Dynamical View of Energy Coupling Mechanisms in Active Membrane Transporters

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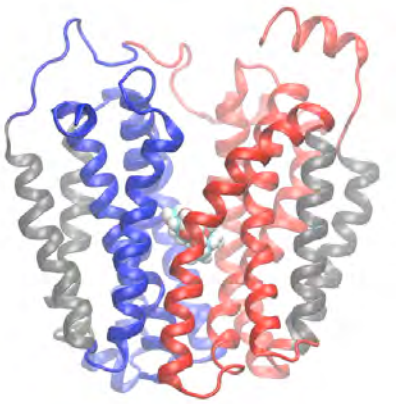
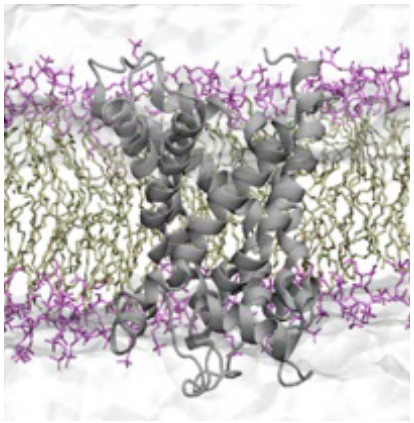
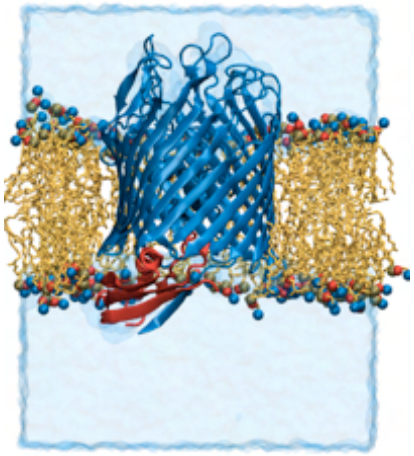


Proton-Driven Sugar Transport in LacY

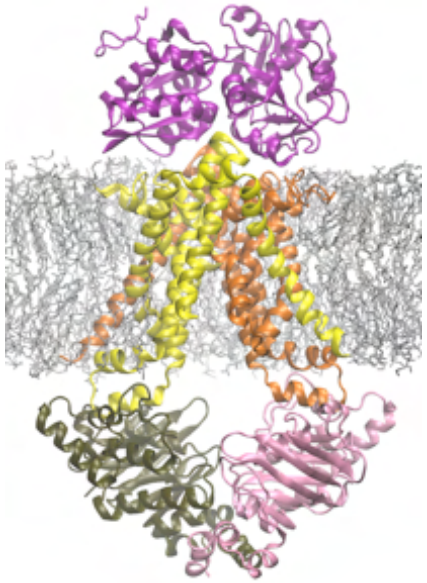


ADP/ATP Exchange in Mitochondria (AAC)

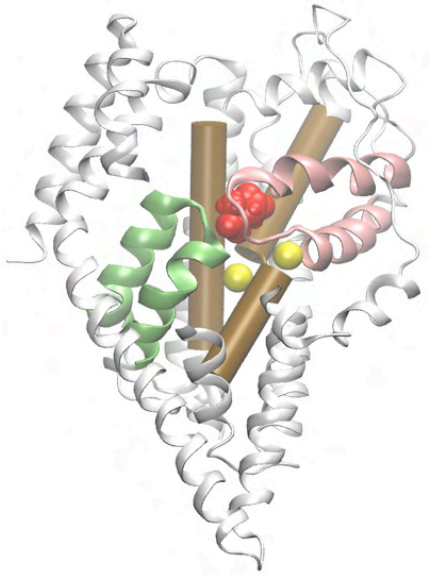
25th Molecular Modeling Workshop
April 2011, Erlangen, Germany



Mechanically(?) Driven Transporters of the Outer Membrane

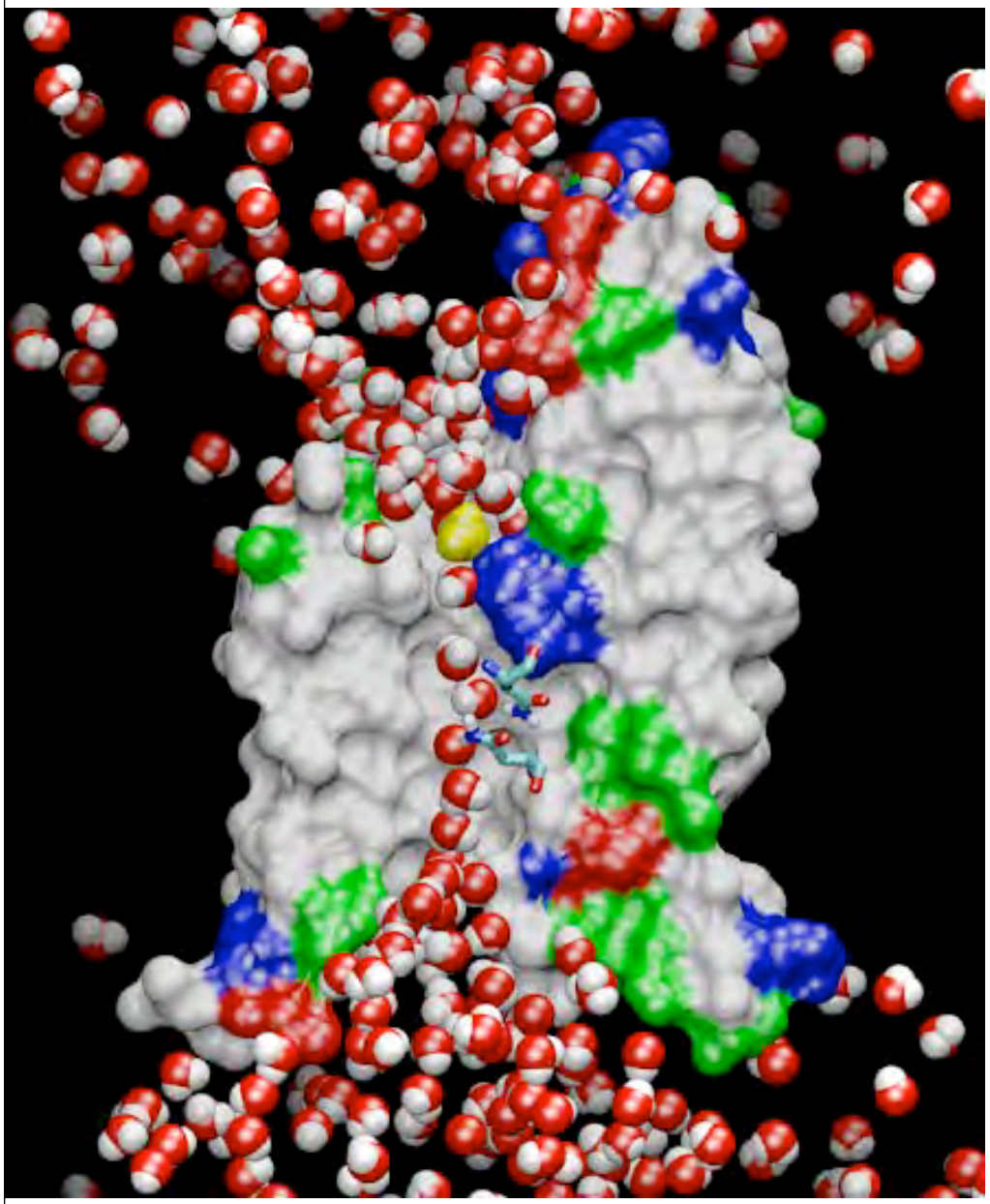


ATP-Driven Transport in ABC Transporters



Na^+-Driven Neurotransmitter Uptake Glutamate Transporter

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

Access to More Computational Power

HP 735 cluster
12 processors
(1993)

SGI Origin 2000
128 processors (1997)

Blue Waters (UIUC)
200,000+ processors (2012)

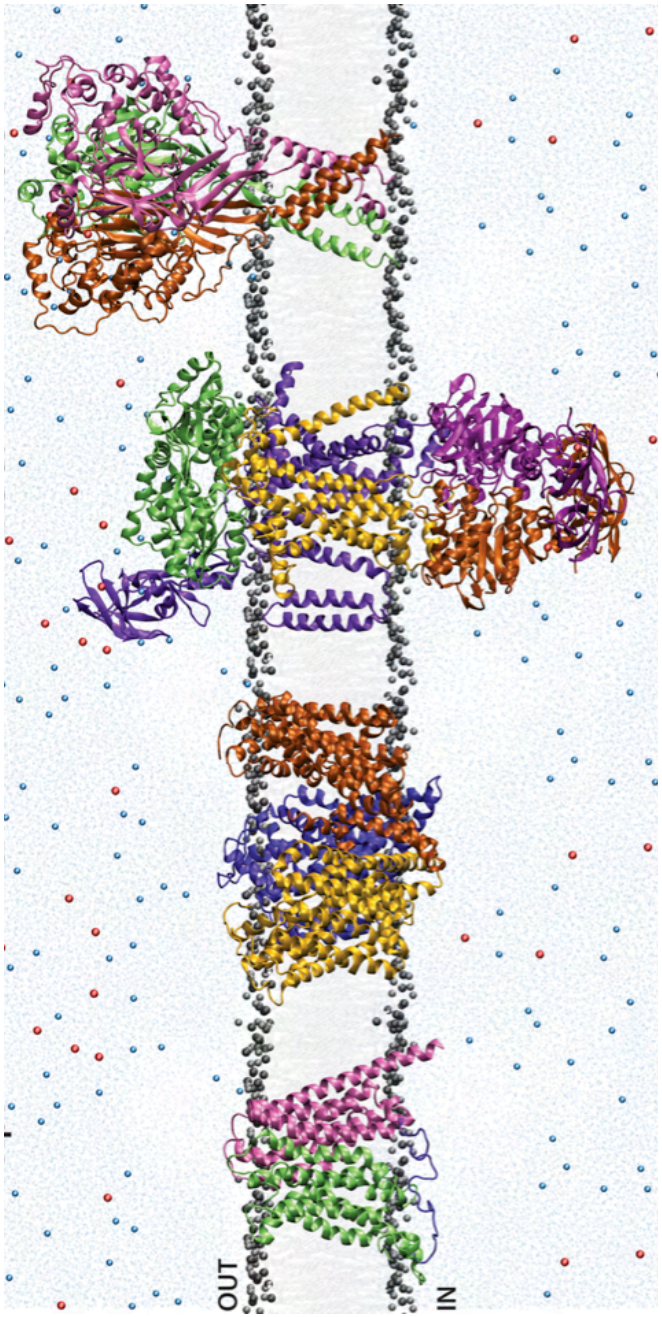
Anton/DESHAW/PSC
512 processors (2010)

PSC LeMieux AlphaServer SC
3000 processors (2002)

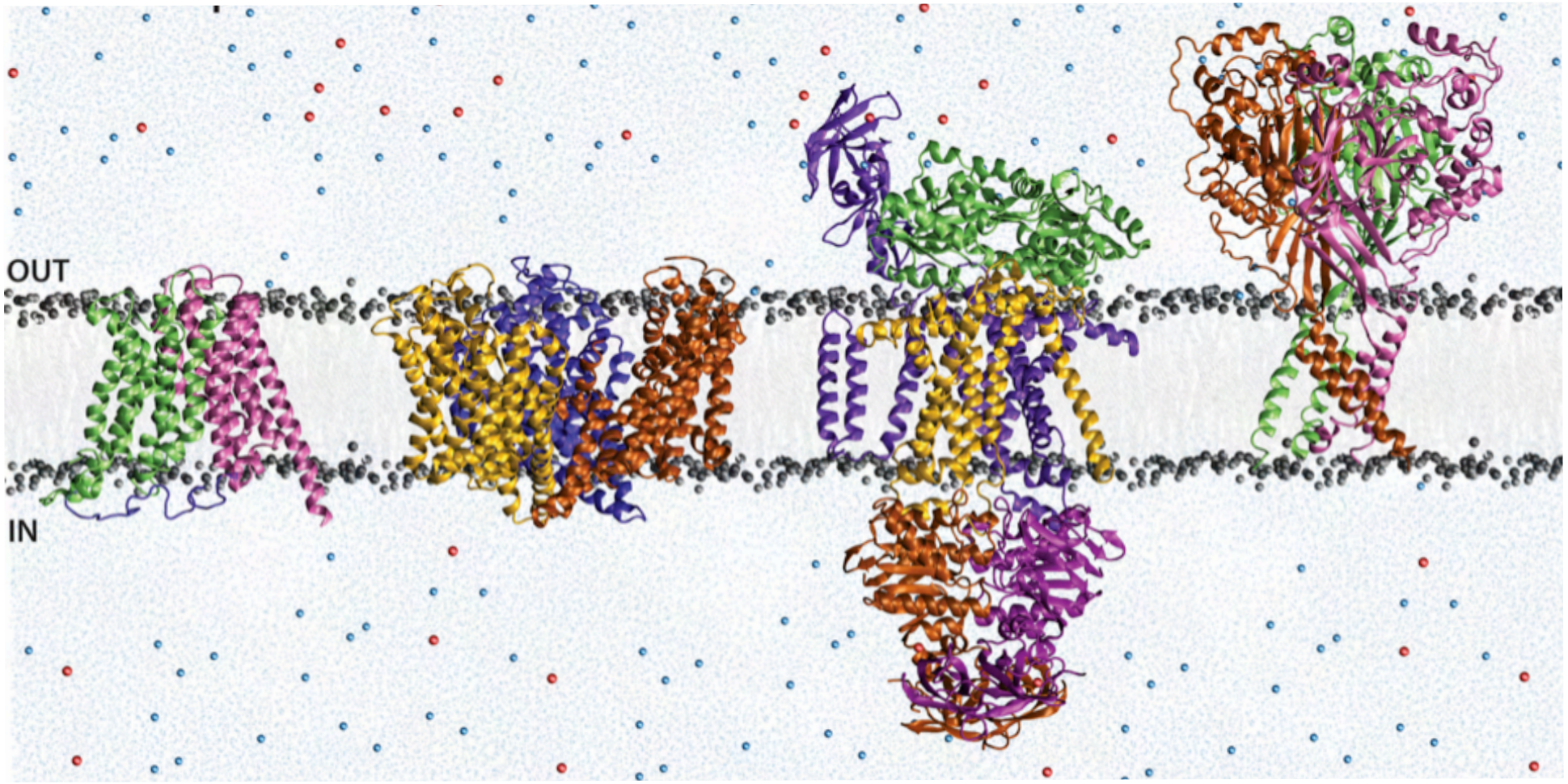
Ranger/Kraken
~60,000 processors (2007)

Complexity of Transporter Function

- Active transport is **coupled** to an energy source in the cell
- Transporters Function on ***μs* and longer** time scales
- Protein conformational changes of various forms and magnitudes coupled to **step-wise** vectorial translocation of the substrate and co-transported materials
- The **sequence** of molecular events is largely unknown

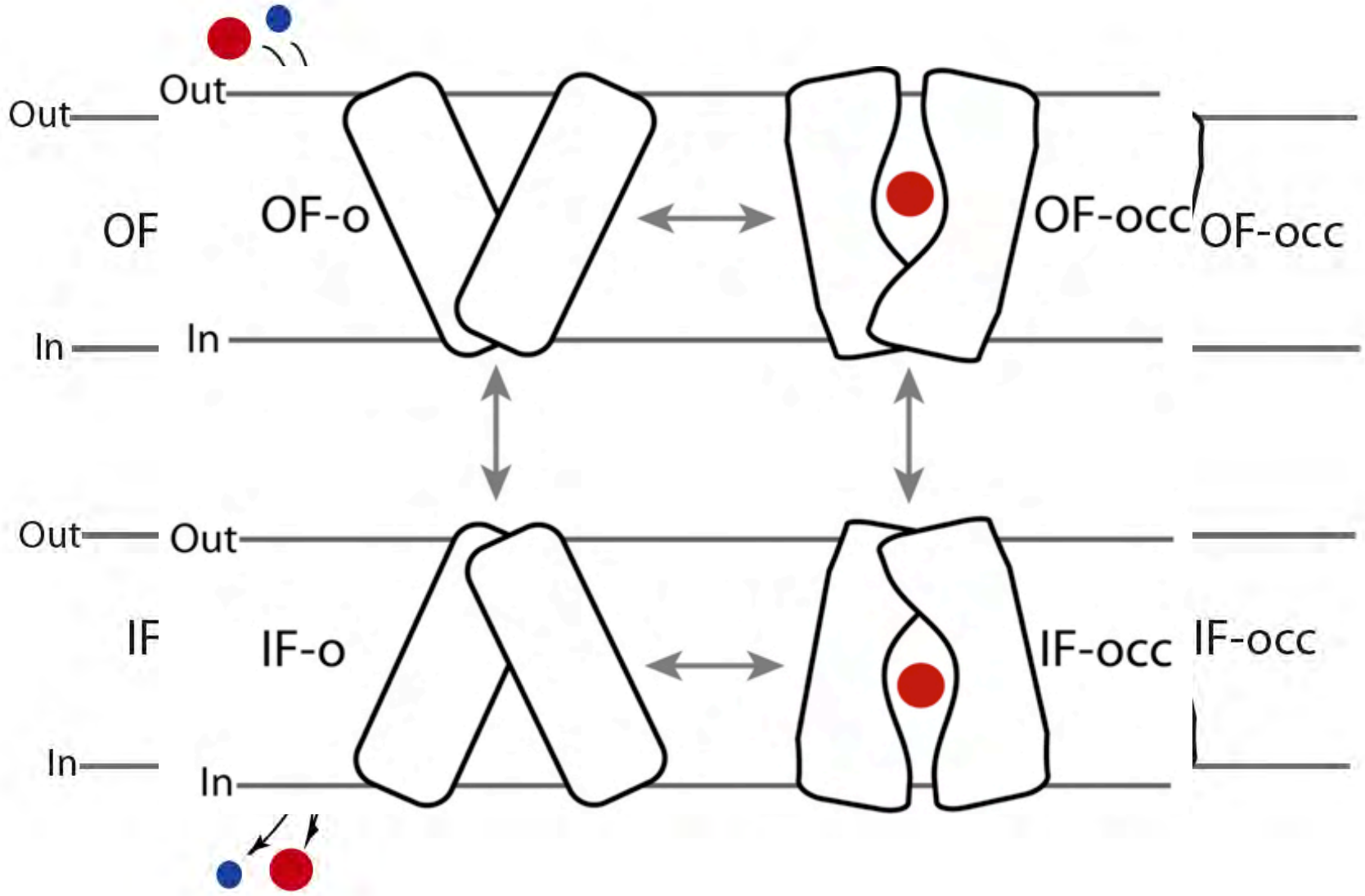


In situ Molecular Dynamics Simulations



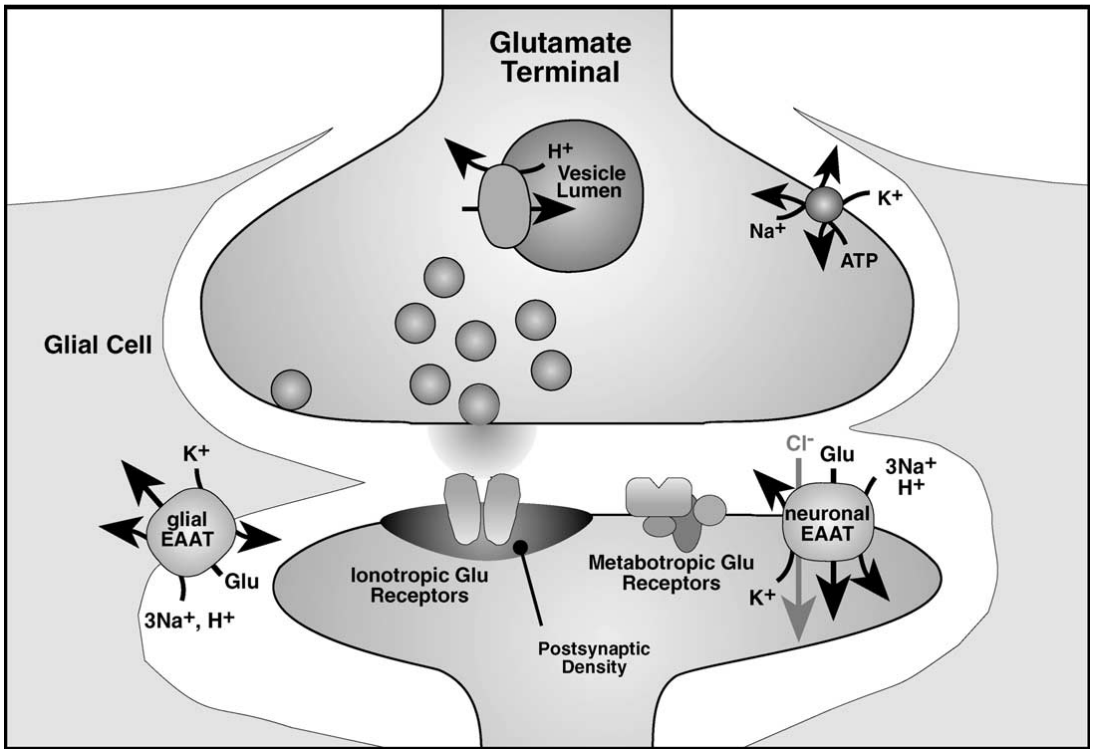
Atom count: 100-500k
~10 ns/day on 128-1024 processors
100-500 ns for each system

Alternating Access Model

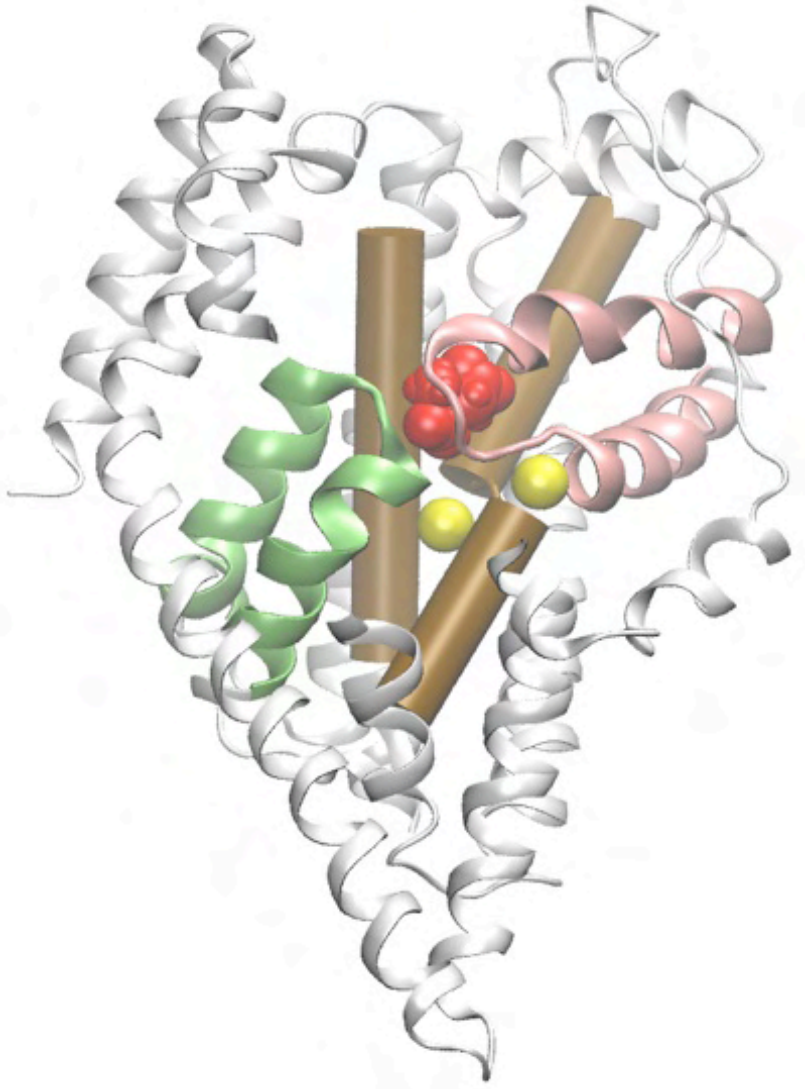


Hard to define the number of (sub)states involved?

