# High-throughput protein stability screens with Aunty

## Introduction

When it comes to protein stability testing, an easy-to-use sample holder is a critical element of the process. Aunty achieves high resolution, sensitivity, and automation readiness with a quartz 96-well plate, making it the fastest, highest throughput stability platform on the planet (Figure 1A). Its full spectrum differential scanning fluorimetry (DSF) lets you choose intrinsic or dye-based methods to determine a protein's melting temperatures. Static and dynamic light scattering (SLS & DLS) run in parallel to each other, and with fluorescence, to spot aggregates at any point of an experiment. The three methods work together with Aunty's one-of-a-kind quartz 96-well plate to give you the full protein stability picture of all the samples every minute and crank out actionable results at breakneck speeds.

96-well microplates are the workhorse of high-throughput assays. Aunty's SBS-format 96-well plate maximizes throughput and minimizes sample consumption, only needing 8 µL of sample per well (Figure 1B). These wells are etched into quartz, which means impeccable optical performance and chemical compatibility with all common biologics reagents. Sealing the plates after loading is easy and protects your samples from evaporation and contamination. Aunty can ramp the temperature from 15–95 °C at rates from 0.1–10 °C/minute or hold the samples at a temperature for hours or days. Thanks to the SBS-format plates with sample input ports in an 8 x 12 arrangement and an API to control experimental set up and operation, Aunty's entire process, from loading samples to running the software, is automation-ready. No matter how you design your experiments, Aunty yields unmatched data resolution without sacrificing experimental flexibility.

Heating up proteins in solution causes them to unfold, exposing the hydrophobic amino acids tryptophan and tyrosine to an aqueous environment. When aromatic amino acids transition from a hydrophobic environment to an aqueous one, they change their

A


B

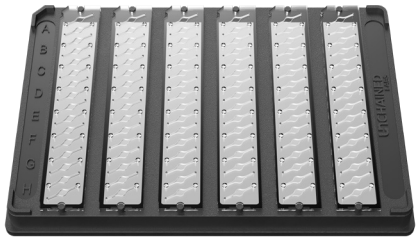

Figure 1: Aunty (**A**) is the world's only flexible, automation-friendly, fit-for-purpose stability platform that uses SBS-format quartz 96-well plates (**B**).

fluorescence behavior – typically the fluorescence maximum decreases and the peak shifts to higher wavelengths (Figure 2), a phenomenon referred to as a "red shift." Tracking the emission change over temperature in a nanoDSF experiment allows you to monitor a protein's conformational stability and determine a melting temperature ($T_m$). You can use this to rank order the conformational stability of different formulations, constructs, mutants, or even conjugates. Aunty's quartz plates, robust 280 nm excitation LED, and high-sensitivity spectrophotometer give it a wide dynamic range from 0.025–300 mg/mL IgG. Full spectrum DSF detection allows you to detect red or blue shifts of the protein's intrisic fluorescence (nanoDSF), plus the freedom to use reporter dyes for 'classic' DSF. You can also use Aunty in thermal shift assays.

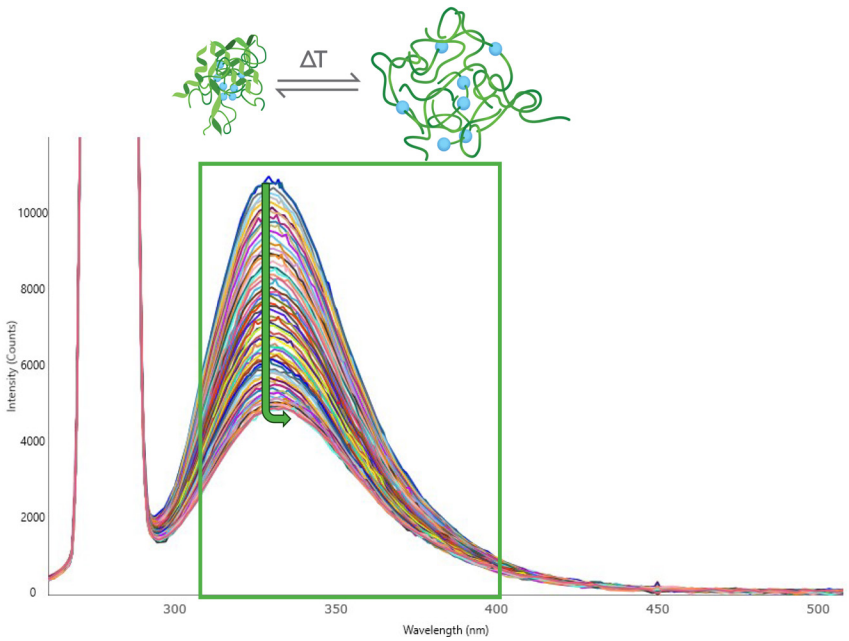


Figure 2: Aunty uses a 280 nm LED to excite intrinsic protein fluorescence. As proteins unfold due to heating, their intrinsic fluorescence (green box) typically decreases and shifts to longer wavelengths.

