WILEY

A Virtual Event Designed For The Masses

Now Available On-Demand!

Scale up your research and translate your results more rapidly and simply than ever before. Welcome to vLC-MS.com - the event for Orbitrap Exploris mass spectrometers and much more!

Tune in to:

- Explore the LC-MS portfolio and meet the expanded Orbitrap Exploris MS system in our staffed Exhibit Hall.
- Learn from mass spectrometry experts, such as Professor Alexander Makarov himself, about Orbitrap mass spectrometry technology and the applications it enables.
- Browse posters and short presentations in our application area.

Event Highlights:

Prof. Alexander Makarov

Dr. Christian Münch

Thomas Moehring

vLC-MS.com

REGISTER NOW

ThermoFisher SCIENTIFIC

Proteomics
Proteomics and Systems Biology
www.proteomics-journal.com

The Origins of Organellar Mapping by Protein Correlation Profiling

Matthias Mann

Cells have a rich inner structure that is commonly explored by microscopy. Classical biochemical methods that break apart the cells and fractionate them along a gradient have now gotten a new lease on life through modern methods of mass spectrometry-based proteomics. Their common principle is to comprehensively measure all the proteins in each of the fractions. The resulting quantitative profile then associates thousands of proteins to their cellular homes. Here, the author recounts how protein correlation profiling, the first such technique, was conceived and how it was applied to answer intricate cell biological questions.

Eukaryotic cells have been visualized for centuries by optical microscopy and for decades by electron microscopy with ever greater resolution. Gross morphology indicates that their interior is far from a homogeneous mass and that it is instead organized into an intricate array of membrane-enclosed and membraneless organelles. The cell nucleus, mitochondria, and lysosomes are prominent examples of the former and the nucleolus and the centrosome of the latter. They are visualized in microscopy via either marker molecules that have an affinity to them, such as mitotracker for mitochondria, or marker proteins that are then tagged or targeted by antibodies. Apart from studying the shape and distribution of these cellular substructures, a central question of cell biology is: "What are the protein constituents of an organelle of interest and how do their dynamics contribute to organellar function?" Antibody tools can also address this question, especially when a nearly universal collection of them is available, such as in the (subcelluar) Human Protein Atlas.[1] Likewise, bioinformatics methods can sometimes suggest members of complexes based on shared sequence features.[2]

Prof. M. Mann
Department of Proteomics and Signal Transduction
Max Planck Institute of Biochemistry
82152 Martinsried, Germany
E-mail: mmann@biochem.mpg.de
Prof. M. Mann
Novo Nordisk Foundation Center for Protein Research
Faculty of Health Sciences
University of Copenhagen
Copenhagen, Denmark

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/pmic.201900330

DOI: 10.1002/pmic.201900330

However, a much more direct, comprehensive, and unbiased approach would be to isolate or purify the organelle of interest and then identify all of its components. With the advent of powerful mass spectrometric methods, this became increasingly feasible in principle.[3–5] As a result, there is a substantial literature reporting increasingly larger compendia of organellar proteomes, such as mitocarta for mitochondria, for instance.[6] However, just purifying or enriching an organelle runs into problems due to inherent resolution limitations of biochemical methods and the exceedingly high sensitivity of modern mass spectrometers. Consequently, in the absence of stringent quantitative information, organellar catalogs of proteins may be dominated by contaminants—such as abundant cytosolic proteins or proteins that belong to other organelles but end up in minute proportions in purified organelles.

In my group, we had faced a very similar or worse problem in the context of interaction proteomics. Pull-downs are inevitably dominated by background proteins binding to beads or to antibodies ostensibly targeting the bait. As we and the Aebersold group showed many years ago, this problem can be side-stepped very efficiently by using quantitative methods: instead of trying to purify protein complexes to homogeneity by MS standards, one uses the excellent quantitative characteristics of this method to look for differences in the pull downs of the bait and a control, or between each bait and all other baits.[7–9]

