Rapid protein stability prediction using deep learning representations

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Abstract Predicting the thermodynamic stability of proteins is a common and widely used step in protein engineering, and when elucidating the molecular mechanisms behind evolution and disease. Here, we present RaSP, a method for making rapid and accurate predictions of changes in protein stability by leveraging deep learning representations. RaSP performs on-par with biophysics-based methods and enables saturation mutagenesis stability predictions in less than a second per residue. We use RaSP to calculate ~ 8.8 million stability changes for nearly all single amino acid changes in 1,381 human proteins, and examine variants observed in the human population. We find that variants that are common in the population are substantially depleted for severe destabilization, and that there are substantial differences between benign and pathogenic variants, highlighting the role of protein stability in genetic diseases. RaSP is freely available and enables large-scale analyses of stability in experimental and predicted protein structures.

Introduction

Protein stability, as measured by the thermodynamic free energy difference between the native and the unfolded state, is an important feature of protein structure and therefore function. Protein stability plays a critical role when trying to understand the molecular mechanisms of evolution and has been found to be an important driver of human disease (***Casadio et al., 2011***; ***Martelli et al., 2016***; ***Nielsen et al., 2020***). Furthermore, optimisation of protein stability is a fundamental part of protein engineering and design (***Rocklin et al., 2017***).

For a given protein, single amino acid substitutions can have substantial effects on protein stability depending on the particular substitution and the surrounding atomistic environment. Assessing such variant effects can be done experimentally for example via thermal or chemical denaturation assays (***Lindorff-Larsen and Teilum, 2021***).

This process, however, may be laborious and time-consuming for even a modest number of amino acid substitutions. In contrast, computational methods have been developed that can predict protein stability changes. Such methods include well-established energy-function-based methods such as for example FoldX (***Schymkowitz et al., 2005***) and Rosetta (***Kellogg et al., 2011***), or methods based on molecular dynamics simulations (***Gapsys et al., 2016***).

Machine learning models have also been developed to predict protein stability and can roughly be split into two types; supervised and self-supervised models. In supervised models, experimental protein stability measurements are used as targets for model predictions (***Li et al., 2020***; ***Benevenuta et al., 2021***; ***Pires et al., 2014***; ***Chen et al., 2020***). Supervised models are immediately appealing as they are trained directly on experimental data and are able to make predictions at the correct absolute scale. Supervised models may, however, suffer from systematic biases, which can be hard to overcome with limited experimental data. These biases relate to issues of model overfitting of the training data, biases present in the experimental data towards destabilising mutations, biases of the types of amino acid changes that have been probed experimentally, and a lack of self-consistency in model predictions (***Yang et al., 2018***; ***Pucci et al., 2018***; ***Usmanova et al., 2018***; ***Fang, 2019***; ***Stein et al., 2019***; ***Caldararu et al., 2020***).

In contrast, self-supervised models can be trained without the use of experimental protein stability measurements. Typically, self-supervised models are trained to predict masked amino acid labels from structure or sequence information, thereby learning a likelihood distribution over possible amino acid types at a particular position. This learned likelihood distribution can subsequently be used directly to predict the effects of mutations on protein stability (***Lui and Tiana, 2013***; ***Boomsma and Frellsen, 2017***; ***Riesselman et al., 2018***; ***Shin et al., 2021***; ***Elnaggar et al., 2021***; ***Meier et al., 2021***). Self-supervised models sidestep many of the challenges associated with training on experimental data and have shown impressive results in recent years. These models, however, often require substantial computational resources during training—and sometimes during evaluation—and generally do not make predictions at an absolute scale, which limits their practical application.

Due to the complimentary strengths and weaknesses of supervised and self-supervised models, we suggest that the two approaches may be combined. Here we present the RaSP (Rapid Stability Prediction) model for rapid protein stability predictions. The RaSP model leverages the combined strengths of supervised and self-supervised models; RaSP is able to make both fast and accurate predictions of protein stability changes and thus enables large-scale, proteome-wide applications.

