# Supporting information

# In-solution IgG titer determination in fermentation broth using affibodies and flow induced dispersion analysis

Morten E. Pedersen[1,2], Jesper Østergaard[2], and Henrik Jensen[1,2]

*[1]FIDA Biosystems ApS, Fruebjergvej 3, 2100 Copenhagen, Denmark,*

*[2]Department of Pharmacy, University of Copenhagen, Universitetsparken 2, 2100 Copenhagen, Denmark.*

Currently, the FIDA binding isotherm has only been reported for 1:1 binding stoichiometries described by the following equilibrium:

$$\mathrm{IA} \rightleftharpoons \mathrm{I} + \mathrm{A} \tag{S1}$$

where IA is the complex between the indicator I (fluorescently labeled affibody in the present work) and the analyte A (Rituximab in the present work). Assuming low indicator concentration and fast kinetics, the binding isotherm can be expressed as[1]:

$$R_{\mathrm{app}} = \frac{1 + \frac{1}{K_{\mathrm{d}}}[\mathrm{A}]}{((R_{\mathrm{I}})^{-1} - (R_{\mathrm{IA}})^{-1}) + \left(1 + \frac{1}{K_{\mathrm{d}}}[\mathrm{A}]\right)(R_{\mathrm{IA}})^{-1}} \tag{S2}$$

where $R_{\mathrm{app}}$ , $R_{\mathrm{I}}$, and $R_{\mathrm{IA}}$ are the apparent, indicator and complex hydrodynamic radii, respectively, $K_{\mathrm{d}}$ is the dissociation constant and [A] is the actual analyte concentration. A low indicator concentration ensures that the actual and formal analyte concentrations are identical under all conditions ($C_{\mathrm{A}}$ = [A], where $C_{\mathrm{A}}$ is the formal analyte concentration).

**Excess indicator model**

In the present case, the affinity between the affibody and antibody is high and consequently the actual and formal analyte concentrations are not identical under all conditions. It is, however, still possible to model the binding curve as described below.

The expression $K_{\mathrm{d}}$ corresponding to S1 can be written as.

$$K_{\mathrm{d}} = \frac{[\mathrm{I}] \cdot [\mathrm{A}]}{[\mathrm{IA}]} \tag{S3}$$

where, [I], [A] and [IA] are the actual indicator, analyte and complex concentrations, respectively.

In terms of formal concentrations of $I$ ($C_{\mathrm{I}}$), $A$ ($C_{\mathrm{A}}$), $K_{\mathrm{d}}$ can be written as:

$$K_{\mathrm{d}} = \frac{(C_{\mathrm{I}} - [\mathrm{IA}]) \cdot (C_{\mathrm{A}} - [\mathrm{IA}])}{[\mathrm{IA}]} \tag{S4}$$

The indicator fraction not bound is termed $x$ and given by:

$$x = \frac{[\mathrm{I}]}{([\mathrm{I}] + [\mathrm{IA}])} = \frac{(C_{\mathrm{I}} - [\mathrm{IA}])}{C_{\mathrm{I}}} \tag{S5}$$

The fraction bound is 1 - *x*, and can be expressed as:

$$1 - x = \frac{[\mathrm{IA}]}{C_\mathrm{I}} \tag{S6}$$

By combining equation S4-S6, $K_\mathrm{d}$ can be expressed as:

$$K_\mathrm{d} = \frac{(x \cdot C_\mathrm{I}) \cdot (C_\mathrm{A} - (1-x) \cdot C_\mathrm{I})}{(1-x) \cdot C_\mathrm{I}} \tag{S7}$$

Equation S7 is a 2. order polynomial equation with respect to *x*, from which the following expressions for *x* and (*1-x*) can be obtained:

$$x = \frac{(C_\mathrm{I} - C_\mathrm{A} - K_\mathrm{d}) + \sqrt{(C_\mathrm{I} + C_\mathrm{A} + K_\mathrm{d})^2 - 4C_\mathrm{A} \cdot C_\mathrm{I})}}{2C_\mathrm{I}} \tag{S8a}$$

$$(1 - x) = \frac{(C_\mathrm{I} + C_\mathrm{A} + K_\mathrm{d}) - \sqrt{(C_\mathrm{I} + C_\mathrm{A} + K_\mathrm{d})^2 - 4C_\mathrm{A} \cdot C_\mathrm{I})}}{2C_\mathrm{I}} \tag{S8b}$$

