Developing a Chaperonin-based Label-free real-time high-throughput assay platform that identifies lead compounds to stabilize misfolded proteins; *Cystic Fibrosis Transmembrane Regulator Nucleotide Binding Domain 1 as a test case*

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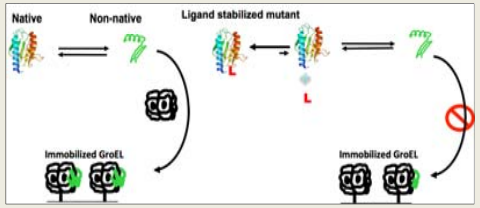
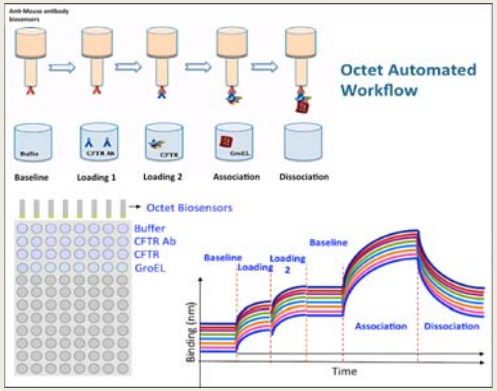
Abstract:

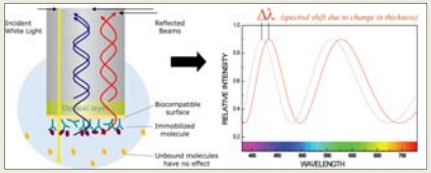
Protein misfolding diseases account for 30-50% of known human diseases. It is possible that chemical ligands that stabilize proteins can be used to treat a significant subset of these folding diseases. Discovery of such lead compounds is however, hampered by slow & expensive screening. We have previously utilized an immobilized high affinity form of *E. coli* chaperonin GroEL as a tool to detect misfolded proteins and identify small molecule ligands to stabilize them (Naik et al, Biopolymers, 2010). Based on our proof of principle success, we have now developed a high-throughput platform using the GroEL chaperonin in conjunction with label free protein interaction techniques such as Surface Plasmon Resonance (SPR) and Bio-layer interferometry (BLI) to detect general protein unfolding and stabilization. We demonstrate that the nucleotide free form of Cystic Fibrosis Nucleotide Binding Domain 1 (CFTR NBD1) undergoes partial unfolding reactions that can be detected with GroEL when the chaperonin is either immobilized or free in solution. These label free techniques allow us to monitor direct binding of GroEL to the transient states of partially folded proteins, providing real time quantitative information on the specificity and affinity of binding as well as the kinetics of the interactions. Any ligands that bind to native protein will shift the dynamic folding equilibrium from their partially folded form toward the native protein fold, decreasing GroEL binding (decreased signal), which can potentially result in the further development and characterization of the potential lead compound(s). A limited set of CFTR correctors tested showed the characteristics of potential protein stabilizers (decreased GroEL binding) for both wild type and delta F508 CFTR variants. In conclusion, the chaperonin screening assay combined with SPR and BLI gives us a broad-range, rapid & real-time HTS system that can be used to screen and validate potential misfolding protein stabilizers.

Introduction:





Bio-layer Interferometry



Results:

Question 1: Can we detect stabilization of CFTR using GroEL and BLI?

Fig.1: Detecting GTP stabilization of CFTR with CFTR NBD1 on biosensors

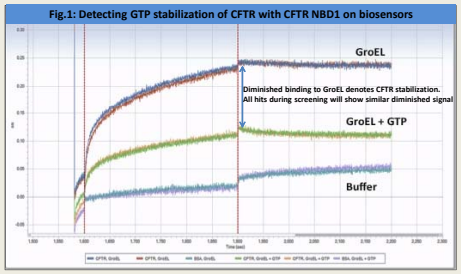
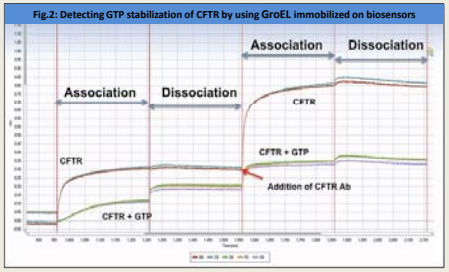


Fig.2: Detecting GTP stabilization of CFTR by using GroEL immobilized on biosensors



Question 2: Can we carry out rapid screening of compounds using this platform to detect CFTR stabilization?

