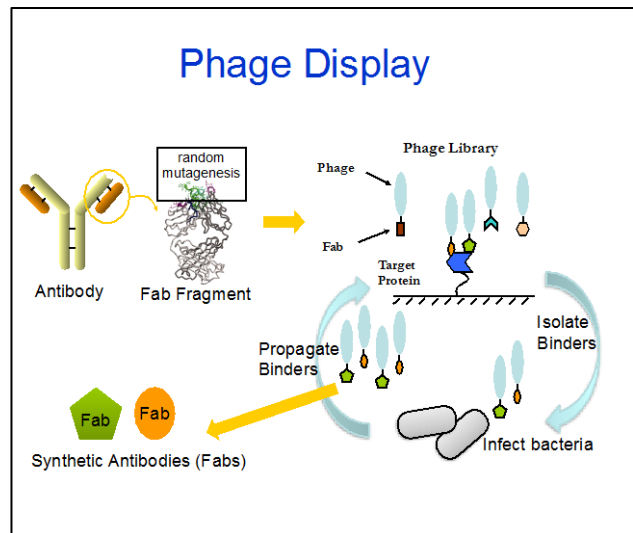


Research in the Rizk Lab

Dynamic Control of protein structure and function:

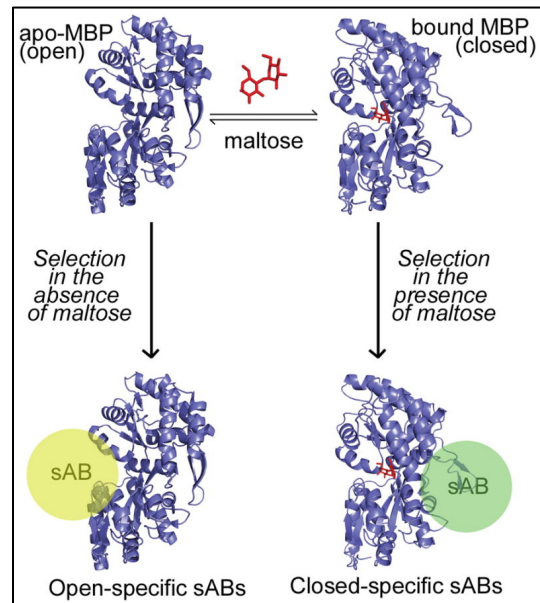
Proteins are tiny machines that carry out all sorts of biological functions. Proteins adopt very precise 3D structures that enable them to carry out their functions. Mutations that disrupt protein structure in most cases result in proteins that are unable to perform their natural function, leading to disease. Our goal in the Rizk lab is to understand the structural basis of protein function; specifically, how to rescue the function of a mutant protein by forcing it to adopt its natural functional 3D structure (or conformation).



We use protein-engineering tools to generate reagents based on antibody fragments that can precisely differentiate between different conformations of the same protein. We utilize a technique known as phage display, which allows the generation of engineered antibody fragments (Fabs), also known as synthetic antigen binders (sABs), with high affinity and specificity for a protein conformation. We have utilized this technique to generate a number of Fabs that can influence the function of proteins by influencing their structure.

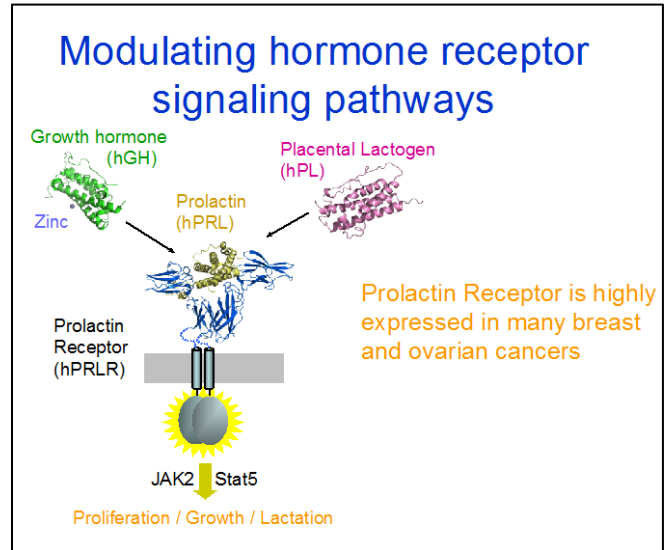
Dynamic regulation of ligand binding by design of allosteric effectors

