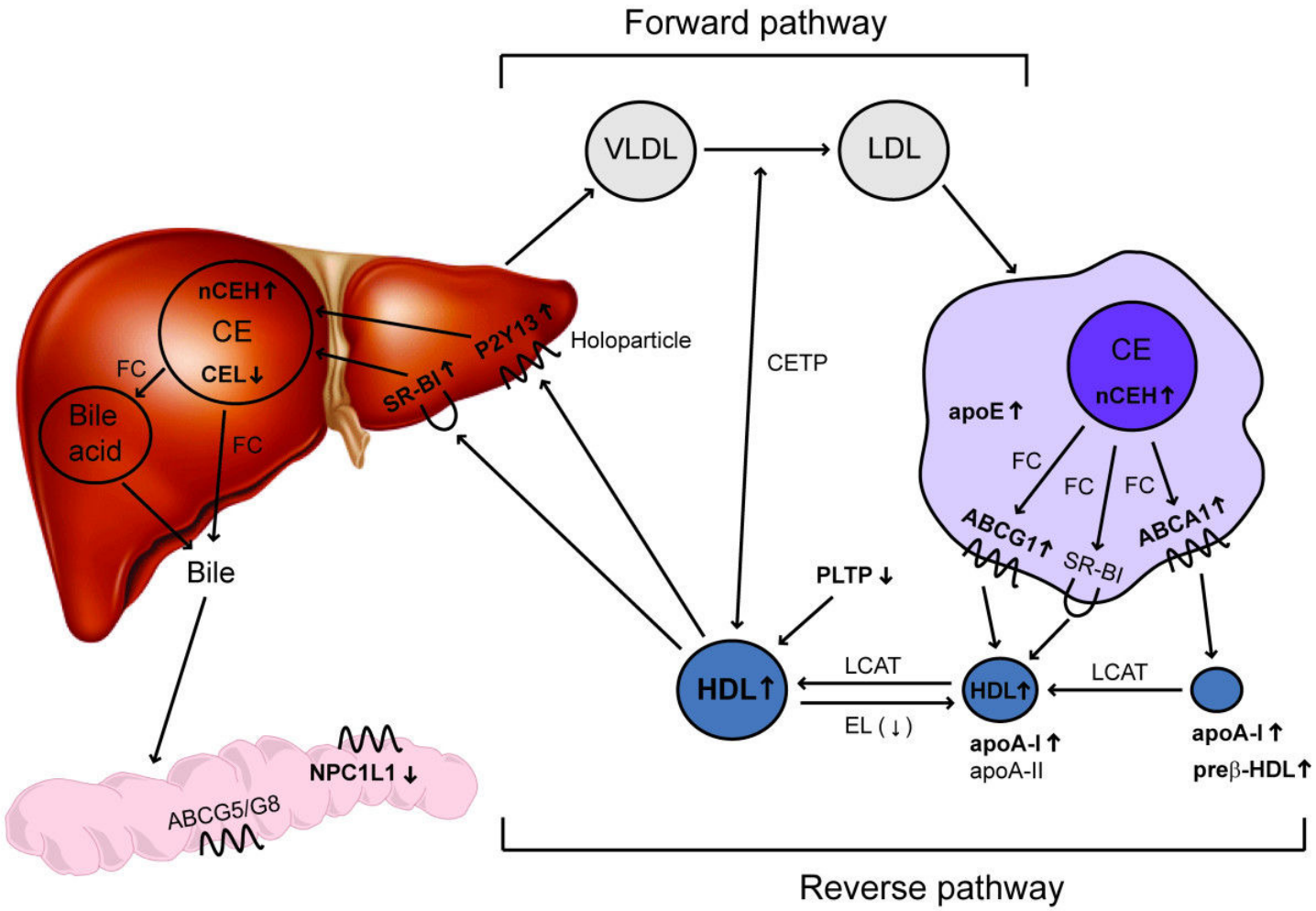
BCH 8107:
Lipids in Health and Disease

- Structural Biology of ABC Sterol Transporters
  (March 2nd, 2022, Virtual Classroom)

Jyh-Yeuan (Eric) Lee, Assistant Professor, BMI

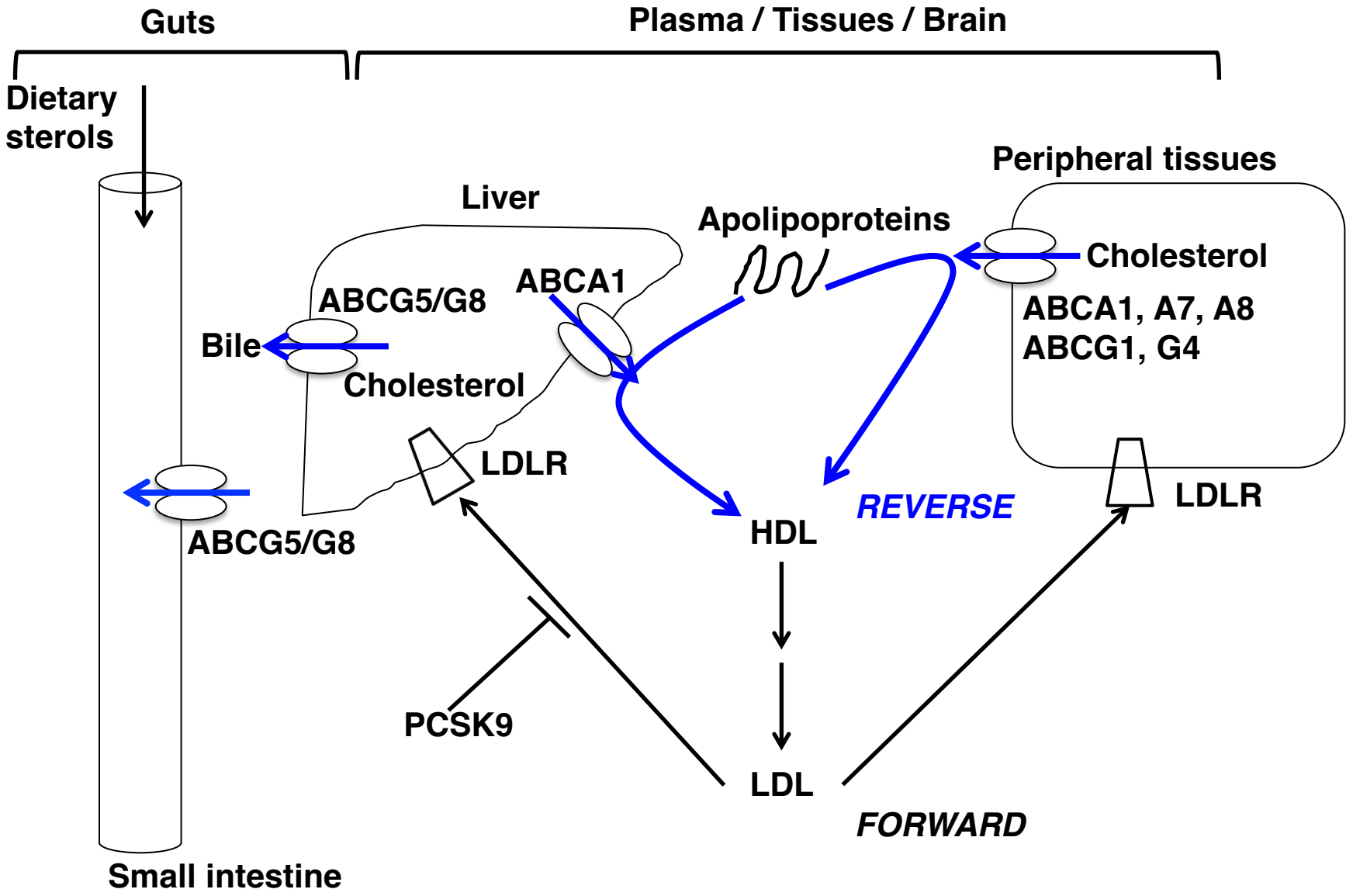
- **Membrane protein-mediated sterol transport**
- ABC sterol transporters in health and disease
- Structural biology approach to study ABC sterol transporters
- Mechanistic models of sterol transport
  - Lessons from ABCG and ABCA

Cholesterol-Trafficking by Transporters Extracellular

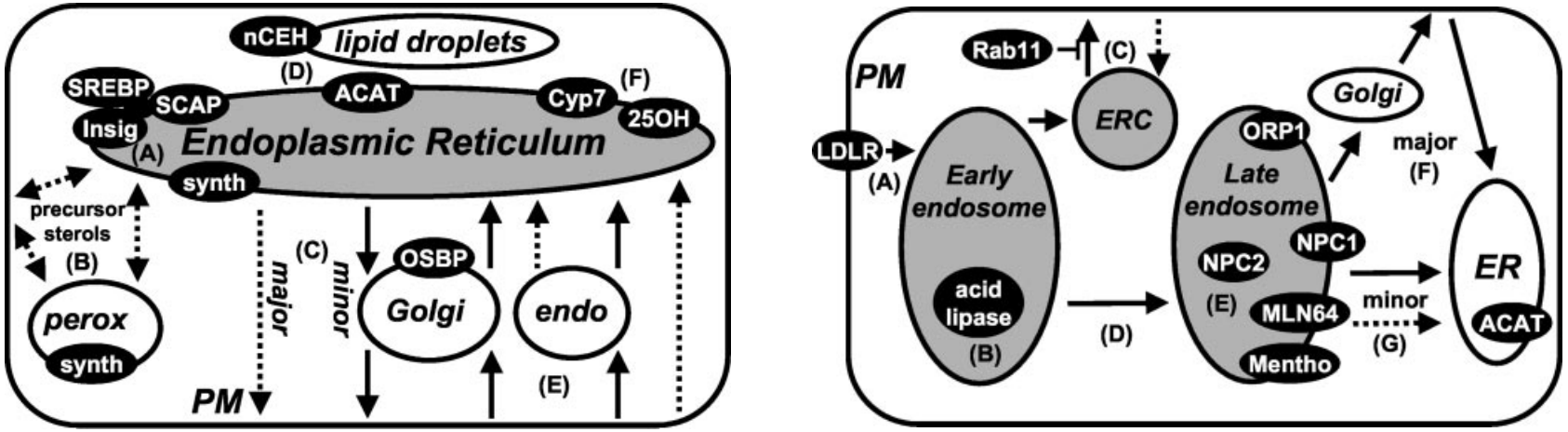


(Annema & Tietge, Nutrition and Metabolism, 2012, 9:25)

ABC Transporters in Cholesterol Trafficking



Cholesterol-Trafficking by Transporters Intracellular



(Soccio & Breslow, ATVB, 2004)

- Membrane protein-mediated sterol transport
- **ABC sterol transporters in health and disease**
- Structural biology approach to study ABC sterol transporters
- Mechanistic models of sterol transport
  - Lessons from ABCG and ABCA

ABCA1: Tangier Disease / HDL deficiency

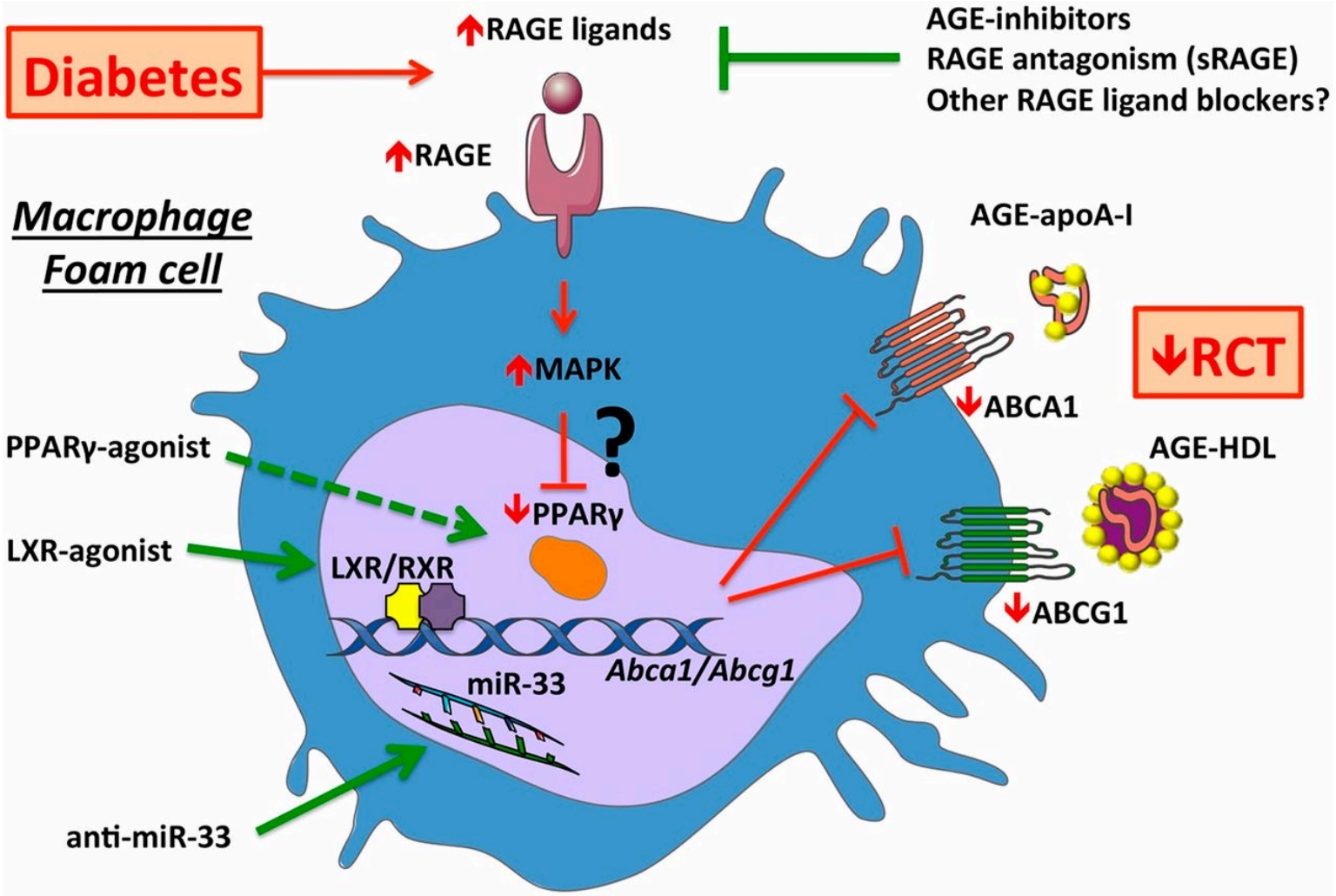
TANGIER DISEASE

MEDREWIND

- Its also known as **Familial HDL deficiency**.
- Its rare Autosomal codominant form of extremely low plasma HDL-C levels that is caused by mutations in gene encoding **ABCA1 (ATP-Binding Cassette transporter A1)**.
- The biochemical signs of this condition are: **plasma HDL < 5mg/dL**, low total plasma cholesterol (below 150mg/dL), and **apoA-1 (<5mg/dL)**.
- Cholesterol accumulates in reticuloendothelial system of these patients, resulting in **Hepatosplenomegaly** and pathognomonic enlarged, **grayish yellow or orange Tonsils**.

- An intermittent peripheral neuropathy (Mononeuritis multiplex) can be seen.

