Selected Topics in Protein Structure and Function

Structural Basis of ABC Transporters

Presented by: Jyh-Yeuan (Eric) Lee, Assistant Professor, BMI

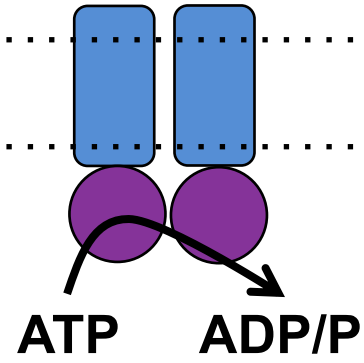
Structural basis: what do we do with membrane protein structures?

1. **Protein-protein interactions**
2. **Ligand recognition**
3. DNA and RNA binding
4. Hydrogen atoms critical for macromolecule functions
5. Kinetics
6. **Enzymatic reaction**
7. Oncogenes and tumor suppressors
8. Drug Design

9. **Lipid-protein interaction**
10. **Structure-function crosstalk in the cellular membranes**
11. …

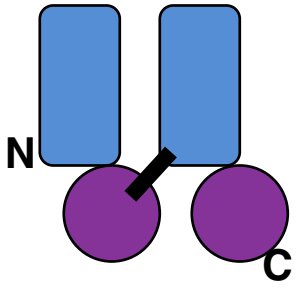
ATP-binding cassette (ABC) transporters

(more than just ABC)

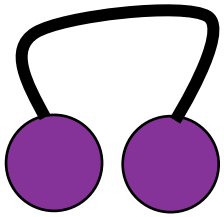


Transmembrane domain (TMD)

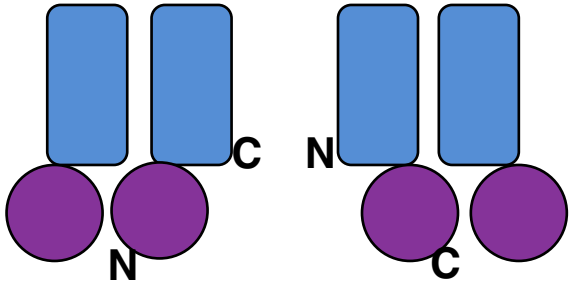
Nucleotide-binding domain (NBD)



Full transporters



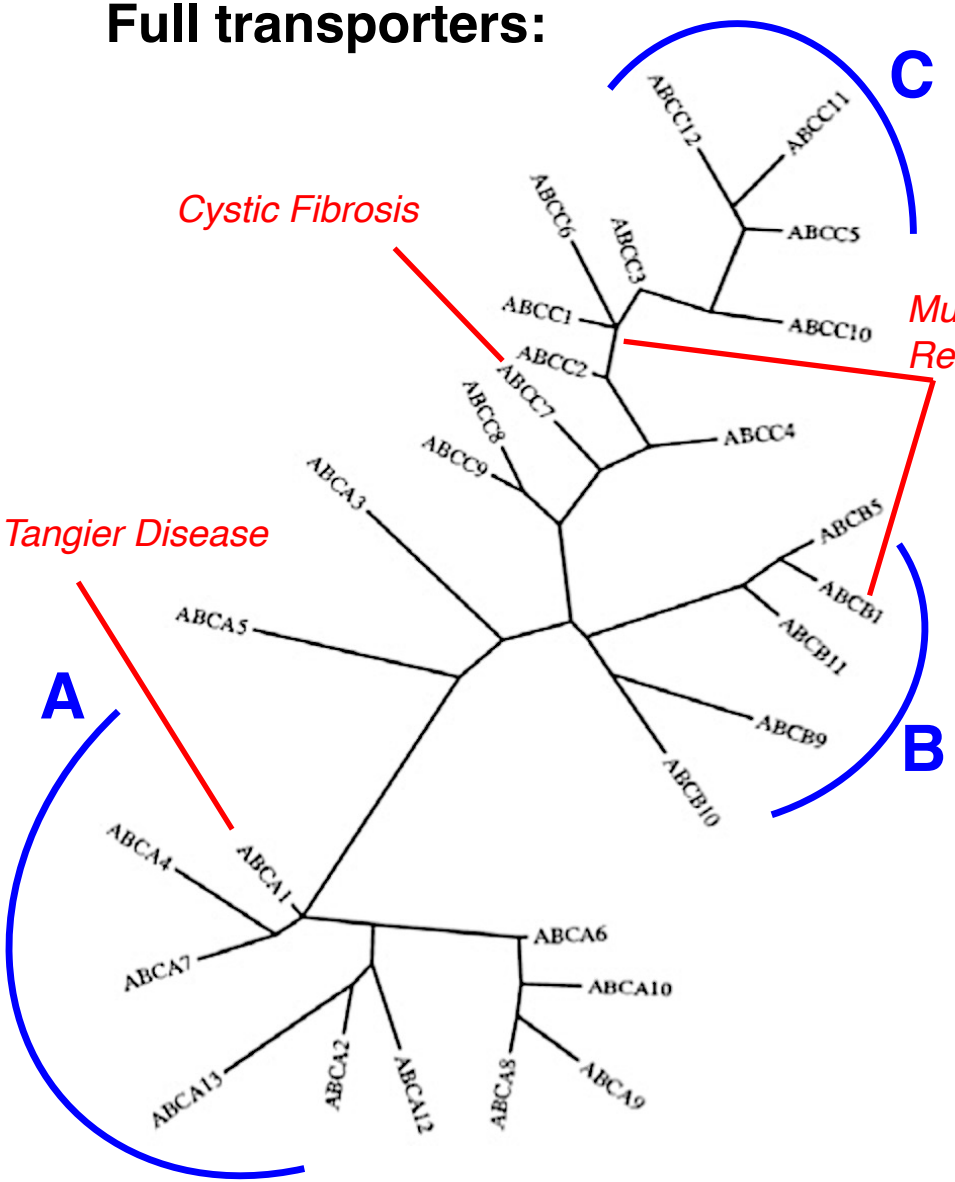
Non-transporters



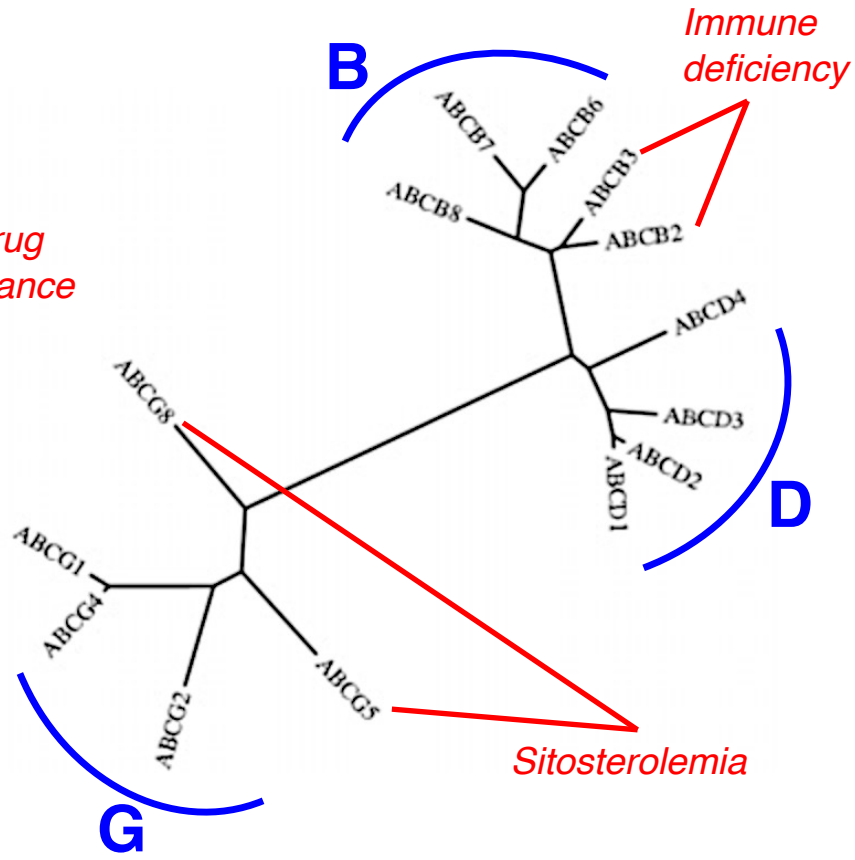
Half transporters
Homo-dimer
Hetero-dimer

ABC Transporters v.s. Human Diseases

Full transporters:

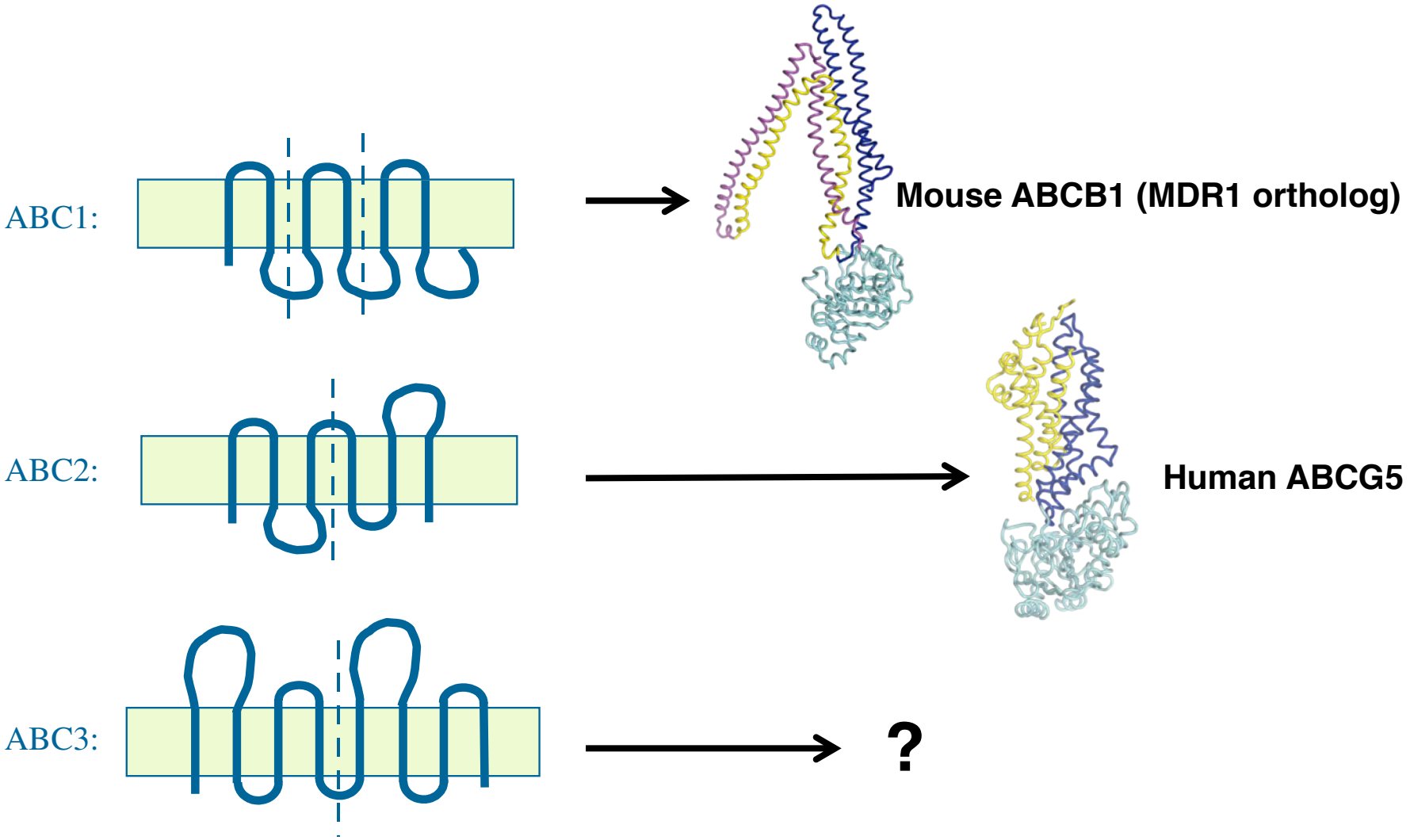


Half transporters:



(Vishwakarma et al, Int J Sci Res, 2012)

Evolutionary Origin of ABC Exporter TMD



(Wang et al, J Membr Biol, 2009)

Human ABC proteins: 48 members, 44 transporters

Transporters
- **B, C, D**
- **A, G**

Non-transporters
- **E, F**

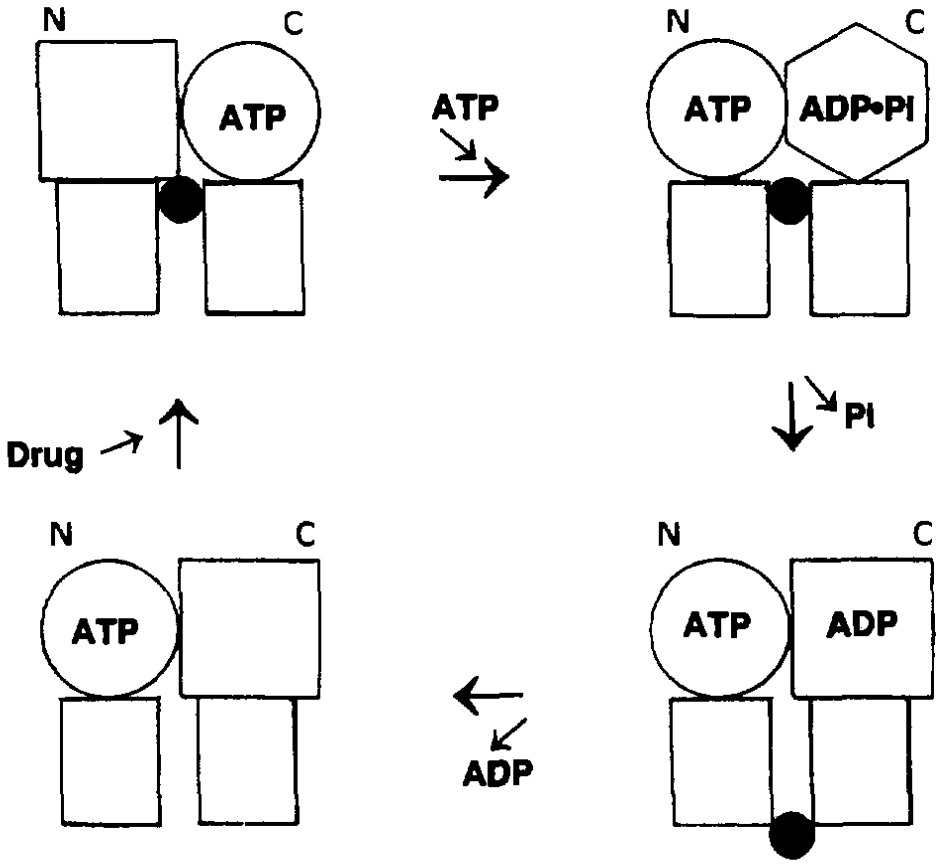
Table 1. Human ATP-binding cassette proteins.

	Subfamily	Human ABC proteins	Physiological role (known and probable)	Disease	Structure	Select citation(s)
ABC1	B: multidrug resistance, MDR, 11 members	ABCB1	Efflux of xenobiotics	Multidrug resistance	Kim and Chen 2018	Riordan et al. 1985
		ABCB2	Peptide transport associated with antigen processing	Immune deficiency	Oldham et al. 2016	Deverson et al. 1990; Monaco et al. 1990; Spies et al. 1990; Trowsdale et al. 1990
		ABCB3				
		ABCB4	Phospholipid excretion into bile	Progressive familial intrahepatic cholestasis type III		Van der Bliek et al. 1987
		ABCB5	Efflux of xenobiotics			Allikmets et al. 1996
		ABCB6	Porphyrin transport			Mitsuhashi et al. 2000
		ABCB7	Transport substrate involved in the mitochondrial iron homeostasis	X-linked sideroblastic anemia with ataxia		Savary et al. 1997
		ABCB8	Mitochondrial iron and glutathione export; efflux of xenobiotics			Allikmets et al. 1996
		ABCB9	Peptide translocation to lysosomes			
		ABCB10	Involved in heme biosynthesis		Shintre et al. 2013	Zhang et al. 2000
		ABCB11	Bile salt secretion into bile	Progressive familial intrahepatic cholestasis type II		Strautnieks et al. 1998
	C: multidrug resistance-associated protein, MRP, 12 members	ABCC1	Multispecific organic anion transport	Multidrug resistance	Martin et al. 2017*a*	Cole et al. 1992
		ABCC2	Renal and biliary elimination of organic anionic substrates	Dublin–Johnson syndrome		Büchler et al. 1996
		ABCC3	Organic anion transport			Kiuchi et al. 1998
		ABCC4	Nucleotide transport; antiviral drug efflux			Kool et al. 1997
		ABCC5	Nucleotide and glutamate conjugate transport			Jedlitschky et al. 2000; Wijnholds et al. 2000
		ABCC6	Transport of organic anions	Pseudoxanthoma elasticum		Kuss et al. 1998
		ABCC7	Epithelial chloride channel	Cystic fibrosis; congenital bilateral absence of the vas deferens	Liu et al. 2017	Riordan et al. 1989
		ABCC8	Modulation of associated potassium channels	Hyperinsulinemic hypoglycemia of infancy	Martin et al. 2017*a*	Aguilar-Bryan et al. 1995
		ABCC9		Cantu syndrome		Chutkow et al. 1996
		ABCC10	Efflux of xenobiotics			Allikmets et al. 1996
		ABCC11	Anionic hydrophobic solute transport	Resistance to anticancer and antiviral nucleoside based drugs		Lagasse and Clerc 1988
		ABCC12	Unknown			Tammur et al. 2001
	D: adrenoleukodystrophy-related protein, ALD, 4 members	ABCD1	Long and very long chain fatty acid transport	Adrenoleukodystrophy		Mosser et al. 1993
		ABCD2				Holzinger et al. 1999
		ABCD3	Branched chain fatty acid transport	Zellweger syndrome		Kamijo et al. 1990
		ABCD4	Possible role in vitamin B12 transport			Holzinger et al. 1997
ABC2	A: 12 members	ABCA1	Cholesterol and phospholipid transport	Tangier disease; familial high-density lipoprotein deficiency	Qian et al. 2017	Luciani et al. 1994
		ABCA2	Phospholipid transport			
		ABCA3	Phospholipid transport	Neonatal surfactant deficiency		Connors et al. 1997
		ABCA4	Transport of retinoid	Stargardt macular degeneration; cone–rod dystrophy		Allikmets et al. 1997
		ABCA5	Nucleotide and glutamate conjugate transport			Arnould et al. 2002
		ABCA6	Role in macrophage lipid homeostasis			Kaminski et al. 2001
		ABCA7	Phospholipid and sphingolipid transport			Kaminski et al. 2000
		ABCA8	Cholesterol and taurocholate transport			Arnould et al. 2002
		ABCA9	Role in macrophage lipid homeostasis			Piehler et al. 2002
		ABCA10	Role in macrophage lipid homeostasis			Wenzel et al. 2003
		ABCA12	Sphingolipid transport	Harlequin ichthyosis		Annilo et al. 2002
		ABCA13	Unknown			Prades et al. 2002
	G: five members	ABCG1	Cholesterol and phospholipid transport			Chen et al. 1996; Savary et al. 1996
		ABCG2	Efflux of xenobiotics	Multidrug resistance	Taylor et al. 2017	Allikmets et al. 1998; Doyle et al. 1998; Miyake et al. 1999
		ABCG4	Cholesterol transport			Annilo et al. 2001; Oldfield et al. 2002
		ABCG5 ABCG8	Cholesterol and plant sterol efflux	β-Sitosterolemia	Lee et al. 2016	Berge et al. 2000
	E: one member	ABCE1	Role in translation initiation and ribosome recycling		Preis et al. 2014; Shao et al. 2016†,‡	Wolkoff et al. 1985
	F: three members	ABCF1	Regulation of innate immune response			Richard et al. 1998
		ABCF2	Role in cell volume regulation			Allikmets et al. 1996
		ABCF3	Probable role in cell proliferation			

Note: The 48 human ABC proteins from the subfamilies ABCA–G can be classified into two gro… Physiological function and disease phenotypes were obtained from www.genecards.org and http… yeast, and rabbit homologs, respectively. …009) and Zheng et al. (2013). …d ‡, which indicate bovine,

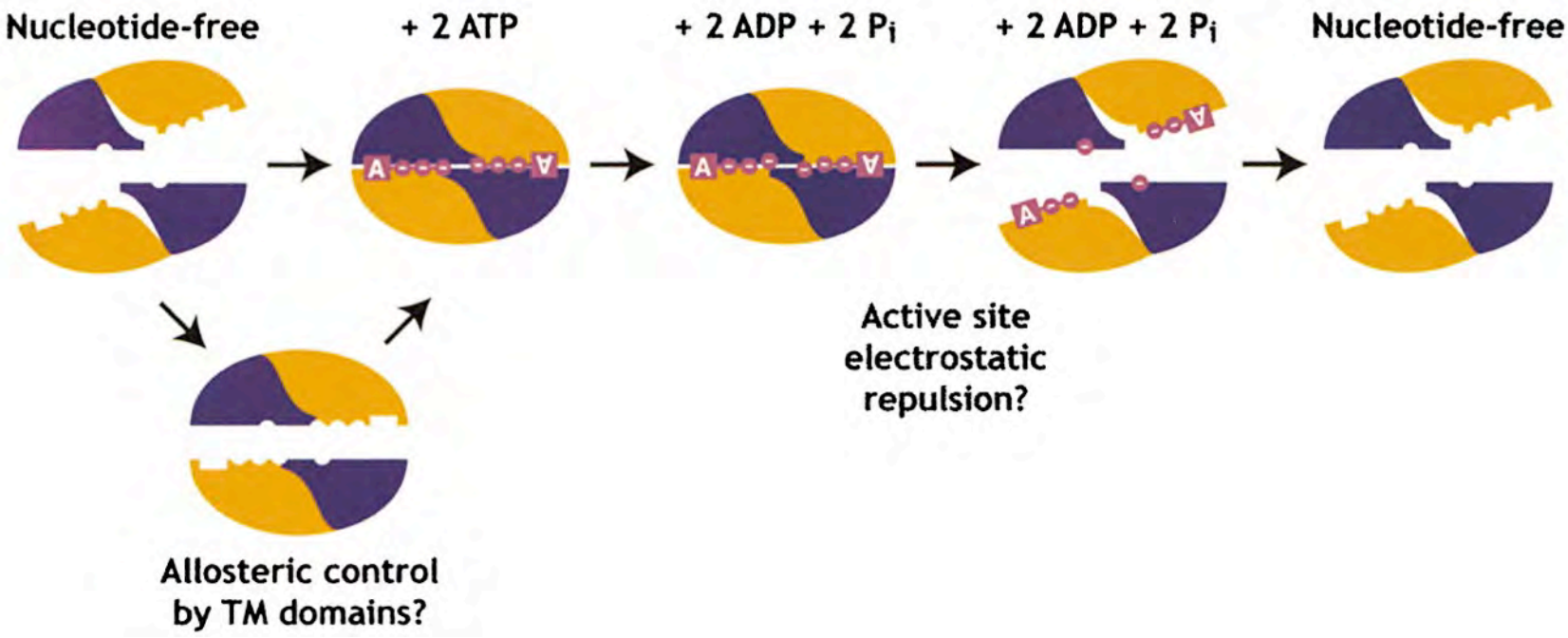
(Xavier et al, BCB, 2019)

