

Structure-based drug discovery case study: CFTR modulators



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Institute of Biomedical Technologies
National Research Council



The Bioinformatics Lab consists of approximately 15 researchers and technologists having a diverse background encompassing bioinformatics, biotechnology, engineering, physics and computer science.

The Lab develops, implements and applies advanced analysis techniques in order to tackle open problems from the Life Sciences realm, with particular attention to key sectors such as genomics, epigenomics, bulk and single-cell transcriptomics, multi-omics data integration, molecular biodiversity, systems biology, molecular modelling and artificial intelligence.

Additionally, biological data banks and bioinformatics tools are designed and developed within the Lab for Big Data analyses in the sectors of biomedicine, nutrition and environmental problems. A Translational Bioinformatics unit also part of this Lab provides preparation of biological samples and performs related *omics analyses so to further support and validate the bioinformatics predictions.

The Lab has built and manages an advanced HPC (High Performance Computing) infrastructure so to support the Lab projects' computational needs from the inside. The infrastructure provides predictable execution times for the computations without needing to rely on external resources.

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Research Topics

 **AUTISM**

 **GENE THERAPY**

 **STRUCTURAL BIOINFORMATICS AND DRUG DISCOVERY**

 **MULTI-OMICS AND INTERACTOME-BASED ANALYSES FOR PRECISION MEDICINE**

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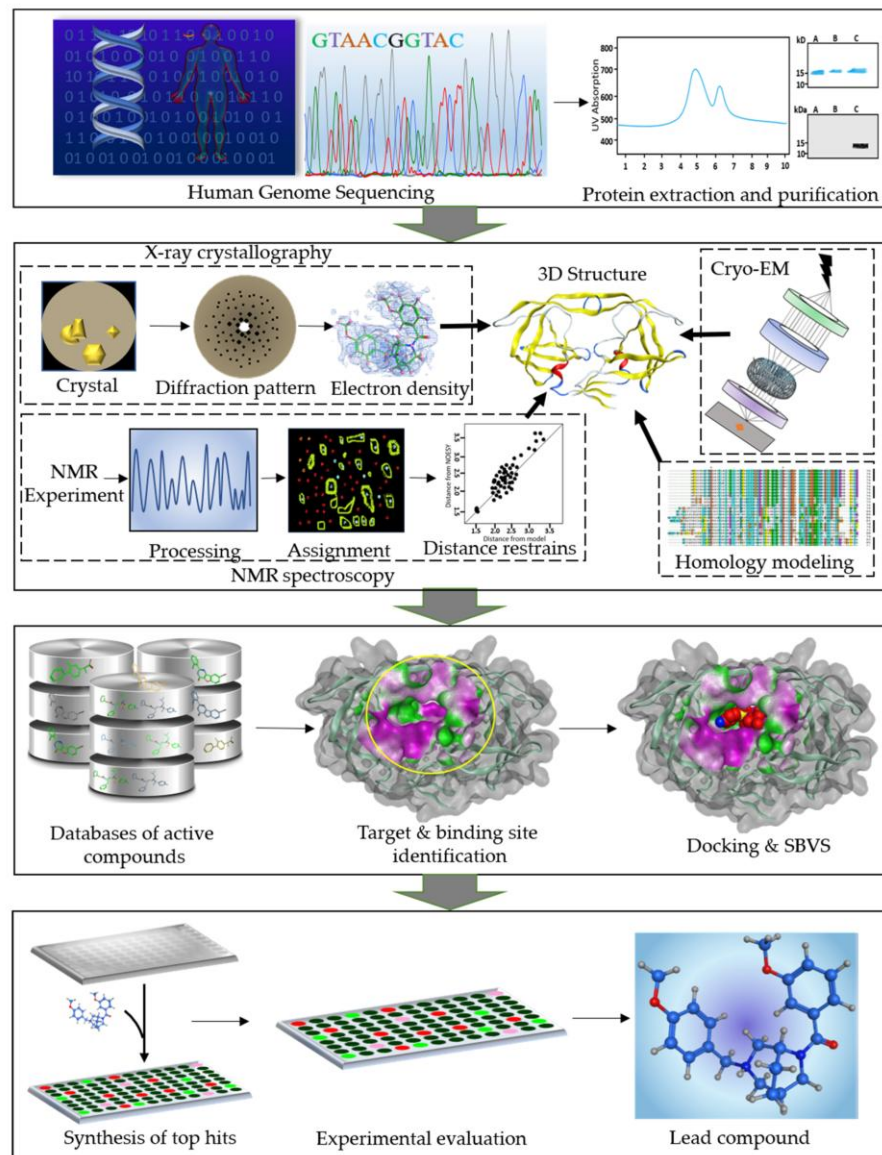


John Hatton



Luciano

A workflow diagram of the structure-based drug discovery process



Human genome sequencing followed by extraction and purification of the target proteins

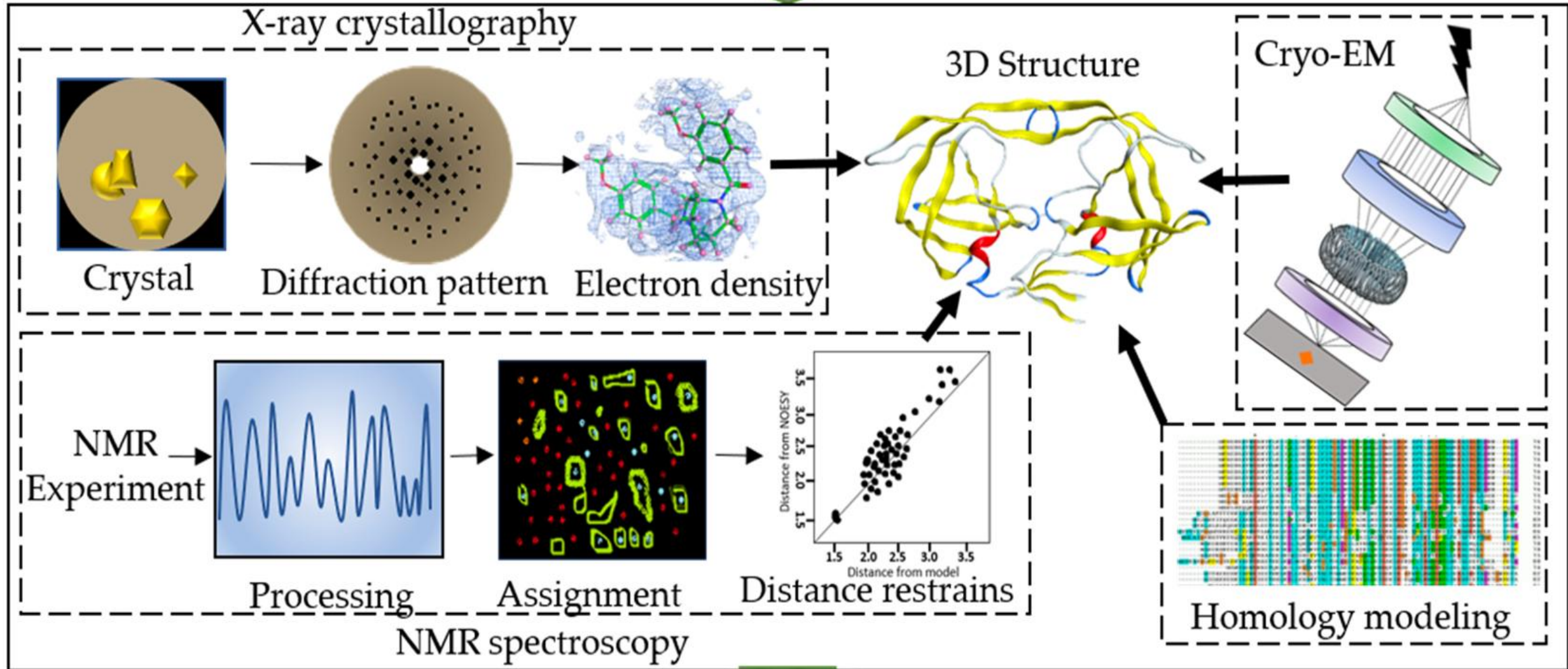
Structure determination of therapeutically important proteins using integrative structural biology approaches.

Database preparation of the active compounds.
Identification of the druggable target protein and its binding site.
Active compound databases are screened and docked into the target protein's binding cavity.

The top hit compounds obtained as a result of virtual screening and docking are synthesized and tested in vitro. Further modifications can be done for the optimization of the lead compound.

Structure-based drug discovery

3D Structure of protein target



New: More Computed Structure Models (CSM) available [Learn more](#)

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RCSB Protein Data Bank (RCSB PDB) enables breakthroughs in science and education by providing access and tools for exploration, visualization, and analysis of:

- Experimentally-determined 3D structures from the **Protein Data Bank (PDB)** archive
- Computed Structure Models (CSM)** from AlphaFold DB and ModelArchive

These data can be explored in context of external annotations providing a structural view of biology.

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8OFW

Crystal structure of the full-length dihydroorotate dehydrogenase from Mycobacterium tuberculosis

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In October 2023, wwPDB will roll out updated CCD data files with

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All members of the American

Publications

Structure prediction methods

Homology modelling

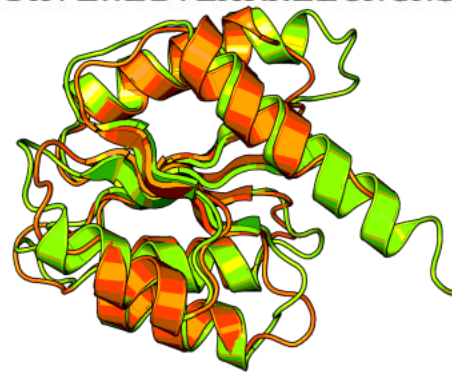
The principle behind it is if two proteins share high sequence similarity.

LUCA
LUCIA
Score= 3

LUC-A
LUCIA
Score equals 4, but the gap is penalized by -0.5,
resulting in a final score of 3.5.

sequence protein A	MNSFSTSAFGPVAFSLGLLLVLPAAFP-APVPPGEDSKDVAAPHRQPLTS
sequence protein B	MKFLSARDFHPVAF-LGLMLVTTTAFPTSQVRRGDFTED-TTPNR-PVYT
sequence protein A	SERIDKQIRYILDGISALRKETCNKSNMCESSKEALAENNLNLPKMAEKD
sequence protein B	TSQVGGLITHVLWEIVEMRKELCNGNSDCMNDDALAENNLKLPEIQRND

They have similar 3D structure



Protein A and protein B are coloured in green and orange respectively and their 3D structures are superimposed

Structure prediction methods

Homology modelling

How is a 3D model created?

Template



Unknown structure



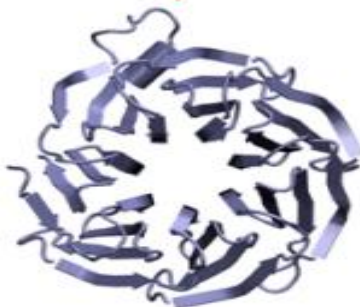
If one of the protein sequences has a known structure, then the structure can be copied to the unknown protein and the structural model is obtained

ELAGIILTVSYIPSAEKIA

ELAIGILTVSYIPSAEKIR

Sequence alignment

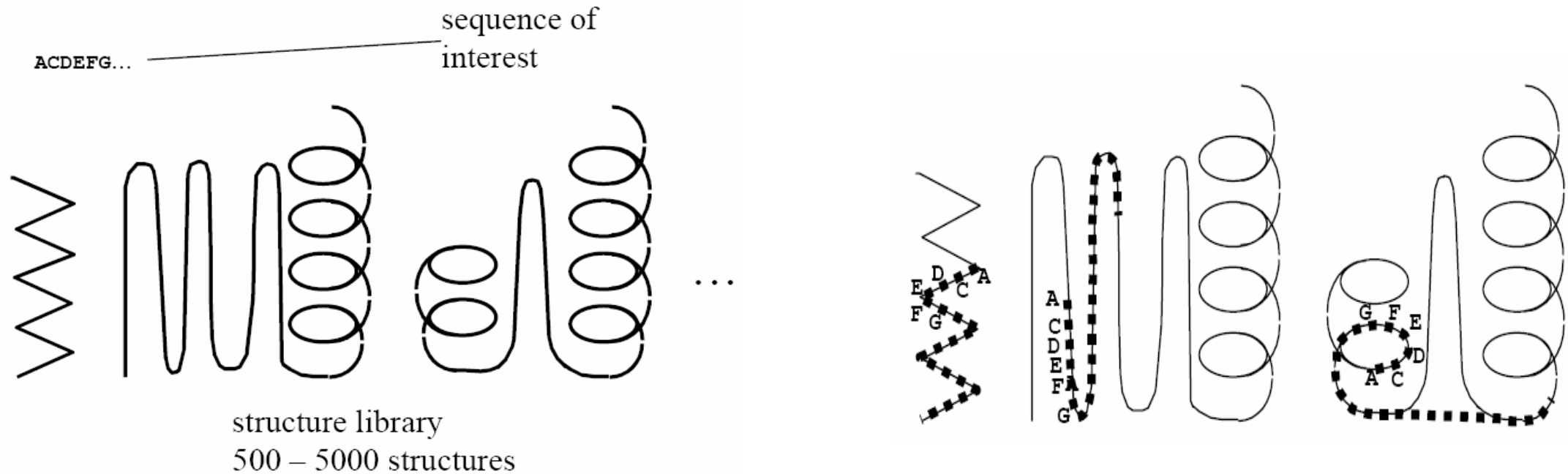
ELAGI-ILGVSYPSEKI-ARACELTI
ELA-IGILTVSYIPSAEKIRAP--ELTI



Structural model

Structure prediction methods

Threading



Threading takes a protein sequence and a library of templates or known structures

The sequence **ACDEFG** is dragged through each location on each template, and then the best matching structural fold is found using energy-based criteria.

Structure prediction methods

Alphafold

The neural network is trained using data related to known proteins (amino acid sequences and 3D structures), in order to generate structural fragments based on the distance between pairs of residues and the angular distribution. In a second step, the results are further refined using the Gradient Descent method, a mathematical technique widely used in machine learning to make progressive and incremental improvements to the solutions obtained.

Deep Learning

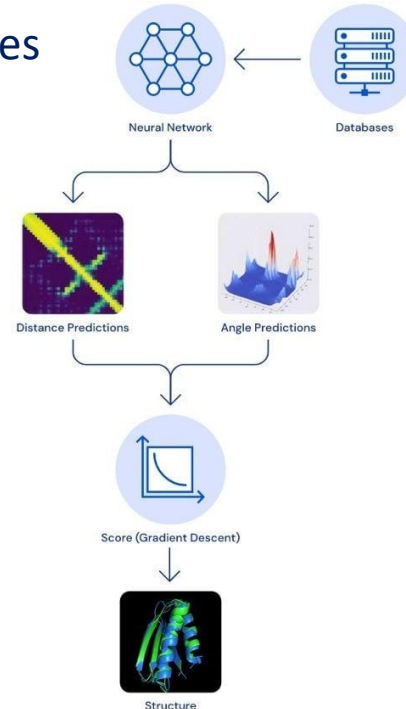
Neural network trained on known protein structures
Detection of co-evolutionary patterns between residues

Geometric Predictions

Distances between residue pairs
Angles and relative orientations in 3D space

Structure Construction

Integration of geometric constraints
Optimisation to generate the most probable 3D structure



Input

Protein amino acid sequence
Evolutionary information from databases
(multiple sequence alignments, MSA)

Output

Atomic-resolution 3D protein model
Per-residue confidence score (pLDDT)

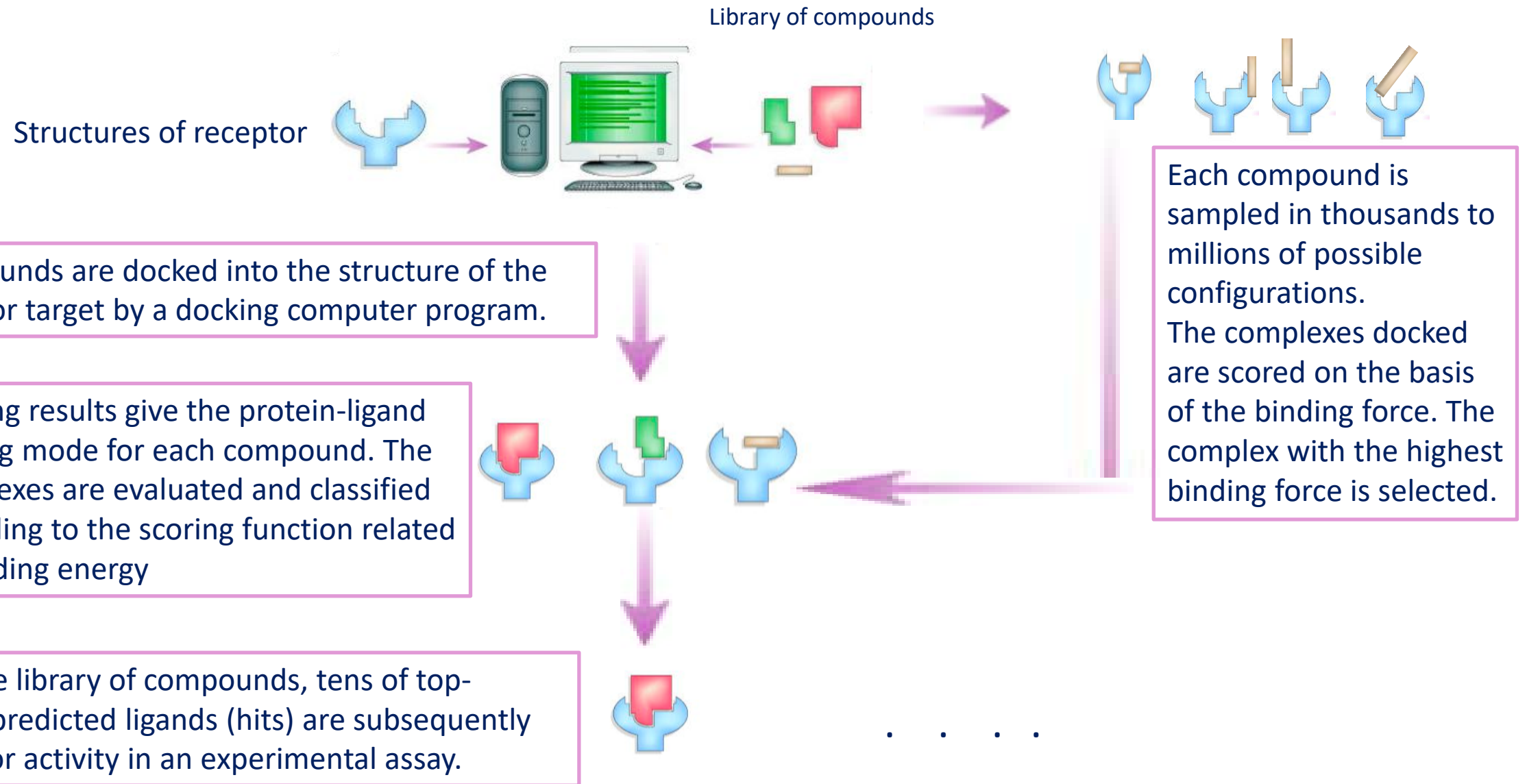
Binding pocket identification

Table 15.1. Available software for pocket identification, binding site comparison and chemical compound database search.

