Mass photometry with detergents

APPLICATION NOTE

Detergents are widely used in biochemistry but display complex behaviour in aqueous solutions. Mass photometry is an effective tool for the study of biomolecules in detergent-containing solutions as well as for the evaluation of molecular aggregation and micelle formation. Here, we describe how detergents affect mass photometry measurements and outline recommendations for how to optimise conditions for mass photometry experiments involving detergents.

Detergents may be used to extract and solubilise membrane proteins, to prevent unspecific binding, or to control protein crystallisation conditions. However, due to their remarkable chemical properties and complex behaviour in aqueous solutions, the presence of detergents significantly limits the downstream use of many analytical technologies. Mass photometry (MP), a powerful novel technology capable of measuring the mass of individual biomolecules, can overcome this challenge. Compatible with a wide range of buffer components, MP eliminates the need for complete detergent removal. It also provides a straightforward way to determine how detergents affect sample solubility and how detergent behaviour varies at different concentrations and in different buffers.

The effect of detergents in mass photometry

Small molecules, including individual detergent molecules, are not detectable by MP because the amount of light they scatter – resulting in their MP contrast, or signal – is largely below the detection threshold. Nonetheless, detergents can generate noise (signal fluctuations) across ratiometric MP images. This noise can be due to water molecules forming large solvation shells around the detergent molecules, detergent molecules creating dynamic structures on the glass surface or other factors that affect the refractive index at the glass-water interface.

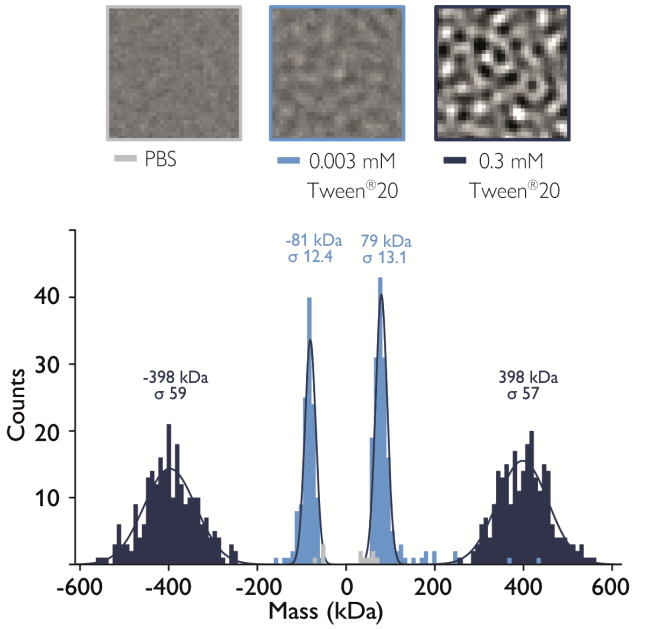

Fig. 1 Typical detergent noise signature. Top: PBS buffer alone and with detergent Tween®20 at two concentrations. Bottom: Superposition of histograms of PBS (grey) with PBS supplemented with Tween®20 at concentrations below (0.003 mM, mid blue) and above (0.3 mM, dark blue) the CMC. Apparent mass and sigma values of Gaussian fits are indicated.

Another way that detergents can affect MP measurements is through the formation of micelles. Detergents arrange themselves into these spherical forms when the detergent concentration in an aqueous solution is above what is called the critical micelle concentration (CMC). Like individual detergent molecules, smaller micelles (those below the MP detection limit) will generate noise in an MP measurement. Larger micelles can be visualised directly[1], in the same way as biomolecules are visualised in MP[2].

