

# Discovering the chloride conducting pathway of the CFTR channel using *in silico* methods

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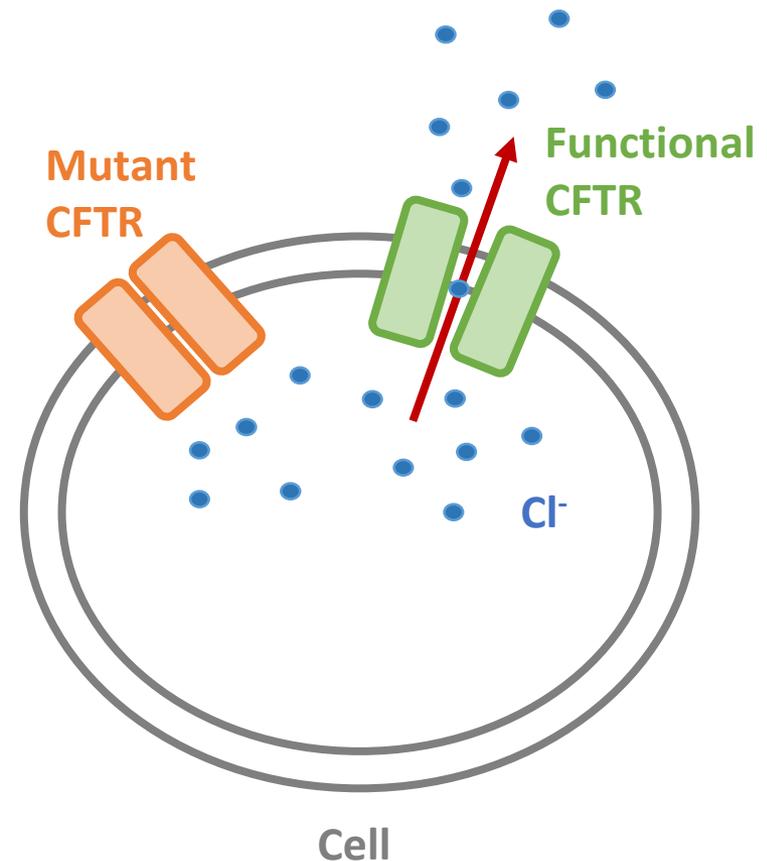
# CFTR protein and CF disease

## Cystic fibrosis transmembrane conductance regulator (CFTR/ABCC7)

- ATP-binding Cassette (ABC) protein superfamily
- Chloride channel
- Apical membrane of epithelial cells

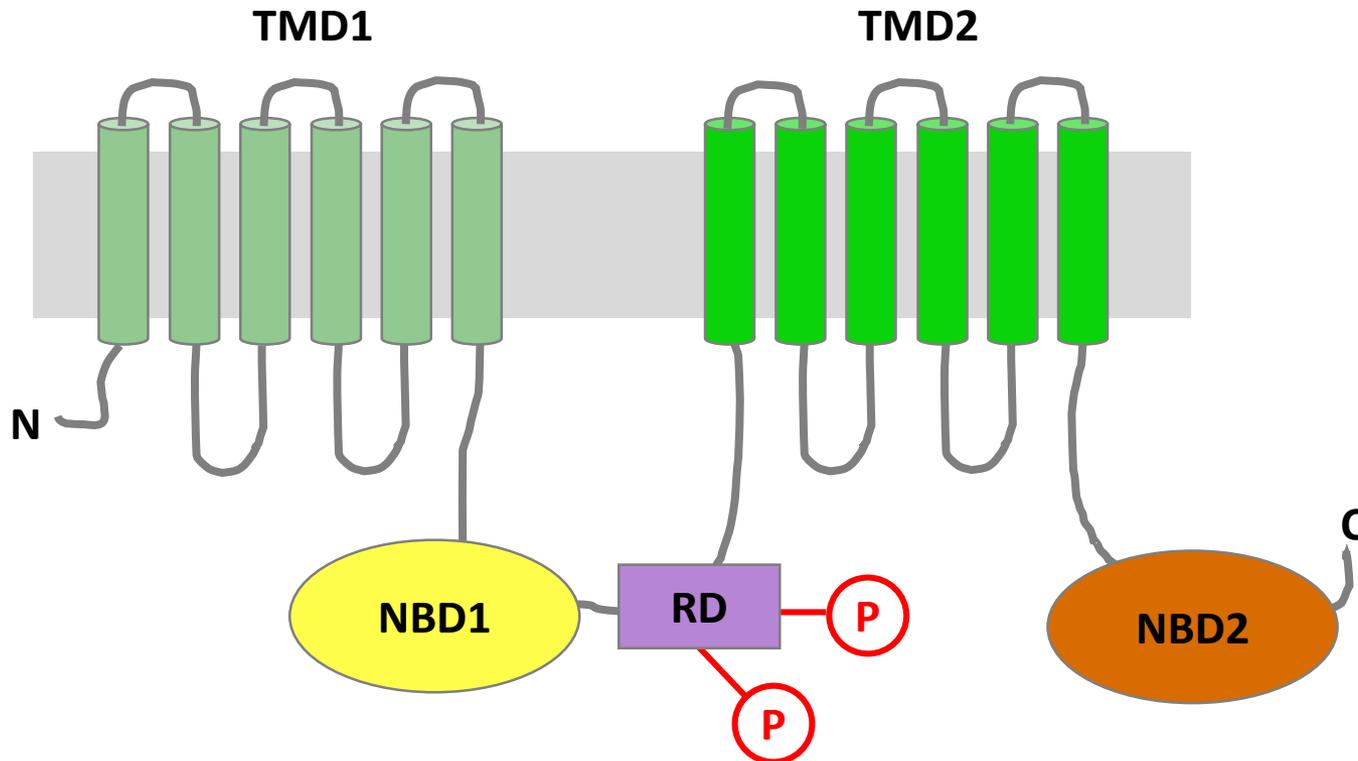
## Cystic fibrosis (CF)

