# *In silico* approaches to assess the structure, dynamics, and function of transmembrane proteins

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# Acknowledgement

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*Tóth, Ágota*

Kellermayer, Miklós
Sarkadi, Balázs

NKFIH K 111678

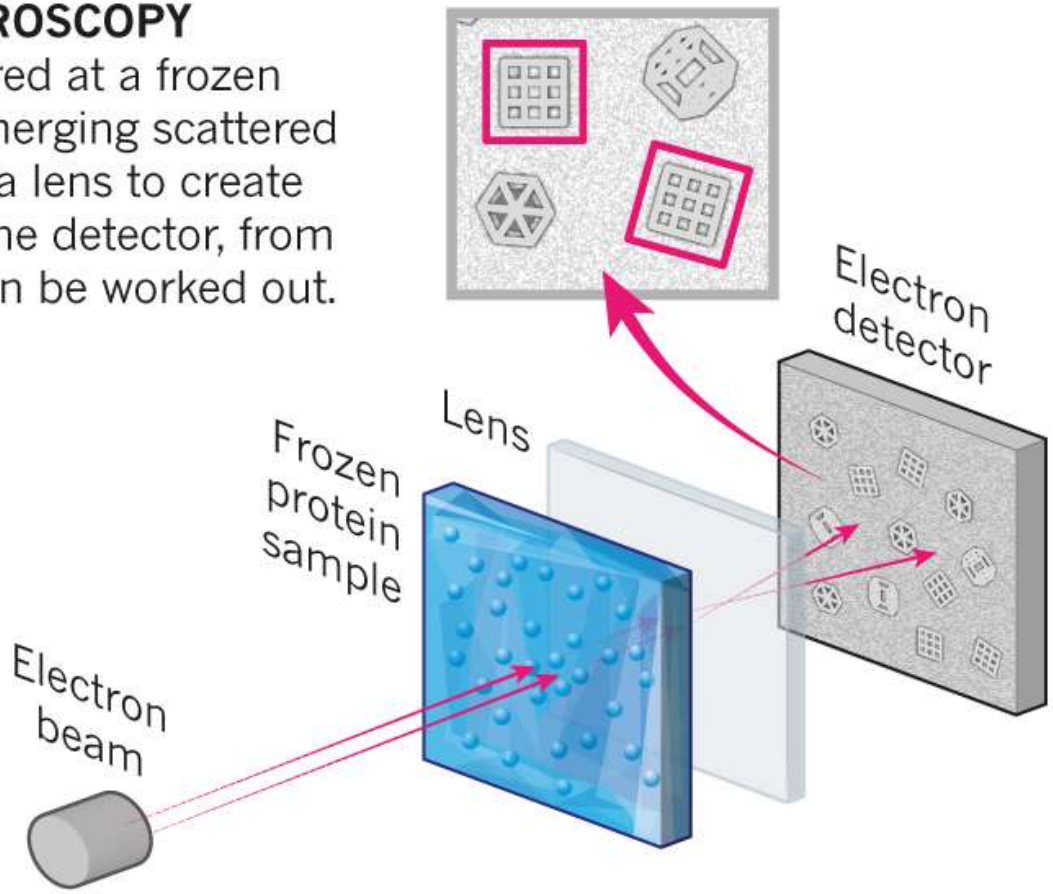
- **Structures**
  - **cryo-EM**
- **Comparison of structures and assessing their validity**
  - **ABC proteins**
- **Effect of mutations on structure and dynamics**
  - **ABCG2**
  - **CFTR**
- **Studying function**
  - **ABCG2**
  - **CFTR**

# Structure determination – „single particle”

## Cryo-electron microscopy

**CRYO-ELECTRON MICROSCOPY**

A beam of electron is fired at a frozen protein solution. The emerging scattered electrons pass through a lens to create a magnified image on the detector, from which their structure can be worked out.

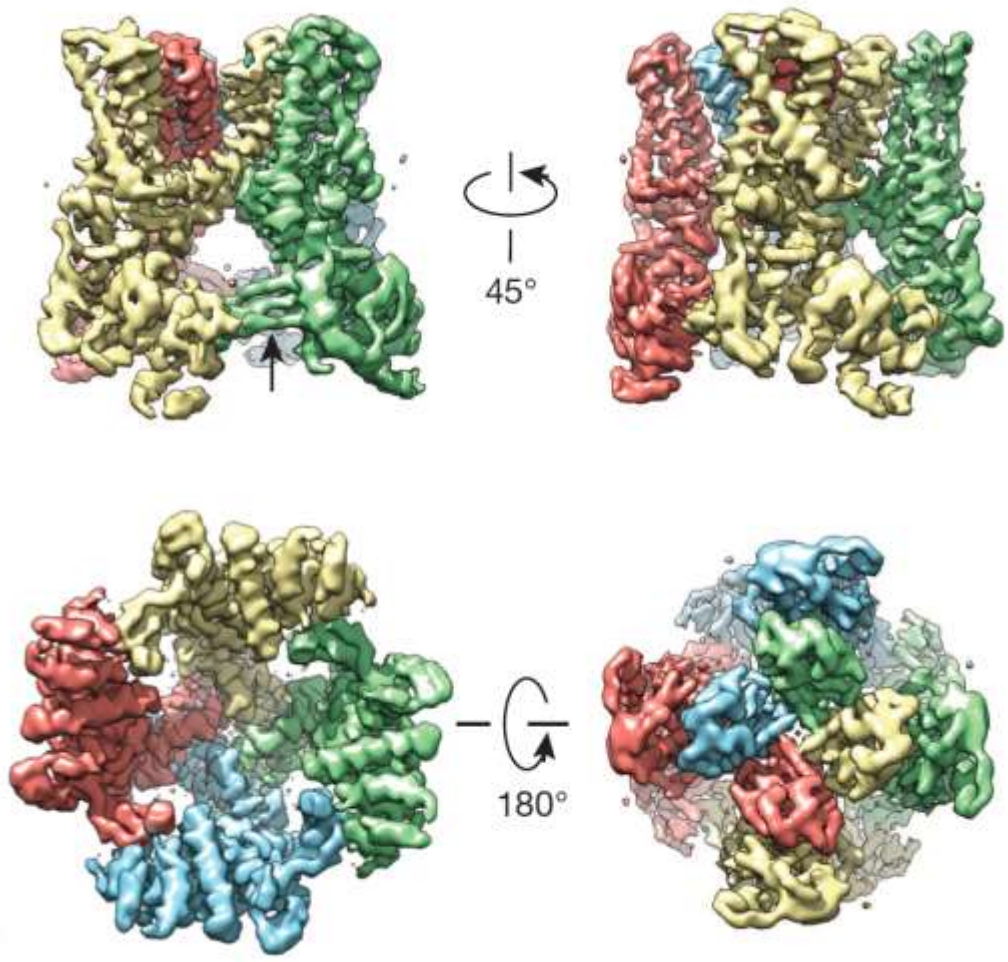



© nature

Ewen Callaway, Nature | News Feature
The revolution will not be crystallized: a new method sweeps through structural biology, 09 September 2015

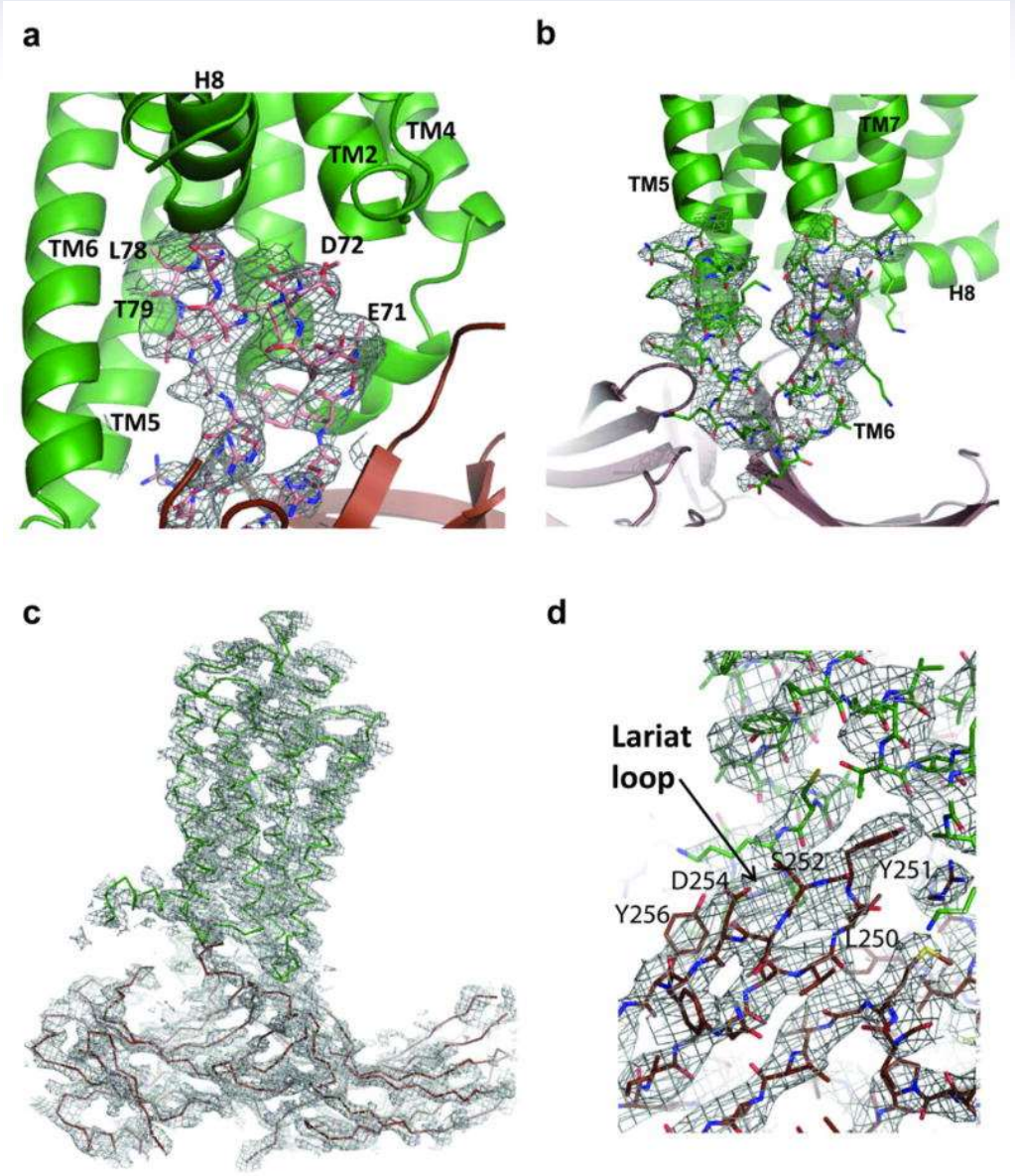
# Structure determination – „single particle”

The TRPV1 channel detects the burn of chilli peppers, and this 3.4-Å structure is considered super-hot in the structural-biology world.



# Szerkezet meghatározás – „single particle”

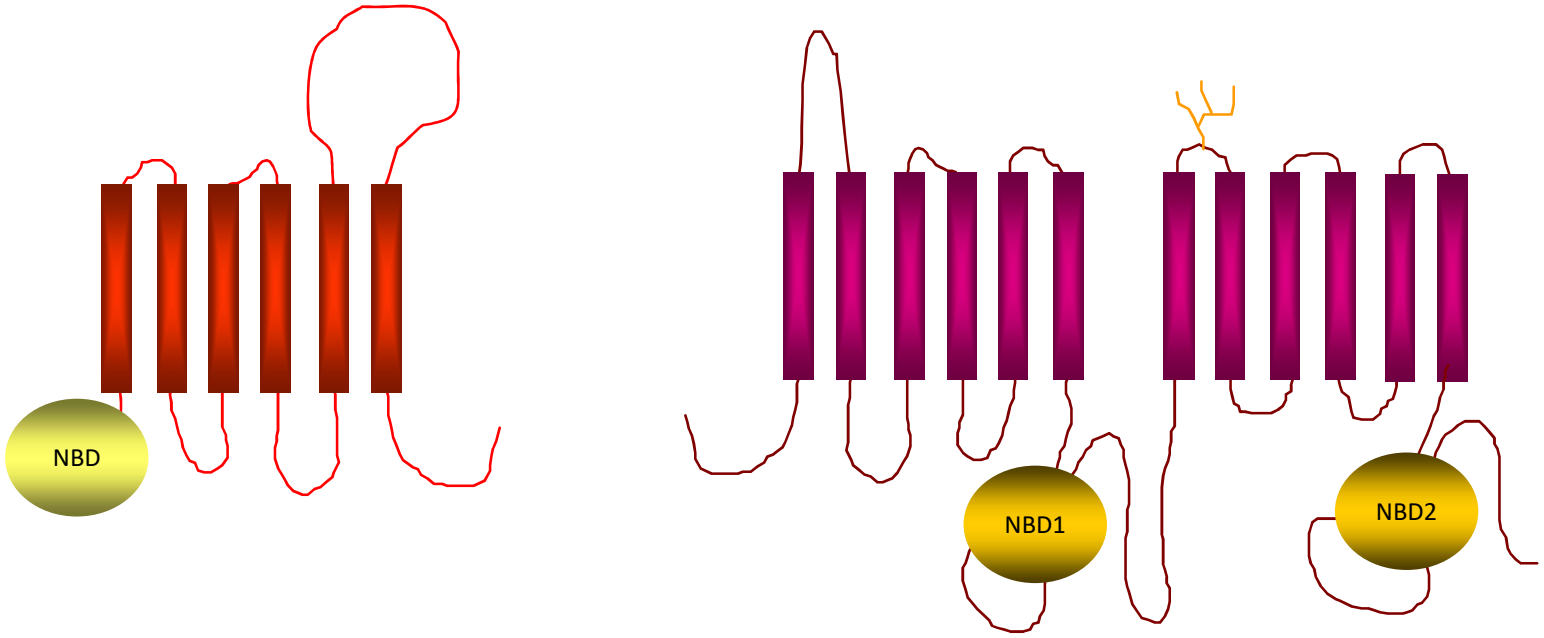
## Free Electron Laser (FEL)



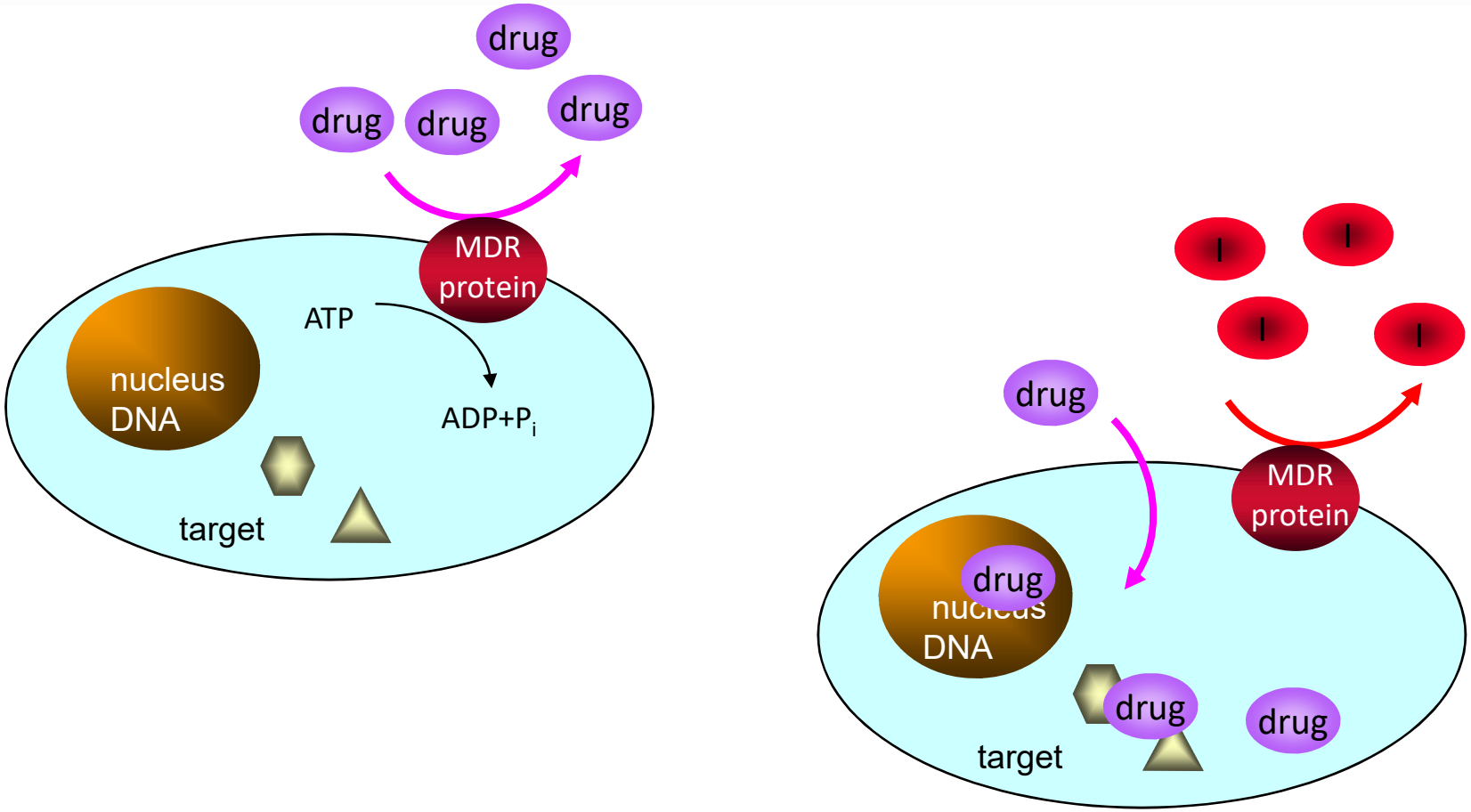

Nature. 2015 Jul 30;523(7562):561-7.

