UNIVERSITÀ DEGLI STUDI DI MILANO
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Cystic Fibrosis the involvement of an atypical ABC transporter: CFTR (ABCC7)
What we know and which are the future directions

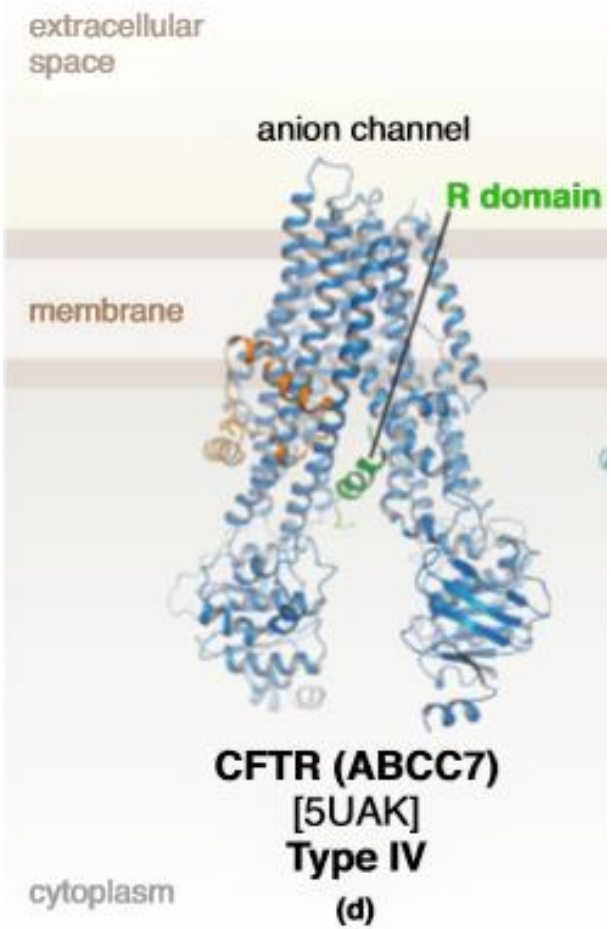
Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)

- CFTR is a CL^- and HCO_3^- channel expressed at the apical surface of many epithelia and codified by the gene CFTR located in the long arm of chromosome 7

- CFTR is a single polypeptide chain formed by 1480 aa and it is belonging to the ABC family C7

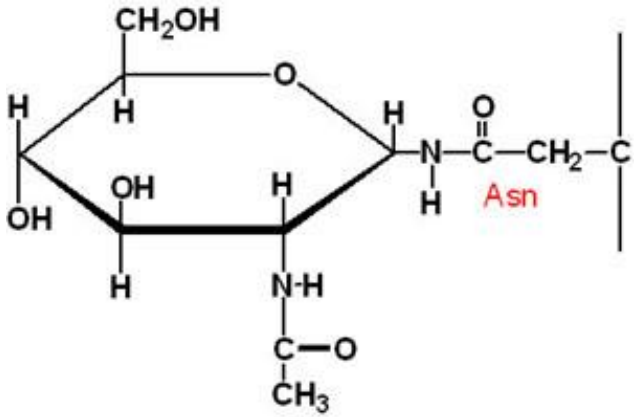
- CFTR synthesis starts in the ER but because of the complex structure of the protein the folding have a low yield, indeed only the 20% of the neobiosynthesized proteins reach the Golgi apparatus for the final maturation and trafficking to plasma membrane.

- The complexity of CFTR is also due to the important and massive post-translational modifications



N-glycoproteins

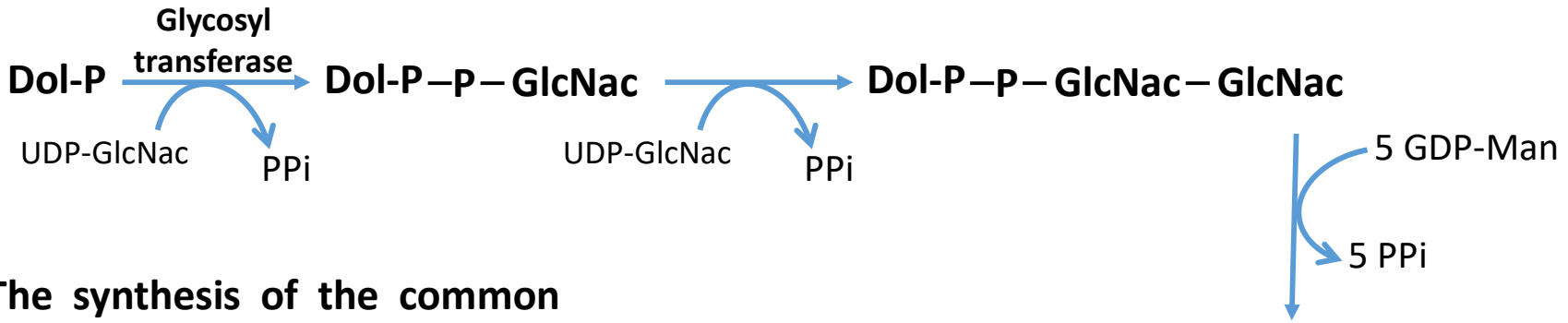
N-glycoproteins are proteins characterized by the presence of a polysaccaride chain, which is linked to an asparagine residues of the protein with an N-acetyl-glucosamine (N-GlcNac)



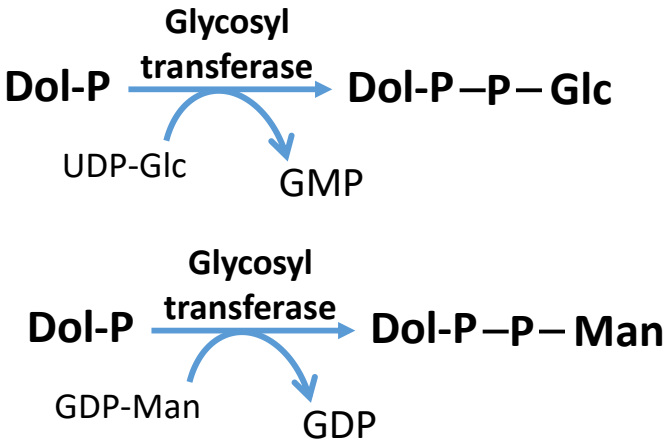
***N*-GlcNAc**

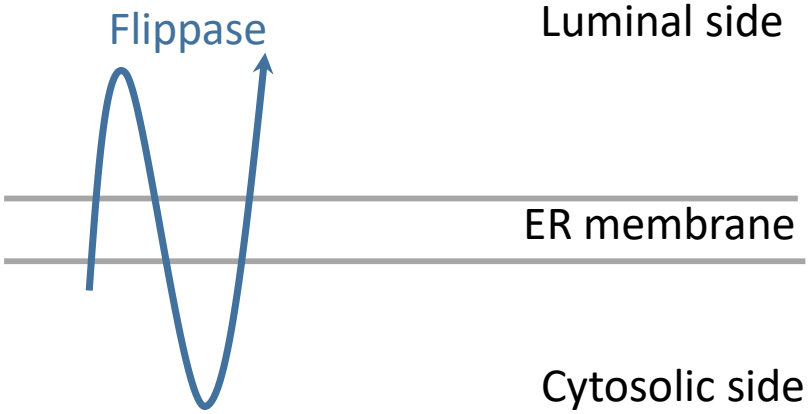
Post-translational modifications of CFTR: N-glycosylation

ER: cytosolic side



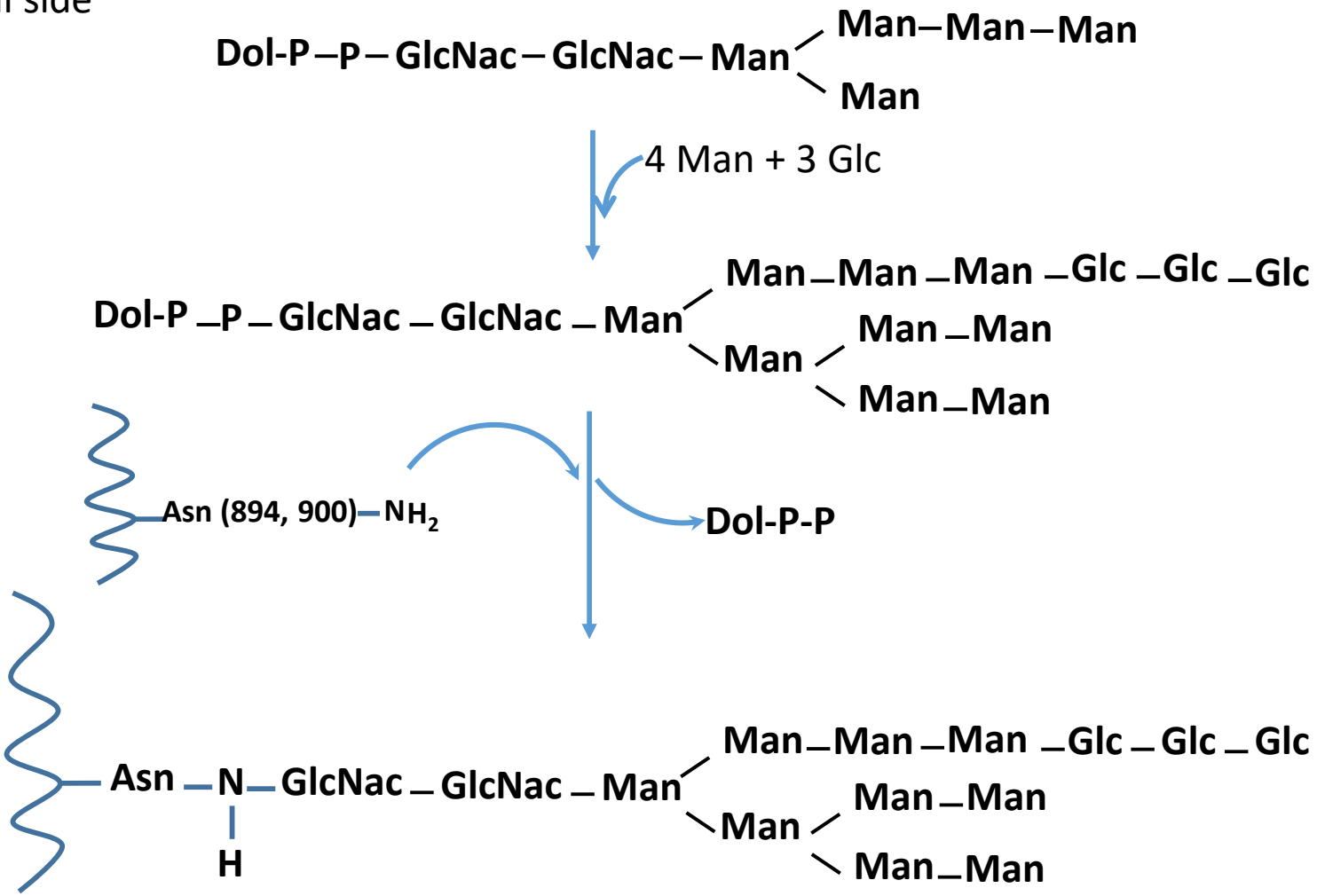
The synthesis of the common ologosaccaridic portion shared by all the N-glycoproteins occur in the ER and is in parallel with the protein biosynthesis.





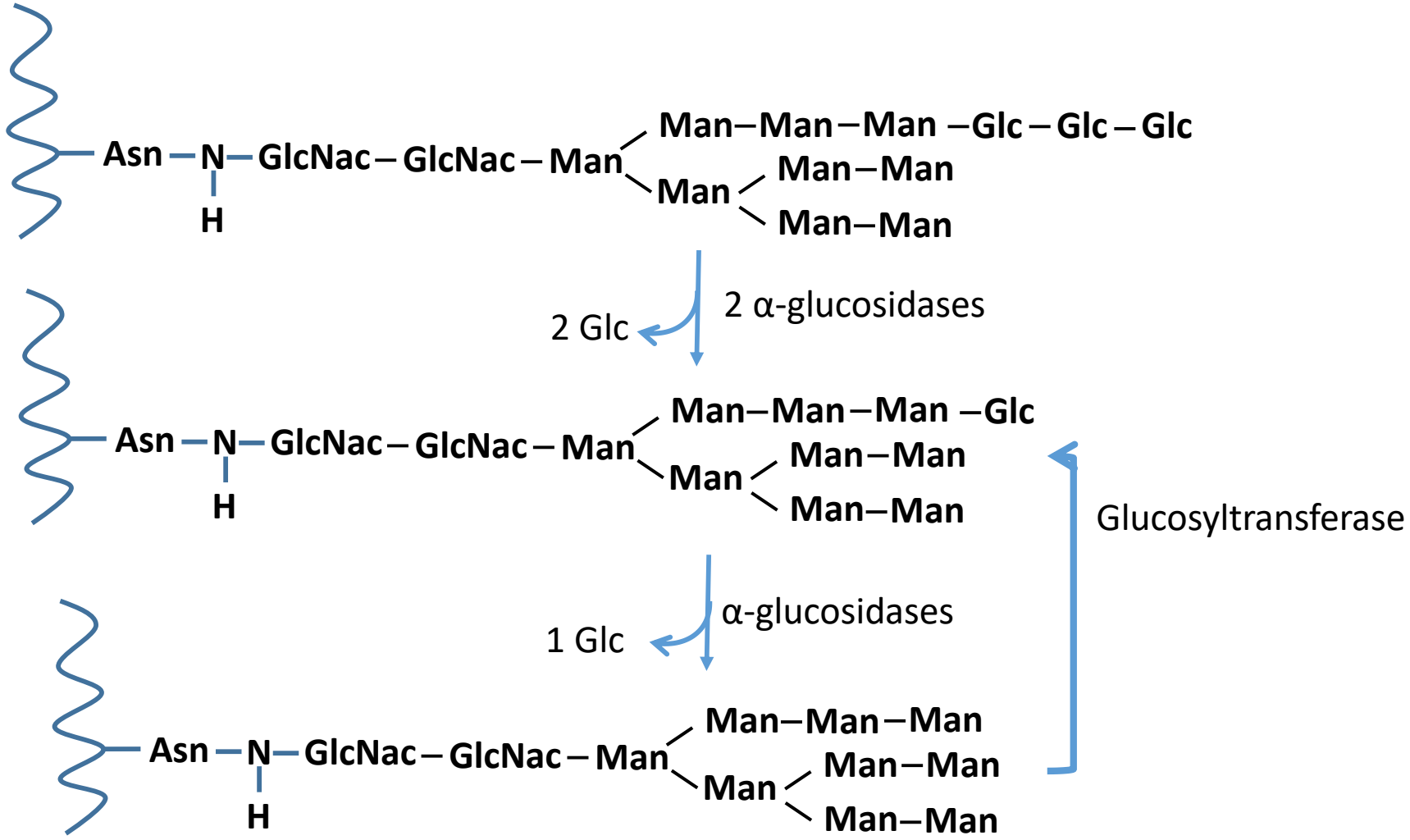
Post-translational modifications of CFTR: N-glycosylation

ER: luminal side

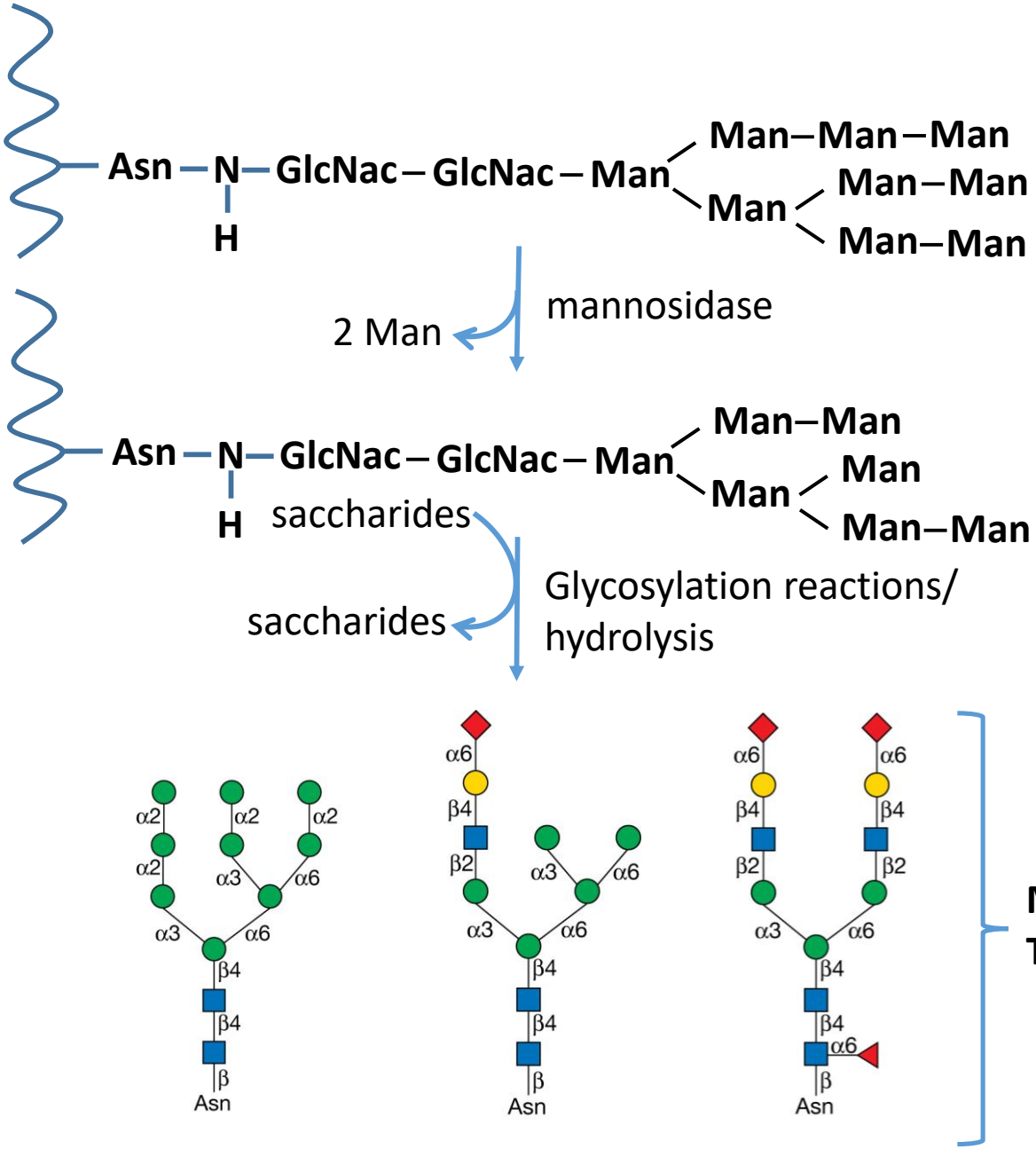


Post-translational modifications of CFTR: N-glycosylation

ER: luminal side

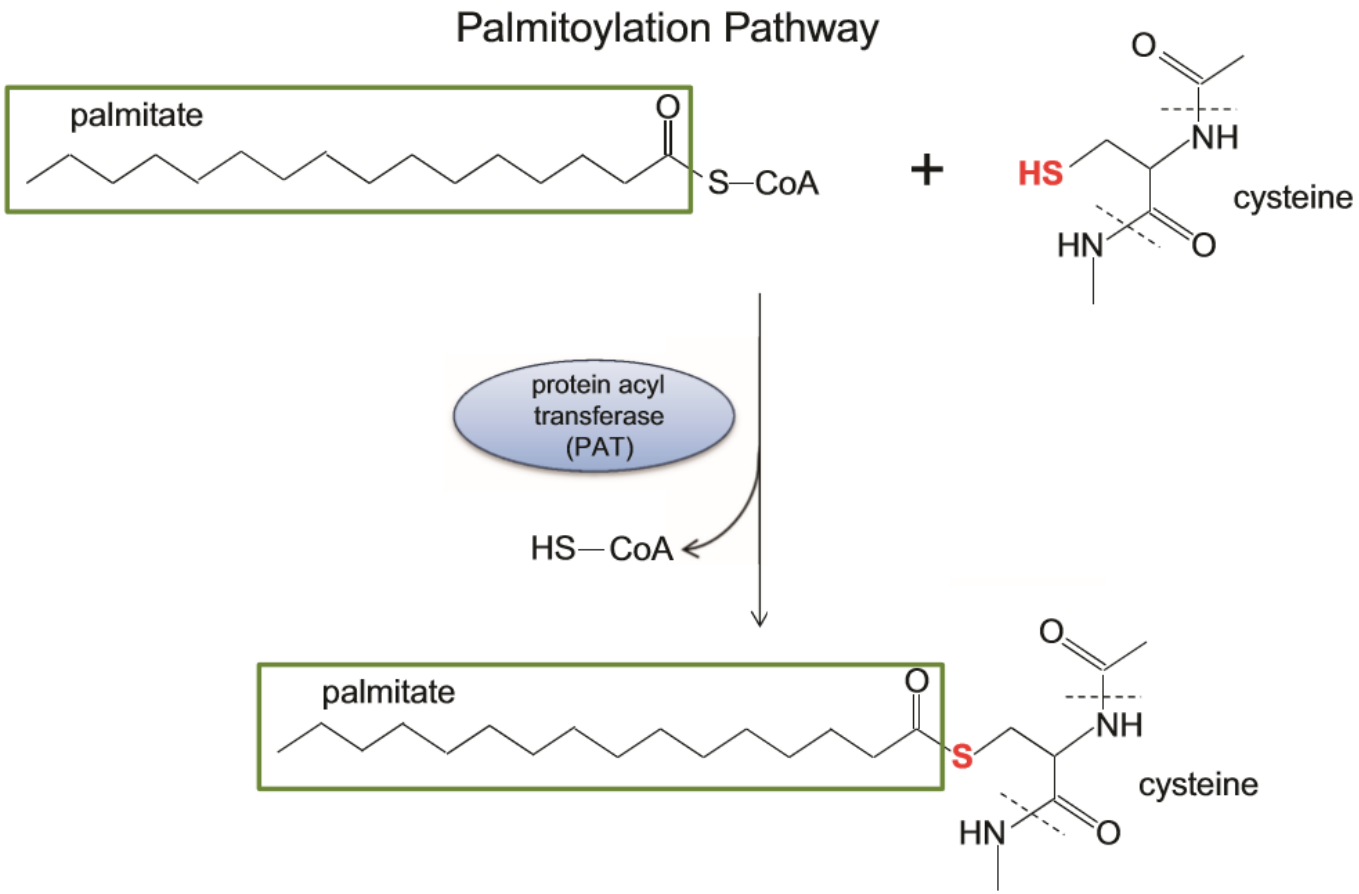


Glycoprotein N-GlcNAc: Biosynthesis



Post-translational modifications of CFTR: Palmitoylation

Palmitoylation: is characterized by the thioester linkage of a 16-carbon saturated fatty acid to cysteine residues (for CFTR in position 524 and 1395).



