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Engineered Streptavidin Mutants with High-Affinities and Reversible Binding Capacities to Biotin- and SBP- Tagged Proteins

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Background

The high-affinity interactions of biotin and streptavidin binding peptide (SBP) with streptavidin are well characterized and have been exploited for a variety of uses in biomedical research. One such application of this technology is the purification of recombinant biotinylated and SBP-tagged proteins via affinity chromatography. There are, however, several limitations that exist with respect to the technologies currently available in this field; wild-type streptavidin columns can only be used in a single round of purification due to the ultra-high affinity interaction between streptavidin and the free biotin in the elution buffers. The present technologies overcome these limitations.

Researchers at the University of Calgary have engineered novel streptavidin mutants that can be re-used in multiple rounds of biotin- and SBP-tagged protein purification. The mutant forms of streptavidin have a high binding affinity for SBP and a reduced binding affinity for biotin. This makes their interactions with biotin reversible and thus makes them viable for multiple rounds of purification. Moreover, they can easily be produced and manufactured using common bacterial expression systems and are readily purified.

Areas of Application

- Affinity purification of biotinylated and SBP-tagged proteins using liquid chromatography
- Immobilization of proteins to biochips, biosensors and enzyme bioreactors coated with the engineered streptavidin mutants
- Conjugated mutants can serve as strippable detecting agents for various blots (ie. Western blots)
- Cell and protei n isolation
- Scaleable up to 300mL

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Competitive Advantages

- Efficient affinity purification of biotin- and SBP-tagged proteins in a re-usable and cost-effective manner; the engineered mutants can be regenerated for multiple rounds of protein purification
- Mild elution conditions preserve the integrity of the molecule of interest
- Easily produced, purified and packaged into a product

Wild-Type Streptavidin	8-aa-loop H127C Mutein Streptavidin	SAVSPBM18 Tetrameric Streptavidin
✓ Highly specific binding	✓ **Highly specific binding**	✓ **Highly specific binding**
× Ultra-high affinity for biotin; irreversible (Kd = 10^{-14} M)	✓ **Reduced binding affinity for biotin (Kd = 1.9 x 10^{-8} M)**	✓ **Reduced binding affinity for biotin (Kd = 11.5 nM)**
× Single round of purification	✓ **Multiple rounds of purification**	✓ **Higher binding affinity for SBP (Kd = 15.7 nM)**
× High associated costs	✓ **Lower associated costs**	✓ **Multiple rounds of purification**
	✓ **Easily produced using *Bacillus subtilus***	✓ **Lower associated costs**
		✓ **Easily produced using *Bacillus subtilus***
		✓ **High recovery (~90%)**
		✓ **Compatible with commonly used buffer and salt solutions**

Intellectual Property Status

- **Issued:** U.S. 7,265,205, U.S. 7,704,708

Publications

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- Barrette-Ng, I. et al. "The structure of the SBP-Tag-streptavidin complex reveals a novel helical scaffold bridging binding pockets on separate subunits." Acta Crystallographica Section D, Vol 69: pp. 879-887. January 2013.