REFEYN

WEIGHING MOLECULES WITH LIGHT

Biomolecular characterisation with mass photometry

BROCHURE

Introducing mass photometry

Mass photometry is a powerful analytical technology that accurately measures the mass of single particles in solution, without labels. Its applications span structural biology, biomolecular function research and bioanalytics.

What is mass photometry?

Mass photometry analyses biomolecules at the single-particle level, in their native state and without needing labels. It measures the light scattered by individual particles, and uses this signal to count the particles and measure their mass (Fig. 1).

What is mass photometry used for?

Mass photometry measurements provide the mass distribution and relative concentrations of all species in a sample. Mass photometry can be used to characterise proteins, nucleic acids and other bioparticles (Fig. 2). It can be applied to biomolecules in solution or on membranes,[1,2] accelerating a variety of experimental workflows, including:

- **Sample characterisation:** direct assessment of sample purity and homogeneity during expression and purification[3]
- **Protein oligomerisation:** easy validation of protein mono- or polydispersity to guide formulation or structural biology studies[3,4]
- **Biomolecular interactions:** simple quantification of high-affinity interactions among biomolecules[5,6]
- **Macromolecular complex assembly:** monitoring of the assembly/disassembly of complexes, over time[7]

With its quantitative and easy-to-interpret results, mass photometry complements existing analytical technologies, providing unique insights or confirmation where needed.

Key benefits of mass photometry

- **Accurate measurement of true native behaviour**
  - In solution, in a variety of buffers and compatible with membrane proteins
  - Label free, without the need to modify samples
- **Information on all sub-populations in samples**
  - Single molecule counting
  - Wide mass range and high dynamic range
- **One assay format delivering multiple results**
  - Homogeneity, structural integrity and activity
  - Quick and simple to use
  - Minimal sample required

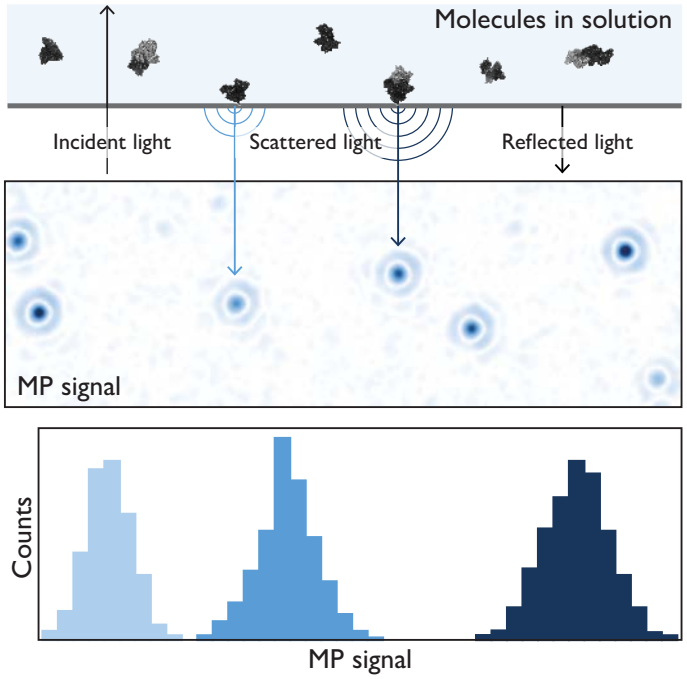

Fig. 1 The principle of mass photometry. The light scattered by a molecule that has landed on a measurement interface interferes with light reflected by that surface. The interference signal (MP signal) scales linearly with the molecule's mass.

