

DynaPro[®] Plate Reader III

Automated dynamic *and* static light scattering in microwell plates for fast, productive development of biologics

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Introduction

Dynamic Light Scattering (DLS) is an important tool in the research and development of proteins, nanoparticles, colloids and macromolecules with sizes ranging from less than one nanometer up to a few micrometers. Where high-throughput analysis and screening of biologics is concerned, Wyatt's DynaPro Plate Reader line of in-plate dynamic light scattering instruments has become the *de facto* standard, relied upon by drug product development scientists across the globe for assessing developability, pre-formulation, aggregation and stability. The DynaPro Plate Reader III (PRIII) is the latest generation of this instrument, offering new capabilities that extend its value even further as the leading screening tool for size and stability.

For development of therapeutic proteins, monoclonal antibodies, antibody-drug conjugates, virus-like particles, vaccines and biosimilars, the PRIII measures multiple essential biophysical properties, simultaneously, robustly and conveniently:

- Size and size distributions
- Molar mass
- Polydispersity
- % large aggregates
- Colloidal stability: k_D , A_2 and their temperature dependence
- Thermal stability: T_m/T_{onset} (unfolding) and T_{agg} (aggregation)
- Solubility
- Viscosity

Beyond biophysical characterization, the DynaPro Plate Reader III also gives a bigger picture: it takes a photo of every well to help identify macroscopic instabilities and provide

measurement diagnostics. Simply put, it gives a comprehensive profile of protein properties and behavior.

One instrument, so much information...



Figure 1. The DynaPro Plate Reader III measures dynamic and static light scattering *in situ* in standard microwell plates.

Who needs a DLS Plate Reader?

DLS measurements are carried out in solution or suspension, quickly and with a small amount of material, to help assess properties such as size, aggregation, stability and purity. However, conventional DLS instruments suffer from one big drawback: manual sample loading and “one-at-a-time” measurements.

Using either disposable or quartz cuvettes, traditional DLS requires significant operator time to make just a few measurements, even when the actual data acquisition only takes a few minutes. Because of this, scientists are deterred from full and proper utilization of DLS, which should include acquisition of multiple repeats, replicate samples, controls and many different conditions. The scientist—and the science—get bogged down. Luckily, an elegant solution is at hand.

The DynaPro Plate Reader is an *automated* DLS instrument that performs measurements on samples in standard 96, 384, or 1536 well plates. Instead of manually replacing each cuvette, just load the plate, place it in the PRIII, click 'Go' and walk away.

Hundreds of pharmaceutical, protein and nanoparticle development scientists have used previous generations of this instrument—the DynaPro Plate Reader Plus and its successor, the DynaPro Plate Reader II—to increase productivity by an order of magnitude or more. They implemented high-content DLS workflows that had been considered impractical until the advent of the DLS plate reader. Biotherapeutic formulation and stability screens, nanoparticle process development and optimization of crystallization buffers have all benefited from the DynaPro DLS Plate Reader.

The [DynaPro Plate Reader III](#) (DynaPro PRIII) is the latest generation in the DynaPro product line. The DynaPro PRIII incorporates multiple technological advances to provide additional, valuable functionality. Perhaps the most significant new feature is in-plate measurement of solution weight-averaged molar mass (M_w) and second virial coefficient (A_2 or B_{22}) by means of static light scattering.

Imagine a Quantum Leap

Science experiences quantum leaps when the automation of previously slow and tedious processes leads to qualitatively new research. From massively parallel computing, to screening of compound libraries in drug discovery, to PCR and genome-sequencing, the benefits of high-throughput automation extend well beyond simply saving time. With high throughput, you can imagine—and carry out—novel studies previously inaccessible to most labs (unless they happened to have bench after bench of “one-at-a-time” instruments attended by legions of technicians).

The DynaPro PRIII gives you the opportunity to dream big dreams for your DLS measurements. Acquire in one day the data that might otherwise take weeks. Test hundreds of samples and thousands of buffer/excipient/temperature conditions with no more effort than that required to load a single microwell plate. Analyze and visualize the entire dataset in one fell swoop using SpectralView™, the heatmap generator in [DYNAMICS®](#) software, then zoom in for a detailed study of the most promising conditions.

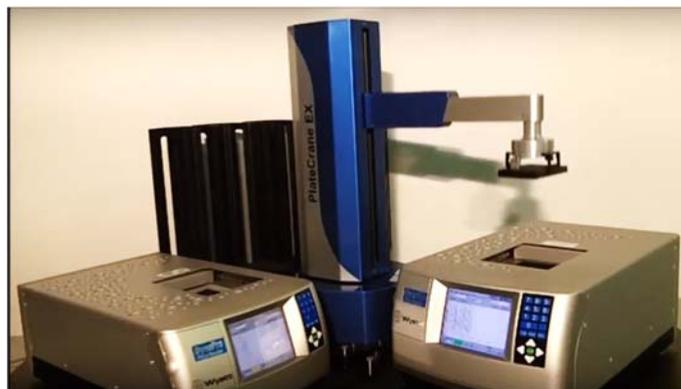


Figure 2. Loading and unloading can be automated, as shown here with a Hudson Plate Crane. The DYNAMICS API provides tools for controlling the instrument and transferring results to external software.

Integrate with robotic liquid handling for even larger-scale automation. The DYNAMICS API provides tools to integrate plate handling, data acquisition, temperature control and additional PRIII features into your fully-automated, plate-based workflow that incorporates liquid handling, sample prep and loading, and other analytical techniques.