However, many standard protocols require high concentrations of detergent, which results in concentrations of micelles that are too high to allow the masses of individual micelles to be quantified by MP; instead, many overlapping events are observed as the micelles encounter the glass-water interface. The overlapping events will

Box 1: In-drop, fast dilution procedure

When performing MP measurements on biomolecules that have been solubilised in a detergent-containing solution, it can be challenging to maintain the biomolecule's solubility while keeping the detergent background signal low. In-drop, fast dilution is a straightforward procedure that enables MP measurements to be performed at a detergent concentration below what is otherwise the minimum for protein stability. This procedure can be used as long as the detergent-protein interaction can remain stable for the duration of the measurement (one minute). The procedure should be performed after a control measurement has been made of the buffer alone at the same detergent concentration, to assess the apparent mass of the detergent noise peak:

1. Load buffer without detergent onto the coverslip where the MP measurement will be made
2. Find the focus
3. Add protein together with detergent and mix gently using the pipette (by slowly aspirating in and out)
4. Perform the MP measurement

Mass photometry with detergents

Table 1 Effective lower detection limits corresponding to relative concentrations of detergents. Estimates of lowest detectable protein mass are based on the noise peak detected at the respective detergent concentration. The detergents were diluted in PBS. The CMC is indicated in grey. N/A: Detection limit of the One^{MP} applies.

	Critical micelle concentration						
Relative concentration	**1**	**5**	**20**	**100**	**500**	**2000**	
SDS	0.082	0.41	1.64	8.2	41	164	mM
	N/A	70	70	170	180	180	kDa
DDM	0.0012	0.006	0.024	0.12	0.6	2.4	mM
	N/A	N/A	N/A	560	560	560	kDa
OG	0.23	1.15	4.6	23	115	460	mM
	N/A	N/A	N/A	220	460	760	kDa
Digitonin	0.004	0.02	0.08	0.4	2	8	mM
	N/A	60	240	900	910	1170	kDa
NP-40	0.0008	0.004	0.016	0.08	0.4	1.6	mM
	N/A	50	90	260	430	430	kDa
Tween®20	0.00059	0.00295	0.0118	0.059	0.295	1.18	mM
	90	120	240	430	430	430	kDa
Triton X-100	0.0035	0.0175	0.07	0.35	1.75	7	mM
	90	110	190	620	620	620	kDa
CHAPS	0.08	0.4	1.6	8	40	160	mM
	N/A	N/A	90	210	210	300	kDa
LMNG	0.0001	0.0005	0.002	0.01	0.05	0.2	mM
	N/A	N/A	60	210	410	500	kDa

produce a pattern of noise similar to that produced by the individual molecules and smaller micelles, but with a stronger signal.

The overall result is a random noise pattern in the ratiometric MP image (Fig. 1, upper panel), which prevents the detection of macromolecules with a signal in the same range or lower, effectively raising the lower limit for mass detection (Table 1). These noise patterns can also lead to spurious MP signals. When the patterns form shapes that are similar to the signals generated by macromolecules, standard MP image analysis will interpret those patterns, incorrectly, as a macromolecule landing on the surface. If these shapes recur, they will give rise to a peak in the histogram with a certain apparent mass but no biological significance. A mirror-image peak – with equivalent, 'negative' apparent mass and the same height – will also be present (Fig. 1, lower panel). This signature mirror-imaging can be used to distinguish peaks that arise from noise from those that represent biomolecules landing on the measurement surface. This is because negative mass results from particles moving away from the glass surface (rather than landing on it). MP measurements of biomolecules, such as proteins, typically yield very small negative peaks or none at all because the biomolecules interact with the glass surface, moving away from it only infrequently.

Using mass photometry to measure a sample in a detergent-containing solution

Only biomolecules with mass significantly greater than the apparent mass corresponding to the detergent noise peak will be observable by MP. A lower detergent concentration will generally result in a lower mass detection limit, as well as improved resolution and accuracy (Fig. 2). Hence, it is recommended that measurements be performed at the lowest possible detergent concentration. In some cases, that concentration will correspond to a mass detection limit that is still too large to permit meaningful measurement by MP. In those cases, if the detergent is firmly attached to the protein, an in-drop, fast dilution procedure (Box 1) can enable MP measurements of proteins at detergent concentrations below what is otherwise the minimum for protein stability.

