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Curcumin/poly(2-methyl-2-oxazoline-b-tetrahydrofuran-b-2-methyl-2-oxazoline) formulation: An improved penetration and biological effect of curcumin in F508del-CFTR cell lines

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1 **Curcumin/poly(2-methyl-2-oxazoline-b-tetrahydrofuran-b-2-methyl-2-oxazoline)**

2 **formulation: An improved penetration and biological effect of curcumin in F508del-**

3 **CFTR cell lines**

4
5 Cristine Gonçalves^{1§}, Jean-Pierre Gomez^{1§}, William Mème¹, Bazoly Rasolonjatovo², David
6 Gosset¹, Steven Nedellec⁵, Philippe Hulin⁵, Cécile Huin², Tony Le Gall⁶, Tristan Montier⁶⁻⁷,
7 Pierre Lehn⁶, Chantal Pichon¹, Philippe Guégan^{3,4}, Hervé Cheradame^{2*} and Patrick Midoux^{1*}.

8
9 ¹ Centre de Biophysique Moléculaire, CNRS UPR4301 and Université d'Orléans, France.

10 ²Laboratoire Analyse et Modélisation pour la Biologie et l'Environnement, CNRS UMR8587
11 Université d'Evry Val d'Essonne, Evry, France.

12 ³Laboratoire de Chimie des Polymères, Sorbonne Universités, UPMC Univ Paris 06, UMR
13 8232, IPCM, Chimie des Polymères, F-75005, Paris, France.

14 ⁴CNRS, UMR 8232, IPCM, Chimie des Polymères, F-75005, Paris, France.

15 ⁵Plateforme MicroPICell IFR26 –IRT, Université de Nantes, Nantes, France.

16 ⁶INSERM 1078, équipe « Transfert de gènes et thérapie génique »; Faculté de Médecine et
17 des Sciences de la Santé, Université de Bretagne Occidentale ; Université Bretagne-Loire, 22
18 avenue Camille Desmoulins, 29238 Brest, France

19 ⁷Laboratoire de génétique moléculaire et d'histocompatibilité, CHRU de Brest, 5 Avenue du
20 Maréchal Foch, 29609 Brest cedex, France

21 *Corresponding authors: Hervé Cheradame: herve.cheradame@univ-evry.fr and Patrick

22 Midoux: patrick.midoux@cns-orleans.fr

23 [§]Both investigators contributed equally.

24 **Running title:** Curcumin/triblock copolymer and F508del-CFTR cells

25 **Keywords:** Triblock copolymer; amphiphilic polymer; Curcumin; CFTR; Cystic fibrosis

26 **Abstract**

27 Neutral amphiphilic triblock ABA copolymers are of great interest to solubilize hydrophobic
28 drugs. We reported that a triblock ABA copolymer consisting of methyl-2-oxazoline (MeOx)
29 and tetrahydrofuran (THF) (MeOx₆-THF₁₉-MeOx₆) (TBCP2) can solubilize curcumin (Cur) a
30 very hydrophobic molecule exhibiting multiple therapeutic effects but whose insolubility and
31 low stability in water is a major drawback for clinical applications. Here, we provide
32 evidences by flow cytometry and confocal microscopy that Cur penetration in normal and
33 ΔF508-CFTR human airway epithelial cell lines is facilitated by TBCP2. When used on
34 ΔF508-CFTR cell lines, the Cur/TBCP2 formulation promotes the restoration of the
35 expression of the CFTR protein in the plasma membrane. Furthermore, patch-clamp and
36 MQAE fluorescence experiments show that this effect is associated with a correction of a Cl⁻
37 selective current at the membrane surface of F508del-CFTR cells. The results show the great
38 potential of the neutral amphiphilic triblock copolymer MeOx₆-THF₁₉-MeOx₆ as carrier for
39 curcumin in a Cystic Fibrosis context. We anticipate that other MeOx_n-THF_m-MeOx_n
40 copolymers could have similar behaviours for other highly insoluble therapeutic drugs or
41 cosmetic active ingredients.

42

43

44 **1. Introduction**

45 Many chemotherapeutic drugs or cosmetic active ingredients are very insoluble in
46 water compromising their effectiveness and clinical applications. Delivery of drugs into the
47 cells relies on many constraints such as solubilization, endocytosis or crossing through the
48 membrane lipid bilayer. In this context, more particularly, polymer-based synthetic vectors
49 offer advantages such as relative simplicity of production, safety and versatility. Polyester/
50 ether ABA triblock copolymers developed as drug delivery system comprise poly(D,L-
51 lactide-block-ethylene oxide-block-D,L-lactide) (PLA-PEO-PLA), poly[(D,L-lactide-co-
52 glycolide)-block-ethylene oxide-block-(D,L-lactide-co-glycolide)] (PLGA-PEO-PLGA) and
53 poly(ϵ -caprolactone-block-ethylene oxide-block- ϵ -caprolactone (PCL-PEO-PCL) [1]. PPO-
54 PEO-PPO copolymers, Pluronic polymers, have been studied quite extensively thanks to the
55 formation of core-shell micelles comprising the polyethylene oxide (PEO) block as the
56 hydrophilic shell of the corona and the polypropylene oxide (PPO) block as the core [2, 3].
57 Such copolymers have already received large attentions to solubilise hydrophobic molecules
58 for drug delivery [4, 5]. Certain Pluronic polymers have shown capacity to pass the blood-
59 brain barrier [2]. They have even demonstrated their ability to increase the biodistribution
60 after oral delivery of molecules poorly soluble in water such as genestein [6]. In a clinical
61 phase I study, doxorubicin bound to a pluronic polymer has demonstrated an increased
62 efficacy compared to free doxorubicin [7]. In the field of immunization with proteins and
63 peptides, neutral amphiphilic copolymers have increased significantly the humoral and
64 cellular responses after intravenous injection [8]. Lutrol has provided efficient gene
65 expression in lung and skeletal muscles upon intratracheal and intramuscular co-injection in
66 mice of plasmid DNA, respectively [9, 10]. Moreover, DNA vaccination has been obtained
67 with DNA/amphiphilic block copolymer nanospheres [11].

68 Poly(2-oxazolines) based polymers are also interesting biomaterials to solubilize water
69 insoluble molecules [12]. Formulations based on poly(2-oxazoline) polymeric micelles and
70 the effect of parameters related to the structure, formulation, additives and toxicity have been
71 described [4, 13-16]. These reported properties allowed to conclude to the high potential of
72 poly(2-oxazoline) block copolymers, for instance in cancer treatments. Poly(2-methyl-2-
73 oxazoline-b-2-butyl-2-oxazoline-b-2-methyl-2-oxazoline) (p(MeOx-b-BuOx-b-MeOx)) has
74 been found to solubilize high quantity of paclitaxel, a very low soluble molecule in aqueous
75 media exhibiting powerful antineoplastic agent, and demonstrated an improved therapeutic
76 effect in xenograft mice tumor models [17, 18]. A clinical phase I study with a rotigotine
77 polyoxazoline conjugate has been initiated from data obtained in Parkinson's disease [19].

78 Recently, we have reported the synthesis and characterization of an original family of
79 poly(2-methyl-2-oxazoline-b-tetrahydrofuran-b-2-methyl-2-oxazoline) (MeOx-THF-MeOx)
80 neutral amphiphilic triblock copolymers and showed that some of them solubilized curcumin
81 [20]. Curcumin (diferuloylmethane) (Cur), the natural component of the plant "*Curcuma*
82 *Longa*" exhibits multiples therapeutic effects but is very insoluble in water [21]. This is a safe
83 drug even at high doses, but is rapidly metabolized and poorly absorbed by the cells [22].
84 Curcumin is proposed for treatment of various pulmonary diseases including Asthmas, cancer
85 and Cystic Fibrosis [23]. In order to evidence the interest of curcumin solubilisation by
86 MeOx-THF-MeOx copolymers, we decided to investigate its influence in a Cystic Fibrosis
87 context. Indeed, curcumin has been proposed to treat Cystic fibrosis (CF), the most lethal
88 genetic disease caused by mutations in the gene encoding the Cystic Fibrosis Transmembrane
89 Conductance Regulator (CFTR) Cl⁻ channel resulting in abnormal chloride transport at the
90 plasma membrane of many tissues and organs [24]. ΔF508 is the most common mutation of
91 CFTR causing a misfolding of the CFTR protein and its retention in the endoplasmic
92 reticulum (ER) for subsequent proteolytic degradation by the ubiquitin-proteasome pathway

93 [25]. Cur can partially compensate the ER retention of the defective CFTR protein, both *in*
94 *vitro* in appropriate cell lines and *in vivo* in F508del-CFTR mice [26, 27]. However, the
95 insolubility and poor stability of Cur in water explain some disappointing results observed in
96 mice treated with Cur [28]. Thus, it appeared for us that curcumin was a good example to test
97 the potential of our amphiphilic copolymers to carry hydrophobic molecules.

98 Here, we report that the MeOx₆-THF₁₉-MeOx₆ (TBPC2) copolymer facilitates
99 penetration of curcumin in F508del-CFTR cells and preserves the capacity of curcumin to
100 restore a functional CFTR protein.

101 **2. Material and Methods**

102 All reagents were purchased from Sigma (St. Quentin Fallavier, France) unless
103 otherwise stated.

104 *2.1. Polymer synthesis.*

105 Poly(2-methyl-2-oxazoline) – *b* – poly(tetrahydrofuran) – *b* – poly(2-methyl-2-
106 oxazoline) triblock copolymer (TBPC2). The copolymer was synthesized as described [20].
107 Briefly, to a 250 mL reaction flask containing 236 mmol (17 g) of dry THF, 13.9 mmol (3.91
108 g) of trifluoromethanesulfonic anhydride (Tf₂O) was added at -9°C. The reaction mixture was
109 stirred during 15 minutes and the polymerization was quenched by adding 55.6 mmol (4.7 g)
110 of MeOx at -9°C. Evaporation of residual THF under reduced pressure yielded to a pTHF
111 prepolymer as a white solid. This solid was dissolved in 40 mL of dry acetonitrile and the
112 temperature was increased to 80°C. MeOx (88.2 mmol; 7.5 g) was added and the solution
113 stirred for 24 hours. The reaction was quenched by 4 mL of 2 M sodium carbonate solution
114 and stirred for 1 hour at room temperature. The copolymer was dried and the crude yield was
115 80%. A chloroform/water extraction was then conducted, the organic phase evaporated and
116 dried for 2 days under vacuum. The triblock copolymer had hydroxyl and ester end functions
117 (47%) as determined by ¹H NMR. Molecular weight of TBPC2 determined by ¹H NMR was

118 2400 g.mol⁻¹ with a p(THF) block containing 19 monomers and 2 p(MeOx) blocks of 6
119 monomers each.

