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# Bypassing CFTR dysfunction in cystic fibrosis with alternative pathways for anion transport

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**ABSTRACT**

One therapeutic strategy for cystic fibrosis (CF) seeks to restore anion transport to affected epithelia by targeting other apical membrane $Cl^-$ channels to bypass dysfunction of the cystic fibrosis transmembrane conductance regulator (CFTR) $Cl^-$ channel. The properties and regulation of the $Ca^{2+}$-activated $Cl^-$ channel TMEM16A argue that long-acting small molecules which target directly TMEM16A are required to overcome CFTR loss. Through genetic studies of lung diseases, SLC26A9, a member of the solute carrier 26 family of anion transporters, has emerged as a promising target to bypass CFTR dysfunction. An alternative strategy to circumvent CFTR dysfunction is to deliver to CF epithelia artificial anion transporters that shuttle $Cl^-$ across the apical membrane. Recently, powerful, non-toxic, biologically-active artificial anion transporters have emerged.

## HIGHLIGHTS

- $Cl^-$ channels distinct from CFTR are found in the apical membrane of CF epithelia.
- TMEM16A and SLC26A9 are promising targets to bypass CFTR dysfunction in CF.
- Long-acting drugs that target directly TMEM16A are required to mimic CFTR activity.
- SLC26A9, a CF disease modifier, supports sustained $Cl^-$ secretion by epithelia.
- Biologically-active artificial anion transporters have been identified.

## INTRODUCTION

The genetic disease cystic fibrosis (CF) is caused by mutations in the epithelial anion channel cystic fibrosis transmembrane conductance regulator (CFTR) [1,2], which plays a pivotal role in transepithelial ion transport [3]. Because of the large number of disease-causing mutations in the *CFTR* gene (http://www.genet.sickkids.on.ca/app) and their complex effects on CFTR expression and function [4], there is significant interest in therapeutic strategies which are mutation-independent.

One mutation-independent therapeutic strategy is to exploit other pathways for anion transport in the apical membrane of CF epithelia to rehydrate epithelial surfaces lining ducts and tubes (Figure 1). Most work in this area has focused on the $Ca^{2+}$-activated $Cl^-$ channel and has included clinical trials of long-acting $P2Y_2$ receptor agonists that act indirectly by increasing the intracellular free $Ca^{2+}$ concentration [5,6]. However, targeting apical membrane $Cl^-$ channels is not the only possible approach. First, inhibition of the epithelial $Na^+$ channel (ENaC) prevents dehydration of CF airway epithelia by ENaC-mediated $Na^+$ absorption [7,8]. Second, activation of basolateral membrane $K^+$ channels enhances the driving force for $Cl^-$ exit across the apical membrane of CF epithelia through either residual CFTR $Cl^-$ channels or other apical membrane $Cl^-$ channels [9,10]. Third, inhibition of the nongastric $H^+/K^+$-ATPase (ATP12A) prevents deleterious acidification of airway surface liquid (ASL), which promotes bacterial growth [11*]. Finally, artificial anion channels and transporters might be delivered to CF epithelia to restore transepithelial $Cl^-$ transport [12] (Figure 1). Here, we examine the utility of targeting apical membrane $Cl^-$ channels to bypass CFTR dysfunction in CF and discuss recent progress in the development of artificial anion transporters.

## TMEM16A: A TARGET TO STIMULATE ANION TRANSPORT BY AIRWAY EPITHELIA

TMEM16A (or anoctamin-1 [ANO1]) is the molecular identity of the $Ca^{2+}$-activated $Cl^-$ channel [13-15]. It mediates the bidirectional flux of $Cl^-$, $HCO_3^-$ and other anions across the apical membrane of epithelia [5]. When compared to CFTR [2], the structure, function and regulation of TMEM16A differ noticeably. Structural information from a distant paralog, the fungus *Nectria haematococca* [16], argue that TMEM16A is a homodimer with each subunit containing at least ten membrane-spanning helices and a $Ca^{2+}$-binding site. Intriguingly, the TMEM16A pore might only in part be delimited by transmembrane helices, the remainder being formed in some way by lipids of the plasma membrane [17*]. This unconventional pore structure arises from the lipid scramblase function of distant paralogs [16]. A cleft on each side of the homodimer forms a pathway through which the heads of phospholipids can translocate from one side of the membrane to the other. Although scramblase activity has been conserved in some family members, such as TMEM16F [18], in TMEM16A and TMEM16B, the pathway for lipid head translocation has evolved into a pore for anions.

TMEM16A is expressed in the airway epithelium, although its localisation appears different to that of CFTR. In the surface epithelium, CFTR is predominantly expressed in ciliated cells, whereas TMEM16A is more abundant in mucus-secreting goblet cells [19,20]. Like CFTR, TMEM16A is also present in submucosal glands of the bronchi [21]. Interestingly, TMEM16A expression is increased under pro-inflammatory conditions (e.g. treatment with cytokines or bacterial components), which lead to mucus hypersecretion [19,21,22].

Physiologically, TMEM16A is activated by stimuli that mobilise intracellular $Ca^{2+}$, such as purinergic agonists. In the surface epithelium, this mechanism might link release of

intracellular ATP [23], caused by mechanical stress (e.g. cough) or other stimuli, to activation of TMEM16A-dependent anion secretion. As revealed by short-circuit current recordings (one measure of transepithelial ion transport), $Ca^{2+}$-dependent secretion is usually characterised by a large transient, attained a few seconds after stimulation, which declines to near resting levels within 15-20 minutes [5] (Figure 2). This behaviour suggests that TMEM16A is involved in rapid adaptation to conditions where increased anion secretion is required. Given its localisation in goblet cells, TMEM16A might play an important role mediating bicarbonate secretion to support mucus release. By contrast, CFTR function has a more "tonic" role. Its activity is maintained for prolonged periods [3] (Figure 2), suggesting that CFTR contributes to basal anion secretion.

