

Elméleti módszerek lipidek és membránfehérjék tanulmányozására

Hegedűs Tamás

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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

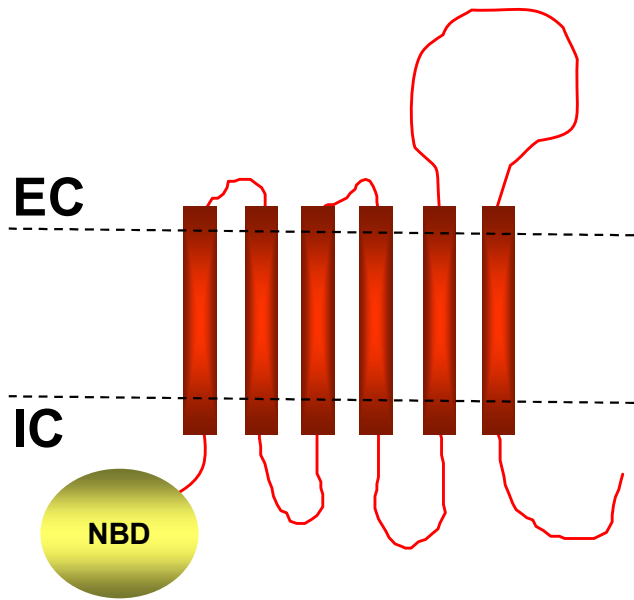
Atomi szintű információt ad mozgásokról.

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

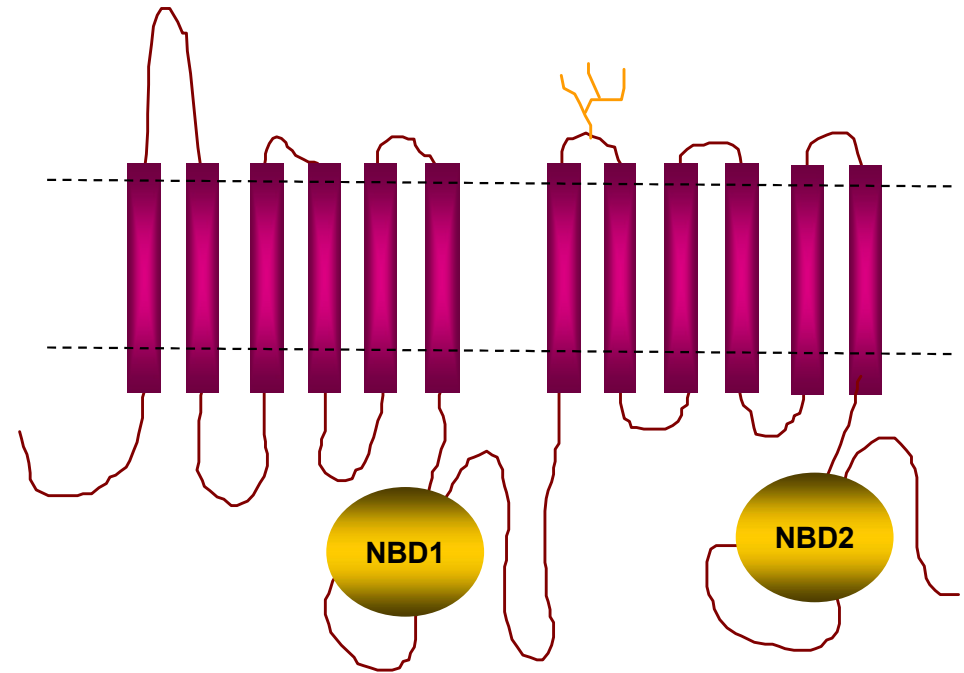
Membránfehérjék szerkezeti modellezése

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

ATP Binding Cassette (ABC) fehérjék



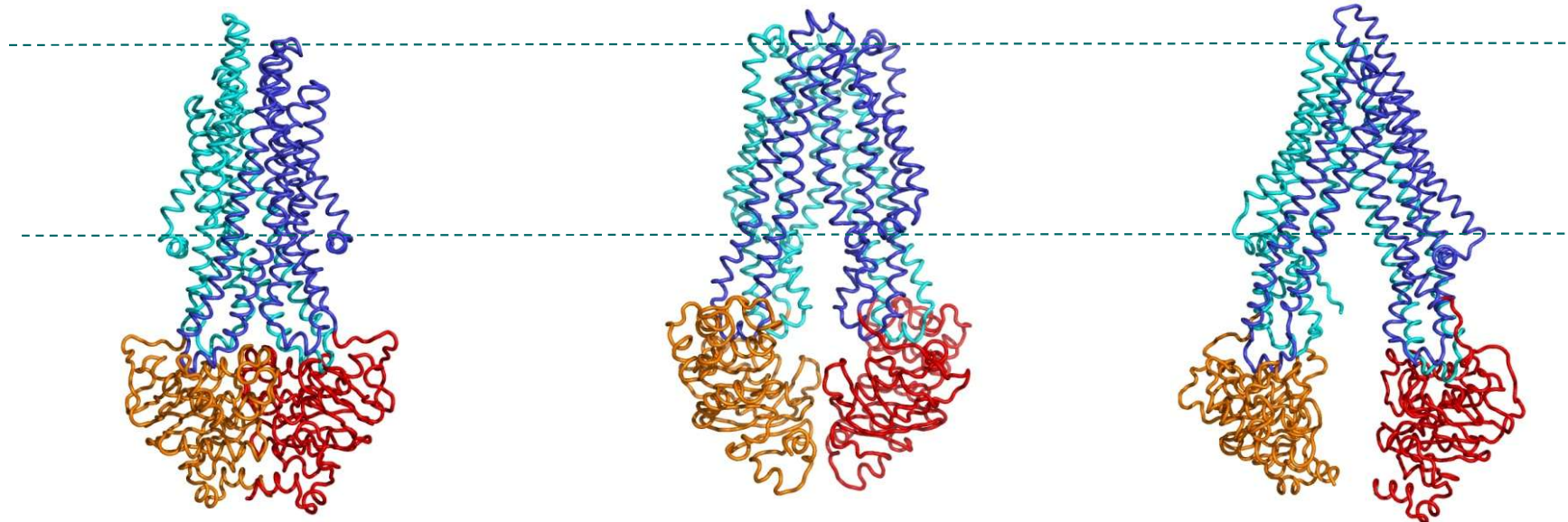
Fél-transzporterek
pl. humán ABCG fehérjék



full transporters
pl. P-glycoprotein, CFTR

ABC fehérjék konformációi

(Pgp-szerű)

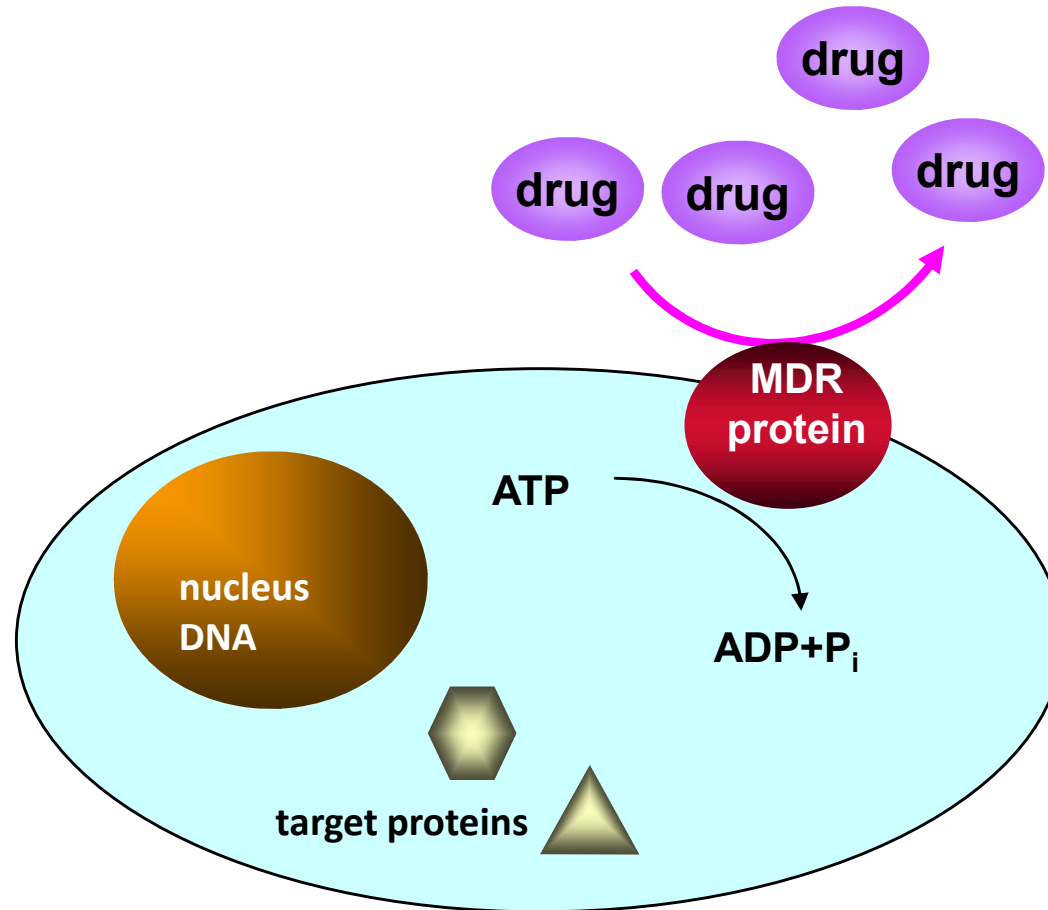


**“bottom-closed”
(+ATP)**

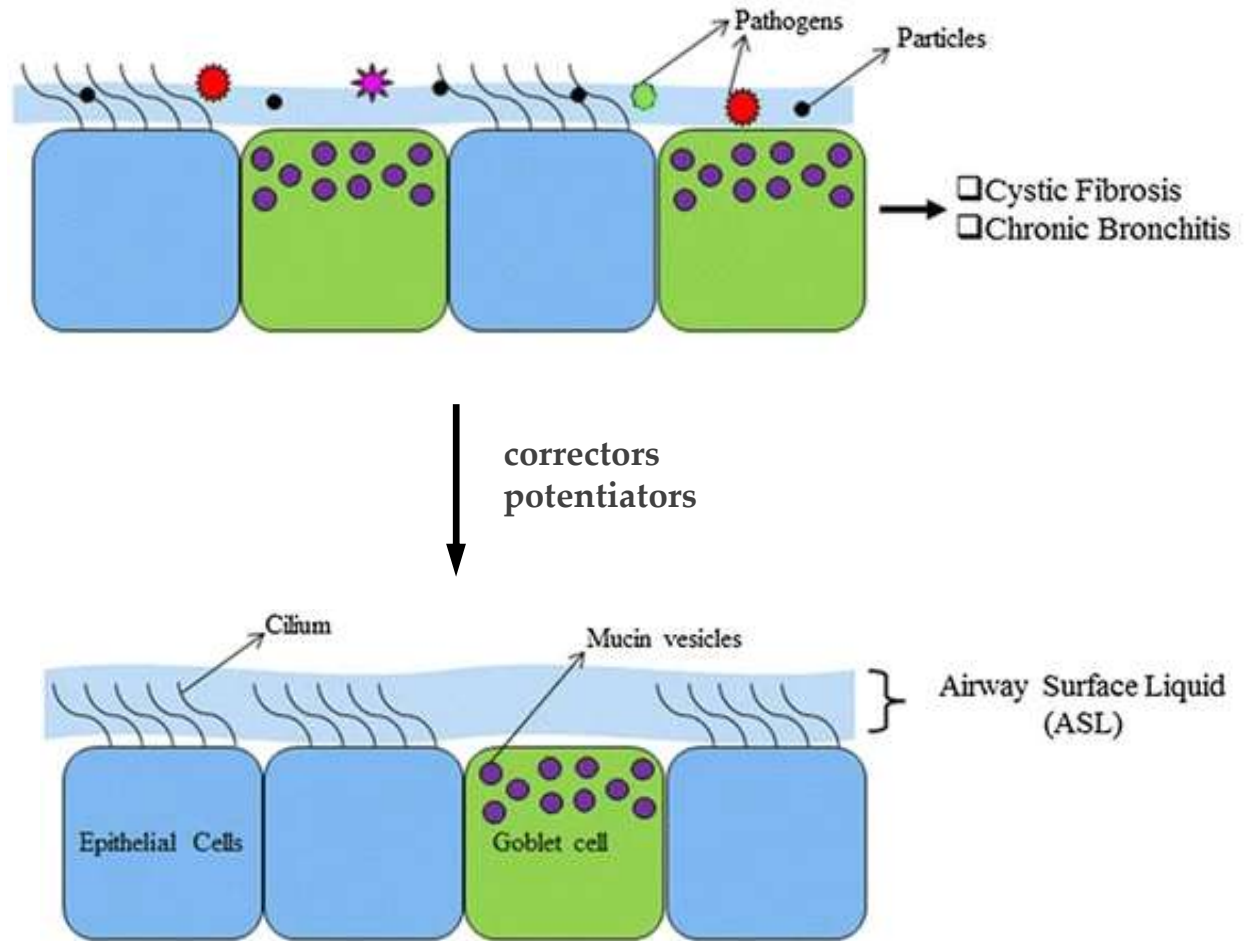
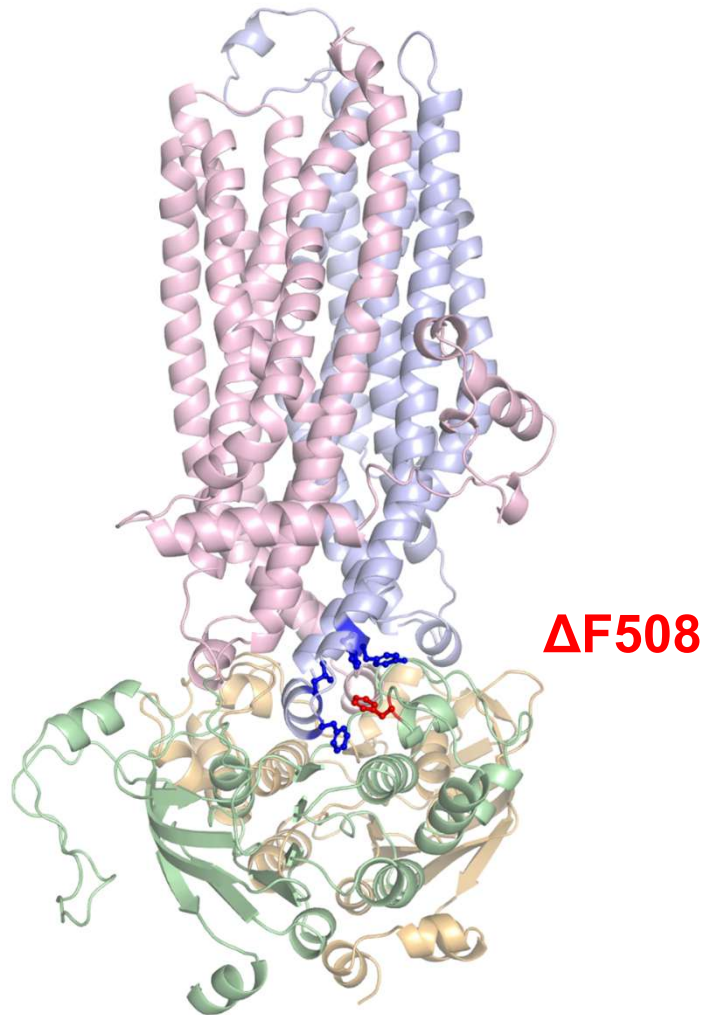
**“bottom-closed” apo
(-ATP)**

**“bottom-open” apo
(-ATP)**

Multidrog transzport – ABC fehérjék

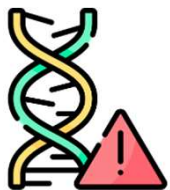


Cisztás fibrózis (CF)

