Knock out same-time protein concentration and quality with Stunner

Introduction

Sensitive, quick methods for evaluating protein quality prior to extended characterization studies or assay development save time and precious sample. Uncover more about your samples with Stunner (Figure 1), which combines high-speed UV/Vis analysis with dynamic light scattering (DLS) to measure the concentration and quality of your biologics. Stunner has a wide dynamic range, measuring proteins from 0.02–200 mg/mL, and applies a wavelength-specific correction that provides more accurate values. At the same time concentration is measured, Stunner uses DLS to measure the hydrodynamic diameter of your samples and identify whether aggregates might give cause for concern.

Figure 1: Stunner: the first and only platform to combine protein quantification and particle sizing.

Stunner uses 2 µL of sample and measures up to 96 samples in just one hour. The micro-volume plate keeps your sample protected from dust, and there's no risk of cross-contamination or evaporation. Measure highly concentrated samples so you can stop wasting time with manual dilutions. Pipet your samples directly into the Stunner plate or use a liquid handler to automate the whole workflow. Add a 21 CFR Part 11 package if you need to nail down compliance. Stunner knocks sample quantity and quality measurements out of the park.

This application note describes how to use the Sizing & Polydispersity application on Stunner. This application is specifically designed to measure protein concentration by UV/Vis and particle size distribution by DLS in a single experiment.

App selection

In Stunner Client software, the Sizing & Polydispersity application can be found by selecting the Protein UV/Vis & DLS button on the home screen (Figure 2). During experimental setup, the DLS settings can be customized, which includes the number of acquisitions collected and the acquisition time. For the UV/Vis measurements, multiple blanks can be measured on one plate in a single experiment. The user can also define sample groups, which enables matching a set of samples with their corresponding blanks. Analytes and buffers are assigned to each sample group to calculate accurate protein concentrations and particle sizes. New analytes and buffers can be created, edited and saved at any point. Stored parameters include E1%, molecular weight, and RI increment values for analytes, along with the refractive index and viscosity values for buffers. The Stunner Plate, equipped with two micro-cuvettes with pathlengths of 0.1 and 0.7 mm, is loaded with 2 µL of each sample and read on Stunner.

Methods

Sample preparation

Nine different proteins spanning a range of sizes (RNase A, IgG, Ovalbumin, Conalbumin, Aldolase, Thyroglobulin, Insulin, Lysozyme, and BSA) were purchased from commercial sources. Protein solutions were prepared by weighing out and

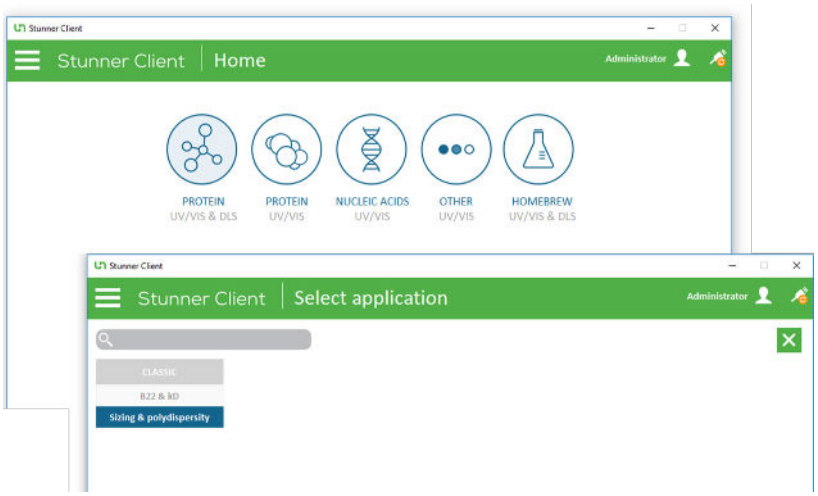

Figure 2: Selection of the Sizing & Polydispersity application in Stunner Client software.

reconstituting lyophilized powder to reach a target concentration of 5 or 10 mg/mL in phosphate buffered saline (PBS), pH 7.4. Two µL of each sample was loaded in octuplicate into a Stunner plate and measured with UV/Vis and DLS, with 4 DLS acquisitions of 5 seconds each. Each protein was entered in as a new analyte, using the appropriate MW (g/mol) and E1% values for each.