Crystal structure of rhodopsin bound to arrestin by femtosecond X-ray laser.

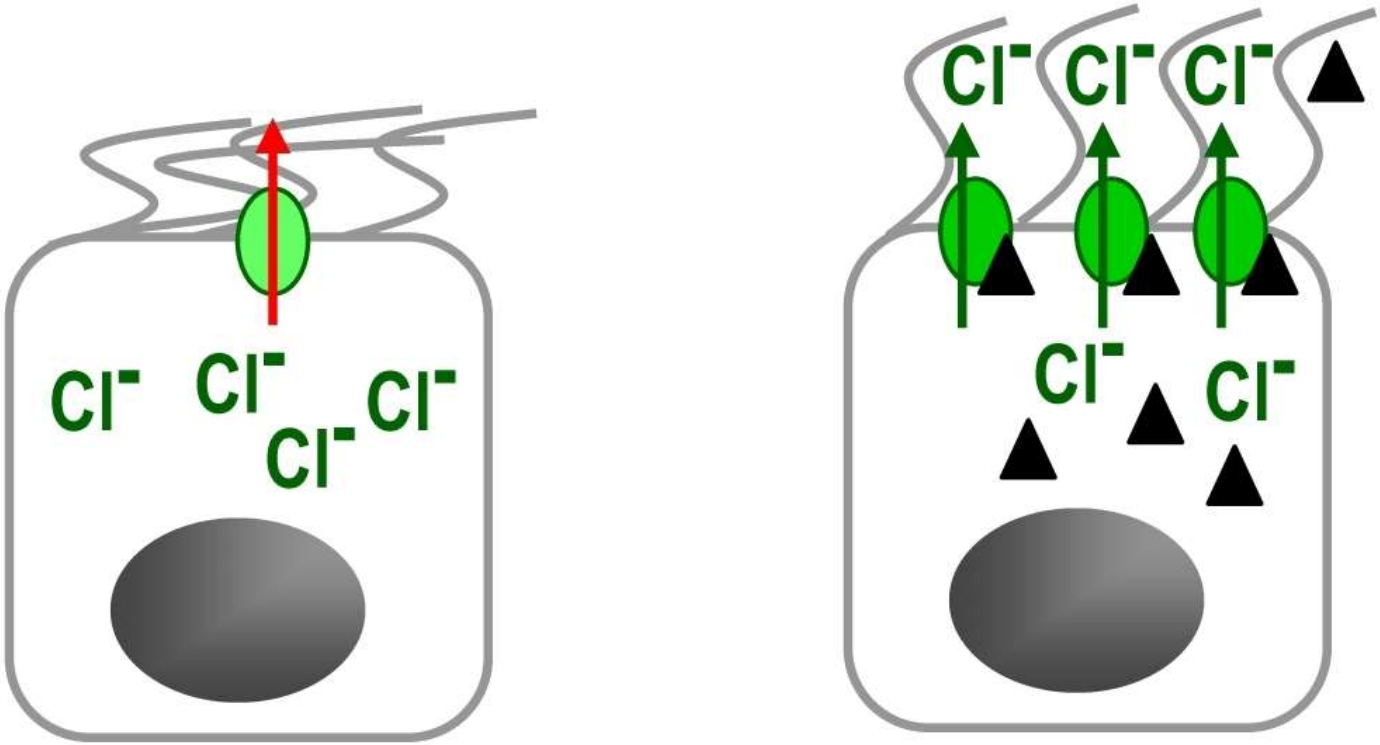
# ATP Binding Cassette (ABC) proteins



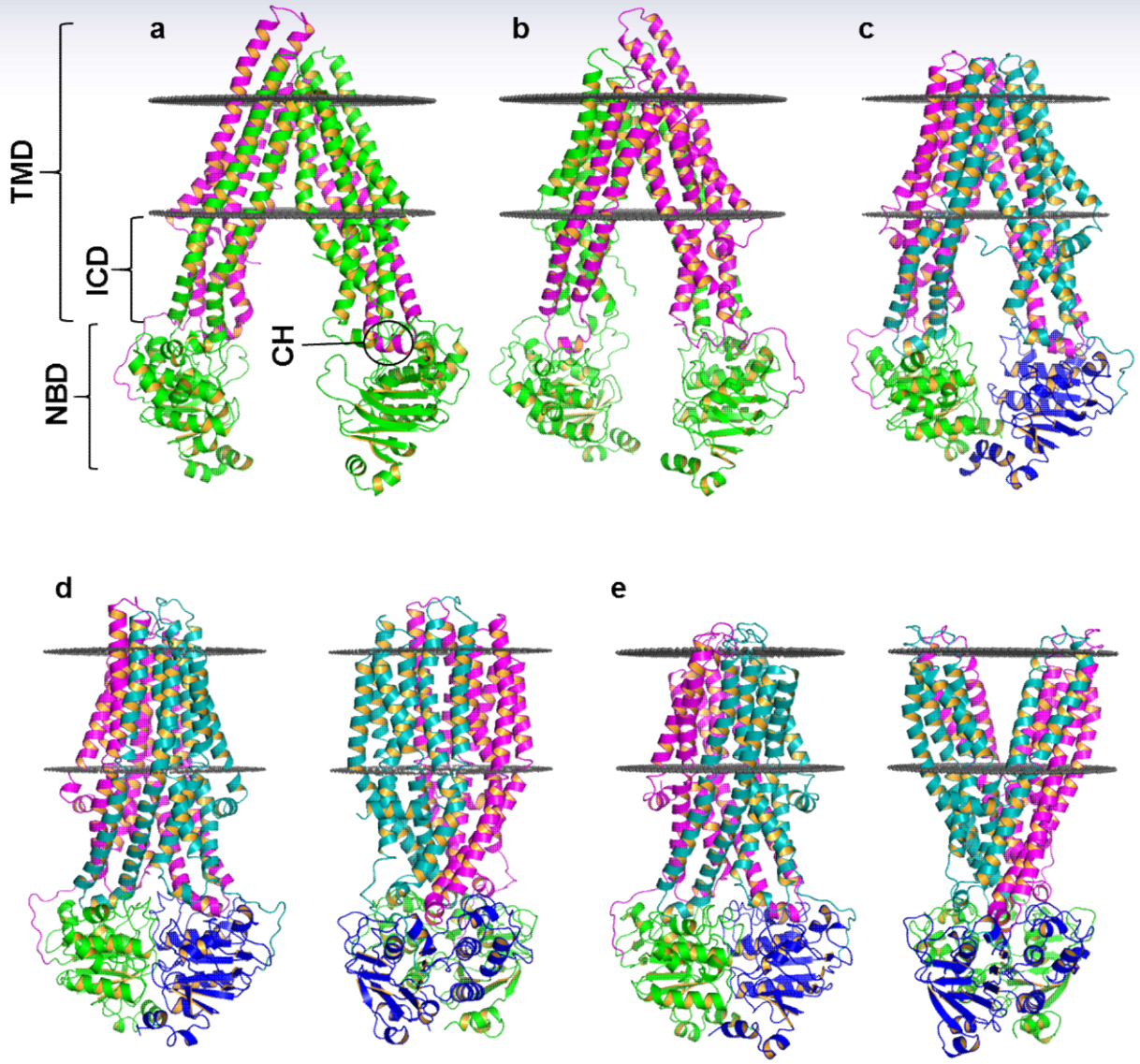
# Multidrug resistance of cancer cells



# Cystic Fibrosis – ABCC7/CFTR

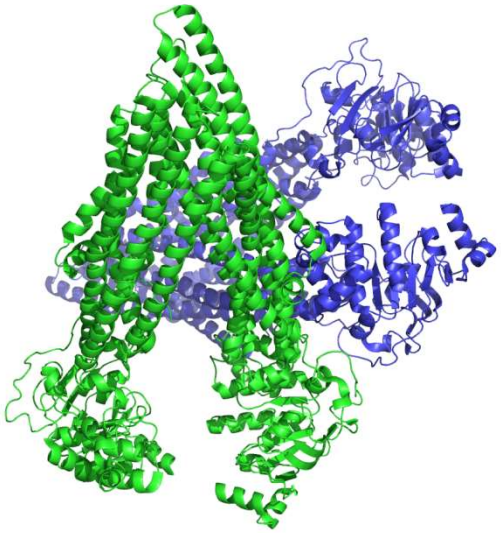


# Conformation of ABC proteins

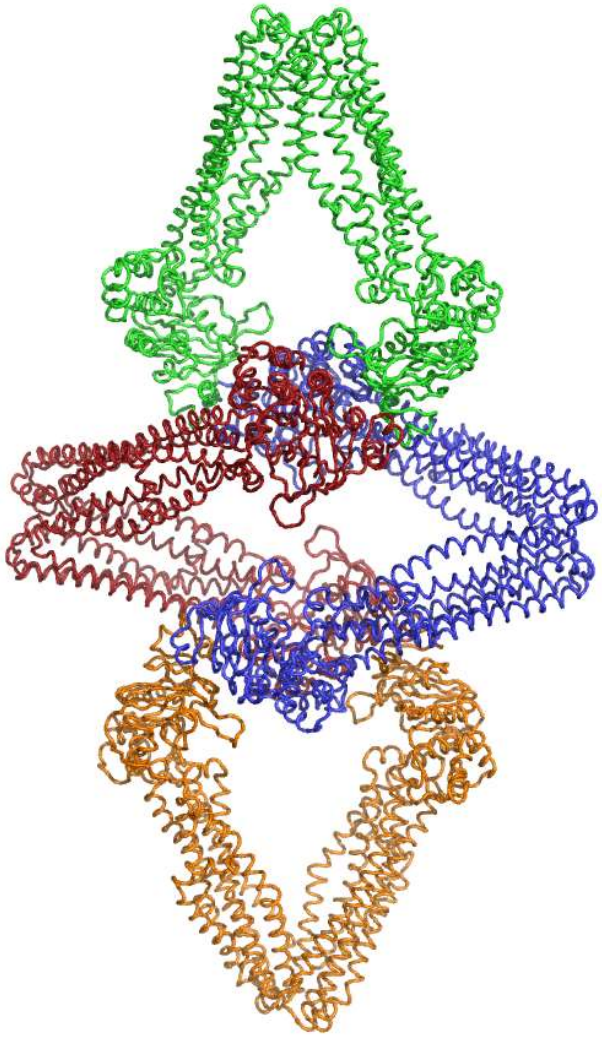


# Bottom-open structures

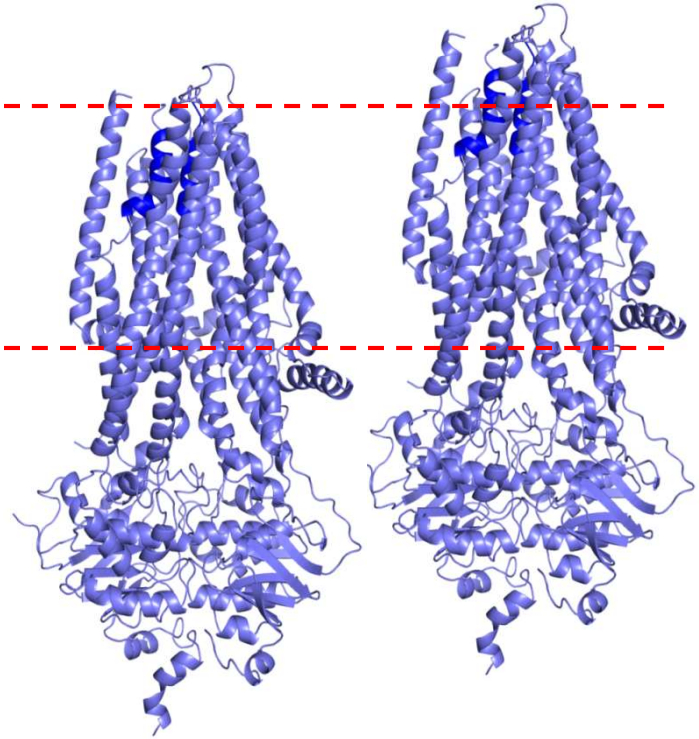
mMDR3, PDBID:3G5U

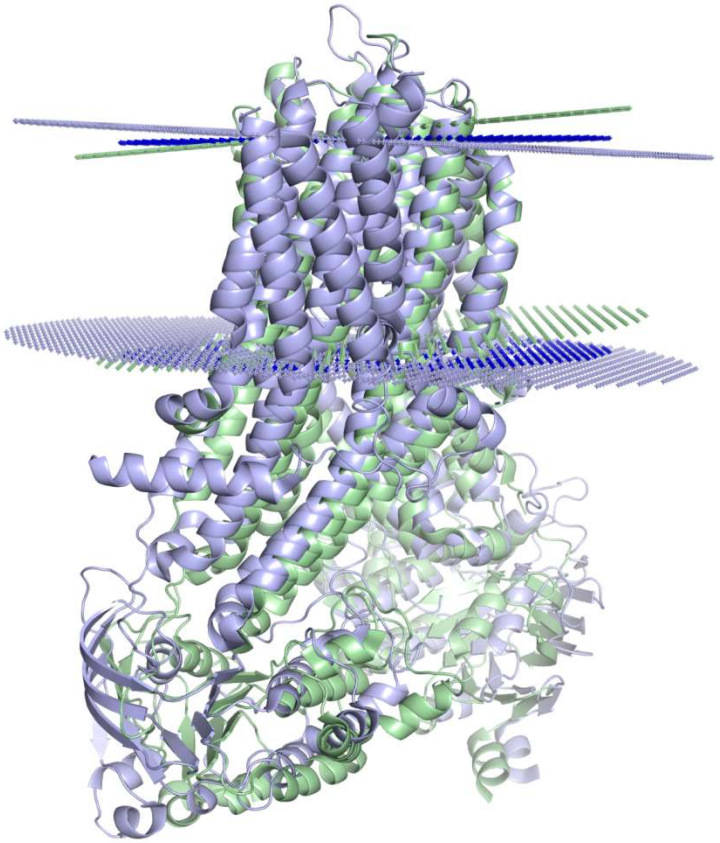


MsbA, PDBID:3B5W

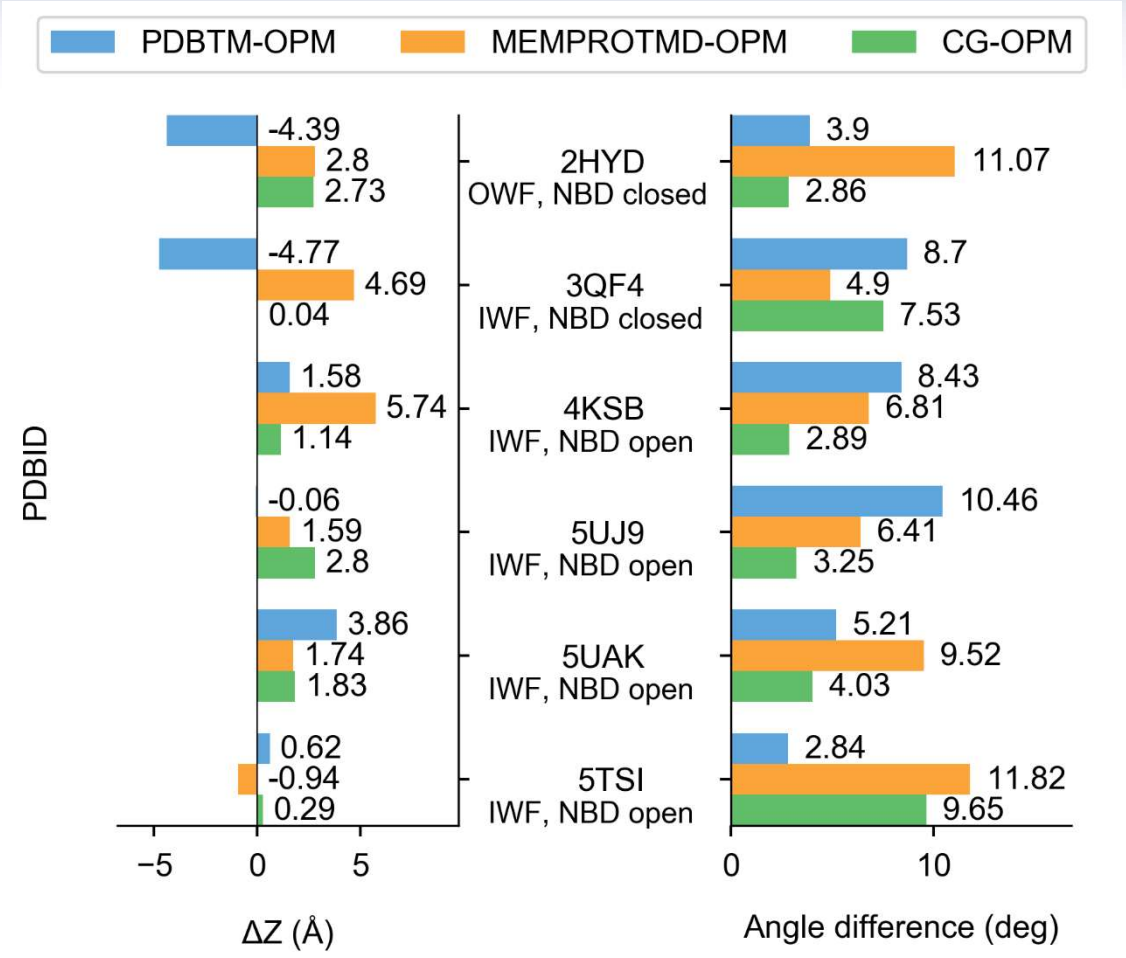


# Localization and tilting in a bilayer





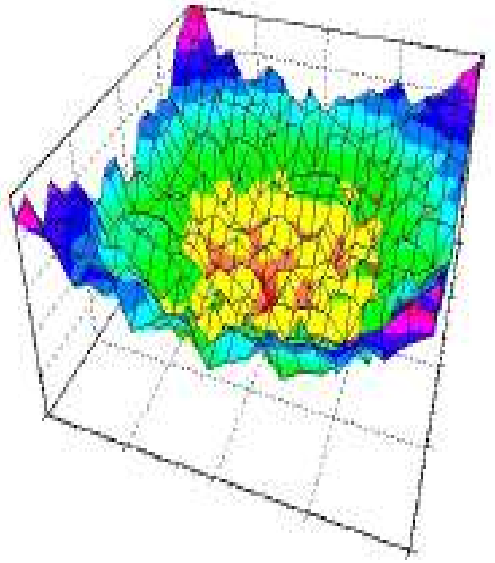
