Structural and energetic insights into CF-causing mutations in CFTR

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Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) result in cystic fibrosis (CF), with various mechanisms for loss of function due to different mutations. In the case of substitutions in the nucleotide binding domains (NBDs) of this ATP binding cassette (ABC) protein, these include destabilization of the domain or the entire channel and perturbations in ATP binding and catalysis, necessary for gating the channel. F508del within the first NBD, the most common and severe mutation, causes defective channel processing and channel gating defects, with our NMR studies pointing to inhibition of NBD dimerization as part of the underlying mechanism. Characterization of the CFTR NBD2 has lagged behind research into the NBD1 domain, because NBD1 contains the F508del mutation and NBD2 is highly unstable. Nonetheless multiple disease-causing mutations reside in NBD2 and the domain is critical to CFTR function, since channel gating involves NBD1:NBD2 dimerization and NBD2 contains the catalytically active ATPase site in CFTR. Using bioinformatics and iterative screening of substitutions, we have produced minimally mutated stable constructs leading to an NBD2 crystal structure and characterized the effects of NBD2 mutations identified in CF patients. We demonstrated that N1303K and G1349D have lower stability, as previously shown for some NBD1 mutations, suggesting a role for NBD2 instability in the pathology of CF. With the recently published cryoEM structures of CFTR, modeling and computational work has pointed to additional mechanisms for defects of the various CF-causing substitutions. Together with our work, these support individualized CF therapeutic strategies for more rare CFTR mutations.