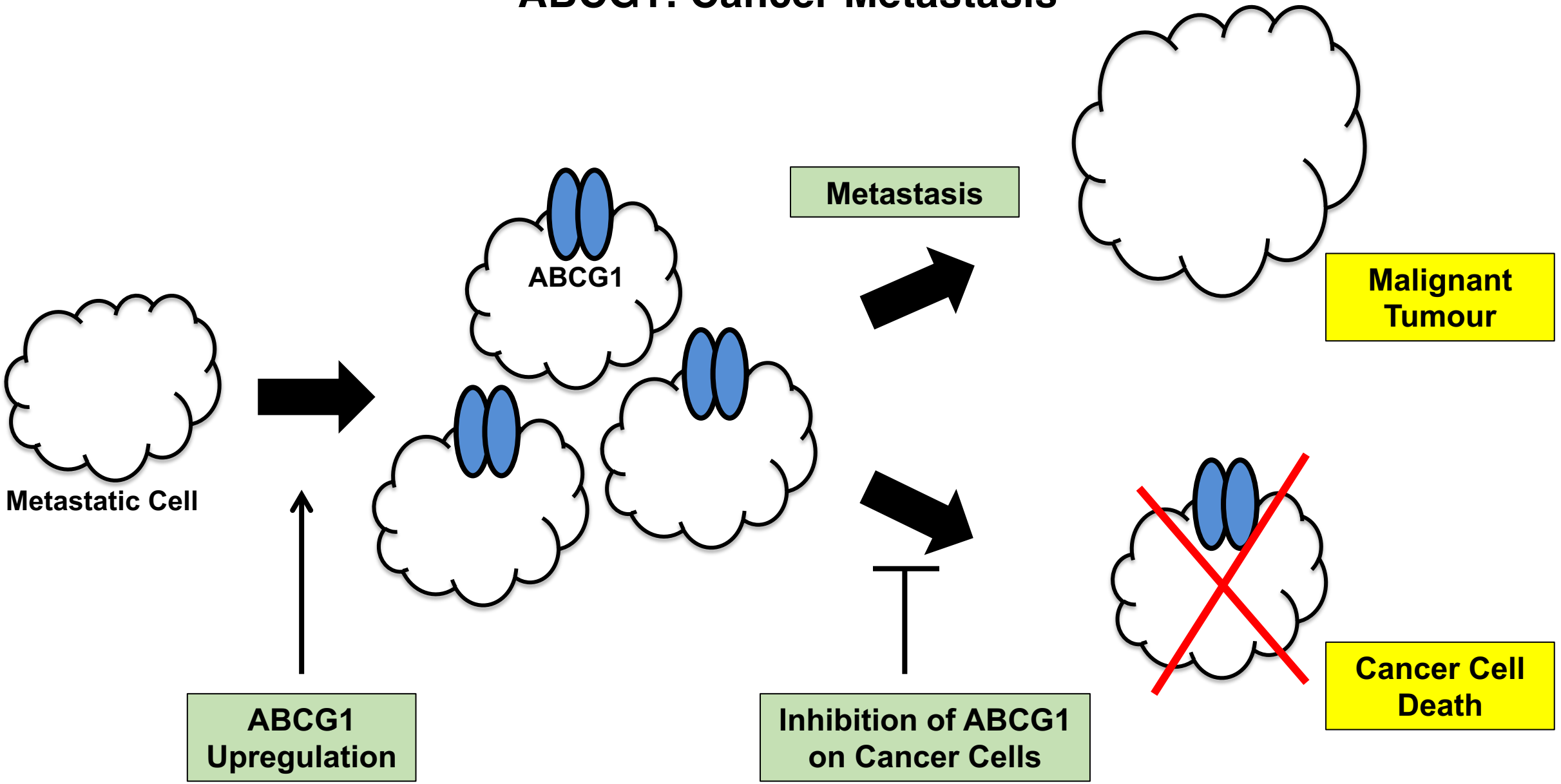
ABCA1 & ABCG1: Diabetes / Atherosclerosis



Terri J. Allen et al. Diabetes 2015;64:3981-3983

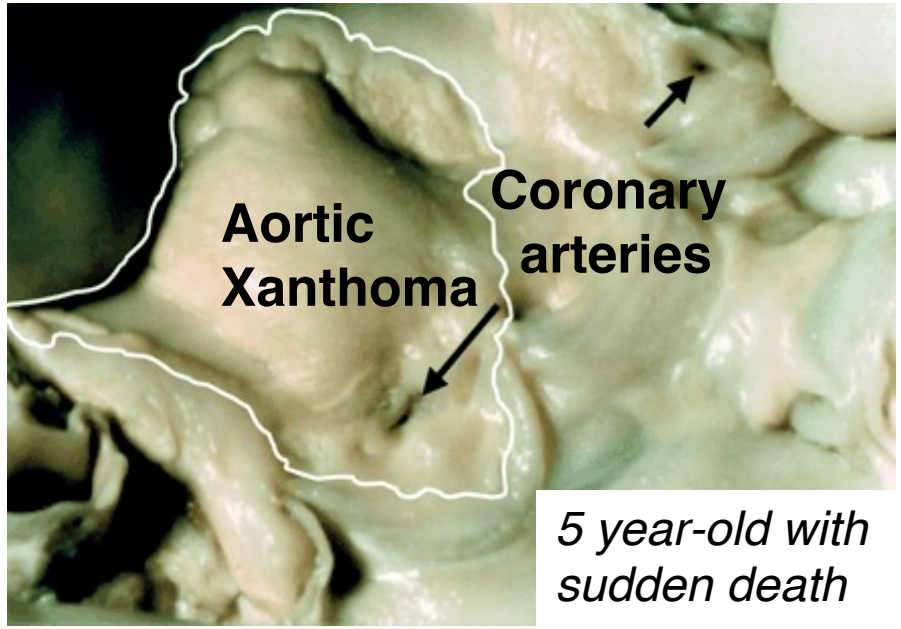
American Diabetes Association.

ABCG1: Cancer Metastasis



ABCG5/ABCG8: Sitosterolemia / Hypercholesterolemia

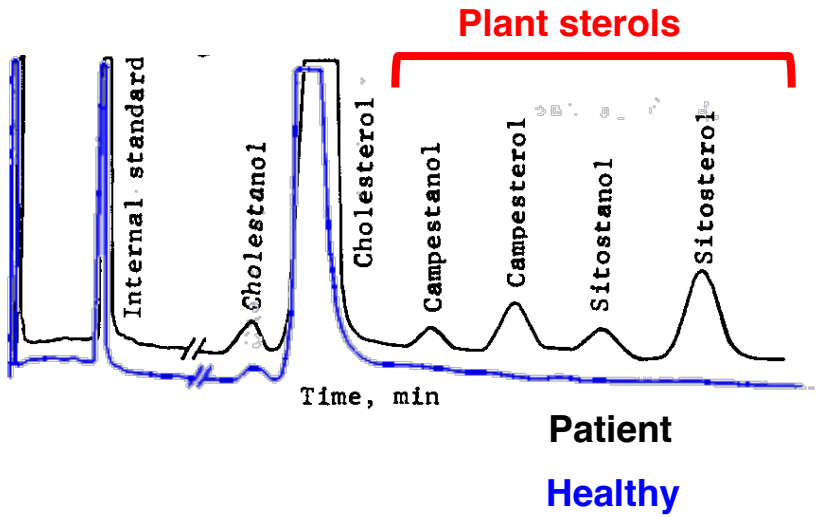
Aortic valve of heart



5 year-old with sudden death

- ↑ Plant sterols
- ↑ Cholesterol
- Premature coronary atherosclerosis

(Mymin et al, Circulation, 2003)



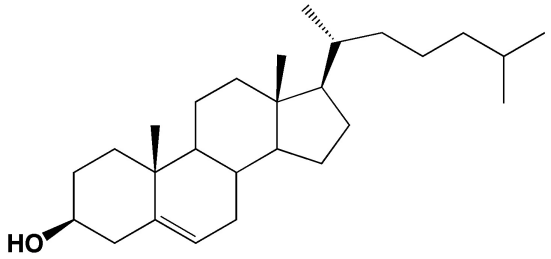
14 patients:

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

(Salen et al, JLR, 1985)

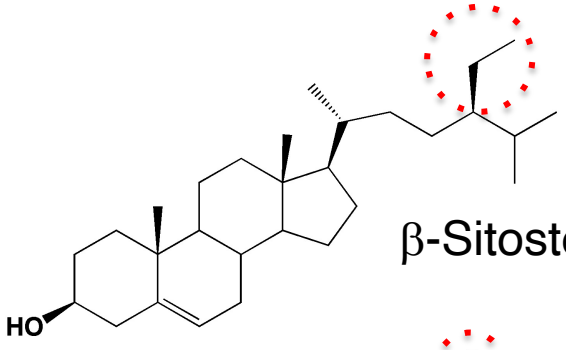
Dietary Sterols

Animal (60%)

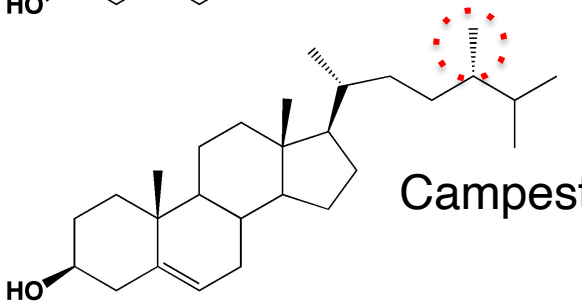


Cholesterol

Plant (40%)



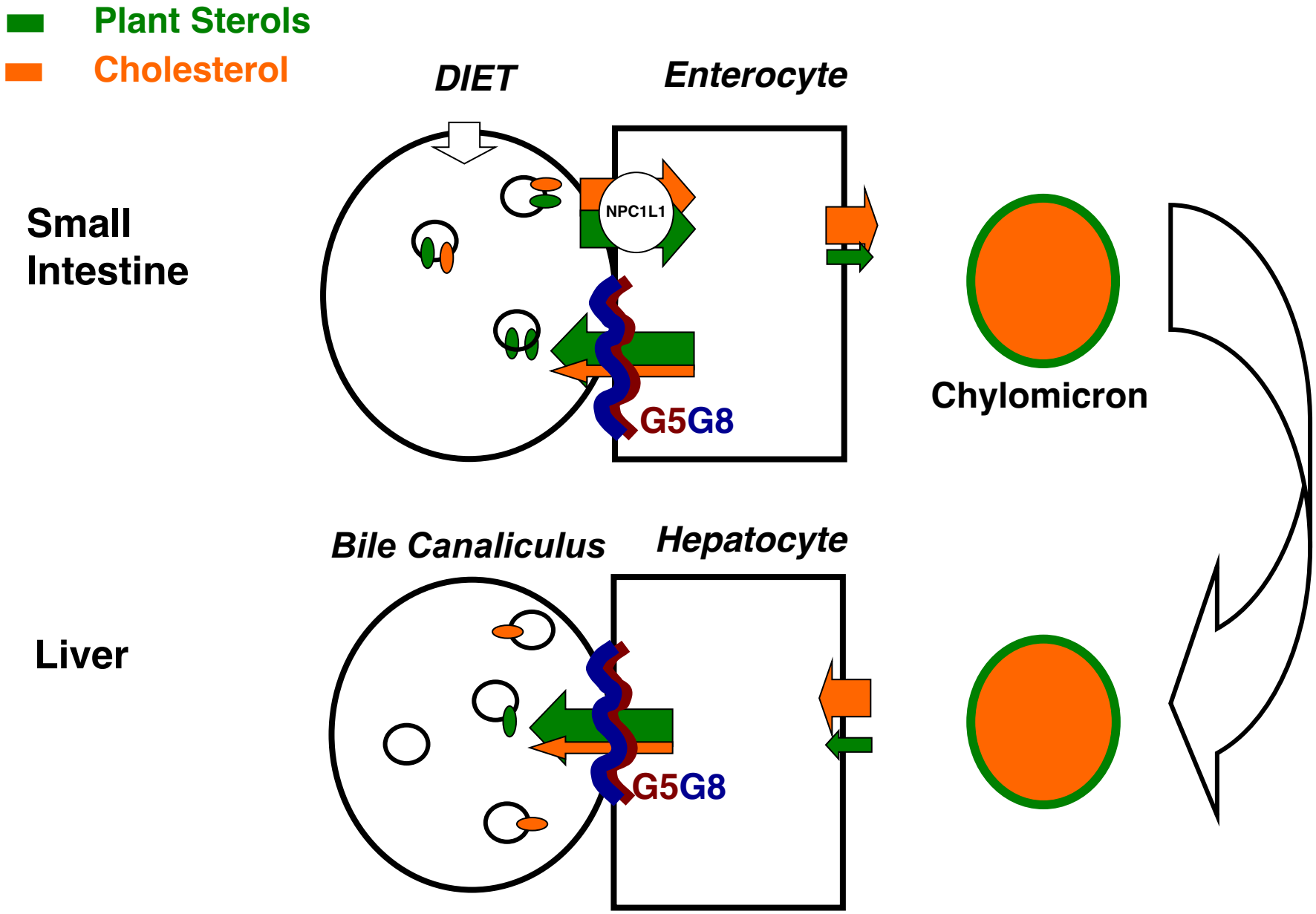
β-Sitosterol



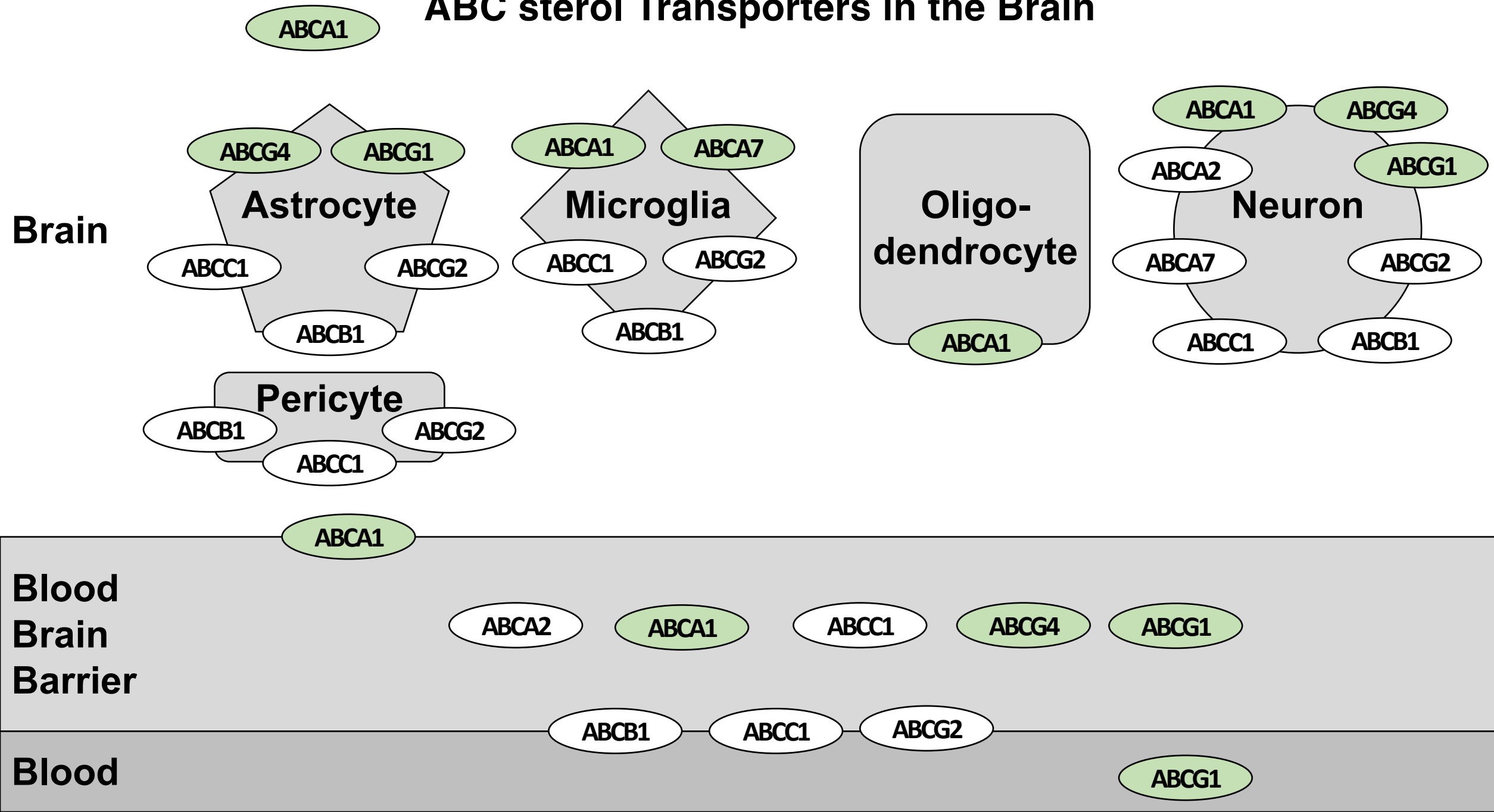
Campesterol

ABSORPTION: ~50% | < 5%

Liver/Intestine-Specific Sterol Transporter



ABC sterol Transporters in the Brain



- Membrane protein-mediated sterol transport
- ABC sterol transporters in health and disease
- **Structural biology approach to study ABC sterol transporters**
- Mechanistic models of sterol transport
  - Lessons from ABCG and ABCA

Structural Biology

- Understanding biology by examining three dimensional (3-D) molecular architectures and their changes.

