Characterizing protein oligomerization with automated mass photometry

Oligomerization can be critical for protein function, so to understand a protein's function requires the ability to quantify its oligomerization state. Mass photometry can measure the mass distribution of a sample in native conditions and at a single-molecule level, with enough sensitivity to detect rare species. Automated mass photometry builds on these capabilities with easy and consistent sample dilution and manipulation, enabling thorough assays of oligomerization behavior.

Oligomerization, self-assembly into specific quaternary structures, is a central process for the function of many proteins. In fact, proteins that function as monomers are a minority according to current databases.[1,2] The formation of oligomers has several relevant physiological roles. For example, enzyme activity can be regulated via allosteric interactions between subunits or by formation of active sites at the subunit interface. Similarly, the regulation of gene expression often relies on homo-oligomeric DNA-binding proteins. Membrane-associated proteins, such as channels and receptors, frequently require oligomerization to provide transport across membranes or facilitate cell-cell adhesion.[2,3] Finally, altered oligomeric states are involved in the development of some diseases, which makes them relevant in the context of translational research.[4] Identifying the conditions that determine the oligomeric state of a given protein can help find mechanisms for stabilizing or interfering with this process, opening up new potential avenues for therapeutic intervention.

Mass photometry and protein oligomerization

Capturing and characterizing protein oligomerization can be challenging, particularly if one or more of the species are only present at very low concentrations. Among currently available analytical techniques, mass photometry is especially suited for assessing oligomerization states and dynamics. Mass photometry is a powerful technique that measures the interference between the light reflected by a glass surface and the light scattered by a molecule in contact with the glass. The magnitude of this interference scales linearly with molecular mass, and it can be measured using very little sample, no labels, and a wide range of buffers.

Mass photometry provides high-resolution distributions of molecular mass with single-molecule sensitivity directly in solution. The information about the mass distribution of a sample can then be used to infer oligomerization states. Moreover, mass photometry is sensitive enough to detect rare species that form less than 1% of the main sample population. Automation through use of Refeyn's Auto platform offers another important advantage that complements the strengths of mass photometry. The Auto robotics unit is compatible with Refeyn's One^{MP} and Two^{MP} instruments, combining the ease and efficiency of automation with the sensitivity and simplicity of mass photometry. This combination makes it ideal for rapid screening and analysis of oligomeric states under varying conditions.

Mass photometry can characterize oligomerization behavior

In this experiment series performed by GSK, the team used the Two^{MP} Auto in a preliminary study to characterize the oligomerization dynamics of a proprietary protein of interest. They evaluated the oligomeric status of the protein at different concentrations. In buffer alone, the target protein formed mainly monomers at lower concentrations. As its concentration increased, homodimers appeared in the mass distribution histogram as a secondary peak at twice the molecular mass of the monomer peak. At a protein concentration of 6.3 nM, for instance, both peaks were visible (Fig. 1).

