Mass photometry of nucleic acids

TECHNICAL NOTE

Mass photometry allows quantification of the mass distribution of biomolecules in solution. Its use in analysing the purity of proteins, characterising their oligomeric states and quantifying their protein-protein interactions is established. However, its application to characterising other biomolecules, including nucleic acids, is less well explored. Here, we show how to use mass photometry to measure the mass, size and abundance of DNA, in the range of 100 to 5000 base pairs.

Mass photometry (MP) is a novel analytical technique that uses the light scattered by individual biomolecules to measure their mass[1]. In an MP measurement, the minute amount of light scattered by single molecules in solution is detected as the molecules encounter the glass-water interface of the mass photometer coverslip. The amount of light scattered scales linearly with the mass of the molecule. So, with a simple calibration, the molecular mass of different species of molecules can be determined precisely.

In this application note, we give an overview of how to apply MP to measure nucleic acids, including steps for preparation and calibration. We also show example measurements for DNA—from low mass ladders to entire plasmids.

Preparation to measure nucleic acids

Both DNA and RNA are negatively charged in solution, meaning they tend to be repelled from untreated coverslip glass. As a consequence, nucleic acids show infrequent and transient binding. This results in few and poorly defined events in the MP data, with as much unbinding as binding (Fig. 1).

To overcome this transient behaviour, the coverslip surface can be functionalised to become positively charged, increasing its interaction with negatively charged nucleic acids. To achieve this functionalisation, the glass surface can be treated with APTES[2] or poly-lysine (PLL).

Coating a glass coverslip with PLL greatly enhances detection, with a dramatic increase in the number of binding events (and a reduction in unbinding) (Fig. 1). All six components of the low-mass DNA ladder, spanning 100 to 2000 base pairs (63 kDa to 1242 kDa) are well resolved. This demonstrates how MP can be used to measure the mass distribution of nucleic acids in solution.

Mass calibration for nucleic acids

MP can be applied to a variety of biomolecules. However, the refractive index will differ for different types of molecules. Therefore, to correctly correlate the measured

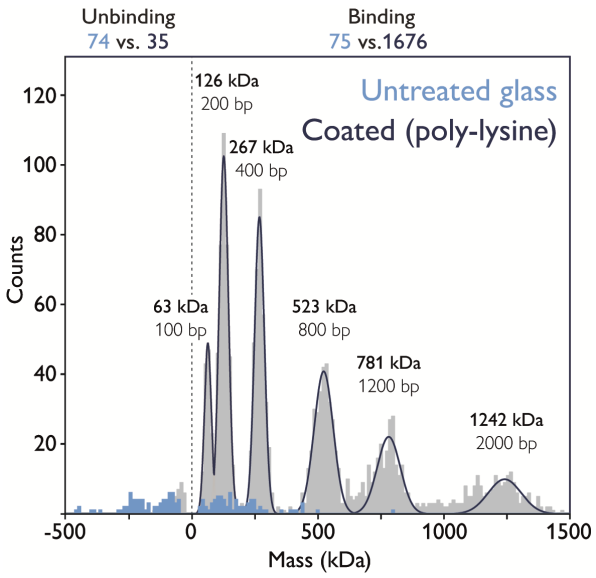

Fig. 1 Measurements of nucleic acids on untreated vs. functionalised glass. Superposition of measurements of a DNA ladder on untreated (mid blue) and poly-lysine coated (grey/dark blue) glass. Molecular weight (kDa) was determined using a DNA calibration, assuming one base pair equals 660 Da.

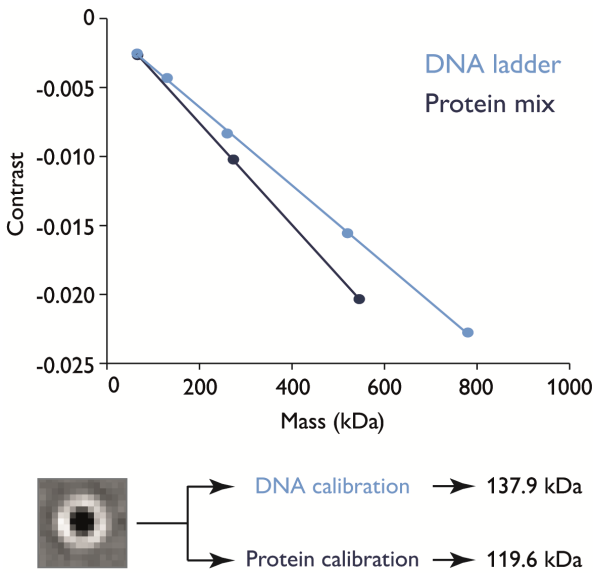

Fig. 2 Comparison of DNA and protein calibration. Different refractive indices for nucleotides and amino acids result in different slopes for DNA and protein calibration curves. This does not affect the measurement but impacts the calculation of the analyte's molecular mass.