- Learning life in action with the eyes of atoms: chemical and physical properties of biological matters.

- Structures of biological molecules determine their functions.

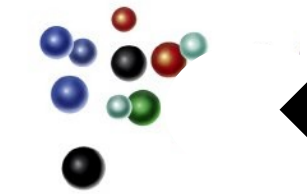
Central dogma:

Sequence ➜ Structure ➜ Function

A major key concept in human physiology (or any organism) is how all the biological matters work in the bodies. From systems (such as gastrointestinal), organs, tissues, to cells, all comes down to operations of biological macromolecules, such as DNA or proteins.

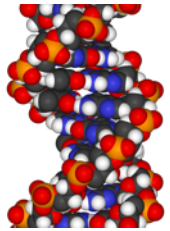
As we discuss the molecular interactions, we are looking at reactions that happen among thousands of atoms that make up individual macromolecules.

This is the spirit of this course. We are looking at how proteins work in our bodies and how they contribute to the physiological functions at "**atomic**" resolution.

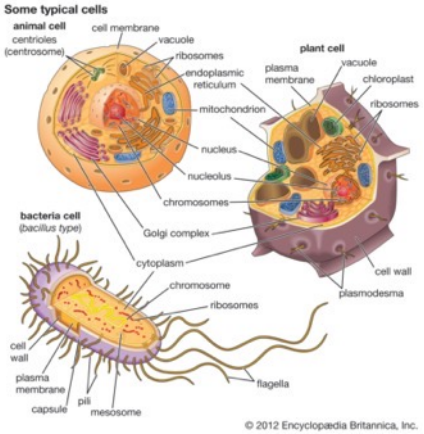


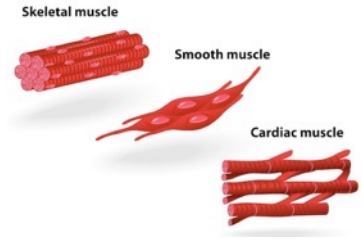
Atoms (C, H, O, N, P)

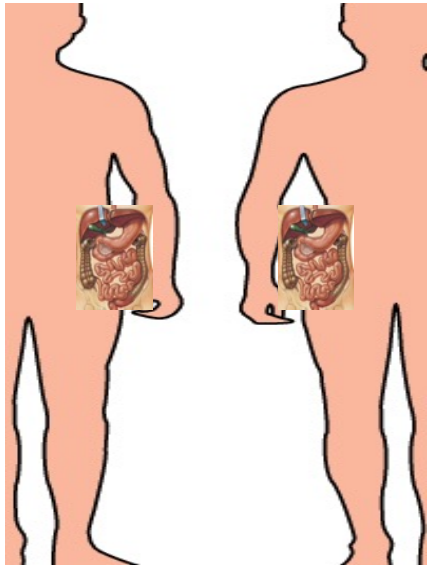
Nucleotides (DNA)

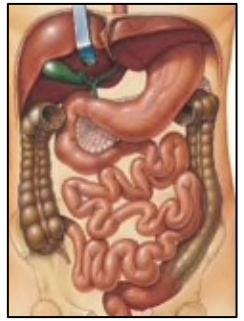


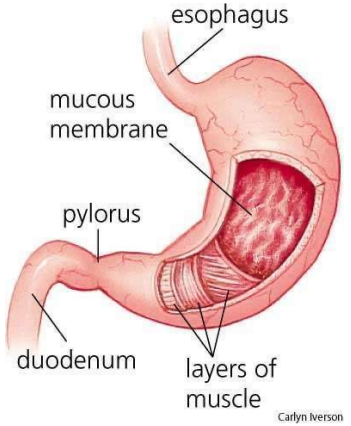
Proteins (tubulin/microtubule)











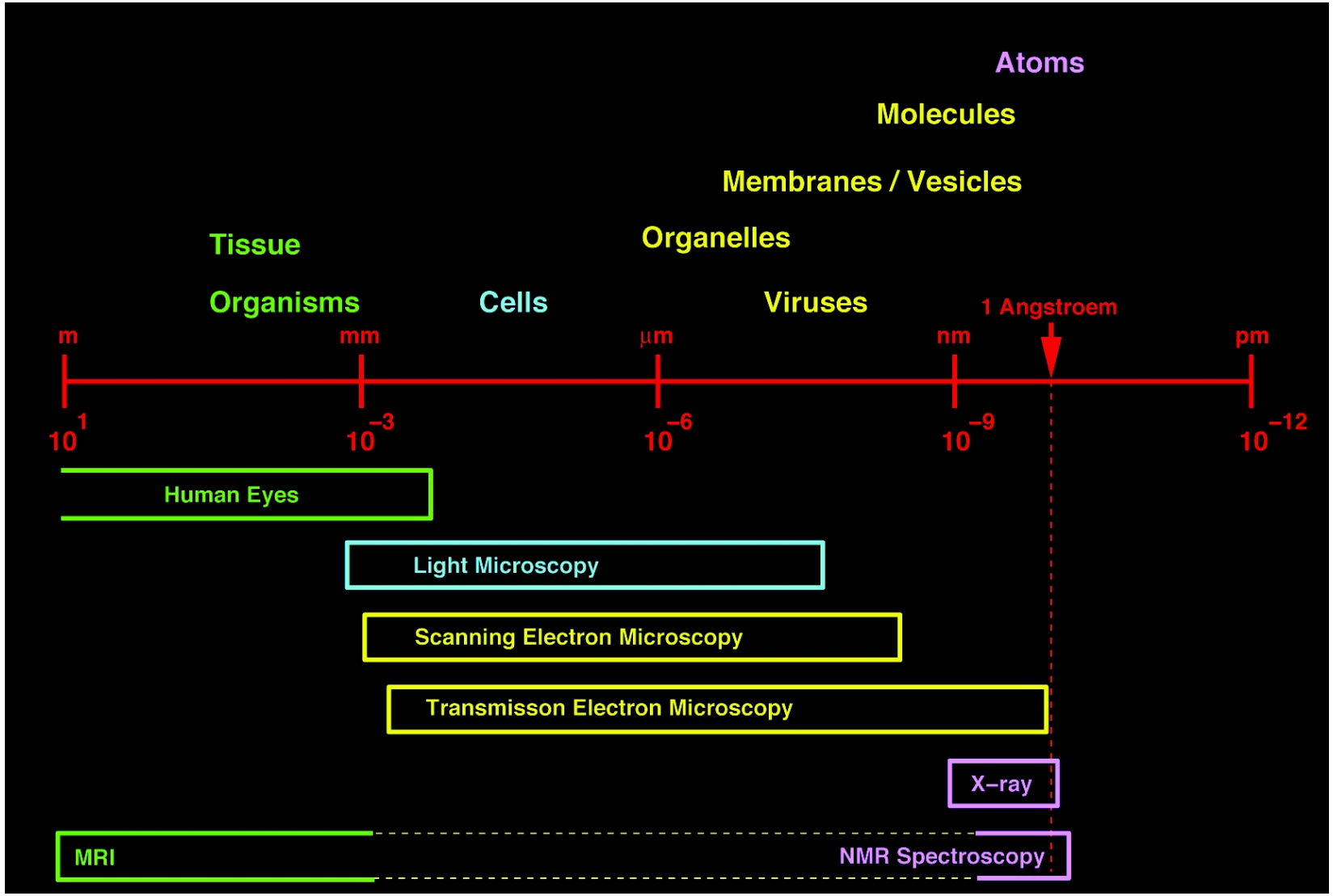
How ”Tiny” Can We See?

From human’s eyes to analytical instruments, we are all limited to how small objects we can see.

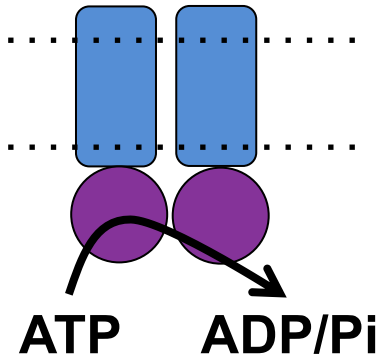
For cells, we can easily observe under a light microscope and with more detailed information using electron microscopes.

To see objects at atomic resolution, so far, we know X-ray crystallography, transmission electron microscopy, and NMR spectroscopy can enable such high-resolution imaging.

This course will selectively focus on these three methodologies that enable vast protein structure-function studies so far.

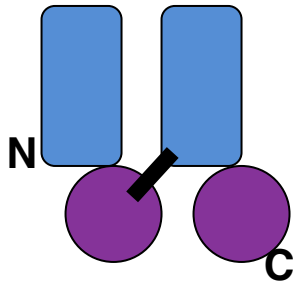


ATP-Binding Cassette (ABC) Proteins

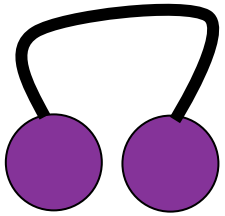


Transmembrane domain (TMD)

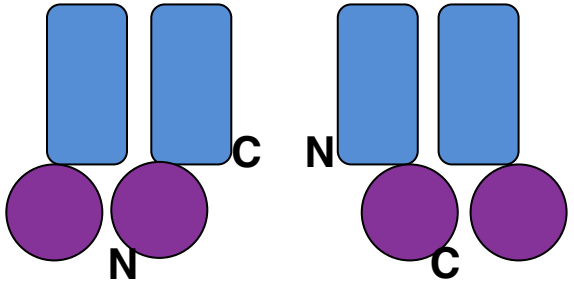
Nucleotide-binding domain (NBD)



Full transporters

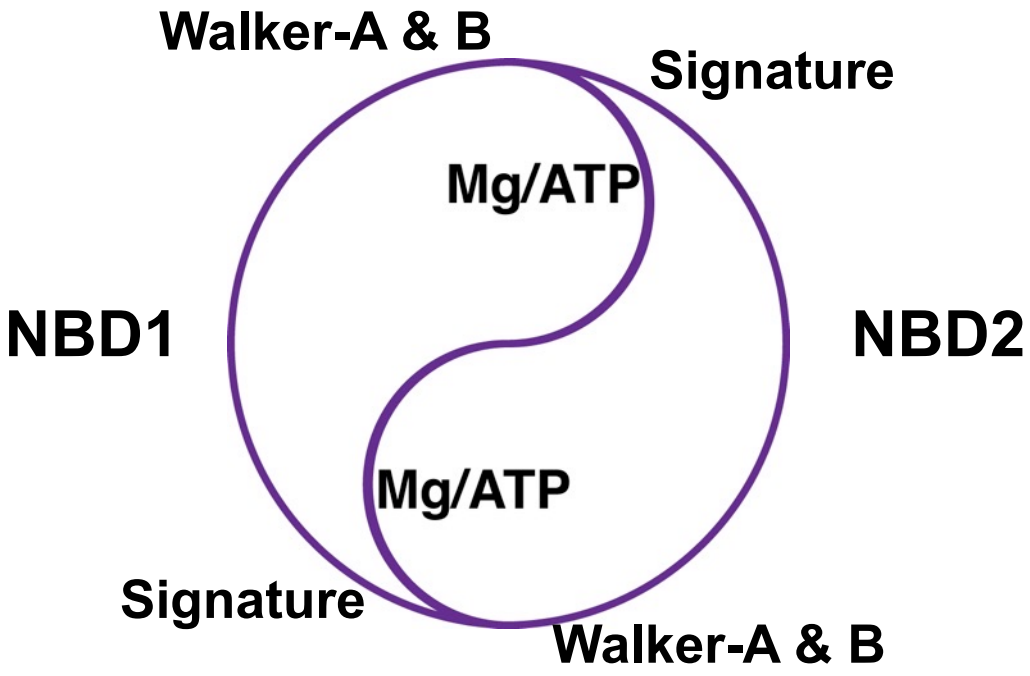


Non-transporters



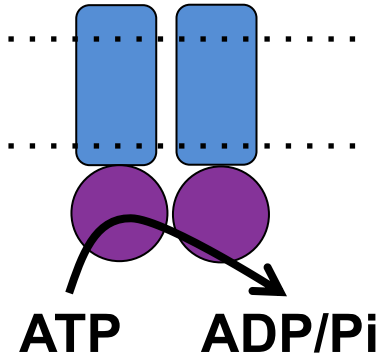
Half transporters
Homo-dimer
Hetero-dimer

ABC coupled transport: a simple idea

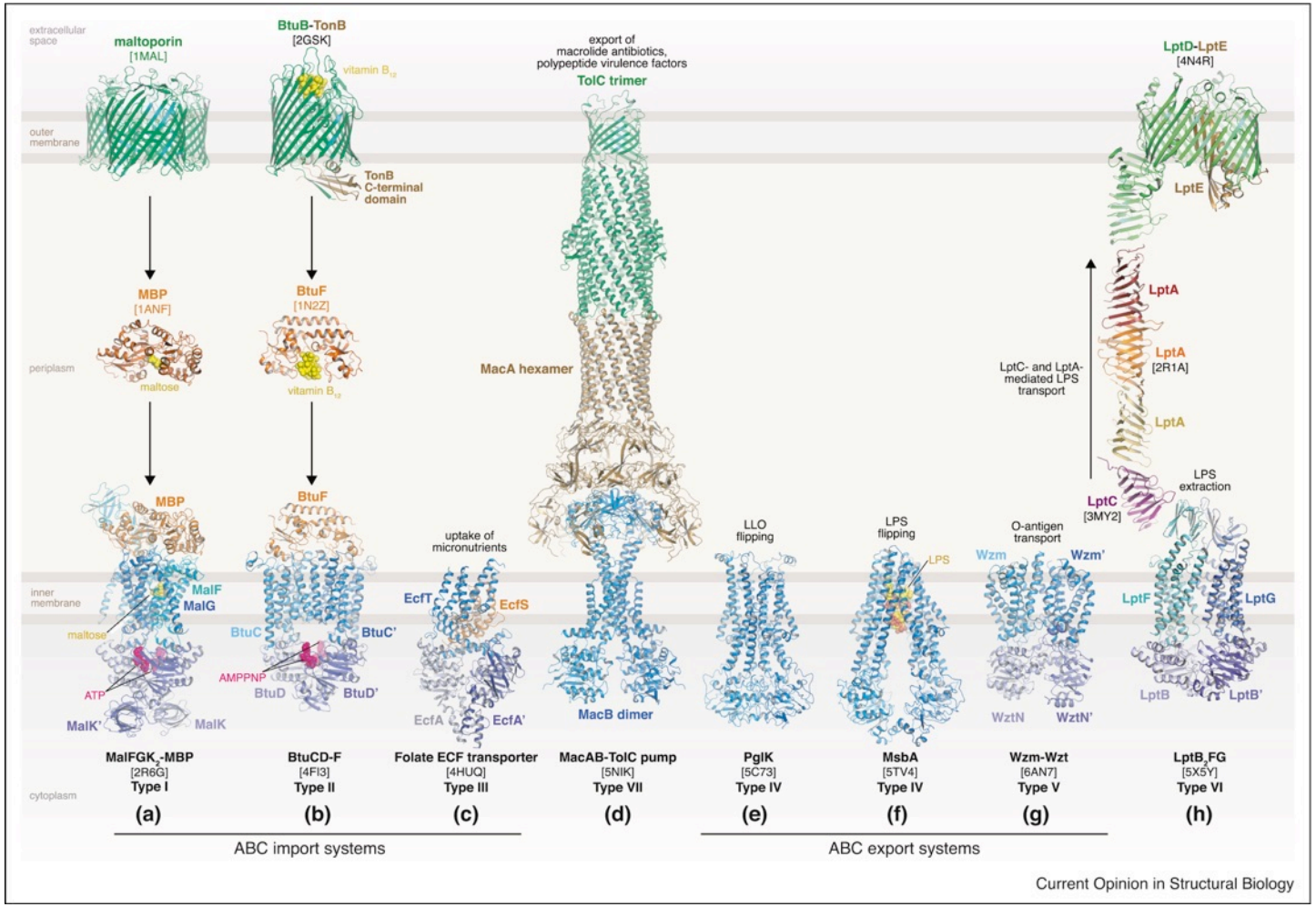


ATP-binding cassette (ABC)

ABC and ATP usage are part of story!