Glutamate Transporter



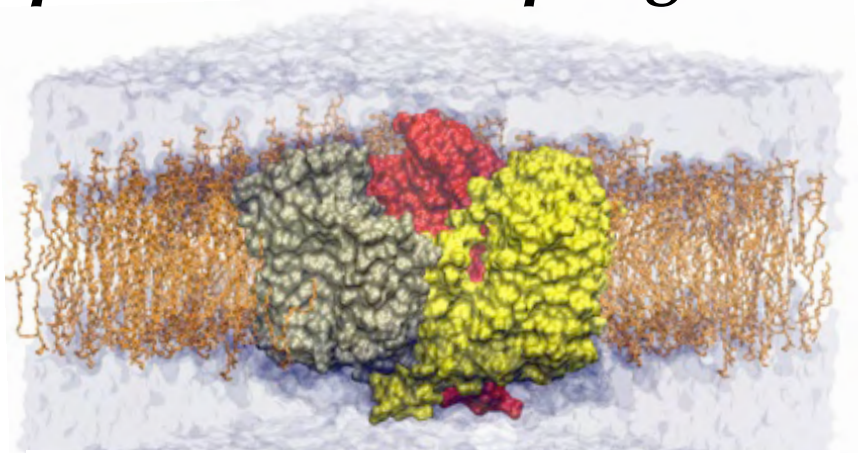
Amara and Fontana, Neurochemisty International 41:313-318 (2002)



Glutamate Transporter (Glt_{ph})

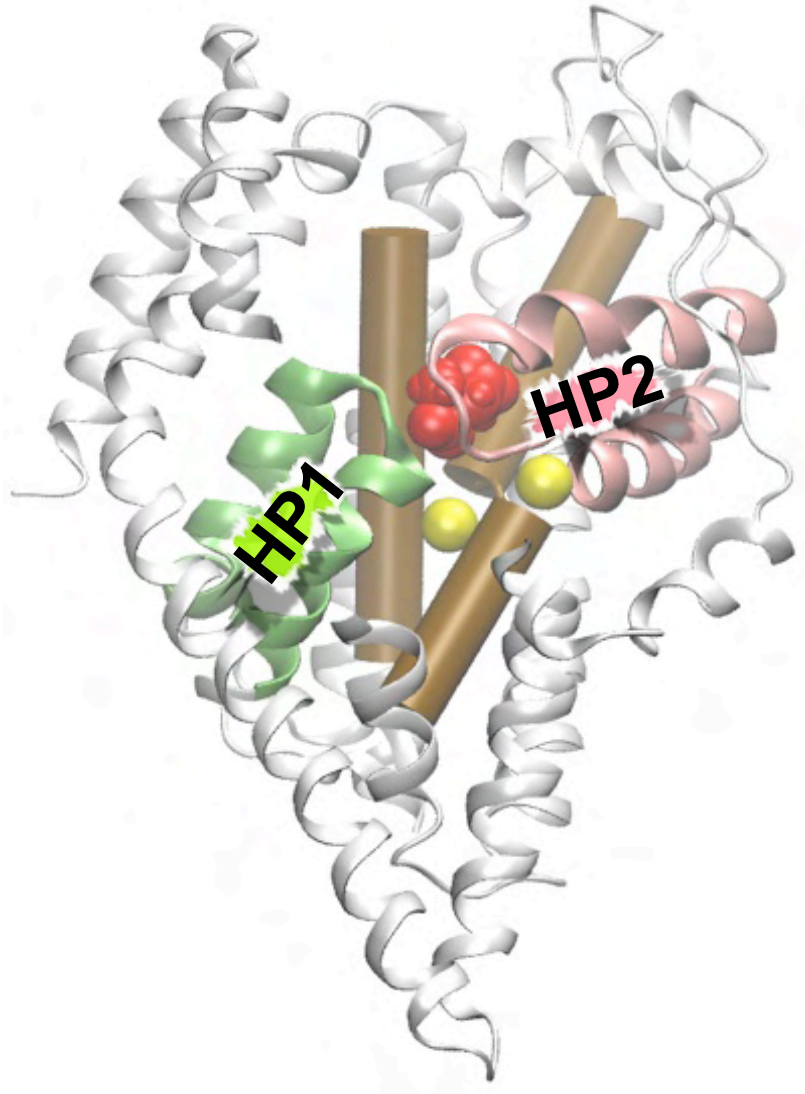
Yernool, Boudker, Jin, Gouaux. Nature, 431: 811–818, **2004**.

Sequence and ***Coupling*** of Events in an Ion-Coupled Transporter



monomer	substrate	Na1	Na2
S1A	+	+	+
S1B	+	+	-
S1C	+	-	+
S2A	+	-	-
S2B	-	-	-
S2C	-	-	-
S3A	-	+	+
S3B	-	+	-
S3C	-	-	+

Z. Huang and E. Tajkhorshid, *Biophysical Journal* **2008**

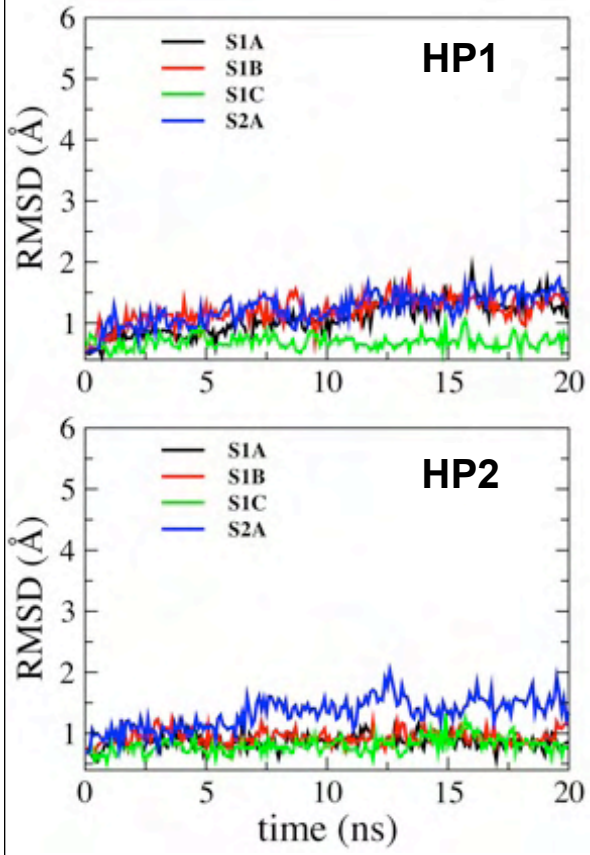


Glutamate Transporter (Glt_{ph})

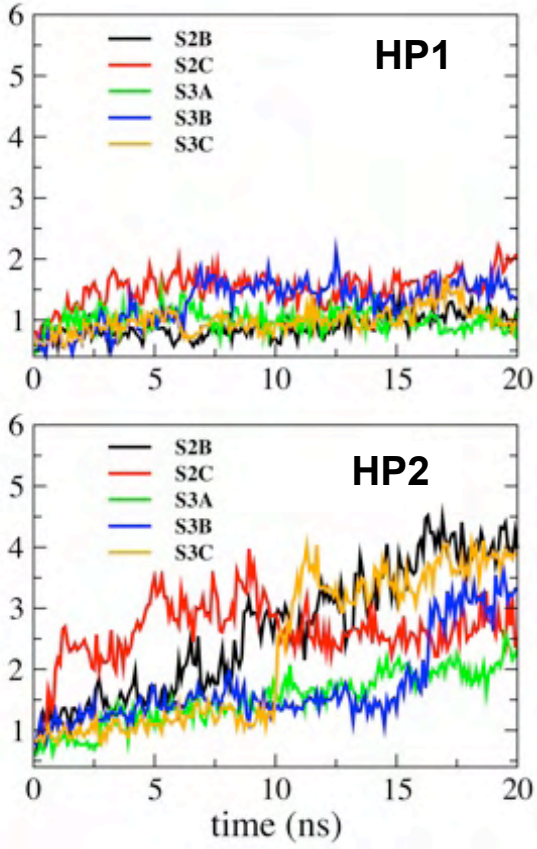
Yernool, Boudker, Jin, Gouaux. Nature, 431: 811–818, **2004**.

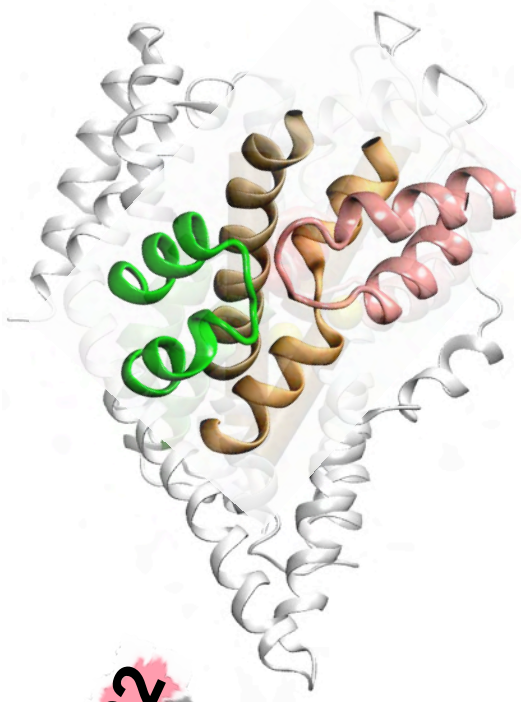
Dynamics of the *Extracellular* Gate

w/ substrate



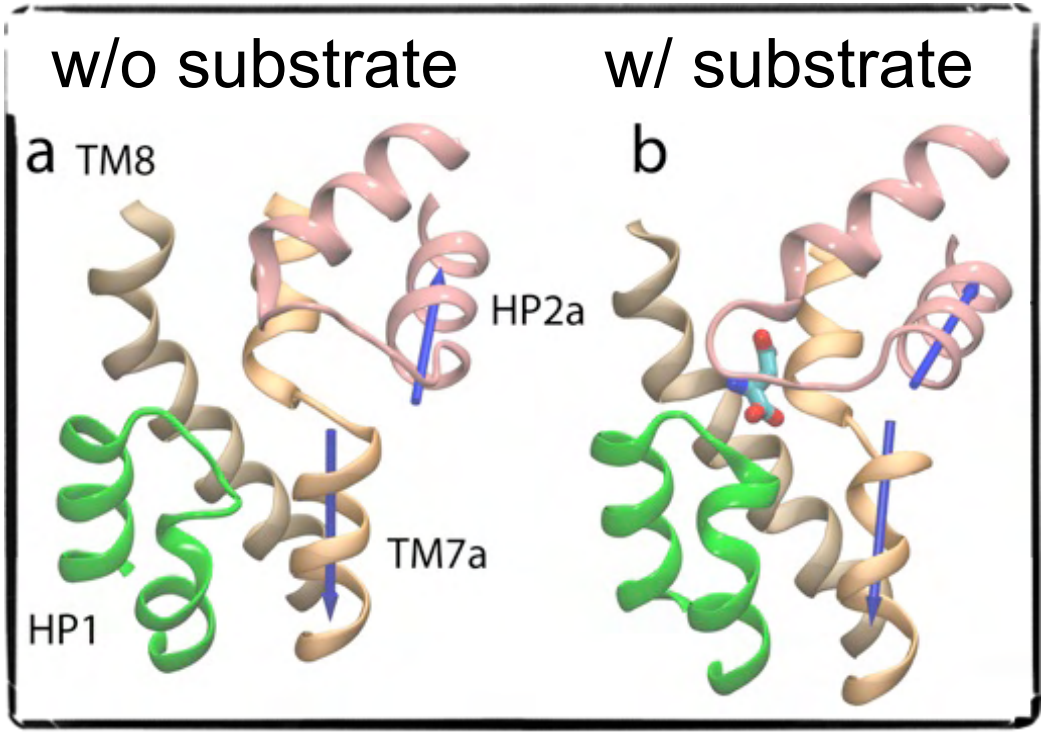
w/o substrate

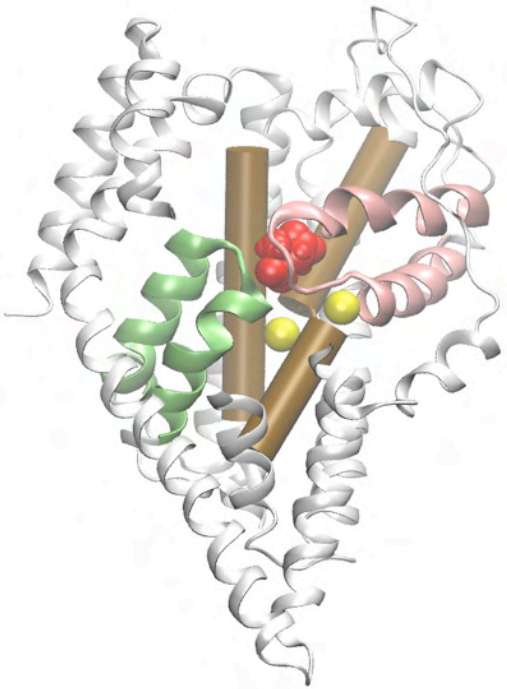




Z. Huang and E. Tajkhorshid, *Biophysical Journal* **2008**

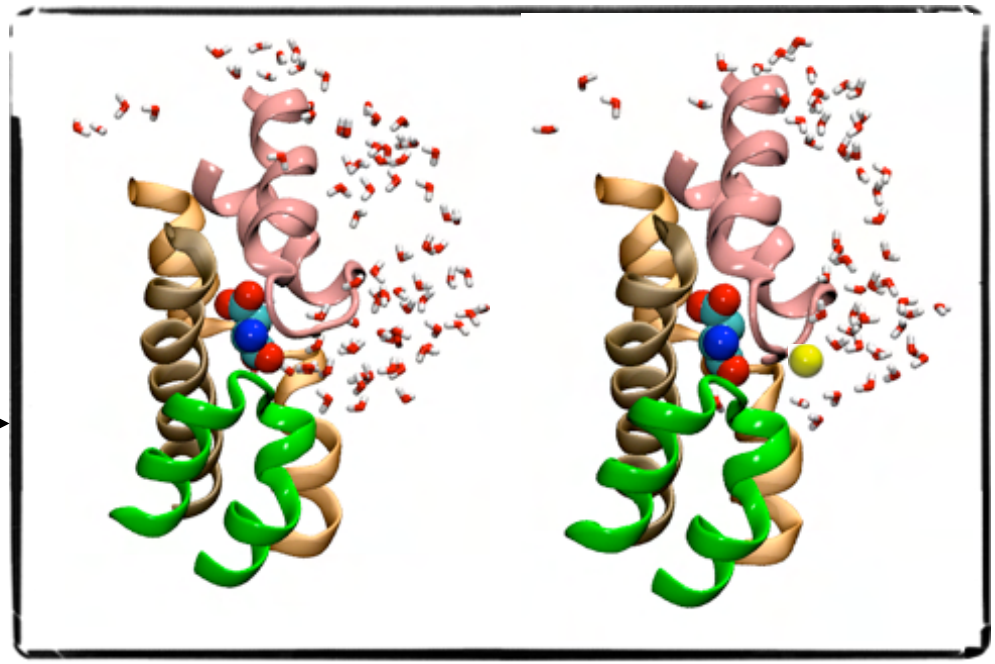
Ion-Substrate Coupling on the Extracellular Side





Substrate binding forms the Na2 binding site

Na2 seals the binding site



Z. Huang and E. Tajkhorshid, *Biophysical Journal* **2008**

Inward-Facing, Occluded Glt_{ph}

doi:10.1038/nature08616 nature

ARTICLES

Transport mechanism of a bacterial homologue of glutamate transporters

Nicolas Reyes[1], Christopher Ginter[1] & Olga Boudker[1]

Glutamate transporters are integral membrane proteins that catalyse a thermodynamically uphill uptake of the neurotransmitter glutamate from the synaptic cleft into the cytoplasm of glia and neuronal cells by harnessing the energy of pre-existing electrochemical gradients of ions. Crucial to the reaction is the conformational transition of the transporters between outward and inward facing states, in which the substrate binding sites are accessible from the extracellular space and the cytoplasm, respectively. Here we describe the crystal structure of a double cysteine mutant of a glutamate transporter homologue from *Pyrococcus horikoshii*, Glt_{Ph}, which is trapped in the inward facing state by cysteine crosslinking. Together with the previously determined crystal structures of Glt_{Ph} in the outward facing state, the structure of the crosslinked mutant allows us to propose a molecular mechanism by which Glt_{Ph} and, by analogy, mammalian glutamate transporters mediate sodium-coupled substrate uptake.

Reyes, et al., Nature **2009**

Different Modes of Cytoplasmic (HP1) and Extracellular (HP2) Gating

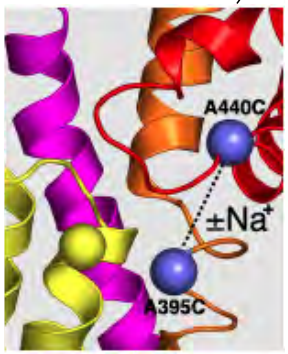
Leighton, et al., JBC (2006)

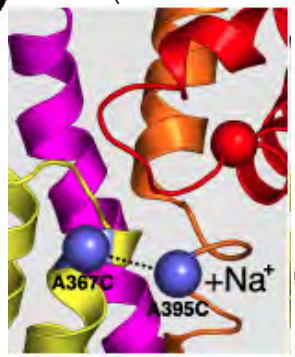
Coupled to substrate release

Na1-controlled

periplasm

***inward*-facing gate**





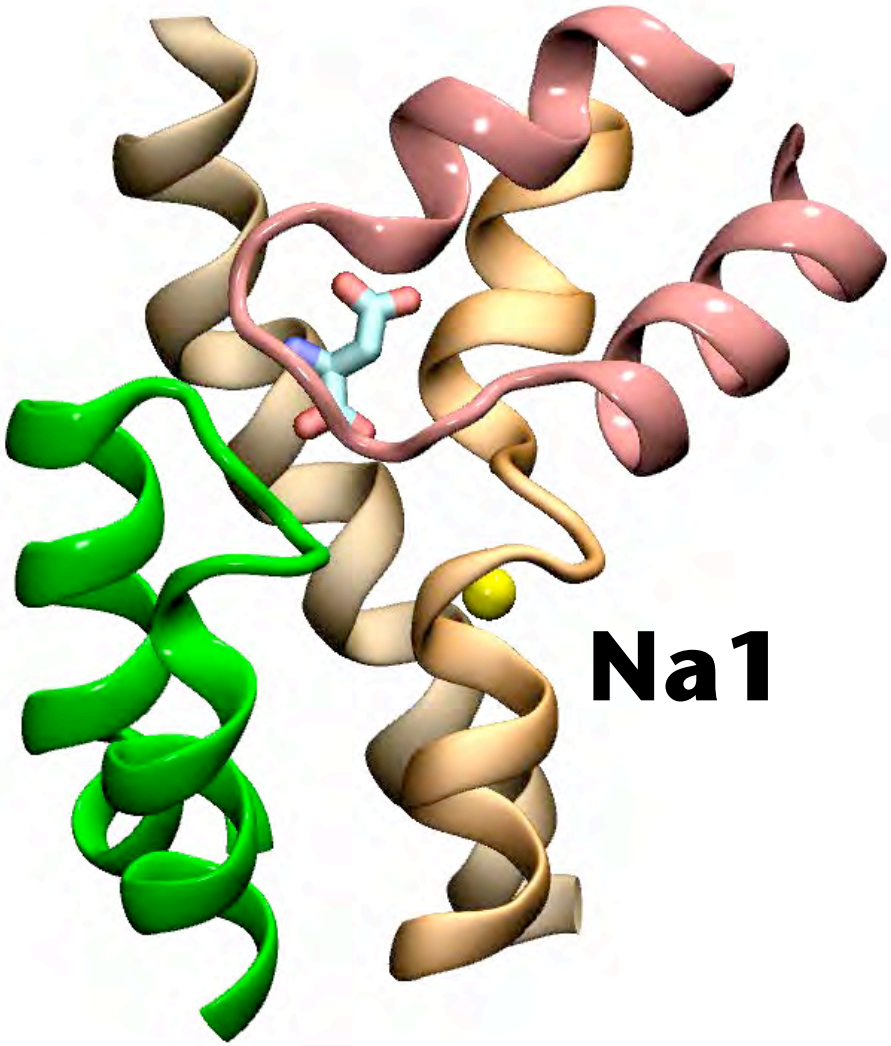
***outward*-facing gate**

Substrate-controlled

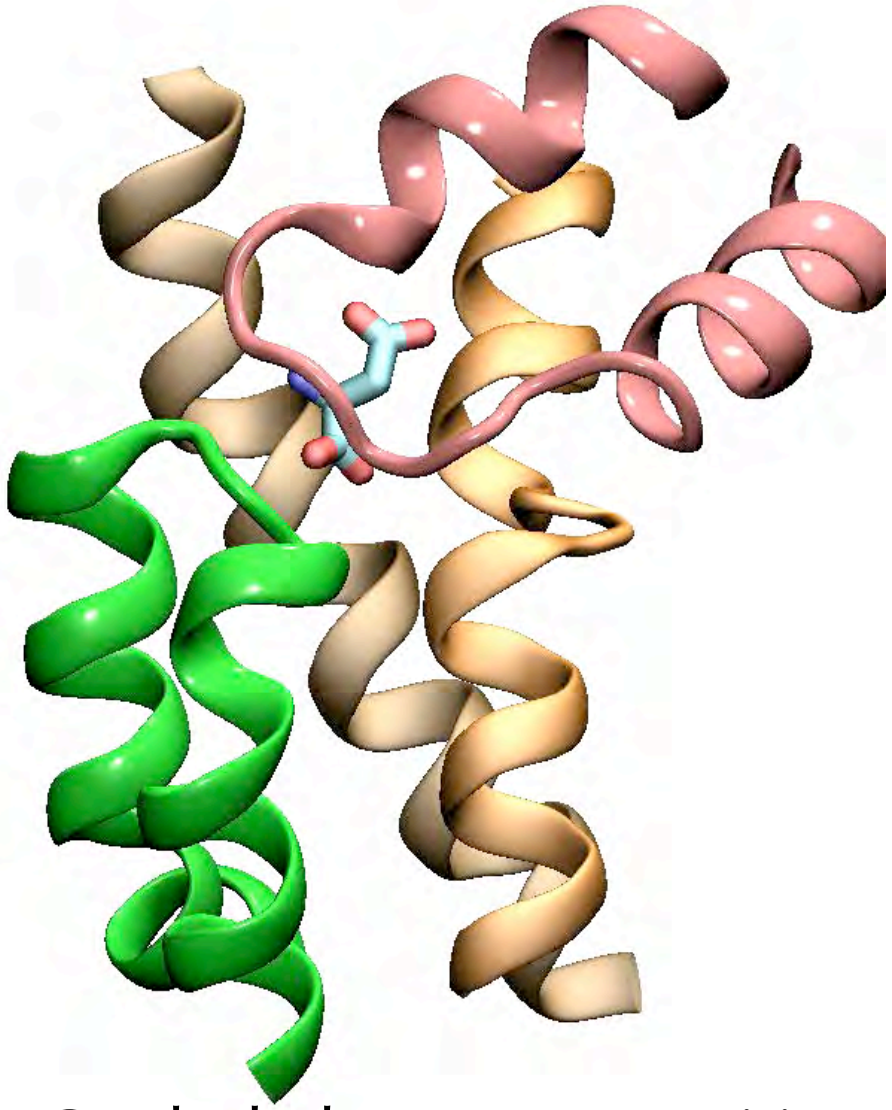
Coupled to Na2 binding

cytoplasm

Na1 Dependence of the Cytoplasmic Gate

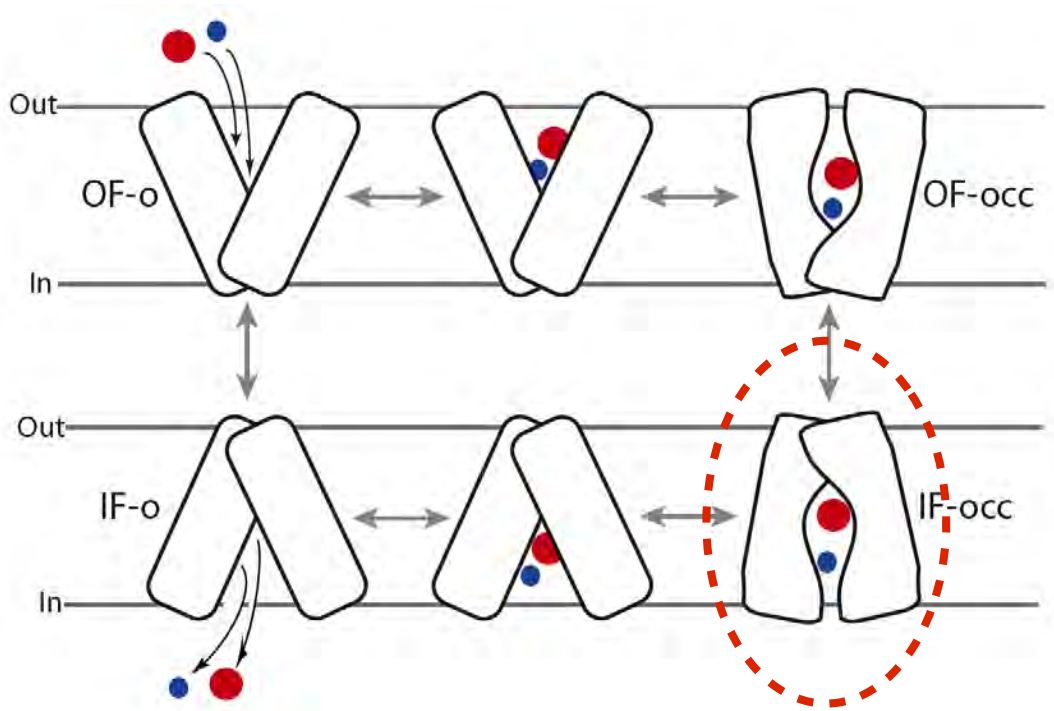


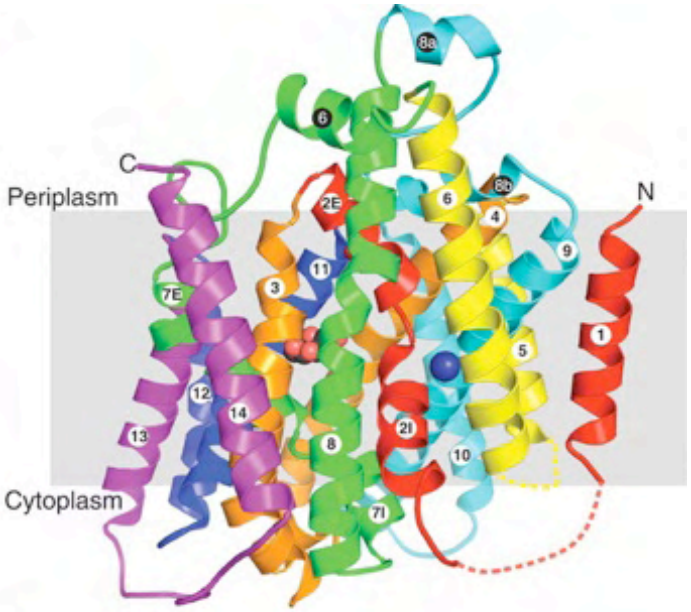
Inward-facing, occluded
with Na1/substrate