We have shown that Fabs can be used to influence the affinity of a protein for its ligand. For example, Fabs that bind to maltose binding protein in its liganded form promote maltose binding and can dynamically increase its affinity without introducing any mutations in the proteins itself. Fabs that bind to the protein in the absence of ligand inhibit ligand binding and decrease the affinity for maltose. The Fabs act as allosteric effectors, meaning, they bind far away from the binding site, but affect the affinity for the ligand by influencing the equilibrium between the two conformations of the protein. We showed our engineered Fabs can be fully functional *in vitro* (in the test tube), on the cellular level, and *in vivo* (in a living organism).



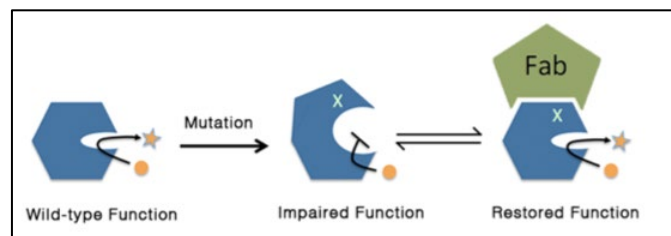
Modulation of hormone receptor specificity

We also show that Fabs can be used to modulate the specificity of a receptor for its hormones. For example, the prolactin receptor also binds to two other hormones, placental lactogen and growth hormone. Even though all three hormones bind to the same receptor, each binding event has a unique biological outcome ranging from growth, cell proliferation and lactation. The differences between the structures adopted by the receptor when bound to each of the hormones are subtle, yet highly structure-specific Fabs can recognize these differences and modulate the specificity of the receptor, increasing the affinity of one hormone relative to another. A structure of the Fab-bound receptor shows an allosteric mechanism that induces a structure more favorable for growth hormone binding over prolactin. Cell-based assays confirm that these Fabs can work on the cellular level to modulate the function of the receptor.



Rescuing enzyme function using Fab chaperons

Current work has shown the ability to engineer Fabs that can modulate the activity of enzymes. The goal is to use these reagents to rescue the function of proteins that have been disrupted by disease causing mutations, and as a tool to find therapies for a large number of inherited genetic disorders. We have demonstrated that Fabs that recognize the wildtype (functional) form of the enzyme IDH1 can be used to rescue the function of a mutant IDH1 found in brain tumors (Rizk, 2017). This process of using Fabs as chaperones for modulating enzyme activity can be expanded to a large number of genetic disorders where a single mutation affecting the function of an enzyme results in disease.



Generating bio-responsive nanostructures

Our work also extends to show that Fabs can be engineered to influence how proteins come together to form higher-order oligomers. Ongoing research with viral proteins that adopt multiple different complexes demonstrates that Fabs can be

engineered to modulate the equilibrium between different complexes of the same protein (*i. e.* monomer to dimer to multimer). We hope to utilize these Fabs as a tool to understand the mechanism of how a single protein can adopt multiple types of higher order complexes and to develop therapies that can prevent those transitions from one complex to another to block viral proliferation.

One of the main areas of research in the lab deals with generating subunits that will polymerize into higher-order nanostructures or disassemble in response to a biological molecule, such as glucose. This work will help us understand the basis for engineering reversible self-assembly for developing biosensors as well as molecular shuttles for targeted drug delivery.

Engineering Biosensors

Another branch of the Rizk lab is to utilize protein-engineering tools to develop protein-based biosensors for the detection of toxins and environmental pollutants. Current work explores the use of bacterial binding proteins as scaffolds for engineering biosensors by redesigning their binding pockets or enhancing a natural ability to bind to a molecule of interest. We are currently designing biosensors for the herbicide glyphosate, the active ingredient in Roundup and a major environmental pollutant in the US.

