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Flow Induced Dispersion Analysis for Probing non-Covalent Interactions and Quantifying large Biomolecules: A new Approach to Ligand Binding Assays

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Abstract: Flow Induced Dispersion Analysis (FIDA) is a new method for quantification of proteins and affinity constants under native conditions in nanoliter volumes. In the assay, the size of a small ligand (indicator) known to bind the protein (analyte) is measured. When the indicator is bound by the analyte the apparent size increases and this change in size can be used to estimate the concentration of protein in the sample. The FIDA assay has pico-nano molar sensitivity and may be completed in minutes. Using assay development software, the development of new assays is fast and requires very limited experimental optimization. In addition to affinity and concentration, FIDA also provides info on hydrodynamic radius of ligand, analyte and complex.

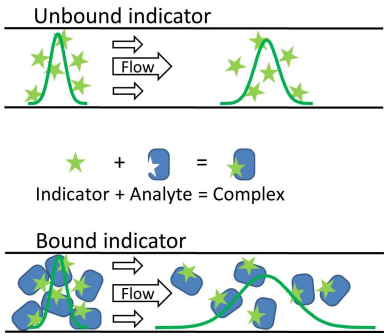


Figure 1. The FIDA principle is based on measuring the change in the apparent size (diffusivity) of an indicator (specific ligand) interacting with the analyte. The apparent indicator size is measured in a hydrodynamic flow system by Taylor dispersion analysis.

Figure 2. The dedicated FIDA instrument is fully automated, and has in-built assay evaluation tools. The FIDA analyser accepts vials and 96 well plates.

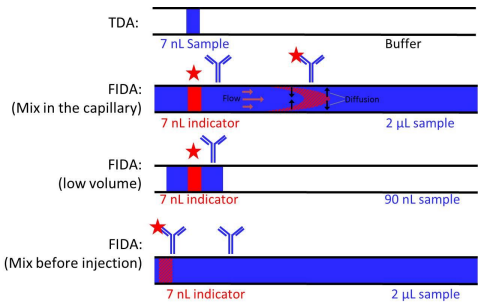


Figure 3. Injection and mixing procedure in the FIDA assay. Each step is fully automated using dedicated instrumentation.

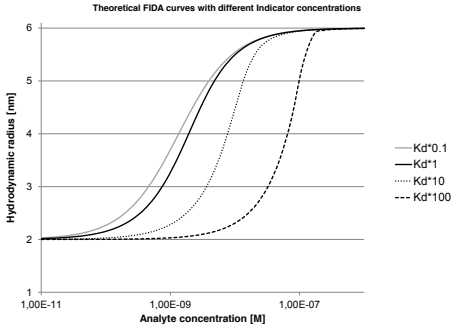


Figure 4. Theoretical binding curved calculated using FIDA software for different values of indicator concentration. The software allows *in silico* assay development resulting in fast assay development with limited experimental optimization.

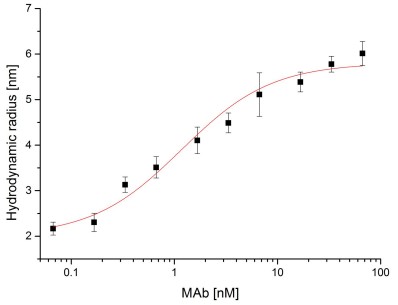
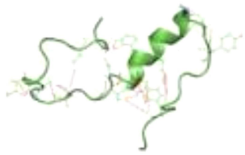


Figure 5. Apparent size of the Abeta-40 peptide as a function of the concentration in 10% plasma of Gantenemumab (an investigative antibody based drug compound to treat Alzheimers disease). The binding constant obtained in the automated FIDA protocol correlates well with other techniques based on e.g. SPR. The dynamic range for quantifying Gantenemumab is 0.4-10 nM for an indicator Abeta concentration of 5 nM.



The A-beta peptide is responsible for development of plaques (aggregates) leading to Alzheimers disease

Conclusion. The FIDA methodology is a new approach for rapid measurement of protein concentration and affinity constants under native conditions. FIDA is characterized by being fast (minutes), by only consuming nanoliter sample material, featuring rapid assay devleopment (thanks to *in silico* assay development software) and using dedicated instrumentation the entire assay protocol is fully automated. In this study the FIDA assay was used for determine the affinity constant between Abeta and Gantenemumab under native conditions. The binding curve may be used to quantify Gantenemumab in plasma in concentrations ranging from 0.4 – 10 nM.

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