Human protein interactome structure prediction at scale with Boltz-2

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ABSTRACT

In humans, protein-protein interactions mediate numerous biological processes and are central to both normal physiology and disease. Extensive research efforts have aimed to elucidate the human protein interactome, and comprehensive databases now catalog these interactions at scale. However, structural coverage of the human protein interactome is limited and remains challenging to resolve through experimental methodology alone. Recent advances in artificial intelligence/machine learning (AI/ML)-based approaches for protein interaction structure prediction present opportunities for large-scale structural characterization of the human interactome. One such model, Boltz-2, which is capable of predicting the structures of protein complexes, may serve this objective. Here, we present *de novo* computed models of 1,394 binary human protein interaction structures predicted using Boltz-2. These structural predictions were based on biochemically determined interaction data sourced from the IntAct database, together with corresponding protein sequences obtained from UniProt. We then assessed the predicted interaction structures through various confidence metrics, which consider both overall structure and the interaction interface. These analyses indicated that prediction confidence tended to be greater for smaller complexes, while increased multiple sequence alignment (MSA) depth tended to improve prediction confidence. Additionally, we performed a limited evaluation against experimentally determined human protein complex structures not included in the Boltz-2 training regimen, which indicated prediction accuracy consistent with AlphaFold3. This work demonstrates the utility of Boltz-2 for *in silico* structural modeling of the human protein interactome, while highlighting both strengths and limitations. Ultimately, such modeling is expected to yield broad structural insights with relevance across multiple domains of biomedical research.

INTRODUCTION

Interactions between biological macromolecules are fundamental to life, mediating numerous processes in both physiologic and pathological contexts. In particular, protein-protein interactions (PPIs) have been extensively studied for the elucidation of a wide variety biological functions [1]. Various large-scale initiatives have focused on systematic identification and annotation of PPIs based on experimental (e.g., biochemical) evidence [2-7]. These efforts have produced data resources, which provide comprehensive characterization of the 'interactomes' of many species including human. In parallel, databases dedicated to sequence annotation and protein structure determination, namely UniProt [8] and the Protein Data Bank (PDB) [9], respectively, have also been critical to systematic profiling of PPIs.

In recent years, metagenomic sequence data and three-dimensional (3D) structure data from such open-access repositories [8-11] have additionally enabled development of artificial intelligence/machine learning (AI/ML)-based approaches for protein structure prediction [12-15], including for prediction of structures of biomolecular interactions [14, 16, 17]. AI/ML models trained to leverage the relationship between sequence and 3D structure [18, 19], including those using multiple sequence alignments (MSAs) [12, 14, 16], have exhibited unprecedented accuracy [20, 21] for prediction of both individual structures and those of multiprotein complexes from amino acid sequence alone. AlphaFold-multimer [17], an initial approach for interaction structure prediction, motivated previous work aimed toward structurally resolving the human interaction network [17]. Since then, other AI/ML models, including AlphaFold3 [16] and more recently Boltz-2 [22], have been developed and exhibit substantially improved performance in predicting the structures of protein complexes.

Boltz-2 has drawn particular interest given its ability to predict binding affinities of small molecules interacting with proteins [22]. Moreover, Boltz-2 performs robustly in terms of predicting structures of protein-protein, protein-DNA, and protein-RNA complexes, as evaluated against other AI/ML models (including but not limited to AlphaFold3) on a wide variety of complexes freely available from the PDB (following the training cut-off date) [22]. Herein, we leverage the protein-protein structure prediction capabilities of Boltz-2 for high-throughput modeling of n = 1,394 binary human protein interaction structures, based on biochemically determined PPIs sourced from the IntAct database. The human protein interactome was the focus of the current study due to direct relevance in biomedical research. We assessed predictions using various confidence metrics, considering both overall complex structure and the interaction interface of protein pairs, and found that prediction confidence tended to be greater for smaller complexes while increased MSA depth tended to improve prediction confidence. These findings demonstrate the utility of Boltz-2, highlighting both strengths and limitations, for capturing the broad structural landscape of the human protein interactome and exemplifying opportunities for large-scale structural and functional insights with applicability across the biomedical sciences.

