One bead one peptide for the identification of protease substrate specificity

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Abstract

A novel bead-based proteolytic profiling assay enabled the rapid determination of protease substrate specificities. The one bead one peptide library of protease substrates was synthesized with FRET method and marked with ladder sequence. The beads were increased the fluorescence by protease activity. The synthesized beads were reacted in protease solution, and then followed by incubation and fluorometric scanning. The scanning detected by Fluorescent Activated Cell Sorting system can be effective. This method is able to provide prolific screening results. Photolabile linker linked the combinatorial peptide beads. The screened bead was treated with UV light. then the segments of peptides were detected by MALDI-TOF. The array is able to provide complete maps of protease specificity for a lot of the unknown substrate proteases. We confirmed the conservation of thrombin specificity with the array system. One bead one peptide system with FRET method provides a rapid way to determine protease substrate specificity information that can be used for the design of selective inhibitors and substrates, the study of evolutionary divergence, and potentially, for diagnostic applications.