- Mutations
  - > 2 000 CF-related
    - reduced expression, impaired function
- Drug molecules
  - High-resolution structure
  - Dynamics



# CFTR topology

- Transmembrane domains (TMD1-2)
- Nucleotide binding domains (NBD1-2)
- Disordered regulatory domain (RD) : Phosphorylation is essential



# CFTR structure determination

## X-ray crystallography

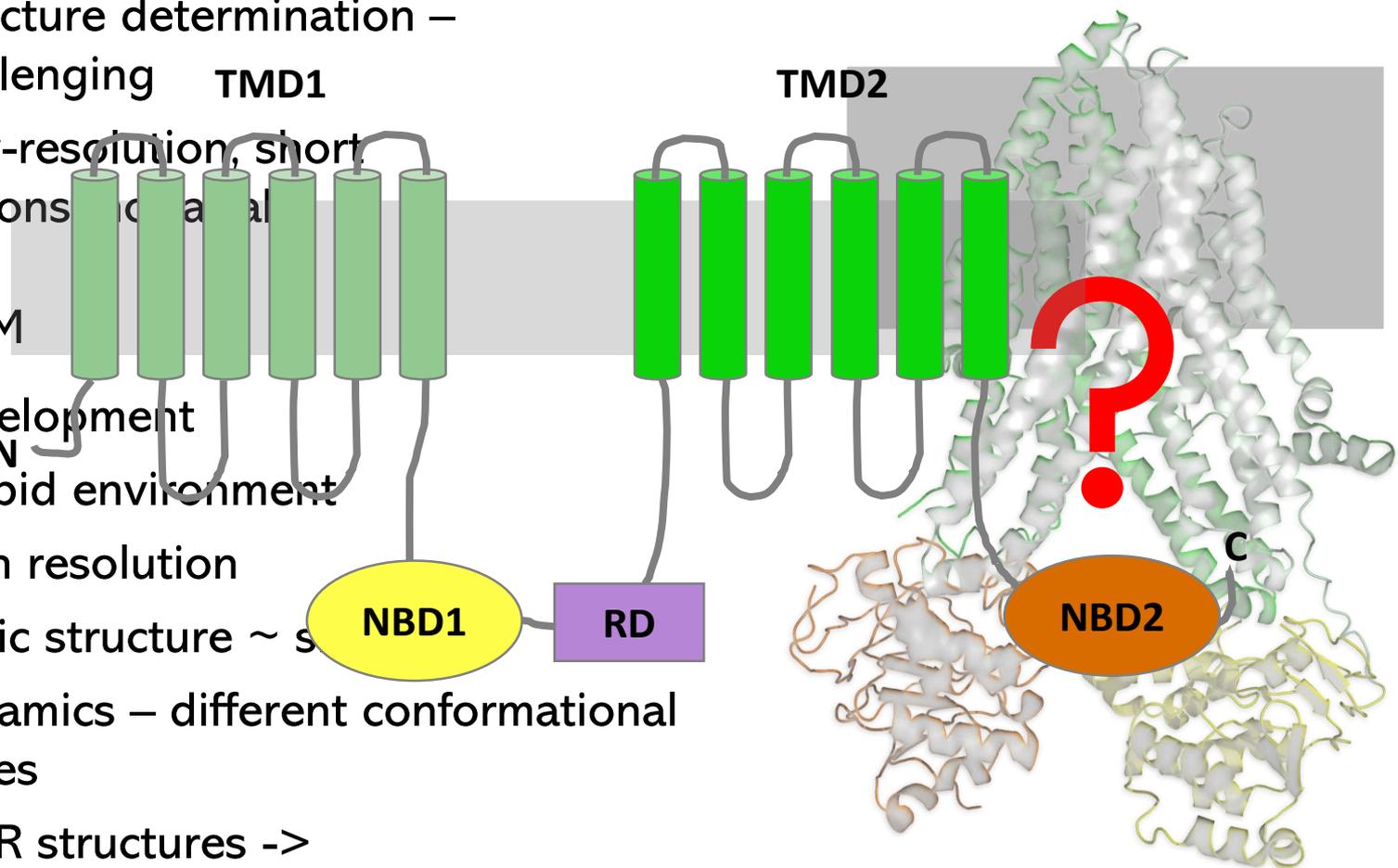
- Structure determination – challenging

- Low-resolution, short regions

## Cryo-EM

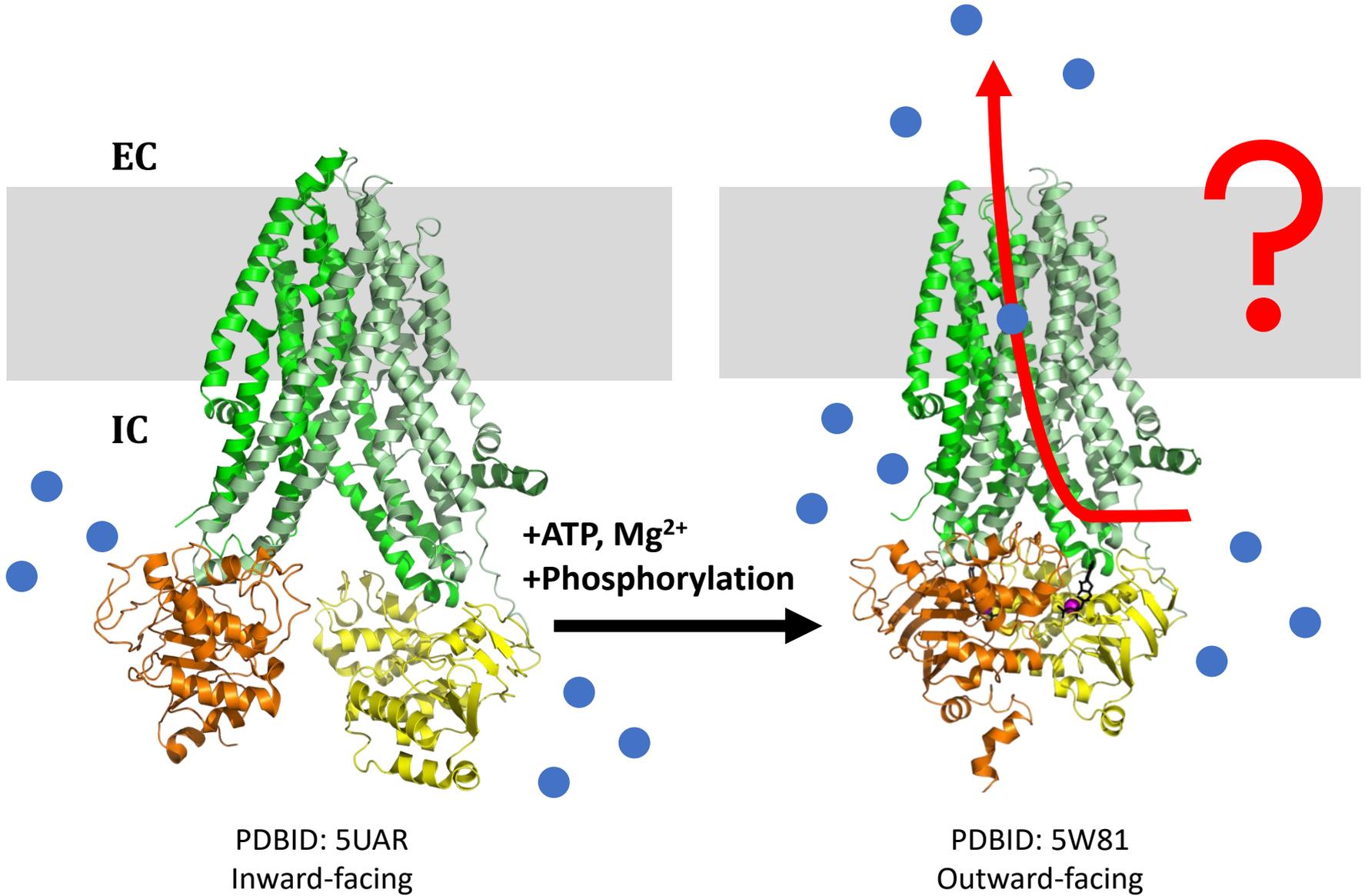
- Development
- In lipid environment
- High resolution
- Static structure ~ s
- Dynamics – different conformational states
- CFTR structures ->

