NIH Biomedical Technology Research Center for Macromolecular Modeling and Bioinformatics

Overview of the Program in Day 3 and 4

Introduction to MD in NAMD
Intro to Molecular Visualization in VMD

Advanced Modeling Tools

Goal:
Making users comfortable with the software environment

Tutorials — MUST MUST MUST

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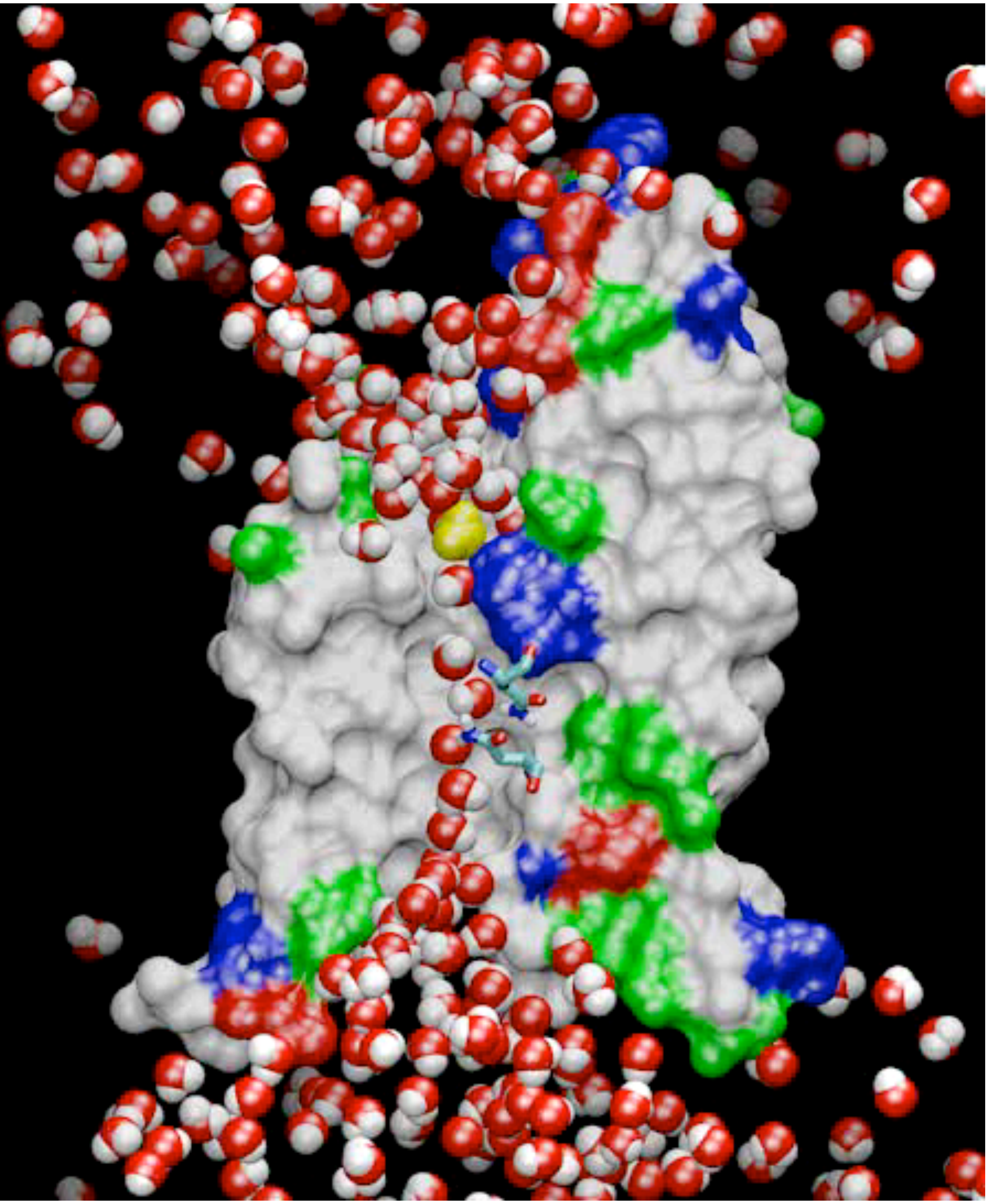
Beckman Institute for Advanced Science and Technology
University of Illinois at Urbana-Champaign

Molecular Dynamics Simulations



Solving the Newtonian equations of motion for all particles at every time step

Major limitations:

- Time scale / sampling
- Force field approximations

SPEED LIMIT

1 fs

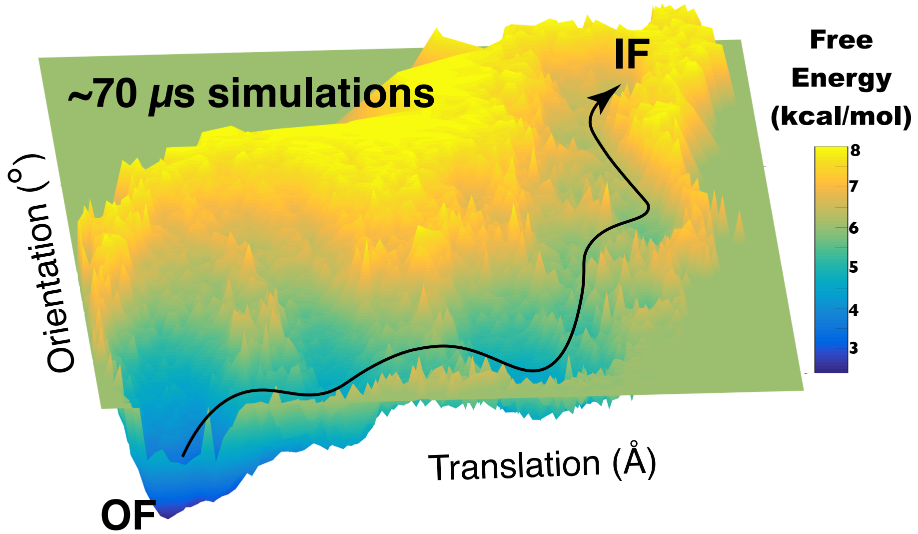
Major advantage:

- Unparalleled spatial and temporal resolutions, simultaneously

Overcoming Timescale limitation

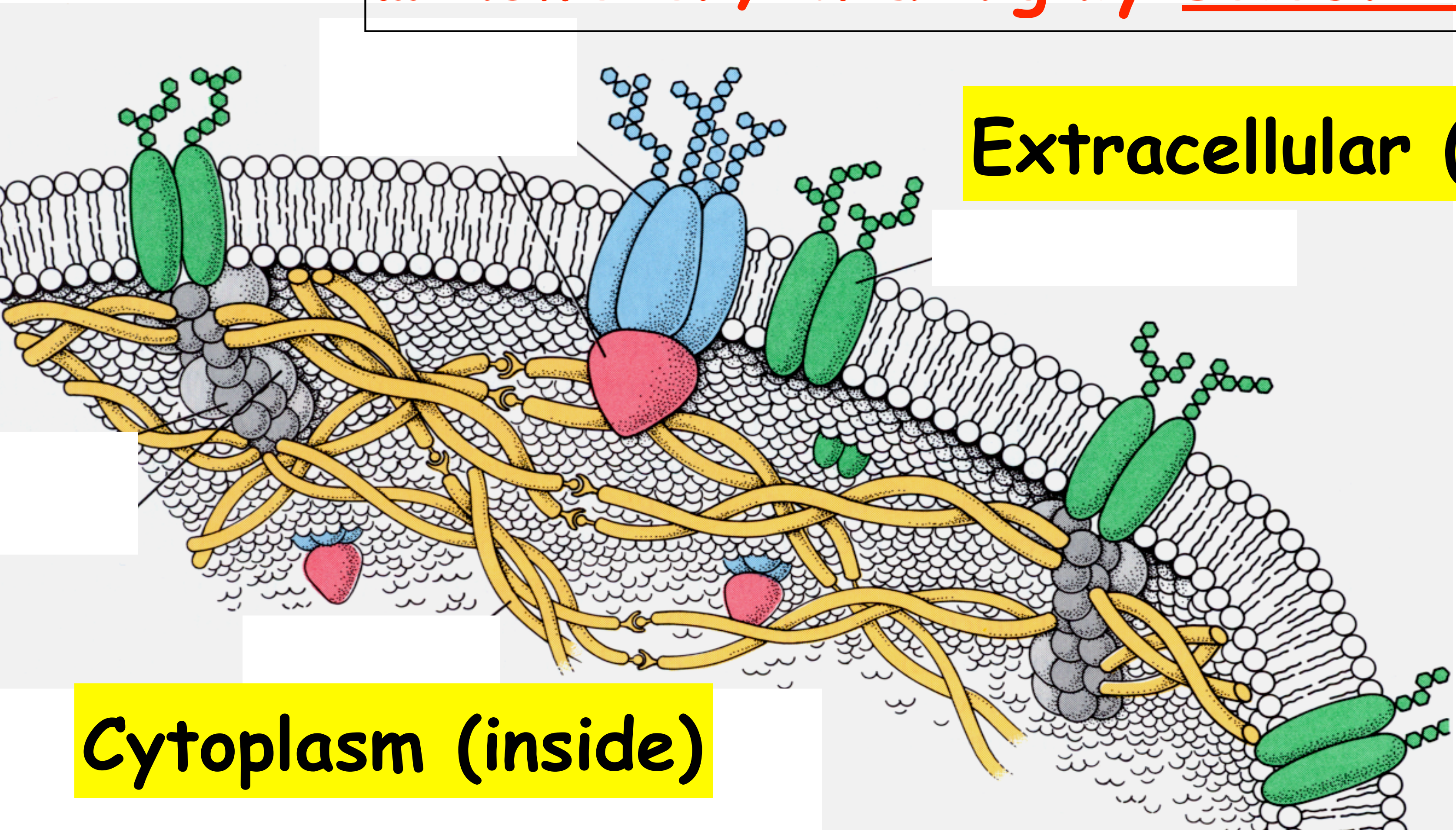
Visiting more regions in the Configuration Space

Enhanced Sampling



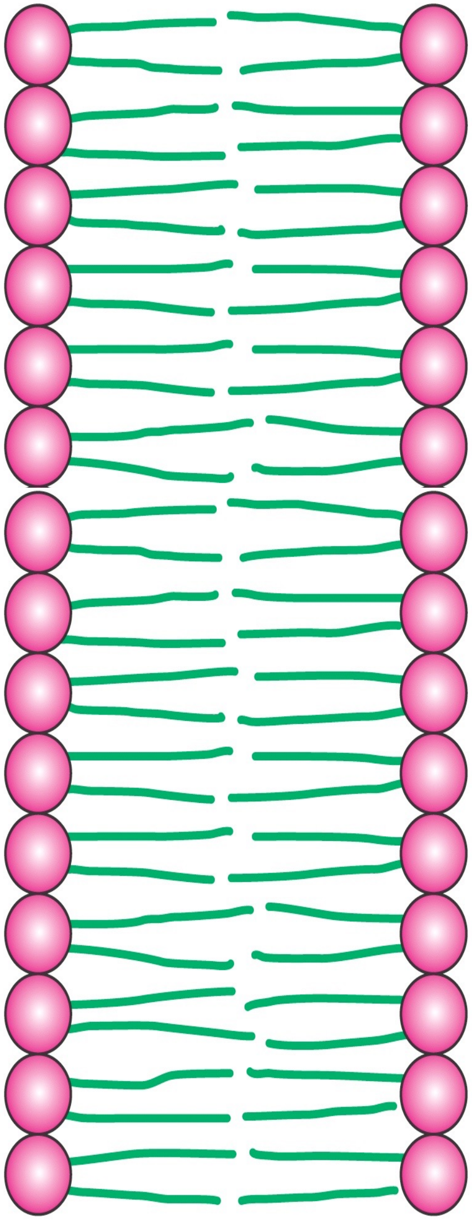
- Living cells also need to exchange materials and information with the outside world

… however, in a highly <u>selective</u> manner.

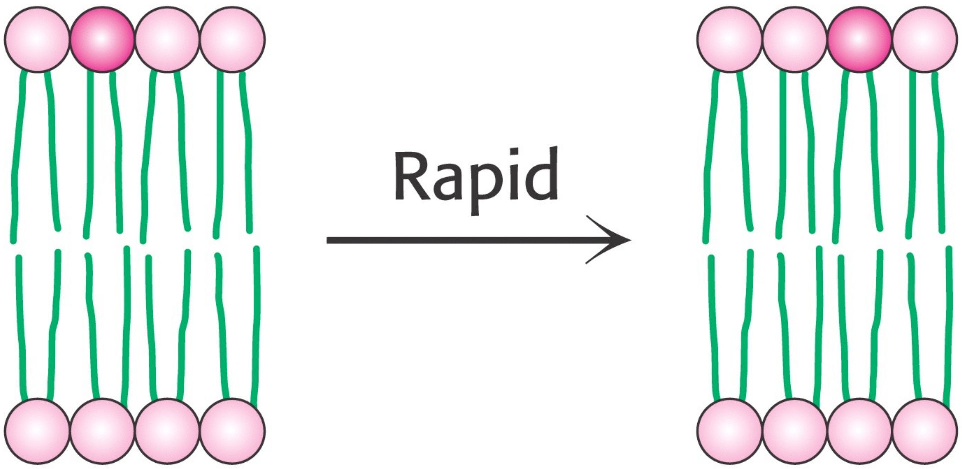


Phospholipid Bilayers Are Excellent Materials For Cell Membranes

- 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



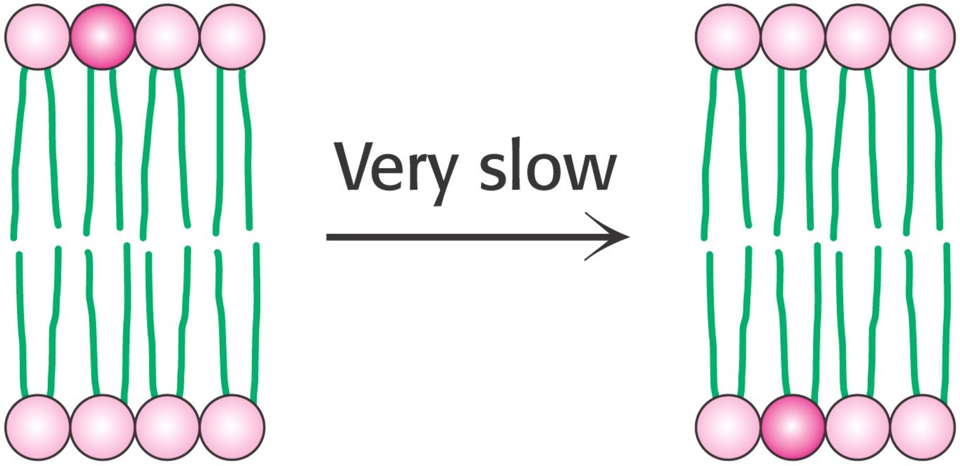
Lateral diffusion

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

(50 Å in ~ 5 x 10^{-6} s)

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

Modeling mixed lipid bilayers!



Tranverse diffusion
(flip-flop)

Once in several hours!

(~ 50 Å in ~ 10^4 s)

~9 orders of magnitude slower
ensuring bilayer asymmetry

Technical difficulties in Simulations of Biological Membranes

- Time scale
- Heterogeneity of biological membranes ☹

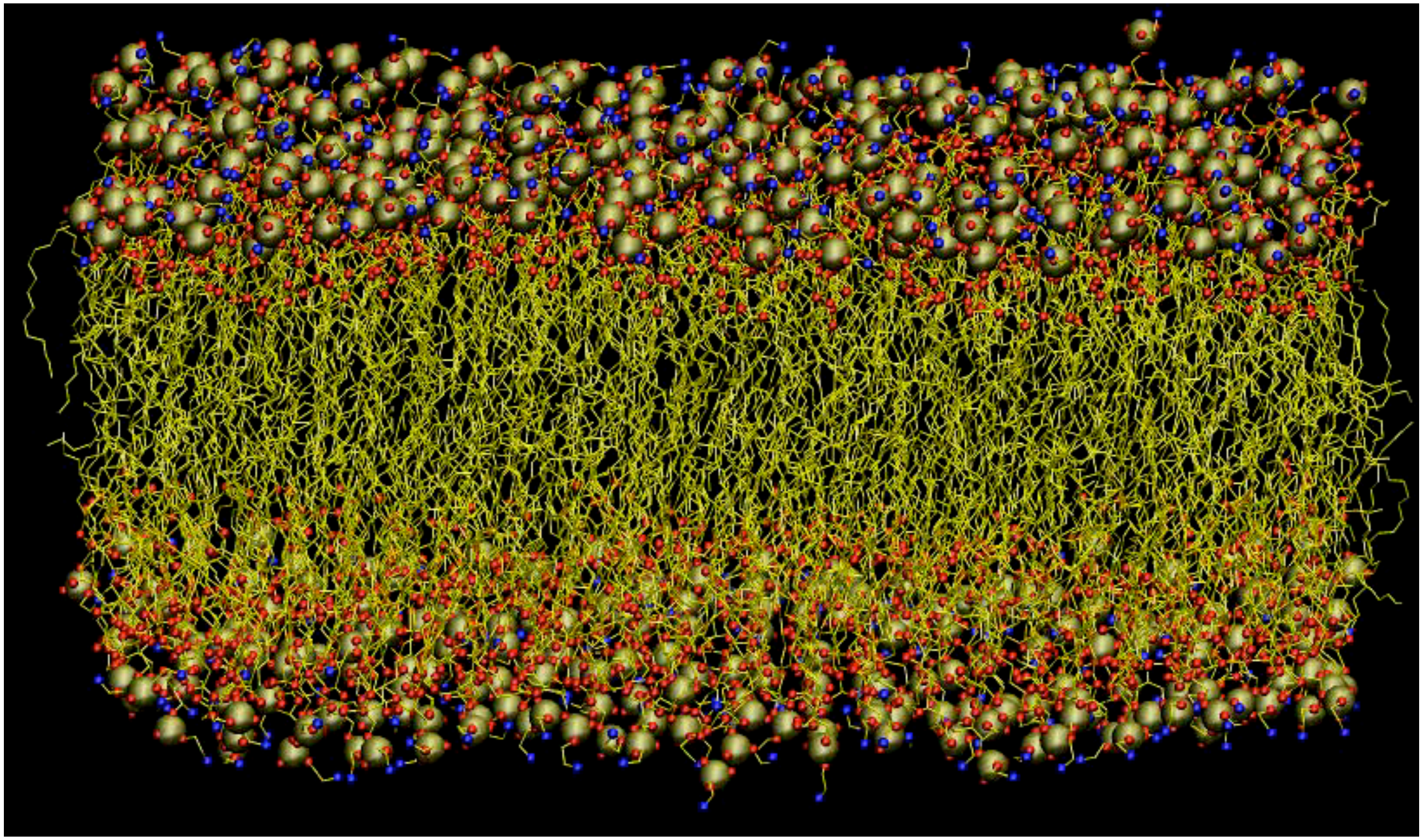
60 x 60 Å

Pure POPE

5 ns

~100,000

atoms



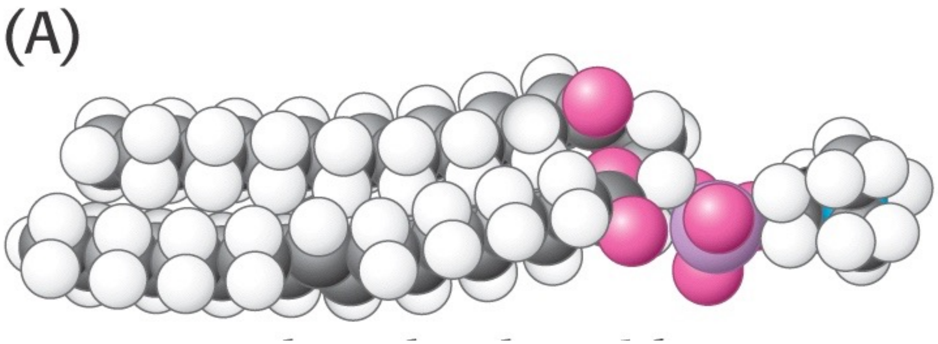
Battling the Timescale - Case I

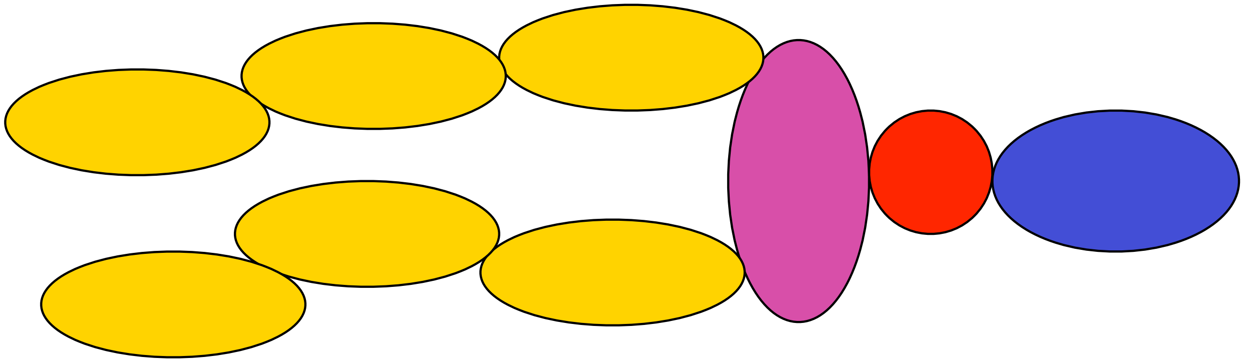
Coarse-grained modeling of lipids

150 particles

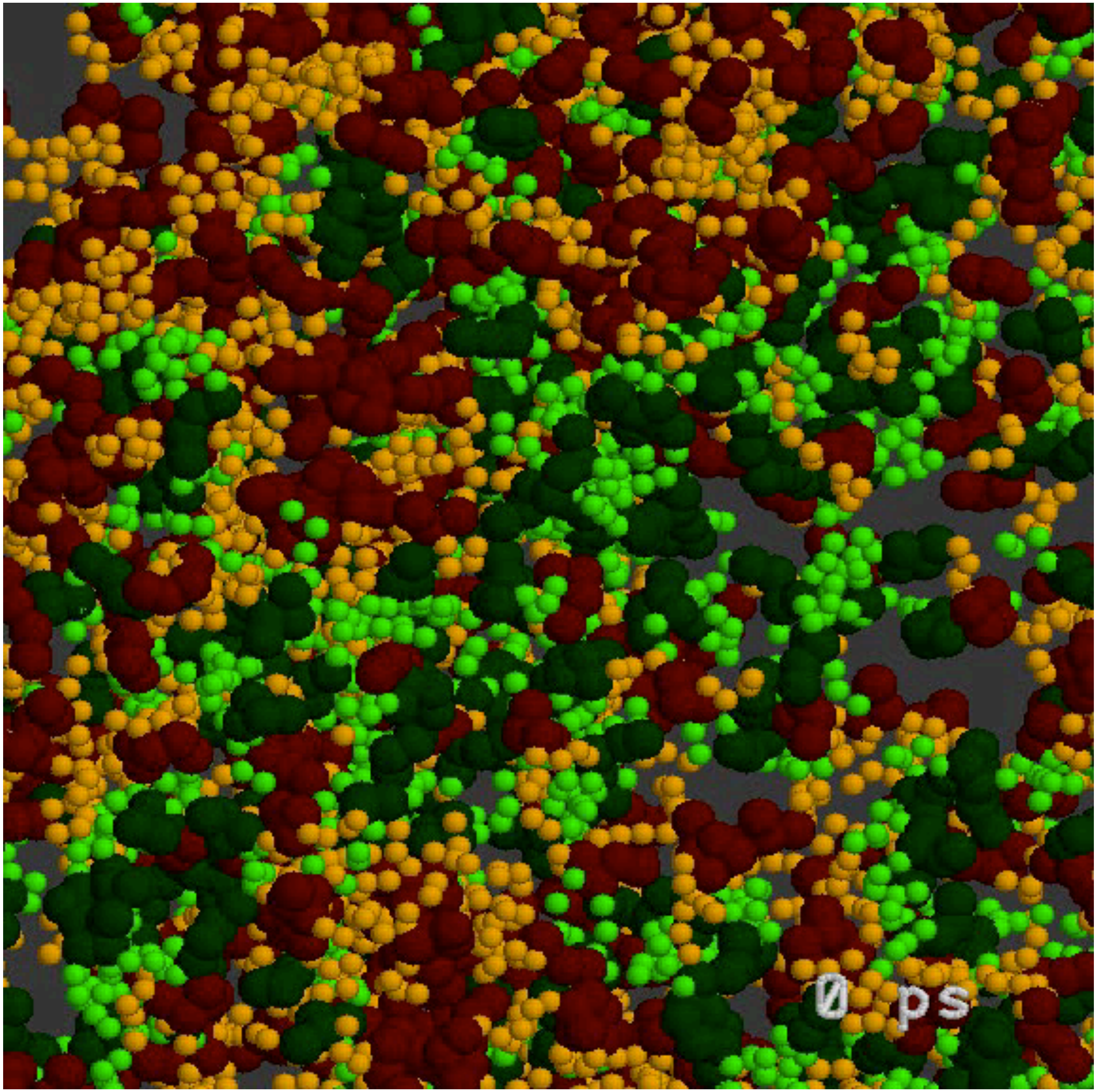
↓

9 particles!





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

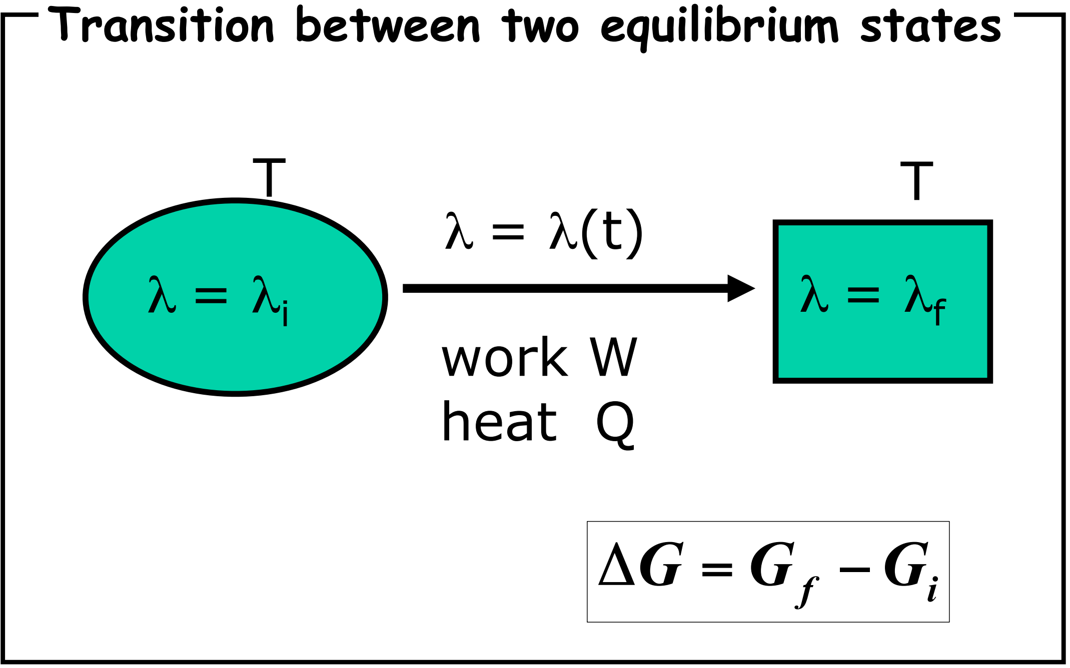
Steered Molecular Dynamics is a non-equilibrium method by nature

- 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.

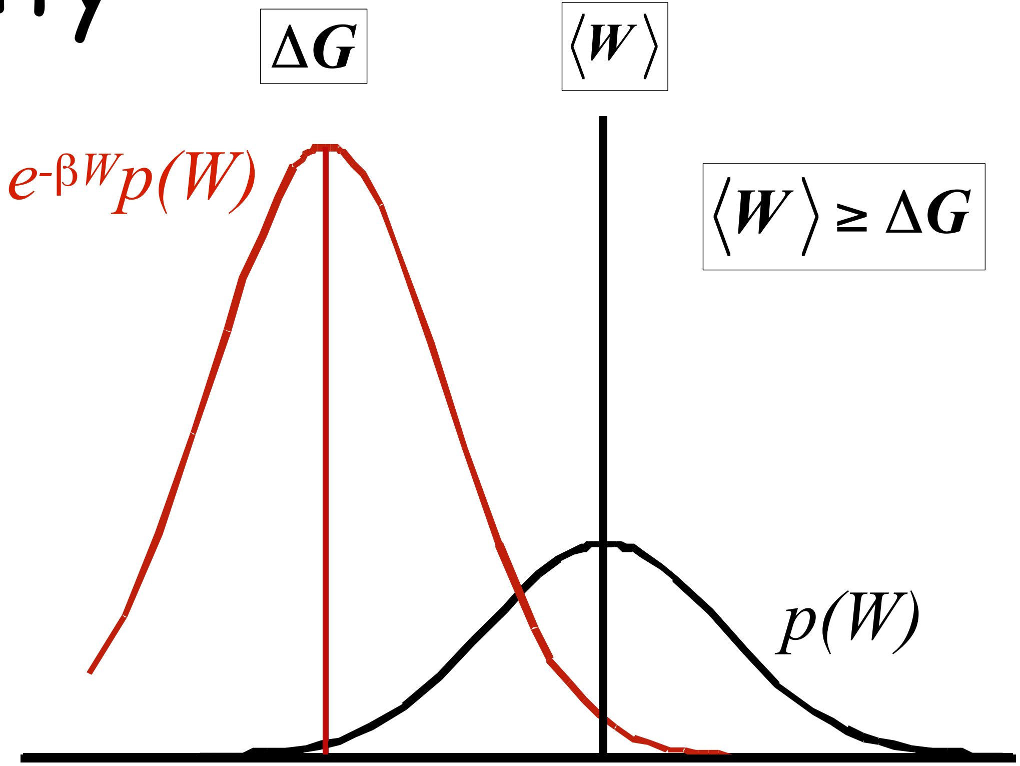
Second law of thermodynamics → $W \geq \Delta G$

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)

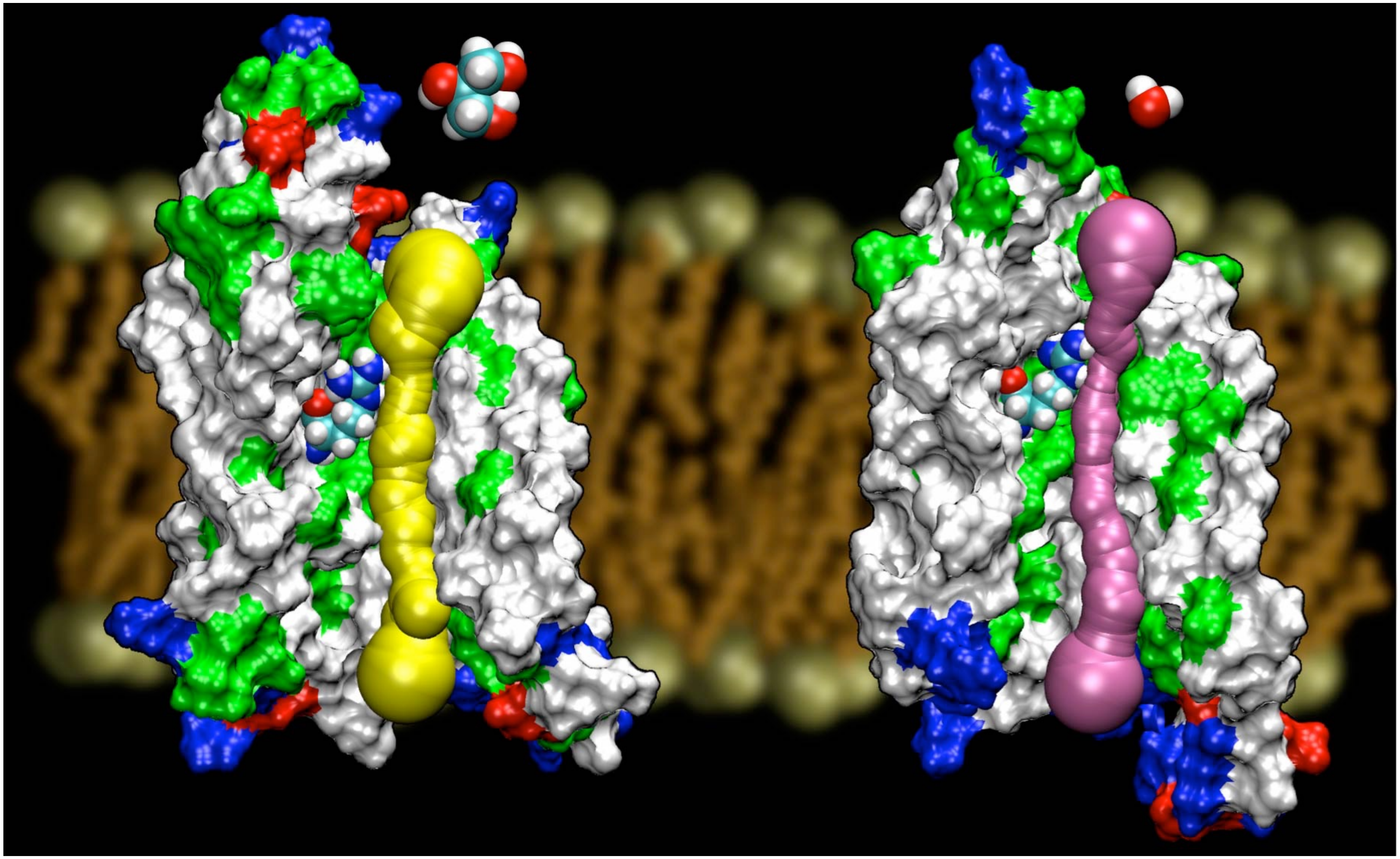
$$\left\langle e^{-\beta W} \right\rangle = e^{-\beta \Delta G}$$

$$\beta = {}^{1}\!/_{k_B T}$$

In principle, it is possible to obtain free energy surfaces from <u>repeated</u> non-equilibrium experiments.