120 Rhodamine-labelled TBCP. First the amine terminated copolymer (diamino-TBCP) was
121 synthesized. The α,ω -diamino-poly(2-methyl-2-oxazoline) – block - poly(tetrahydrofuran) -
122 block - poly(2-methyl-2-oxazoline), was prepared as above but the polymerization was
123 quenched by addition of a large amount of 1,3-diaminopropane instead of MeOx. Molecular
124 weight of diamino-TBCP was 2200 g.mol⁻¹ with a central p(THF) block of 12 monomers and
125 2 external p(MeOx) blocks containing 8 monomers each. Despite these variations we have
126 considered that TBCP2 and diamino-TBCP have similar physicochemical behaviours.
127 Rhodamine-labelled TBCP was prepared by reaction of 33 mg diamino-TBCP in 1.5 ml of 0.1
128 M carbonate bicarbonate buffer, pH 9.3 with 14 mg (~2.6 equivalents) of N-
129 hydroxysuccinimide ester activated rhodamine (Invitrogen) dissolved in 0.5 ml dimethyl
130 sulfoxide. The mixture was stirred at room temperature during 24 hours in the dark. A silica
131 gel thin layer chromatography in chloroform/methanol (1/1 vol : vol) revealed the presence of
132 dye bound to the polymer that did not migrate; the fluorescent polymer did not react with
133 ninhydrin. Then, the polymer was purified by precipitation in dichloromethane; the coloured
134 precipitate was washed in ethyl ether and dried under reduced pressure.

135 2.2. *Curcumin solubilization*

136 Curcumin was purified by crystallisation in hot ethanol. Curcumin (10 g) was
137 solubilized in 20 mL pure ethanol under reflux. The hot solution was filtrated, the filtrate was
138 left to cool down and the Cur was collected by filtration (yield 60 %). A 0.2% TBCP2
139 solution was prepared by adding 5 ml H₂O to 10 mg TBCP2 and vigorous agitation with
140 vortex until complete dissolution. Then 2 mg of Cur were added to the TBCP2 solution, the
141 Cur/TBCP2 (400:2000; $\mu\text{g}:\mu\text{g}$) mixture was vortexed and then sonicated for 5 min at 20°C at

142 37 kHz. The solution was then clarified by centrifugation (14,100 x g for 10 min) to remove
143 any precipitate.

144 2.3. *Cells and cell culture*

145 The CFBE41o- human bronchial epithelial (homozygous for the F508del mutation)
146 [29] and ΣCFTE29o- human tracheal epithelial (homozygous for the F508del mutation) [30]
147 cell lines carrying the F508del *CFTR* mutation (so called CF cells) and the 16HBE14o-
148 normal human bronchial epithelial cell line [31] (generous gifts from Dieter Gruenert, San
149 Francisco, CA, U.S.A.) were cultured at 37°C in a 5% CO₂-humidified incubator in 20 ml
150 MEM with Earle's Salts (PAA Laboratories), 0.4% Penicillin (40 Units/ml)/Streptomycin (40
151 µg/ml) (PAA Laboratories), 2mM L-Glutamine (PAA Laboratories), supplemented with 10%
152 heat-inactivated fetal bovine serum (PAA Laboratories). Tissue culture plastic wares (75 cm²)
153 were coated for 20-30 min at 37°C with MEM with Earle's Salts containing fibronectin (0.01
154 mg/ml), collagen (0.03 mg/ml) and bovine serum albumin (BSA) (0.1 mg/ml). The culture
155 medium was changed every 2 days. The absence of mycoplasma in cell cultures was
156 determined by using MycoAlert® Mycoplasma Detection Kit (Lonza, Levallois Perret,
157 France).

158 2.4. *Curcumin uptake*

159 Two days prior to the experiments, 1.4×10^4 cells were seeded in 2 cm² well of a 4-
160 well plastic culture plate. For curcumin uptake, cells were incubated at 37°C with the
161 curcumin/ TBCP2 solution (stock solution: 1.1 mM Cur in 0.2% TBCP2) at various
162 concentrations. Then, the cells were washed twice with PBS, harvested by trypsin, centrifuged
163 (800 x g for 5 min at 4°C) and suspended in cold PBS. The cell-associated fluorescence
164 intensity was measured with a flow cytometer (FACSort, Becton Dickinson; $\lambda_{ex} = 488$ nm;
165 $\lambda_{em} = 530/30$ nm) before and after treatment with trypan blue (a final concentration of 0.004

166 %) in order to quench the extracellular fluorescence of curcumin. The fluorescence intensity
167 was expressed as the mean value of the fluorescence intensity (MFI) of 10,000 cells.

168 *2.5. Immunofluorescence assays*

169 Immunofluorescence was carried out with the following antibodies: mouse anti-human
170 CFTR antibodies (Clone M3A7, LifeSpan Biosciences) (dilution 1/50), rabbit anti-calreticulin
171 antibodies (LifeSpan Biosciences) (dilution 1/100), goat anti-calnexin antibodies (C20, Santa
172 Cruz Biotechnology) (dilution 1/100), goat anti-ERGIC-53 antibodies (A-18, Santa Cruz
173 Biotechnology) (dilution 1/100), Alexa Fluor 568 goat anti-mouse antibodies (Invitrogen)
174 (dilution 1/200), Alexa Fluor 568 donkey anti-goat antibodies (Invitrogen) (dilution 1/200),
175 Cy5 sheep anti-mouse antibodies (Jackson Immunoresearch) (dilution 1/100).

176 *2.6. Confocal microscopy*

177 The cells (1.4×10^4) were seeded on glass coverslips (14 mm diameter) in 2 cm² well
178 of a 4-well plastic culture plate. Intracellular locations were performed by
179 immunofluorescence. The cells were washed several times with ice-cold PBS, fixed in cold
180 methanol (90% in PBS) for 30 min at -20°C, washed several times with PBS containing 0.5%
181 bovine serum albumin (PBS-0.5%BSA). Coverslips were incubated with primary antibodies
182 in PBS-1%BSA for 1 h at 20°C, washed with PBS-0.5%BSA and then incubated for 45 min at
183 20°C with secondary fluorescent antibodies in PBS-1%BSA. After several washes with PBS,
184 coverslips were mounted in Vectashield (Vector Laboratories, Inc., Burlingame, CA, USA).
185 Confocal laser scanning microscopy (CLSM) was performed using a Zeiss Axiovert 200M
186 microscope coupled with a Zeiss LSM 510 scanning device (Carl Zeiss Co. Ltd., Jena,
187 Germany). The inverted microscope was equipped with a Plan-Apochromat 63X objective
188 (NA=1.4). The fluorescence was measured at either 560 nm upon excitation at 543 nm (Alexa
189 Fluor 568), or 660 nm upon excitation at 633 nm (Cy5).

190 *2.7. Electrophysiological measurements*

191 The cells were cultured on a glass coverslip that was transferred to the experimental
192 chamber of an upright microscope (BX51WI, Olympus Corporation, Tokyo, Japan). Patch-
193 clamp experiments were performed at 21-23°C. Cells were placed in continuously flowing (1-
194 2 ml/min) bath solution containing (mM): 150 NaCl; 6 CsCl; 1 CaCl₂; 1 MgCl₂; 10 D-
195 glucose; 10 HEPES (adjusted to pH 7.4 with Tris) and identified at 60x magnification with a
196 CCD camera (XC-ST70CE, Sony). Somatic whole-cell recordings were performed as
197 previously described [32]. Briefly, low resistance (4-6 MΩ) patch-pipettes pulled on a vertical
198 puller (PB-7, Narishige, Tokyo, Japan) from borosilicate capillaries (Clark Electromedical
199 Instruments, Edenbridge, UK) were filled with internal solution containing (mM): 100 L-
200 aspartic acid; 94 CsOH; 26 CsCl; 14 NaCl; 1 MgCl₂; 3 MgATP; 1 EGTA; 10 HEPES
201 (adjusted to pH 7.3 with Tris). Signals were amplified using the MultiClamp 700B amplifier
202 (Axon Instruments, Foster City, CA). Series resistance was monitored continuously and was
203 typically compensated by 60-70 % in whole-cell configuration. Voltage-clamp recordings
204 were filtered at 4 kHz, sampled at 10 kHz using a data acquisition board (Digidata 1322A,
205 Axon Instruments) operated by Pclamp10 software (Axon Instruments). Off-line analysis was
206 performed using Clampfit10 (Axon Instruments) and Origin8 (Origin Lab Corporation,
207 Northampton, MA). 16HBE14o- and ΣCFTE29o- cells were cultured under control conditions
208 or in the presence of Cur/TBCP2 treatment. Currents were elicited by voltage steps applied
209 from -110 mV to +70 mV (10 mV increments, 400 ms duration) from a holding potential
210 (HP) of -40 mV. Steady-state current amplitude was measured at the end of the pulse and
211 normalized to the cell membrane capacitance (17.6 ± 1.5 pF, n=19 on 16HBE14o- cells; 22.1
212 ± 1.2 pF, n=28 on ΣCFTE29o- cells).

213 2.8. MQAE fluorescence assay

214 The Cl⁻ channel activity of CFTR was assessed on 16HBE14o- and CFBE41o- cells
215 using the halide-sensitive fluorescent probe MQAE [33]. Cells were loaded with MQAE

216 intracellular dye by incubation for 8 min at 37°C in a hypotonic medium (mM) (110 NaI, 1.92
217 K₂HPO₄, 0.64 KH₂PO₄, 8 HEPES , 0.8 CaSO₄, 8 D-Glucose, pH 7.4) containing 10 mM
218 MQAE. Coverslips were mounted on the stage of an inverted microscope (LEICA DMI
219 6000B) equipped for fluorescence, and cell fluorescence was excited at 380 nm. The emitted
220 fluorescence was detected at 470 nm by a CCD camera coolsnap HQ2 (Roper). Cells were
221 maintained at 37°C and continuously perfused with an extracellular bath solution containing
222 (mM): 138 NaI, 2.4 K₂HPO₄, 0.8 KH₂PO₄, 10 HEPES, 1 CaSO₄, 10 D-Glucose, pH 7.4. A
223 superfusion system (Biosciences Tools) allowed rapid change of different extracellular
224 experimental media. Cells were sequentially perfused with 138 mM I⁻ buffer solution, 138
225 mM NO₃⁻ buffer solution, 138 mM NO₃⁻ buffer solution with 0.5 mM 8-(4-chlorophenyl)thio-
226 cyclic AMP (8-CPT-cAMP) and again with 138 mM I⁻ buffer solutions. Single cell
227 fluorescence intensity was plotted against time at an acquisition rate of 1 image *per* 15
228 seconds. Fluorescence intensity was normalized to the initial fluorescence level measured in
229 the presence of I⁻.