RESULTS

Large-scale prediction of human protein interaction structures

To construct a representative dataset of the human interactome suitable for structural prediction, we coupled biochemically determined PPIs with their corresponding amino acid sequences. PPIs were sourced from the IntAct database [2] and sequences from UniProt [8], with a binary arrangement of interactor A paired with interactor B **(Figure 1a)**. PPIs were filtered to include only interactions in which both protein interactors were human and designated as having direct physical binding as determined by pull-down assay using purified proteins. Boltz-2 [22] was then used to perform structural predictions of these binary interactions (see Methods). Predictions were performed for protein pairs with individual interactor sequence lengths up to 1,000 residues, providing a dataset consisting of a total of n = 1,394 predicted protein interaction structures. For example, the predicted complex structure with the greatest confidence score is presented in **Figure 1b**.

With the scope of better characterizing the set of predicted interaction structures, we explored sequence-level attributes of the interacting proteins. Bivariate analysis of the sequence lengths across the protein interactor pairs used for structural prediction revealed a relatively uniform distribution **(Figure 1c)**. This distribution did not indicate any strong bias toward specific sequence length combinations other than most sequence pairs having lengths greater than 100 amino acid residues. For additional biological context, the interactor pair sequences used for structural prediction were queried against the Pfam database [23, 24] to identify co-occurrence of functional protein domains **(Figure 1d)**. In total, there were 2,391 unique domain co-occurrences, highlighting rich biological variability among the predicted structures. The most abundant co-occurring domains were PF00385 (chromatin organization modifier domain) with PF13923 (zinc finger, C3HC4 type (RING finger)) and PF00385 (chromatin organization modifier domain) with PF16207 (RAWUL domain RING finger- and WD40-associated ubiquitin-like), each present in 15 protein interactor pairs. Notably, the domain PF02991 (autophagy protein Atg8 ubiquitin like) was present in 173 protein interactor pairs. It should be noted that the presence of these domains does not imply interaction involvement in the interactor pairs in which they are found—*i.e.* these domains may or may not be located at the interaction interface.