Category	Software	Feature	Web address
Pocket identification	SURFNET	Identifies a pocket with a large and a small probe sphere rolling on the protein surface	www.ebi.ac.uk/thornton-srv/software/SURFNET [104]
	LIGSITEcsc	Identifies a ligand-binding pocket as a spatially semiclosed region by protein surface and shows sequence conservation	http://projects.biotec.tu-dresden.de/pocket [105]
	CAST	Identifies a pocket using the Delaunay triangulation and the α complex	http://sts.bioengr.uic.edu/castp [106]
	PASS	Identifies a pocket as a surface region where small probe spheres fit into	www.ccl.net/ccs/software/UNIX/pass/overview.html [107]
	VisGrid	Identifies a pocket as a surface position with a low visibility	http://kiharalab.org/VisGrid [101]
Binding site comparison	eF-Site	Compares shape and the surface electrostatic potential using a graph matching method	http://ef-site.hgc.jp [108]
	SitesBase	Compares atoms in pockets using geometric hashing	www.modelling.leeds.ac.uk/sb [109]
	Pocket-Surfer	3DZD-based method described in this chapter	http://kiharalab.org/pocket-surfer [103]
Chemical compound database search	SIMCOMP	Subgraph matching method	www.genome.jp/tools/simcomp [110]
	UNITY2D	Fingerprint-based method, a molecule is represented as a Boolean array of features of the molecule	A part of SYBYL package www.tripos.com [111]
	PubChem	Users can draw query molecules or specify formula	http://pubchem.ncbi.nlm.nih.gov [112]

Lead identification approach

Virtual screening



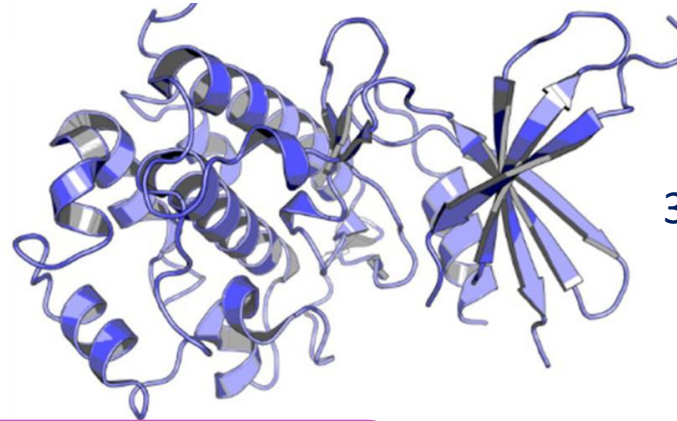
Molecular docking

Outline of molecular docking process

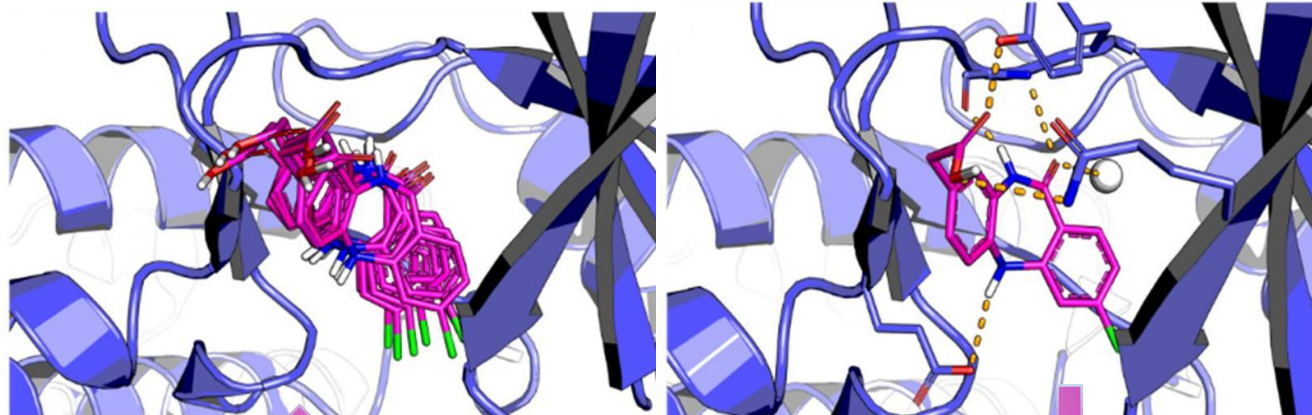
3D structure of ligand



3D structure of receptor



the ligand is docked into the Binding cavity of the receptor and the putative conformations are explored

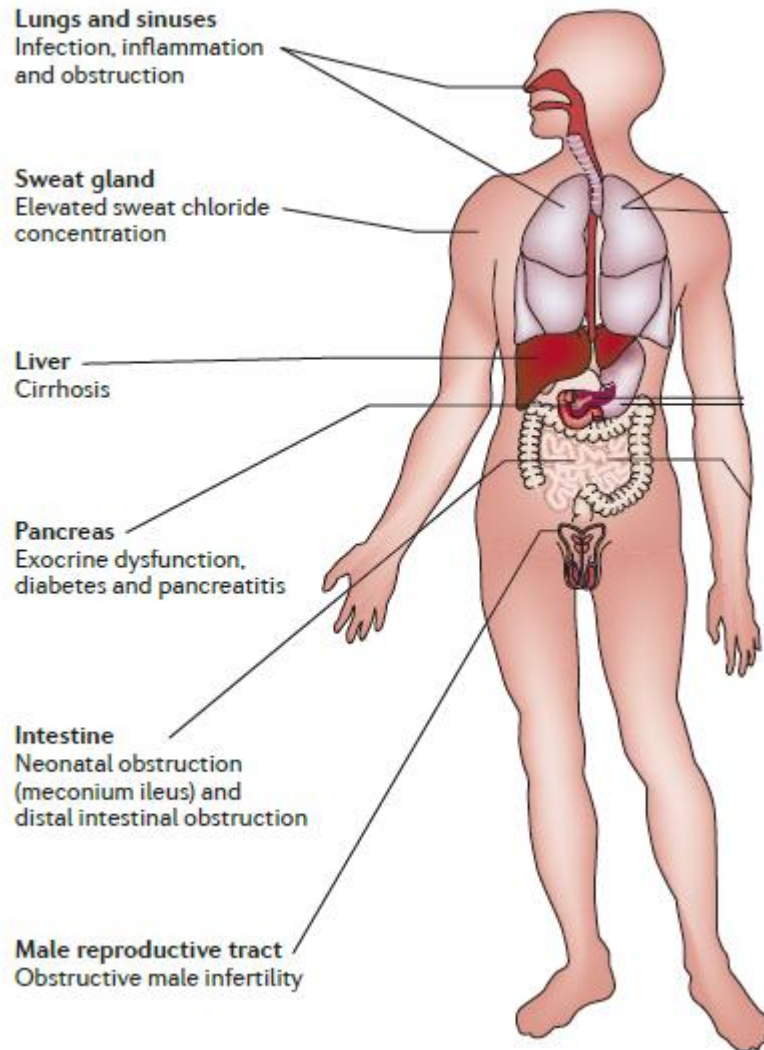


accurate prediction of the interaction energy associated with each of the predicted binding conformations

Docking programs perform these tasks through a cyclical process, in which the ligand conformation is evaluated by scoring function until it converges to the solution of minimum energy

Case study of Cystic fibrosis transmembrane conductance regulator (CFTR) modulators

Cystic fibrosis damages



Cystic fibrosis is a genetic disease related to the alteration of the CFTR (cystic fibrosis transmembrane conductance regulator) protein.

The dysfunction of the CFTR causes abnormal viscous secretions in some organ systems containing epithelia.

CFTR Protein

CFTR is a transmembrane protein that belongs to the ATP-binding cassette (ABC) superfamily.

These proteins carry molecules such as sugars, peptides, inorganic phosphates, chloride ions, and metal cations across the cellular membrane, and their activity is ATP-dependent.

CFTR carries chloride ions across the cellular membrane of the liver, lungs, pancreas, digestive and reproductive tract cells.

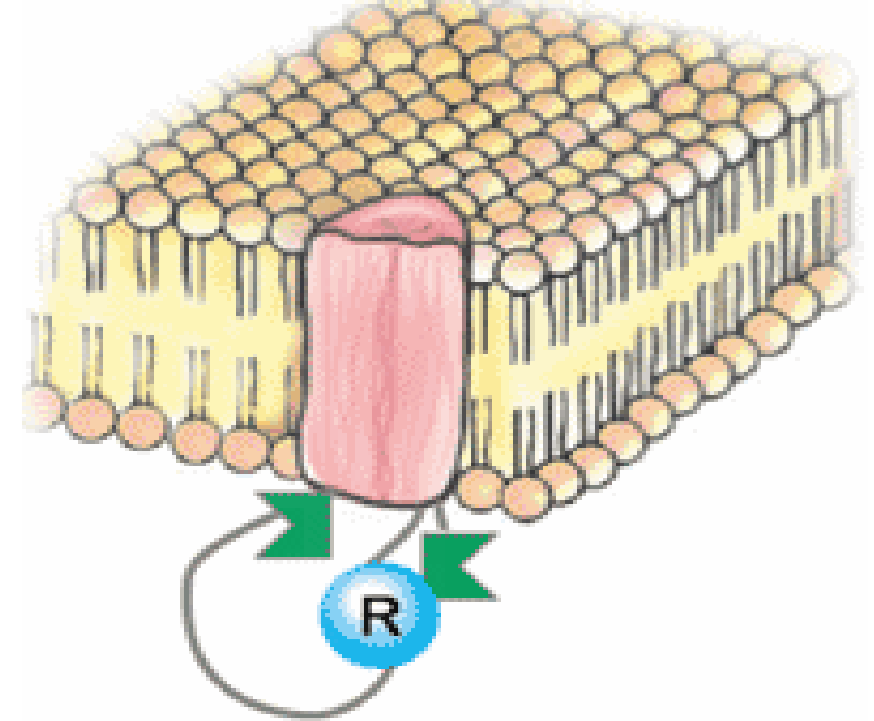
The phosphorylation of serine residues regulates the protein's physiological activity. (stage2)

It allows ATP to bind to nucleotide-binding factor domains. (stage3)

After binding, ATP is hydrolysed. (stage4)

The protein changes conformation, and the channel opens. (stage5)

Il canale è chiuso



stage 1

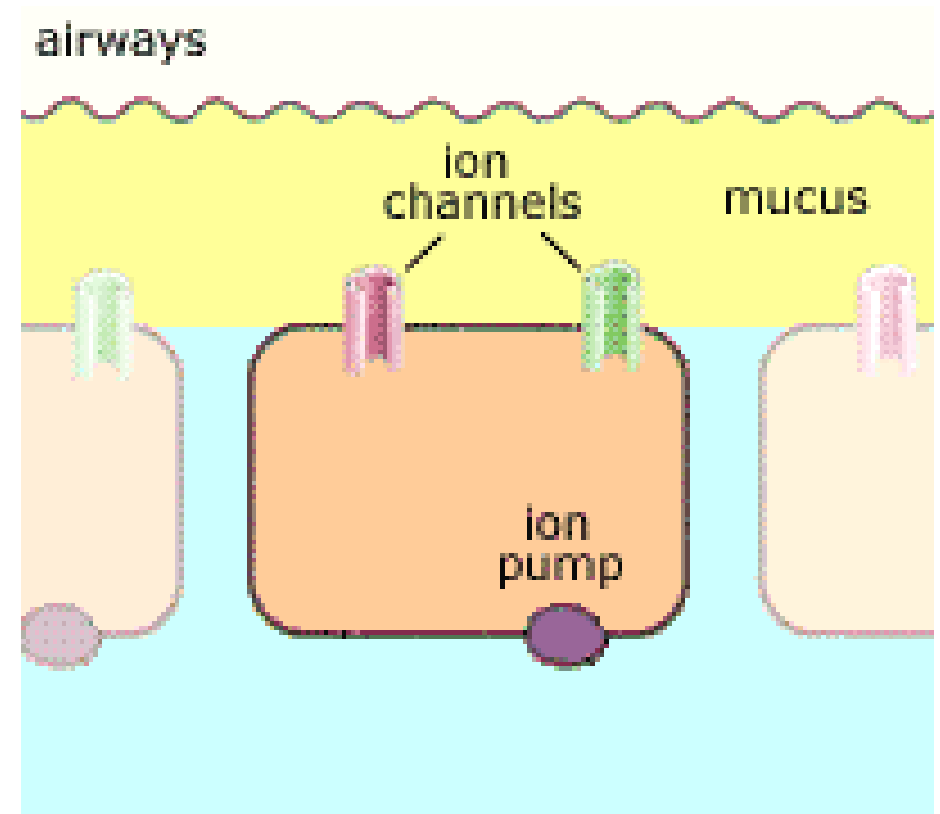
Chloride channel function

Ion channels are present in lung epithelial cells.

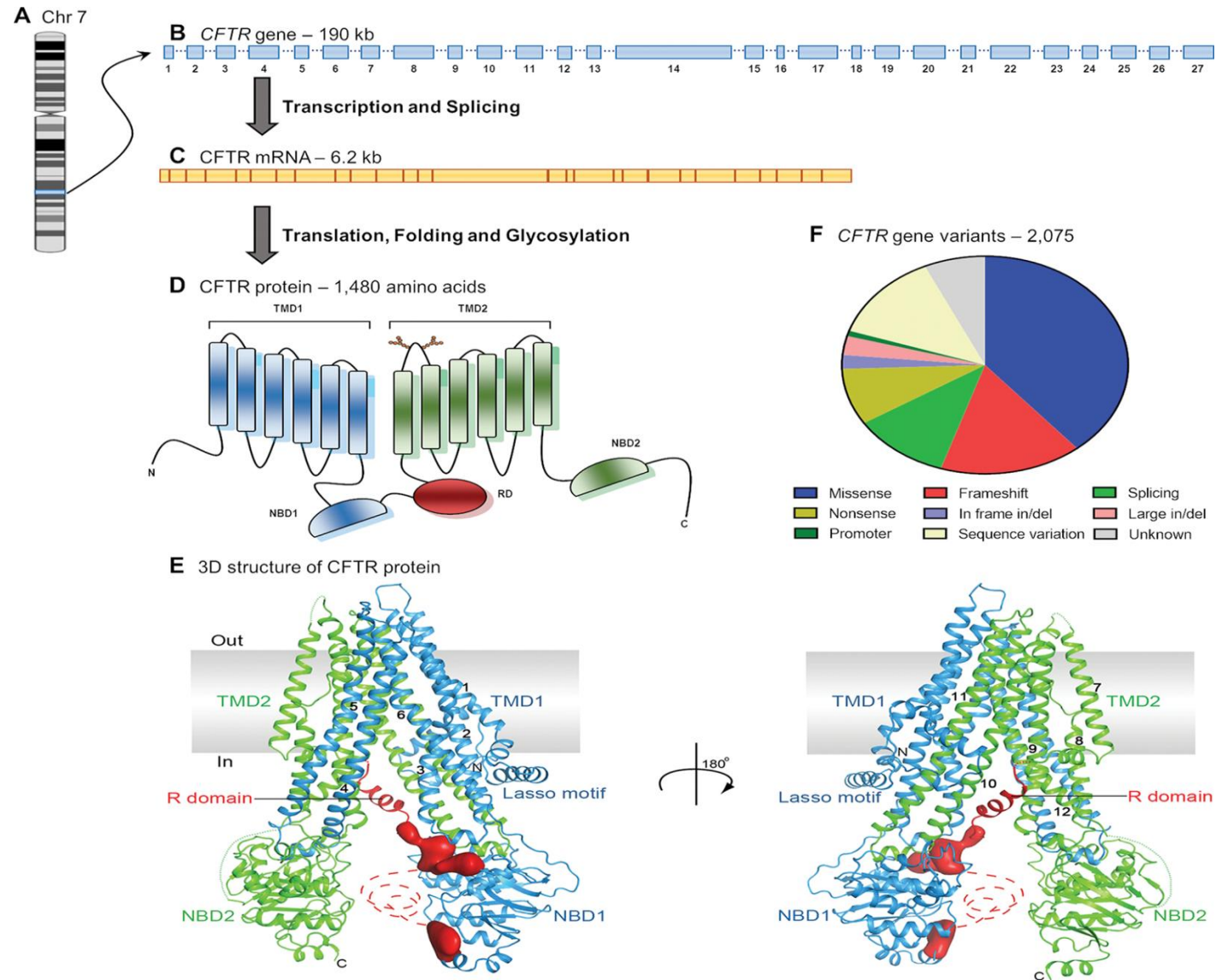
The movement of ions helps water reach the surface and keeps the mucous membrane moist.

A lack of ion flow causes a defect in mucous hydration, which reduces the ciliary movement of mucus.

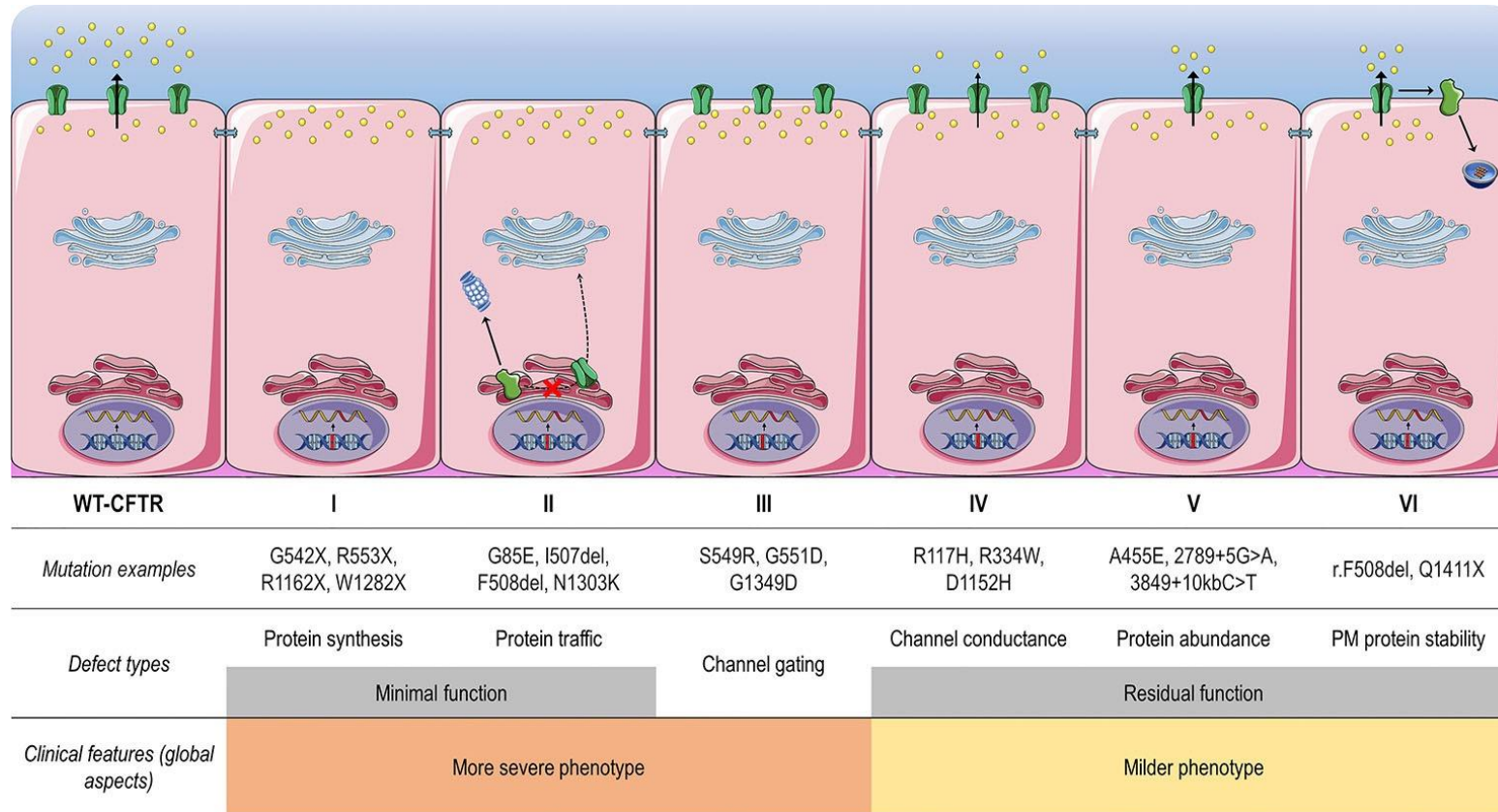
When ciliary movement fails, it results in bacterial infections.