# Tilting and localization in a bilayer

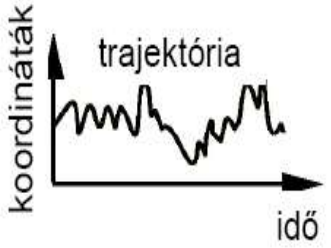


# Molecular dynamics

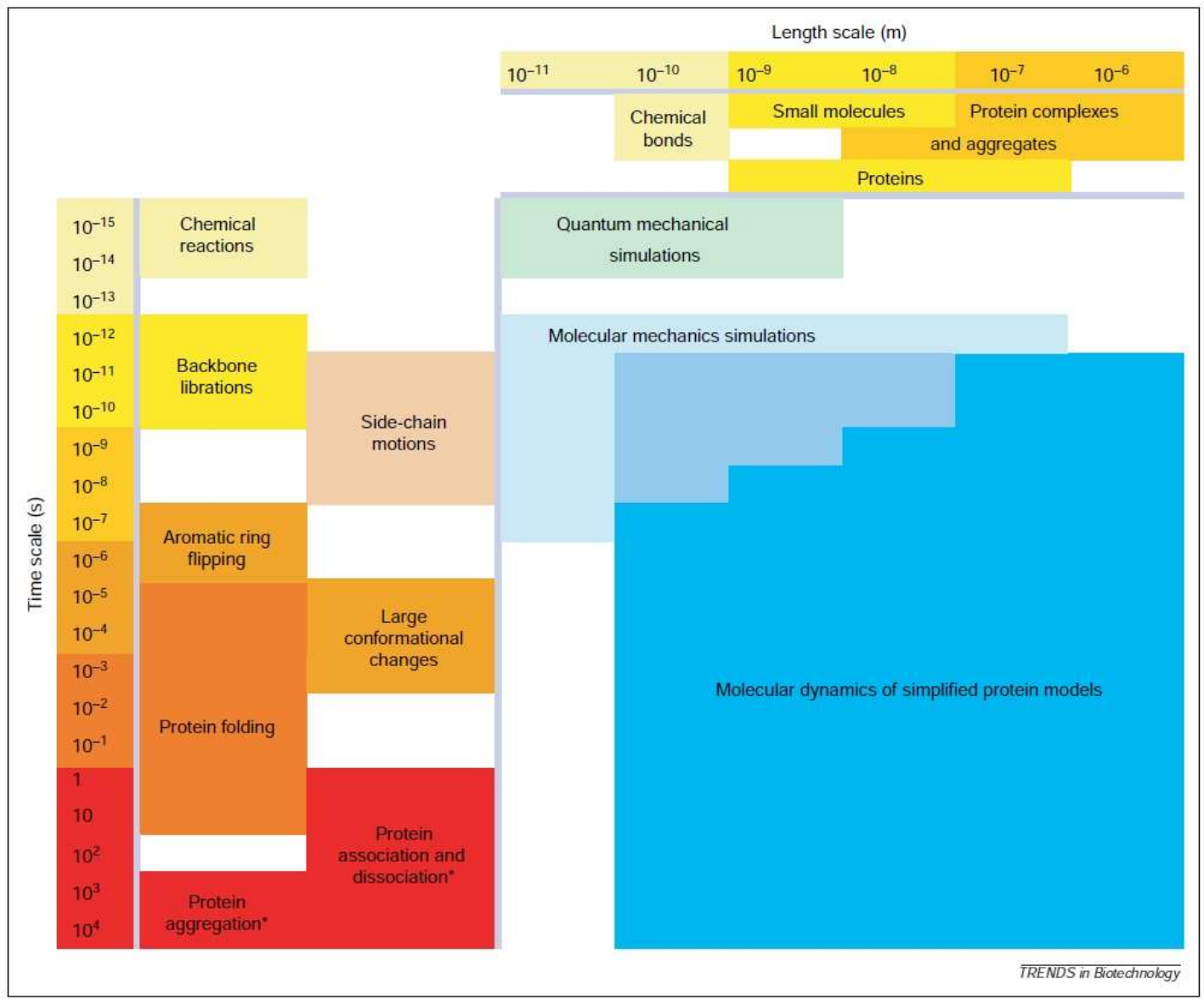
## Molecular dynamics (MD)

- potential surface
- solving the equations of motions numerically
- output: trajectory



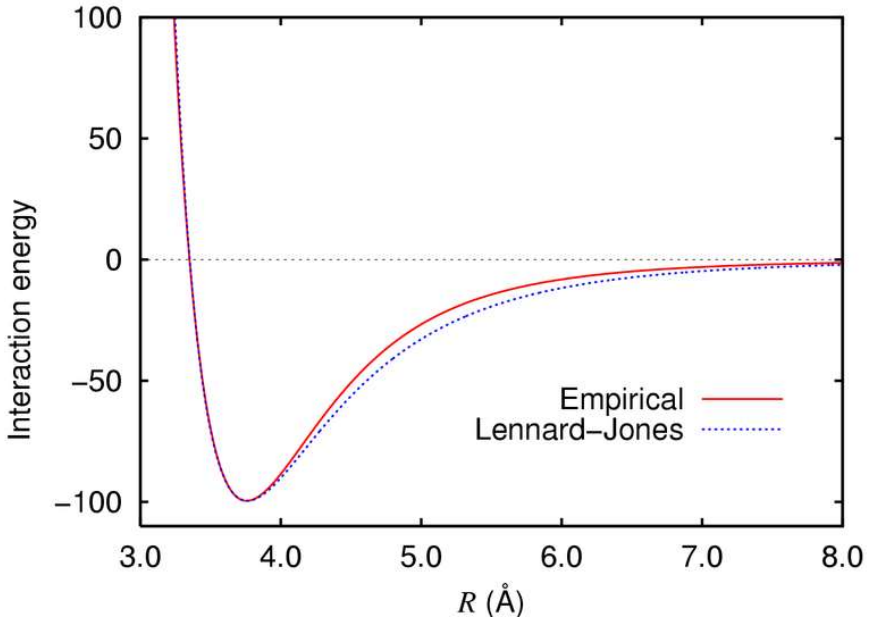


# Timescale of events

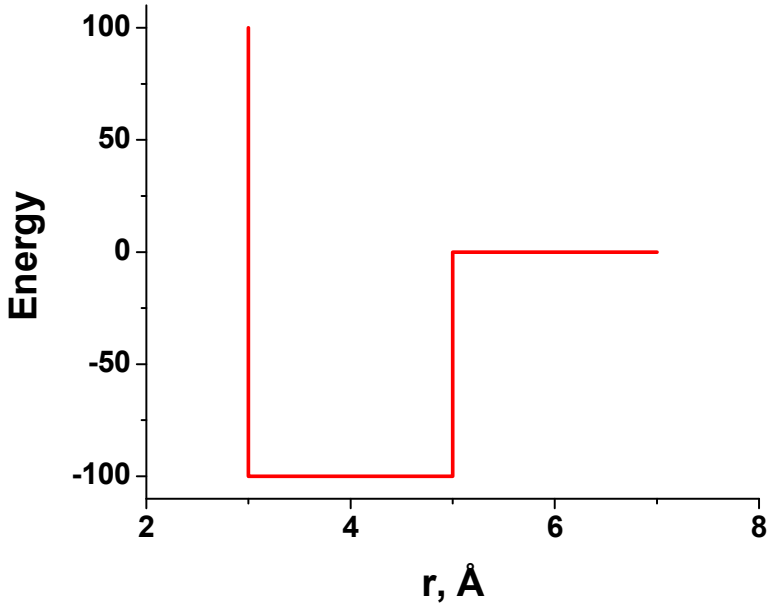



F. Ding and N.V. Dokholyan, TRENDS in Biotechnology, **23:**450 (2005)

# Discrete Molecular Dynamics (DMD)

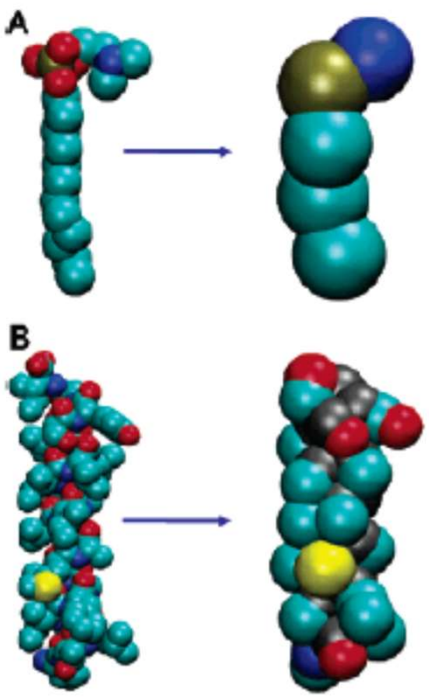



wikipedia




Ding, F., Dokholyan, N. V. PLoS Comput Biol 2:e85

# Coarse Grained models



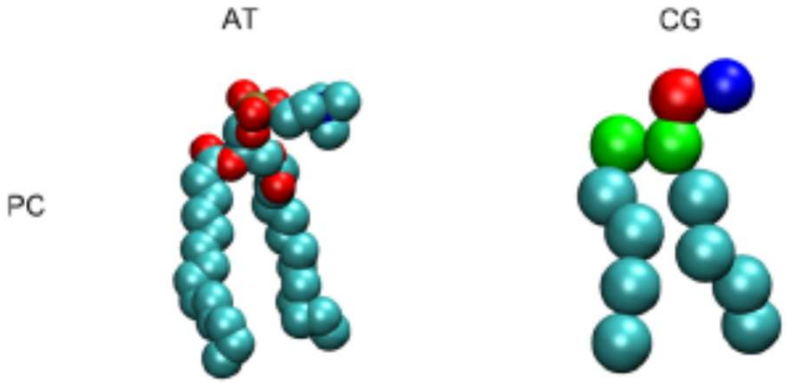
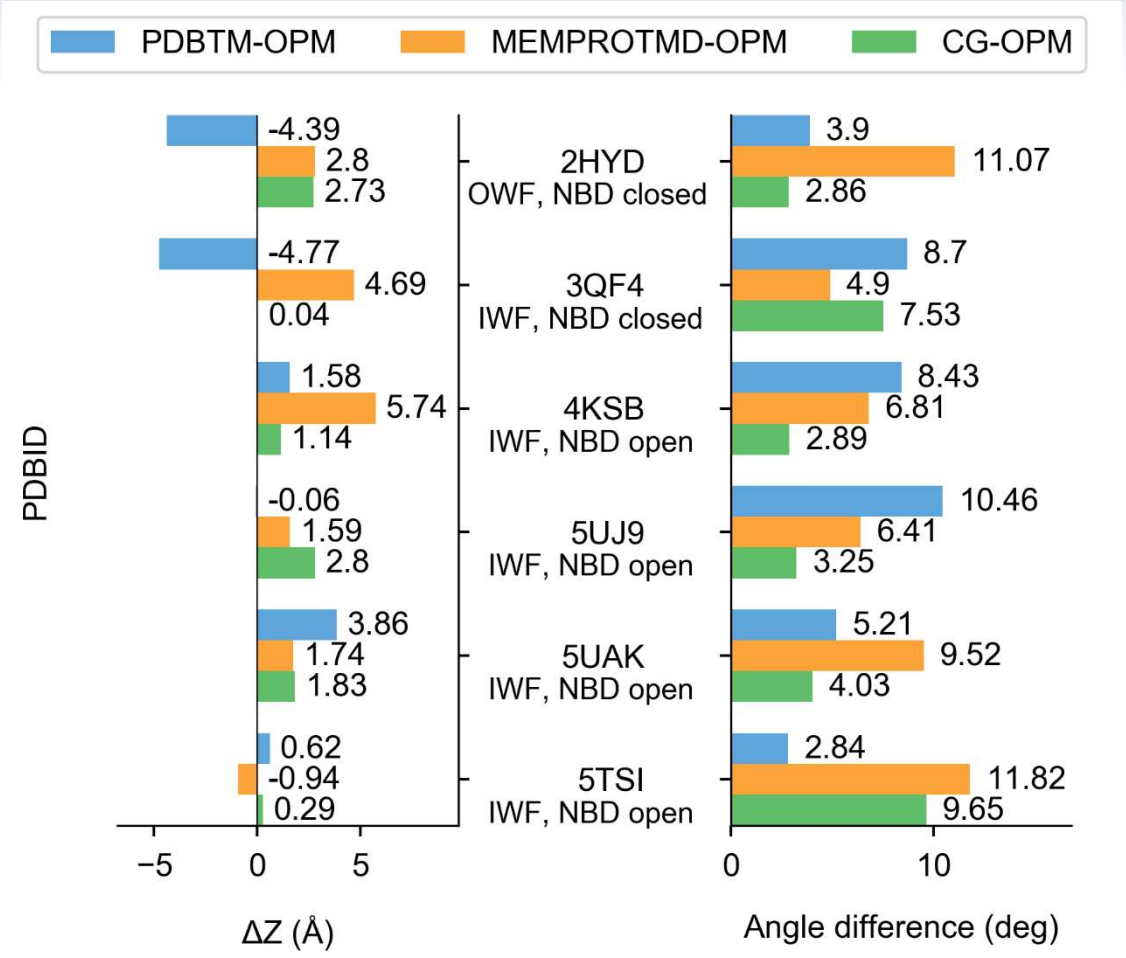




