Characterisation of protein interaction equilibria

APPLICATION NOTE

Protein-protein interactions and protein complex formation are often multi-step reactions involving multiple species. Mass photometry, a novel analytical method, makes it possible to monitor complex equilibrium formation reactions, and to assess how changes in the chemical environment or protein concentration affect equilibria. Mass photometry directly measures the relative concentrations of all protein populations in a sample, in a single molecule fashion.

Protein-protein interactions are pivotal to virtually every cellular process, from DNA replication to cell cycle control. As protein-protein interactions are mainly non-covalent, they usually exist in a state of equilibrium. Assessing how a system regains equilibrium after a perturbation provides insights into the reaction kinetics, while interaction affinities can be determined by measuring the relative concentrations of each species at equilibrium. However, the study of protein-protein interactions can become complicated when the system of interest includes multiple reactions or species at low concentrations. The value of bulk or averaged measurements quickly declines in these conditions. In contrast, mass photometry is a single molecule analytical technology, and so it can directly measure the molecular mass and relative abundance of all populations in a sample[1]. Here, we use mass photometry to study the oligomerisation process of a recombinant protein that forms tetramers.

Monitoring the formation of reaction equilibria

After a protein-protein interaction system is perturbed, such as by a change in total protein concentration, the system re-equilibrates. The recombinant protein studied here primarily forms tetramers at high (>100 µM) concentrations, but we investigated its behaviour in the low nM concentration range, which is more physiologically relevant. After diluting the sample to 10 nM, populations of species with a molecular mass corresponding to monomeric, dimeric and tetrameric forms were detected by mass photometry (Fig. 1). Other species, such as trimers, pentamers and octamers, were also visible, but at low abundance. Over time, the proportion of tetramers decreased, whereas the proportion of monomers increased until equilibrium was reached, after approximately one hour of incubation. The proportion of dimers remained roughly constant over time. For physiological relevance, the sample was incubated at 37° C; the mass photometry measurements were performed at room temperature. Here, mass photometry provided a fast and easy way to assess the incubation time required to ensure that the subsequent experiments were performed at equilibrium.

Quantifying how protein concentrations affect equilibria

Once an oligomerisation system has reached equilibrium, the relative concentrations of each protein species will vary as a function of changes in the total protein concentration. Typically, an increase in the total concentration will favour the formation of higher-order protein complexes, whereas a reduction will favour monomers or lower-order oligomers. Mass photometry data confirmed this expectation for the recombinant protein under study; monomers were the most abundant species at 1-50 nM total protein concentration, and tetramers were the most abundant at 100 nM (Fig. 2). The dimer proportion remained roughly constant despite the changes in total protein concentration.

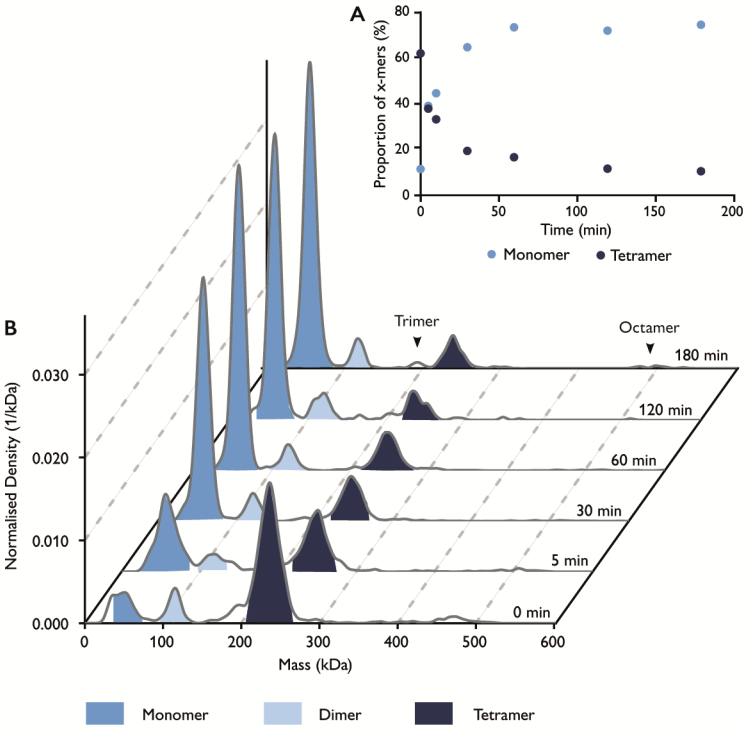

Fig. 1 Mass photometry enables monitoring of the formation of reaction equilibria. A: relative abundance of monomeric and tetrameric forms over time, calculated as the area under a Gaussian function fitted to each population. B: superimposition of normalised mass distributions (shown as kernel density estimates, KDEs) at the same time points, showing the relative abundances of all molecular species detected by mass photometry.

In the above example, mass photometry effectively measured the relative abundances of each oligomeric species across a range of physiologically relevant protein concentrations. This capability is valuable because the oligomeric state often relates directly to protein activity. Consequently, knowledge of the oligomeric states present in a sample can inform decisions on downstream applications.

