Mechanistic Studies of Small Molecule CFTR Modulators that Restore Channel Function to CFTR Variants

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INTRODUCTION

- The genetic disease cystic fibrosis (CF) is caused by mutations that disable or destroy the ion channel cystic fibrosis transmembrane conductance regulator (CFTR).
- The CFTR-targeted therapy elexacaftor/tezacaftor/ivacaftor (E/T/I) has transformed the treatment of people with CF and the most common CFTR variant F508del.
- There are over 2000 CFTR variants, many of which are rare and still need targeted therapies.

AIMS

- To use AI-based modelling and molecular docking tools to identify drug binding sites on CFTR.
- To study the effects of CFTR modulator on the function of CFTR variants using the patch-clamp technique.

Molecular Modelling of CFTR

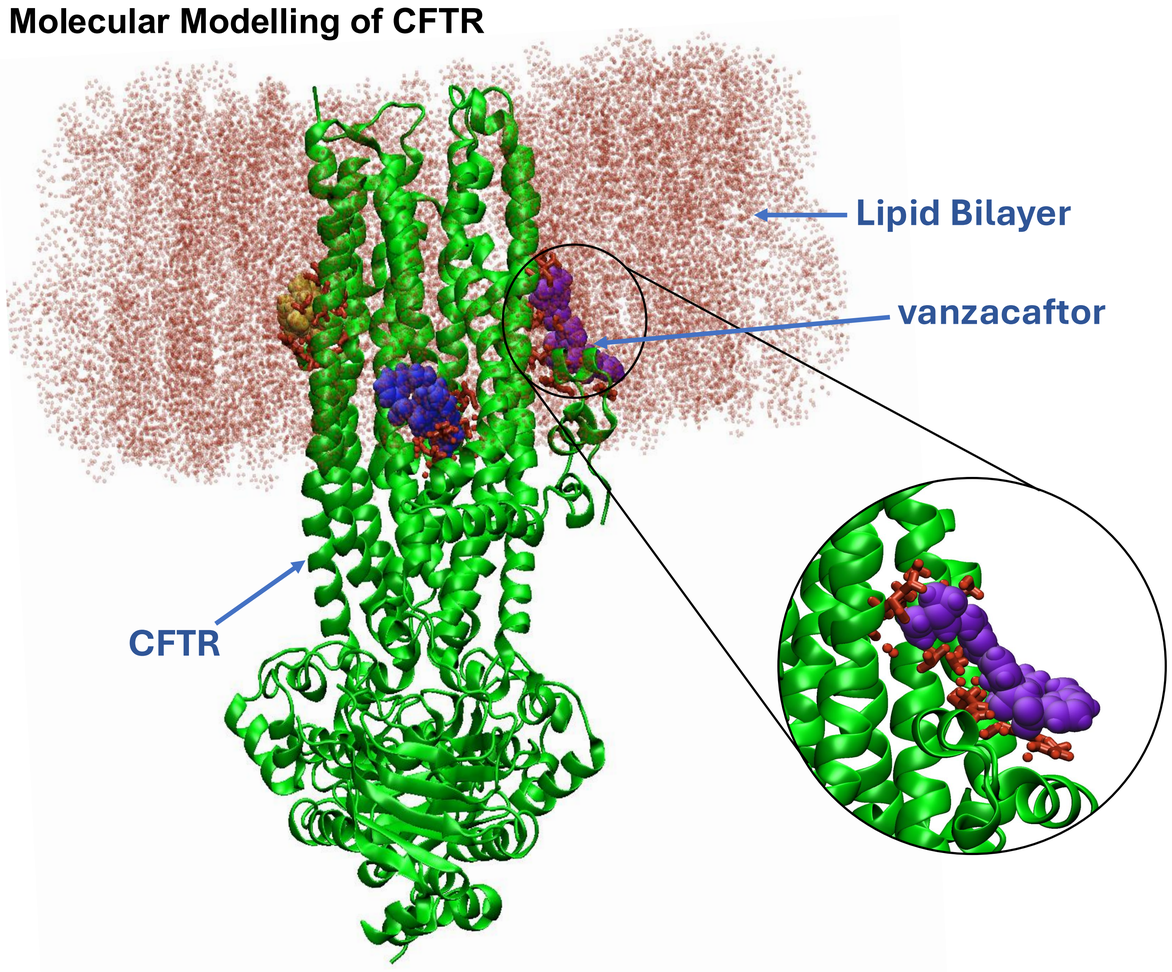

Figure 1. AI based molecular modeling of vanzacaftor bound to CFTR
A molecular model of CFTR in a lipid membrane with the latest CFTR-targeted drug vanzacaftor bound at the three drug-binding pocket of E/T/I. The model is based on the Cryo-EM structure of phosphorylated ATP bound F508del human CFTR complexed with ETI (PDB ID: 8EIQ). The binding poses of vanzacaftor were predicted with GNINA Dock and the best ranked poses selected. The molecules coloured yellow, blue and purple are vanzacaftor bound at the drug binding coordinates of ivacaftor, tezacaftor and elexacftor, respectively. The inset shows vanzacaftor bound at the elexacaftor binding site.

