SINGLE MOLECULE

Weighing single proteins with light

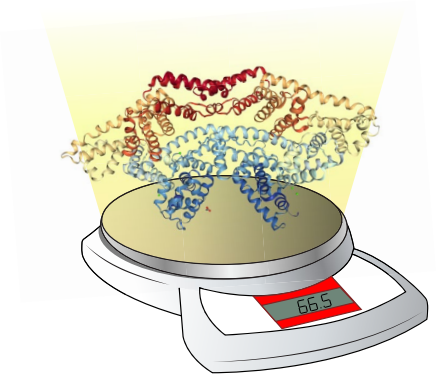
Interferometric scattering microscopy enables quantitative mass imaging and biophysical characterization of single biomolecules in solution.

Investigation of proteins and other biomolecules at the single-molecule scale has long been challenging to accomplish with light microscopy, but great strides were made with the advent of single-molecule fluorescence imaging and super-resolution microscopy methods. However, label-free approaches for studying individual proteins have lagged behind fluorescence-based measurements, despite their promise as a universal means for studying assemblies and interactions, and for quantifying their biophysical properties.

Philipp Kukura at the University of Oxford, whose work has focused on light-scattering-based imaging approaches, was interested in exploiting the possibility of using interferometric scattering microscopy (iSCAT) to study single biomolecules and their interactions. He notes that, "although we've been able to see single proteins through light scattering for four or five years, it was never clear whether we could do it in a quantitative fashion and get biologically relevant structural data out of it." Further, he wondered, "Can we try to achieve what people do with native mass spectrometry in the gas phase with single molecules in solution?"

To address this challenge, Kukura and Justin Benesch, an expert in mass measurement, along with graduate student Gavin Young and postdoctoral fellow Nikolas Hundt, developed interferometric scattering mass spectrometry (iSCAMS), based on highly sensitive iSCAT microscopy. When illuminated with coherent light, biomolecules in solution both scatter and reflect light, with scattered light being the most pronounced. In iSCAT and iSCAMS, the scattered and reflected light interfere at the detector, giving rise to contrast and allowing single particles to be imaged.

The fundamental relationship that underlies iSCAMS is between a particle's scattering signal and its mass. The scattering signal scales with the particle's refractive index and is proportional to the particle volume. Because the refractive indices and volumes vary only slightly among amino acids, a polypeptide's mass is proportional to its scattering signal, which allows the mass of single proteins to be determined with high accuracy on the basis of scattering signal alone.



A cartoon depicting the use of light to measure protein mass. Credit: Marina Corral Spence/ Springer Nature

Although the theoretical basis of the method was well understood, implementation of iSCAMS at levels of accuracy, resolution and precision that would be useful was not trivial. Kukura recalls that, in order to generate the measurements reported in their study, they had to optimize the optical setup to enhance contrast from single molecules while reducing the amount of light that reached the detector; "it's the combination of these two things that allows us to get the accuracy and precision high enough for these measurements," he noted.

Once developed, the approach proved robust and versatile. As a first demonstration, the team showed that they could resolve and thus accurately quantify the number of particles present as monomers, dimers, trimers and tetramers in bovine serum albumin, thereby highlighting the power of iSCAMS for characterizing the mass distribution of a protein, and hence its oligomerization propensity.

To validate the approach, they measured the mass of a range of proteins, and found that the precision and accuracy of their measurements remained high independent of factors such as size and shape. Moving beyond proteins, they showed that the method could accurately measure the masses of various nanodiscs comprising both proteins and lipids, as well as highly glycosylated viral coat complexes. In additional demonstrations, they found that they could monitor both the kinetics of protein oligomerization and the binding of biotin to streptavidin. The latter demonstration suggests that the method is practically useful for studies of small-molecule binding to proteins, a testament to the high precision of the approach.

In a final set of demonstrations, the authors showed that they could monitor the aggregation of α-synuclein and the growth of actin filaments at the single-molecule level in a position-sensitive manner. For the latter system they monitored nanometer changes in length and associated mass increments as the filaments grew, thus highlighting the power of the approach for studies of molecular assembly.

Despite the demonstrated utility of the approach, Kukura called the work one of the most uncertain projects he has done in terms of its likelihood of success, going so far as to say that "there were so many reasons why it was never supposed to work," owing to the limitations associated with accurate detection of single particles without fluorescence. He calls the success and robustness of iSCAMS surprising.

According to Kukura, the next steps for the method are to get it into the hands of as many users as possible in order to push the boundaries of the approach and spur future development. One possible barrier to user uptake is the accessibility of the ultrasensitive optical iSCAT setup needed to record measurements. Kukura has launched a company, Arago Biosciences, to help overcome this barrier. With these tools available, iSCAMS is likely to enable numerous biophysical experiments and should attract interest in the development of methods based on light-scattering microscopy. ❐

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Young, G. et al. Quantitative mass imaging of single biological macromolecules. *Science* **360**, 423–437 (2018).