## 3D structure



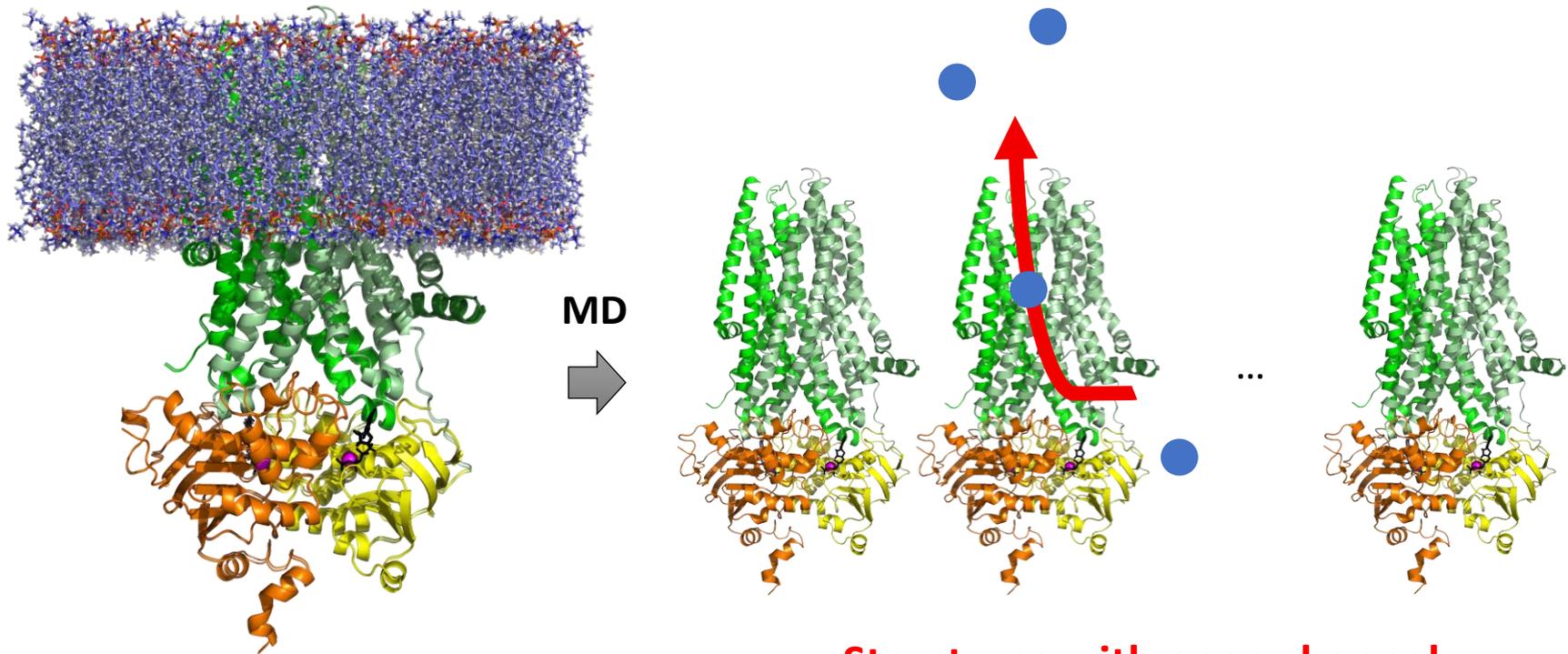
PDBID: 5UAR  
Inward-facing

# CFTR structure and function



# MD simulations to “open” the CFTR channel

22 equilibrium Molecular Dynamics (MD) simulations  
