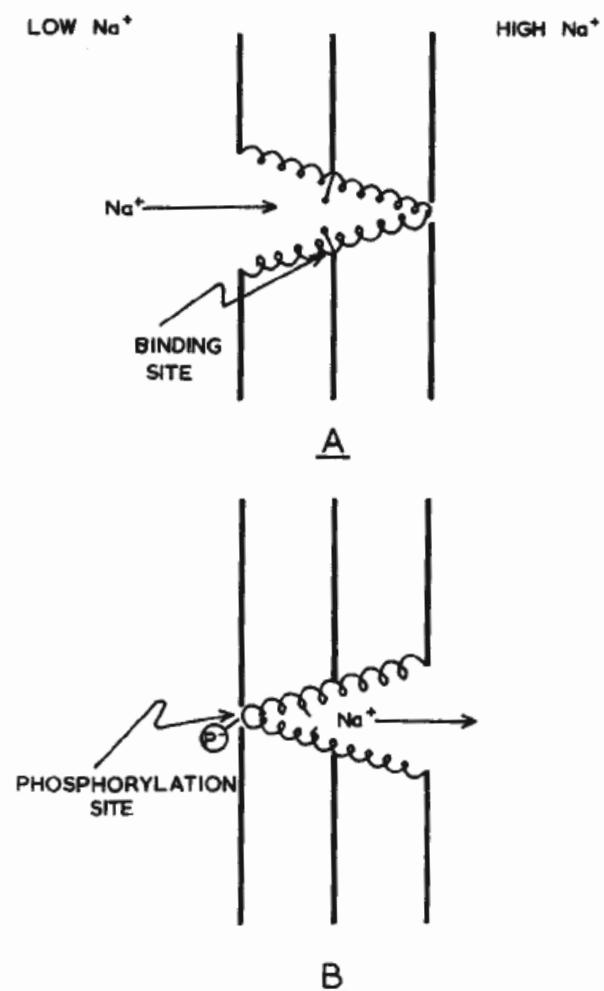


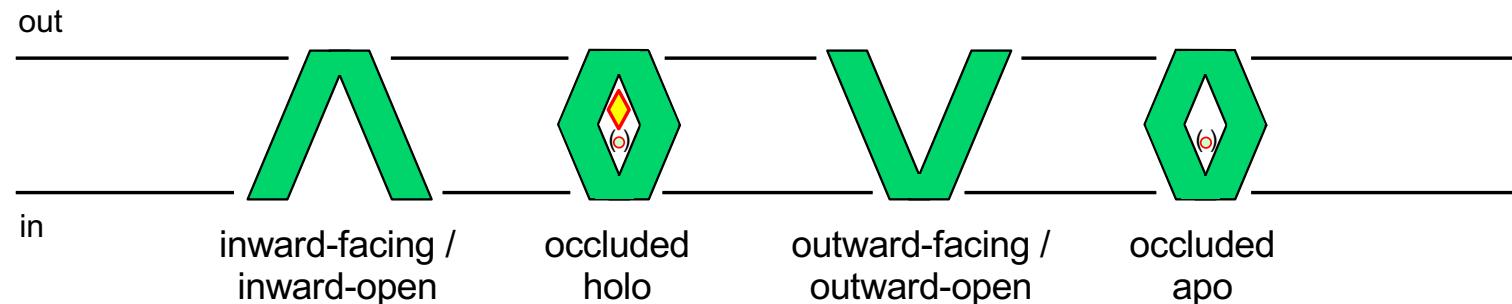
## Alternating access and release mechanism

To function as a pump a polymer molecule must meet three structural conditions only. (1) It must contain a slit or a cavity in the interior of the molecule, large enough to admit a small molecule. (2) It must be able to assume two different configurations, such that the molecular cavity is open to one side of the membrane in one configuration and to the opposite side in the other. (3) It must contain a binding site for the transported species in its molecular cavity, the affinity of which for the transported species is different in the two configurations.



Oleg Jardetzky, *Nature* 211: 969-970 (1966)

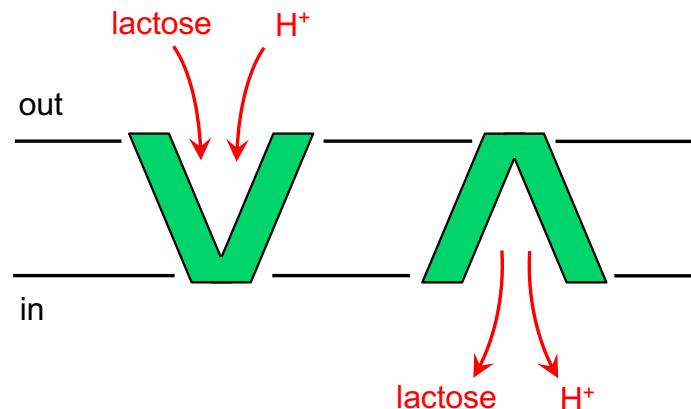
## Alternating access conformations / states in transport proteins



Mechanisms of active transport proteins employing alternating access:

### Bi-directional, reversible

(Secondary active transporters, e.g. major facilitators)

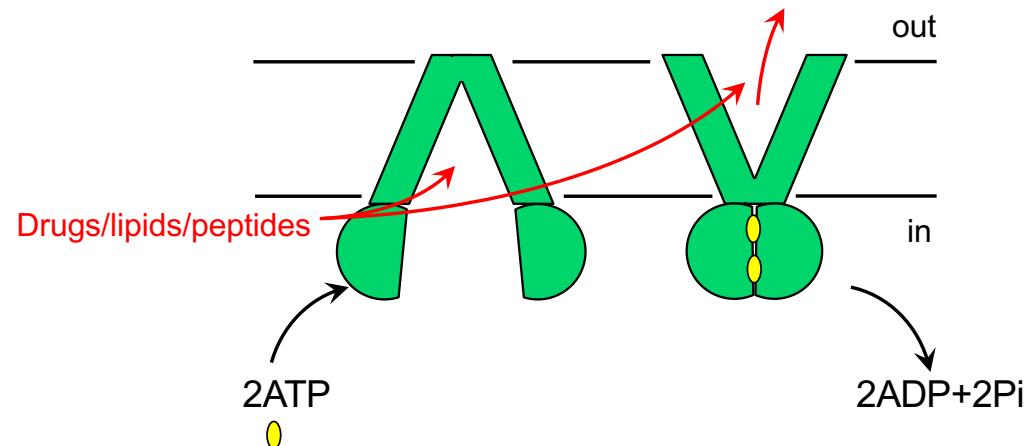


strictly coupled

↓  
it is impossible to transport  
without lactose

### Uni-directional ("irreversible")

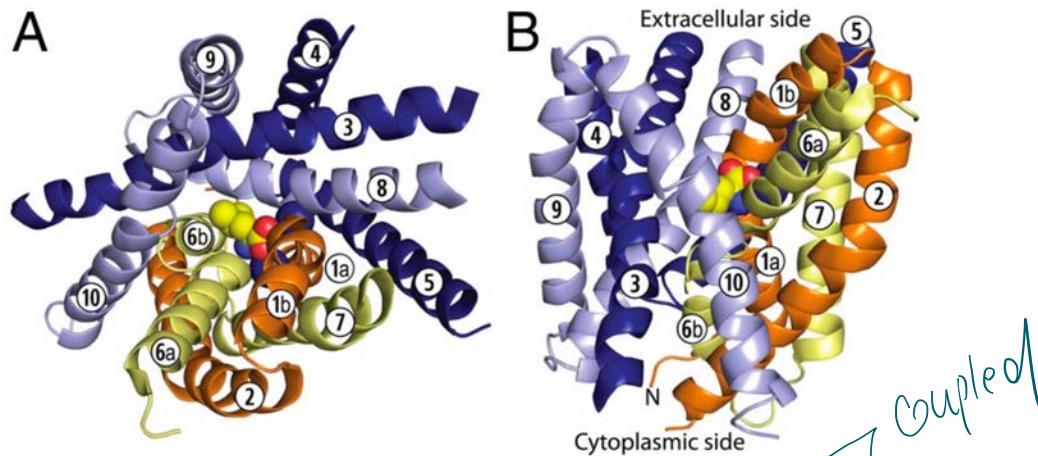
(Primary active transporters, e.g. ABC transporters)



Slipping / uncoupling observed

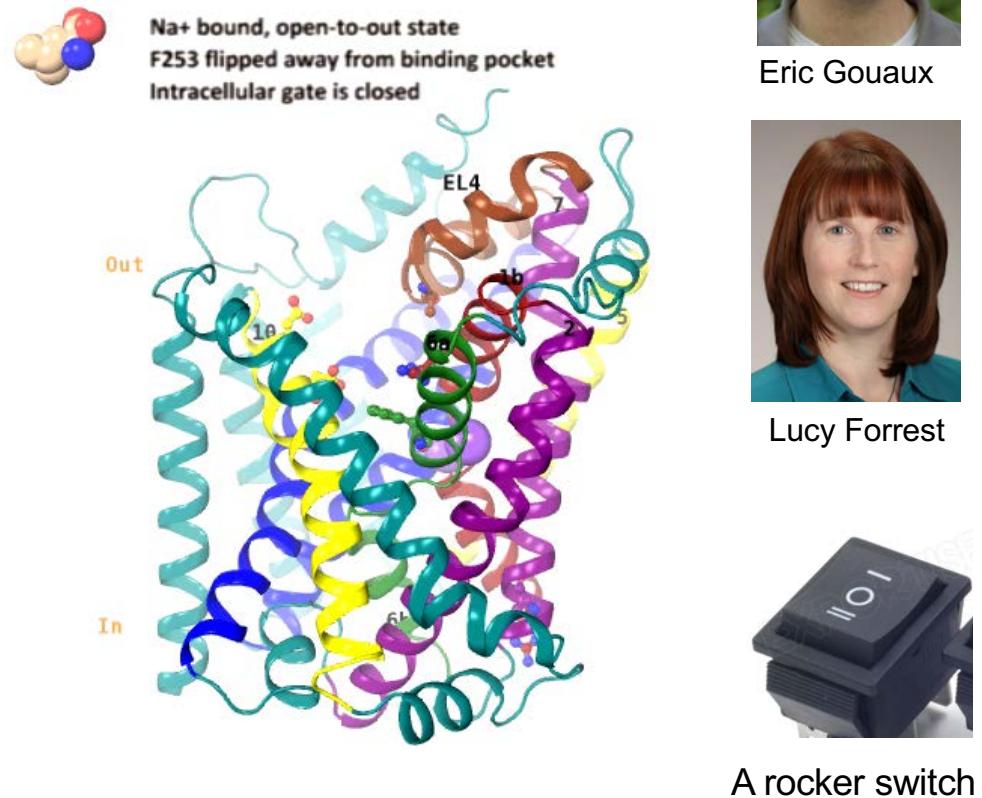
hydrolyze ATP but not  
transport other molecules

# The LeuT fold of sodium-coupled neurotransmitter transporters: Rocker switch-type alternating access



**Fig. 1.** Crystal structure of LeuT viewed from the extracellular side (A) and in the plane of the membrane (B). The leucine substrate and sodium ions are shown in spheres. Transmembrane domains from the first repeat (1–5) are shown in darker colors (orange and dark blue), and those from the second repeat (6–10) are in lighter colors (yellow and pale blue). The two structural repeats are divided up to form a bundle (yellow and orange) cradled within a scaffold (pale and dark blue).

Forrest LR et al., PNAS 105: 10338 (2008)



Eric Gouaux

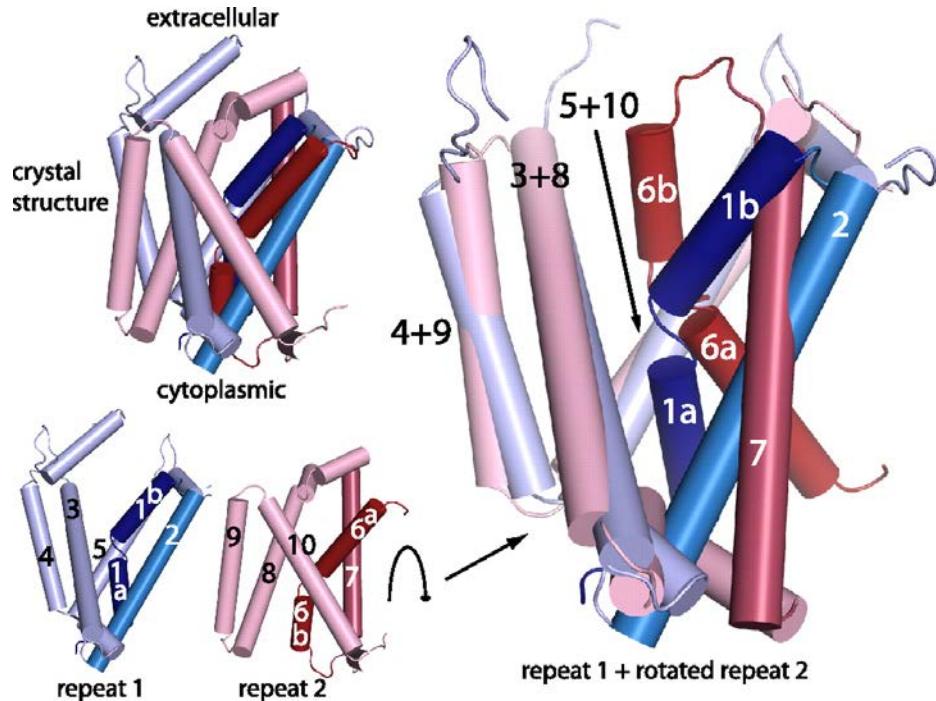


Lucy Forrest

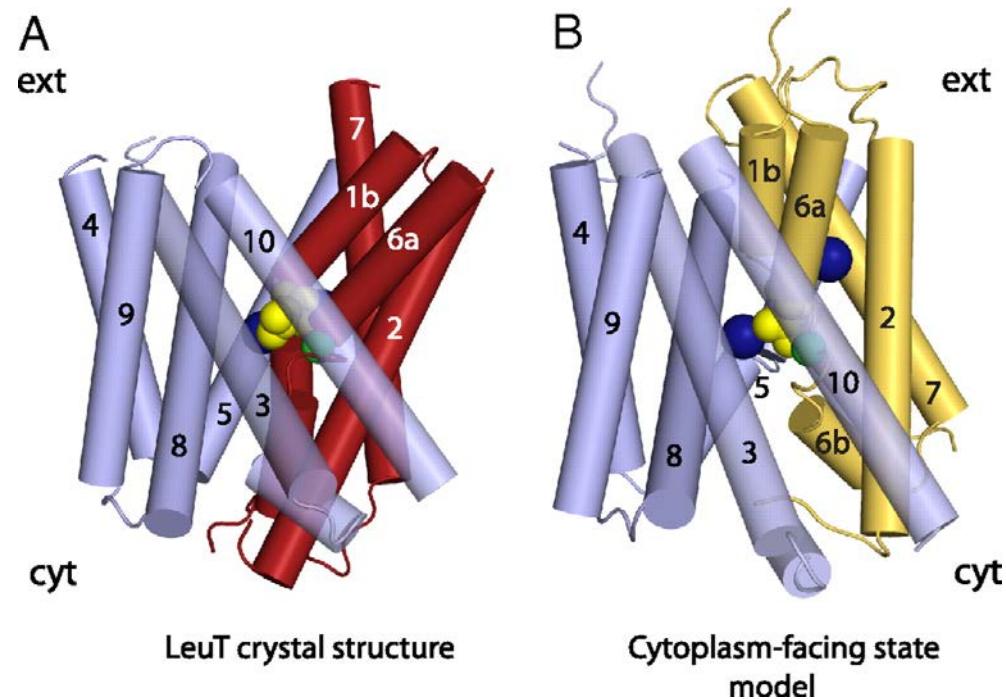


Krishnamurthy & Gouaux, Nature 481: 469 (2012)

## LeuT-fold transporters: rocker switch motion involves rigid-body rearrangement of the two pseudo-symmetrically related halves.



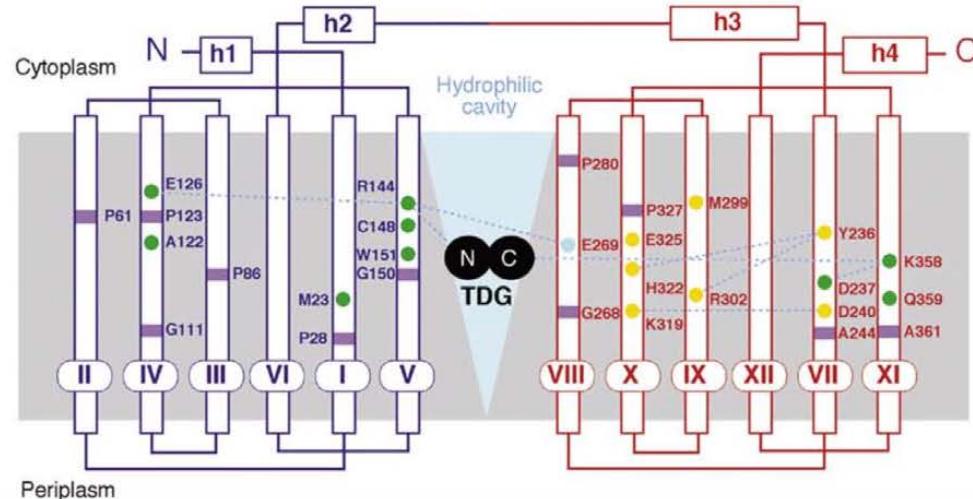
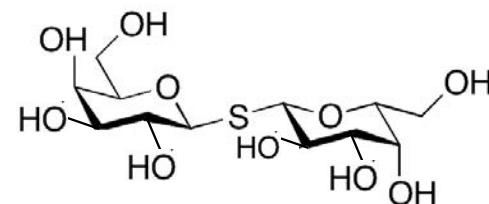
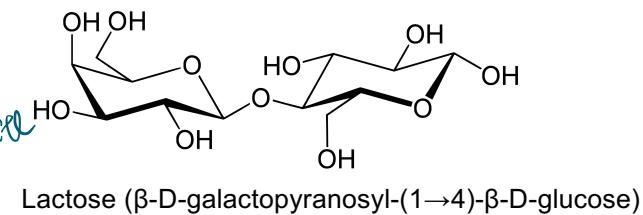
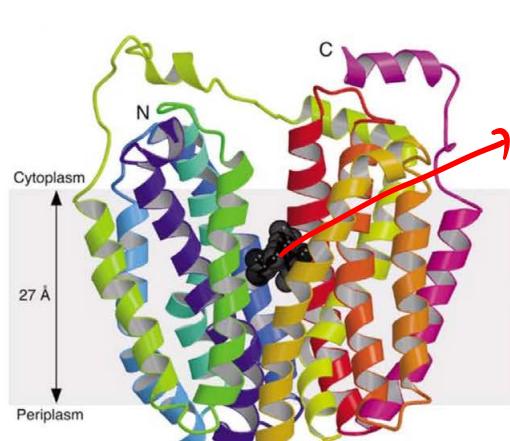
**Fig. 4.** Structural asymmetry of repeats in LeuT. TMs 1–10 of the LeuT crystal structure (*Upper Left*) were separated into the two structural repeats (*Lower Left*). TMs 1–2, dark blue; TMs 3–5, pale blue; TMs 6–7, dark red; TMs 8–10, pale red. The repeats were superimposed by aligning the positions of the last three helices in each repeat (*Right*). This required an  $\approx 180^\circ$  rotation of repeat 2 with respect to repeat 1 as calculated with SKA (45). Note the different orientation of the first two helices (particularly 1 and 6) in each repeat with respect to the other three helices.



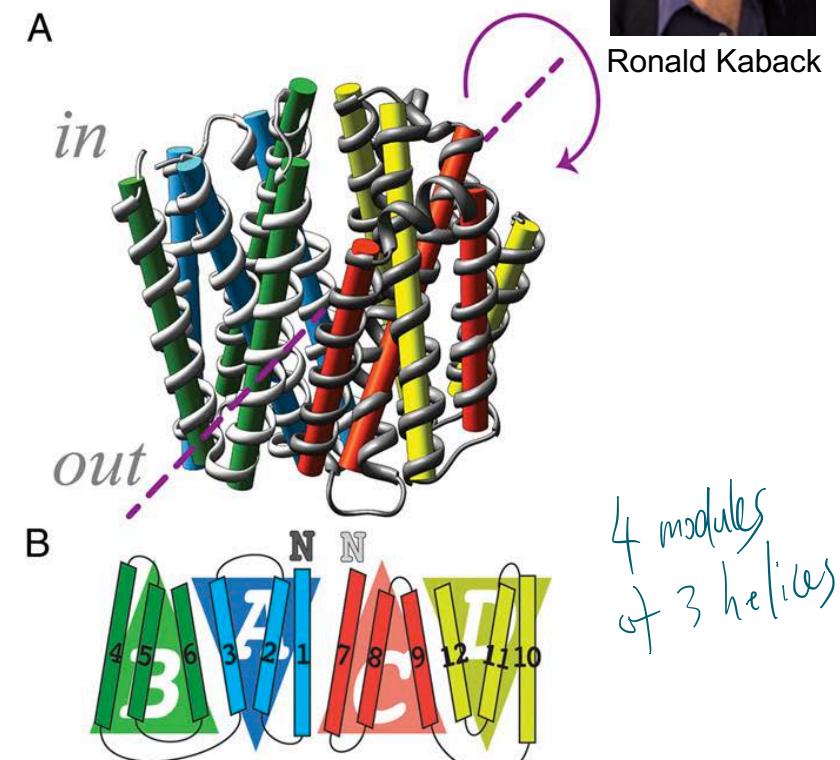
**Fig. 5.** Predicted conformational change in LeuT. The position of the four-helix bundle comprised of TMs 1–2 and 6–7 with respect to the scaffold (in blue) is shown along the plane of the membrane in red in the crystal structure (A) and in yellow in the model of the cytoplasm-facing state (B). The positions of the sodium ions (blue), the leucine substrate (yellow), and the C $\alpha$ -atom of Ser-256 (green) are shown as spheres. For clarity, TM11 and -12 and the extracellular loops are not shown.