230 **2. Results**

231 *3.1. Curcumin solubilisation and penetration into CF and normal airway epithelial cell lines*

232 The MeOx₆-THF₁₉-MeOx₆ ABA copolymer (TBCP2) of 2400 g.mol⁻¹ was composed
233 of 12 poly(2-methyl-2-oxazoline) (MeOx) hydrophilic blocks A and 19 polytetrahydrofuran
234 (THF) hydrophobic blocks B (Fig. 1A). The chemical shifts of the pTHF blocks were at 1.61
235 ppm and 3.40 ppm, those of the pMeOx ones at 2.13 ppm (CH₃ groups) and 3.42 ppm and
236 those of the telechelic CH₂ groups adjacent to the end hydroxyl functions at 3.78 ppm (Fig.
237 1C). The surprising high dispersity ($D = 4.5$) was attributed to the low molar mass of the
238 pTHF block associated to the reversible cationic ring opening polymerization of THF. The
239 standard conditions for MeOx polymerization allowed expecting a much better control of
240 these blocks synthesis as previously reported [34]. The amphiphilic feature in aqueous

241 solution and self-assembling to form micelles of TBCP2 were determined by different
242 techniques including Nile Red fluorescence spectroscopy (F), isothermal titration calorimetry
243 (ITC), dynamic light scattering (DLS) [20]. The critical micellar concentration was 3.2×10^{-3}
244 mol.L^{-1} , $6.5 \times 10^{-4} \text{ mol.L}^{-1}$, $4.2 \times 10^{-4} \text{ mol.L}^{-1}$ to $1 \times 10^{-3} \text{ mol.L}^{-1}$ as determined by F, ITC and
245 DLS, respectively. In a 0.2% solution in water, TBCP2 formed micelles with a hydrodynamic
246 diameter of $16 \pm 1.4 \text{ nm}$ as determined by DLS (Fig. 1D). The solubilisation of 2 mg Cur (400
247 $\mu\text{g/ml}$; 1.1 mM) was achieved by mixing, vortexing and then sonicating 2 mg Cur in 5 ml of
248 0.2% TBCP2 in water (Fig. 1B). In contrast, Cur was completely insoluble in water in the
249 absence of TBCP2 (Fig. 1B). The resulting Cur/TBCP2 formulation (17 wt.%) formed
250 nanoparticles of $255 \pm 30 \text{ nm}$ (Fig. 1D).

251 We then tested whether the Cur/TBCP2 formulation allowed for Cur uptake by the
252 cells. For this purpose, two CF ($\Sigma\text{CFTE29o-}$ and CFBE41o-) and one normal (16HBE14o-)
253 human airway epithelial cell lines were incubated in complete culture medium at 37°C in the
254 presence of Cur solubilized in TBCP2 or TBCP2 without Cur. After 2h incubation, Cur
255 location was analysed by fluorescence microscopy thanks to Cur fluorescence at 520 nm upon
256 excitation at 488 nm. As shown in Figure 2, the fluorescence was in the cytoplasm with
257 Cur/TBCP2 showing penetration of Cur in these cell lines. In contrast, no fluorescence was
258 detected in the cells incubated with TBCP2 in the absence of Cur or the supernatant of Cur in
259 PBS (Fig. 2). The Cur fluorescence intensity associated with the cells was measured by flow
260 cytometry after 2h incubation with various dilution of the Cur/TBCP2 formulation. As shown
261 in Figure 3, the mean fluorescence intensity (MFI) increased with the Cur concentration.
262 Those MFI corresponded to the fluorescence of Cur. Indeed, MFI of the three cell lines
263 incubated with TBCP2 at the concentration used to solubilize $220 \mu\text{M}$ Cur were the same as
264 MFI (2.5 A.U) measured for those cells incubated in the medium without any polymer and
265 Cur. MFI were reduced after treatment with trypan blue which quenched the extracellular

266 curcumin fluorescence and thus the residual MFI were indicative of Cur internalization. The
267 amounts of Cur associated with the three cell lines and that taken up by the three cell lines
268 were comparable. About ~55% of Cur associated with the cells was inside the cells. For
269 information, MFI upon trypan blue treatment of Σ CFTE29o-, 16HBE14o- and CFBE41o-
270 cells incubated with 27.5 μ M Cur solubilized in DMSO were 45, 13 and 18, respectively,
271 indicating that the penetration of Cur was larger than Cur solubilized in TBCP2 micelles. We
272 evaluated by confocal microscopy the uptake of the copolymer by incubating those cell lines
273 for 4h at 37°C with a Cur/Rho-TBCP formulation (Fig. 4). The images showed that the
274 copolymer did not enter deeply into the cytoplasm of Σ CFTE29o- cells while curcumin enters
275 the cells. It was mostly remained close to the cell surface either at and/or under the cell
276 surface while a few amount of polymer was inside the cells. Regarding the bronchial
277 epithelial cells (CFBE41o-and 16HBE14o- cells), the presence of yellow spots in the
278 cytoplasm was indicative of higher TBCP internalization than in the tracheal epithelial cells
279 (Σ CFTE29o- cells). Next, the evaluation of the effect of curcumin on CFTR in F508del-CFTR
280 cells was studied. The CFTR intracellular location was performed by immunofluorescence
281 and confocal microscopy analysis, and its functionality (assessed *via* chloride current
282 measurements) by patch-clamp experiments and MQAE fluorescence assay.

283

284 3.2. *Effect of Cur/TBCP2 on CFTR intracellular location*

285 Before testing the Cur/TBCP2 effect, the intracellular distribution of CFTR was
286 examined in F508del-CFTR and normal cells by immunofluorescence and confocal
287 microscopy. The cells were labelled with anti-CFTR antibodies and also with antibodies
288 directed against calreticulin or calnexin which are two quality-control chaperones that bind to
289 misfolded proteins such as the muted CFTR and prevent them from being exported from the
290 ER to the Golgi apparatus for complete glycosylation. The cells were also stained with
291 antibodies directed against ergic-53 because previous observations suggested that F508del-

292 CFTR was also present in the Endoplasmic reticulum intermediate compartment (ERGIC)
293 [35]. Confocal microscopy images showed that in normal (16HBE14o-) cells, CFTR was
294 located at the periphery of the cells or at the plasma membrane (blue spots) and very few
295 colocations (white spots) were observed with calreticulin or calnexin (Fig. 5). CFTR was
296 logically detected also in ergic-53 en route for its glycosylation in the Golgi. In Σ CFTE29o-
297 cells, the F508del-CFTR was mostly concentrated in the perinuclear region and strongly
298 colocalized with antibodies directed against calnexin and ergic-53 in line with the retention of
299 the mutated protein in the ER/ERGIC compartments of cells expressing the F508del-CFTR
300 (Fig. 5). The retention effect in ERGIC looked stronger in Σ CFTE29o- cells with the presence
301 of many white spots and no blue ones than in CFBE41o- cells. The F508del-CFTR
302 distribution in CFBE41o- looked like that the CFTR in 16HBE14o- cells suggesting
303 localization in the plasma membrane. However, it was reported that the F508del-CFTR was
304 distributed in the cytoplasm of CFBE41o- cells [36]. The CFBE41o- cells are bronchial
305 epithelial cells while the Σ CFTE29o- cells are from tracheal epithelial cells. The intracellular
306 distribution of the F508del-CFTR could vary with the nature of the pulmonary epithelial
307 tissues [37]. Of note, the F508del-CFTR location in the calreticulin compartment looked weak
308 in the CF cell lines.

309 When Σ CFTE29o- cells were incubated overnight with 220 μ M Cur solubilized with
310 TBCP2, CFTR was not located in the ER/ERGIC compartment near the nucleus as in absence
311 of Cur but its distribution was similar as in the normal 16HBE14o- cells indicating CFTR
312 redistribution in the presence of Cur (Fig. 6). This redistribution after incubation of
313 CFBE41o- cells with the Cur/TBCP2 solution was not as demonstrative as in Σ CFTE29o-
314 cells (Fig. 6). This could be explained by the lower retention of F508del-CFTR in ERGIC of
315 CFBE41o- cells comparatively to Σ CFTE29o- cells (Fig 5). As expected, no modification was
316 observed in normal 16HBE14o- cells in which CFTR was mostly at the plasma membrane

317 (Fig. 6). The images reconstitution of several z steps as well as sections of the z-step gallery
318 passing through the middle of a representative Σ CFTE29o-cell showed clearly that F508del-
319 CFTR was concentrated at the nucleus periphery in the absence of Cur while it was
320 distributed throughout the cell and at the plasma membrane in the presence of Cur (Fig. 7).
321 These results evidenced that incubation of F508del-CFTR cells with the Cur/TBCP2 solution
322 promoted the relocation of the CFTR at the plasma membrane.

323 *3.3. CFTR current specific activation by Cur/TBCP2 treatment*

324 Whole-cell patch-clamp experiments were carried out to determine the activation of
325 CFTR chloride current after incubation of CF cells with Cur/TBCP2 solution. First, whole-
326 cell patch-clamp experiments were performed in normal (16HBE14o-) and CF (Σ CFTE29o-)
327 cells in the absence of Cur/TBCP2. The measurement of the associated averaged steady-state
328 current-voltage (I/V) relationships revealed the presence of a high chloride induced current
329 (15.3 ± 2.8 pA/pF) in normal cells (Fig. 8Aa and 8B black squares) whereas in CF cells (Fig.
330 8Ab and 8B, black circles) a weak chloride current (3.1 ± 0.5 pA/pF) was measured at a
331 potential of + 60 mV. In the presence of 10 μ M CFTRinh-172 - a specific CFTR inhibitor -
332 [38] the chloride current was strongly inhibited in 16HBE14o- cells (Fig. 8B, white squares).
333 In contrast the effect was limited in CF cells (Fig. 8B, white circles). The CFTRinh-172
334 current sensitivity in 16HBE14o- cells (9.3 ± 2.9 pA/pF at a potential of + 60 mV) was 7-fold
335 higher than in Σ CFTE29o- cells (1.3 ± 0.3 pA/pF). The chloride current measured in both cell
336 lines was also inhibited by 20 μ M GlyH-101 - another specific CFTR inhibitor - [39] (data not
337 shown). All together these data demonstrated that chloride currents measured on these
338 different cell lines corresponded to a CFTR chloride current and were in line with those as
339 previously described for these two cell lines [40, 41].

