

ASSESSMENT OF SAMPLE QUALITY WITH EVERY MEASUREMENT - FIDA FUNDAMENTALS

Kritika Sahni Ray, Dr. rer. nat.

Introduction

Protein quality control (QC) is the most crucial checkpoint of any protein production and characterisation process. On a frequent basis, many experiments end up unsuccessfully due to low quality of protein sample. This leads not only to generation of non-conclusive datasets but also costs loss of significant amount of time on troubleshooting assay design without any reliable insight into the protein itself. It is therefore highly valuable to gain in-depth confirmation about the protein's state. Historically, in order to gain insights into the state and condition of a given protein sample, a set of different techniques needed to be employed prior to running an assay.

Luckily, new technologies allow to streamline the process, thanks to broad and easy sample quality characterisation. The Fida instrument offers great advantage for deciphering a wide array of QC parameters such as absolute size, aggregation status, labelling efficiency, stickiness, viscosity and sample polydispersity in a labelled or label-free manner. With Flow Induced Dispersion Analysis (FIDA), all these parameters are obtained with every measurement, consuming only nL amount of sample. Altogether, the Fida instrument offers an integrated assessment of sample quality parameters with complete flexibility on choice of buffer composition or even complex matrices, and hence enables robust assay development which can be a challenge with other technologies.

Material and Methods

Experiments were performed on a **Fida Neo** instrument employing 280 nm LED-UV fluorescence and 480 nm LED-fluorescence detection using a high-sensitivity coated capillary (Fida Biosystems). Each data point consumed only 39 nL of protein sample with an analysis time of 5 min. Data analysis was conducted using the Fida software (V 3.0).

Results

FIDA delivers a range of biophysical parameters from a single assay format. This includes direct assessment of protein's state by means of absolute hydrodynamic radius (R_h) measurement, along with additional sample features reflecting the sample quality. With every Fida Neo measurement, clear indications for sample loss, aggregation, viscosity, stickiness, polydispersity, labelling quality and buffer mismatch are obtained.

Specifically, Fida Neo Quality Control Module was used to perform the task in order to streamline the process. The QC Module's dashboard provides an easy-to-grasp overview of all the QC parameters, and allows for data export in two formats. First, PDF reports include graphs and QC overviews ideal for interteam coordination or performance reporting. Second, exporting raw .txt files allows to smoothly move the data to any data analysis software. Both solutions have positive practical implications on quality control workflows.

- Labelling quality
- Absolute size (hydrodynamic radius, R_h)
- Multiple species
- Stickiness
- Aggregation status
- Viscosity
- PDB correlator
- Polydispersity

Conclusions

The Fida instrument enables assessment of sample quality using only 40 nL of sample in under 5 minutes measuring time. Sample parameters like in-solution size, aggregation, stickiness, polydispersity and viscosity can be obtained with every measurement. Unlike other technologies where such sample attributes pose challenges in assay designing and obscure data interpretation, the Fida instrument highlights sample issues and provides users with an opportunity to strategically resolve them.

