SaprotHub: Making Protein Modeling Accessible to All Biologists

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Abstract

Training and deploying deep learning models pose challenges for users without machine learning (ML) expertise. SaprotHub offers a user-friendly platform that democratizes the process of training, utilizing, storing, and sharing protein ML models, fostering collaboration within the biology community—all without requiring extensive machine learning expertise. At its core, Saprot is an advanced, foundational protein language model. Through its ColabSaprot framework, it supports potentially hundreds of protein training and prediction applications, enabling the co-construction and co-sharing of these trained models. This enhances user engagement and drives community-wide innovation.

Proteins, the building blocks of life, are essential for a myriad of biological processes and pivotal in medical breakthroughs, pharmaceutical innovations, and genetic discoveries [5, 22, 11]. Despite their importance, understanding protein structure and function has been a considerable challenge in the scientific community. The success of AlphaFold2 [23] in CASP14 marks a new era in structural biology by achieving accuracy of protein structure prediction at the experimental margin of error. Simultaneously, large-scale protein language models (PLMs) are driving substantial advances in protein function prediction.

In this context, several notable PLMs have emerged, including the ESM models [43, 29, 75, 80], ProtTrans [6], ProstT5 [14], UniRep [1], Tranception [36], ProGen [30, 35], and ProtGPT2 [7], each demonstrating remarkable efficacy in their respective tasks. However, training and deploying these machine learning (ML) models for proteins often pose major challenges for researchers without ML expertise. These include selecting appropriate model architectures, managing coding intricacies, preprocessing large datasets, training model parameters, evaluating and interpreting results. This complexity often deters non-ML researchers, hindering their active involvement in this domain, especially as AI models continue to grow more sophisticated.

Given these challenges, ColabFold [34] emerged as a pioneering initiative by deploying AlphaFold2 predictions on Google Colaboratory, making protein folding accessible to researchers of all backgrounds. However, ColabFold primarily emphasizes model inference, lacking support for researchers in the complex tasks such as training their own ML models.

In response to this need, we present SaprotHub, an easy-to-use platform based on Google Colaboratory tailored for protein function-related predictions. SaprotHub empowers researchers by enabling them to create and train their own task-specific models without requiring advanced ML or coding expertise. Additionally, SaprotHub distinguishes itself by supporting a broad range of protein function predictions (Fig. 1c, 2b), rather than being confined to a specific task.

Furthermore, we introduce the *Open Protein Modeling Consortium* (OPMC) (see Supplementary) with the vision to create a unified repository for decentralized protein prediction models. Within the OPMC framework, researchers can easily share their individually trained models, fostering both the direct use and collaborative construction of protein models. This approach enables continue learning and improvement of

Partial materials of Saprot was reported in [85], following Nature journal's conference proceedings policy, see here.

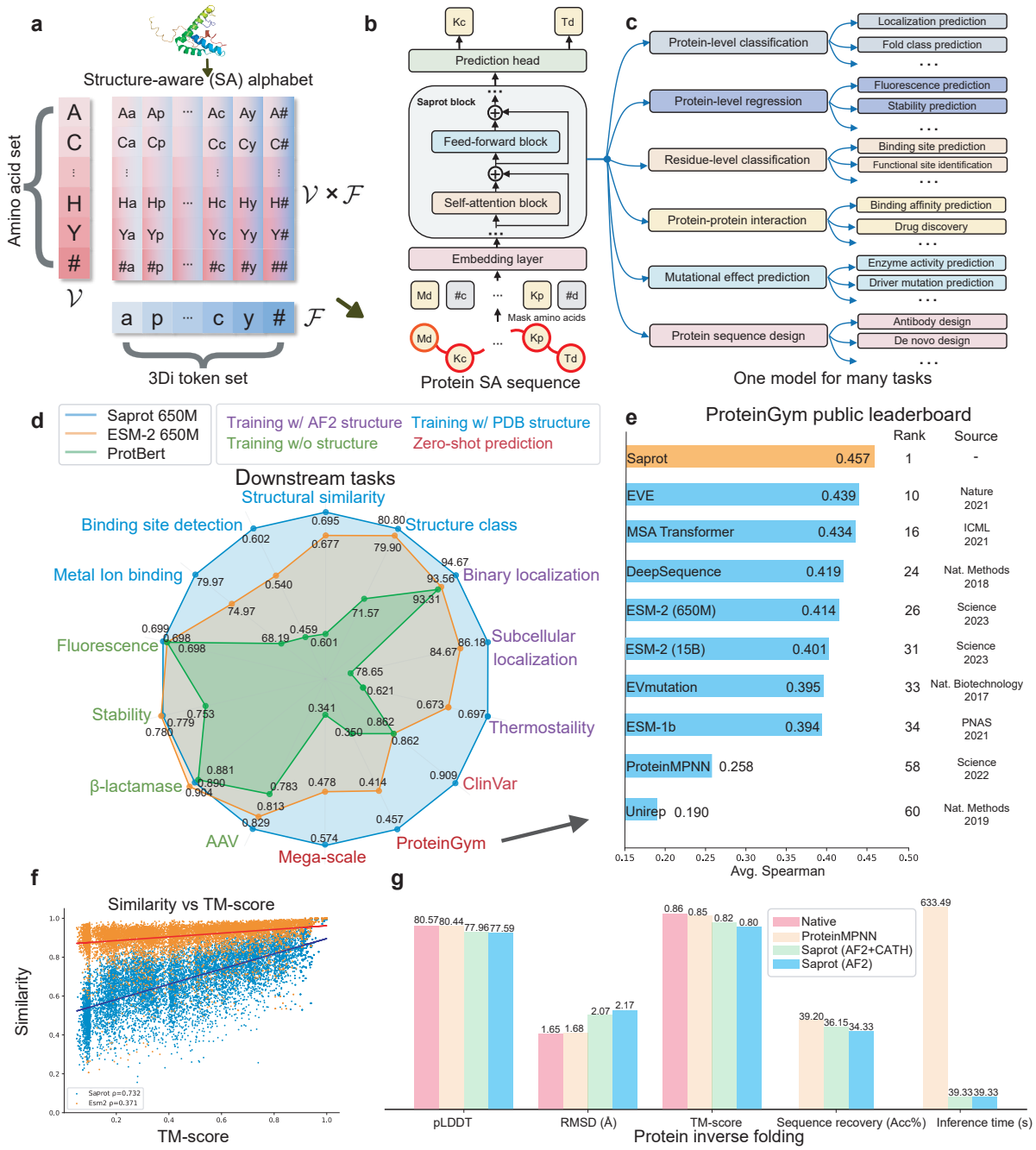

Fig. 1: **Illustration of Saprot. a,** The proposed SA alphabet. #a, #p,...,#y represent only the 3Di token being visible, while A#, C#,..., Y# represent the AA token being visible. **b,** Architecture and pre-training of Saprot. **c,** Saprot supports a wide range of protein prediction tasks, see more specific tasks in Supplementary Table 8. **d,** Performance comparison on 14 diverse prediction tasks. **e,** Saprot (650M) ranked *first* on the ProteinGym blind assessment leaderboard, outperforming 60 single and hybrid methods. Hybrid methods are not shown here (see Supplementary Fig. 1). **f,** Comparison of Saprot (650M) and ESM-2 (650M) in structural representation ability. The y-axis represents the cosine similarity score of the last layer representation for every pair of proteins, while the x-axis represents the TM-score of the protein pairs. Saprot shows higher Pearson correlation coefficient (ρ) between x and y axes, indicating more accurate protein structure encoding. **g,** Performance on protein sequence design. Although Saprot (650M) was not trained with a typical generation loss, its protein design performance rivals that of ProteinMPNN, with over 16 times faster inference speed. "AF2+CATH" indicates that Saprot was pre-trained using AlphaFold2 predicted structures and fine-tuned with experimental structures from the CATH dataset. "AF2" is a version of Saprot trained only with the AlphaFold2 predicted structure. CATH is also used for training ProteinMPNN.

models through collective efforts. SaprotHub, as the first PLM Hub to join OPMC, marks the first step to bring the OPMC vision to fruition.

SaprotHub comprises three novel contributions: a new protein language model called Saprot (Fig. 1a,b), ColabSaprot (Fig. 2a,b,c) for fine-tuning (or re-training) and inference of Saprot using adapter learning techniques [19] on the Colab platform, and a centralized community model repository (Fig. 2d) for saving,

sharing, searching, and collaborating on fine-tuned Saprot models. Through the innovative integration of advanced protein language models, Colab-based cloud computing, and adapter-based fine-tuning, SaprotHub addresses several key challenges. These challenges include the difficulty of sharing and aggregating large-scale models, the risk of catastrophic forgetting during continual learning, and the need to protect proprietary data. Below, we describe these three modules in detail.

We developed Saprot, a cutting-edge, large-scale protein language model that forms the foundation for ColabSaprot and SaprotHub. Saprot introduces a novel protein alphabet and representation method that differs from traditional amino acid (AA) sequences or explicit 3D coordinate structures. This proposed alphabet is structure-aware (SA), where each 'letter' simultaneously encodes both amino acid type and local protein geometry. Formally, the SA alphabet (SAA) is defined as $SAA = \mathcal{V} \times \mathcal{F}$, representing the Cartesian product of $\mathcal{V}$ and $\mathcal{F}$, where $\mathcal{V}$ denotes the 20 amino acids (AA) and $\mathcal{F}$ represents the 20 structural (3Di) letters or tokens (Fig. 1a). These 3Di tokens are derived from protein 3D structures through Foldseek discretization [50]. The SA alphabet encompasses all possible combinations of AA (capital letters) and 3Di tokens (lowercase letters) in SAA, such as $Aa, Ap, Ac, ..., Ya, Yp, Yy$, allowing proteins to be represented as a sequence of SA tokens that captures both primary and tertiary structures (Fig. 1b and Methods 1.1). Despite its conciseness, applying the SA alphabet successfully addresses the key challenges of scalability and overfitting in training on large-scale *AlphaFold2-generated* atomic structures (see Supplementary Note 1 and Supplementary Fig. 4). The use of "AA + discrete structural token" sequences to represent proteins has gained increasing attention in many subsequent studies [27, 48, 39, 9, 28, 67, 21] and shows promise as an emerging paradigm in protein representation.

Saprot uses a bidirectional Transformer [62] architecture (Fig.1b). It is pre-trained on SA sequences with partial token masking (Methods 1.2). The model was trained from scratch on 40 million protein SA sequences, filtered from AlphaFold's 214 million proteins [51] at 50% identity (Methods 1.3). Saprot is available in three sizes: 35M, 650M, and 1.3B parameters (Supplementary Tables 7). The 650M model, used for most evaluations unless otherwise specified (see Supplementary Tables 5,6), was trained for three months using 64 NVIDIA A100-80GB GPUs.

After pre-training, Saprot has evolved into a versatile foundational protein language model that excels across diverse protein prediction tasks, such as various supervised training tasks [20, 79, 73, 88] (both regression and classification), zero-shot mutation effect prediction [15, 8], and protein sequence design [4] (Fig.1 c,d and Supplementary Table 8). Fig. 1d shows Saprot's superior performance across 14 diverse protein prediction tasks compared to two well-known PLMs: ESM-2 [29] and ProtBert [6] (see detailed task, dataset, baseline and experimental setup descriptions in Methods 1.5, 1.8,1.9, 1.11, Supplementary Table 1, and more baseline comparison in Supplementary Table 3). For tasks where protein structural information is available (indicated by purple, blue, and red colors), Saprot consistently surpasses both benchmark models. Even in scenarios without structural data (green tasks), Saprot maintains competitive performance with ESM-2 under the fine-tuning settings. Notably, Saprot substantially outperforms ESM-2 in three zero-shot mutational effect prediction tasks (Methods 1.6, 1.7): Mega-scale [49] (0.574 vs. 0.478), ProteinGym [37] (0.457 vs. 0.414), and ClinVar [26] (0.909 vs. 0.862) (see more results in Supplementary Table 2). Furthermore, despite not being specifically designed for generation, Saprot (trained with masked language modeling) exhibits remarkable capabilities in protein sequence design [4], achieving a 16-fold acceleration in inference speed compared to ProteinMPNN [4](Fig.1g) (also see Methods 1.10 and Supplementary Fig. 2).

After its release, Saprot maintained the first position on the public blind-test ProteinGym leaderboard for over half a year (Fig. 1e, Supplementary Fig. 1). It surpasses more than 60 existing methods, demonstrating marked improvements over established models including ESM-1b [43], ESM-2 (650M, 3B, 15B), EVE [8], ESM-1v [75], MSA Transformer [80], ESM-IF [17], DeepSequence [42], EVmutation [16] and ProGen2 [35]. Impressively, the 35M parameter Saprot model is comparable to the 15B parameter ESM-2 model while being 400 times smaller. Supplementary Fig. 3 shows visualizations of the embeddings extracted from the final hidden layers of Saprot and ESM-2 for direct comparison. Recent studies also indicate Saprot's versatility and effectiveness across diverse applications, including B-cell conformational epitope prediction [21], protein and enzyme engineering [55, 45], fitness prediction [2], protein half-life prediction [44], protein-ligand interaction [52], drug-target interactions [33, 31], binding residue prediction [38], natural language based protein design [3] and molecular understanding [56], etc. These results demonstrate Saprot's 'one-model-fits-many' capability (Figs. 1c,d and Supplementary Table 8), which is critical to achieving SaprotHub's community collaboration vision.