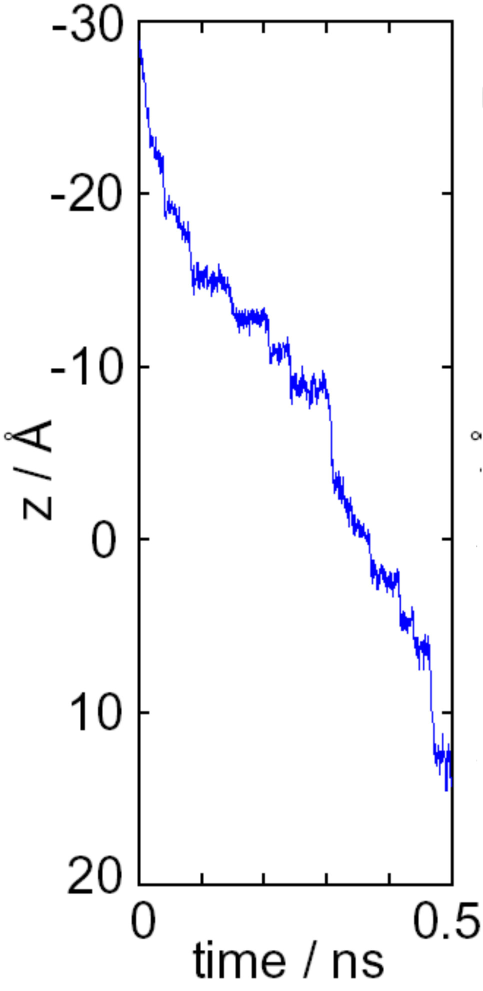
AqpZ *vs*. GlpF

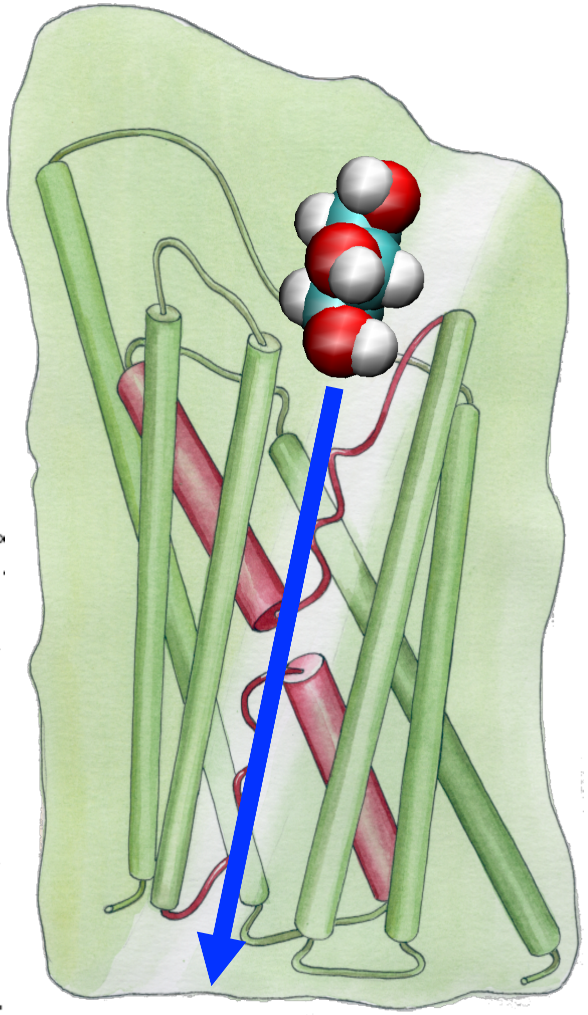
- Both from *E. coli*
- AqpZ is a pure water channel
- GlpF is a glycerol channel
- We have high resolution structures for both channels



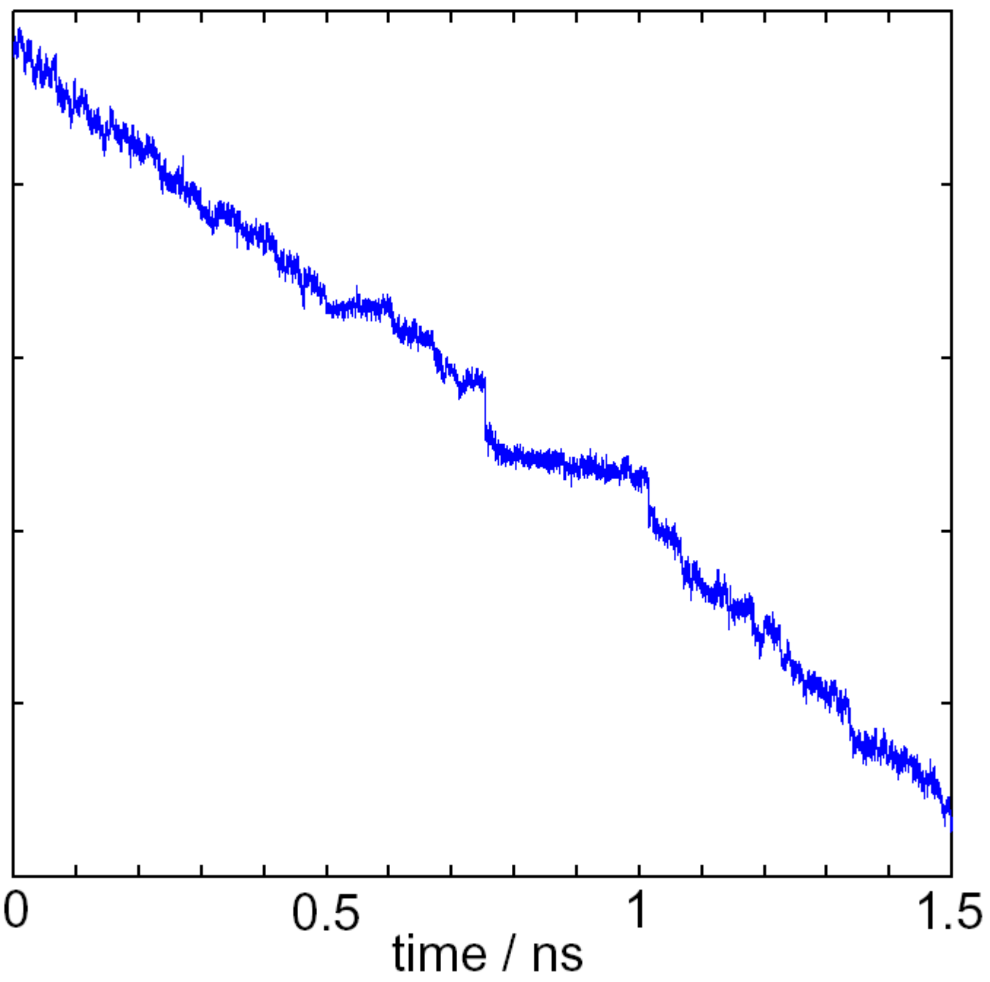
Steered Molecular Dynamics

constant force
(250 pN)

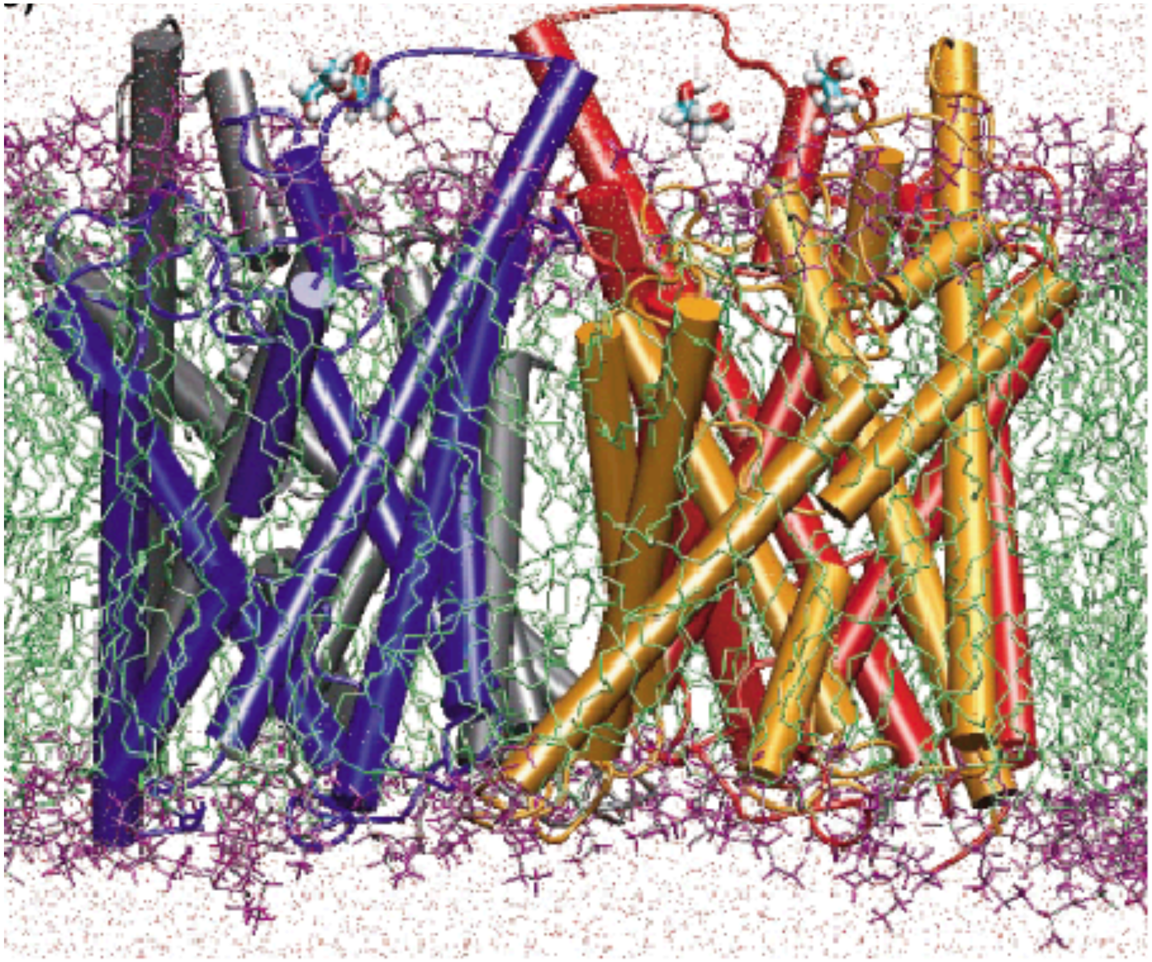


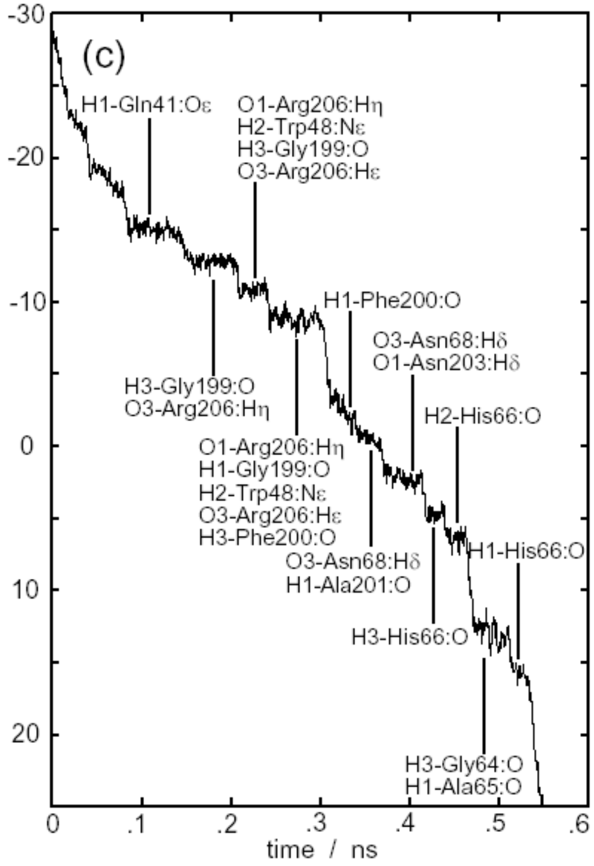


constant velocity
(30 Å/ns)



SMD Simulation of Glycerol Passage





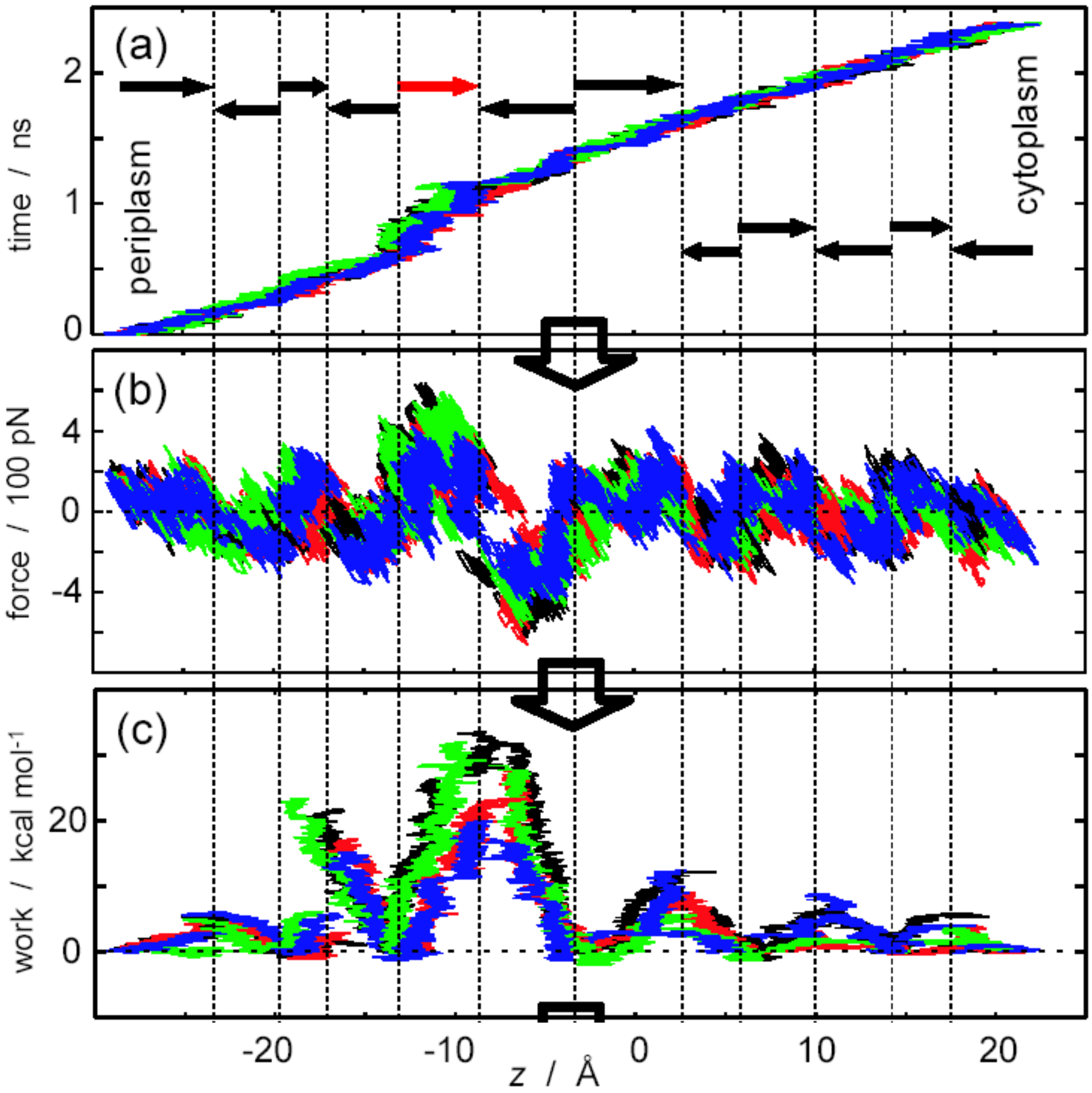
Trajectory of glycerol pulled by **constant force**

Constructing the Potential of Mean Force

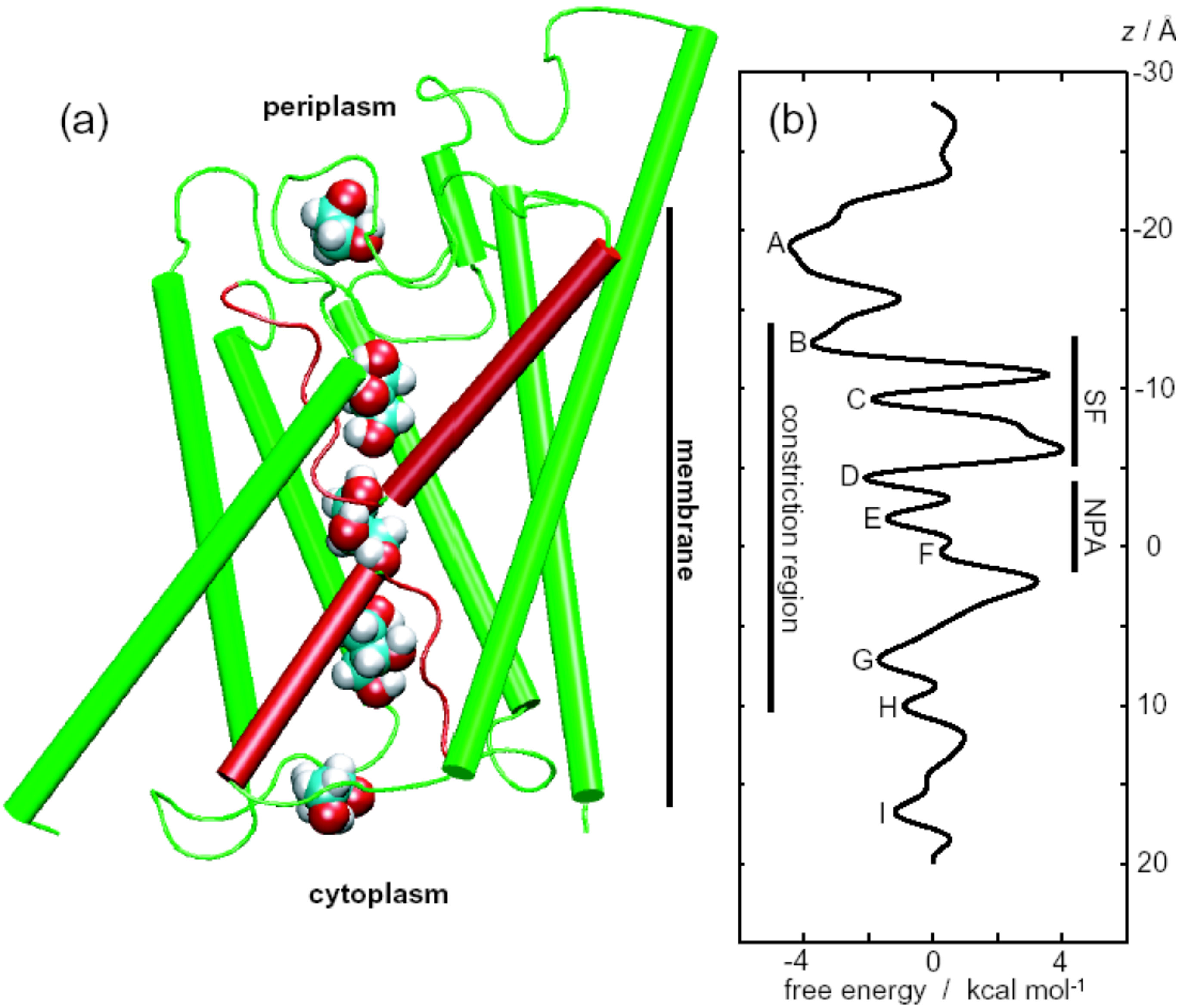
4 trajectories
v = 0.03, 0.015 Å/ps
k = 150 pN/Å

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

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



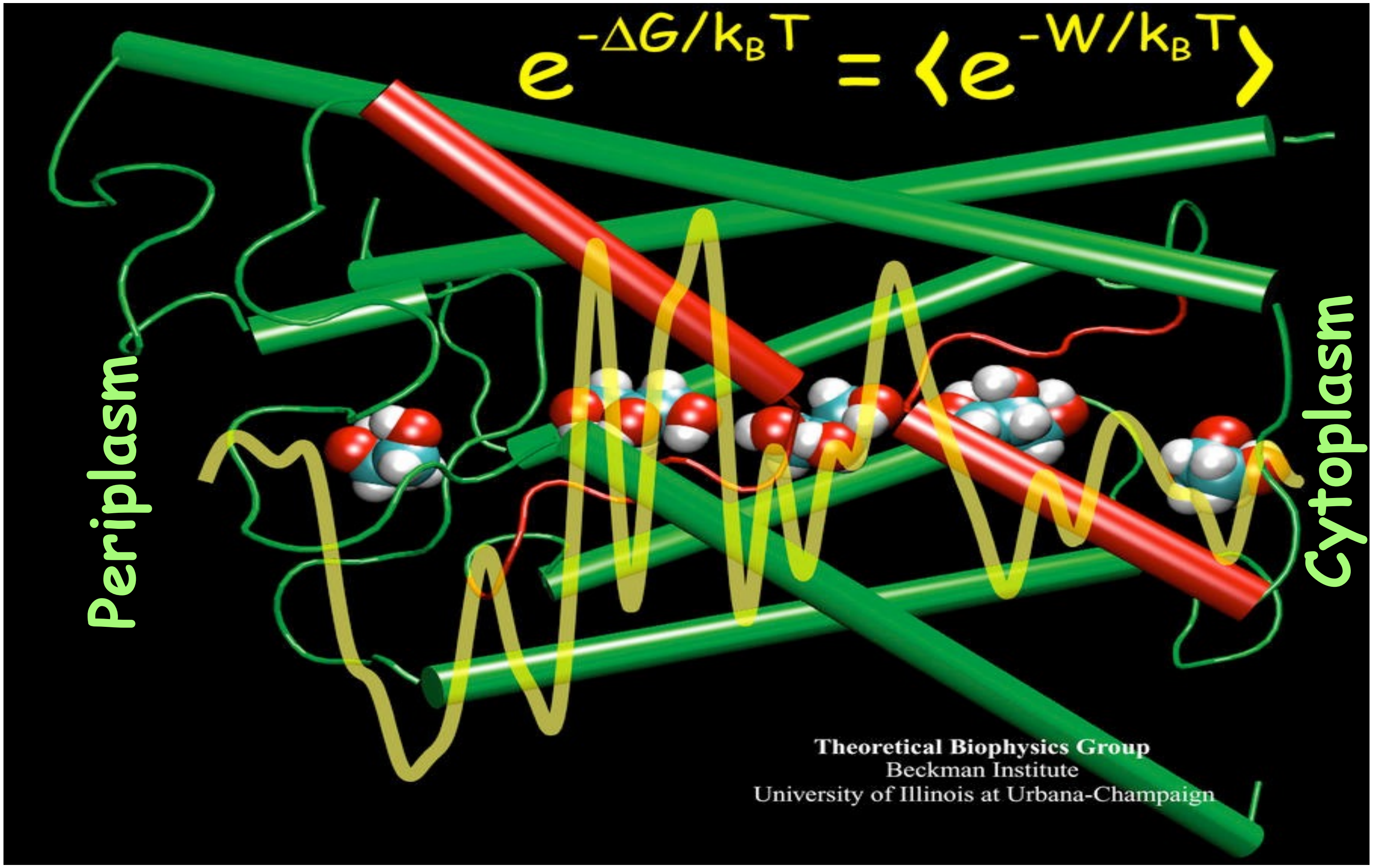
Features of the Potential of Mean Force



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

Jensen et al., *PNAS*, 99:6731-6736, 2002.

Features of the Potential of Mean Force

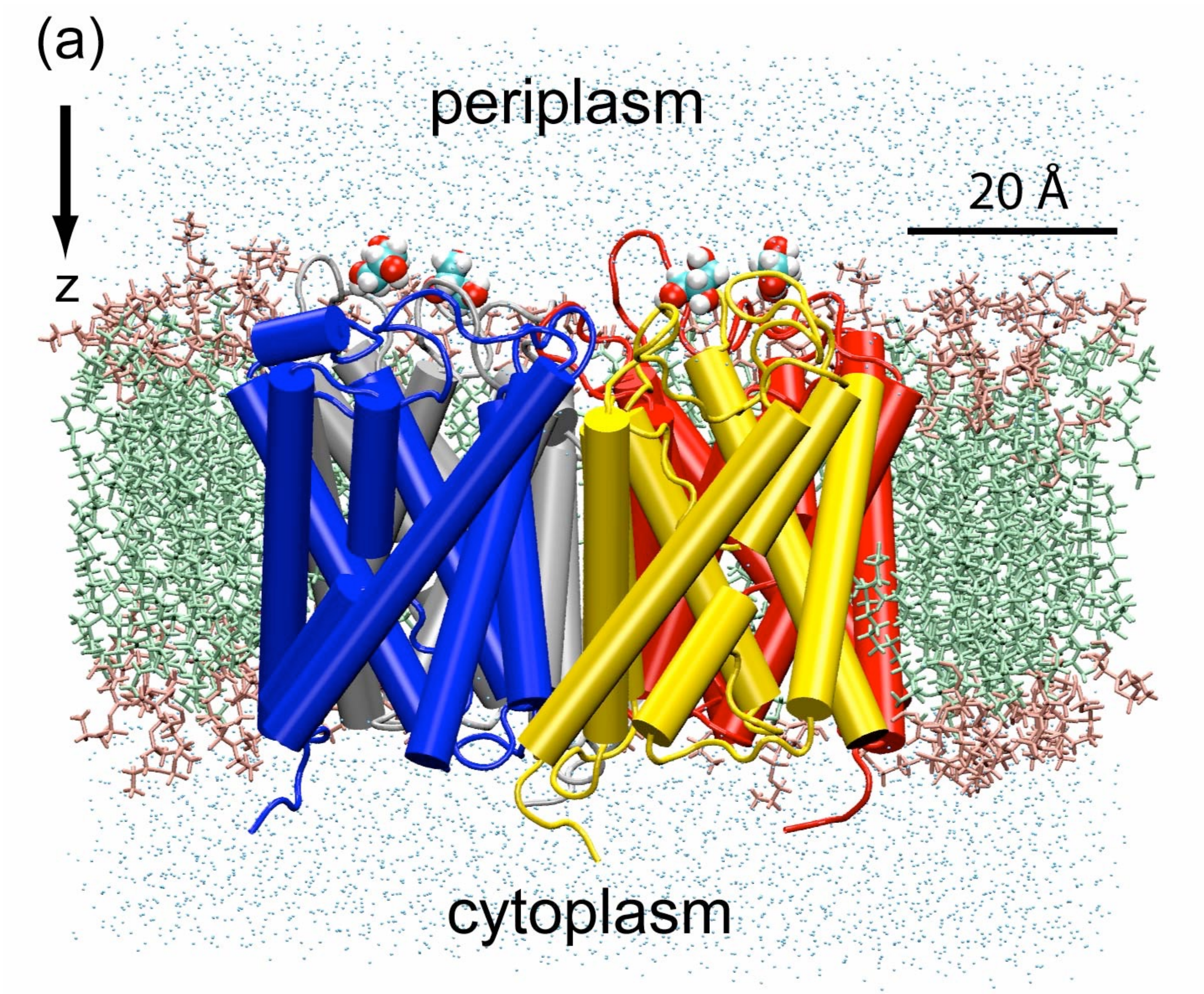


Theoretical Biophysics Group
Beckman Institute
University of Illinois at Urbana-Champaign

Asymmetric Profile in the Vestibules

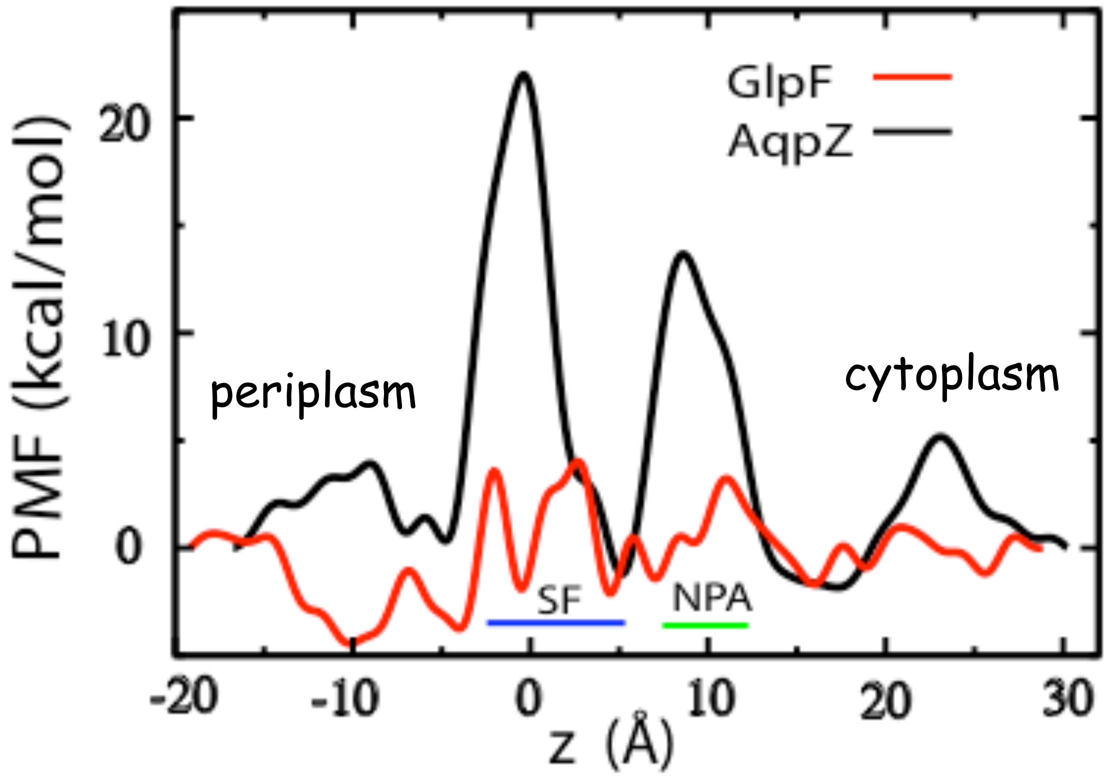
Jensen et al., *PNAS*, 99:6731-6736, 2002.