# Major facilitator superfamily (MFS), e.g. lactose permease

contain both active and passive transporters

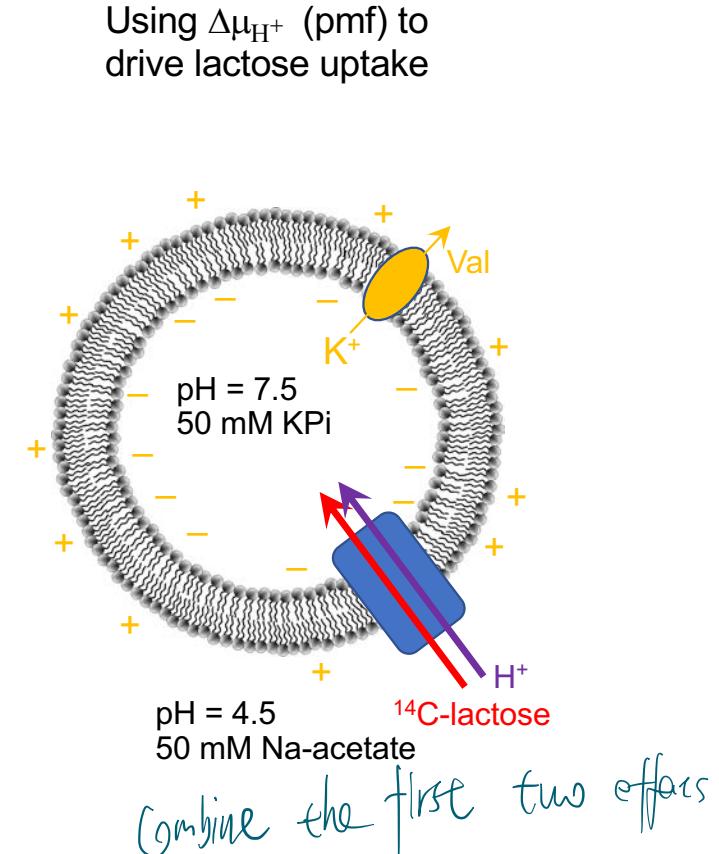
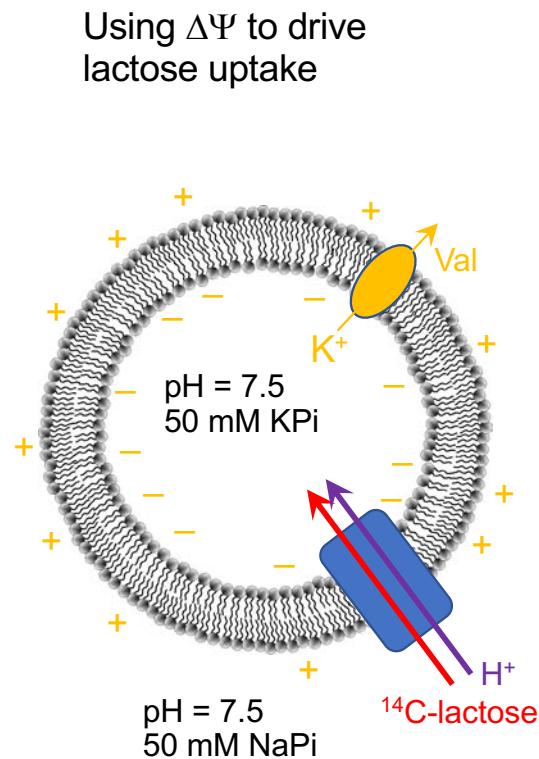
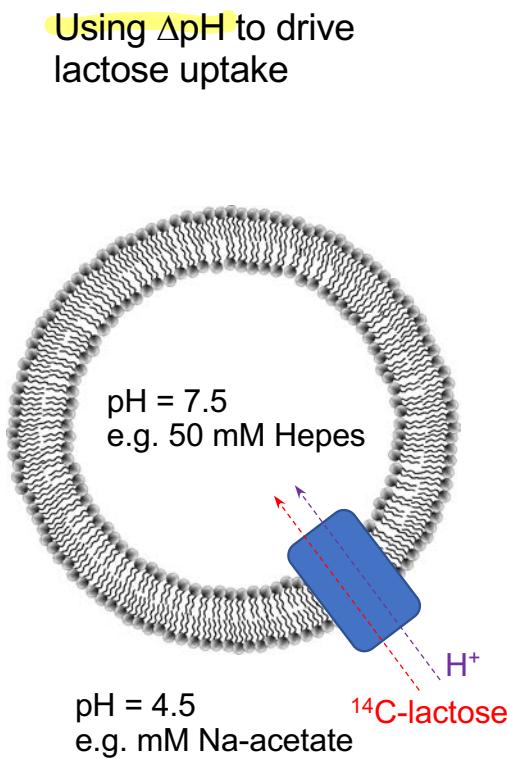


Kaback HR C R Biol 328: 557-567 (2005)

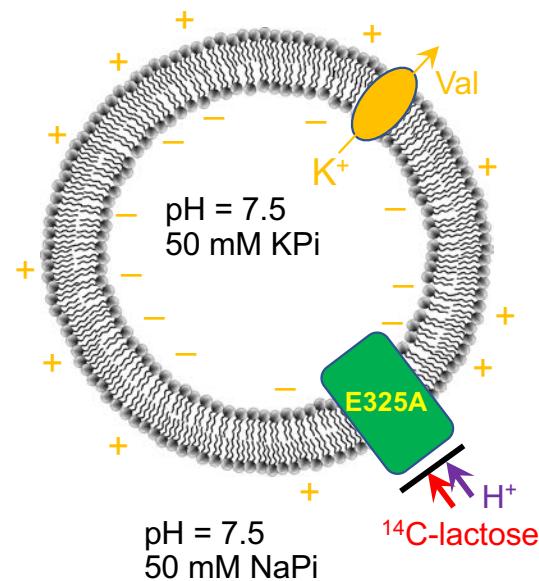
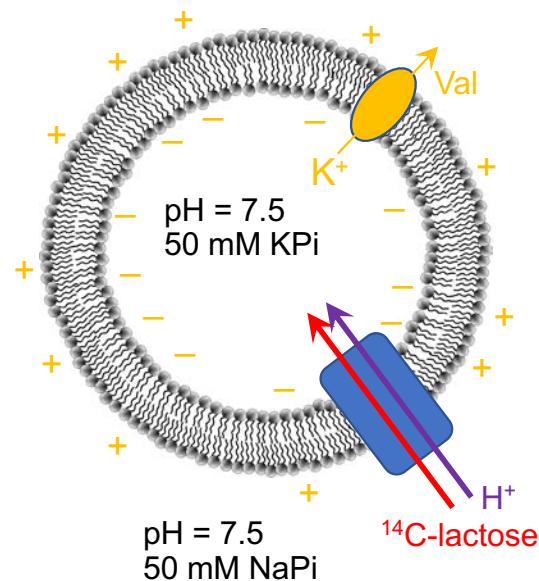


Madej MG et al., PNAS 109: E2970 (2012)

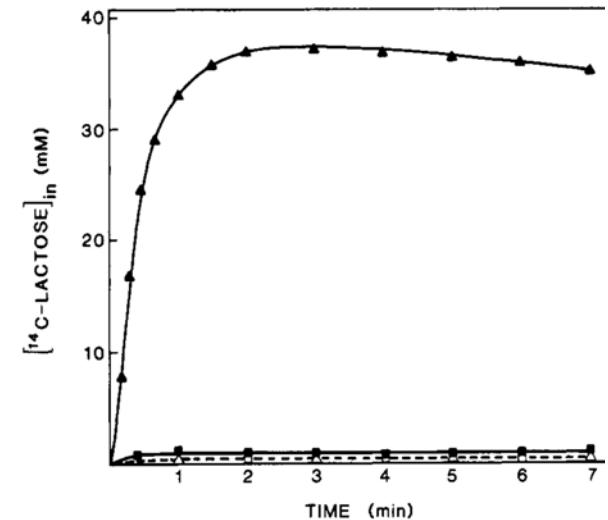
## Lactose permease (LacY) *in vitro* assay: Driving lactose uptake into proteoliposomes



## Lactose permease variant E325A



(lactose and H<sup>+</sup>)  
const go through



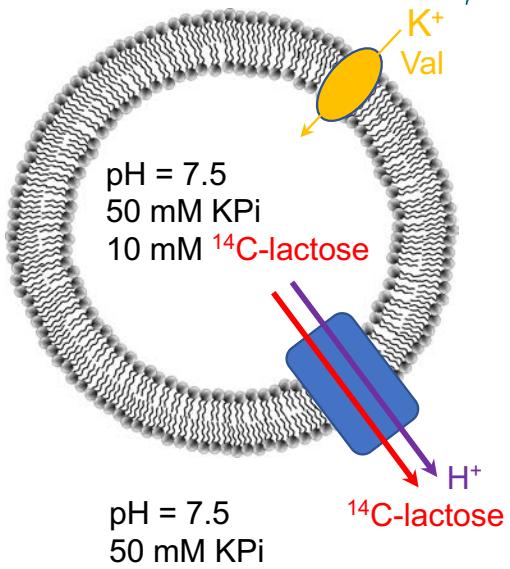
Carrasco N et al., *Biochemistry* **28**: 2533 (1989)

Observation: No more measurable lactose-proton symport (uptake into liposomes).

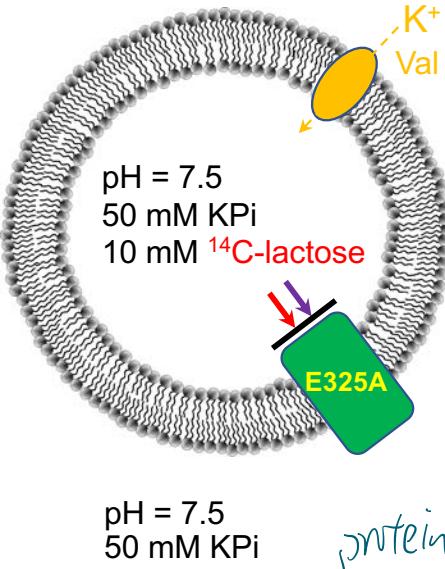
Conclusion: Glutamate 325 is a key residue for proton coupling.

## Lactose permease variant E325A

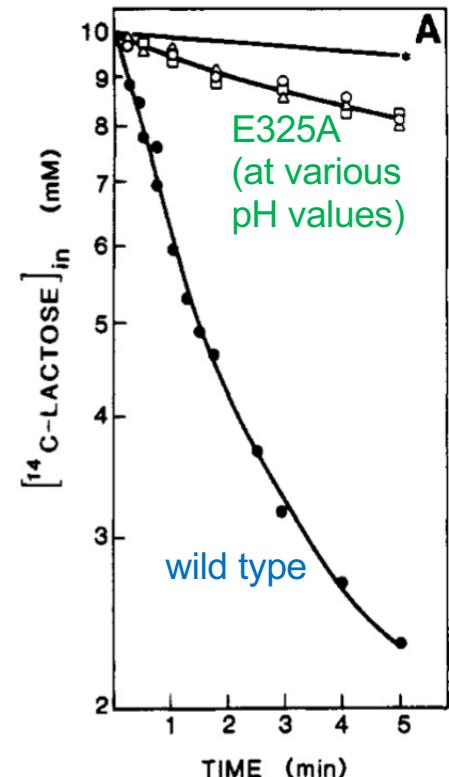
*balance the field caused by  $H^+$  transport*



*the driving force  
is only gradient of lactose*



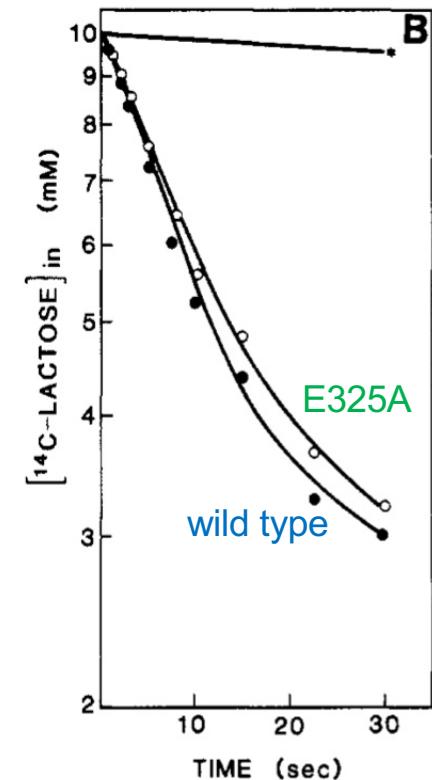
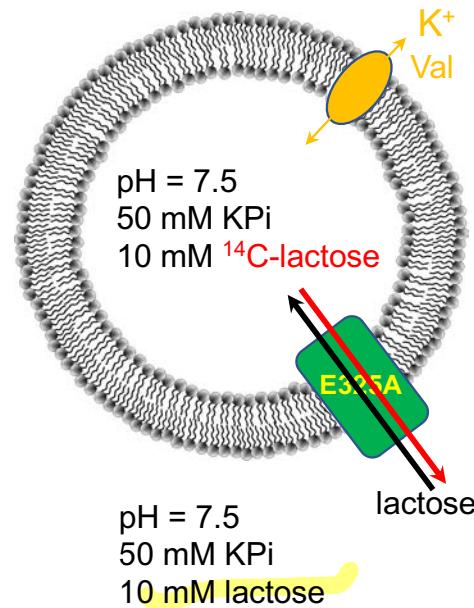
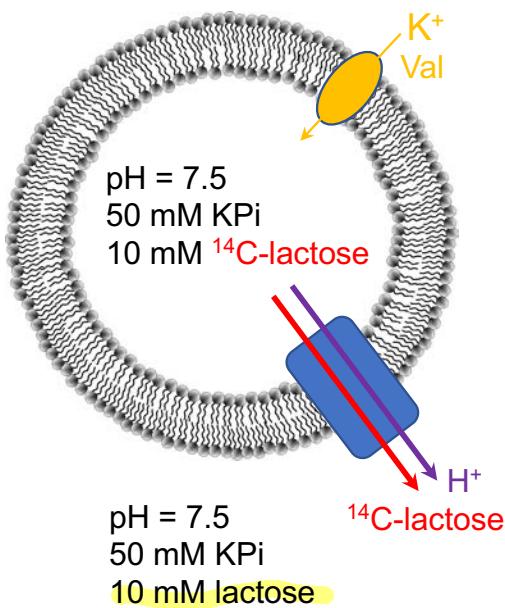
*protein cannot change  
conformation without lactose  
binding*



Carrasco N et al., *Biochemistry* **28**: 2533 (1989)

Observation: Lactose efflux (symport) abolished, efflux (downhill) also abolished!

## Lactose permease variant E325A



Carrasco N et al., *Biochemistry* 28: 2533 (1989)

Observation: Lactose efflux (symport) abolished, efflux (downhill) also abolished. However, exchange (uncoupled) is maintained!  $\Delta\Psi$ -driven lactose transport through wt LacY is as fast as proton-independent lactose exchange through E325A variant, where no net lactose transport activity occurs (one lactose out, one lactose in).

Conclusion: Lactose permease can only flip from inward-open to outward-open with bound lactose or with a negatively charged E325

TDG (higher affinity substrate analog) binds WT LacY and E325A variant equally strongly, except at high pH, where binding affinity to WT drops

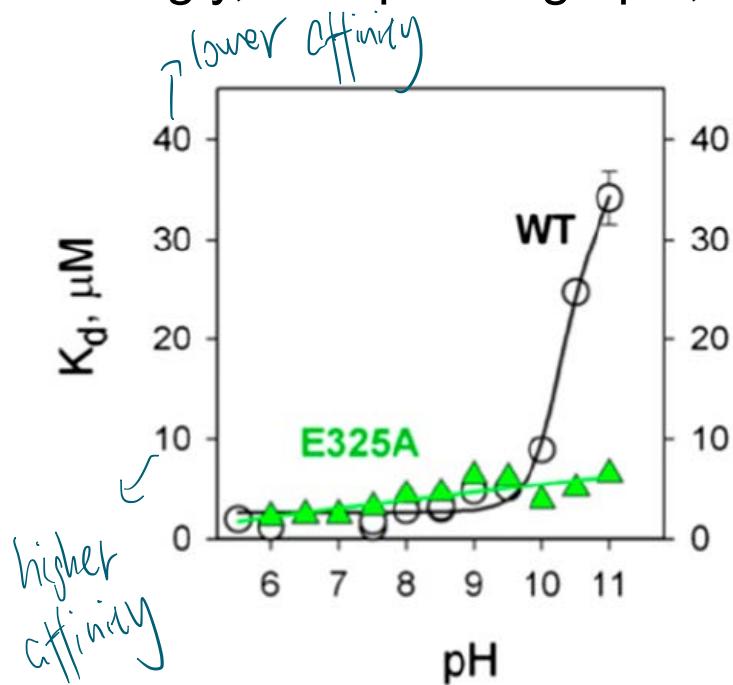
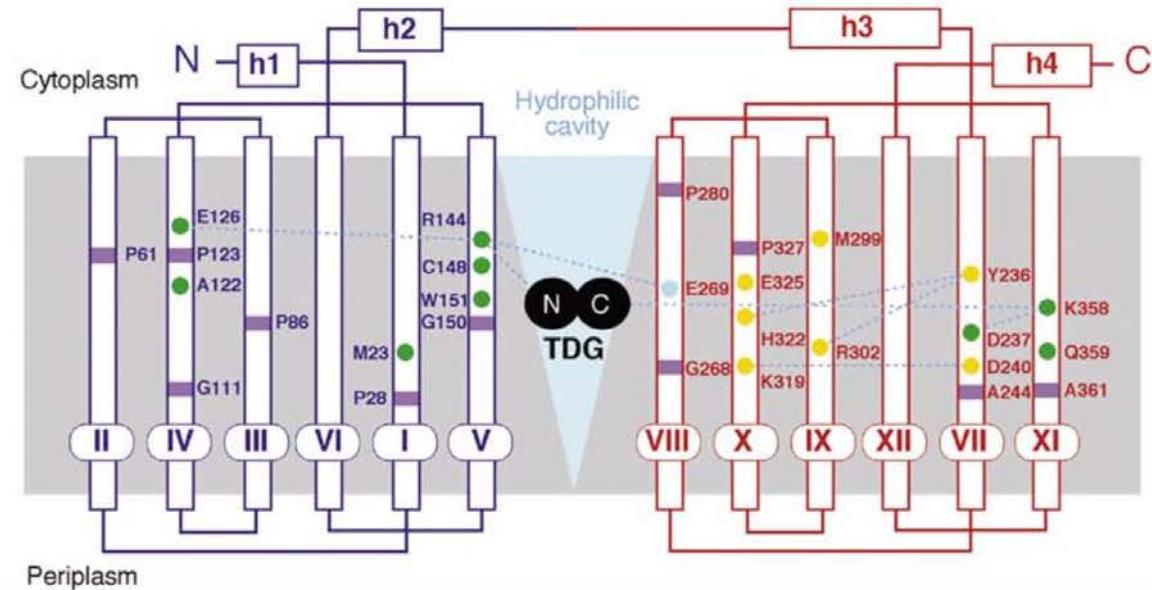


Fig. 7. Effect of pH on the apparent  $K_d$  ( $K_d^{\text{app}}$ ) for TDG binding to WT LacY (black) and the E325A mutant (green).

Conclusion: Apparent pKa of E325 is ~10. Only when (E325 of LacY) is protonated can lactose bind.

Kaback RH, PNAS 112: 1259 (2015)

Kaback HR C R Biol 328: 557-567 (2005)



Residues at the kinks in the transmembrane helices are marked with purple squares; residues marked with green and yellow circles are involved in substrate binding and proton translocation, respectively; while residue E269, colored aqua, is involved in both substrate binding and H<sup>+</sup> translocation.

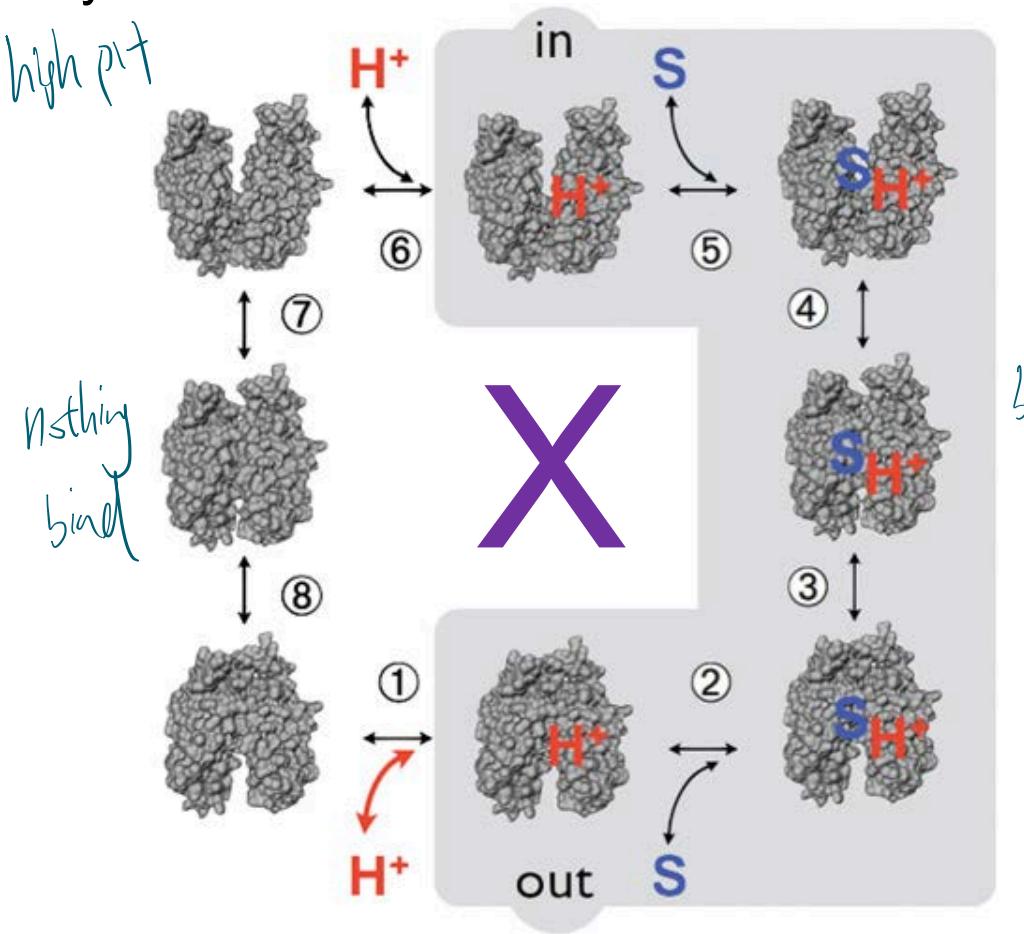
# pymol LacY

PDB ID: 4ZYR.pdb

- Pymol scene 1: Ribbon diagram with p-Nitrophenyl- $\alpha$ -D-galactopyranoside ( $\alpha$ -NPG) bound at binding site (~8x higher affinity than the two-fold symmetric TDG and three orders of magnitude higher affinity than lactose or galactose)
- Pymol scene 2: Electrostatic surface potential, narrow access slit visible
- Pymol scene 3: Side chains of E325 and R302 near substrate binding pocket, but not in direct contact with substrate, nor with each other.

