OneStep® Pulse Injections

Novel SPR Injection for Determining Diffusion Coefficients and Sample Aggregation

Simplifying Progress

SARTORIUS

OneStep® Pulse Injections

Introduction

OneStep® Pulse is a dispersion-based gradient injection requiring a minimal volume of analyte (either 2.5 µL, 5 µL or 10 µL). Dispersion occurs at both the sample front and the tail of the injection, with 50% of the analyte concentration in front of the peak and 50% behind it (Figure 1), forming a Gaussian shaped analyte concentration gradient that is sensitive to both the analyte diffusion coefficient and hydrodynamic radius.

Monomeric and aggregated analytes contained within the sample will diffuse at different rates through the dispersion loop and produce a response curve representative of the entire sample. The area under the pulse curve represents the total protein concentration, comprising of monomeric and aggregate components. The pulse signal can then be deconvoluted and the diffusion coefficient, aggregation number and component concentration empirically determined from the peak width, shape and height.

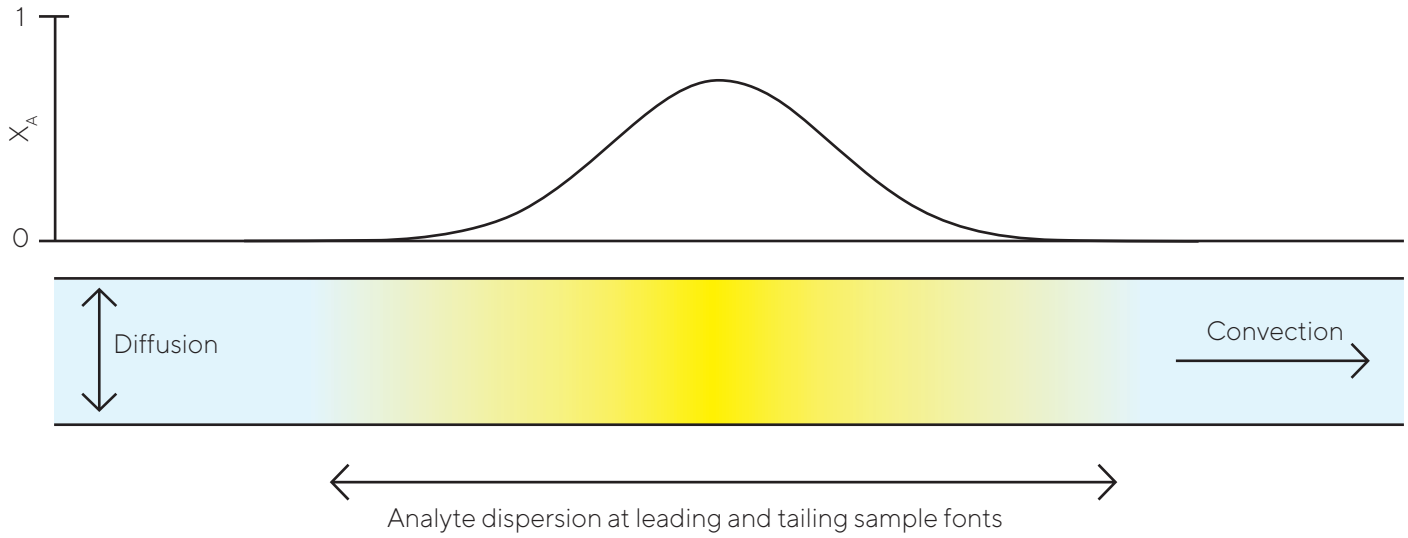
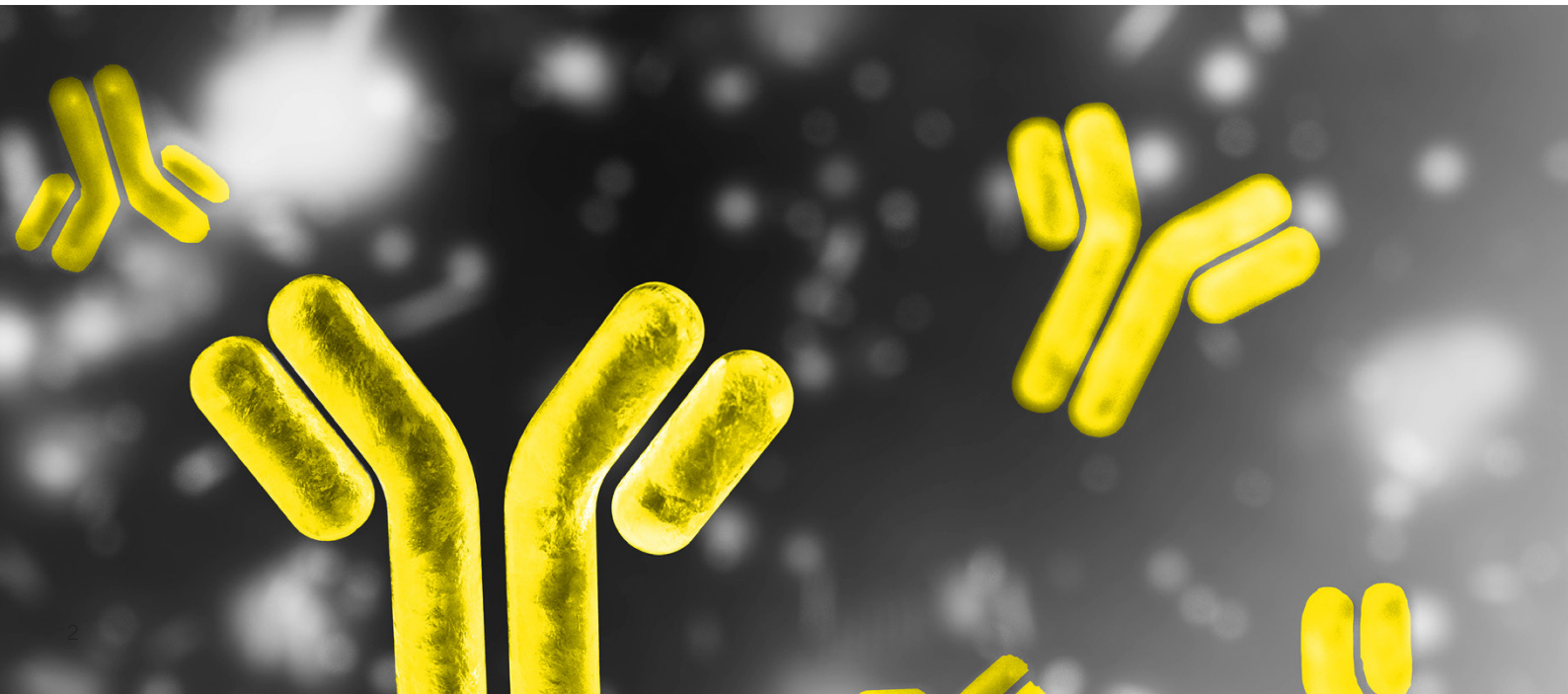


