LambdaPP: Fast and accessible protein-specific phenotype predictions

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Running Title: LambdaPP protein-specific phenotype prediction (<50 characters)
Doc stats: 10 pp, 3370 of 5000 words, Abstract: 147/250 words, Impact: 63/75, SOM: 3 Fig + 4 p, tables: 0, figures: 4/10
Journal: Protein Science
Submitted: Aug 3, 2022

Impact Statement

Here, we introduce LambdaPP, a webserver integrating fast and accurate sequence-only protein feature predictions based on embeddings from protein Language Models (pLMs) in seconds with high-quality protein structure predictions. The intuitive interface invites experts and novices to benefit from the latest machine learning tools. LambdaPP's unique combination of predicted features may help in formulating hypotheses for experiments and as input to bioinformatics pipelines.

Abstract

The availability of accurate and fast Artificial Intelligence (AI) solutions predicting aspects of proteins are revolutionizing experimental and computational molecular biology. The webserver *LambdaPP* aspires to supersede PredictProtein, the first internet server making AI protein predictions available in 1992. Given a protein sequence as input, *LambdaPP* provides easily accessible visualizations of protein 3D structure, along with predictions at the protein level (GeneOntology, subcellular location), and the residue level (binding to metal ions, small molecules, and nucleotides; conservation; intrinsic disorder; secondary structure; alpha-helical and beta-barrel transmembrane segments; signal-peptides; variant effect) in seconds. The structure prediction provided by *LambdaPP* - leveraging *ColabFold and computed in minutes* - is based on *MMseqs2* multiple sequence alignments. All other feature prediction methods are based on the pLM *ProtT5*. Queried by a protein sequence, *LambdaPP* computes protein and residue predictions almost instantly for various phenotypes, including 3D structure and aspects of protein function.

Accessibility Statement

LambdaPP is freely available for everyone to use under embed.predictprotein.org, the interactive results of the described case study can be found under https://embed.predictprotein.org/#/Q9NZC2. The code of LambdaPP can be found on GitHub (github.com/sacdallago/embed.predictprotein.org), and can be freely used and distributed under the academic free use license (AFL-2). For high-throughput applications, all methods can be executed locally via the bio-embeddings (bioembeddings.com) python package, or docker image at ghcr.io/bioembeddings/bio_embeddings.

Key words: protein structure prediction, protein function prediction, protein annotation, protein language models, web server, artificial intelligence.

Abbreviations used: 3D, three-dimensional; **AI**, Artificial Intelligence; AlphaFold2, break-through method for protein 3D structure prediction (30); **API**, application programming interface; **AUC**, area under the receiver operating characteristic curve; **BPO**, biological process ontology; **CAFA**, critical assessment of functional annotation; **CASP**, critical assessment of protein structure prediction; **CATH**, class, architecture, topology, homologous superfamily; **CCO**, cellular component ontology; **CheZOD**, chemical shift Z-scores; **CI**, confidence interval; **CNN**, convolutional neural network; **ColabFold**, protocol for fast execution of *AlphaFold2* (45); **DB**, database; **DMS**, deep mutational scanning; **EI**, evolutionary information; LambdaPP, embedding based protein predictions; **GO**, gene ontology; **GPU**, graphical processing unit; **LA**, light attention; **MFO**, molecular function ontology; **MSA**, multiple sequence alignment; **pLDDT**, predicted Local Distance Difference Test; **pLM**, protein language model; **PP**, PredictProtein; **RI**, Reliability Index; **RMSD**, root-mean-square deviation; **SAV**, single amino variant effect; **SE**, standard error; **SOTA**, state-of-the-art; **TM**, transmembrane; **TMP**, transmembrane protein; **TPU**, tensor processing unit

Introduction

PP protein prediction since dawn of internet. Launched 30 years ago, the PredictProtein (PP) web server provides a comprehensive interface for protein sequence analysis (55; 58; 75; 12). As the first internet server for predicting aspects of protein structure and function, it offers a broad overview of predicted features. Amongst many innovations, PP introduced the combination of evolutionary information (EI) from multiple sequence alignments (MSAs) and machine learning (56), a subset of artificial intelligence (AI), for protein prediction. Nature's 2021 method of the year (43), *AlphaFold2* (30), peaked the innovation by essentially solving the protein structure prediction problem with models approaching experimental high-resolution, inspiring a new era of advancing methods (2; 6; 45) and their application (17; 32; 77). *AlphaFold2* came at a crucial time when more sequences than ever before (2.1 billion proteins in BFD (66)) were met by new algorithms in AI and hardware optimized to AI (NVIDIA's GPUs and Google's TPUs). *PredictProtein* and *AlphaFold2* work great in their domains and integrating both might help in enabling experts and novices alike to experiment, hypothesize, and generate novel insights quickly.

EI+AI top, but not without caveats. Since the release of PredictProtein, the amount of non-annotated sequences has been rapidly increasing (57; 66). In fact, the sequence-annotation gap continues to increase despite experimental advances, e.g., experimental residue binding annotations are currently added for only two sequence-unique proteins per month for any organism and any ligand (39). AI models mitigate this gap. Until 2020, almost all state-of-the-art prediction methods had implemented the concept introduced by PP, namely inputting MSAs into AI. Although super-fast tools relying on algorithmic and hardware advances sped up MSA generation (46; 16), bio-databases continue outgrowing the pace at which computer hardware accelerates (47; 70; 65). This challenge cannot be resolved by advancing computers. On top, MSAs are not always informative, especially for small sequence families, or proteins of the Dark Proteome (50).

