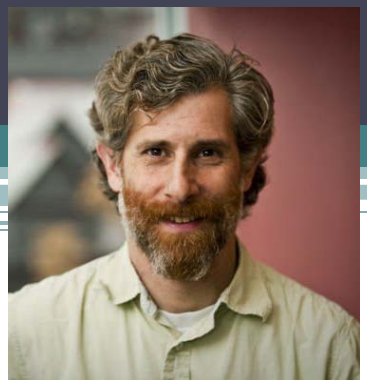
TR&D1

Leader

Ivet Bahar, PhD
Professor and John K. Vries Chair, Computational & Systems Biology, School of Medicine (SOM), Pitt

Co-Investigators

Christopher J. Langmead PhD, Assoc Prof, Computer Sci and Lane Center, Carnegie Mellon University Assoc Director, CMU/ Pitt PhD Program in Comp Biol



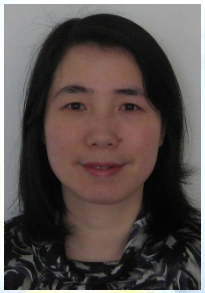
Daniel M. Zuckerman PhD, Assoc Prof, Comp & Systems Biology, Pitt, and Assoc Director, CMU/ Pitt PhD Program in Computational Biology

Troy Wymore, PhD
Senior Scientific Specialist National Res for Biomed Supercomputing (NRBSC) Pittsburgh Supercomputing Center (PSC)

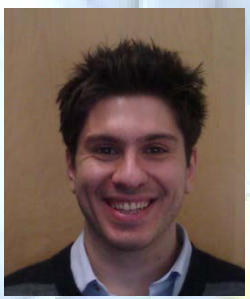
TR&D1:

Molecular-to-Supramolecular Systems Dynamics

Dr. Susan Amara, NIMH

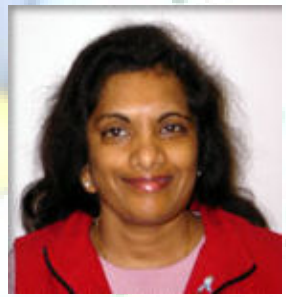


Mary Cheng
CMU/Pitt Comp Biol



Dr. Mert Gur
Comp & Systems Biol,

Dr. Elia Zomot
Comp & Systems Biol,



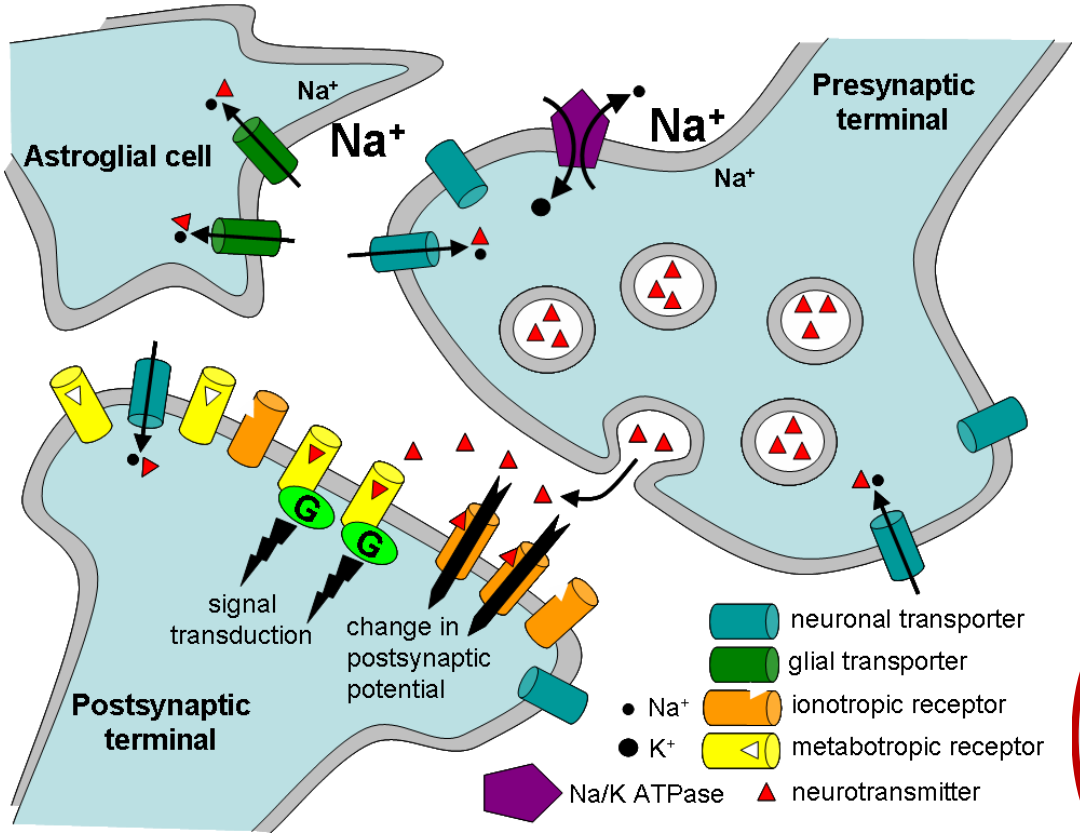
Dr. Indira Shrivastava
Comp & Systems Biol

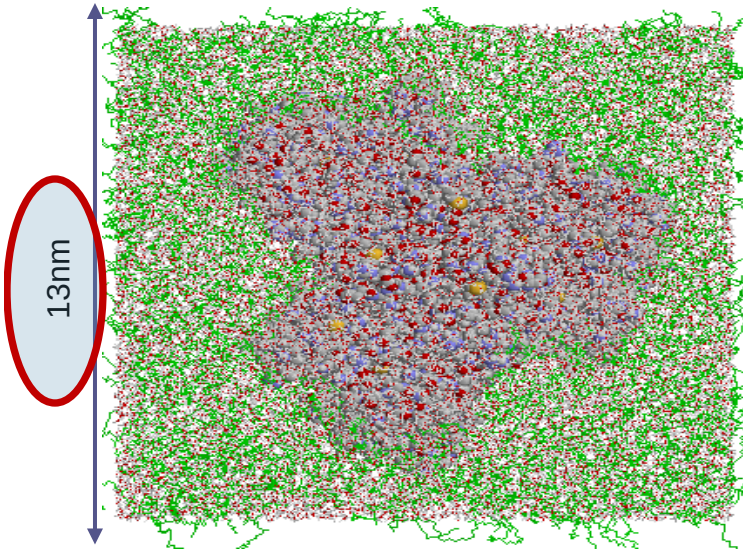
Dr. Filippo Pullara
Comp & Systems Biol,

Dr. Tim Lezon
Comp & Systems Biol,

Dr. Ahmet Bakan
Comp & Systems Biology

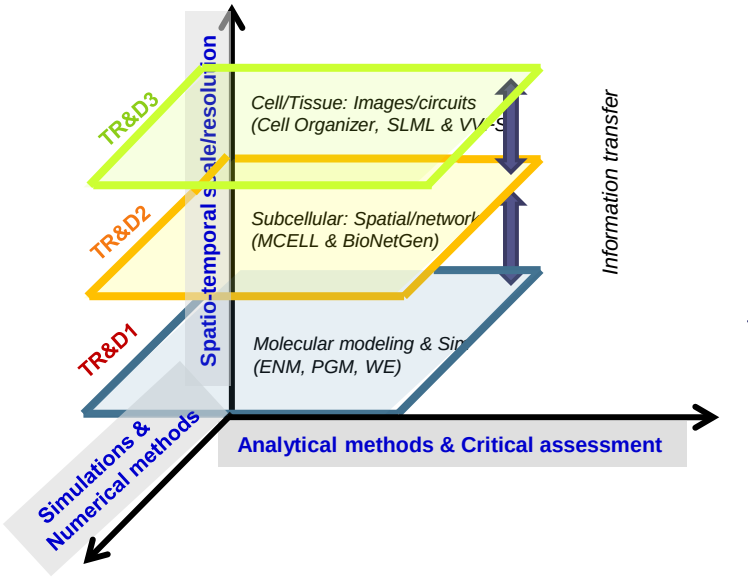
Simulation of neurotransmitter transport





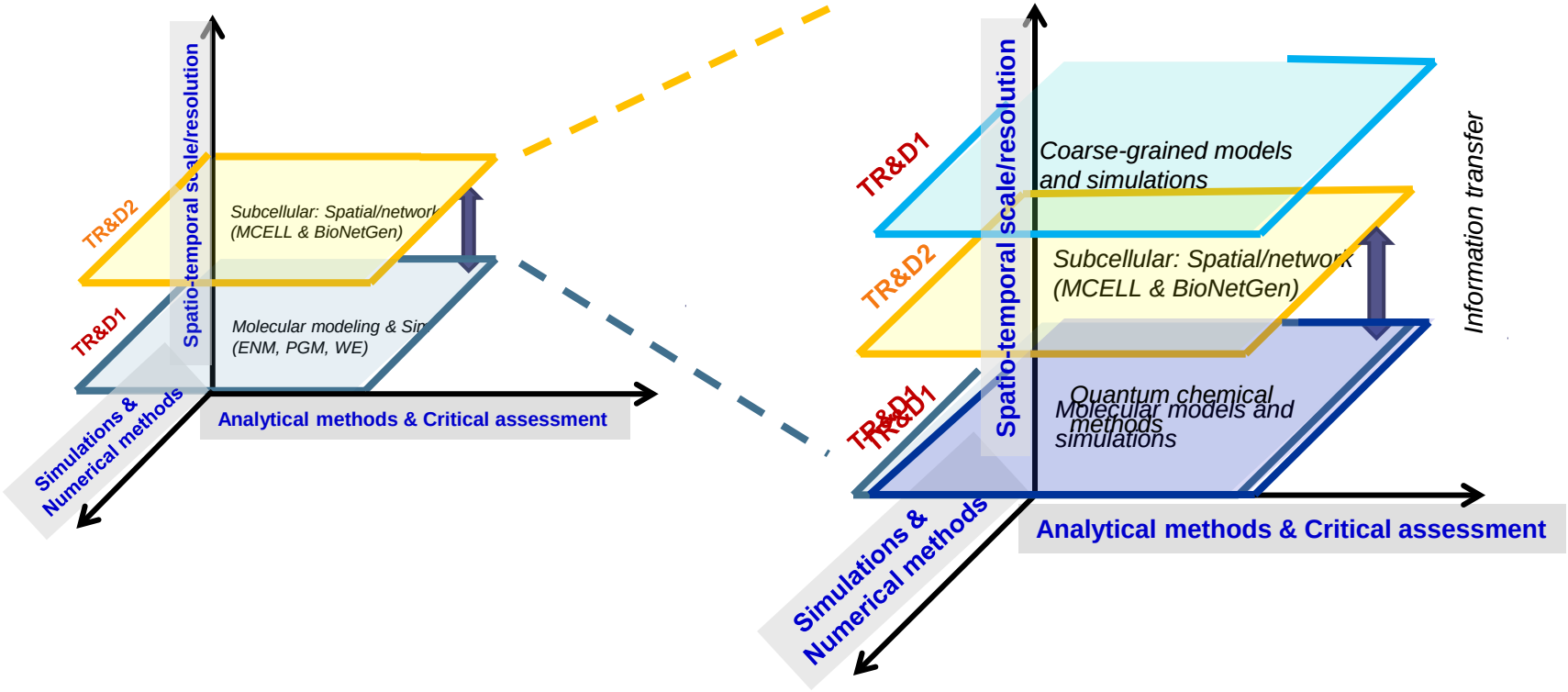
Significance

***Technological**: Integrated software and APIs for biomolecular systems dynamics which will facilitate information transfer across scales*



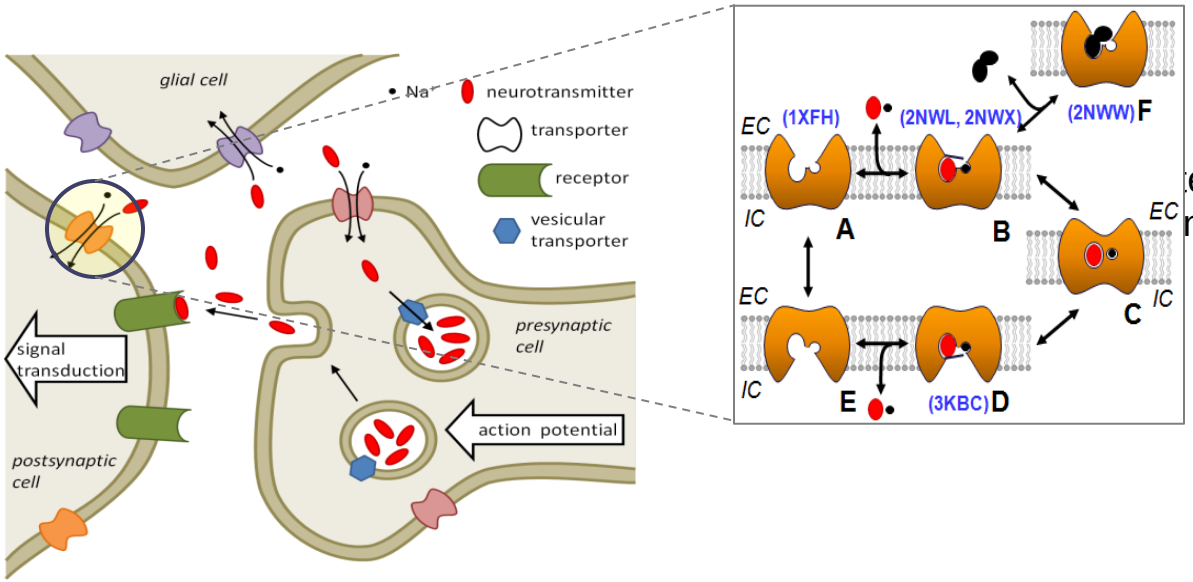
Significance

*3. **Technological**: Integrated software and APIs for biomolecular systems dynamics which will facilitate information transfer across scales*



Amara lab

Molecular and cellular biology of neurotransmitter transporters



ers (DAT, SERT, NET)
nsporters (EAATs or GluTs)

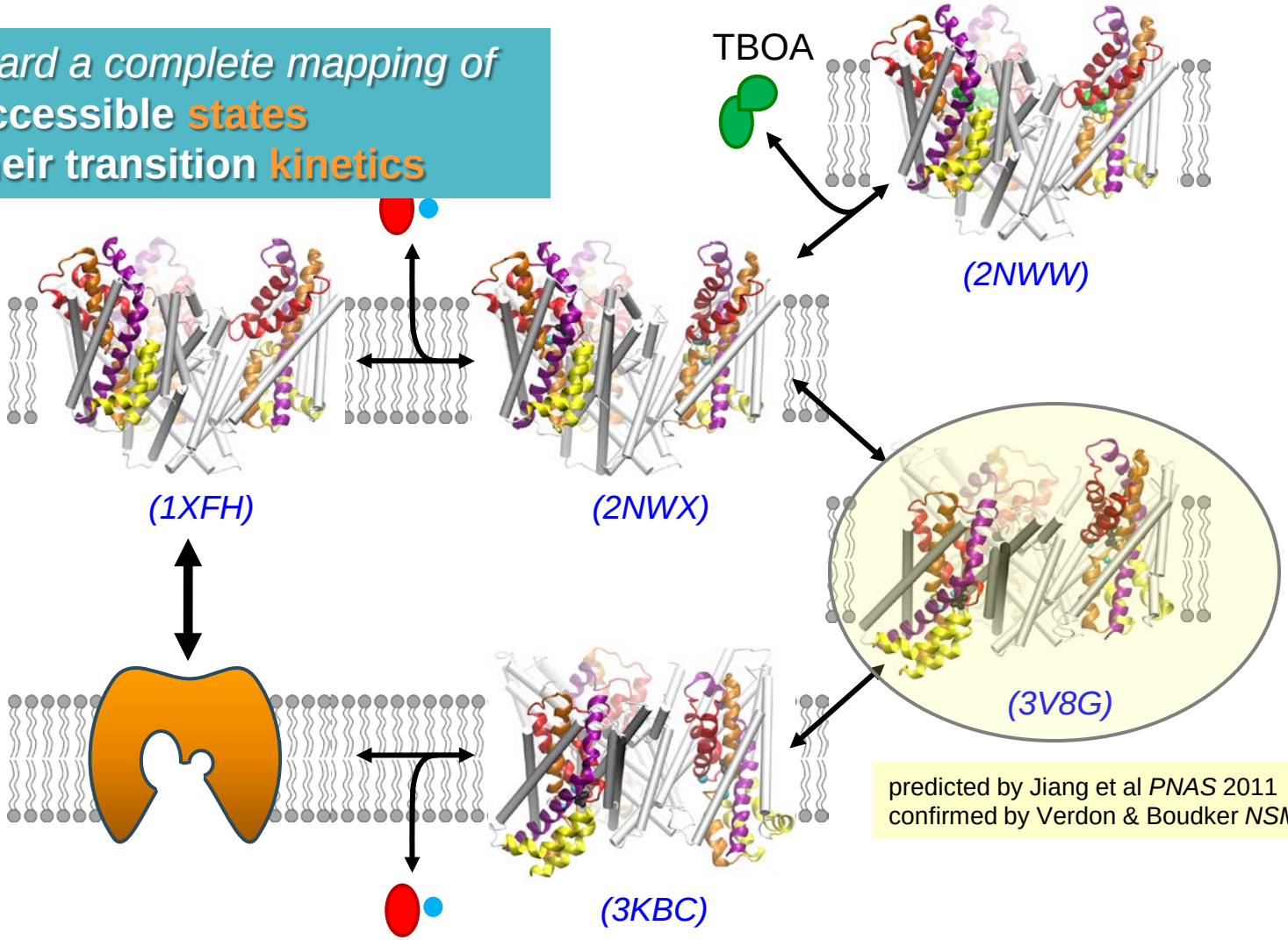
molecular genetic, electrophysiological and cell biological methods

