We present a selection of methods and areas of methodological development worth watching in the coming years.

DIA mass spectrometry

Data-independent acquisition (DIA) mass spectrometry may change how proteomic data are generated.

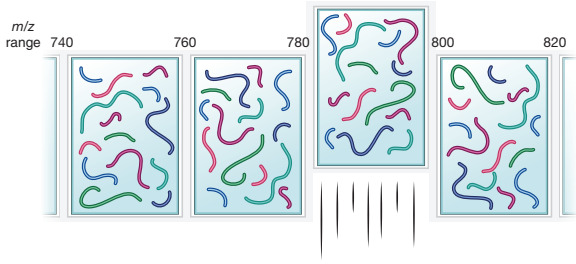
In traditional data-dependent acquisition (DDA), a proteomic sample is digested into peptides, ionized and analyzed by mass spectrometry. Peptide signals that rise above the noise in a full-scan mass spectrum are selected for fragmentation, producing tandem (MS/MS) mass spectra that can be matched to spectra in a database. Although extremely powerful, the mass spectrometer randomly samples peptides for fragmentation and is biased to pick those with the strongest signal. Thus, it remains a challenge to reproducibly quantify especially low-abundance peptides.

In targeted proteomics, most notably selected reaction monitoring (SRM), mass spectrometry assays are deployed to very sensitively detect peptides representing proteins of interest with high quantitative accuracy. Despite also being quite powerful (it was our 2012 Method of the Year), this approach is not suitable for discovery-based applications.

Many eyes in the proteomics community are now trained on data-independent acquisition (DIA), which in theory combines the advantages of DDA and SRM. In a DIA analysis, all peptides within a defined mass-to-charge (m/z) window are subjected to fragmentation; the analysis is repeated as the mass spectrometer marches up the full m/z range. This results in accurate peptide quantification without being limited to profiling predefined peptides of interest.

Although the DIA concept was introduced a decade ago, interest has been rekindled as several practical DIA implementations have recently been developed. Whereas many in the proteomics field are excited about the potential of DIA to overcome the sampling problems seen with DDA, others have yet to be impressed. Further applications to challenging biological questions are needed to showcase the advantages of DIA.

Another requirement for broader adoption of DIA will be the development of robust data analysis tools. Because multiple peptides in an m/z window are fragmented together in DIA, the resulting MS/MS spectra are very complex and require deconvolution. A few software tools were published this year (*Nat. Biotechnol.* **32**, 219–223, 2014; *Nat. Methods* **11**, 167–170, 2014), and others are likely on the horizon. We will be watching to see whether DIA can live up to its potential. **Allison Doerr**



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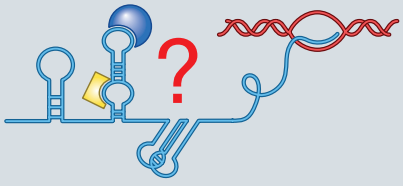
In DIA mass spectrometry, all peptides in a given m/z window are fragmented and analyzed.

Understanding noncoding RNAs

Methods to profile and characterize the function of noncoding RNAs will emerge.

Noncoding RNAs (ncRNAs) are hardly a new discovery: from tRNA characterized in the 1960s to rRNA in the '80s and microRNA in the '90s, biologists have appreciated the important and versatile roles of RNAs that do not encode a protein. The advent of RNA sequencing and derivatives thereof—such as CaptureSeq—have yielded a number of new ncRNA classes, from large (sometimes intergenic) l(i)ncRNAs (defined as longer than 200 nucleotides without an open reading frame) to shorter transcripts derived from regulatory regions such as promoters and enhancers. Some short RNAs, including circular (cRNAs) and competing endogenous RNAs (ceRNAs), act as regulators of other ncRNAs such as microRNAs, but the function of the vast majority of these newly discovered ncRNAs has yet to be determined. Recent efforts to elucidate the role of some lncRNAs include laborious knockout experiments (*eLife* **2**, e01749, 2013). More indirectly but on a larger scale, the study of expression levels, the propensity to degrade targets and the directionality of transcription has yielded insight into whether ncRNAs are signal or noise (*Nat. Commun.* **5**, 5336, 2014), but a comprehensive characterization is still outstanding. Structure and post-translational modifications in addition to binding partners need to be cataloged to understand mechanisms of ncRNA action. Particularly for lncRNAs, a unified nomenclature would help to better place new additions in the rapidly growing list and to define functional relationships. Improved computational tools to predict ncRNA sequences, and databases to compare newly discovered and existing structures, functions and impact on disease, will be invaluable. **Nicole Rusk**



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The many roles of ncRNA.