Combating Cystic Fibrosis: Computational Studies on CFTR

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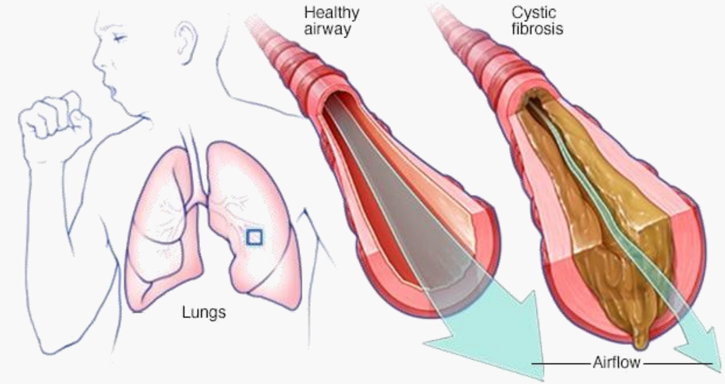
"As Gianni Rodari is often quoted: 'A lesson in which laughter has not been heard is a wasted lesson.'"

Gianni Rodari: 1920-1980

The Cystic Fibrosis Disease

- Most common lethal, inherited disease among people of European descent
- The number of CF patients is estimated at 60,000-165,000 across 94 countries

CF results in pathologies in multiple organs but primarily in the lungs



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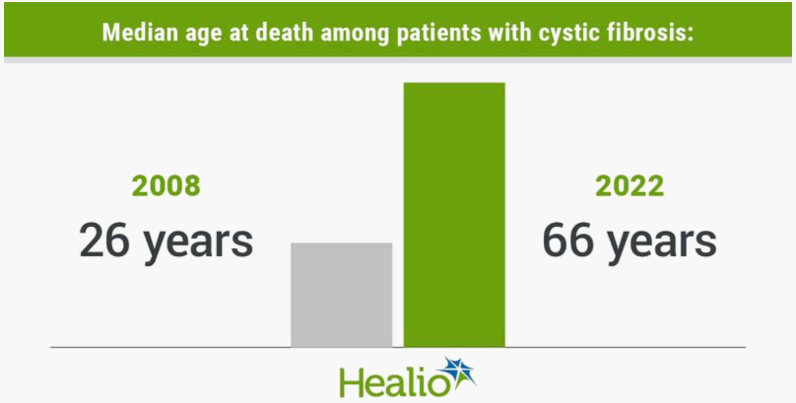
Median survival age of CF patients

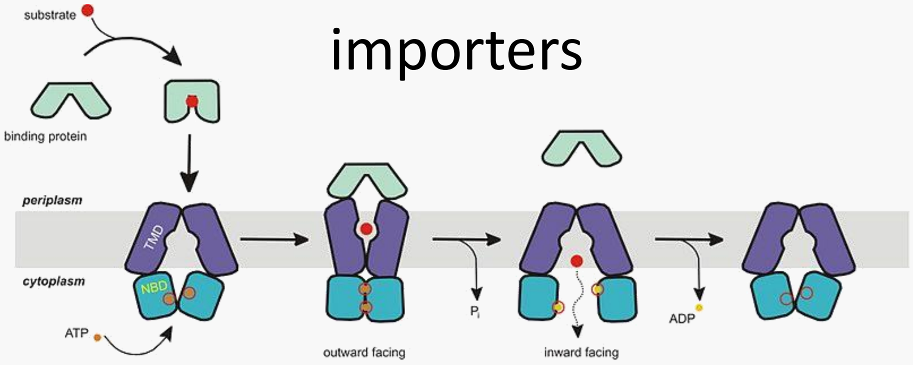


CF is caused by mutations to the CFTR protein

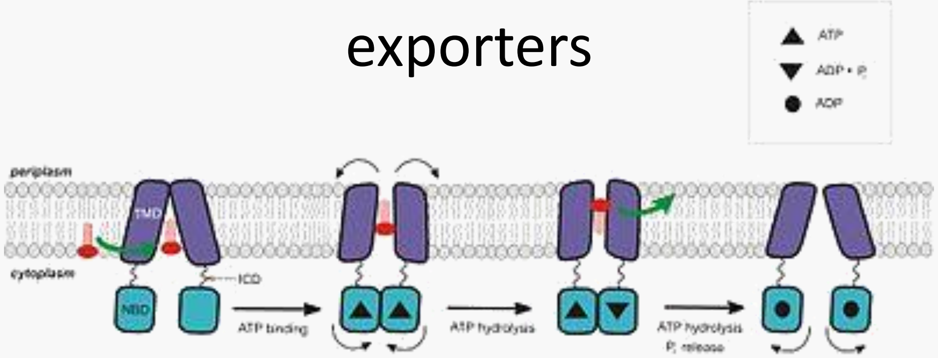
CFTR is an ATP Binding Cassette (ABC) Transporter

- One of the largest and most ancient protein families
  - Membrane proteins
  - Found in prokaryotes and eukaryotes (48 ABC transporters in humans)
  - Harness the power of ATP hydrolysis to mediate substance transport across cell membranes

importers



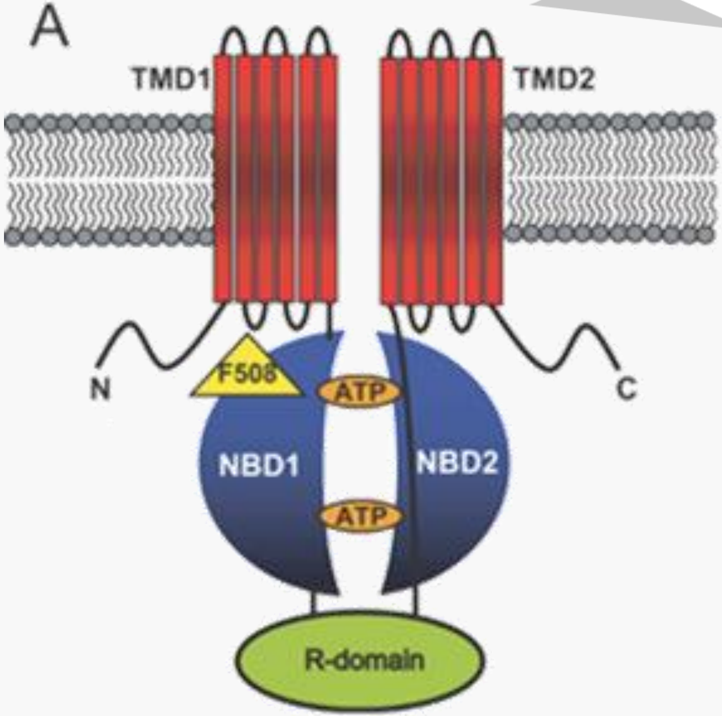
exporters



CFTR is Unique!

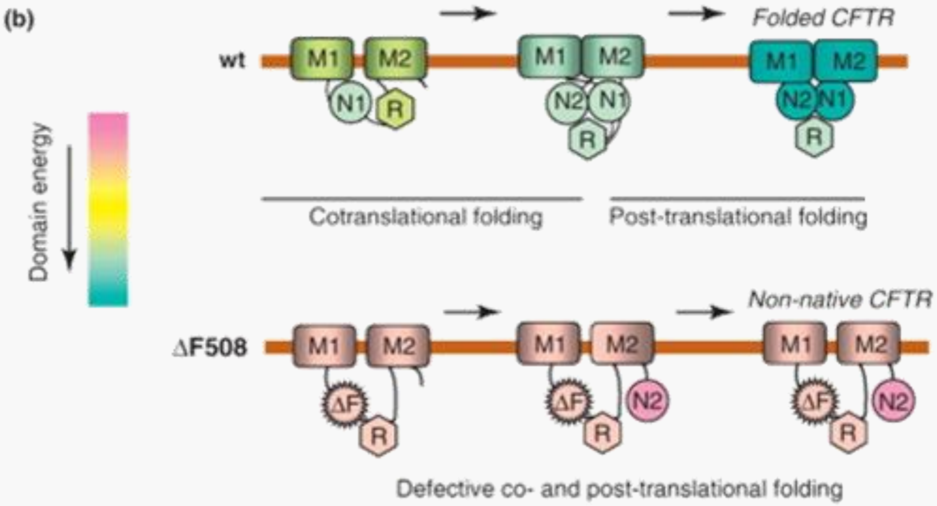
CFTR is the only known ion channel in the ABC family

- Historic perspective
  - Gene cloning: 1989 (35 years ago)
  - First low-resolution structure: 2004
  - First published homology model: 2008
  - First cryo-EM structure: 2016
  - First crystal structure: ????



CFTR Folding

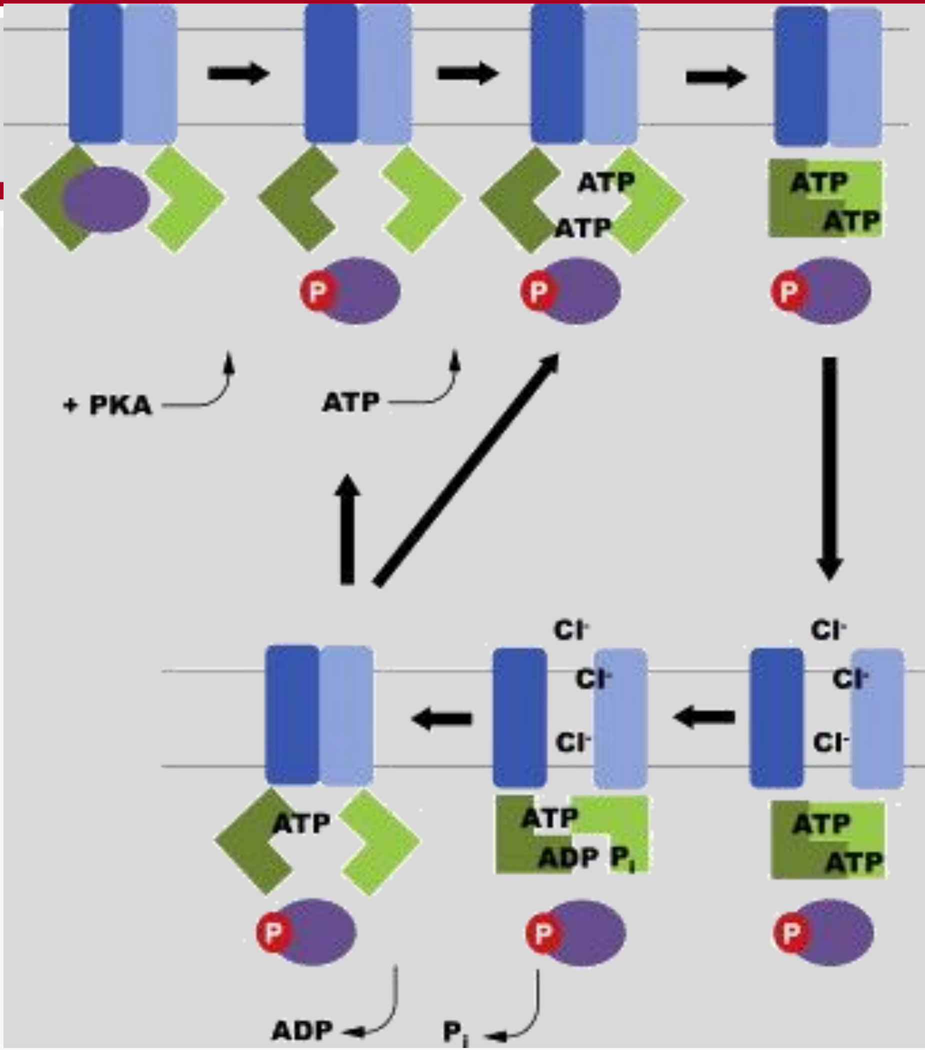
- Individual domains loosely fold and assemble co-translationally
- Compact fold with native MSDs-NBDs interface forms post-translationally
- F508del disrupts the folding process by reducing the hermal stability of NBD1 and disrupting its interactions with ICL4 and ICL1



Lukacs and Verkman, Trend. Mol. Med. 2012

Gating Cycle of CFTR

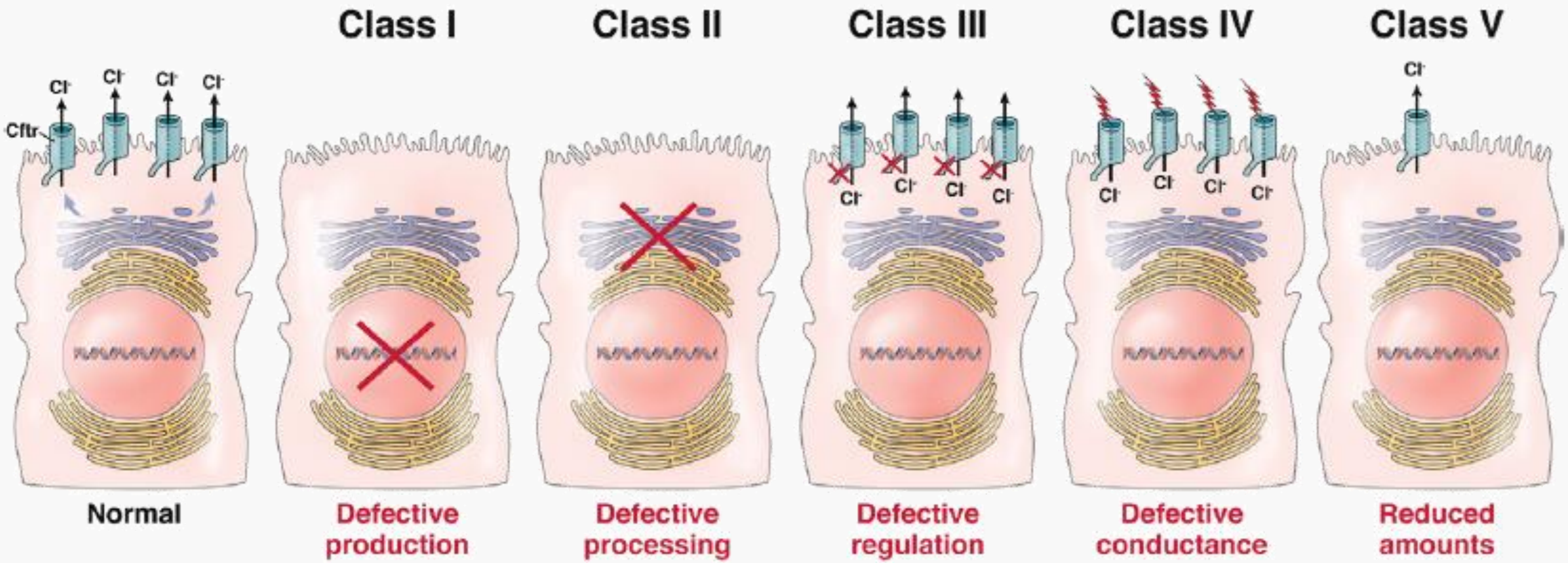
- CFTR likely has multiple states
- Different states may be clinically relevant



Cant et al. Int J Biochem Cell Biol. 2014;52:15–25

CFTR Mutations

- >2000 CFTR mutations (CF-causing: 719; Non CF-causing: 25)
- All mutations compromise the ability of CFTR to conduct Cl^- ions



Gelfond and Borowitz CLINICAL GASTROENTEROLOGY AND HEPATOLOGY 2013;11:333–342

How Do Mutations in CFTR Cause CF?

