Use of self-interaction parameters derived from light scattering experiments for better developability assessment of biologic therapeutics

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Abstract

Self-interaction of therapeutic proteins has an impact on the ultimate success of a biologic candidate. During the development process, candidates are often assayed at concentrations that differ from that of their final delivery form. Understanding how therapeutic proteins behave at higher concentrations is crucial for ensuring their ultimate success in the clinic. Dynamic (DLS) and static (SLS) light scattering techniques help researchers determine and optimize the self-interaction behavior of protein-based therapeutics ahead of scale-up and manufacture. Here we show how the Prometheus Panta measures self-interaction of proteins and allows researchers to prioritize candidates and improve outcomes.

Introduction

Light-scattering techniques give researchers important insight into the biophysical characteristics of their therapeutic protein candidates. One example that is of concern to researchers is **self-interaction**. Therapeutic proteins are often delivered to patients at much higher concentrations than seen during the developmental process, and this can lead to promising candidates failing in the clinical stage after lengthy and costly development[1]. Researchers have small batches of candidates to use during development, and it is not until they pass a series of qualifying tests that they will enter into scale-up and ultimately be used at these higher concentrations. Because of this, researchers must have a way to use low amounts of material to assess how their protein therapeutics will behave once they are at their clinical concentration.

There are many parameters researchers must evaluate their candidates on prior to reaching pre-clinical testing stages[2]. Dynamic light scattering (DLS) and static light scattering (SLS) offer important information about size and dispersity of particles in solution. DLS allows researchers to measure the hydrodynamic radius of a particle as well as the distribution of particle sizes in a solution, and can be used to determine the **self-interaction parameter k_D** of a protein. SLS measures the average molecular weight in solution and can be used to find the **second virial coefficient B_{22}** of a candidate. Researchers aim to minimize the self-interaction of proteins in order to avoid aggregation and therefore effectiveness of candidates once they reach their scaled-up concentrations for the clinic. Together, these parameters inform researchers about their developmental candidates, and can be used to monitor self-interaction of a system and optimize it to avoid aggregation in the final scaled-up formulation.

The Prometheus Panta is a DLS-enabled instrument that uses nano-differential scanning fluorimetry (nanoDSF), backreflection (for turbidity, or large amorphous aggregates), and DLS to enable researchers to measure their candidate's biophysical characteristics using minimal sample consumption[3]. **Merck Darmstadt used the Panta to measure and rank the self-interaction parameters of several protein biologic candidates, and compared those rankings to that of other systems.** The data obtained from Panta agreed with that from other systems, and demonstrated exemplary linearity of measurement.

Methods

Panta DLS Screening

Five candidate molecules were prepared in several different buffer conditions, exhibiting a range of salt concentrations and pHs. Each molecule and buffer system was prepared at six concentrations, from 2.5 to 15 mg/ml. Triplicates of 10 ul each were run in the prototype early access program (EAP) Panta system on High Sensitivity mode. Values for k_Ds were determined using the beta (0.4) Panta Analysis software for EAP.

Experiments were run in duplicates on the Prometheus Panta (NanoTemper Technologies, GmbH) and Wyatt DynaProII (Wyatt Technologies Europe, GmbH; denoted as "plate reader") device to compare the k_Ds for each candidate. Results were compared for both the agreement of the k_D value obtained as well as the deviation between three replicates.

Panta light-scattering experiments

A bi-specific Fc-fusion was prepared in several buffer formulations and examined using the light scattering of the system in an EAP Panta. While EAP Panta was not yet calibrated for publication-standard SLS measurements, the scattering values obtained from the system could be used to provide rankings for SLS-deviation, similar to what was obtained from other systems.

Proteins were prepared as previously[1]; specifically in duplicate at 2.5 to 15 mg/ml in different formulation buffers. Overall scattering intensity was measured and plotted vs. concentration, and a linear regression line was fit. A high-concentration measurement of the same molecule and formulation was then measured at 40 mg/ml. Deviation of the 40 mg/ml measurement from the linear regression line was used as a parameter for ranking candidates. Deviation above the regression line (positive SLS deviation values) shows an increased propensity for aggregation; deviation below the linear regression line (negative SLS deviation values) indicates a lower propensity for aggregation, and therefore a good result for future development.

Experiments were run in duplicates on the Prometheus Panta, Wyatt DynaProII, and Avacta Analytical Optim[1] (Avacta Group plc, UK, denoted as "microcuvette") device to compare the deviation rankings for each candidate. The results were evaluated by the values obtained and the deviation between the duplicate measurements.