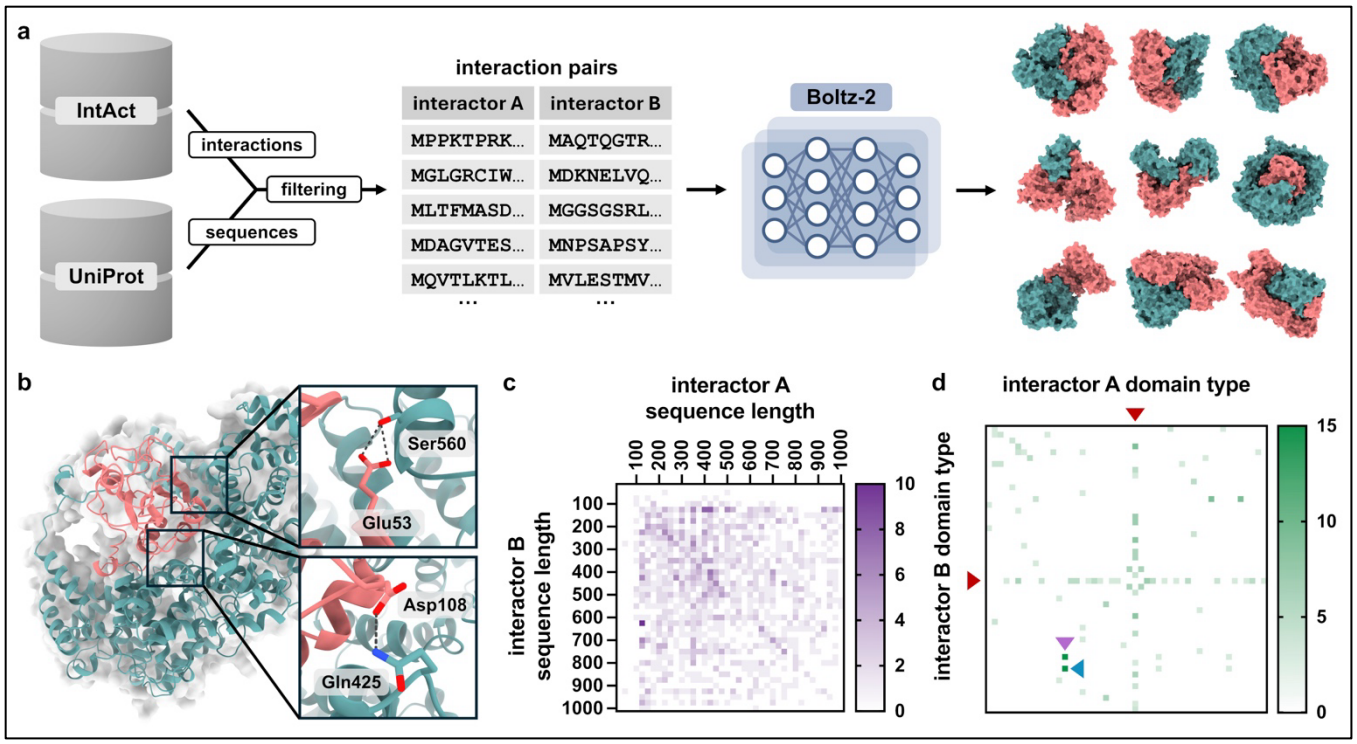

Figure 1. (a) Overview of protein-protein interaction structure prediction workflow, leveraging human biochemical interaction data and amino acid sequence data from the IntAct and UniProt databases, respectively, for binary complex prediction with Boltz-2. **(b)** Boltz-2 structure interaction prediction with the highest confidence score (0.938) among all interaction structures predicted in the current study. Interactor protein A is depicted in teal, interactor protein B is depicted in red, and dashed lines represent hydrogen bonds. The interaction is that of nuclear cap-binding complex protein subunit 1 and protein subunit 2. Insets show various predicted interacting residues between the two proteins. **(c)** Bivariate distribution of sequence lengths of protein interactor pairs, with an upper limit of 1,000 amino acid residues per protein. Data shown as increasing intervals of 25 amino acids, with the scale bar representing number of protein interactor pairs. **(d)** Co-occurrence matrix of Pfam domains across protein interactor pairs. Red arrowheads indicate the most abundant domain, PF02991 (autophagy protein Atg8 ubiquitin like), present in n = 173 pairs. Purple arrowhead indicates co-occurrence of domains PF00385 (chromatin organization modifier domain) and PF13923 (zinc finger, C3HC4 type (RING finger)), n = 15 pairs, while blue arrowhead indicates co-occurrence of domains PF00385 (chromatin organization modifier domain) and PF16207 (RAWUL domain RING finger- and WD40-associated ubiquitin-like), n = 15 pairs. Data shown as bins with a minimum of two protein interactor pairs per bin, with the scale bar representing number of protein interactor pairs.

Prediction confidence and sequence-dependent effects

To gain insight into the quality of the predicted interaction structures, we used confidence metrics for assessment of overall structure as well as the interaction interface. Predicted local distance difference test (pLDDT) [12, 25] and predicted template modelling (pTM) [12, 26] score were used for evaluation of

overall structure. In brief, pLDDT scores residue-level confidence while pTM scores confidence in domain topology. Interaction interface-restricted versions of pLDDT and pTM were used for assessment of interaction-specific structural confidence [16, 17, 22]. The Boltz-2 confidence score, which combines overall pLDDT with interface pTM, was also included for evaluation [22] (see Methods). These metrics each exhibited a wide range of values across the dataset of n = 1,394 predicted interaction structures **(Figure 2a)**. The distribution of these values suggests greater residue-level confidence (as indicated by pLDDT metrics) and lower confidence in domain topology (as indicated by pTM metrics), while the combined confidence score had a median value of 0.583, indicating that more than half of the structures were predicted with moderate confidence.

