Flow Induced Dispersion Analysis (FIDA) quantifies proteins, protein-ligand interactions and immune responses under native conditions

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Aim: In this work we demonstrate the application of FIDA (Flow Induced Dispersion Analysis) for quantification of proteins and protein –ligand interactions under ***native*** conditions using nano-microliter samples amounts.

FIDA is a novel capillary-based technology for assessing protein concentration in complex solutions (e.g. plasma samples) and for measuring in-solution binding under native conditions. FIDA is based on quantifying Taylor dispersion in a pressure driven flow of a ligand (indicator molecule) interacting with the protein of interest (e.g. an antibody-based drug). The indicator appears small (i.e. it has a high apparent diffusivity) when it is not bound to the antibody, but upon binding it will appear larger (i.e. it has a lower apparent diffusivity). The change in apparent diffusivity/size forms the basis for an accurate measure of protein concentration and interaction.[1,2]

In this presentation, FIDA is demonstrated for assessing in-solution protein concentration, quantification of an antibody-based drug compound (sub-nanomolar sensitivity), and for detection of immune responses in patients. The detection of immune responses constitutes a key element in relation to immunogenicity testing of protein-based drugs and for diagnosis of autoimmune diseases. Therefore, FIDA is demonstrated for rapid (minutes) measurement of autoantibodies against dsDNA (a diagnostic marker for Systemic Lupus Erythematosus, SLE) in patient samples[3].

Conclusions: FIDA may be used as an alternative to ELISA and Surface Plasmon Resonance (SPR) based methodologies for protein quantification and for measuring protein interactions such as immunogenicity of biopharmaceuticals under ***native*** conditions using nano- to microliter sample amounts. FIDA is performed on a fully automated platform resembling those used for LC and CE (accepting vials and 96 well plates).

[1] N. N. Poulsen, et al, *Analyst*, 2015, **140**, 4365-4369. [2] Henrik Jensen and Jesper Østergaard; *Analyte Quantification using Flow Induced Dispersion Analysis*, EP2542885B1, US9310359, US20130059313, WO2011107406A1. National Phase. [3] N. N. Poulsen, et al, *Anal. Chem.*, 2016, **88**, 9056-9061.