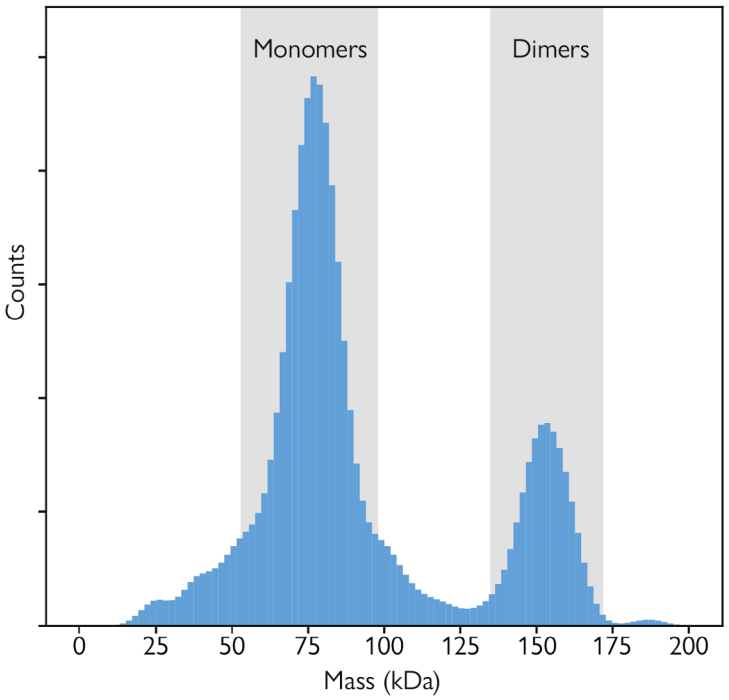


Fig. 1 Measuring oligomerization with mass photometry. Mass distribution of a sample of the target protein measured at a concentration of 6.3 nM. The peak containing the main population (around 80 kDa) corresponds to counted monomers, while the secondary peak (around 160 kDa) corresponds to dimers.

Mass photometry helps study oligomerization effectors

In this experimental series, the effects of varying calcium concentrations as well as adding a candidate inhibitor molecule, hypothesized to prevent oligomerization, were also tested. A series of measurements at increasing protein concentrations was performed after addition of the candidate inhibitor. Both sets of repeated measurements, made faster and easier by automation, were used to calculate the dissociation constants of the homodimers with and without inhibitor (Fig. 2).

The results suggest the candidate inhibitor influences protein oligomerization, with reduced dimer abundance in the presence of inhibitor. This change was quantified by calculating the dissociation constant (K_D) of protein samples without inhibitor (K_D= 3.55 ± 1.2 nM) and with inhibitor (K_D= 9.1 ± 1.1 nM). However, the effect is small, potentially indicating a different mechanism of inhibition.

The presence of calcium also affected oligomerization. Addition of $CaCl_2$ promoted the formation of tetramers, appearing as a peak on the mass distribution histogram at four times the molecular mass of the monomeric species. Conversely, the addition of the calcium chelator EDTA reversed the formation of tetramers, which was reflected on the histogram as the absence of the third peak (Fig. 3).

The effect of calcium on the oligomerization of the target protein was further explored by repeated mass photometry measurements at increasing protein concentrations under constant calcium concentrations. As protein concentration increased, the presence of calcium resulted in a gradual increase in tetramers, with a consequent reduction in monomers (Fig. 4). On the other hand, the proportion of dimers remained constant, in contrast to the behavior observed in the absence of calcium (Fig.2).

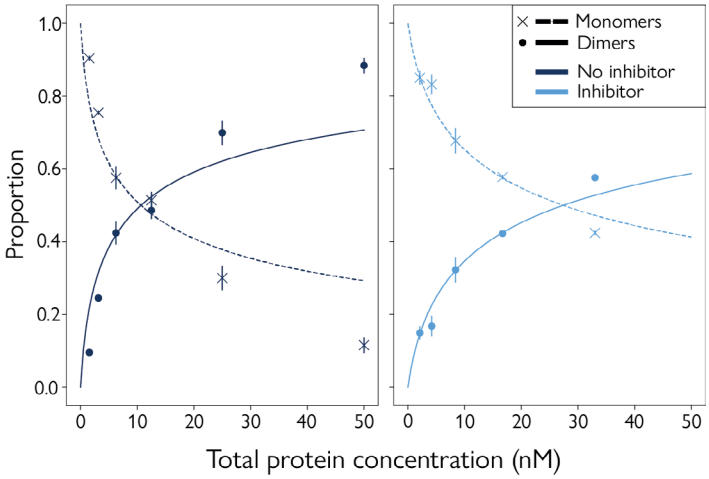


Fig. 2 Characterization of protein oligomerization with and without an inhibitor. Relative proportions of monomers and dimers in the sample, as a function of target protein concentration. Left plot (dark blue) shows measurements in simple buffer, while right plot (mid blue) shows measurements in the presence of an oligomerization inhibitor. Concentrations measured were : 1.55, 3.15, 6.25, 12.5, 25.0 and 50.0 nM for the samples without inhibitor, and 2.1, 4.2, 8.4, 16.7 and 33 nM for the samples with inhibitor.