From gene to CFTR protein structure



Classes of CF transmembrane conductance regulator (CFTR) mutations



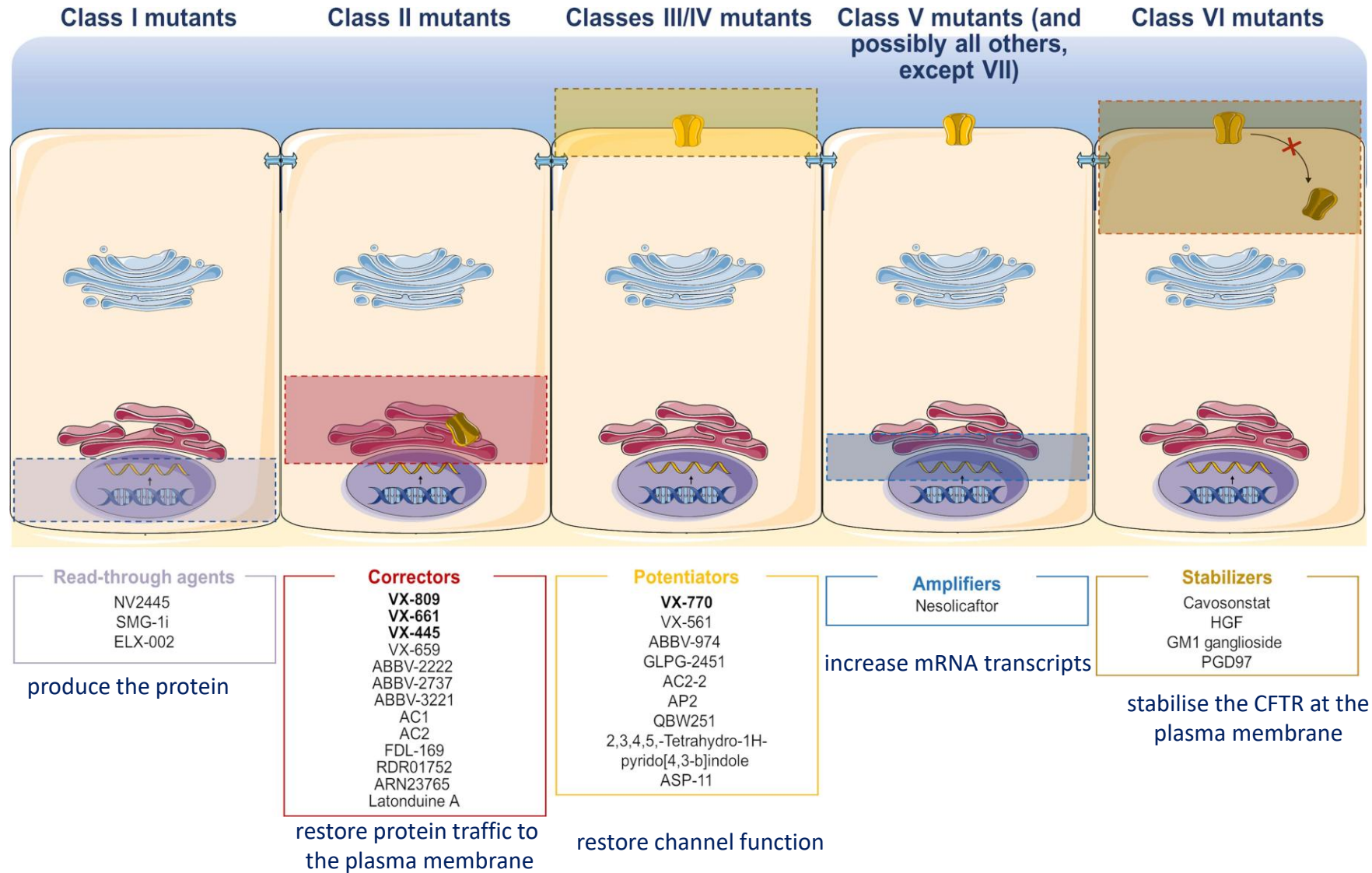
I no production of full-length protein
 II defective folding and trafficking
 III defective gating

IV reduced anion conductance
 V reduced protein production
 VI reduced stability at the PM

High-throughput screening for modulators of CFTR activity

- Numerous assays and high-throughput screens (HTS) have been developed and optimized to screen drug-like compound libraries and identify CFTR modulators.
- These specialized small molecules target the root cause of CF to enhance or even restore the expression, function, and stability of a defective CFTR in distinct manners, and they have been classified into five main groups depending on their effects on CFTR mutations.

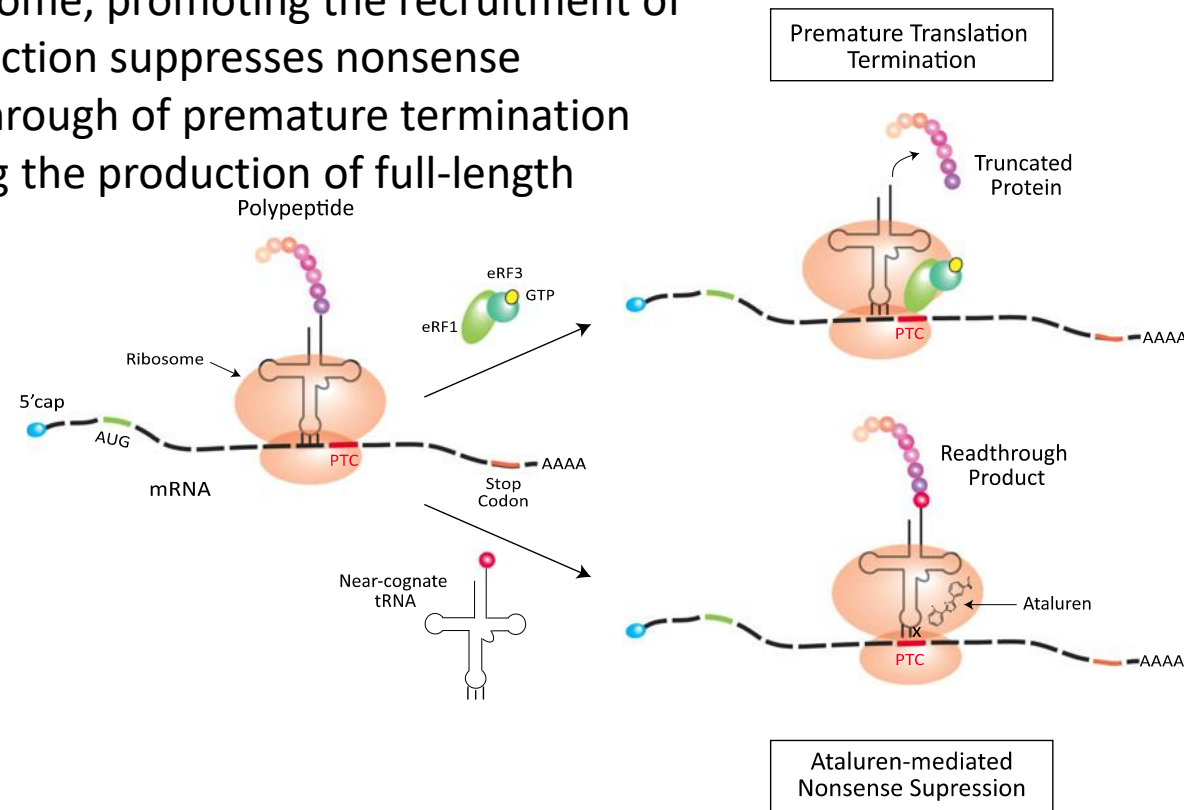
Site of action of the different CFTR modulator drugs



CFTR modulator drugs may be grouped according to their actions on CFTR mutations

Read-through agents

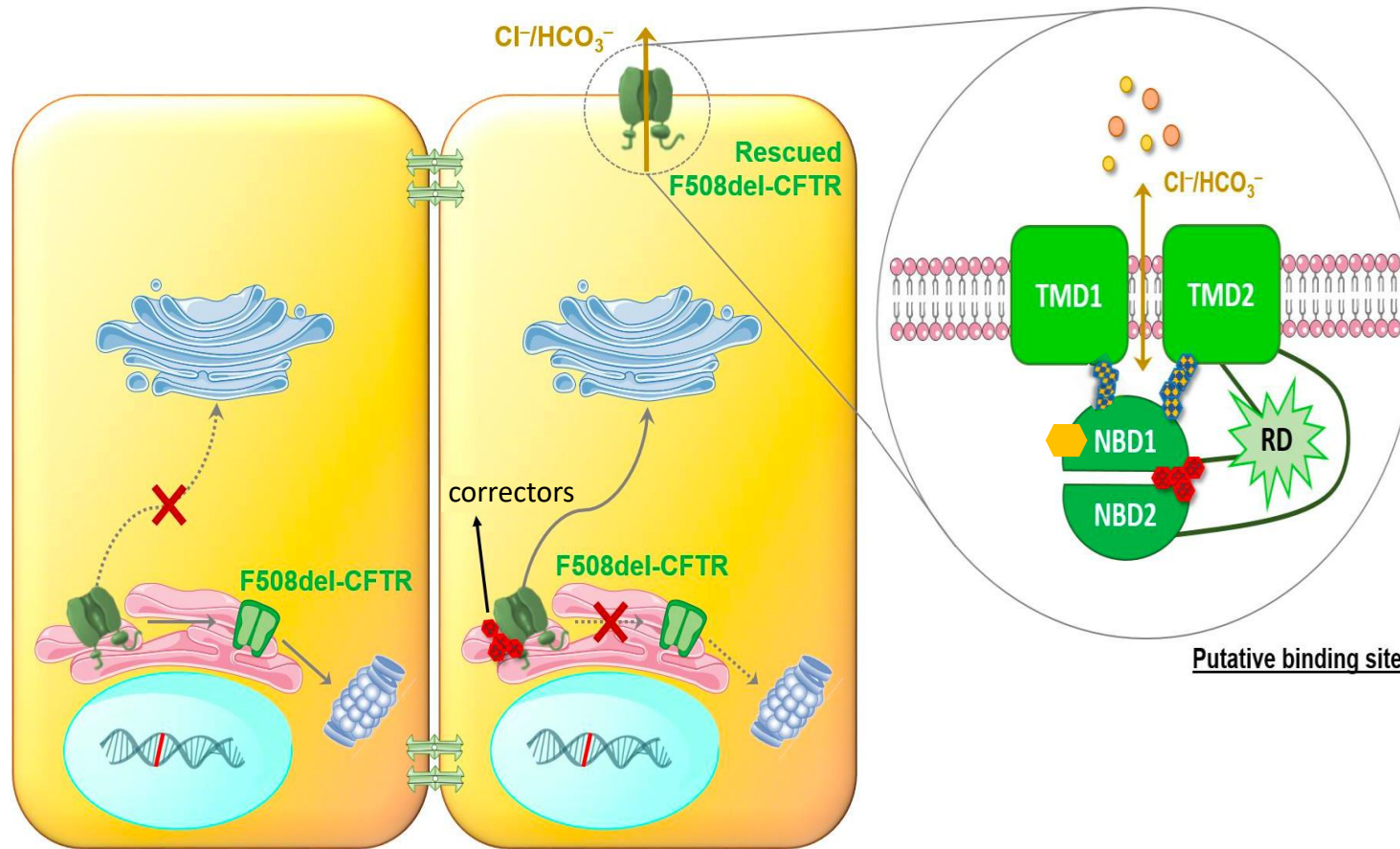
Ataluren binds to the ribosome, promoting the recruitment of near-cognate tRNAs. This action suppresses nonsense mutations, enabling readthrough of premature termination codons (PTCS) and allowing the production of full-length proteins.



Class I mutations occur when a ribosome hits a premature stop codon (PTC) due to a nonsense mutation, eukaryotic release factors in complex with GTP are recruited, and translation is terminated prematurely, generating a truncated protein.

Ataluren interacts with the ribosome and facilitates the recruitment of near-cognate tRNAs. This action suppresses the non-sense mutation and allows for the readthrough of premature termination codons (PTC) and allowing the production of a full-length protein

Correctors



Correctors are classified based on their molecular targets in the CFTR structure



class 1 correctors stabilize NBD1-TMD1 and/or NBD1-TMD2



class 2 correctors stabilize NBD2 and its interfaces with other CFTR domains

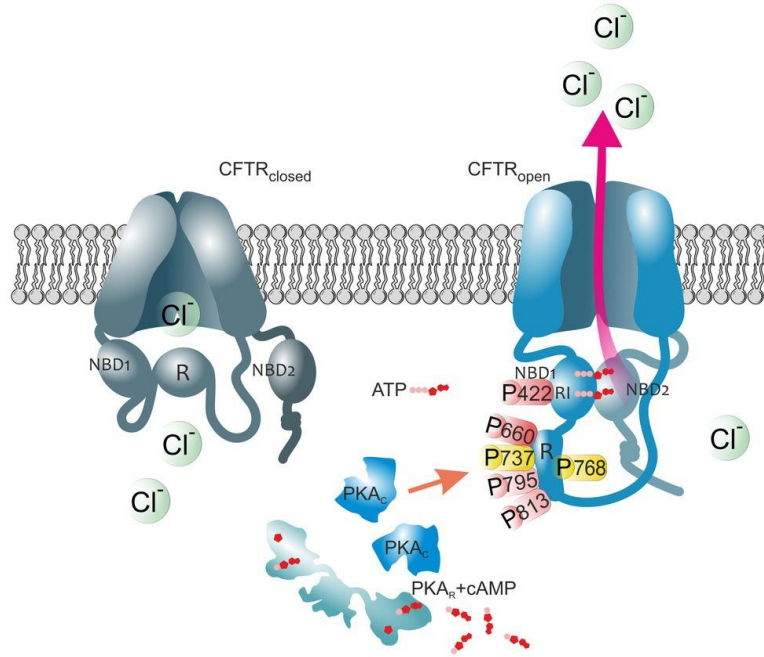


class 3 correctors directly stabilize NBD1

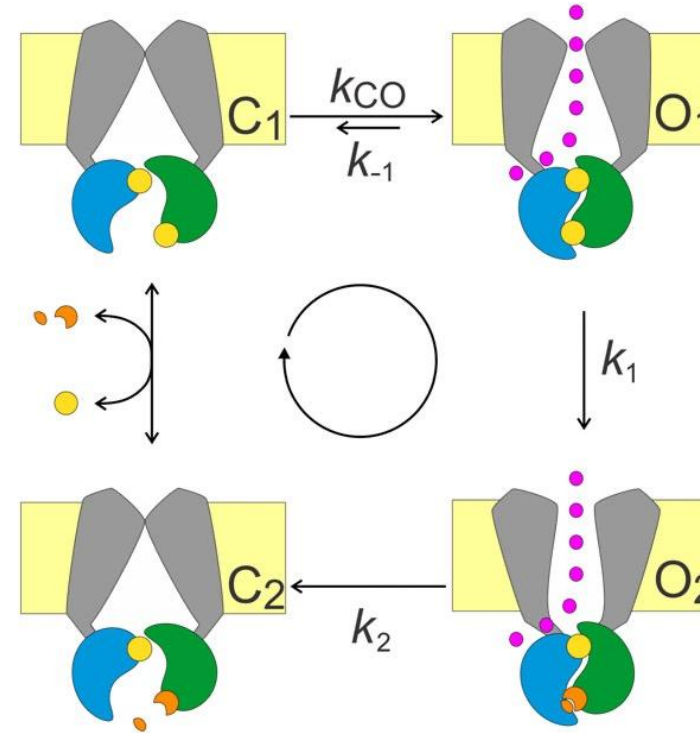
This classification may be useful to evaluate corrector combinations with complementary mechanisms of action

Correctors are small molecules that rescue CFTR mutants with a traffic defect to the PM. These compounds may act by distinct mechanisms, they usually enhance protein conformational stability during the ER folding process. The structure of wild type (WT) CFTR shows that F508 is located on the surface of NBD1, where it makes extensive interactions with the cytosolic region of TM helix 11 and intracellular loop 4. These interactions are critical for both CFTR folding and coupling of ATP dependent NBD dimerization to pore opening, suggesting that disruption of these interactions may underlie both trafficking and functional defects in $\Delta 508$ CFTR.

Phosphorylation in CFTR channel activity regulation

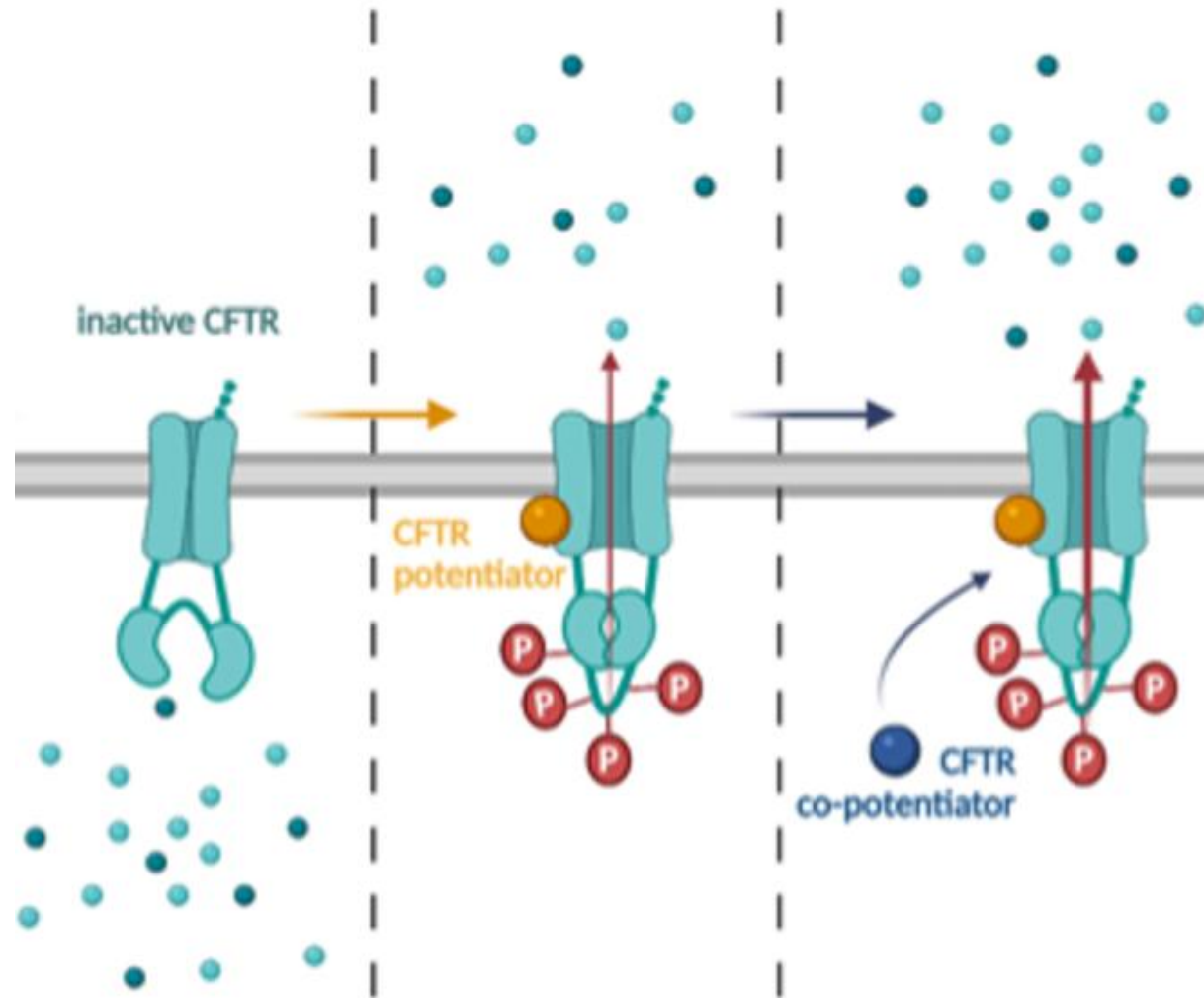


In the closed CFTR state, the NBD1 interacts with the R domain creating steric hindrances that prevent it from dimerization with NBD2. Phosphorylation causes large conformational changes that remove the R region from its inhibitory position and allow NBDs dimerization to occur.



The channel enters a stable open state (O1) following the formation of a head-to-tail NBD dimer, with ATP bound at the two ATP binding sites formed at the interface. Upon hydrolysis of the ATP bound at site 2, the channel closes via a short-lived post-hydrolytic (O2). Mutations disrupt any critical steps in this gating process (opening and closing) and may lead to CFTR dysfunction.