Reference

Jiang J, Shrivastava IH, Watts SD, **Bahar I, Amara SG**. (2011) Large collective motions regulate the functional properties of glutamate transporter trimers *Proc Natl Acad Sci USA* **108**, *15141-6.*

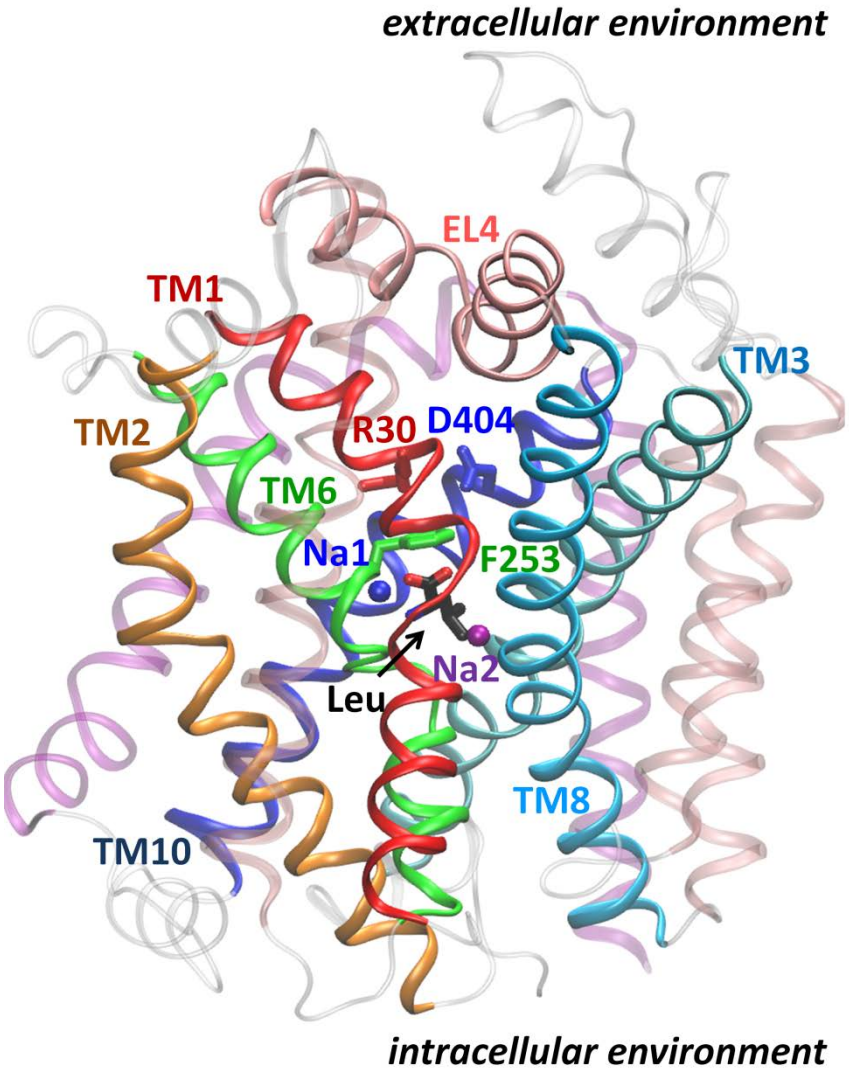
Structural data on membrane proteins

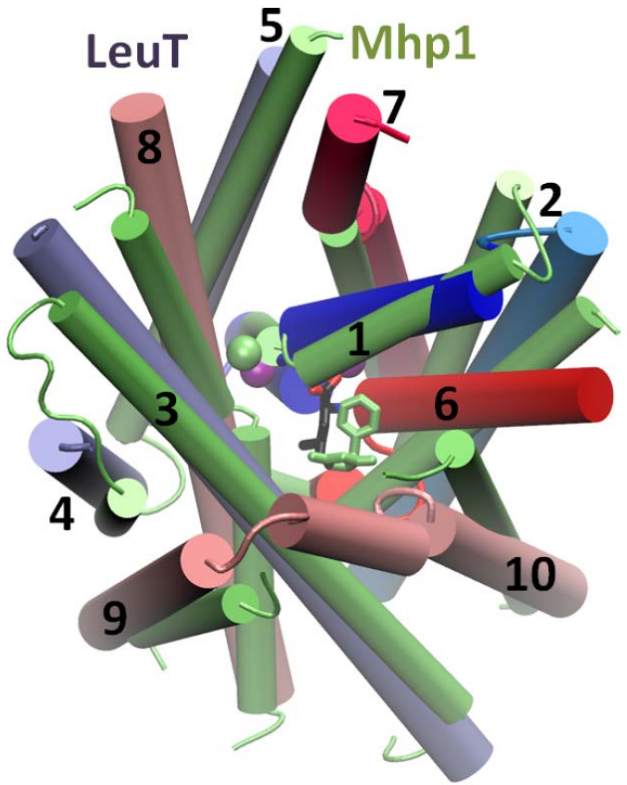
Toward a complete mapping of
- **accessible states**
- **their transition kinetics**



predicted by Jiang et al *PNAS* 2011
confirmed by Verdon & Boudker *NSMB* 2012

Outward-facing structure of Leucine transporter



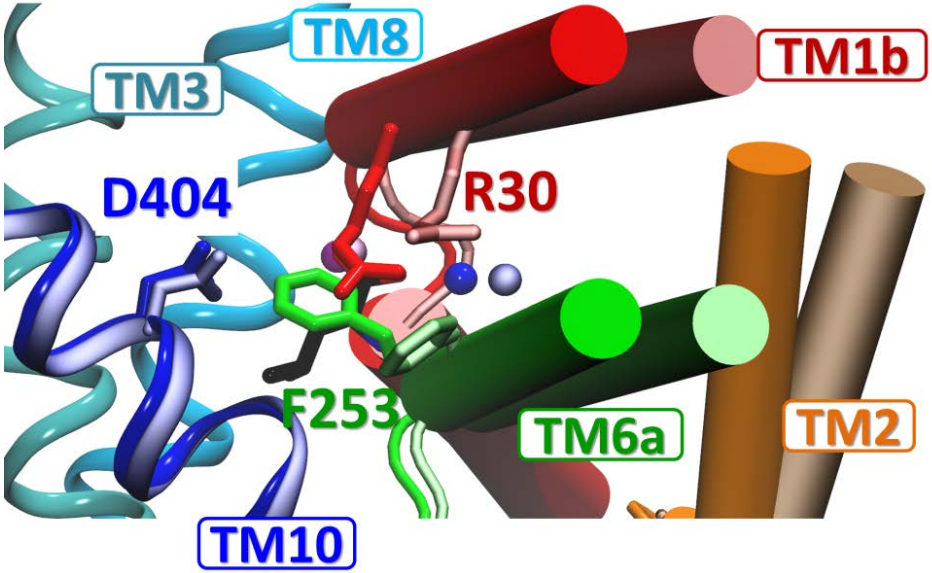


Fold shared by transporters of serotonin, dopamine, and other neurotransmitter: sodium symporters (NSS), as well as other distant transporters, such as Mhp1 shown above.

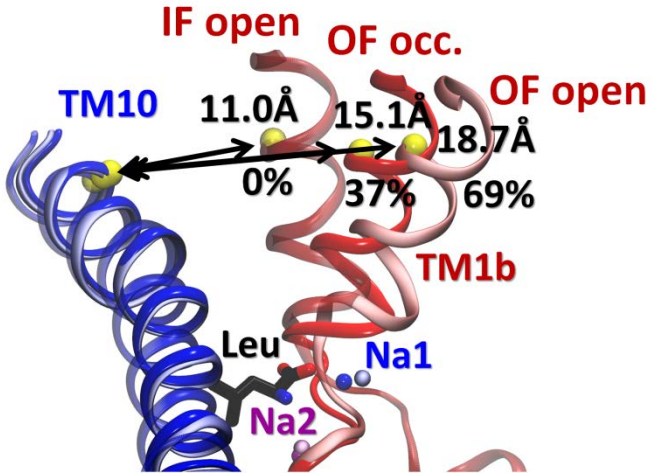
Structures resolved by: (1) Yamashita A, et al. *Nature*. 2005; 437(7056):215-23.
(2) Krishnamurthy H. & Gouaux E. *Nature.* 2012 Jan 9;481(7382):469-74.

Measuring the degree of opening of EC vestibule

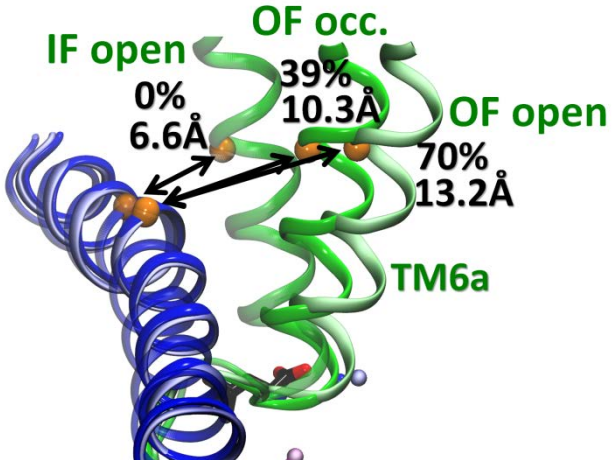
OF open vs. occluded structures
(lighter and darker colors, respectively)



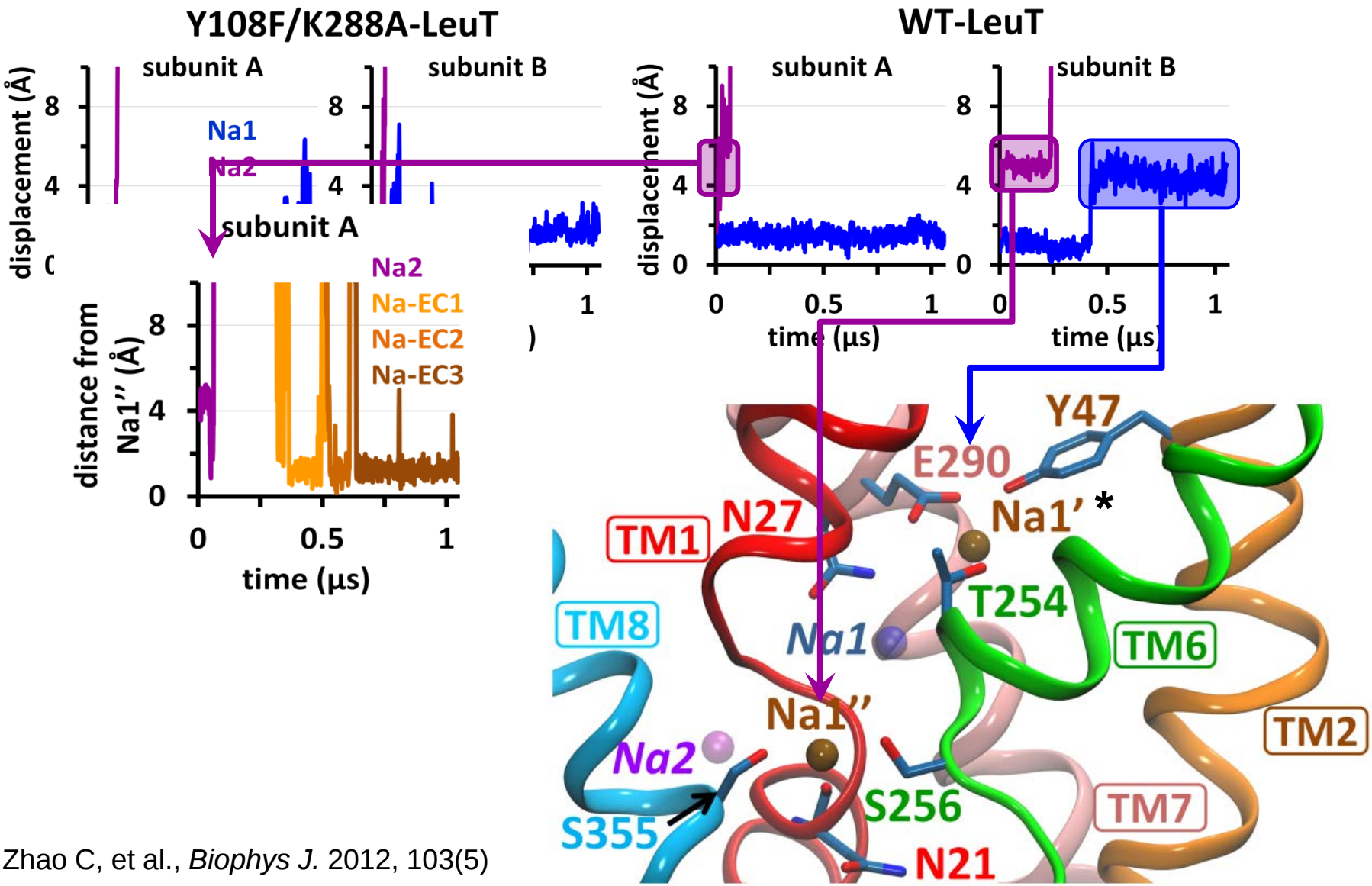
degree of opening in TM1b



degree of opening in TM6a

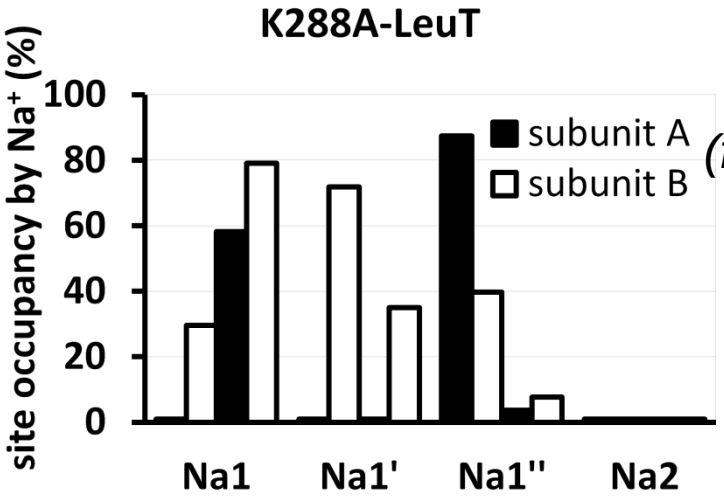


Na+ at Na2 (but not at Na1) leaves into EC vestibule



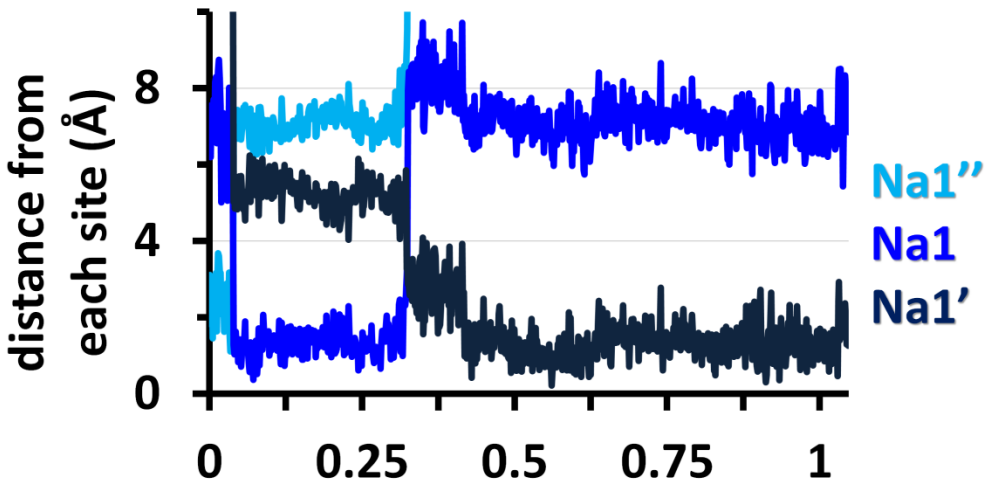
* Zhao C, et al., *Biophys J.* 2012, 103(5)

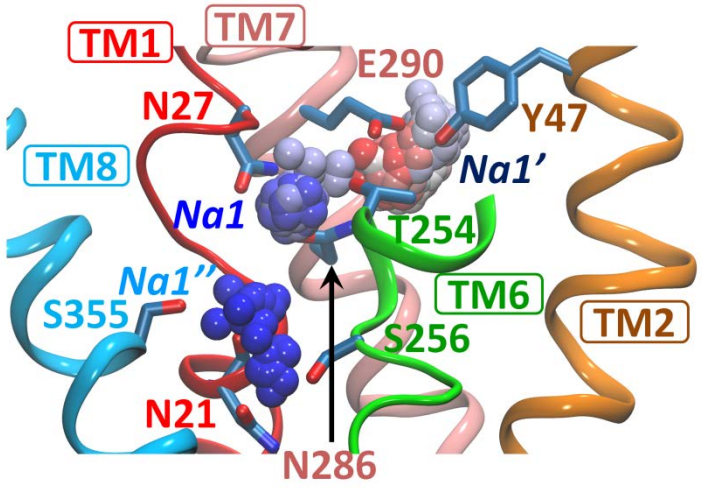
What is the probability of external Na^+ binding to each of these 4sites?