Figure 1

Note. OneStep® Pulse is an SPR injection unique to the Octet® SF3, requiring the injection of a small volume of sample into the dispersion loop, with analyte diffusion occurring in both directions. OneStep® Pulse can detect protein aggregation (diffusion coefficient analysis) and measure aggregate size (hydrodynamic radius), with further aggregation analysis revealing the concentration of monomeric and aggregated analytes at therapeutically relevant concentrations.



Benefits of empirically derived data

The inherent properties of all molecules can significantly vary, and few assays are perfectly homogeneous. A number of variables can therefore influence analyte diffusion and formation of the subsequent concentration gradient, such as a sample's:

- diffusion coefficient
- molecular weight
- hydrodynamic radius
- viscosity

More compact, well-folded lower weight molecules will generally diffuse more rapidly and exhibit a steeper binding gradient compared to larger, poorly folded complexes or aggregates (Figure 2).

Thus, the benefits of using a small volume of analyte to empirically calculate precise values, as opposed to using theoretical online calculators, is invaluable.

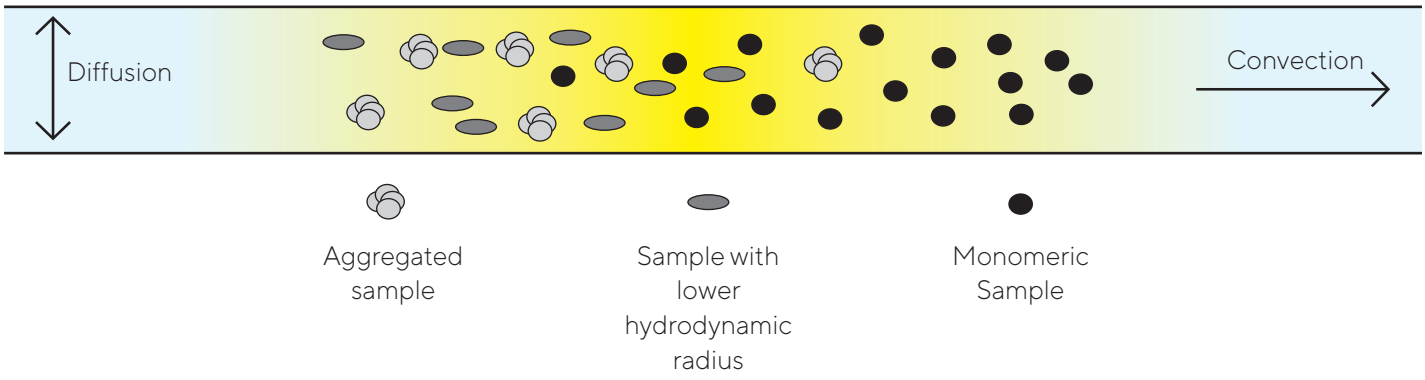
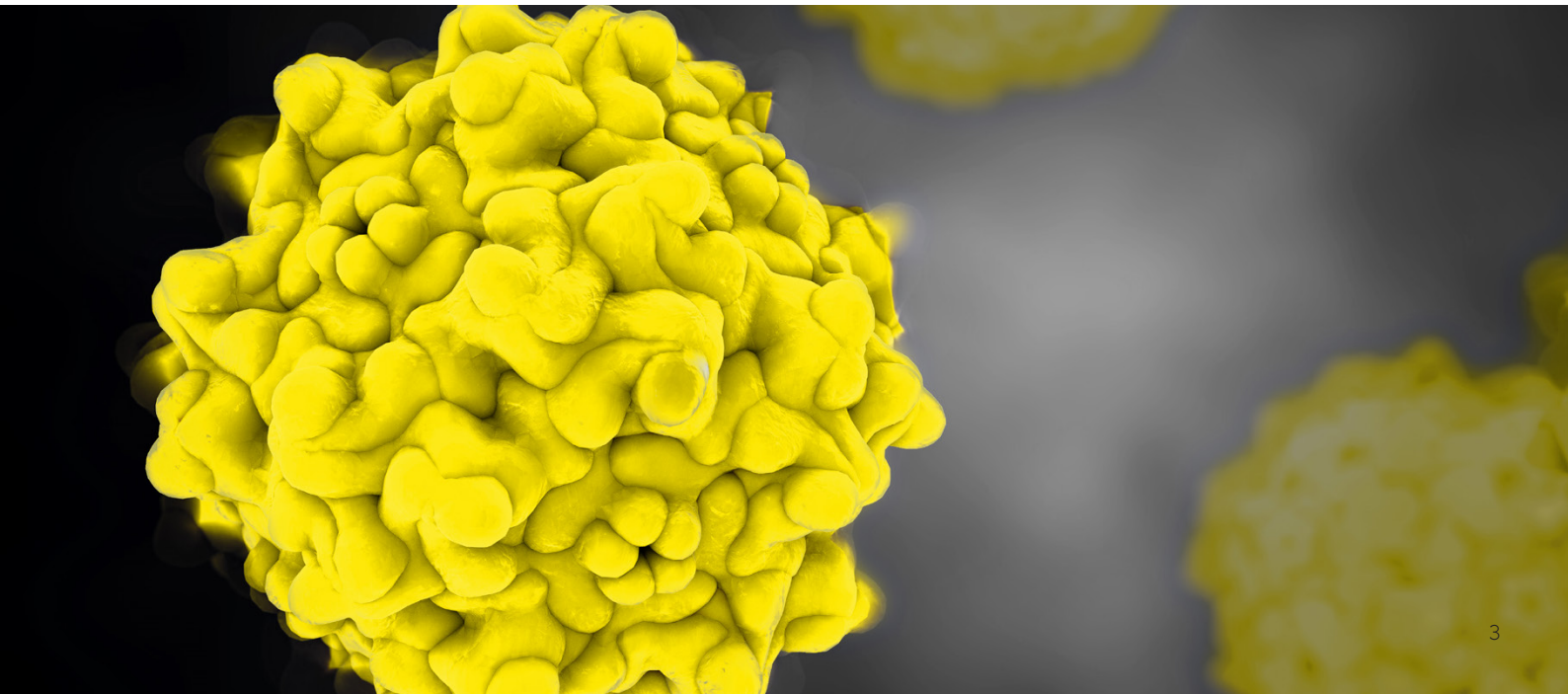


Figure 2

Note. Analyte diffusion within the dispersion loop during a OneStep® Pulse injection. Many samples contain a mix of monomeric and aggregated analyte, both of which diffuse at different rates through the dispersion loop towards the flow cell. Stress treated samples will exhibit a range of hydrodynamic radii which will also diffuse at different rates.



