

Structural Role of ABC Sterol Transporters

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Faculté de médecine | Faculty of Medicine

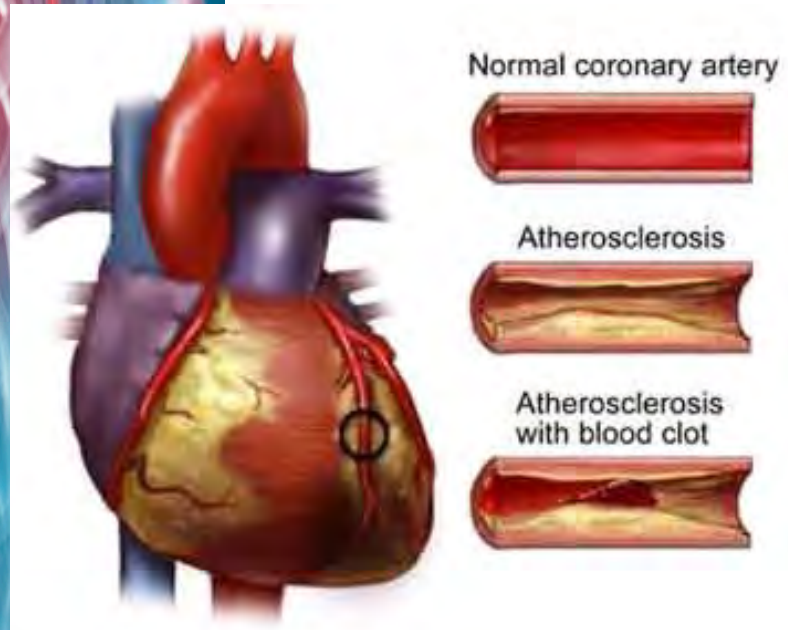
uOttawa.ca



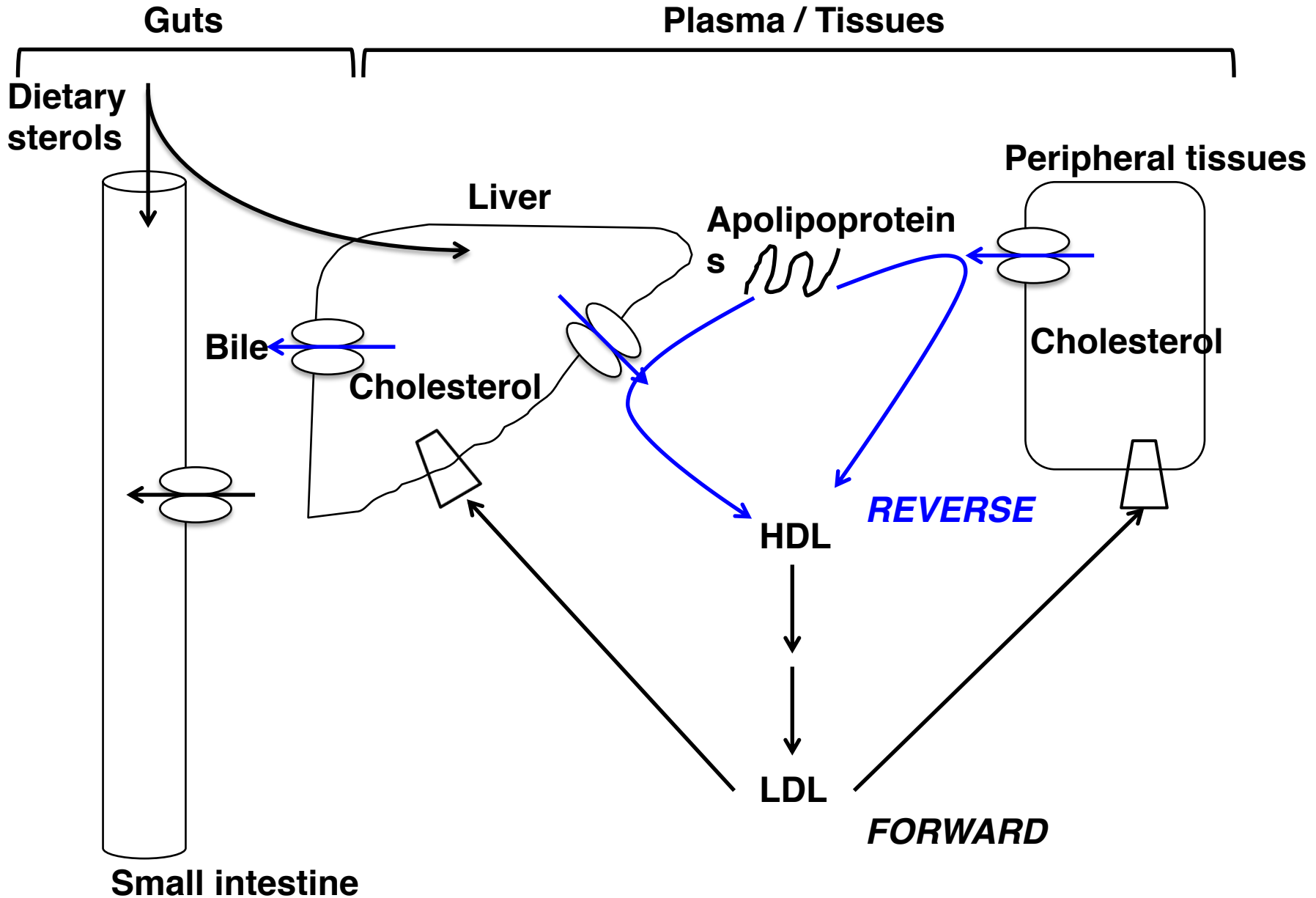
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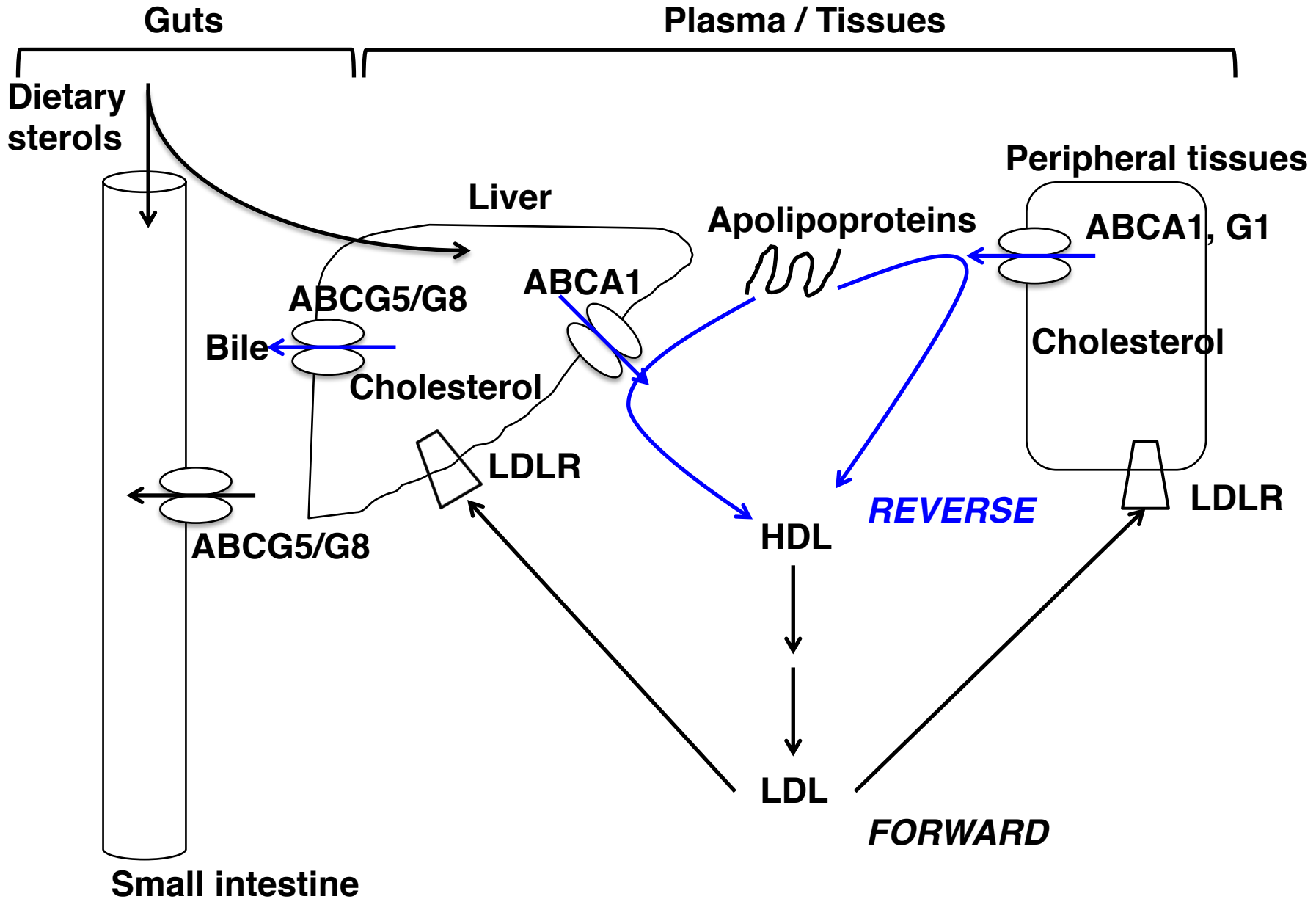
Cholesterol: a Risk Factor of Cardiovascular Diseases



Transporting Cholesterol



Transporting Cholesterol



✓ **Function and structure of ABCG5/ABCG8**

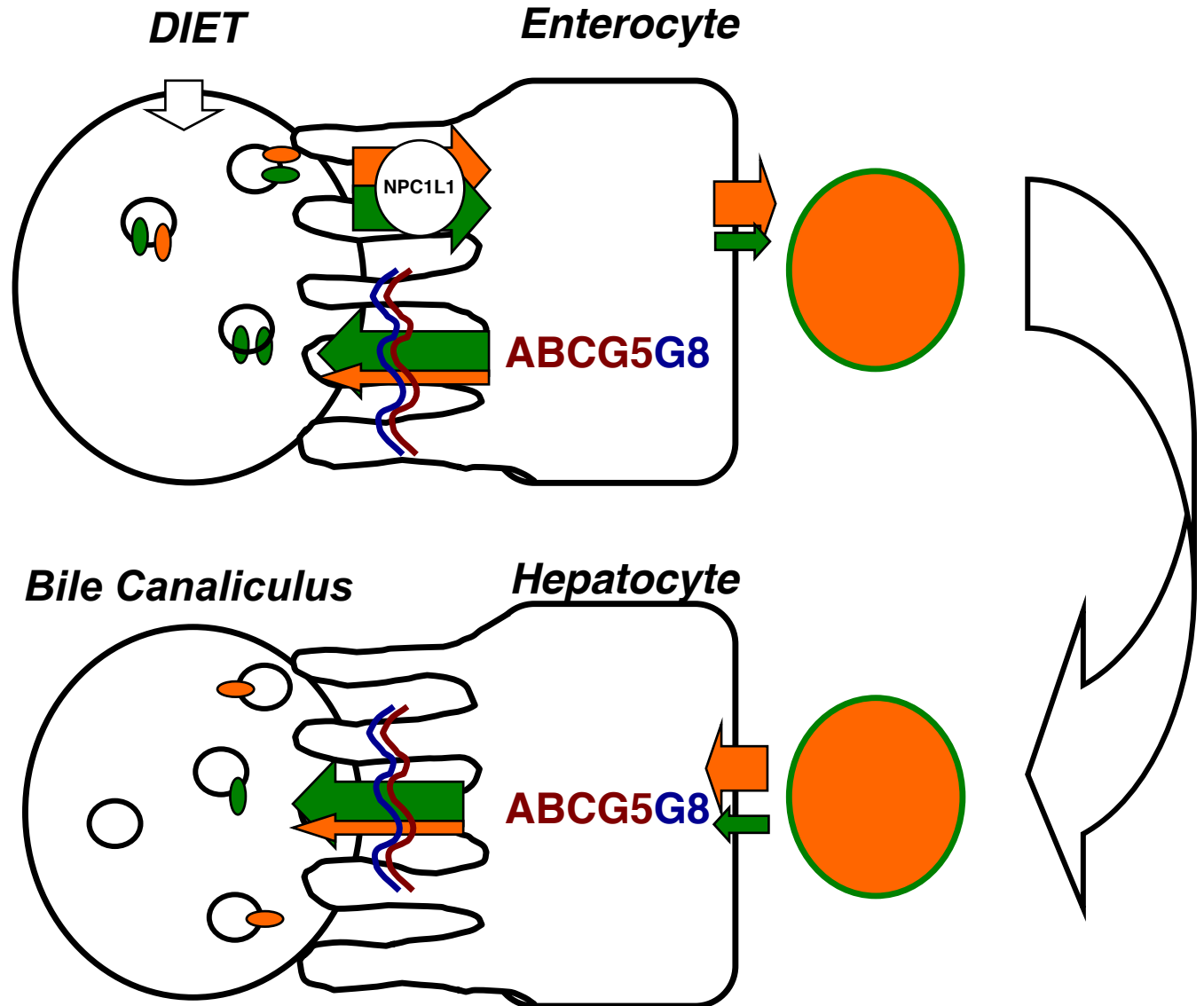
- **What's known & what's not known**
- **Summary, questions, & future directions**