Results

Development of the RaSP model

We trained the RaSP model in two steps (Fig. 1). First, we trained a self-supervised representation model to learn an internal representation of protein structure (***Boomsma and Frellsen, 2017***). Second, we trained a supervised downstream model using the learned structure representation as input to predict protein stability changes on an absolute scale. The supervised downstream model was trained on a set of calculated protein stability changes to enable the generation of a larger and more powerful training data set

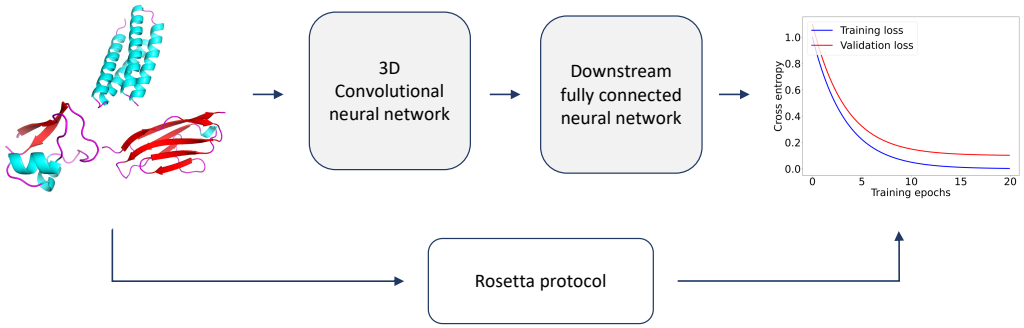

Figure 1. Overview of model training. We trained a self-supervised three-dimensional convolutional neural network to learn internal representations of protein structures by predicting wild-type amino acid labels from protein structures. Using the representations from the convolutional neural network as input, a second downstream and supervised fully connected neural network was trained to predict Rosetta $\Delta\Delta G$ values.

while minimizing the effects from experimental data biases. We chose to estimate protein variant stability values using the Rosetta 'cartesian_ddg' protocol, which has shown to be a reliable and well-established predictor of stability (***Park et al., 2016***; ***Frenz et al., 2020***), and as shown below the model generalizes to a wide range of situations.

Building on earlier work (***Boomsma and Frellsen, 2017***), we first trained a self-supervised 3D convolutional neural network on a large, homology-reduced set of high-resolution structures. This model was trained to predict the wild type amino acid labels of a protein given its structure (see Methods for further details). In the second step of developing RaSP, we trained a downstream supervised fully-connected neural network to predict stability changes; this model uses the internal representation of the 3D convolutional neural network as well as the corresponding wild type and mutant amino acid labels as input. We trained this model on $\Delta\Delta G$ values generated by saturation mutagenesis using Rosetta to limit the effects of biases from experimental assays and amino acid compositions; as described further below we validated the model using a range of different experiments. In the training of the supervised model we used a custom loss function to focus the model on $\Delta\Delta G$ values in the range from approximately -1 to 7 where Rosetta is known to be most accurate (***Kellogg et al., 2011***) and where many loss-of-function variants relevant for human disease can be identified (***Casadio et al., 2011***; ***Cagiada et al., 2021***).

After training (Fig. 2a), the downstream model achieves a Pearson correlation coefficient of 0.82 and a mean absolute error (MAE) of 0.73 kcal/mol on a test data set comprised of 10 proteins with full saturation mutagenesis which was not seen by the model until after it had been developed and trained (Fig. 2b). In practice, the downstream model predictions were made using the median of an ensemble of 10 model predictions with each model trained using different initialization seeds. The accuracy of the model is relatively uniform for the different types of amino acid substitutions, with bigger errors in particular when substituting glycine residues, or when changing residues to proline (Fig. S1).