## Kinetic scheme of lactose permease (LacY): Key factors

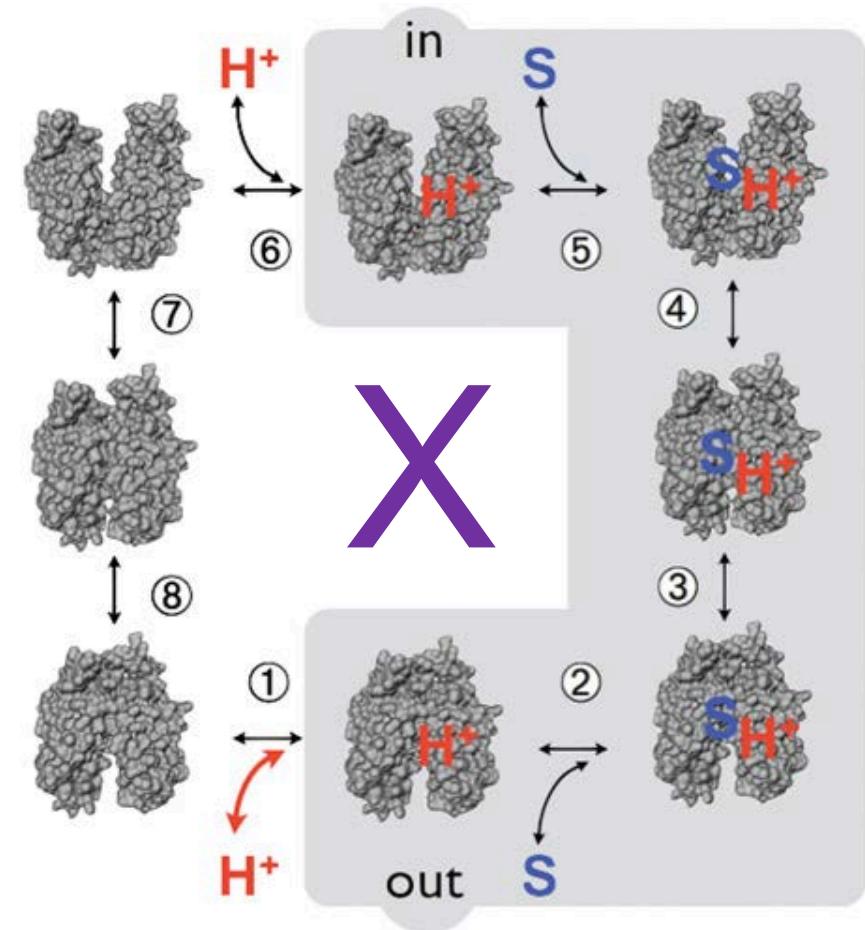
- Substrate binding cannot occur when a negative charge is at E325. In wt LacY, protonation therefore must precede lactose binding.
- Flipping from inward-open to outward-open and vice versa is only possible with bound lactose or with a negative charge at E325.
- Given the extremely high (local) pKa of E325, it must be in an environment that is either highly hydrophobic (remember ATP synthase!) or another negatively charged residue could be nearby. No such residue has been identified.
- The functional properties of LacY variants containing mutations of Arg302 (e.g. to Ala) are similar to those of E325. Arg302 must be part of a proton relay and involved in the key conformational transition of lactose-free LacY.



Kaback RH, PNAS 112: 1259 (2015)

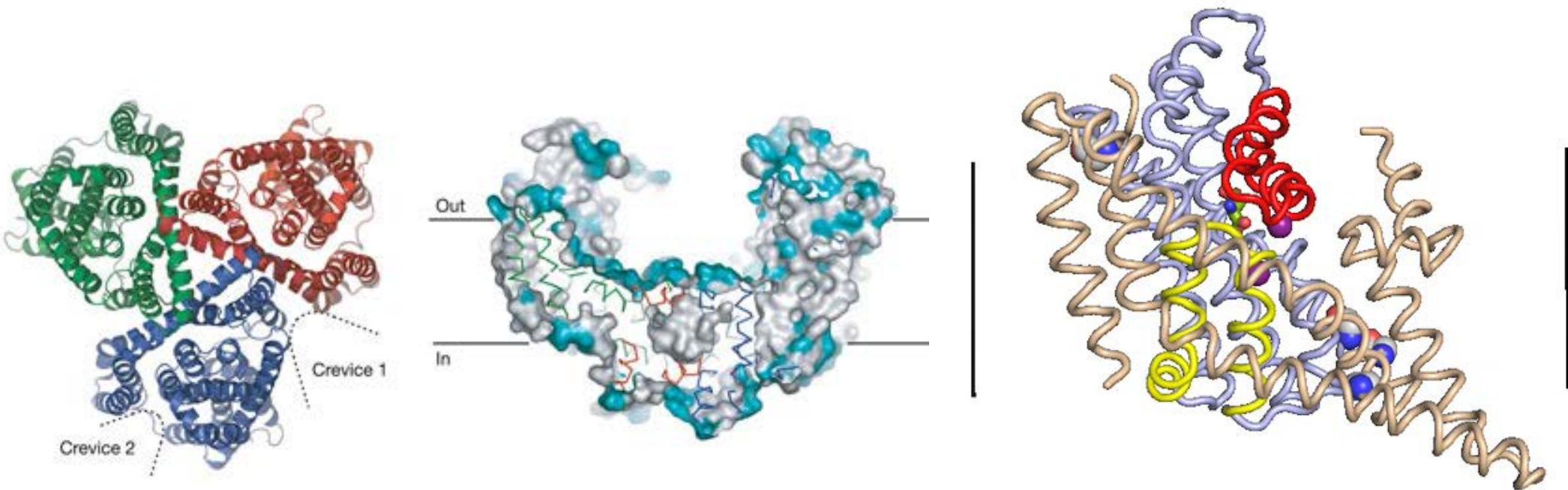
# Mechanism of lactose permease

- Symport starts with protonation of LacY (step 1 or 6 for influx or efflux, respectively), which is required for high-affinity binding of lactose. Sugar (S) binding to protonated LacY (step 2 or 5) allows a conformational change to an occluded state (step 3 or 4), which can relax to either side where sugar dissociates first (step 2 or 5), followed by deprotonation (step 1 or 6)
- The return of unloaded LacY via an apo occluded intermediate (steps 7 and 8) is only possible upon deprotonation. A possible scenario would be that the side chains of Arg302 and E325 approach each other when lactose-free LacY attempts to transition to an occluded conformation.
- Exchange, counterflow: Mutants of E325 replacing carboxyl group with neutral group are still able to catalyze these reactions. LacY moves through the steps in grey shading, but without  $\text{H}^+$  binding.



Kaback RH, PNAS 112: 1259 (2015)

## Glutamate transporters: Trimeric structure and elevator domain mechanism



Yernool et al., *Nature* **431**: 7010 (2004)

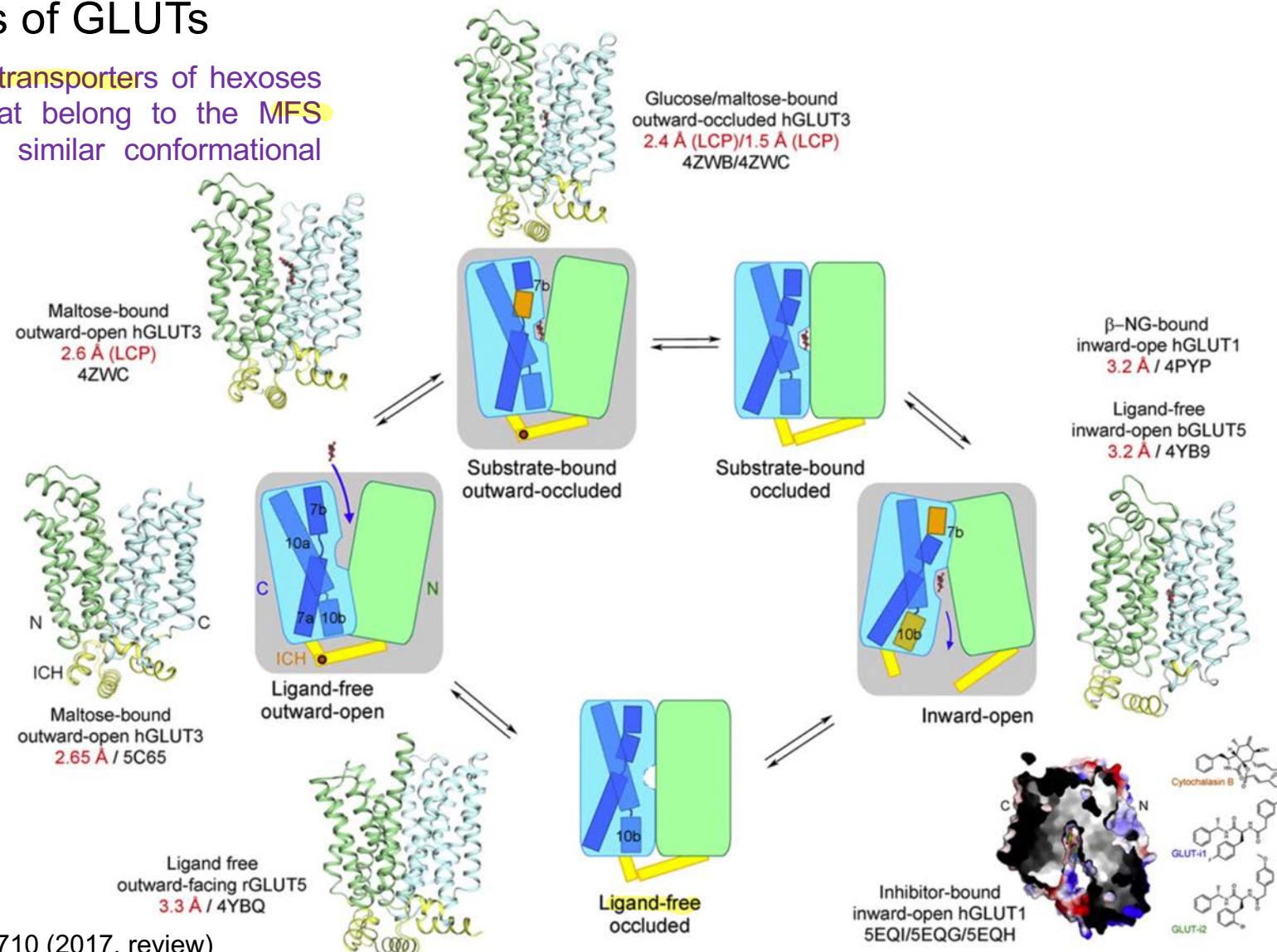
Reyes et al., *Nature* **462**: 7275 (2009)

# Conformations of GLUTs

GLUTs are passive transporters of hexoses (mostly glucose) that belong to the MFS family and undergo similar conformational changes



Nieng Yan



X

## Mitochondrial "carriers": Transporters moving a wide range of substrates

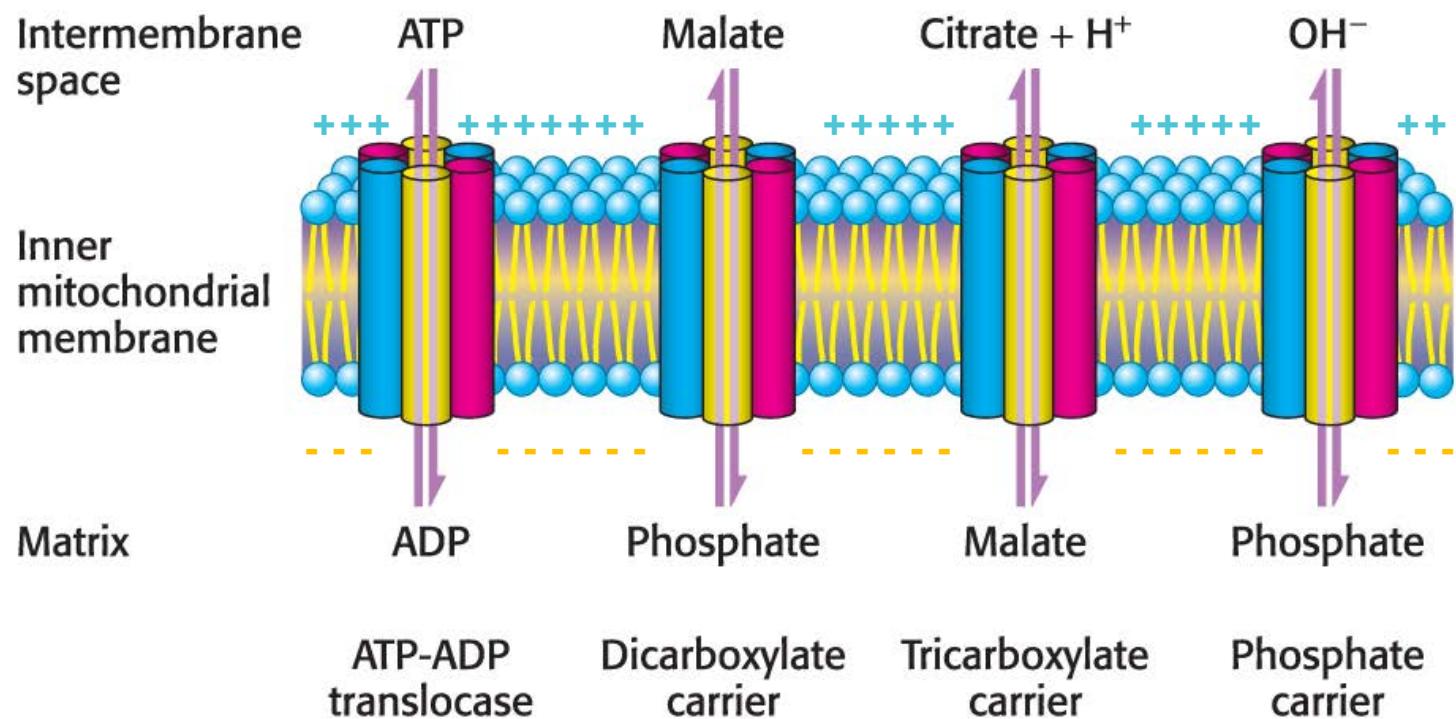
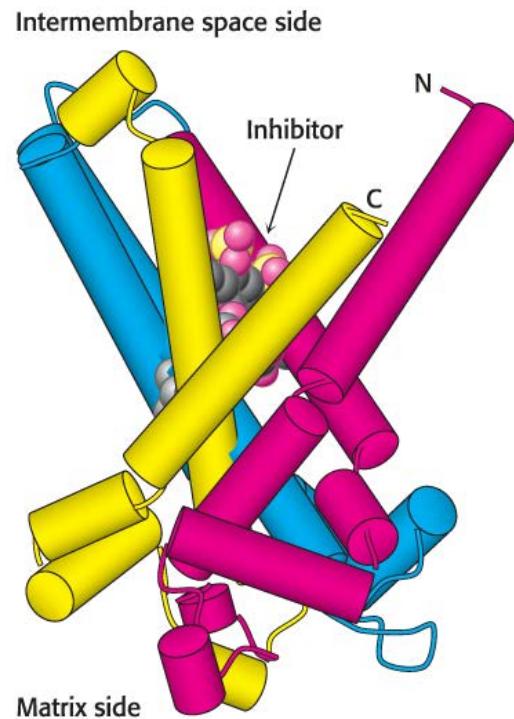
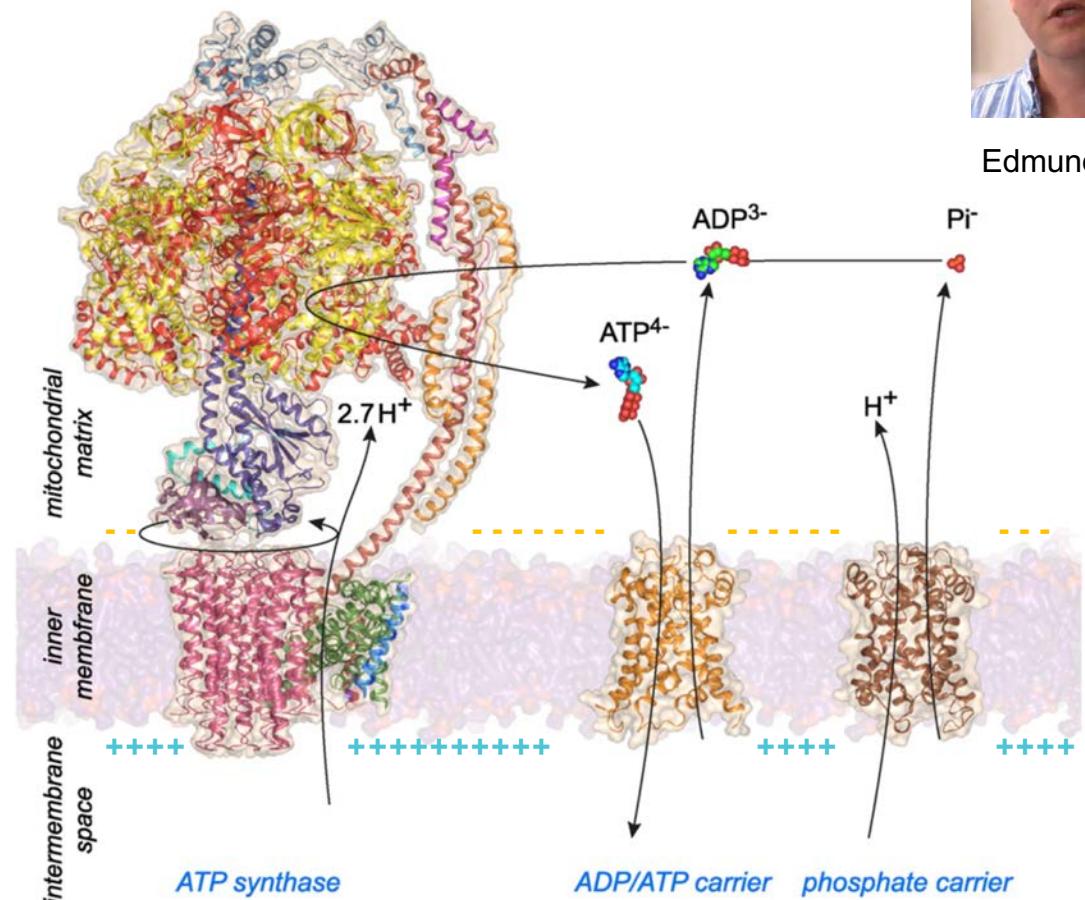
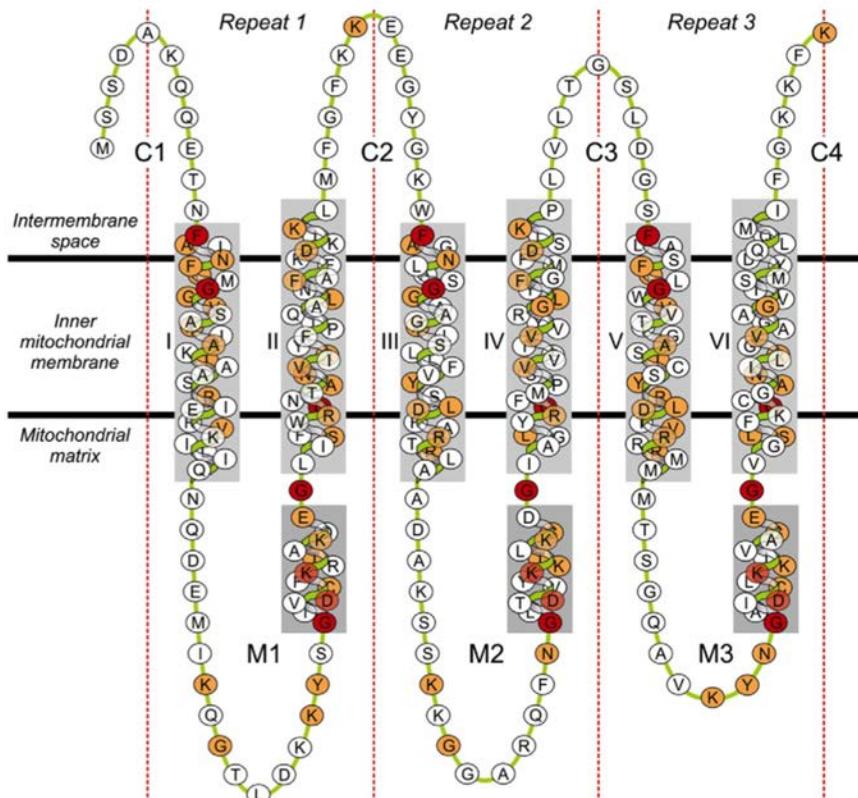


Fig. 18.40, 18.41mod Berg et al., Biochemistry, 9<sup>th</sup> ed.