The challenges with membraneless organelles are obviously even greater than with membrane bound ones. One such organelle is the centrosome, the "microtubular organizing center" that plays a vital role during chromosome segregation in the cell cycle.[10] My future colleague Erich Nigg had long been interested in this important structure and had done beautiful microscopy and cell biology on particular proteins of interest. However, an inventory of its constituent proteins remained enigmatic because it was not clear how one could distinguish true centrosomal proteins from background in the relatively crude enrichments that one could do by biochemistry. Thus our quest was clear but not how to go about solving it. This problem was on my mind and a solution presented itself during a relatively uneventful parent-teacher conference that I had to attend: I was suddenly reminded of a picture in the problem set of Harvery Lodish's textbook, *The Cell*. It has since been pointed out to me that the original idea comes from Nobel Prize winner Christian De Duve.[11] Here, the entire cell was fractionated by density centrifugation and the levels of marker proteins for different organelles were drawn as smooth curves. It occurred to me that if we did this by

quantitative mass spectrometry for all proteins at once and not just the marker proteins, then the organellar proteome would reveal itself by simple correlation of each protein to one of those marker proteins. This strategy was simple to implement and Jens Andersen, postdoc in my Danish group at the time, got it to work beautifully right away on the centrosome. Not only did we capture virtually all of the centrosomal proteins that had been described to that date but we found a treasure trove of new ones. Since many of them had never been described in the literature, we got to name them, too. We were not too imaginative, though, and to this day many of them are still called centrosomal protein (molecular weight), like Cep218, for instance.

Having established the principle—which is usable in any context in which there is a distribution of correlated proteins over a gradient or over fractions—we got more ambitious and asked if one could get an organellar map of the entire cell in this way. Leonard Foster rose to the challenge and established density gradient centrifugation for cellular lysates that balanced resolution with the proteomics capacities then available. He then embarked on a tour de force on mapping as many proteins in his fractions as possible. Interestingly, the longest part of the project was the bioinformatic analysis, which took about a year of number crunching on the desktop computers that we had. However, it was well worth it as this exercise assigned an organellar home to thousands of proteins.[12] Interestingly, it turned out that nearly half of all proteins had not one but two addresses, a finding that has been replicated in numerous studies since.[1]

Taking a step back, the reason that protein correlation profiling (PCP) was so successful is that it combined a decidedly low resolution biochemical method such as density centrifugation, with an extremely quantitatively accurate and specific mass spectrometric read out. Although proteins of an organelle may be spread over half of the fractions, the distinct shape of the distribution still assigns it to that organelle with very high confidence. A striking example of this in further work by my group was the discovery of the MICOS complex, which links the inner boundary membrane and the cristae of mitochondria. This structure had remained elusive until we used PCP to discover a set of proteins whose fractionation profile was minutely but highly consistently offset from other mitochondrial proteins.[13] Upon deletion of these proteins, cristae did not even form.

Shortly after PCP was published, Paul Dupree and Kathryn Lilley described the LOPIT method[14] which also used the correlation of proteins across fractions to to define organellar proteomes.

While PCP has mainly been used in a discovery mode to identify new organellar proteins, for certain organelles there are established and efficient isolation methods and new ones are constantly being developed. However, the challenge of co-purifying proteins persists and can be addressed in a slightly different way: the isolation procedure can simply be performed in a more and deliberately less stringent manner. Quantitative comparison between the two will then show opposite patterns for true organellar proteins versus contaminants, with the former being enriched in the stringent procedure and the latter being de-enriched. This principle also works for cells that are hard to purify completely.[15]

Although the principle is straightforward (or perhaps especially for this reason), PCP and similar technologies can be extended in a wide variety of ways. As MS technology improves, it is now possible to assign not only the main protein form to a particular pattern but increasingly to distinguish the different shapes and therefore localizations of proteoforms.[16] This also extends to post-translationally modified proteins as demonstrated in a recent study from my laboratory in which we discovered major architectural reorganizations of liver cells upon the development of fatty liver (which affects about a quarter of world population.[17,18]) Most dramatically, Natalie Krahmer found by PCP that essentially the entire Golgi apparatus got "stuck" on lipid droplets, as we verified by super-resolution microscopy. In this experiment, we performed PCP on both the protein and phosphoprotein level. This approach has the potential to directly reveal the molecular mechanism by which proteins change localization—for instance, phosphorylation on a residue that regulates intracellular transport.