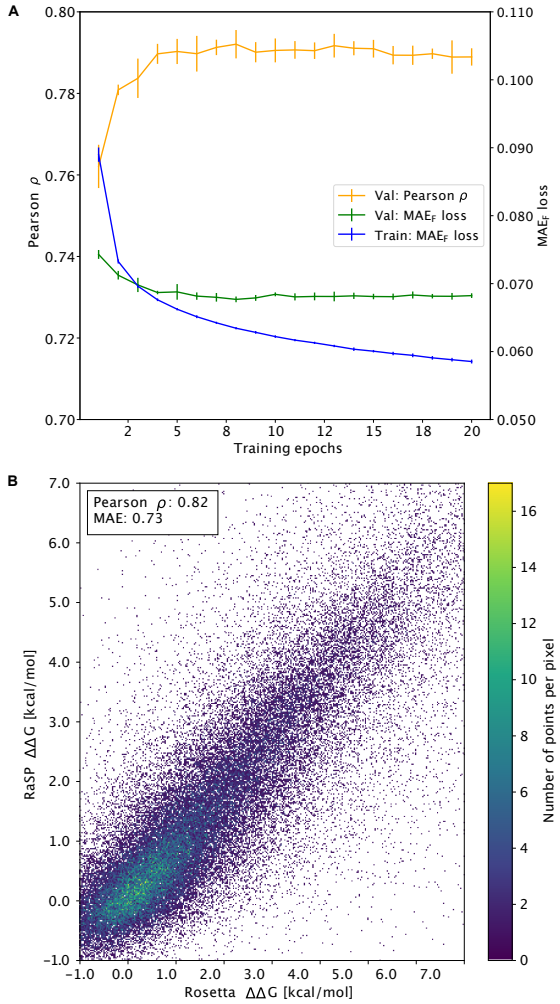

Figure 2. Overview of RaSP downstream model training and testing. (A) Learning curve for training of the RaSP downstream model, with Pearson correlation coefficients (ρ) and mean absolute error (MAE_F) of RasP predictions. During training we transformed the target $\Delta\Delta G$ data using a switching (Fermi) function, and MAE_F refers to this transformed data (see Methods for further details). Error bars represent the standard deviation of 10 independently trained models, that were subsequently used in ensemble averaging. Val: validation set; Train: training set. (B) After training, we applied the RaSP model to an independent test set to predict $\Delta\Delta G$ values for a full saturation mutagenesis of 10 proteins. Pearson correlation coefficients and mean absolute errors (MAE) were for this figure computed using only variants with Rosetta $\Delta\Delta G$ values in the range [-1;7] kcal/mol.

Validating RaSP using experimental data

After having shown that the RaSP model can successfully reproduce Rosetta $\Delta\Delta G$ values, we proceeded to test it on experimental measurements for five proteins. Specifically, we selected four proteins with stability measurements from ProTherm (***Kumar et al., 2006***; ***Ó Conchúir et al., 2015***) and the B1 domain of protein G that where stability measurements are available for almost all possible single amino acid substitutions (***Nisthal et al., 2019***). We compared RaSP predictions for the five test proteins to Rosetta as a baseline (Fig. S2). Pearson correlation coefficients of RASP model predictions versus experimental stability measurements ranged from 0.79 (RNAse H, Rosetta benchmark: 0.68) to 0.61 (lysozyme, Rosetta benchmark: 0.69) (see Table S1 for all comparisons).

Multiplexed assays of variant effects (MAVEs, also known as deep mutational scanning assays) leverage recent developments in high-throughput DNA synthesis and sequencing to probe large (e.g. thousands per protein) libraries of protein variants (***Kinney and McCandlish, 2019***). A particular type of MAVE termed 'Variant Abundance by Massively Parallel sequencing' (VAMP-seq) probes variant effects on cellular protein abundance (***Matreyek et al., 2018***), and correlates with both *in vitro* measurements (***Matreyek et al., 2018***; ***Suiter et al., 2020***) and computational predictions (***Cagiada et al., 2021***) of protein stability. We therefore compared RaSP calculations with VAMP-seq data for three proteins and find that it correlates about as well as Rosetta calculations (Fig. S3).