ABCG5 and ABCG8 in the Guts

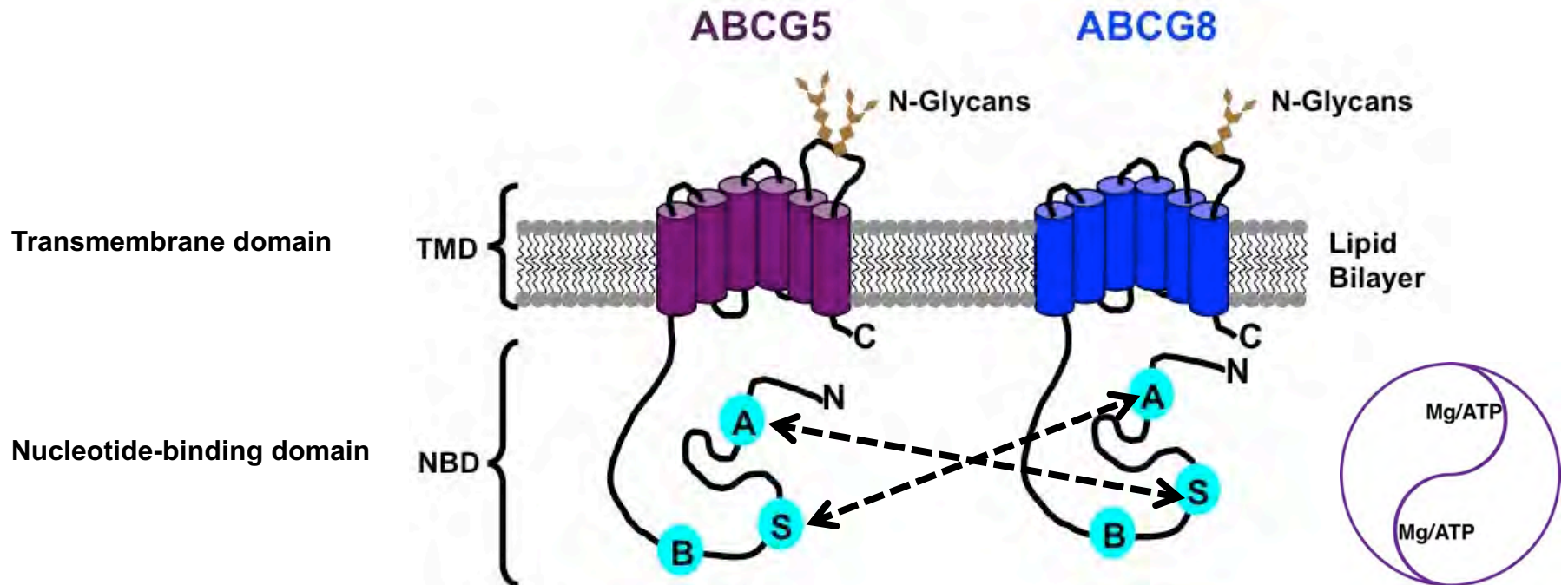
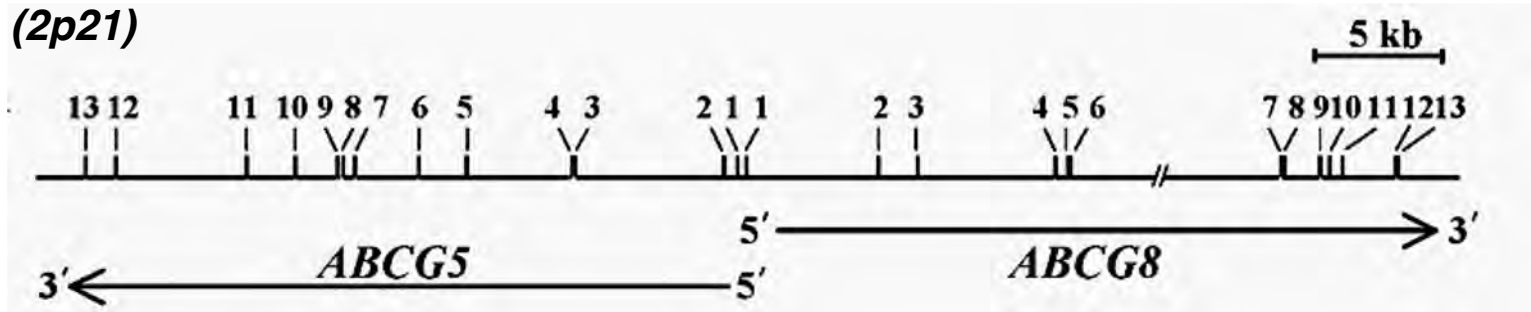
- Plant Sterols
- Cholesterol

Small Intestine



Liver

Heterodimeric ABCG5/ABCG8



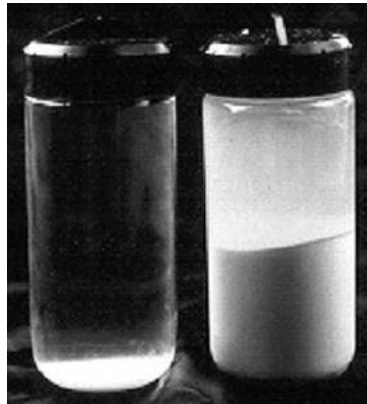
Expression of Human ABCG5/ABCG8

Host: *Pichia pastoris* Yeast



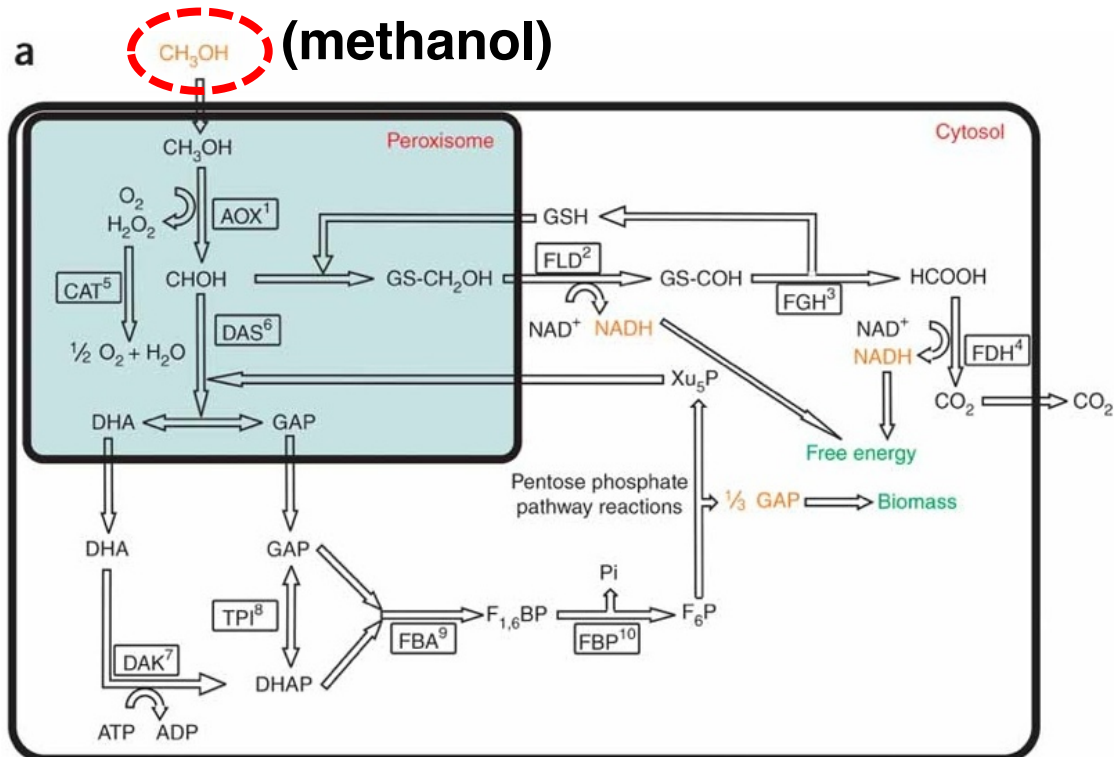
(Shutter et al, Nature Biotechnology, 2009)

(Ina Urbatsch, Texas Tech HSC)

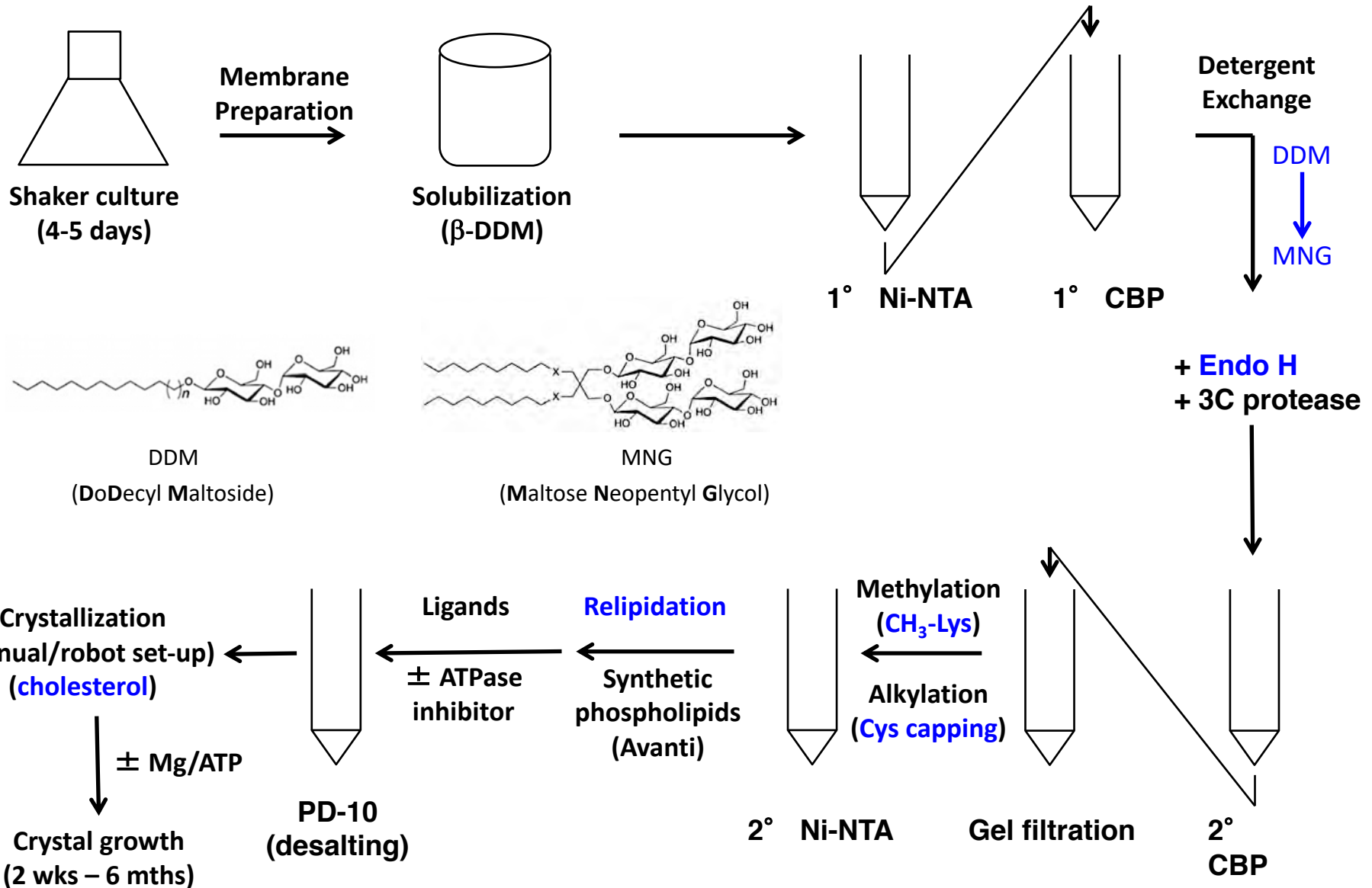


Left: *Saccharomyces* (1.0 OD_{600nm} units/mL)
Right: *Pichia* (400 OD_{600nm} units/mL)

(Cereghino & Cregg, FEMS Microbiol, 2000)

