New and Notable

SpIDA Surveys the Intricate Web of Macromolecular Oligomerization In Situ

Andrew H.A. Clayton[1,*]
[1]Centre for Microphotonics, Faculty of Science, Engineering and Technology, Swinburne University of Technology, Hawthorn, Victoria, Australia

The analysis of the quaternary state of proteins continues to be of importance in biophysics (1). Oligomerization is a key biological control mechanism in the functioning of proteins at the cell membrane and throughout the cellular milieu. For example, receptors involved in cell signaling appear to trigger cellular responses after dimerization or oligomerization from external cues (i.e., after ligand binding) (2). Likewise, an increasing number of diseases have been found to be related to inappropriate aggregation or oligomerization leading to pathological states (3).

Measurement of the oligomeric state of proteins in solution has enjoyed a rich, long history that predates measurements of oligomerization in cells. In solution measurements (e.g., via size-exclusion chromatography, analytical centrifugation, light scattering, nuclear magnetic resonance, mass spectrometry, or fluorescence anisotropy) the experimentalist has the luxury of a purified protein of known concentration, a solvent of defined composition (buffer, pH, gel, solid-phase), the ability to perform measurements under variable conditions (concentration, temperature, native or denaturing, electric field, centrifugal field), and at a high signal/noise (under volume and/or time integration). Generally speaking, these classical techniques measure an apparent weight-average molecular weight in the micromolar to millimolar concentration range of solute.

With the advent of brighter fluorophores, improvements in detectors, and confocal/two-photon microscopy, fluorescence approaches such as single-molecule fluorescence and fluorescence correlation spectroscopy enabled measurement of smaller concentrations in the picomolar to nanomolar range. In single-molecule spectroscopy measurements, fluorescent tags are bleached through a tight focus. The number of detected bleaching steps yields the apparent oligomeric state. In fluorescence correlation spectroscopy, one analyzes the photon stream emanating from molecules as they diffuse through a small focused confocal volume (4). Analysis of the temporal autocorrelation function yields the concentration of species from the zero-time-lag amplitude and the transport properties (and therefore apparent molecular weight) from the shape of the autocorrelation function as a function of time. Alternatively, brightness analysis of the fluorescence intensity trace can be performed through fluorescence intensity distribution analysis (FIDA) (5), photon count histogram (PCH) (6) analysis, or N&B (7). In the FIDA approach, the total photon count over a small time interval (smaller than the diffusion time) is measured repeatedly to obtain a PCH. These FIDA and PCH approaches are useful because they allow oligomerization to be measured for small solute concentrations, and are largely independent of transport properties of the solute in solution.

The measurement and analysis of oligomerization in single cells and subcellular compartments consists of several challenges. The first problem is the cell solvent is not homogenous and the protein interactions with other species (proteins, nucleic acids, carbohydrates, lipids, cytoskeleton, organelles, vesicles) lead to context-dependent local concentration and local oligomeric states. This issue can be overcome by using an imaging approach. The second problem is the relationship between molecular weight and transport coefficient is generally not the same in or on the cell as it is in buffer. This requires a FIDA or PCH-like approach. The third problem is the concentration of solute and fluorescent tag is difficult to precisely control in cells. Labeling and purification of the labeled protein is not possible inside the cell—instead, proteins are generally substoichiometrically labeled. This problem can be solved if the oligomeric distribution and the concentration can be measured concurrently and the influence of labeling can be taken into account.

In this issue of the *Biophysical Journal*, Godin et al. (1) overcome these challenges to ultimately determine the oligomeric state distribution of human proteolipid protein in the endoplasmic reticulum (ER) and plasma membrane of intact single cells. Using spatial intensity distribution analysis (SpIDA), the authors impressively revealed the distribution of monomer, dimer, and tetramer and the concentration (density) of the human proteolipid protein in the plasma membrane and ER compartments. Significantly, mutations in the protein (known to be responsible for pathological effects) led to impaired trafficking, which was linked to the tetrameric form of the protein in the ER.

This article not only provides a biophysical advance but contains important control experiments that serve as lessons for us all. Even genetically encoded probes are not produced with 100% labeling efficiency. However, by using a control protein as done in this work, the extent of unlabeled proteins can be backcalculated and this calculation used to arrive at a true oligomeric distribution of the unknown protein. This caveat of mislabeling also applies to endogenous/labeled protein systems where a genetically encoded protein is transfected into an

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*Correspondence: aclayton@swin.edu.au

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endogenous protein background. In this article, the authors overcame this potential problem with a genetic knock-down system. Alternatively, one can use cells that do not express the endogenous protein of interest. Finally, the use of computer simulations is important when evaluating any new biophysical technique.

There are yet unexplored vistas of SpIDA that one can only speculate upon. By knowing the oligomeric distribution and the total concentration of solute, estimates of in-cell dissociation constants should be possible, leading to estimates of the energetics of protein oligomerization in the cell. Manipulation of the brightness with external agents, i.e., through fluorescence quenching, might give additional insights into accessibility. Whatever the future, you can put away your insect repellent this summer, as SpIDA is here to stay.

REFERENCES

1. Godin, A. G., B. Rappaz, …, P. W. Wiseman. 2015. Spatial intensity distribution analysis reveals abnormal oligomerization of proteins in single cells. *Biophys. J.* 109: 710–721.
2. Clayton, A. H., F. Walker, …, A. W. Burgess. 2005. Ligand-induced dimer-tetramer transition during the activation of the cell surface epidermal growth factor receptor—a multidimensional microscopy analysis. *J. Biol. Chem.* 280:30392–30399.
3. Ciccotosto, G. D., N. Kozer, …, A. H. Clayton. 2013. Aggregation distributions on cells determined by photobleaching image correlation spectroscopy. *Biophys. J.* 104:1056–1064.
4. Magde, D., E. L. Elson, and W. W. Webb. 1972. Thermodynamic fluctuations in a reacting system: measurement by fluorescence correlation spectroscopy. *Phys. Rev. Lett.* 29:705–708.
5. Saffarian, S., Y. Li, …, L. J. Pike. 2007. Oligomerization of the EGF receptor investigated by live cell fluorescence intensity distribution analysis. *Biophys. J.* 93:1021–1031.
6. Chen, Y., J. D. Müller, …, E. Gratton. 1999. The photon counting histogram in fluorescence fluctuation spectroscopy. *Biophys. J.* 77:553–567.
7. Digman, M. A., R. Dalal, …, E. Gratton. 2008. Mapping the number of molecules and brightness in the laser scanning microscope. *Biophys. J.* 94:2320–2332.