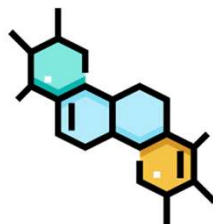


Ghosh, Boucher, Tarran,
CMLS 2015

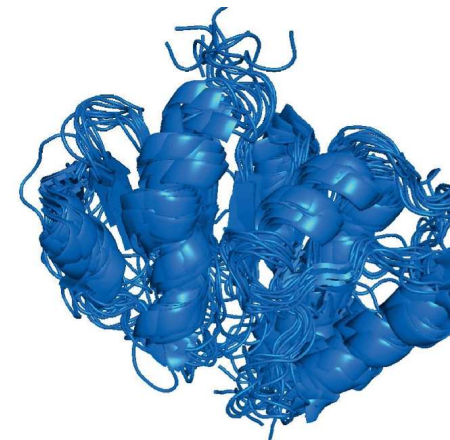
Célkitűzéseink



**Atomic-level basis of
diseases**

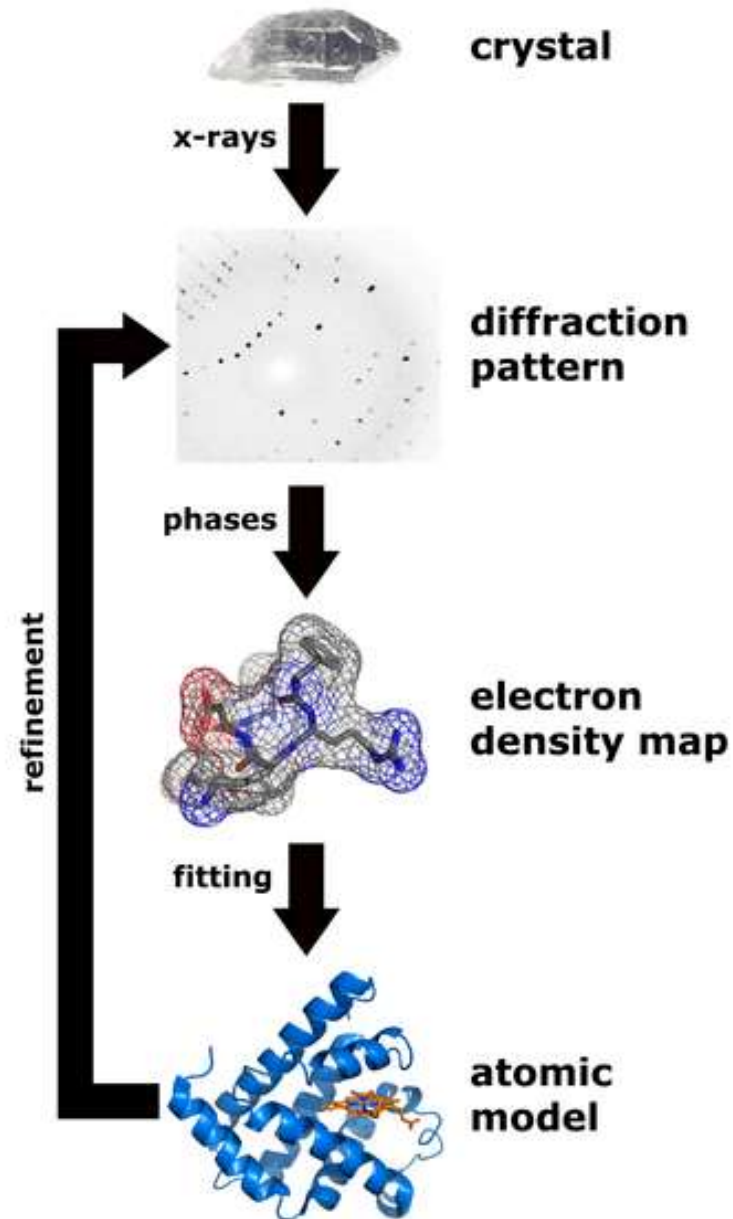


**Identification
of drug binding sites**



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

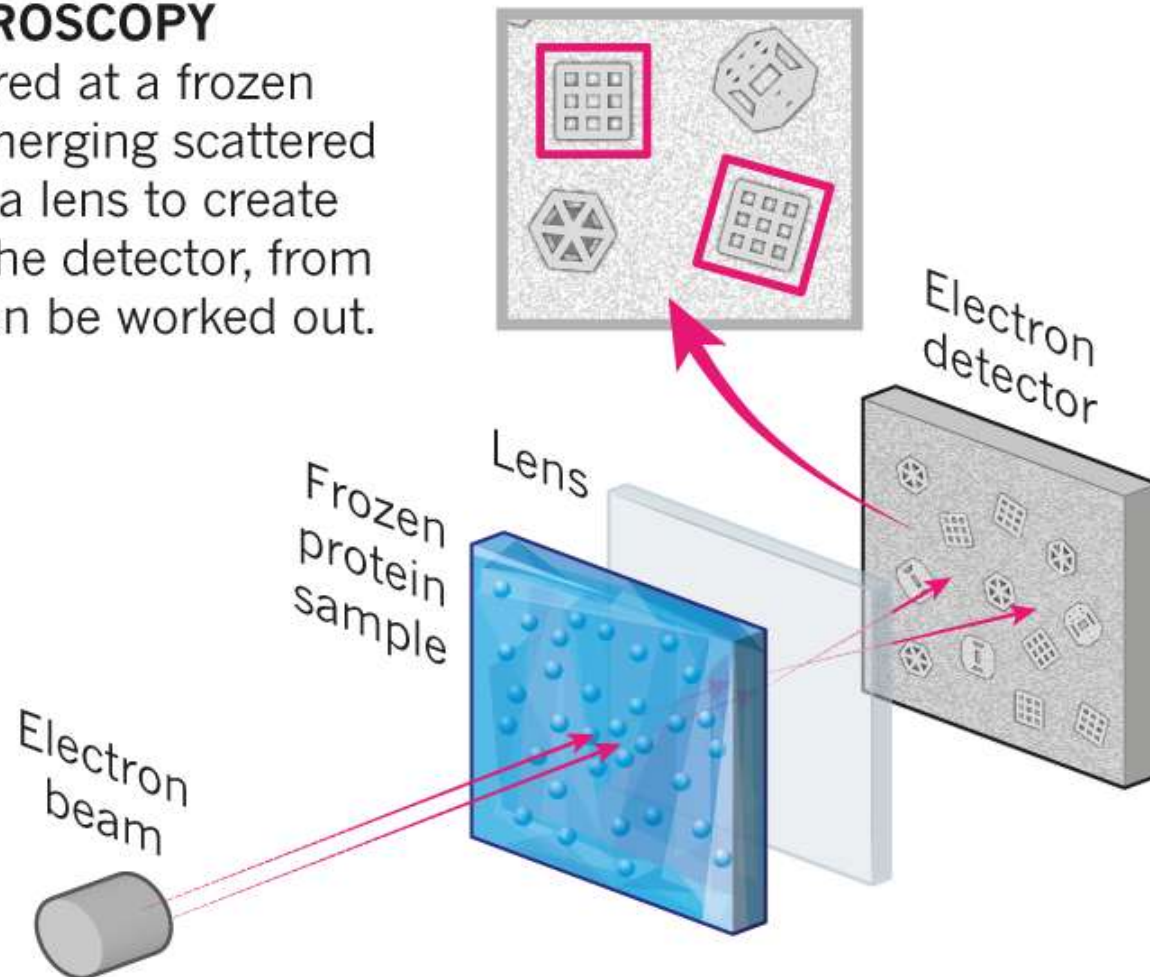
Szerkezet meghatározás – Röntgen kristallográfia



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.



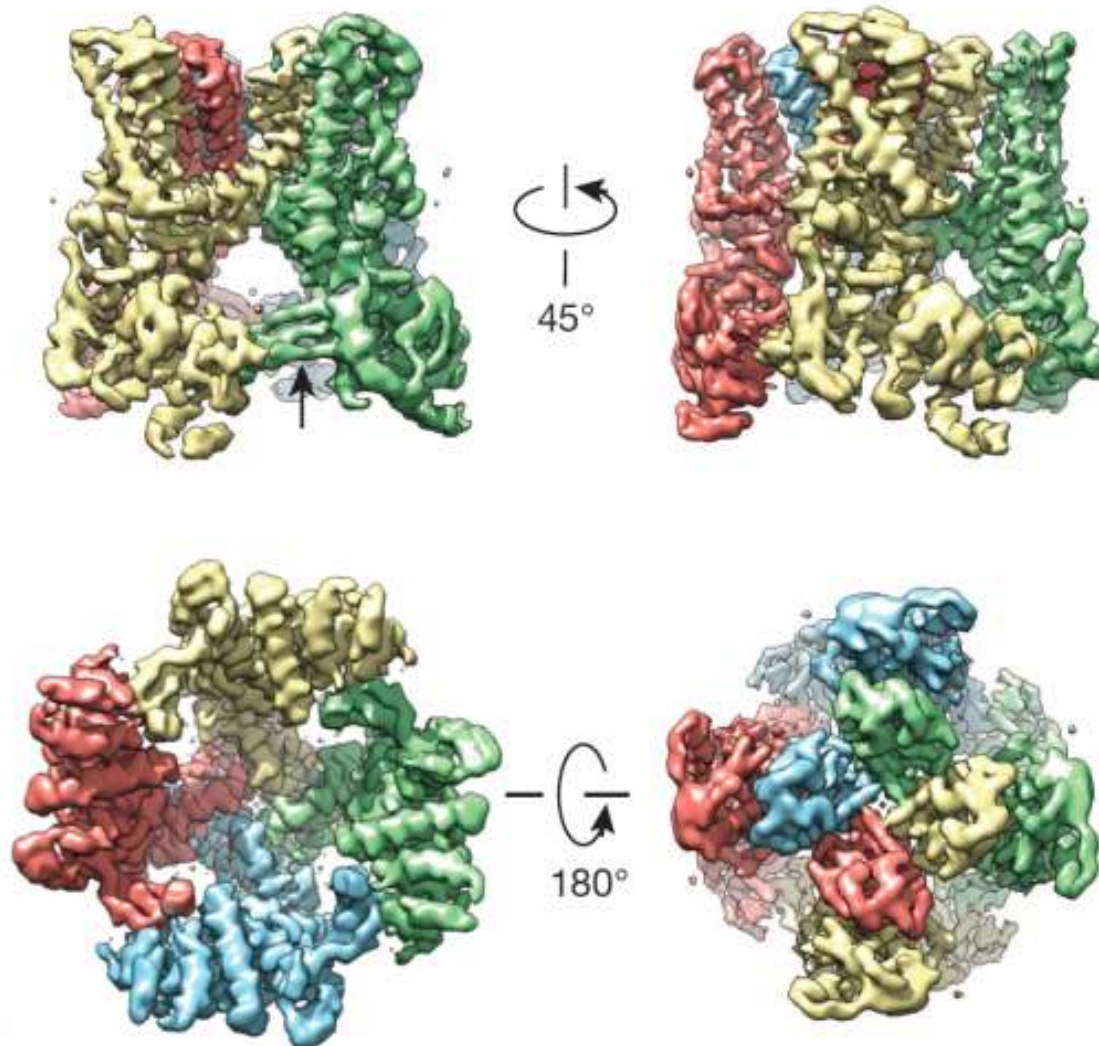
© nature

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.



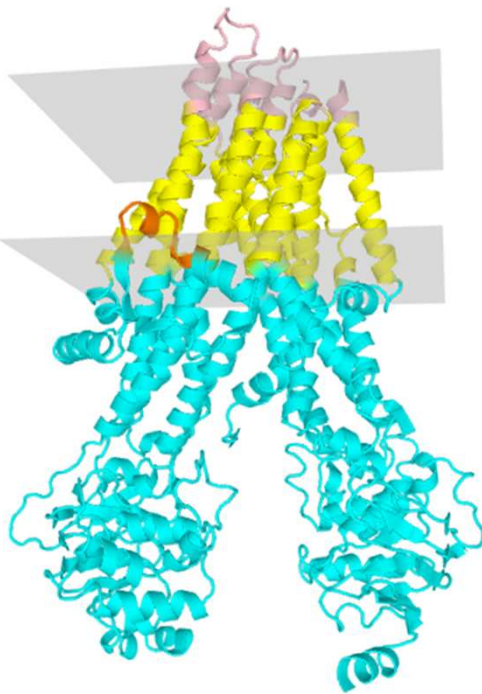
Membrántopológia és TM hélixek predikciója szekvencia alapján

- 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

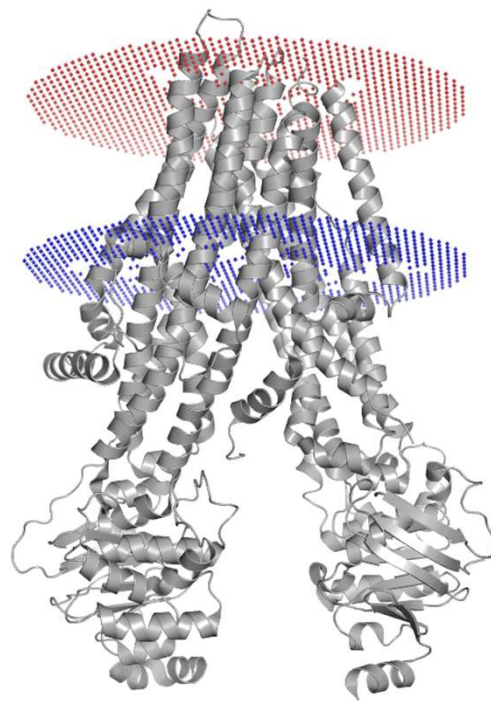
TM hélixek predikciója szerkezet alapján

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

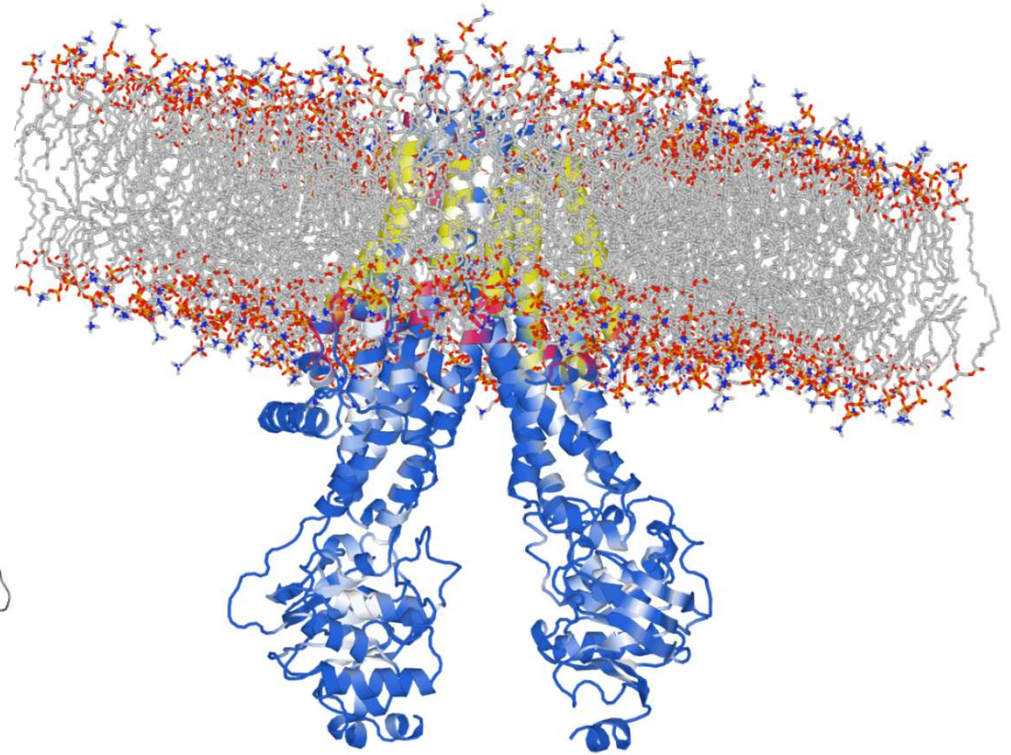
TMDet
PDBTM



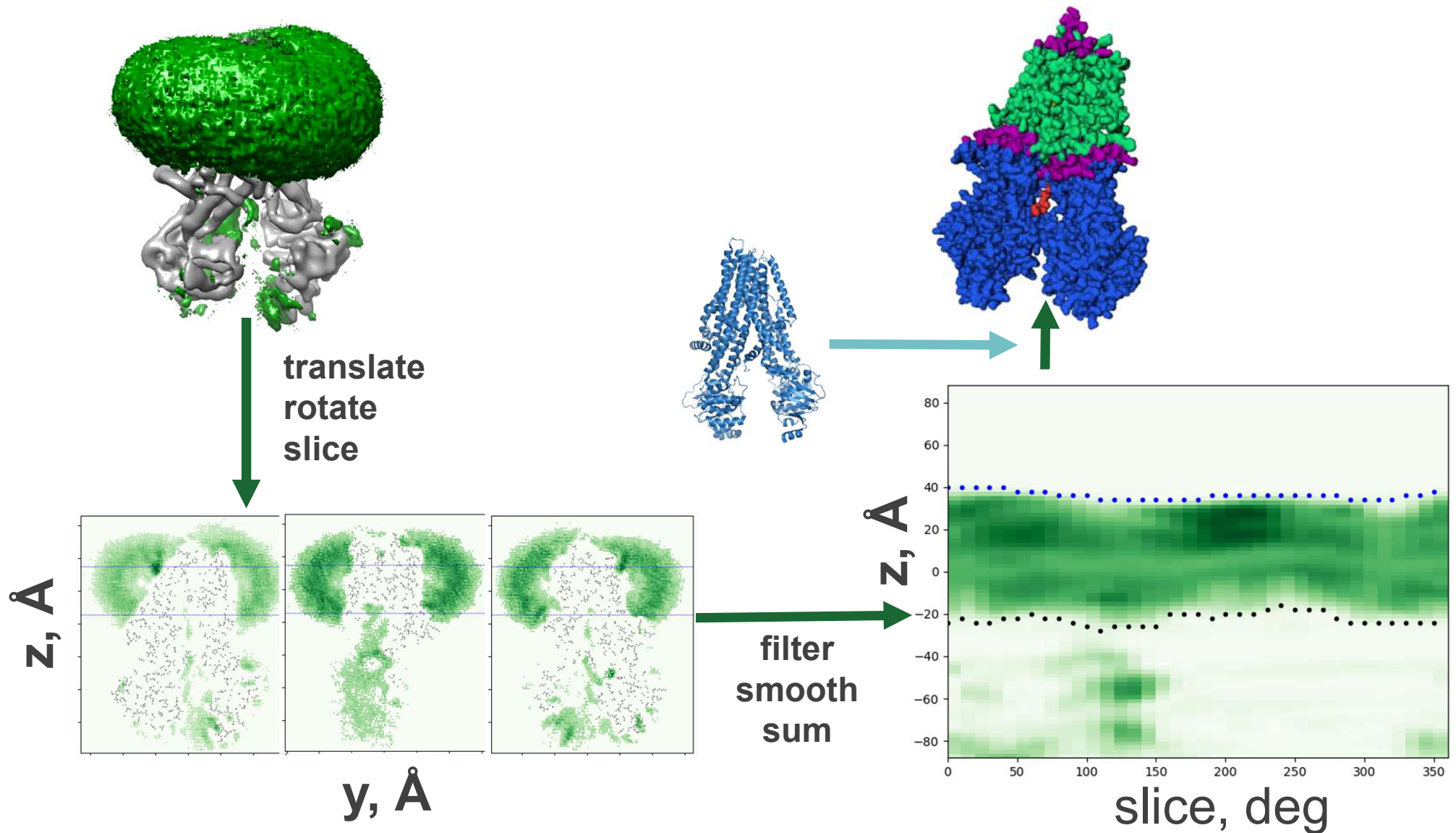
PPM
OPM



MemProtMD

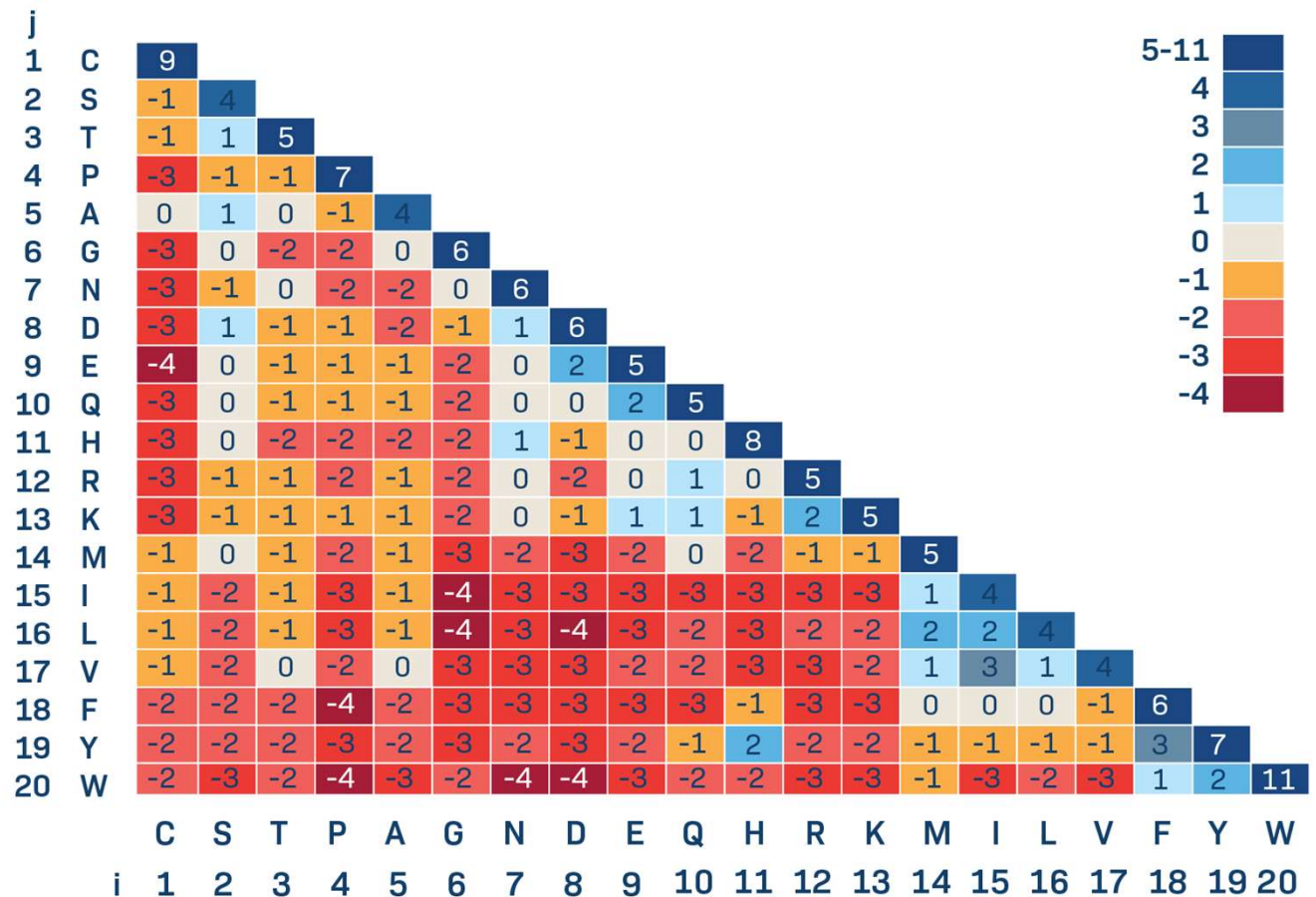


The MemBlob can be converted to membrane boundaries



Homológia modellezés I.