We developed ColabSaprot by integrating Saprot into Google Colab's infrastructure to support PLM training and predicting (Methods 1.15). ColabSaprot enables seamless deployment and execution of various task-specific re-trained Saprot models, eliminating the need for environment setup and code debugging. It also allows researchers to initiate training sessions with just a few clicks (Fig. 2a). ColabSaprot is designed to accommodate all tasks within the original Saprot framework, enabling direct prediction for tasks such as zero-shot mutation effect prediction [13, 8, 42] and protein sequence design [18, 4]. For mutation effect prediction, it implements single-site, multi-site, and whole protein sequence single-site saturation mutation. For sequence design, it can design sequences based on backbone structures or redesign specific protein regions, such as antibody variable regions, while keeping the scaffold fixed.

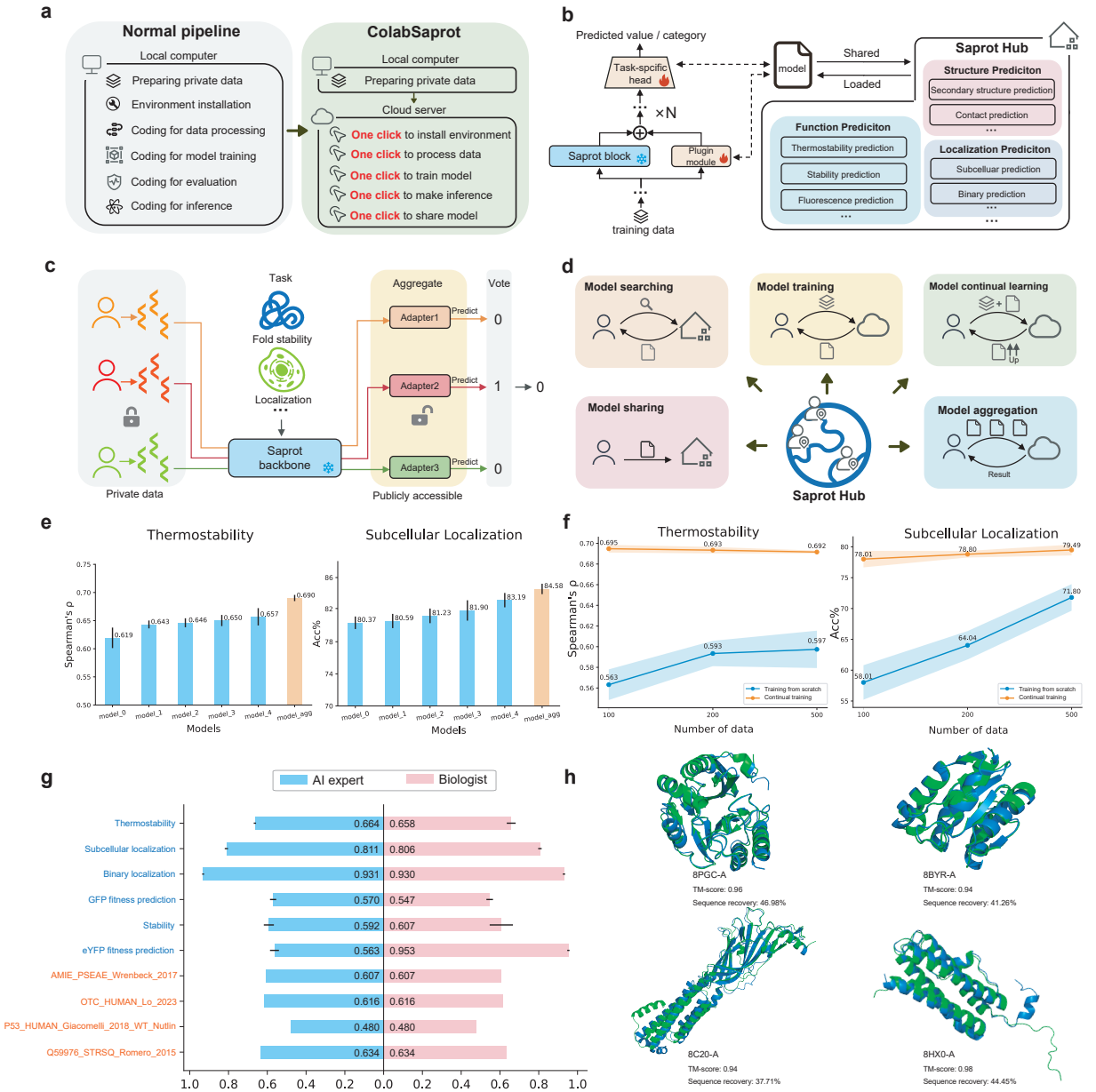

Fig. 2: **Illustration of ColabSaprot and SaprotHub. a,** Comparison of model training and inferring between normal pipeline and ColabSaprot. ColabSaprot streamlines the process, offering simplified procedures for model training and inference with just a few clicks. **b,** A lightweight plugin module (i.e., adapter) is inserted into Saprot for efficient training, sharing and co-construction. **c,** ColabSaprot predicts protein functions by aggregating existing models (or adapters) in SaprotHub without requiring private data. **d,** SaprotHub enables biologists to co-share, co-build, co-use and collaborate within the community. **e,** ColabSaprot's community-wide model collaboration mechanism (see **c**) allows it to achieve higher performance (orange bars) by aggregating individually trained models (blue bars). Each individual model is trained with its own data, which may or may not overlap. **f,** By continually learning on models trained and shared by other biologists, ColabSaprot substantially outperforms those training-from-scratch models, especially when users lack sufficient training data (x-axis represents the number of training examples). **g,** User study on supervised fine-tuning and zero-shot mutation effect prediction tasks. **h,** User study on the inverse folding task. Experimental structures are shown in green, while predicted structures are in blue.

For supervised re-training tasks, users can fine-tune ColabSaprot with their own data. Here, we introduce a parameter-efficient fine-tuning technique by integrating lightweight adapter networks into ColabSaprot (Fig. 2b and Methods 1.13). During training, only adapter parameters are updated, achieving nearly the same accuracy as fine-tuning all Saprot parameters (Supplementary Fig. 5). This design not only enhances optimization efficiency as mentioned in past literature [54, 84], but more importantly, as proposed here, it realizes collaborative and centralized mechanisms for these biologist fine-tuned, task-specific PLMs (or Saprot) within the community, especially in the cloud environment (Fig. 2c,d,e,f). With adapters and the ColabSaprot interface, researchers can also easily store and exchange their re-trained Saprot models in SaprotHub by loading or uploading adapter networks instead of the full pre-trained model. Since adapter

networks contain much fewer parameters (around 1% of the whole Saprot model), this method greatly reduces storage, communication, and management burdens on SaprotHub, making model accessibility, co-construction, co-utilization, and co-sharing possible for a broader scientific community (see Fig. 2b,c,d and Methods 1.14).

Additionally, we developed several key features for ColabSaprot. The interface supports both sequence and structure inputs, offering automated dataset handling capabilities including efficient large-file upload mechanisms, real-time training monitoring with loss visualization, automatic saving and evaluation of the best checkpoints, break-point training resumption. To enhance GPU memory efficiency, we implemented adaptive batch sizing through gradient accumulation [23]. The platform also includes customizable settings, allowing researchers to modify code and adjust training parameters for specific research needs.

Finally, we developed SaprotHub with an integrated search engine, providing a centralized platform for sharing and collaboratively developing peer-retrained Saprot models within the biology community (Fig. 2d). Through the ColabSaprot interface, we implemented features like model storage, sharing, search, continual learning, and aggregation for enhanced performance (Methods 1.14,1.15, and Supplementary Fig. 8). SaprotHub offers three key advantages: First, biologists can share their trained models while retaining their proprietary datasets, facilitating research output sharing without compromising valuable experimental data (Fig. 2c). Second, researchers can leverage shared models in the hub for continual training [24], particularly beneficial in limited-data scenarios, as fine-tuning from superior pre-trained models typically yields enhanced predictive performance (Fig. 2f). Third, as the SaprotHub community expands, it will accumulate a diverse collection of models for various protein prediction tasks, with multiple Saprot models becoming available for specific protein functions. To leverage this growing model collection, we implemented a model aggregation mechanism in ColabSaprot (see Methods 1.14,1.15), enabling users to enhance predictive performance (Fig. 2e) through the integration of multiple existing models.

Saprot and SaprotHub have attracted much attention from the community and demonstrated usefulness through multiple wet lab validations. The Zhang team at a commercial biological company employed ColabSaprot for the zero-shot single-point mutation prediction on a xylanase (NCBI ID: XP_069217686.1) from *Mycothermus thermophilus* and experimentally validated the top 20 predicted variants. Among these, 13 variants exhibited enhanced enzyme activity, with R59S showing a 2.55-fold improvement and F212N demonstrating a 1.88-fold increase in enzyme activity along with enhanced thermostability (see Methods 1.17 and Supplementary Table 10, Figs. 6, and 7). Similarly, the Chang lab employed ColabSaprot to perform zero-shot single-point mutation predictions on TDG, a uracil-N-glycosylase variant. Following experimental validation in HeLa cells, the top 20 variants were incorporated into the nCas9 protein. At the Dicer-1 target site, 17 out of the 20 predicted mutations showed enhanced editing efficiency compared to the wild type, as measured by the percentage of T-to-G substitutions at position T5 (the fifth thymine in the target sequence). Notably, three mutations - L74E, H11K, and L74Q - achieved nearly doubled editing efficiency (see Methods 1.19 and Supplementary Table 9). Another OPMC member recently fine-tuned Saprot using a dataset of approximately 140,000 GFP variants with corresponding fluorescence intensities to predict brighter avGFP variants from a pool of 500 million candidates. Experimental validation revealed that 7 out of the top 9 predicted double-site variants exhibited enhanced fluorescence compared to the wild-type, with one variant reaching more than 8-fold of the wild-type fluorescence intensity (see Methods 1.18 and Supplementary Table 11). Similarly, the Zheng lab shared an eYFP fluorescence prediction model, trained on 100,000 experimentally validated variants, achieved a Spearman correlation (ρ) of 0.94 with experimental fluorescence intensity on an independent test set of 6,000 variants, demonstrating near-experimental accuracy for double-site and triple-site mutations (see Model-EYFP_100K-650M on SaprotHub).

We also conducted a user study by recruiting 12 biology researchers (without an ML background) and compared their performance with that of an AI expert (Methods). The results demonstrated that, with ColabSaprot and SaprotHub, biology researchers can train and use state-of-the-art protein models with performance comparable to that of an AI expert (Figs. 2g,h). In some cases, such as the eYFP fitness prediction task in Fig. 2g, biologists using existing models in SaprotHub achieved higher prediction accuracy than AI experts, especially when training datasets were unavailable or limited (Methods 1.16).

SaprotHub and ColabSaprot enable biology researchers to train and share sophisticated protein ML models for diverse prediction tasks, even without extensive AI expertise. This platform empowers the broader protein research community to contribute and exchange models, facilitating collaborative research and knowledge sharing through peer-trained models. We have made both Saprot and ColabSaprot open-source (see the data and code availability sections 2,3), providing a framework for other PLMs to either develop their own hubs or integrate with SaprotHub. Our OPMC members are currently expanding the ecosystem by incorporating additional PLMs, including ProTrek [47], ProtTrans [6], and METL [65] into the OPMC framework, broadening access to diverse protein language models (see the code and service availability section 3).

This community-wide participation approach to protein modeling aligns with the OPMC vision. Our goal here is to inspire and foster the cooperative construction of open protein prediction models through SaprotHub. We envision SaprotHub as the catalyst that initializes OPMC, driving innovation and collaboration in the field.

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Senior OPMC members made significant contributions to manuscript development through rigorous proofreading, revisions, and conceptual discussions. They provided strategic guidance for SaProtHub and OPMC initiatives, while actively promoting the platform's adoption in protein research competitions and educational programs. More contributions about OPMC senior members are provided in the Supplementary Materials.

Regular OPMC members were instrumental in developing core components including ColabProTrek [47], ColabProtTrans [6], ColabMETL [65] and other PLMHub platforms. They conducted experimental validations of Saprot, performed comprehensive testing of the ColabSaprot platform, and maintained the OPMC infrastructure. These members continue to drive platform innovation and enhancement. More contributions about OPMC regular members are provided in the Supplementary Materials

1 Methods

1.1 Constructing SA protein sequence

The Structure-Aware (SA) vocabulary encompasses both residue and structure information, as illustrated in Fig. 1**a**. Given a protein P, its primary sequence can be denoted as $(s_1, s_2, ..., s_n)$, where $s_i \in \mathcal{V}$ represents the residue at the i_{th} site, and $\mathcal{V}$ represents residue alphabet. Drawing inspiration from the vector quantization learning technique [86], we encode protein 3D structures into discrete residue-like structural tokens. Here, we use Foldseek [50], a fast and accurate protein structure aligner. Through Foldseek, we have a structure alphabet $\mathcal{F}$, wherein P is expressed as the sequence $(f_1, f_2, ..., f_n)$, with $f_j \in \mathcal{F}$ representing the 3Di token for the j_{th} residue site. To maintain simplicity, we adhere to the default configuration of Foldseek, which sets the size m of $\mathcal{F}$ to 20. We then combine the residue and structure tokens per residue site, generating a new structure-aware protein sequence $P = (s_1f_1, s_2f_2, ..., s_nf_n)$, where $s_if_i \in \mathcal{V} \times \mathcal{F}$ is the so-called SA token naturally fusing both residue and geometric conformation information. The SA-token protein sequence can then be fed into a standard Transformer encoder as basic input. It's important to note that we also introduce a mask signal "#" to both residue and structure alphabet, which results in "s_i#" and "#f_i" that indicate only residue or structure information is available. The size of the SA vocabulary is $21 \times 21 = 441$.

