Size matters in Uncle's Sizing with Thermal Ramp app

Introduction

To add to Uncle's already powerful fluorescence and thermal stability capabilities, the Sizing with Thermal Ramp application now packs even more DLS data into every run. With a faster DLS read rate and 2 new metrics to help quantify your results – T_{size} and T_{agg} 660 – you get even more information out of every experiment. These new upgrades add some serious punch to the Sizing with Thermal Ramp application and give you the option to collect orthogonal data when evaluating protein stability on Uncle.

DLS is a highly sensitive tool for measuring the hydrodynamic size of proteins in solution. By measuring the changes in light scattering of a sample over time, DLS quantifies the motion of particles in a solution. If the viscosity of the solution is known, DLS can be used to determine the diffusion coefficient, size, and polydispersity of the particles. DLS tracks protein unfolding and aggregation with the Z-Average Diameter, which represents the average hydrodynamic diameter of particles in the whole sample.

Uncle is an all-in-one solution for protein stability that uses 3 detection methods: full-spectrum fluorescence, static light scattering (SLS), and DLS to fully profile protein stability (Figure 1). Temperature control (15–95 °C) and sealed sample chambers provide greater flexibility in how that profiling can be performed. Multiple measurements such as fluorescence, aggregation, sizing, and size distribution can be performed in just one experiment, allowing you to obtain orthogonal information on protein stability within the same sample. Samples are loaded into low volume, multi-well quartz cuvette chambers requiring only 9 µL of sample. Uncle can measure up to 48 samples simultaneously, enabling greater throughput when characterizing your biologics.

Figure 1: Uncle: an all-in-one biologics stability screening platform.

Uncle's Sizing with Thermal Ramp application determines the thermal stability of your biologic by measuring a protein's hydrodynamic size with DLS as it undergoes conformational changes during thermal stress. An increase in size as measured by DLS can be evidence of unfolding or aggregation. In this application, Uncle identifies T_{size}, the temperature at which the average particle size begins to increase, to make it easy to screen stability of protein formulations or constructs. From the scattering intensity of the DLS signal, Uncle also tracks aggregation and reports a T_{agg} 660 value to let you know when aggregation starts to become a problem. Multiple detection modes allow you to get a clearer picture of unfolding and aggregation to confidently monitor conformational changes in your proteins.

This application note describes how the faster DLS read rate of Uncle can be used for the optimization of formulations, by monitoring sizing and scattering intensity of your biologic during thermal denaturation with the Sizing with Thermal Ramp application.

Methods

Human polyclonal IgG1 antibody was prepared at 4 mg/mL in 1x phosphate buffered saline (PBS), pH 7.4 with 0, 62.5, 125, 250, or 445 mM arginine. Samples were centrifuged in a benchtop centrifuge for 30 seconds at 14,000 rpm to spin down any large particles. 9 µL of each sample was run in triplicate either with the Sizing with Thermal Ramp application or with the T_m & T_{agg} application on Uncle. Full spectra were collected from 250–720 nm during the T_m & T_{agg} run. DLS and SLS at 266 nm results were analyzed with Uncle Analysis v4.0.

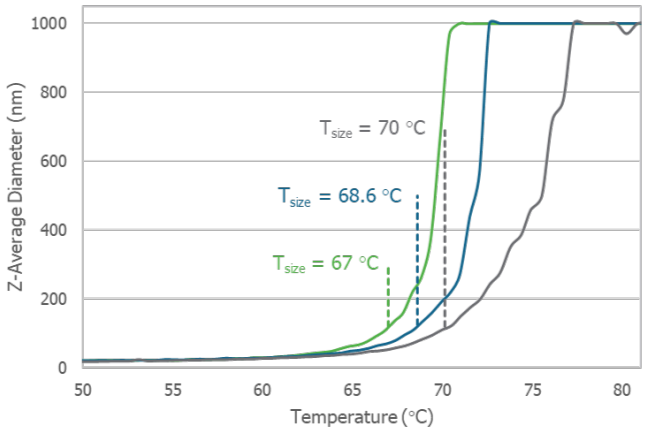

Figure 2: Average particle size of human IgG in PBS with 0 mM (green), 62.5 mM (blue), or 250 mM (gray) arginine during thermal ramp. Dashed lines depict T_{size} for each example.

