

Quantitative Mass Imaging of Actin Nucleation

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Actin filaments are one of the major components of the cytoskeleton and mediate a vast number of cellular processes that regulate shape and motility, such as cell migration, cell adhesion and muscle contraction. The mechanism of actin polymerization has been studied for more than 50 years and a basic mechanism has been formulated where actin subunits form a stable nucleus which is extended into a bipolar filament. This model was largely inferred from the observation of time-dependent increases in scattering or fluorescence of a solution of polymerizing actin filaments or by following macroscopic growth of individual filaments using fluorescence microscopy. None of these methods, however, provides a means of quantifying actin oligomers formed during the nucleation process directly, which would be very useful to verify the existing model and for investigating how actin nucleation is mediated by actin-binding proteins. Our lab has recently developed mass photometry based on interferometric scattering microscopy, which enables direct visualization and size quantification of polydisperse biomolecular distributions in a solution environment. The latest improvements in detection technology and data analysis enable mass resolution to 13 kDa (mass peak FWHM). As a result, we can now generate mass spectra of actin solutions above the critical nucleation concentration allowing us to identify and quantify the oligomers present shortly after initiating polymerization. Since we can quantify the amount of each individual oligomeric species and its evolution with time, we are able to directly compare and validate predictions of oligomeric distributions obtained from kinetic simulations. We find direct evidence for a nucleation mechanism of actin, demonstrating a general methodology for studying nucleation processes in biology.