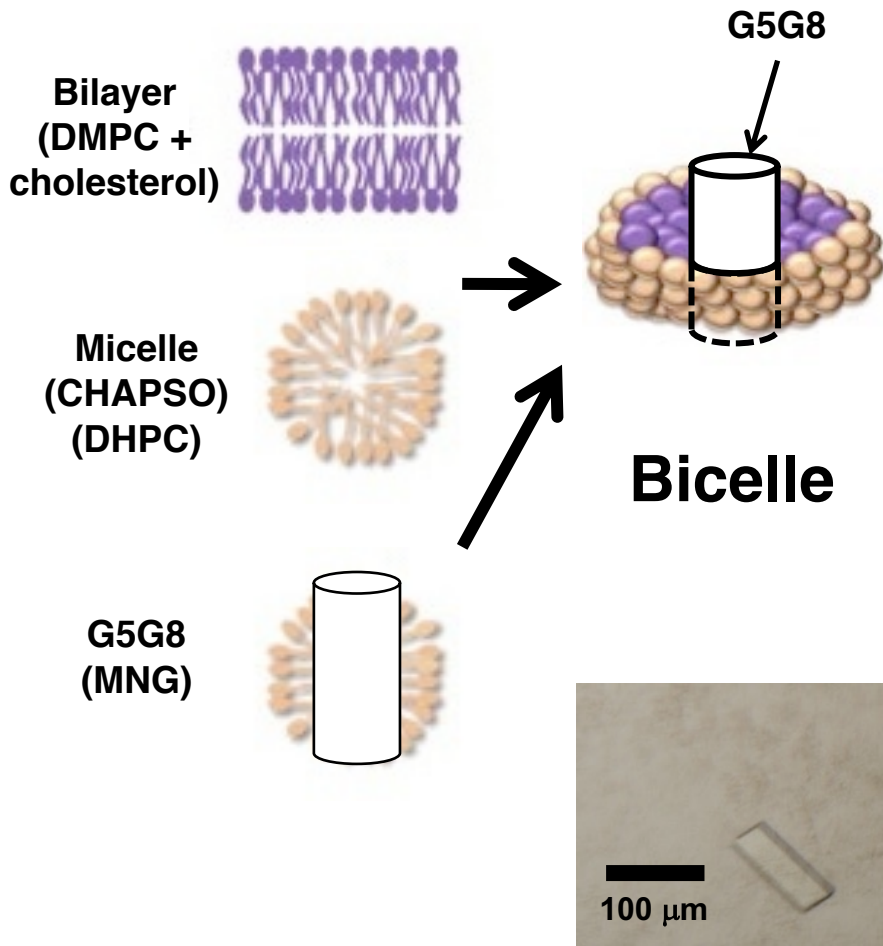


Optimization of Protein Preparation

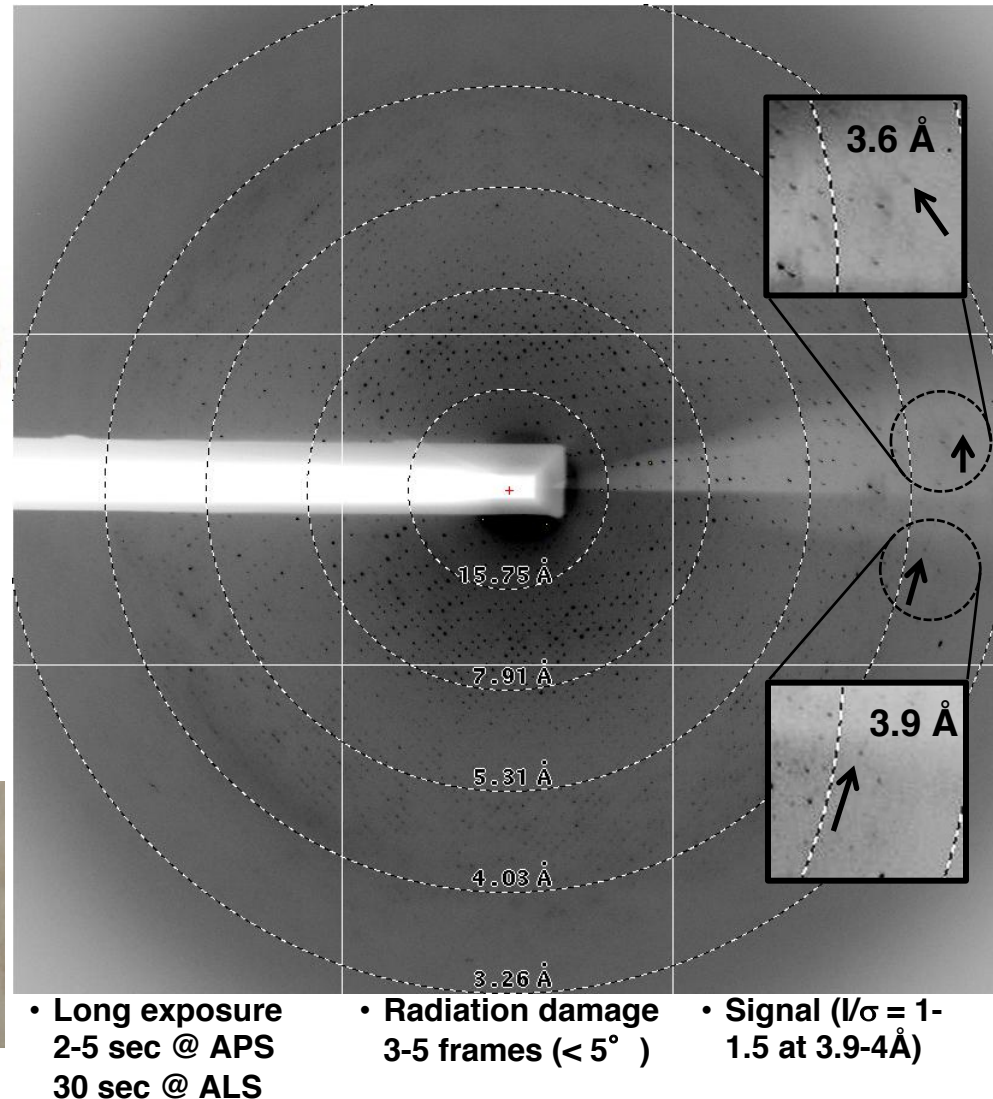


Bicelle Crystallization (Lipid Bilayers)

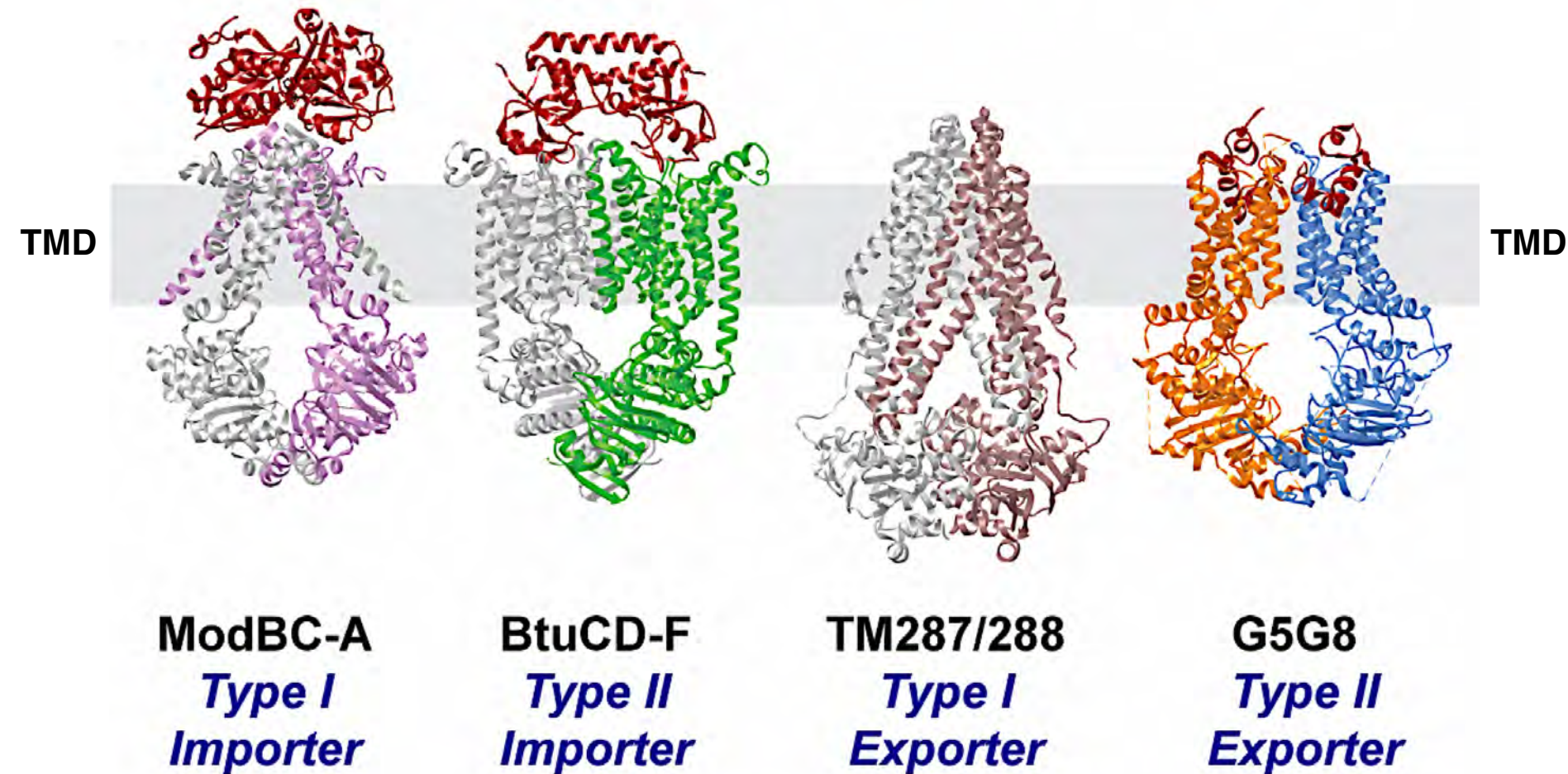
G5G8 bicelle preparation



Crystal growth & X-ray diffraction



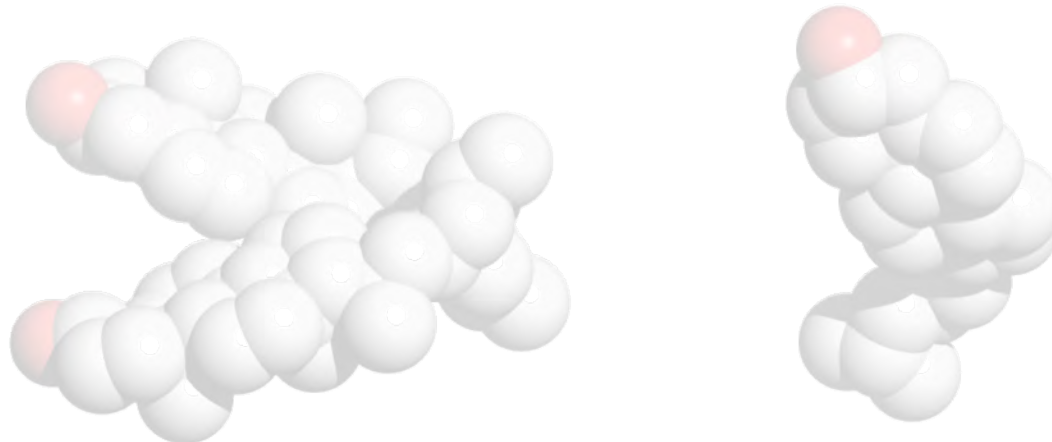
The G5G8 reveals new TMD structural fold for the ABC transporter superfamily.



(Hollenstein et al, Curr Opin Struct Biol, 2007)
(Rees et al, Nature Rev Mol Cell Biol, 2009)

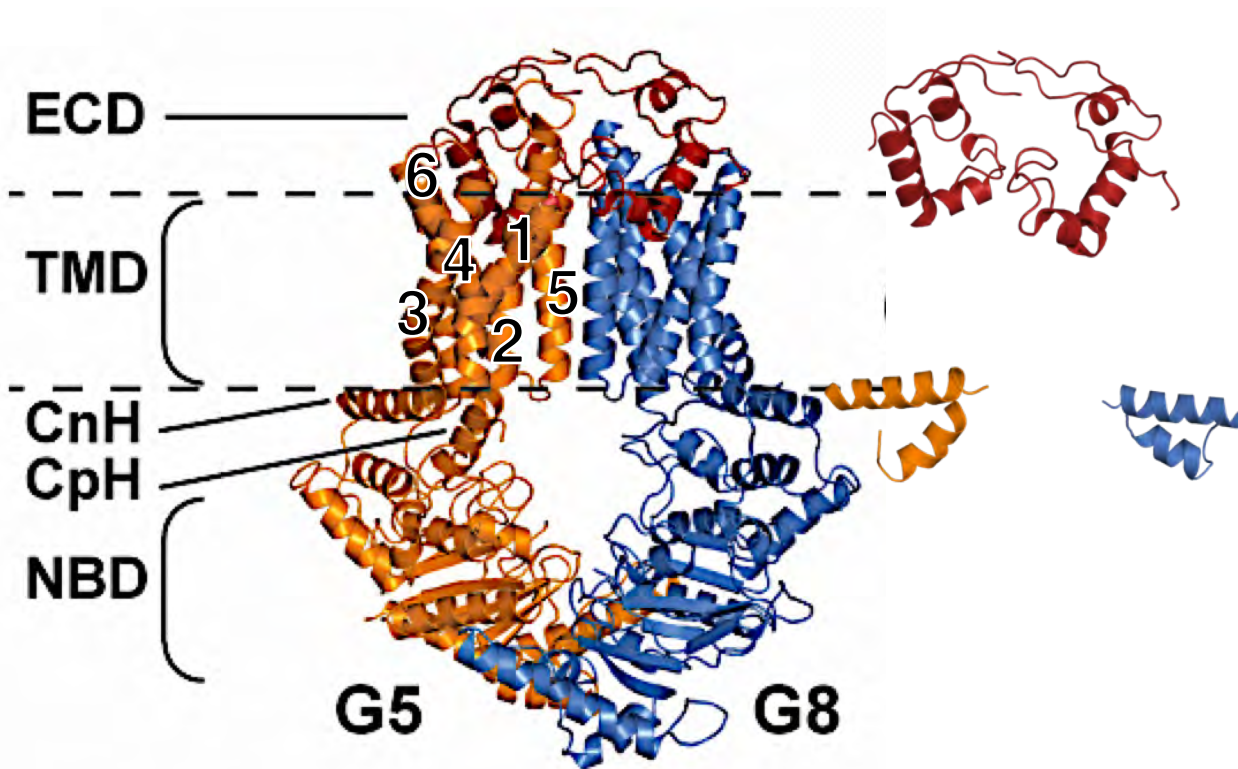
(Lee et al, Nature, 2016)

- **Function and structure of ABCG5/ABCG8**
- ✓ **What's known & what's not known**
- **Summary, questions, & future directions**



ABCG5 and ABCG8 share high structural similarity.