Quantity without sacrificing quality

In the DynaPro Plate Reader III, all measurements are made *in situ* using industry-standard microwell plates from Corning, Greiner and other vendors. With no sample handling necessary (beyond pipetting into the plates) there is no fear of cross-contamination, and huge time savings are realized.

To further extend the analysis, the plates may be transferred seamlessly to spectroscopic plate readers or chromatographic well-Δplate samplers for additional analyses.

Fortunately, automation and high-throughput do not come at the expense of data quality. Even using inexpensive, disposable plates, the sensitivity and robustness engineered into the instrument by Wyatt's uncompromising R&D team are similar to those achieved by other DLS instruments with precision-fabricated (*i.e.*, expensive) quartz cuvettes, and certainly better by far than measurements in expensive capillary arrays.

You can collect more data, in less time, with less labor, and with greater accuracy; with the ability to incorporate multiple replicates effortlessly into every experiment, you can also obtain robust statistical analyses.

Under the hood

Dynamic vs. static light scattering

'Dynamic' and 'static' are technical terms loaded with cultural significance. We all want to be seen as 'dynamic' individuals, agile and forward-looking; nobody wants to be classified as 'static', sedentary and stuck in place. Yet the positive or negative connotations of these words do not hold up in the context of light scattering; *both* dynamic and static light scattering are powerful analytical techniques. The two forms of light scattering measure *different* properties of macromolecules and particles, and combine synergistically to build a fuller understanding of the sample's quality and viability for research or therapeutic use.

Weighing molecules with light

Static light scattering (SLS) provides two key pieces of information about macromolecules and nanoparticles: molar mass and size. Amazingly, it does so from first principles and so is a very sound and robust technique.

An SLS measurements simply consists of illuminating a solution with a laser beam and measuring the intensity of light scattered from the solution relative to the illumination intensity. As long as the particles are much smaller than the wavelength of the illuminating laser (radius less than ~12-15 nm for the PIII), the analysis is quite straightforward: the molar mass is obtained from the ratio of scattered intensity to analyte concentration. If more than one species is present in solution, the calculation provides the weight-averaged molar mass M_w .

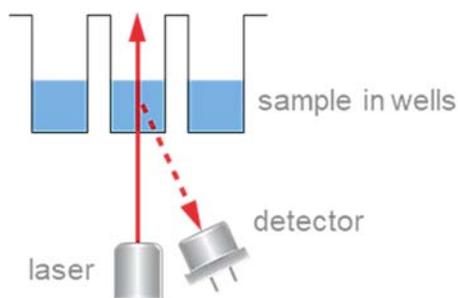


Figure 3. For both static and dynamic light scattering in a well plate, laser illumination and detection take place from below.

For larger particles, the light scattering signals vary with angle so a correction to the SLS is needed; DYNAMICS performs this second-order correction to the molar mass

value reliably for particles up to 50 nm in radius by assuming a molecular conformation.

For greater precision or even larger particles a multi-angle light scattering (MALS) detector is required. The analysis provides both molar mass and the size in terms of the rms radius r_g . A MALS detector such as the **DAWN® HELEOS® II** is most commonly combined with a separation system such as size-exclusion chromatography (**SEC-MALS**) or field-flow fractionation (**FFF-MALS**) to obtain true size distributions. While the PIII is not suitable for measuring the molar mass of these larger particles by SLS, it can determine size from DLS and *estimate* molar mass by assuming a specific conformation.

Information from noise

Dynamic light scattering takes advantage of the Brownian motion of particles in solution or suspension to measure their size. The fluid is illuminated with a narrow laser beam, and each particle in the beam scatters light waves in all directions. As the particles undergo diffusion, they produce fluctuations in the intensity of light scattered into any particular direction. A detector exposed to the scattered light will generate signals that change over time correspondingly. Since the particles' Brownian motion is random, so are the signal changes, which appear as uncorrelated intensity fluctuations on time scales of microseconds to milliseconds, more commonly known as 'noise'. DLS raw data, shown in Figure 4, truly look like detector noise.

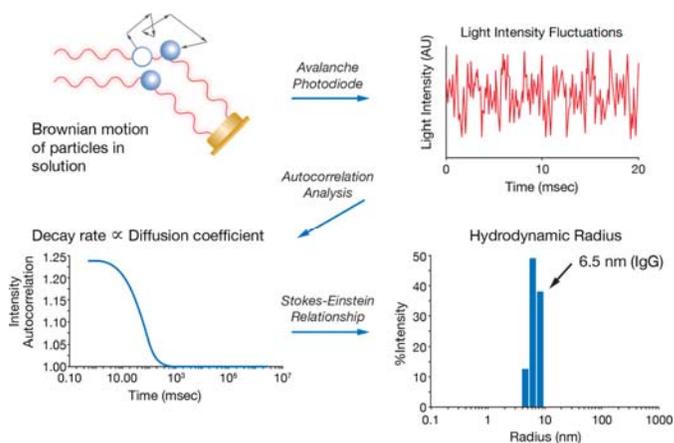


Figure 4. Brownian motion of particles produces dynamic light scattering signals that look like noise. However, these fluctuations do contain useful information: diffusion coefficients that can be converted to size.

However, since the rate of diffusion depends on particle size (in addition to solution viscosity and temperature), the fluctuations in the optical signal do contain some important information. The rate of fluctuation corresponds directly to

the diffusion rate of the scattering particles. Larger particles diffuse more slowly, leading to slow optical fluctuations, while smaller particles diffuse more rapidly, leading to fast optical fluctuations. The diffusion coefficient of the particles D_t can be determined by performing a mathematical algorithm known as “autocorrelation analysis” on the raw optical signals and fitting the resulting autocorrelation function (acf). The particle size is then determined from the diffusion coefficient using the Stokes-Einstein equation.