1.2 Model architecture and pre-training of Saprot

Saprot follows the same network architecture and parameter size as ESM-2 [29], which draws inspiration from the BERT [62] model in Natural Language Processing (NLP). The primary difference lies in the embedding layer: Saprot incorporates 441 structure-aware tokens in place of the conventional 20 amino acid tokens. This nearly identical architecture enables straightforward performance comparison with the ESM model.

Saprot is pre-trained with the Masked Language Modeling (MLM) objective, like ESM-2 and BERT. Formally, for a protein sequence $P = (s_1f_1, s_2f_2, ..., s_nf_n)$, the input and output can be represented as: $input : (s_1f_1, ..., \#f_i, ..., s_nf_n) \rightarrow output : s_if_i$ (Fig. 1b). Given that the 3Di token may not always be accurate for certain regions in predicted structures by AlphaFold2, f_i in $\#f_i$ is made visible during training so as to reduce the emphasis the model places on its predictions.

AlphaFold2 (AF2) predictions include pLDDT confidence scores that indicate the precision of predicted atom coordinates. Therefore, we implement specialized handling for regions with low confidence scores. During pre-training, regions with pLDDT scores below 70 are processed distinctly. When these regions are selected for MLM prediction, we use the "s_i#" token as the prediction target, while masking the input SA sequence with the "##" token. This approach encourages the model to predict residue types based solely on contextual information. When these low-confidence regions are not selected for MLM prediction, we employ the "s_i#" token in the input, ensuring the model relies solely on residue context rather than inaccurate structural information. For downstream tasks, we maintain consistency with the pre-training protocol by applying the same handling to regions with pLDDT scores below 70. These regions are represented using "s_i#" tokens, with only residue information remaining visible.

Saprot 35M and 650M underwent typical pre-training from scratch as described above. In contrast, Saprot 1.3B employed an efficient training strategy [66] by architecturally combining two identical 33-layer Saprot 650M models. The initialization process involved duplicating the pre-trained 650M model's parameters to populate both the lower (layers 1-33) and upper (layers 34-66) sections of the 1.3B architecture. After initialization, Saprot 1.3B was trained following the same training protocol employed for the 35M and 650M models, as described above.

1.3 Processing pre-training data

We followed the procedures outlined in ESM-2 [29] to generate filtered protein data. Subsequently, we acquired all AF2 structures via the AlphaFoldDB website (https://alphafold.ebi.ac.uk/), utilizing the UniProt IDs of protein sequences. Proteins without structures in AlphaFoldDB were not removed. This process yielded a collection of approximately 40 million structures. Employing Foldseek, we encoded these structures into 3Di tokens. Subsequently, we formulated structure-aware (SA) sequences by combining residue and 3Di tokens, treating them as a single SA token at each position. These datasets were used for training all three versions of the SaProt models.

1.4 Hyper-parameters for pre-training

Following ESM-2 and BERT, during training, 15% of the SA tokens in each batch are masked. We replace the SA token s_if_i with the $\#f_i$ token 80% of the time, while 10% of the tokens are replaced with randomly selected tokens, and the other 10% tokens remain unchanged. For the optimization of Saprot, we adopt similar hyper-parameters to those employed in the ESM-2 training phase. Specifically, we employ the AdamW optimizer [74], setting $\beta_1 = 0.9, \beta_2 = 0.98$ and we utilize L_2 weight decay of 0.01. We gradually increase the learning rate from 0 to 4e-4 over the first 2000 steps and linearly lower it to 5e-4 from 150K steps to 1.5M steps. The overall training phase lasts approximately 3M steps. Like the ESM model, we also truncate them

to a maximum of 1024 tokens, and our batch size consists of 512 sequences. Additionally, we also employ mixed precision training to train Saprot.

1.5 Descriptions of baseline models

We compared Saprot to several prominent protein language models (see Supplementary Table 2,3). For supervised learning tasks, we compared Saprot with ESM-2 (the 650M version) [29], ProtBert (the BFD version) [6], MIF-ST [90], and GearNet [92]. The first two models utilize residue sequences as input, while the latter two models incorporate both residue and 3D structures as input. ESM-2 (650M) stands out as the primary baseline for comparison, given its similar model architecture, size, and training approach when compared to Saprot. ESM-2 also offers a 15 billion (B) parameter version, which can be challenging to fine-tune even on GPUs with 80G memory. Therefore, we only conducted comparisons with ESM-2 (15B) for zero-shot mutational effect prediction tasks, which can be achieved without the need for fine-tuning.

For the zero-shot mutational effect prediction task, we compare with the state-of-the-art ESM-2, ProtBert, ESM-1v [75], Tranception L (without MSA retrieval) [76], MSA Transformer [80], and EVE [8] models. For the protein inverse folding task, we compare with ProteinMPNN [61] as basline (see Fig. 1).

1.6 Zero-shot formula

Previous sequence-based protein language models like the ESM models predict mutational effects using the log odds ratio at the mutated position. The calculation can be formalized as follows:

$$\sum_{t \in T} [logP(x_t = s_t^{mt} | x_{\setminus T}) - logP(x_t = s_t^{wt} | x_{\setminus T})] \tag{1}$$

Here T represents all mutations and $s_t \in \mathcal{V}$ is the residue type for mutant and wild-type sequence. We slightly modify the formula above to adapt to the structure-aware vocabulary, where the probability assigned to each residue corresponds to the summation of tokens encompassing that specific residue type, as shown below:

$$\sum_{t \in T} [log \sum_{f \in \mathcal{F}} P(x_t = s_t^{mt} f | x_{\setminus T}) - log \sum_{f \in \mathcal{F}} P(x_t = s_t^{wt} f | x_{\setminus T})] \tag{2}$$

Here $f \in \mathcal{F}$ is the 3Di token generated by Foldseek and $s_t f \in \mathcal{V} \times \mathcal{F}$ is the SA token in our new vocabulary.

1.7 Zero-shot mutational effect prediction tasks and datasets

ProteinGym [37] comprises an extensive collection of Deep Mutational Scanning (DMS) assays, enabling comprehensive comparison among zero-shot predictors. We evaluate all baseline models (see Supplementary Table 2) on ProteinGym's substitution branch, utilizing its provided protein structures and adhering to the standard evaluation protocol outlined in the original paper [37]. We employ Spearman's rank correlation as our evaluation metric.

ClinVar [26] is a publicly accessible repository housing information about human genetic variants and their clinical significance in disease. For our analysis, we utilize data curated from EVE [8], excluding proteins exceeding 1024 residues in length. To ensure data quality, we restrict our analysis to proteins with reliability ratings of 1 "Gold Star" or higher. Following EVE's methodology, we assess model performance using the AUC metric.

Mega-scale [49] employs cDNA display proteolysis to measure thermodynamic folding stability across protein domains. The dataset encompasses all single mutations and selected double mutants in both natural and *de novo* designed proteins. For this dataset, we also utilize Spearman's rank correlation as our evaluation metric.

For each mutation dataset, we provide all variants with the wild-type structure, as AF2 does not reliably distinguish the structural changes induced by single mutations. Additionally, the ClinVar dataset only provides UniProt IDs, so we manually downloaded all AF2 structures and eliminated proteins without structures in AlphaFold DB. Both ProteinGym and Mega-scale datasets provide protein structures, either predicted from AF2 or derived from *de novo* design.

1.8 Supervised fine-tuning tasks and datasets

Fine-tuning Saprot with AF2 structure. These benchmarks include the Thermostability task from FLIP [60] and the Localization Prediction task from DeepLoc [57]. The DeepLoc benchmark has two prediction branches: a multi-class classification with 10 subcellular locations and a binary classification with 2 location categories. We use the datasets provided by these original literature. For structural information, we obtain AlphaFold2-predicted structures for all proteins using their corresponding UniProt IDs.

Fine-tuning Saprot with PDB structure. There are a few tasks providing experimentally determined structures as training data. We evaluate Metal Ion Binding task [70] and a series of tasks from

ProteinShake [73], including Structure Class Prediction, Structural Similarity Prediction and Binding Site Detection. The corresponding datasets are provided in the respective literature.

Fine-tuning Saprot without structure. While Saprot is designed to leverage protein structural information, it can still work in scenarios where structural data is not provided during supervised fine-tuning (also see Methods 1.2). In these cases, we mask the 3Di token in the SA sequence. We evaluate performance on the Fluorescence and Stability prediction datasets from TAPE [79], the AAV dataset from FLIP [60], and the β-lactamase landscape prediction dataset from PEER [88].

Regarding the evaluation metrics, we adopt those established in the original literature. For details on dataset splits, please refer to the next subsection.

1.9 Data split

In prior literature, datasets were typically partitioned based on sequence identity. However, a recent benchmark study, ProteinShake [73], highlighted that protein structures show higher conservation than sequences. They demonstrated that sequences appearing dissimilar in training and test sets might share similar structures, potentially causing data leakage. Consequently, we implement a more rigorous data splitting strategy. As our evaluation primarily focuses on Saprot 650M, which balances accuracy and computational efficiency, we adopt the ProteinShake approach of splitting data based on structural similarity, quantified using the Local Distance Difference Test (LDDT) metric. For datasets with structural information (Methods 1.8), we employ the suggested (by Proteinshake) 70% structural similarity threshold for dataset partitioning. For tasks without protein structures, we maintain the commonly adopted 50% sequence identity based splits, as all models, including Saprot, utilize only sequence data as input. The data is consistently divided into training, validation, and testing sets with a ratio of 80:10:10, regardless of whether the splitting criterion is structural or sequence similarity.

The structure-similarity based data split strategy in ProteinShake [73] is described as below: "*We create a structure-similarity split by utilizing the fast structure alignment software Foldseek. A complete clustering is computationally prohibitive so we resort to an efficient heuristic: test and validation sets are built iteratively by randomly sampling an initial protein and then retrieving all similar instances with Foldseek, constructing structure-based clusters analogously to the sequence-based split. Structural similarity is defined by thresholds on the Local Distance Difference Test (LDDT) metric. Clusters are then sampled until the desired set size is reached. This split procedure serves as a novel and challenging setting that tests the ability of models to generalize in terms of structure and not just sequence homology. All tasks are available with random, sequence, and structure splits, providing different levels of difficulty for generalization. The splits are pre-computed with similarity thresholds between 30%-90% for the sequence split and 50%-90% for the structure split, with a default of 70%.*"

Additionally, to systematically examine performance sensitivity to different splitting criteria, we evaluate Saprot (35M) and ESM (35M) using both highly stringent (30% structural similarity) and relaxed (50% sequence identity) thresholds, as shown in Supplementary Table 5.

1.10 Protein inverse folding

Saprot for protein inverse folding

To use Saprot for inverse folding, we first encode the protein backbone into 3Di tokens $(f_1, f_2, ..., f_n)$. We then mask all residue parts of the SA tokens, forming an SA sequence $(\#f_1, \#f_2, ..., \#f_n)$. This sequence is input into Saprot to predict residue distributions at all positions. In contrast to ProteinMPNN [61] that generates residues in the auto-regressive manner, i.e. generating next token conditioned on all previous outputs, Saprot is able to simultaneously predict all residues with only one forward propagation.

Fine-tuning Saprot on the CATH Dataset We evaluate two variants of Saprot for protein design: one pre-trained using AF2-predicted structures and another further fine-tuned on the CATH dataset [77]. This CATH database, which was also used to train ProteinMPNN [4], is partitioned into training, validation, and test sets using an 80/10/10 split, following Ingraham et al. [71]. For detailed dataset construction procedures, readers are referred to [71].

1.11 Hyper-parameters for supervised fine-tuning tasks

We employ the AdamW [74] optimizer during fine-tuning, setting $\beta_1 = 0.9, \beta_2 = 0.98$, along with L_2 weight decay of 0.01. We use a batch size of 64 for all datasets. We empirically found that the optimal learning rate for most baselines are in the range of 1e-5 to 5e-5. For **Training with AF2 structure** and **Training with PDB structure**, the optimal learning rate was set to 5e-5, whereas for **Training without structure**, it was set to 1e-5. We fine-tuned all model parameters until convergence and selected the best checkpoints based on their performance on the validation set.

1.12 Embedding visualization

We employ t-SNE [87] to visualize the protein representations generated by Saprot and ESM-2 across three datasets (Supplementary Fig. 3). We use the non-redundant version ($PIDE < 40\%$) of the SCOPe(see [58]) database, visualizing all alpha and beta proteins. We additionally visualize proteins in Subcellular Localization and Binary Localization datasets from DeepLoc [57]. To generate protein representations, we adopt the average pooling on the embeddings of the last layer for both Saprot and ESM-2.

1.13 Adapter learning

The adapter learning technique, originated in natural language processing [69, 63, 91], has recently gained traction in protein research. While previous studies have primarily focused on its benefits for model accuracy and GPU memory efficiency [84, 83, 82, 81], our work explores a novel direction. We integrate adapter learning with Google Colab to create a platform that enables model sharing and collaborative development within the biological research community through our online SaprotHub.

In the broader AI community, various types of adapters exist, including Houlsby [69], Pfeiffer [78], Compacter [72], and LoRA [19]. We choose LoRA for its capacity to deliver comparable results with fewer parameters. By integrating learnable low-rank matrices into each Saprot block while freezing the backbone, LoRA enables parameter-efficient fine-tuning and model sharing for these downstream tasks.