Although an established technology, PCP and related technologies of course also have their limitations. The inherently low resolution of biochemical fractionation limits the functional detail that can be read out. Furthermore, it is still not practical to measure the proteome to completeness in each of many fractions. In this regard, PCP will continue to benefit from the ongoing advances in proteomics, imaging, and large-scale functional assays such as CRISPR screens. To my knowledge, PCP has only been applied to the localization of proteins, but the principle should be likewise applicable to other molecules that fractionate with the organelles, such as their constituent small molecules. For instance, this might yield the unique lipid composition of membrane bound organelles. As these mass spectrometric technologies become more and more accessible to cell biologists, they will play an increasing role in the arsenal of technologies that allow us to understand the cell in a systems biological manner.

Conflict of Interest

The author declares no conflict of interest.

Keywords

centrosome, protein correlation profiling, proteomics, mass spectrometry

Received: April 11, 2020
Revised: July 27, 2020
Published online:

[1] P. J. Thul, L. Åkesson, M. Wiking, D. Mahdessian, A. Geladaki, H. Ait Blal, T. Alm, A. Asplund, L. Björk, L. M. Breckels, A. Bäckström, F. Danielsson, L. Fagerberg, J. Fall, L. Gatto, C. Gnann, S. Hober, M. Hjelmare, F. Johansson, S. Lee, C. Lindskog, J. Mulder, C. M. Mulvey, P. Nilsson, P. Oksvold, J. Rockberg, R. Schutten, J. M. Schwenk, Å. Sivertsson, E. Sjöstedt, et al. *Science* **2017**, *356*, eaal3321.
[2] O. Emanuelsson, H. Nielsen, S. Brunak, G. von Heijne, *J. Mol. Biol.* **2000**, *300*, 1005.
[3] R. Aebersold, M. Mann, *Nature* **2003**, *422*, 198.
[4] M. Larance, A. I. Lamond, *Nat. Rev. Mol. Cell Biol.* **2015**, *16*, 269.
[5] E. Lundberg, G. H. H. Borner, *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 285.
[6] D. J. Pagliarini, S. E. Calvo, B. Chang, S. A. Sheth, S. B. Vafai, S. E. Ong, G. A. Walford, C. Sugiana, A. Boneh, W. K. Chen, D. E. Hill, M.

Vidal, J. G. Evans, D. R. Thorburn, S. A. Carr, V. K. Mootha, *Cell* **2008**, *134*, 112.
[7] B. Blagoev, I. Kratchmarova, S. E. Ong, M. Nielsen, L. J. Foster, M. Mann, *Nat. Biotechnol.* **2003**, *21*, 315.
[8] J. A. Ranish, E. C. Yi, D. M. Leslie, S. O. Purvine, D. R. Goodlett, J. Eng, R. Aebersold, *Nat. Genet.* **2003**, *33*, 349.
[9] E. C. Keilhauer, M. Y. Hein, M. Mann, *Mol. Cell. Proteomics* **2015**, *14*, 120.
[10] A. Vertii, H. Hehnly, S. Doxsey, *Cold Spring Harbor Perspect. Biol.* **2016**, *8*, a025049.
[11] C. de Duve, *J. Theor. Biol.* **1964**, *6*, 33.
[12] L. J. Foster, C. L. de Hoog, Y. Zhang, Y. Zhang, X. Xie, V. K. Mootha, M. Mann, *Cell* **2006**, *125*, 187.
[13] M. Harner, C. Körner, D. Walther, D. Mokranjac, J. Kaesmacher, U. Welsch, J. Griffith, M. Mann, F. Reggiori, W. Neupert, *EMBO J.* **2011**, *30*, 4356.
[14] T. P. J. Dunkley, R. Watson, J. L. Griffin, P. Dupree, K. S. Lilley, *Mol. Cell. Proteomics* **2004**, *3*, 1128.
[15] M. Zeiler, M. Moser, M. Mann, *Mol. Cell Proteomics* **2014**, *13*, 3435.
[16] I. Bludau, R. Aebersold, *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 327.
[17] N. Krahmer, B. Najafi, F. Schueder, F. Quagliarini, M. Steger, S. Seitz, R. Kasper, F. Salinas, J. Cox, N. H. Uhlenhaut, T. C. Walther, R. Jungmann, A. Zeigerer, G. H. H. Borner, M. Mann, *Dev. Cell* **2018**, *47*, 205.
[18] N. Krahmer, M. Mann, *Contact* **2019**, https://doi.org/10.1177/2515256419859186.