Automated mass photometry streamlines oligomerization research

By automating sample manipulation, the Two^{MP} Auto avoids the variability associated with manual operation across experiments and between operators. Reproducibility tests of the Two^{MP} Auto show variabilities of less than 1% in measured mass and relative proportion of detected species. This proved useful for the characterization of the candidate inhibitor investigated as a part of the GSK preliminary study.

Another important factor in this experimental series was the 'in-plate' dilution feature of the Two^{MP} Auto. This feature makes it possible to store the protein sample in each well at a higher concentration and dilute it immediately prior to measurement. In-plate dilution helps minimize adsorption to the well surface,

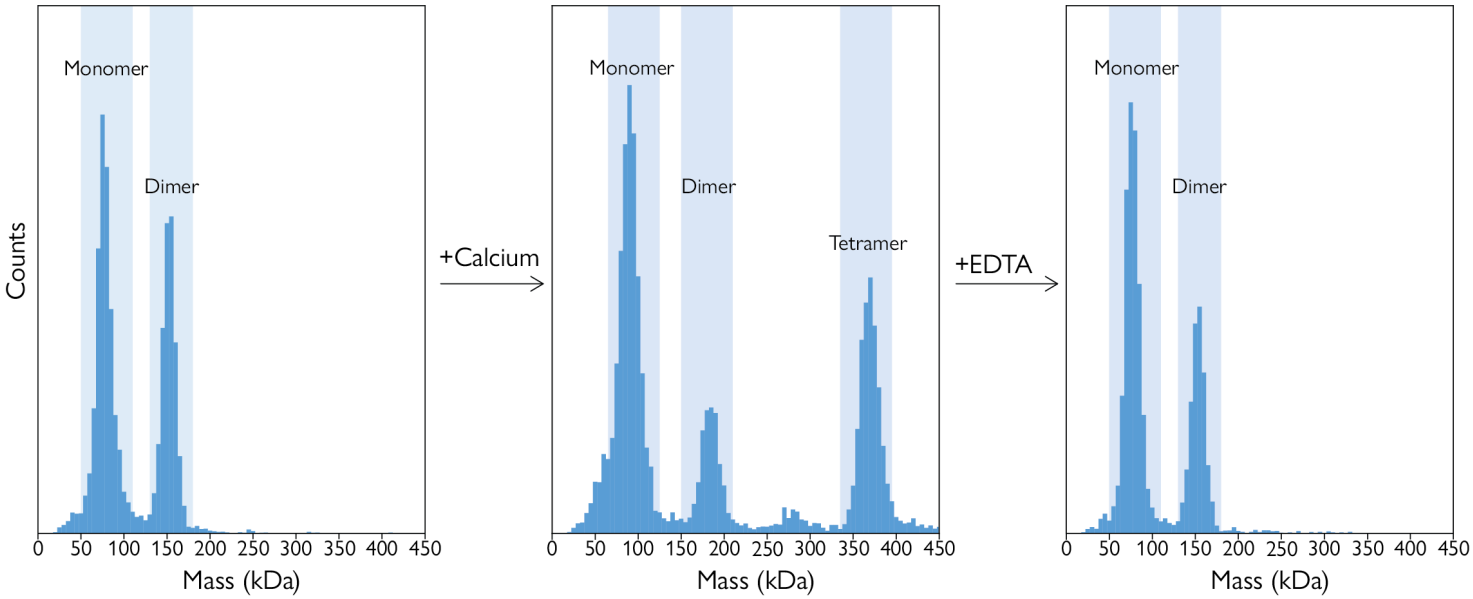


Fig 3 Reversal of tetramer formation. A: A mass histogram of a protein-only sample, with monomers and dimers present. B: Calcium chloride (present in excess) induced the formation of tetramers and reduced the number of dimers. C: Adding a saturating concentration of EDTA reversed this effect, resulting in the disappearance of the tetrameric species.

which can otherwise be significant at low protein concentrations. The in-plate dilution feature also minimizes the requirement for prior manual dilution of samples to the concentration range required for optimal mass photometry measurements. Moreover, the system enables the user to run multiple sets of conditions in every run, reducing screening time. Refeyn's Discover^{MP} software enables all files generated from each run to be analyzed as a single batch, streamlining data analysis and simplifying data reporting.