Protein Language Models (pLMs) reach top. Developments in representation learning (8), particularly in natural language processing (18), have spawned solutions for encoding latent protein information including aspects of evolutionary information. Protein language models (pLMs) are deep learning models pre-trained on large sets of unannotated sequences to generate numerical representations (embeddings) (9; 24; 40; 23; 49; 54). Embeddings from pLMs have been successfully used as input to downstream protein prediction tools (78; 37-39; 41; 42; 44; 64; 14; 25; 27; 63; 72). While some pLM-based methods do not reach the performance of MSA-based methods (23; 38; 72), others exceed those (5; 10; 23; 39; 42; 64; 28; 29; 36). Prediction performance has risen so much that sequence-specific predictions based on pLMs can capture some aspects of structural and functional dynamics better than much more accurate family-averaged solutions even from *AlphaFold2* (35; 72; 74).

pLM-based protein predictions for the web. 30 years ago, PredictProtein offered the first access to a variety of MSA-based AI solutions. Similarly, LambdaPP is now making state-of-the-art solutions for embedding-based protein predictions available. The server outputs

predictions for the entire query protein (per-protein) and for each of its residues (per-residue, Fig. 1). All results are linked to 3D structure visualizations, currently retrieved from the AlphaFold Database (71) or if unavailable predicted using *ColabFold*, easing the access to *AlphaFold2* at similar performance (45). As the only method of LambdaPP not based on pLM inputs, it will soon be complemented by pLM-based solutions (35; 72; 74). As novel AI tools leveraging embeddings emerge, e.g. predicting CATH (62) classes (25), LambdaPP will be updated to extend the breadth of pLM-based predictions offered. All feature prediction methods integrated into the LambdaPP webserver are currently based on ProtT5 embeddings (23) as methods trained on ProtT5 have, so far in our hands, outperformed those trained on ESM-1b (54) and others (4; 9; 24; 23) for numerous different applications (37-39; 42; 64; 13; 25; 72). This consistency also increases speed as the generation of embeddings has become a limiting step.

Results

Access of server. All methods are available through embed.predictprotein.org where users submit amino acid sequences up to 2000 residues. This limit is imposed, at this point, to speed-up response-time (embedding computation is non-linear in protein length). The following formats are currently handled: FASTA format, UniProt accession number, UniProt protein name (69), or a string of residues ("AA format"). Results are displayed immediately if cached or computed on the fly within seconds for pLM-based methods if not. There is no queuing system, as it takes longer to generate the display items on the frontend than to compute the predictions in the backend. Protein 3D structures are fetched from the AlphaFold Database (71) when using UniProt accessions as input, or predicted using ColabFold (45) to a first come, first serve queue (completing within 30 minutes for the average protein with ~350 residues). Due to GPU limitations, the ColabFold 3D structure prediction is restricted to sequences ≤500 residues, but work is underway to transition to pLM-based 3D structure prediction providing fast and accurate predictions for longer sequences. All outputs are cached for 10 days before being deleted to conserve disk space and respect data privacy.

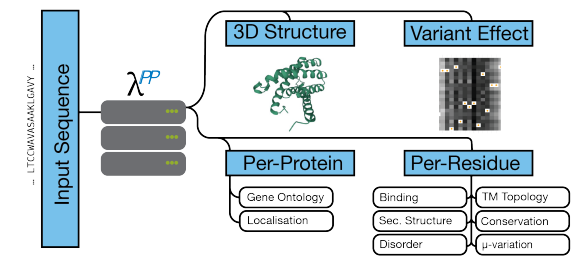

Fig. 1: LambdaPP pipeline. Starting with an amino acid sequence, LambdaPP orchestrates the prediction of (1) protein structure using ColabFold (45); (2) per-protein features: Gene Ontology (GO) annotations using goPredSim (38), subcellular location using LA (64); (3) per-residue features: binding residues using bindEmbed21DL (39), conservation using ProtT5cons (42), disorder using SETH (29), secondary structure using ProtT5-sec (23), helical and barrel transmembrane (TM) regions using TMbed (13); and (4) variant effect scores using VESPAI (42).

Frontend and interface. The main LambdaPP interface displays the results of predictions in thematically ordered sections. Leveraging the *neXtProt* feature viewer (59), per-residue predictions are displayed in one view-pane (Fig. 2). The neXtProt plugin enables to display categorial features, e.g., binding, transmembrane-regions, and secondary structure as colored regions, and continues features, e.g., disorder, variance effect, and conservation, as line plots. An interactive connection between residue-level features and 3D protein structure allows to map the predictions onto the 3D visualization while displaying additional information in tooltips. The *protein-level section* visualizes subcellular location through colored images (19; 64) and GO-term predictions as lists of predicted GO-terms along with scores reflecting reliability (RI) and links to the reference protein used for the annotation transfer (38). The *Single Amino acid Variant (SAV) effect section* features the predictions of how much point mutations (SAVs) negatively affect molecular function. By default, the effect is predicted for all 19 non-native SAVs, i.e., all amino acid point mutations irrespectively of their reachability through single nucleic variants (SNVs or SNPs). Finally, the predicted 3D structure is visualized in the *structure section*, using the Mol* plugin (61). To facilitate users in exploring the predictions, we offer two alternative interfaces: the so-called *print-page*, which displays several residue-level features in a print-friendly display, and the *interactive* page, which displays the neXtProt feature viewer and 3D structure prediction in a single panel to allow easier interactive exploration. These alternative displays can be reached from