ABC: alternating catalytic cycle



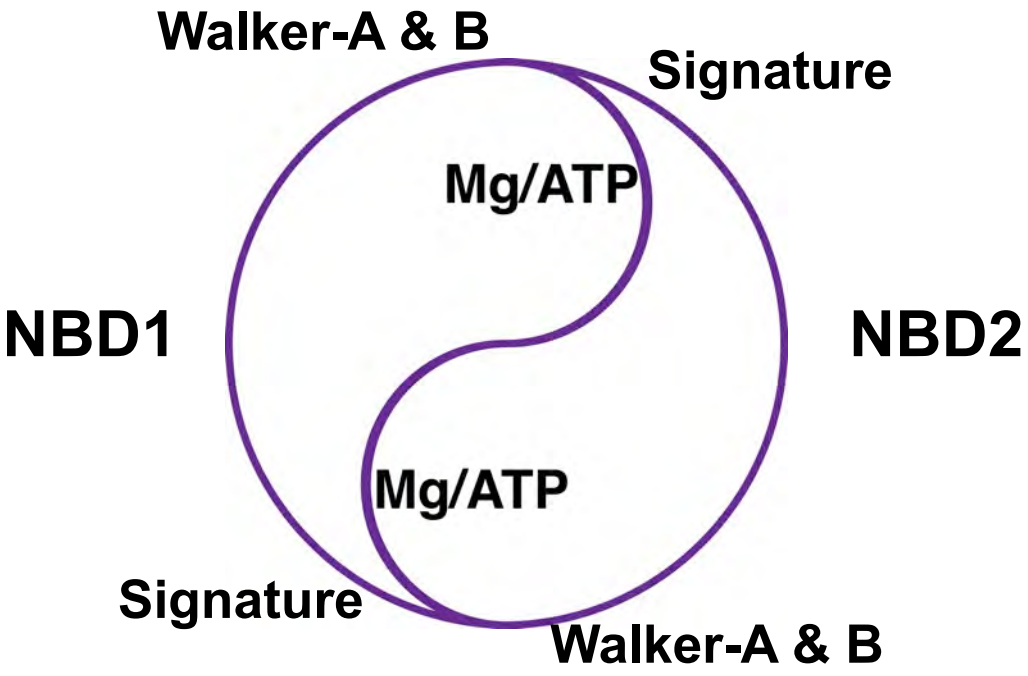
(Senior et al, FEBS Lett, 1995)

ABC: ATP sandwich model



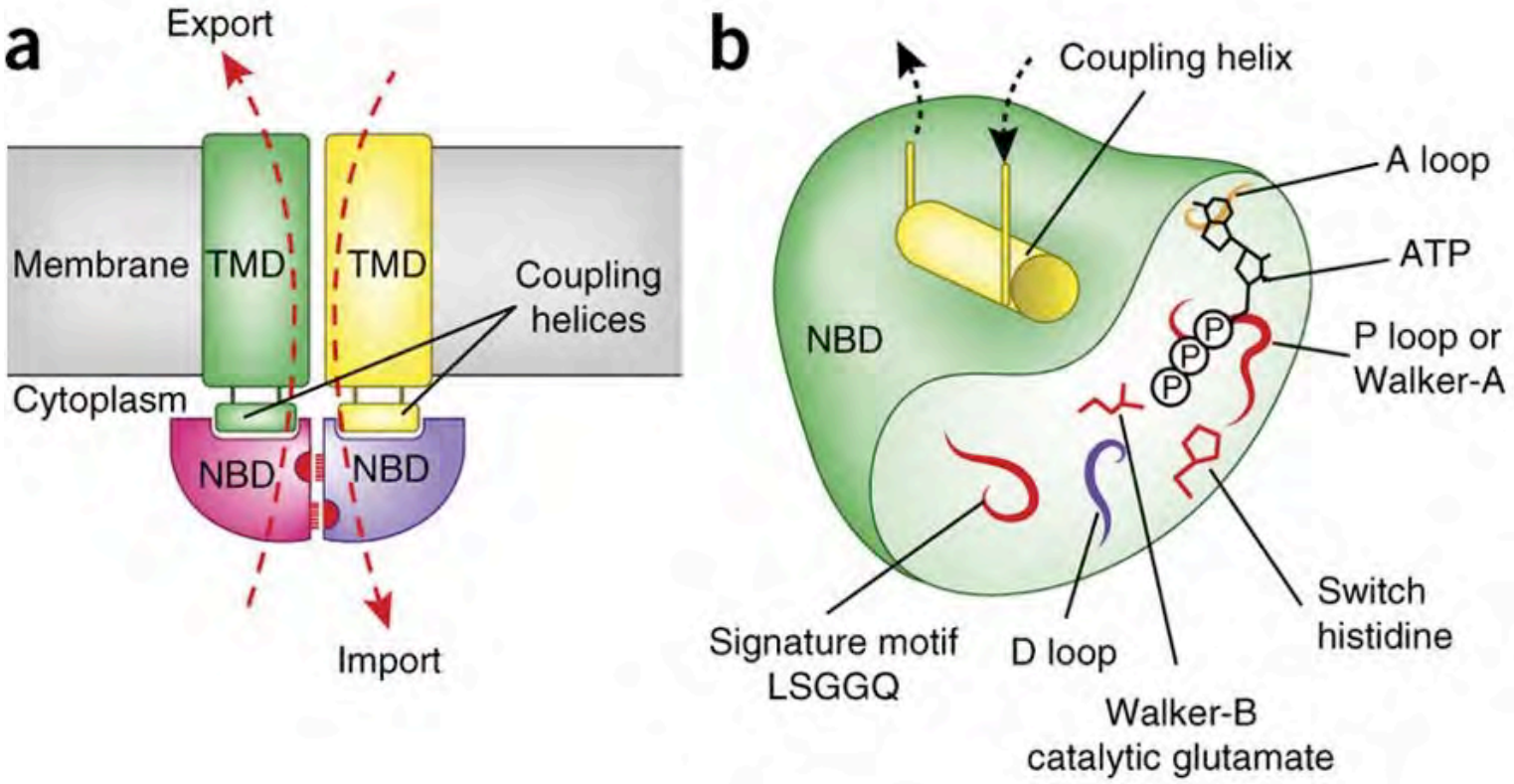
(Smith et al, Mol Cell, 2002)

ABC coupled transport: a simple idea



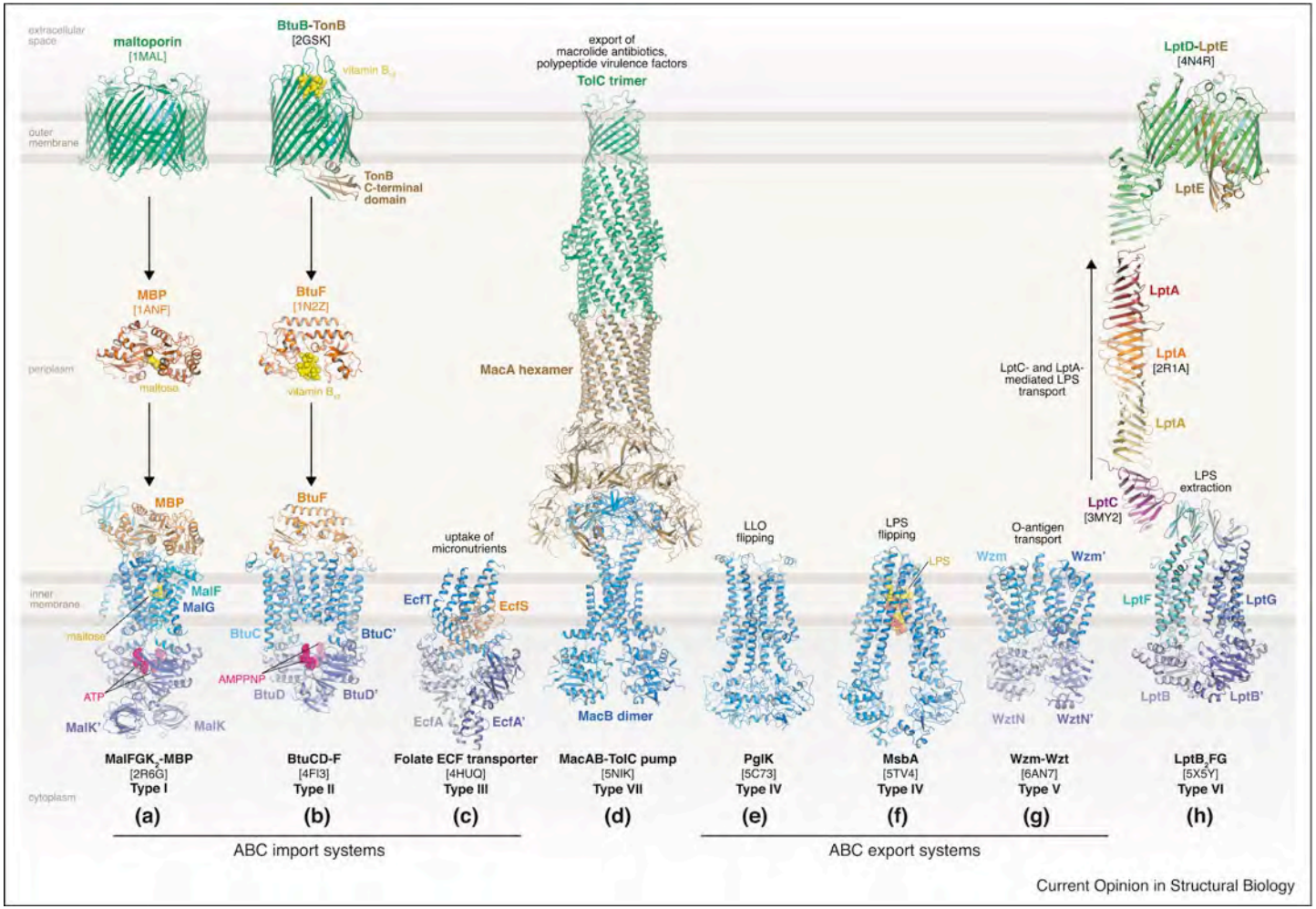
ATP-binding cassette (ABC)

ABC coupled transport: a simple idea



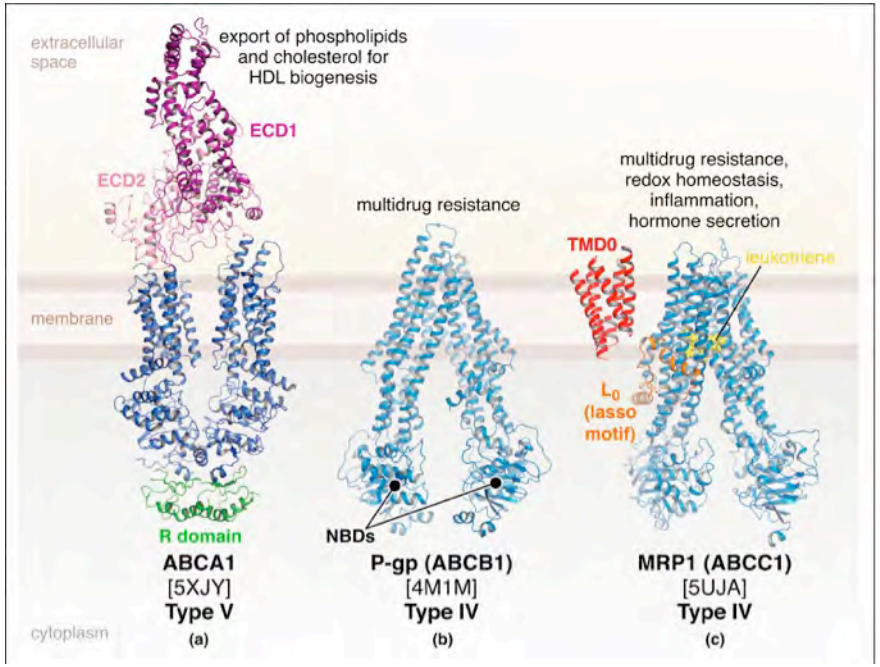
(Locher, Nat Struct Mol Biol, 2016)

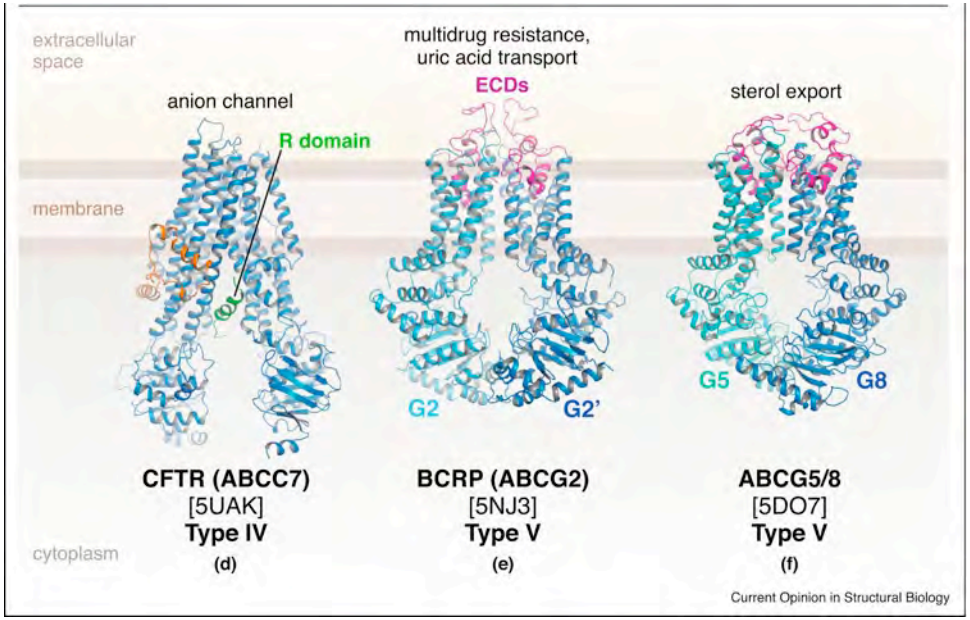
Structural diversity: bacterial point of view



(Thomas & Tampé, Curr Opin Struct Biol, 2018)

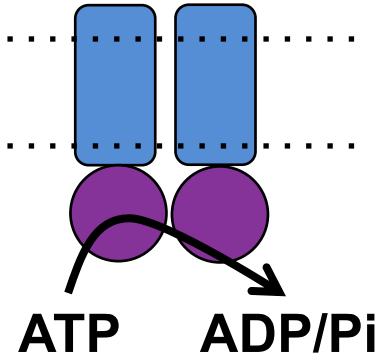
Structural diversity: mammalian point of view



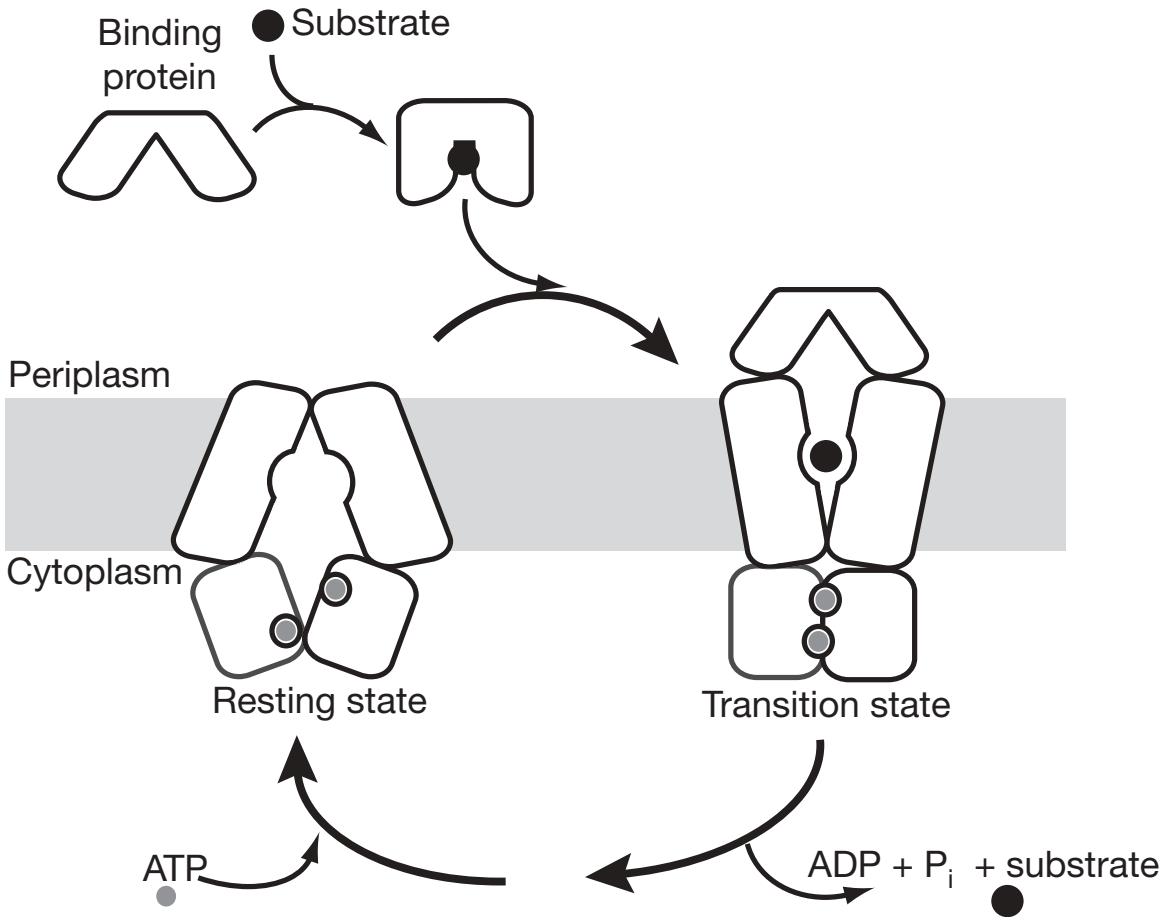


(Thomas & Tampé, Curr Opin Struct Biol, 2018)

ABC and ATP usage are part of story!

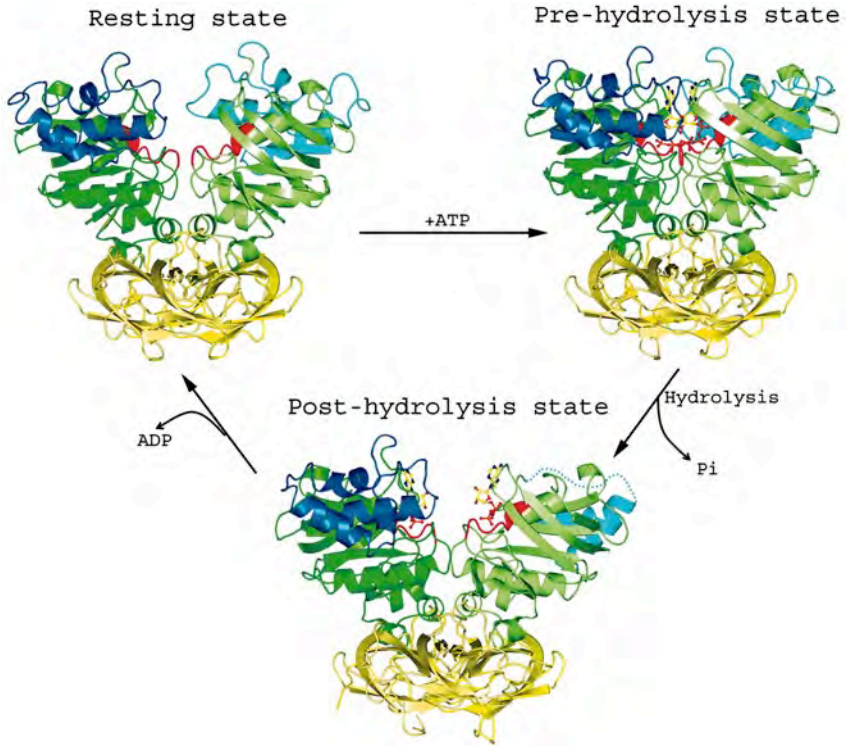


Maltose transporter: a bacterial importer

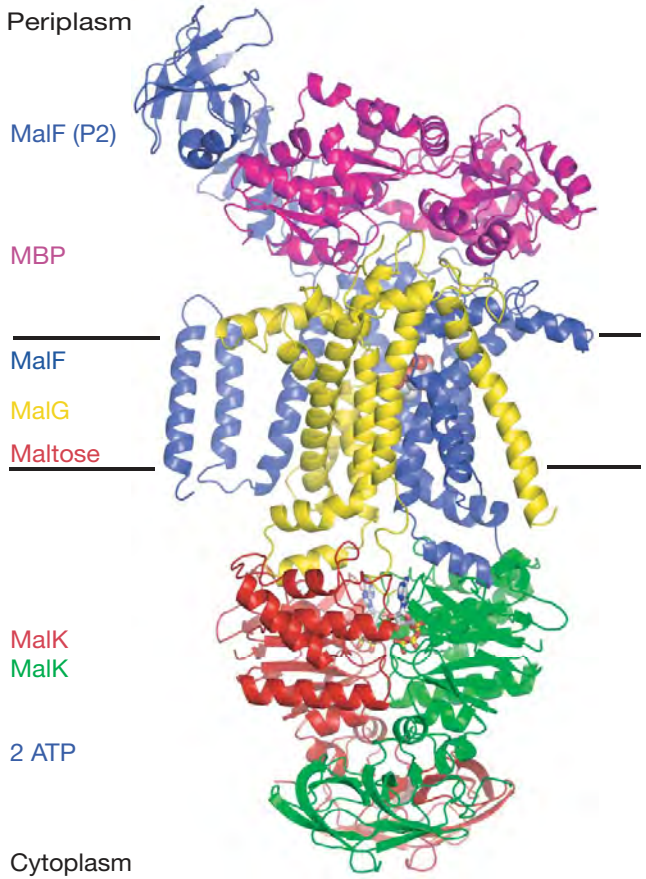


(Olham et al, Nature, 2007)

Maltose transporter: a bacterial importer



(Yu et al, PNAS, 2005)



(Olham et al, Nature, 2007)