*Figure 1.* Atomistic (left-hand) and coarse-grained (right-hand) models compared for (A) a DPC molecule and (B) a GpA helix. Colors for atoms:

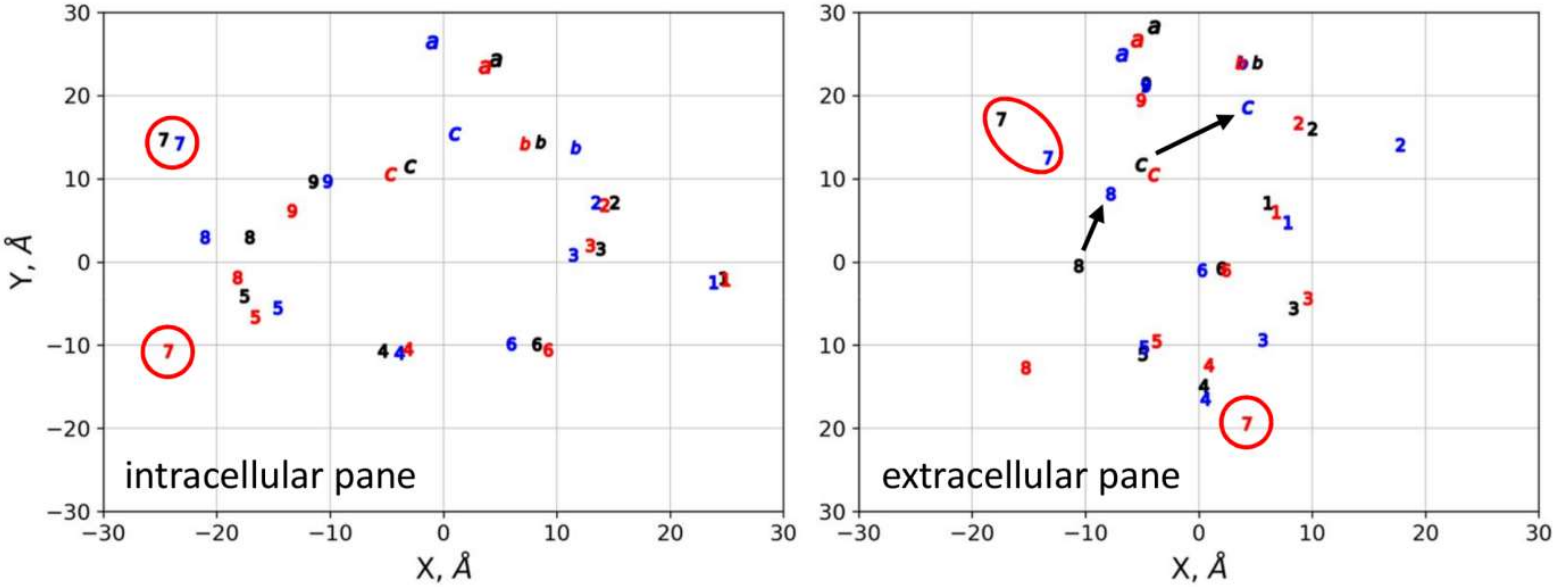
**MARTINI CG force field**

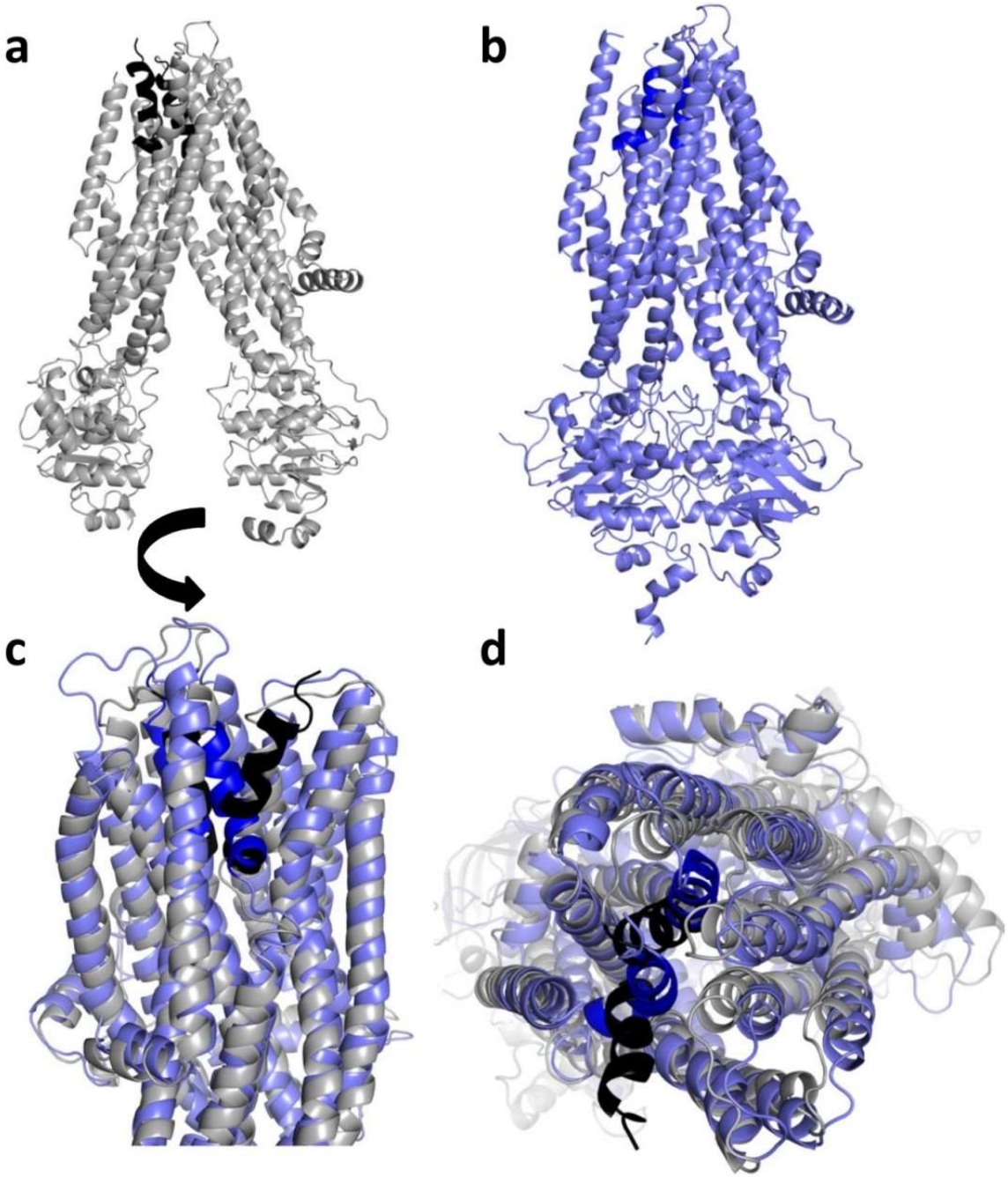
# Bilayer formation around a TM protein

# Tilting and localization in a bilayer

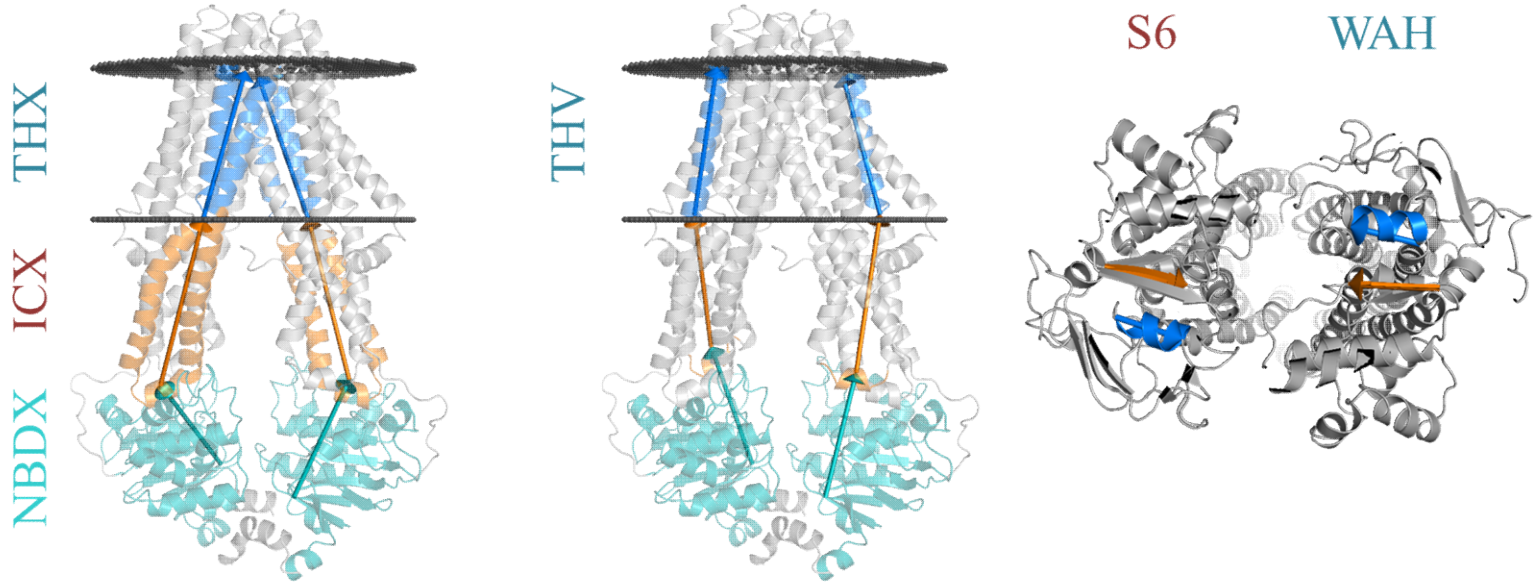


# Tilting and localization in a bilayer CFTR structures



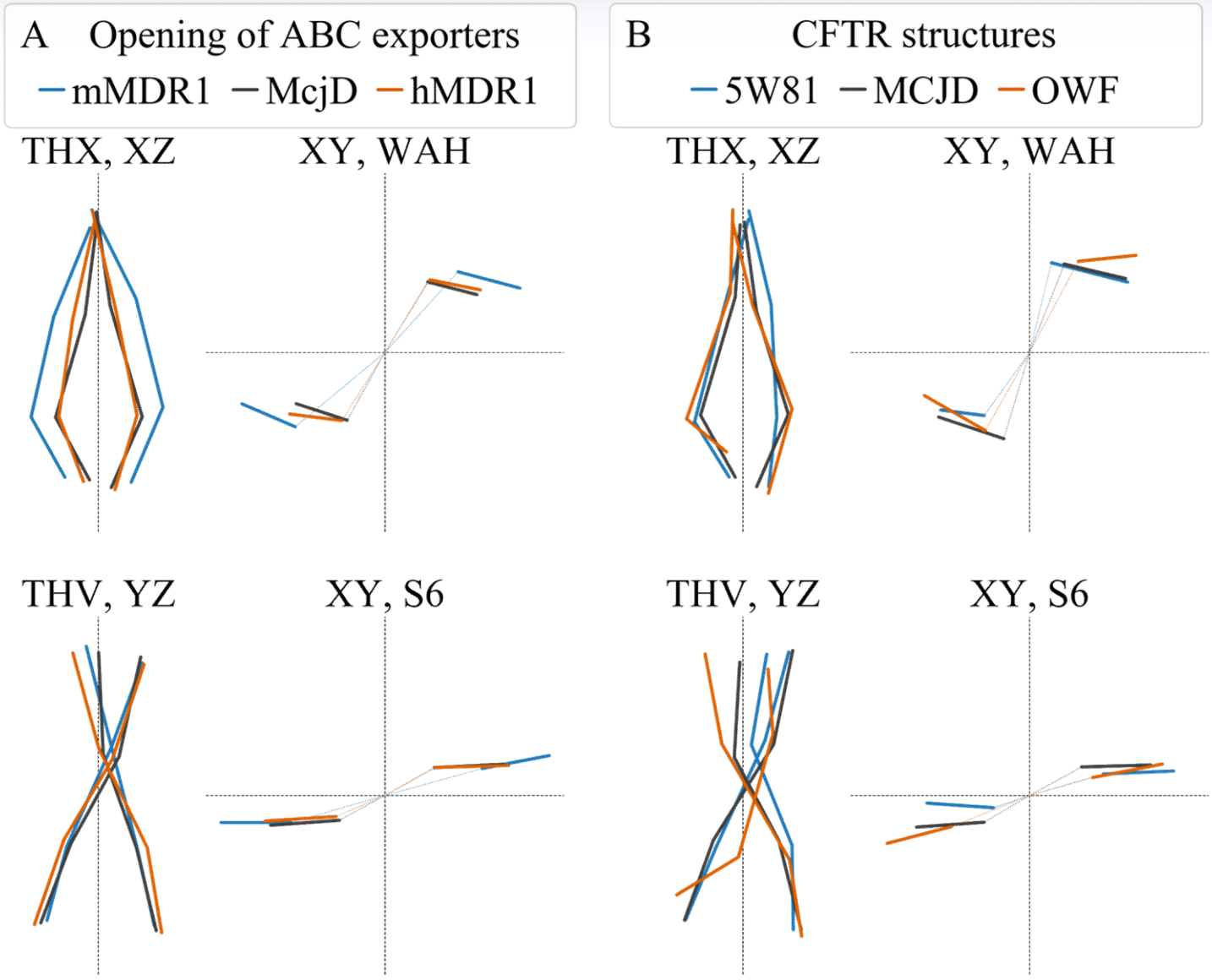

a
b
c
d

# Conftors



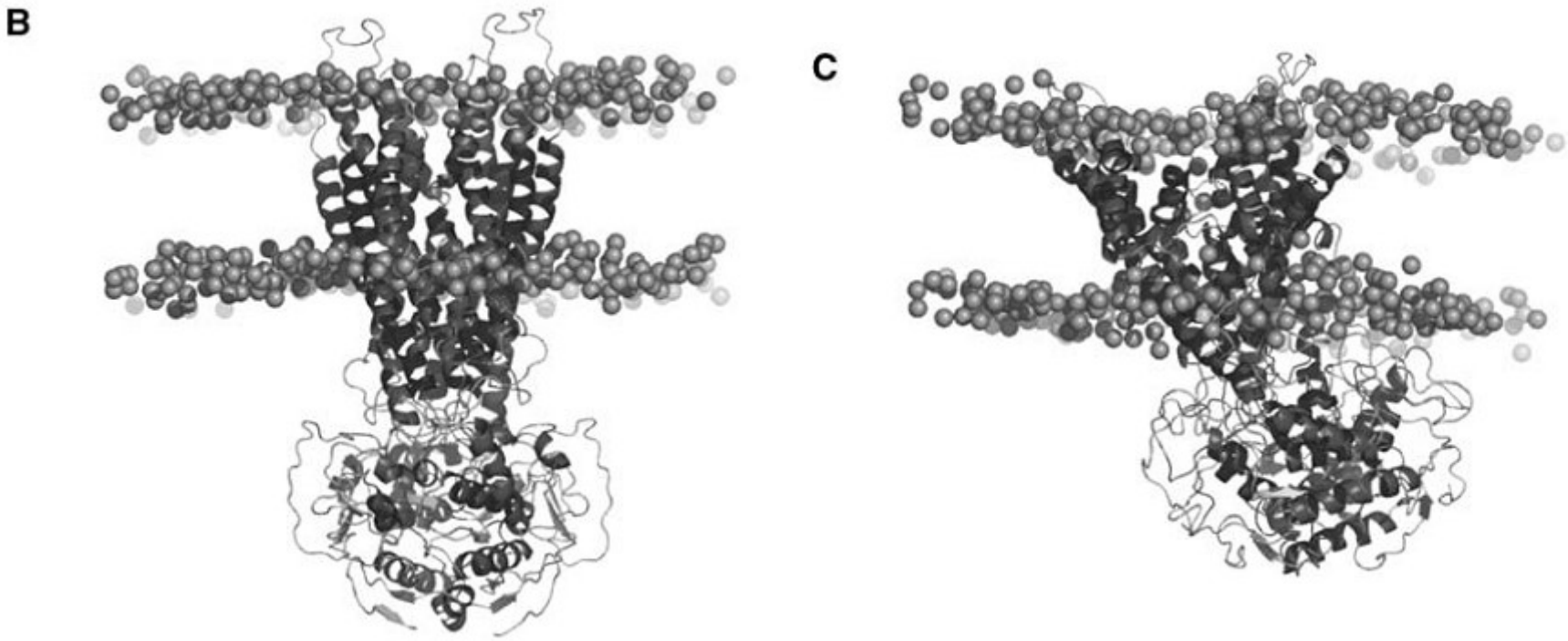

| Conformation | Structures (n) | THX1&THX2 (°) | ICV1&ICV2 (°) |
|---|---|---|---|
| bottom-open, inward-facing | 27 | 46.13 (+/-1.78) | 42.70 (+/-1.24) |
| bottom-closed, inward-facing | 6 | 39.73 (+/-2.35) | 37.96 (+/-3.32) |
| bottom-closed, outward-facing | 4 | 35.04 (+/-2.38) | 60.12 (+/-2.01) |
| bottom-closed, top-closed | 3 | 27.25 (+/-5.91) | 53.39 (+/-2.62) |