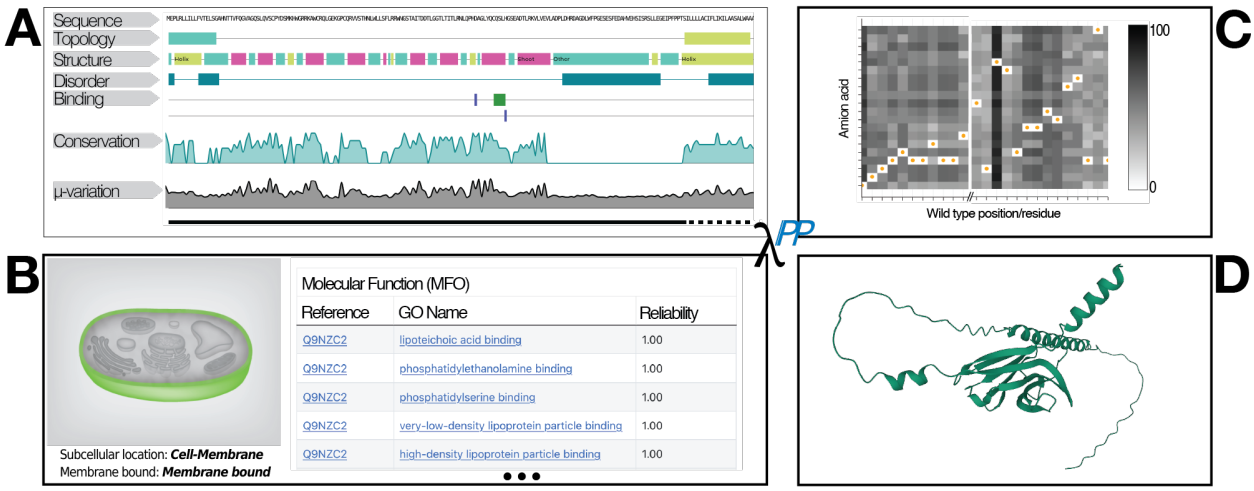

Fig. 2: LambdaPP output for *TREM2_HUMAN*. *Panel A:* residue level features: secondary structure, transmembrane topology, disordered residues, small molecule, nucleic or metal binding residues, residue conservation and average variation (23; 39; 42; 13; 29); *Panel B:* sequence-level features: predicted subcellular localization (64), and an excerpt of predicted GO-annotations (38); *Panel C:* effect of SAVs (wild-type sequence on x-axis, mutations on y-axis; darker color=higher effect) (42); and *Panel D*: predicted 3D structure (45). Interactive version at https://embed.predictprotein.org/#/Q9NZC2.

the main interface by clicking on the suggested alternative display buttons.

Backend and programmatic access. To retrieve prediction results programmatically, users can use the bio-embeddings REST API, with documentation through api.bioembeddings.com/api. Among other things, the interface allows to create ProtT5 embeddings of proteins with up to 2,000 residues (hardware not model restriction), as well as to retrieve the full spectrum of available annotations as generated by the backend, i.e., to download all predictions in JSON format. The backend could be hosted entirely on a standard workstation equipped with a workstation GPU (e.g., Quadro RTX 8000 46GB RAM), delivering results in seconds compared to minutes or hours for multi-node and cluster-based PP (12). However, to counter machine faults and guarantee availability, the hosted LambdaPP runs on different servers at the LCSB in Luxembourg and the TUM in Munich and can process one request at the time on average in four seconds for sequences of 350 residues. The hosted backend can be manually scaled to respond to parallel requests during high demand.

Availability for local deployment. To take advantage of the methods feature on LambdaPP locally, advanced users can rely on the bio-embeddings package (20) (bioembeddings.com). Along with various use cases, it provides a docker image (ghcr.io/bioembeddings/bio_embeddings) for easy deployment on local machines. We recommend installing bio-embeddings locally to avoid length restrictions imposed on LambdaPP.

Use case: TREM2_HUMAN (Q9NZC2). We demonstrated the LambdaPP workflow using the *triggering receptor expressed on myeloid cells 2* (UniProt accession: Q9NZC2) protein and compared results to the expert curated UniProtKB entry (69). We selected Q9NZC2, as it is associated with *Polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy* (PLOSL2) and has a variety of annotations in different regions. The full spectrum of available annotations at the time of writing can be found in the accompanying supporting online material (SOM).

Per-protein: LambdaPP trivially listed most UniProtKB GO annotations with high reliability because the protein was in goPredSim's lookup set. Subcellular location was correctly predicted as cell-membrane, and the structure from the AlphaFold Database (71) aligned well with the structures of 5ELI (3; 31) (RMSD = 0.45 Å,) and 5UD8 (67; 68) (RMSD = 0.37 Å) (SOM: Fig. S4).

Per-residue: LambdaPP marked the first 17 residues as signal peptides, matching the automatic (rule-based) UniProtKB annotation (one residue shorter). The transmembrane stretch

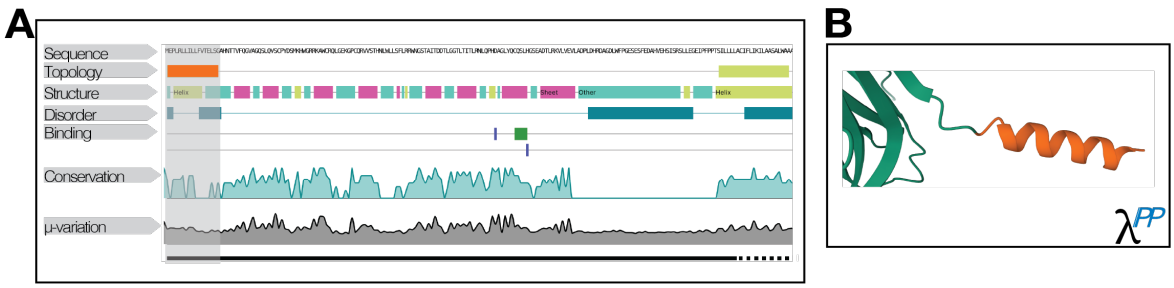

Fig. 3: Interactive connection of 2D and 3D viewer. *Panel A* displays the predicted residue level features (23; 39; 42; 13; 29) for TREM2_HUMAN, with a selected signal peptide prediction. *Panel B* shows how the selection affects the 3D visualization; the structure is turned, zoomed, and highlighted to best display the selected feature. An interactive version of the collated panels can be found at https://embed.predictprotein.org/#/Q9NZC2.

visualized in LambdaPP aligns well with the UniProtKB transmembrane stretch (for residues shorter) (SOM: Fig. S5).

Binding: UniProtKB annotates no metal ion, small molecule, or nucleic acid binding site. However, bindEmbed21DL predicted two metal ion and one small-molecule binding residues with high reliability which might be interesting targets for future experiments (39).