Results

Maximizing the amount of DLS data from a single thermal ramp experiment means you won't miss anything about your protein's behavior. The newest version of Uncle software keeps the time spent moving and adjusting lasers and detectors to a minimum, dedicating more time to gathering data. The results speak for themselves – Sizing with Thermal Ramp now has a 40% faster read rate than before. That means each read is done in about 12 seconds, then it's time to move on the next sample.

By collecting DLS data during a thermal ramp, Uncle determines the average particle size of an IgG sample and monitors the thermal denaturation of the protein. Antibody unfolding and aggregation from heating is shown by an increasing Z-average diameter as temperature increases (Figure 2).

Uncle uses T_{size} as a metric to compare increases in average particle size across multiple samples.

T_{size} is the temperature at which the average particle size begins to increase. Proteins and formulations that resist unfolding and aggregation tend to have higher T_{size} values. In this example, higher concentrations of arginine in the buffer protect the antibody from aggregation and therefore result in higher T_{size} values.[1]

Uncle hunts for aggregation and reports the T_{agg} 660 metric to identify changes in aggregation behavior. T_{agg} 660 uses the intensity of the scattered light from a DLS measurement to track aggregation in the same manner as an SLS measurement. Just like with T_{size} values, T_{agg} 660 values increase with higher concentrations of arginine (Figure 3A).

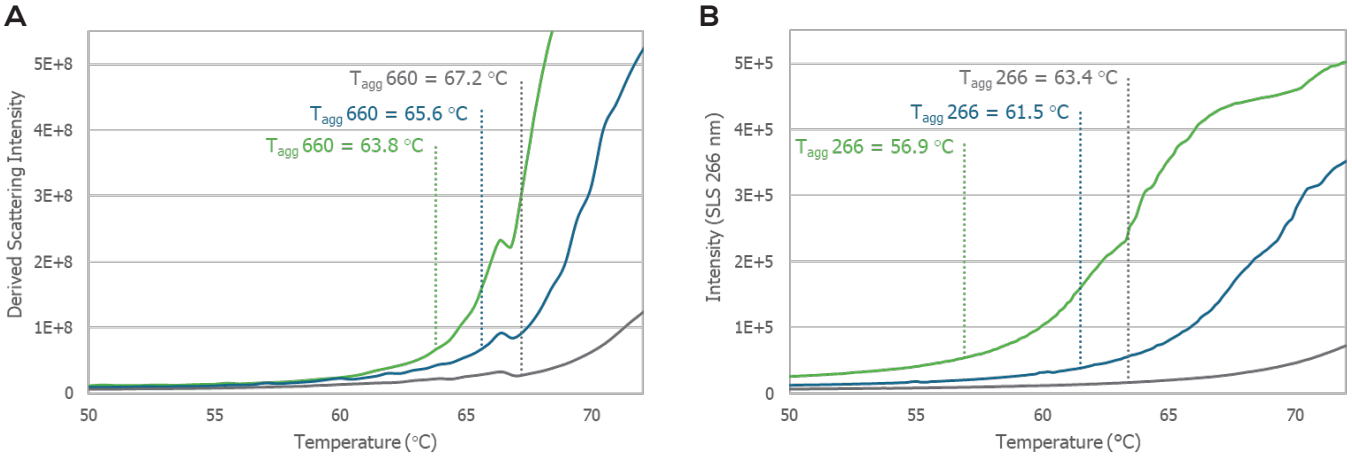

Figure 3: Samples of human IgG in PBS with 0 mM (green), 125 mM (blue), or 445 mM (grey) arginine during thermal ramping analyzed by derived scattering intensity of the 660 nm DLS laser from the Sizing with Thermal Ramp application (**A**) and static light scattering intensity at 266 nm from the T_m & T_{agg} application (**B**). Dotted lines depict T_{agg} determinations.

