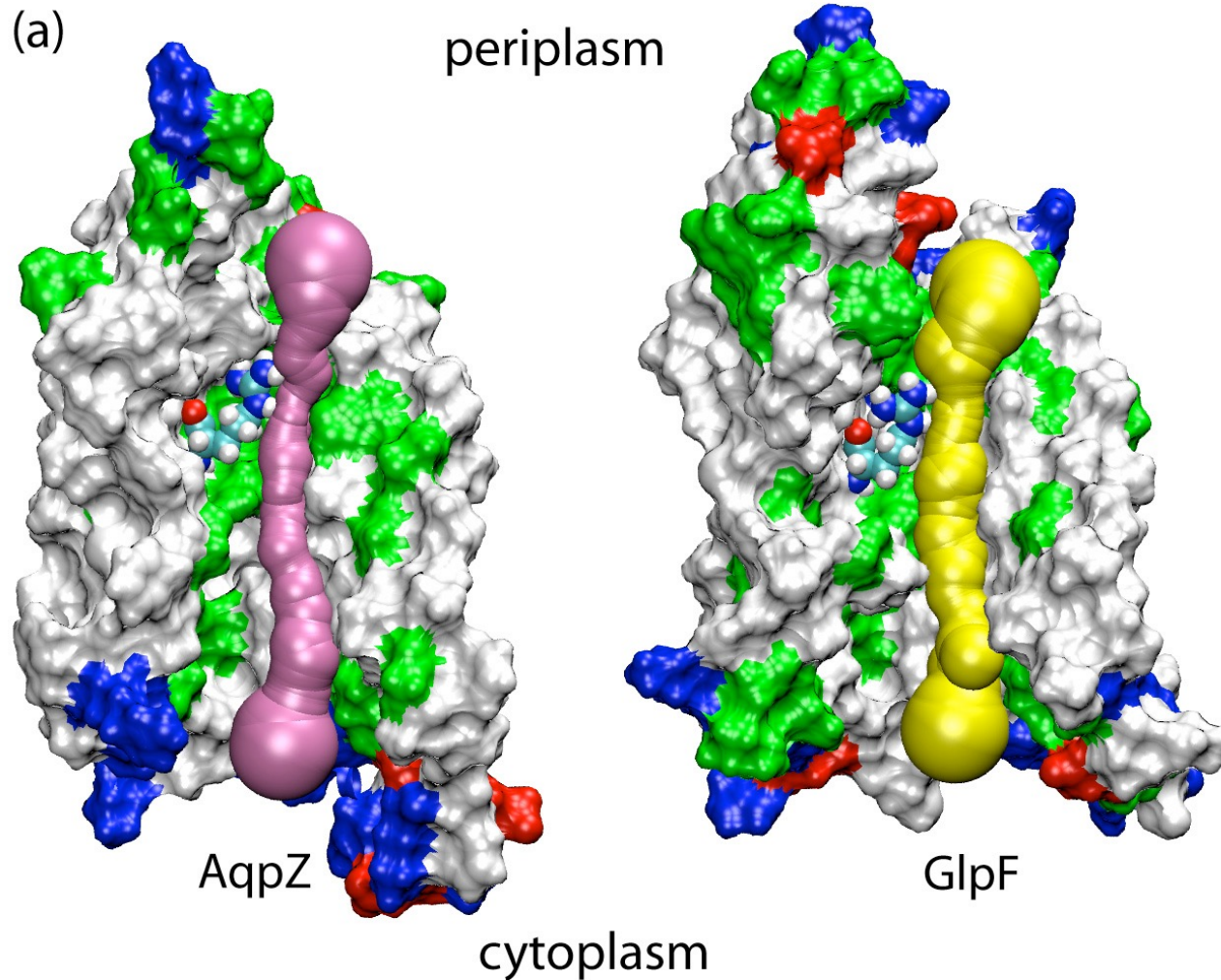


AqpZ 22.8 kcal/mol

GlpF 7.3 kcal/mol

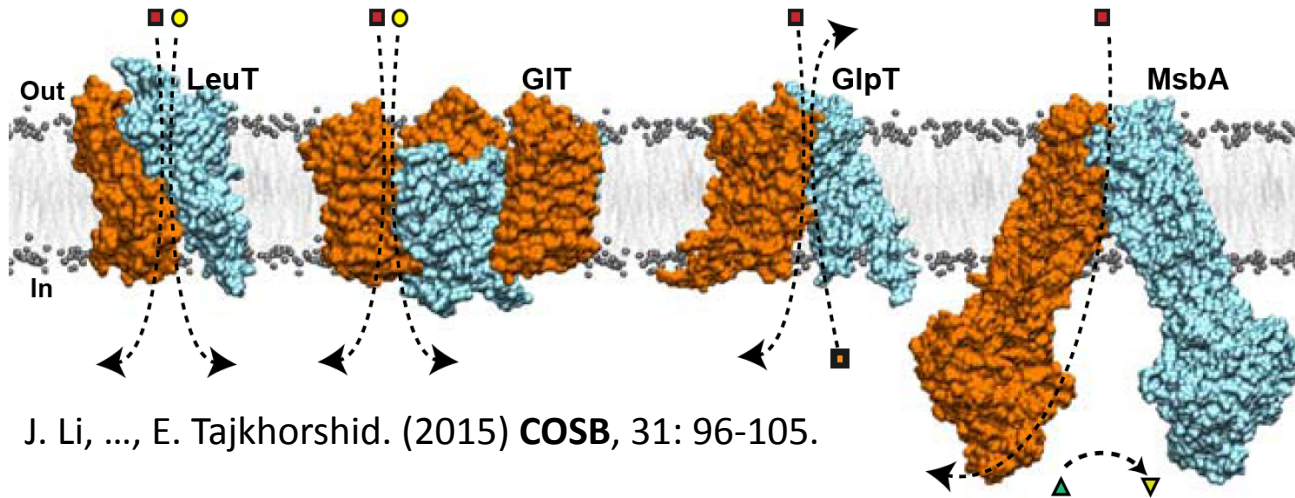


Could it be simply the size?

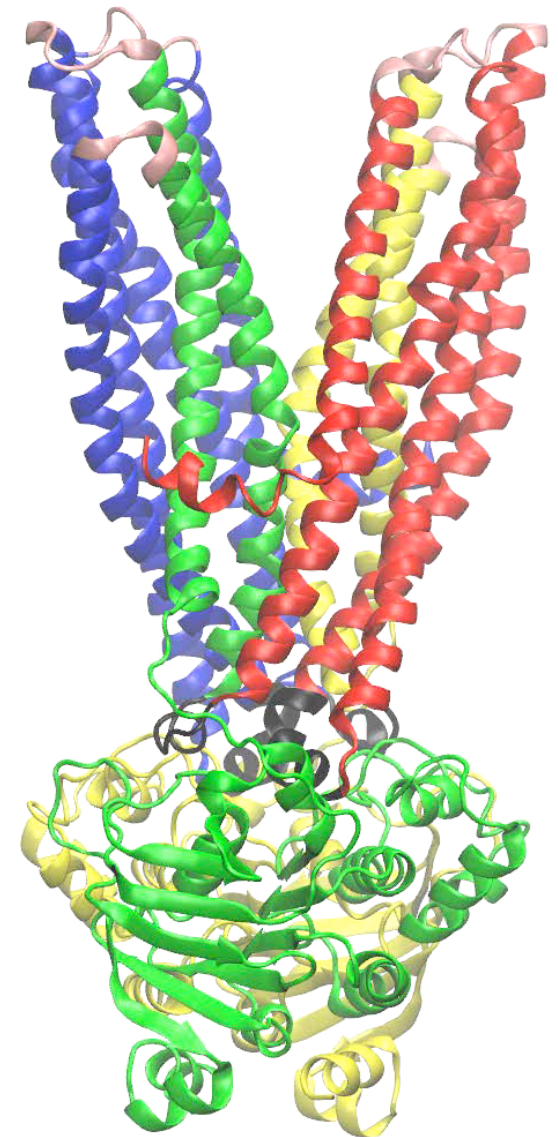


Battling the Timescale - Case III

Biased (nonequilibrium) simulations



J. Li, ..., E. Tajkhorshid. (2015) **COSB**, 31: 96-105.



◆ Neurotransmitter Uptake

» Norepinephrine, serotonin, dopamine, glutamate,...

◆ Gastrointestinal Tract

» Active absorption of nutrients
» Secretion of ions

◆ Kidneys

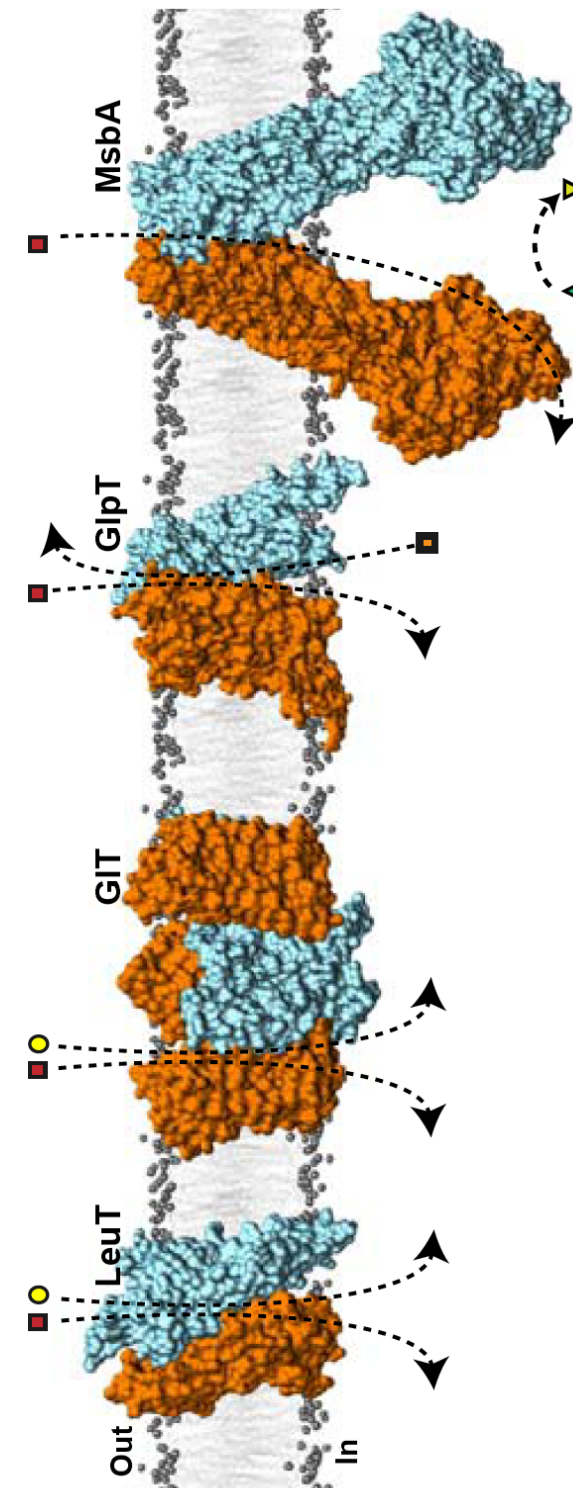
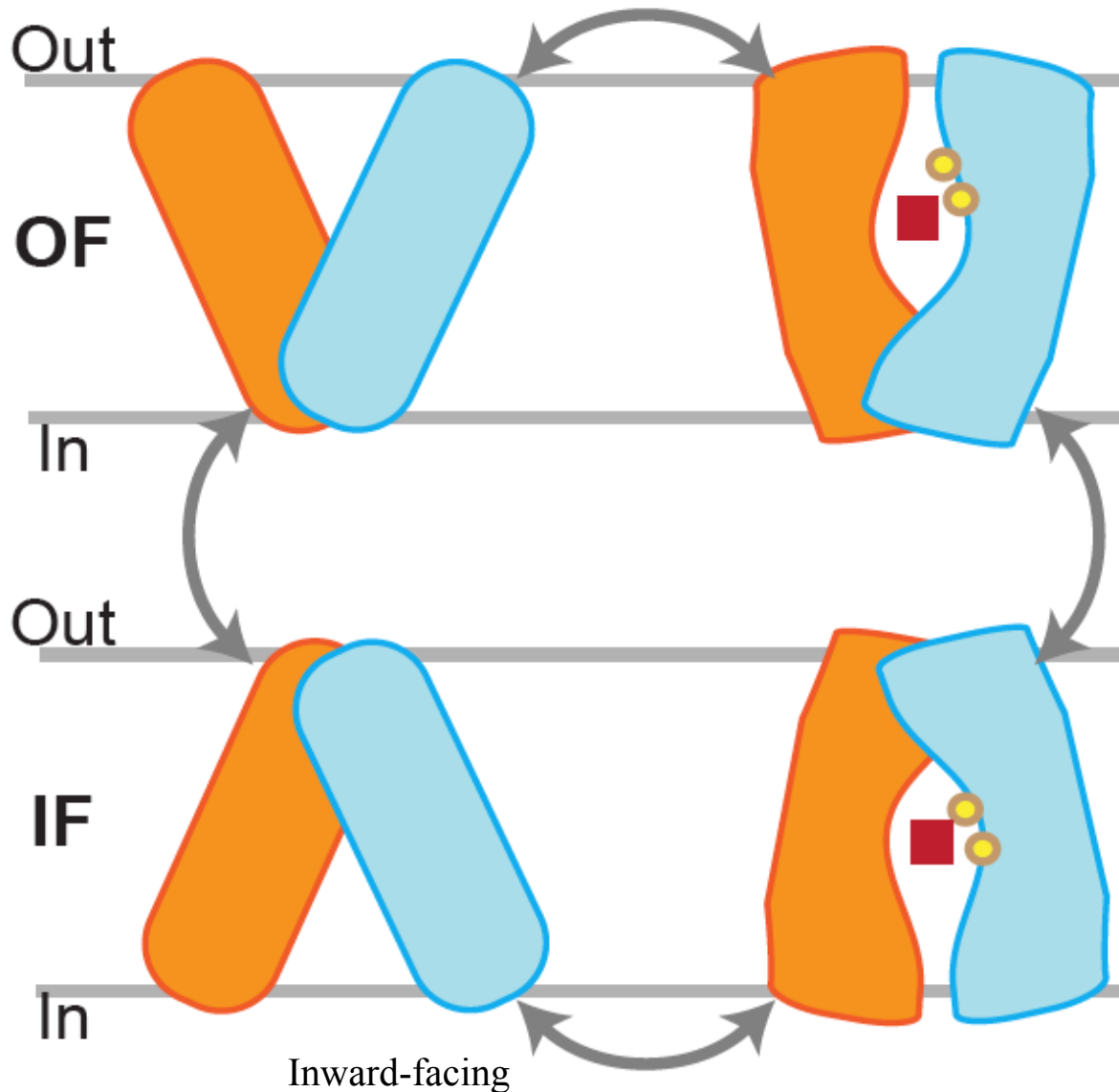
» Reabsorption
» Secretion

◆ Pharmacokinetics of all drugs

» Absorption, distribution, elimination
» Multi-drug resistance in cancer cells

Alternating Access Mechanism

Outward-facing

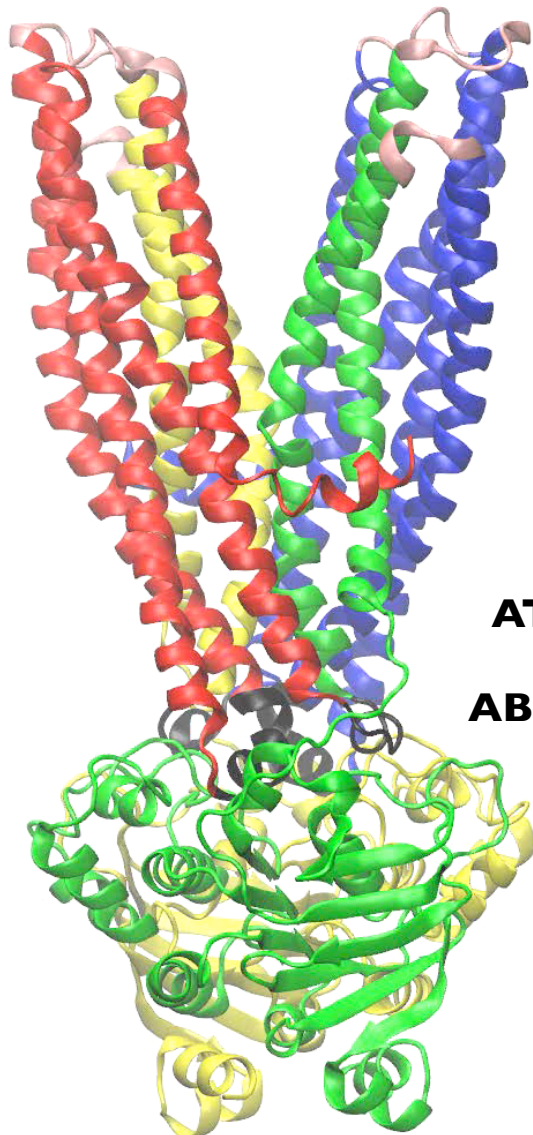


Jardetzky O. *Nature* **211**: 969–970 (1966)

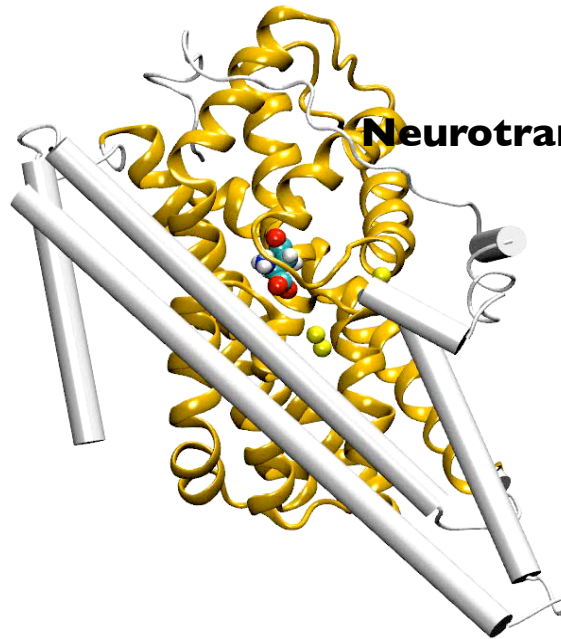
J. Li, ..., E. Tajkhorshid. (2015) *COSB*, 31: 96-105.

COMPLEX

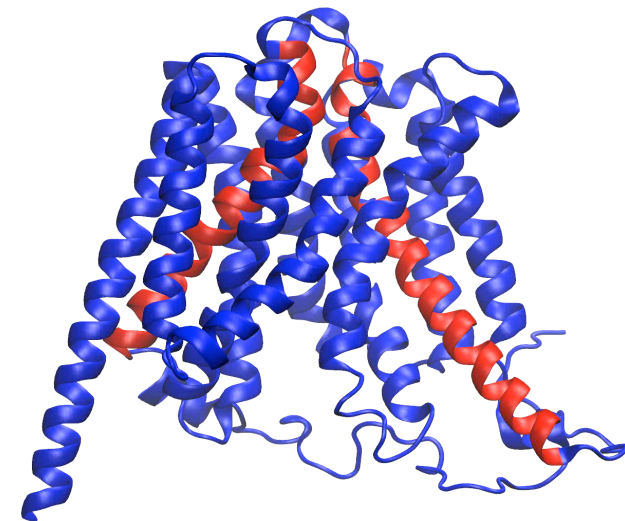
Diverse Structural Transitions Involved



**ATP-Driven
Primary
ABC Exporter**



**Na-coupled
Secondary
Neurotransmitter Transporter**



**Secondary
Phosphate
Antiporter**

NON-EQUILIBRIUM METHODS ARE REQUIRED.

Complex Processes Require Complex Treatments

I.1 Defining Practical Collective Variables

Empirical search for practical collective variables for inducing the conformational changes involved in the transition.

I.2 Optimizing the Biasing Protocols

Systematic search for a practical biasing protocol by using different combinations of collective variables.

II. Optimizing the Transition Pathway

Use all of the conformations available to generate the most reliable transition pathway:

1. Bayesian approach for combining the data
2. Post-hoc string method (analysis tool)
3. String method with swarms of trajectories

III.1 Free Energy Calculations

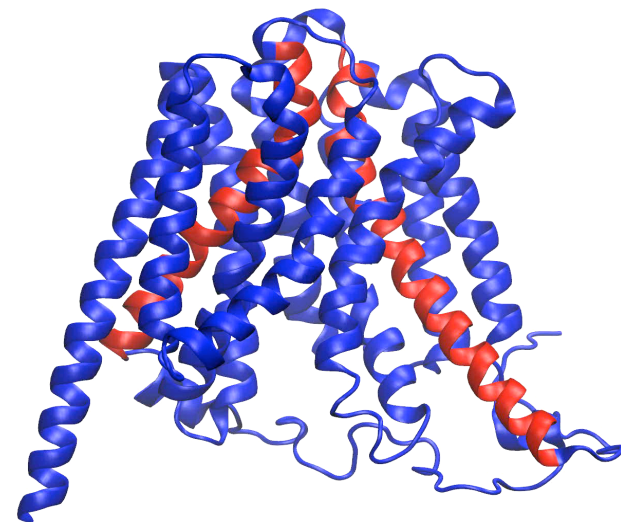
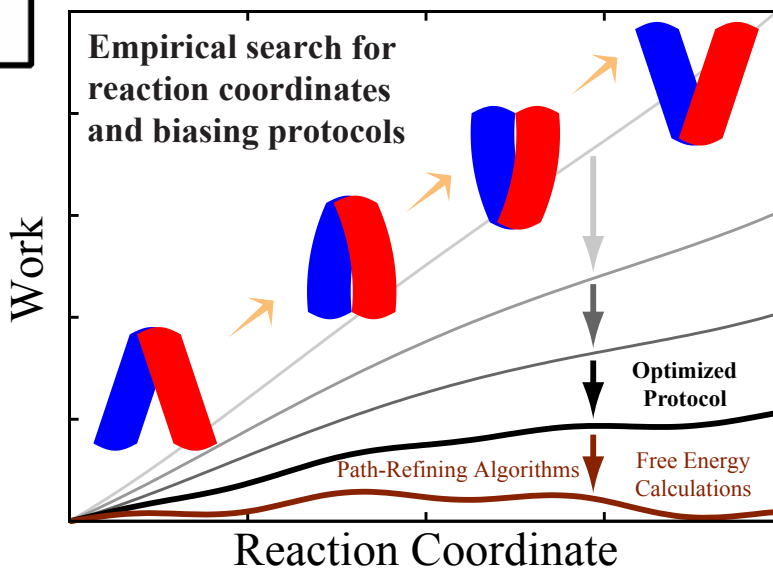
Using the most relevant collective variables (from I.1), biasing protocol (from I.2), and initial conformations (from I.2).

III.2 Assessing the Sampling Efficiency

Detecting the poorly sampled, but potentially important regions, e.g., by using PCA.