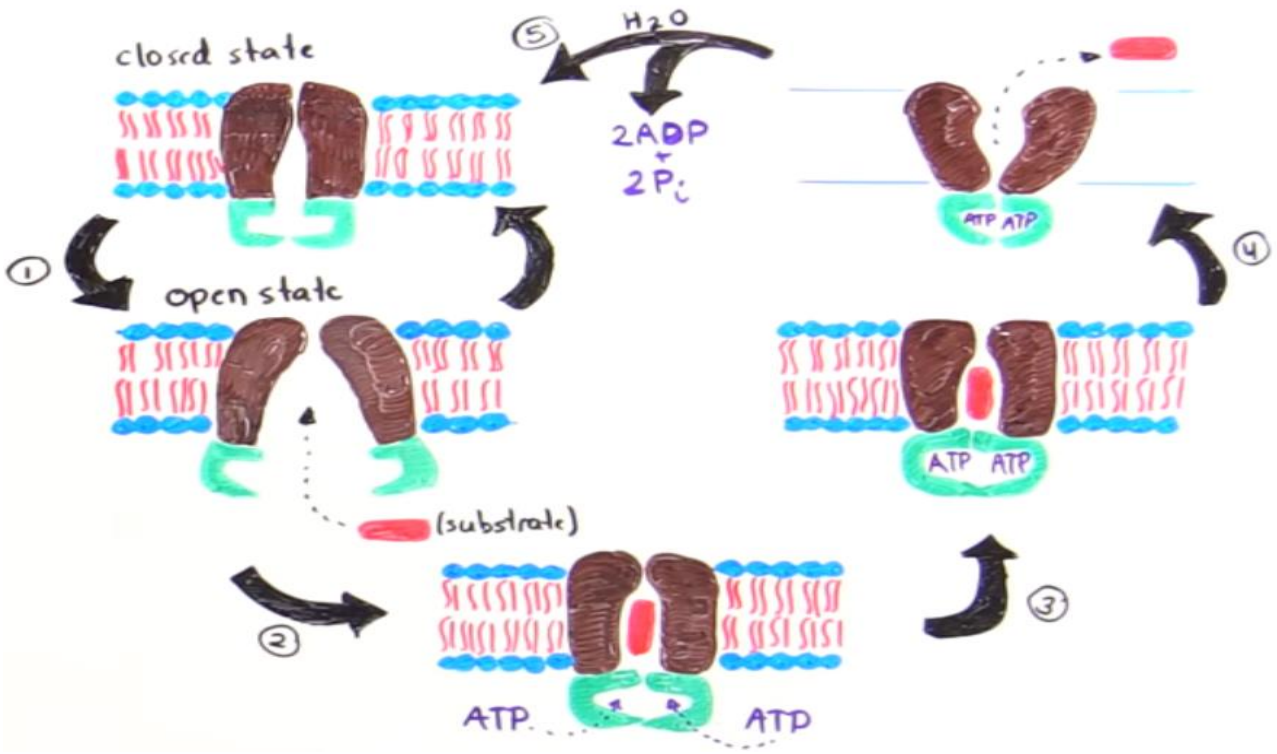
Post-translational modifications of CFTR: Phosphorylation

Table 1. *Effects of kinases shown to phosphorylate CFTR*

Kinase/Target Residue(s)	CFTR Domain	Suggested Consequence of Phosphorylation	References
PKA			
S422	RI	Unknown	44
S660	RE	Channel activation; disrupts the NBD1:NBD2 interface	17, 45, 176
S670	RE	Channel activation; disrupts the NBD1:NBD2 interface	45
S700	RD	Channel activation	68
S737	RD	Channel activation or inhibition	17, 43, 68, 178, 190
S768	RD	Channel inhibition	17, 43, 68, 178, 190
S795	RD	Channel activation	17, 190
S813	RD	Channel activation	17, 68, 89, 190
AMPK			
S737	RD	Channel inhibition; blocks activation from PKA phosphorylation	87, 185
S768	RD	Channel inhibition; blocks activation from PKA phosphorylation	87, 185
S813	RD	Channel inhibition	89
PKC			
S686	RD	Enhances PKA-dependent binding of RD to other CFTR domains	34, 78, 81
S790	RD	Enhances PKA-dependent binding of RD to other CFTR domains	34, 78, 81
CK2			
S422	RI	Stabilizes trafficking and thus activation	109, 173
S511	NBD1	Channel activation; may interact with SYK phosphorylation at Y512	109, 173
T1471	COOH terminal	Decreases chloride conductance by decreasing trafficking	109, 173
SYK			
Y512	NBD1	Reduces expression at the cell surface; regulates phosphorylation by CK2	109, 121
PI3K			
Unknown	—	Promotes trafficking to the cell surface	176
WNK4			
Y512	NBD1	Inhibits SYK phosphorylation; promotes expression at the cell surface	121
CaMKI			
Unknown	—	Unknown	144
p60c-src			
Unknown	—	Regulates channel open probability	49
PKGII			
Unknown	—	Channel activation	144, 177
LMTK2			
S737	RD	Facilitates endocytosis of CFTR from the cell surface	108

CFTR, cystic fibrosis transmembrane conductance regulator; NBD1, nucleotide binding domain; RD, regulatory domain; RI, regulatory insertion.

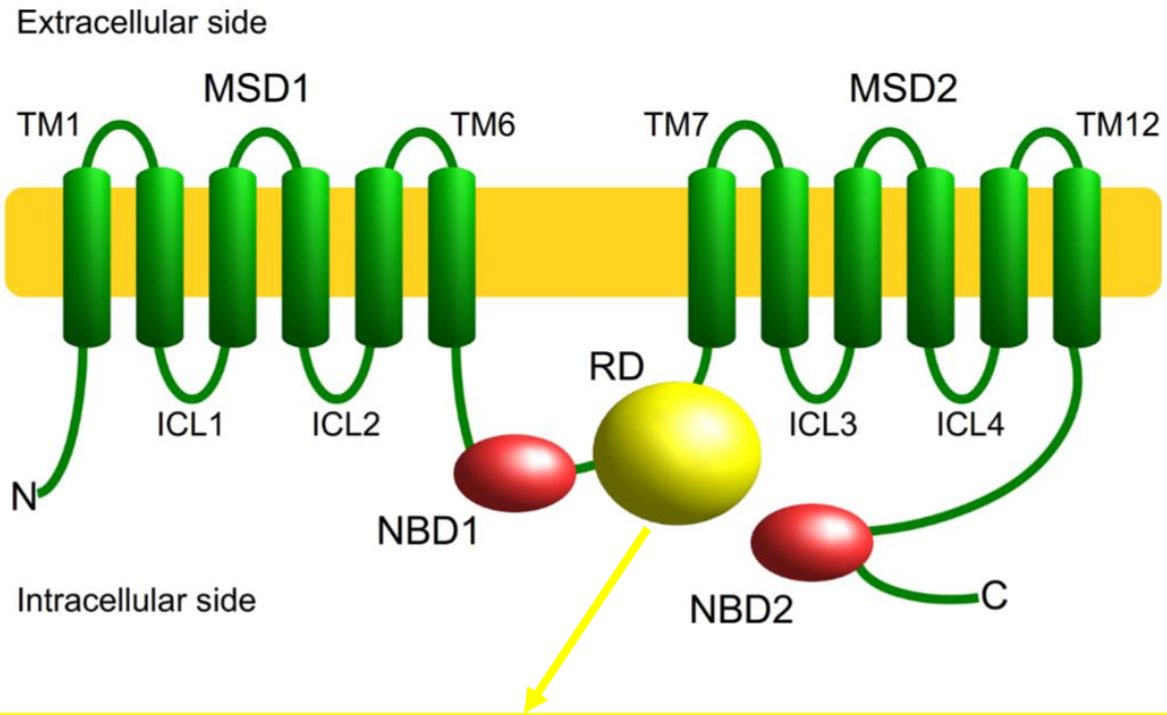
Overview on ABC transporters



The canonical ABC proteins are active pumps, which transport against the concentration gradient of the substrate, there is an important outsider deviating from this type of function called CFTR

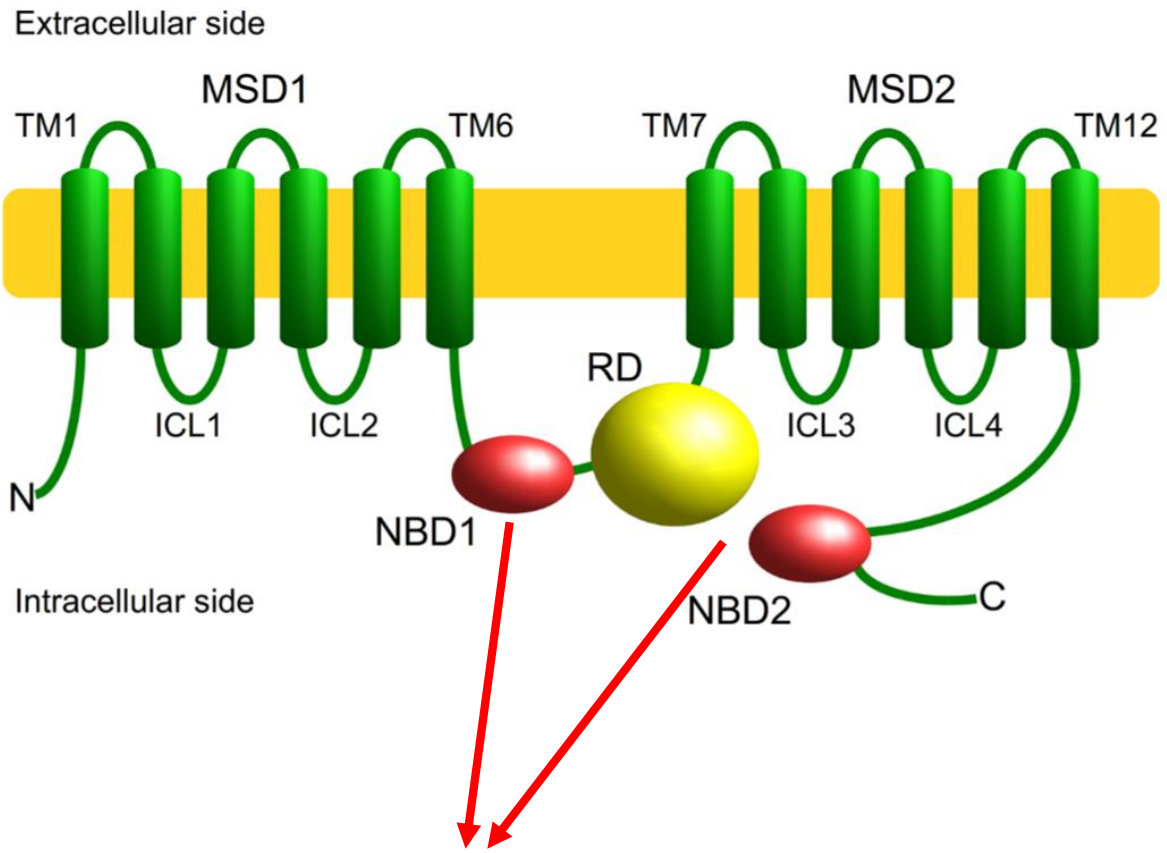
CFTR Structure of R domain

- **CFTR is a channel that allow the ion passage depending on their electrochemical gradient of concentration: ATP hydrolysis is not used to transport chloride**



With respect to the other ABC transporter the unique feature of CFTR is the presence of a lasso and intrinsically disordered portion called regulatory domain linking the two halves of the protein. The Hyper-phosphorylation of RD by PKA and PKC is fundamental for CFTR activation

CFTR Structure of NBDs



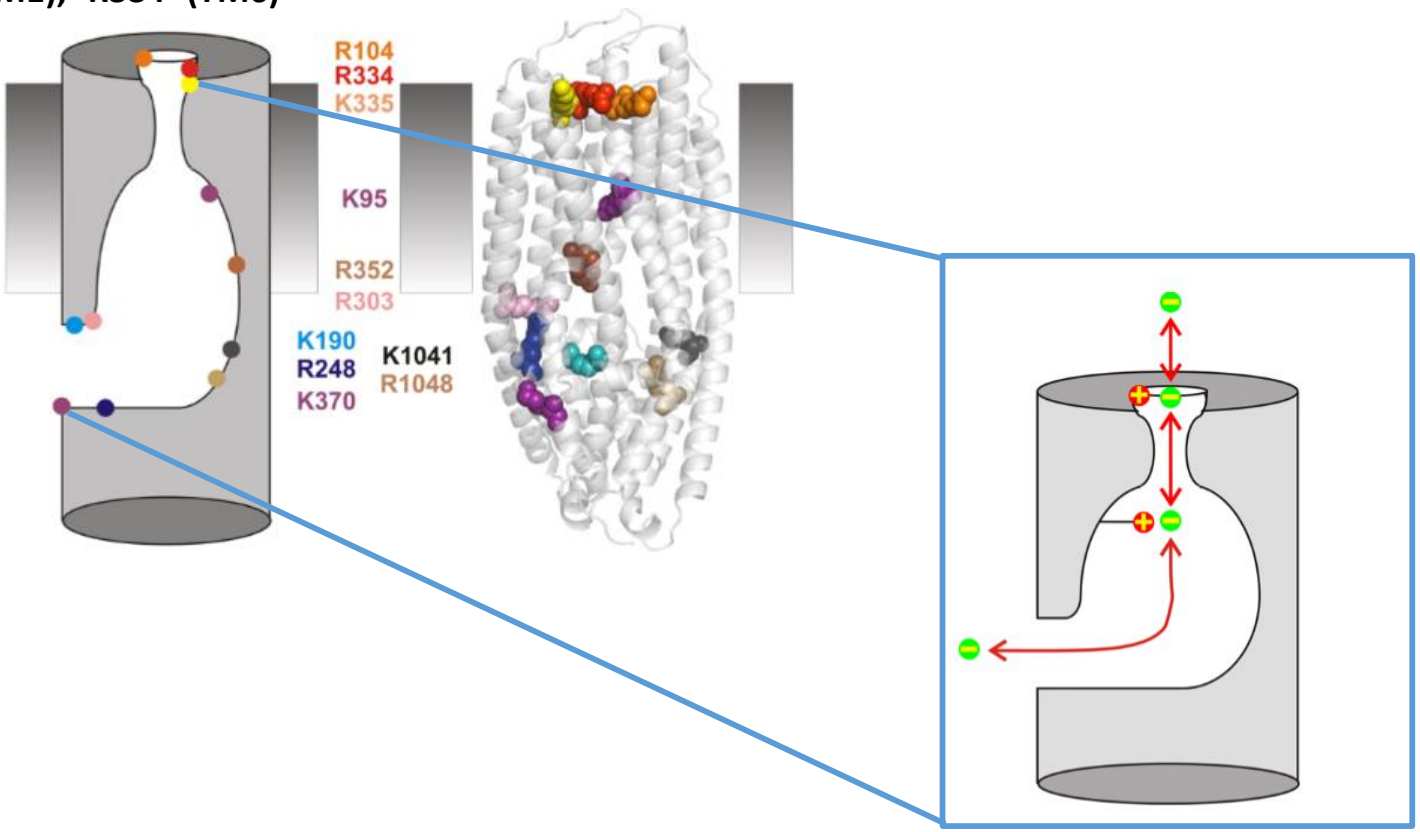
The two domains appear to act independently in the binding and hydrolysis of ATP. NBD1 is a stable ATP-binding site at which hydrolisis occur very slowly (degenerated site). In contrast, at the NBD2 ATP is hydrolysed as rapidly as it is bound and the nucleotide diphosphate hydrolysis product dissociates immediately.

CFTR Structure of the pore

Structure of the pore: a key role is played by positive charged aminoacids found in the outer vestibule, inner vestibule.

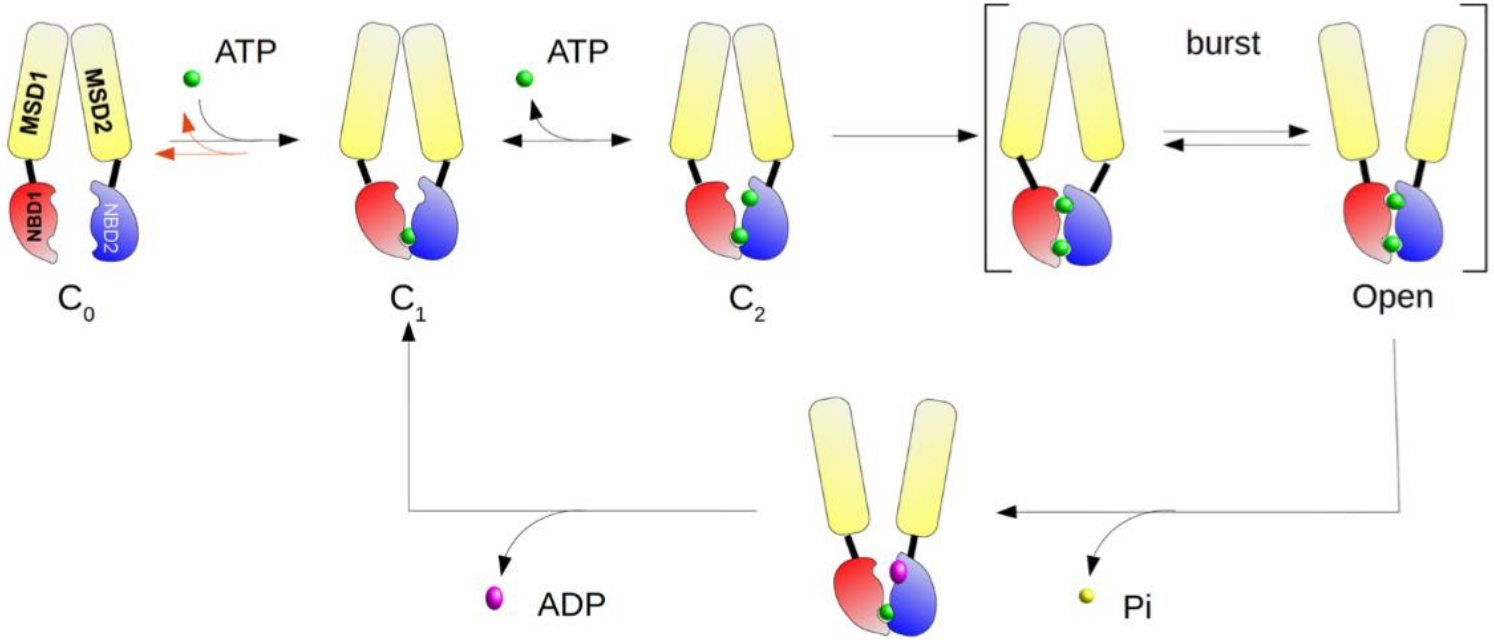
At the outer mouth of the pore, Cl- ions are likely attracted by positively charged amino acid side-chains R104 (TM1), R334 (TM6) and K335 (TM6)

Positively charged side-chains in the intracellular vestibule electrostatically attract cytoplasmic Cl- ions to the internal entrance to the pore



CFTR mechanism of action

First the activation of CFTR by the protein kinase A (PKA)- dependent phosphorylation of multiple sites located at the regulatory domain (RD). The phosphorylation of R domain increase the affinity of NBD1 and NBD2 for ATP. Then binding of ATP promotes the ''dimerization'' of the Nucleotide binding domains, leading to a conformational change at the level of the Multi spanning domains, that in turn leads to channel opening. The hydrolysis of ATP by the enzymatic activity of the Nucleotide binding domains determine channel closure with the release of ADP. In addition, the phosphorylation of R domain seems to be essential to amplify the conformational changes induced by the ATP binding to the MSDs giving a real stroke for the pore opening. Interestingly, as I mentioned before ATP hydrolyses in NBD1 is very slow so the channel could be rapidly activated by the only binding of ATP in NBD2.