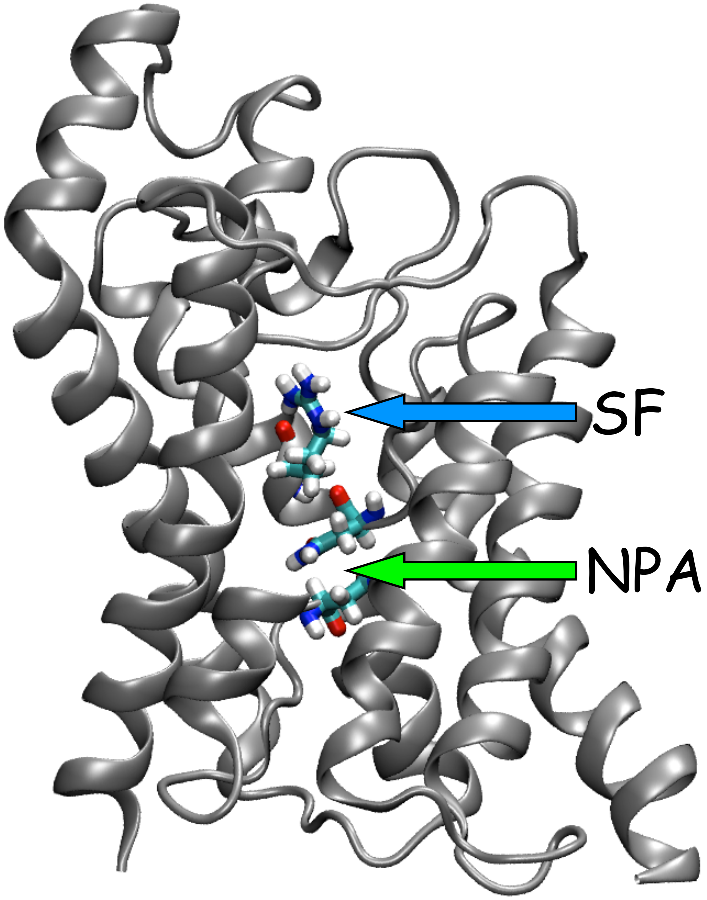
Artificial induction of glycerol conduction through AqpZ



Y. Wang, K. Schulten, and E. Tajkhorshid ***Structure*** 13, 1107 ***(***2005)

Three fold higher barriers

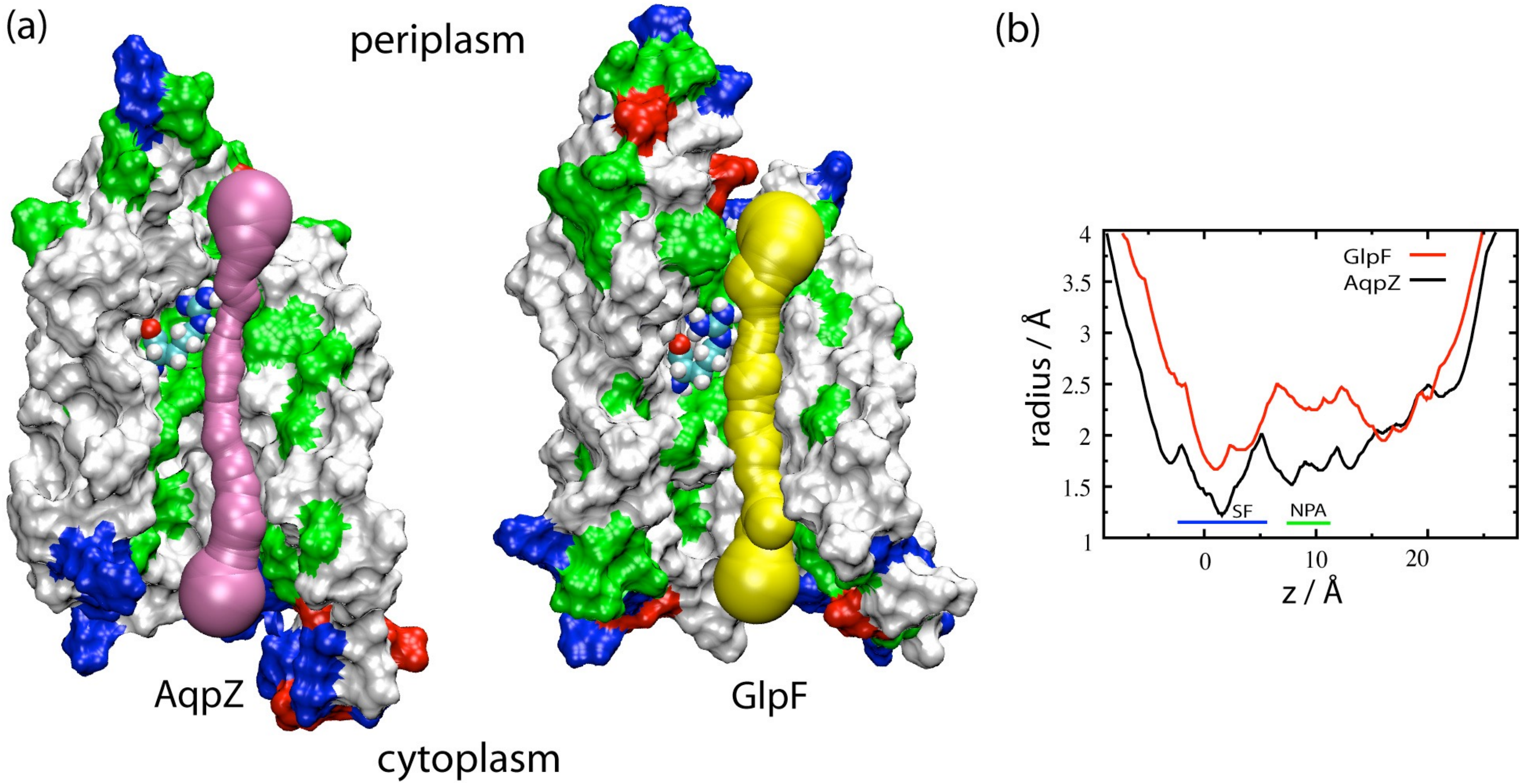




AqpZ 22.8 kcal/mol
GlpF 7.3 kcal/mol

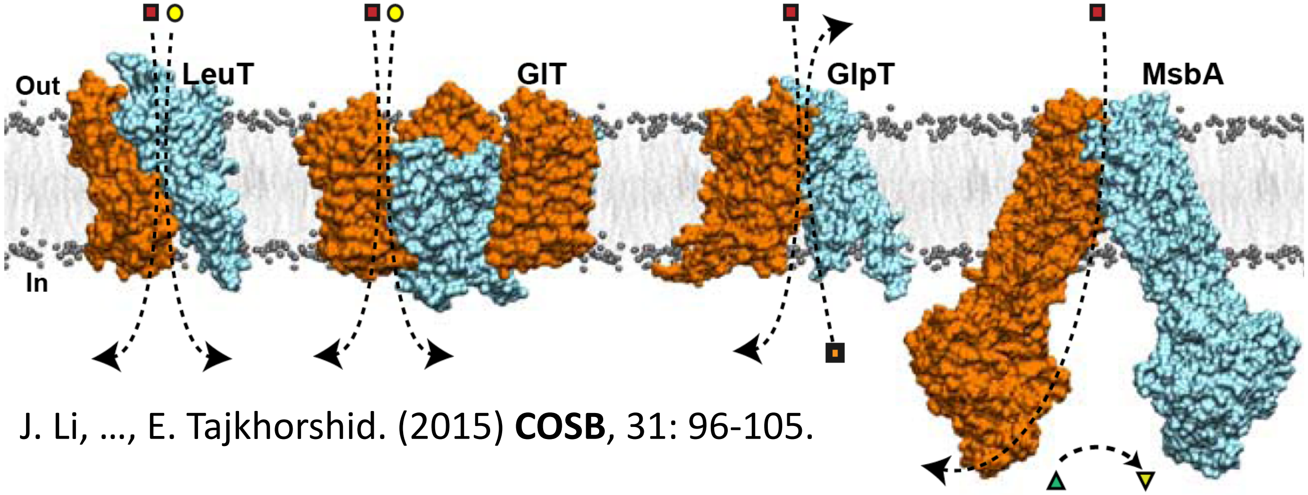
Y. Wang, K. Schulten, and E. Tajkhorshid ***Structure*** 13, 1107 *(*2005)

Could it be simply the size?



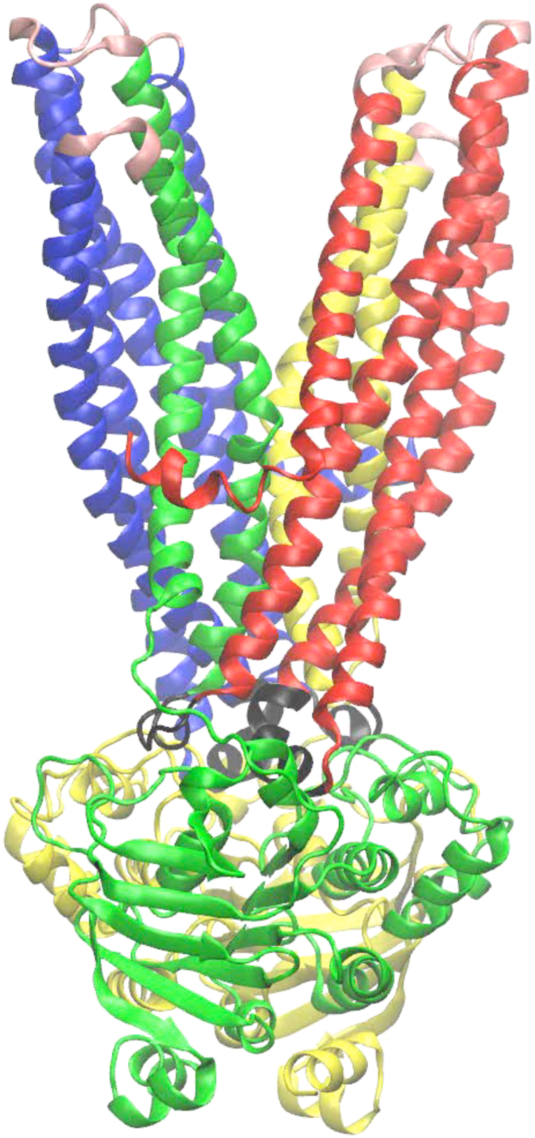
Y. Wang, K. Schulten, and E. Tajkhorshid ***Structure*** 13, 1107 (2005)

Battling the Timescale - Case III
Biased (nonequilibrium) simulations

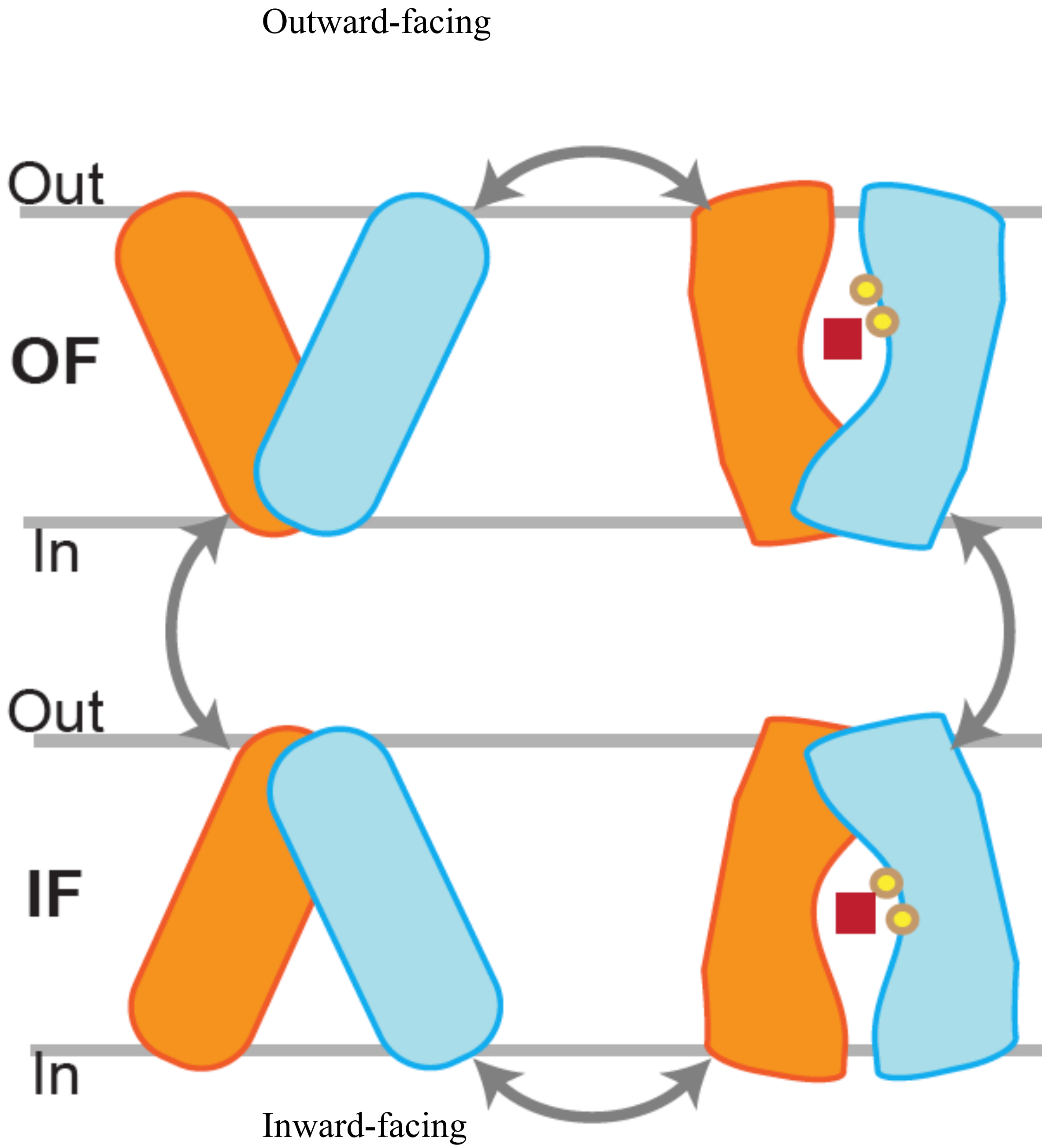


J. Li, ..., E. Tajkhorshid. (2015) **COSB**, 31: 96-105.

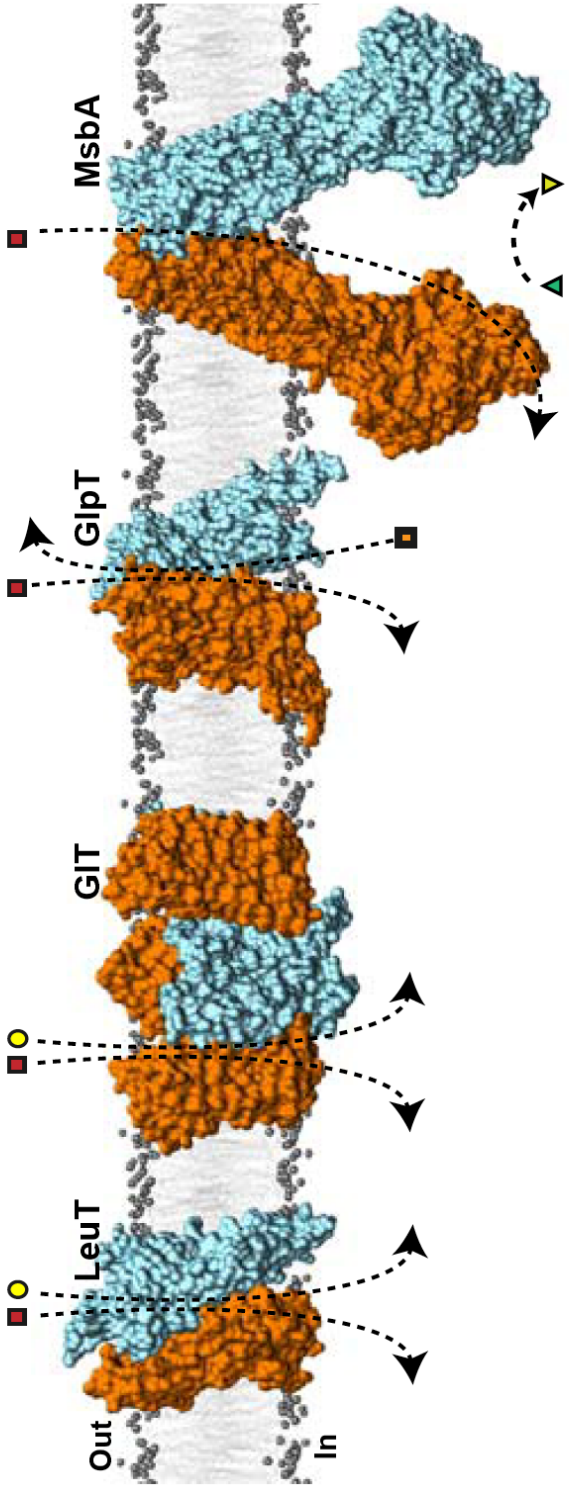
- **Neurotransmitter Uptake**
  - Norepinephrine, serotonin, dopamine, glutamate,…
- **Gastrointestinal Tract**
  - Active absorption of nutrients
  - Secretion of ions
- **Kidneys**
  - Reabsorption
  - Secretion
- **Pharmacokinetics of all drugs**
  - Absorption, distribution, elimination
  - Multi-drug resistance in cancer cells



Alternating Access Mechanism



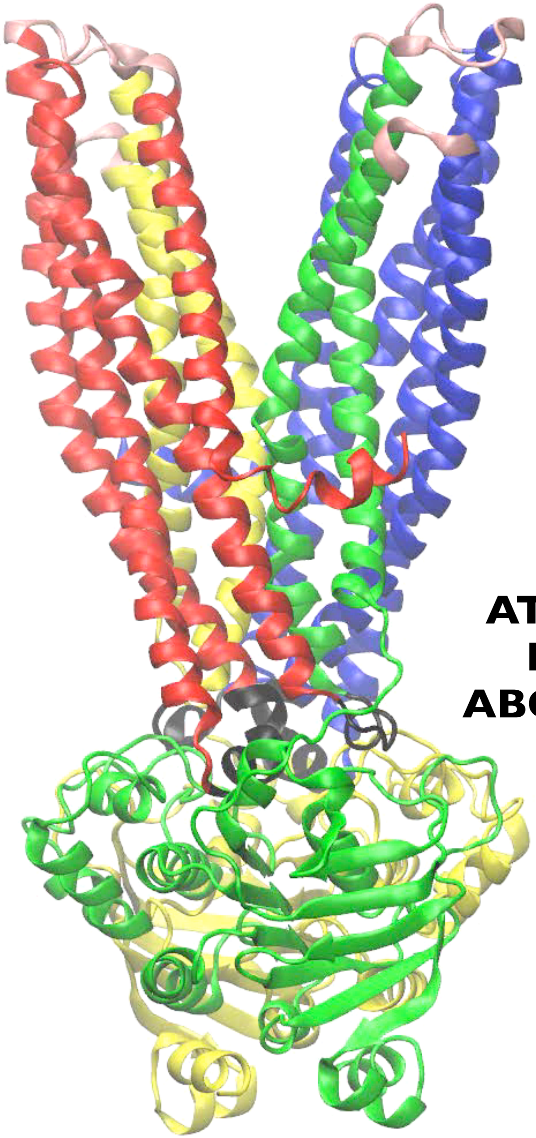
Jardetzky O. *Nature* **211**: 969–970 (1966)



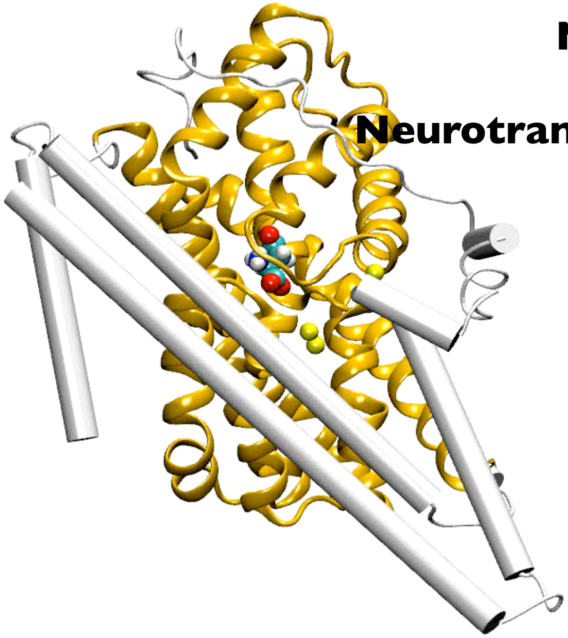
J. Li, …, E. Tajkhorshid. (2015) **COSB**, 31: 96-105.

COMPLEX

Diverse Structural Transitions Involved

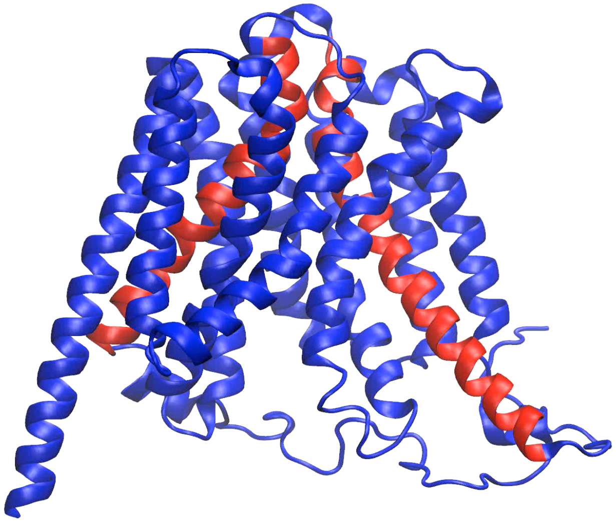


ATP-Driven
Primary
ABC Exporter



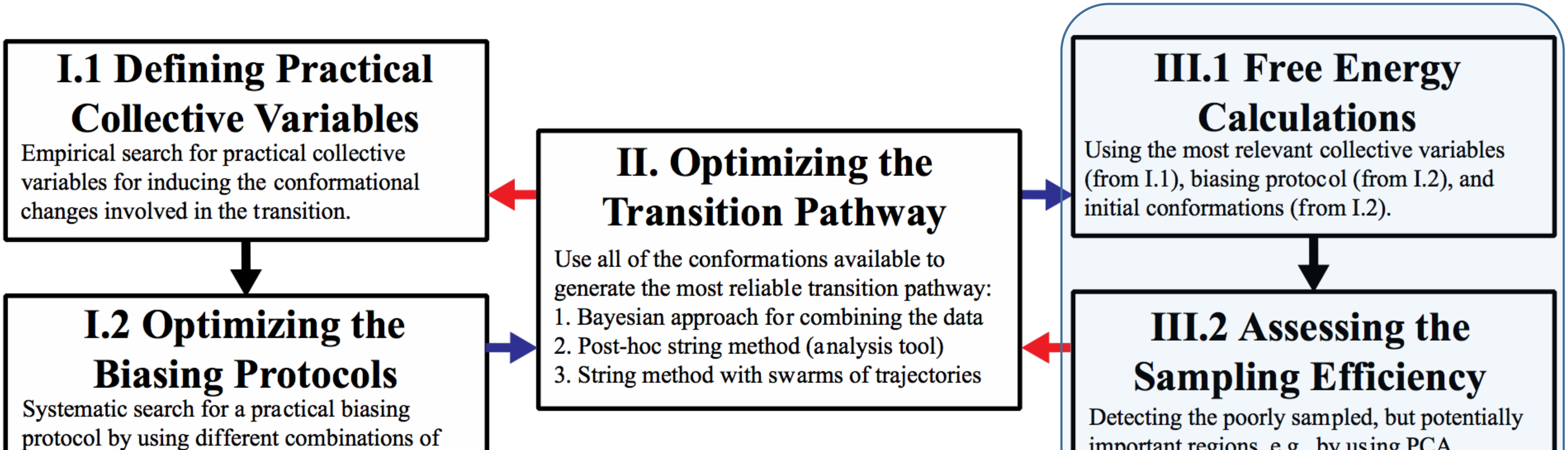
Na-coupled
Secondary
Neurotransmitter Transporter

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:

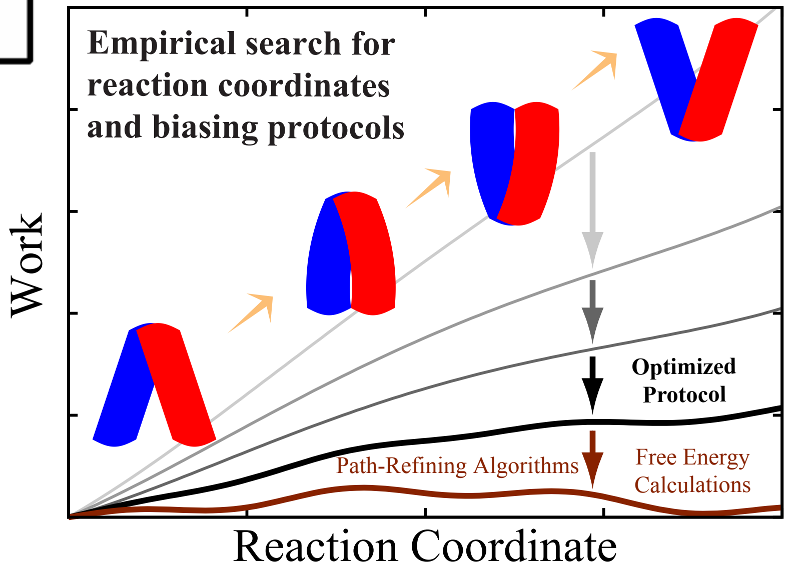
1. Bayesian approach for combining the data
2. Post-hoc string method (analysis tool)
3. String method with swarms of trajectories

III.1 Free Energy Calculations

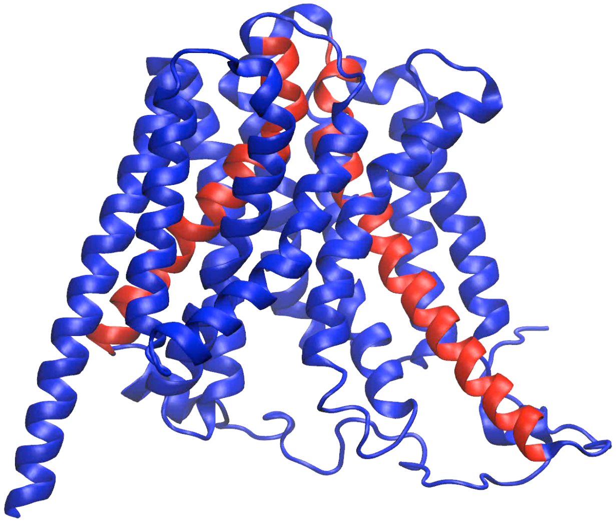
Using the most relevant collective variables (from I.1), biasing protocol (from I.2), and initial conformations (from I.2).

III.2 Assessing the Sampling Efficiency

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

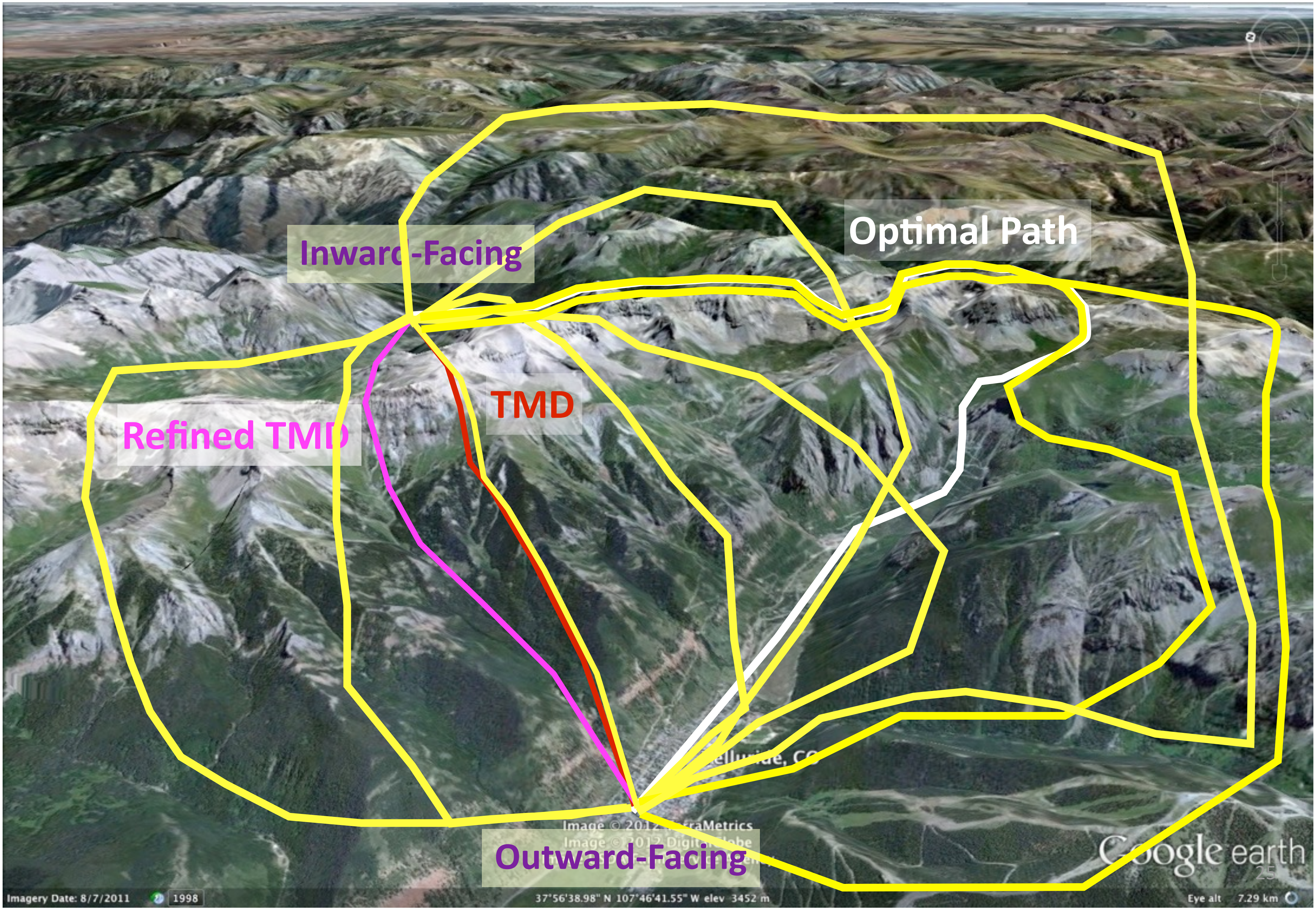


Mahmoud Moradi



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.

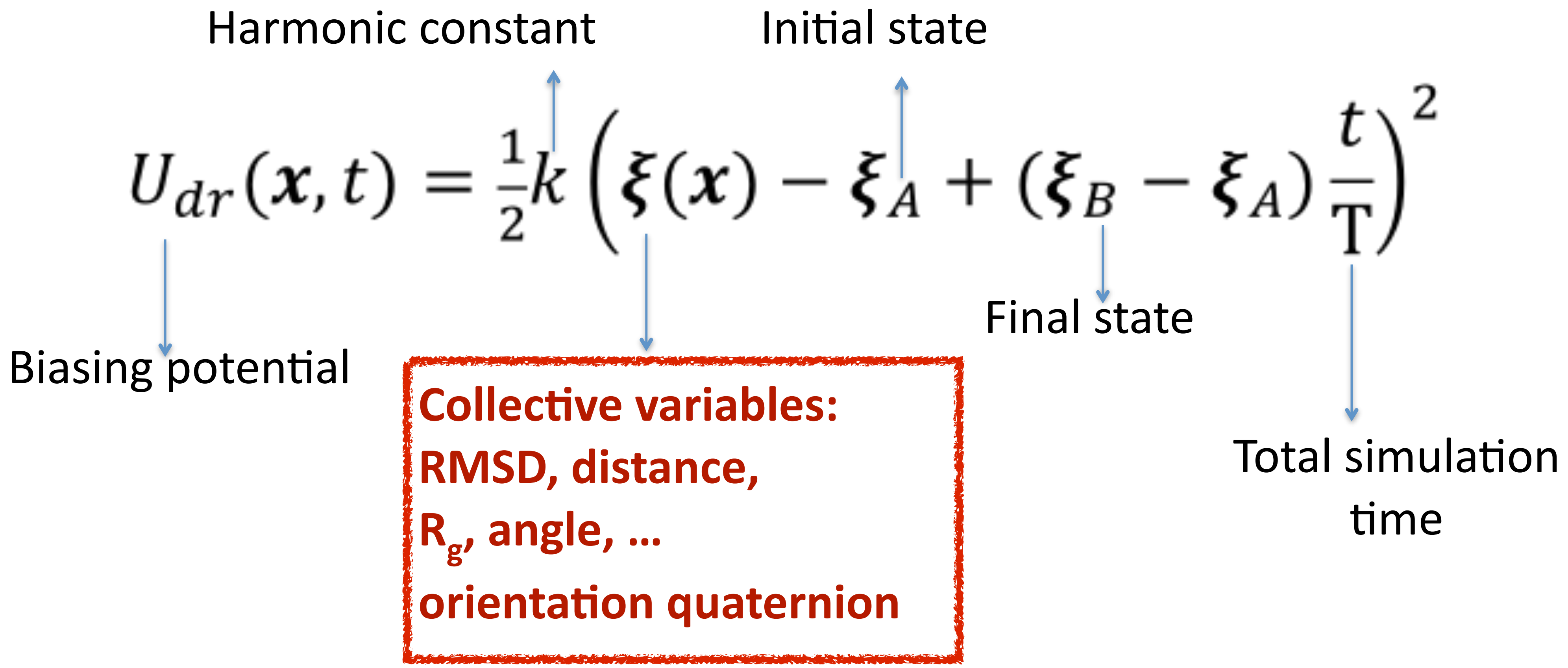
Aggressive Search of the Space



Non-equilibrium Driven Molecular Dynamics:

Applying a time-dependent external force to induce the transition

Along various pathways/mechanisms (collective variables)



Harmonic constant

Initial state

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

Final state

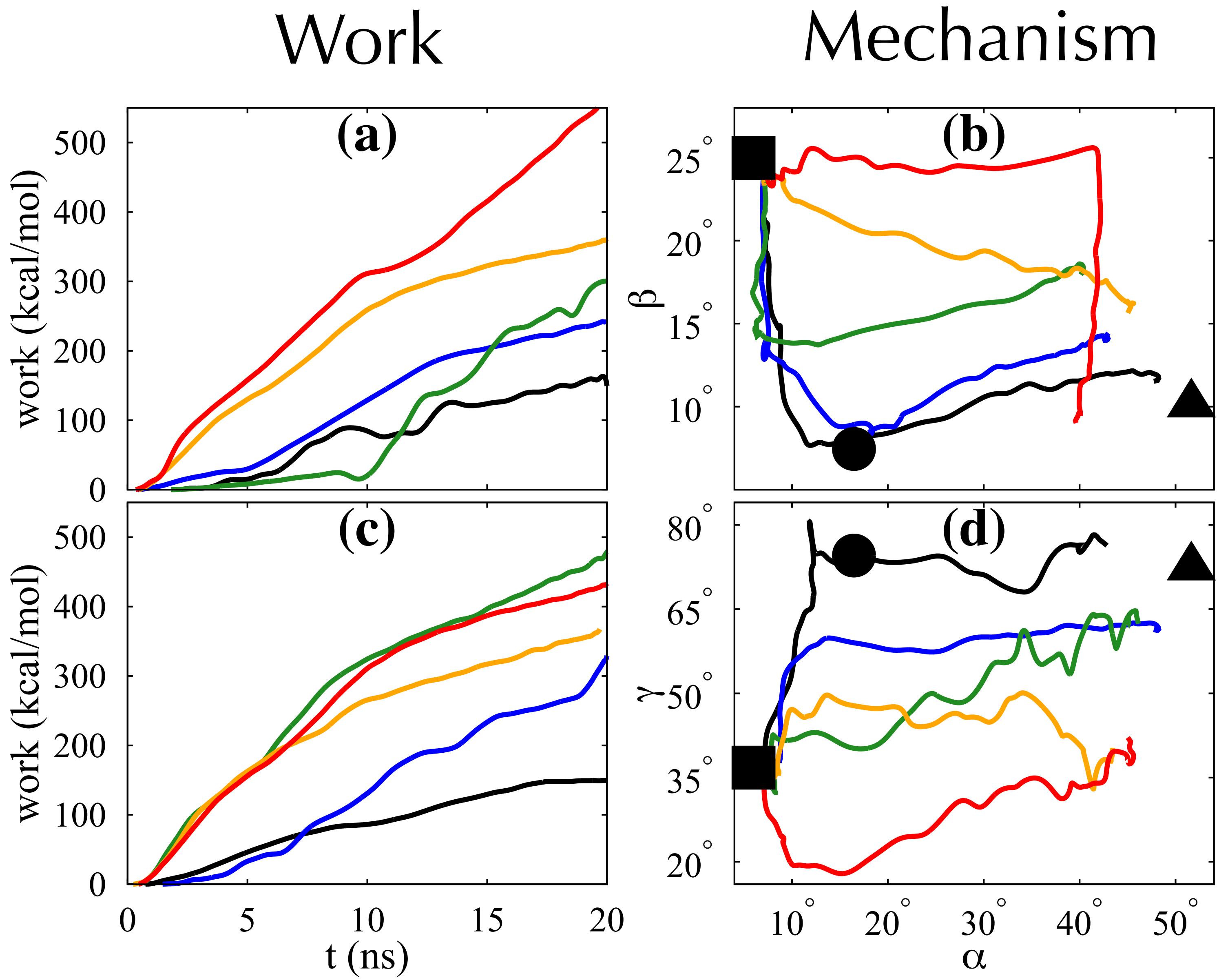
Biasing potential

Collective variables:
RMSD, distance,
R_g, angle, …
orientation quaternion

Total simulation time

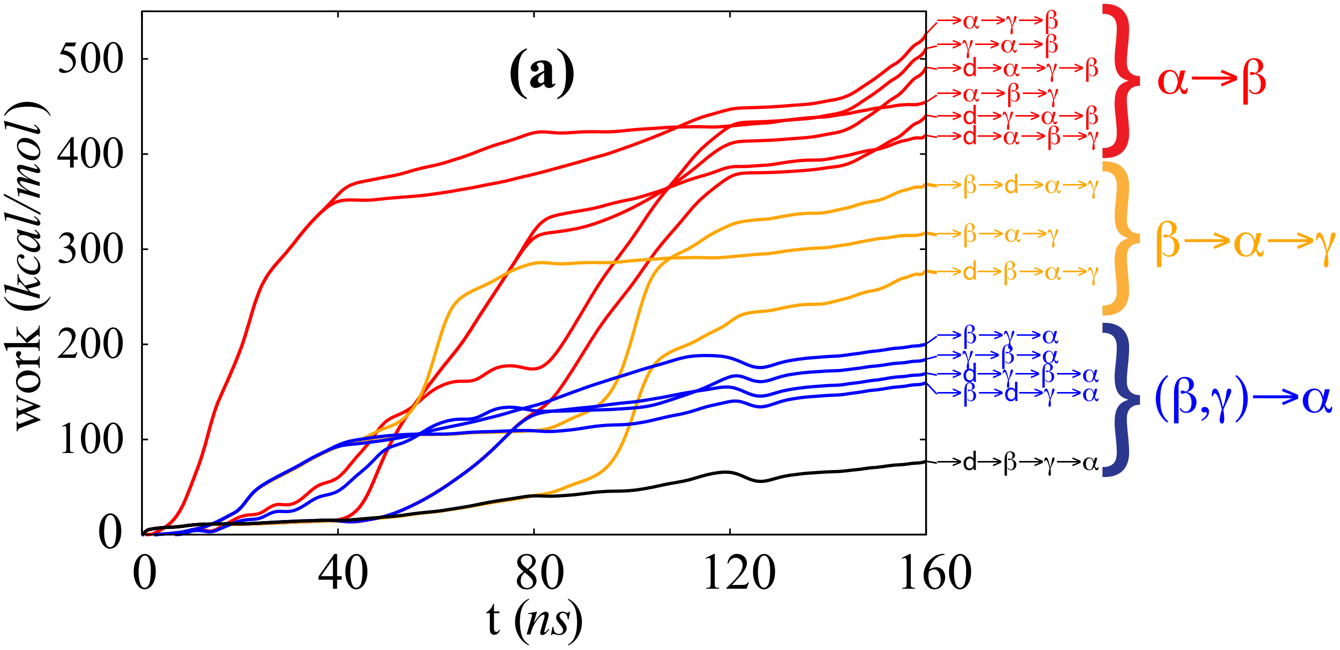
M. Moradi and ET (2013) **PNAS**, 110:18916–18921.
M. Moradi and ET (2014) **JCTC**, 10: 2866–2880.
M. Moradi, G. Enkavi, and ET (2015) **Nature Comm.**, 6:8393.