Maltose transporter: ATP binding

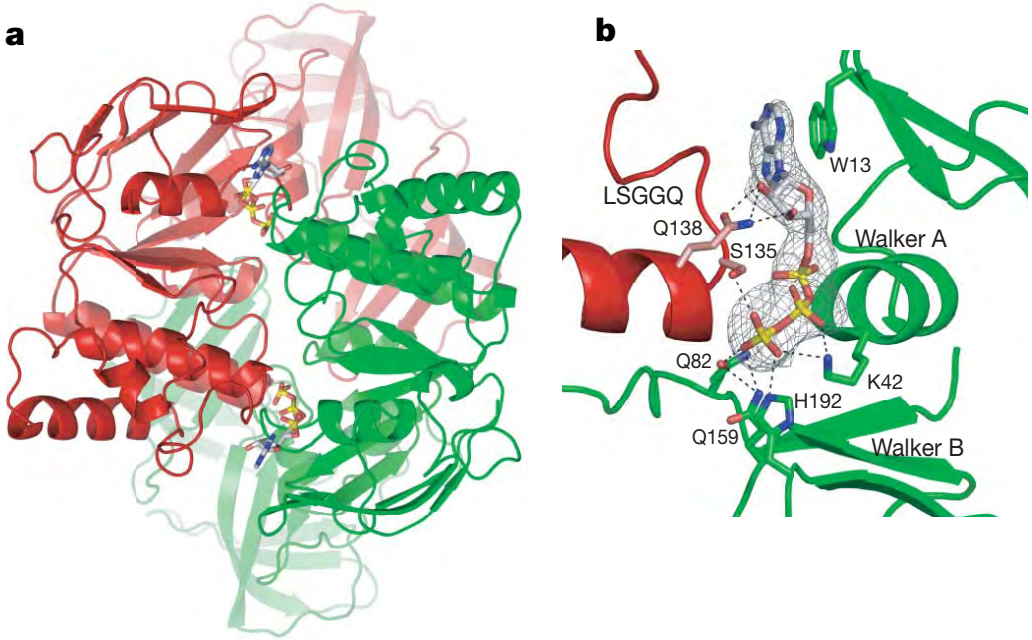
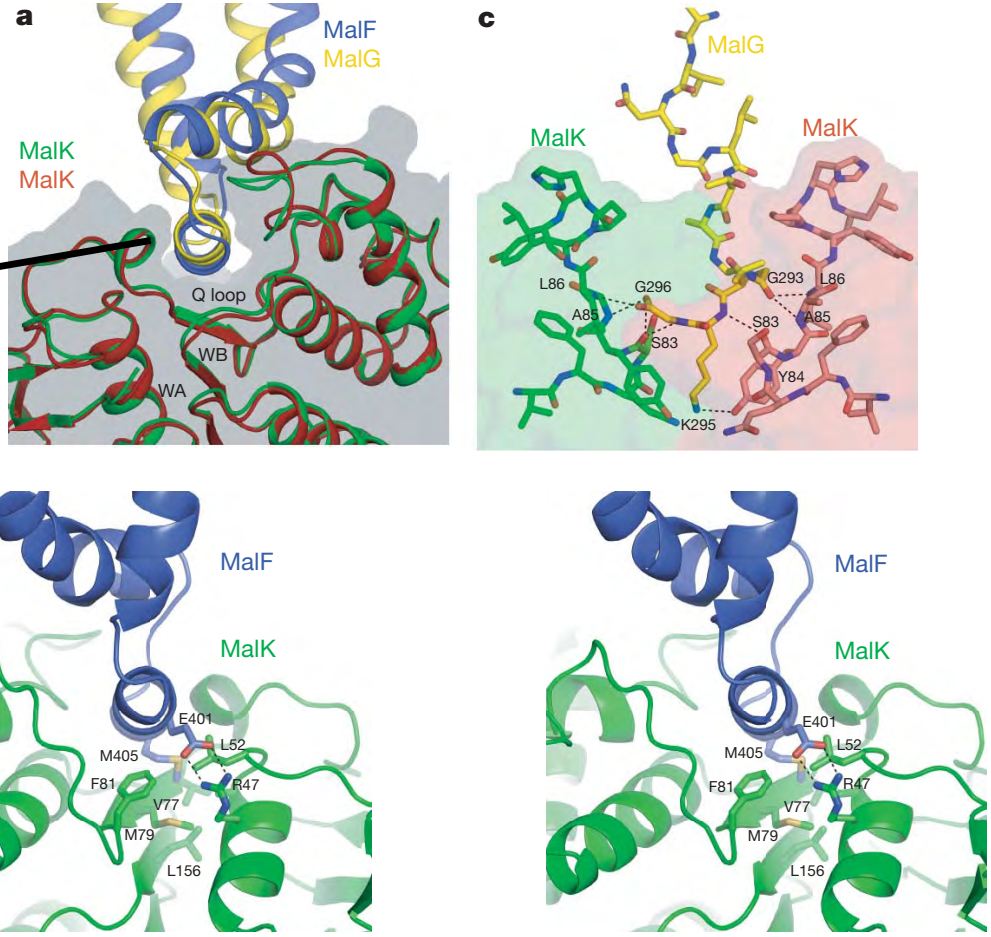


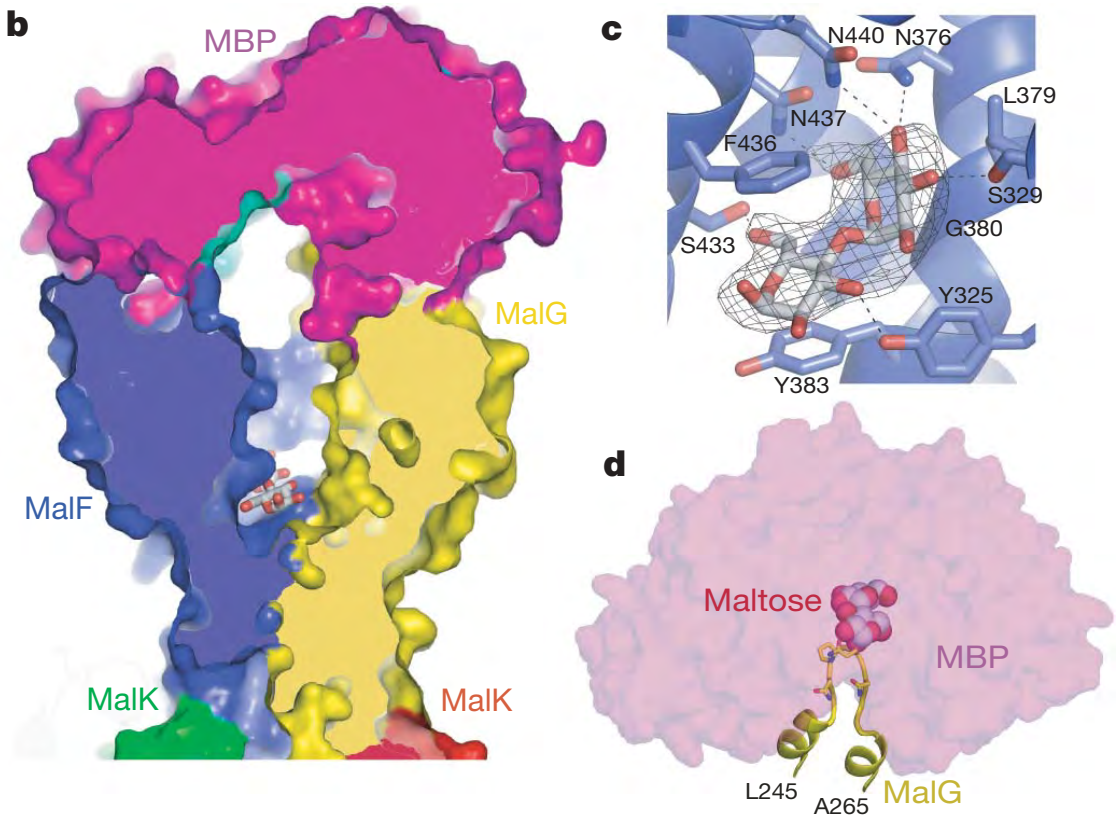
Figure 2 | Ribbon diagram of the MalK subunits with bound ATP. **a**, The MalK dimer viewed down the local twofold axis. The two subunits are coloured in red and green. The ATP is represented in ball-and-stick model. **b**, The ATP-binding site, showing that residues from both MalK subunits are making contact with the ATP. Interacting residues and associated hydrogen bonds (black dashed lines) from residues in the Walker A, Walker B and LSGGQ motifs, and from H192 and Q159, to ATP are indicated. A positive $F_o - F_c$ electron density (contoured at 1.5σ) obtained with ATP omitted in the structure factor calculation is also shown.

Maltose transporter: first molecular view of TMD-NBD interaction

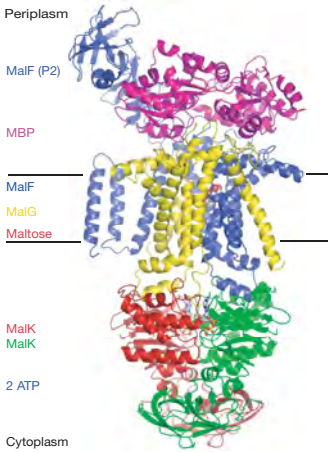
Coupling helix

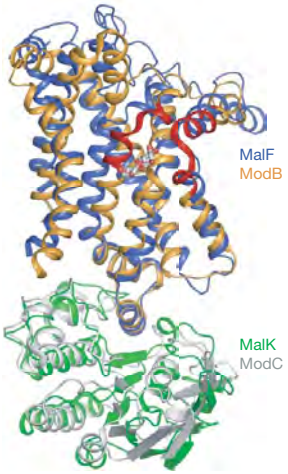


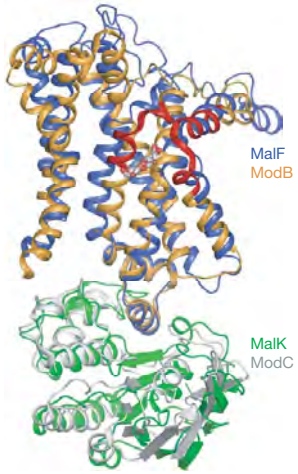
Maltose transporter: transport-substrate binding

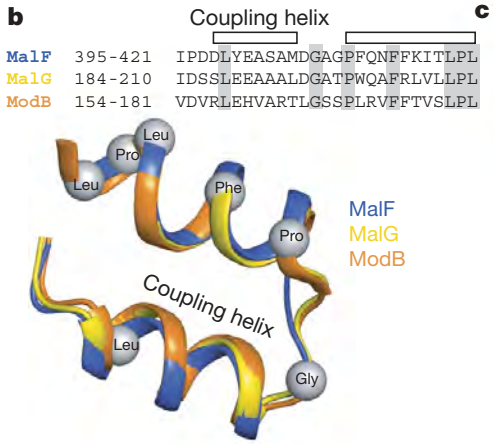


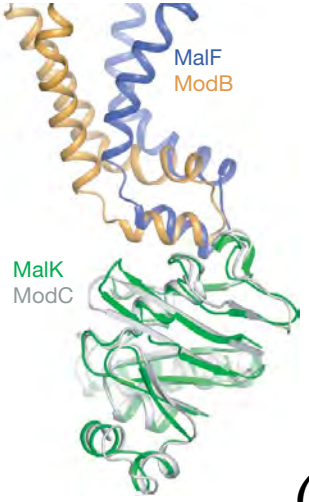
Maltose transporter v.s. other importer(s)

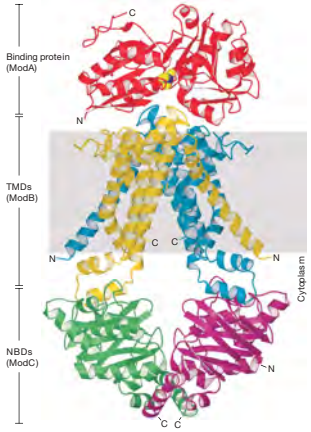








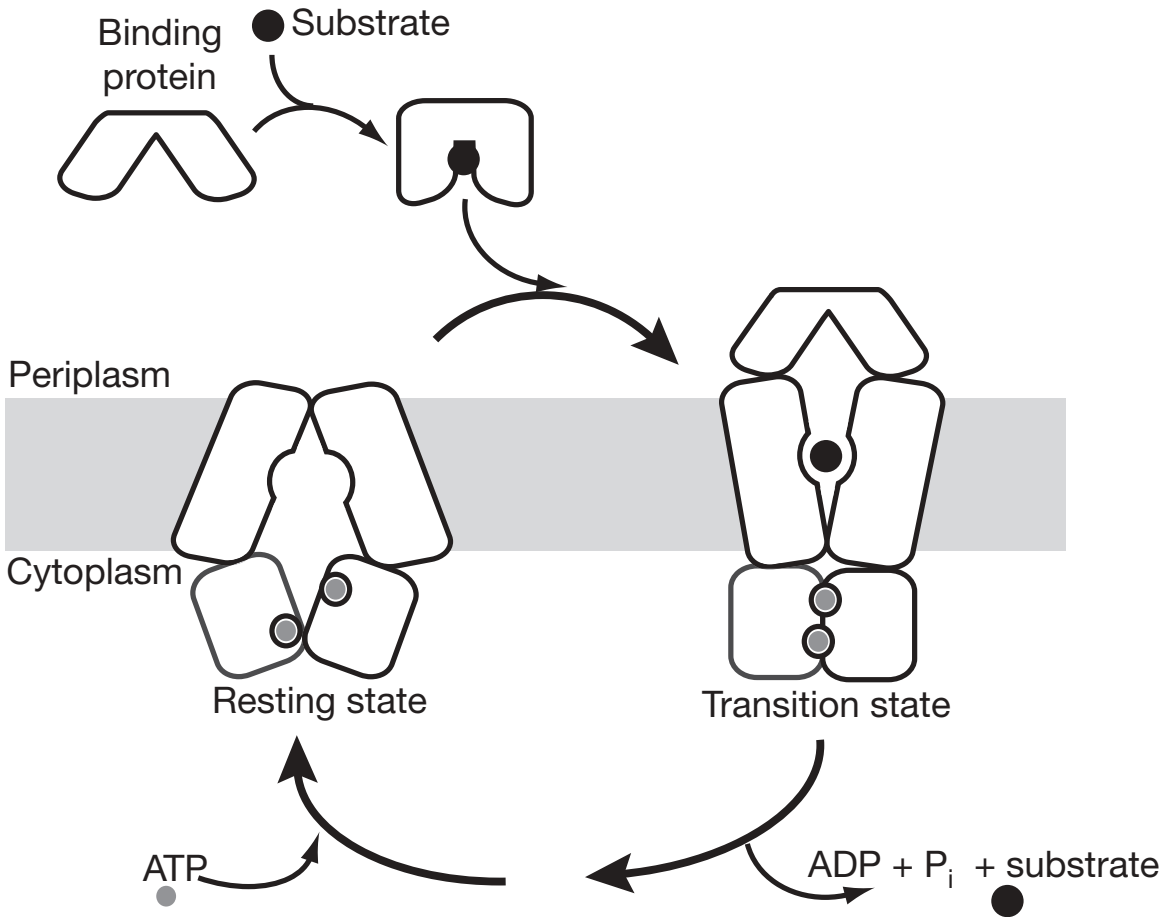




(Olham et al, Nature, 2007)

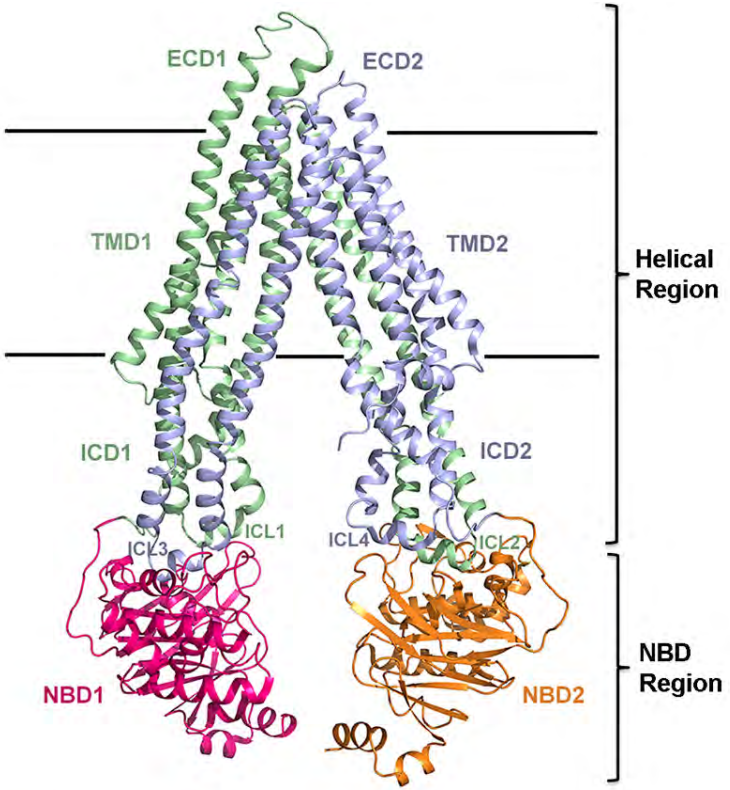
(Hollenstein et al, Nature, 2007)

Maltose transporter: a bacterial importer

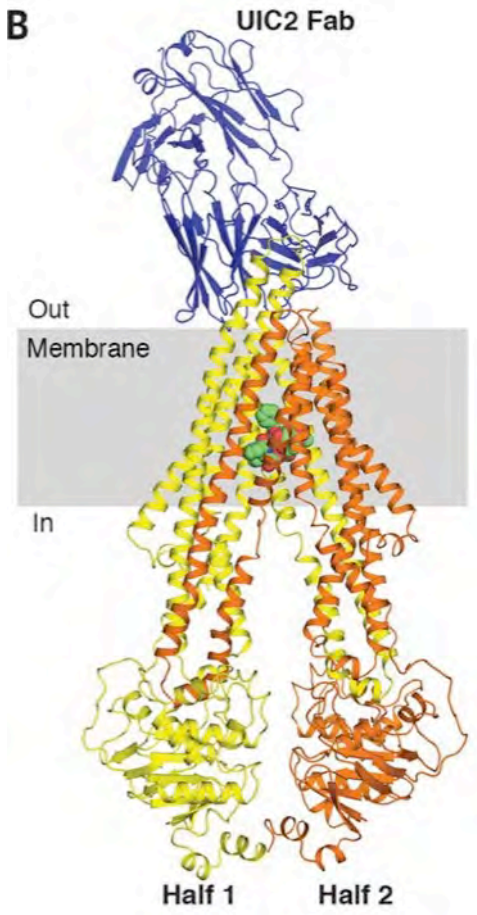


(Olham et al, Nature, 2007)

P-glycoprotein: a multidrug-resistance efflux pump

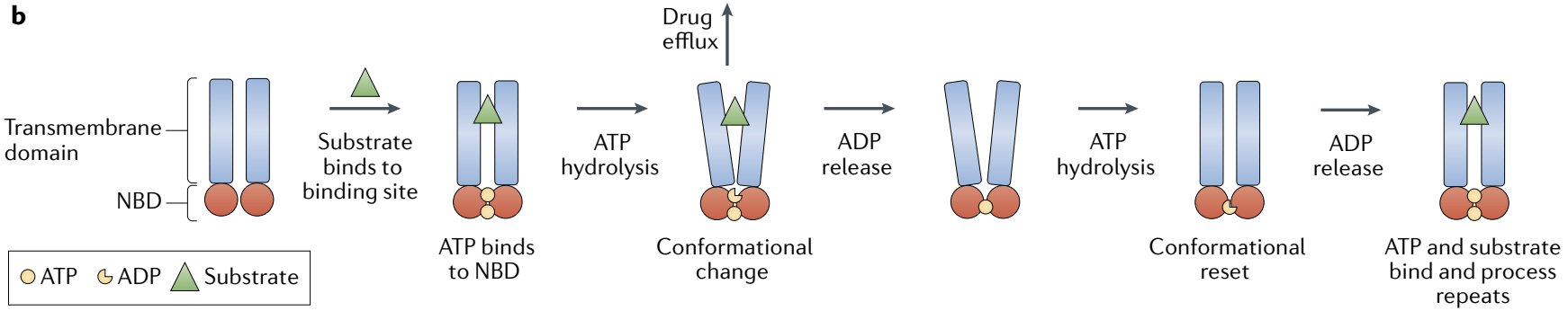


(Esser et al, JBC, 2017)



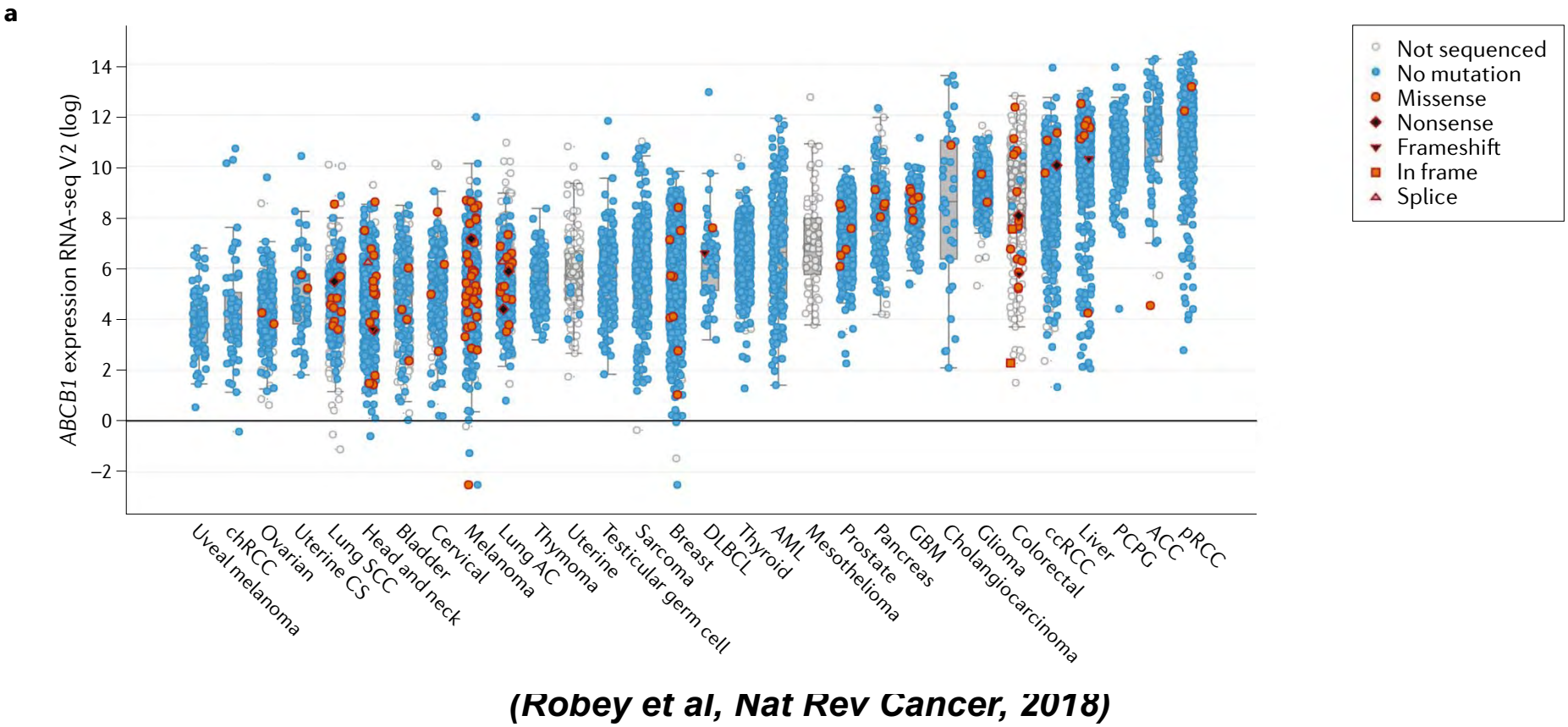
(Alam et al, Science, 2019)

P-glycoprotein: a multidrug-resistance efflux pump



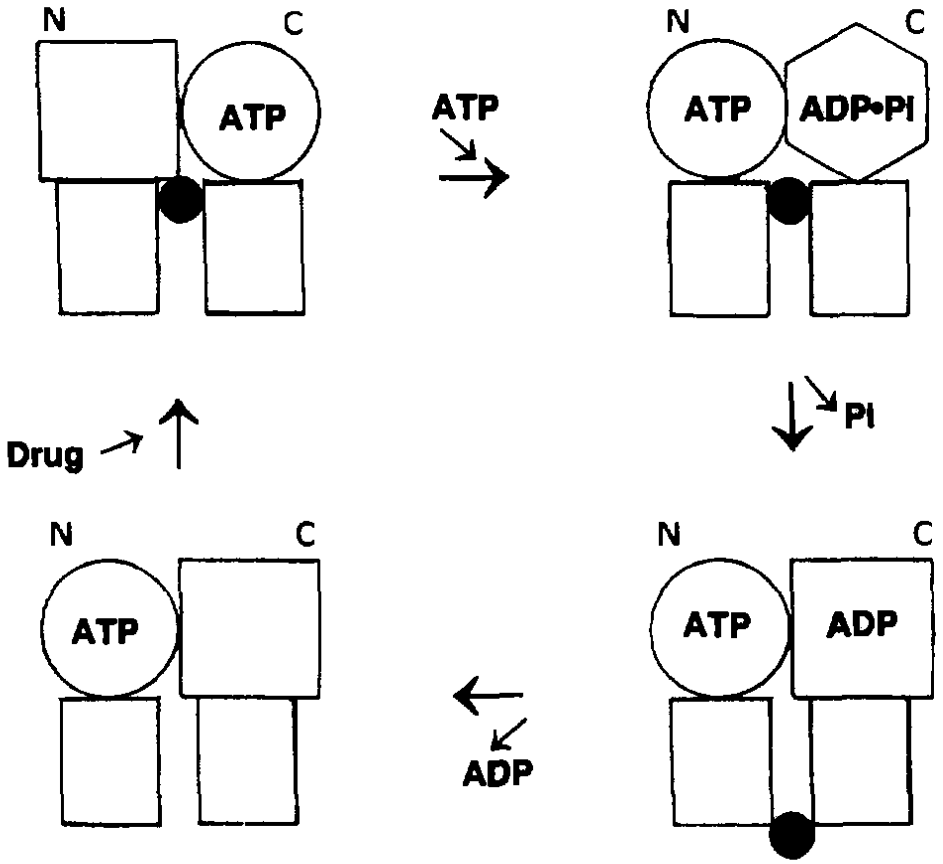
(Robey et al, Nat Rev Cancer, 2018)