(16x35ns, 6x100ns)



**Structures with open channel**

Initial structure, PDBID: 5W81

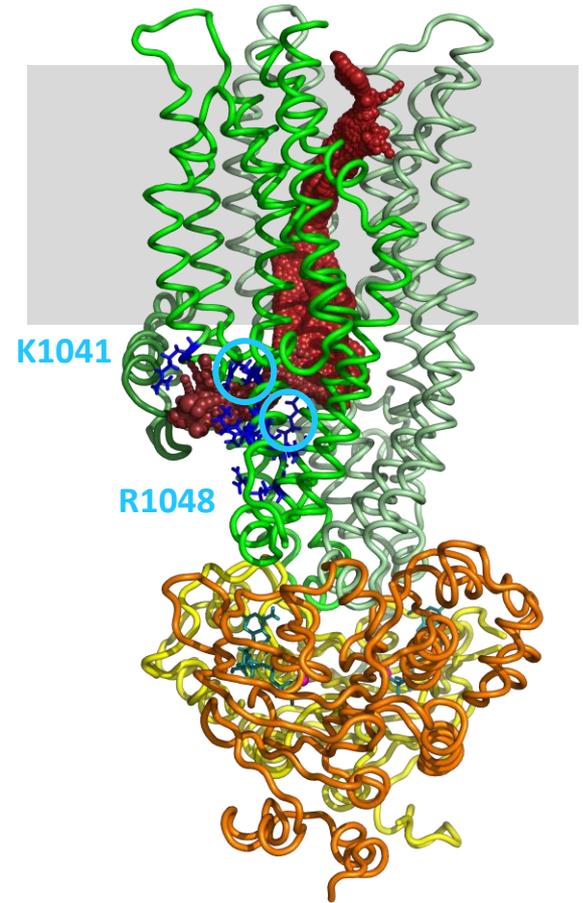
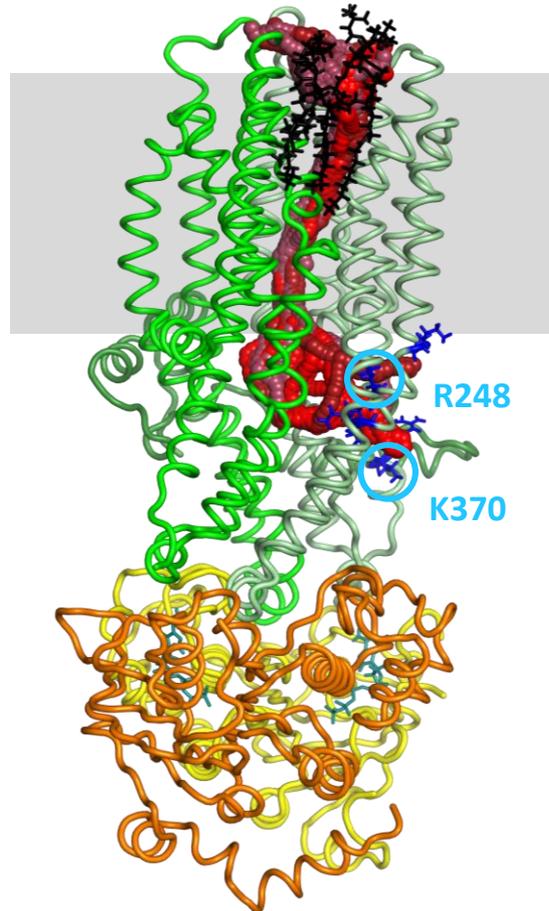
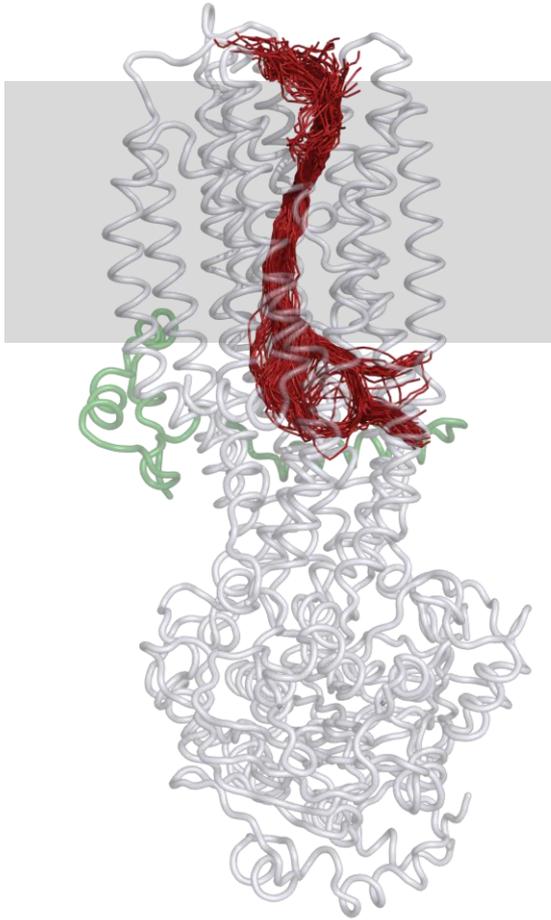
- +ATP, +Phosphorylation