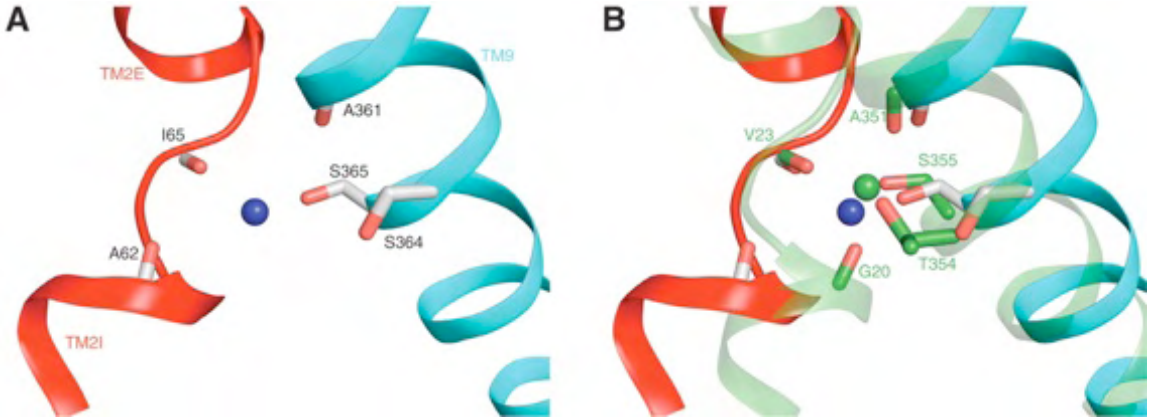


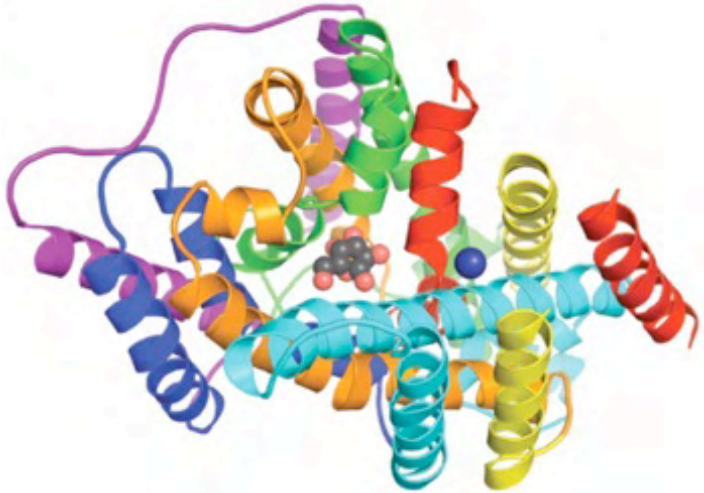
Occluded to open transition
after Na1 release

vSGLT: A Secondary Membrane Transporter in the ***Occluded*** Inward-Facing State





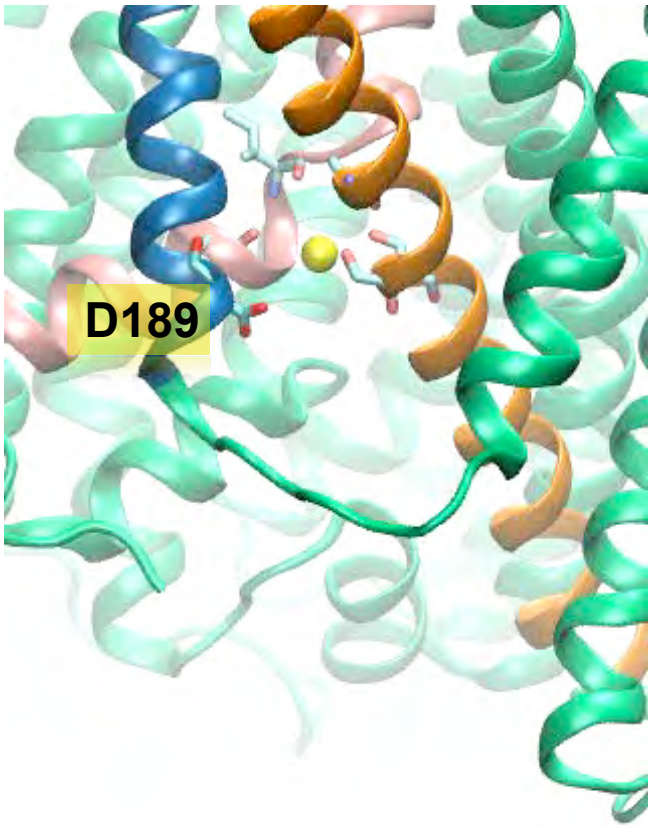


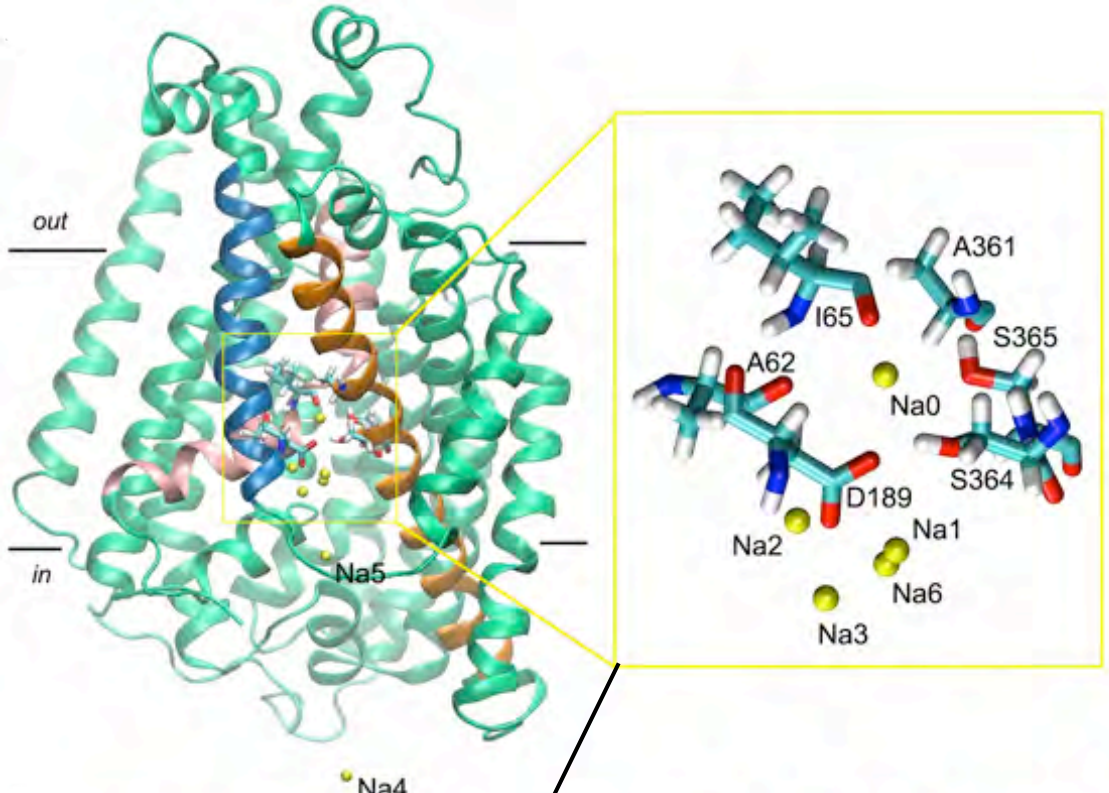


Na^+ was modeled based on LeuT

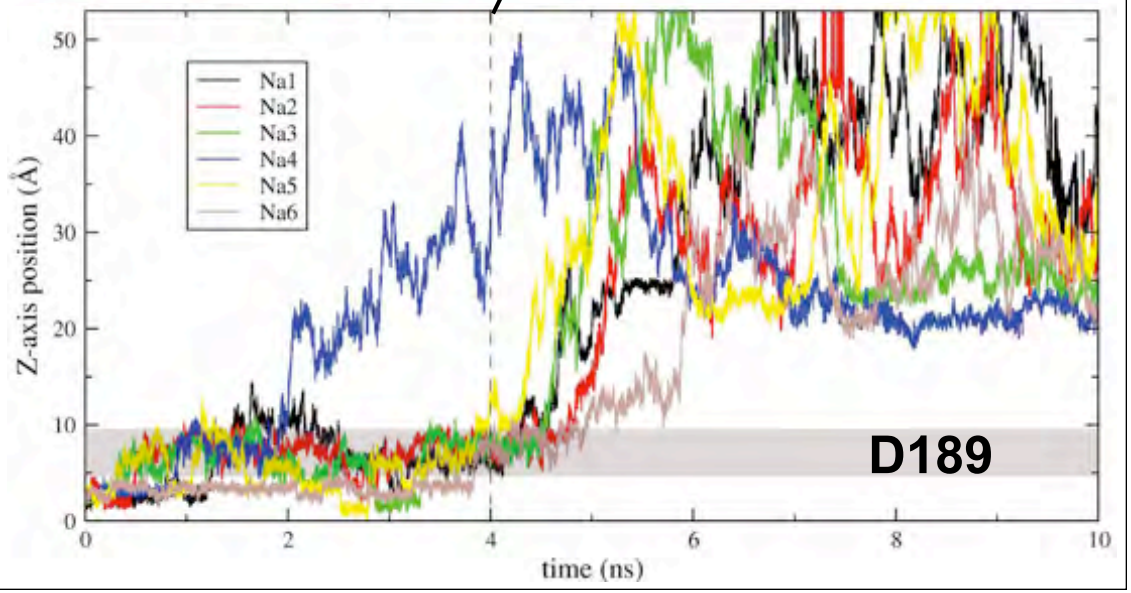
Faham et al., Science, 810-814, 2008

Spontaneous Na^+ Unbinding in Multiple Simulations



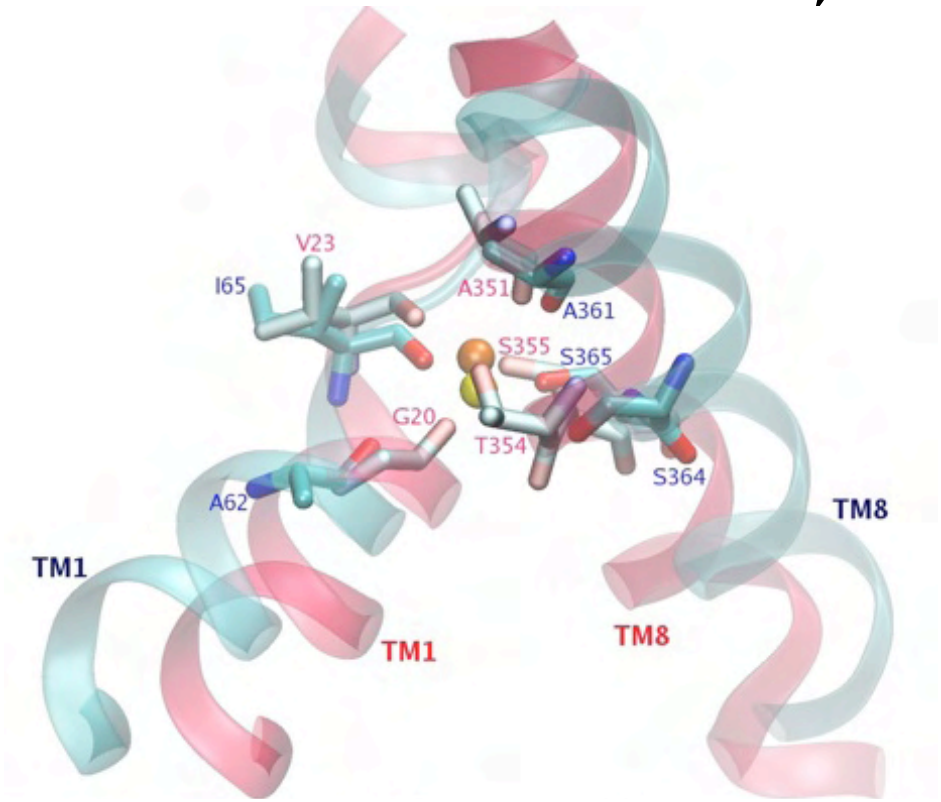


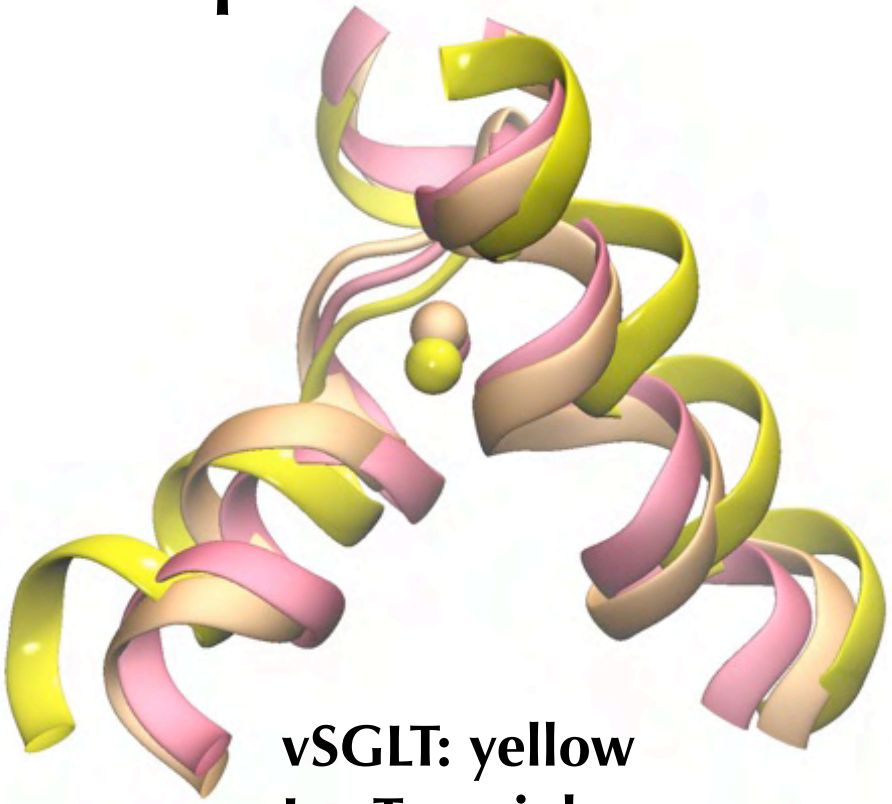
- Several independent simulations, all resulting in Na^+ unbinding
- The crystal structure is not an occluded state, rather an **open** inward-facing state.



J. Li and E. Tajkhorshid, *Biophysical Journal* **2009**

Comparison of the Na^+ Binding Sites in vSGLT, LeuT and Mhp1





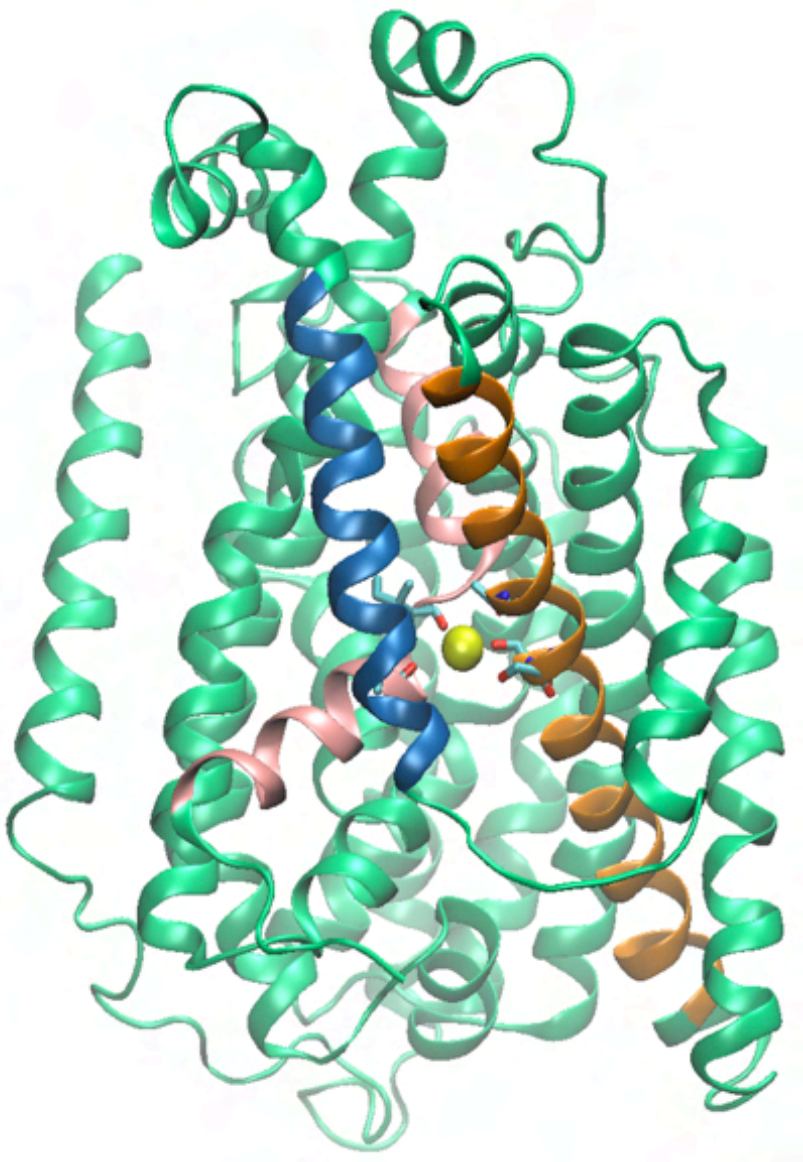
vSGLT: yellow
LeuT: pink
Mhp1: orange

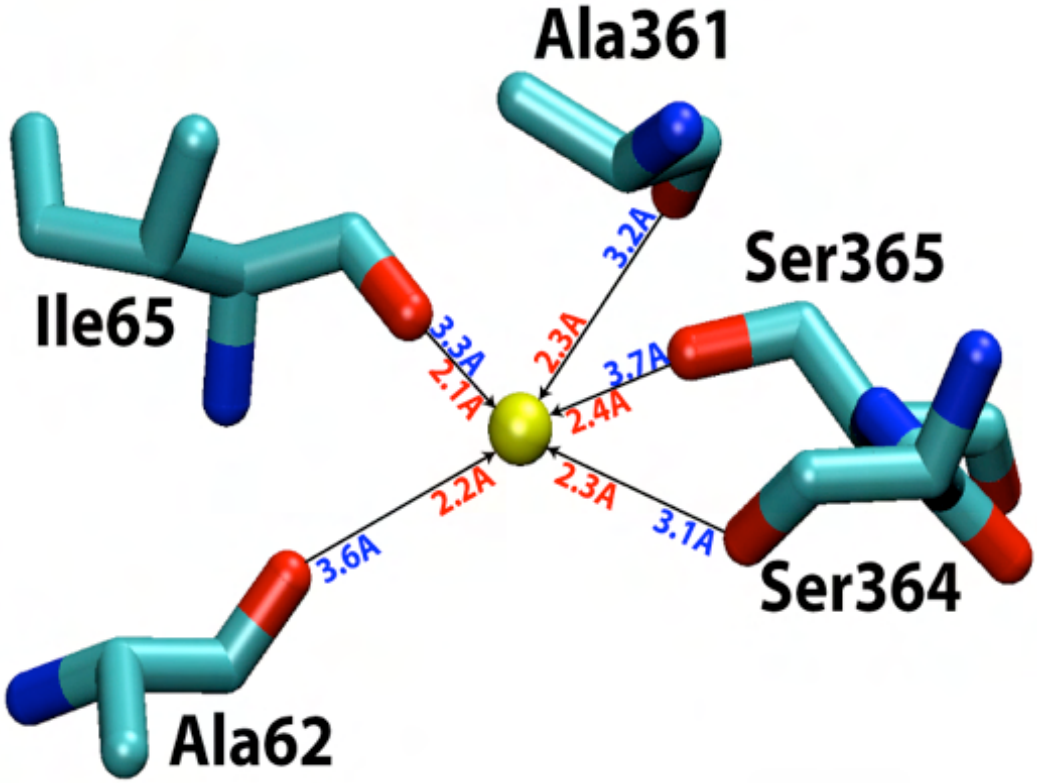
LeuT (PDB:2A65)		vSGLT (PDB:3DH4)	
bond	distance (Å)	bond	distance (Å)
G20(O)–Na	2.23	A62(O)–Na	3.64
V23(O)–Na	2.15	I65(O)–Na	3.32
A351(O)–Na	2.29	A361(O)–Na	3.23
T354(Oγ)–Na	2.25	S364(Oγ)–Na	3.13
S355(Oγ)–Na	2.35	S365(Oγ)–Na	3.68
bond angle	angle (degree)	bond angle	angle (degree)
V23(O)–Na–S355(Oγ)	99.3	I65(O)–Na–S365(Oγ)	60.5
V23(O)–Na–T354(Oγ)	112.8	I65(O)–Na–S364(Oγ)	133.0
T354(Oγ)–Na–S355(Oγ)	147.9	S364(Oγ)–Na–S365(Oγ)	73.5
G20(O)–Na–A351(O)	166.5	A62(O)–Na–A361(O)	150.6

vSGLT: **open** state
LeuT,Mhp1: **occluded** state

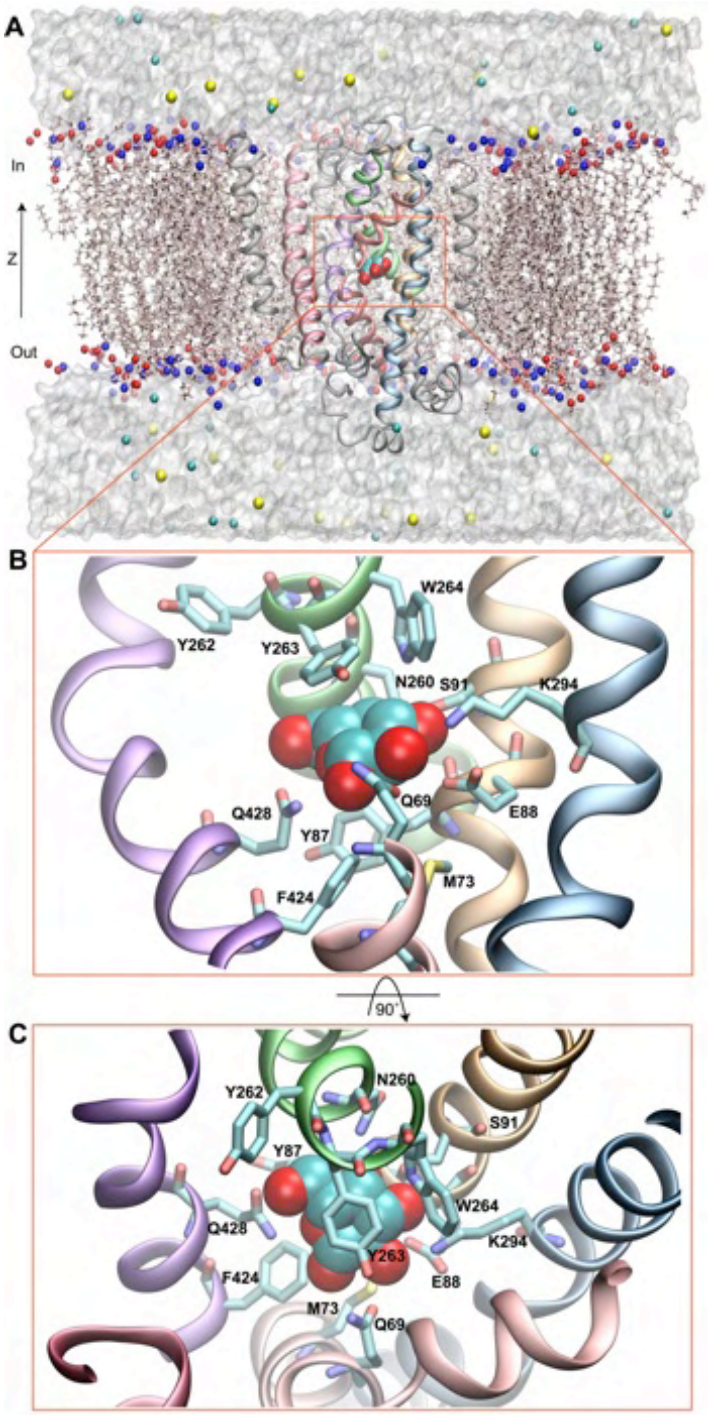
J. Li and E. Tajkhorshid, *Biophysical Journal* **2009**

