Lecture 9, BCH 8102, 2021 Winter

Lipid metabolism and drug resistance: ABC transporters and structural biology

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Lecture Outline

Part I: ABC Transporters

a) From bacteria to mammals
b) The engine
c) The transport
d) A long way to structural understanding

Part II: Structural studies of multidrug resistance transporters

ABCB1 (P-glycoprotein)

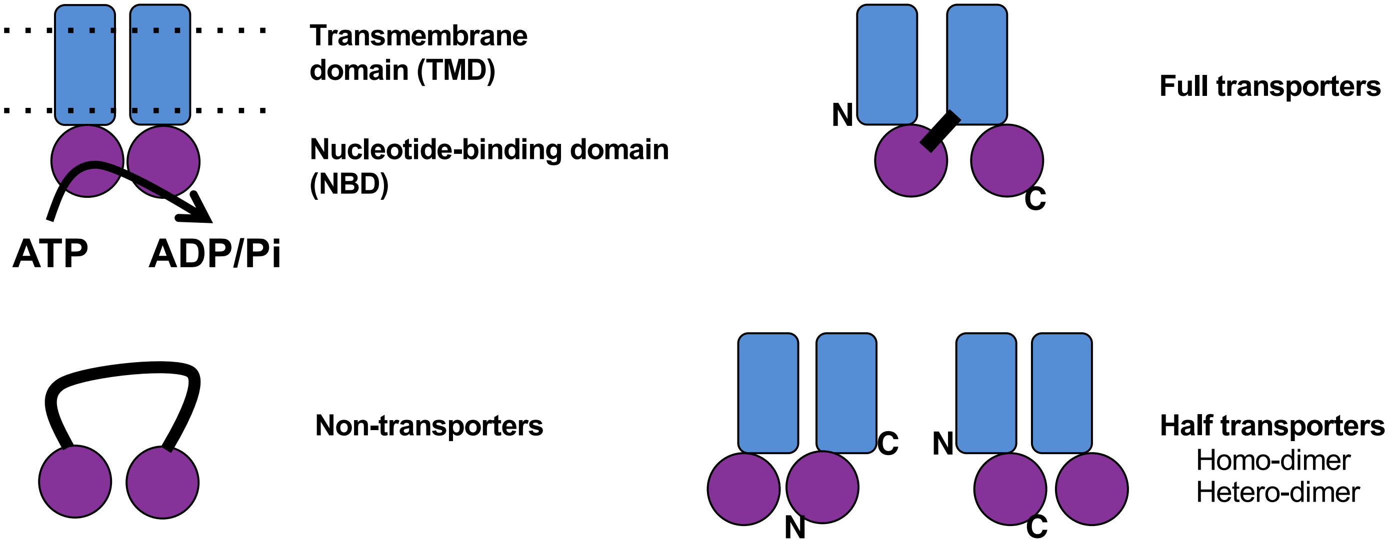
Part III: Structural studies of lipid transporters

ABCG5/G8 (Sterolin)

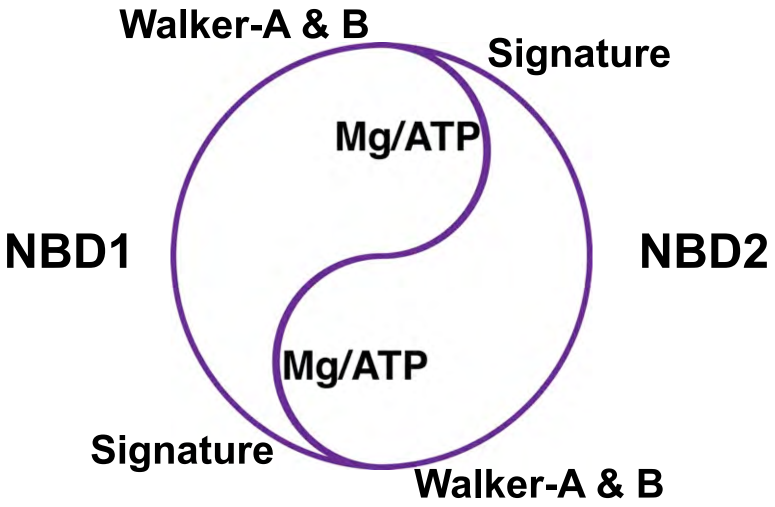
Part I:

ABC TRANSPORTERS

ATP-Binding Cassette (ABC) Proteins

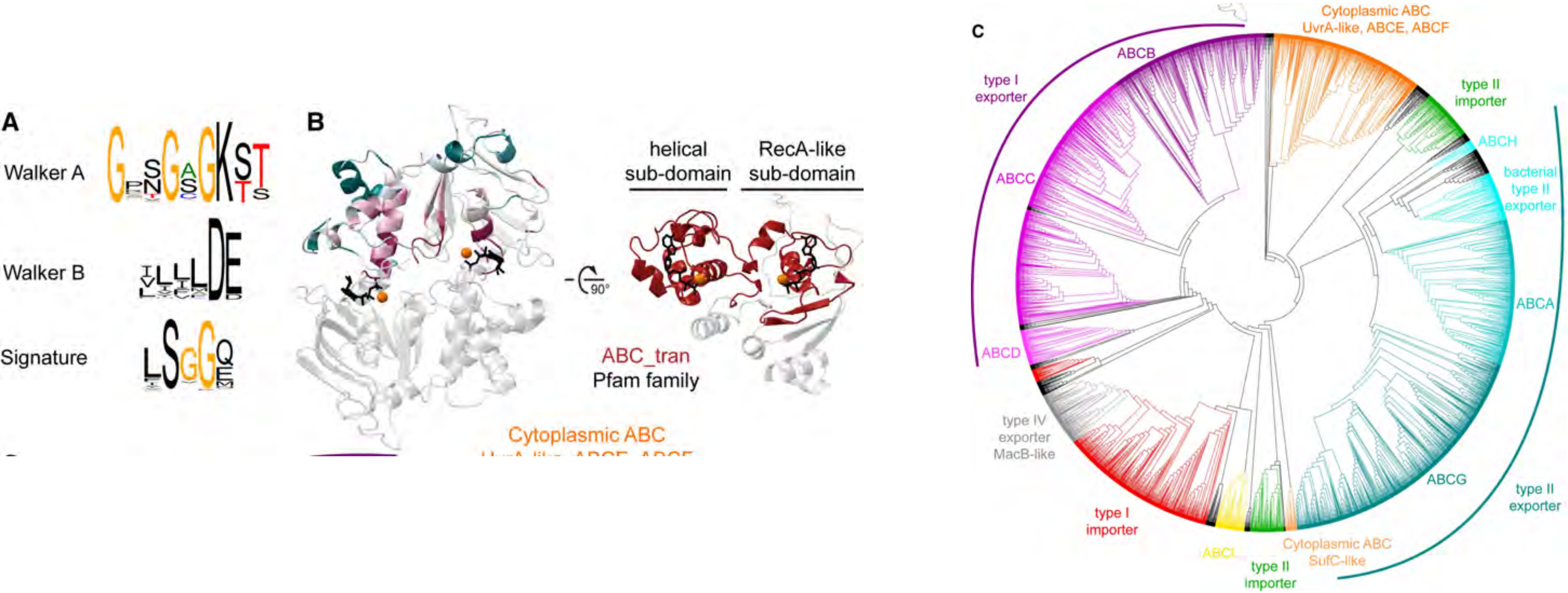


ABC coupled transport: a simple idea



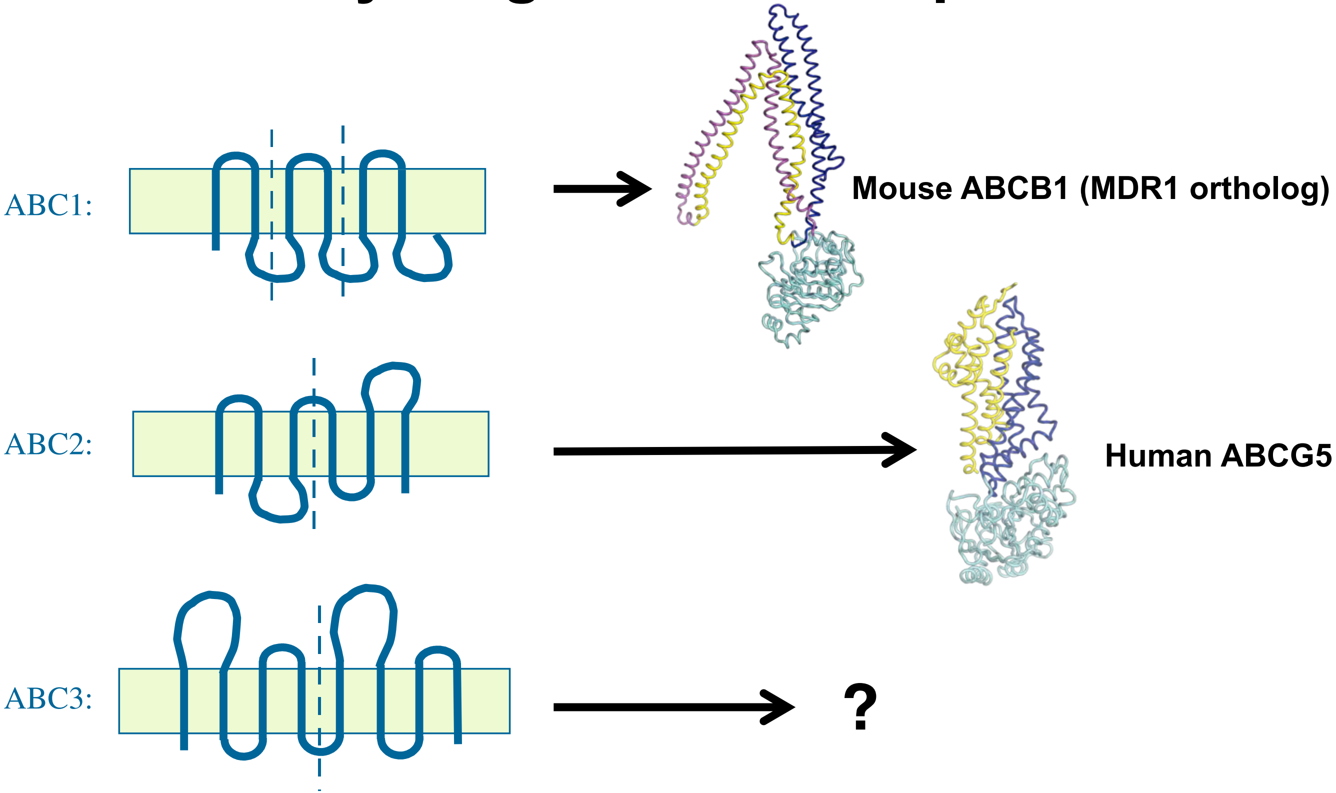
ATP-binding cassette
(ABC)

Evolutionary History of ABC Proteins



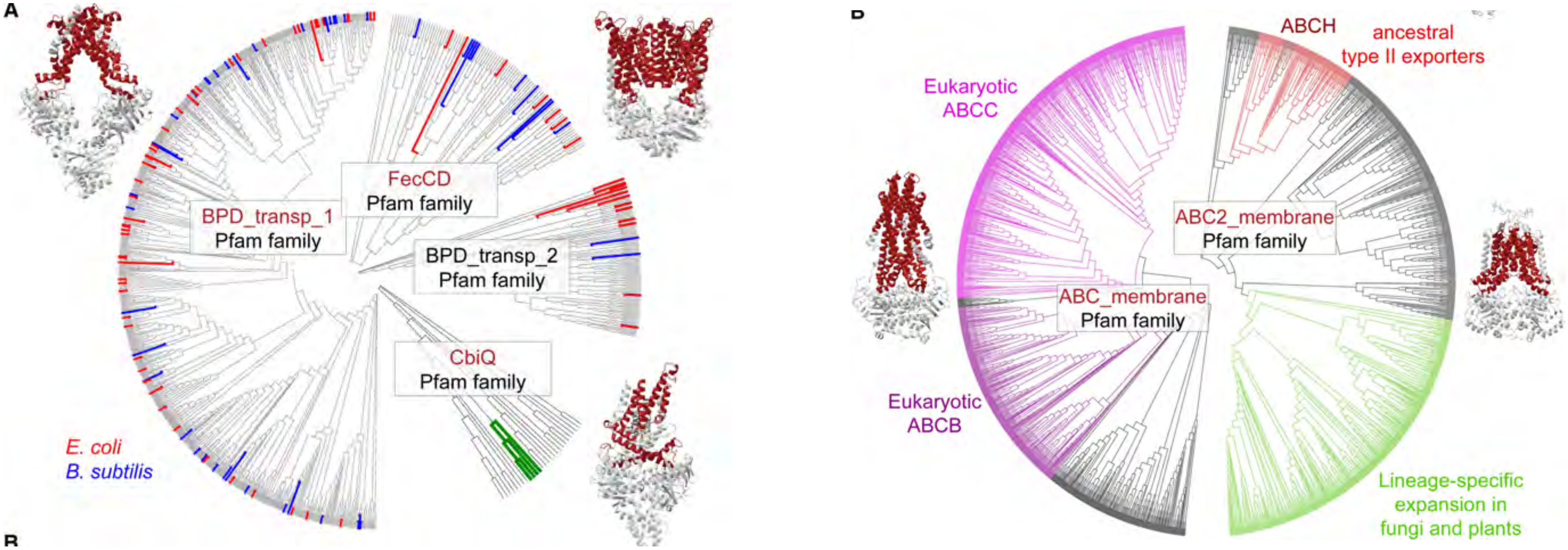
(Srikant, FEBS Lett, 2020)

Evolutionary Origin of ABC Exporter TMD



(Wang et al, J Membr Biol, 2009)

Evolutionary History of ABC Proteins



(Srikant, FEBS Lett, 2020)

Human ABC proteins: 48 members, 44 transporters

Transporters
- B, C, D
- A, G

Non-transporters
- E, F

ABC1

ABC2

Table 1. Human ATP-binding cassette proteins.

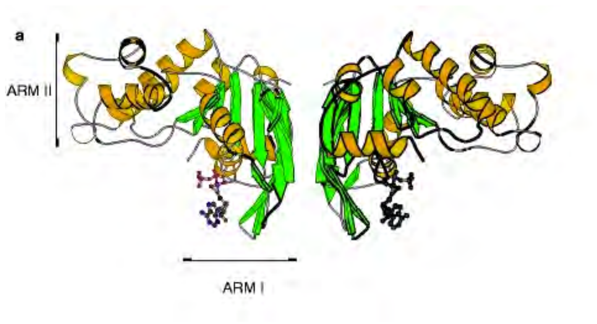
	Subfamily	Human ABC proteins	Physiological role (known and probable)	Disease	Structure	Select citation(s)
ABC1	B: multidrug resistance, MDR, 11 members	ABCB1	Efflux of xenobiotics	Multidrug resistance	Kim and Chen 2018	Riordan et al. 1985
		ABCB2	Peptide transport associated with antigen processing	Immune 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	Cholesterol and plant sterol efflux	β-Sitosterolemia	Lee et al. 2016	Berge et al. 2000
		ABCG8				
	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 grou[…]9) and Zheng et al. (2013). Physiological function and disease phenotypes were obtained from www.genecards.org and https:/[…] ‡, which indicate bovine, yeast, and rabbit homologs, respectively.

(Xavier et al, BCB, 2019)

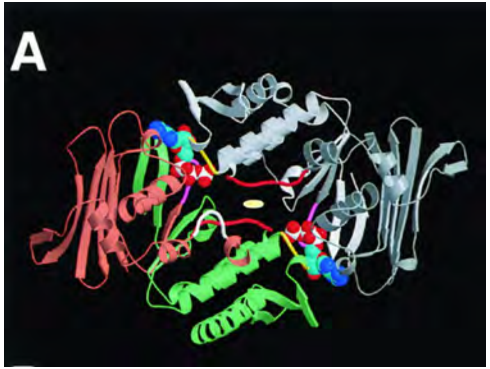
ABC transporter structures have come a long way …

Crystal structure of HisP
- ATP-binding subunit
- Right structure, wrong model



(Hung et al, Nature, 1998)

Crystal structure of Rad50
- ATP-binding cassette
- Right structure, right model

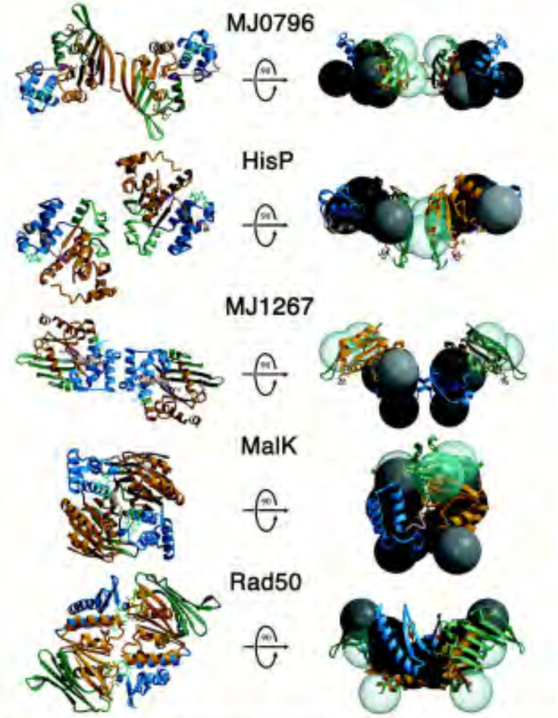


(Hopfner et al, Cell, 2000)

ABC transporter structures have come a long way …

Crystallographic dimer ≠ biological dimer

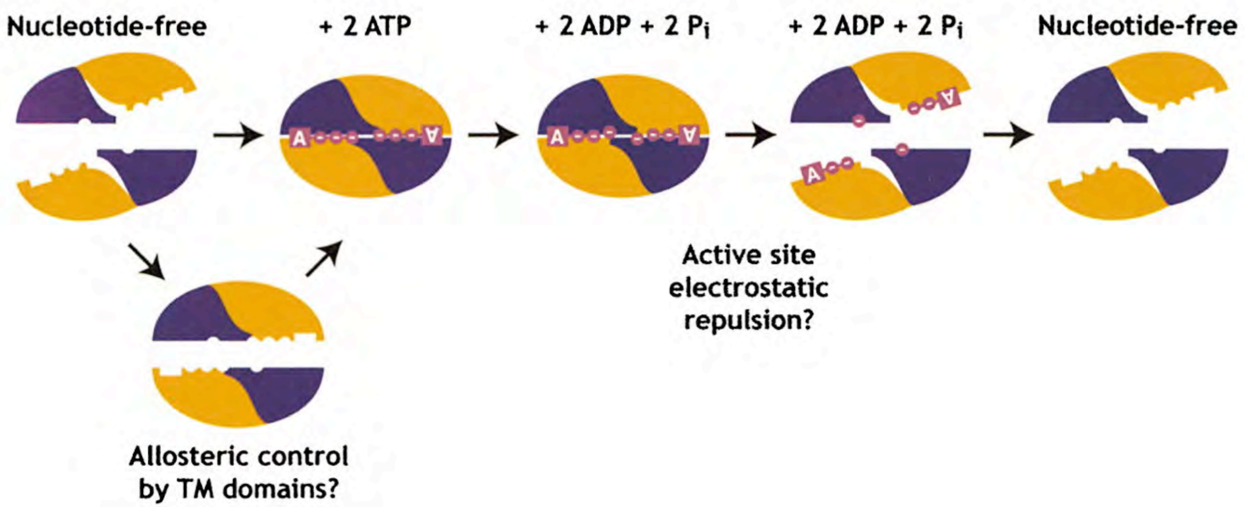
(Yuan et al, J Biol Chem, 2001)



	βabcβ1-βabcβ2	[illegible]	βabcβ3-βabcβ4	[illegible]	[illegible]	[illegible]	βcore4-βcore7	[illegible]	MJ0796 Dimer	HisP Dimer	MJ1267 Dimer	MalK Dimer	Rad50 Dimer
Buried ASA	654	330	255	479	331	351	334	296	406	462	645	1509	1502
Surface Complementarity	0.69	0.74	0.74	0.70	0.75	0.66	0.71	0.75	0.60	0.54	0.74	0.69	0.75
vdW Contacts	54	35	21	69	69	29	30	23	36	39	72	112	146
H-bond Contacts	12	5	8	10	7	4	8	4	4	9	8	12	20

ABC transporter structures have come a long way …

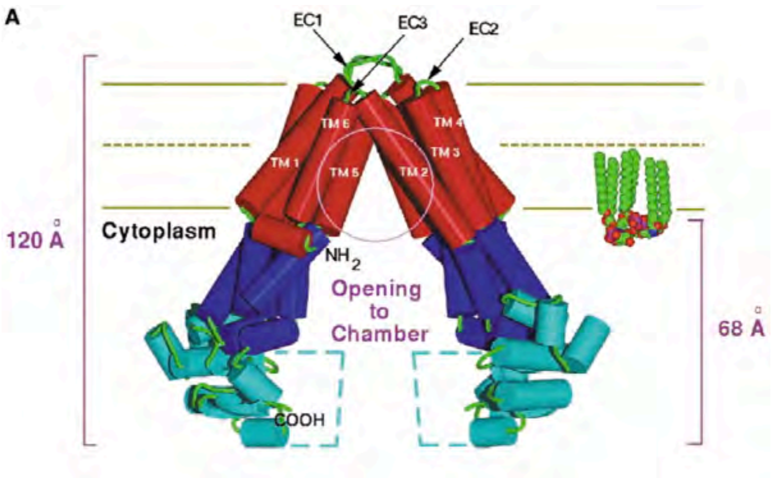
ATP sandwich model



(Smith et al, Mol Cell, 2002)

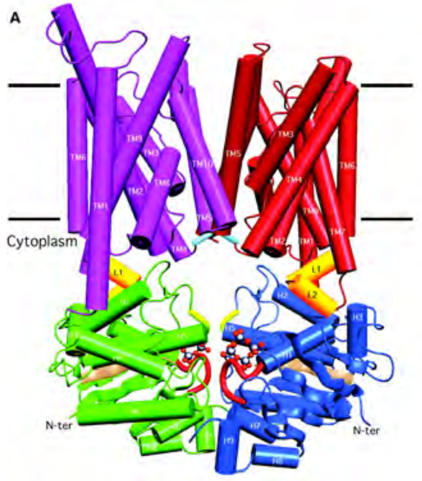
ABC transporter structures have come a long way …

Crystal structure of MsbA
- Full transporter structure
- Wrong model



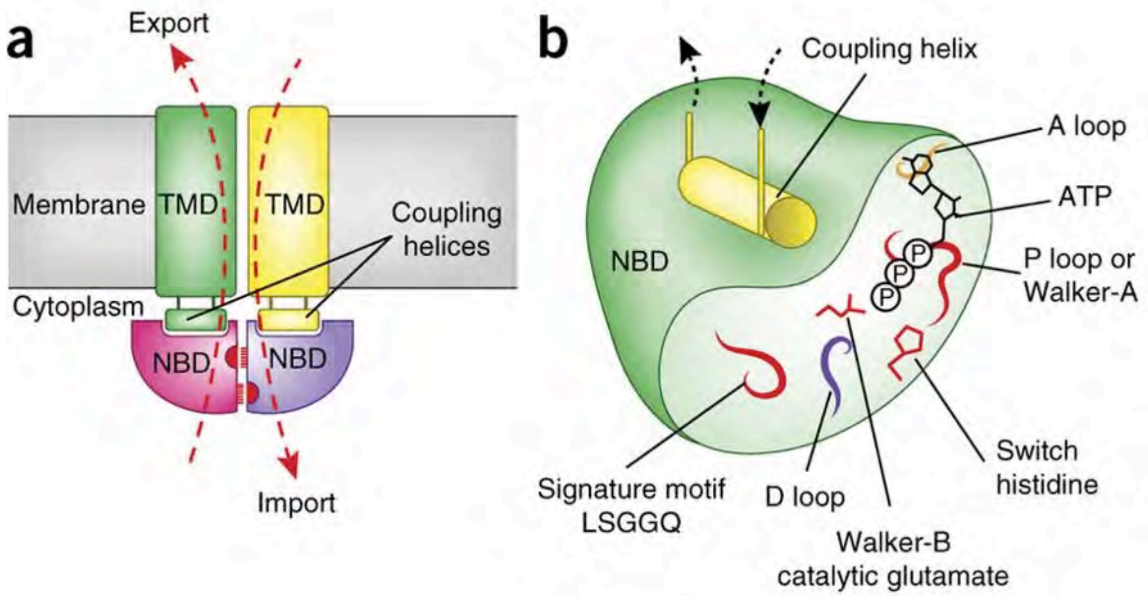
(Chang & Roth, Science, 2001; retracted 2006)