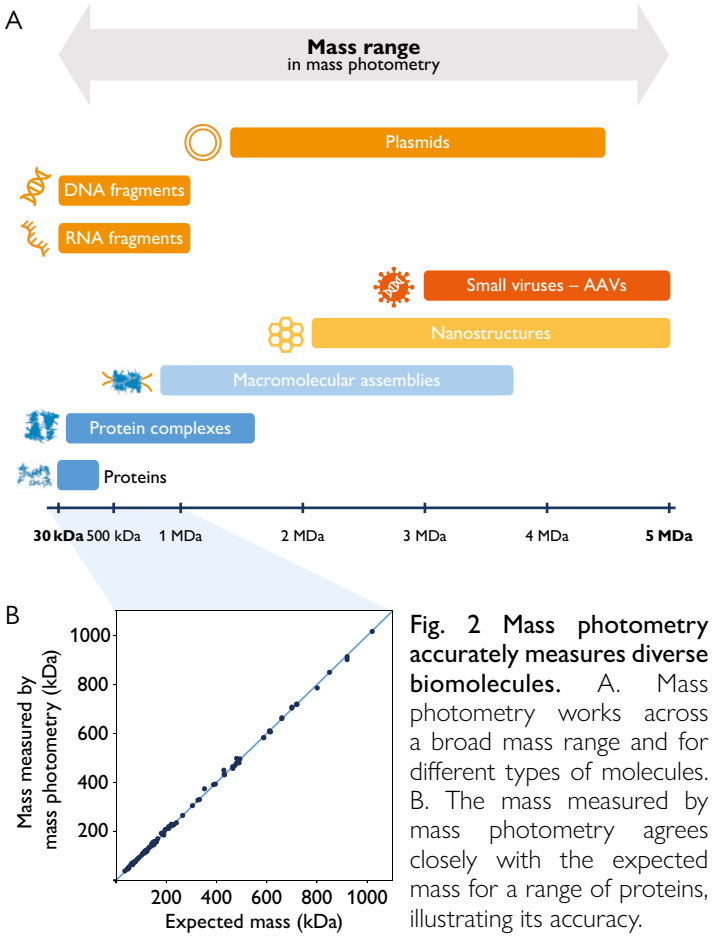

Fig. 2 Mass photometry accurately measures diverse biomolecules. A. Mass photometry works across a broad mass range and for different types of molecules. B. The mass measured by mass photometry agrees closely with the expected mass for a range of proteins, illustrating its accuracy.

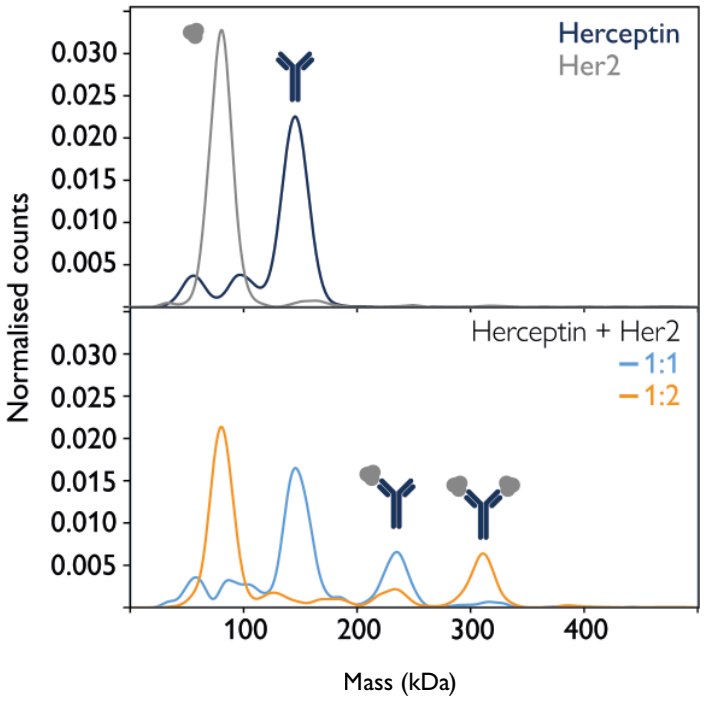

Fig. 3 The Two^{MP} can be used to quantify molecular interactions. The monoclonal antibody Herceptin (trastuzumab) and its target, Her2, were measured individually and in mixtures, demonstrating the instrument's ability to quantify the interactions of individual antibody molecules with target molecules.

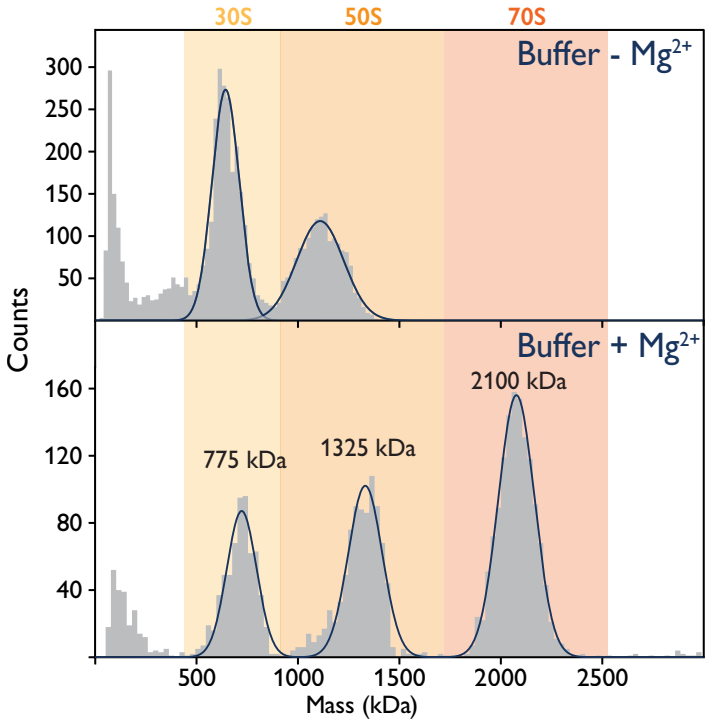

Fig. 4 The assembly of bacterial ribosomal complexes monitored with mass photometry. Mass distributions show that ribosome complexes disassemble completely into two subunits in the absence of Mg^{2+} (top). Complexes assemble in the presence of Mg^{2+} (bottom).