Disorder & conservation: UniProtKB also annotates no intrinsic disorder. Yet, the predicted high disorder content for loop regions next to the transmembrane segment and the high order content outside correlated well with the predicted pLDDT from *AlphaFold2* (Pearson's r: 0.92; Fig. S6) (51; 73). For sequence conservation, we compared the predictions generated by LambdaPP to those in ConSurfDB (7) obtained for 5ELI (3; 31) (mean squared error (MSE) ~9) and 5UD8 (67; 68) (MSE ~4) (Fig. S7).

SAV effects: The predictions of the effects of single SAVs upon molecular function showed a similar trend as the UniProtKB annotations: Q9NZC2 seems susceptible to mutation effects. Zooming into residues relevant for binding to PLOS2, e.g., a mutation at residue 126 (V>G) suggests a strong mutation effect (score 71). Residues marked in LambdaPP with high scores could be an interesting target for future mutational assays (e.g., residues at position 35, 85, 105).

Interactive selection. When users select predicted residue features on the nextProt viewer, the selection is transferred into the 3D viewer (Fig. 3). This eases the identification of relevant structural regions. For instance, selecting the predicted signal peptide for Q9NZC2 (Fig. 3), highlights the region on 3D structure and allows to visually verify the prediction.

Use case: ice nucleation protein. Another use case was an ice nucleation protein from *Pseudomonas syringae* (ICEV_PSESX, O33479). We selected this protein when spotting the AlphaFold2 prediction (Fig. 4A). Interestingly, the predicted intrinsically disordered region (residues 111-165) appeared to agree more with the *AlphaFold2* prediction (Fig. 4A: loop next to top left) than with the three separate regions annotated in UniProtKB.

Use case: potential new superfamily. The choice of the third use case was also triggered by AlphaFold2 predictions for UniRef published in Aug. 2022. The plant protein with UniProt ID Q9S828_ARATH remains uncharacterized by experiment. AlphaFold2's 3D prediction suggests this as a new superfamily (15). SETH's prediction of an intrinsically disordered region agreed with the predicted 3D structure (N-terminal region looping to the lower left (Fig. 4B). Surprising appeared the very low level of conservation.

Conclusions

LambdaPP delivers an important step into the next generation of protein prediction, providing the first lightning fast, all-round protein prediction server based almost exclusively on the outputs of one protein language model – ProtT5, accompanied by high-quality 3D structure predictions from the AlphaFold DB or ColabFold. The web interface allows detailed analyses without requiring users to chip-in high-end servers, AI knowledge, or programming skills. The sub-minute turnaround time from sequence input to predictions of 15 different per-residue and per-protein features coupled with an intuitive user interface allow users to quickly generate an overview for any desired protein sequence, in turn allowing hypothesis

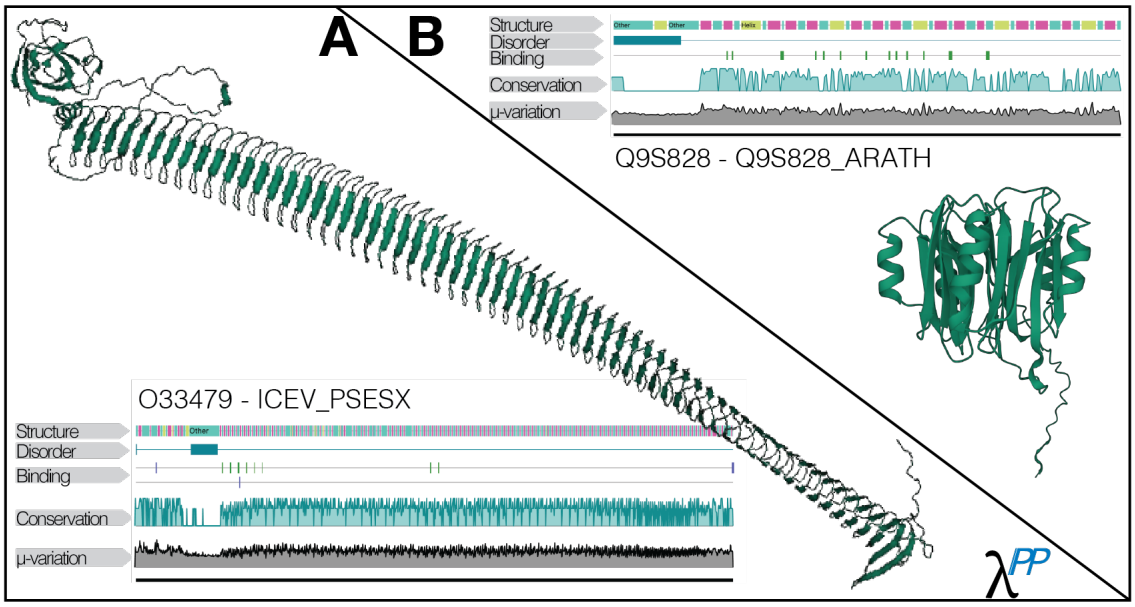

Fig. 4: Remarkable AlphaFold2 predictions. *Panel A* (lower left triangle) displays the 3D structure predicted by AlphaFold2 for the ice nucleation protein ICEV_PSESX. The protein contains 1165 residues and is available through LambdaPP as part of AFDB. *Panel B* (upper right triangle) showcases the AlphaFold2 prediction of what might constitute a novel superfamily for the plant protein with the UniProt identifier Q9S828_ARATH.

generation and analysis. Thereby, LambdaPP might become valuable for bridging the sequence-annotation gap through predictions from high-quality methods, allowing researchers to prioritize experiments and curation efforts.

Materials & Methods

LambdaPP computes protein language model (pLM) representations (embeddings) using ProtT5 (23) from single amino acid sequences. More specifically, the embeddings are derived from the encoder-part of ProtT5 in half-precision, i.e., from float32 model weights to float16 model weights, which speeds up inference at no cost in performance (23). These embeddings are input to all methods provided via LambdaPP except ColabFold (45). Per-protein features predicted solely with ProtT5 embeddings as input currently include subcellular location (64), and Gene Ontology terms (GO) (38). Per-residue predictions solely with ProtT5 embeddings as input include: conservation (42); helical transmembrane regions, transmembrane beta barrels, along with signal peptides (13); binding for various ligands (39); intrinsically disordered regions (29); secondary structure (23). LambdaPP also predicts the effect of introducing single amino acid variants (SAV) in the input sequence upon molecular function, which uses the predicted conservation with a BLOSUM62-score (26) of the SAV as input (42). Additionally, the 3D structure for the query-sequence is currently predicted with ColabFold (45) implementing AlphaFold2 (30), however pLM-based alternatives are in development and will be available online soon (72).

