# Mass photometry with detergents

*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 nonspecific 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, a technology that measures the mass of individual biomolecules in solution, can overcome this challenge. Compatible with a wide range of buffers, mass photometry eliminates the need for complete detergent removal. It also provides a quick and 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

Individual detergent molecules are not detectable by mass photometry because the amount of light they scatter is largely below the detection threshold. Nonetheless, detergents can generate noise (signal fluctuations) across ratiometric mass photometry 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.

Another way that detergents can affect mass photometry measurements is through the formation of micelles 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 detection limit) will generate noise in a mass photometry measurement. Larger micelles can be visualised directly[1] in mass photometry in the same way as biomolecules[2].

However, many standard protocols require high concentrations of detergent, which results in concentrations of micelles that are too high to allow masses of individual micelles to be quantified by mass photometry; instead, many overlapping events are observed as the micelles encounter the glass-water interface. The overlapping events will 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 mass photometry 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 mass photometry signals, similar to the signals generated by macromolecules. Standard mass photometry image analysis will incorrectly interpret those patterns as a macromolecule landing on the surface and, if they 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

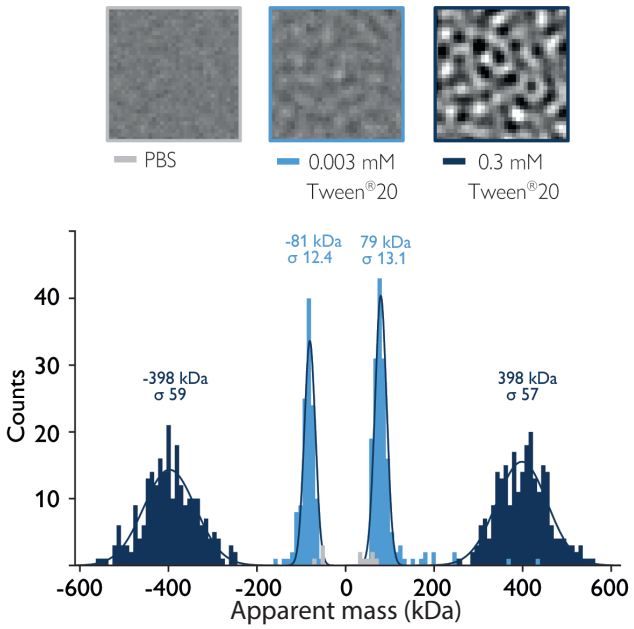


**Fig. 1 Typical detergent noise signature.** Top: PBS buffer alone and with detergent Tween®20 at two concentrations. Bottom: Superposition of histograms of PBS (grey) with PBS supplemented with Tween®20 at concentrations below (0.003 mM, mid blue) and above (0.3 mM, dark blue) the CMC. Apparent mass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

it. Mass photometry 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.

## Measuring samples containing detergents

Only biomolecules with mass significantly greater than the apparent mass corresponding to the detergent noise peak will be observable by mass photometry. Therefore, 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 which, in some cases, will correspond to a mass detection limit that is still too large to permit meaningful measurement by mass photometry. In those cases, if the detergent and protein are adequately bonded, an in-drop, fast dilution procedure (Box 1) can enable mass photometry 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 actual detection limits can vary depending on the ionic strength, pH and other characteristics of the buffer used. Therefore, 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.