(four subunits in two runs)

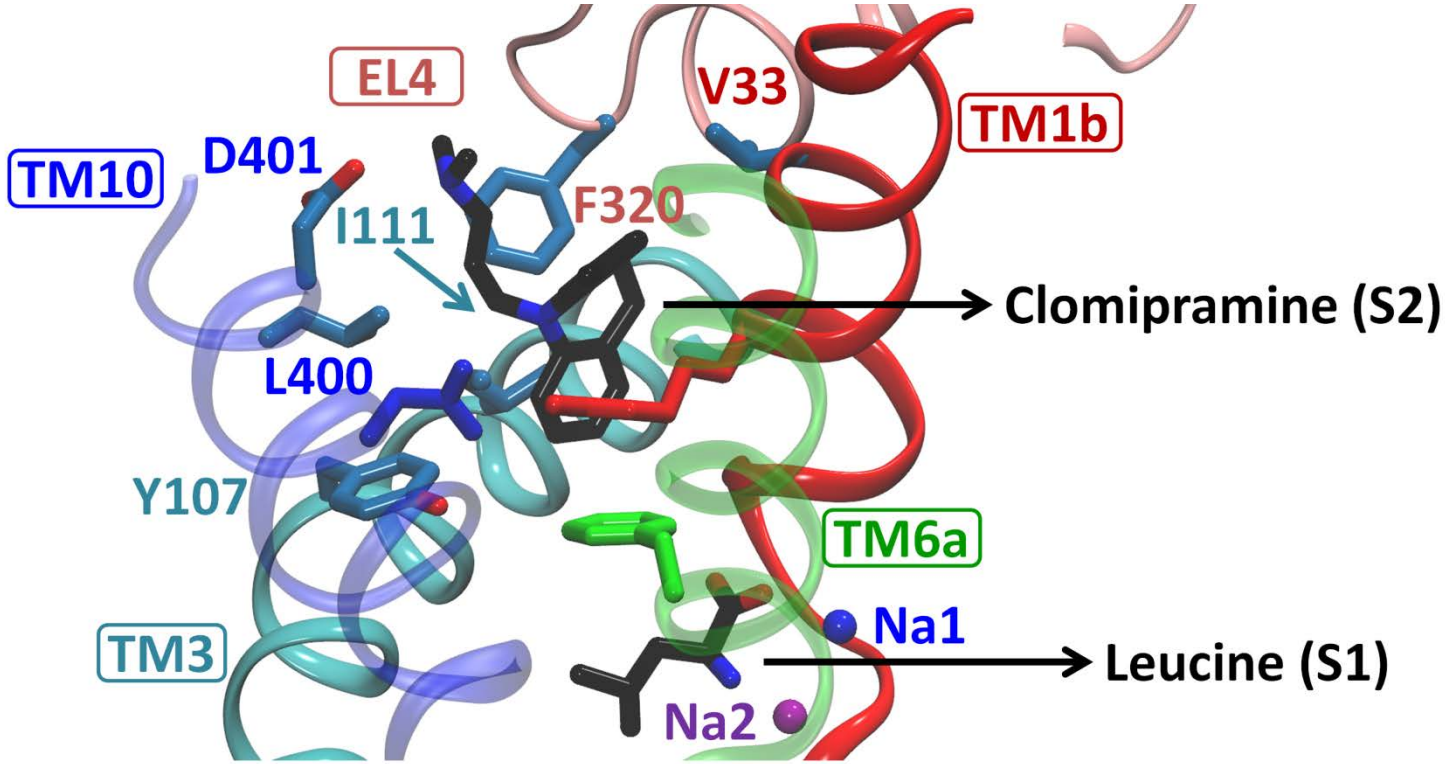
EC sodium first binds at Na1”, followed by Na1 and finally Na1’





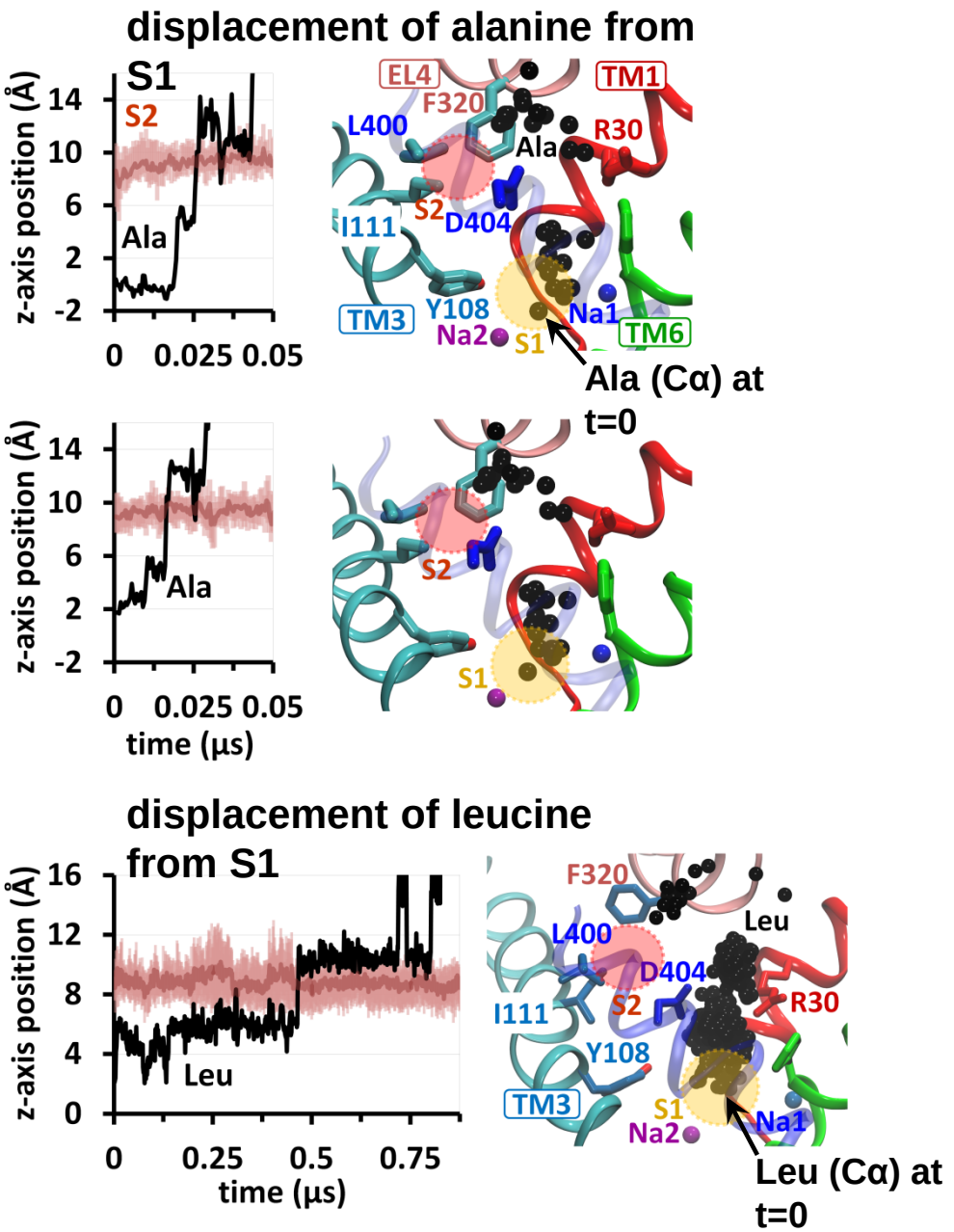
Na^+ colored by time, blue to red

A secondary binding site (S2) in LeuT binds non-competitive inhibitors

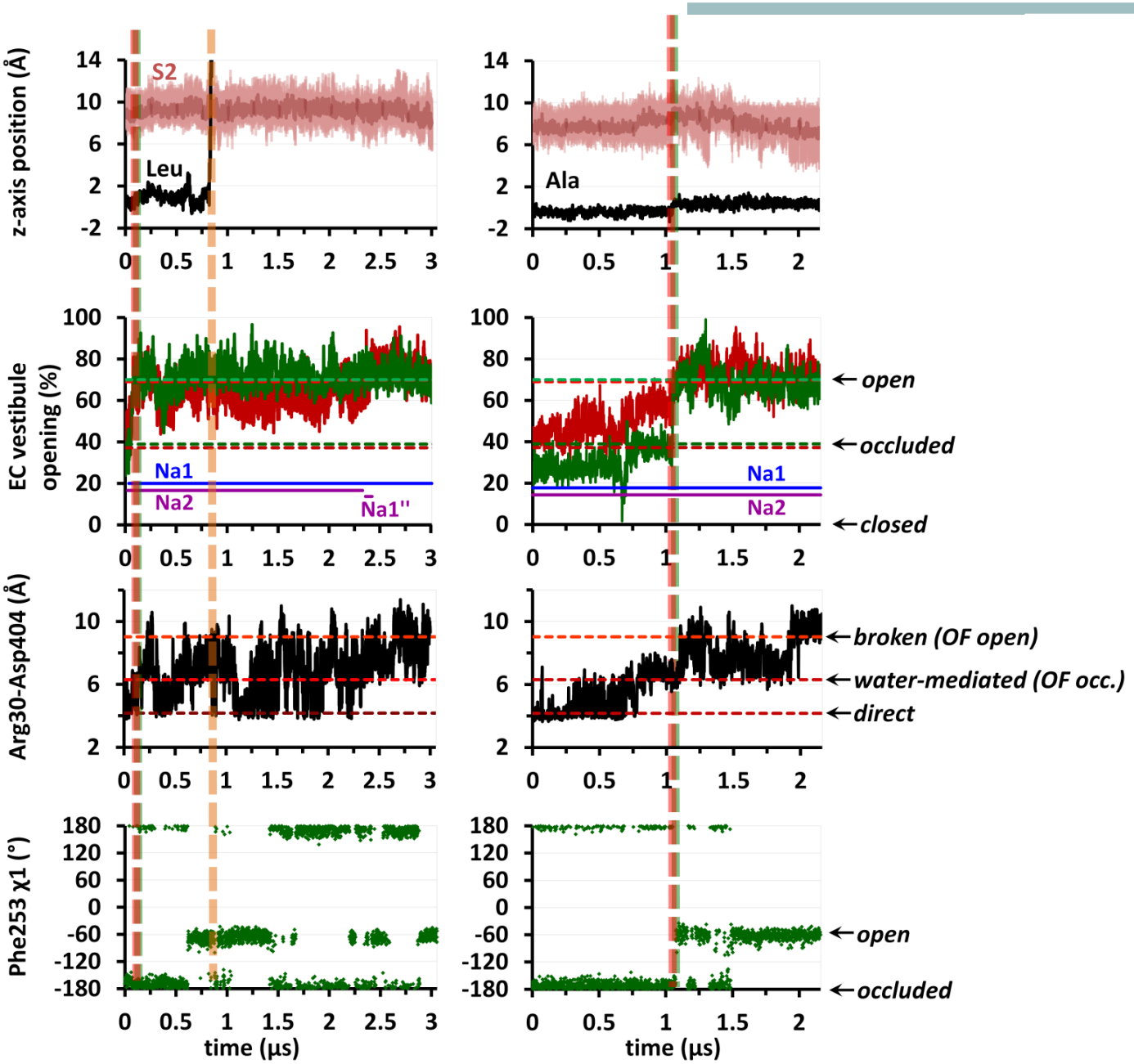


Structure resolved by: Singh SK, et al. *Nature*. 2007; 448(7156):952-6.

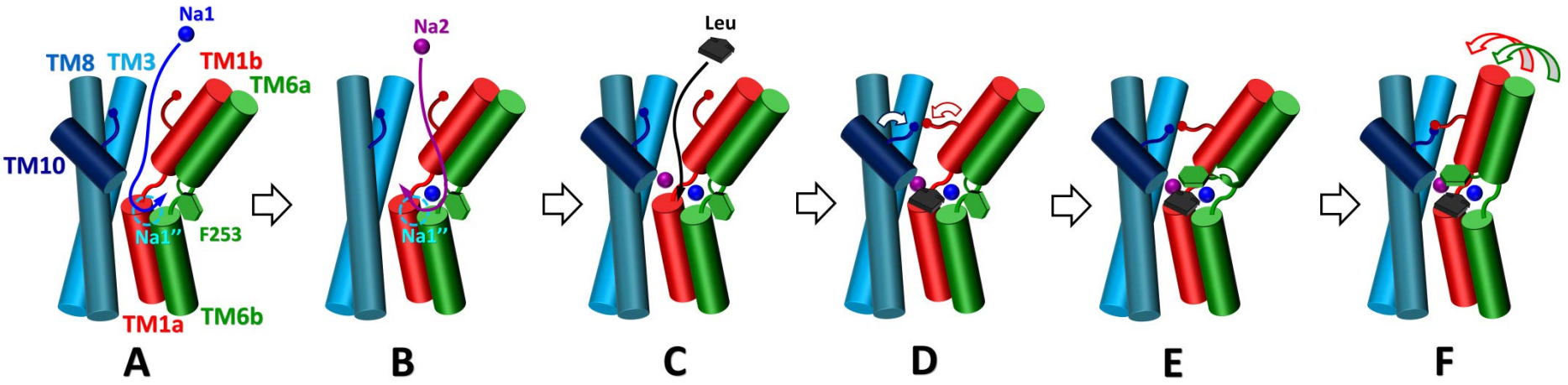
In the OF open form, substrate leaving S1 does not bind to S2



Transition from OF occluded to OF open state



Mechanism of substrate/sodium binding in OF LeuT



Need for experimental validation

Using LeuT or homologues

- Investigate how Na1” site affects affinity for Na^+ using site-specific mutagenesis.

- Examine whether TM1b and 6a move (in)dependently using intra-molecular sulfhydryl-crosslinking.

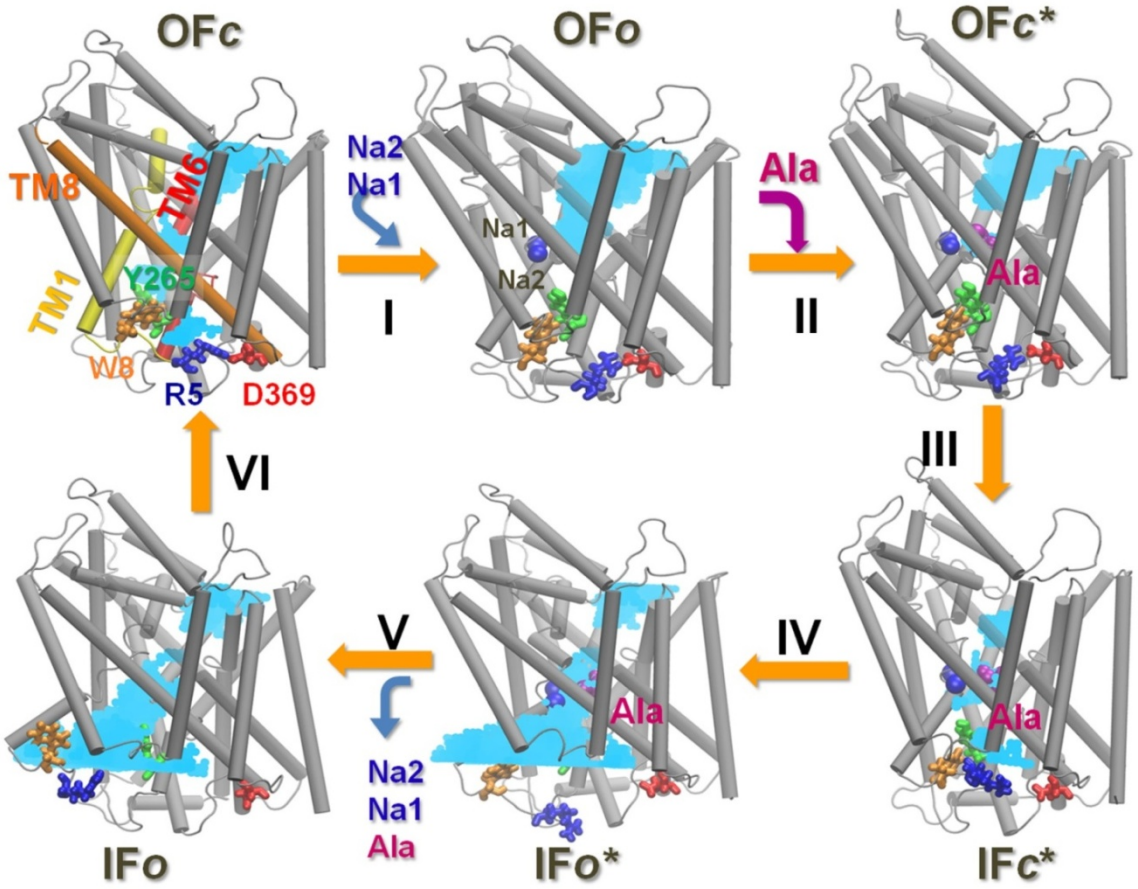
Challenges and Future Directions

- Identification of states and their relative population is a challenge – not enough sampling to have statistically significant, robust results

- Milli-to-microsecond timescales are required to efficiently sample events on the nano-to-microsecond scale in full-atomic detail.

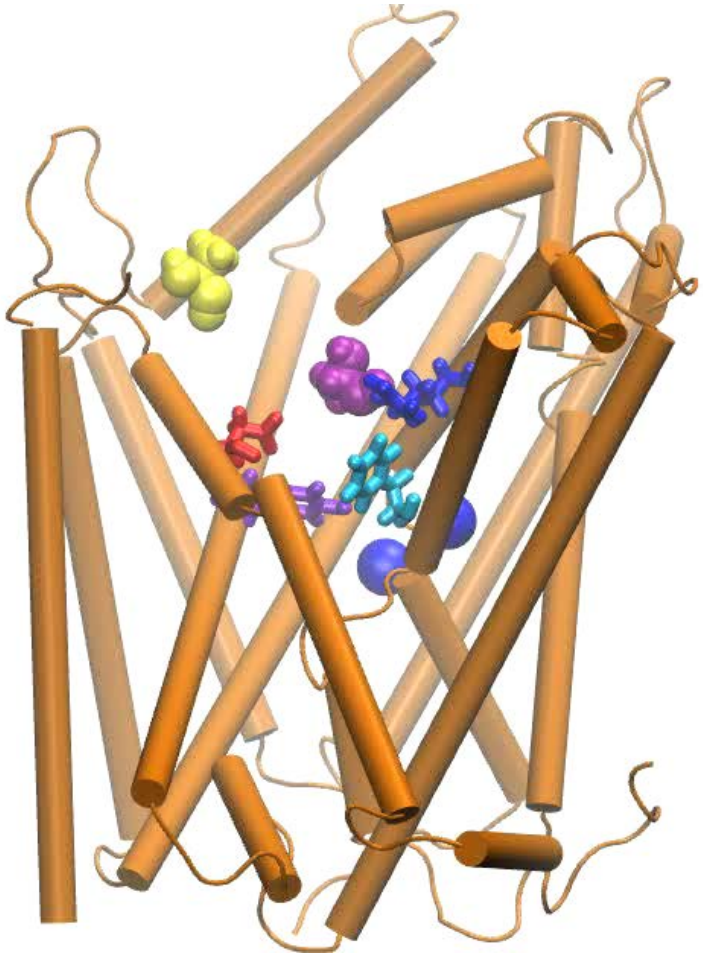
- Microsecond (brute force) simulations do not necessarily provide statistically reliable parameters on transition rates (first passage times) – a challenge to extract parameters for coarse-grained simulations.