In summary, mass photometry is a powerful technique for characterizing oligomer formation and elucidating the factors involved in the promotion and inhibition of this critical biological process. Automation further streamlines the process from sample preparation to data capture, saving operator time for fast, reproducible and cost-effective investigation of oligomerization behaviors.

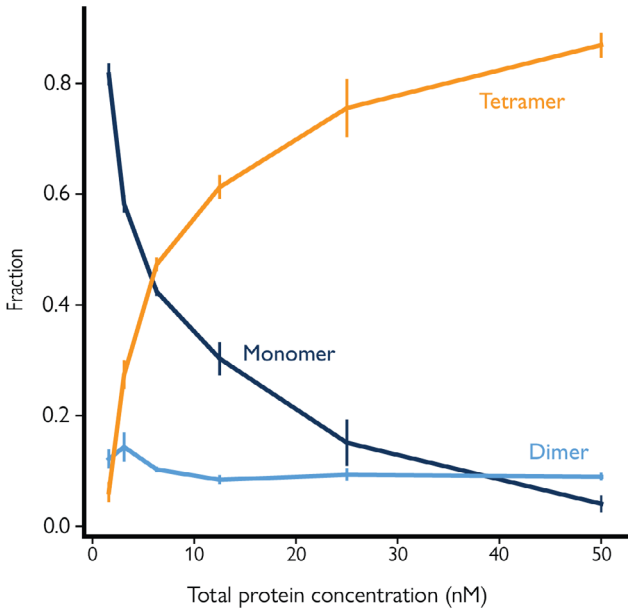
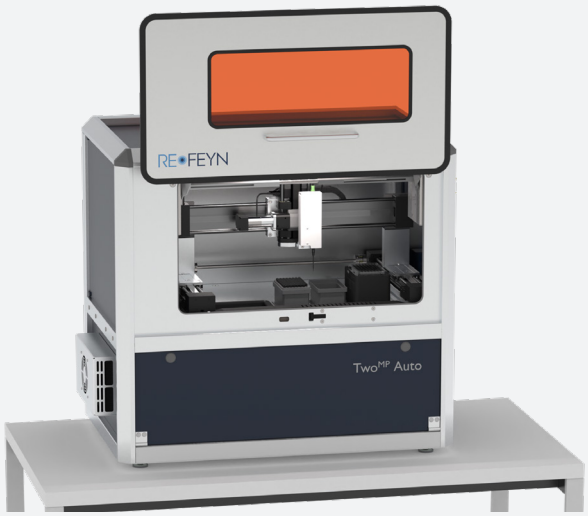


Fig 4. Dilution series showing protein oligomerization under constant $CaCl_2$ concentration. In the presence of 20 mM $CaCl_2$, increasing the target protein concentration resulted in an increase in tetramer formation, while the proportion of dimers in the sample remained small.

Studying oligomerization with mass photometry

- Minimal sample preparation, results within minutes
- Compatible with most buffers, no labels required
- Sensitive to changes in oligomerization status even when sample is scarce
- Detection of rare oligomeric species
- Automation allows rapid, unattended measurement of up to 24 samples
- Ideal for screening and titration, studies of protein interaction and effector dynamics

Related product



Two^{MP} Auto: The automated mass photometer

The experiments shown in this application note were performed with the Refeyn Two^{MP} Auto. This automated mass photometry platform 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.

References

[1] *Danielli et al., Sci Rep 2020*

[2] *Hashimoto and Pachenko, PNAS, 2010*

[3] *Marianayagam et al., Trends Biochem Sci 2004*

[4] *Sato et al. J Biol Chem 2018*

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