Crystal structure of BtuCD
- Full transporter structure
- Correct model



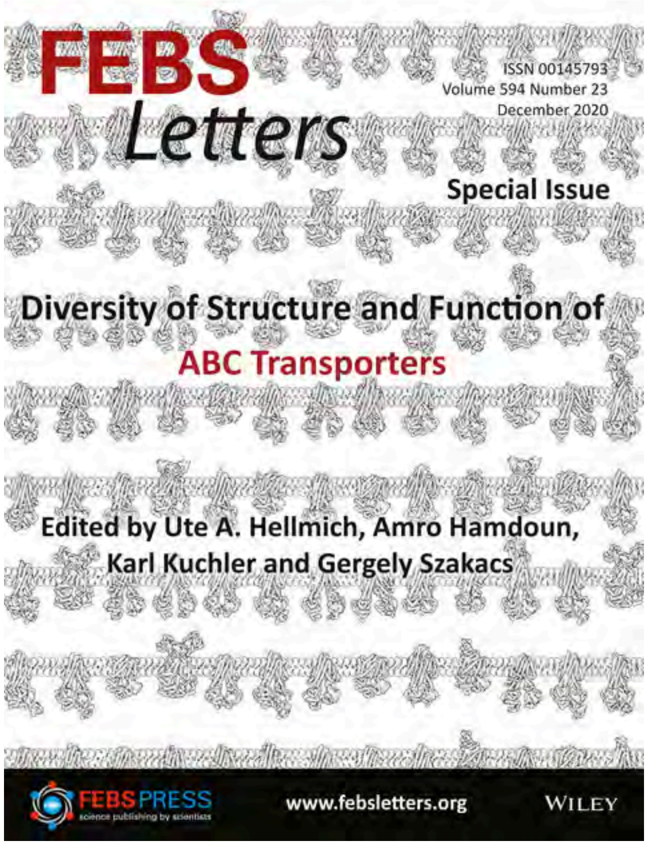
(Locher et al, Science, 2002)

ABC-coupled transport: a simple idea

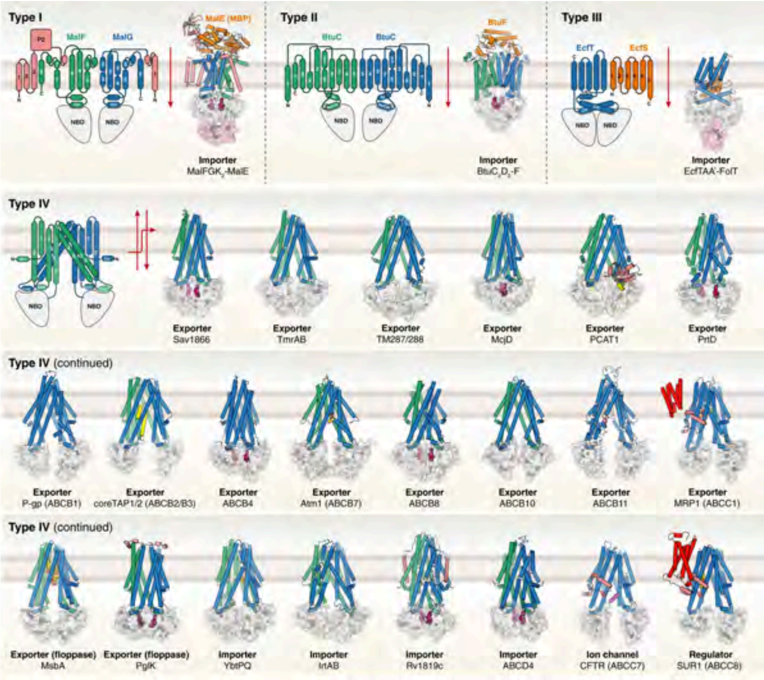


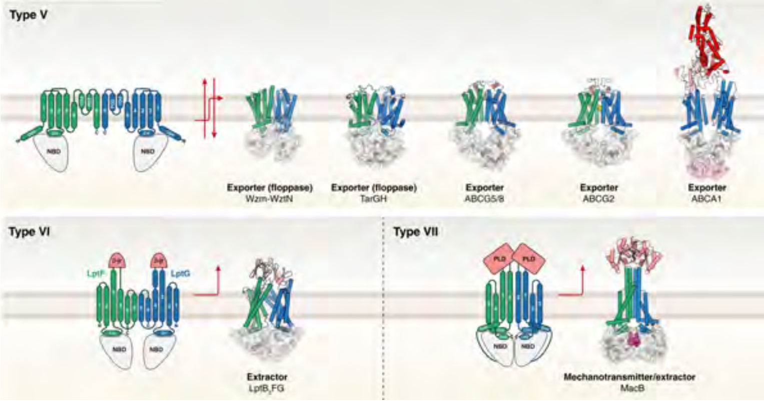
(Locher, Nat Struct Mol Biol, 2016)

ABC-coupled transport: not that simple!



ABC-coupled transport: not that simple!





(Thomas et al, FEBS Lett, 2020)

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.

Part II:

STRUCTURAL STUDIES OF MULTIDRUG RESISTANCE TRANSPORTERS

Multidrug Resistance (MDR) in Mammalian Cells

Biochimica et Biophysica Acta, 455 (1976) 152–162

BBA 77508

A SURFACE GLYCOPROTEIN MODULATING DRUG PERMEABILITY IN CHINESE HAMSTER OVARY CELL MUTANTS

R. L. JULIANO [a, c] and V. LING [b, c]

[a] *Research Institute, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario,* [b] *The Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario and* [c] *The Department of Medical Biophysics, University of Toronto, 500 Sherbourne Street, Toronto, Ontario (Canada)*

(Received April 23rd, 1976)

Multidrug Resistance (MDR) in Mammalian Cells

[CANCER RESEARCH 43, 4413–4419, September 1983]

Daunorubicin-resistant Chinese Hamster Ovary Cells Expressing Multidrug Resistance and a Cell-Surface P-Glycoprotein[1]

Norbert Kartner,[2] Michael Shales,[3] John R. Riordan, and Victor Ling

Ontario Cancer Institute, Princess Margaret Hospital and Department of Medical Biophysics, University of Toronto, Toronto M4X 1K9 [N. K., M. S., V. L.], and Research Institute, The Hospital for Sick Children and Departments of Biochemistry and Clinical Biochemistry, University of Toronto, Toronto M5G 1X8 [J. R. R.], Ontario, Canada

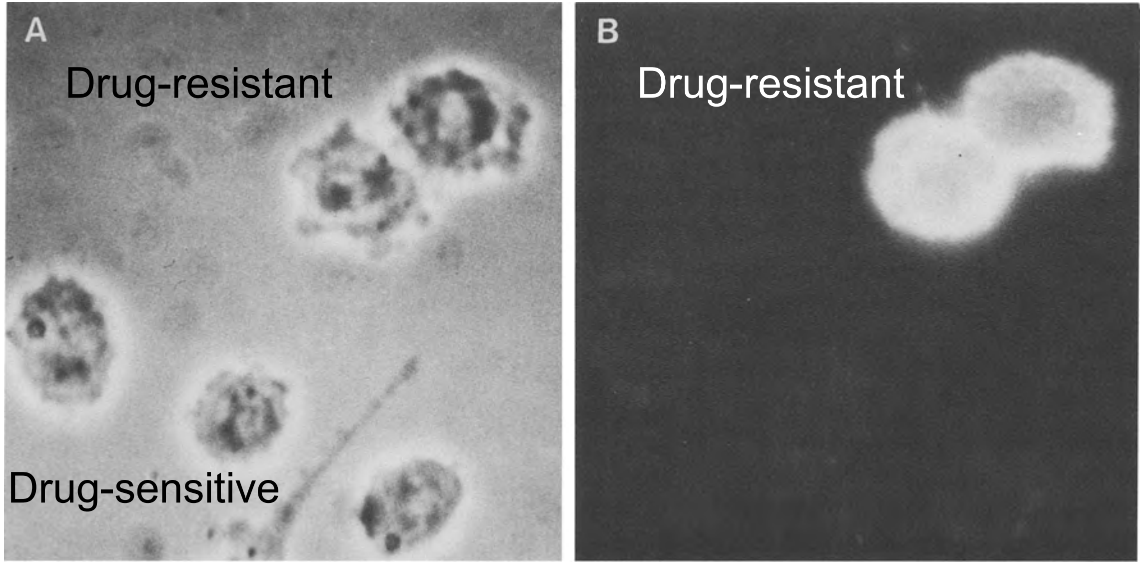
Breast Cancer Research and Treatment, 4, 89–94 (1984)

6th Annual San Antonio Breast Cancer Symposium

Multidrug resistance

V. Ling, J. Gerlach, and N. Kartner
Department of Medical Biophysics, University of Toronto and the Ontario Cancer Institute, 500 Sherbourne Street, Toronto, Ontario, Canada M4X 1K9

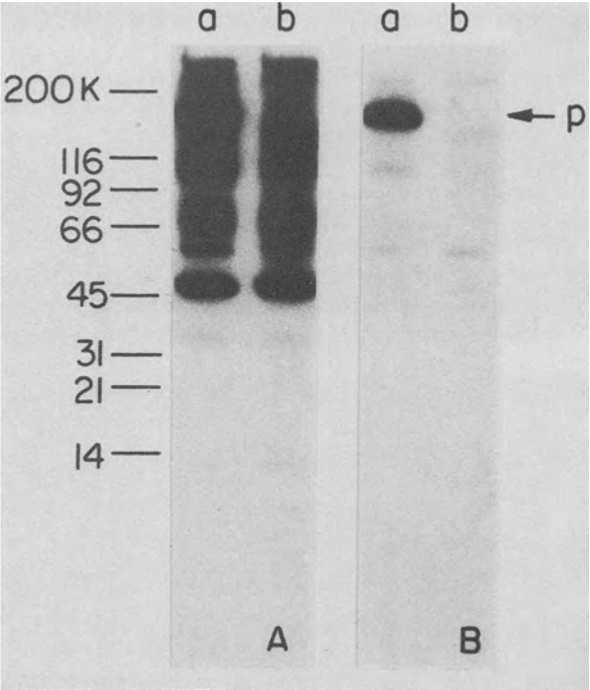
P-glycoprotein (Pgp/MDR1/ABCB1): the first mammalian ABC transporter reported.



(phase contrast) (fluorescence w/ Ab)

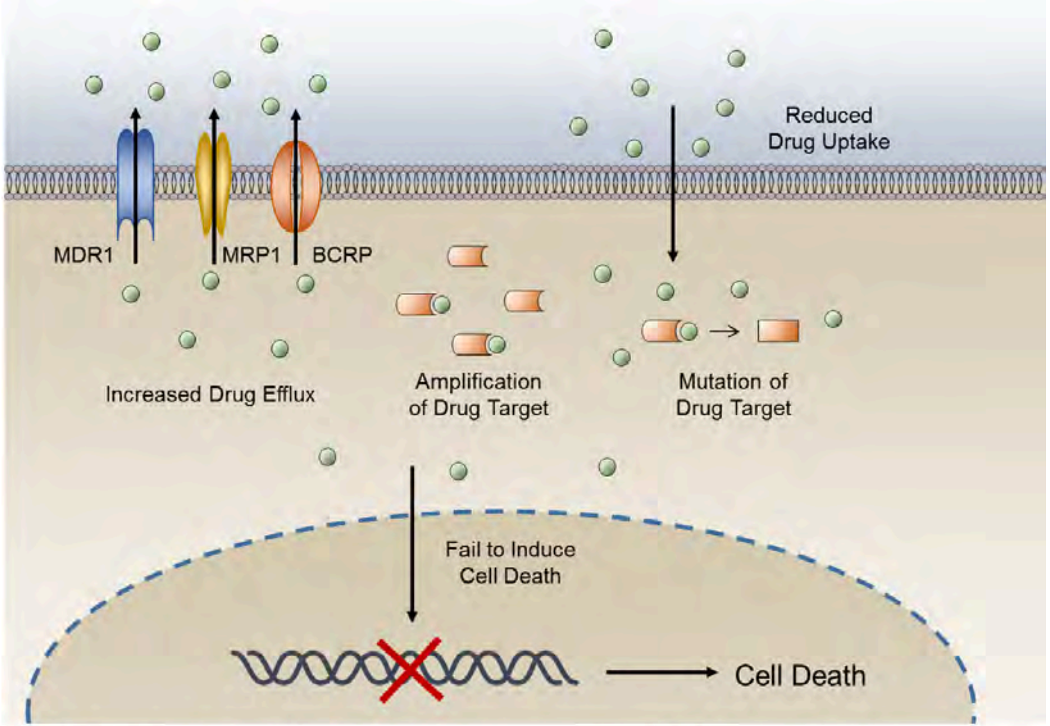
(Ling et al, Breast Cancer Res Treat, 1984)

P-glycoprotein (Pgp/MDR1/ABCB1): the first mammalian ABC transporter reported.



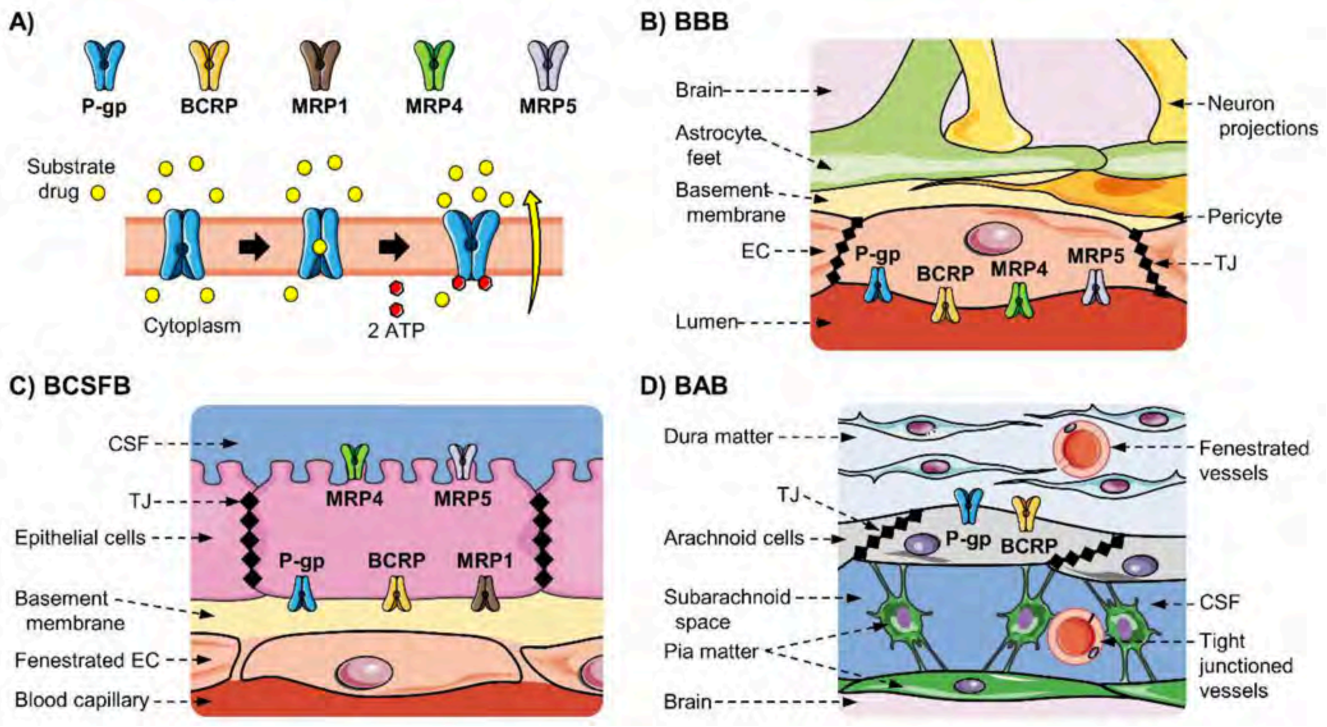
(Ling et al, Breast Cancer Res Treat, 1984)

MDR1 (ABCB1), MRP1 (ABCC1) and BCRP (ABCG2)



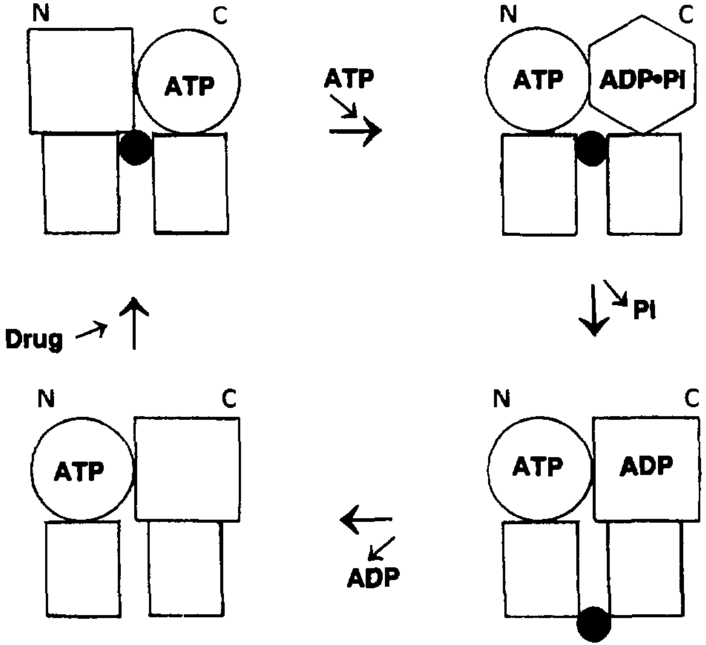
(Liu et al, Oncotarget, 2016)

MDR1 (ABCB1), MRP1 (ABCC1) and BCRP (ABCG2)



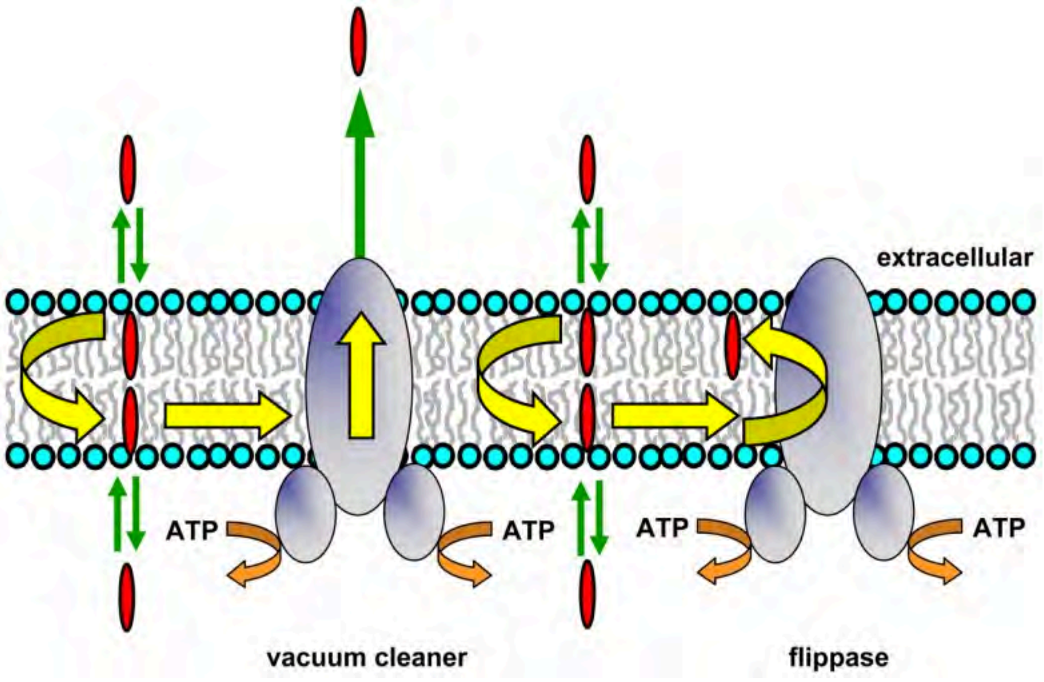
(Gomez-Zepeda et al, Pharmaceutics, 2019)

P-glycoprotein as the model system for MDR



(Senior et al, FEBS Lett, 1995)

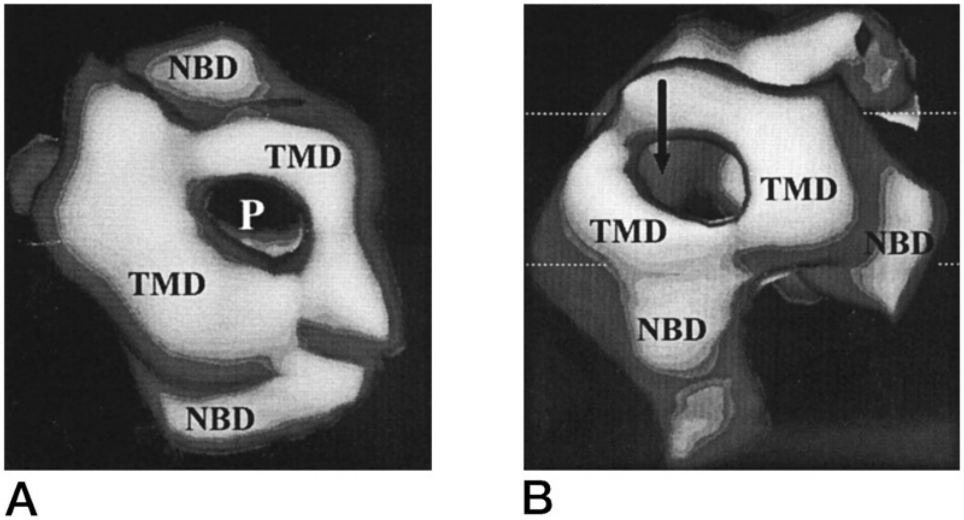
P-glycoprotein as the model system for MDR



(Sharom, Front Oncol, 2014)

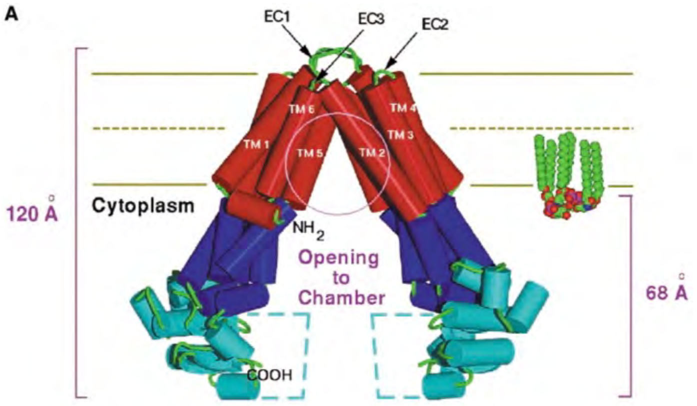
P-glycoprotein as the model system for MDR

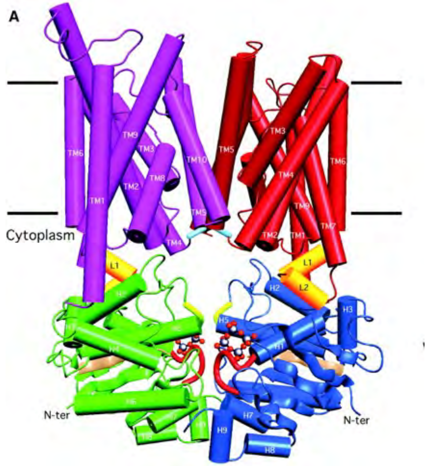
Low-resolution EM models:

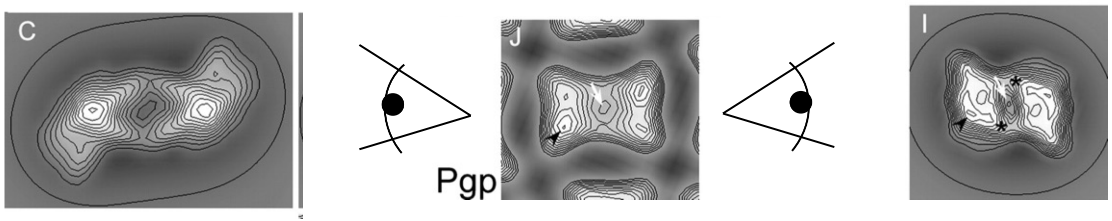


(Rosenberg et al, J Biol Chem, 1997)