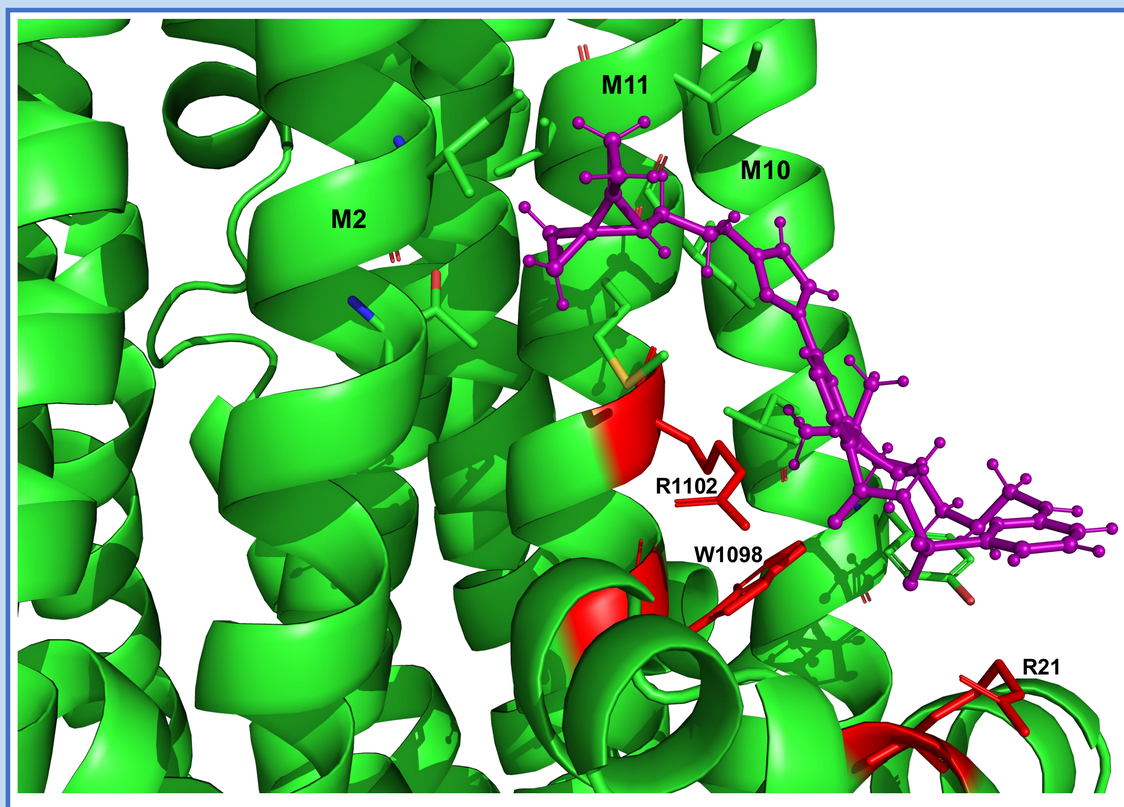

Figure 2. Interactions between vanzacaftor and CFTR.
Vanzacaftor (purple) stabilizes the CFTR protein by interacting with nearby residues (red) in transmembrane segments 2, 10 and 11. The nearby residues involved are R1102, W1098 and R21.

SUMMARY AND SPECULATION

- Vanzacaftor interacts with the drug-binding pockets of E/T/I.
- F508del-CFTR Cl^- channels rescued by E/T/I are stable and deactivate much more slowly in cell-free membrane patches
- The single-channel current amplitude of F508del-CFTR rescued by E/T/I is stable in cell-free membrane patches, like that of wild-type CFTR
- The open probability of F508del-CFTR rescued by E/T/I is stable in cell-free membrane patches, but reduced compared to that of wild-type CFTR
- E/T/I stabilizes the structure of F508del-CFTR without restoring wild-type levels of channel activity.

NEXT STEPS

- Validate the AI modelling hypothesis of vanzacaftor using single-channel recording and other experimental tools. This will help guide the design of variant-specific treatments for people with CF.

