STANDARD OPERATING PROCEDURE (DLS#1_ProtStab_Gre2p)					
IBG-1 KIT Karlsruher Institut für Technologie		**Institute for Biological Interfaces** IBG-1 | Biomolecular Micro- and Nanostructures			
Short Title	Protein stability DLS	**Page**	1	**of**	6
Title	Measure the homogeneity of protein samples using dynamic light scattering (DLS)				
Version	1.0		**Created on**	26.04.2021	
			Related SOP	SOP_UVVIS#2_SpAc_Gre2p SOP_ITC#1_General	
Safety instructions	By following the instructions in this SOP you confirm that you have checked the SDS-sheet of the involved chemicals (also reagents of your specific enzyme system) and will protect yourself accordingly when working with these substances. Also, you confirm that you will follow standard lab safety procedure to protect yourself, others, and the environment.		**Category**	PROTOCOL	
			Purpose	describe how to perform experiments to measure the homogeneity of protein samples after different treatments using DLS.	
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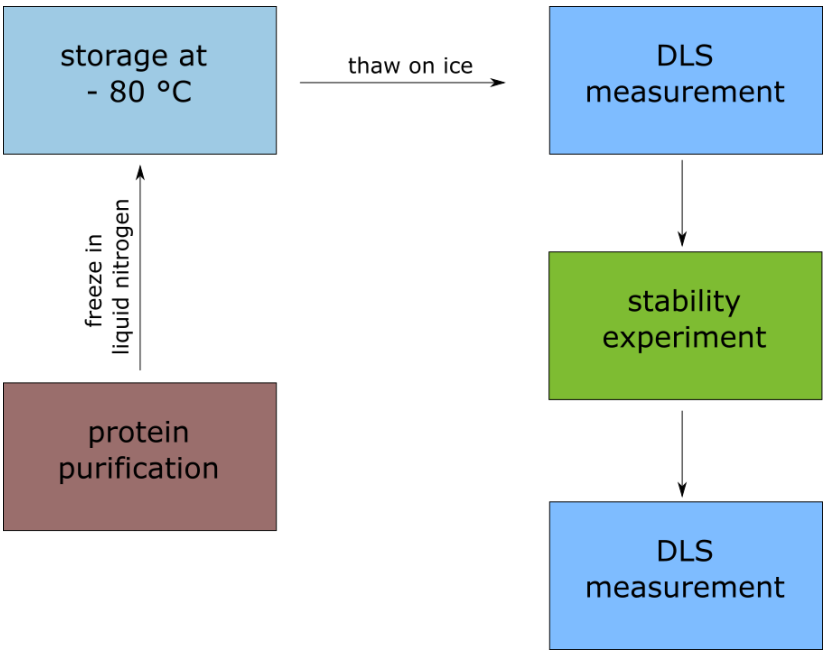

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1) Introduction

The homogeneity (not purity) of enzyme samples can be measured using DLS using a Zetasizer Nano ZSP (Malvern Panalytical, Malvern, Worcestershire, United Kingdom). Z-average and polydispersity index can be used as indicators of sample stability under different conditions, and are indicators of sample quality. If the sample is very heterogenous, or its homogeneity changes considerably before and after storage under different storage conditions, it is advisable to also determine activity of the sample (e.g. using SOP_UVVIS#2_SpAc_Gre2p) to ensure you are working with functional enzyme. Especially if you perform highly sensitive experiments with your enzyme, e.g. ITC experiments (see SOP_ITC#1_General), DLS is a valuable tool to determine sample quality.

2) Preparation

- Prepare the desired buffer and filter it using a bottle top filter with a 0.45 µm pore size SFCA membrane (e.g. Nalgene™ Rapid-Flow™ Sterile single Use Bottle Top Filters, ThermoFisher Scientific, Waltham, MA, USA)
- Thaw stored enzyme/protein on ice
- Dilute the enzyme/protein in buffer to a concentration of 50 µM (you need 200 µL for a total of two measurements, so prepare 200 µL minimum but 300 µL preferably)
- Prepare a DLS experiment using the Zetasizer Nano ZSP (Malvern, currently located in lab 235); 13.01.2021) with the following parameters by clicking "Measure → Manual" and the different categories presented on the left.