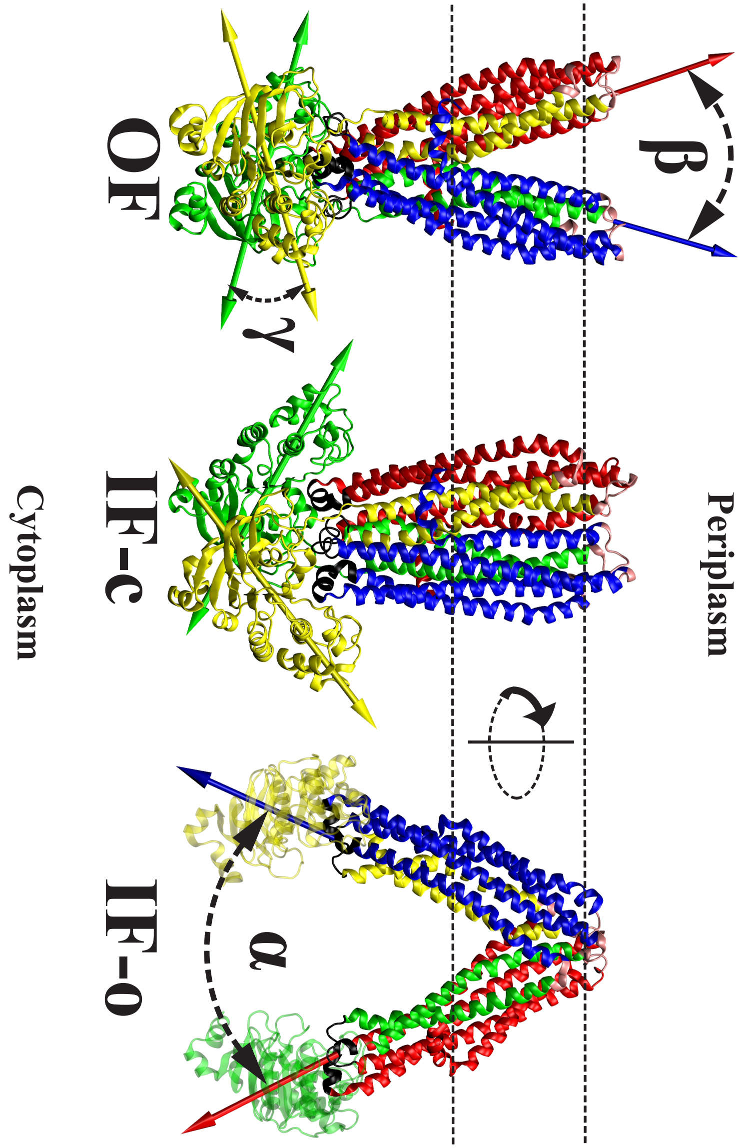
Progressively Optimizing the Biasing Protocol/Collective Variable using non-Equilibrium Work as a Measure of the Path Quality



Example set taken from a subset of 20 ns biased simulations

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

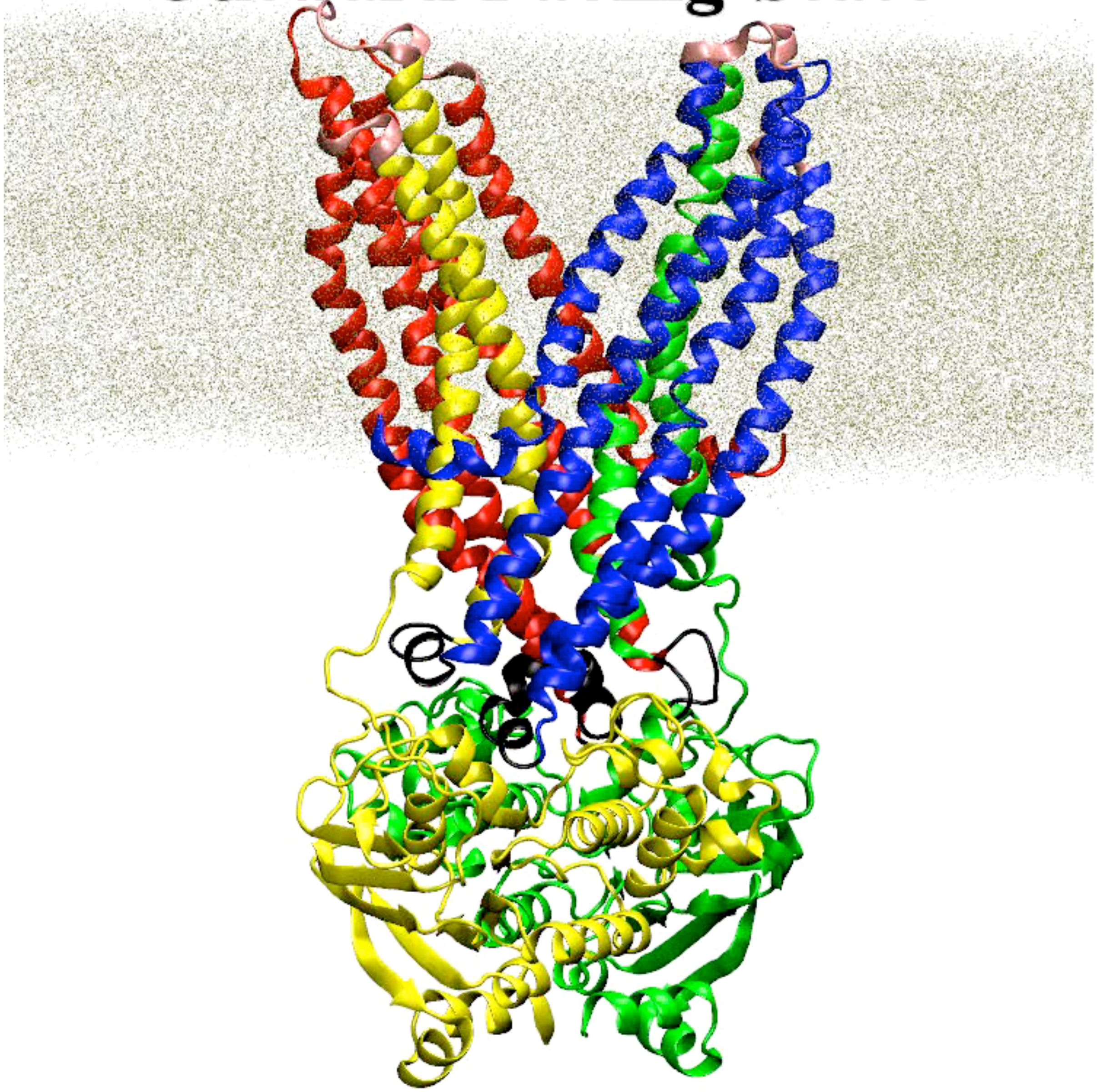




M. Moradi and ET (2013) **PNAS**, 110:18916–18921.

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

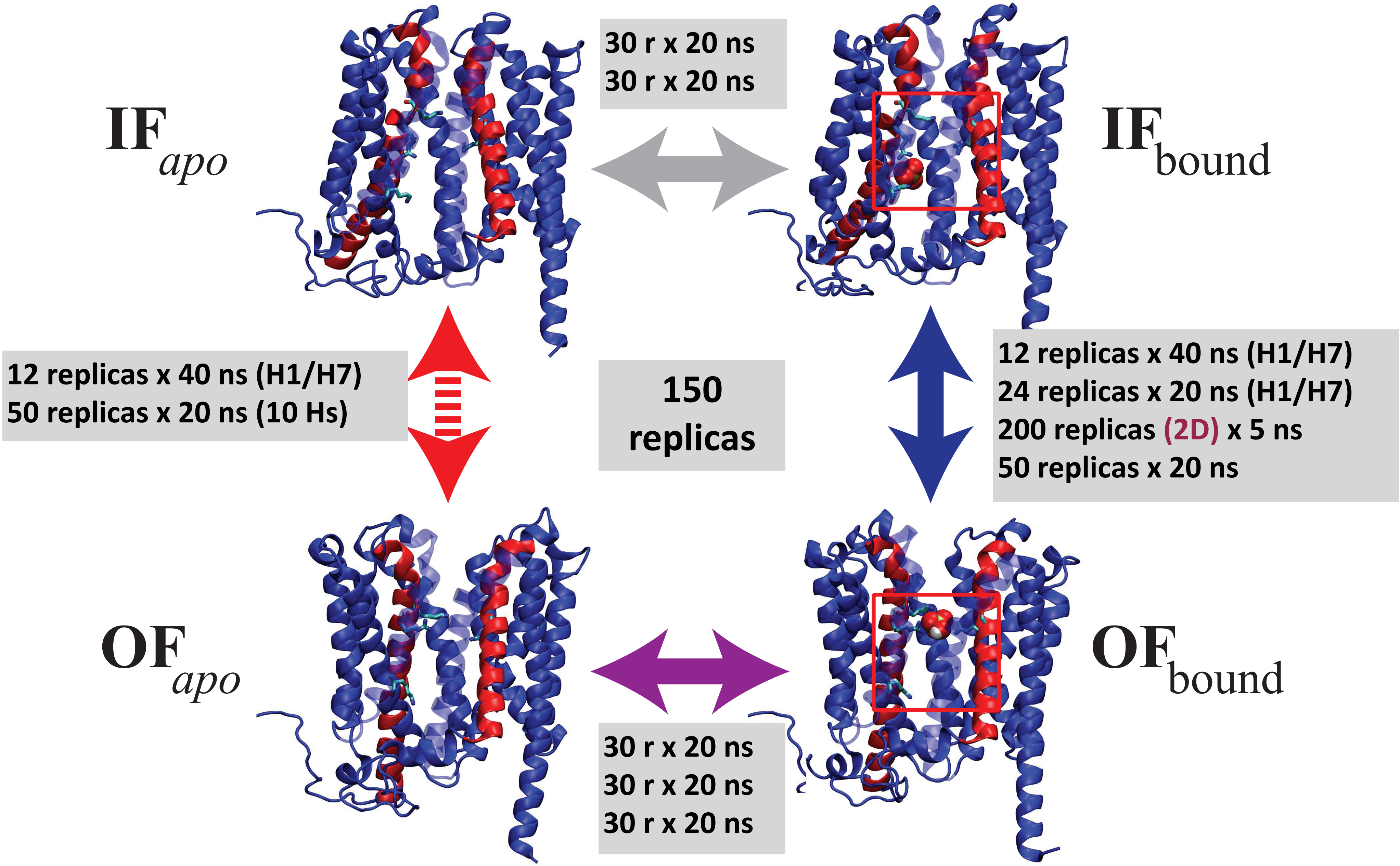
R	T	R	T	R	T	R	T	R

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



30 r x 20 ns
30 r x 20 ns

12 replicas x 40 ns (H1/H7)
50 replicas x 20 ns (10 Hs)

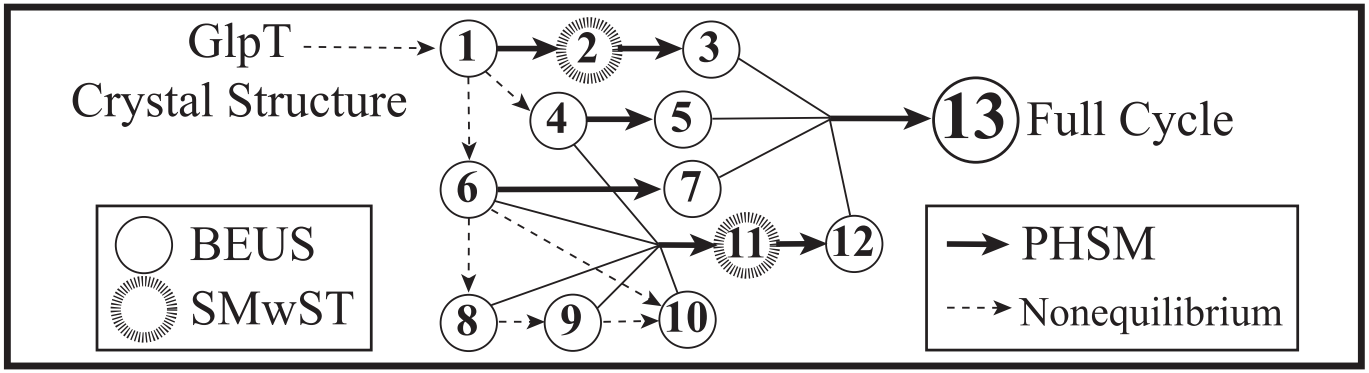
150
replicas

12 replicas x 40 ns (H1/H7)
24 replicas x 20 ns (H1/H7)
200 replicas (2D) x 5 ns
50 replicas x 20 ns

30 r x 20 ns
30 r x 20 ns
30 r x 20 ns

Simulation protocols

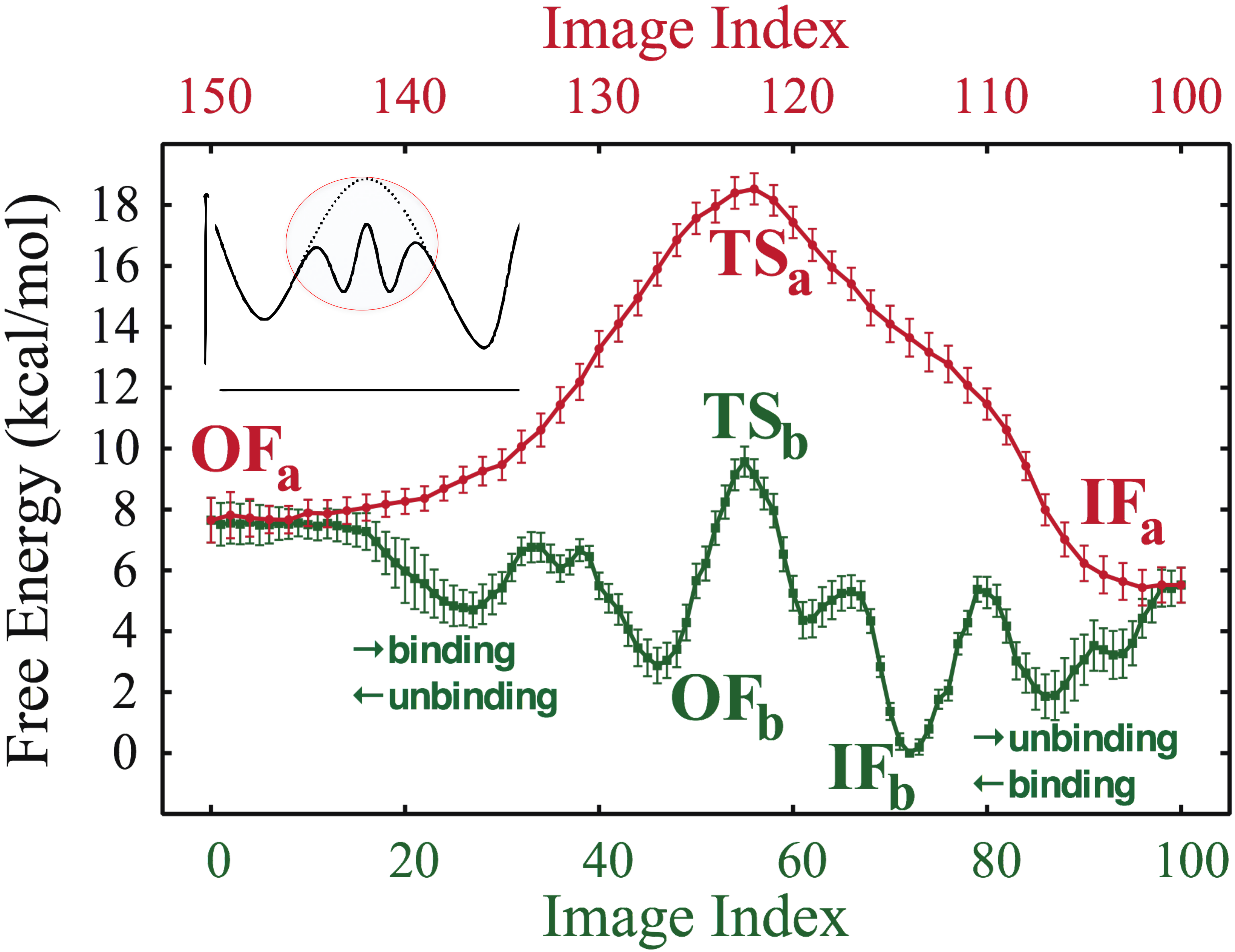
	Transition	Technique	Collective Variables	# of Replicas × Runtime		
1	$IF_a \leftrightarrow OF_a$	BEUS	(Q_1,Q_7)	12 × 40 ns	=	0.5 µs
2		SMwST	$\{Q\}$	1000 × 1 ns	=	1 µs
3		BEUS	$\{Q\}$	50 × 20 ns	=	1 µs
4	$IF_a \leftrightarrow IF_b$	BEUS	Z_{Pi}	30 × 40 ns	=	1.2 µs
5		BEUS	$(\{Q\}, Z_{Pi})$	30 × 40 ns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



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

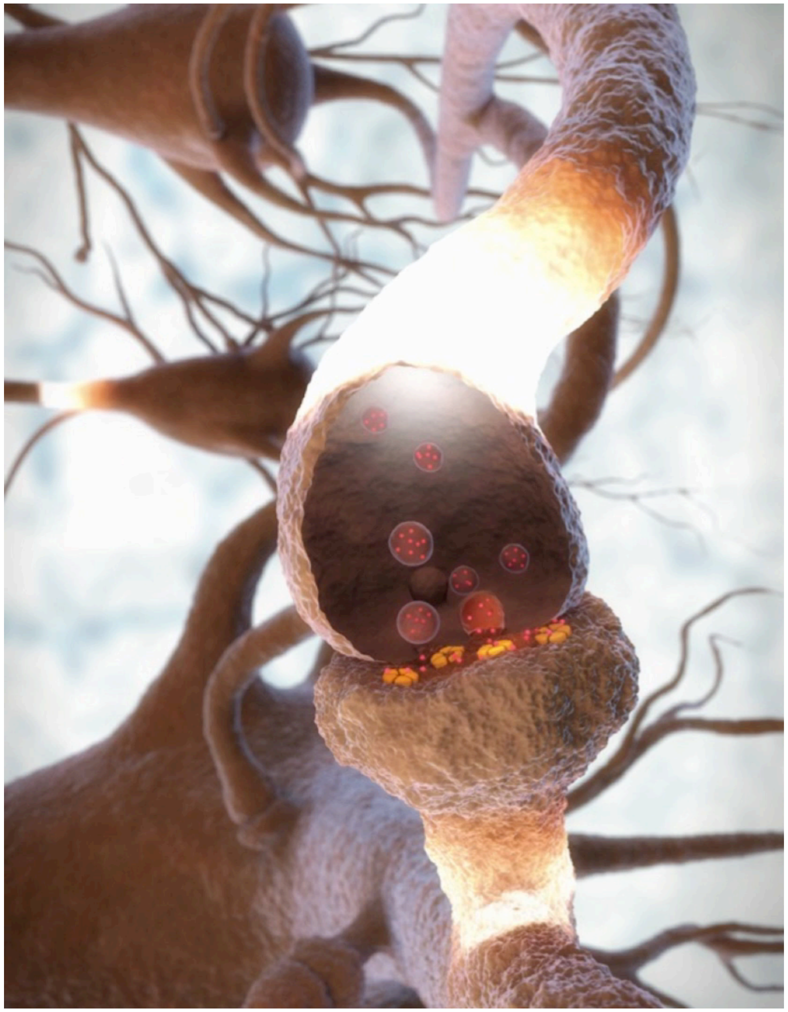
U.S. DEPARTMENT OF ENERGY
INCITE
LEADERSHIP COMPUTING

BLUE WATER NCSA

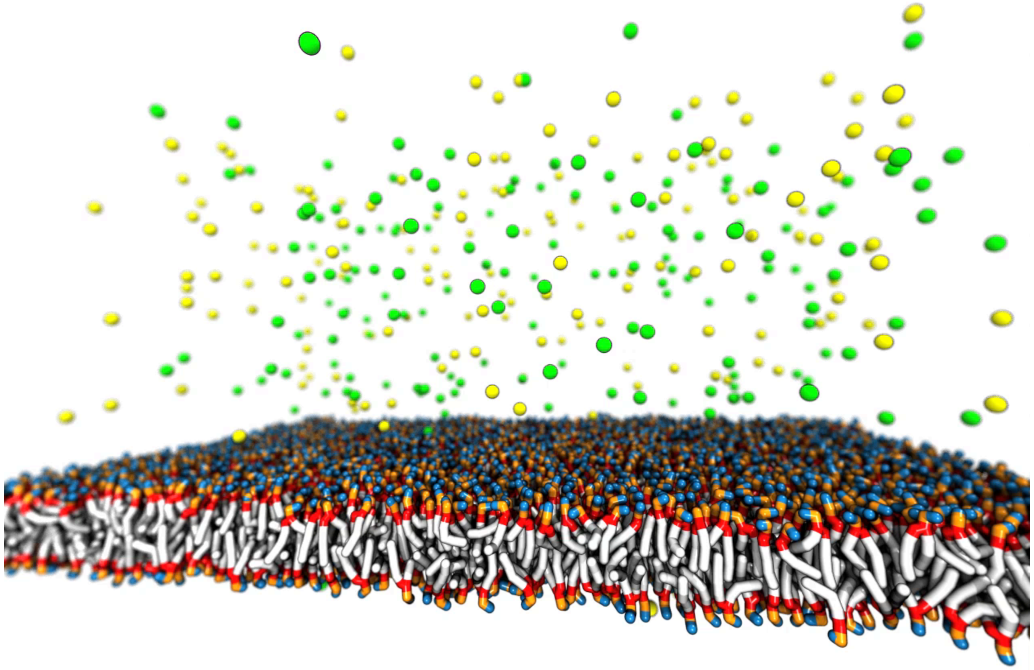


M. Moradi, G. Enkavi, and ET (2015) **Nature Communication**, 6: 8393.

Battling the Timescale - Case IV
Multiscale Simulations



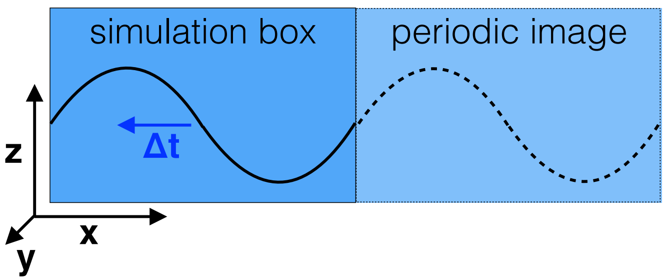
Membrane Budding/Fusion

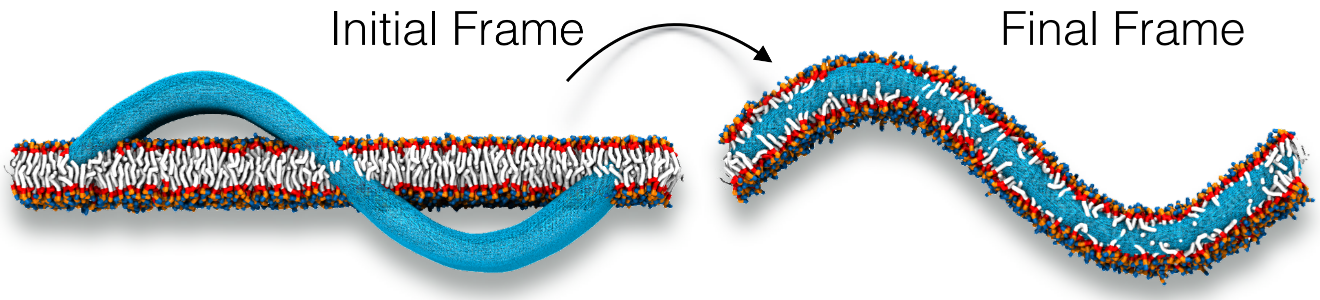


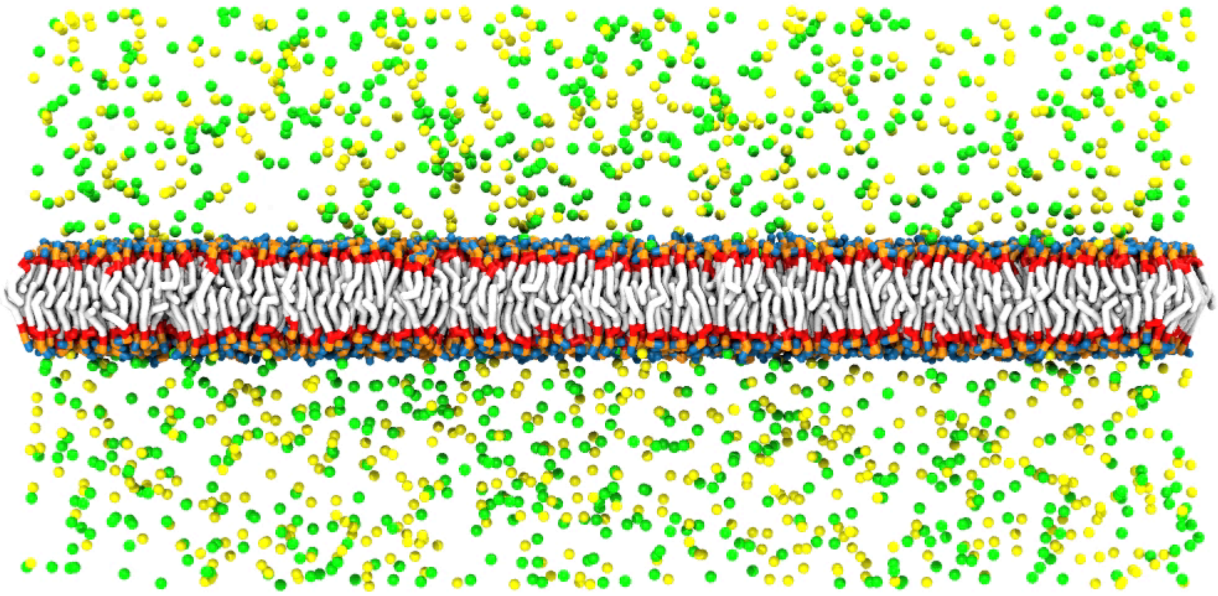
Combining multiple replica simulations and coarse-grained models to describe membrane fusion

