Engineering of the FRB domain of the mechanistic target of rapamycin (mTOR) to probe transient interactions

Sarah Pollock, Erin Kennedy, Chidera Ekeocha, Vanessa Nwaiwu, Danielle Schmitt, Songon An, Minjoung Kyoung (mjkyoung@umbc.edu)
Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, MD 21250

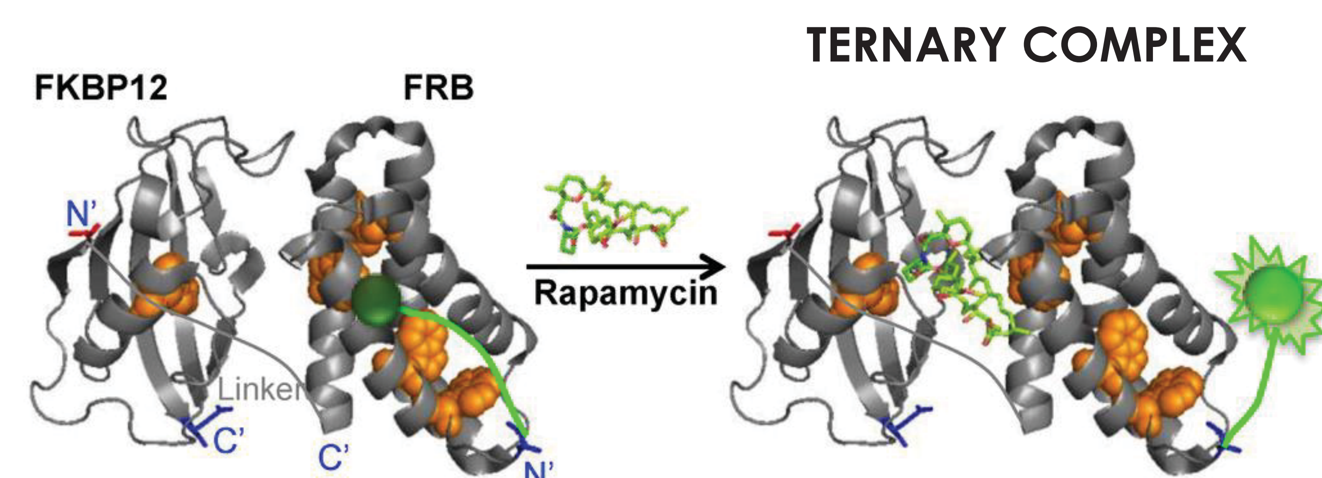
ABSTRACT

This project aims to develop a system to evaluate a novel sensor employing innovative nano-reaction chamber technology, which will track transient biomolecular interactions in real time at a single molecule level. We are evaluating this sensor by studying the interactions between the FK506-binding protein (FKBP) and the FKBP12-rapamycin binding (FRB) domain in the presence of various small molecules such as rapamycin. FKBP bound rapamycin forms complexes with the FRB domain of mTOR, which is an essential regulator of cell growth and survival. We purchased the FKBP gene from Addgene, and inserted it into the pET28b plasmid with a histidine tag for future purification. We have performed site directed mutagenesis on the FRB domain by deleting the endogenous cysteines and exchanging an endogenous serine at a desired location to a cysteine. This will allow us to probe the FKBP and FBR domain interactions by measuring fluorescence resonance energy transfer (FRET) efficiency. The mutated FRB and FKBP proteins will generate a FRET signal upon binding to small molecules. These tertiary interactions between a single FKBP, FRB domain, and a small molecule in our nano-reaction chamber will allow us to evaluate the sensor by analyzing the binding mechanisms and affinity.

BACKGROUND

FRB and FKBP are found at various organelles throughout the cell and are components of the mTOR signaling pathway. mTOR signaling affects most major cellular functions, regulating cell growth and proliferation, and controlling many processes that use energy, which is why it is an important pathway to study.

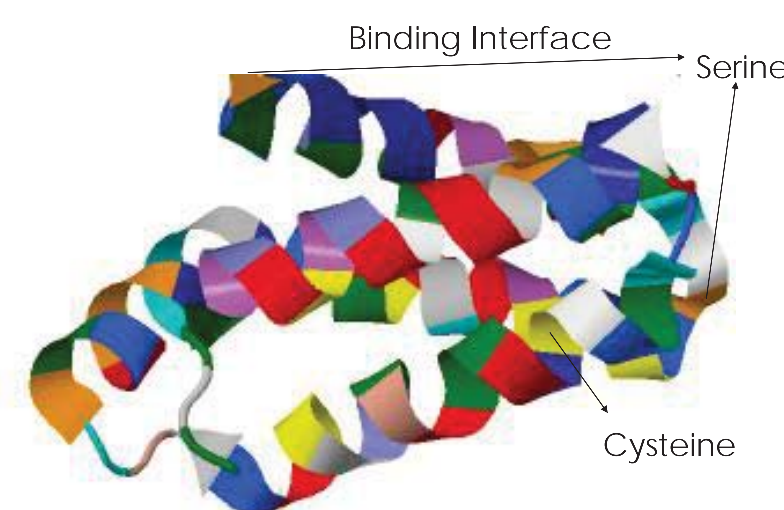
We are focusing on the complex that rapamycin forms with FKBP and FRB. When rapamycin is present it will form a complex with FKBP and FRB.