Prediction of protein stability using computationally modelled structures

For maximal utility for proteome-wide stability predictions, our model should be generally robust to the quality of input protein structures (***Caldararu et al., 2021***). To test this, we used template-based (homology) modelling to generate structures of the four proteins selected from ProTherm that we analysed above. To minimize issues of leakage between training and testing data, we have recently used MODELLER (***Martí-Renom et al., 2000***; ***Webb and Sali, 2016***) to construct models of the four proteins, using templates with decreasing sequence identities to the original structures (***Valanciute et al., 2022***). We used these structures as input to RaSP in order to calculate $\Delta\Delta G$ values to compare with experiments; we also performed similar tests using $\Delta\Delta G$ values calculated using Rosetta (Fig. 3). Overall we find that both RaSP and Rosetta are relatively insensitive to the input models, with some decrease in accuracy for both methods when models are created using more distant homologues.

The development of AlphaFold 2 (***Jumper et al., 2021***) and other protein structure prediction algorithms has enabled large-scale generation of accurate structural models (***Varadi et al., 2022***). Recently, it has been shown that high-confidence models generated by AlphaFold 2 are sufficiently accurate to be used as input for protein stability predictions using for example FoldX and Rosetta (***Akdel et al., 2021***). We therefore tested whether AlphaFold 2 structures could similarly be used as input to RaSP. More specifically, we asked whether $\Delta\Delta G$ could be predicted as accurately from AlphaFold 2 structures as from crystal structures. We selected three proteins that each had two high-resolution crystal structures and used these as well as an AlphaFold 2 model to predict $\Delta\Delta G$. Overall we find a high correlation between the RaSP predictions from the pairs of crystal structures ($\overline{\rho} = 0.97$), only slightly greater than the correlation between values predicted from a crystal structure and an AlphaFold 2 structure ($\overline{\rho} = 0.94$) (Table 1). These results, to-

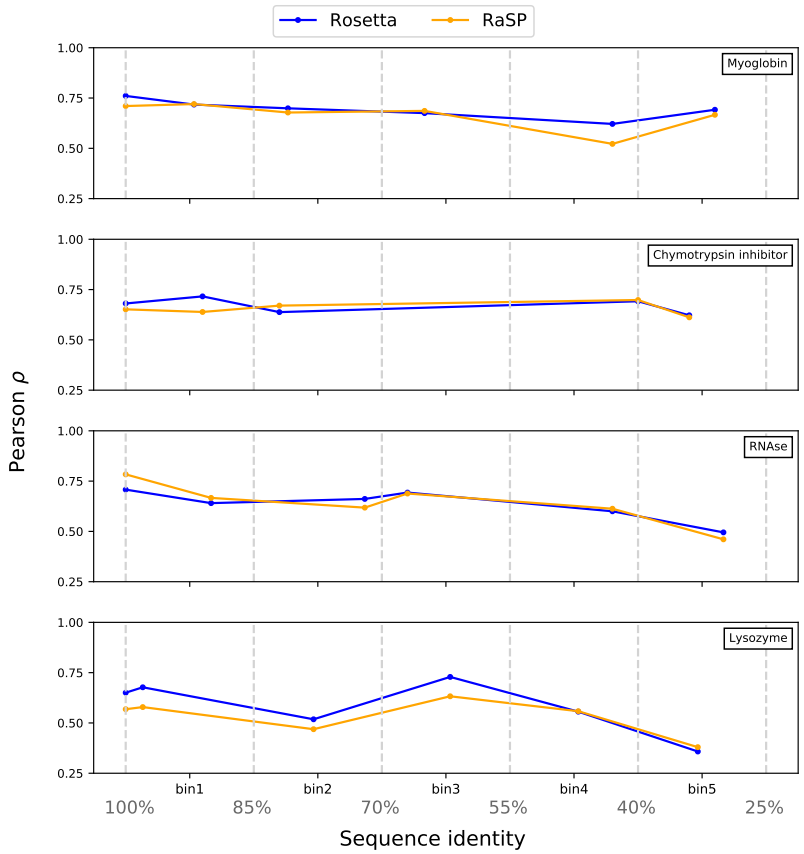

Figure 3. Stability predictions from structures created by template-based modelling. Pearson correlation coefficients (ρ) between experimental stability measurements and predictions using protein homology models with decreasing sequence identity to the target sequence. Pearson correlation coefficients were computed in the range of $\Delta\Delta G$ $[-1; 7]$ kcal/mol.

