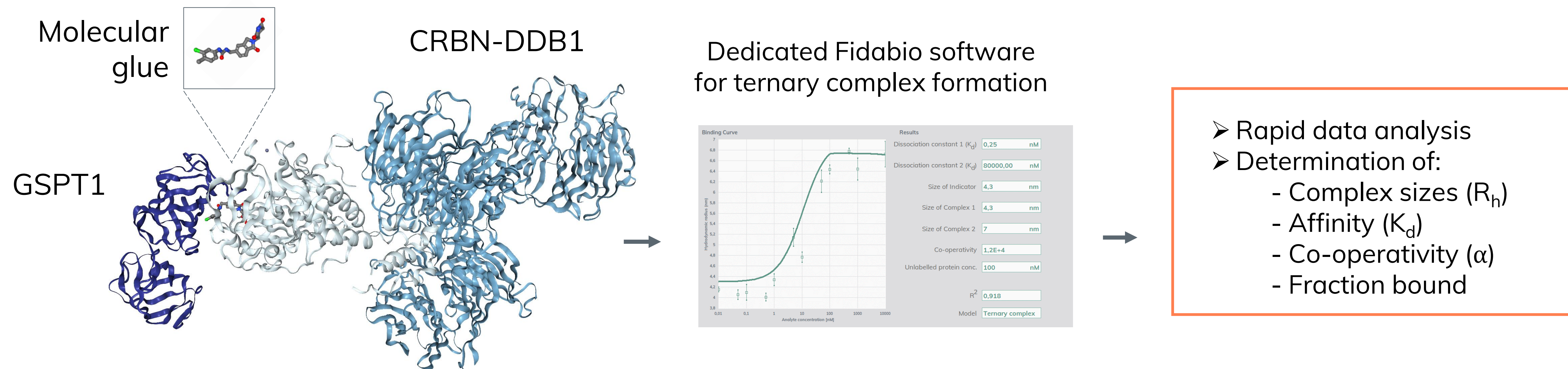


Characterization of Molecular Glues – an automated in-solution platform

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INTRODUCTION

We demonstrate how the Fida 1 can be employed as an effective tool for characterizing novel molecular glue candidates. The Fida 1 provides fast and accurate determination of ternary complex formation as well as determination of complex sizes, affinity constants, and co-operativity using the dedicated Fidabio software. Here, the study for target protein GSPT1, ligase CRBN-DDB1, and Molecular Glue X (MGX) and lenalidomide is presented.