340 Figure 9 presents characteristic whole-cell currents in Cur/TBCP2-treated Σ CFTE29o-
341 cells in the absence and the presence of 10 μ M CFTRinh-172. CFTR currents were

342 equivalently blocked on this cell lines by CFTRinh-172 and GlyH-101, the experiments were
343 then conducted with CFTRinh-172 only. Compared to untreated cells, the incubation with
344 Cur/TBCP2 induced a current enhancement that was strongly inhibited in the presence of
345 CFTRinh-172 (Fig. 9A). The CFTRinh-172 current sensitivity was calculated as the
346 difference between currents measured prior and after CFTR-inh 172 exposure, in the same
347 way as done for Fig. 9B. CFTRinh-172 current sensitivity plotted as current-voltage (I-V)
348 relationships revealed that incubation of Σ CFTE29o- cells with Cur/TBCP2 (120 μ M)
349 induced a statistically significant increase in CFTRinh-172 current sensitivity at all membrane
350 potential tested (Fig. 9B). Cur/TBCP2 induced a linear conductance typical of a Cl^- selective
351 current. At + 30mV, the current density reached 4.5 ± 1 pA/pF with Cur/TBCP2 *versus* $1 \pm$
352 0.6 pA/pF under basal conditions. The observed zero current potential was -39.3 ± 0.1 mV
353 ($n= 19$) a value that closely matched the chloride reversal potential (-33.8 mV) predicted by
354 the Nernst equation with an internal $[\text{Cl}^-]$ of 42 mM and external $[\text{Cl}^-]$ of 160 mM, suggesting
355 channel selectivity for Cl^- . All together, these results strongly supported that Cur/TBCP2
356 induced the expression of a Cl^- selective current at the membrane surface of Σ CFTE29o- cells
357 typical of new functional CFTR channels.

358 2.5. CFTR Cl^- activity assessed by MQAE fluorescence assay

359 The functional measurement of the CFTR expression in the different airway epithelial
360 cells was also assessed by using the chloride fluorescent probe MQAE [33]. The fluorescence
361 of MQAE is quenched in the presence of chloride or iodide ions in CF cells but its
362 fluorescence is restored in response to $\text{I}^- / \text{NO}_3^-$ substitution and cAMP stimulation after CFTR
363 restoration. Figure 10 presents MQAE fluorescence measurements using I^- , NO_3^- , $\text{NO}_3^- +$
364 cAMP (8-CPT-cAMP) solution substitution protocol in normal 16HBE14o-, untreated and
365 Cur/TBCP2-treated CFBE41o- cell monolayers. In contrast to 16HBE14o- cells (Fig. 10A),

366 MQAE fluorescence was not changed in untreated CFBE41o- cells upon I⁻ substitution by
367 NO₃⁻ or was slightly induced by cAMP + NO₃⁻ (amplitude 10.96 ± 2.6) (Fig. 10B).

368 The incubation of the CFBE41o- cell monolayer with Cur/TBCP2 (165 μM)
369 drastically restored the MQAE fluorescence response to I⁻ / NO₃⁻ substitution (amplitude 24.4
370 ± 4.4) and particularly in response to cAMP stimulation (amplitude 39.55 ± 6.6) (Fig. 10C).
371 The fluorescence amplitude variation of the fluorescence intensity used as an index of CFTR
372 activity demonstrated a specific restoration of the CFTR activity in CF cells treated with
373 Cur/TBCP2 close to that observed in normal cells (Fig. 10D). Of note, MQAE fluorescence
374 testing was not performed with ΣCFTE29o- cells because the functional restoration of
375 F508del-CFTR was convincing by Patch-clamp measurements (Fig 9). In contrast it was less
376 demonstrative with CFBE41o- cells.

377 **3. Discussion**

378 Several strategies have been explored to improve solubility and stability of Cur in
379 water. For instance, Cur was conjugated to sugars [42, 43], amino acids [44] and polyethylene
380 glycol [45, 46]. Formulations with carriers were also explored [47, 48]. For instance, Cur
381 solubility and activity were improved upon inclusion in cyclodextrin [49] or encapsulation in
382 liposomes [50-52], chitosan/poly(butyl cyanoacrylate) nanoparticles [53, 54], PLGA
383 nanoparticles [55] or methoxy poly(ethylene glycol)-block-polycaprolactone diblock
384 copolymers micelles [56]. Cur effect was enhanced in CF mice upon oral administration of
385 Cur encapsulated in biodegradable nanoparticles made of an oil-in-water emulsion mixture of
386 poly (lactic-co-glycolic acid) (PLGA) and poly(vinyl alcohol) [57]. ABA copolymers
387 comprising poly(ethylene oxide) (PEO) block as the hydrophilic moiety and poly(propylene
388 oxide) (PPO) block as hydrophobic moiety forming core-shell micelles were also used.
389 However, PEO polymers due either to the polymer itself or a side product formed during its
390 synthesis can generate various unfavourable effects [58]. Polyoxazoline was proposed as an

391 alternative to PEO [15, 59] and a family of poly(2-methyl-2-oxazoline-b-tetrahydrofuran-b-2-
392 methyl-2-oxazoline) (MeOx-THF-MeOx) neutral amphiphilic triblock copolymers (TBCP)
393 have been synthesized [20]. Our Cur/TBCP2 formulation (17 wt.%) was obtained by
394 solubilisation of Cur (0.5 mg) in a water solution of micelles of TBCP2 copolymer (2 mg/ml;
395 2400 g.mol⁻¹). Comparatively, the preparation of 1 mg/ml curcumin with 10 mg/ml of 1,2-
396 dimyristoyl-sn-glycero-3-phosphocholine and 1,2-dimyristoyl-sn-glycero-3-phospho-rac-(1-
397 glycerol) liposomes was described [52]; the preparation of 14% wt.% and 7.6% wt.%
398 curcumin formulations were reported with methoxy poly(ethylene glycol)-block-
399 polycaprolactone diblock copolymers micelles [56] and poly (lactic-co-glycolic acid) (PLGA)
400 and poly(vinyl alcohol) nanoparticles [57], respectively. With another highly hydrophobic
401 drug, the preparation of a 45 wt.% Paclitaxel formulation was successfully achieved with the
402 MeOx₃₇-b-BuOx₂₃-b-MeOx₃₇, a triblock copolymer of 10 000 g.mol⁻¹ [15]. The resulting
403 Cur/TBCP2 solution formed nanoparticles of ~250 nm while a 0.2% TBCP2 in water formed
404 micelles of ~16 nm. The large size of TBCP2/Cur nanoparticles could result from the
405 coalescence of TBCP2 micelles around Cur aggregates. Here, we show that TBCP2 allowed
406 Cur internalization by human airway epithelial cell lines. Yet, the penetration of Cur was
407 lower than when Cur was solubilized in DMSO which allows Cur diffusion through the
408 plasma membrane. The uptake mechanism of Cur in TBCP2 micelles is not yet determined
409 but it could process *via* a transient destabilization of the plasma membrane by TBCP2.
410 Indeed, Rho-TBCP does not enter deeply into the cytoplasm of the tracheal epithelial cells
411 while Cur enters the cells. This suggests that once bound to the plasma membrane, the
412 copolymer would destabilize the lipid bilayer inducing Cur diffusion through the plasma
413 membrane. The copolymer that is more internalized in the bronchial epithelial cells would in
414 addition induce the formation of transient pores in endocytic vesicles allowing Cur diffusion
415 in the cytoplasm. This latter hypothesis is supported by data showing that a neutral

416 amphiphilic diblock copolymer - a poly(ethylene oxide-b-4-vinylpyridine) - was able to form
417 transient pores in a lipid artificial membrane allowing the passage of a plasmid DNA of ~5
418 kbp across the model membrane [60].

419 Here, we have evaluated the biofunctionality of curcumin after solubilisation by
420 TBCP2. For this purpose, we have tested whether the Cur/TBCP2 formulation could restore
421 the expression of a functional CFTR protein at the surface of F508del-CFTR cells. Indeed,
422 F508del-CFTR is not expressed at the plasma membrane because it is retained in the
423 ER/ERGIC compartment by the quality control system involving calreticulin and/or calnexin
424 [61]. Our immunofluorescence analyses performed on two F508del-CFTR cell lines verified
425 that F508del-CFTR colocalizes with calnexin and calreticulin. They confirmed also that
426 F508del-CFTR colocalizes with ERGIC-53, a protein specific of the ERGIC compartment as
427 previously reported [35]. p58/ERGIC-53 is a calcium-dependent lectin recognizing mannose
428 that cycles between the ER and the Golgi apparatus and functions as a cargo receptor for a
429 subset of soluble glycoproteins exported from the ER. The lectin domains of ERGIC-53 and
430 calnexin are structurally similar. F508del-CFTR is not exported to the Golgi for maturation
431 but is driven to the cytosolic ubiquitin-proteasome machinery for degradation. It has been
432 shown that ERGIC compartment accumulates proteins on the way for degradation as the
433 precursor of human asialoglycoprotein receptor H2a and free heavy chains of murine class I
434 major histocompatibility complex (MHC) [62]. Significant amounts of various ER resident
435 proteins have been detected in ERGIC indicating that a leak of calnexin from ER into ERGIC
436 might occur [63]. Thus, calnexin could be transiently present in ERGIC which could be a site
437 for the concentration and retrotranslocation of proteins that are transported to the cytosol.
438 That explains colocalizations between F508del-CFTR and p58/ERGIC-53 observed in the
439 F508del-CFTR cell lines.

440 Cur was shown to partially compensate the ER retention of the defective CFTR
441 protein in appropriate cell lines and in F508del-CFTR mice [26, 27]. We demonstrate that
442 incubation of F508del-CFTR cell lines with Cur/TBCP2 induces the relocation of the mutated
443 CFTR protein at the plasma membrane. This effect resulted likely from the better cellular
444 penetration of Cur upon solubilization in the TBCP2 solution. The functionality of CFTR
445 chloride channels in the apical membrane of CF cells after incubation with Cur/TBCP2 is
446 clearly revealed by whole-cell patch-clamp experiments and MQAE fluorescence assay. In
447 the former, the specificity of the activation of CFTR currents is provided by the use of
448 CFTRinh-172 or GlyH-101, two specific CFTR inhibitors that both induce strong inhibition
449 of restored currents in CF cells treated with Cur/TBCP2. CFTRinh-172 is a well-known and
450 widely used specific inhibitor of CFTR channels, without affecting other chloride
451 conductance such as the Ca^{2+} -dependent Cl^- conductance (CaCC) or the volume-sensitive
452 outwardly rectifying Cl^- conductance (VSORC). GlyH-101 appears less specific as it inhibits
453 both VSORC and CaCC at concentrations close to those used to inhibit CFTR conductance
454 [64]. Therefore, our calculated CFTRinh-172 current sensitivity was due to CFTR current.
455 The fluorescence assay with the chloride sensitive fluorescent probe MQAE which is
456 quenched in the presence chloride or iodide ions is drastically restored in CF cells after
457 Cur/TBCP2 treatment in response to $\text{I}^- / \text{NO}_3^-$ substitution and cAMP stimulation. All
458 together, these results provide strong evidences that the CFTR relocation leads to a functional
459 chloride channel.