Fig.3: CFTR Correctors

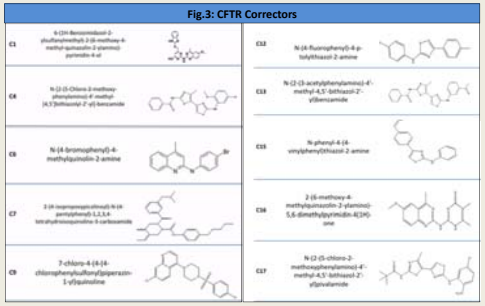


Fig.4: Screening corrector set 1 using CFTR biosensors for their efficacy in stabilizing CFTR

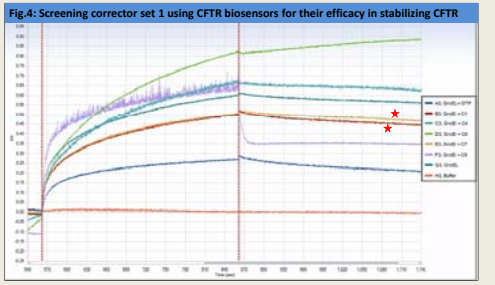


Fig.5: Screening corrector set 2 using CFTR biosensors for their efficacy in in stabilizing CFTR

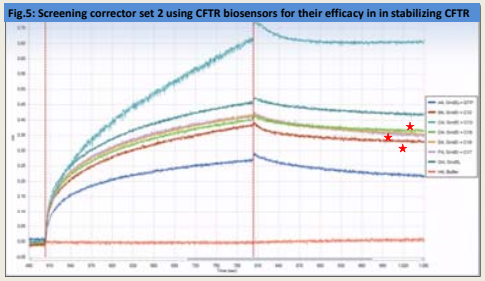
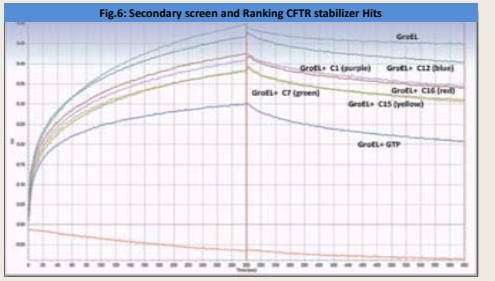


Fig.6: Secondary screen and Ranking CFTR stabilizer Hits



10 corrector compounds were sourced from the Cystic Fibrosis Foundation. We introduced the antibody-antibody-CFTR complex into buffers containing GroEL in the presence of the different stabilizers and observed the change in binding as compared to binding with CFTR alone. The compounds showing binding lower than CFTR NBD1-GroEl binding were designated as hits(★)

Fig.7: Stabilization of mΔF508 CFTR by GTP using GroEL biosensors

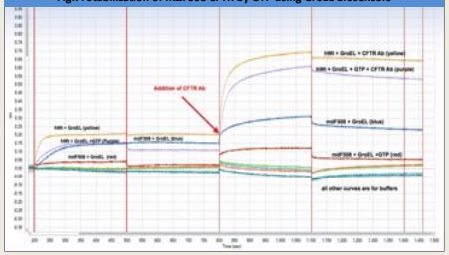
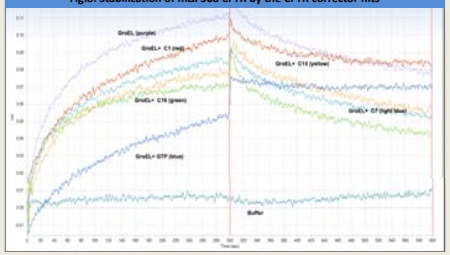


Fig.8: Stabilization of mΔF508 CFTR by the CFTR corrector hits



Since human ΔF508 mutants, which is the most common CF mutation are not available, we compared the Human wt CFTR and mouse ΔF508 mutants for GroEL binding and GTP stabilization. We utilized the top 4 hits to successfully stabilize the mouse ΔF508 mutants

Summary & Conclusions:

- Proof of principle for using the GroEL chaperonin in conjunction with label free technologies as a platform system to detect protein misfolding
- Demonstrates that ligand based stabilization of protein folding can be easily detected using BLI systems
- We utilized the platform to search for potential lead compounds for CFTR NBD1 Out of 10 corrector compounds, 4 showed CFTR stabilizing activity. Some of these hits did not inhibit DHFR binding to GroEL