Per-protein: Gene Ontology (GO). The method goPredSim (38) predicts GO terms by transferring annotations from the closest neighbor in a lookup dataset of proteins with known GO annotations (38). The closest neighbor is defined by the smallest pairwise Euclidean distance calculated between the ProtT5 embeddings of the lookup set and the target. The distance is converted to a Reliability Index (RI) ranging from 0 (weak prediction) to 1 (confident prediction). RI values above 0.35 for biological process ontology (BPO), 0.28 for molecular function ontology (MFO), and 0.29 cellular component ontology (CCO) suggest reliable results. Replicating CAFA3 (79), goPredSim reached F_{max}(BPO)=38±2%, MFO: 52±3%, and CCO: 59±2% using ProtT5 embeddings (12; 38). Tested on proteins annotated after Feb. 2020 and confirmed by CAFA4 (22), results were slightly better (github.com/Rostlab/goPredSim - performance-assessment).

Per-protein: subcellular location. For a given protein sequence, light attention (LA) predicts where in a cell a protein functions, i.e., its subcellular location or cellular compartment (64). Ten subcellular localization classes are differentiated as mapped in DeepLoc (5). The LA network architecture using ProtT5 embeddings as input significantly outperformed MSA-based state-of-the-art (SOTA) methods by about eight percentage points (Q10). For this task, ProtT5 embeddings (23)

significantly outperformed all other pLM embeddings as input for the same architecture (4; 9; 24; 23; 54).

Per-residue: ligand-binding. bindEmbed21DL (39), a two-layer convolutional neural network (CNN) exclusively inputting ProtT5 embeddings, predicts residues binding to metal ions, nucleic acids, or small molecules, distinguishing the three classes. The pLM-based method substantially outperformed its MSA-base predecessor (F1=47±2% vs. F1=34±2%) (60) on binding annotations from BioLiP (76). The seemingly low F1-score hid that the method often outperformed human annotations, in the sense that all strongly predicted (high reliability) residues annotated as non-binding investigated in detail appeared to reveal missing annotations rather than prediction mistakes.

Per-residue: conservation. ProtT5cons (42) is a two-layer CNN, predicting the degree to which a residue is conserved in an MSA without using an MSA as input. The conservation level is scaled from 0 (highly variable) to 8 (highly conserved) similarly to ConSurf-DB (7). ProtT5 embeddings outperformed those from the pLM ProtBERT and performed *on par* with the ESM-1b (54) pLM embeddings. While only taking embeddings as input, the performance of ProtT5cons was similar to ConSeq (11) using MSAs (two-state Matthews Correlation Coefficient MCC(embeddings)= 0.596±0.006 vs. MCC(ConSeq) = 0.608±0.006) when compared to conservation levels of ConSurf-DB.

Per-residue: intrinsic disorder. SETH (29), a two-layer CNN, predicts the degree of intrinsic disorder of a residue as defined by the chemical shift Z-scores (CheZOD) (48), where values below 8 signify disorder and values above 8 signify order. Different pLMs were compared (ProtT5 (23), ProSE(10), ESM-1b (54), ProtBERT (23), SeqVec (24)) with ProtT5 numerically outperforming the others. SETH outperformed all existing SOTA approaches in terms of mean AUC (area under the receiver operating characteristic curve) and Spearman correlation (0.72±0.01 for SETH vs. 0.67±0.01 for next best method ODinPred (21)) as well as similar current solutions operating on ESM-1b embeddings (52).

Per-residue: secondary structure. ProtT5-sec(23), a two-layer CNN, reached a Q3 (three state per-residue accuracy) of 81±1.6% for the CASP12 (1) test set and Q3 of 84±0.5% for a larger data set NEW364 (23) competitive with, or even surpassing, top methods relying on MSAs.

Per-residue: transmembrane helices and strands. TMbed predicts for each residue one of four classes: alpha helical transmembrane (TM) region, transmembrane beta strand, signal peptide, or other (13). For proteins with TM regions, it also predicts the inside/outside orientation within the membrane, i.e., on which side of the membrane the N-terminus begins. The model uses a four-layer CNN combined with a Gaussian filter and a Viterbi decoder. When applied to a non-redundant test set, TMbed correctly predicted 94±8% of beta-barrel transmembrane proteins (TMPs) and 98±1% of alpha-helical TMPs at false positive rates <1%. Furthermore, TMbed placed on average 9 out of 10 transmembrane segments within five residues of the experimental observation. TMbed performed *on par* with or better than SOTA methods. It stood out in terms of its low false positive rate and speed; both making TMbed well suited for high-thruput annotation and filtering, such as annotating millions of AlphaFold2 models.

Per-residue: variant effect and μ-variation. To predict the effect of SAVs, VESPAl (42) takes the nine-state conservation prediction by ProtT5cons, and the BLOSUM62 substitution matrix (26) as input for a logistic regression. The simple architecture of VESPAl was close in performance to SOTA MSA-based methods (53; 34), and the embedding-based ESM-1v (44) on 39 deep mutational scanning (DMS) experiments (with 135,665 SAV) that had not been used for development. The per-residue μ-variation describes the average effect score of the 19 possible substitutions for the respective wild type.