OneStep® Gradient and OneStep® Pulse Injections

OneStep® Gradient Injections

Standard OneStep® Gradient Injections generate an SPR response that is directly proportional to the analyte and ligand concentration (Figure 3A). Analyte diffusion only occurs at the leading edge of the injection as an air bubble at the rear of the injection does not allow tail diffusion. The dissociation constant (k_d) is subsequently calculated as the analyte dissociates from the ligand in the presence of analyte-free running buffer. A range of saturation levels can be achieved from injection volumes as low as 80 µL, irrespective of molecule size and affinity.

OneStep® Pulse Injections

In contrast, OneStep® Pulse Injections analyze the shape of the SPR response curve, as molecules with different molecular weights (MW) and hydrodynamic radii pass through the flow cell. Molecules with lower MW or hydrodynamic radii generate narrow Gaussian distribution responses, whilst complexes or aggregates with larger MW or hydrodynamic radii move more slowly and exhibit a smaller, flatter peak response (Figure 3B).

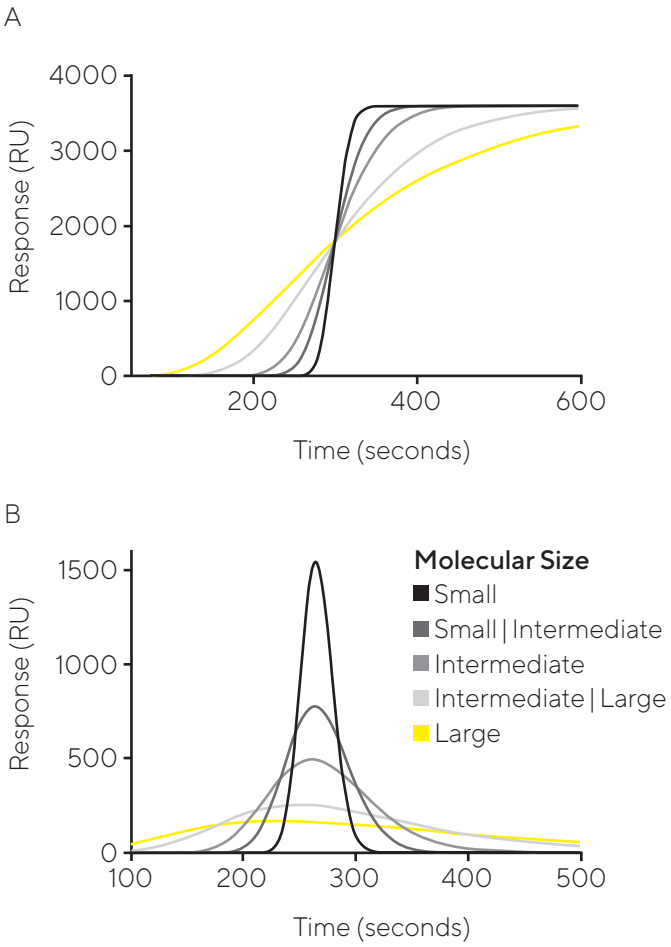
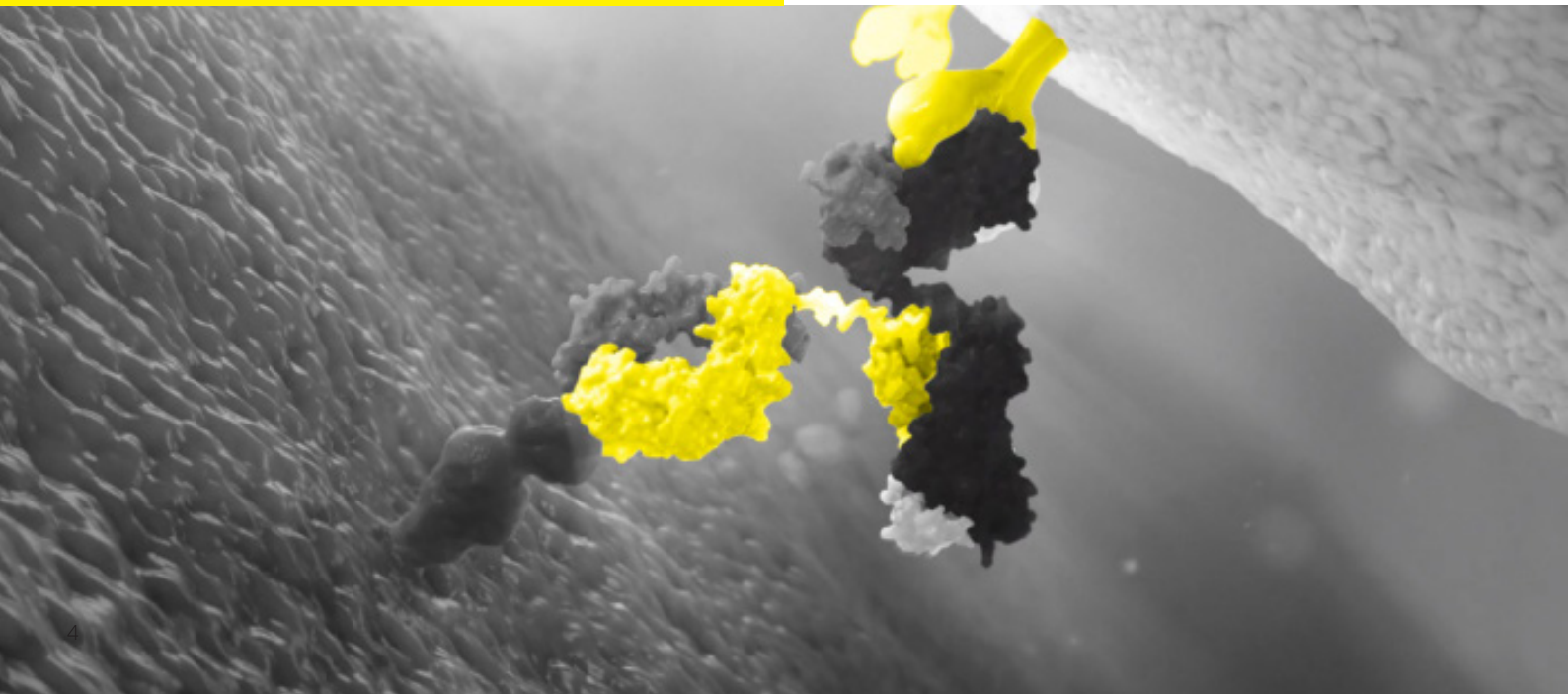