Protein	Uniprot	PDB 1	PDB 2	Pearson ρ		
				PDB 1 vs. PBD 2	PDB 1 vs. AF2	PDB 2 vs. AF2
PRMT5	O14744	6V0P_A	4GQB_A	0.93	0.88	0.95
PKM	P14618	6B6U_A	6NU5_A	0.99	0.93	0.93
FTH1	P02794	4Y08_A	4OYN_A	0.99	0.96	0.97

Table 1. Comparing RaSP predictions from crystal and AlphaFold 2 structures. Pearson correlation coefficients between RaSP $\Delta\Delta G$ predictions using either two different crystal structures or a crystal structure and an AlphaFold 2 structure for three test proteins: PRMT5, PKM and FTH1.

gether with those using the homology models above, indicate that RaSP can also deliver accurate predictions using computationally generated protein structures.

Large-scale stability calculations and analysis of disease-causing missense variants

Making rapid predictions is a critical feature for any model designed for large-scale applications. We designed RaSP to be fast, yet retain the accuracy of the Rosetta model. Indeed, we find that RaSP enables saturation mutagenesis $\Delta\Delta G$ calculations at a speed of ~0.4 seconds per position—independent of total protein length—after pre-processing of the protein structure. At this speed, saturation mutagenesis scans are possible for most proteins in a few minutes—orders of magnitude faster than Rosetta (Table 2; note differences in hardware used for the two sets of calculations).

To demonstrate how the speed of RaSP enables large-scale applications, we used it to perform saturation scans for 1,381 experimentally determined structures of human proteins or domains (see Methods for further details on selection criteria), yielding a data set of ~ 8.8 million $\Delta\Delta G$ values. The $\Delta\Delta G$ values predicted by RaSP follow the expected distribution (***Tokuriki et al., 2007***; ***Nisthal et al., 2019***) with a small number of stabilizing variants, a large number of slightly destabilizing variants and a long tail of very destabilizing variants (Fig. S4).

As an example of the kinds of analyses that these large-scale calculations enable, we examined variants that have been observed in the human population. Specifically, we extracted validated disease-causing and benign variants from ClinVar (***Landrum et al., 2018***) and variants observed more broadly in the human population from the Genome Aggregation Database (gnomAD) (***Karczewski et al., 2020***), and annotated them with their predicted $\Delta\Delta G$ values (Fig. 4). We find that benign variants generally only have a smaller effect on stability (median $\Delta\Delta G$ 0.54 kcal/mol, and 95% within the range -0.9 to 2.7 kcal/mol; Fig. 4A) whereas many disease causing missense variants are predicted to be destabilizing (median $\Delta\Delta G$ 1.4 kcal/mol, and 95% within the range -1.4 to 6.7 kcal/mol; Fig. 4A). Thus, a substantial fraction of the pathogenic variants are predicted to be destabilized to an extent that is likely to cause lowered abundance (***Cagiada et al., 2021***; ***Høie et al., 2022***). Similarly, variants that are common in the human population (i.e. with an allele frequency in gnomAD $> 10^{-2}$) generally only have a small effect on the stability (median $\Delta\Delta G$ 0.41 kcal/mol), whereas rarer variants have a broader distribution including more destabilizing variants (Fig. 4B). Thus, in line with previous observations (***Casadio et al.,***