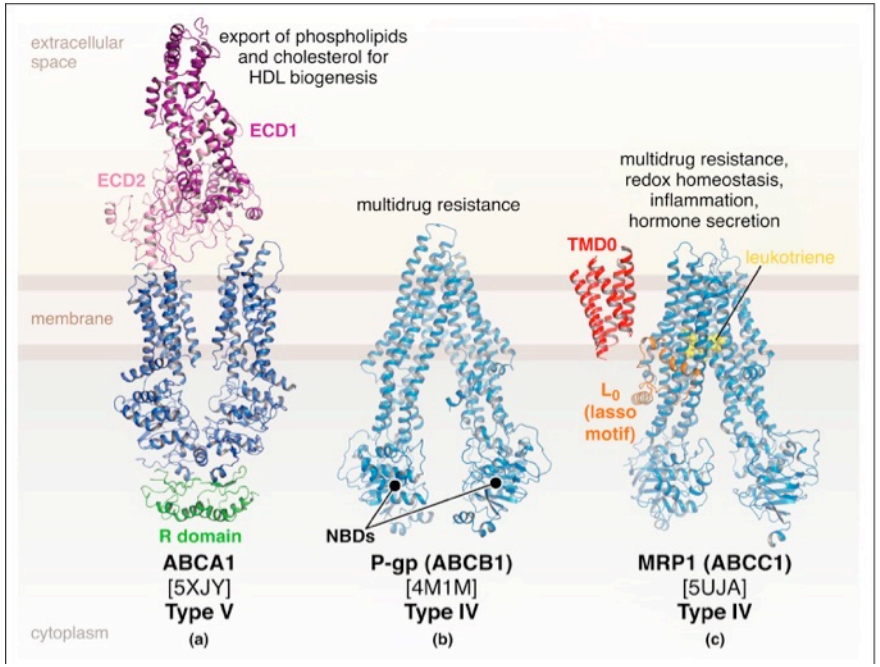


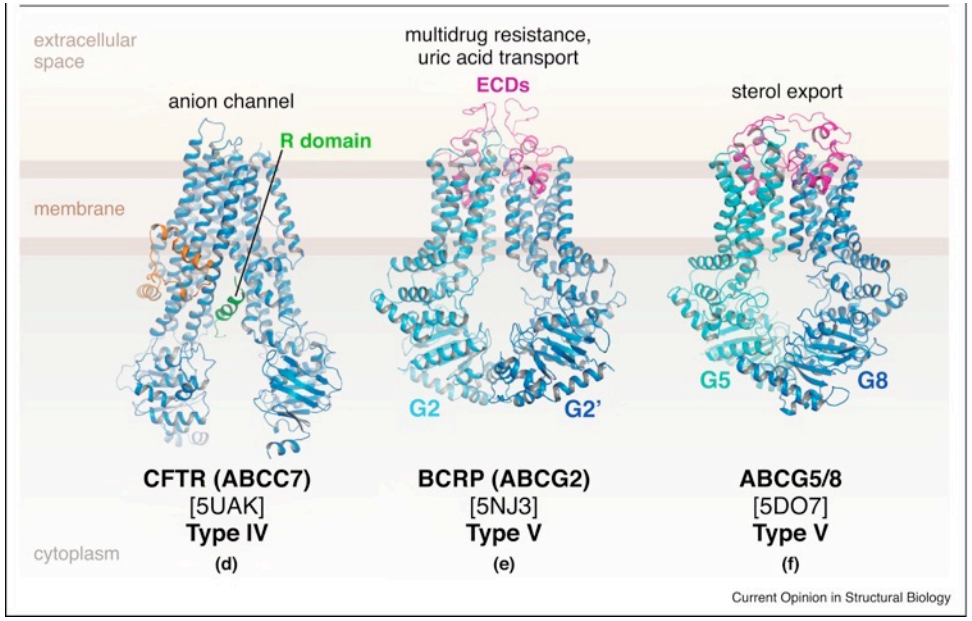
Structural diversity: bacterial point of view



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

Structural diversity: mammalian point of view





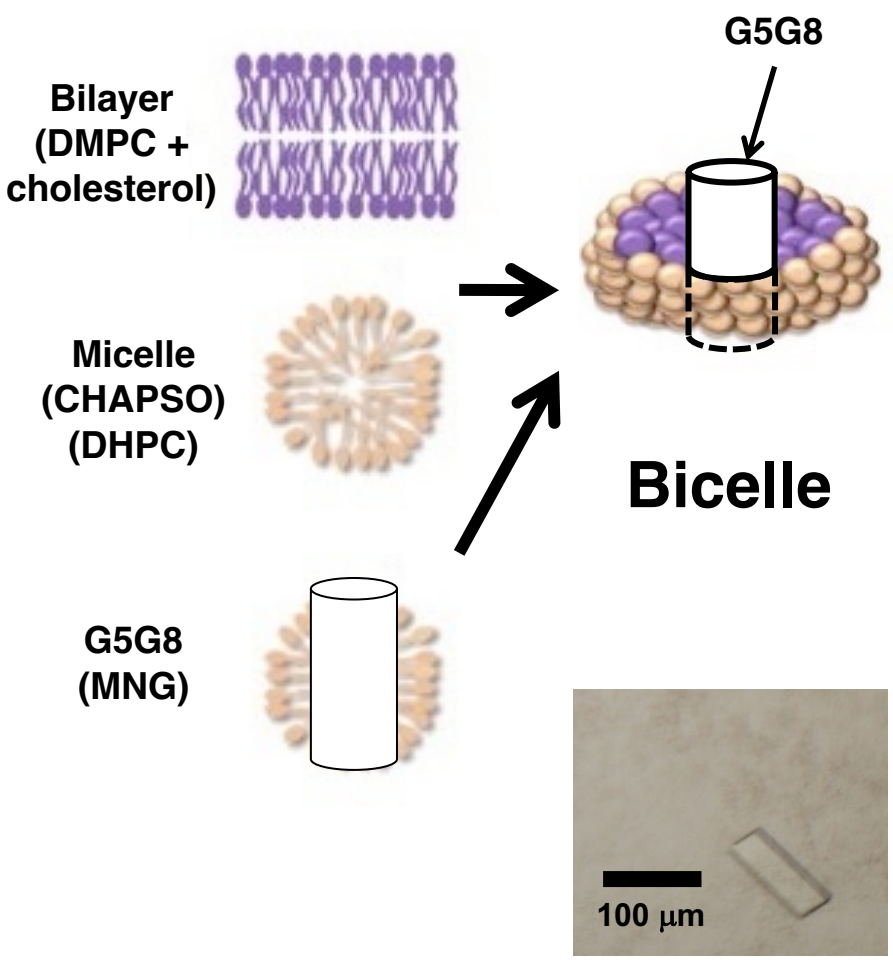
(Thomas & Tampé, Curr Opin Struct Biol, 2018)

So, …

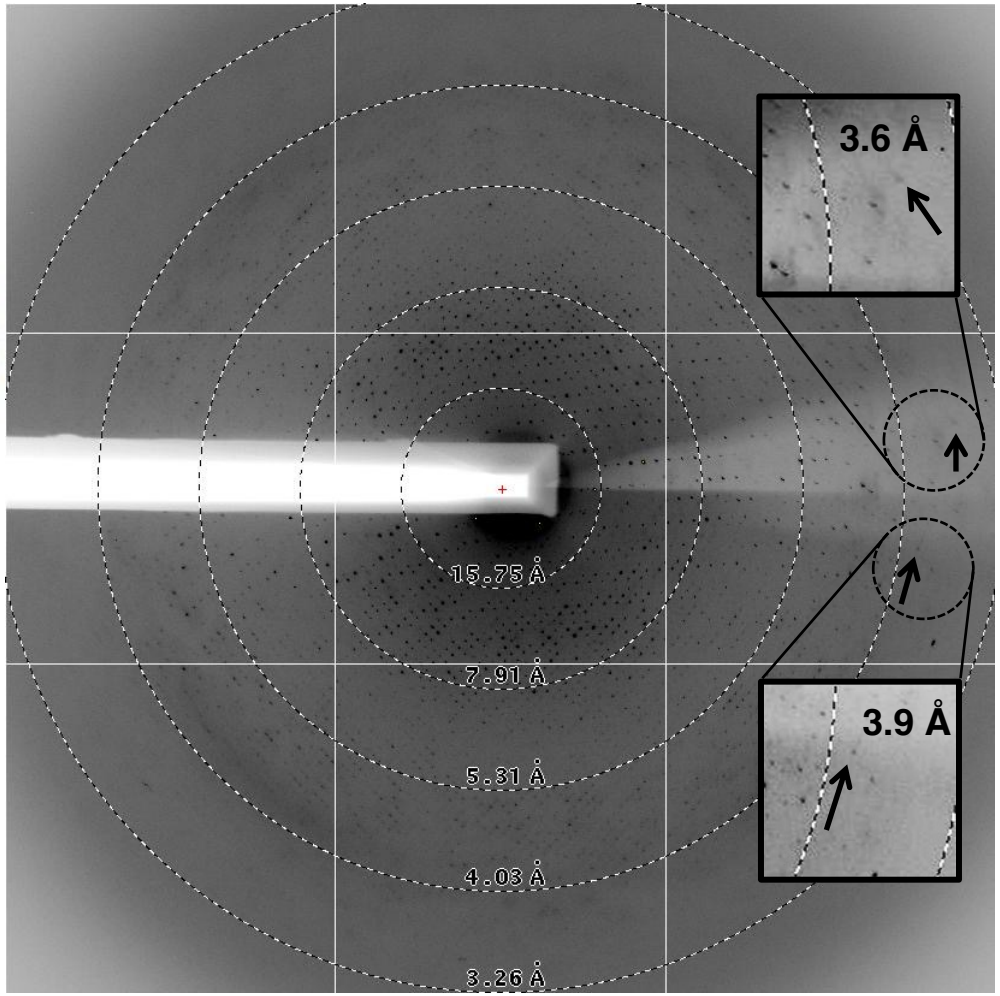
- **High-degree of structural diversity in the transmembrane domains of ABC transporters.**
- **The structural variability (likely) determines the functional diversity of ABC transporters.**
- **Transport mechanism is (likely) individually distinct.**

ABCG5/G8: X-ray Crystallography

G5G8 bicelle preparation



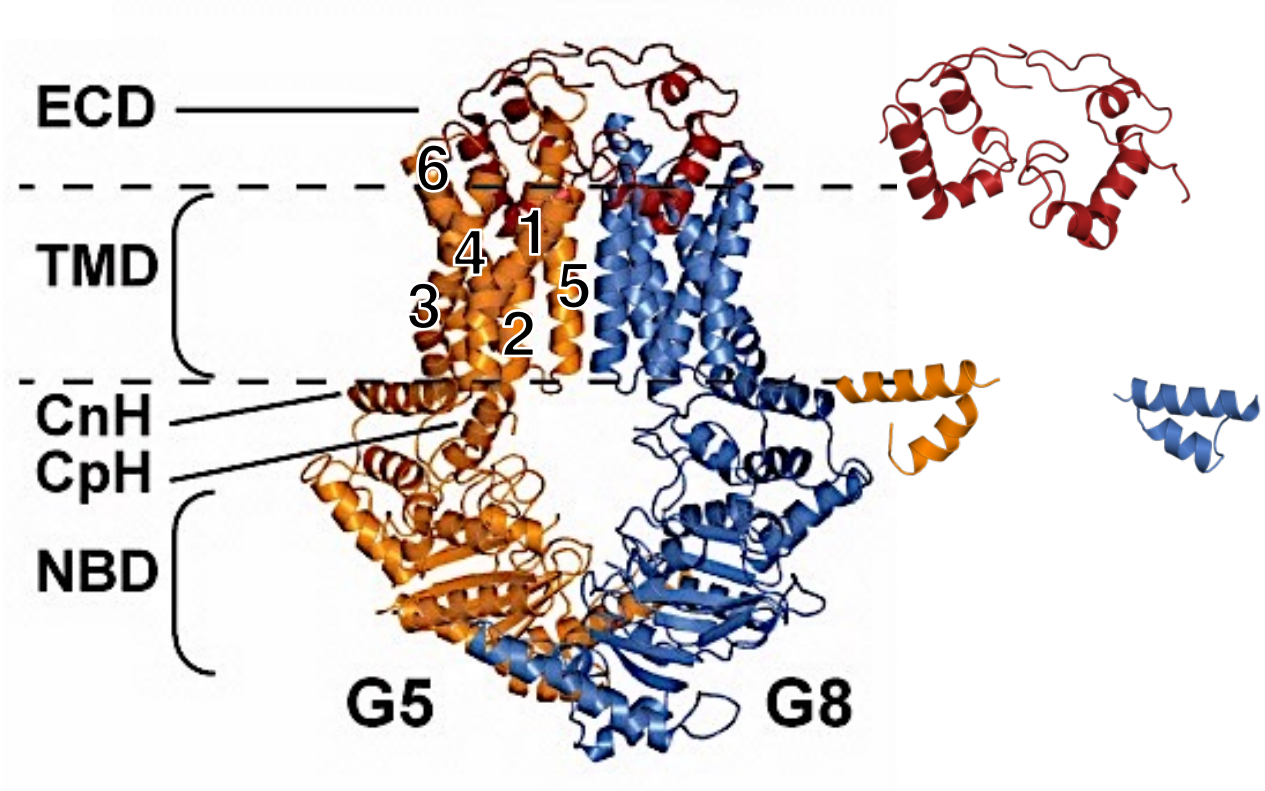
Crystal growth & X-ray diffraction



- 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)

ABCG5/G8: X-ray Crystallography

Domain features



TMD: transmembrane domain

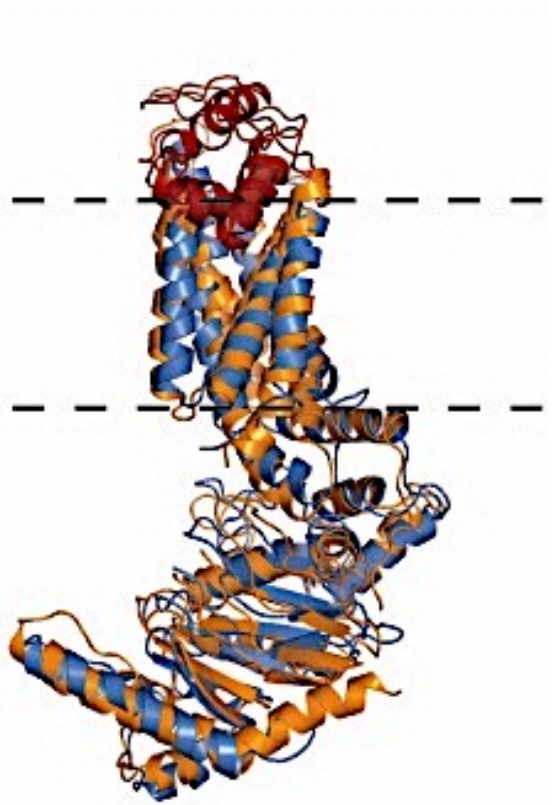
NBD: nucleotide-binding domain

ECD: extracellular domain

CnH: connecting helix

CpH: coupling helix

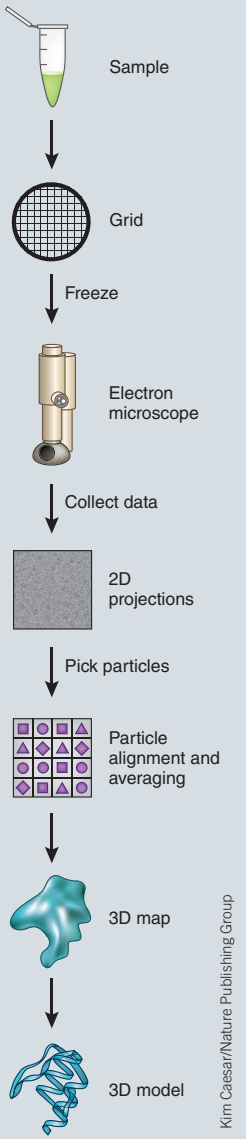
Structural similarity:



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

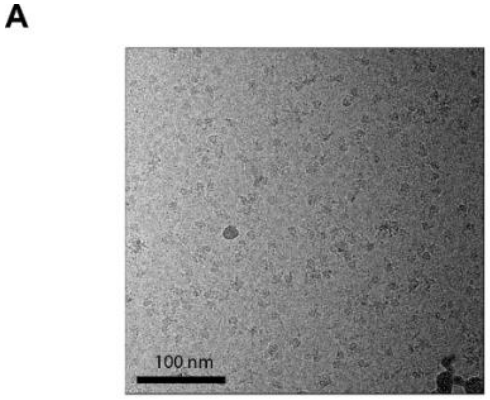
(Lee et al, Nature, 2016)

ABCG5/G8, ABCG1, ABCA1: Single-particle Cryo-EM



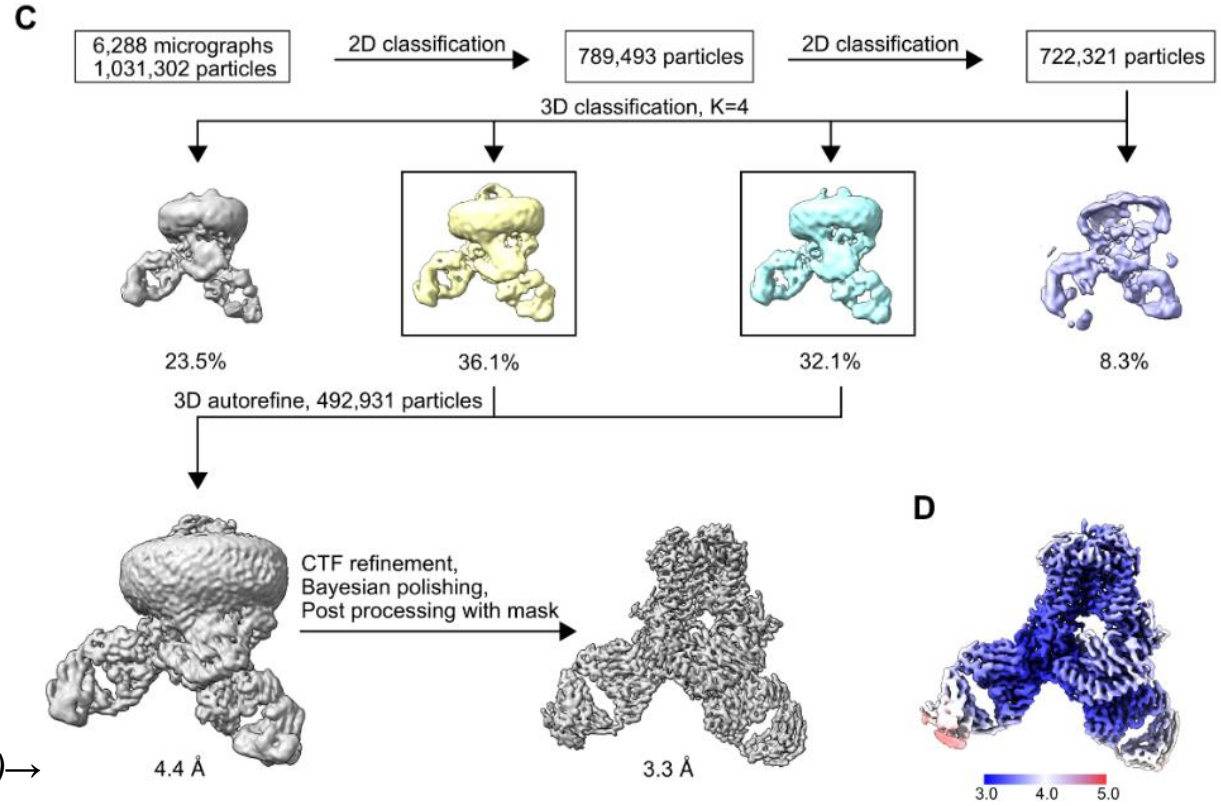
The overall single-particle cryo-EM workflow, from protein sample to 3D model.

Electron micrograph



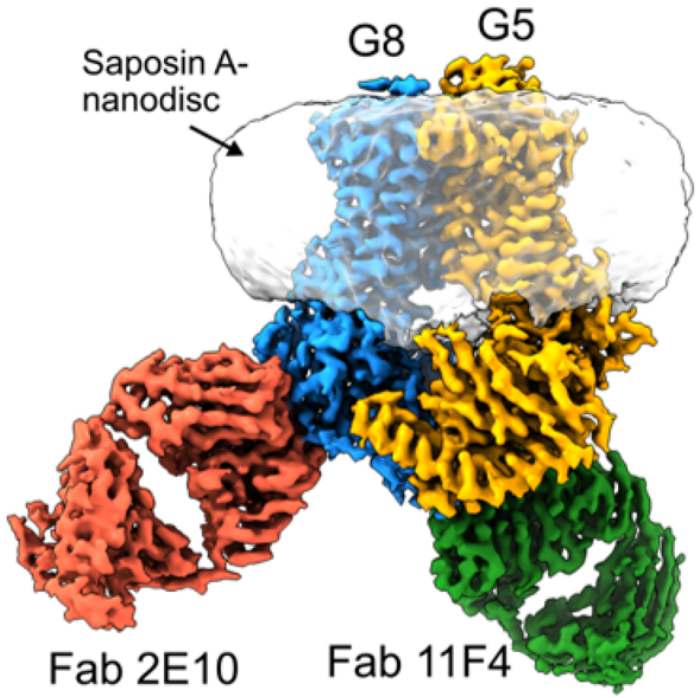
2-D classification

3-D reconstruction & refinement

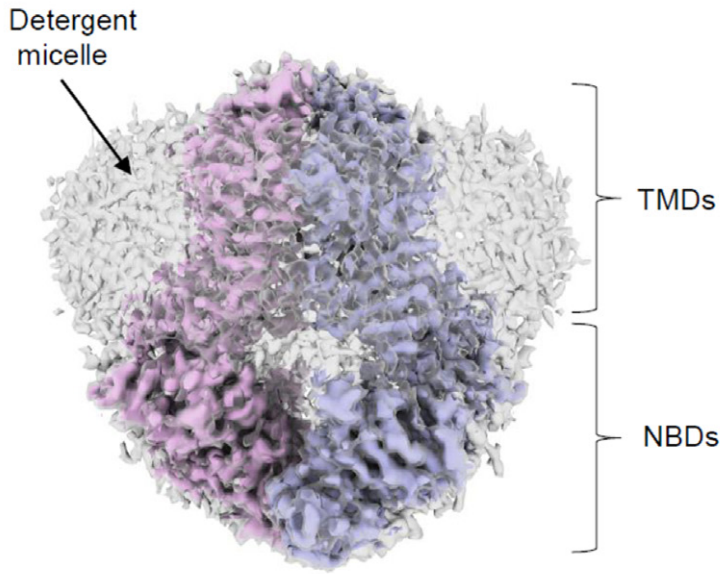


←*(Dörr, Nat Meth, 2016)*
(Zhang et al, Comm Biol, 2021)→

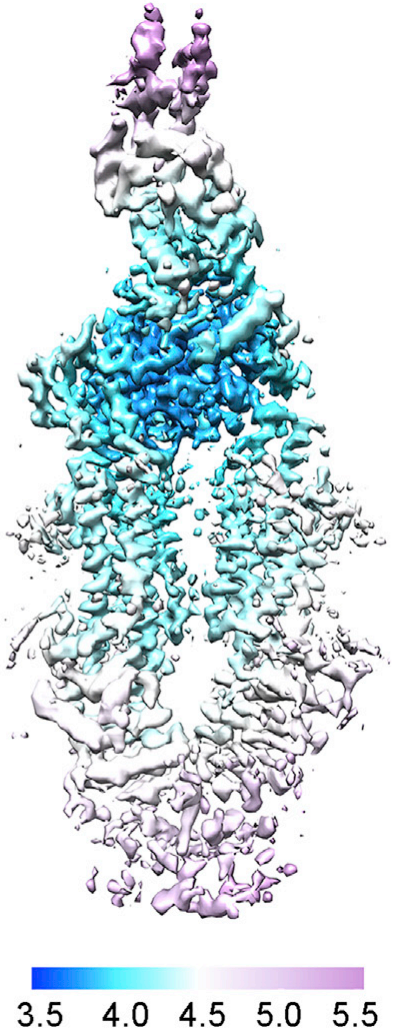
ABCG5/G8, ABCG1, ABCA1: Single-particle Cryo-EM



(Zhang et al, Comm Biol, 2021)

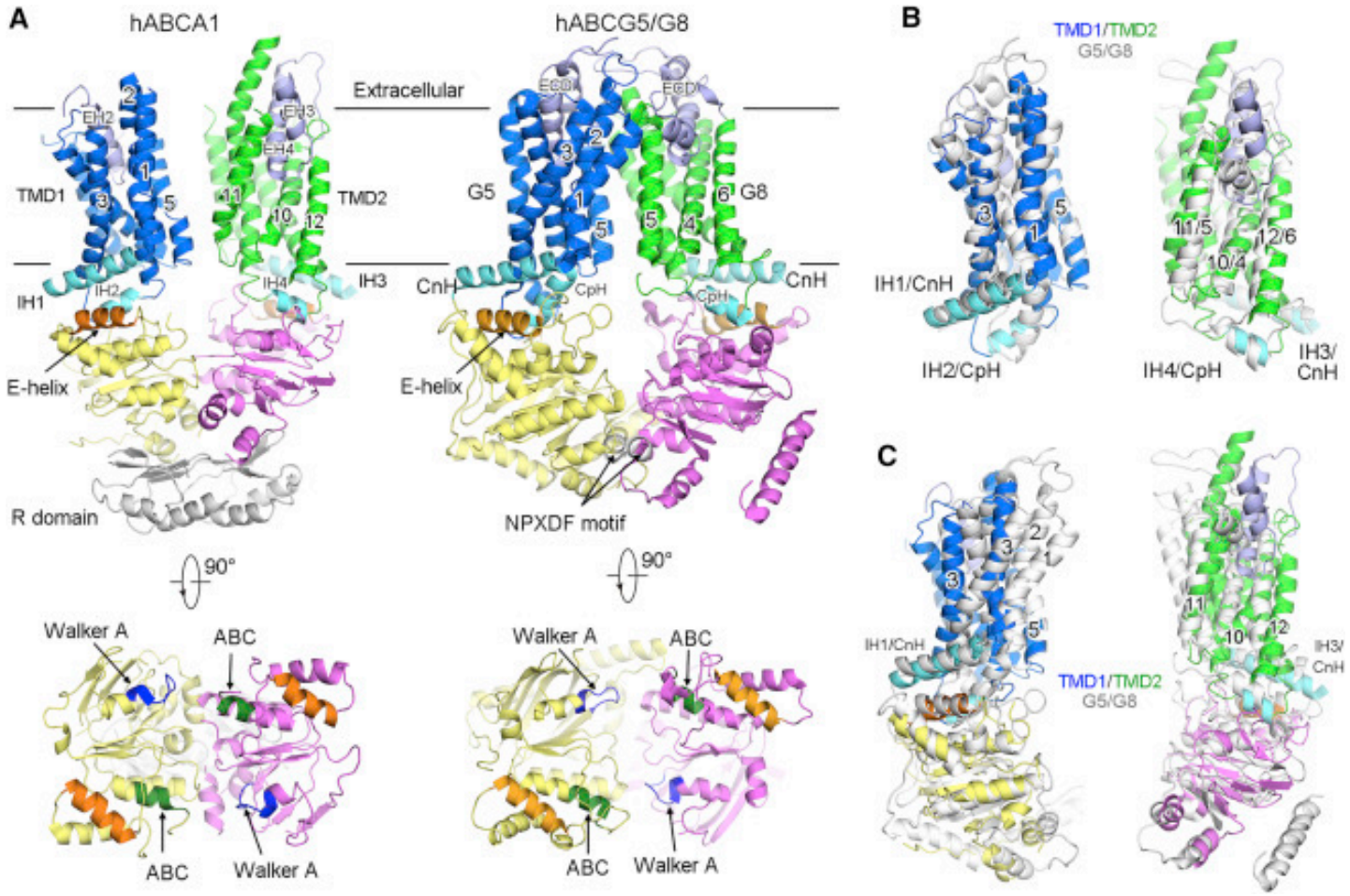


(Skarda et al, JMB, 2021)



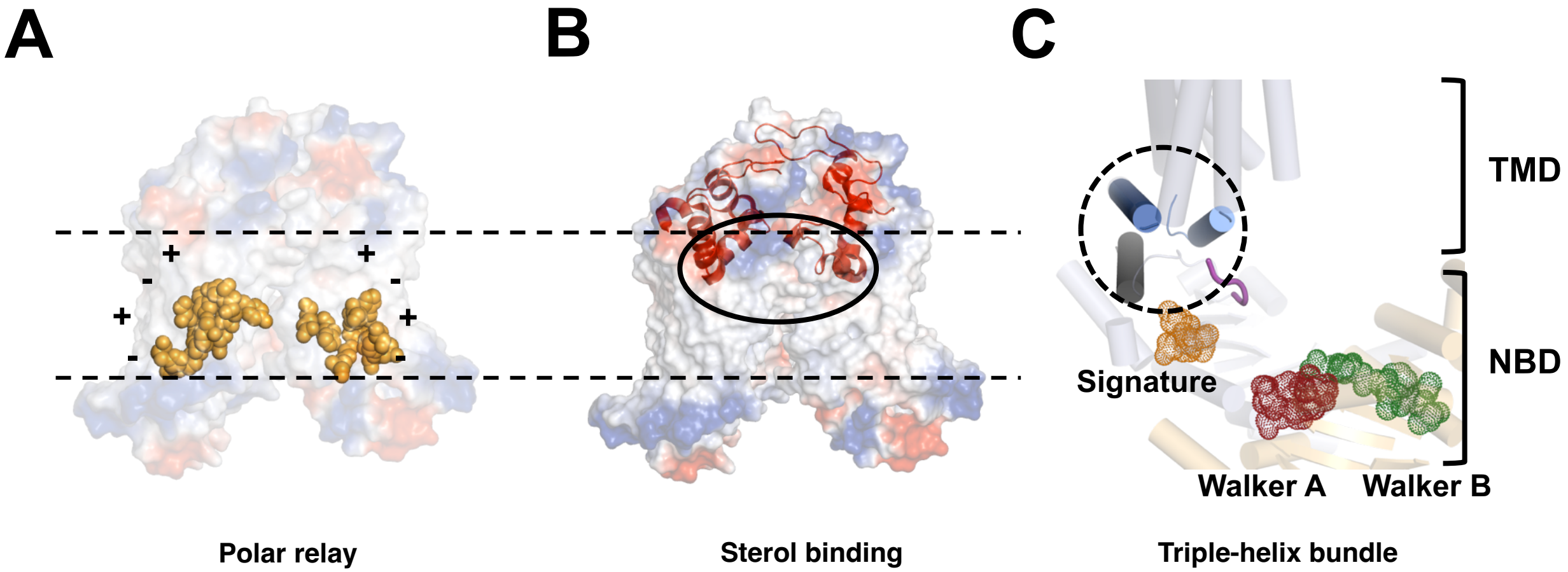
(Qian et al, Cell, 2017)

Shared structural fold in ABCA and ABCG



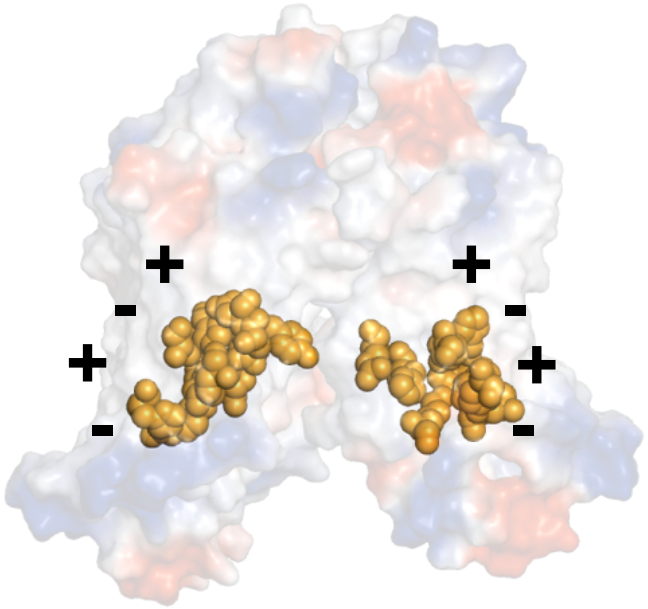
(Qian et al, Cell, 2017)