Workflow for Multi-Scale Modeling

Parametrically Defined Sine Function

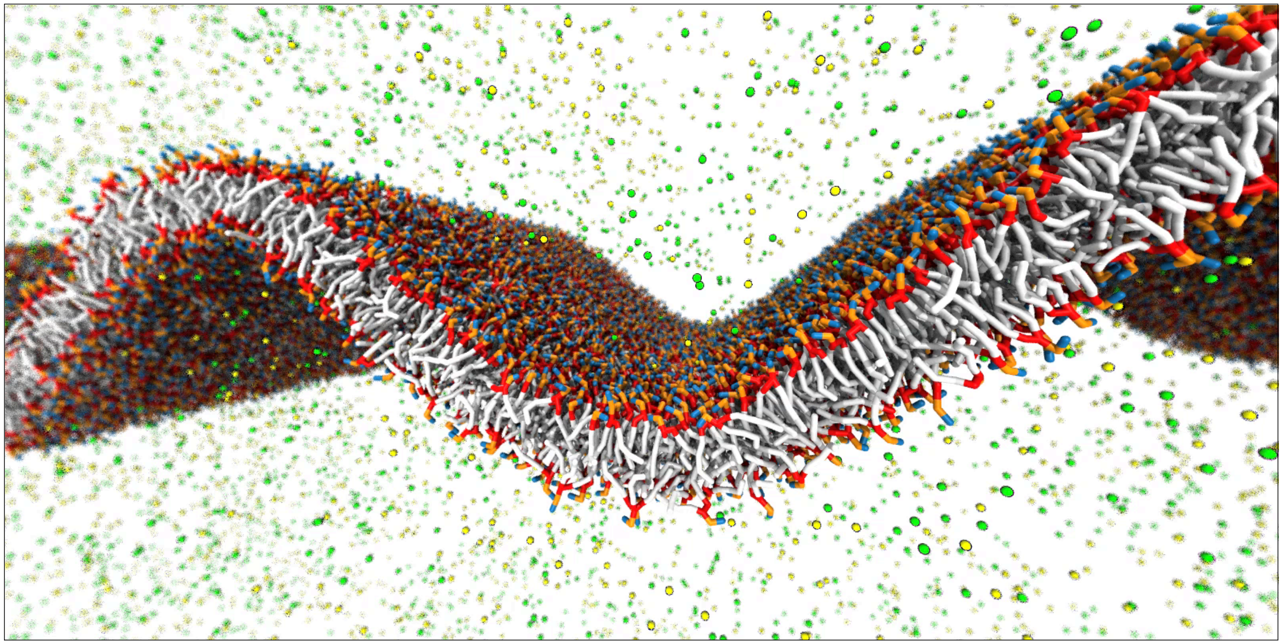






Christopher Mayne, Tajkhorshid Lab

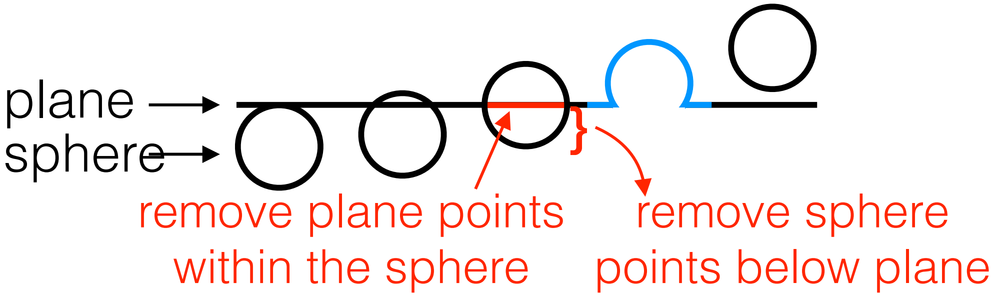
Workflow for Multi-Scale Modeling



Christopher Mayne, Tajkhorshid Lab

Workflow for Multi-Scale Modeling

Grid Design and Construction

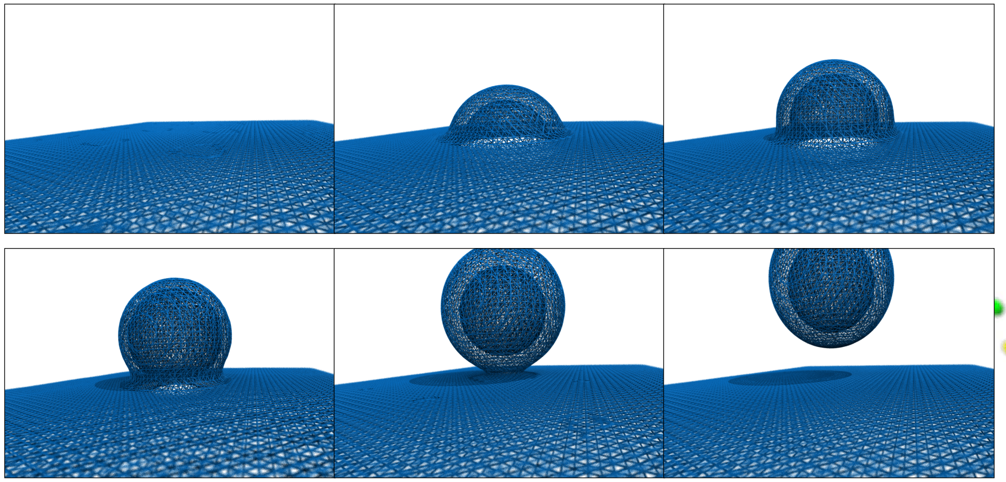


plane

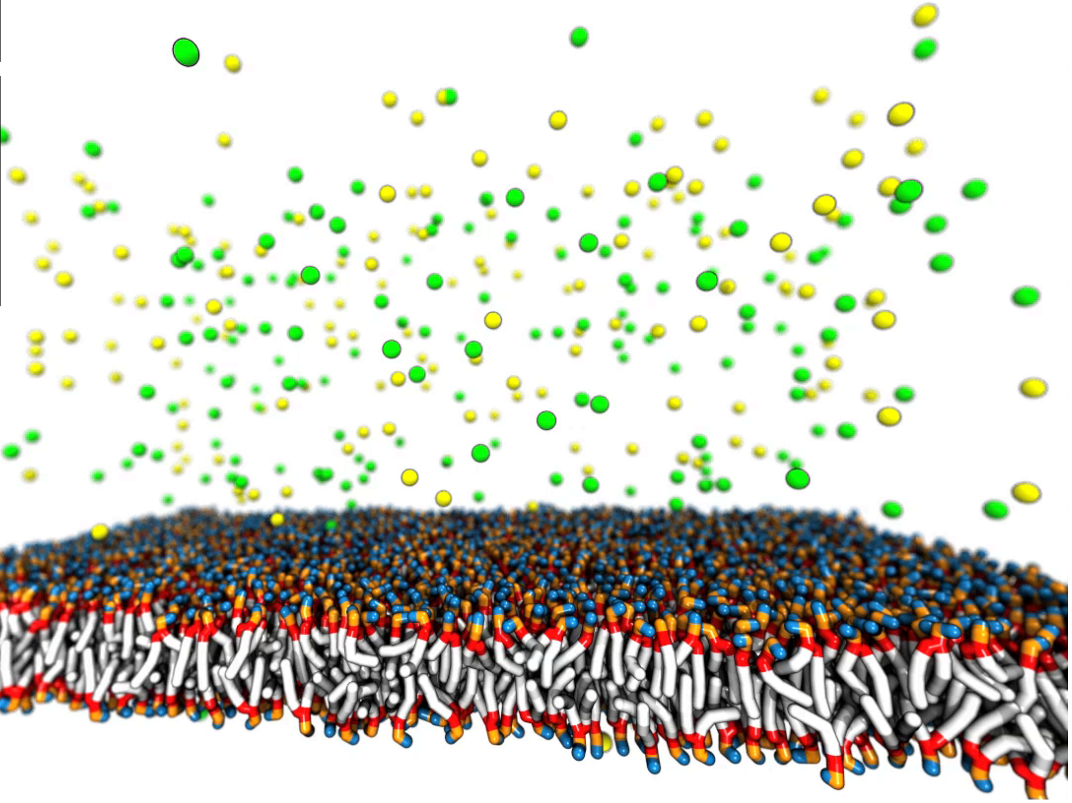
sphere

remove plane points within the sphere

remove sphere points below plane



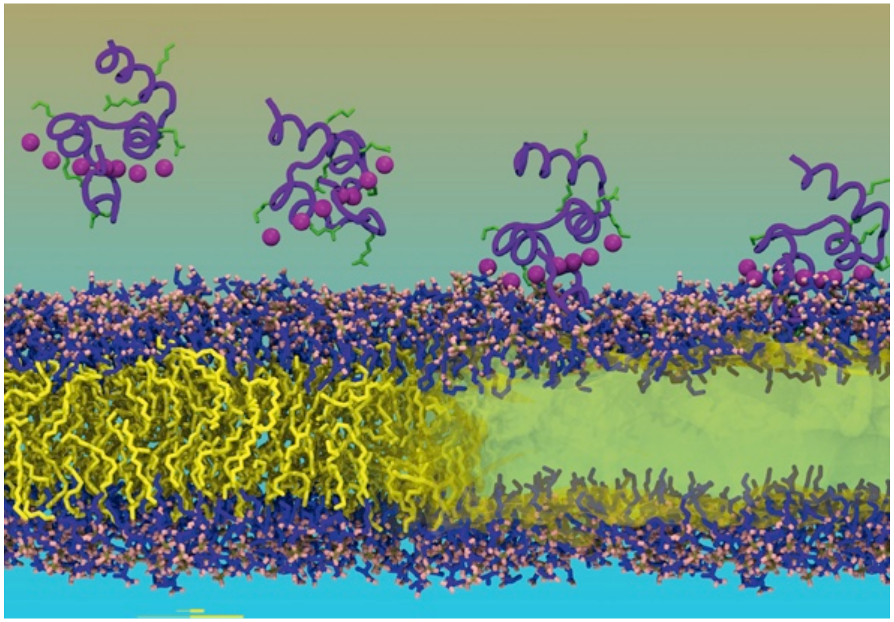
Membrane Budding/Fusion

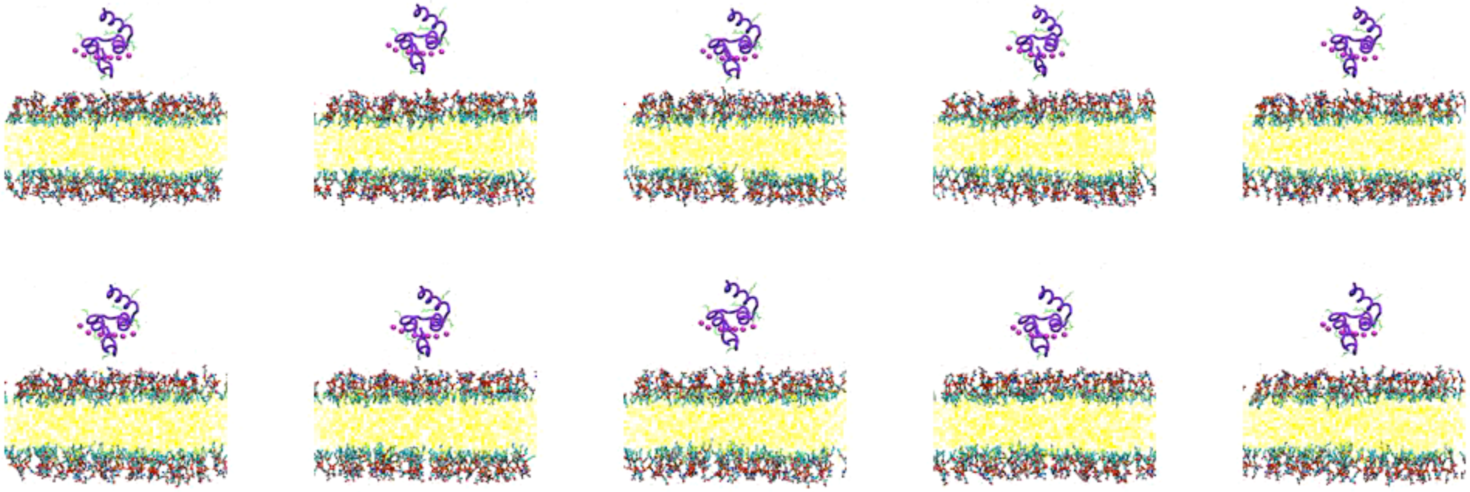


Christopher Mayne, Tajkhorshid Lab

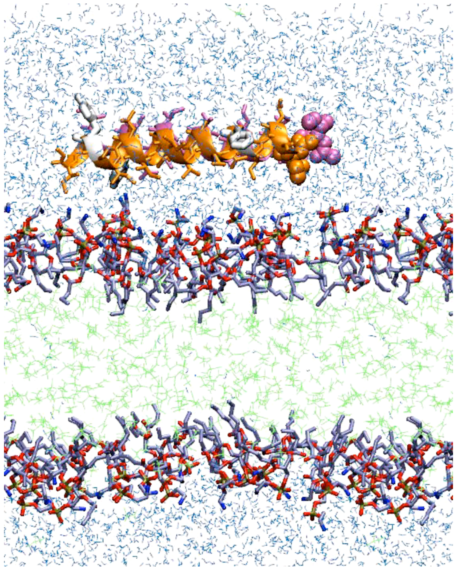
Battling the Timescale - Case V
Reduced Representations

Highly Mobile Membrane Mimetic model





GpA insertion in 12 ns

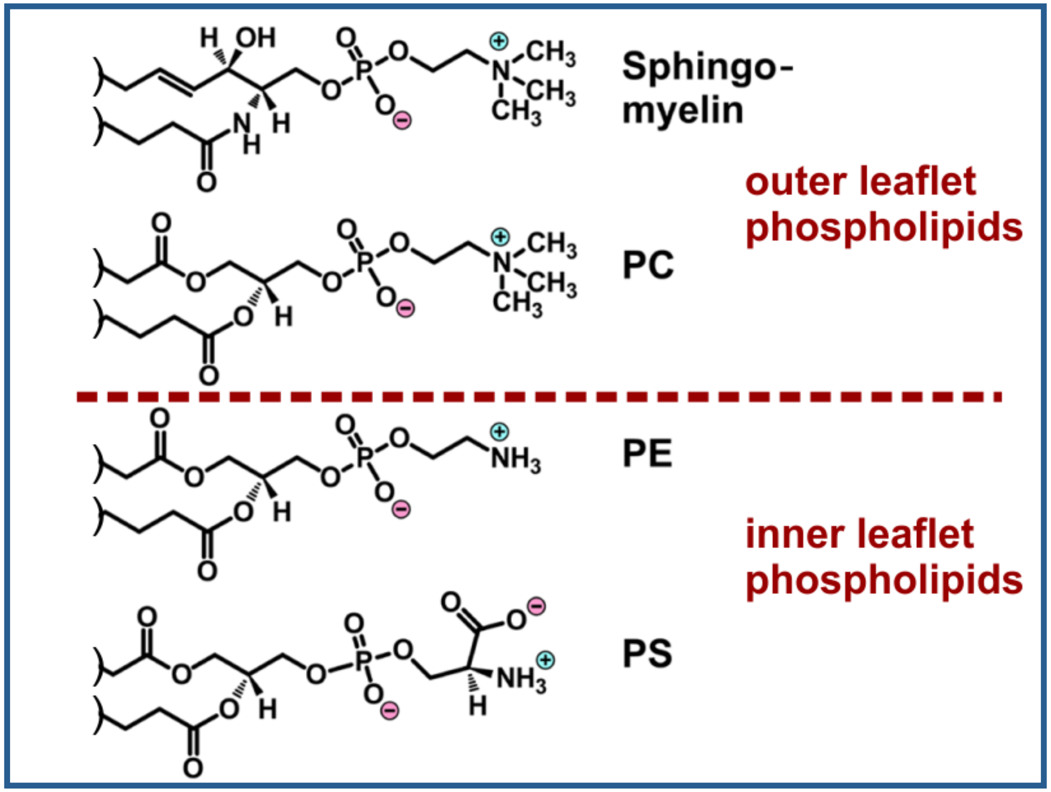


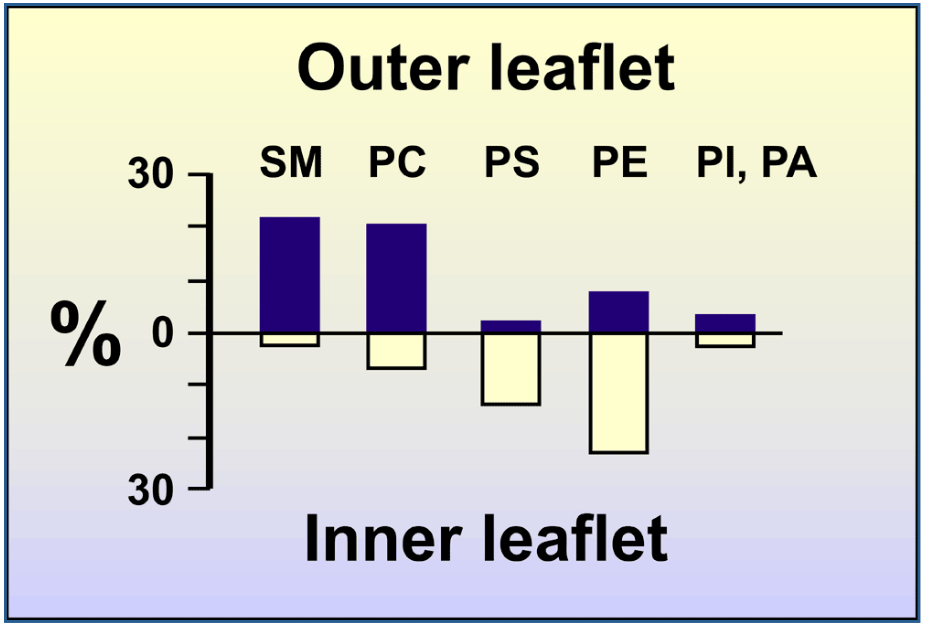
Specific lipids regulate various functional aspects of membrane proteins

Integral membrane proteins

Peripheral membrane proteins

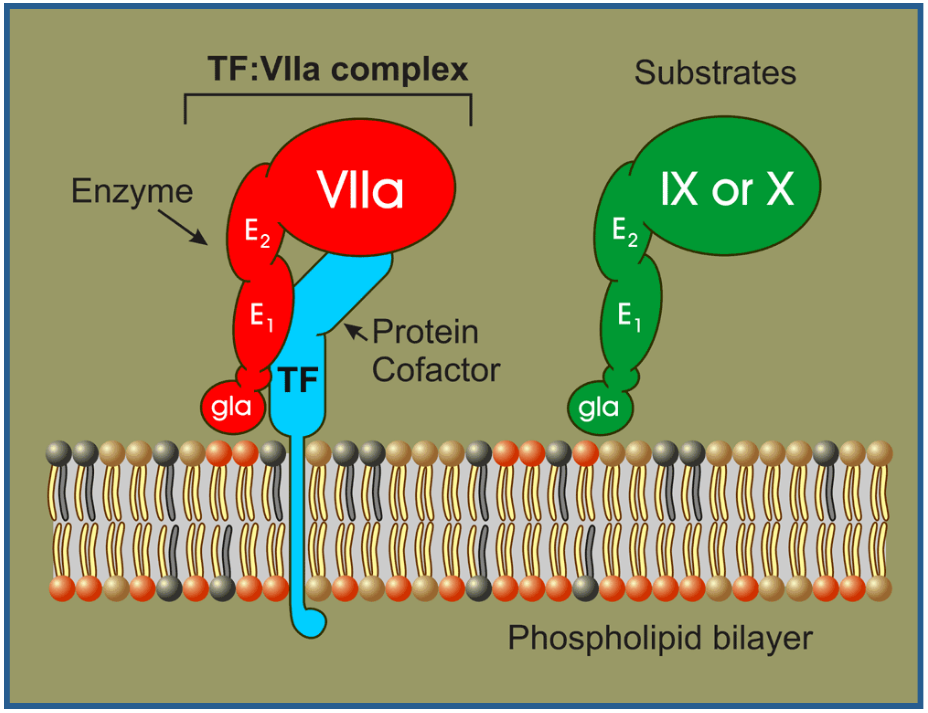
Lipid Dependent Binding and Activation





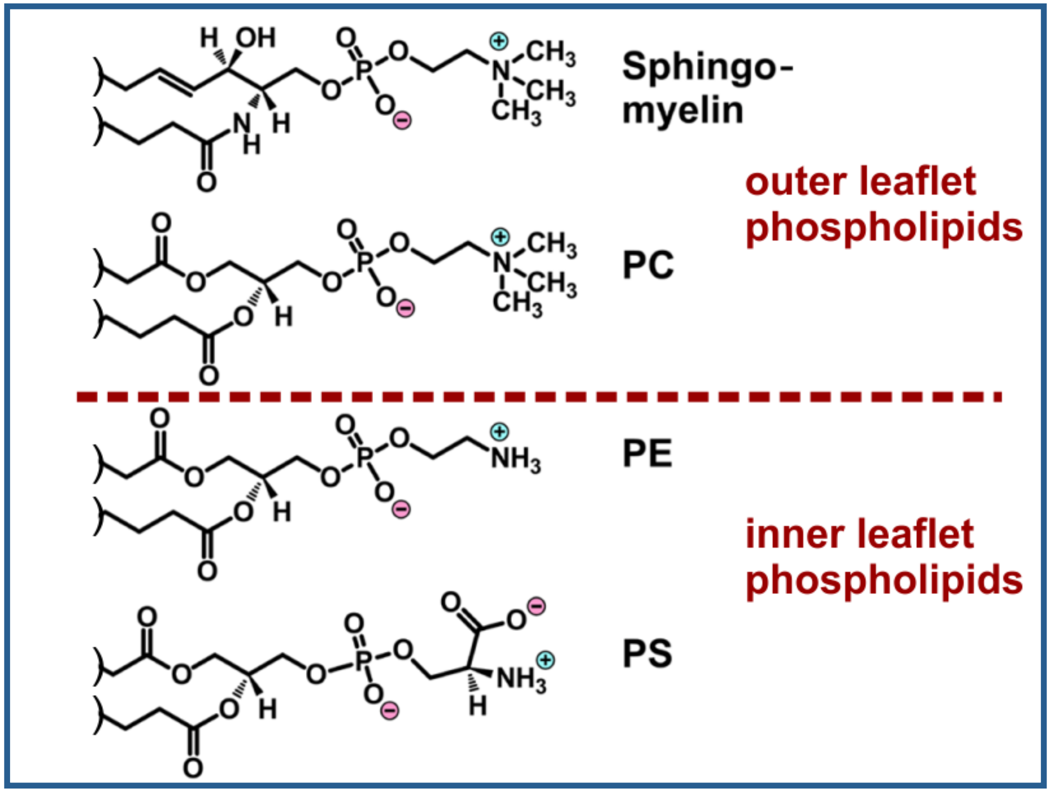
Affinity is controlled by lipid content

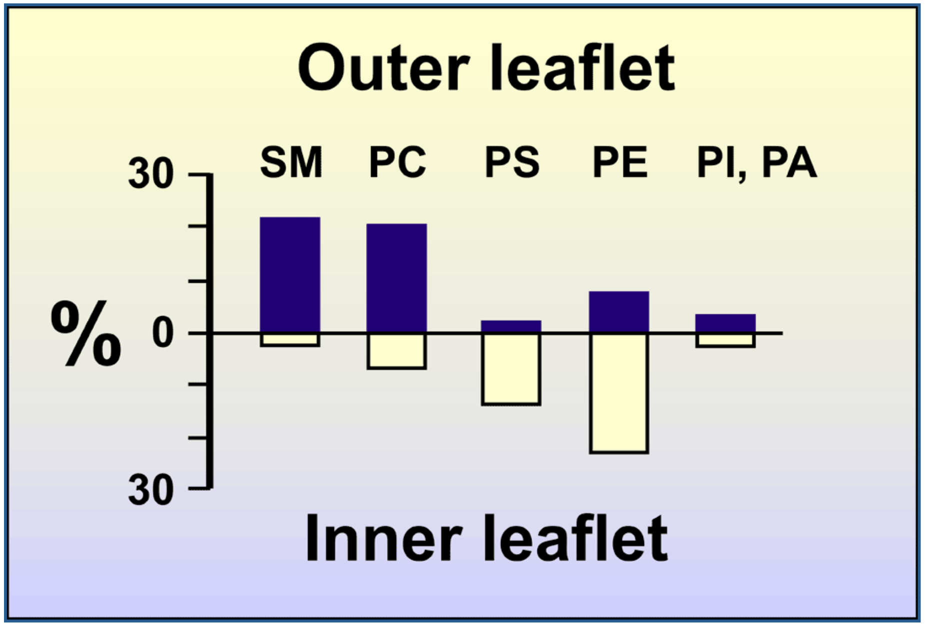
Leaflet asymmetry is vital for coagulation



Courtesy of Jim Morrissey, UIUC

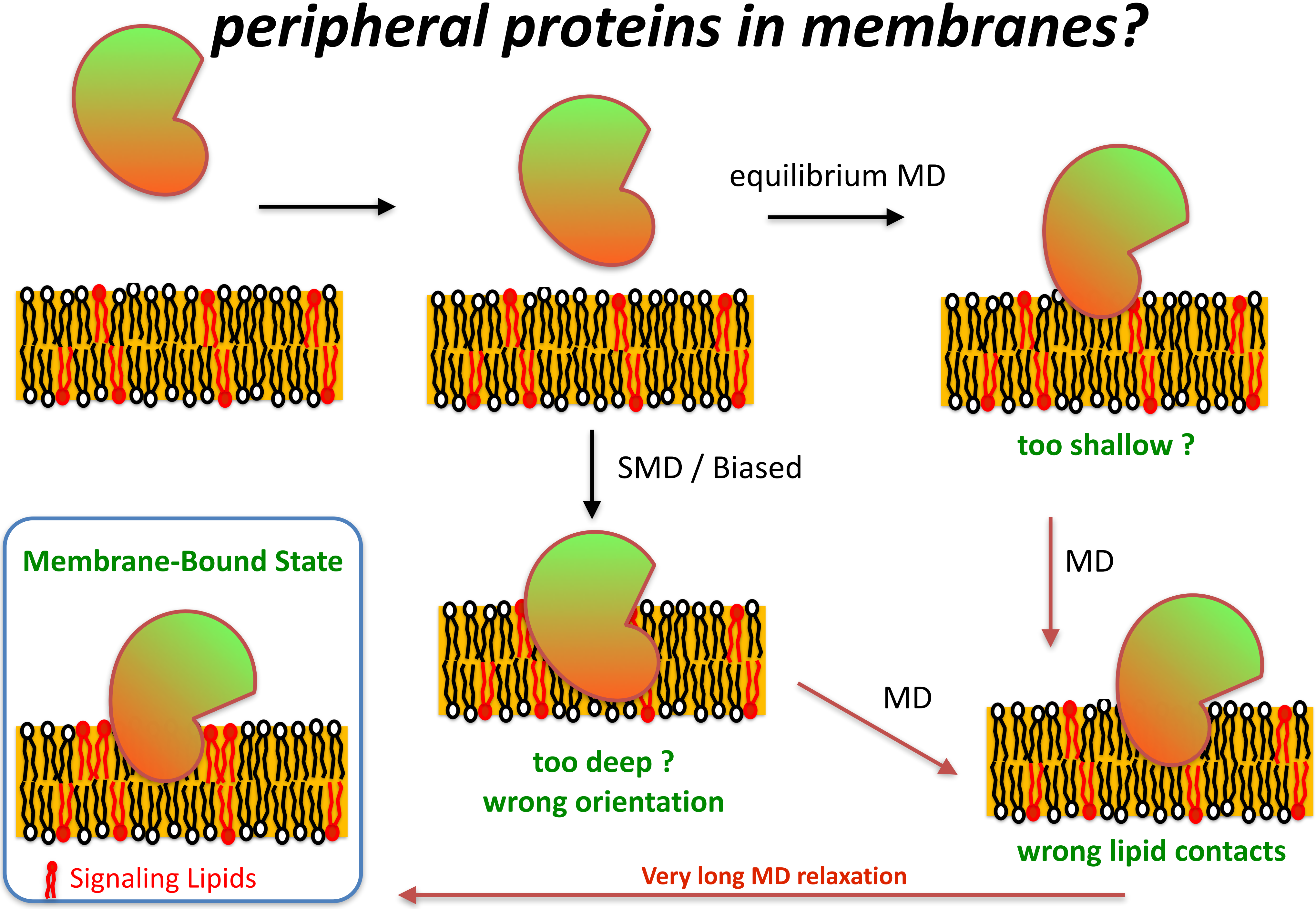
Lipid Dependent Binding and Activation





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?



equilibrium MD

too shallow ?

SMD / Biased

MD

too deep ?
wrong orientation

MD

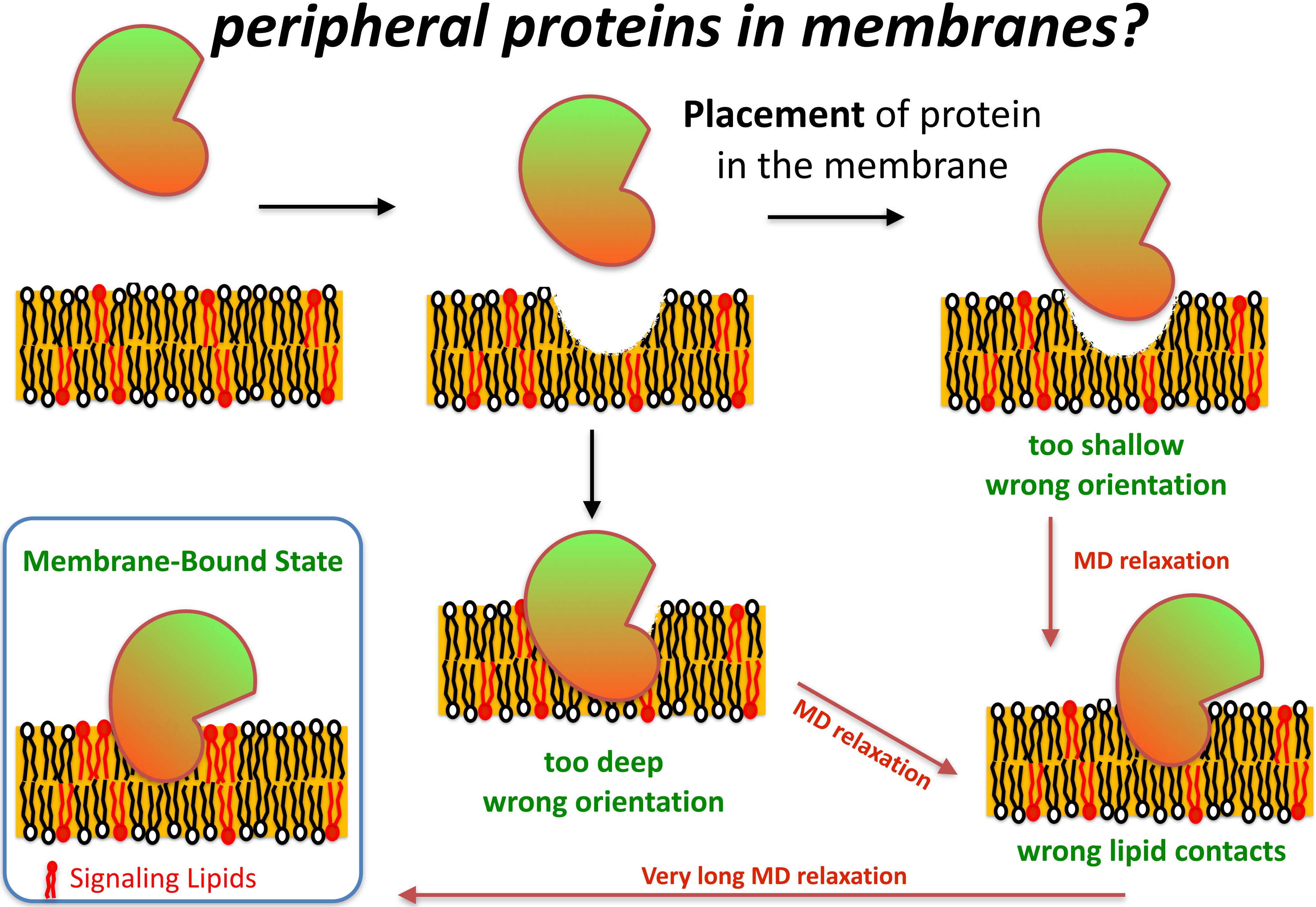
wrong lipid contacts

Very long MD relaxation

Membrane-Bound State

Signaling Lipids

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



Placement of protein
in the membrane

too shallow
wrong orientation

MD relaxation

too deep
wrong orientation

MD relaxation

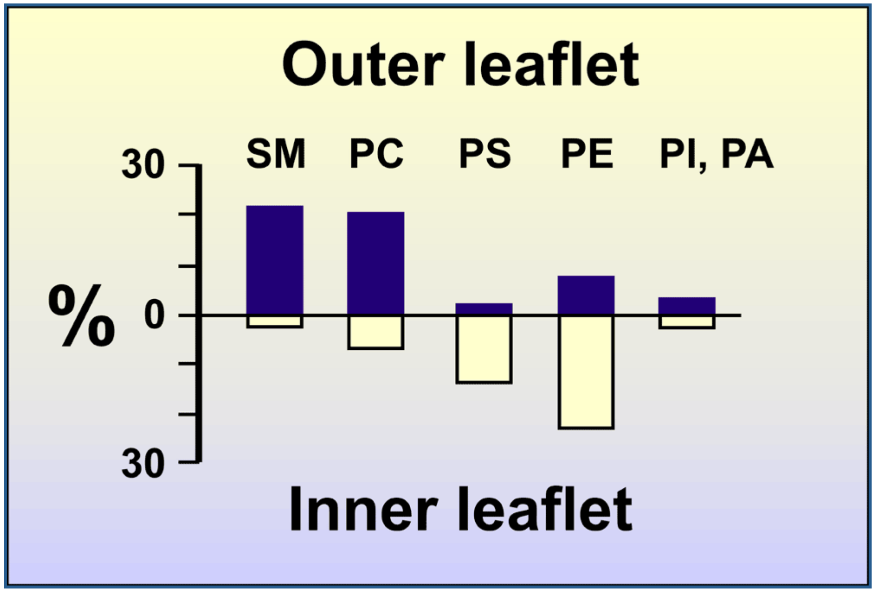
wrong lipid contacts

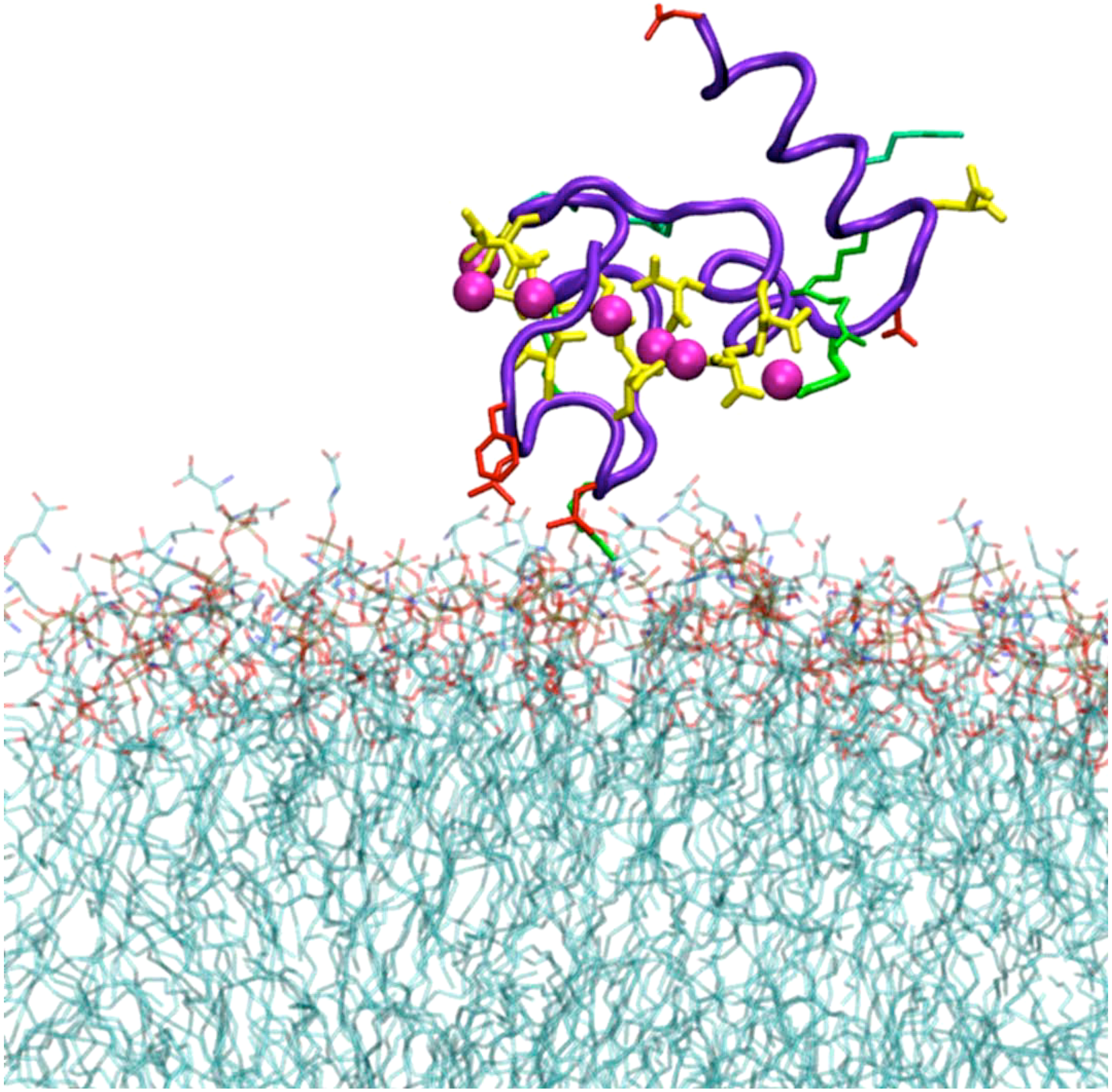
Very long MD relaxation

Membrane-Bound State

Signaling Lipids

Simulation of Binding with Full Membrane Representation





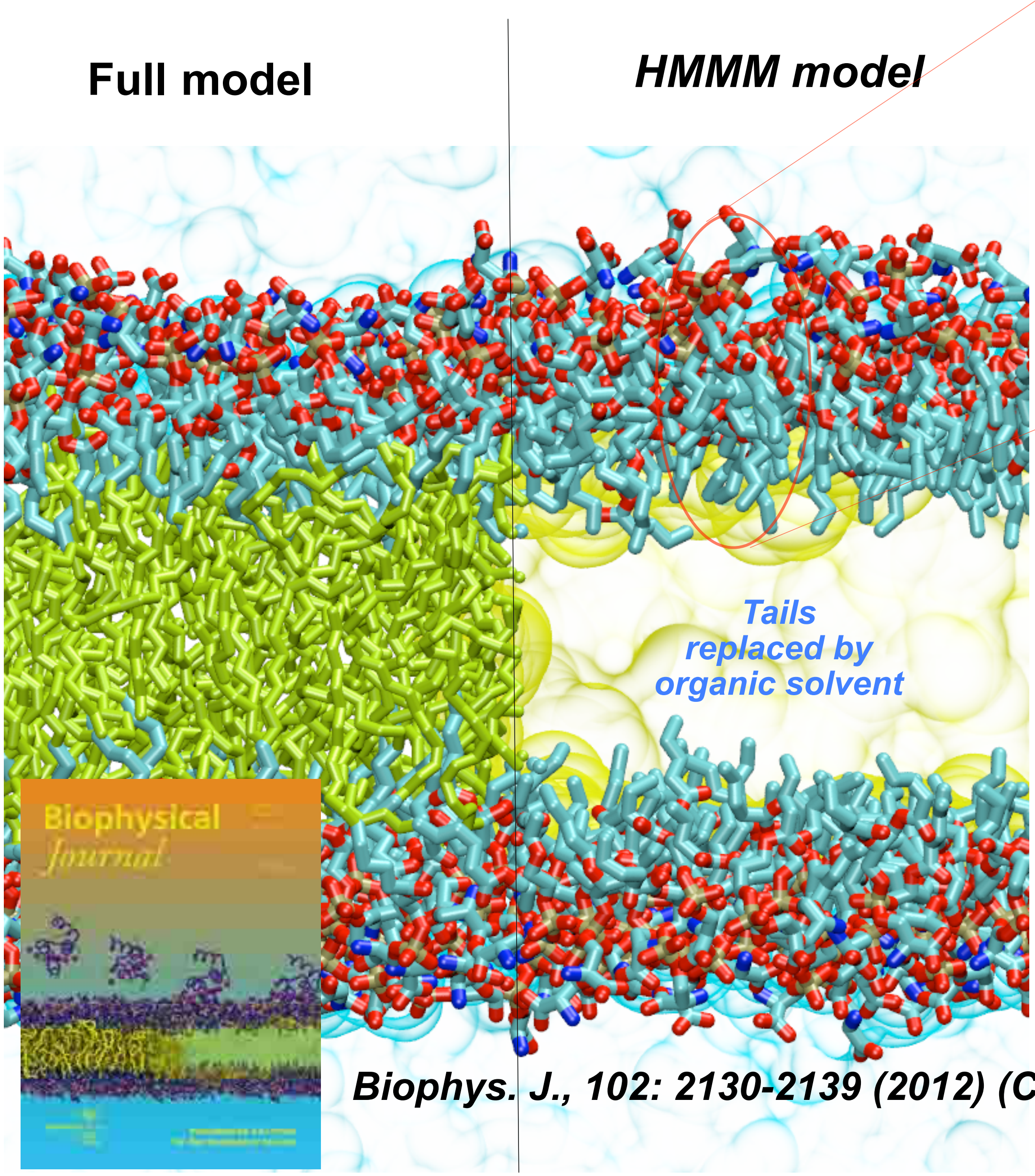
Z. Ohkubo and E. T., **Structure**, 16: 72-81 (2008)

