

# Elméleti módszerek lipidek és membránfehérjék tanulmányozására

Hegedűs Tamás

[tamas@hegelab.org](mailto:tamas@hegelab.org)

MTA-SE Molekuláris Biofizikai Kutatócsoport  
SE Biofizikai és Sugárbiológiai Intézet



# Fehérjék szerkezetének és dinamikájának jelentősége

**A betegség molekuláris szintű oka?  
A gyógyszer-kötő zseb alakja?**

**37°C-on, oldatban nem egy szerkezet létezik,  
hanem egy konformációs sokaság.**

# Számítógépes modellezés jelentősége

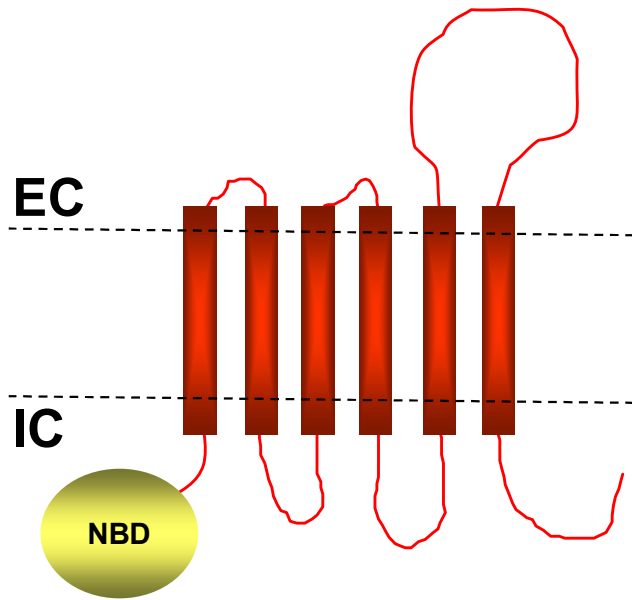
**Atomi szintű információt ad mozgásokról.**

**Kísérletes módszerek  
általában nem szolgáltatnak  
közvetlen információt az atomi szintű történésekről.  
(pl. EPR, X-links: nem, NMR: igen, de korlátoltan)**

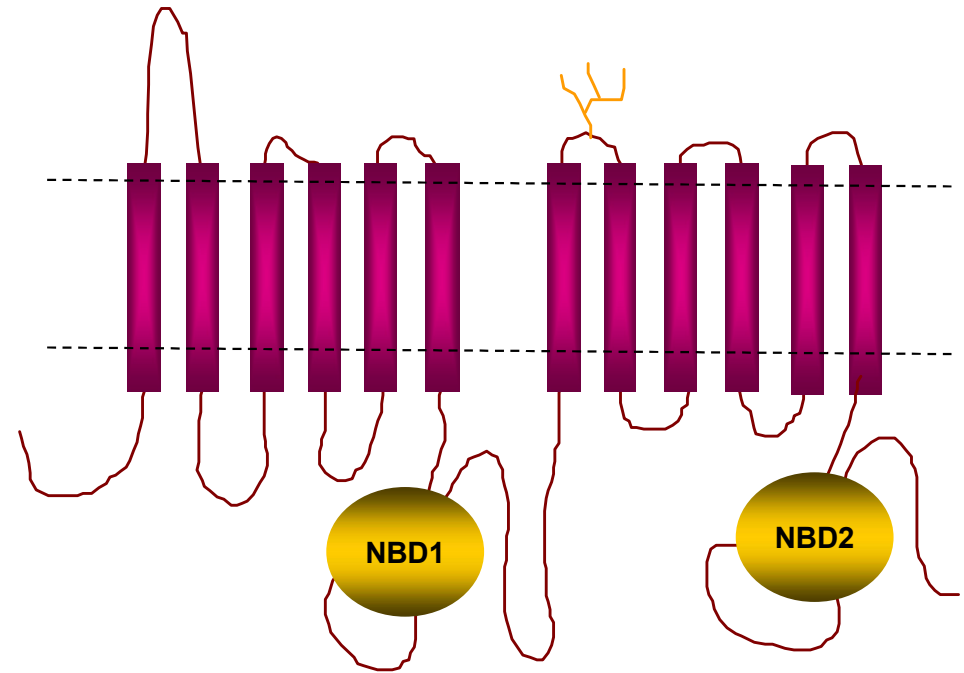
# Membránfehérjék szerkezeti modellezése

- Kísérleti adatok alapján
- Elméleti módszerekkel
  - homológia modellezés
  - géptanulási módszerek

# ATP Binding Cassette (ABC) fehérjék



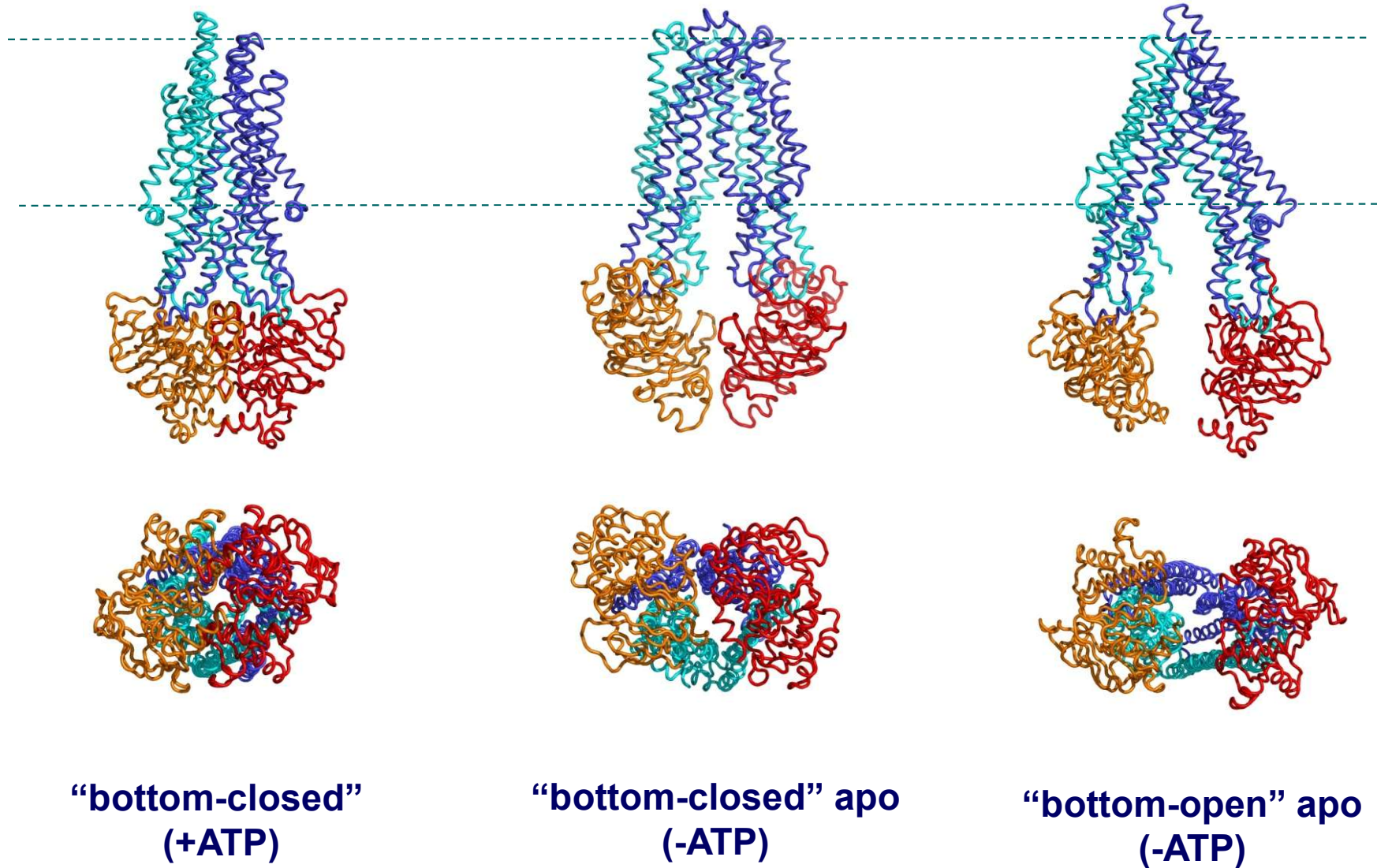
**Fél-transzporterek**  
pl. humán ABCG fehérjék



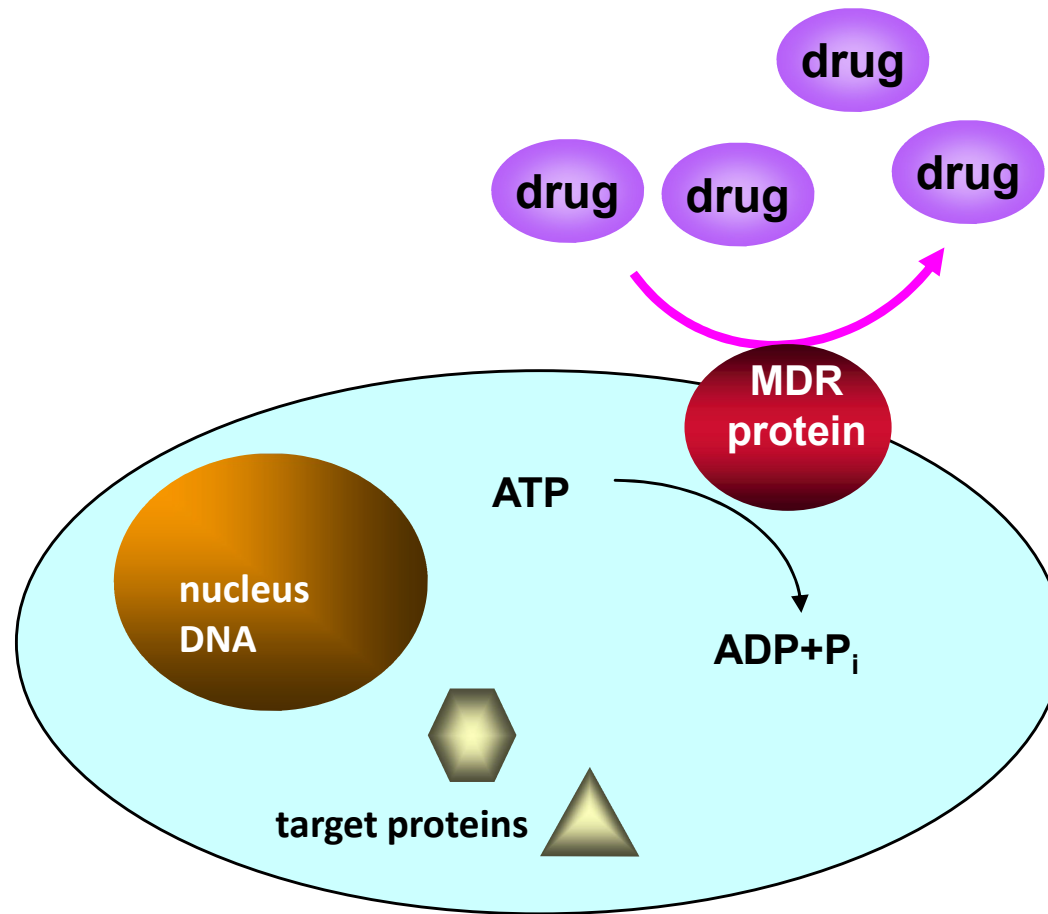
**full transporters**  
pl. P-glycoprotein, CFTR

# ABC fehérjék konformációi

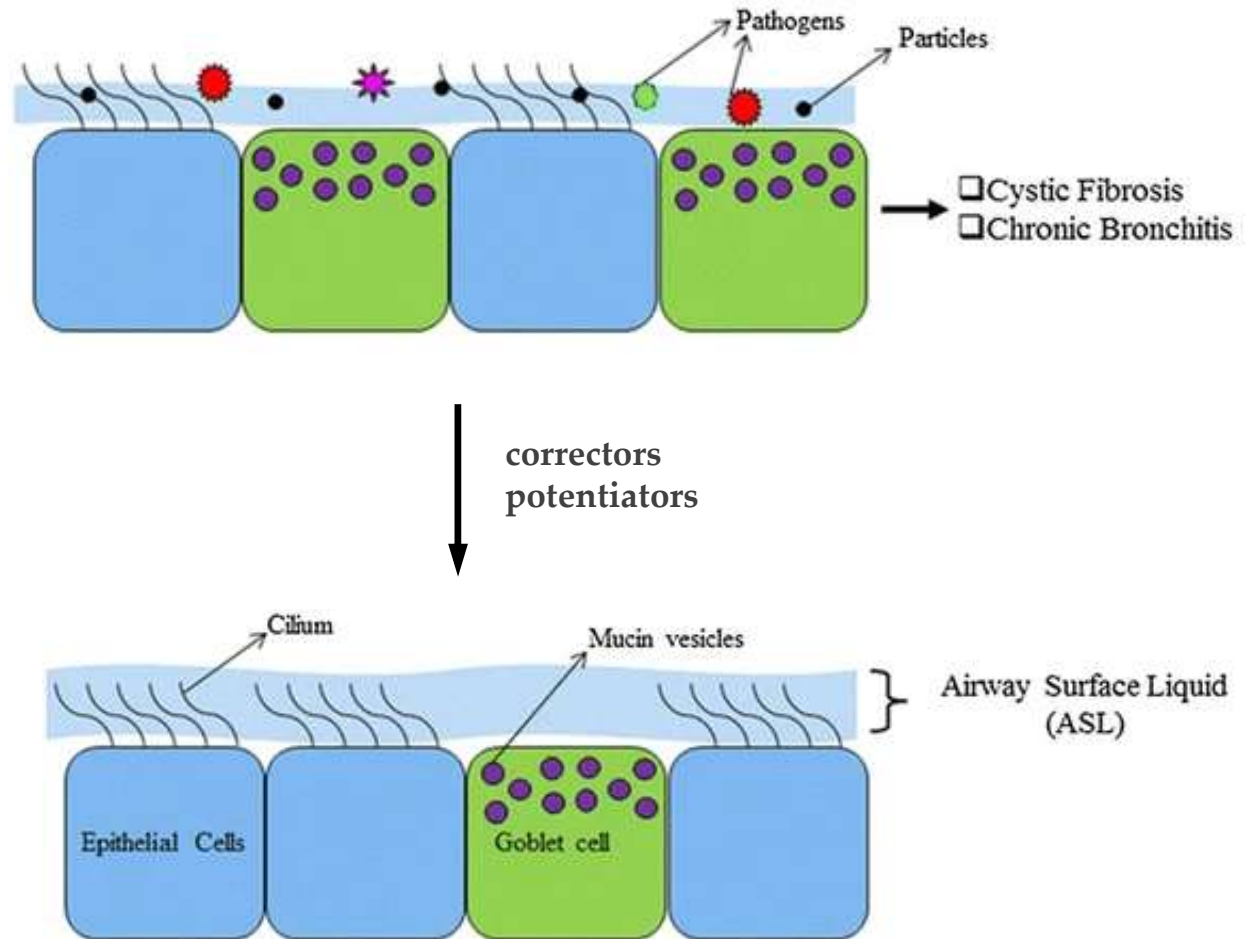
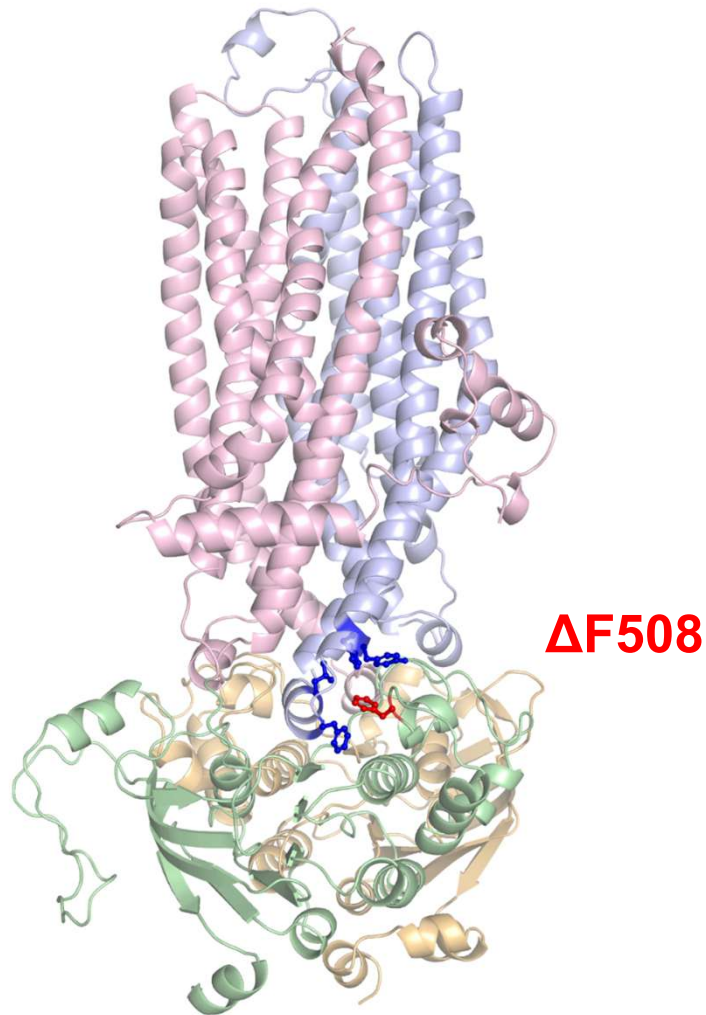
(Pgp-szerű)



# Multidrog transzport – ABC fehérjék



# Cisztás fibrózis (CF)

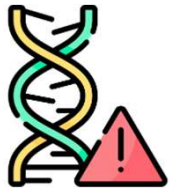


Ghosh, Boucher, Tarran,  
CMLS 2015

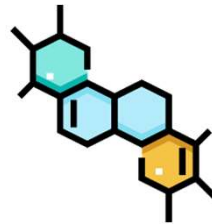
Serohijos, Hegedus, ... Dokholyan, Riordan, PNAS 2008