Table 1 gives approximations of the effective detection limits for different detergents diluted in PBS. This information is provided as a general guide, but will be subject to significant changes depending on the ionic strength, pH and other characteristics of the buffer used. It is recommended that the mass detection limit of detergent-containing solutions be assessed on a case-by-case basis by performing control measurements of the solution in the absence of the biomolecules of interest.

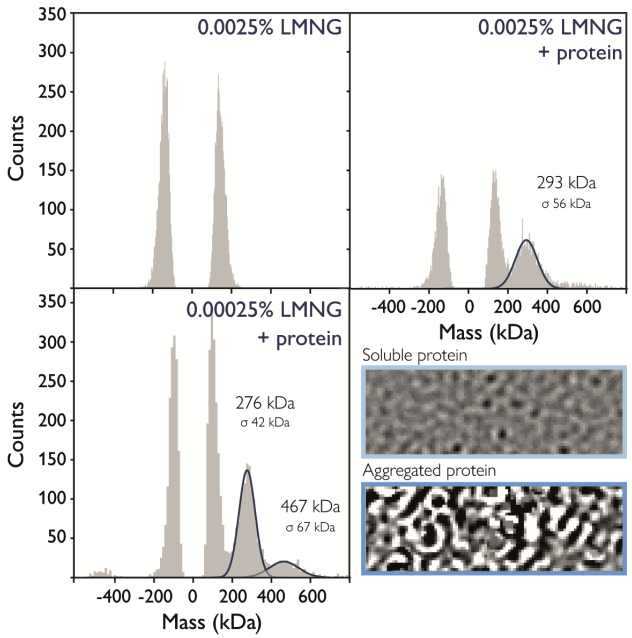

Fig. 2 Mass photometry measurement of a protein in detergent. Histograms represent measurements of buffer with 0.0025% LMNG alone, and 10 nM protein in buffer with 0.0025% and 0.00025% LMNG. Excessive dilution of detergent may result in protein aggregation, as illustrated in ratiometric frames showing soluble protein (light blue) and aggregated protein (mid blue). Data courtesy of Blanca López Méndez and Vadym Tkach, University of Copenhagen.

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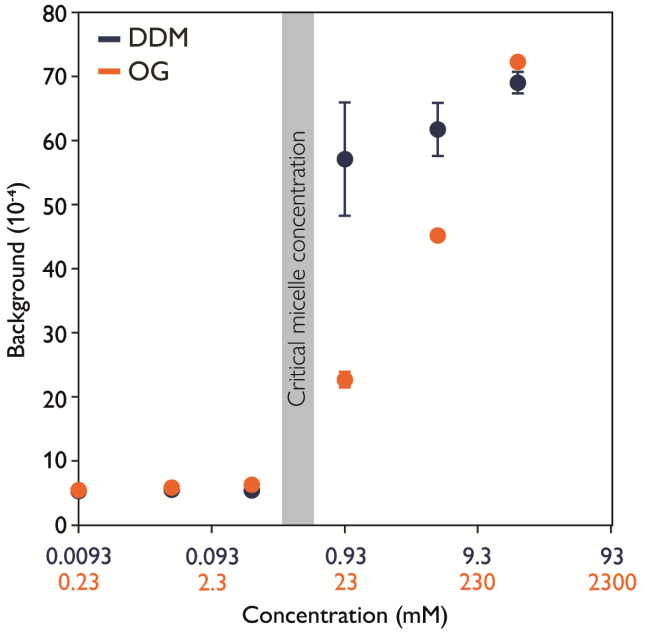

Fig. 3 Detergent behaviour above the CMC varies by detergent. MP measurements of two different detergents, DDM (dark blue) and OG (orange), show sigmoidal (DDM) vs linear (OG) increases in background as detergent concentration is increased above the CMC. The approximate CMC (in PBS) is indicated in grey. Background was quantified as the standard deviation of contrast for each ratiometric image, averaged over 3000 frames.

