Protease Specificity Profiling Identifies Ligands that Substantially Increase Cleavage Rate and Protease Detection Sensitivity

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Proteolytic enzymes play an essential role in regulating and localizing protein activity in many physiological and pathological processes by acquiring high selectively for a protein. The specificity of proteases is characterized by the ability of a specific peptide substrate to recognize, orient, and bind to the active site of the protease. However, other sites on the protease surface in addition to the active site have been discovered to play an essential role in influencing its specificity and activity, including allosteric sites and exosites. The objective of this research is to develop a new methodology to enable quantitative identification of binding peptides that enhance cleavage rate of a defined proteolytic substrate by targeting the active site and other distinct epitopes or domains on the protease. A two-color cellular libraries of peptide substrates (CLiPS) methodology was used to quantitatively screen a 12-mer random library for clones with enhanced cleavage kinetics when incubated with thrombin. Thrombin is a protease which plays a central role in blood coagulation by modulating procoagulant and anticoagulant processes. The identified thrombin binding peptides resulted in enhancing the hydrolysis rate of the thrombin cleavable substrate by about 9 fold. In addition, the binding peptides exhibited similar cleavage kinetics when compared to known thrombin exosite binding peptides as shown in Figure 1. The substrates were then conjugated to the binding peptides to generate FRET beacons, and the beacons resulted in similar kinetic outcomes. Our results demonstrate that this methodology can be applied to determine the specificity of proteases and develop more active and selective substrates for basic research and diagnostic and therapeutic development.

Fig. 1. Measurement of extent of conversion of substrate clones displayed on cell surface. Extent of conversion was measured for control clones (SP GlySer: without a binding ligand and SP PAR1: with a thrombin exosite binding peptide) and enriched clone SP12 after incubating with 0.25 nM thrombin.