Results

DLS measurements to determine k_D

Candidates with positive k_D values indicate repulsive forces between molecules, and were considered a better formulation compared to those with negative k_D values in Figure 1.

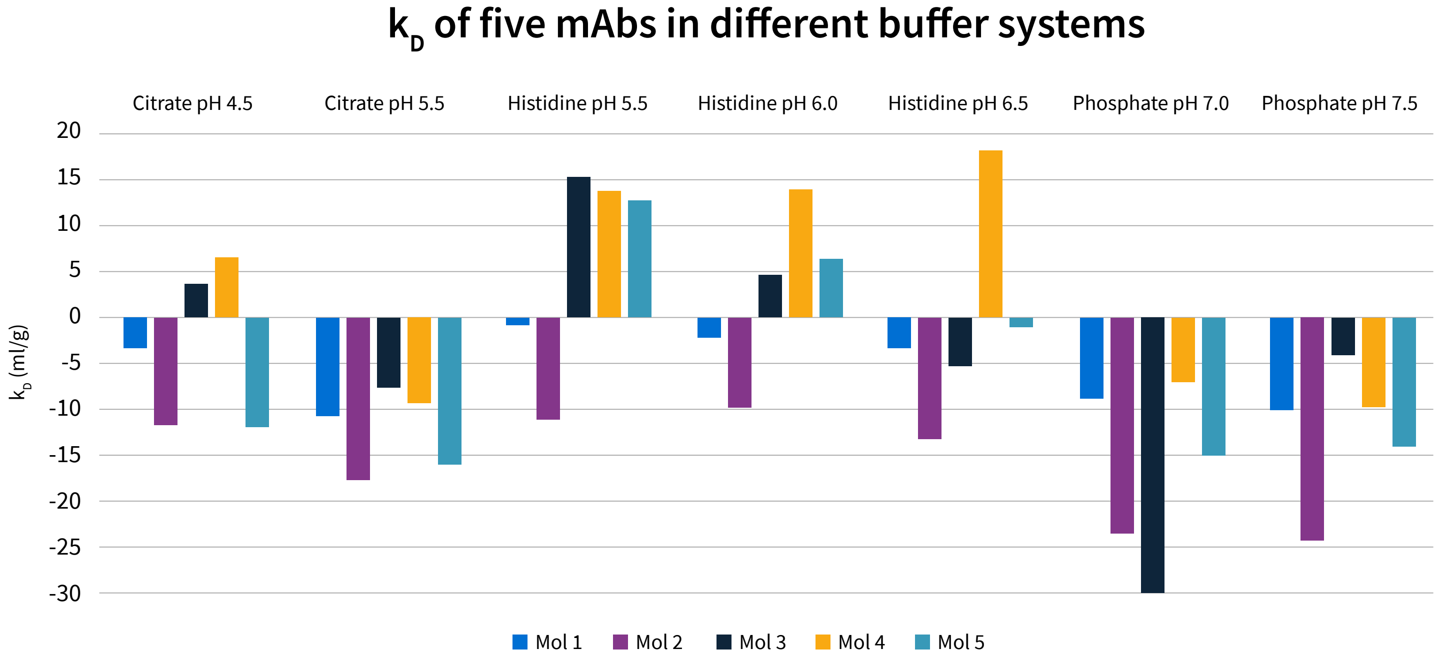


Figure 1: k_D values for five different candidates in seven different buffers. Candidates with positive k_D values are considered optimal for further development due to their repulsive forces, which reduce the chance for aggregation at higher concentrations.

Figure 1 shows that candidate molecules 1 and 2 exhibited negative k_D for all buffer systems. Histidine-based buffers showed the most success for molecules 3-5. This information allows not only the selection of the ideal molecule to move forward with during development, but also the optimal buffer conditions for storage and assays.

These molecules had been previously investigated for their self-interaction behavior using a plate-reading system. As the goal of the EAP project was to ensure that Panta gave accurate measurements that agree with current literature values, the k_D values measured on each system were compared. Figure 2 demonstrates that the results obtained from the Panta are in good agreement with those from previous plate reader experiments.