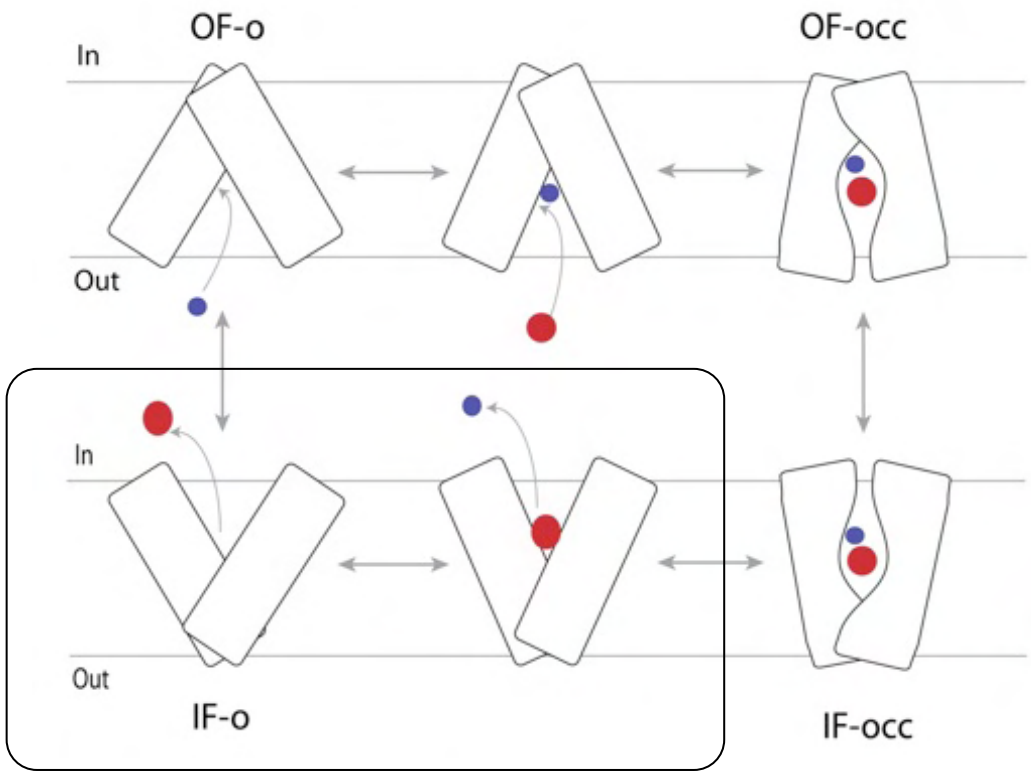
Artificially Recovering the Occluded State





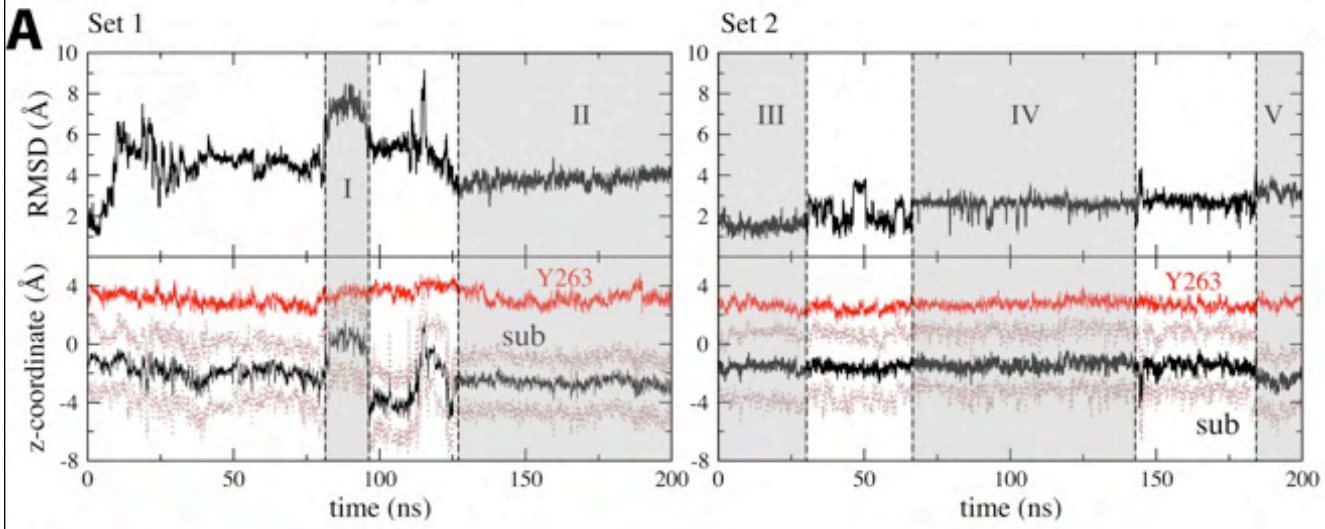
Cytoplasmic Gate?

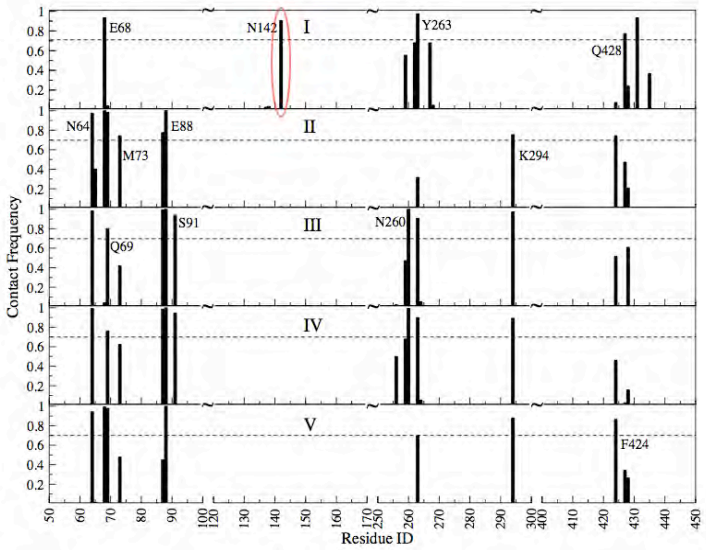


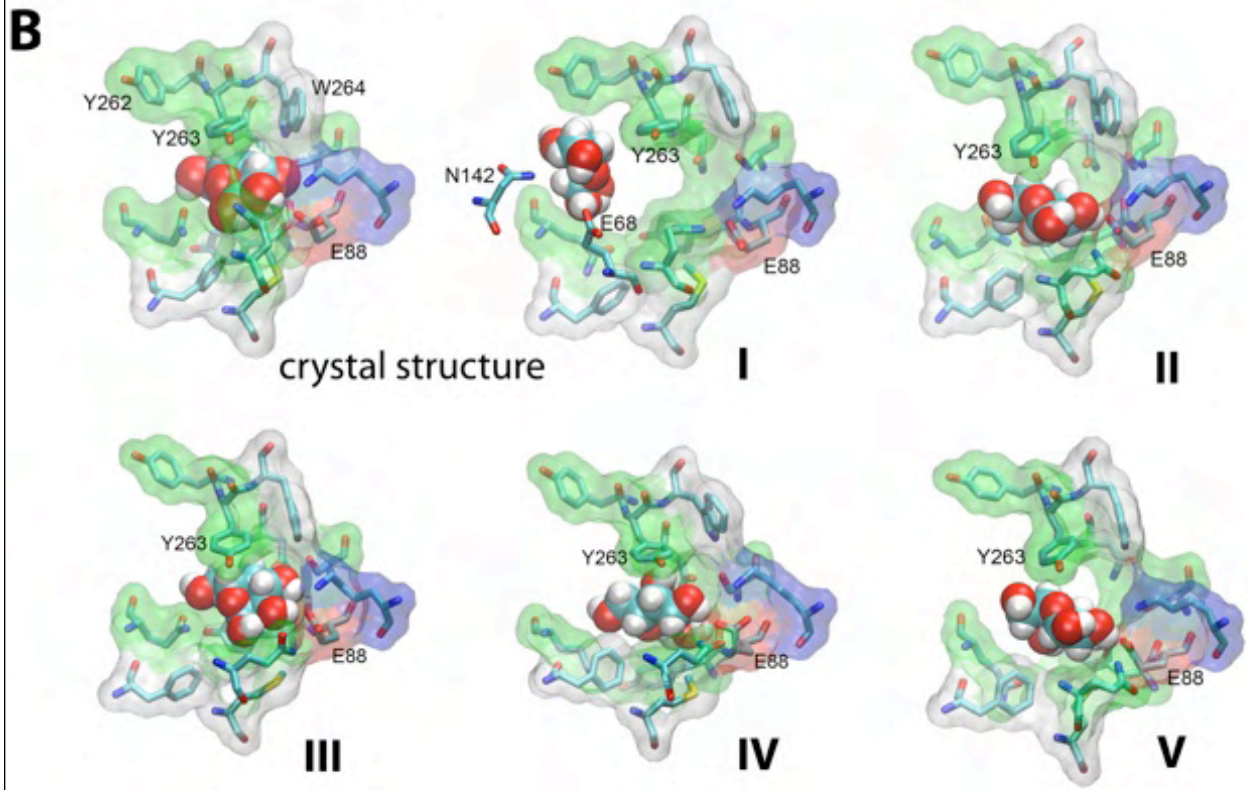


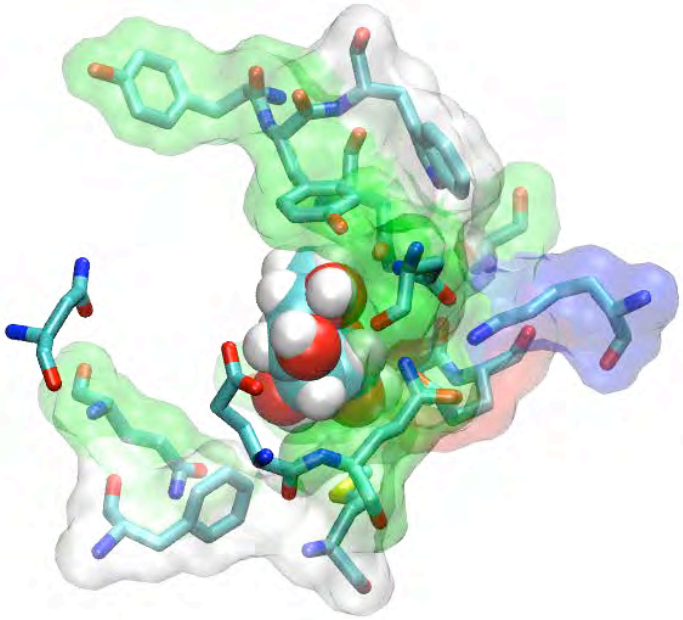
Substrate-bound state → substrate-free state?

Early Stage of Substrate Release Captured by Free MD

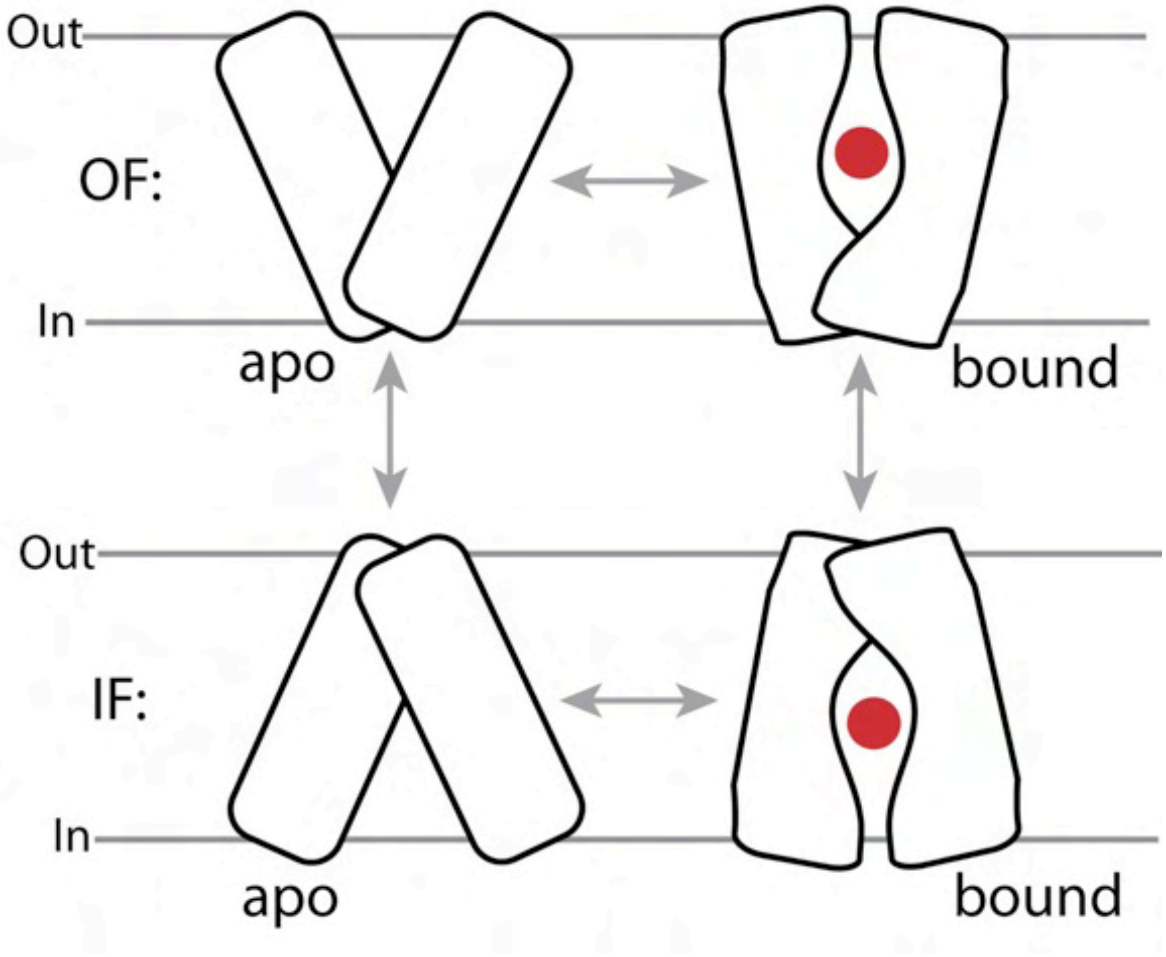






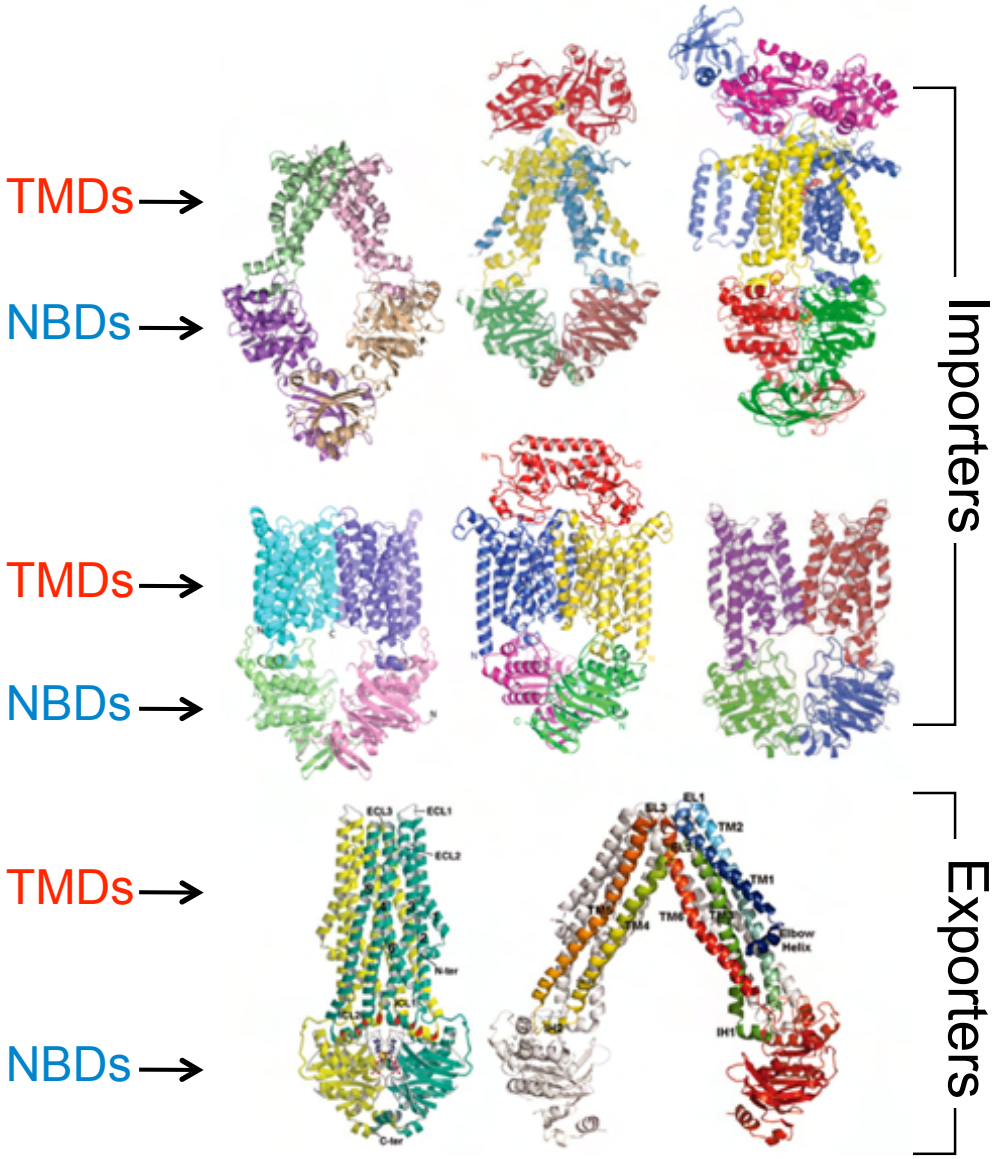


Transporter Dogma: Alternating Access Mechanism



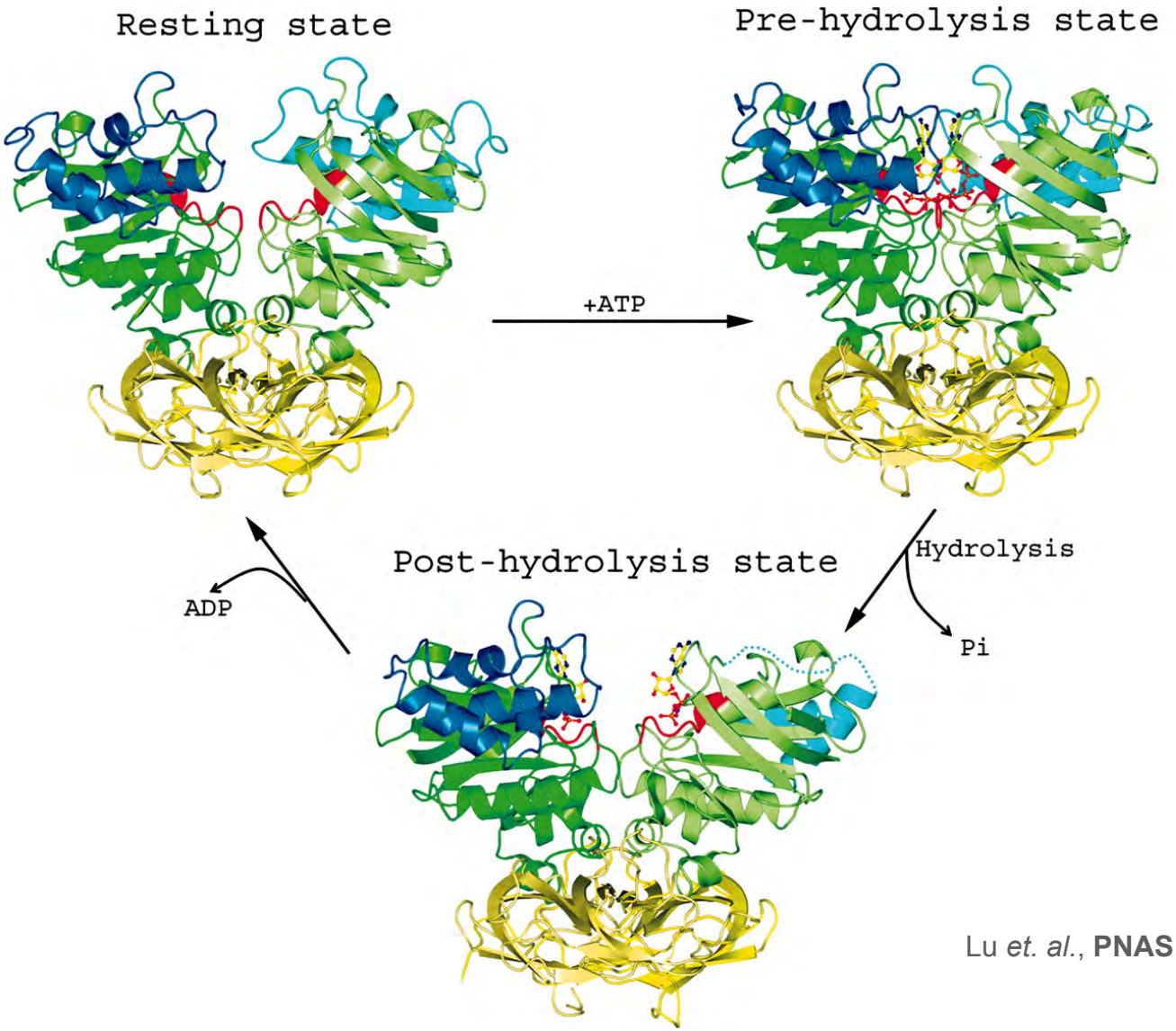
- Transporters switch the substrate access between the two sides.
- The central binding site should **never** be exposed to both sides **simultaneously**.

ATP-Driven Transport in ABC Transporters



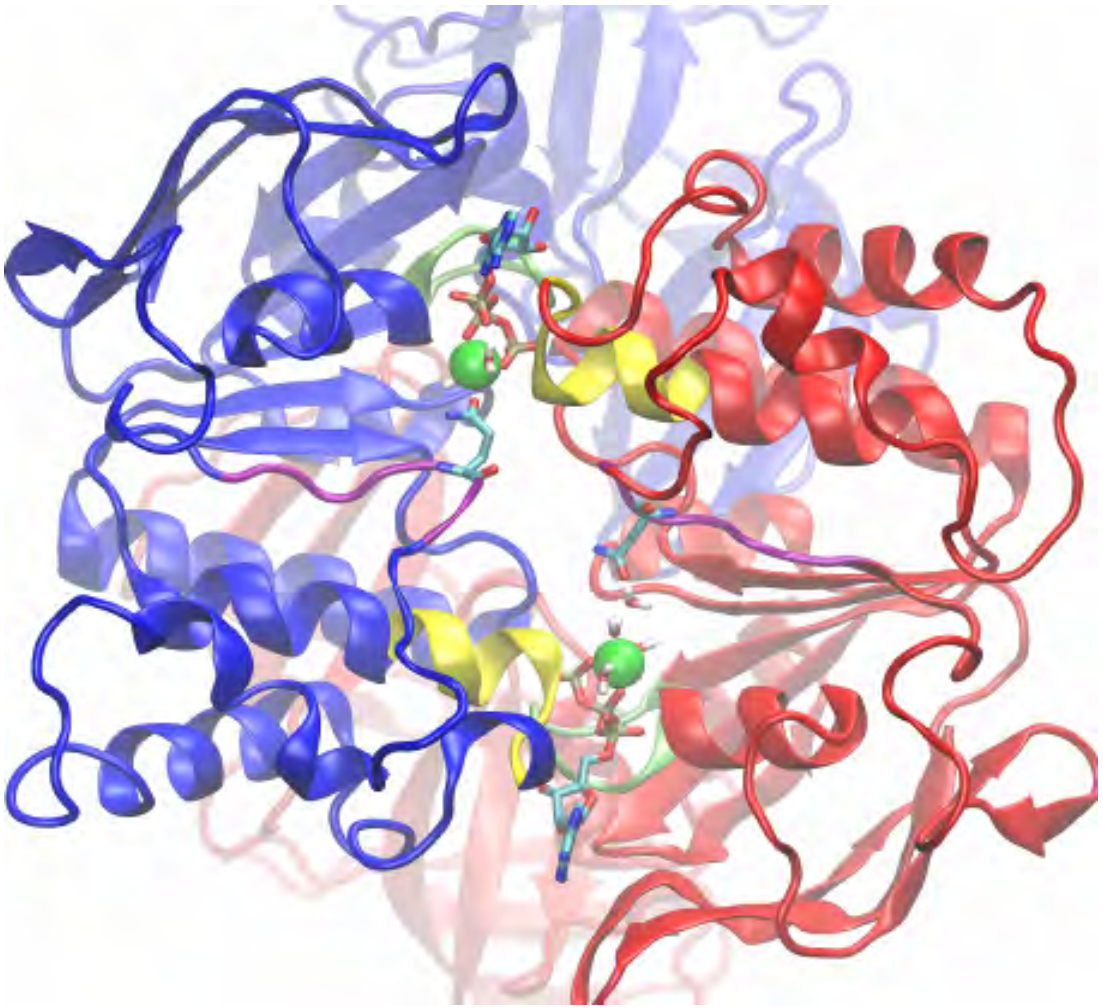
- Architecture
  - 2 NBDs
    - Conserved domains
    - ATPase activity
  - 2 TMDs
    - Diverse sequence and structure
    - Substrate transport
  - 1 BP
    - ABC importers only
    - Substrate recognition and binding

Nucleotide-Dependent State of NBDs



Lu *et. al.*, **PNAS**, (2005)

Simulation Systems



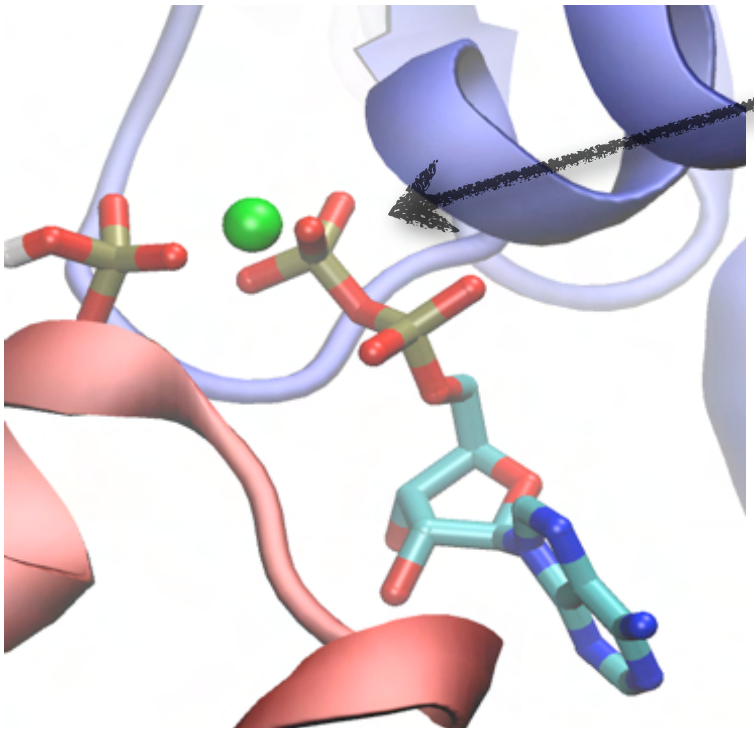
- MalK dimer (1Q12.PDB)
- Placing Mg^{2+}
- Solvate (80,000 atoms)
- Equilibrium MD - 75 ns
- 4 simulation systems
  - **ATP / ATP**
  - **ADP-P_i / ATP**
  - **ATP / ADP-P_i**
  - **ADP-P_i / ADP-P_i**

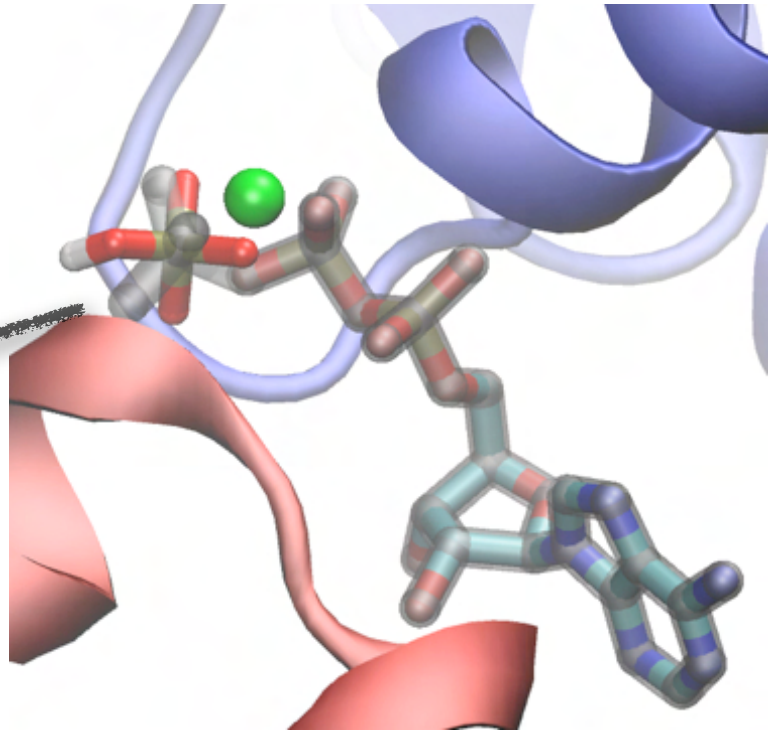
1 or 2 ATP hydrolysis?