## Mitochondrial ATP-ADP translocase structure and function

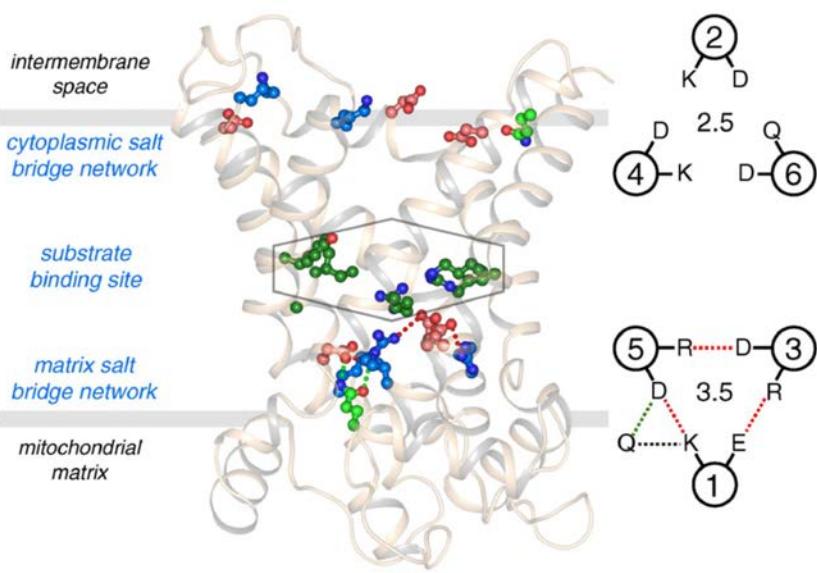


Edmund Kunji

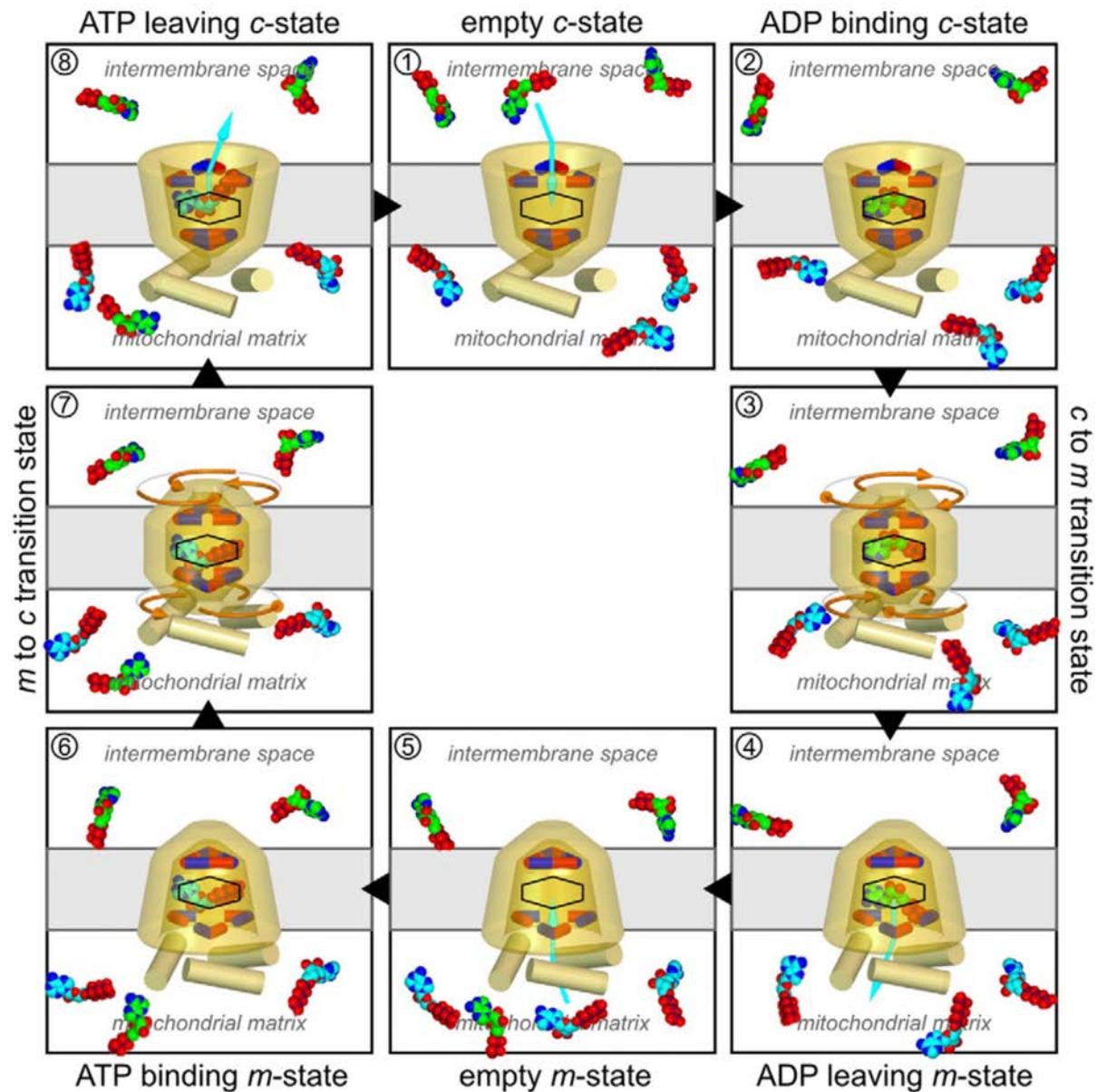


adapted from  
Kunji ERS et al., BBA 1863: 2379-2393 (2016)

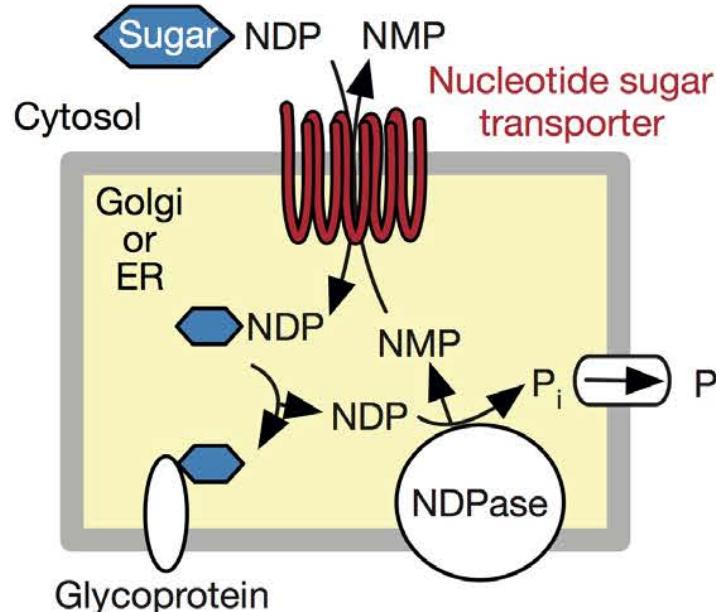
# Mitochondrial ATP-ADP translocase mechanism



Kunji ERS et al., BBA 1863: 2379-2393 (2016)

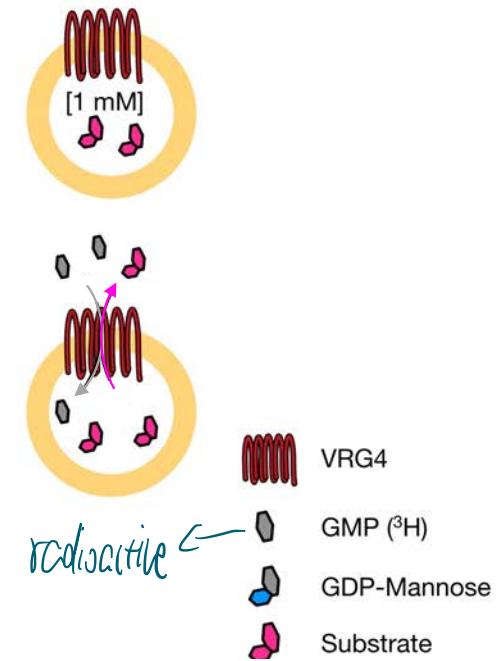
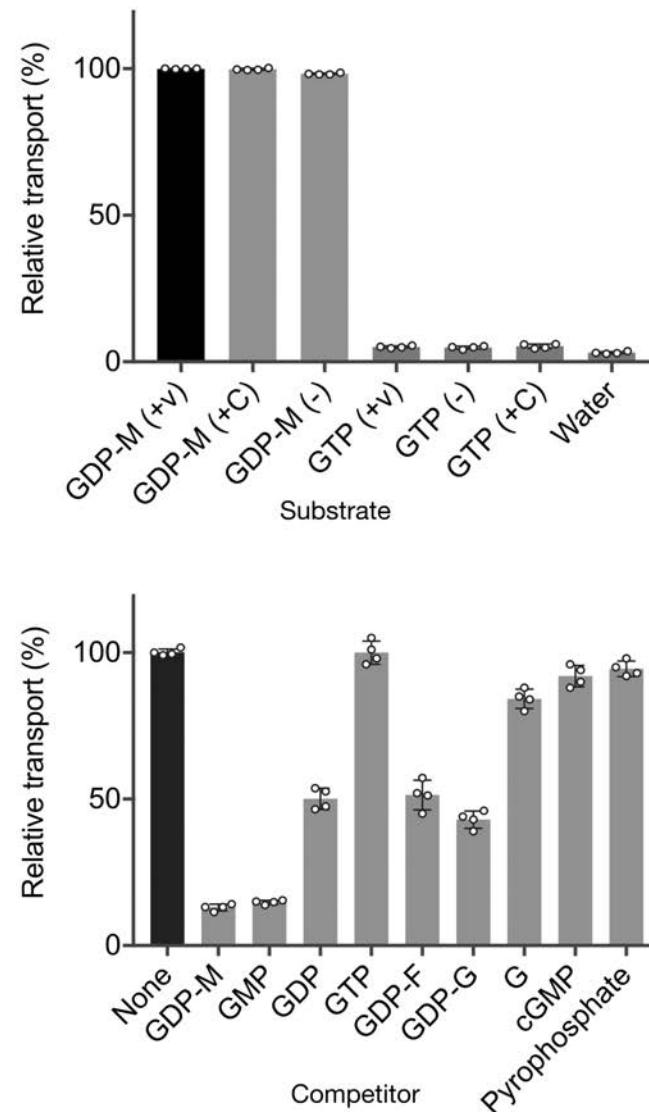


## Nucleotide-sugar transporters in Golgi

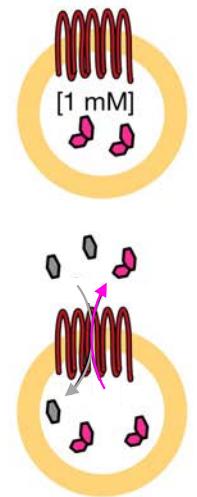


Nucleotide sugar transporters (NSTs) shuttle activated sugar donors (sugar-NDP) across the endoplasmic reticulum (ER) and Golgi membranes. NDP, nucleoside diphosphate; NMP, nucleoside monophosphate.

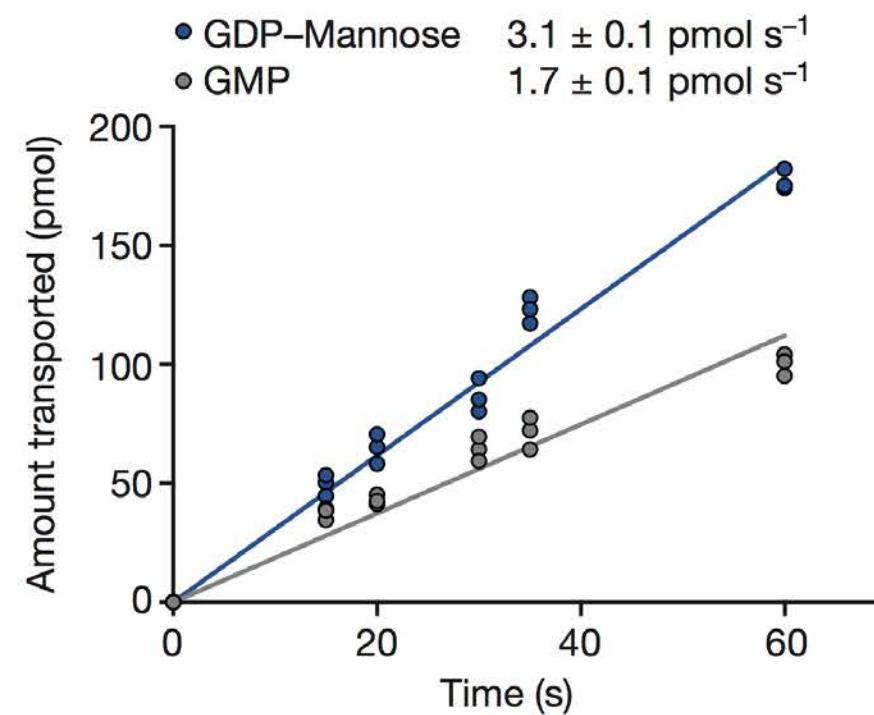
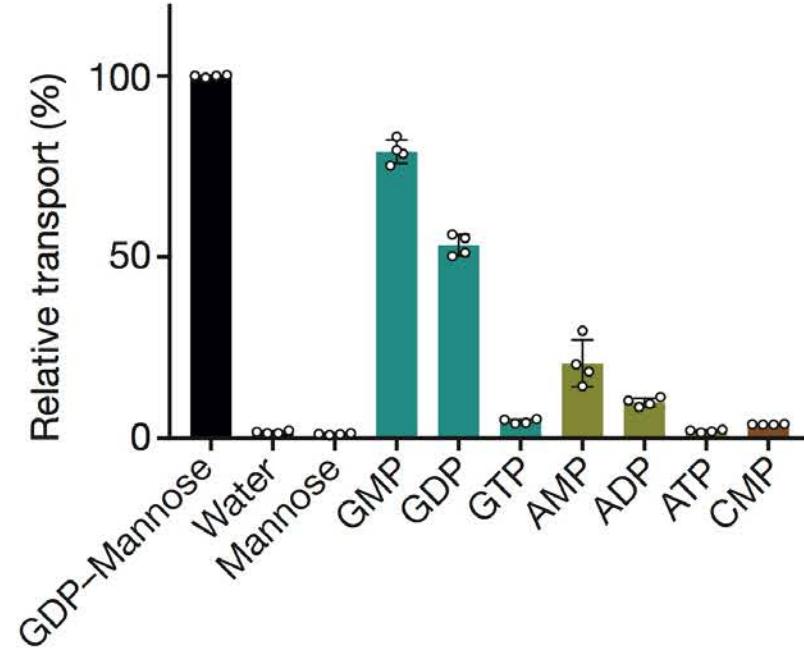
passive transporter but strictly coupled with inward and outward movements (antiporters)