Soya ... Hegedus, Lukacs, Nat. Comm. 2023

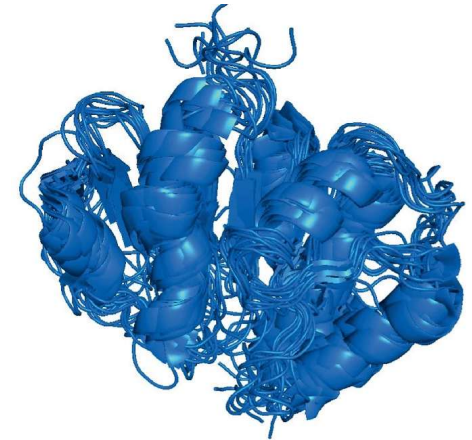
# Célkitűzéseink



**Atomic-level basis of  
diseases**

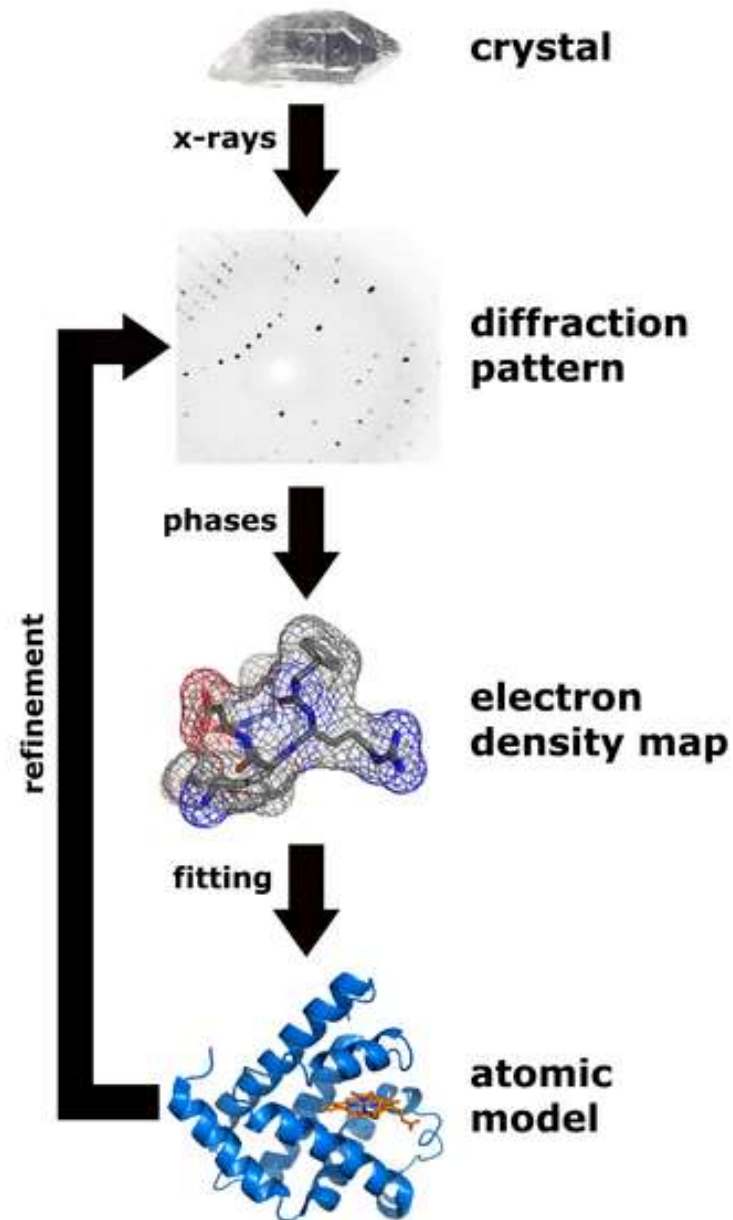


**Identification  
of drug binding sites**



**Not a single structure but  
a conformational  
ensemble**

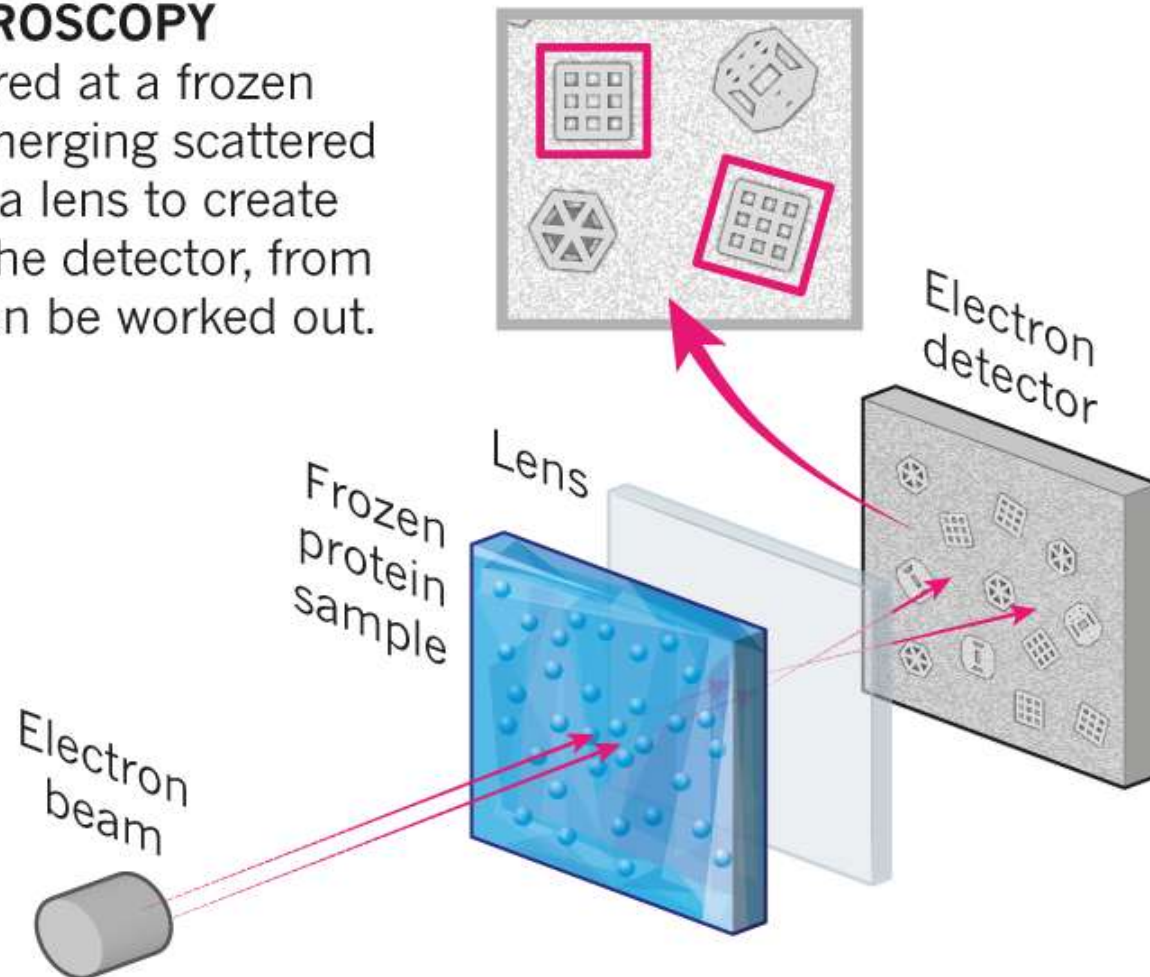
# Szerkezet meghatározás – Röntgen kristallográfia



# Szerkezet meghatározás – krio-EM

## 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.



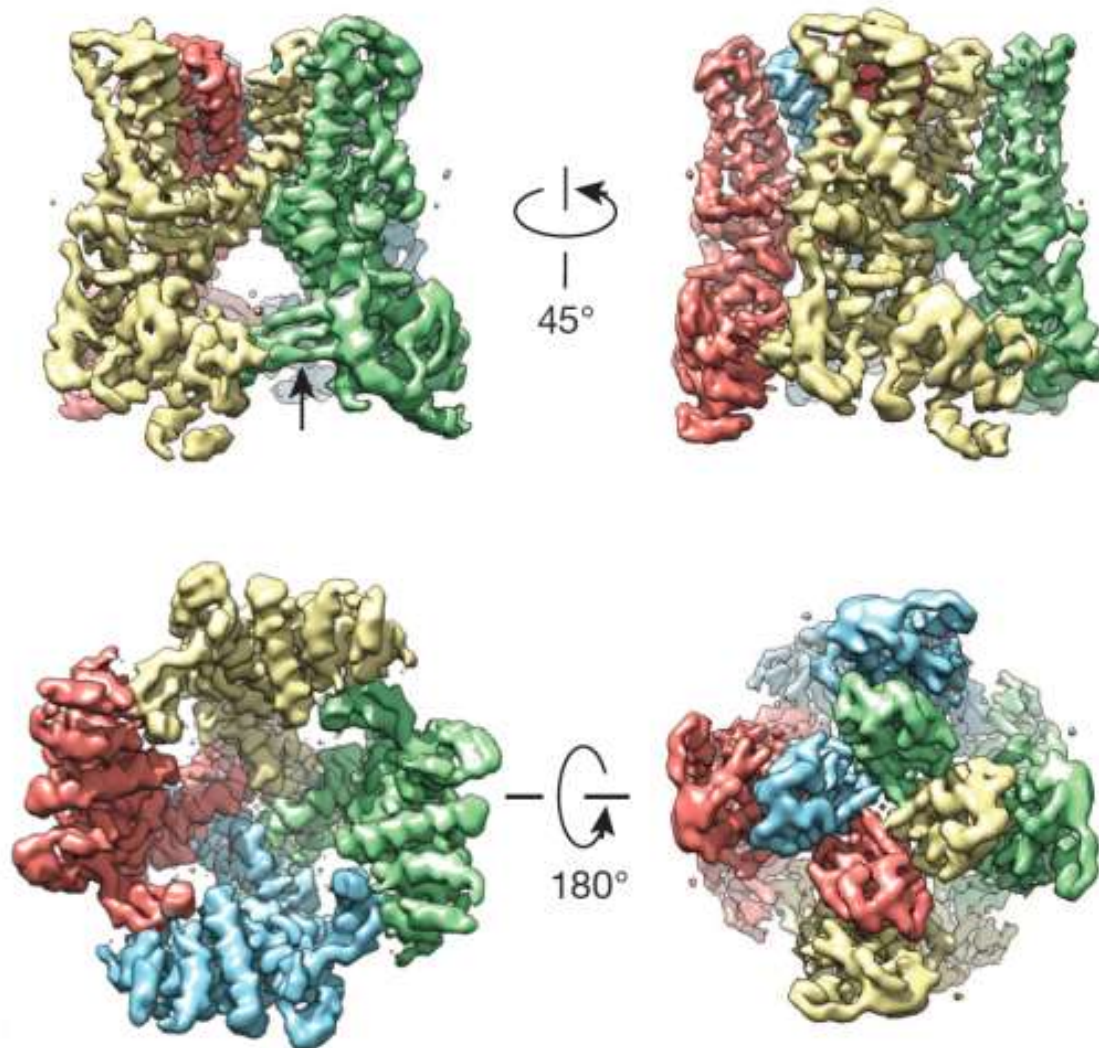
© nature

Ewen Callaway, Nature | News Feature

The revolution will not be crystallized: a new method sweeps through structural biology, 09 September 2015

# Szerkezet meghatározás – „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.



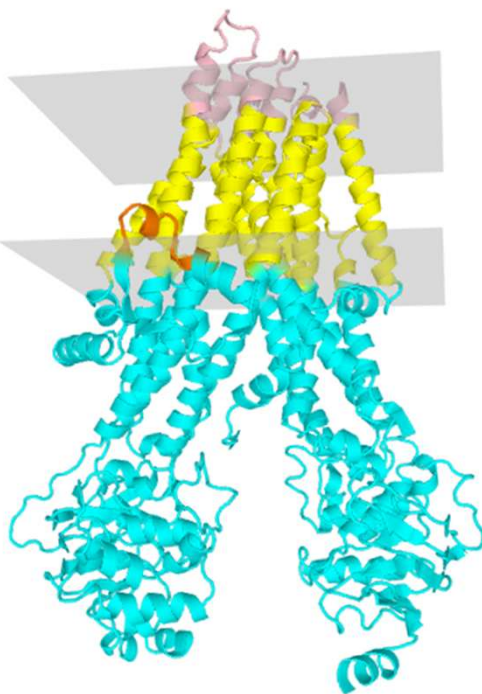
# Membrántopológia és TM hélixek predikciója szekvencia alapján

- Szekvencia alapján
  - *positive-inside rule*
  - TM hélixek és nem-TM részek aminosav eloszlása más
- Biológiai tudás figyelembevételének lehetősége
- Több prediktor integrálása  
pl. CCTOP.enzim.hu

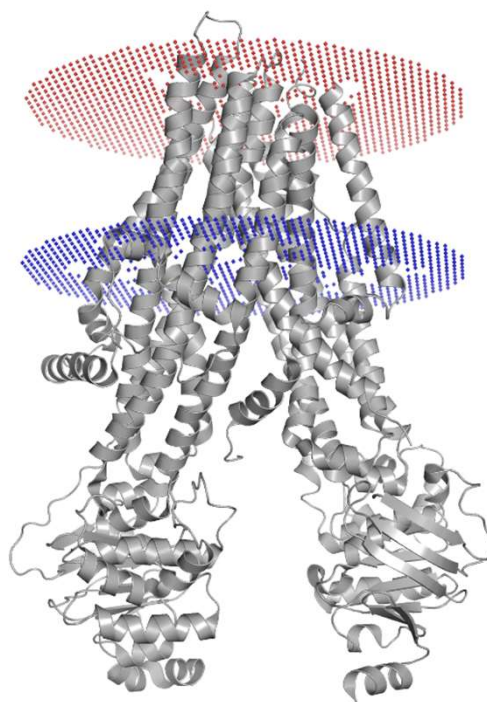
# TM hélixek predikciója szerkezet alapján

1. „water accessible area”, lipid kettősréteg lehetséges helye, pontozás – fitness függvény
2. molekula dinamika szimulációk

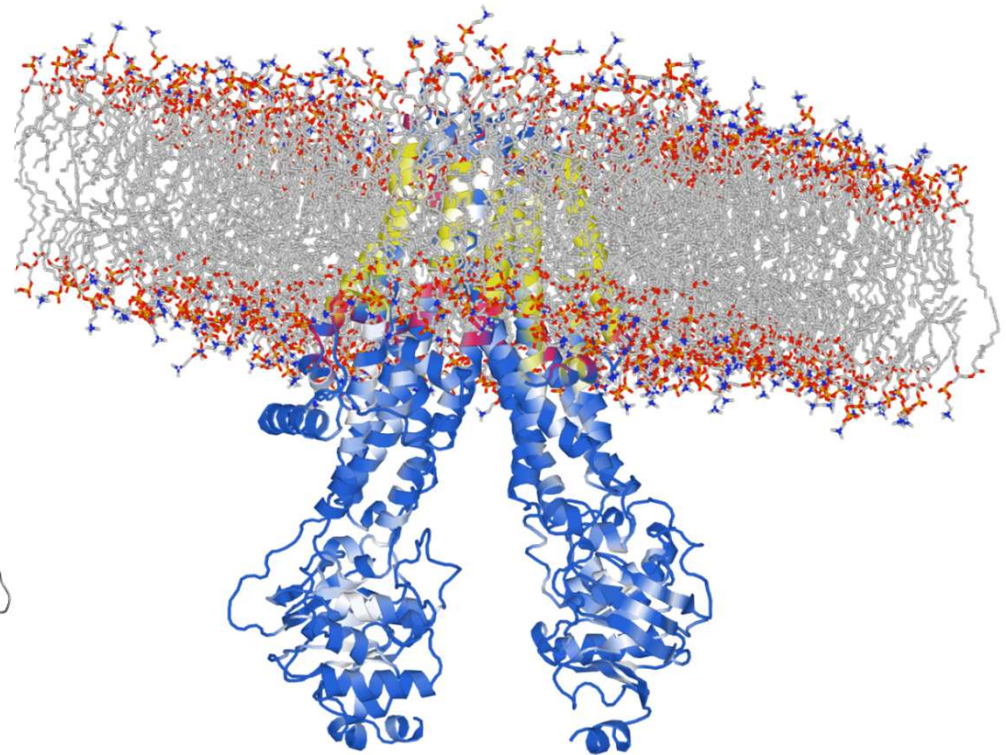
TMDet  
PDBTM



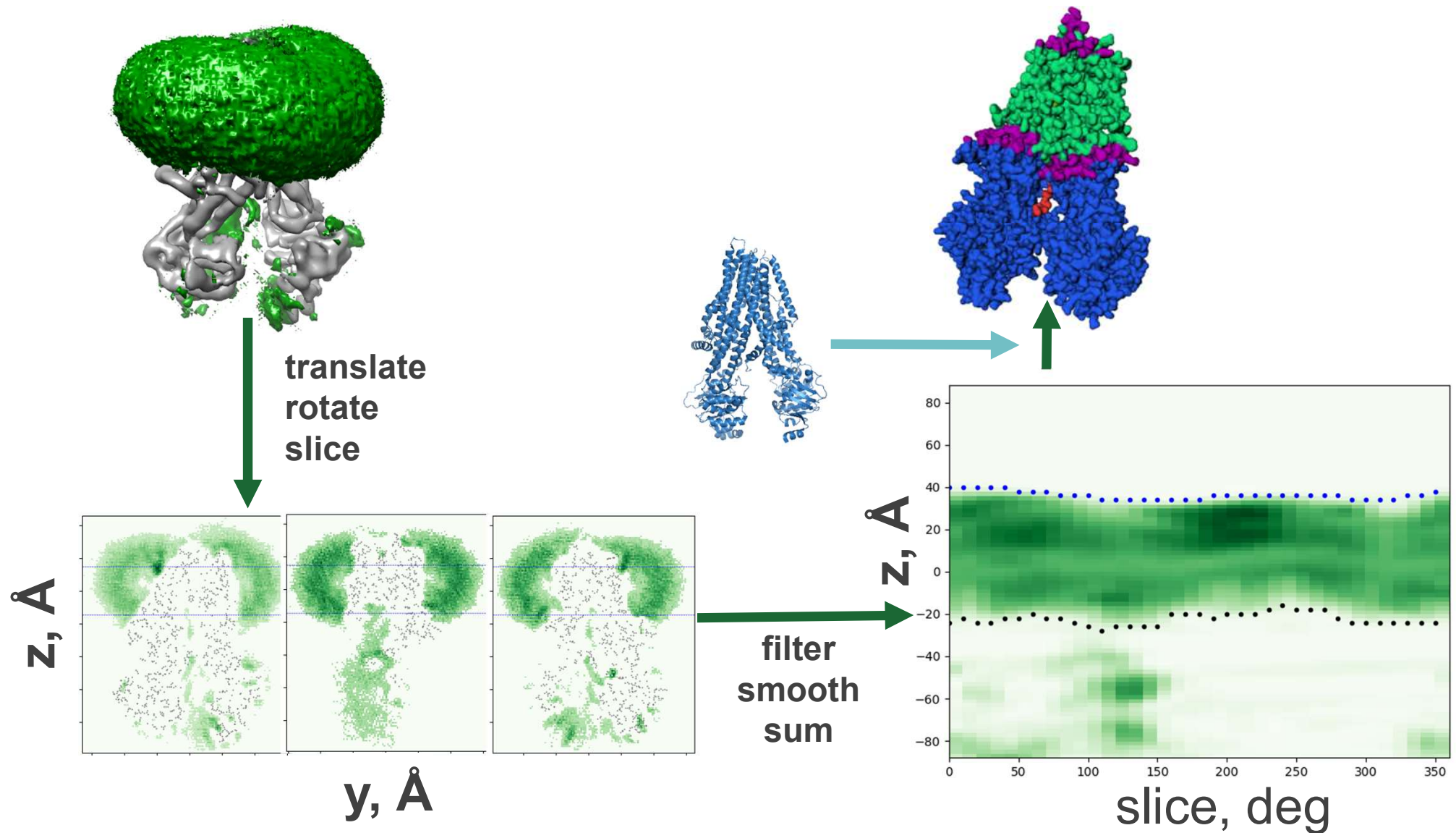
PPM  
OPM



MemProtMD

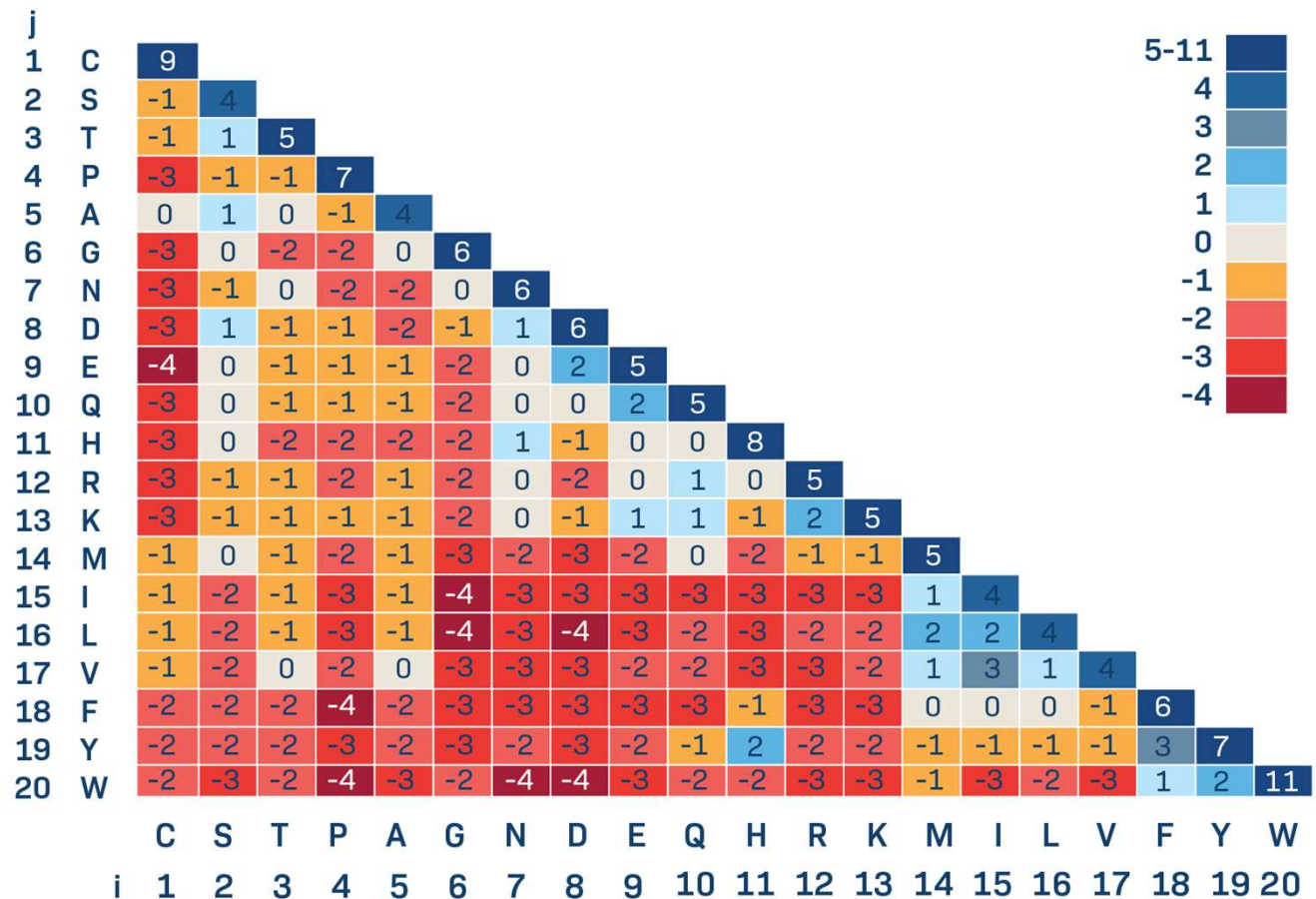


# The MemBlob can be converted to membrane boundaries



# Homológia modellezés I.

- Templát keresés
- Szekvencia illesztés
- Modellezés
- Energia-minimalizálás



BLOSUM62  
(BLOcks of Amino Acid SUBstitution Matrix)

