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Application note

A comparison of Microfluidic Diffusional Sizing (MDS) with Dynamic Light Scattering (DLS) and Taylor Dispersion Analysis (TDA)

Microfluidic Diffusional Sizing is compared to the established techniques of Dynamic Light Scattering and Taylor Dispersion Analysis for protein size measurement. A range of proteins of different sizes and concentrations were evaluated using all three techniques. Results obtained by MDS are not only consistent with other techniques, but exhibit higher accuracy and precision to lower sample concentrations.

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Introduction

A number of well-established methods exist for measuring the size of proteins, such as Dynamic Light Scattering (DLS) and Taylor Dispersion Analysis (TDA).

However, these techniques are subject to various limitations due to inherent features of the techniques, and in some cases can suffer from a reduction in accuracy at the low end of protein concentration. This comparison of Microfluidic Diffusional Sizing (MDS) with DLS and TDA evaluates the accuracy and dynamic range of each method.

Size measurement by DLS is based on the principle that the Brownian motion of particles is proportional to their size. When the particles are illuminated with a laser, the light they scatter fluctuates as they move. This fluctuation can be measured to determine their Brownian motion, and hence size (1).

TDA measures the hydrodynamic radius by assessing the amount of peak broadening, caused by Taylor dispersion, of a sample plug as it travels through a capillary (1).

MDS has been developed from research undertaken in the Knowles group of the University of Cambridge (2, 3) and takes advantage of steady state laminar flow to measure the rate of diffusion of protein molecules and thereby determine size.

All three techniques facilitate analysis of protein size in solution state but use very different underlying technologies to determine that size. Here we compare the measurements taken for a range of proteins with varying molecular weights using the three techniques.

Methods

Dynamic light scattering

DLS measurements were performed on a Zetasizer Nano ZSP (Malvern Panalytical). For this instrument, measurements required 70 µL of sample and took on average 5 minutes for a single measurement.

Taylor Dispersion Analysis

TDA measurements were performed on a Viscosizer TD (Malvern Panalytical). For this instrument, measurements required 15 µL of sample and took approximately 20 minutes for a single measurement, including washing and priming steps.

Microfluidic Diffusional Sizing

MDS measurements were performed on a Fluidity One*. For this instrument measurements required 7 µL of sample and took approximately 9 minutes for a single measurement, including washing and priming steps.

Results

We took a set of 10 standard peptides and proteins, with molecular weights spanning over two orders of magnitude, and measured their hydrodynamic radii using MDS, DLS and TDA. The proteins measured and their molecular weights are shown in Table 1.

Protein	Molecular weight (kDa)
Bacitracin	1.42
Insulin	5.73
Beta-lactoglobulin	18.4
Ovalbumin	42.7
BSA	66.5
Immunoglobulin G	150
Aldolase	161
Thyroglobulin	600

Table 1: List of proteins and their molecular weights

We sized each protein listed in the table using the three different techniques. Experiments were performed in PBS. The results are summarised in Figure 1-A, where the measured hydrodynamic radius is plotted against the molecular weight of each protein. We find that the hydrodynamic radii measured using MDS are consistent with that of DLS and TDA.

In addition, we tested the dynamic range of sizing for each of the techniques above and found that for BSA, MDS allows accurate measurement of hydrodynamic radius down to 13.8 µg/mL protein, as shown in Figure 1-B. This is far lower than the 41.7 µg/mL and 125 µg/mL achieved by DLS and TDA respectively, and shows that MDS may prove particularly useful when working with low concentration samples.

