456a Antibody Affinity Maturation Using Computational Protein Design

Shaun M. Lippow, Bruce Tidor, and K. Dane Wittrup

In the development of a therapeutic protein, it is often necessary to engineer a high-affinity interaction. Computational protein design and directed evolution provide complementary and potentially synergistic approaches to protein engineering. Computations can in principle search a vastly larger sequence space, albeit with an approximate energy description serving as a surrogate for biochemical function. Directed evolution is limited by experimental library size, with random mutagenesis generally covering most single mutations and only sampling larger combinations of mutations.

Here we have explored the evolutionary space available for a case of antibody affinity maturation. Our objective was to explore the binding affinity landscape for mutations in high-affinity antibody/antigen complexes. Computational tools were used to address the following questions: What does the space of all single mutations look like with regard to variations in binding affinity? How much more is gained by covering all double or triple mutations, over the simple accumulation of single mutations? That is, how often does a pair or more of mutations improve binding affinity, where each mutation individually is neutral or detrimental to binding?

The design process uses the dead-end elimination and A* algorithms and an approximate, pairwise-additive energy function to search a discrete rotamer space. The most promising designs are then re-evaluated using successively more accurate models and energy functions, including Poisson-Boltzmann continuum electrostatics. We have incorporated an improved model of the nonpolar component of the hydration free energy, replacing common models based on solvent-accessible surface area.

Initial studies have found that most single mutations are predicted to be neutral or unfavorable and only a few variants are predicted to yield marginal improvements. We have experimentally tested some of the predictions of small improvement and validated the computations. We find that our improvement to the nonpolar component of solvation greatly improves the quality of designs. Work is underway to examine whether substantial, non-additive affinity enhancements are available through larger sets of multiple mutations.