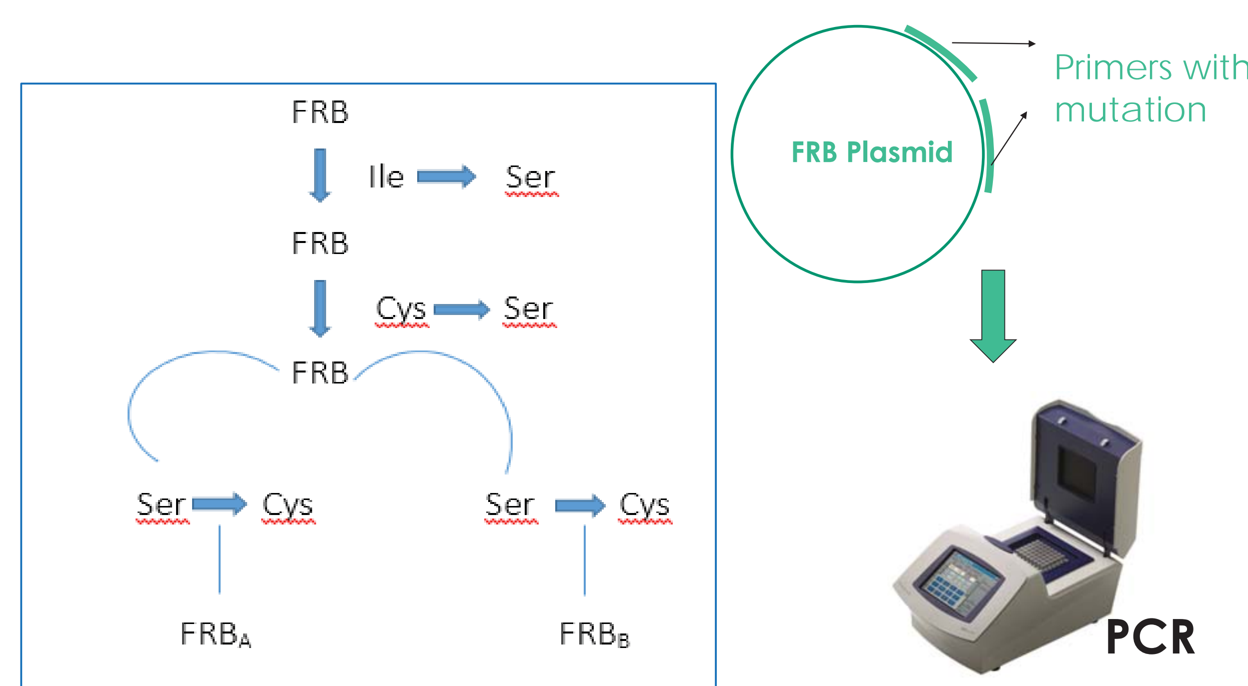


http://www.mdpi.com/2079-6374/5/2/131/htm

SITE DIRECTED MUTAGENESIS

The FRB protein needs to be labelled with a fluorescent dye in order to view its location and dynamics. The fluorescent dye can be attached to cysteine residues that are solvent exposed. After reviewing the protein in JSmol we determined that the one cysteine is not solvent exposed so we would need to mutate a serine residue to cysteine in order to successfully tag the protein. The plasmid that was purchased from Addgene encoded FRB with an isoleucine mutation, so we first changed the isoleucine to serine. Next, we mutated the existing cysteine into a serine so that it would not be accidentally tagged. Then we selected one of two serines that would make a good FRET partner relative to the FKBP label. Both of these serines were mutated into cysteines, leaving us with two options for a FRET partner of the FKBP protein.

