

Label-free tracking and mass measurement of single protein complexes on lipid bilayers

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Biomolecular interactions in and on lipid bilayers are critical for a number of cellular processes, but are difficult to robustly and stoichiometrically quantify. Approaches to visualise and track single proteins on lipid bilayers mostly rely on fluorescent labelling, which has inherent limitations in observation times and the ability to resolve different species and oligomeric states, making the quantitative and stoichiometric characterisation of biomolecular interactions challenging. Here, we demonstrate dynamic mass photometry, a method for label-free imaging, tracking and mass measurement of individual membrane-associated proteins on supported lipid bilayers. We applied our method to the GTPase dynamin1 and demonstrate a mass resolution of < 50 kDa and < 20 nm localisation precision at 330 Hz. By tracking the movement of dynamin1 oligomers on the supported lipid bilayer, we were able to quantify oligomer-dependent mobilities, membrane affinities and (dis)association events of individual complexes. Our results revealed a highly heterogeneous mixture of dimer-based oligomers. Additionally, we observed decreasing diffusion and increasing lipid bilayer affinity with increasing oligomeric state, which suggests an increase in contact area and that the presence of the lipid bilayer induces and/or stabilises assembly of the dimer-based oligomers. Overall, these capabilities of dynamic mass photometry, together with assay-based advances for studying integral membrane proteins, could be transformative for studies of biomolecular mechanisms in and on lipid bilayers.