Nevertheless, TMEM16A might represent an alternative therapeutic target to circumvent CFTR dysfunction in the airway epithelia of CF patients. Pharmacological stimulation of TMEM16A function could help to normalise the properties of airway surface liquid and mucus by increasing chloride and bicarbonate secretion. For example, TMEM16A stimulation might be used as an adjuvant therapy, in combination with CFTR modulators that rescue the apical membrane expression and function of CF mutants. It might be especially valuable to individuals carrying CF mutations unresponsive to CFTR modulator therapy.

An ideal drug stimulating TMEM16A should act directly upon TMEM16A, not work through elevation of intracellular $Ca^{2+}$ [24]. It should also produce long-lasting activation of TMEM16A to mimic the normal behaviour of CFTR. Finally, this drug should be delivered by nebulization to avoid systemic administration, which might elicit unnecessary and potentially harmful TMEM16A activation in other organs and tissues, but it also should avoid activation of TMEM16A in bronchial smooth muscle to prevent bronchial constriction [5]. Of note,

Danahay et al. (Poster 91, 14th European Cystic Fibrosis Society Basic Science Conference, Albufeira, Portugal, March 2017) reported the identification of chemical structures that potentiate TMEM16A independent of elevation of intracellular $Ca^{2+}$. It is also feasible that TMEM16A activators and potentiators might be identified among the chemical structures of TMEM16A inhibitors identified by high throughput screening [5,24]. Lessons from the development of CFTR modulators will no doubt accelerate work to transform small molecule TMEM16A modulators into therapeutics for CF lung disease.

## SLC26A9: AN ALTERNATIVE TARGET TO BYPASS CFTR DYSFUNCTION IN CF

One member of the solute carrier 26 (SLC26) family of anion transporters, SLC26A9 has recently been identified as a promising target to bypass CFTR dysfunction in CF epithelia [25,26]. While most SLC26 proteins function as anion transporters, SLC26A9 is a $Cl^-$-selective channel with limited $HCO_3^-$ conductance [25,27]. Like CFTR, the SLC26A9 $Cl^-$ channel has a linear current-voltage (I-V) relationship with a low single-channel conductance [25,28,29]. However, in marked contrast to CFTR and TMEM16A, which are tightly regulated by cAMP-dependent phosphorylation and the intracellular free $Ca^{2+}$ concentration, respectively [2,5], SLC26A9 is constitutively active, supporting sustained $Cl^-$ secretion once inserted into the apical membrane of airway epithelia [25,26,29,30] (Figure 2). These data argue that SLC26A9 is an important target for mutation-independent therapy in CF.

The predicted topology of SLC26A9 contains a PDZ-binding motif at its C-terminus through which SLC26A9 can interact with CFTR, if both proteins are expressed in the same cell [31-33*]. Consistent with this idea, co-expression of SLC26A9 with wild-type CFTR increased the function of both $Cl^-$ channels, whereas co-expression of SLC26A9 with the CF mutant F508del-CFTR, which fails to reach the plasma membrane, reduced SLC26A9 function

[25,33*,34]. These reciprocal interactions of CFTR and SLC26A9 are likely relevant for the pathophysiology and pharmacotherapy of CF lung disease. First, inhibition of constitutive SLC26A9 activity in airway epithelia by F508del-CFTR might abrogate an important fail-safe mechanism for $Cl^-$ secretion, thus aggravating the dehydration of epithelial surfaces in CF patients carrying the F508del-CFTR mutation. Conversely, SLC26A9 might augment the residual function and pharmacological rescue of CF mutants present at the apical membrane (e.g. the gating mutation G551D-CFTR). Consistent with these ideas, Strug *et al*. [35*] found an association between a single nucleotide polymorphism in the *SLC26A9* gene and the magnitude of treatment response in CF patients with gating mutations treated with the CFTR potentiator ivacaftor. The authors also found improved transepithelial $Cl^-$ transport in F508del homozygous primary bronchial epithelial cultures treated with the CFTR corrector lumacaftor. These and other data [36] argue that SLC26A9 acts as a modifier of CF lung disease severity and the response to CFTR modulator therapy.

Further evidence supporting SLC26A9 as a promising therapeutic target for muco-obstructive lung diseases was obtained using SLC26A9-deficient mice challenged with intrapulmonary installation of the Th2 cytokine IL-13 to induce goblet cell metaplasia and mucus hypersecretion [30]. Like TMEM16A [13], SLC26A9 function is induced by IL-13 treatment [30]. In wild-type mice treated with IL-13, SLC26A9-mediated $Cl^-$ secretion prevented airway mucus obstruction despite substantial mucus hypersecretion, whereas in SLC26A9-deficient mice treated with IL-13, which lacked this $Cl^-$ secretory capacity, severe airway mucus plugging developed [30]. Interestingly, studies of CFTR-deficient mice revealed that concomitant absence of SLC26A9 causes a substantial increase in gastrointestinal tract-related mortality, indicating that SLC26A9 is a genetic modifier of meconium ileus-like intestinal obstruction in CF mice [37]. Consistent with this idea, SLC26A9 polymorphisms

have also been associated with the risk to develop meconium ileus and early exocrine pancreatic disease in CF patients [38,39].

In summary, SLC26A9 has emerged as a promising therapeutic target to bypass CFTR dysfunction in the respiratory airways, gastrointestinal tract and pancreas of CF patients. However, current knowledge of the SLC26A9 $Cl^-$ channel is limited. Considerable research is therefore required before SLC26A9 can be tested as a therapeutic target in patients with CF and potentially other muco-obstructive lung diseases. The crystal structure of an SLC26 homolog from the bacterium *Deinocoocus geothermalis*, a proton-driven fumarate transporter [40] should accelerate progress towards this goal.