460 The exact mechanism by which Cur allows the F508del-CFTR relocation is not fully
461 understood. Cur is an inhibitor of the sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase
462 (SERCA) involved in the translocation of calcium from the cytosol to the sarcoplasmic
463 reticulum lumen [65-67]. The lectin-like recognition of calreticulin, calnexin and ERGIC-53
464 that binds to the terminal glucose of the N-oligosaccharide structure of misfolded

465 glycoproteins is calcium-dependent. SERCA inhibition by Cur *via* the modulation of
466 sarcoplasmic reticulum calcium content could prevent retention of F508del-CFTR in the
467 ER/ERGIC favouring its delivery in the Golgi apparatus for maturation and exocytosis to the
468 plasma membrane. It has also been reported that the trafficking of CFTR to the plasma
469 membrane involves the keratin 8/keratin 18 network of intermediate filaments [68, 69]. An
470 important remodelling in the keratin 18 network has been indeed observed in Cur-treated cells
471 with an increase in the phosphorylation of K18 Ser52 in a calcium-independent manner [70].
472 This reorganization reduces the intracellular trafficking of organelles mediated by the
473 intermediate filaments and the turnover of some membrane proteins as CFTR. Inhibitions by
474 Cur of the ER retention of F508del-CFTR and its turnover from the plasma membrane could
475 contribute to the restoration of a functional CFTR in the plasma membrane. Curcumin was
476 proposed to treat Cystic Fibrosis but its multiple therapeutic effects as well as the absence of
477 fully understanding mechanism (s) of action delay its FDA agreement. For this reason,
478 curcumin is often named as pan-assay interference (PAIN) compound, classes of compounds
479 that can interfere with bioassays *via* a number of different mechanisms [71].

480 **4. Conclusion**

481 We demonstrated the great potential of the MeOx₆-THF₁₉-MeOx₆ copolymer to
482 solubilize a very insoluble molecule such as curcumin. In a cystic fibrosis context, we showed
483 that curcumin better penetrated normal and F508del-CFTR human airway epithelial cells and
484 promoted a functional expression of the mutated CFTR protein in the plasma membrane of
485 the CF cells. More generally, MeOx_n-THF_m-MeOx_n copolymers could help solubilisation of
486 other water insoluble drugs or cosmetic ingredients contributing to their better applications.

487

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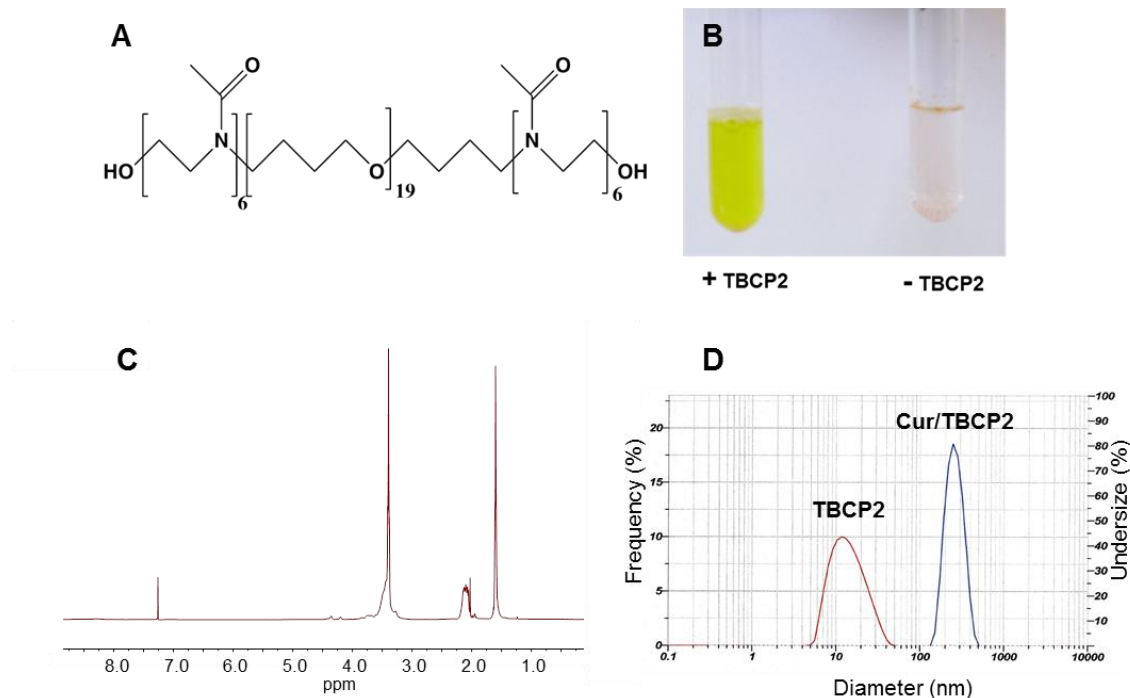
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Figure 1: (A) Chemical structure of the amphiphilic triblock copolymer MeO_x₆-THF₁₉-MeO_x₆ (TBCP2). (B) Cur solubilisation in a TBCP2 aqueous solution. Two mg Cur was added to 5 ml of a 0.2% TBCP2 solution in water (+TBCP2) or in 5 ml water (-TBCP2). (C) ¹H NMR spectrum of TBCP2. (D) Size of TBCP2 micelles and Cur/TBCP2 formulation.

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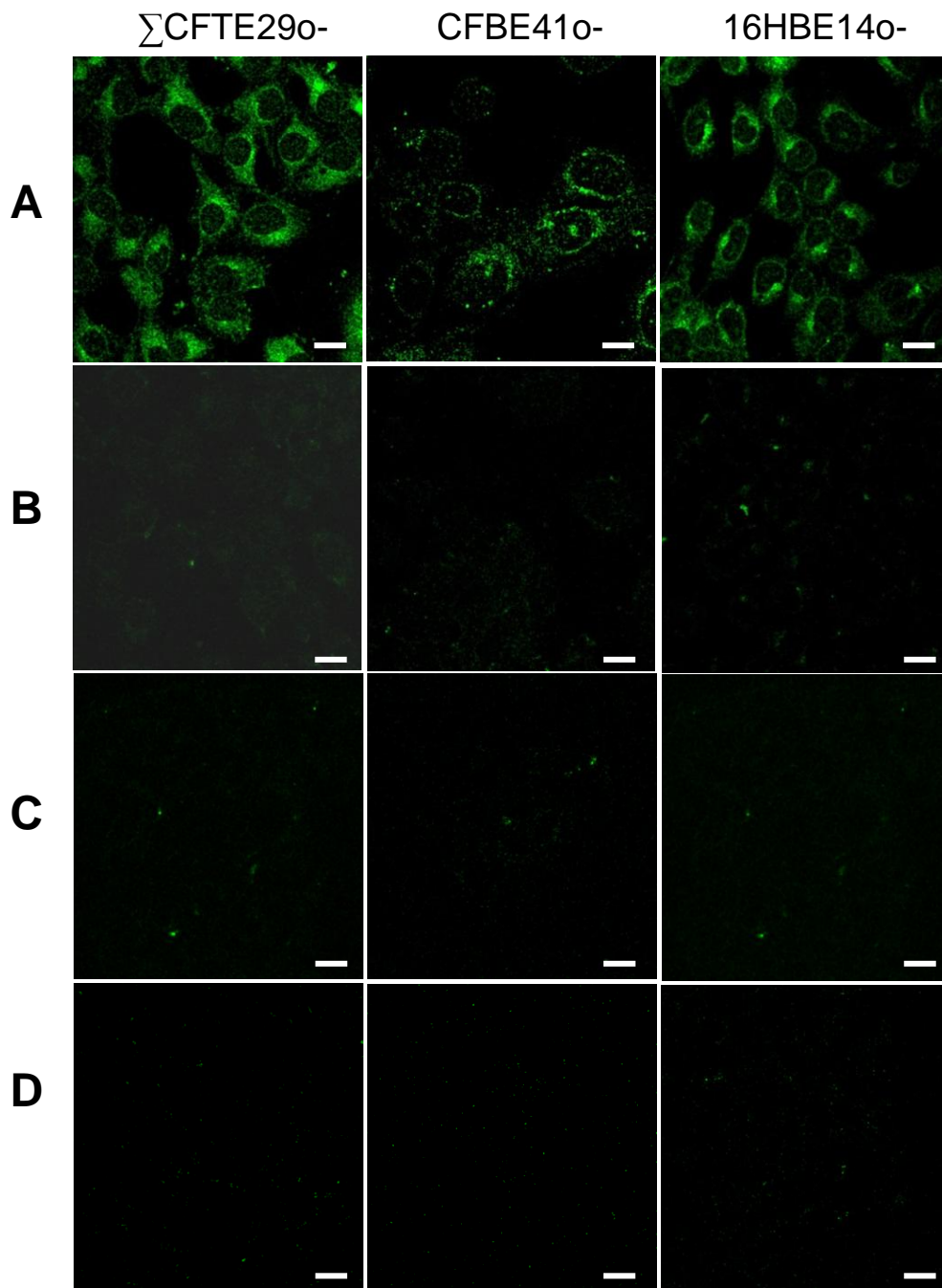
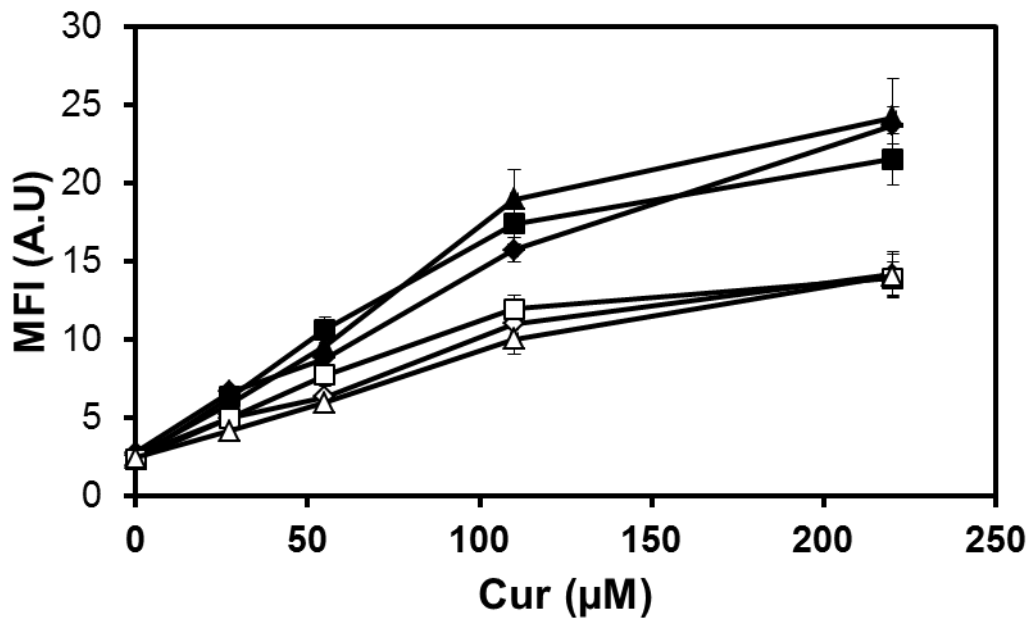


Figure 2: TBCP2 allows Cur penetration in the cells. Σ CFTE29o-, 16HBE14o- and CFBE41o- cells were incubated at 37°C in the presence of (A) 220 μ M Cur solubilized in TBCP2, (B) supernatant of 220 μ M Cur in PBS or (C) TBCP2 without Cur. (D) as control, the cells were incubated without any Cur/TBCP2 formulation. After 2 h, the cell fluorescence was analyzed by confocal laser scanning microscope (λ_{ex} : 488 nm; λ_{em} : 520 nm). Bar scale: 20 μ m.