As protein molecules diffuse, the amount of light they scatter fluctuates (Figure 3). SLS looks at the overall average intensity of scattering and depends on the molecular weight and concentration of the protein particles. DLS, on the other hand, looks at how fast the intensity changes over time and helps determine the hydrodynamic size of particles, the z-average diameter, the polydispersity, and the distribution of particle sizes in the solution. When proteins aggregate, both the SLS intensity and z-average diameter increase. Aunty uses high-sensitivity optics to achieve a lower limit of detection for SLS of 50 µg/mL of IgG and for DLS of 0.1 mg/mL lysozyme.

This technical note shows how Aunty combines fluorescence, SLS, and DLS with high-quality quartz 96-well plates and intuitive data analysis software to perform high-throughput protein stability screens.

## Results

Parallel monitoring of protein unfolding and aggregation is the key to thermal stability screening of buffers, constructs, and excipients. Aunty's combination of fluorescence, SLS, and DLS detection in low volume 96-well plates lets you power through experiments to find the optimal protein formulation. Sometimes that means finding the right balance between different excipient properties. For example, arginine is a common excipient which limits aggregation but also drives protein unfolding.[1] Aunty lets you assess how much of an impact those different effects have on the stability of your antibody of interest. Aunty determined the $T_m$s of 1 mg/mL bovine IgG in PBS with and without 500 mM arginine from the peaks in the 1st derivative of the barycentric mean of the protein's intrinsic fluorescence (Figure 4). Adding arginine decreased the $T_m$ by 3.5 °C. The temperature of the onset of aggregation, $T_{agg}$, of bovine IgG with arginine was higher than that of bovine IgG in PBS alone (73.4 versus 68.5 °C). Side-by-side DLS measurements during the thermal ramp showed a temperature with a 2-fold increase from the baseline hydrodynamic diameter ($T_{size}$) of IgG in PBS at 70.2 °C. The $T_{size}$ of IgG with arginine was 72.7 °C. Aunty's plate-based stability screening readily showed that adding arginine delayed aggregation of this polyclonal antibody but accelerated its unfolding. Overlaying the graphs lets you compare results directly and draw the right conclusions regarding stability of mutants, modifications, conjugations, or conditions.