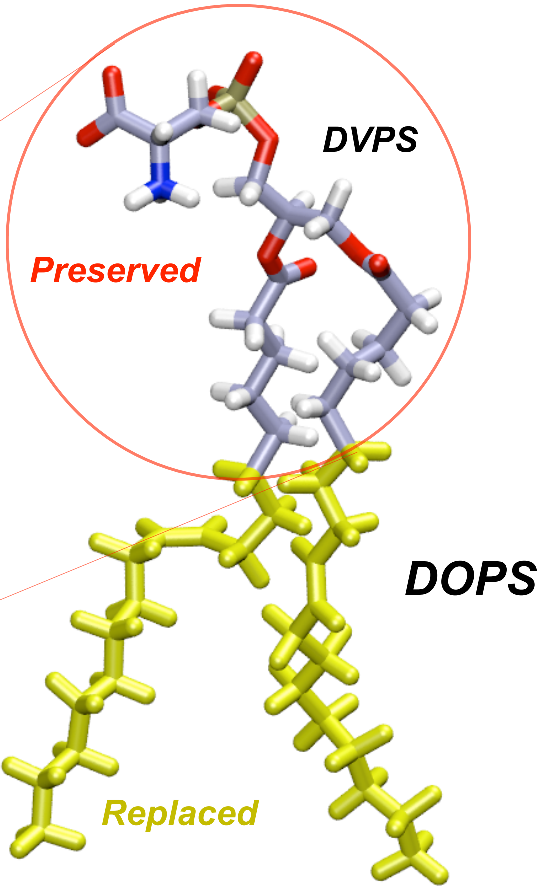
Partial list of technical problems:

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

HMMM model

Highly **M**obile **M**embrane **M**imetic **model**





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

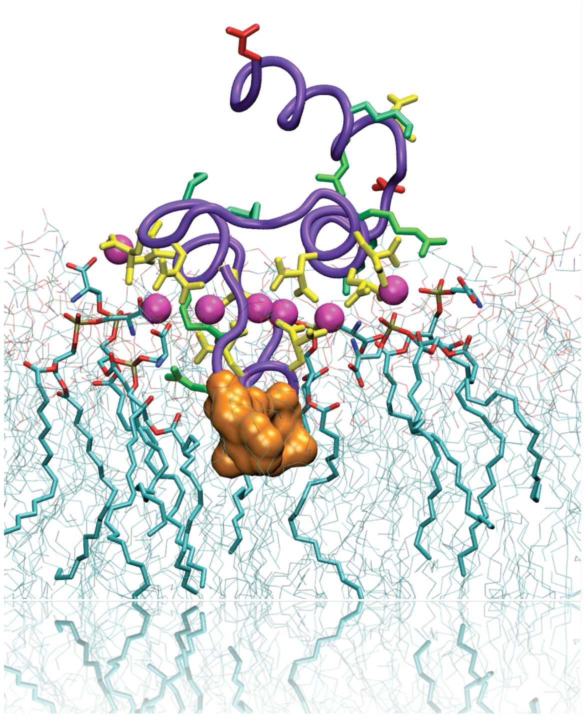
Josh Vermaas

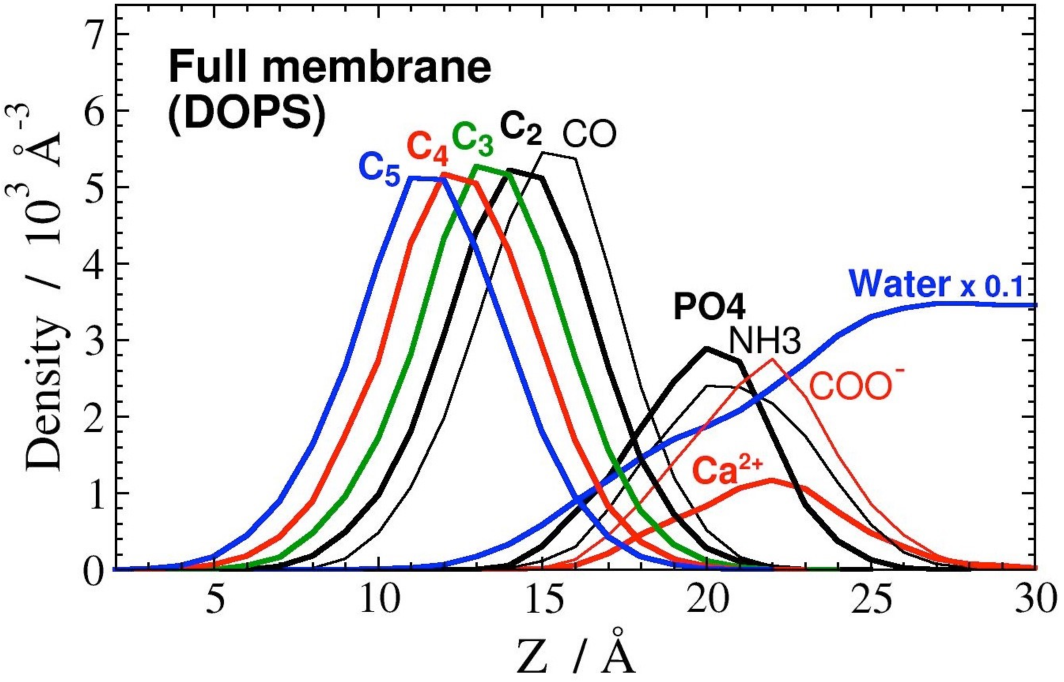
Javier Baylon

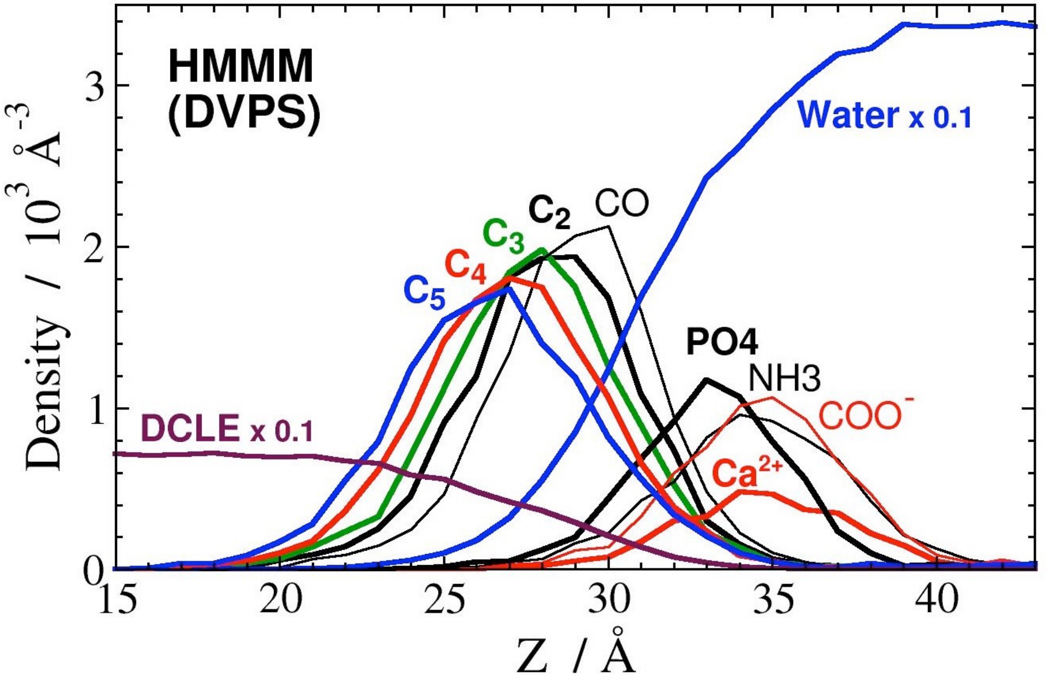
HMMM- Preserving the "Face" of the Lipid Bilayer

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

Critical for proper description of lipid protein interactions

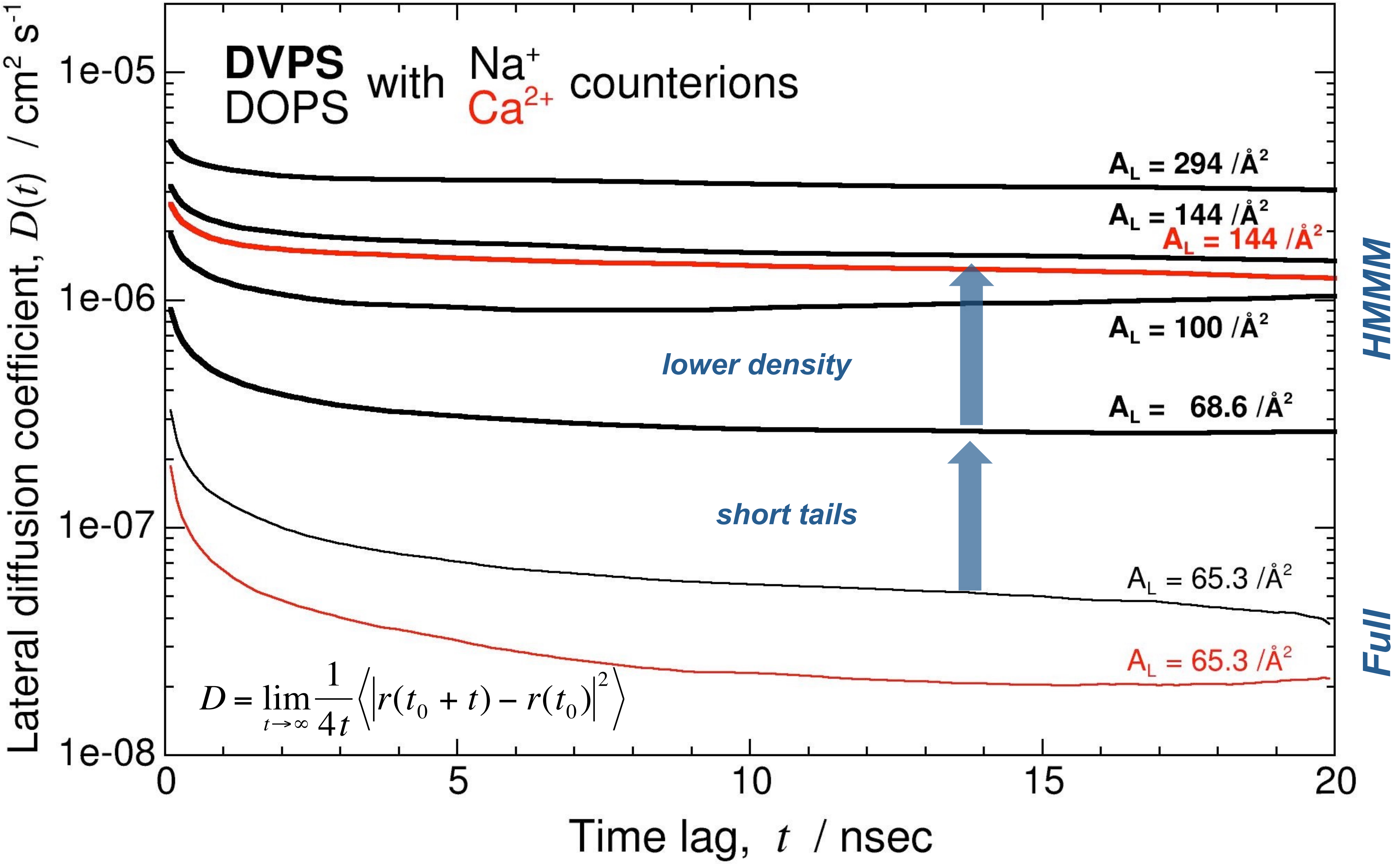






Enhanced Lipid Lateral Diffusion

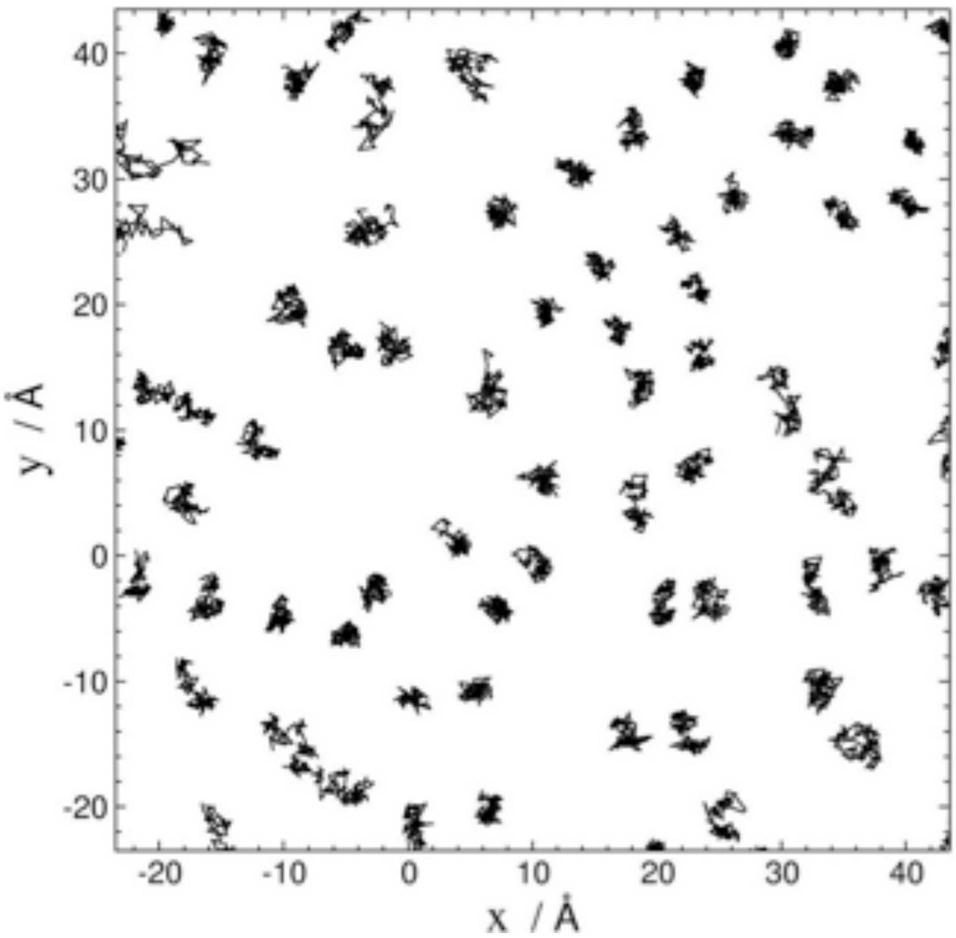
Without Compromising Atomic Details of the Headgroups



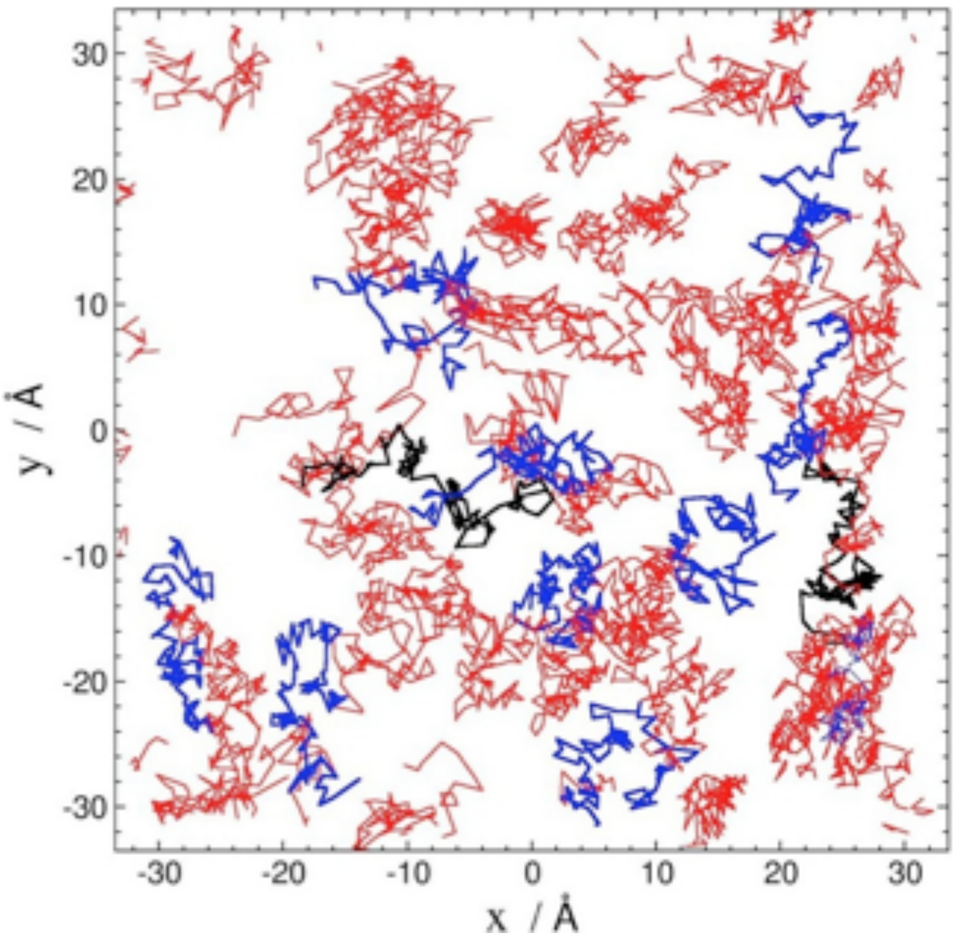
$$D = \lim_{t \to \infty} \frac{1}{4t} \left\langle \left| r(t_0 + t) - r(t_0) \right|^2 \right\rangle$$

Enhanced Lipid Lateral Diffusion

Without Compromising Atomic Details of the Headgroups



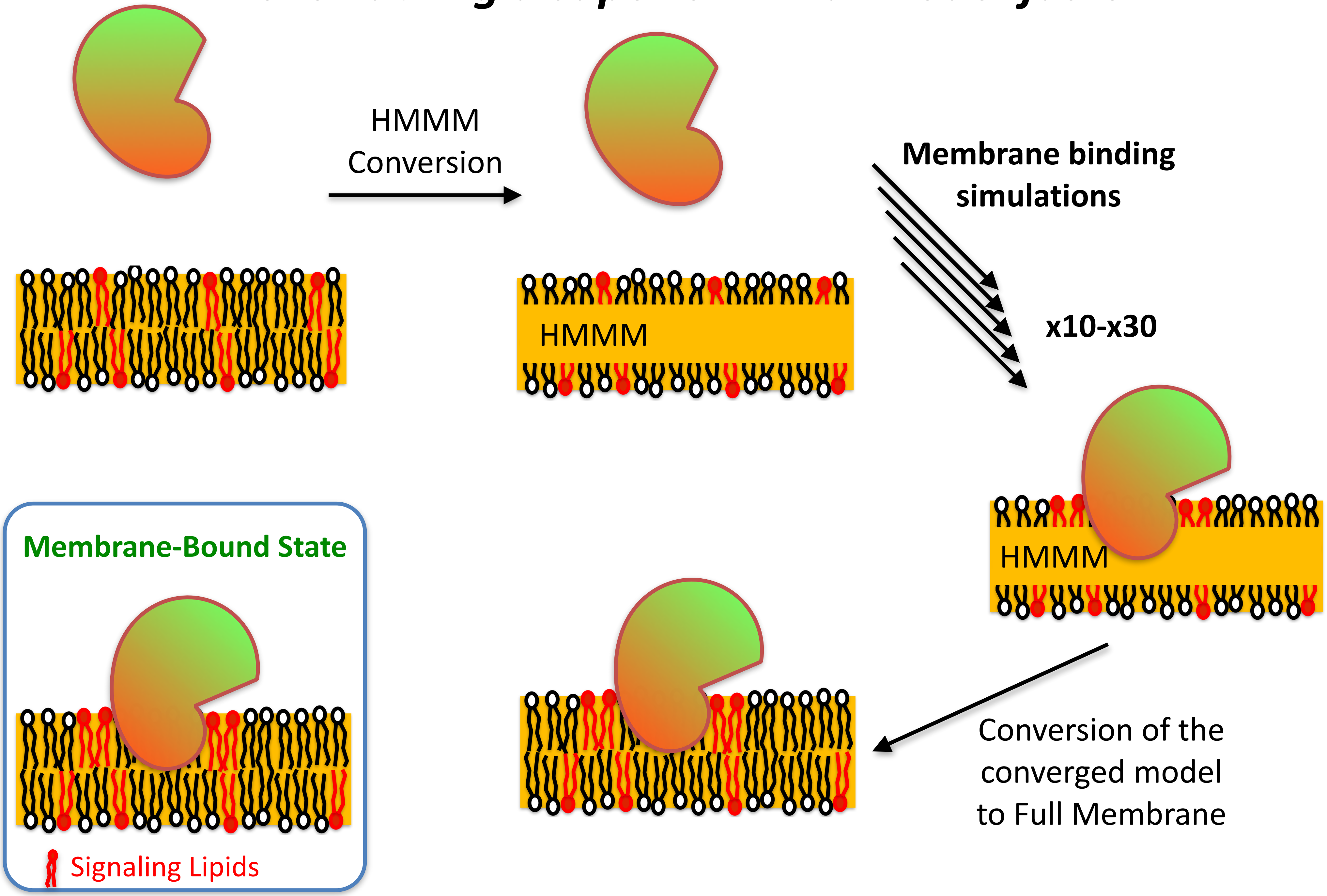
Conventional membrane (10 ns)



HMMM membrane (1 ns)

HMMM accelerated sampling of lipid-protein interactions

Constructing a superior initial model faster



HMMM Conversion

Membrane binding simulations

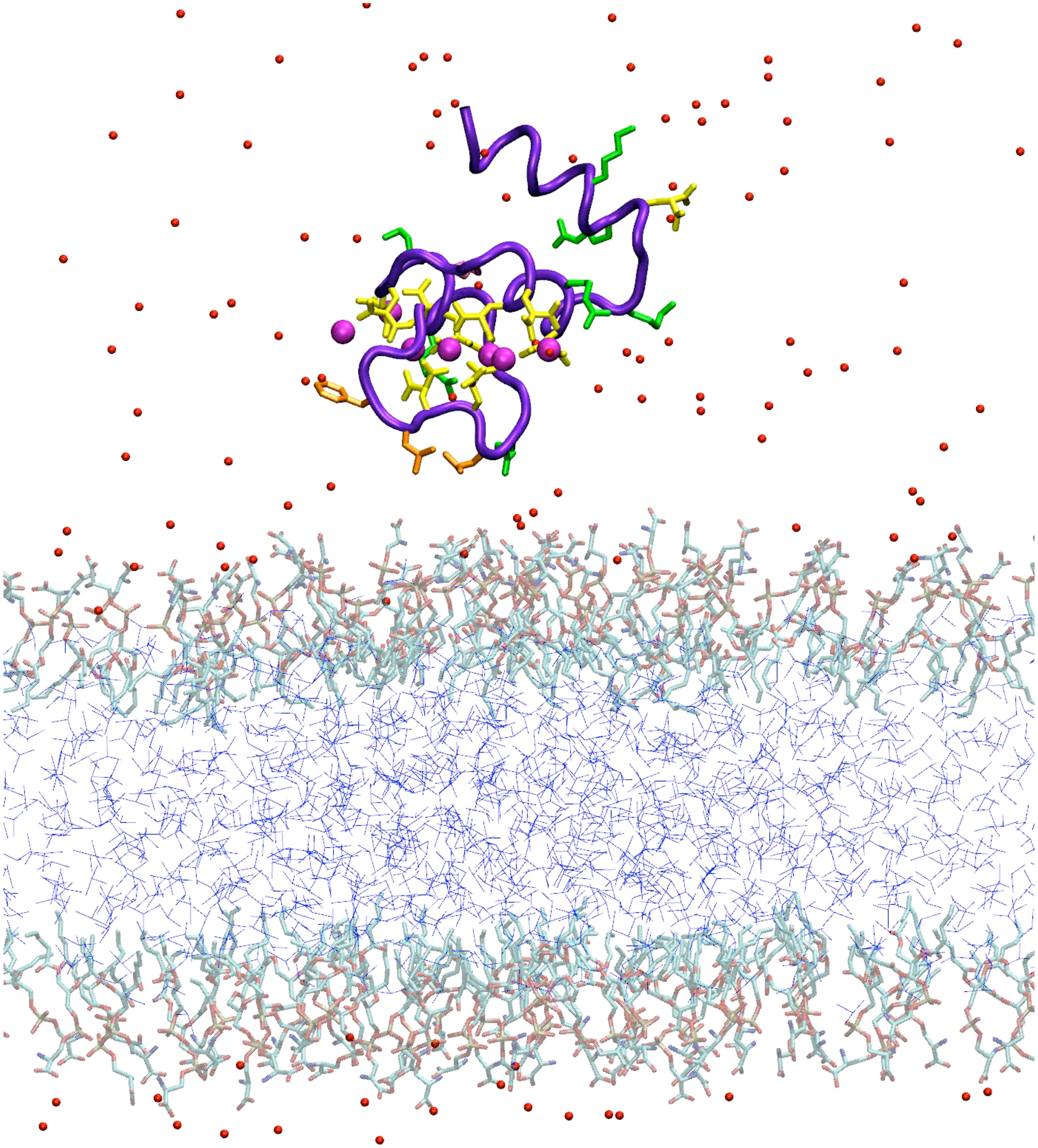
x10-x30

Conversion of the converged model to Full Membrane

Membrane-Bound State

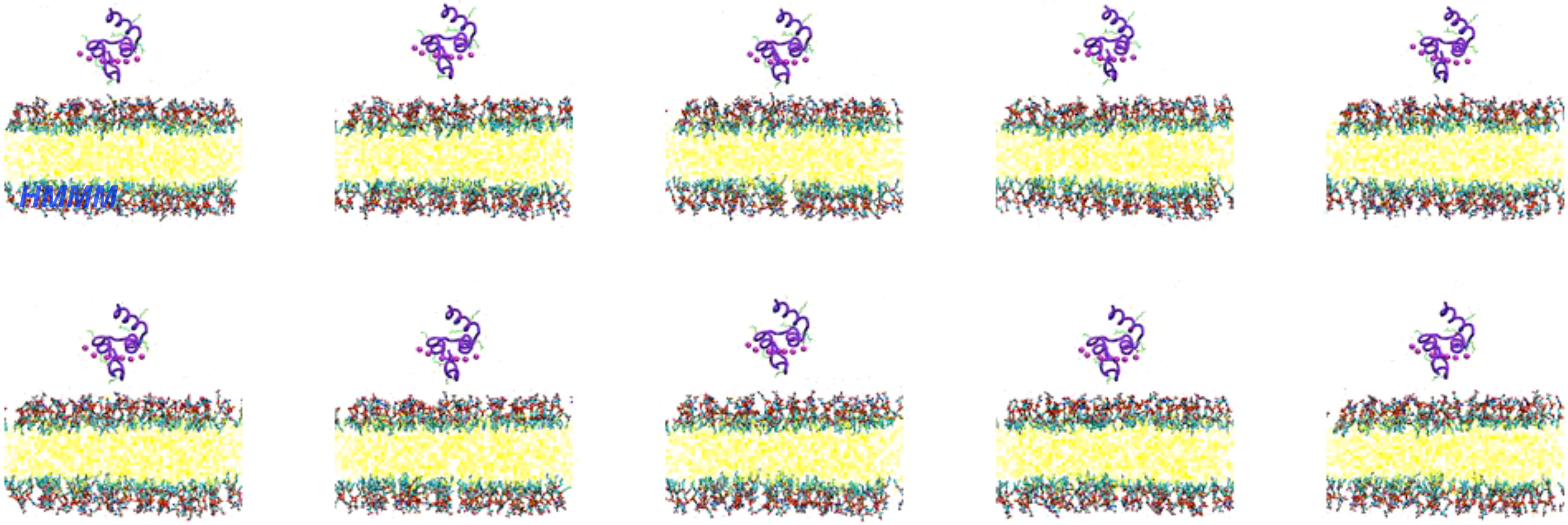
Signaling Lipids

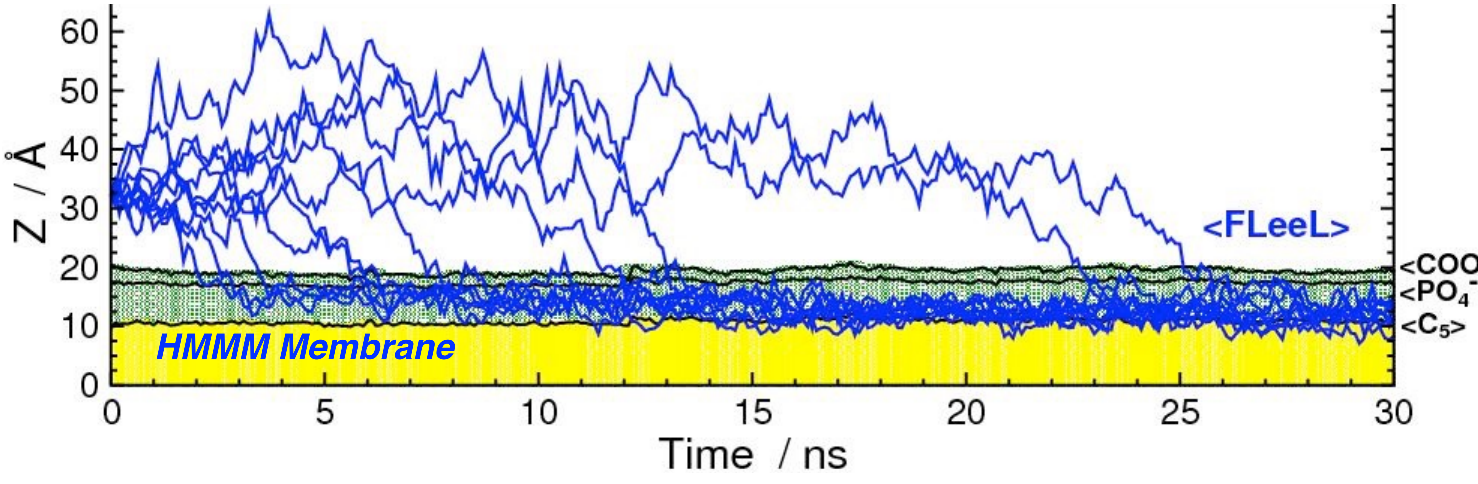
PS-Dependent Spontaneous Insertion of FVII-GLA