Patch-clamp Studies of CFTR

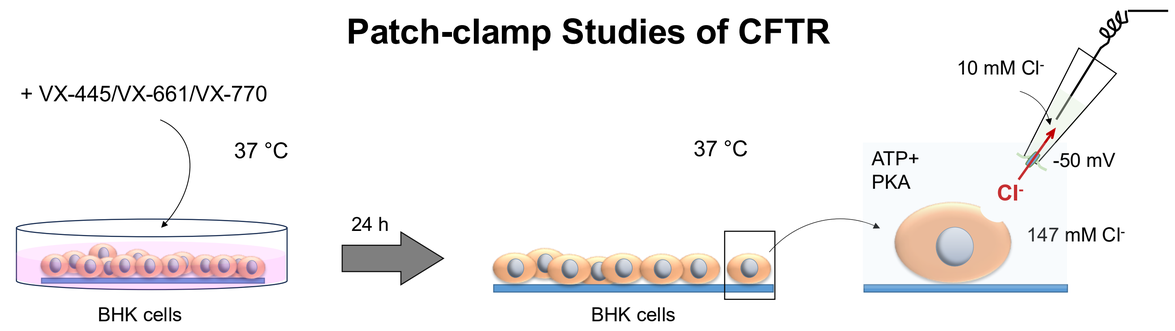

Figure 3. Single-Channel Patch-Clamp Studies of CFTR.
F508del-CFTR-expressing baby hamster kidney (BHK) cells were treated with elexacaftor (VX-445; 2 μM), tezacaftor (VX-661; 3 μM) and ivacaftor (VX-770; 1 μM) for 24 h at 37 °C before single-channels were studied at 37 °C and –50 mV in the presence of a Cl^- concentration using excised inside-out membrane patches. Protein kinase A (75 nM) and ATP (1 mM) were continuously present in the intracellular solution.

Elexacaftor/Tezacaftor/Ivacaftor Prolong F508del-CFTR Cl^- Channel Activity in Cell-Free Membrane Patches

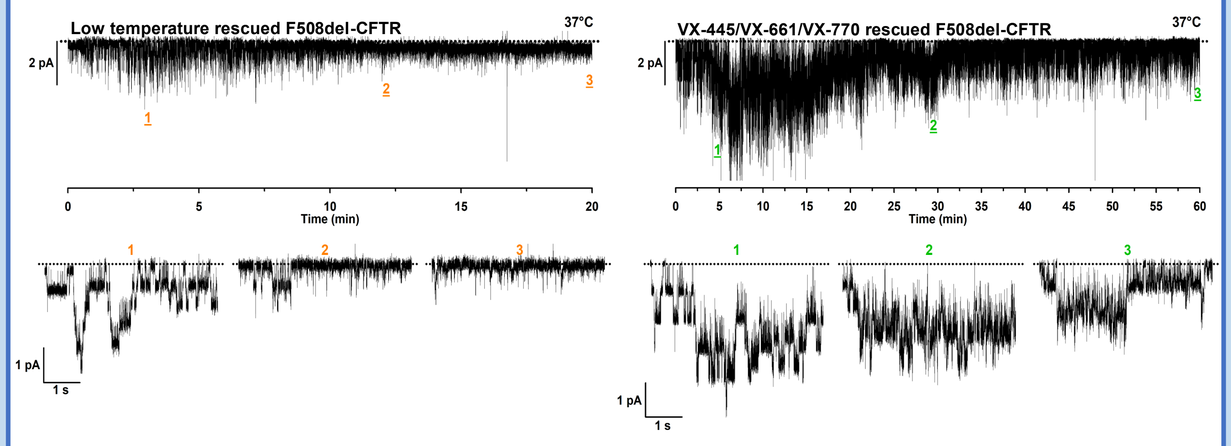

Figure 4. Elexacaftor/Tezacaftor/Ivacaftor Prolong CFTR Cl^- Channel Activity in Cell-Free Membrane Patches. Representative recording of F508del-CFTR Cl^- channels in excised inside-out membrane patches from CFTR-expressing BHK cells rescued by either low temperature incubation at 27 °C for 24 h (left traces) or treated with E/T/I (VX-445/VX-661/VX-770) for 24 h at 37 °C (right traces). The recordings were acquired at 37 ºC with ATP (1 mM) and PKA (75 nM) continuously present in the intracellular solution. The top traces are 20- and 60-minute recordings, while the recordings underneath labelled 1 to 3 show the 5-second portions indicated by the bars on an expanded time scale, to demonstrate single-channel activity at the beginning, in the middle and at the end of the experiment. Dotted lines indicate the closed channel state and downward deflections correspond to channel openings.