As previously described[S1], the measured apparent hydrodynamic radius (eq S9) can be expressed as a weighted average of the fractions of bound and unbound indicator:

$$R_\mathrm{app} = ((R_\mathrm{I})^{-1} \cdot x + (R_\mathrm{IA})^{-1} \cdot (1 - x))^{-1} \tag{S9}$$

Combining equation S8a, S8b and S9 provides the excess indicator binding isotherm:

$$R_\mathrm{app} = \left( \begin{array}{l} (R_\mathrm{IA})^{-1} \cdot \left( \frac{(C_\mathrm{I} + C_\mathrm{A} + K_\mathrm{d}) - \sqrt{(C_\mathrm{I} + C_\mathrm{A} + K_\mathrm{d})^2 - 4C_\mathrm{A} \cdot C_\mathrm{I})}}{2C_\mathrm{I}} \right) \\ +(R_\mathrm{I})^{-1} \cdot \left( \frac{(C_\mathrm{I} - C_\mathrm{A} - K_\mathrm{d}) + \sqrt{(C_\mathrm{I} + C_\mathrm{A} + K_\mathrm{d})^2 - 4C_\mathrm{A} \cdot C_\mathrm{I})}}{2C_\mathrm{I}} \right) \end{array} \right)^{-1} \tag{S10}$$

**Development of FIDA method**

When the indicator concentration is in the same range as the $K_d$, it is expected that the fraction bound will be dependent on the indicator concentration[S2]. The is experimentally confirmed in the present case where a shift in the binding curve is observed upon changing the affibody concentration from 20 nM to 5 nM (figure S1).