<https://www.labxchange.org>

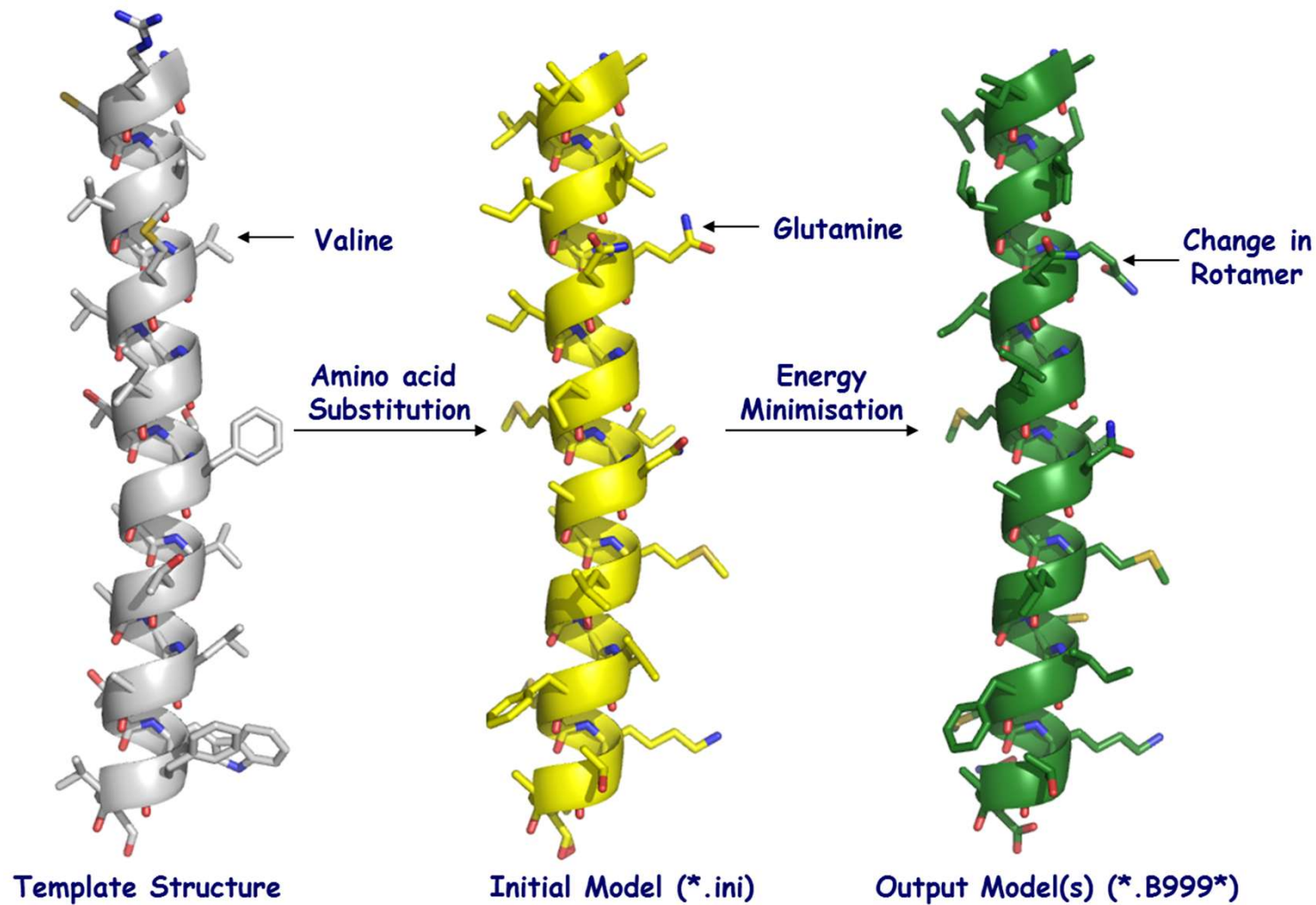
CLUSTAL W (1.83) multiple sequence alignment

Alignment – pl. ClustalW

```

2HYD      -----MIKRYLQFVK-----PYKYRIFATIIVGIIKFGIPMLIP
3B5X      -----WQTFKRLWTYIR-----LYKAGLVVSTIALVINAAADTYMI
CFTR_HUMAN MQRSPLEKASVVS KLFFSWTRPILRKGYRQRLELSDIYQIPSVDSADNLS
              *      :      :      *      :      :      *      :      :
    
```

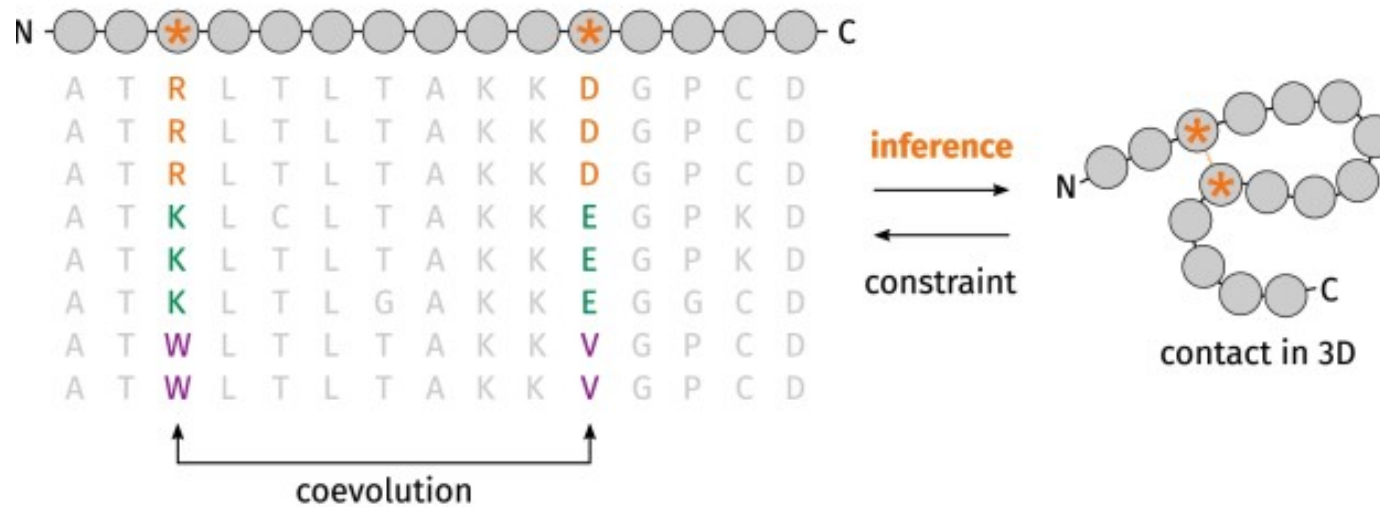
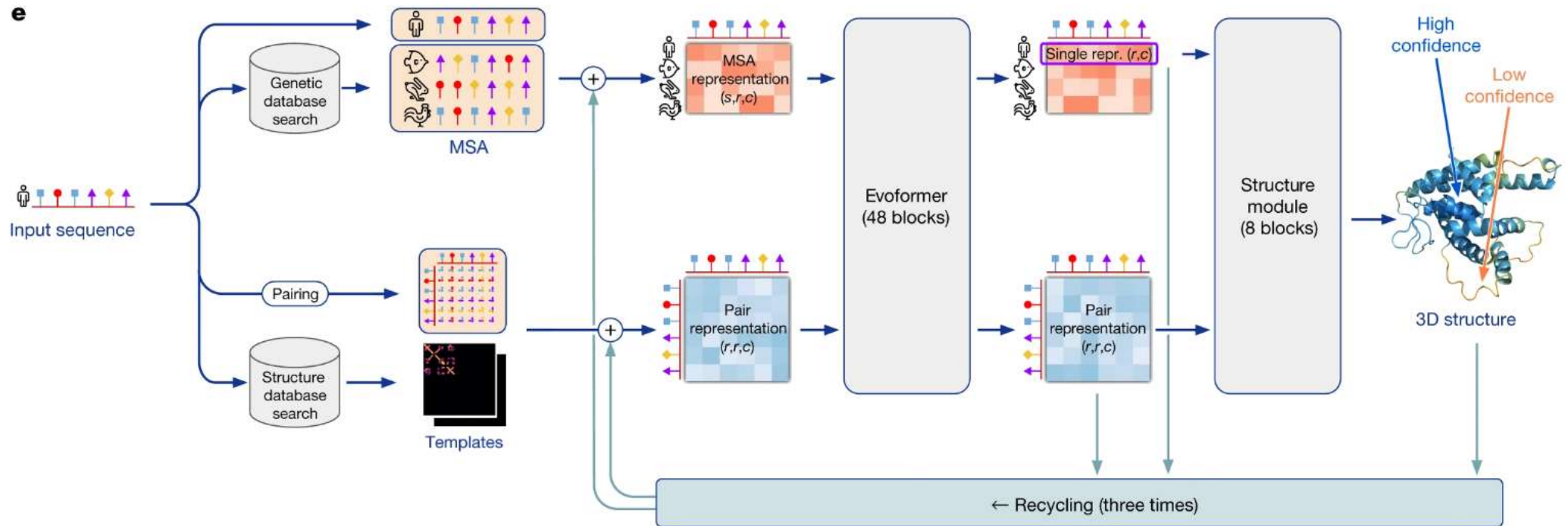
# Homológia modellezés II.



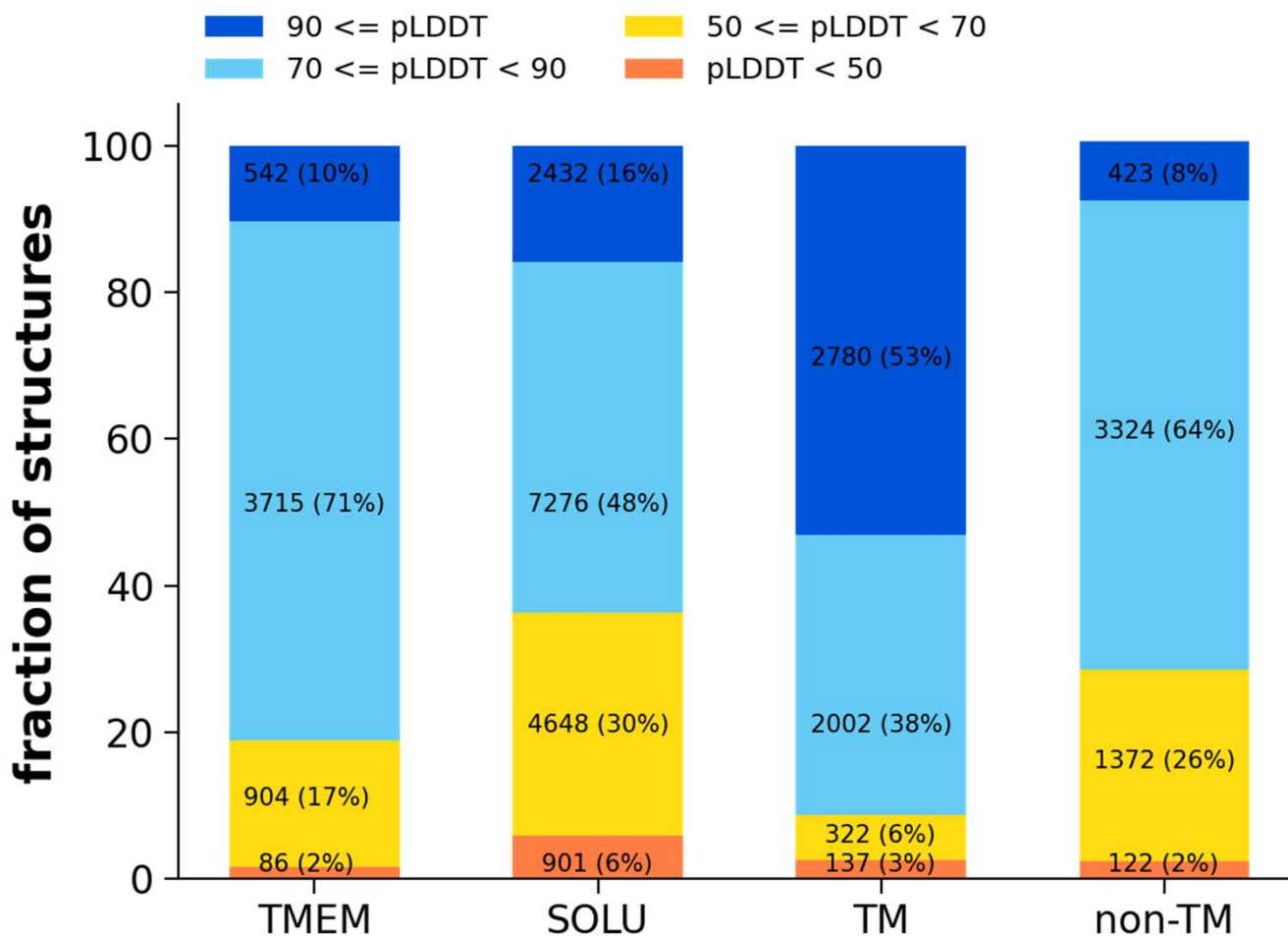
# AlphaFold2

machine learning, deep learning, AI

Jumper et al. Nat 2021

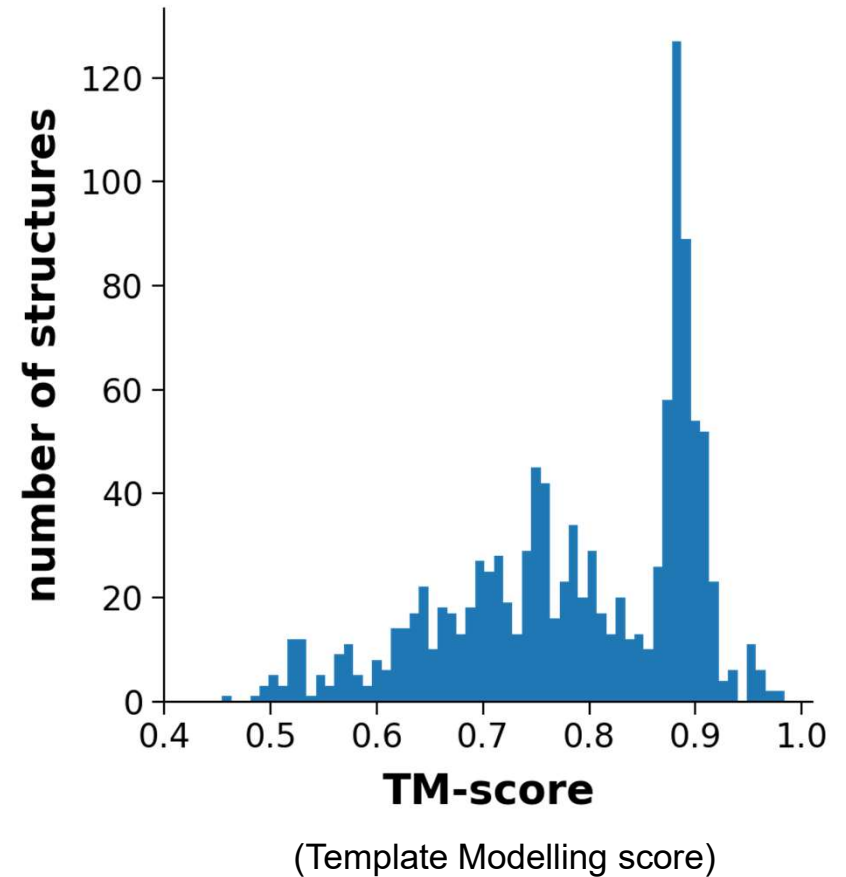
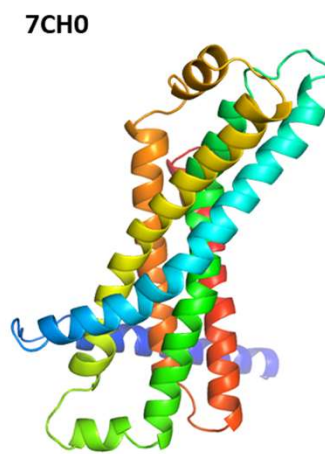
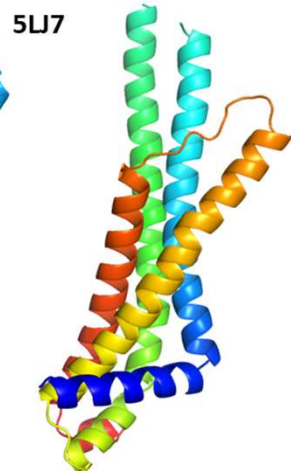
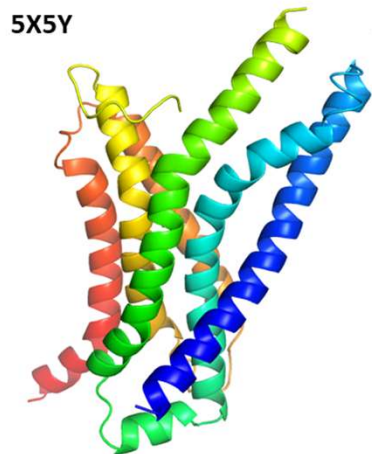
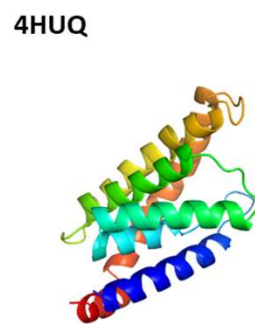
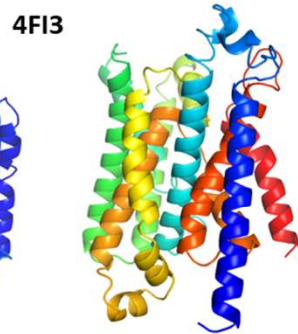
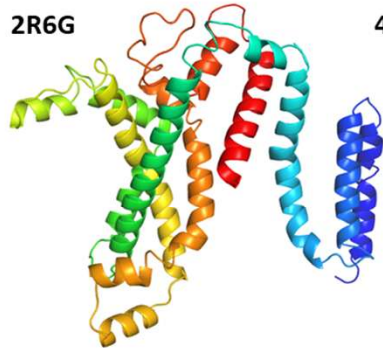
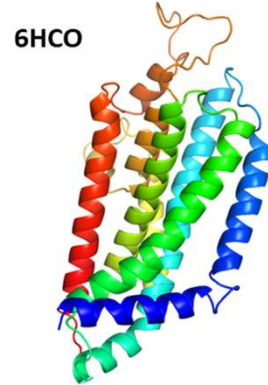
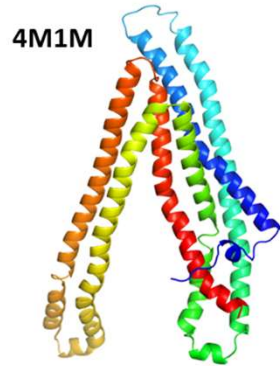


