



APPLYING FIDA FOR

IN-SOLUTION BINDING KINETICS

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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.

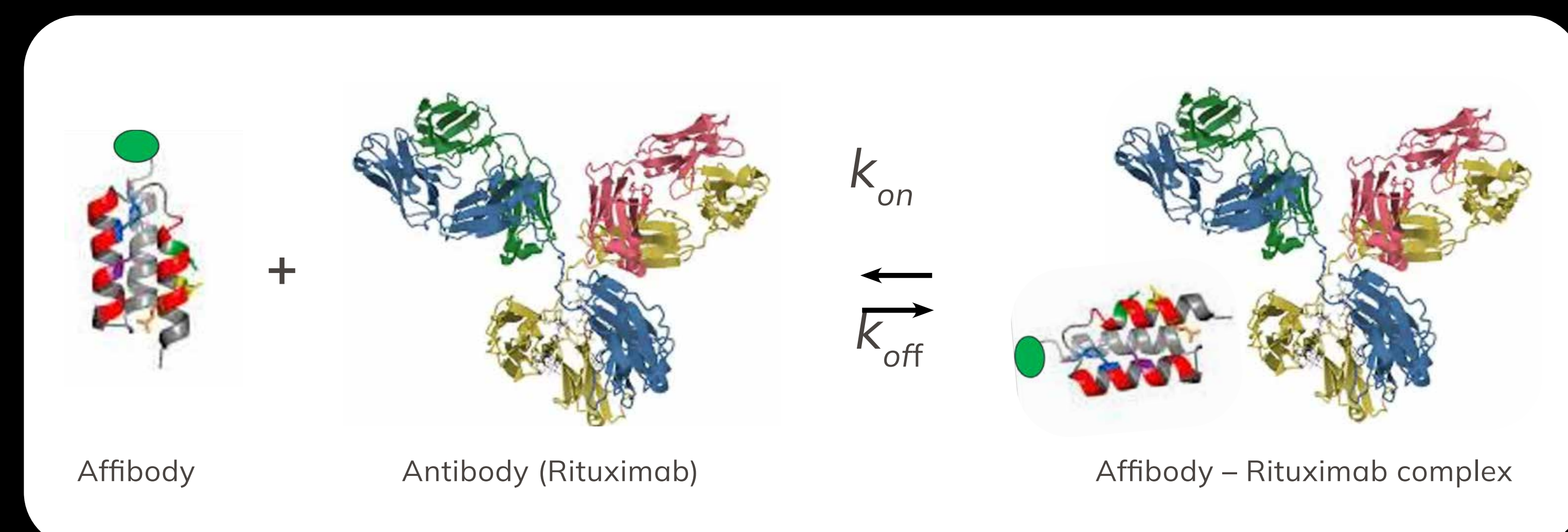
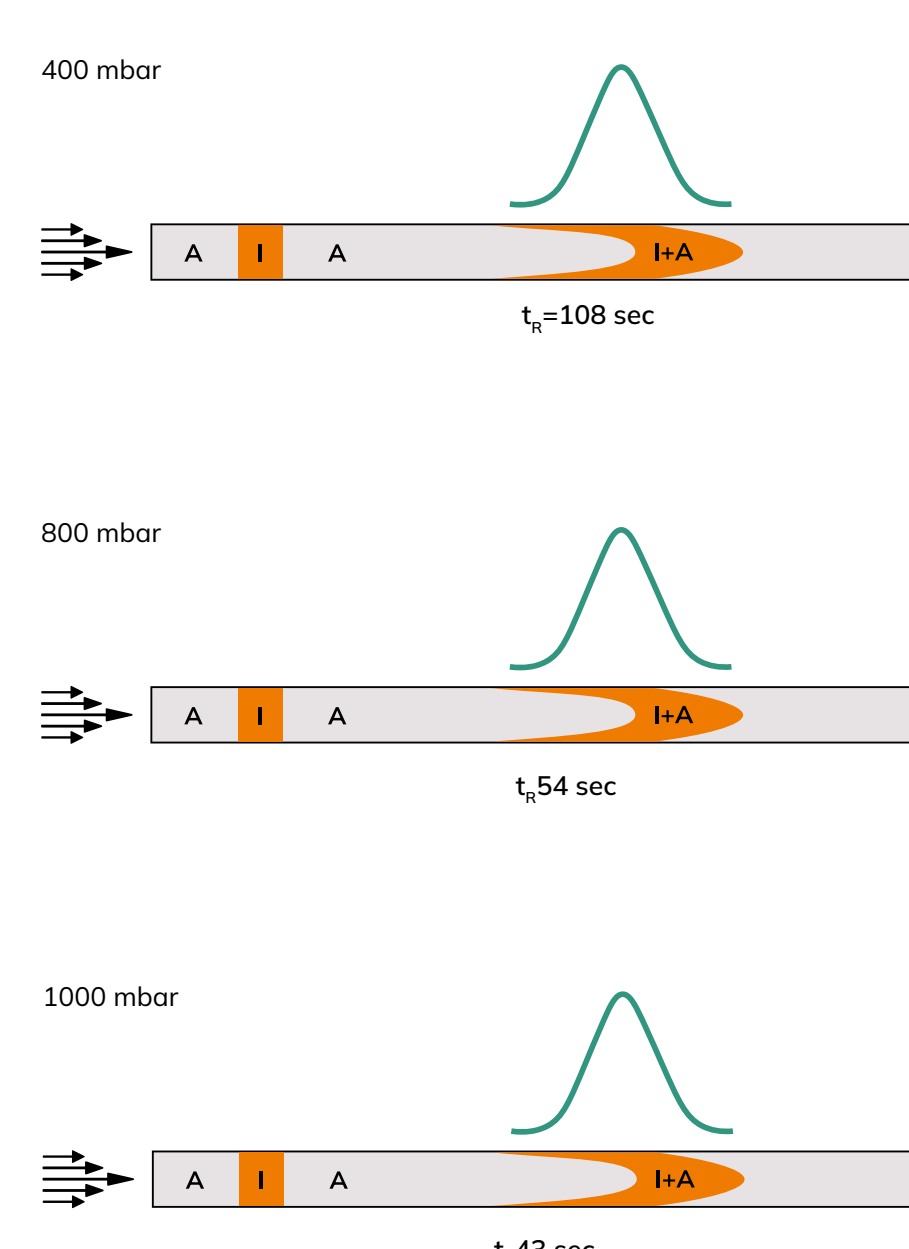