***3D* structure prediction.** LambdaPP also includes SOTA MSA-based 3D structure predictions. If UniProt accessions are used as an input, the 3D structure is retrieved from the AlphaFold Database (AFDB) (71). If the structure is unavailable in AFDB or the input is not a UniProt accession number, ColabFold (45) is employed to predict the 3D structure, easing access to AlphaFold2 (30). Toward this end, MSAs are generated by searching with MMseqs2 (46) against UniRef 30 and ColabFoldDB (69). For single predictions, this combination is 20-30 times faster than the original AlphaFold2 at little loss of performance on the CASP14 (33) targets (46). Further parameters are an early stop criterion of a prediction certainty (pLDDT) above 85 or below 40, a default recycle count of 3 and a compilation of only the best performing out of five AlphaFold2 models. As the goal of LambdaPP is to provide a single reference for pLM-based predictions, 3D structure will soon be predicted using tools just recently presented in the literature (35; 72; 74), which will allow structure prediction to happen in seconds rather than in minutes, at accuracy comparable with MSA-based methods.

Supplementary Material (SOM)

The Supplementary Online Material (SOM) provides additional display items, SOM_figures.pdf contains the UniProtKB Annotations at submission (Figure S1), a full print-out of LambdaPP predictions for TREM2_HUMAN (Figure S2), an example for the structure exploration pane for TREM2_HUMAN (Figure S3), and aligned UniProtKB and LambdaPP annotations (Figure S5).

Additional details are provided in the main document (SOM.pdf).

Acknowledgements

Thanks primarily to Tim Karl (TUM) for invaluable help with hardware and software and to Inga Weise (TUM) for support with many other aspects of this work. Thanks to John Jumper and his team at DeepMind for the breakthrough development of AlphaFold2 and for making code and predictions freely available. Last, not least, thanks to all who deposit experimental data in public databases, to those who maintain these databases, and those who make methods available that allow enriching the experimental data.

Funding

This work was supported by Bavarian Ministry of Education through funding to the TUM and by a grant from the Alexander von Humboldt foundation through the German Ministry for Research and Education (BMBF: Bundesministerium für Bildung und Forschung); BMBF [031L0168 and program 'Software Campus 2.0 (TUM) 2.0' 01IS17049]; Deutsche Forschungsgemeinschaft [DFG-GZ: RO1320/4-1]. The authors declare no conflicts of interest.

References

1. Abriata LA, Tamò GE, Monastyrskyy B, Kryshtafovych A, Dal Peraro M (2018) Assessment of hard target modeling in CASP12 reveals an emerging role of alignment-based contact prediction methods. Proteins 86 Suppl 1:97-112.
2. Ahdritz G, Bouatta N, Kadyan S, Xia Q, Gerecke W, AlQuraishi M. OpenFold. (2021).
3. Alexander-Brett JM, Kober DL (2015) Triggering receptor expressed on myeloid cells 2.
4. Alley EC, Khimulya G, Biswas S, AlQuraishi M, Church GM (2019) Unified rational protein engineering with sequence-based deep representation learning. Nat Methods 16:1315-1322.
5. Almagro Armenteros JJ, Sønderby CK, Sønderby SK, Nielsen H, Winther O (2017) DeepLoc: prediction of protein subcellular localization using deep learning. Bioinformatics 33:3387-3395.
6. Baek M, Baker D (2022) Deep learning and protein structure modeling. Nat Methods 19:13-14.
7. Ben Chorin A, Masrati G, Kessel A, Narunsky A, Sprinzak J, Lahav S, Ashkenazy H, Ben-Tal N (2020) ConSurf-DB: An accessible repository for the evolutionary conservation patterns of the majority of PDB proteins. Protein Science 29:258-267.
8. Bengio Y, Courville A, Vincent P (2013) Representation Learning: A Review and New Perspectives. IEEE TPAMI 35:1798-1828.
9. Bepler T, Berger B. Learning protein sequence embeddings using information from structure. (2019). arXiv.
10. - (2021) Learning the protein language: Evolution, structure, and function. Cell Systems 12:654-669.e653.
11. Berezin C, Glaser F, Rosenberg J, Paz I, Pupko T, Fariselli P, Casadio R, Ben-Tal N (2004) ConSeq: the identification of functionally and structurally important residues in protein sequences. Bioinformatics 20:1322-1324.
12. Bernhofer M, Dallago C, Karl T, Satagopam V, Heinzinger M, Littmann M, Olenyi T, Qiu J, Schütze K, Yachdav G et al. (2021) PredictProtein - Predicting Protein Structure and Function for 29 Years. NAR 49:W535-W540.
13. Bernhofer M, Rost B. TMbed – Transmembrane proteins predicted through Language Model embeddings. (2022). bioRxiv.
14. Bileschi ML, Belanger D, Bryant DH, Sanderson T, Carter B, Sculley D, Bateman A, DePristo MA, Colwell LJ (2022) Using deep learning to annotate the protein universe. Nat Biotechnol 40:932-937.
15. Bordin N, Sillitoe I, Nallapareddy V, Rauer C, Lam SD, Waman VP, Sen N, Heinzinger M, Littmann M, Kim S et al. (2022) AlphaFold2 reveals commonalities and novelties in protein structure space for 21 model organisms. bioRxiv.
16. Buchfink B, Reuter K, Drost H-G (2021) Sensitive protein alignments at tree-of-life scale using DIAMOND. Nat Methods 18:366-368.
17. Cardim-Pires TR, Sant'Anna R, Foguel D (2021) Peptides derived from gp43, the most antigenic protein from Paracoccidioides brasiliensis, form amyloid fibrils in vitro: implications for vaccine development. Sci Rep 11:23440.
18. Chowdhary KR. Natural Language Processing. In: Chowdhary KR, Ed. (2020) Fundamentals of Artificial Intelligence. Springer India, New Delhi, pp. 603-649.
19. Dallago C, Goldberg T, Andrade-Navarro MA, Alanis-Lobato G, Rost B (2020) Visualizing Human Protein-Protein Interactions and Subcellular Localizations on Cell Images Through CellMap. Current Protocols Bioinf 69:e97.
20. Dallago C, Schütze K, Heinzinger M, Olenyi T, Littmann M, Lu AX, Yang KK, Min S, Yoon S, Morton JT et al. (2021) Learned Embeddings from Deep Learning to Visualize and Predict Protein Sets. Current Protocols 1:e113.
21. Dass R, Mulder FAA, Nielsen JT (2020) ODiNPred: comprehensive prediction of protein order and disorder. Sci Rep 10:14780.
22. El-Mabrouk N, Slonim DK (2020) ISMB 2020 proceedings. Bioinformatics 36:i1-i2.
23. Elnaggar A, Heinzinger M, Dallago C, Rehawi G, Wang Y, Jones L, Gibbs T, Feher T, Angerer C, Steinegger M et al. (2021) ProtTrans: Towards Cracking the Language of Lifes Code Through Self-Supervised Deep Learning and High Performance Computing. IEEE Transactions on Pattern Analysis and Machine Intelligence:1-1.
24. Heinzinger M, Elnaggar A, Wang Y, Dallago C, Nechaev D, Matthes F, Rost B (2019) Modeling aspects of the language of life through transfer-

