Developing Comprehensive Specificity Profiling of Promiscuous Proteases

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Proteases are a rich source for the development of powerful therapeutics that can inactivate disease-causing proteins. While most drugs are binders and act at a stoichiometric ratio, proteases are capable of catalytic turnover. Therapeutic proteases are currently used to treat thrombosis, sepsis, and coagulation, neuromuscular and digestion disorders. Significantly, all of these applications required identification of an existing protease in nature with the desired activity. Unfortunately, most potential therapeutic targets are not currently addressable because proteases with suitable specificities have not been identified. We aim to overcome this limitation by first developing a high-throughput method for assaying protease specificity. This technique will be invaluable for scanning natural proteases for activity that can be efficiently repurposed for therapeutic applications. Additionally, current methods are unable to reliably predict off-target cleavage within the human proteome. However, our unique approach will screen all possible octa-peptide substrates in a single experiment by combining our mRNA display technology, next-generation sequencing, LC-MS/MS, and computational analysis.