

# Assessing In Solution Binding Kinetics with $\mu\text{L}$ Sample Volume Using Flow-Induced Dispersion Analysis (FIDA)

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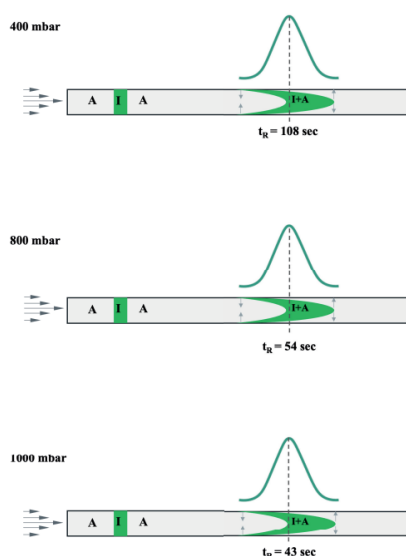
Understanding binding kinetics is crucial in the development and characterization of protein-based drugs. Currently, state-of-the-art methods for assessing binding kinetics rely on surface-based technologies such as Bio-Layer Interferometry (BLI) and Surface Plasmon Resonance (SPR). However, surface immobilization can pose challenges, requiring optimization of surface chemistries, and slow off-rates may result in inadequate surface regeneration.

Here, we introduce a novel in-solution methodology, Flow-Induced Dispersion Analysis (FIDA), designed to measure binding kinetics using only nano-microliters of samples. This methodology can be applied to any 1-1 protein interaction within various liquid sample matrices, including human serum or plasma. Its setup is straightforward, and both measurements and analysis are fully automated.

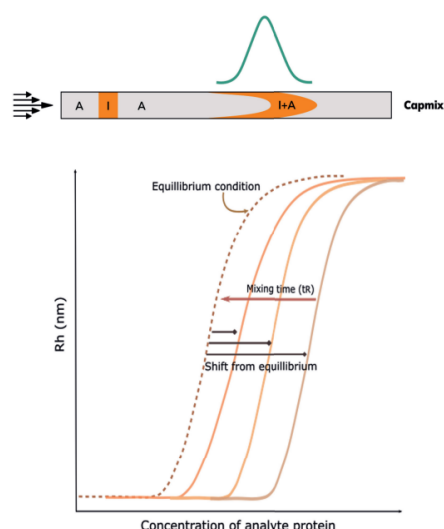
In addition to providing information on binding kinetics ( $k_{\text{on}}$  and  $k_{\text{off}}$ ), FIDA also yields equilibrium binding constants ( $K_D$ ) and hydrodynamic radius ( $R_h$ ). Data obtained through FIDA demonstrate good agreement with results obtained using SPR.

This approach offers a promising surface free alternative for assessing binding kinetics with minimal sample volume, paving the way for advancements in protein-based drug development and characterization.

## a. Fida capillary mix assay



## b. Effect of manipulating in-capillary reaction times.



**Figure 1** Principle of FIDA in-solution kinetics. The indicator is mixed with the analyte inside a thin microfluidic capillary (a). Reaction time can be controlled by varying pressure (b).