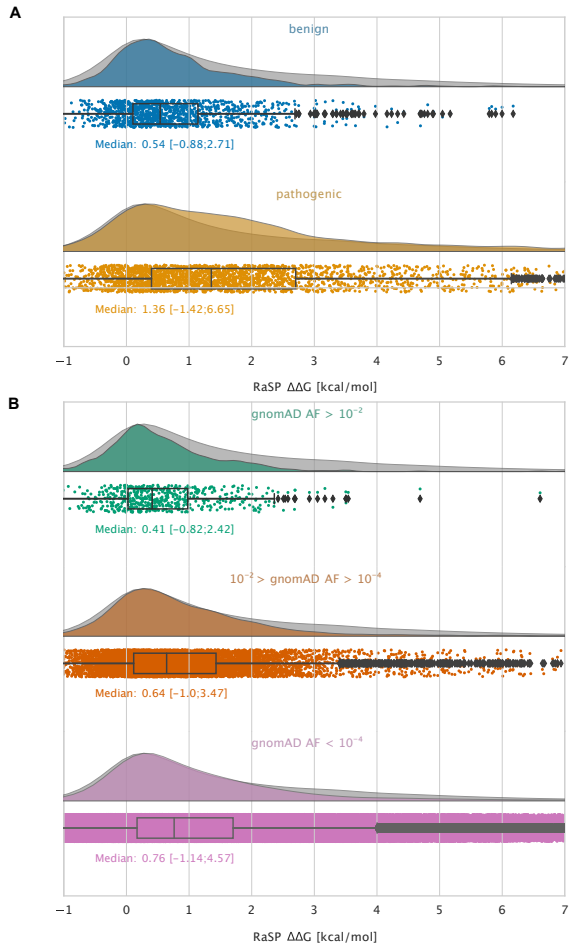

Figure 4. Large-scale analysis of disease-causing variants and variants observed in the population. The grey distribution shown in the background of all plots represents the distribution of $\Delta\Delta G$ values calculated using RaSP for all single amino acid changes in the 1,366 proteins that we analysed (15 of the 1,381 proteins that we calculated $\Delta\Delta G$ for did not have variants in ClinVar or gnomAD). Each plot is also labelled with the median $\Delta\Delta G$ of the subset analysed as well as a range of $\Delta\Delta G$ values that cover 95% of the data in that subset. The plots only show values between -1 and 7 kcal/mol (for the full range see Fig. S5.). (A) Distribution of RaSP $\Delta\Delta G$ values for (blue) benign and (tan) pathogenic variants extracted from the ClinVar database (***Landrum et al., 2018***). We observe that the median RaSP $\Delta\Delta G$ value is higher for pathogenic variants compared to benign variants. (B) Distribution of RaSP $\Delta\Delta G$ values for variants with different allele frequencies (AF) extracted from the gnomAD database (***Karczewski et al., 2020***). We observe a gradual shift in the median RaSP $\Delta\Delta G$ going from common variants (AF> 10^{-2}) towards rarer ones (AF< 10^{-4}).

Protein	Uniprot	PDB	Chain	Residues	Computation	CPU/GPU-time [s]	
						RaSP	Rosetta
ELOB	Q15370	4AJY	B	105	Pre-processing	7	25,620
					$\Delta\Delta G$ full protein	41	3,685,020
					$\Delta\Delta G$ residue avg.	0.4	35,095.4
					Total	48	3,710,640
GCK	P35557	4DCH	A	434	Pre-processing	11	274,020
					$\Delta\Delta G$ full protein	173	38,808,780
					$\Delta\Delta G$ residue avg.	0.4	89,421.2
					Total	183	39,082,800
F8	P00451	2R7E	A	693	Pre-processing	20	617,220
					$\Delta\Delta G$ full protein	270	62,383,800
					$\Delta\Delta G$ residue avg.	0.4	90,019.9
					Total	290	63,001,020

Table 2. Run-time comparison of RaSP model versus Rosetta for three test proteins ELOB, GCK and F8. The RaSP model is in total $\sim 8 \cdot 10^4$–$2 \cdot 10^5$ times faster than Rosetta. RaSP computations were made on a single NVIDIA V100 16 GB GPU and Rosetta computations were made on a server using 64 2.6 GHz AMD Opteron 6380 CPUs (total CPU seconds reported). In practice, the wall-clock time for Rosetta calculations can be decreased by parallelizing the $\Delta\Delta G$ calculations over multiple CPUs.