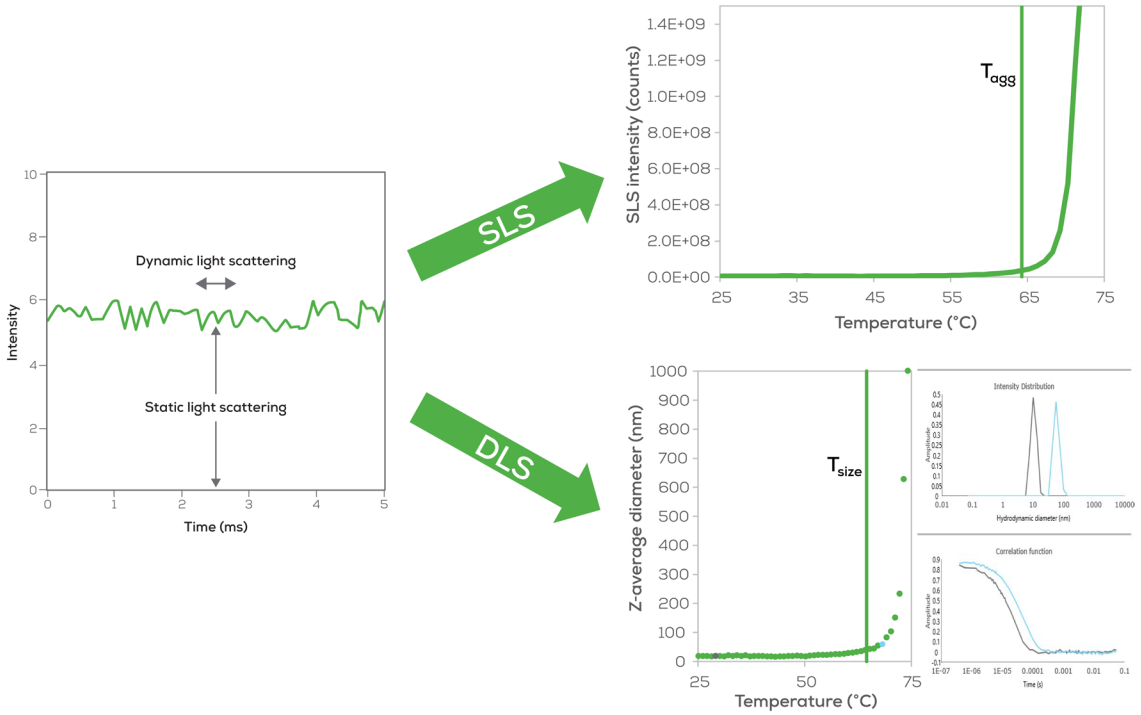


Figure 3: The light scattering of particles diffusing through a solution fluctuates over time. SLS is the average of these intensity fluctuations; DLS looks at how fast the scattered light intensity changes over time. Aunty puts both to use side-by-side in a thermal ramp to, for example, detect the onset of aggregation by SLS and the change in size of particles using DLS.

Maximizing throughput while minimizing sample consumption are major goals of biologics characterization, but so are high levels of accuracy and precision. The $T_m$s of 96 replicates of 1 mg/mL bovine IgG in PBS determined on Aunty had an average of 69.6 °C with a CV of 0.4% (Figure 5). Simultaneously collected $T_{agg}$s had an average of 4.8 °C with a CV of 0.5%. Reproducibility is no sweat with Aunty; its $T_m$s have CVs <1% and it heats with temperature accuracy ±0.1 °C.

Identifying stable biologics and formulations with Aunty is a snap. When you use Aunty's Analysis software to graph $T_m$ versus $T_{agg}$ you can immediately tell which samples tend to have good conformational stability, colloidal stability, or both.

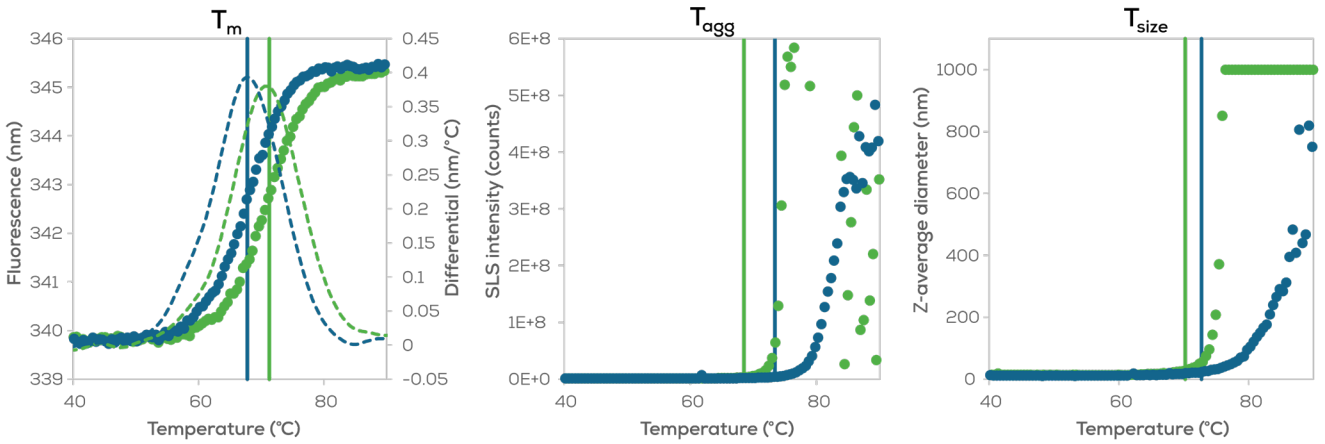


Figure 4: 1 mg/mL bovine IgG in PBS (green) or in PBS with 500 mM arginine (blue) were heated from 25–95 °C at a rate of 1 °C/minute. Fluorescence (left), SLS (middle), and DLS (right) measurements were taken every 30 seconds using Aunty's $T_m$ & $T_{agg}$ including sizing app. $T_m$s, $T_{agg}$s, and $T_{size}$s (vertical lines) were assigned based on peaks in the fluorescence differential (dashed lines) and at sustained increases in SLS intensity and z-average diameter, respectively. Graphs are representative of triplicates.