# 2D conftors – a simplified representation



# Stability investigated using MD

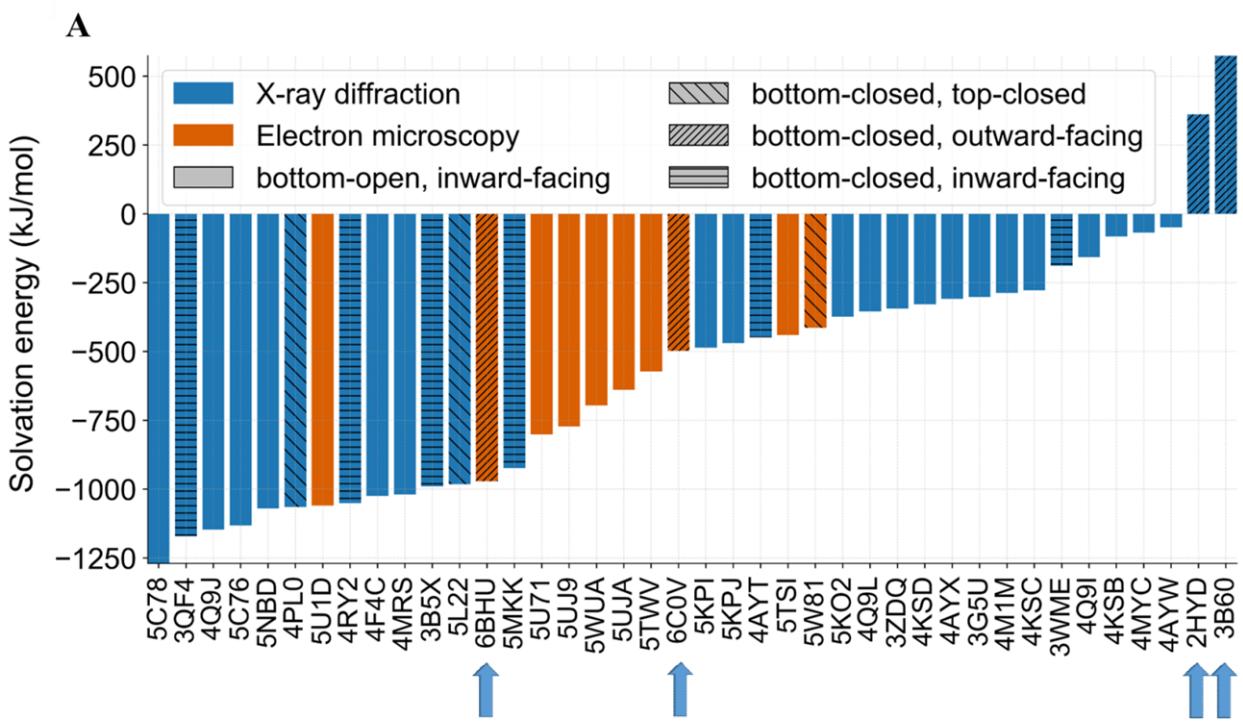
Eur Biophys J (2008) 37:403–409



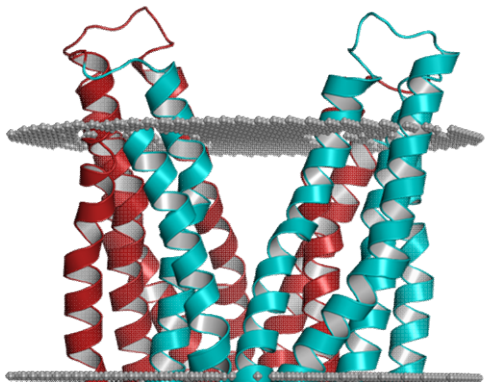

0 ns

20 ns

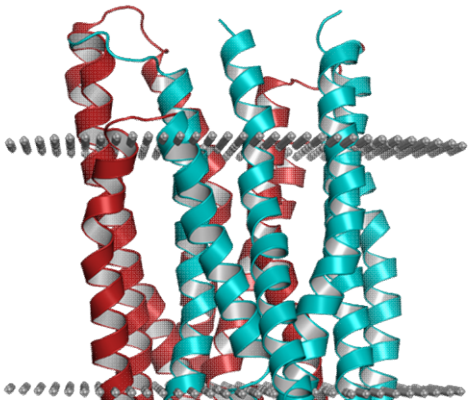
# APBS – membrane solvation energies




SAV1866



hMDR1



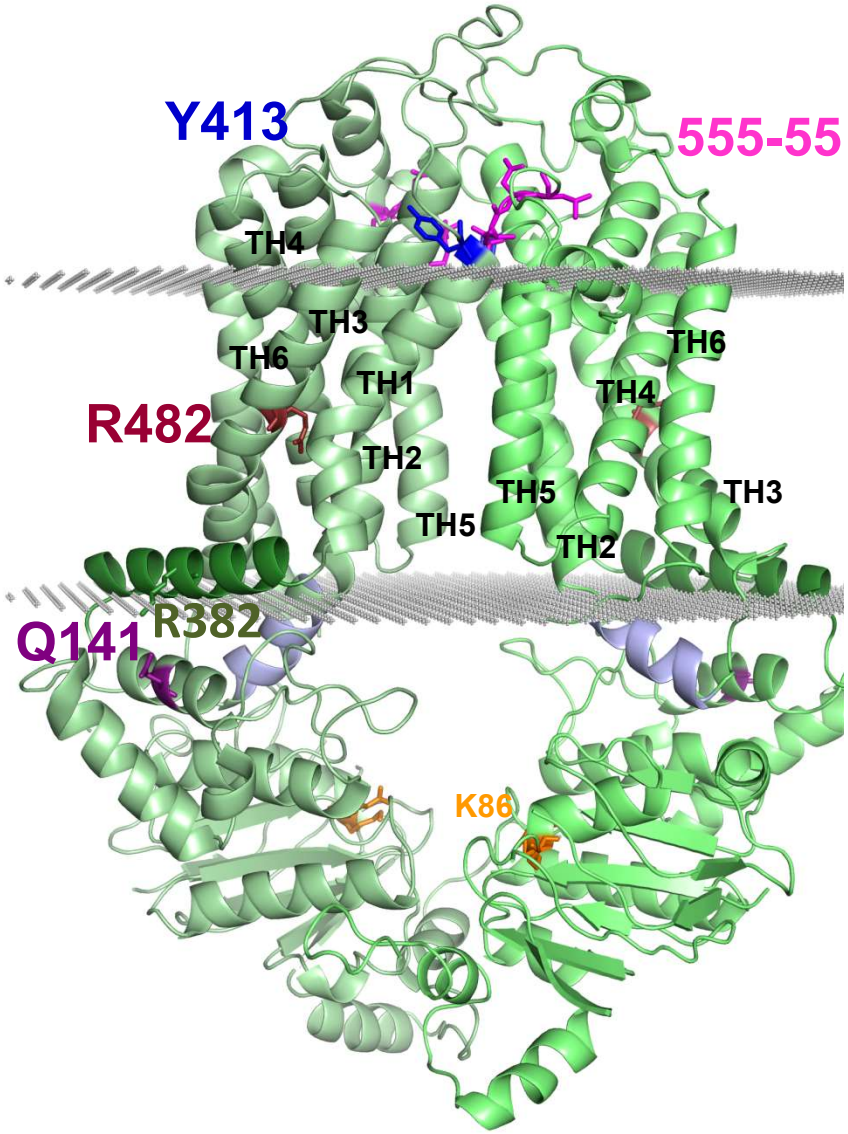
# Summary I

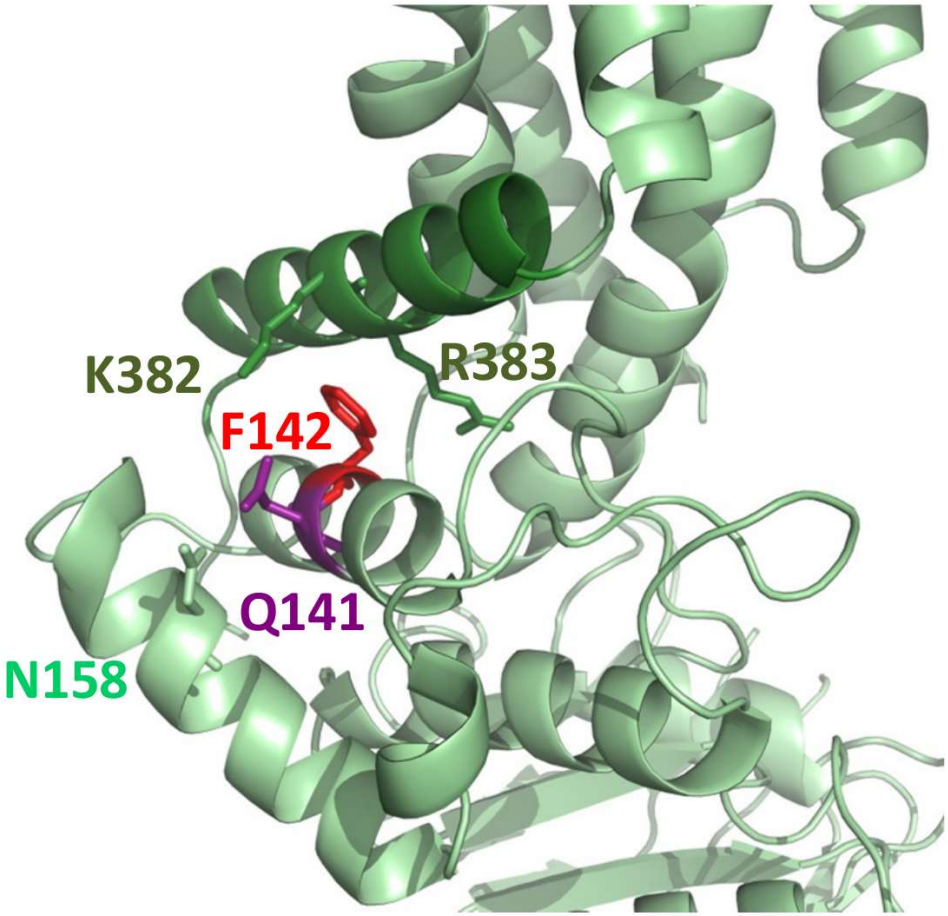
- Metrics for comparing structures
- Information on function of mechanism
- Some membrane protein structures might be biased by the lipid environment

# Effect of mutations

- Folding
- Trafficking
- Function
- Combinations of these

# The Q141K variant of ABCG2

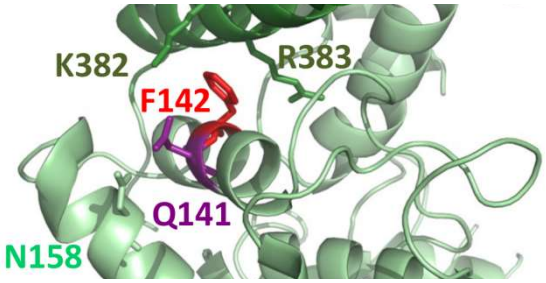


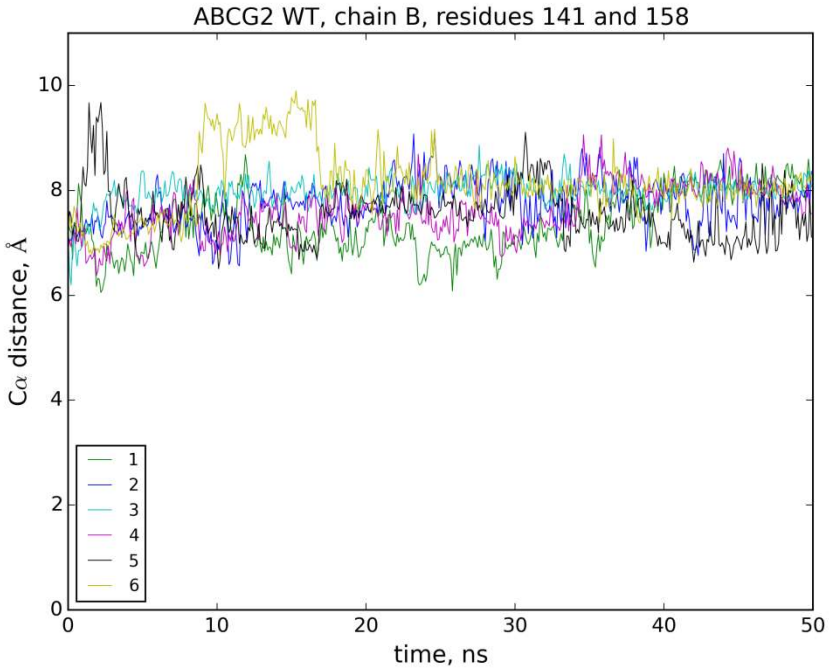



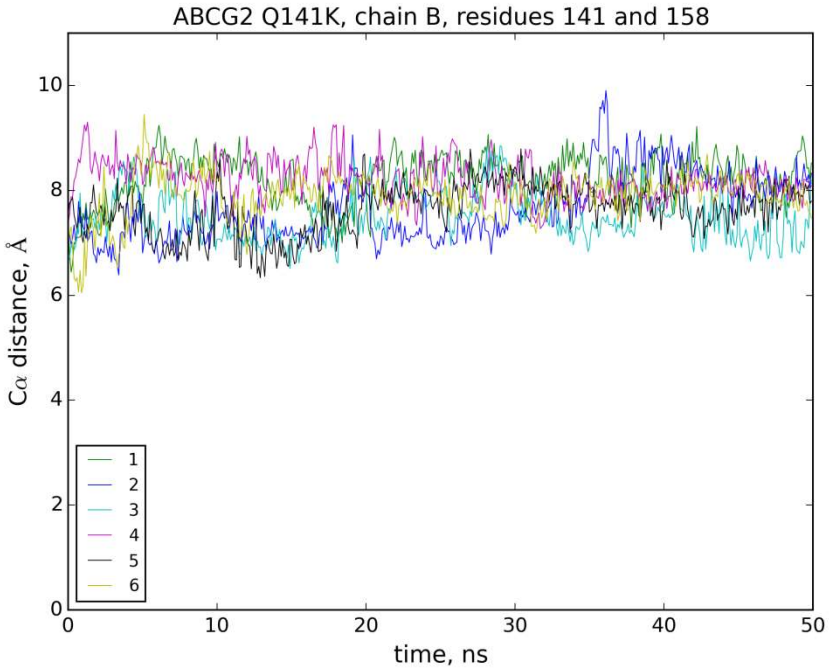
# MD simulations

- **Insertion of ABCG2 into a membrane (POPC) bilayer**
- **Optimizing the packing of a.a. side chains, lipids, and water molecules:**
  - energy minimization
  - equilibration (6 parallel with random velocities)
  - minimal backbone movements (position constrains)
- **Production run**
  - no constraints
  - 50 ns x 6 = 300 ns;
- **Comparing the WT and the variants**
  (e.g. Q141K, R482G)