Hydrolysis or release of products?

Simulating the Immediate Effect of ATP Hydrolysis

ADP/Pi-Bound

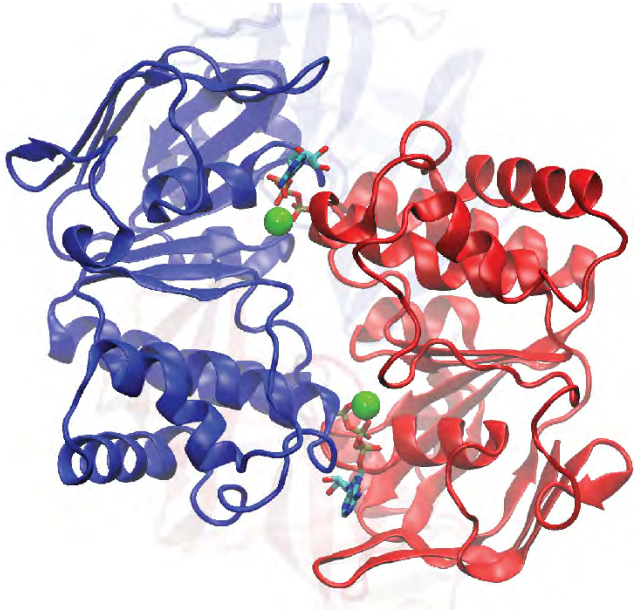




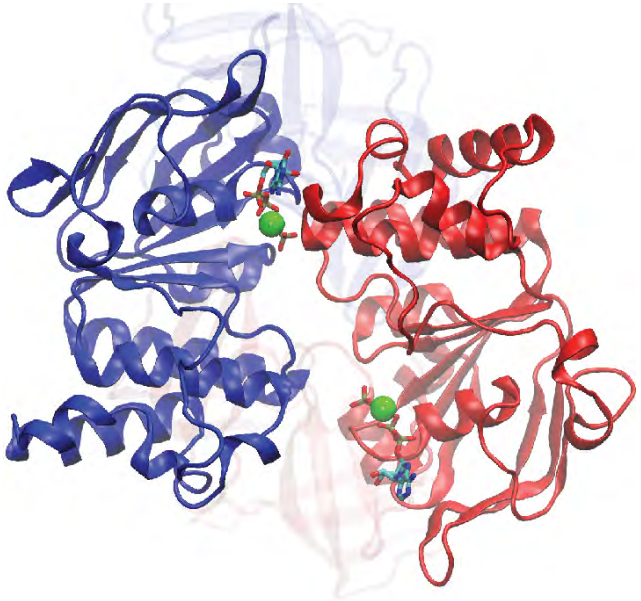
ATP-Bound

P. Wen and E. Tajkhorshid, *Biophysical Journal* **2008**

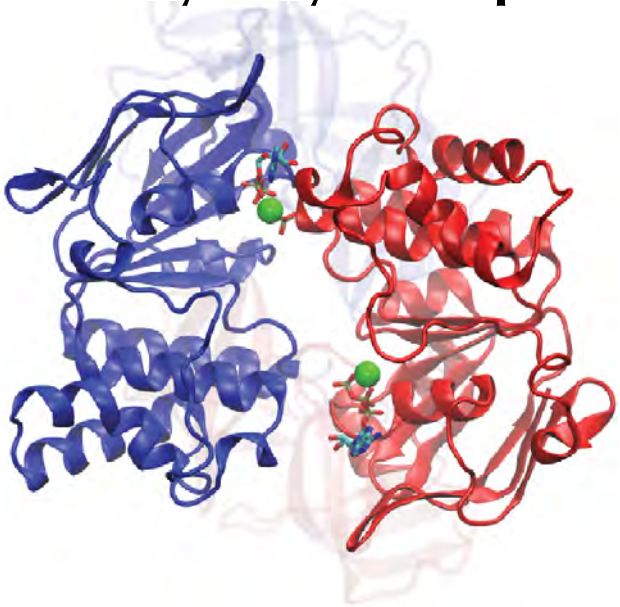
ATP/ATP



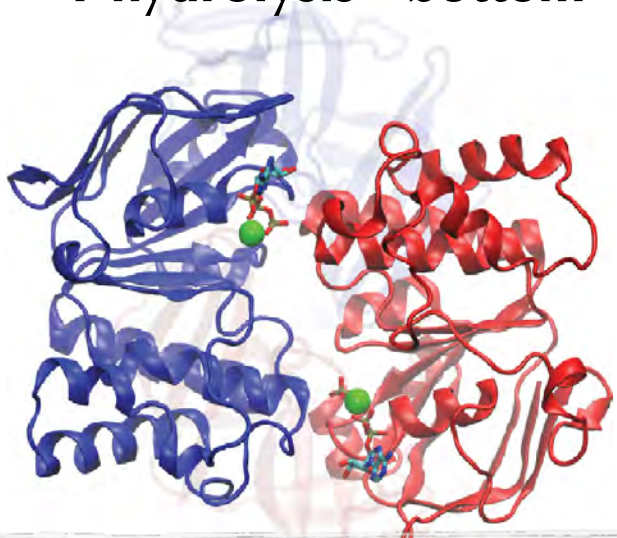
2 Hydrolysis



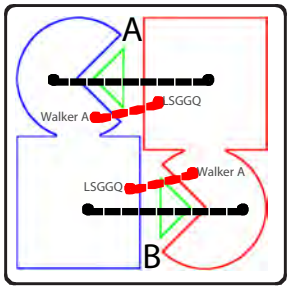
1 hydrolysis - top

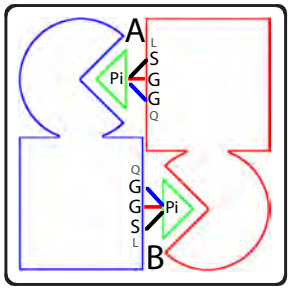


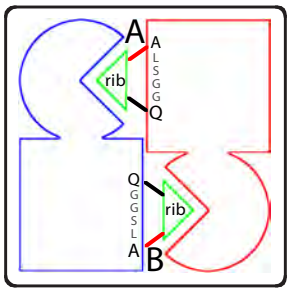
1 hydrolysis - bottom

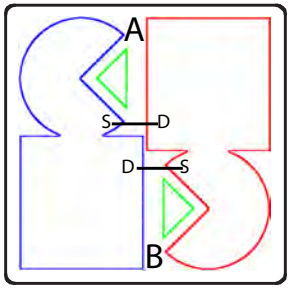


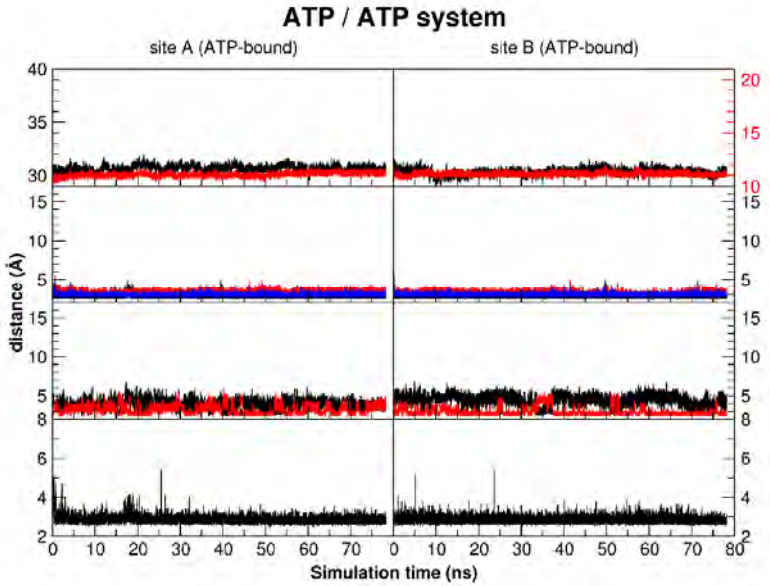
P. Wen and E. Tajkhorshid, *Biophysical Journal* **2008**

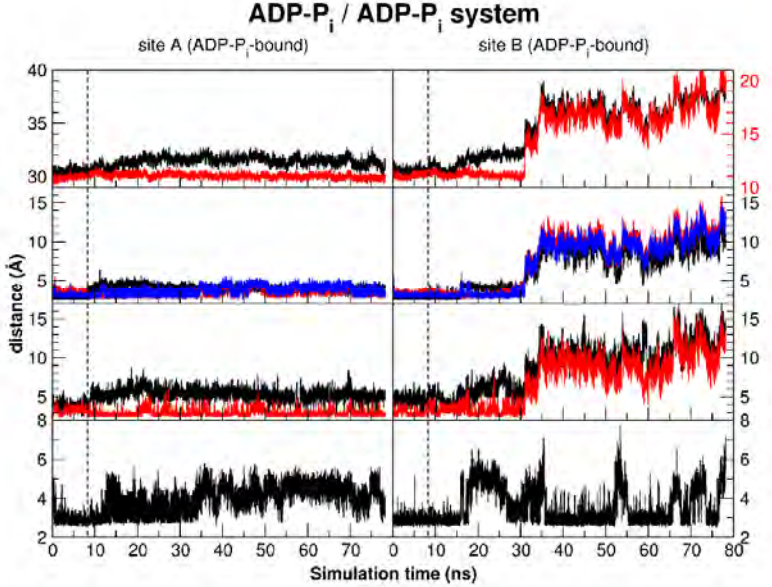


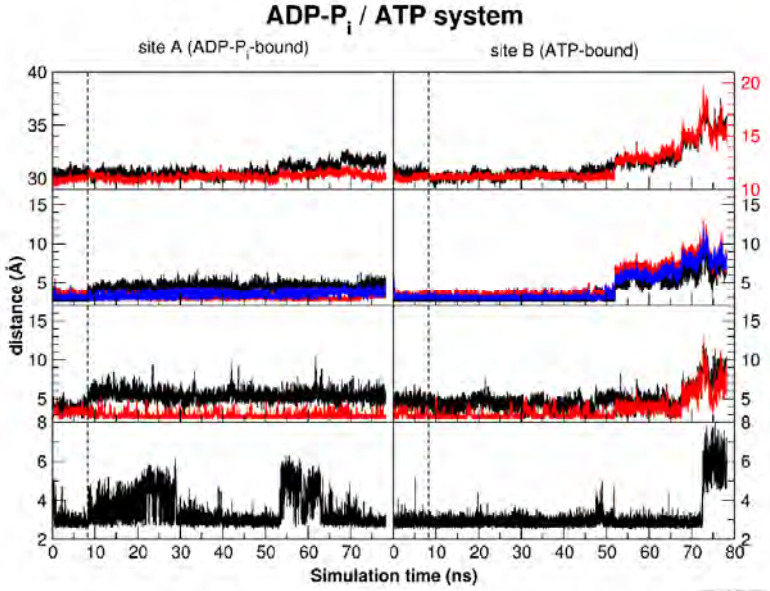


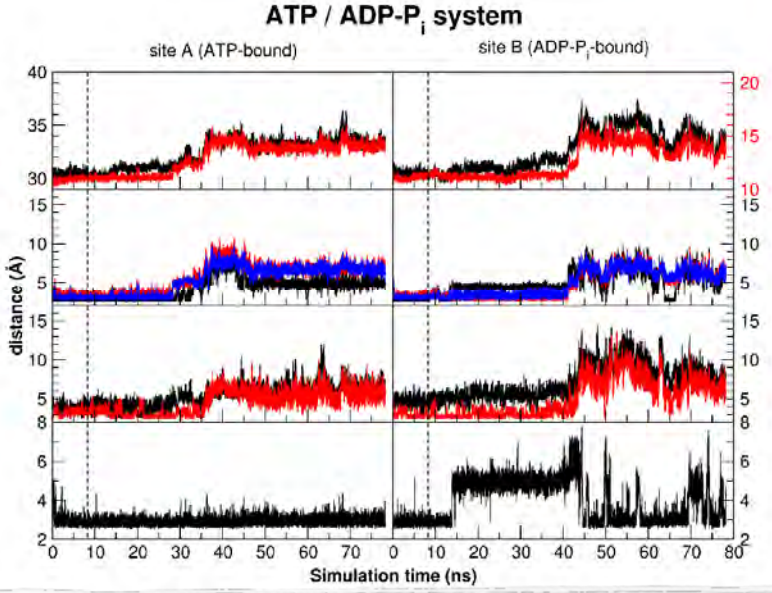






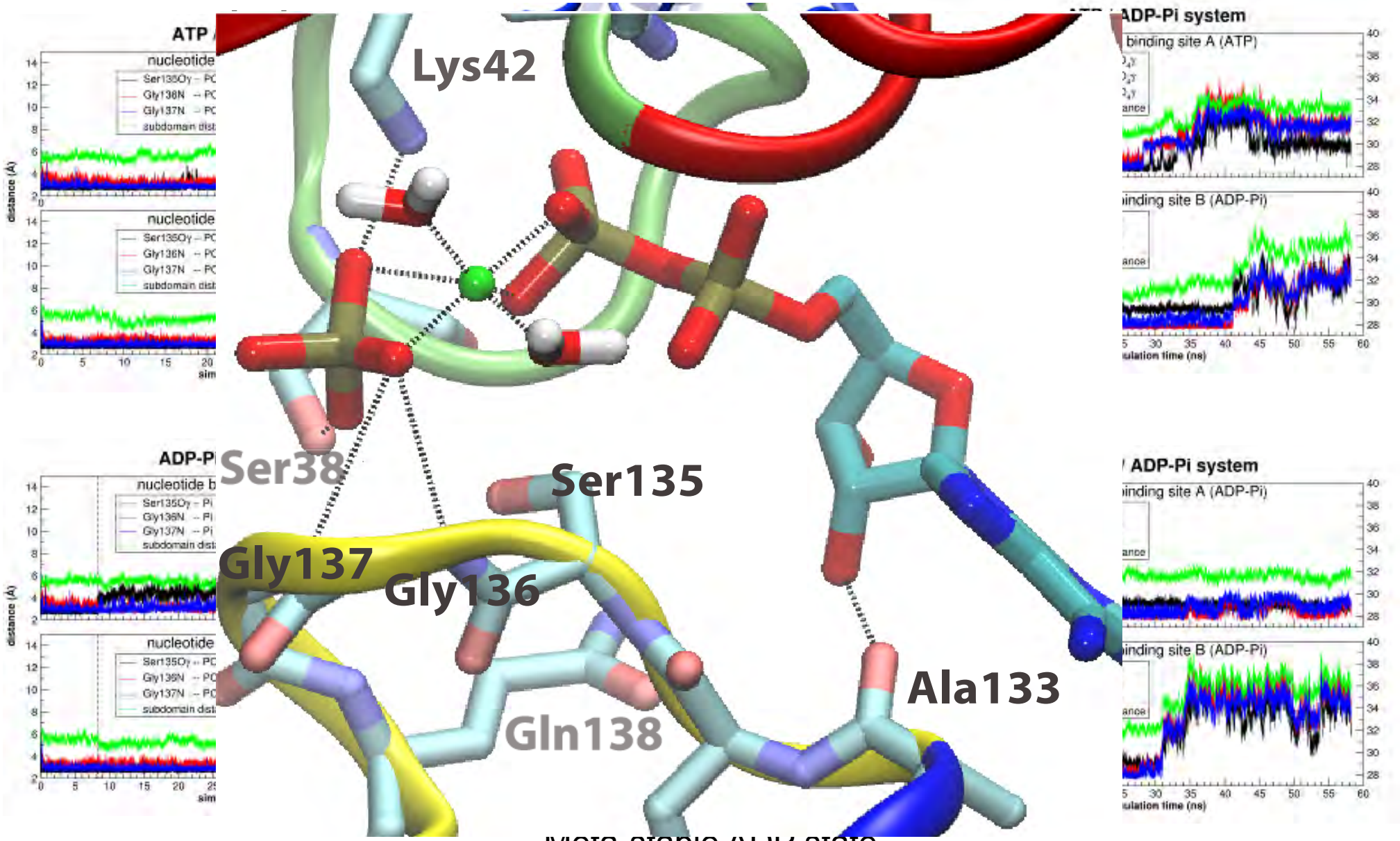






P. Wen and E. Tajkhorshid, *Biophysical Journal* **2008**

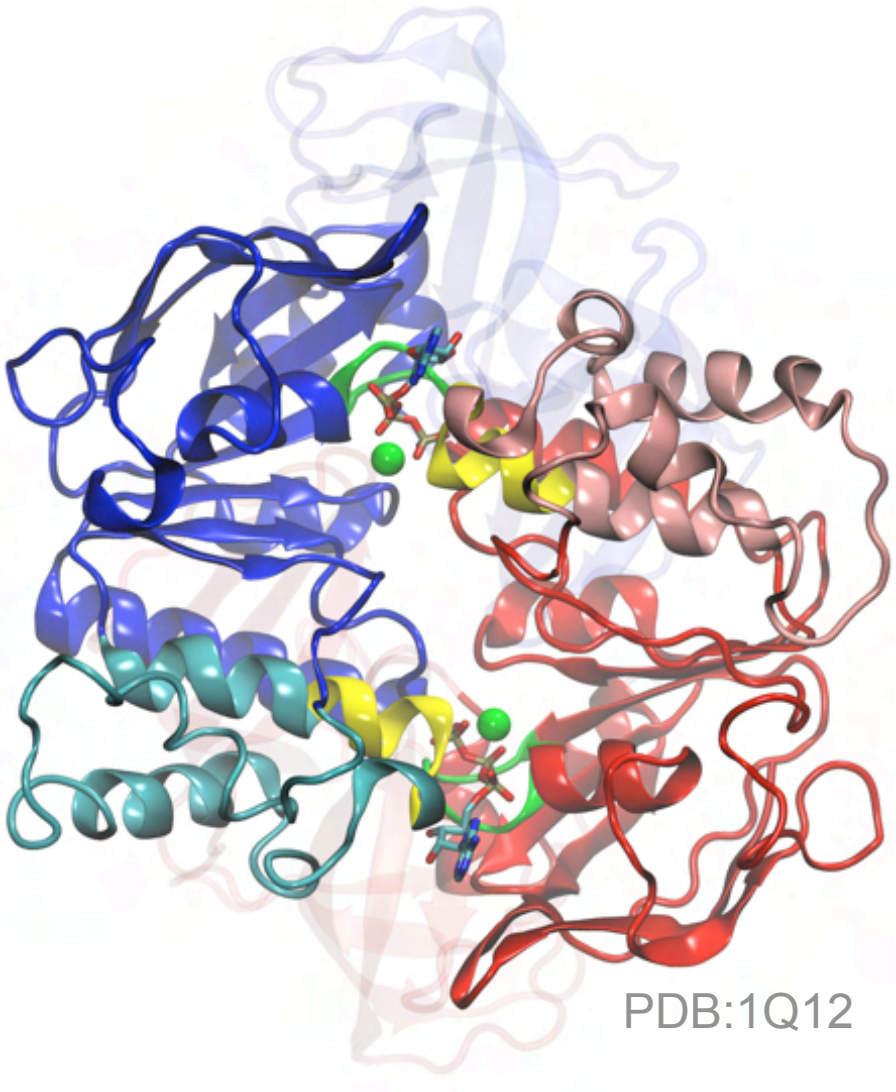
Pinpointing the Mechanism



Meta-stable ADP state

P. Wen and ET, ***Biophys. J.*** , 2008.

Nucleotide Binding Domains



- Two subdomains
  - RecA-like subdomain
    - Majority of ATP binding site
    - Walker A motif
  - Helical subdomain
    - Complimentary to ATP binding
    - Signature "LSGGQ" motif
- Two nucleotide binding sites
  - Both at the dimer interface
  - "Nucleotide-sandwiched" dimer

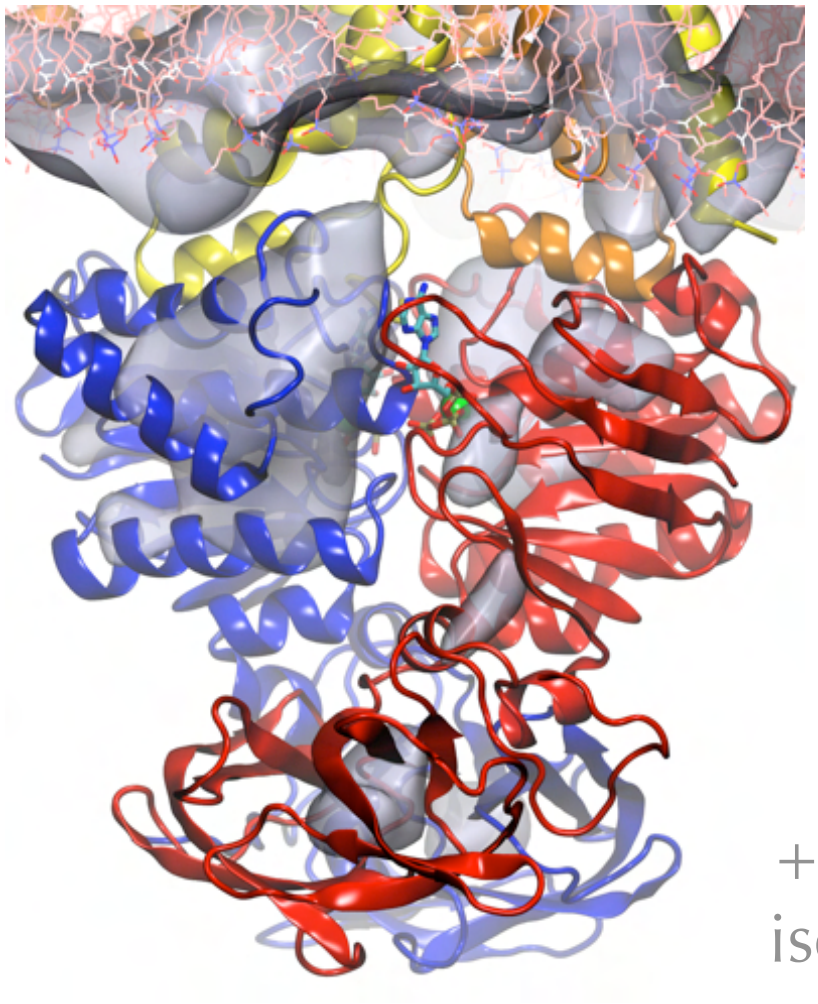
ATP binding → dimerization

Hydrolysis → dimer opening

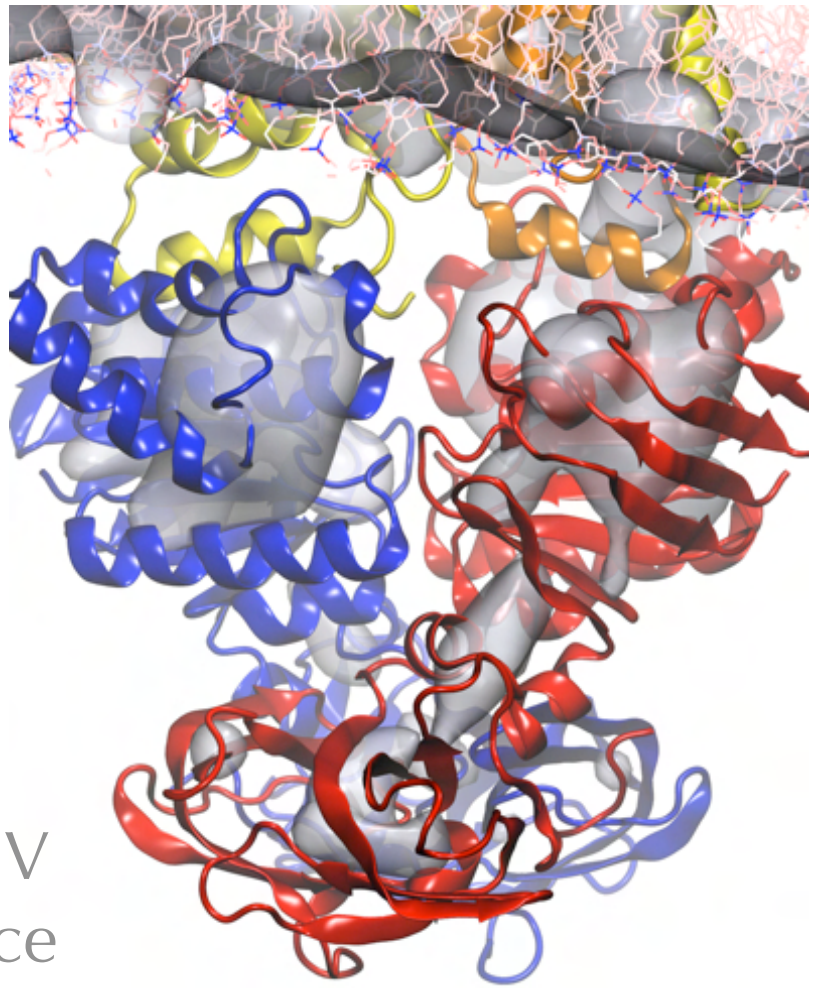
Why?