Zenmei Ohkubo

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

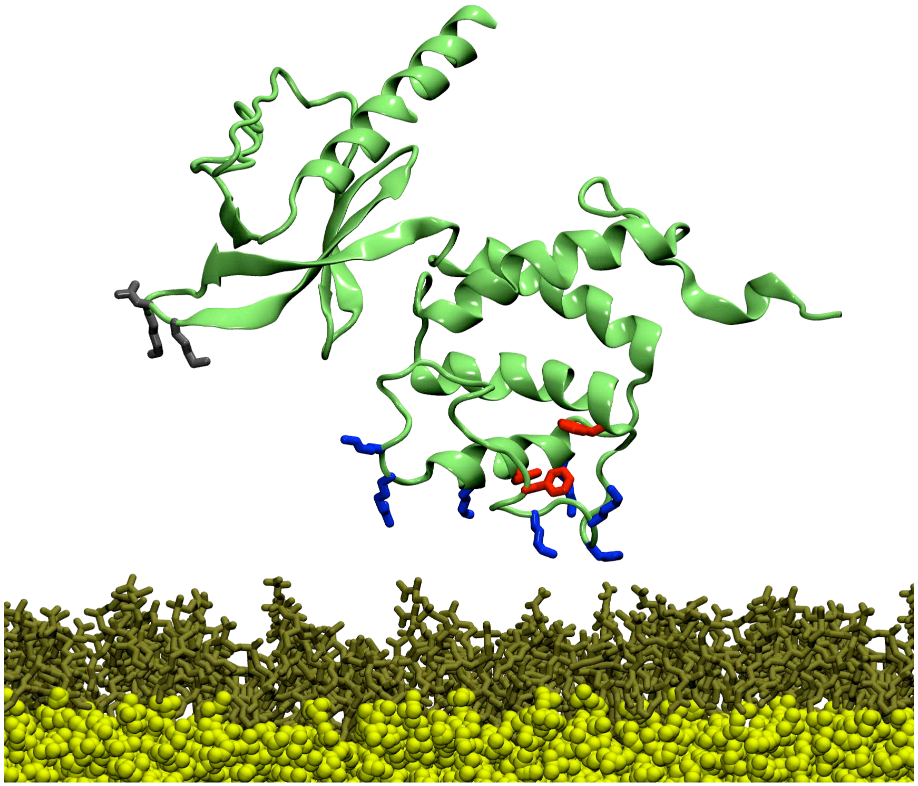




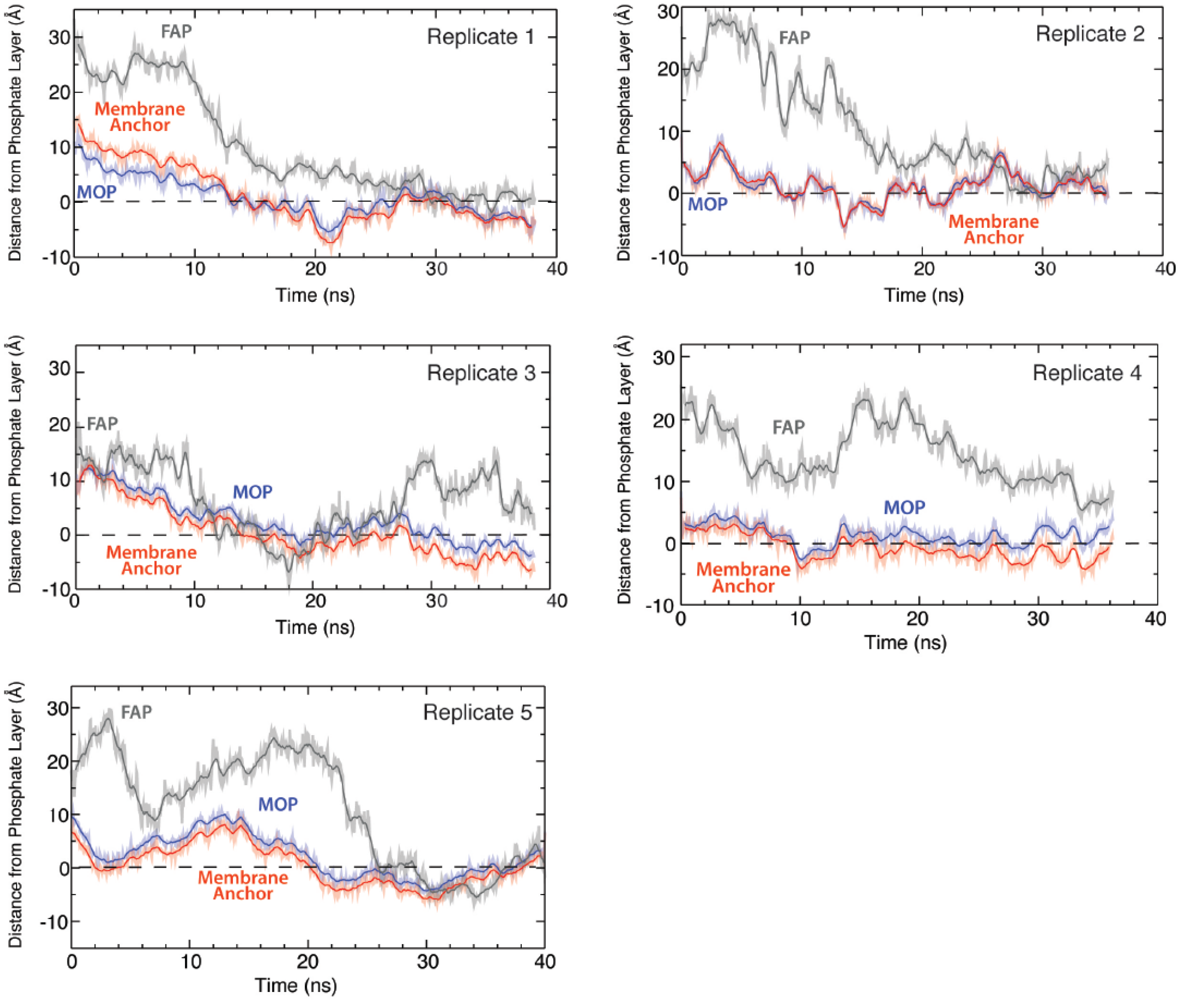
Z. Ohkubo, …, E.T., **Biophys. J.**, 102: 2130-2139 (2012) (Cover Article)

PS-Dependent Membrane Binding of Talin

Mark Arcario



Five independent membrane binding simulations

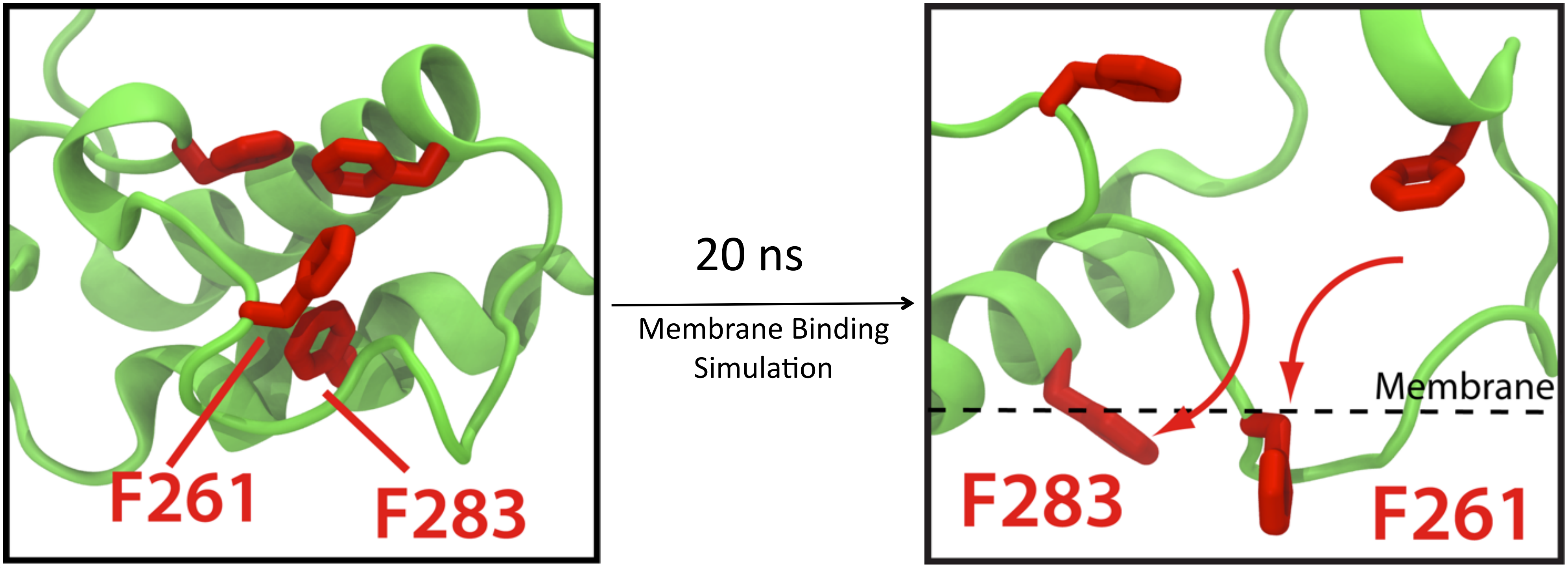


Final model converted to **full membrane**

Stable in 100 ns simulations

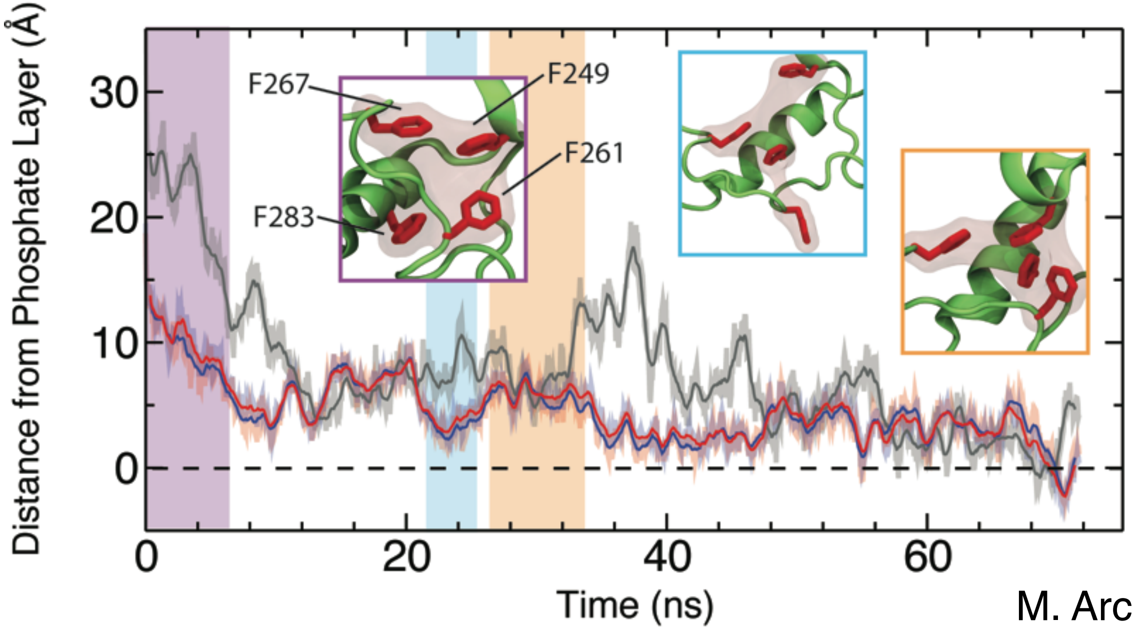
M. Arcario and ET, **Biophys. J.**, 107: 2059–2069 (2014).

Revealing the ***Hydrophobic Anchor***



20 ns

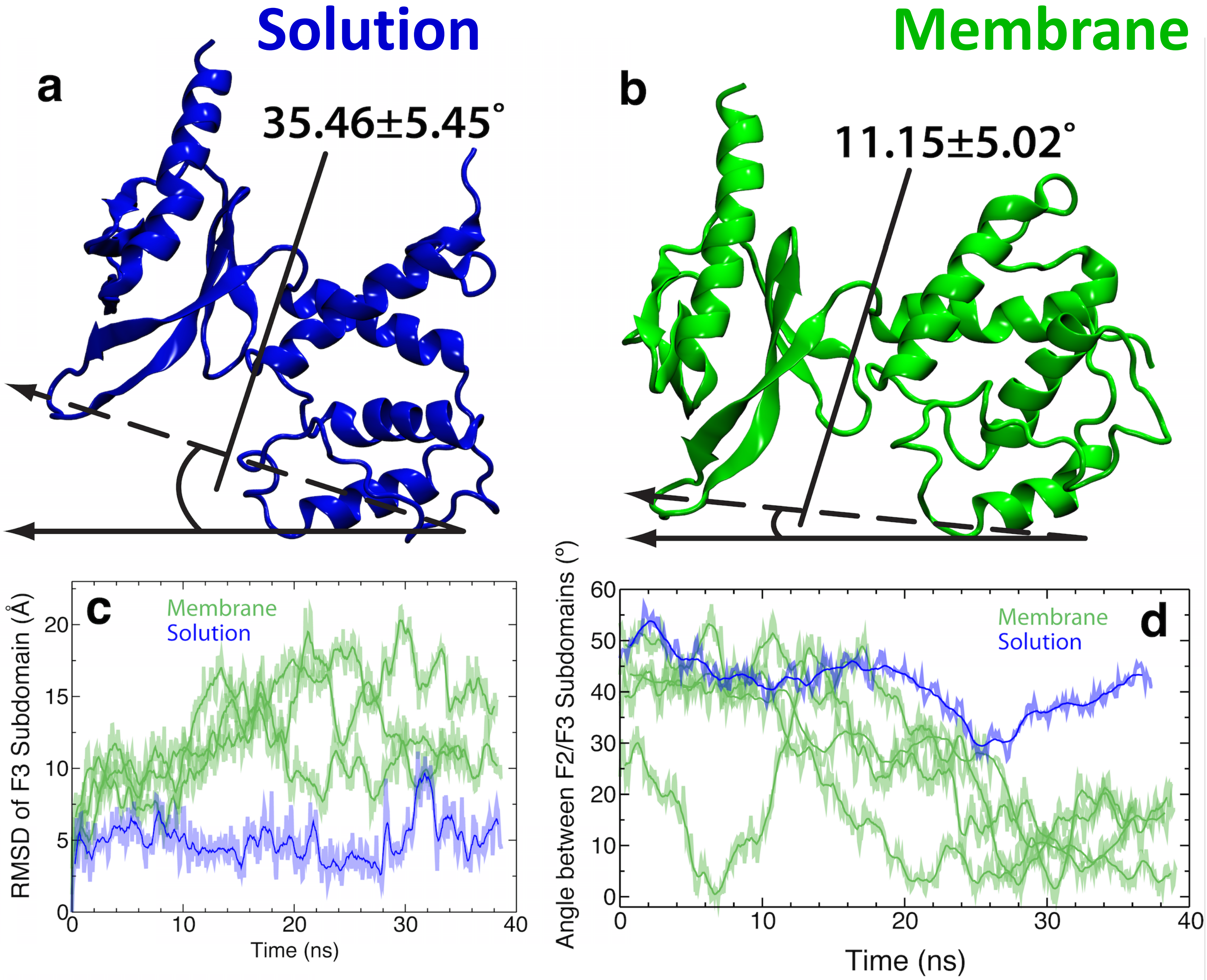
Membrane Binding Simulation



- 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

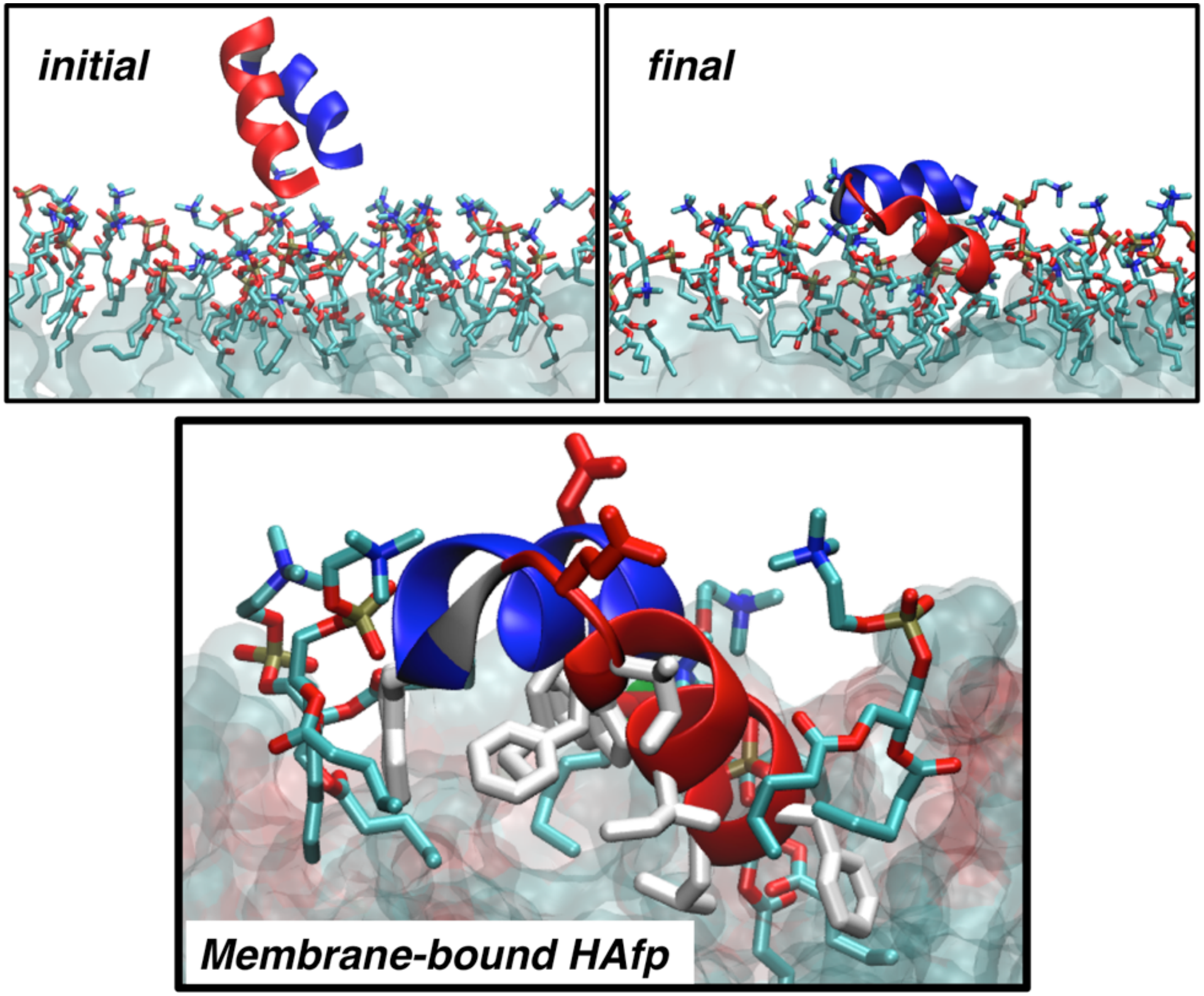
M. Arcario and ET, **Biophys. J.**, 107: 2059–2069 (2014).

Membrane Induced Domain Rearrangement of Talin



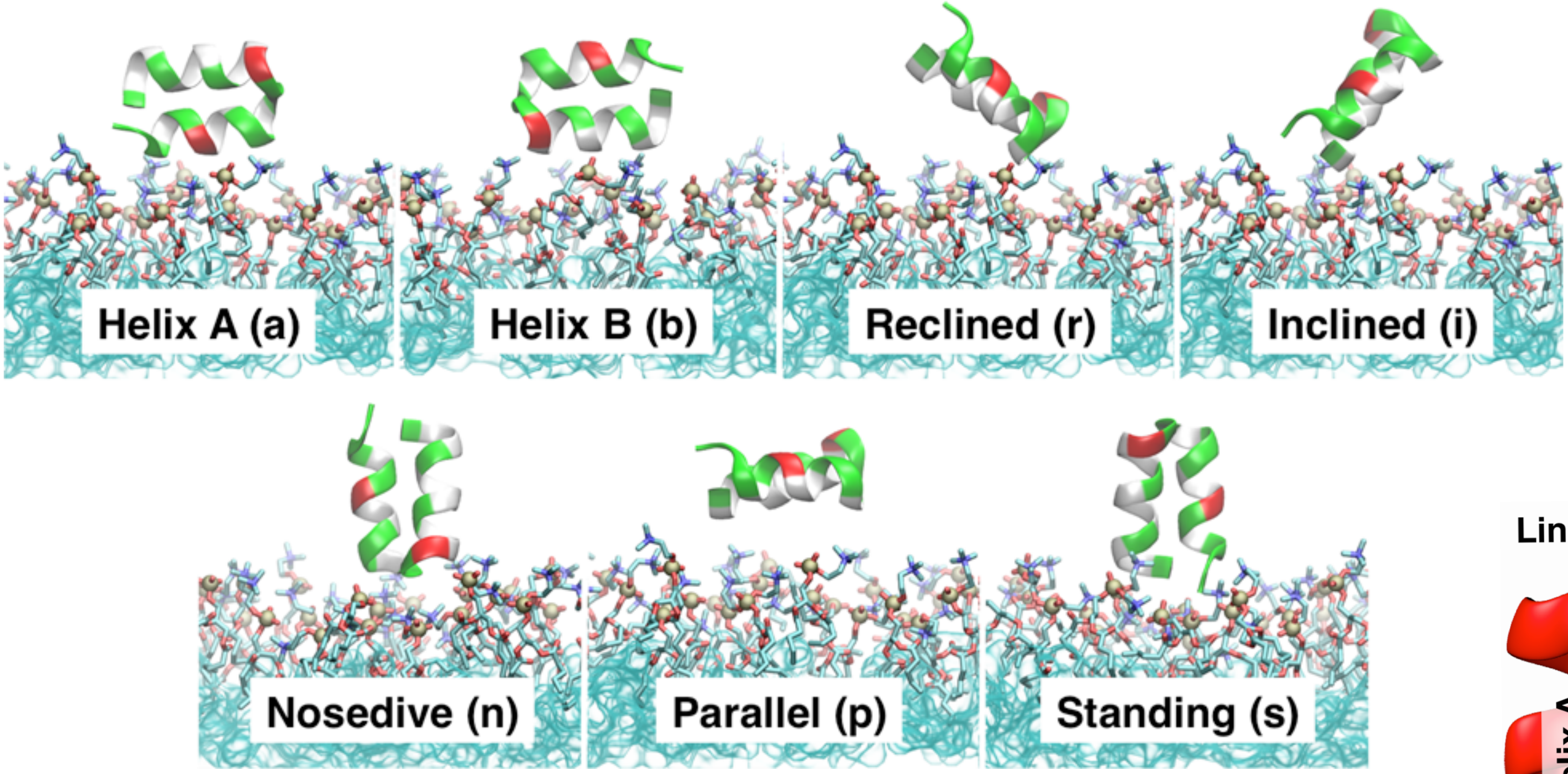
M. Arcario and ET, **Biophys. J.**, 107: 2059–2069 (2014).

Membrane Binding of Influenza Hemagglutinin Fusion Peptide

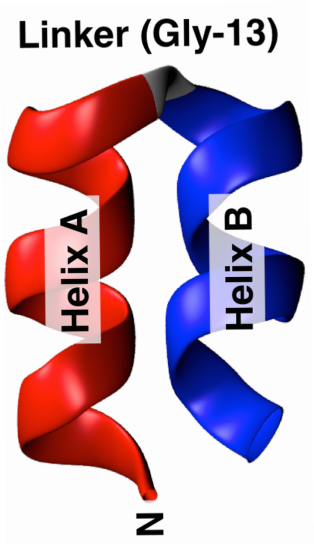


J. Baylon and E. T., **J. Phys. Chem.B**, 2015, in press.

Membrane Binding of Influenza Hemagglutinin Fusion Peptide



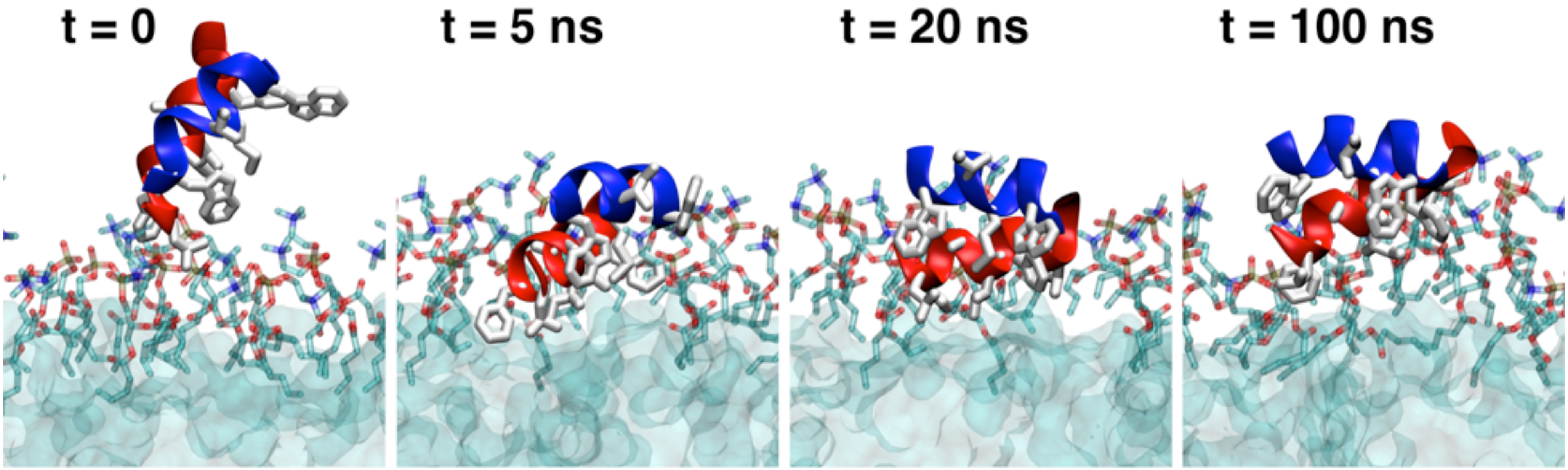
7 different initial orientation
each simulated 3 times



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

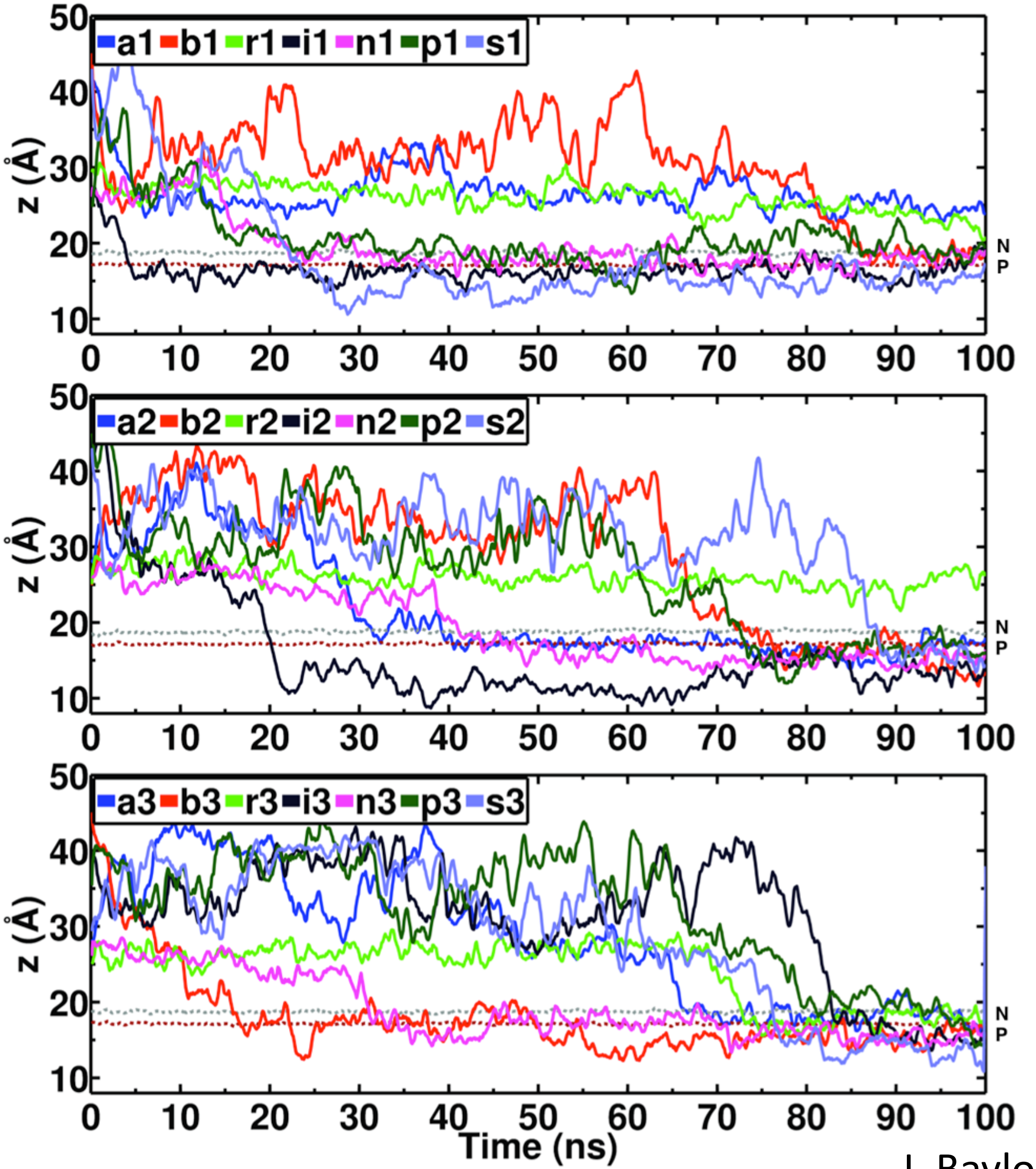
Membrane Binding of Influenza Hemagglutinin Fusion Peptide

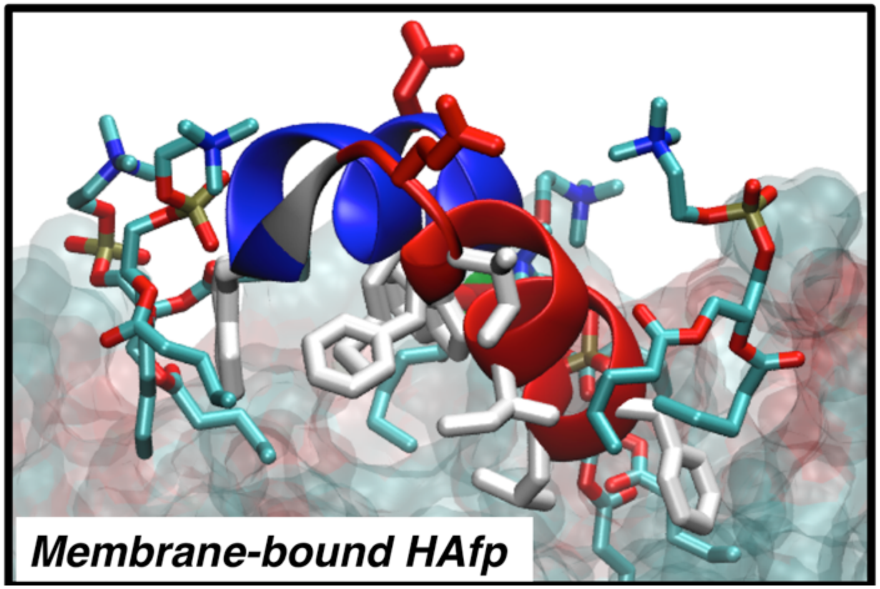
Spontaneous binding observed in the majority of the simulations:
    21 independent simulations starting from 7 different orientations