Yet More Information

An interesting feature included with the DynaPro Plate Reader III is the onboard camera that captures an image of each well. Every scientist who has made DLS measurements has, at some point, run into odd data that resulted from dust, precipitation or a bubble in the sample. The only way to find the source of the bad data was to hold the cuvette up to the light and examine the sample with a jeweler’s loupe... assuming, of course, that you hadn’t yet discarded or emptied the cuvette. Thanks to the PRIII’s camera, when strange results show up, you can simply pull up the image that was taken automatically and verify if there was precipitation, crystallization, debris or a bubble. No more guesswork, or squinting, required—just lots more information on your sample’s behavior.

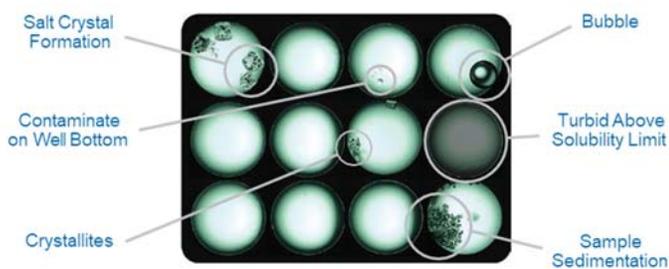


Figure 5. Well-bottom images captured with the DynaPro Plate Reader’s onboard camera show sample behavior beyond nanoparticle size distributions.

So, what’s new?

Weigh the plate

A common misbelief holds that DLS can be used to determine molar mass of proteins, but unfortunately this is not supported by physics. DLS determines diffusion coefficient and size; molecular weight can then be *estimated* by assuming a specific molecular conformation such as globular for proteins or random coil for polymers. Nature

often does not cooperate, though, in these assumptions, and produces non-globular proteins, branched polymers, and other non-conforming species which invalidate the assumptions.

The PRIII adds several new features, the first of which is true molecular weight measurements of proteins and other macromolecules, in the plate, using static light scattering. SLS does not require assumptions of conformation, and therefore is appropriate even when Nature does not cooperate. All that is needed for a proper measurement of molar mass is knowledge of the sample concentration and the refractive increment dn/dc , plus a highly linear detector (usually a well-known constant for standard proteins and buffers, dn/dc can also be measured, if necessary, for modified proteins and/or non-standard buffers).

Sounds easy to pull off, but it turns out that there is a tradeoff between speed, sensitivity and linearity of detector modules. Standard p-i-n photodiodes (such as those used in a MALS detector) are highly linear over many orders of magnitude of signal, but when operated at high gain, have a slow response—too slow for DLS. Avalanche photodiodes, typically used in DLS instruments (such as the DynaPro), have high gain and high speed with sub-microsecond response times, but are usually quite nonlinear.

In the DynaPro PRIII, the standard APD was replaced with a new module that maintains linearity over a sizeable range of signals. This range is suitable for analyzing common proteins and polymers, at concentrations typical of DLS and SLS measurements. When the sample is pure and monodisperse, SLS measurement in the plate will provide a true molecular weight value; when the sample is a mixture of species, SLS will determine the weight-average molar mass. The PRIII is the first (and only) plate reader to determine molecular weights in a highly automated, low-volume format, making this quite an exciting development.

B22 and A2, too

Non-specific protein-protein interactions, arising from surface moieties such as charged or hydrophobic residues shown in Figure 6, impact the colloidal stability and propensity for aggregation of biotherapeutics. One of the primary thermodynamic parameters describing these interactions is the second virial coefficient A_2 , a.k.a B_{22} ; and a primary, first-principles methods for measuring A_2 is SLS.

Conventional SLS requires fairly large quantities of protein, though, which are often unavailable in early stages of biotherapeutic development. In recent years, researchers working on early-stage biologics have utilized DLS to measure the diffusion interaction parameter k_D as a reasonably reliable proxy for A_2 . While not a purely thermodynamic quality— k_D incorporates both hydrodynamic and thermodynamic properties—it could be measured by DLS, using much smaller sample quantities than SLS. Even better, it could be measured in a highly automated fashion using the DynaPro Plate Reader, and has been shown to correlate reasonably well to aggregation propensity and the viscosity of high-concentration monoclonal antibodies.

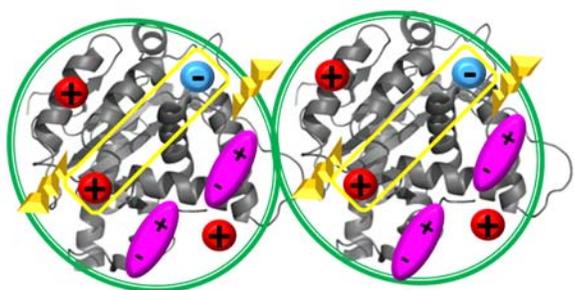


Figure 6. Non-specific protein-protein interactions arise from hard-core repulsion, net charge, charge homogeneities, local dipoles, hydrophobic residues and other source. To first order they are quantified by the second virial coefficient, A_2 (measured by SLS), or its proxy, k_D (measured by DLS).

This new capability of the PRIII extends automated, high-throughput screening of multiple formulations or candidates to include A_2 , nicely complementing k_D measurements. In combination with thermal and conformational stability screening using temperature ramps, denaturant series or accelerated stability tests at elevated temperatures, A_2 analysis affords the PRIII comprehensive stability screening and prediction of biotherapeutic drug product viability.