learning protein sequences. BMC Bioinformatics 20:723.
25. Heinzinger M, Littmann M, Sillitoe I, Bordin N, Orengo C, Rost B (2022) Contrastive learning on protein embeddings enlightens midnight zone. NAR Gen Bioinf 4:lqac043.
26. Henikoff S, Henikoff JG (1992) Amino acid substitution matrices from protein blocks. PNAS 89:10915-10919.
27. Hie BL, Yang KK, Kim PS (2022) Evolutionary velocity with protein language models predicts evolutionary dynamics of diverse proteins. Cell Systems 13:274-285.e276.
28. Høie MH, Kiehl EN, Petersen B, Nielsen M, Winther O, Nielsen H, Hallgren J, Marcatili P (2022) NetSurfP-3.0: accurate and fast prediction of protein structural features by protein language models and deep learning. NAR:gkac439.
29. Ilzhoefer D, Heinzinger M, Rost B. SETH predicts nuances of residue disorder from protein embeddings. (2022). bioRxiv.
30. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Žídek A, Potapenko A et al. (2021) Highly accurate protein structure prediction with AlphaFold. Nature 596:583-589.
31. Kober DL, Alexander-Brett JM, Karch CM, Cruchaga C, Colonna M, Holtzman MJ, Brett TJ (2016) Neurodegenerative disease mutations in TREM2 reveal a functional surface and distinct loss-of-function mechanisms. eLife 5:e20391.
32. Kouba T, Vogel D, Thorkelsson SR, Quemin ERJ, Williams HM, Milewski M, Busch C, Günther S, Grünewald K, Rosenthal M et al. (2021) Conformational changes in Lassa virus L protein associated with promoter binding and RNA synthesis activity. Nat Commun 12:7018.
33. Kryshtafovych A, Schwede T, Topf M, Fidelis K, Moult J (2021) Critical assessment of methods of protein structure prediction (CASP)—Round XIV. Proteins 89:1607-1617.
34. Laine E, Karami Y, Carbone A (2019) GEMME: A Simple and Fast Global Epistatic Model Predicting Mutational Effects. Molecular Biology and Evolution 36:2604-2619.
35. Lin Z, Akin H, Rao R, Hie B, Zhu Z, Lu W, Santos Costa Ad, Fazel-Zarandi M, Sercu T, Candido S et al. (2022) Language models of protein sequences at the scale of evolution enable accurate structure prediction. bioRxiv:2022.2007.2020.500902.
36. Lin Z, Akin H, Rao R, Hie BL, Zhu Z, Lu W, dos Santos Costa A, Fazel-Zarandi M, Sercu T, Candido S et al. (2022) Language models of protein sequences at the scale of evolution enable accurate structure prediction. bioRxiv.
37. Littmann M, Bordin N, Heinzinger M, Schütze K, Dallago C, Orengo C, Rost B (2021) Clustering FunFams using sequence embeddings improves EC purity. Bioinformatics 37:3449-3455.
38. Littmann M, Heinzinger M, Dallago C, Olenyi T, Rost B (2021) Embeddings from deep learning transfer GO annotations beyond homology. Sci Rep 11:1160.
39. Littmann M, Heinzinger M, Dallago C, Weissenow K, Rost B (2021) Protein embeddings and deep learning predict binding residues for various ligand classes. Sci Rep 11:23916.
40. Madani A, McCann B, Naik N, Keskar NS, Anand N, Eguchi RR, Huang P-S, Socher R. ProGen: Language Modeling for Protein Generation. (2020). bioRxiv.
41. Marks C, Hummer AM, Chin M, Deane CM (2021) Humanization of antibodies using a machine learning approach on large-scale repertoire data. Bioinformatics. PMID: 34110413 {Medline}
42. Marquet C, Heinzinger M, Olenyi T, Dallago C, Erckert K, Bernhofer M, Nechaev D, Rost B (2021) Embeddings from protein language models predict conservation and variant effects. Hum Genet.
43. Marx V (2022) Method of the Year: protein structure prediction. Nat Methods 19:5-10. PMID: 35017741 {Medline}
44. Meier J, Rao R, Verkuil R, Liu J, Sercu T, Rives A. Language models enable zero-shot prediction of the effects of mutations on protein function. (2021). Curran Associates, Inc., pp. 29287-29303.
45. Mirdita M, Schütze K, Moriwaki Y, Heo L, Ovchinnikov S, Steinegger M (2022) ColabFold: making protein folding accessible to all. Nat Methods 19:679-682.
46. Mirdita M, Steinegger M, Söding J (2019) MMseqs2 desktop and local web server app for fast, interactive sequence searches. Bioinformatics 35:2856-2858.
47. Moore GE (1965) Cramming more components onto integrated circuits. Electronics 38:114.
48. Nielsen JT, Mulder FAA (2020) Quantitative Protein Disorder Assessment Using NMR Chemical Shifts. Methods Mol Biol 2141:303-317.
49. Ofer D, Brandes N, Linial M (2021) The language of proteins: NLP, machine learning & protein sequences. Computational and Structural Biotechnology Journal 19:1750-1758.
50. Perdigao N, Heinrich J, Stolte C, Sabir KS, Buckley MJ, Tabor B, Signal B, Gloss BS, Hammang CJ, Rost B et al. (2015) Unexpected features of the dark proteome. Proc Natl Acad Sci U S A. PMID: 26578815 {Medline}
51. Piovesan D, Monzon AM, Tosatto SCE (2022) Intrinsic Protein Disorder, Conditional Folding and AlphaFold2. bioRxiv:2022.2003.2003.482768.
52. Redl I, Fisicaro C, Dutton O, Hoffmann F, Henderson L, Owens BMJ, Heberling M, Paci E, Tamiola K (2022) ADOPT: intrinsic protein disorder prediction through deep bidirectional transformers. bioRxiv:2022.2005.2025.493416.
53. Riesselman AJ, Ingraham JB, Marks DS (2018) Deep generative models of genetic variation capture the effects of mutations. Nat Methods 15:816-822.
54. Rives A, Meier J, Sercu T, Goyal S, Lin Z, Liu J, Guo D, Ott M, Zitnick CL, Ma J et al. (2021) Biological structure and function emerge from scaling