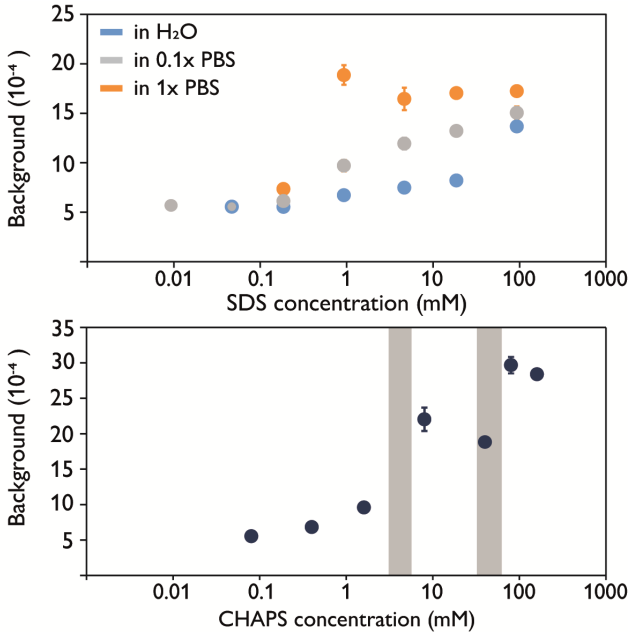

Fig. 4 Detergent micelle formation may be sensitive to buffer composition or display complex behaviour. Top: Background measured using MP for increasing concentrations of SDS in water (blue), 0.1x PBS (grey) and 1x PBS (orange). Bottom: Background measured using MP for increasing concentrations of CHAPS in PBS. The CMCs reported in the literature[3] are indicated as grey areas. Background was quantified as in Fig. 3.

Using mass photometry to screen for protein aggregation

Some proteins are soluble only above a certain detergent concentration, below which they form large aggregates. Specific conditions for each protein/detergent combination depend on many factors and are difficult to anticipate. As a result, they need to be assessed experimentally in each case. MP is an advantageous method for screening solubility conditions as it can be done quickly and uses minimal sample. Aggregates can be easily identified in a ratiometric MP movie (Fig. 2).

Using mass photometry to assess the CMC

Typically, detergents generate low MP background below the CMC, with the background increasing sharply above the CMC. The background intensity can then plateau for detergents that form micelles of a single size, such as DDM (n-dodecyl-ß-D-maltoside), or it can continue increasing if the micelle size increases with concentration, as it does for OG (octyl glucoside) (Fig. 3). The CMC depends on the pH and ionic strength of the buffer, the nature of the biomolecules, and other factors. As a result, the CMC observed for a detergent in experimental conditions can vary significantly from the CMC reported for that detergent in water. This variability is particularly evident in ionic detergents, such as SDS (sodium dodecyl sulfate)[3] (Fig. 4, upper panel). Detergents can also display complex micelle formation behaviour. For instance, the detergent CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate) has been reported to have two different CMCs (around 7 and 32 mM) and to form micelles 1.8 times larger above the second CMC[4] (Fig. 4, lower panel).

Having the ability to monitor micelle formation in any given set of experimental conditions is valuable because it enables one to optimise the detergent concentration, using no more detergent than necessary. However, in practice, measuring the CMC is difficult and experiments are typically conducted with detergent concentrations far above the CMC. MP offers a possible solution to this problem. By providing a convenient way to assess detergent behaviour under exact experimental conditions, MP makes it easy to establish the optimal conditions for any given experiment.

References

[1] *Lebedeva et al., ACS Nano 2020*
[2] *Young et al., Science 2018*
[3] *Danov et al., Adv Colloid Interface Sci 2014*
[4] *Qin et al., J Phys Chem B 2010*

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