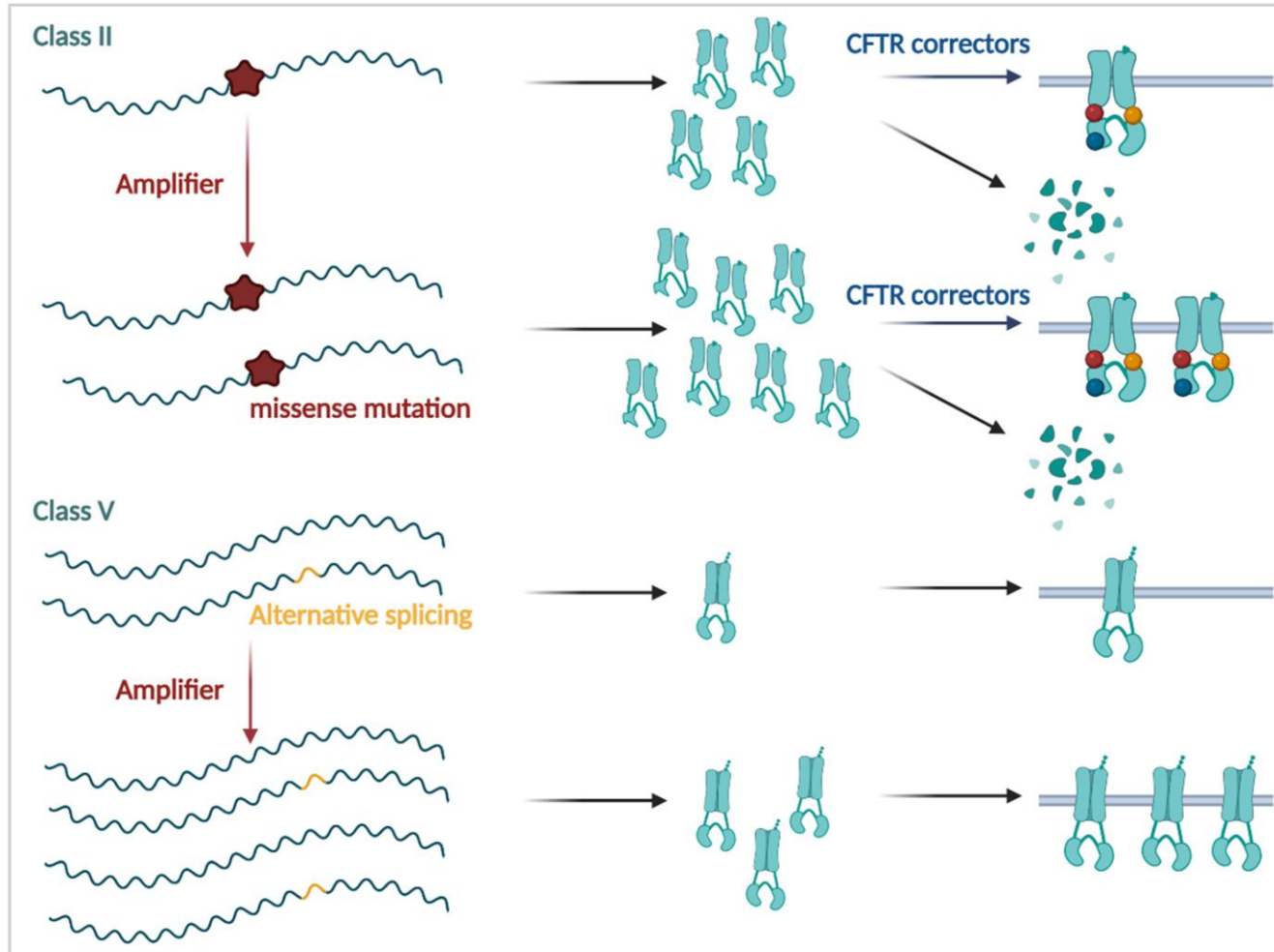
Potentiators



CFTR potentiators interact directly with CFTR to promote function in a phosphorylation-dependent, but ATP-independent manner. Co-potentiators also interact with CFTR to further stimulate function, but only when CFTR potentiators are present.

Amplifier

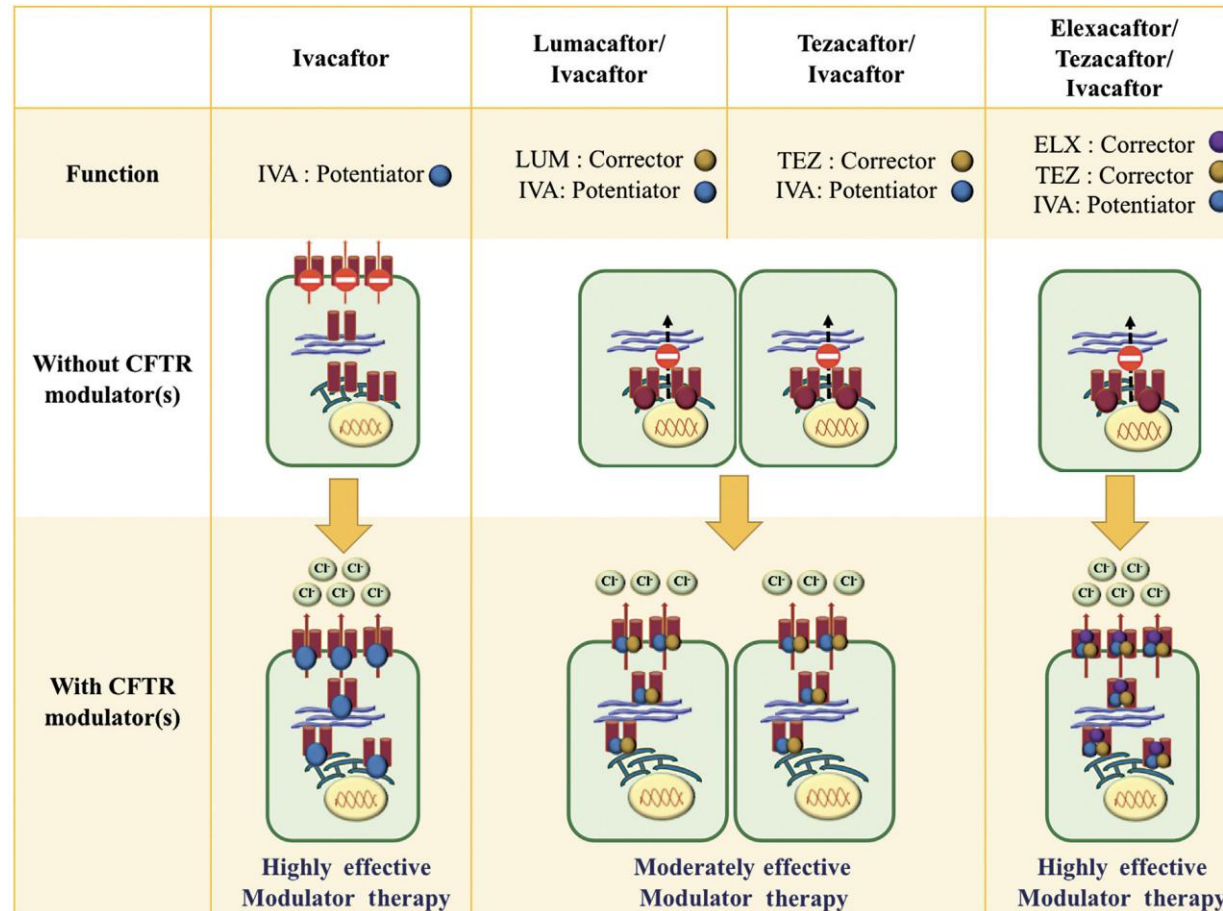
The amplifier stabilizes the mRNA, which results in an increase in available CFTR mRNA transcripts.



Class II mutations are misfolded and only a fraction reaches the plasma membrane, even in the presence of CFTR correctors. Amplifiers provide more immature protein that can be rescued with CFTR modulators.

Class V introduce cryptic splice sites, resulting in a mix of normal and alternatively spliced CFTR mRNA. Enhancing the number of correct mRNA transcripts results in more CFTR protein at the plasma membrane.

Mechanisms of action of CFTR modulators approved for people with CF

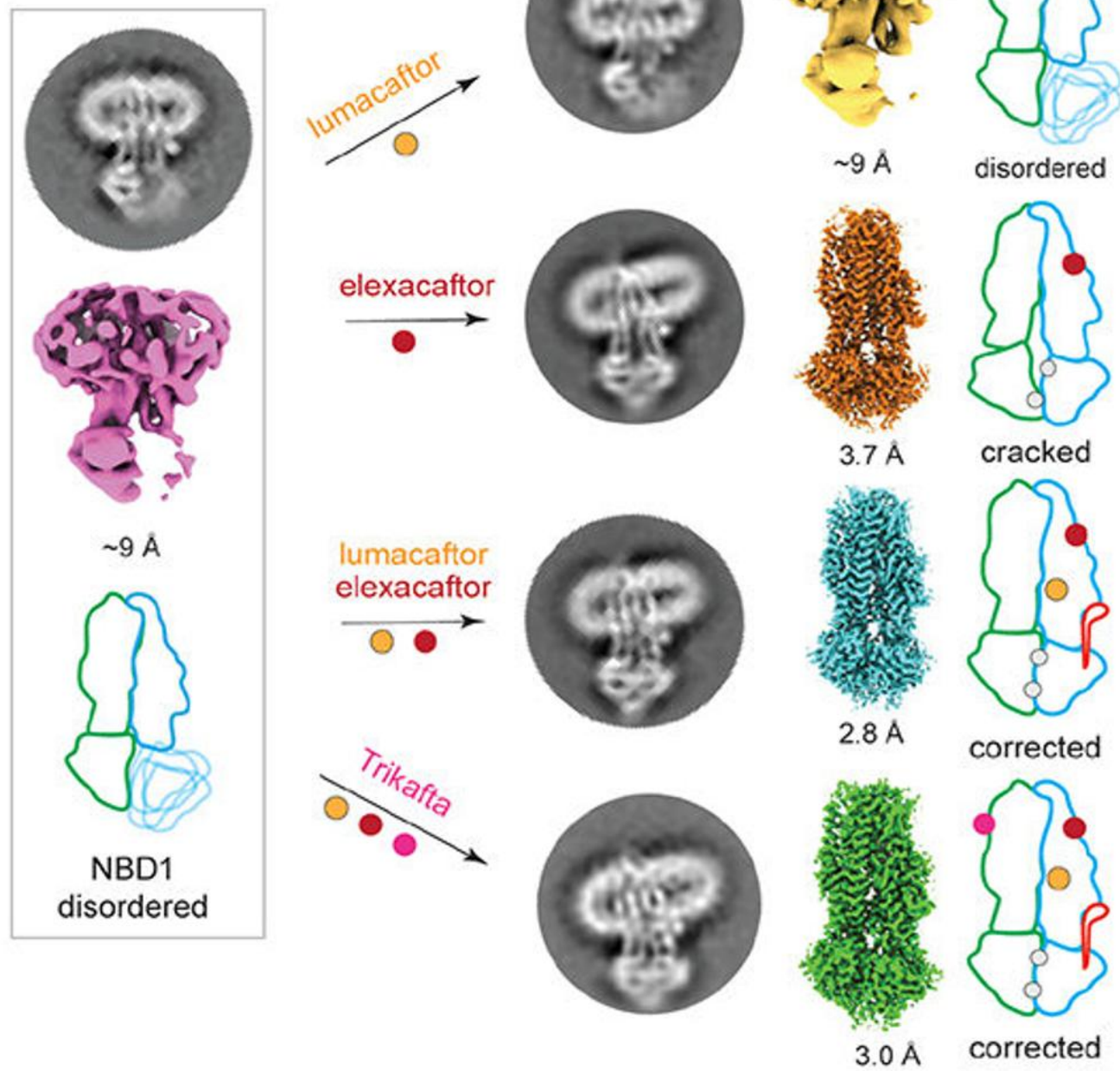


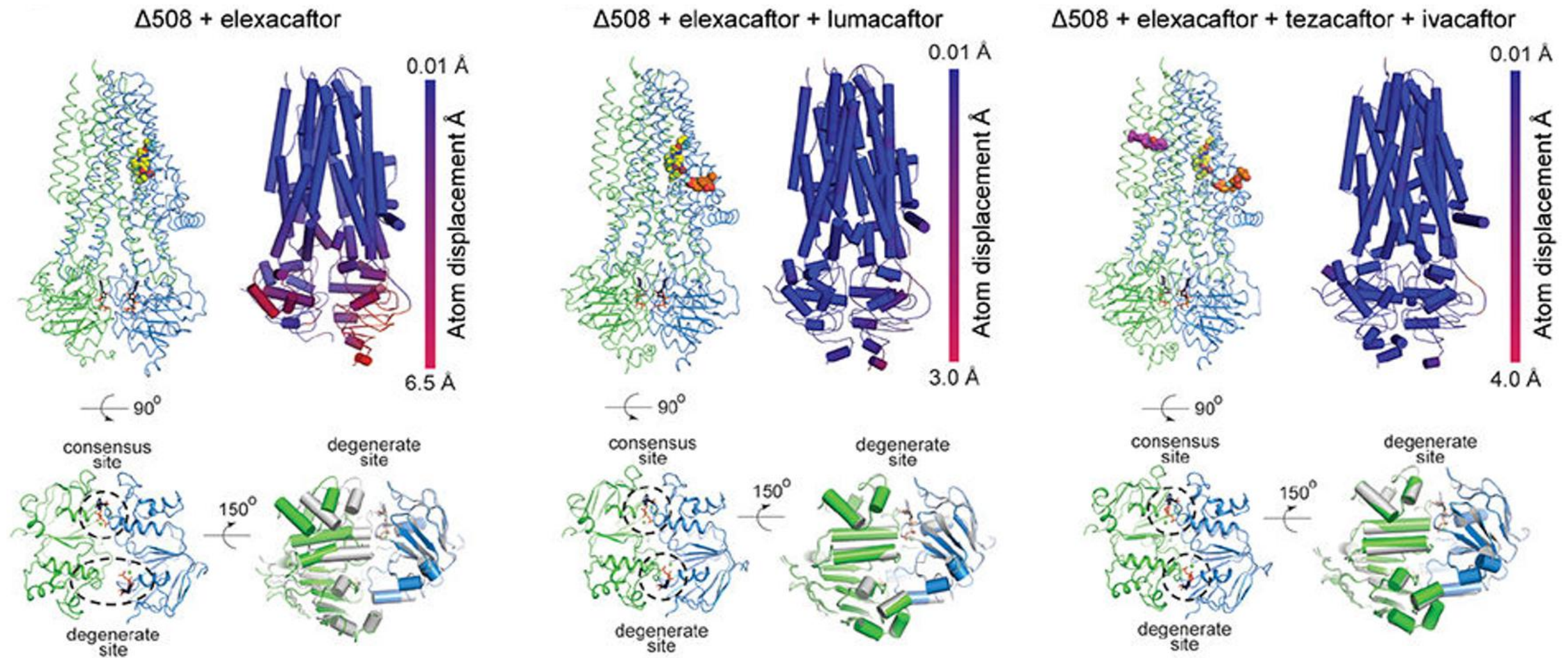
IVA increases CFTR opening frequency and ion conductance.

LUM, TEZ, and ELEX are correctors that have an additive effect to facilitate the intracellular maturation and trafficking of the CFTR protein to increase the amount of CFTR proteins brought to the cell surface.

LUM/IVA and TEZ/IVA are moderately effective in F508del homozygous patients, but TEZ/IVA is highly effective in patients with one F508del mutation.

A triple combination of ELX/TEZ/IVA is considered highly effective: high level of CFTR function restoration (major decrease in sweat chloride concentration) and rapid and major clinical improvement





The structures of CFTR F508del with modulators coloured by Ca displacement values of the $\Delta 508$ compared to the WT.



Projects funded by Fondazione Fibrosi Cistica



FFC#10/2019

Rescuing defective CFTR by applying a drug repositioning strategy based on computational studies, surface plasmon resonance and cell-based assays.

FFC#11/2018

Rescuing defective CFTR by applying a drug repositioning strategy based on computational studies, surface plasmon resonance and cell-based assays.

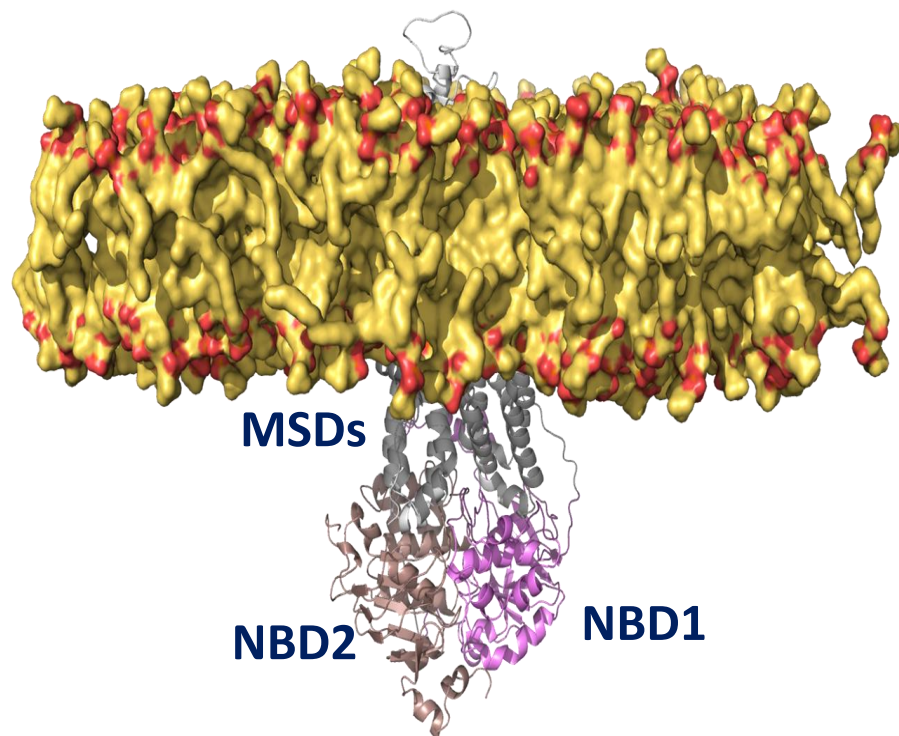
FFC#6/2014

Development of novel methodologies for the identification of cftr-targeted drugs: a multidisciplinary approach using real-time surface plasmon resonance interaction assay supported by bioinformatic strategies on HPC infrastructures

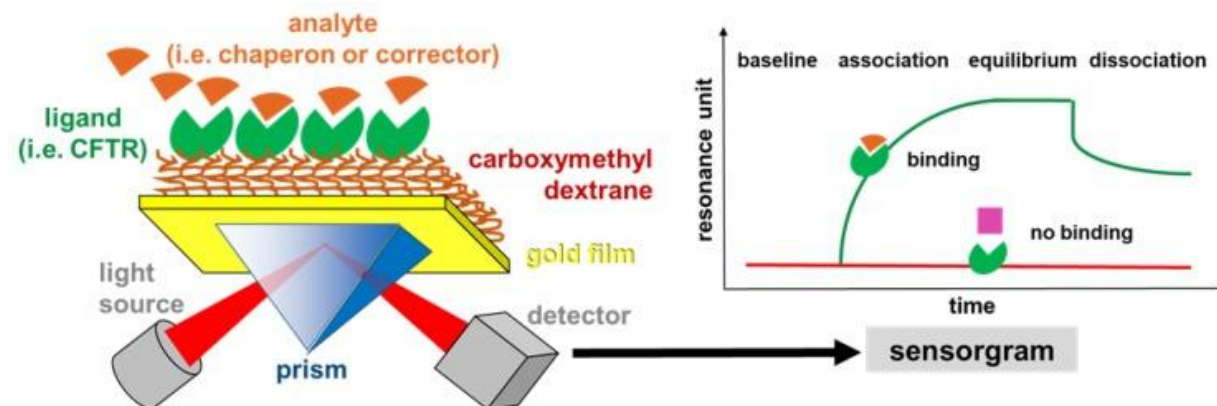
PI: Prof. Marco Rusnati

Centres Involved: University of Brescia, University of Genova and CNR

Novel methodologies for the identification of cftr-targeted drugs were developed by a multidisciplinary approach using real-time surface plasmon resonance interaction assay supported by bioinformatic strategies on HPC infrastructures