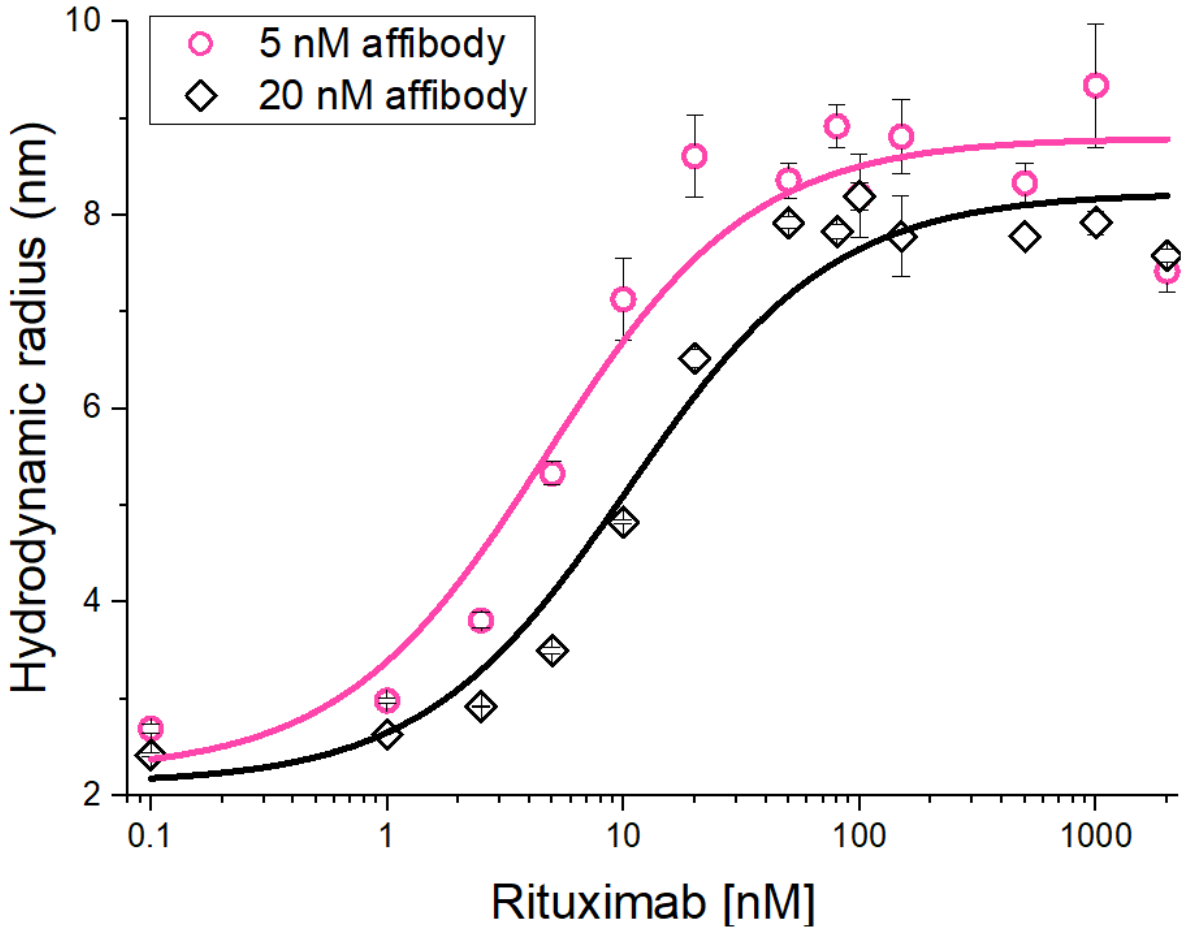


*Figure S1: Apparent hydrodynamic radius of anti-IgG affibody-alexa488 as function of rituximab concentration (0-2000 nM) determined by FIDA at 25 °C. The black open diamonds and magenta open circles represent measurements with 5 and 20 nM anti-IgG affibody-alexa488, respectively (n = 3, error bars represent standard deviation). The solid black line and solid magenta line represents fitting to the 1:1 binding isotherm (eq S2).*

The area of the affibody signal was observed to depend on the fermentation broth concentration as seen in figure S2.