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



BLOSUM62
(BLOCKS of Amino Acid SUBstitution Matrix)

<https://www.labxchange.org>

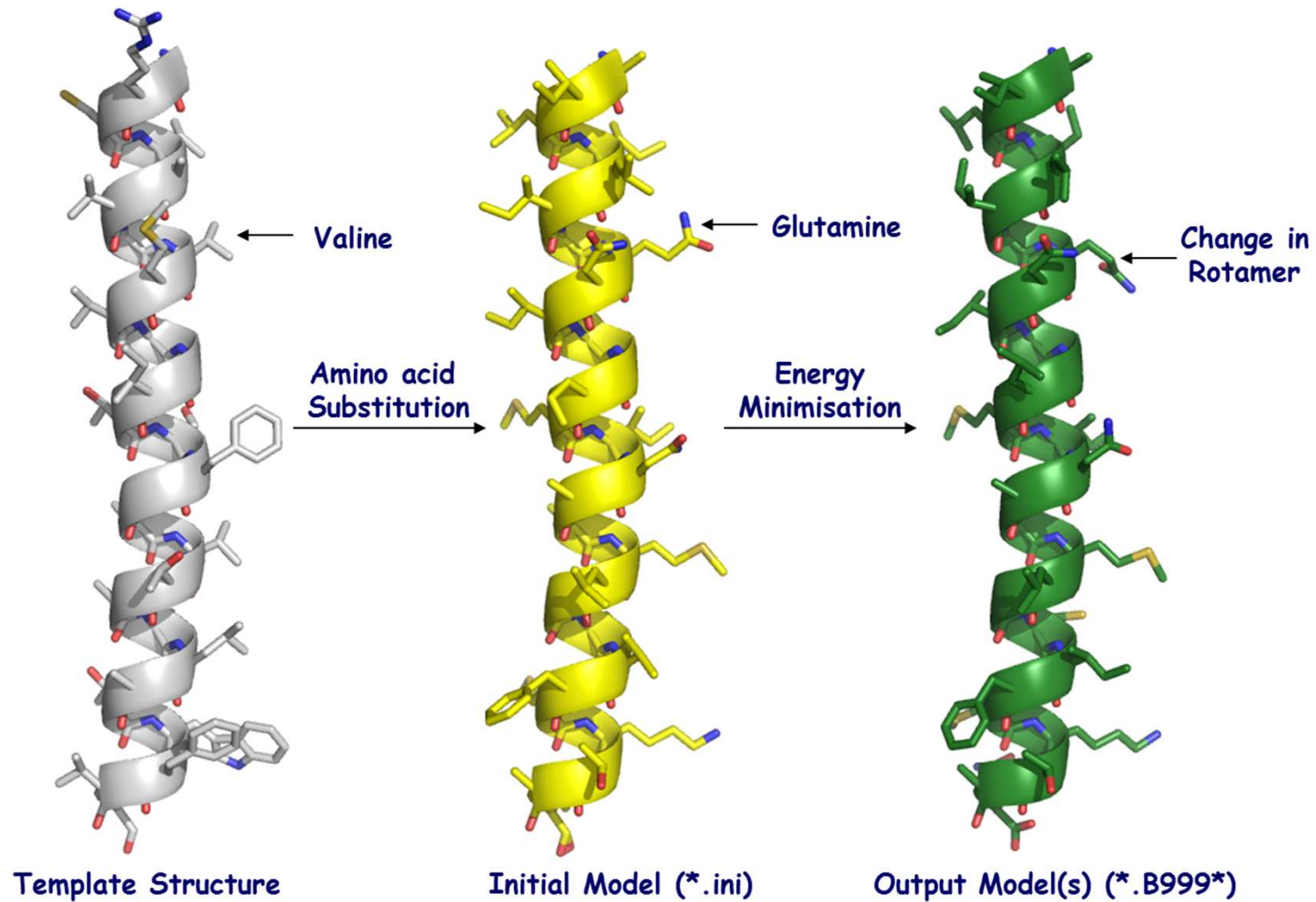
Alignment – pl. ClustalW

CLUSTAL W (1.83) multiple sequence alignment

```

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

Homológia modellezés II.



AlphaFold2

machine learning, deep learning, AI

Jumper et al. Nat 2021

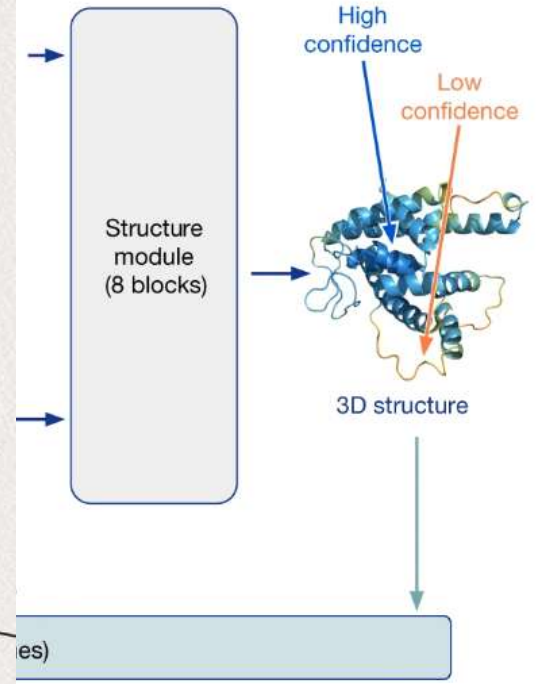
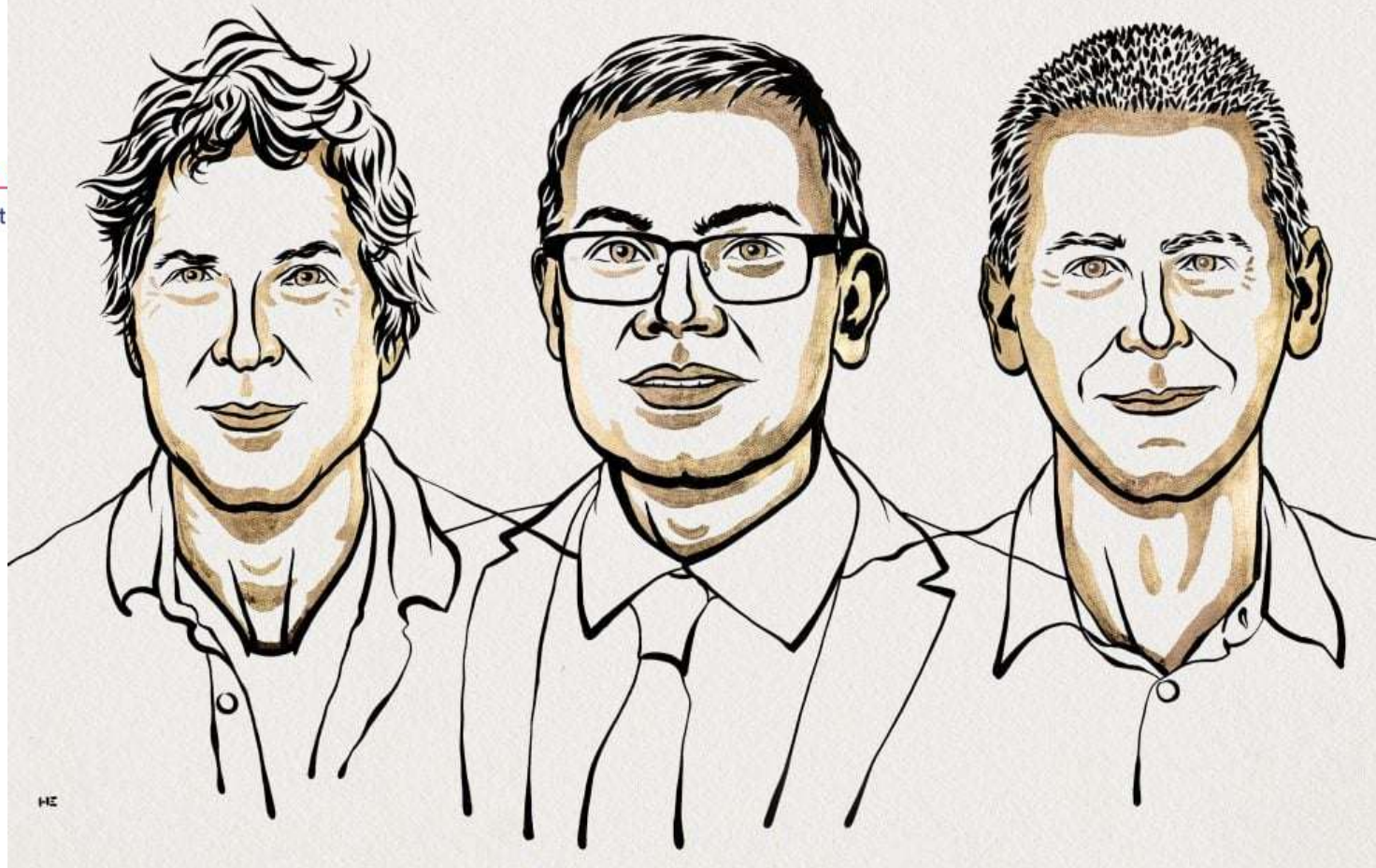
e

D. Baker

D. Hassabis

J. Jumper

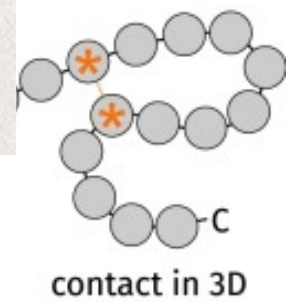
Input



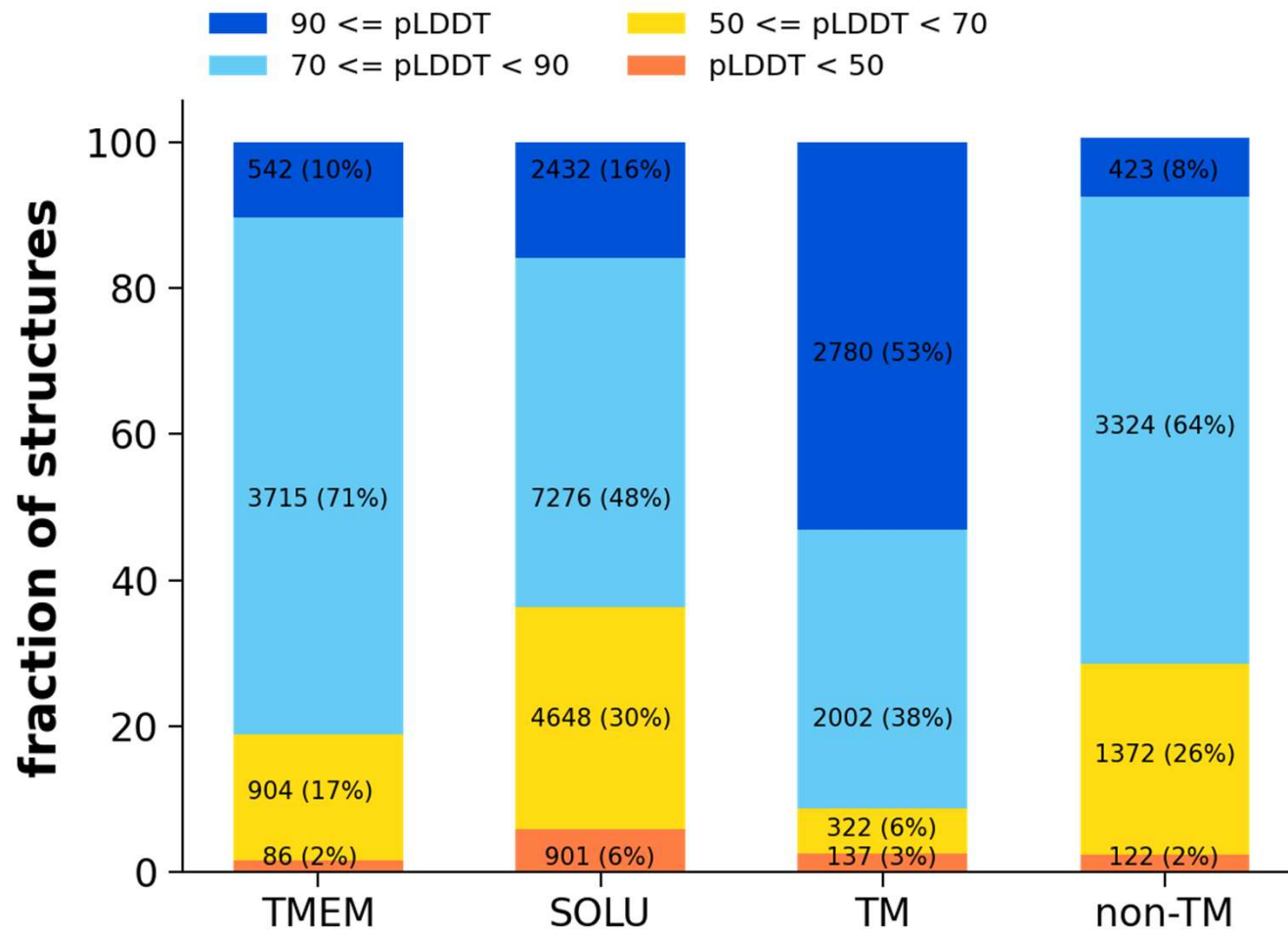
A	T	K	L	T	L	T	A	K	K	E	G	P	K	D
A	T	K	L	T	L	G	A	K	K	E	G	G	C	D
A	T	W	L	T	L	T	A	K	K	V	G	P	C	D
A	T	W	L	T	L	T	A	K	K	V	G	P	C	D

← constraint

↑ coevolution ↑

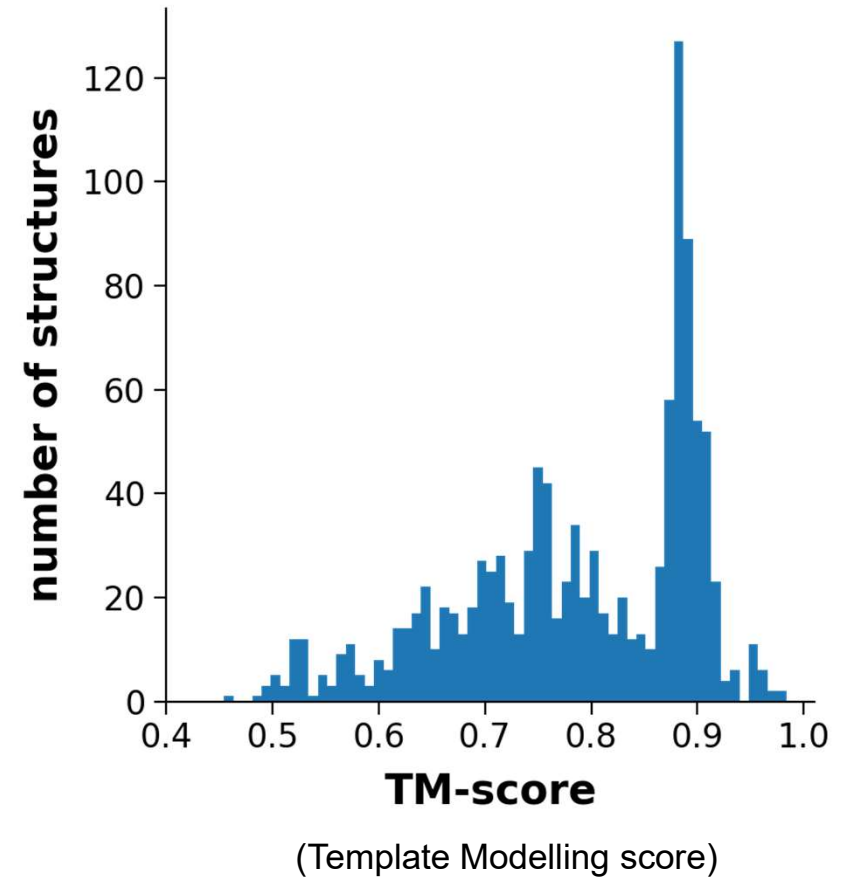
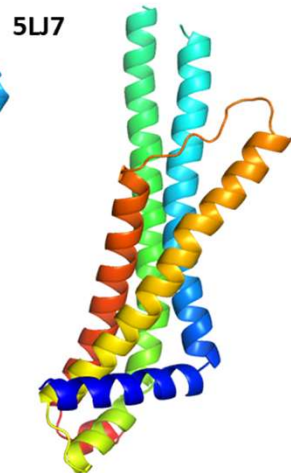
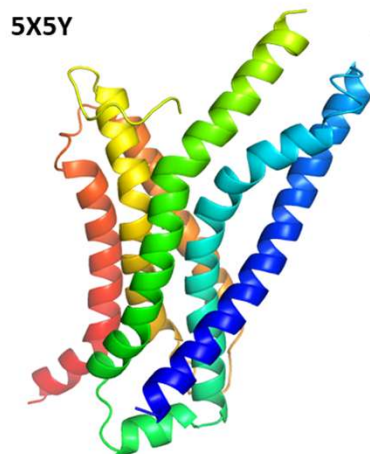
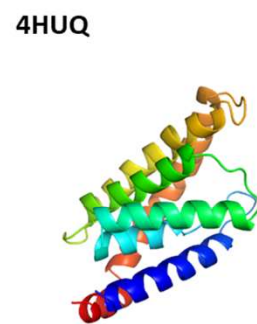
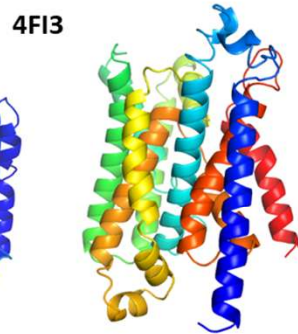
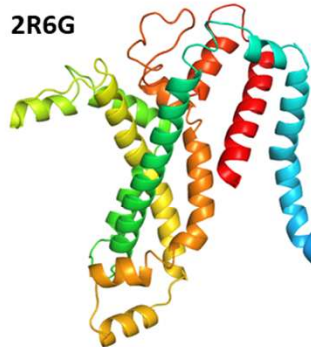