## BIOLOGICALLY-ACTIVE ARTIFICIAL ANION TRANSPORTERS

An alternative approach to CFTR bypass therapy is to create artificial pathways for anion transport that can be inserted into the apical membrane of CF epithelia to mediate transepithelial $Cl^-$ transport. Transmembrane $Cl^-$ transport might be achieved using static pathways for anion movement (synthetic anion channels) or small molecules, which shuttle anions across cells membranes (artificial anion transporters or anionophores) (Figure 1). In an early proof of principle study, Wallace *et al*. [41] demonstrated that a synthetic peptide derived from the glycine receptor induced sustained transepithelial $Cl^-$ secretion across cultured renal epithelia. Subsequently, self-assembled non-peptide synthetic anion channels have been developed and their biological activity assessed [12,42]. Of note, Jiang *et al*. [43] demonstrated that a squalamine analogue, functioning as a synthetic $Cl^-$ channel, partially corrected loss of CFTR function in CF airway cells, epithelia and the nasal epithelium of a CF mouse model. Subsequently, Shen *et al*. [44] showed that a synthetic $Cl^-$ channel based on a $C_2$-symmetric isophthalamide scaffold conferred a $Cl^-$ conductance on CF airway epithelial cells, while a

modified $C_2$-symmetric isophthalamide scaffold has been identified, which transports $Cl^-$ and $HCO_3^-$ across synthetic lipid vesicles and airway epithelia and confers $Cl^-$ transport on CF airway epithelia [45].

The successful development of biologically-active anionophores depends greatly on their chemical structure. This chemical structure should bind anions at the interface between the aqueous extracellular environment and the plasma membrane, translocate them across the lipid bilayer before release on the intracellular side (Figure 1). Anionophores therefore require solubility within the lipid bilayer, while forming a binding site for anions shielded from the lipid bilayer. These characteristics are well illustrated by cholapods, steroid-based scaffolds derived from cholic acid [46-48]. The lipophilic nature of cholic acid promotes solubility in lipid membranes, while its rigid structure has well-spaced side groups that can be modified to form a high-affinity $Cl^-$ binding site (for review, see [48]) (Figure 3). When tested in synthetic lipid vesicles, cholapods proved to be powerful anion transporters, some exhibiting anion transport at cholapod to lipid ratios as low as 1:250,000 (i.e. one or two anionophores per lipid vesicle) [47,48]. The observation that the central part of the cholapod structure contained most of its anion transport activity [48] motivated the development of a second family of anionophores, termed decalins [49] (Figure 3). Of note, decalins have improved drug-like properties, including smaller molecular weight and reduced lipophilicity, making them easier to partition from aqueous to lipid environments. Moreover, some decalins achieve impressive transport rates in synthetic lipid vesicles, approaching that of CFTR in natural membranes [50].

Using cells engineered to express the halide-sensitive fluorophore YFP-H148Q/I152L [51], Li *et al*. [52*] tested the biological activity of 15 anionophores representative of different chemical scaffolds, including cholapods [46,47], decalins [49,50] and cyclohexanes [53]. One

decalin, a bis-ureiododecalin demonstrated especially promising activity, including (i) delivery to cell membranes from the aqueous solution, (ii) potency at low micromolar concentrations, (iii) sustained activity, transporting anions for up to 2 hours and (iv) lack of cytotoxic effects when tested on three different epithelial cell lines [52*]. These data argue that anionophores are worthy of further investigation as a CFTR bypass therapy for CF. However, important challenges remain to be addressed, including optimisation of chemical structure, regulation and delivery to airway epithelia *in vivo*. Encouragingly, recent data argue that anionophores with encapsulated $Cl^-$ binding sites will likely achieve transmembrane $Cl^-$ transport with high $Cl^-$ selectivity [54*]. Given the importance of $HCO_3^-$ for epithelial physiology (e.g. [11*,55]), future studies should also seek to develop anionophores capable of transmembrane $HCO_3^-$ transport.

## CONCLUSIONS

Mutation-independent therapies that circumvent CFTR dysfunction have a key role to play in the armamentarium of innovative new therapies that tackle the root cause of CF. These therapies are likely to be particularly useful for individuals carrying mutations that are not amenable to treatment with small molecule CFTR modulators. The molecular identification of TMEM16A has accelerated the search for long-acting small molecule activators and potentiators of the epithelial $Ca^{2+}$-activated $Cl^-$ channel to achieve sustained transepithelial $Cl^-$ secretion. SLC26A9 is an attractive target to circumvent CFTR dysfunction in CF, but much remains to be learnt about this epithelial $Cl^-$ channel before therapeutically-active small molecule SLC26A9 activators and potentiators are developed. Similarly, work to develop therapeutically-active anionophores is at an early stage, but the identification of powerful, non-toxic biologically-active anionophores augurs well for the future. Evaluation of CFTR bypass strategies using animal models (ferrets and pigs) resembling CF disease in humans and

organotypic cell cultures will be important for their development as therapeutic tools for CF lung disease.

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## CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

**REFERENCES**


1. Riordan JR, Rommens JM, Kerem B-S, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou J-L *et al.*: **Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA.** *Science* 1989, **245**:1066-1073.

2. Gadsby DC, Vergani P, Csanády L: **The ABC protein turned chloride channel whose failure causes cystic fibrosis.** *Nature* 2006, **440**:477-483.

3. Frizzell RA, Hanrahan JW: **Physiology of epithelial chloride and fluid secretion.** *Cold Spring Harb Perspect Med* 2012, **2**:a009563.

4. Veit G, Avramescu RG, Chiang AN, Houck SA, Cai Z, Peters KW, Hong JS, Pollard HB, Guggino WB, Balch WE *et al.*: **From CFTR biology towards combinatorial pharmacotherapy: expanded classification of cystic fibrosis mutations.** *Mol Biol Cell* 2016, **27**:424-433.

5. Pedemonte N, Galietta LJV: **Structure and function of TMEM16 proteins (anoctamins).** *Physiol Rev* 2014, **94**:419-459.