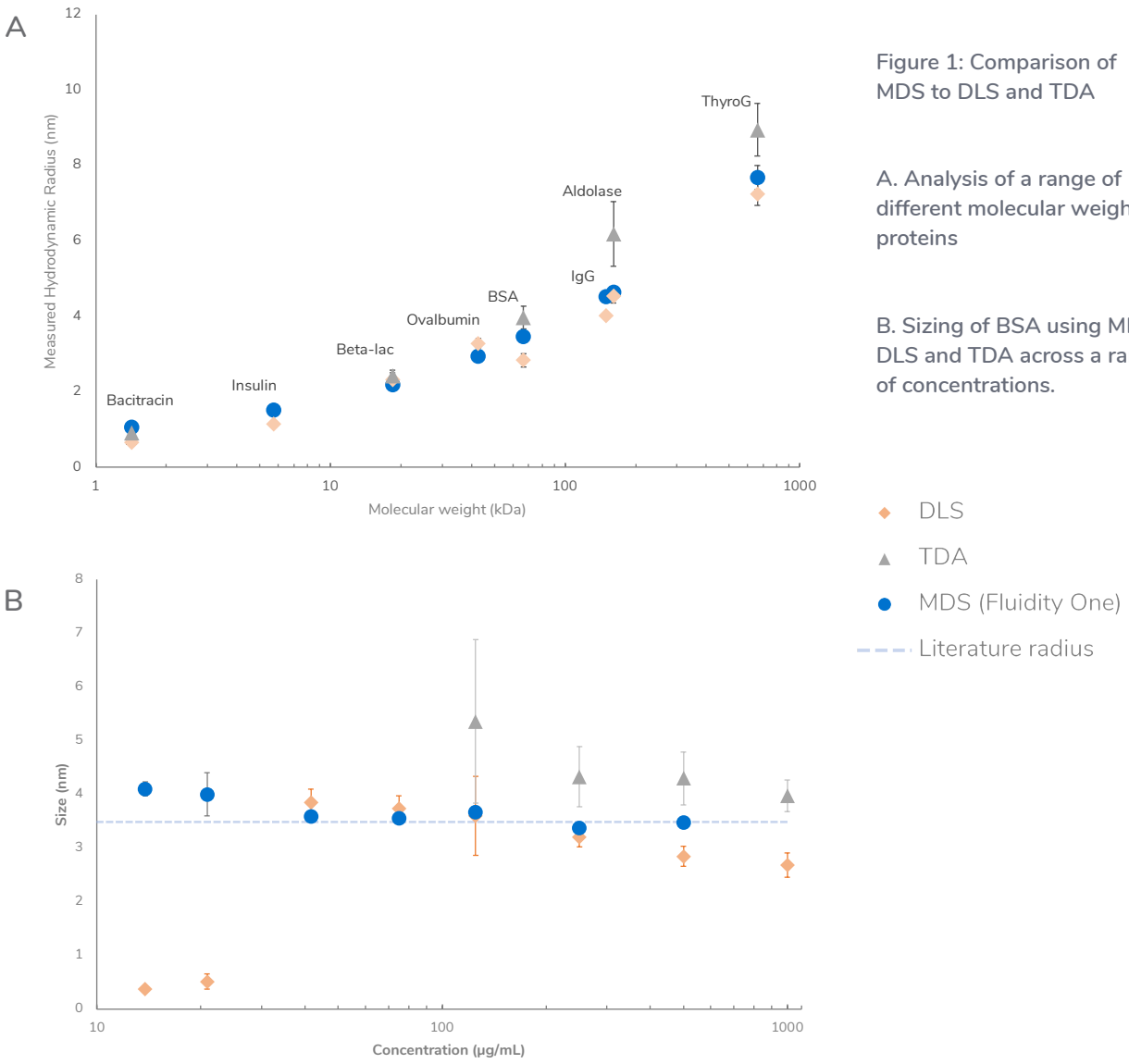

Figure 1: Comparison of MDS to DLS and TDA

A. Analysis of a range of different molecular weight proteins

B. Sizing of BSA using MDS, DLS and TDA across a range of concentrations.

Conclusion

When measuring hydrodynamic radius MDS analysis is shown to provide accurate data across a range of protein sizes which correlates with results obtained using other techniques.

In testing samples across a concentration range, results obtained by MDS were consistent even at low concentrations (<50 µg/mL), while measurements at these concentrations were either not possible with TDA or were inaccurate with DLS.

In summary, protein analysis by MDS on the Fluidity One offers an orthogonal data point and is a robust alternative to DLS and TDA for determination of unlabelled protein size in solution.

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***Please note that the data collected for this application note was obtained using a pre-release Fluidity One prototype.**

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