CFTR-dependent diseases

CFTR Hyperactivation: Secretory Diarrhea

When the gut lumen is exposed to certain types of stimuli (e.g., toxins secreted by the colonizing pathogenic microorganisms Escherichia coli, Vibrio cholera), the intracellular second messengers cAMP and/or cGMP are excessively produced, causing the hyperactivation of CFTR channel.

This hyperactivation increases the electrical and osmotic driving forces for the parallel flows of Na+ and water and inhibits the fluid absorption processes mediated by Na+/H+ exchangers and epithelial sodium channel.

The net result is the excessive fluid secretion into the intestine lumen, which overwhelms the reabsorbing capacity of the colon and leads to fluid loss and dehydration.

CFTR-dependent diseases

CFTR loss of function: Cystic Fibrosis

- **The most common life-limiting autosomal recessive disease among Caucasians, affecting approximately 1 every 2,500-4,000 newborns**
- **More than 2,000 mutations are found associated with CFTR gene so far and among these 200 have been proved to be disease-causing. 70% of patients carry the mutation F508del. On the basis of the effect on CFTR, the mutations are divided into 6 major classes**

-Class I: mutations preventing the production of a full-length CFTR protein

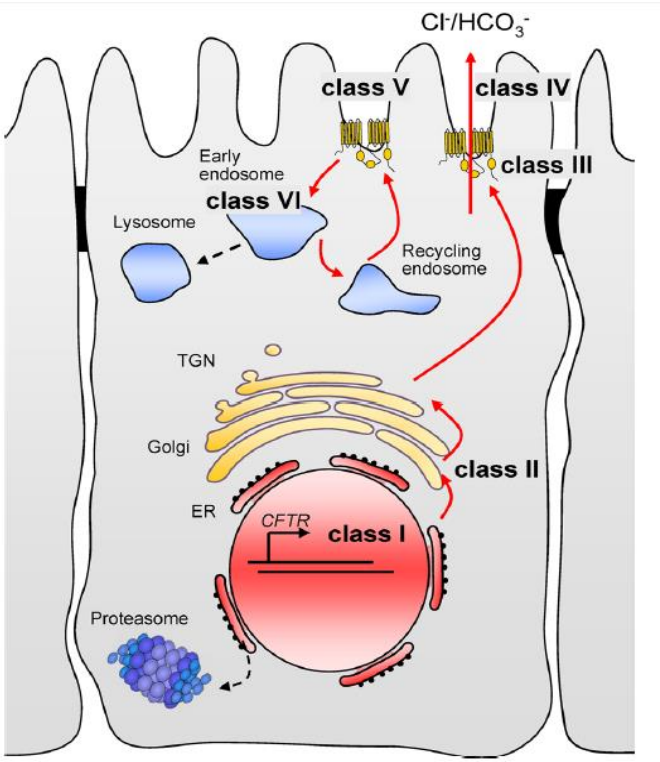
-Class II: mutations altering the cellular processing of the protein

-Class III: mutations disturbing the regulation of the Cl^- channel

-Class IV: mutations altering the conduction of the Cl^- channel

-Class V: mutations reducing the amount of functional CFTR protein

-Class VI: mutations destabilize the channel in post-ER compartments and/or at the PM



Clinical Feature of cystic fibrosis

The faulty secretion of chloride from epithelial cells alter the electrolytes equilibrium, reduced recall of water and hydration of the mucins present in the lumen of different organs such as lungs, pancreas, gut and testis.

The final results is the production of hyperviscous mucus that alters the organs functionality.

Indeed, even if the pulmonary manifestations are the most severe, CF is considered a multi systemic disease.

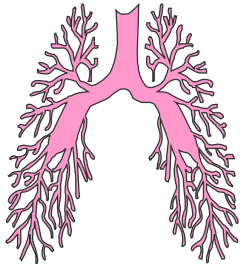
CF lung disease: the main leading cause of death for CF patients

Is caused by the pulmonary hyper-inflammation as a consequence of the formation of a very viscous mucus that does not allow to eliminate bacteria from the lung.

Persistent high-intensity inflammation leads to structural damage of the airways and impaired lung function that may result in respiratory failure and death.

Cystic Fibrosis: therapy

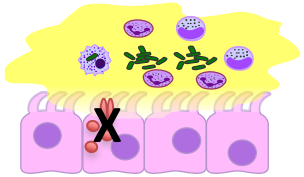
Bronchiestasis & lung insufficiency



Respiratory physiotherapy

Infection & inflammation

Persistent inflammation

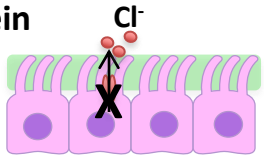


Recurrent infections

CFTR $^{-/-}$

Mucolytics
Antibiotics
Anti-inflammatories

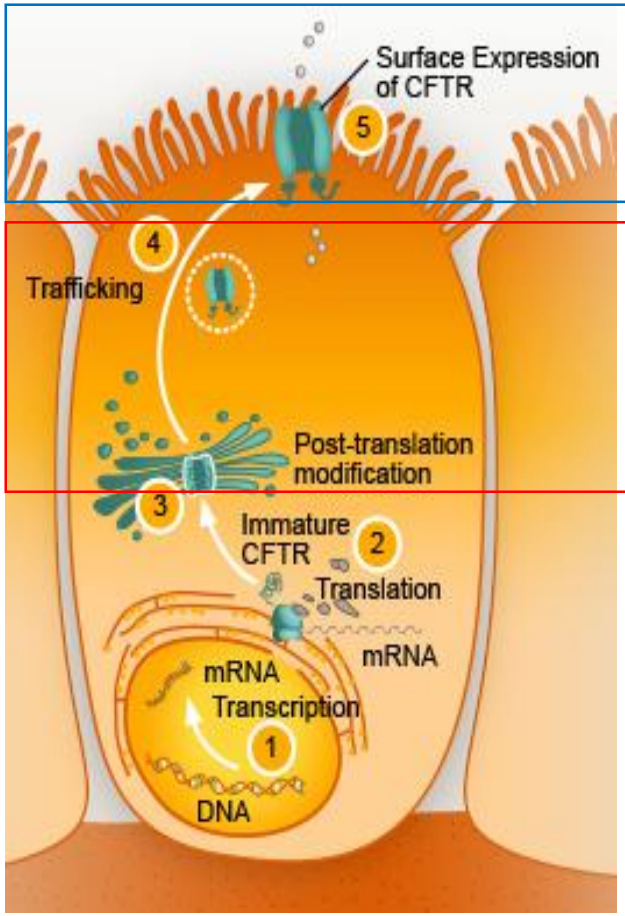
Defective protein



CFTR $^{-/-}$

CFTR-modulators

CFTR-modulators: mechanism of action and outcomes



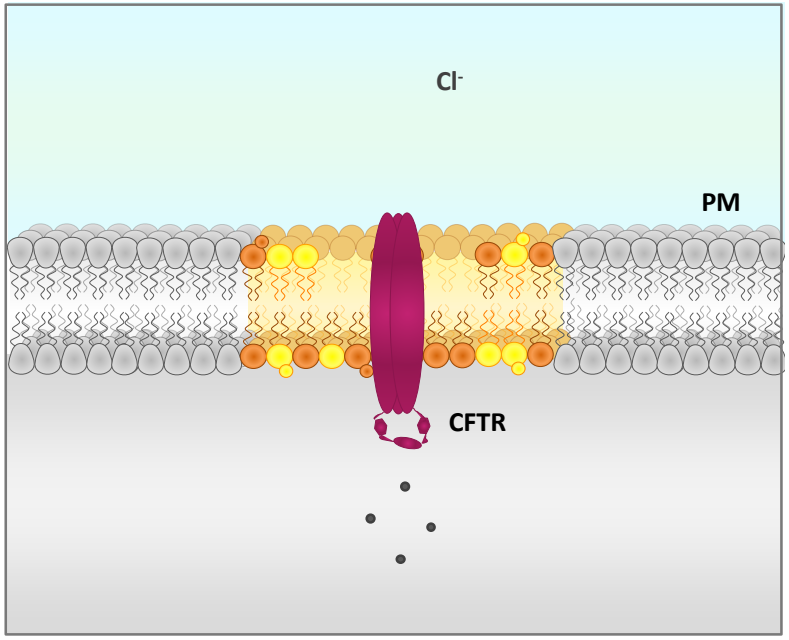
Potentiators
Increase the gating of CFTR, resulting in higher ion flow

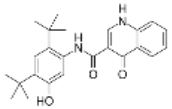
Correctors
Improve the delivery of CFTR to cell plasma membrane

The correctors such as lumacaftor can correct mutated CFTR structure and the potentiators like Ivacaftor improve the channel activity directly at PM. Unfortunately, even if these small molecules work very well for the patients who carry the mutation G551D, for the most common CF-causing mutation their efficacy seems to be time-limited.

CFTR-modulators: mechanism of action on different mutations

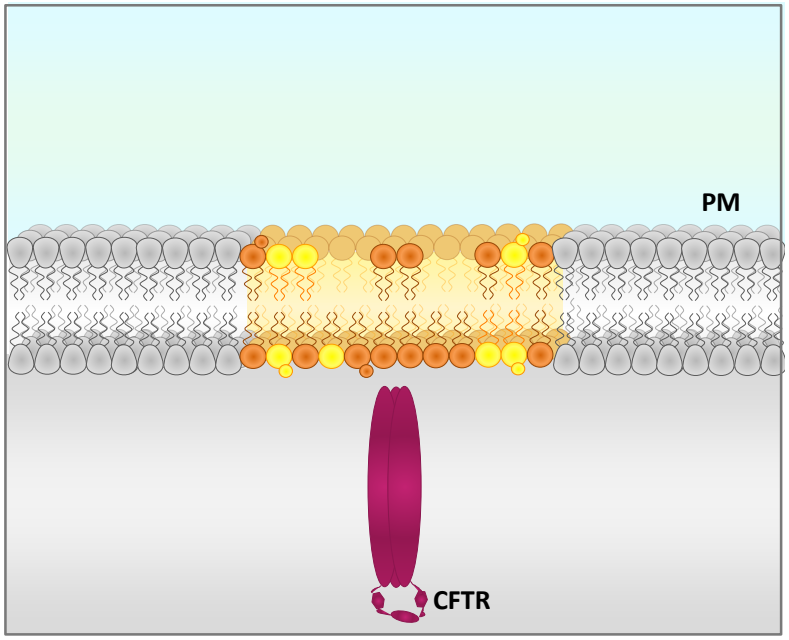
Gating mutation e.g. G551D

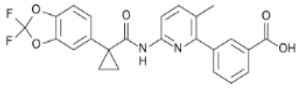




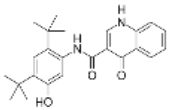
VX-770

Folding mutation e.g. F508del





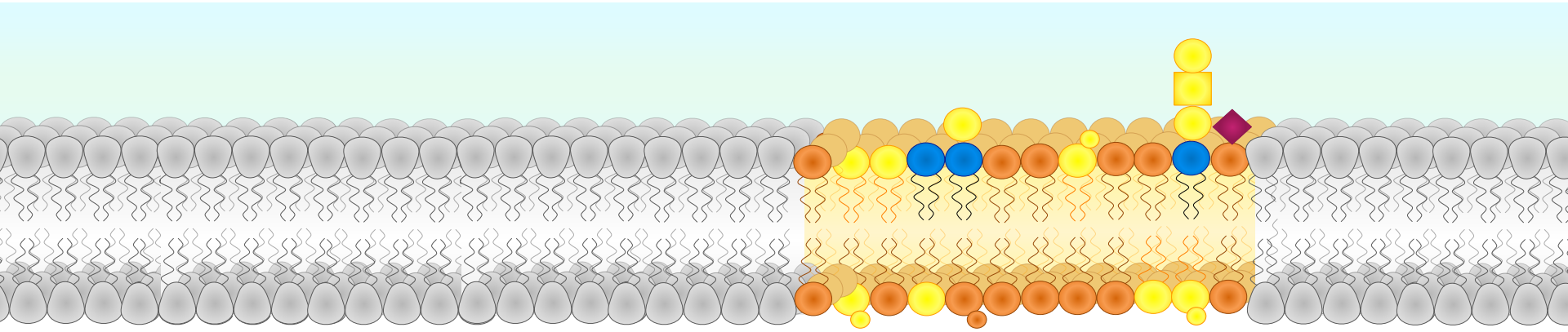
VX-809

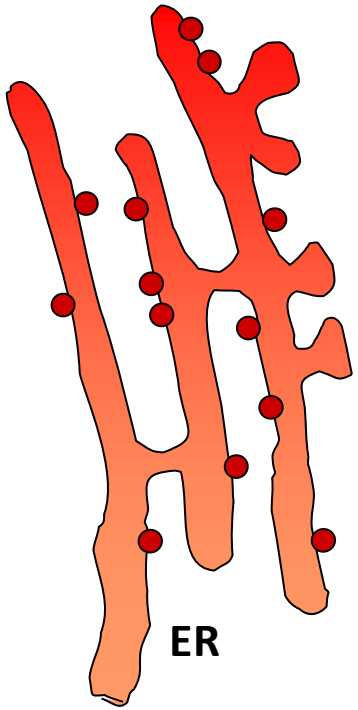


VX-770

In the case of gating mutations, such as G551D where CFTR is in the PM but does not exert its channel function, the therapy with the potentiator VX-770 restore the channel activity. Whereas when the mutations cause the lack of CFTR from the cell surface principally for problem of folding such as the case of F508del, the therapy with corrector VX-809 and potentiator (VX-770) promotes the trafficking of CFTR with the PM but CFTR is not stable and it is immediately internalized and degraded