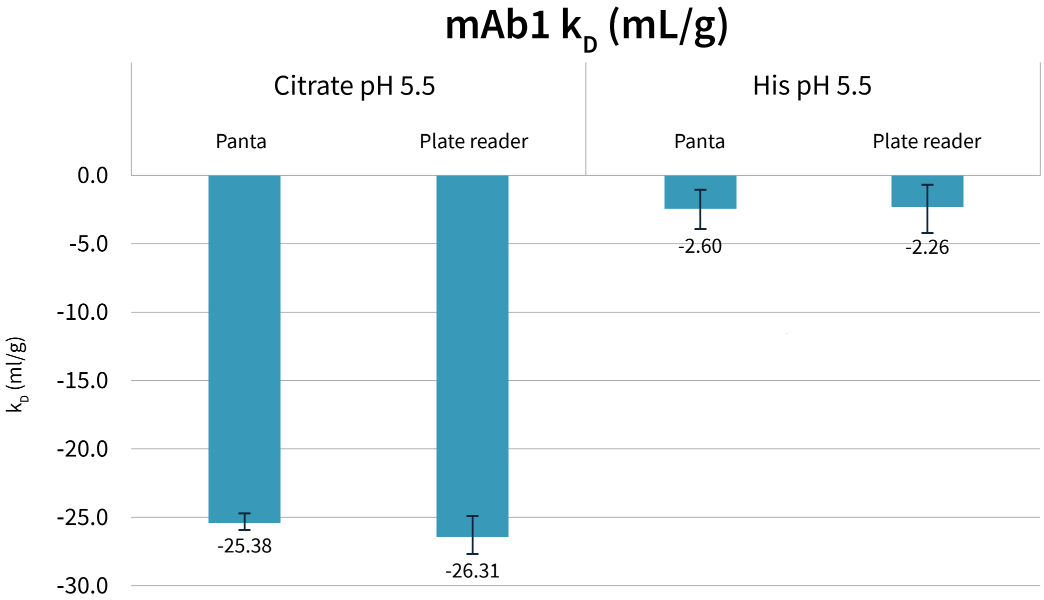


Figure 2: Representative comparison of DLS k_D values between Prometheus Panta (EAP) and a plate reader system. The values agree within standard deviation; the Panta demonstrated less variation between triplicates.

The k_D values obtained from Panta DLS quickly and efficiently show which candidates and formulations have optimal self-association values to decrease the potential for aggregation at higher concentrations. The values obtained from the Panta also line up well with previously published data, demonstrating how it can be reliably instituted into existing workflows.

Static light scattering experiments

Though the EAP Panta was not yet optimized for SLS experiments, research has demonstrated the importance of looking at the SLS deviation from linearity as a more reliable method for predicting aggregation behavior at high concentrations[1]. Using scattering intensity, it was possible to build a ranking system similar to previous SLS deviation experiments, and compare those to data obtained from other systems. Figure 3 shows the results of these experiments for a bispecific Fc-fusion protein[1].

