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 $\times$ 47.5 $\times$ 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 $\alpha$-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.

<table>
<thead>
<tr>
<th>αB-crystallin wild type - 24meric oligomer (7)</th>
<th>Fibrinogen (8)</th>
<th>α-synuclein (9)</th>
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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.
References


