Side-scattering spectroscopy of biological aggregates

Zita Salajkova[1,2*], *Lorenzo Barolo*[3], *Paola Baiocco*[3] , *Barbara Ruzicka*[4], *Alberto Boffi*[1,3], *Vincenzo Ricco*[2], *Giancarlo Ruocco*[1,4], *Marco Leonetti*[2,5]

[1]Center for Life Nano- and Neuro-Science, Istituto Italiano di Tecnologia, Viale Regina Elena 291, 00161, Rome, Italy
[2]D-TAILS srl, Via di Torre Rossa, 66, 00165, Rome, Italy
[3]Department of Biochemical Sciences "Alessandro Rossi Fanelli", Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy.
[4]Department of Physics, Sapienza University of Rome, Piazzale Aldo Moro, 5, 00185, Rome, Italy
[5]Institute of Nanotechnology of the National Research Council of Italy, CNR-NANOTEC, Rome Unit, Piazzale A. Moro 5, I-00185, Rome, Italy

Abstract. In this study, we introduce a novel optical setup tailored for measuring scattering spectra of small biological aggregates with minimal sample volumes. Calibration was achieved using Polystyrene beads (PS beads) based on Mie scattering principles, enabling accurate measurements of scattering intensities. Bovine serum albumin (BSA) served as a model for studying protein aggregation due to its predictable aggregation behaviour at elevated temperatures. Analysis of non-aggregated and aggregated BSA solutions revealed significant differences in particle size, underscoring the setup's capability to detect variations in aggregation states. Key findings demonstrate the system's efficacy in monitoring protein aggregation processes, which is critical for biochemical and pharmaceutical research. The precise calibration method and the use of BSA as a validation tool highlight the setup's sensitivity and accuracy in quantifying changes in particle concentration and size due to aggregation. This study provides a framework for analysing protein aggregation and offers insights into the aggregation's impact on scattering properties.

1 Side Scattering Spectroscopy

1.1 Experimental Setup Design.

The custom bench optical setup is designed for acquiring scattering spectra of small protein aggregates with minimal sample volumes, typically a few hundred microliters, as shown in Figure 1. Key to this setup is the subtraction of light source specular reflection from optical components to accurately measure the weaker scattering light within the same wavelength range. The setup features an OSL2 Fiber Illuminator (Thorlabs, Inc.) whose light is collimated and focused through a series of lenses and a pinhole, ensuring a homogeneous light spot of 0.6 mm diameter on the sample in a disposable capillary. A custom 3D-printed holder facilitates microliter volume analysis and capillary alignment. Light interaction with the sample is analysed through two paths, with back-reflected and side-scattered light collected for spectrum acquisition and focus monitoring, using a spectrometer setup for spectral image creation. Spectrum calibration is achieved with the use of short band filters and normalisation by black/white spectrum, ensuring accuracy in measurement while minimising liquid volume and light interference.

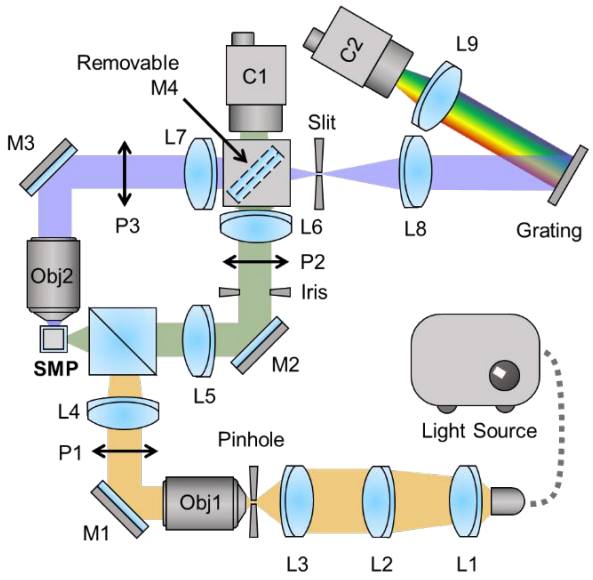

Fig. 1. Sketch of bench set-up: Light Source; 9 achromatic lenses with focal lengths of 25 mm (L1, L3), 100 mm (L2), 50 mm (L4, L8, L9) and 75 mm (L5, L6, L7); 2 objectives (Obj1) and (Obj2); 3 mirrors M1, M2 and M3; 3 linear polariser filter P1, P2 and P3; customised 3D printed capillary holder; Pinhole; Iris; Slit; Grating and 2 cameras (C1 and C2). All the optical components are suitable for the 400-700 nm wavelength range.

* Corresponding author: zita.salajkova@iit.it

1.2 Total Side Scattering Power Calculation.

The calibration of the system was performed using side scattering intensity measurements from solutions with varying concentrations of Polystyrene beads (PS beads), selected for their analytically calculable scattering cross-section via Mie scattering. Before actual measurements, Mie scattering calculations were conducted using the MiePlot v4.6 software [1]. These PS bead solutions were then examined with our side-scattering spectroscope to identify the concentration range where single scattering predominates, as indicated by a linear increase in intensity with PS bead concentration (see Fig. 2).