P-glycoprotein as the model system for MDR

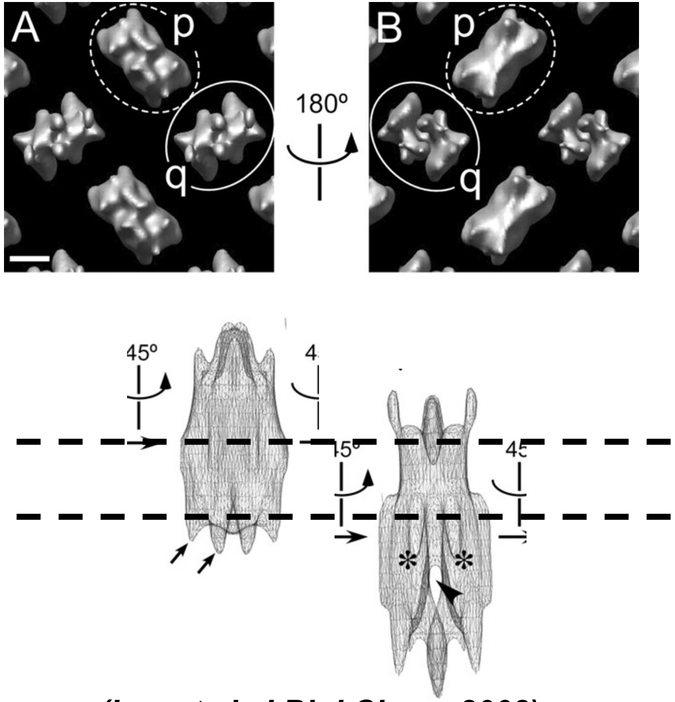




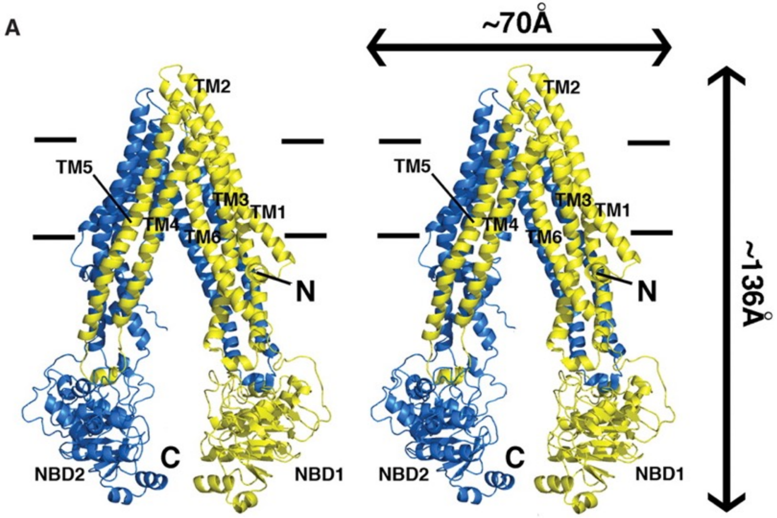


(Lee et al, J Biol Chem, 2002)

P-glycoprotein as the model system for MDR



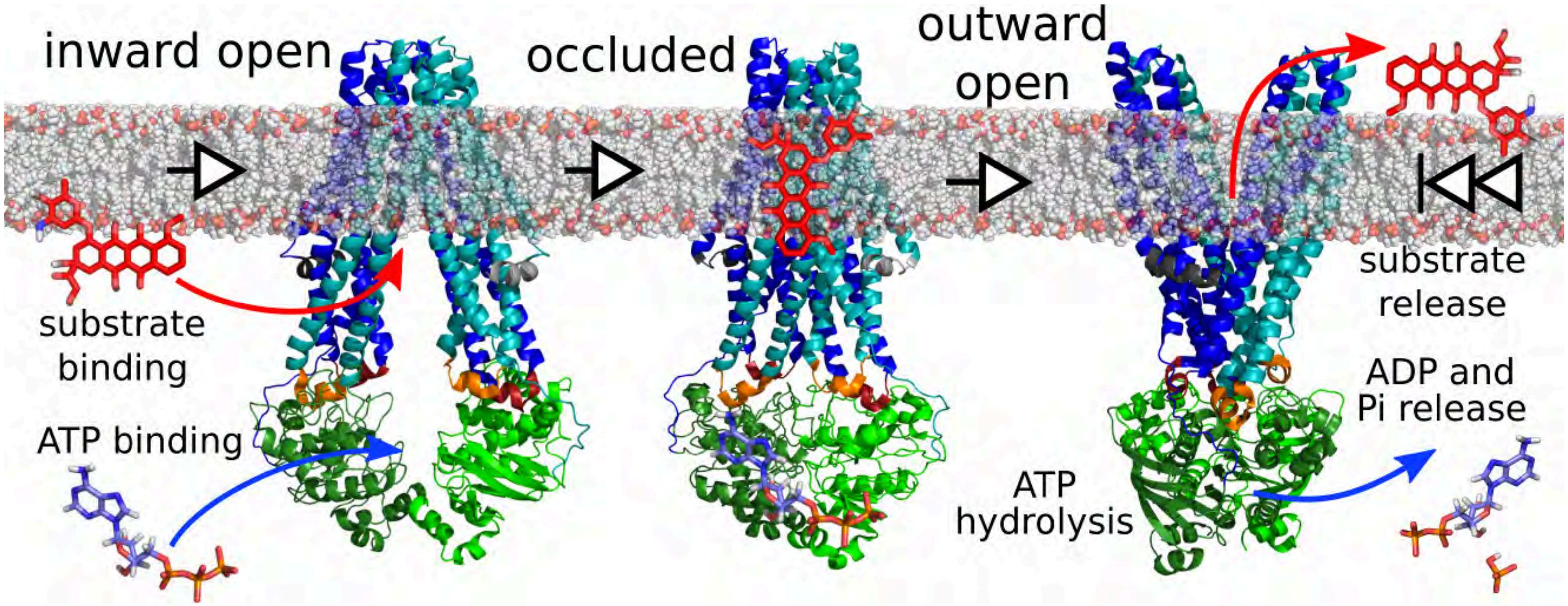
(Lee et al, J Biol Chem, 2008)



(Aller et al, Science, 2009)

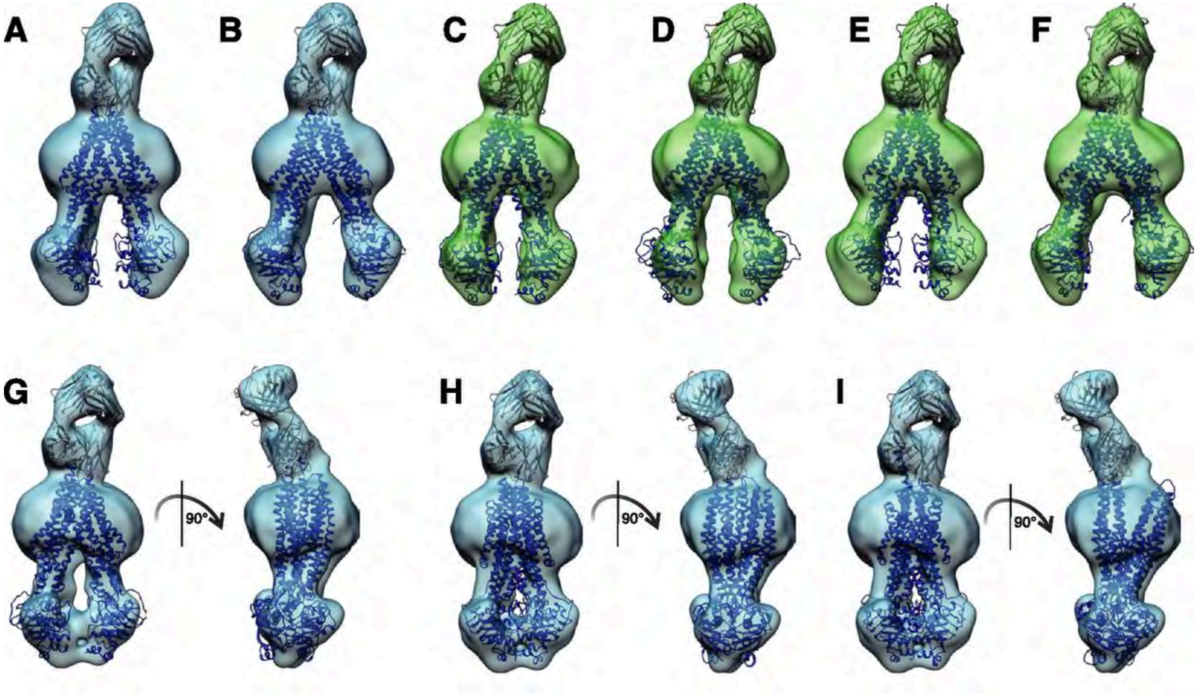
Conformational Landscape of P-glycoprotein

(Bacterial homolog MsbA)



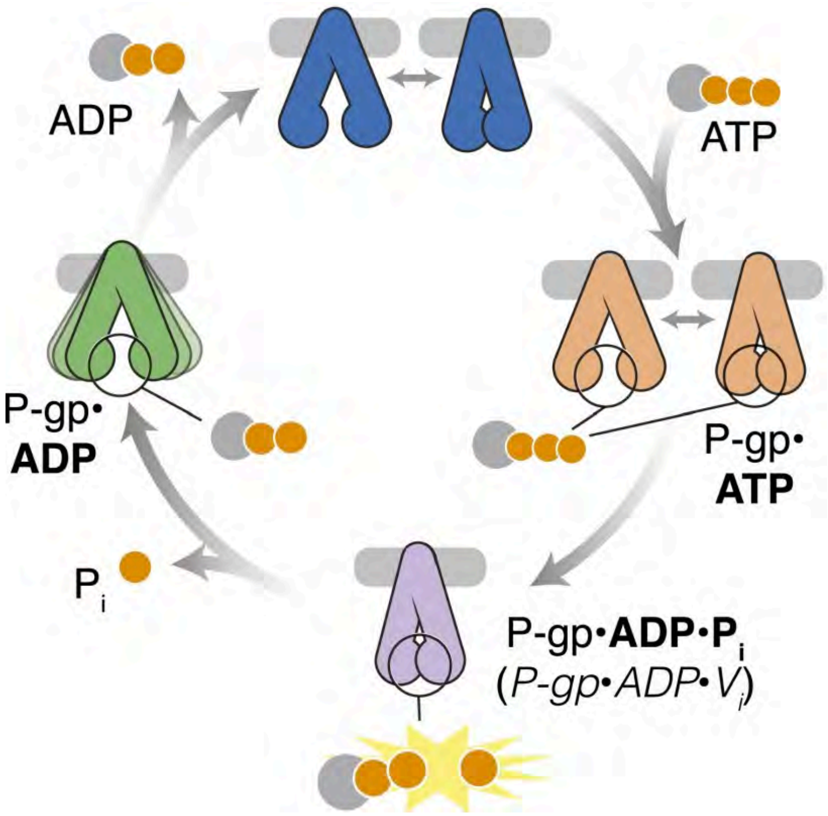
(Szöllősi et al, Biochim Biophys Acta Biomembr, 2018)

Conformational Landscape of P-glycoprotein



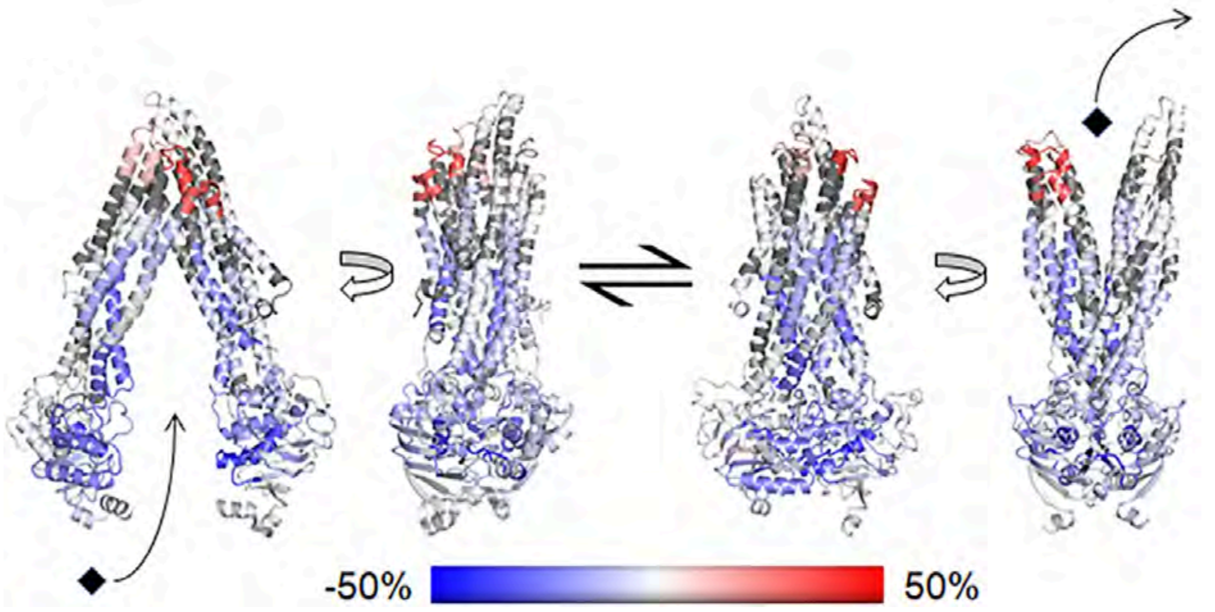
(Frank et al, Mol Pharmacol, 2016)

Conformational Landscape of P-glycoprotein



Dynamics of P-glycoprotein

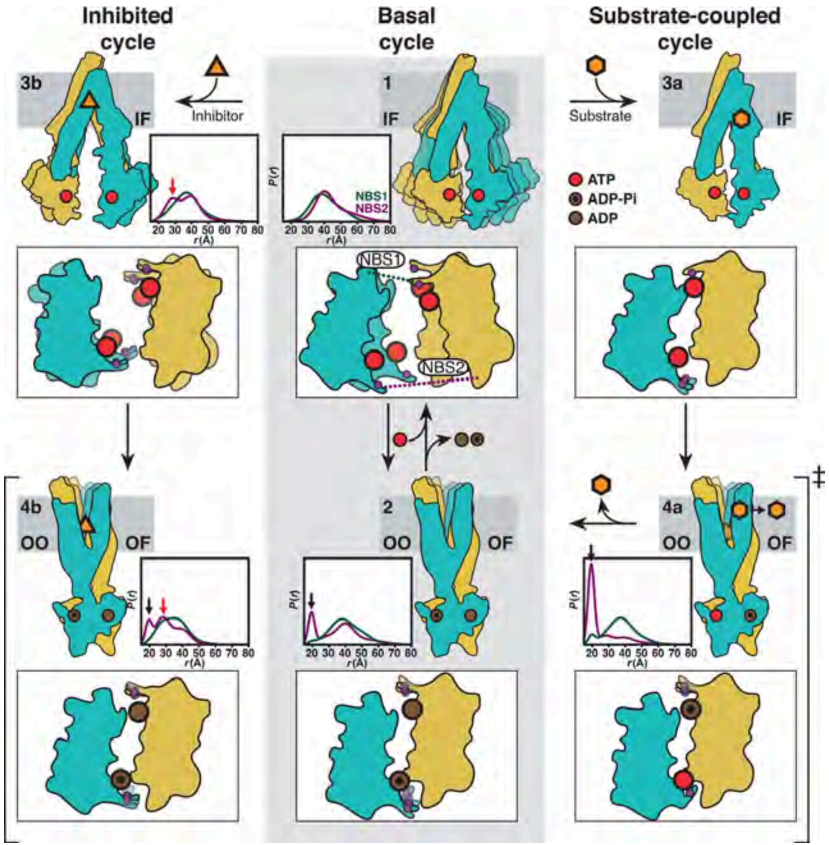
hydrogen-deuterium exchange mass spectrometry (HDX-MS)



(Kopcho et al, Sci Rep, 2019)

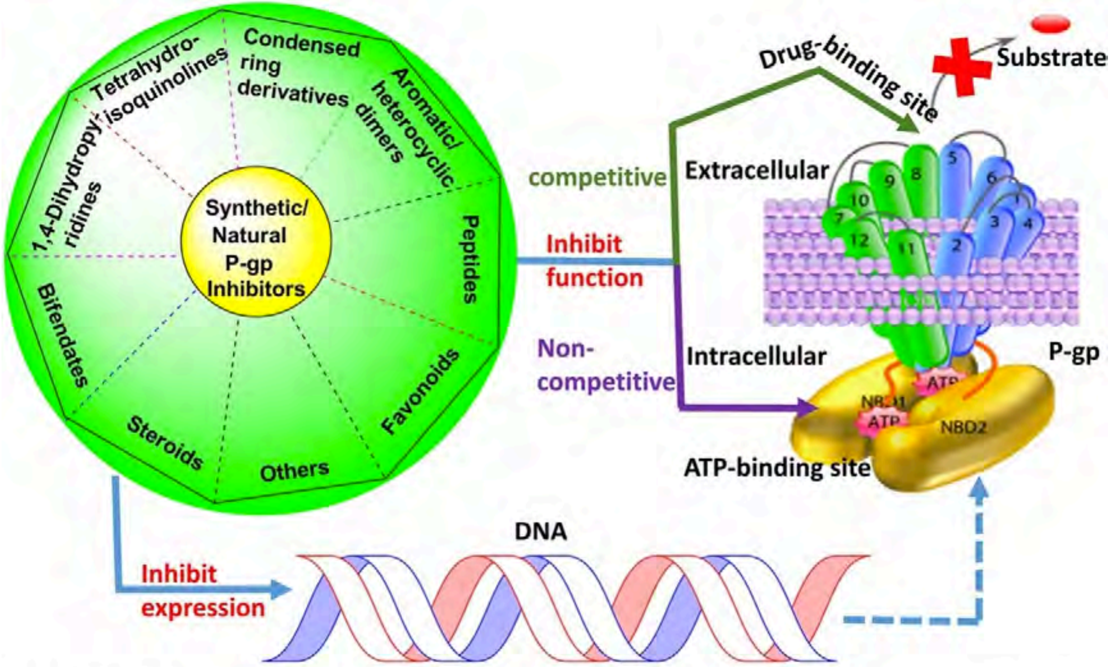
Dynamics of P-glycoprotein

Double electron electron resonance (DEER) spectroscopy



(Dastvan et al, Science, 2019)

P-glycoprotein Inhibition-based Drug Discovery

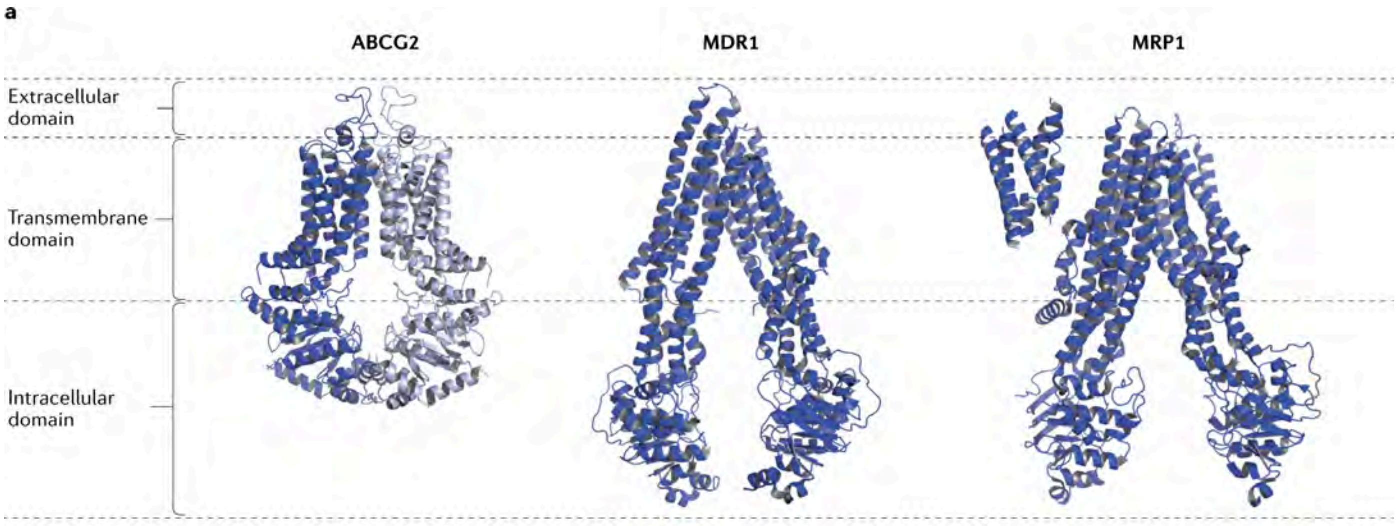


A wide variety of chemically diverse compounds could reverse MDR in resistant cancer cells *via* inhibition of P-gp function/expression.

(Dong et al, Drug Resist Updat, 2020)

Beyond P-glycoprotein

ABCG2/BCRP/MXR **ABCC1/MRP1**

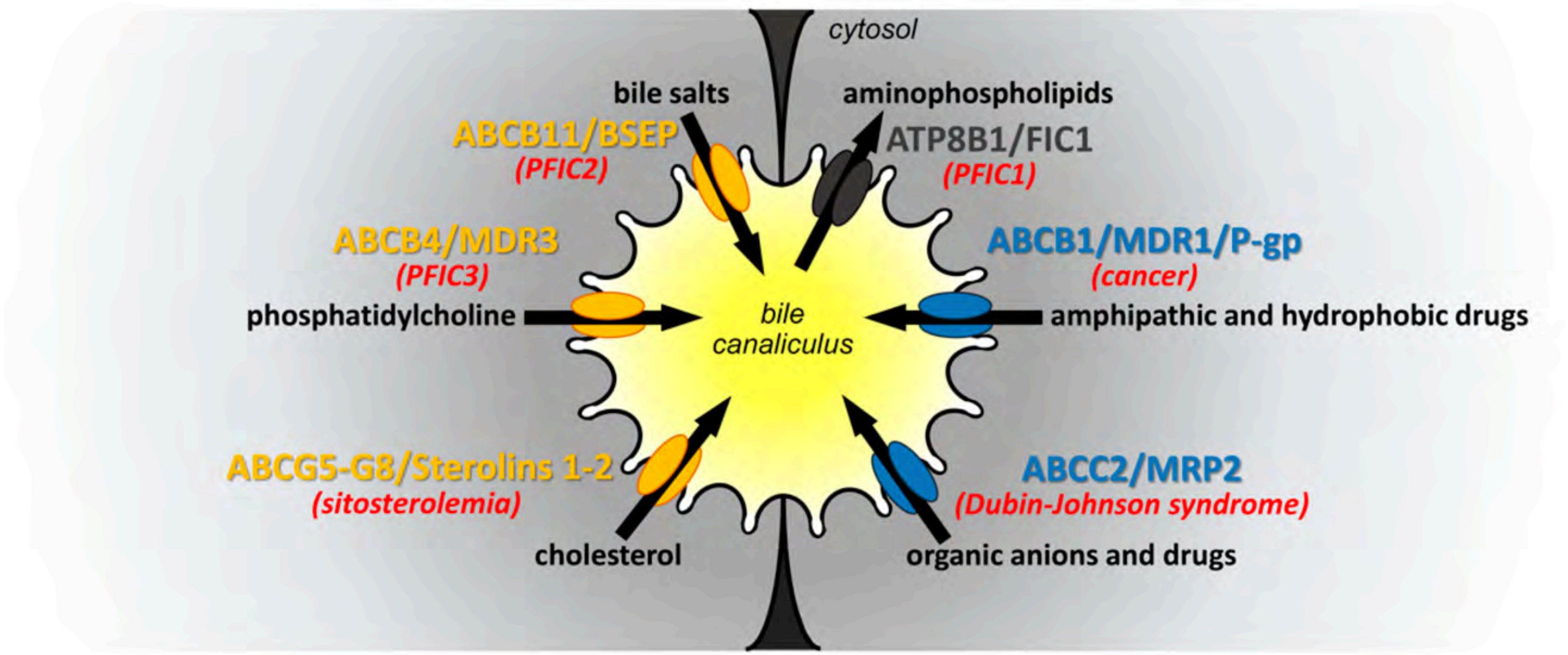


(Robey et al, Nat Rev Cancer, 2018)

Part III:

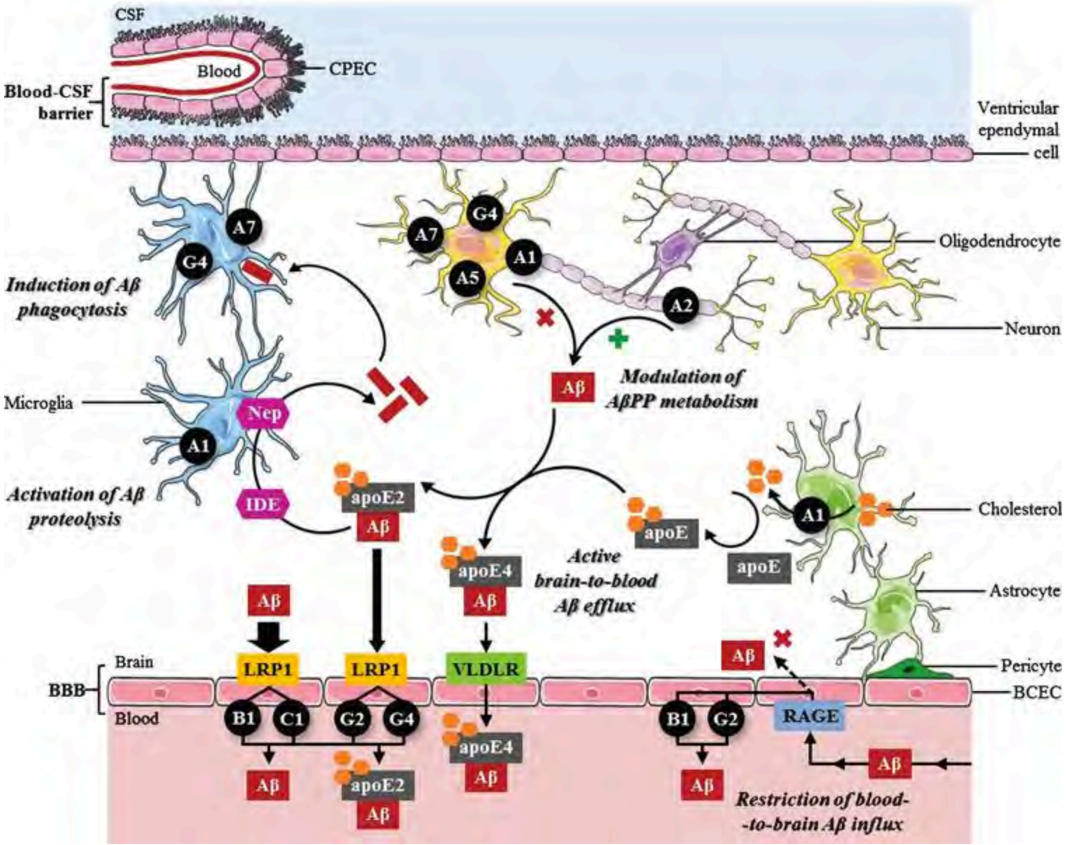
STRUCTURAL STUDIES OF LIPID TRANSPORTERS

Canalicular ABC Transporters



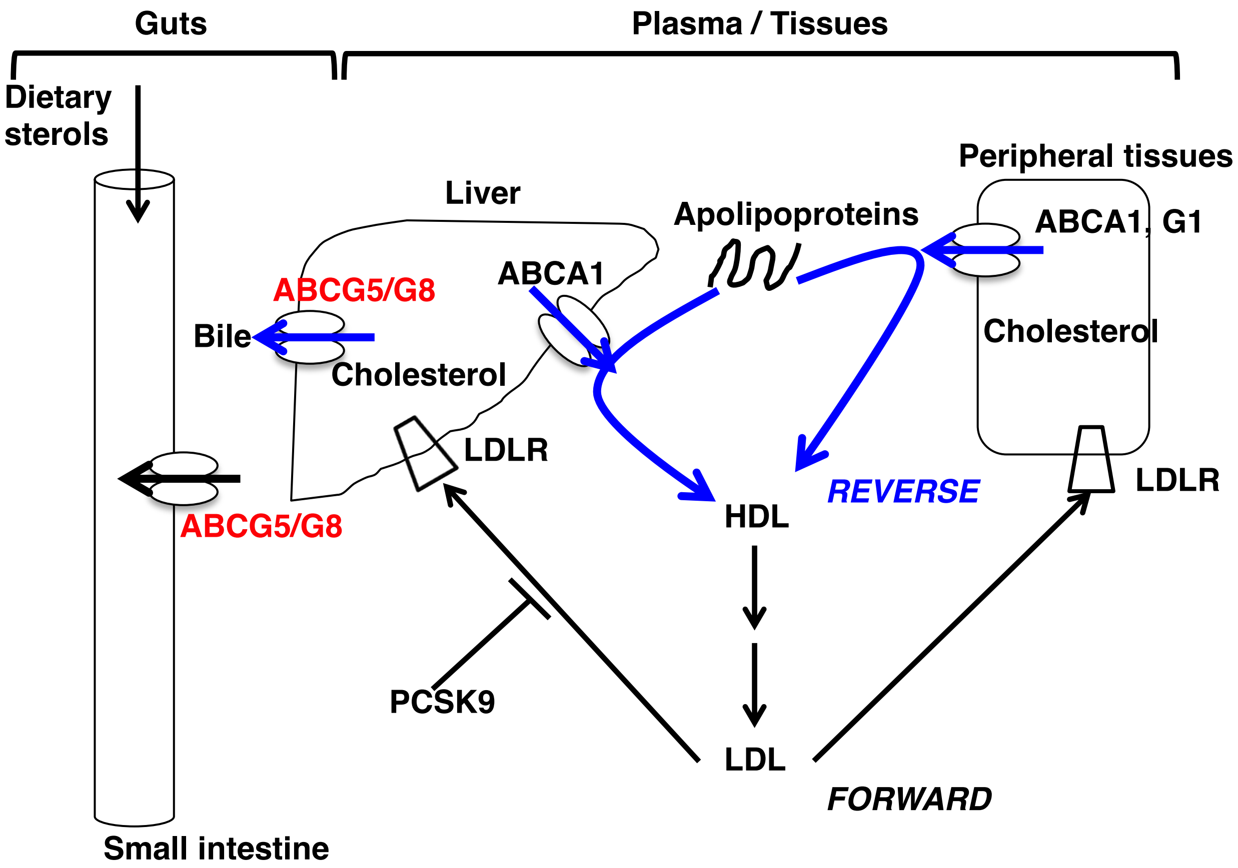
(Saad et al, Int J Mol Sci, 2021)

ABC Transporters in the Brain

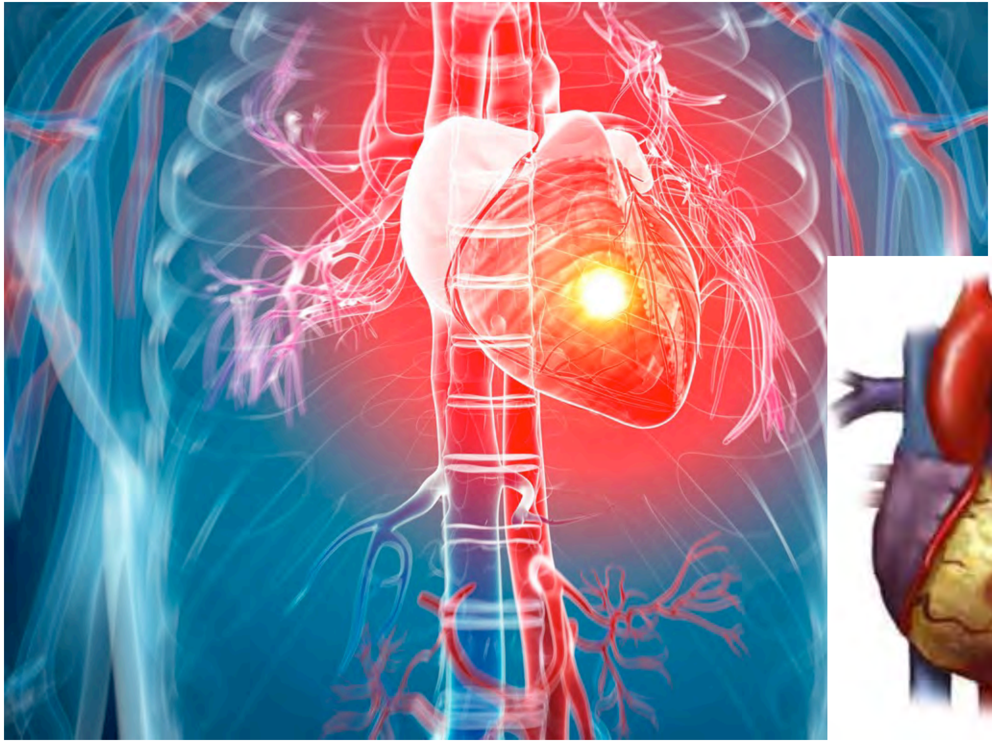


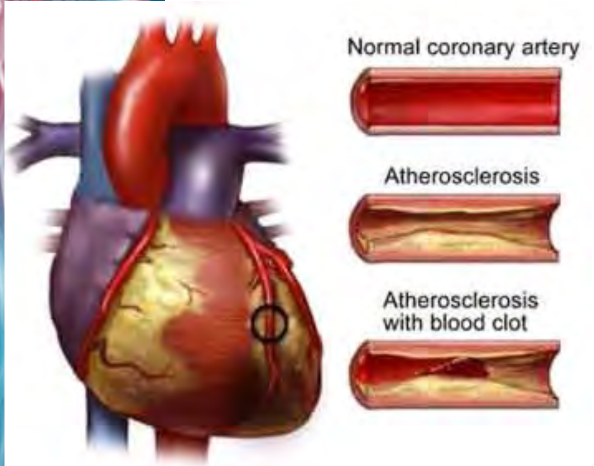
(Pereira et al, J Alzheimers Dis, 2018)

Transporting Cholesterol (A Simple View)



Cholesterol: a Risk Factor of Cardiovascular Diseases



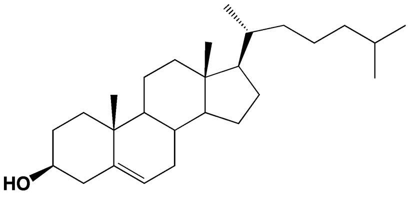


ABCG5/G8: patients, genetics, animal model

ABCG5/G8: human nutrition

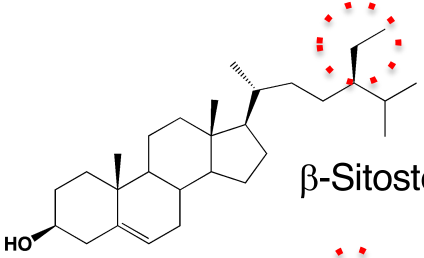
Dietary sterols

Animal (60%)

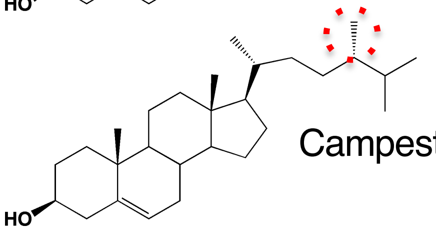


Cholesterol

Plant (40%)



β-Sitosterol



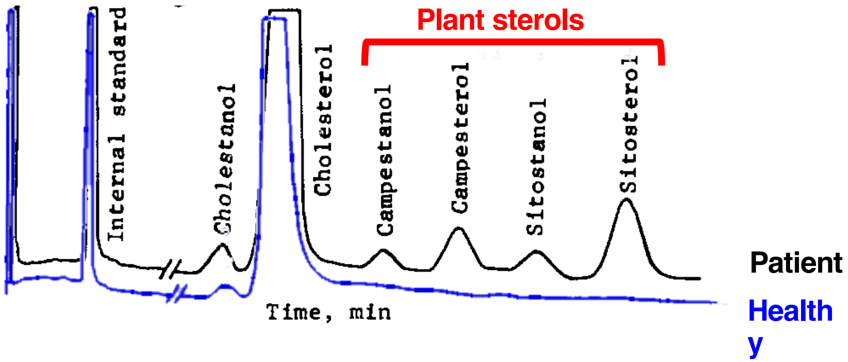
Campesterol

ABSORPTION: ~50% | < 5%

ABCG5/G8: patients

Sitosterolemia

(Bhattacharyya & Conner, 1974, JCI)



<u>14 patients:</u>

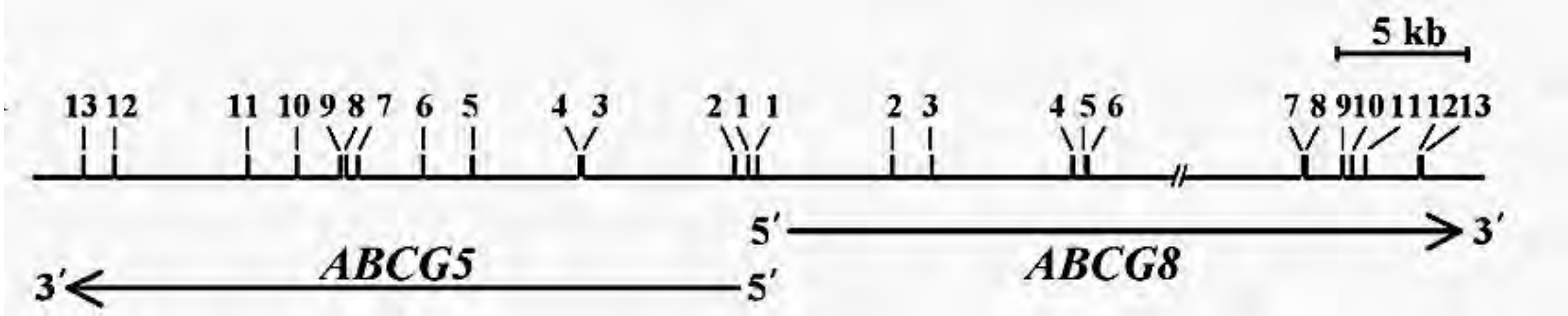
	Healthy	Sitosterolemia
Sitosterol (mg/dL)	0.3 ± 0.3	**35 ± 16 (50-120x)**
Cholesterol (mg/dL)	187 ± 29	258 ± 29

(Salen et al, J Lipid Res, 1985)

ABCG5/G8: genetics

Autosomal recessive & rare genetic disorder

(2p21)

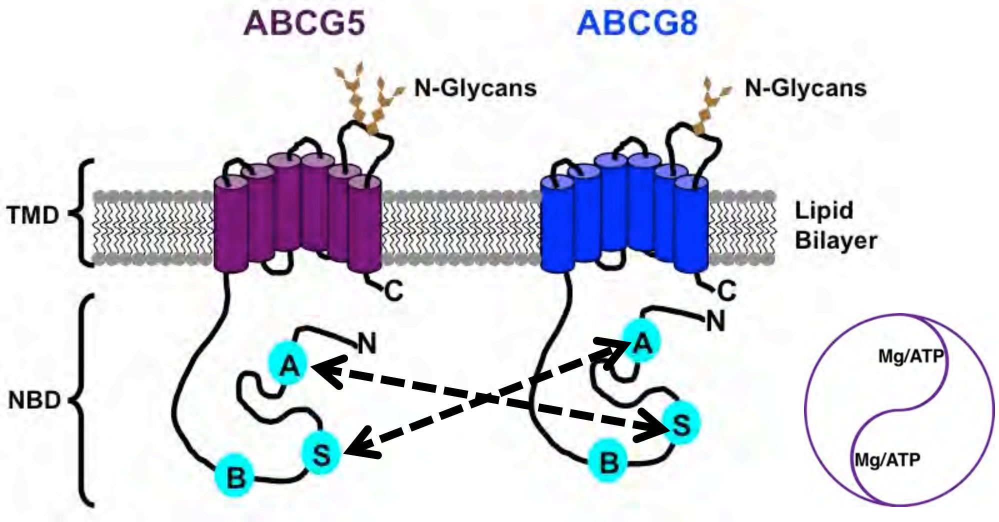


(Berge et al, 2000, Science; Lee et al, 2001, Nat Genet; Lu et al, 2001, AJMG)

ABCG5 and ABCG8 are half ABC transporters.

Transmembrane domain

Nucleotide-binding domain



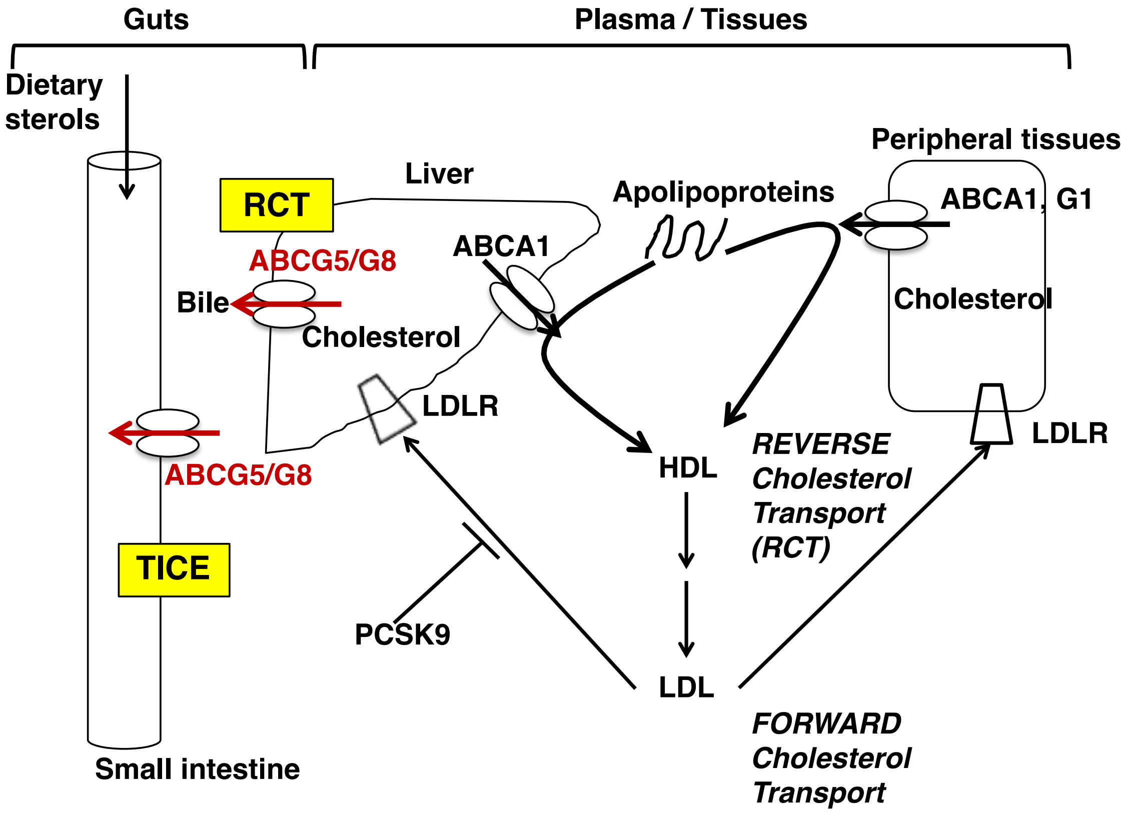
A: Walker A motif
(GxxGxGKS/T)
B: Walker B motif
(φφφφDE)

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

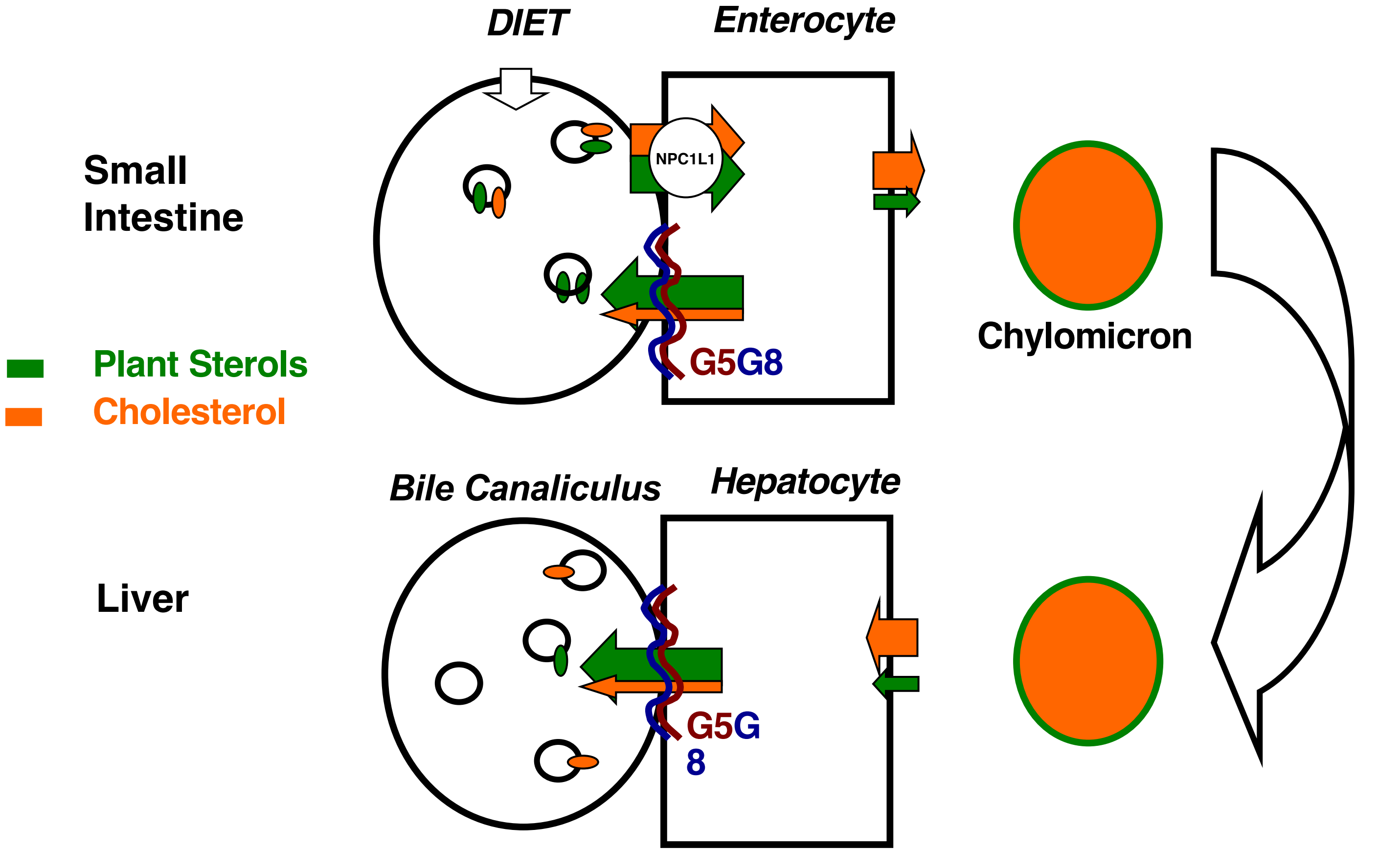
ABCG5/G8: genetics

Last stop of reverse cholesterol transport (RCT)

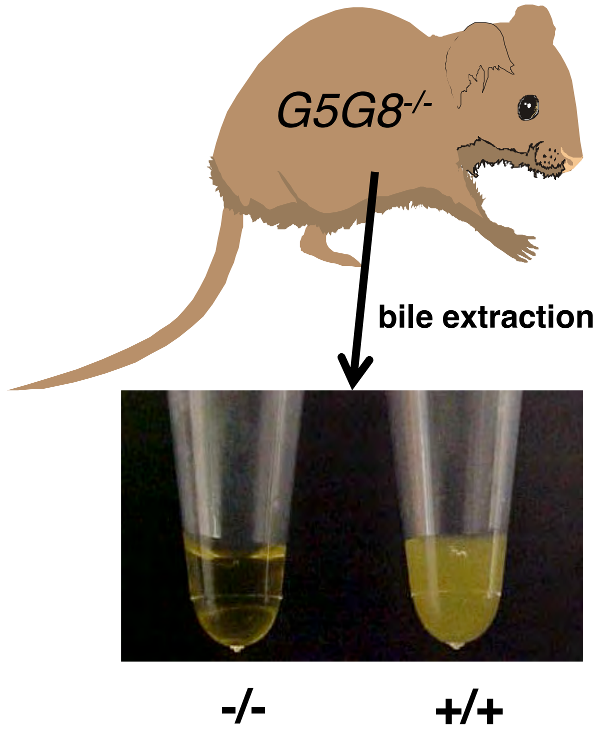
Trans-intestinal cholesterol efflux (TICE)



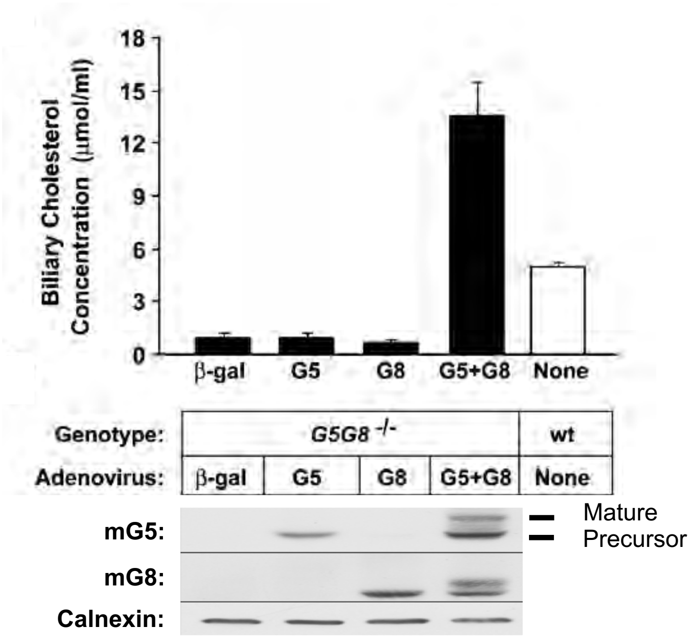
ABCG5/G8 promotes biliary and intestinal sterol secretion (liver/small intestine specific).