2011; ***Martelli et al., 2016***; ***Stein et al., 2019***) we find that loss of protein stability is likely to be the underlying cause for loss of function for a large fraction of pathogenic variants.

Discussion

Here, we have presented a novel deep-learning-based model for making rapid predictions of protein stability changes using a combination of supervised and self-supervised methods. Our model uses the latent-space representation of a 3D convolutional neural network for protein structures as input to a supervised model to predict $\Delta\Delta G$. Our results show that the RaSP model performs on-par with the Rosetta protocol that we used as target for our optimization, but at a speed that is several orders of magnitude faster. We analysed RaSP's sensitivity towards using predicted protein structures, and applied it to generate a unique and large set of stability predictions for 8.8 million variants of 1,381 human proteins. As an example of the kinds of applications that RaSP may enable, we analysed the role of protein stability for genetic diseases, and hope that this data and the RaSP model will be a broadly useful tool to study the effects of amino acid changes on protein stability.

Methods

A convolutional neural network for protein structures

We use a 3D convolutional neural network as a model to represent protein structures (***Boomsma and Frellsen, 2017***). During training, the model is tasked with predicting the type of an amino acid given its 3D environment. Specifically, for each residue in the

polypeptide chain, the model aims to predict the residue type based on the surrounding atomistic environment encoded as a $(18\text{Å})^3$ cube (voxel) of the structure centered on the target residue to be predicted. Each voxel has five channels corresponding to the detection of C, N, O, H, and S atoms. To avoid overfitting, we apply Gaussian blurring to the input voxel before the convolutional layers. The model consists of a series of three convolutional layers, connected using leaky ReLU activation (***He et al., 2015***), max pooling (***Riesenhuber and Poggio, 1999***) and batch normalization functions (***Ioffe and Szegedy, 2015***). After the convolutional layers, the output is flattened and the dimensionality is reduced by two fully connected layers with 100 and 20 nodes, respectively. Model predictions are optimized by minimizing the cross-entropy between the predicted 20-length vector and the one-hot encoded amino acid label.

We trained the above model on a data set of 2,315 high resolution protein structures obtained using the PISCES server with maximal sequence identity set to 30% (***Wang and Dunbrack, 2003***). All structures were pre-processed using the Reduce program (***Word et al., 1999***) to add missing hydrogen atoms and OpenMM PDBFixer (***Eastman et al., 2013***) to correct for common PDB errors. Out of the total 2,315 proteins, 2,083 proteins were used for training while 232 were withheld for validation. Optimization was performed using Adam (***Kingma and Ba, 2014***) with a learning rate of $3 \cdot 10^{-4}$ and a batch size of 100 residues. After training, the representation model achieves a wild-type amino acid classification accuracy of 63% on a validation set (Fig. S6).

Downstream model architecture and training

We used the convolutional neural network described above to represent protein structures when predicting protein stability changes. Specifically, for each variant, the downstream model takes as input (i) a flattened 100-dimensional representation of the atomistic environment extracted for the wild-type structure using the self-supervised model, (ii) one-hot encoded labels of the wild type and variant amino acid, and (iii) the amino acid frequencies of the wild type and variant amino acid in the PISCES set of proteins. These inputs are concatenated and passed through three fully connected layers with 128, 64 and 16 nodes, respectively, resulting in a scalar value. During training, this value is passed through a sigmoid function corresponding to the transformation of the target data described below. Between each layer, we apply batch normalization (***Ioffe and Szegedy, 2015***) and leaky ReLU activation (***He et al., 2015***). We train the model by minimizing the mean absolute error between the predicted stability values and Rosetta $\Delta\Delta G$ values, after being transformed using a switching function (see below).