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contrast with molecular mass, an appropriate calibration should be conducted, using a mix of molecules of known mass and the same molecular class as the analyte. Such calibrations for different molecular classes all show the universal linear correlation between contrast and mass, but with different slopes (Fig. 2).

As for calibrating measurements of proteins, commercially available standards can be used to calibrate DNA measurements. A single measurement of a DNA ladder for gel electrophoresis (1 base pair = 660 daltons) is sufficient for accurate calibration (Fig. 2). With these simple preparations, MP can be used to measure the mass (or number of base pairs) of DNA samples of unknown composition.

Measuring nucleic acids

To demonstrate the utility of MP measurements for nucleic acids, we determined the mass and homogeneity of a PCR product, using minimal sample (3 ng). The measurement revealed a single symmetric peak at 218 kDa, equivalent to 331 bp (Fig. 3) and in close agreement with the expected length of 335 bp.

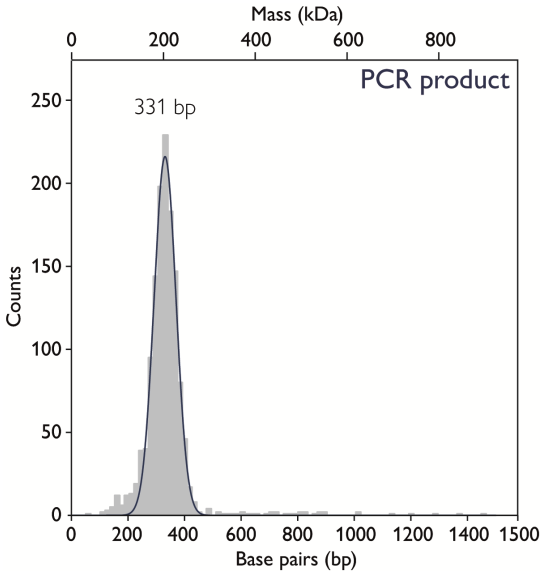

Fig. 3 Validation of a PCR product reveals its homogeneity. A single peak with a molecular mass of 218 kDa was obtained for the MP measurement of a PCR product. Calibration with a DNA standard curve revealed a length of 331 bp (expected length: 335 bp).

To test whether larger DNA species can be analysed with similar precision, we measured the plasmid pUC18 (Fig. 4). The most abundant peak was observed at 2683 bp, equivalent to 1773 kDa. DNA species with approximately 5000 bp were also detected, as were shorter DNA pieces, with fewer than 1000 bp.

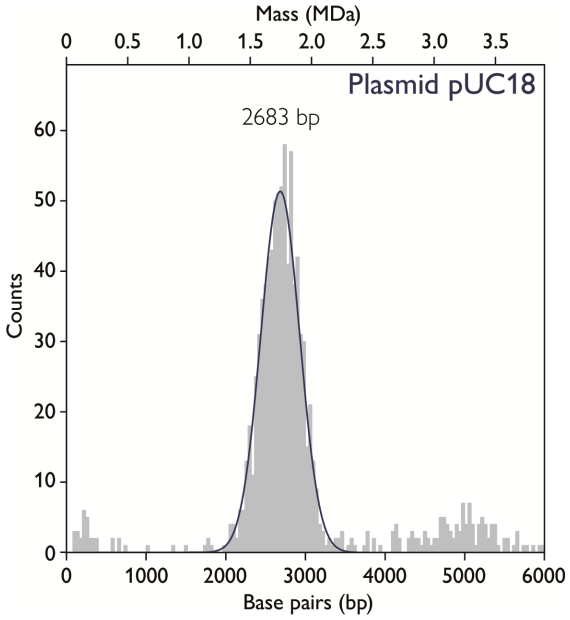

Fig. 4 Validation of a plasmid reveals its heterogeneity. An MP measurement of the plasmid pUC18 shows a major peak with a molecular weight of 1773 kDa. Calibration using a DNA standard curve reveals a length of 2683 bp for the intact plasmid (expected length: 2686 bp). In addition, DNA species with both higher and lower mass were detected.

This measurement highlights the wide mass range amenable to MP, and the high dynamic range afforded by the single molecule nature of the measurement.

In summary, MP is emerging as an exciting alternative to existing techniques for the quantification of nucleic acids. It allows mass determination and purity assessment using very little sample, within just a few minutes, and without the need for any stains.

Experimental details

- Low mass DNA ladder (Invitrogen) was measured using 39.2 ng in 10 µl PBS
- PCR product was measured using 33 ng in 10 µl PBS
- Plasmid pUC18 was measured using 50 ng in 10 µl PBS
- Clean glass was functionalised by applying 7 µl of a 0.01% PLL solution and incubating for 30 s
- MP measurements were made on a One^{MP}
- Data was analysed using Discover^{MP} v 2.0

References

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

[2] *Li et al., Nucleic Acids Res 2020*

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