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POINT OF VIEW

Is Proteomics Possible Without Mass Spectrometry?

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Proteomics was already being performed for many years before the term was coined in 1994. For protein identification after separating proteomes by two-dimensional electrophoresis, we used to rely on N-terminal protein sequencing by chemical methods, since mass spectrometry was not practical for proteomics yet. However, successful identification of a few proteins could take weeks or even months. With the advent of mass spectrometers devoted to protein analysis in the ’90s, proteomics gained huge momentum. We continued to use two-dimensional electrophoresis to separate proteomes for a while but began using mass spectrometry to identify proteins through peptide mass fingerprinting. Later, liquid chromatography linked on-line to mass spectrometry became gradually more popular; therefore, two-dimensional electrophoresis is infrequently used nowadays, and mostly for specific applications. In current proteomic laboratories modern mass spectrometers with higher resolutions, accuracies, sensitivities, speeds, and throughputs allow for sequencing thousands of proteins in a few hours. We can categorize proteomics into two main modes, namely bottom-up and top-down, though there are currently several proteomic strategies and abundant protocols. Mass spectrometry is the central technique for all these approaches. However, one can be curious as to whether or not mass spectrometry is going to remain the primary technique for proteomics in the future. What else could substitute mass spectrometry as the dominant technique?

In 2002, I came across an interesting paper. Maybe that was not the first paper in the field, but it was the one that caught my attention immediately. Its title is “Macromolecular Architecture in Eukaryotic Cells Visualized by Cryoelectron Tomography” [1]. I could see beautiful images of the actin network in the cytoskeleton, membranes, and cytoplasmic macromolecular complexes obtained by electron microscopy of tomograms of *Dictyostelium* cells under cryogenic temperatures. The authors called the technique “cryoelectron tomography”. I then searched the literature for other papers and reviews using these keywords. Other insightful papers and reviews showed up. In the last 20 years, the development of high-resolution microscopy has been astounding. Cryoelectron microscopy has been increasingly used to resolve tertiary and quaternary protein structures. The Nobel Prize for Chemistry in 2017 was awarded to three scientists who developed cryoelectron microscopy for the high-resolution structure determination of biomolecules in solution (https://www.nobelprize.org/uploads/2018/06/advanced-chemistryprize2017-1.pdf). Cryoelectron microscopy now competes with crystallography and nuclear magnetic resonance as the most powerful and adequate technique for protein structural studies. Recently, proteins of the new SARS-CoV-2 coronavirus had their structures quickly resolved in record times by groups that have mastered cryoelectron microscopy.

From my point of view, cryoelectron tomography will replace mass spectrometry as the principal technique for performing proteomics in the future. See yourself obtaining an image with all the proteome of a cell totally identified in a single shot. If you can have the 3D structures of proteins, you can also automatically get the protein identities. You will also have all the protein quantities, all the post-translational modifications, all the sequencing positions, all the protein subcellular locations, all the architecture of the complexes, all the 3D structures, all the molecular interactions. You will have everything!

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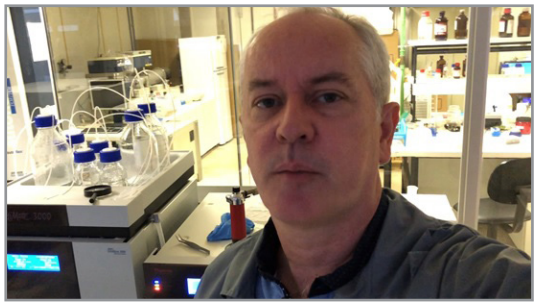
The major constraints against the rapid inclusion of cryoelectron tomography in modern proteomics are: 1) Cost of equipment. High-resolution electron microscopes are much more expensive than high-resolution mass spectrometers; 2) Technical difficulties in sample preparation. There are several accessory devices and technical details needed to prepare samples for cryoelectron microscopy experiments; 3) Lack of expertise. Proteomic scientists have mastered mass spectrometry over the years, but have not yet acquired intimacy with electron microscopy; 4) Bias against the unknown. Having not mastered cryoelectron microscopy yet, the proteomic community is impeded in going for it with greater confidence. However, needless to say that all the above obstacles are amenable to be surpassed, as mass spectrometry drawbacks were overcome by proteomic researchers in the past.

I am a molecular biologist (in the *lato sensu* of the term) who received training in protein chemistry and biochemistry from my first supervisor, Prof. Lauro Morhy. In the ’80s, he used to say that protein sciences would be extremely dependent on mass spectrometry, as he had used it for small molecules analysis in the ’60s. He was right. After completing my PhD in 1991 and coming back home, I quickly introduced mass spectrometry for the initial works in proteomics at the University of Brasilia in the early ’90s. Since then, I have been using mass spectrometry every day. I am a great fan of the fantastic mass spectrometry. However, I have also to admit that proteomics will be extremely dependent on electron microscopy in the future.

1. Medalia, O.; Weber, I.; Frangakis, A. S.; Nicastro, D.; Gerisch, G.; Baumeister, W. Macromolecular architecture in eukaryotic cells visualized by cryoelectron tomography. *Science*, **2002**, *298* (5596), pp 1209-1213 (http://dx.doi.org/10.1126/science.1076184).



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