Elexacaftor/Tezacaftor/Ivacaftor Does Not Restore Wild-Type Levels of Channel Activity to F508del-CFTR

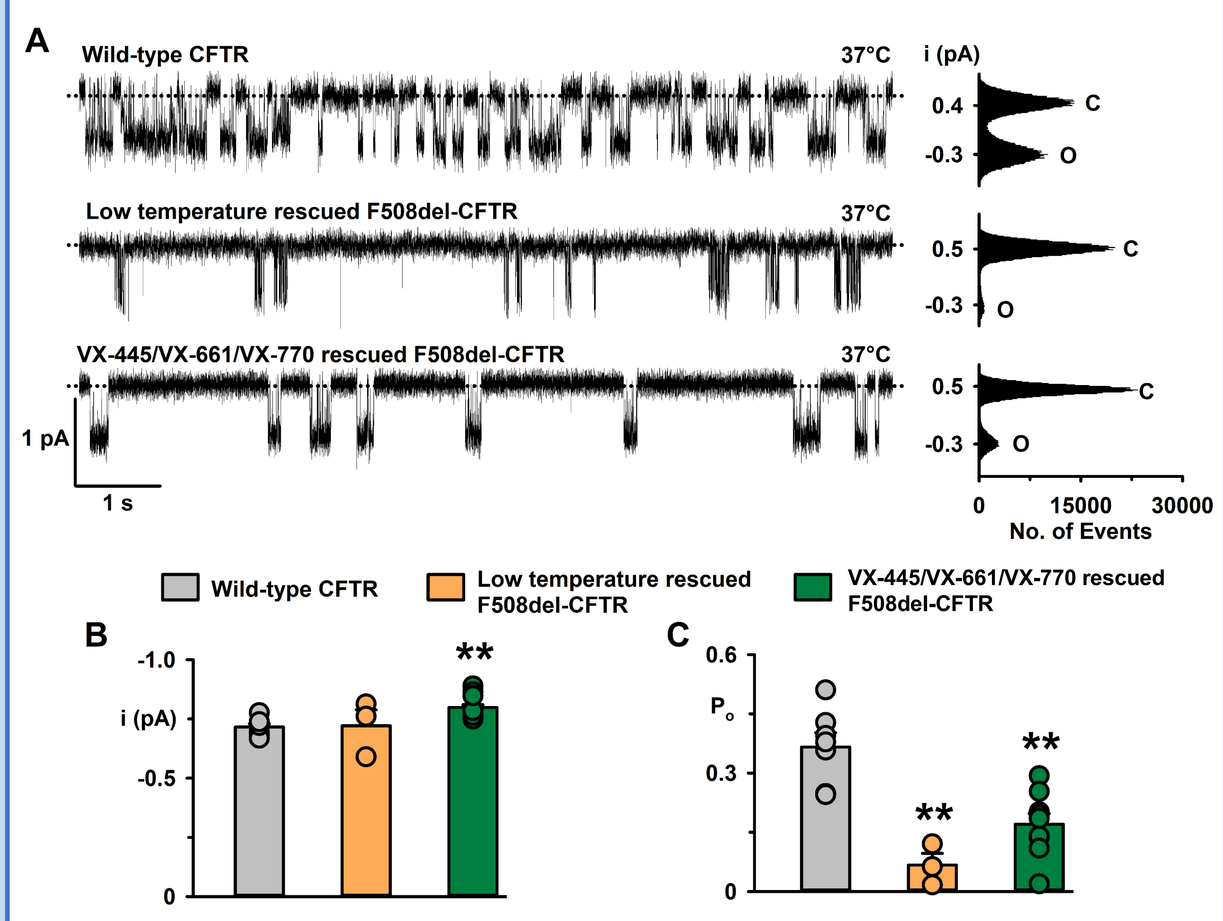

Figure 5. Elexacaftor/Tezacaftor/Ivacaftor Does Not Restore Wild-Type Levels of Channel Activity to F508del-CFTR. A, single-channel recordings and current amplitude histograms of wild-type and F508del-CFTR in excised inside-out membrane patches from CFTR-expressing C127 and BHK cells using the same conditions as Figure 4. **B and C,** single-channel current amplitude (i) and open probability (P_o) of wild-type, low temperature- and E/T/I (VX-445/VX-661/VX-770)-rescued F508del-CFTR Cl^- channels. Data are means ± S.E. with circles representing individual values (wild-type, n = 7; low temperature-rescued, n = 3; VX-445/VX-661/VX-770, n = 9); **, $P < 0.01$ vs. wild-type CFTR.

ACKNOWLEDGEMENTS

This work was supported by the Cystic Fibrosis Trust.