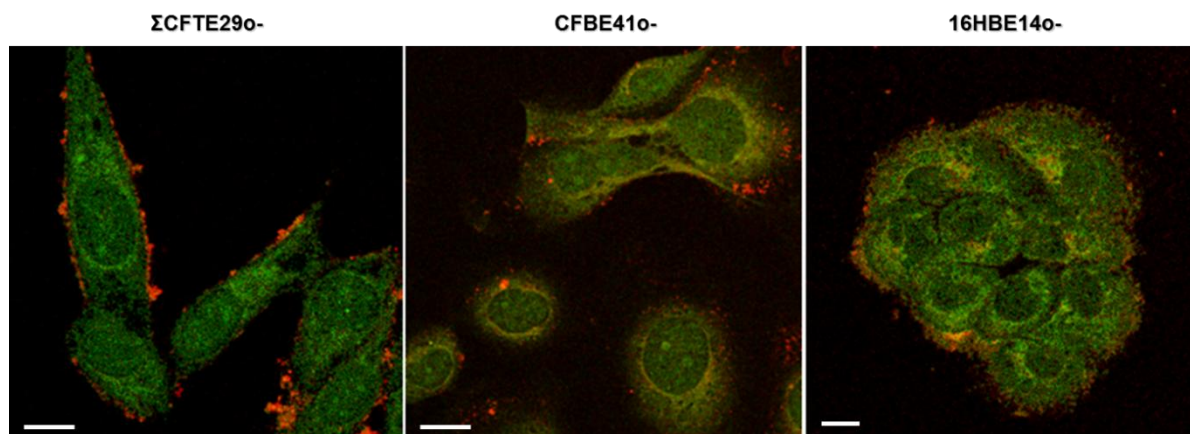
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770 **Figure 3: Uptake of Cur.** (◆, ◇) ΣCFTE29o-, (■, □) 16HBE14o- and (▲, Δ) CFBE41o-
 771 cells were incubated for 2 h at 37°C in the presence of various dilutions of the Cur/TBCP2
 772 formulation. The mean fluorescence intensity (MFI) of the cells was measured by flow
 773 cytometry (λ_{ex} : 488 nm; λ_{em} : 530 ± 30 nm) before (black symbols) and after (white symbols)
 774 treatment with trypan blue. The fluorescence intensity is expressed as MFI value of 10,000
 775 cells.

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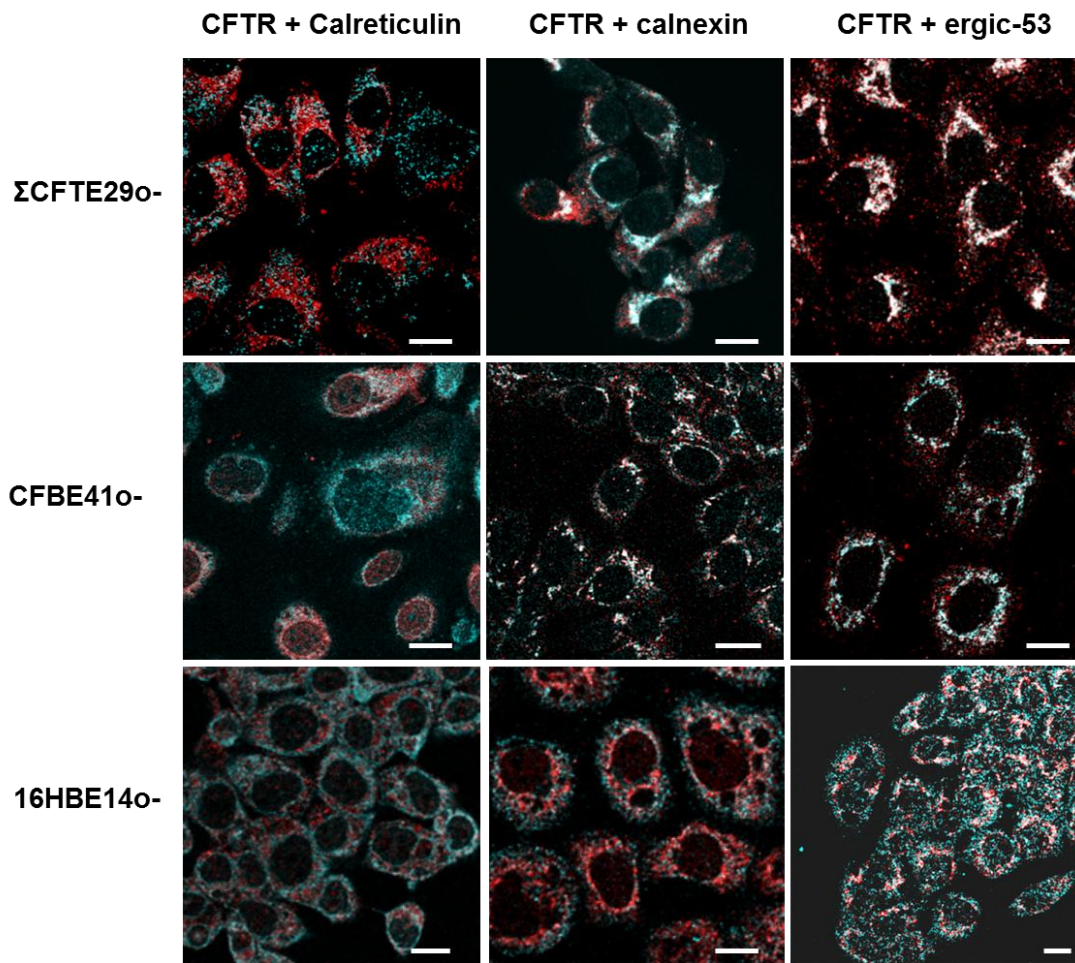
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Figure 4: Curcumin and copolymer cellular distribution. ΣCFTE290-, 16HBE140- and CFBE410- cells (1.4×10^4) were seeded on glass coverslips in 2 cm² well of a 4-well plastic culture plate. Two days after, the cells were incubated at 37°C in the presence of 40 μM curcumin solubilised with Rho-TBCP. Upon 4h incubation, cells were washed with PBS coverslips were mounted in Vectashield and analysed by confocal laser scanning microscopy. The fluorescence of curcumin and rhodamine were measured at 520 nm upon excitation at 488 nm and 560 nm upon excitation at 543 nm, respectively. Scale bar: 20 μm.

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796 **Figure 5: Confocal microscopy images of CFTR intracellular location.** Σ CFTE29o-,
797 16HBE14o- and CFBE41o- cells were labelled with mouse anti-hCFTR antibodies and either
798 with anti-calreticulin anti-calnexin or anti-ERGIC-53 antibodies. Anti-hCFTR antibodies
799 were revealed with Cy5 sheep anti-mouse antibodies (blue) and the other antibodies with
800 Alexa Fluor 568 secondary antibodies (red). Scale bar: 20 μ m.

