Dynamical View of Energy Coupling Mechanisms in Active Membrane Transporters

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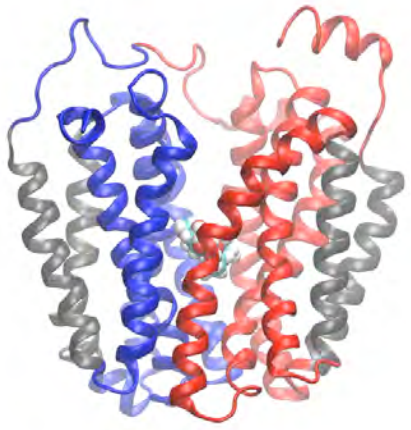
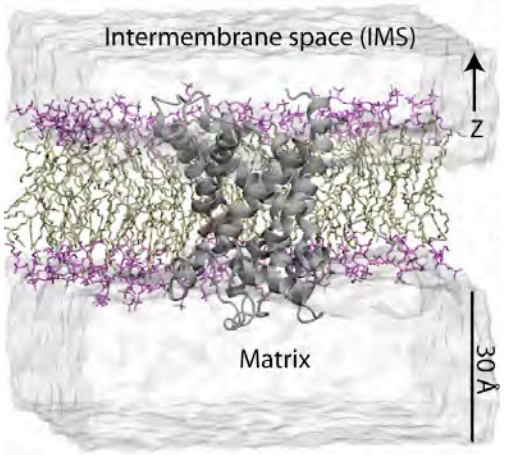
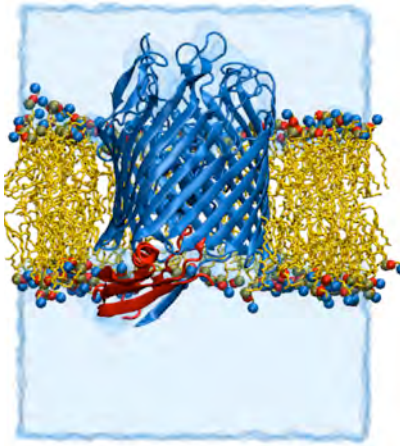


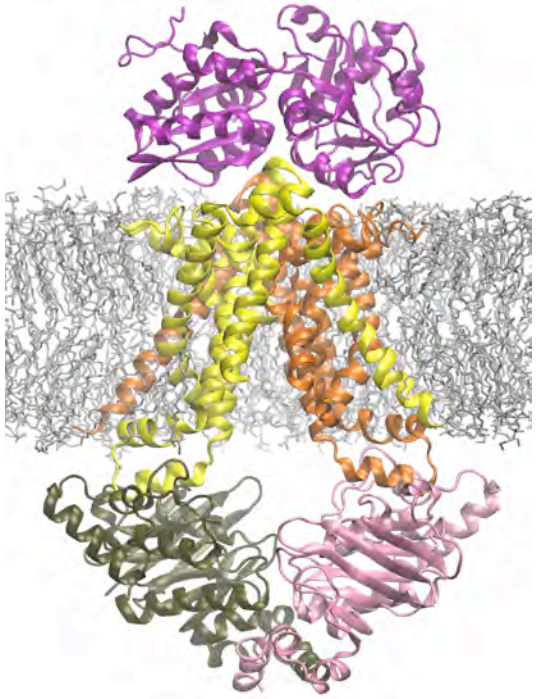
Probing Permeation Pathway in Lactose Permease



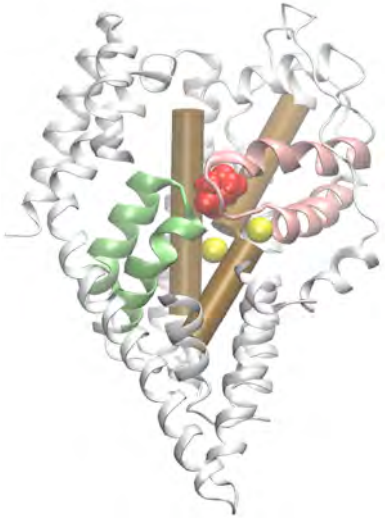
Nucleotide Exchange Across Mitochondrial Membrane



Energy transduction in outer membrane transporters

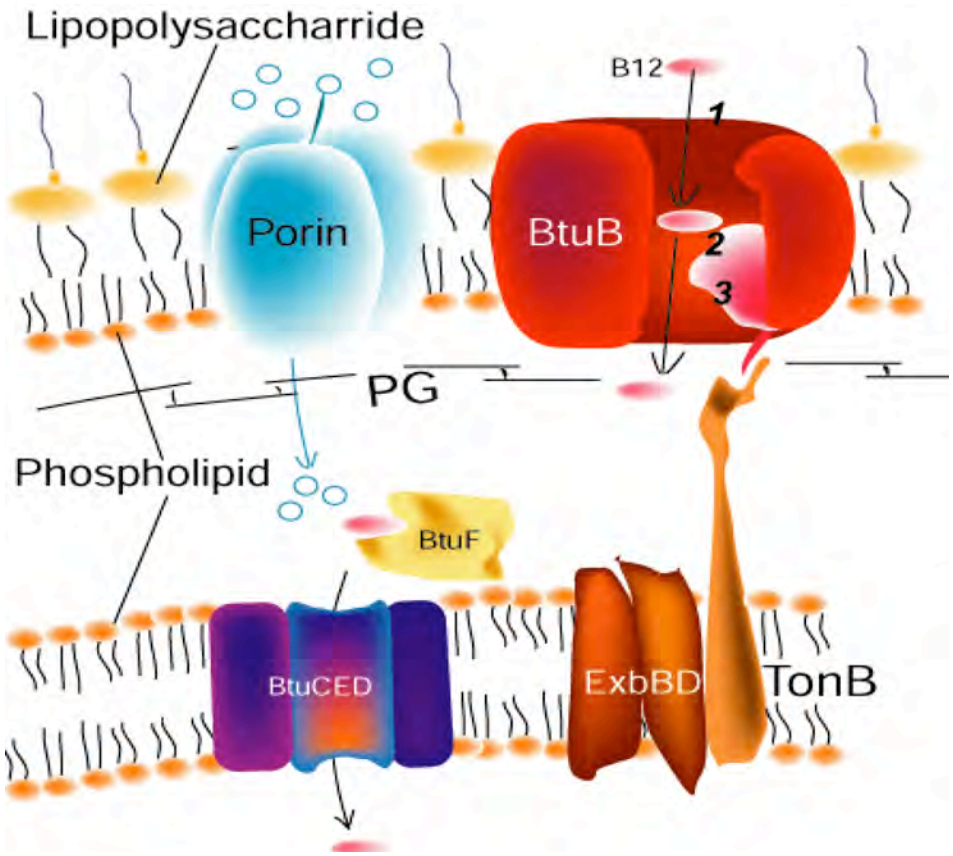


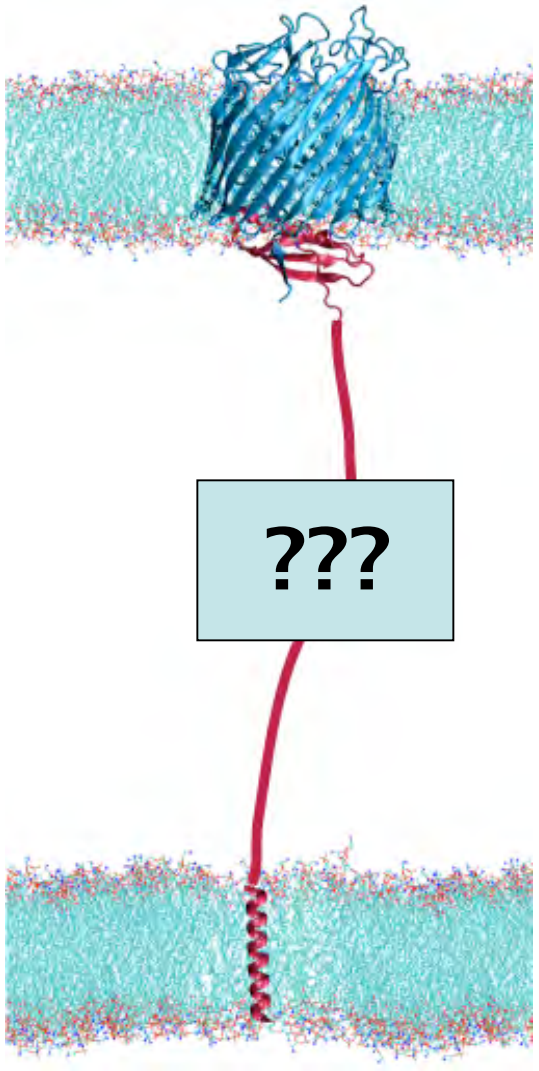
ATP Driven Transport in ABC Transporters



Neurotransmitter uptake by GluT

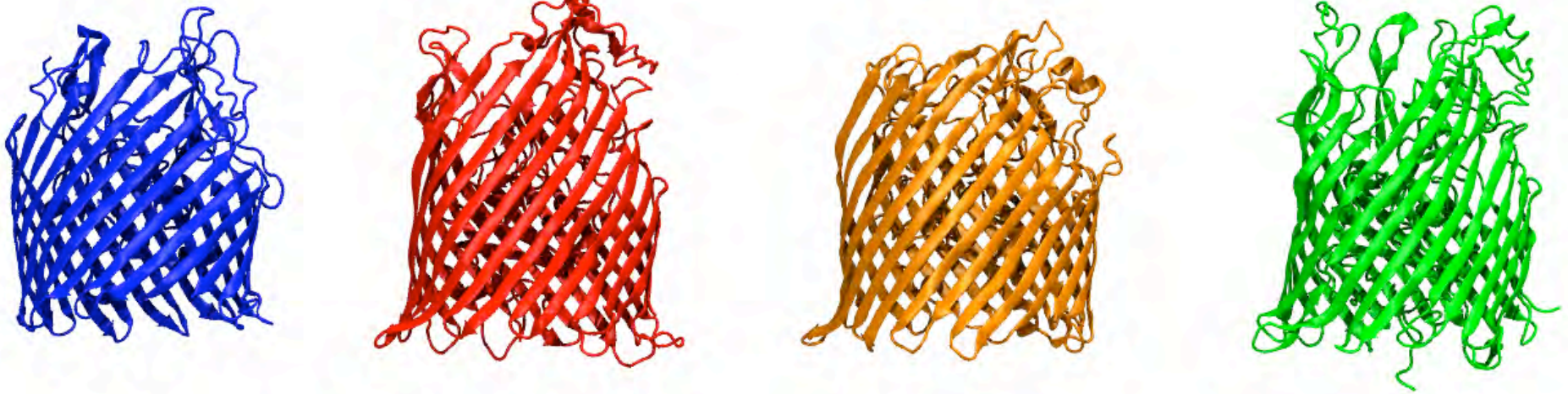
Force-Induced Activation in Outer Membrane Transporters





