Fidabio

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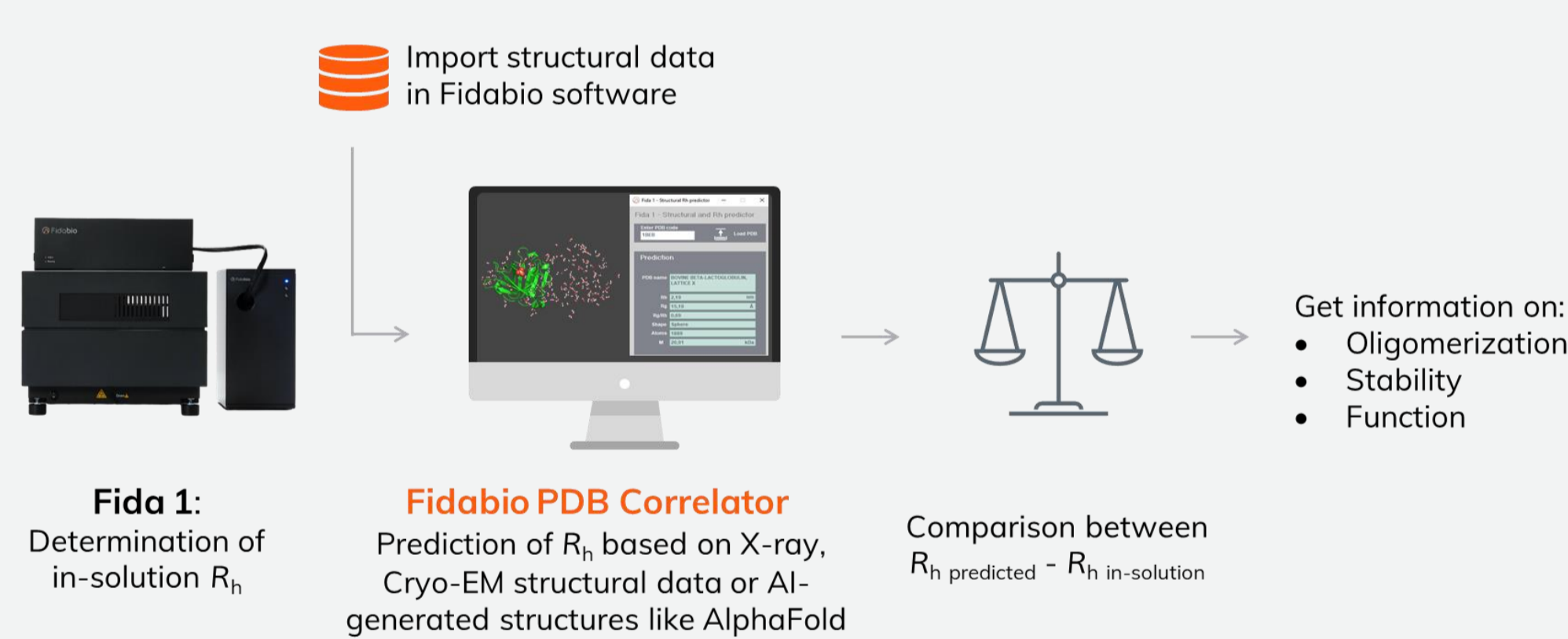
In the present work, we use the Fidabio PDB Correlator to predict the in-solution R_h of bovine β-lactoglobulin, human serum albumin, bovine serum albumin and chicken lysozyme. Subsequently, using Fida 1 equipped with a UV fluorescence detector, the R_h of label-free proteins are experimentally measured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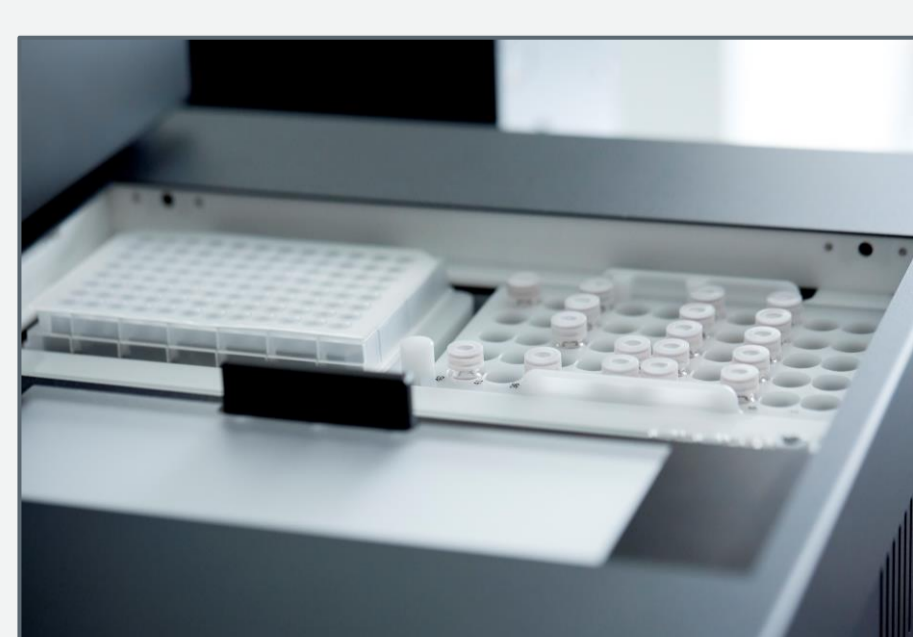
METHOD