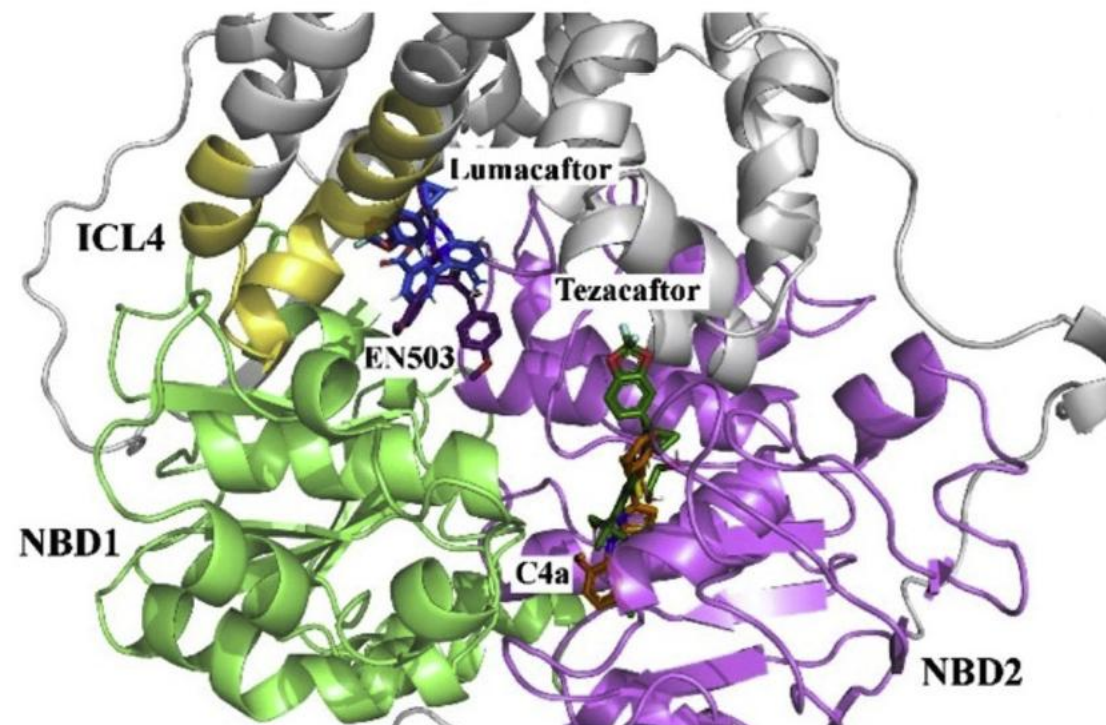
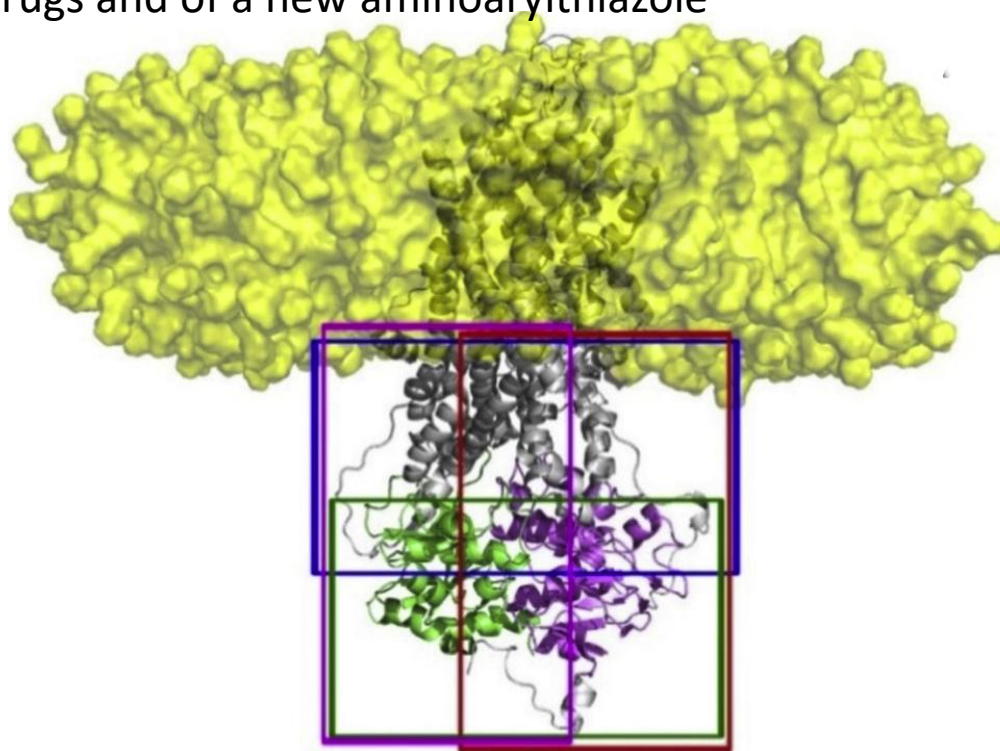


A computational model of F508del CFTR obtained by homology modelling and molecular dynamics.
The F508del CFTR is embedded in the lipid environment



The biosensor includes F508del-CFTR in a lipid environment and has a wider analytical potential than the previous one based on CFTR

The computational model was used to explore the binding modes of some known F508del-CFTR-rescuing drugs and of a new aminoarylthiazole



The multiple boxes method can cover the entire protein to explore the binding site of ligand. Red box: NBD1-MSDs region partially overlapping NBD2-MSDs; purple box: NBD2-MSDs region partially overlapping NBD1-MSDs; green box: NBD1-NBD2 region; blue box: MSDs, NBD1 and NBD2 regions.

Docking Poses of compounds into apo F508del-CFTR. Lumacaftor (blue), Tezacaftor (dark green), C4a (orange) and EN503A (purple) are shown as stick, NBD1 (green), NBD2 (violet), ICL4 (gold) as cartoon

Exploring the stability of ligand binding modes to CFTR by molecular dynamics simulation

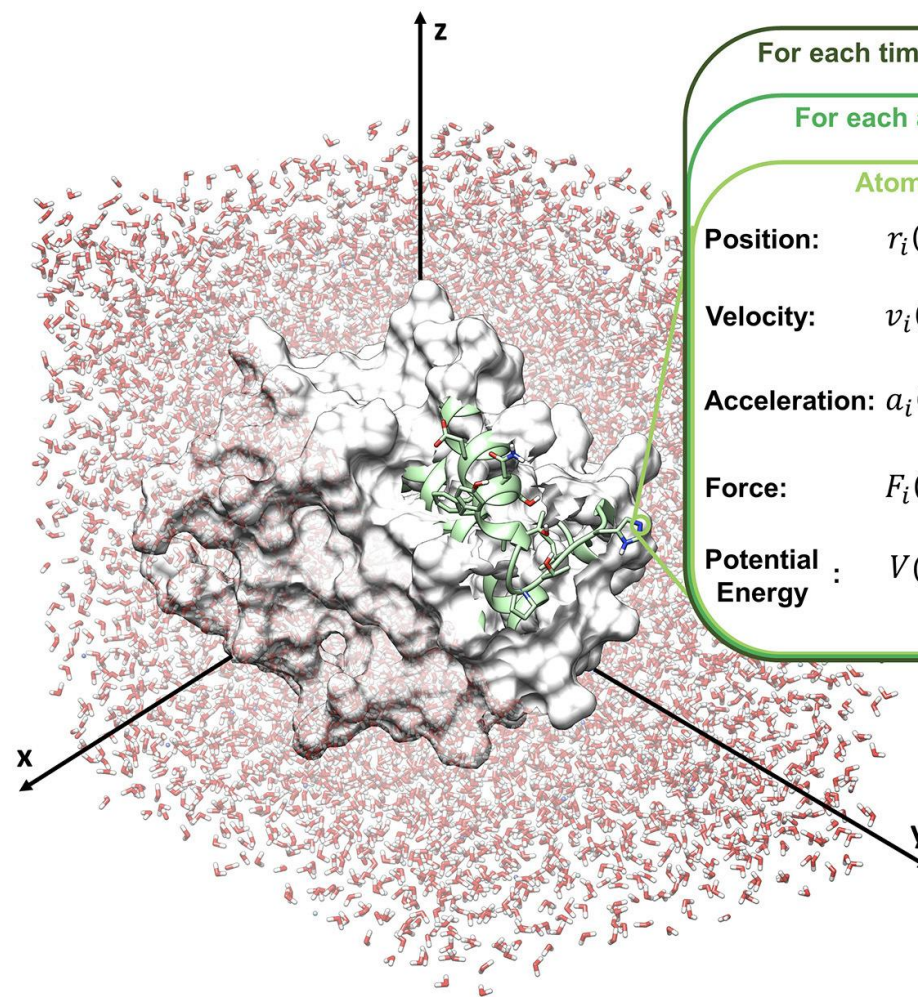
Molecular dynamics (MD) is a computational technique that simulates the dynamic behaviour of molecular systems as a function of time. The integration of Newton's equations calculates the movements of atoms over time:

$F_i(t)$ force exerted on atom i at time t

$r_i(t)$ vector position of the atom i at time t

m_i mass of the atom

$$\frac{d^2 r_i(t)}{dt^2} = \frac{F_i(t)}{m_i}$$



For each timestep (δt):

For each atom:

Atom i at time t :

Position: $r_i(t) = (x_i(t), y_i(t), z_i(t))$

Velocity: $v_i(t) = \frac{dr_i(t)}{dt}$

Acceleration: $a_i(t) = \frac{d^2 r_i(t)}{dt^2} = \frac{F_i(t)}{m_i}$

Force: $F_i(t) = - \frac{dV(r(t))}{dr_i(t)}$

Potential Energy : $V(r(t))$

Exploring the stability of ligand binding modes to CFTR by molecular dynamics simulation

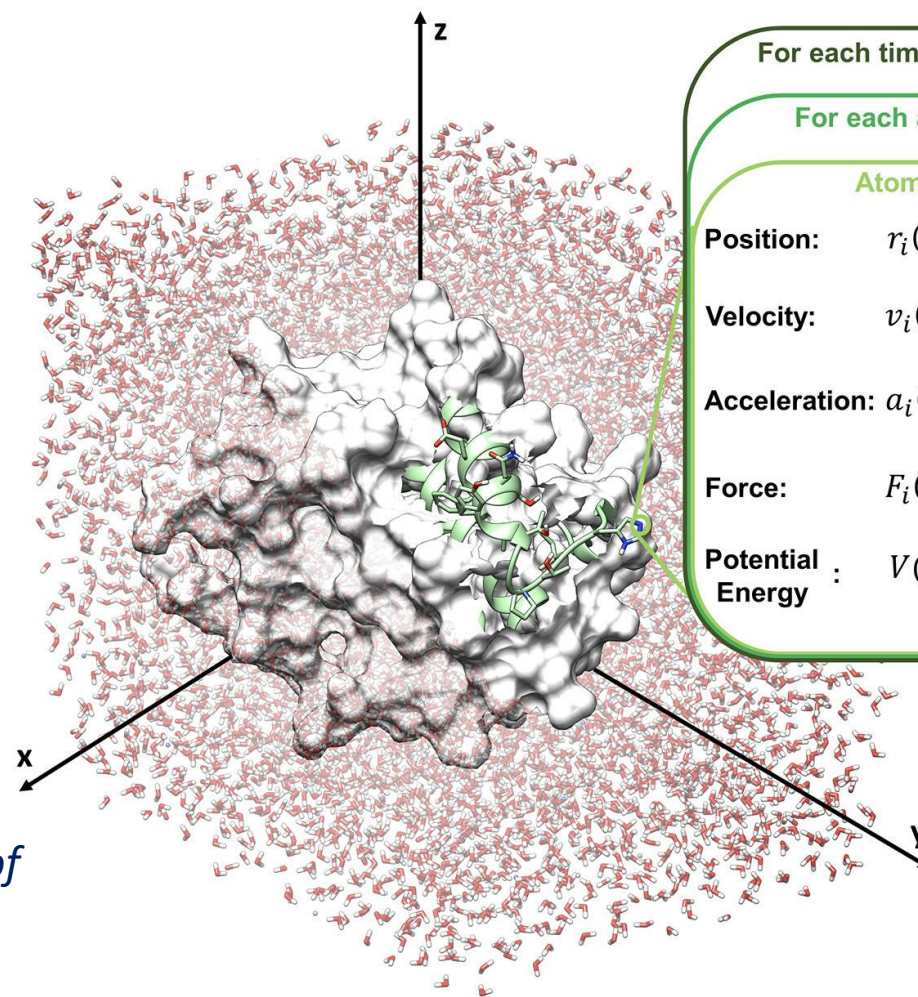
Time is partitioned into time steps (δt), which are used to propagate the system forward in time. Several integration algorithms are available, which derive Newton's equations by a discrete-time numerical approximation. An example to compute the position and velocity of an atom i at the time step $t+\delta t$, starting from step t :

$$r_i(t + \delta t) = r_i(t) + v_i(t) \delta t + \frac{1}{2} a_i(t) \delta t^2$$

$$v_i(t + \delta t) = v_i(t) + \frac{1}{2} [a_i(t) + a_i(t + \delta t)] \delta t$$

$r_i(t)$, $v_i(t)$ and $a_i(t)$ are the position, velocity and acceleration of atom i at time t

$r_i(t+\delta t)$, $v_i(t+\delta t)$ and $a_i(t+\delta t)$ are the position, velocity and acceleration of atom i at time $t+\delta t$.



For each timestep (δt):

For each atom:

Atom i at time t :

Position: $r_i(t) = (x_i(t), y_i(t), z_i(t))$

Velocity: $v_i(t) = \frac{dr_i(t)}{dt}$

Acceleration: $a_i(t) = \frac{d^2 r_i(t)}{dt^2} = \frac{F_i(t)}{m_i}$

Force: $F_i(t) = - \frac{dV(r(t))}{dr_i(t)}$

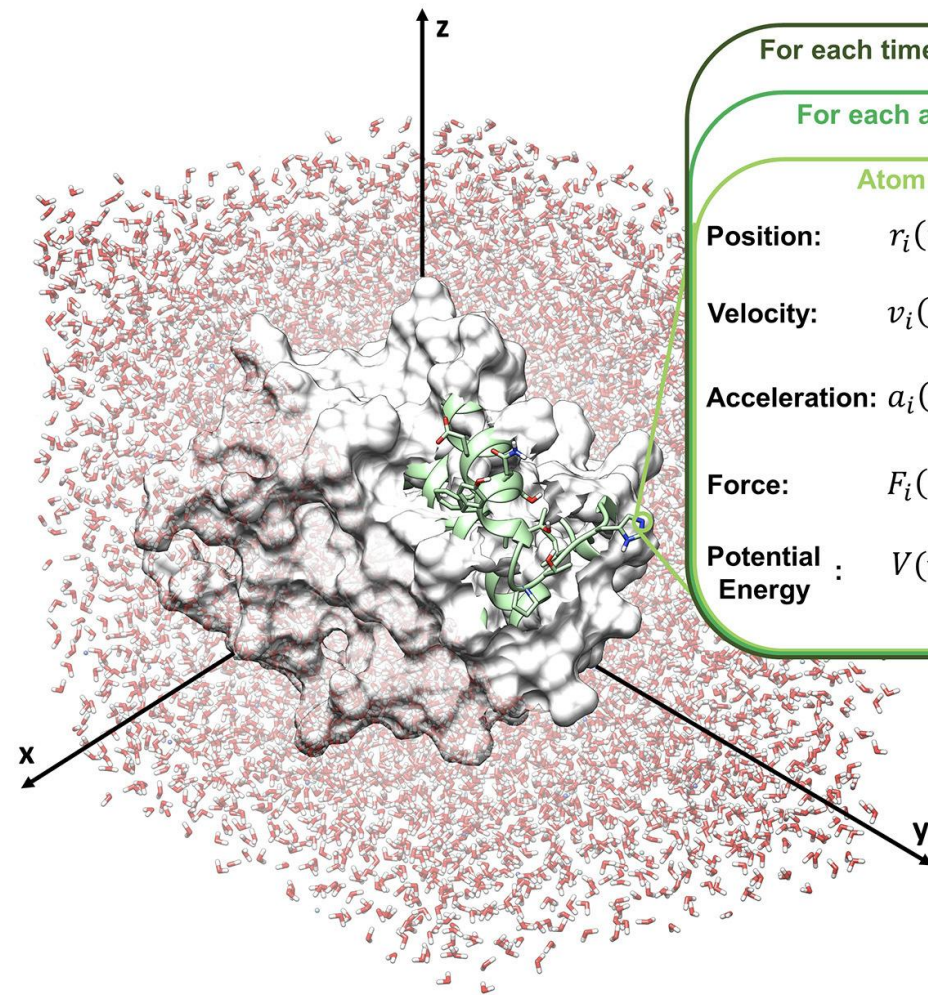
Potential Energy : $V(r(t))$

Exploring the stability of ligand binding modes to CFTR by molecular dynamics simulation

Acceleration is calculated from the forces acting on atom i according to Newton's second law, and forces are computed from the force field, according to the following equation:

$$a_i(t) = \frac{d^2 r_i(t)}{dt^2} = \frac{F_i(t)}{m_i} = - \frac{dV(r(t))}{m_i dr_i(t)}$$

$V(r(t))$ is the potential energy function retrieved by the force field



For each timestep (δt):

For each atom:

Atom i at time t :

Position: $r_i(t) = (x_i(t), y_i(t), z_i(t))$

Velocity: $v_i(t) = \frac{dr_i(t)}{dt}$

Acceleration: $a_i(t) = \frac{d^2 r_i(t)}{dt^2} = \frac{F_i(t)}{m_i}$

Force: $F_i(t) = - \frac{dV(r(t))}{dr_i(t)}$

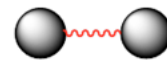
Potential Energy: $V(r(t))$

Exploring the stability of ligand binding modes to CFTR by molecular dynamics simulation

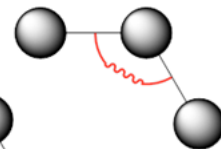
The potential energy function used to derive atomic forces for molecular movement is the sum of bonded (intramolecular) and non-bonded energy terms.

$$\begin{aligned}
 V(r(t)) = & \sum_{\text{bonds}} k_r (r - r_{eq})^2 \\
 & + \sum_{\text{angles}} k_\theta (\theta - \theta_{eq})^2 \\
 & + \sum_{\text{dihedrals}} k_\phi (1 + \cos[n\phi - \gamma]) \\
 & + \sum_{\text{impropers}} k_\omega (\omega - \omega_{eq})^2 \\
 & + \sum_{i < j}^{atoms} \epsilon_{ij} \left[\left(\frac{r_m}{r_{ij}} \right)^{12} - 2 \left(\frac{r_m}{r_{ij}} \right)^6 \right] \\
 & + \sum_{i < j}^{atoms} \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}}
 \end{aligned}$$

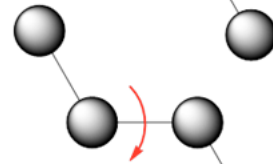
bond



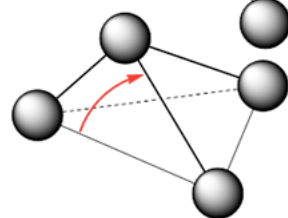
angle



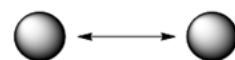
dihedral



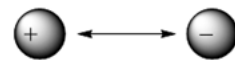
improper



van der Waals



electrostatic



r : bond length;

θ : atomic angle;

ϕ : dihedral angle;

ω : improper dihedral angle;

r_{ij} : the distance in between atom i and j ;

k_r , k_θ , k_ϕ , and k_ω : force constants;

r_{eq} , θ_{eq} and ω_{eq} are equilibrium positions;

dihedral term is a periodic term characterized by a force constant (k_ω), multiplicity (n), and phase shift (γ);

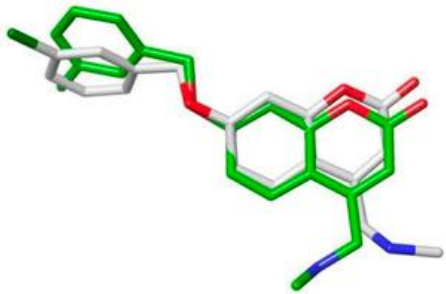
ϵ_{ij} is related to the Lennard–Jones well depth;
 r_m is the distance at which the potential reaches its minimum;

q_i and q_j are the charges on the respective atoms;
 ϵ_0 is the dielectric constant.