We trained the downstream model using $\Delta\Delta G$ values from saturation mutagenesis of 35 training proteins and 10 validation proteins, and tested using 10 different proteins. Before training, we transformed all Rosetta $\Delta\Delta G$ values using a switch (Fermi) function to focus model training on the $\Delta\Delta G$ range from -1 to 7, as this is where Rosetta is expected to be most accurate (***Kellogg et al., 2011***), where most experimental values lie (***Kumar et al., 2006***; ***Ó Conchúir et al., 2015***; ***Nisthal et al., 2019***), and covering an important range for detecting missense variants that cause disease via loss of protein stability and abundance (***Stein et al., 2019***; ***Cagiada et al., 2021***; ***Høie et al., 2022***). The Fermi function was defined as:

$$F(\Delta\Delta G) = \frac{1}{1 + e^{-\beta(\Delta\Delta G - \alpha)}} \tag{1}$$

with $\beta = 0.4$ and $\alpha = 3.0$. Furthermore, stability changes for (disulfide-bonded) cystine

residues were removed from the training and validation set as they are not predicted well using the Rosetta protocol. Optimization was performed using Adam (***Kingma and Ba, 2014***) with a learning rate of $5 \cdot 10^{-4}$ and a batch size of 40 variants.

Rosetta protocol

We used Rosetta (GitHub SHA1 99d33ec59ce9fcecc5e4f3800c778a54afdf8504) and the the Cartesian $\Delta\Delta G$ protocol (***Park et al., 2016***) to predict $\Delta\Delta G$ values. Values from Rosetta values were divided by 2.9 to convert to a scale corresponding to kcal/mol (***Park et al., 2016***).

Selection of proteins and structures of for large-scale analysis

Our selection of experimental structures was based on 5,557 human proteins annotated with cytosolic location. To make this list, we extracted UniProt identifiers from the human genome assembly GRCh38.p13 filtered for the Gene Ontology (***Ashburner et al., 2000***) term 'cytosol' (GO:0005829). We aligned the sequences of all chains of all PDB entries in the SIFTS map (***Dana et al., 2019***) (June 2021) to the UniProt sequences for the cytosolic proteins and calculated how much the protein structure in each PDB file covered the UniProt entries (explicitly considering mismatches, indels, residues that are not resolved in the structure, and modified residues). Based on these features, we calculated a score for each protein structure and the highest scoring structure was selected to represent a PDB entry. When calculating the score, we gave the highest priority to the coverage of the UniProt sequence and the experimental method. Specifically, given that RaSP was initially developed and benchmarked using crystal structures, we prioritized methods in the following order: (i) X-ray, (ii) cryo-electron microscopy, (iii) NMR. For our final selection, we chose 1,381 structures from the above selection, where the structure was of high quality in a canonical PDB format and where at least 50% of the UniProt sequence was covered by the structure.

Selection of ClinVar and gnomAD variants

The extraction of variants from ClinVar and gnomAD is based on ***Tiemann et al.*** (***2022***). Briefly, we extracted data from gnomAD using an in-house database (scripts available at: https://github.com/KULL-Centre/PRISM/tree/main/software/make_prism_files, release-tag v0.1.1). This was constructed using exome data from gnomAD v2 and whole-genome data from gnomAD v3, selecting exome GRCh38 liftover for v2 and whole-genome files for v3. Allele frequencies were computed as the sum of all DNA variants leading to the same protein-level variant. Similarly, ClinVar data were extracted from an in-house database constructed using the NCBI data source: https://ftp.ncbi.nlm.nih.gov/pub/clinvar/tab_delimited/variant_summary.txt.gz (May 2021). For our analysis, only single nucleotide variants with a mapping to GRCh38 and with a rating of at least one star was used. We did not find any variants in gnomAD or ClinVar for 15 of the 1,381 proteins that we calculated $\Delta\Delta G$ for; the data shown in Fig. 4 thus refers only to 1,366 proteins.

Data availability

Scripts and data to repeat our analyses are available via: https://github.com/KULL-Centre/papers/tree/master/2022/ML-ddG-Blaabjerg-et-al.

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