We also considered whether sequence length and MSA depth might influence prediction confidence. The relationship between combined sequence length of interaction structures (defined here as the geometric mean of the two interactor proteins) and the overall confidence score indicates that structures with shorter sequences were predicted somewhat more confidently, though this negative correlation was relatively weak (r = -0.261) **(Figure 2b)**. Comparing of MSA depth with the overall confidence score revealed a weak-to-moderate positive correlation (r = 0.353), suggesting that increased MSA depth may improve prediction confidence **(Figure 2c)**. Further analyses comparing MSA depth with overall **(Figure 2d)** and interface **(Figure 2e)** pLDDT and pTM metrics showed varying degrees of prediction confidence improvement with increased MSA depth, although relatively limited in correlation strength.

Additionally, we performed a limited evaluation of prediction accuracy using experimentally resolved structures as a reference. Given that the training cutoff for Boltz-2 was 06/01/2023, we searched the PDB [9] for structures deposited after the cutoff date for entries consisting of binary protein complexes corresponding to those predicted. While only three such structures were identified, they provide a means for experimental comparison. These experimentally determined complex structures were compared to the corresponding interaction structures predicted by Boltz-2 and by AlphaFold3 **(Figure 3)**. DockQ [27] scores and interface RMSD were computed between experimentally determined and predicted complex structures, with the predicted structures generated by both models ranging in scores from medium quality (DockQ of 0.49 – 0.80) **(Figure 3a)**, acceptable quality (DockQ of 0.23 – 0.49) **(Figure 3b)**, and incorrect (DockQ of 0 – 0.23) **(Figure 3c)**. Taken together, these results highlight various strengths and limitations of large-scale protein interaction structure prediction with Boltz-2, including favorable residue-level prediction confidence contrasted by relative uncertainty in domain topology prediction. Furthermore, the availability of additional sequence context through increased MSA depth, though not found to be a requirement for high prediction confidence, may have a moderately positive effect.