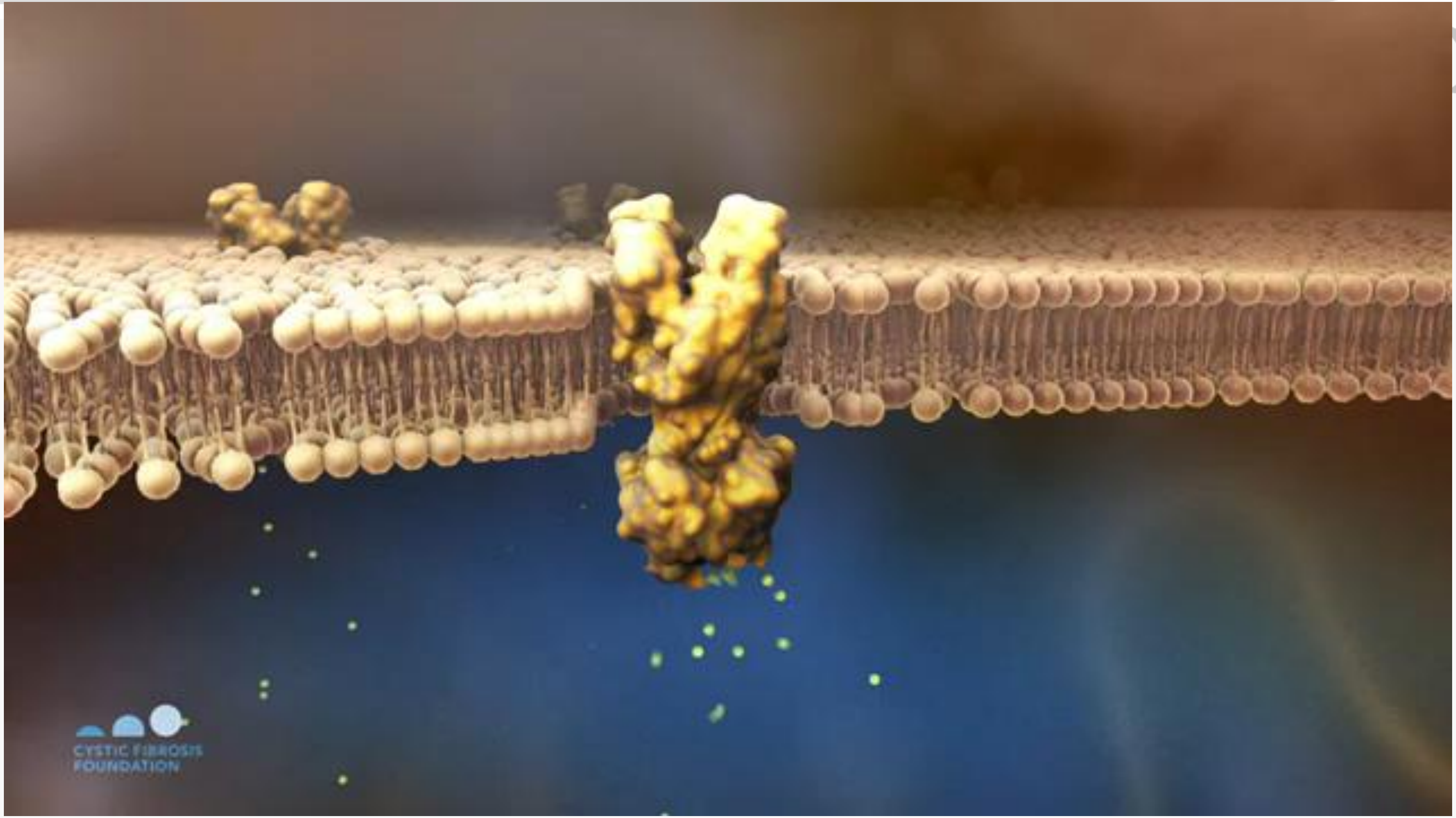
Treatment Hypothesis

Restoring Cl^- conductance to “normal” levels will ameliorate CF pathologies

Current ~ [# channels] * [open probability]

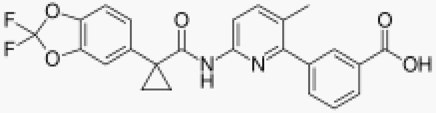
- **CFTR corrector**: Corrects folding defect and increases number of CFTR channels at cell membrane
- **CFTR potentiator**: Increases open probability of CFTR channels at the membrane
- **Combo therapy:** Does both

Treatment Hypothesis

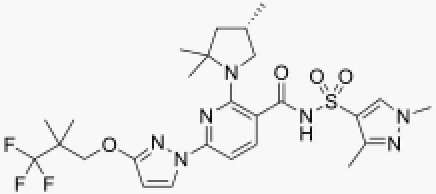


Available CFTR Modulators

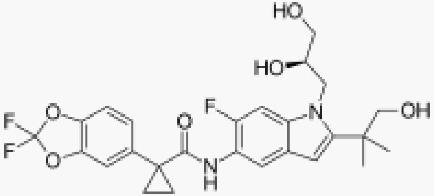
Lumacaftor (corrector)



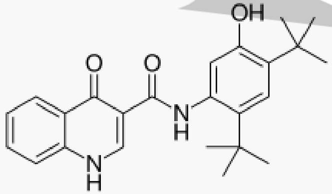
Elexacaftor (corrector)



Tezacaftor (corrector)



Ivacaftor (potentiator)



Therapy	**Luma**	**Elexa**	**Teza**	**Iva**	**Indication**
TriKafta		🟨	🟨	🟨	F508del or 177 specific mutations
Symdeko			🟨	🟨	F508del/F508del + 154 specific mutations
Orkambi	🟨			🟨	F508del/F508del
Kalydeco				🟨	97 specific mutations

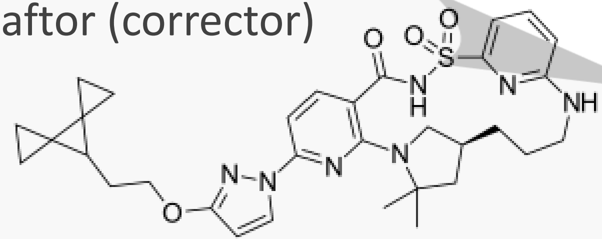
~90% of CF patients are treatable; ~10% are not

Available CFTR Modulators

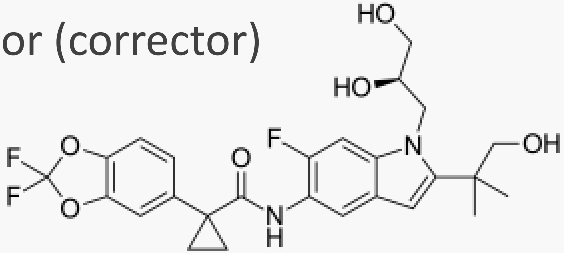
Alyftrek (vanzacaftor/tezacaftor/deutivacaftor):

- Approved in December 2024
- individuals aged 6 and older with at least one F508del mutation or other responsive mutations

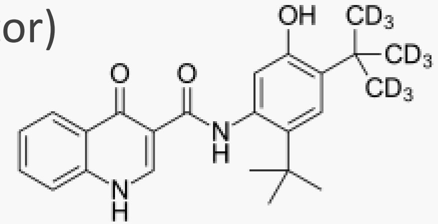
Vanzacaftor (corrector)



Tezacaftor (corrector)



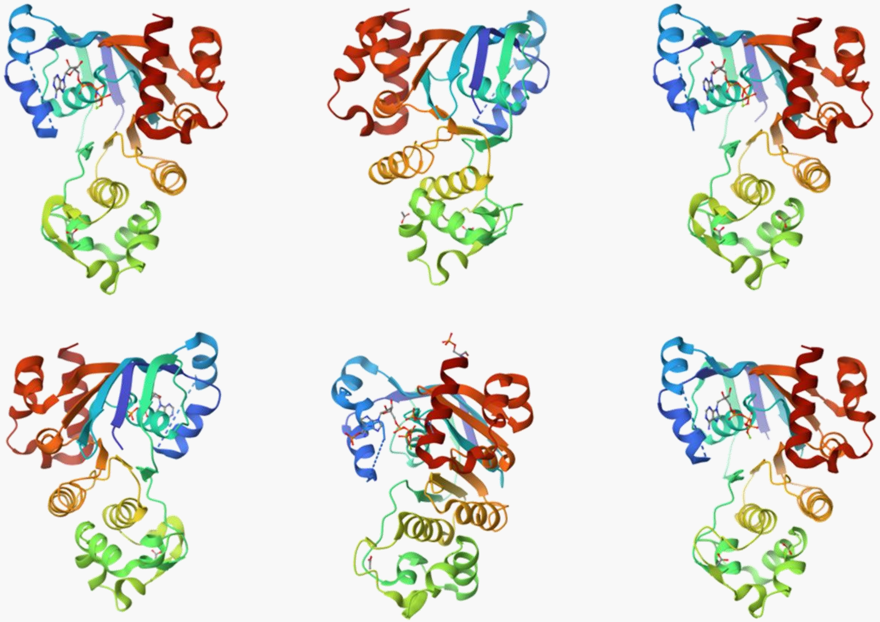
Deutivacaftor (potentiator)



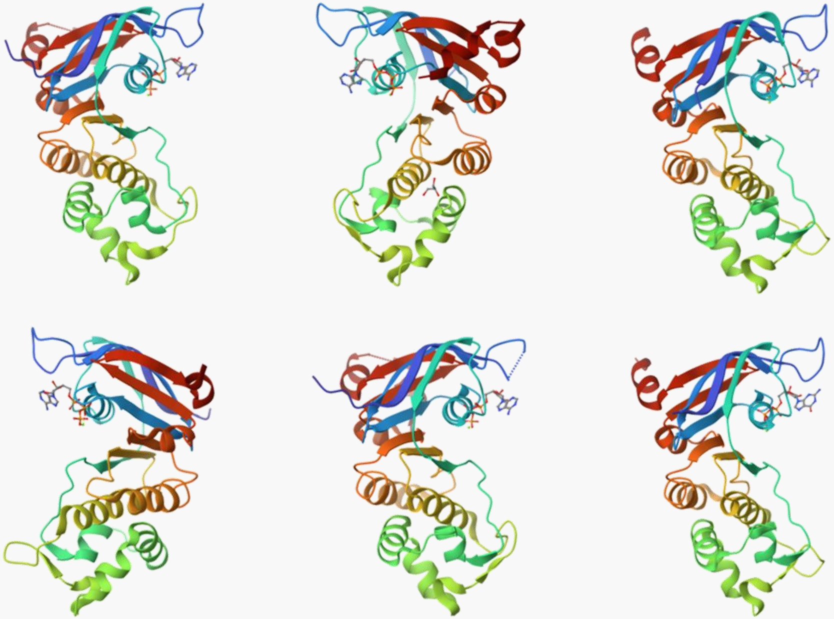
Structural Information on NBD1

NBD1 is considered a hot-spot for CF causing mutations

2004: 6 structures, Resolution: 2.2-3.0Å

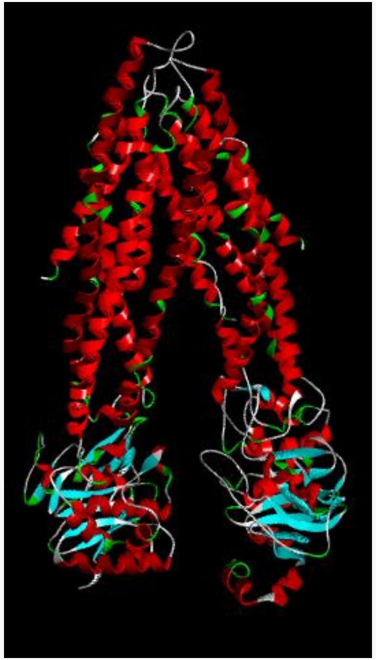


Today: 36 structures, Resolution: 1.7-3.1Å



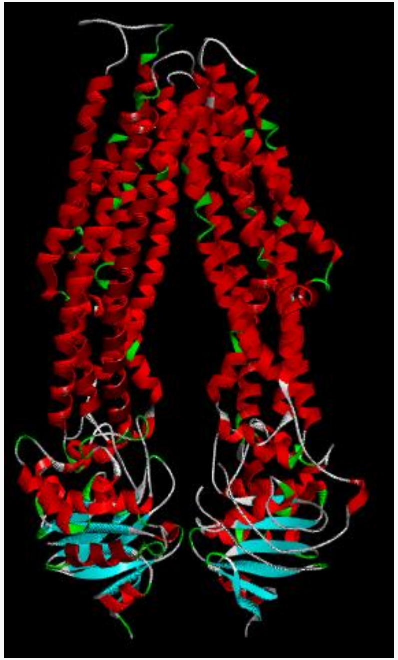
CFTR Models

Pgp

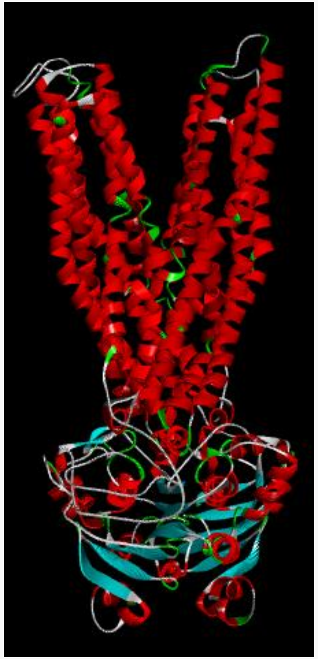


McjD

ABC-B10



Sav1866



TM287-288

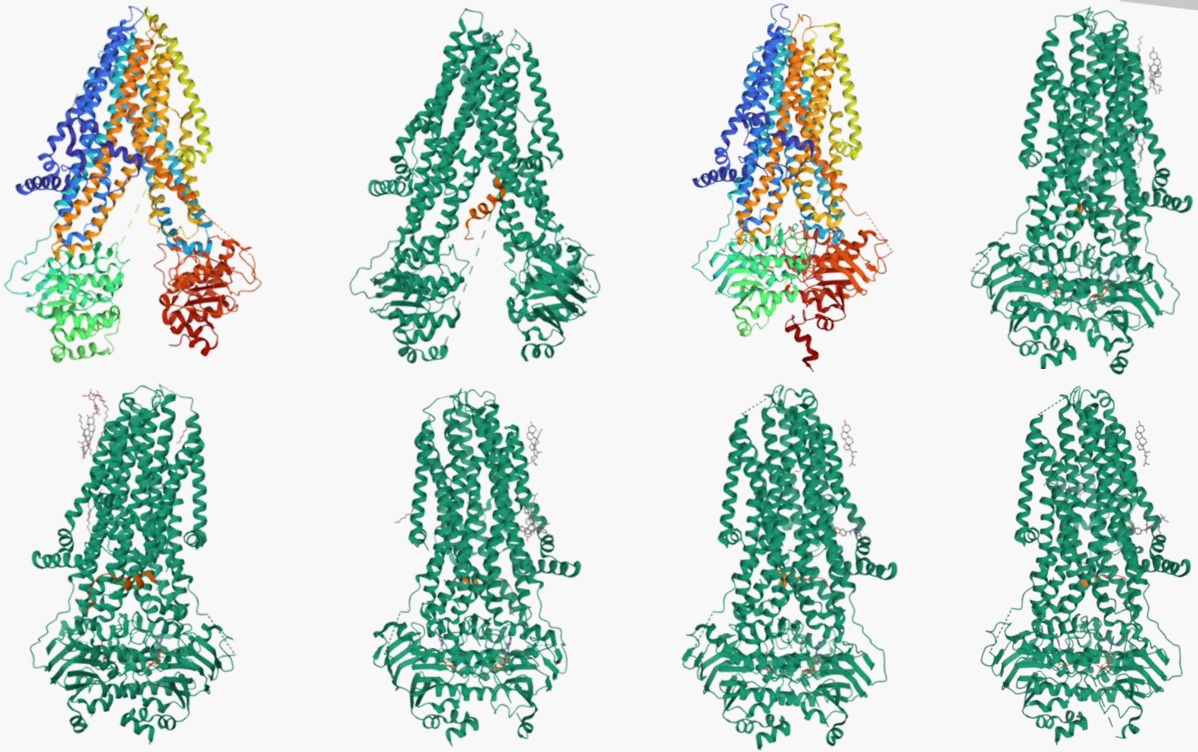
Adapted from: Rahman et al. PLoS One. 2013;8(9):e74574, Corradi et al. J Biol Chem. 2015;290(38):22891–906, Mornon et al. Cell Mol Life Sci. 2015;72:1377–1403

Structural Information on CFTR

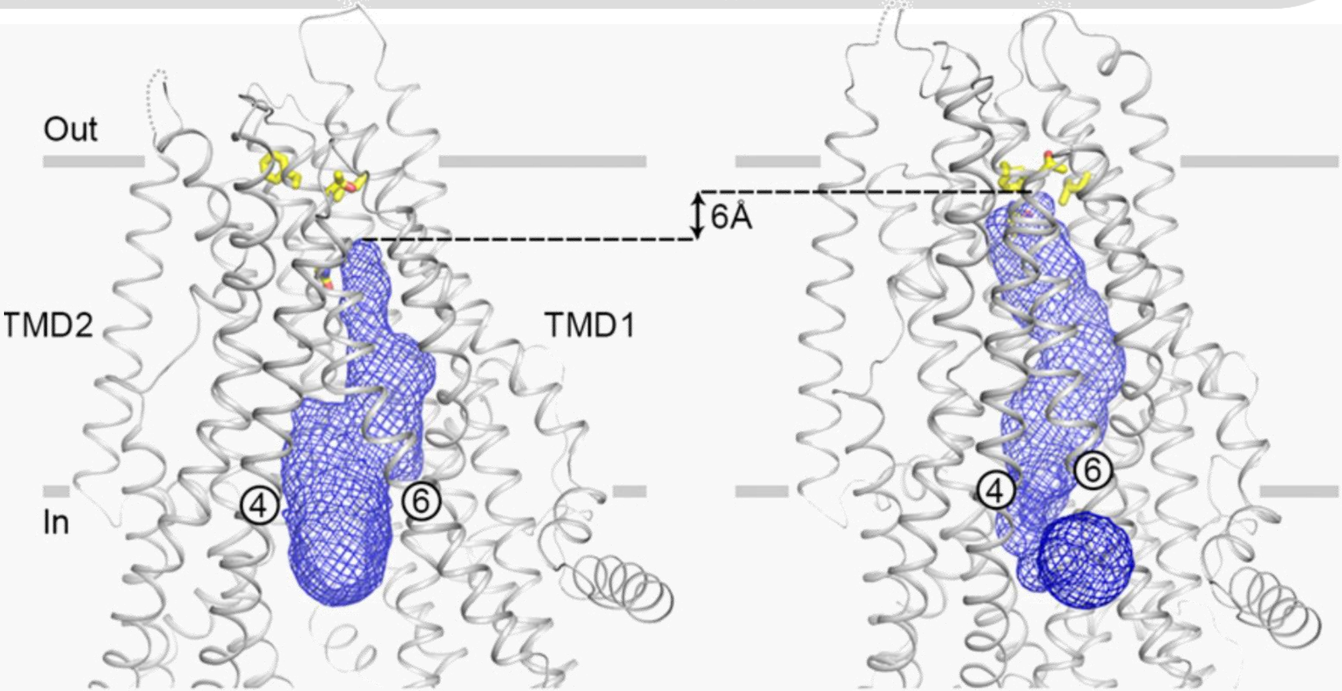
~30 cryo-EM structures (resolution 2.7-9.0Å) from different species, and representing different conformational states

Inward facing (closed state)

Outward facing (open state)