For in-solution determination of R_h, we used the Fida 1 instrument employing 280 nm LED fluorescence detection. In this Fida 1 assay, the Bovine β-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 R_h 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

Import structural data in Fidabio software

Fida 1:
Determination of in-solution R_h

Fidabio PDB Correlator
Prediction of R_h based on X-ray, Cryo-EM structural data or AI-generated structures like AlphaFold

Comparison between $R_{h\ predicted}$ ~ $R_{h\ in-solution}$

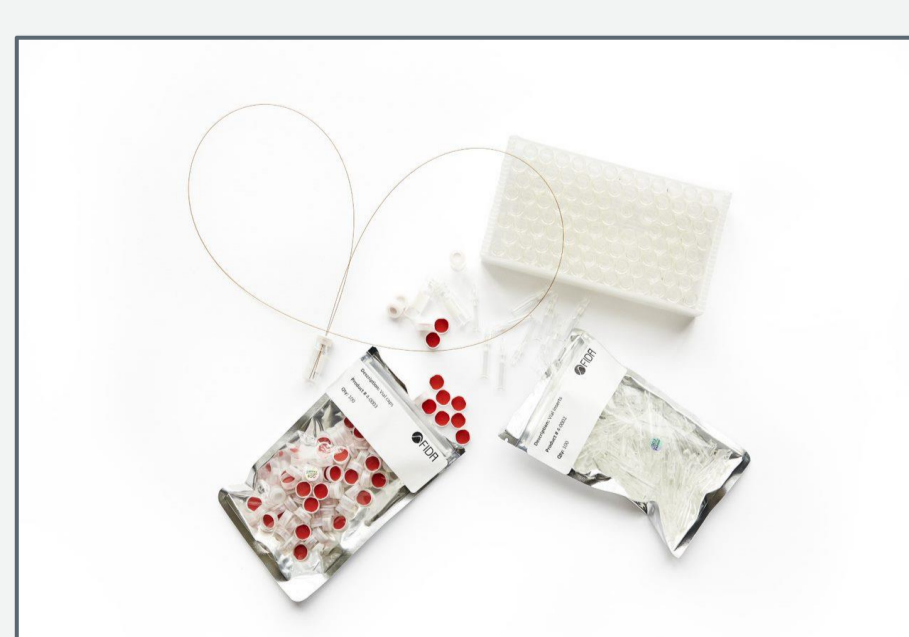Get information on:
• Oligomerization
• Stability
• Function