Effect of the corrector VX-809

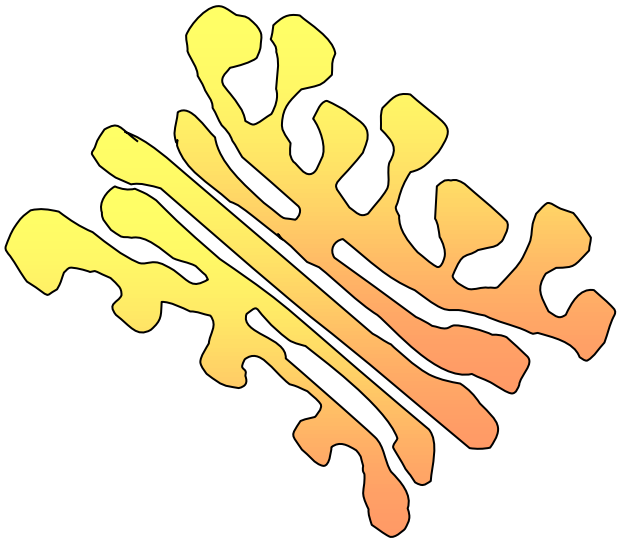




ER

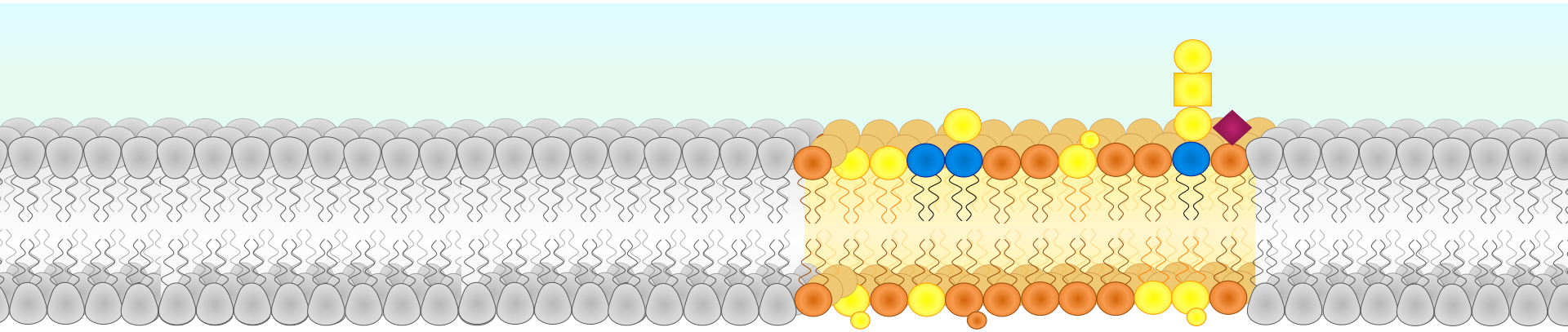
VX-809

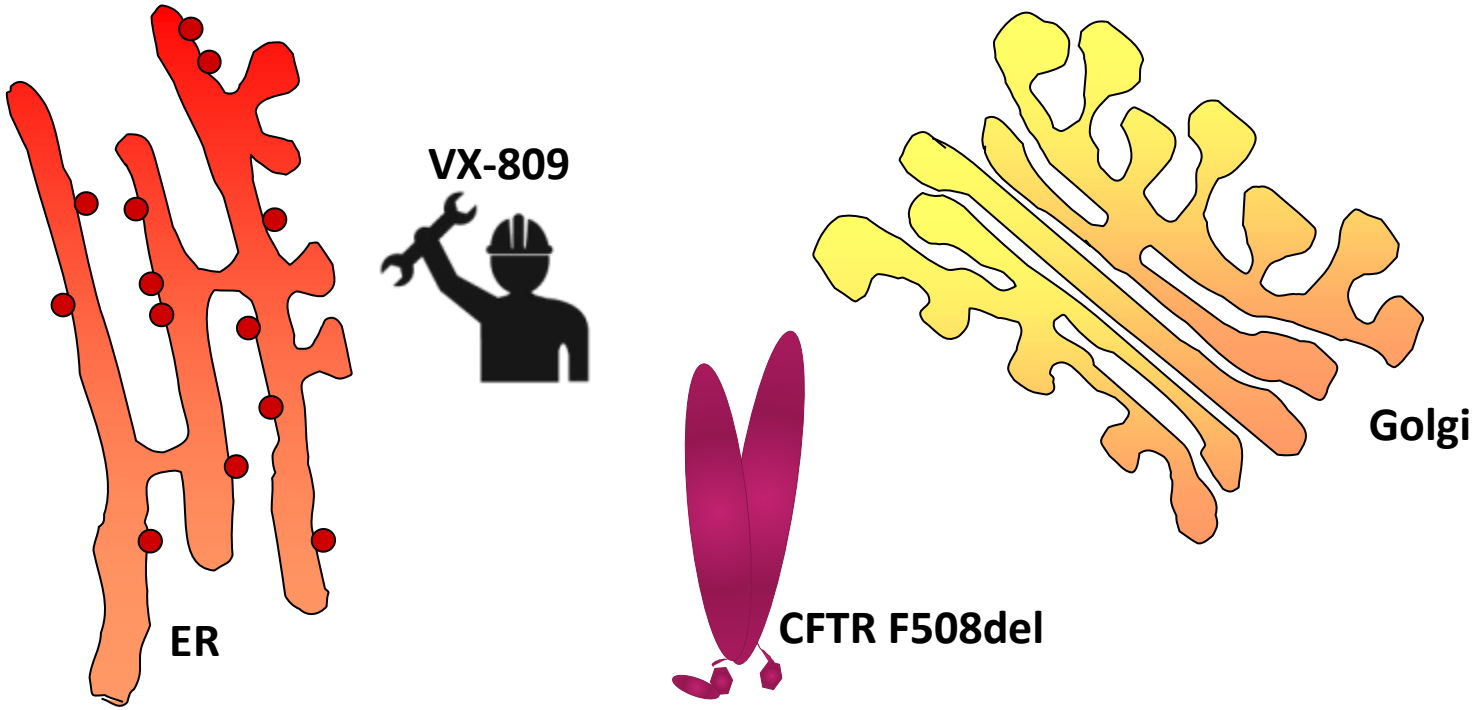
CFTR F508del



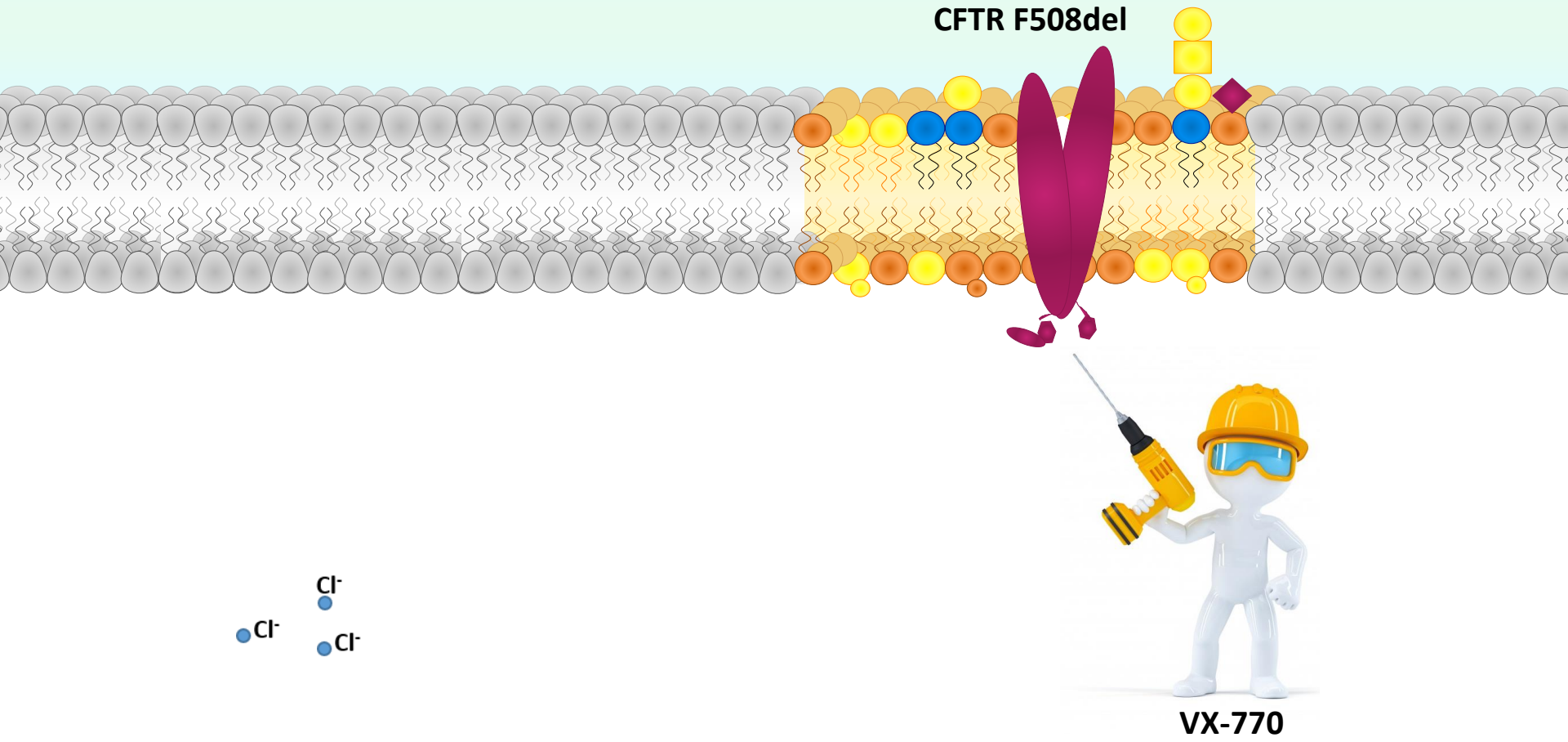
Golgi

Effect of the corrector VX-809

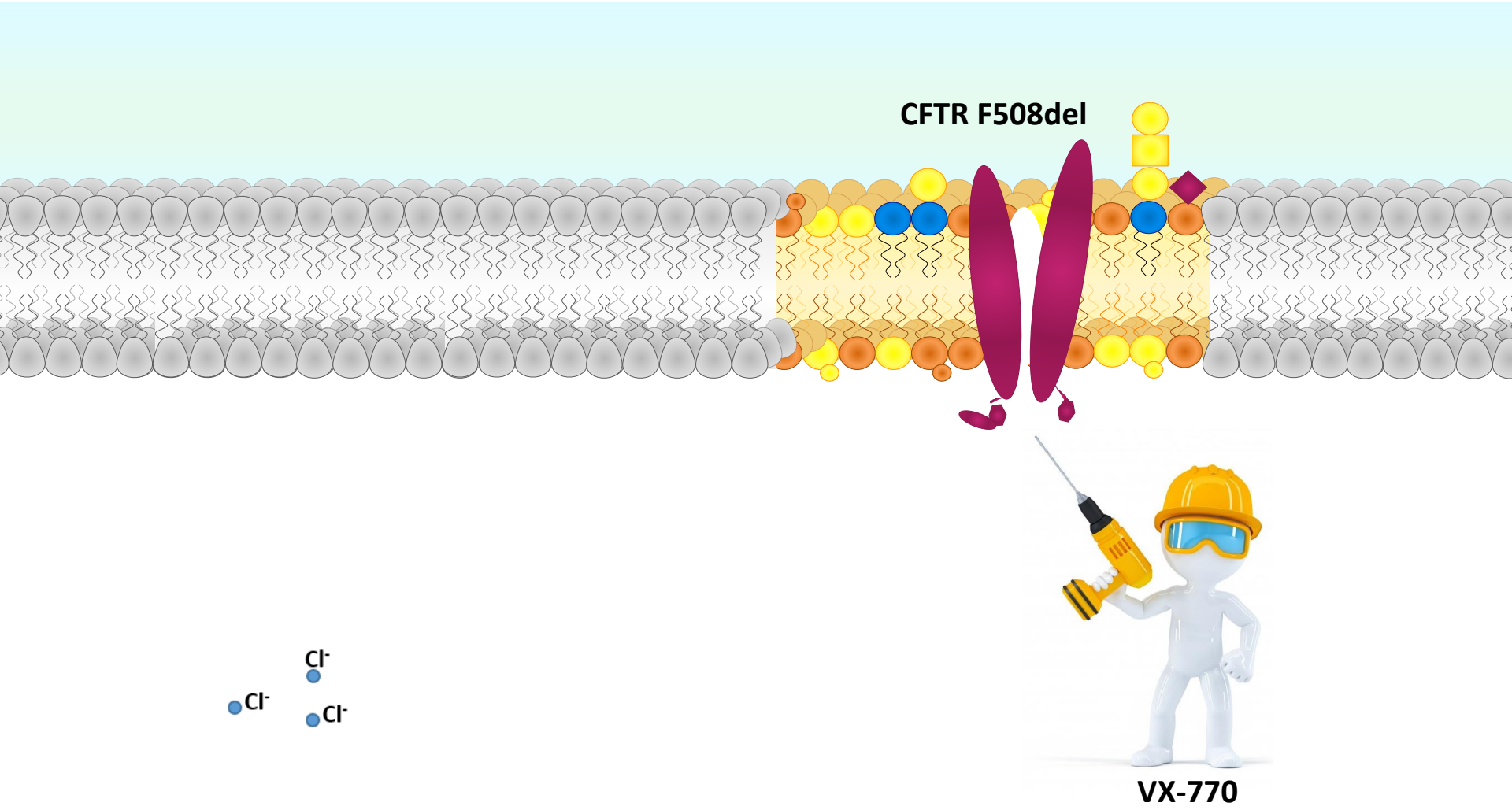




Effect of the potentiator VX-770

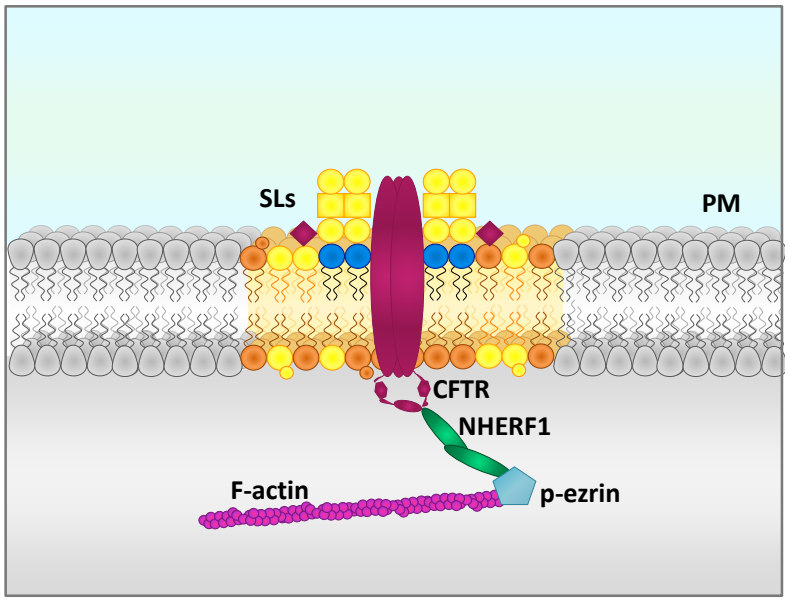


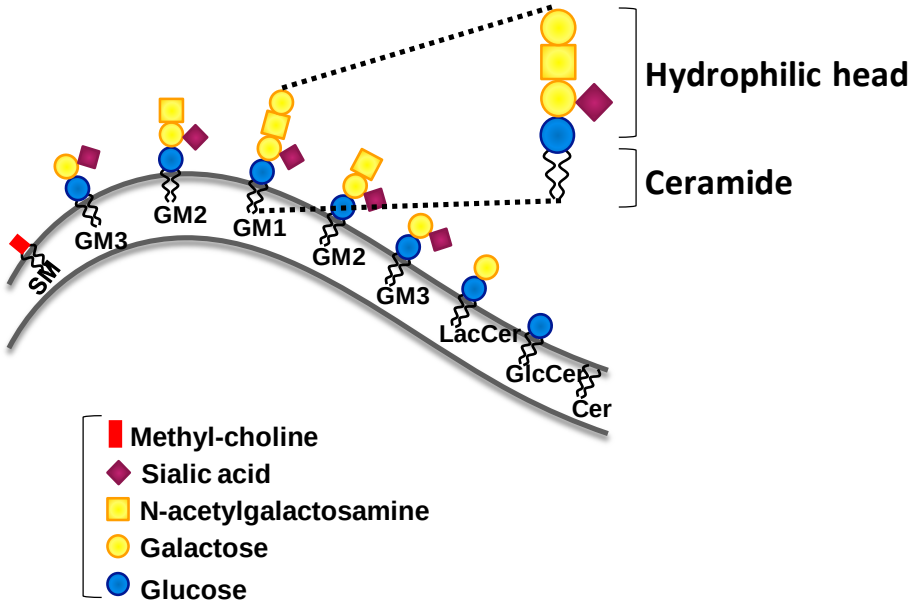
Effect of the potentiator VX-770



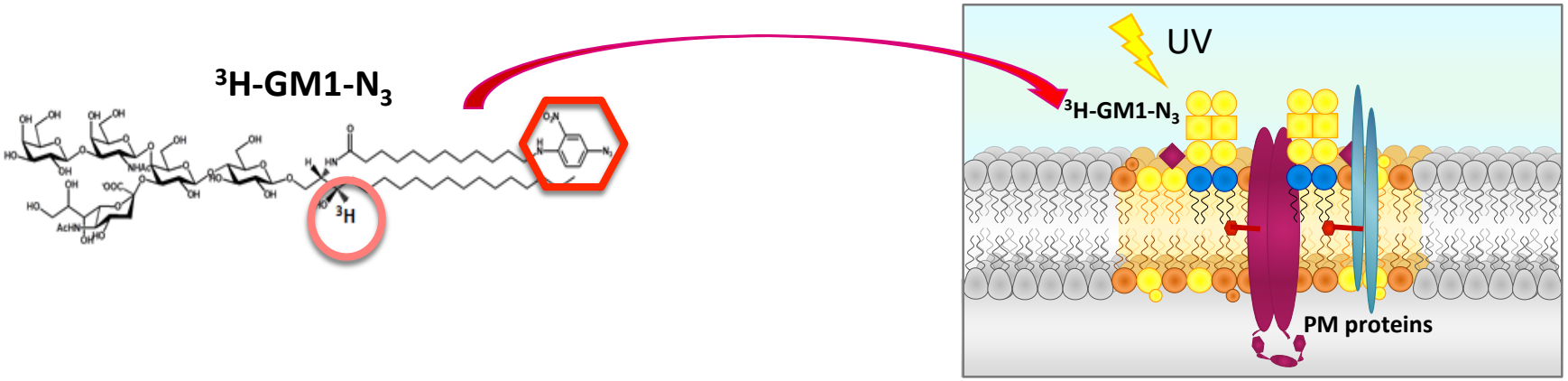
CFTR plasma membrane microenvironment

In bronchial epithelial cells, CFTR plasma membrane stability and function depend on the organization of a multi-protein complex involving F-actin, the scaffolding proteins NHERF1 and p-ezrin. This protein complex stabilizes CFTR in highly restricted membrane domains, known as **lipid rafts**, enriched in **sphingolipids**

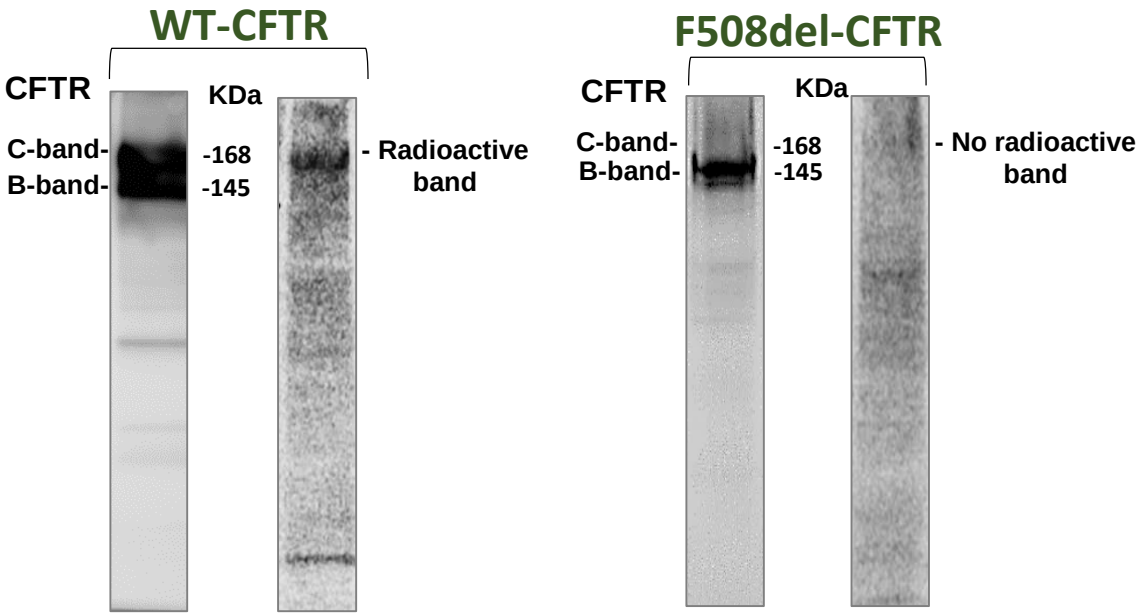




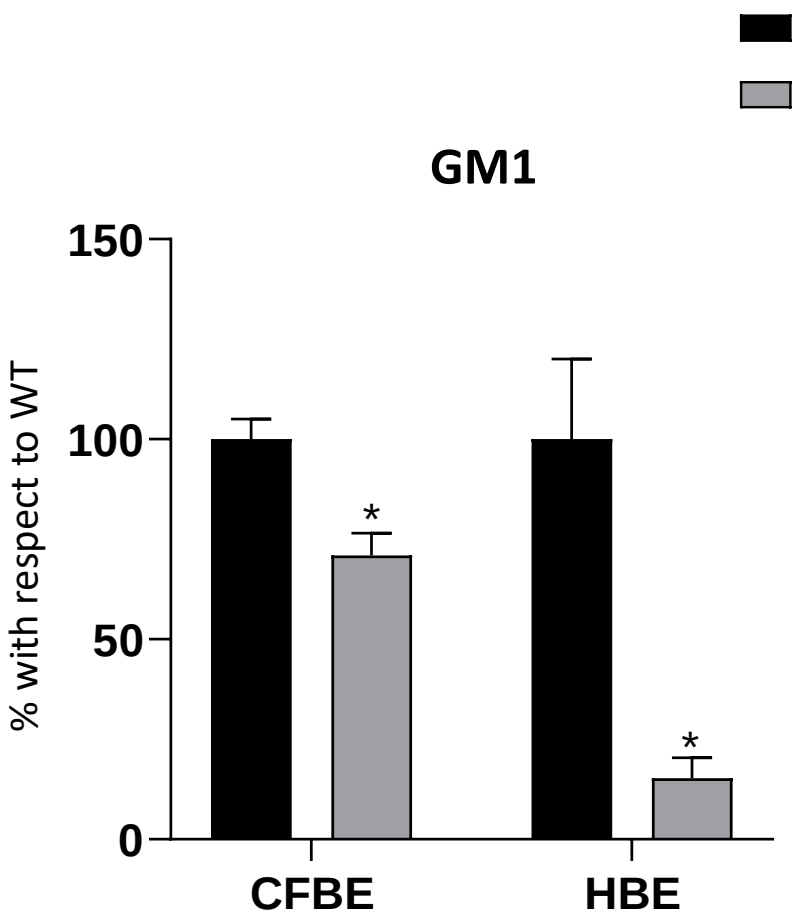
CFTR and GM1 localize in the same PM microenvironment

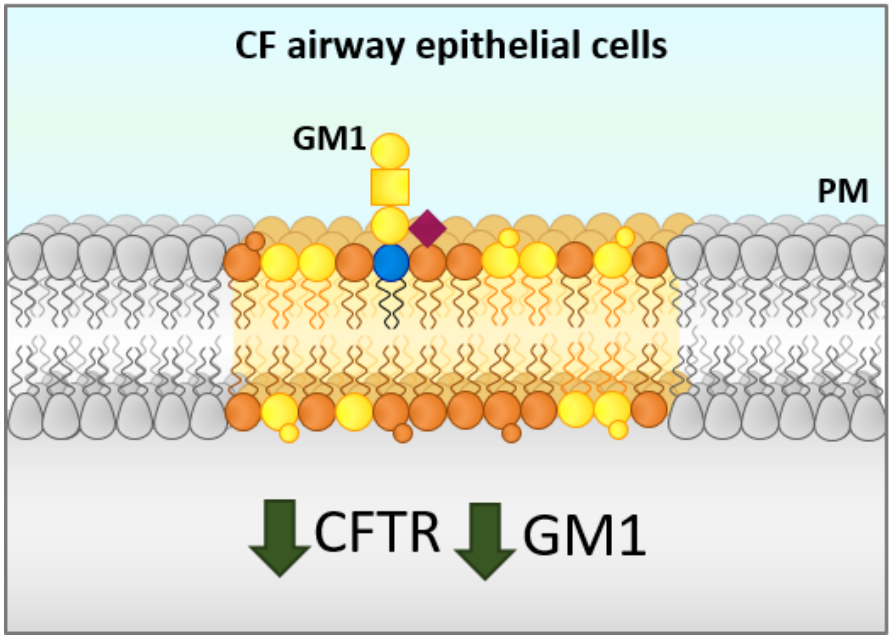


CFBE cells overexpressing



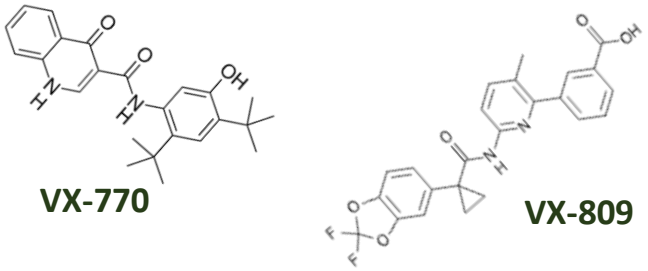
In airway cells the lack of CFTR is associated with a decrease of GM1

