

# SMALL MOLECULE INTERACTIONS WITH MEMBRANE

# PROTEINS USING FLOW INDUCED DISPERSION ANALYSIS

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## Introduction

Characterising Membrane protein interactions in-vitro presents a significant challenge, primarily due to the subtle mass changes between the ligand-free and bound states of membrane proteins. This proves an obstacle for methods relying on a change in molecular mass for detection such as SPR, BLI, mass photometry, analytical ultracentrifugation, and MST. In this work, we outline how Flow Induced Dispersion Analysis (FIDA) provides a selection of approaches to study interactions between small molecules and membrane proteins.

## FIDA

Based on microfluidics and building on Taylor Dispersion and Stokes-Einstein equation, FIDA is a unique technology for characterising biomolecular size and interactions. The raw data entails a wide range of information including size measured as hydrodynamic radius, Rh and BRIC (Binding Related Intensity Change). It should be stressed that these two readouts are completely orthogonal measurements stemming from a single set of raw data.

Full details on the FIDA technology can be found on [Fidabio.com](http://Fidabio.com).

## Method

The assays were performed label-free on the Fida Instrument equipped with a 275 nm LED fluorescence detector and a Fida PC capillary (L: 1 m, ID: 75  $\mu$ m, Leff: 84 cm).

The sample tray in the autosampler was kept at 5 °C during the entire run and the capillary chamber was set to 10 °C.

All experiments were performed in capillary mixture (capmix) mode, reducing the membrane protein sample consumption to 1.44  $\mu$ L per titration curve.

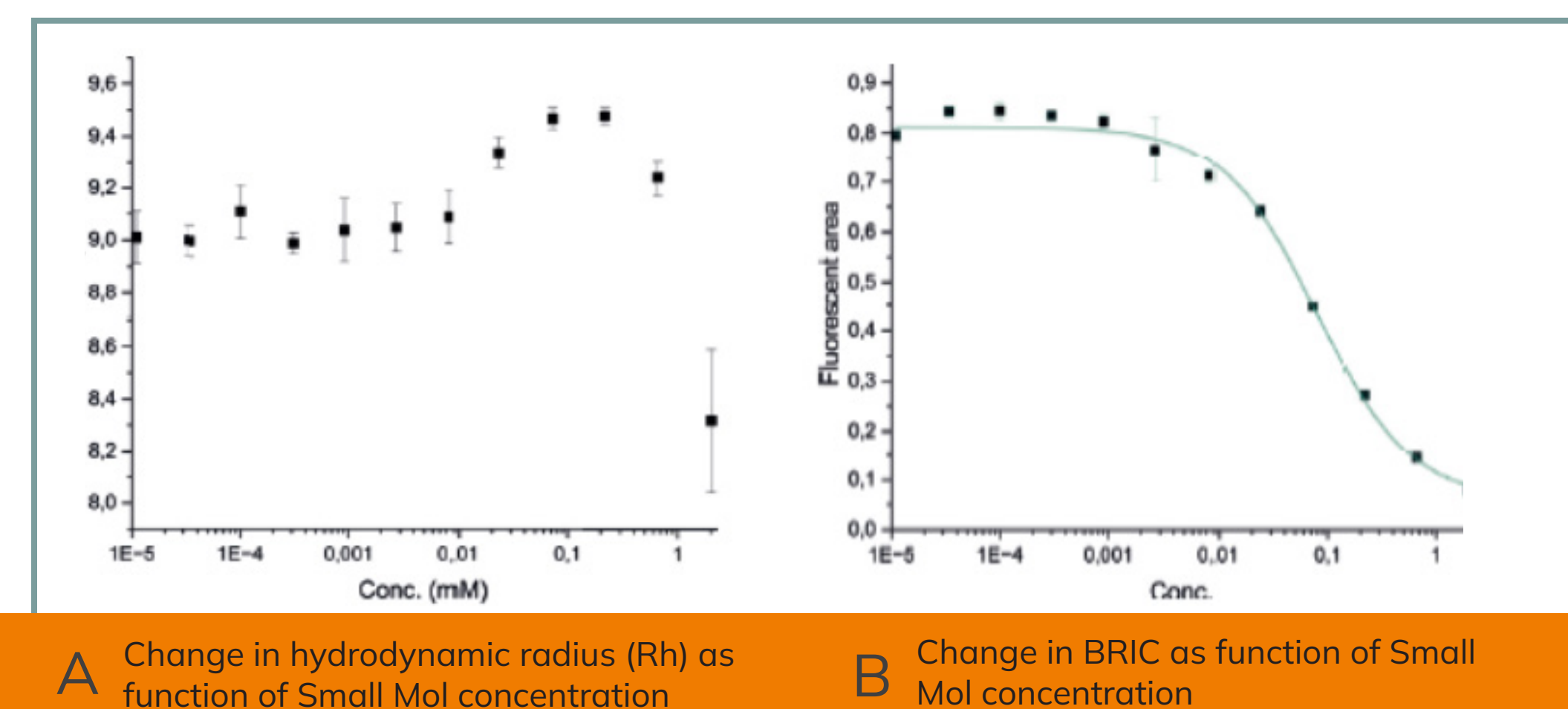
The indicator was a detergent solubilized membrane protein at 0.8 g/L, assayed in buffer X containing detergent Y (details to be disclosed upon peer reviewed publication).