ABCG5/G8: animal model



(Yu et al, 2002, PNAS)



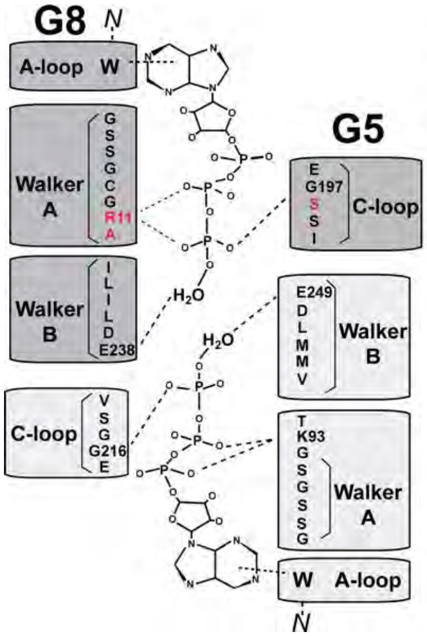
(Graf et al, 2003, JBC)

ABCG5/G8: animal model

Functional asymmetry

NBS1 (inactive)

NBS2 (active)

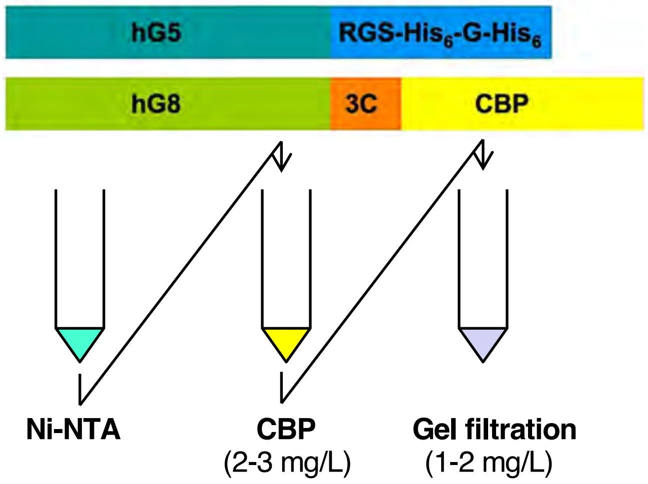


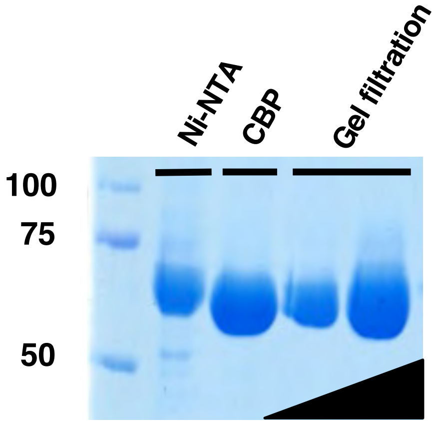
	Walker A	Q-loop	C-loop	Walker B	D-loop	Switch
	G8	G8	G5	G8	G5	G8
Human	GSSGCGRA	VRQHN	ISTGE	ILILDE	TGLD	SLHQP
Mouse	GSSGCGRA	VRQHD	ISSGE	ILILDE	TGLD	SLHQP
Cow	GSSGCGRA	VRQHD	ISHGE	ILILDE	TGLD	SIHQP
Opossum	GSSGYGKS	VRQHD	ISNGE	ILILDE	TGLD	SLHQP
Chicken	GSTAGGKT	VRQDD	ISGGE	ILILDE	TGLD	SLHQP
Frog	GNTGCGKT	VRQDD	ISSGE	ILILDE	TGLD	SIHQP
Zebrafish	GSSGCGKT	VRQDD	ISGGE	ILILDE	TGLD	SVHQP
	G5	G5	G8	G5	G8	G5
Human	GSSGSGKT	VLQSD	LSGGE	VMLFDE	SGLD	TIHQP
Mouse	GSSGSGKT	VLQSD	VSGGE	VMMLDE	SGLD	TIHQP
Cow	GSSGSGKT	VLQSD	VSGGE	VMLFDE	SGLD	TIHQP
Opossum	GSSGSGKT	ALQNE	VSGGE	VMLFDE	SGLD	TIHQP
Chicken	GNSGSGKT	VPQND	ISGGE	VMLLDE	SGLD	TIHQP
Frog	GNSGSGKT	VLQHD	VSGGE	IILLDE	SGLD	SIHQP
Zebrafish	GNSGSGKT	VLQSD	VSGGE	VILLDE	SGLD	TIHQP
CONSENSUS	GXXGXGKS/T	XXQXX	φSGGQ	φφφφD	S/TXLD	XXHXX

(Zhang et al, 2006, JBC; Wang et al, 2011, JBC)

Large-scale Purification of Human G5G8

Tandem Affinity Chromatography:
(*Pichia pastoris* yeast)



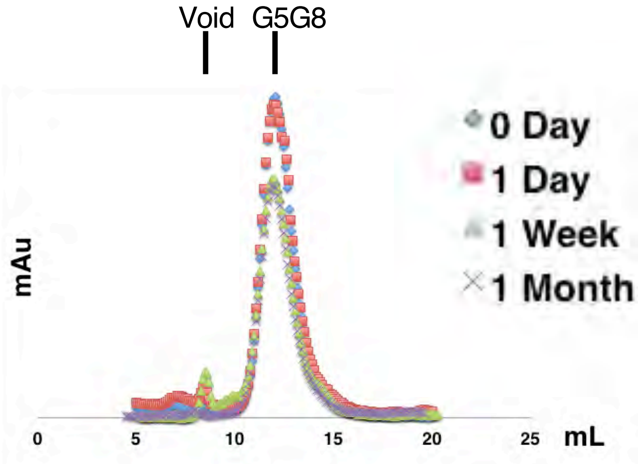


3C: 3C cleavage site

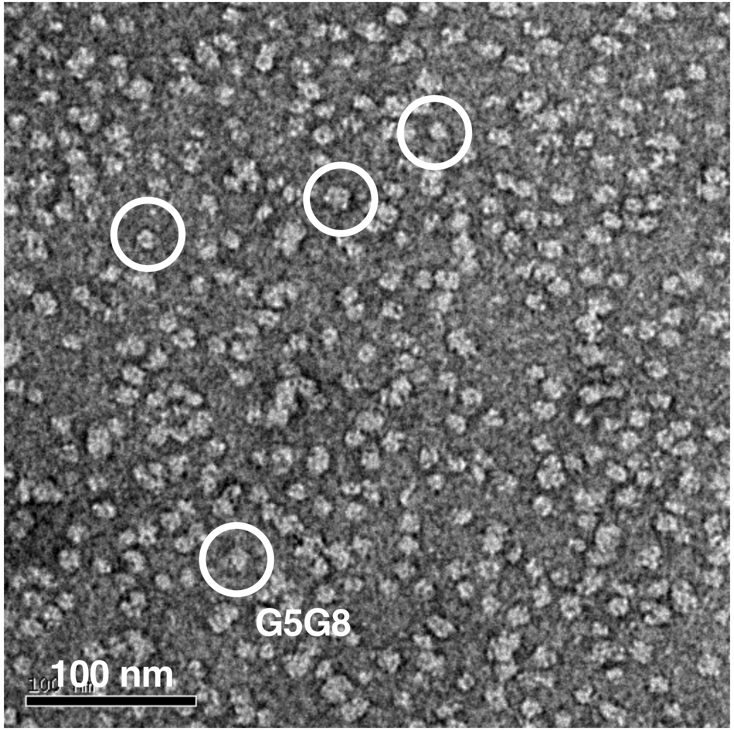
CBP: calmodulin-binding peptide

Stable and Monodisperse G5G8

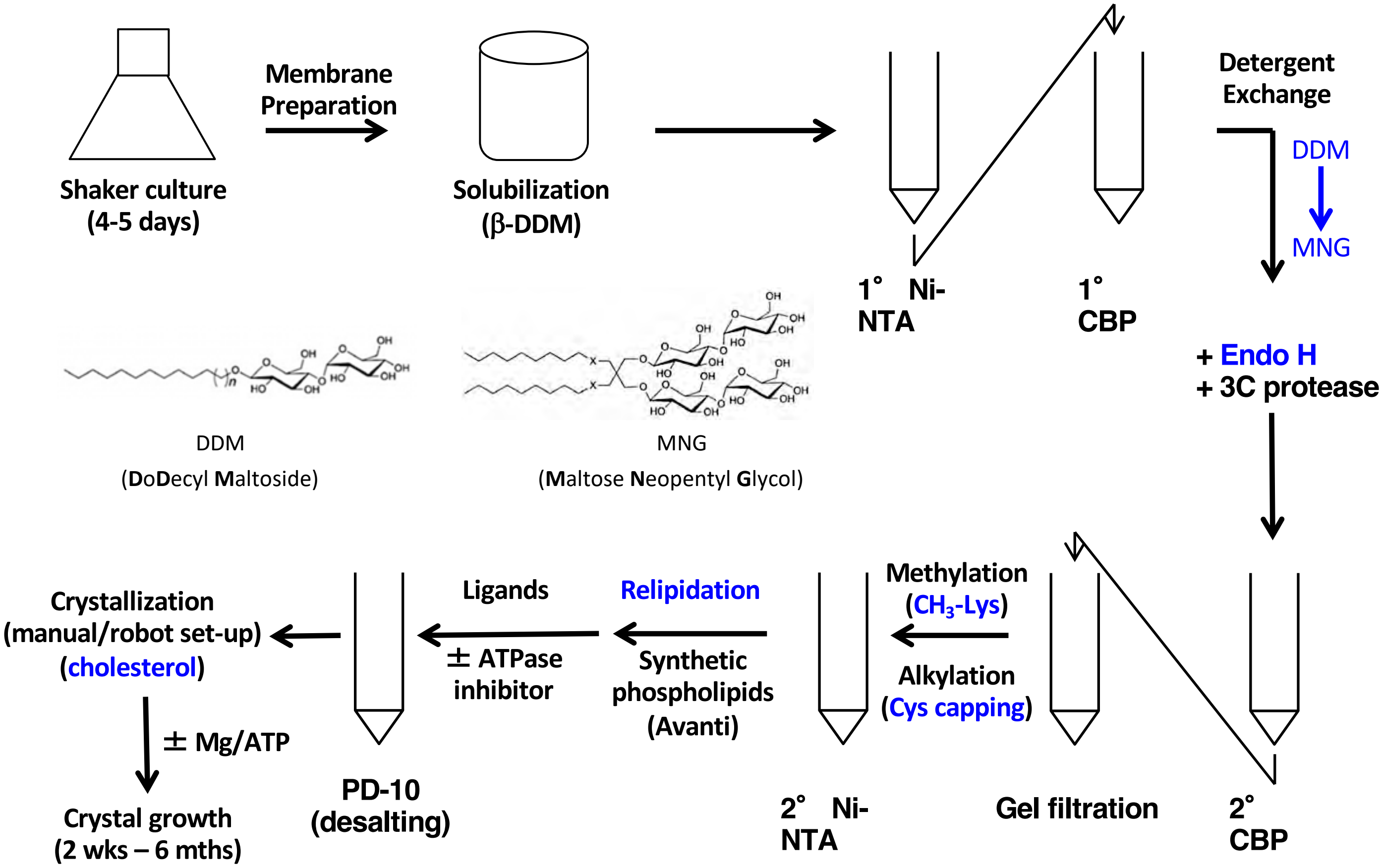
Analytical gel filtration:



Negative-stained TEM single particles:
(FEI Tecnai G2)

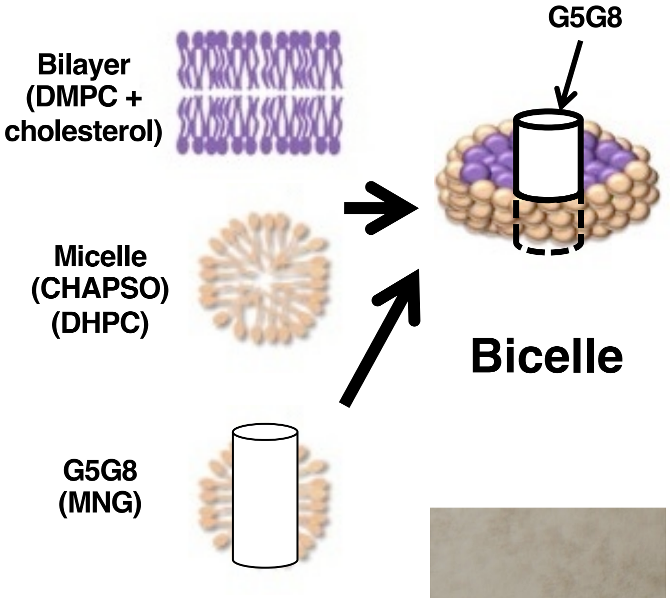


Optimization of Protein Preparation

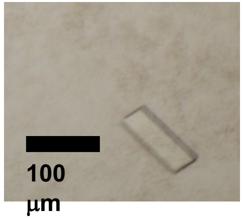


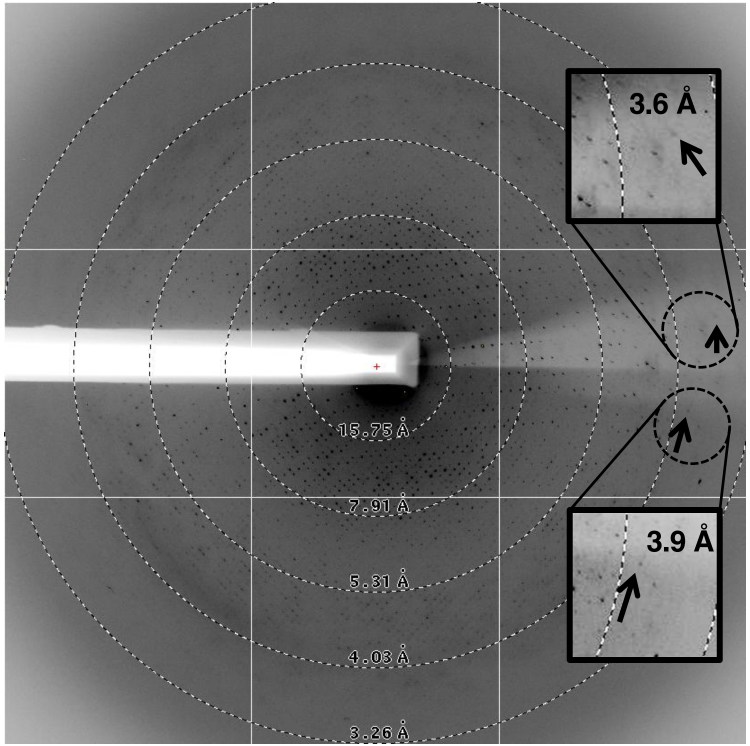
Bicelle Crystallization (Lipid Bilayers)

G5G8 bicelle preparation



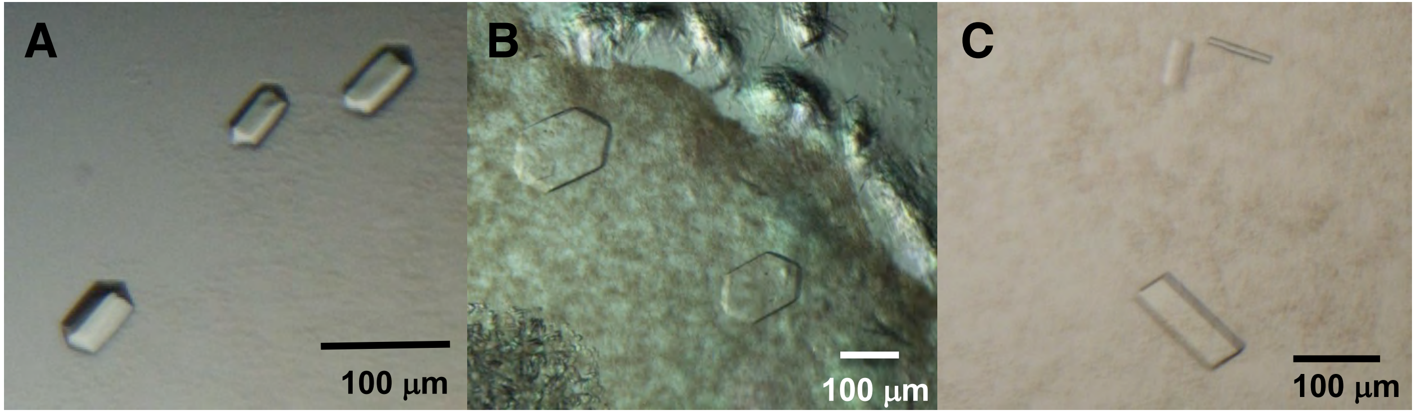
Crystal growth & X-ray diffraction



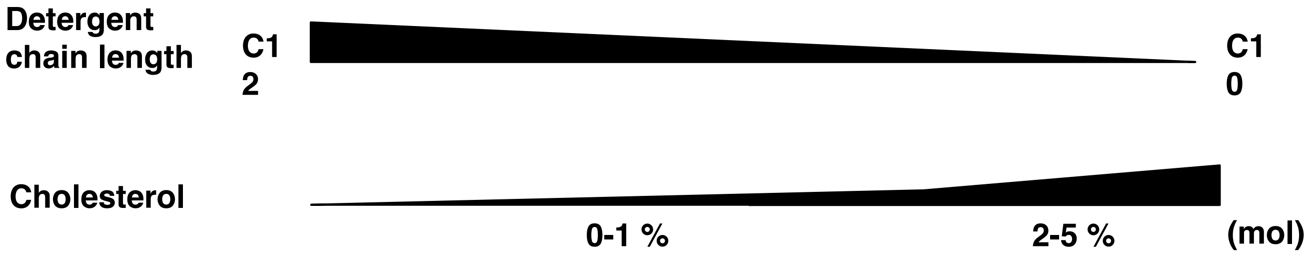


- Long exposure 2-5 sec @ APS 30 sec @ ALS
- Radiation damage 3-5 frames (< 5°)
- Signal (I/σ = 1-1.5 at 3.9-4Å)

Optimization of Crystal Growth



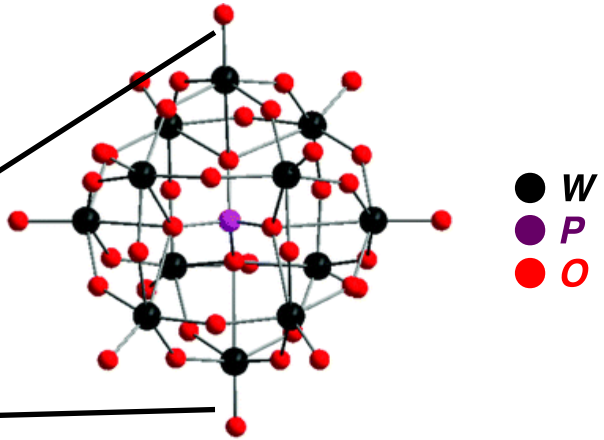
50-500 μm	100-300 μm	50-150 μm
25-30 Å	7-10 Å	3.5-4 Å



Single-wavelength Anomalous Diffraction (SAD)

W-SAD

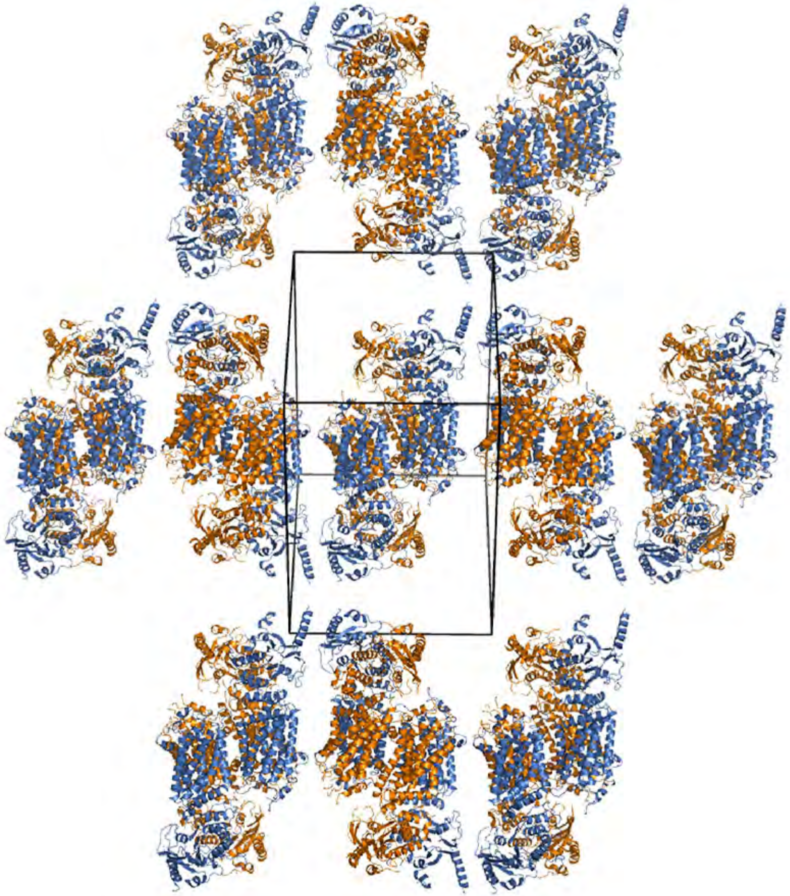
W: dodeca-tungsten cluster
(phosphotungstate: $PW_{12}O_{40}^{3-}$)



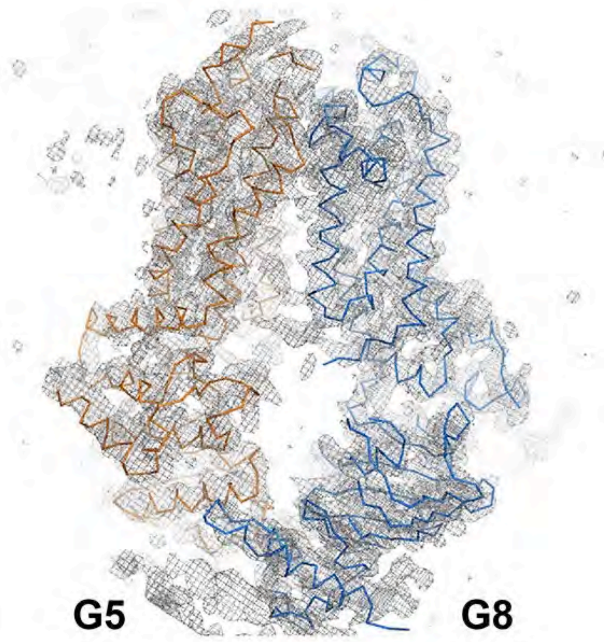
Keggin, Proc R Soc Lond A, 1934
Zhang & Wang, RSC Adv, 2012

(Lee et al, Nature, 2016)

Crystal Packing



Model Building for the G5G8 Structure

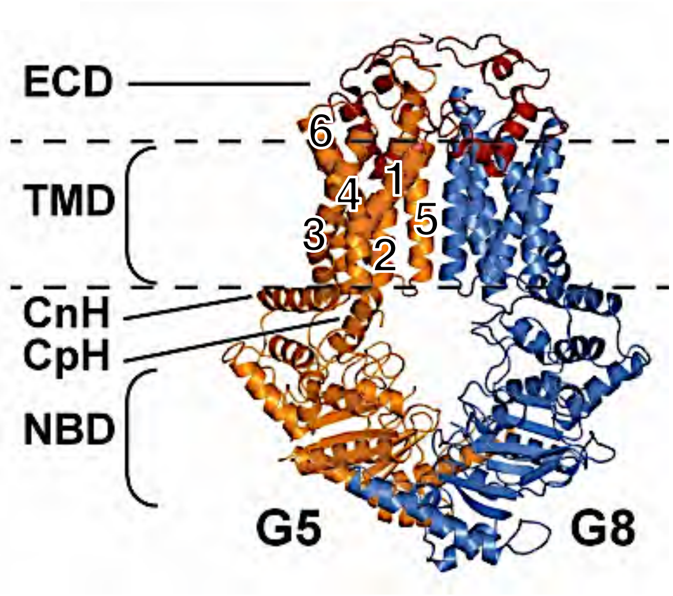


Merge: 19 datasets (least amorphous)
(Collect: ~200 native datasets)

Space group	I 222
Cell dimensions	
a, *b*, *c* (Å)	173.6, 224.8, 253.3
Resolution (Å)	50-3.9 (3.93-3.9)
R_{sym} or R_{merge}	16.1 (NA)
$\langle I \rangle / \langle \sigma I \rangle$	8.8 (0.15)
Completeness (%)	99.4 (84.2)
Redundancy	18.9 (2.5)
Refinement	
Resolution (Å)	25-3.94
No. reflections	34889
R_{work}/ R_{free}	24.5 / 32.9
No. atoms	
Protein	18151
R.m.s deviations	
Bond lengths (Å)	0.010
Bond angles (°)	1.64

ABCG5 and ABCG8 share high structural similarity.