Mahmoud Moradi

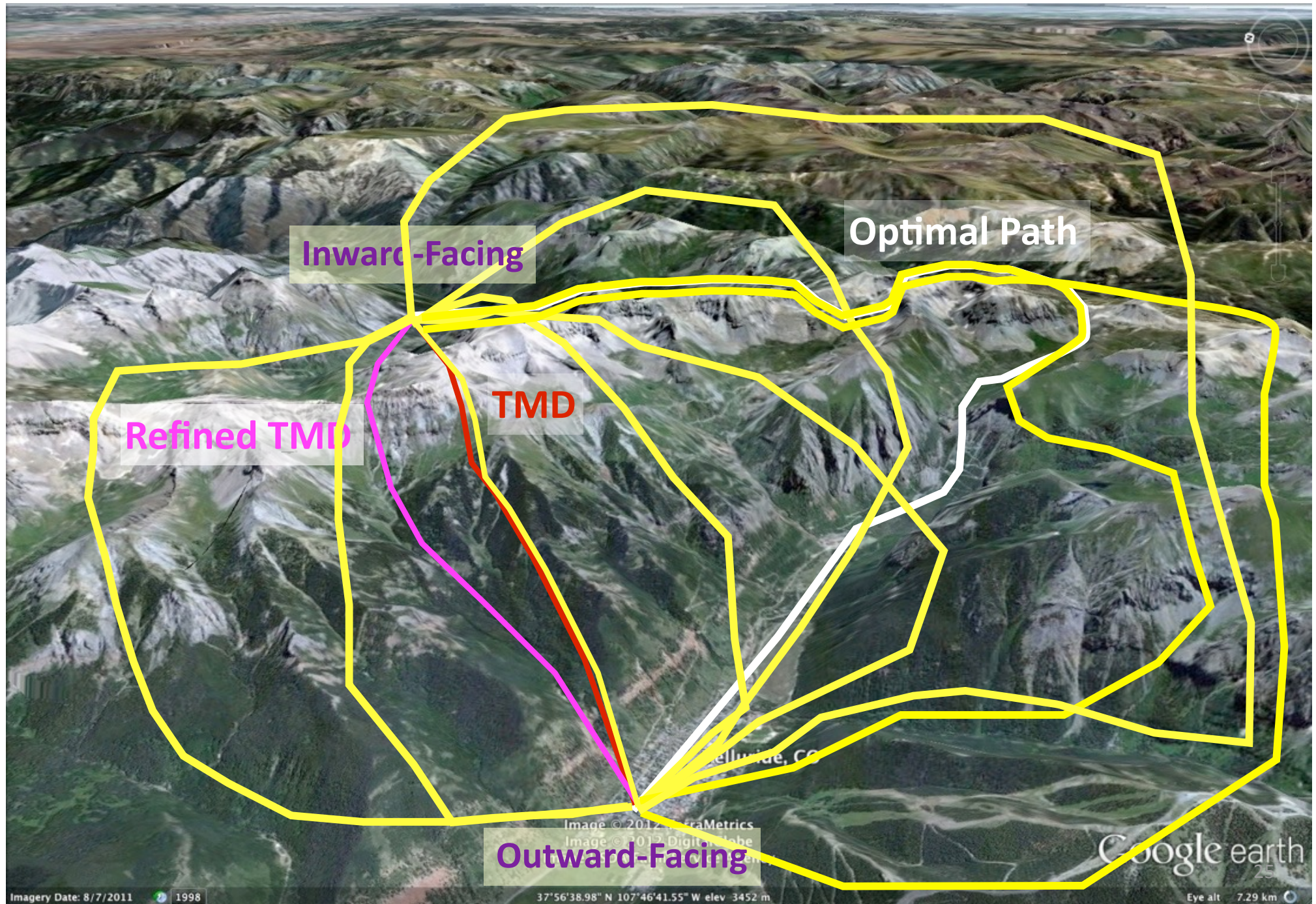


M. Moradi and ET (2013) **PNAS**, 110:18916–18921.

M. Moradi and ET (2014) **JCTC**, 10: 2866–2880.

M. Moradi, G. Enkavi, and ET (2015) **Nature Comm.**, 6:8393.

Aggressive Search of the Space



Non-equilibrium Driven Molecular Dynamics:

Applying a time-dependent external force to induce the transition

Along various pathways/mechanisms (collective variables)

$$U_{dr}(\mathbf{x}, t) = \frac{1}{2}k \left(\xi(\mathbf{x}) - \xi_A + (\xi_B - \xi_A) \frac{t}{T} \right)^2$$

Diagram illustrating the components of the non-equilibrium driven potential $U_{dr}(\mathbf{x}, t)$:

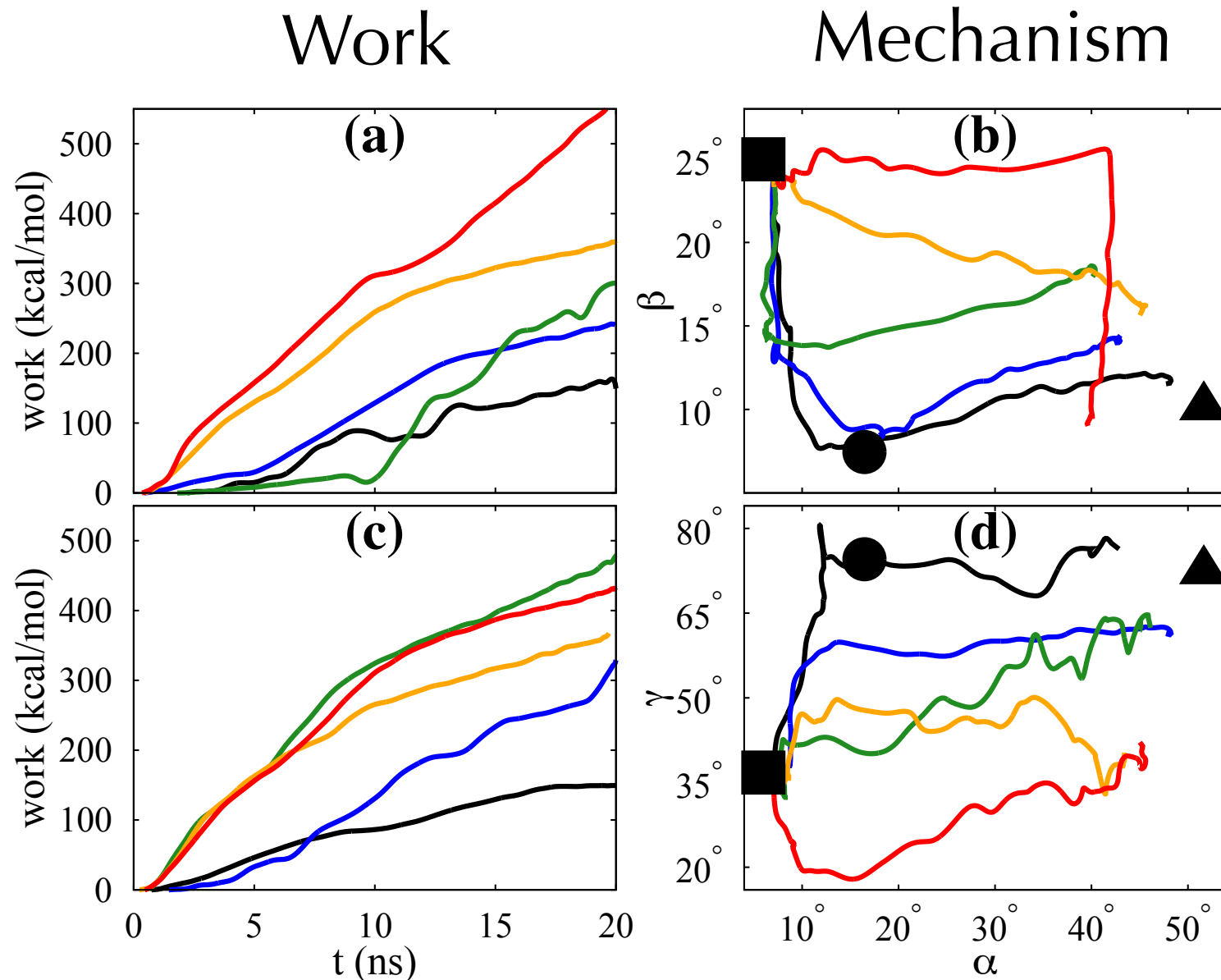
- Harmonic constant**: Points to k .
- Initial state**: Points to ξ_A .
- Final state**: Points to ξ_B .
- Biasing potential**: Points to $U_{dr}(\mathbf{x}, t)$.
- Collective variables**: Points to $\xi(\mathbf{x})$. The box lists: **RMSD, distance, R_g , angle, ... orientation quaternion**.
- Total simulation time**: Points to T .

M. Moradi and ET (2013) **PNAS**, 110:18916–18921.

M. Moradi and ET (2014) **JCTC**, 10: 2866–2880.

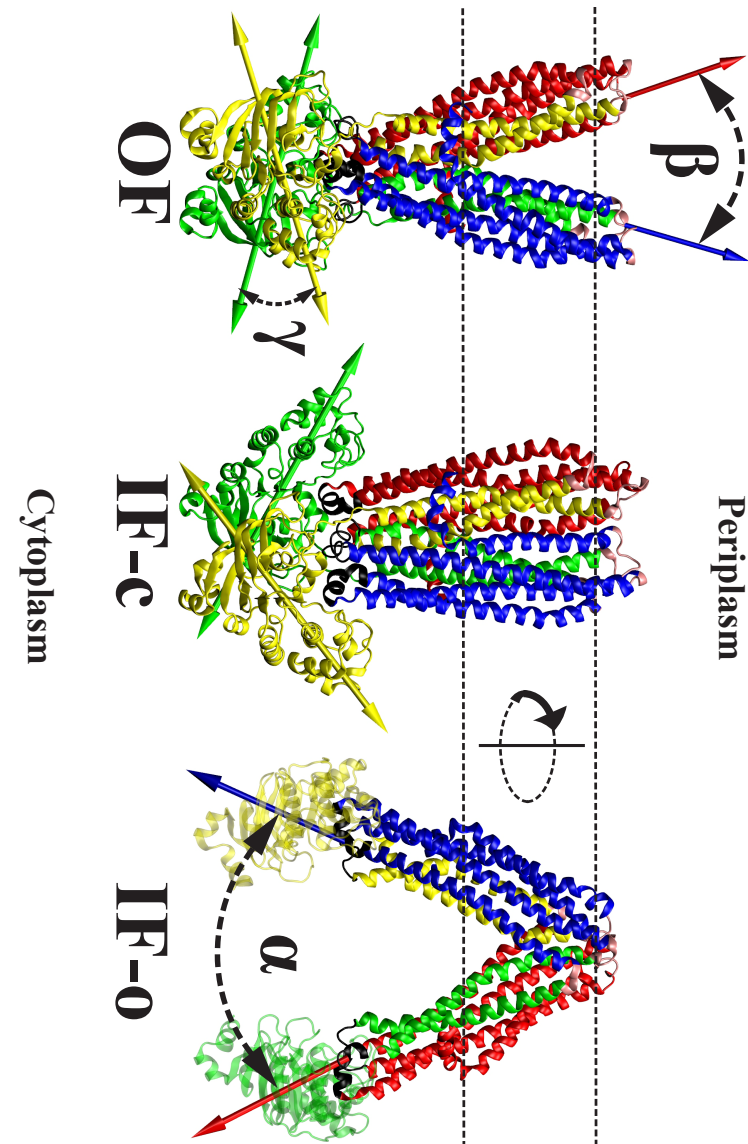
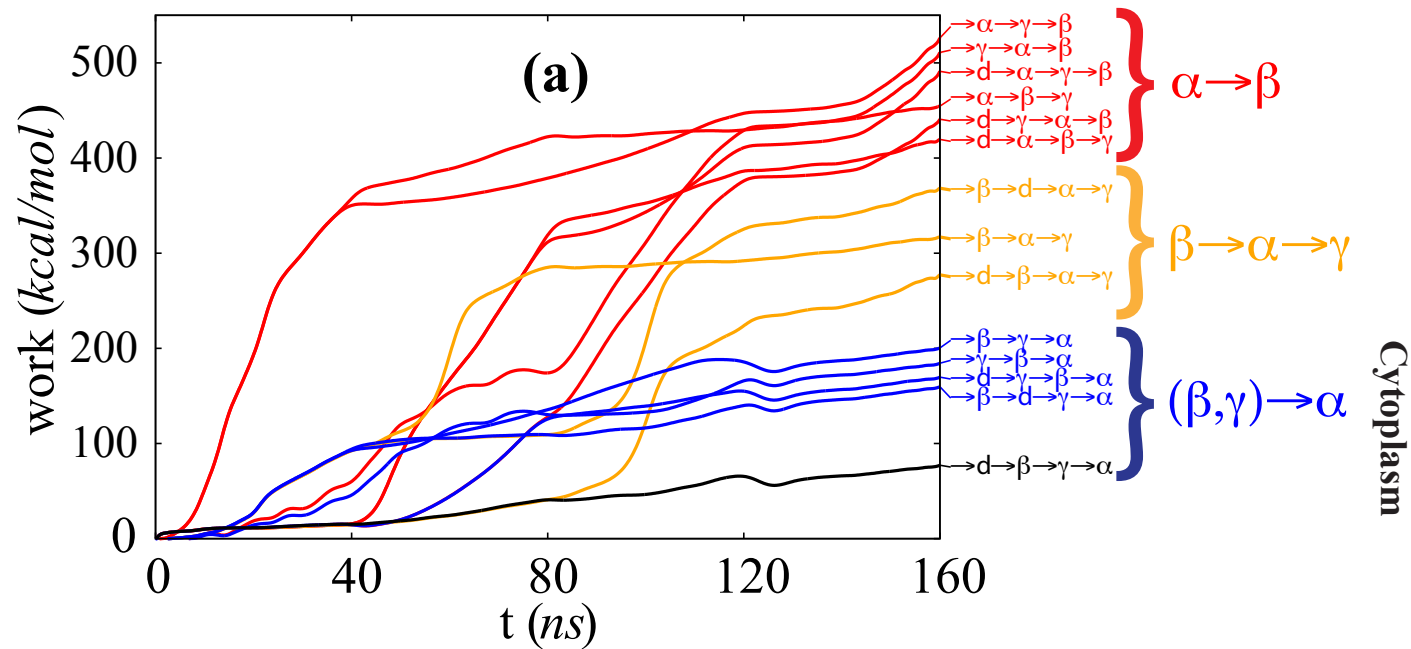
M. Moradi, G. Enkavi, and ET (2015) **Nature Comm.**, 6:8393.

Progressively Optimizing the Biasing Protocol/Collective Variable using non-Equilibrium Work as a Measure of the Path Quality



Example set taken from a subset of 20 ns biased simulations

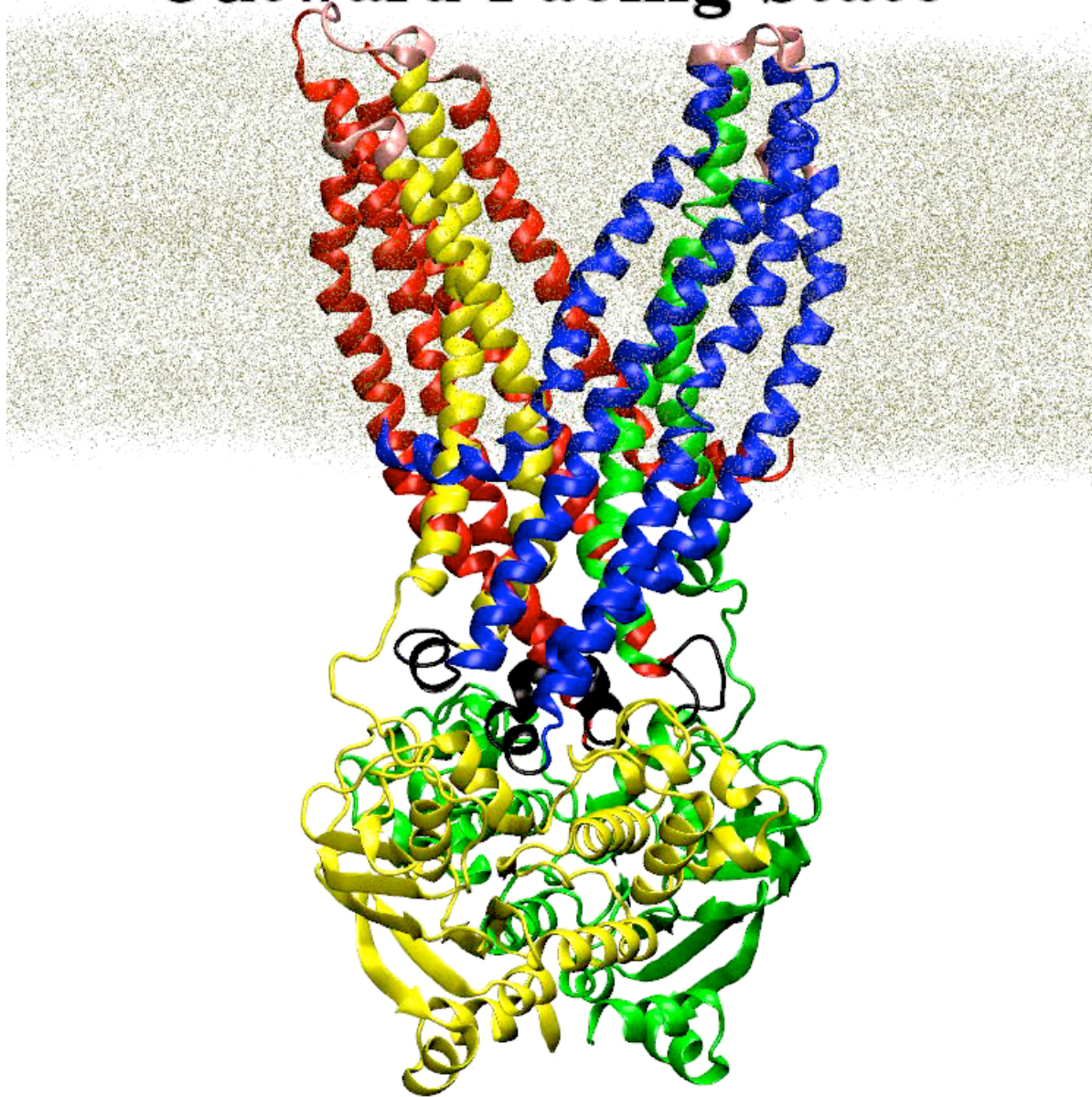
Mechanistic Insight From Transition Pathways in ABC exporters from Non-Equilibrium Simulations



M. Moradi and ET (2013) **PNAS**, 110:18916–18921.

M. Moradi and ET (2014) **JCTC**, 10: 2866–2880.

Outward-Facing State



OF → IF

NBD Dissociation

Periplasmic Closure

NBD Twist

Cytoplasmic Opening

IF → OF

Cytoplasmic Closure

NBD Twist

Periplasmic Opening

NBD Dimerization

R T R T R T R T R

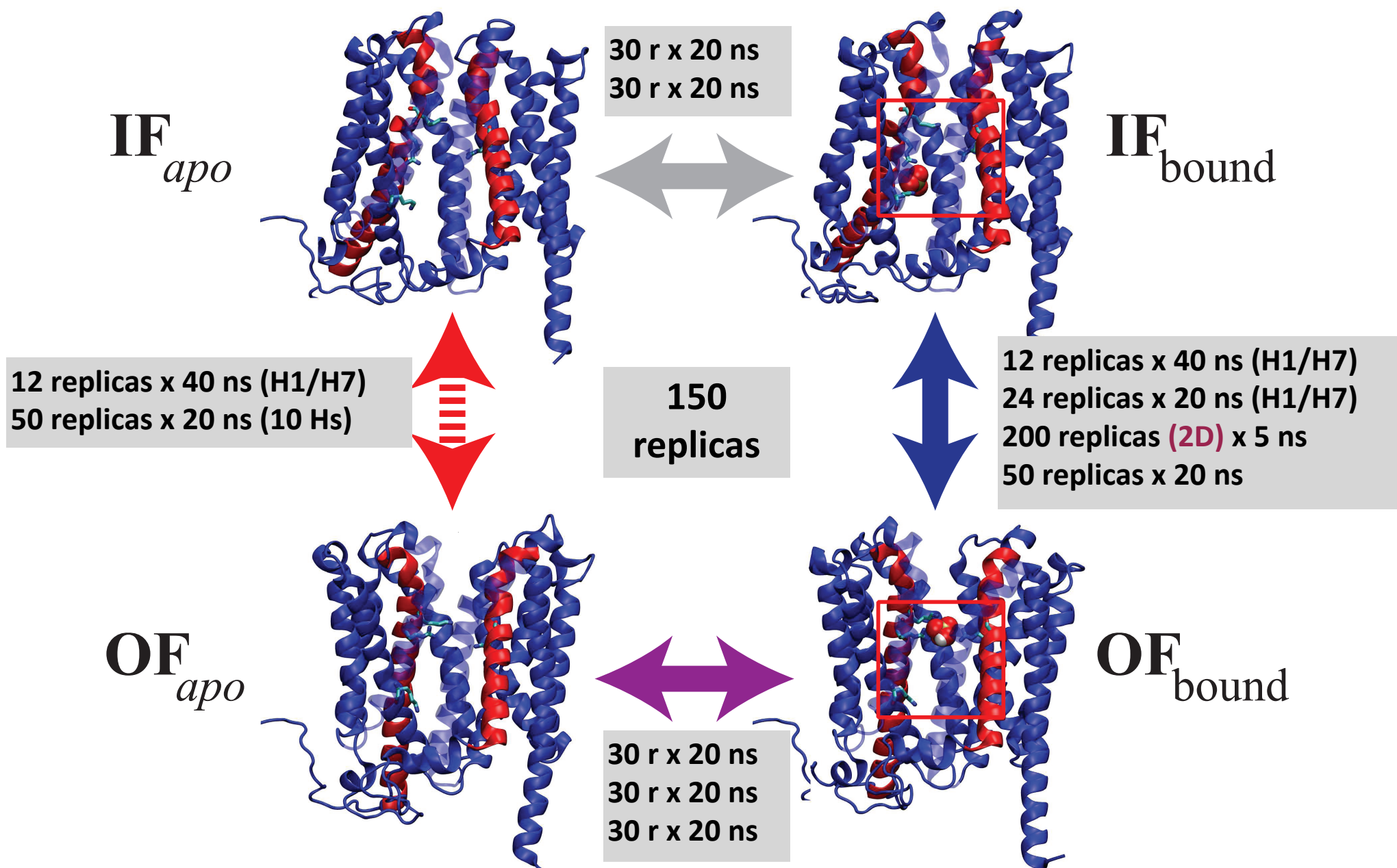
T Transition

R Relaxation

NBD Doorknob Mechanism

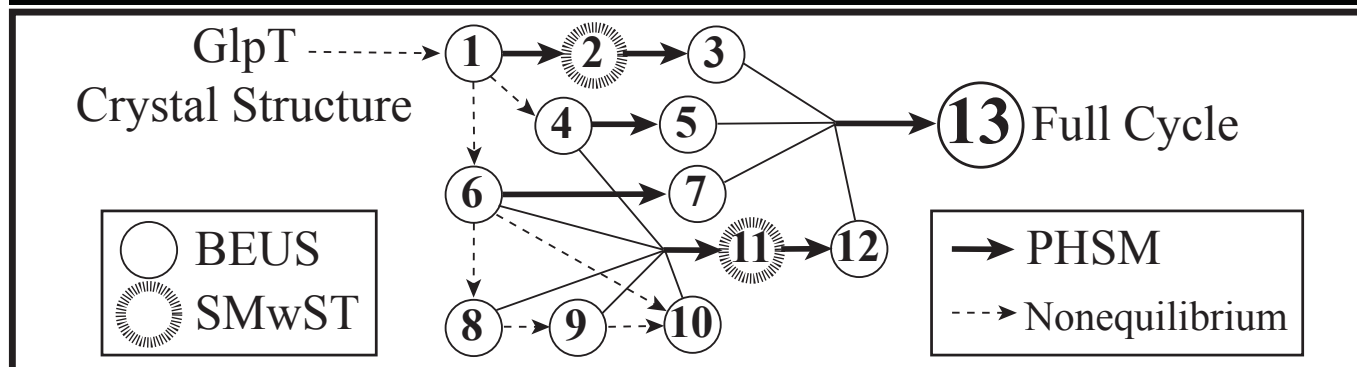
M. Moradi and ET (2013) **PNAS**, 110:18916–18921.

