ENGINEERING CLPS FOR ENHANCED N-TERMINAL AMINO ACID BINDING AND USE IN PEPTIDE SEQUENCING

Jennifer Tullman, National Institute of Standards and Technology (NIST), Institute for Bioscience and Biotechnology Research (IBBR)
tullmanj@ibbr.umd.edu
Nicholas Callahan, University of Maryland, IBBR
Zvi Kelman, NIST, IBBR
John Marino, NIST, IBBR

Key Words: protein engineering, peptide sequencing, biosensor, N-end Rule Pathway

As different single-molecule protein sequencing technologies emerge, the need for reagents that can selectively recognized and detect amino acids with high affinity has become apparent. Naturally occurring proteins that function through recognition of amino (N)-terminal amino acids (NAAs), such as the N-end rule pathway adaptor protein ClpS can be engineered for enhanced affinity and specificity to meet this requirement. The native ClpS protein has a high specificity albeit modest affinity for the amino acid Phe at the N-terminus but also recognizes other residues at the N-terminal position. We employed directed evolution methods to select for ClpS variants with enhanced affinity and selectivity for NAAs. In addition, we combined these mutations with rationally designed mutations to improve the thermal stability of the protein. The results and their possible implication to peptide sequencing will be presented.

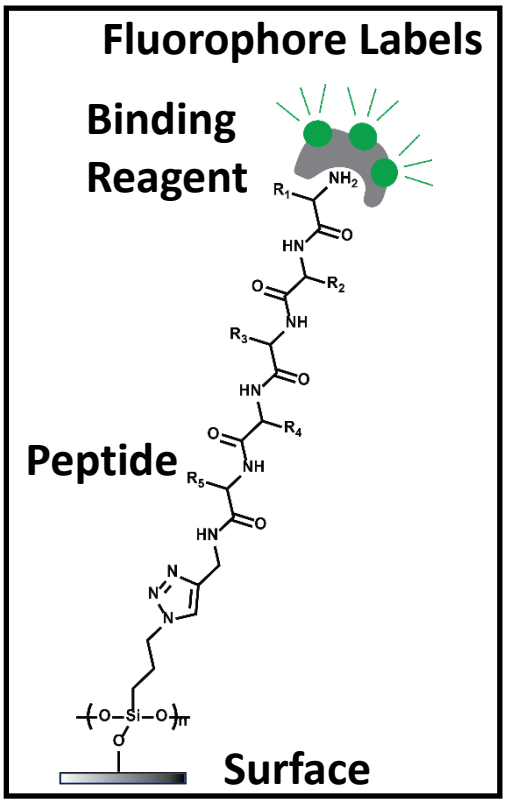

Figure 1 – Example of a binding reagent employed in detection of a surface adhered peptide