# Transzmembrán fehérjék szerkezeti predikciója



# ABC fehérje szerkezetek

| fold class | reference PDB |
|------------|---------------|
| Pgp-like   | 4M1M          |
| ABCG2-like | 6HCO          |
| MalFG-like | 2R6G          |
| BtuC-like  | 4FI3          |
| EcfT-like  | 4HUQ          |
| LptFG-like | 5X5Y          |
| MacB-like  | 5LJ7          |
| MlaE-like  | 7CH0          |



# CFTR TM8

Farkas *et al.* CMLS 2019

Hegedus *et al.* CMLS 2022

cryo-EM

1/22 simulations,  $r \geq 1.8$

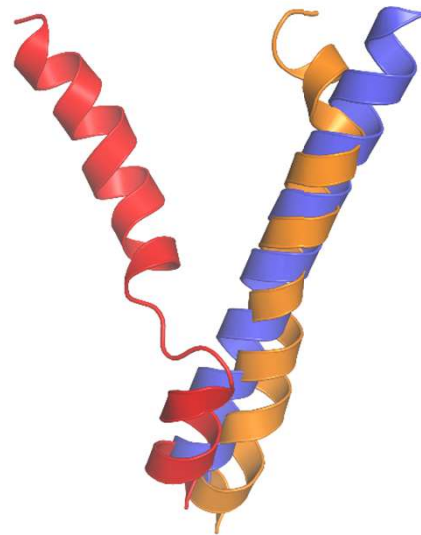
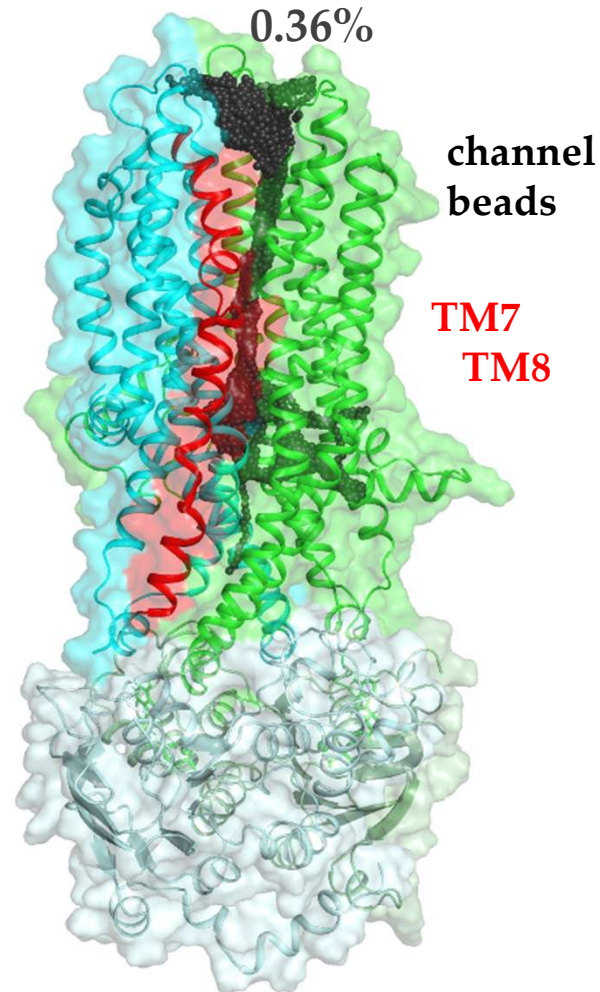
Å

0.36%

cryo-EM

AF2

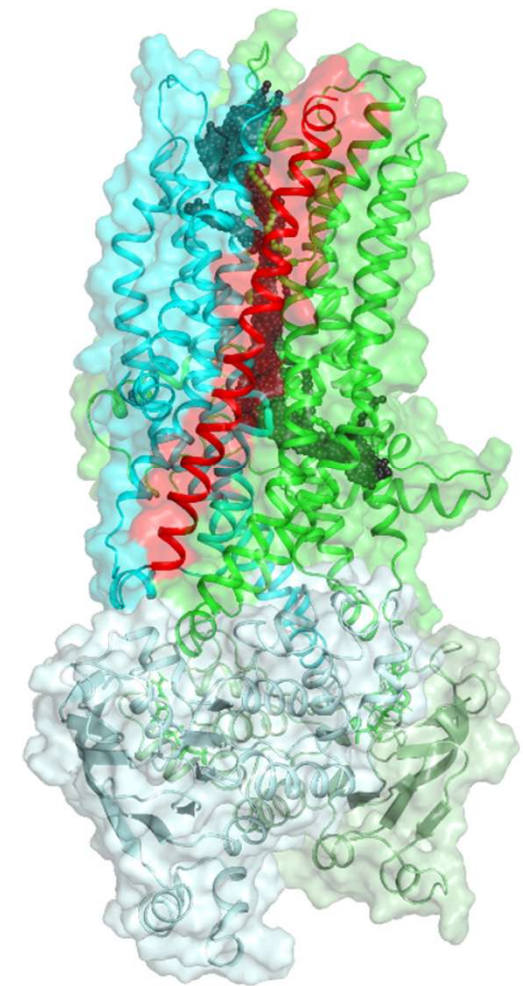
homo



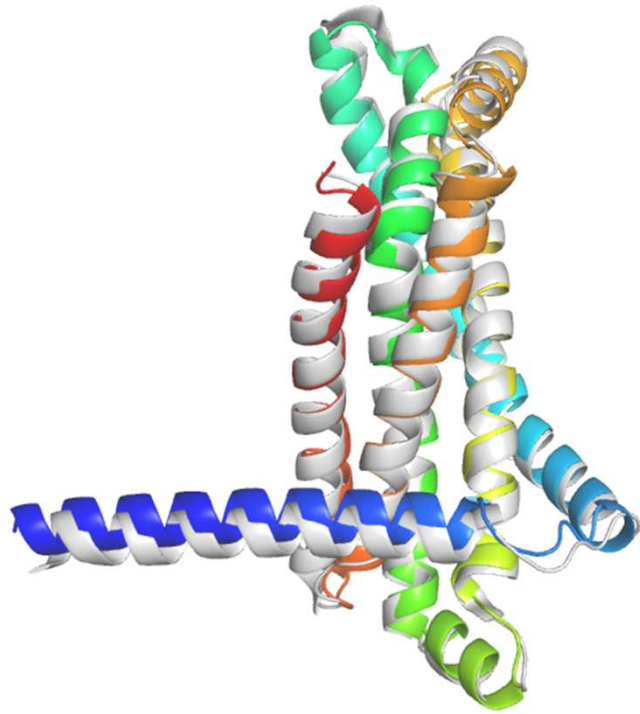
homology modelling

5/6 simulations

3.74%



# Új transzmembrán szerkezetek predikciója



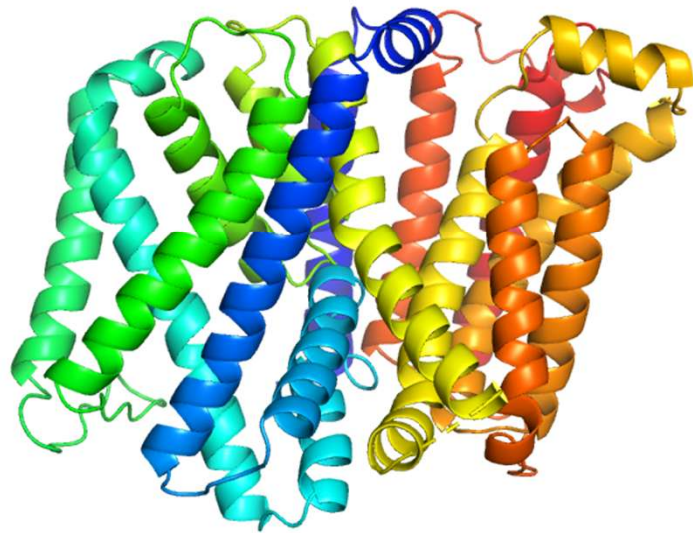
MlaE-like fold  
PDBID: 7ch0  
RMSD of 1.28 Å



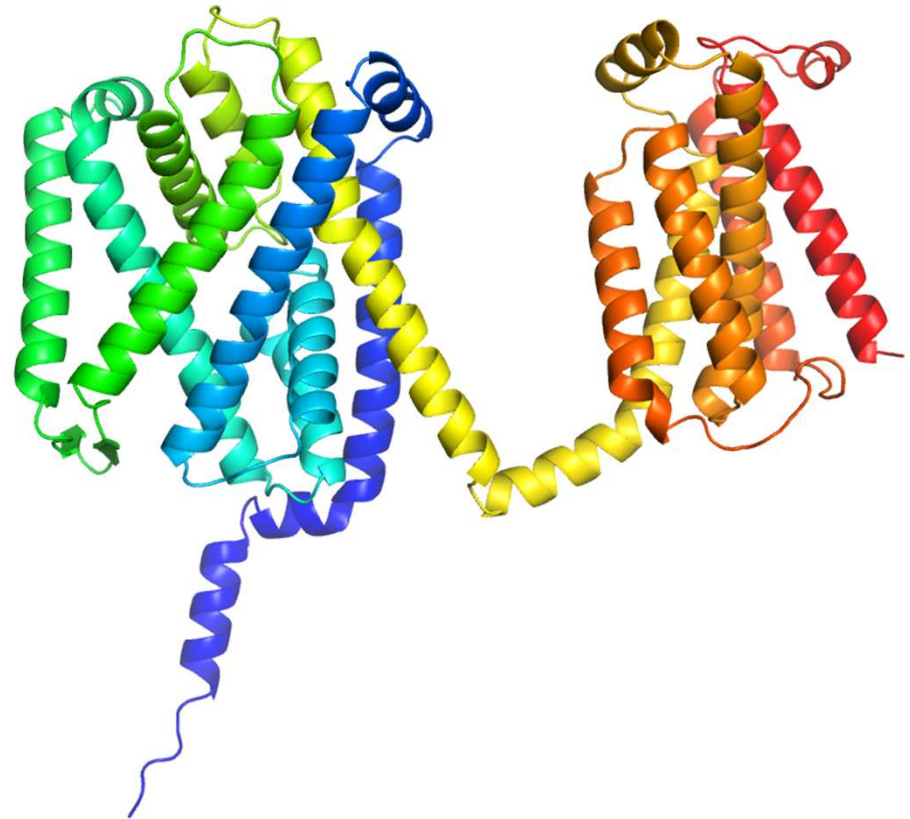
ER membrane protein complex subunit 6  
PDBID: 6ww7  
RMSD of 0.96 Å

# Új transzmembrán szerkezetek predikciója

MprF (PDBID: 7DUW)



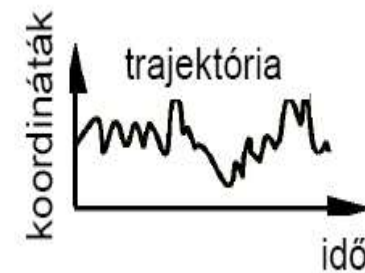
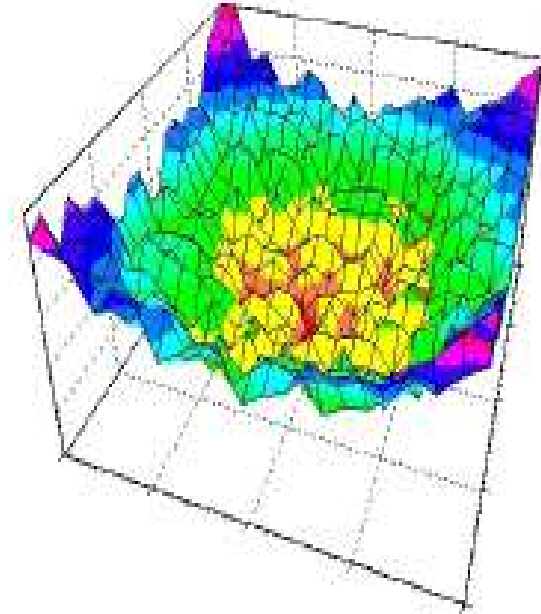
AF2



**Van szerkezet, mozgassuk meg!**

# Molekula dinamika/Molecular dynamics

- valós potenciálfelület
- mozgásegyenletek  
idő-lépésenkénti numerikus megoldása
- trajektória



# A „force field”

Baker (2007)

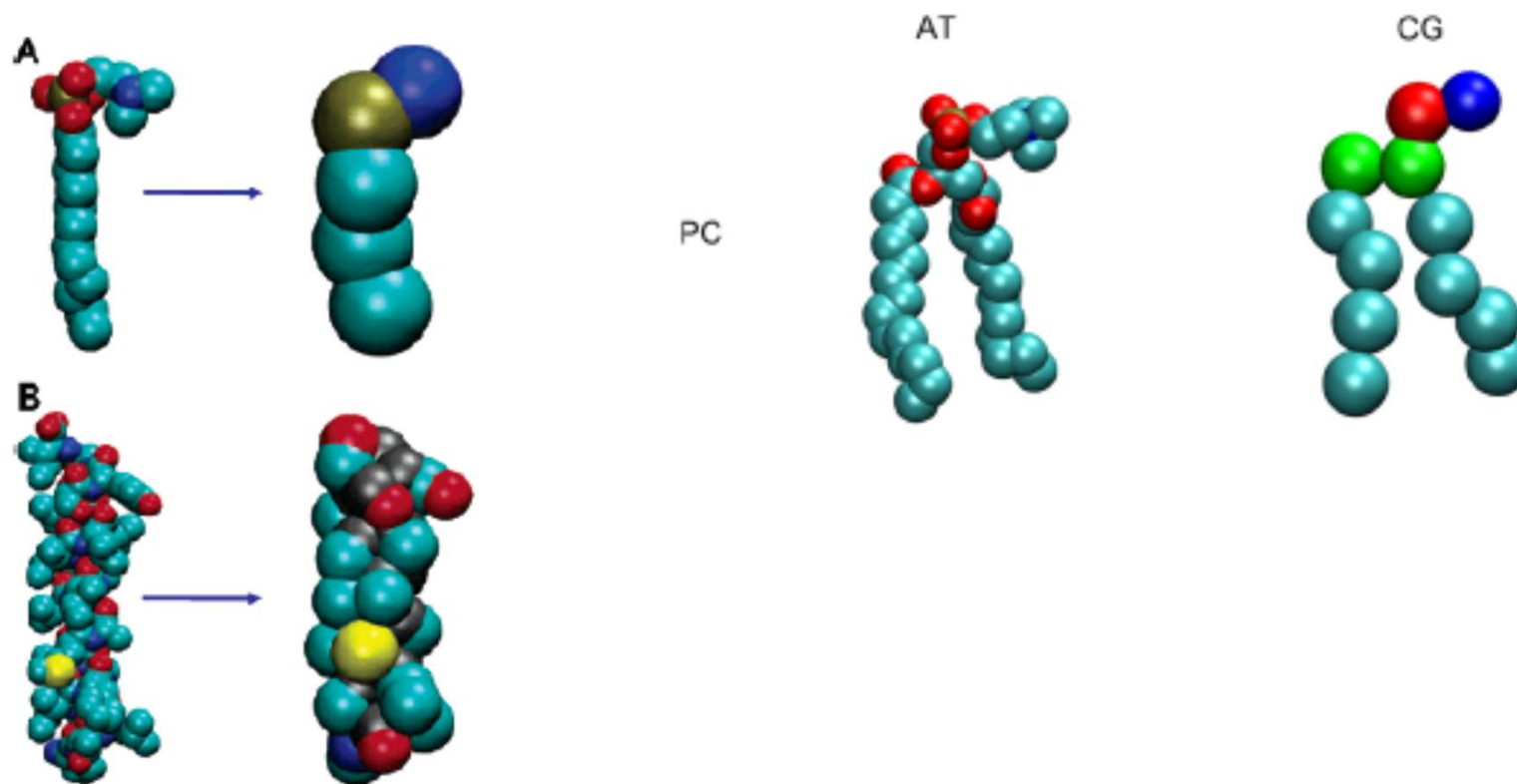
$$E_{\text{prot}} = W_{\text{rot}} E_{\text{rot}} + W_{\text{atr}} E_{\text{atr}} + W_{\text{rep}} E_{\text{rep}} + W_{\text{solv}} E_{\text{solv}} + W_{\text{pair}} E_{\text{pair}} \\ + W_{\text{mbenv}} E_{\text{mbenv}} + W_{\text{hbond}} E_{\text{hbond}} - E_{\text{ref}}$$

$$E_{\text{mbenv}} = \sum_i^{\text{natom}} \Delta G_i^{\text{ref}}(z') \quad \Delta G_i^{\text{ref}}(z') = (1 - f(z')) * (\Delta G_i^{\text{ref, chex}} - \Delta G_i^{\text{ref, water}})$$