P-glycoprotein: high expression in cancer cells (cBioPortal + TCGA)



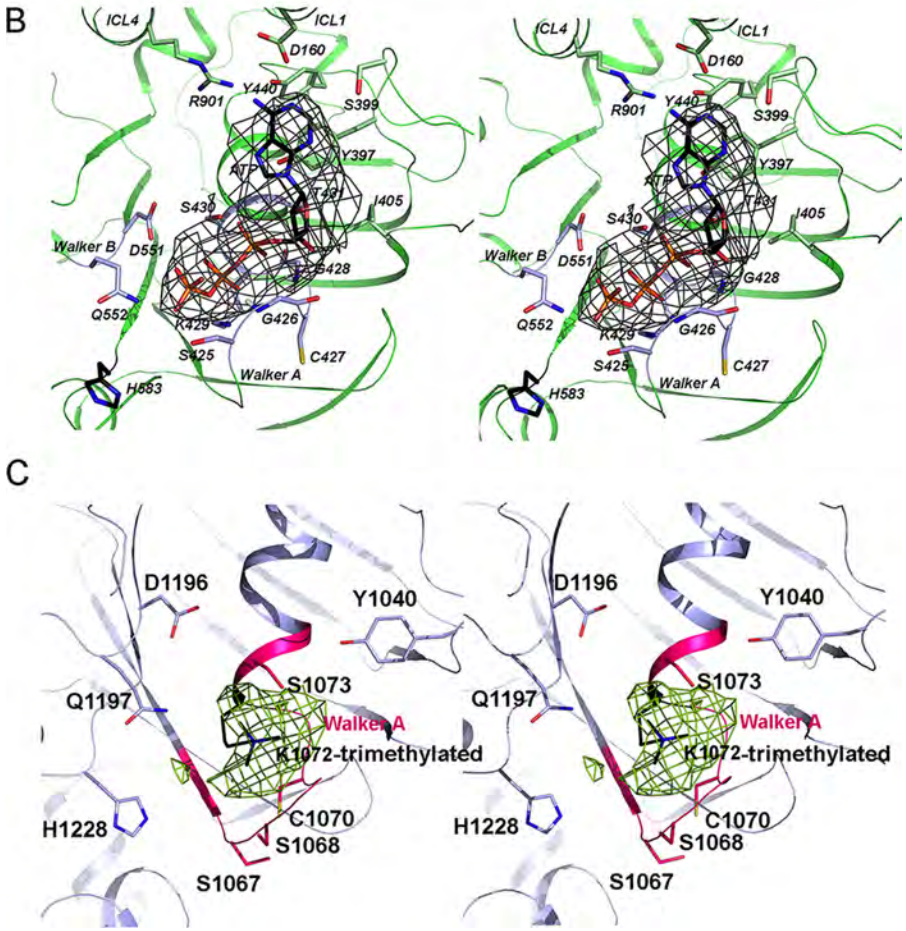
(Robey et al, Nat Rev Cancer, 2018)

ABC: alternating catalytic cycle



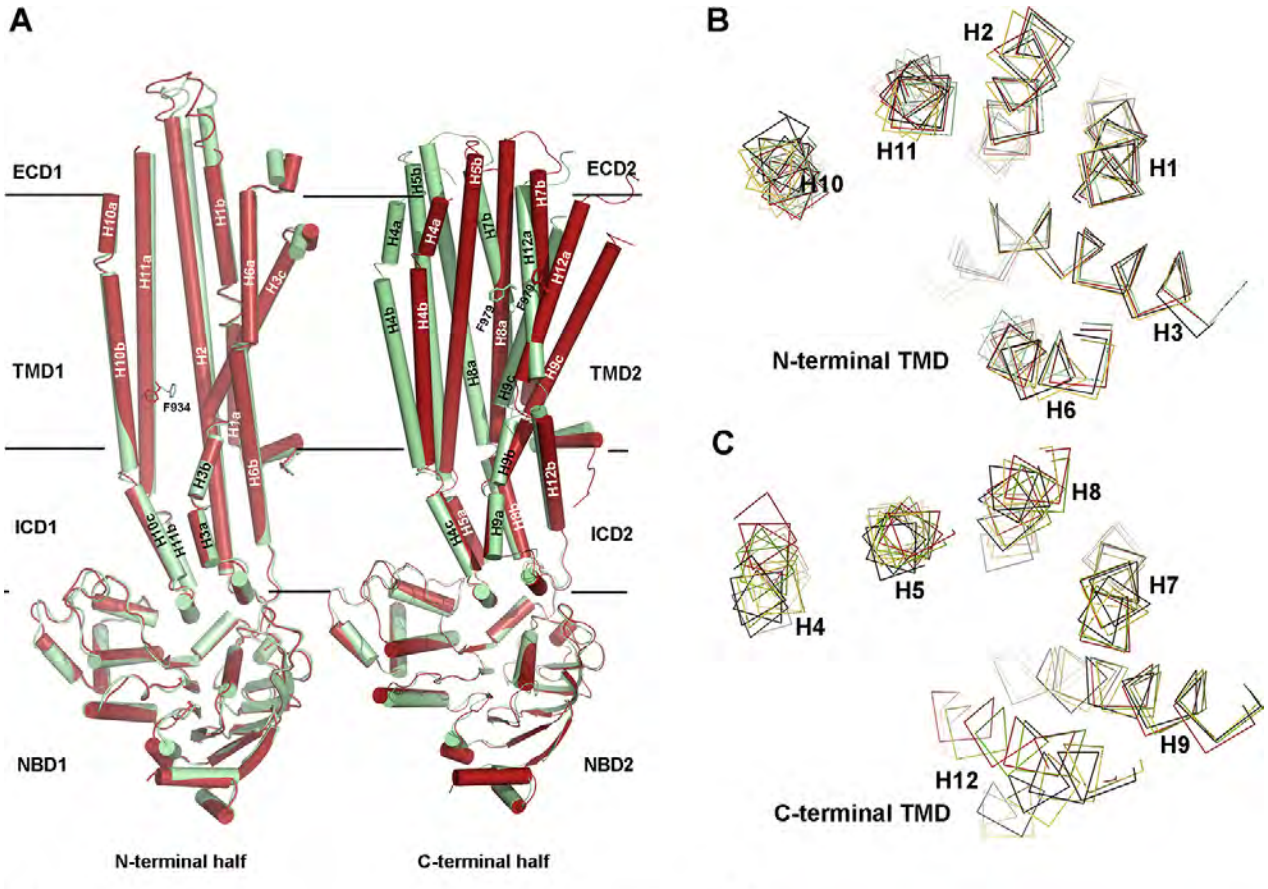
(Senior et al, FEBS Lett, 1995)

P-glycoprotein: asymmetric ATP binding



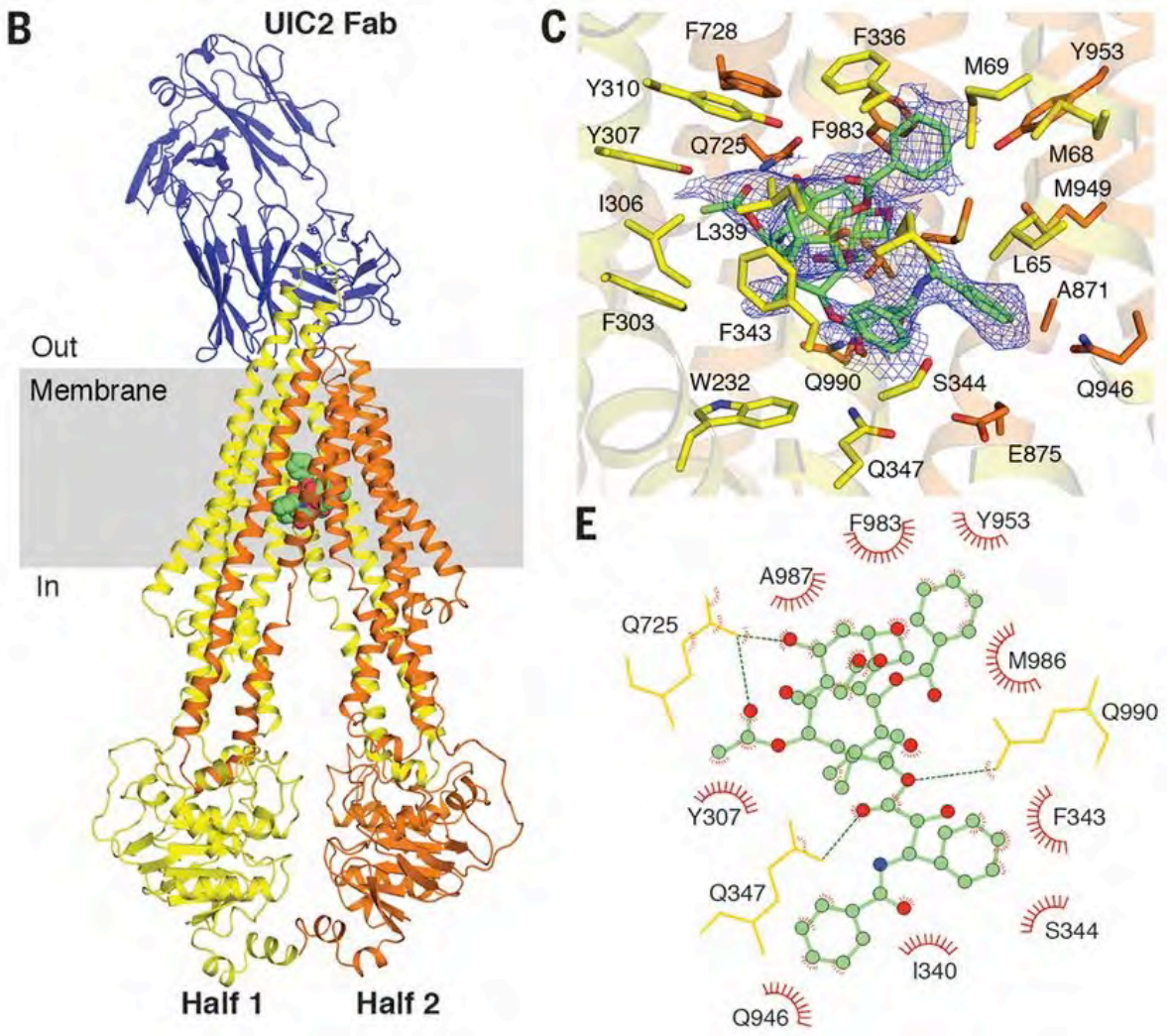
(Esser et al, JBC, 2017)

P-glycoprotein: flexible TMD



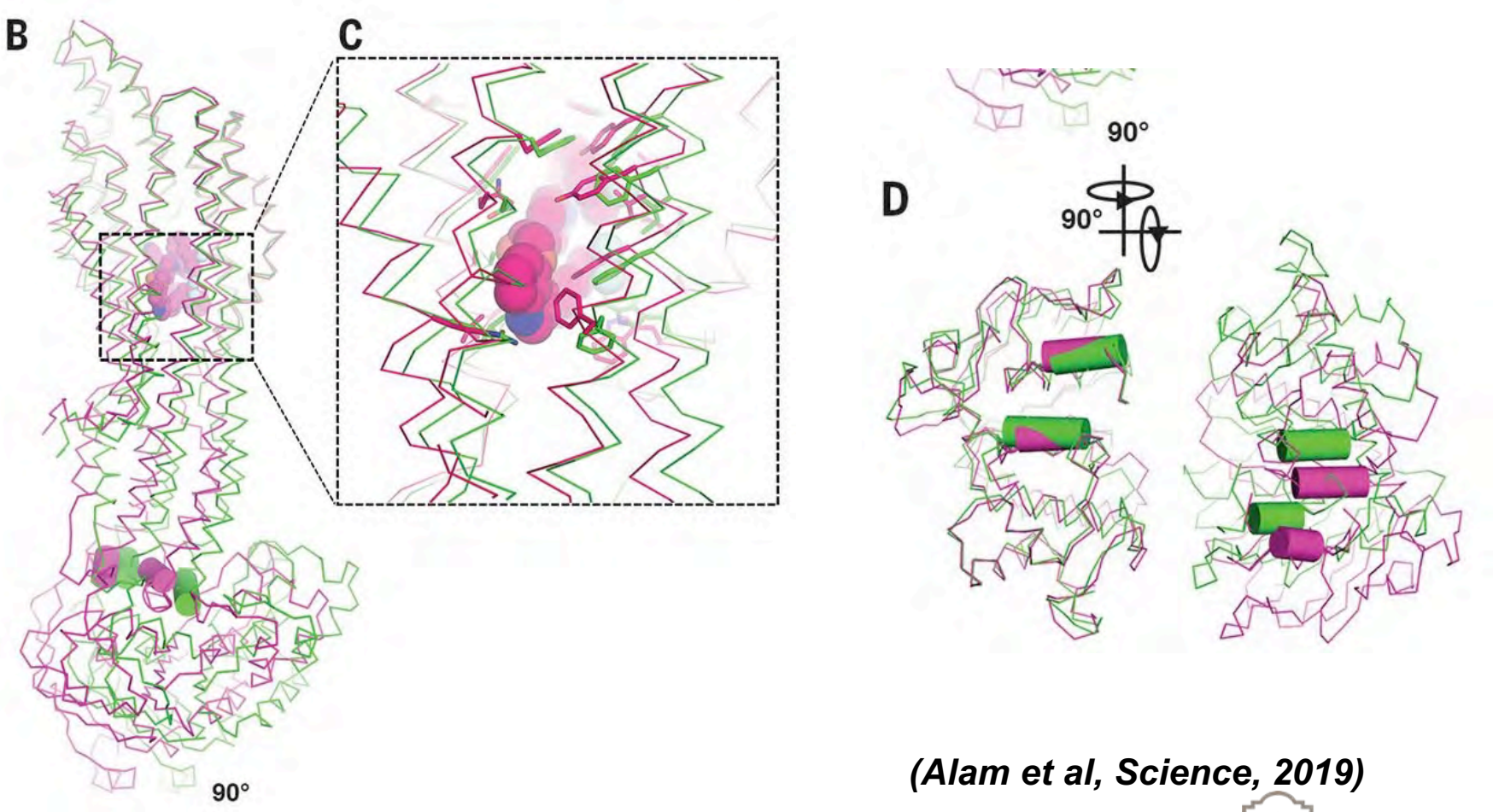
(Esser et al, JBC, 2017)

P-glycoprotein: inhibitor v.s. drug (Taxol)



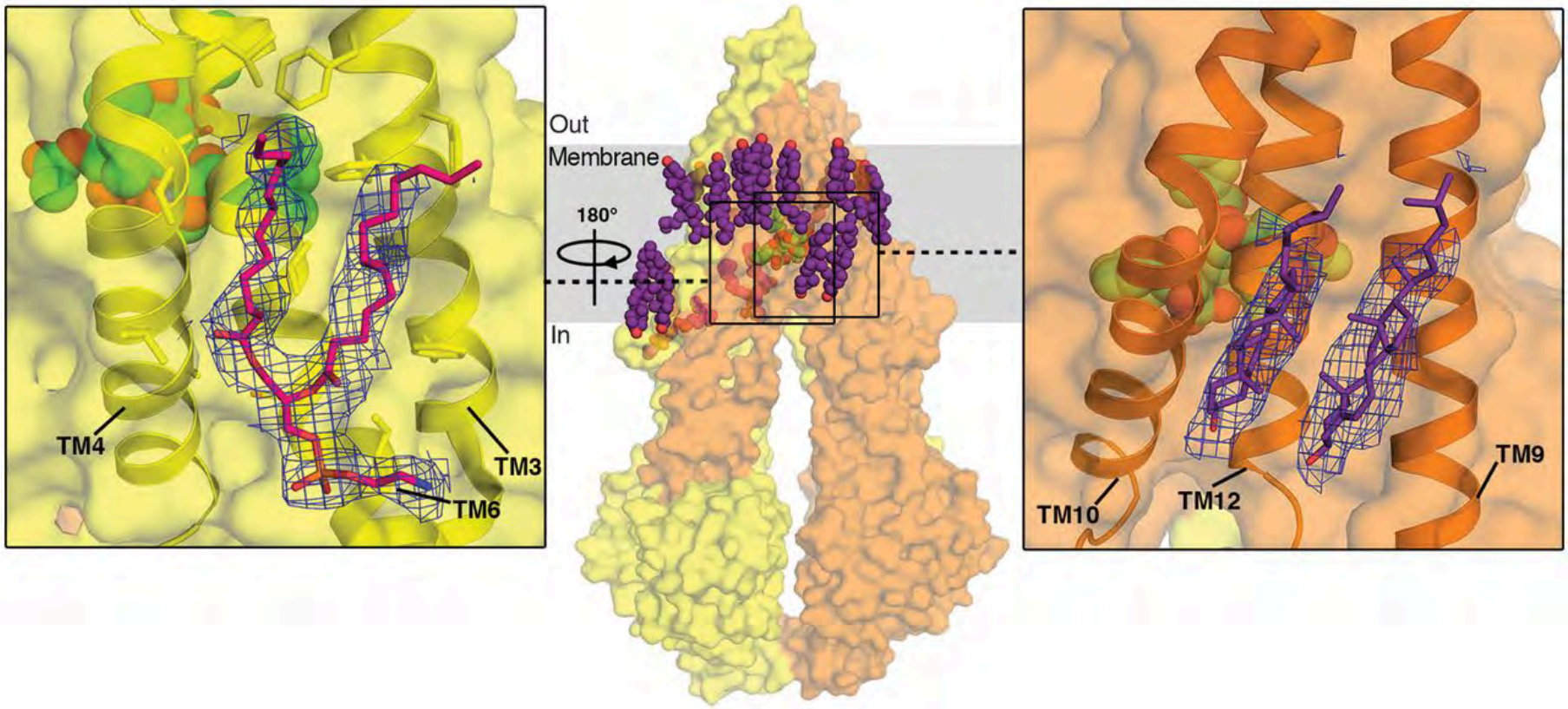
(Alam et al, Science, 2019)

P-glycoprotein: transport substrates affect ATP usage



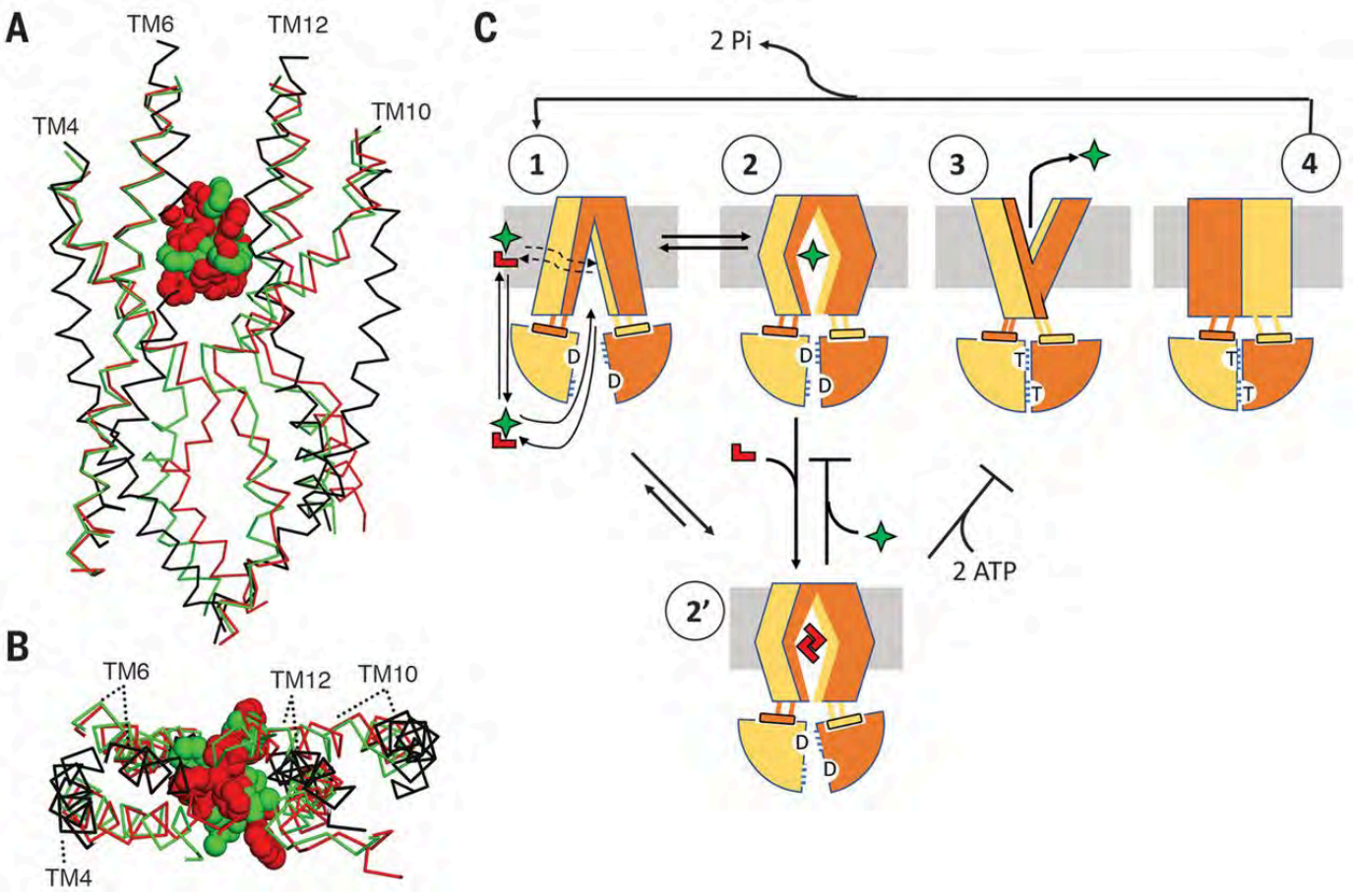
(Alam et al, Science, 2019)

P-glycoprotein: lipid-protein interaction



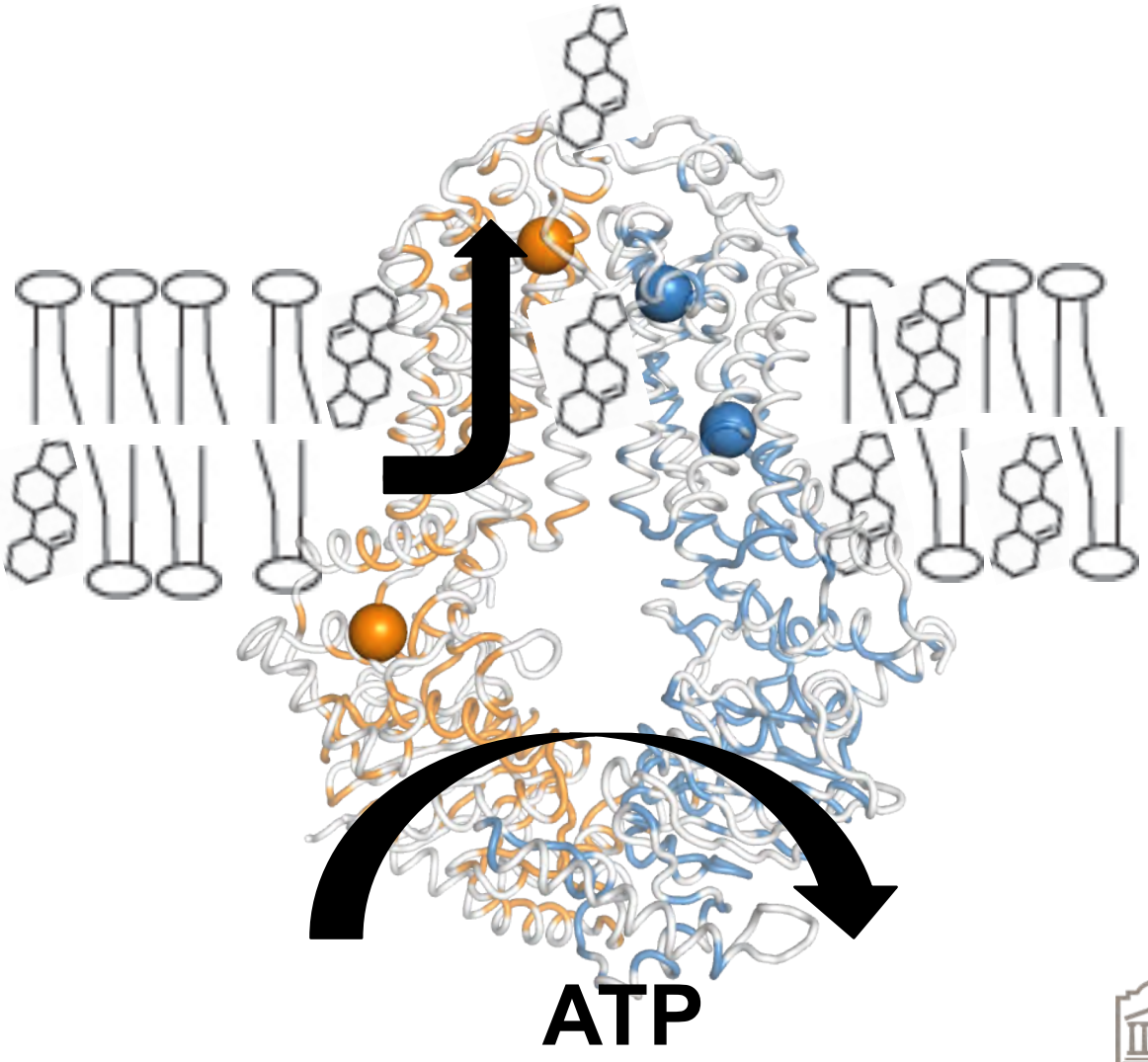
(Alam et al, Science, 2019)

P-glycoprotein: multidrug resistance?