Domain features



Structural similarity:

TMD: transmembrane domain

NBD: nucleotide-binding domain

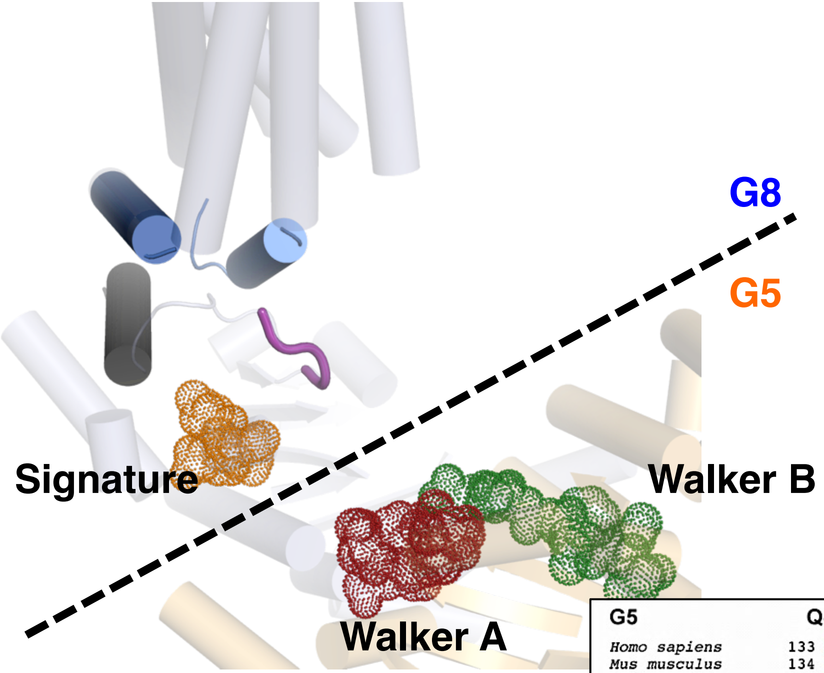
ECD: extracellular domain

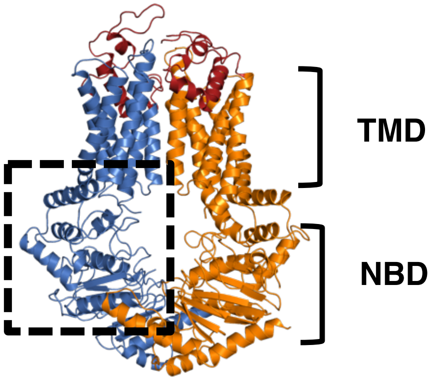
CnH: connecting helix

CpH: coupling helix

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

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





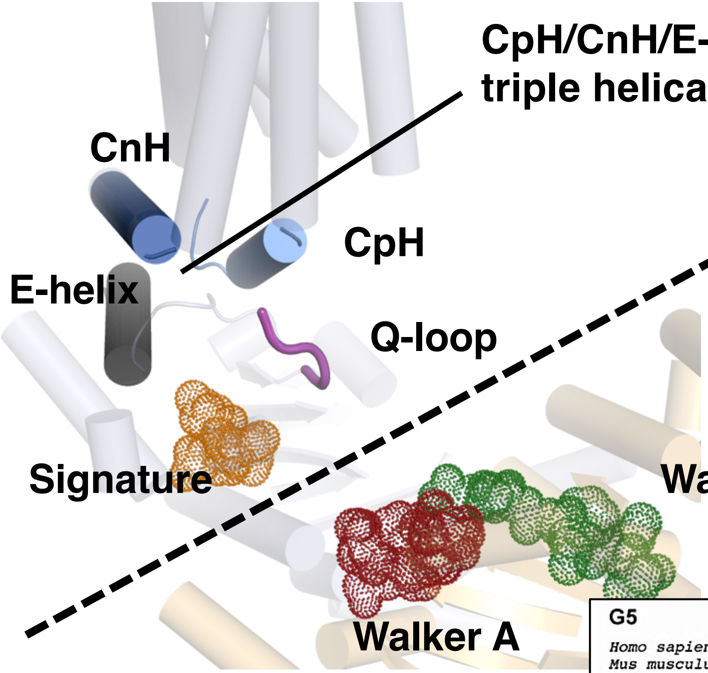

```
G5                          Q-loop E-helix           CnH              CpH
Homo sapiens           133 LQSDT~VRETLHYTALLA---~FSKLGVLLRRVTRNLVRN~LRAVSDQESQDGLY 458
Mus musculus           134 LQSDV~VRETLRYTAMLA---~FGKLGVLLRRVTRNLMRN~LRAVSDQESQDGLY 459
Gallus gallus          126 PQNDA~IEESLTYTALLA---~ISKLWFLLRRITRNFSKD~LRAISDQESKDGLY 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
                           **     ***       *     *       **  ** ** **     * *   *
```

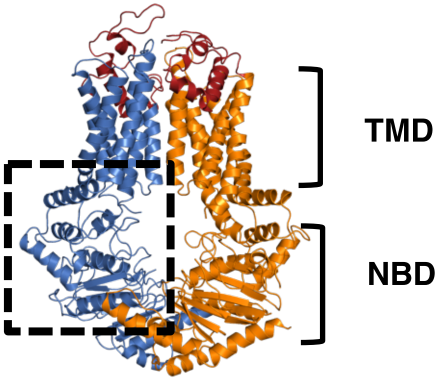
CnH: connecting helix

CpH: coupling helix

★: conserved polar residues

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





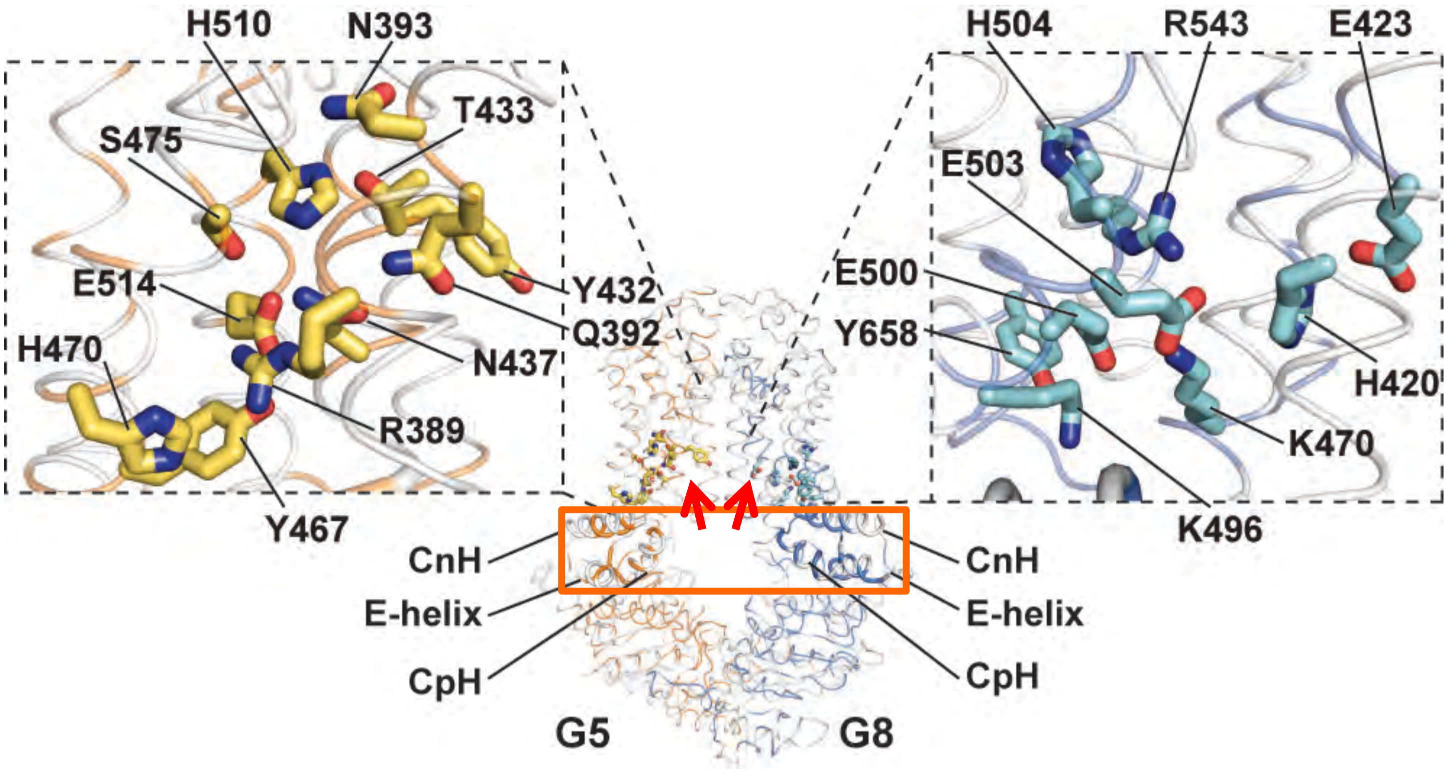

```
G5                            Q-loop  E-helix                CnH              CpH
Homo sapiens         133 LQSDT~VRETLHYTALLA---FSKLGVLLRRVTRNLVRN~LRAVSDQESQDGLY 458
Mus musculus         134 LQSDV~VRETLRYTAMLA---FGKLGVLLRRVTRNLMRN~LRAVSDQESQDGLY 459
Gallus gallus        126 PQNDA~IEESLTYTALLA---ISKLWFLLRRITRNFSKD~LRAISDQESKDGLY 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
                         **    ***    *     *     **  ** ** **      * *  *
```

CnH: connecting helix

CpH: coupling helix

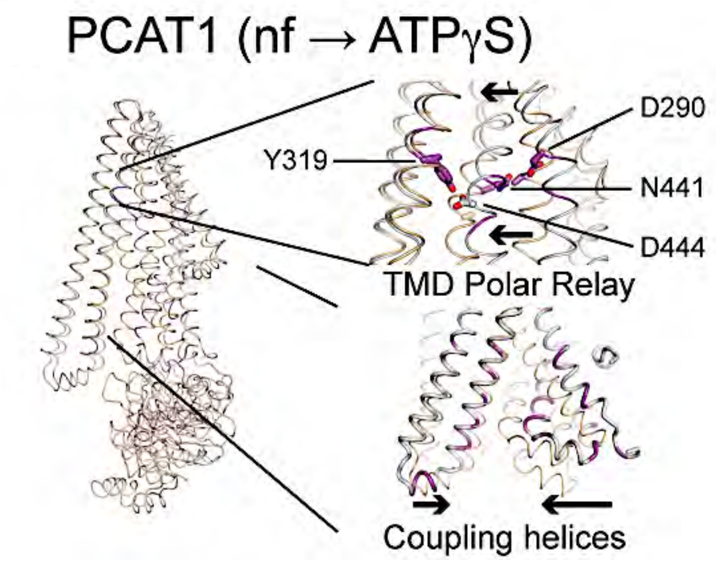
★: conserved polar residues

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



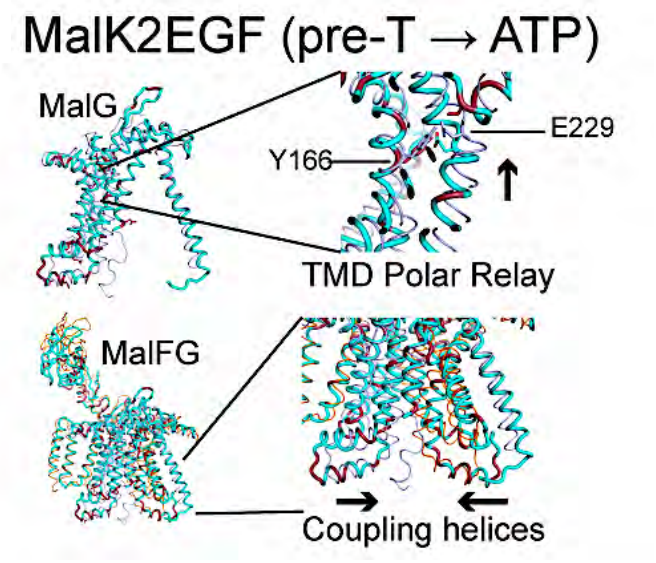
TMD Polar Relays: a General Feature?

Polypeptide processing and secretion transporter:



(Lin et al, Nature, 2015)

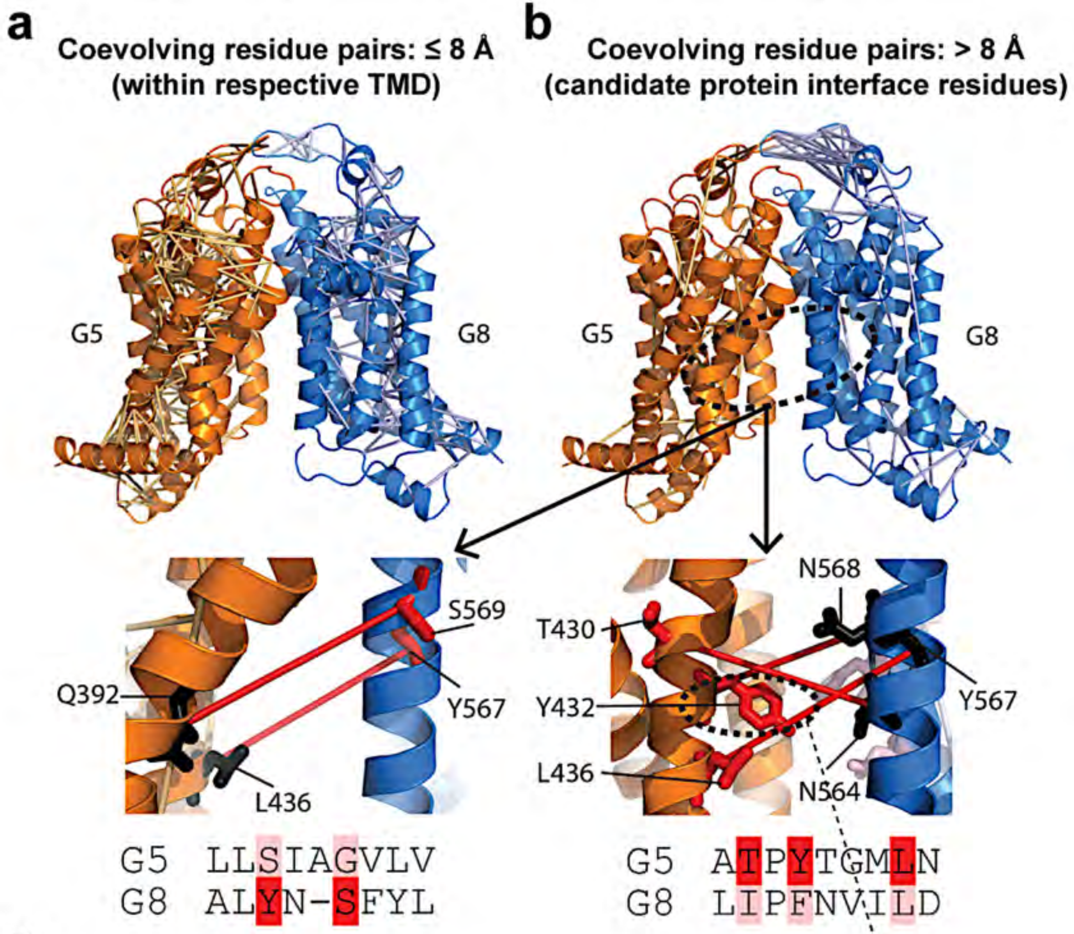
Maltose transporter:

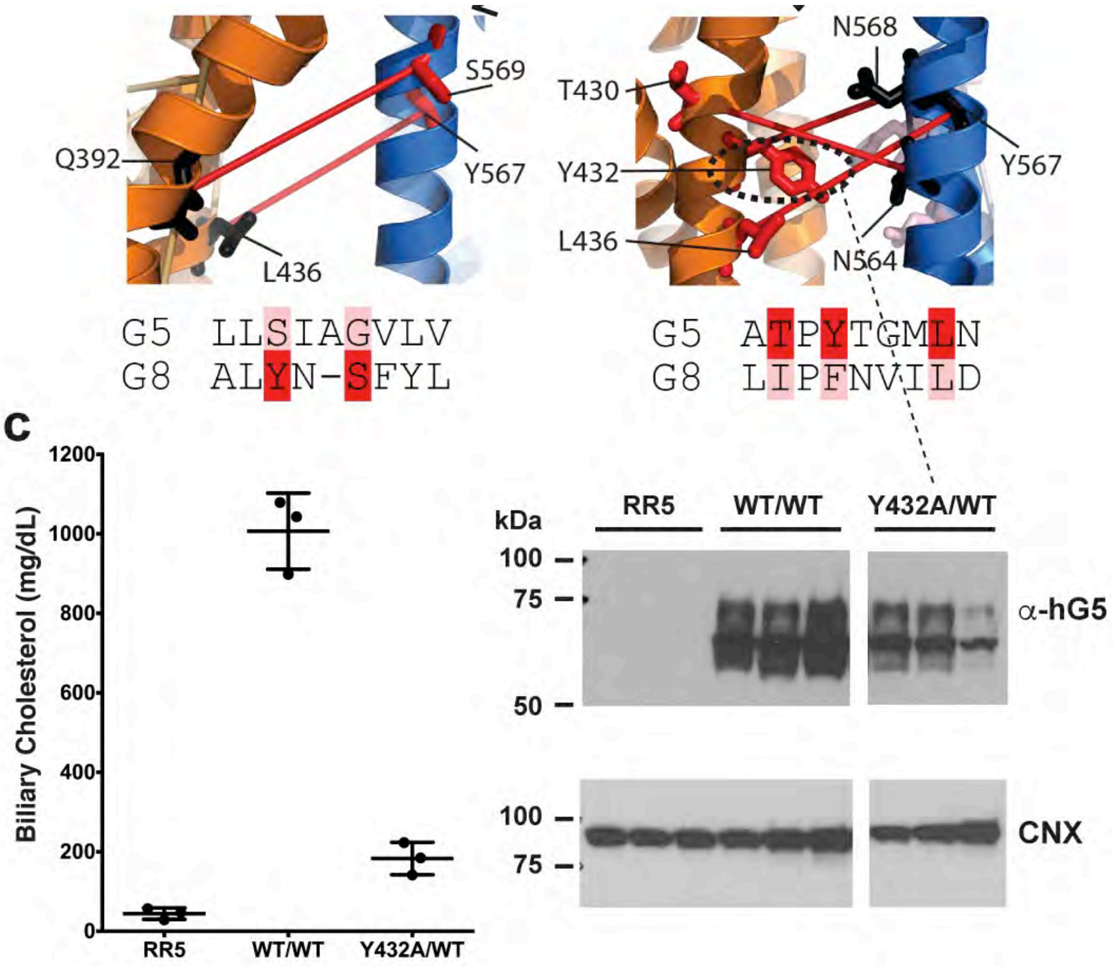


(Oldham et al, Nature, 2007)
(Oldham & Chen, Science, 2011)

(Lee et al, Nature, 2016)

Co-Evolution Analysis

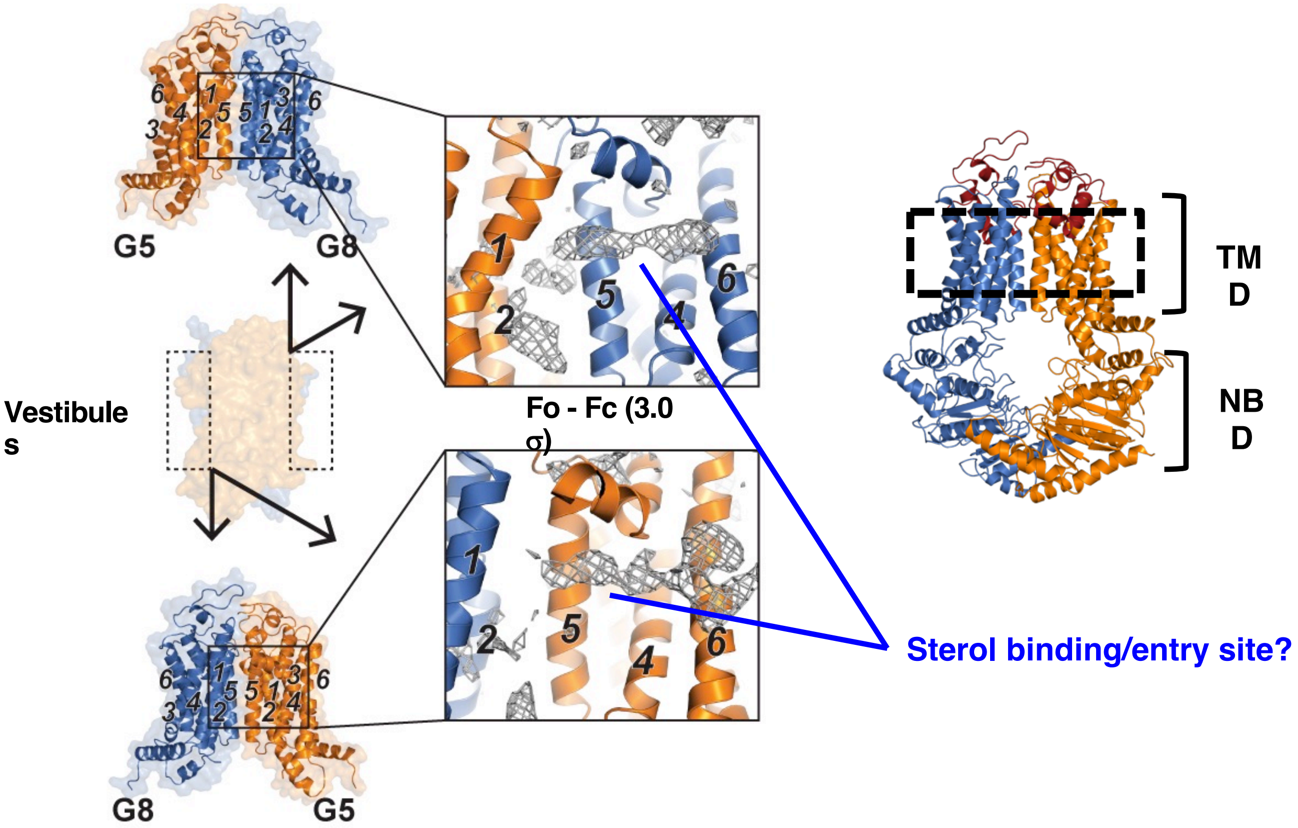




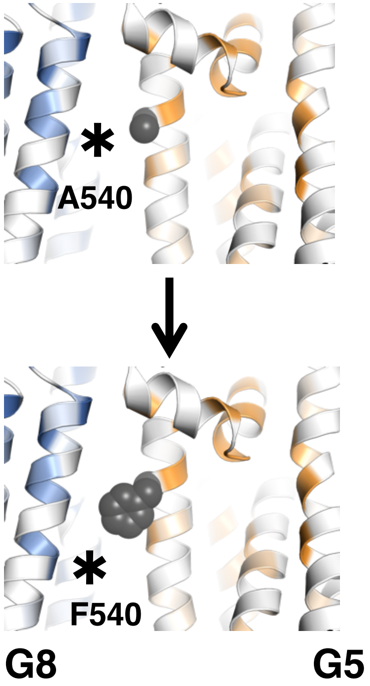
N568
S569
T430
Q392
Y567
Y432
Y567
L436
L436
N564
G5 LLSIAGVLV
G8 ALYN-SFYL
G5 ATPYTGMLN
G8 LIPFNVILD
c
Biliary Cholesterol (mg/dL)
1200
1000
800
600
400
200
0
RR5
WT/WT
Y432A/WT
kDa
RR5
WT/WT
Y432A/WT
100
75
50
α-hG5
100
75
CNX

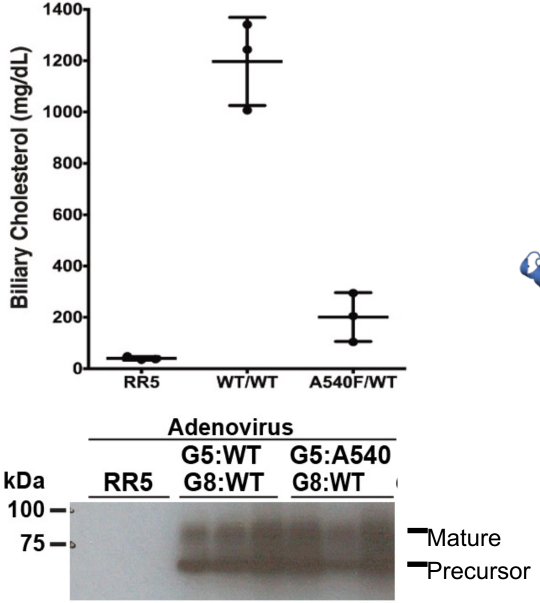
How do sterols move across the lipid-bilayer membranes on the TMD?

