Fidabio

FULLY AUTOMATED CHARACTERIZATION OF TERNARY COMPLEX FORMATION USING FLOW INDUCED DISPERSION ANALYSIS

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Introduction

We demonstrate how the Fida 1 can be used for full characterization of ternary complex formation for targeted protein degradation. The Fida 1 platform only consumes

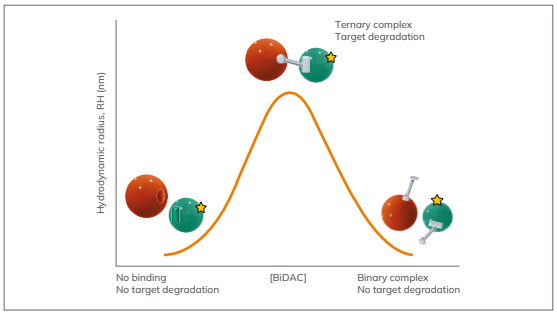
40 nL of sample for one measurement

and thus allows elaborate condition screening using very small amounts of sample material. The present work focuses on quantifying critical quality parameters such as **absolute in-solution size determinations,** affinity for both interaction partners (Kd), as well as cooperativity. The methodology is fully automated allowing **screening of up to two 96 well plates under full temperature control.**

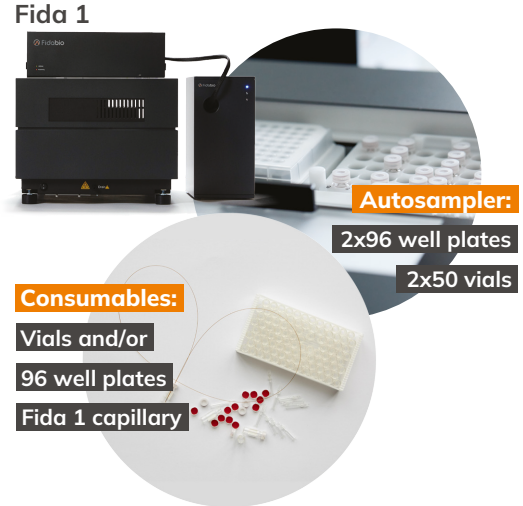
Methods

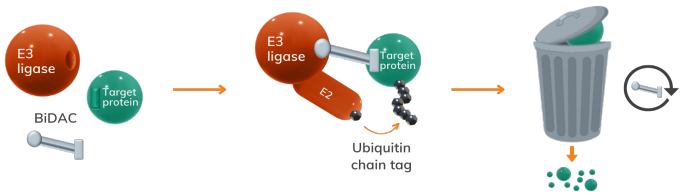
The experiments were performed on a Fida 1 instrument employing 480 nm LED- fluorescence detection.
Flow Induced Dispersion Analysis (FIDA) is a capillary-based technology which measures biomolecular size of fluorescently-labelled protein/complexes, by converting dispersion profiles to a size readout of hydrodynamic radius, R_h.

In this Fida 1 assay, fluorescently labelled Protein of Interest (POI) serves as fluorescent "indicator" and the BiDACTM serves as "analyte". BiDACTM is titrated against constant concentration of target POI and E3 Ligase. Size changes are measured throughout the titrations vrevealing the formation of ternary and binary complexes. A bell-shaped binding curve is a key characteristic of the ternary complex.



Fida 1





Results

FULL CHARACTERIZATION OF TERNARY COMPLEX FORMATION WITH THE DEDICATED FIDABIO TPD SOFTWARE

Before the BiDAC titration, the size of the labelled POI alone, the POI in the presence of E3, and the unlabelled E3 were measured:

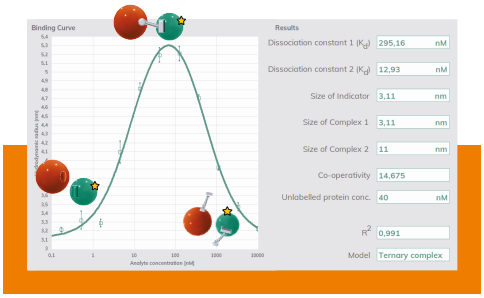
Labelled POI
R_h =3.11 nm

E3 Ligase
R_h =5.8 nm

Labelled POI in the presence of E3
R_h =3.11 nm

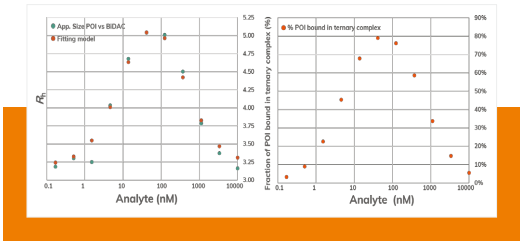
The size did not change in the presence of E3, indicating that the POI and E3 do not interact in the absence of BiDAC.

Subsequently, the BiDAC was titrated against the POI and Fida 1 monitored the size of the labeled POI. The titrations of BiDACs display an initial size increase followed by a size decrease at higher concentrations, resulting in a bell-shaped curve, as predicted by the models in the literature.[1,2]



The Fidabio model describing ternary complex formation is incorporated in the software, and is used to calculate cooperativity, dissociation constant between the POI and the BIDAC, dissociation constant between the BIDAC and the E3, and the ternary complex size. Notably the cooperativity is 14.7, significantly higher than 1, meaning that this BiDACTM is a good promoter of ternary complex formation.

By measuring the diffusion coefficient and the Rh of these components, Fida 1 can subsequently calculate the fraction of POI bound in the ternary complex. Learn more about the Fidabio model for ternary complex formation in the Application Note.



If the structure of the ternary complex is known, the Fidabio PDB Correlator, integrated in the software, can be used for orthogonal calculation of the hydrodynamic radius based on X-ray, Cryo-EM or AlphaFold structures. To learn more about the PDB Correlator click here.

Conclusions

Fida 1 provides in-solution characterization of ternary complex formation generating multiple parameters from just a few nL of sample. Its ability to determine **size, affinities, and cooperativity** in a single run makes it ideal for drug screening as well as for orthogonal characterization.

[1]S. L. Fisher, A. J. Phillips, Targeted protein degradation and the enzymology of degraders. Curr Opin Chem Biol 2018, 44, 47–55.
[2]Douglass EF, Miller CJ, Sparer G, Shapiro H, Spiegel DA. A comprehensive mathematical model for three-body binding equilibria. J Am Chem Soc. 2013;135(16):6092–9.