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

Remarkable convergence of membrane binding simulations

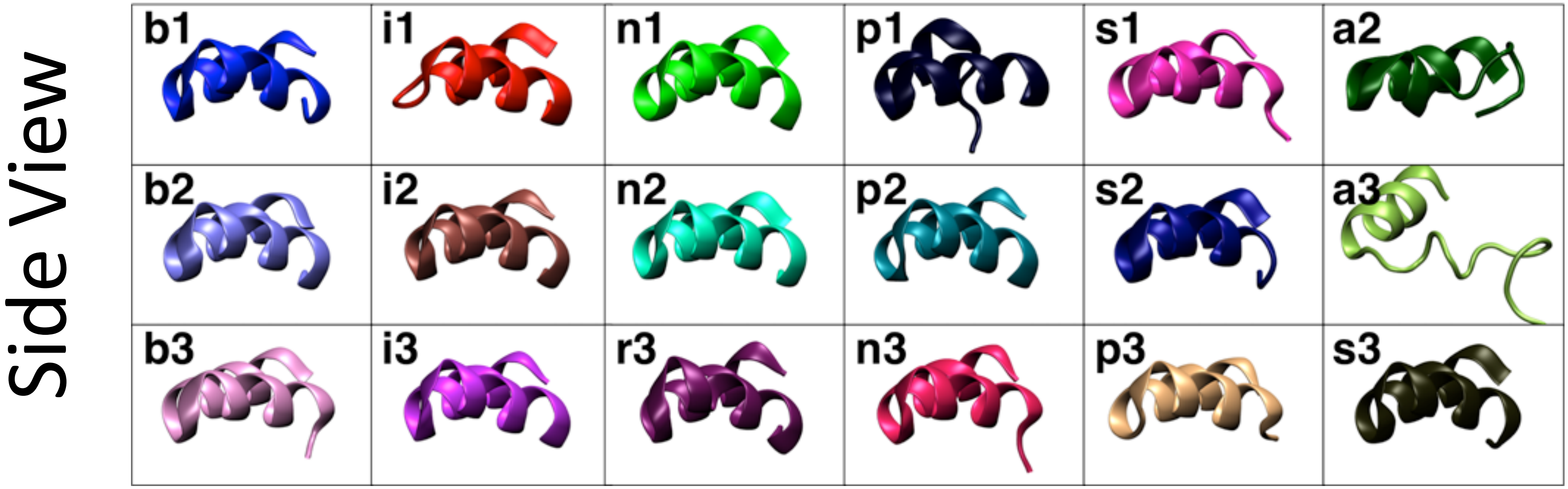


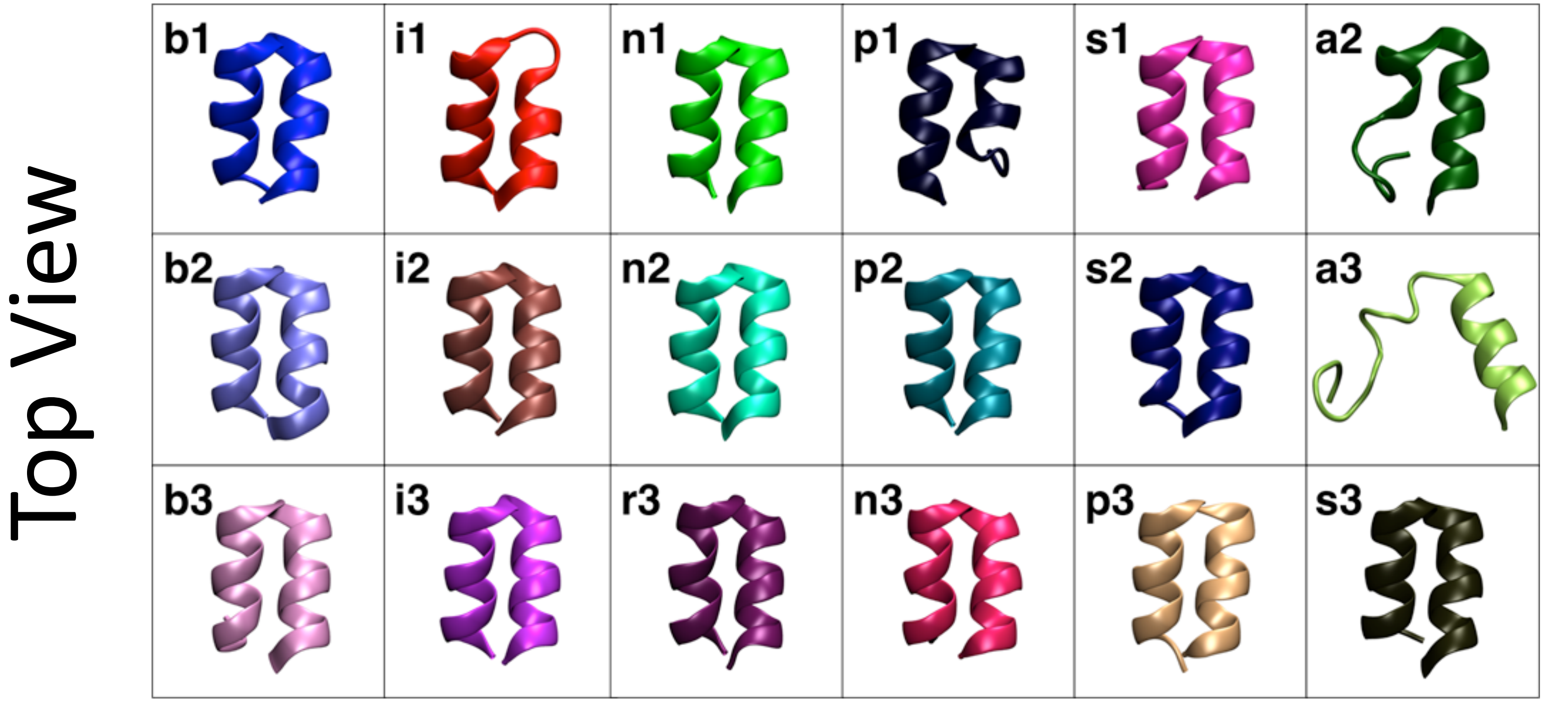


Membrane-bound HAfp

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

Remarkable convergence of membrane binding simulations

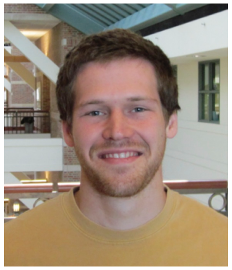




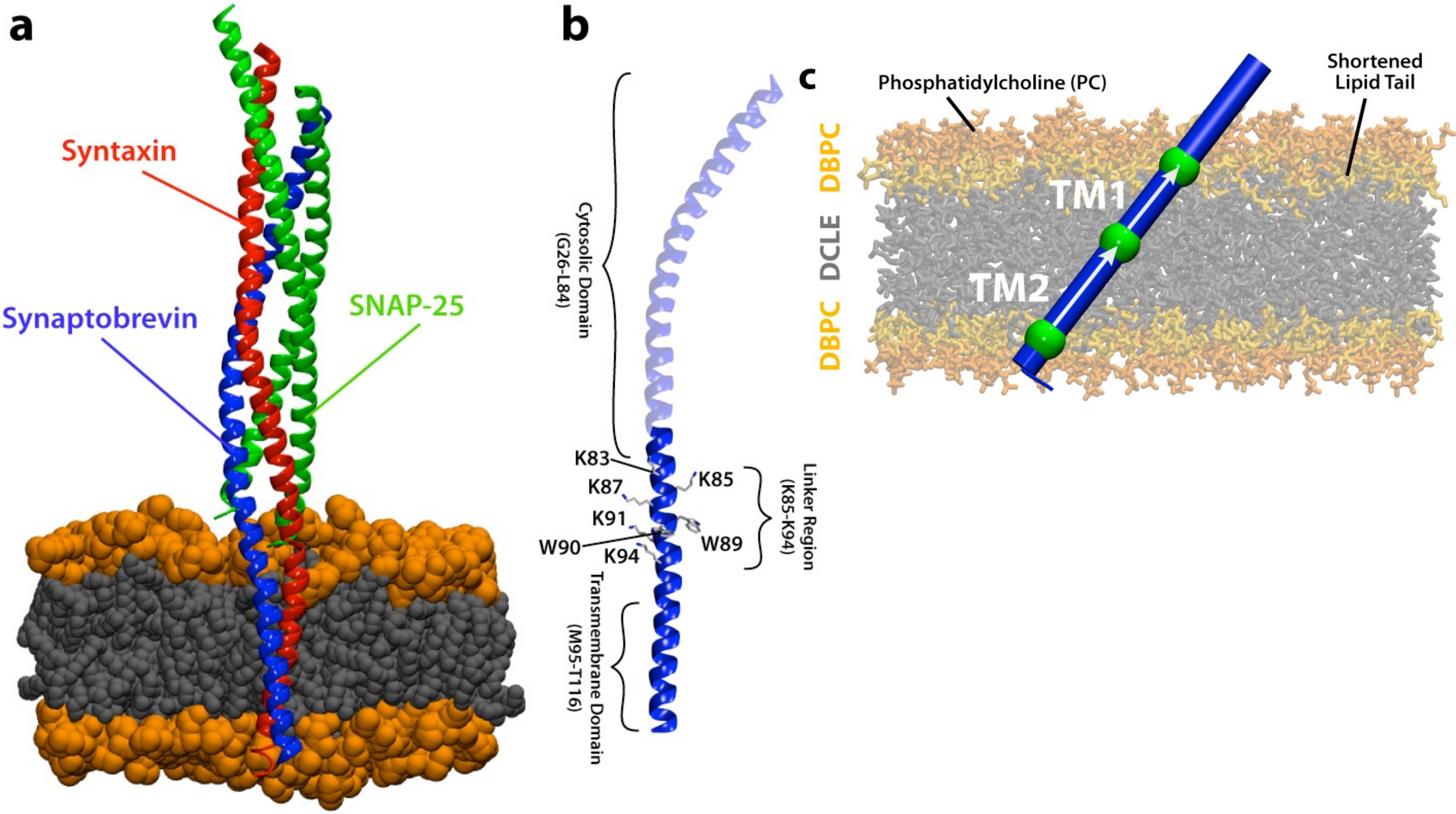
J. Baylon and E. T., **J. Phys. Chem.B**, 2015.

Robust Tilting of the Anchor Domain in Snare Protein Synaptobrevin

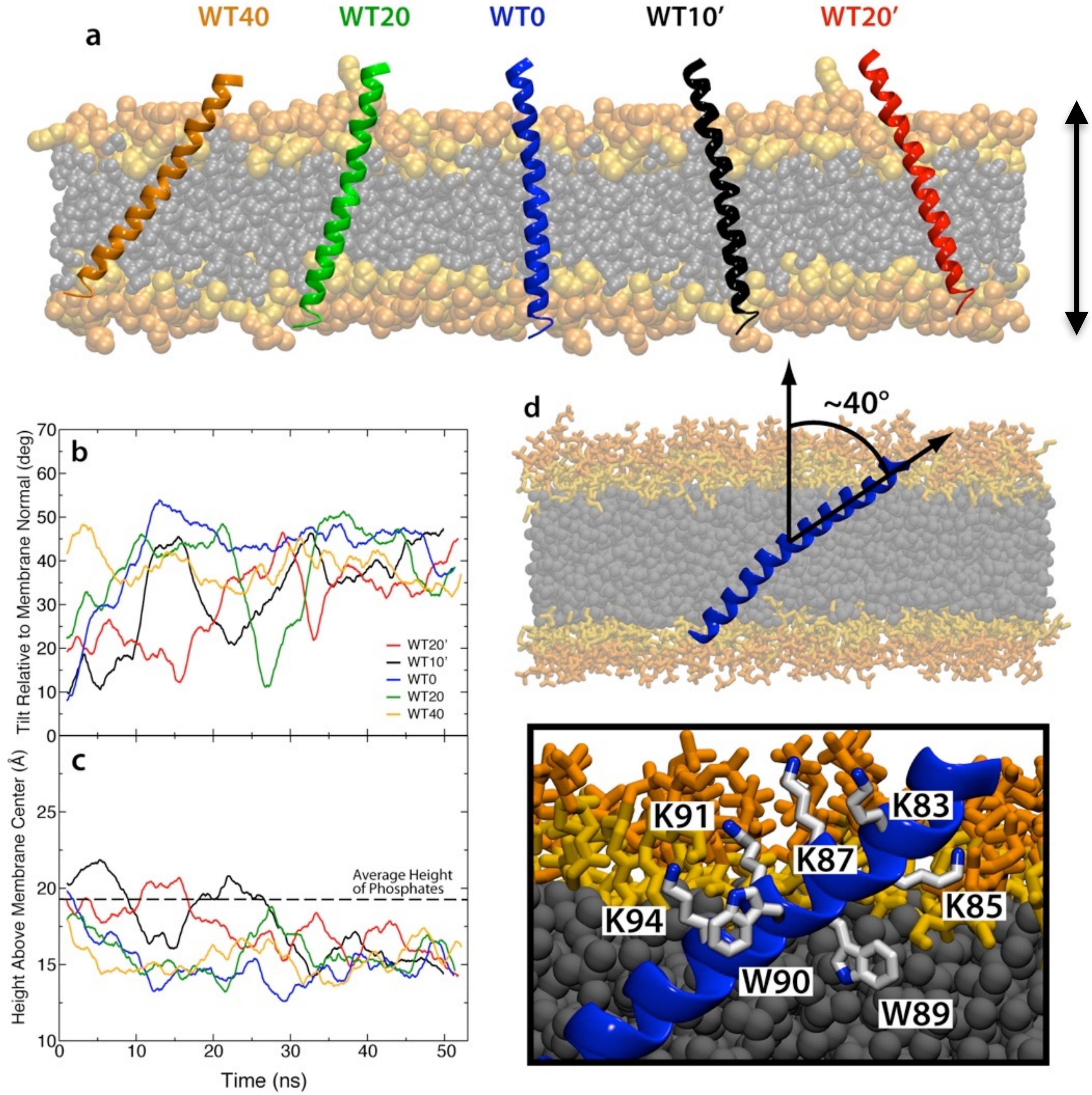
Mark Arcario



Andrew Blanchard



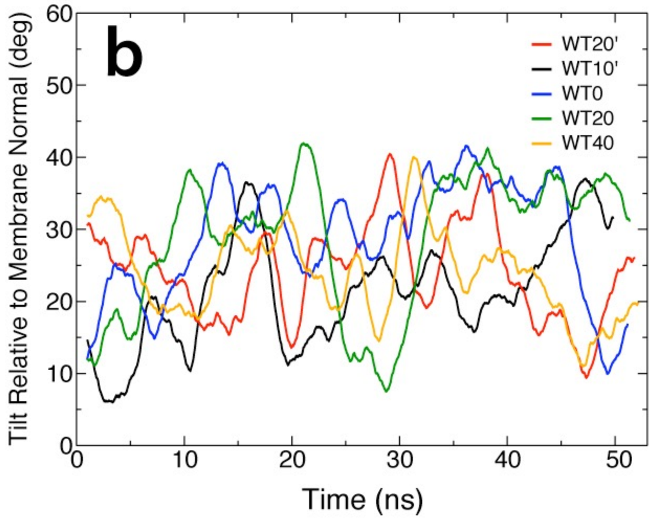
Robust Tilt Observed in Synaptobrevin

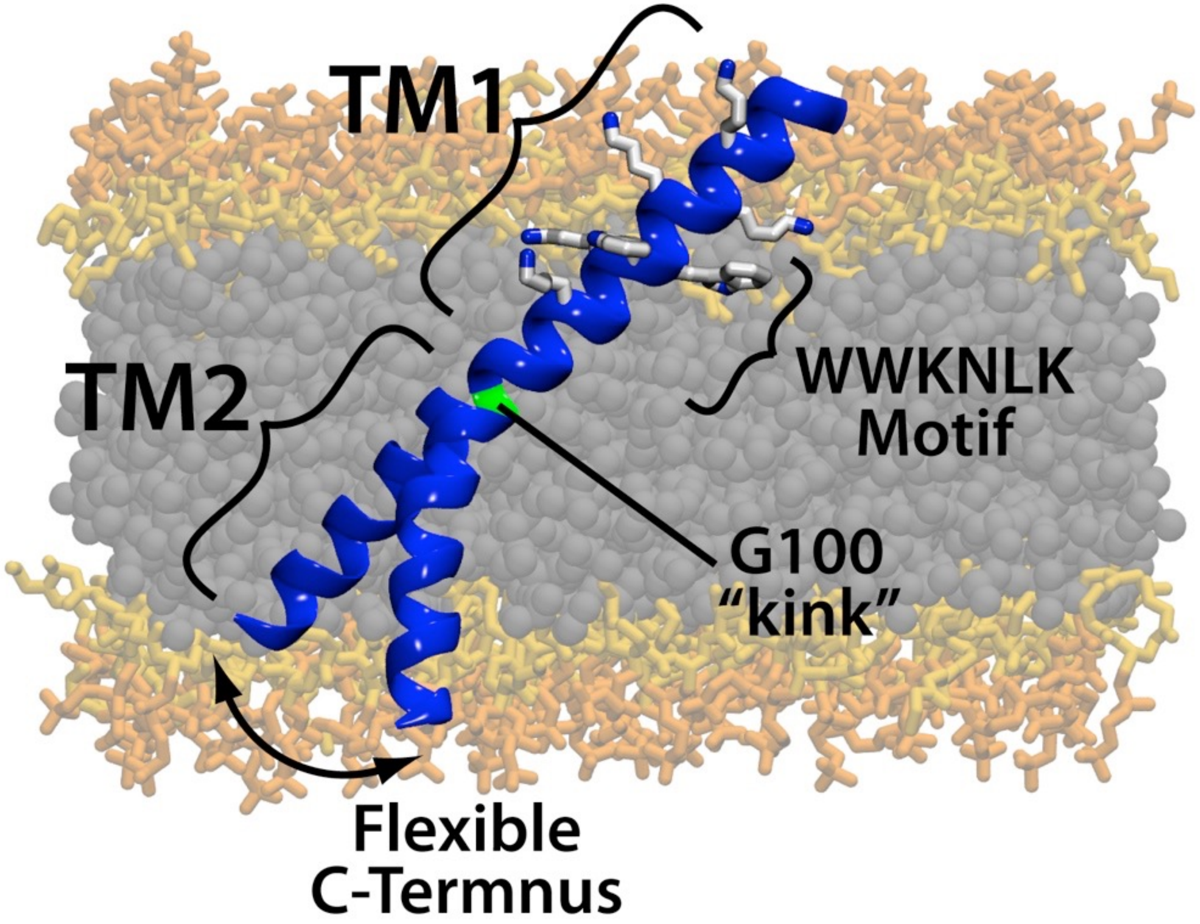


Membrane thickness mildly restrained:
one carbon/tail
k = 0.05 kcal/mole/A2

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

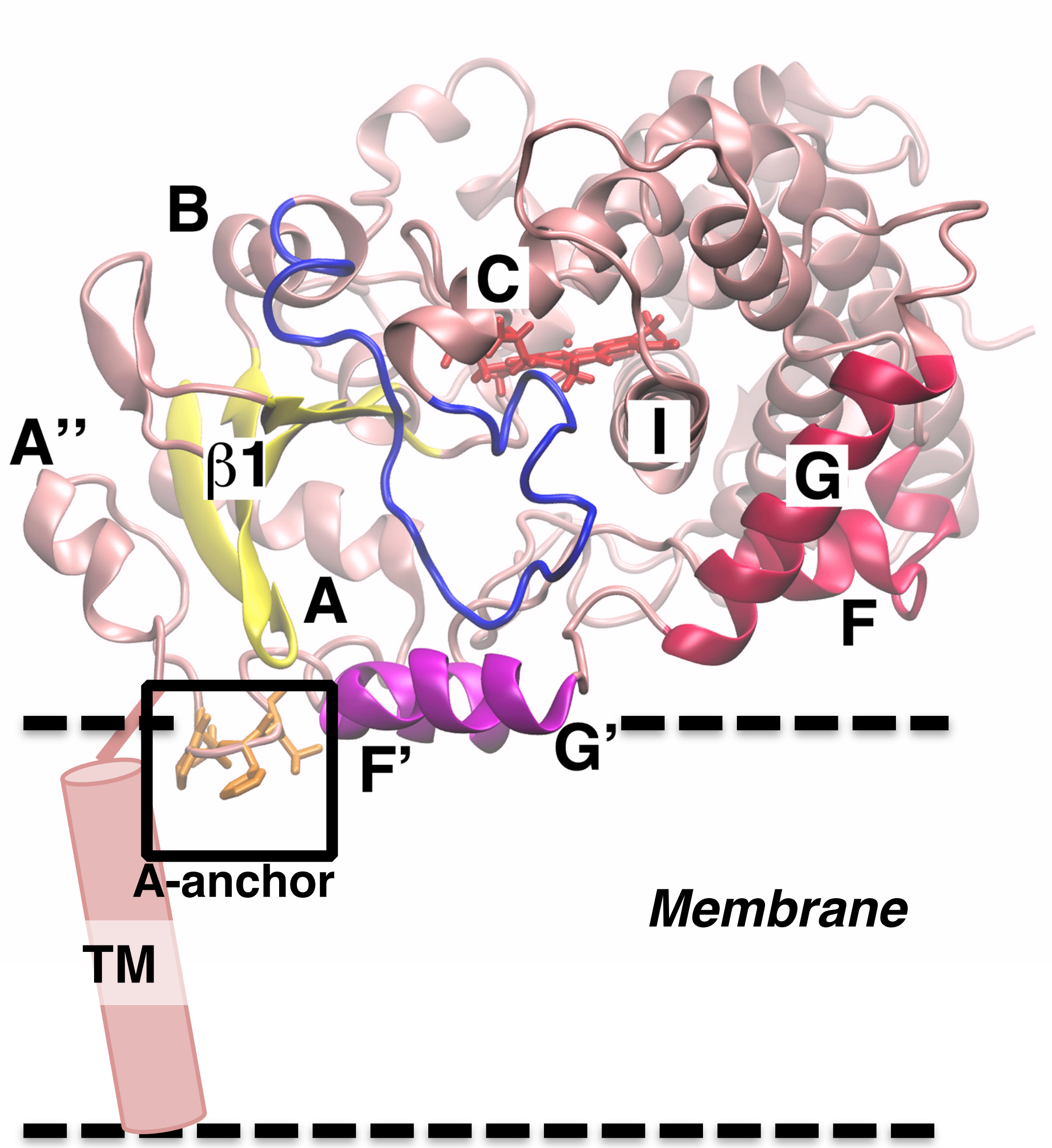
Identifying a **Hinge**





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

Cytochrome P450 3A4 (CYP3A4)

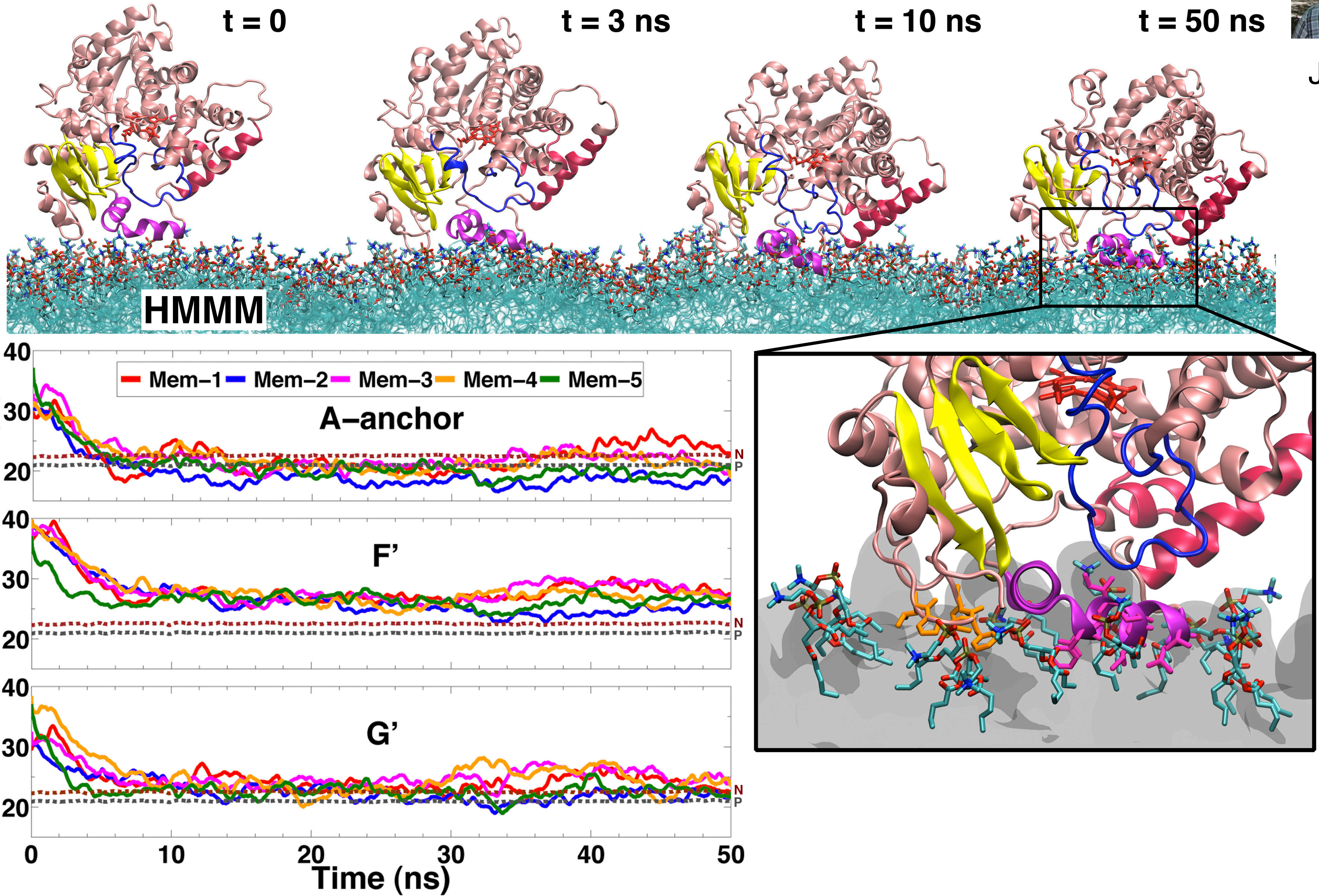


Yano *et al.*, J Biol Chem, 279: 38091-38094, 2004

- 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

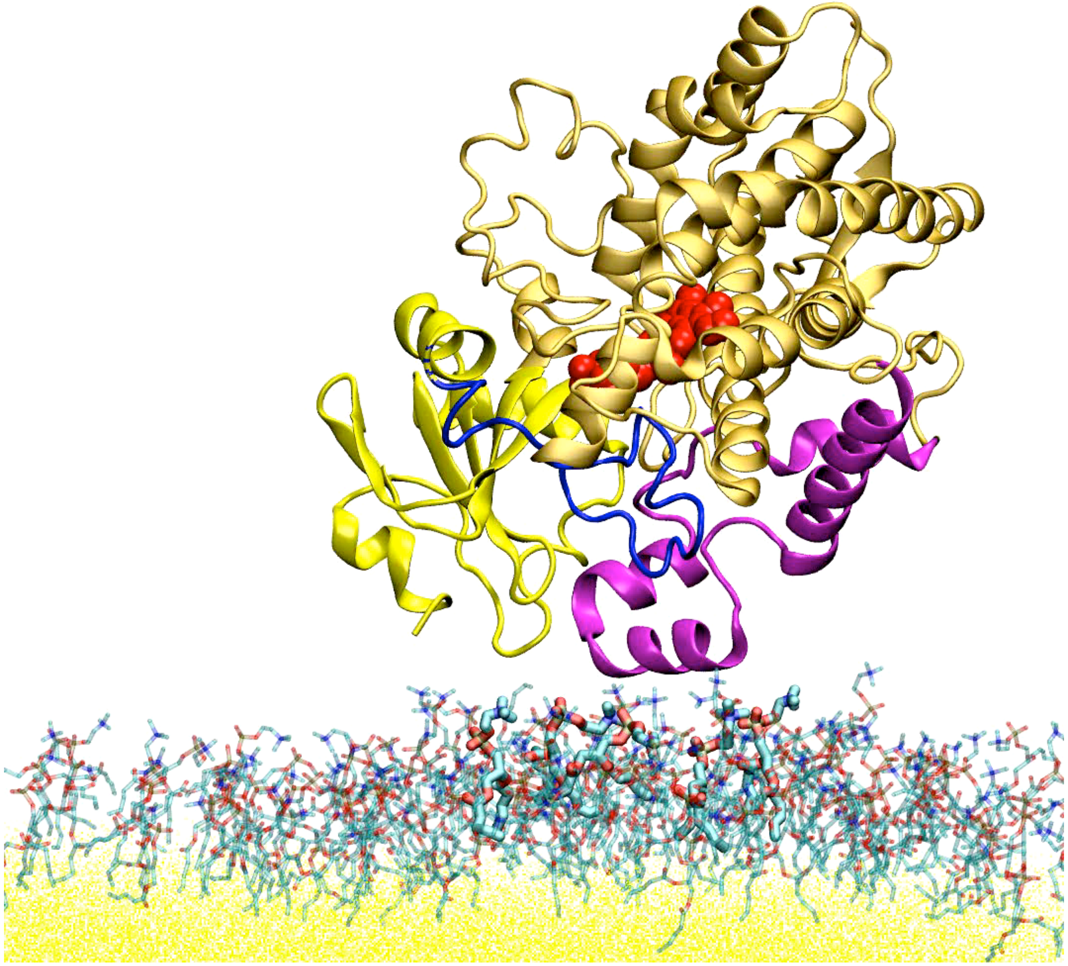
Javier Baylon

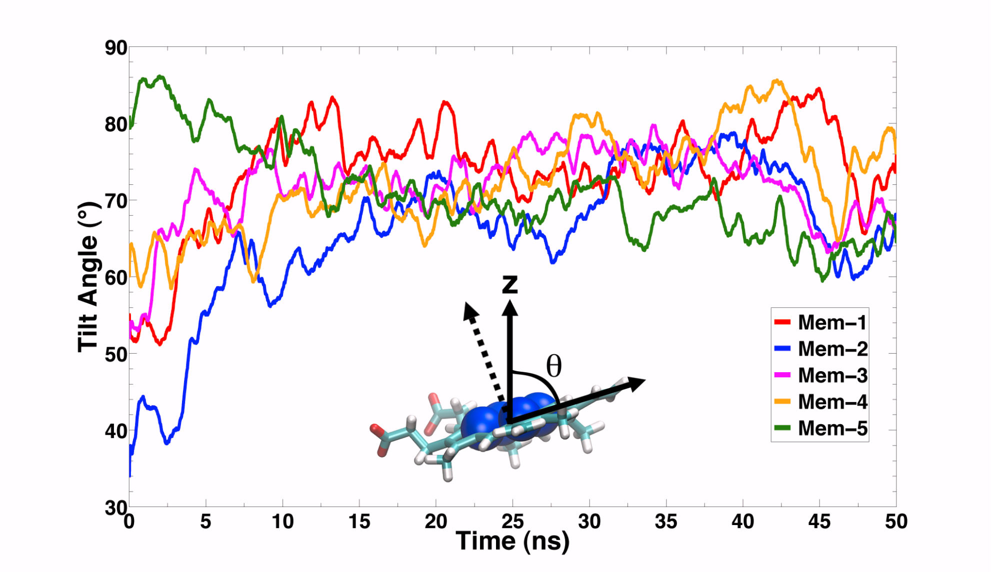


J. Baylon, I. Lenov, S. Sligar and ET, **JACS**, 135: 8542–8551 (2013)

Insertion and Membrane-Induced Conformational Change of Cytochrome P450

Javier Baylon



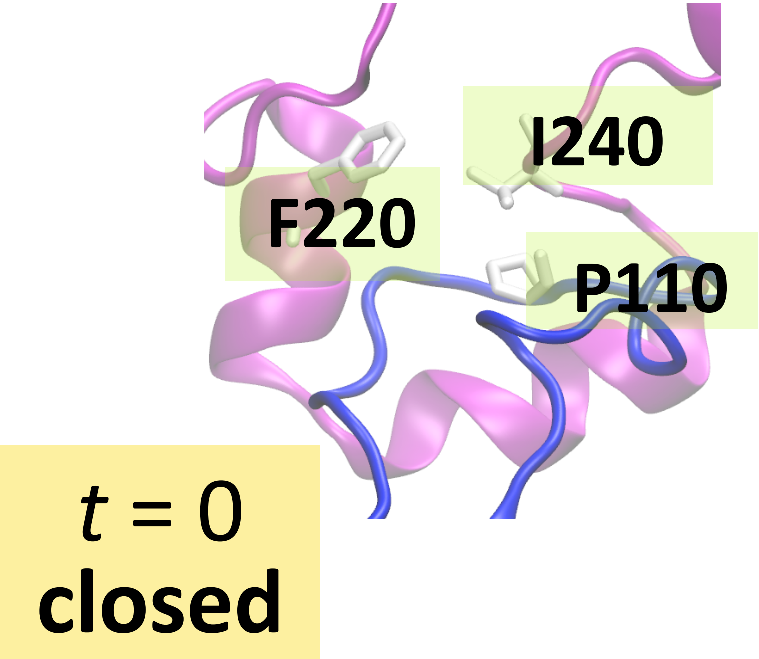


Within 10 degrees of experimental measurement of the tilt angle (S. Sligar)

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

Insertion and Membrane-Induced Conformational Change of Cytochrome P450

Javier Baylon



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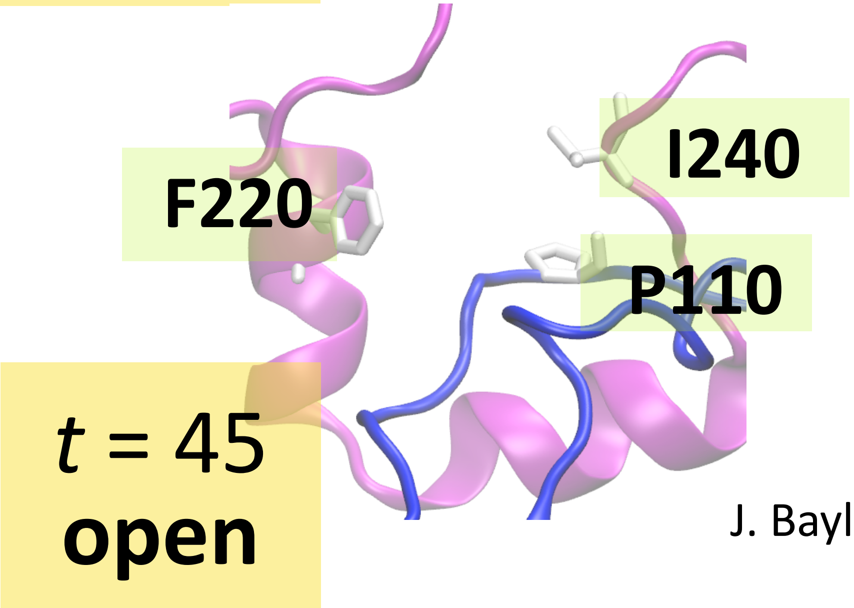

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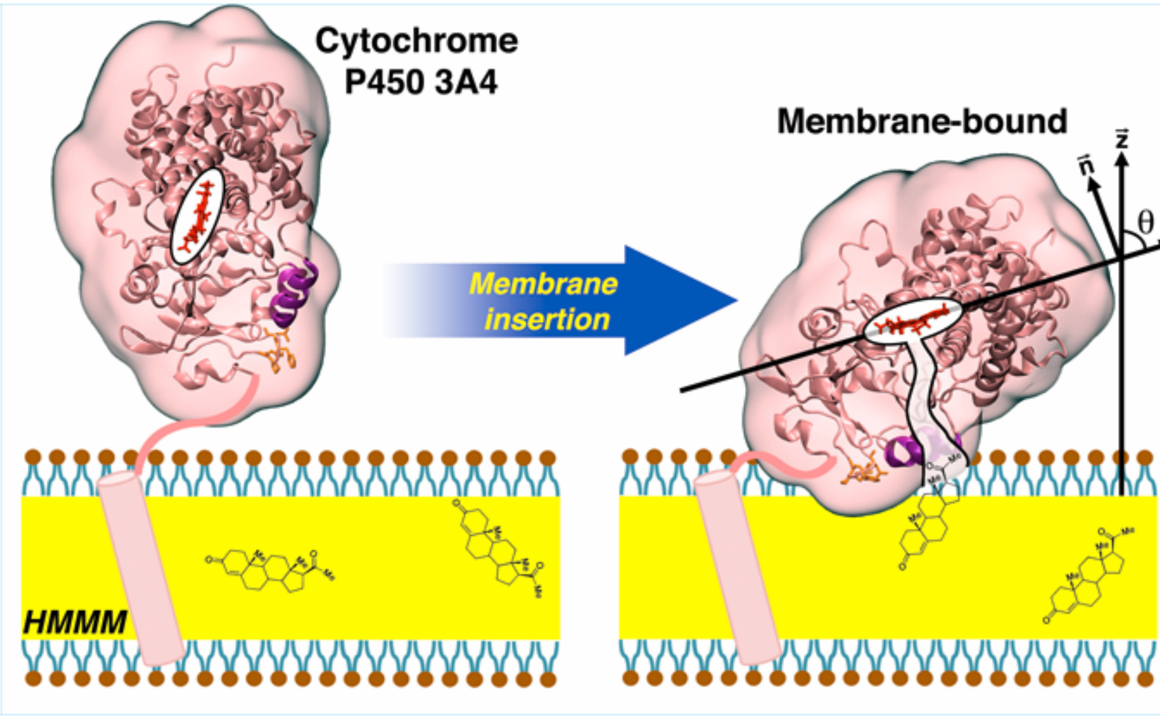

J. Baylon, I. Lenov, S. Sligar and ET, JACS, 135: 8542–8551 (2013)