The antimicrobial peptide Esc(1–21) potentiates CFTR channel via direct targeting of the nucleotide binding domains

https://doi.org/10.17952/38EPS.2026.P564

Maria Luisa Mangoni[1], Loretta Ferrera[2], Giacomo Cappella[1], Mario Passalacqua[3], Mattia Mori[4], Debora Baroni[5]

[1]Department of Biochemical Sciences, Sapienza University of Rome, Laboratory affiliated to Pasteur-Italia Fondazione Cenci Bolognetti; [2]U.O.C. Genetica Medica, Istituto Di Ricovero e Cura a Carattere Scientifico, Istituto Giannina Gaslini, Genova; [3]Department of Experimental Medicine, University of Genova, 16132, Genova ; [4]Department of Biotechnology, Chemistry and Pharmacy, University of Siena; [5]Istitute Institute of Biophysics, National Research Council, Genoa

BACKGROUND

Cystic fibrosis (CF) is caused by mutations in the CFTR ion channel, leading to impaired chloride transport, chronic lung infections, and progressive respiratory damage[1]. The most common mutation is the loss of Phe 508 (F508del-CFTR). Although CFTR modulators have improved patient outcomes, a subset of individuals remains unresponsive or continues to suffer from persistent infections [2], highlighting the need for alternative therapeutic strategies. We identified frog skin–derived antimicrobial peptides (Esc peptides) that reduce bacterial burden in murine models of *Pseudomonas aeruginosa* lung infection. Notably, Esc(1-21) also acts as a potentiator of F508del-CFTR, enhancing chloride transport in both cell lines and primary bronchial epithelial cells [3] **(Figure 1).**

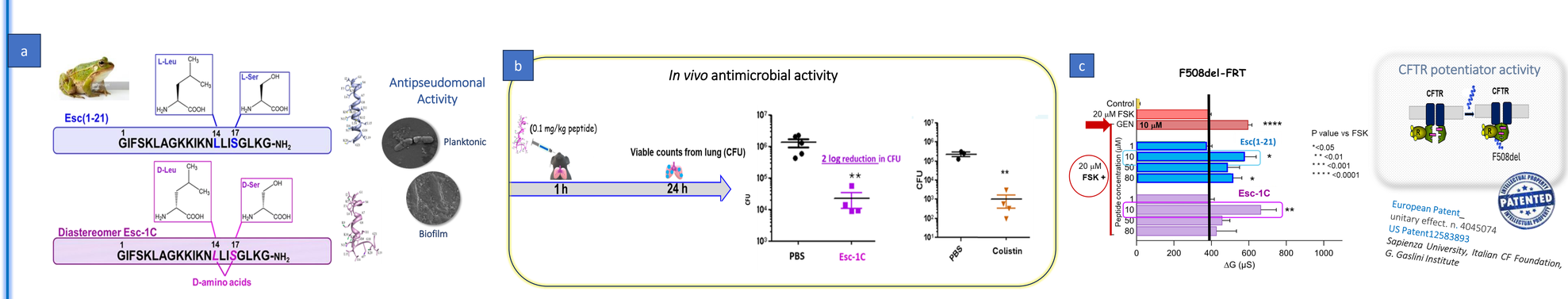


Figure 1. Schematic representation of biological properties of Esc peptides. **a,** *Esc peptides* primary structure. **b,** *In vivo* efficacy of Esc-1C upon intratracheal administration in mice with *P. aeruginosa* lung infection. The number of viable bacterial cells was evaluated after 24 h treatment and compared to that of colistin. **, p < 0.01; ***c***, Transepithelial-electrical-conductance, experiments showing the CFTR potentiator activity of Esc peptides in the presence of forskolin (FSK) on Fisher Rat Thyroid (FRT) cells expressing F508del-CFTR, compared to genistein (GEN)