Uncle determines the values of both metrics for every sample when you use the Sizing with Thermal Ramp application, providing complementary metrics for evaluating protein stability.

Along with the Sizing with Thermal Ramp application, Uncle's T_m & T_{agg} with Optional DLS application includes SLS measurements at 266 nm, giving a T_{agg} 266. Light scattering measurements with shorter light wavelengths are more sensitive to the formation of small particles, while longer light wavelengths can detect larger particles. Thus, T_{agg} 266 and 660 can identify the onset of aggregation of smaller and larger protein aggregates, respectively. Formulations with higher concentrations of arginine had higher T_{agg} 266 values, indicating delayed formation of small aggregates (Figure 3B). Since SLS at 266 nm detects the formation of small aggregates early in thermal denaturation, T_{agg} 660 was higher than T_{agg} 266 in all 3 formulations. Uncle's toolbox of applications sharpen your view of your protein's unfolding and aggregation behavior.

Uncle determines a unique correlation function, intensity distribution, and mass distribution for every sample at each temperature measured during a Sizing with Thermal Ramp run. So, in addition to the full picture of thermal degradation, you can access individual snapshots of your sample's condition. Comparing the intensity distributions, Z-average particle sizes, and polydispersity of a specific sample at 3 temperatures provides sample quality information below, near, and above T_{size} (Figure 4). Below T_{size}, the antibody sample was relatively monodisperse, but as the temperature increased and the antibody unfolded both the Z-average diameter and PDI increased. At higher temperatures the antibody appears to have significant aggregation and an average particle size more than 40x the initial diameter.

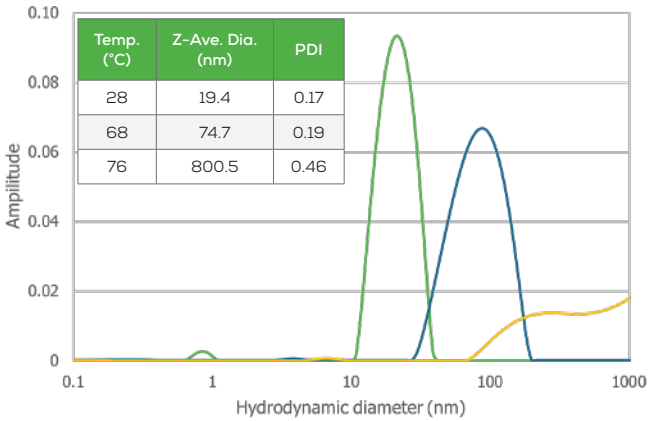

Figure 4: Intensity distributions of human IgG in PBS with 250 mM arginine at 28 °C (green), 68 °C (blue), and 76 °C (yellow). Average particle size and polydispersity index at each temperature (inset table).

Conclusion

Uncle's newest software makes the Sizing with Thermal Ramp application pack a punch with new tools to read faster, report more metrics, and give you better insight into each DLS read. Protein stability metrics such as T_{size} and T_{agg} 660 can be collected in a single experiment to more fully characterize a protein. Along with its world class DLS data, Uncle still has all of the fluorescence-based applications – offering all the best stability tools in one package. More DLS data and the new T_{size} and T_{agg} 660 metrics make Sizing with Thermal Ramp a powerful and orthogonal technique to Uncle's fluorescence applications when looking to evaluate protein stability and aggregation.

References

1. Role of arginine in the stabilization of proteins against aggregation, BM Baynes, et al., *Biochemistry*, 2005; 44(12):4919–25.

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