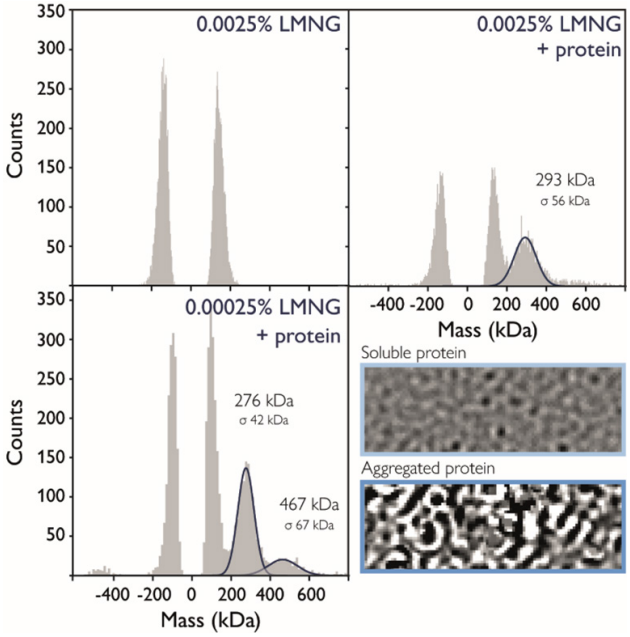


**Fig. 2 Mass photometry measurement of a protein in detergent.** Histograms represent measurements of buffer with 0.0025% LMNG alone, and 10 nM protein in buffer with 0.0025% and 0.00025% LMNG. Excessive dilution of detergent may result in protein aggregation, as illustrated in ratiometric frames showing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

**Table 1 Effective lower detection limits corresponding to relative concentrations of detergents.** Estimates of lowest detectable protein mass in kDa for One$^{MP}$(light blue) and Two$^{MP}$ (mid blue) are based on the noise peak detected at the respective detergent concentration (nM gray gradient). Detergents were diluted in PBS. CMC is indicated in grey. N/A: Detection limit of the instrument applies.

| %CMC | 1% | 5% | 20% | 100% | 500% | 2000% | One$^{MP}$ / Two$^{MP}$ |
|---|---|---|---|---|---|---|---|
| SDS | 82E-3 | 0.41 | 1.6 | 8.2 | 41 | 160 | [mM] |
| | N/A | 70 | 70 | 170 | 180 | 180 | kDa |
| | N/A | 110 | 120 | 120 | 230 | 230 | kDa |
| DDM | 1.2E-3 | 6E-3 | 24E-3 | 0.12 | 0.6 | 2.4 | [mM] |
| | N/A | N/A | N/A | 560 | 560 | 560 | kDa |
| | N/A | N/A | N/A | 120 | 480 | 480 | kDa |
| OG | 0.23 | 1.2 | 4.6 | 23 | 120 | 460 | [mM] |
| | N/A | N/A | N/A | 220 | 460 | 760 | kDa |
| | N/A | N/A | 40 | 250 | 250 | 330 | kDa |
| NP-40 | 0.8E-3 | 4E-3 | 16E-3 | 0.08 | 0.4 | 1.6 | [mM] |
| | N/A | 50 | 90 | 260 | 430 | 430 | kDa |
| | N/A | N/A | N/A | 60 | 500 | 500 | kDa |
| Tween 20 | 0.6E-3 | 3E-3 | 12E-3 | 0.06 | 0.3 | 1.2 | [mM] |
| | 90 | 120 | 240 | 430 | 430 | 430 | kDa |
| | 100 | 110 | 210 | 270 | 270 | 270 | kDa |
| Triton X-100 | 3.5E-3 | 18E-3 | 0.07 | 0.35 | 1.8 | 7 | [mM] |
| | 90 | 110 | 190 | 620 | 620 | 620 | kDa |
| | 30 | 50 | 210 | 480 | 480 | 480 | kDa |
| CHAPS | 0.08 | 0.4 | 1.6 | 8.0 | 40 | 160 | [mM] |
| | N/A | N/A | 90 | 210 | 210 | 300 | kDa |
| | 70 | 80 | 100 | 230 | 230 | 320 | kDa |
| LMNG | 0.1E-3 | 0.5E-3 | 2E-3 | 0.01 | 0.05 | 0.2 | [mM] |
| | N/A | N/A | 60 | 210 | 410 | 500 | kDa |
| | N/A | N/A | 280 | 280 | 400 | 550 | kDa |

## Screening for protein aggregation

Some proteins are soluble only above a certain detergent concentration, below which they form large aggregates. The optimal conditions for each protein/detergent combination depend on many factors and are difficult to anticipate. As a result, they should be assessed experimentally on a case-by-case basis. Mass photometry 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 mass photometry movie (Fig. 2).