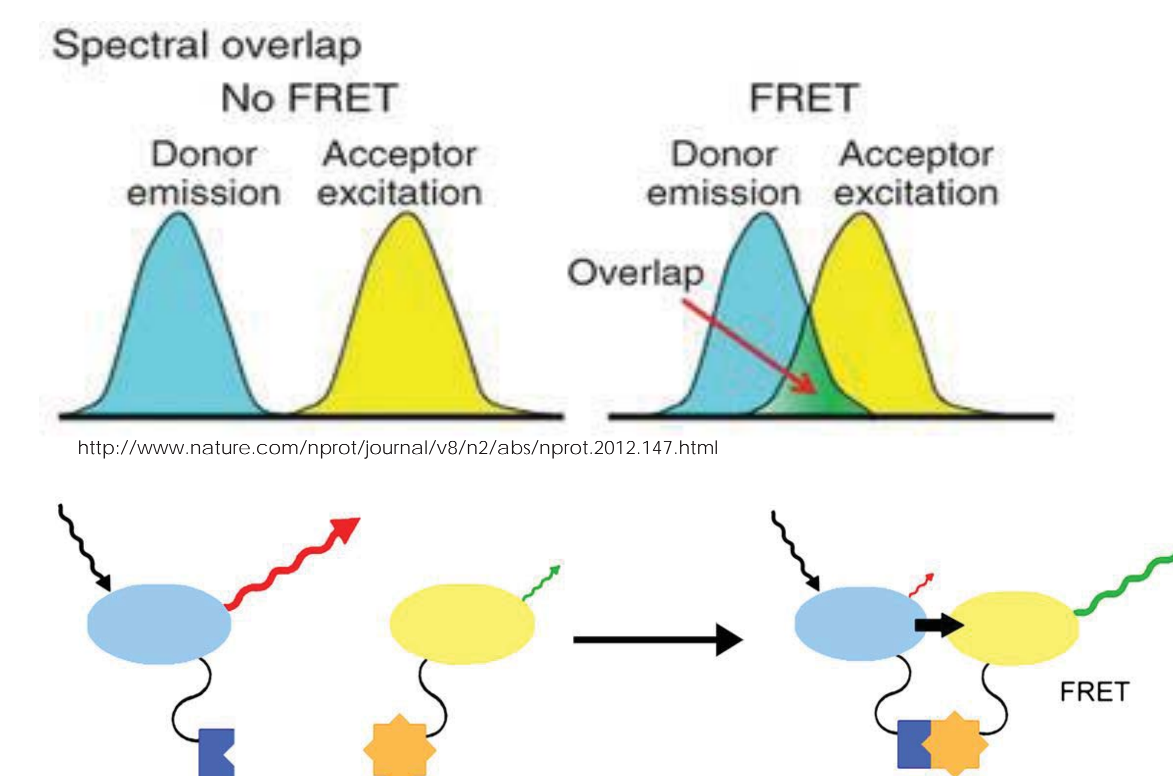




https://www.bioke.com/Products/New%20products/applications/PCR%20-%20Amplification

Process: The FRB plasmid containing primers with the mutation went through PCR. The PCR products were then transformed into competent cells and the colonies went through MiniPrep (Qiagen: 27106 and Thermo Scientific: K0502). Finally, the PCR products are sent for sequencing. Once the mutations are finished, the FRB protein will be fluorescently tagged.

FLUORESCENCE RESONANCE ENERGY TRANSFER



https://neuroamer.wordpress.com/2011/09/08/g-protein-coupled-receptors-rule-the-mind-through-phosphoinositide-signals/

The FRB protein will be fluorescently tagged, allowing us to measure the FRET signal upon formation of the FKBP/FRB/rapamycin complex.

FUTURE STEPS: NANO-REACTION CHAMBER

Development of this nano-reaction chamber will facilitate studies of short-lived protein interactions. The proteins will be in a smaller volume which will promote contact between proteins. We will also be able to obtain high concentrations at specific points in the proteins, monitor very weak interactions, sequentially add encapsulated proteins, and improve the strength and number of interactions. Our system is based on charge-charge interactions and yeast snare proteins.

ACKNOWLEDGEMENT

This investigation was supported in part by a MARC Undergraduate Student Training in Academic Research (U-STAR) Institutional Research Training Grant (2 T34 GM008663) from the National Institutes of Health, National Institute for General Medical Sciences and UMBC Startups and UMBC-Special Research/Assistantship Initiative Support (SRAIS) Award

REFERENCES

Jeong, Hee-Jin, Itayama, Shuya, Ued, Hiroshi. "*A Signal –On Fluoresensor Based on Quench-Release Principle for Sensitive Detection of Antibiotic Rapamycin.*" Biosensors 5.2 (2015): 131-140. Web. 11 Apr. 2016.

FRB ID: Chain B, Atomic structure of the Rapamycin Analogs in Complex with both Human FKBP12 and FRB Domain of FRAP

Bioke.; PCR –Amplification; *Labcycler*; Bioke; Web; 11 Apr. 2016.

Broussard, Joshua, Rappaz, Benjamin, Webb, Donna, Brown, Claire. "*Fluorescence resonance energy transfer microscopy as demonstrated by measuring the activation of the serine/threonine kinase Akt*." Nature Protocols 8 (2013): 265-281. Web. 18 Apr. 2016.

Wordpress.com; Neuroamer, G-protein Coupled Receptors Rule the Mind through Phosphoinositide Signals, 08 Sept. 2011; Web; 18 Apr. 2016.