Concentration with sizing & polydispersity

With the combination of UV/Vis and DLS, Stunner takes the guess-work out of deciding whether your protein is still good to go or has aggregated. Whether quantifying low or high protein concentrations, the Stunner plate can handle it, measuring samples with viscosity values up to 40 cP. Solution turbidity caused by aggregation is also accounted for with a wavelength-specific background correction (Rayleigh scattering) applied directly to A280 measurements in Stunner software.

DLS is a highly sensitive technique used to verify the expected size and polydispersity of proteins or other macromolecules in solution. Meeting industry standards, Stunner measures a hydrodynamic diameter range from 0.3 to 1000 nm and can detect small proteins like lysozyme down to 0.1 mg/mL, and larger proteins at even lower concentrations. Stunner helps uncover the presence of aggregates or contaminants in your sample that would be missed by UV/Vis detection or other quantification methods alone. The polydispersity index (PDI) is a unitless parameter that indicates the level of heterogeneity of particle diameters in the sample. Non-uniform particle sizes in solution give a high PDI, while uniform particle sizes yield a low PDI. In a single experiment, get the concentration of your samples by UV/Vis and quality information (e.g. hydrodynamic diameter and polydispersity values) by DLS.

Results

Plate overview

In Stunner Analysis software, the absorbance spectra, intensity and mass distribution data from the whole plate can be immediately viewed in the Overview tab (Figure 3). Toggling between these modes provides a quick way to weigh in on the protein concentration, average hydrodynamic diameter, and extent of aggregation of each sample. Clicking on an individual sample will open the detailed reporting of the sample measurement in the Details tab.

Detailed reporting

The UV/Vis and DLS results for each sample can be viewed in the Details tab in Stunner Analysis software (Figure 4). The user can toggle between the Absorbance, Correlation function, and Intensity and mass distribution curves to expand the graphs. Replicates can be overlaid by clicking the sample name or sample group in the list on the

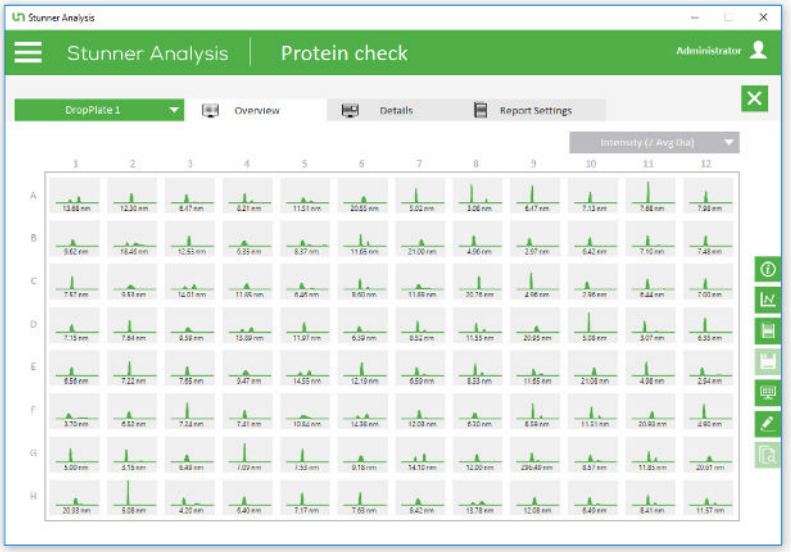

Figure 3: Stunner Analysis software is streamlined, making it easy to toggle between the absorbance spectrum, intensity and mass distribution graphs. In the intensity view mode shown here, the Z. Ave. Diameter is reported below each graph.