# Identification of open channels sufficient for Cl<sup>-</sup>

■ Channel ( $r \geq 1.8 \text{ \AA}$ )

■ Positively charged residues

■ Lipid molecules

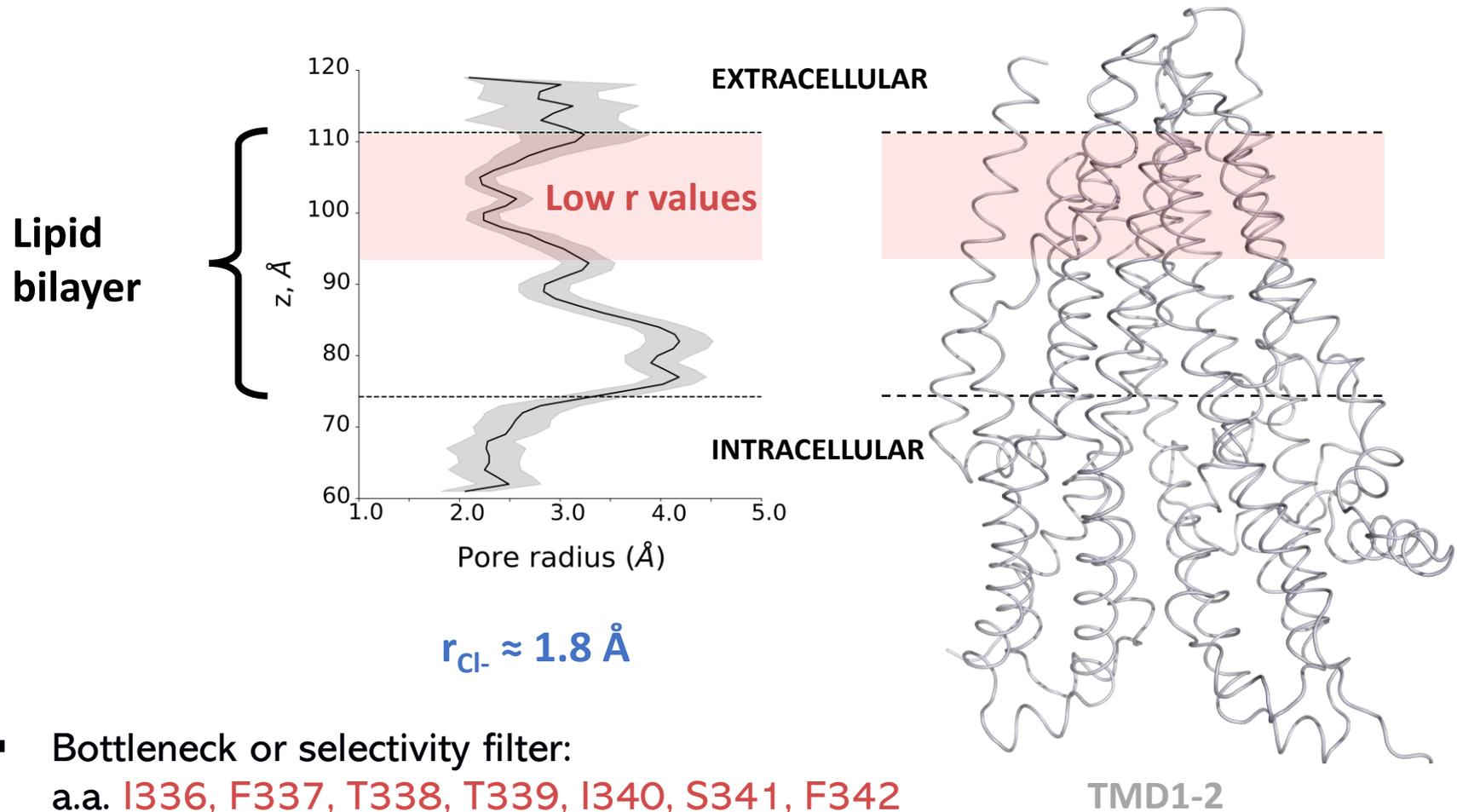


**Primary pathway entry: TM4-6**

**Secondary pathway entry: TM10-12**

# Characterization of the channel profile

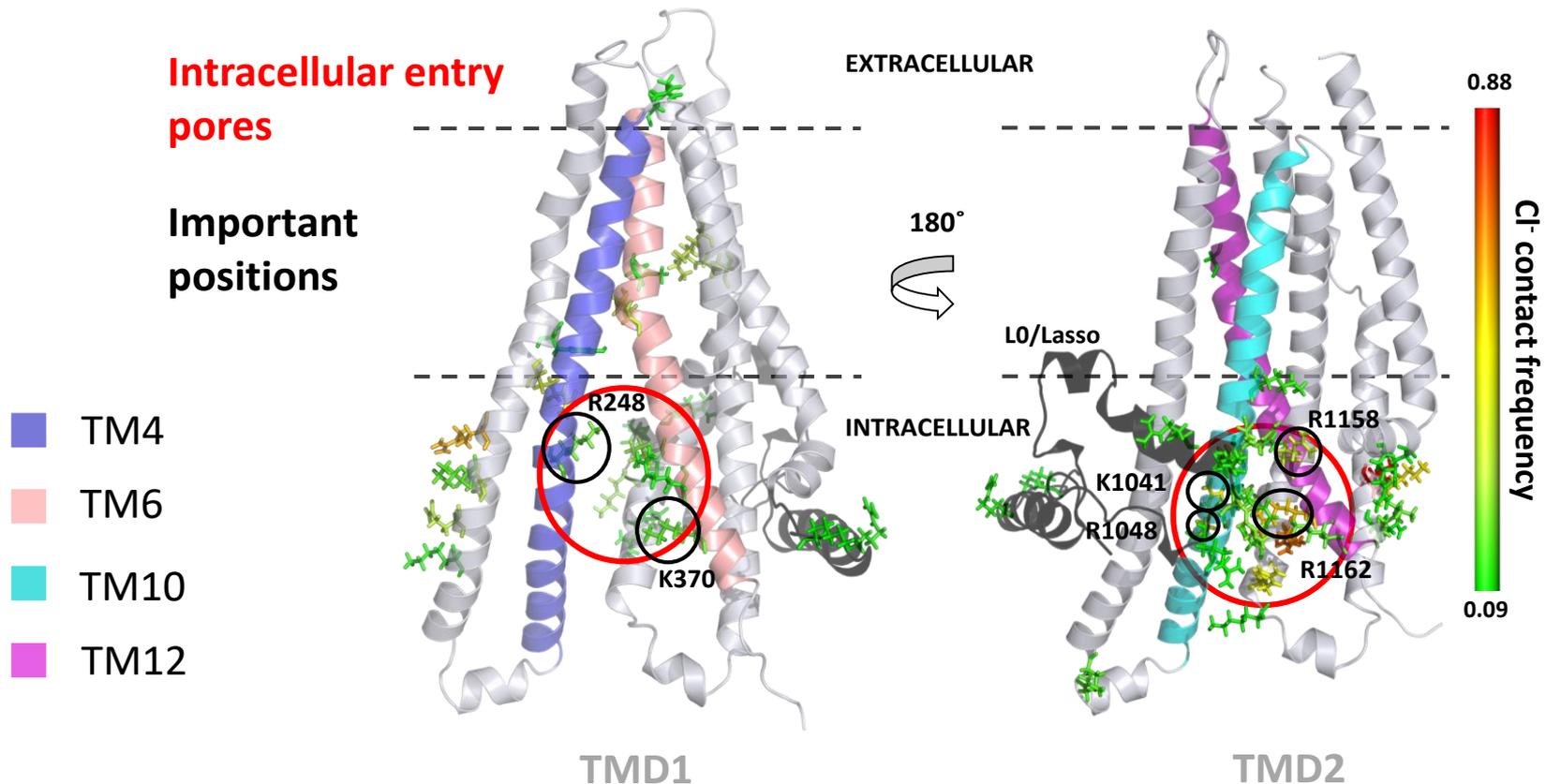
Structural characteristics, presence of bottleneck regions