6. Moss RB: **Pitfalls of drug development: lessons learned from trials of denufosol in cystic fibrosis.** *J Pediatr* 2013, **162**:676-680.

7. Coote KJ, Paisley D, Czarnecki S, Tweed M, Watson H, Young A, Sugar R, Vyas M, Smith NJ, Baettig U *et al.*: **NVP-QBE170: an inhaled blocker of the epithelial sodium**

**channel with a reduced potential to induce hyperkalaemia.** *Br J Pharmacol* 2015, **172**:2814-2826.

8. Scott DW, Walker MP, Sesma J, Wu B, Stuhlmiller TJ, Sabater JR, Abraham WM, Crowder TM, Christensen DJ, Tarran R: **SPX-101 is a novel ENaC-targeted therapeutic for cystic fibrosis that restores mucus transport.** *Am J Respir Crit Care Med* 2017, doi: 10.1164/rccm.201612-2445OC.

9. Devor DC, Bridges RJ, Pilewski JM: **Pharmacological modulation of ion transport across wild-type and ΔF508 CFTR-expressing human bronchial epithelia.** *Am J Physiol Cell Physiol* 2000, **279**:C461-C479.

10. Mall M, Wissner A, Schreiber R, Kuehr J, Seydewitz HH, Brandis M, Greger R, Kunzelmann K: **Role of $K_V$LQT1 in cyclic adenosine monophosphate-mediated $Cl^-$ secretion in human airway epithelia.** *Am J Respir Cell Mol Biol* 2000, **23**:283-289.

11. Shah VS, Meyerholz DK, Tang XX, Reznikov L, Abou Alaiwa M, Ernst SE, Karp PH, Wohlford-Lenane CL, Heilmann KP, Leidinger MR *et al*.: **Airway acidification initiates host defense abnormalities in cystic fibrosis mice.** *Science* 2016, **351**:503-507.

(*) The authors demonstrate that the nongastric $H^+/K^+$-ATPase (ATP12A) is expressed in human and pig (but not mouse) airway epithelia, where it mediates $H^+$ secretion. Inhibition of ATP12A restores bacterial killing to human and pig airway epithelia, whereas expression of ATP12A in mouse airway epithelia impairs the killing of bacteria. The data highlight the therapeutic potential of ATP12A inhibitors in CF.

12. Davis AP, Sheppard DN, Smith BD: **Development of synthetic membrane transporters for anions.** *Chem Soc Rev* 2007, **36**:348-357.

13. Caputo A, Caci E, Ferrera L, Pedemonte N, Barsanti C, Sondo E, Pfeffer U, Ravazzolo R, Zegarra-Moran O, Galietta LJV: **TMEM16A, a membrane protein associated with calcium-dependent chloride channel activity.** *Science* 2008, **322**:590-594.

14. Schroeder BC, Cheng T, Jan YN, Jan LY: **Expression cloning of TMEM16A as a calcium-activated chloride channel subunit.** *Cell* 2008, **134**:1019-1029.

15. Yang YD, Cho H, Koo JY, Tak MH, Cho Y, Shim W-S, Park SP, Lee J, Lee B, Kim B-M *et al.*: **TMEM16A confers receptor-activated calcium-dependent chloride conductance.** *Nature* 2008, **455**:1210-1215.

16. Brunner JD, Lim NK, Schenck S, Duerst A, Dutzler R: **X-ray structure of a calcium-activated TMEM16 lipid scramblase.** *Nature* 2014, **516**:207-212.

17. Whitlock JM, Hartzell HC: **A pore idea: the ion conduction pathway of TMEM16/ANO proteins is composed partly of lipid.** *Pflügers Archiv* 2016, **468**:455-473.

(*) The authors integrate structural and functional data to propose the innovative idea that the pathway for transmembrane $Cl^-$ flow in TMEM16A is formed in part by the lipid bilayer.

18. Suzuki J, Umeda M, Sims PJ, Nagata S: **Calcium-dependent phospholipid scrambling by TMEM16F.** *Nature* 2010, **468**:834-838.

19. Huang F, Zhang H, Wu M, Yang H, Kudo M, Peters CJ, Woodruff PG, Solberg OD, Donne ML, Huang X *et al.*: **Calcium-activated chloride channel TMEM16A modulates mucin secretion and airway smooth muscle contraction.** *Proc Natl Acad Sci U S A* 2012, **109**:16354-16359.

20. Scudieri P, Caci E, Bruno S, Ferrera L, Schiavon M, Sondo E, Tomati V, Gianotti A, Zegarra-Moran O, Pedemonte N *et al.*: **Association of TMEM16A chloride channel overexpression with airway goblet cell metaplasia.** *J Physiol* 2012, **590**:6141-6155.

21. Caci E, Scudieri P, Di Carlo E, Morelli P, Bruno S, De Fino I, Bragonzi A, Gianotti A, Sondo E, Ferrera L *et al.*: **Upregulation of TMEM16A protein in bronchial epithelial cells by bacterial pyocyanin.** *PLoS ONE* 2015, **10**:e0131775.

22. Gorrieri G, Scudieri P, Caci E, Schiavon M, Tomati V, Sirci F, Napolitano F, Carrella D, Gianotti A, Musante I *et al.*: **Goblet cell hyperplasia requires high bicarbonate transport to support mucin release.** *Sci Rep* 2016, **6**:36016.

23. Button B, Okada SF, Frederick CB, Thelin WR, Boucher RC: **Mechanosensitive ATP release maintains proper mucus hydration of airways.** *Sci Signal* 2013, **6**:ra46.

24. Namkung W, Yao Z, Finkbeiner WE, Verkman AS: **Small-molecule activators of TMEM16A, a calcium-activated chloride channel, stimulate epithelial chloride secretion and intestinal contraction.** *FASEB J* 2011, **25**:4048-4062.