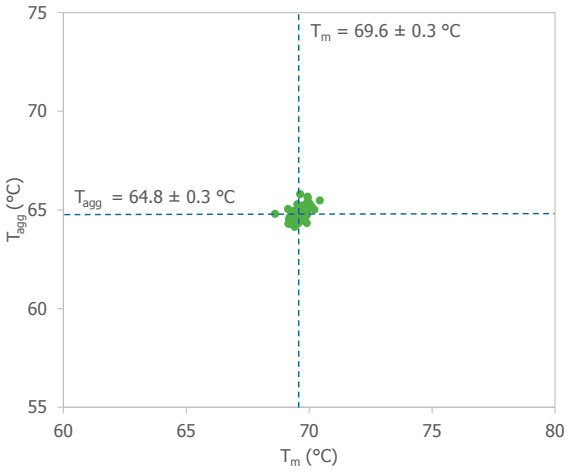


Figure 5: 96 replicates of 1 mg/mL bovine IgG were heated in an Aunty plate from 25–95 °C at a rate of 1 °C/minute. Fluorescence and SLS measurements were taken every minute using Aunty's $T_m$ & $T_{agg}$ app. The $T_m$ & $T_{agg}$ of each replicate is shown.

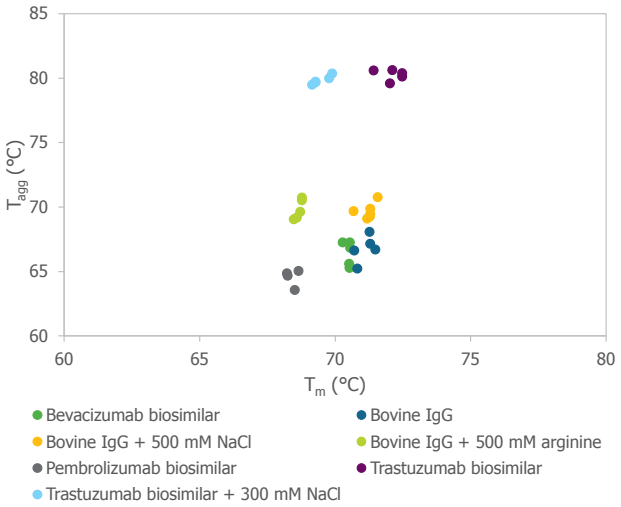


Figure 6: 1 mg/mL antibodies in PBS with the indicated excipients were heated in an Aunty plate from 25–95 °C at a rate of 1 °C/minute. Fluorescence and SLS measurements were taken using Aunty's $T_m$ & $T_{agg}$ app. The $T_m$ & $T_{agg}$ of each replicate is shown.

You can also see the spread between the replicates of each sample, giving you confidence that you're making the right call.

7 combinations of 1 mg/mL antibodies in PBS with various excipients were run in a single $T_m$ & $T_{agg}$ experiment with 4–8 replicates and default settings (Figure 6). A trastuzumab biosimilar in PBS without excipients (purple) had the highest $T_m$ and $T_{agg}$ while a pembrolizumab biosimilar in PBS (gray) had the lowest. Adding NaCl to the trastuzumab biosimilar (light blue) had little impact on its $T_{agg}$ but decreased its $T_m$ by 2.6 °C. NaCl is, therefore, most likely an inappropriate excipient for trastuzumab. However, adding NaCl to bovine IgG in PBS (yellow) did not significantly change its $T_m$ but increased its $T_{agg}$ by 2.8 °C, relative to PBS alone (dark blue). Unlike trastuzumab, NaCl may stabilize this bovine IgG. Any biologic will have its own formulation preference and Aunty's all-quartz 96-well plate makes it the fastest, highest throughput tool to find this individual sweet spot.

## Conclusion

Aunty is the Queen of protein stability with the combination of full spectrum fluorescence, high sensitivity SLS and DLS, precise thermal control, and a 96-well SBS-format plate. Aunty maximizes your throughput while minimizing sample consumption to just 8 µL per well. Fast read rates and unprecedented data resolution let you see the full stability picture of your candidate. All 3 detection methods can run together or separately, on one sample or many, so experiments can be tailor-fit to your needs. Temperature measurements are highly reproducible with $T_m$ CVs <1% and temperature accuracy ±0.1 °C. By combining $T_m$, $T_{agg}$, and $T_{size}$, you'll blast through any number of replicates and quickly recognize the samples with optimal stability profiles to accelerate developability and formulation screening. All this reduces the time it takes to bring novel, life-saving therapies to the clinic.

## References

1 Protein-excipient interactions: Mechanisms and biophysical characterization applied to protein formulation development. TJ Kamerzell, et al. Advanced Drug Delivery Reviews. 2011; 63(13):1118–1159.

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Rev B