In-gel profiling of protease cleavage specificity

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Determination of protease specificity is of crucial importance for understanding protease function and also for the design of selective synthetic peptide substrates. Despite several available proteomic methodologies protease cleavage specificity profiling still remains a labor intensive and experimentally challenging task. To tackle these drawbacks, we developed a gel-based label-free proteomic approach for reliable determination of protease cleavage specificity (Vidmar et al., 2017, EMBOJ).

The presented methodology is based on in-gel digestion of the gel-separated proteome with the studied protease, enrichment of cleaved peptides by gel extraction and subsequent mass spectrometry analysis combined with a length-limited unspecific database search. We developed an optimized and easy-to-implement workflow that enables the identification of both N- and C-terminal cleavage sequences, which are aligned to establish a global protease cleavage specificity profile. The methodology was validated by profiling ten proteases from highly specific caspase-7, legumain, endoproteinase GluC and trypsin to broad specific matrix-metalloproteinase-3, thermolysin and cathepsins K, L, S and V. Specificity profiling of thermolysin was performed at its optimal temperature of 75eC, which confirmed the applicability of DIPPS to extreme experimental conditions. Furthermore, DIPPS enabled us to perform a first global cleavage specificity profile of legumain at a pH as low as 4.0, which confirmed a pH-dependent cleavage specificity switch.

The obtained data of >50 000 cleavages showed excellent correlation with known protease specificities, demonstrating the applicability of this approach to all major mechanistic classes of proteases under a broad range of experimental conditions. This approach can be easily implemented by any proteomic laboratory and thus provides an excellent starting point for protease characterization and development of specific protease substrates and inhibitors.