Exploring the stability of ligand binding modes to CFTR by molecular dynamics simulation

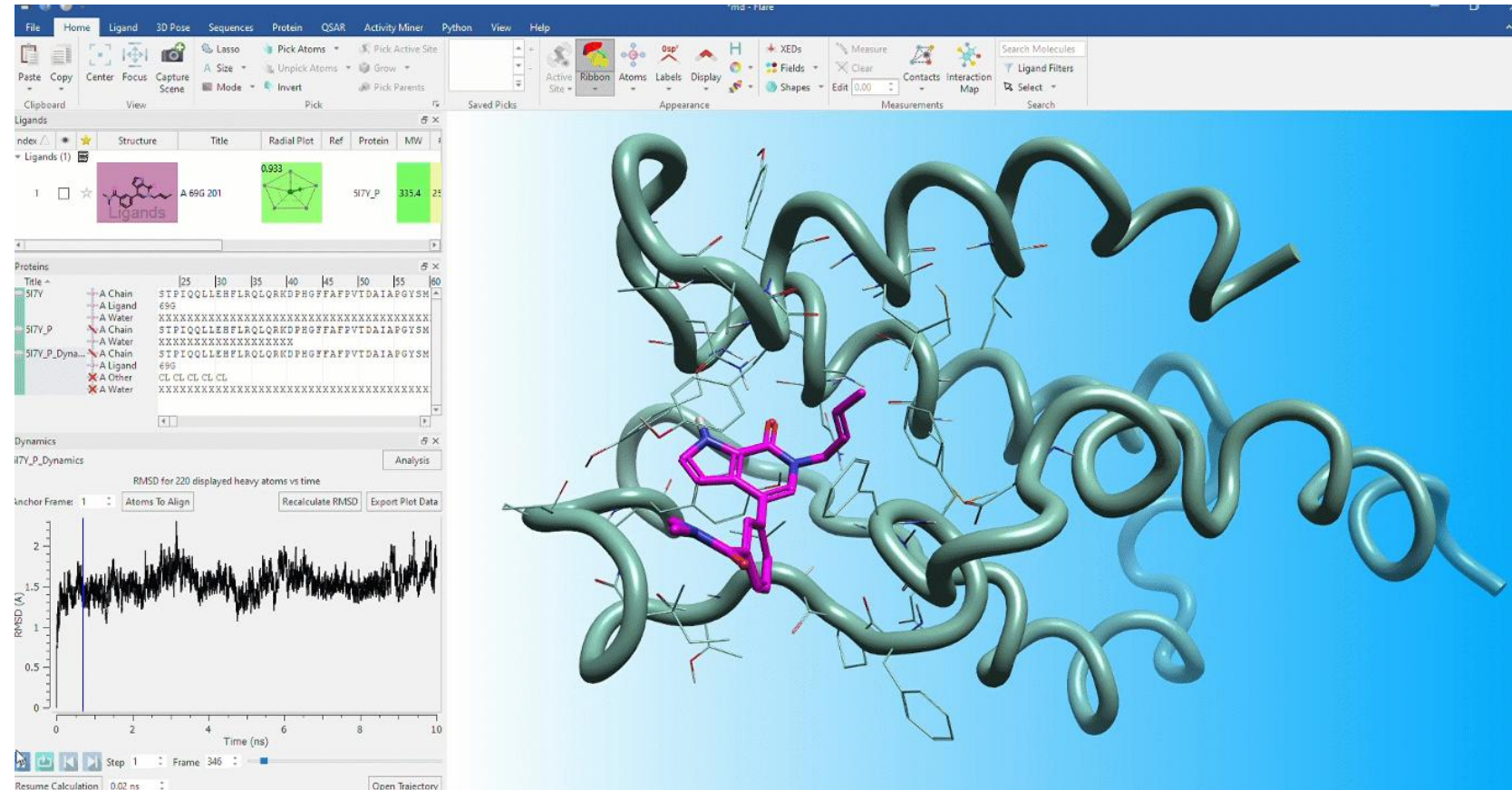
Forces acting on individual atoms are then used for the calculation of accelerations and velocities by Newton's law of motion and atom positions are updated after each time step

RMSD function quantifies the difference between two poses of the same molecule



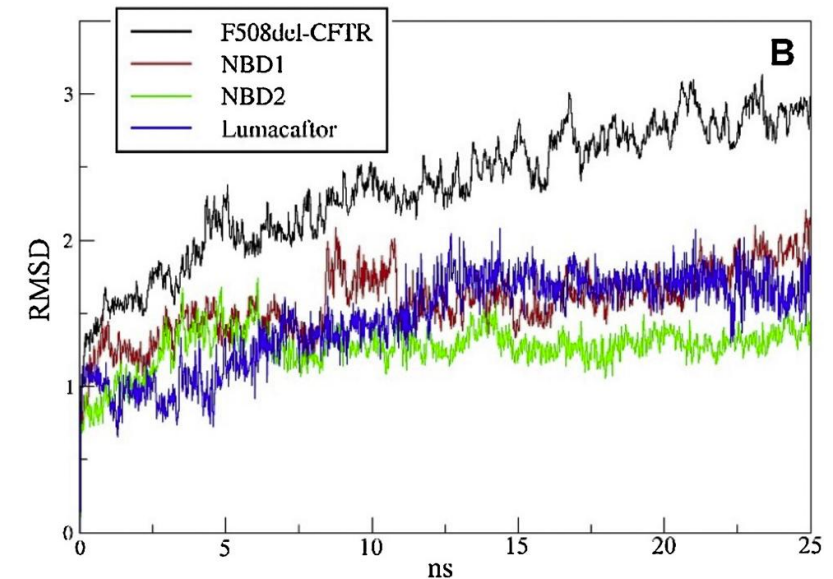
$$\text{RMSD} = \sqrt{\frac{1}{N} \sum_{i=1}^N \delta_i^2}$$

δ_i is the distance between atom i and either a reference structure



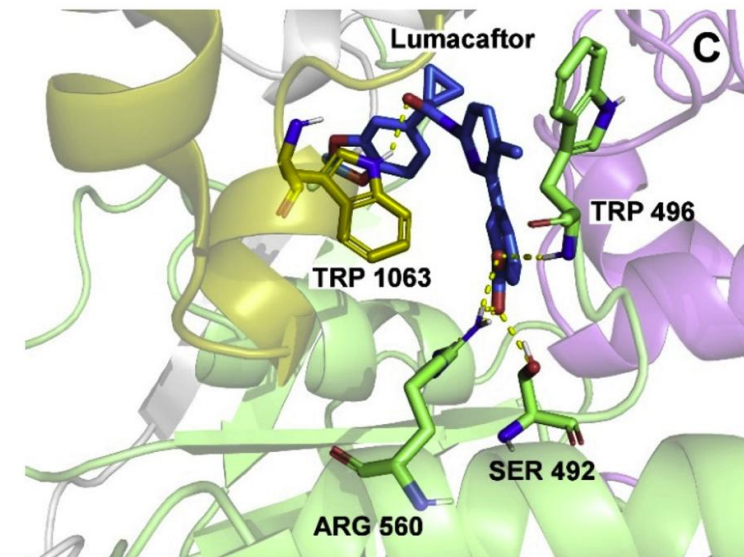
Exploring the stability of ligand binding modes to CFTR by molecular dynamics simulation

RMSD of the F508del-CFTR/Lumacaftor complex shows that it reaches stability at 12 ns

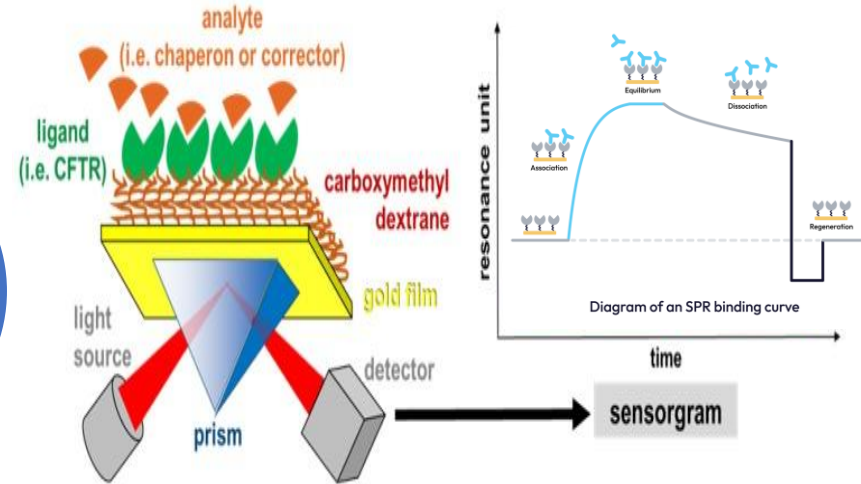
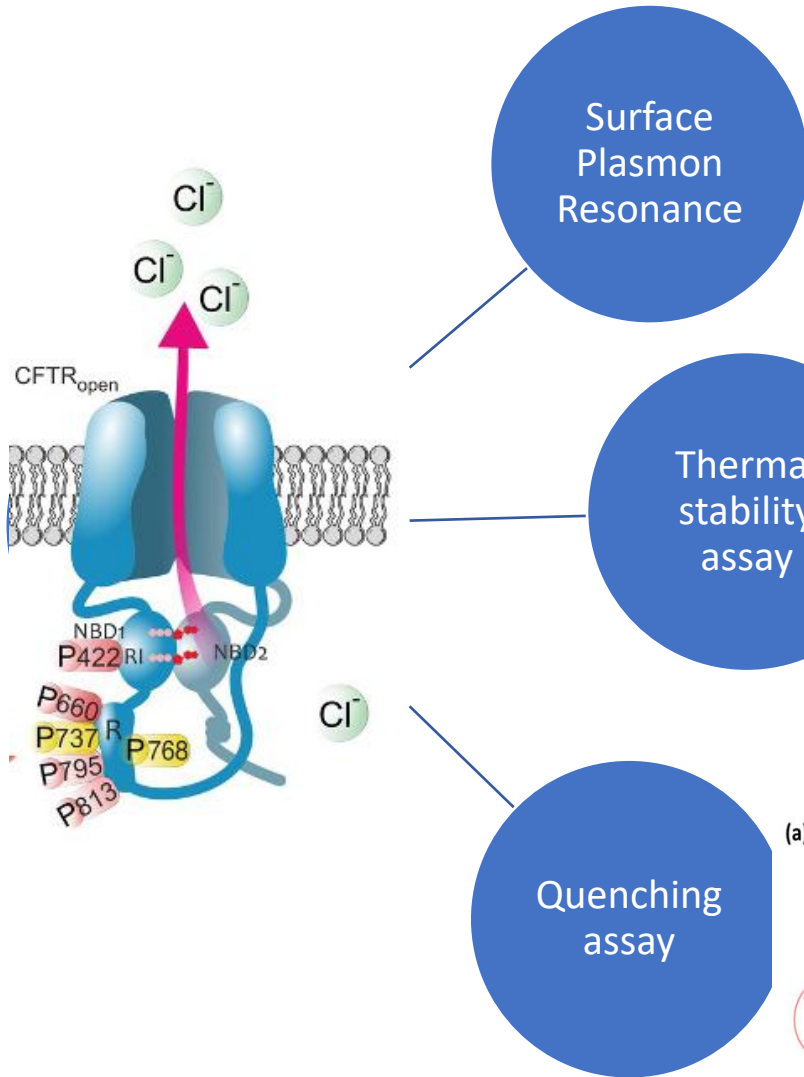


Lumacaftor interacts with an S492, W496, R560 of NBD1 and with W1063 of ICL4. These predictions are supported by recent experimental data suggesting a binding pocket for Lumacaftor between NBD1 and ICL4

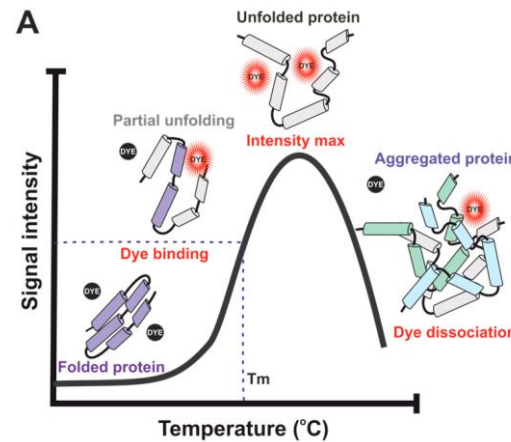
(R.P. Hudson and et al. Direct binding of the corrector VX-809 to human CFTR NBD1: evidence of an allosteric coupling between the binding site and the NBD1:ICL4 interface, Mol. Pharmacol. 92 (2) (2017) 124–135)



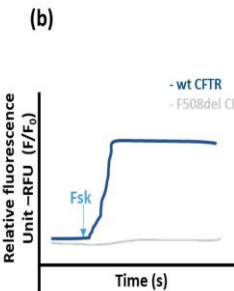
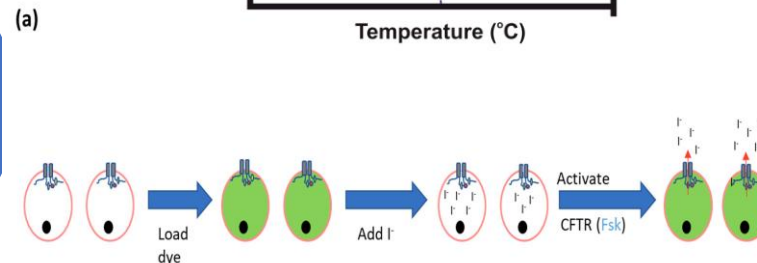
Experimental methods



Light refracts by a prism; the angle changes with molecular binding. A sensorgram plots this interaction over time. The analyte binds to the immobilised ligand, followed steady-state, at the end, a dissociation phase occurs when the analyte solution is replaced by wash buffer, breaking bonds between analytes and ligands.

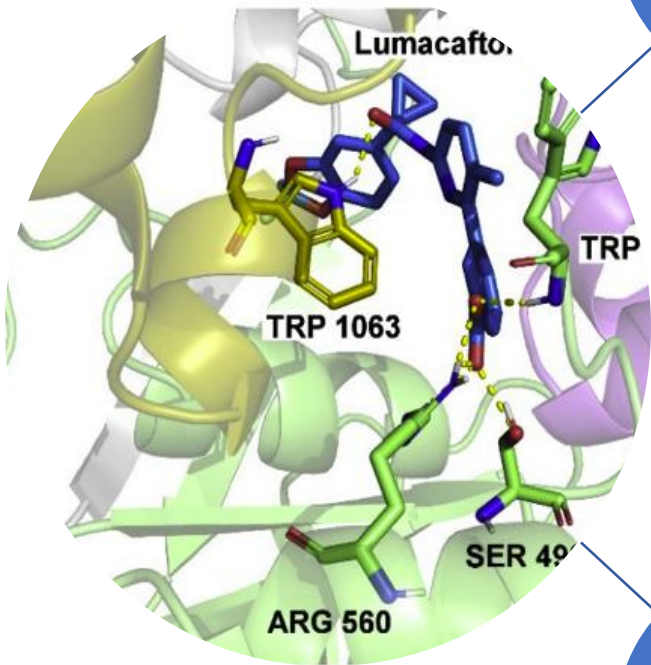


Thermal stability assay data use an external fluorescent dye. As temperature increases, the protein unfolds, allowing the dye to bind and fluorescence to rise. A half-maximum signal indicates the melting temperature (T_m); further temperature increases lead to dye dissociation from protein aggregation.



Cells are loaded with fluorescent probes that induce fluorescence. Then, iodide is added, causing quenching (a decrease in fluorescence). After CFTR stimulation by forskolin (Fsk), iodide comes out through CFTR and increases cell fluorescence again if the CFTR channel is active. The fluorescence measured over time indicates CFTR function.

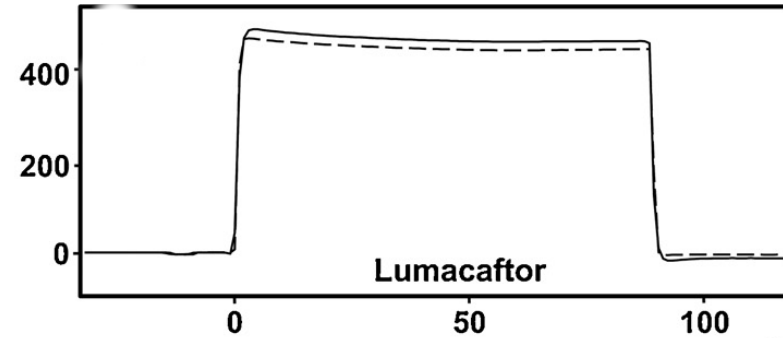
Experimental validation



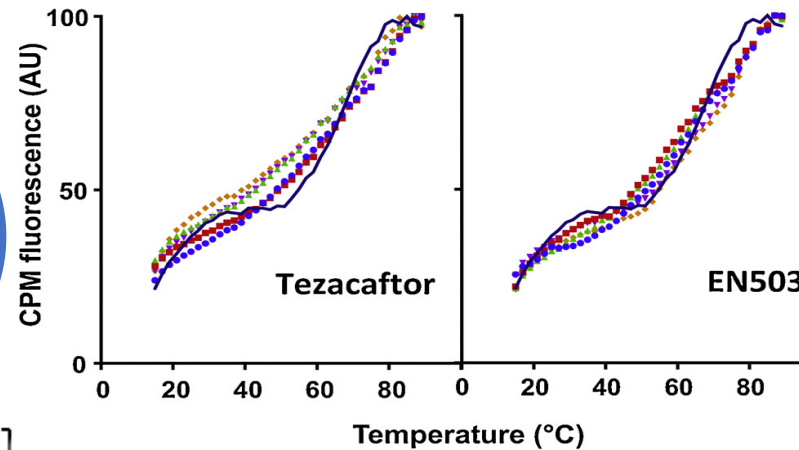
Surface
Plasmon
Resonance

Thermal
stability

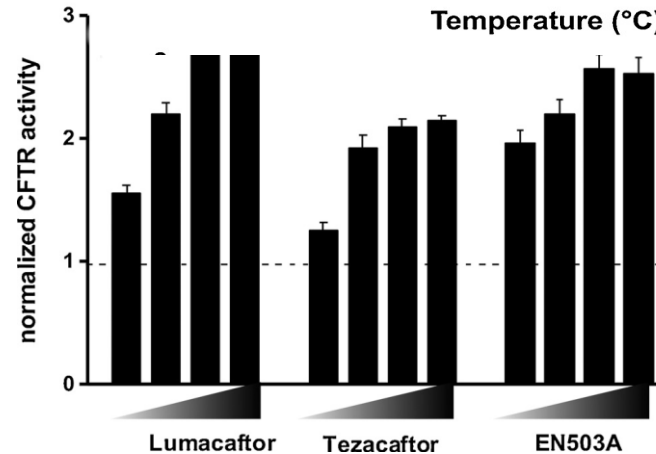
F508del-
CFTR activity
in cells



Lumacaftor sensorgram



Concentration of compounds:
blue circles: 10 μ M;
red squares 40 μ M;
green triangles: 80 μ M;
purple inverted triangles: 120 μ M;
orange diamonds: 180 μ M.



Correctors activity in CFBE41o-cells
at 0.08, 0.4, 2.0 and 10 μ M.
The dashed line indicates the level of
activity in cells treated with a vehicle alone.



Projects funded by the Cystic Fibrosis Foundation

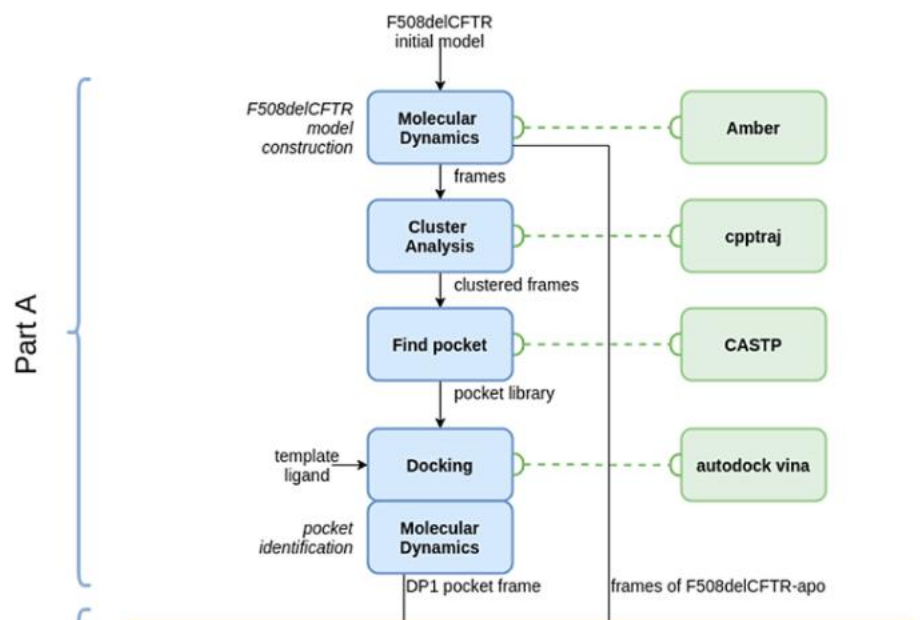


Rescuing defective CFTR by applying a drug repositioning strategy based on computational studies, surface plasmon resonance and cell-based assays.