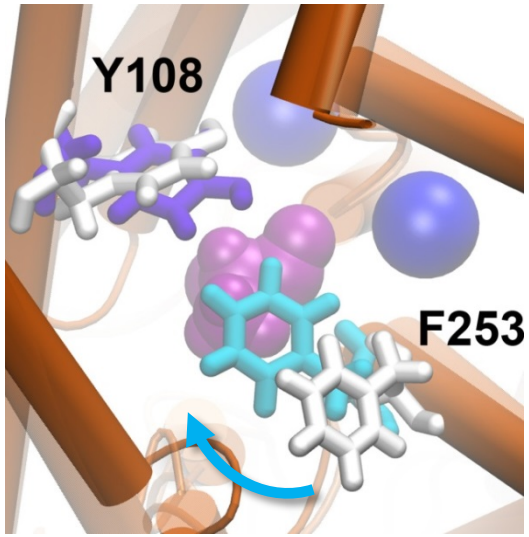
Multi-scale modeling of complete transport cycle

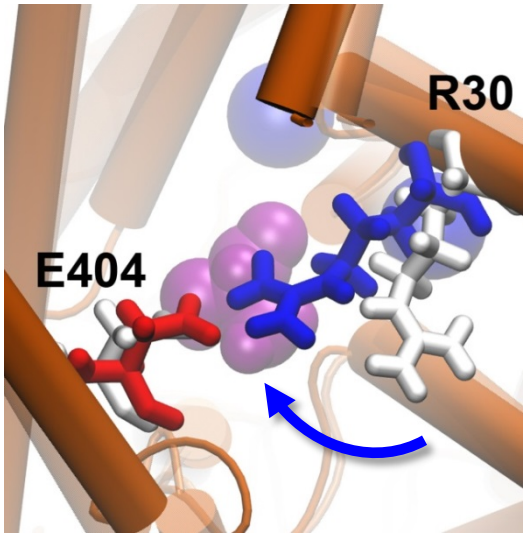


Cheng and Bahar, *Biophys J* 105; 630-639;2013.
Cheng and Bahar, to be submitted.

Substrate binding facilitates closing of EC gates



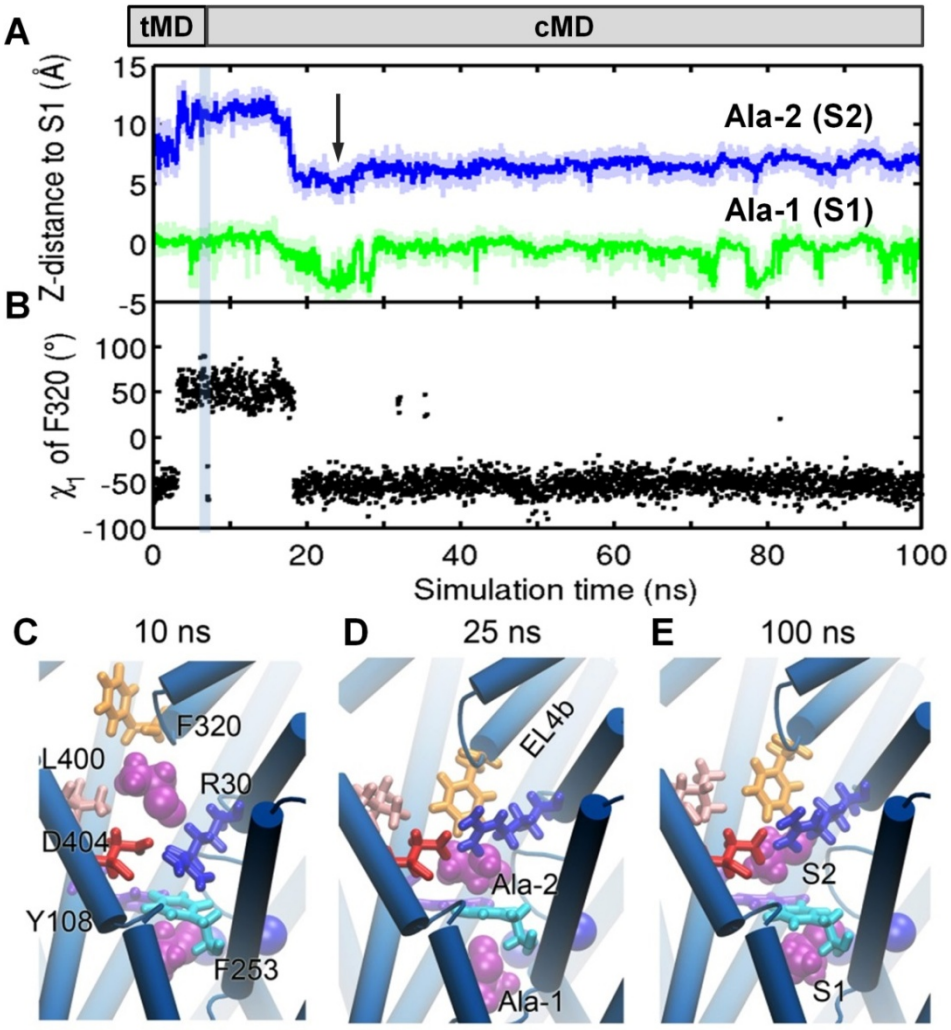




Cheng and Bahar, *Biophys J* 105; 630-639;2013.

60 ns aMD

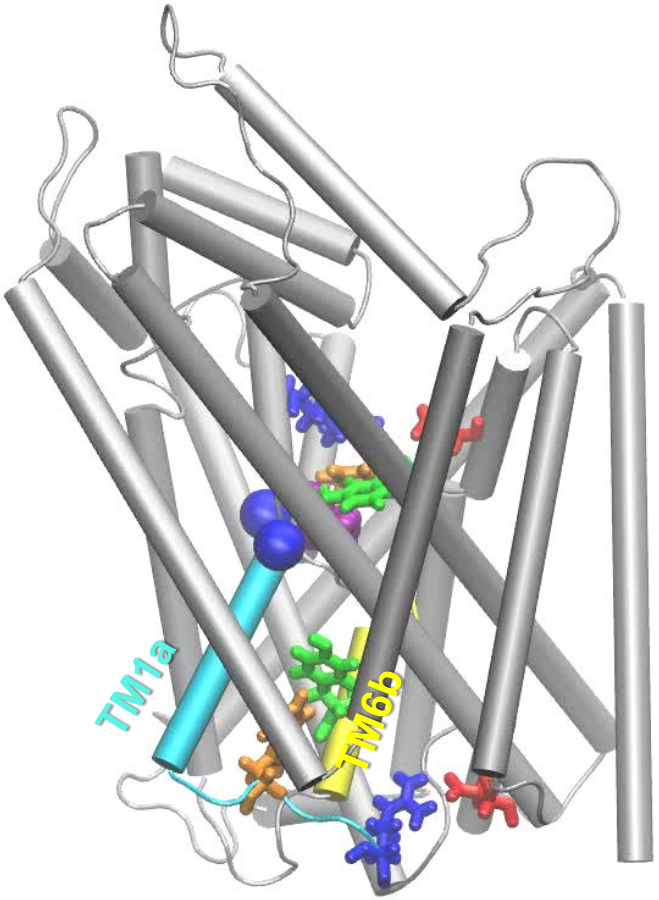
Secondary binding site for substrate, S2



IF state

Cheng and Bahar, *Biophys J* 105; 630-639;2013.

Release of substrate & sodium ions



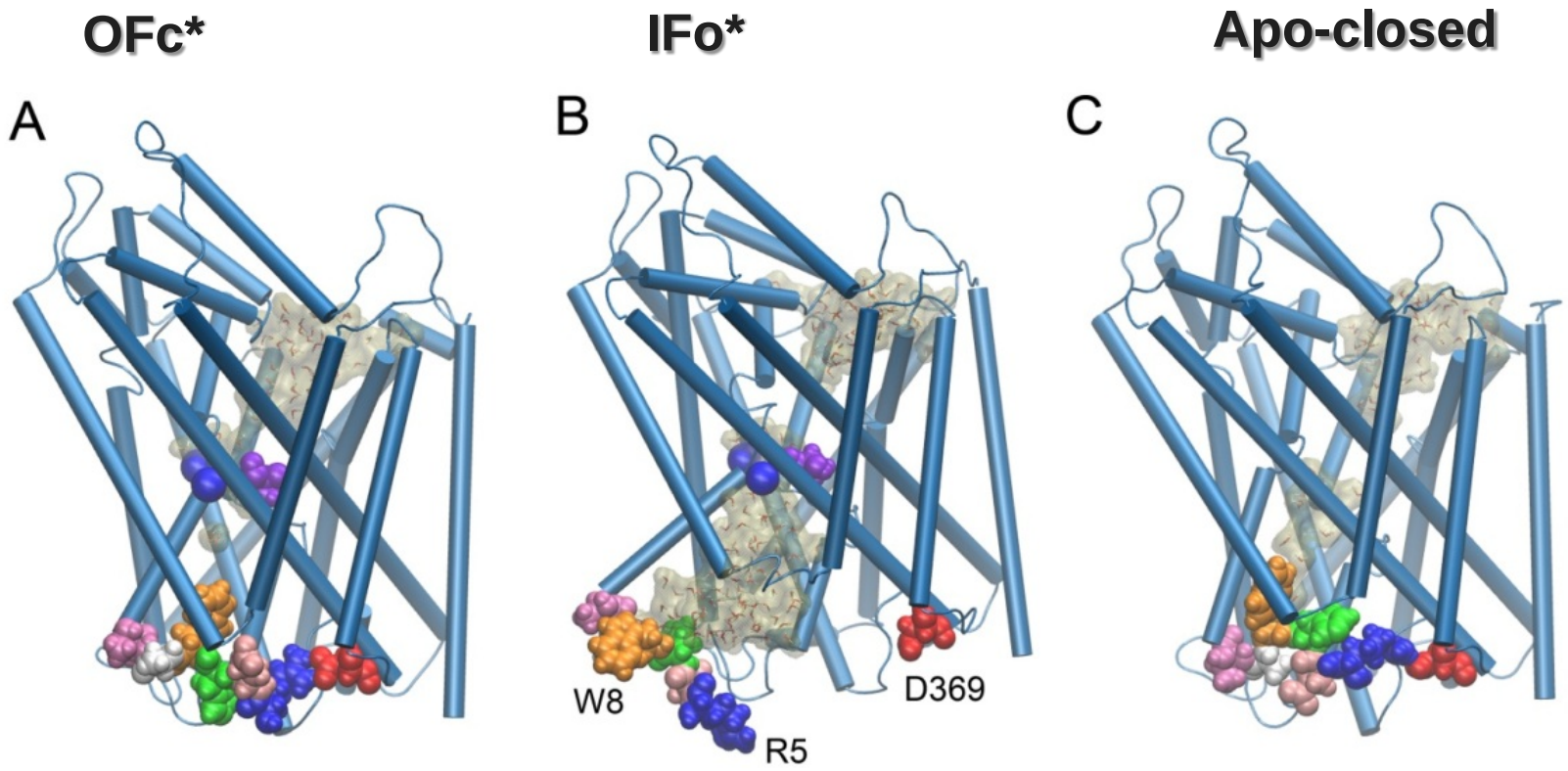
92 ns cMD run:

- destabilization of binding site
- influx of IC water
- TM1a outward tilting
- opening of IC gating residues
- breaking/ formation of salt-bridges and cation-π interactions
- Release of substrate
- Release of ions
- Closure of IC gate

putative four IC gate residues:
R5, **D369**, **Y265**, and **W8**

Cheng and Bahar, to be submitted

N-terminus regulates transport



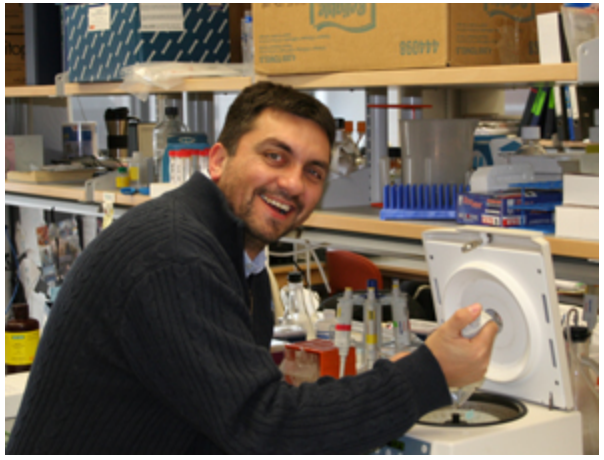
R5 and W8 regulate the opening/closing of the IC gate and the restoration of the OF state.[1] The Sorkin lab showed that removal of DAT N-terminus promotes endocytosis.[2]

[1]**Cheng and Bahar, to be submitted** [2]Sorkina et al. *J Neurosci* **2009,** *29*, 1361-1374.

Proposed experimental collaborators

Using dopamine transporters

Sorkin, Alexander D. (Pitt, Cell Biol & Phys)	5R01DA014204-11	DOPAMINE TRANSPORTER REGULATION BY ENDOCYTOSIS
Torres, Gonzalo E. (U of Pittsburgh)	5R01DA0212130 6	PHYSICAL & FUNCTIONAL LINK OF THE DOPAMINE TRANSPORTER WITH SYNAPTIC Proteins PROTEINS (b)



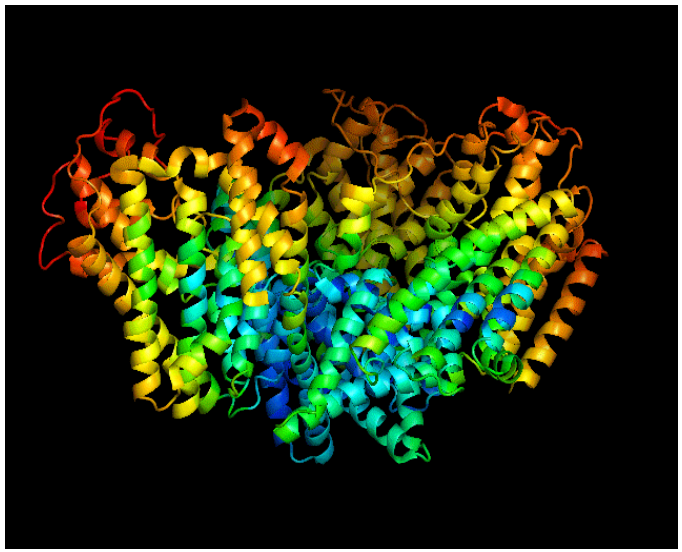
Presidential Early Career Award in 2009

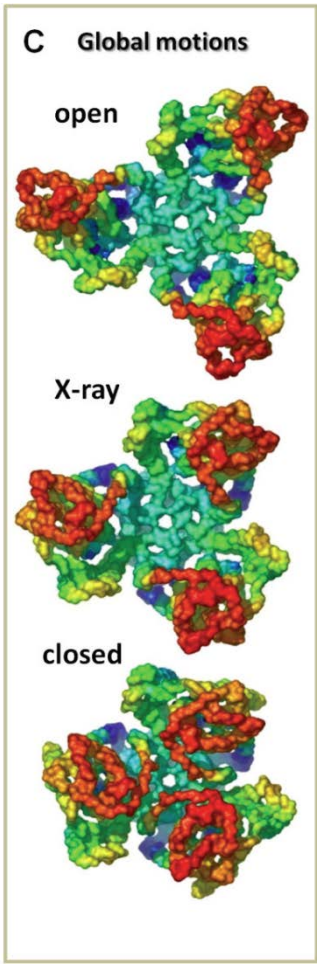
Cellular & Molecular Regulation of Monoamine Transporters

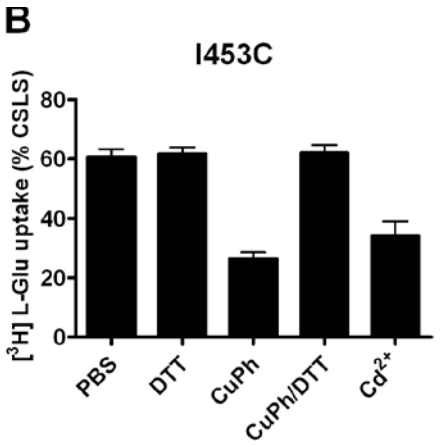
G-protein G$\beta\gamma$ inhibits dopaminergic signaling through interaction with DAT C-terminus.[1] *Its inhibitory effect is further modulated by AMPH.*

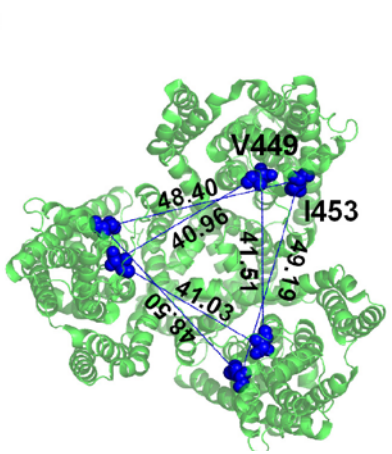
[1]Garcia-Olivares et al *PLoS. One.* **2013,** *8* (3), e59788.

