

Quantifying binding affinities, kinetics and stoichiometry of biomolecular complexes with mass photometry

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The characterisation of biomolecular complexes and their interactions is essential for understanding the function and regulation of cellular processes. Many of the existing biosensor techniques, despite their maturity, are often hampered by the requirement for surface immobilisation, introduction of tags and the inability to differentiate and quantify co-existing species. Here, we show that mass photometry based on interferometric scattering microscopy (iSCAT), can determine the binding affinities, kinetics and stoichiometries of biomolecular complexes in a label-free fashion in solution at the single molecule level. We illustrate these capabilities by quantifying antibody-antigen and antibody-receptor interactions ranging in strength over 4 orders of magnitude ($K_D = 0.1 - 1000$ nM) and showing the simplicity of differentiating between co-existing species. The ease and speed of use (< 1 min), low sample consumption ($< \text{picomole protein/run}$), minimal sample and assay preparation in combination with clear avenues to high-throughput and data analysis makes mass photometry a powerful new method for characterising biomolecular interactions and dynamics *in vitro*.