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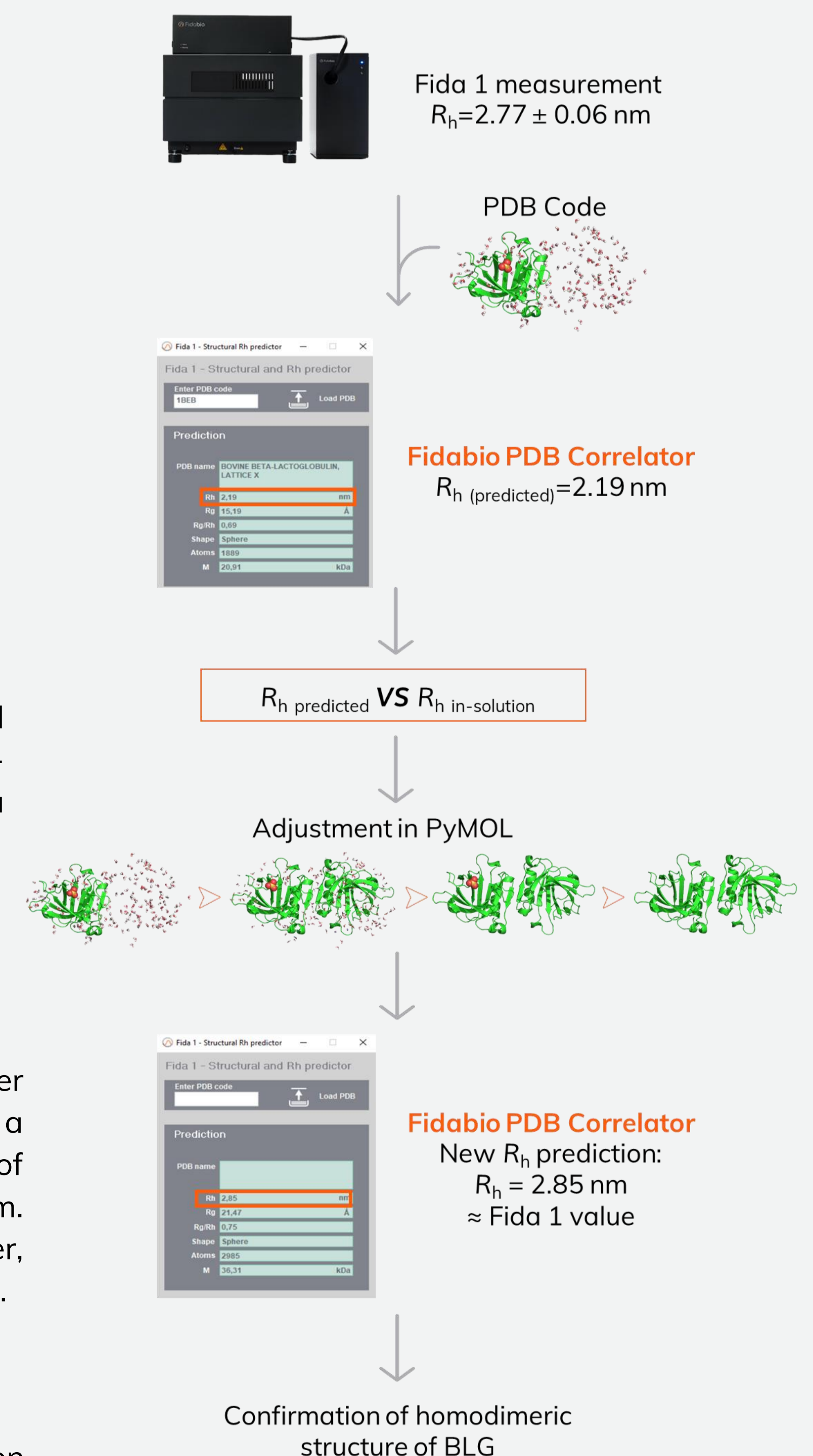
Fida 1 is used for in-solution determination of BLG R_h which is measured to be 2.77 ± 0.06 nm.

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 R_h is 2.19 nm.

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

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].

More data are presented in the Application Note which can be accessed scanning the QR code at the upper left-hand corner.

Fida 1 measurement
R_h=2.77 ± 0.06 nm

PDB Code

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

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

Adjustment in PyMOL

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

Confirmation of homodimeric structure of BLG

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.

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