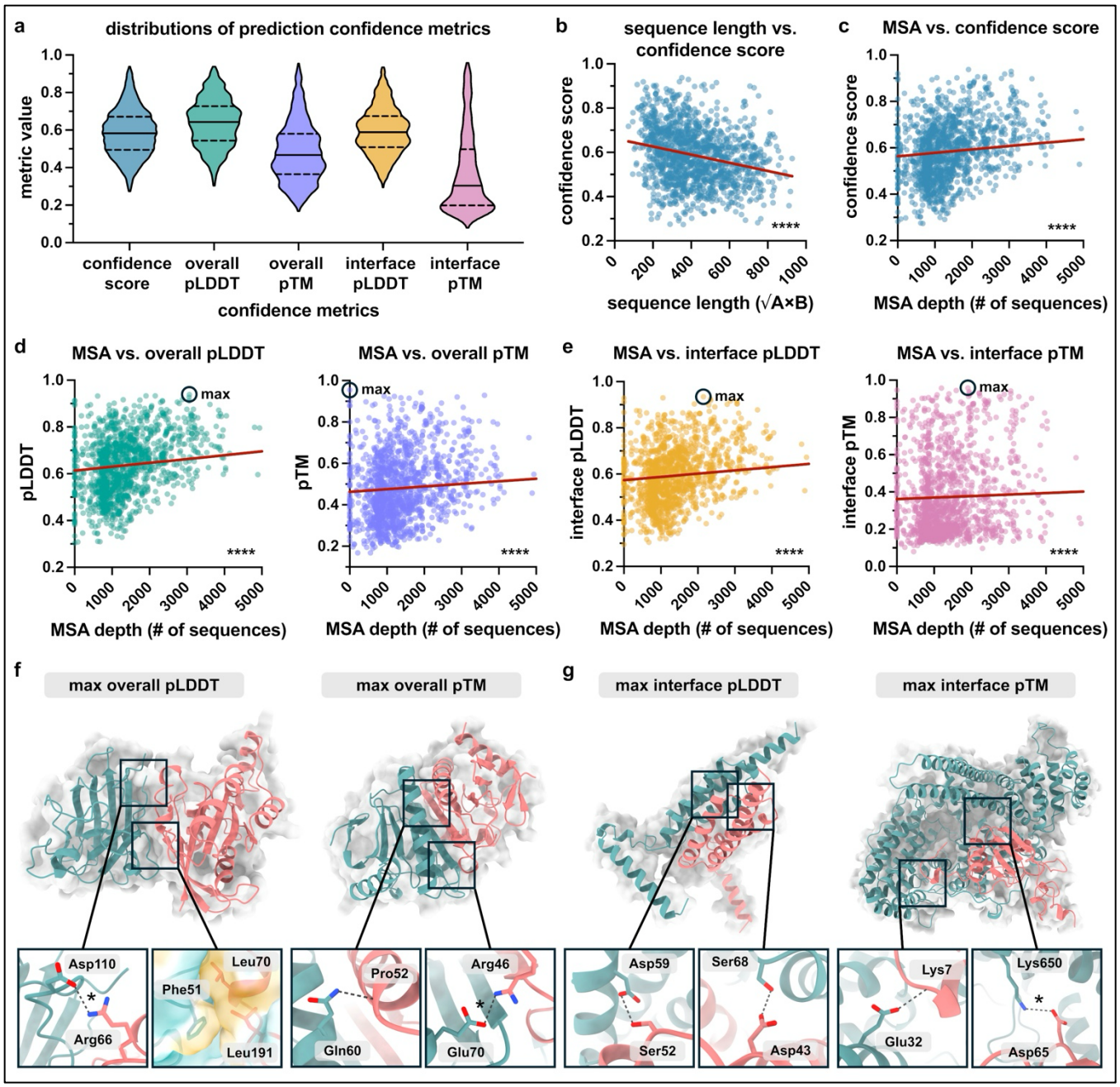

Figure 2. (a) Prediction confidence metric distributions across all protein interaction structures predicted (n = 1,394). All metrics were determined by Boltz-2 on a normalized scale of 0 to 1, enabling direct comparison (see Methods). Solid lines represent medians and dashed lines represent first and third quartiles. **(b)** Relationship between combined sequence length (the geometric mean of interactor sequence A and interactor sequence B) and confidence score of the predicted interaction structures. Spearman correlation r = -0.261, ****p <0.0001. **(c)** Relationship between MSA depth and confidence score of the predicted interaction structures. Spearman correlation r = 0.353, ****p <0.0001. Predicted interaction structures with MSA depth greater than 5000 (n = 25 structures) are not graphically depicted here, but were included in statistical analyses for panels c-e. **(d)** Relationship between MSA depth and entire interaction complex (overall) pLDDT and pTM. Spearman correlation r = 0.369 for overall pLDDT, Spearman correlation r = 0.266 for overall pTM, ****p <0.0001 **(e)** Relationship between MSA depth and interaction interface-aggregated pLDDT and pTM. Spearman correlation r = 0.331 for interface

pLDDT, Spearman correlation $r = 0.139$ for interface pTM, ****$p < 0.0001$. Lines determined by linear regression are depicted in red in panels b-e. **(f-g)** Predicted interaction structures which exhibited the greatest overall (f) and interface (g) metrics. Interactor protein A is depicted in teal and interactor protein B is depicted in red. Dashed lines represent hydrogen bonds, dashed lines with an asterisk represent salt bridges, and yellow surfaces represent hydrophobic regions.