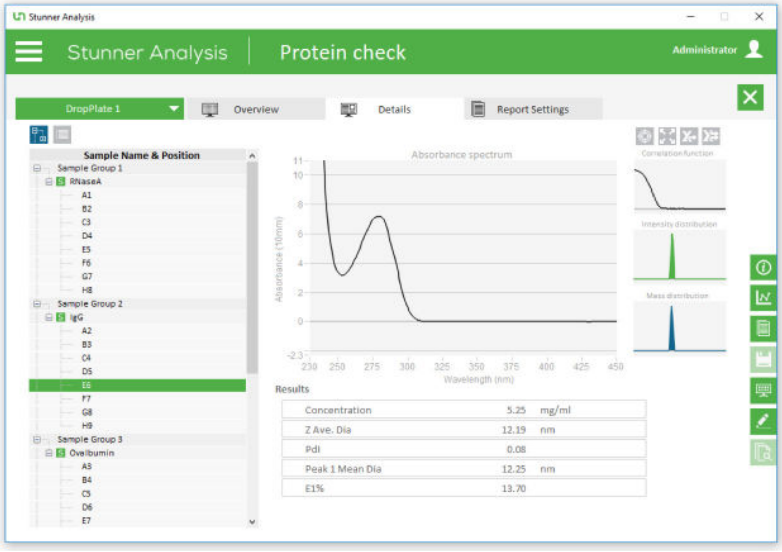

Figure 4: Stunner gives the whole story by reporting the protein concentration and particle sizing results, in addition to showing the full UV/Vis spectrum and DLS curves.

right of the screen. The Z. Ave. Diameter, polydispersity index, and peak mean diameters are reported in the results table, at the bottom of the screen.

The Sizing & Polydispersity application measures the UV/Vis spectrum and DLS data from the sample, and reports the following:

- **Absorbance spectrum**: The concentration is calculated using the A280 peak value and divided by the weight coefficient, E1% ($L{\cdot}g^{-1}cm^{-1}$), which can be measured directly by obtaining the absorbance value of a 1% solution of the same purified protein. Alternatively, to convert to E1% from the theoretical molar extinction coefficient, ε ($L{\cdot}mol^{-1}cm^{-1}$), use the following equation, where MW is the molecular weight (g/mol) of the protein:

$$E1\% = \frac{10 \times \varepsilon}{MW}$$

- **Correlation function**: Describes the overall DLS data quality
- **Z. Ave. Diameter**: Gives the average hydrodynamic diameter of particles in the whole sample
- **Polydispersity Index (PDI)**: Describes the heterogeneity of particle sizes in the whole sample.

  - PDI values < 0.1 indicate highly monodisperse samples.
  - PDI values from 0.1 to 0.2 indicate intermediate samples, for which some aggregates may be present.
  - For highly polydisperse samples containing significant aggregation, PDI values are typically > 0.2.
- **Intensity distribution** (shown in green): Gives the relative scattering of each peak
- **Mass distribution** (shown in blue): Gives the relative proportion of each peak

Reports

A variety of report types are generated for exporting and visualizing the data: HTML, XML, TXT, XLSX, PDF and CSV files. In addition, the Stunner platform enables customization of reports with an assortment of content to select from.

Case Study

To perform quick checks of nine different protein stocks with a range of expected sizes and quality, samples were measured and analyzed on Stunner. In just under one hour, protein concentration, hydrodynamic diameter, and polydispersity index measurements were obtained for 8 replicates of each protein, using 2 µL of sample in each well (Table 1). The exact protein concentrations were measured, which matched with expected concentrations near 5 or 10 mg/mL. The DLS results indicate the presence of both small and large particle sizes in solution for 7 of the 9 protein samples, which is consistent with a polydisperse size distribution in the range of 0.1-0.4. The high polydispersity index (PDI > 0.15) for RNase A, conalbumin, and aldolase shows that these protein solutions contain significant aggregation. In contrast, BSA and insulin, and to a lesser degree, IgG, Lysozyme, and Thyroglobulin, are fairly monodisperse, as indicated by a relatively low polydispersity index (PDI < 0.15). Since there are multiple peaks in the intensity distribution graphs, the Peak 1 mean diameter is reported to identify the particle diameter of the monomer in each sample.