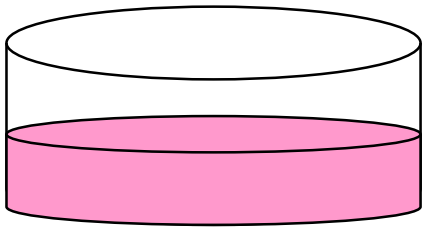




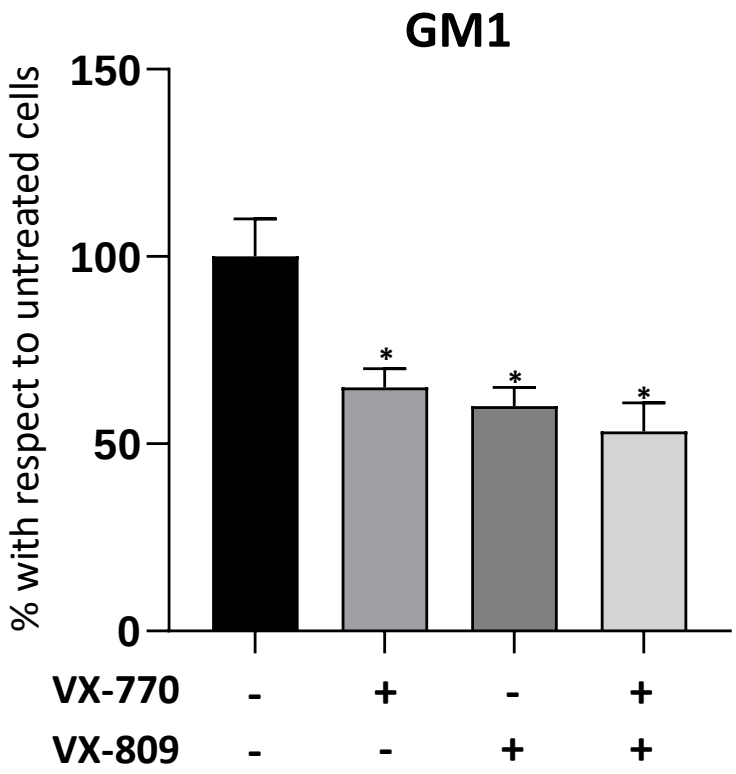
*p<0.01 vs WT

Effects of VX-770 and VX-809 on the GM1 levels



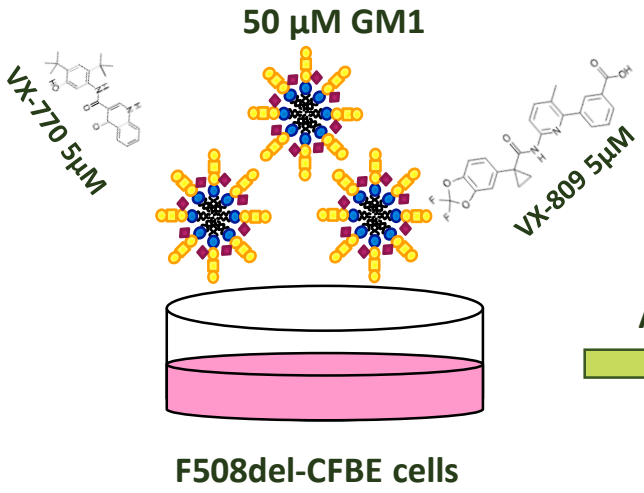


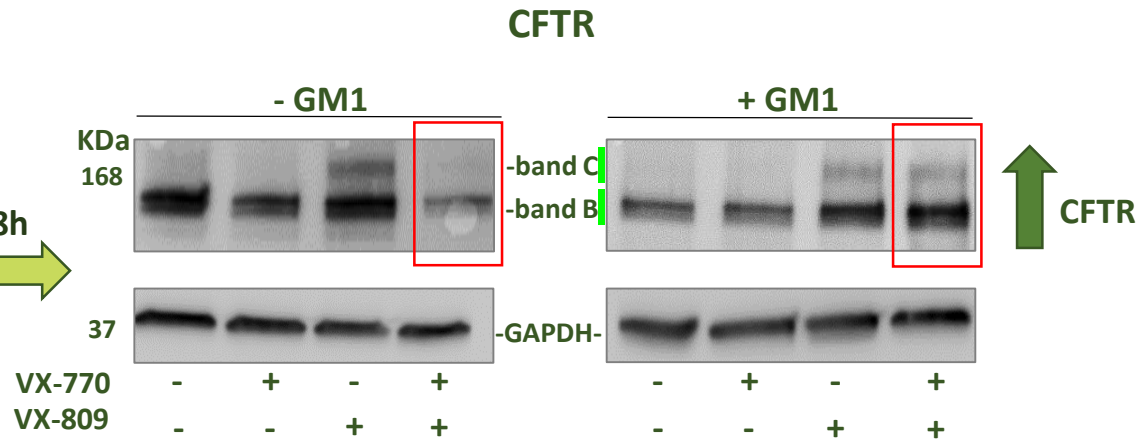
F508del-CFBE cells

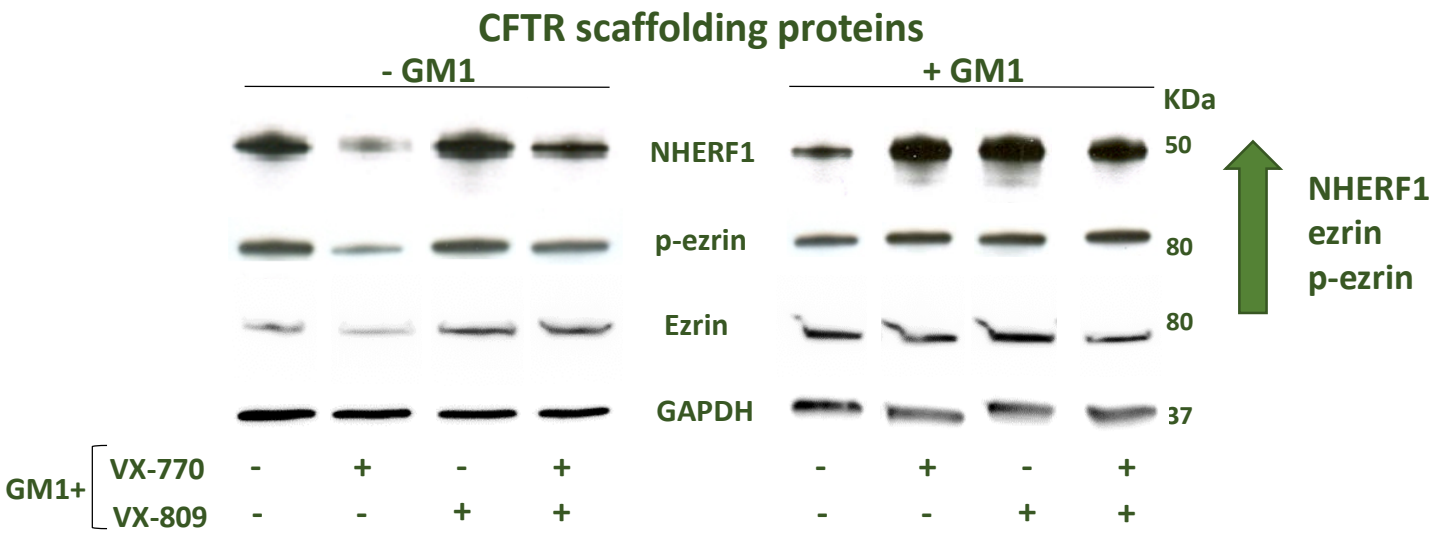


*p<0.01 vs CTRL

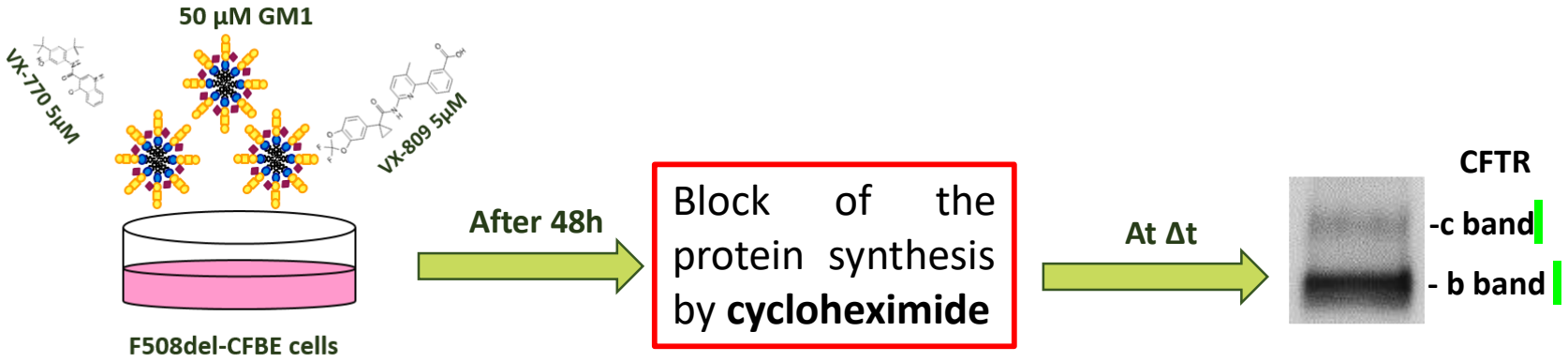
GM1 stabilizes F508del-CFTR rescued by VX-809

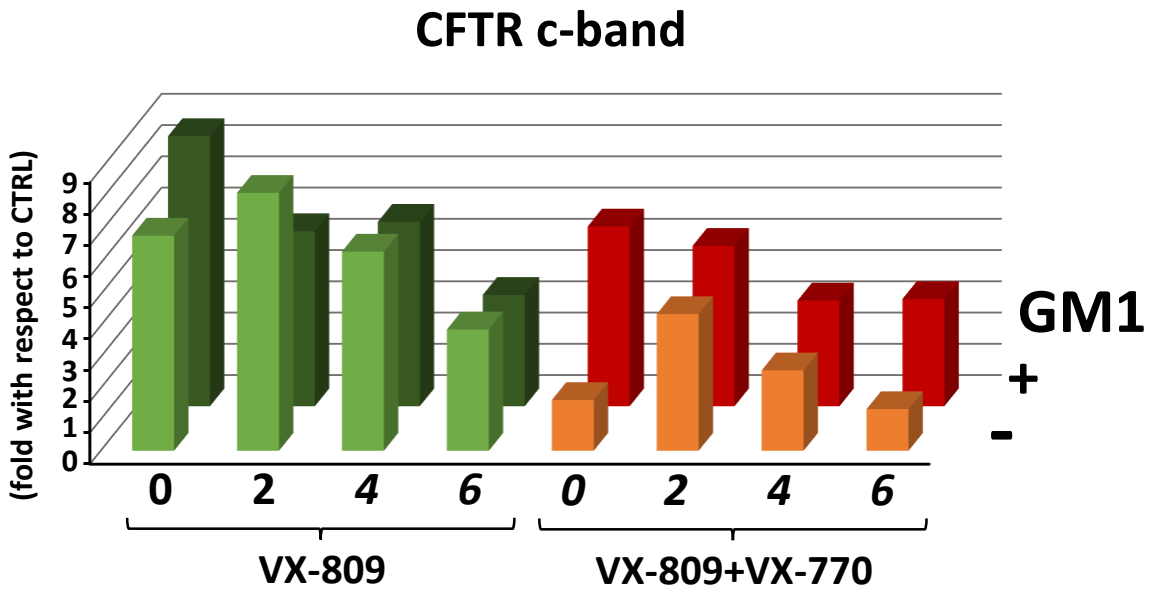




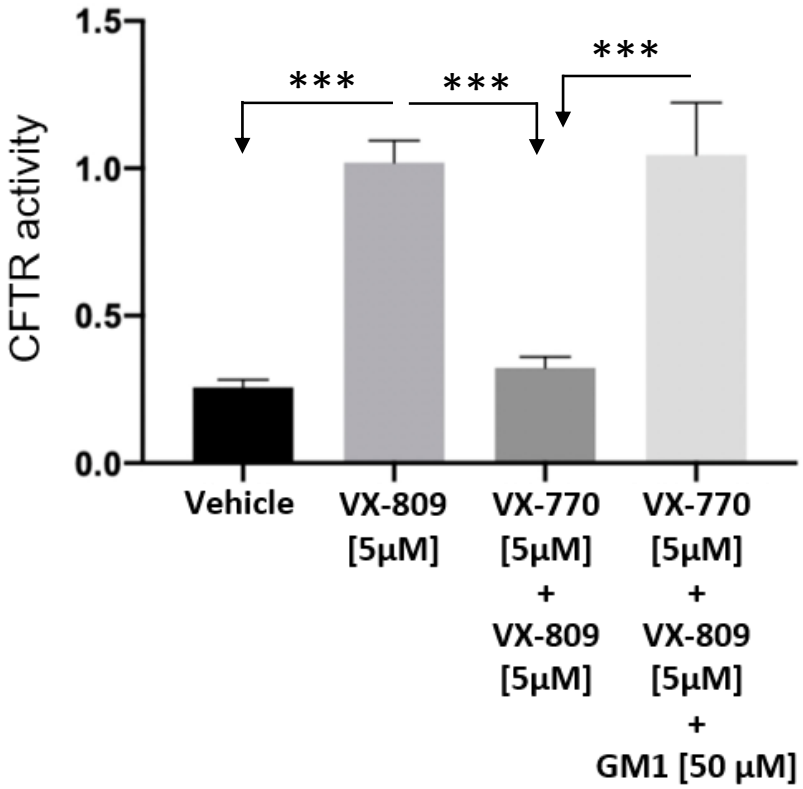


GM1 antagonizes the destabilizing effect of VX-770





CFTR functional assay: Cell-based screening assay of halide transport using CFBE-YFP F508del-CFTR cells



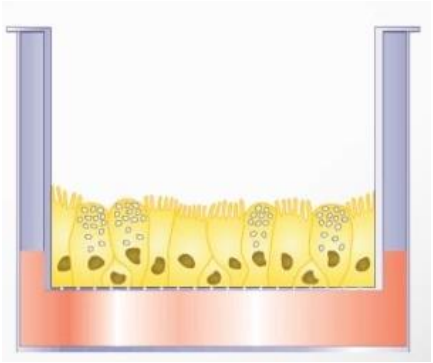
Green fluorescent protein-based halide indicators with improved chloride and iodide affinities

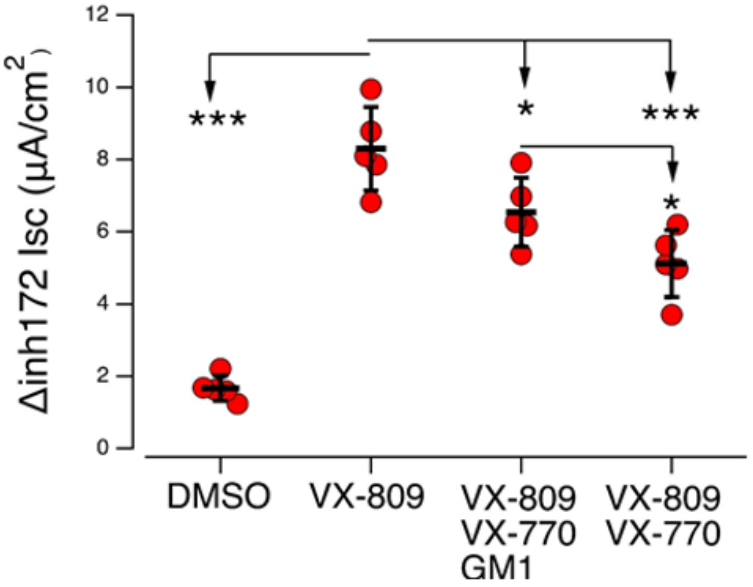
Luis J.V. Galietta[1], Peter M. Haggie[1], A.S. Verkman*

*** p<0.0001

GM1 increases the function of corrected and potentiated F508del CFTR in primary human bronchial cell differentiated at ALI

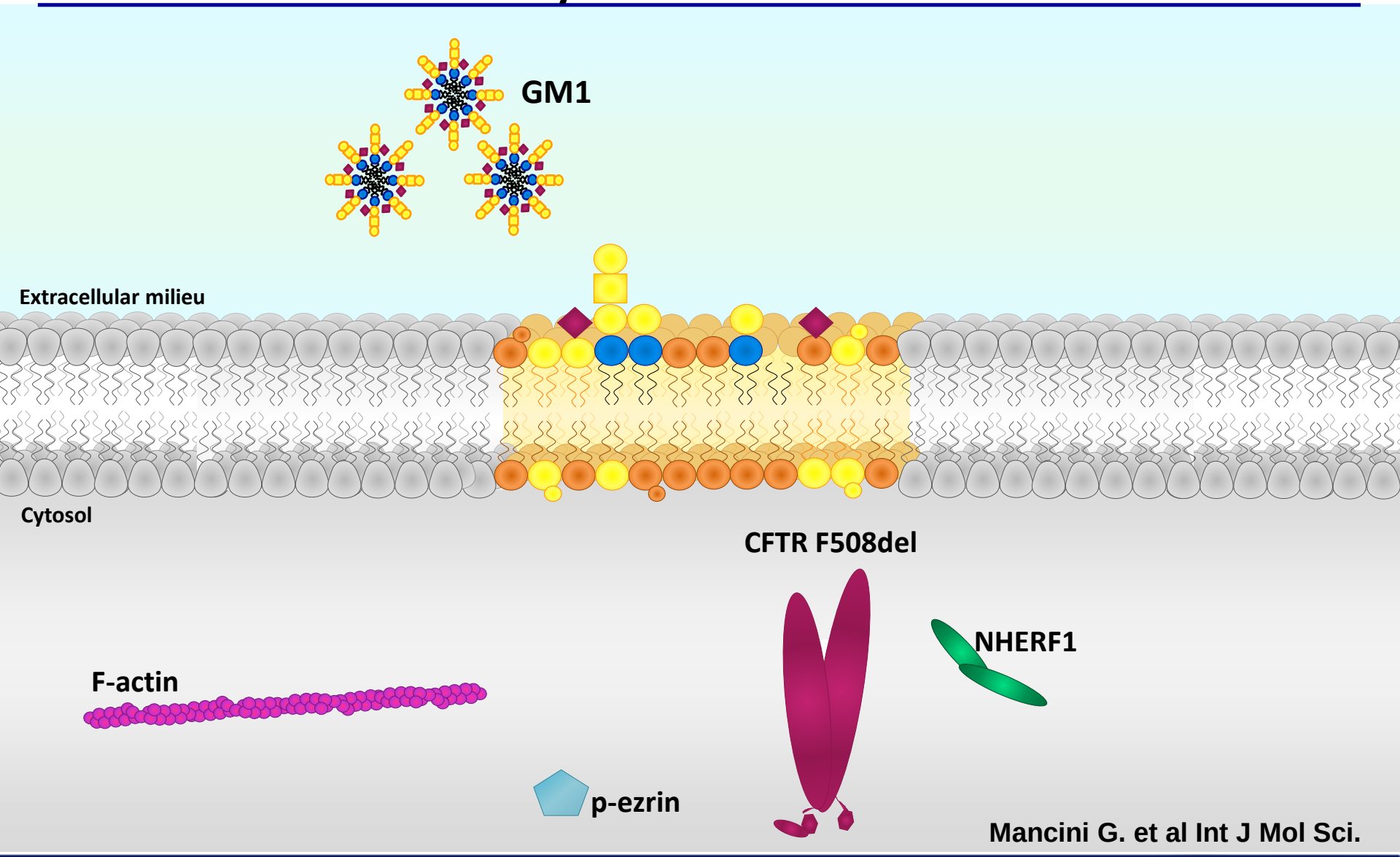
CF-HBE-ALI (F508del)
Primary human bronchial cells





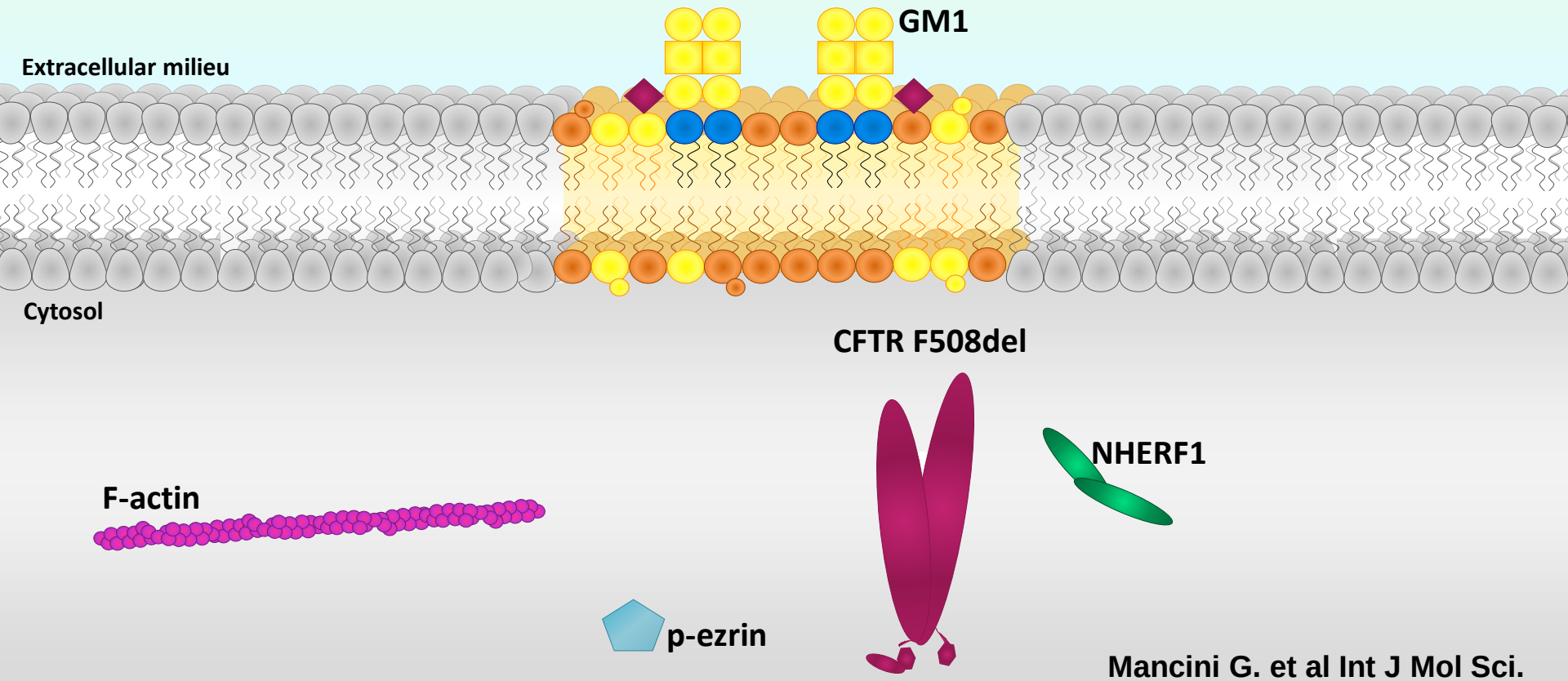
***p < 0.0001, *p < 0.05

Restoring GM1 levels promotes the stability of the F508del CFTR rescued by the VX-809 and VX-770