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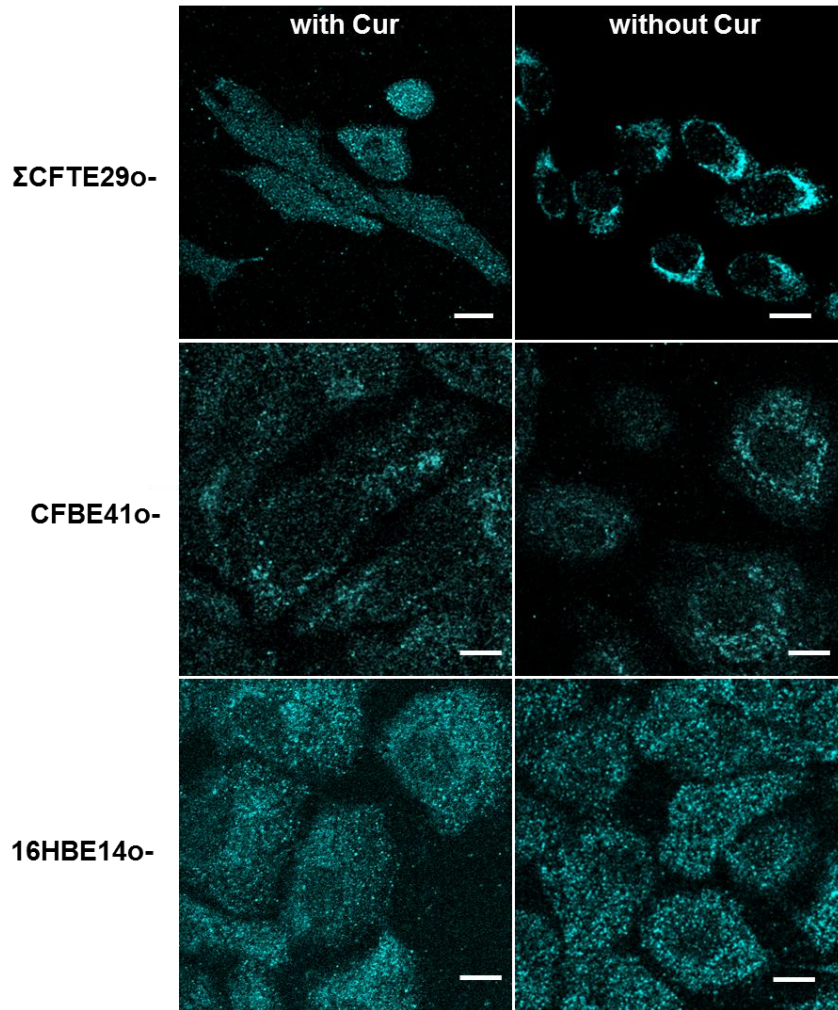
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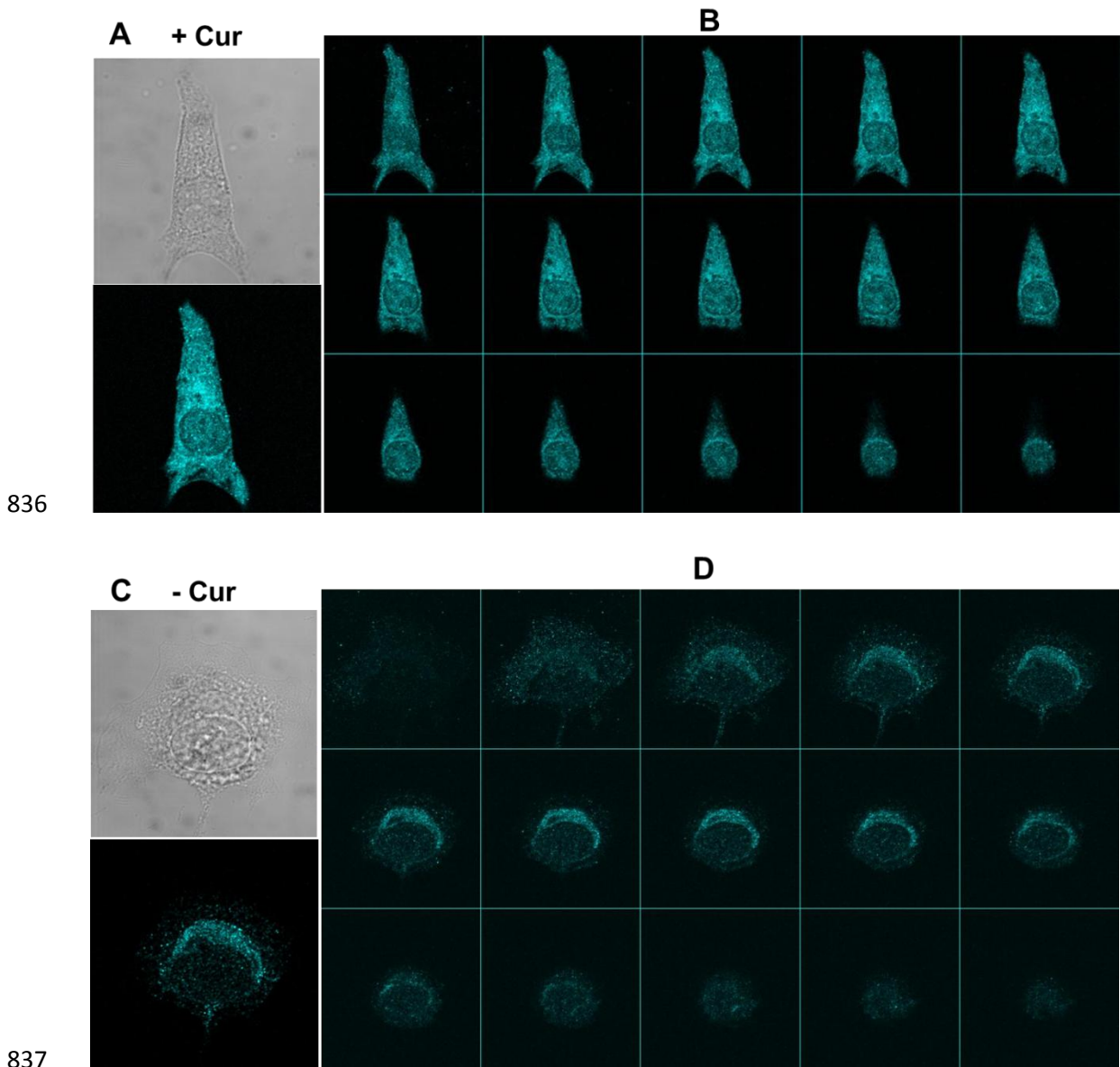
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824 **Figure 6: Effect of Cur/TBCP2 treatment on the F508del-CFTR location.** Σ CFTE29o-,
825 CFBE41o- and 16HBE14o- cells were cultured for 16 h in the absence (without Cur) or the
826 presence (with Cur) of 220 μ M Cur/TBCP2. The cells were stained with mouse anti-hCFTR
827 antibody followed by Cy5-sheep anti-mouse antibodies. The fluorescence of the cells was
828 analysed by confocal microscopy at 660 nm upon excitation at 633 nm (Cy5). Scale bar: 20
829 μ m.

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841 **Figure 7:** Images reconstitution of several z steps (**A, C**) and z-step gallery (**B, D**) after (**A, B**)
 842 and before (**C, D**) Cur/TBCP2 treatment of Σ CFTE29o- cells.

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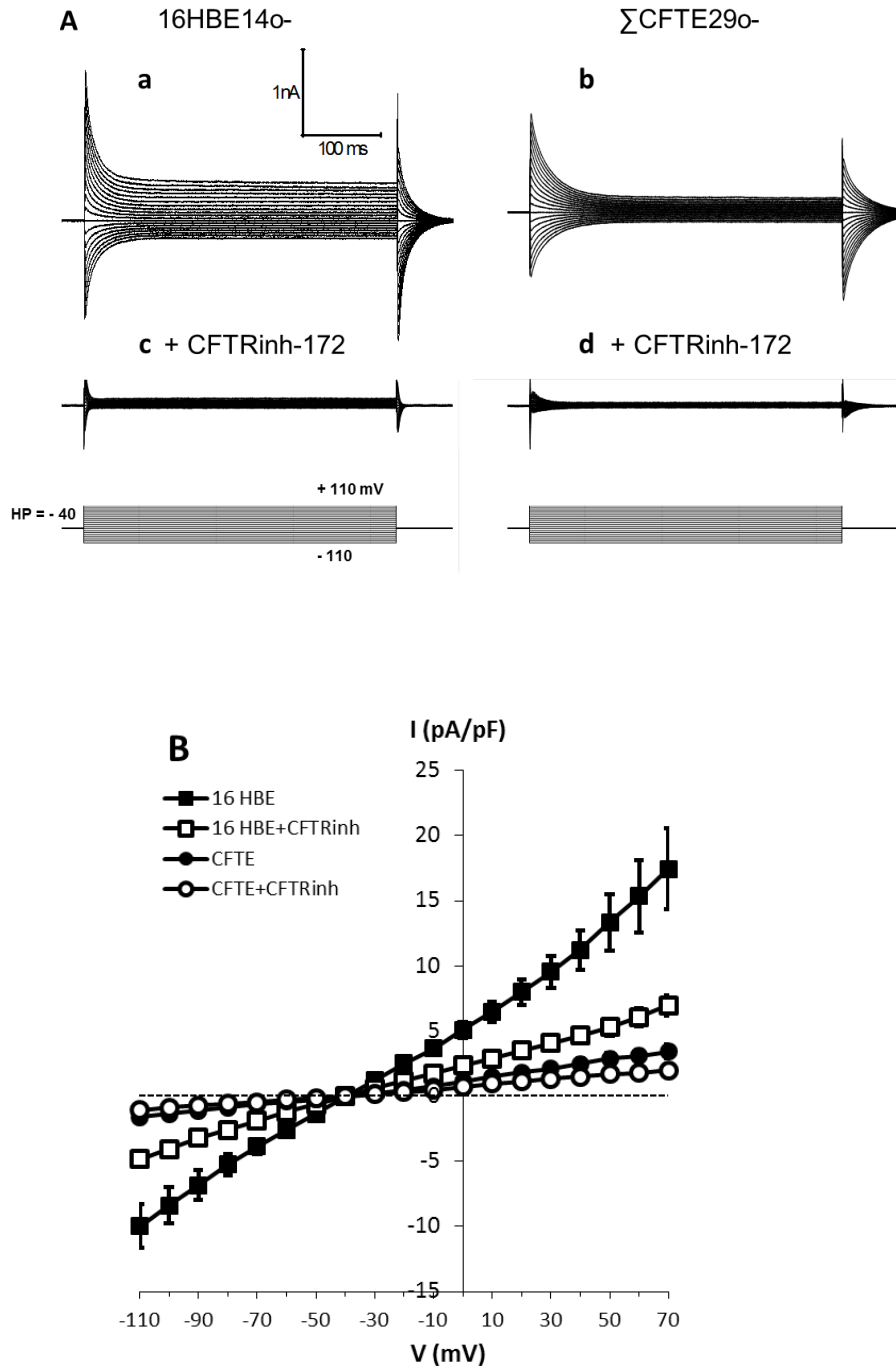


Figure 8: Patch-clamp characterization of CFTR current in normal and CF epithelial cells. (A) Representative whole-cell Cl^- current recordings in (a) 16HBE14o- and (b) Σ CFTE29o- cultured cells under control conditions or (c and d) after addition of the selective CFTR-inhibitor CFTRinh-172 (10 μM) for 10 min into the bath solution. (B) Plots of averaged steady-state current-voltage relationships (I/V curves) of Cl^- currents in the absence (black squares: 16HBE14o- cells, n=5; black circles: Σ CFTE29o- cells, n=9) or after addition of 10 μM CFTRinh-172 for 10 min (white symbols). All results were expressed as mean \pm SEM.