The intensity and mass distribution graphs for two proteins at similar concentrations, BSA and RNase A, are shown in Figure 5. The low PDI value and the presence of a single peak in the intensity distribution for BSA indicates that the sample is fairly monodisperse. On the other hand, the high PDI value and the multiple peaks in the intensity distribution graph for RNase A indicates that the sample contains some aggregates. The relative intensities for RNase A are reported as 33.4 % for peak 1 and 66.6 % for peak 2. The mass distribution graph for RNase A shows that the sample contains mostly monomer by mass, with a small relative population (~0.5 %) of aggregates. These results demonstrate Stunner is sensitive and picks up large differences of particle sizes in solution.

Protein sample	E1% used	MW (kDa)	Conc (mg/mL)	Peak 1 Mean Dia (nm)	PDI
Insulin	9.87	5.80	5.21 ± 0.02	5.20	0.04
BSA	6.67	66.5	10.07 ± 0.04	8.00	0.07
IgG	13.7	150	5.28 ± 0.02	13.06	0.12
Thyroglobulin	11.2	660	4.80 ± 0.06	22.91	0.13
Lysozyme	26.4	14.6	10.07 ± 0.08	3.20	0.14
Ovalbumin	7.52	42.7	5.14 ± 0.01	6.85	0.16
Conalbumin	11.9	76.0	5.00 ± 0.01	7.98	0.22
Aldolase	8.91	36.0	4.61 ± 0.01	10.45	0.25
RNase A	7.22	13.7	9.58 ± 0.05	4.41	0.33

Table 1: Sizing & Polydispersity results for nine different proteins. The average values of eight replicates are listed for protein concentration, peak 1 mean diameter, and polydispersity measurements.

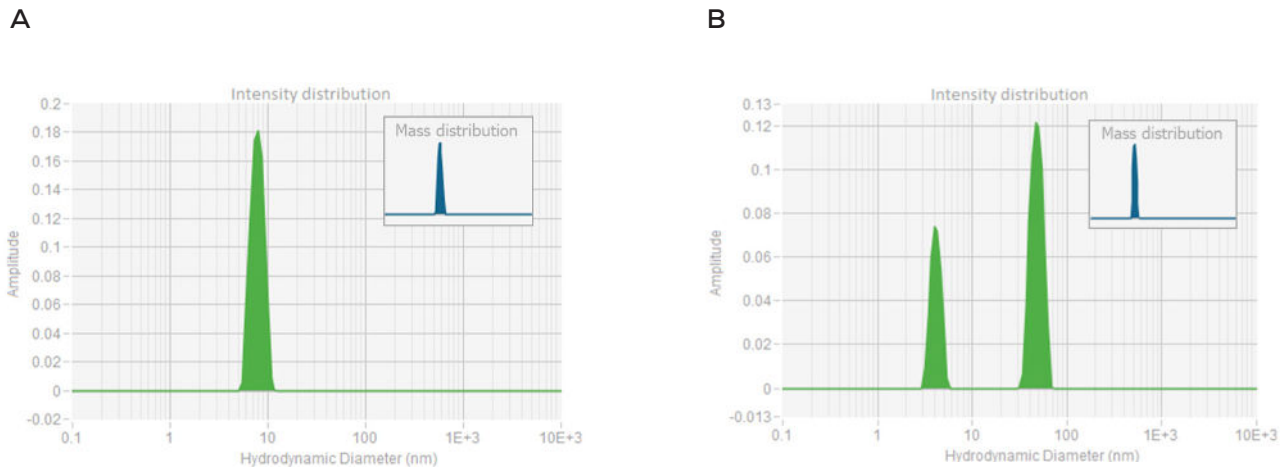

Figure 5: Comparison of BSA and RNAse A, showing the intensity distribution graphs with inset mass distribution graphs. **A:** BSA is monodisperse, indicated by a single peak with a low PDI value. **B:** RNase A is a polydisperse sample, as shown by the presence of two peaks in the intensity distribution and a large PDI value.

Conclusion

Combining high-speed UV/Vis spectral analysis with cutting-edge DLS, Stunner is your ticket for quantifying proteins and performing stability measurements at the same time. Using just 2 µL, Stunner accurately measures even highly concentrated samples, without the need for dilutions and zero clean-up. Measure up to 96 samples in an hour to get the full picture. Stunner is the first and only system that performs simultaneous quantification and particle size distribution, so you can get the info you need in a snap.

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