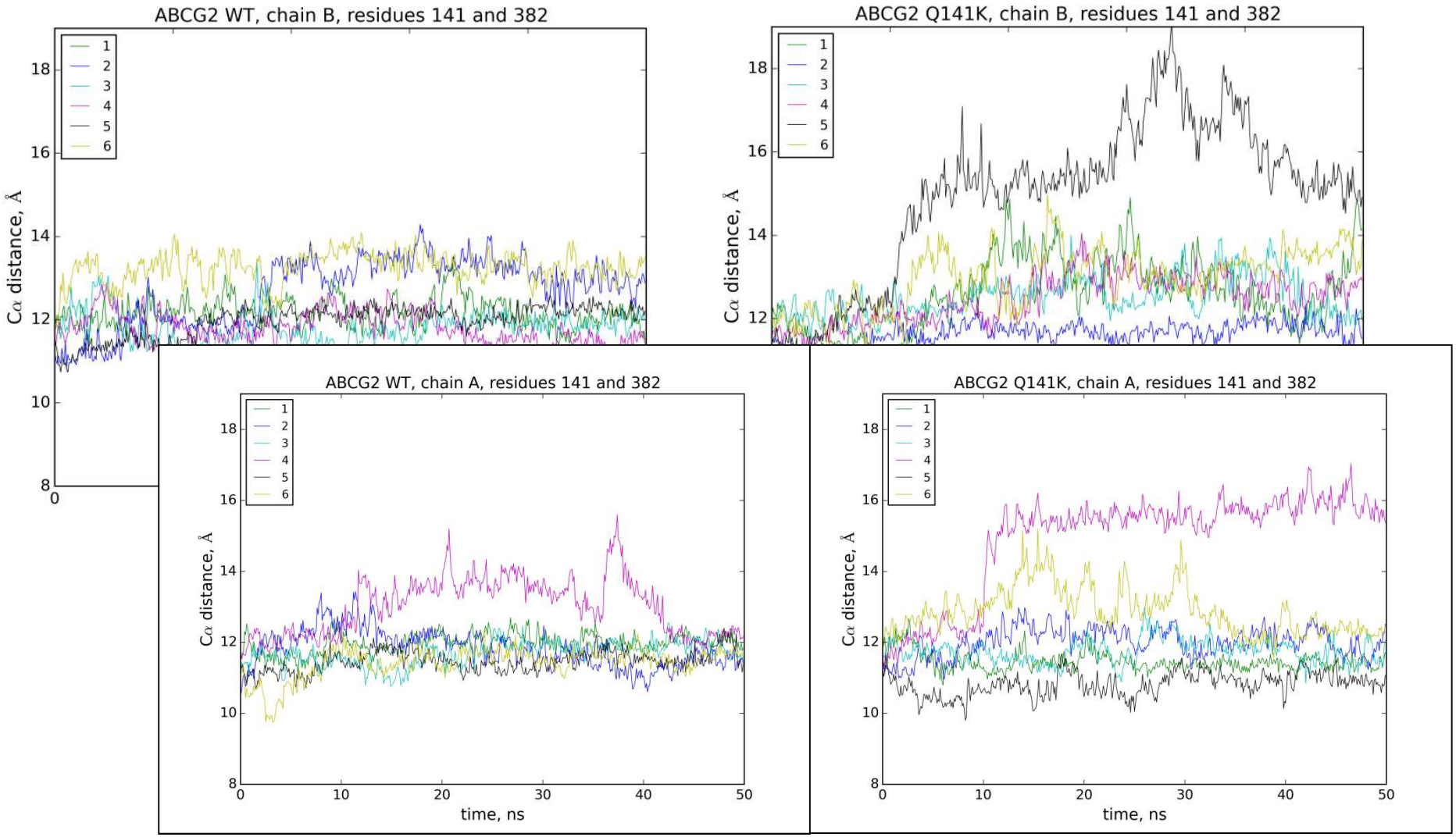
# Effects of Q141K on the 141/158 interaction



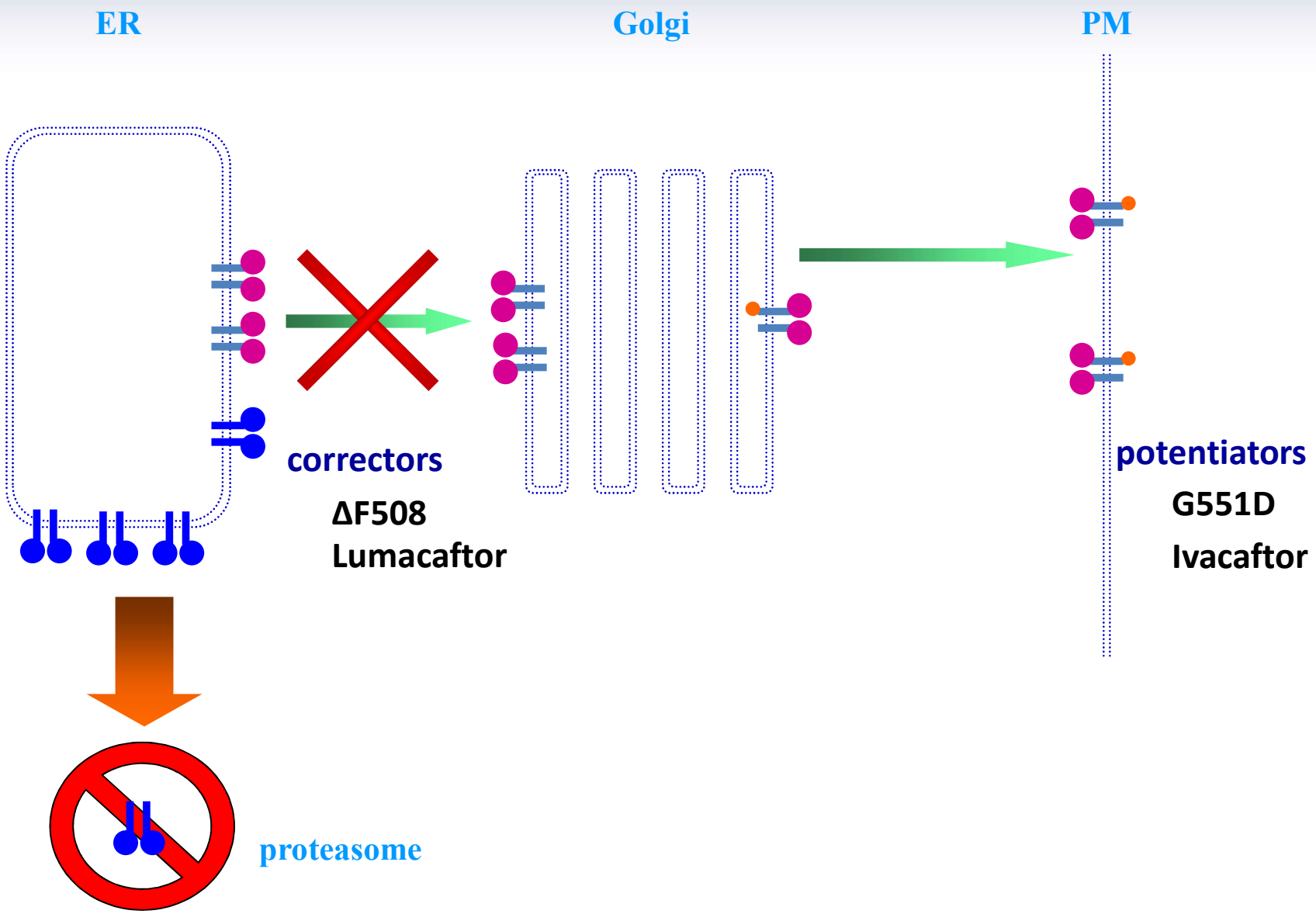



# Effect of Q141K on the 141/382 (NBD/TMD) interaction



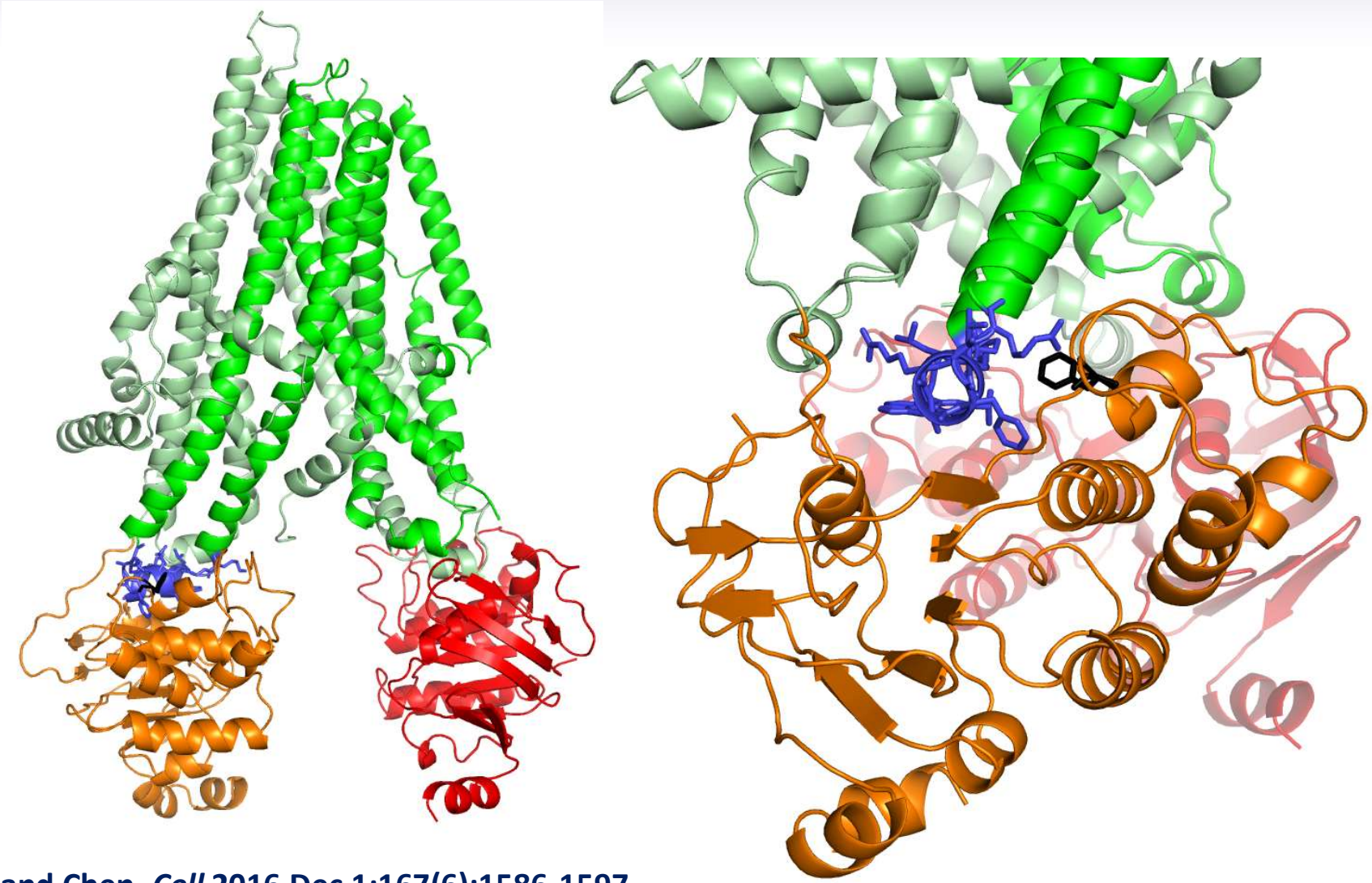
# Maturation of the CFTR protein



# Structural background of ΔF508 CFTR

*domain-domain interactions*

**hCFTR (PDBID:5U71)**



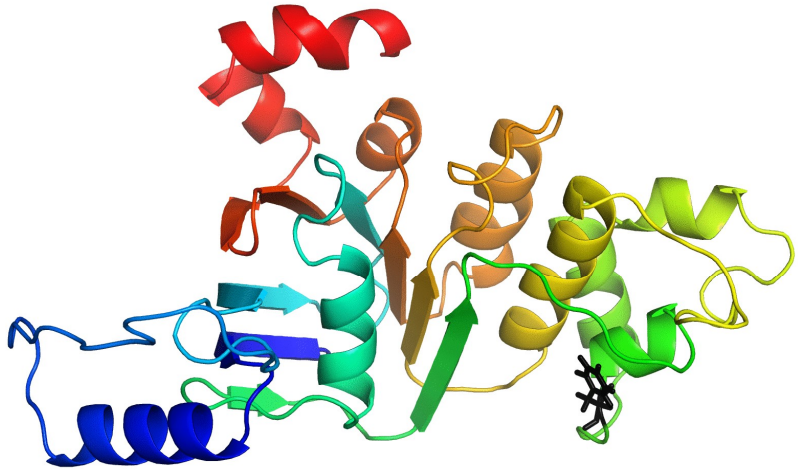
Zhang and Chen, *Cell* 2016 Dec 1;167(6):1586-1597

Liu *et al. Cell* 2017 Mar 23;169(1):85-95

# *In silico* study of NBD1 destabilization

1. Molecular dynamics (MD) simulations
   - WT and mutants
   - 100-100 ns
   - *all-atom force field*

2. Analysis of motions in NBD1
   - correlations in motions (pairwise)
   - building a graph
   - analysis of the graph



# Correlation in motions

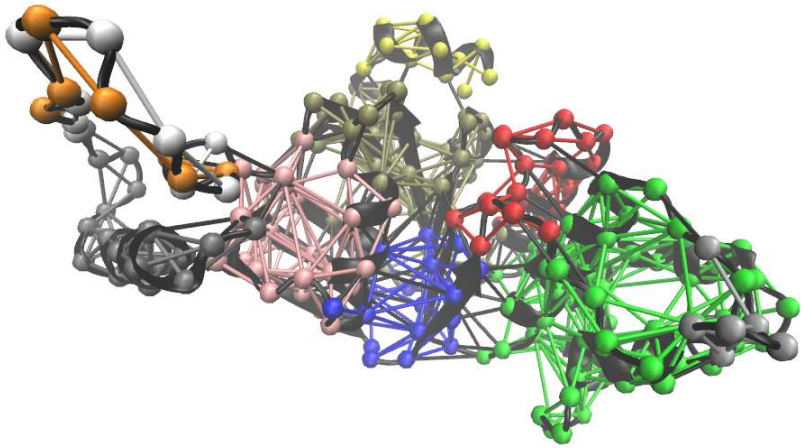
**Nodes: amino acids**

**Edges:**

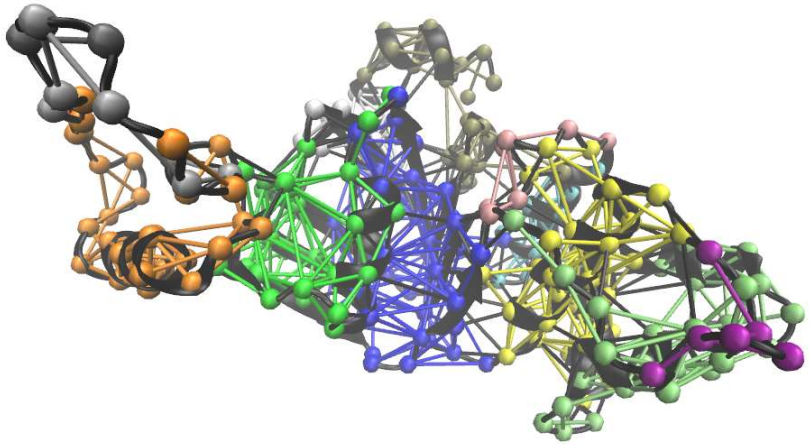
- **displacement Vector Correlation Coefficient**
- **Contacts in 75% over the simulation time**

$$\mathrm{VCC} = \frac{\langle (\mathbf{A} - \langle \mathbf{A} \rangle)(\mathbf{B} - \langle \mathbf{B} \rangle) \rangle}{\sqrt{\langle (\mathbf{A} - \langle \mathbf{A} \rangle)^2 \rangle \langle (\mathbf{B} - \langle \mathbf{B} \rangle)^2 \rangle}}$$

**Community analysis (e.g. critical nodes)**



**WT**



**ΔF508**

# Distance Correlation Coefficient

G. J. Szekely *et al.* (2007), Annals of Statistics, 35 (6): 2769–2794.

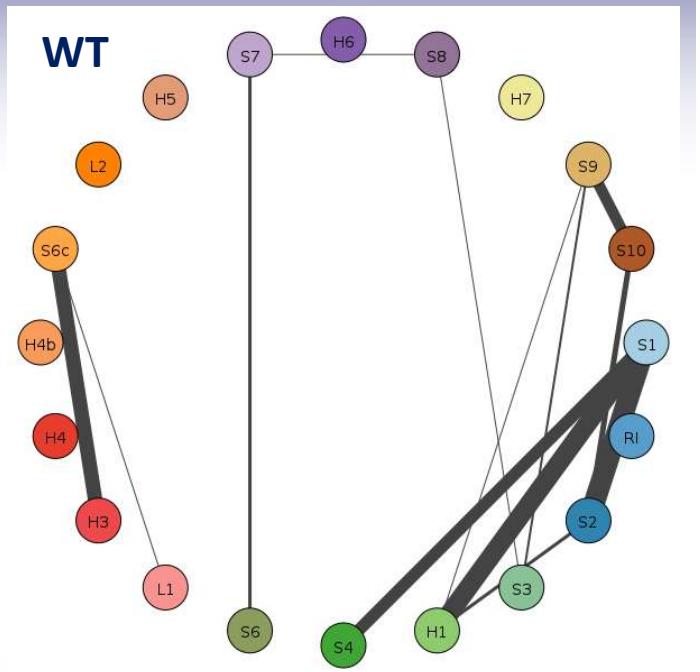
$$\mathrm{DiCC} = \frac{\nu(\mathbf{A}, \mathbf{B})}{\sqrt{\nu(\mathbf{A}, \mathbf{A})\nu(\mathbf{B}, \mathbf{B})}}$$