# Cl<sup>-</sup> interaction sites

Chlorides in the system - Information on important chloride interaction sites

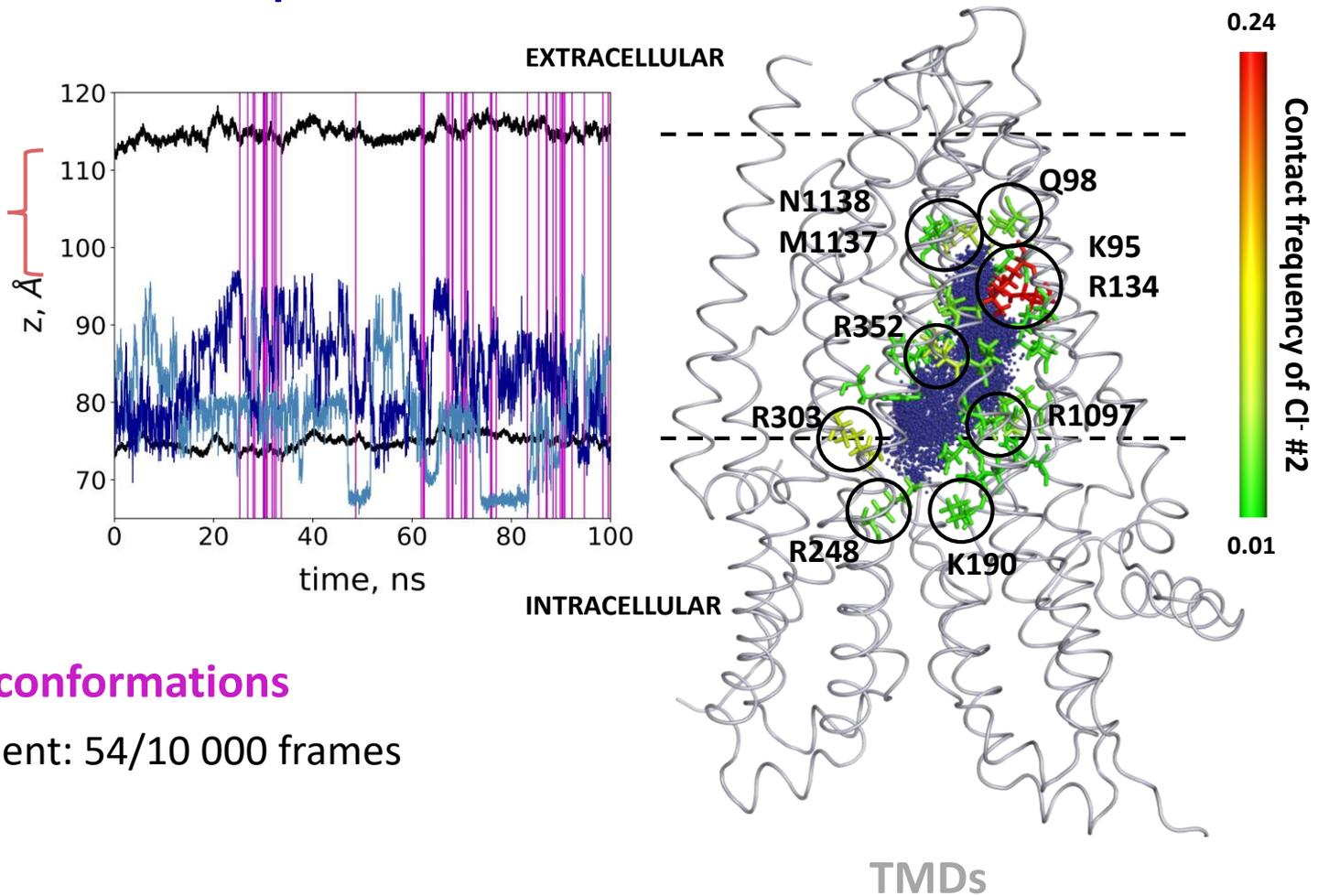
- Potential intracellular entry sites
  - Contact map
  - Intensive interactions in TMD2
- Interaction sites - *in silico* entry sites



# Cl<sup>-</sup> ions entered the channel without transition

## Cl<sup>-</sup> positions

Bottleneck



Open conformations

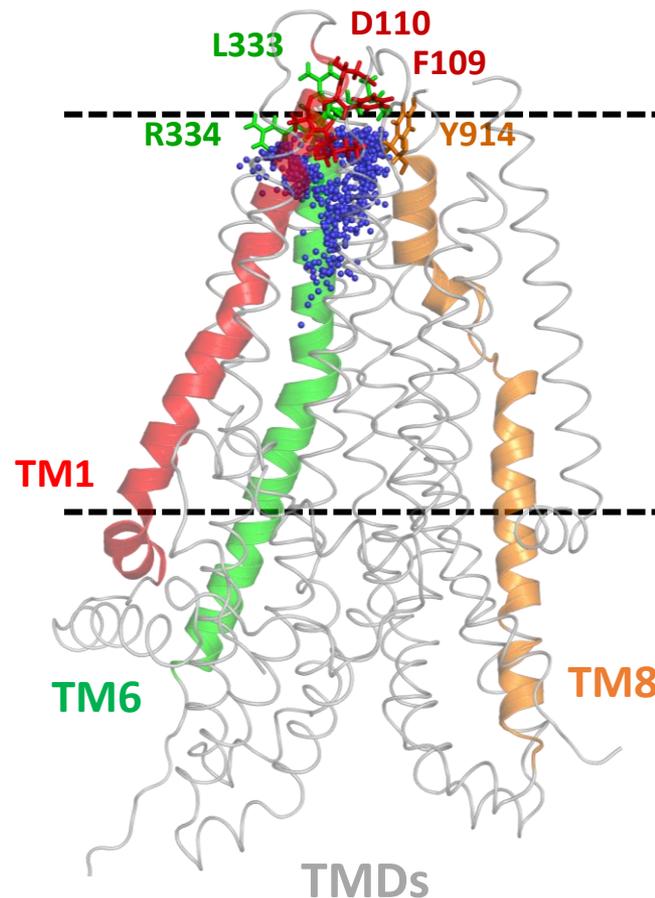
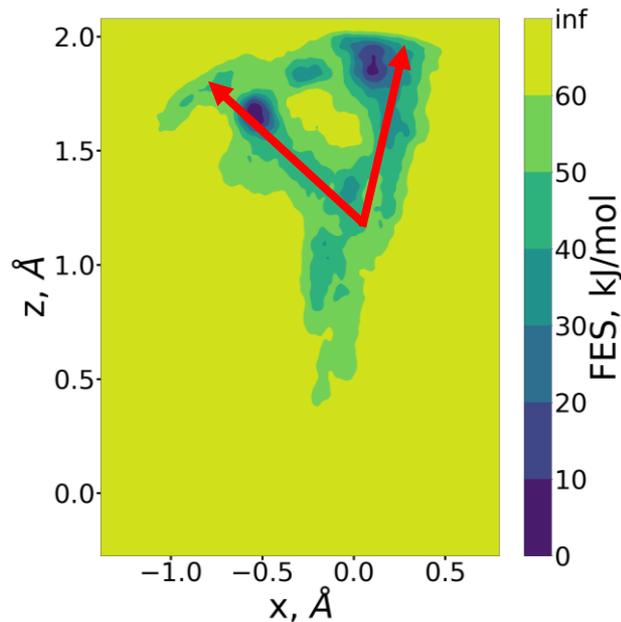
Infrequent: 54/10 000 frames