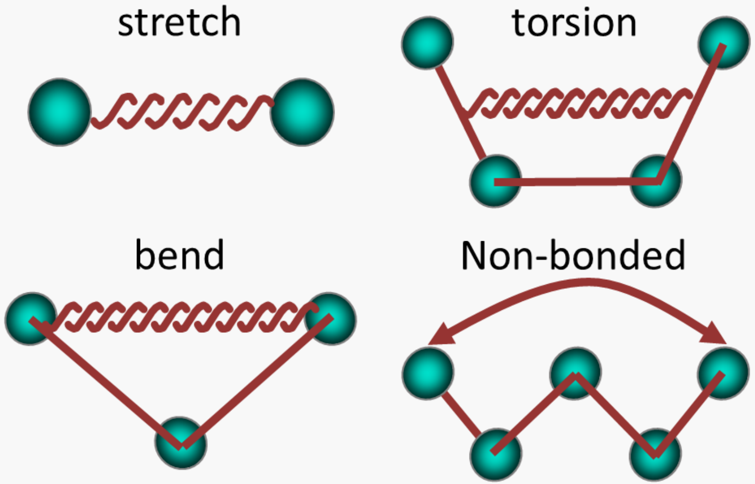


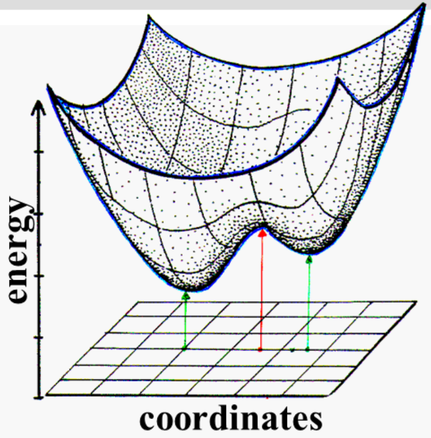
The Structure of the CFTR Pore



Most structures are excellent starting points for MD simulations

Force Field and the Potential Energy Surface



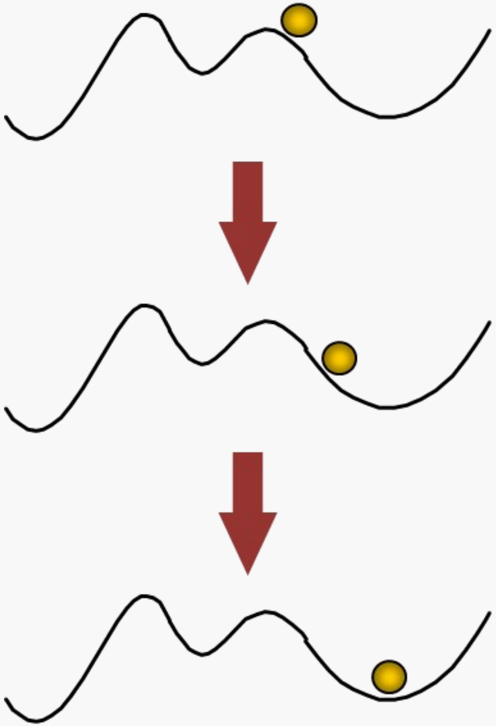


- Energy minimization
- Conformational search
- Molecular dynamics

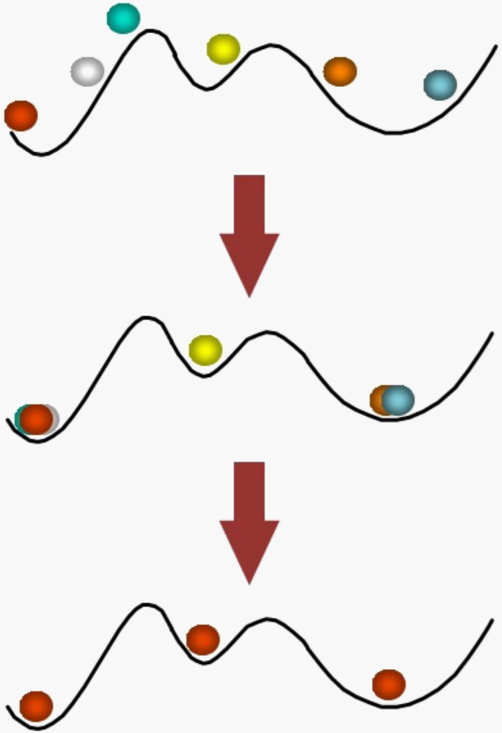
$$V(r^N) = \sum_{bonds} \frac{k_i}{2}(l_i - l_{i,0})^2 + \sum_{angles} \frac{k_i}{2}(\theta_i - \theta_{i,0})^2 + \sum_{torsions} \frac{V_n}{2}(1+\cos(n\omega-\gamma)) + \sum_{i=1}^{N} \sum_{j=i+1}^{N} \left(4\varepsilon_{ij} \left[\left(\frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] + \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}} \right) + \text{cross terms}$$

Energy Minimization and Conformational Search

Minimization



Conformational Search



Molecular Dynamics (MD) Simulations

The basic equations:

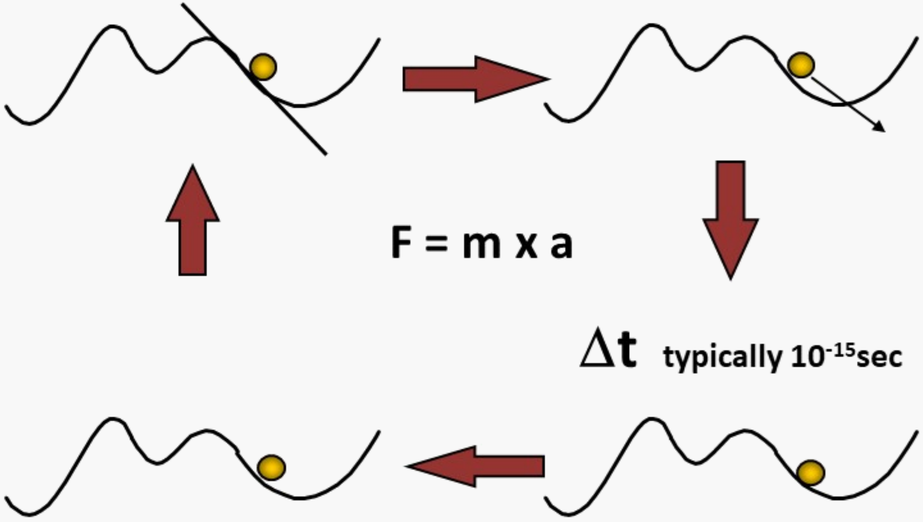
$$v(t)=\frac{dr(t)}{dt}$$

$$F=m\cdot a(t)=m\cdot\frac{dv(t)}{dt}$$

Numeric integration

$$\mathbf{r}(t+\delta t)=\mathbf{r}(t)+\delta t\,\mathbf{v}(t)+\frac{1}{2}\delta t^{2}\frac{1}{m}\mathbf{F}(t)$$

$$\mathbf{v}(t+\delta t)=\mathbf{v}(t)+\frac{1}{2}\delta t\frac{1}{m}[\mathbf{F}(t)+\mathbf{F}(t+\delta t)]$$