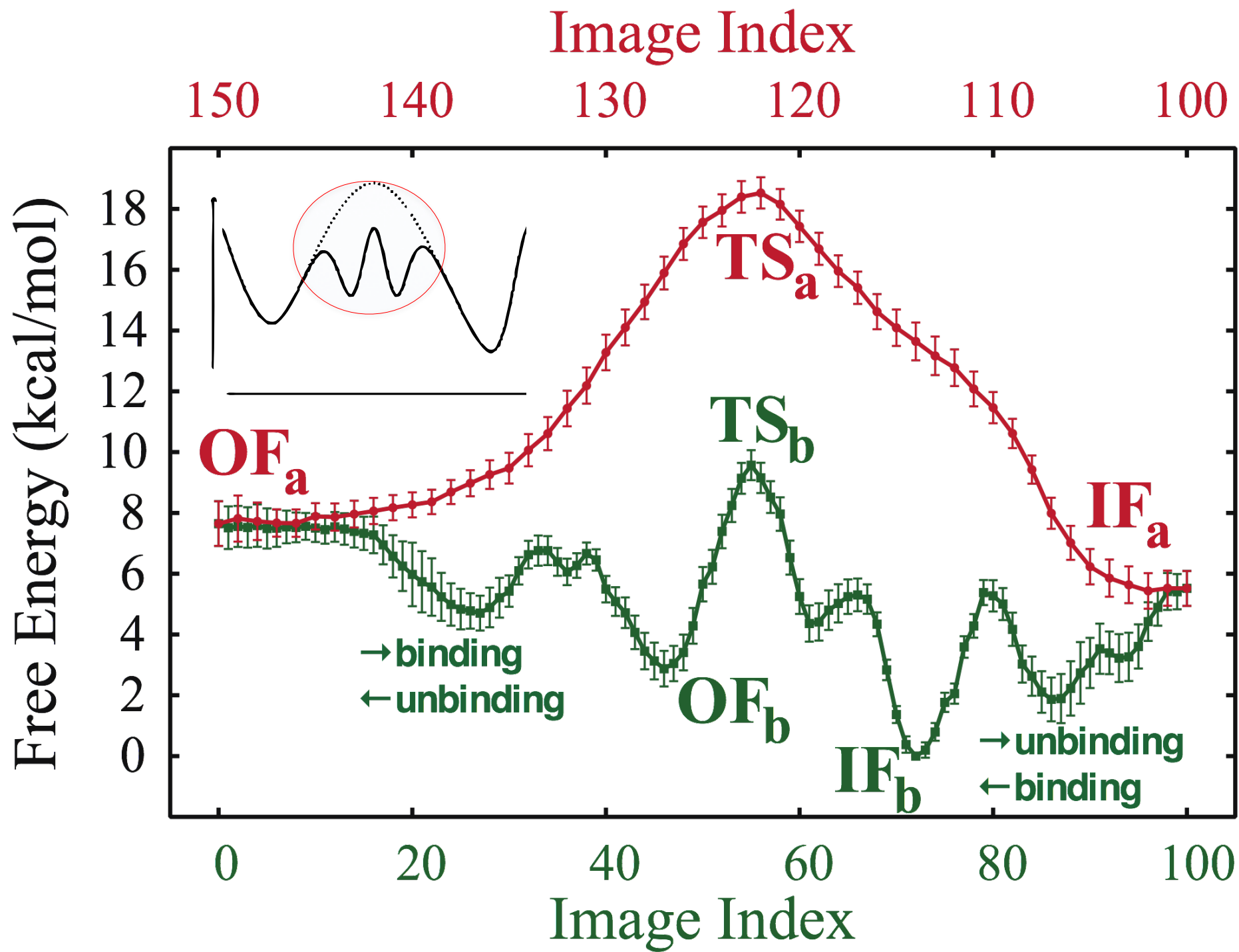
Describing a Complete Cycle (Adding Substrate) Requiring a Combination of **Multiple Collective Variables**



Simulation protocols

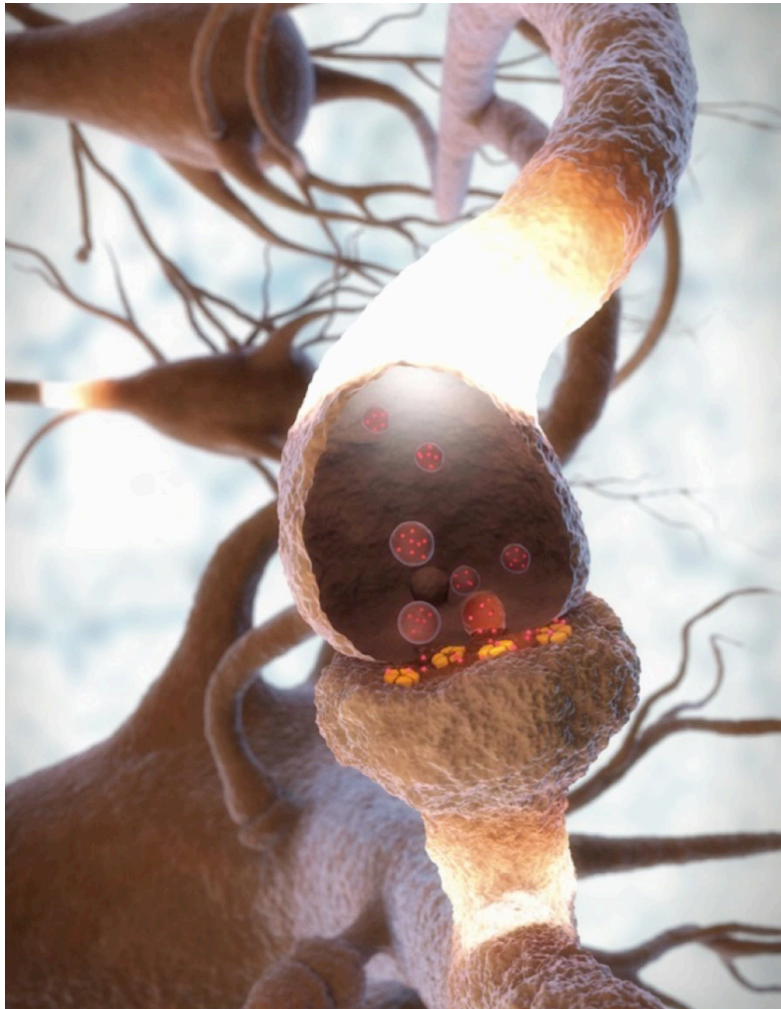
	Transition	Technique	Collective Variables	# of Replicas × Runtime	
1	$IF_a \leftrightarrow OF_a$	BEUS	(Q_1, Q_7)	$12 \times 40 \text{ ns}$	$= 0.5 \mu\text{s}$
2		SMwST	$\{Q\}$	$1000 \times 1 \text{ ns}$	$= 1 \mu\text{s}$
3		BEUS	$\{Q\}$	$50 \times 20 \text{ ns}$	$= 1 \mu\text{s}$
4	$IF_a \leftrightarrow IF_b$	BEUS	Z_{Pi}	$30 \times 40 \text{ ns}$	$= 1.2 \mu\text{s}$
5		BEUS	$(\{Q\}, Z_{Pi})$	$30 \times 40 \text{ ns}$	$= 1.2 \mu\text{s}$
6	$OF_a \leftrightarrow OF_b$	BEUS	Z_{Pi}	$30 \times 40 \text{ ns}$	$= 1.2 \mu\text{s}$
7		BEUS	$(\{Q\}, Z_{Pi})$	$30 \times 40 \text{ ns}$	$= 1.2 \mu\text{s}$
8	$IF_b \leftrightarrow OF_b$	BEUS	(Q_1, Q_7)	$24 \times 20 \text{ ns}$	$= 0.5 \mu\text{s}$
9		BEUS	Z_{Pi}	$15 \times 30 \text{ ns}$	$= 0.5 \mu\text{s}$
10		2D BEUS	$(\Delta\text{RMSD}, Z_{Pi})$	$200 \times 5 \text{ ns}$	$= 1 \mu\text{s}$
11		SMwST	$(\{Q\}, Z_{Pi})$	$1000 \times 1 \text{ ns}$	$= 1 \mu\text{s}$
12		BEUS	$(\{Q\}, Z_{Pi})$	$50 \times 20 \text{ ns}$	$= 1 \mu\text{s}$
13	Full Cycle	BEUS	$(\{Q\}, Z_{Pi})$	$150 \times 50 \text{ ns}$	$= 7.5 \mu\text{s}$
Total Simulation Time					18.7 μs



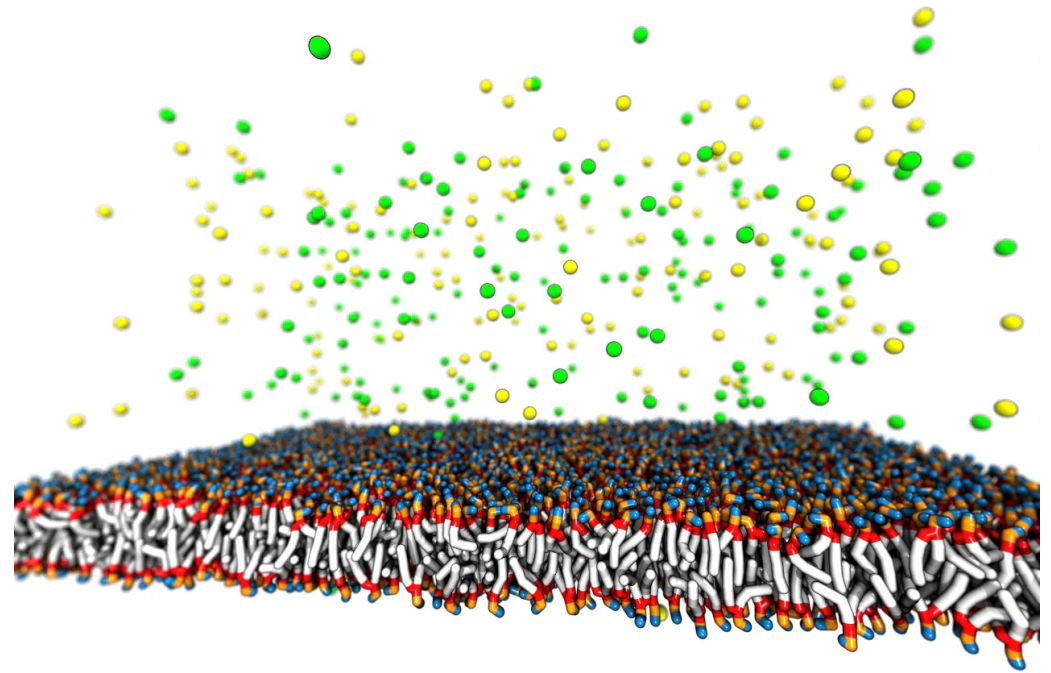


Battling the Timescale - Case IV

Multiscale Simulations



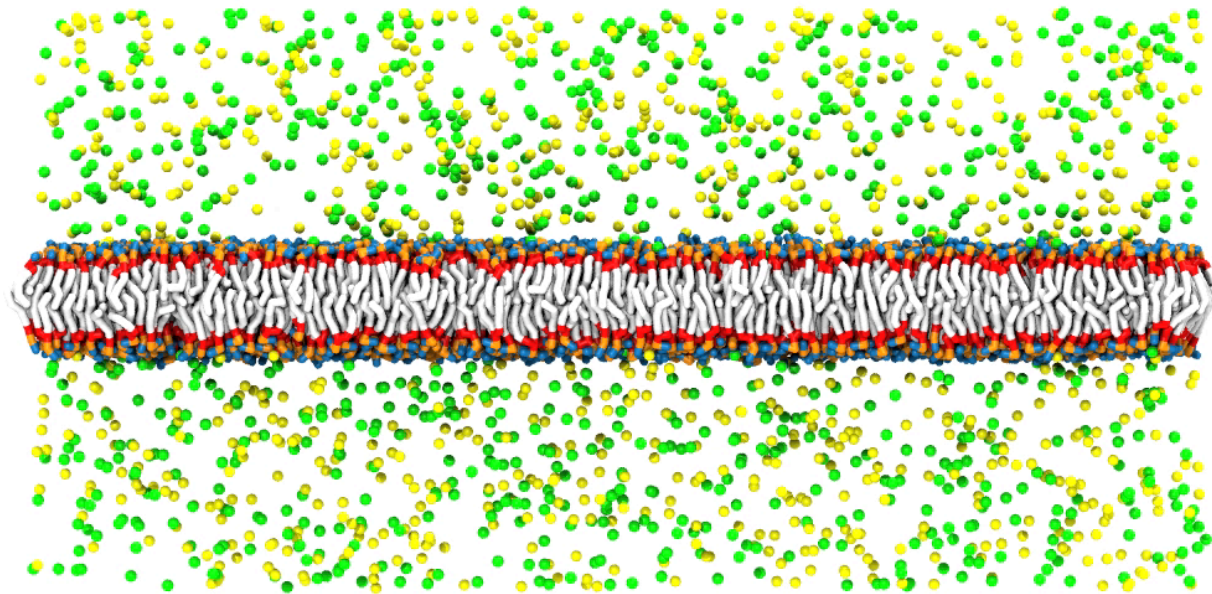
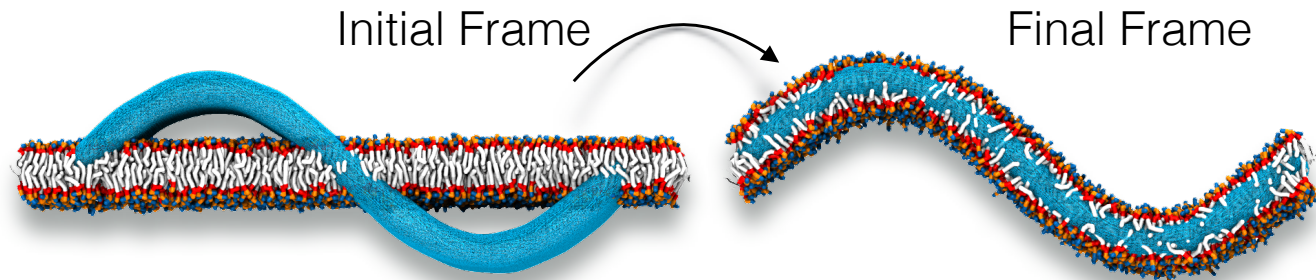
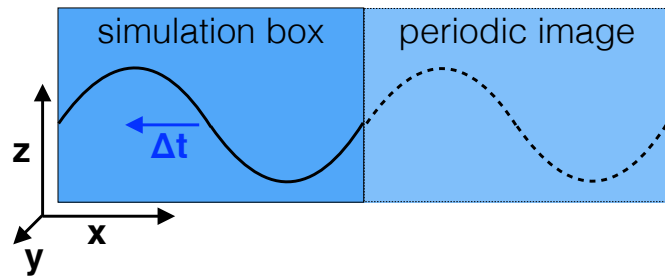
Membrane Budding/Fusion



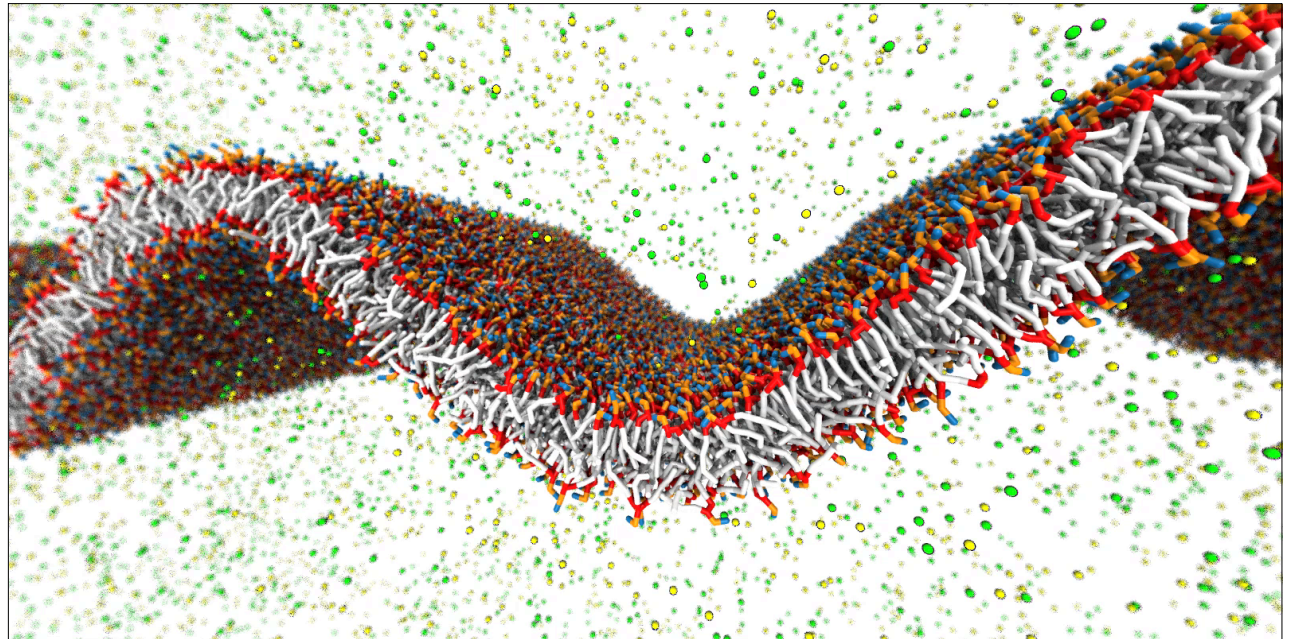
Combining multiple replica simulations and coarse-grained models to describe membrane fusion

Workflow for Multi-Scale Modeling

Parametrically Defined Sine Function

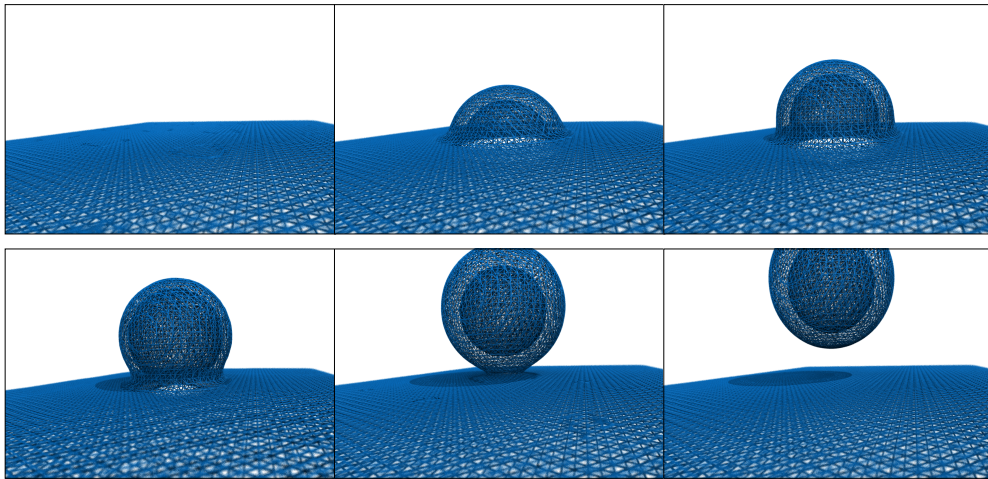
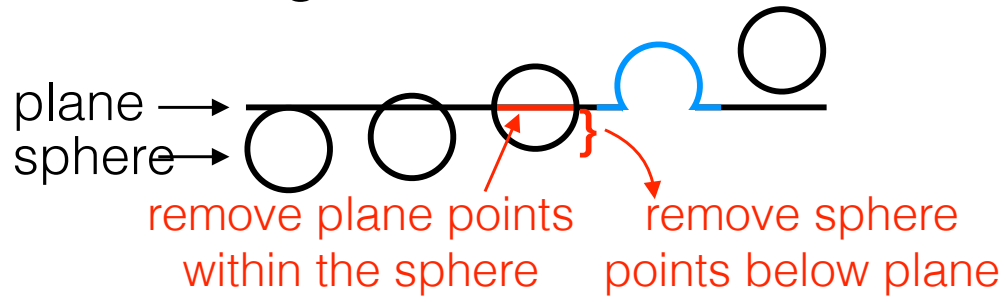


Workflow for Multi-Scale Modeling

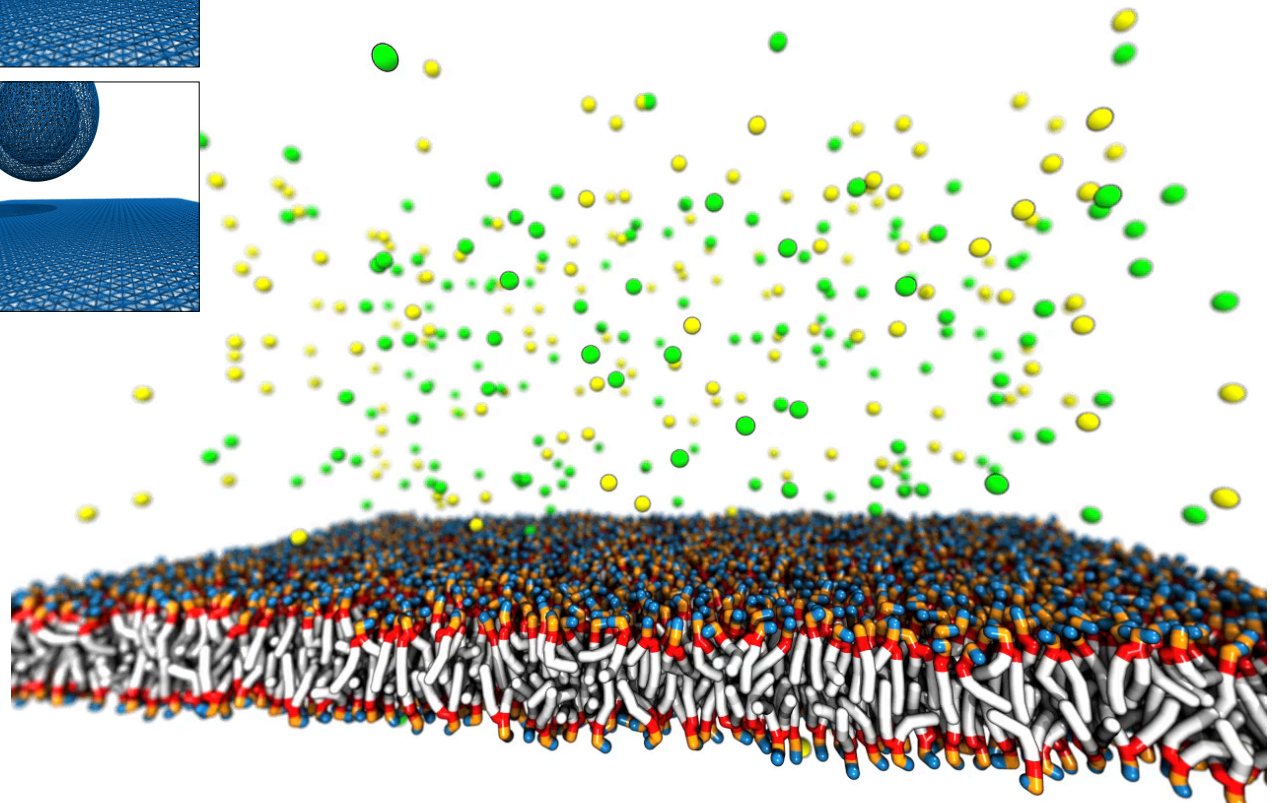


Workflow for Multi-Scale Modeling

Grid Design and Construction



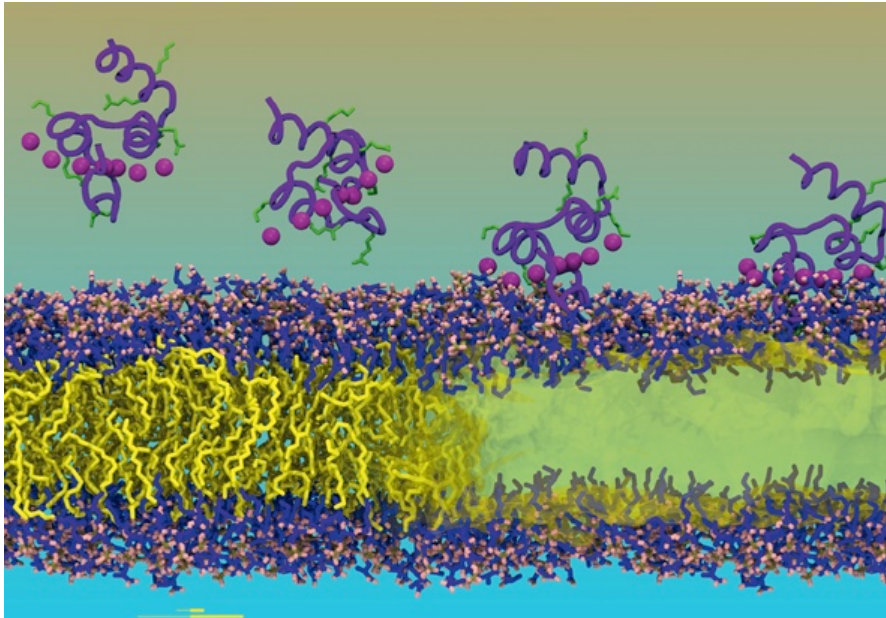
Membrane Budding/Fusion



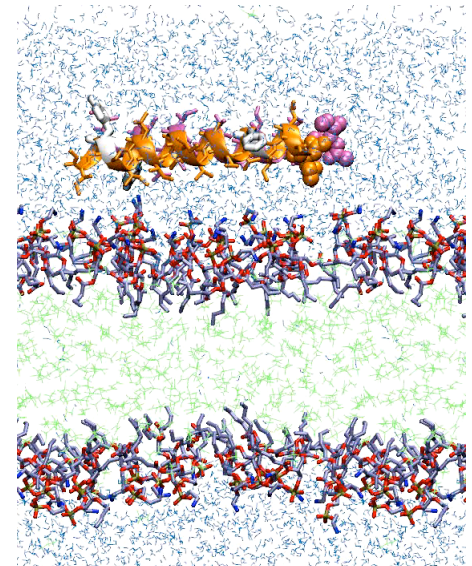
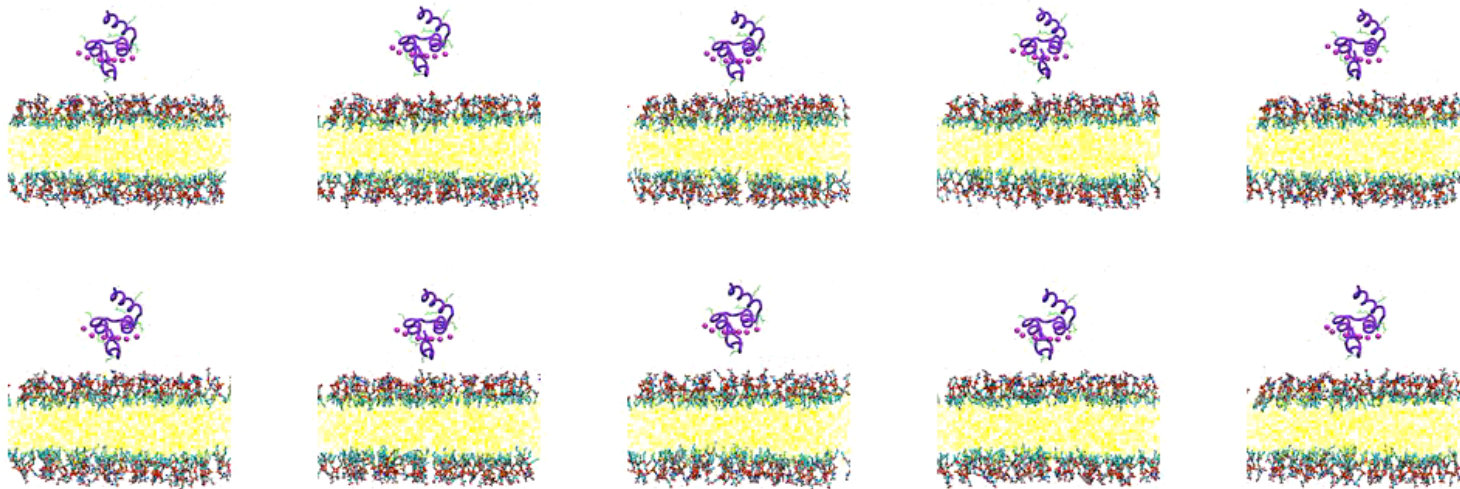
Battling the Timescale - Case V