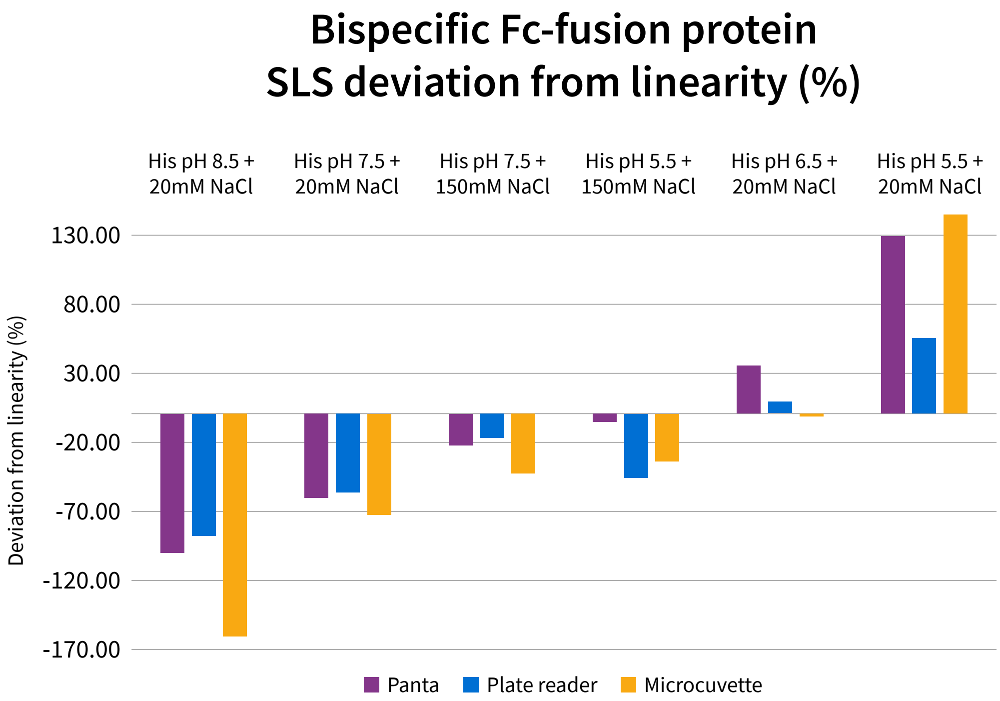


Figure 3: Systems that demonstrate a negative deviation from linearity via light scattering measurements are considered better candidates than those that exhibit positive deviation. The evaluated rankings of a bispecific Fc-fusion for each formulation are the same for all three instruments[1].

As research has shown, it is important to have an alternative method beyond k_D for evaluating self-interaction of candidate molecules[1]. **SLS deviation from linearity is a parameter that allows direct measurement of high-concentration samples in order to use this evaluation method as a predictive model for high-concentration scale-up.**

Prometheus Panta allows measurement of the deviation from linearity using an absolute measurement of scattered light. The EAP instrument used here was not yet calibrated for full SLS analysis and molecular weight measurements, but scattering results in Figure 4 show that the deviation from linearity for scattered light lines up well with other instruments in the field.

