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A new high affinity immobilization tag for generating recombinant antibodies by phage display library selection

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A critical element in a phage display sorting experiment is immobilization of the target protein in functional conformation. There are inherent issues associated with the current methods of immobilization, especially for proteins of limited stability. To circumvent these problems, we have developed a mutant form (N5A) of calmodulin binding peptide (CBP) as an immobilization tag for phage display sorting. The immobilization relies on the ultra high affinity of calmodulin to the mutant CBP in the presence of calcium, which can then be reversed by adding calcium chelators like EDTA allowing "capture and release" of the specific binders in a controlled manner. To evaluate the capabilities of this system, we chose a set of challenging cases that had failed in selection using traditional immobilization. In virtually all cases, we were able to generate synthetic antibodies (sABs) for these targets using CBP fused constructs in selection campaigns. The sABs are of high affinity and have been successfully used to selectively recognize antigens in cell-based experiments. Some of these targets were problematic even without any tag, so the fact that all led to successful selection endpoints means that borderline cases can be worked on with a high probability of a positive outcome. Taken together, we feel the evidence indicates that the CBP tag embodies all the attributes of covalent immobilization tags, but do not suffer from some of their well-documented drawbacks.

Biography

Somnath Mukherjee is currently a Postdoctoral Research Scholar working on Protein and Antibody Engineering in the group of Professor Anthony A Kossiakoff at University of Chicago. He has completed his PhD from Indian Institute of Science and Technology (IIT) Kharagpur, India. He has more than 15 peer reviewed publications in journals of international repute.

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