Novel structural motifs in ABCA and ABCG

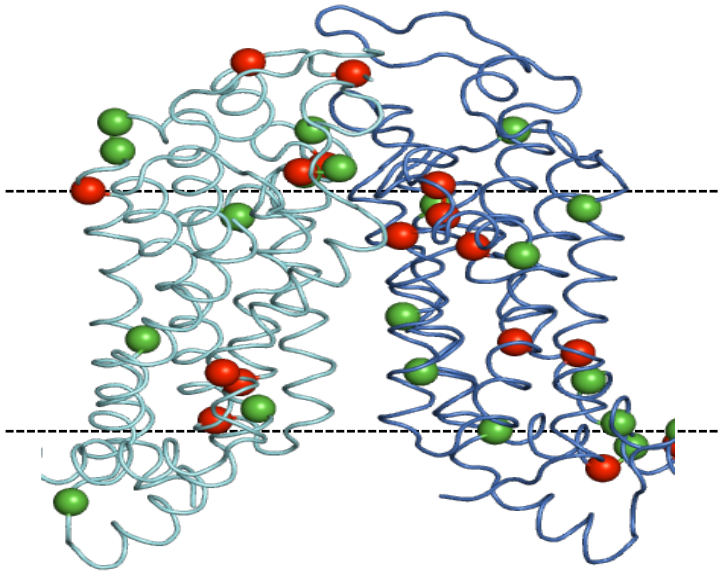


(Lee et al, Nature, 2016; Xavier et al, BCB, 2019)

Transmembrane Domain of ABC Cholesterol Transporters: a Pathogenic Hot Spot

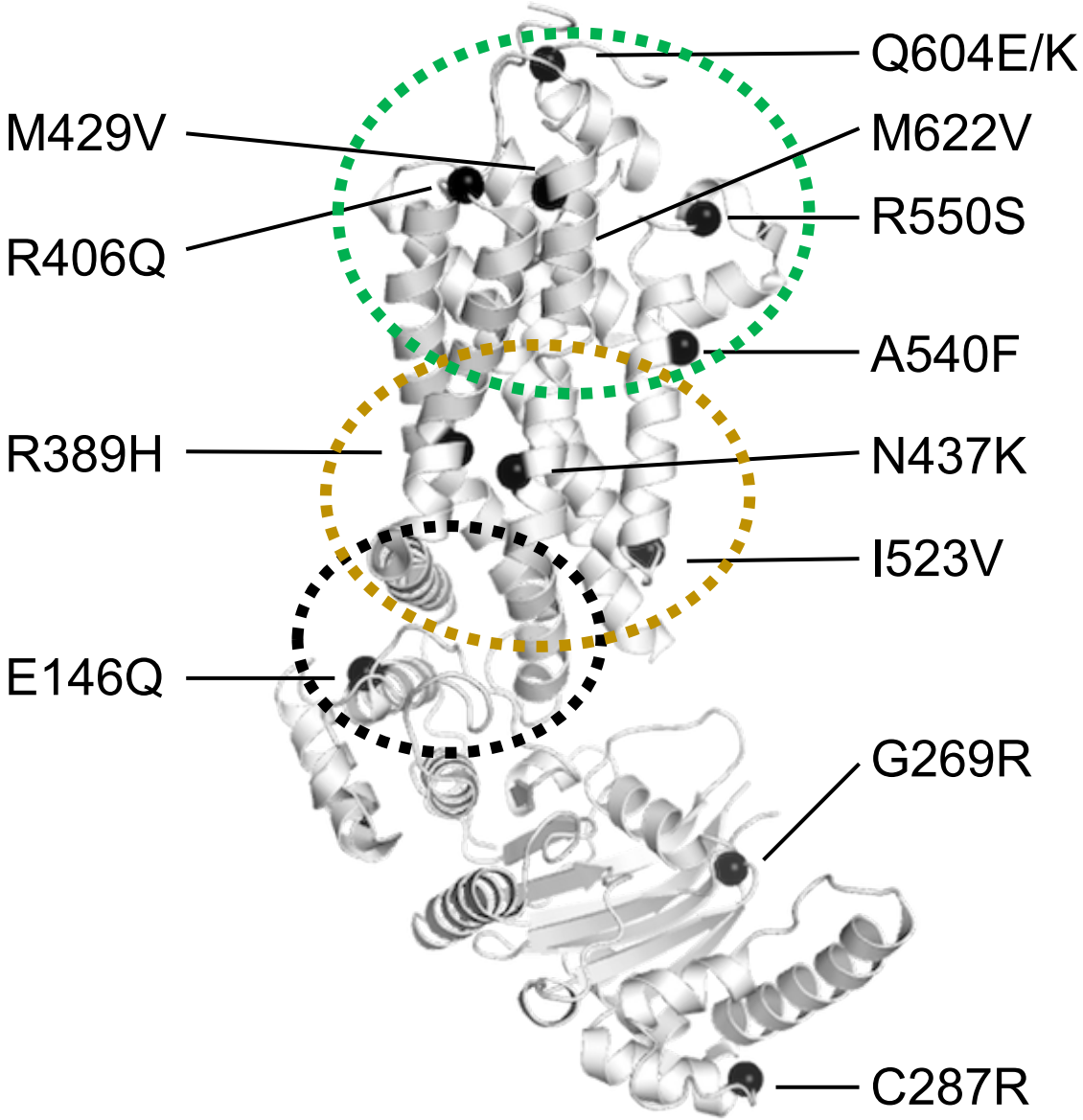


Polar relay

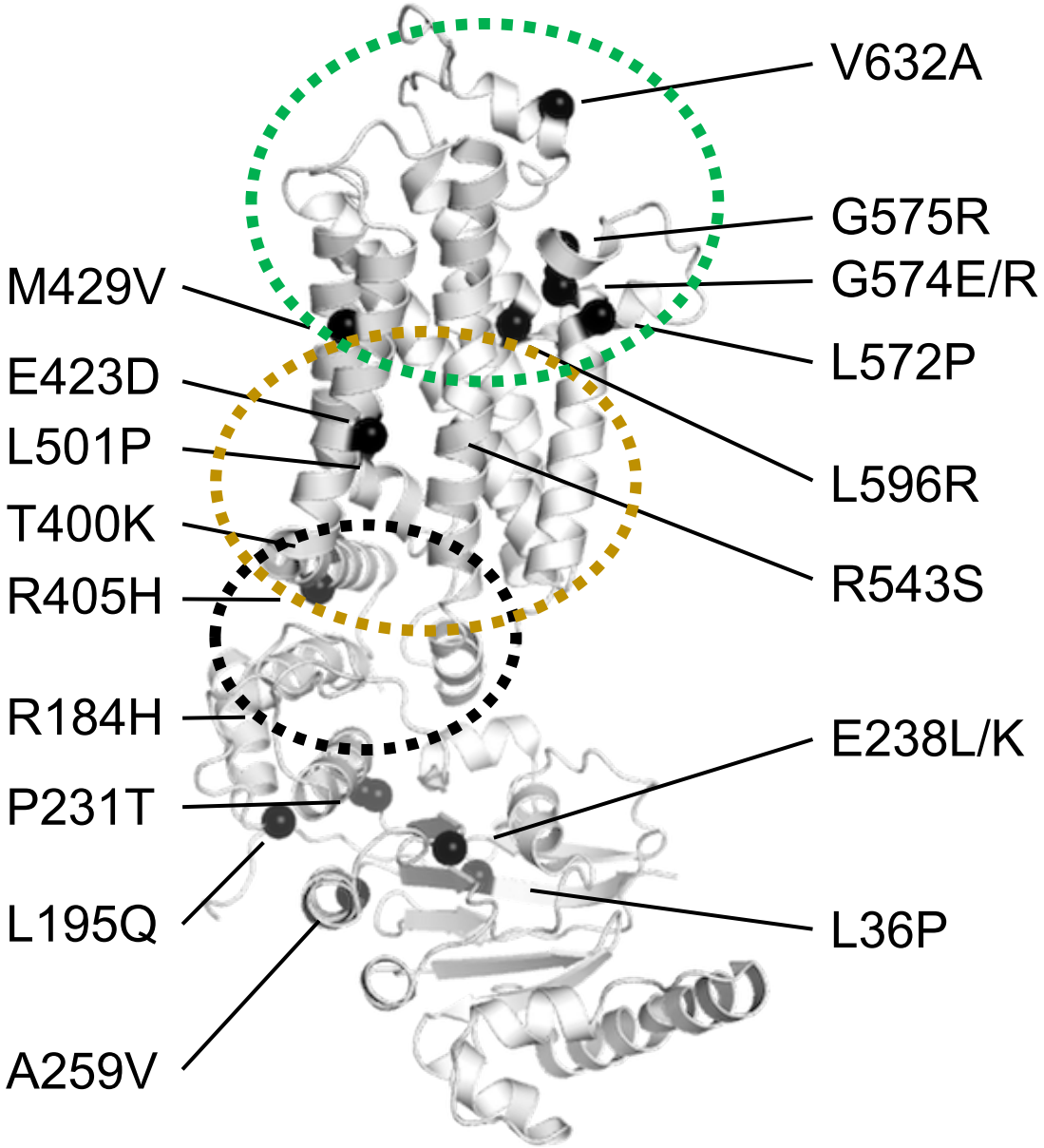


Pathogenic residues:
G5G8 (red), A1 (green)

(Xavier et al, BCB, 2019)



ABCG5

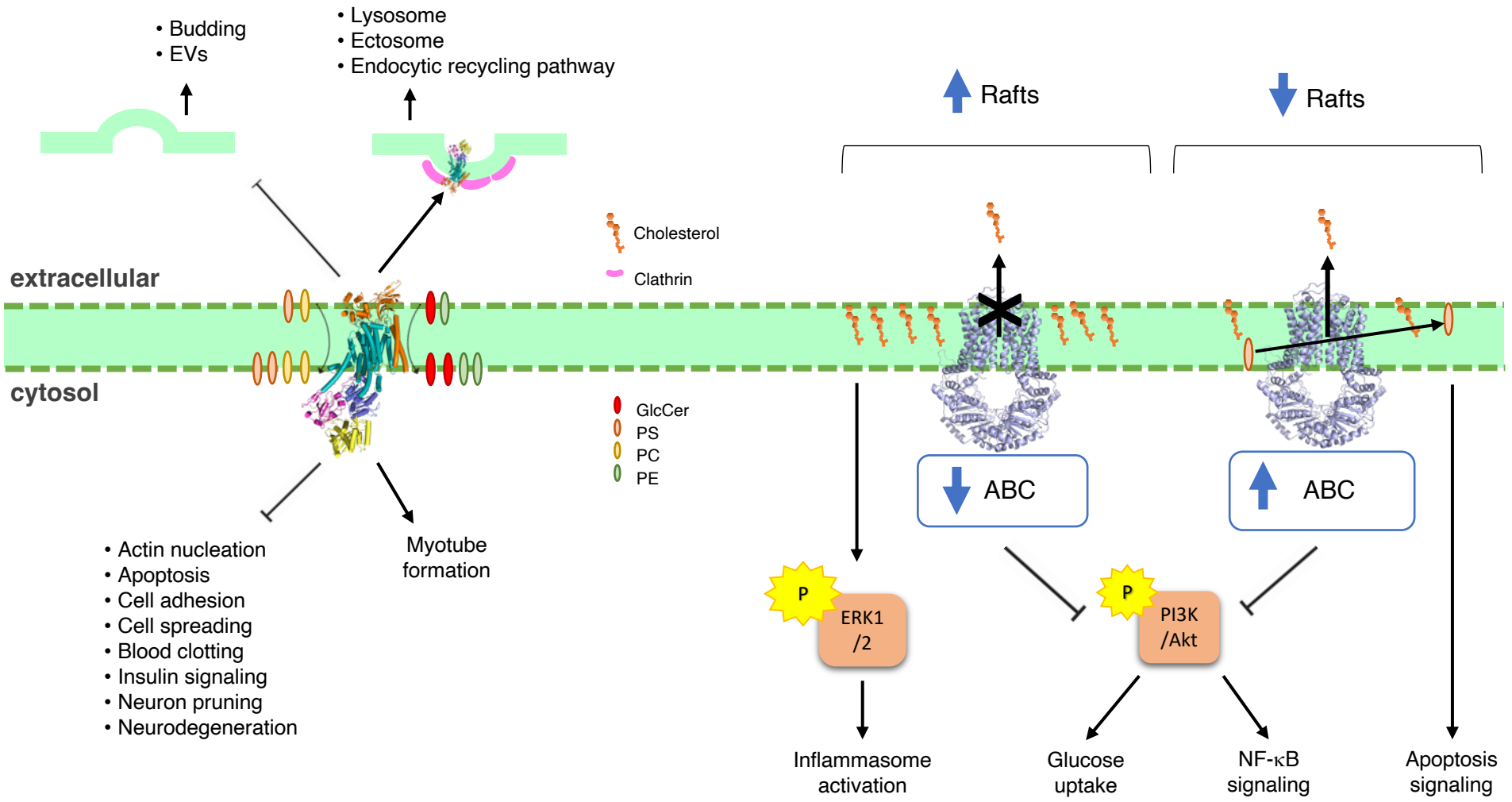


ABCG8

(Xavier et al, IJMS, 2020)

- Membrane protein-mediated sterol transport
- ABC sterol transporters in health and disease
- Structural biology approach to study ABC sterol transporters
- **Mechanistic models of sterol transport**
  - **Lessons from ABCG and ABCA**