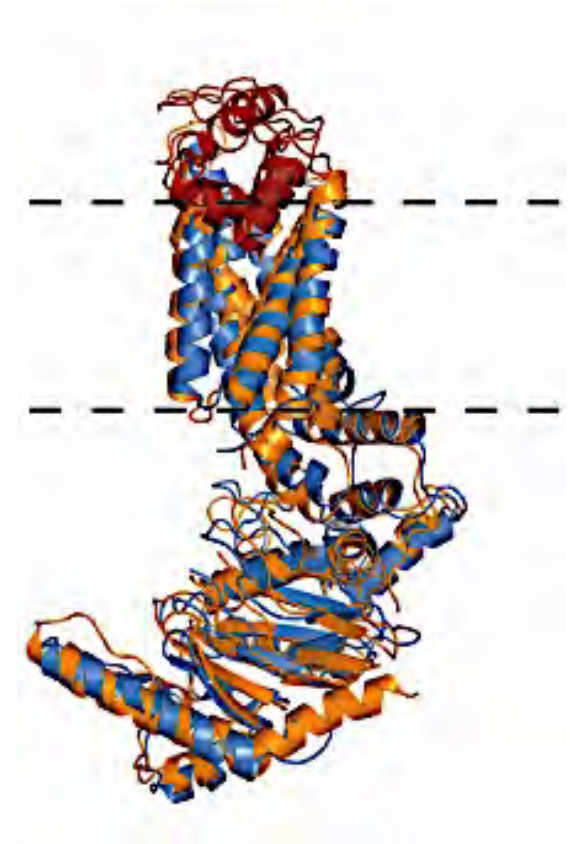
Domain features



TMD: transmembrane domain
NBD: nucleotide-binding domain

ECD: extracellular domain
CnH: connecting helix
CpH: coupling helix

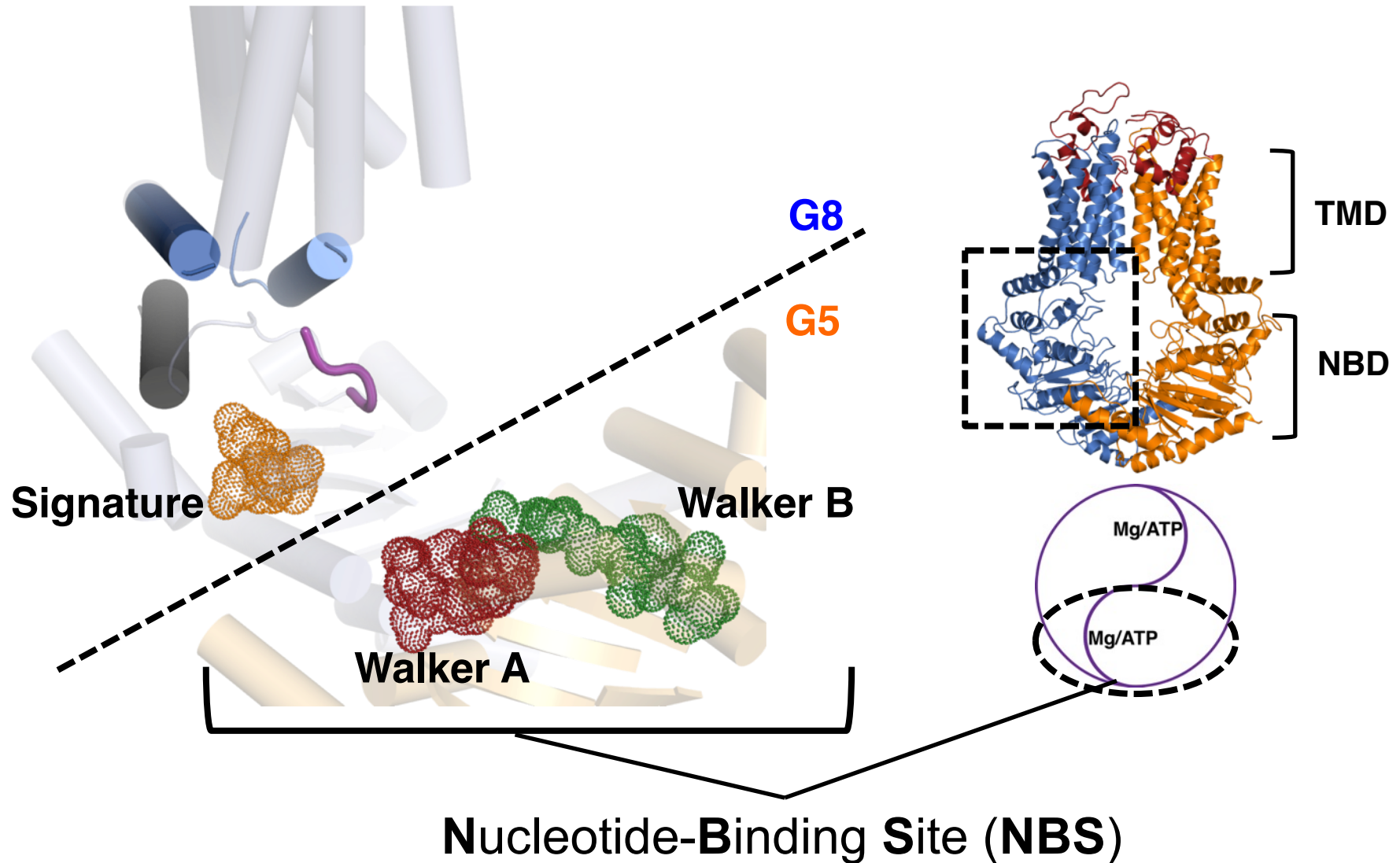
Structural similarity:



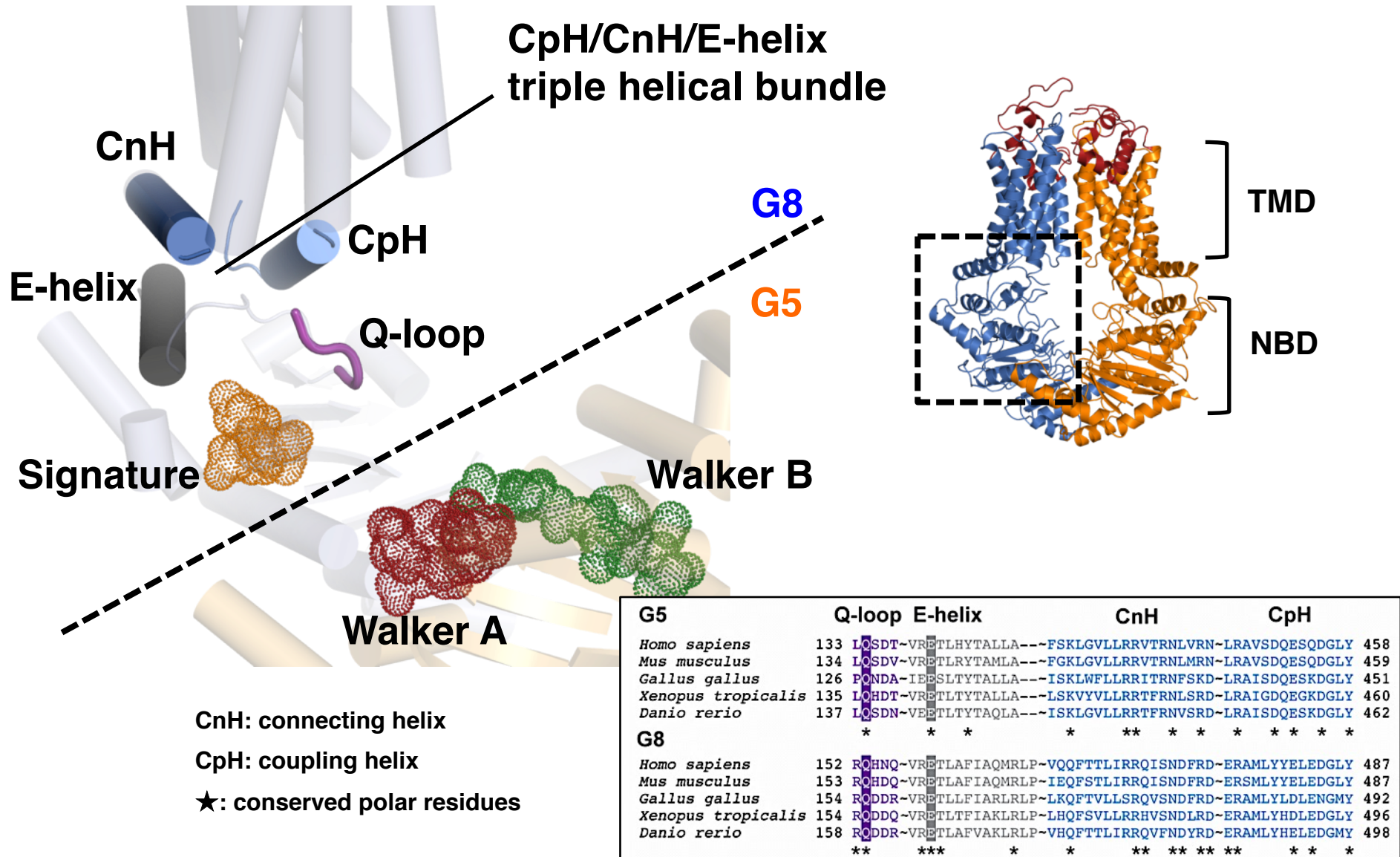
RMSD (C α) ~ 2Å
(~28% sequence identity)

(Lee et al, Nature, 2016)

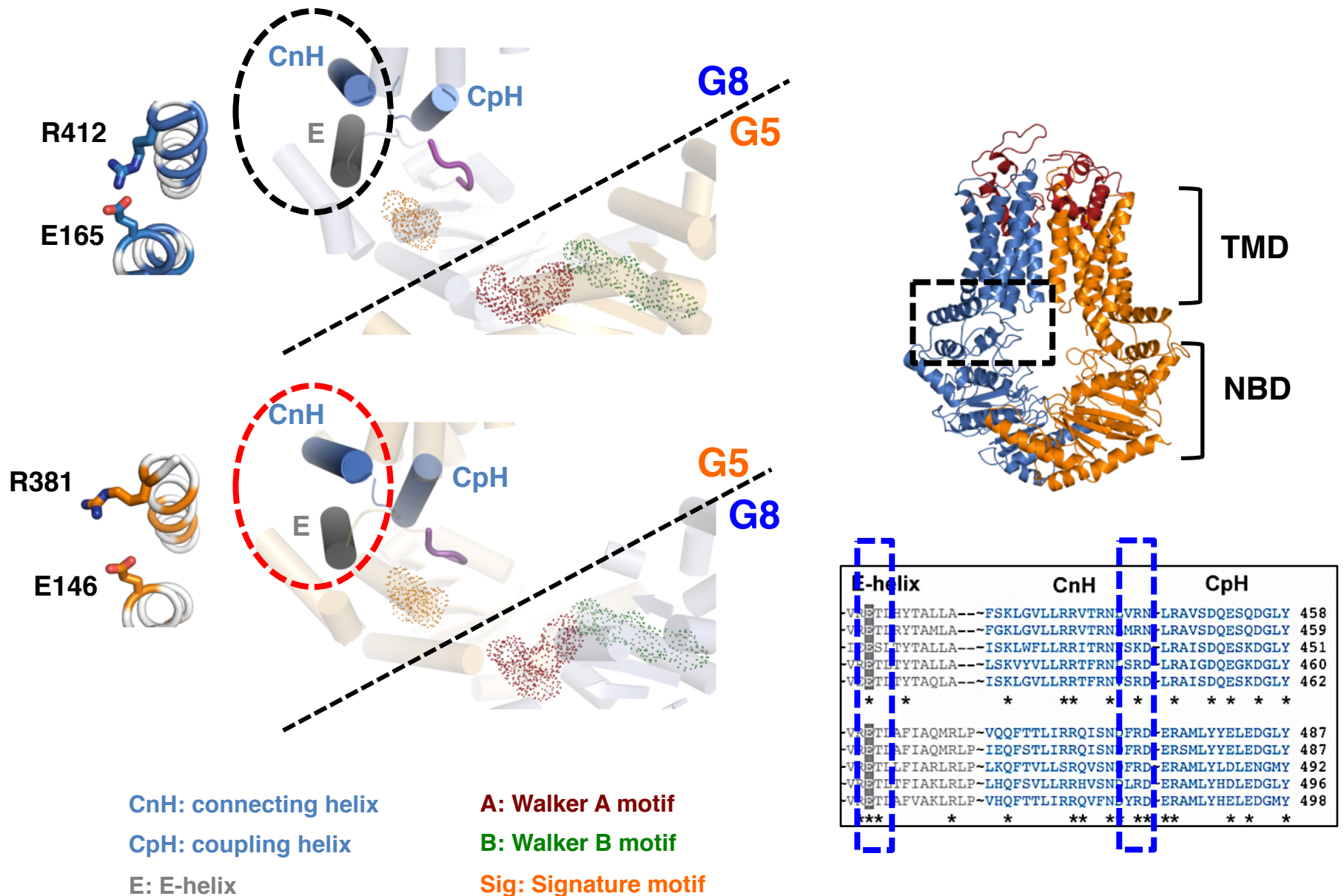
How does the catalytic NBD communicate with sterol transporting TMD?