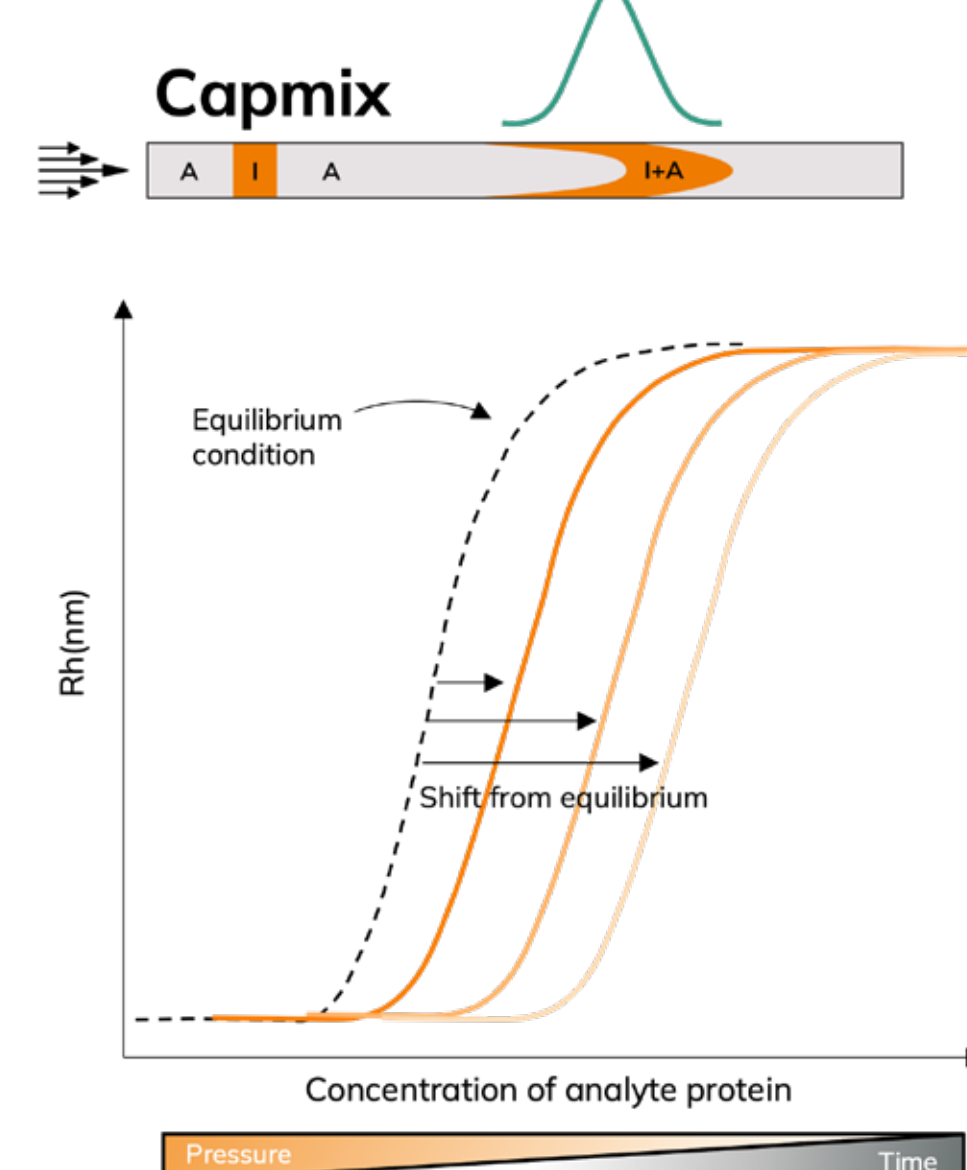
Figure 1: The model system is composed of an Affibody (indicator) binding the constant region of an antibody (Analyte/Rituximab). The affibody is fluorescently labelled.

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).

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
Affibody - Rituximab	0,30	0,24	$2,6 \times 10^6$	$4,7 \times 10^5$	0,00076	0,00011
Protein 1 – Protein 2	6,7	18	$6,37 \times 10^5$	$2,5 \times 10^5$	0,00446	0,004

Comparison data between FIDA and SPR on kinetics for protein – protein interactions.

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.

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_b (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.