**TABLE II. Atomic Contribution to the Solvation Free Energy in Water and Cyclohexane**

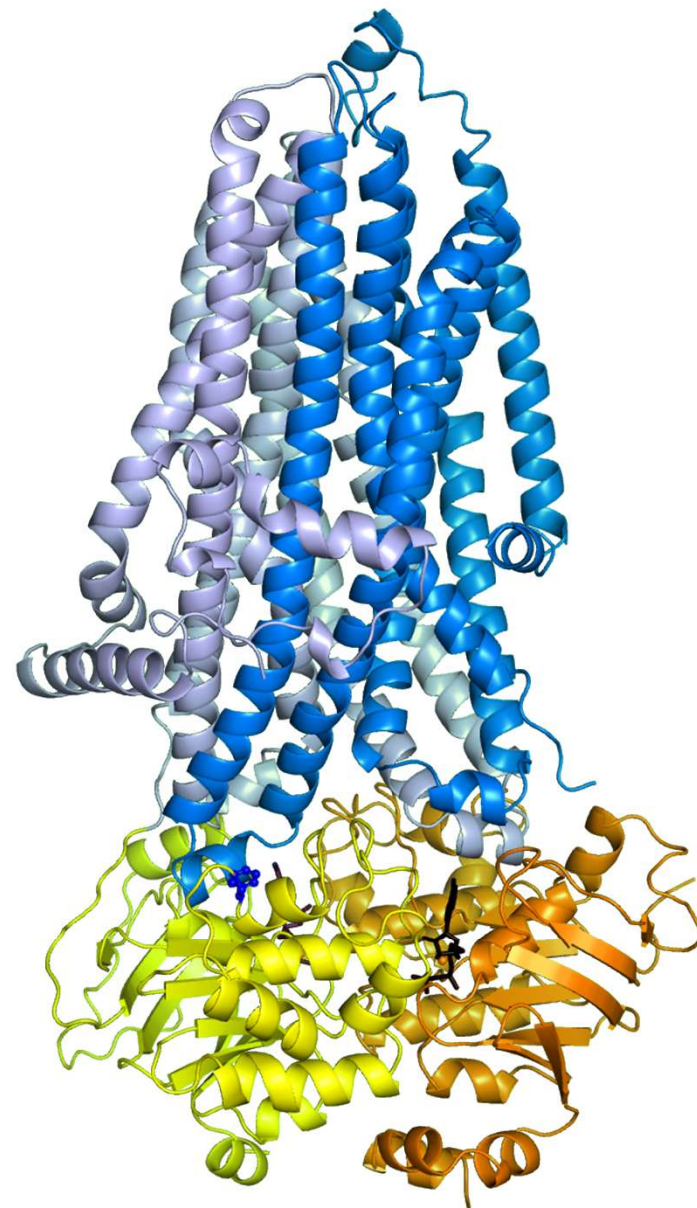
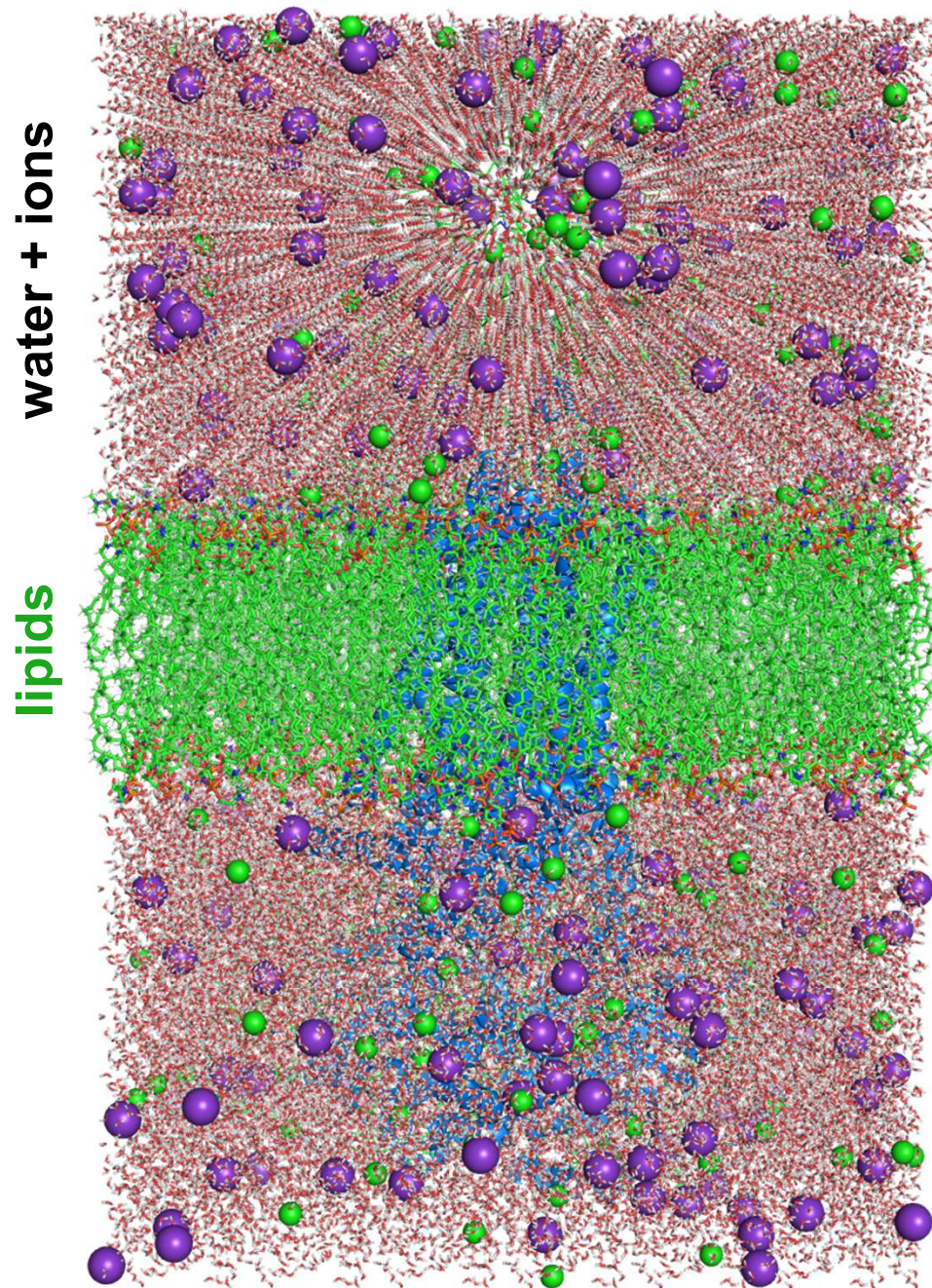
|      | Water  | Chex   |      | Water   | Chex   |
|------|--------|--------|------|---------|--------|
| CR   | −0.890 | −1.350 | NH3  | −20.000 | −1.145 |
| CH1E | −0.187 | −0.645 | NC2  | −10.000 | −0.200 |
| CH2E | 0.372  | −0.720 | N    | −1.000  | −1.145 |
| CH3E | 1.089  | −0.665 | OH1  | −5.920  | −0.960 |
| CR1E | 0.057  | −0.410 | O    | −5.330  | −1.270 |
| NH1  | −5.950 | −1.145 | OC   | −10.000 | −0.900 |
| NR   | −3.820 | −1.630 | S    | −3.240  | −1.780 |
| NH2  | −5.450 | −1.145 | SH1E | −2.050  | −1.855 |

# Egyszerűsített (Coarse Grain) modellek



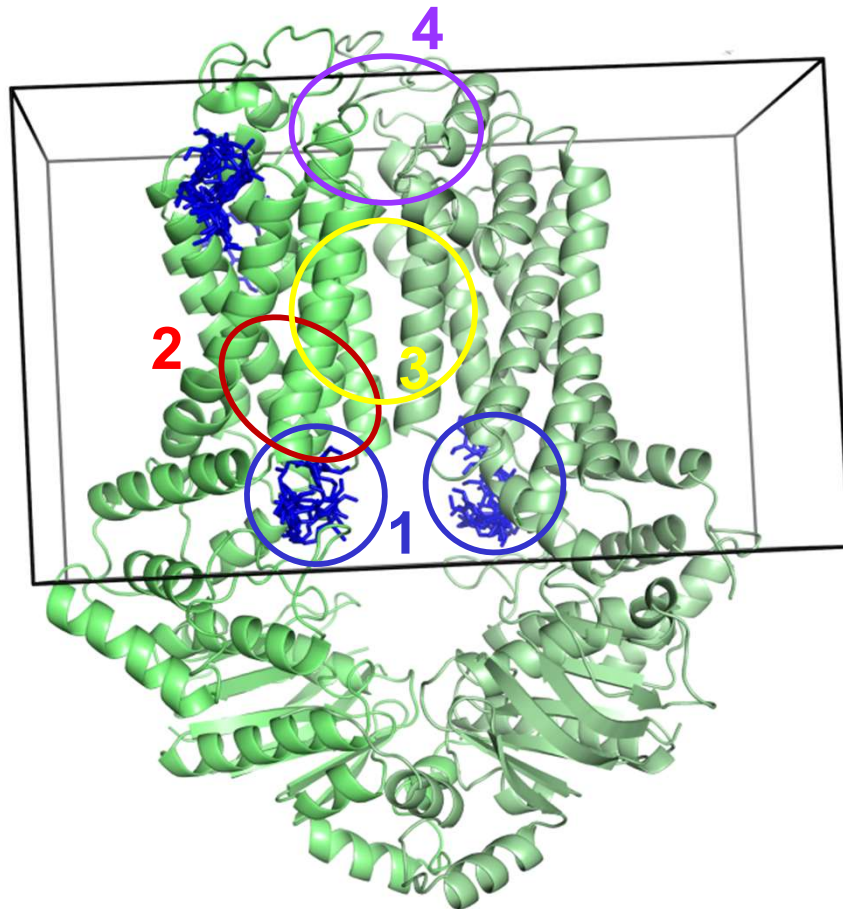
*Figure 1.* Atomistic (left-hand) and coarse-grained (right-hand) models compared for (A) a DPC molecule and (B) a GpA helix. Colors for atoms:

# „All-atom” szerkezetek

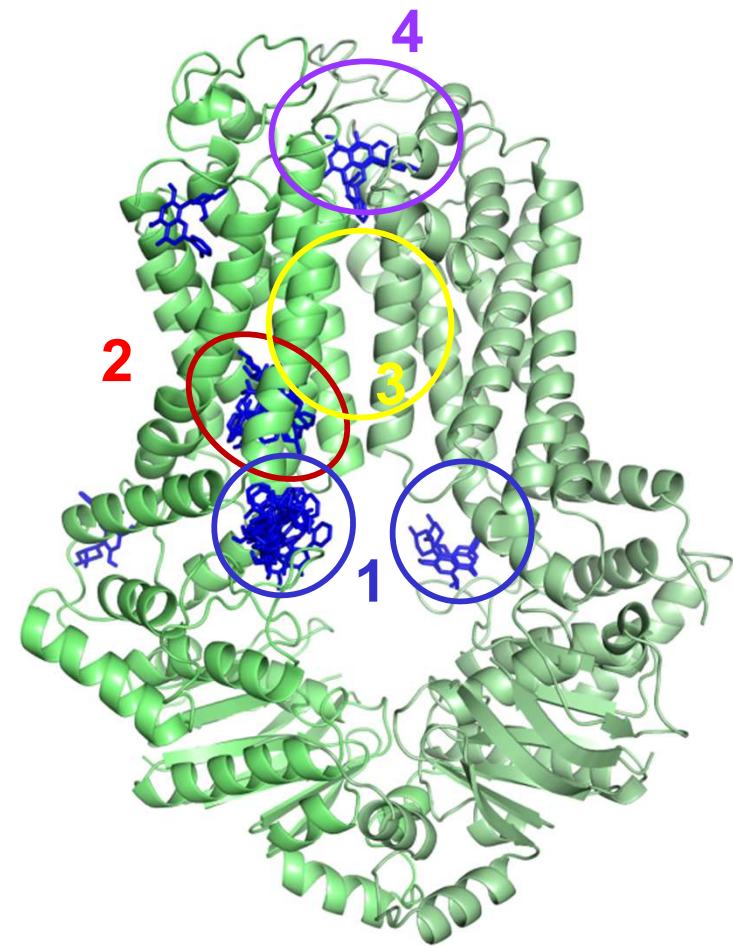


# Drogkötő helyek vizsgálata *in silico* dokkolással

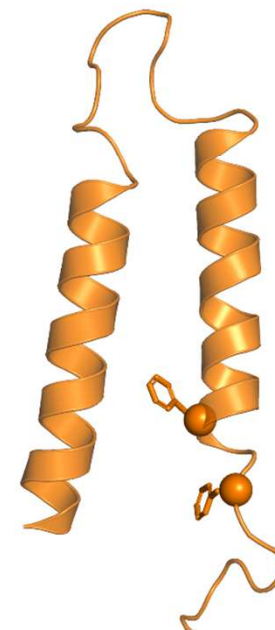
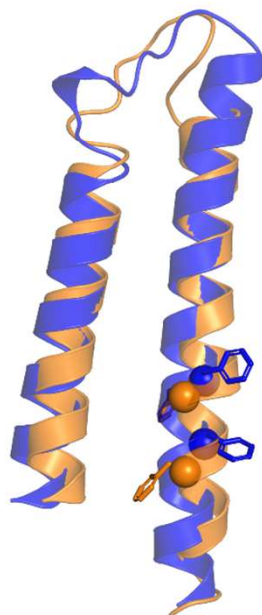
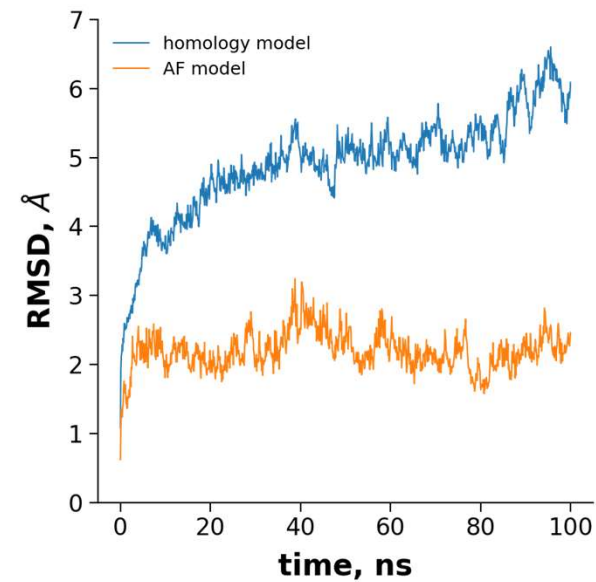
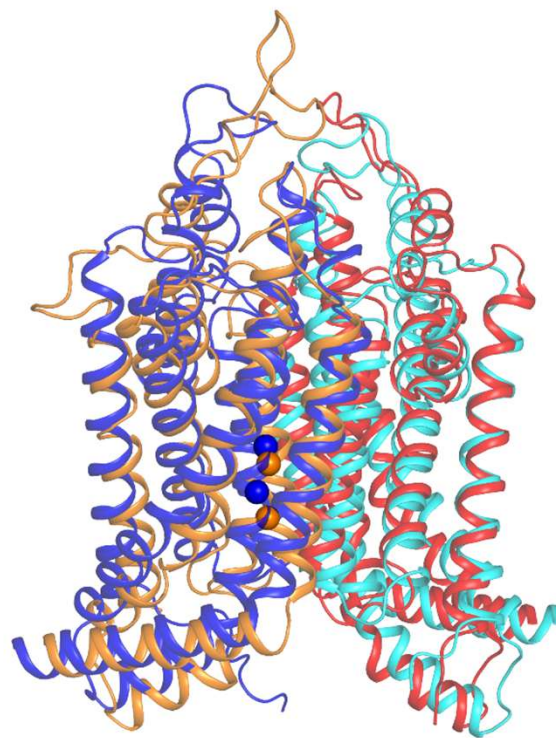
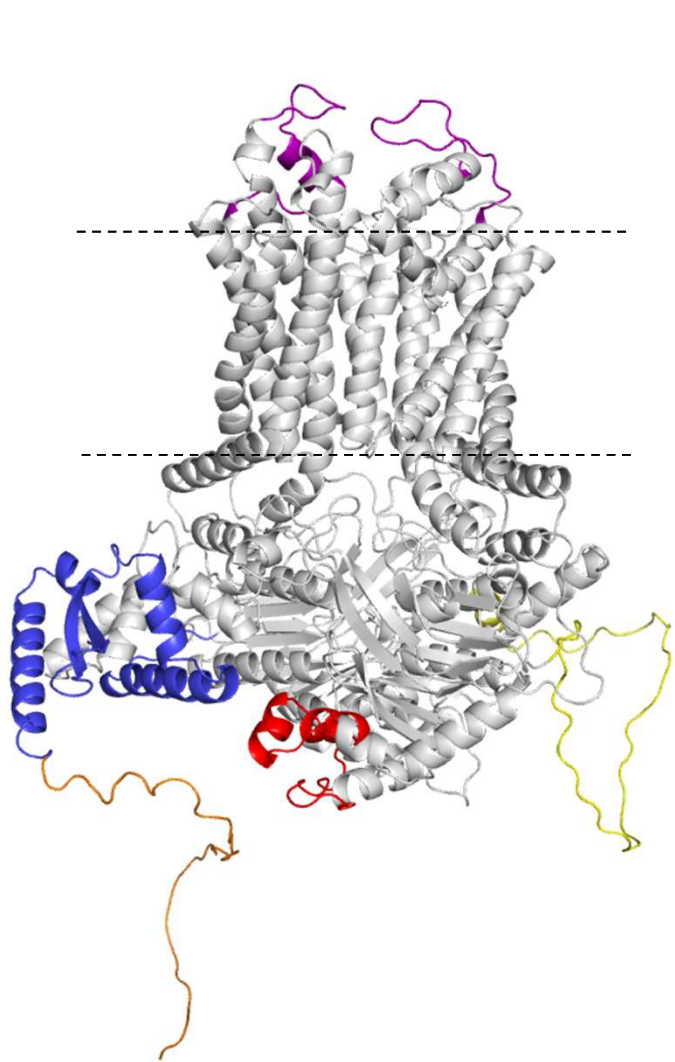
**verapamil**



**flavopiridol**

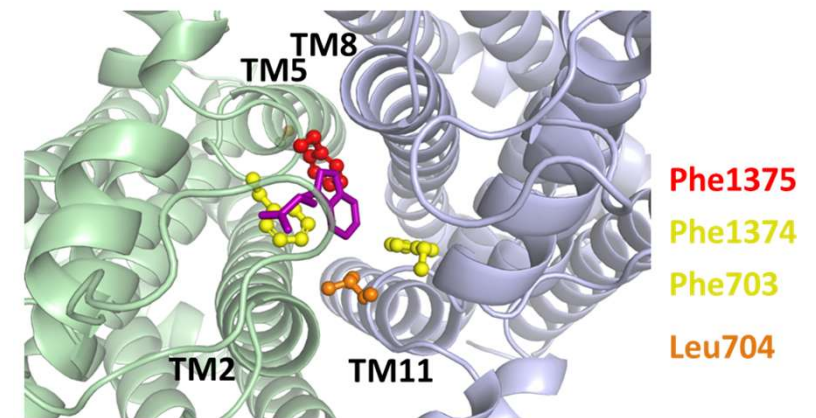
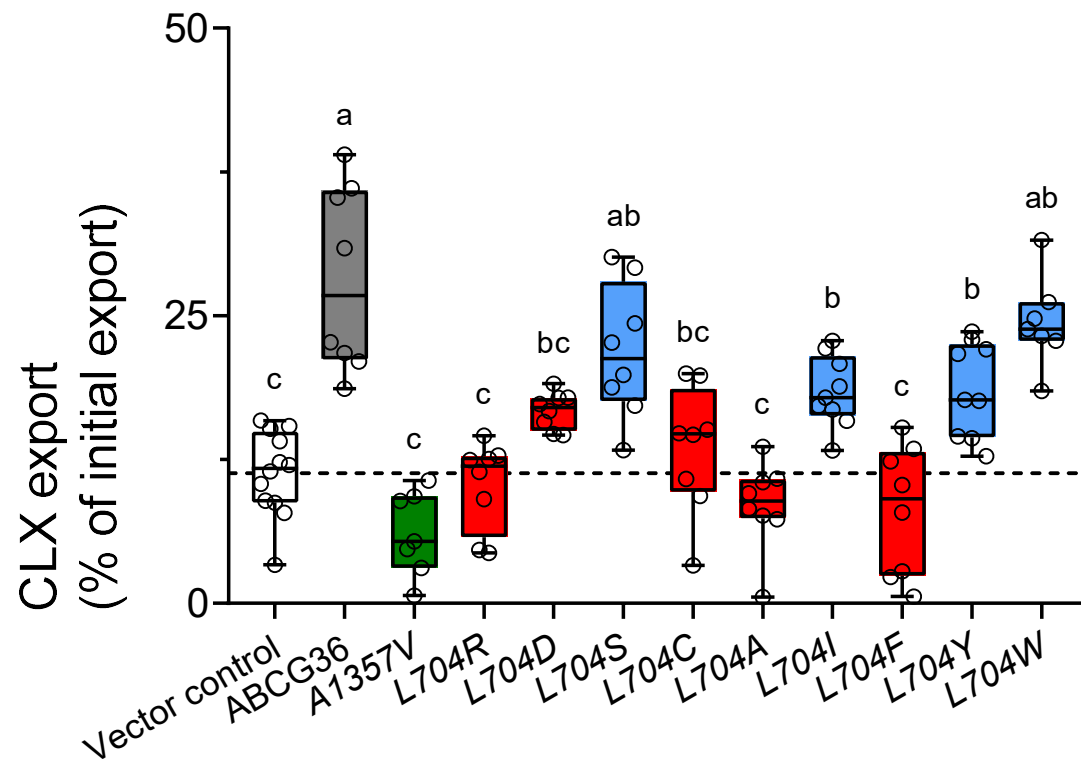
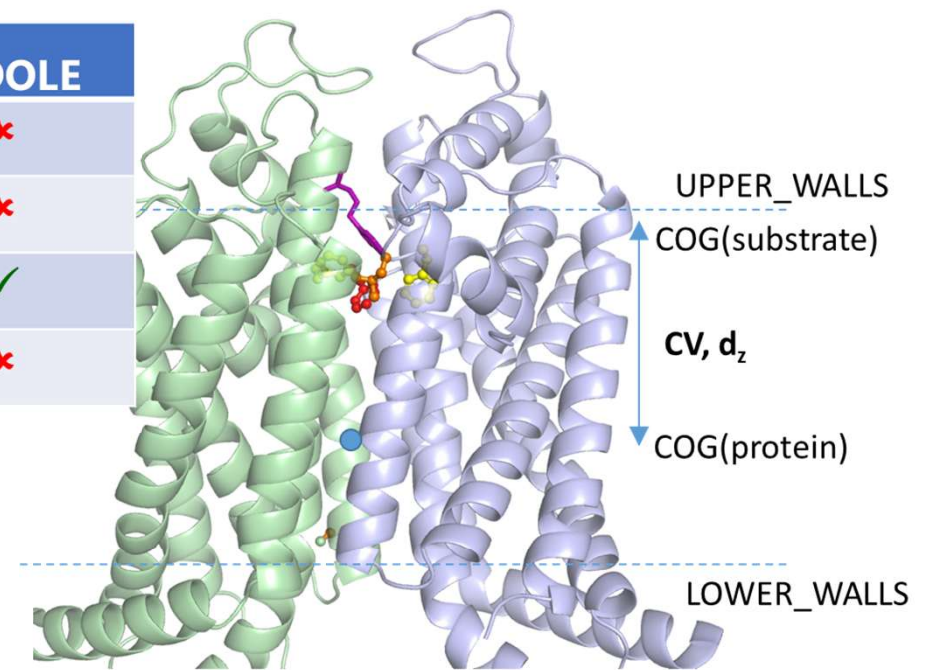


