Computer Modelling the Root Cause of Cystic Fibrosis.

Miro A. Astore ACFRT Final Report 2023

Lay Project Description:

The root cause of Cystic Fibrosis is the malfunction of a protein called the Cystic Fibrosis Transmembrane Conductance Regulator. This protein forms an ion channel, which must conduct negative ions like chloride and bicarbonate in order to avoid disease. In my PhD research I combined physics and biology to better understand the function of this protein. This was done by simulating the motion of CFTR on a supercomputer, to see how it moved the atoms in the diseased protein moved differently to the healthy one.

With my collaborators at the molecular and integrative cystic fibrosis research centre, we were able to test my *in silico* predictions in patient organoids, to help understand Cystic Fibrosis disease in patients from the molecular level.

Through my simulations I was able to model new parts of CFTR and discover how it functioned, including how ions move through the channel and how it moves between an open and a closed state. These results will help inform the design of novel therapies which treat the root cause of the disease.

Research Progress:

To date, I have published four peer reviewed articles on rare cystic fibrosis mutations with my collaborators. These publications focussed on the study of four rare mutations: R352Q, I37R, S945L and Q1291H. Each publication involved a sophisticated set of simulation techniques which I developed to tackle the complicated CFTR protein system.

The R352Q publication centred on the study of the conductance defect caused by this mutation. The confirmation of this class of defect indicated that the mutation could be treated by potentiator class drugs. The insights from this paper will help future studies better understand the intricate mechanism of ion conduction through the inner vestibule of CFTR. These results were complementary to experiments with lung and gut patient derived organoids which demonstrated a response to potentiator class drugs.

Our publication concerning the I37R mutation involved extensive molecular dynamics simulations to test the stability of the mutated lasso motif, followed by a verification of our predictions by the Alphafold2 AI by DeepMind. Our simulation results indicated the protein exhibited a gating defect, which supports the finding of the companion *ex vivo* organoid studies of our collaborators which determined this mutation responded to potentiator class drugs.

The simulations in our publication concerning the rare S945L mutation indicated a significant folding defect to the protein. The concurrent experiments in patient derived organoids showed a response to a combination therapy of both potentiators and correctors.

The most recent publication arising from my collaboration with the miCF research centre concerned the Q1291H mutation. This publication combined results from studying several mutations, G551D, Q1291R and Q1291H. We noticed that in simulations, each of these mutations exhibited a similar motif which was characteristic of a response to potentiator class drugs. However, the experimental component of this study displayed no such benefit from potentiators. It was later determined that this was due to a splicing error introduced by the Q1291H mutation, rendering them unable to respond to conventional potentiators and correctors. Our results thus indicated that potentiator drugs could be a complementary therapy in future for such a patient, upon the development of clinically effective read through compounds.

Finally, I have a fifth publication in preparation for submission. This publication represents roughly half of the computational and time investment from my PhD studies. Here I used newly developed simulation techniques to discover an open conformation of the WT-CFTR ion channel. Upon discovering this open structure, I steered both bicarbonate and chloride ions through the channel to demonstrate that it was capable of conducting ions. I was able to compare the energy required for ions to pass through the channel in both the WT and R334W-CFTR variants of the protein. An understanding of the fully open conformation of the CFTR protein will assist with the development of future potentiator class drugs.

Alongside the more in depth studies I have outlined in this report we have built a library of molecular dynamics simulations for more than 40 rare mutations. As mutations are uncovered in the Australian CF community, we can draw on this library of data and understand these rare mutations at the molecular level.

References:

[1]

K. M. Allan *et al.*, “S945L-CFTR molecular dynamics, functional characterization and tezacaftor/ivacaftor efficacy in vivo and in vitro in matched pediatric patient-derived cell models,” *Frontiers in Pediatrics*, vol. 10, 2022, Accessed: Jun. 05, 2023. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fped.2022.1062766

[2]

S. L. Wong *et al.*, “Molecular Dynamics and Theratyping in Airway and Gut Organoids Reveal R352Q-CFTR Conductance Defect,” *Am J Respir Cell Mol Biol*, vol. 67, no. 1, pp. 99–111, Jul. 2022, doi: 10.1165/rcmb.2021-0337OC.

[3]

S. L. Wong *et al.*, “Molecular dynamics and functional characterization of I37R-CFTR lasso mutation provide insights into channel gating activity,” *iScience*, vol. 25, no. 1, p. 103710, Jan. 2022, doi: 10.1016/j.isci.2021.103710.

[4]

K. M. Allan *et al.*, “Q1291H-CFTR molecular dynamics simulations and ex vivo theratyping in nasal epithelial models and clinical response to elexacaftor/tezacaftor/ivacaftor in a Q1291H/F508del patient,” *Frontiers in Molecular*

Biosciences, vol. 10, 2023, Accessed: Jun. 05, 2023. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fmolb.2023.1148501