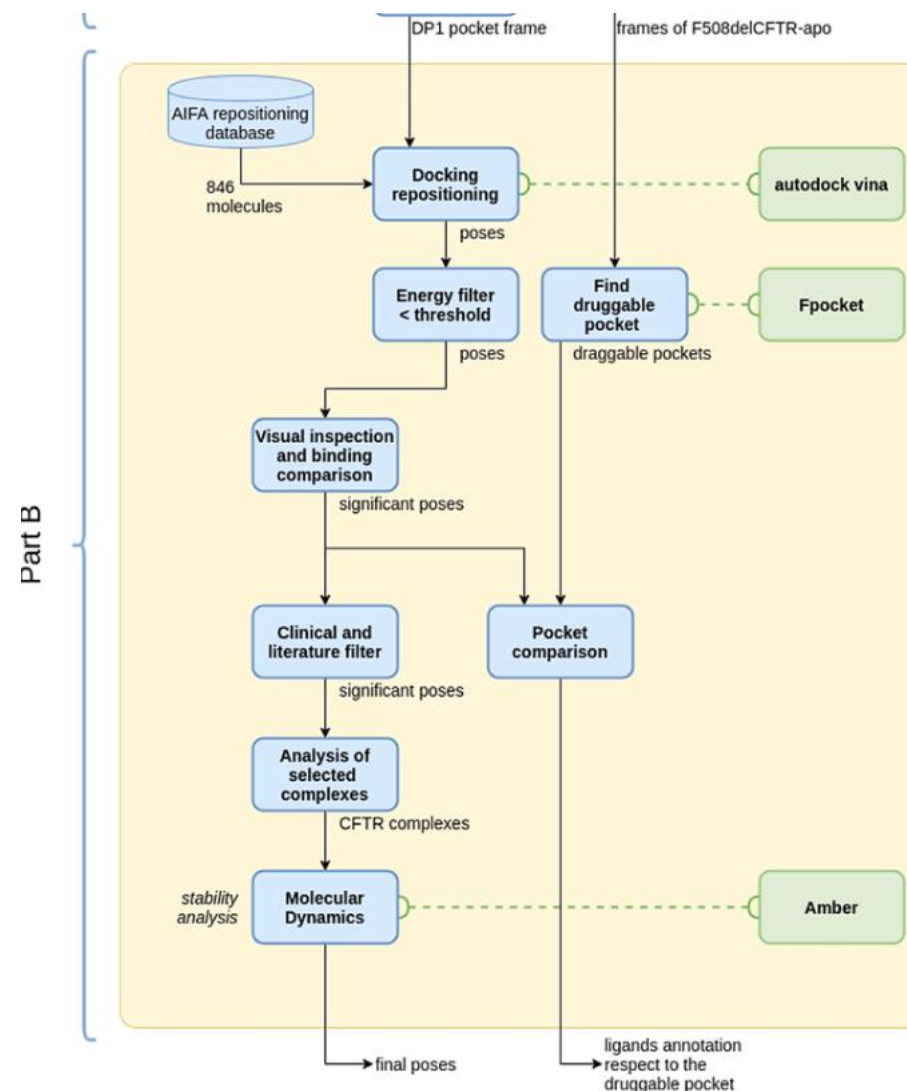
Drug repositioning

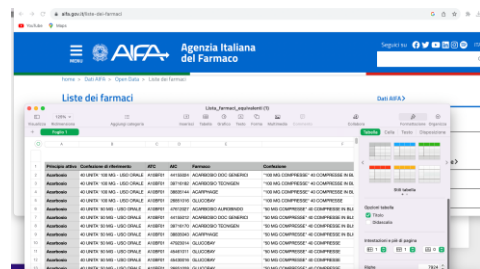
Drug repositioning is a strategy that accelerates the drug development process by identifying new indications for already approved drugs, rather than discovering de novo active compounds.

The drug repositioning method reduces the cost and the time required for drug development, as information regarding the safety, pharmacokinetics, and formulation of existing drugs is already available.



The druggable Pocket1 described before was used in the drug's repositioning pipeline to screen 846 compounds of the AIFA database. Docking repositioning results were firstly filtered by energy, taking into account only those poses with docking energies like that of Lumafactor.



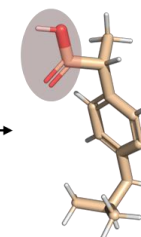


smiles format of the molecule

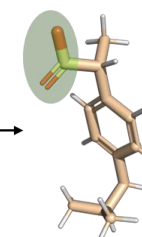
Simplified Molecular Input Line Entry System

SMILES:
CC(C)C1=CC=C(C=C1)C(C)C(=O)O

3D
modeling



Charging the structure
at physiological pH 7,4

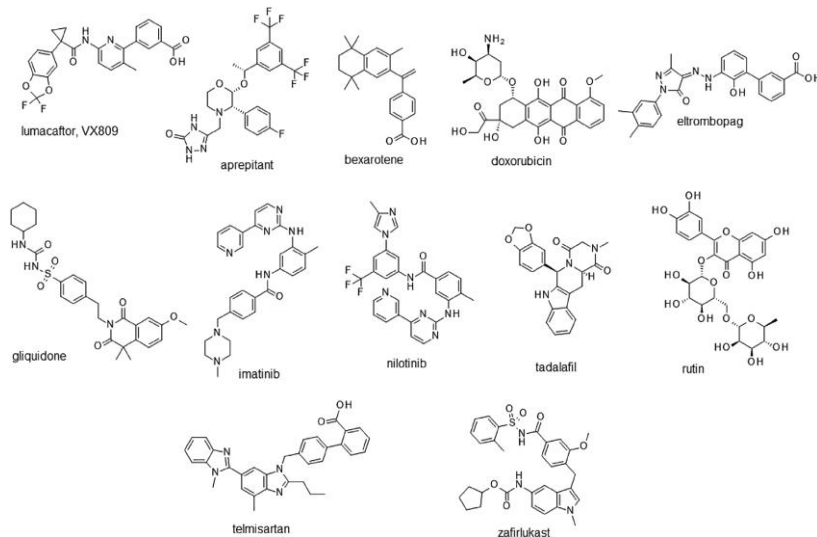


An in-house ligand dataset was manually curated. The molecules were retrieved from the Agenzia Italiana del Farmaco – AIFA and filtered excluding the not appropriate ones, such as hormones, inorganic salts, contrast agents, peptides, and proteins.

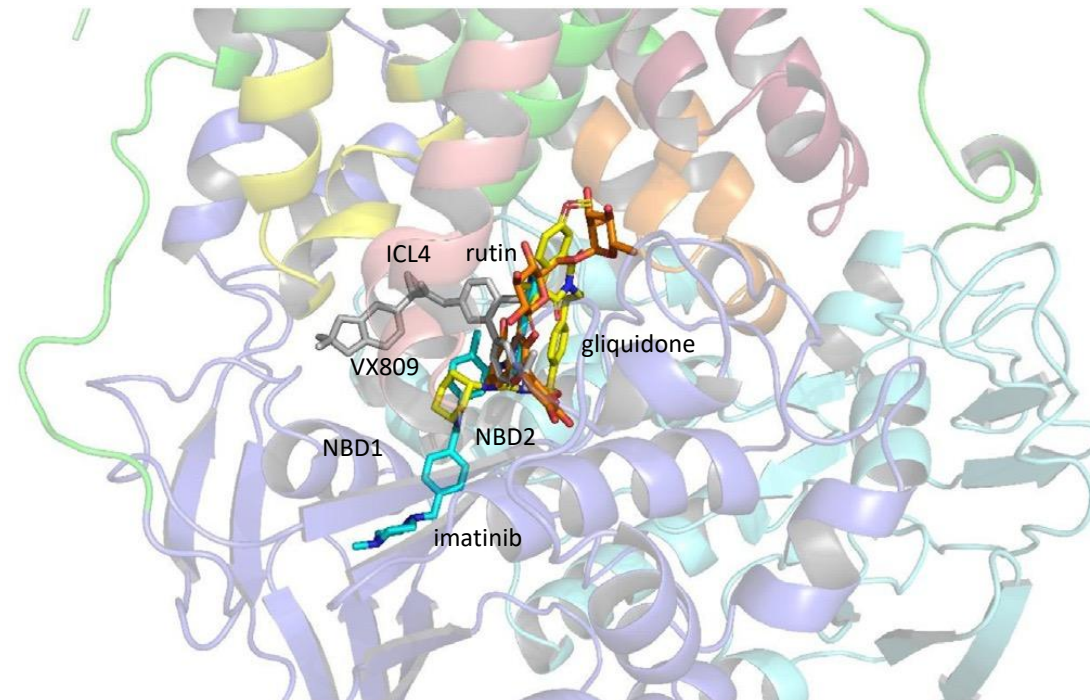
ManXDR database



846 molecules



Chemical structures of the 11 drug repositioned



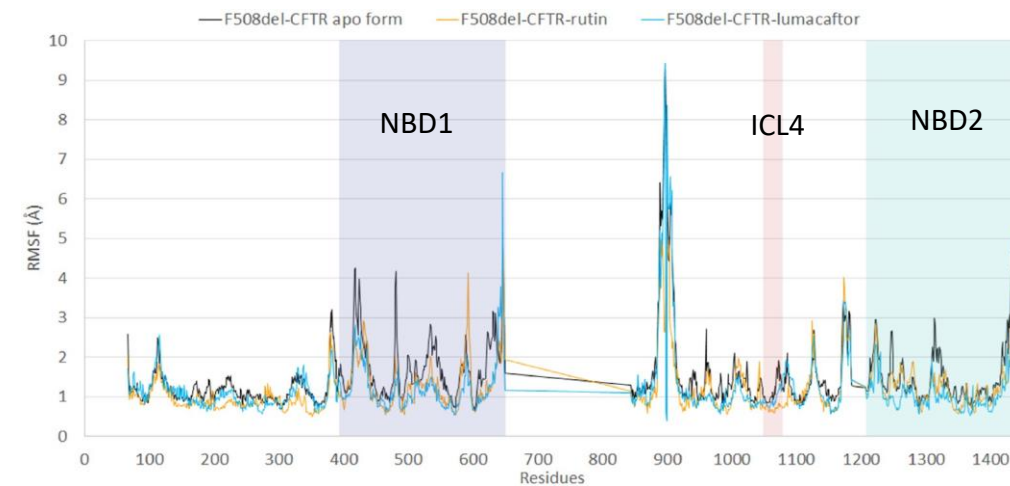
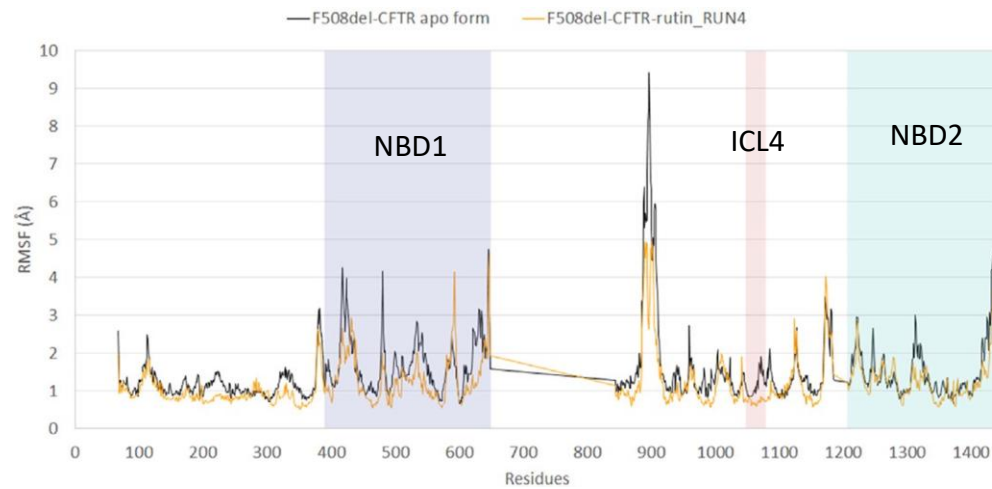
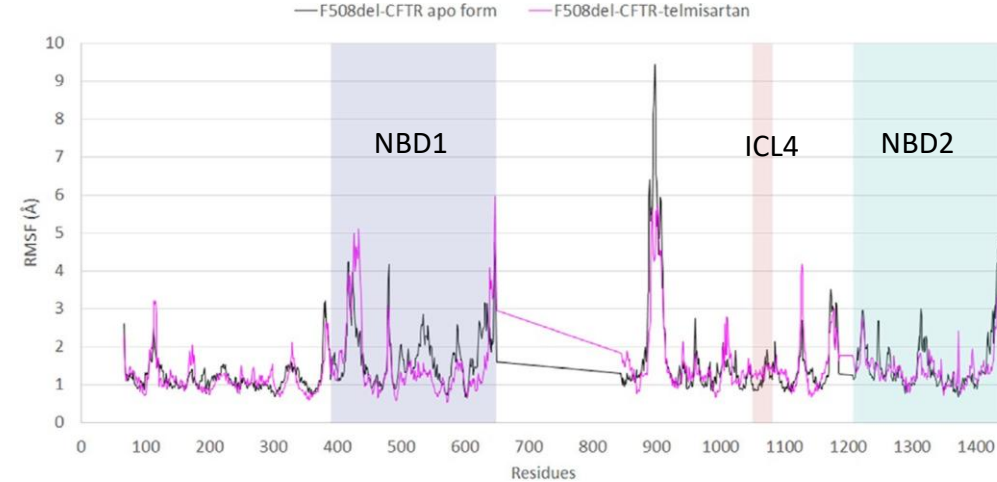
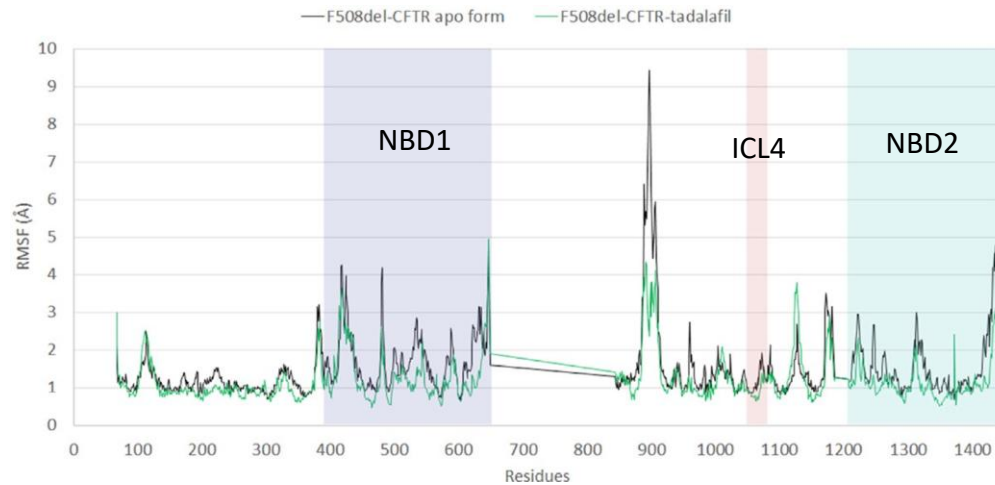
F508del-CFTR in cartoon: NBD1 in slate blue, NBD2 in cyan, ICL1 in yellow, ICL2 in orange, ICL3 in raspberry red, ICL4 in salmon pink, and TMs in green.

Three typologies of drugs can be defined depending on the location of residues involved in the binding:

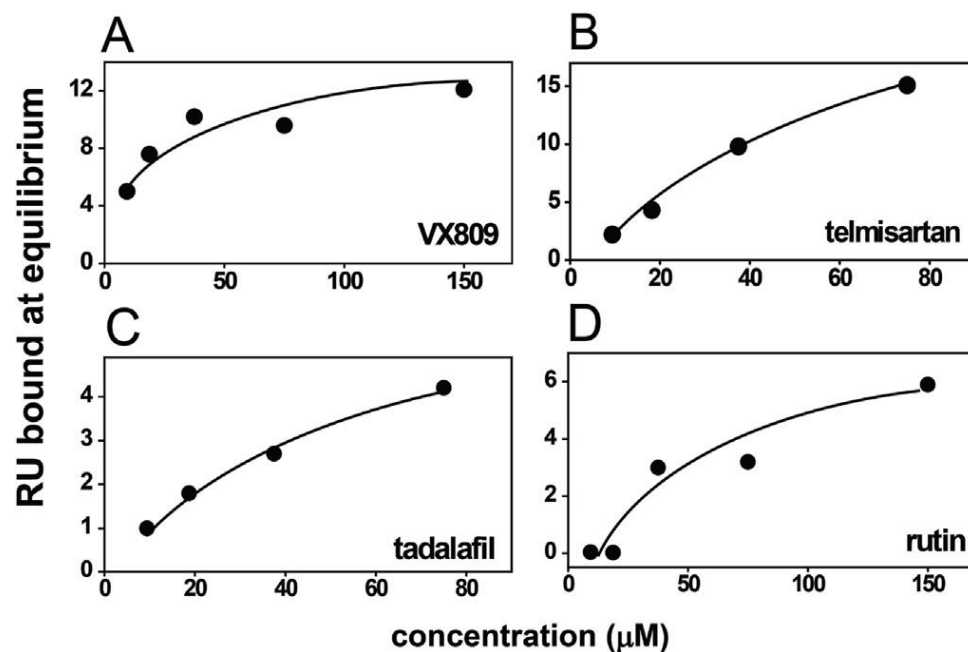
- **VX809** , doxorubicin, nilotinib, aprepitant, bexarotene, eltrombopag, Zafirlukast and **rutin** bind the residues of the **NBD1** and **NBD2** and **ICL4**,
- **imatinib**, tadalafil and telmisartan bind the residues of **NBD1** and **ICL4**,
- **gliquidone** interacts only with the apical region of **NBD1**.

The binding energy ranking of the repositioned drugs was combined with their clinical assessment, and only **telmisartan**, **tadalafil**, and **rutin** were chosen for further computational and experimental evaluation.

RMSF evaluates how much a residue moves during a simulation. In addition, higher RMSF values indicate that the protein has more flexible domains.



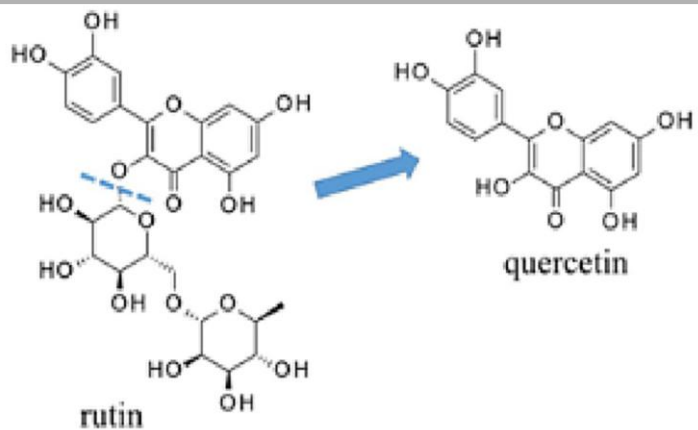
Tadalafil, telmisartan, and rutin show a lower or equal fluctuation than apo F508del-CFTR. Tadalafil, telmisartan, and rutin stabilize part of the NBD1 domain, while part of NBD2 is stabilised only by rutin and tadalafil. Rutin is the only compound able to stabilize the ICL4 domain, while telmisartan produces a higher ICL4 fluctuation when compared to the apo form.



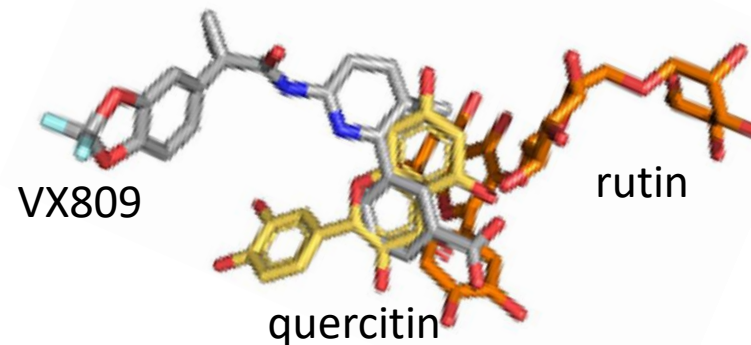
Binding of the compounds to sensorchip-immobilized F508del-CFTR. K_d values are reported in μM ± S.E.M. The number of repeated independent calculations are indicated in brackets.

Compound	K _d (μM)
VX809 (template)	47.8 18.36 (5)
telmisartan	189.3 ± 48.5 (3)
tadalafil	175.0 ± 50.4 (4)
Rutin	65.8 ± 27.3 (7)
quercetin	25.6 ± 10.2 (3)

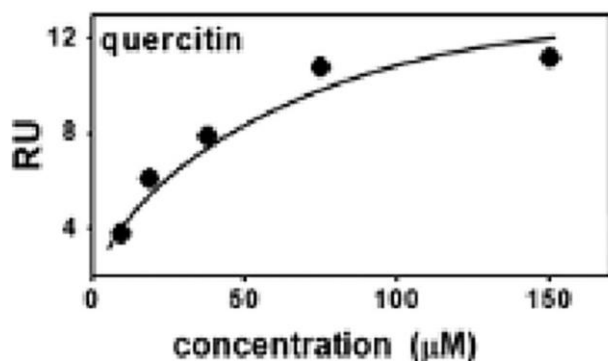
SPR analysis of the binding of the repositioned drugs to F508del-CFTR



Rutin is bio-converted in the quercetin by human gut bacteria

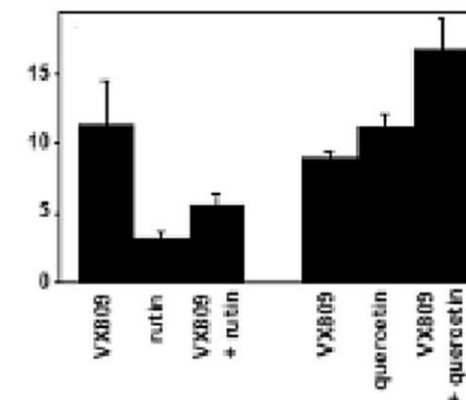


Overlapping of the binding poses of VX809, rutin and quercetin

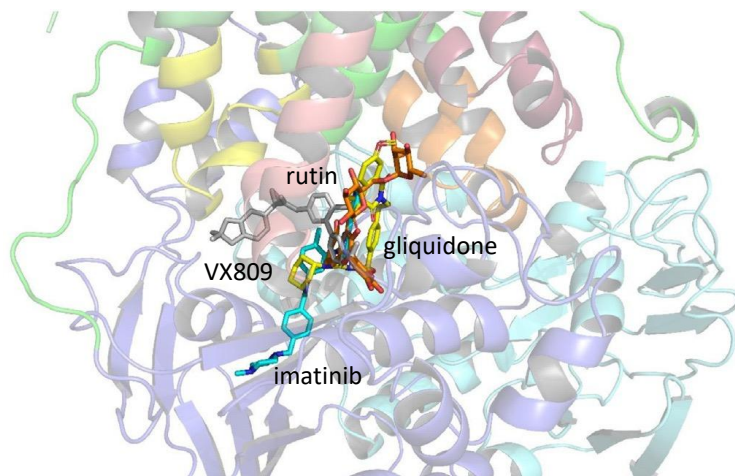


Binding of the compounds to sensorchip-immobilized F508del-CFTR. K_d values are reported in $\mu\text{M} \pm \text{S.E.M.}$ The number of repeated independent calculations are indicated in brackets.