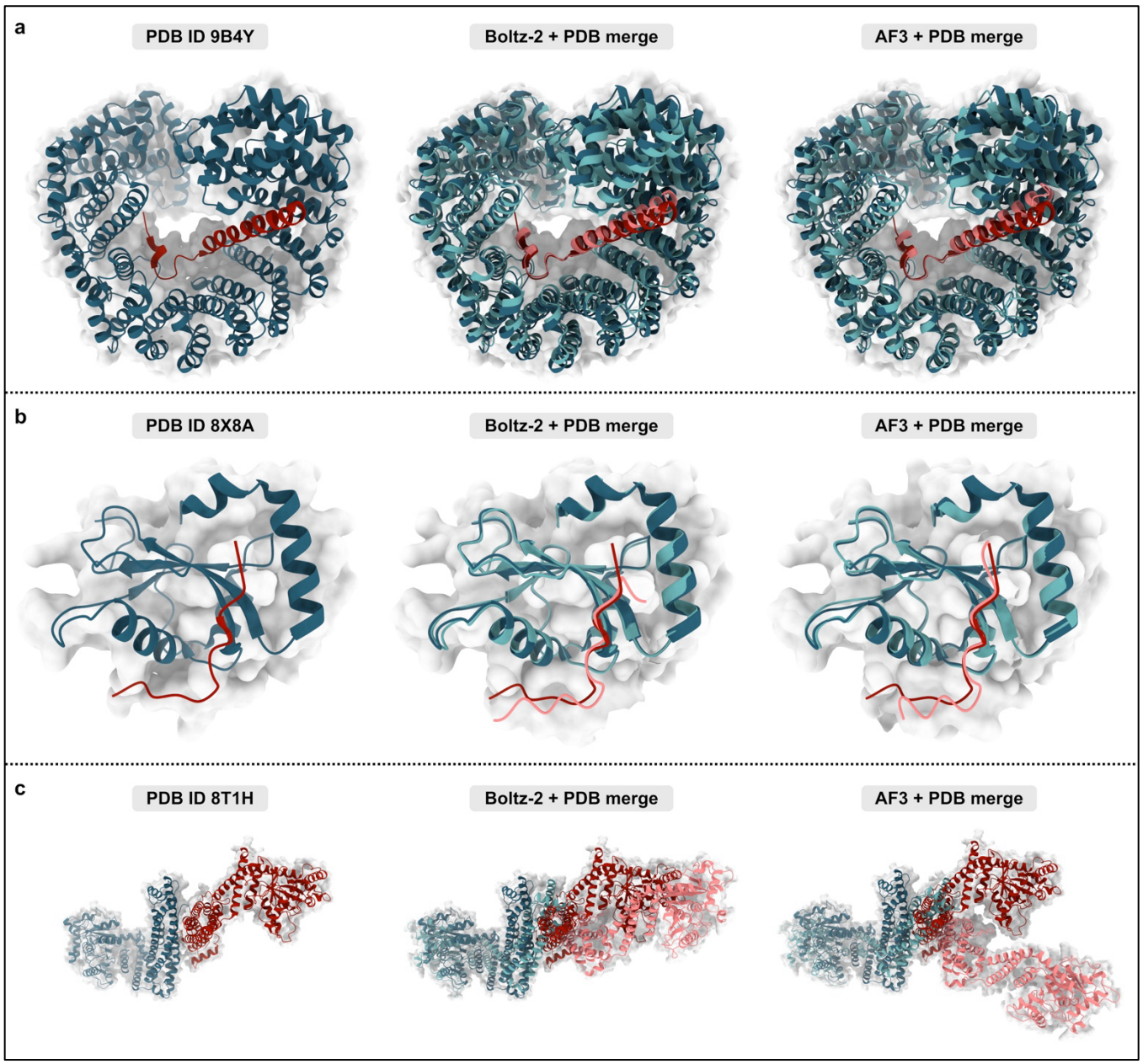

Figure 3. (a-c) Experimentally determined interaction structures deposited in the PDB beyond the Boltz-2 training cutoff date compared to interaction structures predicted by Boltz-2 and AlphaFold3. Interactor protein A is depicted in teal and interactor protein B is depicted in red, with darker shading for experimentally determined structures and lighter shading for predicted structures. **(a)** CryoEM structure of importin subunit alpha-1 in complex with importin subunit beta-1. Measures of accuracy between the PDB structure and predicted structures were DockQ = 0.755