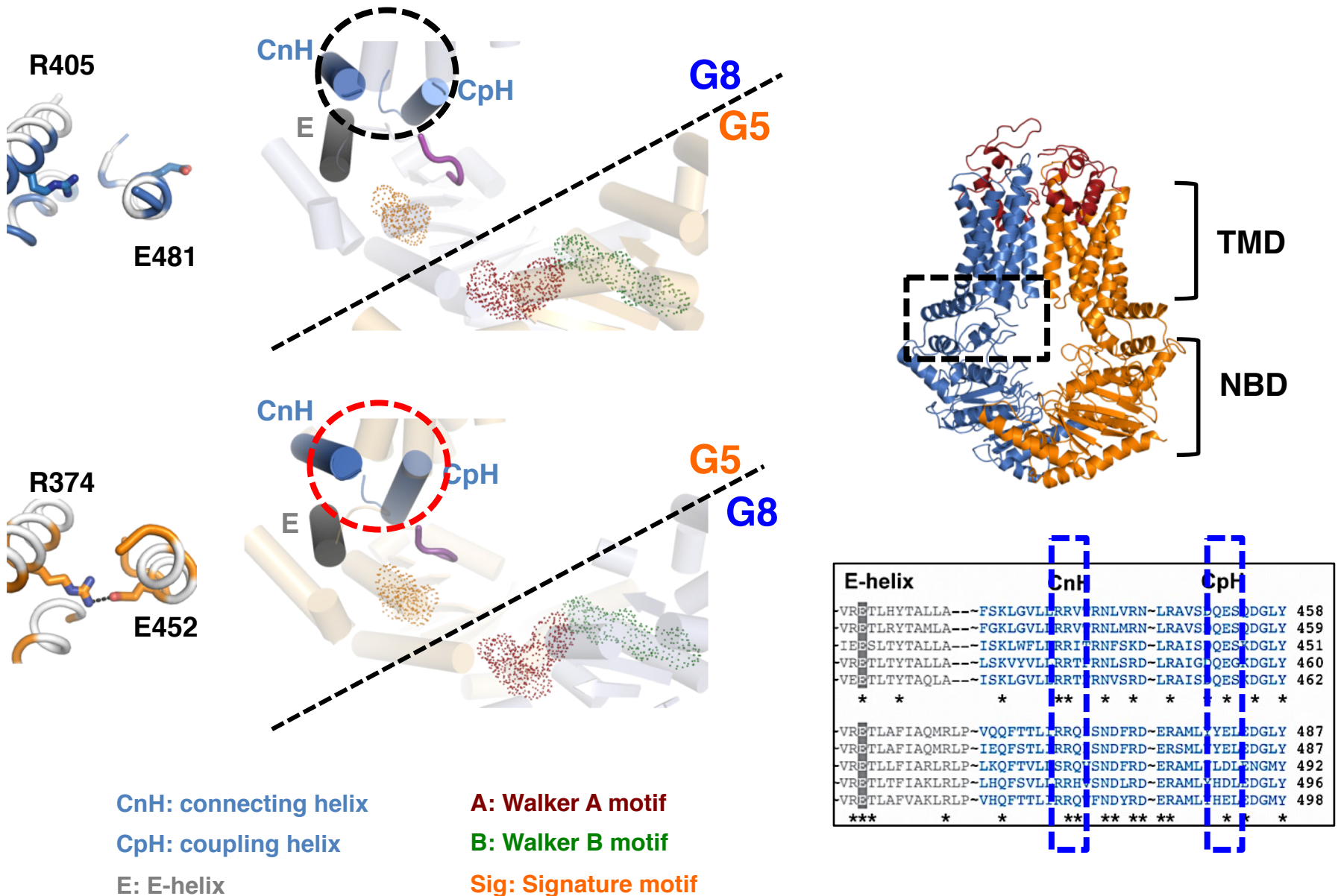
How does the catalytic NBD communicate with sterol transporting TMD?



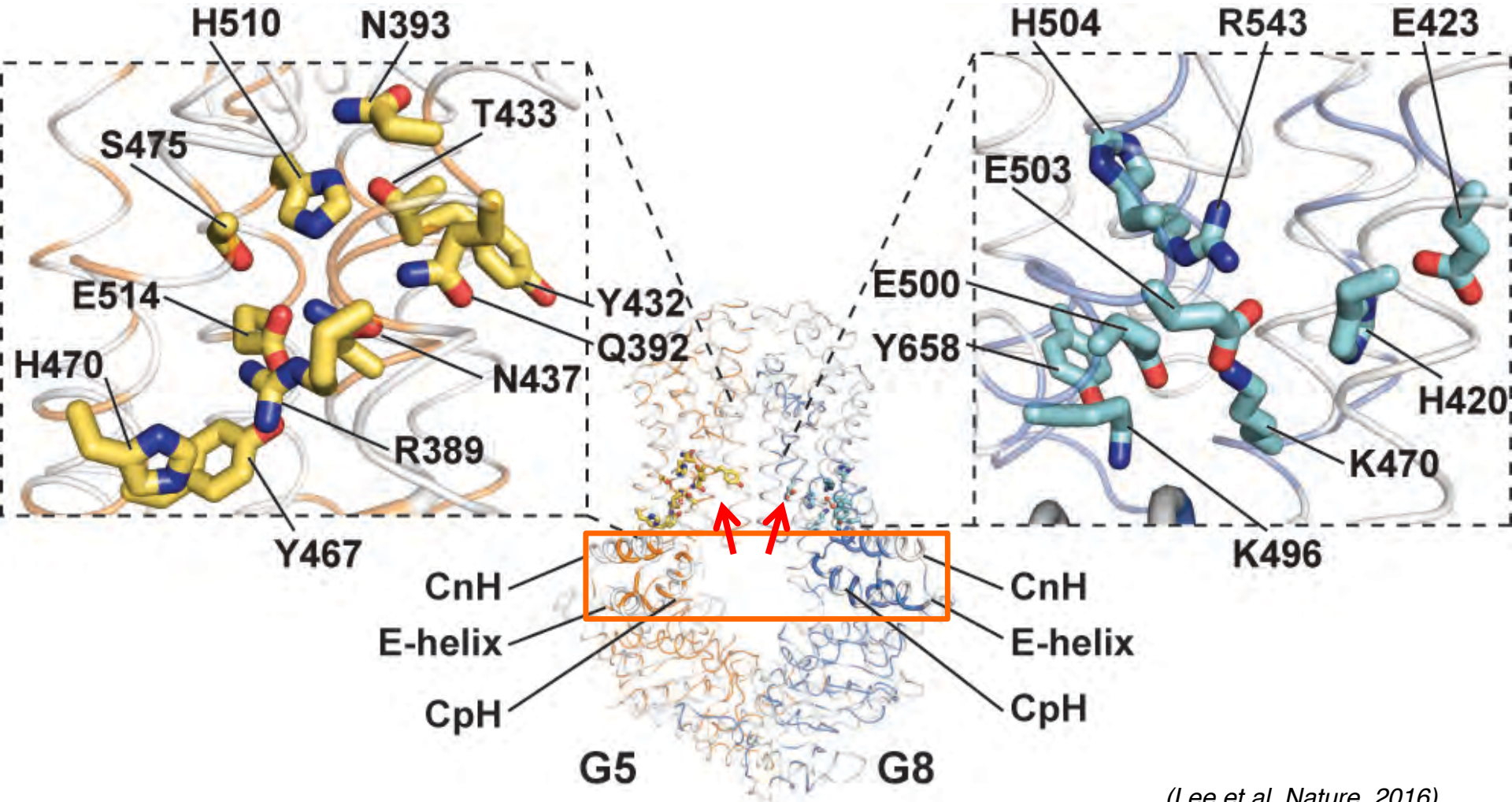
Interactions within the Triple Helical Bundle



Interactions within the Triple Helical Bundle

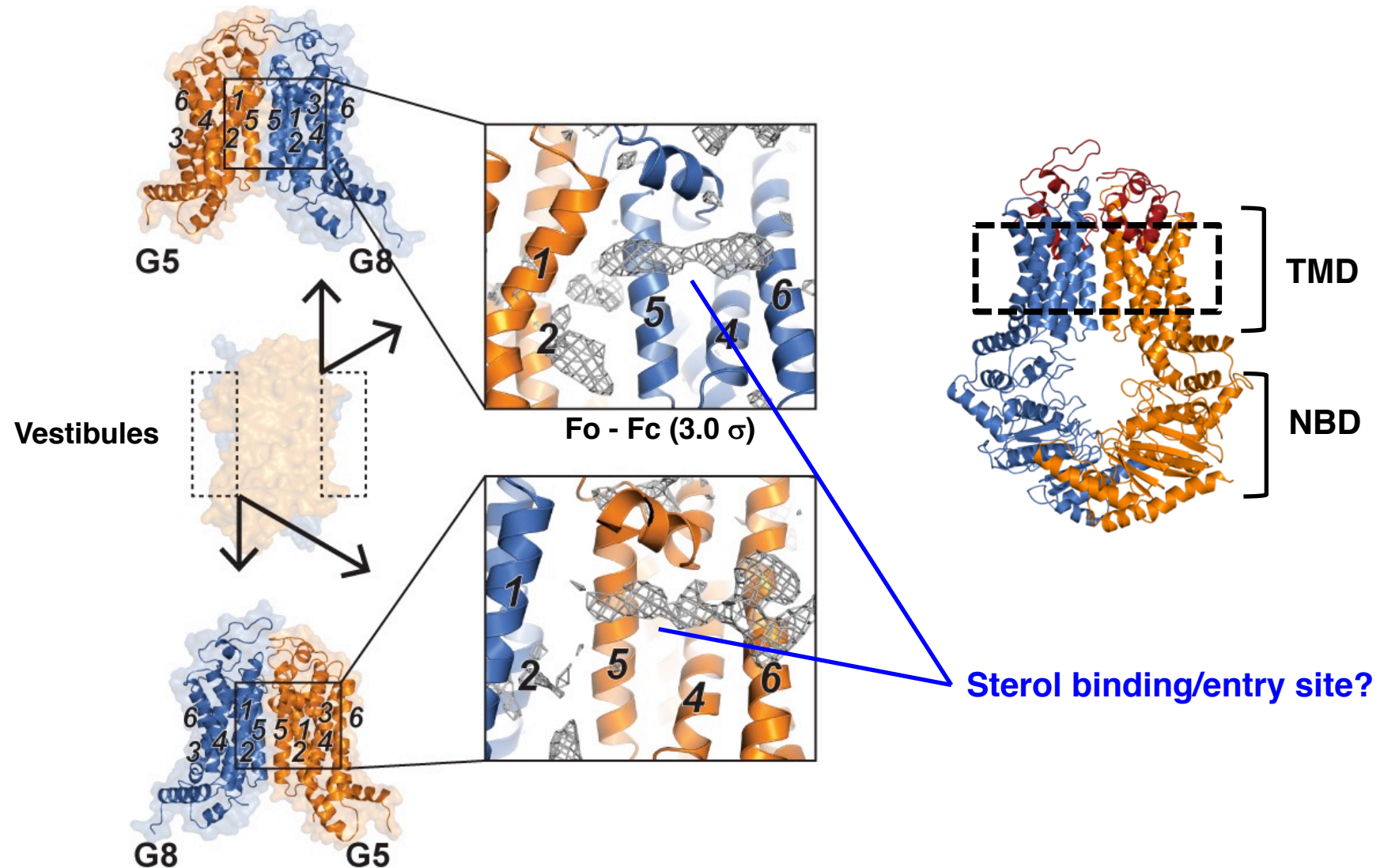


The TMD polar relay connects the triple helical bundle to the TMD.