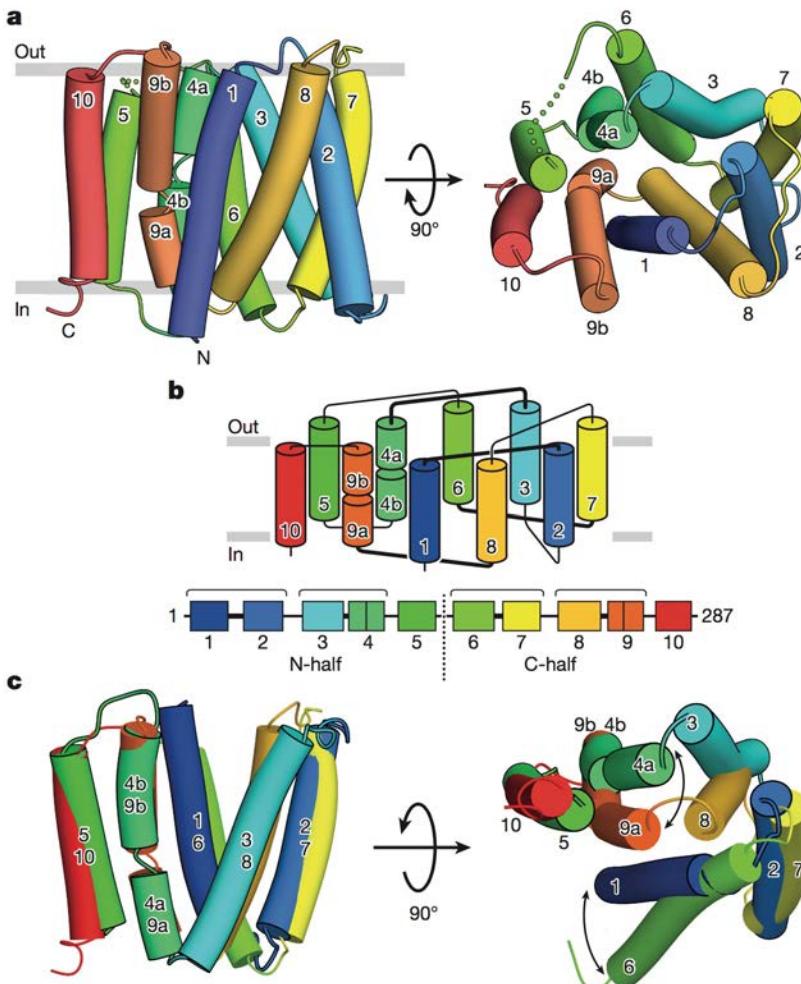
# Nucleotide-sugar transporters in Golgi



~~~~~ VRG4  
~~~~~ GMP ( $^3\text{H}$ )  
~~~~~ Substrate



# Golgi NSTs: Members of the DMT superfamily

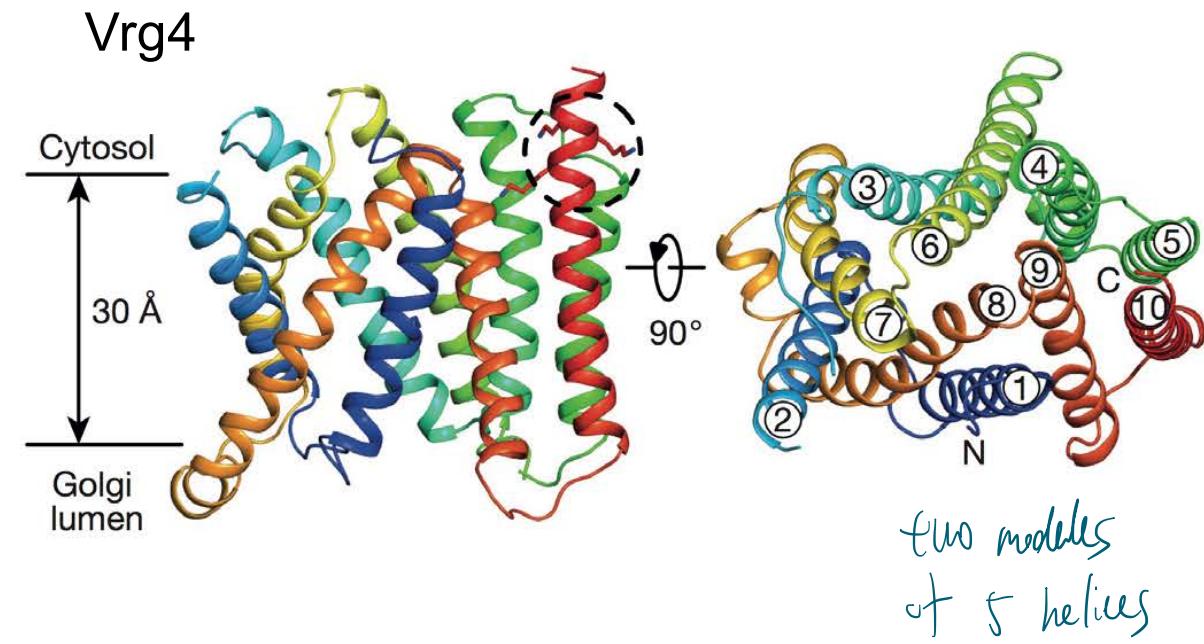


Tsuchiya H et al., *Nature* 534: 417-420 (2016)

DMTs (drug / metabolite transporter) are a large protein superfamily are ubiquitously distributed in eukaryotes, bacteria and archaea. They mediate the translocation (often export from the cytoplasm) of a wide range of substrates, including drugs and metabolites, and DMT proteins.



Simon Newstead



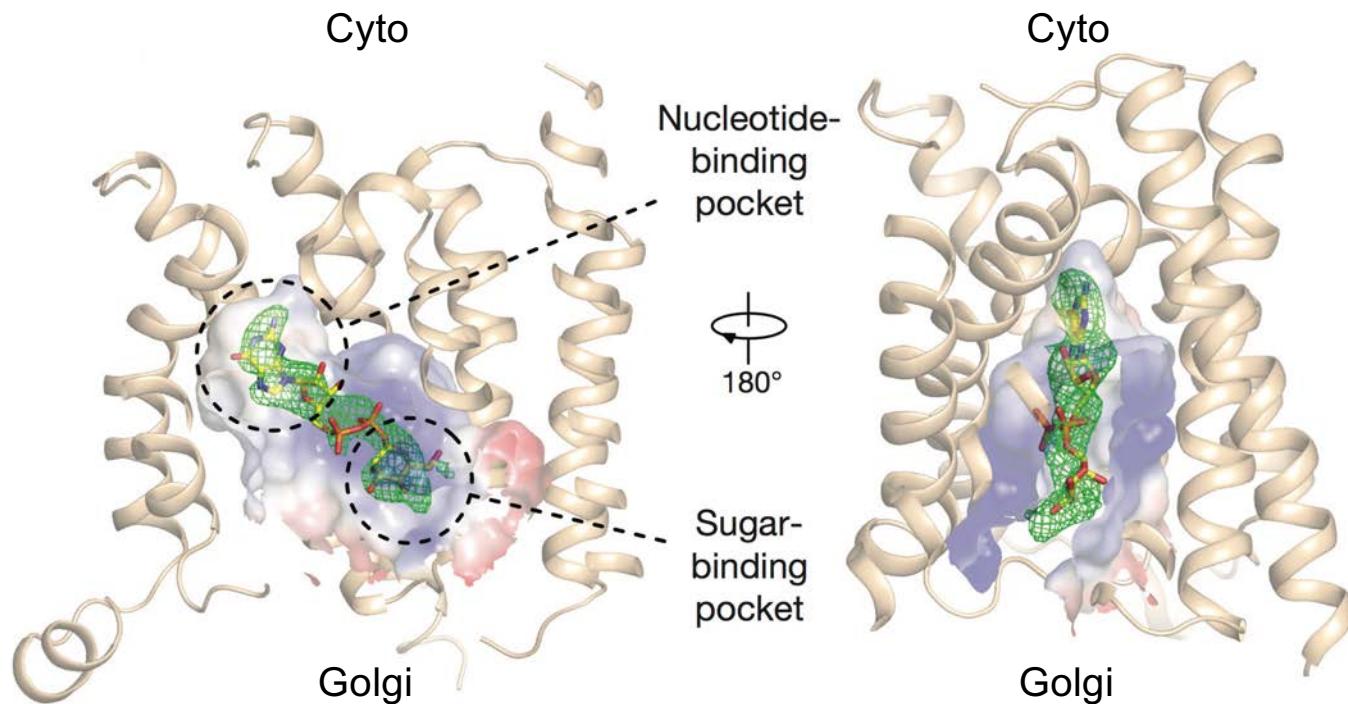
Parker JL, Newstead S *Nature* 551: 521-524 (2017)

# pymol Vrg4

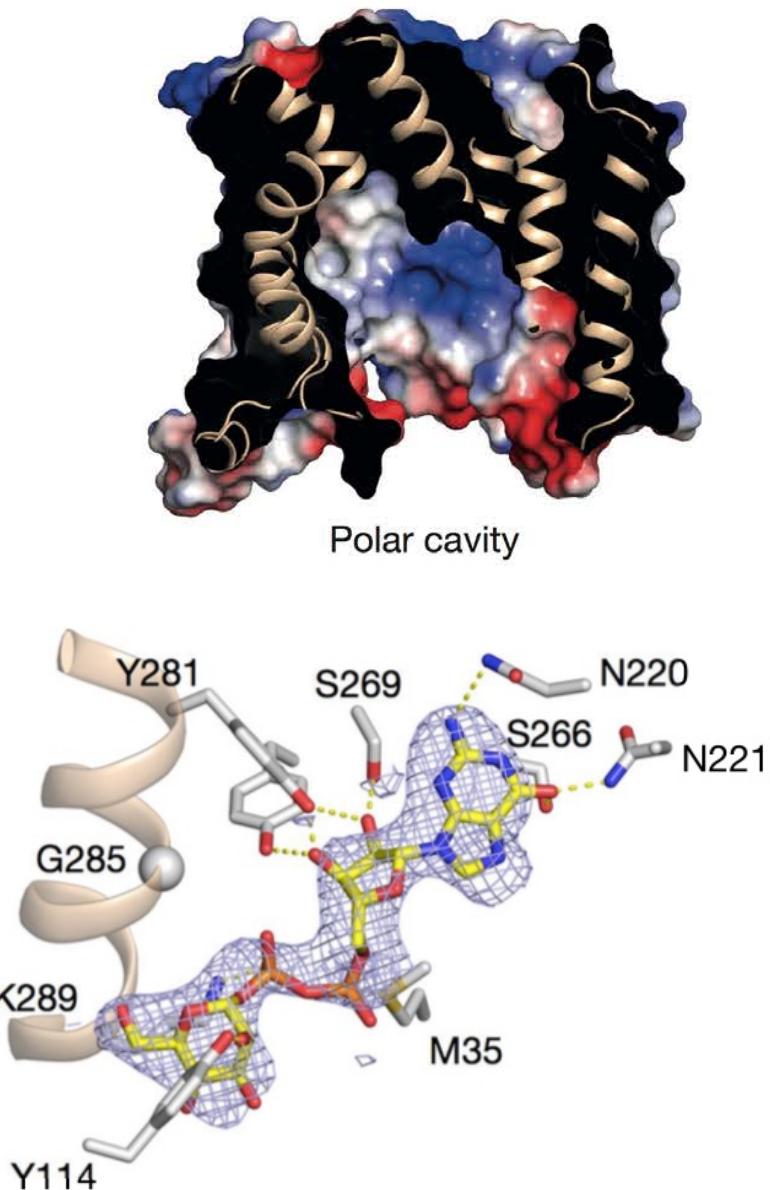
PDB ID: 5OGE.pdb

- Pymol scene 1: Ribbon diagram 10 TM helices, Golgi top, Cyto bottom
- Pymol scene 2: Repeat 1
- Pymol scene 3: Repeat 2, use align to show pseudo-two-fold symmetry
- Pymol scene 4: View from Golgi into large pocket hydrophilic ( bhd to G DP - Manose )
- Pymol scene 5: Electrostatic surface potential, large opening to Golgi, inside positive rule

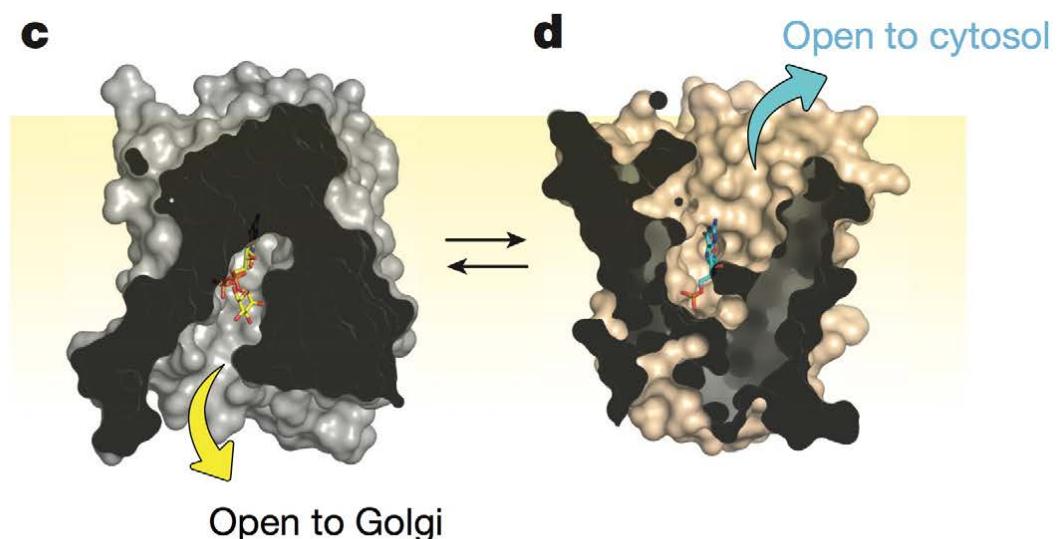
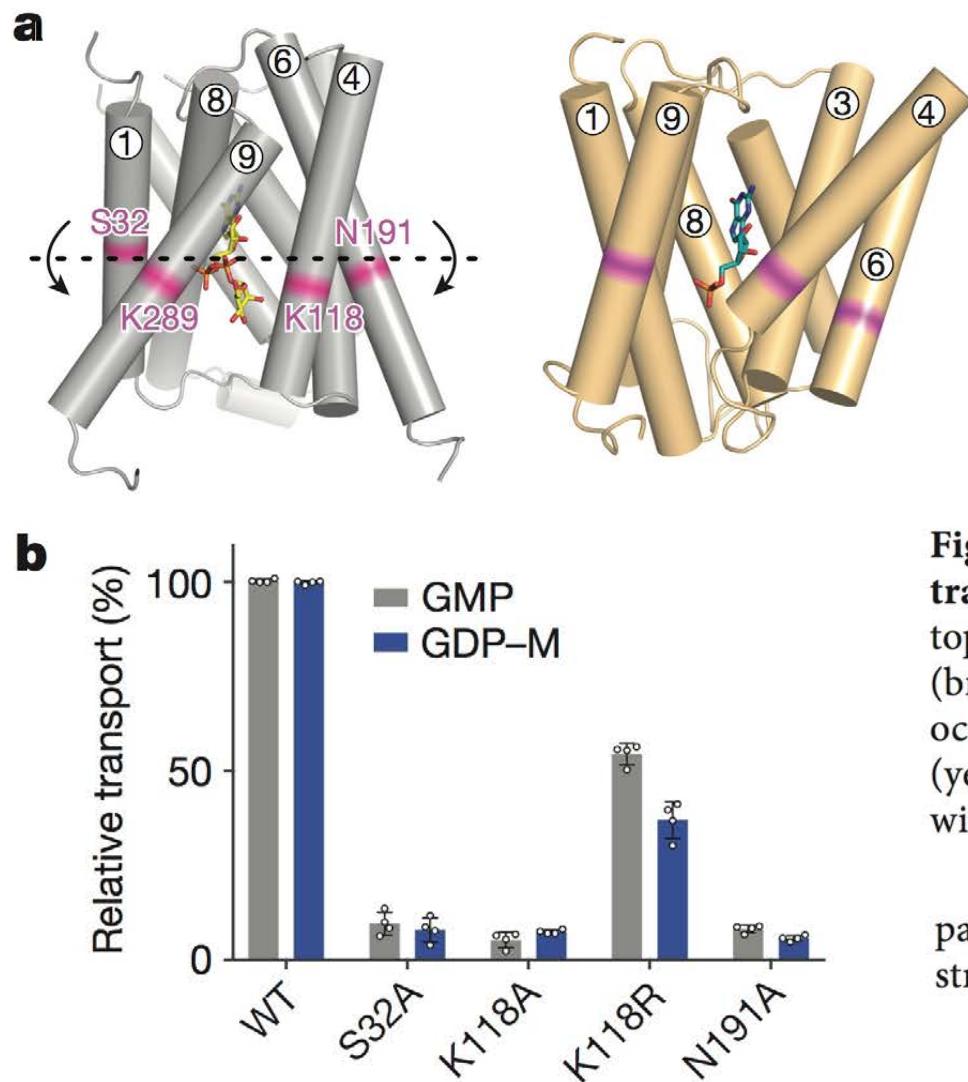
## Vrg4: Substrate binding



The guanosine moiety (in particular the guanine base) is bound at the apex of the cavity, which explains why both GDP and GDP-Man are recognized and transported.



## Vrg4: Alternating access mechanism

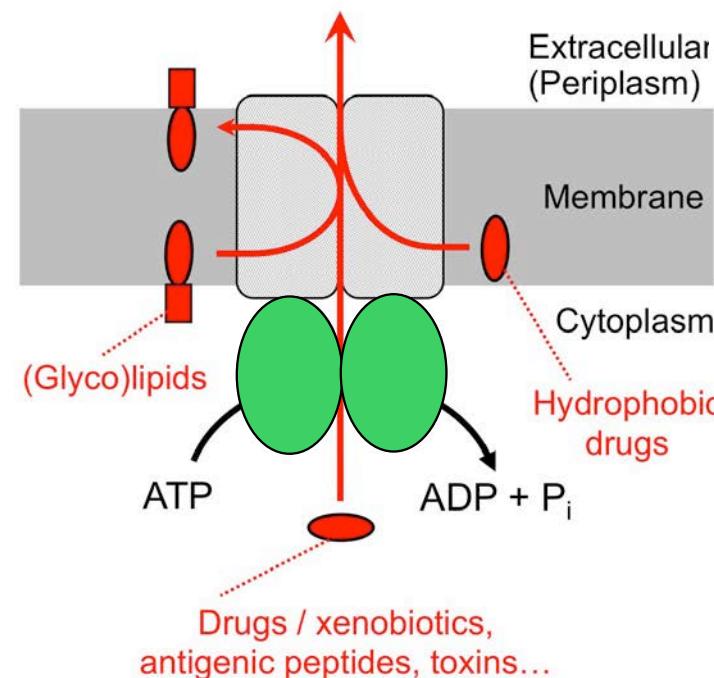
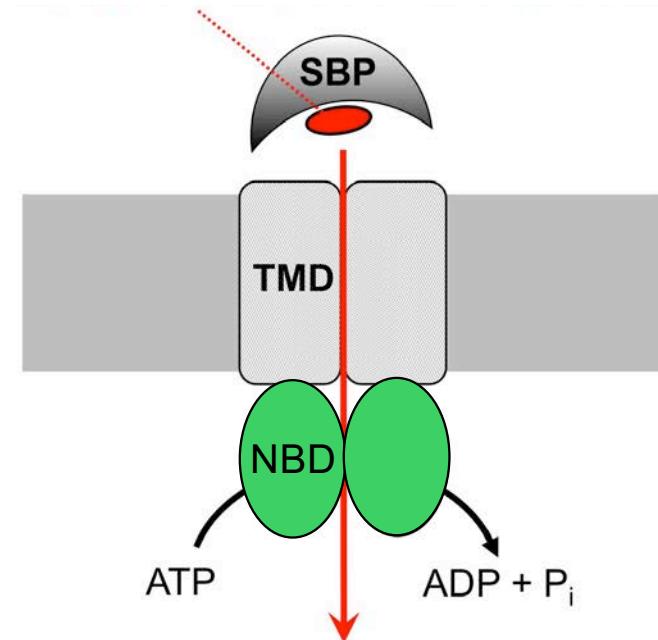
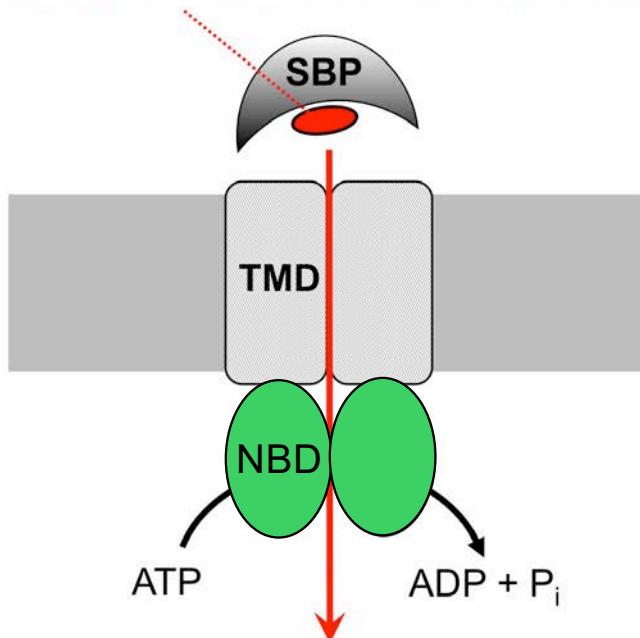


**Figure 4 | Alternating-access transport mechanism of nucleotide sugar transporters.** **a**, Interacting residues (magenta) between the inverted-topology repeats are highlighted. A model of the open-to-cytoplasm state (brown) reveals that the reorientation of the transporter (black arrows) occurs at the same plane (dashed line) as the bound GDP-mannose (yellow) and GMP (cyan). **b**, Effect of replacing the interacting residues with alanine on transport.