Protein dynamics
using elastic network models (ENM / ANM)







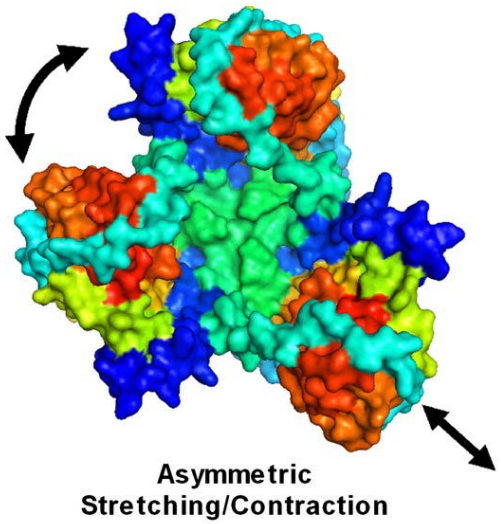


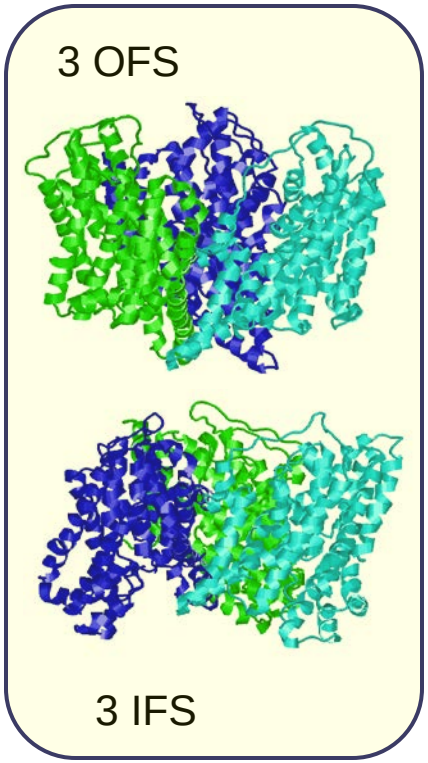
Glutamate uptake blocked by disulfide cross-linking between ‘distant’ pairs

Jiang et al (2011) *Proc Natl Acad Sci* **108**:15141-6.

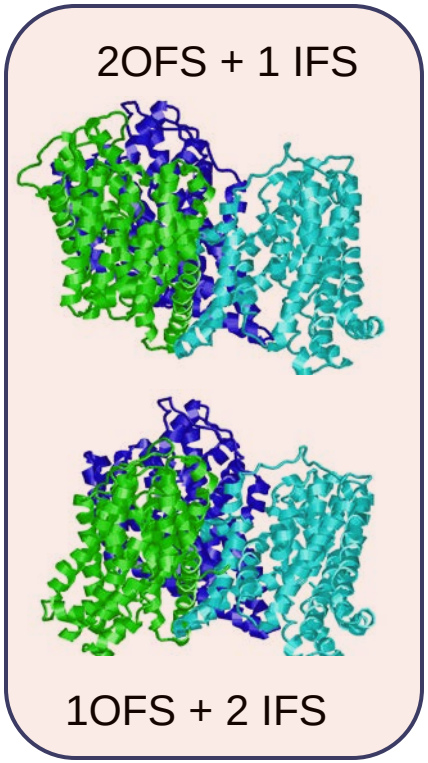
Transition of one subunit at a time:

a mechanism revealed by our experiments & computations





X-ray crystallography

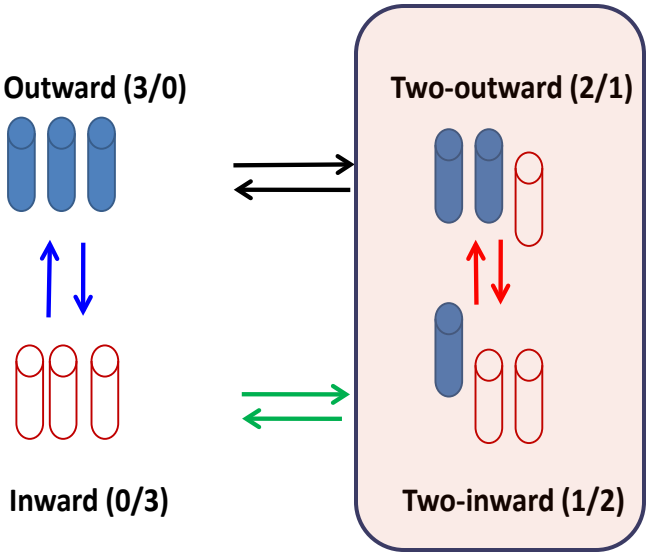


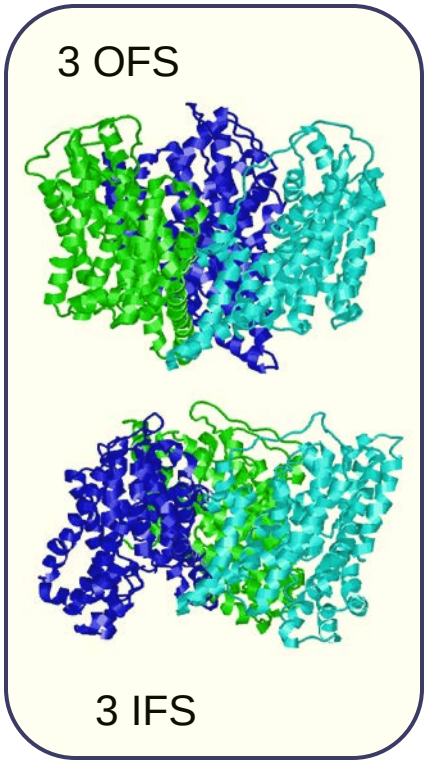
ANM-predicted

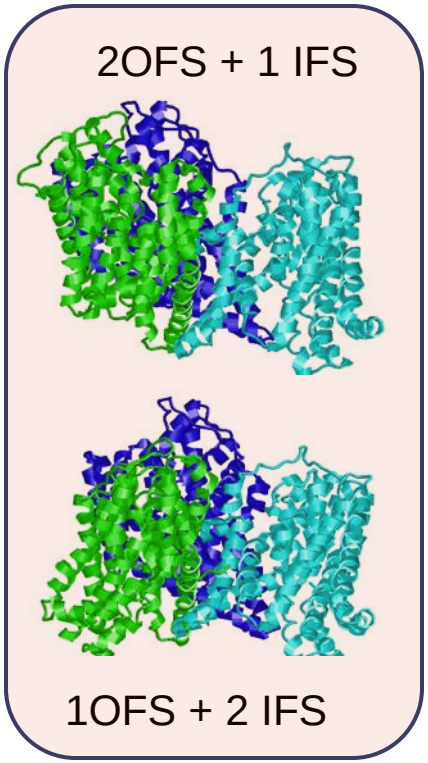
Jiang et al (2011) *Proc Natl Acad Sci* **108**:15141-6.

Transition of one subunit at a time:

a mechanism revealed by our experiments & computations



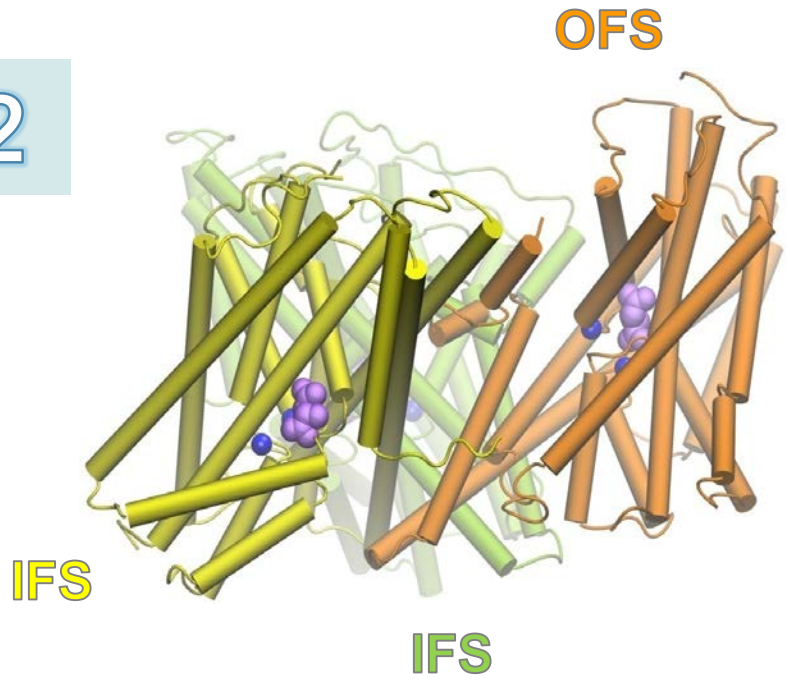


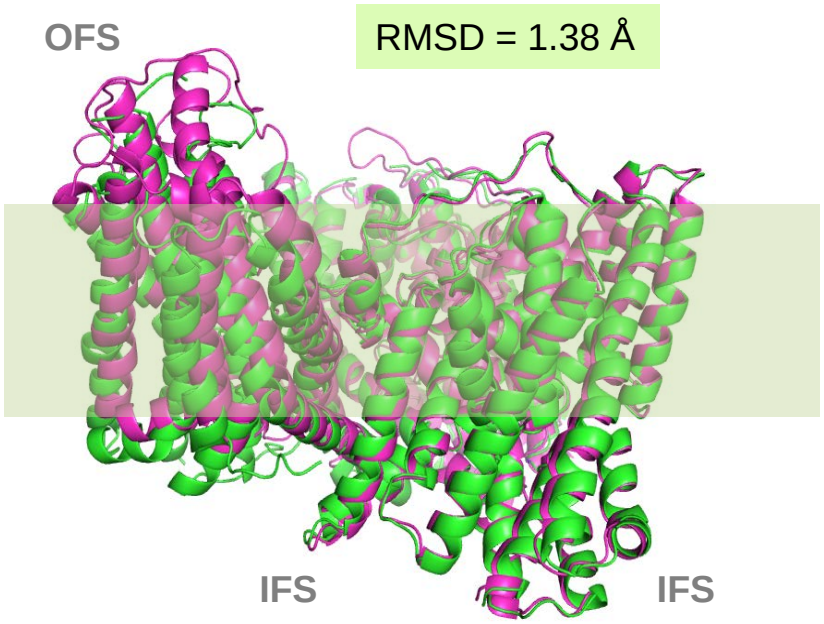


Jiang et al (2011) *Proc Natl Acad Sci* **108**:15141-6.

New X-ray crystallographic data confirmed our prediction!

2



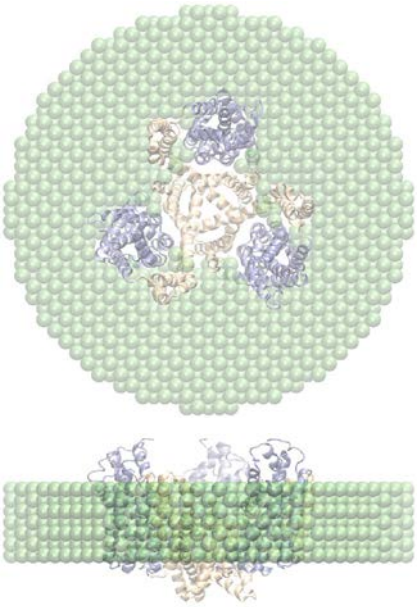


Asymmetric Glt_{Ph} trimer

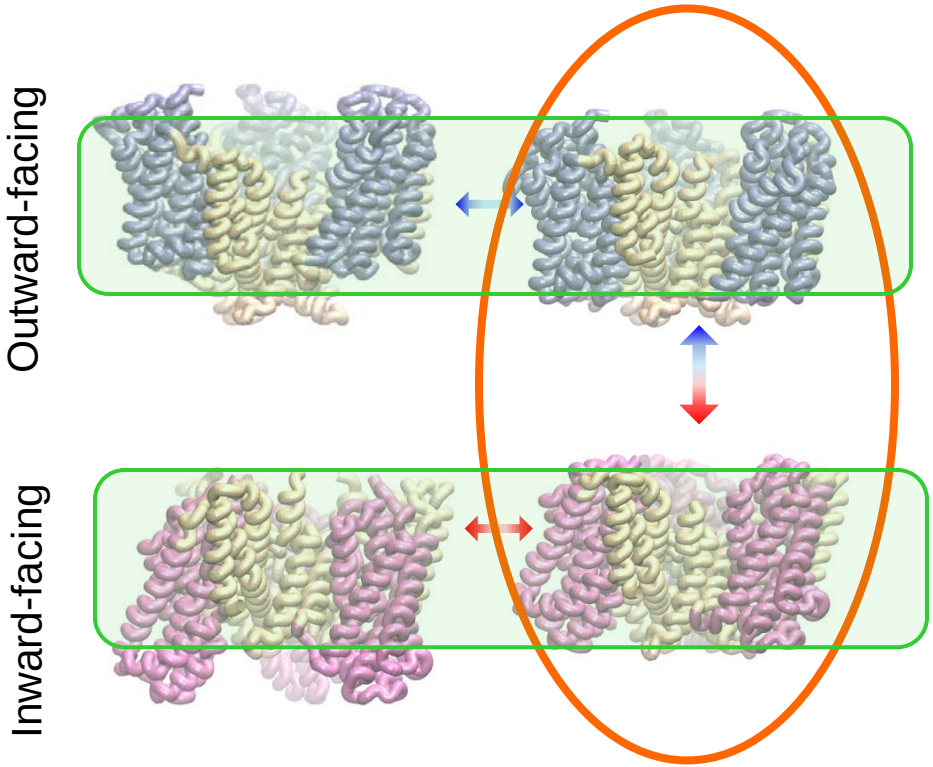
(Verdon & Boudker (2012) *Nat Struct Mol Bol* **19**, *355.*

Goal: Incorporating membrane effect into ENM/ANM

3



Tim Lezon

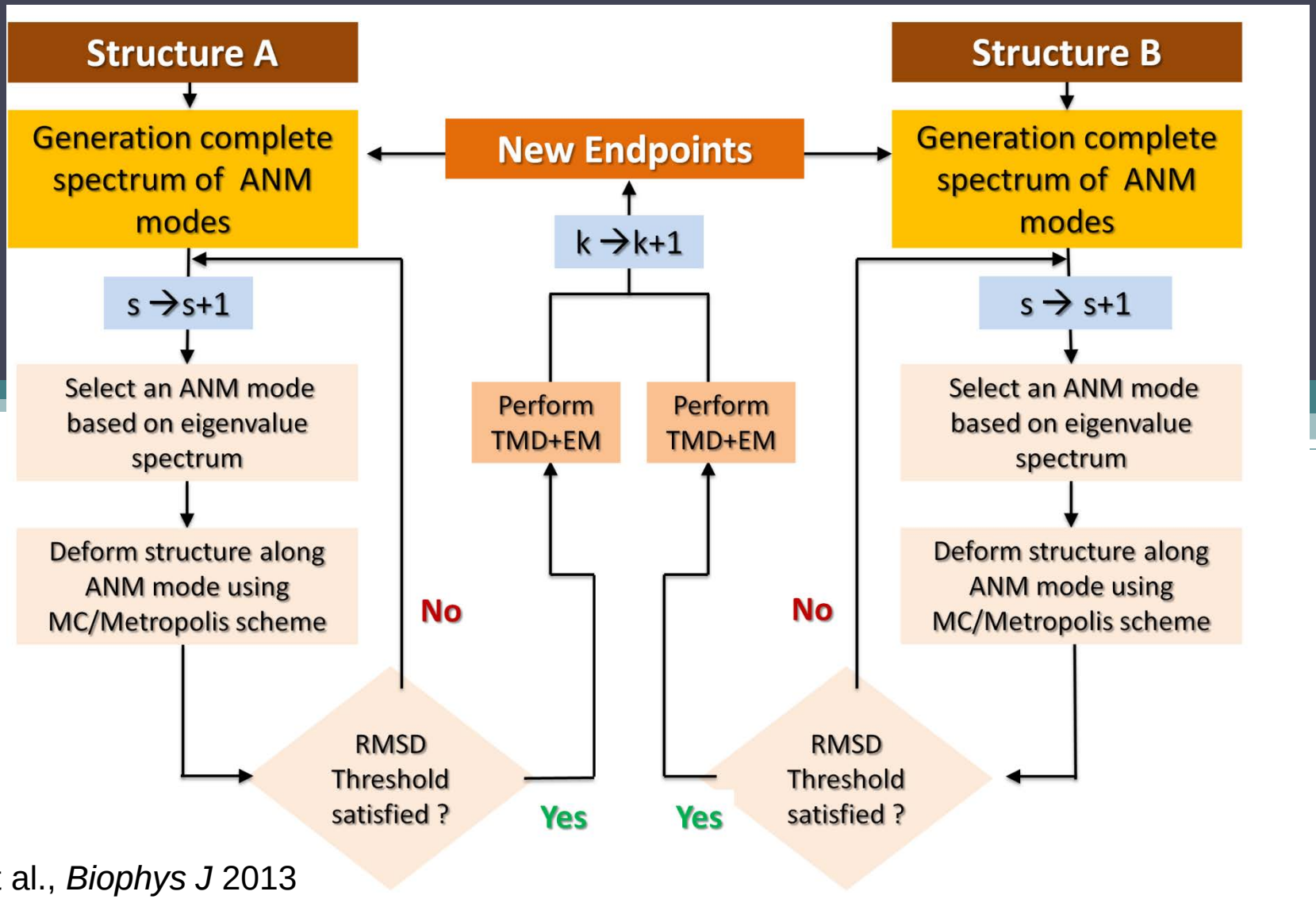


in the presence of lipid constraints

Reference:

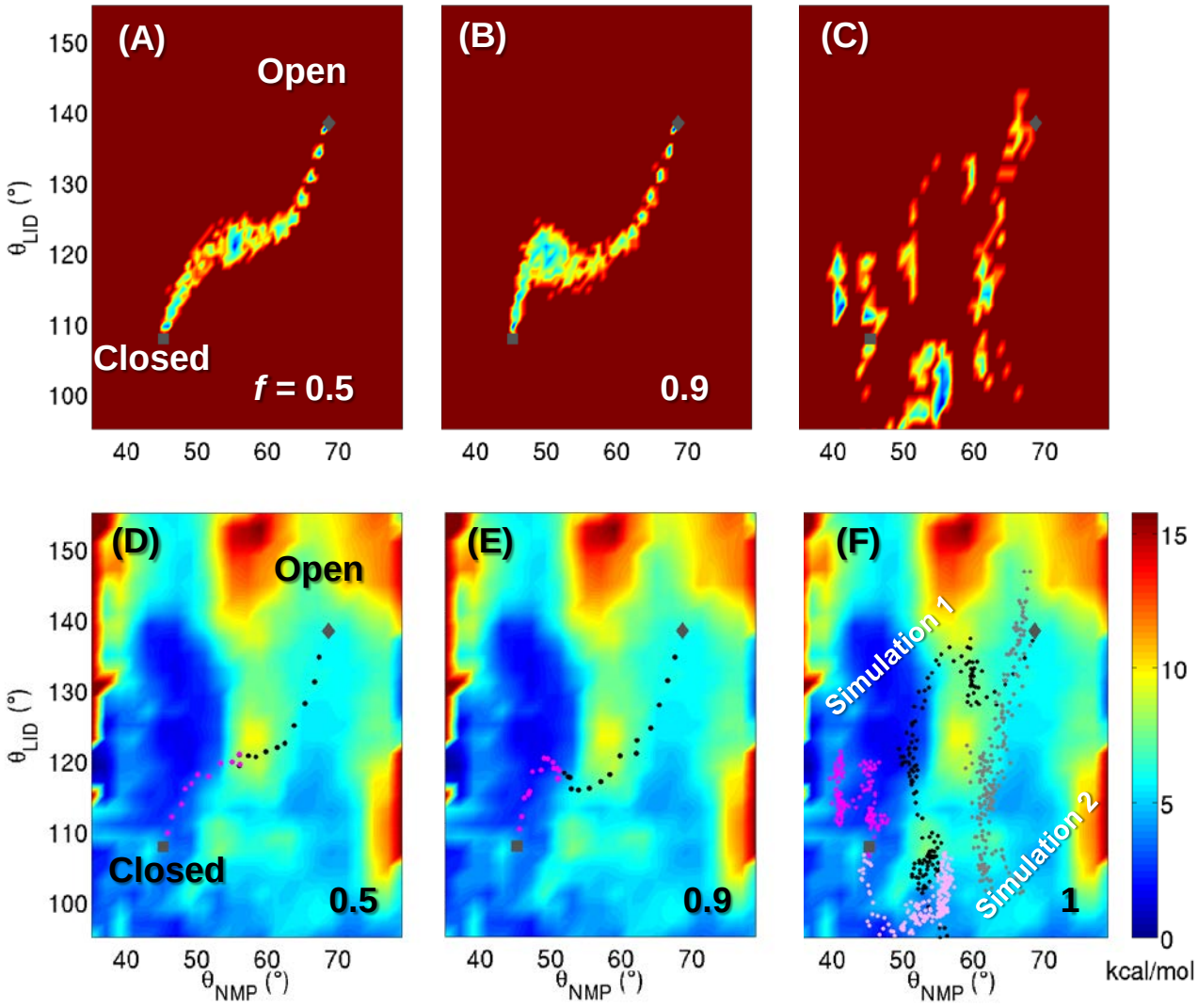
Lezon TR, Bahar I. (2012) Constraints imposed by the membrane selectively guide the alternating access dynamics of the glutamate transporter Glt_{Ph}. *Biophys J*. **102**:1331-40.

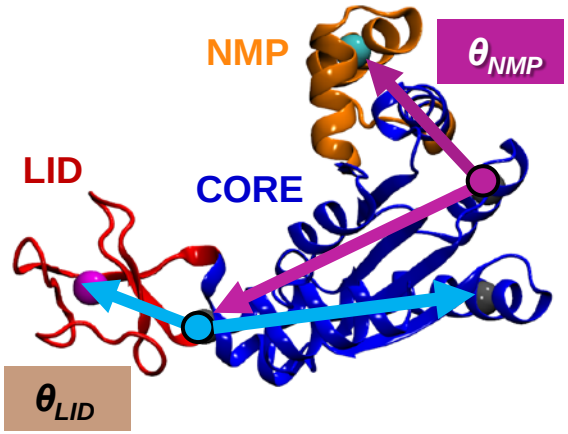
Development of a Hybrid Methodology that combines ANM, MC and MD
Collective Molecular Dynamics (*co*MD)

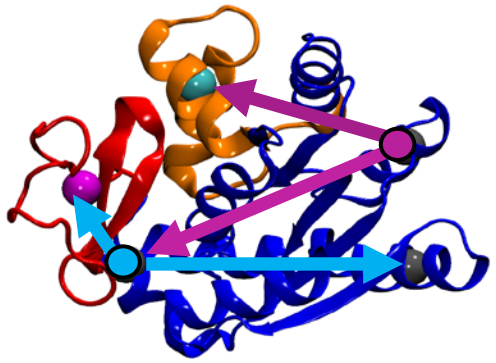


Gur et al., *Biophys J* 2013

How do coMD trajectoriesy compare with energy surfaces from full atomic MD?





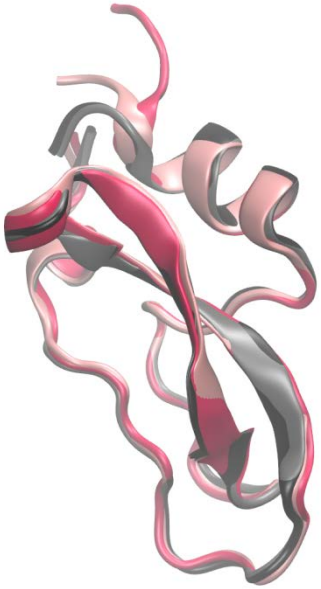


Gur et al (2013) Biophys J.

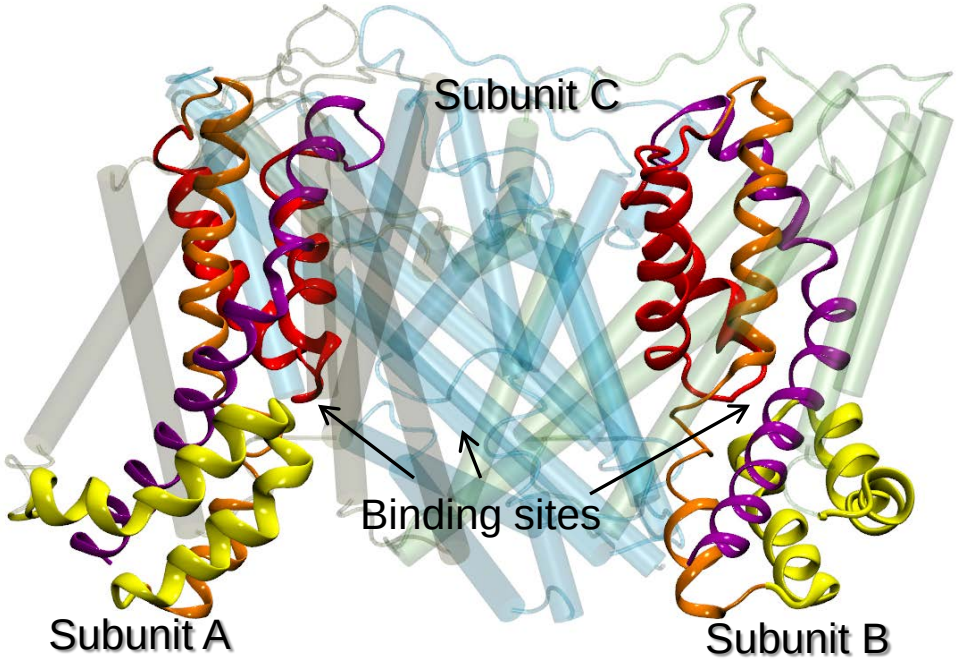
PMF surface from Beckstein, et al. J. Mol. Biol. 394: 160-176. (2009)

Global Motions Extracted from Micro- to Milliseconds Simulations Comparison with Anisotropic Network Model Predictions[1]

1.013 millisecond on the native state dynamics of bovine pancreatic trypsin inhibitor (BPTI).[2]



12 microseconds on the gating mechanism of the inward facing archaeal aspartate transporter, GltPh.[3]



Anisotropic Network Model (ANM)[4] is a simple physics-based model of beads and springs, which exclusively depends on inter-residue contact topology.

1. Gur M., E. Zomot, and I. Bahar. J. Chem.Phys. 139:121912 (2013)
2. D. E. Shaw, et al., Science 330, 341 (2010)
3. E. Zomot and I. Bahar, J. Biol. Chem. 288, 8231 (2013)
4. A. R. Atilgan, S. R. Durell, R. L. Jernigan, M. C. Demirel, O. Keskin and I. Bahar, Biophys. J. 80, 505 (2001)

BPTI dynamics and transitions are well defined by ANM

Perform Principal Component Analysis

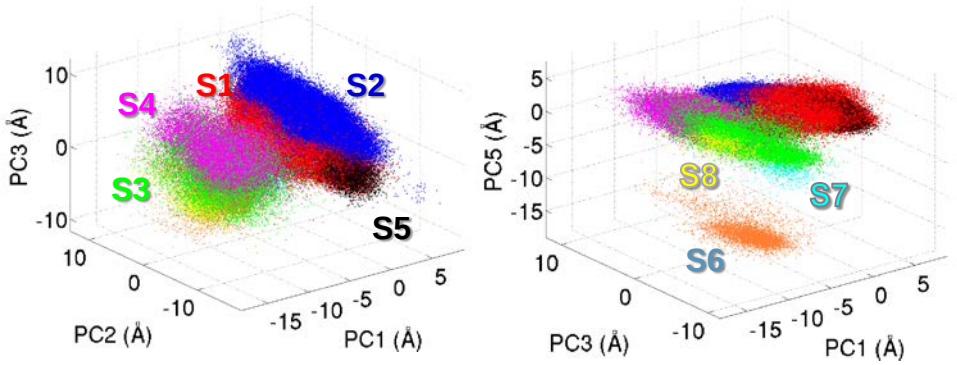
Project on Principal Components

Identify Substates Based on Distributions

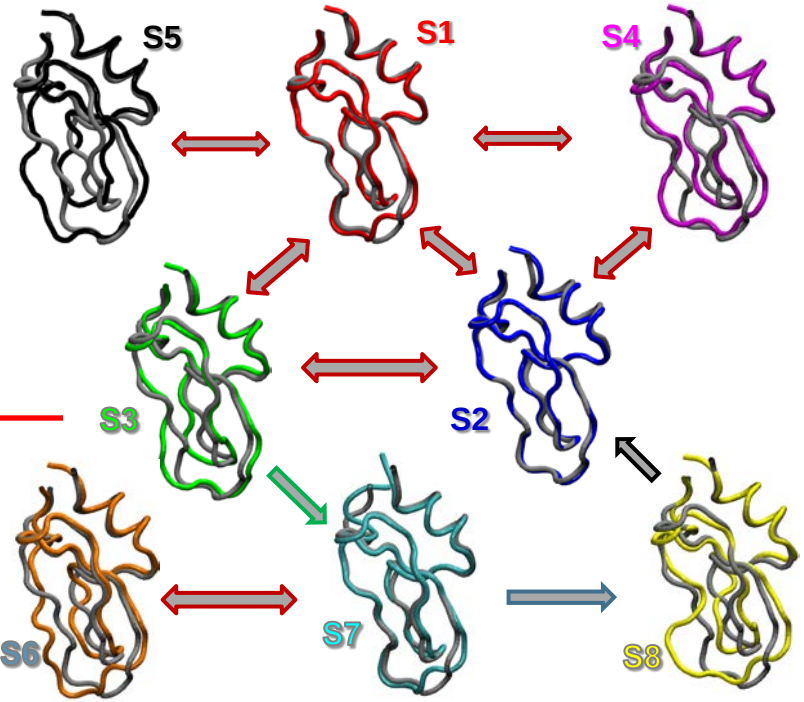
Perform ANM

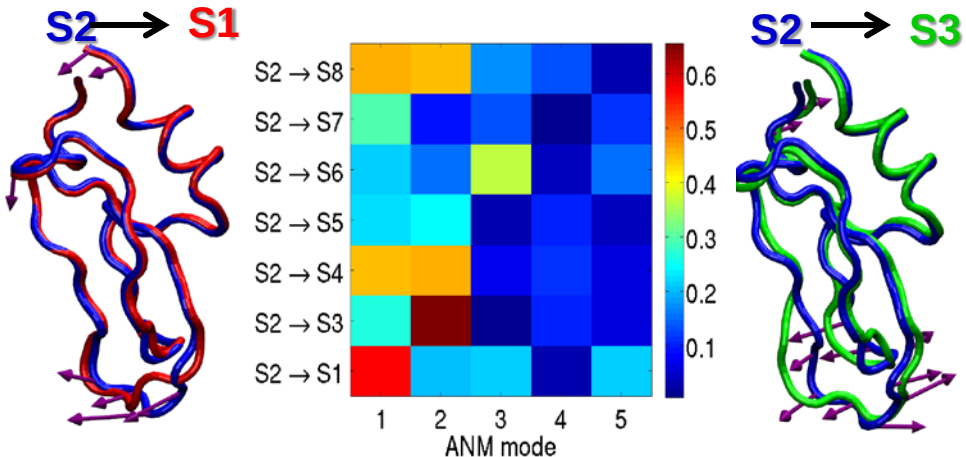
Global Motions from MD and ANM are similar

ANM modes predict transitions between substates

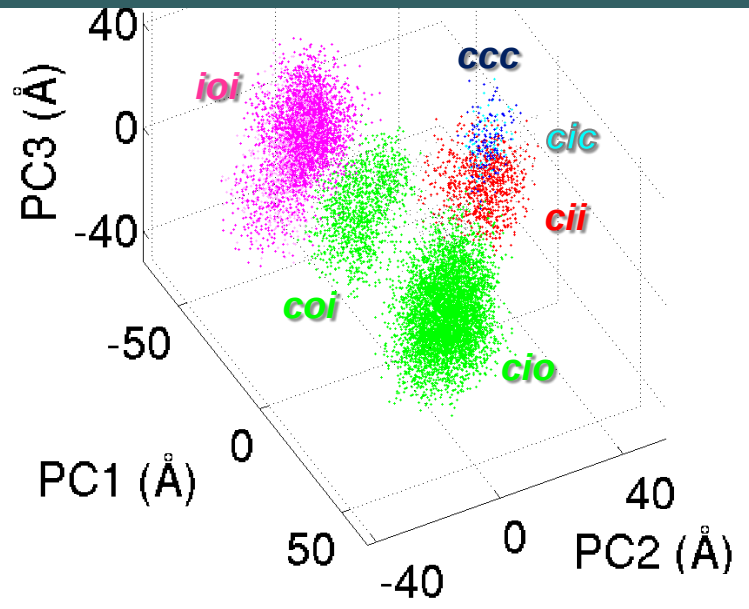


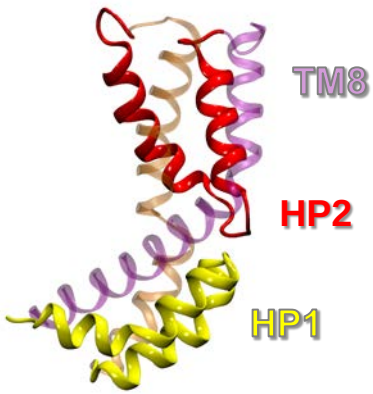
Calculate transition Rates



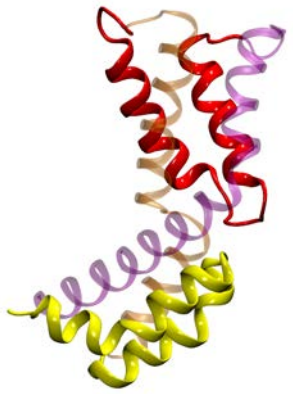


Gating mechanism of GltPh can be mapped by a few ANM modes

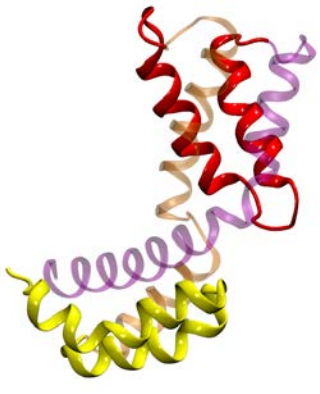




closed (c)



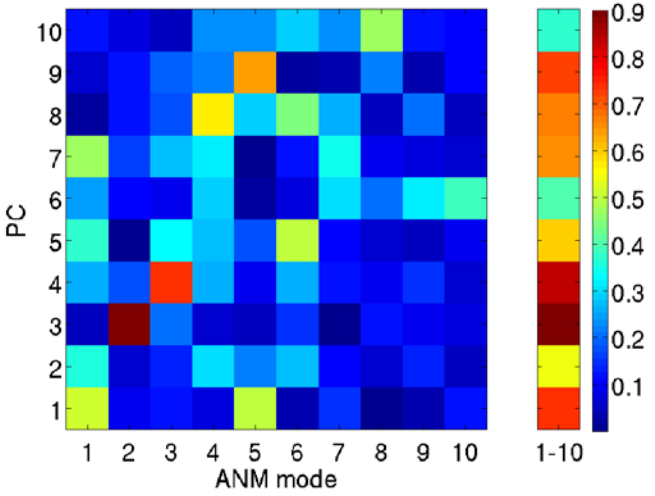
intermediate (i)

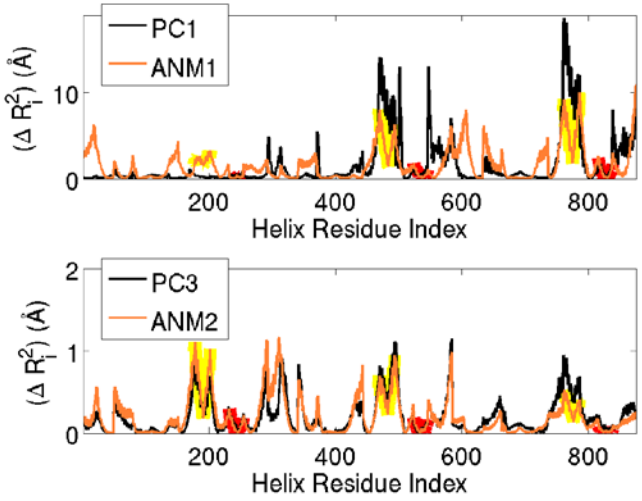


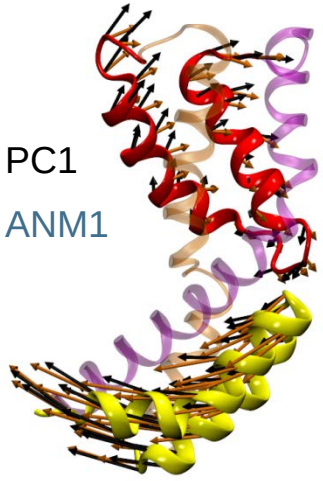
open (o)

Substates are characterized by the degree of opening of the binding site

Global Motions from MD and ANM exhibit overlaps





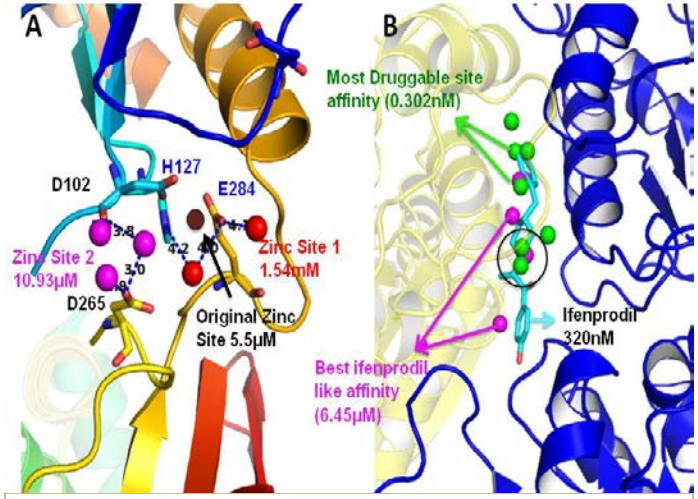


Challenges

- Multiple paths – which one? Collaboration with Emad Tajkhorshid for assessing the work involved in different paths
- Detailed Balance is not satisfied for transitions.
- Poor statistics for rare events (Dan’s WE method)

Development of a robust framework for efficient QC/MM calculations

Wymore



Druggability simulations performed for NMDA receptor NTD. (A) Hot spots near the zinc binding site of monomer N2B. Two high affinity sites are predicted, indicated by *red* and *magenta* dots. (B) Hot spots at the interface between the N2B and N1 monomers, consistent with the ifenprodil binding site, but with higher affinity.