Calculate force based on surface curvature

Calculate velocities based on force

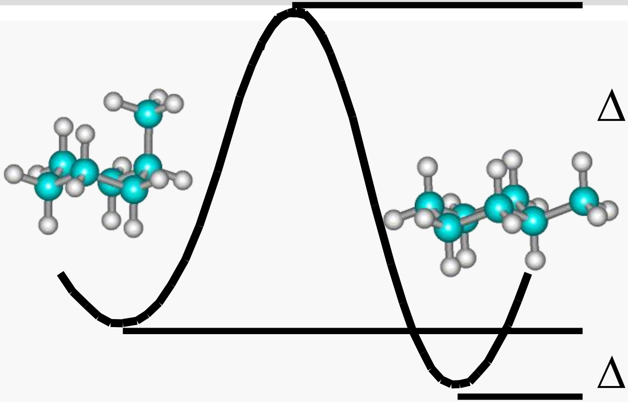
F = m x a

Δt typically 10^{-15}sec

Calculate new position

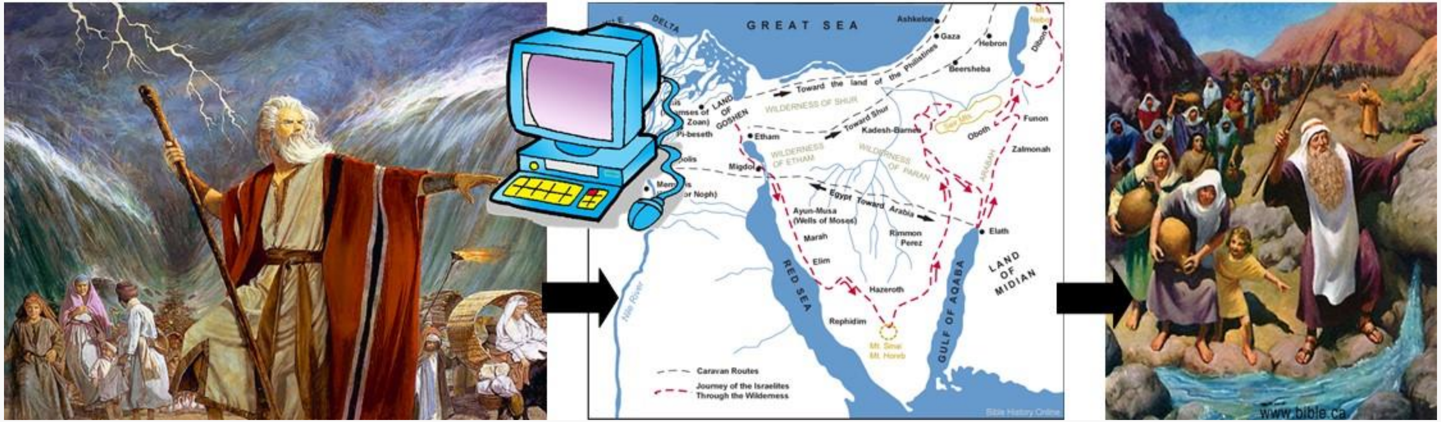
Save energy and geometry for average

Convergence Problems in MD

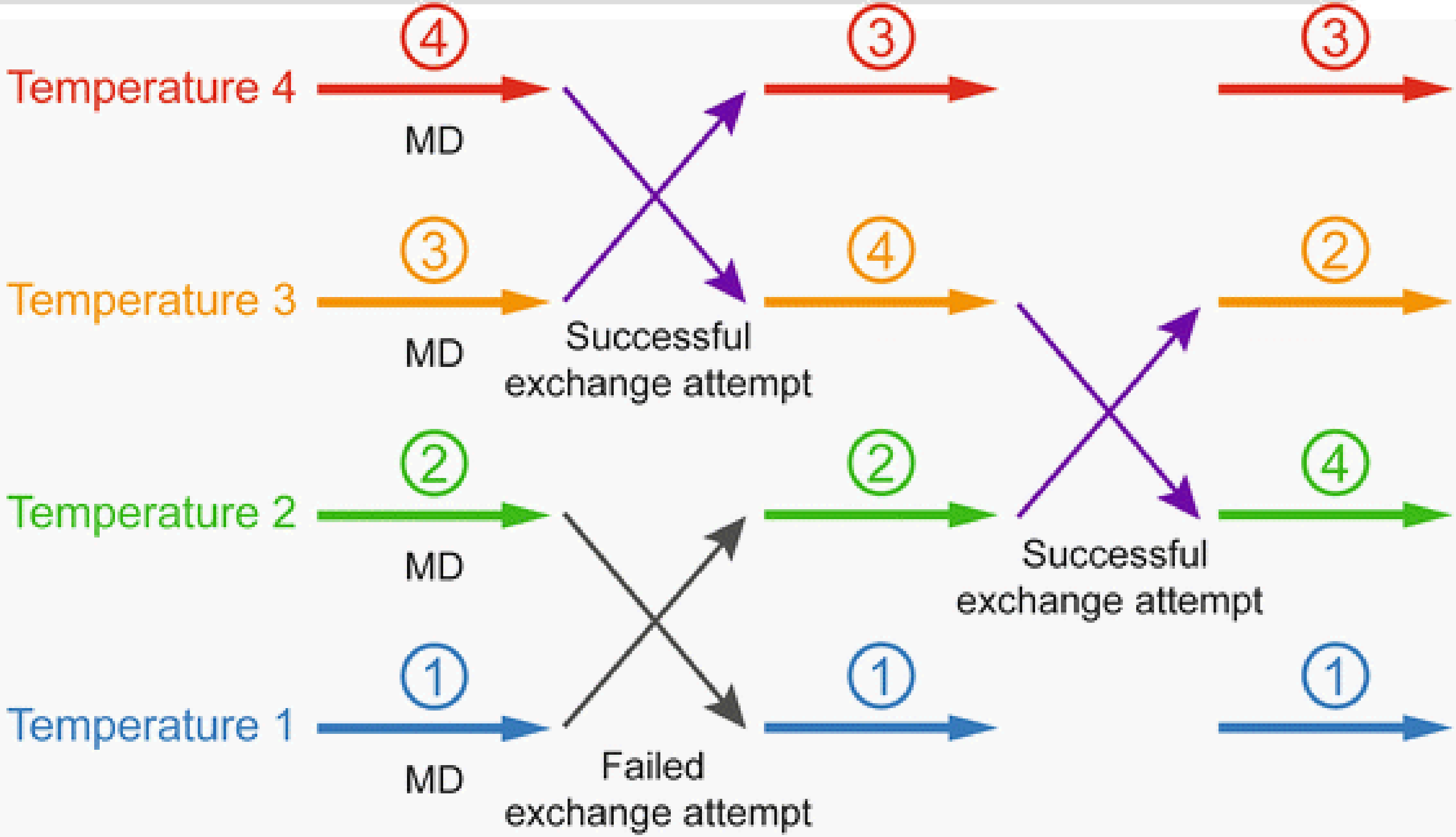


ΔG ~ 10 kcal/mol

ΔG ~ 1.7 kcal/mol



Replica Exchange MD

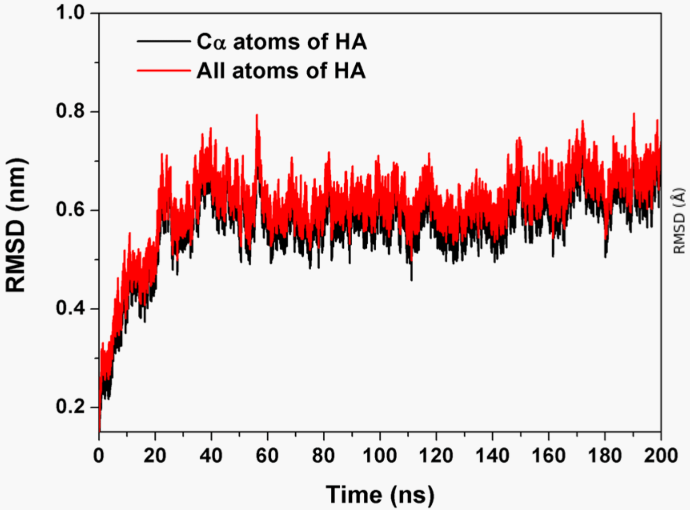


Analyzing MD Simulations

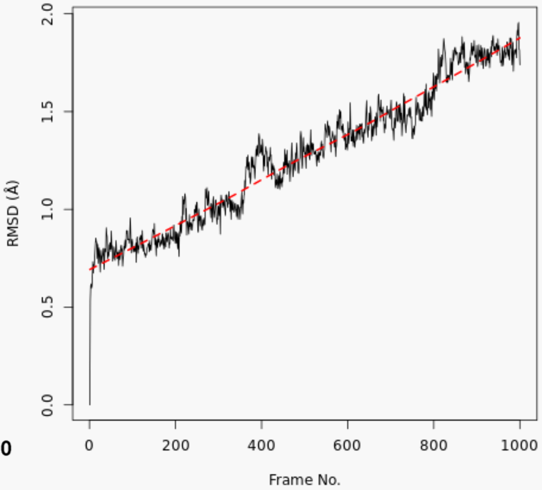
Always look on the bright side of life

Analyzing MD Simulations: RMSD and RMSF

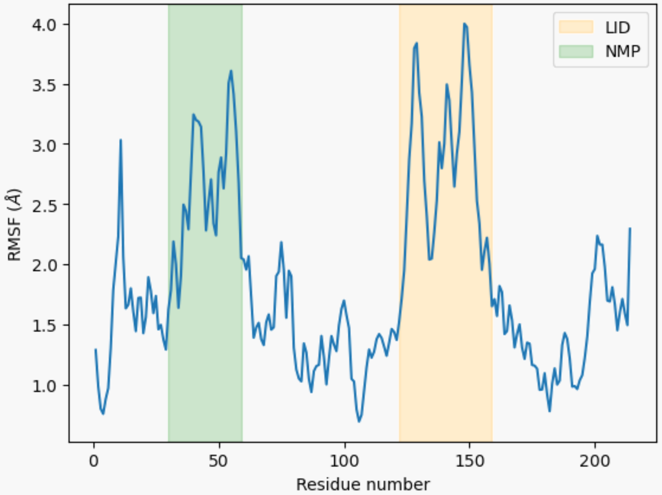
“Good” RMSD Plot



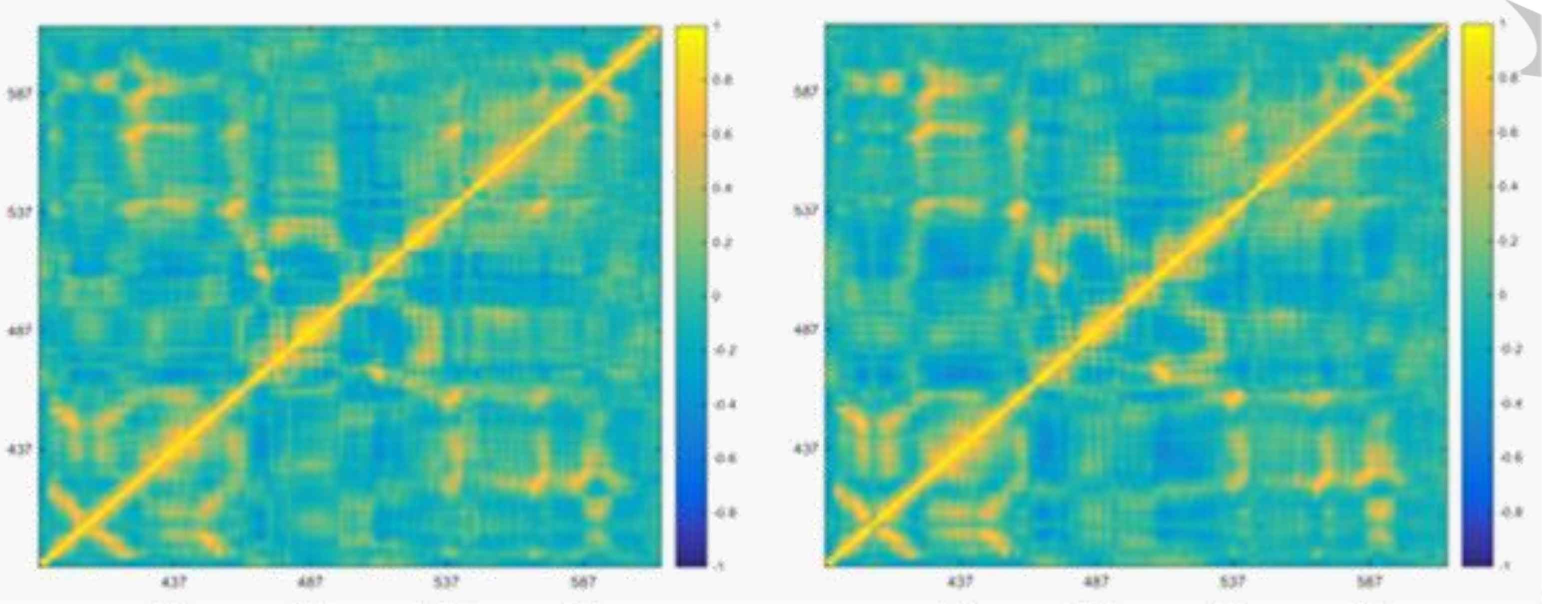
“Bad” RMSD Plot



RMSF Plot

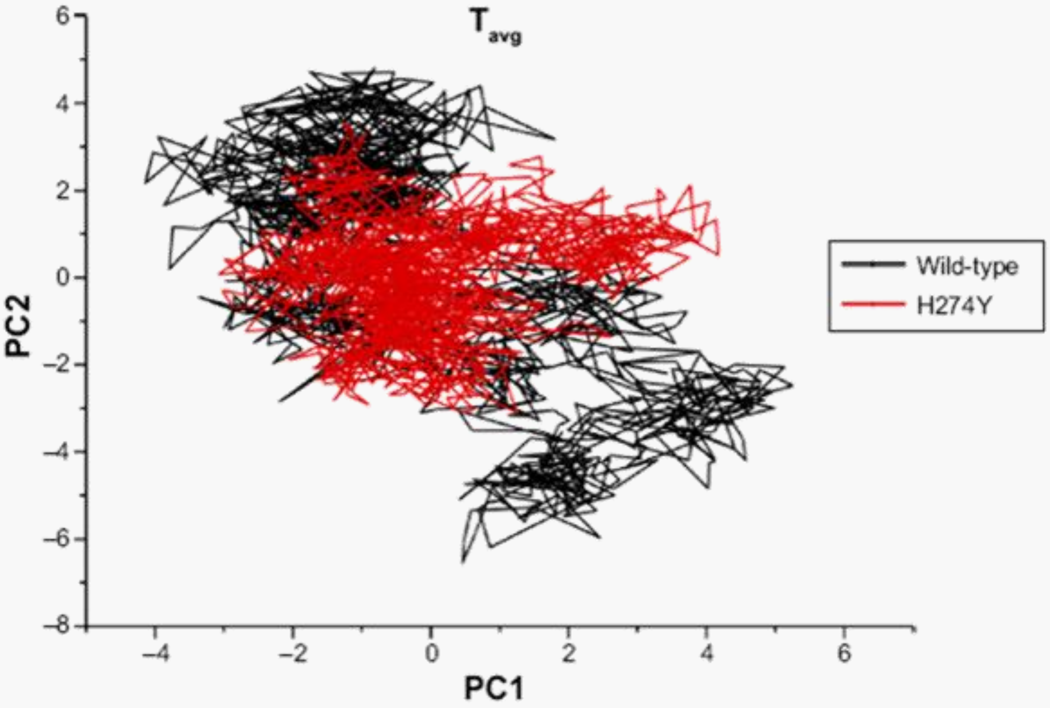


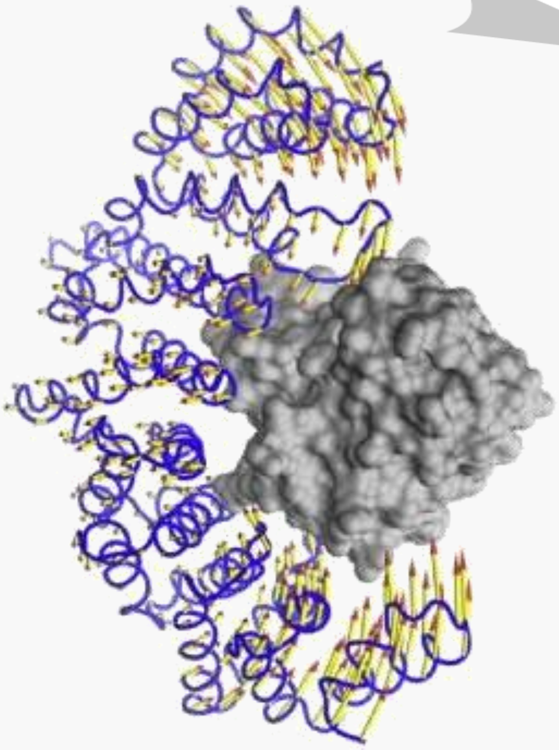
Analyzing MD Simulations: Covariance Matrix



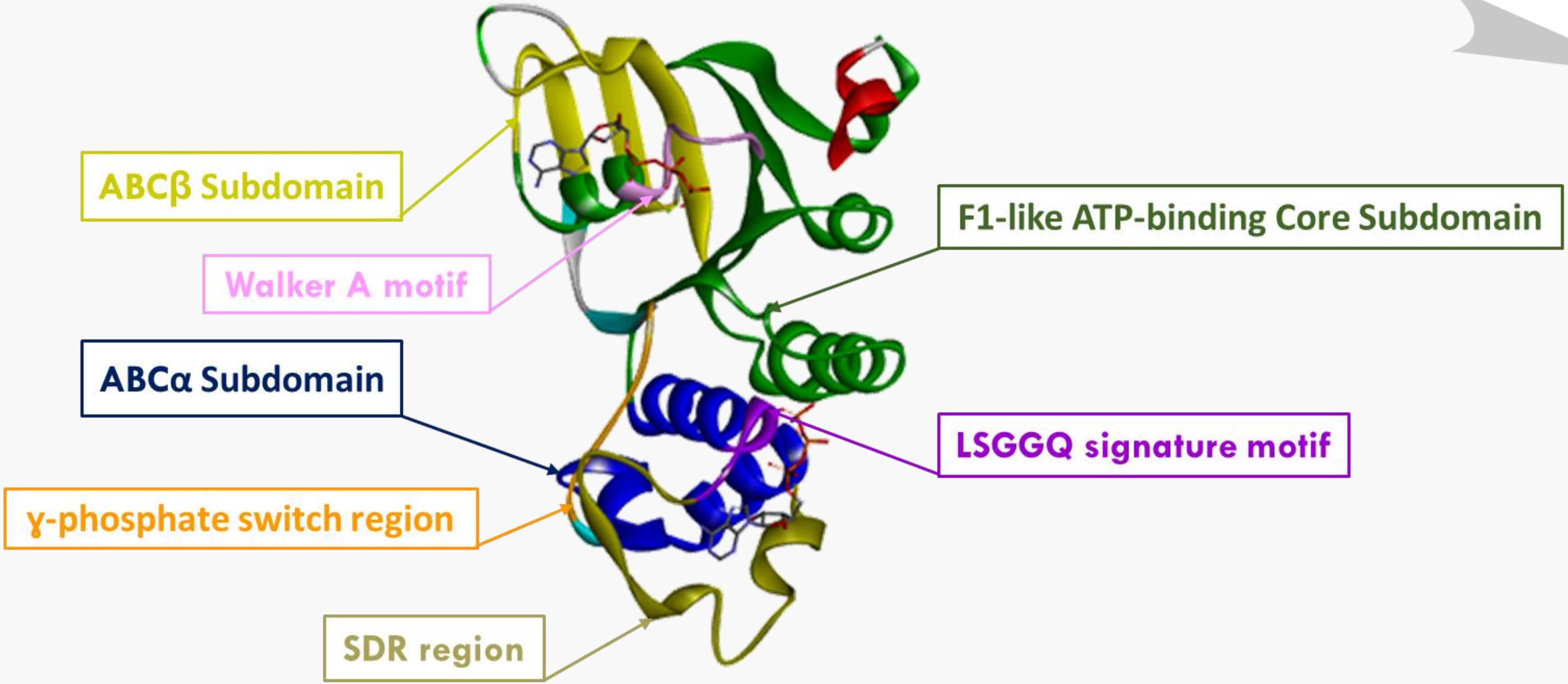
$$N_{\mathrm{cov}(X,Y)} \frac{\sum((X_i - < X >)\cdot(Y_i - < Y >))}{\sqrt{\sum(X_i - < X >)^2 \cdot \sum(Y_i - < Y >)^2}}$$

Analyzing MD Simulations: PCA

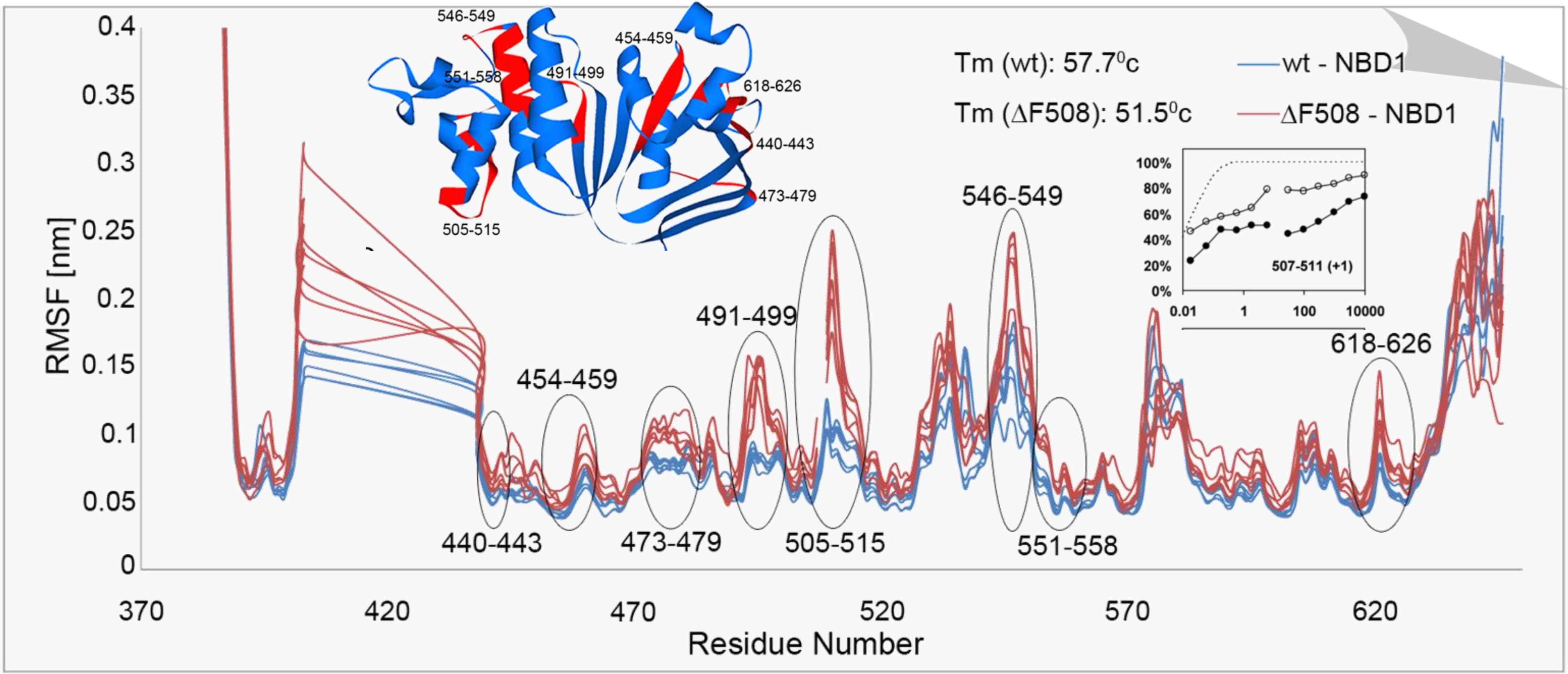




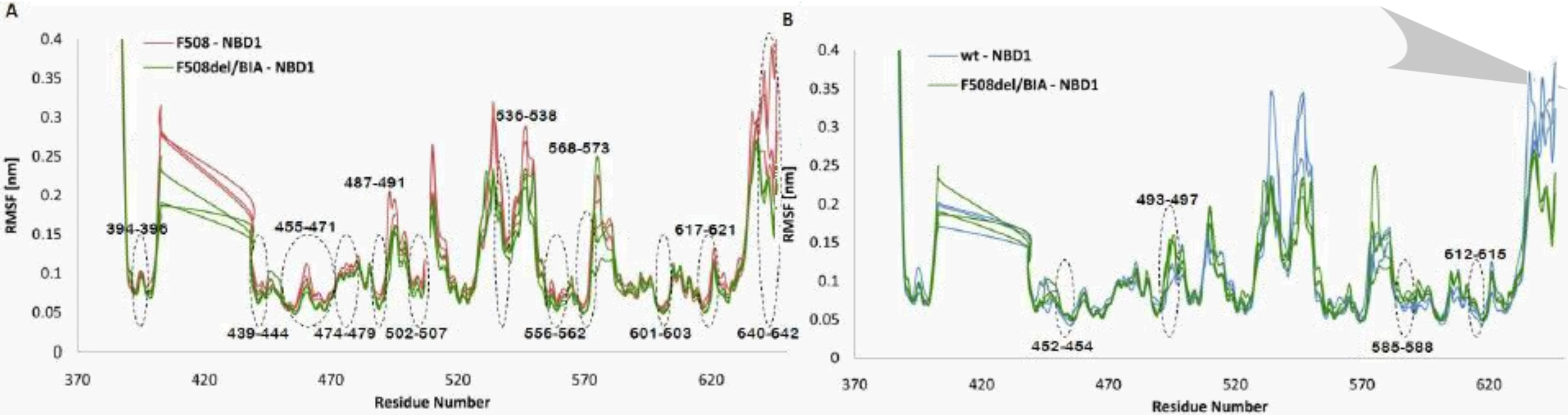
Detailed Structure of NBD1

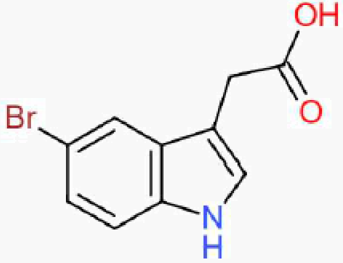


The Dynamics of WT and F508del NBD1



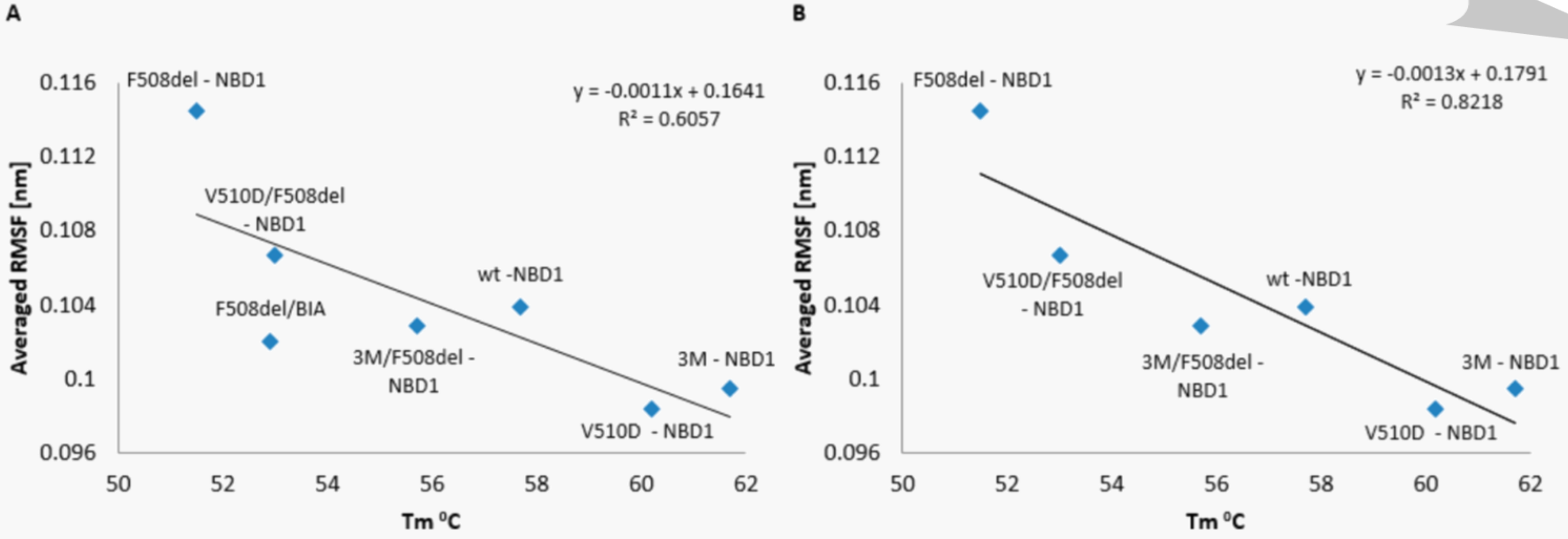
NBD1 in Complex with BIA





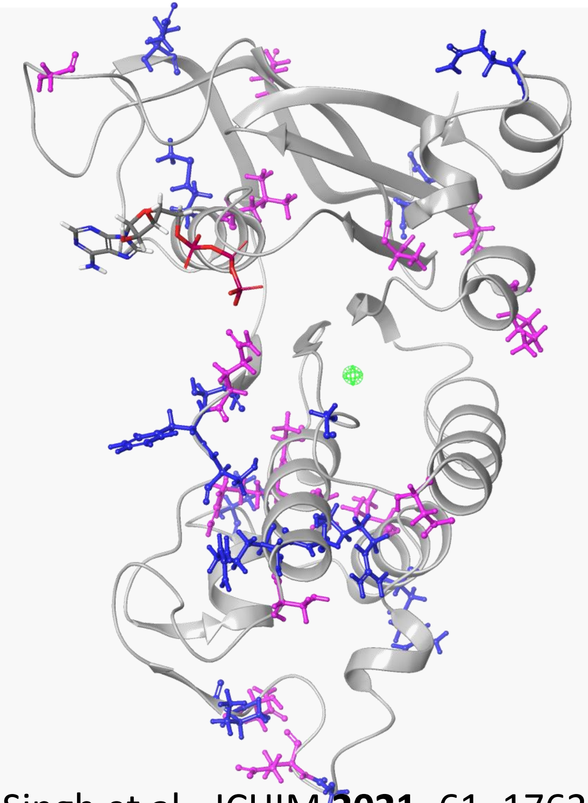
Zhenin et al., JCIM **2015,** 55, 2349-2364

