Fidabio

APPLYING FIDA FOR IN-SOLUTION BINDING KINETICS

Book a discovery call

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Binding kinetics is an integrated part of developing and characterising protein based drugs. Current state of the art for binding kinetics includes surface based technologies based on BLI and SPR.

Surface immobilisation may be challenging as surface chemistries need optimisation and slow off rates can lead to poor surface regeneration.

Here we present a new in-solution methodology (FIDA) for measuring binding kinetics using only nano-to-microliter of samples. The methodology can be applied to any 1-1 protein interaction in any liquid sample matrix. It is easy to set up and the measurements and analysis is fully automated.

In addition to binding kinetics (k_{on} and k_{off}) it also reports equilibrium binding constants (K_D) and hydrodynamic size (R_h). The data obtained using the new FIDA methodology are in good agreement with SPR.

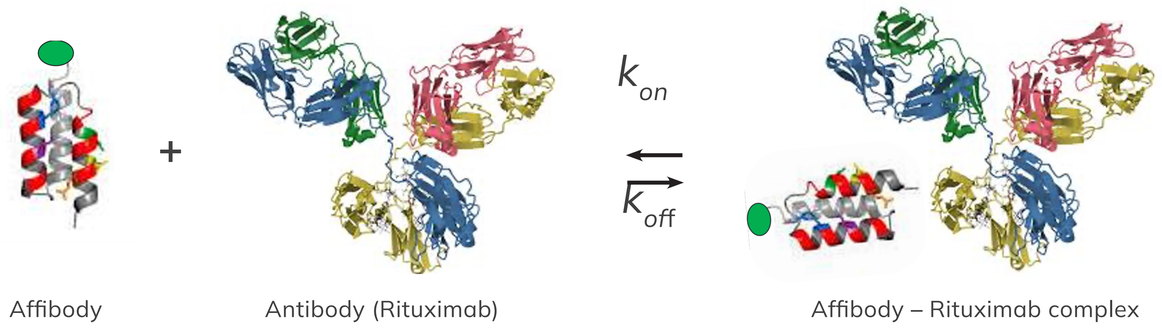


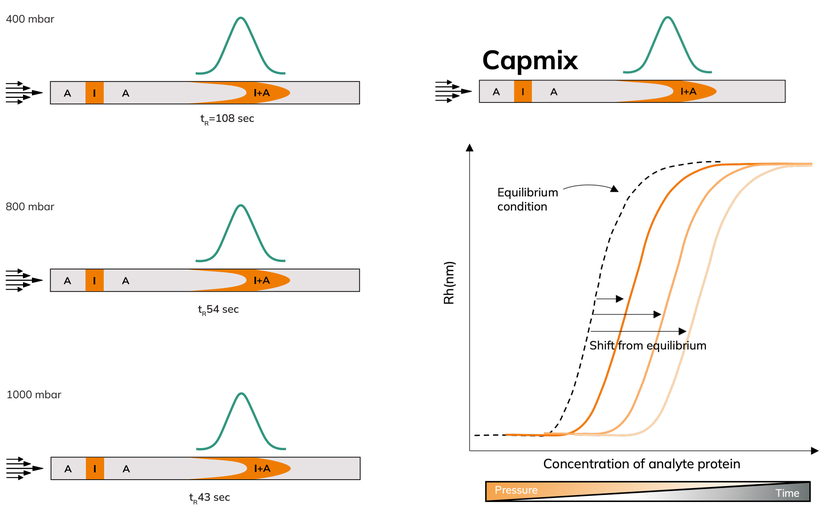
Figure 1: The model system is composed of an Affibody (indicator) binding the constant region of an antibody (Analyte/Rituximab). The affibody is fluorescently labelled.

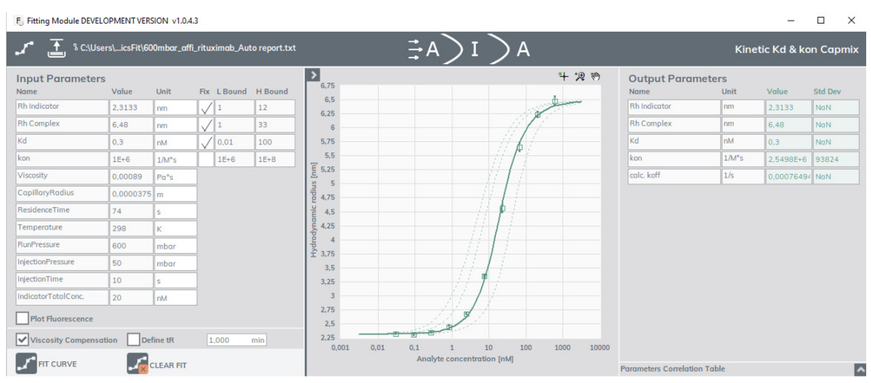
The underlying principle for FIDA in-solution kinetics.

A. Fida capillary mix assay

B. Effect of manipulating in-capillary reaction times.

C. Dedicated kinetic data analysis module.





The indicator is mixed with the analyte inside a thin microfluidic capillary. The indicator/analyte mixture is passed through the capillary by pressure and detected at a fixed point by fluorescence detection.

The detection time can be varied using different pressures (a).

The fraction bound of the indicator changes depending on concentration, kinetic constants, and K_D (b).

The Fidabio data analysis software module fits the data to determine the kinetic rate constants (c).

Figure 2: The underlying principle for FIDA in-solution kinetics.

