PLI-Analyzer: A Novel Platform for Quantitative Assessment of Structural and Biological Accuracy in AI-Predicted Protein-Protein Complexes

Summary

Creation of PLI-Analyzer Platform

AlphaFold 3 & Boltz 2 Benchmarking

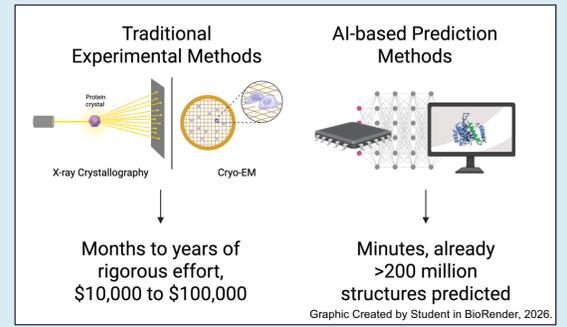Development of PLI-Analyzer Web App & Applications

PLI-Analyzer is a critical, data-driven validation tool that enables researchers to distinguish accurate predictions and to iteratively improve the generative modeling
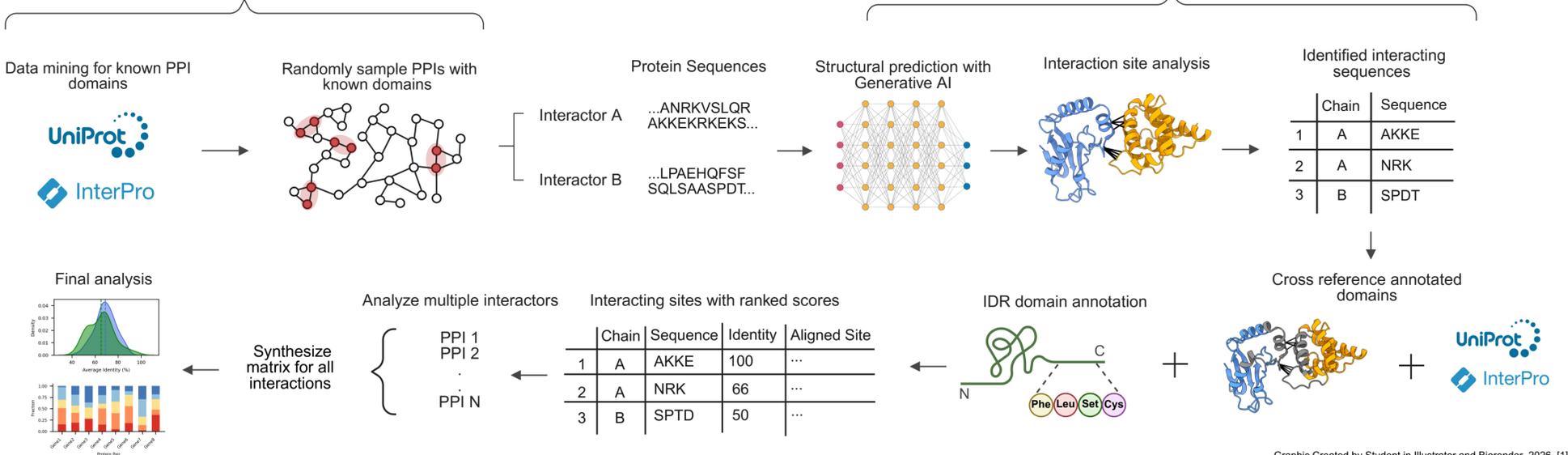
Introduction