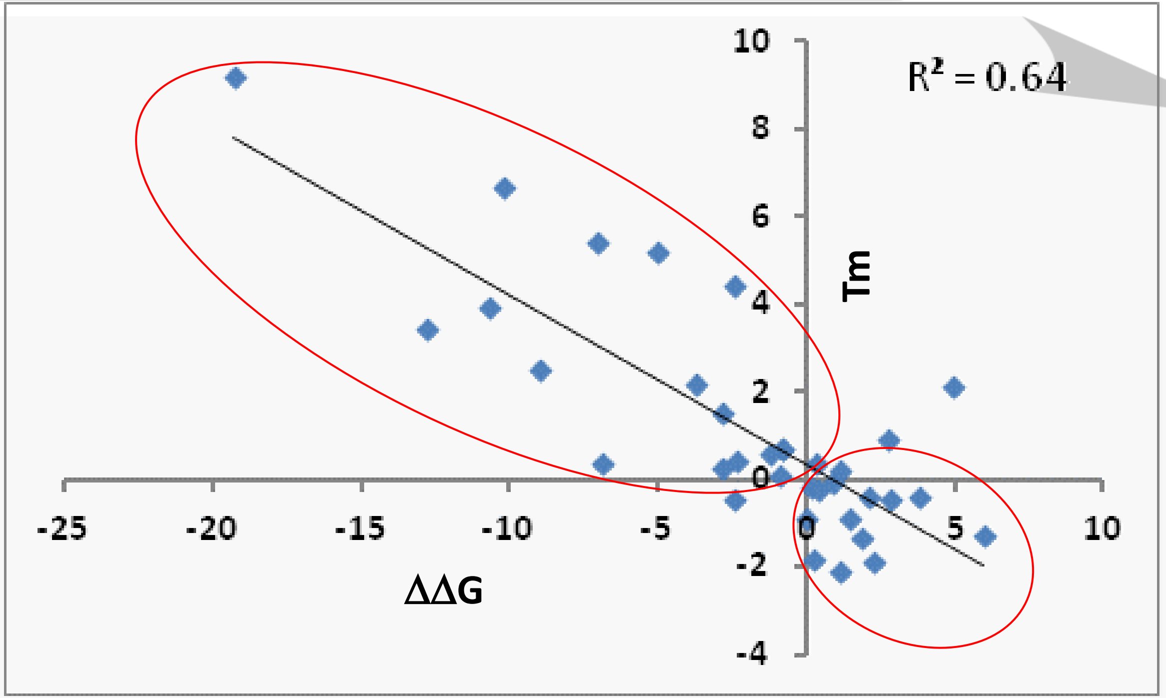
Correlating RMSF Profiles with Thermal Stability



RMSF profiles are indicative of thermal instability in NBD1 constructs of hCFTR

Predicting Thermal Stability with FoldX



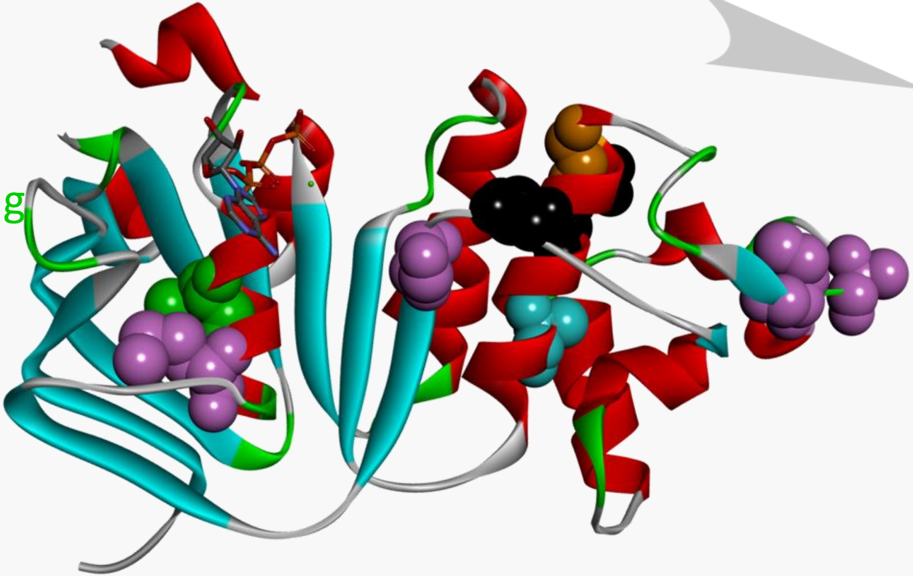


- Stabilizing mutations benefit from better H-bonds
- Destabilizing mutations suffer from steric clashes

Singh et al., JCHIM **2021**, 61, 1762

MD Simulations at Elevated Temperatures

- WT
- G551D (LSGGQ, +0.22°C): CF-causing
- A559T (ABCα, -10.70°C): CF-causing
- L467P (F1 ATP binding core,-19.30°C): CF-causing
- 6SS (+17.50°C): Stabilizing
- 2PT/M470V (+9.30°C): Stabilizing

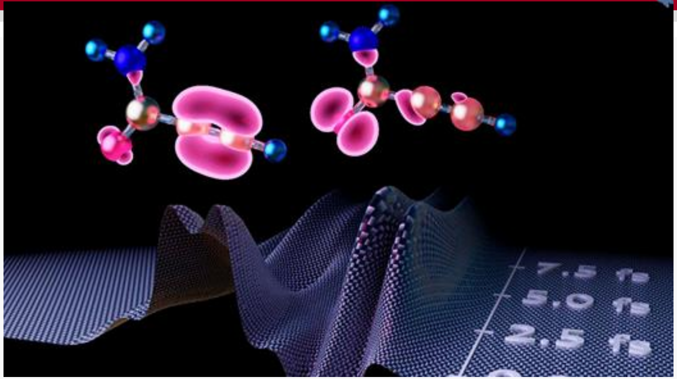


Correlate computational predictions with experimental observations
Mechanistic insights

Lublin et al., manuscript submitted

Study Design

FAST. FLEXIBLE. FREE.
GROMACS



Microsecond long MD simulations at an elevated temperature of 410K (1.8 µs * 6 constructs * 3 replicas) = 32.4 µs

Starting point 2PZE
Protein preparation wizard
Mutate residue
Gromacs 2021.1
AMBER99SB-ILDN FF
Leap-Frog integrator, 2 fs

SCHRÖDINGER.

WT-NBD1
L467P-NBD1
A559T-NBD1
G551D-NBD1
6SS-NBD1
2PT/M470V-NBD1

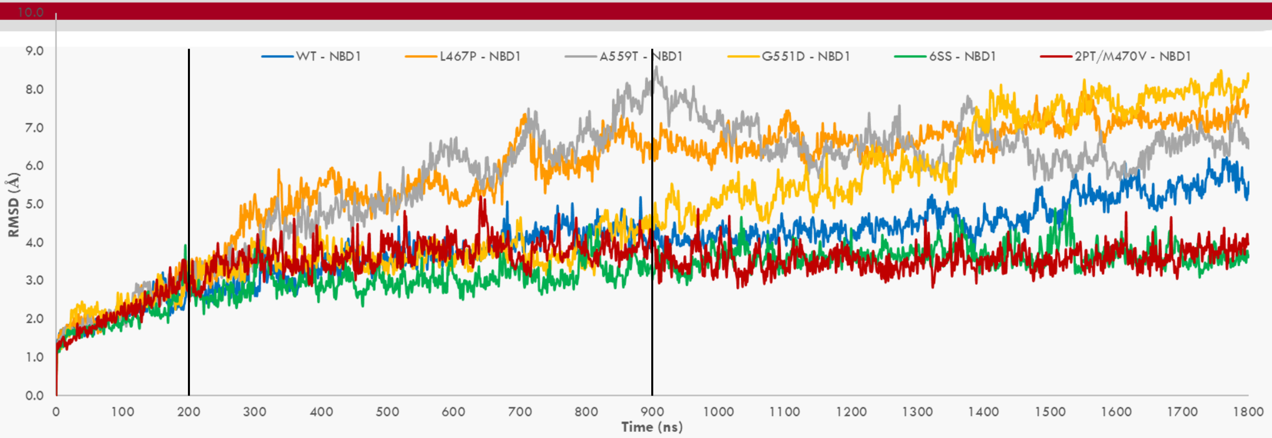
Minimization
NVT [Berendsen thermostat]
NPT [Parrinello-Rahman, Nose-Hoover thermostat]