1.14 Community-wide collaboration

As the SaprotHub community grows, more Saprot models become available for each protein function, facilitating collaborative development among biologists. To demonstrate this advantage (Fig. 2e), we adopt the following approach: For each task, we randomly partition the training data into five subsets and train one model on each subset, yielding models model_0 through model_4, simulating models shared by different researchers. For regression tasks (e.g., Thermostability), the final prediction (model_agg) is computed as the mean of all model outputs. For classification tasks (e.g., Subcellular Localization), we implement majority voting, where the final prediction (model_agg) is determined by the most frequent class prediction across the ensemble. The lightweight adapter technique is crucial for model aggregation, addressing the challenges of loading and sharing multiple large pre-trained models.

To evaluate the effectiveness of incremental learning (Fig. 2f), we randomly sampled 100, 200, and 500 training instances per task to simulate researchers' private data. We then performed fine-tuning using two base models, updating only their adapter parameters: the official Saprot model (blue) and a shared model from SaprotHub (orange). The latter represents models previously trained by researchers with larger or higher-quality datasets. Results demonstrate that continuing training from existing well-trained models substantially outperforms training from scratch with limited data. Note that the official Saprot model (blue) is pretrained on large-scale protein sequence and structure data but has not undergone supervised fine-tuning for specific tasks. This highlights a key advantage of SaprotHub: researchers can build upon others' achievements.

1.15 ColabSaprot notebooks

ColabSaprot consists of three key components, with the first focusing on model training. This component enables researchers to rapidly configure the runtime environment, process training data, and fine-tune Saprot efficiently. We provide access to four base models: Saprot-35M, Saprot-650M, custom fine-tuned Saprot models, community-shared fine-tuned models from SaprotHub. Additionally, we offer advanced hyper-parameter customization options, allowing researchers to tailor their training strategies to specific requirements. These customizable parameters include batch size, learning rate, and training steps etc.

The second component focuses on prediction capabilities. ColabSaprot supports a diverse range of prediction tasks (see Supplementary Table 8) using both community-shared models from SaprotHub and locally fine-tuned models. This includes protein-level, residue-level, and protein-protein interaction predictions, as well as zero-shot mutational effect predictions and protein design. Each task category offers multiple configuration options to accommodate different research requirements. Moreover, ColabSaprot enhances prediction accuracy by implementing ensemble methods that aggregate multiple models from SaprotHub, delivering more robust and reliable results. This collaborative approach facilitates a community-driven model ecosystem where researchers can leverage and combine multiple models to achieve superior performance.

The third component enables model sharing, searching (implemented in SaprotHub, see Supplementary Fig 8) and community collaboration. Upon completion of training, researchers can contribute their model weights (specifically the adapter weights) to the SaprotHub community repository, making them accessible to the broader biological research community. Through SaprotHub's specialized search engine, researchers can efficiently locate and utilize relevant models. These shared models can be leveraged for continual learning, direct application, or model aggregation to achieve enhanced performance.

1.16 User study

We evaluated ColabSaprot through eight protein prediction tasks with twelve participants who, based on brief conversations and background checks (see details through the provided link in Data Availability section 2), had biological backgrounds but no prior involvement in coding or machine learning projects. The evaluation encompassed supervised fine-tuning, zero-shot mutation effect prediction, and protein inverse folding tasks. Each participant had three days to complete their assignments (task descriptions are attached through the provided link in Data Availability section 2) and received compensation upon submitting either their results or documentation of encountered obstacles via screenshots or recordings.

For comparison, we engaged an AI expert - a third-year PhD student specializing in machine learning with over two years of research experience in protein-focused AI applications. The expert executed these tasks using the Saprot codebase from our GitHub repository, conducting five independent runs with optimized hyperparameters using different random seeds.

The twelve participants were organized as follows: one was assigned to zero-shot prediction, another to protein design, and the remaining ten were randomly divided into two groups of five. Each group handled three supervised fine-tuning tasks, covering six tasks in total. In other words, each supervised fine-tuning task was performed by 5 participants. We evaluate the average accuracy of them including even those who cannot finish the task.

For the supervised fine-tuning tasks, we used five public datasets for predicting: thermostability, subcellular and binary localization, GFP fluorescence, and stability (see Supplementary Table 1). We also included a proprietary eYFP fitness prediction dataset from Zheng's lab, containing 3,087 validation and 3,088 test samples, bringing the total to six supervised fine-tuning tasks. To reduce training time and computing power consumption, we randomly selected 1,000 samples from the training set of each dataset while keeping the validation and test sets unchanged.

For model evaluation, all participants generated predictions on designated test sets. In the eYFP task, biology participants leveraged continual learning on a pre-trained model from SaprotHub (Model-EYFP-650M (search model name via https://huggingface.co/spaces/SaProtHub/SaprotHub-search), achieving 0.95 Spearman's ρ on test data), while the AI expert used the base SaProt 650M model. This setup is used to show how SaprotHub enables researchers with limited data to build upon existing fine-tuned models. For other tasks, both biology participants and the AI expert trained their models using the same SaProt_650M_AF2 base model with identical training and test sets, ensuring fair comparison.

For the zero-shot mutational effect prediction, we randomly selected four mutation datasets from ProteinGym benchmark [37] for evaluation. Three of these datasets focus on the impact of mutations on enzyme activity, while the fourth addresses drug resistance. We assigned one participant to conduct predictions on these datasets using ColabSaprot in a zero-shot manner.

For the protein inverse folding task, we assigned one participant to use ColabSaprot to generate protein sequences based on given structures. Subsequently, the participant employed ESMFold (an interface provided by ColabSaprot) to predict the structures of the generated sequences. To assess Saprot's ability on new proteins, we selected these recently released structures (see Fig. 2h).

More materials about participants and task descriptions are available via link in the Data Availability section (section 2). Participants were provided with related protein datasets (including training and test sets), GPU-enabled Google Colab accounts, and detailed task instructions. Participants had three days to complete the assignments on their own using ColabSaprot. To encourage honest feedback and thorough documentation, compensation was guaranteed to all participants who documented their challenges through screenshots or recordings, regardless of whether they completed the assigned tasks.

1.17 Experimental validation on the xylanase

There are several steps. Let's name the protein (NCBI ID: XP_069217686.1) Mth first:

(1) Use AlphaFold3 (https://golgi.sandbox.google.com/) to get the protein structure of Mth;

(2) Use Foldseek or ColabSaprot to get the SA sequence of Mth;

(3) Use ColabSaprot (650M) to perform zero-shot (single-point) mutation effect prediction for the entire SA sequence;

(4) Choose the 20 highest-ranked variants (see Supplementary Table 10) excluding A15G and I13P; A15G and I13P were not selected because they are located in the signal peptide region of the protein, which would be removed during the process of protein secretion in *Pichia pastoris*.

(5) Construct mutants. See below for details:

- The gene sequence of Mth was optimized according to the codon preference of *P. pastoris*, and the plasmid pPIC9K-Mth was synthesised by GenScript (Nanjing, China).
- Mutations of Mth were generated by site-directed mutagenesis via PCR, using the plasmid pPIC9K-Mth as a template. The primers are listed in Supplementary Table 12.
- After confirmation by DNA sequencing, the wild-type and mutated plasmids were linearized with *Sal*I and used to transform *P. pastoris* strain GS115. The recombinant strains were selected on MD plates

(1.34% YNB (yeast nitrogen base without amino acids), 2% glucose, 2% agar) and verified by PCR and sequencing.

(6) Enzyme activity assay of wild-type Mth and mutants. See below for details:

- The positive transformants were cultivated in liquid medium BMGY [1% yeast extract, 2% peptone, 1.34% YNB, 1% glycerol, 100 mM potassium phosphate (pH 6.0)] for 20-24 h. The cells were collected by centrifugation at 6000 rpm and 4°C for 5 min, then transferred to 250 mL shake flasks containing 25 mL BMMY [1% yeast extract, 2% peptone, 1.34% YNB, 1% methanol, 100 mM potassium phosphate (pH 6.0)] with initial OD_{600} =0.5. Fed-batch fermentation was proceeded to express xylanase by feeding 1% methanol per 24 h. All liquid cultures were performed at 30°C and 250 rpm. After 120 h of cultivation, the supernatants were harvested by centrifugation at 6000 rpm and 4°C for 5 min and tested for xylanase activity.
- Xylanase activity assay: The reaction mixture contained 0.1 mL of 1% (w/v) beechwood xylan and 0.1 mL of a suitably diluted enzyme solution (100 mM acetate buffer, pH 5.0) incubated at 60°C for 30 min. The amount of reducing sugar released was determined using the 3,5-dinitrosalicylic acid (DNS) method, with xylose as the standard. One unit (U) of xylanase activity was defined as the amount of enzyme that catalyzes the release of 1 µmol of xylose equivalent per minute under the assay conditions. The enzyme activity was measured at 40–70°C to determine the optimal temperature of the enzymes, with the pH of the reaction maintained at 5.0. After incubation at 60°C for various time periods, the residual enzyme activity was measured to assess the thermostability of the enzymes.

1.18 Experimental validation on GFP variants

The objective of this task was to engineer brighter avGFP variants as part of the 2024 Critical Assessment of Protein Engineering (CAPE) competition [64]. CAPE, a student-focused challenge modeled after the Critical Assessment of Structure Prediction (CASP) competition, emphasizes protein function design and variant effect prediction. The parent GFP sequence is based on avGFP (see the amino acid sequence information in Supplementary Table 13) derived from Aequorea victoria (UniProtKB: P42212). These top-ranked predicted variants by Saprot were experimentally validated by the CAPE organizers.

The process of fine-tuning Saprot (35M) and subsequent prediction consisted of the following steps:
Step 1: Data Preparation: The CAPE organizers provided a dataset of 140,000 GFP variants (including avGFP, amacGFP, cgreGFP, and ppluGFP2) (see Supplementary Table 13) along with their corresponding fitness scores and PDB structures. Our OPMC member Xuting Zhang utilized Foldseek to generate 3Di tokens for these four GFP variants, which were then converted to SA tokens.
Step 2: Model Fine-tuning: The Saprot model underwent full parameter fine-tuning using the SA token sequences of these variants.
Step 3: Variant Prediction and Validation: A pool of 500 million avGFP double-site mutants was generated through random mutagenesis. The fine-tuned Saprot model from Step 2 was used to predict their fitness scores. The top-9 ranked variants were selected for experimental validation.

Experimental validation was conducted using the robotic biofoundries at the Shenzhen Infrastructure for Synthetic Biology, following the below procedure:
Step 1: Expression plasmid pET28a vectors containing designed mutant GFP sequences were ordered from Genescript Biotech (Nanjing, China) and used to transform chemically competent E. coli BL21(DE3) cells through heat shock at 42°C. Two independent clones for each mutant were randomly selected to inoculate 1 mL of non-inducing LB+Kan medium (supplemented with 50 µg mL-1 kanamycin for plasmid maintenance by antibiotic selection) in 96-well microtiter plates to prepare seed cultures, which were grown at 37°C for 16-20 h.
Step 2: For inducible protein expression, 40 µL of stationary-phase seed cultures were used to re-inoculate 4 mL of fresh LB+Kan medium in 24-well microtiter plates, followed by approximately 4-h incubation at 37°C to reach the exponential growth phase (OD_{600}=0.6-0.8). Then, IPTG was added to achieve a final concentration of 1 mM for inducible expression at 18°C for 20 h.
Step 3: Biomass growth was monitored by using OD_{600} measurement and GFP fluorescence was assessed with excitation at 488 nm and emission at 520 nm in 96-well flat-bottomed plates. For each strain, GFP signal intensities was divided by OD_{600} values to calculate biomass-normalized fluorescence.

1.19 Experimental validation of the TDG variants

The Chang lab utilized ColabSaprot (650M) for zero-shot prediction of single-site mutation effects of a uracil-N-glycosylase variant (TDG) (see Supplementary Table 13 for its amino acid sequence). They input only the amino acid sequence with all structural tokens masked into ColabSaprot to identify the top 20 highest-ranked variants. Note that while these wet experiments yielded good results (Supplementary Table 9), we generally recommend incorporating structures when available. For predicted structures generated by AF2/AF3, regions with lower PLDDT scores should employ the mask design outlined in Methods 1.2, which

is automatically implemented in our current ColabSaprot (version: v2) version. Overall, Saprot and ColabSaprot perform better for zero-shot mutation effect prediction when a reliable wild-type protein structure is provided.

2 Data availability

The pre-training dataset for training Saprot is available at https://huggingface.co/datasets/westlake-repl/AF2_UniRef50. Downstream task datasets are all stored at https://huggingface.co/SaProtHub. Data for user study analysis is available at https://drive.google.com/file/d/1LdGRnwt2lttnszNBAJ0F967A8rguPq8b/view?usp=sharing.

3 Code and service availability

Saprot is an open-sourced model with MIT license. The code is available at https://github.com/westlake-repl/Saprot. The code implementation of ColabSaprot notebook is available at https://github.com/westlake-repl/SaProtHub. ColabSaprot service (latest version: v2) is available at https://colab.research.google.com/github/westlake-repl/SaprotHub/blob/main/colab/SaprotHub_v2.ipynb?hl=en (the previous v1 version is still maintained in the official SaprotHub github webpape). All fine-tuned Saprot models can be obtained through SaprotHub https://huggingface.co/SaProtHub by the dedicated search engine https://huggingface.co/spaces/SaProtHub/SaprotHub-search.

Our OPMC members have also implemented ColabProTrek (https://colab.research.google.com/github/westlake-repl/SaprotHub/blob/main/colab/ColabProTrek.ipynb), ColabProtT5 (https://colab.research.google.com/github/westlake-repl/SaprotHub/blob/main/colab/ColabProtT5.ipynb) and ColabMETL (https://github.com/gitter-lab/metl?tab=readme-ov-file#mutational-effect-transfer-learning). Given the substantial development efforts involved, these ColabPLMs will be continuously updated and maintained.