Proteins' 3D structures and interactions drive most biological processes

No effective methods currently exist to identify or evaluate the accuracy of AI-generated PPI complexes, significantly limiting their practical applications
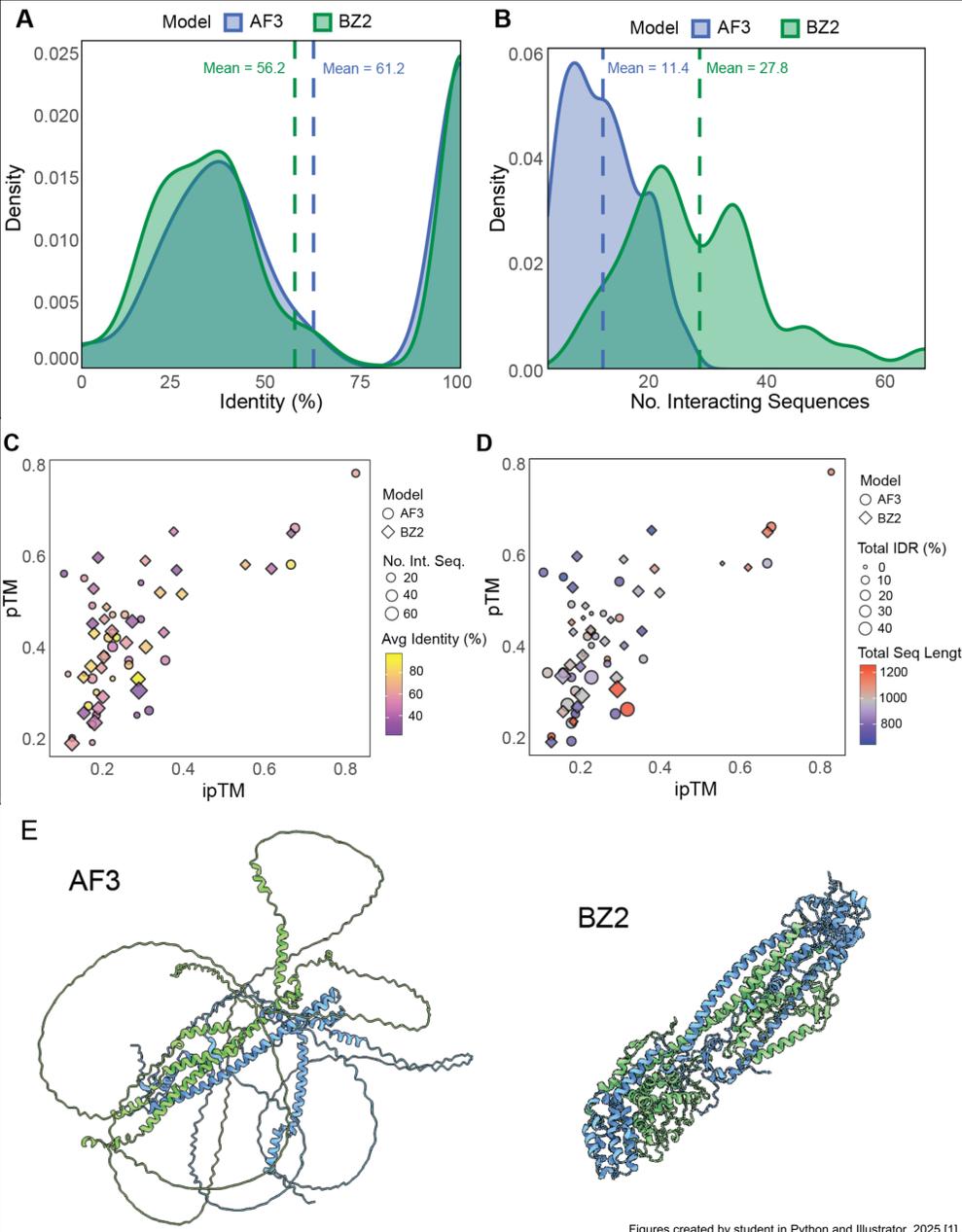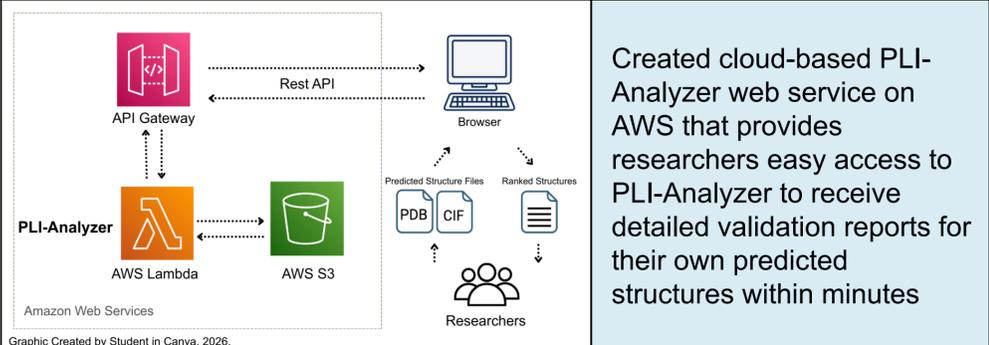
Protein Structure Determination

Traditional Experimental Methods

X-ray Crystallography Cryo-EM

Months to years of rigorous effort, $10,000 to $100,000

AI-based Prediction Methods

Minutes, already >200 million structures predicted

Graphic Created by Student in BioRender, 2026.