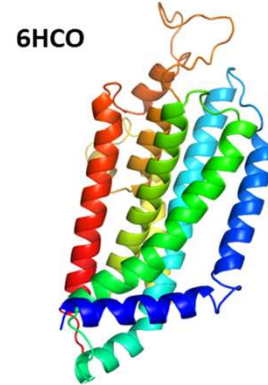


Transzmembrán fehérjék szerkezeti predikciója

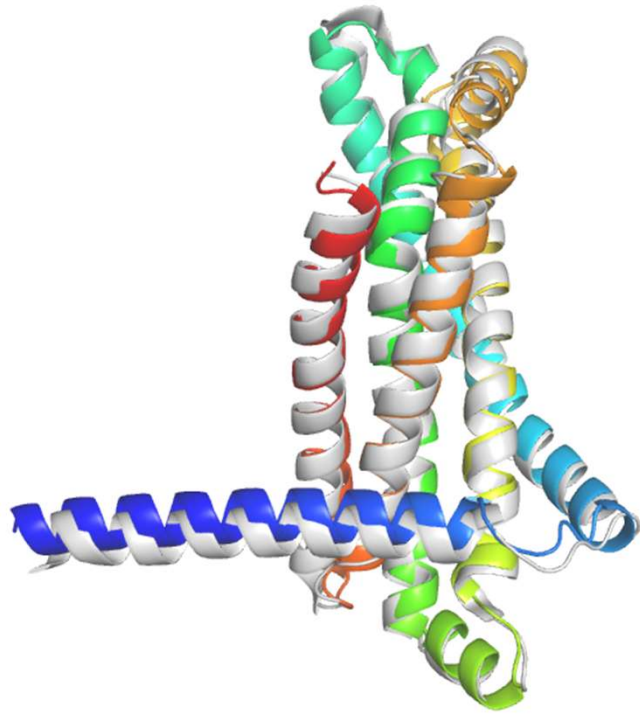


ABC fehérje szerkezetek

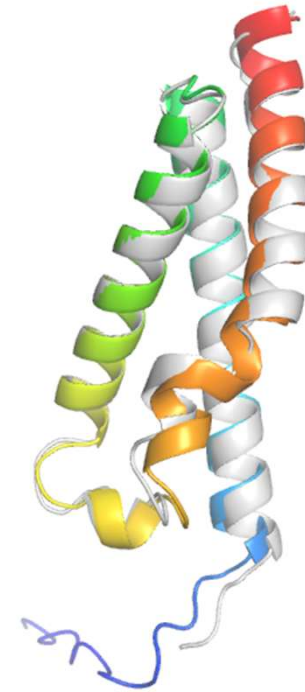
fold class	reference PDB
Pgp-like	4M1M
ABCG2-like	6HCO
MalFG-like	2R6G
BtuC-like	4F13
EcfT-like	4HUQ
LptFG-like	5X5Y
MacB-like	5LJ7
MlaE-like	7CHO



Új transzmembrán szerkezetek predikciója



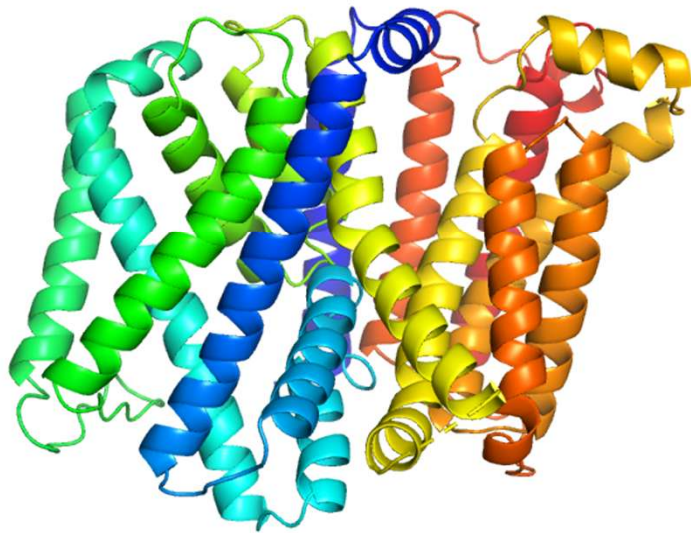
MlaE-like fold
PDBID: 7ch0
RMSD of 1.28 Å



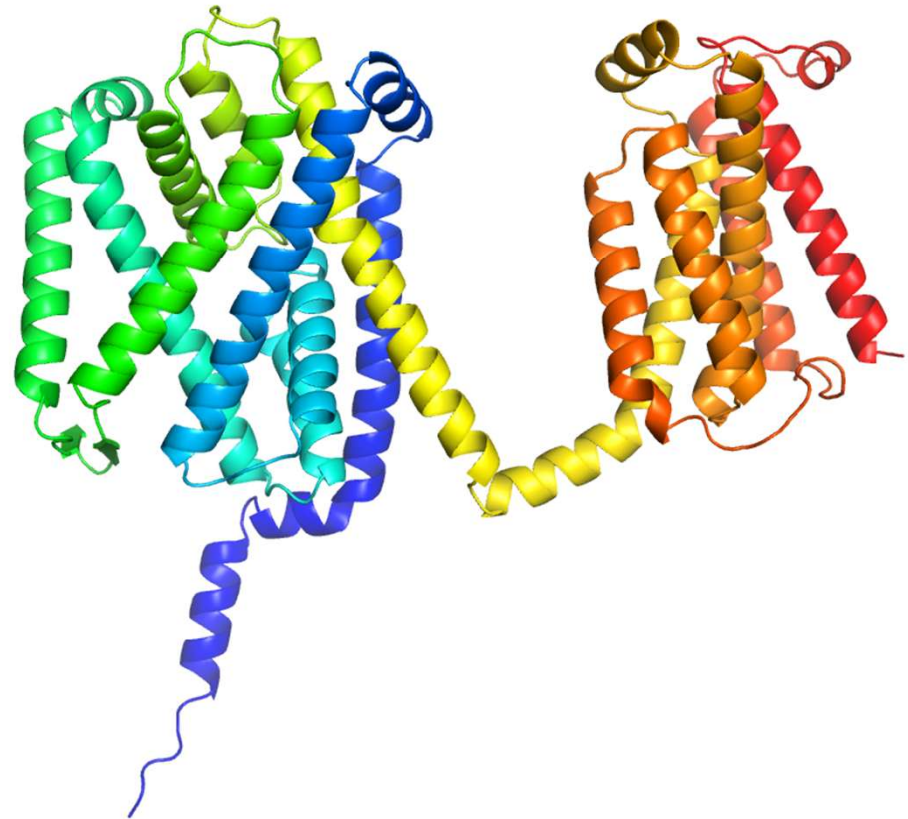
ER membrane protein complex subunit 6
PDBID: 6ww7
RMSD of 0.96 Å

Új transzmembrán szerkezetek predikciója

MprF (PDBID: 7DUW)



AF2



CFTR TM8

Farkas *et al.* CMLS 2019

Hegedus *et al.* CMLS 2022

cryo-EM

homology modelling

1/22 simulations, $r \geq 1.8$

5/6 simulations

Å

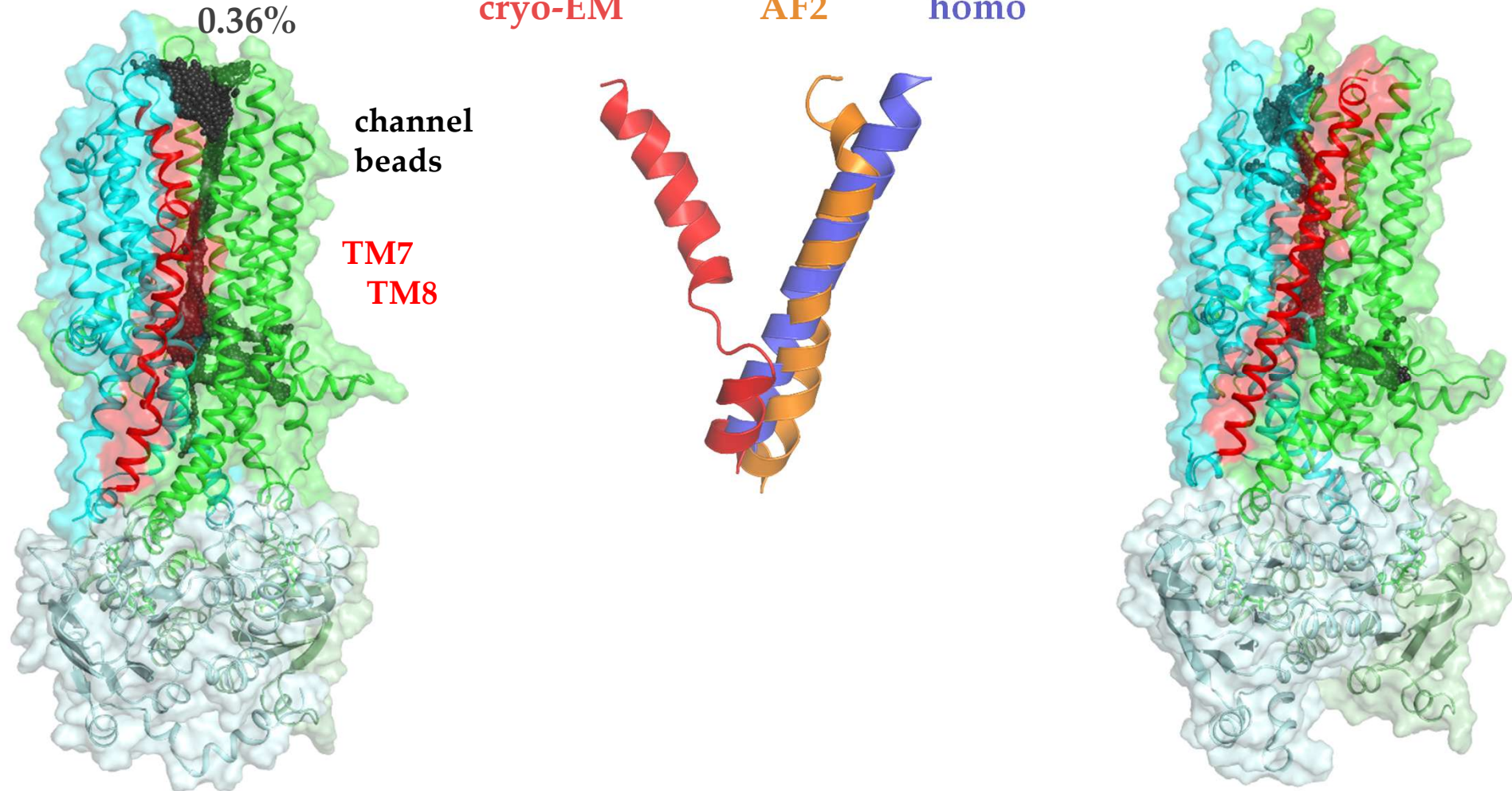
3.74%

0.36%

cryo-EM

AF2

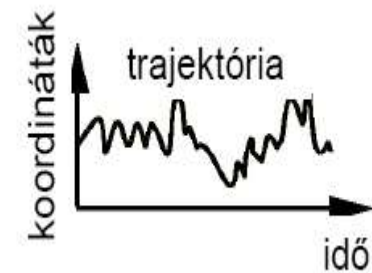
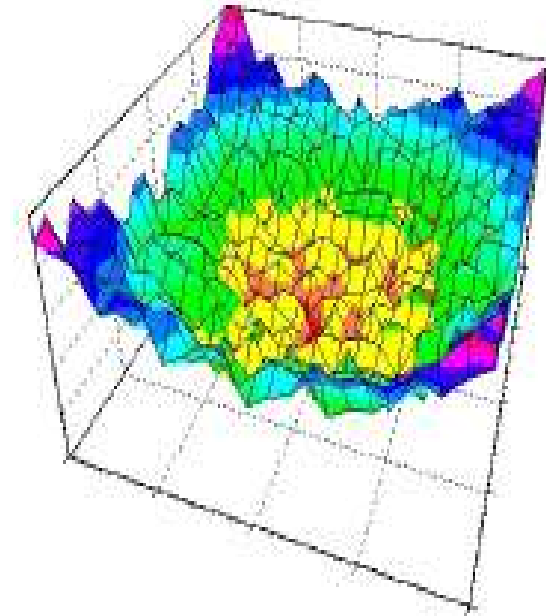
homo



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

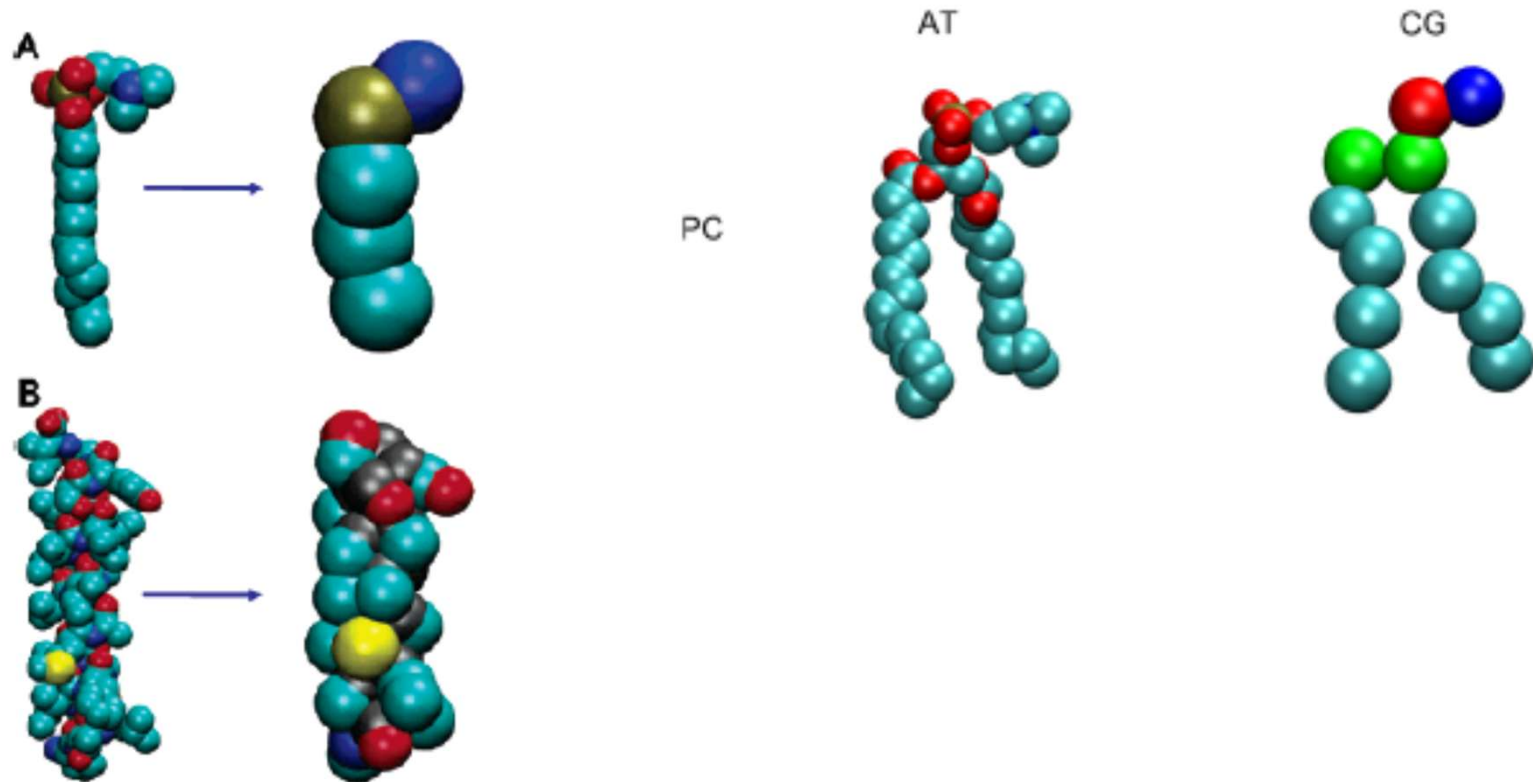