Other efforts are focused on the design of phage libraries for engineering protein-small molecule interactions to utilize directed evolution methods for the development of biosensors. Stay tuned...

Selected Publications:

- 1. Engineered synthetic antibodies as probes to quantify the energetic contributions of ligand binding to conformational changes in proteins**
Mukherjee S, Griffin DH, Horn JR, **Rizk SS**, Nocula- Lugowska M, Malmqvist M, Kim SS, Kossiakoff AA
J Biol Chem. 2018, doi: 10.1074/jbc.RA117.000656
- 2. Targeted rescue of cancer-associated IDH1 mutant activity using an engineered synthetic antibody**
Rizk SS, Mukherjee S, Koide A, Koide S, Kossiakoff AA
Sci Rep. 2017, 7(1):556
- 3. A molecular mechanism of artemisinin resistance in *Plasmodium falciparum* malaria**
Mbengue, A, Bhattacharjee, S, Pandharkar, T, Liu, H, Estiu, G, Stahelin, RV, **Rizk, SS**, Njimoh, DL, Ryan, Y, Chotivanich, K, Nguon, C, Ghorbal, M, Lopez-Rubio, JJ, Pfrender, M, Emrich, S, Mohandas, N, Dondorp, AM, Wiest, O, Haldar, K
Nature, 2015, 520(7549):683-7

4. **Engineering synthetic antibody binders for allosteric inhibition of prolactin receptor signaling**
Rizk, SS, Kouadio JL, Szymborska A, Duguid EM, Mukherjee S, Zheng J, Clevenger CV, Kossiakoff AA
Cell Commun Signal. 2015, 13(1):1-5.
5. **Generating conformation-specific synthetic antibodies to trap proteins in selected functional states**
Paduch M, Koide A, Uysal S, Rizk SS, Koide S, Kossiakoff AA
Methods, 2013, 60(1):3-14.
6. **Substance P variants as versatile tools for specific delivery of various types of bio-molecular cargo**
Rizk SS, Misiura A, Paduch M, Kossiakoff AA
Bioconjug Chem, 2012, 23(1):42-6.
7. **Allosteric Control of Ligand-Binding Affinity Using Engineered Conformation-Specific Effector Proteins**
Rizk SS, Paduch M, Heithaus JH, Duguid EM, Sandstrom A, Kossiakoff AA
Nat Struct Mol Biol, 2011, 18(4): 437-42.
8. **Keeping signaling in check**
Kossiakoff AA, Rizk S.
Structure, 2010, 18(3): 275-6.
9. **An engineered substance P variant for receptor-mediated delivery of synthetic antibodies into tumor cells**
Rizk SS, Luchniak A, Uysal S, Brawley CM, Rock RS, Kossiakoff AA.
Proc Natl Acad Sci USA, 2009, 106(27): 11011-5.
10. **Identification of cognate ligands for the *Escherichia coli* phnD protein product and engineering of a reagentless fluorescent biosensor for phosphonates**
Rizk SS, Cuneo MJ, Hellinga HW.
Protein Sci. 2006, 15(7): 1745-51.
11. **Computational design of receptors for an organophosphate surrogate of the nerve agent soman**
Allert M, Rizk SS, Looger LL and Hellinga HW.
Proc Natl Acad Sci USA, 2004, 101(27): 7907-12.
12. **Control of a biomolecular motor-powered nanodevice with an engineered chemical switch**
Liu H, Schmidt JJ, Bachand GD, Rizk SS, Looger LL, Hellinga HW and Montemagno CD.
Nat Mater. 2002, 1(3): 173-7.
13. **Construction of a fluorescent biosensor family**
de Lorimier RM, Smith JJ, Dwyer MA, Looger LL, Sali KM, Paavola CD, Rizk SS, Sadigov S, Conrad DW, Loew L, Hellinga HW.
Protein Sci. 2002, 11(11): 2655-75.