# Arabidopsis ABCG36



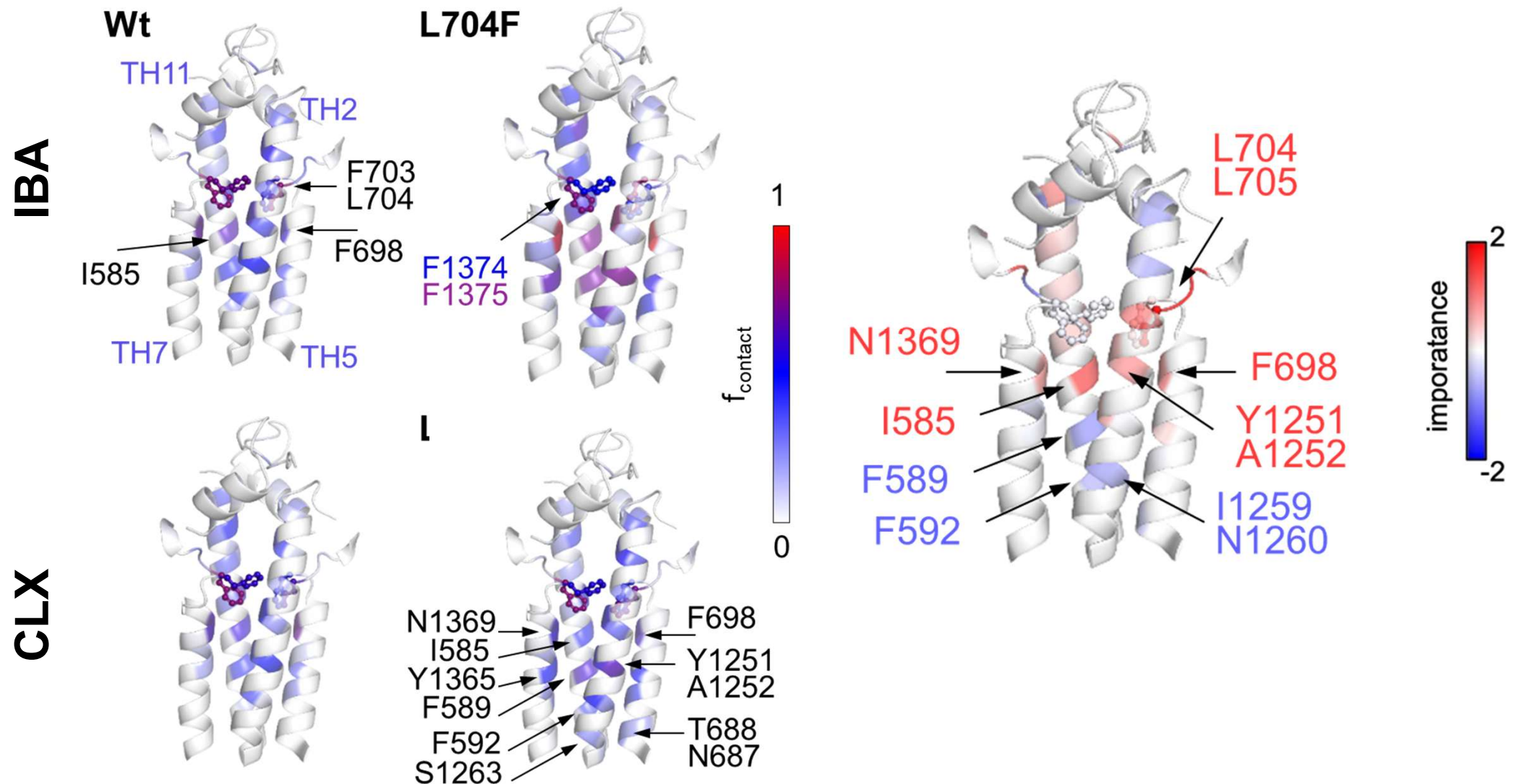
# Arabidopsis ABCG36

| ABG36 | IAA | CLX | IBA | INDOLE |
|-------|-----|-----|-----|--------|
| Wt    | ✗   | ✓   | ✓   | ✗      |
| L704S | ✓   | ✓   | ✓   | ✗      |
| L704Y | ✓   | ✓   | ✓   | ✓      |
| L704F | ✗   | ✗   | ✓   | ✗      |



Xia et al. Nat. Comm. accepted

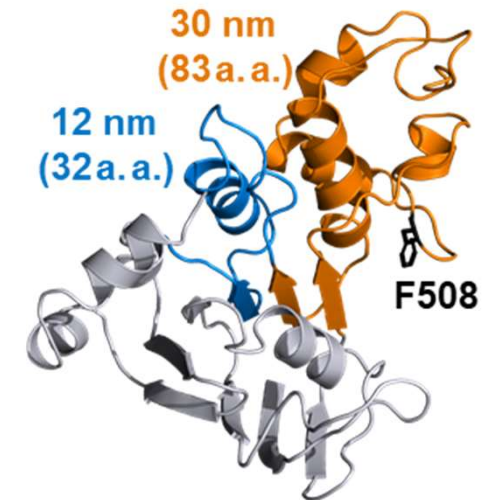
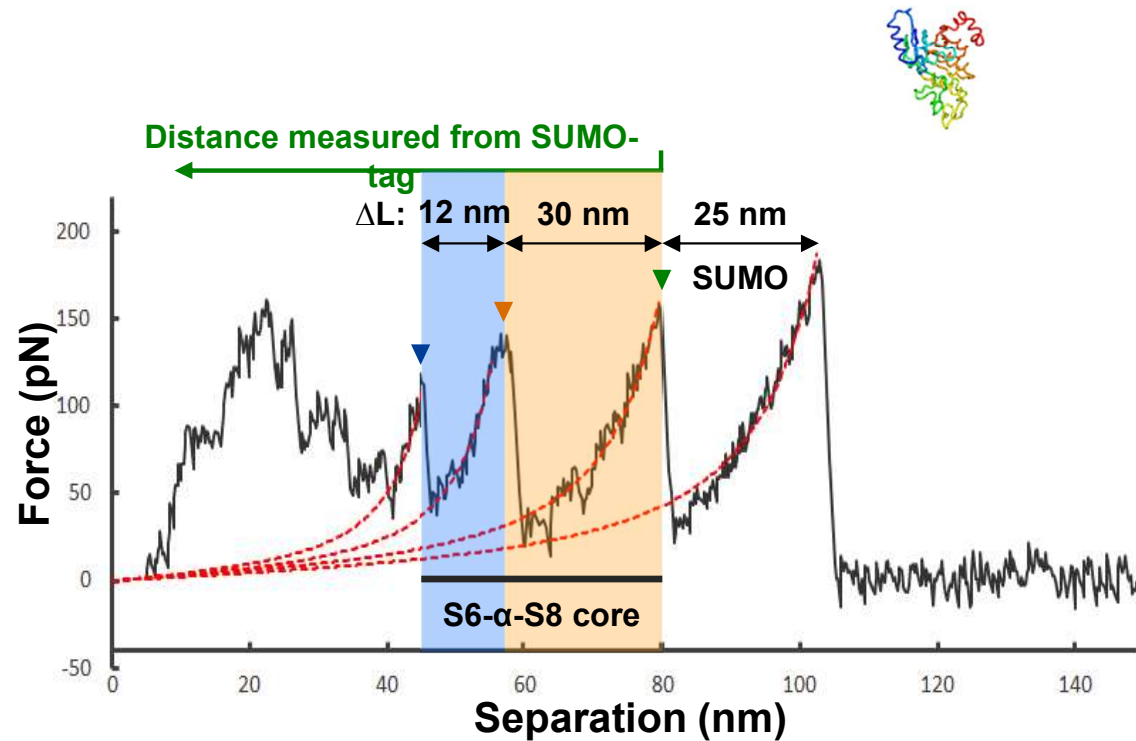
# Arabidopsis ABCG36



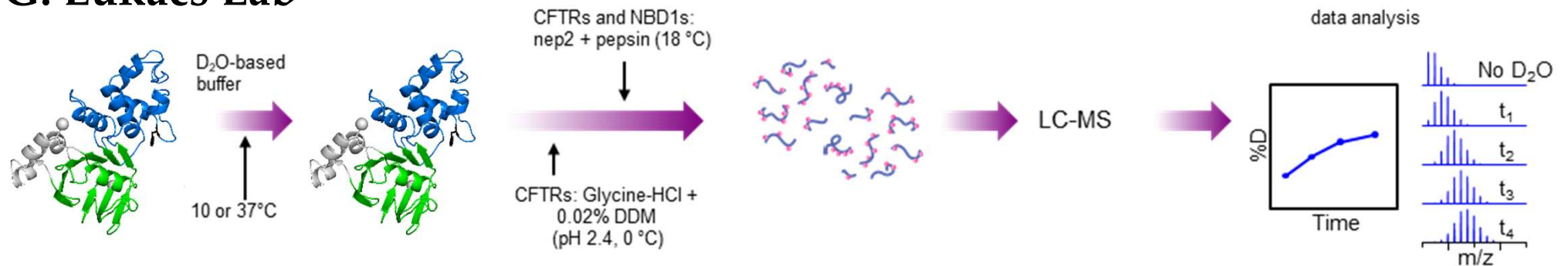
# Experiments for BIA site identification

pulling with AFM, HDX

Padanyi *et al.* Comp. Struct. Biol. 2022



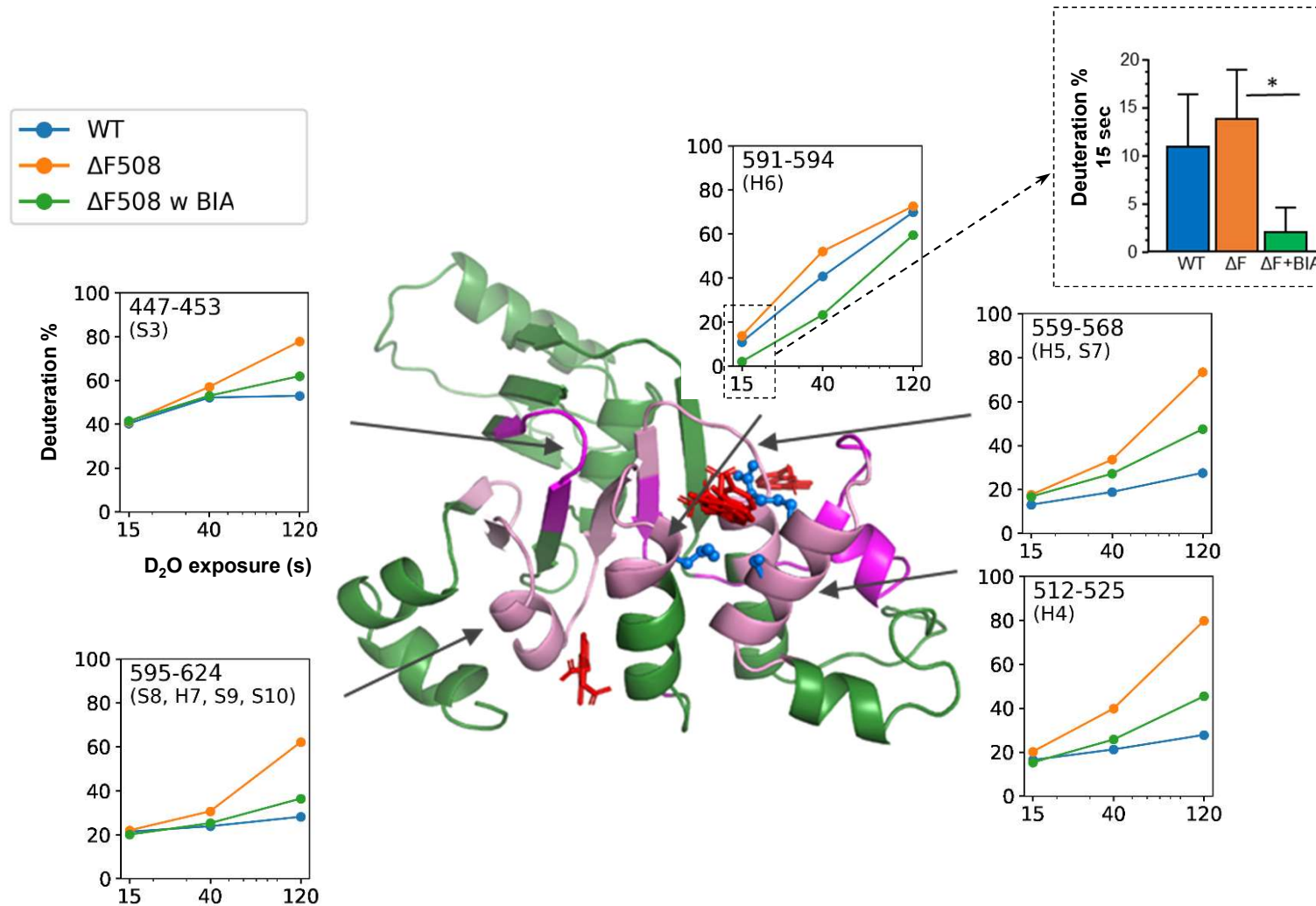
## G. Lukács Lab



# BIA binding site – HDX with NBD1

G. Lukács Lab

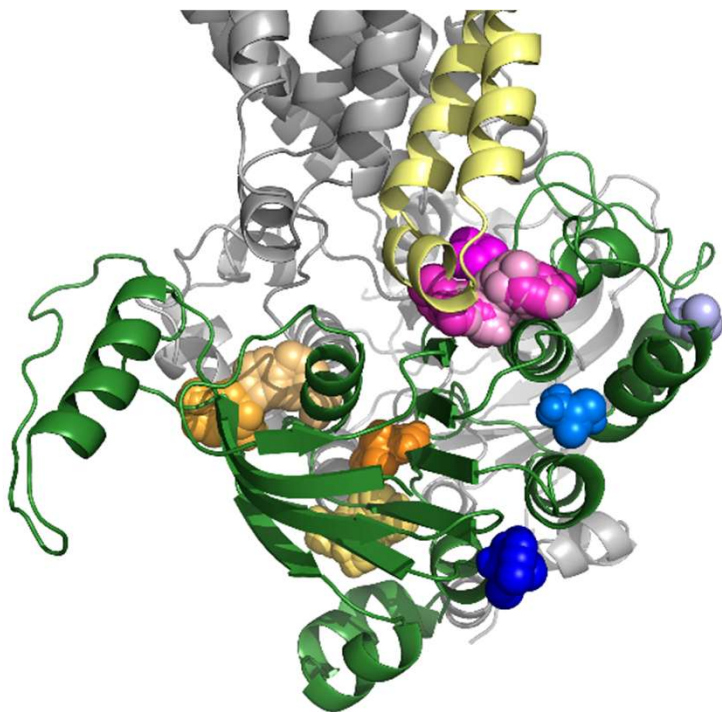
Padanyi *et al.* Comp. Struct. Biol. 2022



