Analysing protein aggregation with DAISY nanoparticle tracking analysis

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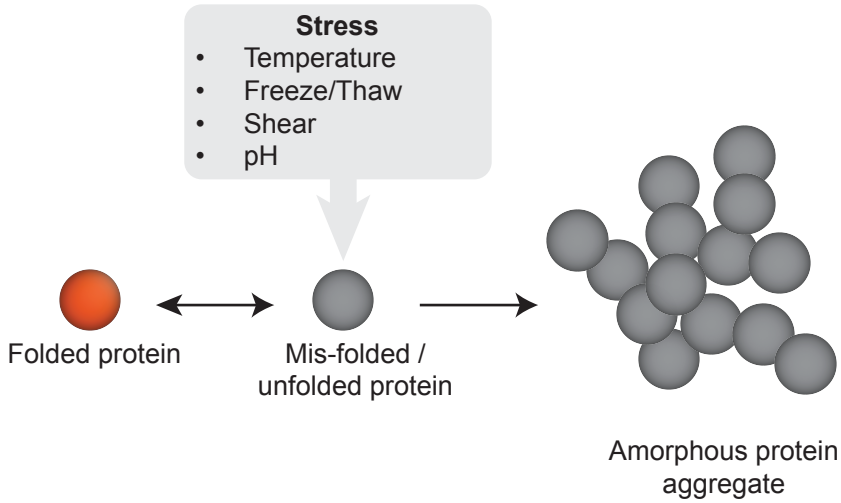
Introduction

Numerous stressors can disrupt protein folding and stability, leading to the accumulation of partially mis-folded or unfolded proteins[1]. These disrupted proteins can in turn come together to form large (nm to mm) insoluble aggregates. As well as reducing the effective concentration of functional protein in solution, the presence of aggregates in biopharmaceuticals can lead to adverse events such as immune responses and the induction of anti-drug antibody responses[2]. Thus, the inhibition of aggregation or removal of aggregates can potentially improve the activity of biotherapeutics and help to avoid unwanted adverse reactions. Detection and monitoring of aggregate formation is therefore of great importance in biotherapeutic manufacturing and formulation. In this application note we describe the capabilities and benefits of the DAISY nanoparticle tracking analysis (DAISY-NTA) approach for the detection and analysis of protein aggregates.

Contents:

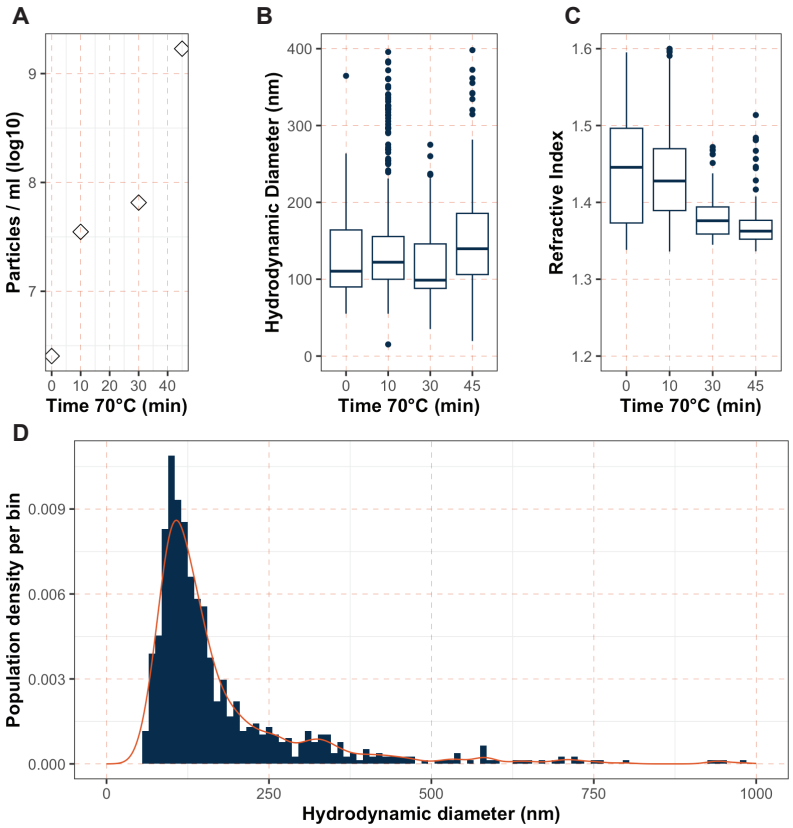
Figure 1. protein aggregation in response to stress



