# The Road to the Structure of CFTR and Pharmacological Correction of the Major Cystic Fibrosis Defect: Is There an Actual Binding Pocket?

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The Cystic Fibrosis (CF) Transmembrane Conductance Regulator (CFTR) is a unique member of the ATP-Binding Cassette (ABC) superfamily in functioning as an ion channel rather than a classical transporter. Loss-of-function mutations of CFTR directly cause the cystic fibrosis (CF) disease. The most prevalent mutation, accounting for up to ~70% of all defective CFTR alleles, is a deletion (F508del) in the first nucleotide binding domain (NBD1), resulting in rapid degradation of virtually all CFTR protein. In contrast, normal CFTR contributes to cholera toxin-associated secretory diarrhea that kills thousands worldwide annually. CFTR has been extensively characterized since the original cloning of the gene in 1989 by Francis Collins and colleagues. Electrophysiological assays, mutagenesis, crosslinking, biophysical studies, cellular localization and low-resolution three-dimensional (3D) structures have shed light on CFTR folding, gating and channel processing. Recent achievements in developing small molecule correctors and modulators showed that CFTR defects can be partially repaired through direct drug-binding to the protein, likely in several distal locations on the molecule. However, progress in answering several critical questions about CFTR will remain limited until high-resolution three-dimensional structure can be achieved, such as: 1) What is the precise amino acid composition and structure of the chloride pore? 2) How well defined is the chloride selectivity filter? 3) How does the physical contact between the regulatory (R) region and rest of the channel regulate channel activity? 4) What is the role of Phe508 in CFTR folding? 5) How does phosphorylation and nucleotide binding regulate chloride gating? and 6) Where do CFTR correctors bind and how do they help fold CFTR-F508del? We are now able to express and purify functional CFTR protein from yeast in sufficiently large amounts for x-ray crystal structure determination. A portion of the innovative proposal employs novel synthetic antigen-binding fragments (SynFabs) to address issues specific to CFTR for forming well-ordered crystals, including immobilizing known regions of disorder and trapping channels in discrete conformations. Taking advantage of existing structures of related ABC proteins, we have created a special homology model to understand problems surrounding the folding of CFTR-F508del. The homology model is sufficiently accurate to allow structure-based *in silico* drug screening for pharmacological "correctors" of CFTR-F508del in primary human CF airway epithelial cells. Results will help us overcome the current efficacy barrier of CF drugs and increase lifespan of CF patients.