## Using mass photometry to assess the CMC

Typically, detergents generate low mass photometry 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

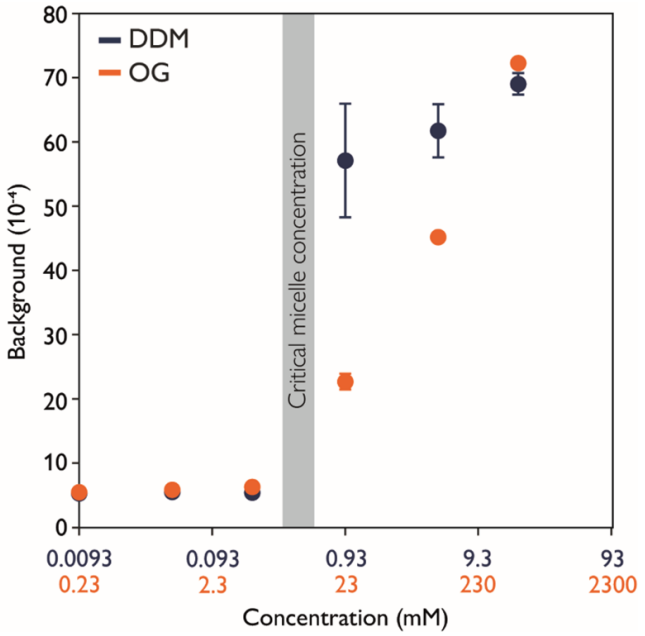


**Fig. 3 Detergent behaviour above the CMC varies by detergent.** Mass photometry measurements of two different detergents, DDM (dark blue) and OG (orange), show sigmoidal (DDM) vs linear (OG) increases in background as detergent concentration is increased above the CMC. The approximate CMC (in PBS)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

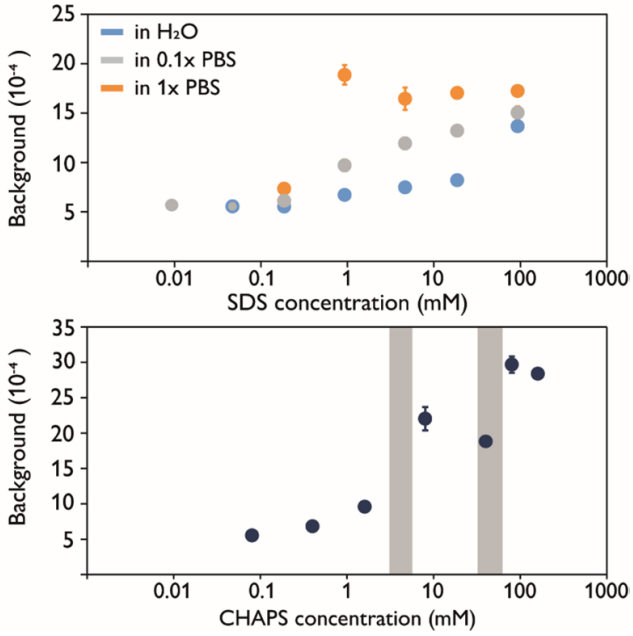


**Fig. 4 Detergent micelle formation may be sensitive to buffer composition or display complex behaviour.** Top: The background measured using mass photometry for increasing concentrations of SDS in water (blue), 0.1x PBS (grey) and 1x PBS (orange). Bottom: Similar measurements for increasing concentrations of CHAPS in PBS. CMCs reported in the literature[3] are indicated as grey areas. The background was quantified as in Fig. 3. Values measured on the One$^{MP}$.

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, top). Detergents can also display complex micelle formation behaviour. For instance, 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, bottom.).

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 it. Mass photometry offers a possible solution to this problem. By providing a fast, convenient way to assess detergent behaviour under different experimental conditions, mass photometry makes it easy to establish the optimal parameters for any given experiment.

## Box 1: In-drop, fast dilution procedure

This straightforward procedure enables mass photometry measurements below the detergent concentrations required for protein stability[5]. It can only be used if the detergent-protein interaction remains stable for the duration of the measurement (one minute) and the protein does not aggregate.

1. Load buffer with no detergent onto the coverslip, find the focus
2. Add protein with detergent to the original buffer and mix by gently aspirating in and out with the pipette
3. Perform the mass photometry measurement

The procedure should be performed after a control measurement of the buffer at the same detergent concentration, to assess the apparent mass of the detergent noise peak.


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*
[5] *Olerinyova et al., Chem 2021*





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