Evaporation, begone

The PRIII is an extraordinarily versatile platform for stability testing and process development. Often these assays require extended temperature ramps or long-term storage at room or elevated temperatures, all of which subject the solutions in the plate to evaporation. With just microliters per sample, it doesn't take much evaporation to greatly increase sample concentration and possibly induce precipitation or aggregation.

The preferred means of preventing evaporation in the DynaPro Plate Reader has been capping the wells with

mineral or silicone oil. Oil is actually less hydrophobic than air and creates a milder interface for proteins with respect to denaturation. It is also beneficial to the optical system since it creates a clean and perfectly smooth surface, far from the detection volume, that does not scatter the laser light projected from below the well.

However, oil can be messy and capping the wells requires additional fluid handling. Surfactants may draw some of the oil into the solution, changing the formulation.

Transparent sealing tape is also an effective method for preventing evaporation that is easier to use and less messy than oil (foil cannot be used because it blocks the camera lighting). The difficulty with transparent sealing tape in previous models of the DynaPro Plate Reader has been condensation of vapors on the tape; the droplets formed scatter copious amounts of light and degrade the DLS signals.

The condensation issue has been solved completely in the PRIII. When specified in the method, the instrument turns on a very gentle heating element that elevates the temperature of the tape by 2-3 °C above that of the plate. This is sufficient to prevent condensation and ameliorate any adverse optical effects without impacting the temperature of the plate contents.

Hence in the PRIII either oil capping or transparent sealing tape may be utilized to prevent evaporation, with no adverse impact on optical performance. You are free to choose your preferred method.

Looks count for something, too

The PRIII, like all Wyatt detectors, incorporates a graphic touch screen display on the front panel which is useful for manual operation of the instrument, quickly reviewing the history of signals and temperature, and diagnosing alarms or faults. All it takes is a quick look to determine what is going on.

New for the PRIII is an improved front panel display that makes it even simpler to identify at a glance key system parameters and operation details. Need more? Just tap the screen to navigate the charts and other displays, for a deeper look.

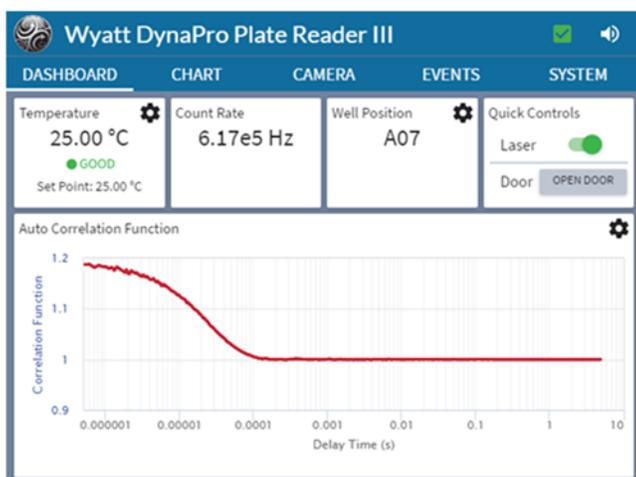


Figure 7. Front panel display on the DynaPro Plate Reader III.

Building experiments, from novice to expert

DYNAMICS is a powerful software package for comprehensive data analysis, and its Event Schedule scripting language enables the construction of complex methods for scanning wells in plates over multiple temperature profiles. It has been great for experts who need lots of options. But what about novices?

Yes, we'll admit that we made DYNAMICS more complex than necessary for novices who just want to perform basic partial-plate scans and temperature profiles. We apologize. And now we've fixed it.

The Experiment Builder, first implemented in DYNAMICS version 7.7, makes it easy to create a basic method in a few simple steps:

- 1) Select a temperature profile (fixed, ramped, or discrete steps) and specify the desired temperature(s)
- 2) Graphically select the wells to be measured and identify their contents
- 3) Specify additional information such as the number of repeat measurements, time to wait between measurements, and whether to acquire camera images.
- 4) Press Go, and bring the DynaPro Plate Reader to life!

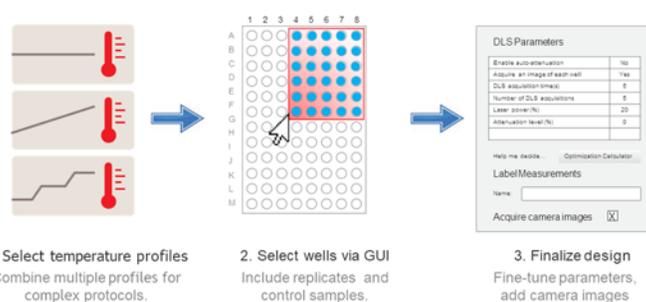


Figure 8. The Experiment Builder in DYNAMICS 7.7 and above allows novices and experts alike to painlessly create methods for the DynaPro Plate Reader.

Sound too easy? Not to worry, the Event Schedule feature is still available if you need more complex methods such as temperature cycling. We believe that ease of use need not come at the expense of versatility.

Applications, old and new

The basics

Protein and particle sizing

Size and aggregation of sub-micron particles, proteins, viruses, liposomes and other macromolecules are bread and butter for DLS. Effortlessly running dozens or hundreds of samples is more like caviar and pralines, a rare luxury. Unless, of course, *you* are the one who has to stand there day in and day out, spoon-feeding a cuvette-based DLS instrument, in which case automated measurements using a DynaPro Plate Reader are absolutely essential.