25. Bertrand CA, Zhang R, Pilewski JM, Frizzell RA: **SLC26A9 is a constitutively active, CFTR-regulated anion conductance in human bronchial epithelia.** *J Gen Physiol* 2009, **133**:421-438.

26. Mall MA, Galietta LJV: **Targeting ion channels in cystic fibrosis.** *J Cyst Fibros* 2015, **14**:561-570.

27. Loriol C, Dulong S, Avella M, Gabillat N, Boulukos K, Borgese F, Ehrenfeld J: **Characterization of SLC26A9, facilitation of $Cl^-$ transport by bicarbonate.** *Cell Physiol Biochem* 2008, **22**:15-30.

28. Chang M-H, Plata C, Zandi-Nejad K, Sindić A, Sussman CR, Mercado A, Broumand V, Raghuram V, Mount DB, Romero MF: **Slc26a9–anion exchanger, channel and $Na^+$ transporter.** *J Membr Biol* 2009, **228**:125-140.

29. Salomon JJ, Spahn S, Wang X, Füllekrug J, Bertrand CA, Mall MA: **Generation and functional characterization of epithelial cells with stable expression of SLC26A9 $Cl^-$ channels.** *Am J Physiol Lung Cell Mol Physiol* 2016, **310**:L593-L602.

30. Anagnostopoulou P, Riederer B, Duerr J, Michel S, Binia A, Agrawal R, Liu X, Kalitzki K, Xiao F, Chen M *et al*.: **SLC26A9-mediated chloride secretion prevents mucus obstruction in airway inflammation.** *J Clin Invest* 2012, **122**:3629-3634.

31. Chang M-H, Plata C, Sindic A, Ranatunga WK, Chen A-P, Zandi-Nejad K, Chan KW, Thompson J, Mount DB, Romero MF: **Slc26a9 is inhibited by the R-region of the cystic**

**fibrosis transmembrane conductance regulator via the STAS domain.** *J Biol Chem* 2009, **284**:28306-28318.

32. El Khouri E, Touré A: **Functional interaction of the cystic fibrosis transmembrane conductance regulator with members of the SLC26 family of anion transporters (SLC26A8 and SLC26A9): physiological and pathophysiological relevance.** *Int J Biochem Cell Biol* 2014, **52**:58-67.

33. Bertrand CA, Mitra S, Mishra SK, Wang X, Zhao Y, Pilewski JM, Madden DR, Frizzell RA: **The CFTR trafficking mutation F508del inhibits the constitutive activity of SLC26A9.** *Am J Physiol Lung Cell Mol Physiol* 2017, **312**:L912-L925.

(*) The most common CF mutation F508del-CFTR disrupts SLC26A9-CFTR interactions mediated by PDZ domains, leading to decreased plasma membrane expression of SLC26A9. The CFTR corrector lumacaftor rescues SLC26A9 by trafficking F508del-CFTR to the plasma membrane.

34. Avella M, Loriol C, Boulukos K, Borgese F, Ehrenfeld J: **SLC26A9 stimulates CFTR expression and function in human bronchial cell lines.** *J Cell Physiol* 2010, **226**:212-223.

35. Strug LJ, Gonska T, He G, Keenan K, Ip W, Boëlle P-Y, Lin F, Panjwani N, Gong J, Li W *et al*.: **Cystic fibrosis gene modifier *SLC26A9* modulates airway response to CFTR-directed therapeutics.** *Hum Mol Genet* 2016, **25**:4590-4600.

(*) In CF patients with gating mutations, there is variability in the improvement in lung function achieved using the CFTR potentiator ivacaftor. The authors demonstrate that the CF modifier gene *SLC26A9* contributes in part to this variability.

36. Bakouh N, Bienvenu T, Thomas A, Ehrenfeld J, Liote H, Roussel D, Duquesnoy P, Farman N, Viel M, Cherif-Zahar B *et al*.: **Characterization of SLC26A9 in patients with CF-like lung disease.** *Hum Mutat* 2013, **34**:1404-1414.

37. Liu X, Li T, Riederer B, Lenzen H, Ludolph L, Yeruva S, Tuo B, Soleimani M, Seidler U: **Loss of Slc26a9 anion transporter alters intestinal electrolyte and $HCO_3^-$ transport and reduces survival in CFTR-deficient mice.** *Pflügers Archiv* 2015, **467**:1261-1275.

38. Sun L, Rommens JM, Corvol H, Li W, Li X, Chiang TA, Lin F, Dorfman R, Busson P-F, Parekh RV *et al*.: **Multiple apical plasma membrane constituents are associated with susceptibility to meconium ileus in individuals with cystic fibrosis.** *Nat Genet* 2012, **44**:562-569.

39. Miller MR, Soave D, Li W, Gong J, Pace RG, Boëlle P-Y, Cutting GR, Drumm ML, Knowles MR, Sun L *et al*.: **Variants in solute carrier *SLC26A9* modify prenatal exocrine pancreatic damage in cystic fibrosis.** *J Pediatr* 2015, **166**:1152-1157.

40. Geertsma ER, Chang Y-N, Shaik FR, Neldner Y, Pardon E, Steyaert J, Dutzler R: **Structure of a prokaryotic fumarate transporter reveals the architecture of the SLC26 family.** *Nat Struct Mol Biol* 2015, **22**:803-808.

41. Wallace DP, Tomich JM, Iwamoto T, Henderson K, Grantham JJ, Sullivan LP: **A synthetic peptide derived from glycine-gated $Cl^-$ channel induces transepithelial $Cl^-$ and fluid secretion.** *Am J Physiol Cell Physiol* 1997, **272**:C1672-C1679.

42. Sidorov V, Kotch FW, Abdrakhmanova G, Mizani R, Fettinger JC, Davis JT: **Ion channel formation from a calix[4]arene amide that binds HCl.** *J Am Chem Soc* 2002, **124**:2267-2278.