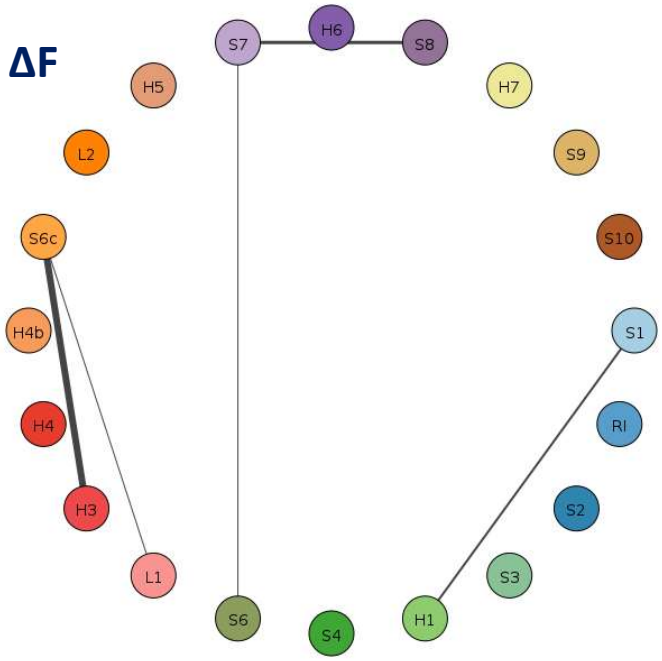
**Nodes:**

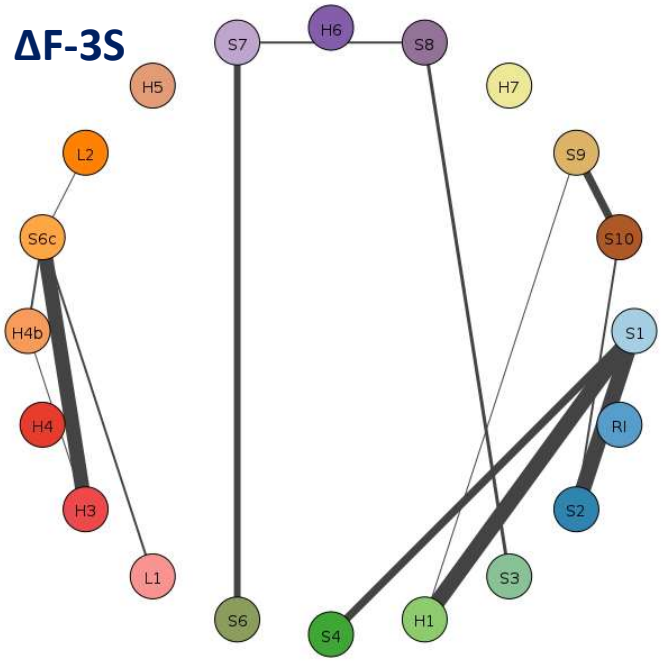
secondary structure elements

**Edges:**

if DiCC > 0.85

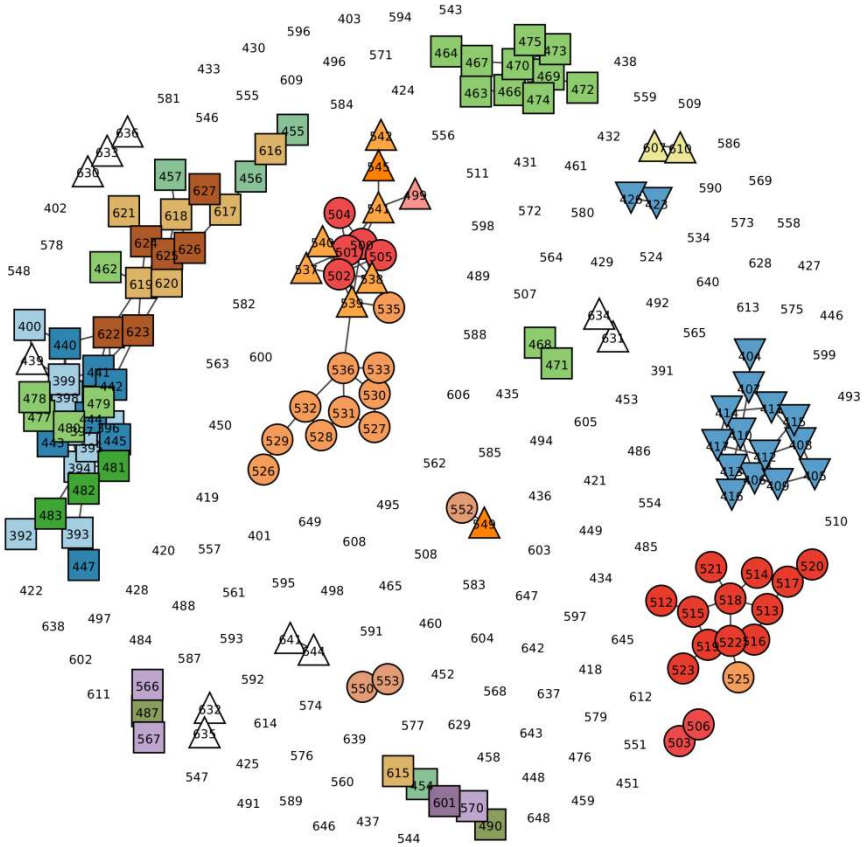


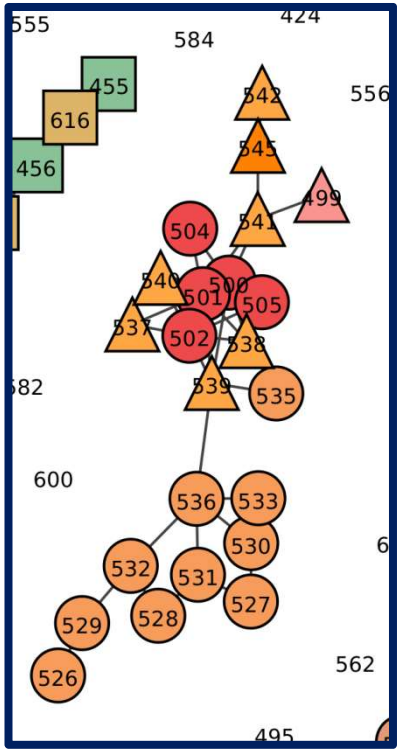



# Identification of critical amino acids

## WT





# Summary II

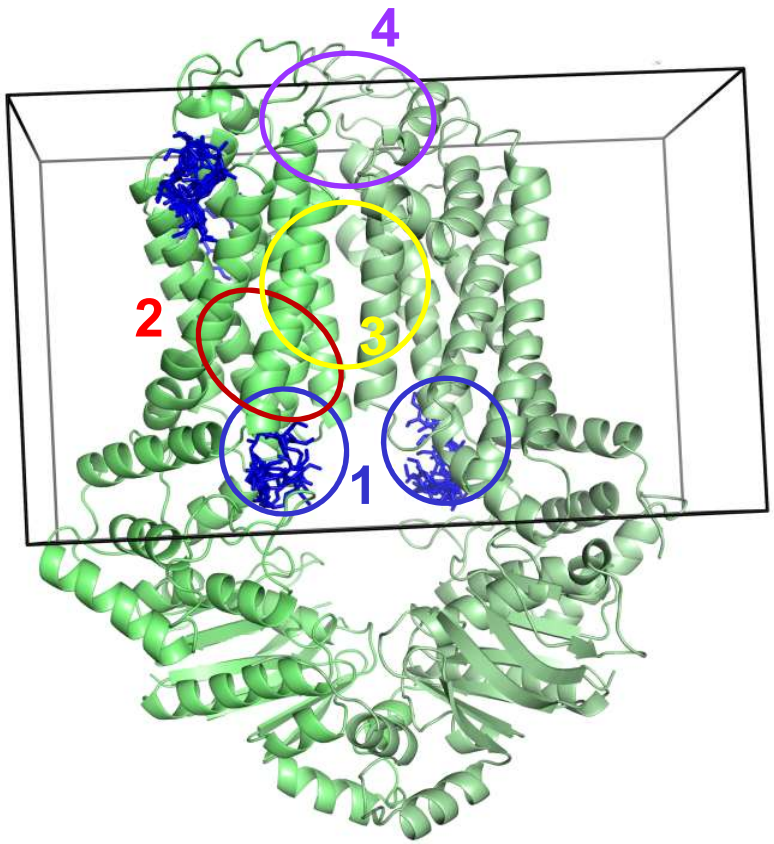
- Even relatively short MD simulations can reveal effects of mutations on structure and dynamics
- Understanding the changes contribute to better treatments

# Function

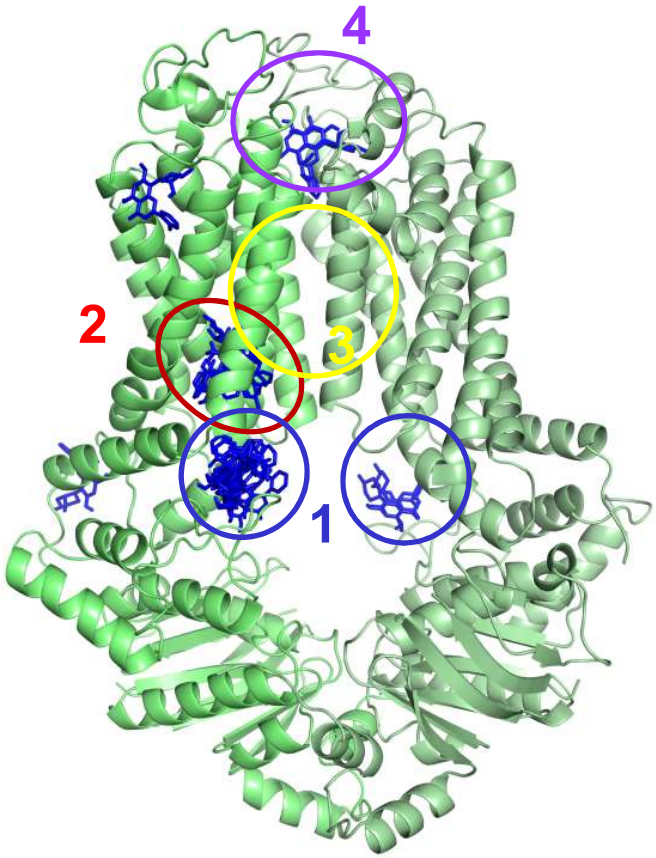
- Large conformational transitions on the ms timescale
- Small but characteristics motions from MD simulations
- Special simulation techniques

# Identification of drug binding sites using *in silico* docking

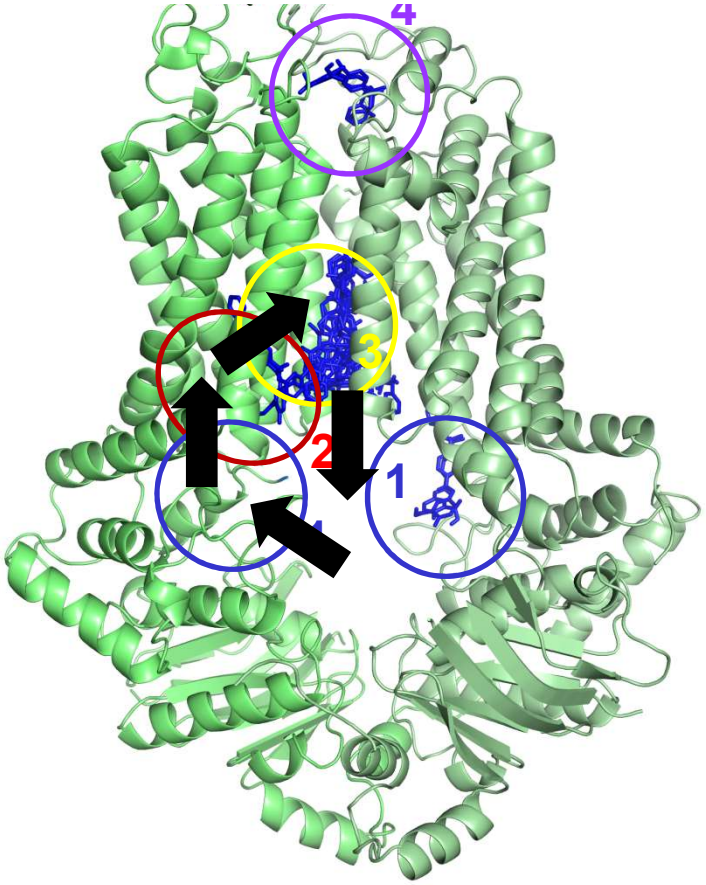
verapamil



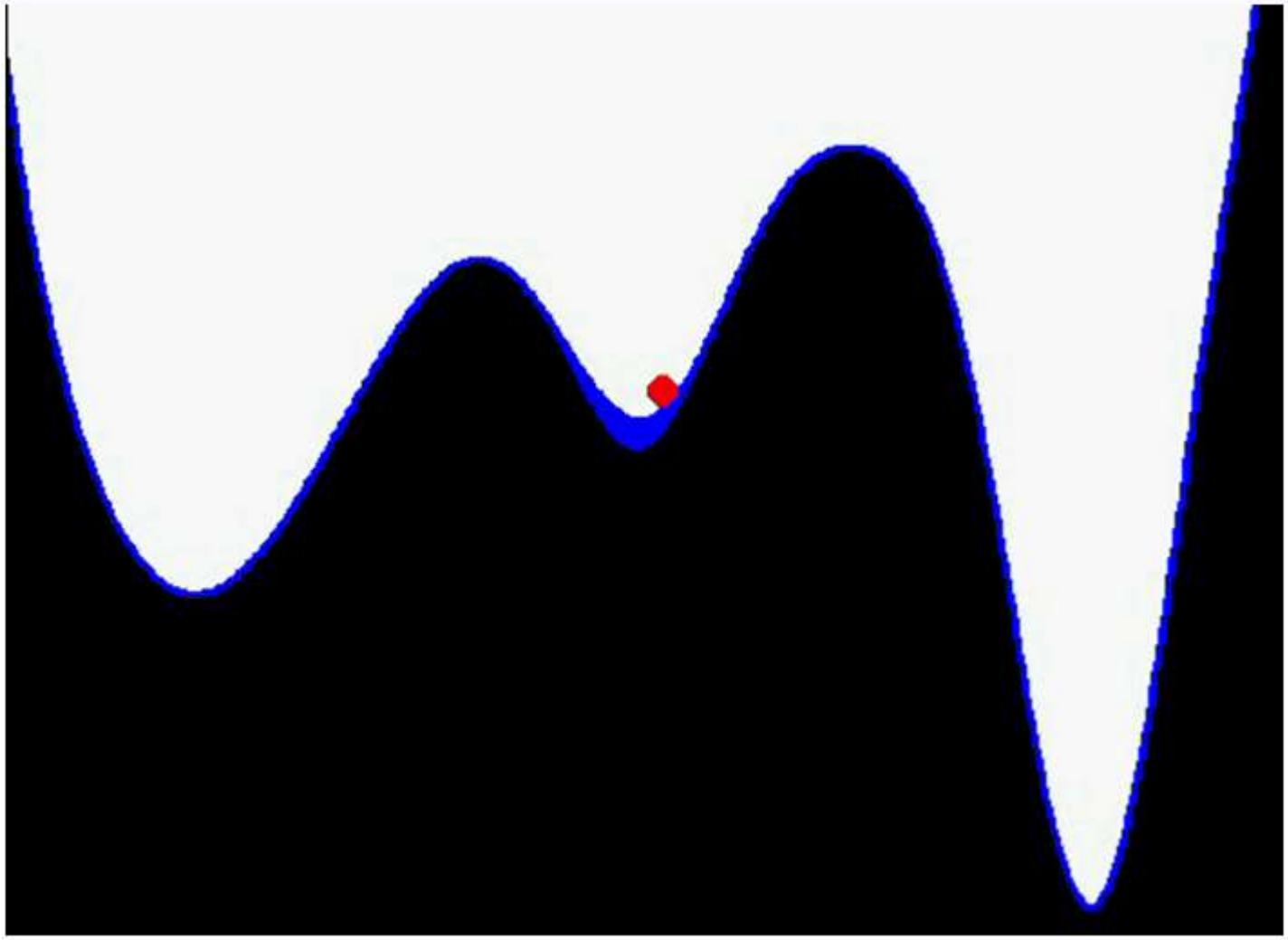

flavopiridol



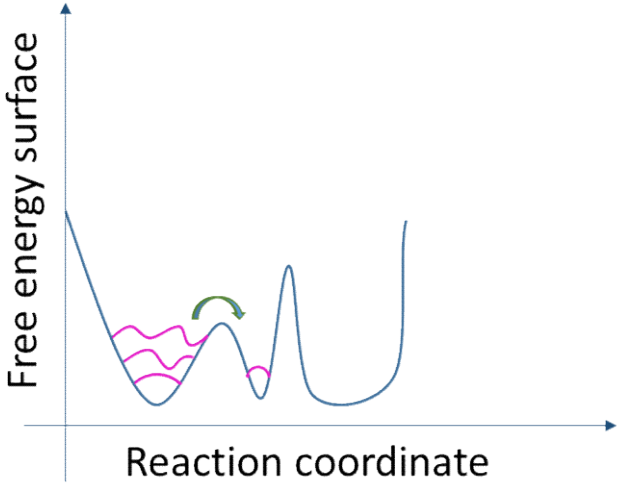
# Exploring drug binding sites by biased MD simulations