TonB-dependent Transporters

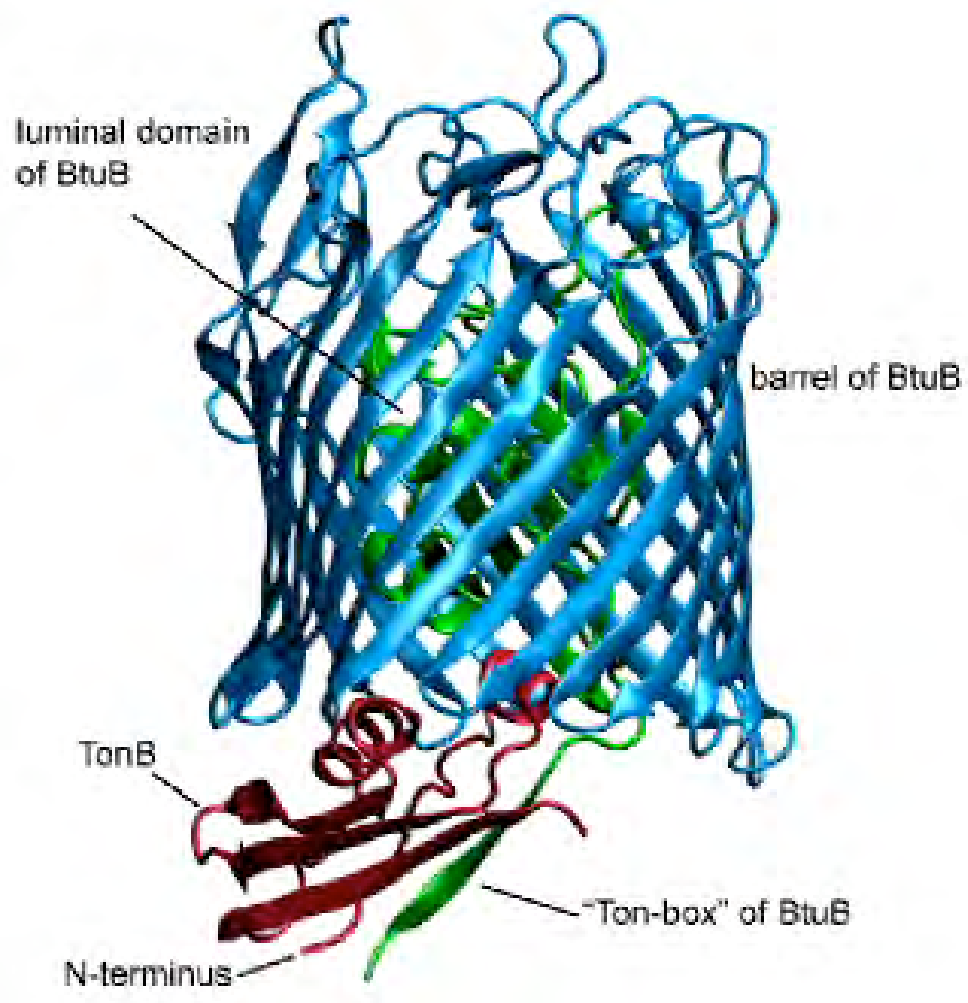
BtuB **FhuA** **FepA** **FpvA**



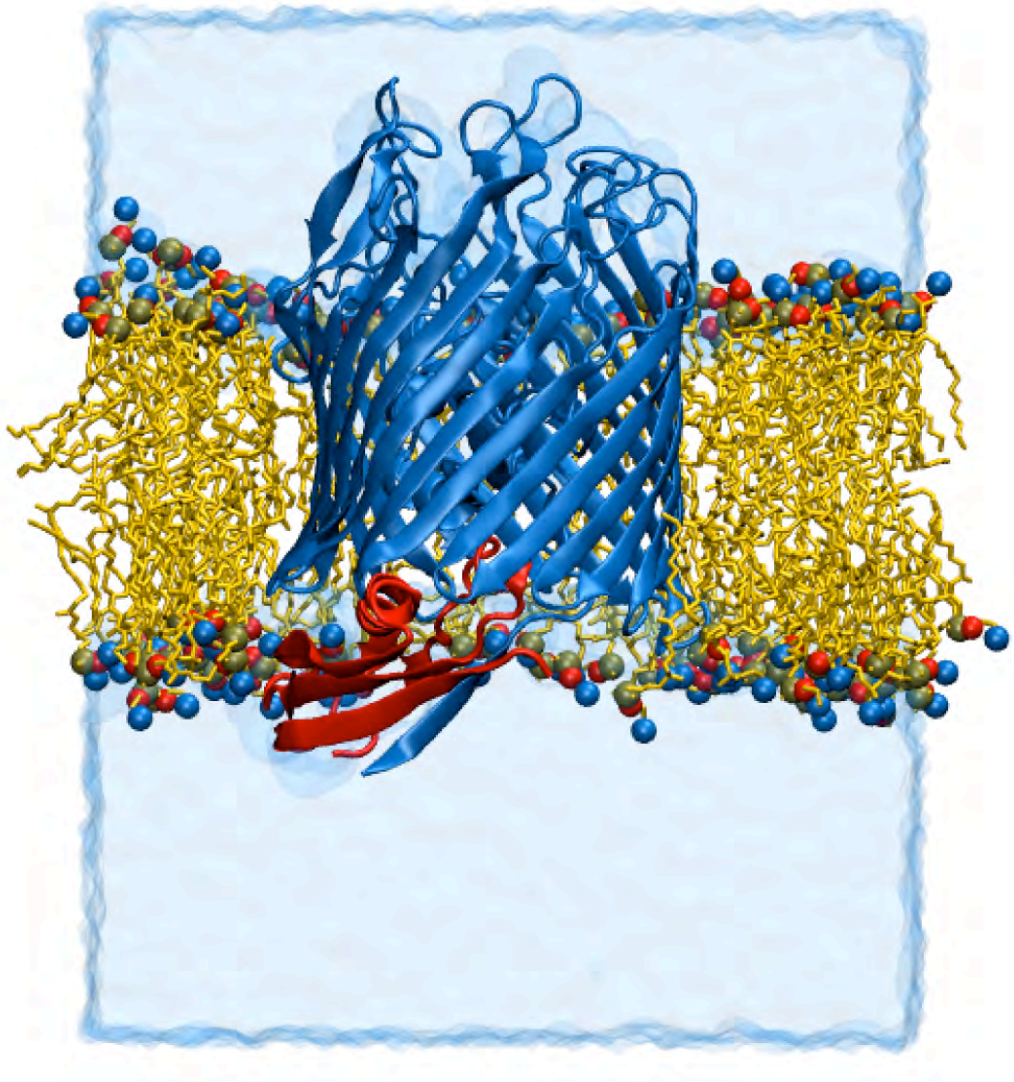
Substrates

B12 **ferrichrome** **ferric citrate** **pyoverdine**

BtuB – Communication in Action



BtuB – Communication in Action



lipid bilayer, water, 100 mM ions

~100,000 atoms

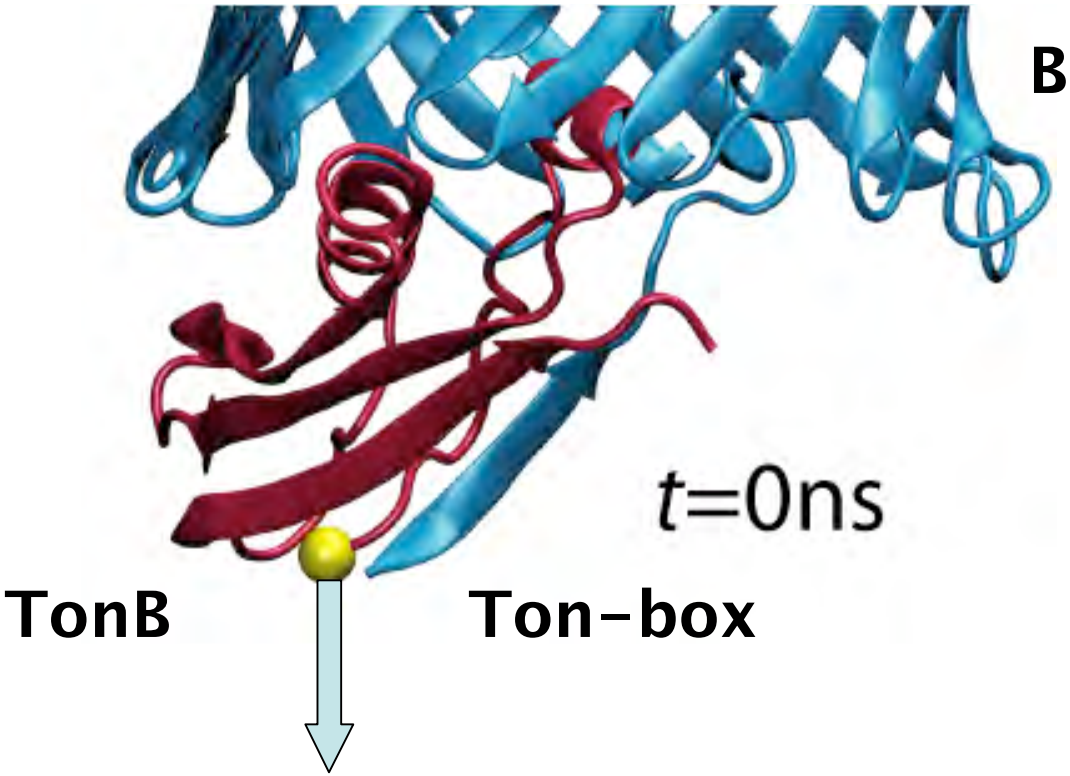
Simulations performed with NAMD2, CHARMM27 forcefield

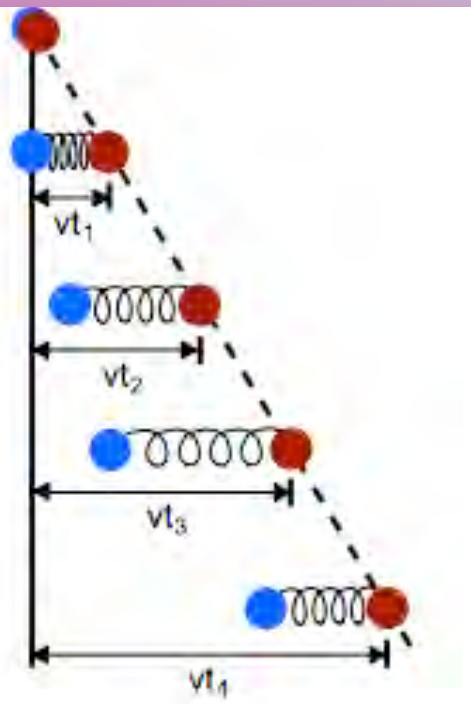
T = 310 K, Periodic system

Total simulation time of over 100 ns

J. Gumbart, et al., ***Biophys. J.,*** 2007.

Mechanical strength of the complex



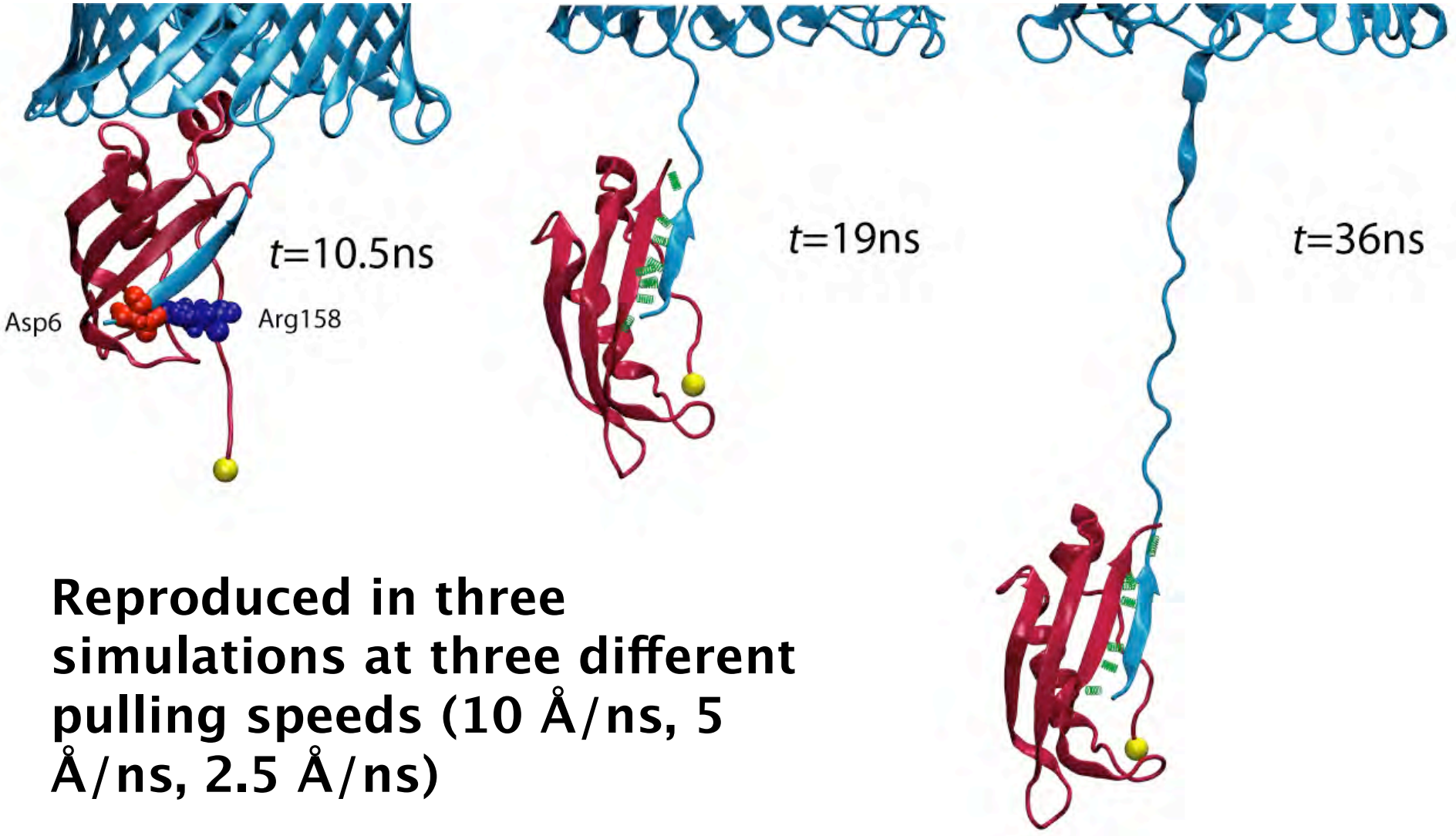


Pulled N-terminus down, toward cytoplasmic membrane

Will the two proteins separate immediately?