Table 1: Experimental parameters using the Zetasizer Nano ZSP. The parameters are suitable for a protein sample dissolved in water.

Parameter		**Value**
Material		Protein (RI: 1.450; Absorption: 0.001)
Dispersant		Water (Viscosity: 0.8872 cP; RI: 1.330)
Temperature		25 °C
Equilibration time (s)		120
Cell type		Disposable cuvettes (ZEN0040)
Measurement angle		173° Backscatter
Measurement duration		Automatic
Number of measurements		3
Delay between measurements (s)		0
Positioning method		Seek for optimum position
Automatic attenuation selection		Yes
Analysis model		General purpose (normal resolution)
Mark-Houwink Parameters	A Parameter	0.428
	K Parameter (cm^2/s):	7.67e-05

The default value was used for parameters not mentioned in the table. Some values mentioned in the table are default parameters.

3) Experimental procedure

- Perform triplicate measurements:
    a. Sample 1: Split the volume of 300 µL into two
        i. Measure DLS with 100 µL ("before treatment" sample)
            - Treat the other 200 µL: e.g. Shaking (500 rpm) at 25 °C)
    a. Sample 2: Split the volume of 300 µL into two
        i. Measure DLS with 100 µL ("before treatment" sample)
            - Treat the other 200 µL: e.g. Shaking (500 rpm) at 25 °
    b. Sample 3: Split the volume of 300 µL into two
        i. Measure DLS with 100 µL ("before treatment" sample)
            - Treat the other 200 µL: e.g. Shaking (500 rpm) at 25 °C
- After the treatment (approx. 40 minutes), 100 µL of the treated sample is measured in the same way as described below.

Measurement:

1. Set parameters for the measurements to match the parameters in Table 1. Every measurement takes approx. 10 minutes.
2. Pipet 100 µL of the enzyme solution into a UV-cuvette (e.g. by Brand, micro, centre height 8,5 mm "UV-Küvette mikro, Zentrumshöhe 8,5 mm, Füllvolumen 70 µL bis 850 µl" (Figure 1))
3. Insert the cuvette in the DLS-Instrument (Malvern, Zetasizer Nano ZSP) with the arrow/triangle on the top of the cuvette (see photo, marked in red) facing towards you and close the lid of the Instrument

Figure 1: Cuvette used for the experiments. The arrow/triangle to indicate the correct orientation is marked red. This arrow/triangle has to face the user when inserting the cuvette.

Other possible treatments are: Storage on ice, Storage at room temperature, Stirring (500 rpm) at RT (performed in 2 mL reaction tubes with 12 - 15 mm long stir bars), or incubation with buffer containing different amounts of tween (0.0 %, 0.01% , 0.1% and 1.0%).

4) Analysis

- The values which are necessary for analysis are:
  - Z-average (the primary result)
  - Polydispersity index (PDI)
- Data quality:
  - Cumulants Fit Error (necessary for sorting out measurements of bad quality. Use only measurements with a Cumulants Fir Error < 0,05)
  - Intercept (necessary for sorting out measurements of bad quality. Use only measurements with an intercept > 0,5)

Before calculation the means and standard deviation of the measured z-averages or PDIs, the measurements of poor quality (“data quality”) are discarded.

5) Troubleshooting

- When the cumulants fit error is too high or the Intercept too low you can try the following:
  - Make sure that the cuvette is oriented correctly
  - Use a different cuvette
  - Was the buffer you are using filtered?

Activities of the treated sample can be determined using **SOP_ UVVIS#1_SpAc_Gre2p.**