Facilitate accurate construction of MM and QC descriptions of molecules & reactions

- developing ParamIT toolkit for MM force field parameterization of small molecules
- developing corrections to SMO methods for use in quantitatively-accurate free energy simulations
- building GUIs in Pymol and VMD for QC/MM model construction (leveraging Schulten's BTRC)

Computer Simulation of Biomolecular Dynamics and Reactions Workshop : June 5 - 8, 2012

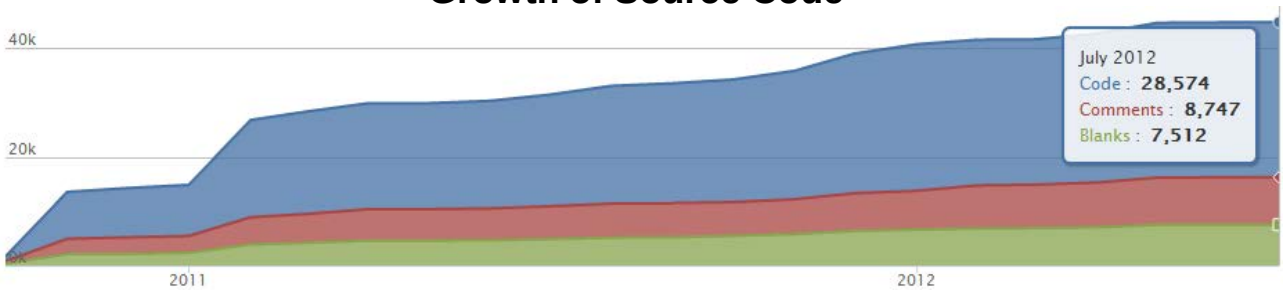
References

Field,M.J. 2008. The pDynamo library for molecular simulations using hybrid quantum mechanical and molecular mechanical potentials. *J. Chem Theory Comp* **4**:1151-1161; **Wymore** et al. 2007 Mechanistic implications of the cysteine-nicotinamide adduct in aldehyde dehydrogenase based on quantum mechanical/molecular mechanical simulations. *Biochemistry* **46**, 9495-9506.

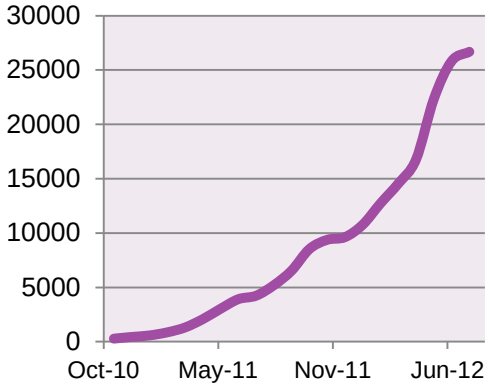
ProDy Code Analysis & Usage Stats

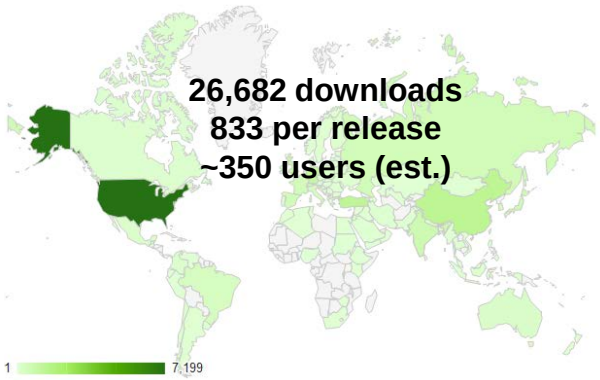
ProDy
Protein Dynamics Analysis in Python

Growth of Source Code

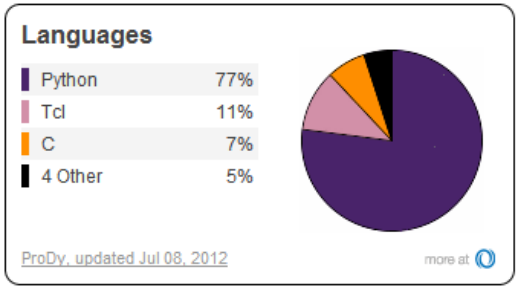


Cumulative Downloads





Source http://pypi.python.org/pypi/ProDy



Pages / Visit: 4.10

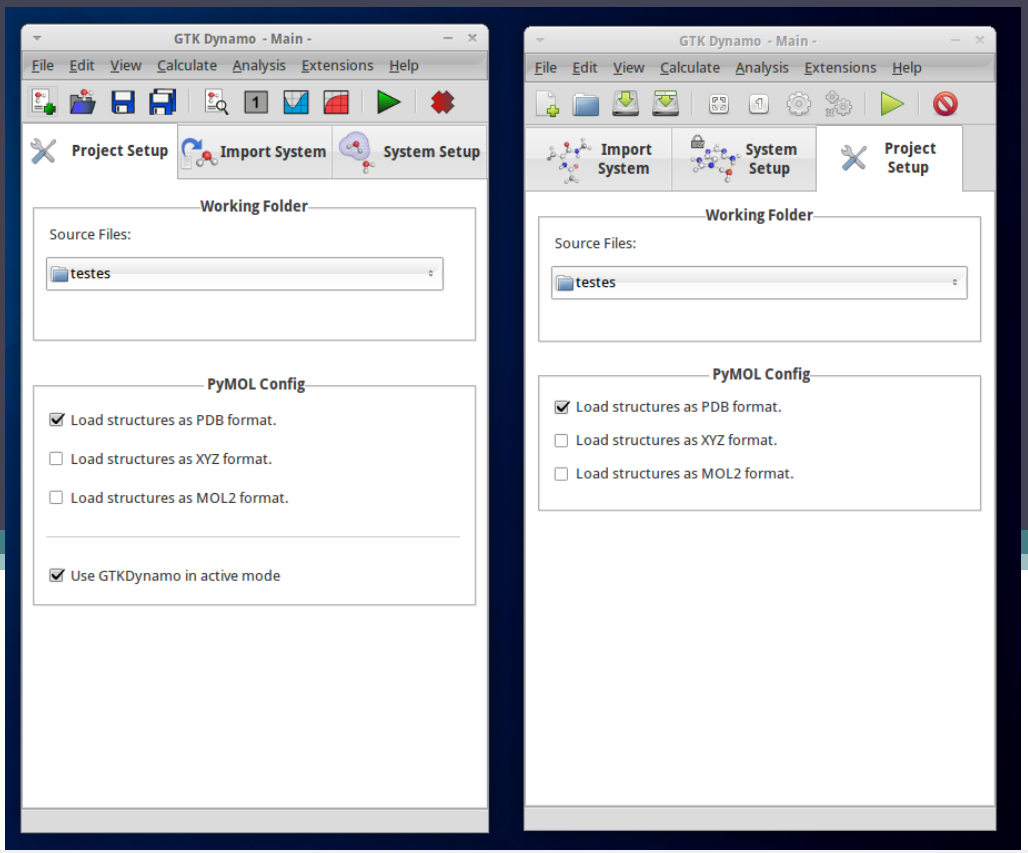
Avg. Visit Duration: 00:05:27

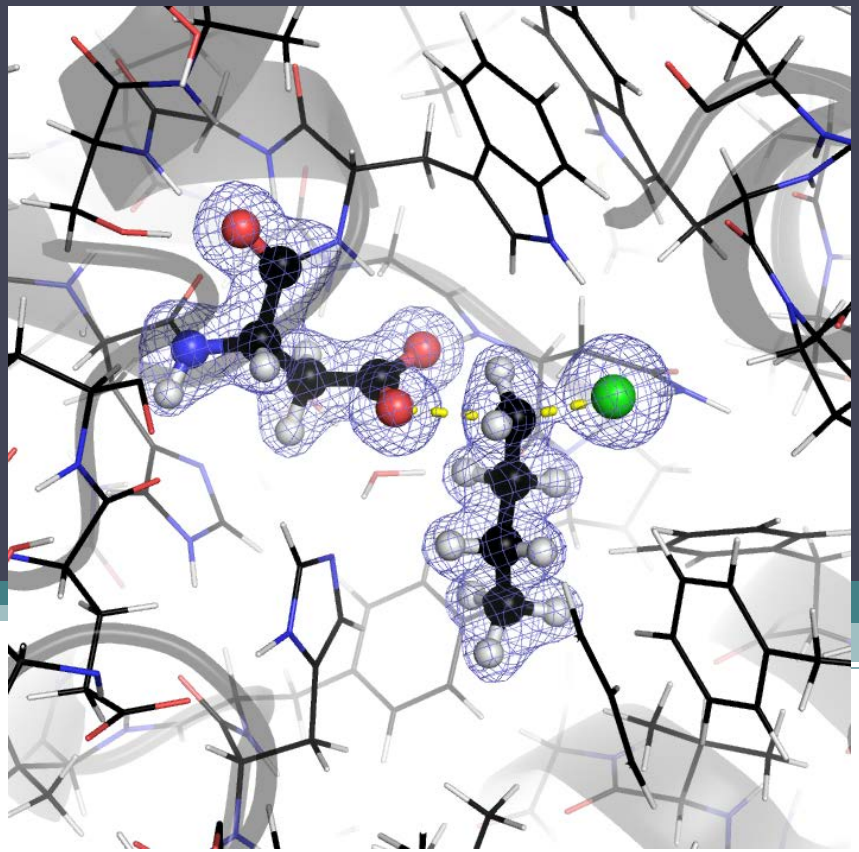
Source http://www.google.com/analytics/

Reference:

Bakan A, Meireles LM**, Bahar I**. (2011) ProDy: Protein dynamics inferred from theory and experiments *Bioinformatics* **27**:1575-7

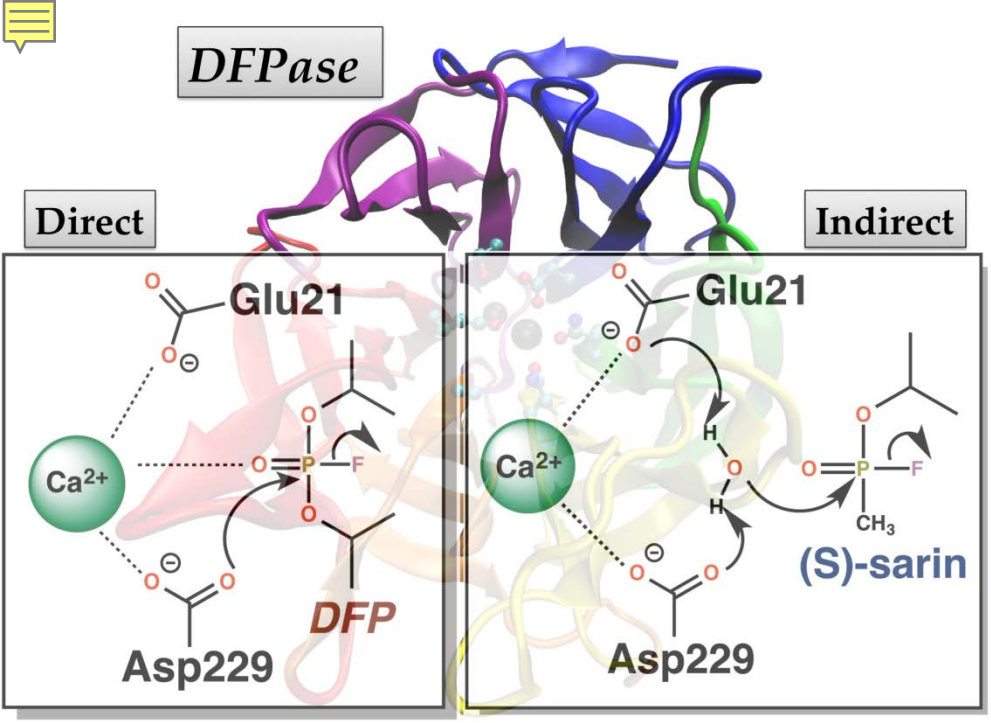
Development and Application of Computational Tools for Simulating Enzyme Reactions

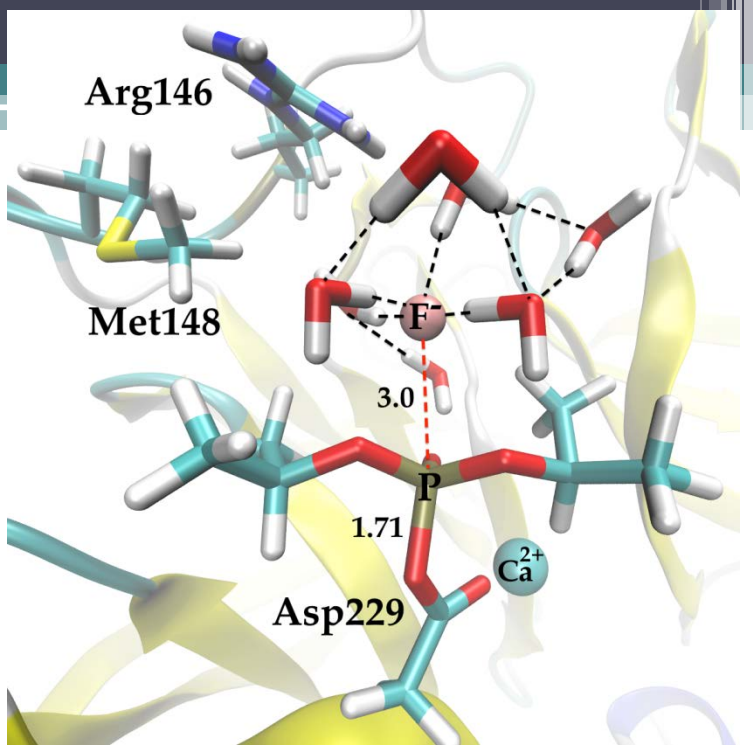




GTKDynamo: a PyMOL plug-in for QC/MM hybrid potential simulations. J. F. R. Bachega, L. Fernando, S.M. Timmers, L. Assirati, L. R. Bachega, M. J. Field, T. Wymore **(***Journal of Computational Chemistry***, *34*:2190, 2013).**

https://sites.google.com/site/gtkdynamo/





Hydrolysis of DFP and the Nerve Agent (*S*)-Sarin by DFPase Proceed Along Two Different Reaction Pathways: Implications for Engineering Bioscavengers. Troy Wymore, Martin J. Field, Paul Langan, Jeremy C. Smith and Jerry M. Parks (manuscript submitted)

Integrating Python programs for Protein Design

ProDy → **PyRosetta** ↔ **pDynamo**

Conformations **MC Sampling** **QC/MM Scoring**

Collaboration & Service Projects

C&SP#	BTRC PI/co-I (Institution)	Collaborators and institution(s)		Collaboration & Service Project Title
1	Bahar (Pitt)	Altman Gierasch	Stanford U (Simbios) U. of Mass.	Structural dynamics of biomolecular machines: Application to bacterial chaperonins and HSP70 chaperones (collaboration with *Simbios* lab)
2	Bahar (Pitt)	Roux, Schulten, Weinstein, Tajkhorshid, Perozo, Greger	U of Chicago, U of Cambridge (UK), Cornell U, U of Illinois at UC	*Membrane Proteins Structure and Dynamics Consortium (MPSDC)* Computational Core, and AMPA receptors structural dynamics
3	Langmead (CMU)	Bailey-Kellogg, Ramakrishnan, Friedman	Dartmouth, Virginia Tech, Purdue	Integration, prediction, and generation of mixed mode information using graphical models, with applications to protein-protein interactions