(interface RMSD = 1.516 Å) for Boltz-2, and DockQ = 0.717 (interface RMSD = 1.811 Å) for AlphaFold3. **(b)** Crystal structure of gamma-aminobutyric acid receptor-associated protein-like 1 in complex with starch-binding domain-containing protein 1. Measures of accuracy between the PDB structure and predicted structures were DockQ = 0.348 (interface RMSD = 5.688 Å) for Boltz-2 and DockQ = 0.372 (interface RMSD = 5.588 Å) for AlphaFold3. **(c)** CryoEM structure of a homodimeric complex of dynamin-1-like protein. Measures of accuracy between the PDB structure and predicted structures were DockQ = 0.210 (interface RMSD = 3.907 Å) for Boltz-2 and DockQ = 0.201 (interface RMSD = 4.125 Å) for AlphaFold3. In all panels, only residues present in the experimental PDB structures are shown for the corresponding predicted structures.

DISCUSSION

This work demonstrates the utility of Boltz-2 for structural modeling of the human interactome through large-scale prediction of binary interaction structures based on biochemical data from IntAct in combination with sequence data from UniProt. Although we predicted structures of a constrained representative subset of human PPIs, the scalability of this approach supports application to larger datasets. Our analyses revealed that over half of the structures were predicted with moderate confidence, with residue-level confidence appearing to outperform confidence in domain topology. Furthermore, we found that increased MSA depth was associated with greater prediction confidence, though only moderately. Another trend we observed, albeit weakly correlated, was decreased prediction confidence with increased sequence length, indicating a potential limitation for the prediction of larger protein complexes.

Notably, in the limited evaluation comparing predicted complex structures to those experimentally determined **(Figure 3)**, both Boltz-2 and AlphaFold3 performed strongly and struggled on the same cases. This observation could indicate that certain structural classes or interface types remain inherently challenging to predict. Novel training strategies and architecture refinements may serve to improve predictive accuracy for currently challenging targets. Nevertheless, caution should be exercised when interpreting predicted complex structures generated with either computational tool, and confidence metrics may be useful indicators in this regard.

Looking ahead, it is reasonable to propose that Boltz-2 could be used to model multi-protein complexes of the human interactome, going beyond binary interactions. Boltz-2 is capable of performing predictions of structures with more than two chains, and such modeling has previously been explored for protein complexes with AlphaFold-multimer [28]. Moreover, large-scale modeling of interactions with disease-relevant mutations as well as interactions with pathogenic proteins would be particularly informative for understanding mechanisms of disease and guide targeted therapeutic development. There are multiple additional avenues of modeling that merit further research. The current work suggests Boltz-2 is a valuable tool for human interactome protein structure prediction and related work, though potential

limitations related to protein complex size and domain-level accuracy remain important considerations. These AI/ML-based modeling approaches are anticipated to aid multiple areas of biomedical research through broadened characterization of structural interactions.

METHODS

Curation and processing of interaction and sequence data

The *Homo sapiens* species-specific dataset, which lists binary interaction pairs by UniProt ID, was downloaded from the IntAct database in tabular format [2]. The dataset was filtered as follows: (1) removal of all interaction pairs for which one of the two interacting proteins were non-human; (2) exclusive retention of interaction pairs which contained the “Direct interaction” designation; and (3) exclusive retention of interaction pairs which determined by pull down as the experimental interaction detection method. Corresponding sequences of interaction pairs were obtained by UniProt ID using the UniProt website REST API [8]. Corresponding Pfam domains were obtained using the InterPro Rest API [23]. Twelve interaction pairs were excluded from data analysis. Three interaction pairs were excluded due to their sequences containing the amino acid selenocysteine. Additionally, nine interaction pairs for which Boltz-2 failed to generate predictions, despite multiple attempts, were also excluded. With all of the above curation and exclusion, a total of n = 1,394 binary interaction pairs were analyzed in the current work.