Reduced Representations

Highly Mobile Membrane Mimetic model



*GpA insertion
in 12 ns*

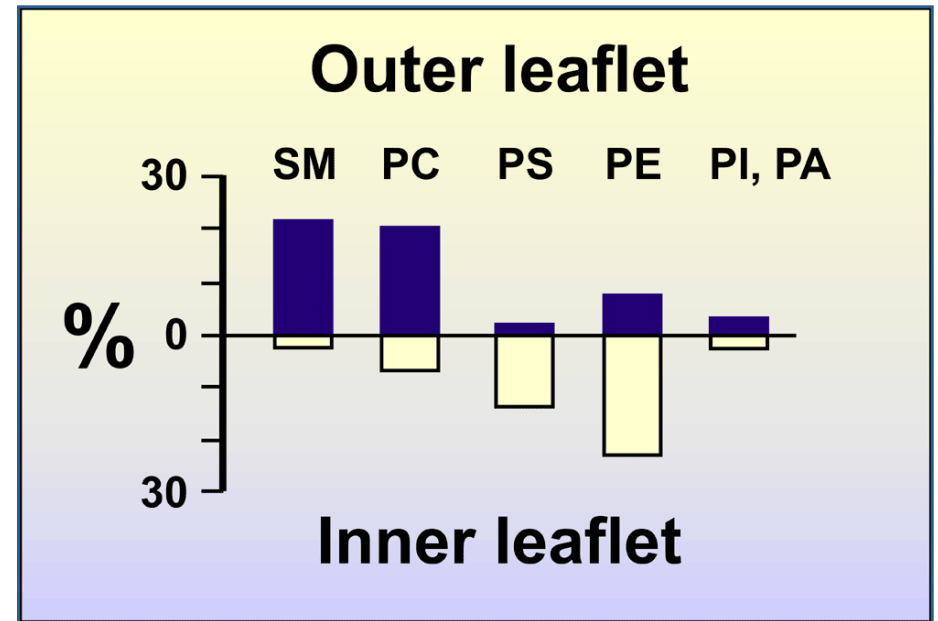
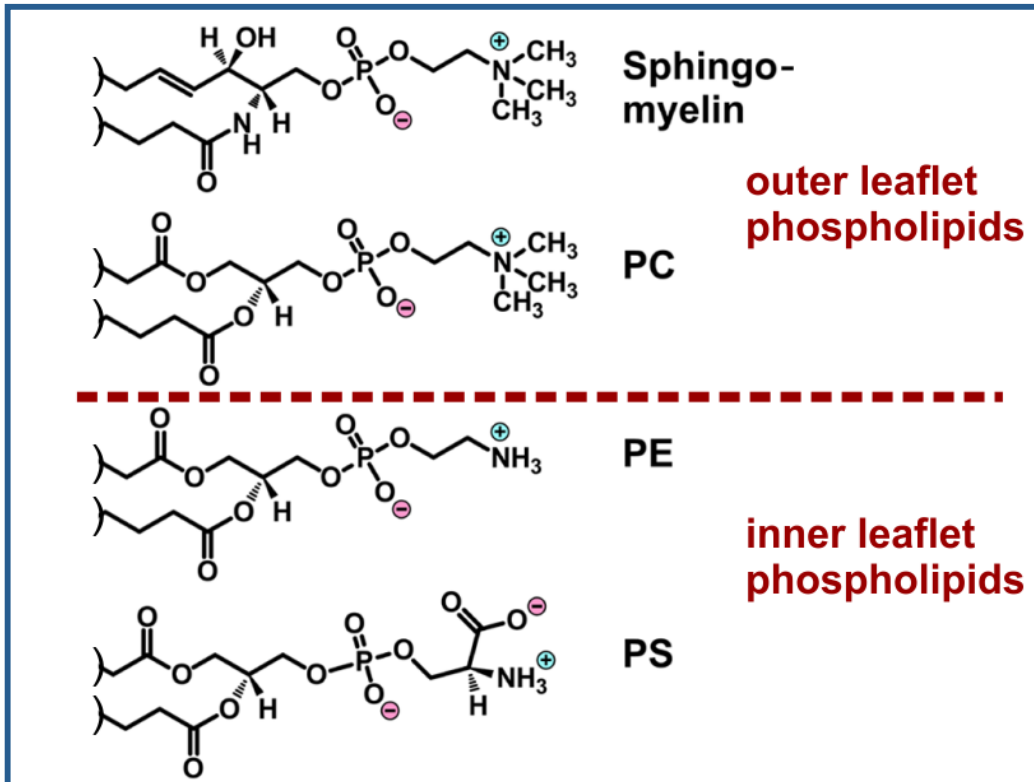


Specific lipids regulate various functional aspects of membrane proteins

Integral membrane proteins

Peripheral membrane proteins

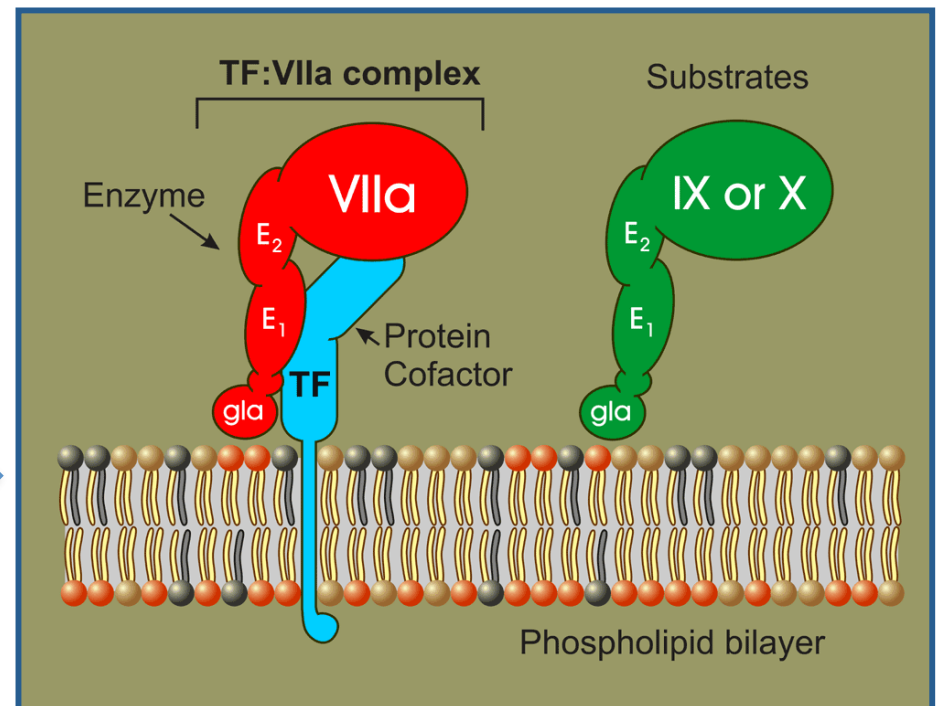
Lipid Dependent Binding and Activation



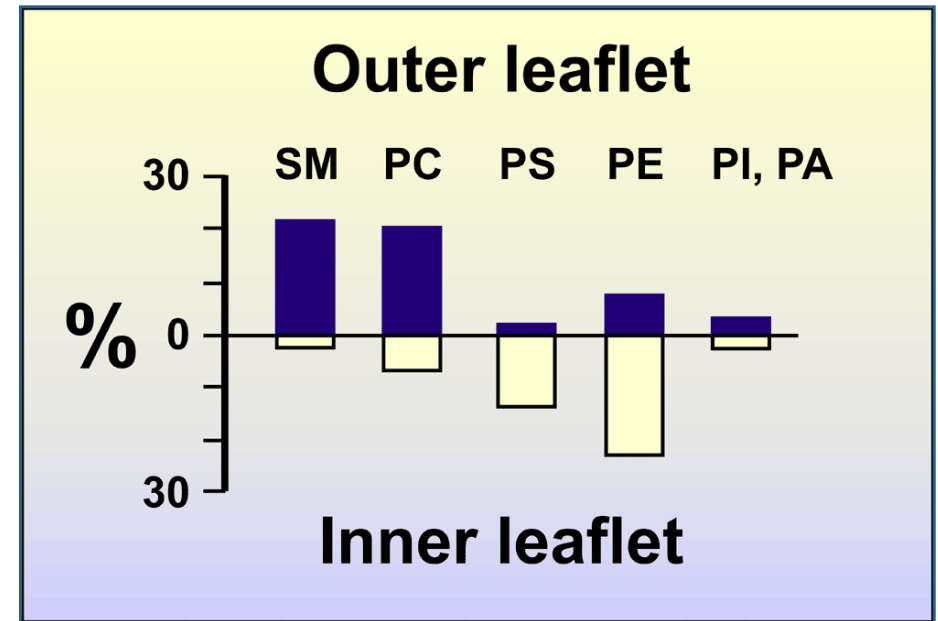
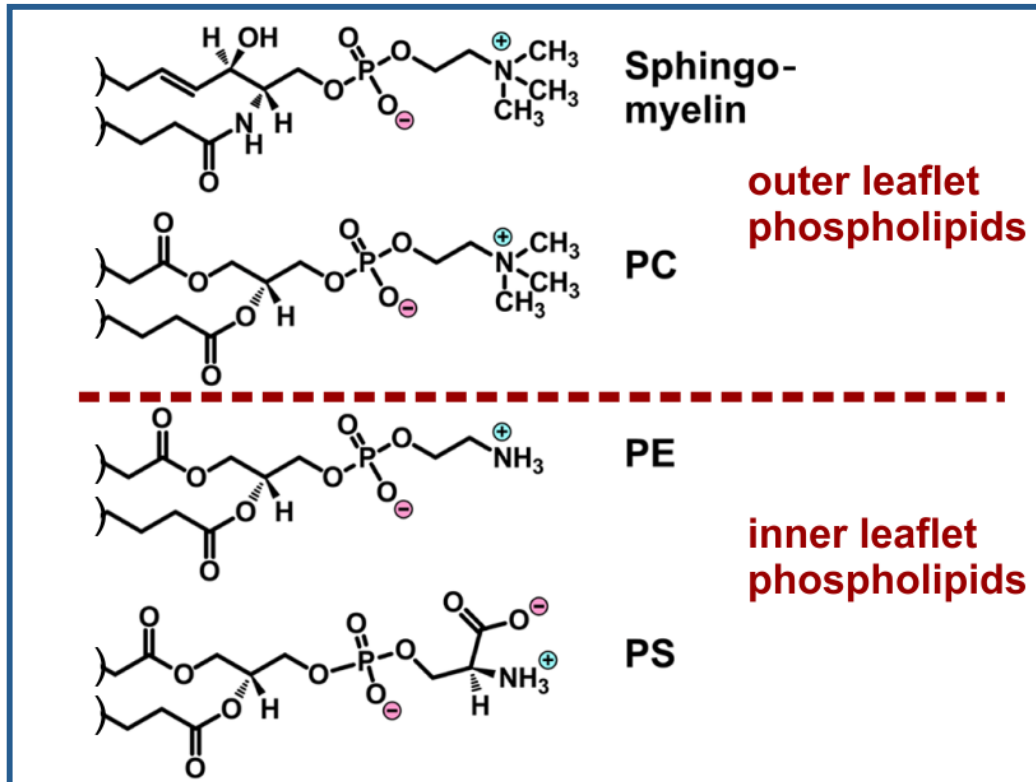
Affinity is controlled by lipid content

Leaflet asymmetry is vital for coagulation

Courtesy of Jim Morrissey, UIUC

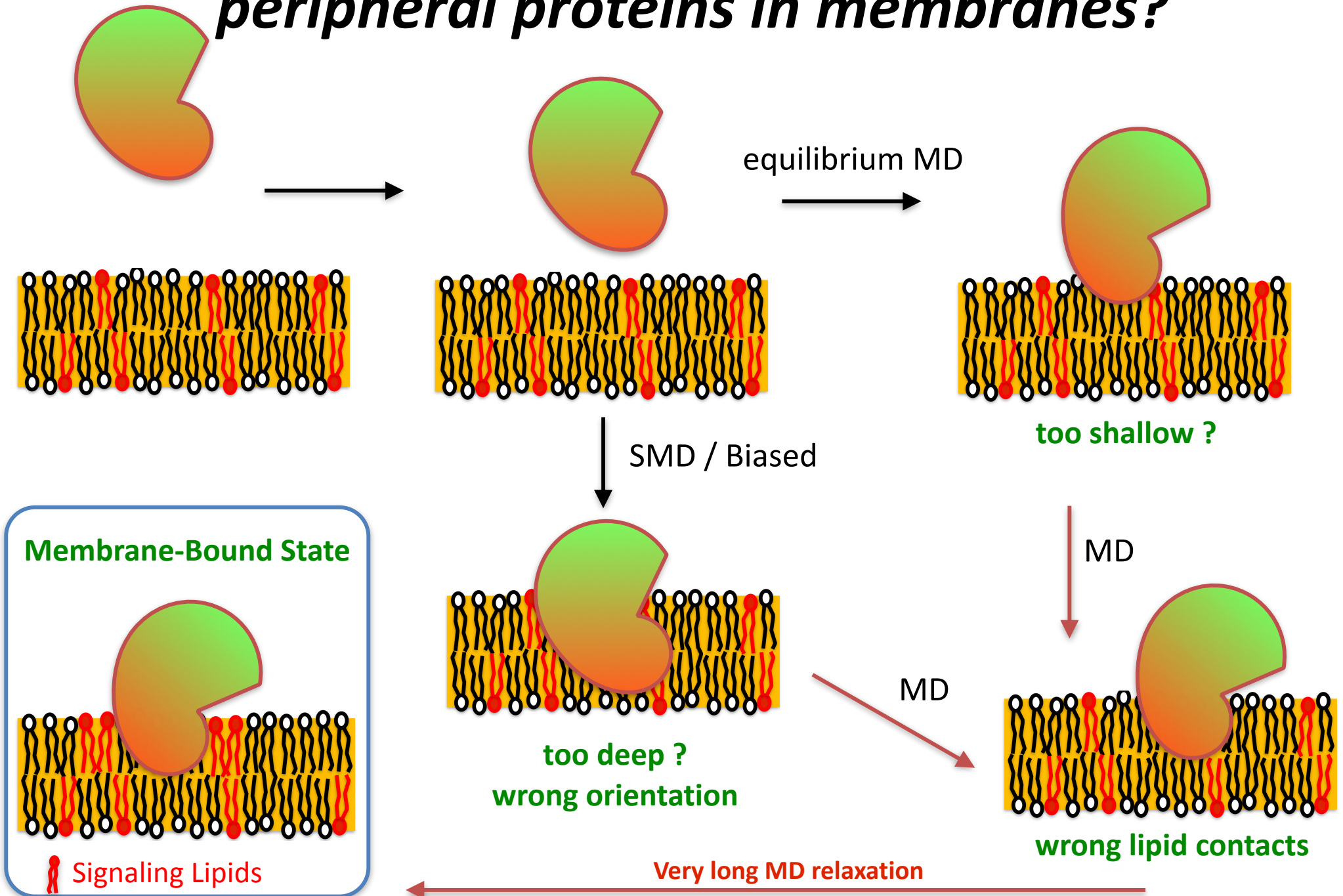


Lipid Dependent Binding and Activation

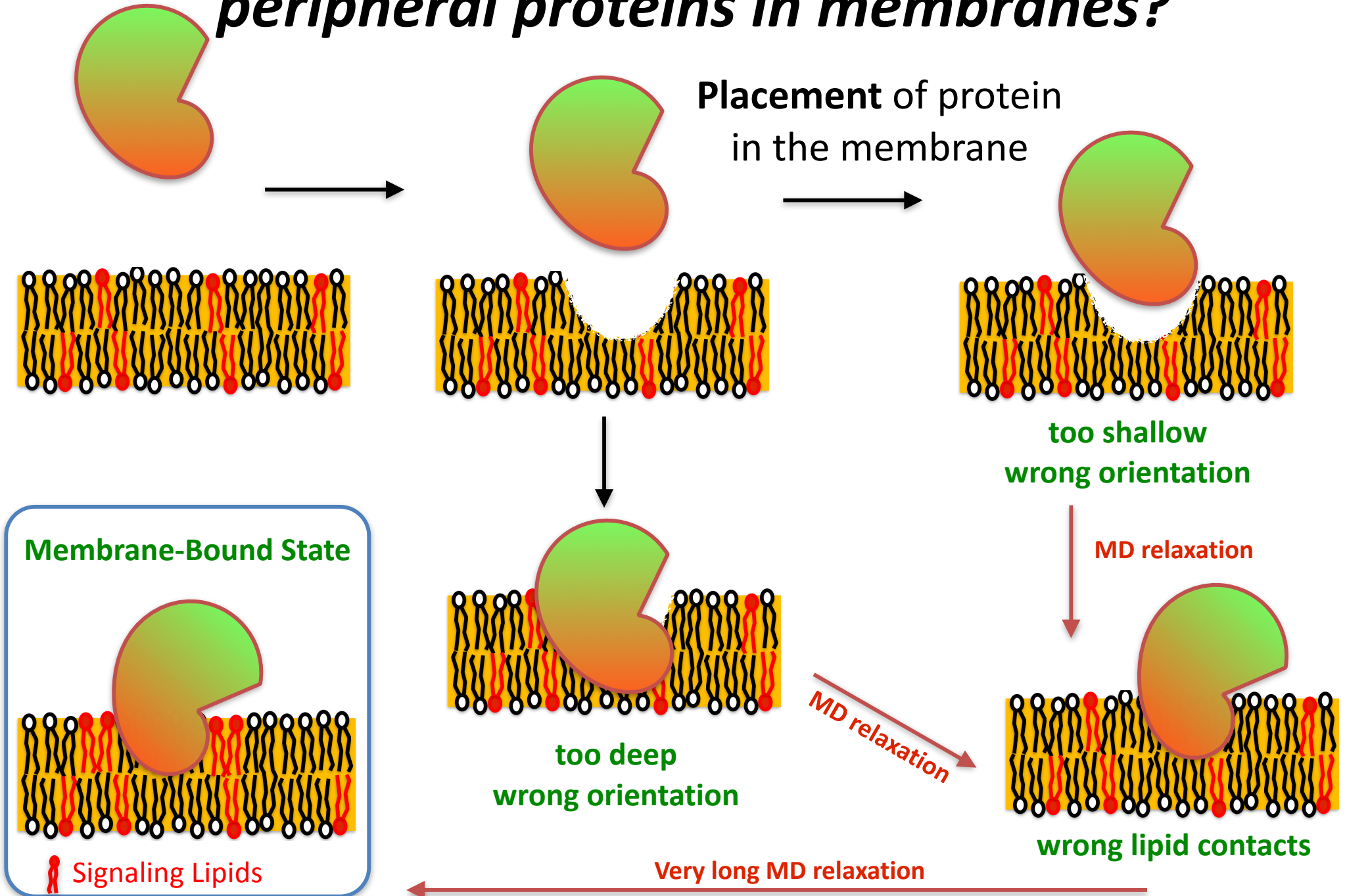


Mode and specificity of lipid-protein interactions constitute one of the main mechanistic aspects

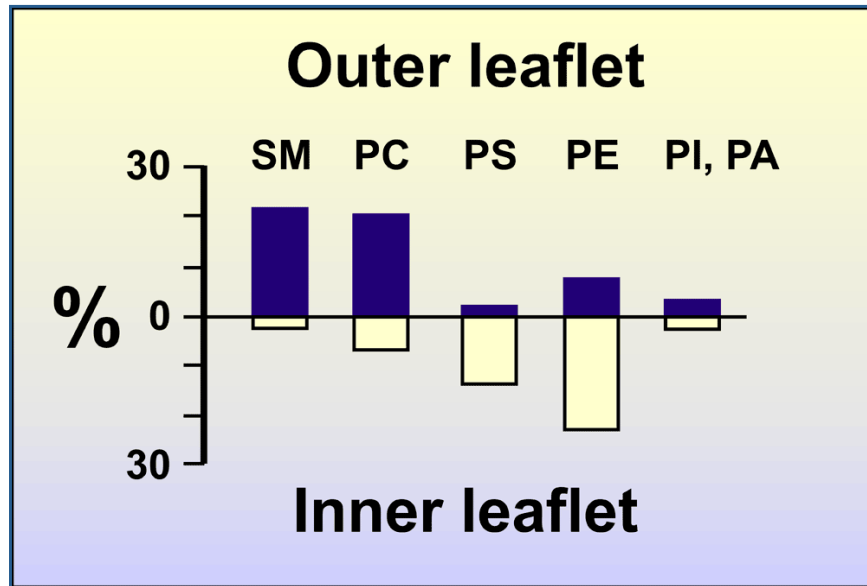
How do we construct an initial model for peripheral proteins in membranes?



How do we construct an initial model for peripheral proteins in membranes?