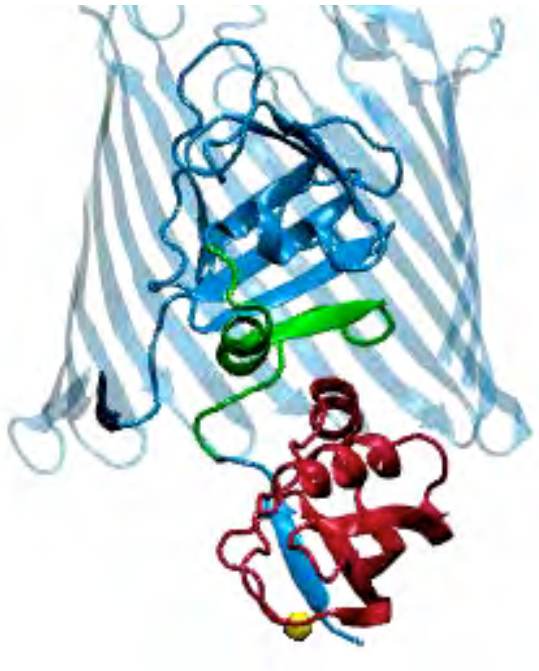
J. Gumbart, et al., ***Biophys. J.,*** 2007.

A small but strong connection

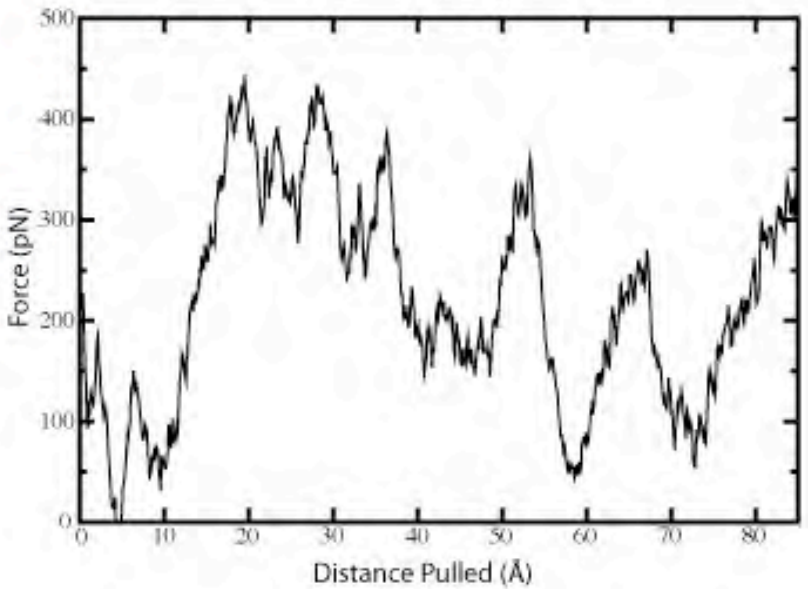


Reproduced in three simulations at three different pulling speeds (10 Å/ns, 5 Å/ns, 2.5 Å/ns)

J. Gumbart, et al., ***Biophys. J.,*** 2007.



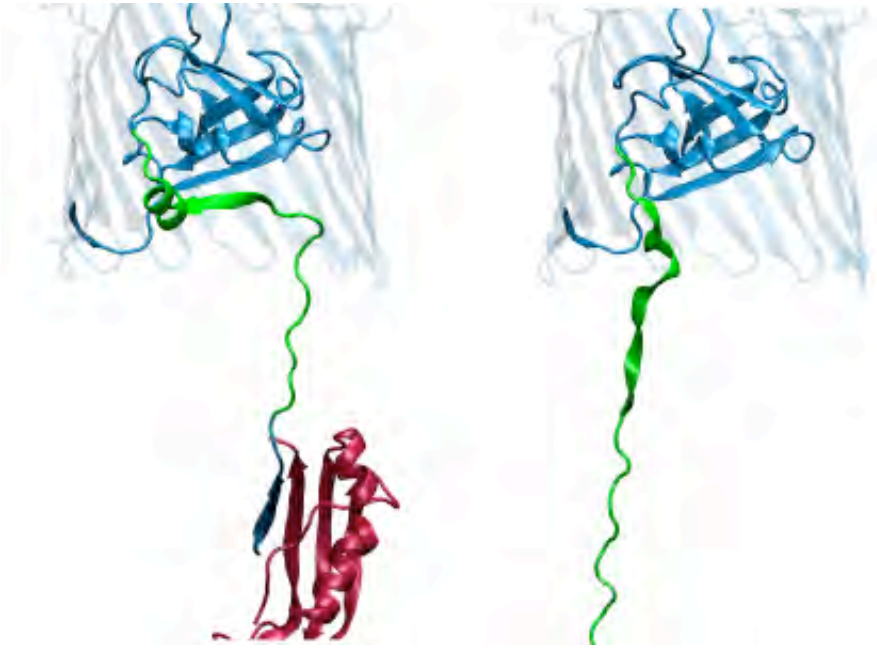
Primary response of the luminal domain to mechanical stress

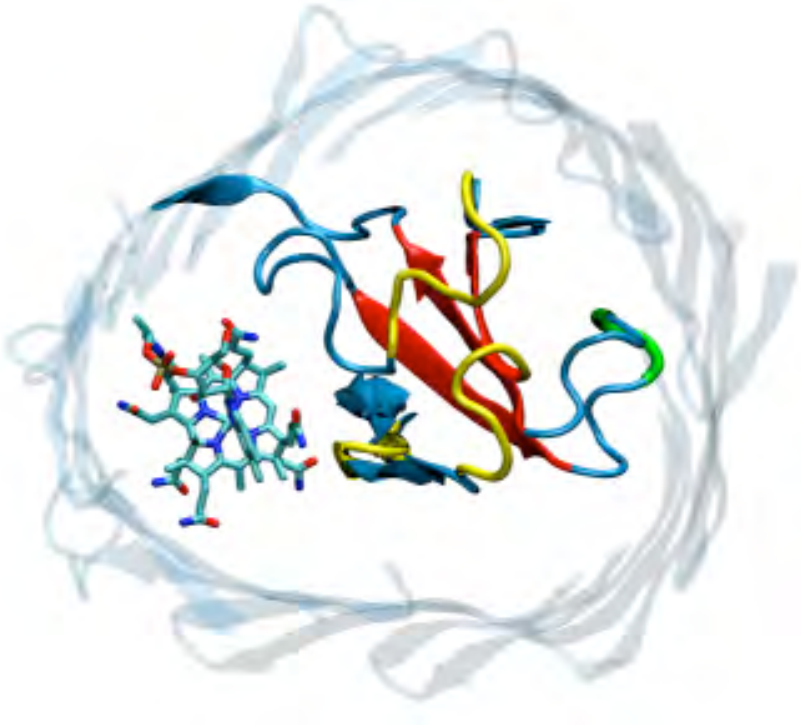


Max Force: 450 pN

J. Gumbart, et al., ***Biophys. J.,*** 2007.

Primary response of the luminal domain to mechanical stress

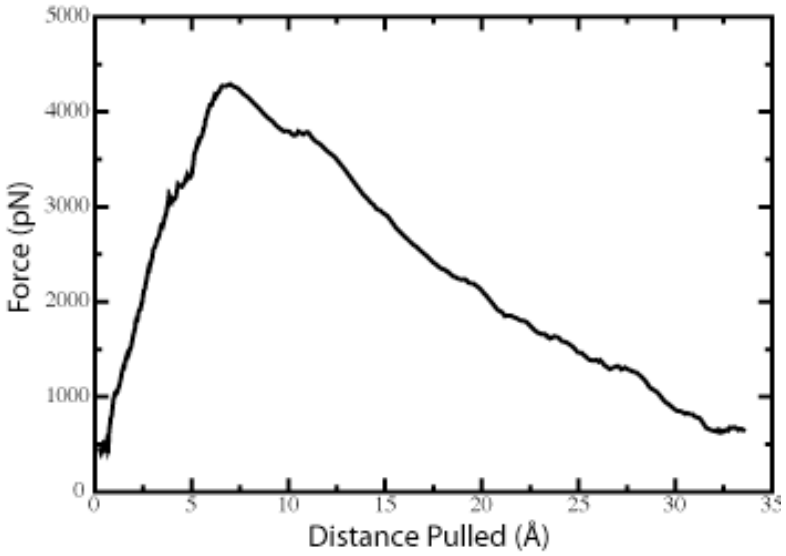




Experimental results strongly suggest the luminal domain leaves the barrel

Ma et al. (2007) *JBC*, **282**: 397–406.

Another way to open(?): “Unplugging”

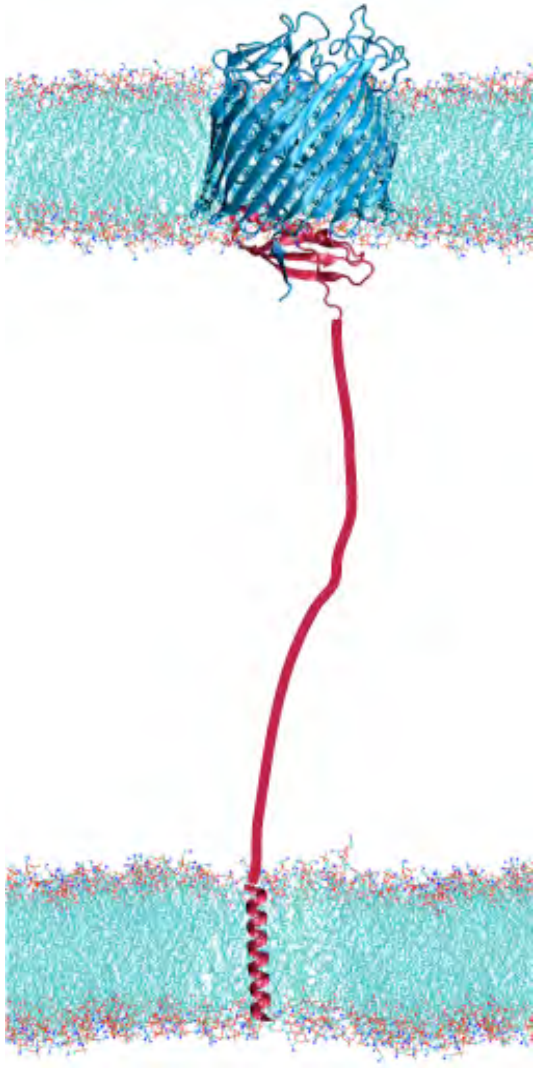


Max Force: 4500 pN,
10x unfolding!

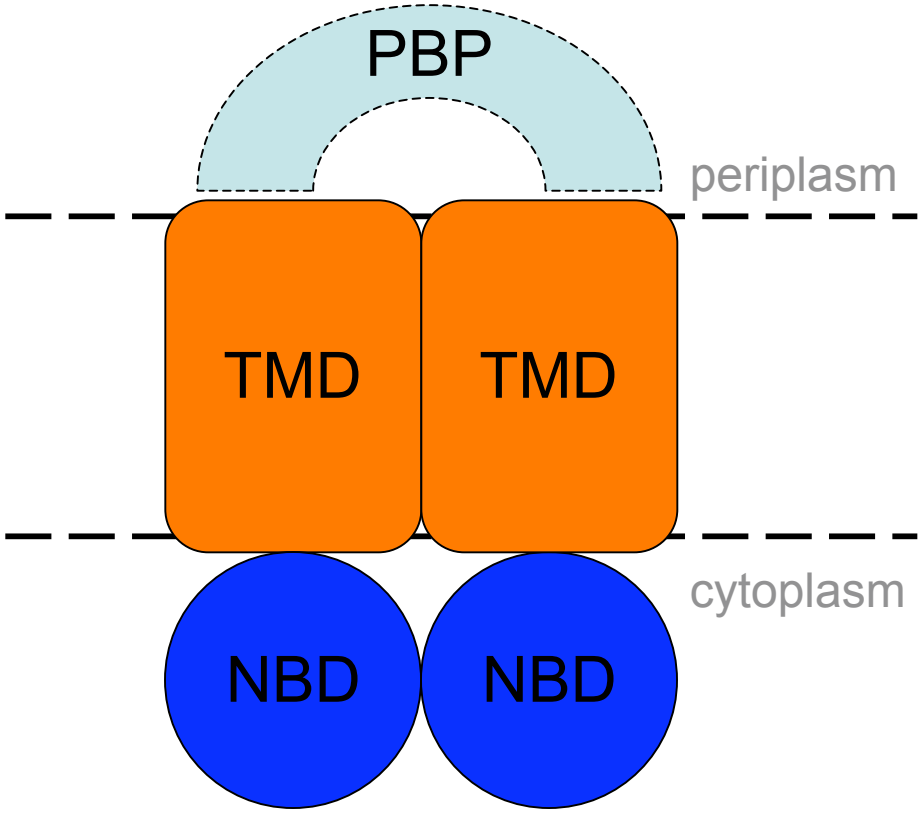
J. Gumbart, et al., ***Biophys. J.,*** 2007.