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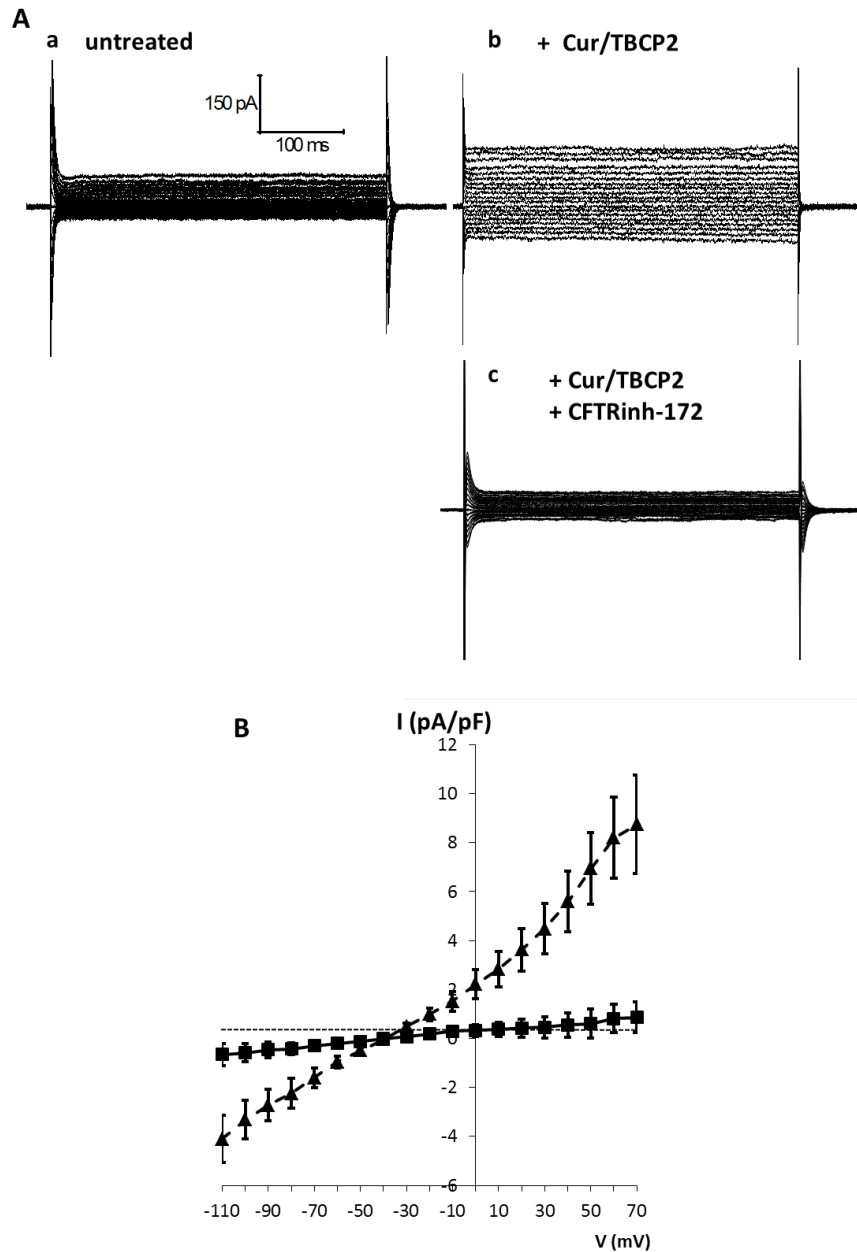
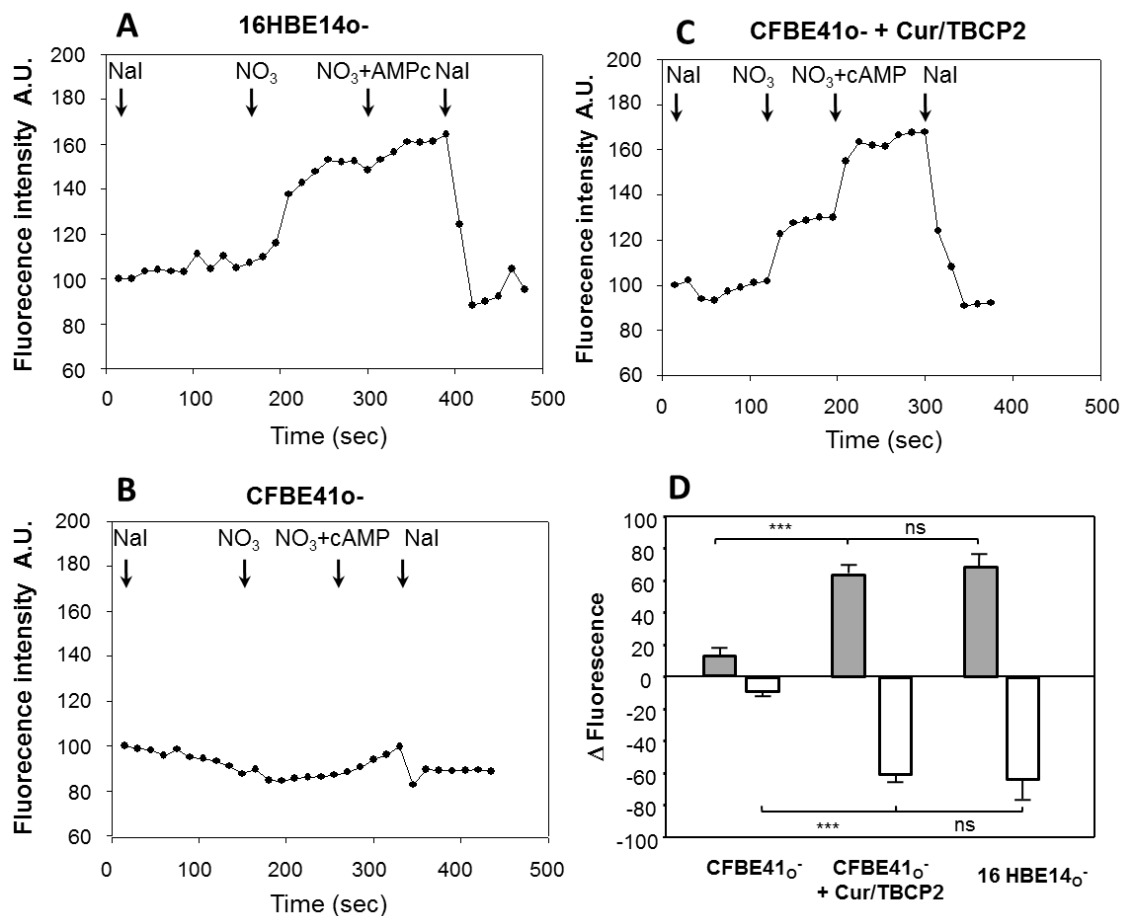


Figure 9: Patch-clamp measurements of Cur/TBCP2-induced activation of CFTRinh-172 sensitive current in Σ CFTE290- cells. (A) Representative whole cell CFTR current traces recorded after cell culture (a) in basal conditions or after 16 h treatment with (b) Cur/TBCP2 (120 μ M) or (c) after addition of the selective CFTR-inhibitor CFTRinh-172 (10 μ M) for 10 min into the bath solution in cells treated with Cur/TBCP2. (B) Plots of averaged steady-state current-voltage relationships (I/V curves) of CFTRinh-172 sensitive currents. I/V curves on cells before treatment (black squares, n= 8), and 16 h treatment with Cur/TBCP2 (black triangles, n= 19). Steady-state current amplitude was measured at the end of the pulse and normalized to the cell capacitance. All results were expressed as mean \pm SEM. Statistical significance was assessed using Mann-Whitney non-parametric test, and the significance level was established at $p < 0.05$ between CFTRinh-172 sensitive currents measured with Cur/TBCP2.



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921 **Figure 10: Cur/TBCP2 treatment influence on the CFTR-mediated anion transport on**
 922 **different epithelial cell monolayers measured by MQAE fluorescence.** CFBE41o⁻ and
 923 16HBE14o⁻ cells loaded with MQAE were sequentially perfused with I⁻, NO₃⁻, NO₃⁻ with
 924 cAMP and again with I⁻ buffer solutions. Representative MQAE fluorescence intensity was
 925 plotted as a function of time in (A) 16HBE14o⁻, (B) untreated CFBE41o⁻ cells or (C)
 926 treated CFBE41o⁻ cells for 16 h with Cur/TBCP2 (165 μM). (D) Histograms of fluorescence
 927 amplitude variation (Δ fluorescence) in cell monolayer without or with Cur/TBCP2
 928 treatment, in the presence of NO₃⁻ and NO₃⁻ + cAMP (grey filled histograms) or NaI⁻ (white
 929 histograms) expressed as the difference between the maximal fluorescence intensity
 930 measured at steady state before and after the change of the respective solutions. Data
 931 are means ± SEM. Statistical significance was assessed using a Student's *t*-test, and
 932 the significance level was established at p < 0.05 (*: p < 0.05; **: p < 0.01; ***: p < 0.001).
 933 ns: non-significant.

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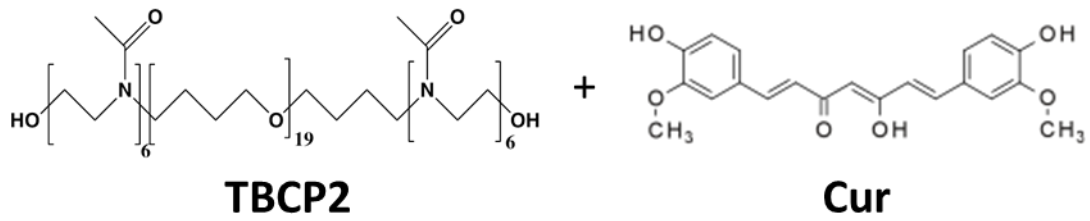
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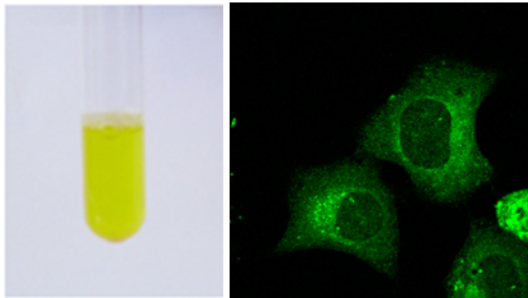
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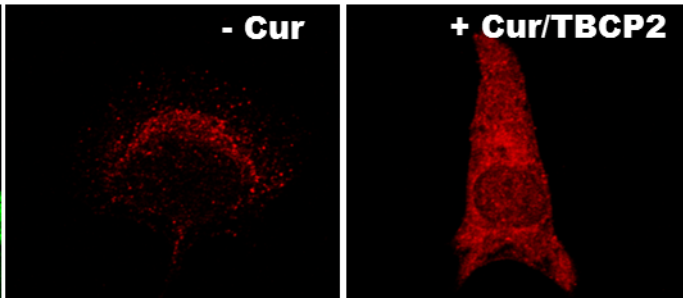
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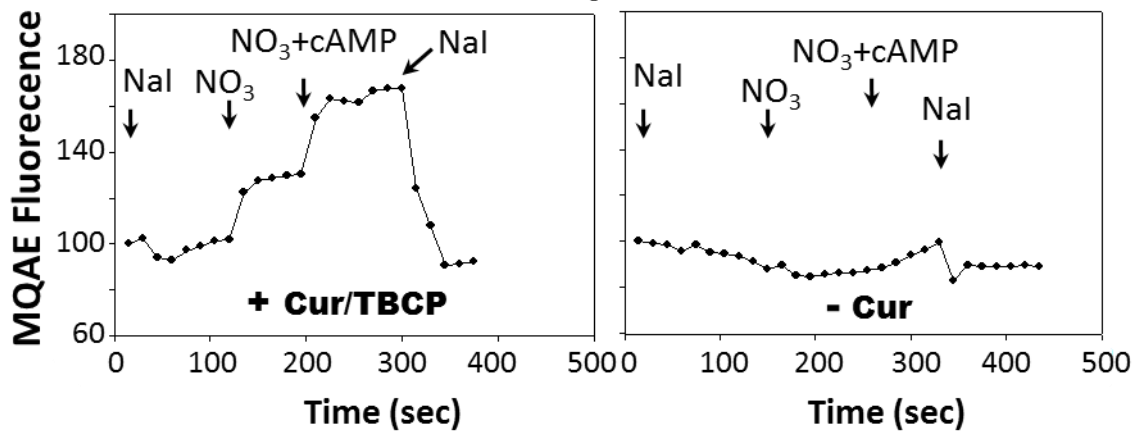
solubilization Cell penetration



CFTR relocalisation



Chloride transport restoration



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Graphical Abstract