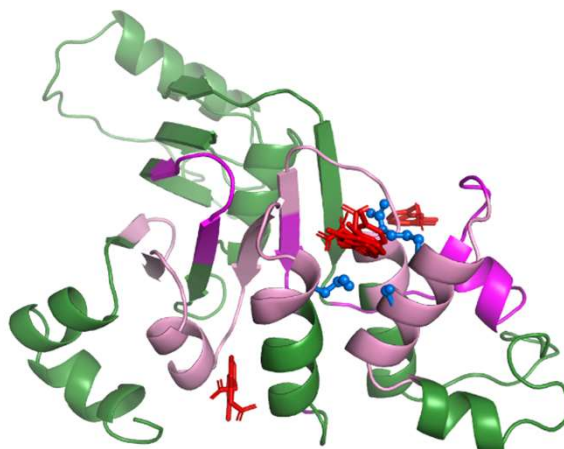
# BIA binding site – *in silico*

Padanyi *et al.* Comp. Struct. Biol. 2022

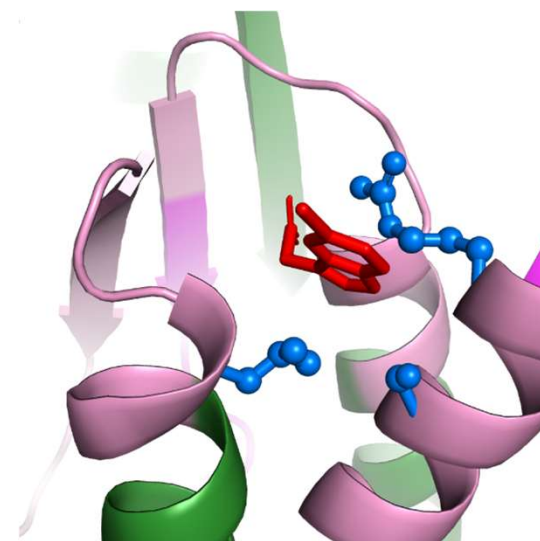
pocket detection  
fpocket



docking  
AutoDock Vina

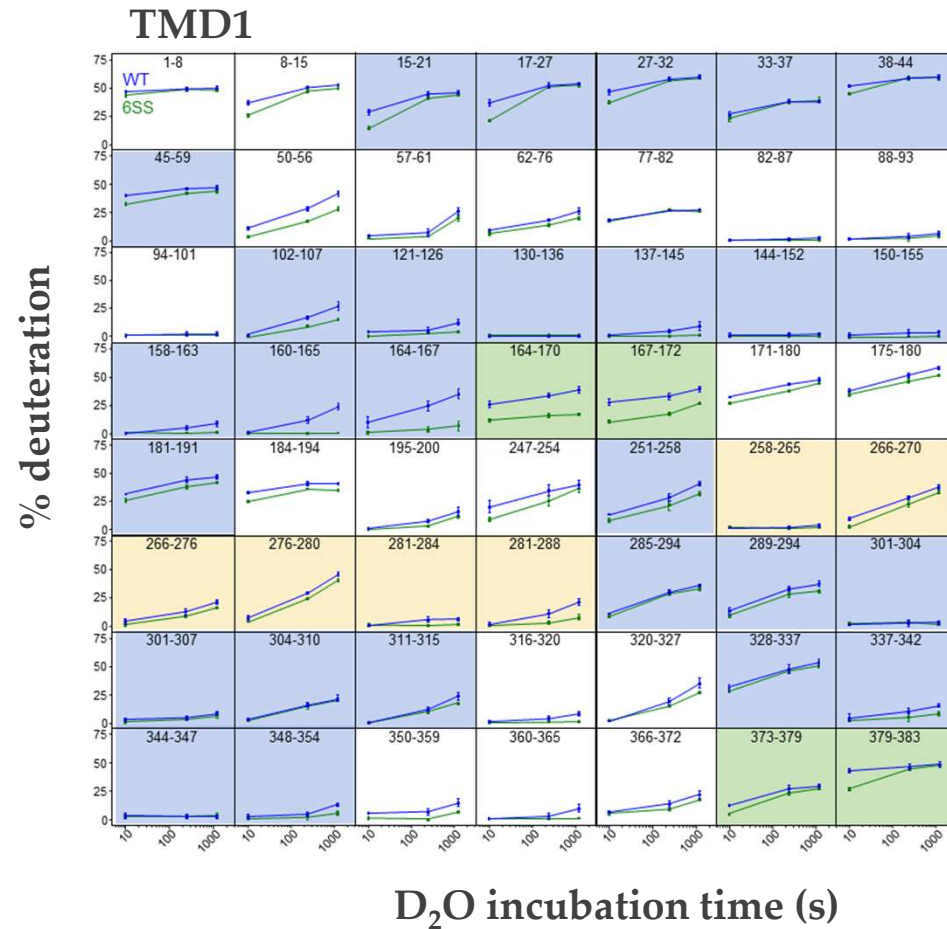
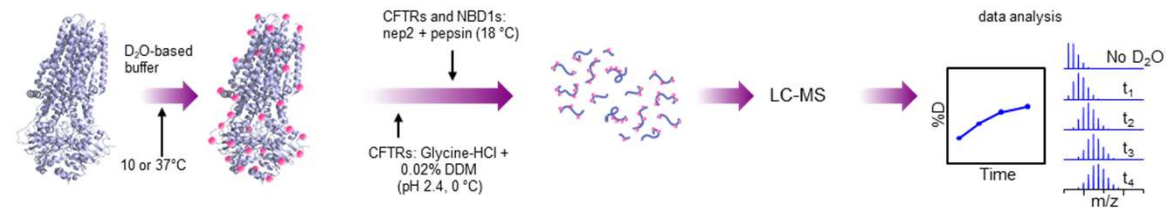


concluded site



# Folding/maturation/stability of the full length CFTR

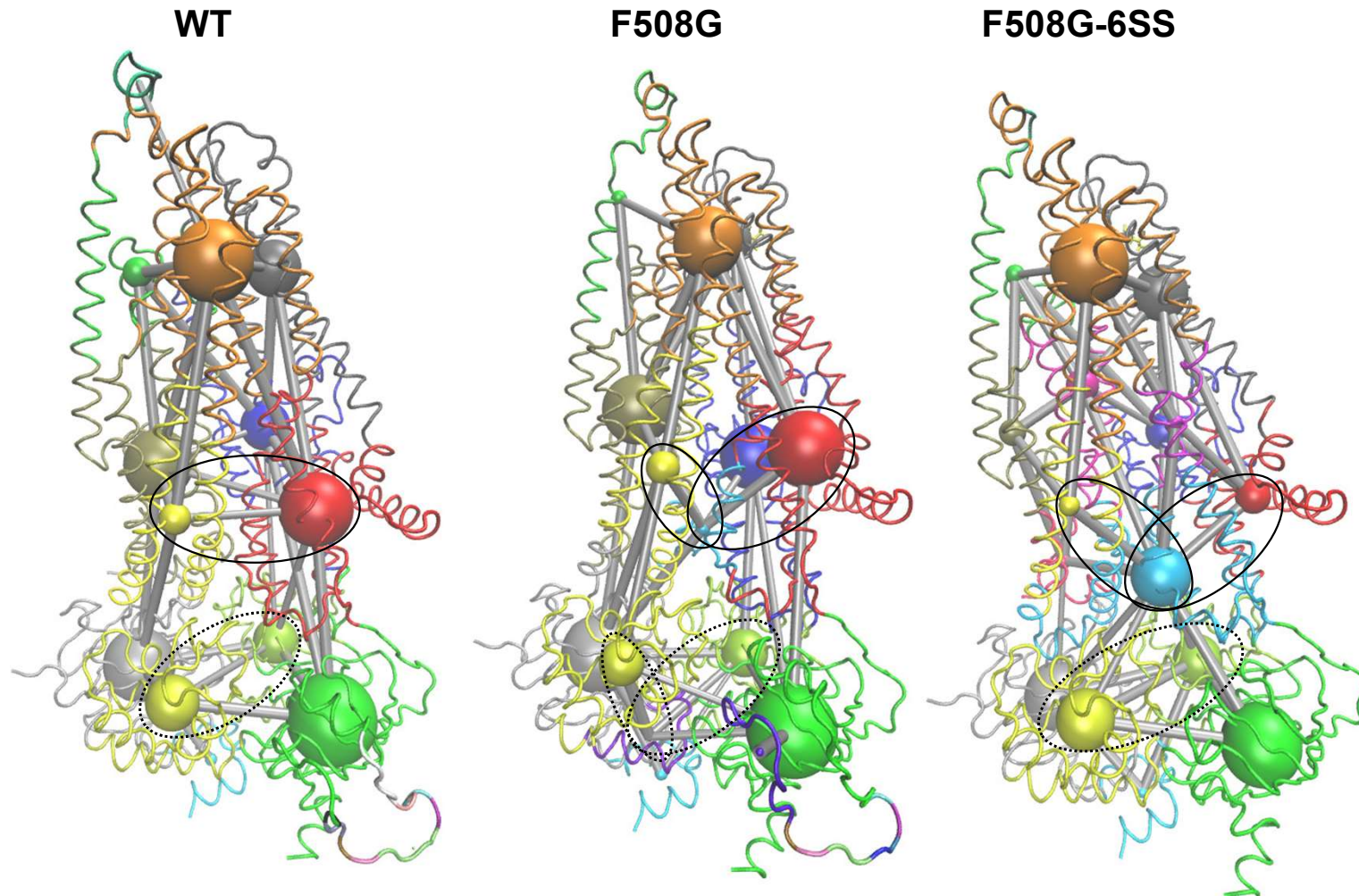
Soya *et al.* Nat. Comm. 2023



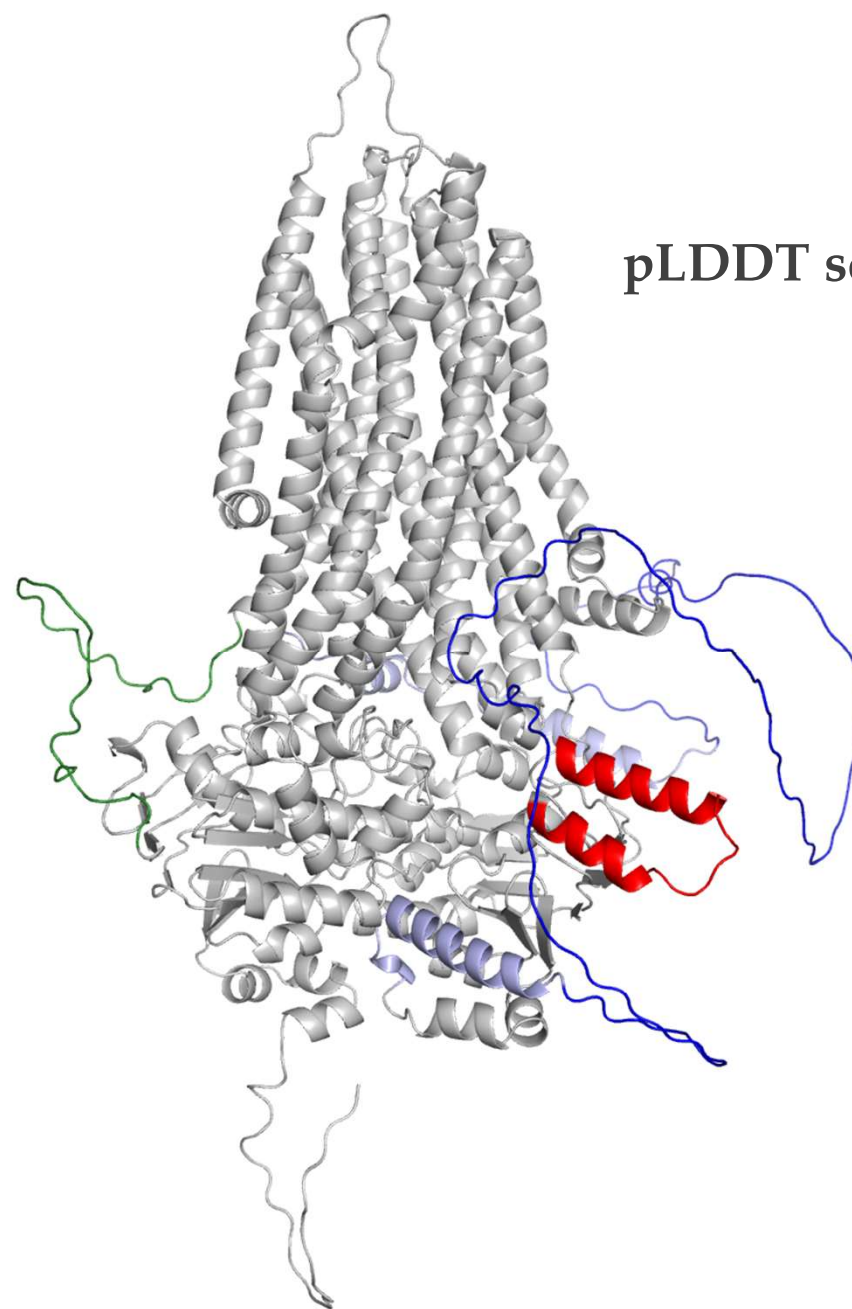
# Allosteric stabilization of TM<sub>IC</sub>

Soya *et al.* Nat. Comm. 2023

- Folded structure
- Correlated motions
- Network
- Community analysis



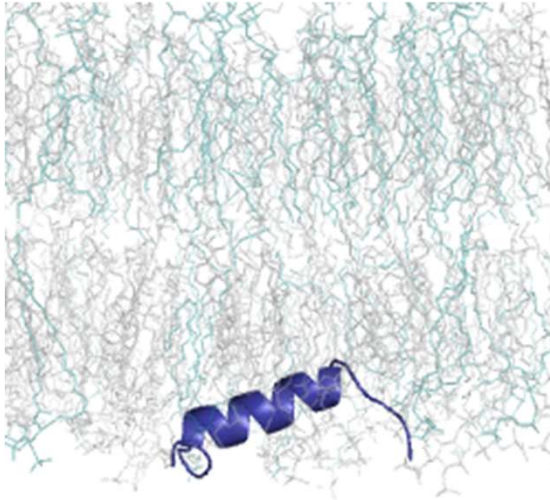
# CFTR – rendezetlen régiók



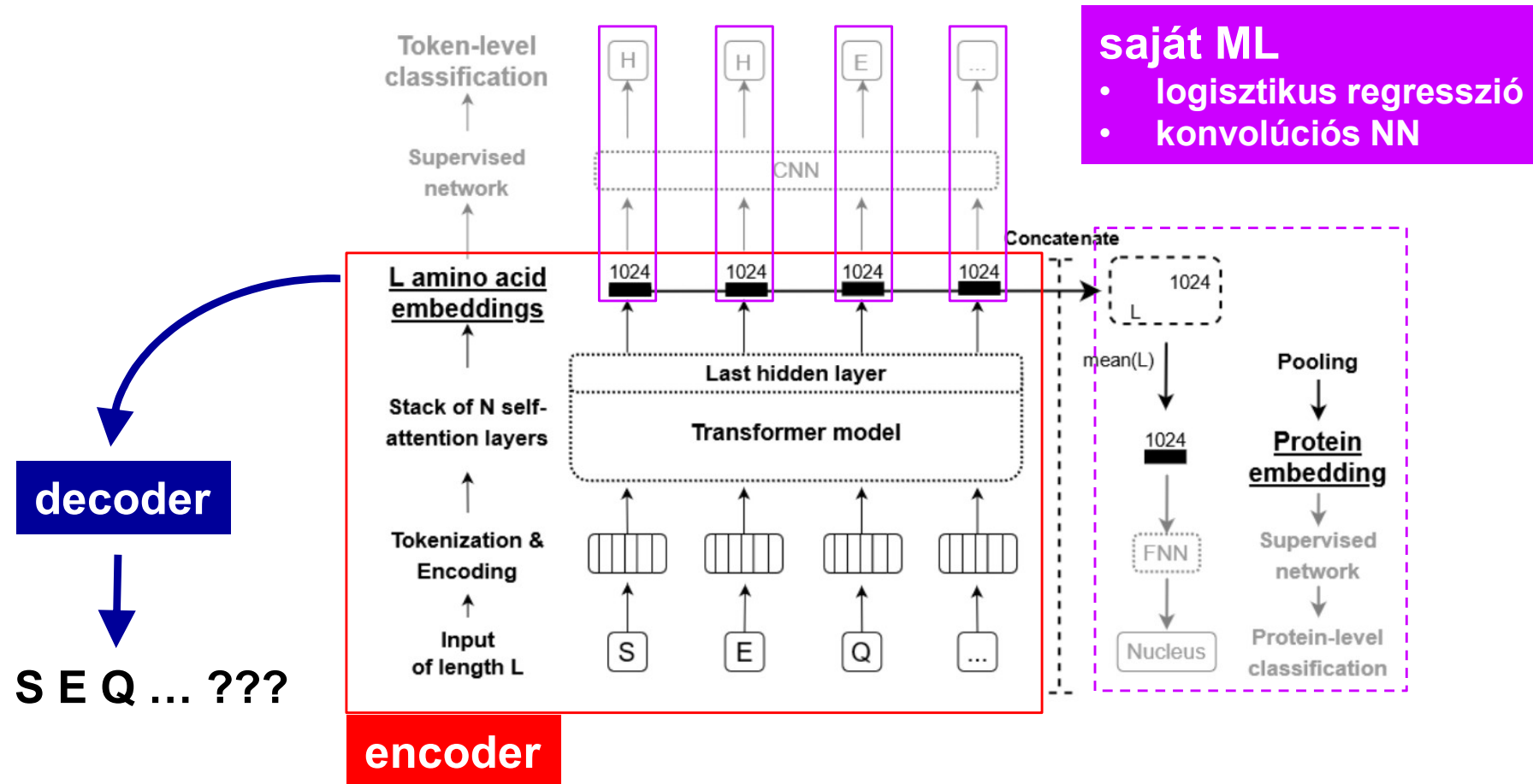
pLDDT score - IDR prediction

# MemMoRFs

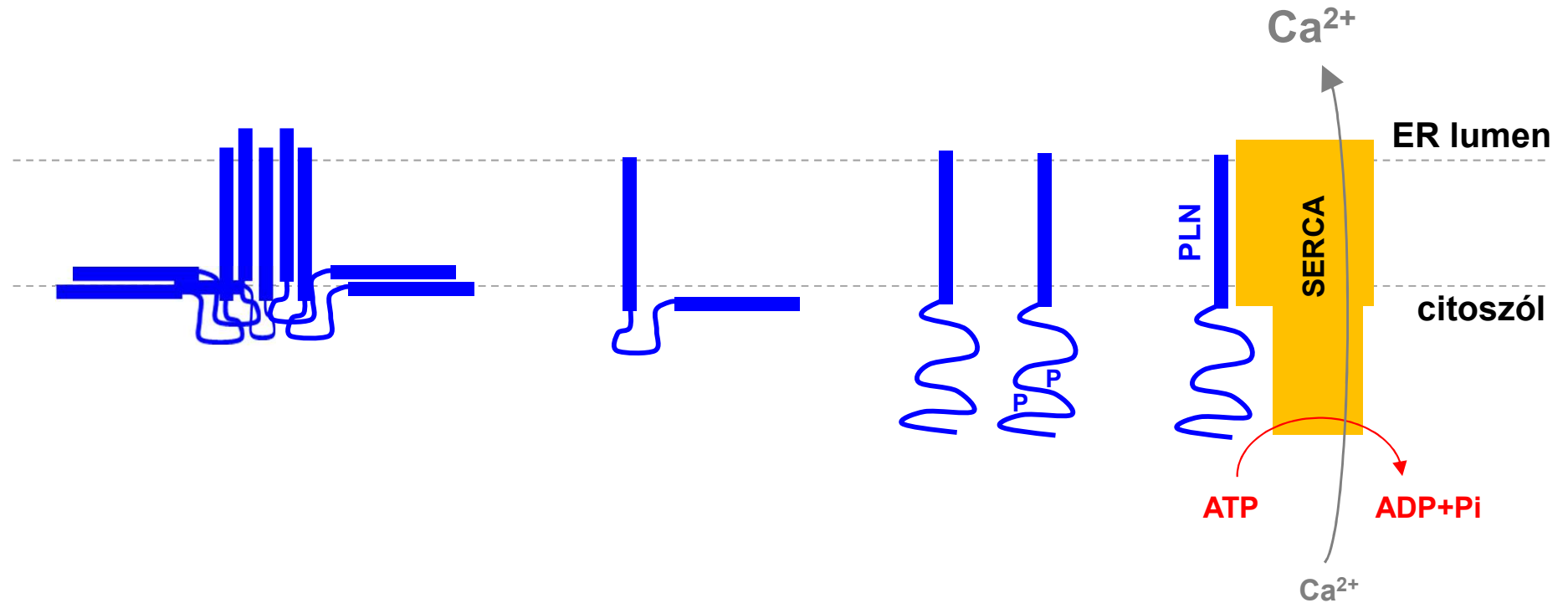
Membrane Molecular Recognition Features, <https://memmorf.hegelab.org>



# MemMoRF prediktor protein Language Models



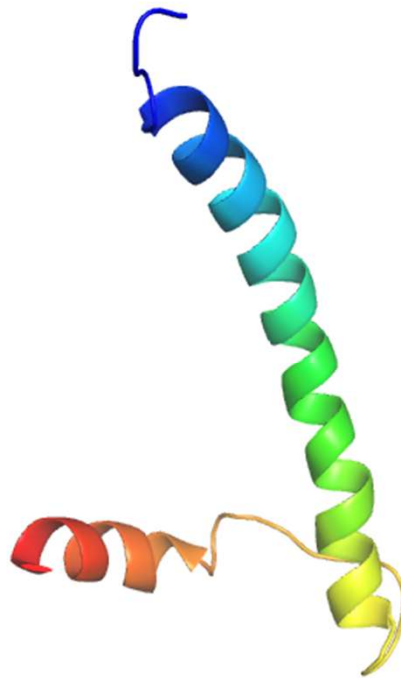
# Kalcium ATP-ázok (SERCA, PMCA)



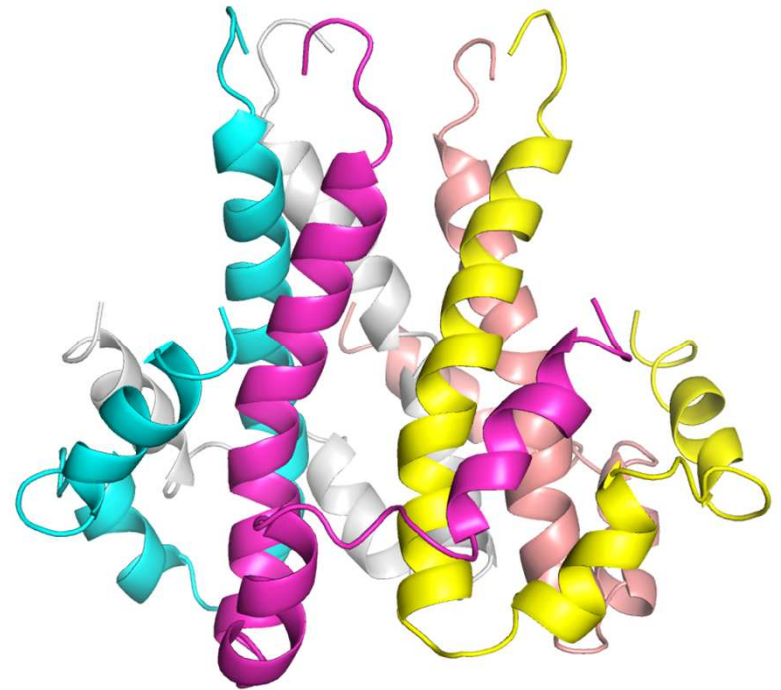
# Regulinok és exoregulinok

SLN  
ELN  
ALN  
Dwarf  
???