unsupervised learning to 250 million protein sequences. PNAS 118:e2016239118.
55. Rost B, Sander C (1992) Jury returns on structure prediction. Nature 360:540.
56. - (1993) Prediction of protein secondary structure at better than 70% accuracy. J Mol Biol 232:584-599.
57. Rost B, Sander C (1996) Bridging the protein sequence-structure gap by structure predictions. Annu Rev Biophys Biomol Struct 25:113-136.
58. Rost B, Sander C, Schneider R (1994) PHD-an automatic mail server for protein secondary structure prediction. Bioinformatics 10:53-60.
59. Schaeffer M, Teixeira D, neXtProt T, Nikitin F, Amos B. calipho-sib/feature-viewer: Feature-viewer - DOI (v1.0.0). (2017). Zenodo.
60. Schelling M, Hopf TA, Rost B (2018) Evolutionary couplings and sequence variation effect predict protein binding sites. Proteins 86:1064-1074.
61. Sehnal D, Bittrich S, Deshpande M, Svobodová R, Berka K, Bazgier V, Velankar S, Burley SK, Koča J, Rose AS (2021) Mol* Viewer: modern web app for 3D visualization and analysis of large biomolecular structures. NAR 49:W431-W437.
62. Sillitoe I, Bordin N, Dawson N, Waman VP, Ashford P, Scholes HM, Pang CSM, Woodridge L, Rauer C, Sen N et al. (2021) CATH: increased structural coverage of functional space. NAR 49:D266—D273.
63. Singh J, Paliwal K, Litfin T, Singh J, Zhou Y (2022) Reaching alignment-profile-based accuracy in predicting protein secondary and tertiary structural properties without alignment. Sci Rep 12:7607.
64. Stärk H, Dallago C, Heinzinger M, Rost B (2021) Light attention predicts protein location from the language of life. Bioinformatics Advances 1:vbab035.
65. Steinegger M, Mirdita M, Soding J (2019) Protein-level assembly increases protein sequence recovery from metagenomic samples manyfold. Nat Methods 16:603-606. PMID: 31235882 {Medline}
66. Steinegger M, Söding J (2018) Clustering huge protein sequence sets in linear time. Nat Commun 9:2542.
67. Sudom A, Talreja S, Danao J, Bragg E, Kegel R, Min X, Richardson J, Zhang Z, Sharkov N, Marcora E et al. (2016) Crystal Structure of Mutant Ig-like Domain.
68. - (2018) Molecular basis for the loss-of-function effects of the Alzheimer's disease–associated R47H variant of the immune receptor TREM2. J Biol Chem 293:12634-12646.
69. The UniProt Consortium, Bateman A, Martin M-J, Orchard S, Magrane M, Agivetova R, Ahmad S, Alpi E, Bowler-Barnett EH, Britto R et al. (2021) UniProt: the universal protein knowledgebase in 2021. NAR 49:D480-D489.
70. Theis TN, Wong HSP (2017) The End of Moore's Law: A New Beginning for Information Technology. Computing in Science & Engineering 19:41-50.
71. Varadi M, Anyango S, Deshpande M, Nair S, Natassia C, Yordanova G, Yuan D, Stroe O, Wood G, Laydon A et al. (2022) AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. NAR 50:D439-D444.
72. Weissenow K, Heinzinger M, Rost B (2022) Protein language model embeddings for fast, accurate, alignment-free protein structure prediction. Structure:2021.2007.2031.454572.
73. Wilson CJ, Choy W-Y, Karttunen M (2022) AlphaFold2: A Role for Disordered Protein/Region Prediction? International Journal of Molecular Sciences 23:4591. PMID: doi:10.3390/ijms23094591 {Medline}
74. Wu R, Ding F, Wang R, Shen R, Zhang X, Luo S, Su C, Wu Z, Xie Q, Berger B et al. (2022) High-resolution de novo structure prediction from primary sequence. bioRxiv:2022.2007.2021.500999.
75. Yachdav G, Kloppmann E, Kajan L, Hecht M, Goldberg T, Hamp T, Hönigschmid P, Schafferhans A, Roos M, Bernhofer M et al. (2014) PredictProtein--an open resource for online prediction of protein structural and functional features. NAR 42:W337-343.
76. Yang J, Roy A, Zhang Y (2013) BioLiP: a semi-manually curated database for biologically relevant ligand–protein interactions. NAR 41:D1096-D1103.
77. Zhao D, Liu W, Chen K, Wu Z, Yang H, Xu Y (2021) Structure of the human RNA polymerase I elongation complex. Cell Discovery 7:97.
78. Zhou G, Chen M, Ju CJT, Wang Z, Jiang J-Y, Wang W (2020) Mutation effect estimation on protein–protein interactions using deep contextualized representation learning. NAR Gen Bioinf 2:lqaa015.
79. Zhou N, Jiang Y, Bergquist TR, Lee AJ, Kacsoh BZ, Crocker AW, Lewis KA, Georghiou G, Nguyen HN, Hamid MN et al. (2019) The CAFA challenge reports improved protein function prediction and new functional annotations for hundreds of genes through experimental screens. Genome Biol 20:244.