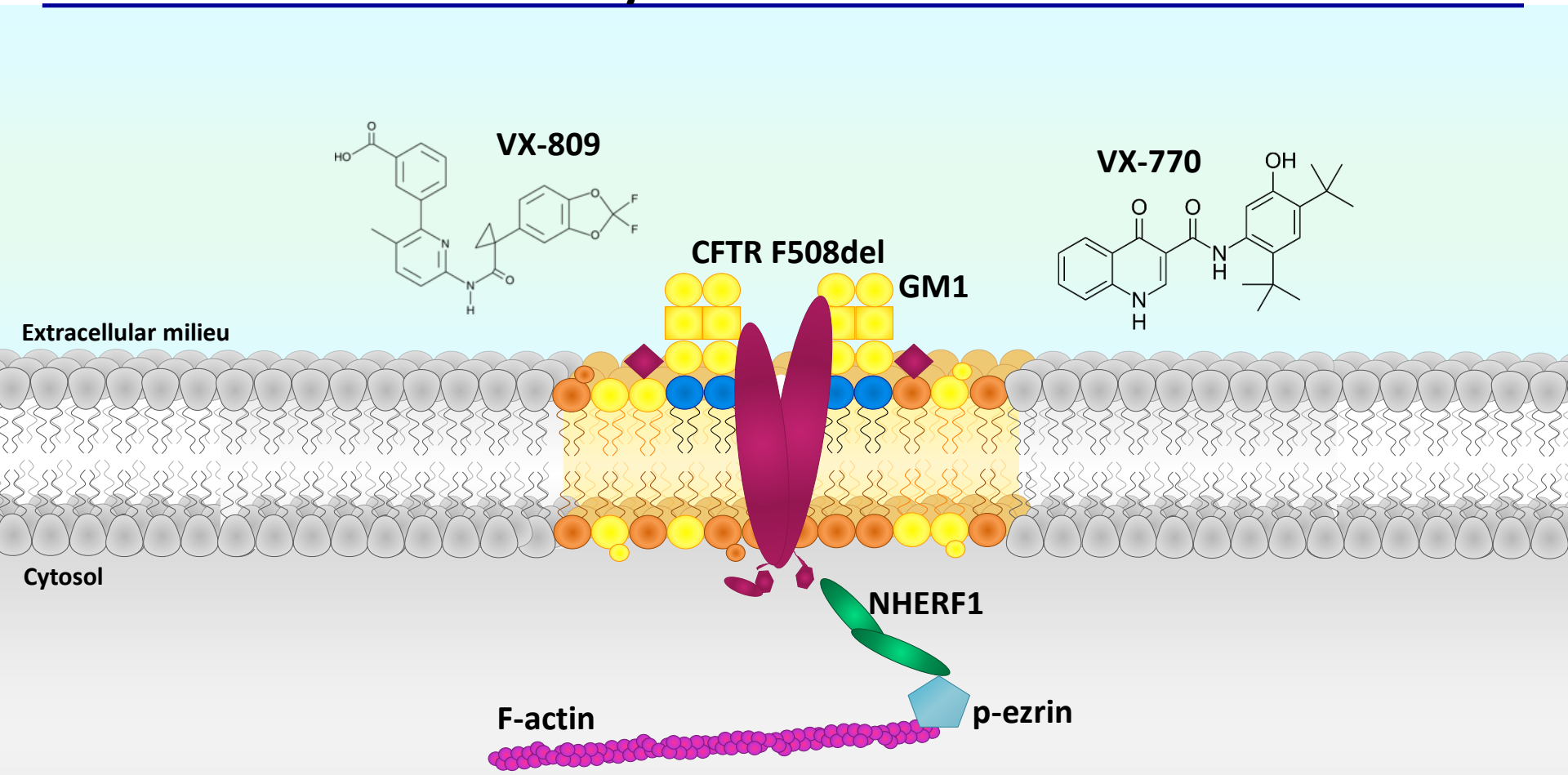
Mancini G. et al Int J Mol Sci.

Restoring GM1 levels promotes the stability of the F508del CFTR rescued by the VX-809 and VX-770



Mancini G. et al Int J Mol Sci.

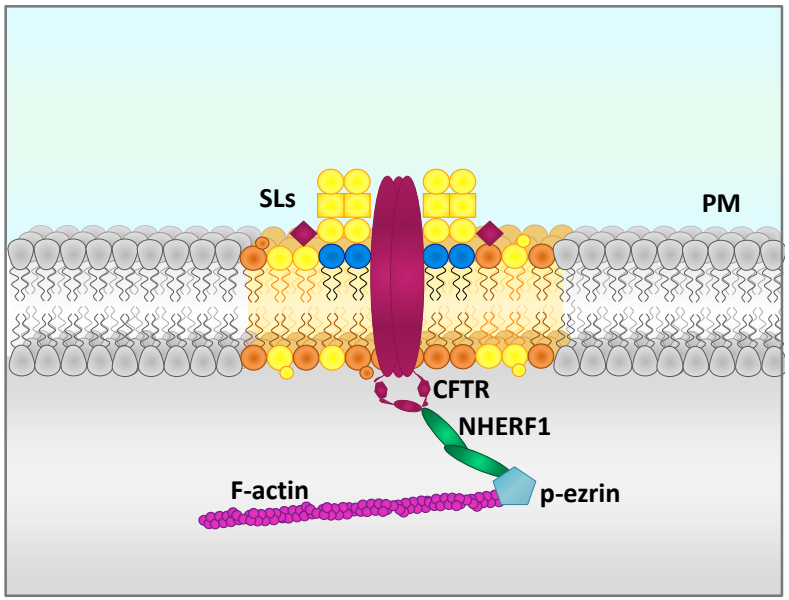
Restoring GM1 levels promotes the stability of the F508del CFTR rescued by the VX-809 and VX-770

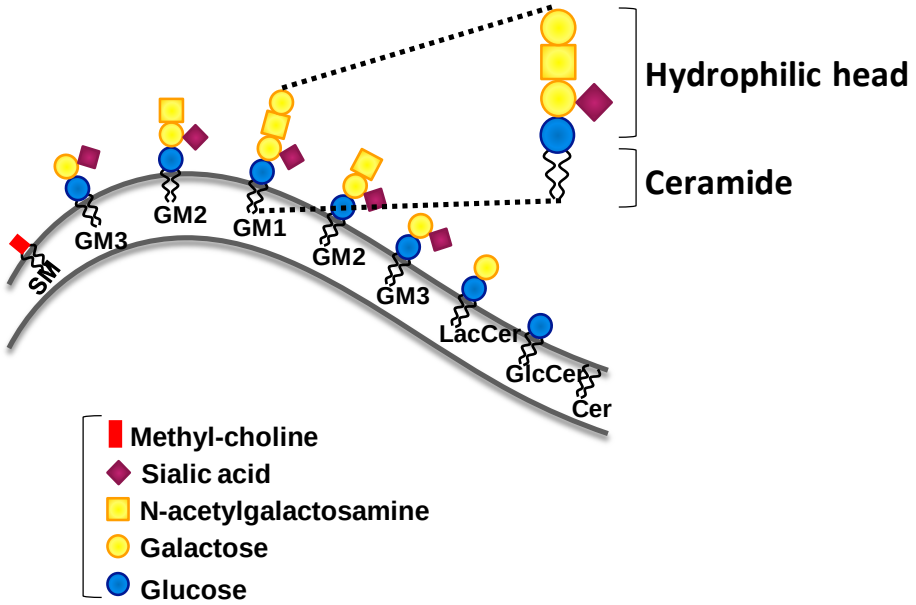


Mancini G. et al Int J Mol Sci.

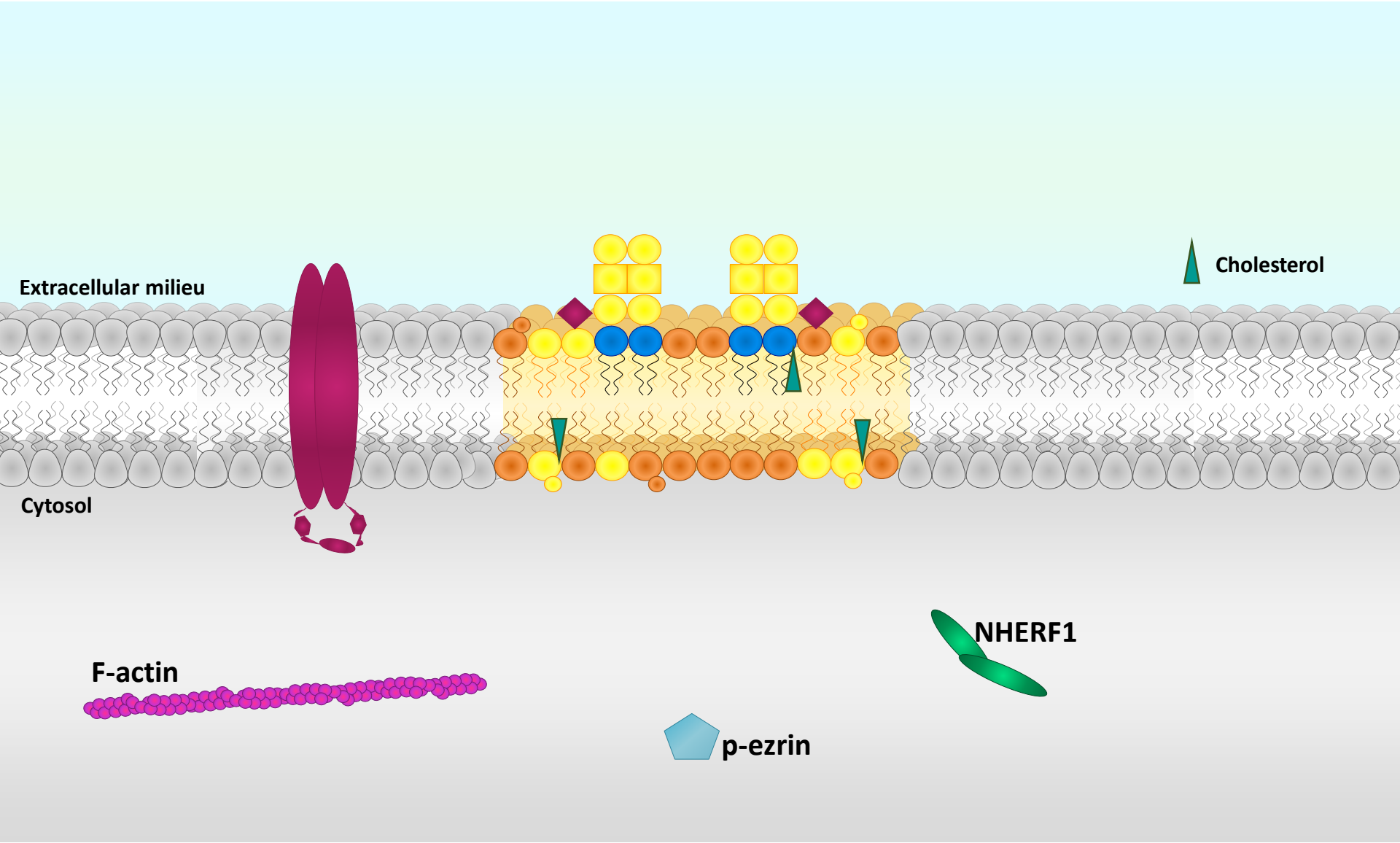
CFTR plasma membrane microenvironment

In bronchial epithelial cells, CFTR plasma membrane stability and function depend on the organization of a multi-protein complex involving F-actin, the scaffolding proteins NHERF1 and p-ezrin. This protein complex stabilizes CFTR in highly restricted membrane domains, known as **lipid rafts**, enriched in **sphingolipids**

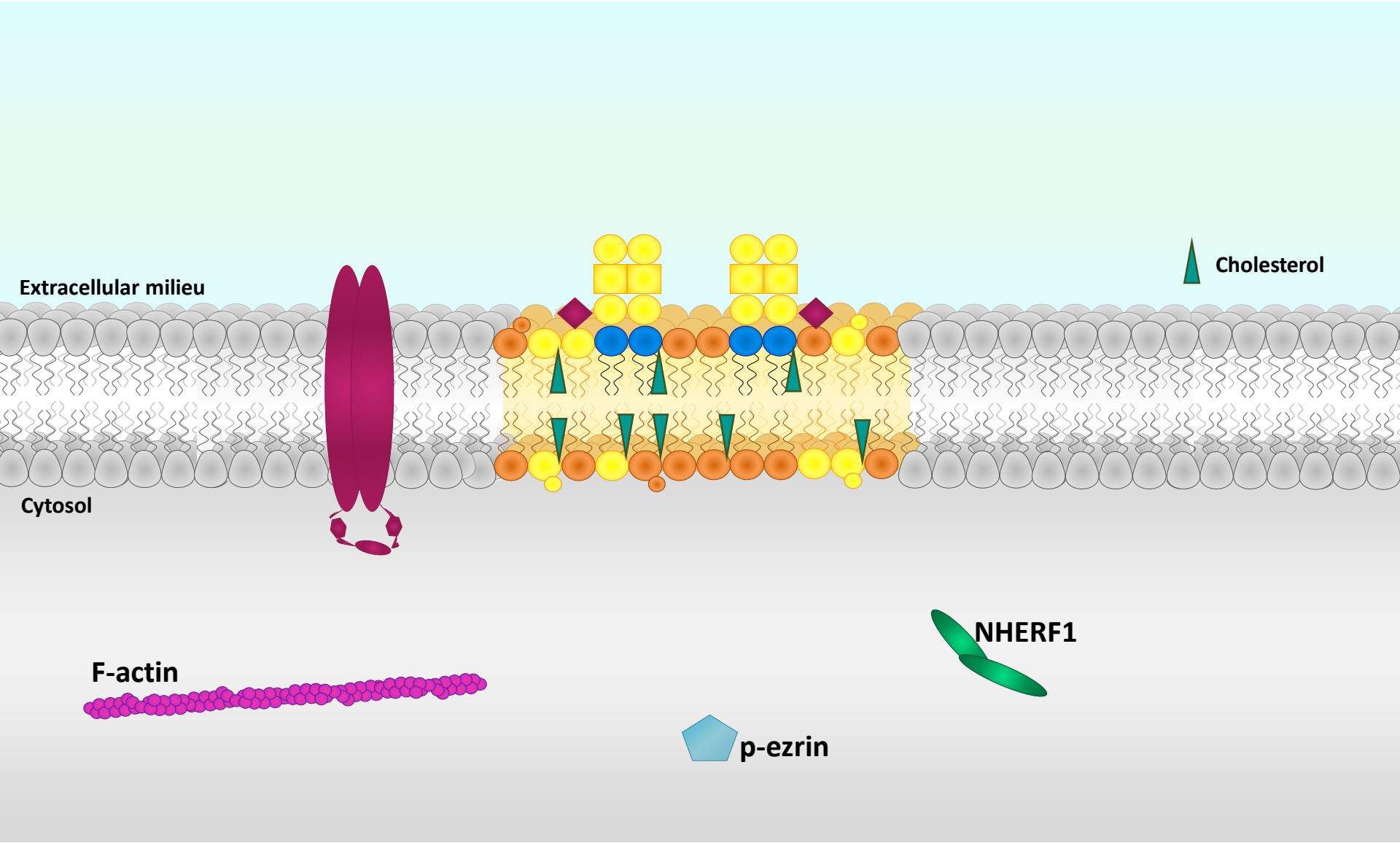




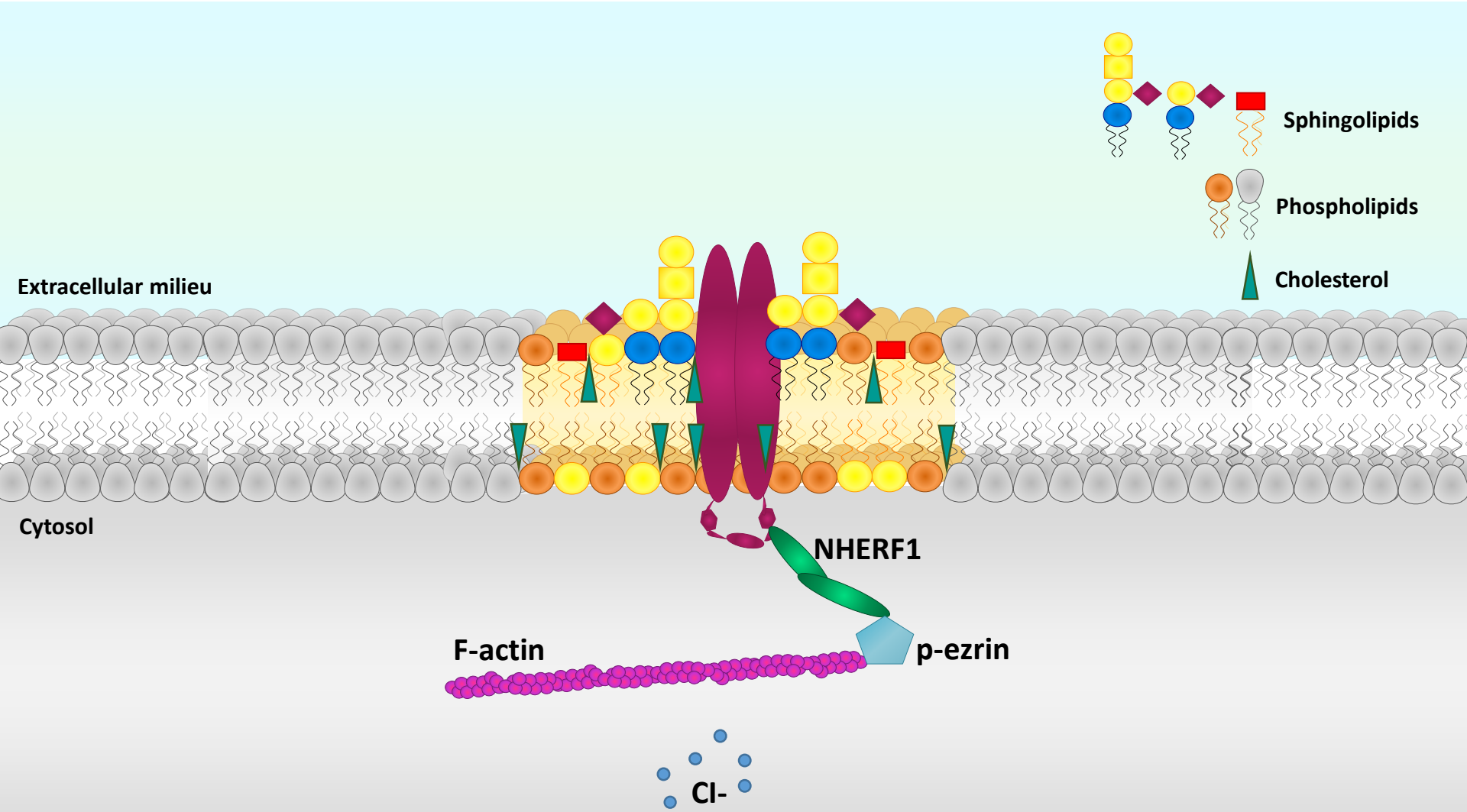
Cholesterol clusterizes CFTR at the PM and increases its open probability



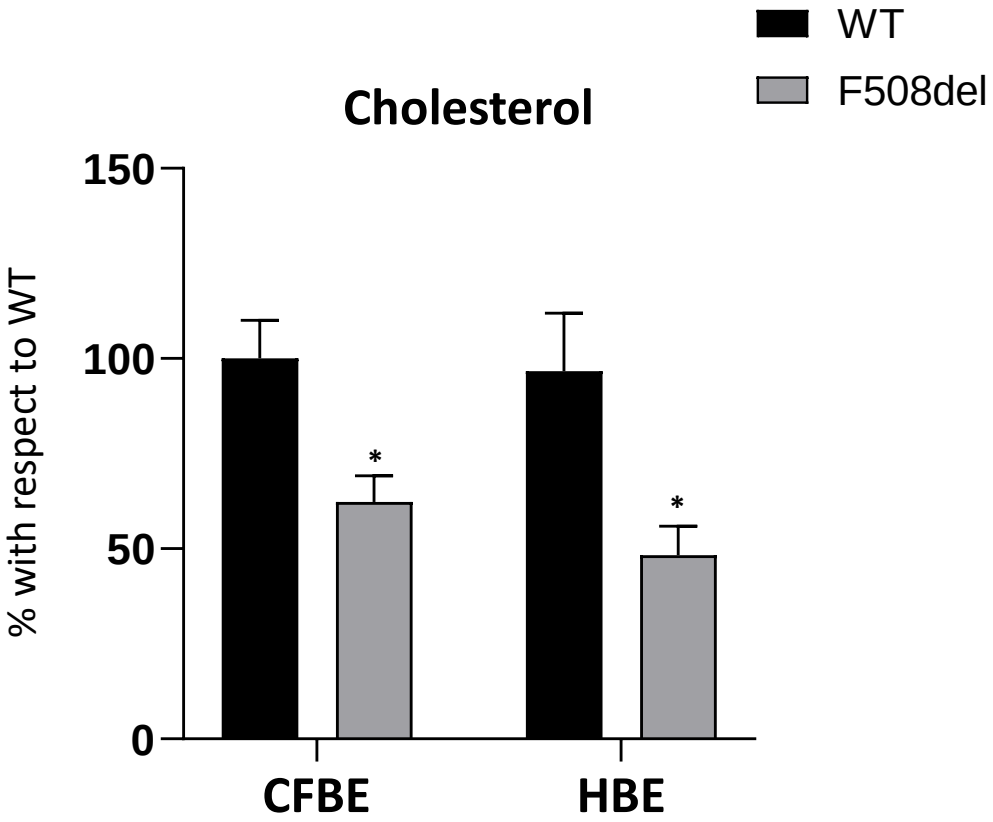
Cholesterol clusterizes CFTR at the PM and increases its open probability