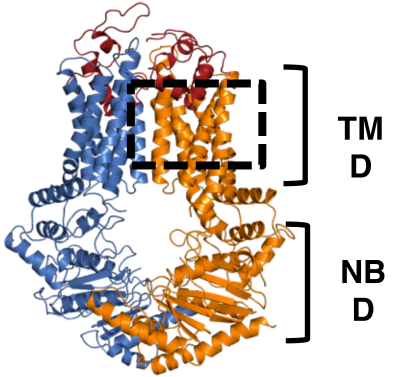
Vestibules at the TMD-membrane interface



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

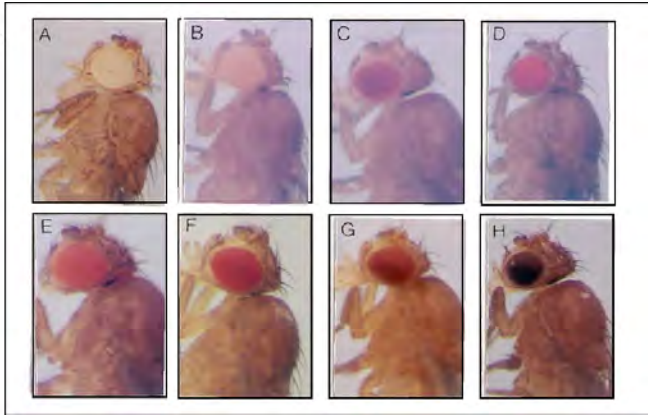




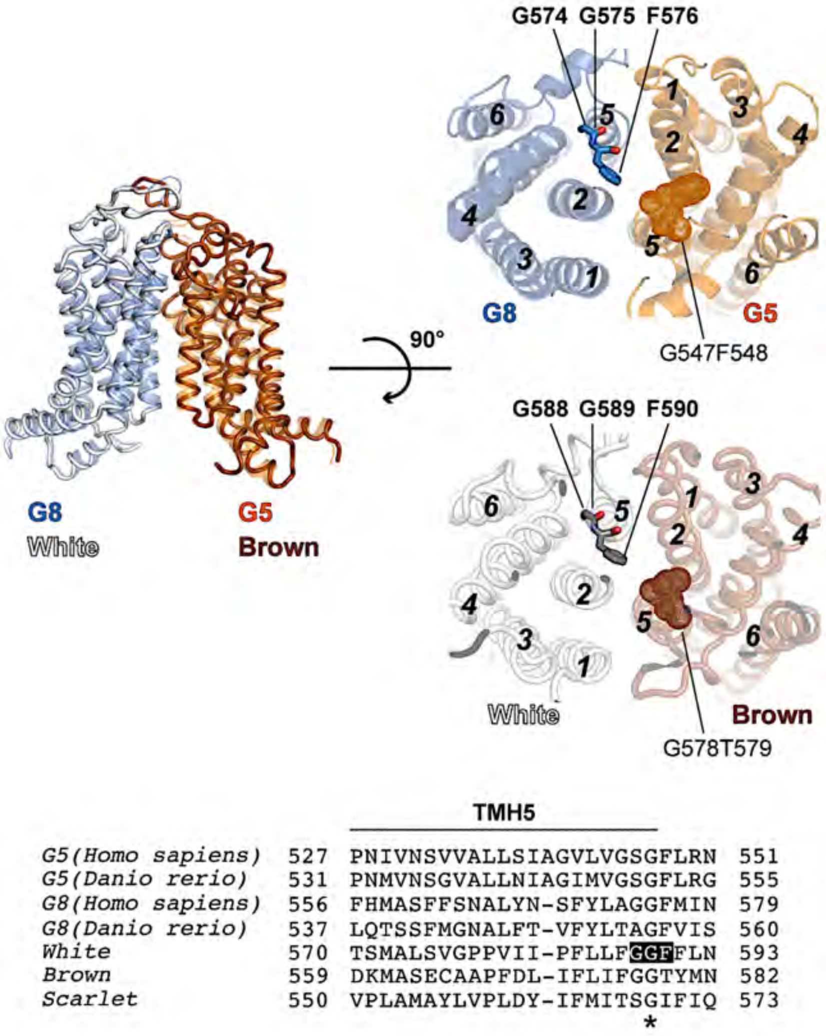


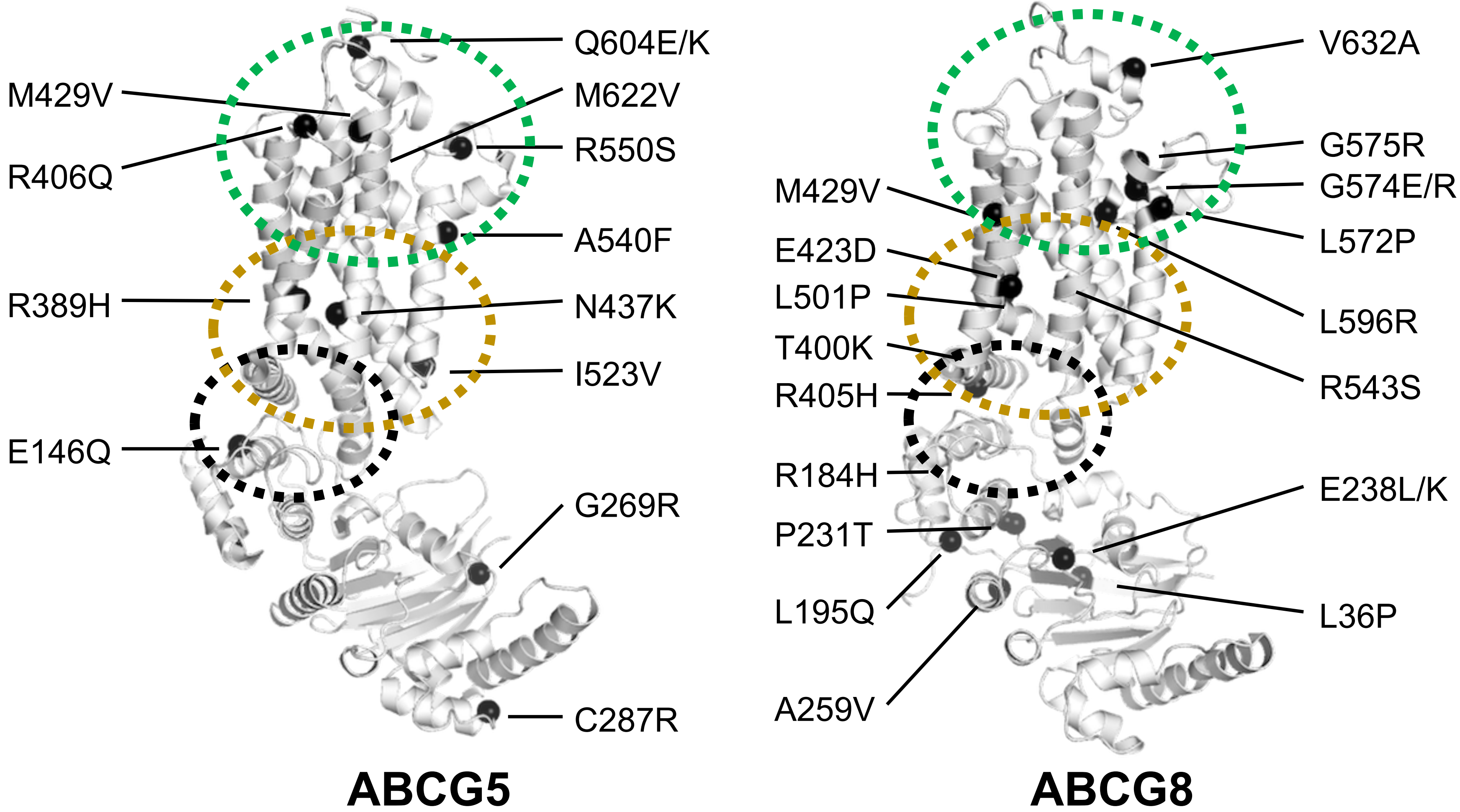
(Lee et al, Nature, 2016)

Figure 2A-H.
Eye colour mutants:
(A) white
(B) white apricot
(C) brown
(D) scarlet
(E) cinnabar
(F) vermilion
(G) rosy
(H) sepia.



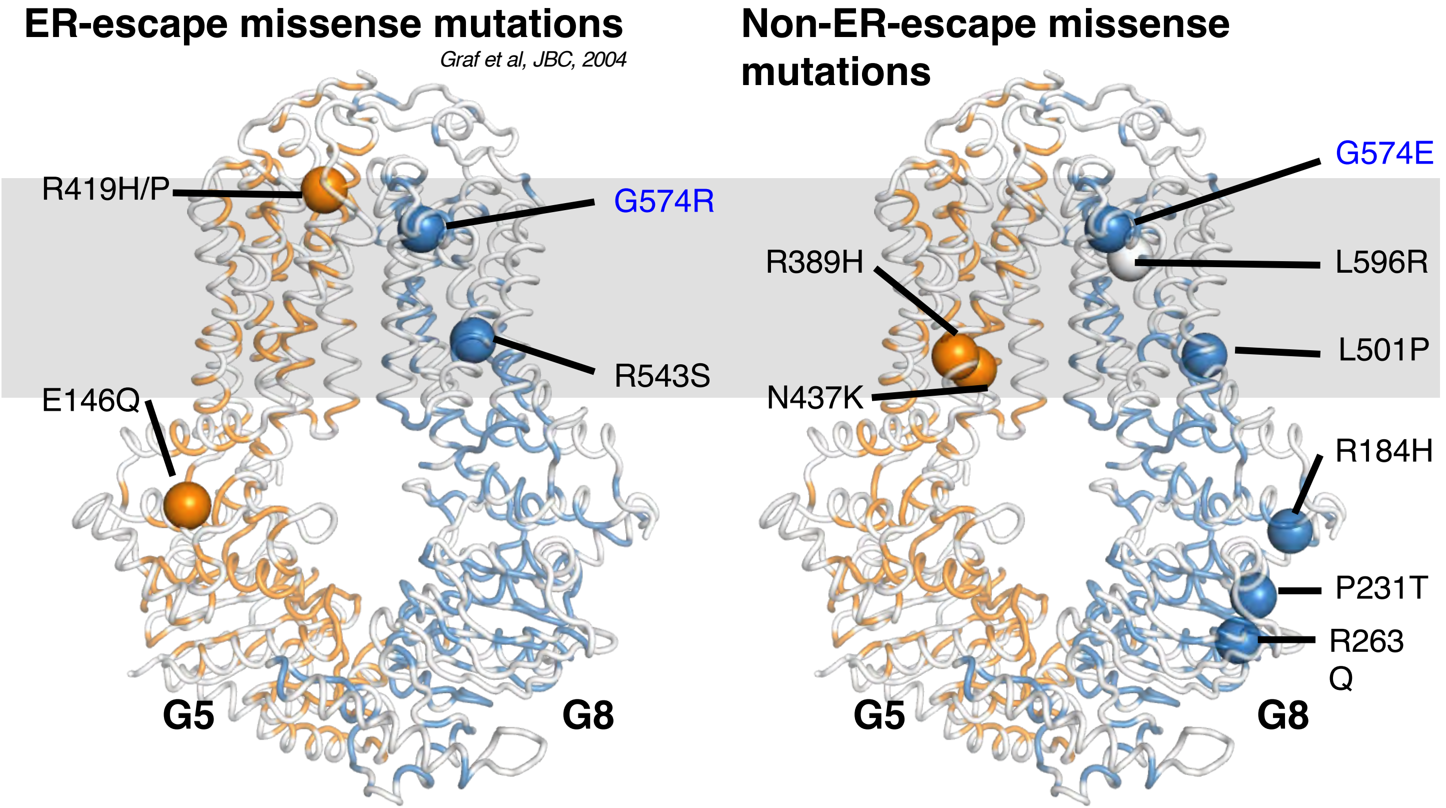
(Ranganath & Tanuja, 1999)
"Teaching and learning genetics with Drosophila. 2. Mutant phenotypes of Drosophila melanogaster"





(Xavier et al, Int J Mol Sci, 2020)

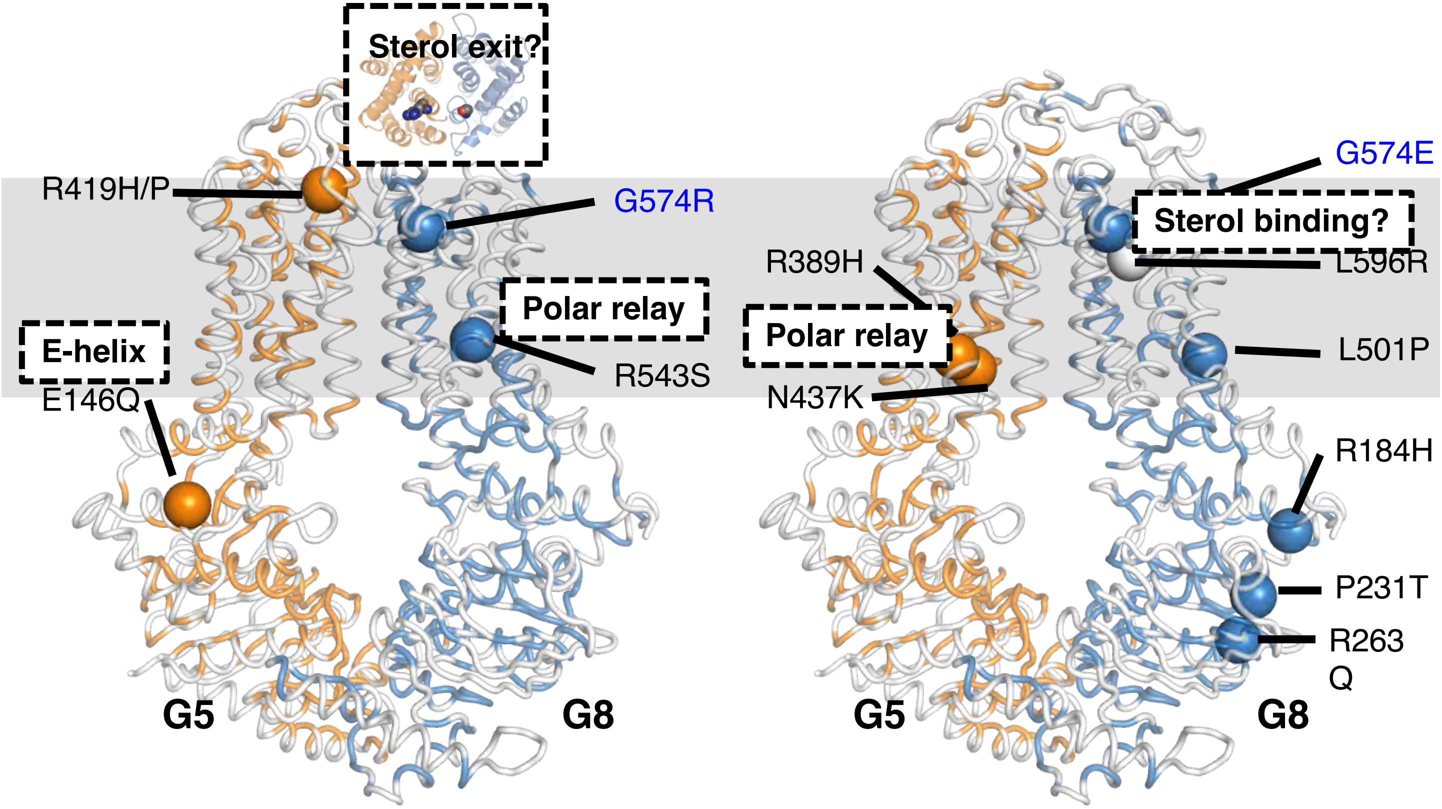
Location of the residues with the disease-causing missense mutations of sitosterolemia.



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

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

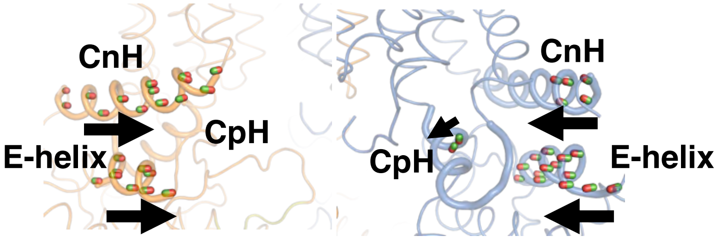
Disease-causing mutations cluster in the conserved functional domains in G5G8.



Trajectory of Domain Movement

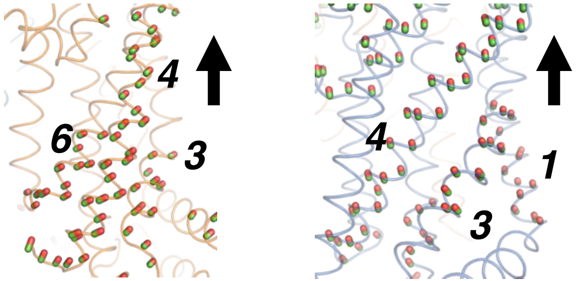
Molecular Dynamics Simulation

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



G5 **G8**

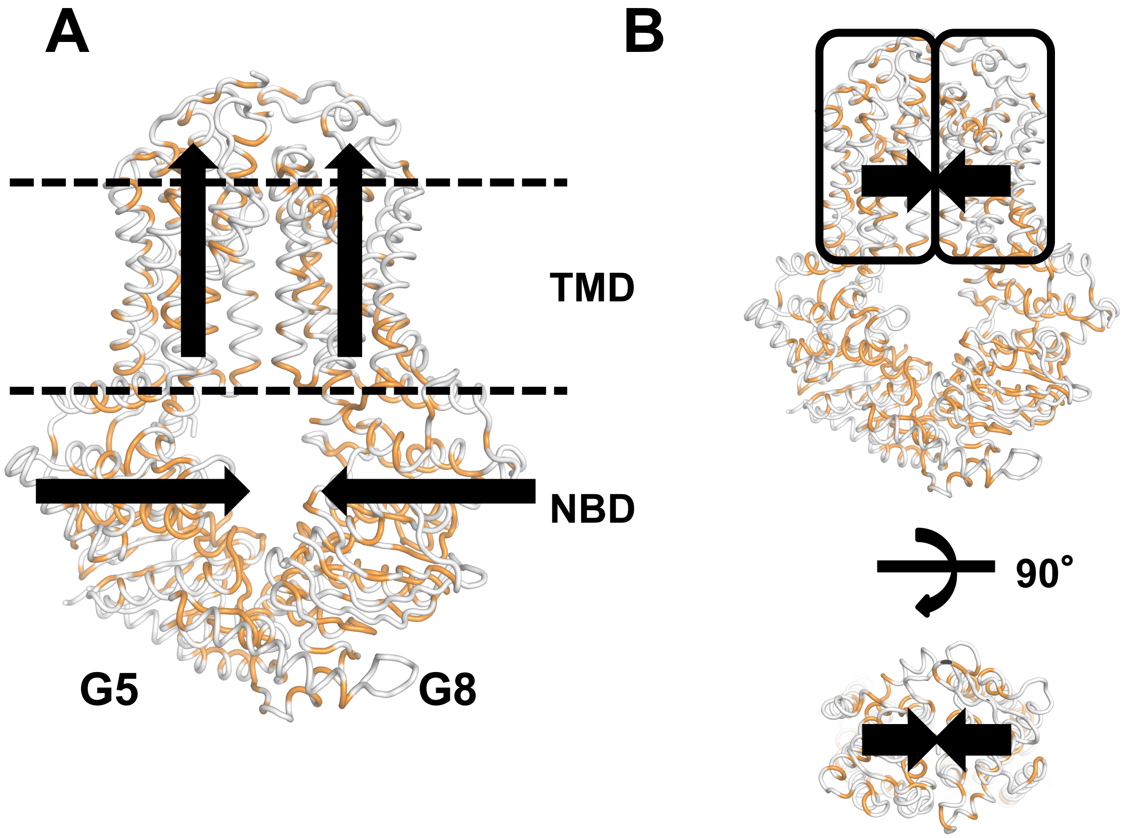
Upward movement
(TM helices)



G5 **G8**

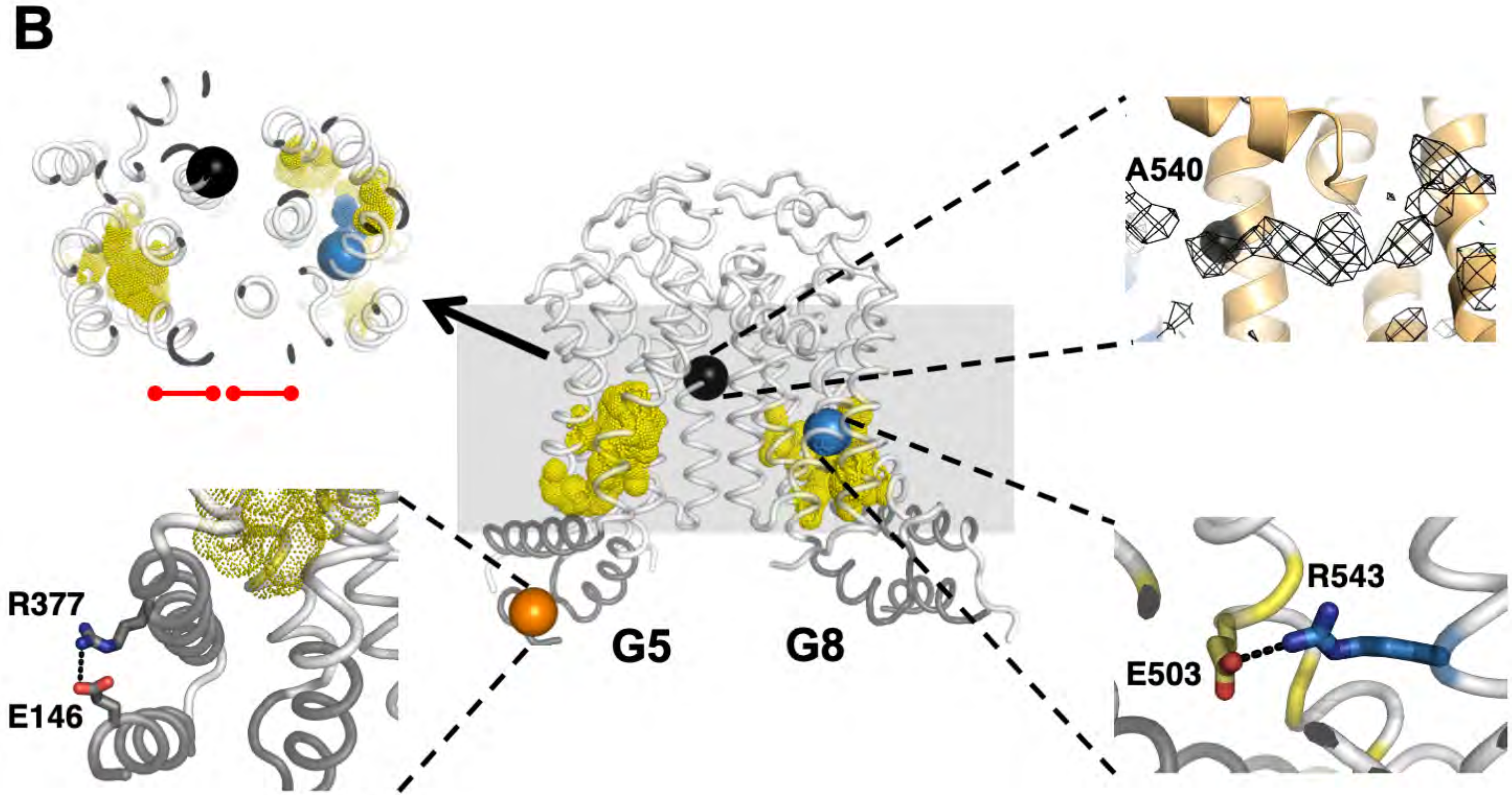
(Lee et al, Nature, 2016)