Figure 9 shows the results of a 45-minute, hands-off experiment, measuring 96 samples in a single well plate. SpectralView color-codes the plate representation according to criteria set by the user, e.g. average size, average molecular weight, polydispersity, %mass above $R_h=10$ nm—and those are just the more common choices. Each well can then be examined in more detail to view the size distribution of its contents, and the entire result set exported in spreadsheet format.

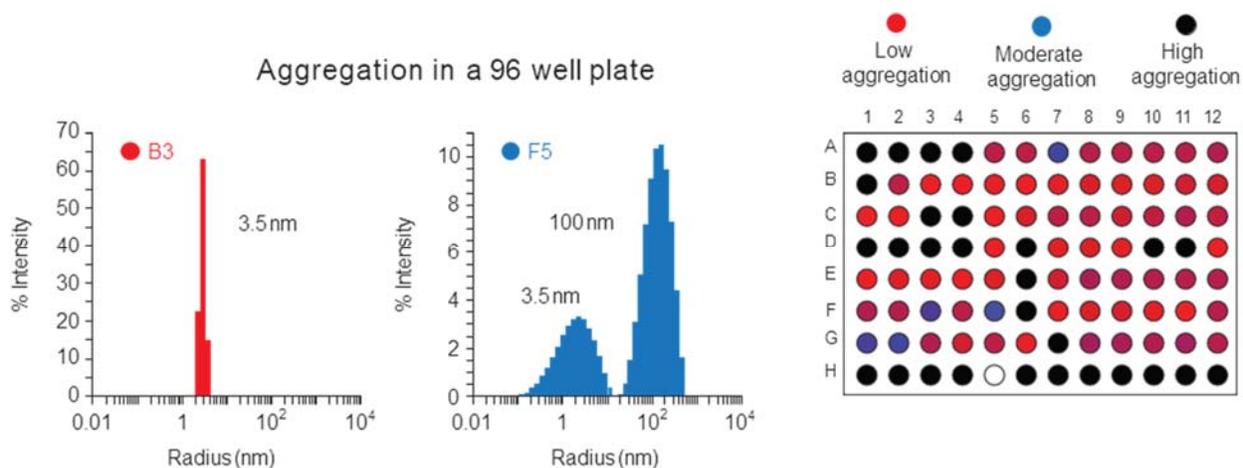


Figure 9. Automated screening of size and aggregation in a 96-well plate takes just 45 minutes. The results are displayed in a SpectralView heat map as well as individual size distributions. (Courtesy Sabin Inst.)

Protein crystallization

Finding the right buffer for protein crystallization can be time- and material-consuming as well as frustrating. You may try hundreds or thousands of conditions, and either a crystal forms, or it doesn't (mostly the latter).

Often the process can be rationalized and made to converge much more quickly using DLS to map out the degree of aggregation and polydispersity for each condition. It is often possible to find the sweet spot for crystallization by interpolation, even if you don't nail exactly the right condition the first time. Naturally this only makes sense in the context of high-throughput DLS—you wouldn't dream of making all those measurements manually!

Protein stability characterization

Automated, high-throughput aggregation screening for formulations and developability

Design of Experiment (DoE) is a smart way to set up formulation screens for implementing Quality by Design. DoE helps to optimize formulations over a large design space, but ultimately you are still limited, by the number of samples and statistical validity of the measurements, in the total parameter set you can explore.

Using a 1536-well plate, the DynaPro PRIII requires as little as 4 µL of solution per well. This promises an extended DLS/SLS aggregation screen over many therapeutic candidates, buffers, excipients and conditions, with multiple replicates, while consuming reasonable quantities of protein. You will have enough data to be confident that you have made the right choice for your product.

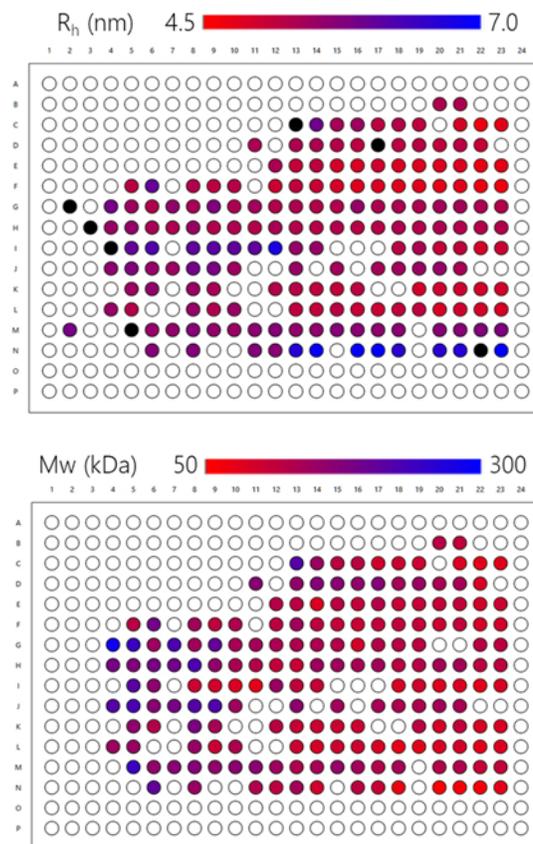


Figure 10. Formulation screening: average size (top) and apparent molecular weight (bottom) of 2 monoclonal antibodies in 6 buffers, measured in a 384 well plate. The apparent molecular weight differs from the true molecular weight as a result of concentration-dependent protein-protein interactions. Each well can be further examined to determine the size and distribution of large aggregates as well as polydispersity index for small aggregates. Some invalid data, due to precipitation, not shown.