Is this how TonB-dependent transport really happens?

- The coupling between TonB and BtuB is strong enough for mechanical activation of the transporter
- The primary response of the luminal domain to mechanical force is unfolding
- Very unlikely that an extension of about 100 A takes place in the periplasm



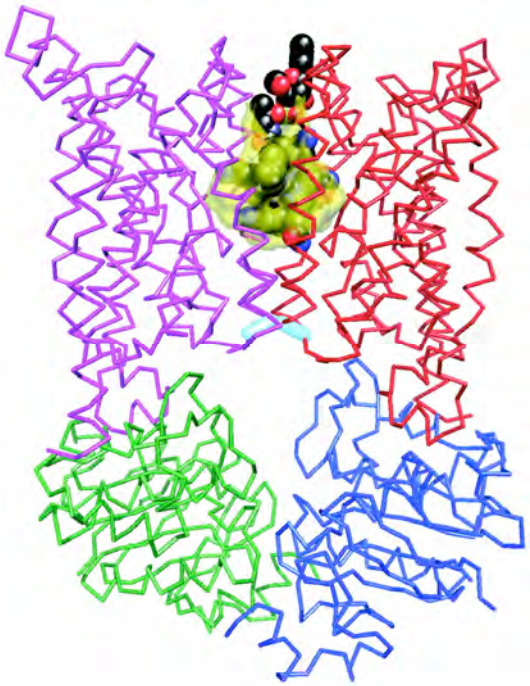
ABC Transporters



- Architecture
  - 2 NBDs
    - Conserved sequence
    - ATPase activity
  - 2 TMDs
    - Diverse sequence
    - Substrate transport
  - 1 PBP
    - Importer only
    - Substrate recognition and binding
- Domain arrangement
  - 1, 2 or 4 polypeptide chains for NBDs & TMDs

Crystal Structures of ABC Importers

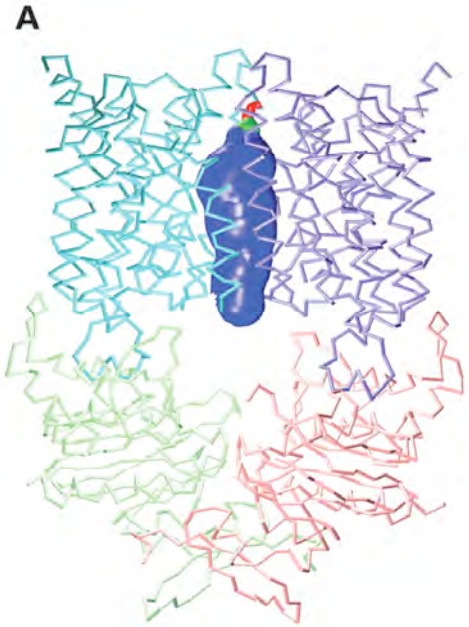
B_{12} importer



Locher *et. al.*, **Science,** (2002)

Periplasmic open

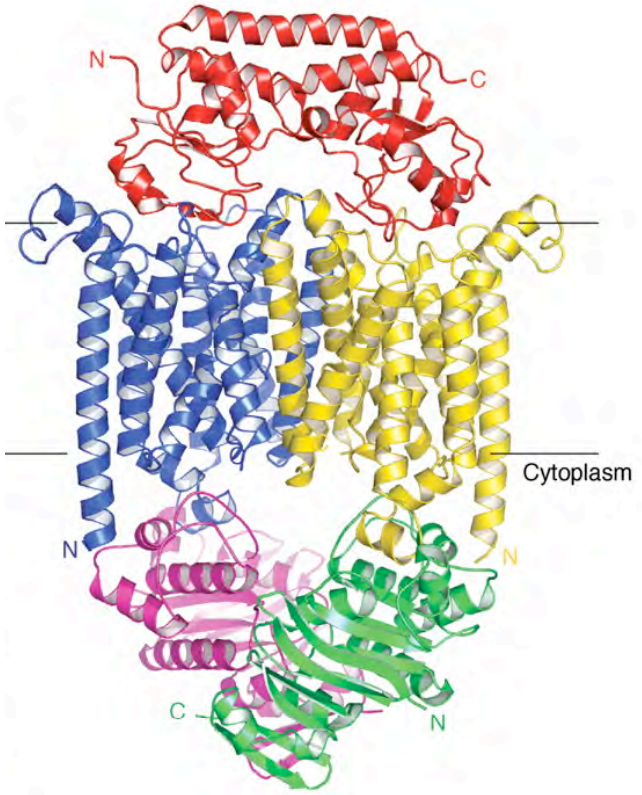
Metal importer



Pinkett *et. al.*, **Science**, (2007)

Cytoplasmic open

B_{12} importer

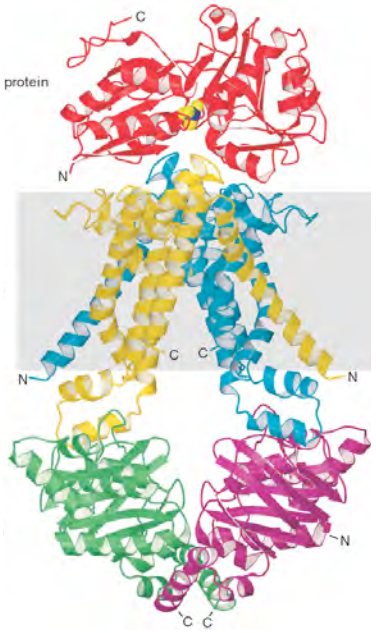


Hvorup *et. al.*, **Science**, (2007)

Occluded

Crystal Structures of ABC Importers

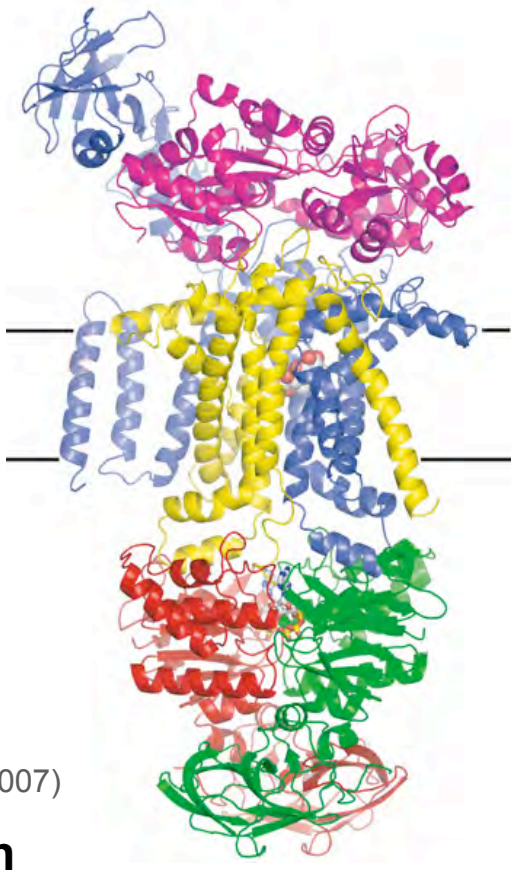
MoO_4^{2-} importer



Hollenstein *et. al.*, **Nature**, (2007)

Cytoplasmic open

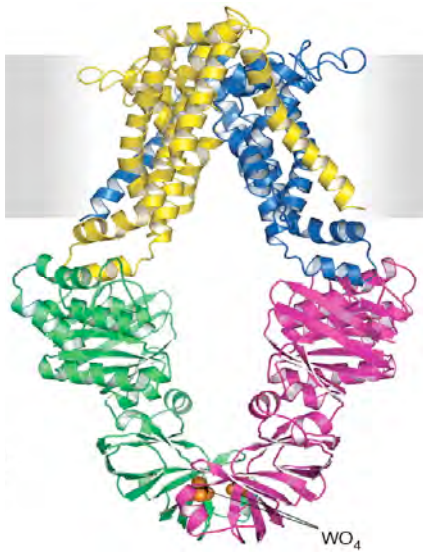
Maltose importer



Oldham *et. al.*, **Nature**, (2007)

Periplasmic open

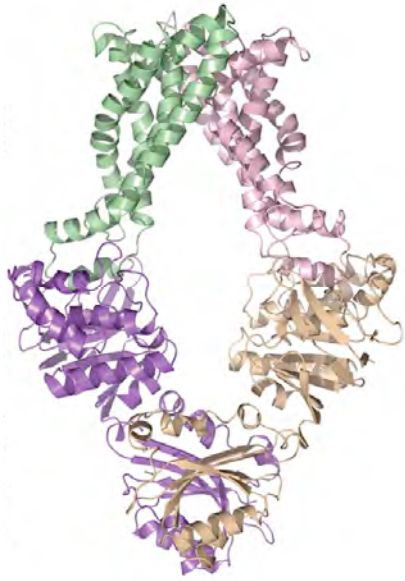
MoO_4^{2-} importer



Gerber *et. al.*, **Science**, (2008)

Cytoplasmic open

Methionine importer



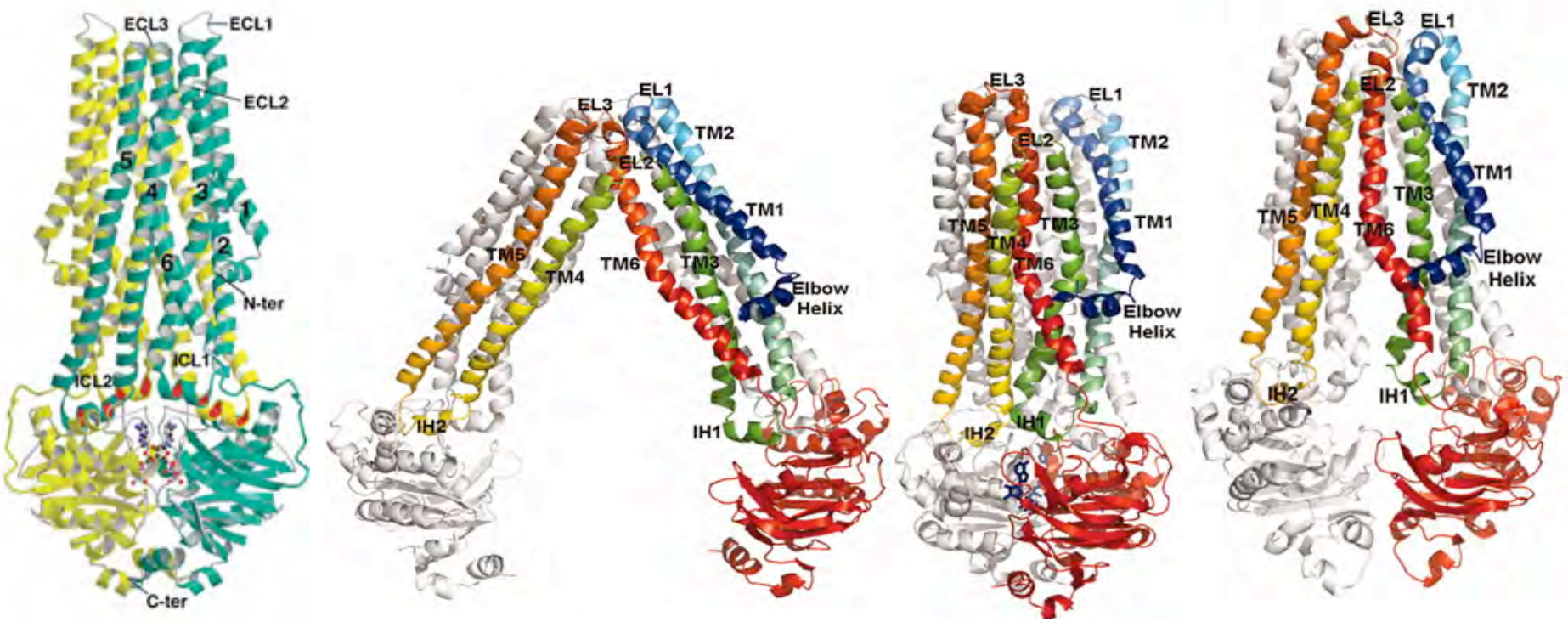
Kabada *et. al.*, **Science**, (2008)