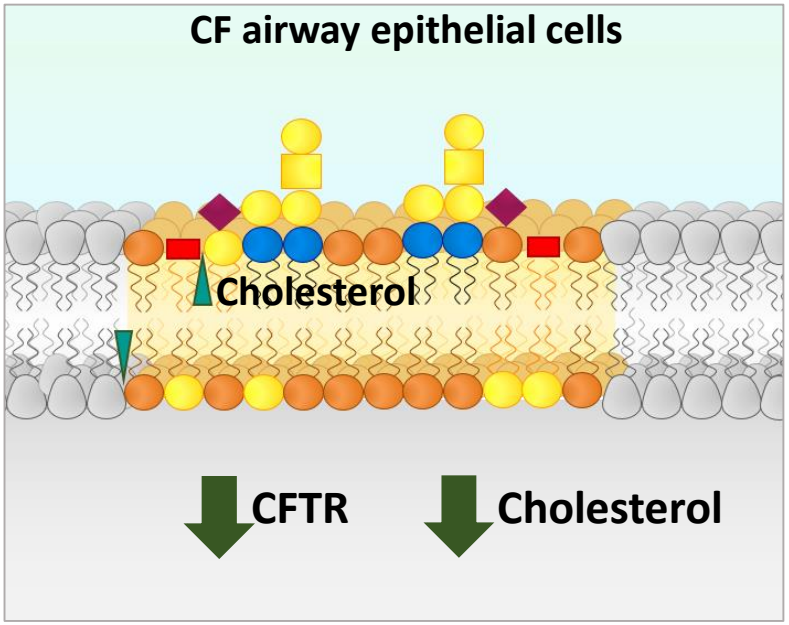


Cholesterol clusterizes CFTR at the PM and increases its open probability



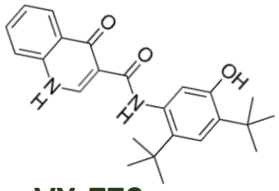
In airway cells the lack of CFTR is associated with a decrease of cholesterol



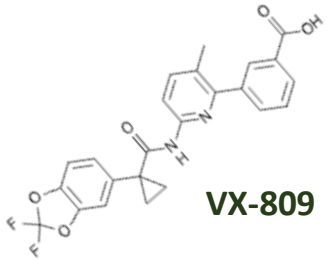


*p<0.01 vs WT

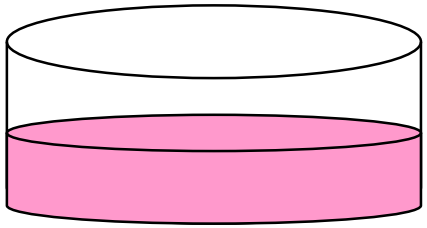
Effects of VX-770 and VX-809 on the cholesterol levels



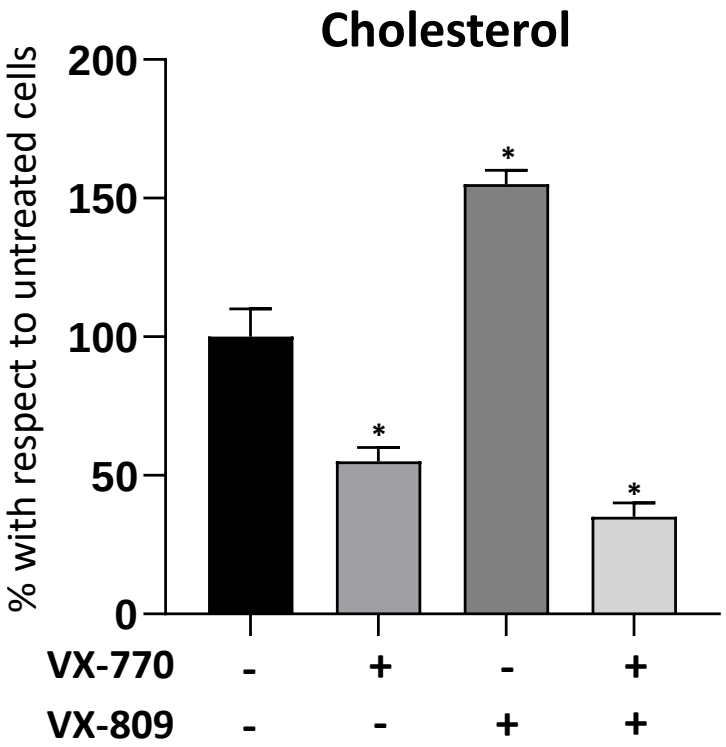
VX-770



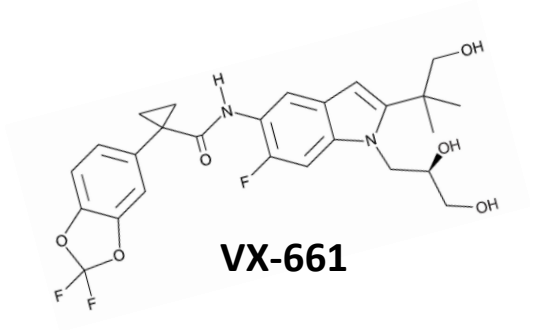
VX-809



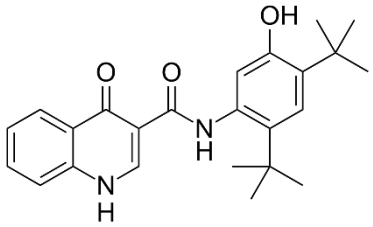
F508del-CFBE cells



*p<0.01 vs CTRL



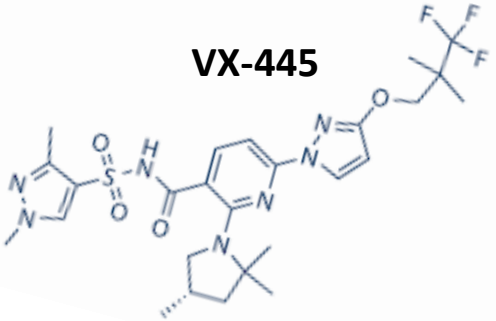
VX-661



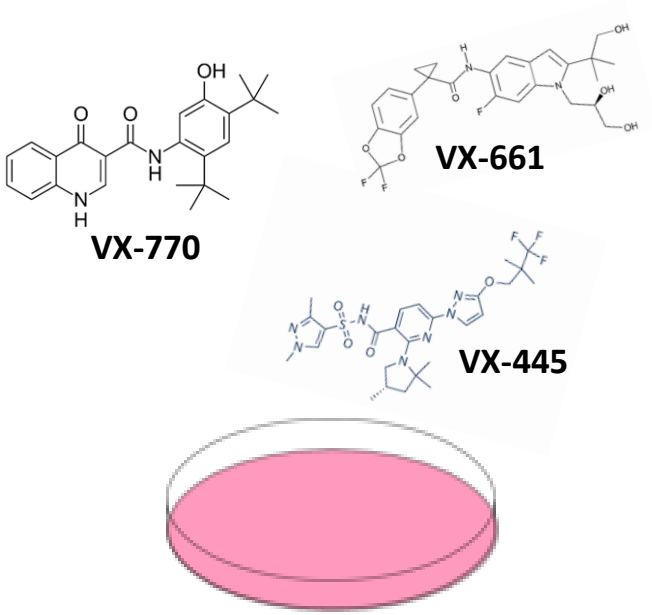
VX-770

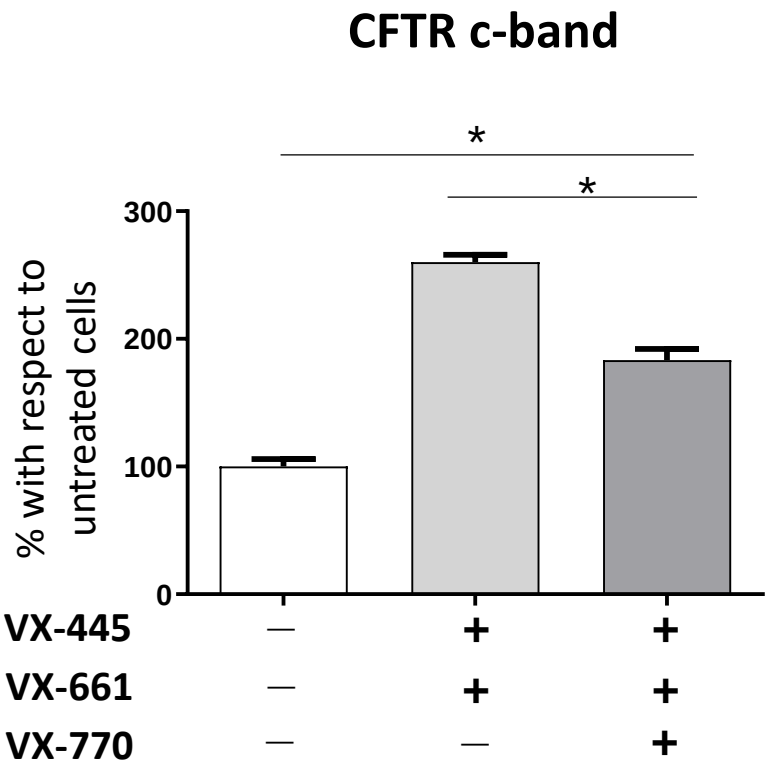
The era of Trikafta®

VX-445



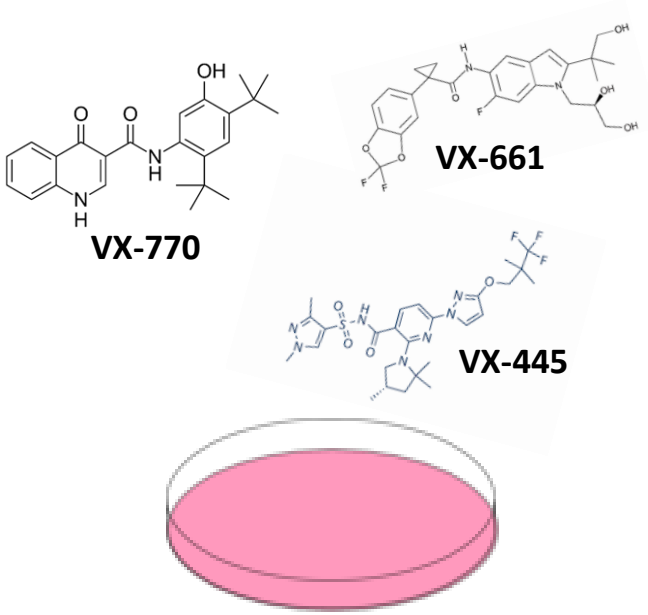
Effects of Trikafta® on the maturation of CFTR



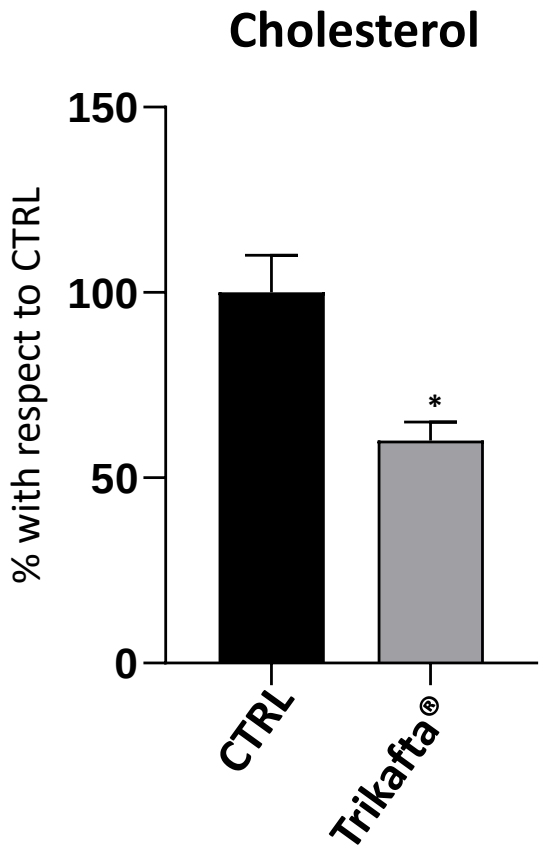


*p<0.01

Effects of Trikafta® on cholesterol levels

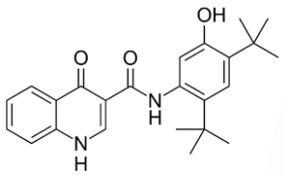


F508del-CFBE cells

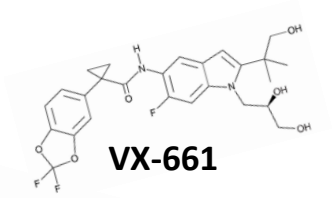


**p<0.01 vs CTRL*

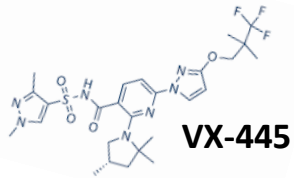
Effects of Trikafta® on the sphingolipids content



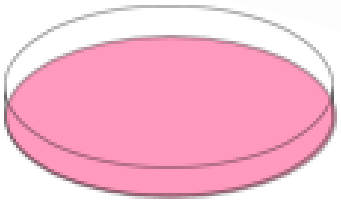
VX-770



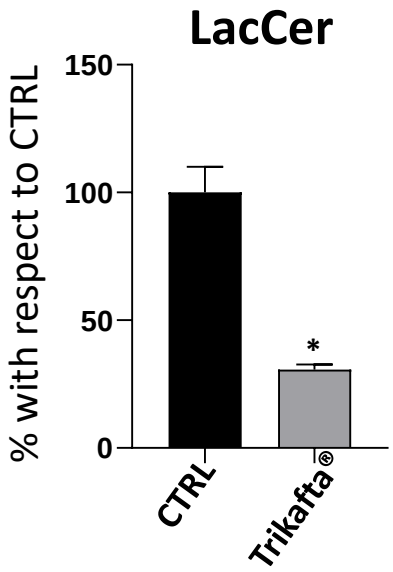
VX-661

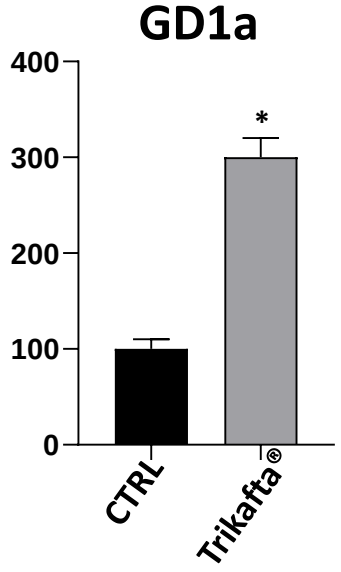


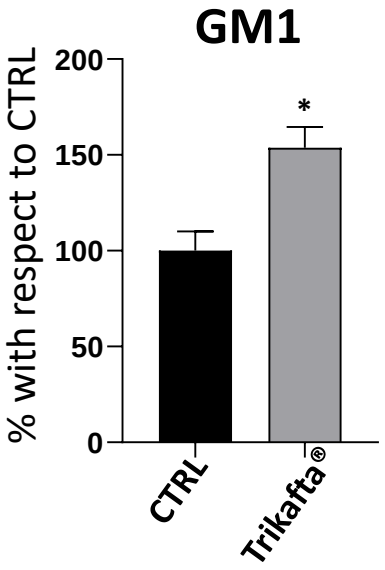
VX-445



F508del-CFBE cells

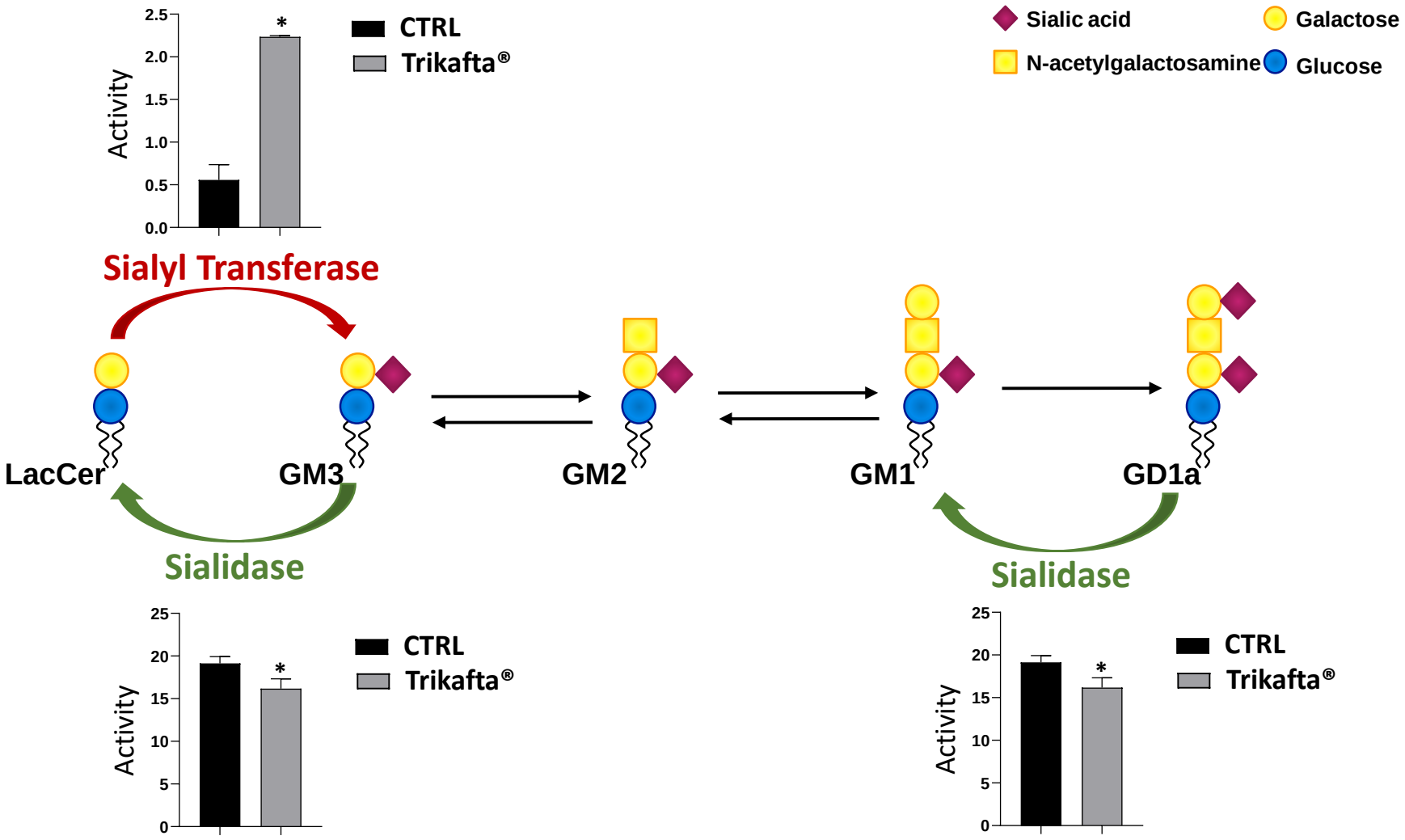






**p<0.01 vs CTRL*

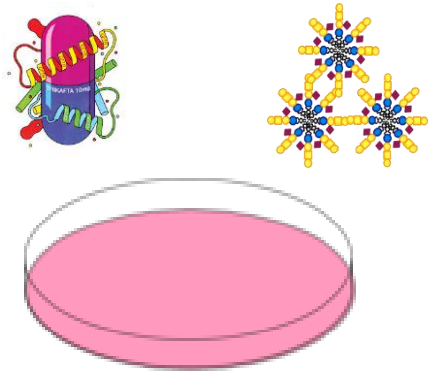
Effects of Trikafta® on the sphingolipids metabolism



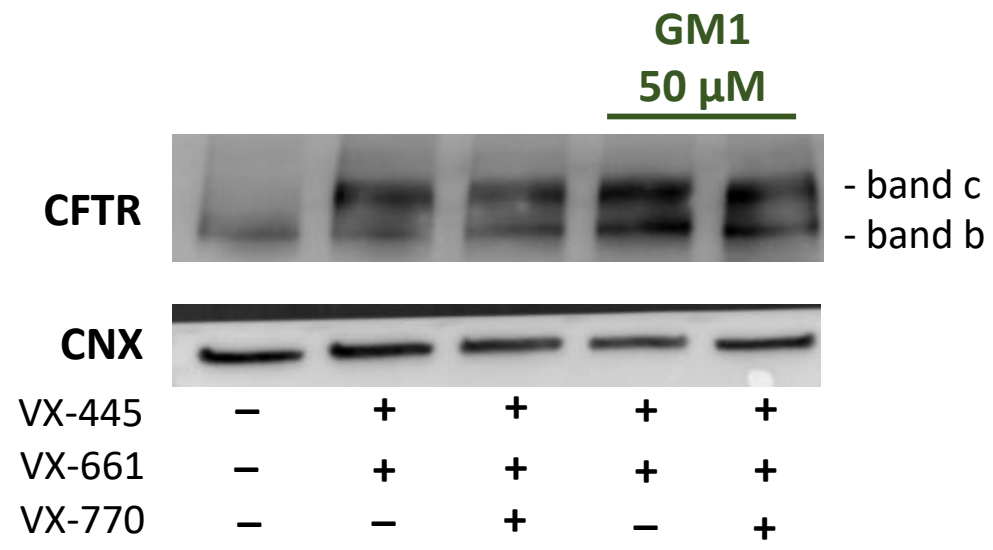
*p<0.01 vs CTRL

Effect of GM1 in the rescue of F508del-CFTR in cells treated or not with Trikafta®