Accelerated stress and aggregation rate

The PRIII is especially well-suited to accelerated stress studies:

- Samples may be incubated at reduced or elevated temperatures directly in the instrument
- Plates may be transferred to temperature chambers, freeze-thaw chambers or shaking/stirring devices and then returned for analysis
- The plates may be transferred to other plate-based analytical instruments or to plate samplers for additional/orthogonal study

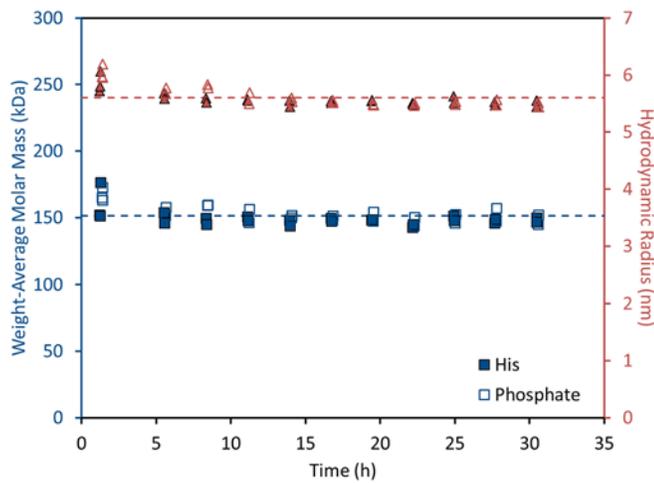


Figure 11. Time course at 40 °C showing a highly stable mAb reference standard provided by NIST. Both weight-average solution molar mass and size are unchanged under these accelerated stress conditions.

Colloidal stability: A_2 and k_D

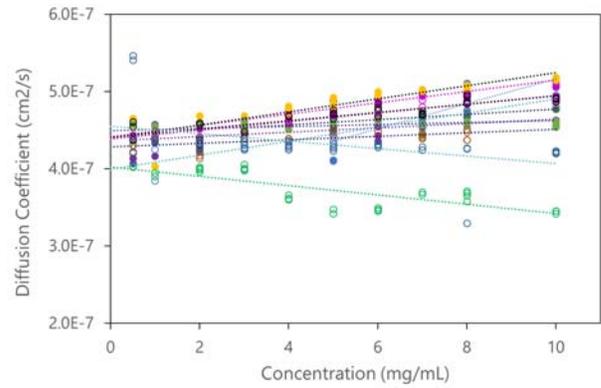
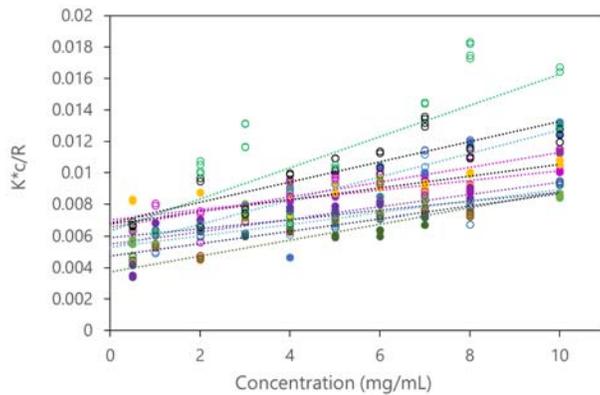


Figure 12. Data utilized to calculate A_2 and k_D of multiple conditions in each well plate include apparent molecular weight (top) and diffusion coefficient (bottom) vs. concentration. Data shown correspond to the measurements of Figure 10.

A single 384-well plate suffices to measure the second virial coefficient A_2 and diffusion interaction parameter k_D by means of a protein concentration series, for a dozen formulation conditions, *in triplicate*. After the DynaPro PRIII has scanned the plate and acquired the data (in as little as 1.5 hours), DYNAMICS will automatically overlay the graphs and analyze the results to determine these indicators of colloidal stability, for each condition.

Thermal stability: temperatures of unfolding and aggregation

Thermal denaturation is another stability-indicating measurement commonly utilized to assess candidate biologics for developability and formulations for optimal stability. There are many ways of determining thermal stability, each of which analyzes a different physical property of the molecule. Many of these techniques rely on extrinsic probes such as fluorescent dyes, or indirect signals such as intrinsic fluorescence (IF) and differential scanning calorimetry (DSC).

Only light scattering provides direct biophysical evidence of thermally-induced changes:

- Molar mass by SLS is *direct* evidence of aggregation
- Size by DLS is *direct* evidence of unfolding and/or aggregation

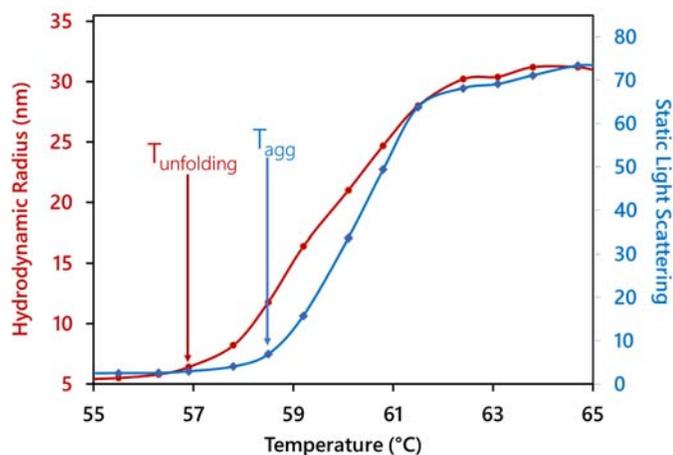


Figure 13. Simultaneous determination of two transition temperatures of an IgG for aggregation (from SLS) and unfolding (from R_h). Since aggregation occurs, a true melting temperature cannot be determined for this protein.