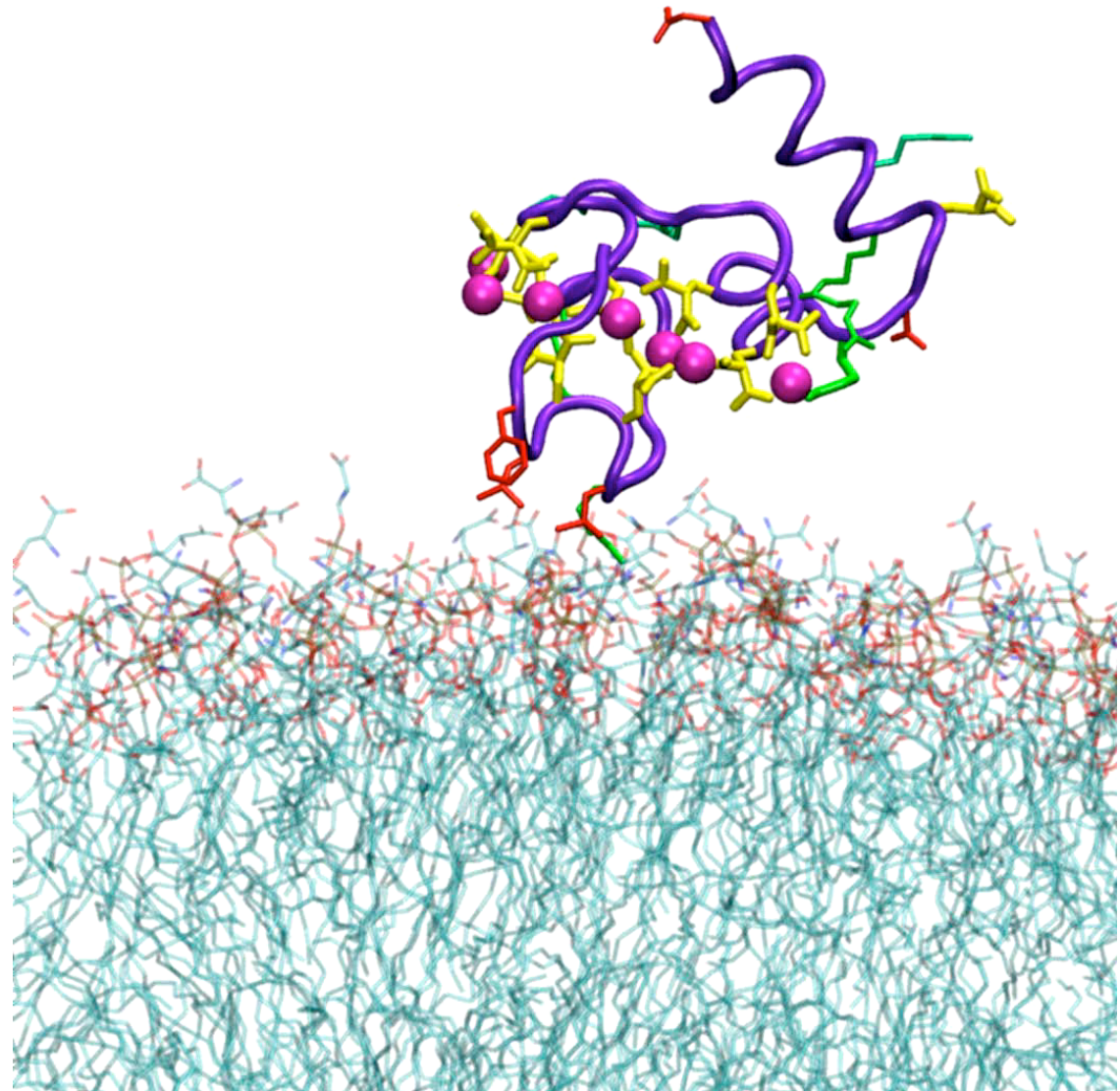


Simulation of Binding with Full Membrane Representation



Partial list of technical problems:

- Biased simulations
- Unknown depth of insertion
- Single binding event
- Frequently failing
- **Minimal lipid reorganization**



Z. Ohkubo and E. T., **Structure**, 16: 72-81 (2008)

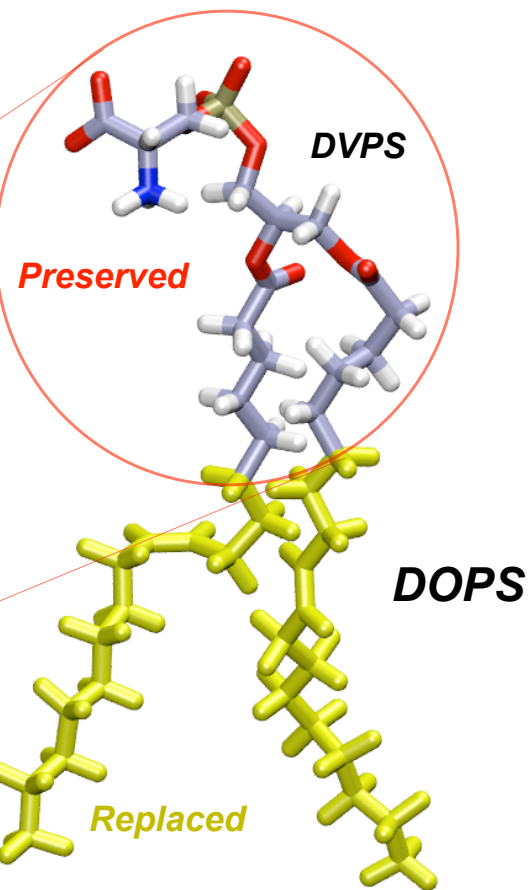
HMMM model

Highly Mobile Membrane Mimetic model

Full model

HMMM model

Tails
replaced by
organic solvent



Advantages

Increased mobility of lipids

**Retain explicit headgroups
allowing for atomic details**

Biophys. J., 102: 2130-2139 (2012) (Cover Article)



Zenmei Ohkubo



Mark Arcario



Taras Pogorelov



Josh Vermaas

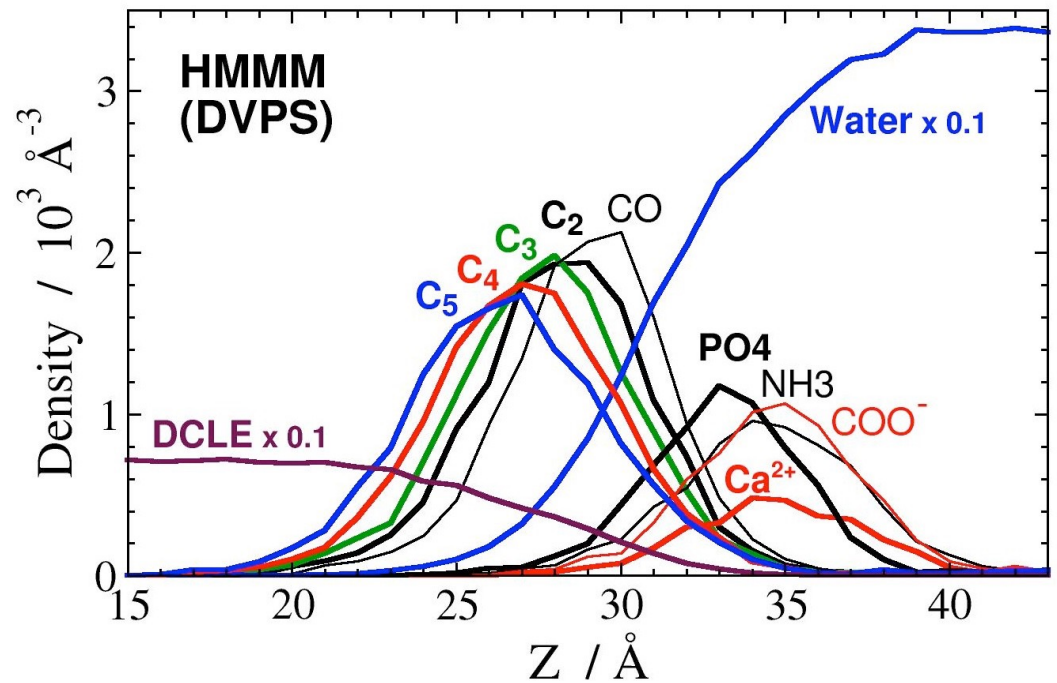
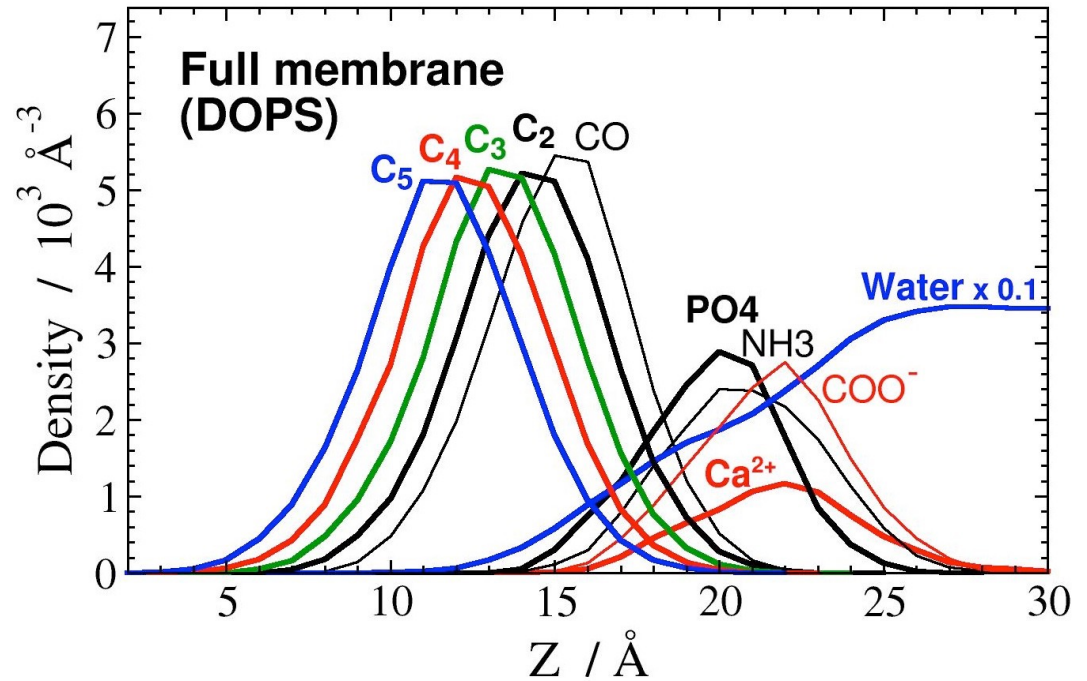
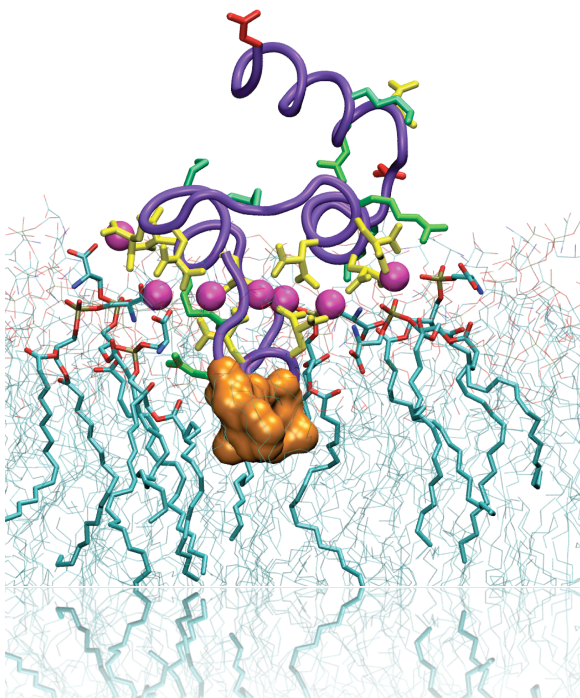


Javier Baylon

HMMM- Preserving the “Face” of the Lipid Bilayer

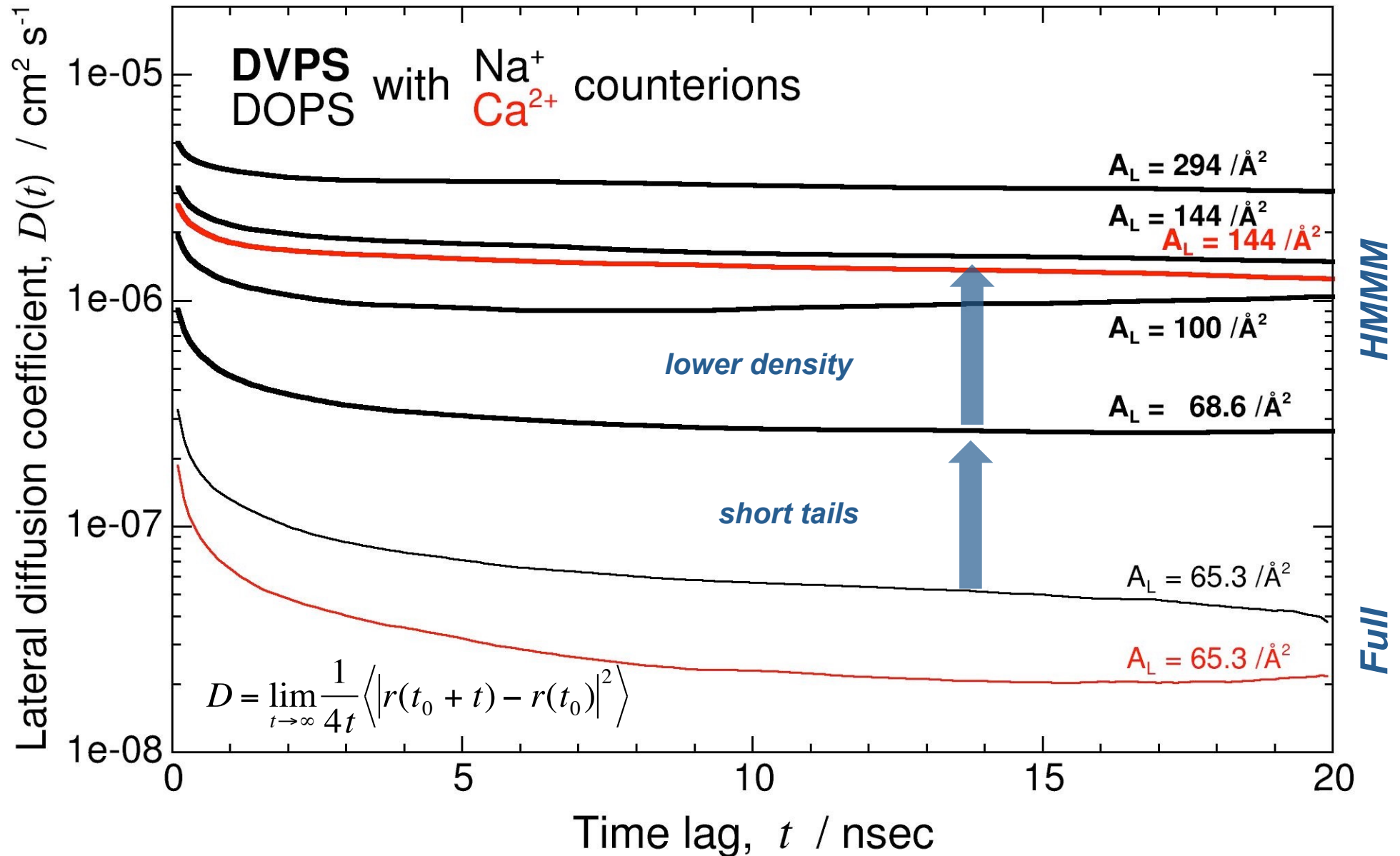
Perfect match in the
membrane profile
particularly in the head
group region

Critical for proper
description of lipid protein
interactions



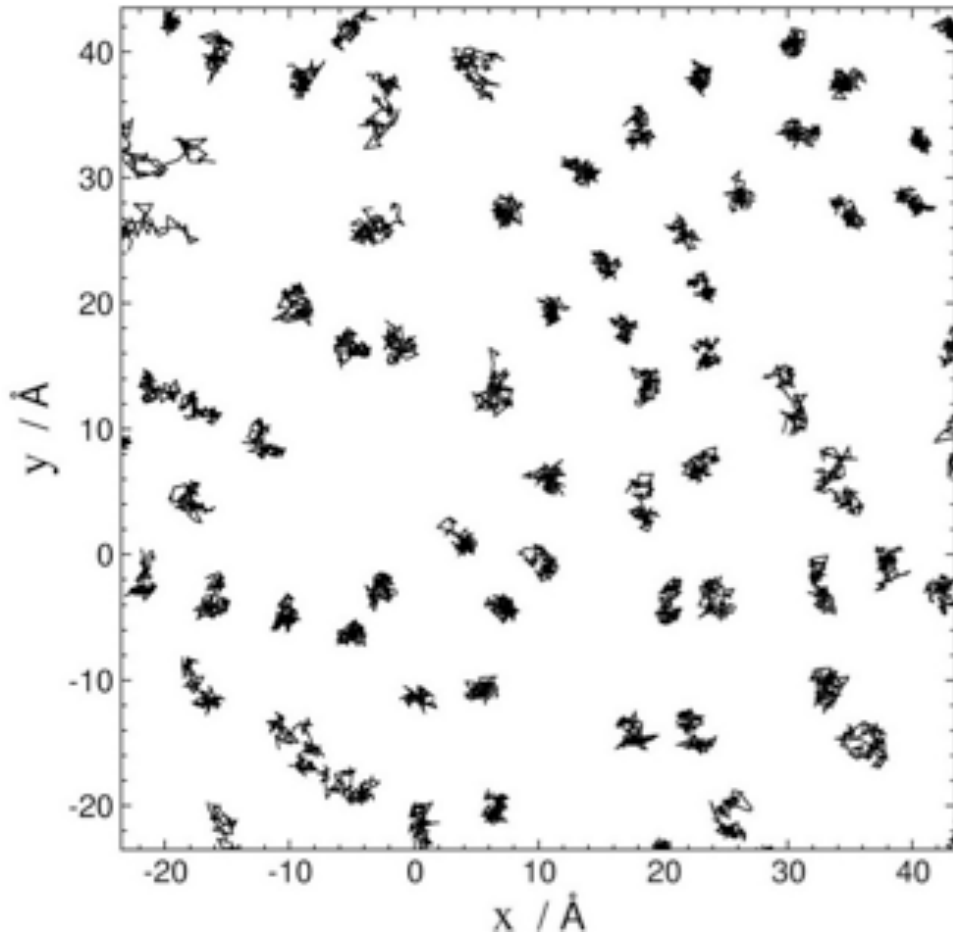
Enhanced Lipid Lateral Diffusion

Without Compromising Atomic Details of the Headgroups

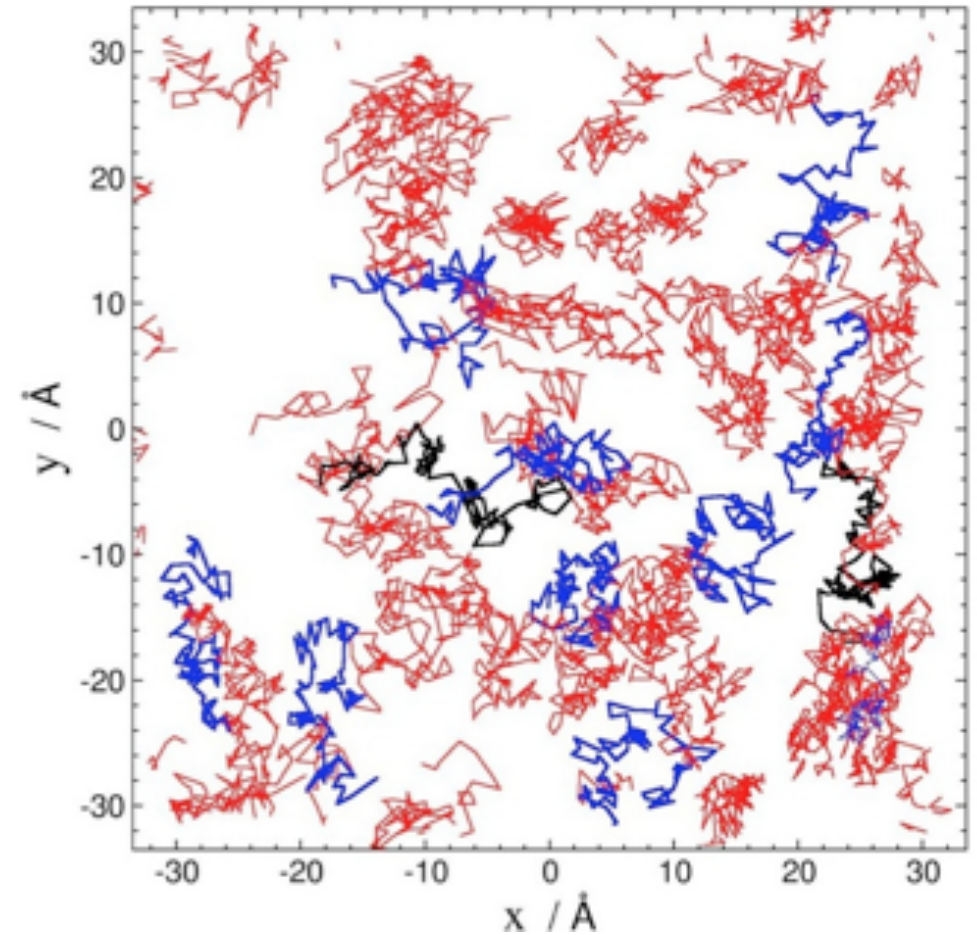


Enhanced Lipid Lateral Diffusion

Without Compromising Atomic Details of the Headgroups



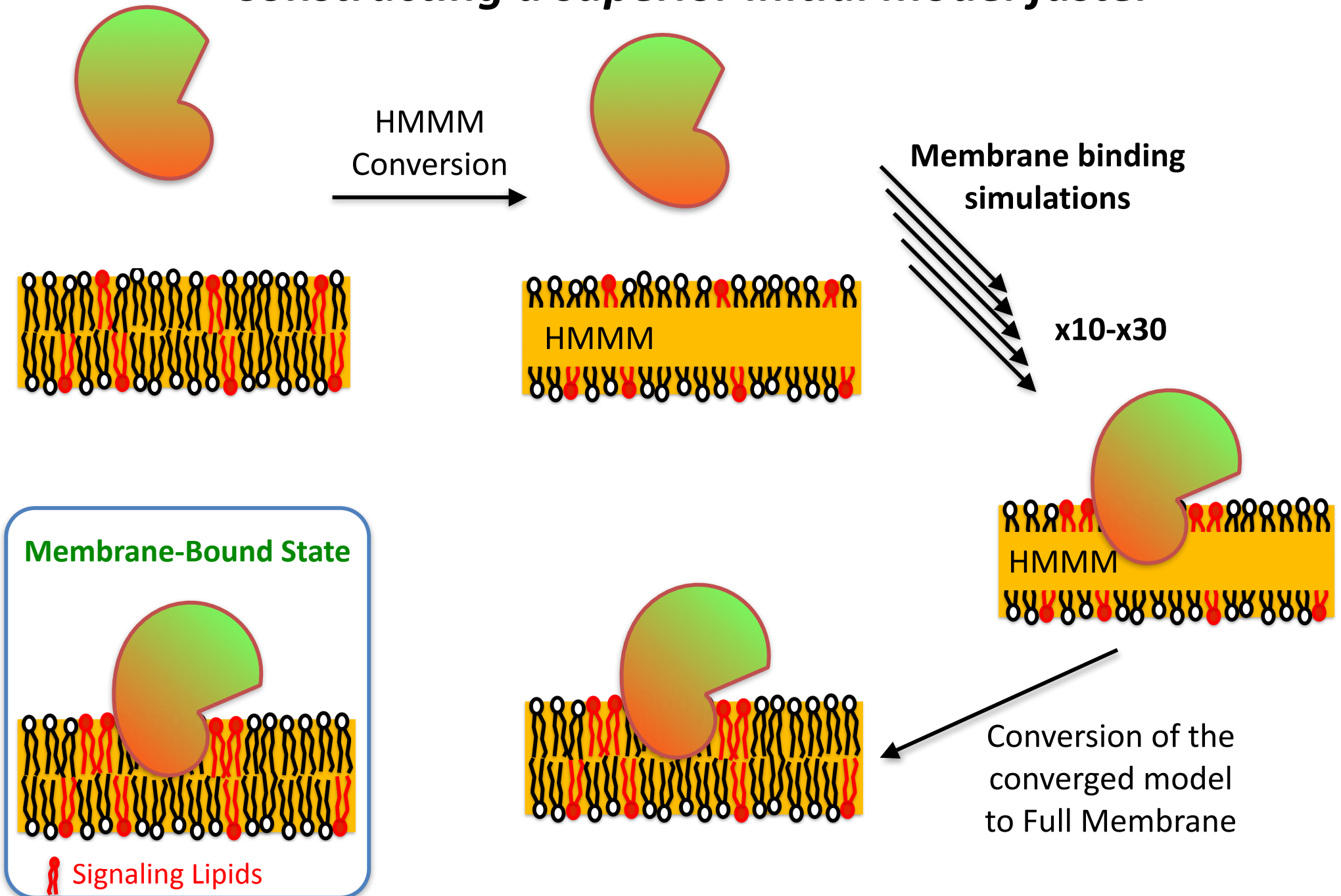
Conventional membrane (10 ns)



HMMM membrane (1 ns)