Mass photometry quantifies binding and assembly

Mass photometry enables **quantitative studies of biomolecular interactions** (e.g. protein-protein or DNA-protein) in solution. As it reveals the relative concentrations of bound and unbound species (Fig. 3), mass photometry can be used to monitor interactions and determine binding constants for high-affinity interactions[5].

Mass photometry can analyse **complex, multistep interactions** because it readily detects sub-species. This makes it possible to determine intermediate binding constants[8], and to easily optimise experimental conditions. Due to its broad mass range (Fig. 2), mass photometry is ideal for measuring the **assembly or disassembly** of large, multicomponent complexes (Fig. 4) and for following assembly kinetics[7,8].

Mass photometry is useful for quality control

Molecular mass is a universal readout that informs on **sample homogeneity, and on the structural integrity and activity of biomolecules and biomolecular complexes**. Mass photometry provides the complete mass distribution of a sample in just minutes, while maintaining a near-native aqueous environment.

Because mass photometry **works quickly and requires very little sample**, it is ideal for any applications that would benefit from frequent sample characterisation—to ensure the highest quality results throughout challenging analytical workflows[3].

Quality control applications include protein purification optimisation and the preparation of complex samples for analysis by cryoEM[3]. Mass photometry also offers a simple way to assess biomolecular **oligomerisation behaviour**, enabling fast optimisation of, for example, buffer conditions[4].

How does mass photometry work?

Mass photometry can weigh single molecules using light because the amount of light scattered by the molecules correlates with their mass. The scattering scales linearly with the particle's volume and refractive index. Because the optical properties and density of biomolecules vary by just a few percent, the scattering signal is directly proportional to their mass.

To detect the minuscule amount of light scattered by single molecules, mass photometry relies on a combination of carefully controlled illumination, novel spatial filtering in the detection beam path[9] and image analysis[8]. Developed at Oxford University, mass photometry builds on the principles of interference reflection microscopy[10] and interferometric scattering microscopy[11].

References

[1] *Foley et al., Nat Methods 2021*
[2] *Heermann et al., Nat Methods 2021*
[3] *Sonn-Segev et al., Nat Comm 2020*
[4] *Haeussermann et al., Angew Chem Int 2019*
[5] *Wu et al., Anal Biochem 2020*
[6] *Soltermann et al., Angew Chem Int Ed 2020*
[7] *Malay et al., Nature 2019*
[8] *Young et al., Science 2018*
[9] *Cole et al., ACS Photonics 2017*
[10] *Verschueren, J Cell Sci 1985*
[11] *Ortega-Arroyo et al., Phys Chem Chem Phys 2012*

PRODUCTS

Mass photometry solutions for analytics in the life sciences

Refeyn mass photometers deliver single-particle mass measurements with sensitivity, speed and simplicity of use. They suit a range of applications, from discovery science to quality control and manufacturing.

Two^{MP}: Bringing biomolecular characterisation to the bench

The Refeyn Two^{MP} mass photometer enables label-free mass measurement of single biomolecules, directly in solution. Fitting on a lab bench and with minimal installation requirements, the Two^{MP} is also easy to operate.

Two^{MP} Auto: The automated mass photometer

The Two^{MP} Auto combines the ease and efficiency of automation with the sensitivity and simplicity of mass photometry. It enables rapid measurement of multiple samples with high reproducibility.

Samux^{MP}: A mass photometer for AAV analytics

The Samux^{MP}, designed specifically for adeno-associated virus (AAV) development and quality control, is an essential tool for labs working with AAVs. It measures the empty/full capsid ratio for AAVs of any serotype in just minutes.

Intuitive software

Refeyn mass photometers all come with Refeyn's software, which has been specifically developed to fit the needs of each instrument. The included software packages are **Acquire^{MP}** for data acquisition and **Discover^{MP}** for data analysis. Intuitive user interfaces make it easy to collect, analyse and visualise data.

Accessories and consumables

Also available are accessories and consumables for hassle-free mass photometry measurements. On top of increasing convenience and reducing the steps needed to prepare for each measurement, these products give users confidence in their data by helping maintain consistent measurement conditions.

Refeyn's range of accessories and consumables includes:

- Ready-to-use sample carrier slides
- Sample well cassettes
- An alignment tool and tweezers
- Magnetic slide holders

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