**c, d**, Molecular surface representations showing entry and exit pathways to the central ligand binding site, for the Golgi lumen (crystal structure, **c**), and the cytoplasmic-facing state (repeat-swapped model, **d**).

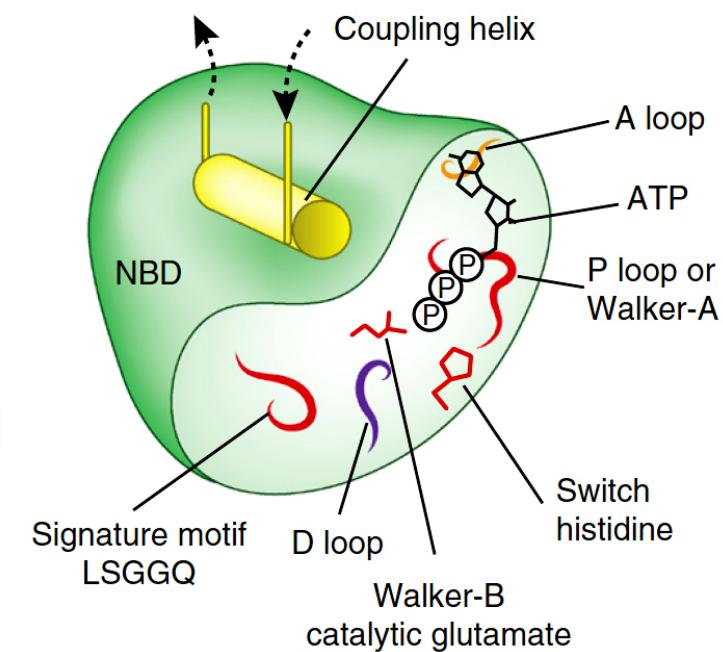
# ATP-binding cassette (ABC) transporter architectures and functions

(Micro)nutrients: Amino acids, sugars, peptides, ions, siderophores, B12,...



*Importers (bacteria, archaea)*  
High specificity due to binding protein

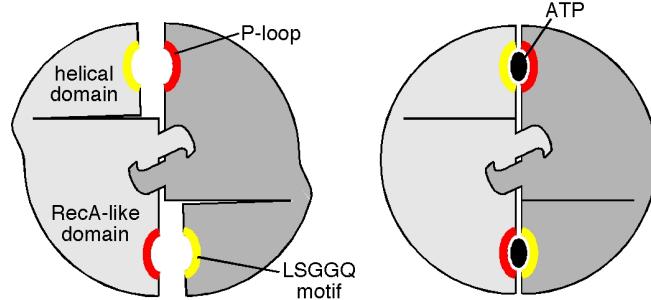
*Exporters (ubiquitous)*  
Lower specificity



Locher KP NSMB 23: 487 (2016)

binding two molecules

NBD "dimerization" traps ATP molecules at shared interface



phosphoel binding motif

P-loop / Walker-A  
(...GxxGxGKST...)

Walker-B

Q-loop

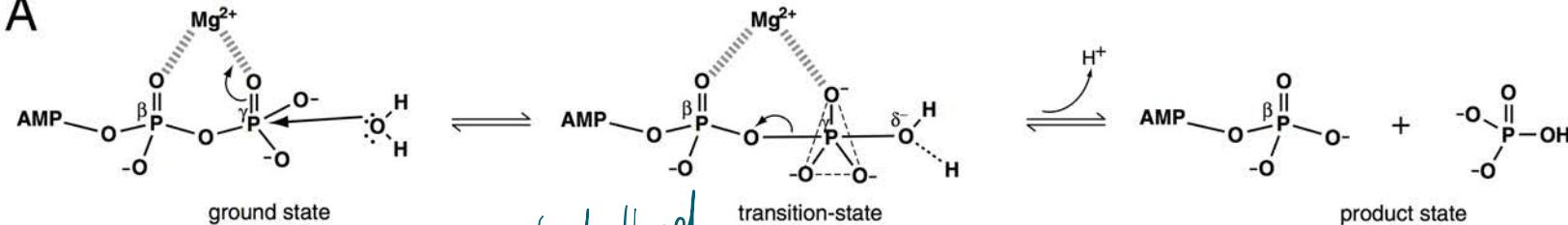
LSGGQ motif / ABC signature motif

AMP-PNP

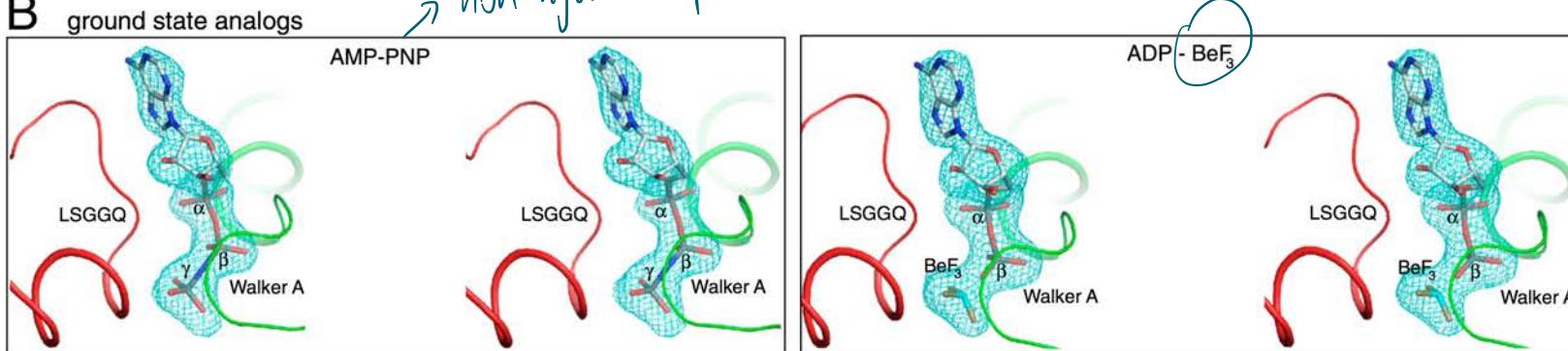
Dawson RJ & Locher KP *FEBS Lett* **581**: 935 (2007)

## Structures of inhibited states: Insight into nucleotide binding and hydrolysis

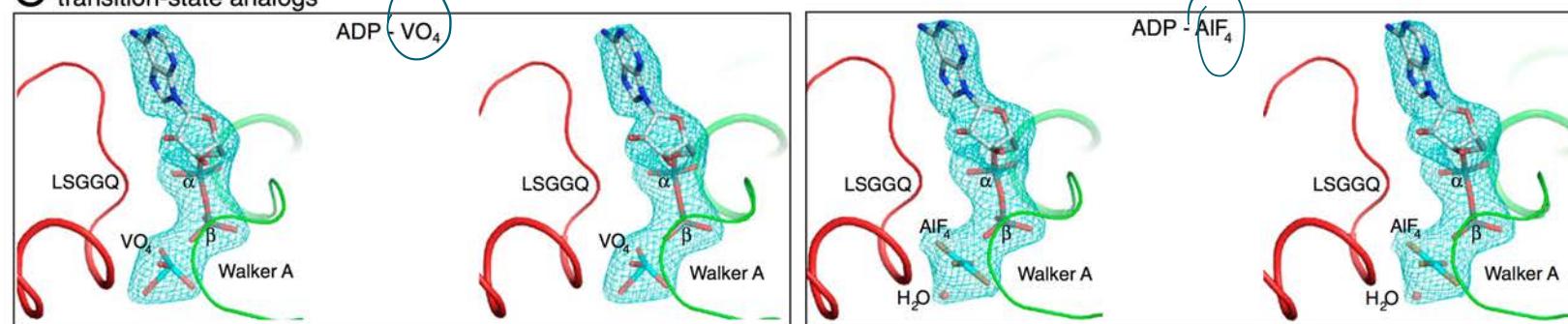
A



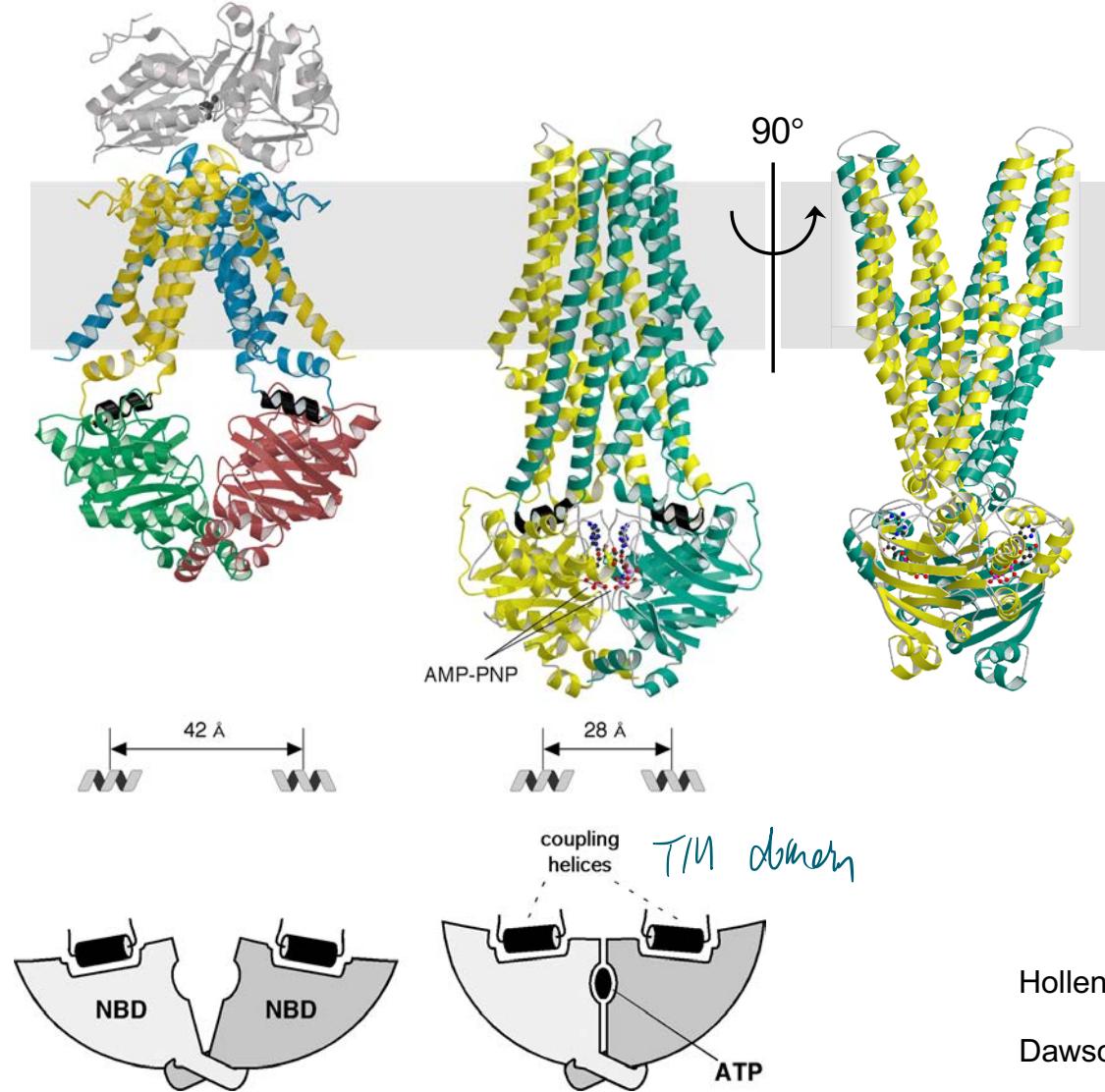
B



C transition-state analogs



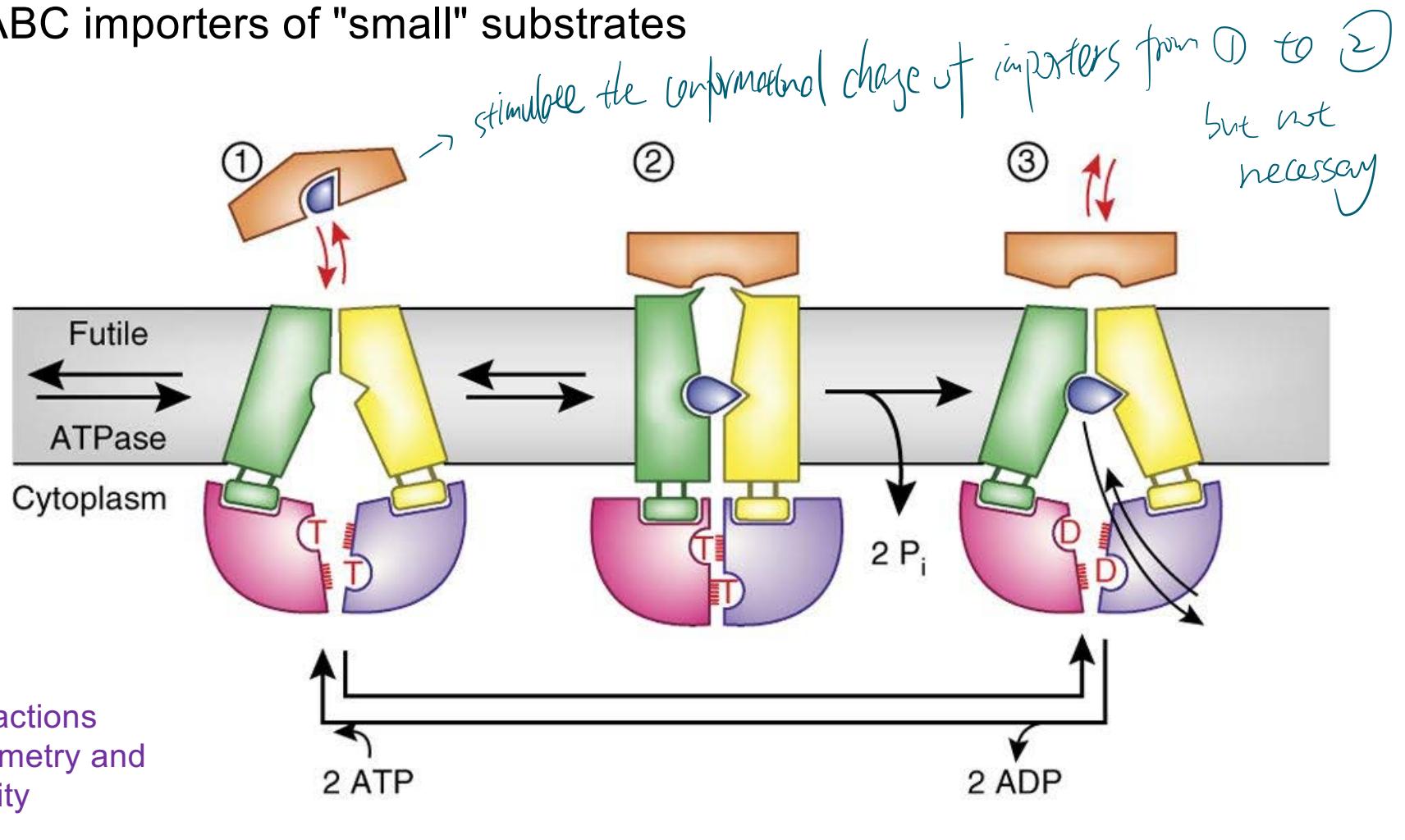
## Coupling helices and alternating access



Hollenstein K et al., *Curr Op Struct Biol* **17**: 412 (2007)

Dawson RJ et al., *Mol Microbiol* **65**: 250 (2007)

## Mechanism of ABC importers of "small" substrates



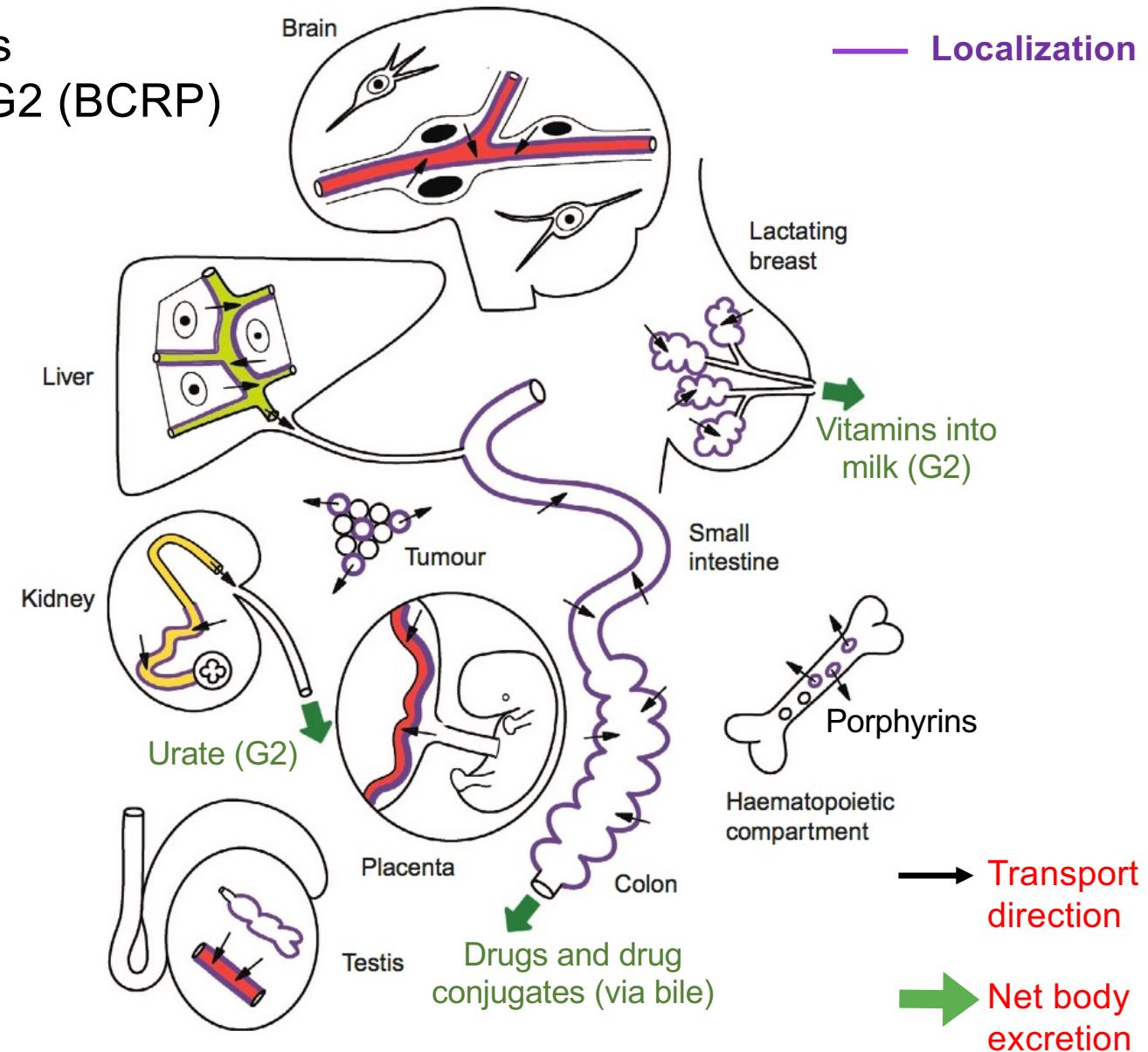
- Selectivity, side reactions
- Coupling / stoichiometry and futile ATPase activity
- Overall rate

# Human multidrug ABC transporters ABCB1 (P-glycoprotein) and ABCG2 (BCRP)

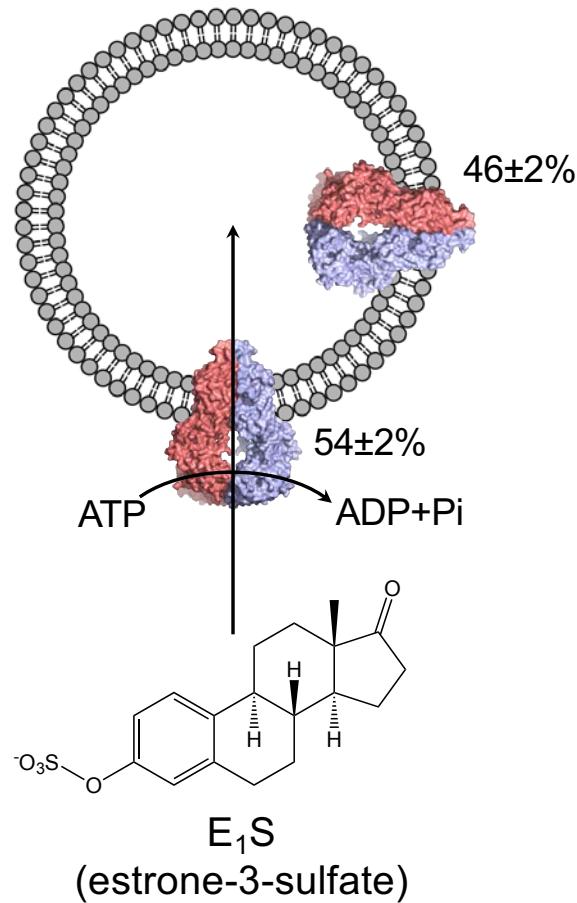
- Essential transporters, expressed in the membranes of many tissues (incl. placenta) and in the fetus throughout gestation.