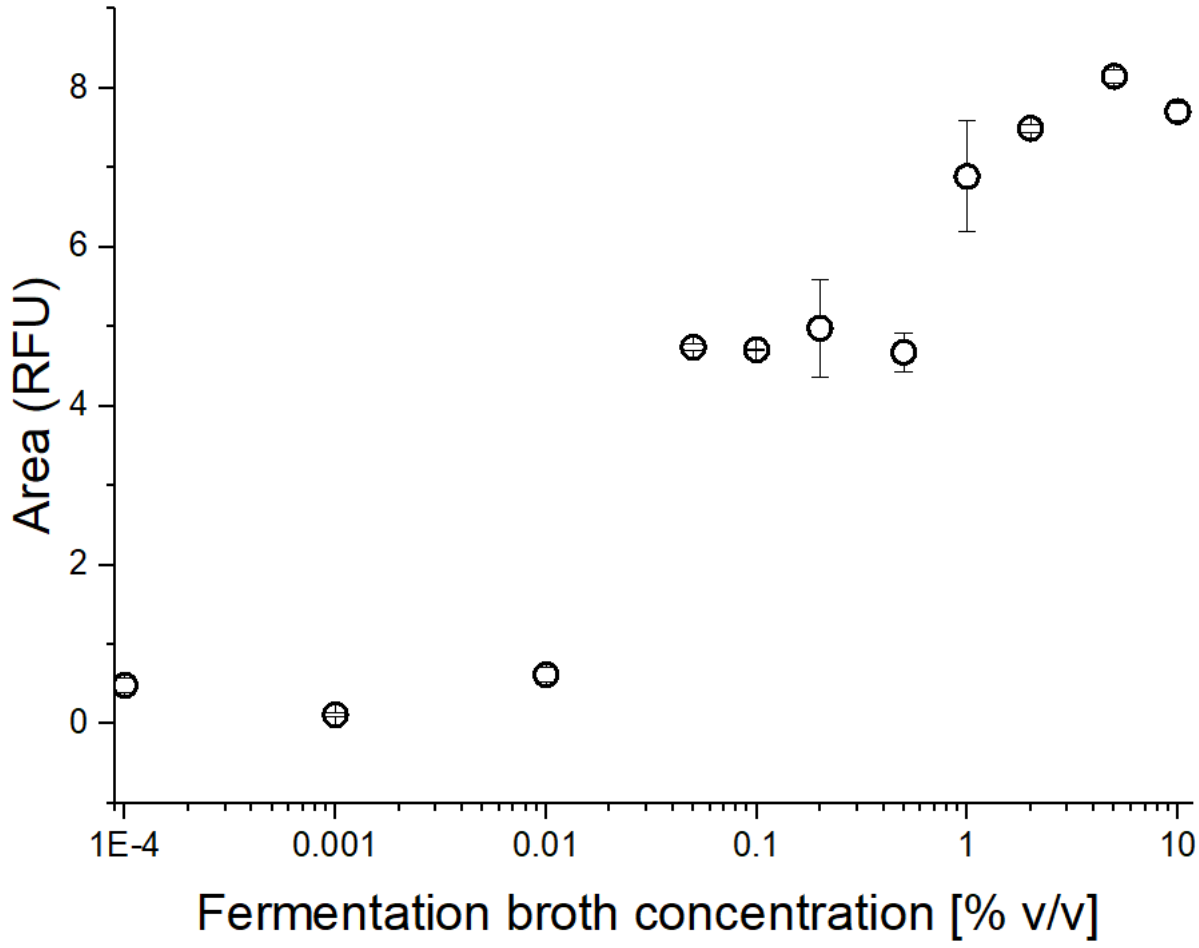


*Figure S2: Peak area of 20 nM anti-IgG affibody-alexa488 as function of fermentation broth concentration (PM02 (containing rituximab), 0-10 % v/v) determined by FIDA at 25 °C (black open circles, n = 3, error bars represent standard deviation).*

The cause of the anti-IgG affibody-alexa488 peak area dependence on the fermentation broth concentration is related to adsorption of the affibody to the labware (e.g. glass vials and/or capillary surface). At high levels of fermentation broth, components of the fermentation broth apparently competes with the affibody adsorption, ultimately preventing affibody adsorption and thereby leading to a higher area of the signal. As the FIDA methodology is based on signal shape rather than signal area, this phenomenon has no impact on the determination of the apparent hydrodynamic radius as long as the signal to noise ratio is sufficient for curve fitting.

## Taylor conditions

The Taylor conditions (*Pe* and $\tau$) were evaluated by the FIDA software as described previously[S1]. As shown in table S1, even the most extreme experiments are shown to be within the Taylor conditions[S3].

**Table S1**. Evaluation of Taylor conditions (*Pe* and *t*)

| **Apparent affibody size (nm)** | ***Pe*** | $\boldsymbol{\tau}$ |
|---|---|---|
| Limit | > 69 | > 1.4 |
| 2.24 | 2705 | 8.28 |
| 8.23 | 9937 | 2.25 |

## References

(S1) Pedersen, M. E.; Gad, S. I.; Østergaard, J.; Jensen, H. Protein Characterization in 3D: Size, Folding, and Functional Assessment in a Unified Approach. Anal. Chem. 2019, 91 (8), 4975–4979. https://doi.org/10.1021/acs.analchem.9b00537.

(S2) Poulsen, N. N.; Andersen, N. Z.; Østergaard, J.; Zhuang, G.; Petersen, N. J.; Jensen, H. Flow Induced Dispersion Analysis Rapidly Quantifies Proteins in Human Plasma Samples. Analyst 2015, 140 (13), 4365–4369. https://doi.org/10.1039/C5AN00697J.

(S3) Taylor, G. Dispersion of Soluble Matter in Solvent Flowing Slowly through a Tube. Proc. R. Soc. A Math. Phys. Eng. Sci. 1953, 219, 186–203. https://doi.org/10.1098/rspa.1953.0139.