Figure 3

Note. (A) OneStep® Gradient Injection of different analytes with varying molecular weights, and (B) OneStep® Pulse Injections using the same analytes as Figure 3A. The black line corresponds to the smallest molecular weight molecule, while the yellow line highlights molecules with higher molecular weights or greater sample aggregation.



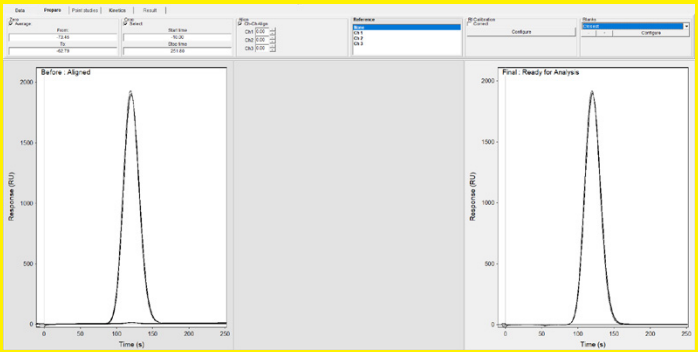
OneStep® Pulse Injection Example

OneStep® pulse injections contain a reference standard molecule to allow accurate characterization of unknown analytes. Here, a 3% (87 mM) solution of sucrose prepared in HBS-EP+ was injected as an example solution using a OneStep® pulse injection (10 µL). As OneStep® pulse injections do not contain a surface binding component, the use of a separate reference channel is not required.

The 3% sucrose was fitted to the diffusion model within the analysis software and from the resultant gaussian curve, the software calculates the peak width providing the apparent diffusion coefficient (Dapp) and the aggregation number (Nagg).

The determined apparent diffusion coefficient (Dapp) of 7.06×10^{10} is in excellent agreement with published values and the aggregation number (Nagg) is equal to 1, indicating that the analyte is a monomer of its ideal molecular weight. Analyte concentration can be determined using the peak height and an aggregation model is also available to quantify the relative concentrations and diffusion coefficients of samples that comprise of at least two analyte components (i.e. monomer and aggregate components).