The analyte was buffer X containing the assayed small molecules at concentrations ranging from 0-500 mM or urea at concentrations ranging from 0-7.2 M. The mobilisation pressure was 400 mbar.



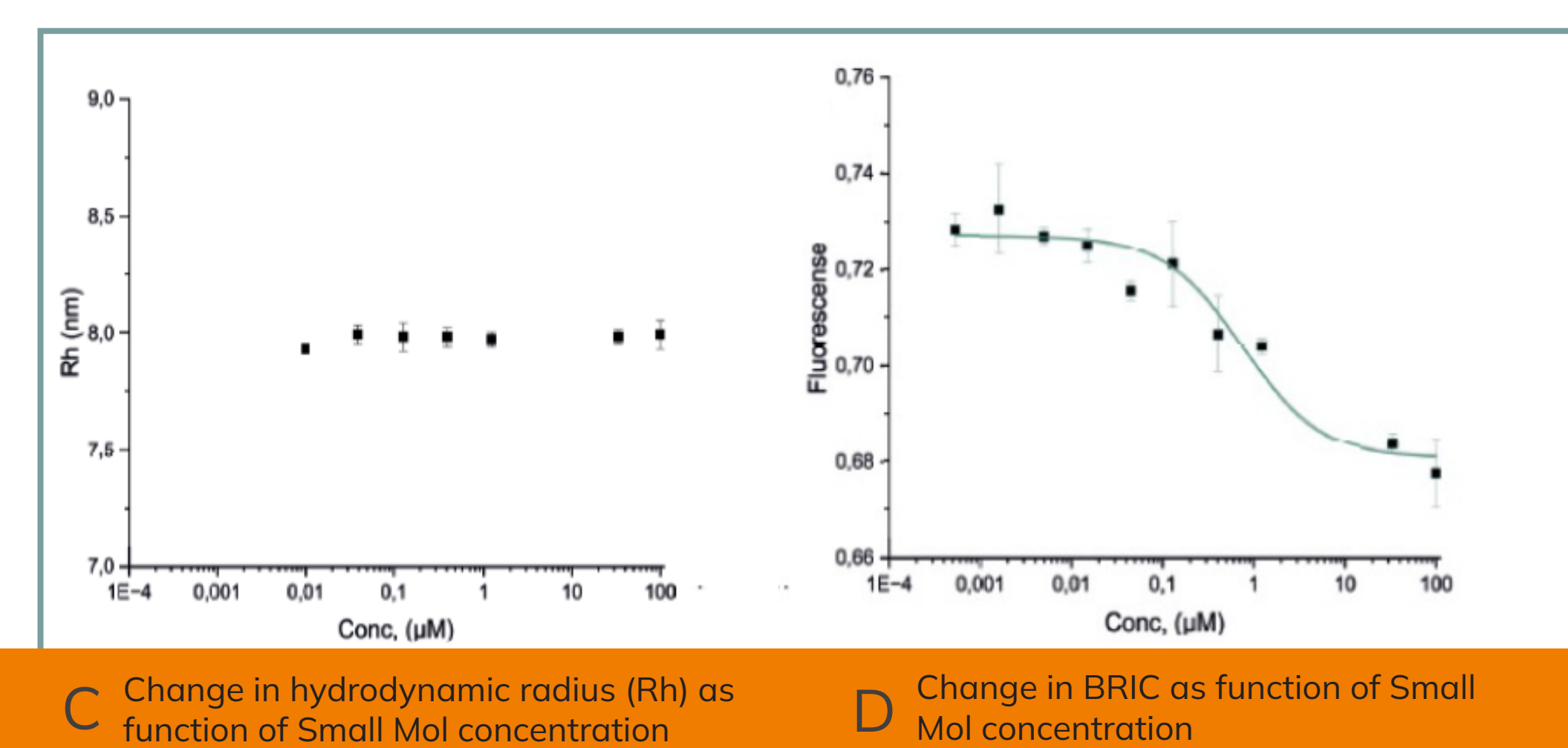
## Results

### CASE 1: BINDING RESULTING IN DETECTABLE SIGNAL OF BOTH SIZE (Rh) and BRIC



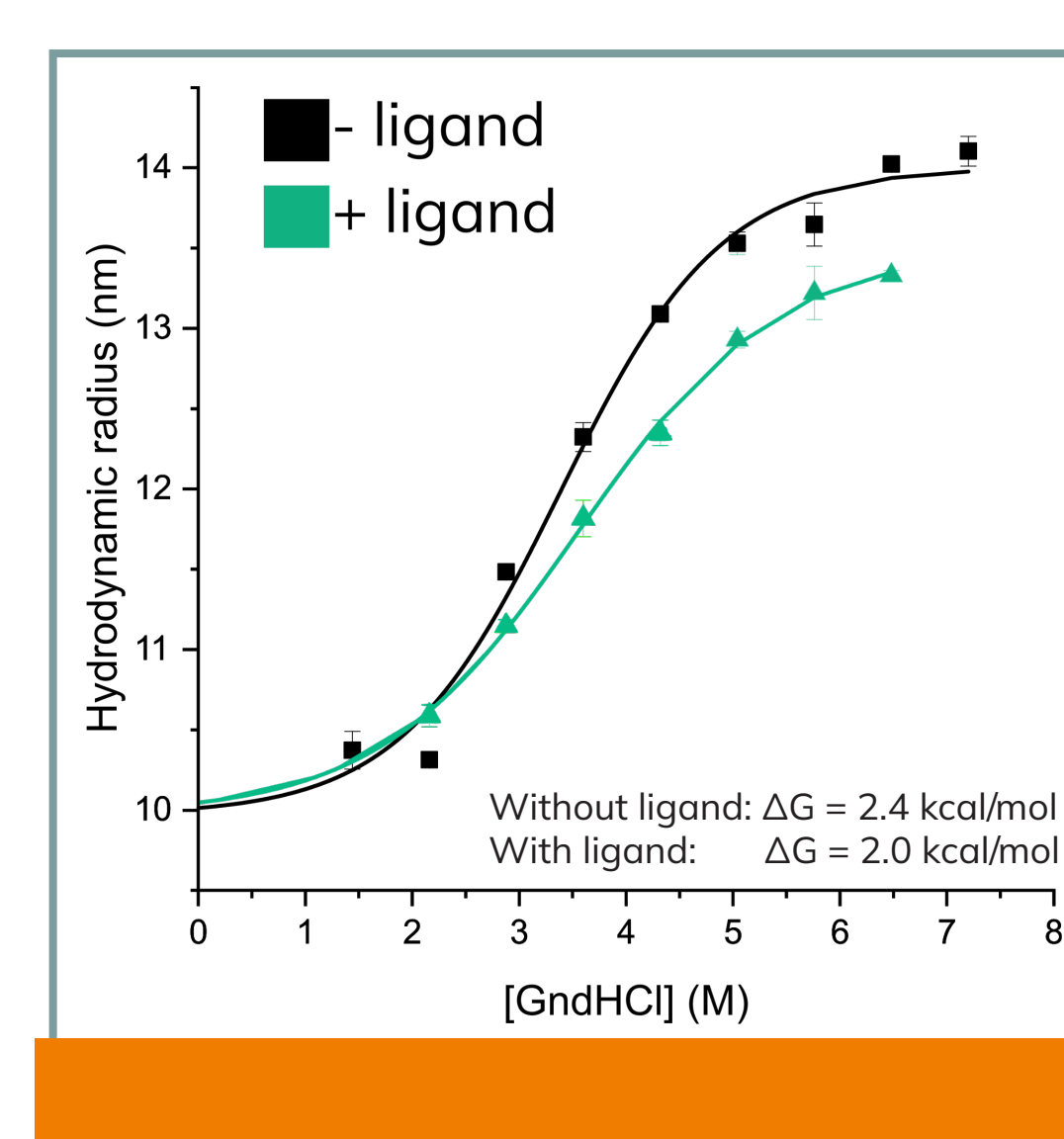
The size development indicates an initial increase in Rh upon binding. At 0.3 mM a decrease in size is observed which could be a collapse of the micelle and/or a second binding of small molecule. The BRIC signal shows a clear concentration dependence.  $K_d$ 's are deductible but not shown here (awaiting final publication).

### CASE 2: CHANGE IN BRIC BUT NO CHANGE IN SIZE



The size measure shows no impact on hydrodynamic radius (Rh) even at high concentrations of the small molecule in this case. The BRIC signal again shows a clear concentration dependence similar to Case 1. So, without a "Global" structural change in Rh, a "Local" change in the vicinity of the intrinsic fluorescence is still detectable for this binding.

### CASE 3: BINDING VALIDATION WHEN NO CHANGE IN SIZE OR BRIC



If a small molecule is added to the membrane protein and no change in size nor BRIC is observed, one might reasonably assume that no interaction occurs. However, it is possible that the small molecule is interacting with the membrane protein in a position that does not cause a conformational change and where there is no nearby tryptophan. Such an interaction should change the energy of folding of the protein and can be determined by titrating the membrane protein in the presence and absence of the small molecule with either urea or guanidinium hydrochloride. A difference in Gibbs free energy will reveal if binding has occurred.

## Conclusions

The present study demonstrates that the Fida Instrument offers an easy-to-use in-solution assay platform for characterising Small Molecule binding to Membrane Protein via orthogonal readouts of size and BRIC (Binding Related Intensity Change) or via unfolding experiments.

In the case at hand, data is generated for an undisclosed detergent solubilised Membrane Protein and its interaction with different Small Molecules, respectively. The described data has been acquired label-free based on intrinsic tryptophan fluorescence, consuming only few  $\mu$ L of Membrane Protein solution for each binding curve.

The added benefit compared to conventional technologies is that not only is FIDA assessing if binding takes place and can provide  $K_d$  values, FIDA also entails structural information on conformational changes upon binding.