Cytoplasmic open

Crystal Structures of ABC Exporters

Bacterial exporter / MDR

Lipid A flippase / MDR

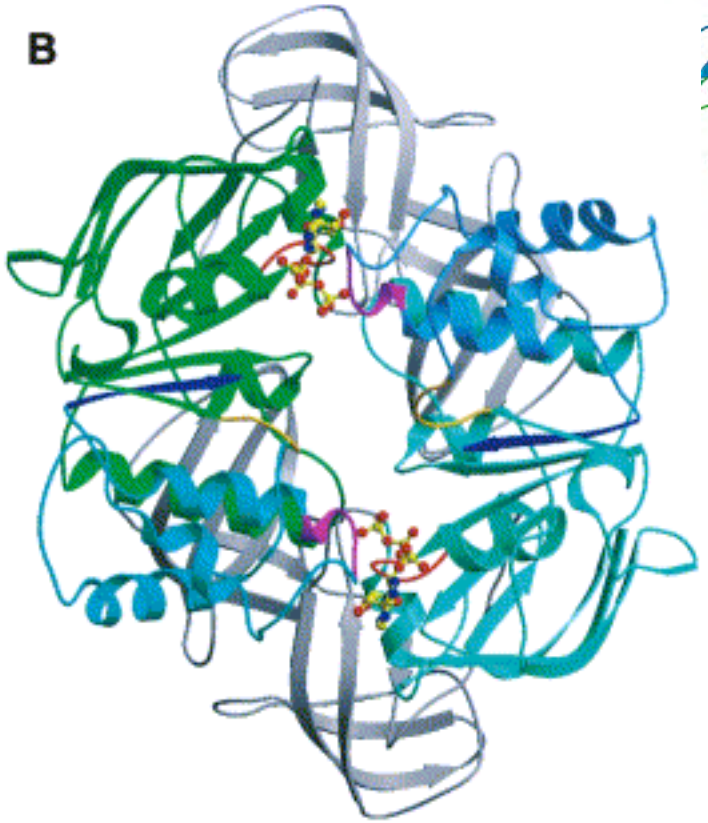


Periplasmic open **Cytoplasmic open** **Periplasmic open** **Semi-Occluded**

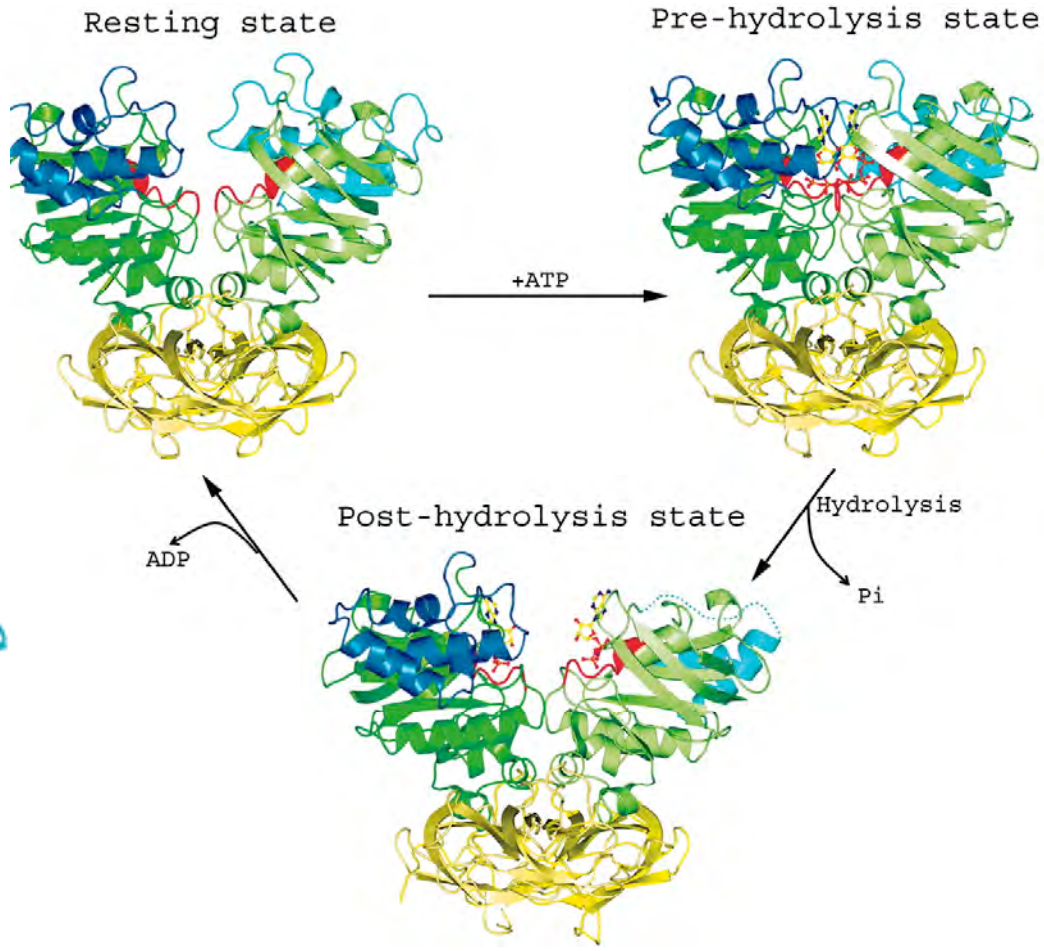
Ward *et. al.*, **PNAS**, (2007)

Dawson and Locher, **Nature**, (2006)

Mechanism revealed by MalK crystal structures

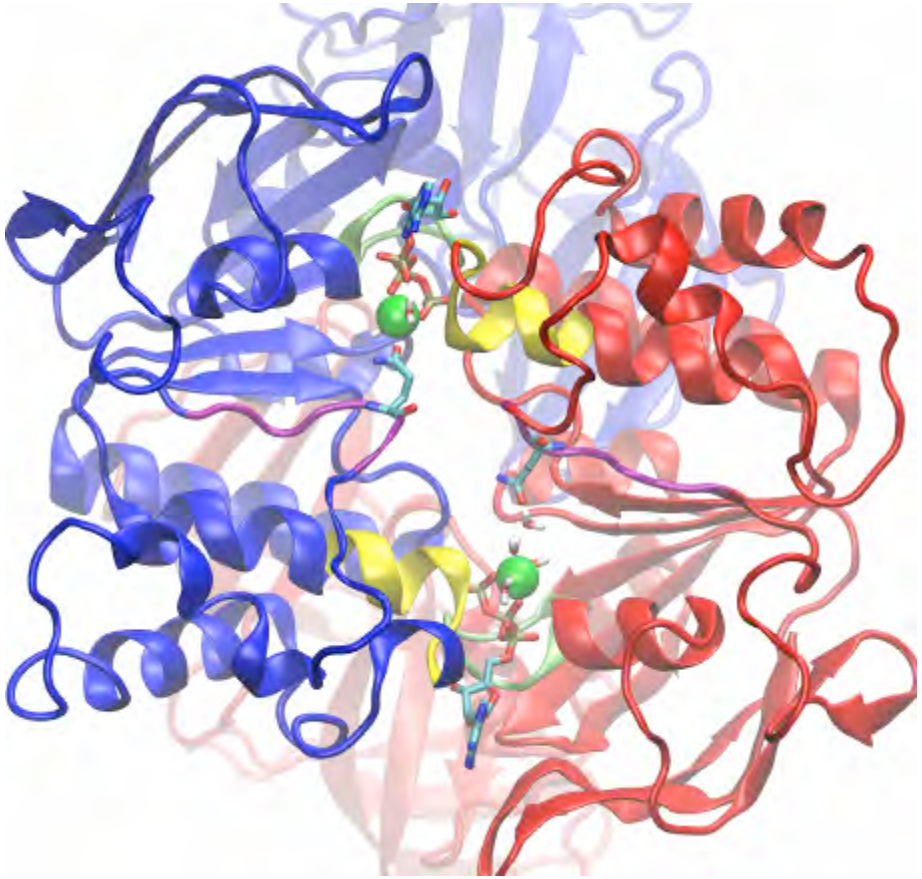


Chen *et. al.*, **Mol. Cell**, (2003)



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

Simulation Systems

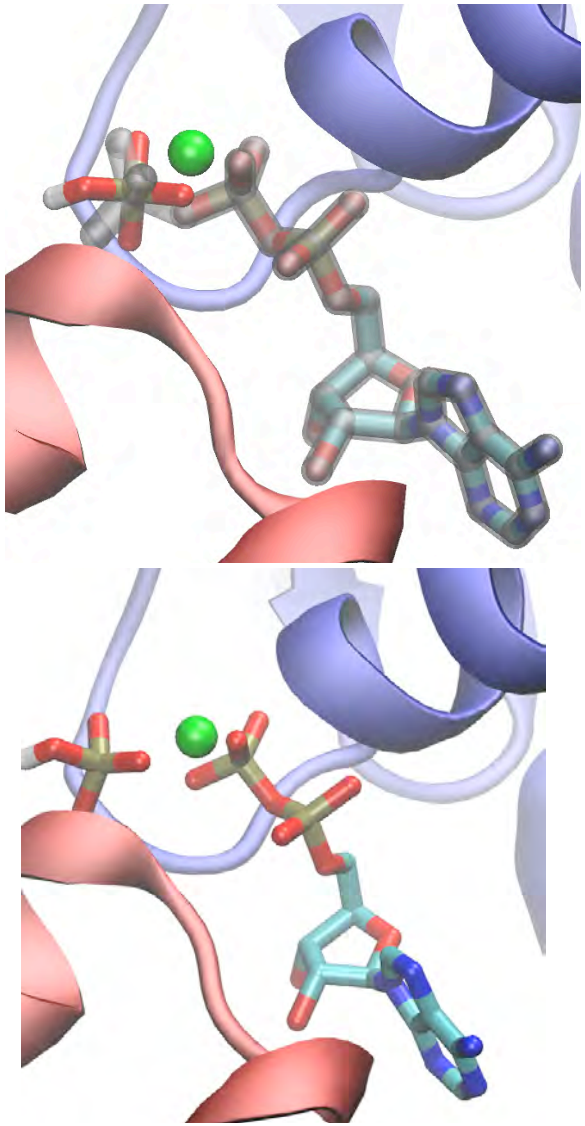


- MalK dimer (1Q12.PDB)
- Placing Mg^{2+}
- Solvate (80,000 atoms)
- Equilibrium MD - 75 ns
- 4 simulation systems
  - **ATP / ATP**
  - **ADP-P_i / ATP**
  - **ATP / ADP-P_i**
  - **ADP-P_i / ADP-P_i**

1 or 2 ATP hydrolysis?

Hydrolysis or release of products?

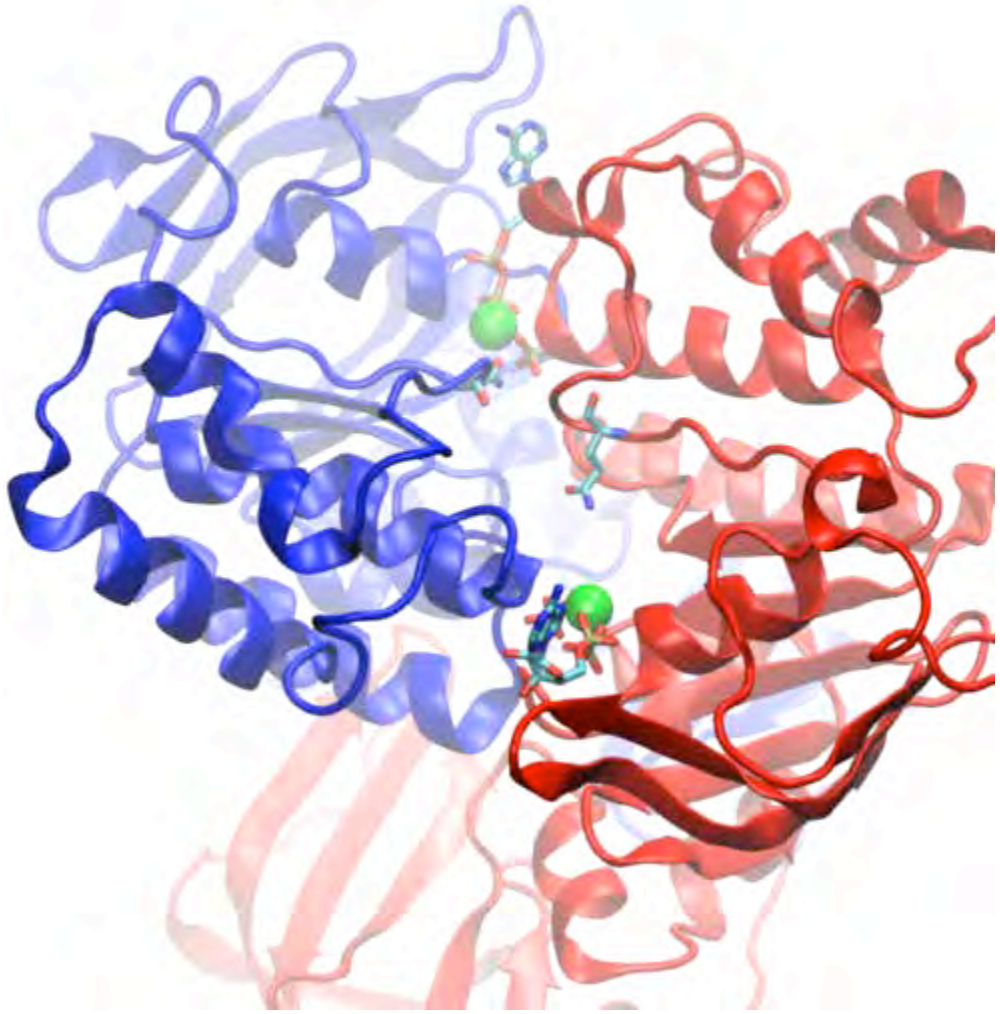
Simulating the Immediate Effect of ATP Hydrolysis