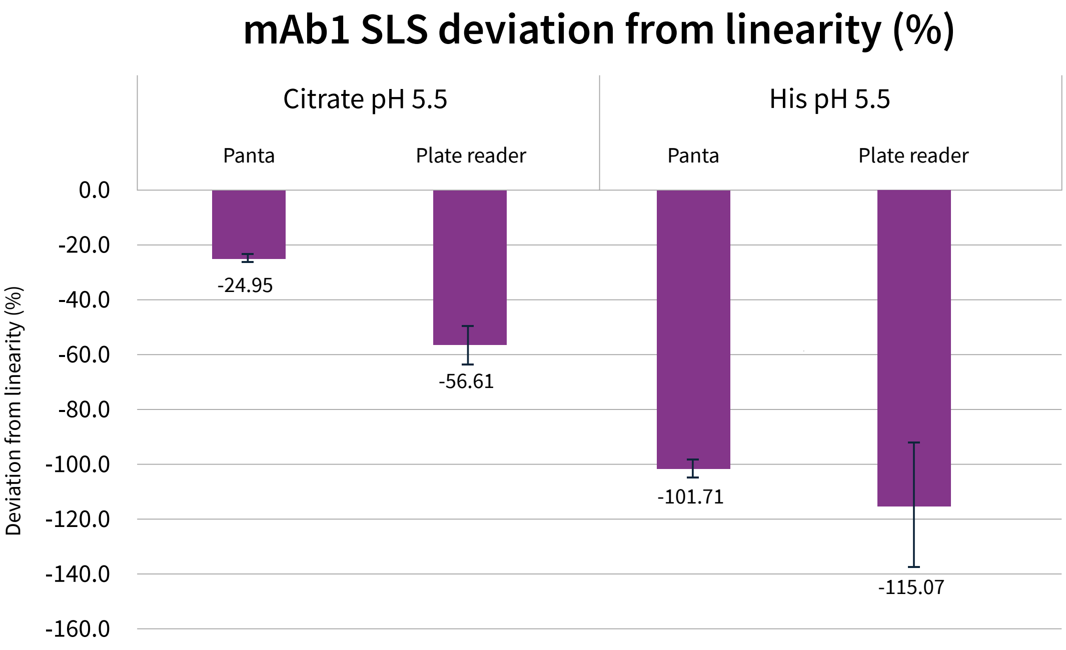
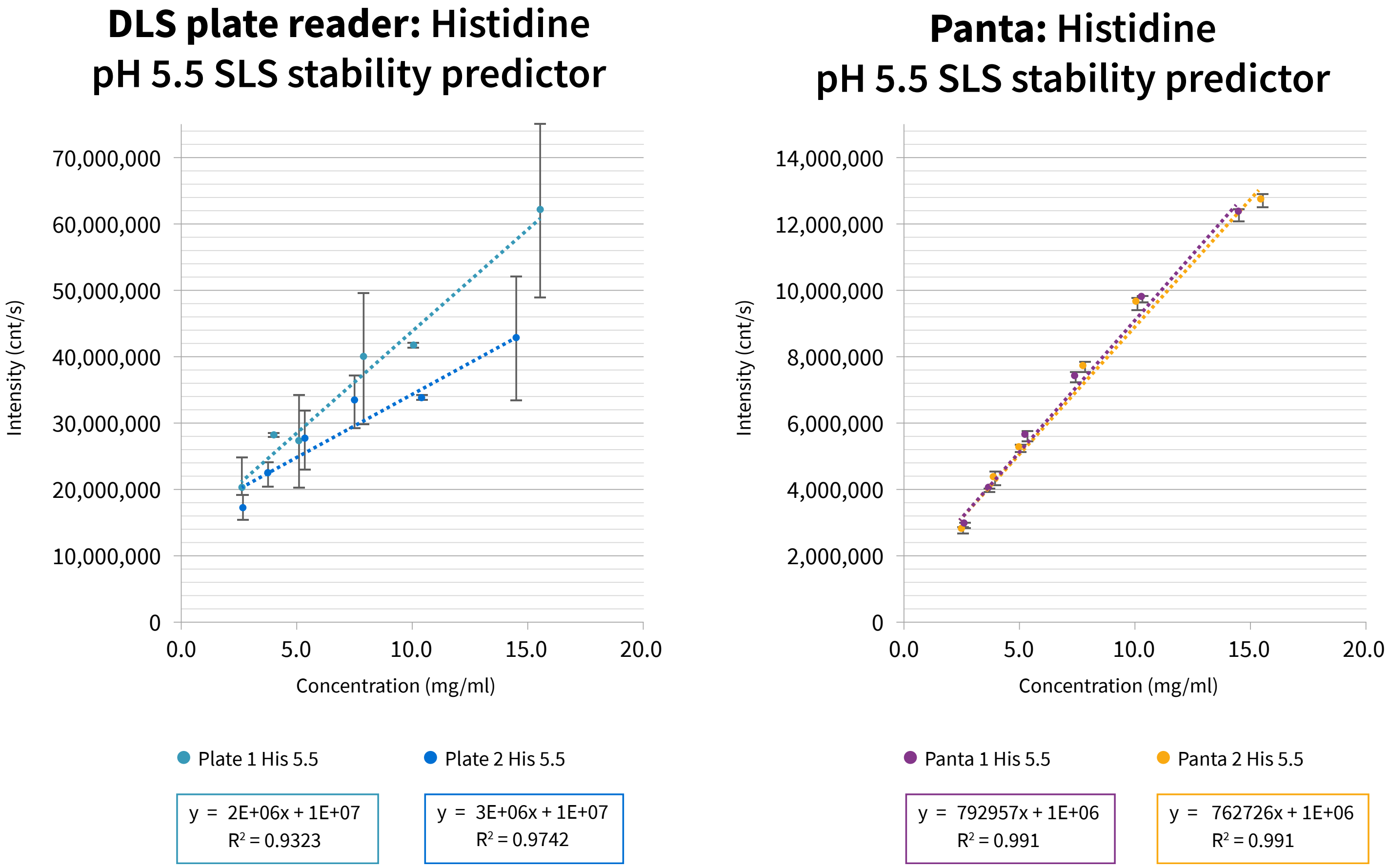


Figure 4: Comparison of SLS deviation from linearity for Prometheus Panta vs plate reader system. The Panta gave systematically lower values compared to the alternative, but with less standard deviation.



DLS plate reader: Histidine pH 5.5 SLS stability predictor

Panta: Histidine pH 5.5 SLS stability predictor

Figure 5: Example plots for determining deviation from linearity for plate reader (left) vs Panta (right). The Panta exhibits exemplary repeatability between measurements.

Though the Panta gave systematically lower SLS stability prediction values, it is also of note to examine the reproducbility between measurements. In Figure 5, we see that the linear regression lines from Panta demonstrate great agreement with each other, as well as exceedingly high R^2 values, indicating exemplary linear fits.

