4aq Directed Evolution of Proteins for Biomedical Application

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The overall theme of my thesis research is to apply directed evolution to engineer proteins for biomedical application and investigate the protein structure-function relationship. I have been working on two proteins: human estrogen receptor and homing endonuclease as described below.

1. Engineering of human estrogen receptor (hER) with altered ligand specificity: We developed a sensitive yeast two-hybrid system to screen for hERa ligand binding domain (hERaLBD) variants with increased transactivation potency toward testosterone. After two rounds of directed evolution, we identified five hERa variants with dramatically improved transactivation potency toward testosterone in both yeast and mammalian cells. These variants showed up to 7,600-fold improvement in the binding affinity for testosterone and only slightly reduced affinity toward 17b-estradiol.

2. Creation of a novel protein function by in vitro coevolution: The transactivation activity of hERa in response to corticosterone is not readily detectable in our yeast two-hybrid system, as well as in a standard mammalian cell transient transactivation assay. Thus we introduced two steroids, testosterone and progesterone that provide a progressive structural bridge between 17b-estradiol and corticosterone, to assist the directed evolution of hERaLBD. Four rounds of random mutagenesis resulted in two hERaLBD variants that respond to corticosterone. Creation of this new ligand activity required the presence of four simultaneous mutations. Such method, which involves the design of a hypothetical pathway for the target function followed by stepwise directed evolution of the corresponding protein along the pathway, can find applications in many other directed evolution experiments.

3. Directed evolution of homing endonuclease with novel sequence specificity: Here we report the development of a highly sensitive selection method for the directed evolution of homing endonucleases. This system links the DNA cleavage event by homing endonuclease with the survival of an E. coli cell under specific conditions. Using I-SceI as a model homing endonuclease, we have demonstrated that cells with wild type I-SceI showed a high cell survival rate of 80-100 % in the presence of the original I-SceI recognition site, whereas cells without I-SceI showed a survival rate less than 0.003%. Currently, we are using this selection method in combination with in vitro coevolution, to engineer I-SceI variants that can selectively cleave a target sequence found in a mutant transmembrane conductance regulator (CFTR) gene involved in cystic fibrosis. This mutant gene contains a deletion (□F508) and is responsible for most of the cases of cystic fibrosis, which affects one in 2500 to one in 1600 Caucasians. The engineered I-SceI may be used to correct this gene in a gene therapy regime.