43. Jiang C, Lee ER, Lane MB, Xiao Y-F, Harris DJ, Cheng SH: **Partial correction of defective $Cl^-$ secretion in cystic fibrosis epithelial cells by an analog of squalamine.** *Am J Physiol Lung Cell Mol Physiol* 2001, **281**:L1164-L1172.

44. Shen B, Li X, Wang F, Yao X, Yang D. **A synthetic chloride channel restores chloride conductance in human cystic fibrosis epithelial cells.** *PLoS ONE* 2012, **7**:e34694.

45.Liu PY, Li ST, Shen FF, Ko WH, Yao XQ, Yang D. **A small synthetic molecule functions as a chloride-bicarbonate dual-transporter and induces chloride secretion in cells.** *Chem Commun* 2016; **52**:7380-7383.

46. Koulov AV, Lambert TN, Shukla R, Jain M, Boon JM, Smith BD, Li H, Sheppard DN, Joos J-B, Clare JP, *et al*.: **Chloride transport across vesicle and cell membranes by steriod-based receptors.** *Angew Chem Int Ed Engl* 2003, **42**:4931-4933.

47. McNally BA, Koulov AV, Lambert TN, Smith BD, Joos J-B, Sisson AL, Clare JP, Sgarlata V, Judd LW, Magro G, *et al*.: **Structure-activity relationships in cholapod anion carriers: enhanced transmembrane chloride transport through substituent tuning.** *Chemistry* 2008, **14**:9599-9606.

48. Valkenier H, Davis AP: **Making a match for valinomycin: steroidal scaffolds in the design of electroneutral, electrogenic anion carriers.** *Acc Chem Res* 2013, **46**:2898-2909.

49. Hussain S, Brotherhood PR, Judd LW, Davis AP: **Diaxial diureido decalins as compact, efficient, and tunable anion transporters.** *J Am Chem Soc* 2011, **133**:1614-1617.

50. Valkenier H, Judd LW, Li H, Hussain S, Sheppard DN, Davis AP: **Preorganized bis-thioureas as powerful anion carriers: chloride transport by single molecules in large unilamellar vesicles.** *J Am Chem Soc* 2014, **136**:12507-12512.

51. Galietta LJV, Haggie PM, Verkman AS: **Green fluorescent protein-based halide indicators with improved chloride and iodide affinities.** *FEBS Lett* 2001, **499**:220-224.

52. Li H, Valkenier H, Judd LW, Brotherhood PR, Hussain S, Cooper JA, Jurček O, Sparkes HA, Sheppard DN, Davis AP: **Efficient, non-toxic anion transport by synthetic carriers in cells and epithelia.** *Nat Chem* 2016, **8**:24-32.

(*) Using halide-sensitive YFP to monitor anion transport, the authors identify an artificial anion transporter with promising biological activity, including delivery to cell membranes, potency, persistence and lack of cytotoxic effects.

53. Cooper JA, Street STG, Davis AP: **A flexible solution to anion transport: powerful anionophores based on a cyclohexane scaffold.** *Angew Chem Int Ed Engl* 2014, **53**:5609-5613.

54. Wu X, Judd LW, Howe ENW, Withecombe AM, Soto-Cerrato V, Li H, Busschaert N, Valkenier H, Pérez-Tomás R, Sheppard DN *et al.*: **Nonprotonophoric electrogenic $Cl^-$ transport mediated by valinomycin-like carriers.** *Chem* 2016, **1**:127-146.

(*) The authors identify new anionophores, which promote electrogenic $Cl^-$ transport mimicking electrogenic $K^+$ transport by valinomycin. The data argue that encapsulation of the $Cl^-$ binding site is important for the design of anionophores with optimal $Cl^-$ transport properties.

55. Garcia MA, Yang N, Quinton PM. **Normal mouse intestinal mucus release requires cystic fibrosis transmembrane regulator-dependent bicarbonate secretion.** *J Clin Invest* 2009, **119**:2613-2622.