- 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**

ATP hydrolysis induces domain opening in NBDs

ADP- P_i



ADP- P_i

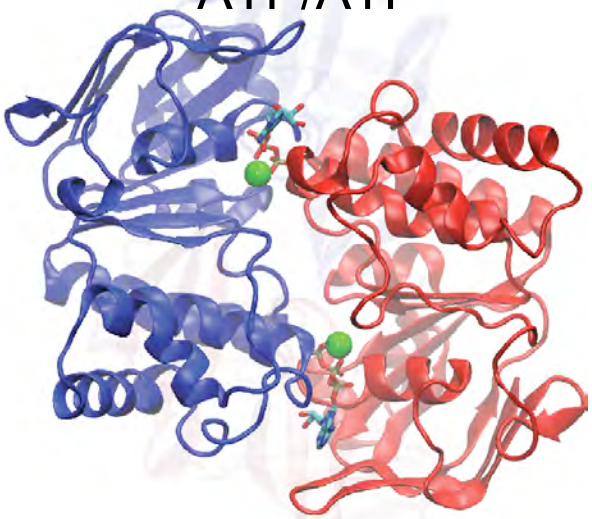
Single ATP hydrolysis Also induces domain opening

ATP

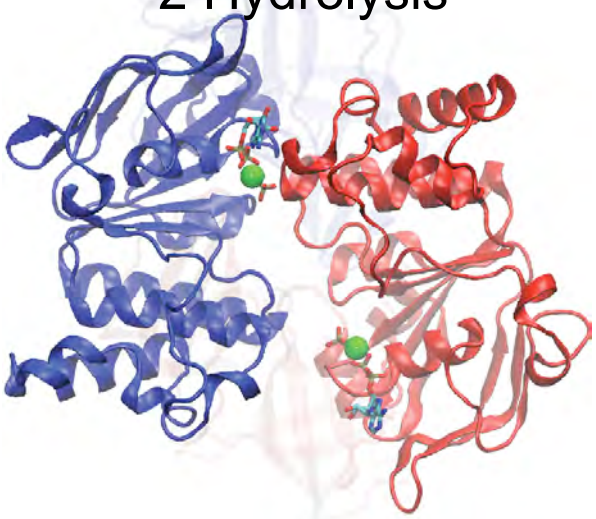
ADP- P_i

Simulation results

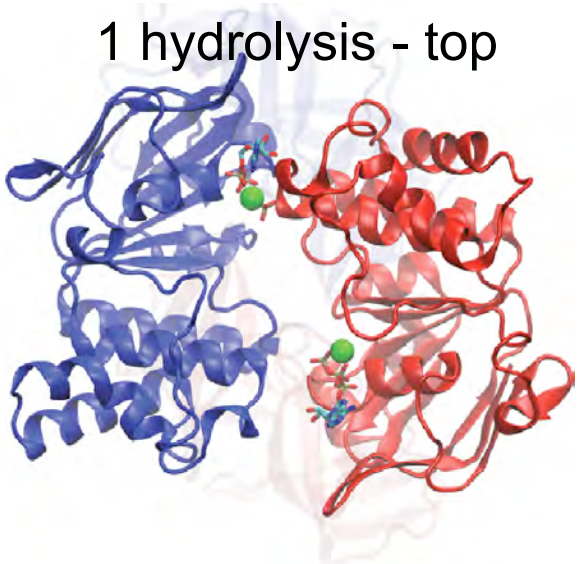
ATP/ATP



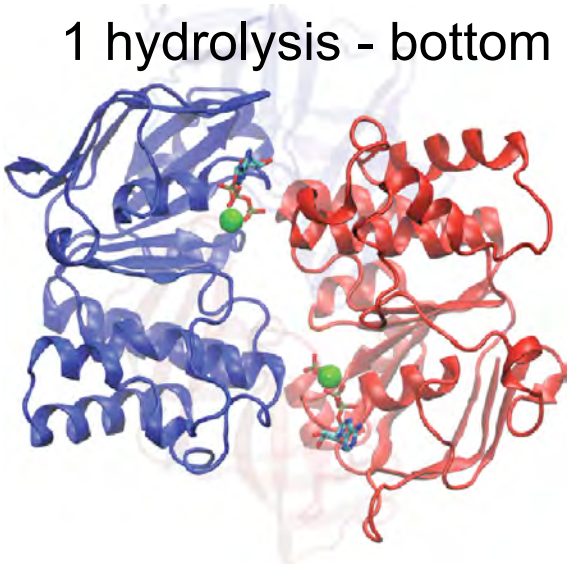
2 Hydrolysis



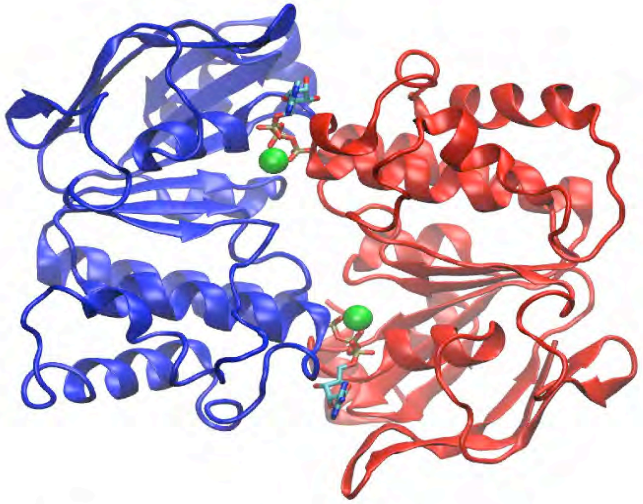
1 hydrolysis - top

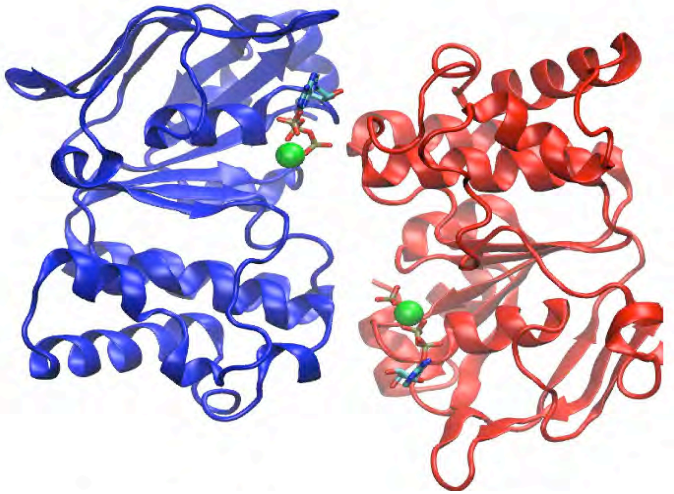


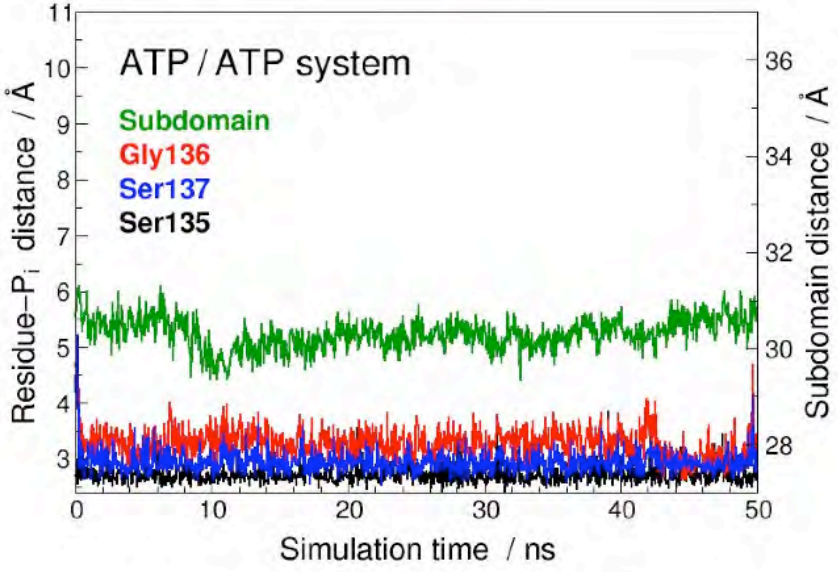
1 hydrolysis - bottom

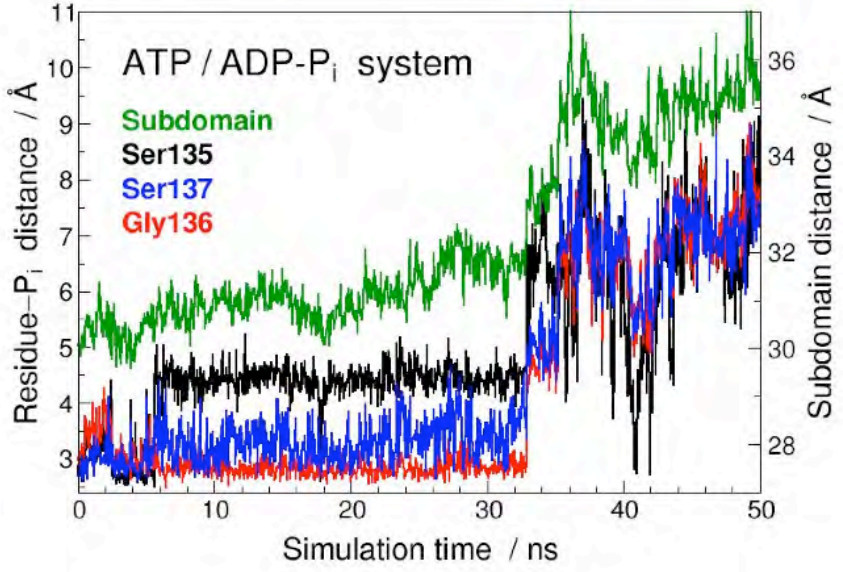


Hydrolysis-Induced NBD Opening



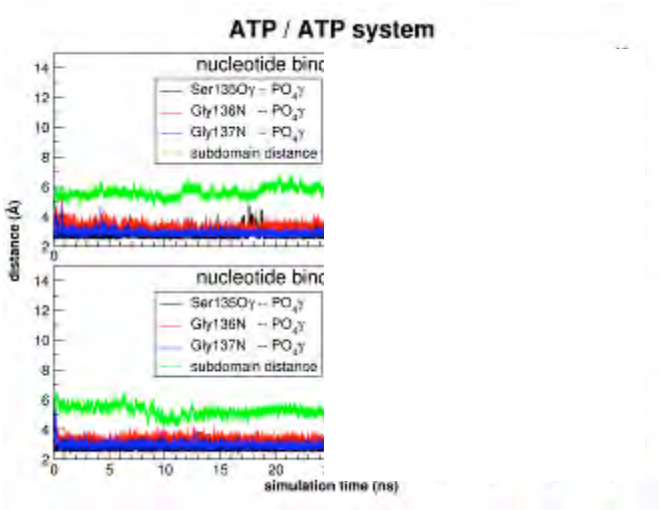


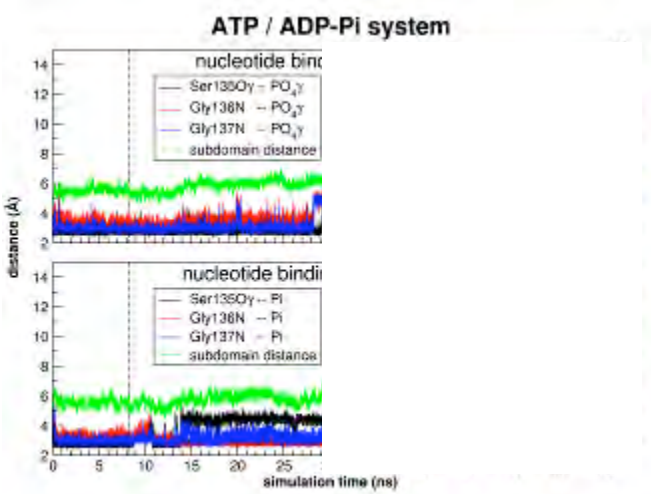


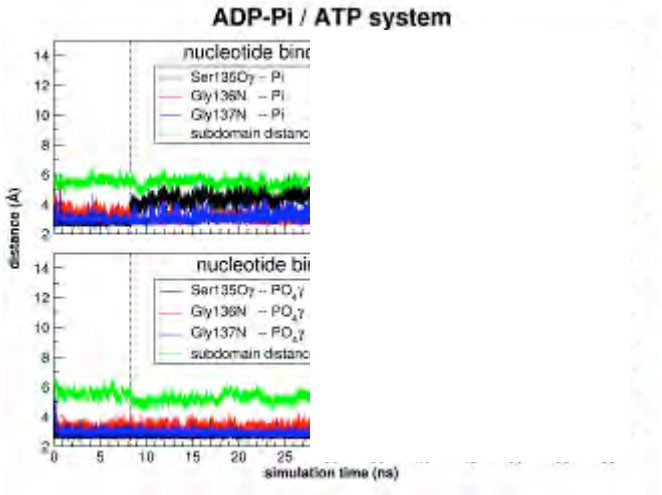


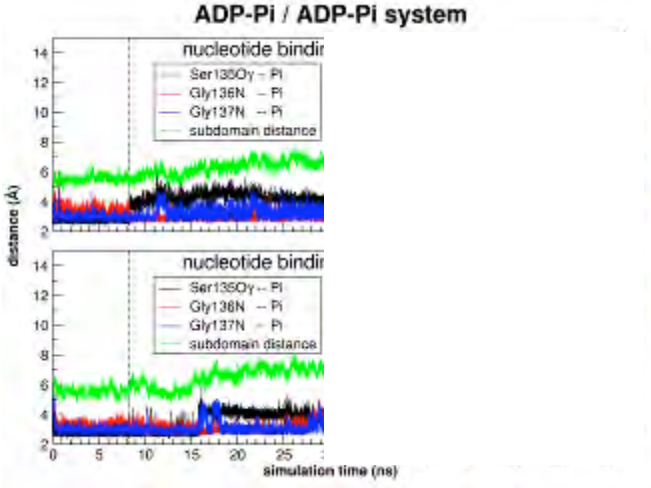