- Impact on pharmacokinetics of drugs and their metabolites and on clinically relevant drug disposition and drug-drug interactions.
- Contribute to MDR of some tumors. ABCB1 and ABCG2 overexpression often correlate with poor prognosis and outcome in patients.

Durmus S, Hendrikx JJ, Schinkel AH  
*Adv Cancer Res* **125**: 1-41 (2015).

Vlaming MLH, Lags JS, Schinkel AH  
*Adv Drug Deliv Rev* **61**: 14-25 (2009).



## *In vitro* transport function of purified ABCG2



1. Add ATP / Mg<sup>2+</sup> and radio-labeled drug to ABCG2 proteoliposomes



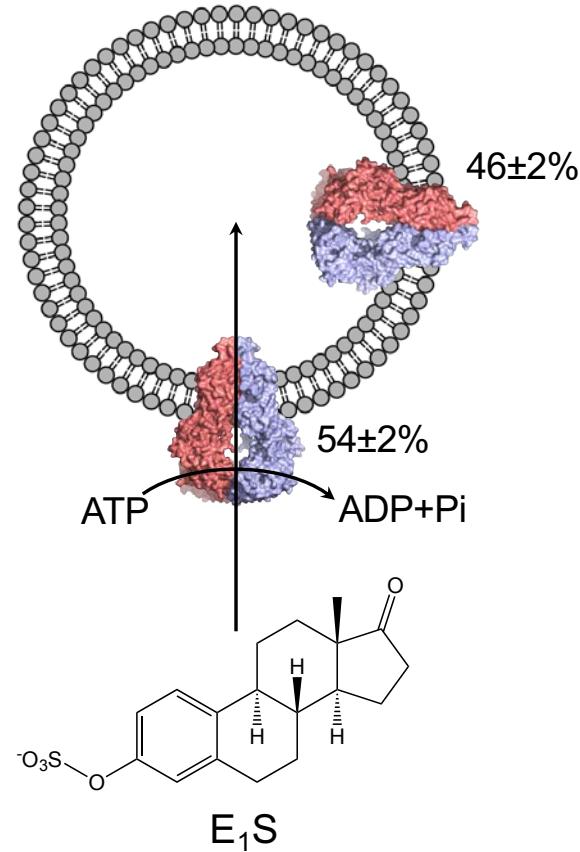
2. Stop reaction at given time points and transfer to filter plate



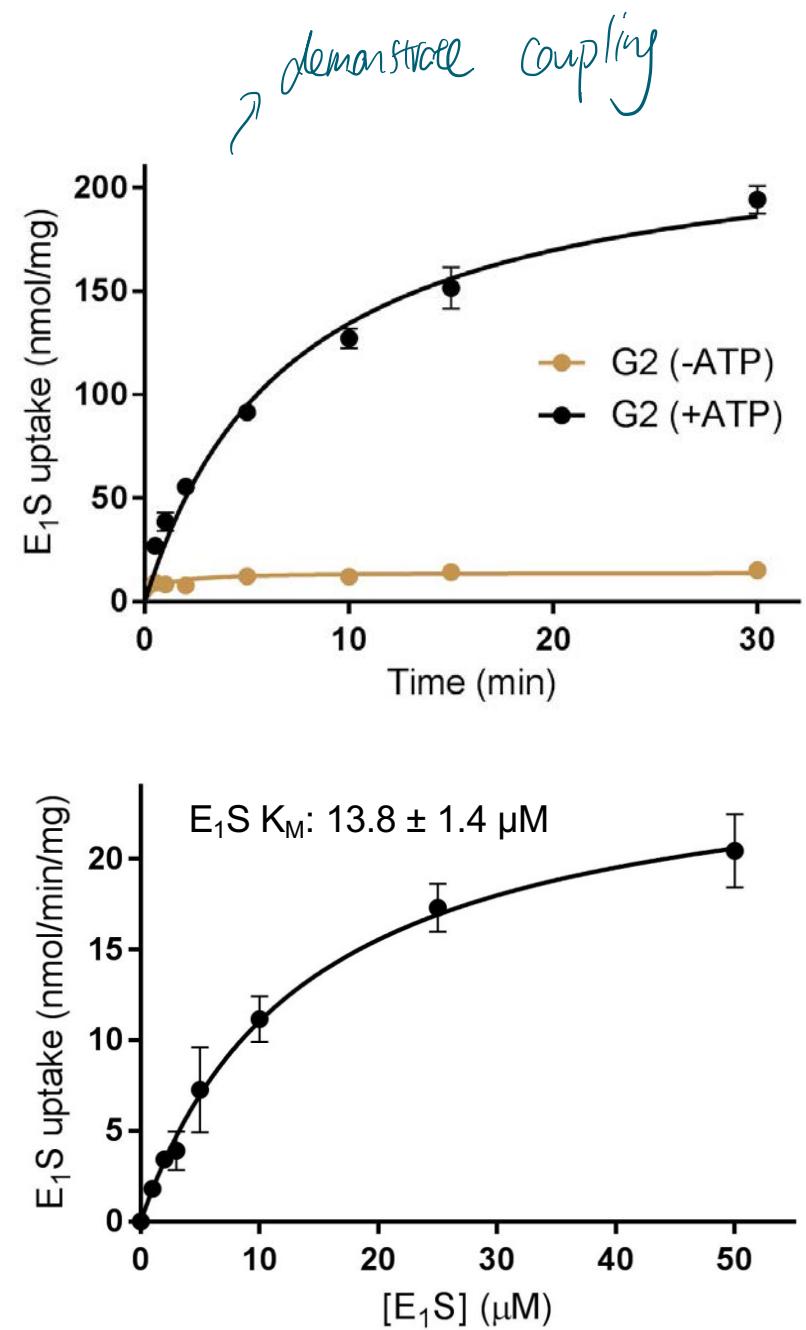
3. Separate proteoliposomes from medium by filtration and washing

4. Count radioactivity associated with proteoliposomes in scintillation counter

## *In vitro* transport function of purified ABCG2

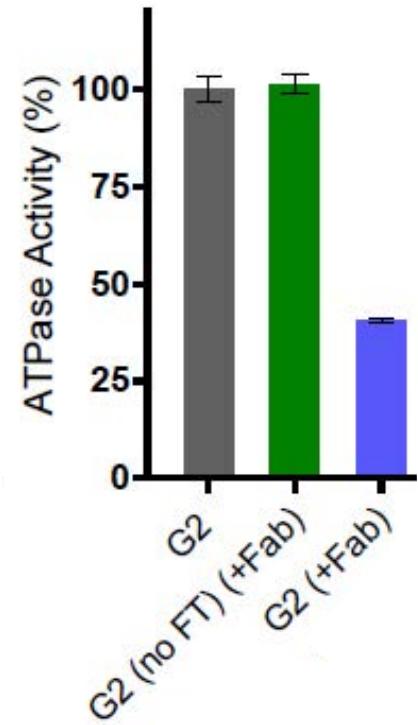
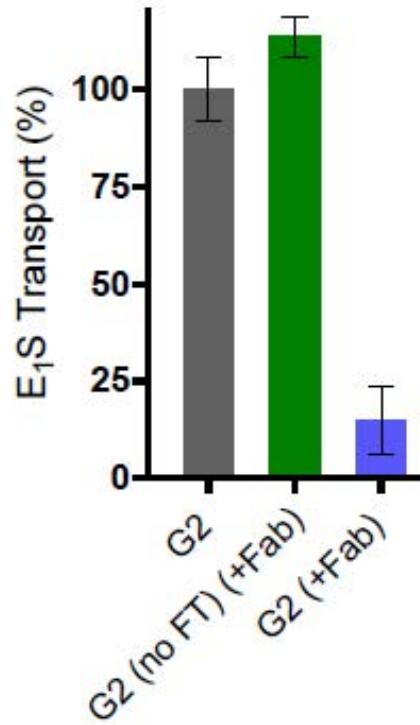
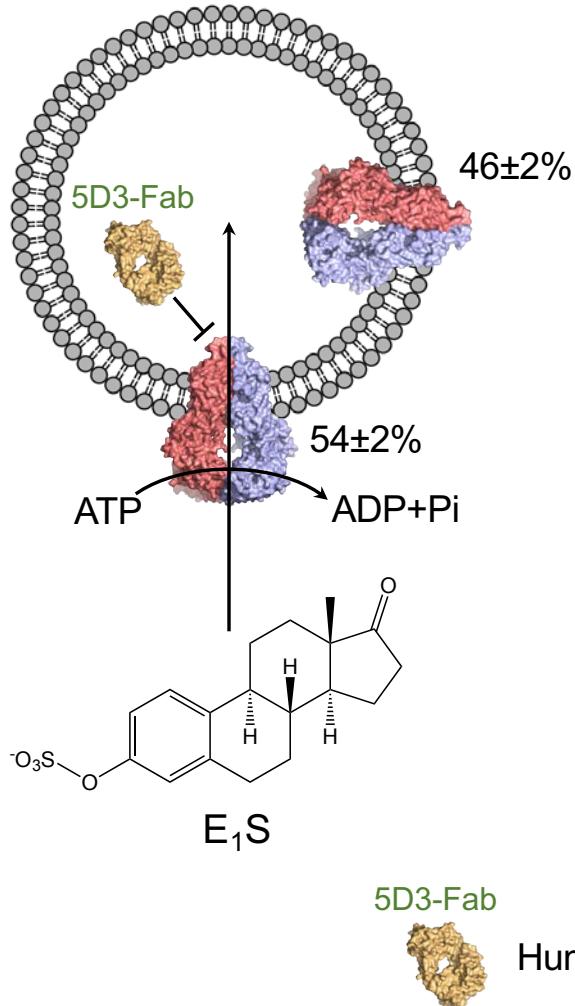


Transport rate: ~3 molecules of E<sub>1</sub>S per ABCG2 dimer and minute.



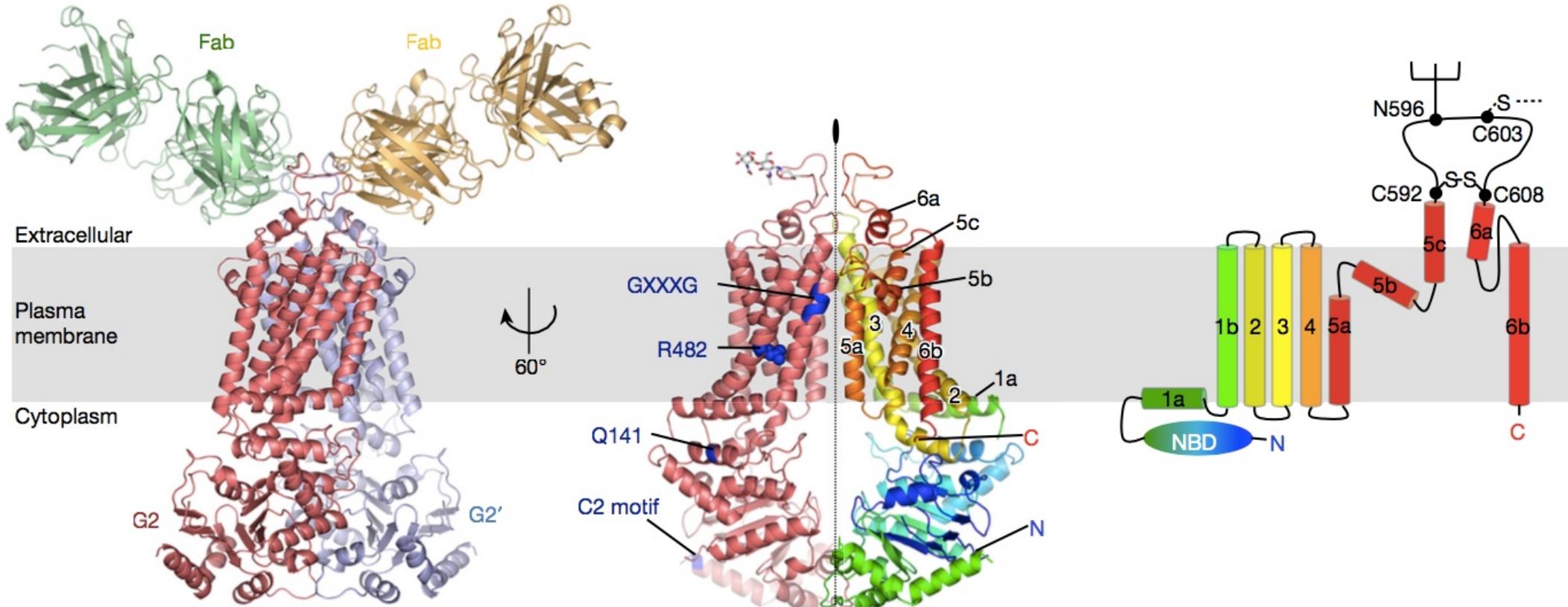
X

## In vitro transport function of purified ABCG2



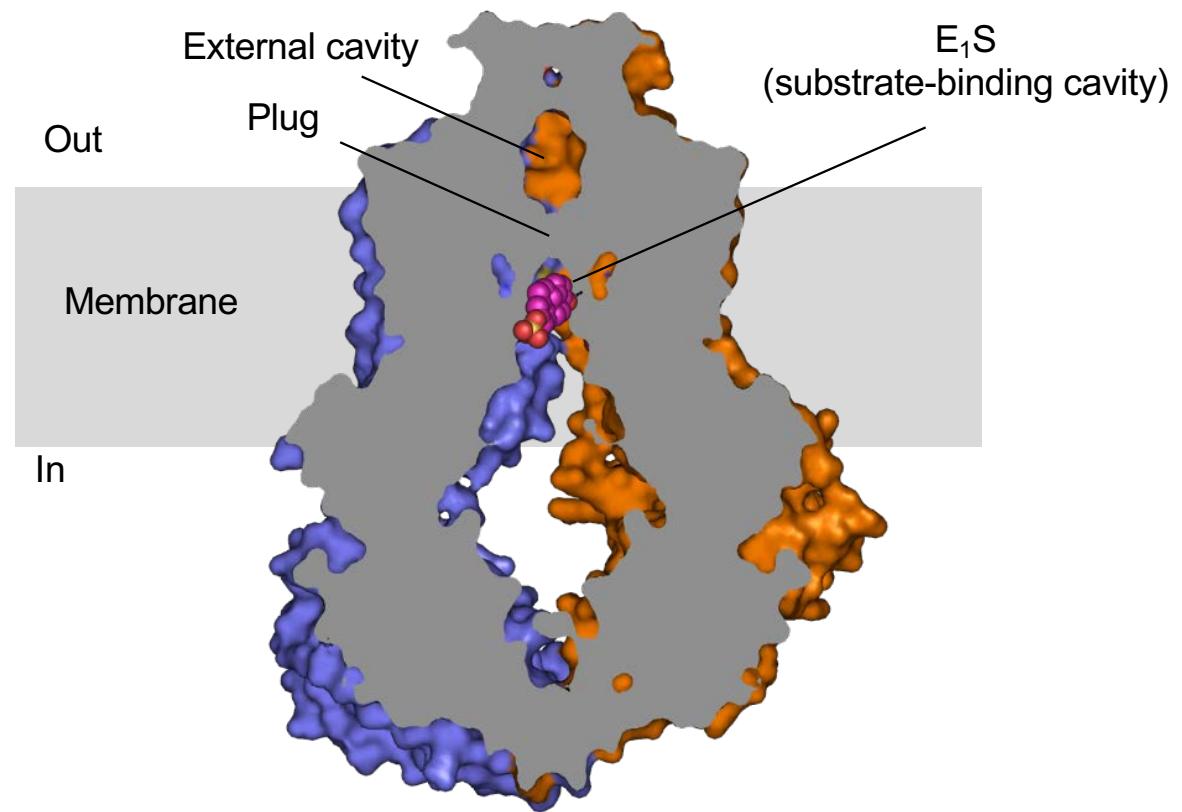
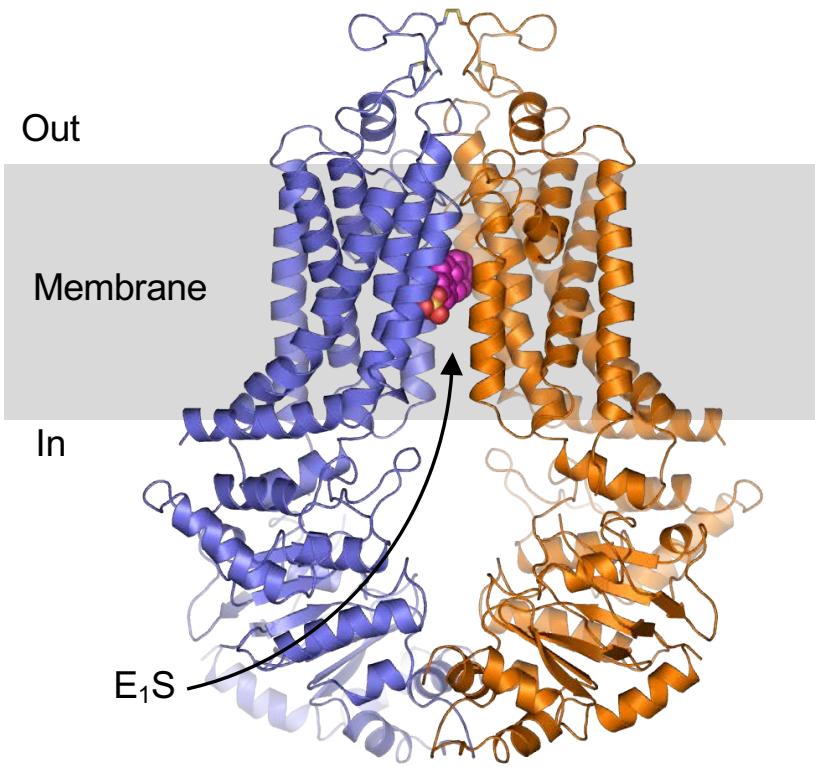
Human ABCG2-specific, inhibitory antibody 5D3: Zhou S, et al. *Nat Med* 7: 1028 (2001).

## Architecture of ABCG2 homodimer and binding site of inhibitory 5D3 antibody



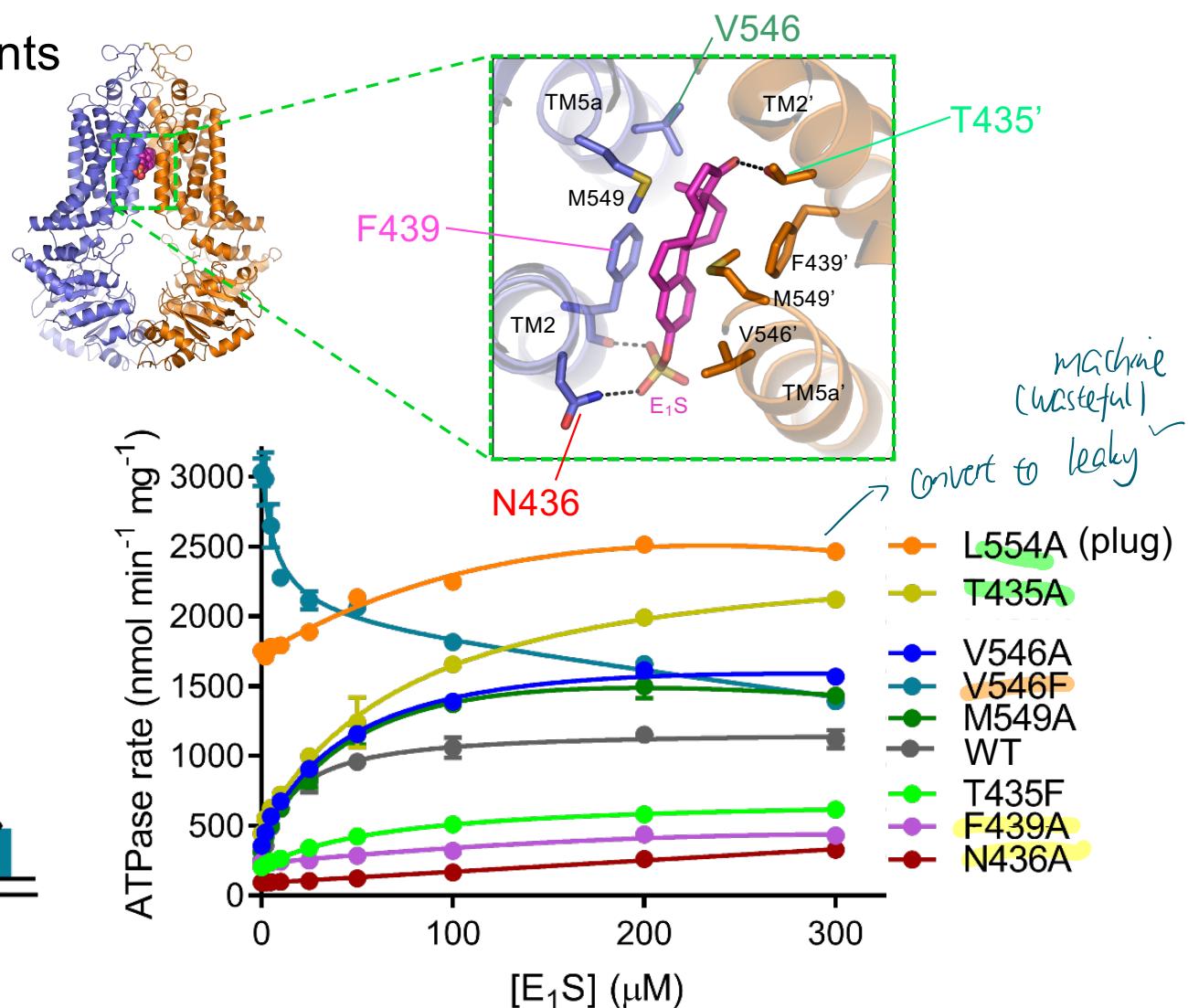
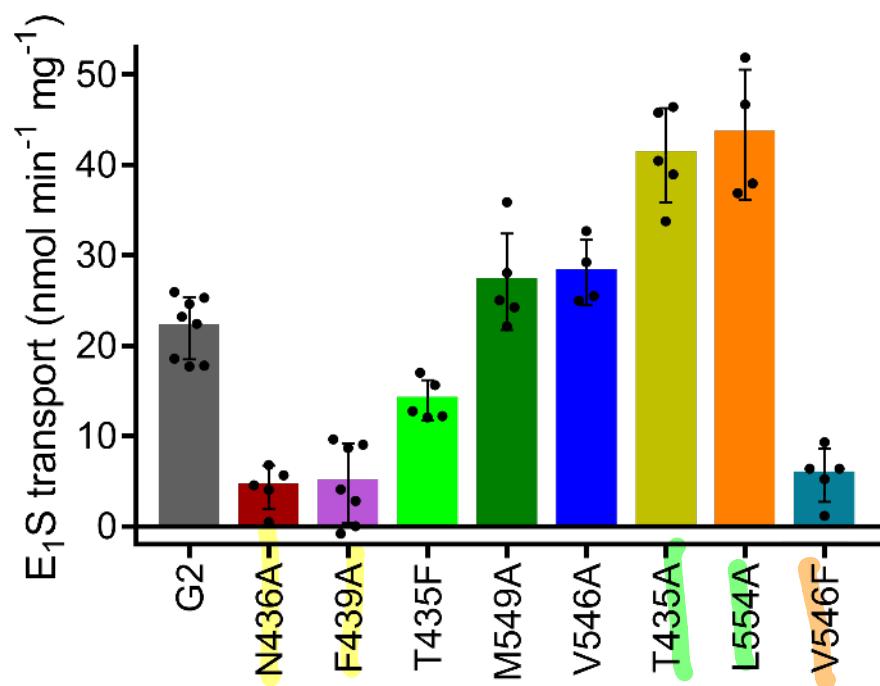
Taylor NMI, Manolaridis I, Jackson SM et al. *Nature* 546: 504-509 (2017).

## Structure of estrone sulfate-bound ABCG2



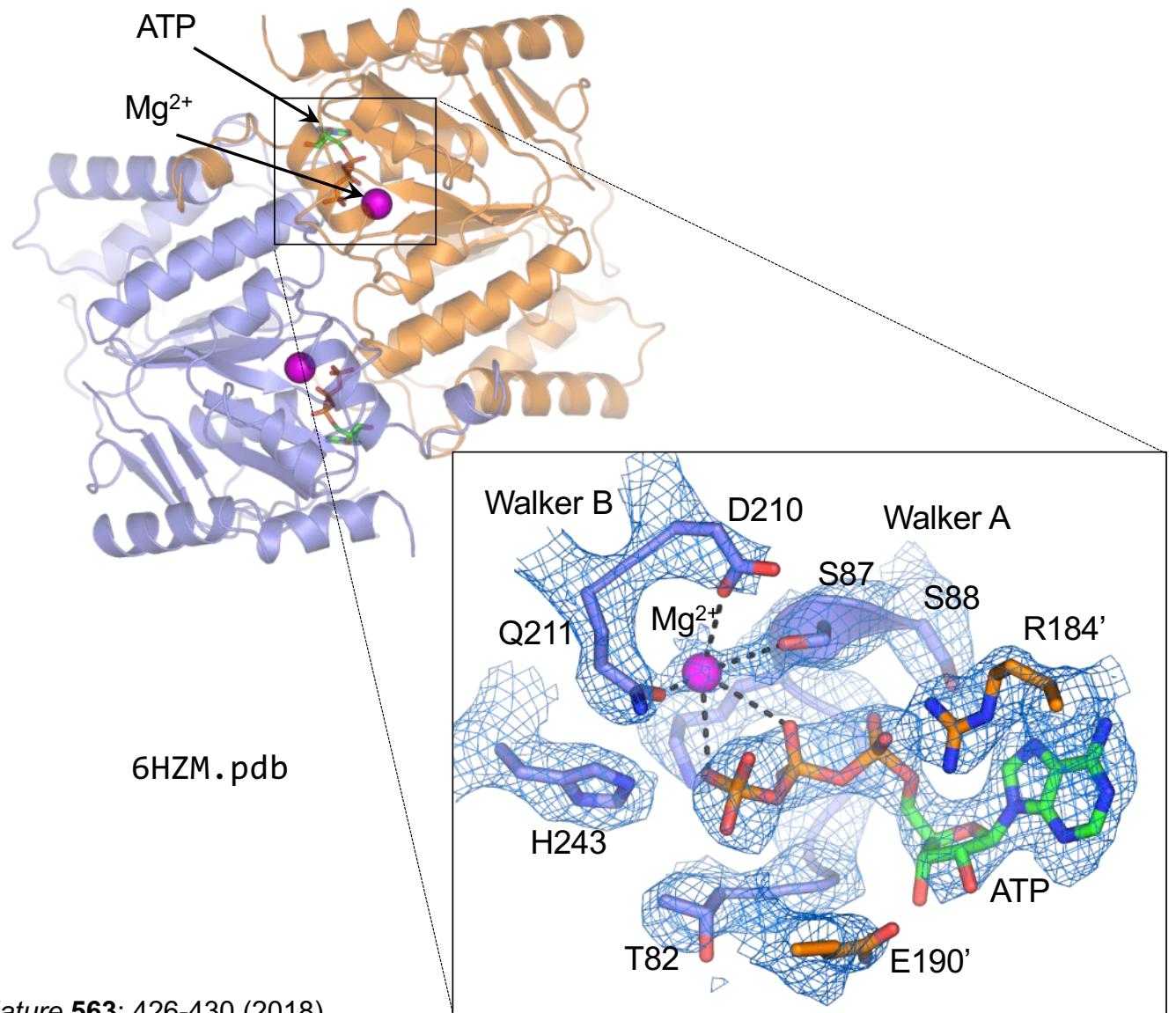
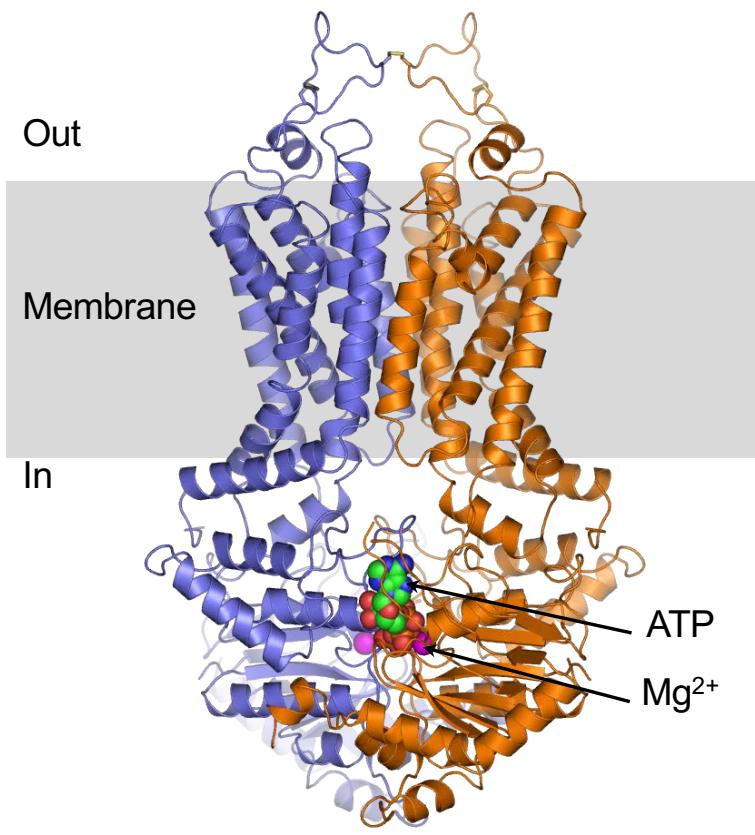
Manolaridis I, Jackson SM, Taylor NMI, Kowal J et al. *Nature* **563**: 426-430 (2018).