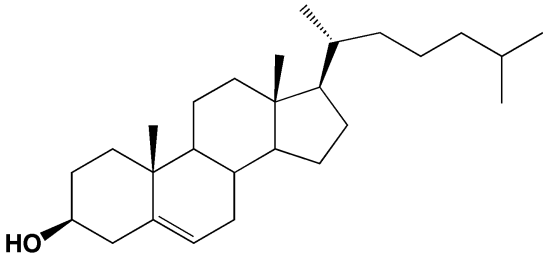
(Alam et al, Science, 2019)

ABCG5/G8: a sterol/cholesterol eflfux pump



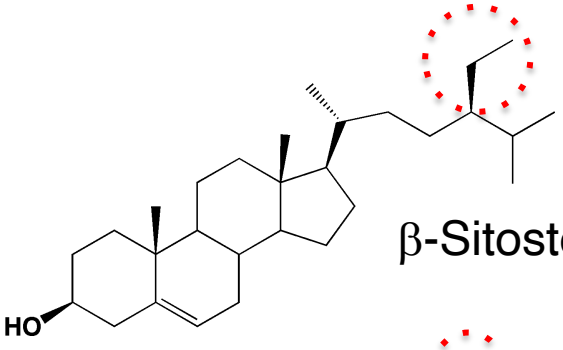
Dietary Sterols

Animal (60%)

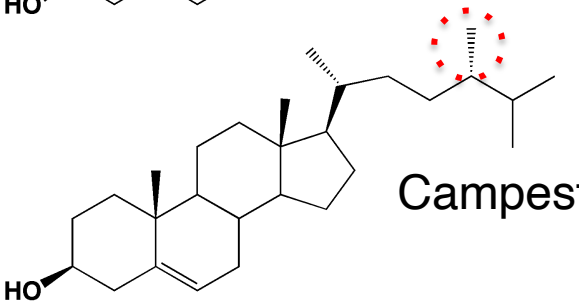


Cholesterol

Plant (40%)



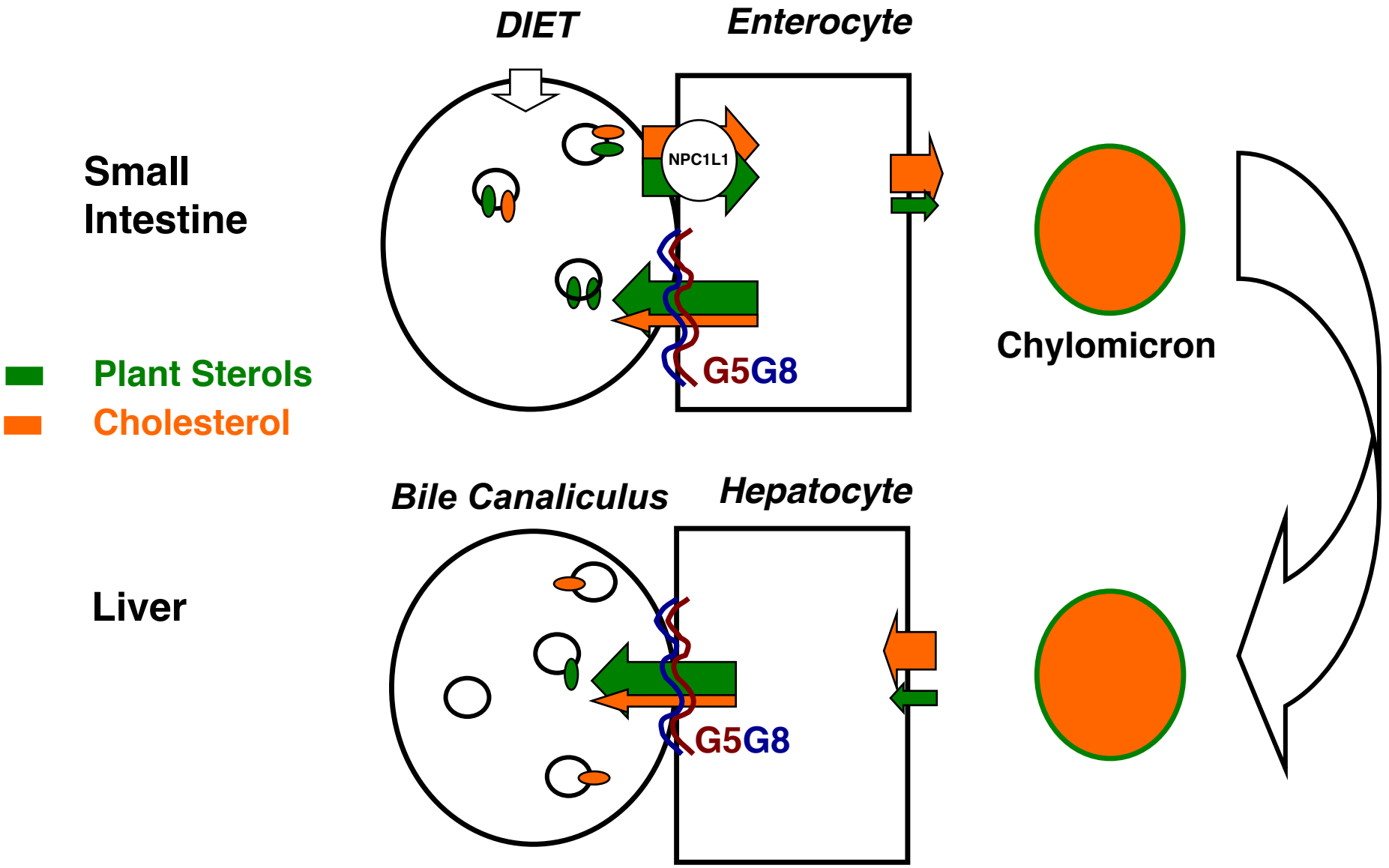
β-Sitosterol



Campesterol

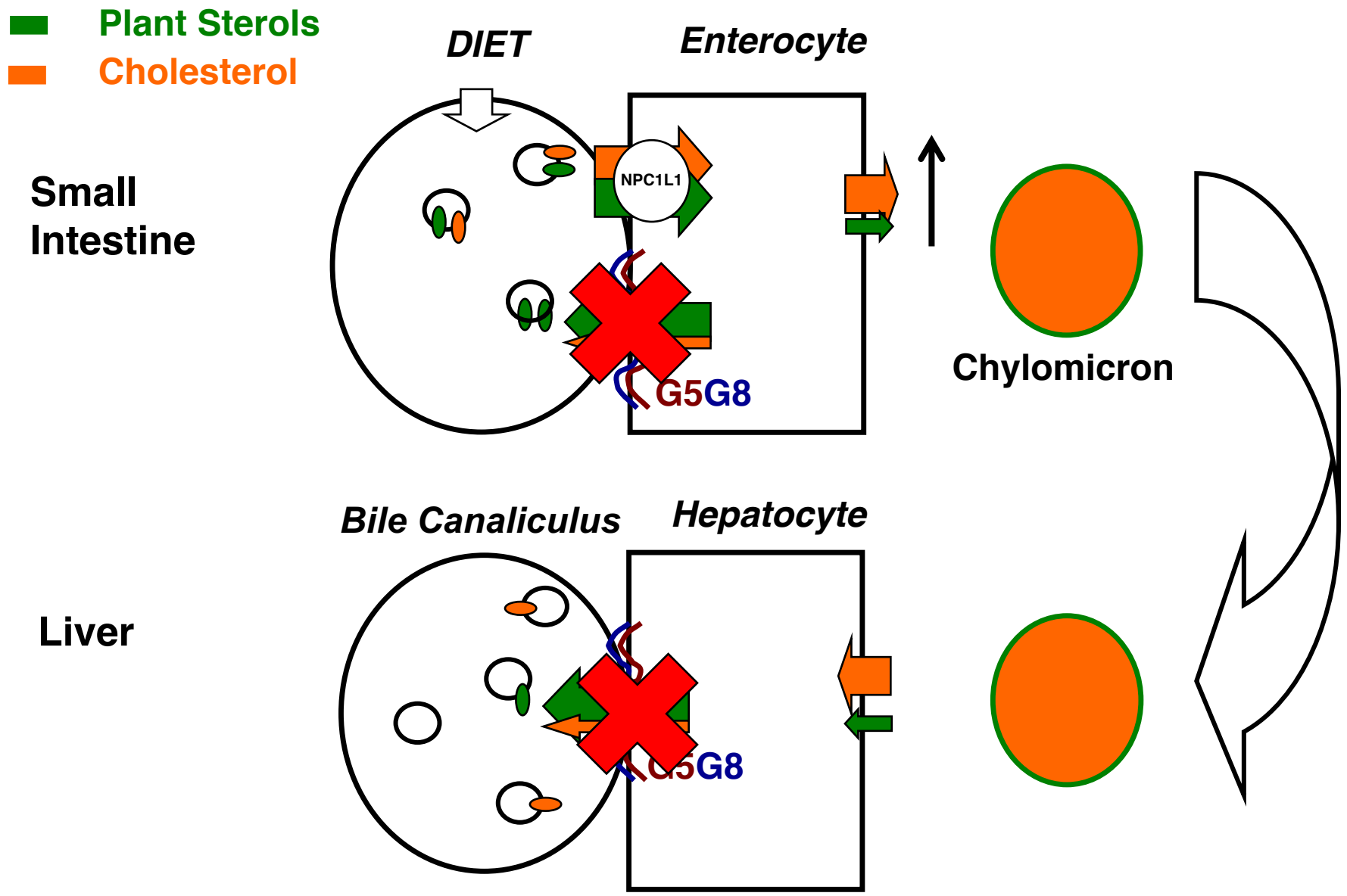
ABSORPTION: ~50% | < 5%

ABCG5/G8 promotes biliary and intestinal sterol secretion (liver/small intestine specific).



G5G8 protects against plant sterol accumulation.

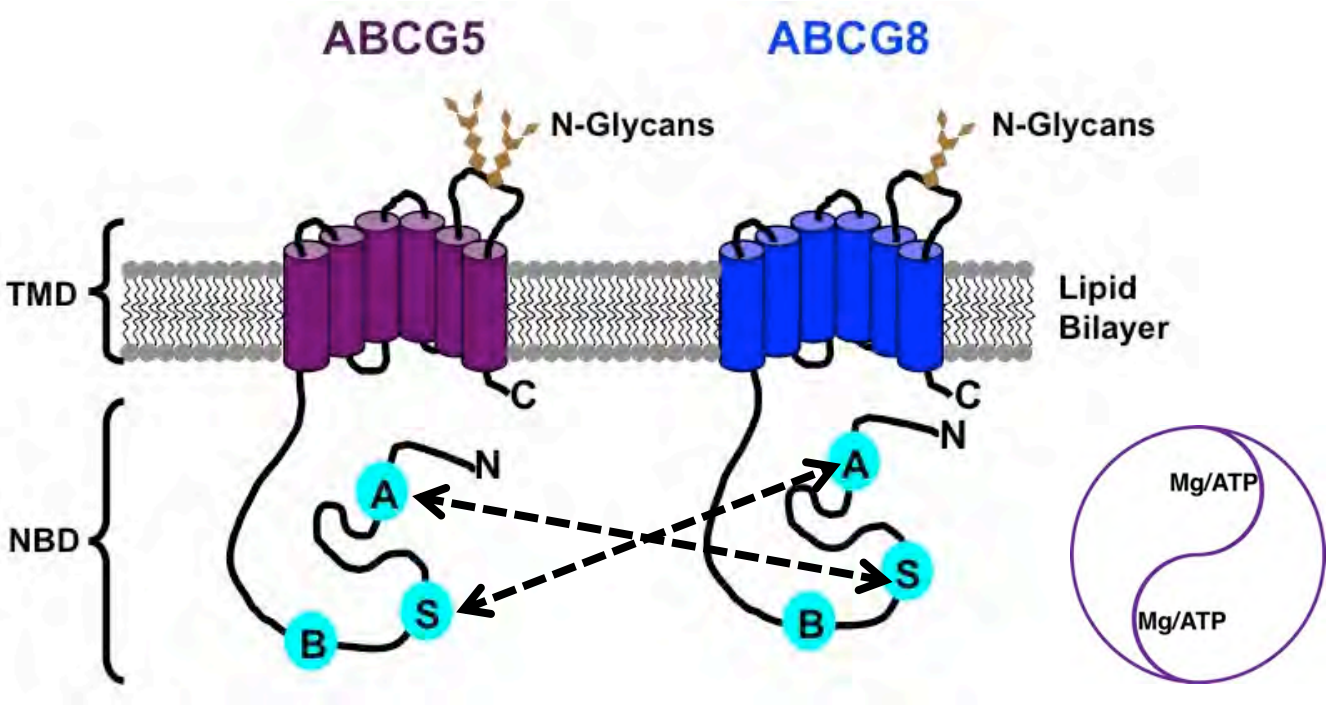
(Sitosterolemia)



ABCG5 and ABCG8 are half ABC transporters.

Transmembrane domain

Nucleotide-binding domain

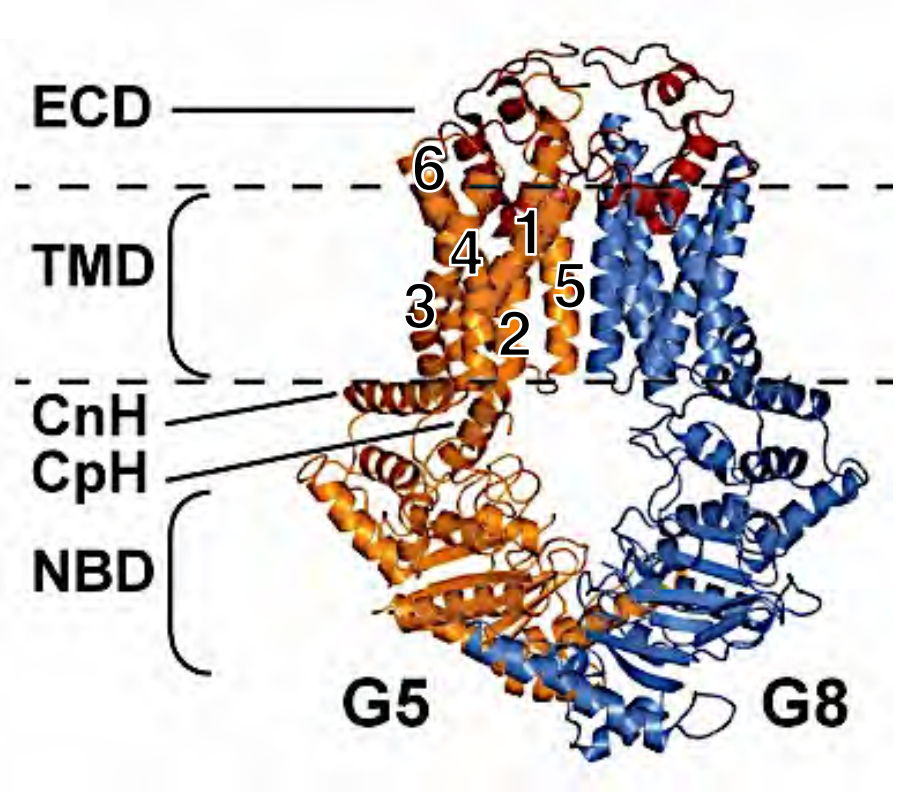


A: Walker A motif (GxxGxGKS/T)
B: Walker B motif (φφφφDE)

S: ABC signature motif (φSGGQ/E)
φ: hydrophobic amino acids

ABCG5 and ABCG8 share high structural similarity.

Domain features



TMD: transmembrane domain

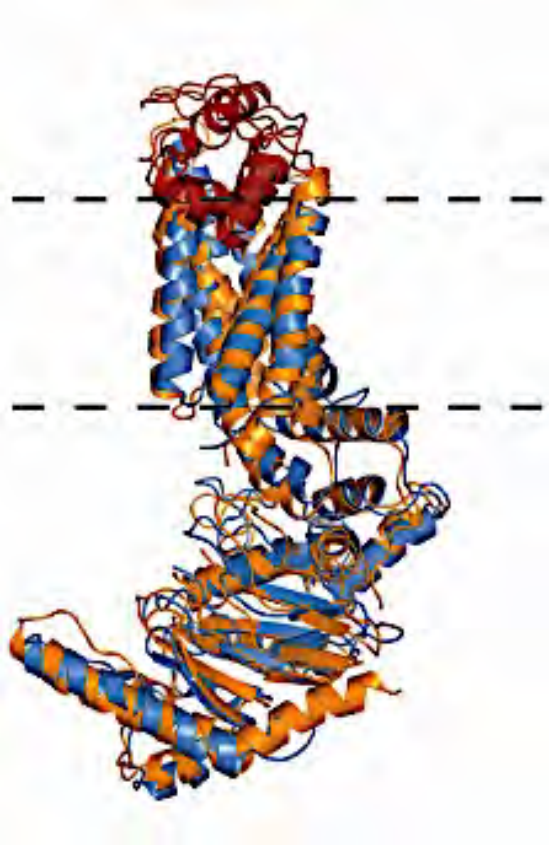
NBD: nucleotide-binding domain

ECD: extracellular domain

CnH: connecting helix

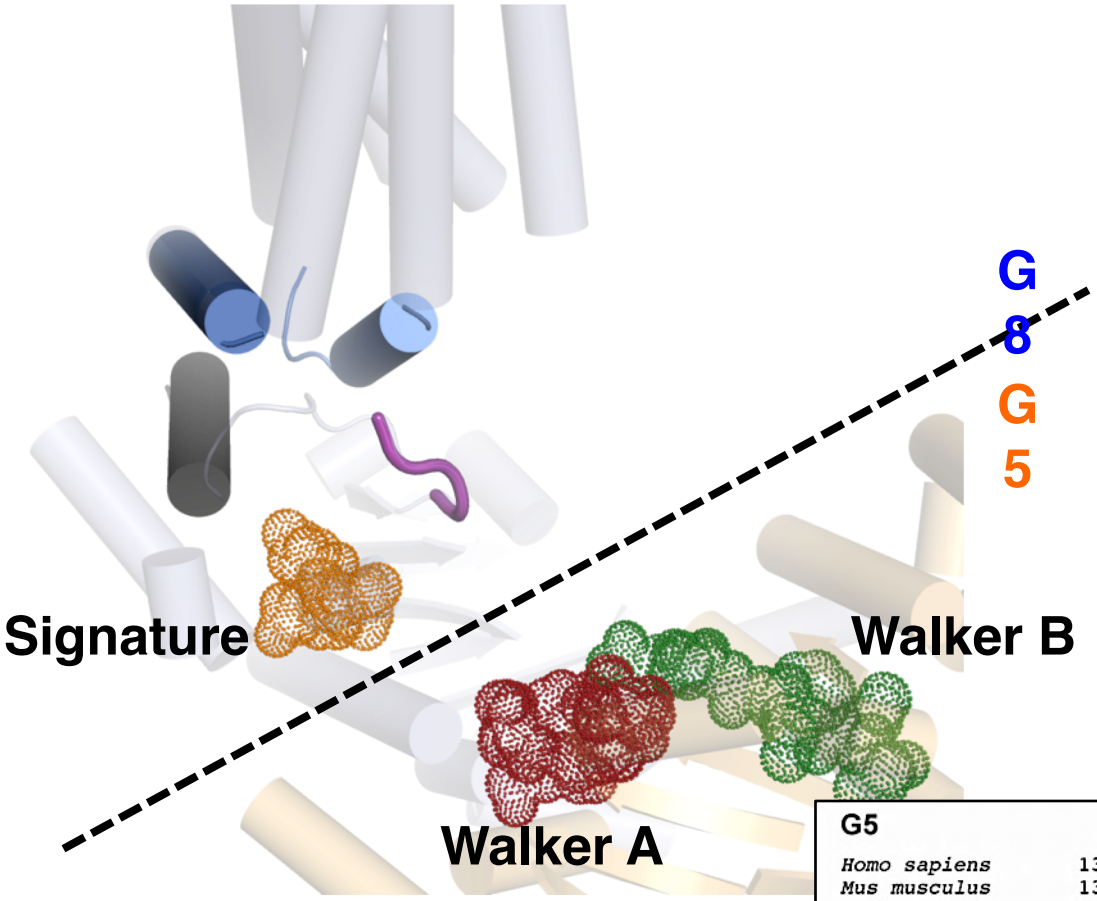
CpH: coupling helix

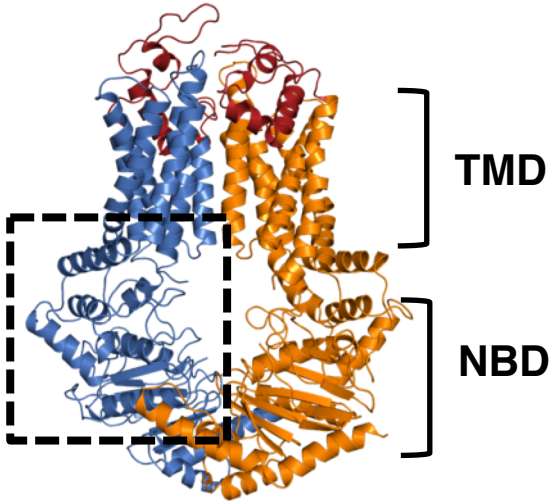
Structural similarity:



RMSD (Cα) ~ 2Å
(~28% sequence identity)

Triple Helical Bundle: Connecting the ATP-Binding Cassette to the Transmembrane Domain





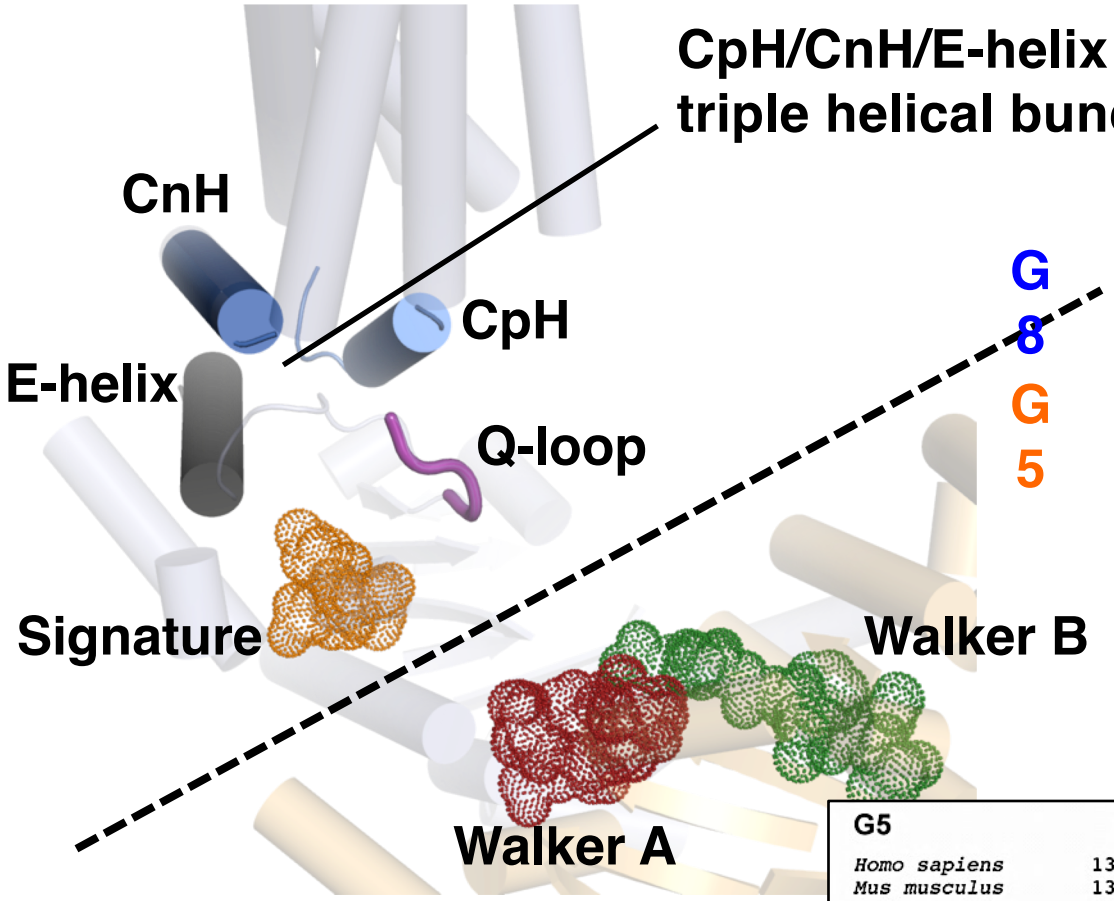
CnH: connecting helix

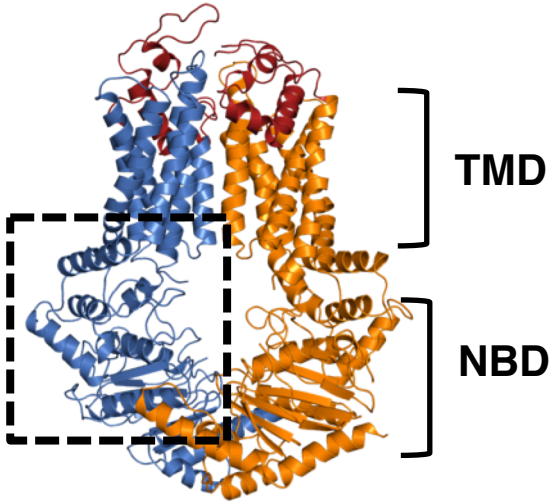
CpH: coupling helix

★: conserved polar residues

```
G5                Q-loop  E-helix                CnH                CpH
Homo sapiens      133 LQSDT~VRETLHYTALLA---~FSKLGVLLRRVTRNLVRN~LRAVSDQESQDGLY 458
Mus musculus      134 LQSDV~VRETLRYTAMLA---~FGKLGVLLRRVTRNLMRN~LRAVSDQESQDGLY 459
Gallus gallus     126 PQNDA~IEESLTYTALLA---~ISKLWFLLRRITRNFSKD~LRAISDQESKDGLY 451
Xenopus tropicalis 135 LQHDT~VRETLTYTALLA---~LSKVYVLLRRTFRNLSRD~LRAIGDQEGKDGLY 460
Danio rerio       137 LQSDN~VEETLTYTAQLA---~ISKLGVLLRRTFRNVSRD~LRAISDQESKDGLY 462
                      *     *  *         *     **  *  *   *  * *  *  *
G8
Homo sapiens      152 RQHNQ~VRETLAFIAQMRLP~VQQFTTLIRRQISNDFRD~ERAMLYYELEDGLY 487
Mus musculus      153 RQHDQ~VRETLAFIAQMRLP~IEQFSTLIRRQISNDFRD~ERSMLYYELEDGLY 487
Gallus gallus     154 RQDDR~VRETLLFIARLRLP~LKQFTVLLSRQVSNDFRD~ERAMLYLDLENGMY 492
Xenopus tropicalis 154 RQDDQ~VRETLTFIAKLRLP~LHQFSVLLRRHVSNDLRD~ERAMLYHDLEDGLY 496
Danio rerio       158 RQDDR~VRETLAFVAKLRLP~VHQFTTLIRRQVFNDYRD~ERAMLYHELEDGMY 498
                     **    ***       *     *      **  ** ** **    * *   *
```

Triple Helical Bundle: Connecting the ATP-Binding Cassette to the Transmembrane Domain





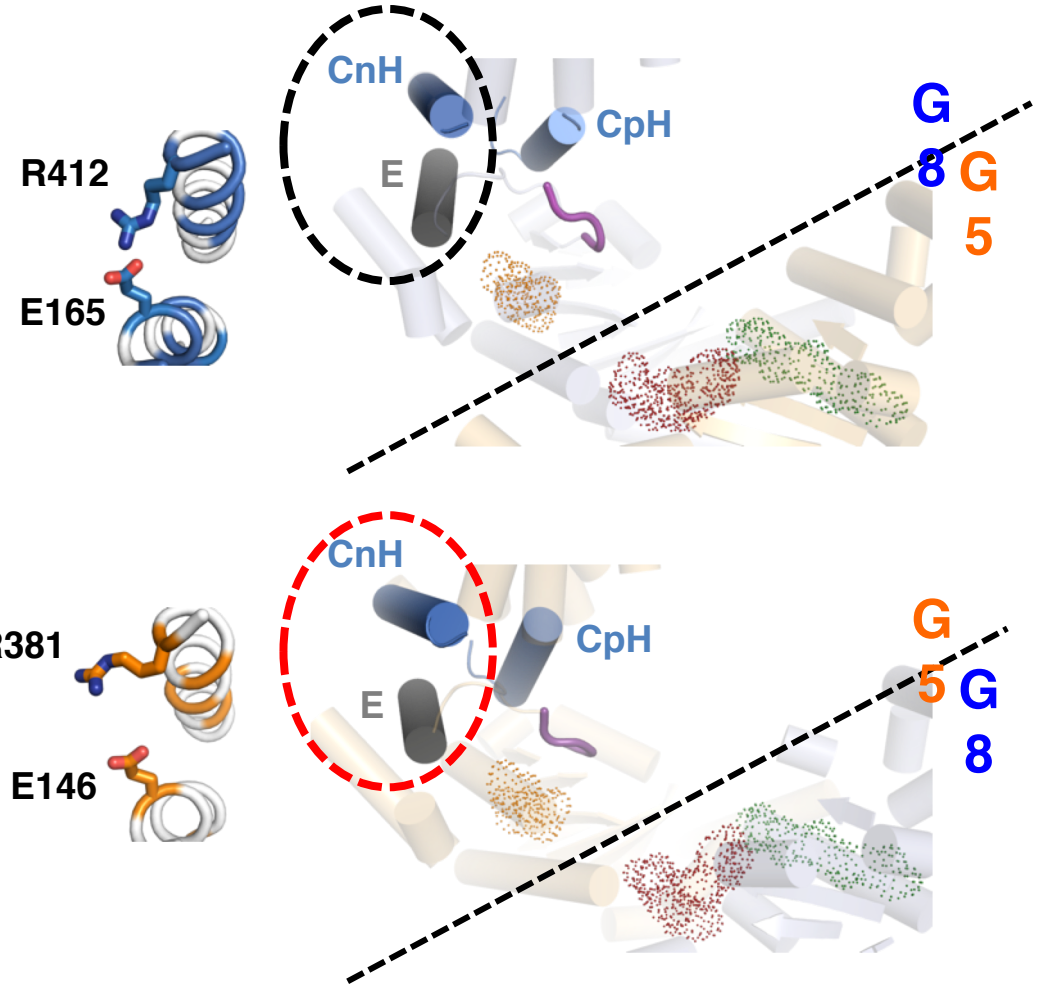
CnH: connecting helix

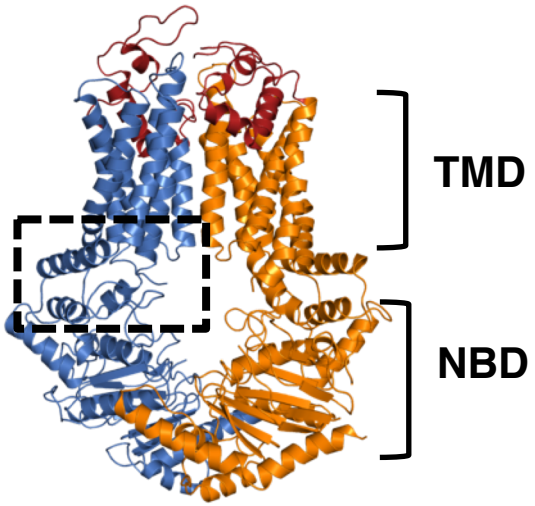
CpH: coupling helix

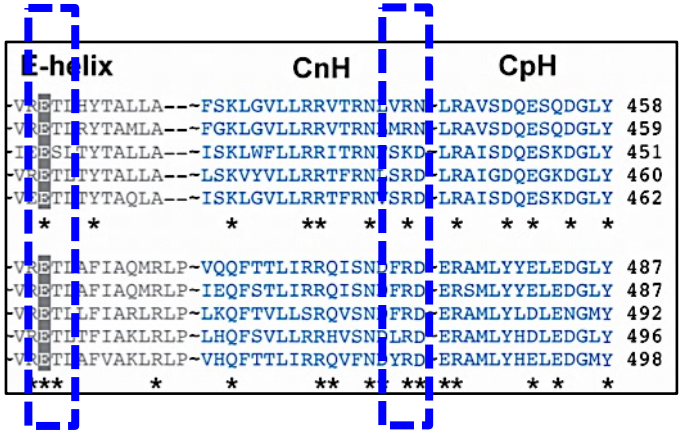
★: conserved polar residues

```
G5                    Q-loop  E-helix              CnH                CpH
Homo sapiens      133 LQSDT~VRETLHYTALLA---~FSKLGVLLRRVTRNLVRN~LRAVSDQESQDGLY 458
Mus musculus      134 LQSDV~VRETLRYTAMLA---~FGKLGVLLRRVTRNLMRN~LRAVSDQESQDGLY 459
Gallus gallus     126 PQNDA~IEESLTYTALLA---~ISKLWFLLRRITRNFSKD~LRAISDQESKDGLY 451
Xenopus tropicalis 135 LQHDT~VRETLTYTALLA---~LSKVYVLLRRTFRNLSRD~LRAIGDQEGKDGLY 460
Danio rerio       137 LQSDN~VEETLTYTAQLA---~ISKLGVLLRRTFRNVSRD~LRAISDQESKDGLY 462
                        *     *  *         *     **  *  *   *  * *  *  *
G8
Homo sapiens      152 RQHNQ~VRETLAFIAQMRLP~VQQFTTLIRRQISNDFRD~ERAMLYYELEDGLY 487
Mus musculus      153 RQHDQ~VRETLAFIAQMRLP~IEQFSTLIRRQISNDFRD~ERSMLYYELEDGLY 487
Gallus gallus     154 RQDDR~VRETLLFIARLRLP~LKQFTVLLSRQVSNDFRD~ERAMLYLDLENGMY 492
Xenopus tropicalis 154 RQDDQ~VRETLTFIAKLRLP~LHQFSVLLRRHVSNDLRD~ERAMLYHDLEDGLY 496
Danio rerio       158 RQDDR~VRETLAFVAKLRLP~VHQFTTLIRRQVFNDYRD~ERAMLYHELEDGMY 498
                       **    ***       *     *      **  ** ** **    * *   *
```

Interactions within the Triple Helical Bundle







CnH: connecting helix

CpH: coupling helix

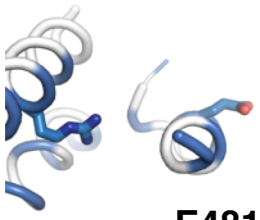
E: E-helix

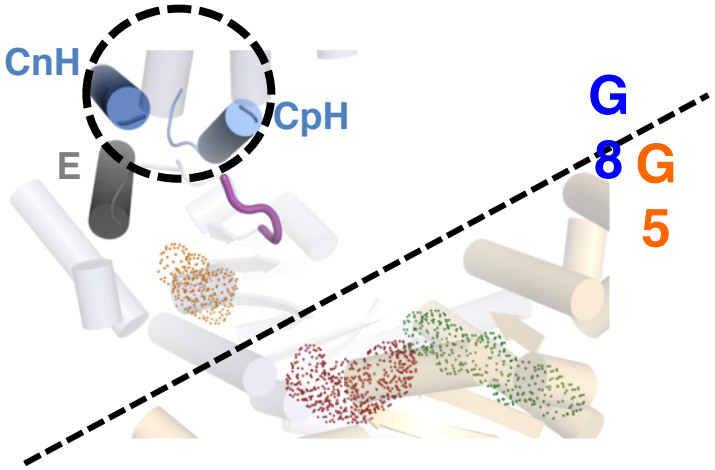
A: Walker A motif

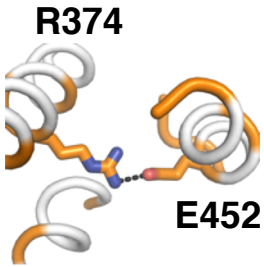
B: Walker B motif

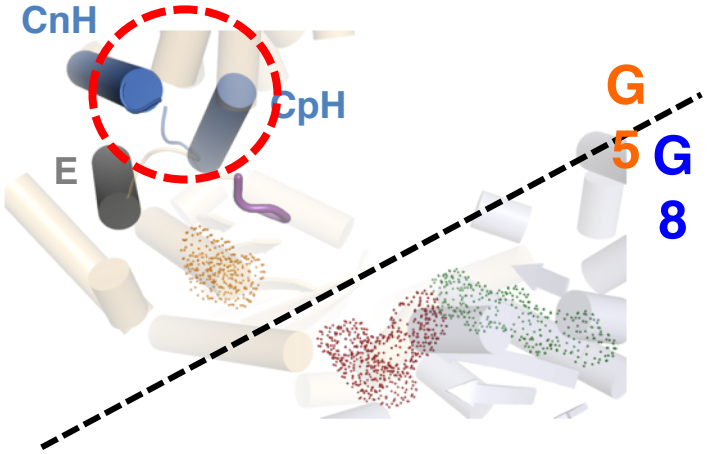
Sig: Signature motif

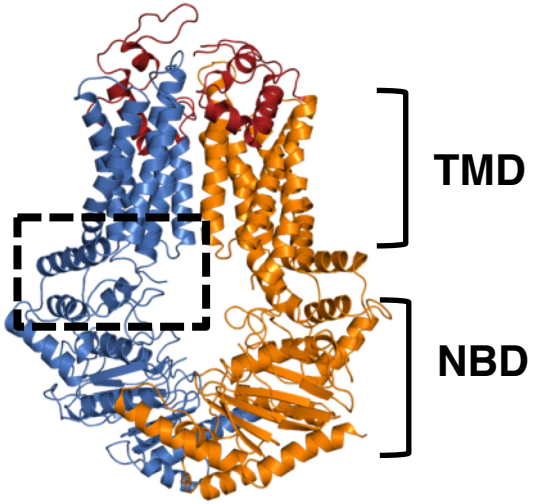
Interactions within the Triple Helical Bundle

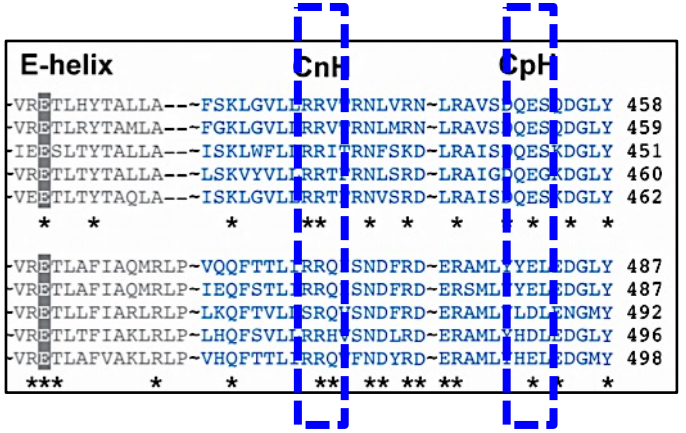












CnH: connecting helix

CpH: coupling helix

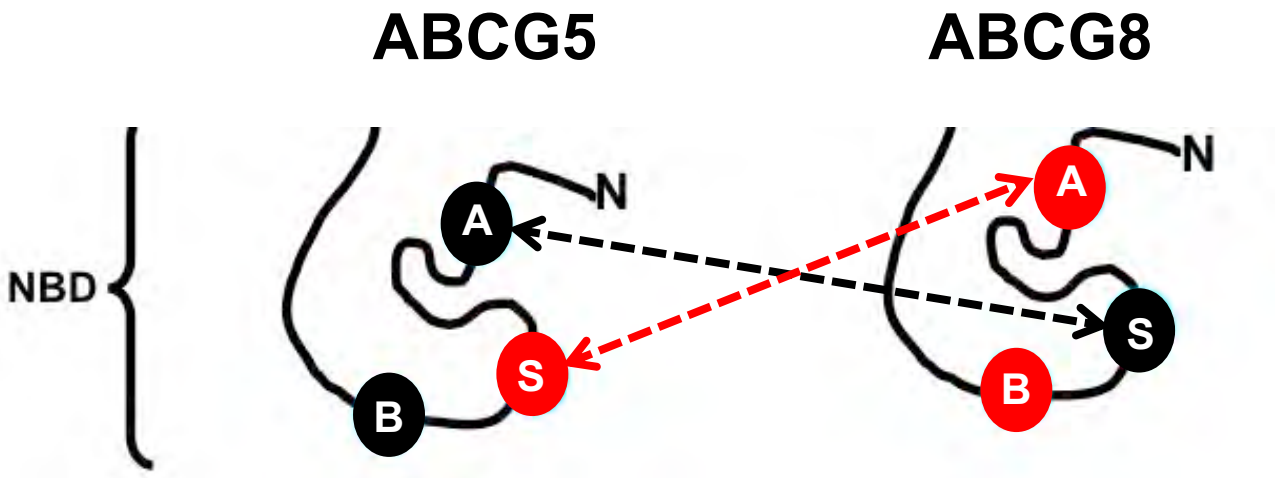
E: E-helix

A: Walker A motif

B: Walker B motif

Sig: Signature motif

The ATPase in G5G8 is catalytically asymmetric.