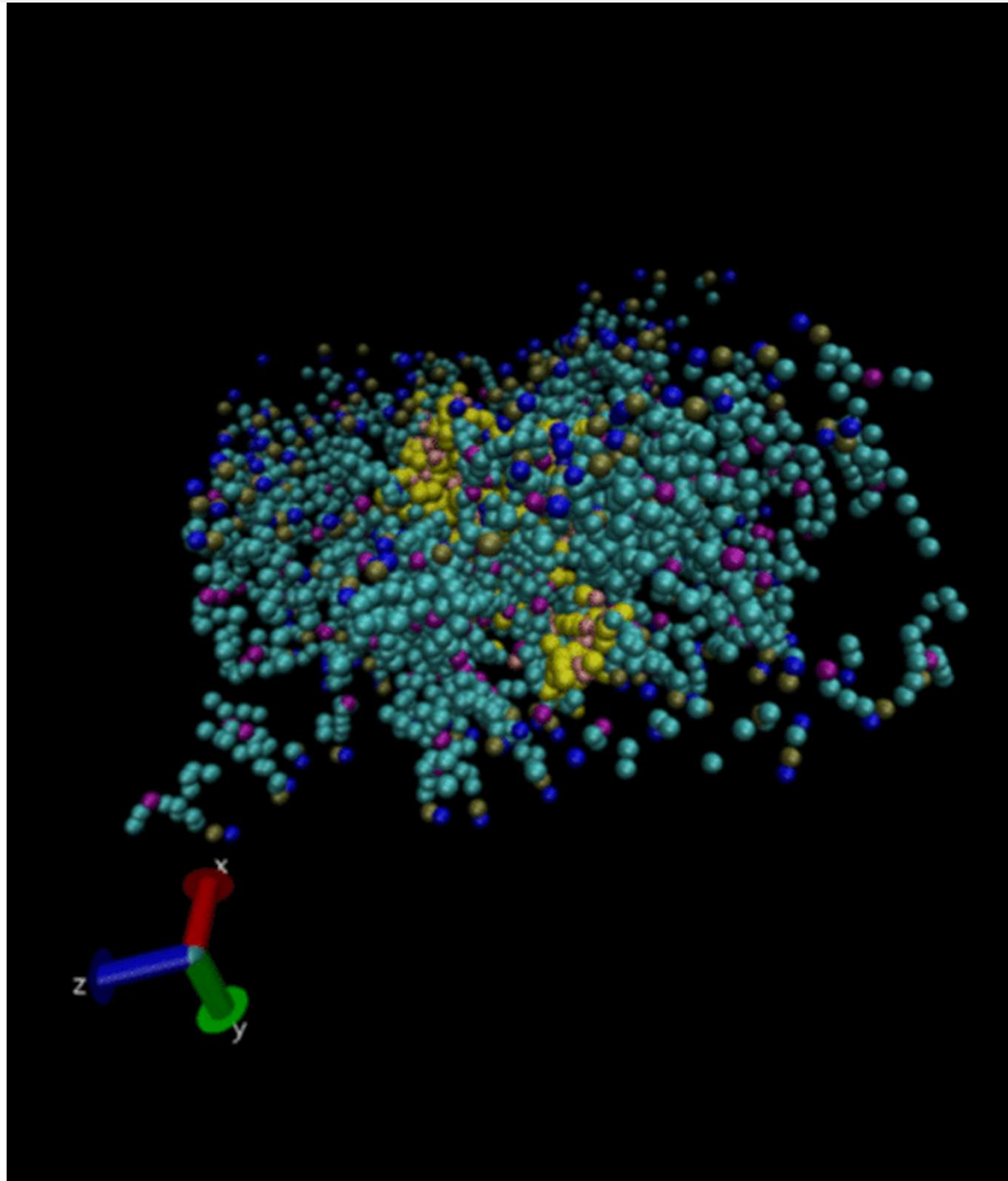


Figure 1. Atomistic (left-hand) and coarse-grained (right-hand) models compared for (A) a DPC molecule and (B) a GpA helix. Colors for atoms:

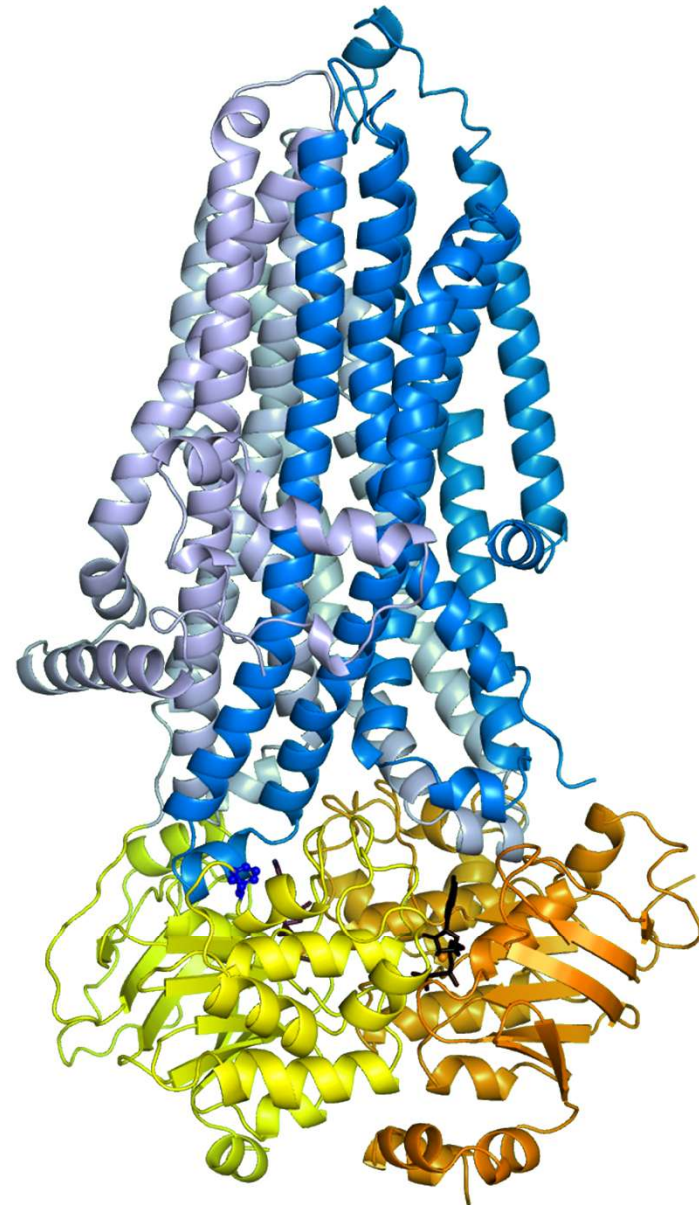
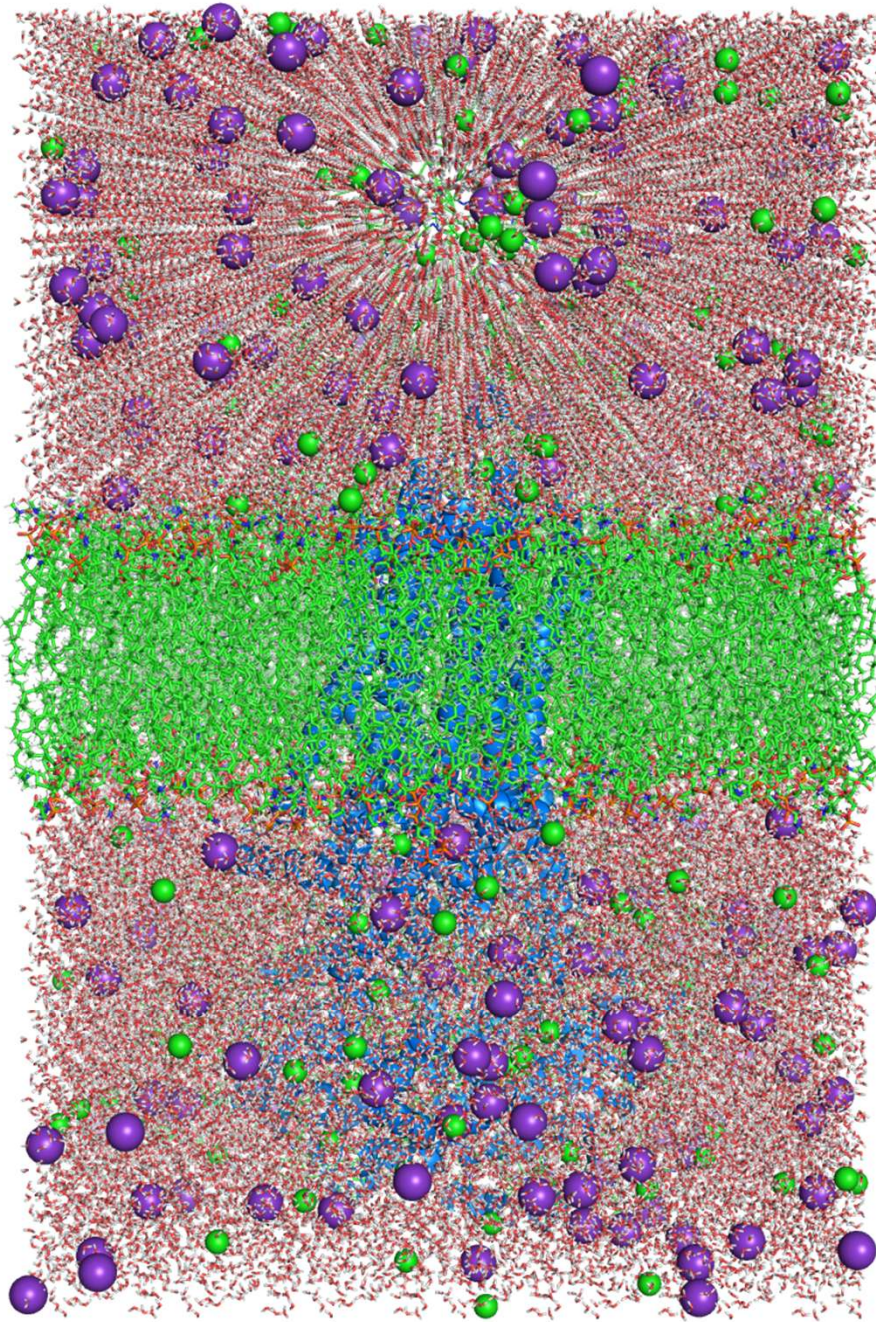
Membrán kettősréteg kialakulása



„All-atom” szerkezetek

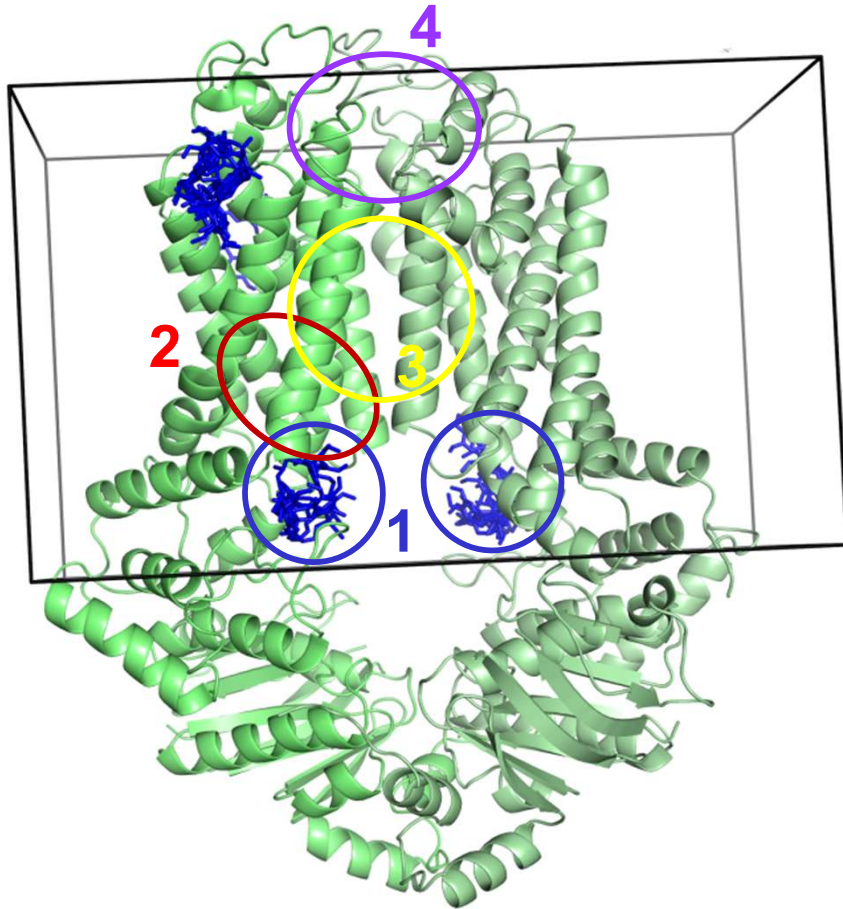
water + ions

lipids

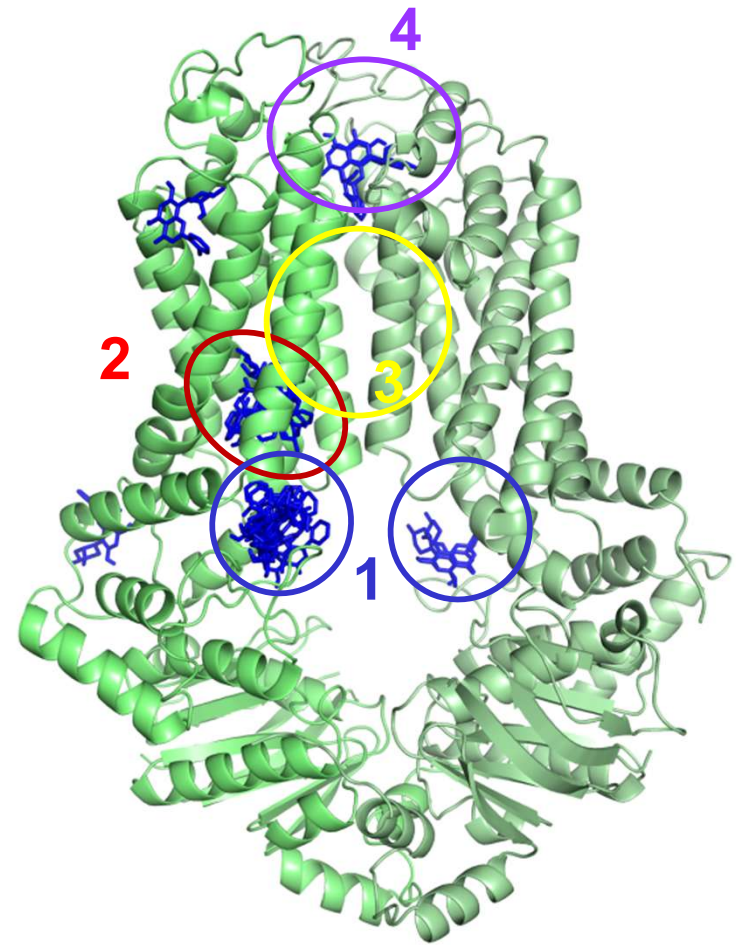


Drogkötő helyek vizsgálata *in silico* dokkolással

verapamil

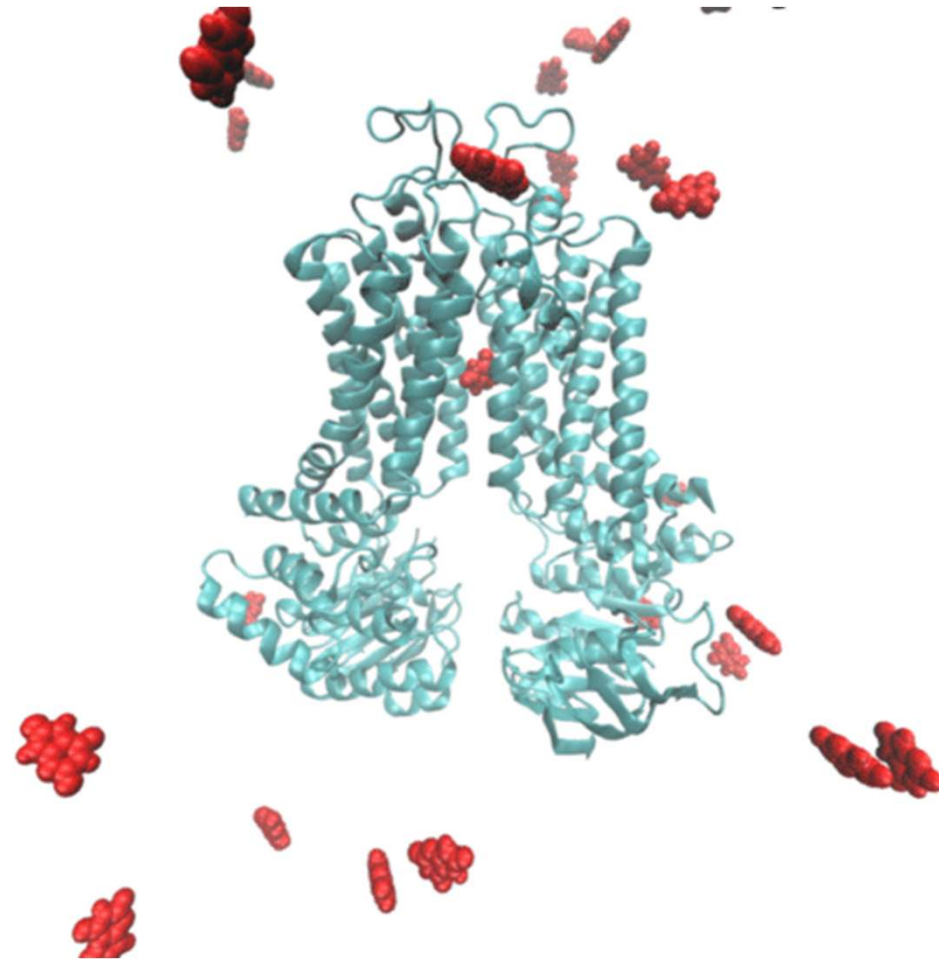


flavopiridol



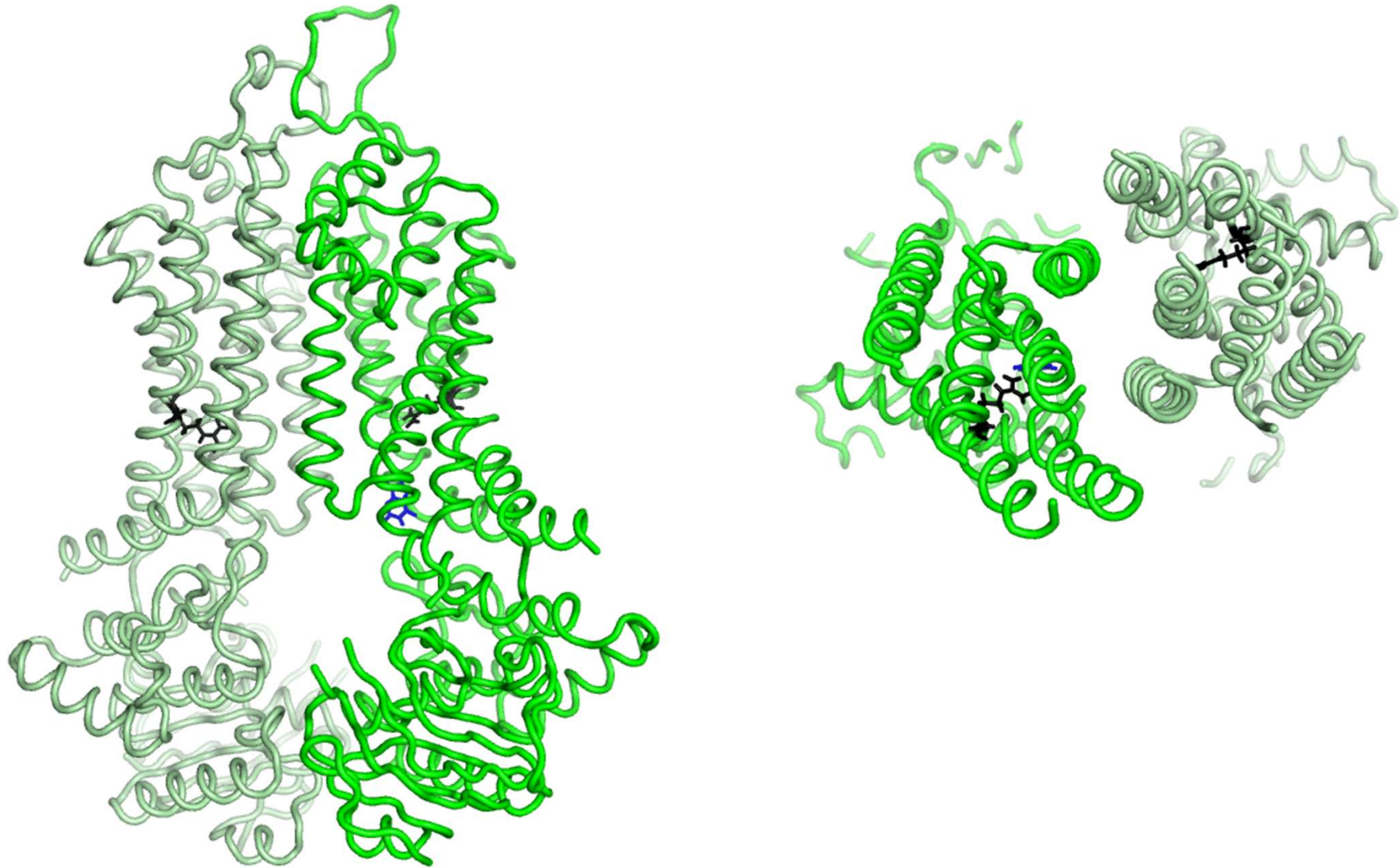
Describing the transport using MD

equilibrium simulations, uric acid molecules

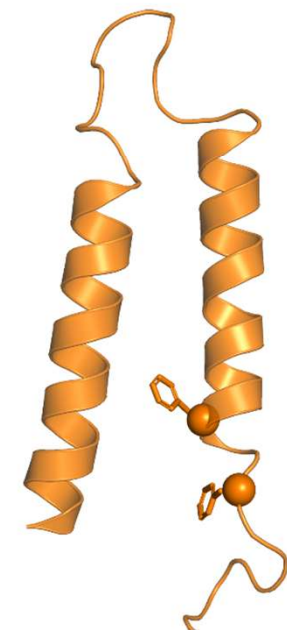
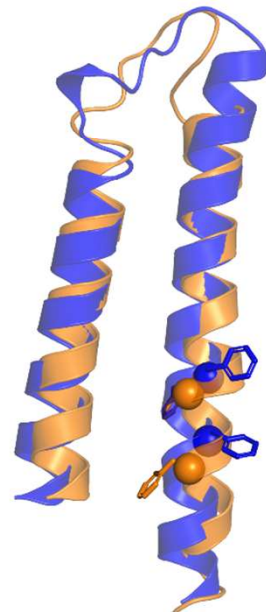
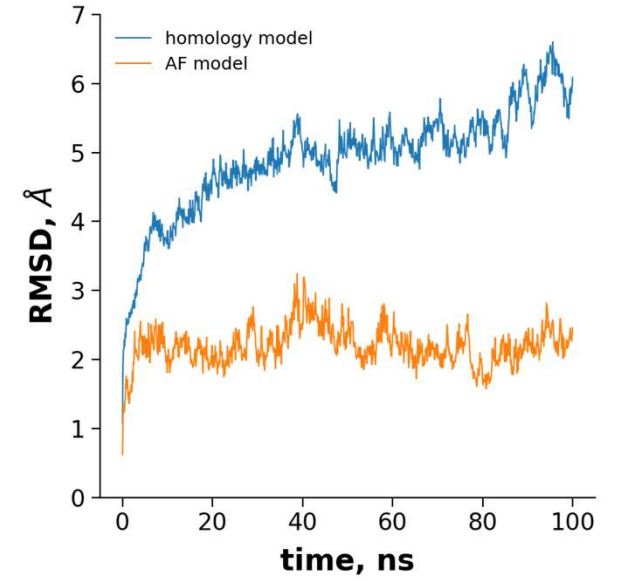
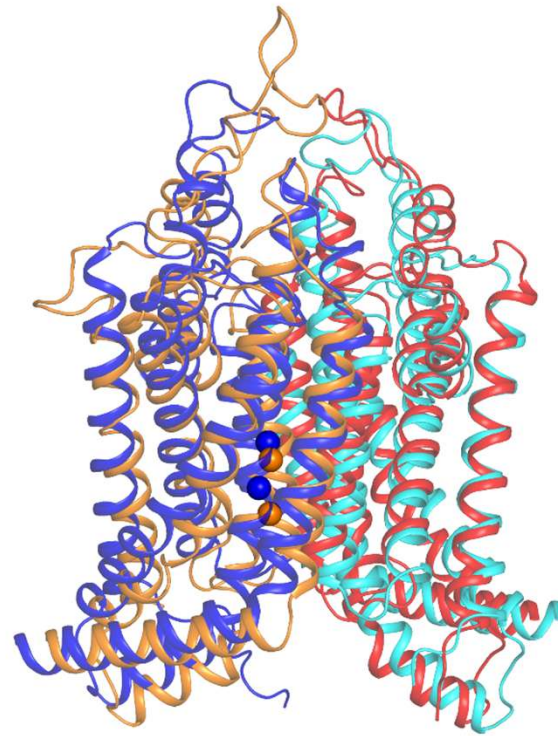
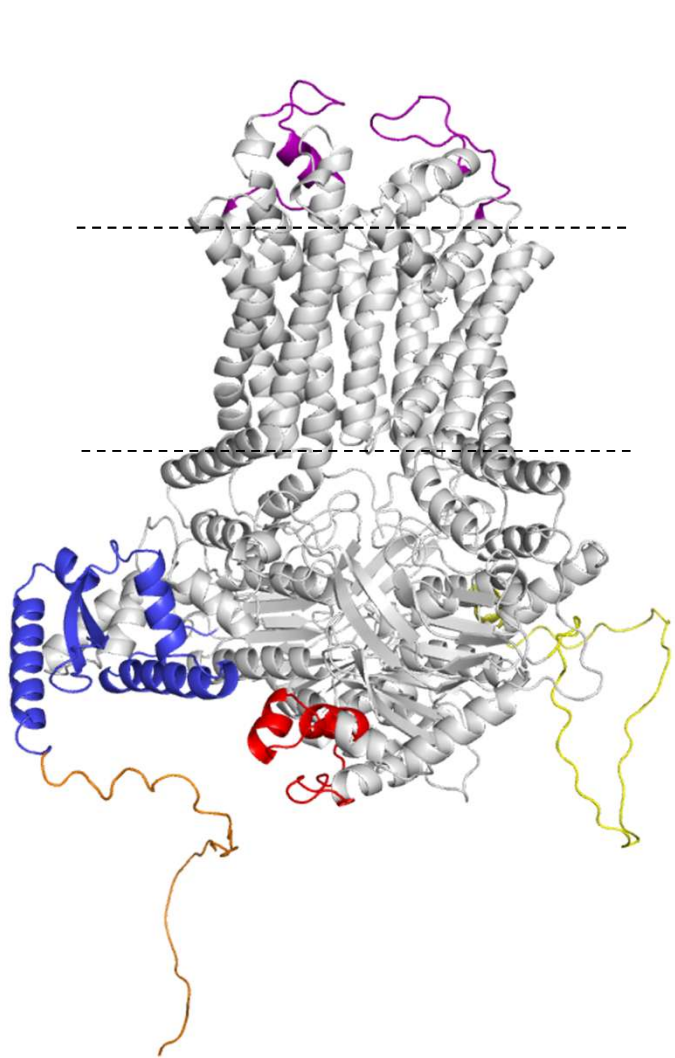


Describing the transport using MD

equilibrium simulations, uric acid molecules

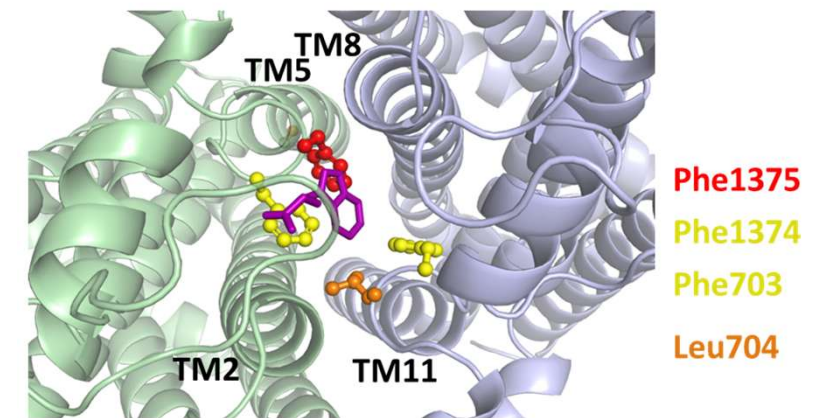