Discovery of Buried Charges

in the maltose transporter



+500 mV
isosurface

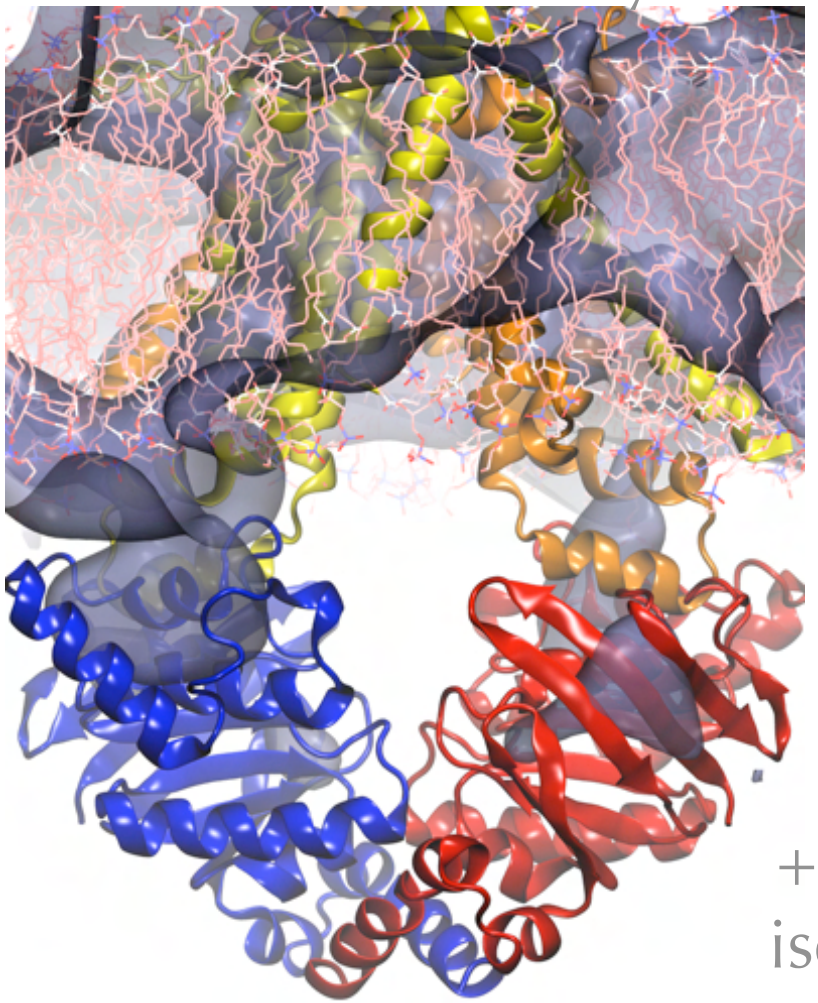


MalEFGK, ATP-bound
~320,000 atoms
averaged between
t = 70-80 ns

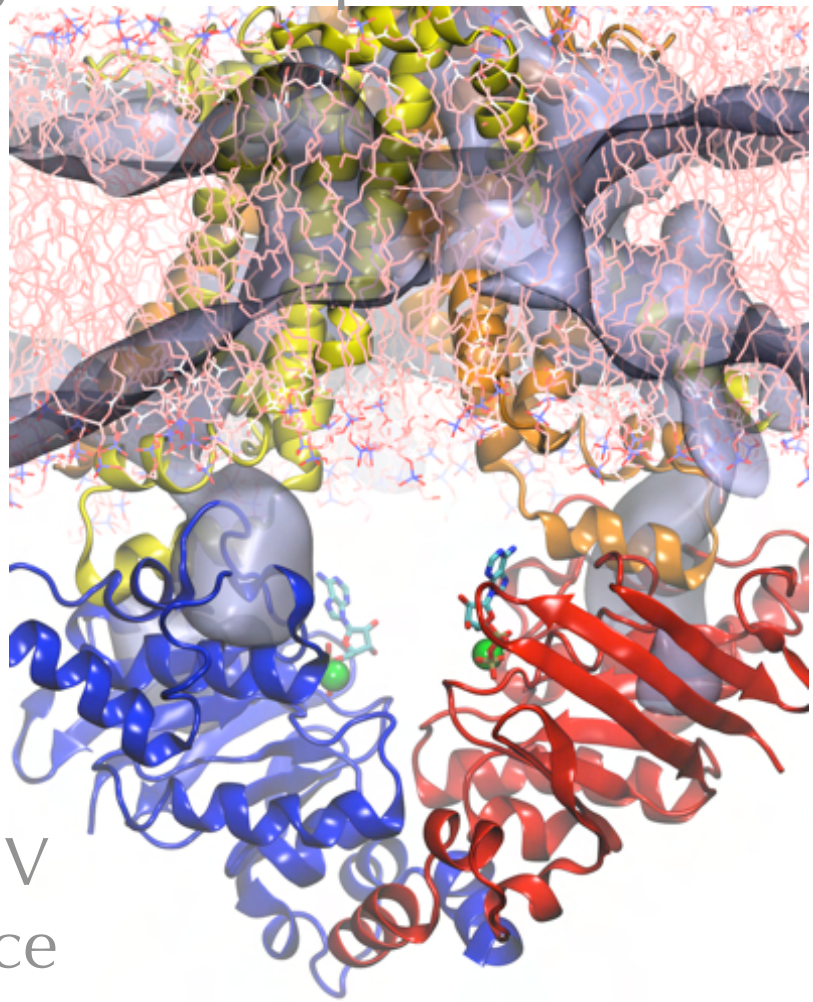
MalEFGK, Nucleotide-Free
~320,000 atoms
averaged between
t = 70-80 ns

Discovery of Buried Charges

in the molybdate/tungstate transporter



+500 mV
isosurface

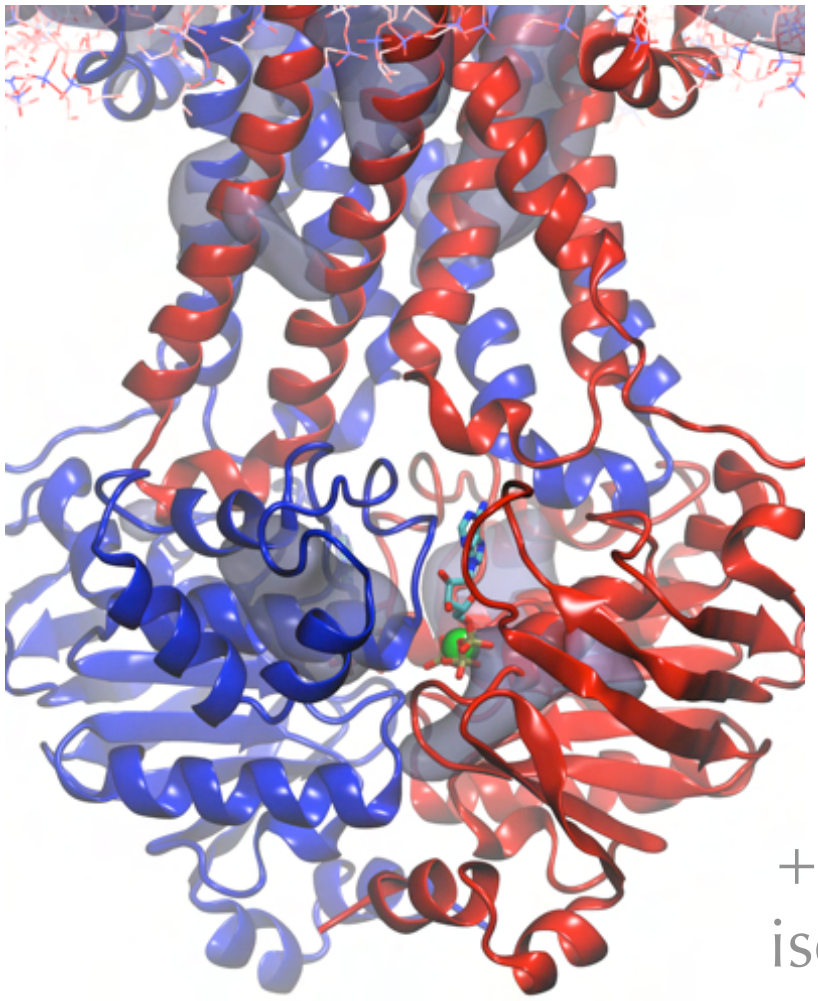


ModABC Nucleotide-Free
~220,000 atoms
averaged between
t = 0-10 ns

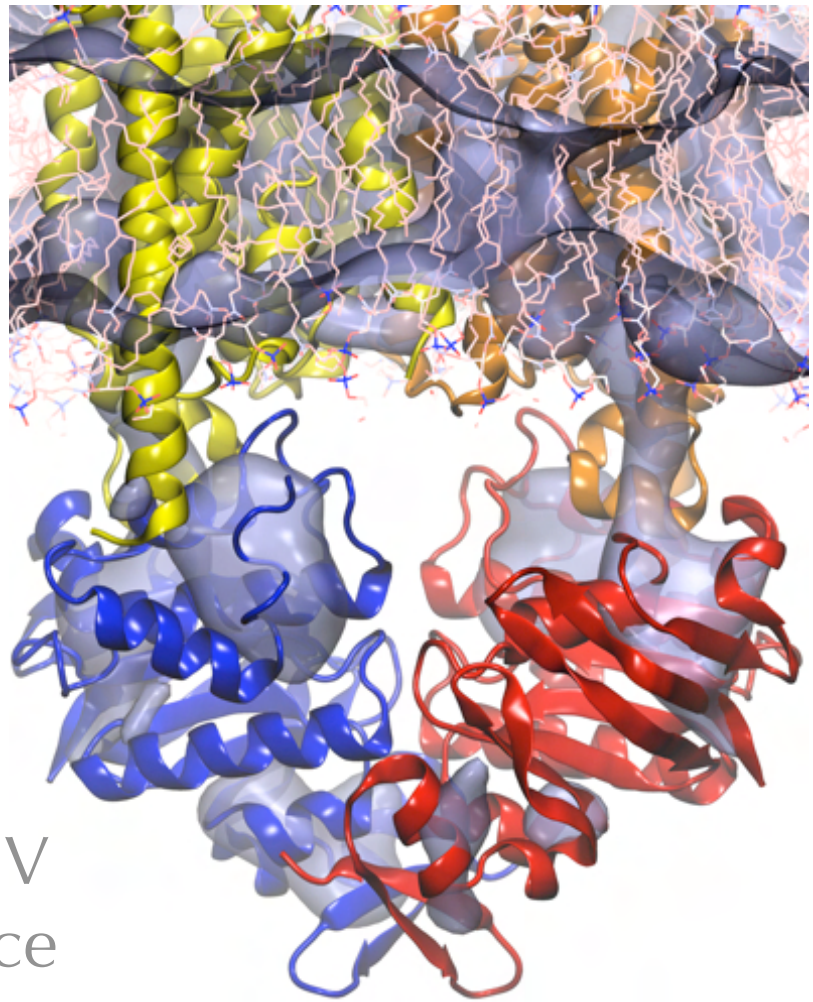
ModABC, ADP docked
~220,000 atoms
averaged between
t = 0-10 ns

Discovery of Buried Charges

in other ABC transporters



+500 mV
isosurface

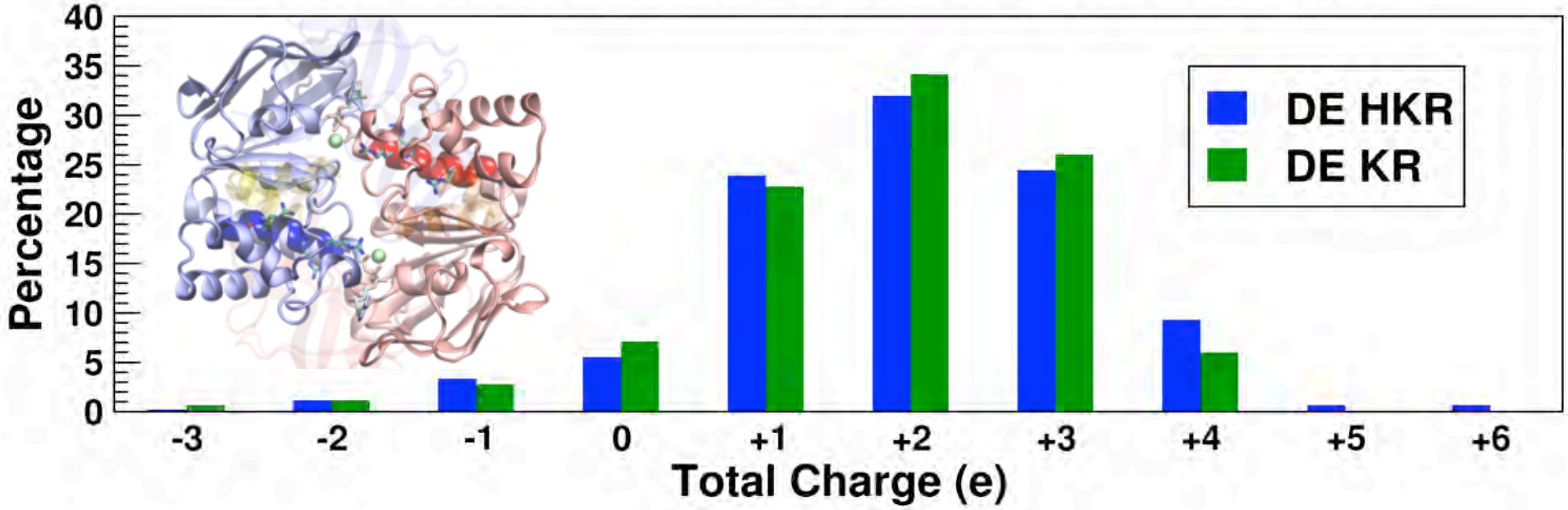


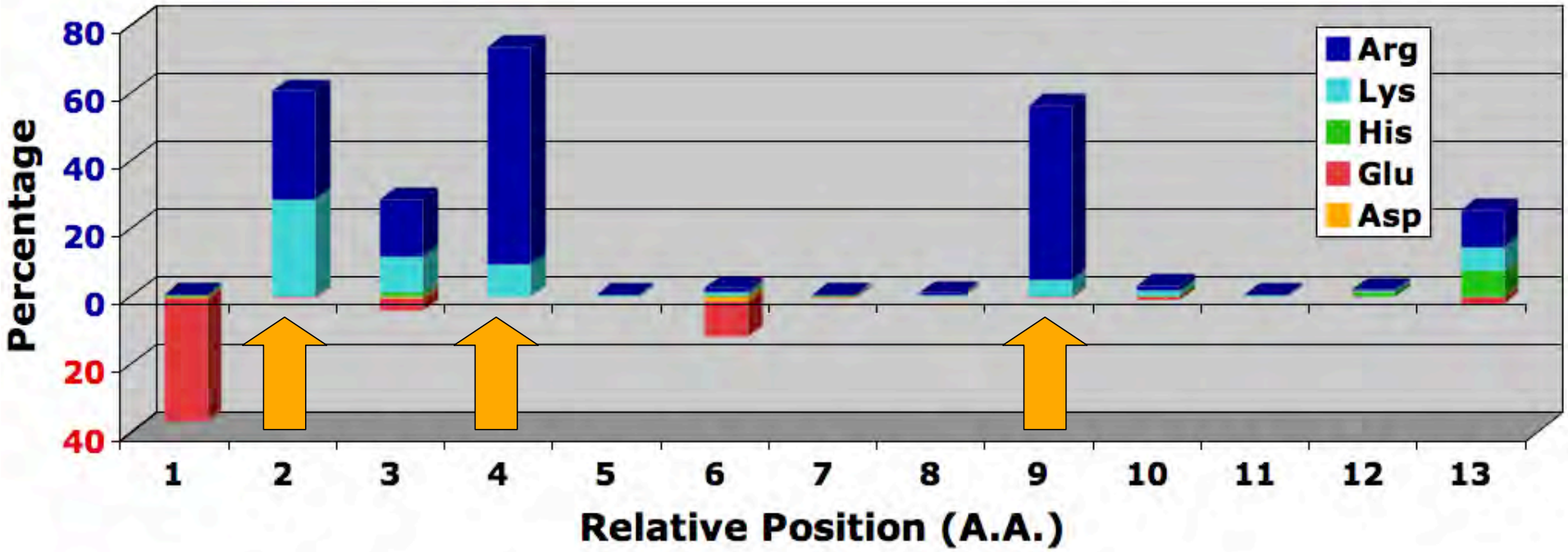
MsbA ATP-bound
~200,000 atoms
averaged between
t = 0-10 ns

BtuCDF, Nucleotide-Free
~220,000 atoms
averaged between
t = 0-10 ns

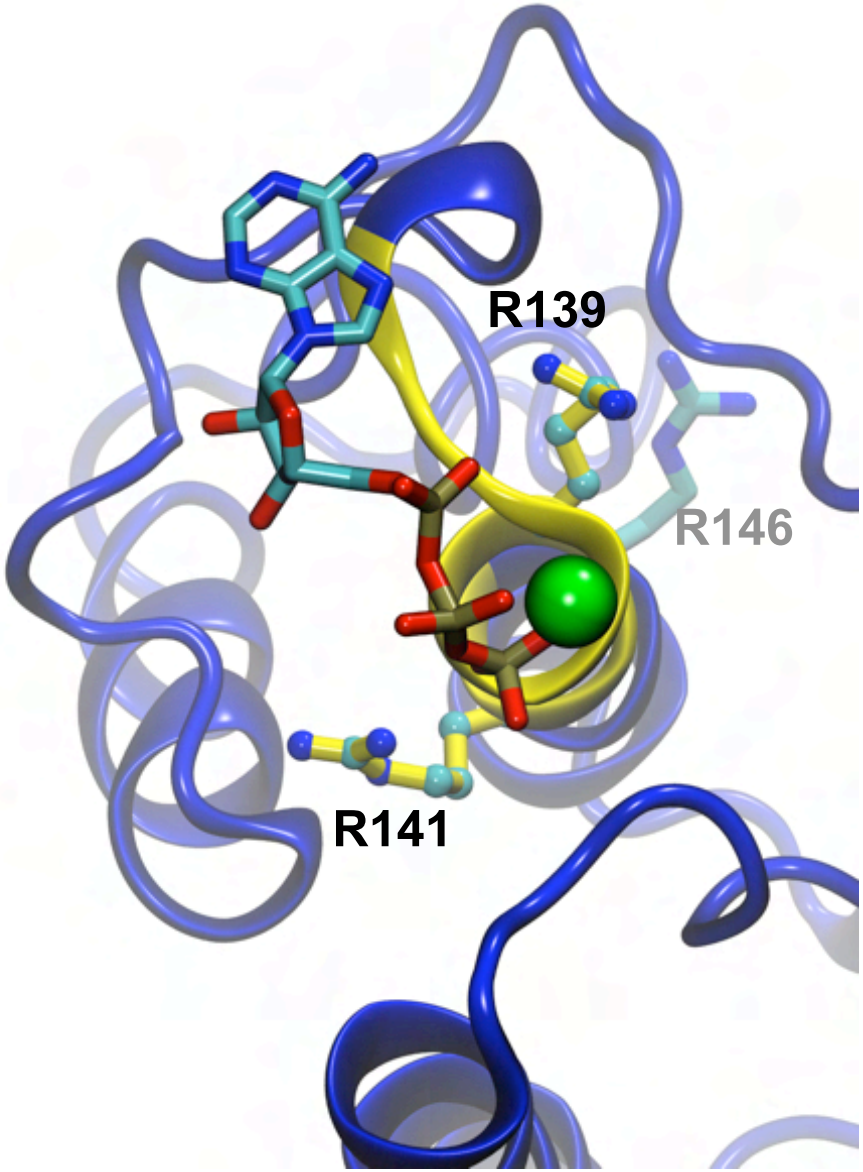
Charge Conservation at the Core of the Helical Subdomain

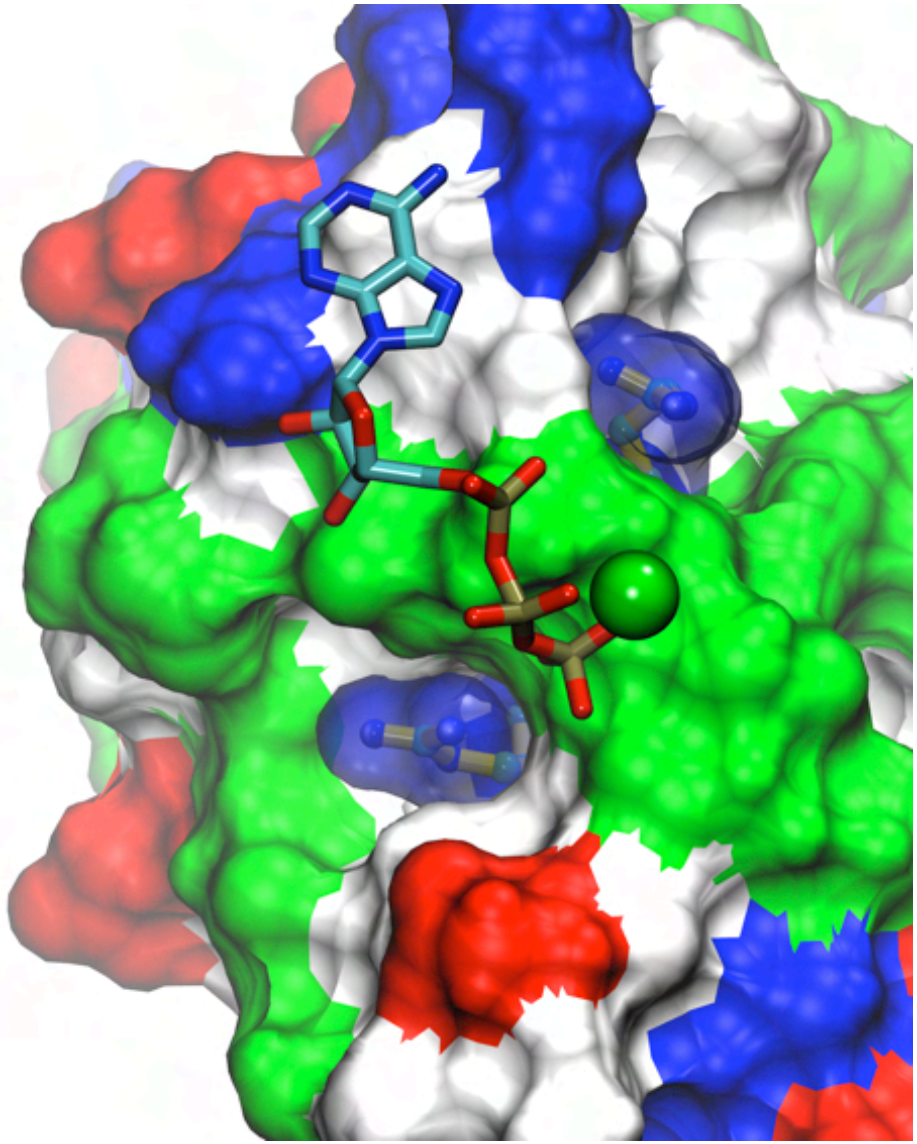
Analyzed from all 185 representative sequences in NCBI CDD #cd00267