Highlights

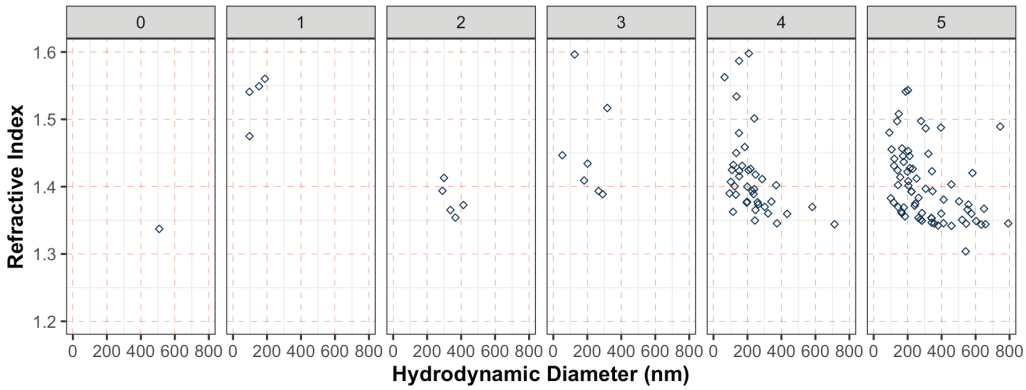
- DAISY analysis is suitable for protein aggregates with low refractive index difference vs. the sample media
- Accurate quantification of protein aggregate concentration, size, refractive index, and geometry
- Reliably differentiate between particle types with overlapping size distributions within the same sample
- For amorphous aggregates, DAISY provides comparative data on internal mass distribution
- DAISY allows real-time assessment of aggregate formation and growth