The PRIII can determine, in a single temperature ramp, *multiple* transition temperatures for *multiple* candidates or formulations:

- T_{agg} is the onset temperature for onset of aggregation, determined by SLS
- T_{onset} is the onset temperature for unfolding, determined by DLS, and
- T_m is the melting temperature, or the midpoint in a transition between folded and denatured states, determined by DLS with confirmation from SLS that aggregation has not occurred.

Intrinsic fluorescence requires the presence of appropriate fluorophores, usually tryptophan or tyrosine, and may not report the unfolding of domains that do not contain these amino acids. In addition, IF requires the use of short-wavelength UV excitation which is actually known to *induce* aggregation in various biotherapeutics, especially antibody-drug conjugates (ADCs). Therefore, an instrument relying on UV scattering to quantify aggregation may in truth be the culprit in creating aggregation. The infrared wavelength used in the DynaPro Plate Reader, 830 nm, is safe and does not induce aggregation.

Temperature onset of interaction

Something IF and DSC *cannot* do is indicate what changes occur in protein-protein interactions in the course of thermal transitions, even if you have good reason to believe that their signals indicate unfolding and structural instability. Light scattering, though, can.

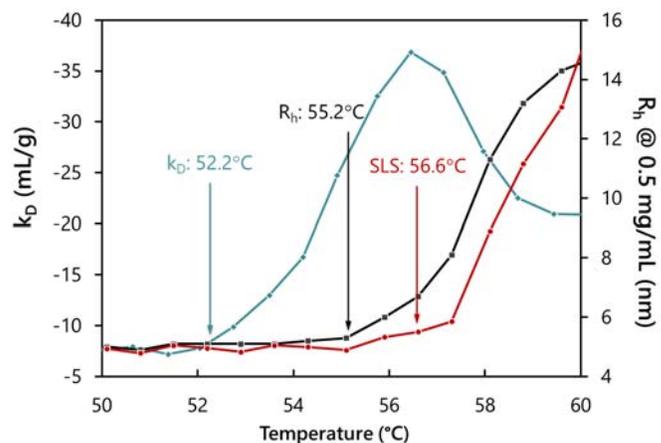


Figure 14. Simultaneous determination of three transition temperatures of an IgG for aggregation (from SLS), unfolding (from R_h) and interactions (from k_D). The increasingly negative value of k_D indicates strong colloidal attraction, well before the onset of unfolding or aggregation.

A_2 and k_D are *direct* evidence of protein-protein interactions. By measuring their dependence on temperature, you will not only know at what temperature the protein structure has changed; you will know if the change leads to increasingly attractive interactions (and hence instability), increasingly repulsive interactions (and hence stability), or neither.

In some cases, it has been observed that the transition temperature for protein-protein interactions is quite distinct from the onset temperatures for unfolding or aggregation. It is likely that these three temperatures correlate to different aspects of protein stability, so their measurement provides additional insight into the properties of each protein candidate or formulation.

Viscosity

Whether for sub-cutaneous injection or ocular delivery, formulation of monoclonal antibodies and other biologics is increasingly occurring at high concentrations. Since solution viscosity under these conditions can be detrimental to injectability as well as to production, processing and storage, it is imperative to identify and mitigate high viscosity as early as possible in the development pipeline.

DLS in microwell plates is ideally suited to high-throughput, low-volume viscosity screening. All that is required is suspension of a small quantity of sub-micron particles of known size, such as 100 nm polystyrene latex spheres or PEG-coated gold nanoparticles, into each well. Since the diffusion coefficient of the particle is inversely related to the solution viscosity η , DYNAMICS can compare the measured diffusion coefficient with the coefficient measured in water

(or calculated from the known size) to immediately provide the value of η . Values measured by DLS correlate closely with those measured by conventional, low-throughput/high-volume techniques.

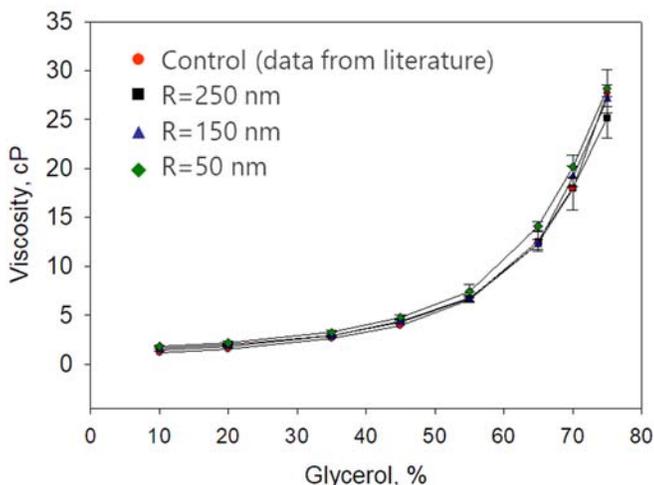


Figure 15. Comparison of viscosity measurement by DLS with conventional measurements. R refers to the size of the polystyrene latex bead used in the DLS viscosity determination.

Solubility by turbidity

Solubility is commonly assayed by measuring turbidity, either as a reduction in transmission or an increase in scattering. The DynaPro PRIII offers a means of high-throughput, low-volume solubility measurements in plates by means of static light scattering, which can be calibrated in terms of nephelometric units if so desired, or presented simply as normalized scattering intensity.

