EASTERN MICHIGAN UNIVERSITY

Chemistry Department Seminar

Monday November 24, 2014

4:00 p.m.

Halle Library Auditorium

Third Biochemistry Faculty Candidate

Optimization of protein bioconjugation reactions using combinatorial peptide librariesse

Chemical reactions that facilitate the attachment of synthetic groups to proteins are useful tools for a wide range of fields, including cell biology studies, the construction of new biomaterials, and the development of novel therapeutics. In order to create well-defined protein bioconjugates, methods of site-specfic protein modification are required. One such reaction is a pyridoxal 5'-phosphate (PLP)-mediated transamination reaction that site-specifically oxidizes the N-terminal amine of a protein to afford a ketone or an aldehyde. This bioorthogonal functional group can then be used to attach a reagent of choice through oxime formation. Since the initial report of PLP-mediated transamination, it has been found that the N-terminal sequence of the protein can significantly influence the overall success of this strategy. In order to identify sequences that result in high levels of modification, we developed a high-throughput method of optimizing protein bioconjugation reactions using a combinatorial peptide library in which short peptides served as a model for protein reactivity. The library screening was achieved using a one-bead-one-compound peptide library, a colorimetric detection scheme to identify high-yielding sequences, and a deconvolution method using a built-in peptide truncation ladder for rapid mass spectrometry sequencing of hit beads. An N-terminal sequence with high reactivity was identified which appears to provide a significant improvement in the reliability with which the PLP-mediated bioconjugation reaction can be used. The results also provide a demonstration of how synthetic peptide libraries can accelerate the discovery and optimization of protein bioconjugation strategies.