NBS1
(degenerated / inactive)

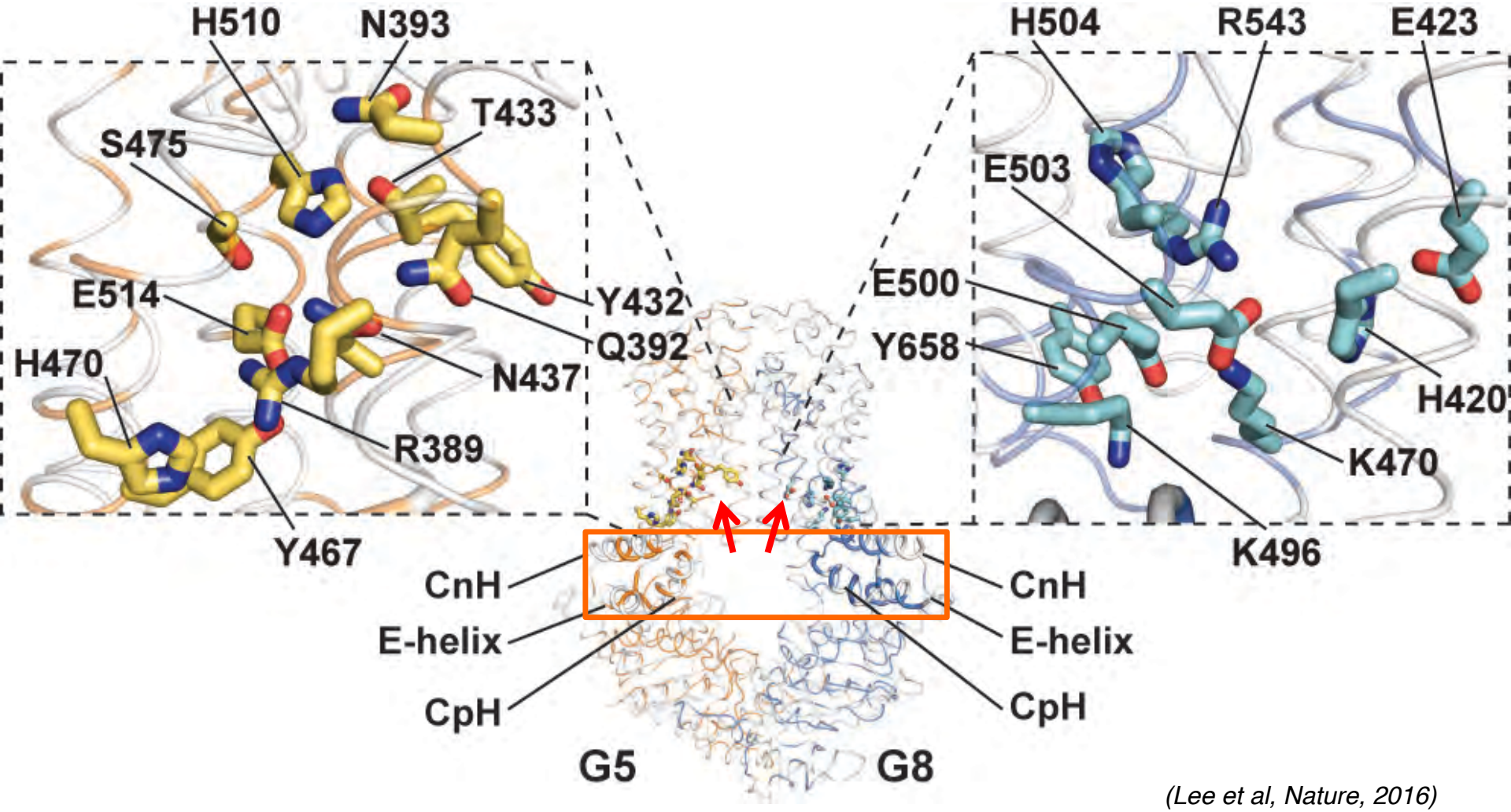
NBS2
(conserved / active)

Zhang et al, JBC, 2006
Wang et al, JBC, 2011

			Consensus Sequence
A: Walker A motif	GSSGSGKT	GSSGCG**RA**	GxxGxGKS/T
S: Signature motif	IS**T**GE	LSGGE	φSGGQ/E
B: Walker B motif	VMLFDE	ILILDE	φφφφDE

(φ: hydrophobic amino acids)

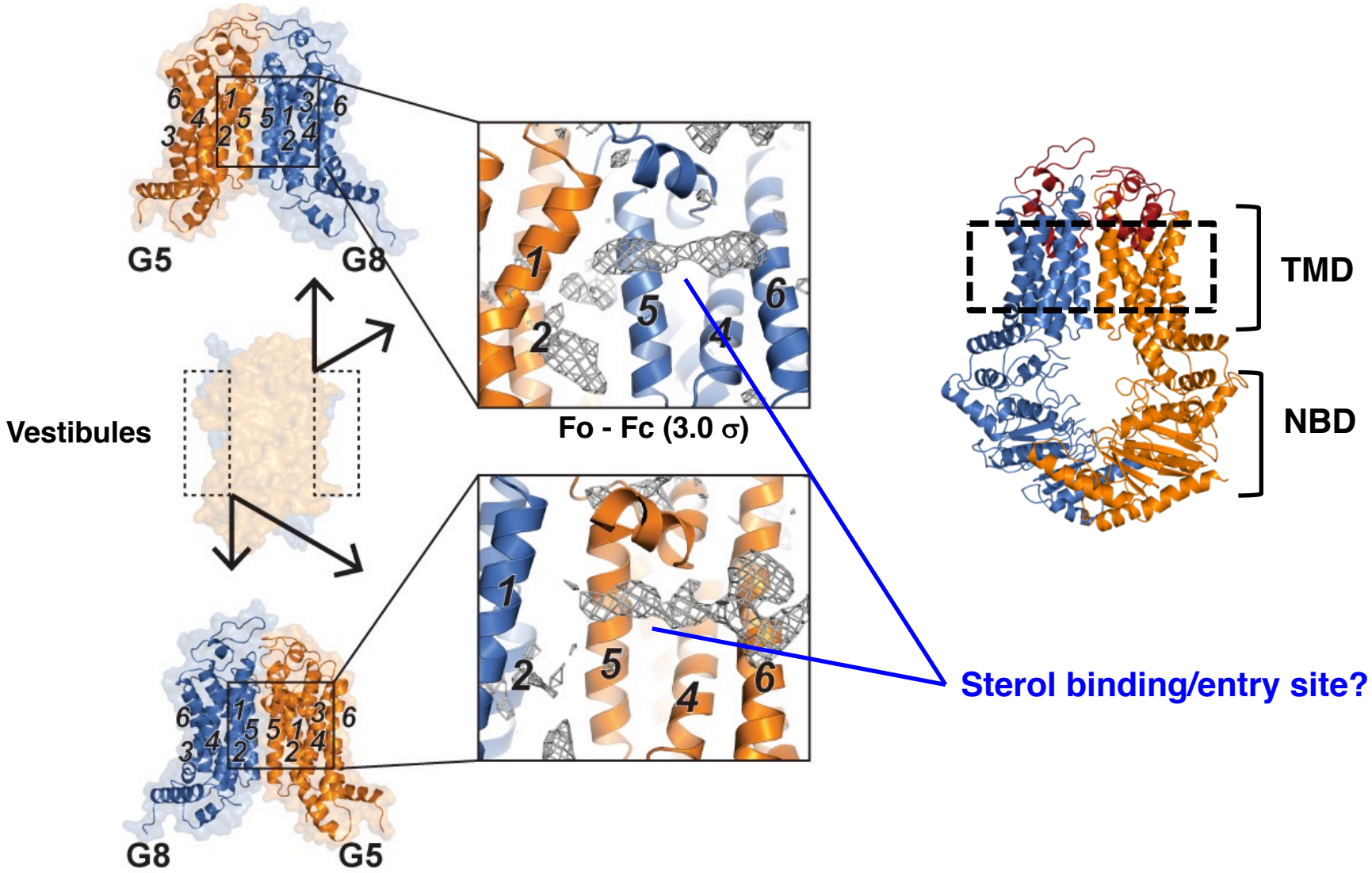
The TMD polar relay connects the triple helical bundle to the TMD.



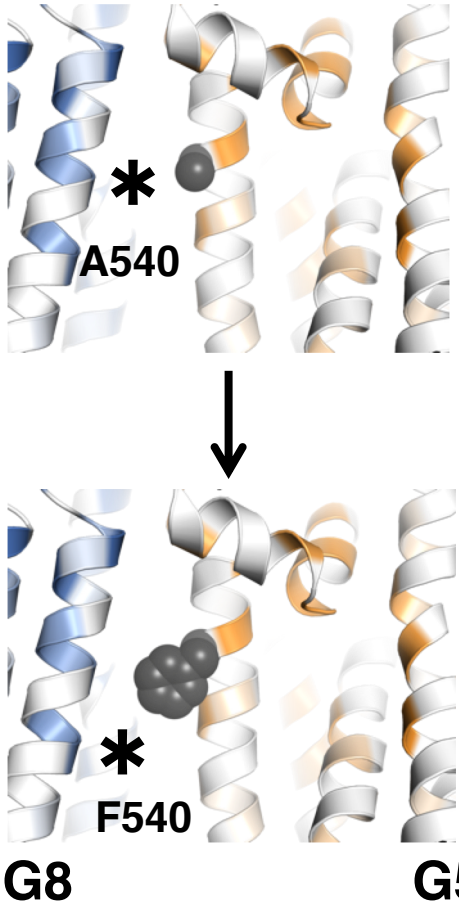
(Lee et al, Nature, 2016)

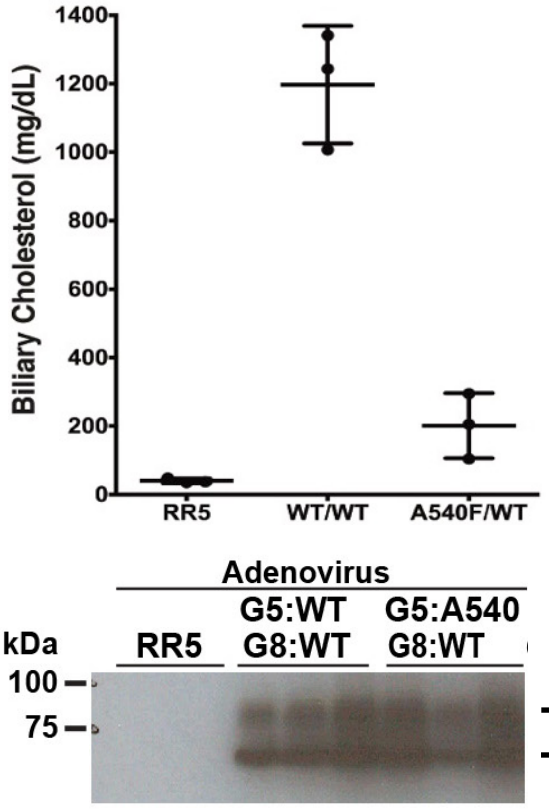
How do sterols move across the lipid-bilayer membranes on the TMD?

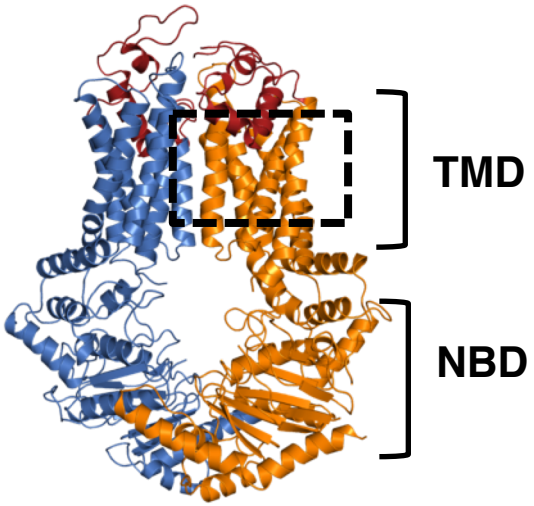
Vestibules at the TMD-membrane interface



How do sterols move across the lipid-bilayer membranes on the TMD?





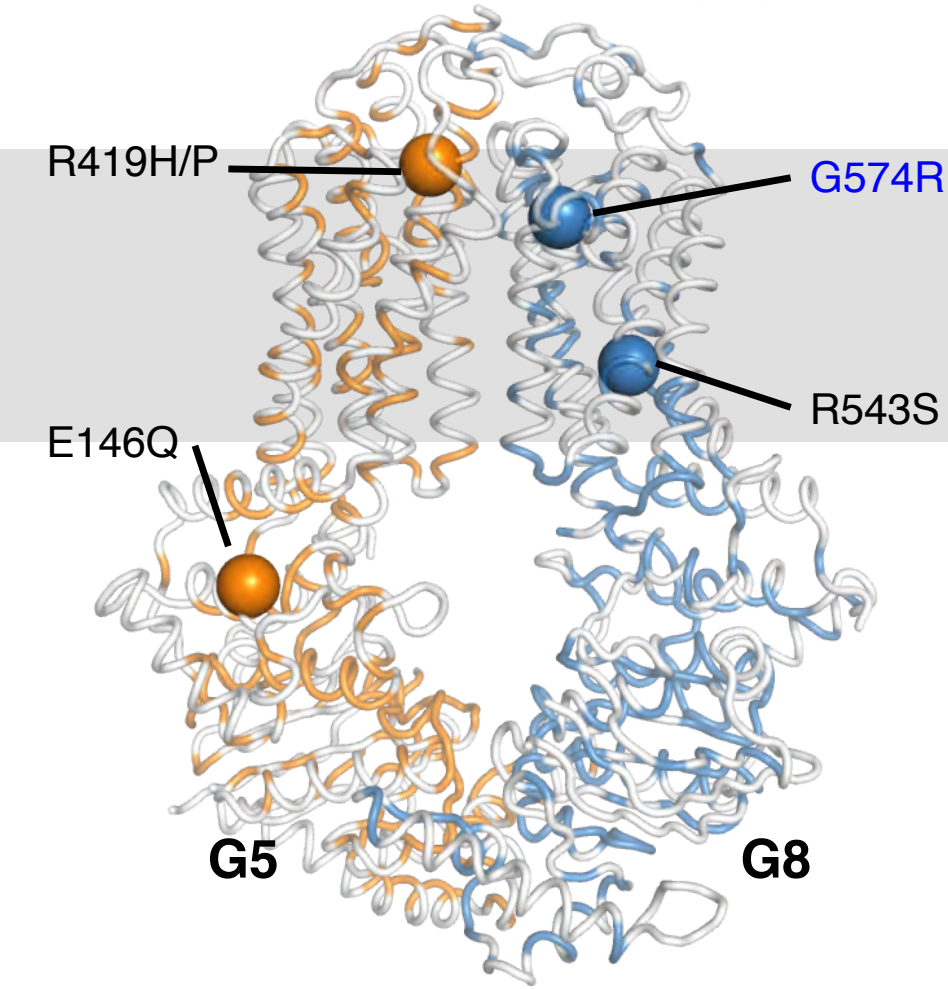


(Lee et al, Nature, 2016)

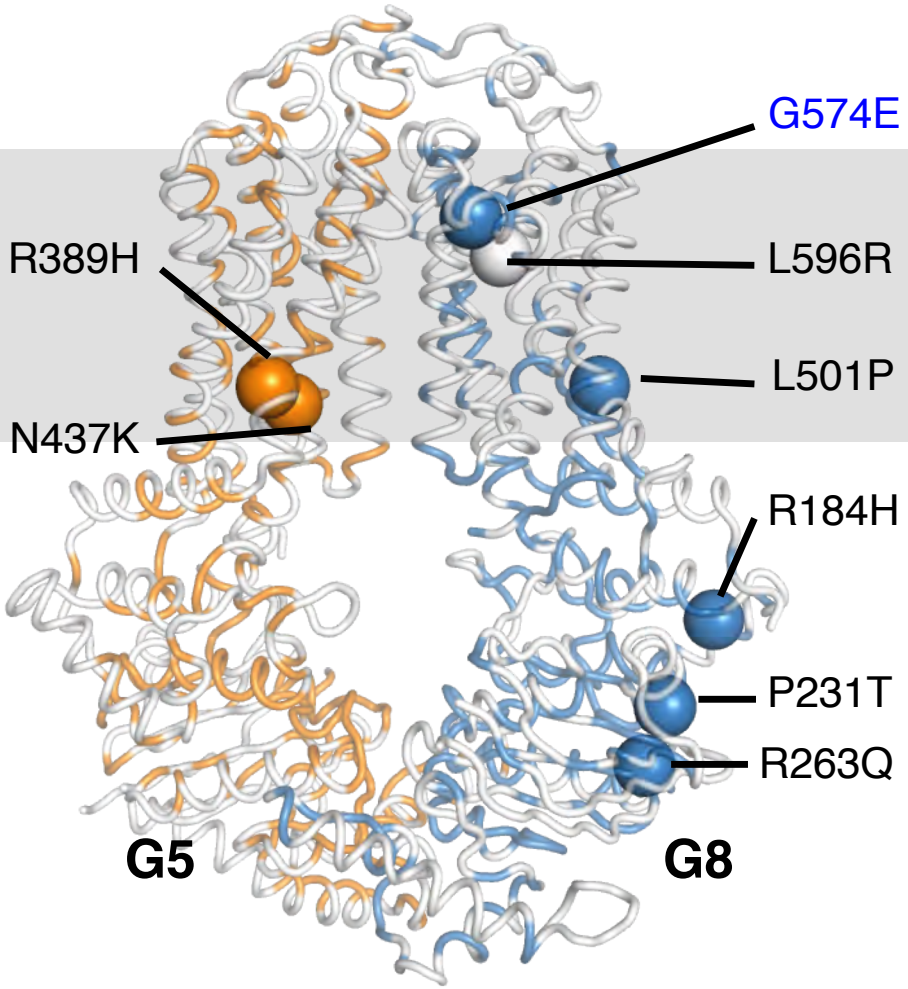
Location of the residues with the disease-causing missense mutations of sitosterolemia.

ER-escape missense mutations

Graf et al, JBC, 2004



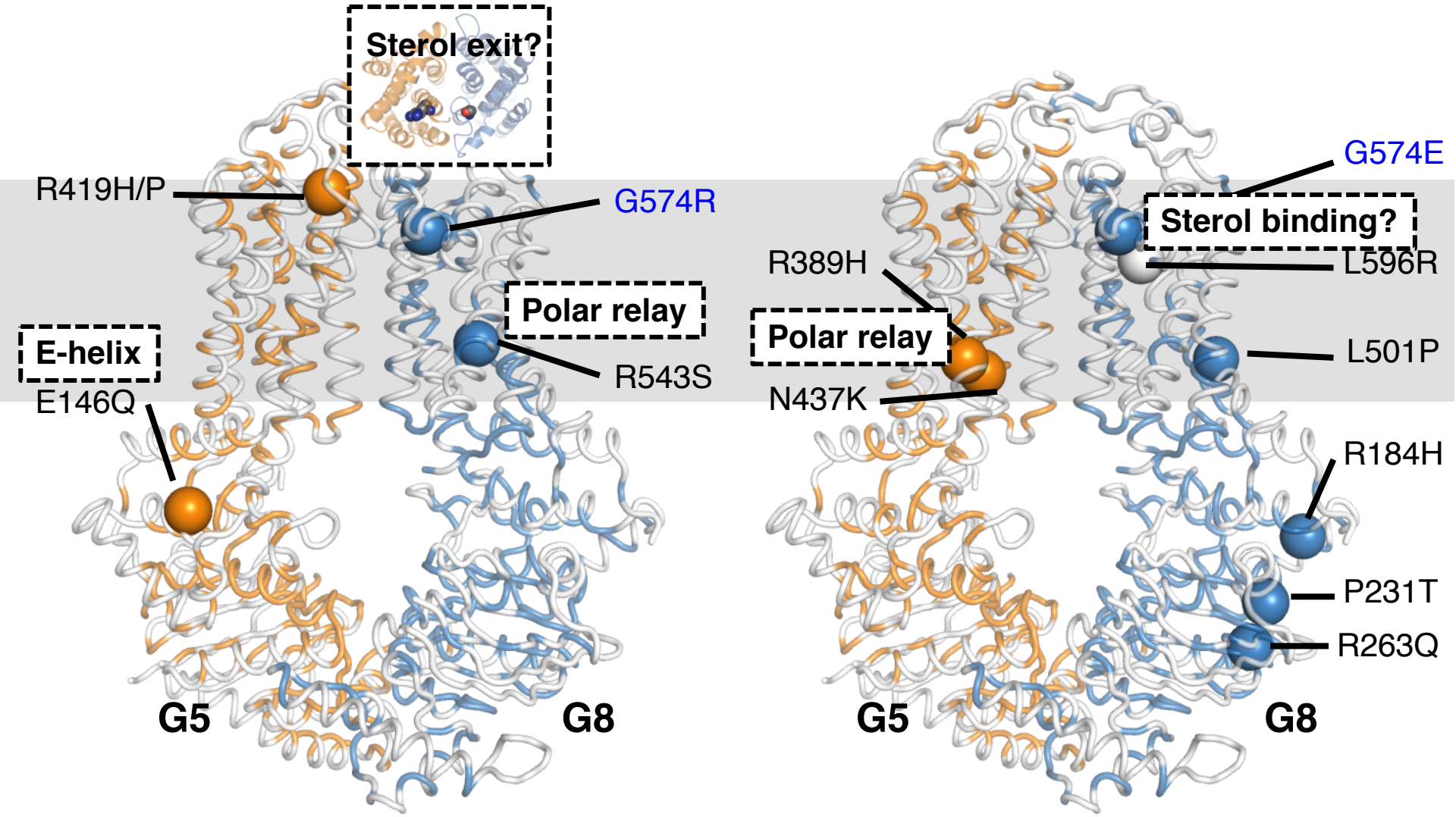
Non-ER-escape missense mutations



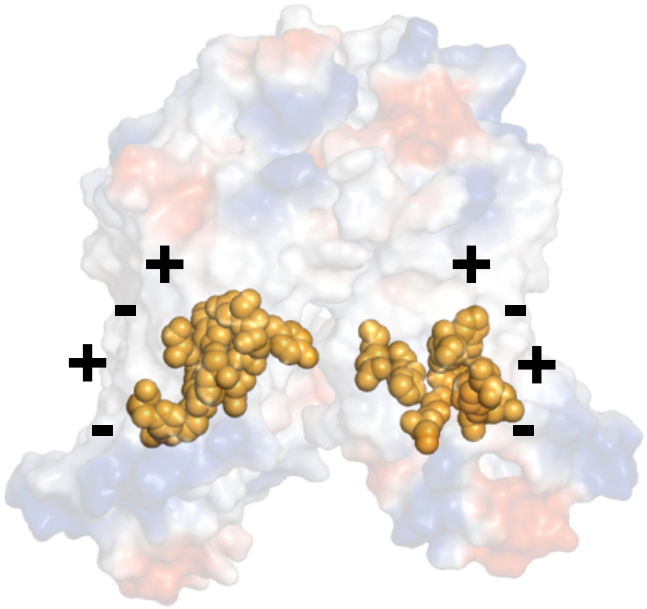
Color: conserved (multiple sequence alignment (MSA) value ≥ 7)

White: less/non-conserved (MSA < 7)

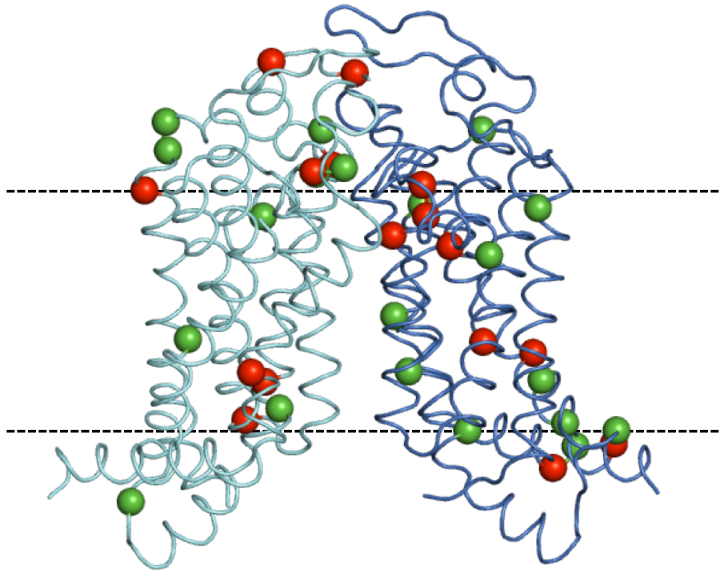
Disease-causing mutations cluster in the conserved functional domains in G5G8.