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Author contributions

F.Y. conceived and led this research. J.S. performed the main research and managed technical implementation. J.S., F.Y., and J.S. (Junjie) designed the SA token and Saprot. Z.L. and J.S. developed the ColabSaprot and SaprotHub. F.Y., J.S., and S.O. designed ColabSaprot and SaprotHub. S.O. and M.S. engaged in discussions of ColabSaprot and SaprotHub, provided many constructive suggestions, and revised the manuscript. C.H. conducted partial zero-shot mutation experiments. Y.Z. collected the AFDB dataset. Y.H., X.C. and X.Z. conducted experiments on eTDG. S.J. conducted some experimental result analysis. D.M. participated in early wet lab experiments. J.S. and F.Y. wrote the manuscript. OPMC authors contributed as described in the paper, including manuscript proofreading, idea discussions, expert advice, promoting, testing and development of ColabSaprot.

Supplementary information

OPMC

The Open Protein Modeling Consortium (OPMC) is a collaborative initiative designed to unify the efforts of the protein research community. Its mission is to facilitate the sharing and co-construction of resources, with a particular focus on individually trained decentralized models, thereby advancing protein modeling through collective contributions. OPMC offers a platform that supports a wide range of protein function predictions, aiming to make advanced protein modeling accessible to researchers irrespective of their machine learning expertise.

Here are some Q&A related to OPMC that we have gathered from our steering committee members and other researchers: https://theopmc.github.io/

Be an OPMC member

The complete list of OPMC authors will be finalized in the last version of the paper. OPMC encompasses two membership categories: the steering committee and regular members.

Steering Committee: The steering committee comprises esteemed researchers with doctoral degrees who assume advisory roles within OPMC. Their primary responsibilities include:

- Providing constructive suggestions for OPMC and SaprotHub.
- Supporting academic activities such as organizing workshops, tutorials, and participating in peer review processes.
- Encouraging direct involvement of team members in the development of SaprotHub or OPMC.
- Participating in the review process for new members joining OPMC.
- Contributing high-quality open-source datasets and models to enhance OPMC's resources.
- Making direct contributions to this paper.
- Making other contributions or providing academic services that drive the progress of OPMC.

Regular Members: Regular members actively contribute to OPMC through practical means. This category includes independent researchers and professionals who:

- Participate in research, development, and collaborative efforts to advance OPMC's goals and objectives.
- Create innovative adapters for SaprotHub, trained on either private or publicly available data.
- Contributing more high-quality datasets to enrich the available resources.
- Aid in the development and administration of SaprotHub or ColabSaprot.
- Wet lab experiments on ColabSaprot.

Membership Eligibility: We enthusiastically welcome individuals who make substantial contributions to OPMC and SaprotHub. The eligibility of regular members will be determined by the steering committee. If you are interested in becoming a member of OPMC, please reach out to us. Note that all OPMC members will be included as authors in the final version of this paper. The final list of members is expected to be finalized within the next 4 to 12 months.

Supplementary Note 1

To realize the vision of community-wide collaboration at SaprotHub, the protein language model (PLM) Saprot should have both advanced performance and support for a diverse range of tasks. This necessitates that its pre-trained representations achieve a high degree of generalizability. Contrastive learning (CL) and BERT-style masked language modeling (MLM) are currently the two predominant pre-training approaches for generalized representation learning, however, CL primarily focuses on protein-level representation learning with limited effectiveness in residue-level tasks. For instance, GearNet [93] and 3D-PLM [68], both trained using CL, cannot be directly applied to predict amino acid mutation effects. Therefore, we decided to adopt the BERT-style MLM loss for training Saprot. However, a problem arises when directly training the AF2-predicted structure using the MLM loss. Here, we demonstrate this issue by applying two common methods (see Supplementary Fig. 4) to train PLMs on AF2-predicted structures with the MLM loss function.

Method 1: following MIF [89], we represented AF2-predicted structures as graphs and utilized Graph Neural Networks (GNNs) for modeling. Note that the original MIF model was pre-trained using only experimental structures, so the effectiveness of incorporating a large number of AF2 predicted structures remains uncertain.

Method 2: we extracted pairwise residue distance and angle information from structures to incorporate as structural bias in the Transformer attention maps, an approach previously implemented in Uni-Mol [94] and Evoformer. Also note that the two models were originally designed for different tasks with different loss functions, and here we would like to evaluate their performance using the AF2-predicted structures and the MLM loss.

The training loss curves are shown in Supplementary Fig. 4. Our experimental results demonstrate that PLMs directly trained on AF2-predicted structures by using the MLM loss (i.e., the two models on the left side in Supplementary Fig. 4) yields poor performance on validation sets containing experimental structures. Notably, while the loss decreases on predicted structures during training, we observe no corresponding improvement in performance on experimental structures. This discrepancy suggests that the PLM may have detected some potential traces or bias from AF2 predictions rather than genuine structural features. Consequently, despite the apparent decrease in training loss, the model fails to acquire meaningful representations of real protein structures. By contrast, the Saprot model show consistent loss decrease in both AF2-predicted structures and experimental structures. More details were reported in the ICLR version of our Saprot paper, see [85] Supplementary Table 8 and section 3.1.

These results explain why we considered training Saprot with discrete structural token rather than explicit 3D structures. A similar idea was recently adopted by ESM-3 [67], further demonstrating that the use of discrete structural token is an effective way to scale PLMs with a large number of structures. By contrast, exiting PLMs [59, 59, 68] that incorporate explicit 3D structural information often utilize only a very limited number of protein structures during pre-training, and have not demonstrated that scaling with more AF-predicted structures improves performance, in particular with the MLM loss function.

Dataset	Evaluation Metric	Train	Valid	Test
Training with AF2 structure				
Thermostability [60]	Spearman's ρ	5310	706	706
Subcellular localization [57]	Acc	10414	1368	1368
Binary localization [57]	Acc	6707	698	807
Training with PDB structure				
Structural similarity [73]	Spearman's ρ	300699	4559	4850
Structure class [73]	Acc	7990	955	1005
Metal ion binding [70]	Acc	5797	719	719
Binding site detection [73]	Mcc	2368	442	464
Training without structure				
Fluorescence [79]	Spearman's ρ	20963	5235	25517
Stability [79]	Spearman's ρ	53614	2512	12851
AAV [60]	Spearman's ρ	22246	2462	50432
β-lactamase [88]	Spearman's ρ	4158	520	520

Supplementary Table 1: Supervised downstream dataset descriptions after data preprocessing. For **Training with AF2 structure** and **Training with PDB structure**, the train/validation/test splits are based on structural similarity, using a default threshold of 70% suggested by the ProteinShake paper. For **Training without structure**, train/validation/test splits are based sequence identity split with 50% as commonly adopted in literature. For **Training with AF2 structure**, proteins lacking AlphaFoldDB structural information had been excluded from both training and testing evaluations. Acc represents classification accuracy, while Mcc denotes the Matthews Correlation Coefficient.

Model	ProteinGym	Mega-scale	ClinVar
	Spearman's ρ	Spearman's ρ	AUC
ESM-2 (650M) [29] (Science, 2023)	0.414	0.478	0.862
ESM-2 (15B) [29] (Science, 2023)	0.401	0.467	0.843
ProtBert [6] (TPAMI, 2021)	0.350	0.341	0.862
ESM-1v [75] (NeurIPS, 2021)	0.374	0.374	0.891
Tranception L [76] (ICML, 2022)	0.374	0.307	0.845
MSA Transformer [80] (ICML, 2021)	0.421	0.423	0.854
EVE [8] (Nature, 2021)	0.433	0.260	0.878
Saprot (650M)	**0.457**	**0.574**	**0.909**

Supplementary Table 2: More baseline results on zero-shot mutational effect prediction. Note that several ensemble models are reported in ProteinGym; here we only show single model results for MSA Transformer and EVE. Note that Saprot ranks first even when compared to these ensemble models. Saprot can also achieve better results by combining MSA (see Table 1 for the ICLR version of Saprot) data and ensemble methods (see Figure 2e). For additional details, please also refer to: https://proteingym.org/benchmarks.

Task	Category	Metric	ESM-2	ProtBert	MIF-ST [90]	GearNet[92]	**Saprot**
Thermostability	Training w/ AF2 structure	Spearman's ρ	0.673	0.621	0.678	0.454	0.697
Subcellular localization		Acc%	84.67	78.65	82.46	75.73	86.18
Binary localization		Acc%	93.56	93.31	92.08	90.72	94.67
Structure class	Training w/ PDB structure	Acc%	79.90	71.57	71.63	74.52	80.80
Structural similarity		Spearman's ρ	0.677	0.601	0.555	0.101	0.695
Binding site detection		Mcc	0.540	0.459	0.504	0.415	0.602
Metal Ion binding		Acc%	74.97	68.19	69.58	67.61	79.97
Fluorescence	Training w/o structure	Spearman's ρ	0.698	0.698	-	-	0.699
Stability		Spearman's ρ	0.780	0.753	-	-	0.779
β-lactamase		Spearman's ρ	0.904	0.881	-	-	0.890
AAV		Spearman's ρ	0.813	0.783	-	-	0.829

Supplementary Table 3: More baseline results on supervised learning tasks. MIF-ST and GearNet are not applicable when protein structures are not available as input. ESM-2 and Saprot both have 650M parameters, while ProtBert has 400M parameters.

Task / Dataset	**Metric**	ProstT5 (1.2B)	**Saprot (650M)**
ClinVar	AUC	0.620	**0.909**
Mega-scale	Spearman's ρ	0.194	**0.574**
Subcellular localization	Acc%	83.26	**86.18**
Binary localization	Acc%	94.11	**94.67**
Protein inverse folding	pLDDT	76.24	**77.96**
	RMSD	2.28	**2.07**
	TM-score	0.78	**0.82**
	Sequence recovery	33.72	**36.15**

Supplementary Table 4: Comparison between ProstT5 and Saprot in several tasks. ProstT5 is a seq2seq T5 model where the input, if it is an amino acid sequence, corresponds to the output of the 3Di sequence, and vice versa. In general, Saprot is comparable to ProstT5 in the protein inverse folding task, but substantially outperform it on other tasks, e.g., zero-shot mutational effect prediction and supervised learning tasks.

Split by 50% sequence identity				
Task	**Metric**	ESM-2 (35M)	Saprot-Seq (35M)	**Saprot (35M)**
Thermostability	Spearman's ρ	0.669	0.672	**0.692**
Metal ion binding	Acc%	73.08	73.23	**74.29**
Subcellular localization	Acc%	76.58	76.67	**78.09**
Binary localization	Acc%	91.60	91.16	**91.97**
Binding site detection	Mcc	0.609	0.507	**0.627**
Structural similarity	Spearman's ρ	0.676	0.657	**0.718**
Structure class	Acc%	68.73	66.63	**69.02**

Split by 30% structure similarity				
Task	**Metric**	ESM-2 (35M)	Saprot-Seq (35M)	**Saprot (35M)**
Thermostability	Spearman's ρ	0.618	0.623	**0.629**
Metal ion binding	Acc%	68.75	65.56	**72.22**
Subcellular localization	Acc%	76.61	76.39	**77.19**
Binary localization	Acc%	91.47	91.71	**91.82**
Binding site detection	Mcc	0.271	0.283	**0.434**
Structural similarity	Spearman's ρ	0.719	0.707	**0.754**
Structure class	Acc%	36.55	34.46	**37.55**

Supplementary Table 5: Comparison of Saprot (35M) and ESM-2 (35M) across seven fine-tuning tasks (with protein structures), employing two data splitting strategies: a conventional 50% sequence identity split widely adopted in literature and a 30% structure similarity split introduced by of ProteinShake. Note that the original ProteinShake paper suggests 70% as a default structure similarity threshold, which has been applied in our evaluation of Saprot (650M). Here, we implement a more stringent threshold (i.e., 30%) for a comprehensive comparison. To demonstrate the effectiveness of SA tokens, we also evaluated Saprot-Seq, a variant that maintains identical architecture but trained exclusively on amino acid sequences by masking structural components (3Di information) with "#" symbols. We report additional results on more tasks (including the zero-shot mutation effect task and tasks beyond this paper) in our the official github (https://github.com/westlake-repl/SaProt) of Saprot, where Saprot (35M) also substantially outperform ESM-2 (35M).

Task	**Metric**	Saprot (650M)	Saprot (1.3B)
ProteinGym	Spearman's ρ	0.457	**0.460**
Mega-scale	Spearman's ρ	0.574	**0.588**
ClinVar	AUC	0.909	**0.910**
Thermostability	Spearman's ρ	0.659	**0.660**
Subcellular localization	Acc%	82.89	**83.11**
Stability	Spearman's ρ	**0.735**	0.732
Binary localization	Acc%	87.24	**89.59**

Supplementary Table 6: Performance comparison of Saprot with 650M and 1.3B versions. Due to computational and memory constraints, we evaluated the Saprot 650M and 1.3B models on seven randomly selected tasks (three mutation effect prediction tasks and four supervised fine-tuning tasks). For the supervised tasks, we implemented a lightweight fine-tuning approach that only retrains the prediction heads while keeping the backbones frozen. This approach was necessary as the computational requirements of Saprot 1.3B made both standard full fine-tuning and LoRA-based methods difficult, even with 80GB GPU memory available. Additionally, considering the relatively small performance gains observed and computational resource constraints, we did not pursue further comparative analyses on more tasks. These findings suggest that merely scaling up model size in protein language models may encounter diminishing returns, potentially indicating a performance ceiling. Scaling AF2's structure predictions to billions of proteins could potentially yield larger improvements with a 1.3B parameter model; however, this exploration extends beyond the scope of this paper.