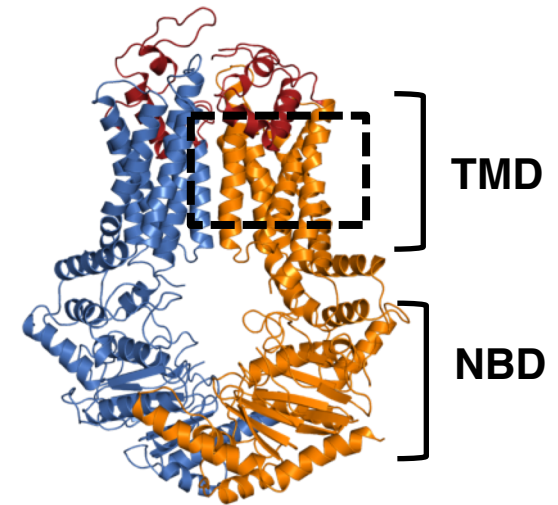
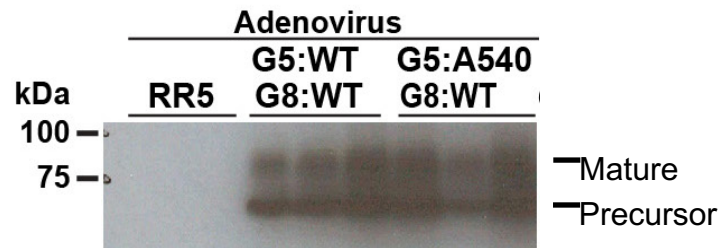
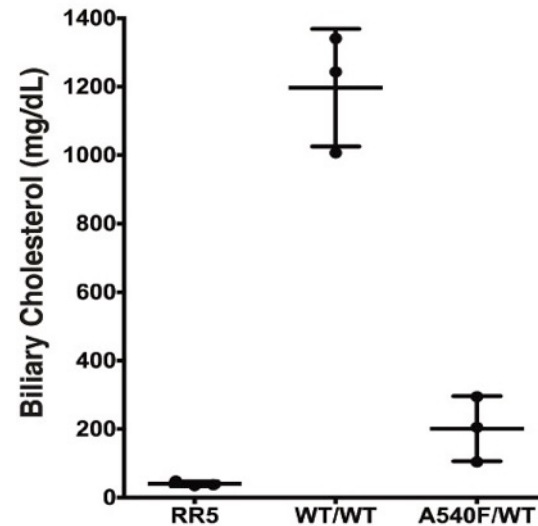
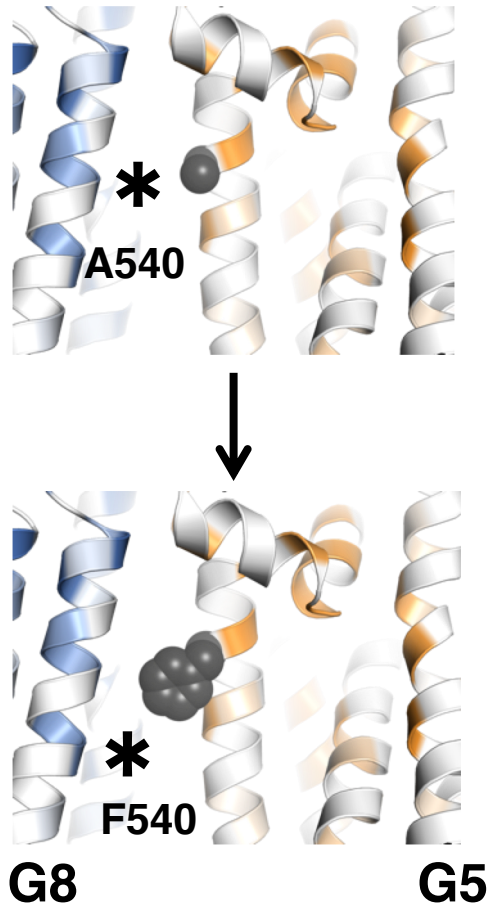


How do sterols move across the lipid-bilayer membranes on the TMD?

Vestibules at the TMD-membrane interface

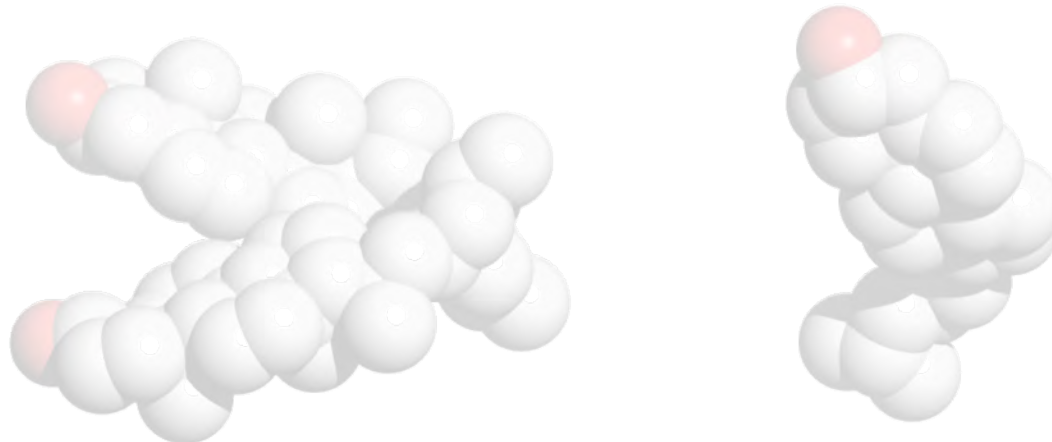


How do sterols move across the lipid-bilayer membranes on the TMD?



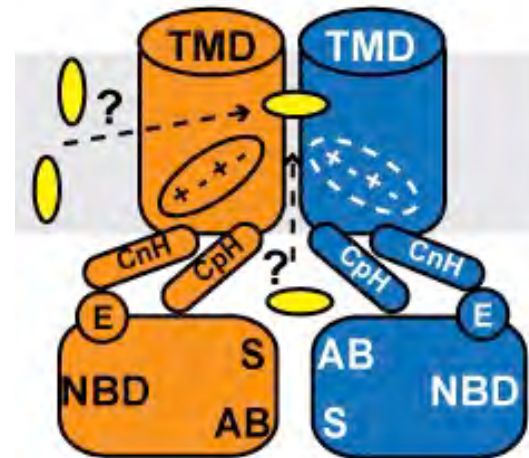
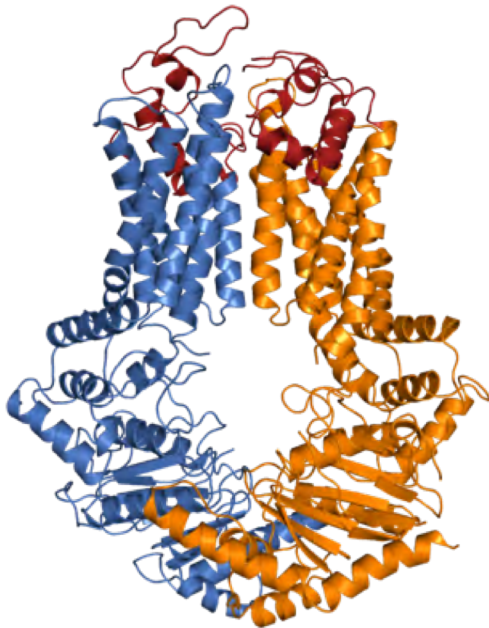
(Lee et al, Nature, 2016)

- **Function and structure of ABCG5/ABCG8**
- **What's known & what's not known**
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Summary

- New TM structural fold for ABC transporters
ABC2 superfamily: G5, G8, G2, A1, ...
- Triple helical bundle b/w NBD and TM helices
- Polar relay
- Sterol “binding” sites



S: ABC Signature

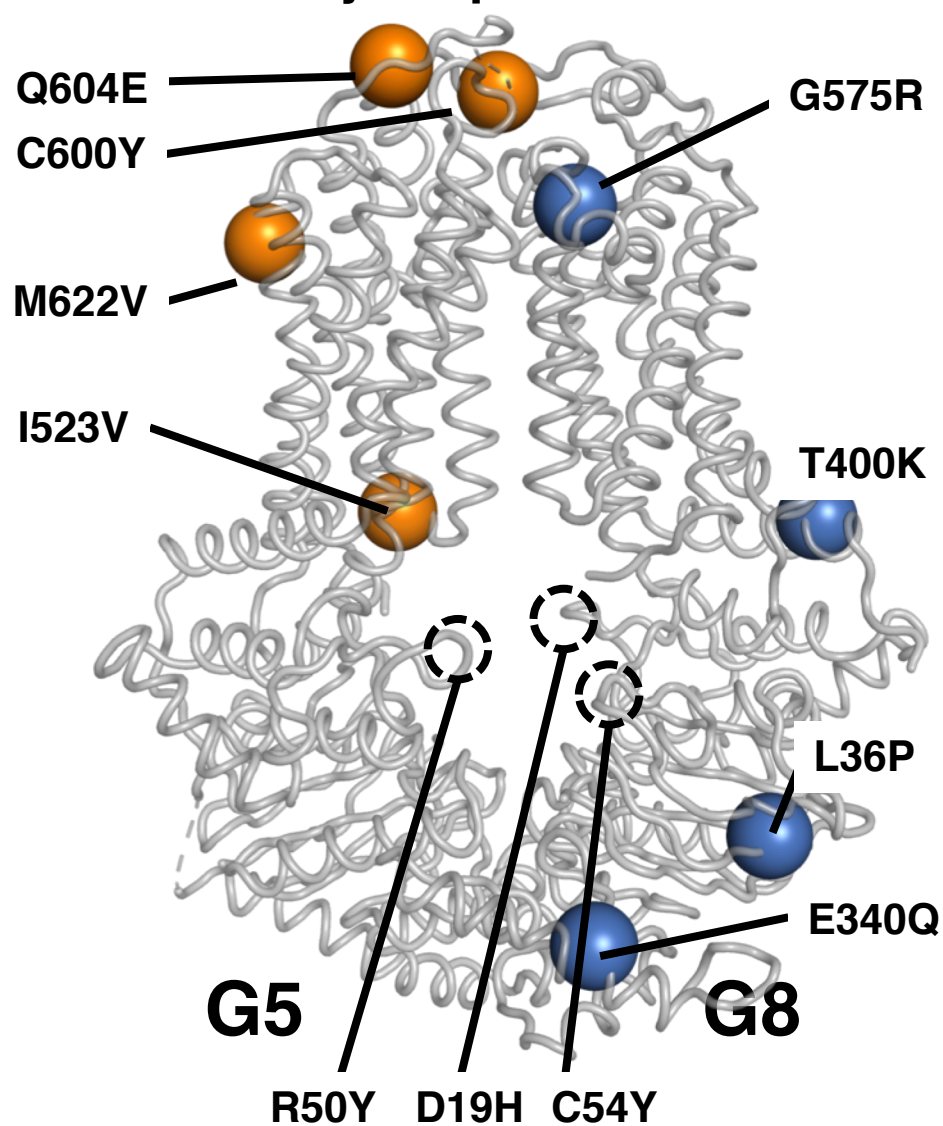
AB: Walker A & B

Questions:

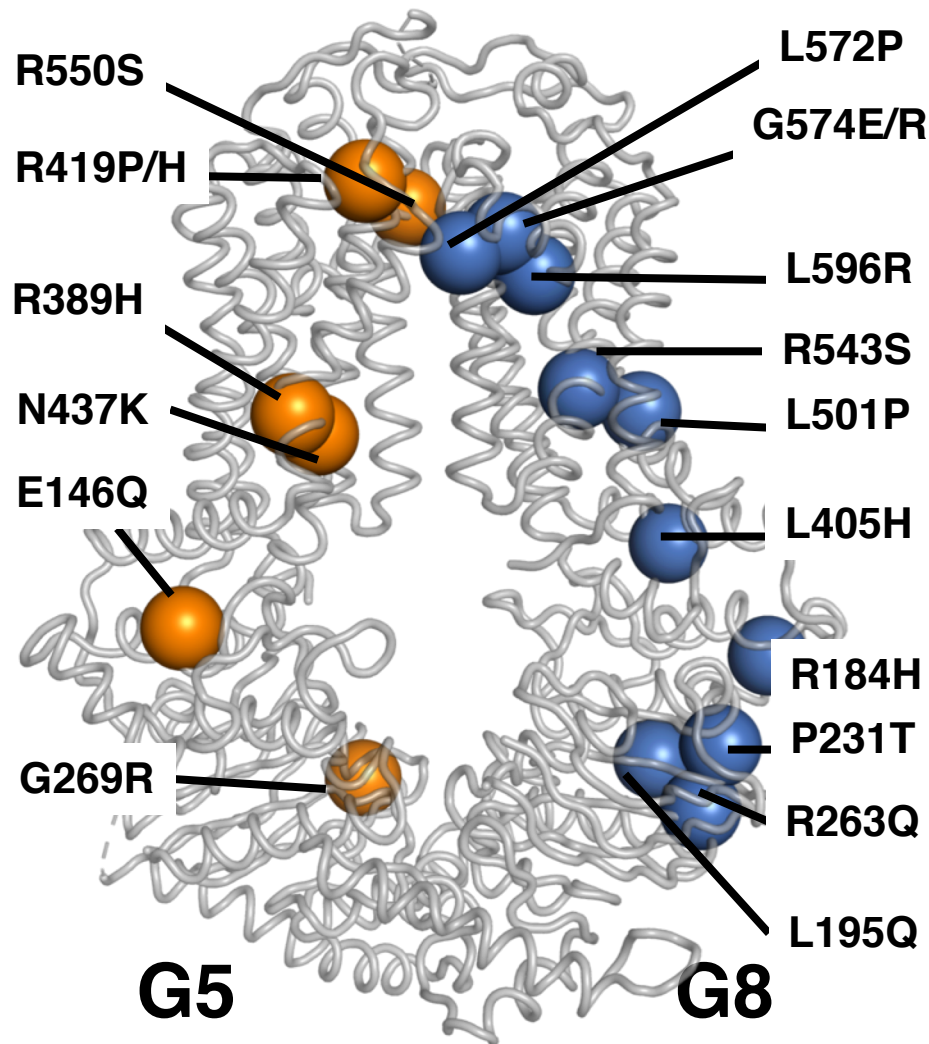
- Impact on medical conditions?