Construction of Validation Data

PLI-Analyzer

Data mining for known PPI domains — UniProt, InterPro

Randomly sample PPIs with known domains

Protein Sequences
Interactor A: ...ANRKVSLQR AKKEKRKEKS...
Interactor B: ...LPAEHQFSF SQLSAASPDT...

Structural prediction with Generative AI

Interaction site analysis

Identified interacting sequences

	Chain	Sequence
1	A	AKKE
2	A	NRK
3	B	SPDT

Cross reference annotated domains — UniProt, InterPro

IDR domain annotation

Interacting sites with ranked scores

	Chain	Sequence	Identity	Aligned Site
1	A	AKKE	100	...
2	A	NRK	66	...
3	B	SPTD	50	...

Analyze multiple interactors: PPI 1, PPI 2, ... PPI N

Synthesize matrix for all interactions

Final analysis

Graphic Created by Student in Illustrator and Biorender, 2026. [1]

Results

A — Model AF3, BZ2; Mean = 56.2, Mean = 61.2; Density vs Identity (%)

B — Model AF3, BZ2; Mean = 11.4, Mean = 27.8; Density vs No. Interacting Sequences

C — pTM vs ipTM; Model AF3, BZ2; No. Int. Seq. 20, 40, 60; Avg Identity (%) 80, 60, 40

D — pTM vs ipTM; Model AF3, BZ2; Total IDR (%) 0, 10, 20, 30, 40; Total Seq Length

E — AF3, BZ2

Figures created by student in Python and Illustrator, 2025 [1].

PLI-Analyzer Webapp

Rest API — API Gateway — Browser

PLI-Analyzer — AWS Lambda — AWS S3

Predicted Structure Files — PDB, CIF — Ranked Structures

Amazon Web Services — Researchers

Graphic Created by Student in Canva, 2026.

Created cloud-based PLI-Analyzer web service on AWS that provides researchers easy access to PLI-Analyzer to receive detailed validation reports for their own predicted structures within minutes
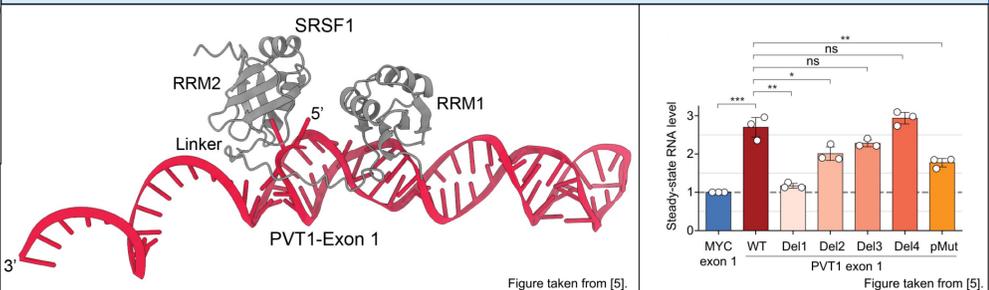
Application to RNA-Protein Interaction

- RNA structure prediction harder than PPIs due to high conformational flexibility
- Sparse experimental data: ~25x fewer resolved RNA structures than protein structures
- Applied to Stanford study published in Cell on RNA-protein interaction in ecDNA driven cancers

SRSF1, RRM2, RRM1, Linker, PVT1-Exon 1, 5', 3'

Figure taken from [5].

Steady-state RNA level — MYC exon 1, WT, Del1, Del2, Del3, Del4, pMut — PVT1 exon 1

Figure taken from [5].

Conclusion

- Current AI models generate structures quickly, but are often biologically inconsistent, especially for multimeric structures
- PLI-Analyzer offers a scalable approach of validating AI-generated structures
- Researchers need to be aware of the strengths and weaknesses of specific AI when interpreting predicted protein-protein complexes

Key Findings from Analysis

- 50-60% Average Identity Alignment
- High Variation in Average Identity From PPI to PPI
- Poor correlation between traditional metrics and pTM & ipTM
- Large differences between AF3 and BZ2 in No. interacting sequences and structuring of intrinsically disordered regions (IDRs)

References

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