# Metadynamics to describe the energetics of the bottleneck region

Potential exit routes, a.a. narrowing the pathway

- Metadynamics: exit from local minima by increasing potential

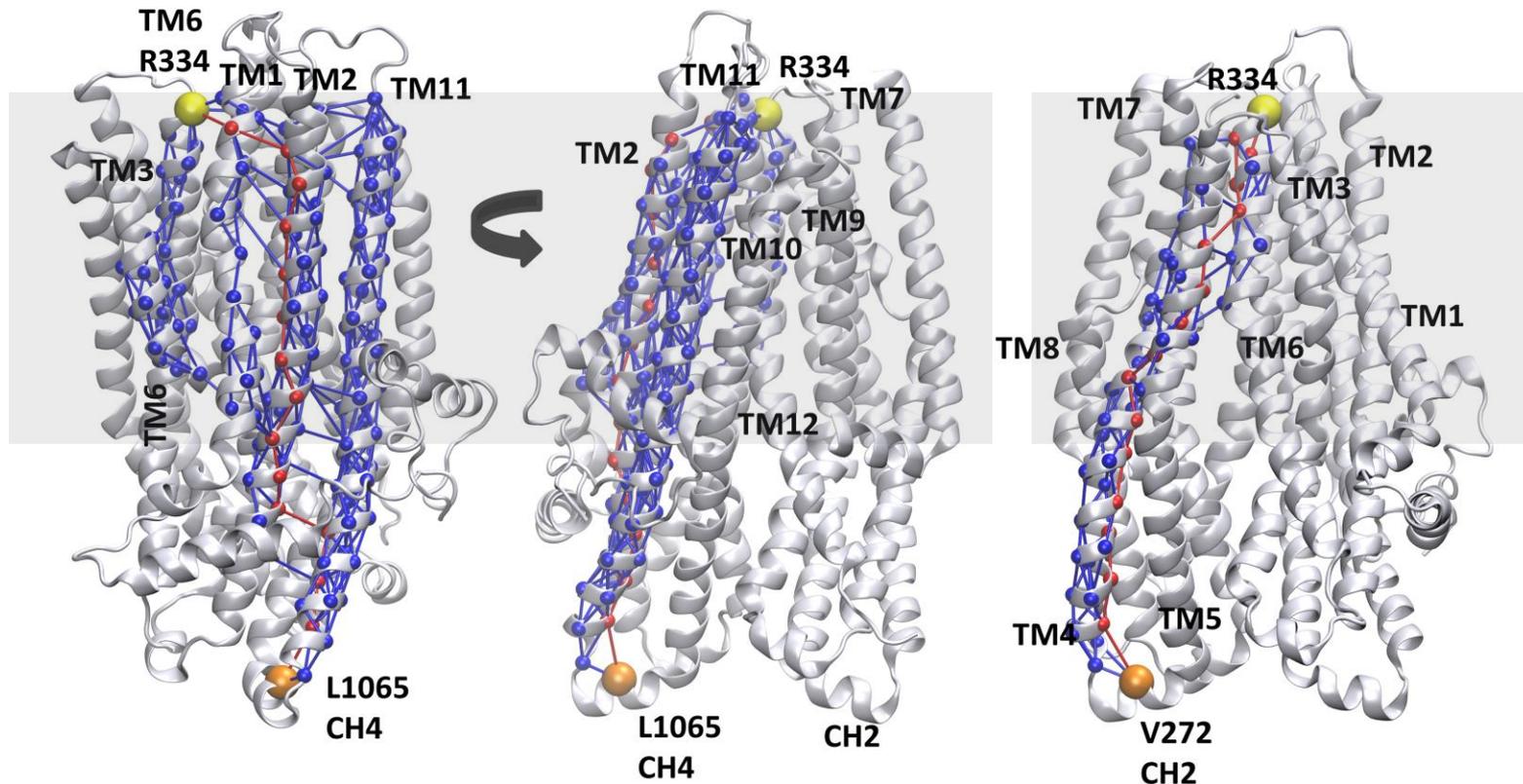
2D free energy surface along x/z



# Asymmetric allosteric coupling

Allosteric communication between the NBDs and the EC end of TMDs: network analysis based on correlations in pairwise residue motions

Sink: EC end of TMDs ( close to the bottleneck region )



Source: IC end of TMDs

# Conclusions

- **Description of the chloride channel at atomic level**
  - Characterization of channel-forming residues
  - There are two alternative exit routes at the EC side of the pathway
- **Important Cl<sup>-</sup> interaction sites**
  - Along the channel, entry sites
- **Lipid molecules participate in the channel formation**
  - Influencing the channel function
- **CH<sub>4</sub> dynamics is strongly coupled to the bottleneck region**
  - May facilitating the opening
- **Our *in silico* results are in good agreement with experimental data**

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