Localization of Missense Mutations and Polymorphisms

Polymorphisms



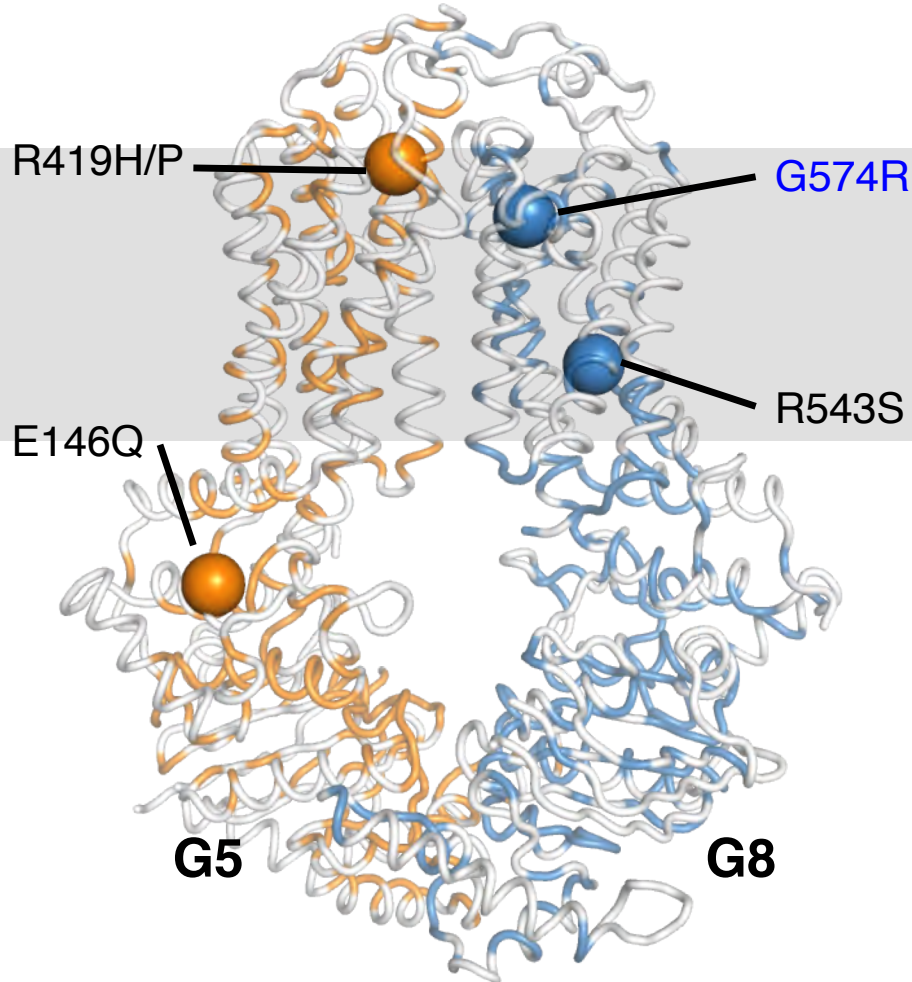
Missense mutations



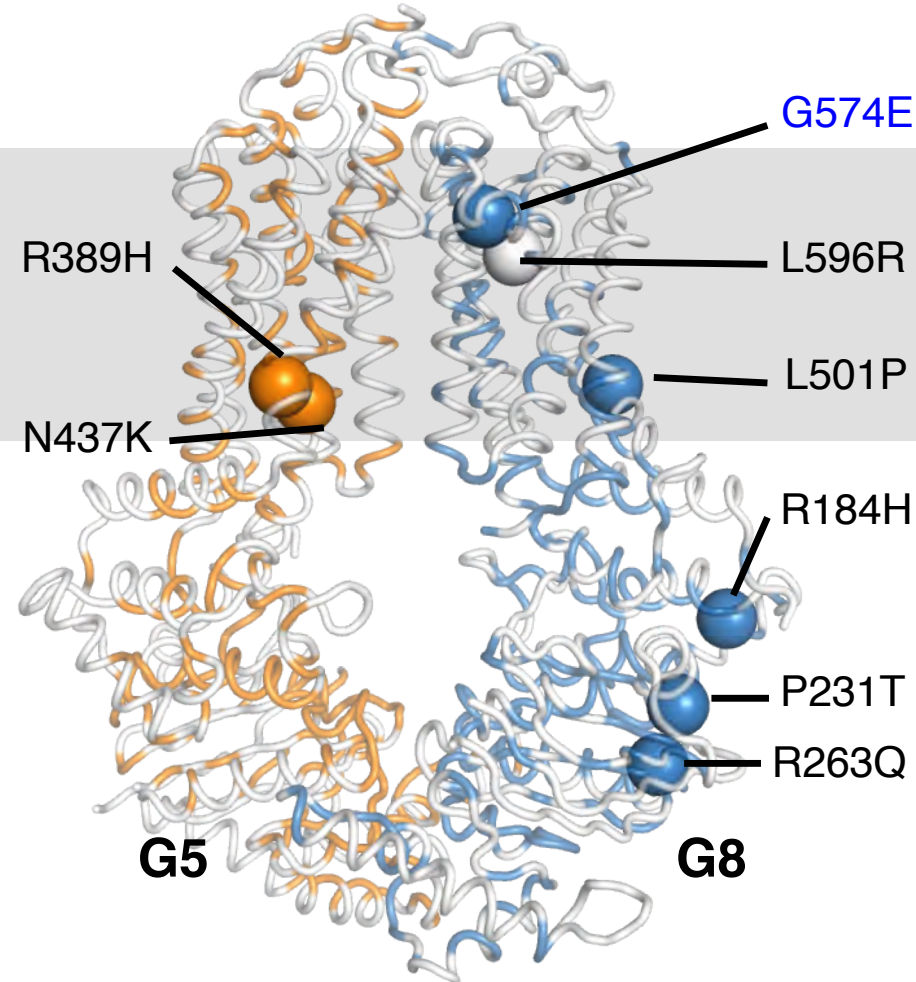
Location of the residues with the disease-causing missense mutations of **sitosterolemia**.

ER-escape missense mutations

Graf et al, JBC, 2004



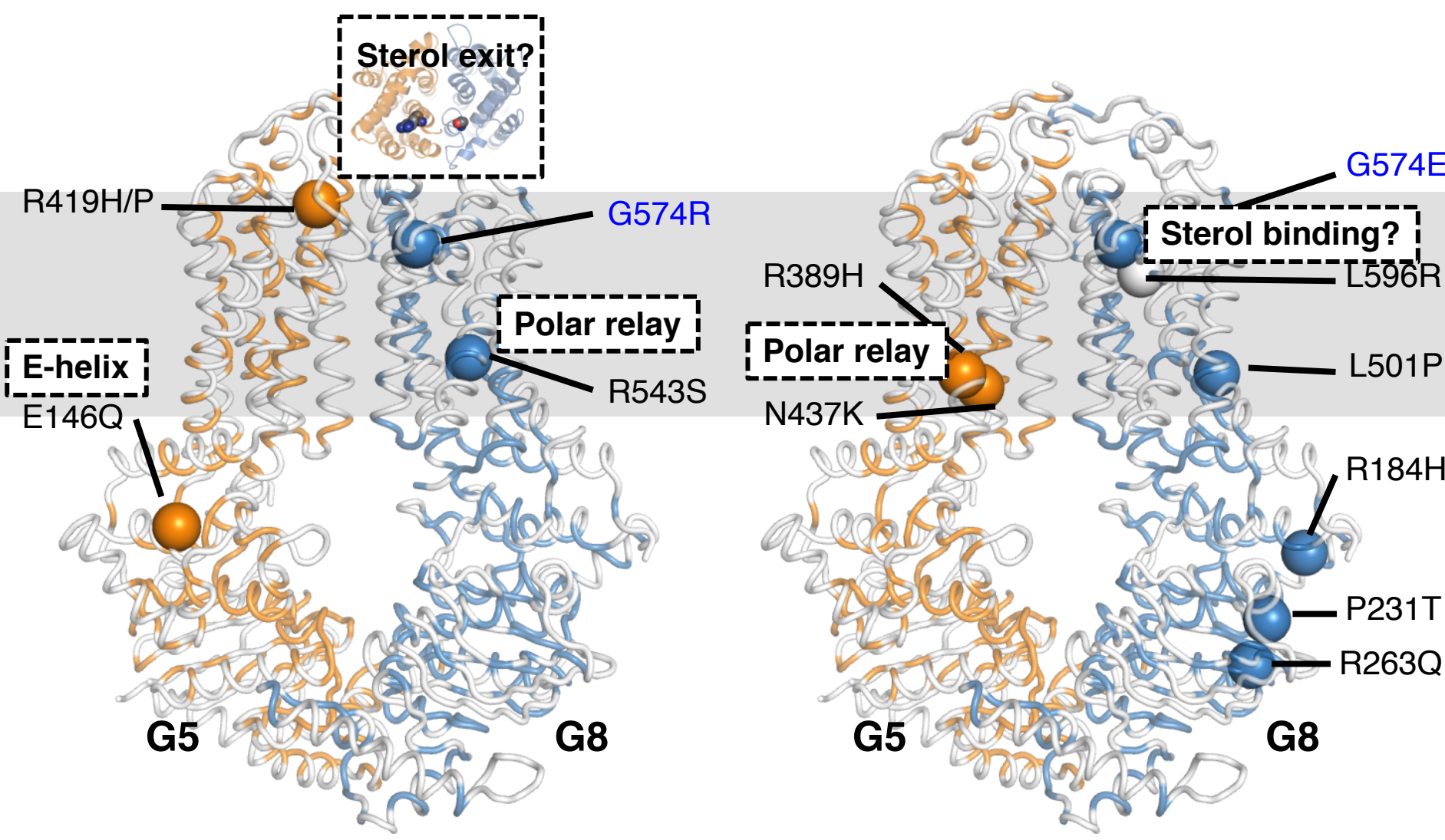
Non-ER-escape missense mutations



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.



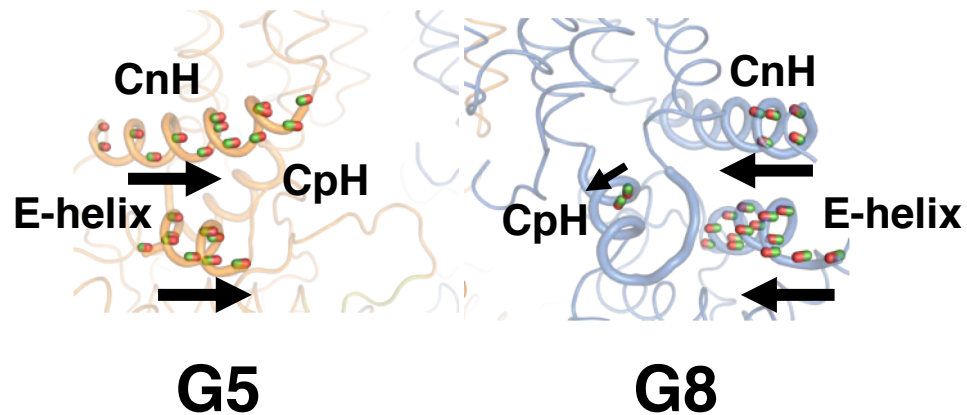
Questions:

- Impact on medical conditions?
- Energy-coupled conformational changes?