P. Wen and E. Tajkhorshid, ***Biophys. J.*** , 2008.

Simulation Time Matters!



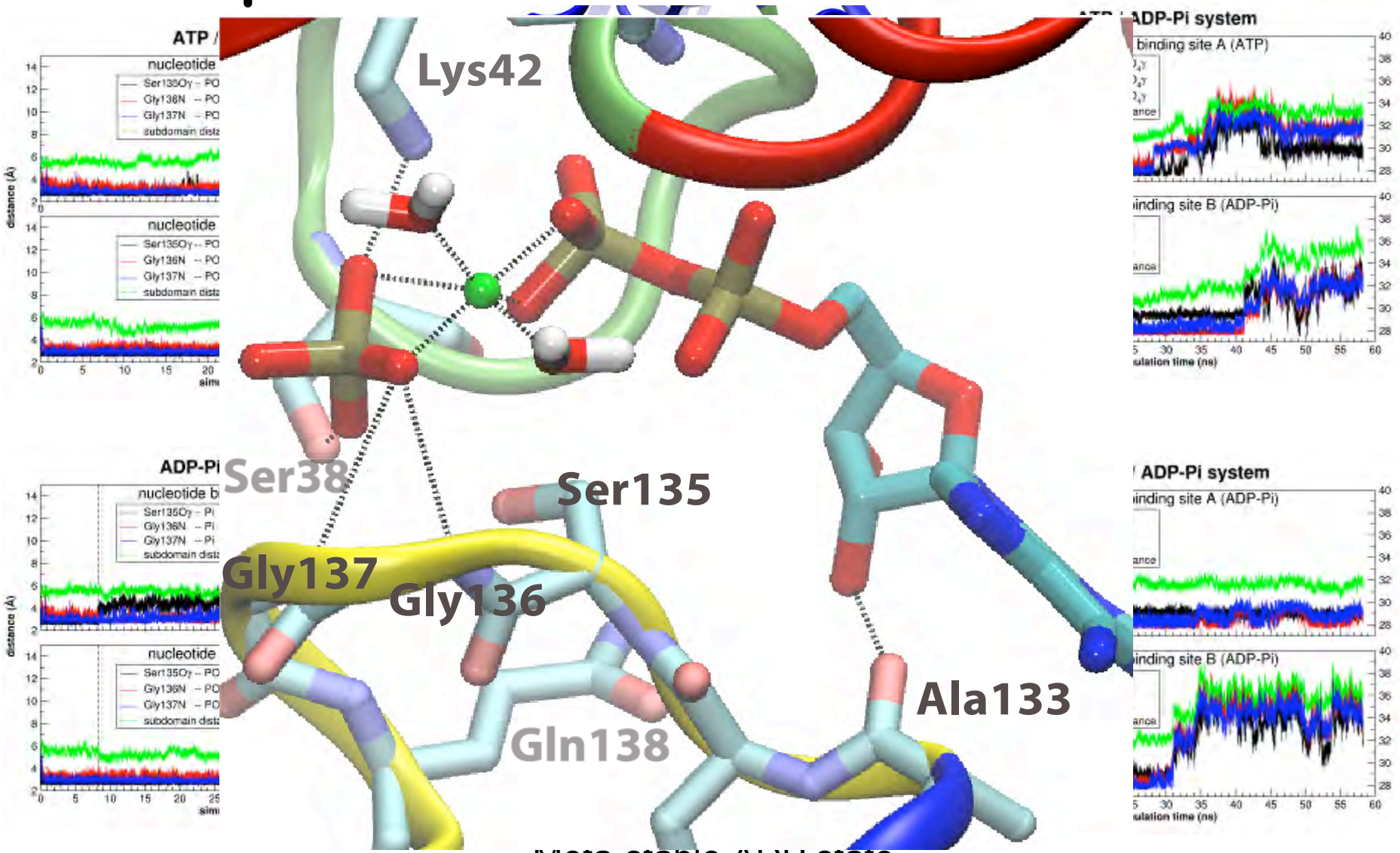






P. Wen and E. Tajkhorshid, ***Biophys. J. ,*** 2008.

Deep Look into the Active Site

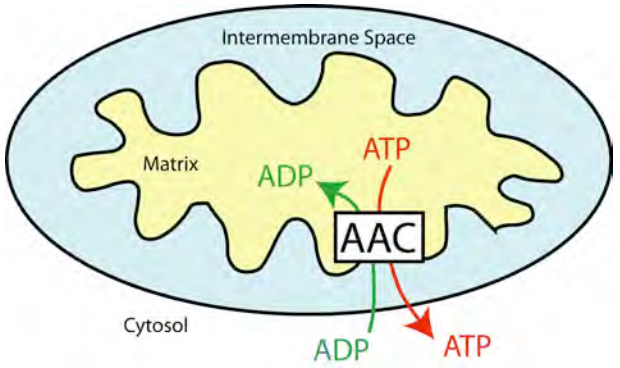


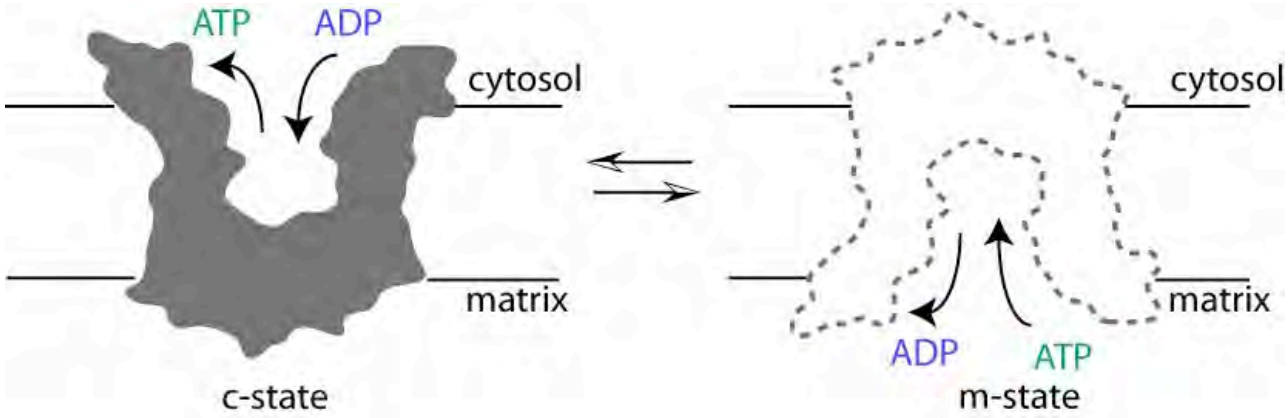
Meta-stable ADP state

P. Wen and E. Tajkhorshid, ***Biophys. J.*** , 2008.

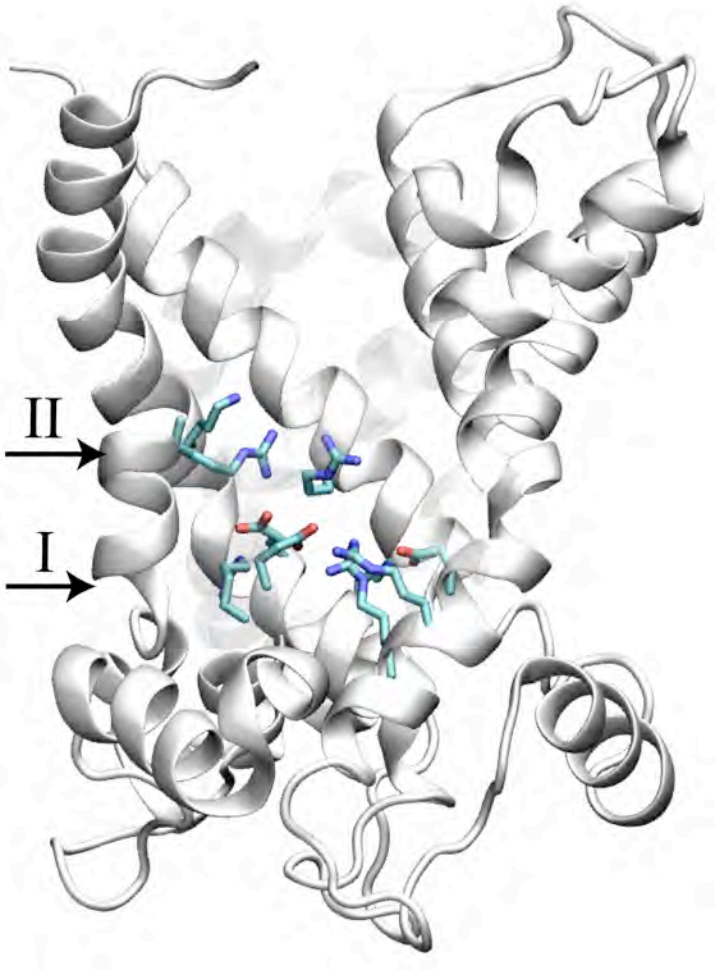
ADP/ATP Carrier (AAC)

- Belongs to the Mitochondrial Carrier Family (MCF)
  - Three repeats of ~100 aa
  - MCF motif PX(D/E)XX(K/R)
- Two conformational states
- Unknowns:
  - ADP binding and biding site
  - Transition between the states

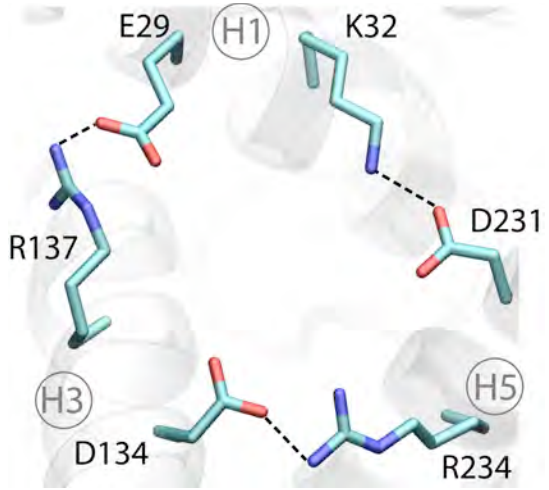




Key Structural Features

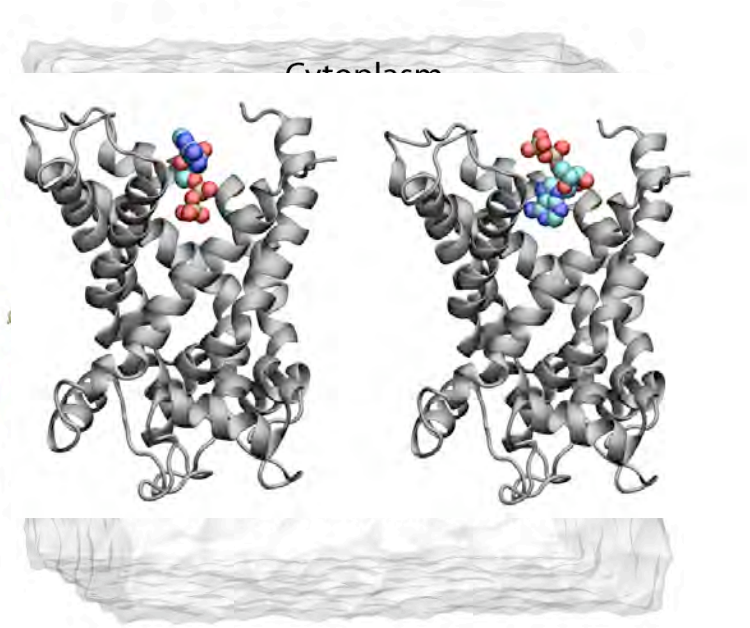


Pebay-Peyroula, *et al*. (2003) *Nature*, 426:39-44.



