REFEYN®
WEIGHING MOLECULES WITH LIGHT

APPLICATION NOTE

Mass photometry analysis of samples at micromolar concentration

Analyzing biomolecular interactions is straightforward with mass photometry. The technology enables the quantitative analysis of binding, oligomerization and macromolecular assembly – in solution and without the need for labels. Now, Refeyn's microfluidics system, MassFluidix® HC, significantly expands the range of sample concentrations amenable to investigation by mass photometry, enabling applications such as the characterization of low-affinity interactions.

Expanding the scope of mass photometry analysis

Mass photometry is a bioanalytic technology that measures the mass distribution of biomolecules in a sample in just one minute. Operating in solution and without the need for labels, the technology has valuable applications in assessing binding, oligomerization, sample purity and more. For optimal mass photometry measurements, the concentration of the sample to be measured should typically be less than 100 nM. While this concentration is suitable for many measurement contexts, in some cases it is necessary to analyze sample behavior at a higher concentration. For example, complexes formed through low-affinity interactions dissociate at low concentrations, meaning they can often be reliably detected only at higher concentrations.

To address this issue, Refeyn's microfluidics system MassFluidix HC significantly expands the scope of samples that can be measured by mass photometry. MassFluidix HC raises the upper sample concentration limit from the nanomolar to the micromolar range. Here, we explain how the system works and demonstrate its use in measuring interactions between an immunoglobulin G (IgG) antibody and the IgG neonatal Fc receptor (FcRn).[1]

Characterization of low-affinity interactions

The MassFluidix HC system enables a measurement to be made at a concentration that is optimal for mass photometry, while capturing the state of the biomolecular interactions at micromolar concentration. The system works by rapidly diluting the sample and flowing it across the measurement surface very quickly – before the biomolecular interaction equilibrium has been disrupted by the dilution. The microfluidic system can dilute a sample up to 10,000-fold, passing the sample directly onto the measurement surface. The sample reaches the observation window within <50 ms of the beginning of the dilution process. Under these conditions, even for low-affinity (μM K_D) interactions with dissociation rates as fast as 1 s^{-1}, 98% of the complex will remain intact when the sample is measured.[2,3]

Samples that undergo rapid dilution via MassFluidix HC maintain the properties they had when they were more concentrated. As a result, the system makes it possible to use mass photometry to characterize biomolecular activity that occurs only at higher concentrations, such as low-affinity interactions.

The MassFluidix HC system

MassFluidix HC includes fluid controls and a rapid dilution chip, and is controlled by a computer. Dilution occurs on the MassFluidix HC chip when the sample and buffer are combined in a reverse Tesla valve mixer, then the diluted sample flows through the observation area before leaving the chip through an outlet (Fig. 1A). In the overall system, a computer, a central unit, a pump, pressure regulators and flow rate monitors work together to ensure that the sample and buffer enter the chip at the correct flow rates (Fig. 1B). This enables rapid sample dilution and then the mass photometry measurement.

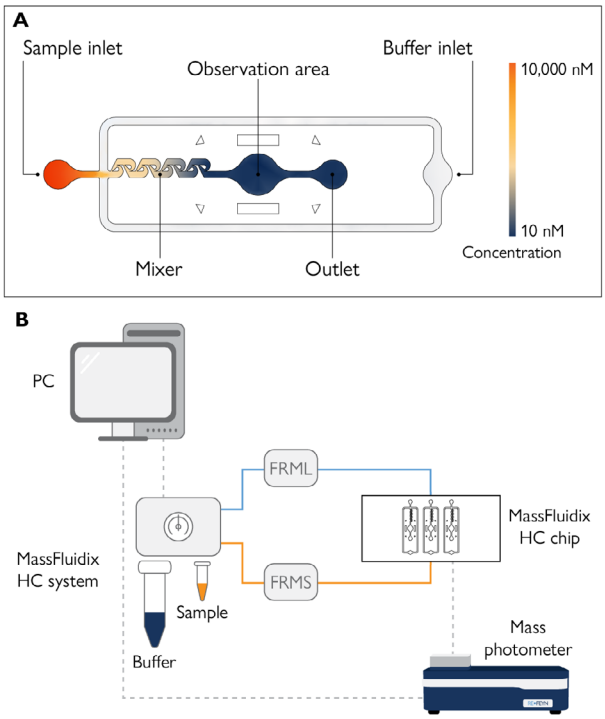

Fig . 1 The MassFluidix HC system is designed for rapid dilution. A: Illustration of the MassFluidix HC chip. The undiluted sample (orange) and buffer (grey) enter through inlets on opposite sides of the chip. The sample is diluted where the channels meet and in the mixer, reaching the target concentration (dark blue) near the observation area. Fluid color indicates concentration (see colorbar). B: The computer (top left) is connected to MassFluidix HC central unit (shown adjacent to the buffer and sample tubes) and the mass photometer (bottom right). Flow through the sample line and buffer line is monitored by the small flow rate monitor (FRMS) and the large flow rate monitor (FRML), respectively. The sample and buffer line tubing connects to a channel on the MassFluidix HC chip, which is placed inside the mass photometer.