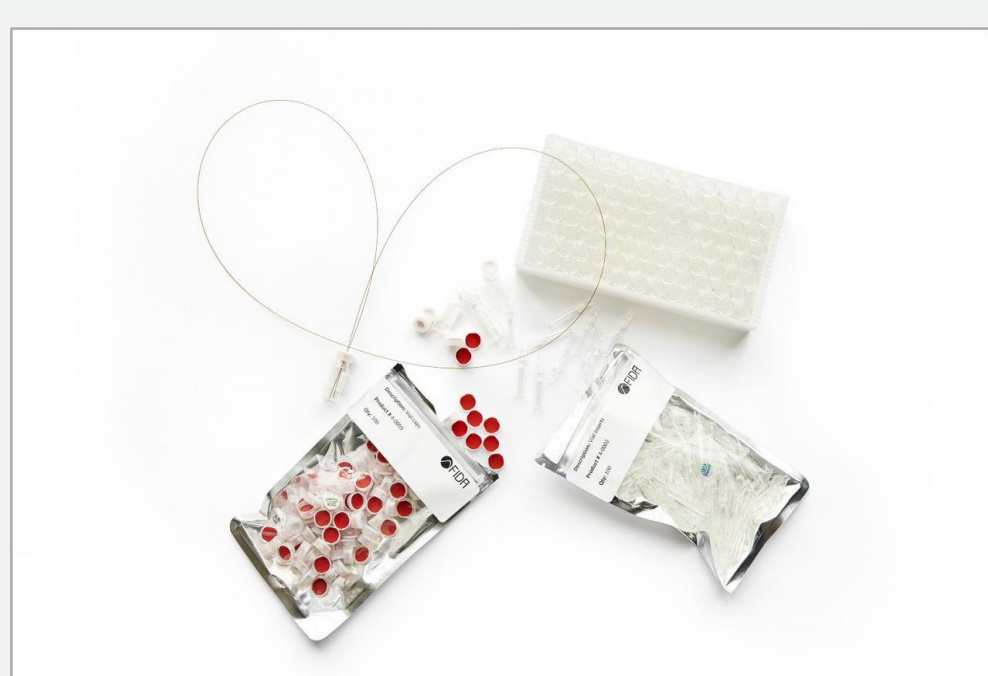