- Region I: salt bridge ring
- Region II: K22, R79, R279

MD Simulation Setup

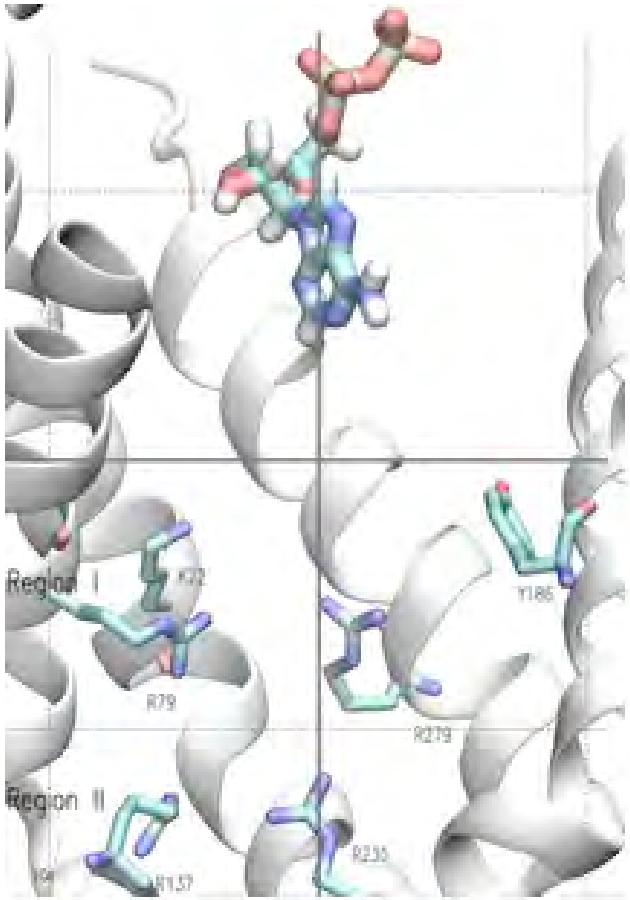


80,000 atoms

	Time (ns)	Ensemble
NB1	200	NP_zT
NB2	260	NP_zT
NB3	36	NP_zT
NB4	193	NP_zT

Four sets of simulations are performed with *NAMD*. Altogether 0.7 μs, ~150 days on 96 processors (0.22 day/ns).

Spontaneous Binding of ADP

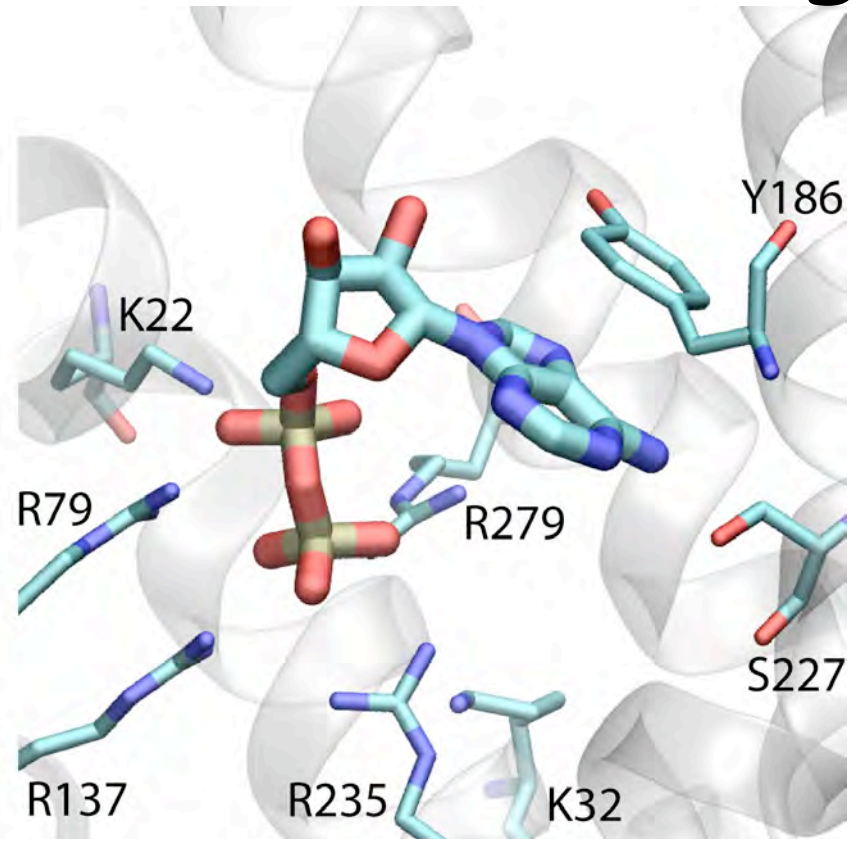


- First complete ligand binding to a protein revealed by unbiased MD simulations.
- Spontaneous binding (<10ns)
- No biasing potential

0.1 μs ADP binding simulation

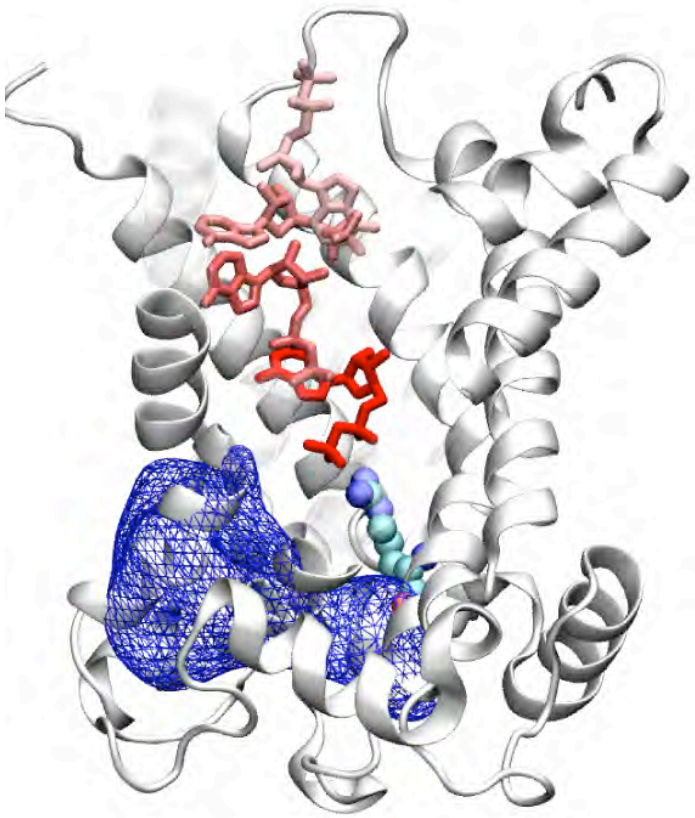
Y. Wang and E. Tajkhorshid, ***PNAS,*** 2008.

Putative ADP Binding Site

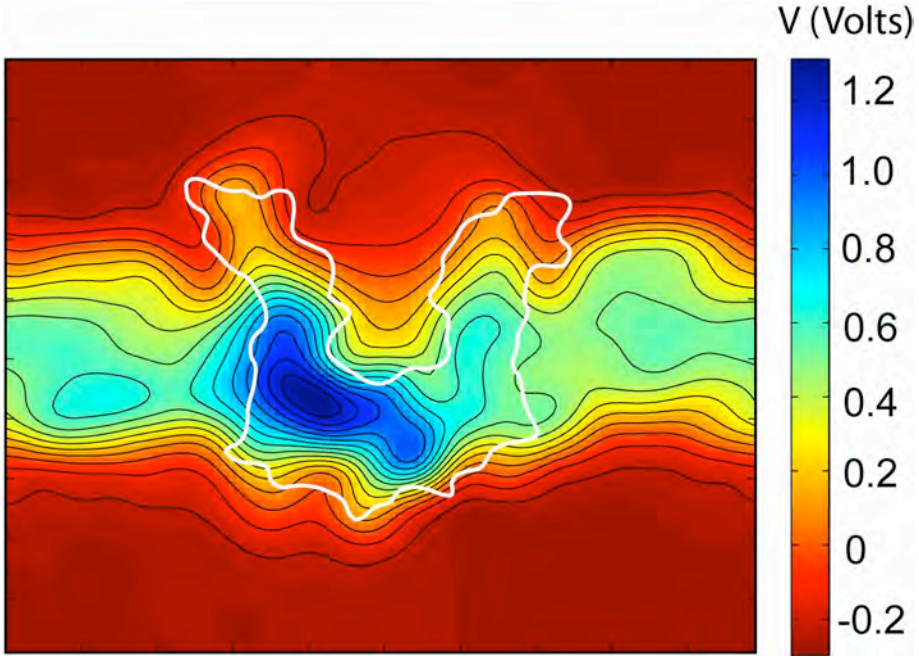


- Phosphate groups: K22, R79, R279, R235
- Adenine ring: stacking interaction with Y186
- ADP binding brings together region I and region II residues.

Unusually Strong Electrostatic Potential



Snapshots of a 0.1 μs ADP binding simulaiton. Blue mesh: the 1.0V electrostatic potential isosurface.



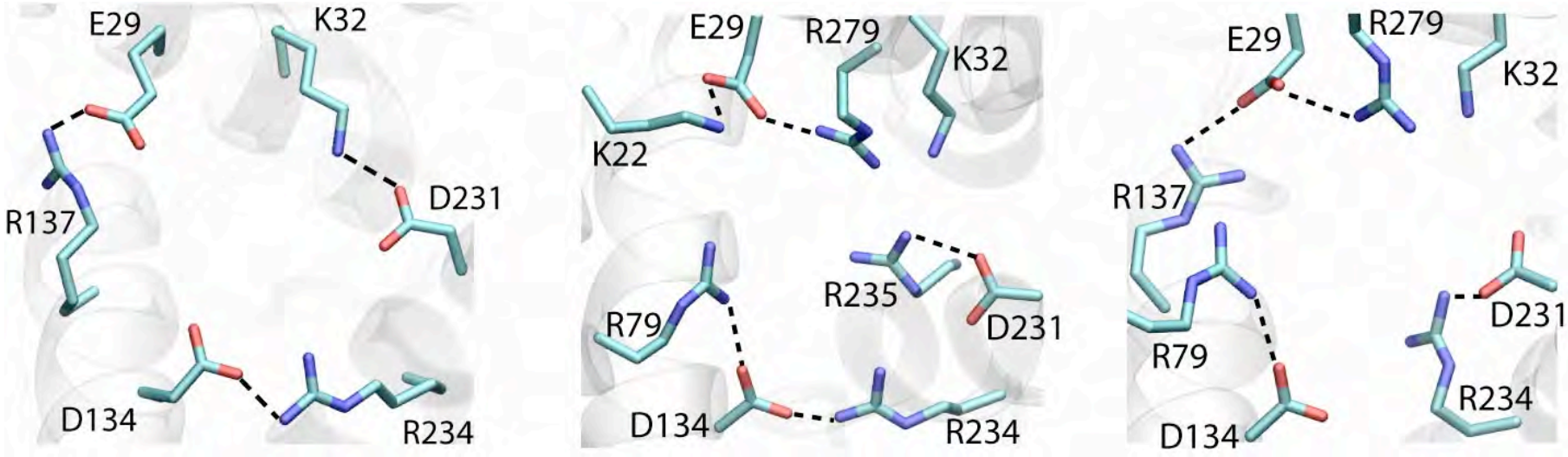
Average electrostatic potential of AAC

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

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

Unlocking of AAC by ADP

- ADP binding unlocks AAC by completely disrupting the salt bridge ring.



Original salt bridge ring (Crystal structure)

Perturbed salt bridge ring in ADP translocation (simulation NT1) (simulation NT2)

Commonality of Electrostatic Features in MCF Members

- The majority of yeast MCF members have a net positive charge.

- AVG (32 MCFs) = +15e

  AVG (1066 yeast membrane proteins) = +0.3e

- Many substrates of MCFs are negatively charged.
  - Substrate recruitment
  - Anchoring the proteins into the negatively charged inner mitochondrial membrane.

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	—