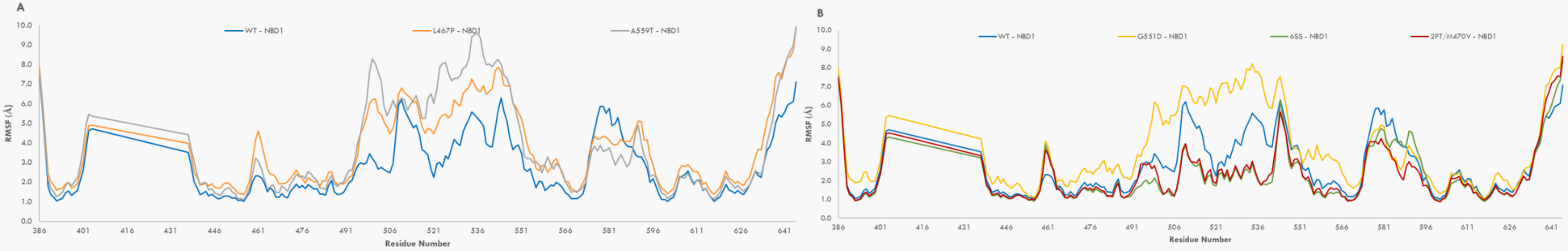
Production run

① Heating from 310K to 410K at a rate of 2ns/1K

② Maintaining at 410K under NPT conditions for 1.6 µs

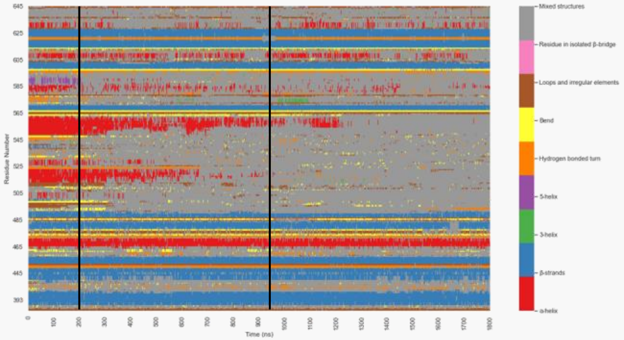
RMSD and RMSF



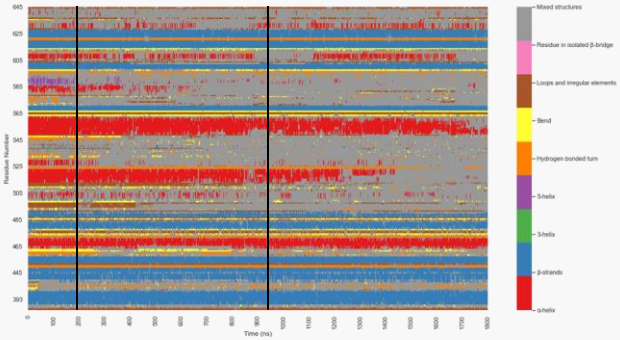


DSSP

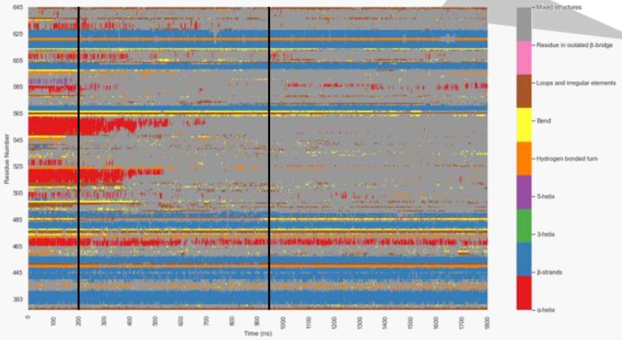
A559T-NBD1



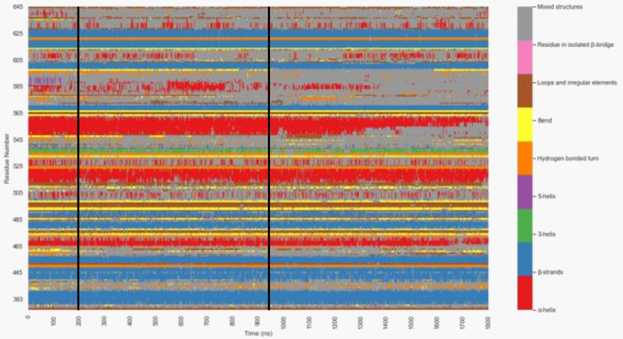
WT-NBD1



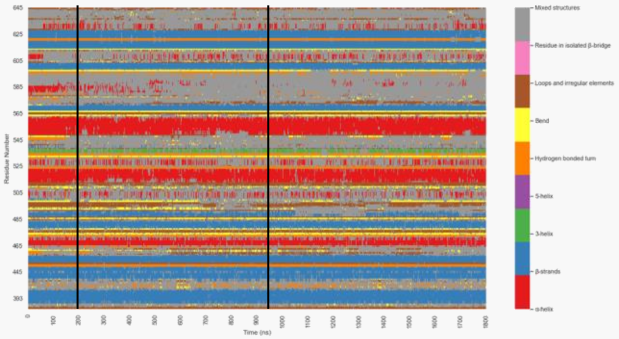
L467P-NBD1



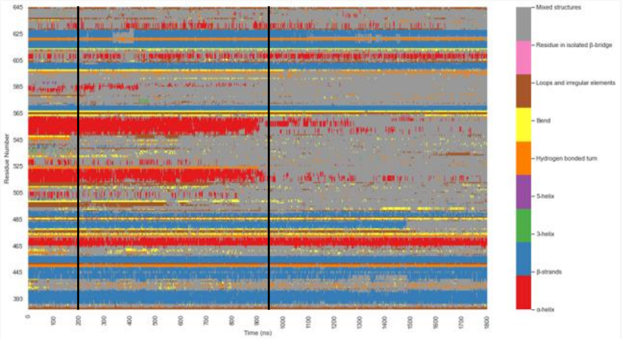
6SS-NBD1



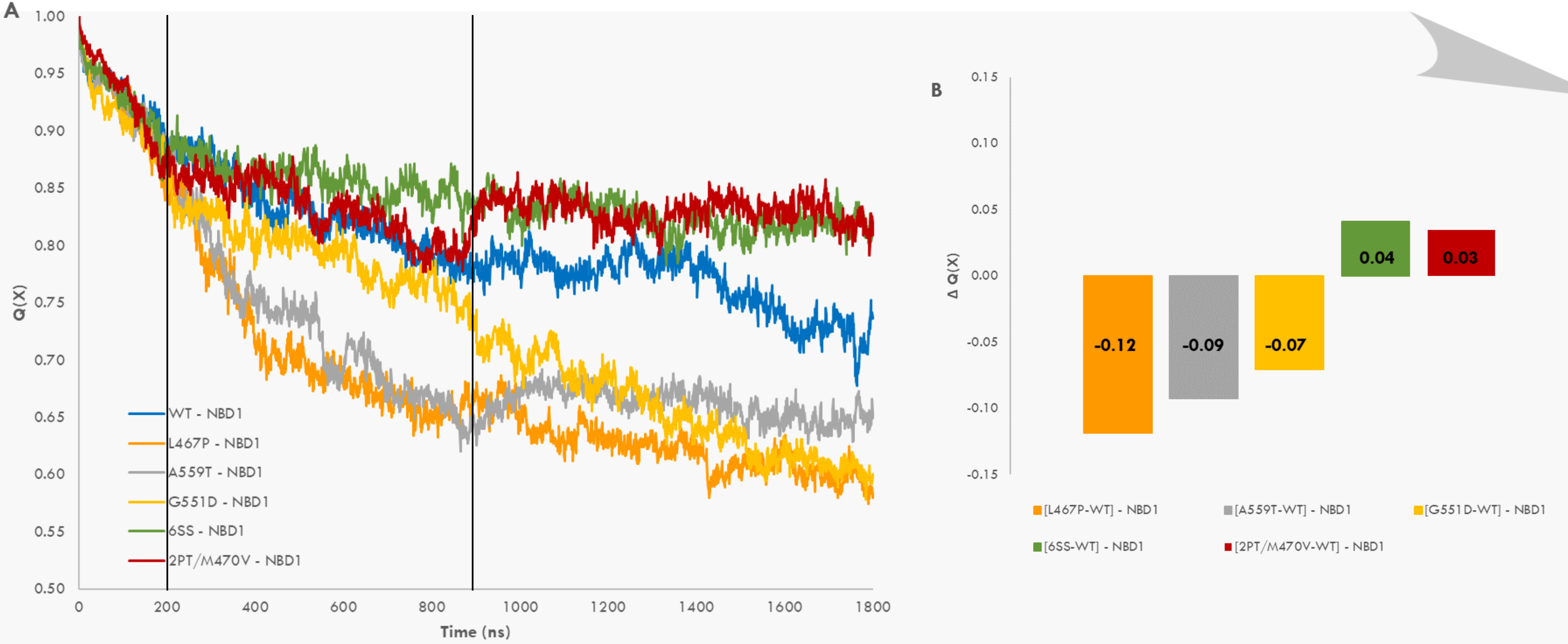
2PT/M470V-NBD1



G551D-NBD1



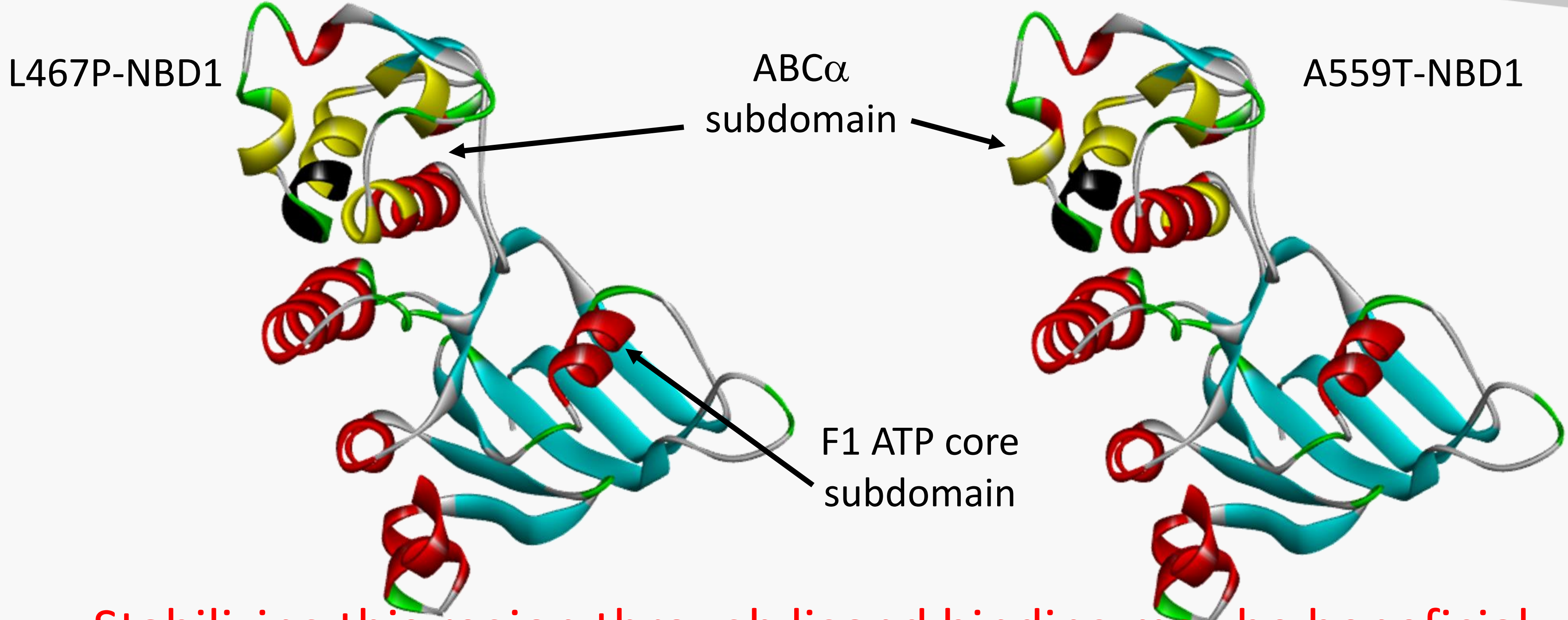
Fraction of Native Contacts



Computational metrics agree with experiment (except G551D)

Mechanistic Insights I

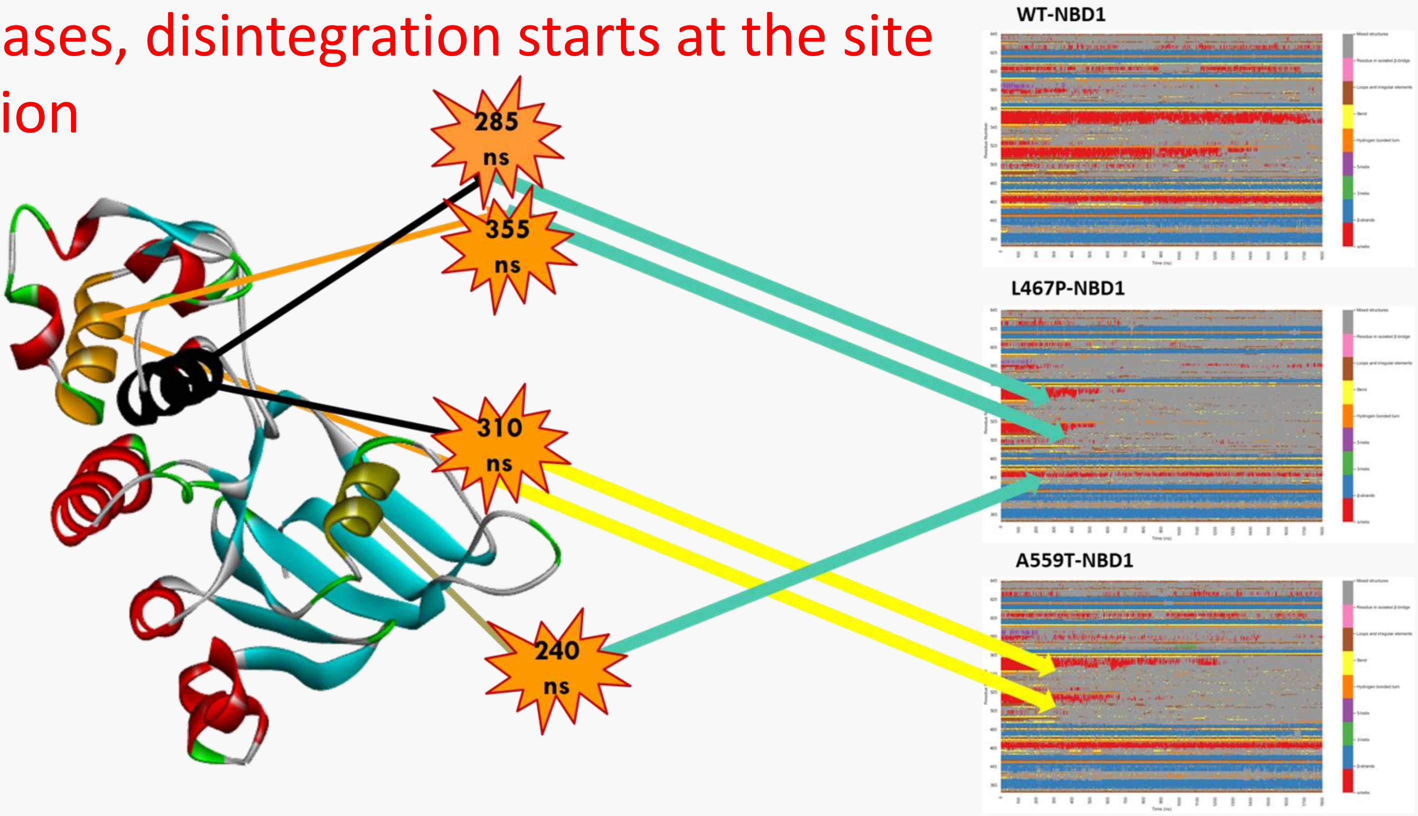
Highly destabilized regions in L467P-NBD1 and A559T-NBD1



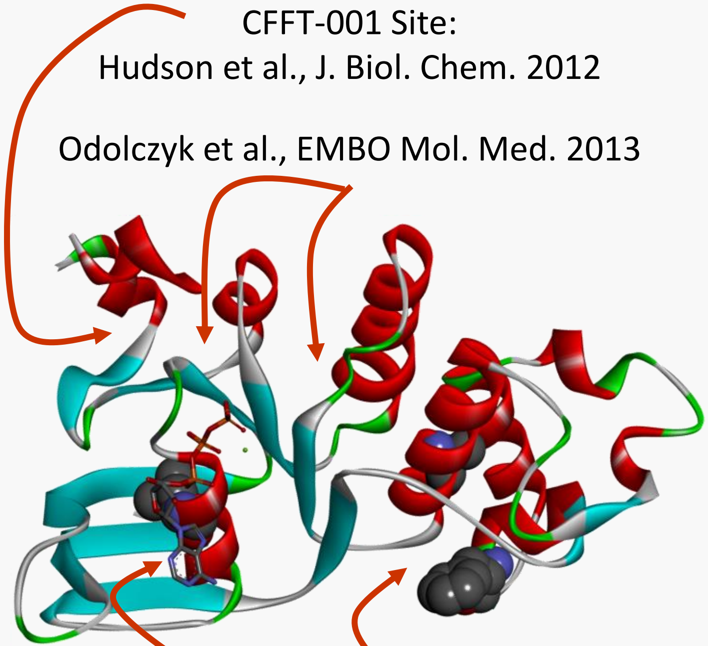
Stabilizing this region through ligand binding may be beneficial

Mechanistic Insights II: First Points of Disintegration

In both cases, disintegration starts at the site of mutation



Binding Sites on NBD1

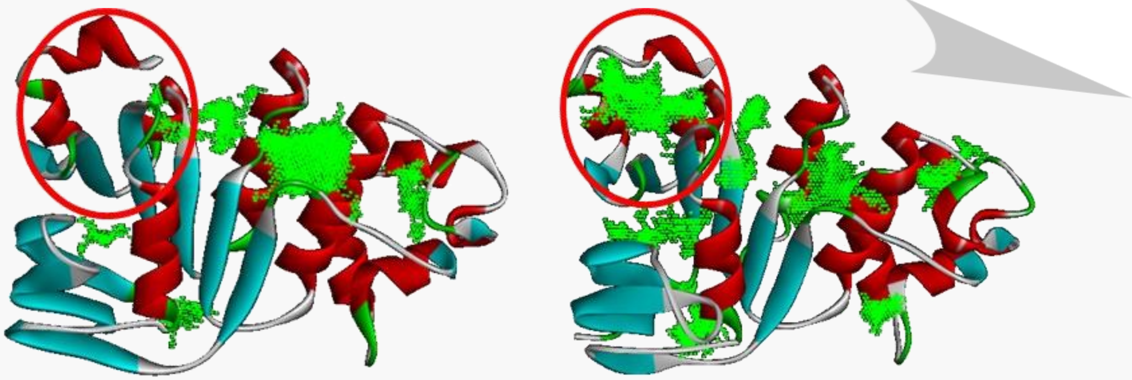


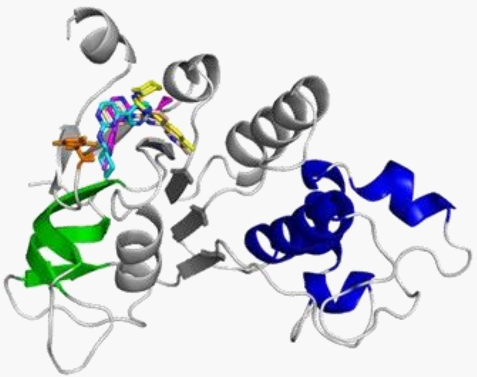
CFFT-001 Site:
Hudson et al., J. Biol. Chem. 2012

Odolczyk et al., EMBO Mol. Med. 2013

ATP Site
(L467P)

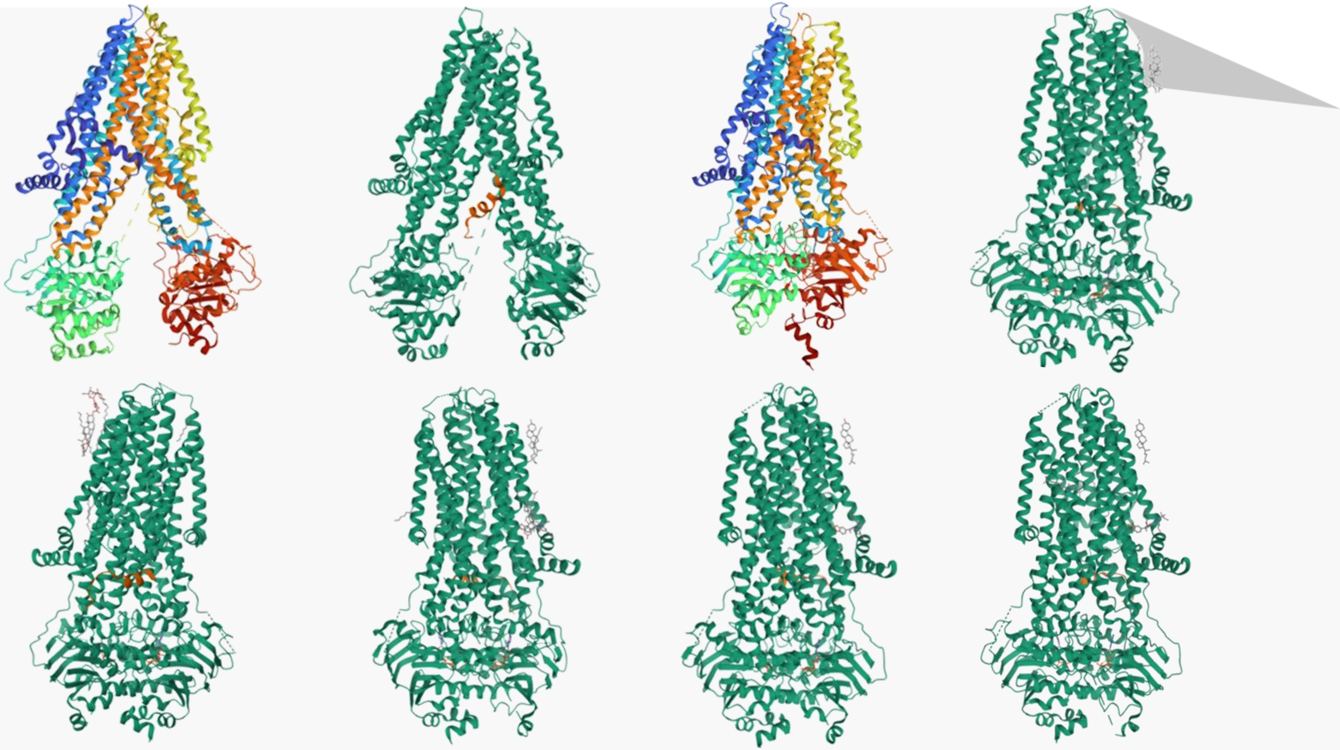
F508-ICL4 Site:
Serohijos et al., PNAS 2008
(BIA; A559T)





Computational Studies on full-length CFTR

~30 cryo-EM structures (resolution 2.7-6.9Å) from different species, and representing different conformational states