Compound	K_d (μM)
VX809 (template)	47.8 18.36 (5)
telmisartan	189.3 $\pm$ 48.5 (3)
tadalafil	175.0 $\pm$ 50.4 (4)
Rutin	65.8 $\pm$ 27.3 (7)
quercetin	25.6 $\pm$ 10.2 (3)

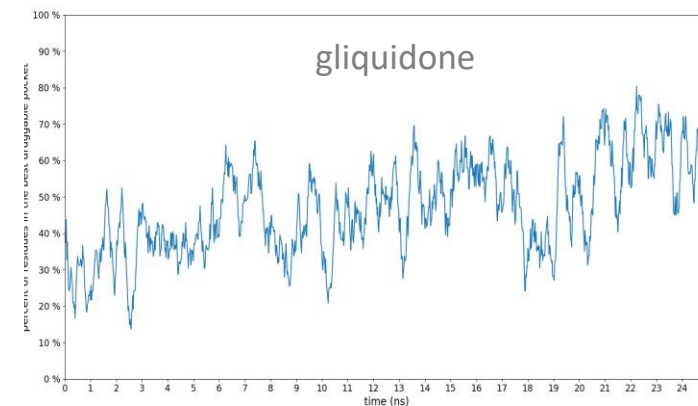
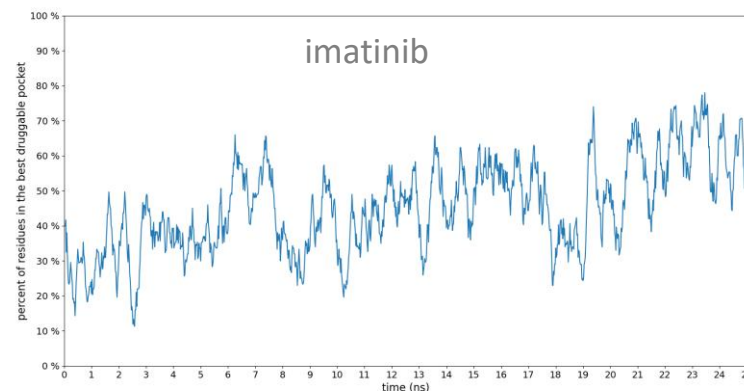
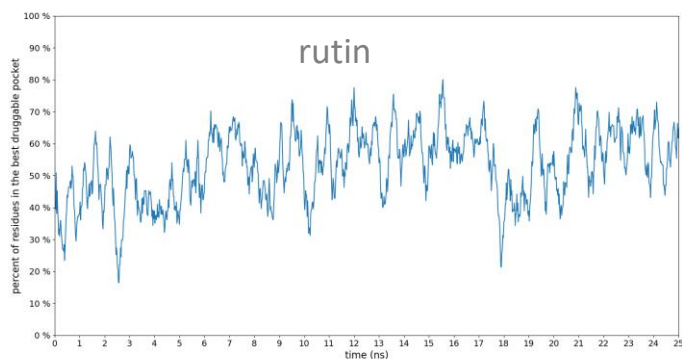


Quercetin binds F508del-CFTR in a dose-dependent and saturable manner with an affinity that is comparable to VX809, as demonstrated by SPR analysis. Competition binding assay shows that rutin partially inhibits VX809 to F508del-CFTR, while quercetin shows an additive effect due to its small dimension



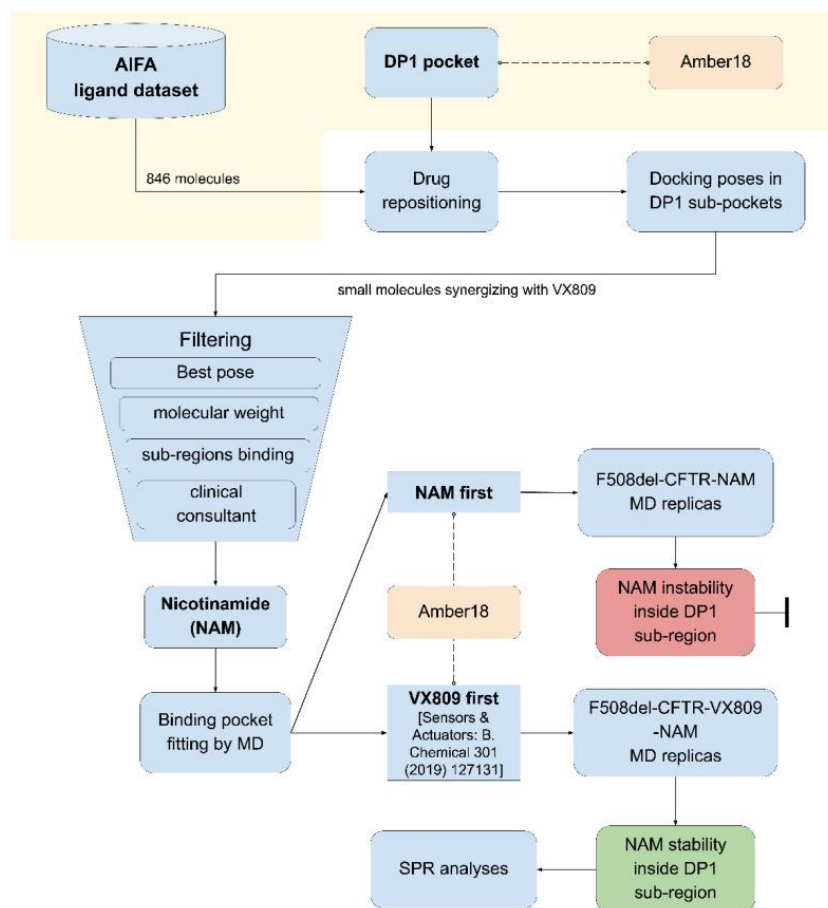
Three sub-regions around the aromatic acid part VX809 are not occupied by the lumafactor. Other drugs could occupy these sub-regions, which could enhance the effect of the corrector VX809.

Fpocket analysis evaluated the druggability score of the sub-regions.



The plots show that the residues surrounding imatinib, rutin and gliquidone are endowed with high druggability scores along the molecular dynamics simulations. For these residues, a druggability score of more than 70% is observed.

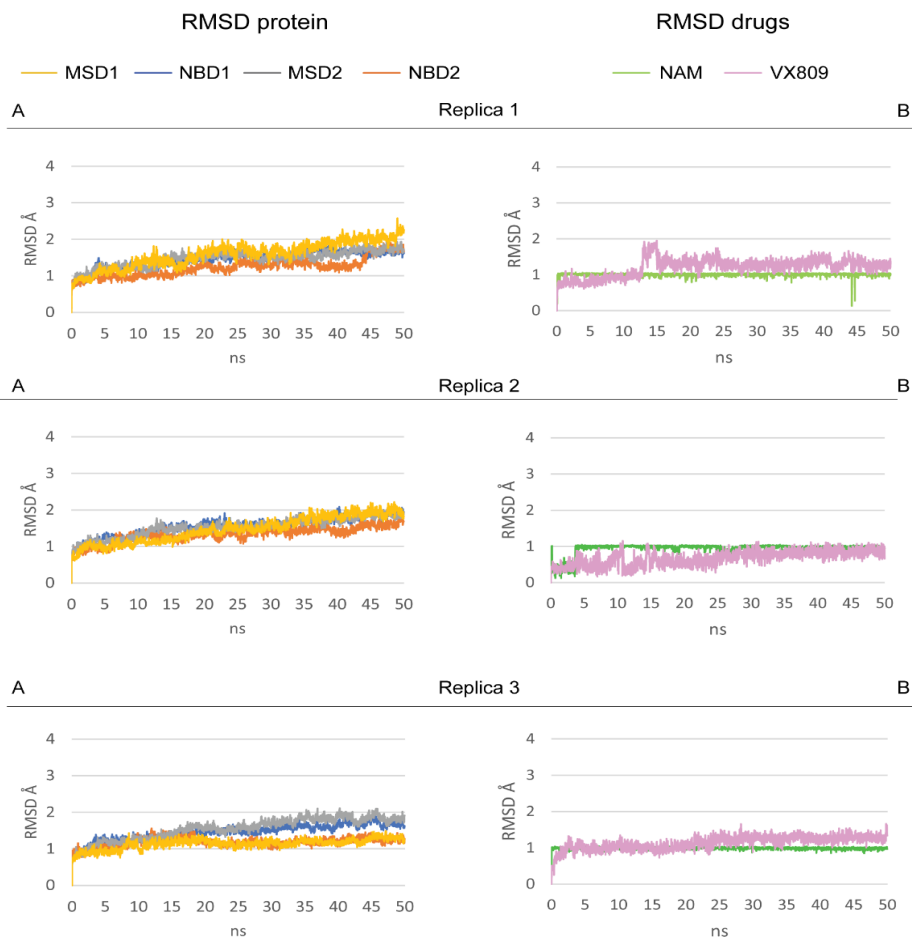
Pipeline update to identify small molecules with additive effect with lumafactor



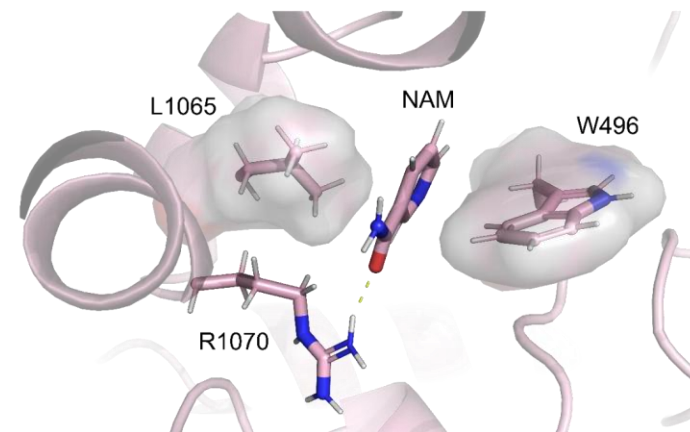
The docking results were filtered based on their binding into the druggable DP1 sub-regions and ranked based on binding energy value and MW.

Pipeline update to identify small molecules with additive effect with lumafactor

Complex stability



RMSD analysis of F508del-CFTR four domains (A), and NAM and lumacaftor (B) for the three replicas.

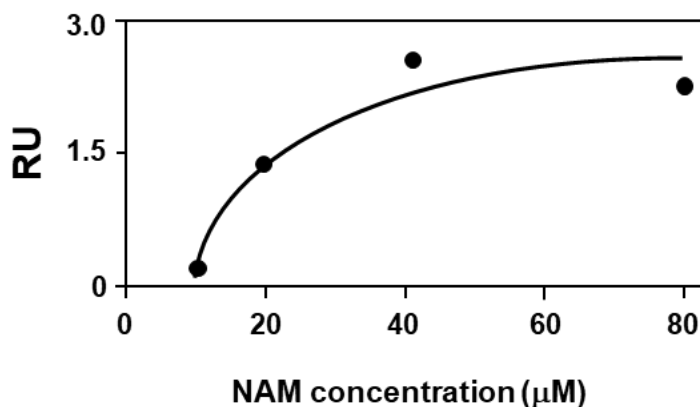


NAM contributes to generate contacts with W496 of NBD1 and L1065 and R1070 of ICL4

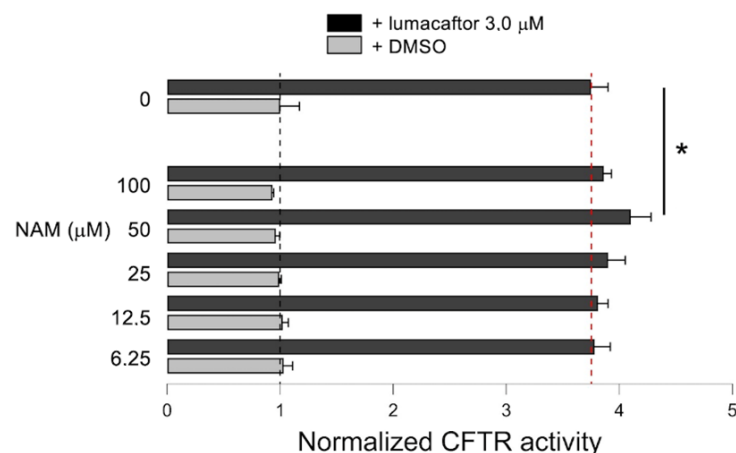
NAM binding pose in the F508del-CFTR-lumacaftor-NAM complex. The H-bond is shown in yellow dotted lines. Residues involved in hydrophobic interactions with NAM are depicted with their hydrophobic surfaces.

Pipeline update to identify small molecules with additive effect with lumacaftor

Experimental validation



Surface plasmon resonance binding analysis confirmed that NAM effectively binds to F508del-CFTR

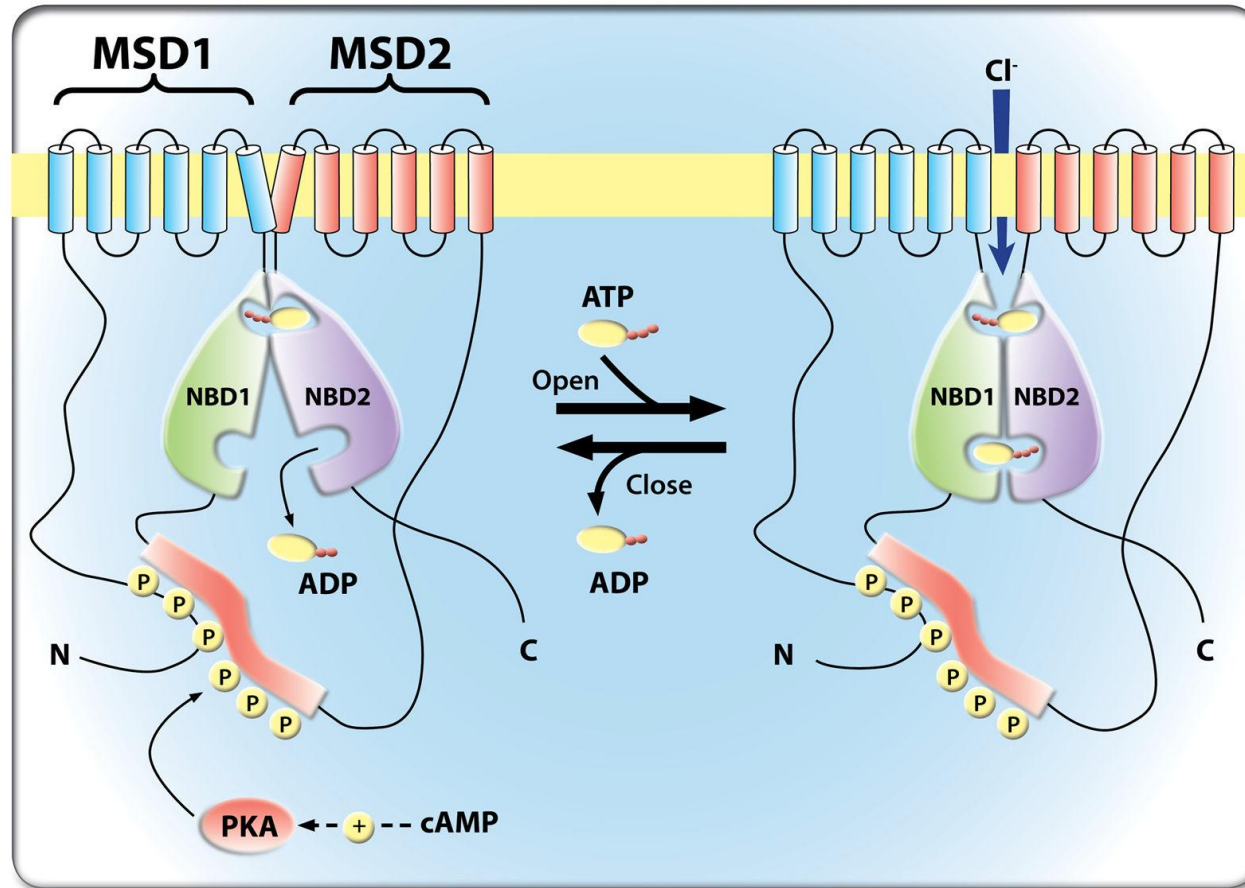


*Effect of NAM-lumacaftor co-treatment on mutant F508del-CFTR rescue. The bar graphs show F508del-CFTR activity in CFBE41o-cells stably expressing the HS-YFP. CFTR activity was determined as a function of the YFP quenching rate following iodide influx in cells treated for 24 h with DMSO in the absence (vehicle) or in the presence of lumacaftor (3.0 μM) as a single agent or combined with the indicated concentrations of NAM. * $p < 0.05$.*



THANK YOU

Regulation of CFTR gating

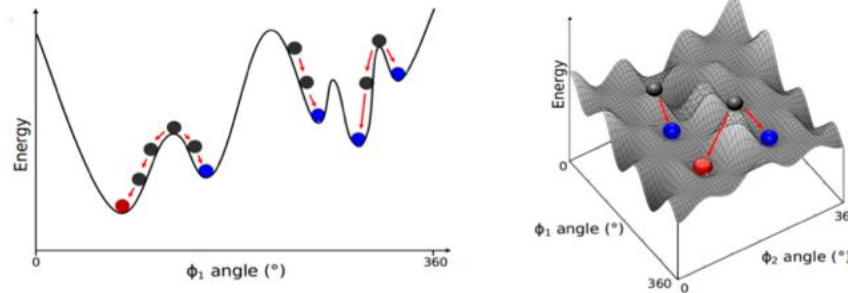


CFTR gating is regulated by the R domain, which has several PKA and PKC phosphorylation sites and physically interacts with NBD1. CFTR uses the energy of ATP binding and hydrolysis to drive ligand-induced conformational changes in the protein that lead to the regulated opening and closing (gating) of the channel 'pore'.

Molecular docking

Conformational research of the ligand

systematic search algorithm

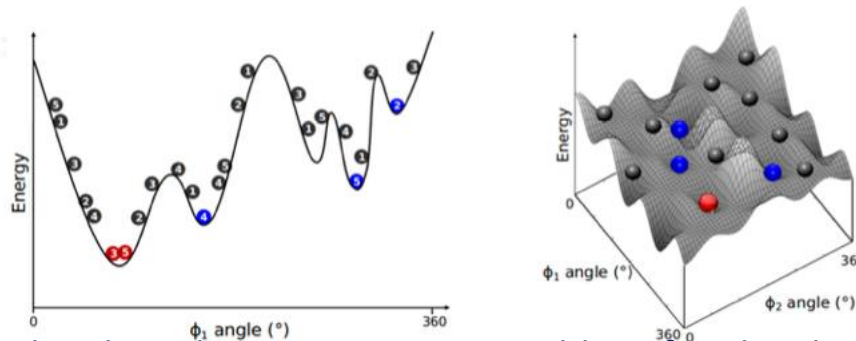


Systematic search methods promote slight variations in the structural parameters of the ligand, gradually changing the conformation of the ligands.

The algorithm explores the energy landscape of the conformational space and, after numerous search and evaluation cycles, converges to the minimum energy solution corresponding to the most likely binding mode. Exploration of the energy landscape is obtained starting from different points of the energy landscape, each of these corresponds to distinct conformations.

structural parameters of the ligands, such as torsional (dihedral), translational and rotational degrees of freedom, are incrementally modified

Stochastic search algorithm



Stochastic methods carry out the conformational search by randomly modifying the structural parameters of the ligands.

The algorithm generates ensembles of molecular conformations and populates a wide range of the energy landscape. This strategy avoids trapping the final solution at a local energy minimum and increases the probability of finding a global minimum