Trajectory of Domain Movement



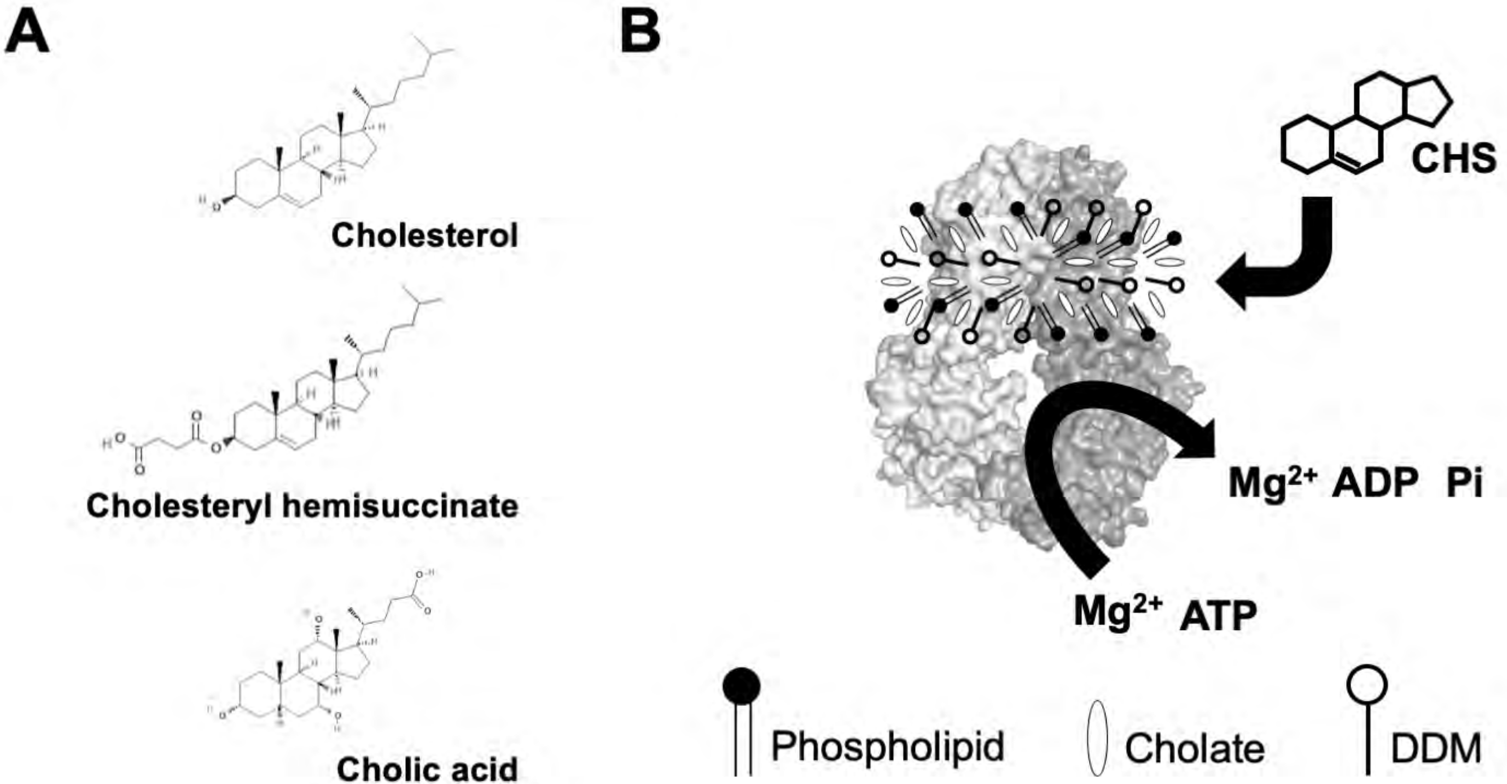
(*Zein et al, Biochem Soc Trans, 2019*)

Using disease mutations to gain mechanistic understanding

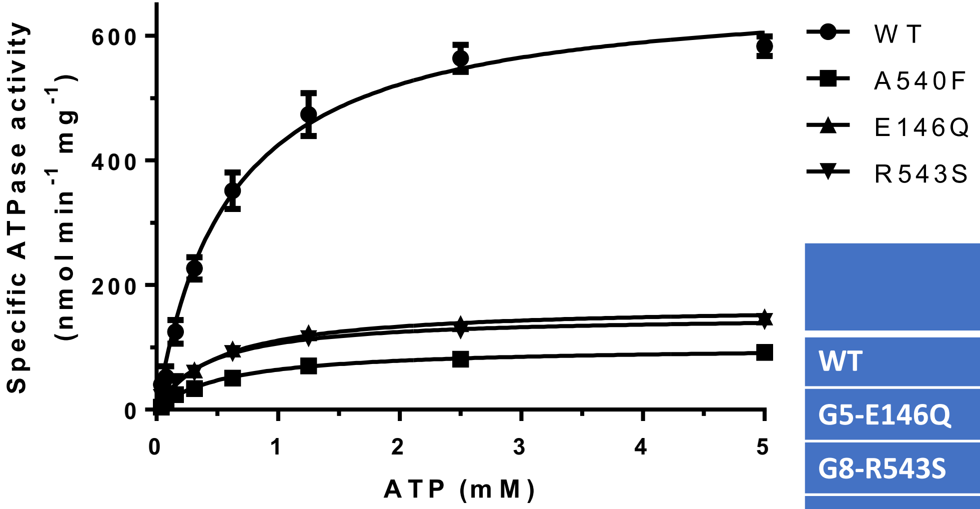


(Xavier et al, Int J Mol Sci, 2020)

CHS-Stimulated ATPase Activity of ABCG5/G8



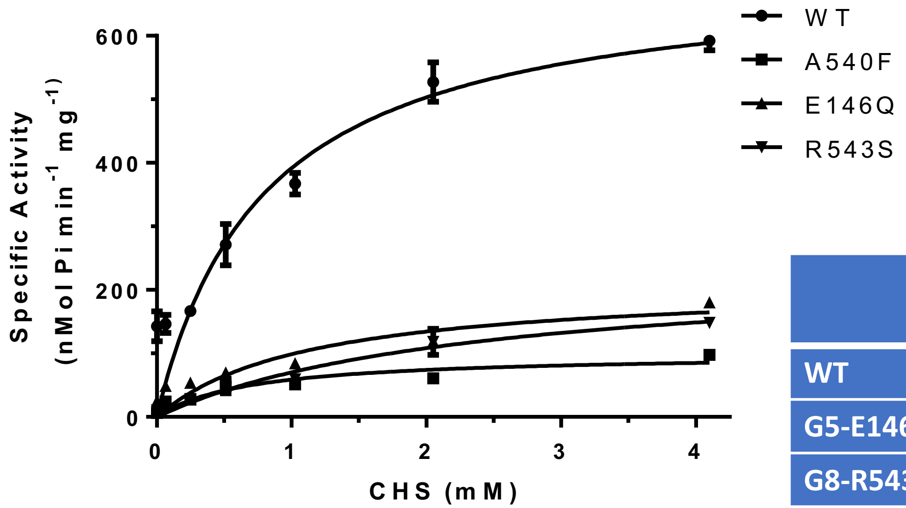
ATP Dependence of ABCG5/G8



	V_{max} (nmol/min/mg)	K_M (ATP) (mM)	$k_{cat}{}^{a}$ (s^{-1})	k_{cat}/K_M ($M^{-1}s^{-1}$)
WT	677.1 ± 25.6	0.60	1.69	$2.8x10^3$
G5-E146Q	167.1 ± 0.05	0.51	0.41	$0.82x10^3$
G8-R543S	150.7 ± 3.7	0.42	0.38	$0.90x10^3$
G5-A540F	101.2 ± 4.2	0.58	0.25	$0.43x10^3$

(Xavier et al, Int J Mol Sci, 2020)

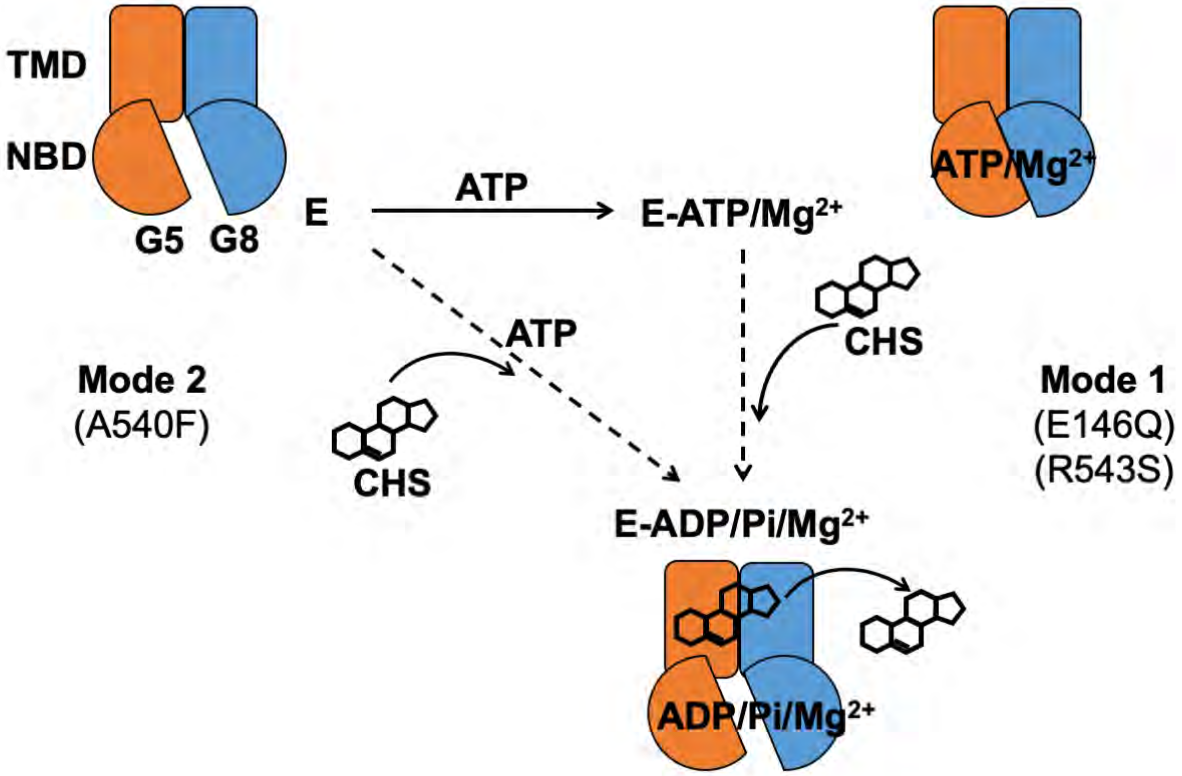
CHS Dependence of ABCG5/G8



	V_{max} (nmol/min/mg)	K_M (CHS) (mM)	k_{cat}^a (s^{-1})	k_{cat}/K_M ($M^{-1}s^{-1}$)
WT	702.9 ± 50.7	0.8	1.74	$2.2x10^3$
G5-E146Q	210.0 ± 33.2	1.13	0.52	$0.46x10^3$
G8-R543S	237.1 ± 33.4	2.38	0.59	$0.25x10^3$
G5-A540F	99.8 ± 11.4	0.70	0.25	$0.36x10^3$

(Xavier et al, Int J Mol Sci, 2020)

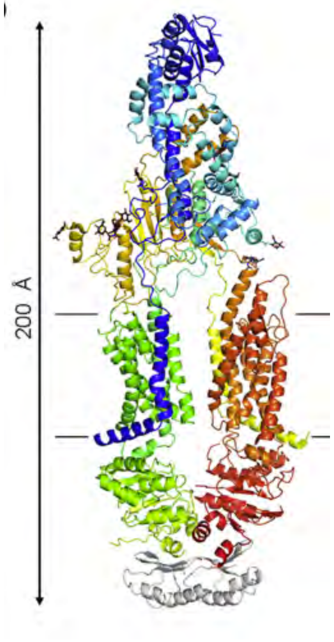
A Catalytic Model of ABCG5/G8



(Xavier et al, Int J Mol Sci, 2020)

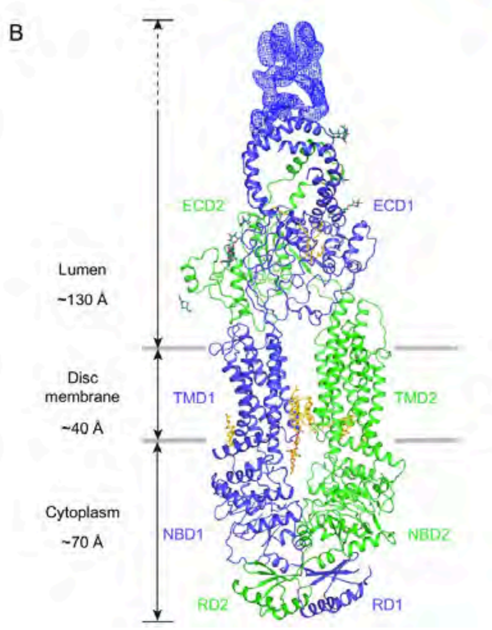
Beyond ABCG5/G8

ABCA1
- cholesterol



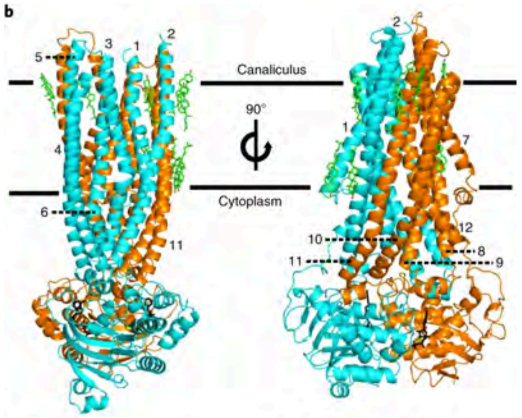
(Qian et al, Cell, 2017)

ABCA4
- phospholipid import

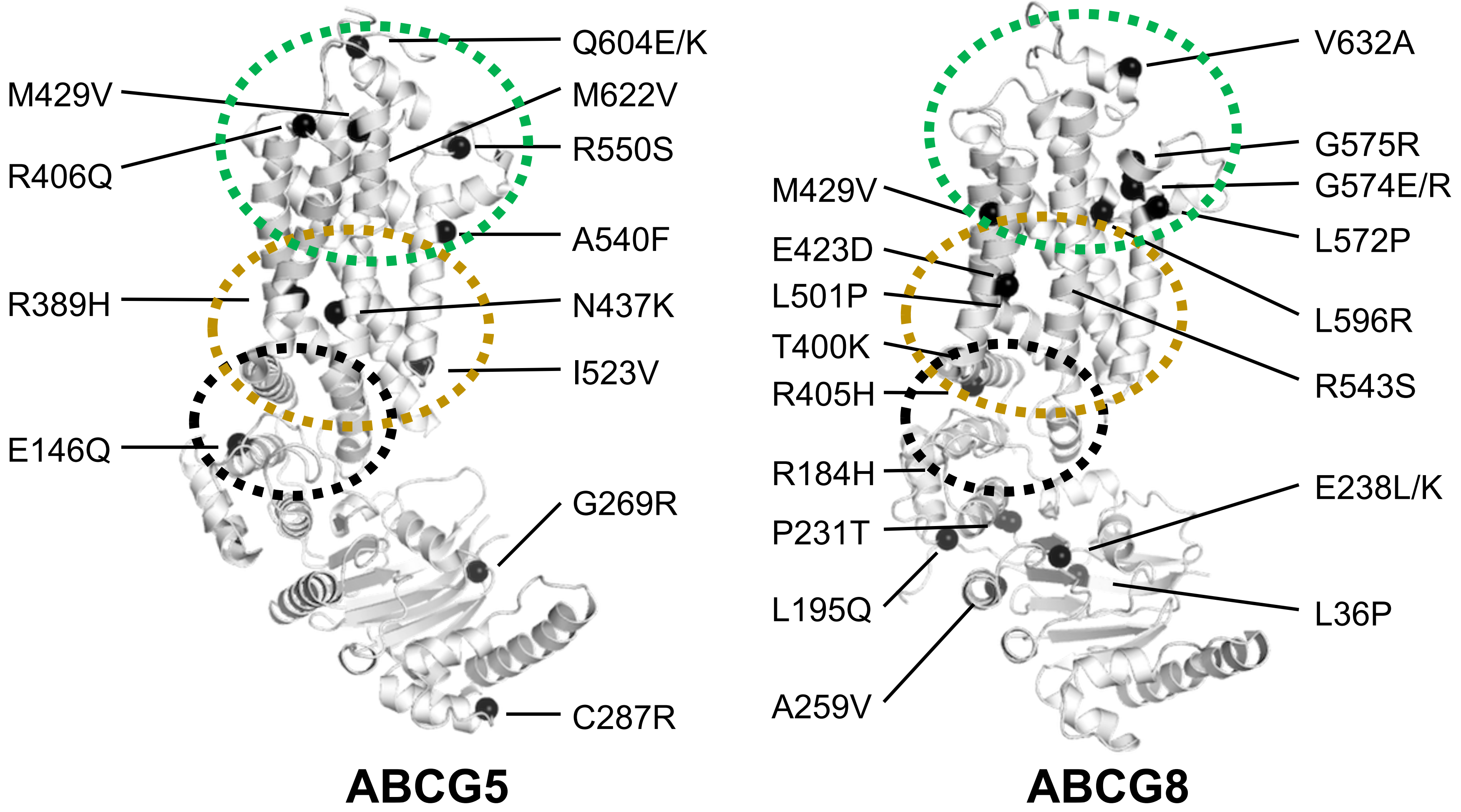


(Liu et al, eLife, 2021)

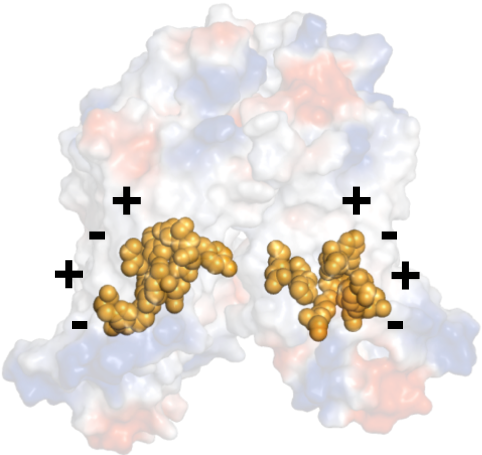
ABCB4
- phospholipid export



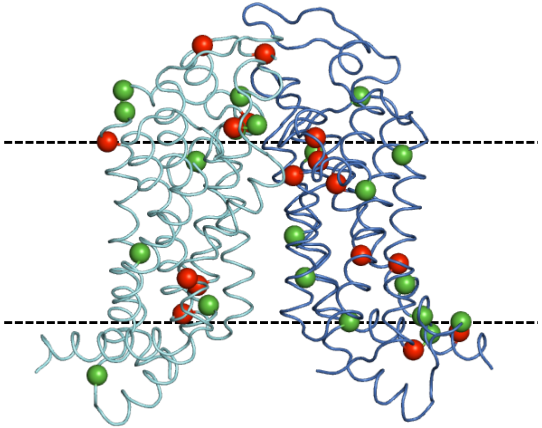
(Olsen et al, Nat Struct Mol Biol, 2020)


Q604E/K
M429V
M622V
R550S
R406Q
A540F
R389H
N437K
I523V
E146Q
G269R
C287R
ABCG5
V632A
G575R
G574E/R
M429V
L572P
E423D
L501P
L596R
T400K
R405H
R543S
R184H
E238L/K
P231T
L195Q
L36P
A259V
ABCG8

Transmembrane Domain of ABC Cholesterol Transporters: a Pathogenic Hot Spot



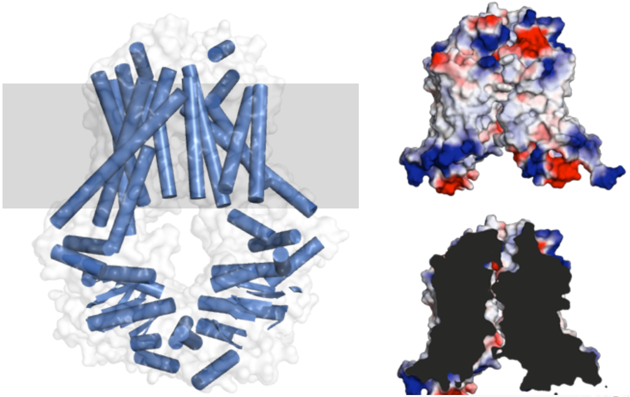
Polar relay

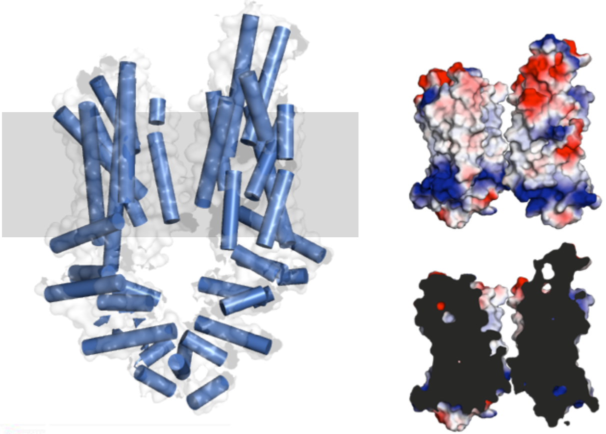


Pathogenic residues:
G5G8 (red), A1 (green)

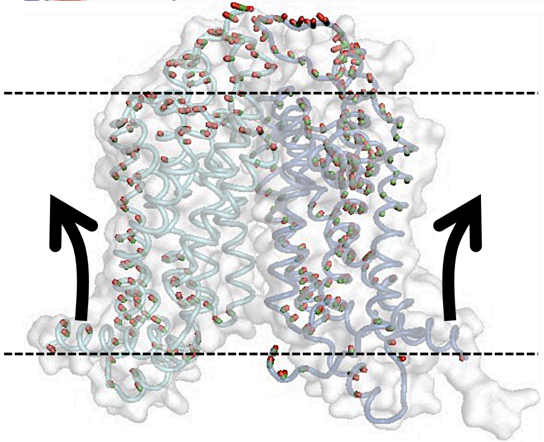
(Xavier et al, Biochem Cell Biol, 2019)

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





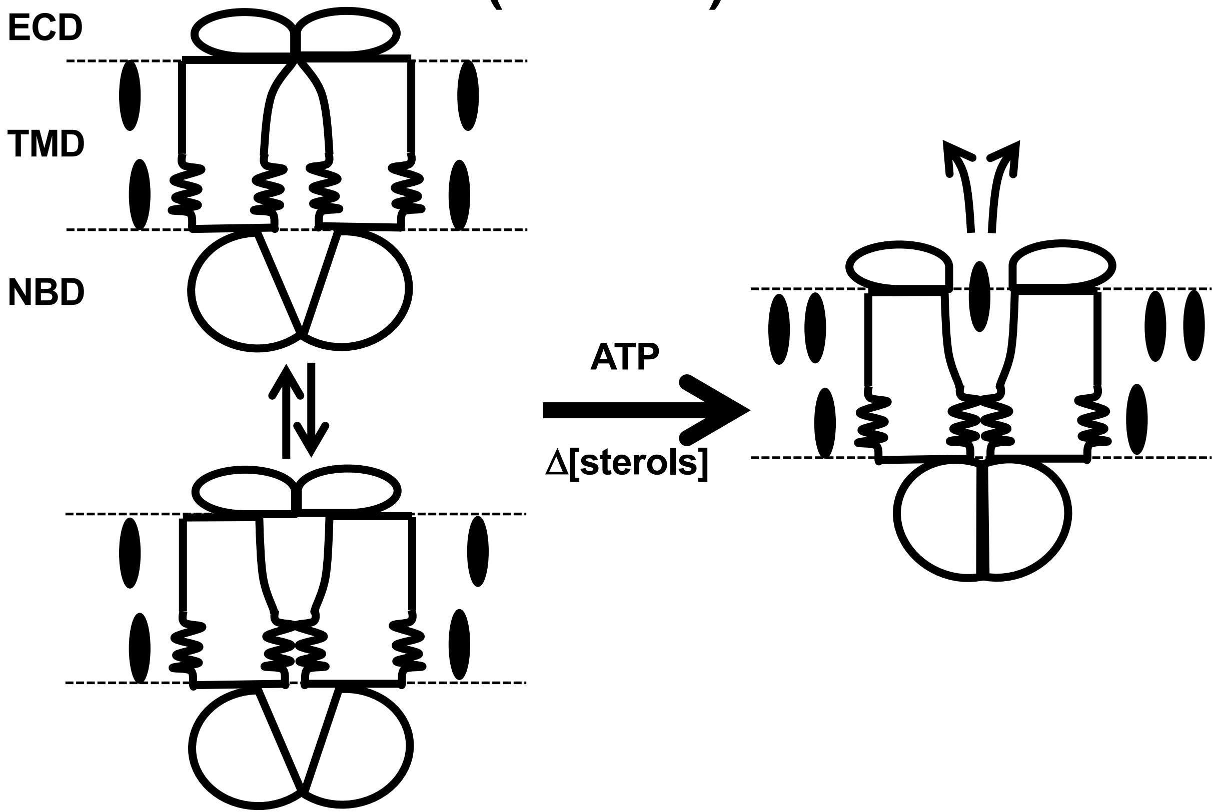
ABCG5/G8
(nf, inward)



ABCA1
(nf, outward)

(Xavier et al, Biochem Cell Biol, 2019)

Working Model of ABC Sterol Transporters (Cellular)



(Xavier et al, Biochem Cell Biol, 2019)