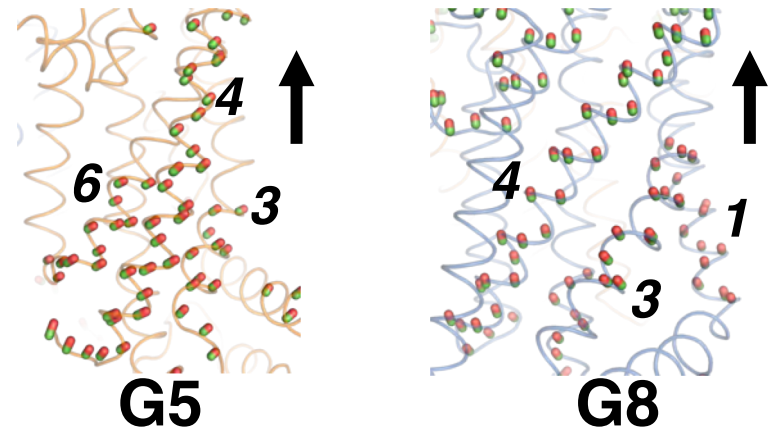
Molecular Dynamics Simulation

(Junmei Wang, Pittsburgh)

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

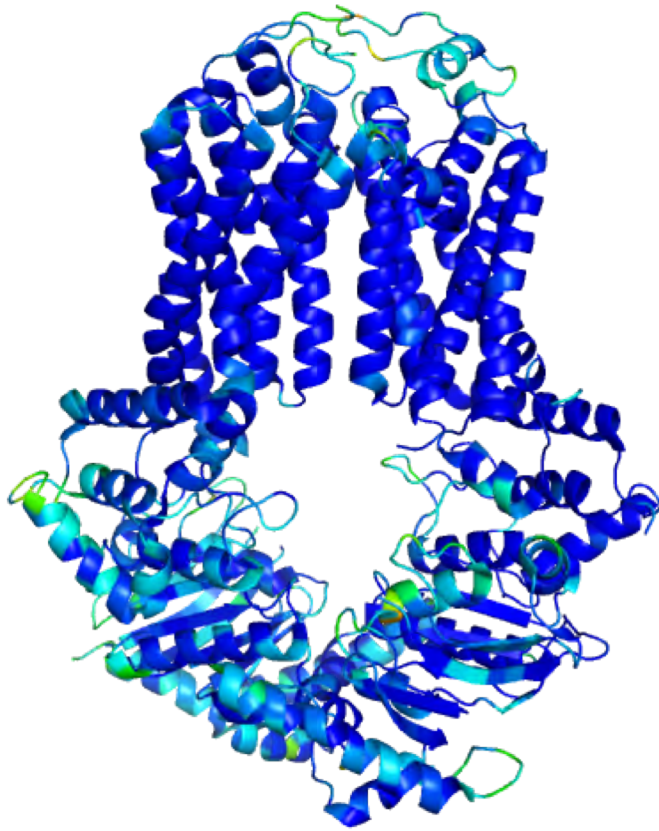


**Upward movement
(TM helices)**

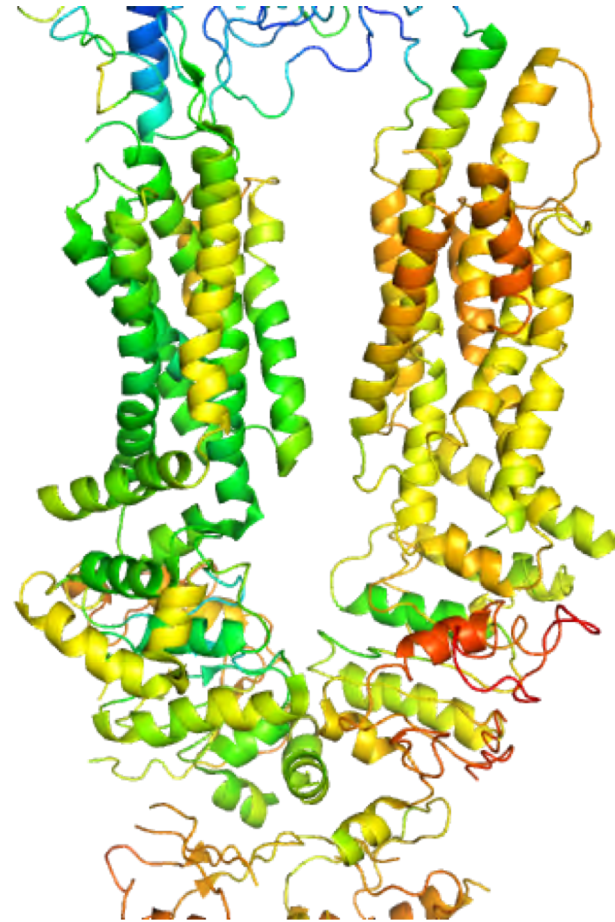


ABC Sterol/lipid Transporters

(Nucleotide-free apo states)



ABCG5/G8

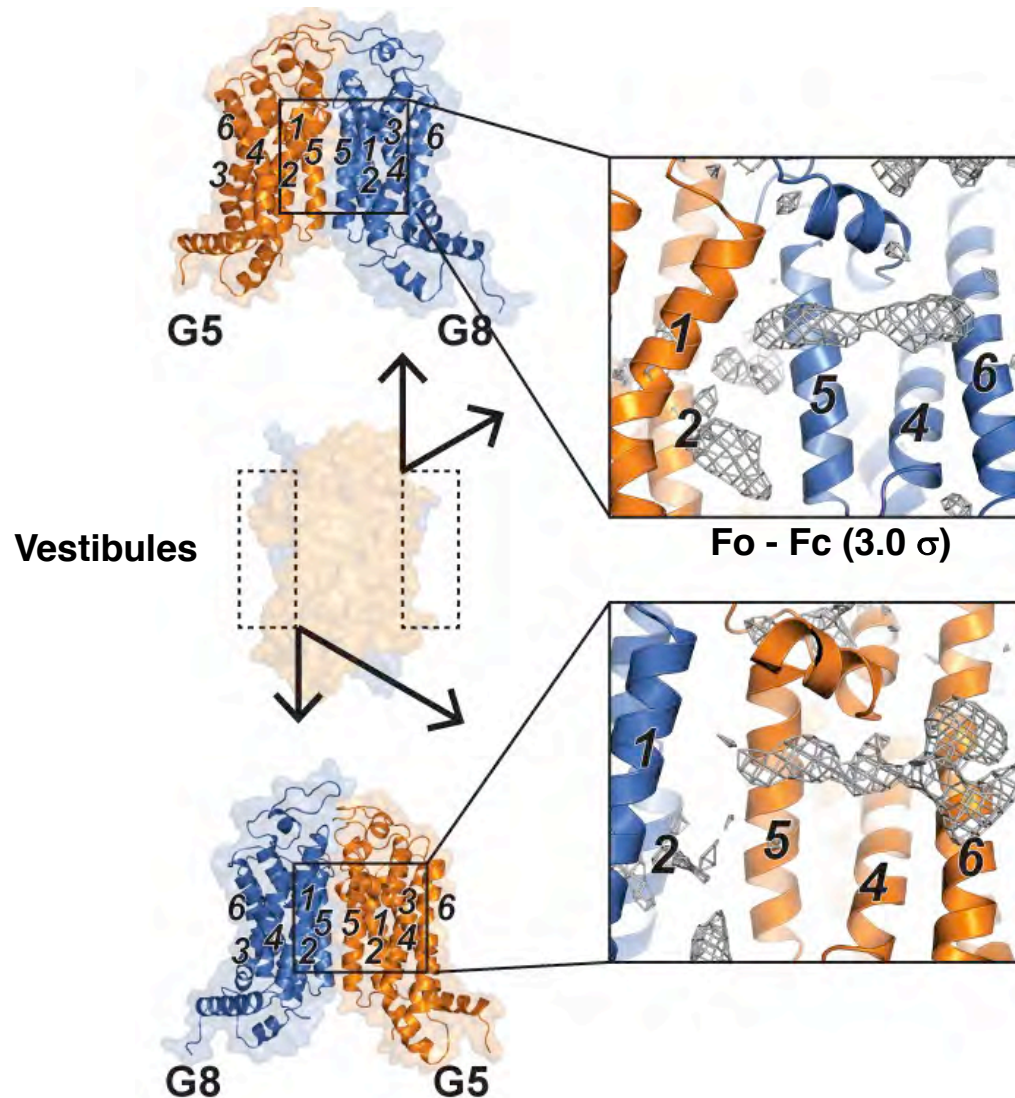


ABCA1

Questions:

- Impact on medical conditions?
- Energy-coupled conformational changes?
- Extracellular domains/loops?
- Sterol/lipid translocation pathway?

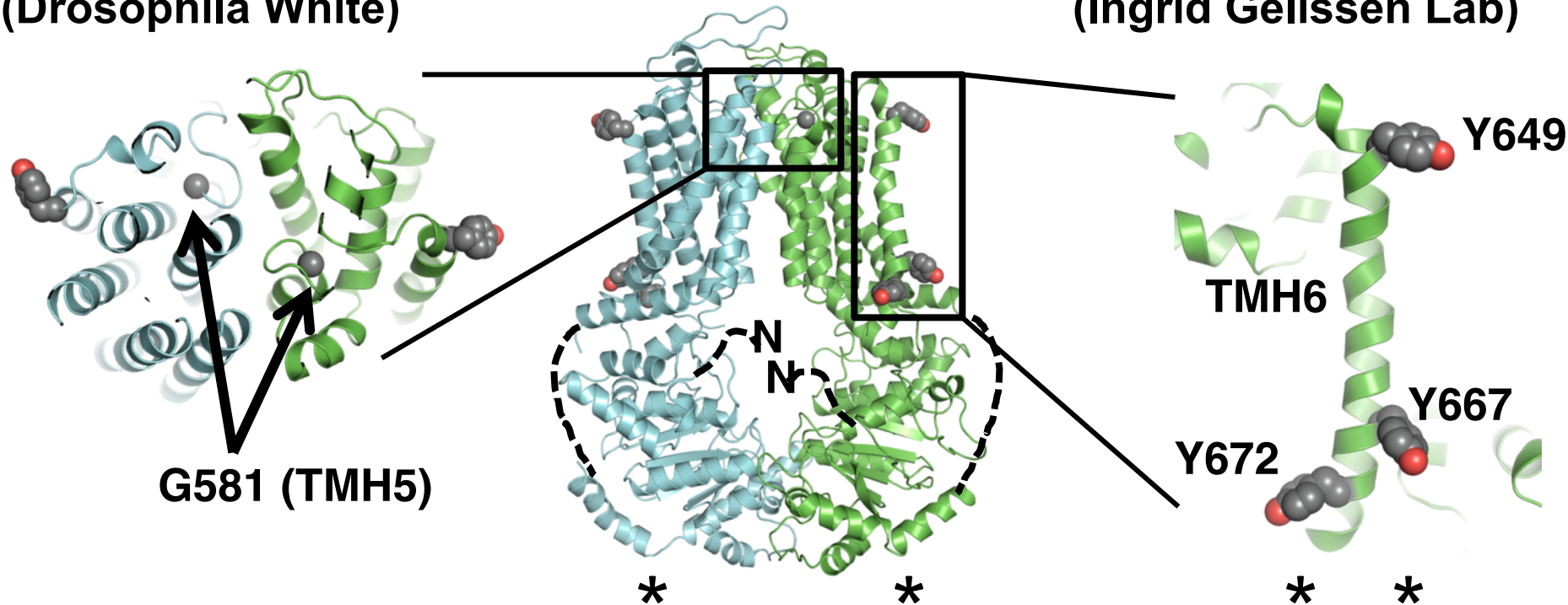
Cholesterol Binding/Translocation?



Homology Model of ABCG1

Substrate exit:
(Drosophila White)

Sterol-Sensing:
(Ingrid Gelissen Lab)



ABCG1	562	LQVATFVG	TAIPVLLFS	G FFVS	--//--	L YLDFIVLG	IFFISLR	LI A Y FVLR	Y KI	674
ABCG2	534	VSVATLLM	TI-CFVFMM	IFSGLLVN	--//--	LWKNHVAL	ACMIVIF	LTIAYLK	LLFLK	652
ABCG4	531	LQVATFVG	TAIPVLLFS	SGFFVS	--//--	LYMDFLVL	GIFFLAL	RLLAYLV	LRYRV	642
ABCG5	527	PNIVNSV	VALLSIAG	VLVGSGFL	RN--//--	FTMNFLIL	YSFIPAL	VILGIVV	FKIRD	646
ABCG8	556	FHMASFF	SNA-LYNS	FYLAGGF	MIN--//--	LYAIYLIV	IGLSGGF	MVLYYV	SLRFIK	666
White	570	TSMALSV	GPP-VIIP	FLLFGGFF	LN--//--	LPLDYVGL	AILIVSF	RVLAYLA	LRLRA	683
TMH5					TMH6					

Questions:

- Impact on medical conditions?
- Energy-coupled conformational changes?
- Extracellular domains/loops?
- Sterol/lipid translocation pathway?
- Functional oligomeric states?

Previous works on A1, G2, Pdr5, ...