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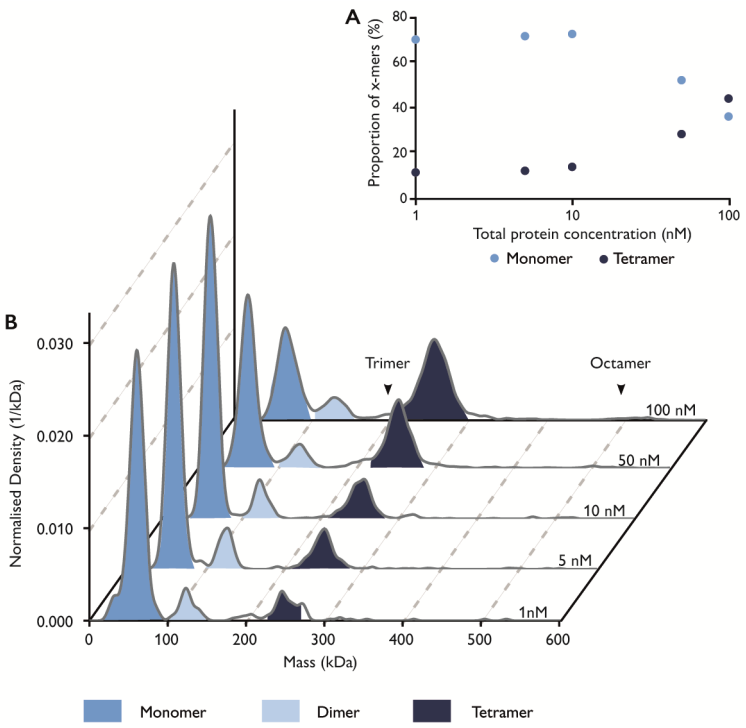

Fig. 2 Mass photometry tracks shifts in the reaction equilibria caused by changes in the total protein concentration. A: relative abundance of monomeric and tetrameric forms as a function of total protein concentration, quantified by calculating the area under a Gaussian function fitted to each population. B: superimposition of normalised mass distributions (shown as KDEs) at the same total protein concentrations, showing the relative abundances of all the molecular species detected by mass photometry.

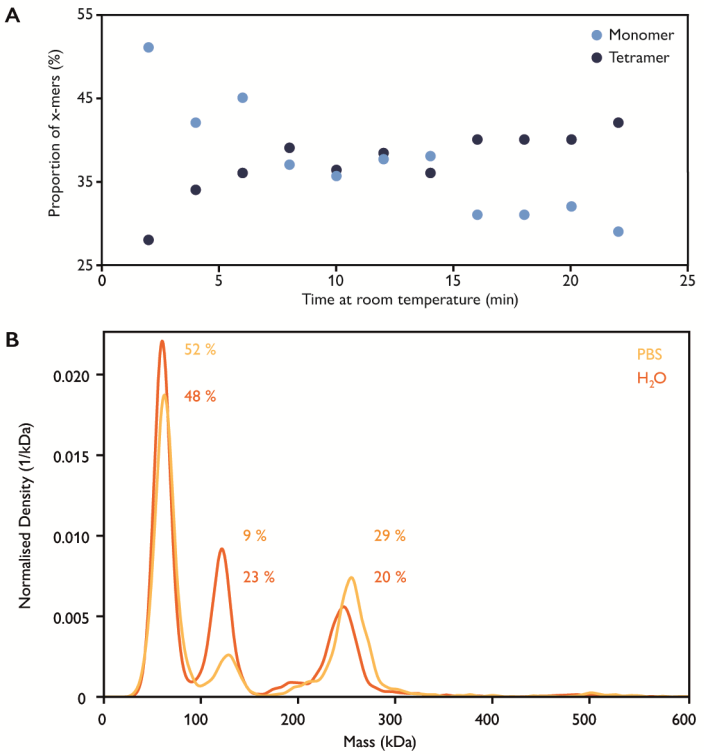

Fig. 3 Environmental factors can perturb reaction equilibria. A: relative abundance of monomeric and tetrameric forms as a function of the amount of time the sample has been incubated at a lower temperature (room temperature), quantified by calculating the area under a Gaussian function fitted to each population. B: superimposition of KDEs of the recombinant protein diluted in water or PBS. Percentages of molecules in each population relative to the total number of molecules are indicated.

Assessing how environmental factors affect equilibria

Physical and chemical properties of the environment – such as temperature, ionic strength or pH – can affect the equilibria of protein-protein interactions. Shifting equilibrated recombinant protein samples from 37°C to a lower temperature (room temperature) perturbed the system's equilibrium, causing the relative proportion of tetramers to increase over time (Fig. 3A). Again, the proportion of dimers remained constant (data not shown). These results highlight the importance of tightly controlling temperatures and incubation time during experiments, and demonstrate that rapid mass photometry measurements are valuable for verifying the state of a system under study.

In contrast, when the recombinant protein was incubated in water instead of PBS, changes in the monomer and tetramer populations were less pronounced. Unexpectedly, we observed a significant increase in the dimer population – which had previously remained constant in the PBS-diluted samples despite other perturbations (Fig. 3B) – illustrating the importance of quantifying each species individually through single molecule approaches.

Overall, the three sets of measurements on the recombinant protein show how using mass photometry to monitor all protein species in a sample at a single molecule level can give valuable insights into difficult-to-analyse, multi-step reactions.

Conclusion

Systems involving protein-protein interactions and other macromolecular interactions are often complex, making bulk measurements difficult to interpret. Mass photometry overcomes this challenge by providing information on all species in a sample, thanks to its single molecule nature (provided that there are sufficient differences in molecular mass between the species of interest). Mass photometry enables the relative concentrations of all components of a sample in solution to be easily monitored over a range of times or conditions, without any need for protein labelling or immobilisation. Building on this approach, the calculation of equilibrium constants from mass photometry data has been described in detail in recent publications[2,3].

References

[1] *Young et al., Science 2018*

[2] *Soltermann et al., Angew Chemie - Int Ed. 2020*

[3] *Wu et al., Anal Biochem. 2020*

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