Integrated structural biology provides new clues for cystic fibrosis treatment

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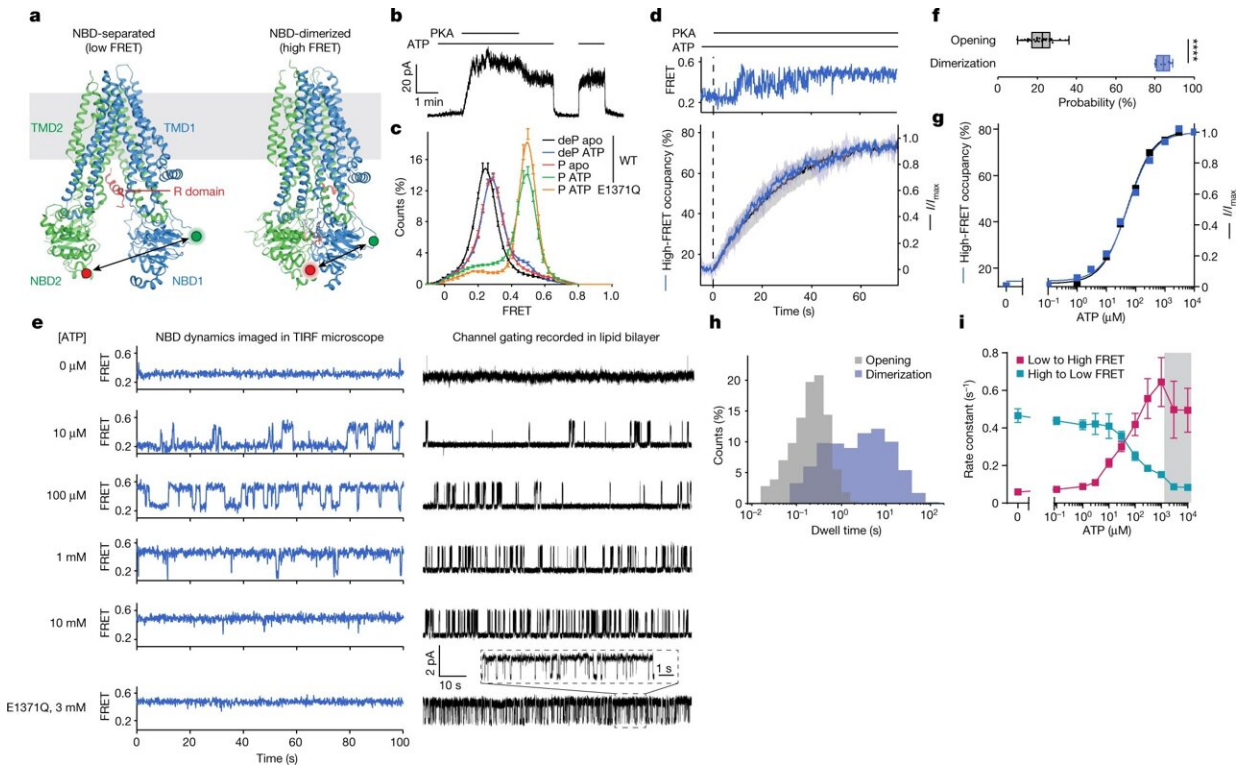

Dependence of CFTR pore opening and NBD dimerization on phosphorylation and ATP. Credit: *Nature* (2023). DOI: 10.1038/s41586-023-05854-7

Scientists at St. Jude Children's Research Hospital and Rockefeller University have combined their expertise to gain a better understanding of the cystic fibrosis transmembrane conductance regulator (CFTR). Mutations in CFTR cause cystic fibrosis, a fatal disease with no cure.

Current therapies using a drug called a potentiator can enhance CFTR functions in some patients; but how the potentiators work is not well understood. The new findings reveal how CFTR functions mechanistically and how disease mutations and potentiators affect those functions. With this information, researchers may be able to design more effective therapies for cystic fibrosis. The study was published today in *Nature*.

Cystic fibrosis is a genetic disorder that causes people to produce mucus that is too thick and sticky. This can block airways and lead to lung damage as well as cause problems with digestion. The disease affects about 35,000 people in the United States. CFTR is an anion channel, a passageway that maintains the right balance of salts and fluid across epithelial and other membranes. Mutations in CFTR are what cause cystic fibrosis, but these mutations can affect CFTR function differently. Therefore, some drugs used to treat the disease can only partially restore function of specific mutant forms of CFTR.

Structures of CFTR, previously captured in the laboratory of Jue Chen, Ph.D., and colleagues at Rockefeller University, revealed two distinct conformations (shapes). Those static images allowed researchers to see the channel when it is open or when it is closed, but the transition between states has been incompletely understood.

Conformational changes were thus inferred to be important for opening and closing the channel, accounting for the electrophysiological properties of CFTR, which have been analyzed for decades. Those findings fueled interest in directly visualizing the structural transitions of CFTR in real time and examining how conformational changes are affected by disease mutations and by drugs used to enhance CFTR function in patients.

"Through this collaboration, we had the opportunity to really dial into

the relationship between structure and function," said co-corresponding author Scott Blanchard, Ph.D., St. Jude Department of Structural Biology. "Our lab's prior work on ribosomes and G-protein coupled receptors had shown this is possible, but there are very few single proteins that are more relevant for the treatment of disease than CFTR because treatments for cystic fibrosis are aimed at ameliorating the defects in the mutant forms of this protein."

"The ability to make biophysical measurements and get these types of quantitative insights is one of the advances of single-molecular imaging that never ceases to amaze me."

Collaboration leads to a breakthrough

The complementary expertise of the research groups was key to making their discoveries. Through electrophysiology and structural studies, the Rockefeller team was able to guide the placement of single-molecule probes by the St. Jude team. By deploying single-molecule fluorescence resonance energy transfer (smFRET) the St. Jude team was able to provide new insights into the moving pieces of the CFTR machinery.

Through the integration of cryo-electron microscopy, electrophysiology and smFRET, the research group was able to draw the connections needed to better understand how CFTR works.

"There is potential here to help cystic fibrosis patients by learning about the structure and behavior of CFTR," said first author Jesper Levring, Rockefeller University. "Looking at these molecules one at a time using these methods—single-channel electrophysiology and smFRET—we could correlate the function of the channel with the conformational changes and relate it back to the underlying structural biology."

What the researchers found is that CFTR exhibits a hierarchical gating

mechanism. The two nucleotide-binding domains of the CFTR dimerize (combine) prior to channel opening. Conformational changes within the dimerized channel, related to ATP hydrolysis (a reaction where energy is released), regulate chloride conductance.

The significance of this mechanistic insight was further revealed by the finding that the potentiator drugs Ivacaftor and GLPG1837 enhance channel activity by increasing pore opening while the nucleotide-binding domains are dimerized. Mutations that cause cystic fibrosis can reduce the efficiency of the dimerization. These insights will be useful for informing the search for more effective clinical therapies.

"The most satisfying thing about this work is that we have answered a question about how CFTR works that has been a subject of debate in the field for many years," said Chen, who is co-corresponding author of the study.

"Every individual method has limitations, so you can have good data but still not have the answers. By combining approaches, we have gotten to a unified mechanism that gives us insight into how this molecule works. With this understanding we can then test how mutations or drugs affect the function, which is ultimately how we'll get to better therapeutics."

More information: Jue Chen, CFTR function, pathology and pharmacology at single-molecule resolution, *Nature* (2023). DOI: 10.1038/s41586-023-05854-7.
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