Poster Presentation

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Ordered Single-Site Immobilization of Enzymes enabled by Linchpin Directed Modification

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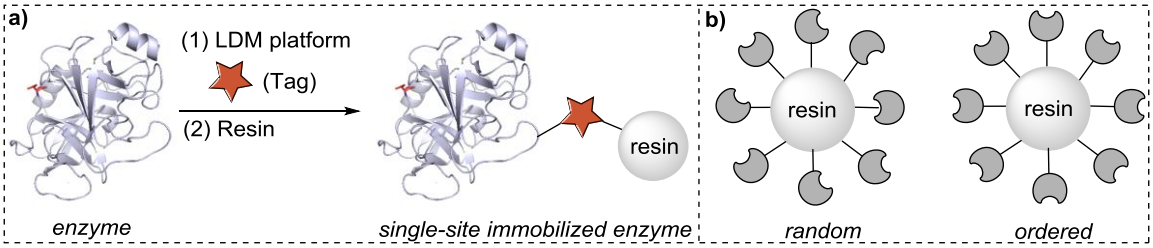
Enzymes are biomolecules with the capability to catalyze chemical transformations with remarkable efficiency even at low concentration.[1] Immobilization of these biomolecules has been useful in the development of various biosensors and bioreactors.[2] Further, regulation of the orientation during the process of immobilization promises to increase the catalytic activity and thermal stability of enzymes.[2,3] We have developed a method for ordered immobilization of enzymes hinged on our linchpin directed modification (LDM) platform.[4] At first, we synthesized a library of LDM reagents. Subsequently, we identified the reagent for single-site labeling of trypsin and α-chymotrypsin. Interestingly, the structure and activity of the enzymes remain conserved post-modification. Next, we immobilized the enzyme from the specific site on an appropriately functionalized resin. We are investigating the effect on the thermal stability, catalytic activity, and self-digestion propensity for both the enzymes. We anticipate that the immobilized enzyme would allow us to resolve the challenges related to protein sequencing. The digestion of protein of interest (POI) using immobilized enzyme would reduce the signal suppression from peptides culminating from enzyme auto-digest. At the same time, we will be able to use thermal denaturation of POI. It would eliminate the requirement of additives like urea and dithiothreitol which is responsible for the signal suppression during peptide mapping and subsequent MS-MS.



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