PROTEOMICS

Peptides aplenty

A large library of peptides and phosphopeptides, along with their tandem mass spectra, serves as a resource for the proteomics field.

The best approach to identify an unknown molecule is to compare its analytical properties to those of a synthetic reference standard. This has not been practical in proteomics, however, because of the sheer size of the proteome. Rather than comparing peptide mass spectra to those of synthetic standards, scientists in the field rely largely on computational tools to match experimental mass spectra to theoretical spectra generated using information from protein sequence databases.

Small synthetic peptide libraries have been used to characterize liquid chromatography–tandem mass spectrometry platforms and data analysis methods, but small peptide sets are not likely to be representative of the full proteome. To fill this gap, a group of researchers led by Bernhard Kuster of the Technische Universität München in Germany has created a large, carefully designed library of more than 100,000 synthetic unmodified peptides and their phosphorylated equivalents, soon to be made commercially available. The team members also provide as a data resource their tandem mass spectra generated on an Orbitrap Velos instrument using electron-transfer dissociation (ETD) and beam-type collision-induced dissociation (HCD)-based fragmentation.

The mass spectral library allowed Kuster and his colleagues to perform several systematic analyses. For example, they rigorously benchmarked two peptide identification algorithms and three phosphorylation-site localization tools. "While these tools have become quite sophisticated, we felt that it was about time that some of the tools and concepts were tested on a large set of known analytes," says Kuster. Not only does the mass spectral library allow systematic comparisons of proteome analysis tools, it should also help bioinformaticians improve the performance of their existing tools and identify opportunities to develop new tools.

Besides comparing the performance of ETD and HCD fragmentation, the physical peptide library should be useful for many other applications as well. "For example, scientists may want to explore novel peptide separation methods, answer questions about the merits of widely used current chromatographic techniques as well as explore more experimental techniques," says Kuster. The resource is neither complete nor perfect, of course, but Kuster's team hopes that it will serve as inspiration for others to generate further peptide libraries.

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RESEARCH PAPERS

Marx, H. *et al.* A large synthetic peptide and phosphopeptide reference library for mass spectrometry–based proteomics. *Nat. Biotechnol.* **31**, 557–564 (2013).

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