HMMM accelerated sampling of lipid-protein interactions

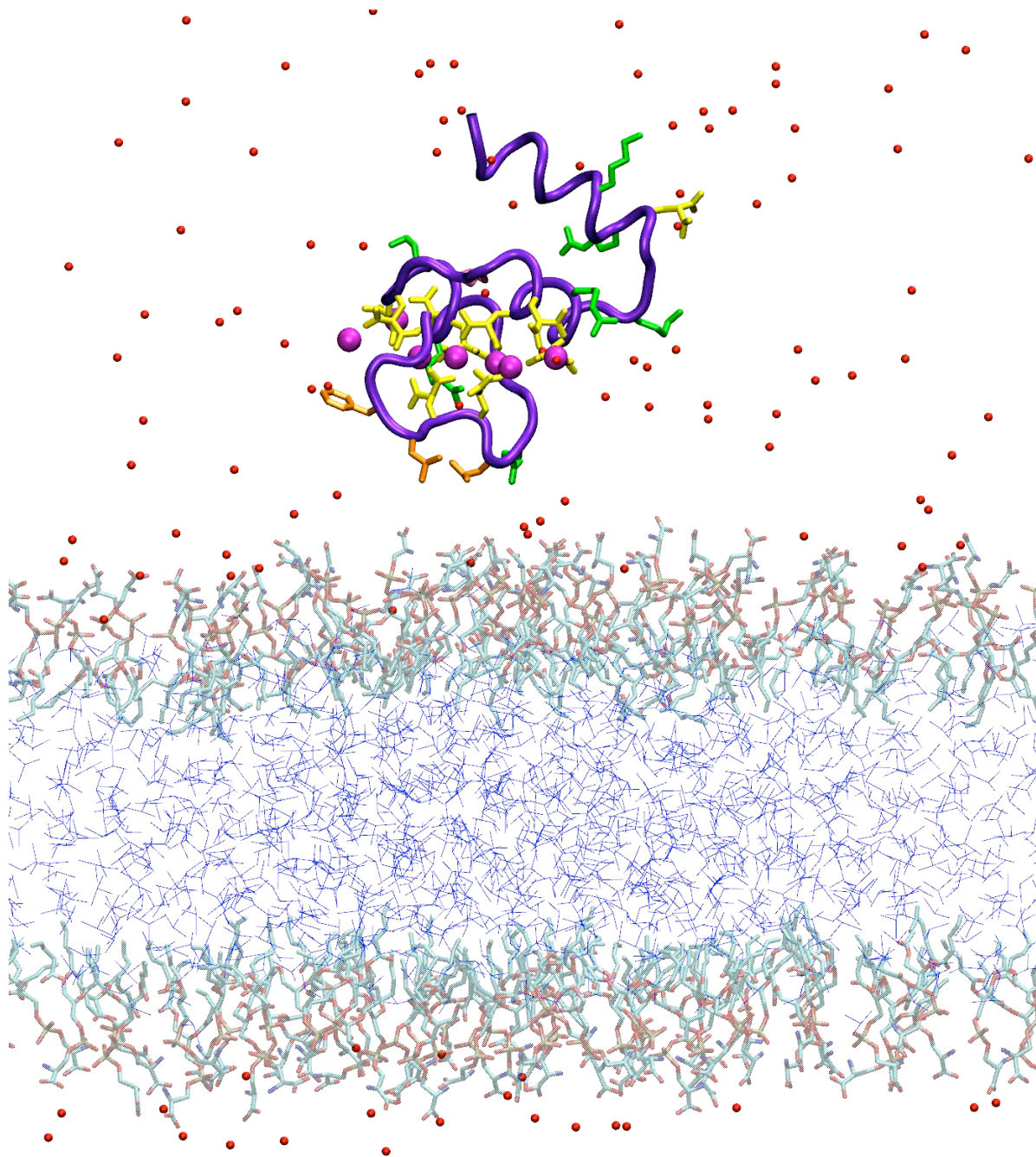
Constructing a superior initial model faster



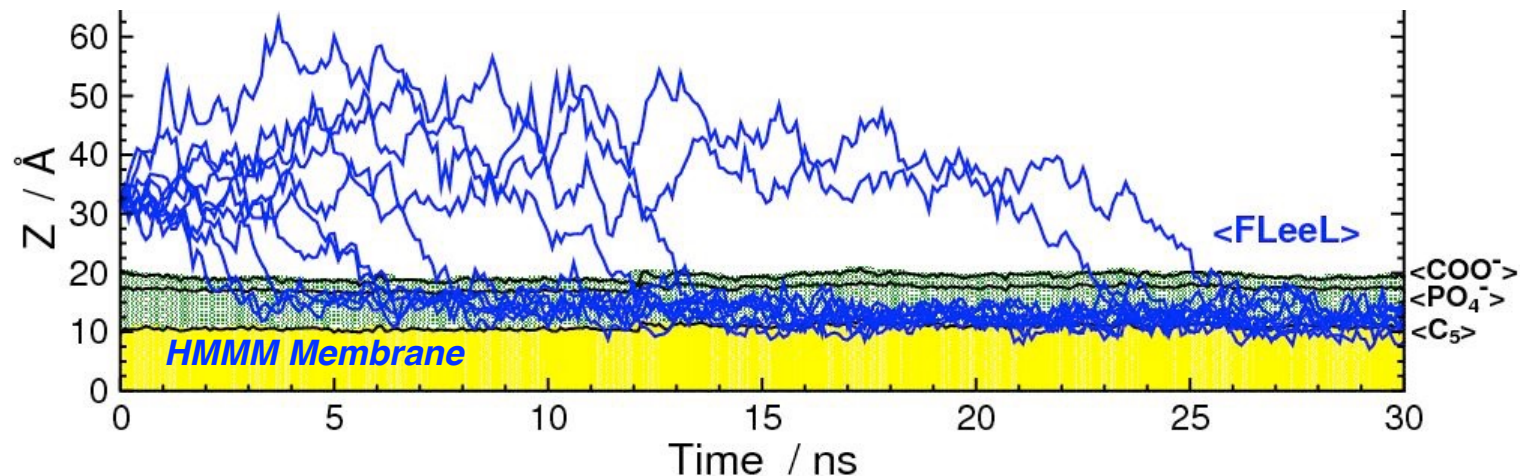
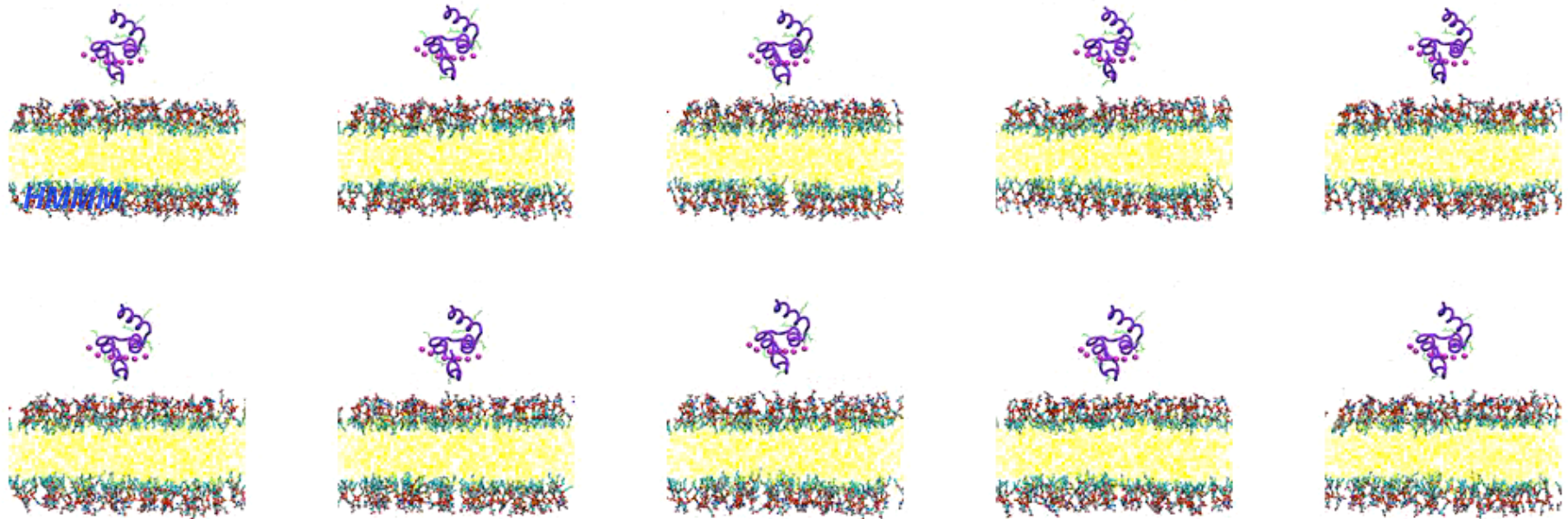
PS-Dependent Spontaneous Insertion of FVII-GLA



Zenmei Ohkubo



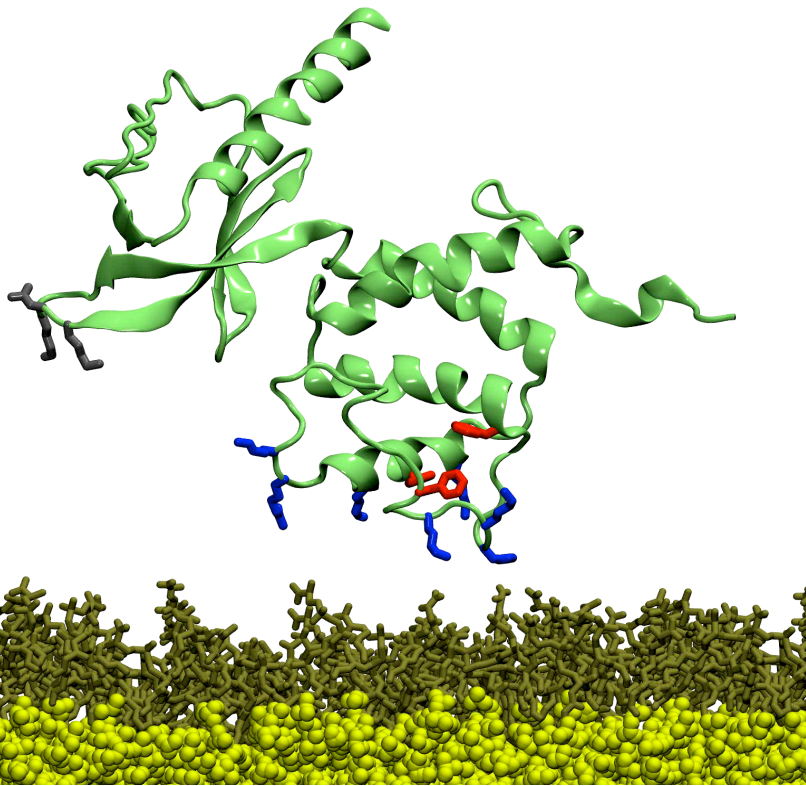
Spontaneous, Unbiased Membrane Binding Accelerated Process Allows for better sampling ($n = 10$)



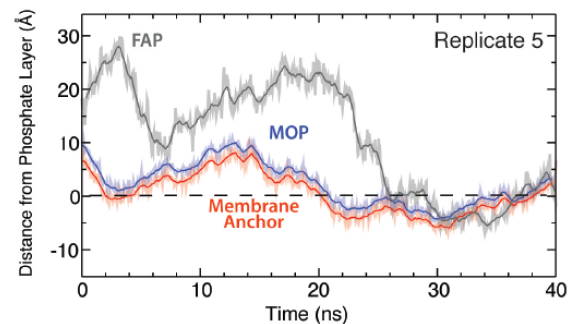
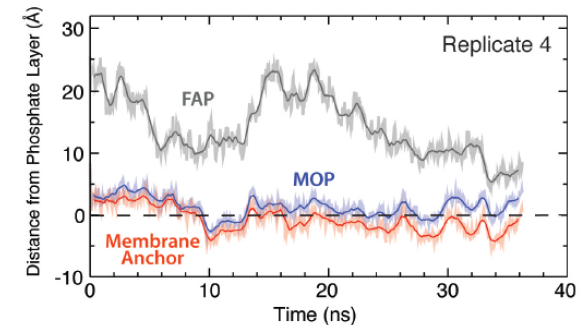
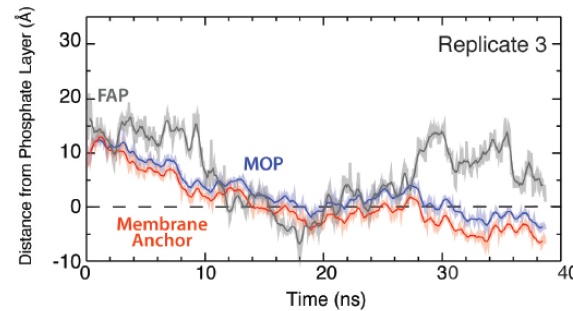
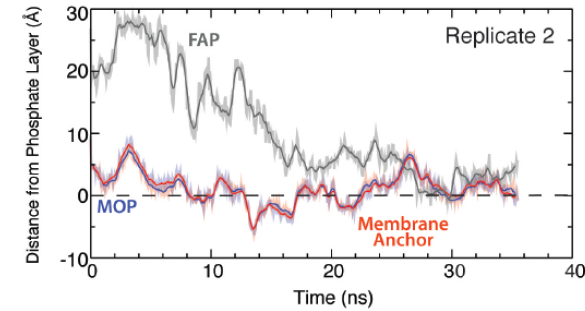
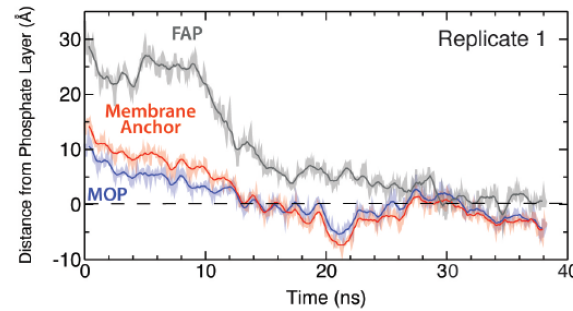
PS-Dependent Membrane Binding of Talin



Mark Arcario



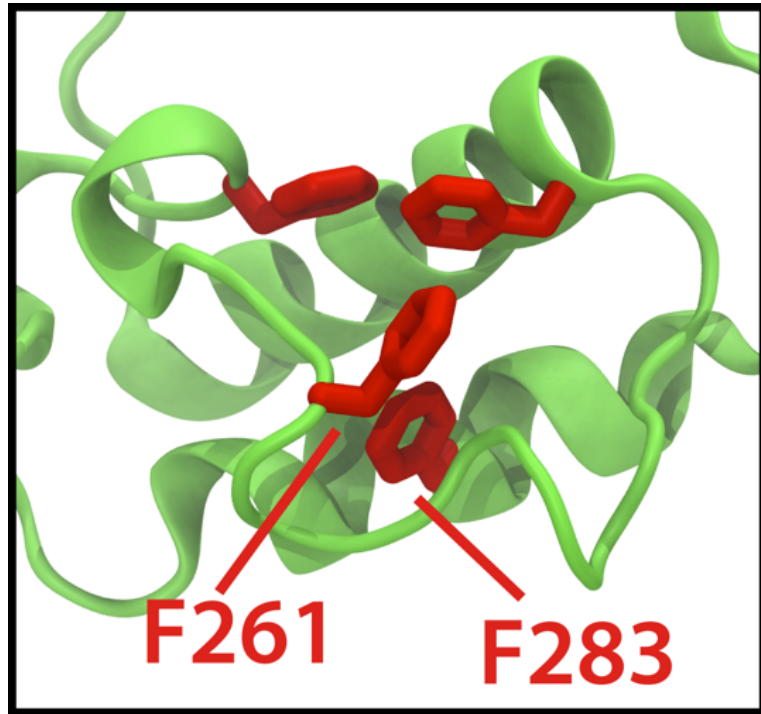
Five independent membrane binding simulations



Final model converted to **full membrane**
Stable in 100 ns simulations

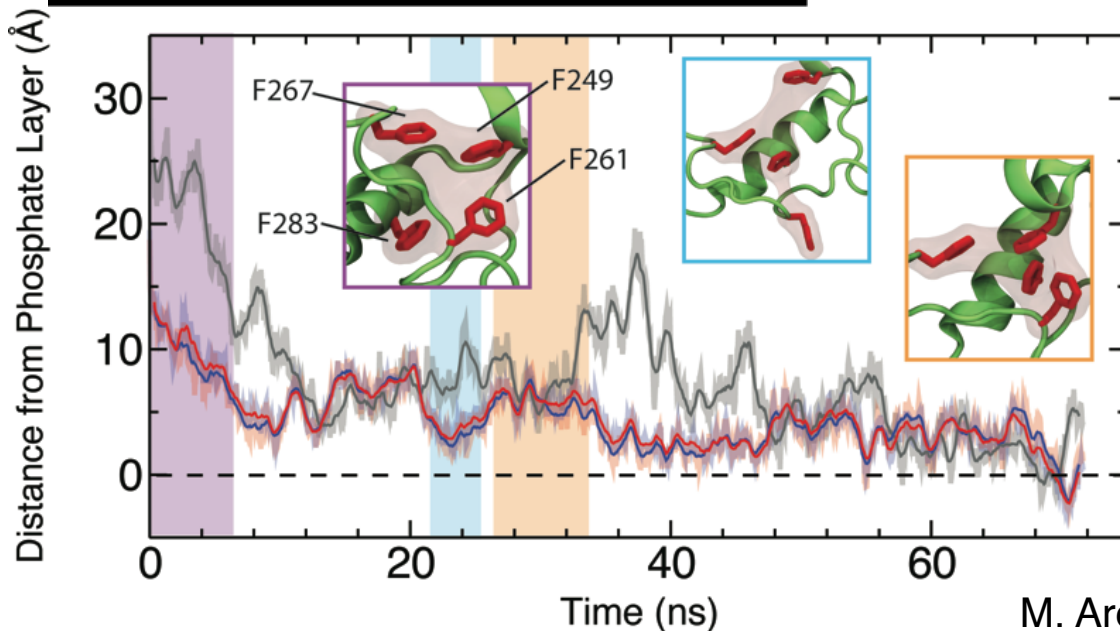
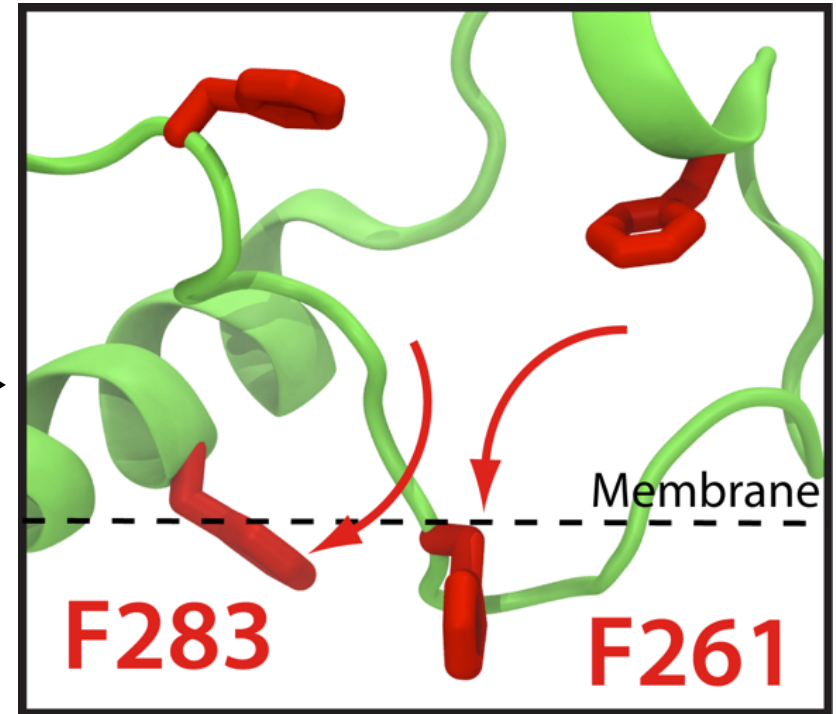
M. Arcario and ET, **Biophys. J.**, 107: 2059–2069 (2014).

Revealing the *Hydrophobic Anchor*



20 ns

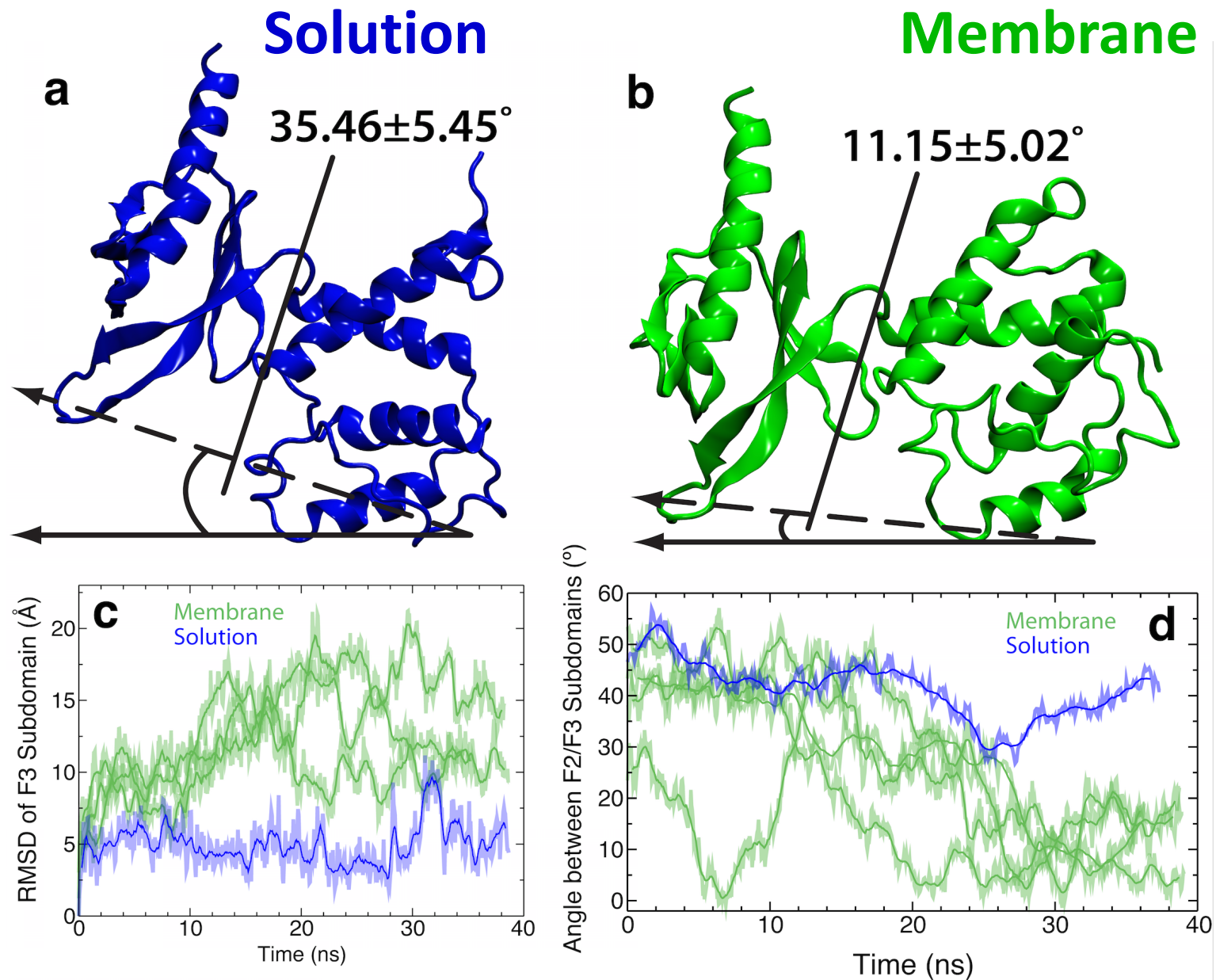
Membrane Binding
Simulation



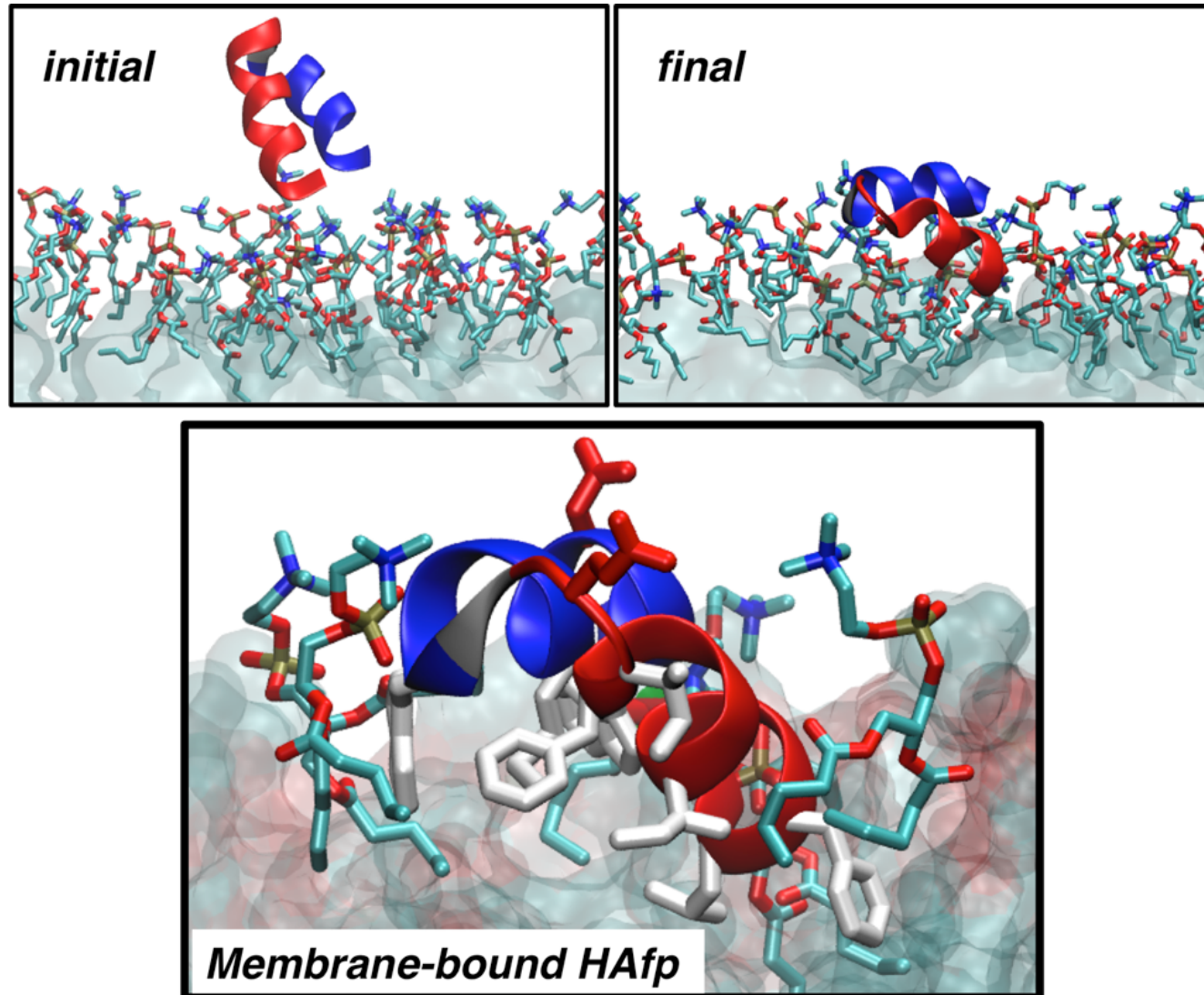
- Snorkeling of lysine acts as a switch which releases a conserved phenylalanine anchor (F261 & F283) into the membrane
- Reformation of the hydrophobic pocket causes looser binding of talin; suggests a mechanism for unbinding of protein