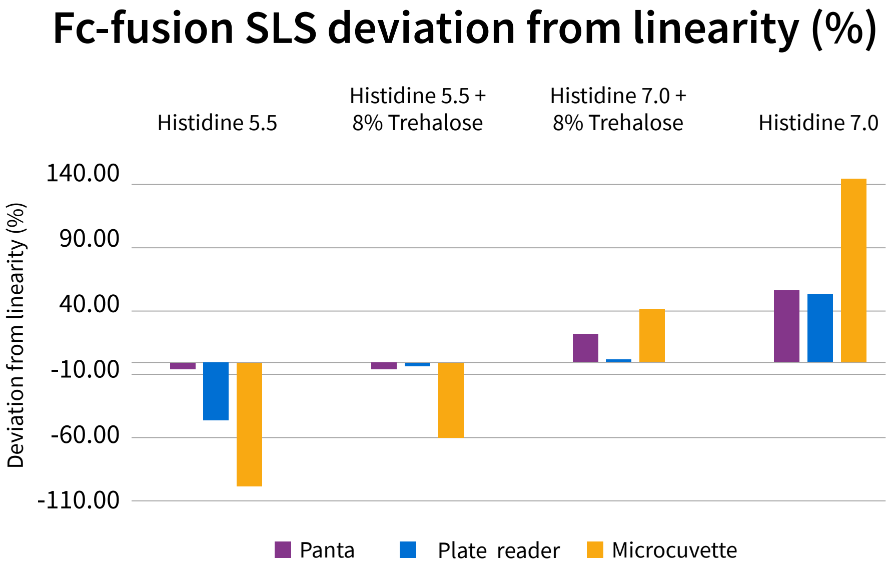


Figure 6: Systems that demonstrate a negative deviation from linearity via light scattering measurements are considered better candidates than those that exhibit positive deviation. The evaluated rankings of a bispecific Fc-fusion for each formulation are the same for all three instruments[1].

To further validate the use of the scatting data from the EAP Panta, researchers examined if the ranking of candidates was consistent for a different Fc-fusion construct. Figure 6 shows that for all sample types and buffer formulations the trends were the same, enabling the EAP Panta to be used for candidate profiling for scalability[1]. Further refinement of the SLS optics and algorithm will enable true SLS measurements in future Panta devices.

Together, these results demonstrate the importance of monitoring self-interaction of candidate protein molecules for better long-term success in the development of biologics[1].

Conclusions

Protein-protein interactions are a crucial aspect for the ultimate success of a protein-based therapeutic. Accurate predictive models of high-concentration behavior of biologic therapeutics are needed to improve candidate outcomes and market success. Current predictive models using DLS-derived k_D values or SLS-derived B_{22} values measured at low (<20 mg/ml) concentrations have been shown to be only moderately good predictors of high-concentration behavior and aggregation propensity.

Deviation from linearity using static light scattering or absolute scattering intensity is a reliable method for measuring high-concentration behavior, as it does a direct measurement with a highly concentrated sample. The ability to use low sample volume for this measurement is crucial in early development stages, when candidate sample is precious and scarce.

Here we have shown that the EAP (prototype) Prometheus Panta gives k_D values that agree well with previous data. Furthermore, while the SLS capabilities of the EAP Panta were not fully developed, the raw intensity scattering from the instrumentation allows for measurements parallel to the SLS deviation from linearity. These results also resulted in the same ranking selections as other instruments in the field. **Additionally, the Panta demonstrated exemplary repeatability between measurements, and therefore lower standard deviations between replicates.**

Both the DLS and impending SLS capabilities of the Panta showcase the importance of a full biophysical profile of candidate molecules, and exhibit how low-volume, low-concentration measurements can be used to predict behaviour in higher concentration systems. This is a benefit to researchers, especially those in early development, who are often working with limited sample quantities for all of their assays.

An added benefit of the Prometheus Panta system not addressed here is its ability to measure thermal denaturation and turbidity. Furthermore, DLS measurements can be run in parallel along the same temperature denaturation ramp, giving additional stability parameters such as T_{size} (the temperature at which a protein begins to increase in size) and $T_{scattering}$ (the temperature at which scattering intensity, and therefore size, begins to increase.) **The Panta gave additional insight that most of the candidate molecules do not show a major increase in turbidity, but there is a given temperature at which T_{size} begins to increase, indicating soluble aggregation (data not shown).**

Merck Healthcare KGaA Darmstadt used the EAP Prometheus Panta to make precise measurements on their biologic samples. The information obtained from the Panta allowed selection of an ideal candidate, as well as the ideal buffer formulation to work in with their candidates. These results show that the Prometheus Panta gives exemplary, reproducible data that corroborates previously obtained ranking parameters.

References

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