A



B

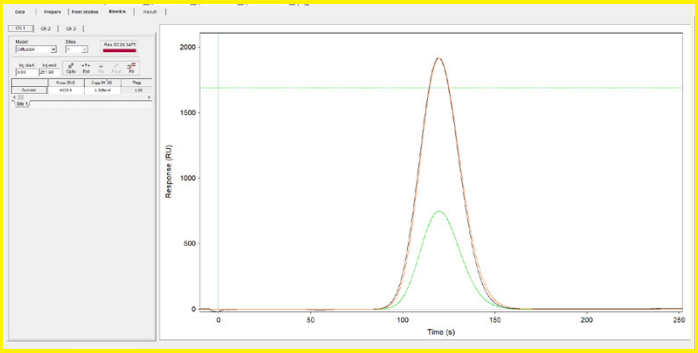
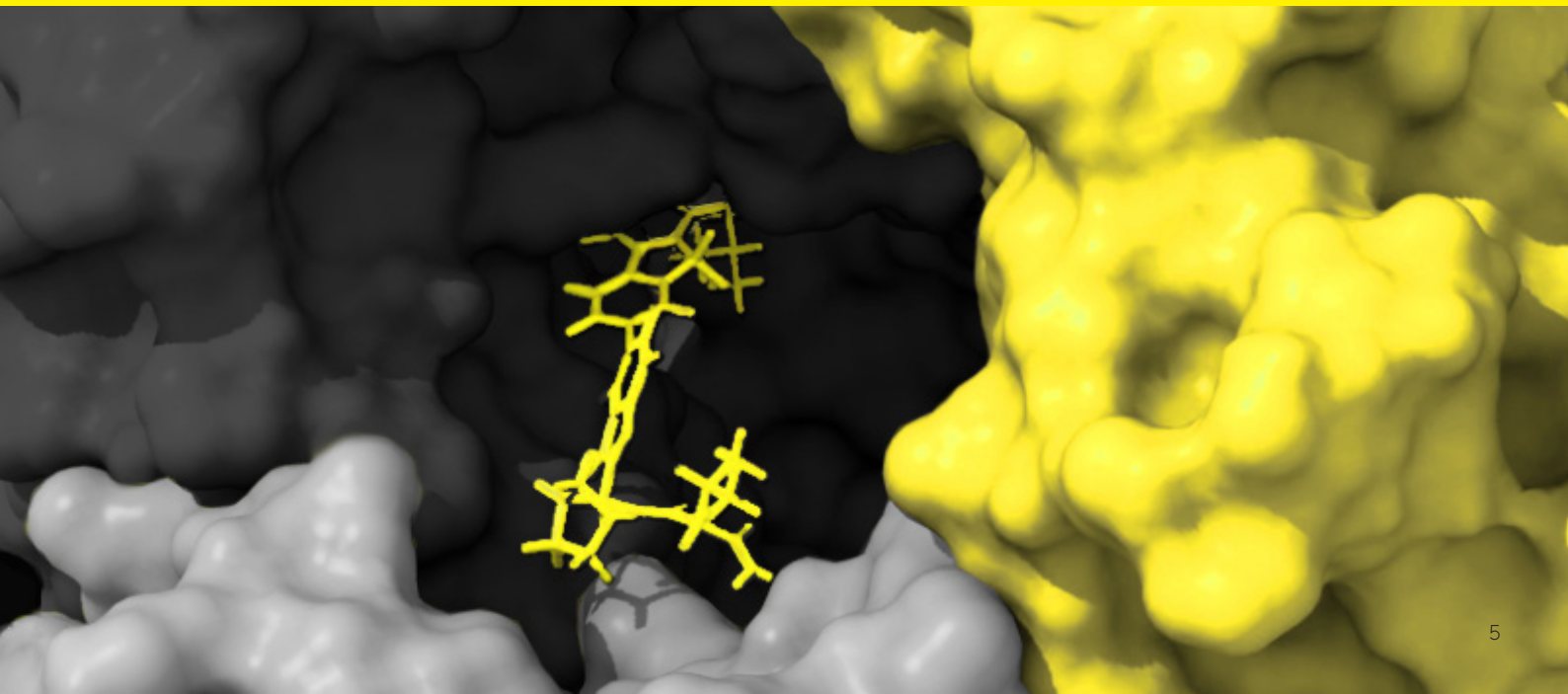


Figure 4

Note. Octet SPR analysis software allows the user to rapidly take (A) raw data, through to (B) fitted data, which can determine the apparent diffusion coefficient and the aggregation number of the analyte.



