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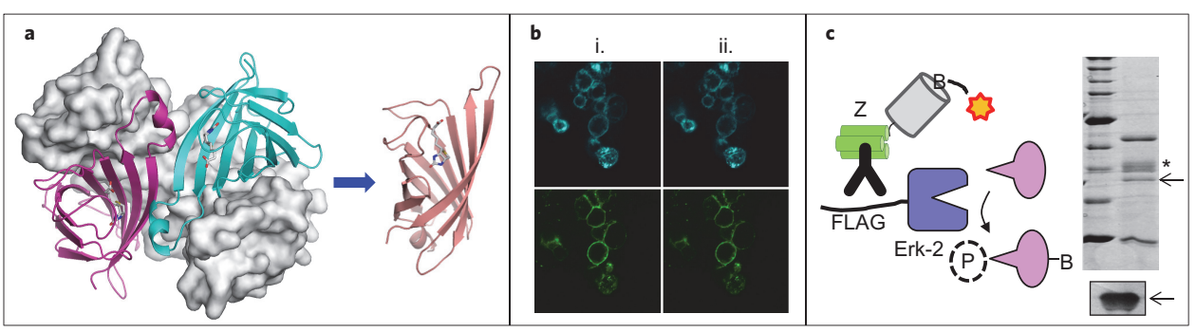
PRESS RELEASE

Engineered monomeric streptavidin for cell labeling and proximity dependent biotinylation

March 6, 2016 — A novel streptavidin variant with improved biotin binding characteristics allows stable monovalent detection of biotinylated targets for imaging applications and can be recombinantly fused to introduce a biotin binding tag.



(a) Streptavidin tetramer (left) was engineered to form a monomer that binds biotin with low nM affinity (right). (b) HEK293 cells displaying biotinylated CFP (top) were fluorescently labeled with Alexa 488 conjugated mSA (bottom). Fluorescent images taken at i) t = 0 and ii) 50 min indicate little dissociation of bound mSA. (c) mSA fused to the Z domain was used to form a noncovalent complex with antibody and recruit reactive biotin to a protein of interest, Erk-2. Biotinylated proteins are affinity purified using immobilized streptavidin and analyzed (e.g. SDS-PAGE, Western blot, mass spectrometry) to identify all proteins that interact with Erk-2.

A team of researchers from the University at Buffalo in Amherst, NY have successfully engineered a novel variant of streptavidin that forms a stable monomer and is capable of monovalent biotin detection. Streptavidin is broadly used for detection of biotinylated ligands but may cause target crosslinking that can interfere with observations at a molecular level. The engineered streptavidin monomer (mSA) binds biotinylated targets without crosslinking because it has only one biotin binding site. As a structural monomer, mSA can also be genetically fused to another protein to form a biotin binding tag. The binding of a biotinylated ligand to the fused mSA domain is useful in various biochemical and cell biology studies. The optimization of mSA design and its potential applications in labeling and protein-protein interaction studies are described in the forthcoming 2016 issue of the journal *TECHNOLOGY*.

"The native streptavidin-biotin interaction has limitations stemming from the obligate tetrameric structure of streptavidin. A small, single domain protein that binds biotin, such as mSA, extends the utility of the streptavidin-biotin system by

allowing monovalent biotin detection and creation of genetic fusions," says Professor Sheldon Park, Ph.D., of the University at Buffalo and Principal Investigator on the paper.

Streptavidin and its homologs all form oligomers, making it difficult to use them as genetic fusions. The group had previously reported the engineering of a stable monomeric protein that binds biotin with low nM affinity and demonstrated that the molecule can be fused to GFP to create a bi-functional molecule. In this study, the group optimized the design and demonstrated how the engineered molecule may be used in biotechnology. A significant improvement in the binding characteristics of mSA includes stabilization of the biotin bound complex to achieve consistent labeling of biotinylated targets over time. For example, using mSA conjugated to a fluorophore, biotinylated receptors on the cell surface can be stably, and specifically, labeled over a period of 1 hour with little loss of signal, which should be useful during fluorescence microscopy studies.

Unlike oligomeric streptavidin, mSA can be readily fused to a protein of interest (POI) to construct a recombinant tag for biotin binding. The current study explores the use of mSA fusion in proximity dependent biotinylation — a technique that has been shown to be useful in proteomics research. Proximity dependent biotinylation utilizes enzymatic or chemical methods to selectively biotinylate the molecules that interact with POI, so that they can be affinity purified using immobilized streptavidin. The authors demonstrated that the mSA tag can be used to recruit a reactive biotin species and achieve specific biotinylation of the molecules that directly or indirectly interact with. Since only co-localization with mSA is required for this to work, direct fusion to mSA is not needed. Instead, mSA may be recruited to the POI through a series of noncovalent interactions, for example, with an antibody, in order to target interacting molecules for biotinylation. The design affords a high level of modularity and works on molecules that interact only transiently, such as an enzyme and its substrate.

The team from UB is working to further reduce the rate of biotin dissociation in order to optimize the labeling efficiency even more. To this end, the crystal structure of mSA (PDB 4JNJ) is used to model the effects of various mutations before they are tested in the lab. The improved biochemical characteristics of mSA and its significantly smaller size (roughtly 25% of streptavidin) create new opportunities in biotechnology by leveraging existing infrastructure and ubiquity of the streptavidin-biotin system to study new interactions.

Additional co-authors of the *TECHNOLOGY* paper are Jasdeep K. Mann, Daniel Demonte, and Christopher M. Dundas, all from the Department of Chemical and Biological Engineering at the University at Buffalo.

Corresponding author for this study in TECHNOLOGY is Professor Sheldon Park, Ph.D., sjpark6@buffalo.edu.

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