Figure 2. concentration, size and refractive index of thermal aggregates of BSA



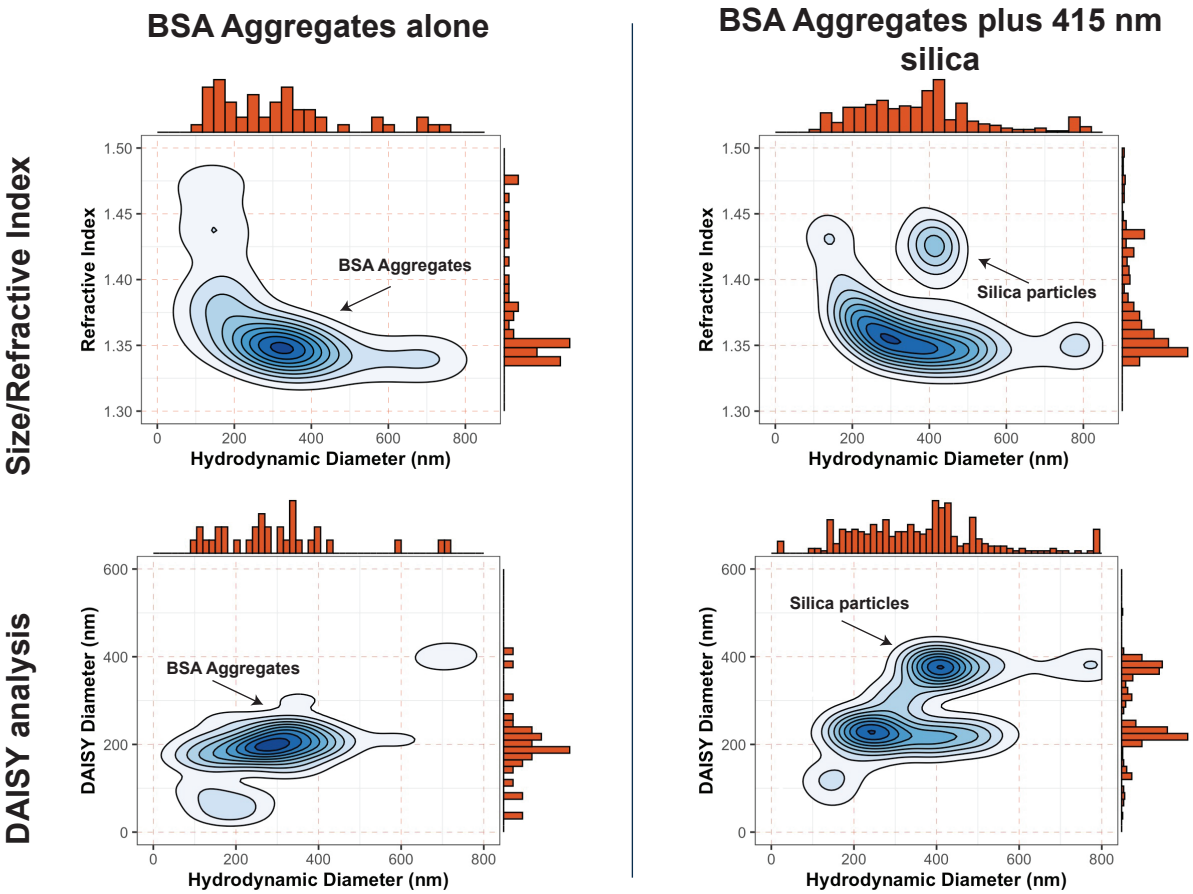
Bovine Serum Albumin (0.5%) in 50 mM Tris buffer (pH7.4) was filtered through a 0.1 µm filter and then kept at room temperature (21° C) for one hour or heated to 70° C for the indicated time in order to induce protein aggregation. DAISY-NTA analysis detects an increase in BSA aggregate concentration within 10 minutes of heating (**A**). The concentration of BSA aggregates in solution continues to rise throughout the time course of heating. Over the time course of heating a trend is seen towards larger aggregates (**B**) with a lower refractive index (**C**), suggesting larger aggregates are less densely packed than smaller aggregates. The full size distribution of BSA aggregates formed following 45 min at 70° C is shown in panel **D**. The data presented in these plots are derived from 25 seconds of recording for each sample using a sample volume of 10 µl.

Figure 3. Freeze/thaw and BGG



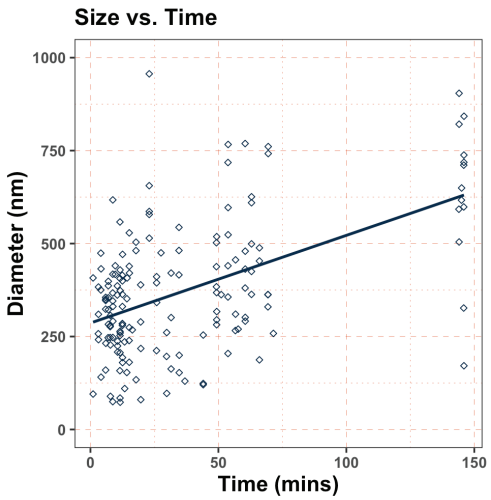
Bovine gamma globulin (BGG) is used as a surrogate to study immunoglobulin behaviour. Immunoglobulins are prone to aggregation during repeated freeze/thaw cycles[3]. In biopharmaceutical applications this aggregation can cause loss of efficacy or increased risk of adverse events. Samples of BGG solution were subjected to repeated freeze/thaw by cycling between -80° C and +25° C for five minutes. Prior to freeze/thaw, particulate concentration was extremely low. The concentration of particles observed increased dramatically within 3 freeze/thaw cycles. Tracking particle concentration, size, and refractive index over consecutive freeze/thaw cycles is straightforward with DAISY analysis.