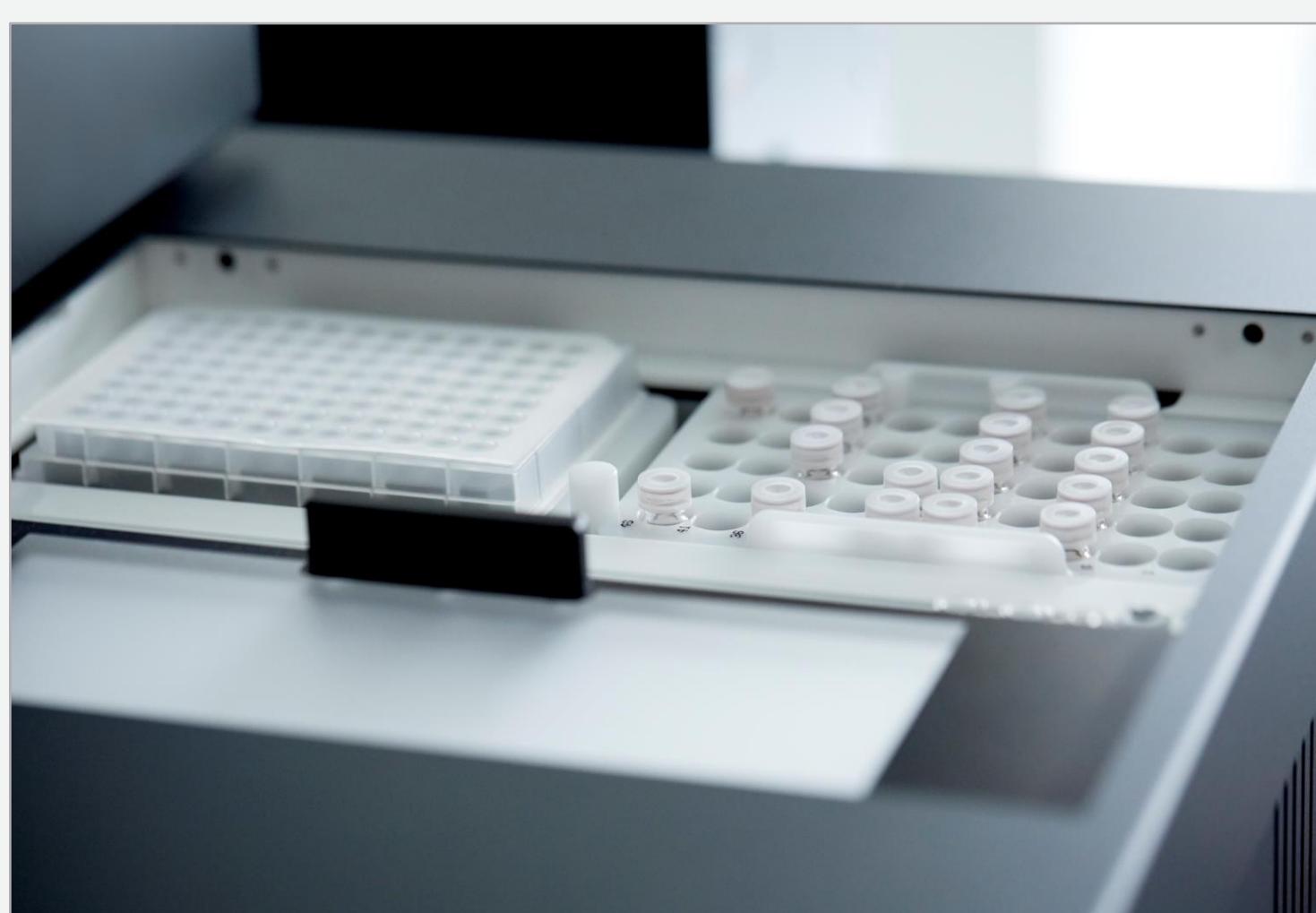
METHOD

