NATURE-INSPIRED ANTIBODY DESIGN AND OPTIMIZATION

Peter Tessier, Rensselaer Polytechnic Institute
tessier@rpi.edu
Mark Julian, Rensselaer Polytechnic Institute
Kathryn Tiller, Rensselaer Polytechnic Institute
Lilia Rabia, Rensselaer Polytechnic Institute
Lijuan Li, Rensselaer Polytechnic Institute
Seth Ludwig, Rensselaer Polytechnic Institute
Shekhar Garde, Rensselaer Polytechnic Institute
Sandeep Kumar, Pfizer Inc.
Ratul Chowdhury, Pennsylvania State University
Costas Maranas, Pennsylvania State University

Key Words: antibody, directed evolution, protein design, affinity, stability.

The biotech industry has seen an explosion in the development of therapeutic antibodies in the last decade. The advantages of antibodies as therapeutics – namely their high affinity, specificity, potency, stability, manufacturability and low toxicity – are compelling. Nevertheless, there are many challenges associated with antibody discovery and development that require key technical advances to improve the rational and reliable generation of potent antibody therapeutics. We have made three key discoveries that address some of these fundamental challenges related to the design and selection of antibodies with high affinity, specificity, stability and solubility. First, we find that the accumulation of affinity-enhancing mutations in the complementaritydetermining regions (CDRs) during affinity maturation is often a destabilizing process. Surprisingly, mutations that enhance antibody binding affinity are commonly destabilizing. Second, we have developed novel yeast surface display methods for co-evolving antibody affinity and stability to address the general problem of antibody destabilization during affinity maturation. Our approach simultaneously evaluates antibody binding to both antigen and a conformational ligand that acts as a folding sensor to rapidly identify sets of mutations that promote both high antibody affinity and stability. This methodology has enabled us to identify novel compensatory mutations that offset the destabilizing effects of affinity-enhancing mutations and lead to affinitymaturated antibodies with high thermodynamic stability. Interestingly, our directed evolution method appears to mimic some aspects of natural antibody evolution, as natural antibodies also accumulate similar types of compensatory mutations to maintain thermodynamic stability during in vivo affinity maturation. Third, we have developed novel antibody library design and selection methods for generating antibodies with high specificity. It is common for antibody specificity to be compromised during in vitro affinity maturation. We have developed innovative methods for designing antibody libraries based on natural antibody diversity to simultaneously sample residues at many sites in the CDRs and framework regions that are most likely to promote high specificity. By coupling these nature-inspired antibody libraries with novel positive and negative selection methods, we have isolated antibodies with specificities that rival those of natural antibodies and which are much higher than typical antibodies identified using in vitro selection methods. Interestingly, we find that antibodies with improved specificity also possess excellent biophysical properties, including high solubility and stability. We are currently using computational methods to understand how rare antibody variants are able to maintain high specificity and stability during affinity maturation. Our long-term goal is to develop systematic and robust design methods to rapidly generate and optimize antibodies for use in a range of diagnostic and therapeutic applications.