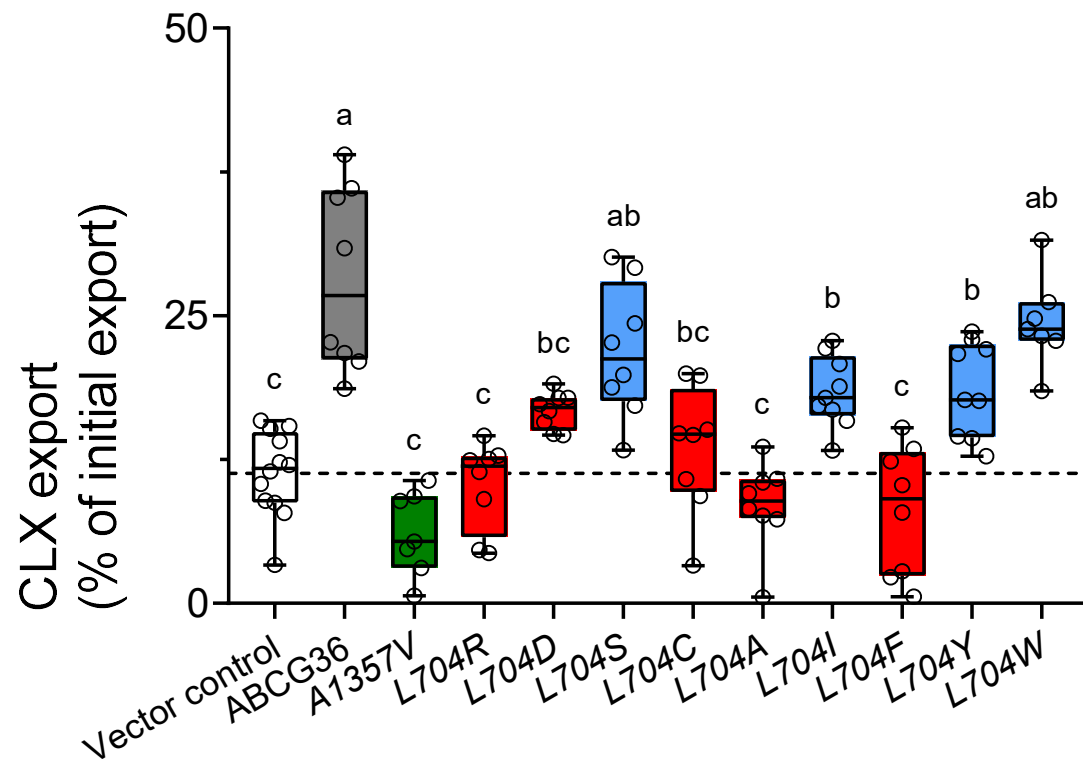
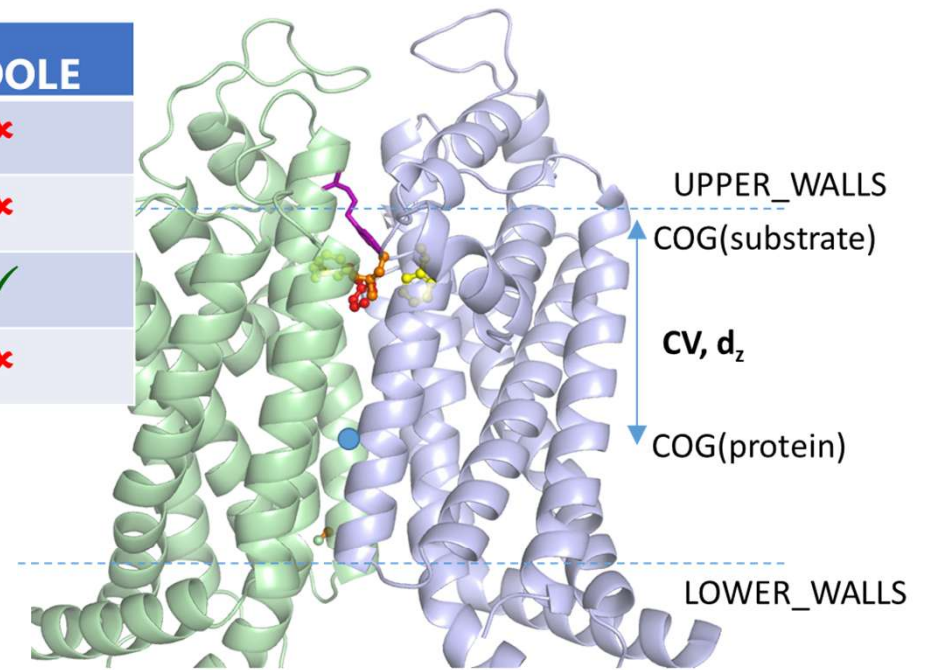


Arabidopsis ABCG36

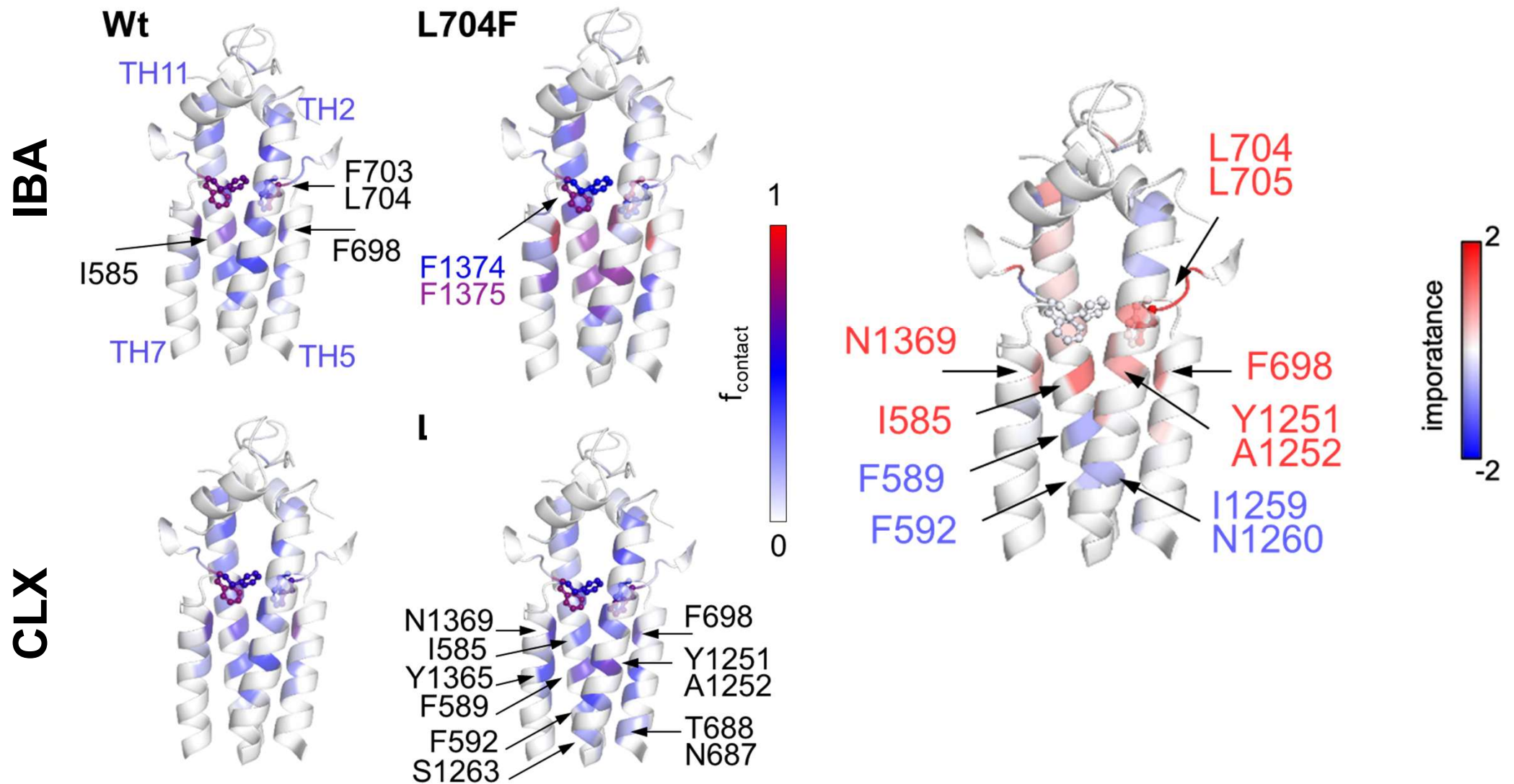


Arabidopsis ABCG36

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



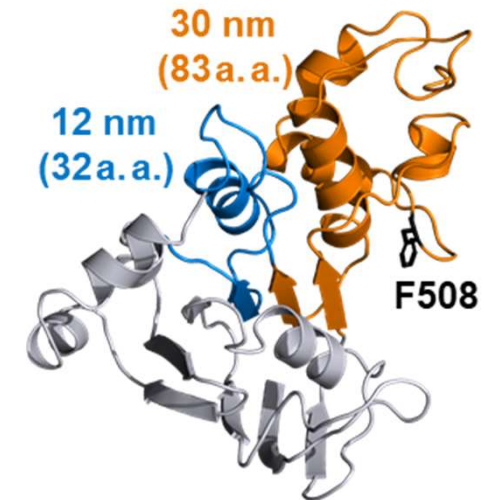
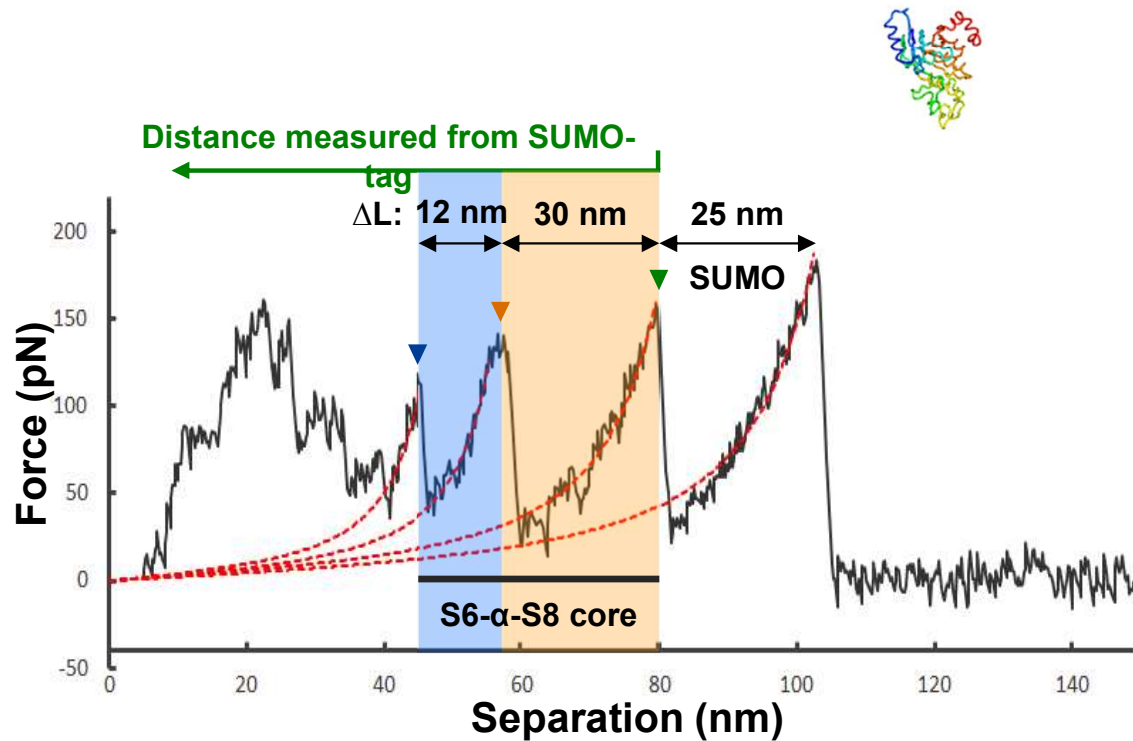
Arabidopsis ABCG36



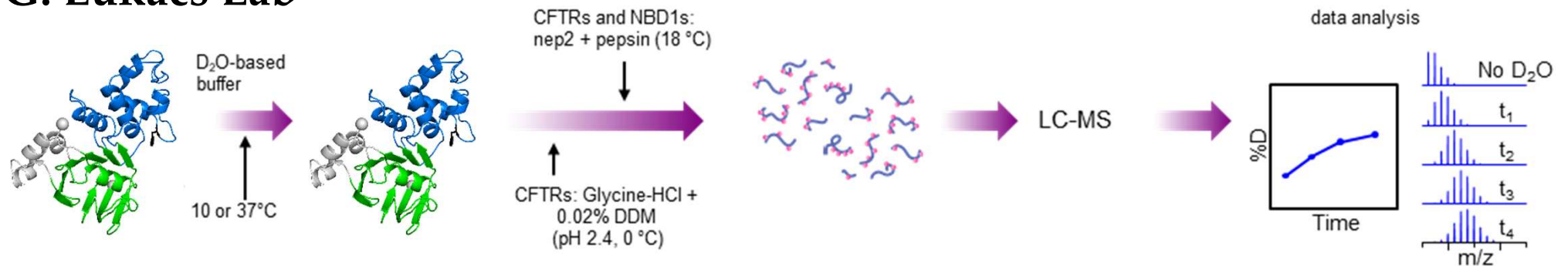
Experiments for BIA site identification

pulling with AFM, HDX

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



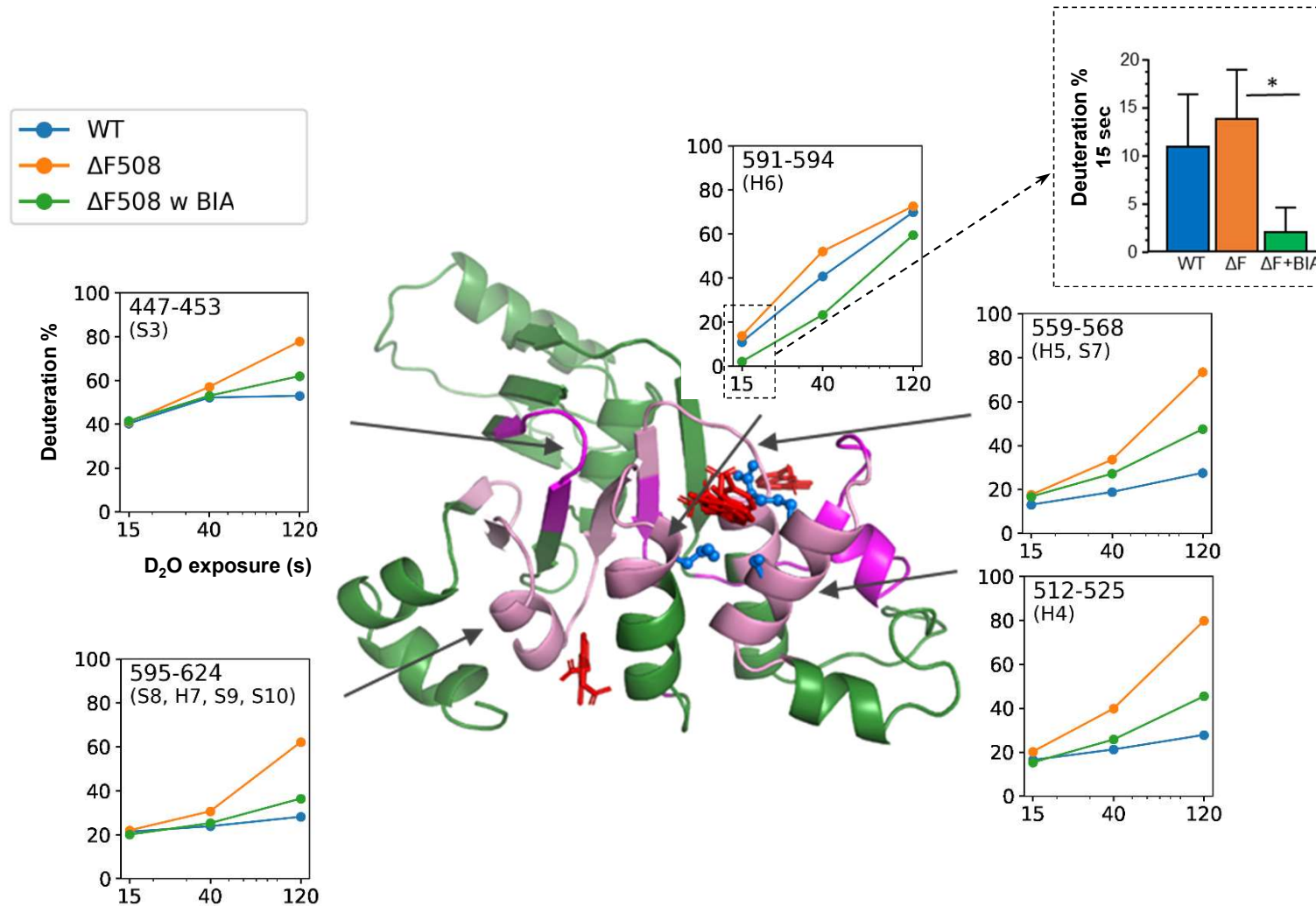
G. Lukács Lab



BIA binding site – HDX with NBD1

G. Lukács Lab

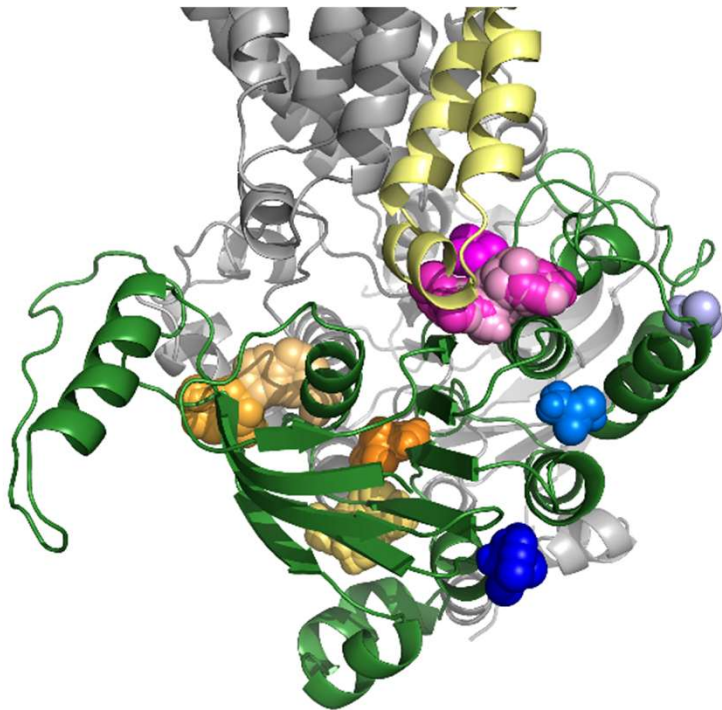
Padanyi *et al.* Comp. Struct. Biol. 2022



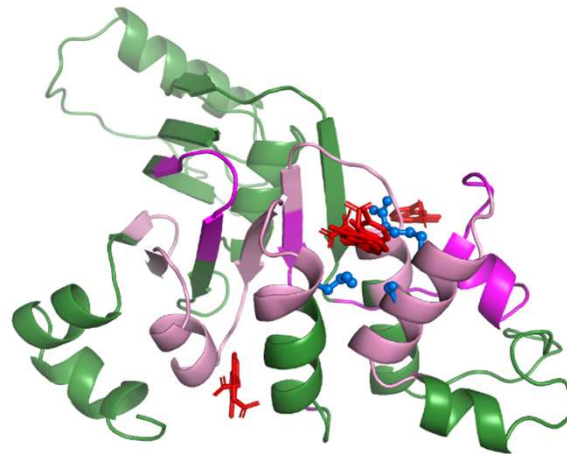
BIA binding site – *in silico*

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

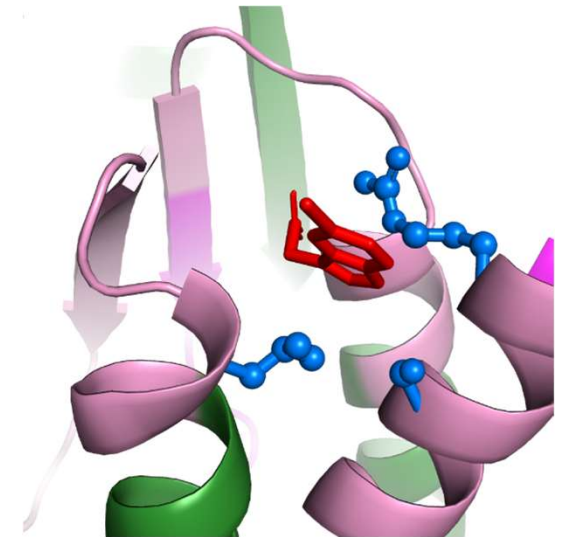
pocket detection
fpocket



docking
AutoDock Vina

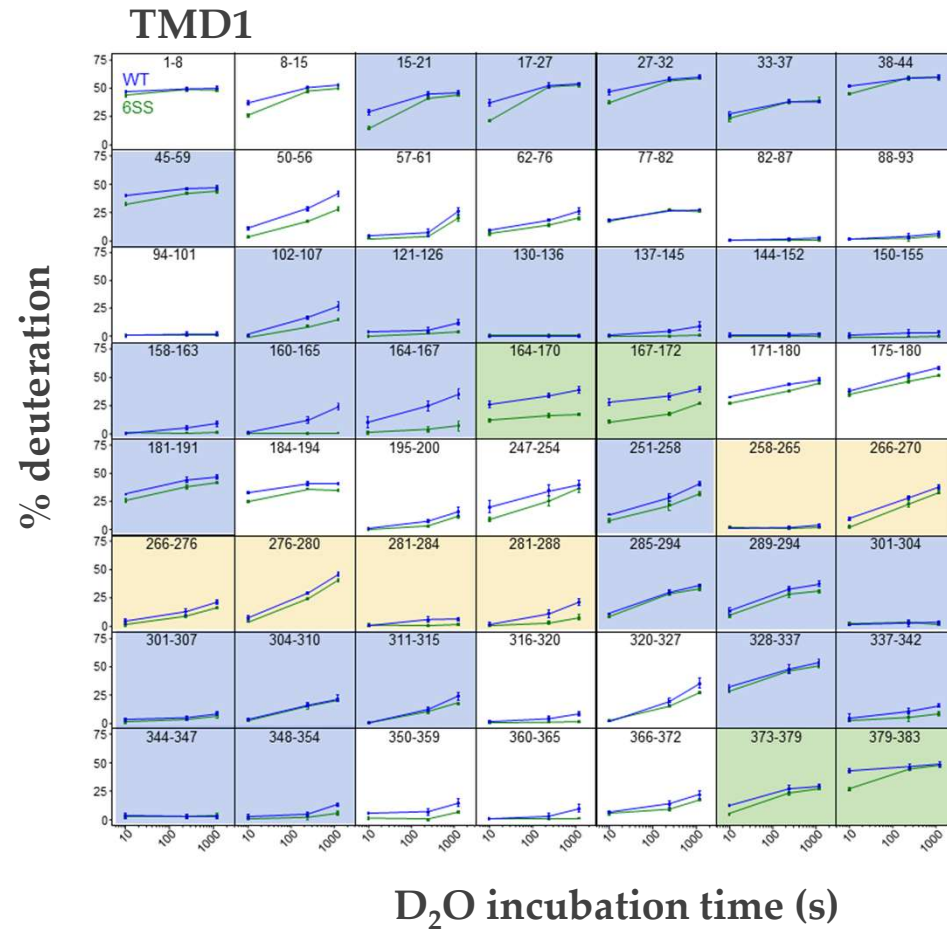
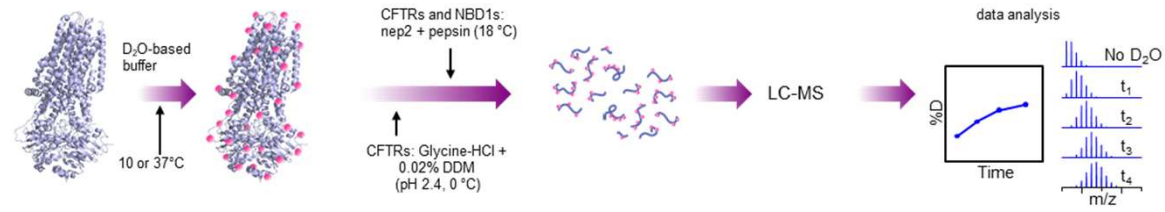


concluded site



Folding/maturation/stability of the full length CFTR