GSPT1 was labelled with DY-490 and used as fluorescent indicator.

Experiments were performed on a Fida 1 instrument employing 480 nm LED detection using a high-sensitivity coated capillary (Fida Biosystems). Working buffer was 20 mM HEPES, 20 mM NaCl, 1 mM TCEP, 0.2 M EDTA, 1% DMSO, 0.05 % Pluronic F127, pH 8.0.

Flow Induced Dispersion Analysis was performed by flushing the capillary with 4 μ L of analyte sample (molecular glue + CRBN-DDB1), followed by an indicator injection of 39 nL (GSPT1-DY490 + molecular glue + CRBN-DDB1).

Fida 1

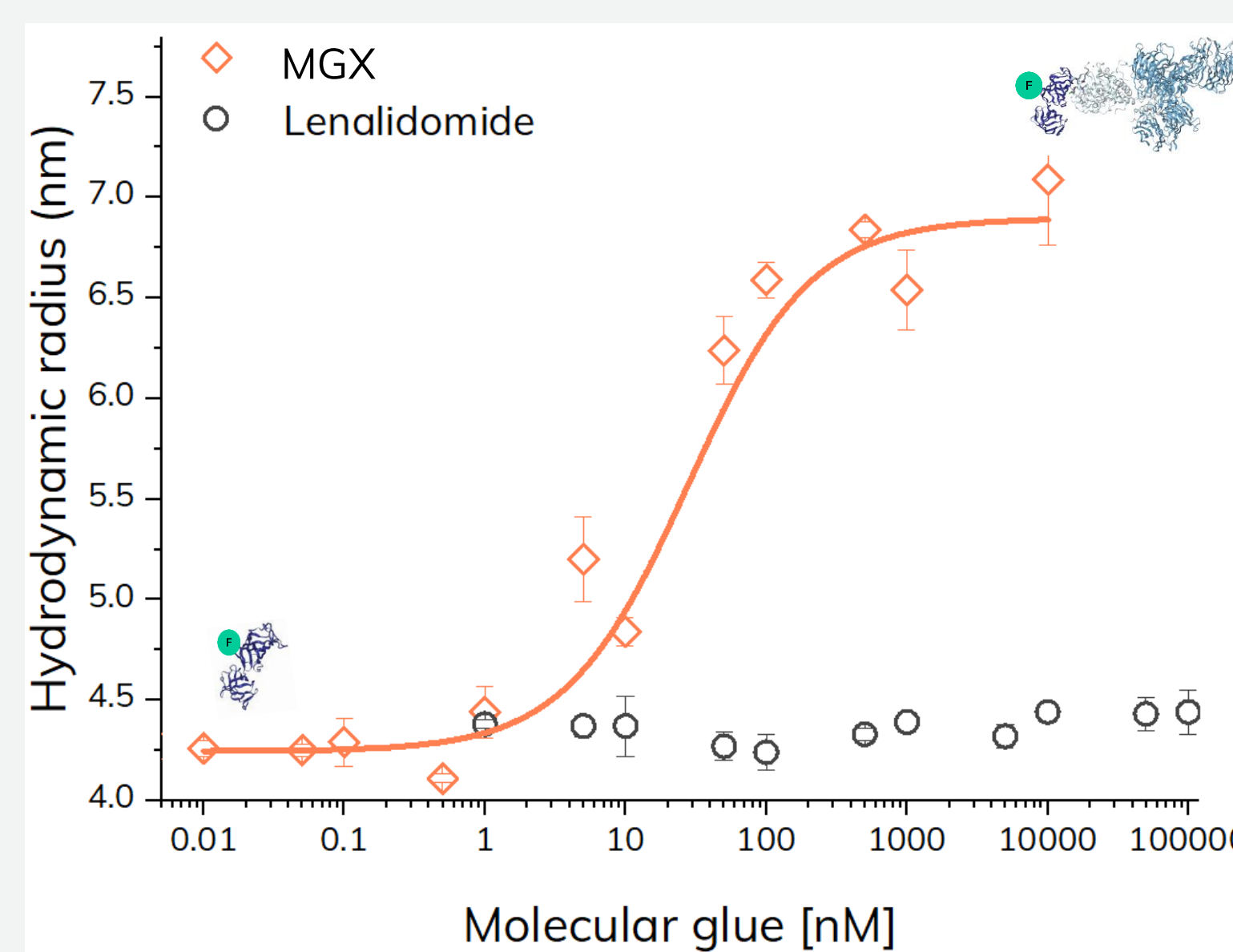


Consumables:

- Vials and/or
- 96 well plates
- HS-capillary

RESULTS

TERNARY COMPLEX FORMATION BETWEEN GSPT1, MOL GLUE AND CRBN-DDB1

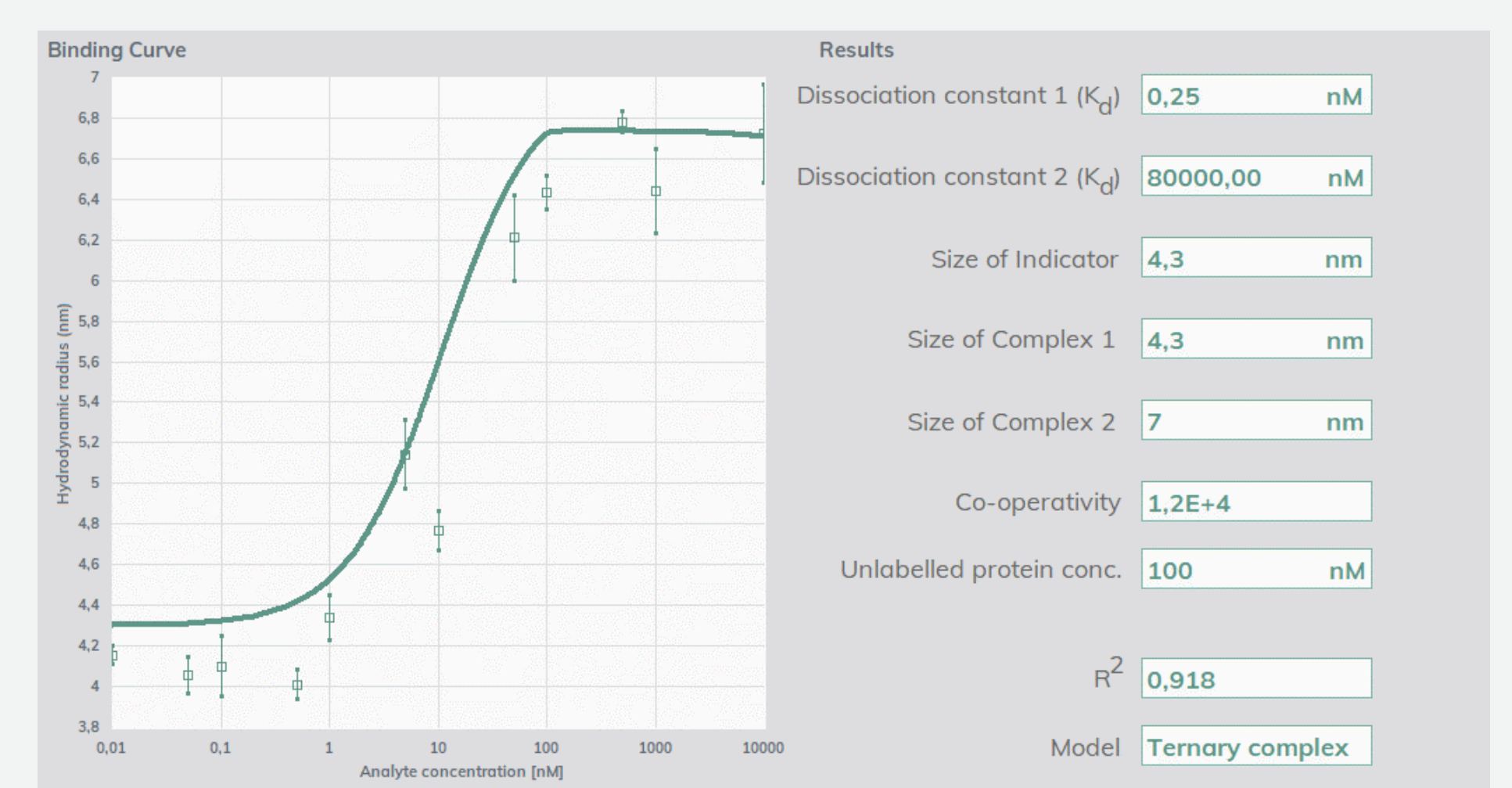


Size of unbound GSPT1 was determined to 4.27 ± 0.06 nm. Titration with mixtures of fixed 100 nM CRBN-DDB1 in increasing concentration of Mol Glue X generated a sigmoid-shaped binding curve confirming ternary complex formation with overall affinity of 11.1 nM. Identical titration with CRBN-DDB1 and increasing concentration of lenalidomide did not increase the size of GSPT1, i.e., indicating no formation of ternary complex.