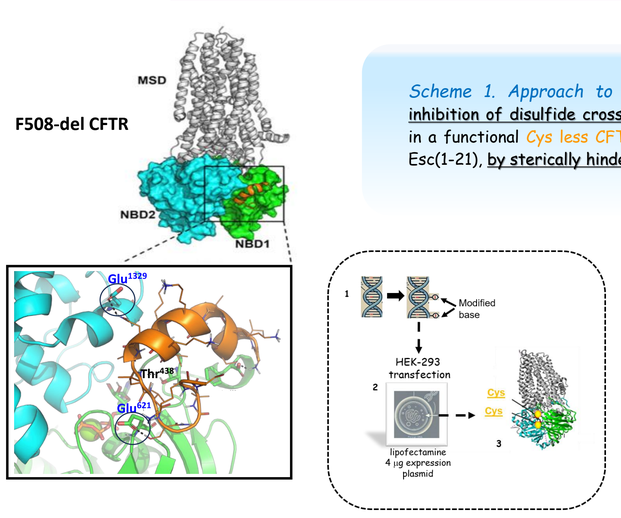
AIMS

1. Identify the binding site of Esc(1-21) on CFTR
2. Validate the peptide interaction by disulfide cross-linking experiments
3. Determine whether Esc(1-21) enters the cells and reaches CFTR

RESULTS

By combining molecular dynamics, mutagenesis, and disulfide cross-linking assays **(See Scheme 1, Figures 2 and 3)**, we show that Esc(1-21) directly interacts with the CFTR nucleotide-binding domain interface. Confocal imaging confirms peptide internalization and co-localization with CFTR **(Figure 4).** These findings identify Esc peptides as multifunctional molecules with antimicrobial and CFTR-modulating activities, supporting their development as novel therapeutic candidates for CF [4] .

Computational studies & Cross-linking



Scheme 1. Approach to verify peptides' binding region to CFTR: inhibition of disulfide cross-linking between 2 cysteines (Cys) introduced in a functional Cys less CFTR mutant, at the predicted binding region of Esc(1-21), by sterically hindering the access of the cross-linker

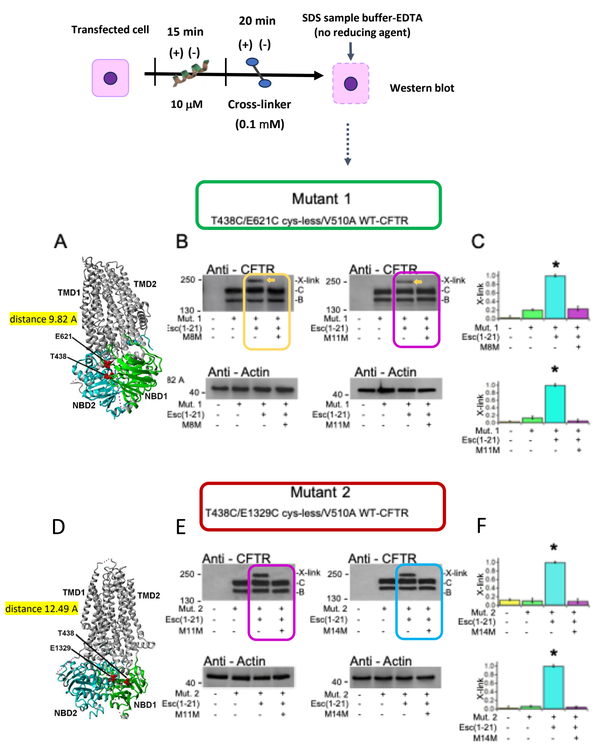


Figure 2. Effect of Esc(1-21) on disulfide cross-linking of CFTR double-cys mutants at the NBD1-NBD2 interface. **Panels A, D**: MD-based structural models of the cys-less/V510A WT-CFTR scaffold showing positions of reintroduced cys residues in mutant 1 (T438C/E621C), mutant 2 (T438C/E1329C); cys residues are in red, with median inter-residue distance indicated. **Panels B, E:** HEK-t cells expressing mutants were incubated with (+) or without (–) 10 μM Esc(1-21) for 15 min and treated (+) or not (–) with 0.1 mM M8M, M11M, M14M cross-linkers, as indicated for 20 min. Lysates were analysed by immunoblotting with anti-CFTR (upper blots) and anti-actin (lower blots) antibodies. Immature (B), mature (C) and cross-linked CFTR species (X-link) are indicated. **Panels C, F**: Bar graphs showing relative X-link abundance, normalized to the X-link signal detected in lysates treated with the cross-linker alone. Data are expressed as mean ± SD.