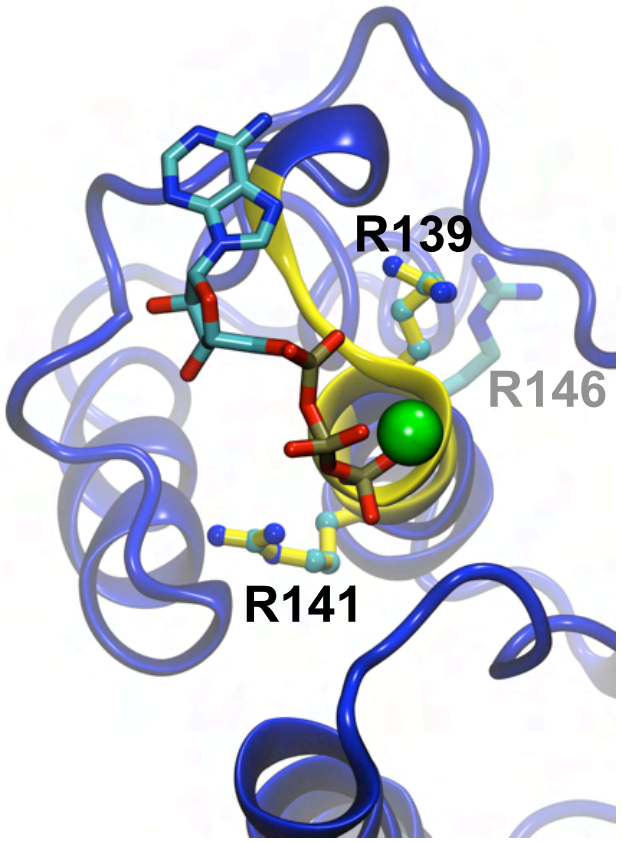


Conserved Arginines in the Helical Subdomain

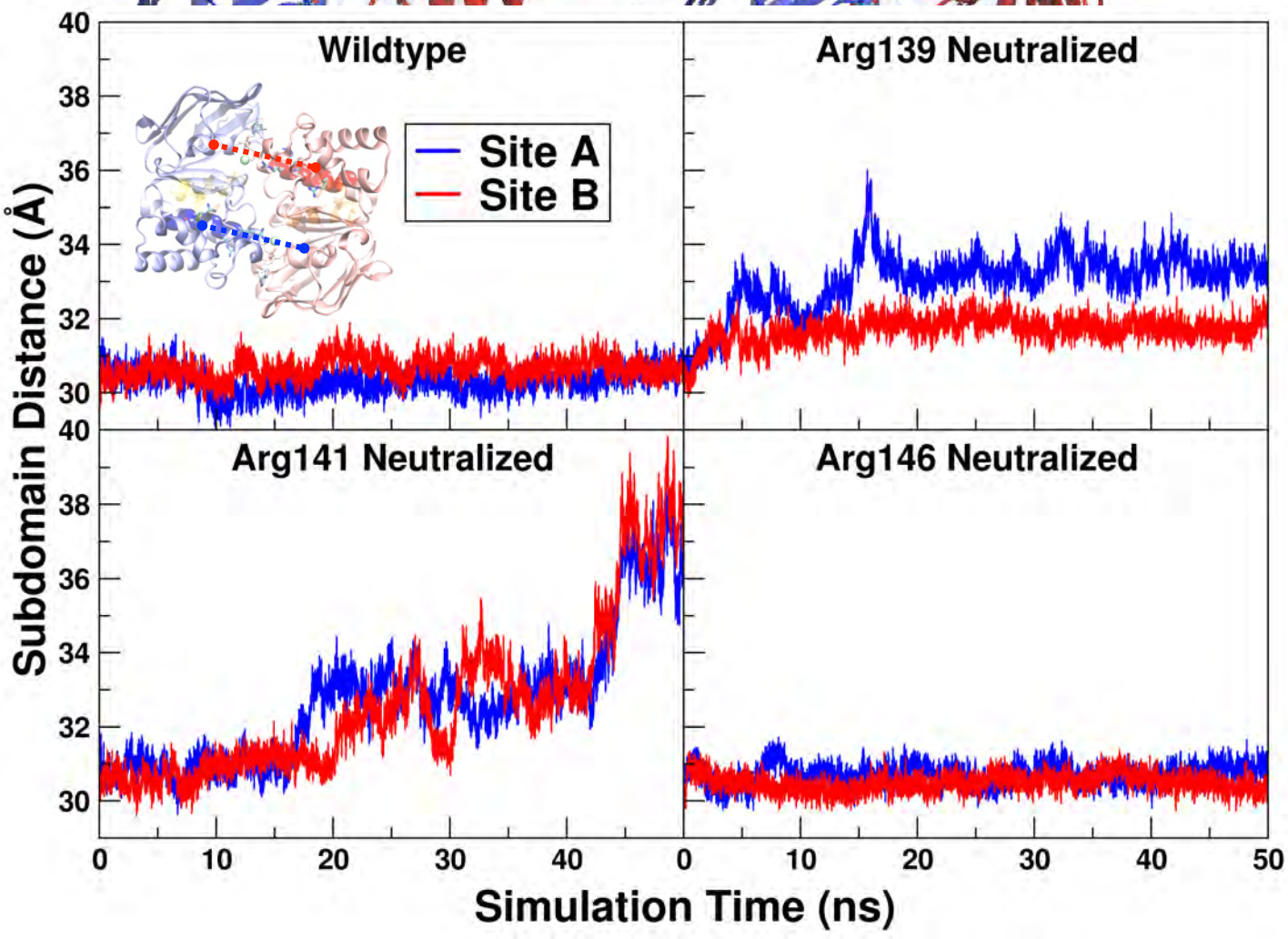




Key Role of Buried Charges in NBD Dimerization



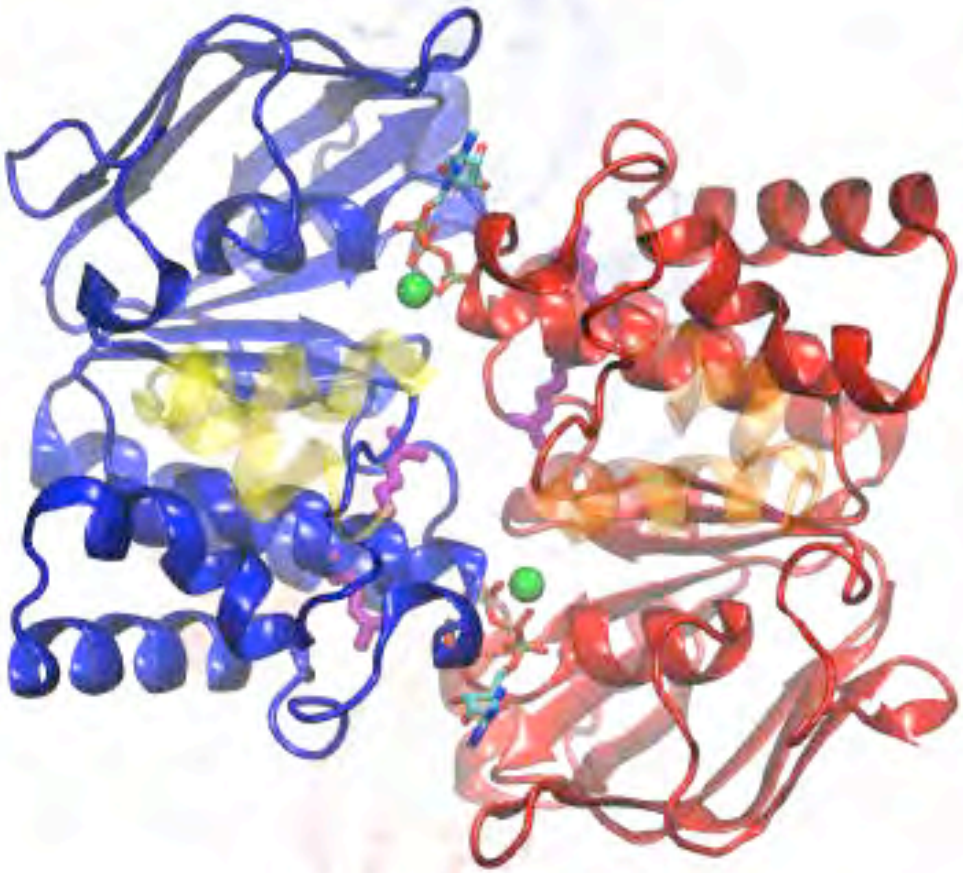
MalK dimers
All ATP Bound
t = 50 ns

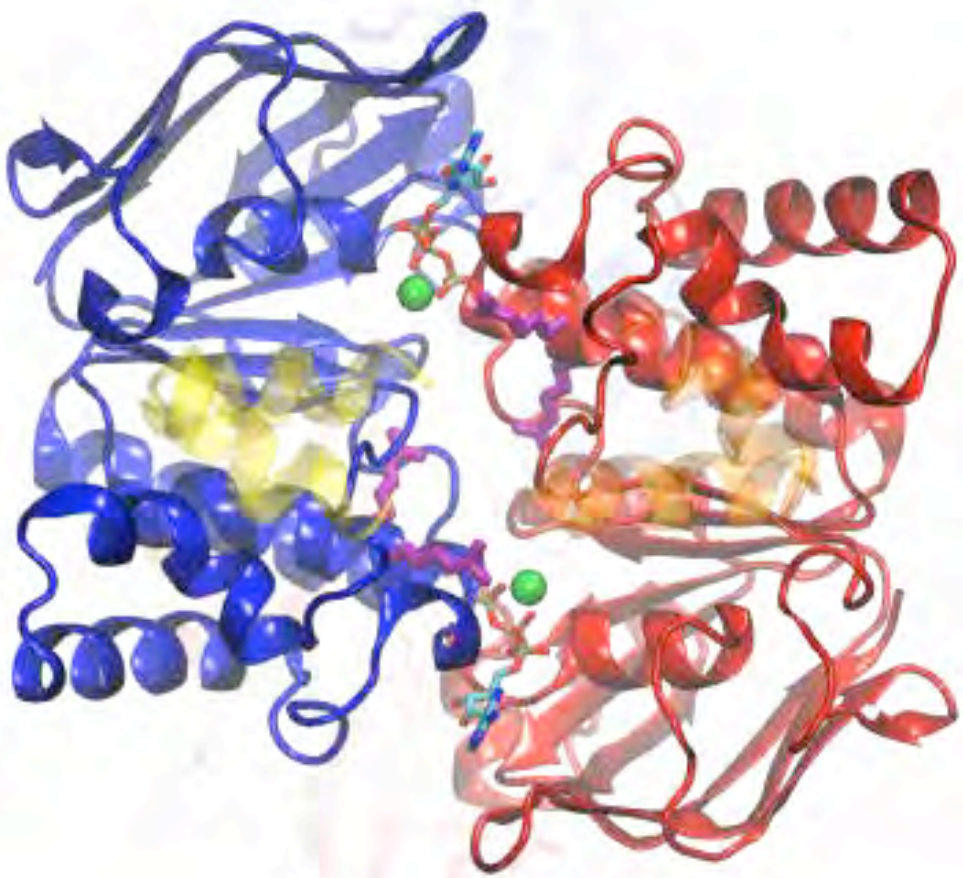


Arg141 neutralized

Arg146 neutralized

Recovery of NBD Dimerization in the Mutants



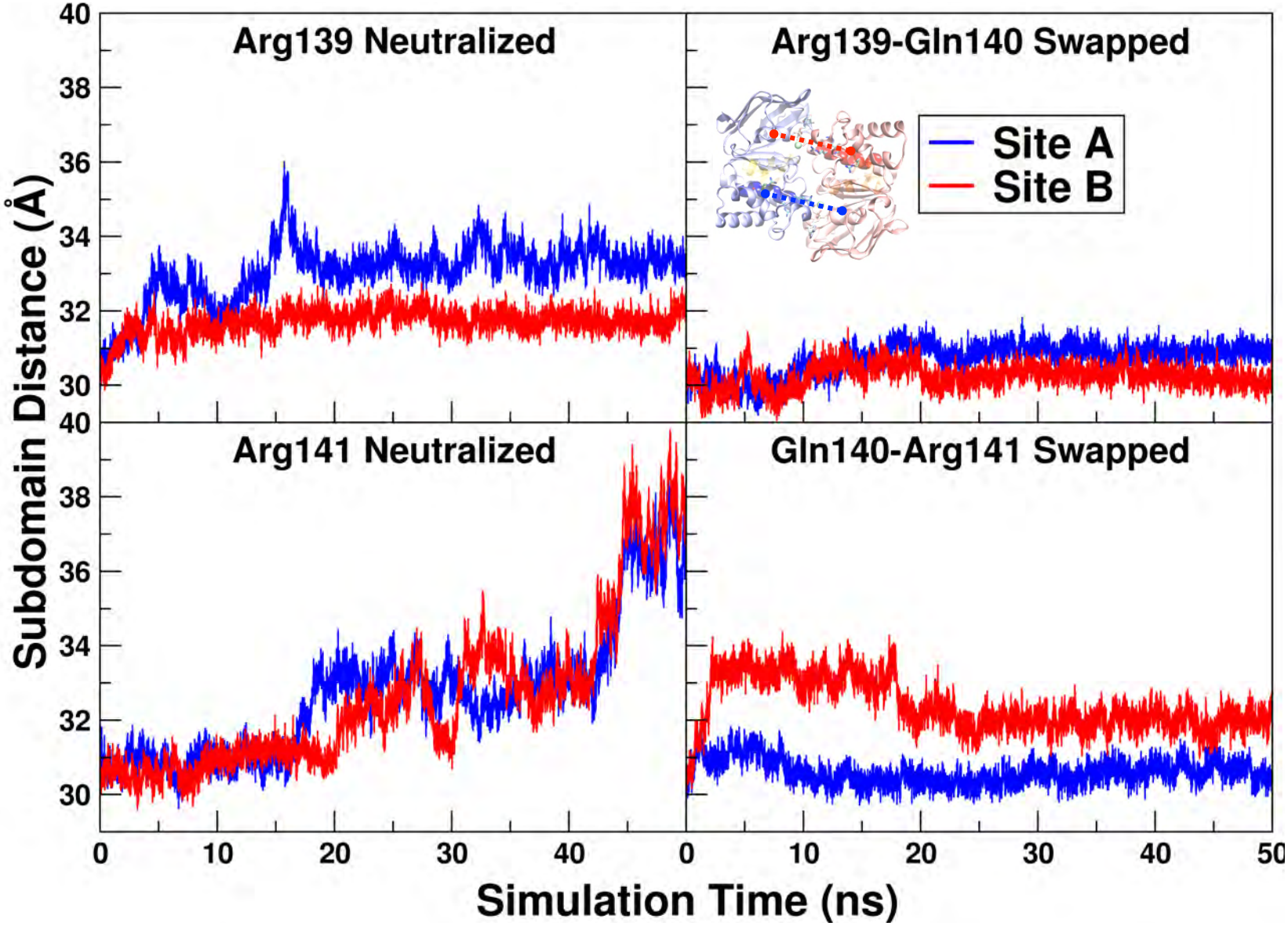


Arg^{139}-Arg^{140}-Gln^{141}

Gln^{139}-Arg^{140}-Arg^{141}

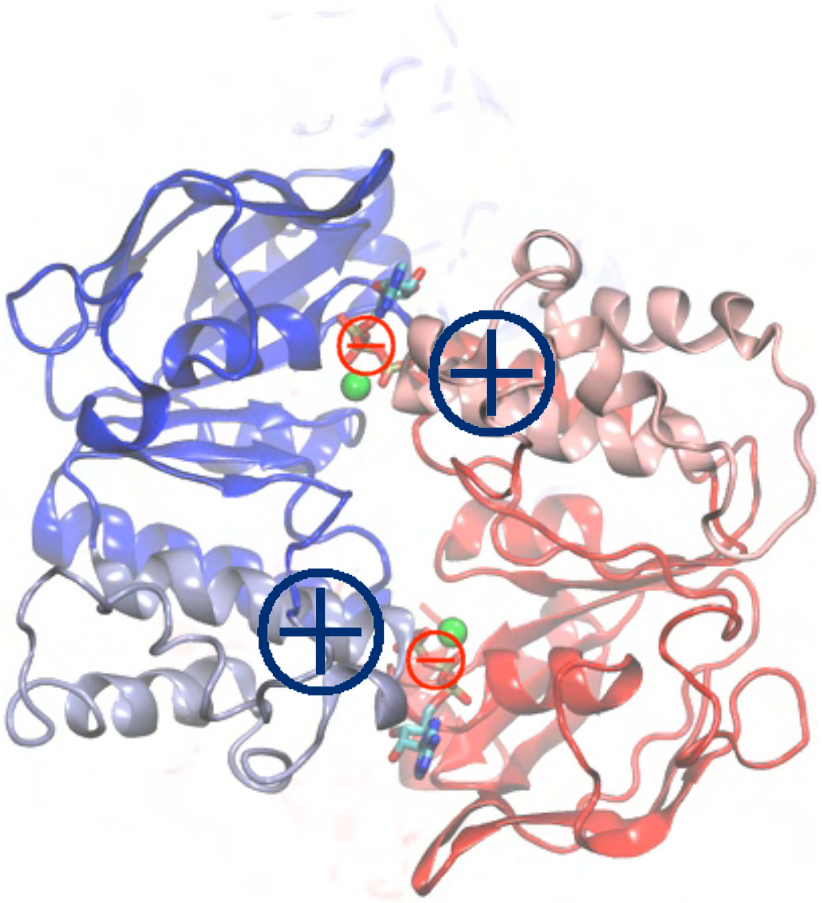
(wt = Arg^{139}-Gln^{140}-Arg^{141})

Recovery of NBD Dimerization in the Mutants



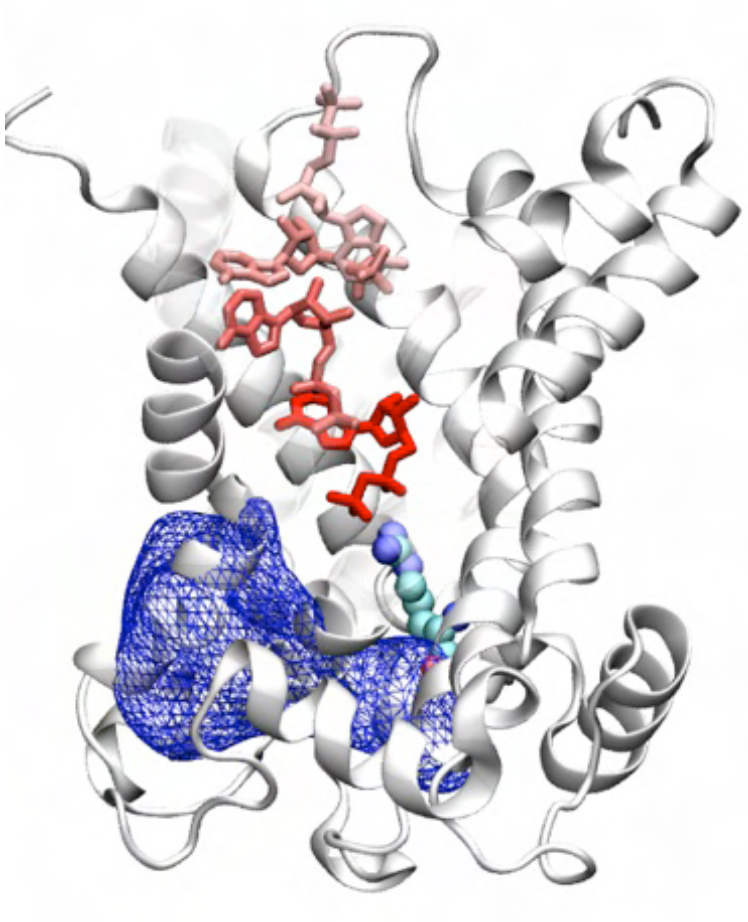
Conclusion: Buried positive charges in the helical subdomains are essential for NBD dimerization

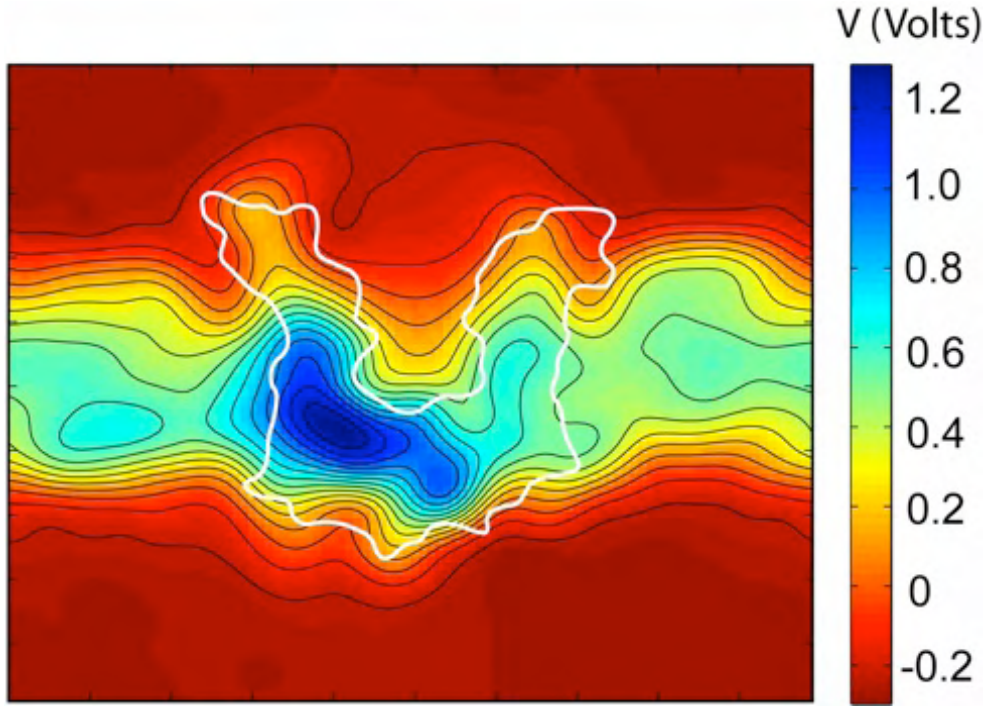
So Why/How Do NBDs Dimerize?



- Three factors determine the NBD dimerization
  - Binding
    - Walker A motif binds ATP
  - Attracting
    - Positive charged residues in the helical subdomain
    - Optimal: 2 positive charges near the LSGGQ motif
    - Location, location, location!
  - Locking
    - Network of hydrogen bonds between the ATP and the LSGGQ motif

Unusually Strong Electrostatic Potential



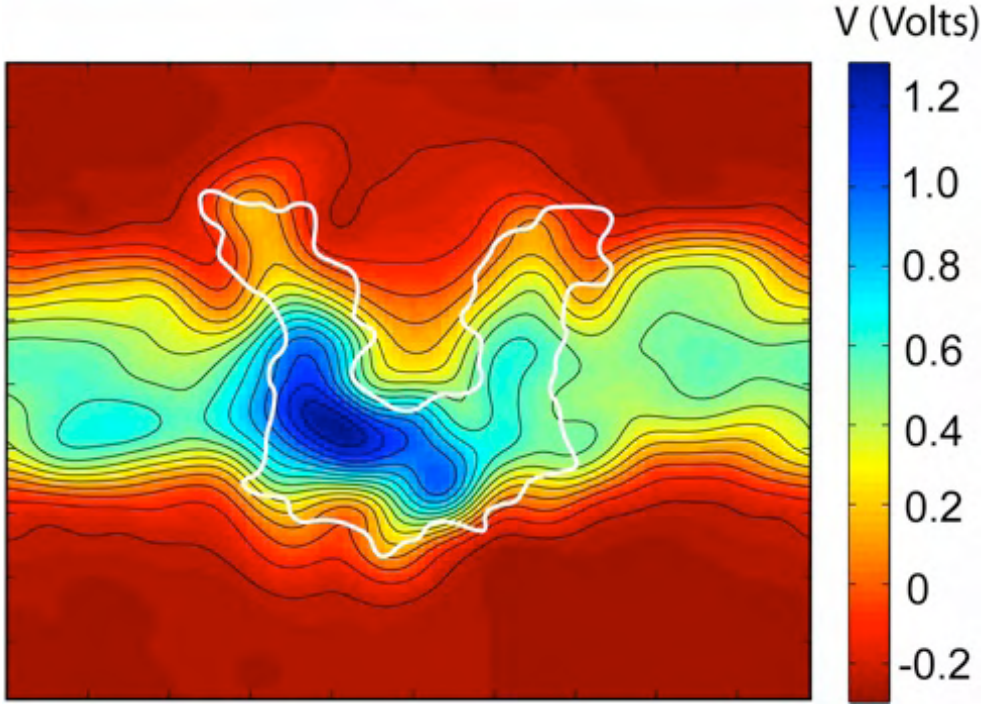


Average electrostatic potential of AAC

- Very strong (~1.4V) positive potential at the AAC basin provides the driving force for ADP binding.