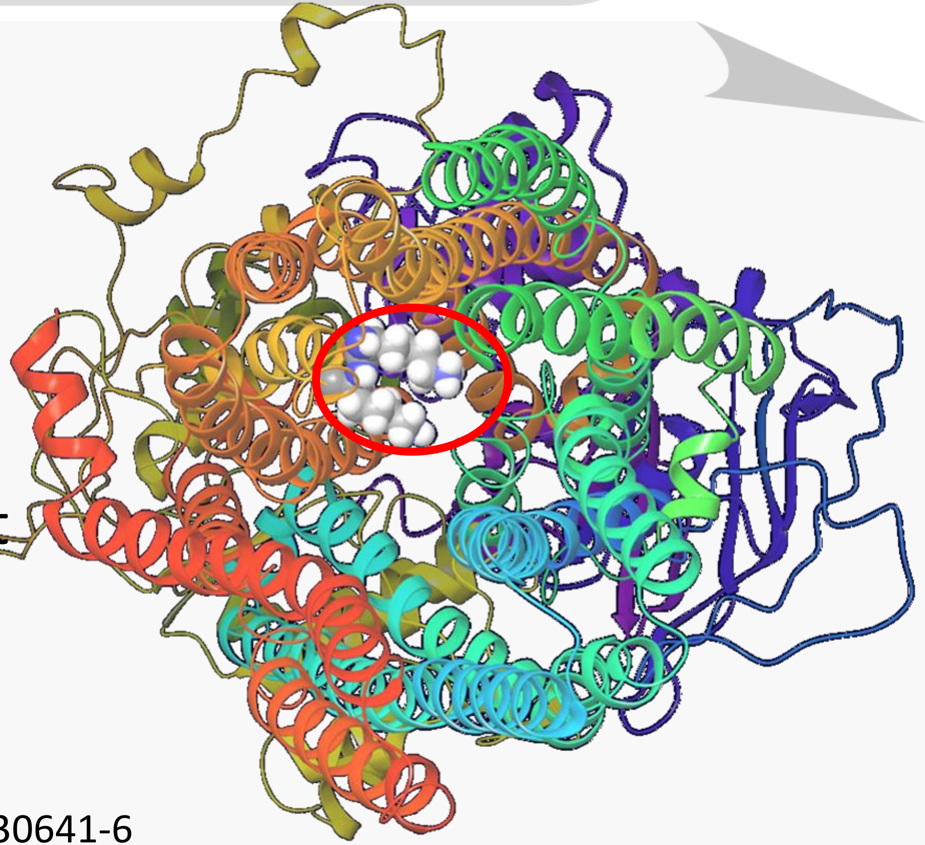


Most structures are excellent starting points for MD simulations

The Q359K / T360K mutation

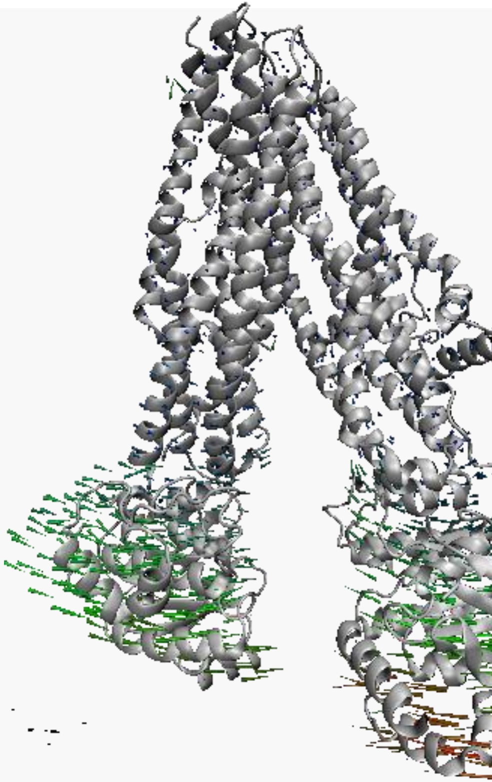
- Described in Jewish CF patients of Georgian decent
- Results in severe CF phenotype albeit with residual early CFTR function

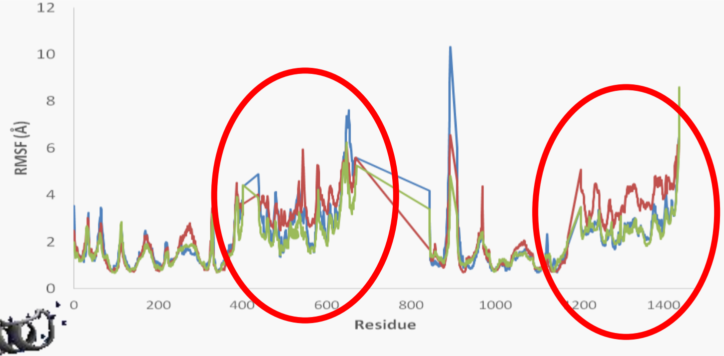
- No predicted de-stabilization effect
- Pore hindrance
- “electrostatic trap” (?)

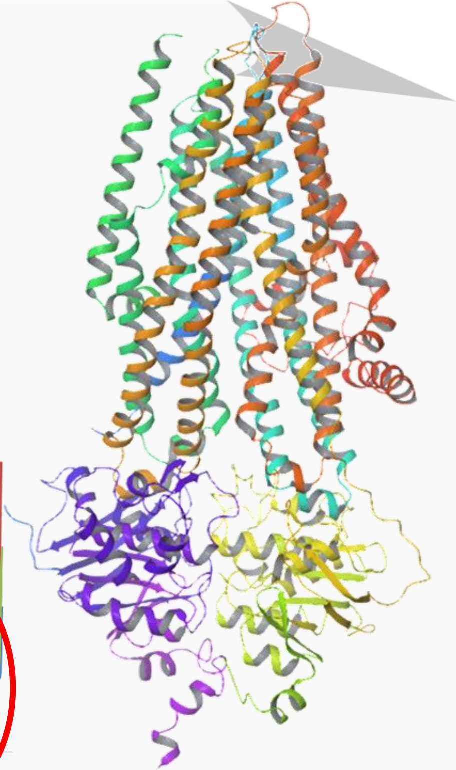


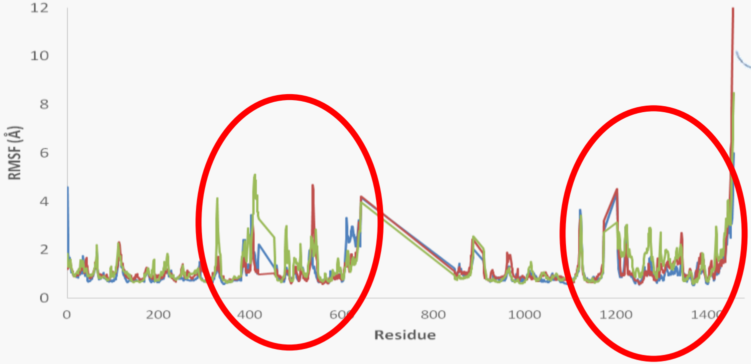
Mei-Zahav et al., J Cyst Fibros. 2018 pii: S1569-1993(18)30641-6

MD Simulations of WT-CFTR







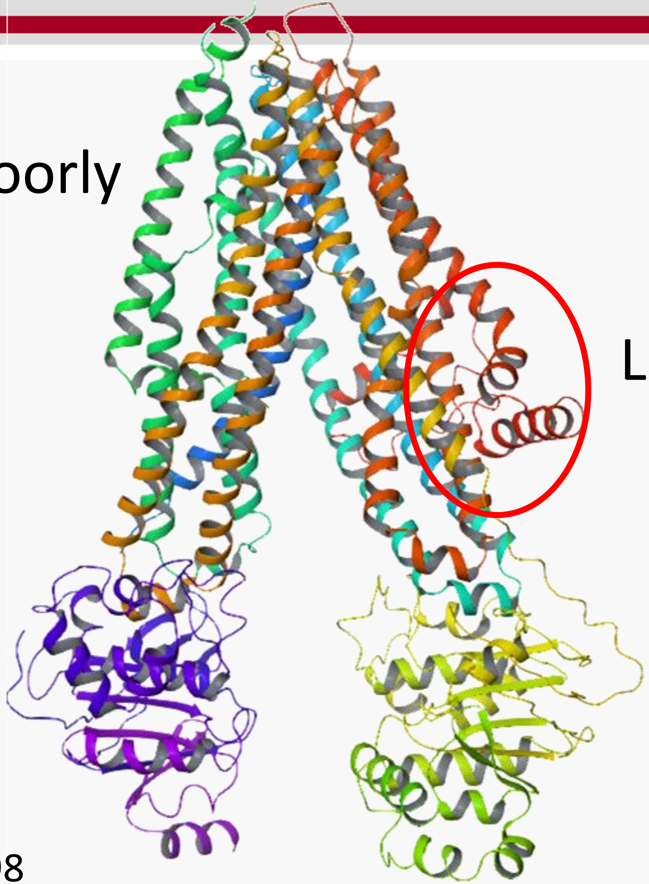


Unpublished results

P67L-CFTR

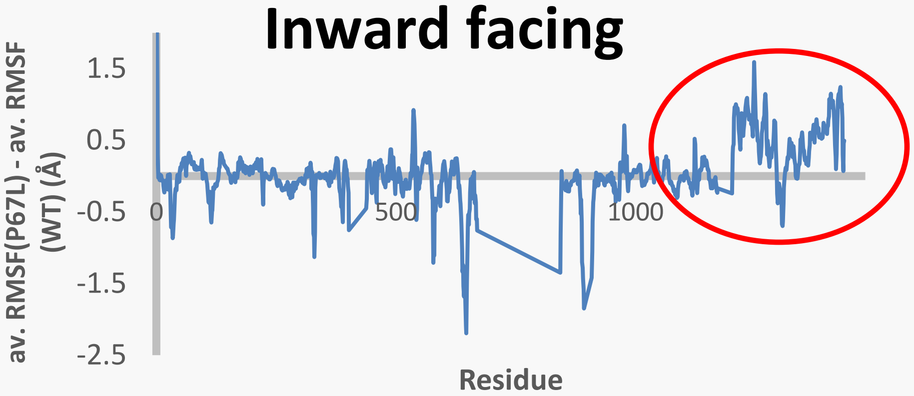
- Rare yet severe mutation
- Molecular consequences poorly characterized
- Correctable by Lumacaftor
- Potentiated by Ivacaftor

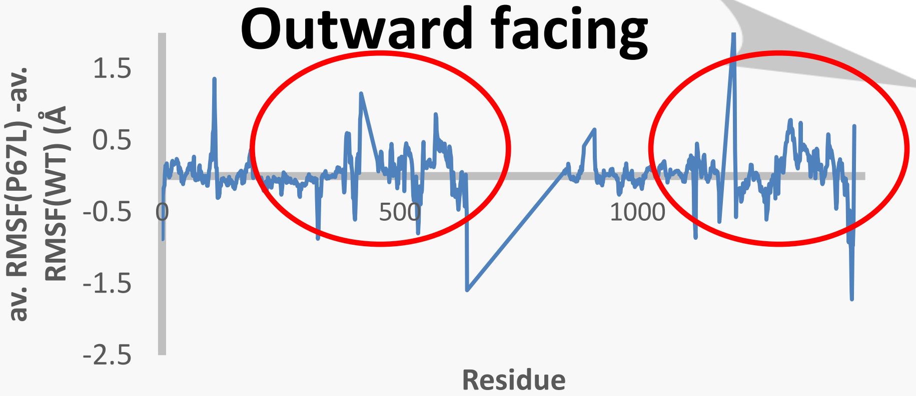
- What does it do?

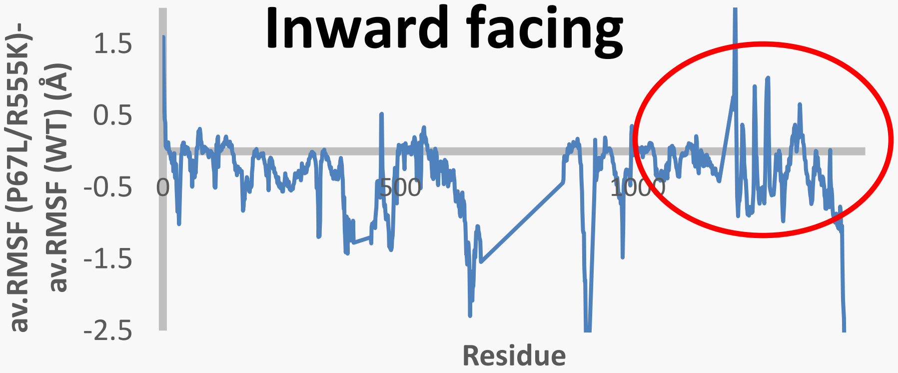


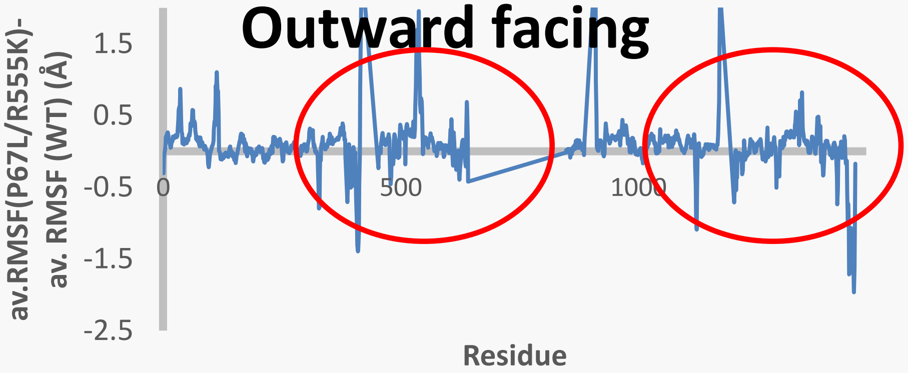
Sabusap et al., J. Biol. Chem., 2021, 296, 100598

P67L-CFTR and P67L/R555K-CFTR


Inward facing

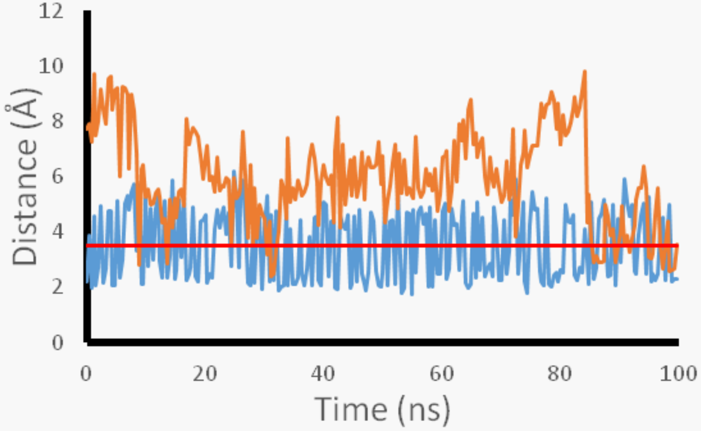

Outward facing

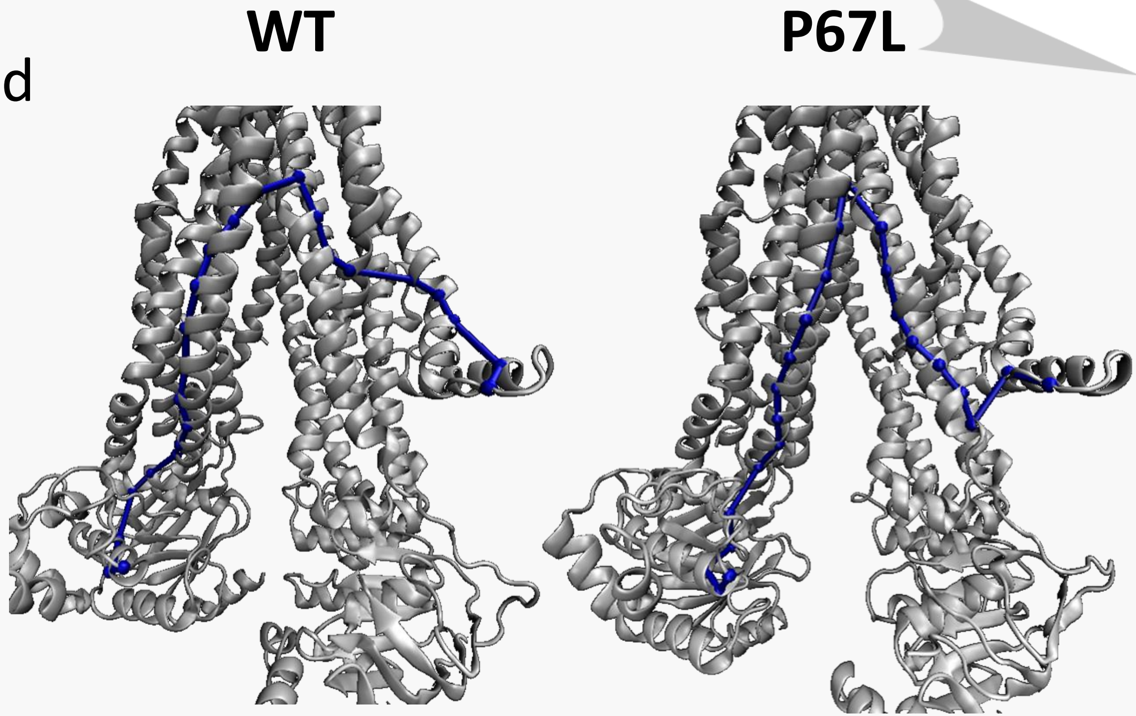

Inward facing


Outward facing

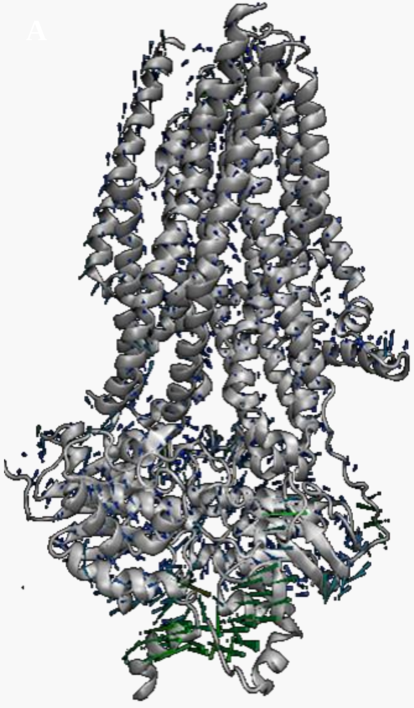
Alostericity

- WT CFTR: Stable H-bond between Y275 (MSD1) and C1355 (NBD2)
- P67L: No such stabilizing interactions

