C-1-06

Screening and Design of Small Molecules that Reversibly Bind to Streptavidin

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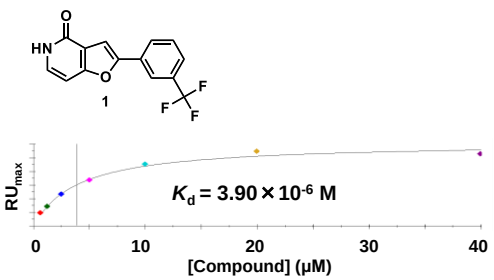
Keywords: Ligand docking, Protein-small molecule interaction

The interaction between streptavidin (from *Streptmyces avidinii*) and biotin is known as the strongest non-covalent interaction in nature (K_d = 10^{-13} M)[1]. Streptavidin-biotin system is used for many applications in biotechnology, such as affinity column chromatography (isolation), SPR sensor chip (immobilization) or fluorescence imaging (detection). However, for some applications, a few disadvantages of this system have been pointed out. One of the major limitations of this system is the almost irreversible binding under physiological conditions. To disrupt a streptavidin-biotin complex, it is necessary to denature the streptavidin using harsh condition.

In order to make the binding of biotin with streptavidin more easily reversible under mild conditions, much work has been done to modify streptavidin by genetic mutations. In contrast, another approach to develop biotin analogs with reduced affinity to streptavidin has been hardly reported, and the reported biotin analogs provide only partial reversibility. In this research, we screen a large chemical library to search for novel chemical scaffolds that bind to streptavidin, and aim to optimize the hit compounds based on information from X-ray crystal structure.

First, we performed the screening for avidin (from hen egg) which has identical tertiary structure with streptavidin. 166,480 compounds were screened by using fluorescence polarization, and we selected 773 compounds as primary hits. These compounds were subsequently screened for streptavidin, dose-dependently assayed, and analyzed by SPR (Surface Plasmon Resonance). Many biotin analogs were detected as hits, but we excluded these compounds. Finally, 6 compounds were selected as novel ligand scaffolds for streptavidin which exhibited fully reversible binding to the protein (K_d = 10^{-4}~10^{-6} M). We characterized the binding mode of compound 1 which showed the strongest affinity (Figure). Based on information from X-ray crystal structure and derivative synthesis, the amide moiety fixed in *cis* form was most likely to be essential for the binding. We synthesize further derivatives of compound 1 and optimizing the affinity to streptavidin. Then we will demonstrate the utility of the new reversible scaffold by applying it for affinity column chromatography.



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