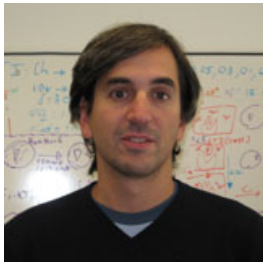
A network of conserved and coevolving residues mediate allosteric communication in Hsp70

Ignacio J. General[1], Ying Liu [1], Mandy E. Blackburn[2], Lila M. Gierasch[2,3] and Ivet Bahar[1]

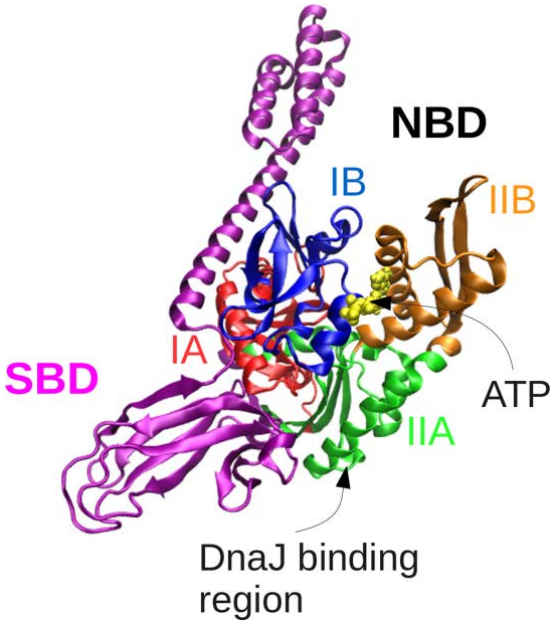
[1]*Department of Computational and Systems Biology, School of Medicine, University of Pittsburgh, Pennsylvania, USA,*

[2]*Department sof Biochemistry, and Chemistry, University of Massachusetts Amherst, Massachusetts, USA.*

Dr. Lila Gierasch



Dr. Ignacio General

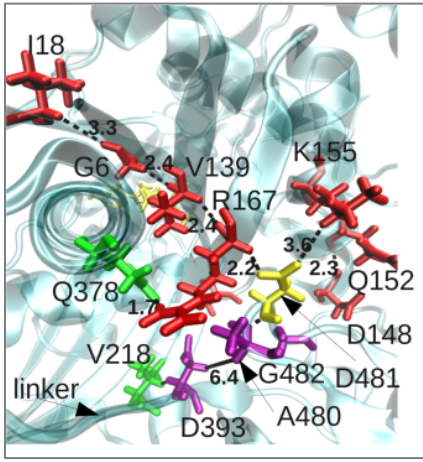


We identified two networks of closely interacting conserved residues, in subdomains **IA** and **IIA** of the nucleotide binding domain (NBD), which are proposed to play a role in propagating allosteric signals between the nucleotide-binding and the substrate-binding domains.

Residues in subdomain IIA show strong co-evolving tendencies, consistent with the needed adaptability to DnaJ recognition.

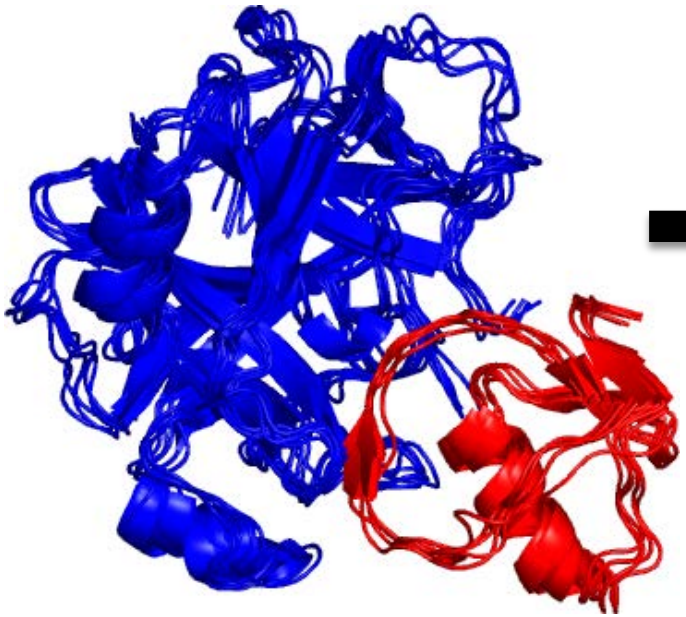
Functional assays in the Gierasch lab verified the critical role of a number of residues – mutations at those sites abolished interdomain communications

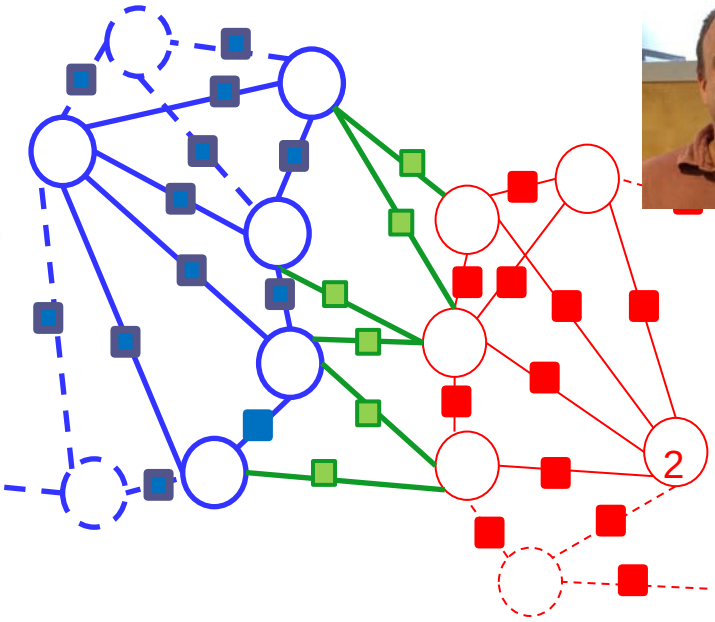
Perturbation Response Scanning (PRS) is used to evaluate the **sensitivity** and **influence** of each residue. Linker residues are unique, as they serve both as *sensors* (near DnaJ-binding site) and *effectors.*



* submitted

PGMs for intermolecular interactions





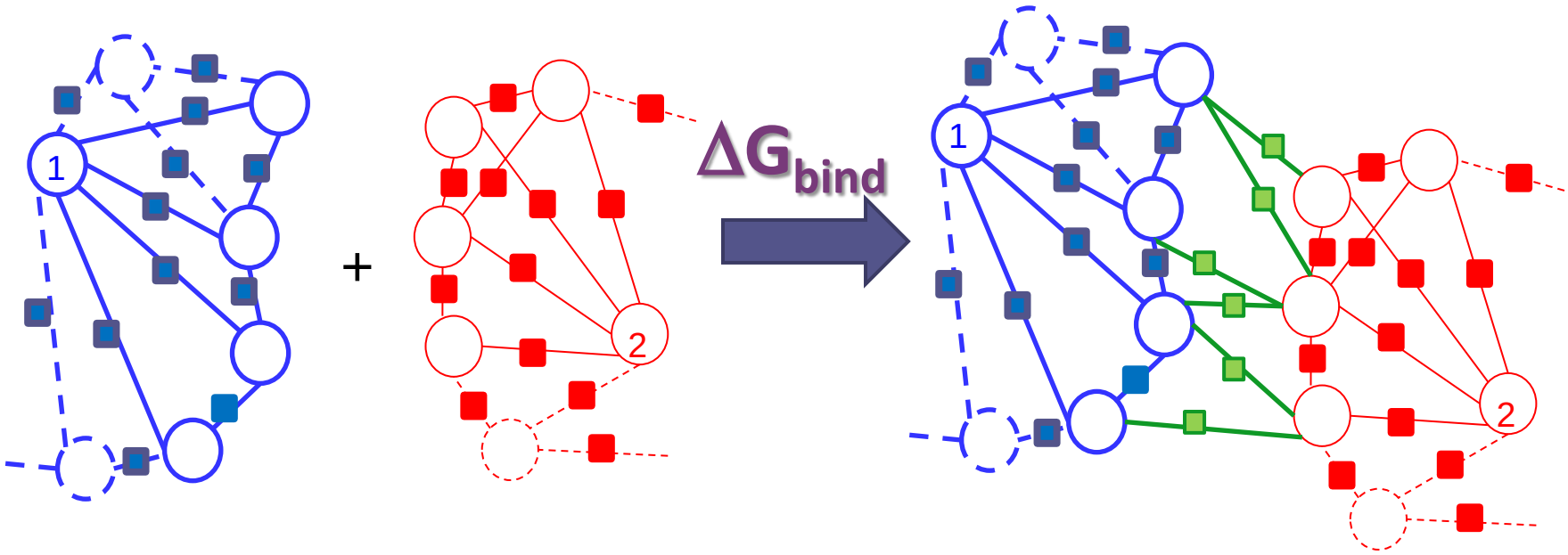
Langmead Lab

Intermolecular interactions
Intramolecular interactions
Intramolecular interactions

The topology and potentials will be

- learned from simulations (MD, WE), or
- approximated from a single structure

PGMs for intermolecular interactions



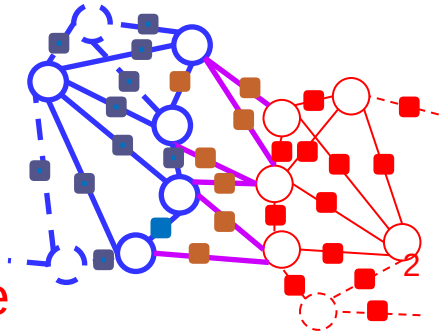
using $\Delta G_{bind} = - RT \ln [Z_{complex} / Z_1 Z_2]$,

DBP2

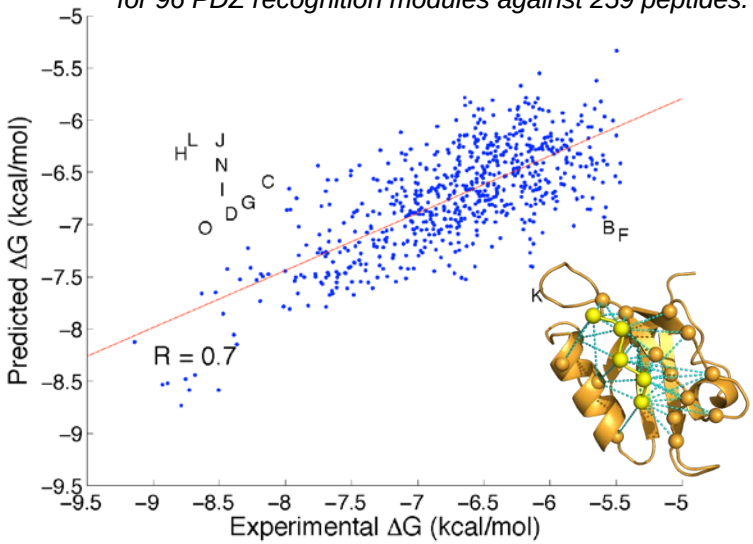
where Z is the partition function $Z = \sum_X \{ \prod_{a \in \Phi} \phi_a(x_a) \}$

PGMs of Protein-Protein Interactions

- Funding: NSF
- Collaborating Investigators:
  - **Langmead** (CMU), Bailey-Kellogg (Dartmouth), Ramakrishnan (V. Tech), Friedman (Purdue)
- Goals: Develop PGMs over sequence *and* structure
  - Applications: Design of protein-protein interactions

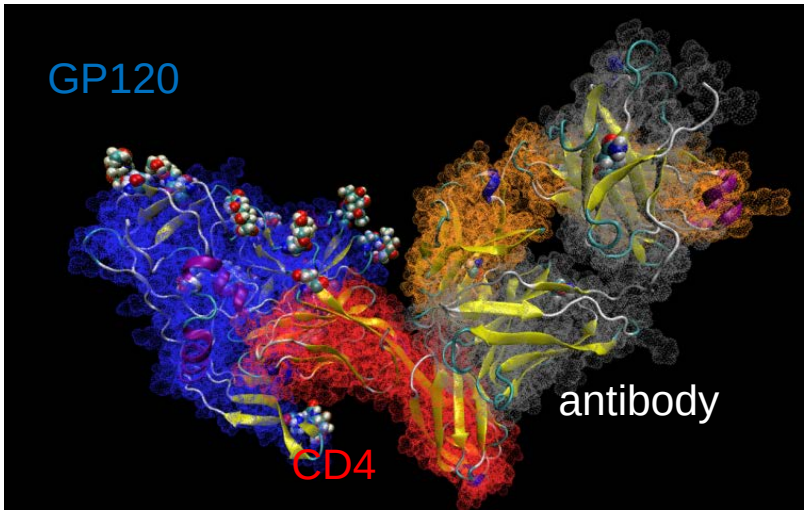


for 96 PDZ recognition modules against 259 peptides.



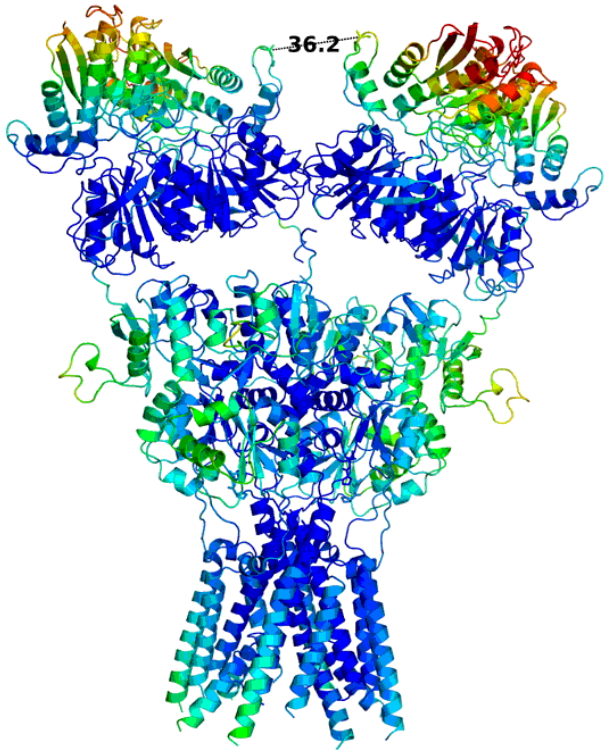
Ex (1) Binding Free Energy Calculations

against MDR mutations in the HIV envelope protein gp120.



Ex (2) Re-design of HIV entry inhibitor

ENM-based methods help infer **functional mechanisms**



- cooperative mechanisms of substrate binding/unbinding, translocation, and **signaling**
- accessible substates and passages
- effect of **multimerization**, or complex/assembly formation
- sites/interactions of functional significance

Anindita Dutta

C&S2 Signaling dynamics of AMPARs and NMDARs

Sukumaran et al (2011) *EMBO J* (collaboration with Ingo Greger, Cambridge, UK); Dutta et al (2012) *Structure*

Challenges and Future Directions

- Development of models capable of simulating membrane remodeling, vesicle formation, endocytosis
- Integration of coarse-grained for membranes with coarse-grained for proteins (ENMs + Martini), or coarse-grained NAMD and adaptive ANM
- Deriving quantitative data from coarse-grained approaches
- Closer examination of druggability properties

Challenge: Integration with coarse-grained modeling of membrane dynamics

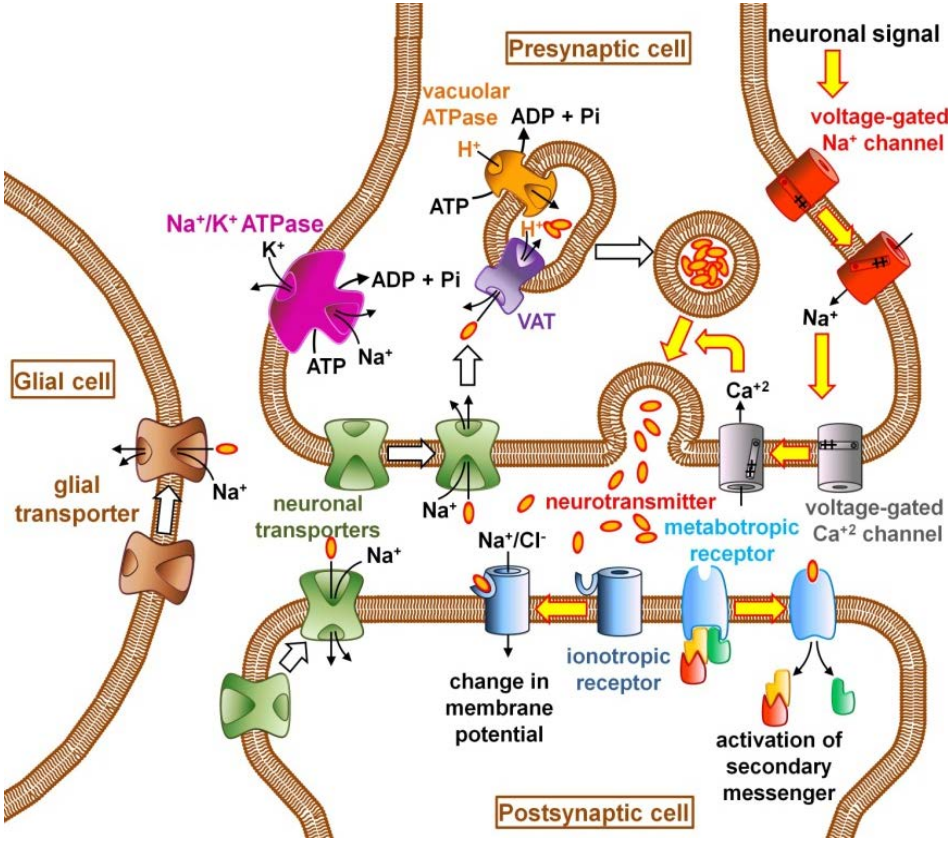


Figure: Courtesy of Elia Zomot

Potential collaborators

Markus Deserno,
Assoc Prof, Physics, CMU

Schulten lab