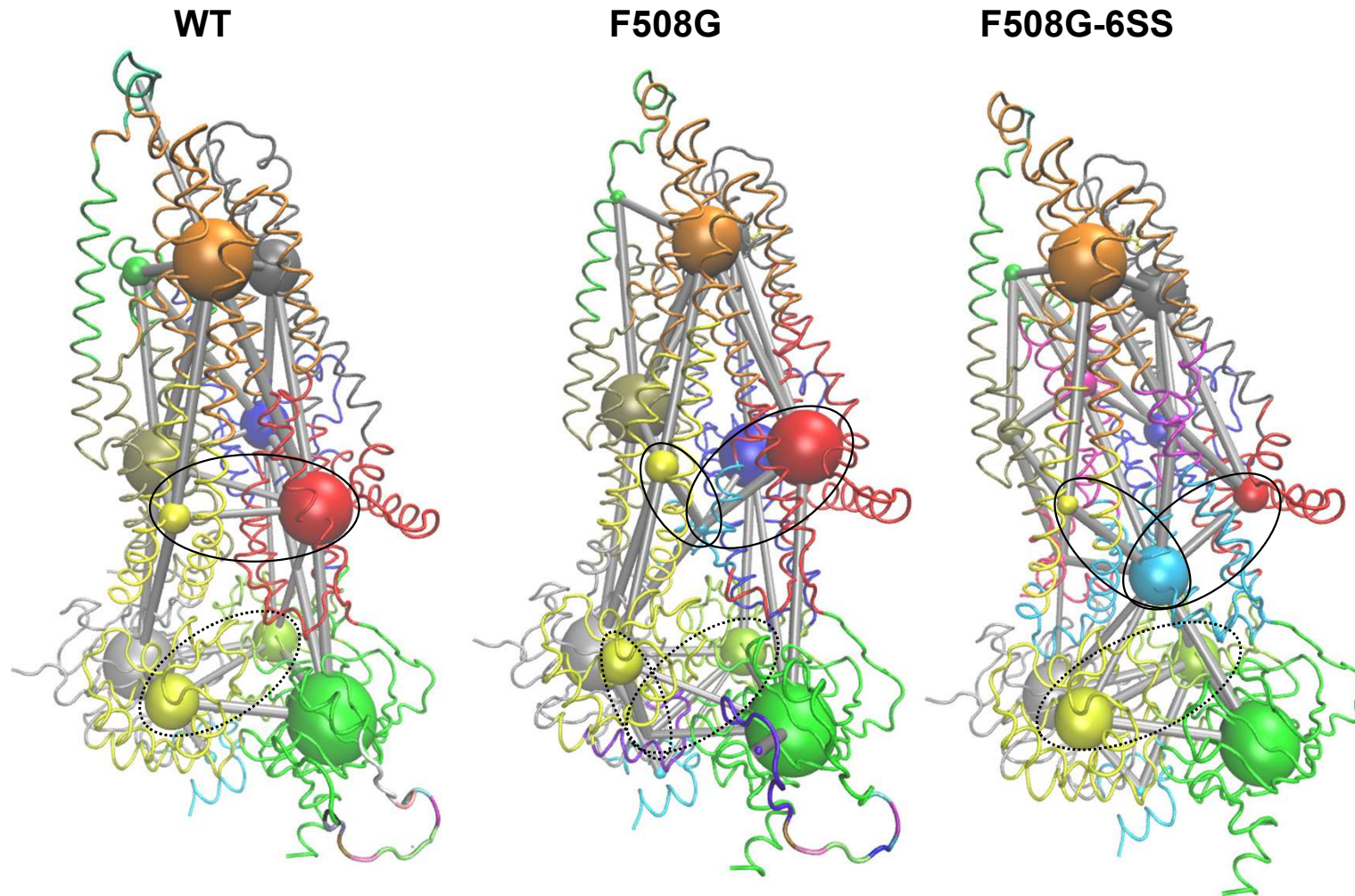
Soya *et al.* Nat. Comm. 2023



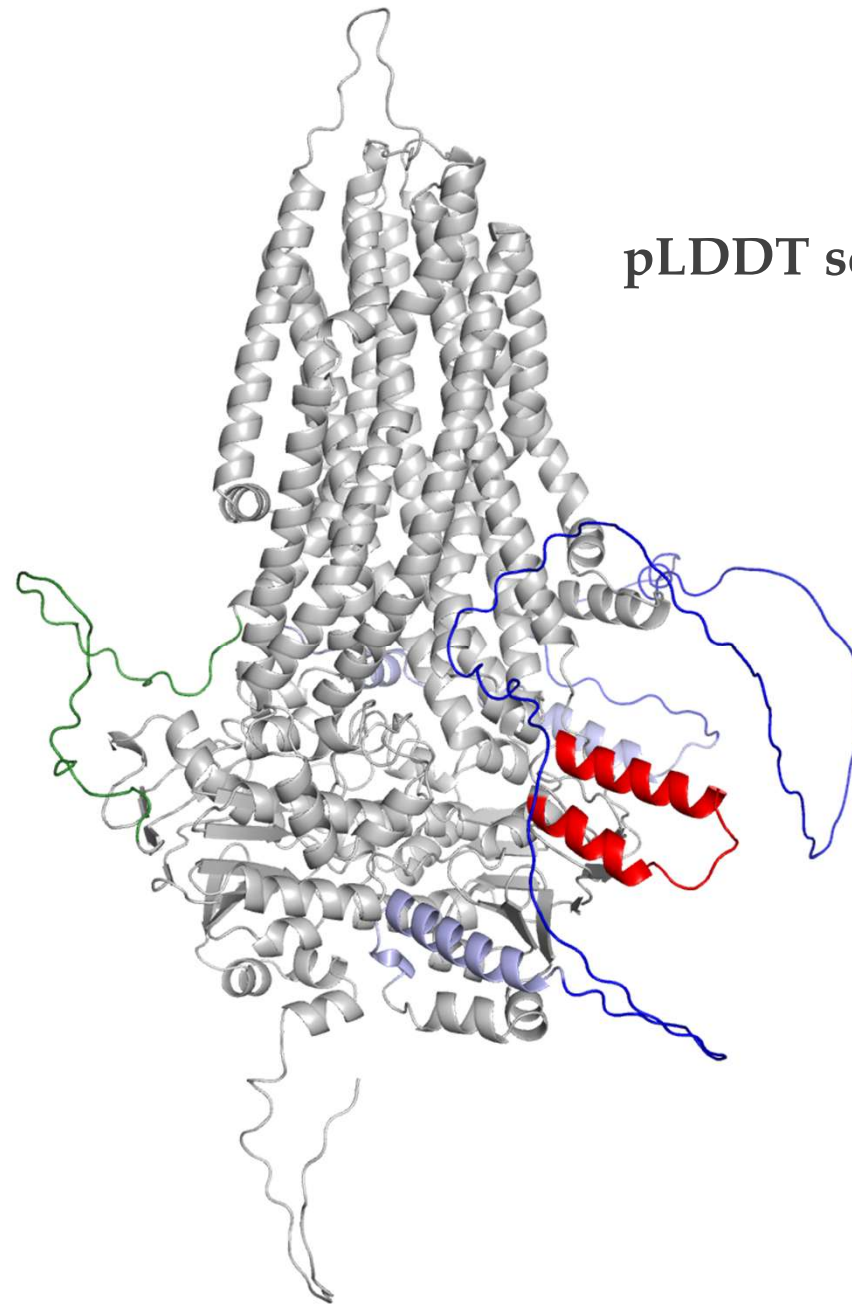
Allosteric stabilization of TM_{IC}

Soya *et al.* Nat. Comm. 2023

- Folded structure
- Correlated motions
- Network
- Community analysis



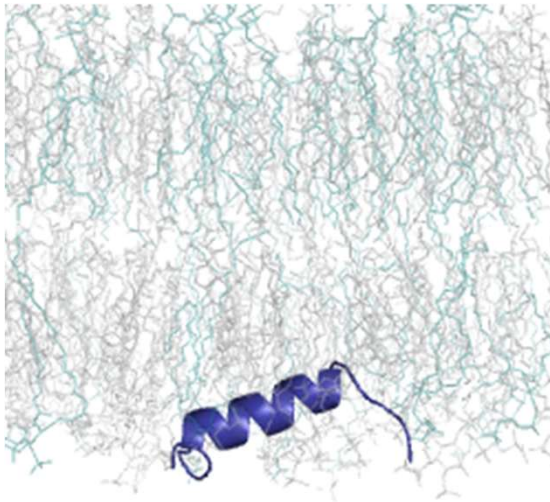
CFTR – rendezetlen régiók



pLDDT score - IDR prediction

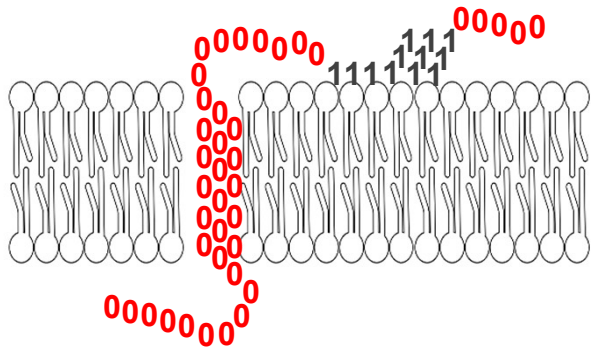
MemMoRFs

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



MemMoRF prediktor

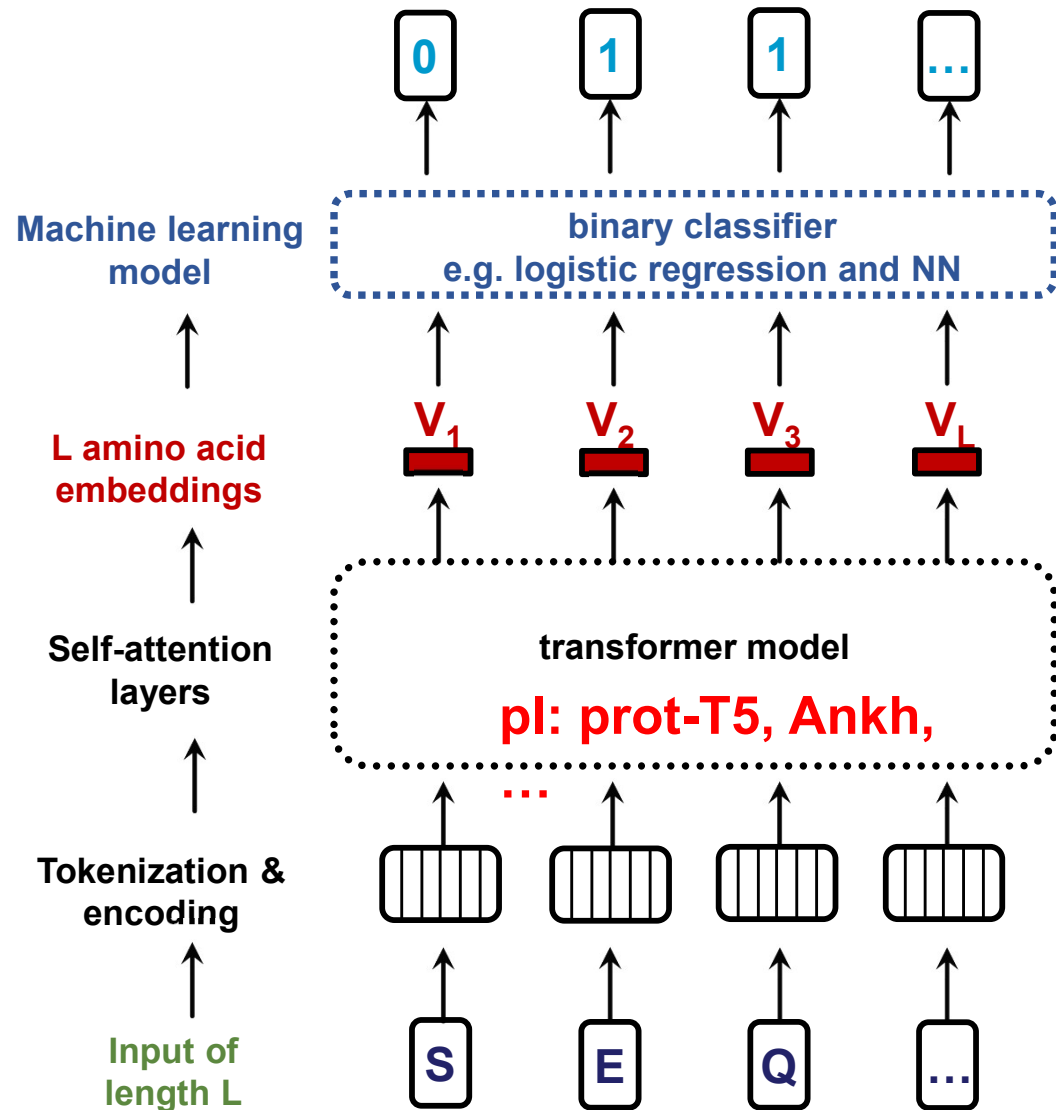
protein Language Models



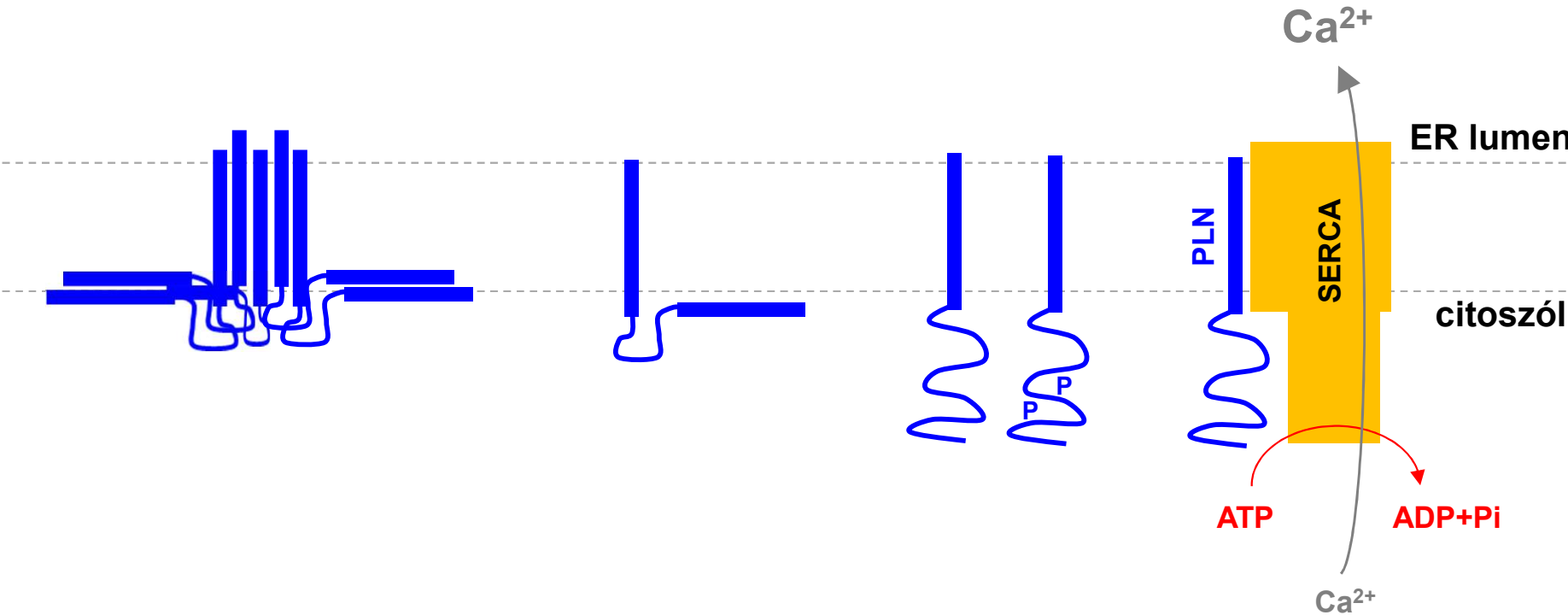
pLMMoRF.hegelab.org
Csepi et al. J Mol Biol 2025

decoder

encoder



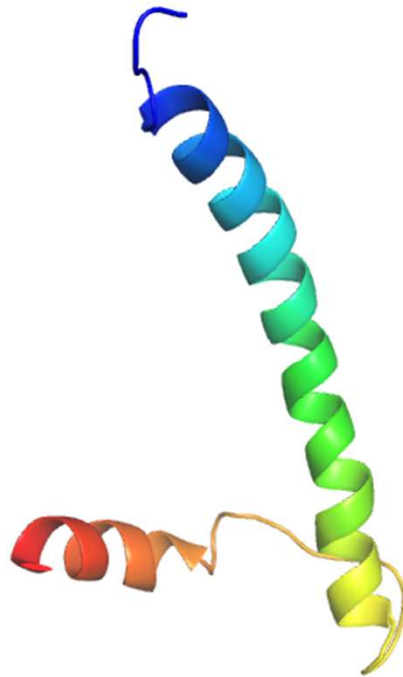
Kalcium ATP-ázok (SERCA, PMCA)



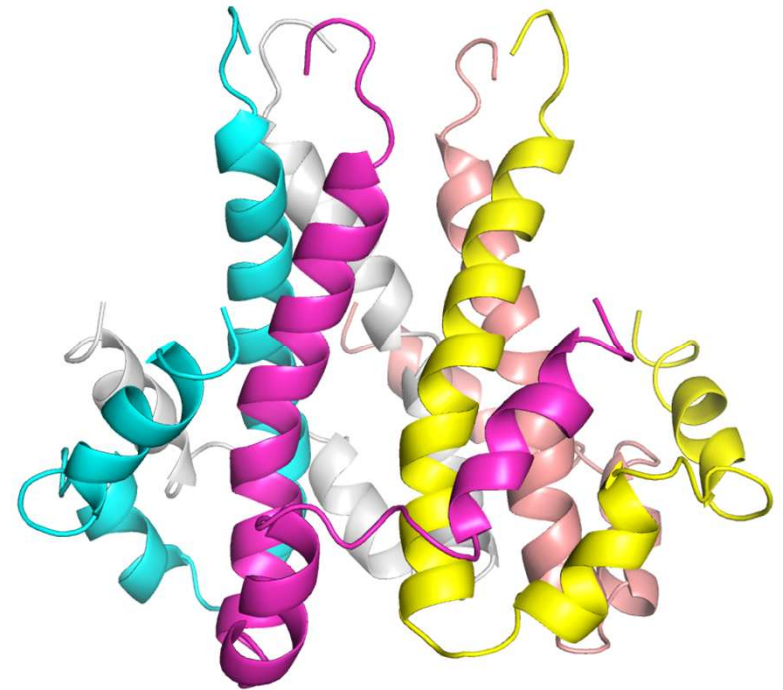
Regulinok és exoregulinok

SLN
ELN
ALN
Dworf
???

SARS Cov-2
Envelope (E) protein

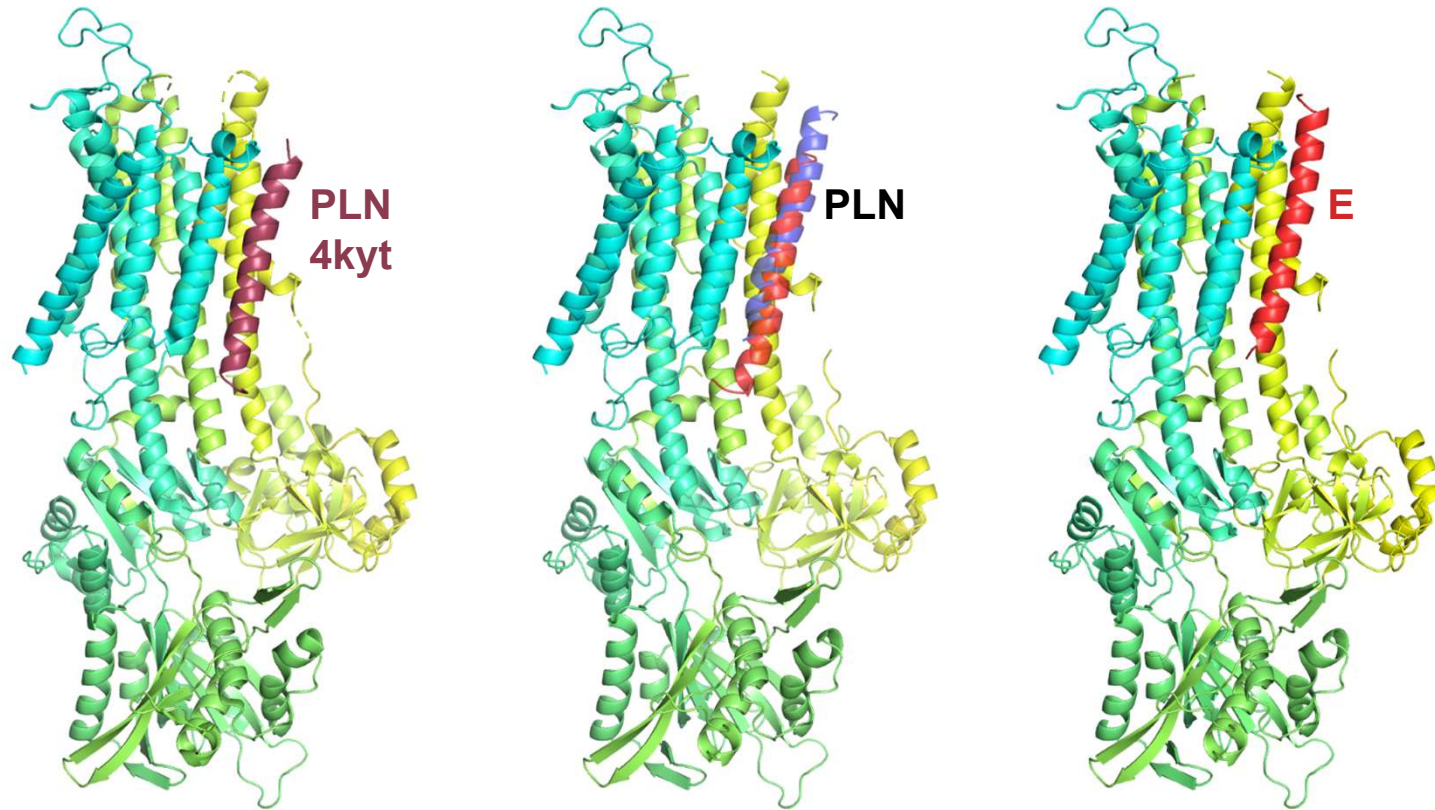


- ERGIC
- viroporin
- PDZ-kötés
- egyéb PPI?



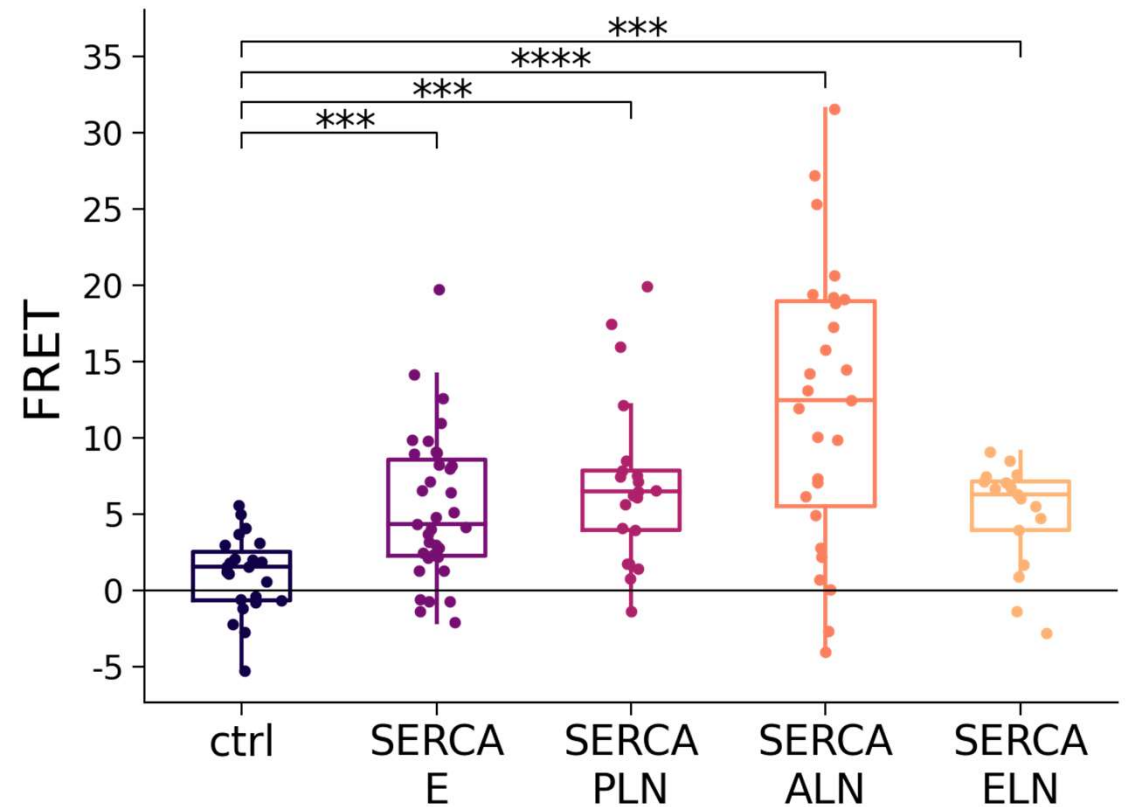
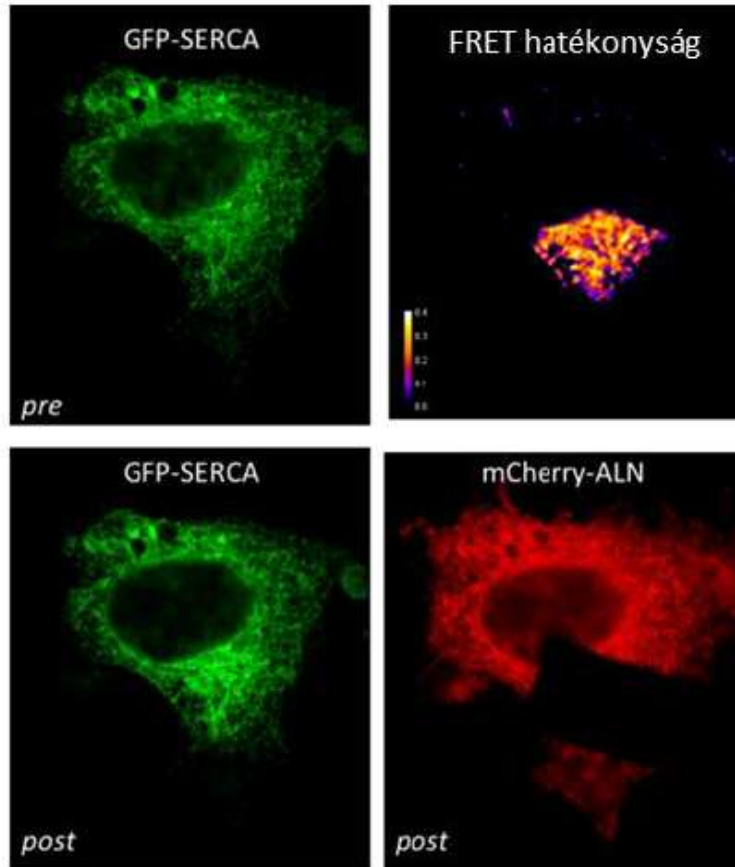