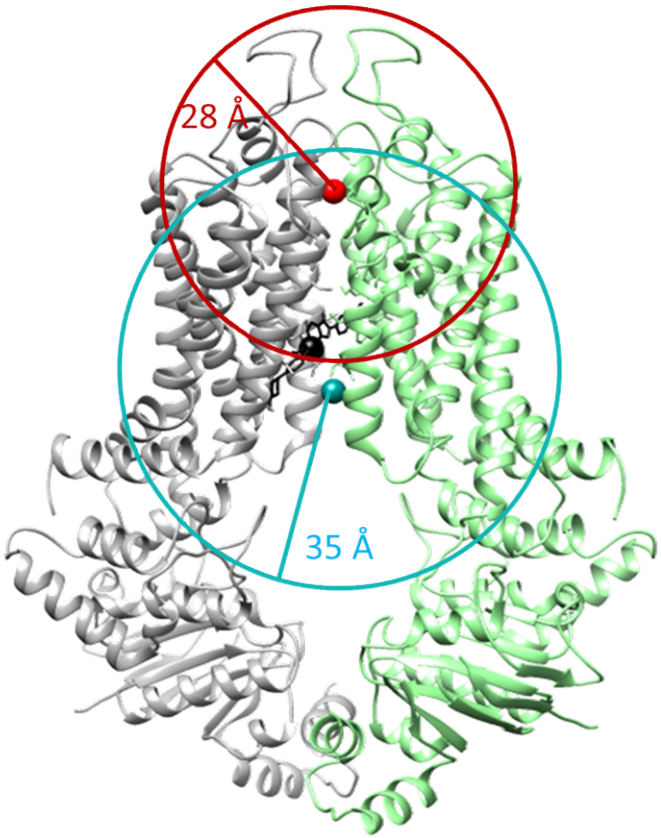


# Exploring drug binding sites using METADYNAMICS

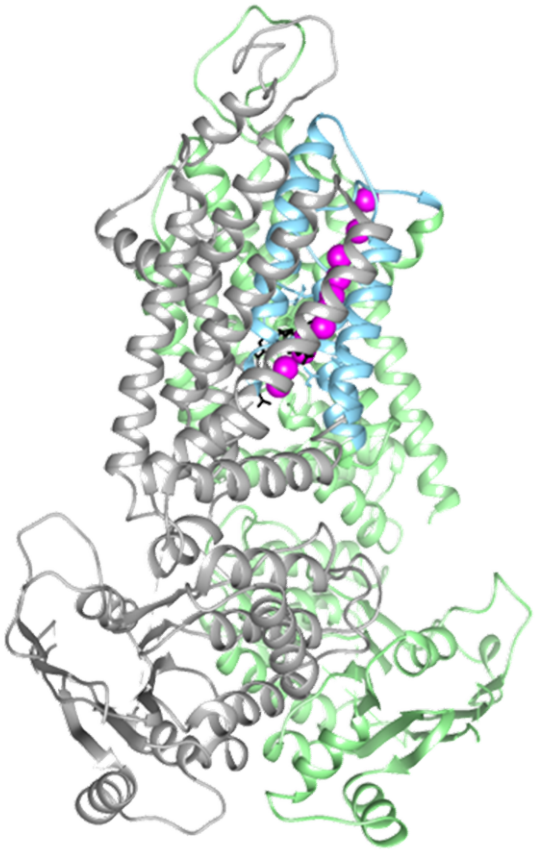


# Exploring drug binding sites using METADYNAMICS

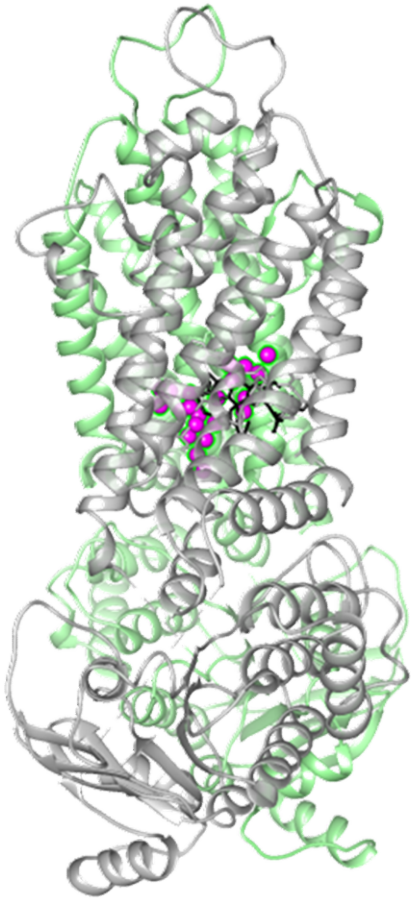


# METADYNAMICS with substrates and non-substrates

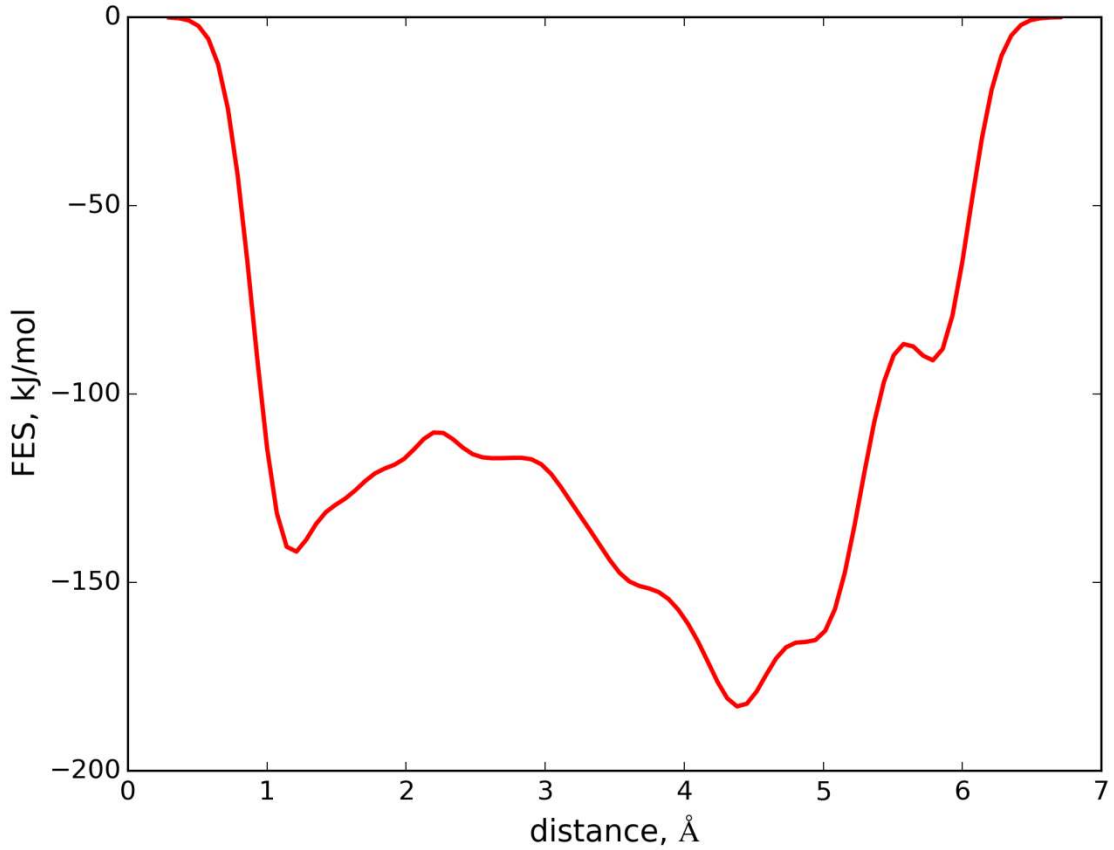


methotrexate

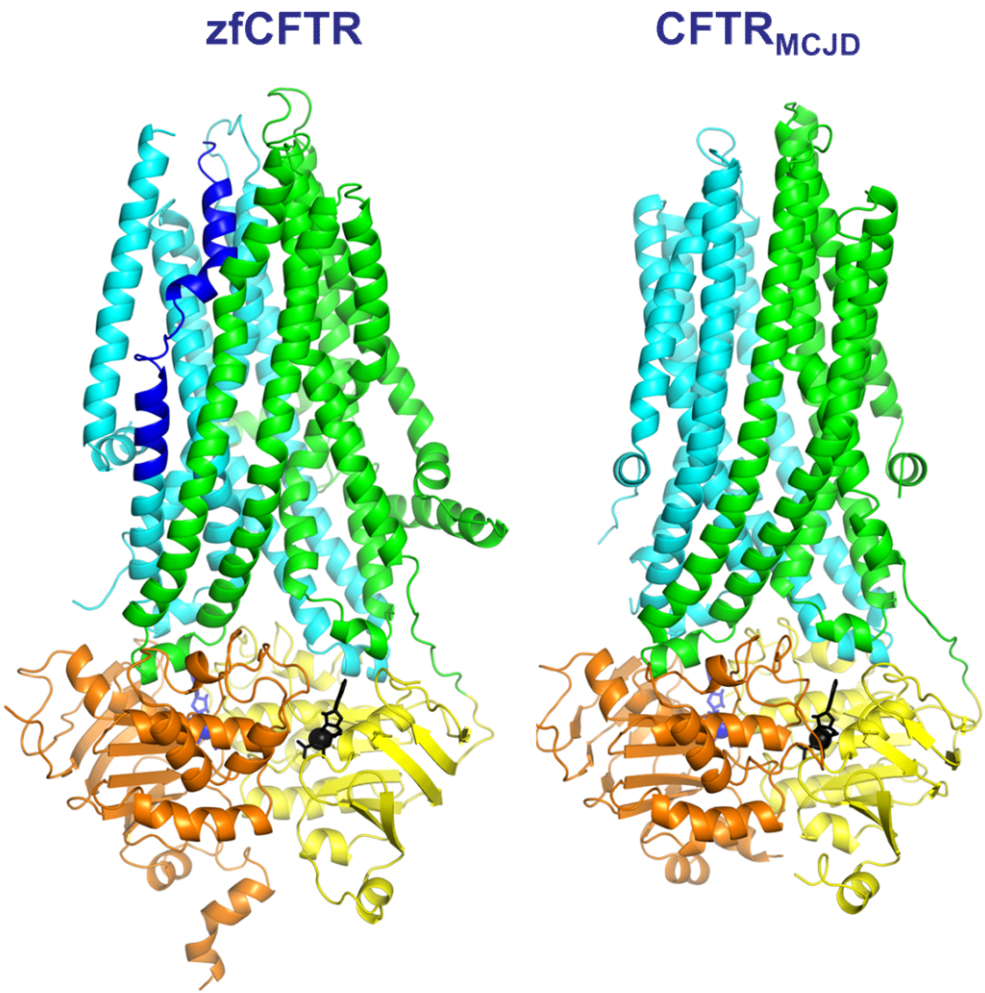


calcein

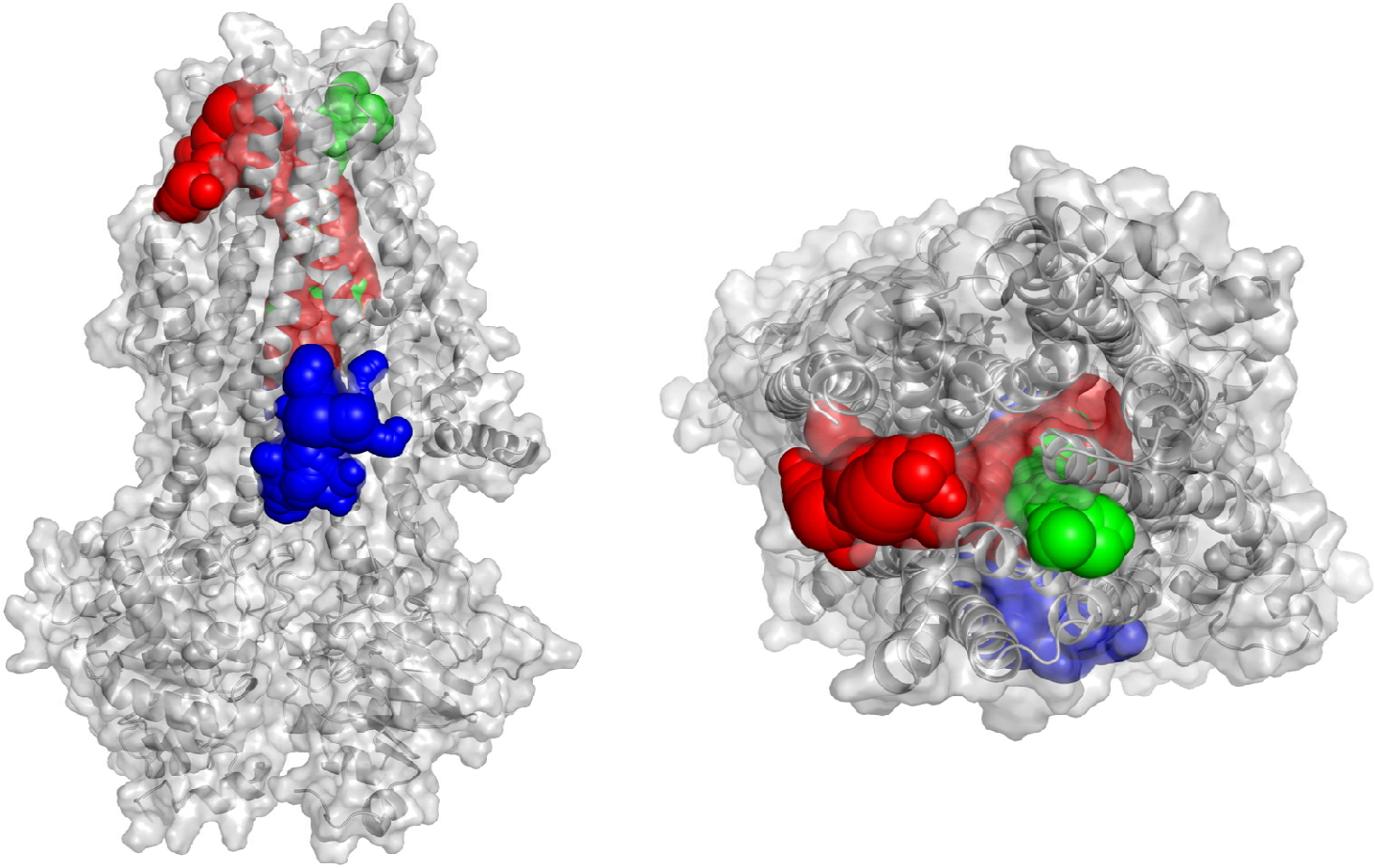
# FES - Free Energy Surface



# The gating of the CFTR chloride channel



# Where is the channel?
# Caver



# Summary III

- Tricks are needed to model events happening on longer time scales
- Well-tempered metadynamics may be sufficient to detect translocation pathways and conformational changes
- Do we believe experiments or computation?
  CFTR TH7 and TH8 are possibly mobile