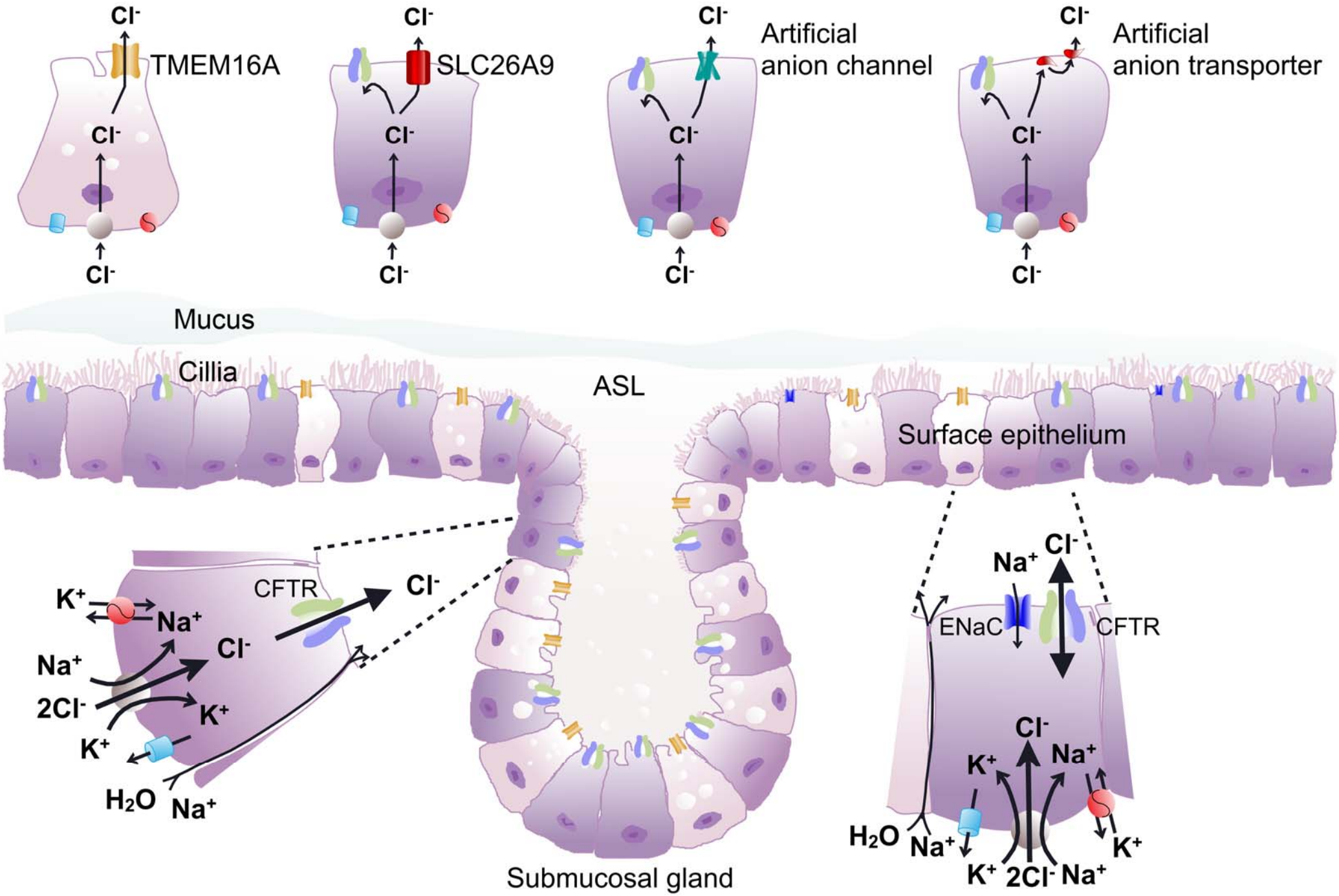
## FIGURE LEGENDS

**Figure 1: Pharmacological rescue of epithelial $Cl^-$ transport in CF by targeting alternative $Cl^-$ channels or using artificial anion channels and transporters** The schematic shows a thin layer of airway surface liquid (ASL) covering the airway surface epithelium and a submucosal gland. The activity of CFTR and the epithelial $Na^+$ channel (ENaC) in the apical membrane controls the quantity and composition of ASL. Submucosal glands deliver mucus and antimicrobial factors to the ASL. The insets show the cellular mechanism of CFTR-dependent $Cl^-$ secretion and mechanisms to bypass CFTR dysfunction in CF: activation of TMEM16A and SLC26A9, $Cl^-$ channels endogenously expressed in the apical membrane of goblet and ciliated cells of the airway epithelia and artificial anion channels and transporters that might be delivered to airway epithelia by nebulisation. Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; ENaC, epithelial $Na^+$ channel; SLC26A9, solute carrier 26 member A9; TMEM16A, transmembrane member 16A. For further information, see the text.

**Figure 2: Time course of $Cl^-$ current mediated by CFTR, TMEM16A, SLC26A9 and a bis-ureiododecalin anionophore** (**A – E**) Time courses of transepithelial $Cl^-$ current recorded with the Ussing chamber technique. The recordings are from human bronchial epithelia endogenously expressing CFTR and TMEM16A (A, B); Fischer rat thyroid (FRT) epithelia expressing recombinant SLC26A9 and control transfected epithelia (C); Madin Darby canine (MDCK) epithelia endogenously expressing canine CFTR (D) and FRT epithelia treated with a bis-ureiododecalin anionophore (2 μM) or the vehicle POPC (20 μM) (E). All recordings were made in the presence of a $Cl^-$ concentration gradient with $[Cl^-]_{basolateral} > [Cl^-]_{apical}$. In A-C, the epithelium was intact, whereas in D and E, the basolateral membrane was permeabilised with nystatin (0.36 mg $ml^{-1}$; Sigma-Aldrich). Arrows indicate the addition of the cAMP

agonists CPT-cAMP (100 μM) (A), forskolin (Fsk; 1 μM) with 3-isobutyl-1-methylxanthine (IBMX; 100 μM) (C) and forskolin (10 μM) (D); the $P2Y_2$ agonist UTP (100 μM) (B) and the start of the recording (C). Recordings were ended without using inhibitors to return current to baseline except A and E, where this was achieved using $CFTR_{inh}$-172 (I172; 10 μM) and by removing the $[Cl^-]$ gradient (E); dashed lines indicate the basal current level. For further information, see the text. Reproduced, with permission, from Salomon *et al*. [29] and Li *et al*. [50*].

**Figure 3: Cholapod and decalin anionophores** The chemical structures of some biologically-active cholapods (where R = $CF_3$ and $NO_2$; X = $OCH_3$) and decalins (where R = $CF_3$ and $NO_2$; X = $OC_2H_5$) are shown together with the crystal structure of the bis-ureiododecalin with $Me_4N^+Cl^-$. In the crystal structure, the dashed lines denote hydrogen bonds that bind the $Cl^-$ ion; the $Me_4N^+$ cation is shown in pink. Modified, with permission, from Li *et al*. [50*].