One difference between MassFluidix HC and conventional mass photometry is that the molecules are under flow. We verified, theoretically and empirically, that the flow has a negligible effect on the measurement. Theoretically, no-slip boundary conditions mean that the fluid velocity at the measurement surface is zero; it remains negligible across the space where biomolecules are measured (up to ~20 nm from the surface). There is also no concentration variation across the channel, as the sample is homogeneously mixed when it reaches the measurement surface. Empirically, measurements of a range of proteins in the system confirmed that counts accumulate consistently, as in conventional measurements.

MassFluidix HC reveals low-affinity complexes

The MassFluidix HC system was used to investigate the binding of the IgG monoclonal antibody trastuzumab to the soluble domain of the FcRn.[1] A standard mass photometry measurement was made of a sample containing both proteins in a 1:10 ratio (IgG at 2 µM and FcRn at 20 µM) that was manually diluted to 10 nM. Consistent with previously published data,[1] two main peaks were observed, corresponding to FcRn monomers and unbound IgG (Fig. 2). IgG-FcRn complexes were not clearly present.

In contrast, FcRn-IgG complexes were clearly present when the same proteins were measured using the MassFluidix HC system. In this measurement, the sample had an initial concentration of 22 µM and was rapidly diluted 2000-fold to 11 nM in the system. In addition to peaks corresponding to FcRn monomers and unbound IgG, peaks corresponding to 1:1 FcRn-IgG complexes and 2:1 FcRn-IgG complexes were also clearly visible (Fig. 2).

In summary, mass photometry measurements with MassFluidix HC enabled detection of low-affinity complexes that had not previously been observed. Because lower-affinity complexes cannot form unless the interacting biomolecules are present at higher concentrations, the results suggest that FcRn-IgG complexes have relatively low binding affinities. The evident differences in complex formation at 10 nM vs. 22 µM demonstrate the value of studying biomolecular interactions at higher concentrations to gain a more complete understanding of biomolecular system dynamics. The MassFluidix HC system makes it possible to run such measurements using mass photometry.

References

[1] *Soltermann et al., Angew. Chem. Int. Ed., 2020*

[2] *Pollard T.D., MBoC, 2017*

[3] *Jarmoskaite et al., eLife, 2020*

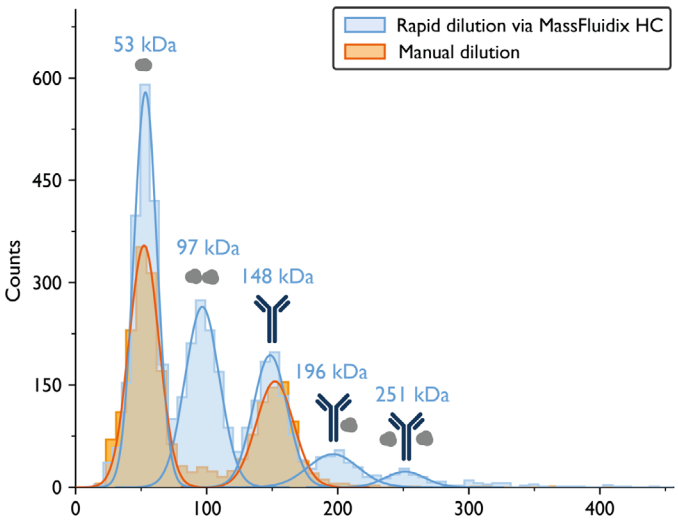

Fig. 2 Mass histograms reveal low-affinity complexes only after rapid sample dilution. Mass histograms and corresponding best-fit Gaussian distributions are shown for measurements of samples containing IgG and FcRn (mixed at 1:10 ratio) following manual dilution (orange) or rapid dilution via the Microfluidix HC system (blue). After manual dilution, peaks corresponding to FcRn monomers and IgG monomers were observed. After rapid dilution, in addition to the FcRn and IgG monomers, peaks corresponding to FcRn dimers and IgG-FcRn complexes with 1:1 and 1:2 stoichiometry were also clearly observed.

Conclusion

The MassFluidix HC system uses rapid dilution to expand the range of sample concentrations that are amenable to analysis using Refeyn's One^{MP} and Two^{MP} mass photometers. Mass photometry makes it easy to quickly measure the mass distribution of biomolecular samples in solution – without labels and using very little sample. It facilitates studies of biomolecular assembly, oligomerization and interactions, and the assessment of sample purity. As demonstrated above, MassFluidix HC makes it possible to characterize low-affinity interactions, further broadening the applicability of mass photometry.

Experimental details

- The coverslip was coated with cationic coating
- Samples were at pH 5.0 and diluted in PBS, pH 5.0
- Flow rates were 0.5 µL/min (sample) and 1 mL/min (buffer)
- The measurement time was 60 seconds
- A Two^{MP} mass photometer was used for all data collection

Unit 9, Trade City, Sandy Lane West, Oxford OX4 6FF, United Kingdom

For information on products, demos and ordering, write to info@refeyn.com

refeyn.com

@refeynit

Refeyn

Refeyn