Lipid/Sterol Transporters *v.s.* Cell Signaling

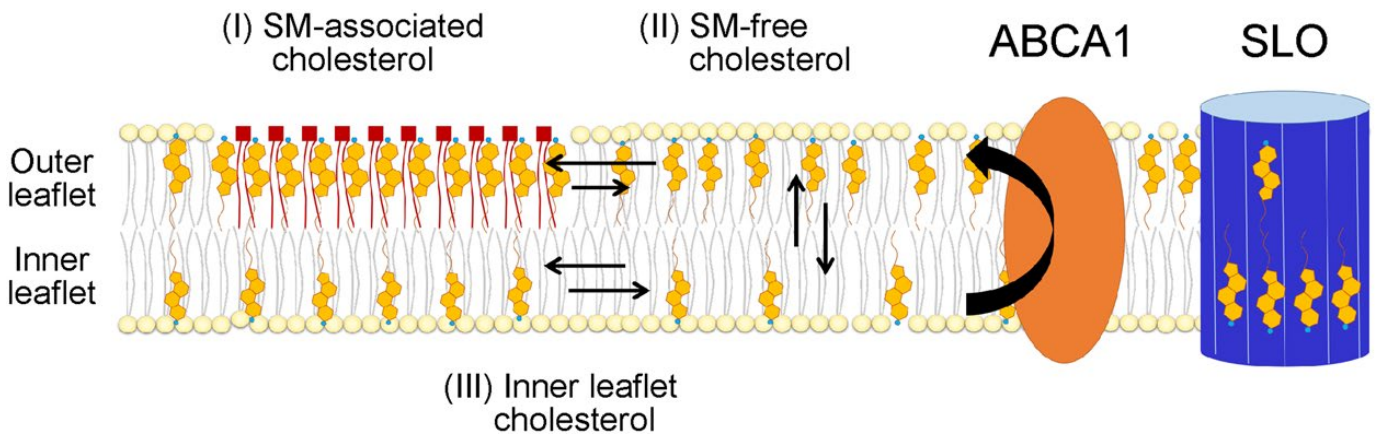


P4-ATPase Phospholipid Transporter

ABC Cholesterol Transporter

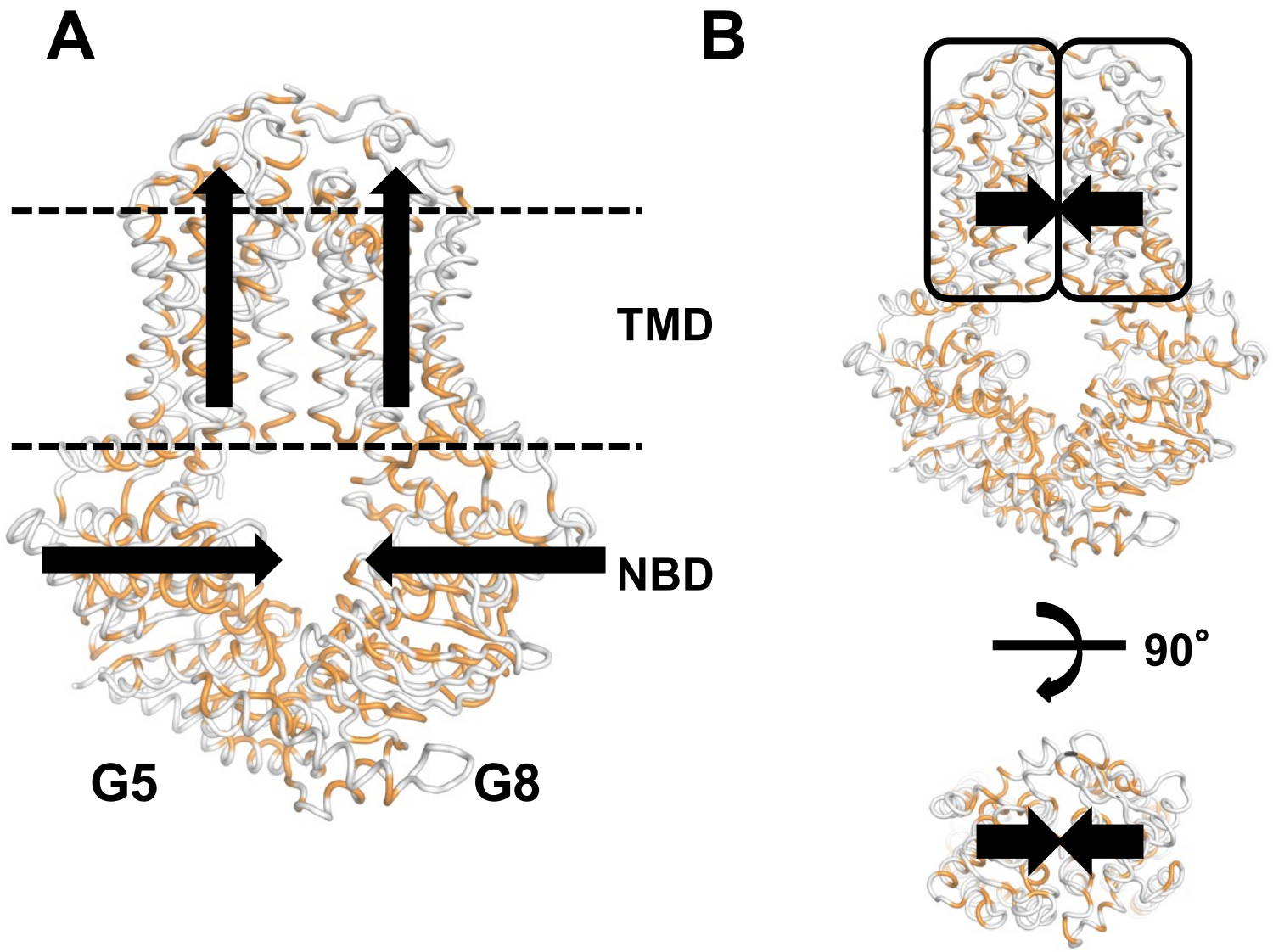
(Ristovski et al, Membranes, 2021)

Asymmetric Cholesterol Distribution by ABC Sterol Transporters



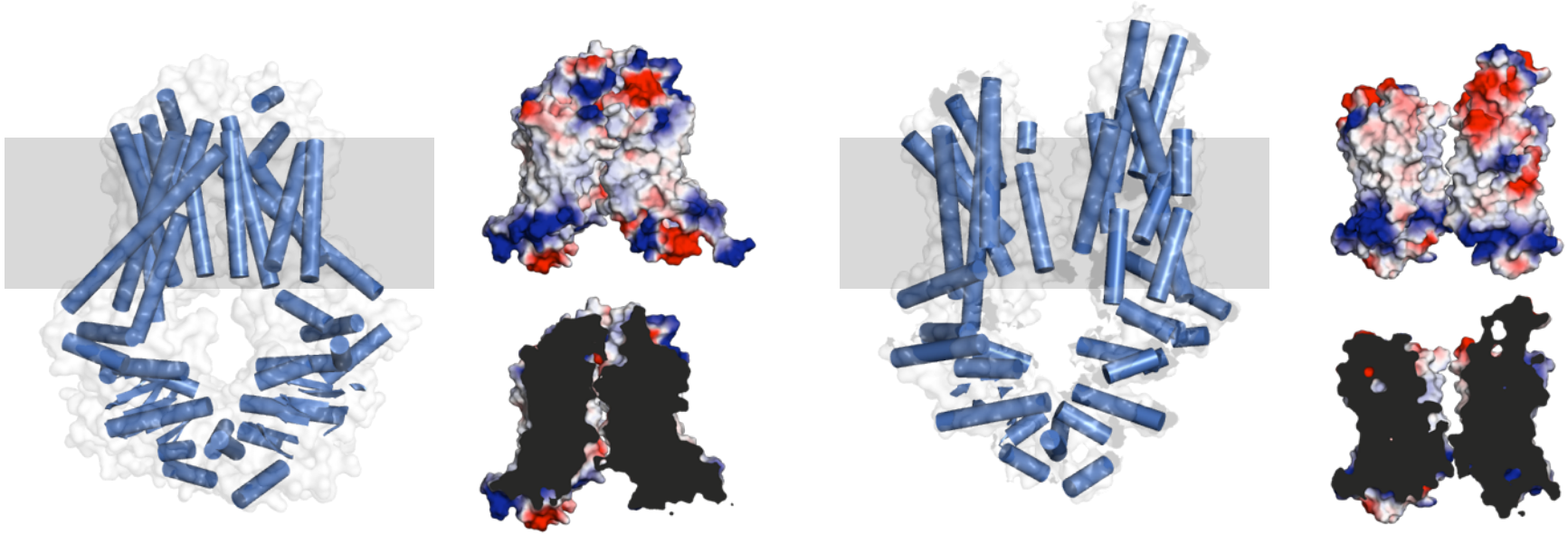
(Ogasawara et al, Sci Rep, 2019)

Simulation of domain movement in ABCG5/G8.

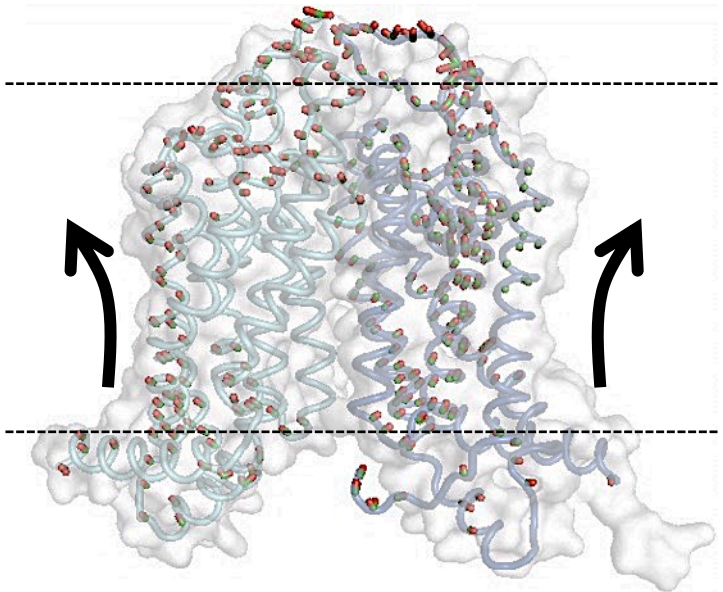


(Zein et al, Biochem Soc Trans, 2019)

Transmembrane conformations
Apo state (no catalytic ligand)



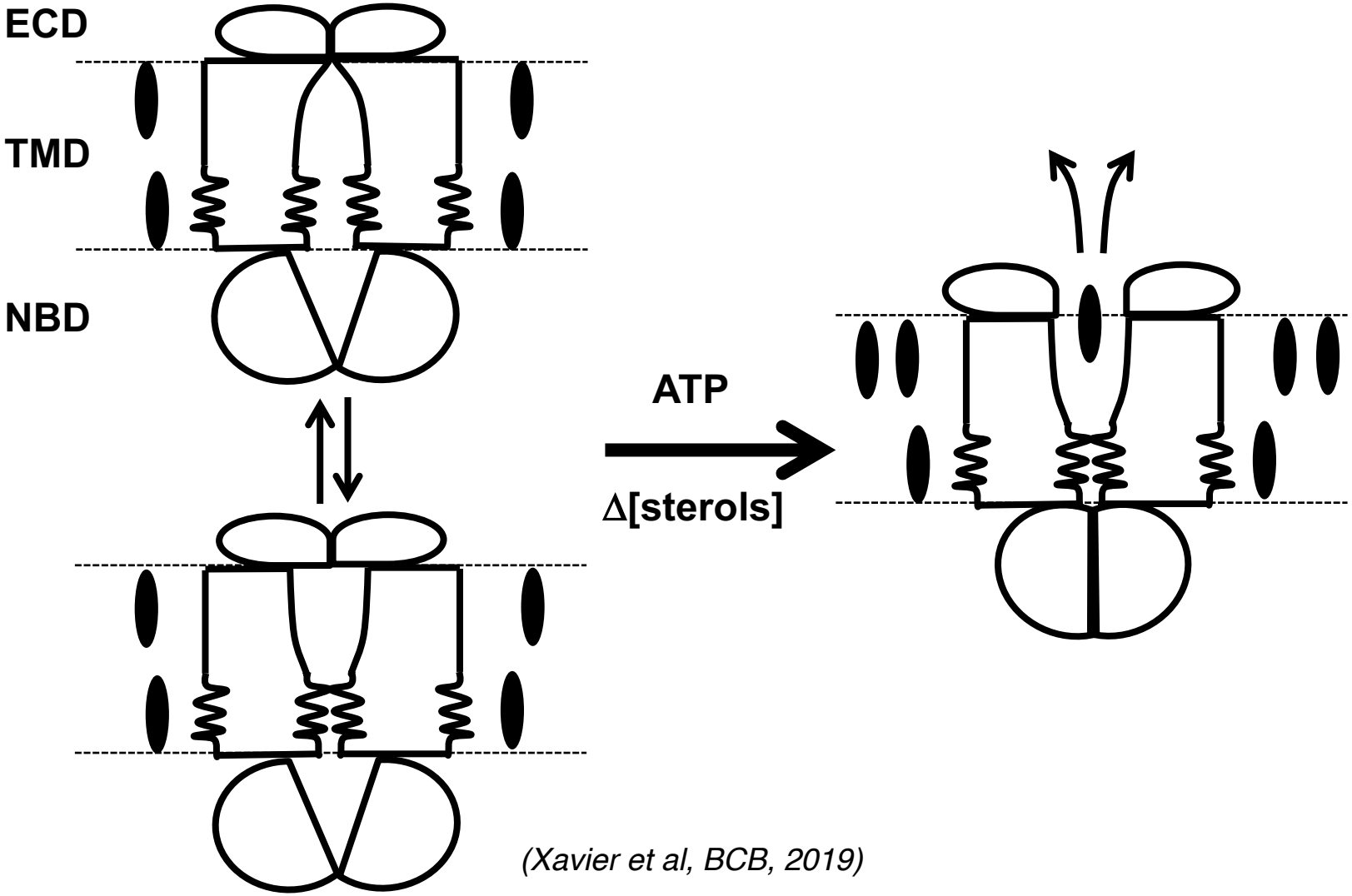
ABCG5/G8
(nf, inward)



ABCA1
(nf, outward)

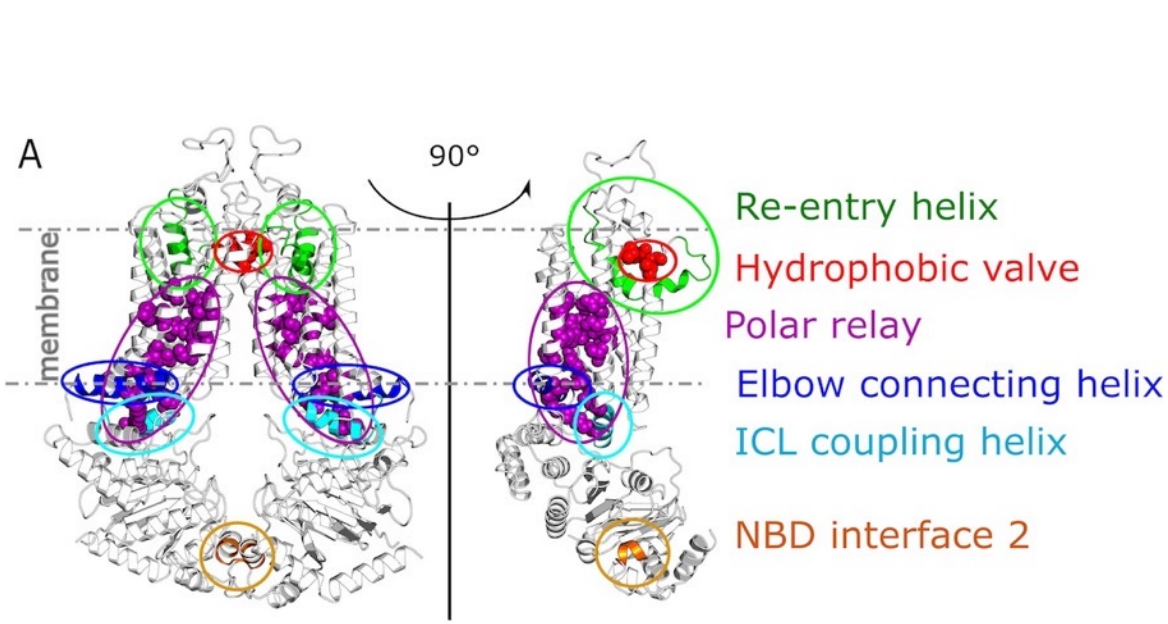
(Xavier et al, BCB, 2019)

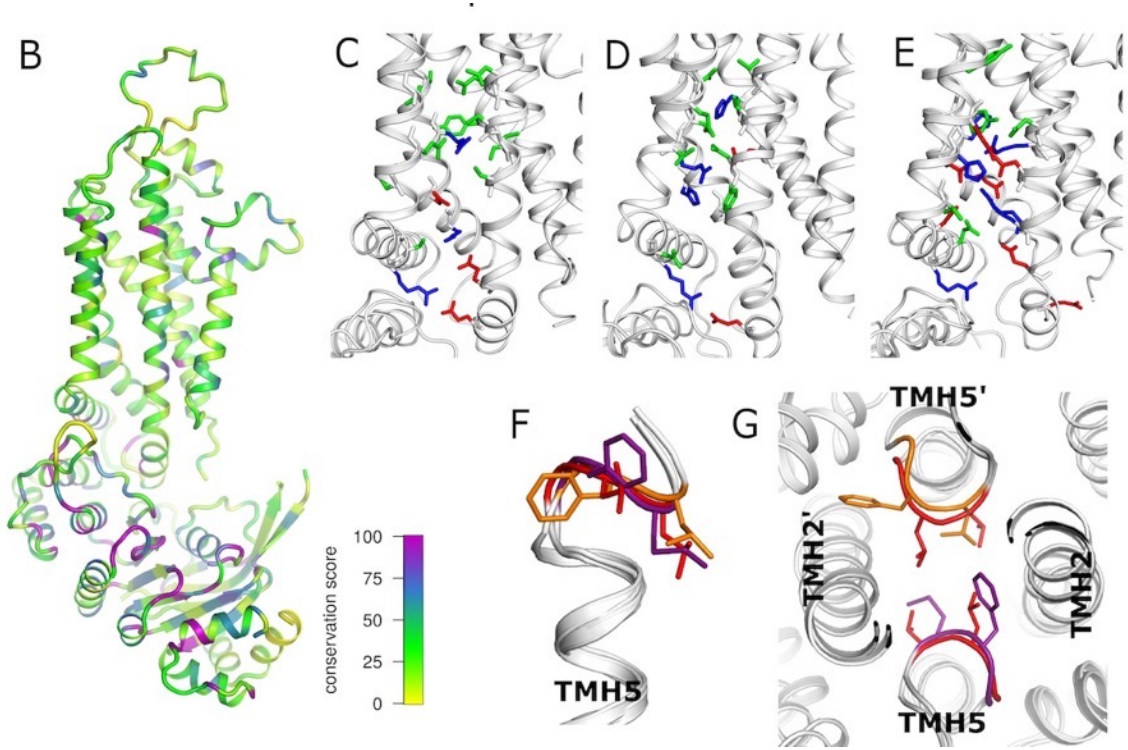
Working Model of ABC Sterol Transporters (Cellular)



