PROTEIN BIOCHEMISTRY

Streptavidin surprises

Reengineered streptavidin mutants bind biotin-4-fluorescein with extremely tight yet reversible binding affinity.

The terrifically tight noncovalent interaction between biotin and streptavidin has made this small molecule–protein pair one of the most practical and widely used systems for affinity labeling. "It's the best ligand-protein binding pair in all of biology," remarks Charles Cantor, who has pursued research on the system for nearly two decades.

The most recent effort by Cantor' laboratory was an attempt to create streptavidin mutants with a wide range of biotin binding affinities, as well as to reengineer the normally tetrameric protein into a stable, single-chain dimer, amenable to phage and chip display (Aslan *et al.*, 2005). Streptavidin is composed of four separate chains arranged into two dimers, which interact very tightly to form the tetramer. Previous attempts to stabilize the dimer have involved engineering strategies to destabilize the dimer-dimer interface, but at a price in the loss of biotin binding affinity. Uniquely, the streptavidin binding site is composed of portions of both dimers, so when the tetramer is broken apart, part of the biotin binding pocket is lost.

A reduction in binding affinity, however, was just what the researchers were after, in endeavoring to engineer mutant streptavidin dimers with a broad spectrum of affinities for biotin. But the result was unexpected. The group was surprised to discover that their mutant constructs bound biotin-4-fluorescein (B4F) with a 100,000-fold higher affinity than they bound biotin. "If anyone had asked, 'What is the best-understood tight ligand-receptor binding interaction known?' the answer is, 'Biotin-streptavidin'. That we produced a surprise out of a system that everyone thought they completely understood is what made this result so exciting," says Cantor.

What makes these new streptavidin mutants so handy is that, unlike the traditional system, the binding of B4F is reversible. "Streptavidin binding to biotin is so strong, that once it is captured, the conditions that you need for release are draconian," explains Cantor. The streptavidin mutants prefer B4F over biotin by a huge advantage, but adding enough excess biotin interrupts the B4F-streptavidin interaction. These novel affinity labels with extremely strong yet reversible binding could have practical applications across many fields, as Cantor anticipates, "This will allow for the possibility of more complex biotin-streptavidin assays."

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RESEARCH PAPERS

Aslan, F.M. *et al*. Engineered single-chain dimeric streptavidins with an unexpected strong preference for biotin-4-fluorescein. *Proc. Natl. Acad. Sci. USA* **102**, 8507–8512 (2005).