Model	Structural information	Parameters	Layers	Embedding size
Saprot	True	35M	12	480
		650M	33	1280
		1.3B	66	1280
Saprot-Seq	False	35M	12	480
ESM-2	False	35M	12	480
		650M	33	1280

Supplementary Table 7: Model architectures for Saprot (35M, 650M, and 1.3B) and ESM-2 (35M and 650M) models. Saprot-Seq (35M) was primarily used for ablation studies, employing the same training procedure (including all hyperparameters, network architecture, and training dataset) as Saprot (35M), but with only the AA tokens visible (i.e., masking all 3Di token components).

Supported Tasks	
Protein-level Classification	Active Site Prediction
Fold Class Prediction	Post-Translational Modification (PTM) Site Prediction
Localization Prediction	Mutation Impact Prediction
Subcellular Localization	Transmembrane Region Prediction
Domain Classification	Conservation Score Prediction
Membrane Protein Classification	Interface Residue Prediction
Enzyme Classification	Enzyme Binding Site Prediction
Post-translational Modification Site Classification	Metal Binding Site Prediction
Protein Stability Classification	Disease-Associated Variant Prediction
Protein Solubility Classification	Immune Receptor Binding Prediction
Antigenicity Classification	Glycosylation Site Prediction
Protein Aggregation Classification	**Protein-protein Classification**
Protein Family Classification	Protein-Protein Interaction (PPI) Prediction
Protein Structural Class Prediction	Interaction Type Classification
Protein Subunit Interface Classification	Disease-Associated Interaction Prediction
Protein Motif Classification	Mutational Impact Classification
Protein Epitope Classification	Functional Annotation Classification
Protein Interaction Network Classification	Interaction Network Module Detection
Protein Toxicity Classification	Interaction Stability Prediction
Protein Expression Level Classification	**Protien-protein Regression**
Protein Dynamics Classification	Interaction Strength Prediction
Protein Flexibility Classification	Binding Free Energy Calculation
Protein Function Annotation	Interaction Affinity Prediction
Protein Interaction Type Classification	Mutational Impact Quantification
Protein Disease Association Classification	Interaction Kinetics Prediction
Protein Functional Domain Classification	**Zero-shot mutational Effect prediction**
Protein-level Regression	Enzyme Activity Prediction
Thermal Stability Prediction	Virus Fitness Prediction
Fluorescence Intensity Prediction	Driver Mutation Prediction
Binding Affinity Prediction	Protein Stability Prediction
Solubility Prediction	Immune Escape Prediction
Expression Level Prediction	MHC Binding Prediction
Enzymatic Activity Prediction	Pathogenicity Prediction
Hydrophobicity Prediction	Membrane Protein Function Prediction
Protease Sensitivity Prediction	Functional Domain Impact Prediction
Protein Interaction Strength Prediction	**Protein Sequence Design**
Drug Binding Affinity Prediction	Enzyme Function Optimization
Protein Stability Change upon Mutation Prediction	Protein Stability Enhancement
Protein Ligand Binding Kinetics Prediction	Protein Folding Prediction
Protein Stability in Different Temperatures Prediction	Antibody Design
Protein Stability in Different Solvents Prediction	Development of Novel Protein Drugs
Residue-level Classification	Vaccine Design
Secondary Structure Prediction	Protein Engineering
Binding Site Prediction	Synthetic Antibody Fragments Design

Supplementary Table 8: Tasks that Saprot potentially support. As a large-scale foundational protein language model, Saprot potentially supports hundreds of protein prediction tasks (also see Fig. 1 c,d). These tasks include, but are not limited to: protein-level classification, protein-level regression, residue-level classification, protein-protein classification, protein-protein regression, zero-shot mutational effect prediction, and protein sequence design.

Mutation	Predicted score by ColabSaprot	T>G% (wildtype=10.3865)	Enhancement factor
I37P	4.8993	15.7850	1.5198
L74A	4.7470	12.9784	1.2495
R8M	3.8026	14.7522	1.4203
L74E	3.6393	**20.4948**	**1.9732**
S5M	3.6230	15.0282	1.4469
P43K	3.3585	14.7687	1.4219
P6M	3.3499	15.5822	1.5002
P43R	3.2832	13.6750	1.3166
I37S	3.2649	16.4798	1.5866
I103E	3.2008	11.9370	1.1493
H11P	3.1735	14.6748	1.4129
H11K	3.1643	**18.0260**	**1.7355**
I37R	3.1442	14.8948	1.4341
I103D	3.1406	6.9425	0.6684
F1M	3.0996	6.0477	0.5823
L74Q	3.0893	**19.1058**	**1.8395**
H11A	2.9147	17.1734	1.6534
P4M	2.7864	15.2689	1.4701
I37A	2.7000	16.3456	1.5737
K252L	2.6721	1.2497	0.1203

Supplementary Table 9: Validation of the top 20 TDG variants predicted by ColabSaprot. The top 20 TDG variants predicted by ColabSaprot were inserted into the nCas9 protein and co-transfected into HeLa cells along with Dicer-1 sgRNA. Five days after transfection, T>G substitutions were analyzed using high-throughput sequencing (HTS). Among the top 20 variants, 17 exhibited higher editing efficiencies compared to the wild-type. Specifically, three variants (L74E, L74Q, and H11K) demonstrated nearly double the editing efficiency relative to the wild-type.

Mutation	Predicted score	Specific activity (U/ml) (wild type=5.9101)	Enhancement factor
F212Q	7.5327	4.4419	0.7516
R59T	5.3584	0.4921	0.0833
F212M	4.6898	4.4720	0.7567
F212R	4.3376	3.9598	0.6700
N236P	4.2207	8.6526	1.4640
S165T	4.0865	7.0325	1.1899
A15G	3.9353	-	-
R59S	3.8820	**15.1057**	**2.5559**
M184V	3.8267	1.0006	0.1693
A194F	3.7584	**11.5114**	**1.9478**
F212N	3.7492	**11.1590**	**1.8881**
S225N	3.7452	**12.9281**	**2.1875**
A194L	3.6962	10.4406	1.7666
F212L	3.6802	1.1437	0.1935
M184T	3.5455	0.4883	0.0826
R59Q	3.4463	10.8399	1.8341
G22A	3.4332	**14.6741**	**2.4829**
N236G	3.3842	8.0005	1.3537
G22V	3.3259	10.1135	1.7112
I13P	3.2729	-	-
F212S	3.2339	8.0726	1.3659
D44N	3.2112	7.8896	1.3349

Supplementary Table 10: The 20 highest-ranked Mth (xylanase) variants predicted by ColabSaprot were expressed in *Pichia pastoris*. Results showed that 13 mutants exhibited increased specific activity compared to the wild-type enzyme. The most effective variant, R59S, reached 2.55-fold of the wild-type (WT) activity. Several other variants, including S225N, G22A, A194F, and F212N, reached approximately 2-fold of the wild-type activity. All of the assays were repeated at least three times.

Designed mutations	Fluorescence intensities
K158Q, V163G	8.45
K158A, I171L	6.87
V163G, I171T	6.78
Y39N, K158Q	4.45
K158N, I171V	3.21
V11W, V163A	2.77
K158A, V163G	2.72
WT(avGFP, UniProtKB: P42212)	1.00
V163A, K214E	0.31
V163G, K214E	0.30

Supplementary Table 11: Top 9 avGFP mutants predicted by Sarot and experimental performance. Fluorescence intensities were normalized relative to the wild-type (WT) parent, which was set as 1. The best-performing variant reached over 8-fold of the WT fluorescence intensity.

Primer	Sequence (5'-3')
MT_G22_U_A	CGGAGCAGCCAGCGCAGTTG
MT_G22A_D_S	CAACTGCGCTGGCTGCTCCGGCTGAACTACCAGGAATGCACCT
MT_G22V_D_S	CAACTGCGCTGGCTGCTCCGGTAGAACTACCAGGAATGCACCT
MT_D44_U_A	ATGAGTACCTGTAGCACTTT
MT_D44N_D_S	AAAGTGCTACAGGTACTCATAATGGCTACTATTTTTCGTTT
MT_R59_U_A	AACATTGGGACCGCCATCCG
MT_R59Q_D_S	CGGATGGCGGTCCCAATGTTCAATACACTAATGAAGCCGGCG
MT_R59S_D_S	CGGATGGCGGTCCCAATGTTAGTTACACTAATGAAGCCGGCGG
MT_R59T_D_S	CGGATGGCGGTCCCAATGTTACTTACACTAATGAAGCCGGCGG
MT_S165_U_A	TCTCGTGCCCTCTATGGAAG
MT_S165T_D_S	CTTCCATAGAGGGCACGAGAACCTTCGATCAATATTGGTCGGT
MT_M184_U_A	ATCCACCGATCCCCCGACCC
MT_M184T_D_S	GGGTCGGGGGATCGGTGGATACGAACGCGCATTTCAACGCGT
MT_M184V_D_S	GGGTCGGGGGATCGGTGGATGTTAACGCGCATTTCAACGCGTG
MT_A194_U_A	TTGCCGCCACGCGTTGAAAT
MT_A194F_D_S	ATTTCAACGCGTGGCGGCAATTCGGCTTAACACTTGGAACTCA
MT_A194L_D_S	ATTTCAACGCGTGGCGGCAATTAGGCTTAACACTTGGAACTCA
MT_F212_U_A	ATATCCTTCGGTTGCAACAA
MT_F212L_D_S	TTGTTGCAACCGAAGGATATCTTAGCTCAGGGTCGGCACGC
MT_F212M_D_S	TTGTTGCAACCGAAGGATATATGAGCTCAGGGTCGGCACGCAT
MT_F212N_D_S	TTGTTGCAACCGAAGGATATAATAGCTCAGGGTCGGCACGCA
MT_F212Q_D_S	TTGTTGCAACCGAAGGATATCAGAGCTCAGGGTCGGCACGCAT
MT_F212R_D_S	TTGTTGCAACCGAAGGATATAGGAGCTCAGGGTCGGCACGCAT
MT_F212S_D_S	TTGTTGCAACCGAAGGATATTCAAGCTCAGGGTCGGCACGCAT
MT_N236_U_A	GTTCCCTCCGCCATTGTTAC
MT_N236G_D_S	GTAACAATGGCGGAGGGAACGGCGGAAACCCAGGTGGTAATC
MT_N236P_D_S	GTAACAATGGCGGAGGGAACCCCGGAAACCCAGGTGGTAATCC
MT_S225_U_A	GCCTCCGCCTACATTGATGC
MT_S225N_D_S	GCATCAATGTAGGCGGAGGCAACACGGGGGGTGGTAACAATG

Supplementary Table 12: Site-directed mutation primers used in this study.

Name	Sequence
TDG	FFSPSPARKRHAPSPEPAVQGTGVAGVPEESGDAAAIPAKKAPAGQEEPGTPPSSPLSAEQ LDRIQRNKAAALLRLAARNVPVGFGESWKKHLSGEFGKPYFIKLMGFVAEERKHYTVYPPP HQVFTWTQMCDIKDVKVVILGQDPAHGPNQAHGLCFSVQRPVPPPPSLENIYKELSTDIED FVHPGHGDLSGWAKQGVLLLNAVLTVRAHQANSHKERGWEQFTDAVVSWLNQNSNGLVFLL WGSYAQKKGSAIDRKRHHVLQTAHPSPLSVYRGFFGCRHFSKTNELLQKSGKKPIDWKEL
Mth	MVAFSSLFLG ASIAATALAA PGELPGMHLN KRQTYTQSAT GTHDGYYFSF WTDGGPNVRY TNEAGGQYSV TWSGNGNWVG GKGWNPGAAR TINYTGTYQP NGNSYLAVYG WTRNPLVEYY VVENFGTYDP STGAQRLGSI NVDGSTYNVY RTRRTNAPSI EGTRSFDQYW SVRVNKRVGG SVDMNAHFNA WRQAGLTLGT HDYQIVATEG YFSSGSARIN VGGGSTGGGN NGGGNNGNPG GNPGGNPGGN PGGNPGGNCS PRWGQCGGQG WNGPTCCEAG TTCRQQNQWY SQCL
avGFP	MSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVP WPTLVTTLSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEV KFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIR HNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFV TAAGITHGMDELYK
amacGFP	MSKGEELFTGIVPVLIELDGDVHGHKFSVRGEGEGDADYGKLEIKFICTTGKLPVP WPTLVTTLSYGILCFARYPEHMKMNDFFKSAMPEGYIQERTIFFQDDGKYKTRGEV KFEGDTLVNRIELKGMDFKEDGNILGHKLEYNFNSHNVYIMPDKANNGLKVNFKIR HNIEGGGVQLADHYQTNVPLGDGPVLIPINHYLSCQTAISKDRNETRDHMVFLEFF SACGHTHGMDELYK
cgreGFP	MTALTEGAKLFEKEIPYITELEGDVEGMKFIIKGEGTGDATTGTIKAKYICTTGDL PVPWATILSSLSYGVFCFAKYPRHIADFFKSTQPDGYSQDRIISFDNDGQYDVKAK VTYENGTLYNRVTVKGTGFKSNGNILGMRVLYHSPPHAVYILPDRKNGGMKIEYNK AFDVMGGGHQMARHAQFNKPLGAWEEDYPLYHHLTVWTSFGKDPDDDETDHLTIVE VIKAVDLETYR
ppluGFP2	MPAMKIECRITGTLNGVEFELVGGGEGTPEQGRMTNKMKSTKGALTFSPYLLSHVM GYGFYHFGTYPSGYENPFLHAINNGGYTNTRIEKYEDGGVLHVSFSYRYEAGRVIG DFKVVGTGFPEDSVIFTDKIIRSNATVEHLHPMGDNVLVGSFARTFSLRDGGYYSF VVDSHMHFKSAIHPSILQNGGPMFAFRRVEELHSNTELGIVEYQHAFKTPIAFA
eYFP	MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWPTLV TTFGYGLQCFARYPDHMKLHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNR IELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTP IGDGPVLLPDNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK

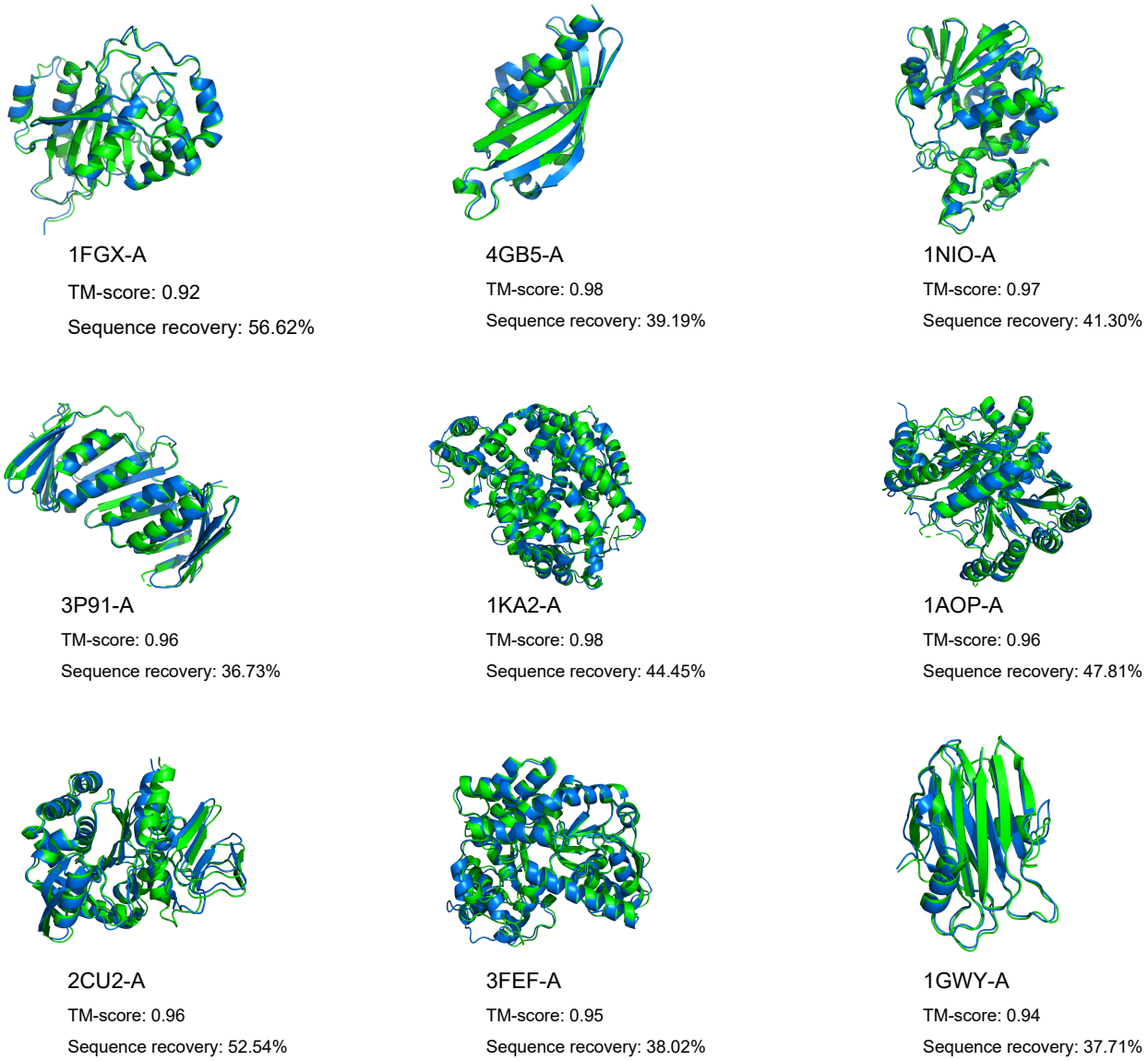
Supplementary Table 13: Amino acid sequence of the wild-type protein studied in this experiment.

Benchmark Scores

https://proteingym.org/benchmarks

Rank▲	Model name	Model type	Avg. Spearman
1	SaProt (650M)	Protein language model	0.457
2	TranceptEVE L	Hybrid - Alignment & PLM	0.456
3	TranceptEVE M	Hybrid - Alignment & PLM	0.455
4	GEMME	Alignment-based model	0.455
5	TranceptEVE S	Hybrid - Alignment & PLM	0.452
6	ProtSSN (ensemble)	Hybrid - Structure & PLM	0.449
7	ProtSSN (k=20 h=1280)	Hybrid - Structure & PLM	0.442
8	ProtSSN (k=20 h=512)	Hybrid - Structure & PLM	0.441
9	ProtSSN (k=20 h=768)	Hybrid - Structure & PLM	0.44
10	EVE (ensemble)	Alignment-based model	0.439
11	ProtSSN (k=30 h=512)	Hybrid - Structure & PLM	0.438
12	ProtSSN (k=30 h=768)	Hybrid - Structure & PLM	0.438
13	ProtSSN (k=30 h=1280)	Hybrid - Structure & PLM	0.438
14	VESPA	Protein language model	0.436
15	Tranception L	Hybrid - Alignment & PLM	0.434
16	MSA Transformer (ensemble)	Hybrid - Alignment & PLM	0.434
17	ProtSSN (k=10 h=1280)	Hybrid - Structure & PLM	0.433
18	EVE (single)	Alignment-based model	0.433
19	ProtSSN (k=10 h=512)	Hybrid - Structure & PLM	0.43
20	Tranception M	Hybrid - Alignment & PLM	0.427
21	ProtSSN (k=10 h=768)	Hybrid - Structure & PLM	0.423
22	ESM-IF1	Inverse folding model	0.422
23	MSA Transformer (single)	Hybrid - Alignment & PLM	0.421
24	DeepSequence (ensemble)	Alignment-based model	0.419
25	Tranception S	Hybrid - Alignment & PLM	0.418
26	ESM2 (650M)	Protein language model	0.414
27	DeepSequence (single)	Alignment-based model	0.407
28	ESM-1v (ensemble)	Protein language model	0.407
29	SaProt (35M)	Protein language model	0.406
30	ESM2 (3B)	Protein language model	0.406
31	ESM2 (15B)	Protein language model	0.401
32	MIF-ST	Hybrid - Structure & PLM	0.4
33	EVmutation	Alignment-based model	0.395
34	ESM-1b	Protein language model	0.394
35	VESPAl	Protein language model	0.394
36	Progen2 XL	Protein language model	0.391
37	ESM2 (150M)	Protein language model	0.387
38	MIF	Inverse folding model	0.383
39	Progen2 L	Protein language model	0.38
40	Progen2 M	Protein language model	0.379

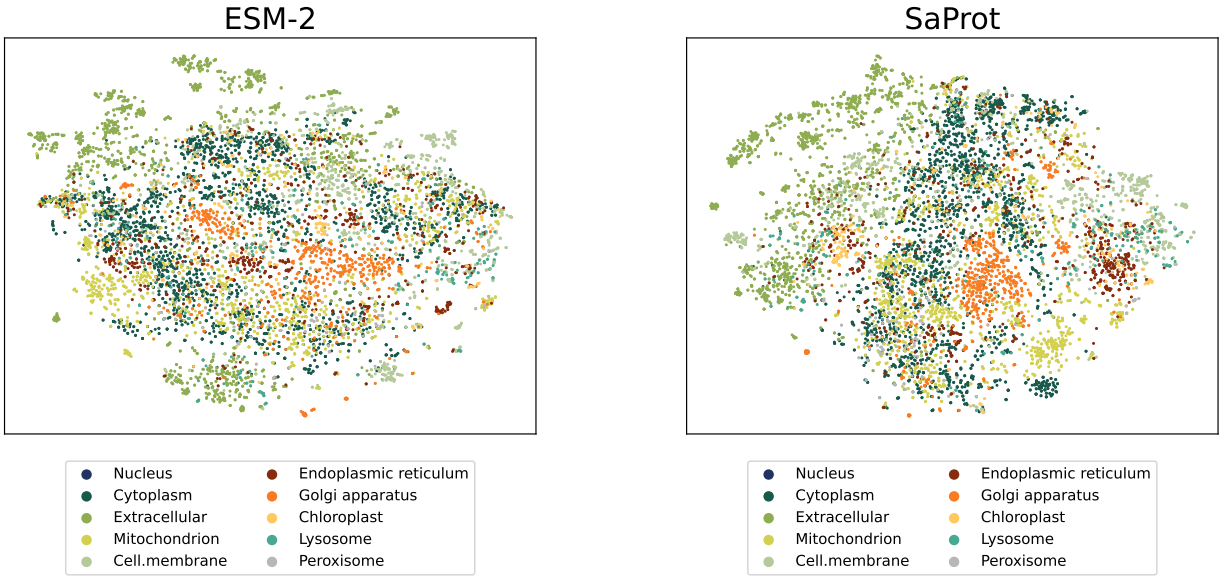
Supplementary Fig. 1: Saprot (checkpoint: https://huggingface.co/westlake-repl/SaProt_650M_AF2) ranked first on the public ProteinGym leaderboard until May 2024, surpassing over 60 well-known methods. Note that Saprot is a single model, while most other top-ranked models are hybrid models.



Supplementary Fig. 2: Case studies of protein inverse folding using ColabSaprot (650M). The blue-colored protein structures are predicted using ESMFold, with residue sequences generated by Saprot, and the green-colored structures are the experimentally determined structures. Given the backbone, Saprot is capable of predicting diverse sequences with few residue overlap while maintaining almost the same 3D structure.

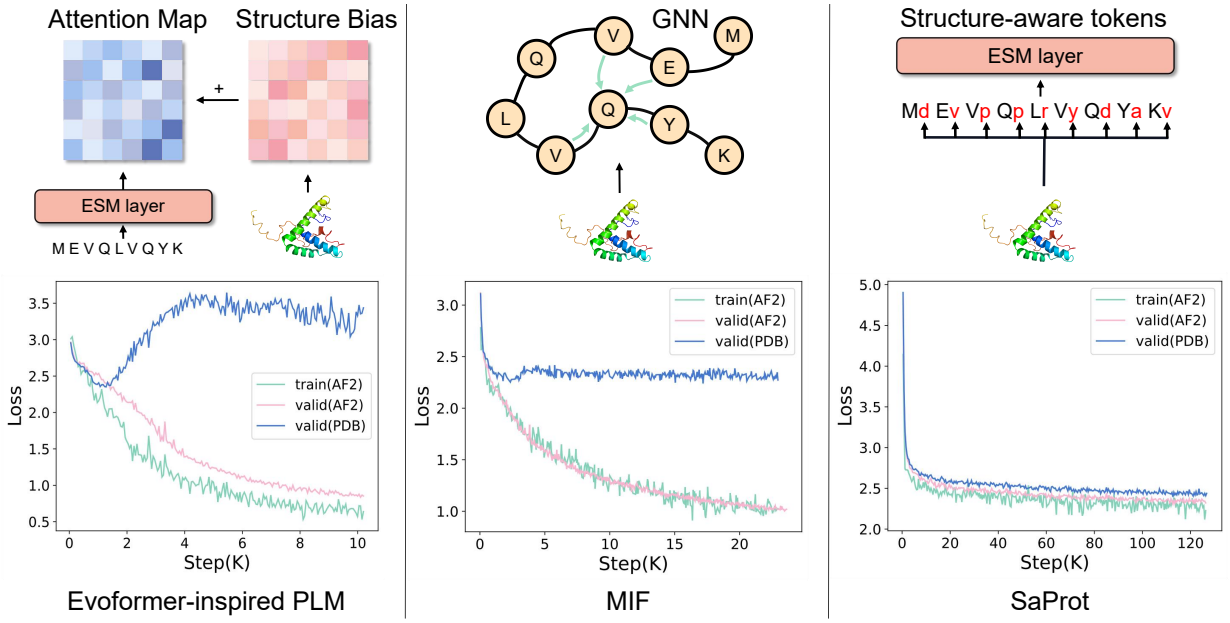
(a) Embedding visualizations of ESM-2 650M version and Saprot 650M version on SCOPe database. We use the non-redundant version ($PIDE < 40\%$) of the SCOPe database.

(b) Embedding visualizations of ESM-2 and Saprot on binary localization dataset.