Figure 4. Differentiation based on optical properties



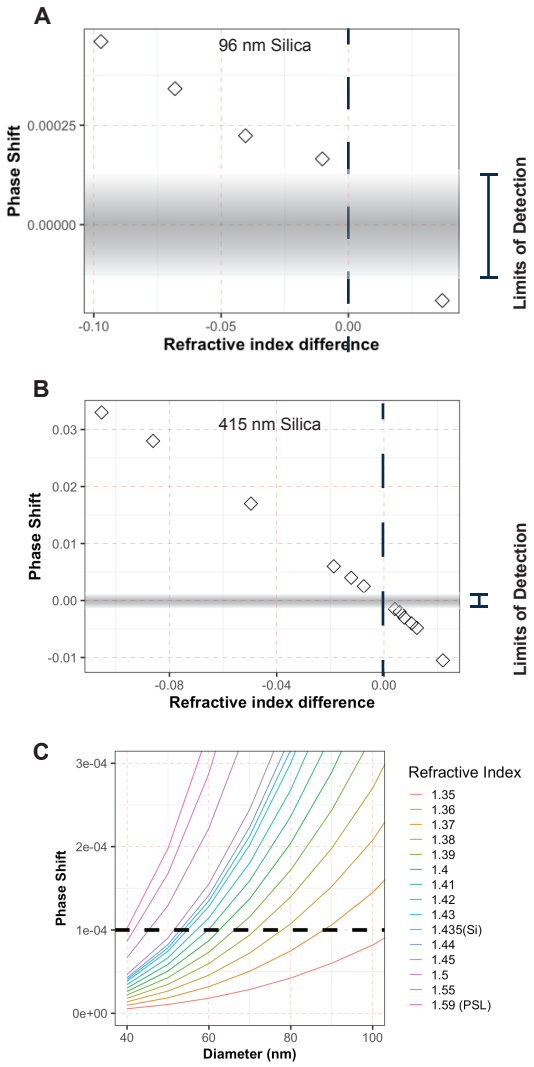
Differentiating different types of nanoparticle is an essential step in identifying their source, estimating the associated risk, and eliminating deleterious particles from the final product or sample. In biopharmaceutical products common contaminating particles besides protein aggregates can be silicon oils and micro or nano-plastics from contact surfaces[4]. DAISY-NTA allows easy differentiation between these types of particles based on differences in their refractive indices. The top panels show thermal aggregates of BSA either alone (left panel) or with a spike in of 415 nm silica particles (right panel). These particle types are readily differentiated by their measured refractive index. The lower panels show that even for particles with overlapping size distributions, their inherent mass distribution differences can be used to differentiate them according to the relationship between their optical size and their hydrodynamic size. Again silica particles are readily distinguished from protein aggregates.

Figure 5. Time course of aggregate formation and growth



Studying the kinetics of particle formation and growth in real time is easy with DAISY. The fine flow control of the DAISY instrument microfluidic system makes it easy to acquire data from the same sample over an extended time course. Particle formation and growth can be measured over multiple hours without any need to disturb the sample. An aggregating protein mixture was incubated at room temperature and recordings were taken across a time course of 2.5 hours. DAISY analysis shows the relationship between incubation time, particle concentration, and particle size throughout this incubation, without the need to change sample or disturb the apparatus.

Figure 6. Detection limits



As with any light scattering microscopy-based approach, the detection limits of DAISY are related to particle size and refractive index. Altering the refractive index of the surrounding media, and therefore the effective refractive index of the particles, objectively demonstrates the detection limits of our instrument.

Our experimentally determined detection limit, in terms of refractive index difference, for 96 nm particles is ~0.01 refractive index unit (**A**). For larger particles, such as 415 nm silica (**B**) this detectable refractive index difference drops to 0.005 refractive index units or below.

Combining this experimentally derived information with well validated optical simulations (**C**) demonstrates detection limits in terms of size for particles with a wide range of refractive indices.

For the detection of protein aggregates, which usually have a relatively low refractive index difference vs. water (between 0.02 and 0.05 refractive index units), the lower size limit for detection is in the range of 75 to 100 nm, depending on exact refractive index.

Rigorous experimental determination of precise detection limits allows researchers to make a fully informed choice about the suitability of DAISY-NTA for their samples.

References

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