## Functional analysis of ABCG2 variants



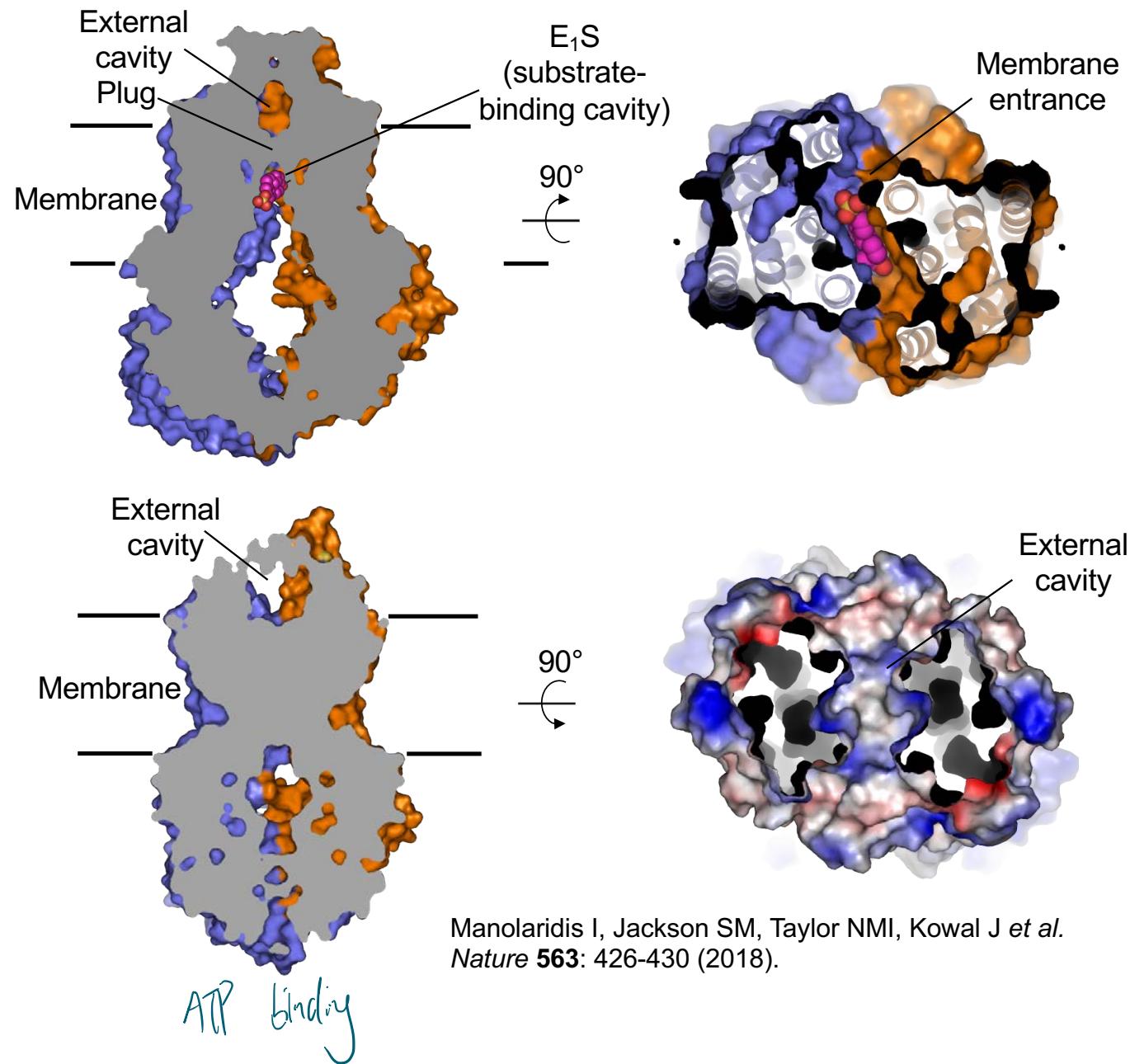
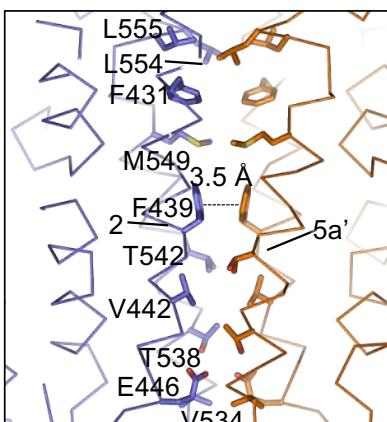
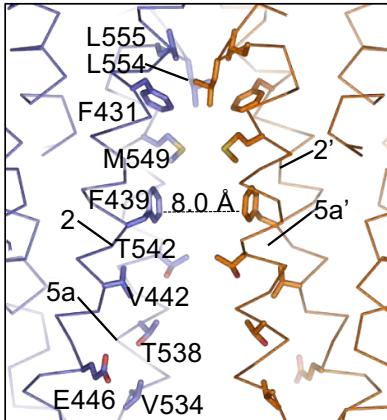
Manolaridis I, Jackson SM, Taylor NMI, Kowal J et al. *Nature* 563: 426-430 (2018).

## Structure of ATP-bound ABCG2



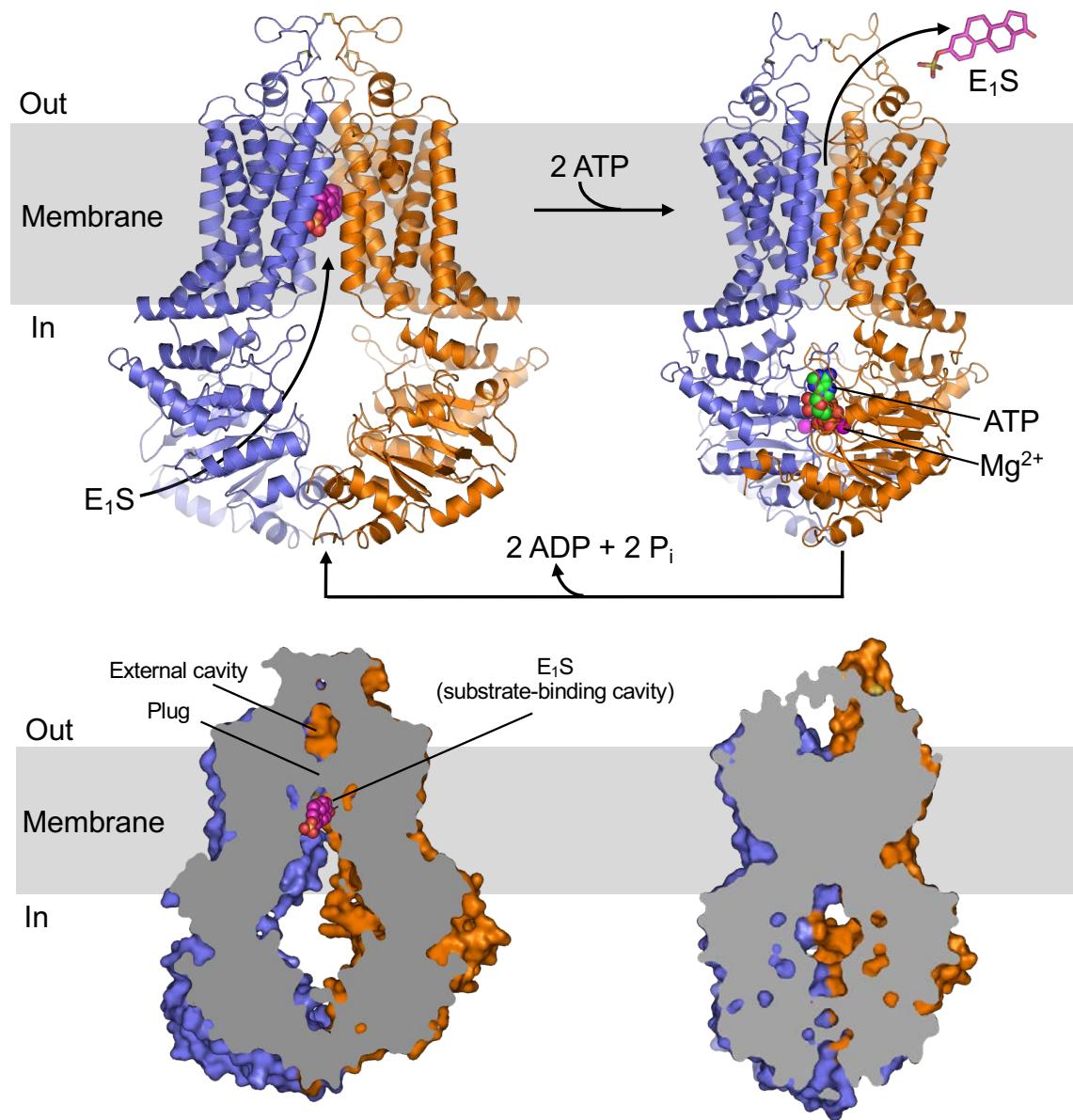
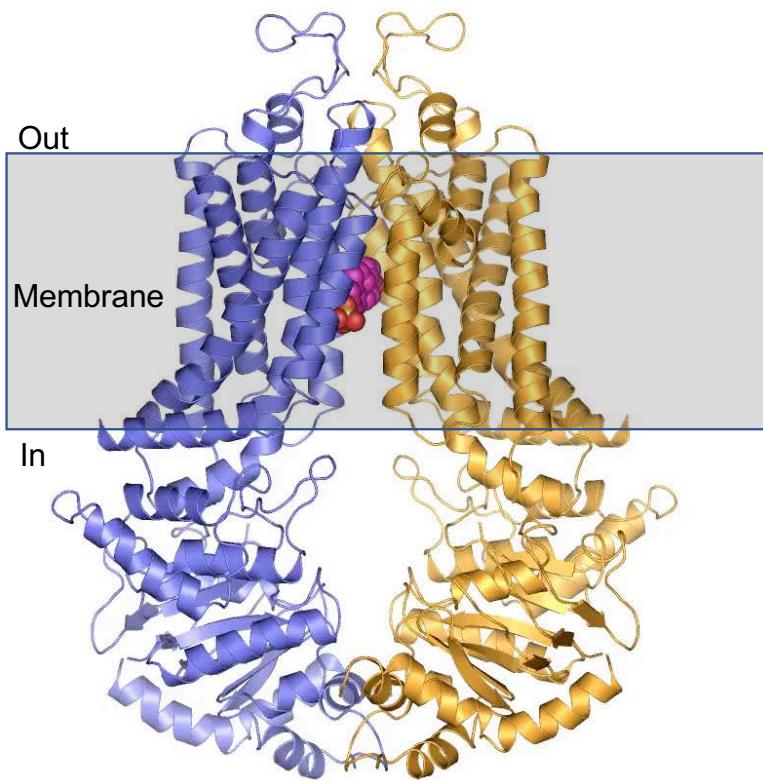
Manolaridis I, Jackson SM, Taylor NMI, Kowal J et al. *Nature* 563: 426-430 (2018).

## Changes in translocation pathway upon ATP binding



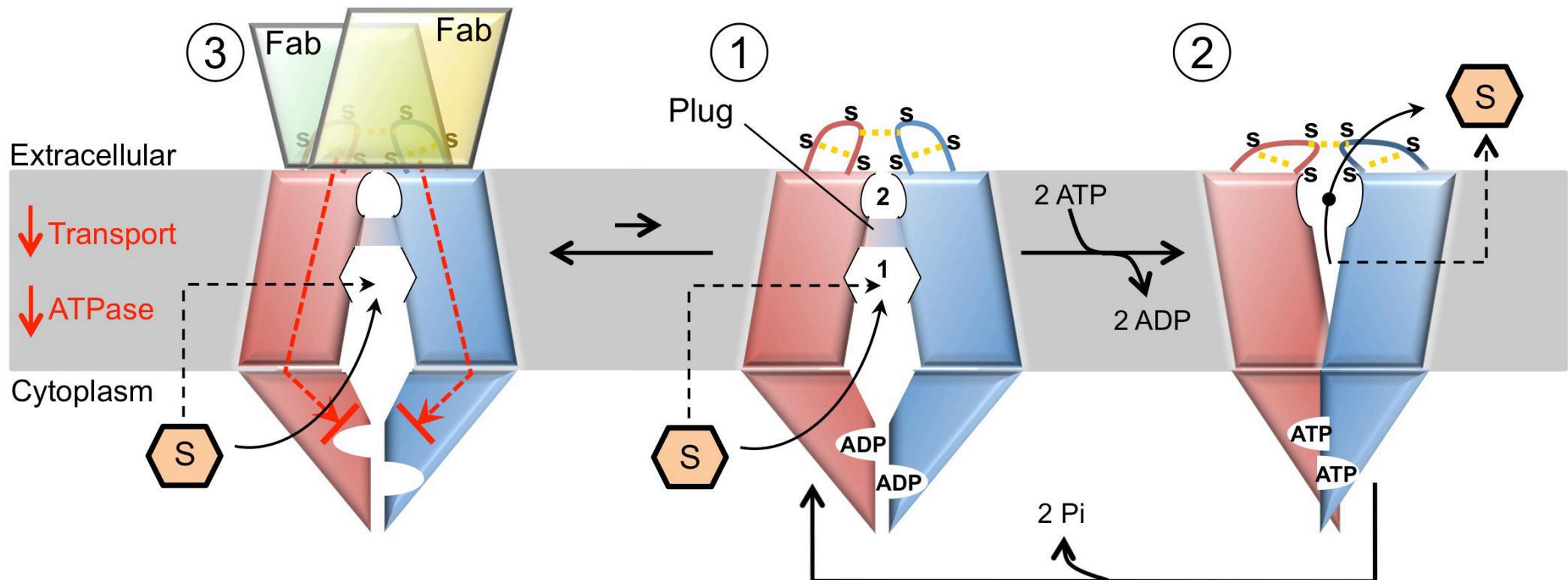
Manolaridis I, Jackson SM, Taylor NMI, Kowal J et al.  
*Nature* **563**: 426-430 (2018).

# ATP-driven substrate extrusion in ABCG2



Manolaridis I, Jackson SM, Taylor NMI, Kowal J et al.  
*Nature*, **563**: 426-430 (2018).

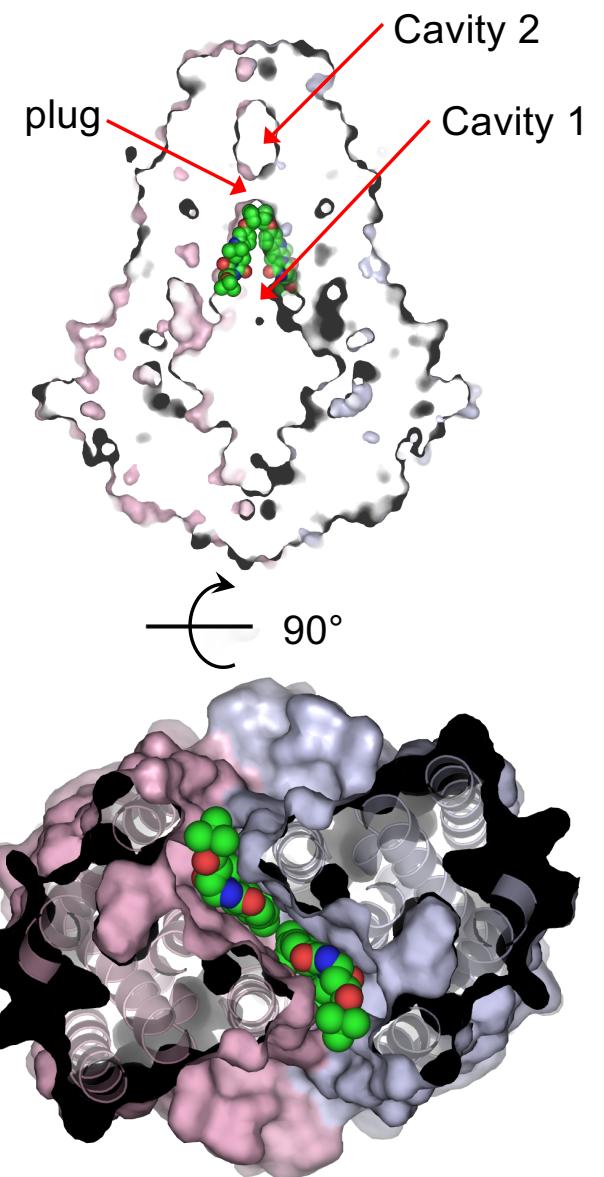
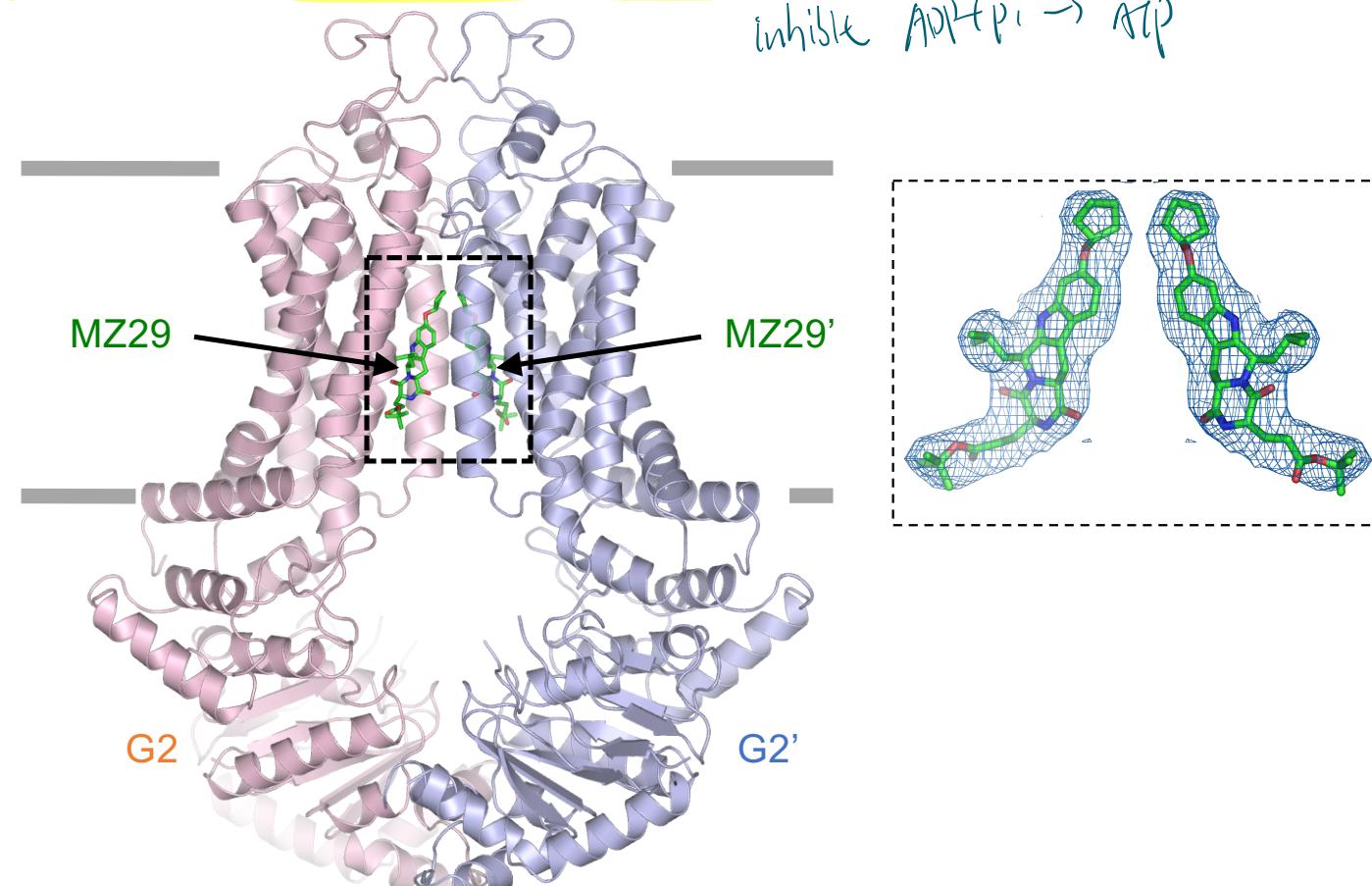
## ABCG2 mechanism: ATP-driven alternating access



- Substrates enter cavity 1.
- ATP binding causes NBD interface closing, converting ABCG2 to outward-facing. substrate escapes to the outside via cavity 2.
- Inorganic Pi release is accompanied by the conversion to state 1.
- 5D3-Fabs clamp extracellular loops of ABCG2, inhibiting transport and (allosterically) ATPase activity.

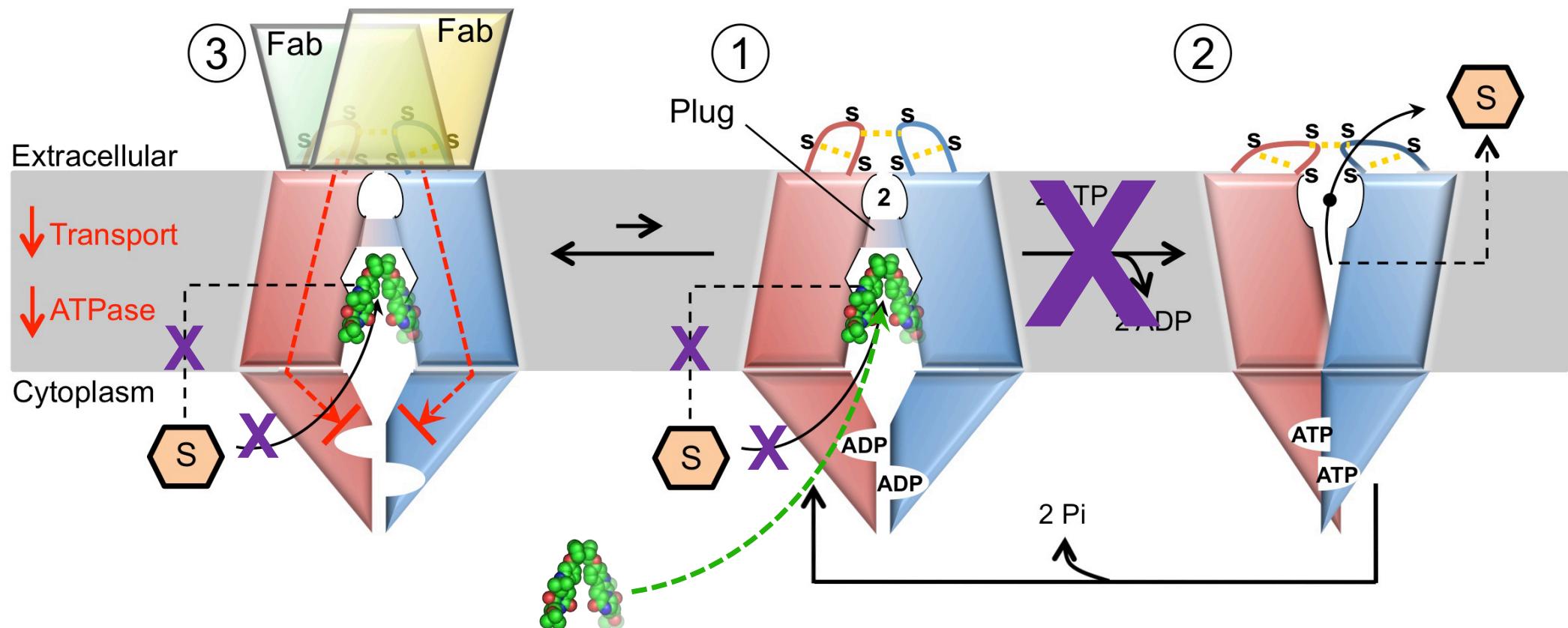
## Inhibitor-bound ABCG2 structure

Two molecules of MZ29 (a fumitremorgin-C derivative) bind to cavity 1 and prevent closing of cytoplasmic ends of TMDs (foot in the door mechanism)



Jackson SM, Manolaridis I, Kowal J, Zechner M, Taylor NMI et al. *Nat Struct Mol Biol* **25**: 333-340 (2018).

## ABCG2 mechanism: ATP-driven alternating access



- ABCG2 inhibition by small molecule compounds occurs competitively by blocking access of lower affinity substrates to cavity 1, preventing TMD and NBD dimer closing and the conversion to the outward-open conformation. Depending on the size of the inhibitor, one or two molecules may bind.