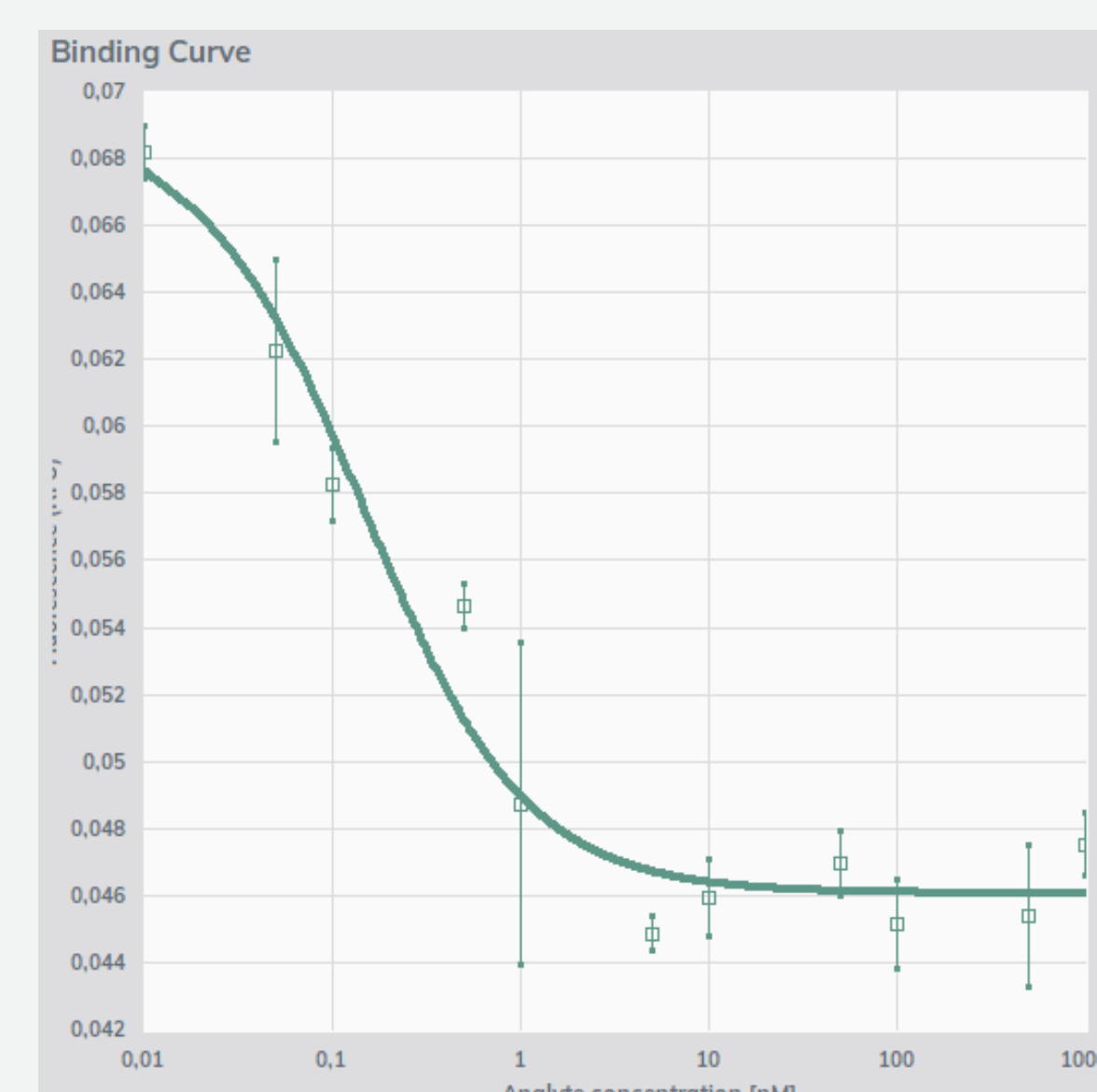
INTEGRATED SOFTWARE FOR ANALYZING TERNARY COMPLEX FORMATION

Parameters identified by fitting the experimental dataset in the software:

- K_d 1 (affinity GSPT1 and molecular glue)
- K_d 2 (affinity CRBN-DDB1 and molecular glue)
- Co-operativity factor (α)
- Size (R_h) of indicator (GSPT1)
- Size (R_h) of binary complex (GSPT1-molecular glue)
- Size (R_h) of ternary complex (GSPT1-molecular glue-CRBN-DDB1)



BINARY BINDING CHARACTERIZATION VIA BRIC (Binding Related Intensity Change)



In addition to the readout of hydrodynamic radius (R_h), Fida 1 provides an inherent, orthogonal measure of fluorescence intensity. Binding taking place in proximity of a fluorescent label can via conformational change and variation in solvent exposure, impact the fluorescence intensity of the label.

The figure shows the BRIC signal of GSPT1-DY490 (25 nM) as function of molecular glue concentration in the presence of 5 nM CRBN-DDB1. The binding curve resulting from the BRIC signal reflects GSPT1 interacting with Mol Glue X revealing an apparent affinity of 0.2 nM

CONCLUSIONS

The present study verifies that the Fida 1 instrument offers an easy-to-use in-solution assay platform for characterizing ternary complex formation via orthogonal readouts of size and BRIC (Binding Related Intensity Change).

In the case at hand, data is generated for Molecular Glue X and its interaction with the target protein and protease, respectively. The experimental data was fitted using the Fidabio model for ternary complex formation and for a sufficiently large data set all parameters (K_d 's, α and complex sizes – including size of the ternary complex) can be obtained.

Not shown is the Fida 1 software functionality for in-solution PDB correlation. This feature converts PDB files into a R_h which can be compared with the Fida 1 measurement.