The effect of heat stress on aggregation formation in BSA

Samples can be manipulated under different stress conditions to determine the effects of variables such as heat, acidification and oxidation on sample integrity. As shown, 3% sucrose provides a suitable bulk reference standard and was therefore subsequently used to determine the effect of heat stress on Bovine Serum Albumin (BSA) aggregation (Figure 5A). Samples were incubated at 70°C for varying lengths of time and OneStep® Pulse Injections used to determine the effect on aggregate formation.

Results show that the longer each BSA sample was kept at 70°C, the lower the peak and the wider the area shape of the curve, indicative of a lower hydrodynamic radius as the aggregate percentage increases, as well as a lower monomeric BSA concentration.

Figure 5B shows the associated increase in aggregate concentration (Ca) and decrease in monomer population (Cm) upon 70°C heat stress determined using an aggregation model.

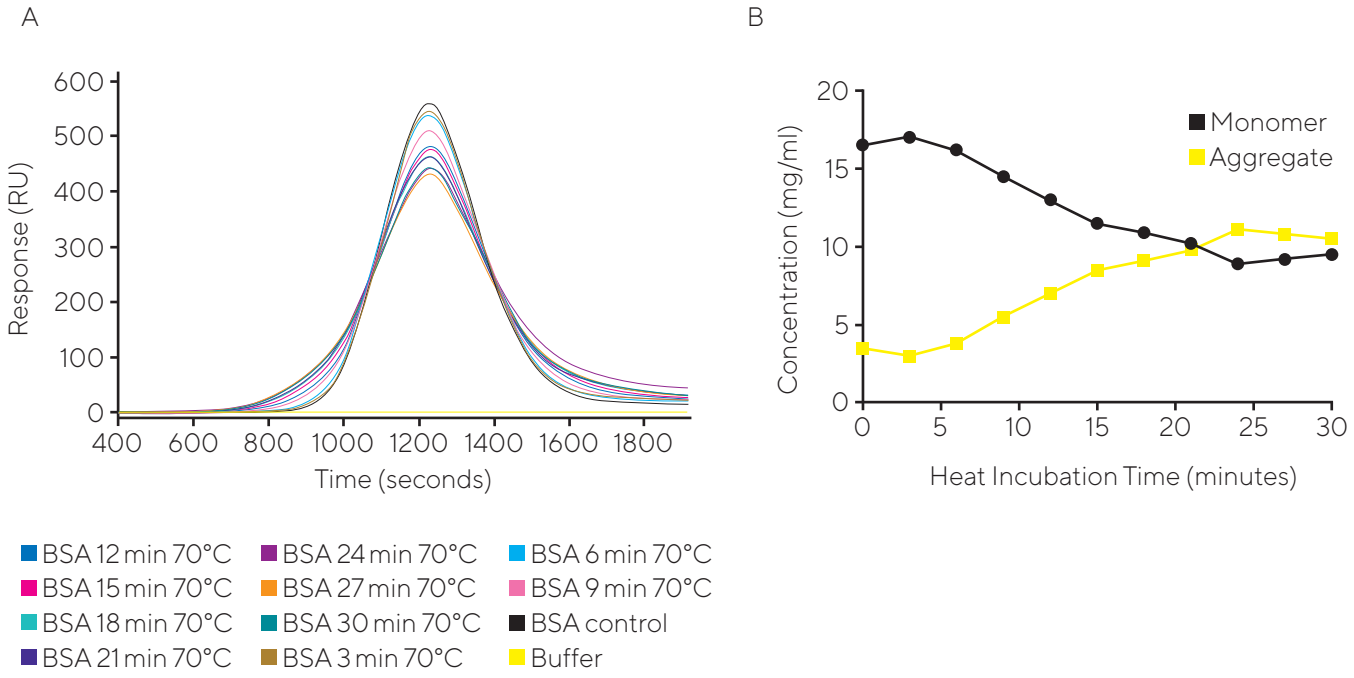
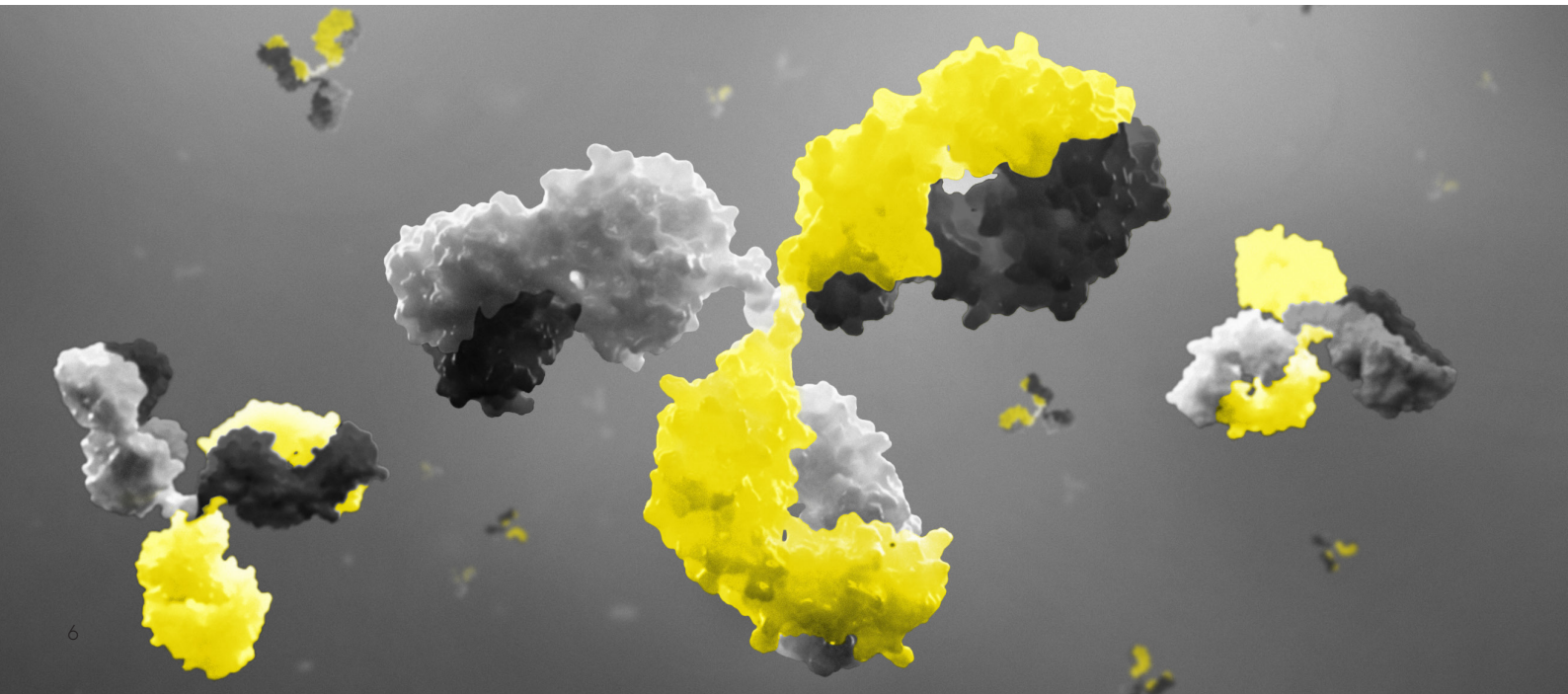


Figure 5

Note. (A) OneStep® Pulse Injection of BSA treated at 70°C for varying lengths of time, and (B) the associated increase in aggregate population and decrease in monomer population upon 70°C heat stress.



OneStep® Pulse Injection – Advantages and Benefits

OneStep® Pulse Injections can rapidly determine a sample's:
- total concentration
- diffusion coefficient
- aggregation number
- monomeric | aggregate component concentration

OneStep® Pulse is an early detection method for the formation of aggregates (validated by SEC). The diffusion coefficient is inversely proportional to the rate of aggregate formation

Based on novel and proprietary Gradient Injection Technology, OneStep® Pulse provides a faster assessment of aggregate formation and size – more quickly than industry standards of measuring aggregation including SEC-HPLC, AUC and DLS

OneStep® Pulse can quantify both protein aggregation and size at therapeutically-relevant concentrations (tested up to 200 mg/mL)

The Octet® SF3 is the only SPR system available on the market to accurately detect protein aggregation events in a single experiment

Discover more information than ever before with any available SPR biosensor. Diffusion coefficients, kinetics and affinities from a single OneStep® experiment

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