LINKING STRUCTURE AND FUNCTION USE OF PDB DATA AS BASELINE FOR IN-SOLUTION CHARACTERIZATION

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Introduction

We present the Fidabio PDB (Protein Data Bank) Correlator which predicts the hydrodynamic radius (R_h) of proteins based on X-ray, Cryo-EM structural data or AI-generated structures like AlphaFold. The comparison of the R_h predictions with in-solution R_h measurements offers structural and functional information about the protein of interest.

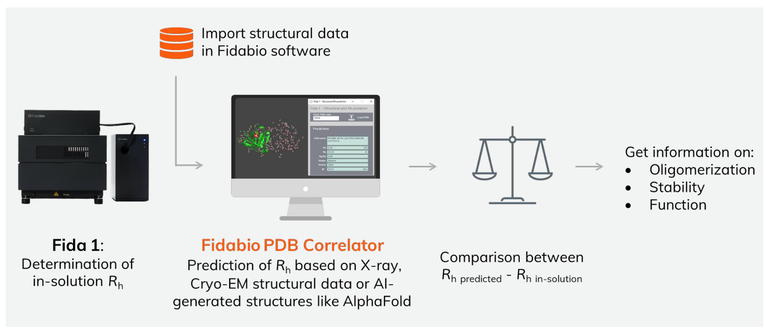
In the present work, we use the Fidabio PDB Correlator to predict the in-solution Rh of bovine ß-lactoglobulin, human serum albumin, bovine serum albumin and chicken lysozyme. Subsequently, using Fida 1 equipped with a UV fluorescence detector, the R_h of label-free proteins are experimentally measured in-solution and compared with the predicted R_h.

Method

For in-solution determination of Rh, we used the Fida 1 instrument employing 280 nm LED fluorescence detection. In this Fida 1 assay, the Bovine ß-lactoglobulin (BLG), Human Serum Albumin (HSA), Bovine Serum Albumin (BSA) and Chicken lysozyme (LYZ) serve as (intrinsically) fluorescent “indicators”. The buffer serves as the “analyte”.

For predicting the Rh based on PDB structural data, we used the Fidabio PDB Correlator, integrated in the Fidabio software. Crystal structures were acquired from the Protein Data Bank (https://www.rcsb.org/).

Fidabio PDB Correlator



Fida 1

Autosampler:

- 2x96 well plates
- 2x50 vials

Consumables:

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

Results

Using the Fidabio PDB Correlator, the PDB data are loaded into the Fidabio software whichsubsequently gives the predicted Rh and mass.

Protein	PDB code	Predicted R_h (nm)	Apparent R_h (nm)	Predicted Mw (kDa)	Theoretical Mw (kDa)
LYZ	1IEE	1.97	1.91 ± 0.01	14.51	14.31
BLG	1BEB	2.85	2.77 ± 0.06	36.21	36.58
HSA	1AO6	3.5	3.81 ± 0.04	65.76	66.47
BSA	4F5S	3.52	3.53 ± 0.04	66.77	66.43

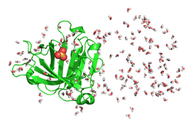
Insights in BLG structure using the fidabio PDB correlator

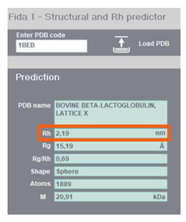
Fida 1 is used for in-solution determination of BLG R_h which is measured to be 2.77 ± 0.06 nm.

Fida 1 measurement
R_h = 2.77 ± 0.06 nm.

The PDB code of BLG (1BEB) is loaded into the Fidabio PDB Correlator which predicts the R_h based on the imported structural data. The predicted Rh is 2.19 nm.

PDB Code





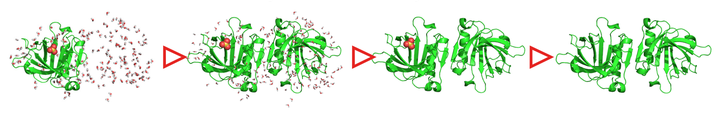
Fidabio PDB Correlator
$R_{h\ (predicted)}$ = 2.19 nm

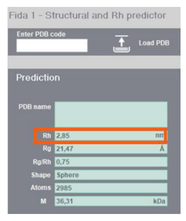
The discrepancy between predicted R_h and experiment R_h values indicates that the insolution BLG structure might not be a monomer.

$R_{h\ predicted}$ *VS* $R_{h\ in\text{-}solution}$

Cleaning up the file and using a homodimer structure, the Fidabio PDB Correlator gives a new R_h of 2.85 nm, which is within 2.8% of the experimental value of 2.77± 0.06 nm. This indicates that BLG is a homodimer, which is in agreement with the literature [1].

Adjustment in PyMOL





Fidabio PDB Correlator
New R_h prediction:
R_h = 2.85 nm
≈ Fida 1 value

Confirmation of homodimeric structure of BLG

[1]Brownlow S. et al.; Bovine beta-lactoglobulin at 1.8 A resolution--still an enigmatic lipocalin., 1997, Structure; Vol 5, p. 481-495

Conclusions

In the present work, we show how the Fidabio PDB Correlator provides an accurate correlation (<9% error) between a protein’s size in-solution (hydrodynamic radius) and structure. Structural data can be supplied both by experimentally solved and AI-generated structures. The correlation can generate information on the in-solution state of the protein of interest, like oligomerization and integrity, as well as insights on functionality.