Y. Wang and E. Tajkhorshid, *PNAS* **2008**

Commonality of Electrostatic Features in MCF Members

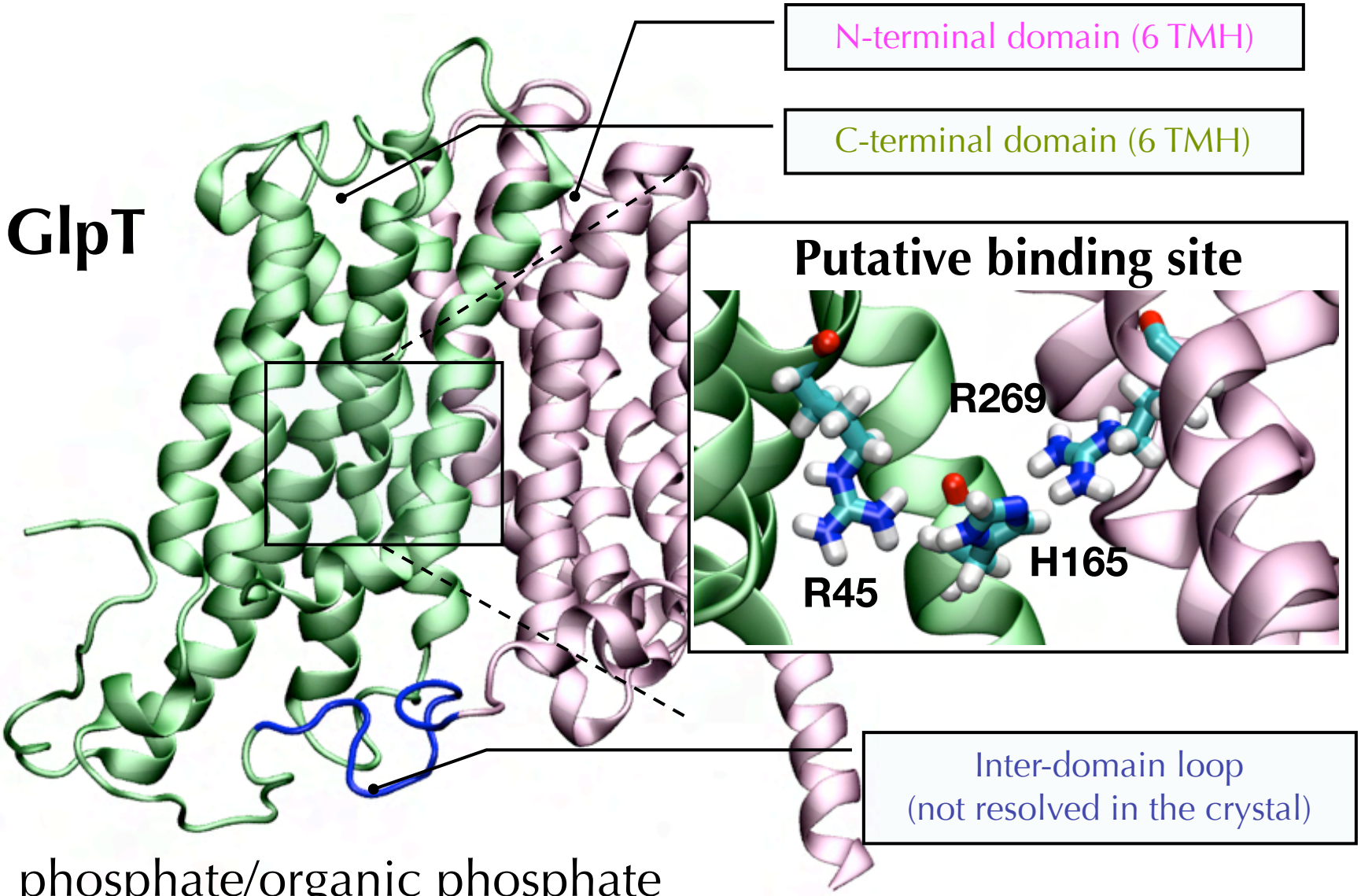


Carrier	P_e	Substrate	S_e
Aac1p	+16	ADP/ATP	-3/-4
Aac2p	+20	ADP/ATP	-3/-4
Aac3p	+20	ADP/ATP	-3/-4
Sal1p†	+15	Mg-ATP/P_i	-2/-3
Leu5p	+17	*C_oA	-4
Flx1p	+18	*FAD	-2
Rim2p	+18	Py(d)NDP/Py(d)NTP	-3/-4
Ndt1p	+5	NAD^+	-1
Ndt2p	+16	NAD^+	-1
Ggc1p	+19	GDP/GTP	-3/-4
Tpc1p	+17	ThPP	-1
Ant1p	−6	AMP/ADP/ATP	-2/-3/-4
Mir1p	+9	P_i	-3
Pic2p	+17	P_i	-3
Oac1p	+13	oxaloacetate	-2
Dic1p	+14	malate	-2
Odc1p	+19	2-oxoglutarate	-2
Odc2p	+19	2-oxoglutarate	-2
Sfc1p	+19	succinate/fumarate	-2
Ctp1p	+14	citrate	-3
Agc1p†	+14	aspartate/glutamate-H^+	-1/0
Crc1p	+17	carnitine	0
Ort1p	+10	ornithine	0
Pet8p	+13	S-adenosyl methionine	0
Mrs3p	+4	*Fe^{+2}	+2
Mrs4p	+2	*Fe^{+2}	+2
Yhm2p	+18	Unknown	—
Ymc2p	+9	Unknown	—
Yfr045wp	+17	Unknown	—
Ypr011cp	+13	Unknown	—
Ymc1p	+10	Unknown	—
Ydl119cp	+18	Unknown	—
Ymr166	+7	Unknown	—
Mtm1p	+15	Unknown	—

- Almost all MCF members are strongly charged (positive).
- Most substrates of MCFs are negatively charged.
  - Substrate recruitment
  - Anchoring the proteins into the negatively charged inner mitochondrial membrane.

Y. Wang and E. Tajkhorshid, *PNAS* **2009**

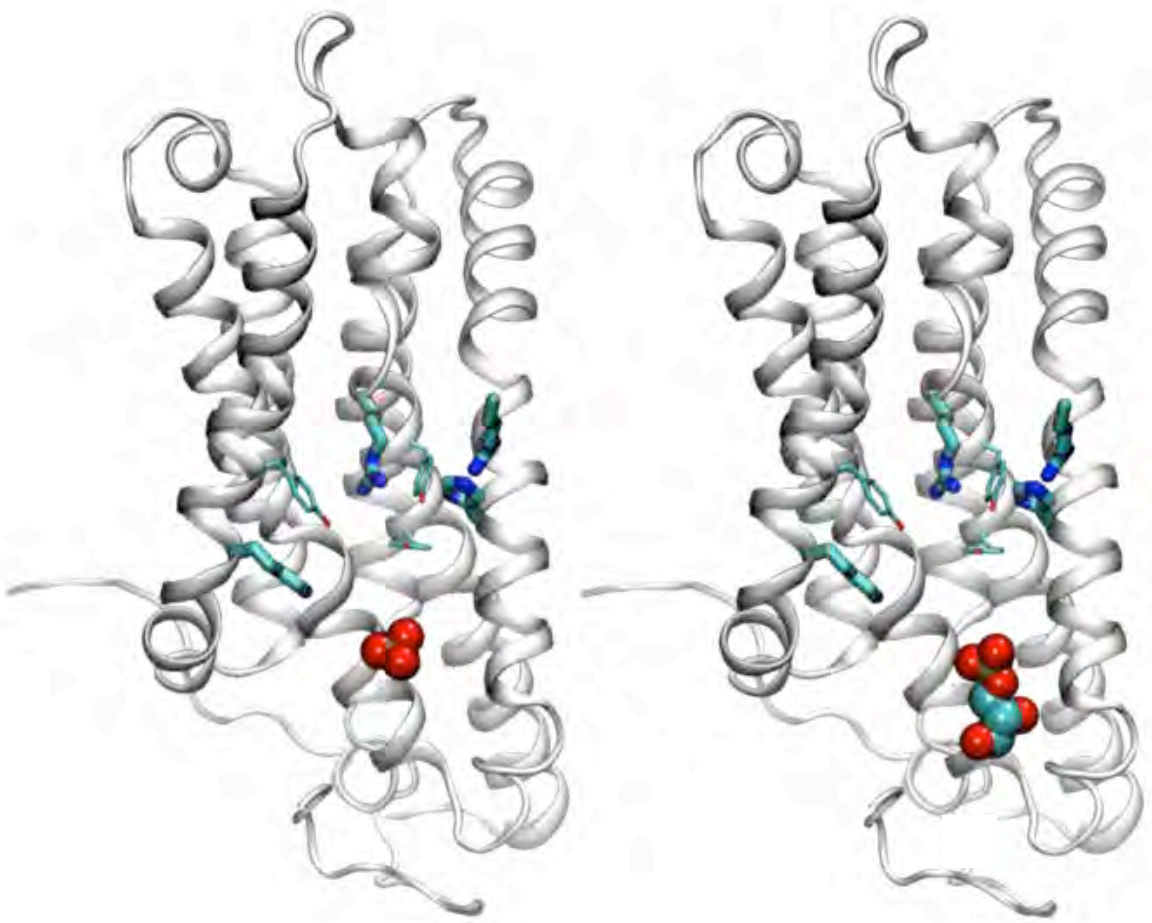
Capturing Substrate Binding - Case II

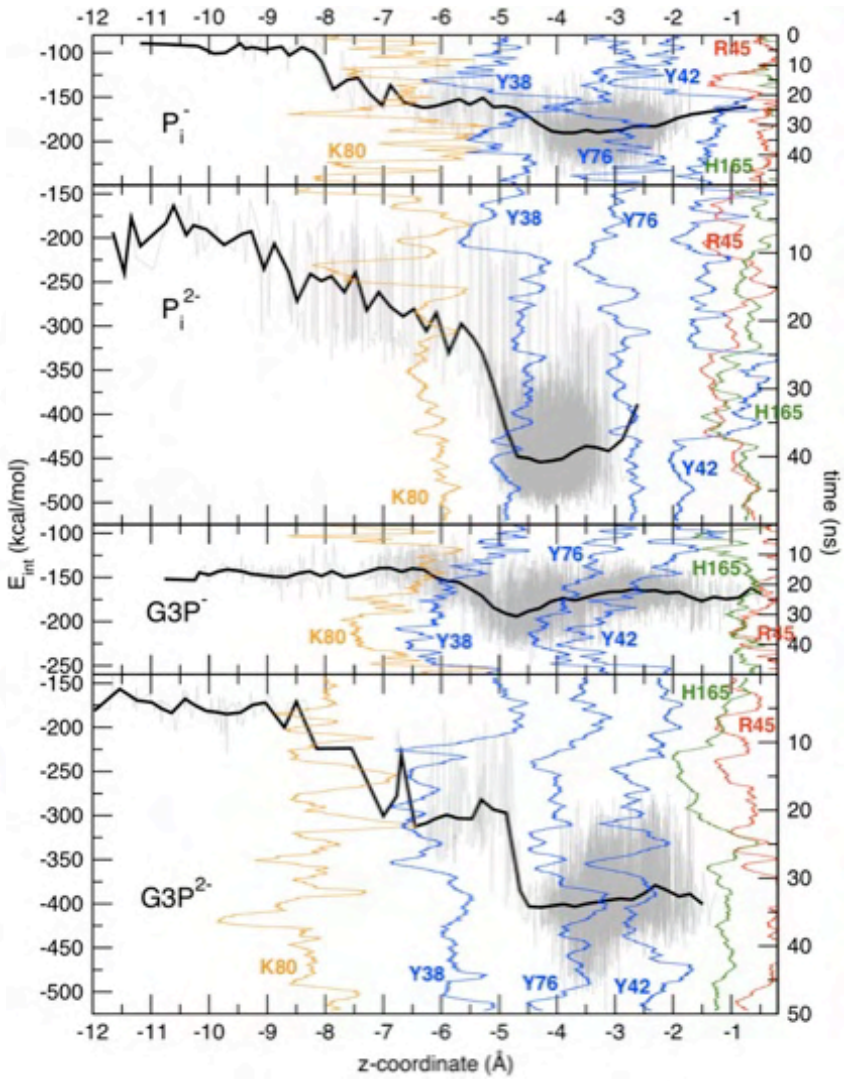


phosphate/organic phosphate antiporter

Huang, et al., *Science* **301**, 616-620 (2003)

Capturing Substrate Binding - Case II

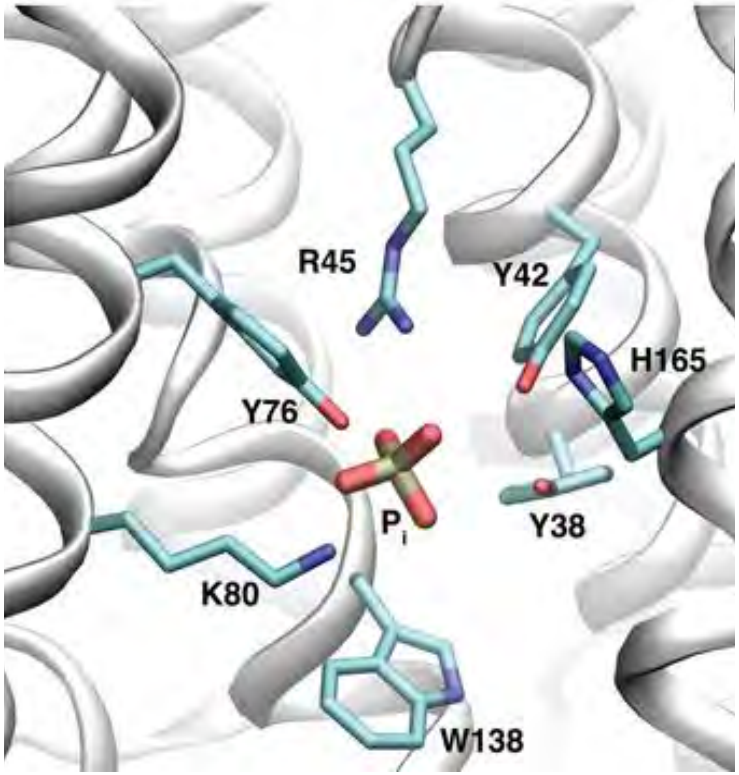




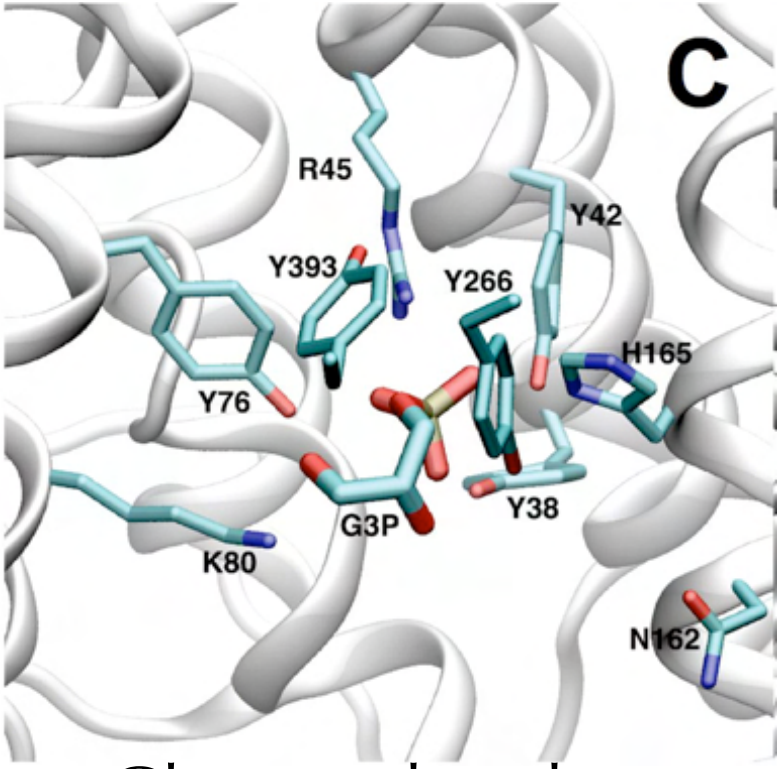
Ch. Law, G. Enkavi, D.-N. Wang and E. Tajkhorshid, Biophysical Journal **2009**

G. Enkavi and E. Tajkhorshid, *Biochemistry* **2010**

Characterizing Substrate Selectivity



Phosphate Binding Site



Glycero-phosphate Binding Site

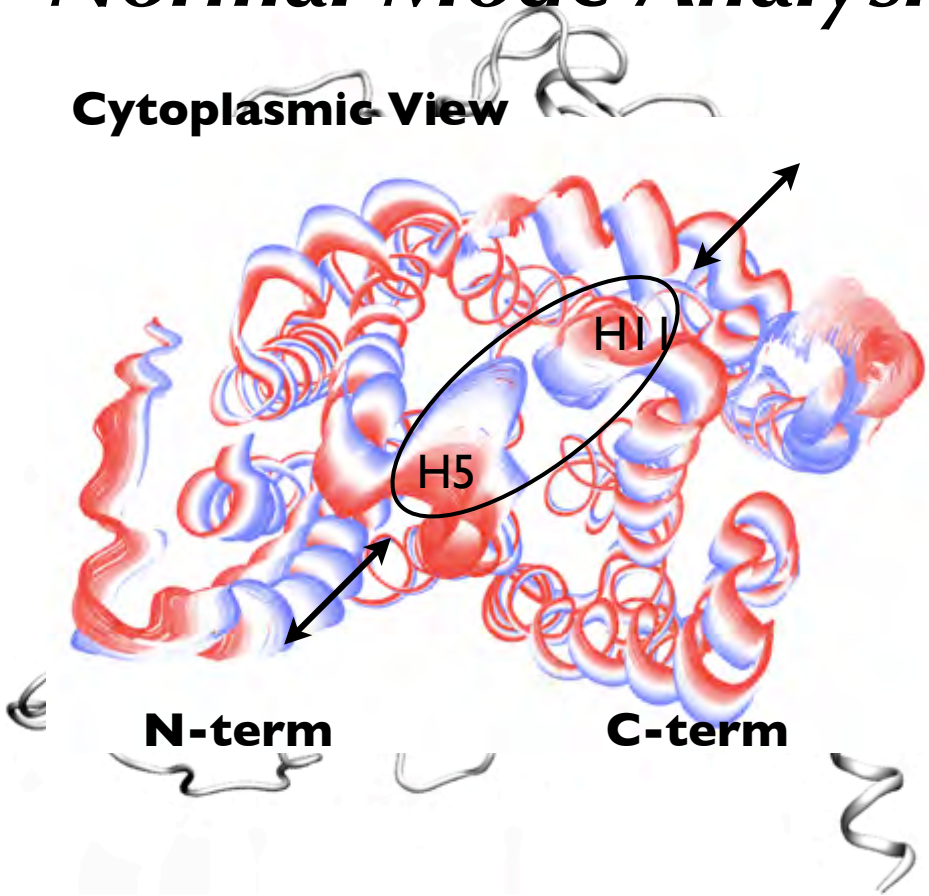
GlpT protein	K_d P_i binding (μM)	K_d G3P binding (μM)	K_d phosphomycin binding (μM)	G3P-P_i exchange transport activity?
Wild-type	7.4 ± 0.4	0.8 ± 0.2	0.18 ± 0.02	Yes
N162A	4.4 ± 1.4	No binding	No binding	n.d.
Y266F	1.7 ± 0.4	No binding	No binding	No
Y393F	8.6 ± 0.7	No binding	43 ± 6	n.d.

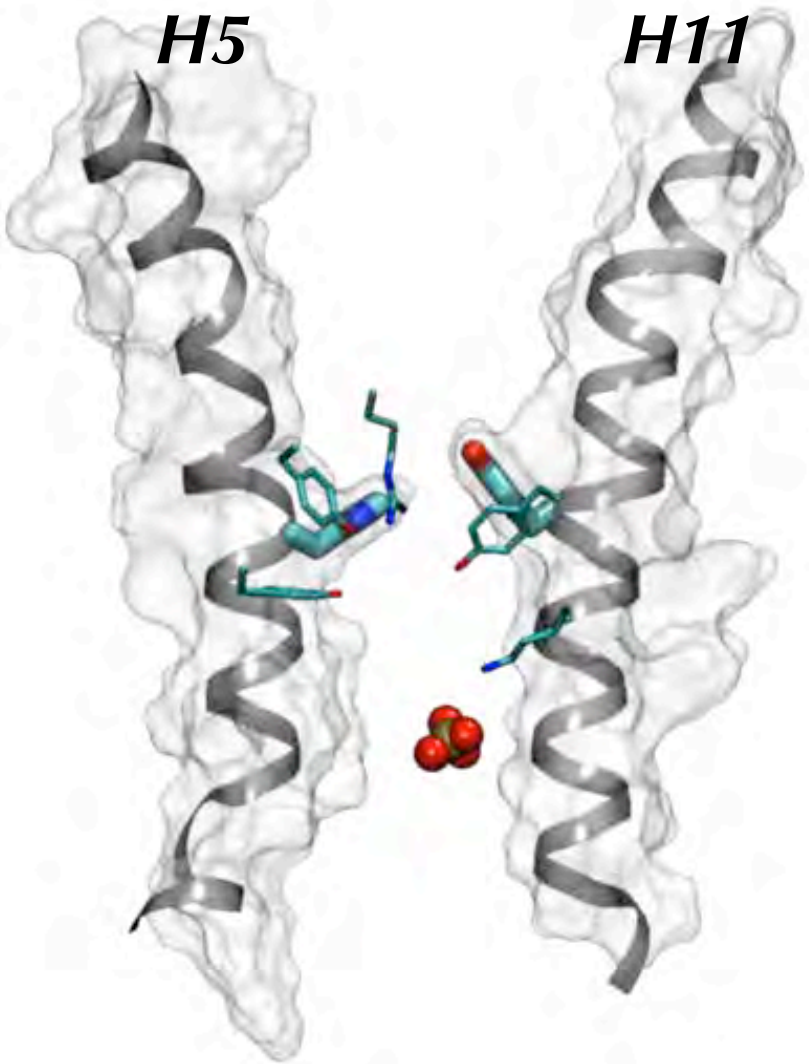
Ch. Law, G. Enkavi, D.-N. Wang and E. Tajkhorshid, Biophysical Journal **2009**.

Capturing the Initial Steps of the Rocker-Switch Mechanism

Substrate induced straightening of Helices 5 and 11

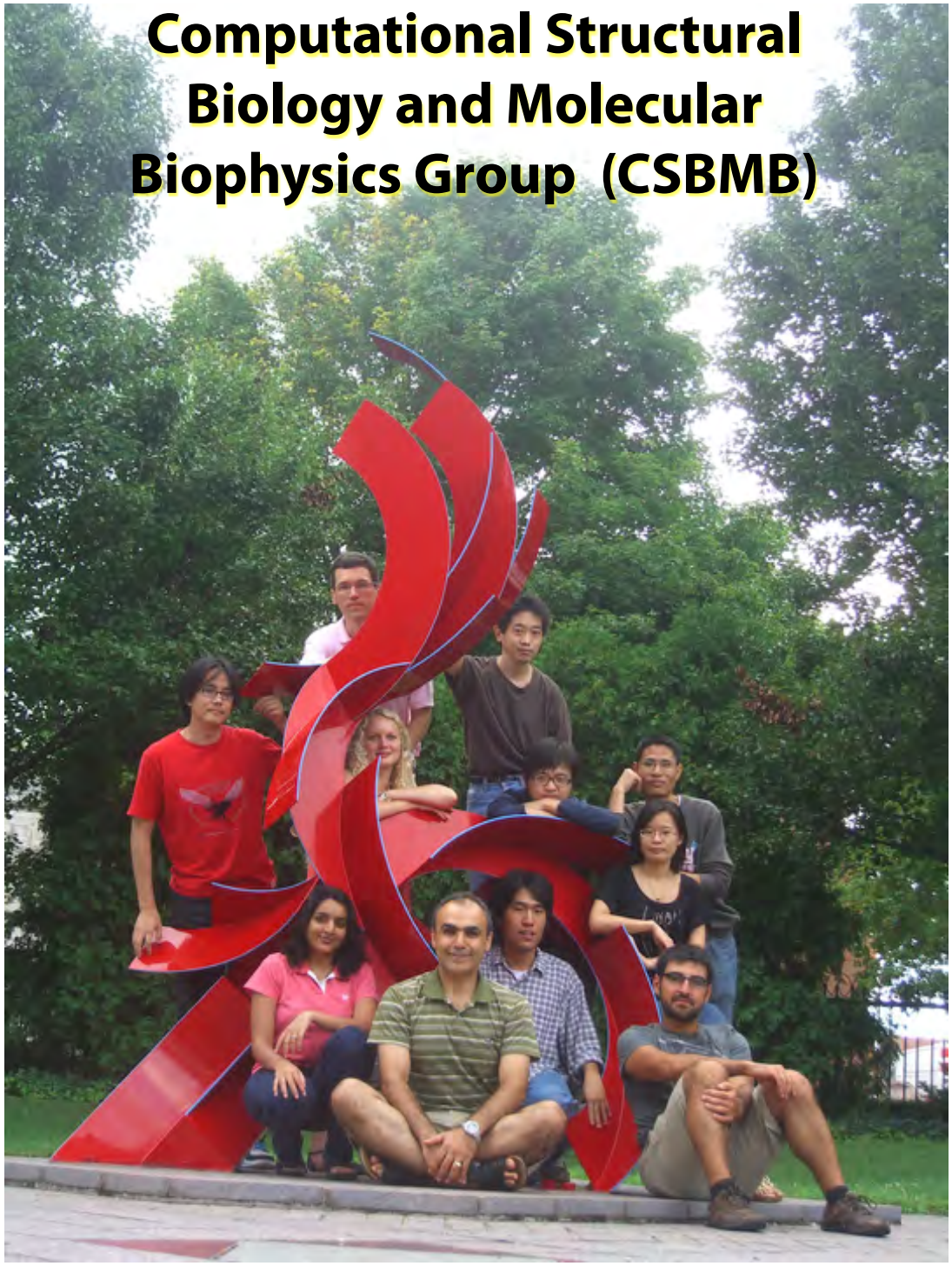
Normal Mode Analysis





G. Enkavi and E. Tajkhorshid, *Biochemistry* **2010**

Computational Structural Biology and Molecular Biophysics Group (CSBMB)



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