Weighing ribosomes with MS

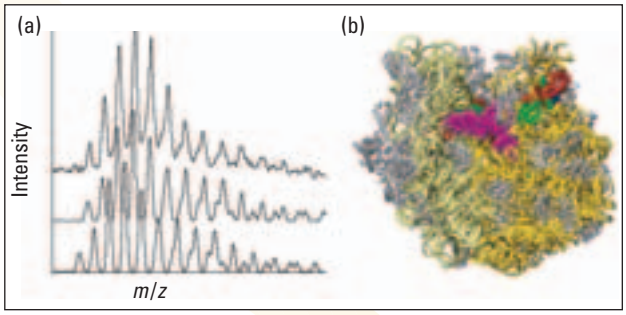
Researchers develop a method to accurately measure the masses of large, noncovalent complexes.

Because they are predicted to tip the scales at molecular masses in the megadalton range, intact ribosomes have been difficult to analyze by MS. However, Carol Robinson and colleagues at the University of Cambridge (U.K.) report a new method for obtaining accurate mass measurements of large, noncovalent complexes, such as ribosomes, with MS (*J. Am. Chem. Soc.* **2006**, *128*, 11,433–11,442).

Gentle ionization techniques such as ESI enable MS analyses of noncovalent complexes by preserving native interactions, but spectra from very large complexes are difficult to interpret. According to Robinson, "We knew that we could maintain intact ribosomes in the mass spectrometer, but because the peaks in the spectra were always very broad, it was quite difficult to extract information."

The major causes of peak broadening are small-molecule adducts and the incomplete binding of proteins in a complex. During ESI, inadequate removal of solvent or buffer ions can cause a substantial mass increase and broadening of MS peaks, because large protein complexes can form adducts with salt ions. In addition, substoichiometric binding of some proteins in a complex complicates the analysis, because the spectrum is actually a composite of spectra from two or more heterogeneous populations. The researchers devised a way to correct for these factors.

The researchers examined adduct formation by using a panel of noncovalent complexes with known molecular compositions. MS analysis revealed masses that were shifted to higher-than-expected values. After studying the peaks, the researchers discovered that each mass shift was linearly correlated with the average width of the peaks in the mass spectrum. This relationship allowed them to correct for adduct formation solely on the basis of the average peak width, without any prior knowledge of the complex's composition or structure.



(a) Simulated spectra for heterogeneous ribosomal populations were summed (bottom) and shifted (middle) to correct for ion adducts. Intensities of simulated spectra were adjusted to fit the experimental data (top). (b) X-ray crystal structure of the *T. thermophilus* 70S ribosome.

Next, Robinson and colleagues addressed the issue of protein-complex heterogeneity with an MS analysis of the *Thermus thermophilus* 30S ribosomal subunit (~800 kDa). After correcting for adducts, the researchers found that the mass of the 30S subunit predicted from protein and RNA sequences was ~28.5 kDa larger than the measured value. X-ray crystal structures had revealed that *T. thermophilus* 30S subunits sometimes lack the S1 protein component. In addition, Robinson's lab had shown previously that excitation of the 30S subunit during MS could cause dissociation of the S6 protein. The researchers hypothesized, therefore, that the observed mass spectrum could be a composite of 4 charge-state series, including contributions from the full protein complement and the 30S subunit without S1, S6, or both components.

To test this hypothesis, Robinson and colleagues generated computer simulations of these charge-state distributions, then summed the spectra and adjusted the respective intensities. The resulting simulation coincided well with the observed mass spectrum. By examining the relative intensities of the 4 components, the researchers estimated that ~50% of the 30S ribosomal subunits contained the S1 protein. Tandem MS of the 30S subunit confirmed the presence of 2 major populations, likely corresponding to the intact 30S subunit and the 30S subunit without S1. Finally, the researchers applied these techniques to the analysis of the massive 70S ribosome, a ~2.3-MDa complex composed of a 30S and a 50S subunit. They observed two major populations of 70S ribosomes, reflecting incomplete binding of S1, as seen for the 30S subunit.

Joseph Loo of the University of California, Los Angeles, comments, "The simulation and modeling strategy presented in this manuscript is really impressive work, and it opens the door to analyze other large protein machines by MS, such as viruses." David Muddiman of North Carolina State University says, "In the absence of suitable molecular-weight standards to validate the method, one is forced to take this elegant yet empirical approach to elucidate the masses of these challenging systems."

Robinson notes that the method allows researchers to probe stoichiometries of proteins in a large complex. She says, "Typically, if you were examining the ribosome by MS, you'd use a proteomics approach, separating all the proteins out. But then you don't know the stoichiometry of the proteins in the complex." The method is also independent of the structure of the complex and of instrumental parameters. These features, along with relative ease of implementation, enable it to be applied to a variety of large, noncovalent complexes. ■

—Laura Tomky Cassiday