Trikafta® + GM1

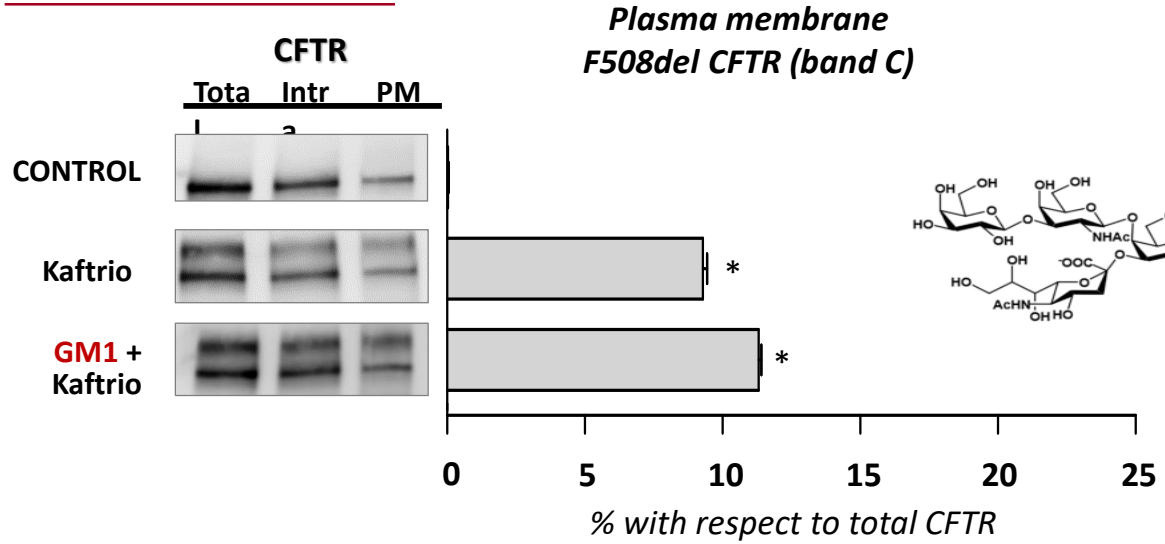


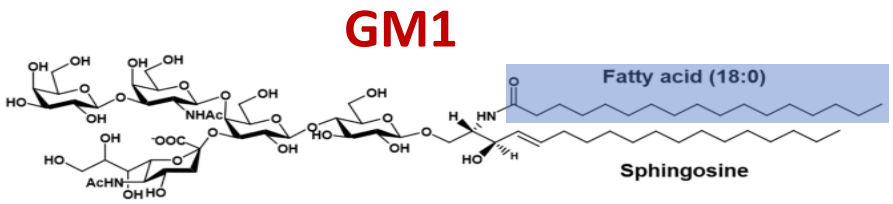
F508del-CFBE cells



*p<0.01 vs CTRL

F508del CFTR CFBE cells



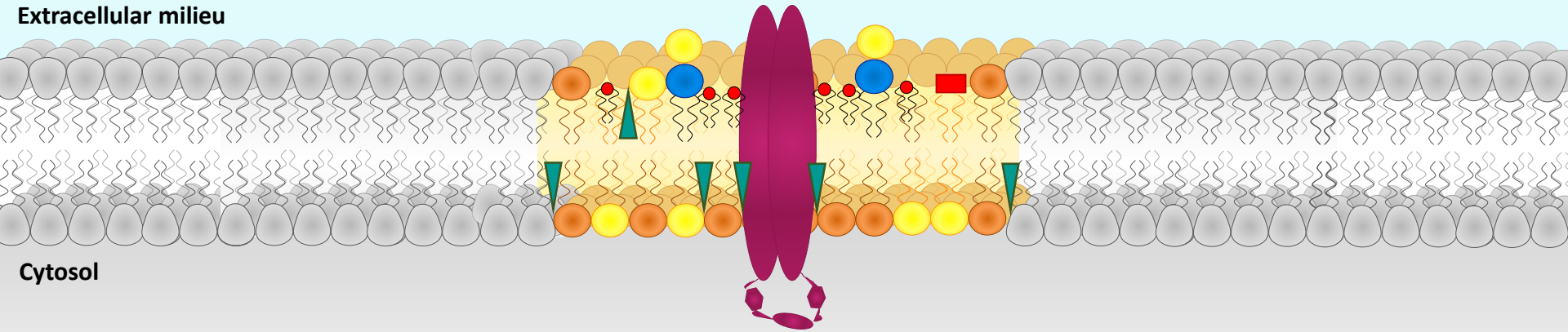


The exogenous administration of GM1 increases the amount of fully mature CFTR at the cell surface GM1 molecular species with shorter acyl chain are more effective

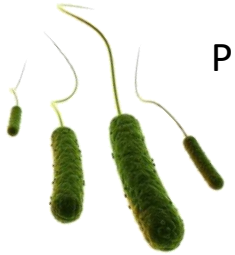
**p<0.001 vs Kaftrio*

P. Aeruginosa infection induces a decrease of CFTR at the PM

P. aeruginosa infection

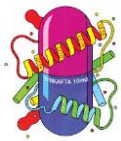


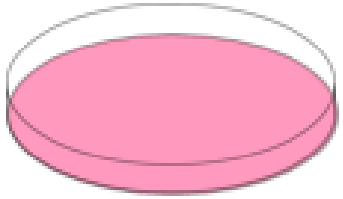
GM1 seems to partially antagonize the effect of P. aeruginosa infection on the F508del-CFTR rescued by Trikafta®



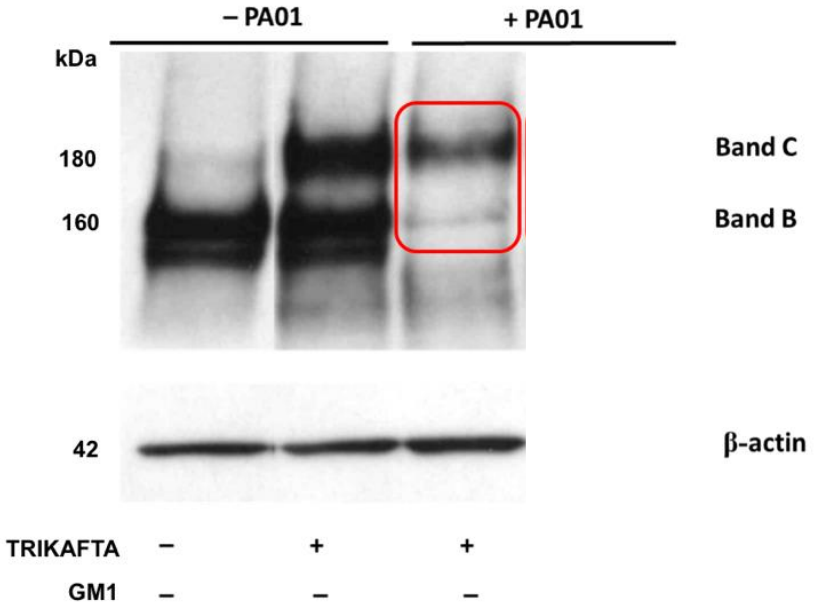
P. aeruginosa infection

Trikafta®



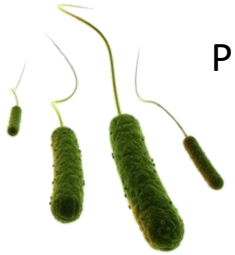


F508del-CFBE cells



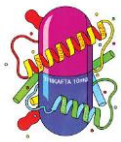
*p<0.01 vs CTRL

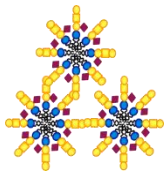
GM1 seems to partially antagonize the effect of P. aeruginosa infection on the F508del-CFTR rescued by Trikafta®

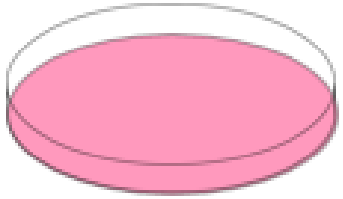


P. aeruginosa infection

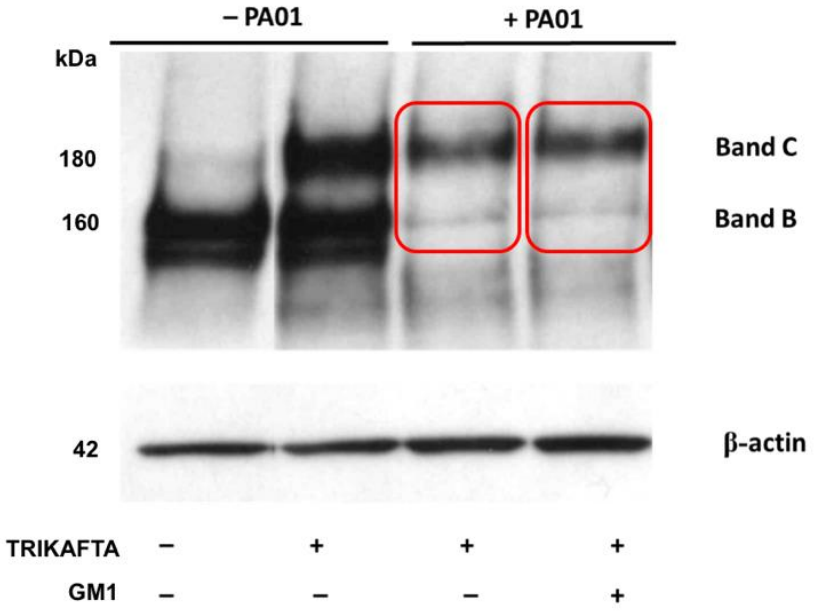
Trikafta® + **GM1**





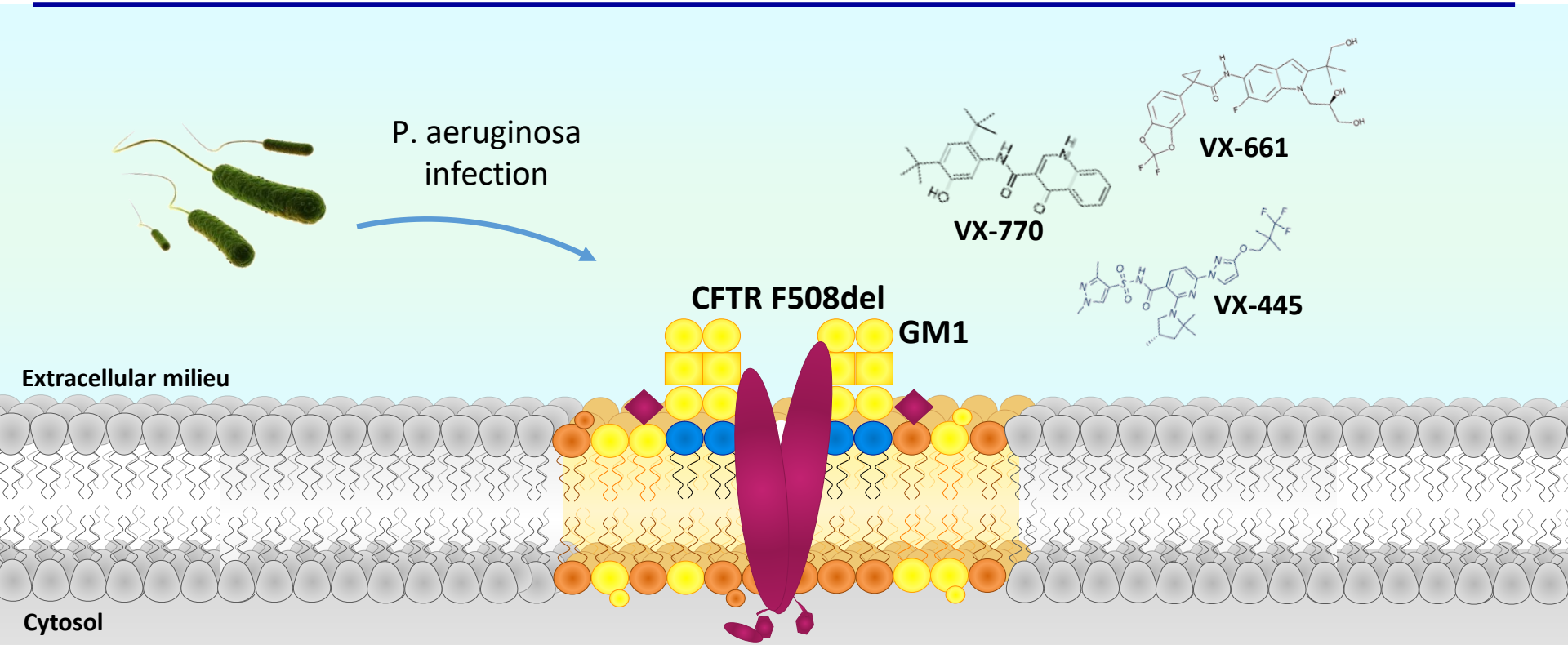


F508del-CFBE cells



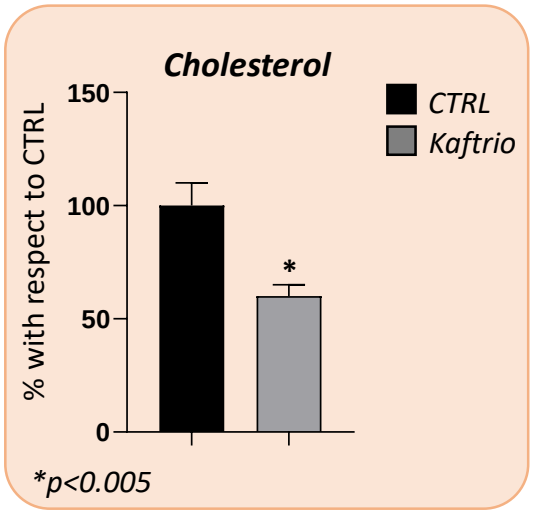
*p<0.01 vs CTRL

Conclusion

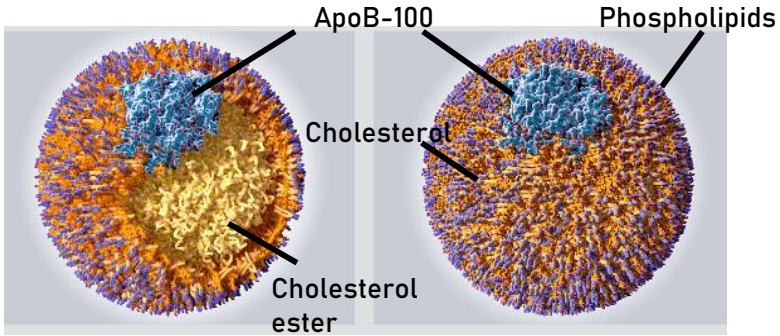


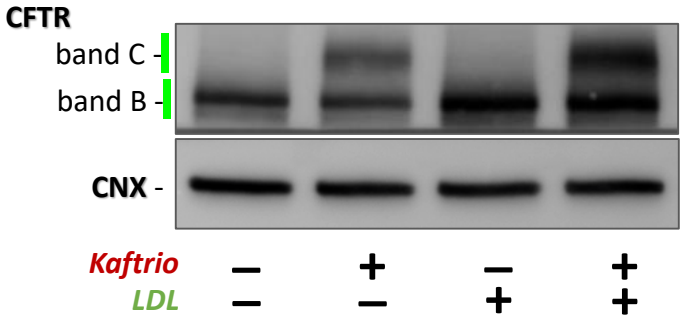
And now what about cholesterol?

F508del CFTR CFBE cells

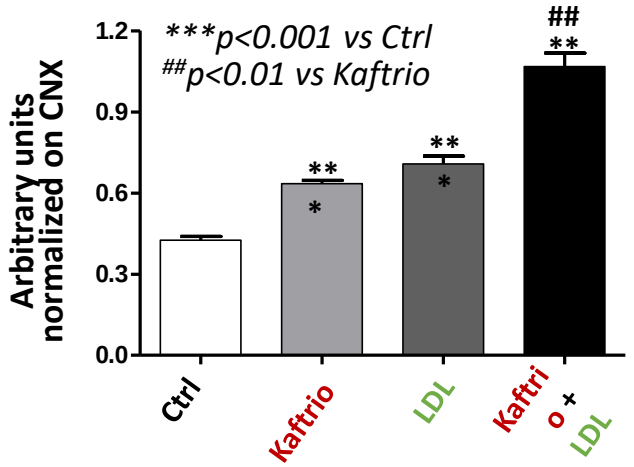


Human LDL
(400 µg/ml)
for 48h
±
Kaftrio
for 24h

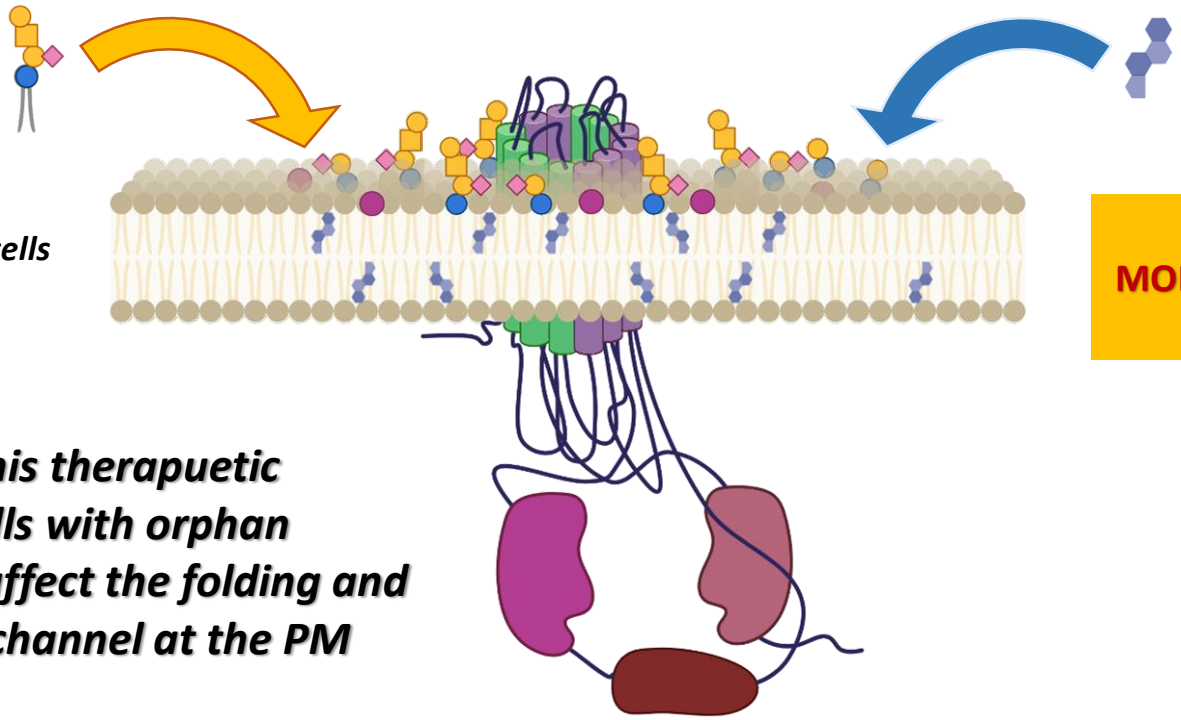




Total F508del CFTR

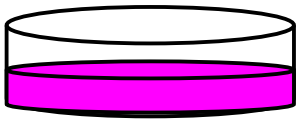


Can we consider this as an adjuvant approach for the treatment of CFTR defect



Can we apply this therapuetic approach on cells with orphan mutation that affect the folding and stability of the channel at the PM

Through personalized medicine



Primary cells from patient

Identification of the Mutation

In case of a new mutation biochemical and physiological characterization of the effect of the channel

(Try to) Found a therapy

Different combinations of corredctors and/or potentiators :

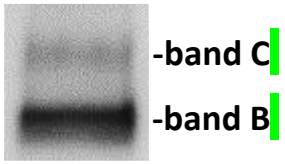
VX-661

OR

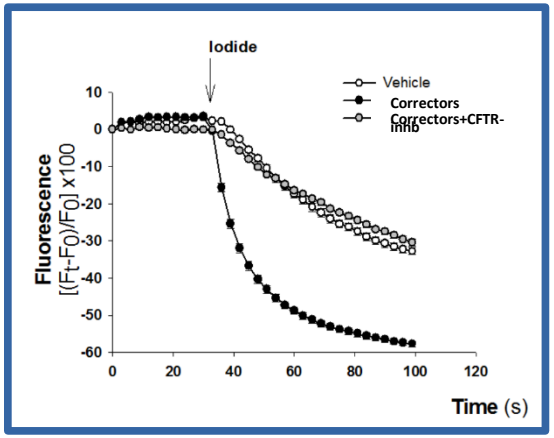
VX-809

AND

VX-770



CFTR function



Thank you for your attention