Comparison of Fida and SPR kinetics data

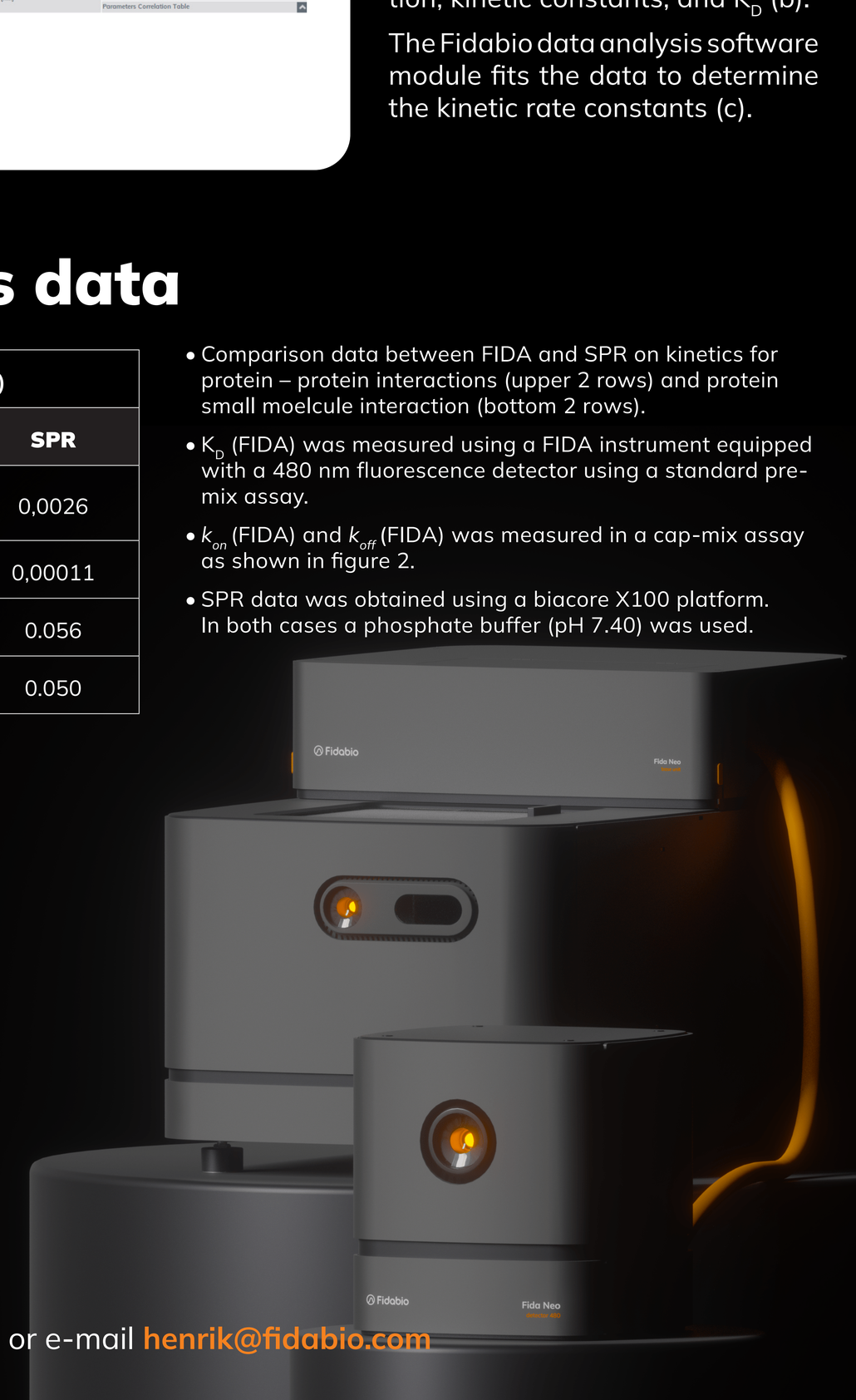
	K_D (nM)		k_{on} ($M^{-1}s^{-1}$)		k_{off} (s^{-1})	
	FIDA	**SPR**	**FIDA**	**SPR**	**FIDA**	**SPR**
ß2-microglobulin – anti-ß2-microglobulin	1.00	2.30	2.19×10^6	1.1×10^6	0,0022	0,0026
Affibody – Rituximab	0.30	0.24	2.60×10^6	4.7×10^5	0,00076	0,00011
Carbonic anhydrase – AZA	22.1	19.0	1.54×10^7	2.9×10^6	0,034	0.056
Carbonic anhydrase – Furosemide	256	513	2.99×10^4	9.65×10^4	0.0078	0.050

- Comparison data between FIDA and SPR on kinetics for protein – protein interactions (upper 2 rows) and protein small moelcule interaction (bottom 2 rows).
- K_D (FIDA) was measured using a FIDA instrument equipped with a 480 nm fluorescence detector using a standard pre-mix assay.
- k_{on} (FIDA) and k_{off} (FIDA) was measured in a cap-mix assay as shown in figure 2.
- SPR data was obtained using a biacore X100 platform. In both cases a phosphate buffer (pH 7.40) was used.

Results for model systems agrees well with SPR data
Causes for Deviations: (1) Structural integrity may be compromised by surface immobilization;
(2) Different biophysical technologies can generate different data

FIDA Kinetics - The Key Points

- In solution kinetics in nanolitre to microliter samples (no surface immobilization required)
- Rapid assay development – also in complicated sample matrixes!
- Simultaneous R_h (binding stoichiometry), fluorescence intensity and QC parameters in every measurement.
- Accessible kinetics : 5-10 seconds (estimated) to hours (half lives).
- Rapid k_{off} determination – no surface regeneration needed.



For more information, please visit: www.fidabio.com or e-mail henrik@fidabio.com