PEG-induced precipitation is one more common stability assay that can be implemented in the PRIII, to assess the solubility of a series of formulations. In addition to nephelometry by scattering, precipitation can be visualized using the camera images.

Formulation phase diagrams

With so many properties to characterize for biologics, and so many formulation variables such as pH, ionic strength, type of salt used as well as additional excipients including various types of sugars and surfactants, it is not surprising that researchers have looked for ways to organize all the information in a digestible manner. One approach is phase diagrams such as those in Figure 17.

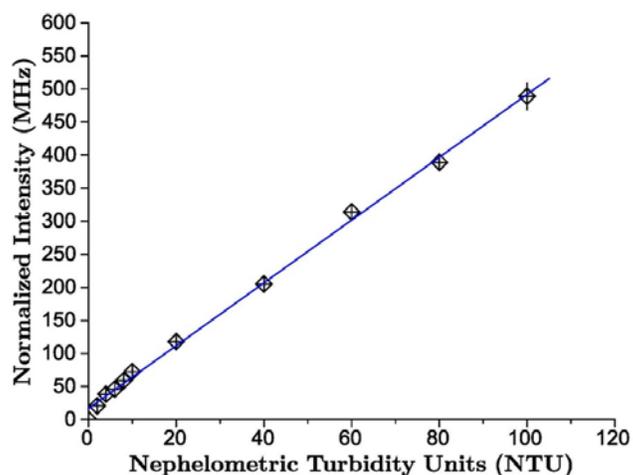


Figure 16. SLS measurements can be calibrated in terms of NTUs

The phase diagram combines two formulation variables (such as pH, salt concentration or protein concentration), with a matrix of experiments that determine two or more solution properties (such as T_{agg} , k_D or viscosity). Boundary lines separate formulation variable ranges that produce acceptable vs. unacceptable ranges for each property, with the intersection of acceptable ranges highlighted in white. This multivariate analysis helps determine the optimal formulation design space.

The real power of the DynaPro Plate Reader III is that it can, in a single run, map out multiple essential biophysical properties, robustly and conveniently:

- Average size
- Average molar mass
- Polydispersity Index
- Presence and quantity of large aggregates
- Diffusion interaction parameter k_D
- Second virial coefficient A_2
- Onset temperature of unfolding T_{onset}
- Onset temperature of aggregation T_{agg}
- Onset temperature of interactions
- Solubility
- Solution viscosity.

What's more, it does so in standard plates, over a large set of conditions, to produce phase diagrams and even more complex multivariate analyses. One instrument, so much data.

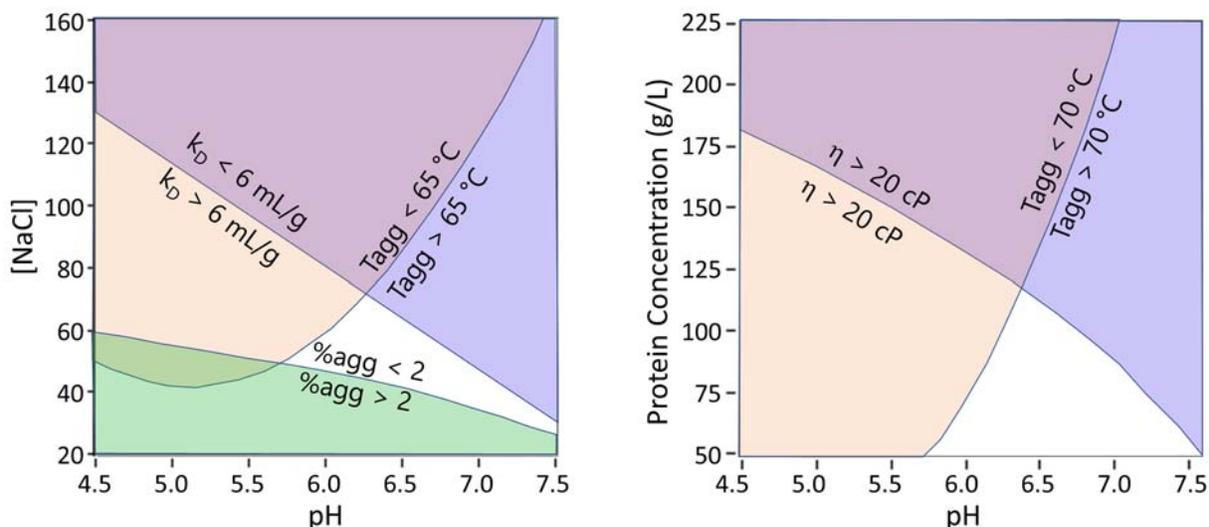


Figure 17. Phase diagrams used to map out optimal formulation space. Top: white space corresponds to k_D values above 6 mL/g for colloidal stability and T_{agg} values above 65 °C for conformational stability and %large aggregates at 25 °C below 2%. Bottom: White space corresponds to viscosity η below 20 cP for injectability and T_{agg} above 70 °C for thermal stability.

Epilogue: be productive

When getting biologics to market faster is your goal, productivity is the name of the game. You need more protein quality and stability analyses, faster, covering more indicators. The DynaPro Plate Reader III helps to realize this dream. It provides the confidence that can only be acquired by robust, comprehensive measurements of dynamic and static light scattering, over many samples, conditions, and replicates. And it does all this automatically and conveniently

in standard microwell plates, with high throughput, so you can be productive in other activities while DLS and SLS measurements are taking place.

More information on the DynaPro Plate Reader is available from Wyatt Technology or at www.wyatt.com/HT-DLS. Contact info@wyatt.com to discuss with a DLS/SLS application expert or knowledgeable sales engineer.