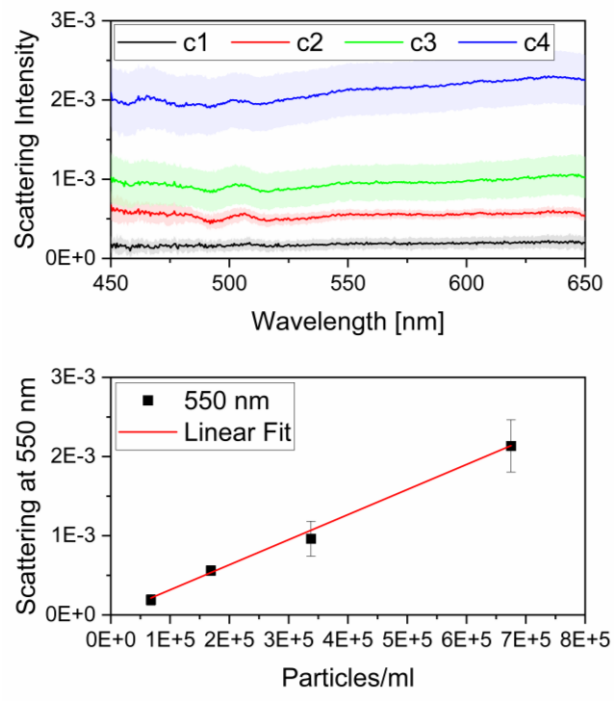

Fig. 2. Measured scattering intensity of PSB at 4 various concentrations: c1 = 2. 67500, c2 = 168750, c3 = 337500 and c4 = 675000 particles/ml. Bellow: the scattering at 550 nm shows the linear growth with particle concentration.

The scattering from a single bead was calculated, serving as a normalisation factor for subsequent analyses. This approach allowed for the quantification of measured intensity based on the scattering properties of the particles, as described by the following equation:

$$I_{exp}^{PSbeads} = n_{PSbeads} V I_{inc}\, \sigma_{90}^{PSbeads} \qquad (1)$$

where σ_{90} cross-section scattering at 90 degrees that was calculated by Mie theory, n particle concentration, V volume of solution participating in scattering, I_{exp} detected intensity, I_{inc} initial light intensity.

A similar equation can be written for the measured intensity of any scattering particles in the solution:

$$I_{exp}^{Protein} = n_{Protein} V I_{inc}\, \sigma_{90}^{Protein} \qquad (2)$$

where $\sigma_{90}^{Protein}$ and $n_{Protein}$ are unknown, and their value is aimed to be measured during the performed experiments. By combining eq.(1) and eq.(2), we obtain the multiplication of $\sigma_{90}^{Protein}$ and $n_{Protein}$, which we call total side scattering power Ω and it is defined by the following equation:

$$\Omega = \sigma_{90}^{Protein} n_{Protein} =$$

$$= \frac{I_{exp}^{Protein} n_{PSbeads} \sigma_{90}^{PSbeads}}{I_{exp}^{PSbeads}} = I_{exp}^{Protein}\, k \qquad (3)$$

where k is the constant assigning the measured intensity value physical meaning

2 BSA scattering power

We used bovine serum albumin (BSA) solutions at fixed concentrations to study its aggregation, chosen for BSA's rapid and controllable aggregate formation at high temperatures [2]. Our research involved two types of BSA solutions: non-aggregated and aggregated (after 5 hours at 63°C). DLS measurement reviled that the radius of non-aggregated BSA was (4.43 ± 0.03) nm, while aggregated BSA measured (10.9 ± 0.7) nm. We then acquired the total side scattering for these samples, preparing each solution in triplicate for accuracy and calculating the standard deviation. As shown in Fig. 3, spectral intensity increases with aggregation, particularly at lower wavelengths. This indicates that our method effectively monitors aggregation, with wavelength data reflecting the size and quantity of scatterers.

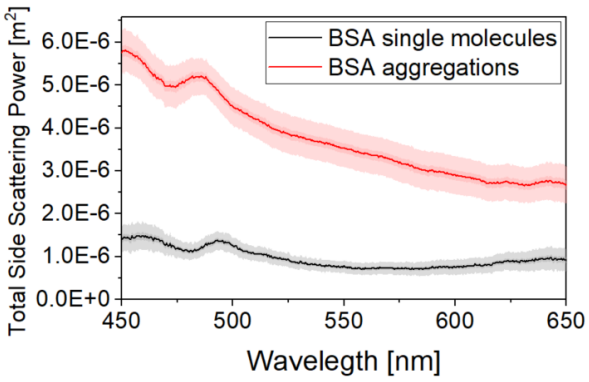

Fig. 3. Total side scattering power Ω of BSA and its aggregated state.

References

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