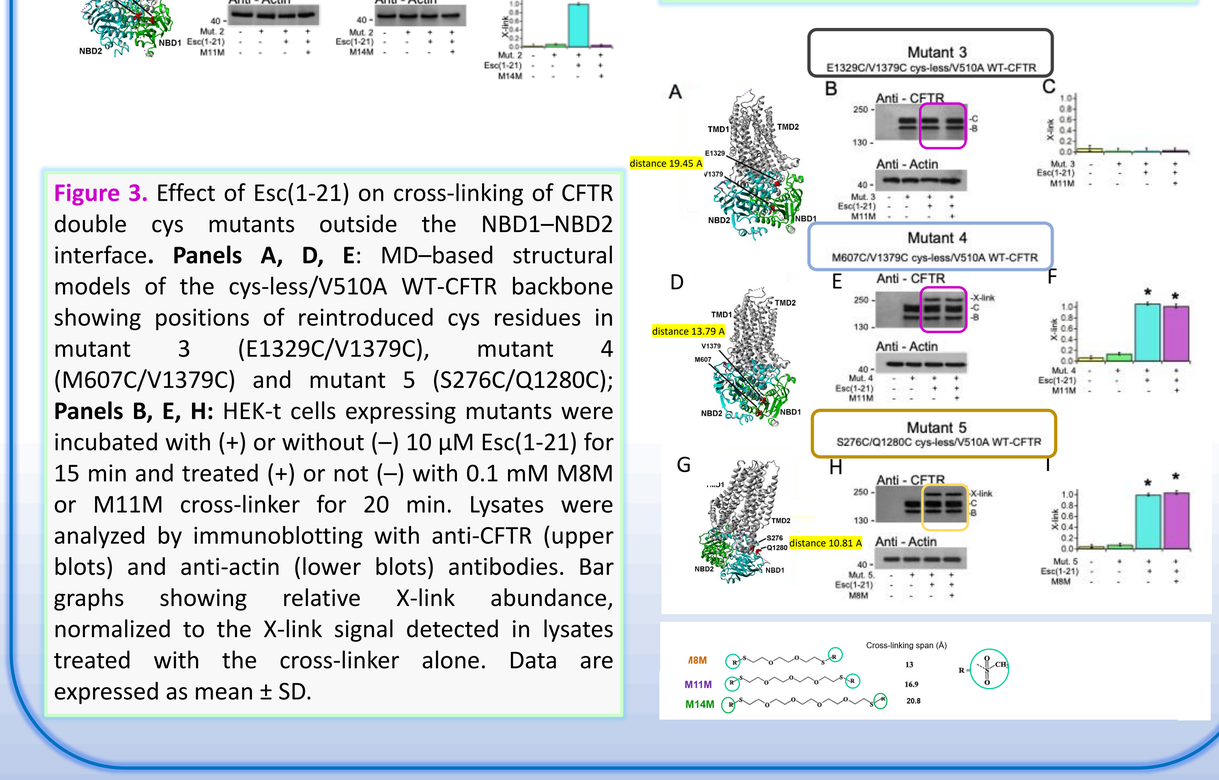
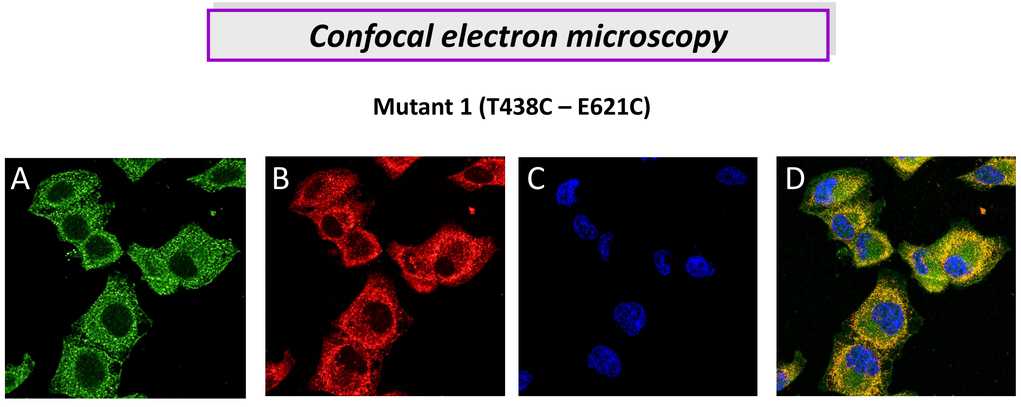


