530a Bacterial Substrate Display: a New Method for Profiling Protease Activity

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Traditionally, protease substrate specificity has been defined by the alignment of naturally occurring substrates. Using this method enteropeptidase was found to specifically cleave the peptide sequence DDDDK. Studies performed since this assertion have elucidated other peptide sequences that enteropeptidase will hydrolyze, rendering it unsuitable for some protein purification applications. This example illustrates the need for improved characterization techniques for protease specificity. A quantitative library screening methodology has been developed using bacterial surface display to screen for protease substrates. Bacterial substrate display in conjunction with fluorescence activated cell sorting enabled screening for efficiently cleaved peptides from libraries as large as 108 peptides. Using bacterial substrate display, peptide substrates of the catalytic subunit of enteropeptidase were identified that were cleaved roughly five-fold faster than the canonical substrate sequence DDDDK. To further demonstrate the generality of this method, Caspase-3 was characterized by bacterial substrate display and the preferred substrates selected were comparable to those found by substrate phage. This new protease profiling method can be used as an alternative to substrate phage methodology and offers the advantage of quantitative library screening.