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Application note

Protein Size as an Indicator of Structure

Molecular weight (M_w) is a commonly used, and for many scientists a readily understood, parameter to describe the size of a protein or complex. Here we show how hydrodynamic radius (R_h) can be used in combination with M_w to provide insights into the shape and structure of proteins and illustrate how M_w alone may not always provide a complete picture.

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Introduction

The molecular weight of proteins is an important analytical parameter, but it does not provide information on the crucial secondary, tertiary or quaternary structural components which are often vital for their proper function. By observing the size in solution as well as molecular weight, we can gain insights into the conformation of proteins and their complexes.

Here we observe the relationship between M_w and R_h (as measured by microfluidic diffusional sizing, MDS) in a range of proteins, and note how this relationship can indicate structure.

Methods

A range of proteins, both globular and irregular in nature, were measured to determine R_h. Analysis by MDS was performed using a Fluidity One prototype (Fluidic Analytics).

For the globular proteins, experiments were performed in PBS. Each test was performed in triplicate, the data reported below being an average of the three results.

For the irregular proteins, α-synuclein and fibrinogen were tested in PBS in triplicate, αB-crystallin was assessed in 4 mM potassium borate at pH 10 with 4 repeats. Again, the average of the repeats is reported below. Alpha-synuclein was then measured in different buffers in triplicate; PBS pH 7.4, sodium phosphate pH 6.5 and MES pH 5.5, to assess the effect of changing environmental conditions, as discussed in the results section.

Results

Globular Proteins

A range of globular proteins were sized using MDS and the measured R_h plotted against the calculated M_w for each protein, as shown in Figure 1.

The R_h/M_w relationship across the range of globular proteins tested was consistent with a power law, with the high R^2 value indicating a strong degree of dependence between the two parameters.

Irregular Proteins

Three irregular (non-globular) proteins were subjected to the same analysis. It is immediately apparent that the irregular proteins do not fall on the R_h/M_w relationship for globular proteins.

The data suggest that there are three distinct ways in which they differ from the standard globular monomers.

Quaternary Structure - Oligomerization

In the case of αB-crystallin, the deviation from the standard R_h/M_w relationship can be explained by oligomerization.

The measured R_h for αB-crystallin (5.87 nm) is much larger than a globular monomer of this M_w (20.16 kDa) is predicted (1) to give (2.25 nm). Even in a fully unfolded monomer, the R_h is predicted to be 4.32 nm.

If we instead consider oligomerization, the measured R_h does correspond to a globular multimer in the order of approximately 24 sub-units.

This in in agreement with findings reported by Challa et al (2). The measured R_h plotted against both the monomer and 24-mer weight are visualized on Figure 1 to illustrate this change.

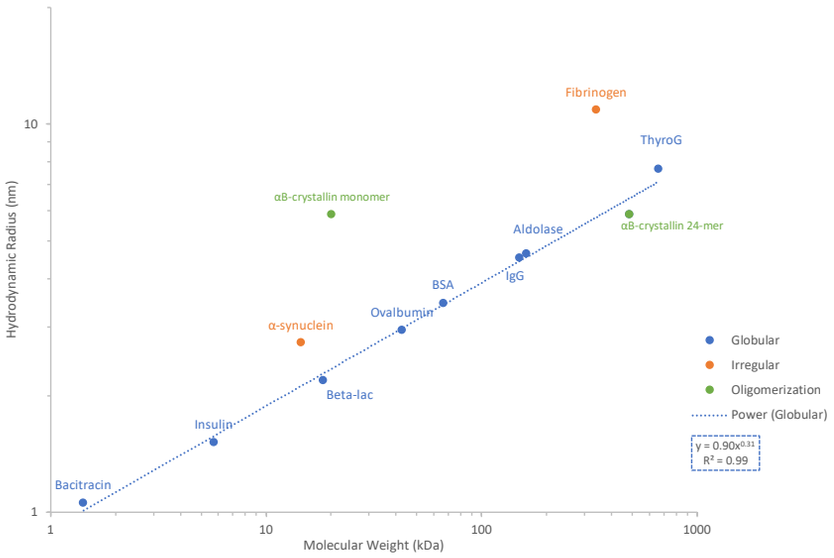


Figure 1: Measured hydrodynamic radius for various globular and irregular proteins of varying molecular weights.

The measured R_h of aB-crystallin is plotted against the M_w for both the monomer and 24-mer, to illustrate the relative distance from the standard R_h/M_w relationship each form has.

Tertiary Structure - Shape

The high R_h for the M_w of Fibrinogen is due to the elongated rod-like structure.

This structure results in the observed R_h of 10.9 nm, which is higher than the predicted globular R_h value (6.2 nm) the calculator (1) would predict for this M_w (340 kDa). When the known dimensions of fibrinogen (9 × 47.5 × 6 nm, (3)) are used to predict the Stoke's radius accounting for the rod-like structure (4), an expected R_h of 10.7 nm is obtained. The measured size of 10.9 nm agrees closely with this prediction.

Secondary Structure - Intrinsically Disordered regions

Sizing of α-synuclein results in a slightly high R_h/M_w compared to the globular species that can be attributed to the disordered C-terminal tail of this protein. By increasing the salt concentration to enable electrostatic screening or by reducing the pH to lower charge on the C-terminal region the disordered domain adopts a more compact state, reducing the measured R_h (5)(6). Figure 2 shows some examples of these changes.

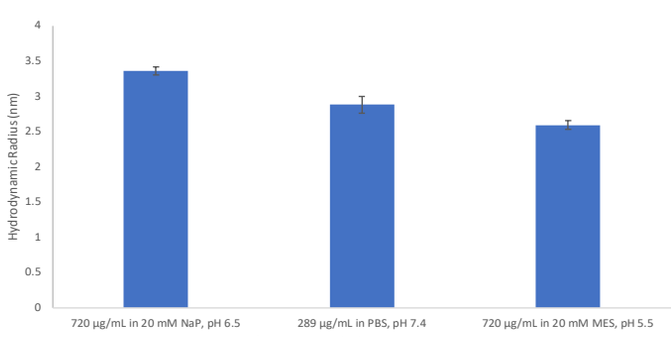


Figure 2: The measured R_h of α-synuclein under changing conditions

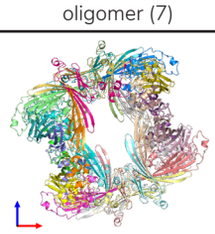
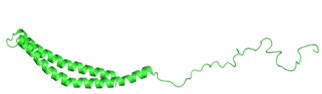
αB-crystallin wild type - 24meric oligomer (7)	Fibrinogen (8)	α-synuclein (9)
		

Table 1: The structures of the irregular (non-globular) proteins tested in this study

Conclusion

By observing the R_h to M_w relationship, we can elicit structural clues that can highlight clear differences between monomeric globular proteins and those that assume other conformations.

If the M_w of a sample is known, the R_h can be predicted with a M_w/R_h calculator (1) which can assume a globular or fully unfolded form. If the measured R_h is then markedly different from the predicted R_h, we can infer that the sample is not globular. As observed there may be several reasons for the difference, including oligomerization, shape and disordered structure.

The use of MDS in elucidating the M_w/R_h relationship in proteins where shape is a critical aspect of function provides a rapid yet insightful method for protein scientists.

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