Transmembrane Domain of ABC Cholesterol Transporters: a Pathogenic Hot Spot



Polar relay

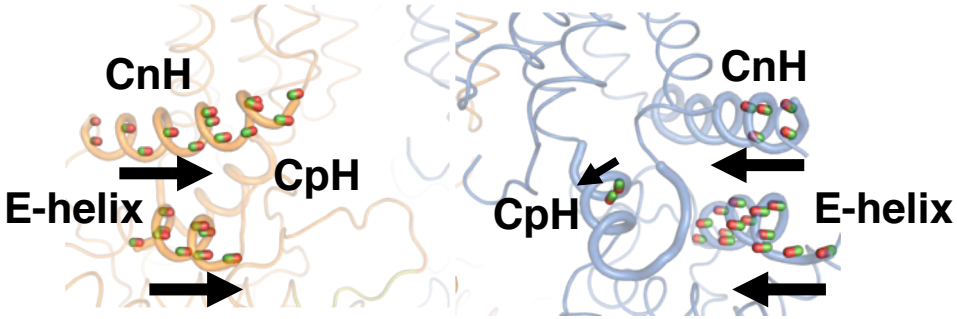


Pathogenic residues:
G5G8 (red), A1 (green)

G5G8-Mediated Sterol Transport

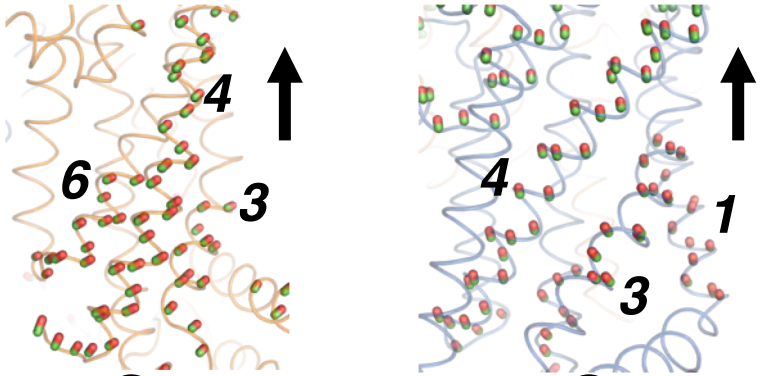
Molecular Dynamics Simulation

Inward movement (CpH/CnH/E-helix bundle)



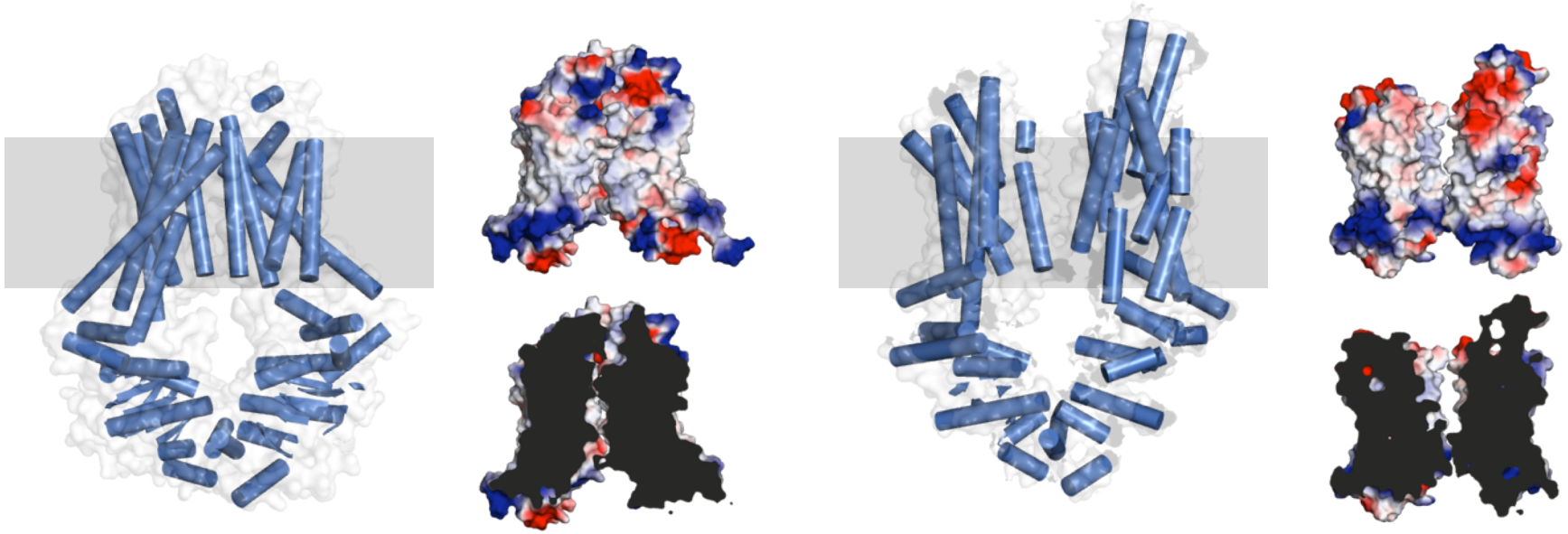
G5 G8

Upward movement (TM helices)

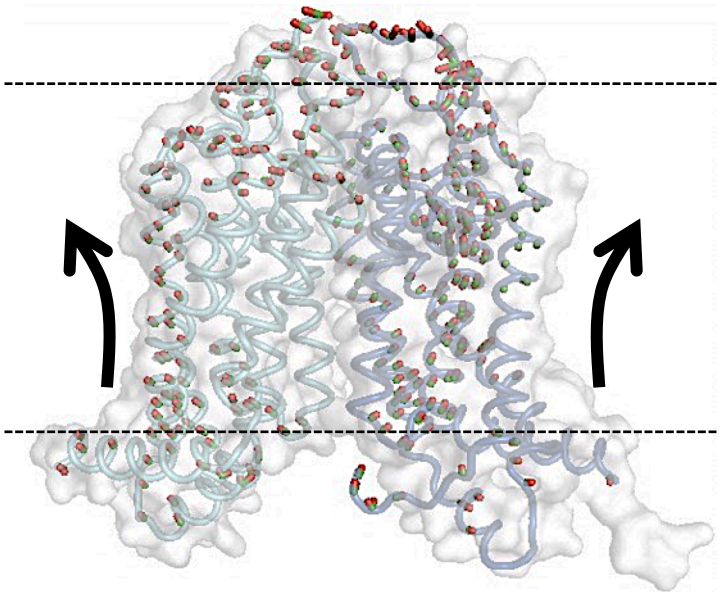


G5 G8

Transmembrane Domain: the Dynamic Nature (Probably at an ATP-Prehydrolytic state)

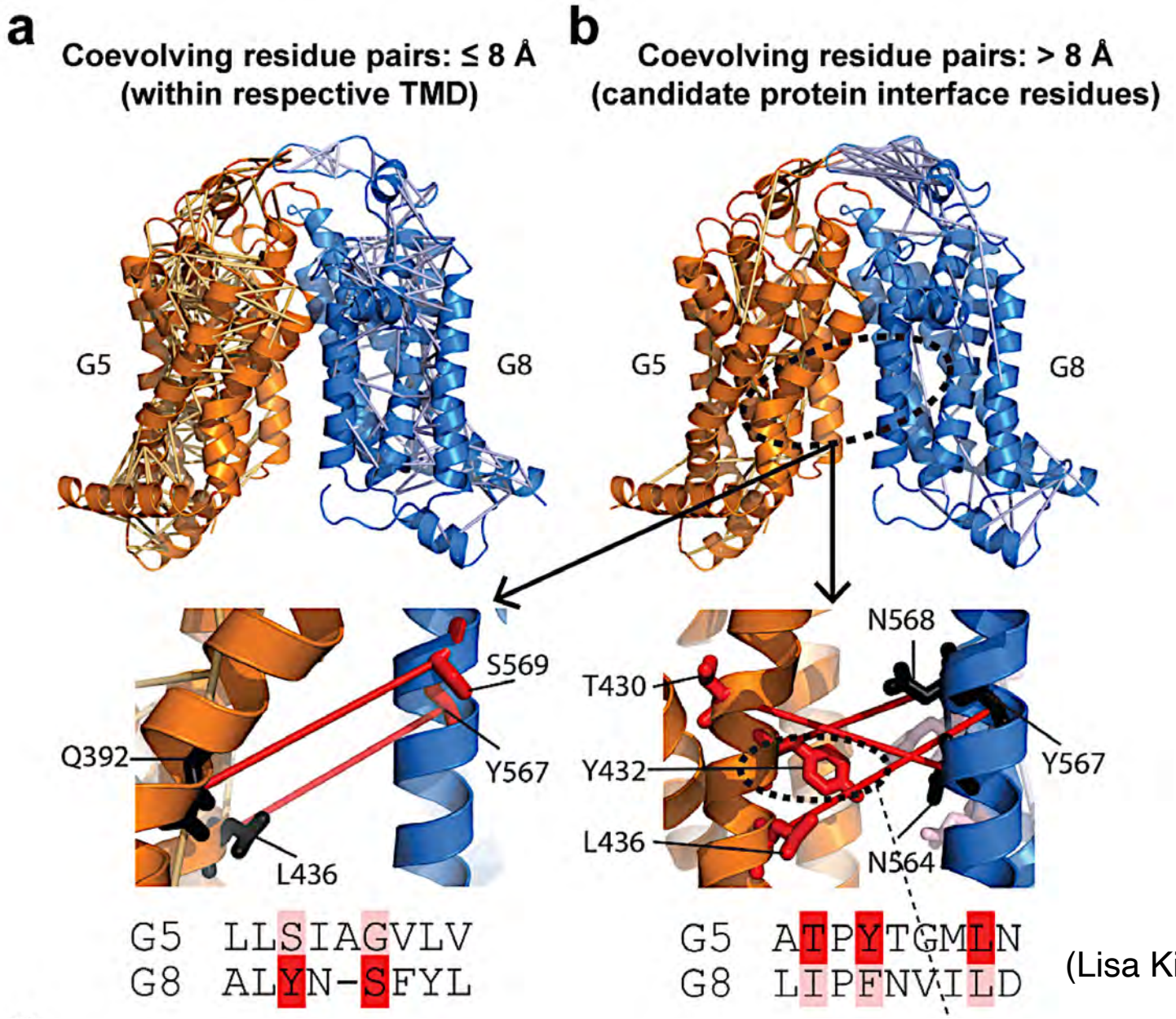


ABCG5/G8
(nf, inward)

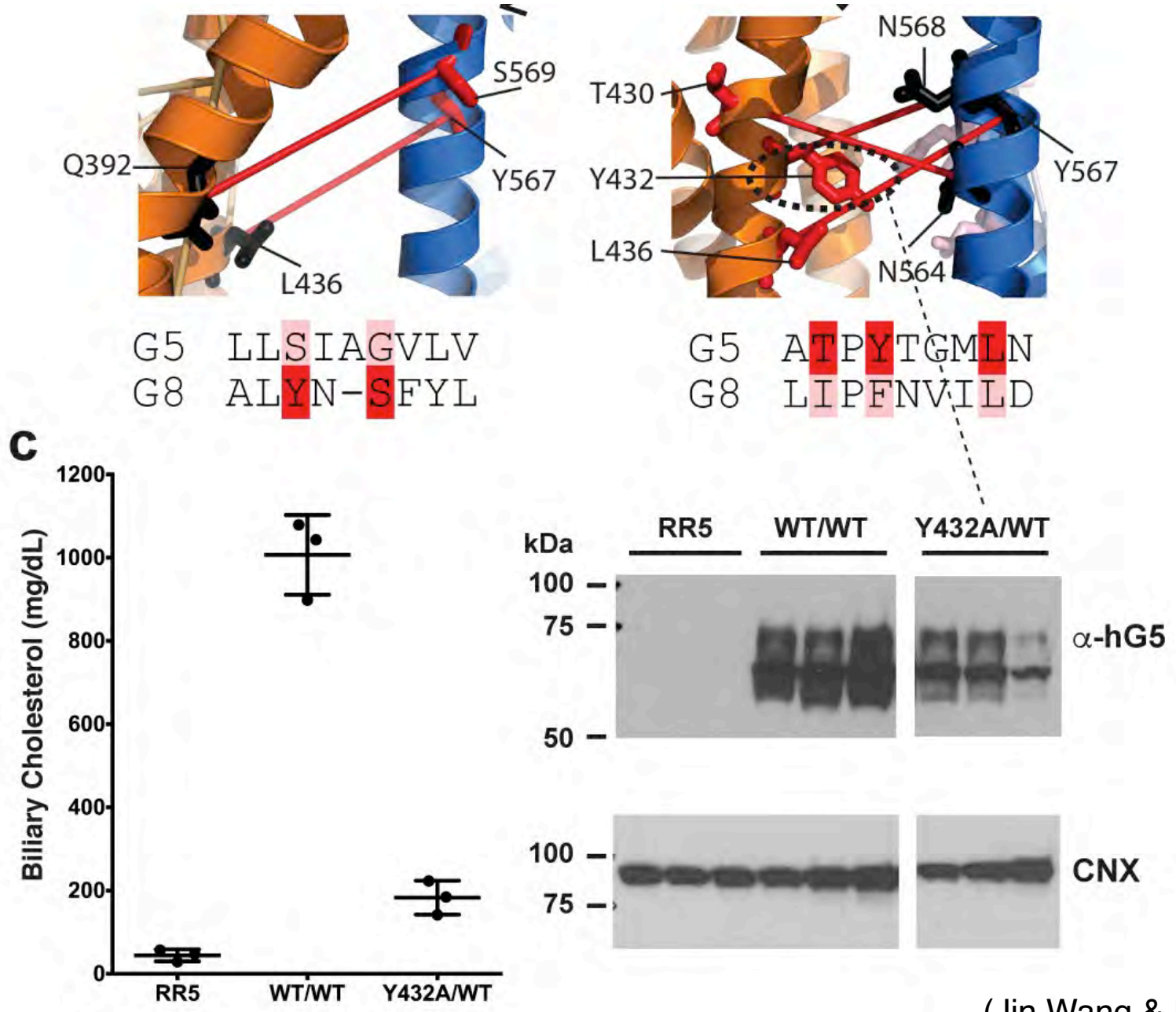


ABCA1
(nf, outward)

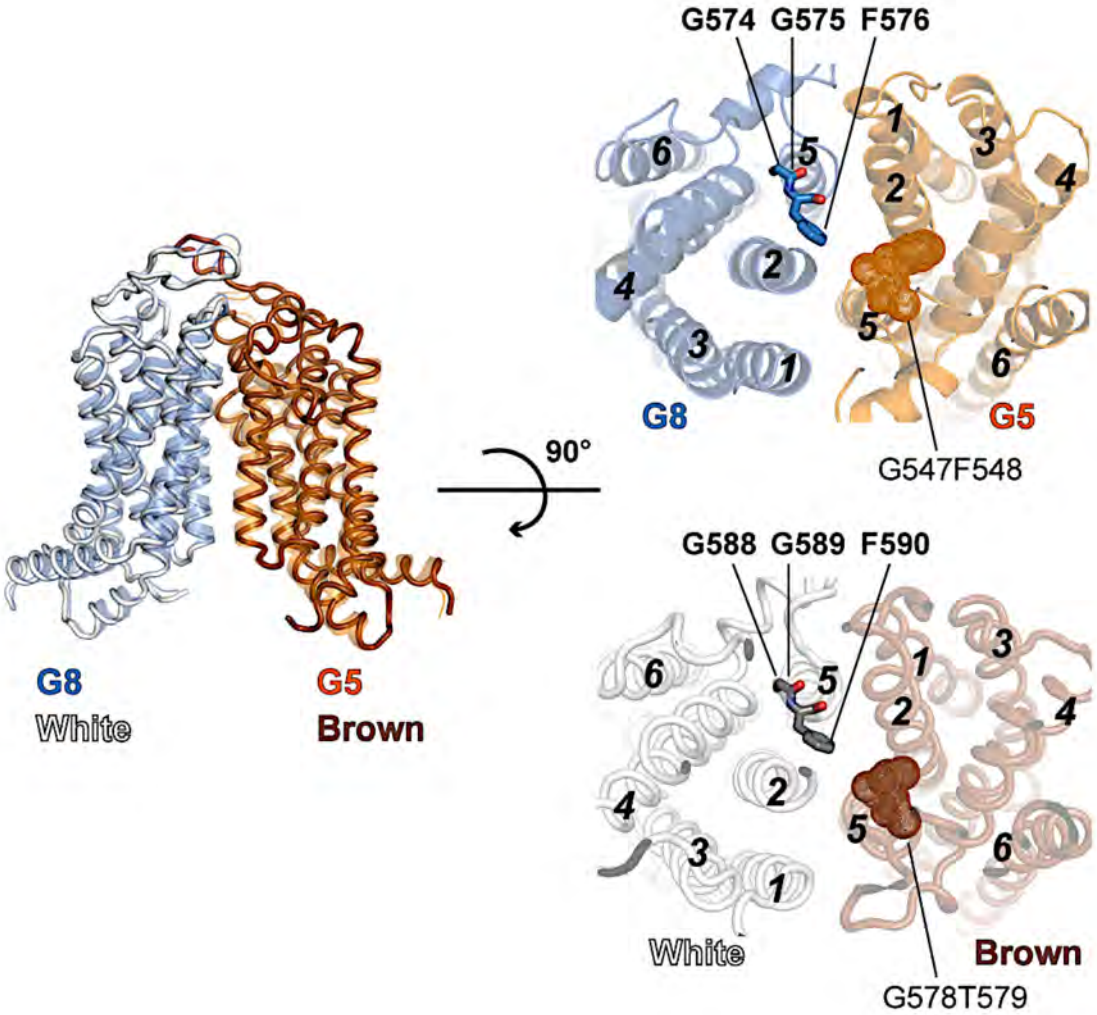
Co-Evolution Analysis



(Lisa Kinch)



(Jin Wang & Fang Xu)

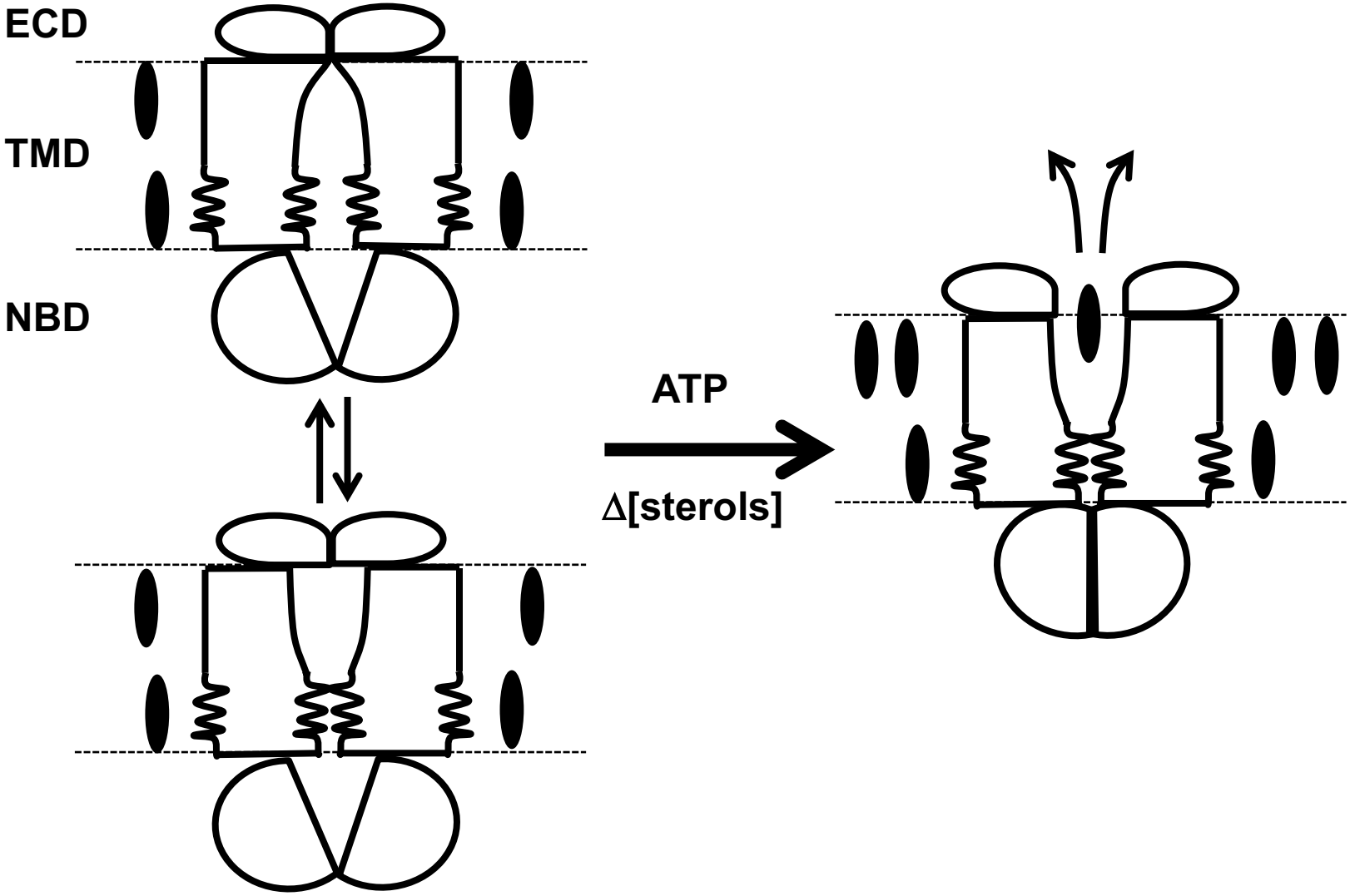


TMH5

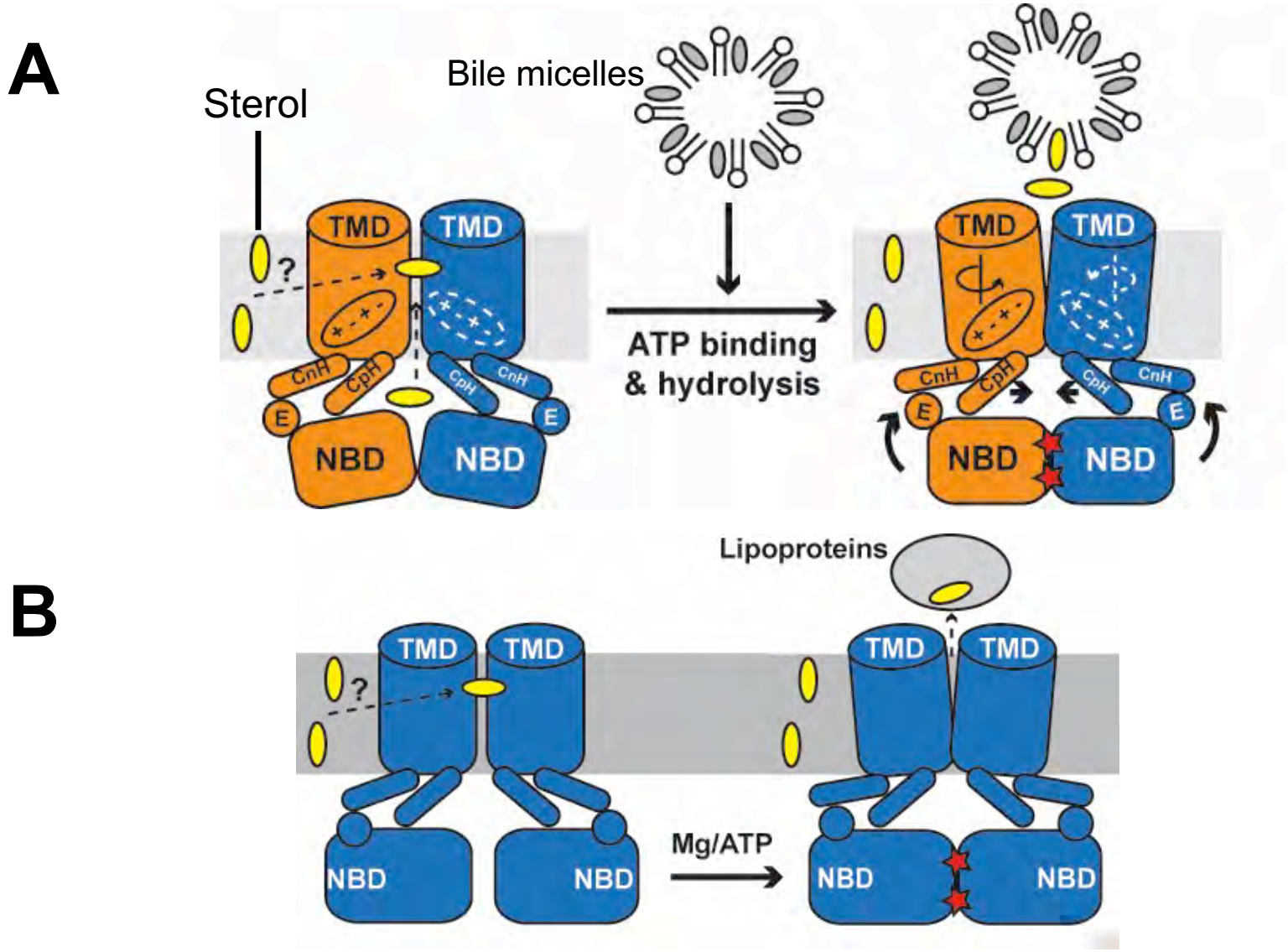
G5(Homo sapiens)	527	PNIVNSVVALLSIAGVLVGSGFLRN	551
G5(Danio rerio)	531	PNMVNSGVALLNIAGIMVGSGFLRG	555
G8(Homo sapiens)	556	FHMASFFSNALYN-SFYLAGGFMIN	579
G8(Danio rerio)	537	LQTSSFMGNALFT-VFYLTAGFVIS	560
White	570	TSMALSVGPPVII-PFLLFGGFFLN	593
Brown	559	DKMASECAAPFDL-IFLIFGGTYMN	582
Scarlet	550	VPLAMAYLVPLDY-IFMITSGIFIQ	573

*

Working Model of ABC Sterol Transporters (Cellular)



Working Model of ABC Sterol Transporters (Molecular)



So, …

- High-degree of structural diversity in the transmembrane domains of ABC transporters.
- The structural variability (likely) determines the functional diversity of ABC transporters.
- Transport mechanism is (likely) individually distinct.