Interaction structure prediction and analyses

Structure predictions were performed using Boltz-2 [22] version 2.1.1 in the Google Colab environment. Hardware configurations were set to A100 GPU and High-RAM. The paired interaction protein sequences were used as input for protein complex prediction. Default Boltz-2 settings were used, except for MSA server use set to True, as follows:

diffusion_samples = 1
recycling_steps = 3
sampling_steps = 200
step_scale = 1.638
use_msa_server = True
msa_server_url = https://api.colabfold.com
max_msa_seqs = 8192

subsample_msa = False
msa_pairing_strategy = greedy

With MSA server use, Boltz-2 relies on the MMseqs2 server [29-31] for automatic MSA generation. Output from Boltz-2 used for analyses include predicted structures in CIF format, MSAs, and confidence metrics (confidence score, overall pLDDT, overall pTM, interface pLDDT, interface pTM), as described in the Boltz-2 documentation available on GitHub (https://github.com/jwohlwend/boltz). The Boltz-2 confidence score is calculated as follows: confidence score = 0.8 × overall pLDDT + 0.2 × interface pTM.

For comparison to experimental structures, the RCSB PDB Search API [9] was used to search for structures corresponding to the UniProt IDs of the *n* = 1,394 predicted structures that were deposited after the Boltz-2 model training cutoff date of 06/01/2023 [22]. Search criteria were set to include only structures determined by X-ray crystallography or CryoEM, without mutations, protein only, and containing exactly two chains (corresponding to the two interactor proteins), which retrieved a total of three structures **(Figure 3)**. Corresponding AlphaFold3 structures were predicted using the Google DeepMind AlphaFold server (https://alphafoldserver.com/) [16]. DockQ scores and interface RMSD were determined using DockQ v2 [27]. All other molecular analyses, including hydrogen bond and salt bridge determination, hydrophobicity determination, and structure visualization were performed using ChimeraX [32]. In ChimeraX, hydrogen bonds were determined with the H-bonds tool using default settings (*i.e.,* radius = 0.075 Å and without relaxed distance/angle criteria) and selection of salt bridge only setting allowed for salt bridge determination. Hydrophobic surfaces were determined using the automated molecular lipophilicity potential (MLP) command.

Statistical Analyses and Graphical Representation

Statistical analyses of medians, quartiles, Spearman correlations, linear regression, as well as graphical representations shown in Figures 1 and 2, were performed using GraphPad Prism 10. In Figure 2c-e, *n* = 25 interaction structures which had MSA depths greater than 5000 sequences are not shown on the graphs but were not excluded from statistical analyses. The complete set of data used for statistical analyses is presented in Supplementary Table 1.

Data Availability

All predicted complex structure models described herein are freely available online at https://github.com/structural-interactome/human-interactome.

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AUTHOR CONTRIBUTIONS

A.M.I., C.M., S.K.B., R.P., and W.A. conceptualization; A.M.I. and C.M. methodology; A.M.I. investigation; A.M.I., C.M., S.K.B., R.P., and W.A. formal analysis; A.M.I. and C.M. writing—original draft; S.K.B., R.P., and W.A. writing—review & editing; S.K.B., R.P., and W.A. funding acquisition; S.K.B., R.P. and W.A. overall project supervision.

COMPETING INTERESTS

A.M.I. is a founder and partner of North Horizon, which is engaged in the development of artificial intelligence-based software. R.P. and W.A. are founders and equity shareholders of PhageNova Bio. R.P. is Chief Scientific Officer and a paid consultant of PhageNova Bio. R.P. and W.A are founders and equity shareholders of MBrace Therapeutics. R.P. and W.A. serve as paid consultants for MBrace Therapeutics. R.P. and W.A. have Sponsored Research Agreements (SRAs) in place with PhageNova Bio, MBrace Therapeutics, and Alnylam Pharmaceuticals; this study falls outside of the scope of these SRAs. These arrangements are managed in accordance with the established institutional conflict-of-interest policies of Rutgers, The State University of New Jersey. C.M. and S.K.B. declare no competing interests.

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