Cl-
TMEM16A
Cl-
Cl-
Cl-
SLC26A9
Cl-
Cl-
Cl-
Artificial
anion channel
Cl-
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Cl-
Artificial
anion transporter
Cl-
Cl-
Mucus
Cillia
ASL
Surface epithelium
K+
Na+
CFTR
Cl-
Na+
Cl-
2Cl-
K+
K+
H2O
Na+
Submucosal gland
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Cl-
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K+
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H2O
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K+
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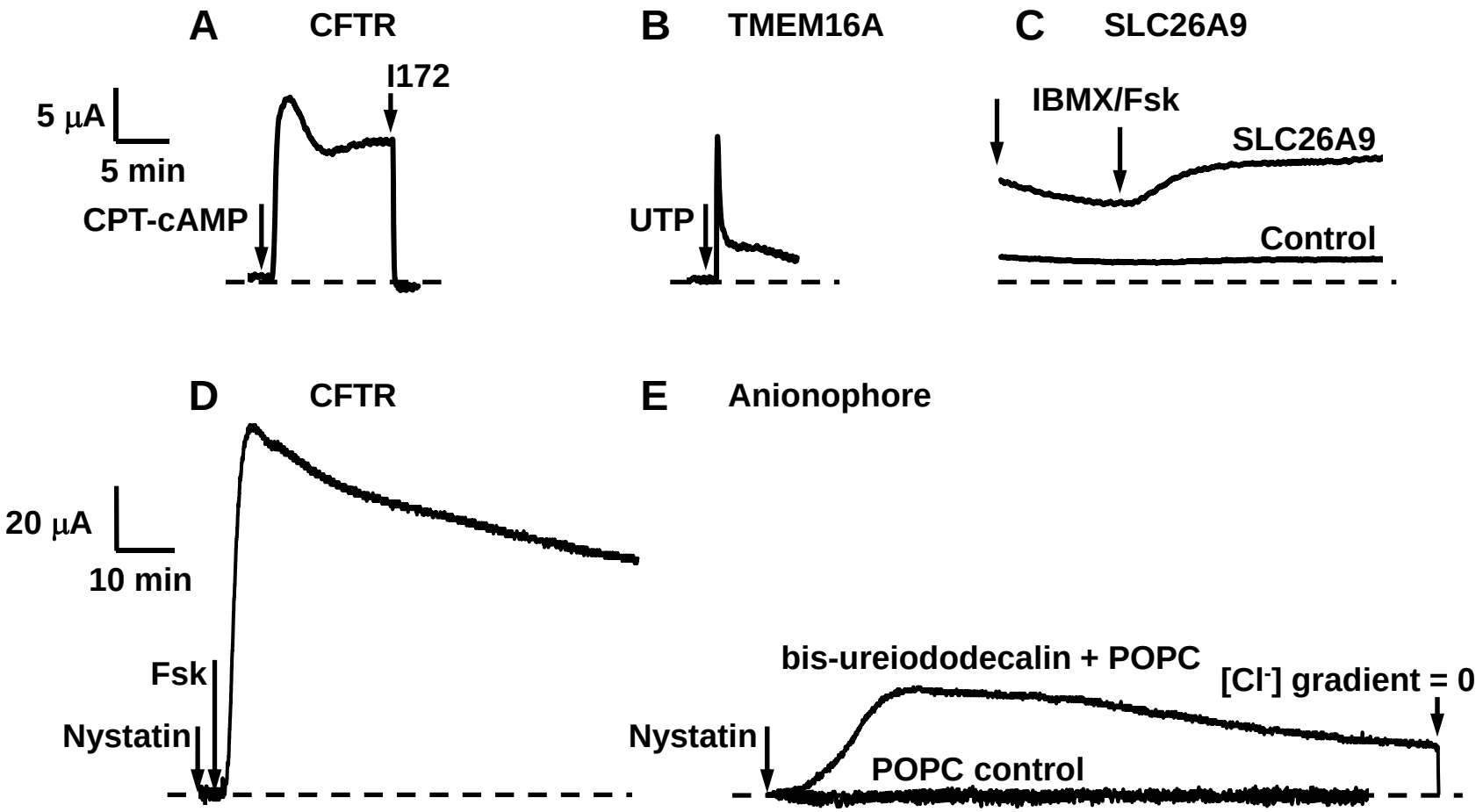
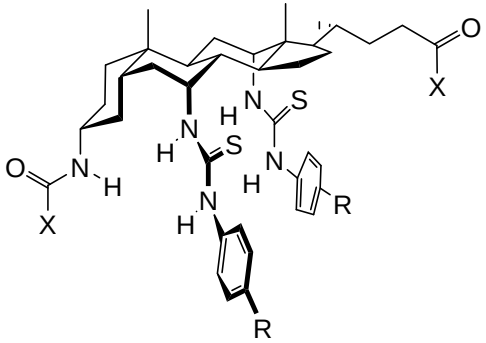
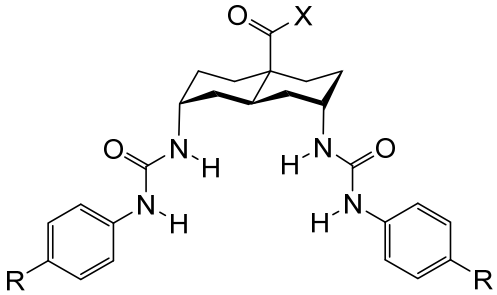

A
CFTR
I172
5 μA
5 min
CPT-cAMP
B
TMEM16A
UTP
C
SLC26A9
IBMX/Fsk
SLC26A9
Control
D
CFTR
20 μA
10 min
Fsk
Nystatin
E
Anionophore
bis-ureiododecalin + POPC
[Cl-] gradient = 0
Nystatin
POPC control

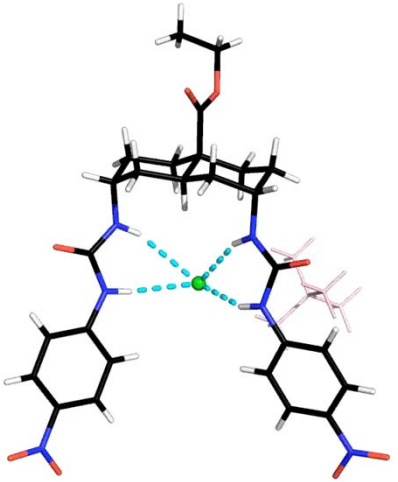
Figure 3




**Cholapod**




**Decalin**



**bis-ureiodecalin + $Me_4N^+Cl^-$**