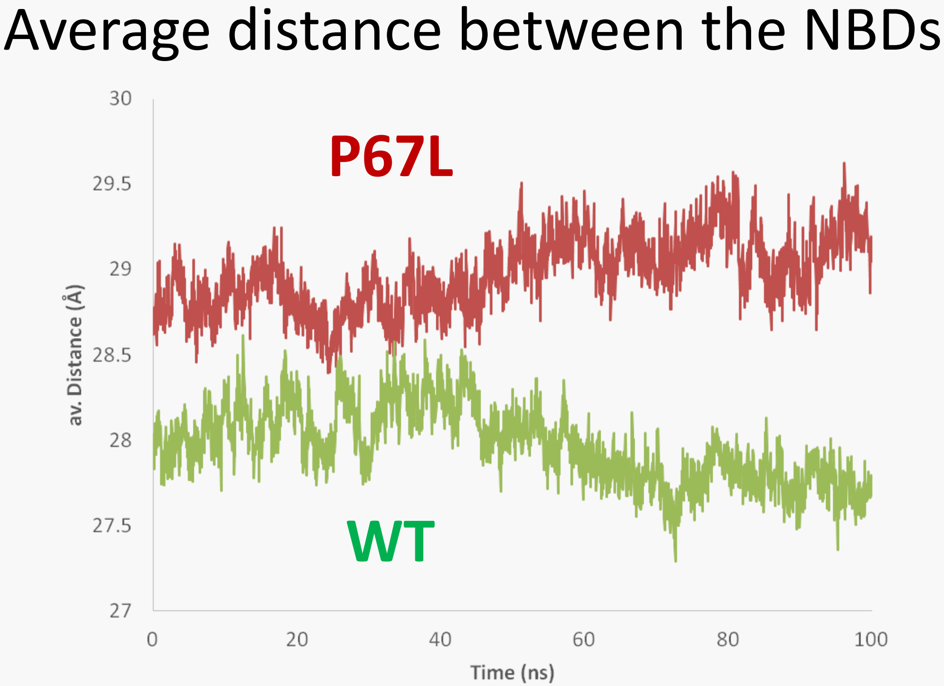


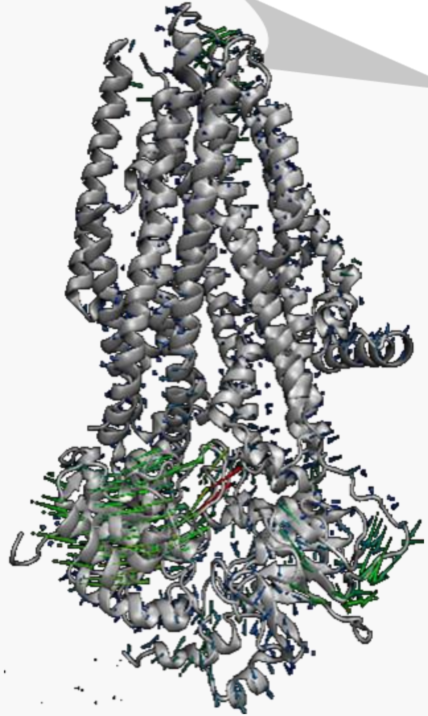


P67L-CFTR: Also, a Gating Mutation (?)



WT



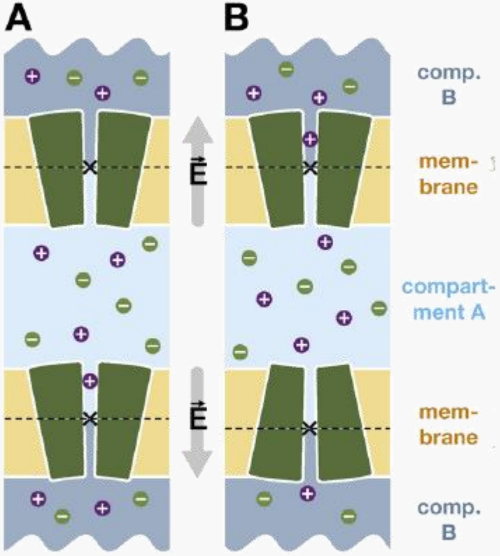


P67L

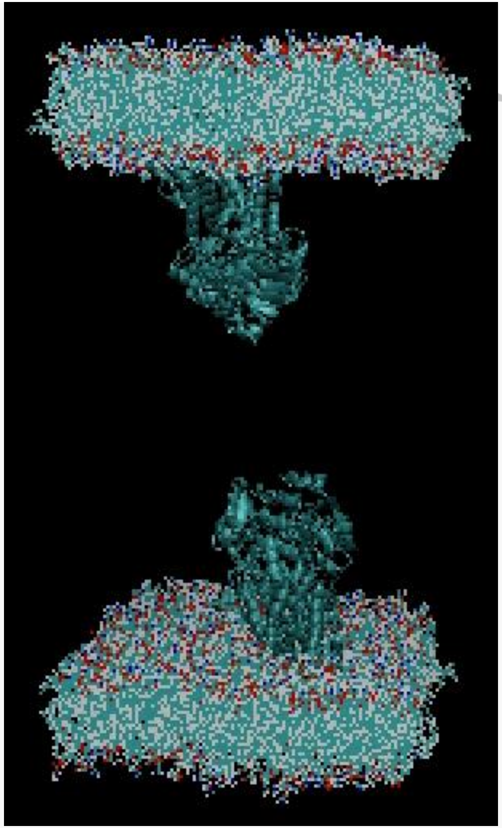
Sabusap et al., J. Biol. Chem., 2021, 296, 100598

Computational Electrophysiology Simulations

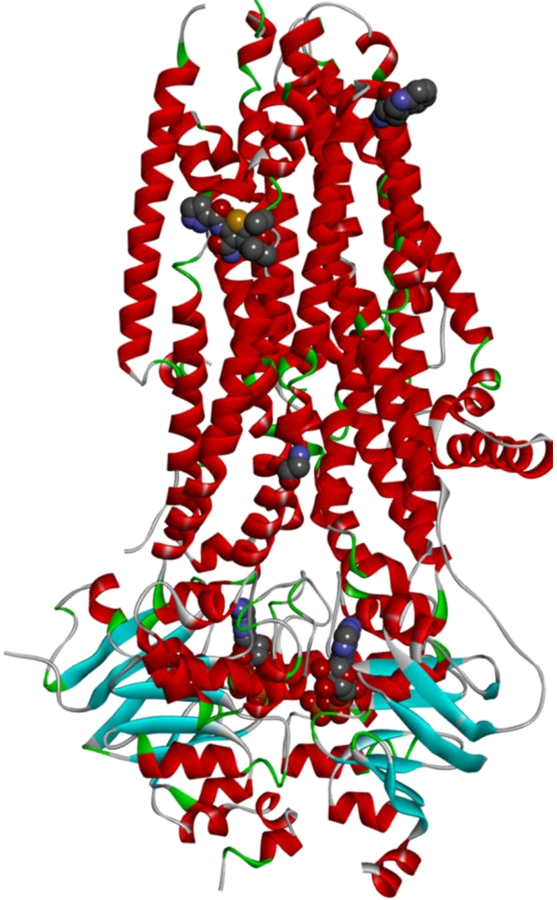
- Simulating chloride conductance under near-physiological conditions
  - ❖ Maintaining relevant gradient of chloride ions across the membrane (using a double-membrane setup)



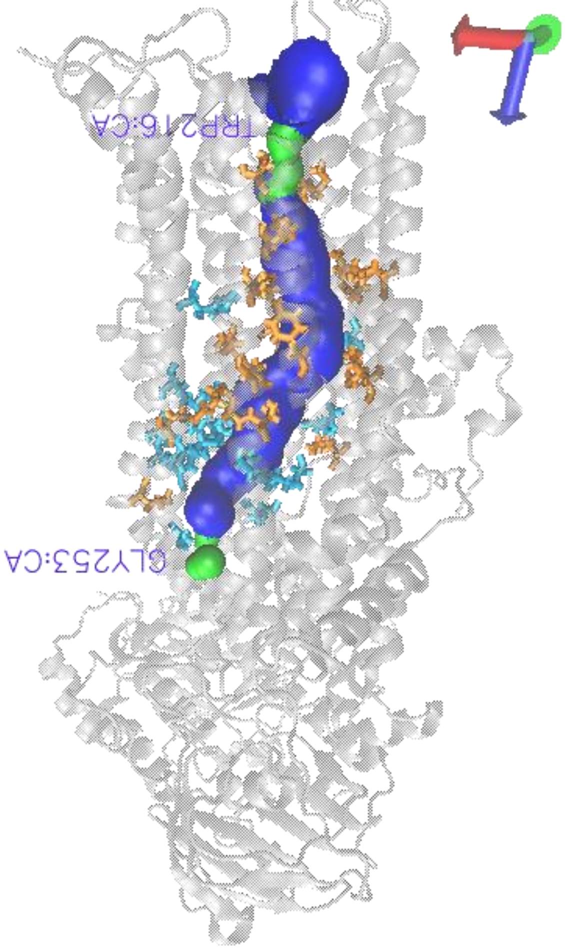
> 10^6 atoms



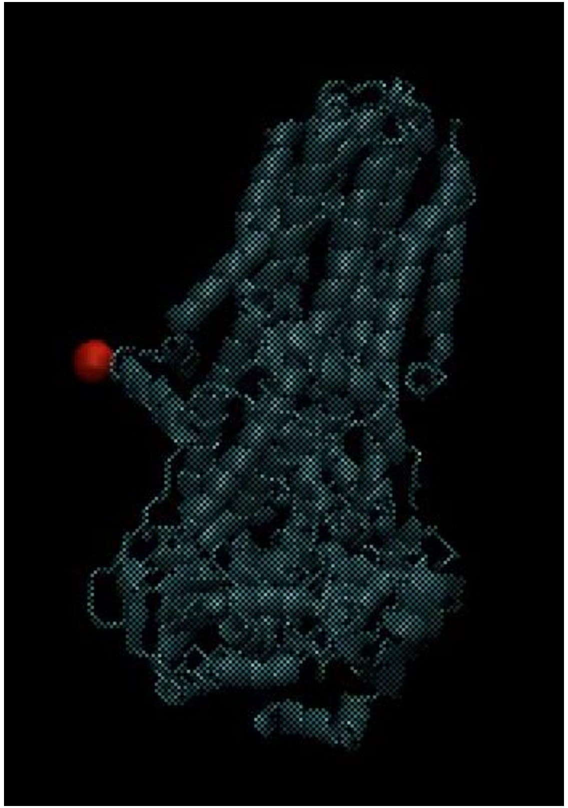
6O1V/GLPG1837



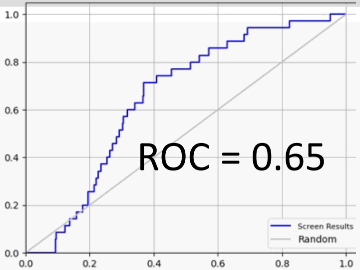
HOLE

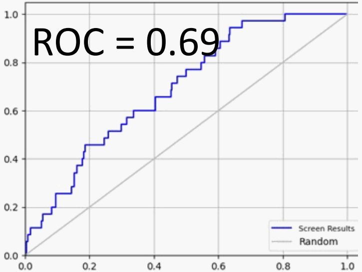


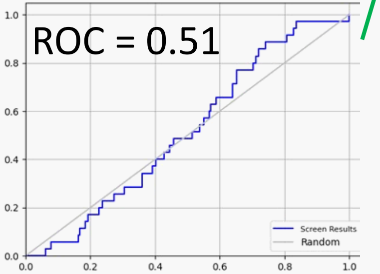
CE

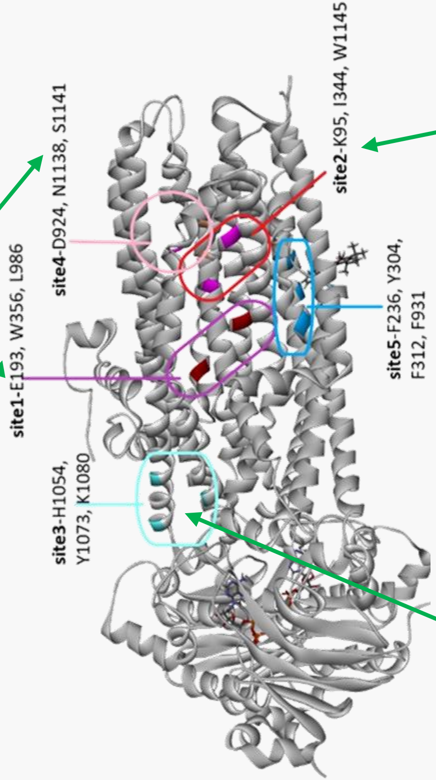


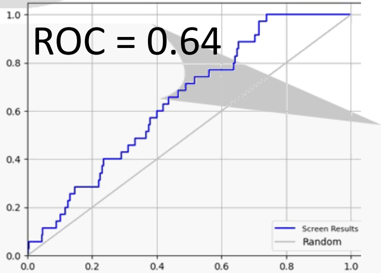
Where on CFTR does Ivacaftor Bind?

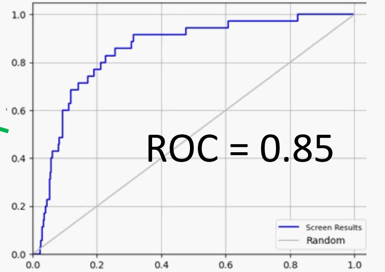


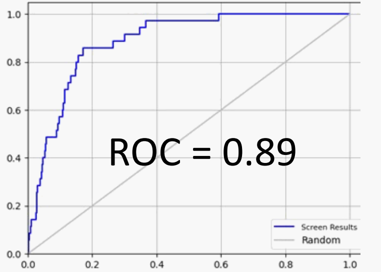






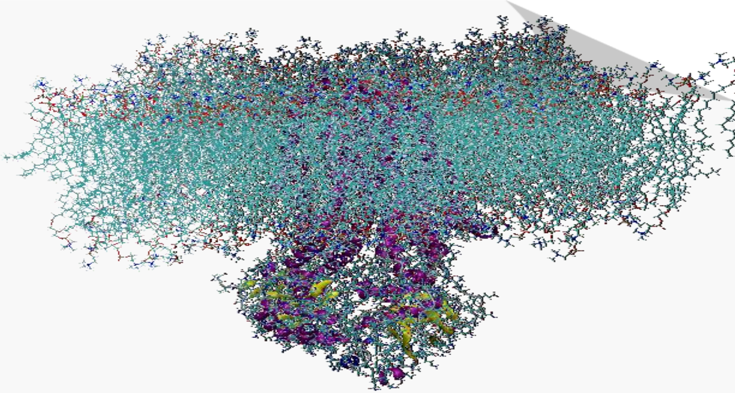






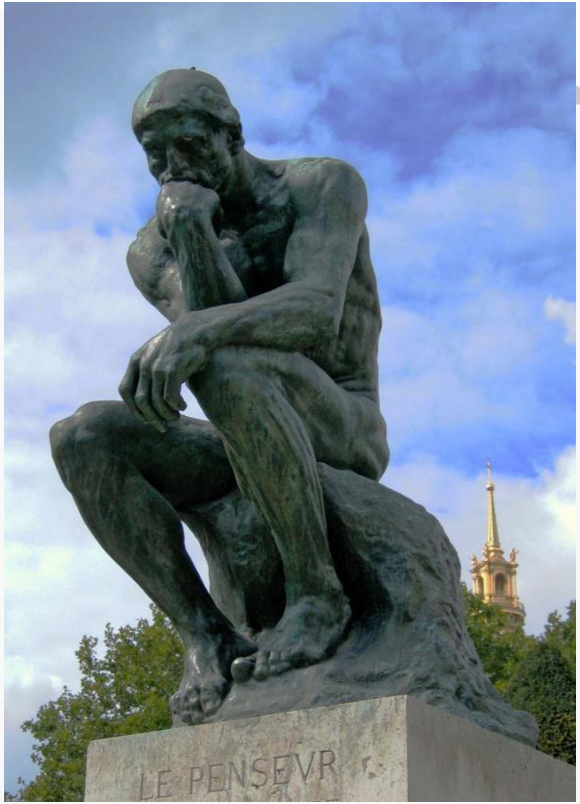
Conclusions

- CFTR structures are useful
    - Interpretation of data
    - Hypothesis generators
    - Drug design
- More Structures are needed
- Simulations provide insight into the dynamics of WT and mutant CFTR
- For specific mutations, simulations suggest atomic level insights into potential mechanisms of action



But wait… A Moment of Philosophy…

- Always look at your data
  - Don't just rely on numbers
- Its bad practice to deduce anything from a single simulation
  - Results very and also depend on the simulations setup
- Hold yourself to the same standards you require from experimentalists
  - Multiple repeat, positive and negative controls



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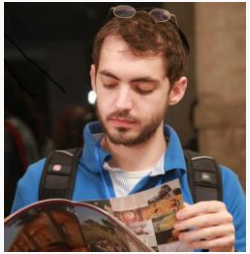

Lior Lublin

Malkeet Singh

All Members of the CFTR Consortium

Many Many members of the CF Community

CYSTIC FIBROSIS FOUNDATION
THERAPEUTICS

BSF
United States – Israel
Binational Science Foundation