Fidabio

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.

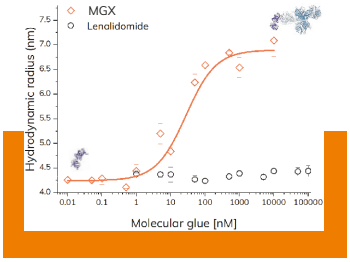
Methods

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 µL of analyte sample (molecular glue + CRBN-DDB1), followed by an indicator injection of 39 nL (GSPT1-DY490 + molecular glue + CRBN-DDB1).

Fida 1

Results

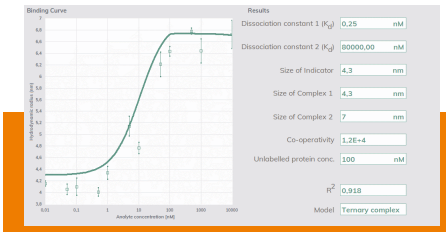
1 TERNARY COMPLEX FORMATION BETWEEN GSPT1, MOL GLUE AND CRBN-DDB1



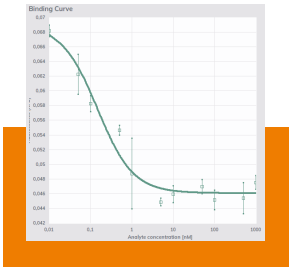
Size of unbound GSPT1 was determined to 4.27 ± 0.06 nm. Titration with mixtures of fixed 100 nM CRBN-DDB1 in increasing concentration of Mol Glue X generated a sigmoid-shaped binding curve confirming ternary complex formation with overall affinity of 11.1 nM. Identical titration with CRBN-DDB1 and increasing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

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)

2 INTEGRATED SOFTWARE FOR ANALYZING TERNARY COMPLEX FORMATION



3 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_ds, 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.