M. Arcario and ET, **Biophys. J.**, 107: 2059–2069 (2014).

Membrane Induced Domain Rearrangement of Talin

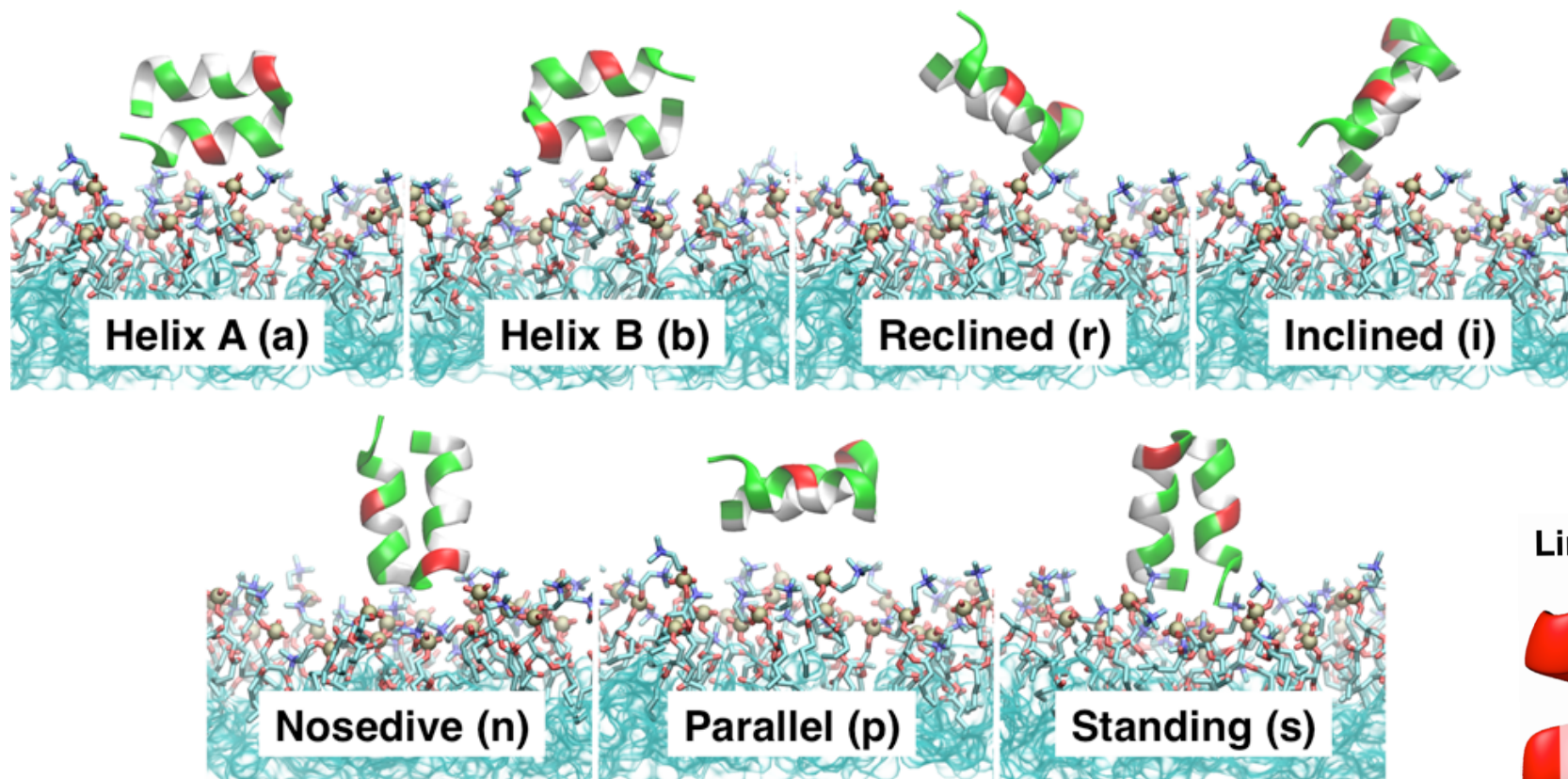


Membrane Binding of Influenza Hemagglutinin Fusion Peptide

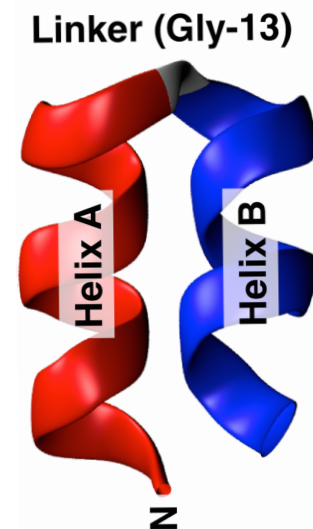


J. Baylon and E. T., *J. Phys. Chem.B*, 2015, in press.

Membrane Binding of Influenza Hemagglutinin Fusion Peptide



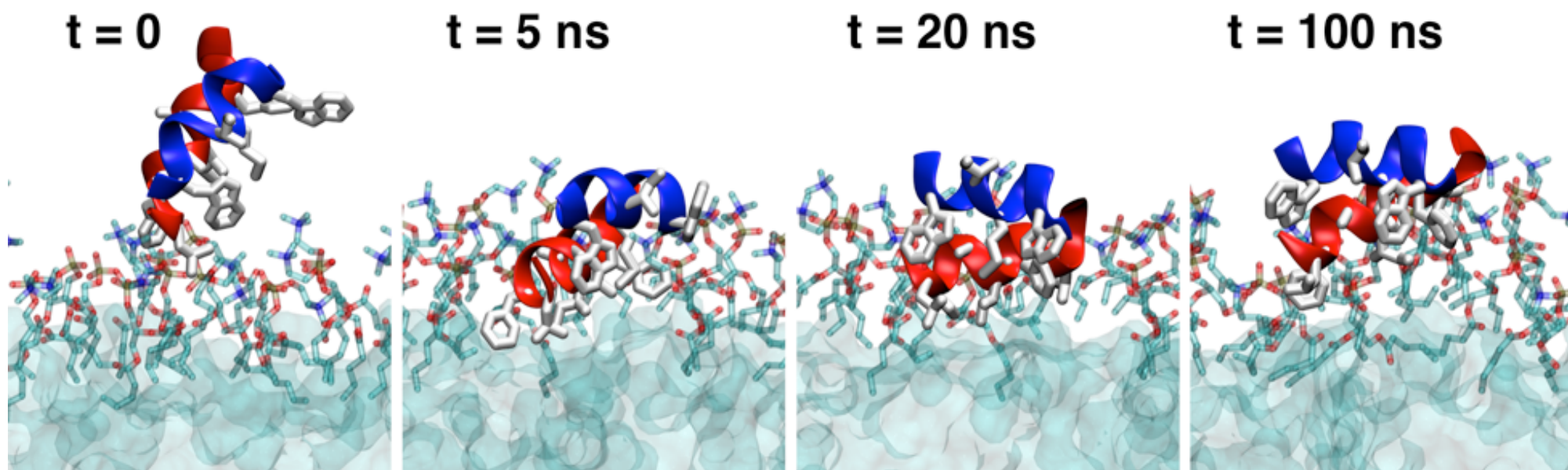
7 different initial orientation
each simulated 3 times



J. Baylon and E. T., *J. Phys. Chem.B*, 2015.

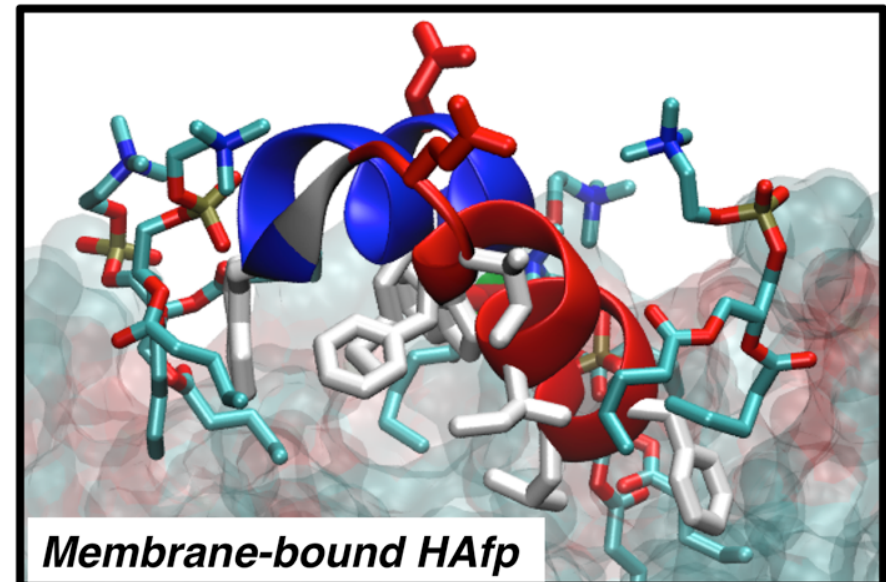
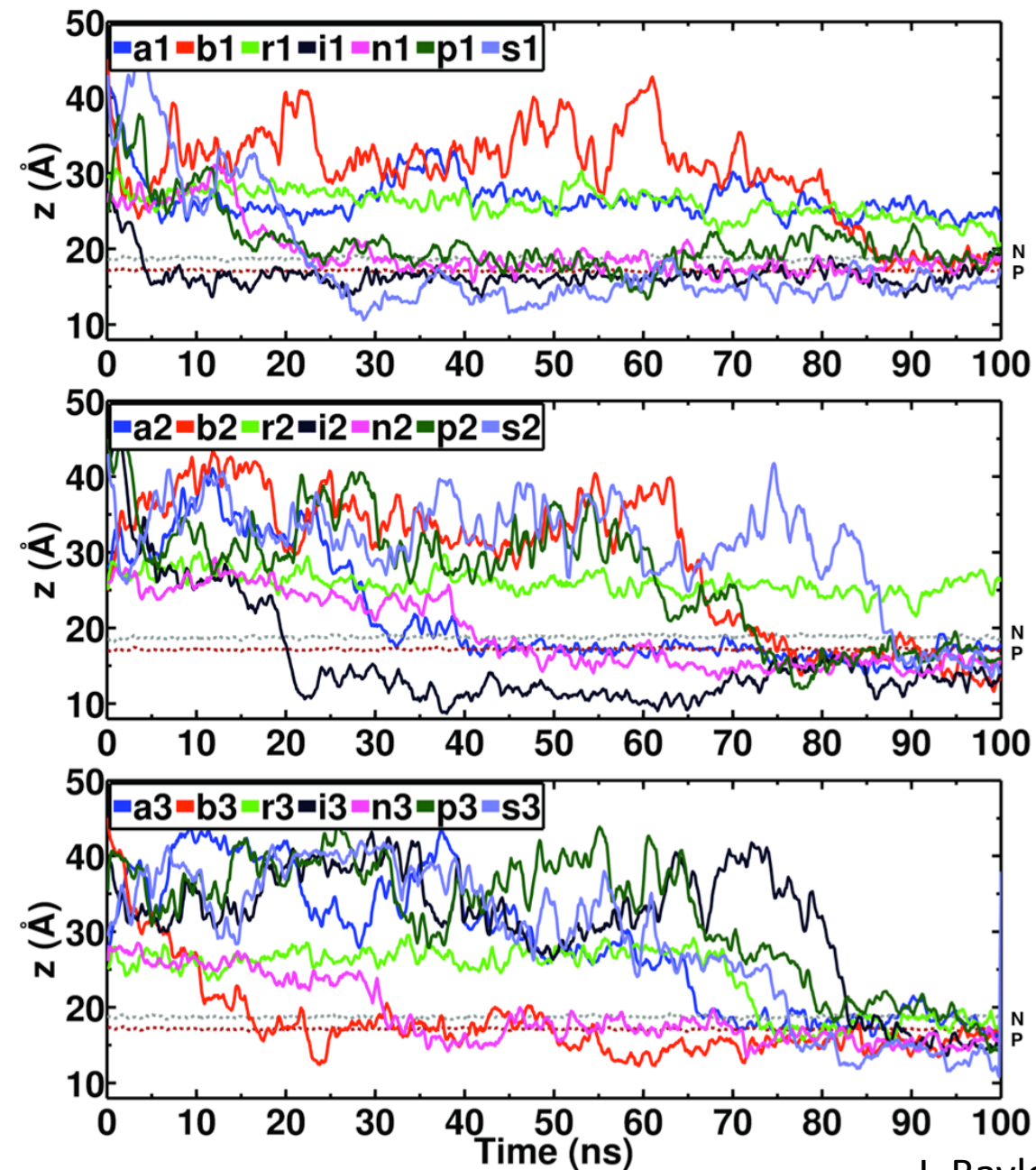
Membrane Binding of Influenza Hemagglutinin Fusion Peptide

Spontaneous binding observed in the majority of the simulations:
21 independent simulations starting from 7 different orientations



J. Baylon and E. T., **J. Phys. Chem.B**, 2015.

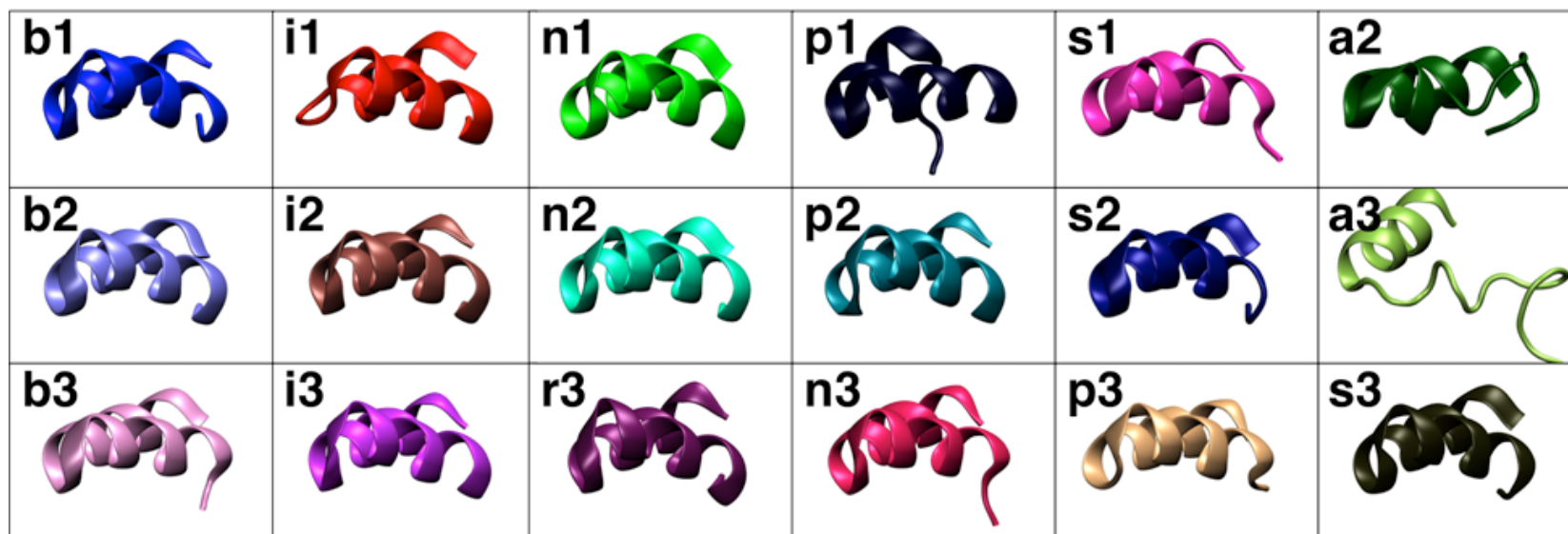
Remarkable convergence of membrane binding simulations



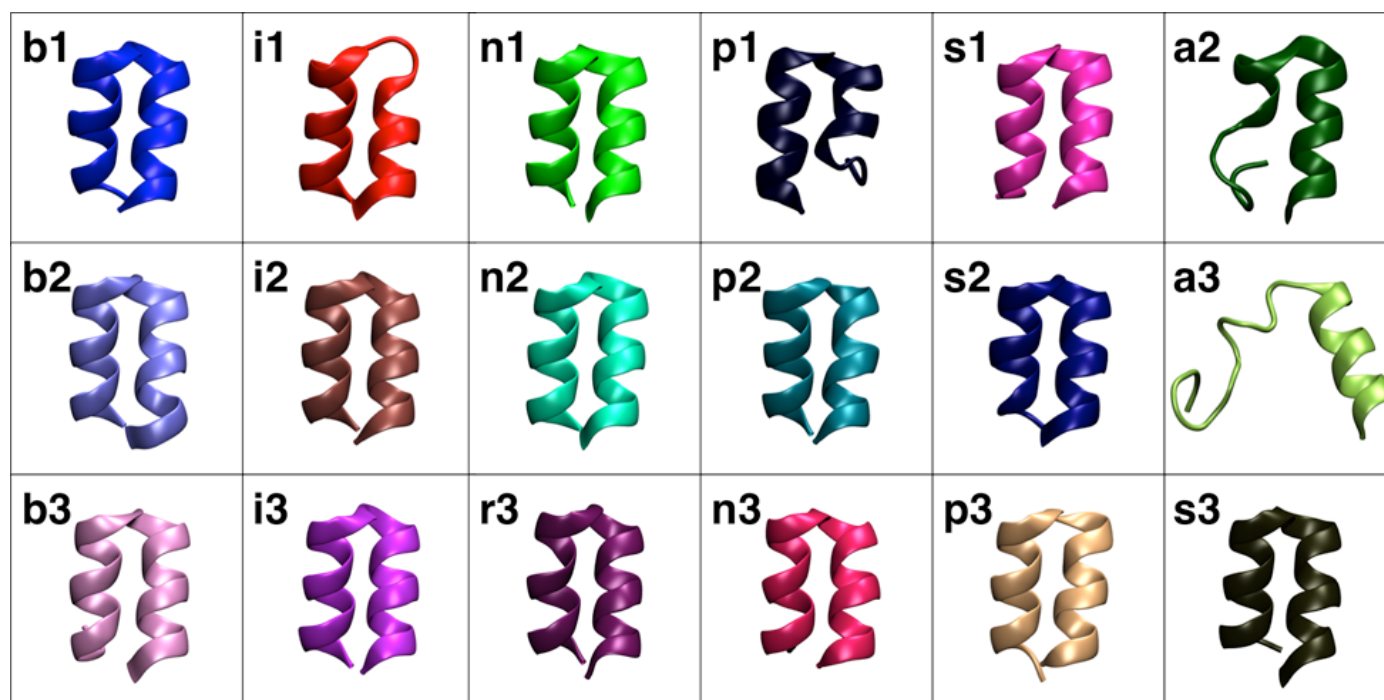
J. Baylon and E. T., *J. Phys. Chem.B*, 2015, in press.

Remarkable convergence of membrane binding simulations

Side View



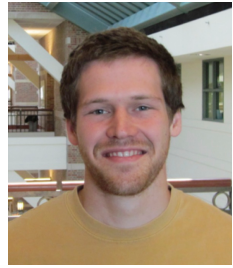
Top View



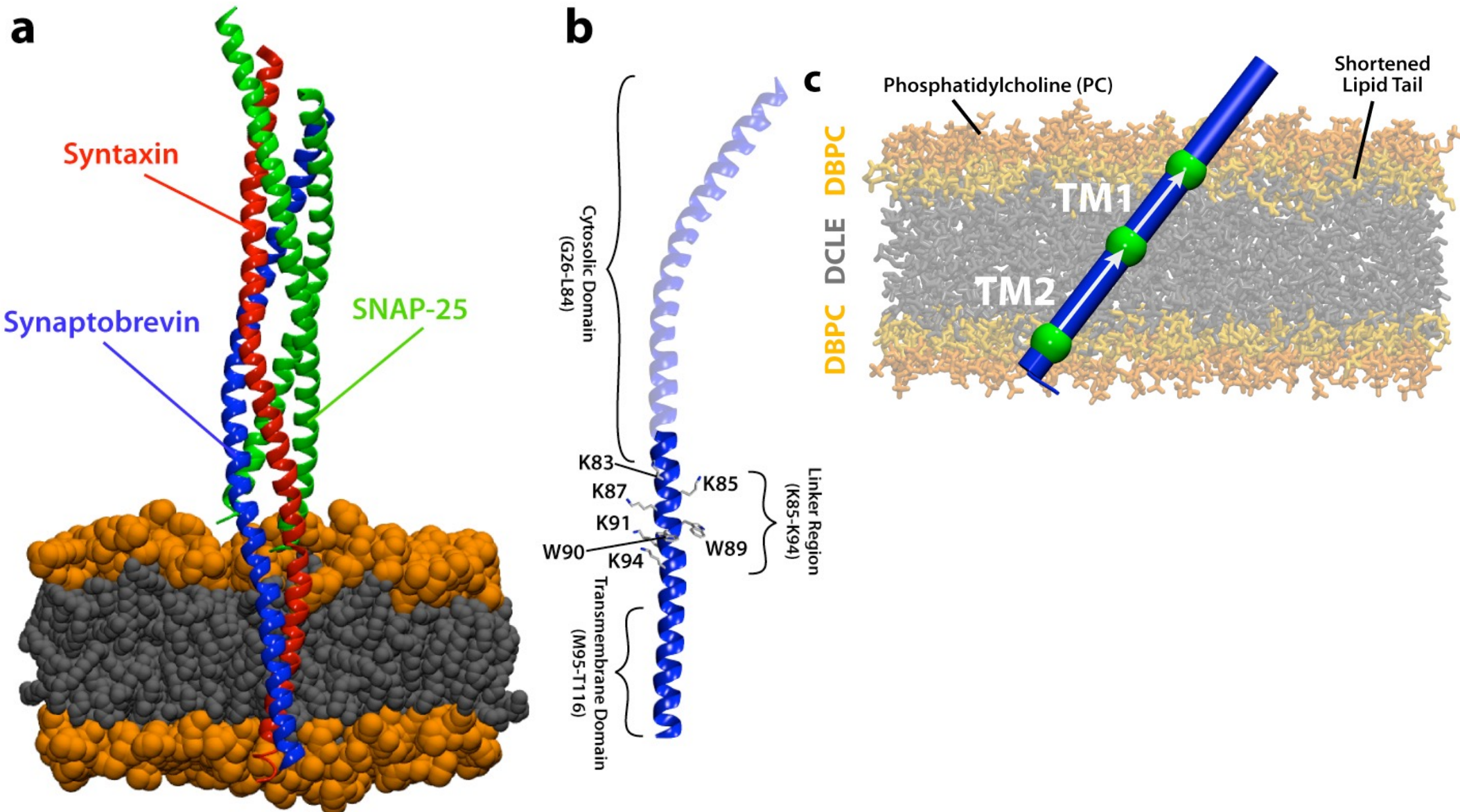
Robust Tilting of the Anchor Domain in Snare Protein Synaptobrevin