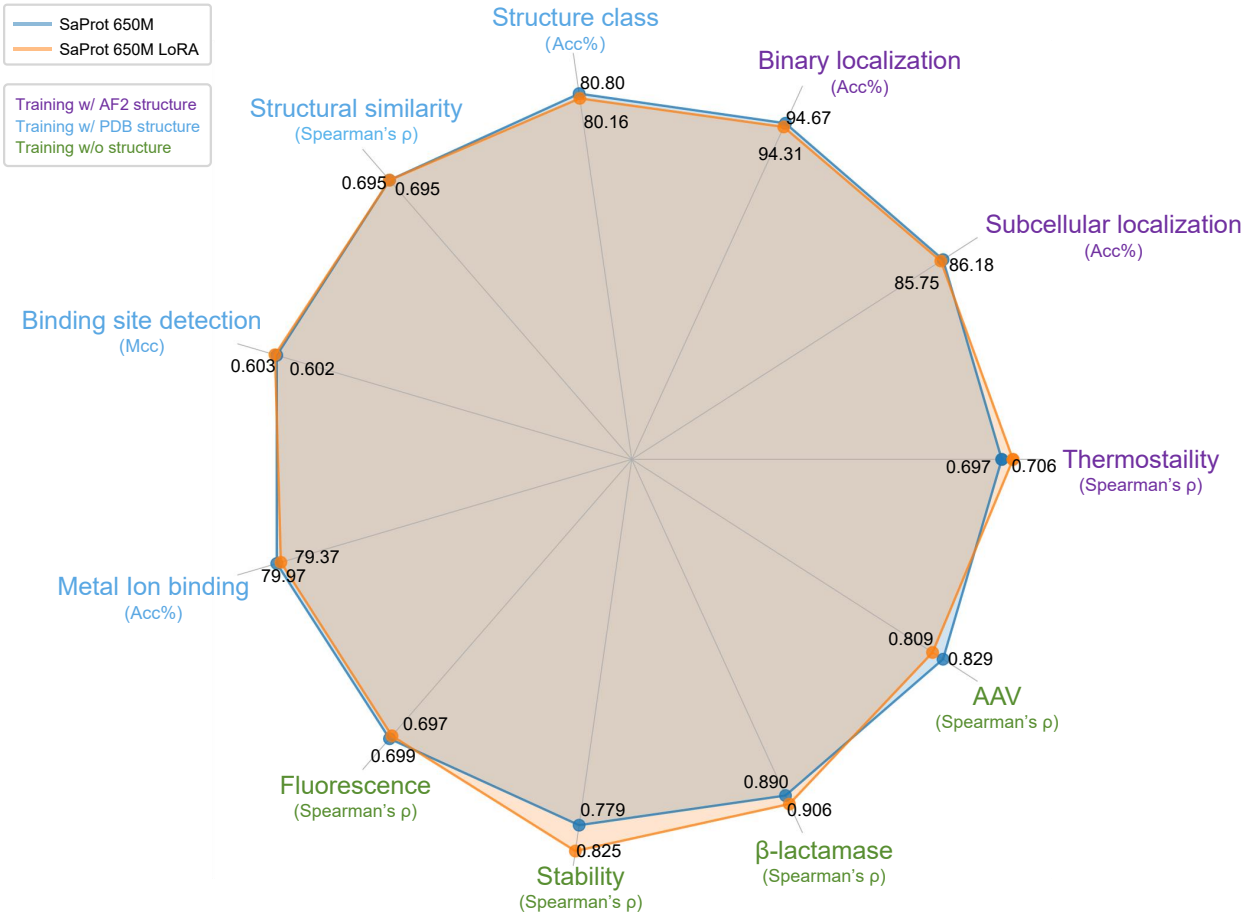


(c) Embedding visualizations of ESM-2 and Saprot on subcellular localization dataset.

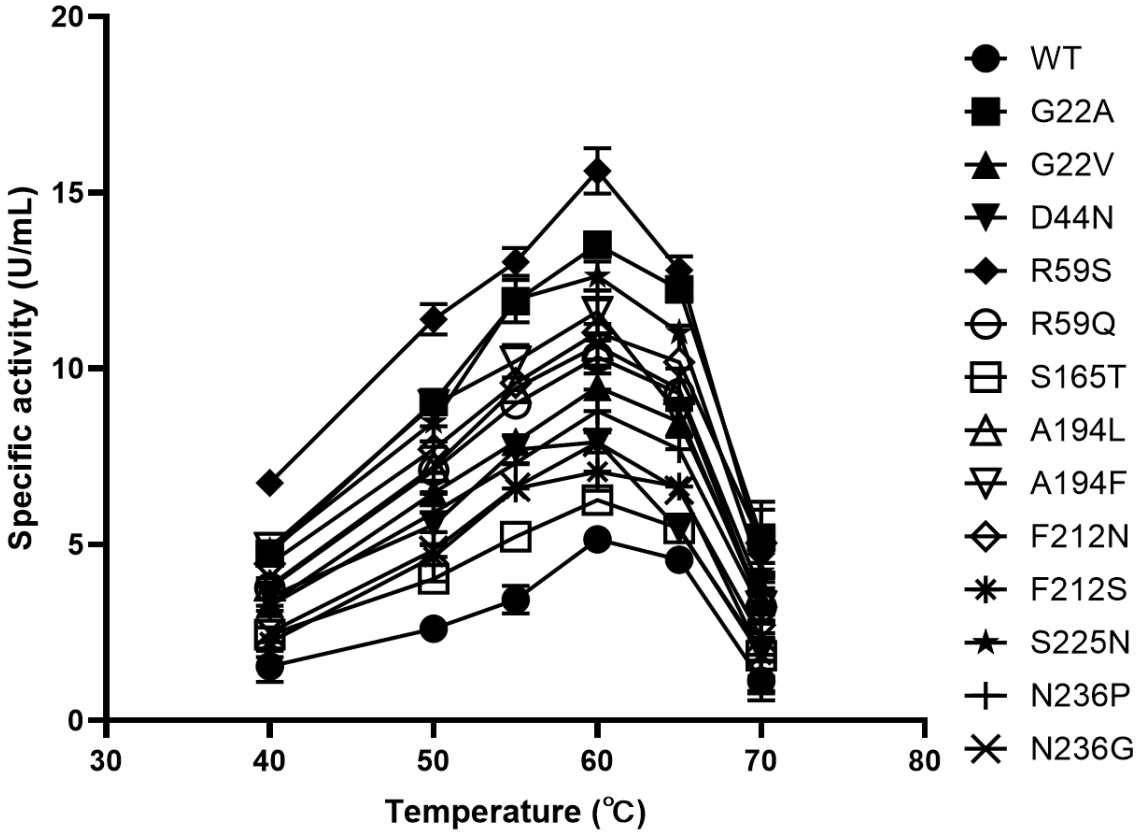
Supplementary Fig. 3: Embedding visualization of Saprot (650M) and ESM-2 (650M))



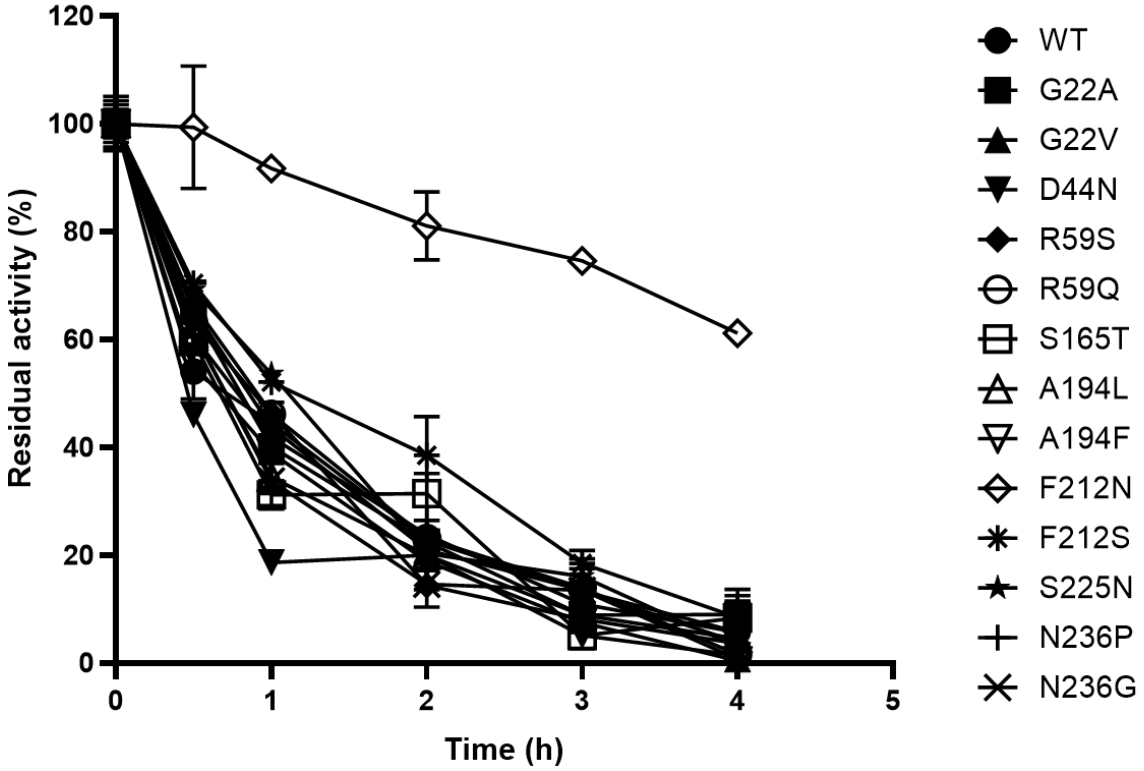
Supplementary Fig. 4: Loss trends for three structure-aware PLMs. The training set is the AF2-predicted structures, while in the validation set, one is the AF2 structure and the other is the experimental structure from PDB. The figure is from our Saprot conference version in ICLR2024 [44].



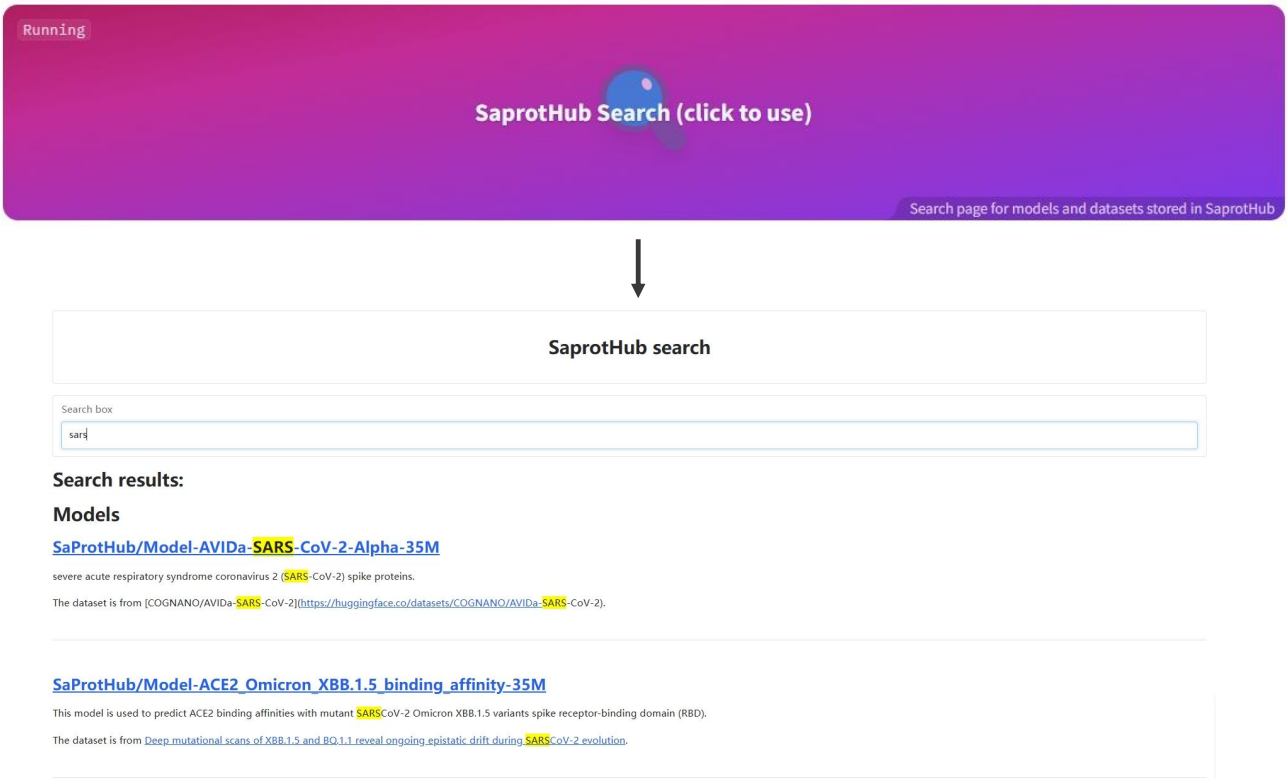
Supplementary Fig. 5: Comparison between full model fine-tuning and parameter efficient fine-tuning. While greatly reducing trainable parameters, fine-tuning Saprot using adapter module achieves competitive performance on all experimental settings, which demonstrates its applicability on various biological tasks.



Supplementary Fig. 6: The optimal reaction temperature for both the top-ranked mutants and the wild type (WT) was 60°C. Error bars represent standard deviations, and two replicates were performed.



Supplementary Fig. 7: Mutant F212N exhibited a 1.88-fold increase in specific activity compared to the wild-type (WT) enzyme, along with significantly improved thermal stability. After a 4-hour incubation at 60°C, F212N retained over 60% of its initial activity, while the WT enzyme was almost completely inactivated under the same conditions. Error bars represent standard deviations, and two replicates were performed.



Supplementary Fig. 8: The model and dataset search engine (https://huggingface.co/spaces/SaProtHub/SaprotHub-search) on the SaprotHub webpage (https://huggingface.co/SaProtHub).