(Xavier et al, BCB, 2019)

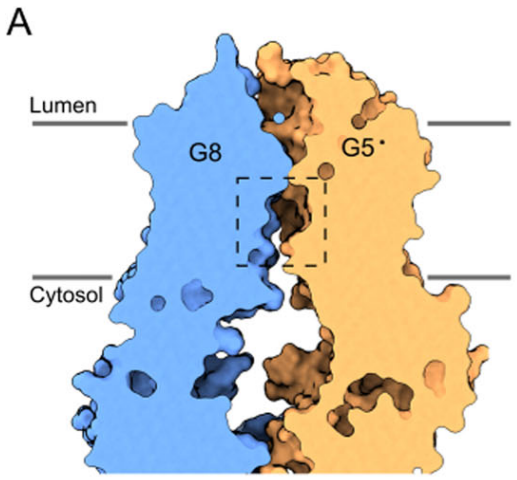
Further Structural Analysis → Hydrophobic valve/gate & …



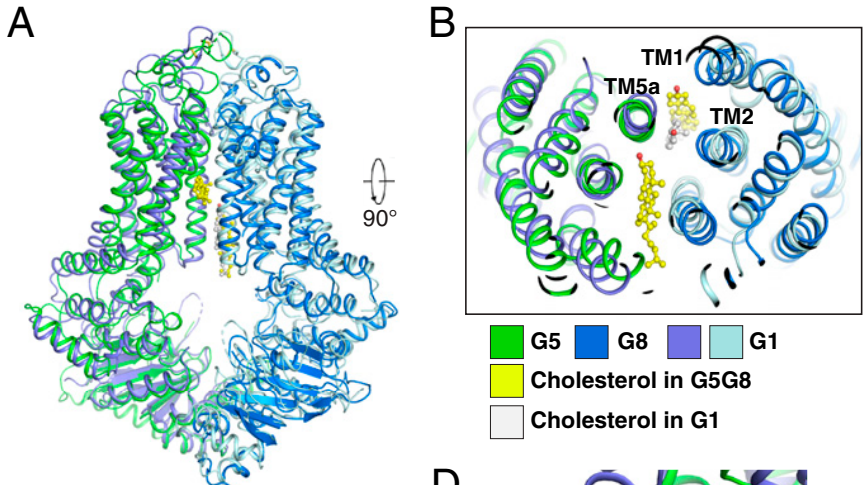


(Khunweeraphong et al, FEBS Lett, 2020)

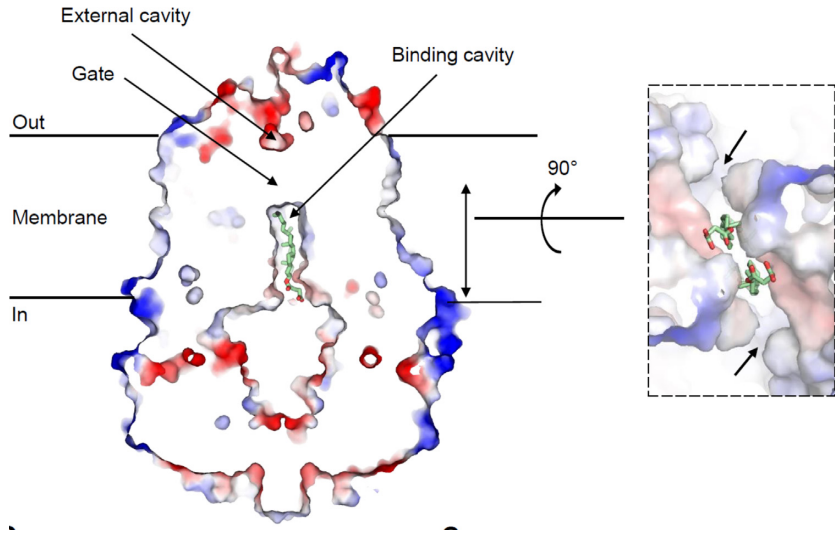
Cholesterol-binding pocket(s): different models, different proposals



(Zhang et al, Comm Biol, 2021)

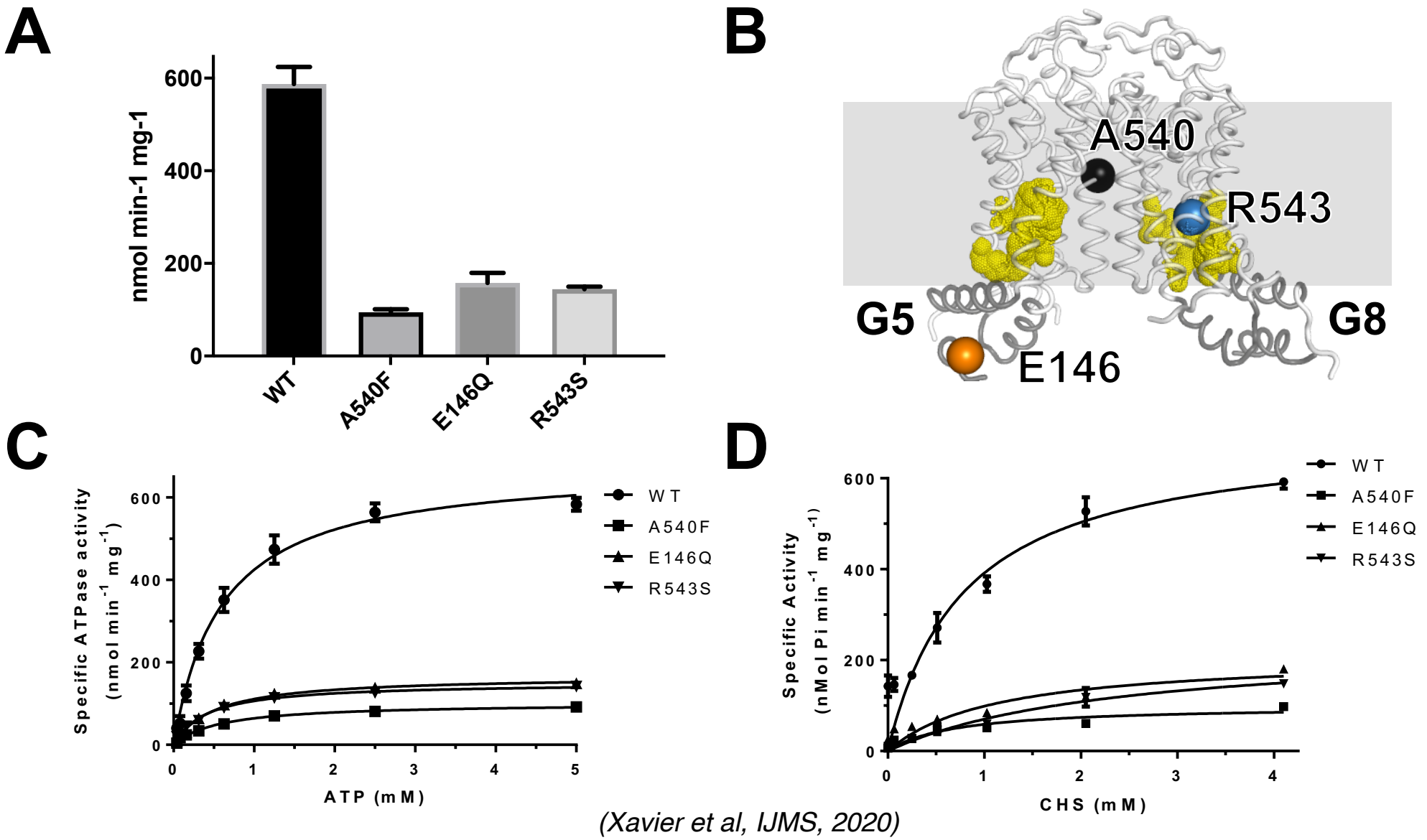


(Sun et al, PNAS, 2021)



(Skarda et al, JMB, 2021)

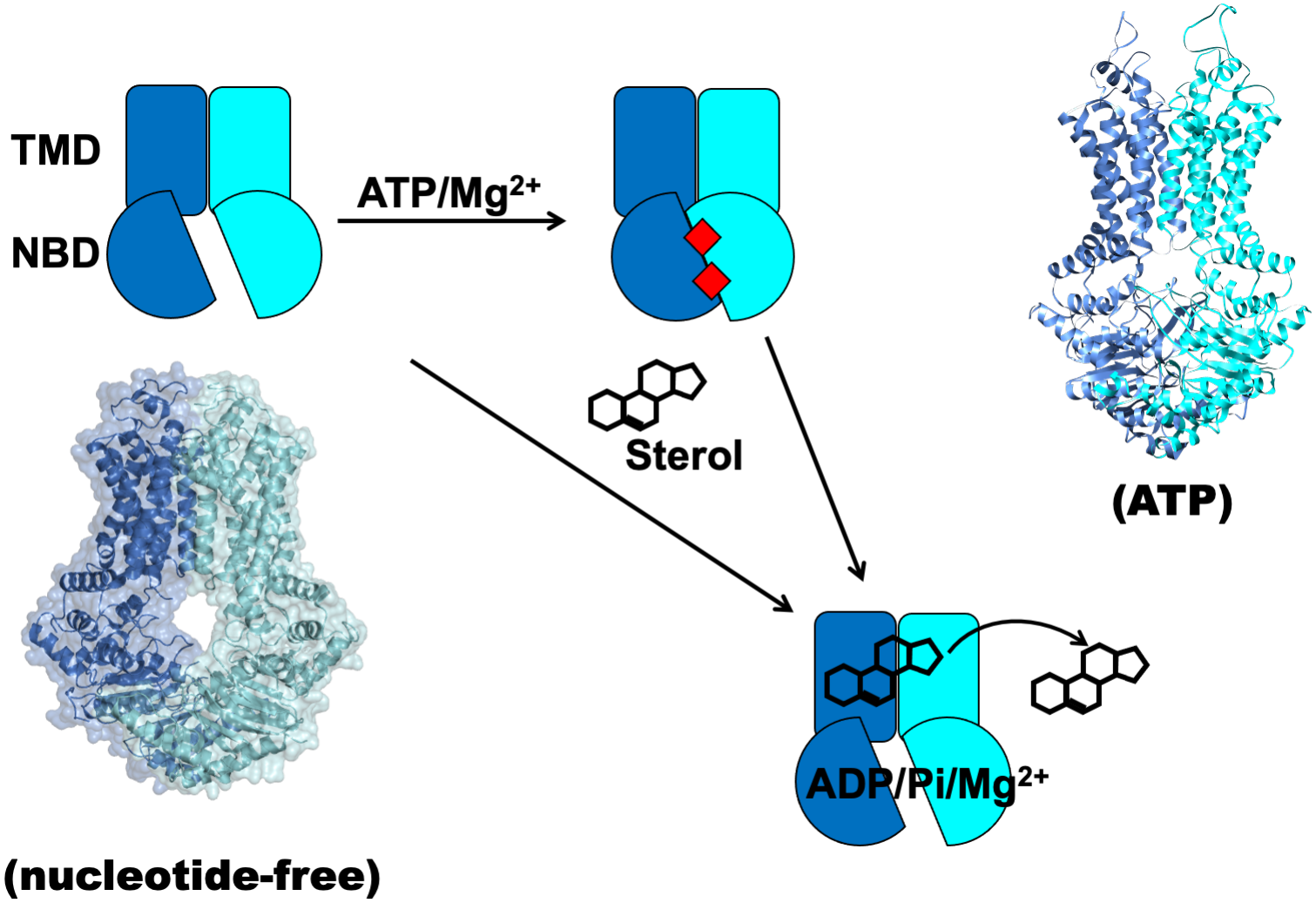
Enzymatic Analysis → Allosteric Regulation



(Xavier et al, IJMS, 2020)

Working Model of ABC Sterol Transporters (Molecular/Atomic)

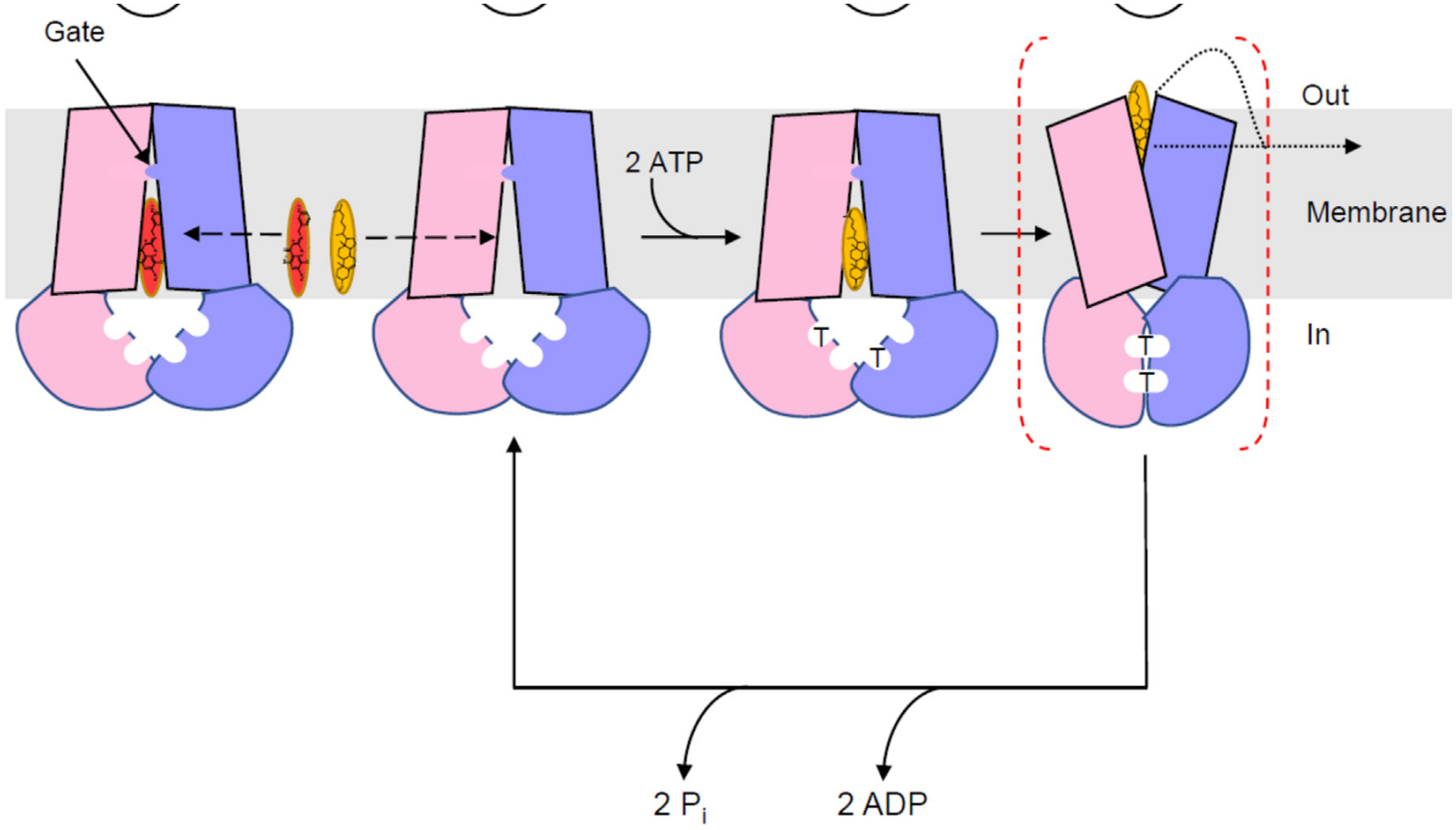
ABCG5/G8



(Xavier et al, IJMS, 2020)

Working Model of ABC Sterol Transporters (Molecular/Atomic)

ABCG1



(Skarda et al, JMB, 2021)