Figure 3. Effect of Esc(1-21) on cross-linking of CFTR double cys mutants outside the NBD1–NBD2 interface**. Panels A, D, E**: MD–based structural models of the cys-less/V510A WT-CFTR backbone showing positions of reintroduced cys residues in mutant 3 (E1329C/V1379C), mutant 4 (M607C/V1379C) and mutant 5 (S276C/Q1280C); **Panels B, E, H:** HEK-t cells expressing mutants were incubated with (+) or without (–) 10 μM Esc(1-21) for 15 min and treated (+) or not (–) with 0.1 mM M8M or M11M cross-linker for 20 min. Lysates were analyzed by immunoblotting with anti-CFTR (upper blots) and anti-actin (lower blots) antibodies. Bar graphs showing relative X-link abundance, normalized to the X-link signal detected in lysates treated with the cross-linker alone. Data are expressed as mean ± SD.

Confocal electron microscopy

Mutant 1 (T438C – E621C)



Rabbit anti-Esc(1-21) Secondary Ab: Anti-rabbit IgG, conjugated to Alexa Fluor 594	Mouse anti-CFTR Secondary Ab: Anti-mouse IgG conjugated to Alexa Fluor® 488	DAPI for nuclei detection	Merge image: co-localization CFTR/Esc(1-21)

Esc(1-21) interferes with cross-linking at the predicted binding site and colocalizes with CFTR

Figure 4. Confocal immunofluorescence analysis of co-localization between cys-less CFTR and the Esc(1-21) peptide. Representative confocal images of highly transfectable embryonic kidney 293 (HEK-t) cells expressing the T438C/E621C cys-less/V510A WT-CFTR construct following incubation with Esc(1-21). **(A)** CFTR cys-less mutant immunostaining (green) detected using a monoclonal anti-CFTR antibody and an Alexa Fluor 488-conjugated secondary antibody. **(B)** Esc(1-21) peptide localization (red), revealed using a rabbit polyclonal anti-peptide primary antibody and an Alexa Fluor 568-conjugated secondary antibody. **(C)** Nuclear counterstaining with 4',6-diamidino-2-phenylindole (DAPI, blue). **(D)** Merged image highlighting CFTR cys-less-peptide co-localization (yellow).

CONCLUSIONS

These results support:

- *Direct interaction of Esc(1-21) with the NBDs interface*
- *Intracellular localization of Esc(1-21) and colocalization with CFTR*

→ Esc(1-21) as potential candidate for the development of new as dual-function candidates for CF therapy

References

1. Veit G, et al. Mol Biol Cell 27: 424-433. 10.1091/mbc.E14-04-0935
2. Almughem FA, et al. Pharmaceutics 12: 10.3390/pharmaceutics12070616
3. Ferrera L et al. Cell Mol Life Sci 82: 121. 10.1007/s00018-025-05633-9
4. Baroni D. et al. ACS Omega in press

Acknowledgments:

Fondazione per la Ricerca sulla Fibrosi Cistica - ETS
italian cystic fibrosis research foundation

FFC Project 6/2025 adopted by:
-GRUPPO DI SOSTEGNO FFC RICERCA DI SCAURI – MINTURNO
-DELEGAZIONE FFC RICERCA DI MONCALVO – ASTI
-DELEGAZIONE FFC RICERCA DI DONGO - COMO