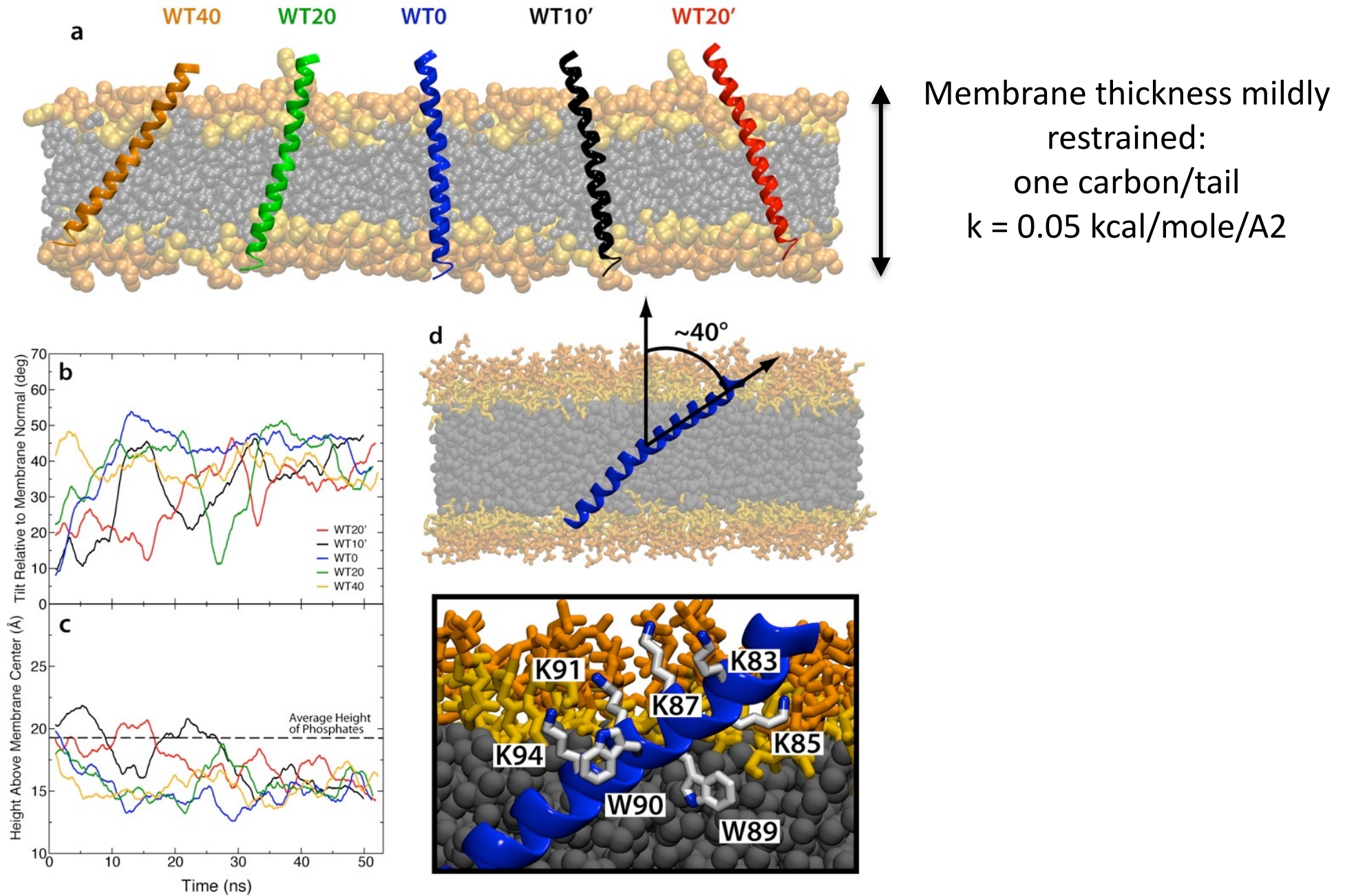
Mark Arcario



Andrew Blanchard

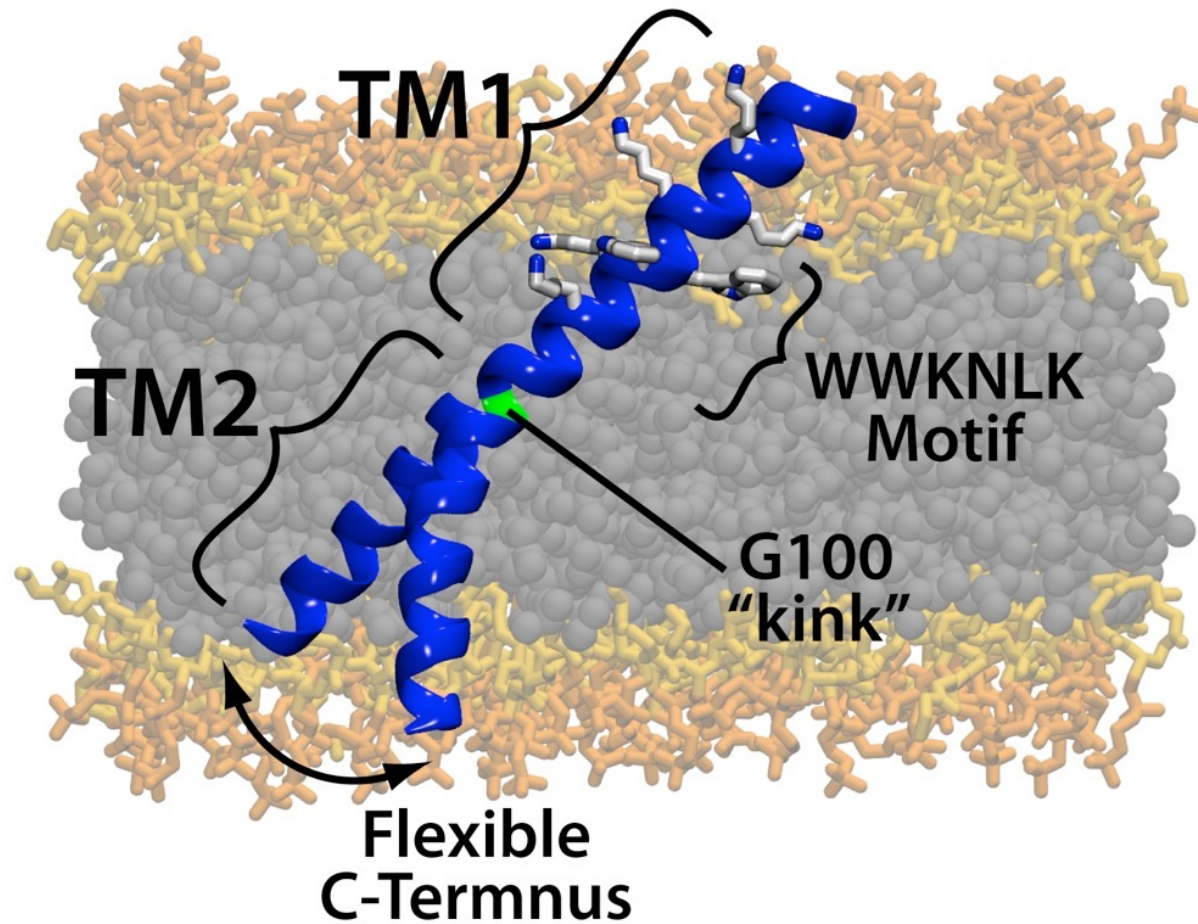
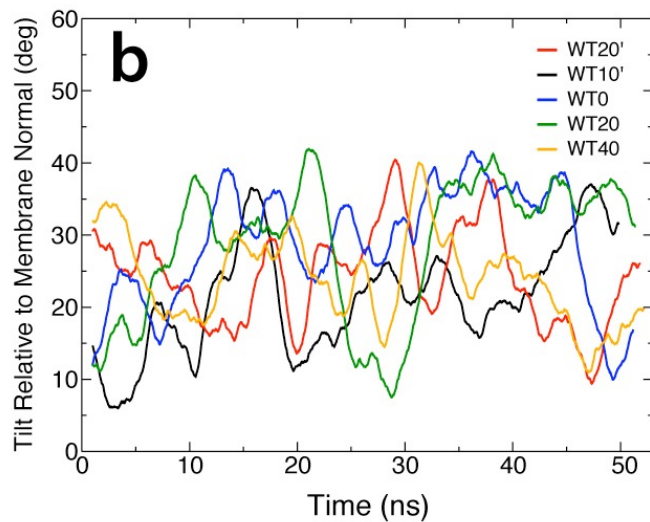


Robust Tilt Observed in Synaptobrevin

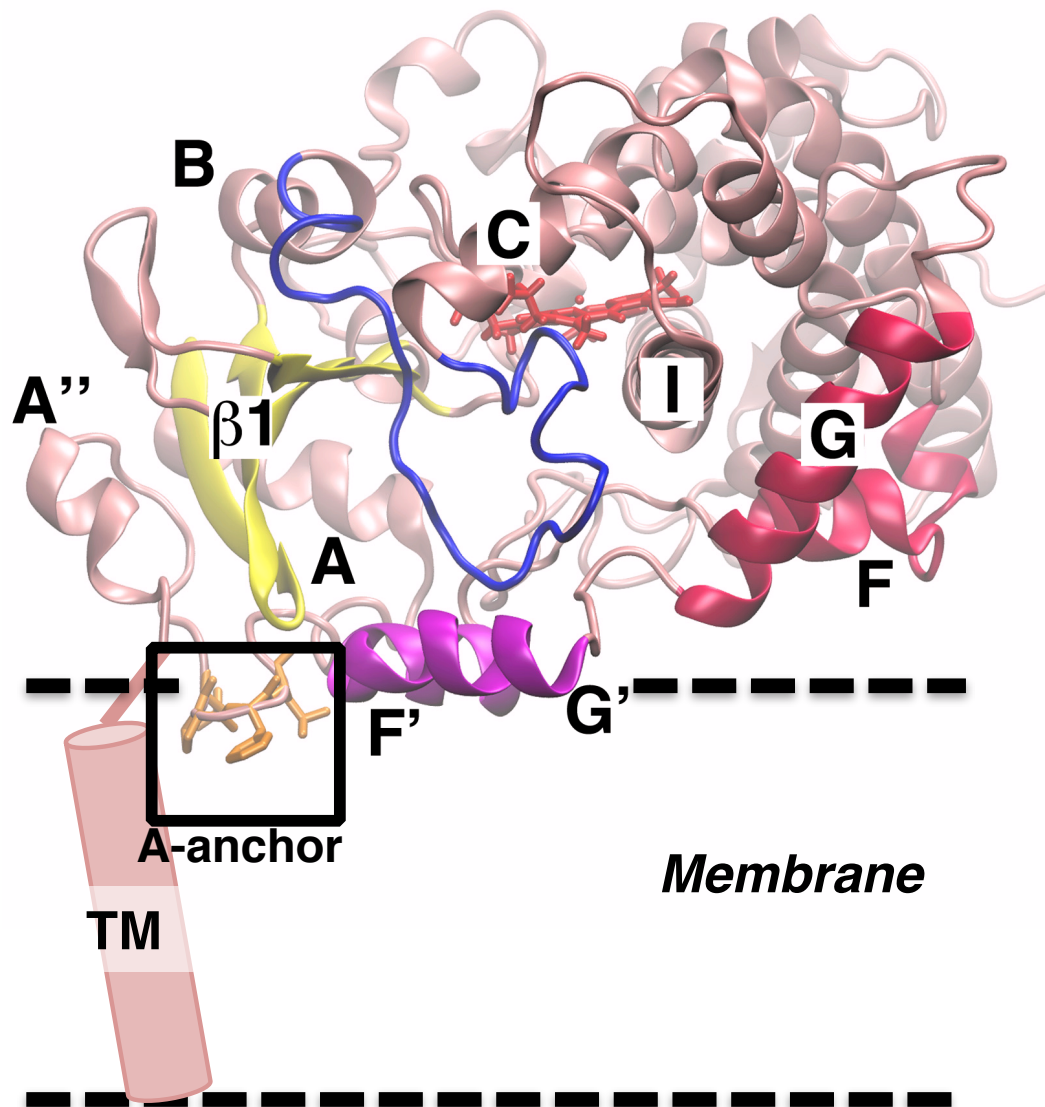


A. Blanchard*, M. Arcario*, K. Schulten, and ET, **Biophys. J.**, 107: 2112–21 (2014)

Identifying a Hinge



Cytochrome P450 3A4 (CYP3A4)

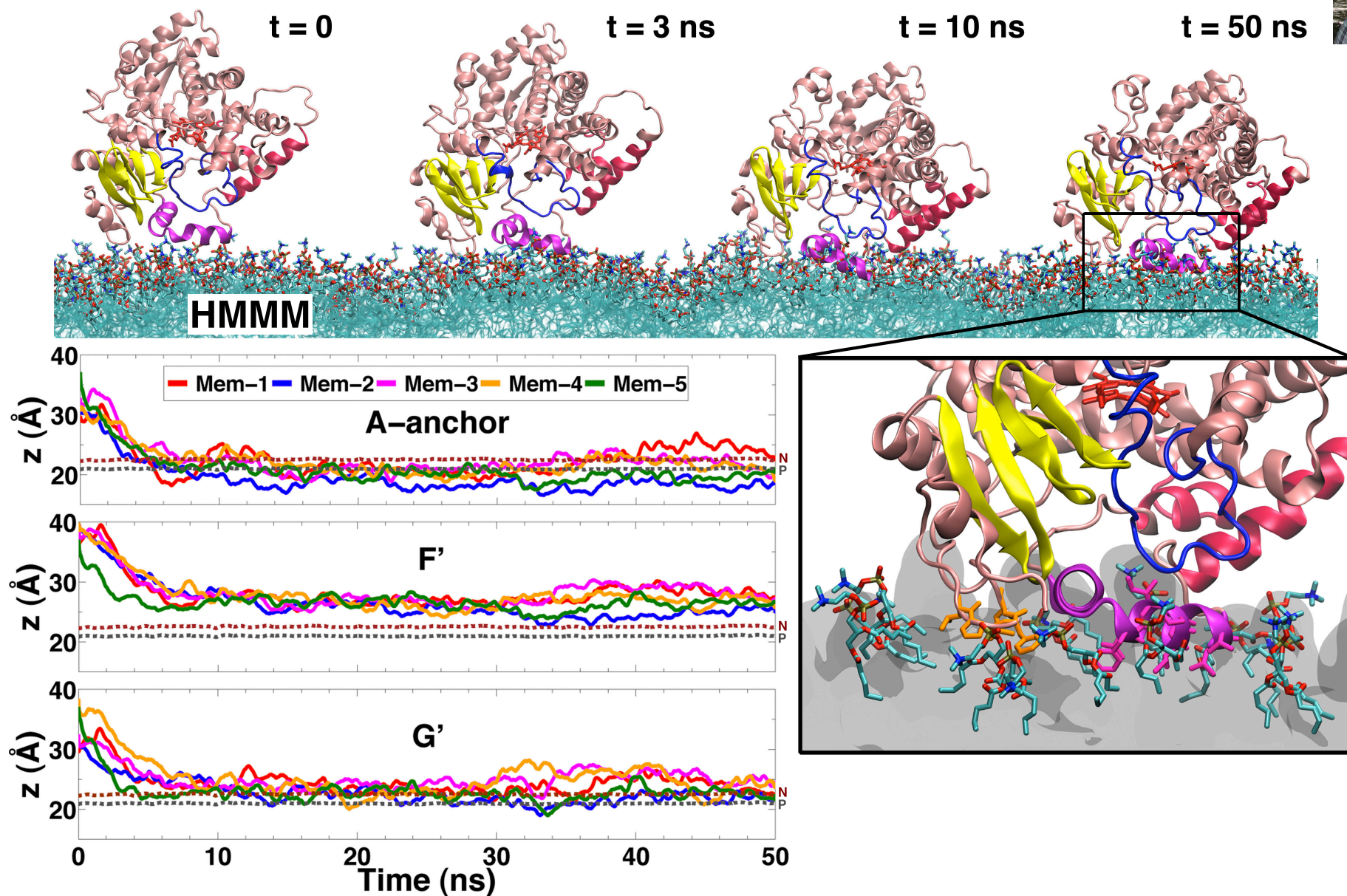


- Enzymes essential for the metabolism of xenobiotics and other compounds, found in all domains of life.
- In the human body, CYPs are membrane-bound proteins.
- The interaction with membrane mediates binding of substrates.
- **CYP3A4**: most abundant CYP in the human body, metabolizes about 50%- 60% of drugs that are metabolized in the body.

Insertion and Membrane-Induced Conformational Change of Cytochrome P450



Javier Baylon

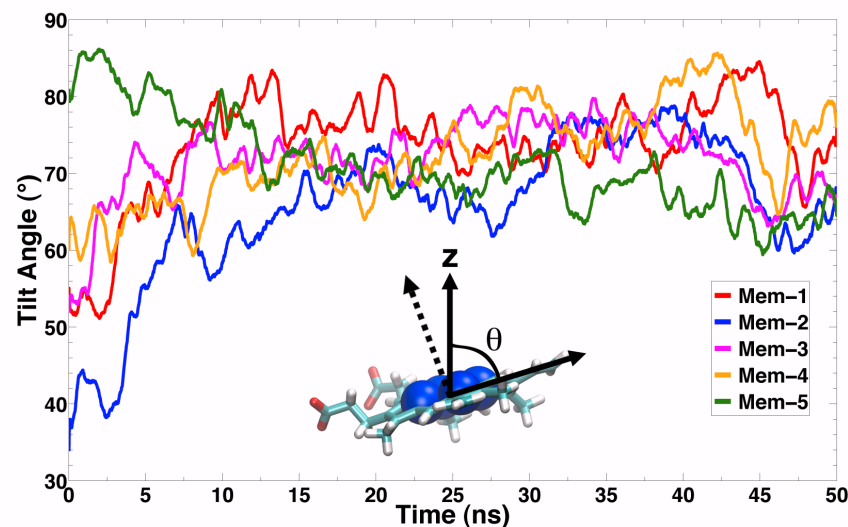
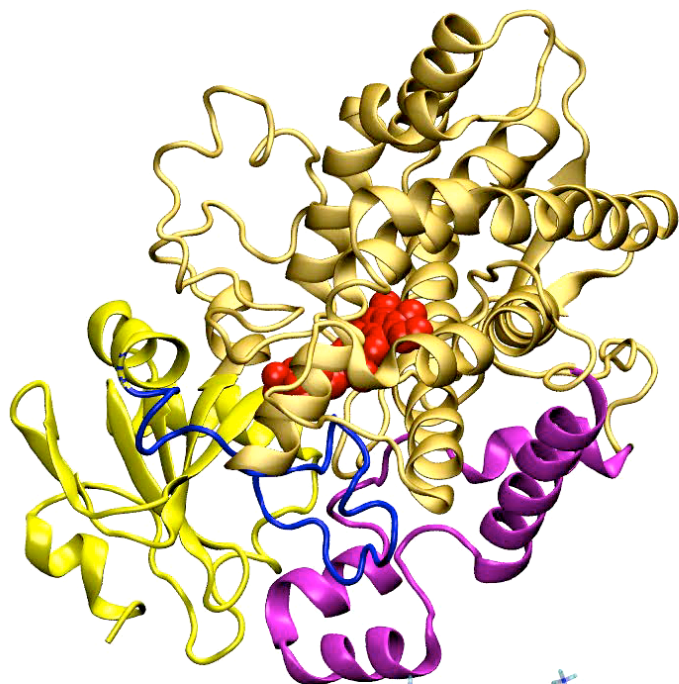


J. Baylon, I. Lenov, S. Sligar and ET, **JACS**, 135: 8542–8551 (2013)

Insertion and Membrane-Induced Conformational Change of Cytochrome P450



Javier Baylon



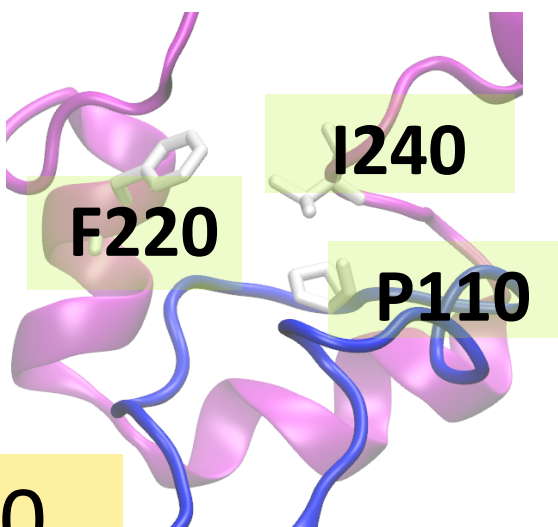
**Within 10 degrees of
experimental measurement of
the tilt angle (S. Sligar)**

J. Baylon, I. Lenov, S. Sligar and ET, JACS, 135: 8542–8551 (2013)

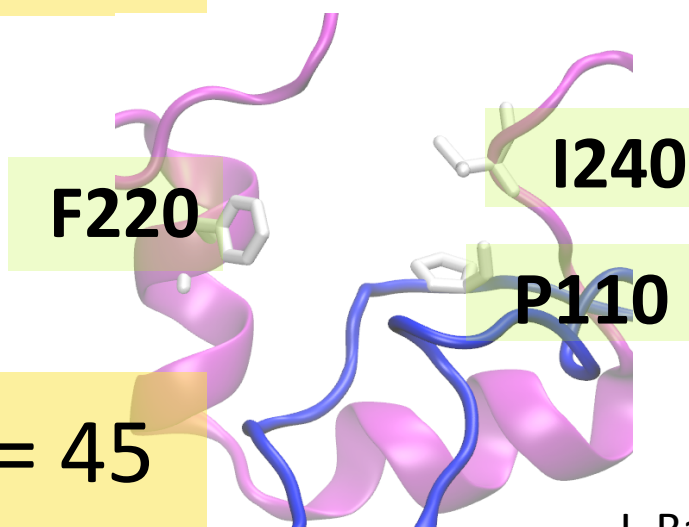
Insertion and Membrane-Induced Conformational Change of Cytochrome P450



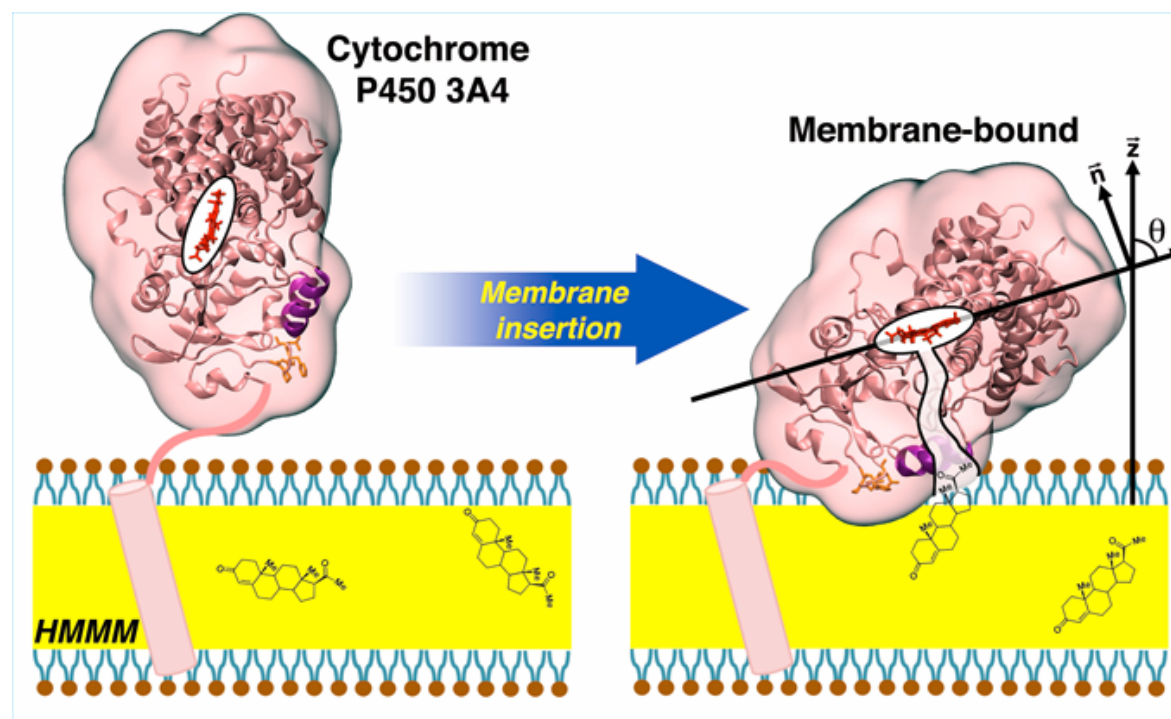
Javier Baylon



$t = 0$
closed



$t = 45$
open



J. Baylon, I. Lenov, S. Sligar and ET, JACS, 135: 8542–8551 (2013)