A SERCA / E protein komplex szerkezete

AlphaFold-Multimer
PIPER/ClusPro



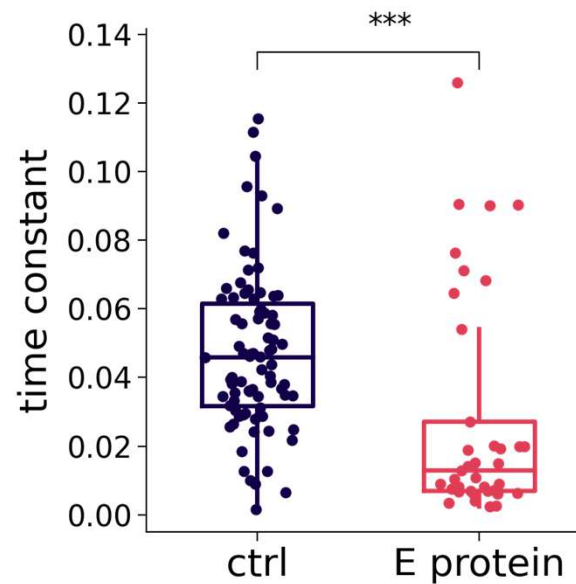
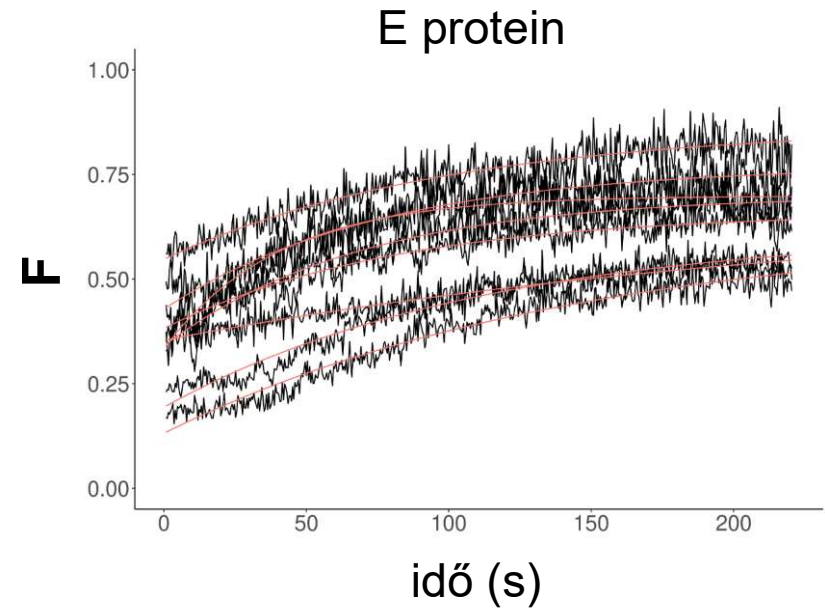
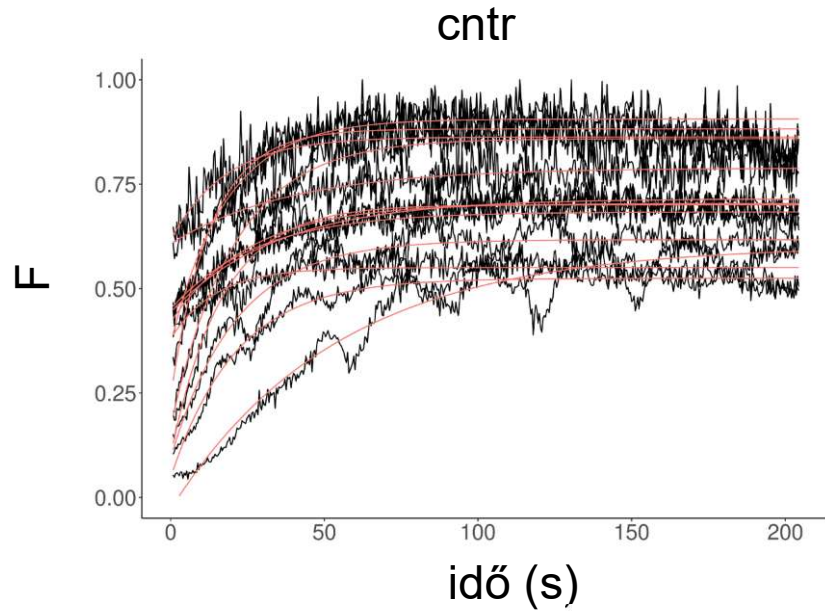
Az E fehérje kölcsönhat a SERCA pumpával

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



Az E fehérje gátolja a SERCA működését

nincs EC Ca^{2+}
ER ürítése
+ Ca^{2+}



AlphaMissense.hegelab.org

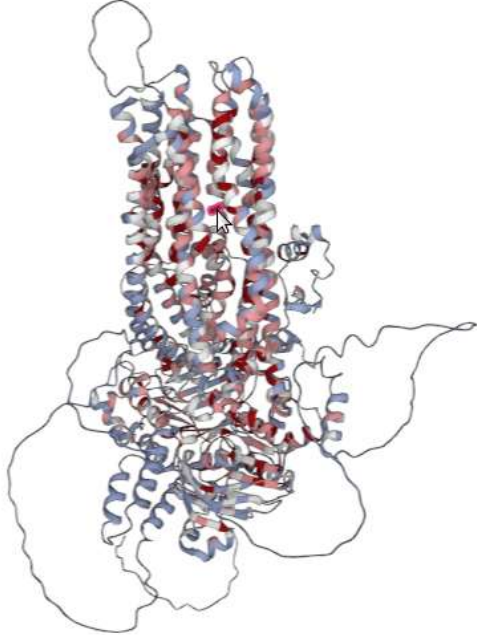
~4,000 users per month

Home Search Hotspot Download Help

Spot mutations

Identifier:

CFTR (CFTR_HUMAN, P13569, ENST00000003084.10)



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

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:F,I,V		2:D,H,P	0.286

Δ -embeddings

An embedding with $f_1, f_2, \dots, f_{1536}$ features

X : $\Delta\text{embedding} = \text{emb}(\text{WT})_i - \text{emb}(\text{MUT})_i$
 y : label (neutral/0, pathogen/1)

Összefoglalás

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

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