SARS Cov-2  
Envelope (E) protein

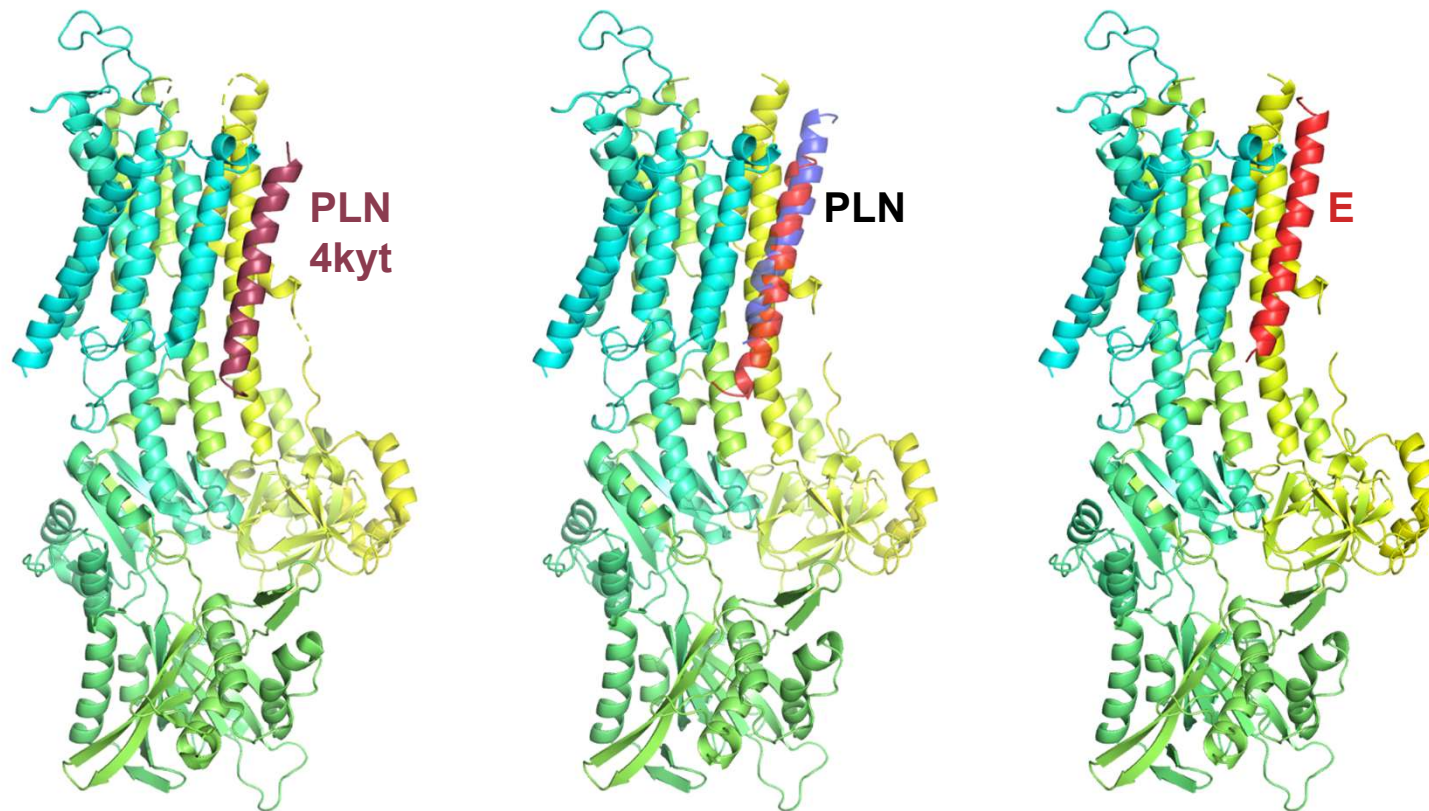


- ERGIC
- viroporin
- PDZ-kötés
- egyéb PPI?



# A SERCA / E protein komplex szerkezete

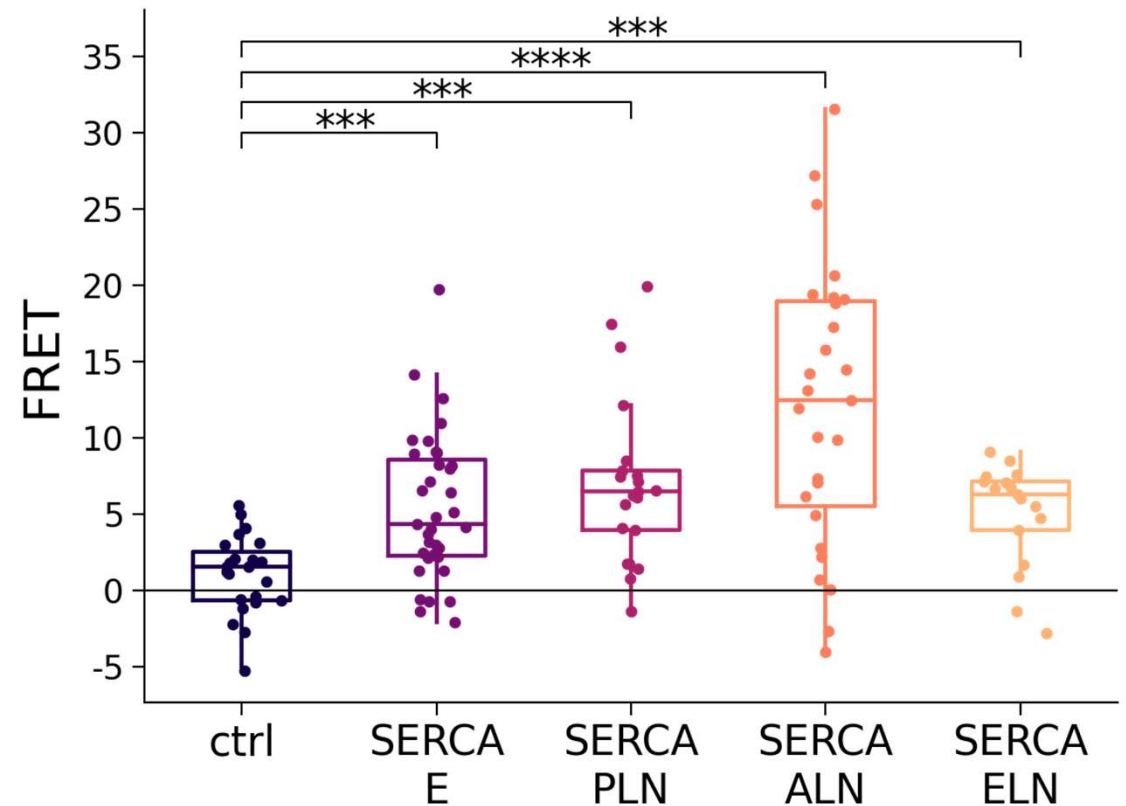
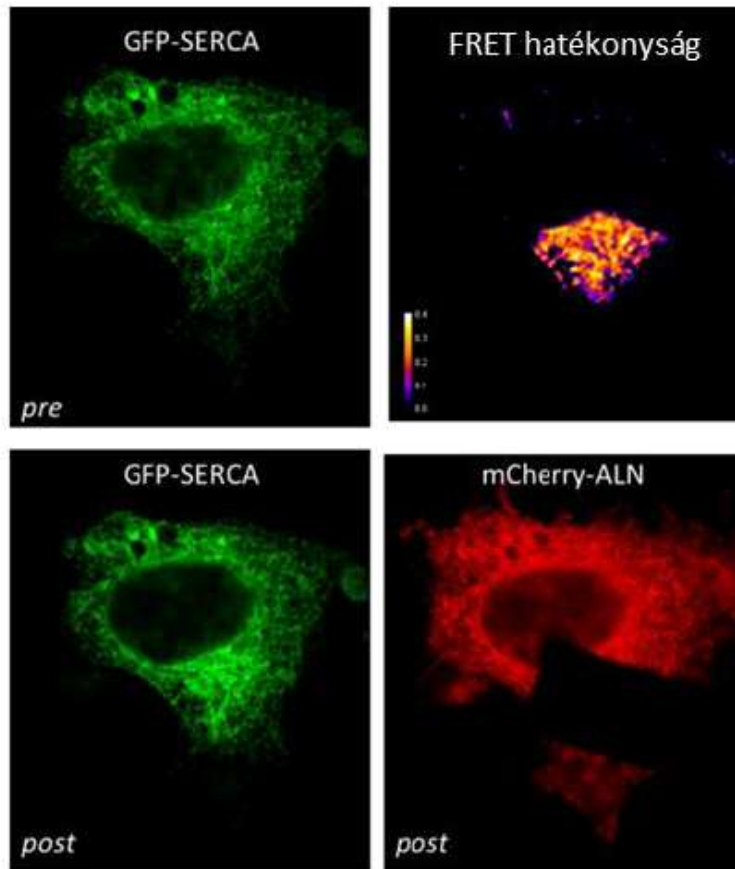
AlphaFold-Multimer  
PIPER/ClusPro



# Az E fehérje kölcsönhat a SERCA pumpával

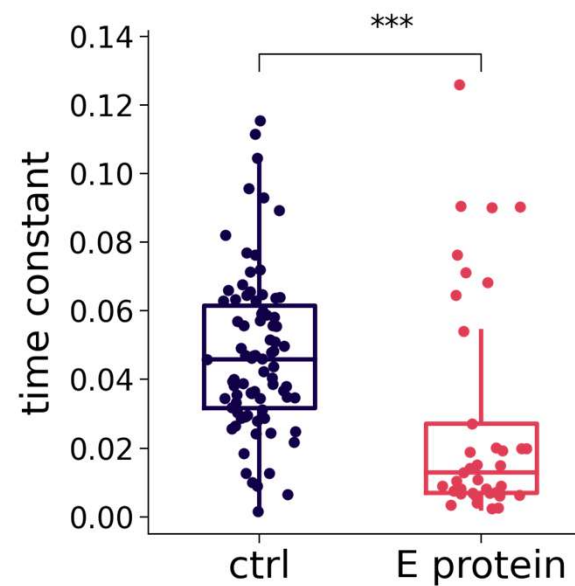
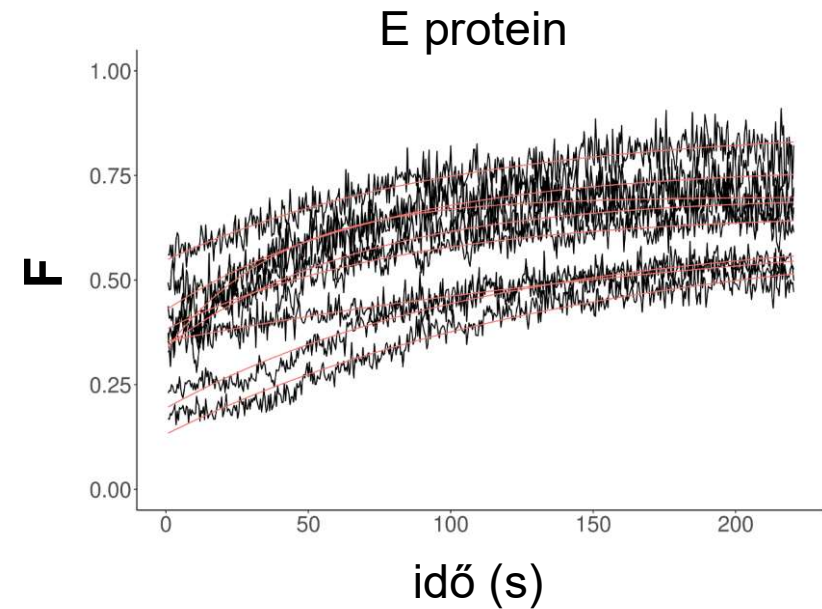
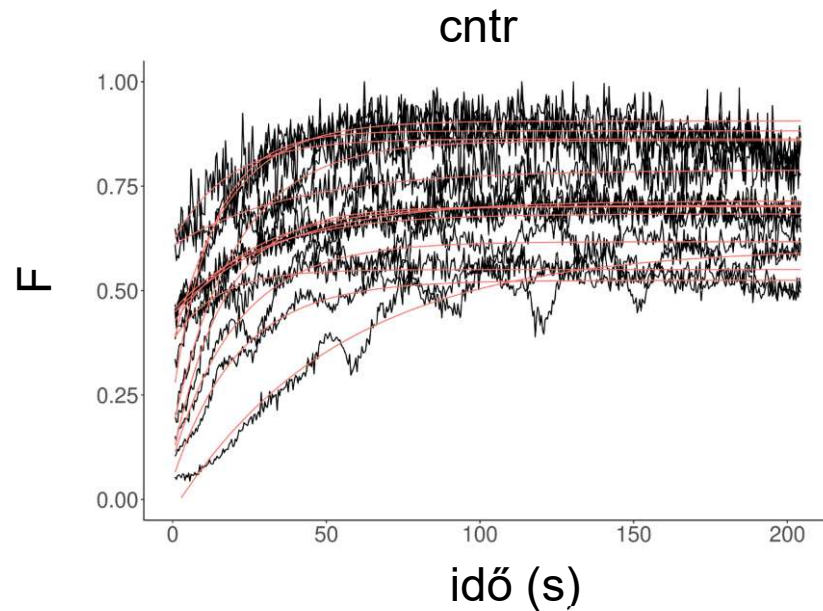
- GFP és mCherry jelölt fehérjék
- koexpresszió HeLa sejtekben
- Lokalizáció átfedés
- AP-FRET

PR: P45



# Az E fehérje gátolja a SERCA működését

nincs EC  $\text{Ca}^{2+}$   
ER ürítése  
+  $\text{Ca}^{2+}$



# AlphaMissense.hegelab.org

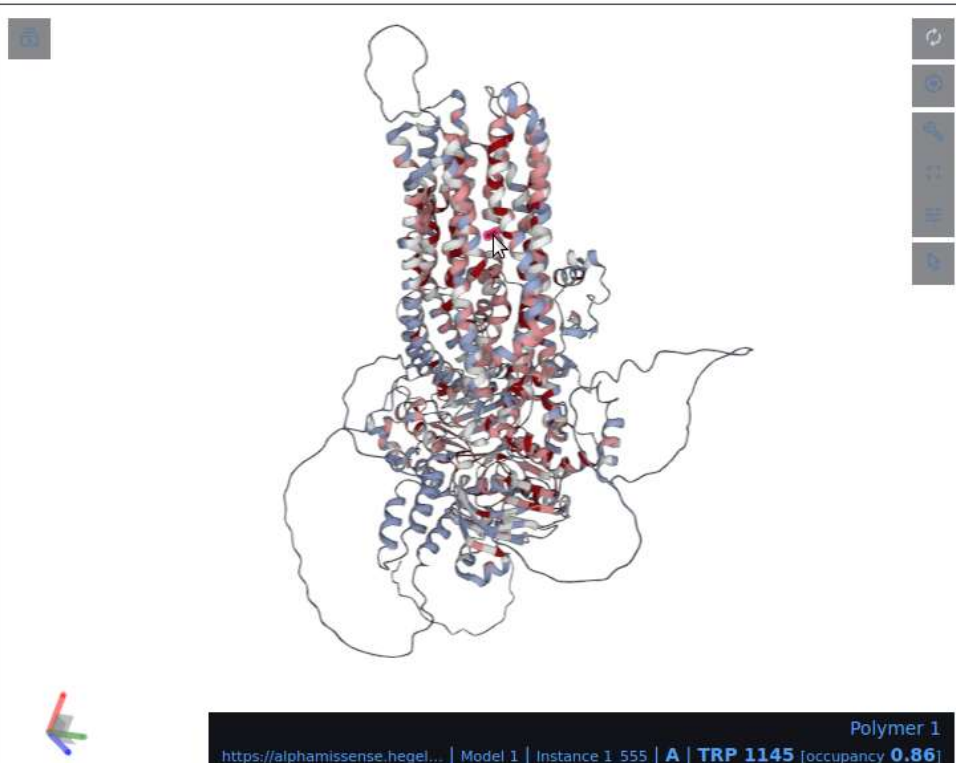
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## Spot mutations

Identifier:  [Get](#)

**CFTR** (CFTR\_HUMAN, P13569, ENST00000003084.10) [Download](#)



https://alphamissense.hegel... | Model 1 | Instance 1\_555 | **A** | **TRP 1145** [occupancy **0.86**] **Polymer 1**

| a.a. | benign        | ambiguous | pathogenic  | mean  |
|------|---------------|-----------|-------------|-------|
| Q2   | 5:K,R,L,P,E   | 1:H       |             | 0.268 |
| R3   | 3:T,K,G       | 3:S,W,M   |             | 0.338 |
| S4   | 2:T,A         |           | 3:L,W,P     | 0.492 |
| P5   |               | 1:A       | 5:T,R,L,S,H | 0.603 |
| L6   | 4:V,M,Q,R     | 1:P       |             | 0.237 |
| E7   | 3:D,Q,G       | 2:V,A     | 1:K         | 0.403 |
| K8   | 6:T,R,N,Q,E,M |           |             | 0.156 |
| A9   | 1:S           | 1:G       | 4:D,V,P,T   | 0.622 |
| S10  | 4:N,T,C,G     | 2:I,R     |             | 0.217 |
| V11  | 6:I,F,L,D,A,G |           |             | 0.139 |
| V12  | 5:I,F,L,A,G   |           | 1:D         | 0.189 |
| S13  | 1:A           | 2:T,C     | 3:F,P,Y     | 0.525 |
| K14  | 5:I,T,R,Q,E   | 1:N       |             | 0.265 |
| I15  | 2:E,I,V       |           | 2:D,H,P     | 0.286 |

# Összefoglalás

- Fehérje szerkezet
- Fehérje dinamika
- Számításos módszerek igen fontosak
- Fehérjék működésének és mutációk hatásának megismerése
- Gyógyszertervezés

[www.hegelab.org](http://www.hegelab.org)  
[hegedus.tamas@hegelab.org](mailto:hegedus.tamas@hegelab.org)