Quantitative Missense Variant Effect Prediction Using Large-Scale Mutagenesis Data

Graphical Abstract

Collect and annotate mutagenesis datasets

Train predictive regression models

Predict new mutations

Highlights

- Large-scale, quantitative mutagenesis data offer a novel source of training data
- Envision outperforms other missense variant effect predictors on independent data
- More mutagenesis data will improve Envision’s predictive performance
- Envision predictions are available for download: https://envision.gs.washington.edu

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In Brief
We present Envision, an accurate predictor of protein variant molecular effect, trained using large-scale experimental mutagenesis data.
Quantitative Missense Variant Effect Prediction Using Large-Scale Mutagenesis Data

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SUMMARY

Large datasets describing the quantitative effects of mutations on protein function are becoming increasingly available. Here, we leverage these datasets to develop Envision, which predicts the magnitude of a missense variant’s molecular effect. Envision combines 21,026 variant effect measurements from nine large-scale experimental mutagenesis datasets, a hitherto untapped training resource, with a supervised, stochastic gradient boosting learning algorithm. Envision outperforms other missense variant effect predictors both on large-scale mutagenesis data and on an independent test dataset comprising 2,312 TP53 variants whose effects were measured using a low-throughput approach. This dataset was never used for hyperparameter tuning or model training and thus serves as an independent validation set. Envision prediction accuracy is also more consistent across amino acids than other predictors. Finally, we demonstrate that Envision’s performance improves as more large-scale mutagenesis data are incorporated. We precompute Envision predictions for every possible single amino acid variant in human, mouse, frog, zebrafish, fruit fly, worm, and yeast proteomes (https://envision.gs.washington.edu/).

INTRODUCTION

Mutations have the power to reshape protein structure, stability, or activity and can have drastic effects on evolutionary fitness, protein function, and human health. For example, mutations were used to improve the pharmacokinetic and pharmacodynamic properties of insulin (Vigneri et al., 2010). Moreover, a recent survey of genetic variation in humans revealed that each individual harbors ~50 private missense variants, most of which are of unknown effect (Karczewski et al., 2017; Zou et al., 2016). This example highlights how DNA sequencing advances have facilitated detection of genetic variation. However, in both laboratory and clinical settings, determining the impact of a missense variant on a protein’s function remains a challenge (MacArthur et al., 2014).

Experiments can reveal a variant’s molecular effect, and recent advances in multiplex assays have enabled the assessment of large numbers of variants (Fowler and Fields, 2014; Gasperini et al., 2016). However, we are far from having a comprehensive atlas of missense variant effects in the human proteome, and such an atlas is a distant goal for model organisms. Thus, variant effect predictors such as PolyPhen2 (Adzhubei et al., 2010), SIFT (Sim et al., 2012), SNAP2 (Hecht et al., 2015), Evolutionary Action (Katsonis and Lichtarge, 2014), CADD (Kircher et al., 2014), and a host of others (Tang and Thomas, 2016) will continue to be widely used to predict missense variant effects. Some predictors are products of sophisticated supervised machine learning algorithms, and are developed using features and training data that make them suited for a particular type of prediction problem. For instance, the PolyPhen2 HumDiv model is a support vector machine trained on thousands of human Mendelian disease-associated and neutral variants, and is thus optimized to predict clinical variant effects (Adzhubei et al., 2010). SNP2, an ensemble of neural network models, is trained on human pathogenic and neutral variants as well as variants that affect molecular function (Hecht et al., 2015). Given the breadth of training data, SNP2 predictions encompass both the clinical and molecular effects of missense variants. Conversely, SIFT and Evolutionary Action are not products of machine learning but instead rely on evolutionary patterns to predict variant effects. Despite their simplicity, SIFT and Evolutionary Action perform similarly to PolyPhen2 and SNAP2 (Katsonis and Lichtarge, 2014), which highlights the importance of evolutionary information to successful variant effect prediction. A recently described unsupervised method, EVmutation, leverages evolutionary signatures of epistasis to predict variant effects, and has demonstrated enhanced accuracy over SIFT and PolyPhen2 for both molecular and clinical effect prediction (Hopf et al., 2017). These tools are all used to prioritize variants in clinical and laboratory settings.

Current predictors face two major limitations. First, most are optimized to predict categorical variant effects (e.g., damaging versus benign), and cannot accurately predict effect magnitude. This limitation arises primarily from the structure of variant effect databases used to train predictors. For example, the Human Gene Mutation Database (Stenson et al., 2012), Online Mendelian Inheritance of Man (Amberger et al., 2015), and ClinVar (Landrum et al., 2013) all categorize variants as clinically deleterious or benign. Swiss-Prot and the Protein Mutant Database contain categorical measures of variant effects in laboratory
assays. Second, most predictors focus on predicting the clinical effect of human variants rather than the molecular effects on protein function (Adzhubei et al., 2010; Sim et al., 2012). However, the relationship between molecular effect and clinical effect is complex, and most predictors do not deal well with this complexity. For example, both gain- and loss-of-function variants of BRAF can be pathogenic (Rodriguez-Viciana et al., 2006; Wan et al., 2004). Variants of PTEN variants can drive carcinogenesis when they occur somatically, or can cause autism or a tumor syndrome when they occur in the germline (Mester and Eng, 2013). Thus, we suggest that accurate clinical effect prediction should start with accurate, quantitative predictions of molecular effect whose subsequent interpretation is guided by specific knowledge about gene-disease associations.

Here, we address the need for an accurate, quantitative predictor of molecular effect by leveraging deep mutational scanning data. In a deep mutational scan, selection for protein function among a library of nearly all possible single amino acid variants of a protein is coupled to high-throughput DNA sequencing (Fowler and Fields, 2014; Fowler et al., 2014). Sequencing reveals how each variant’s frequency changes during selection, yielding quantitative scores that describe the functional effect of each variant in the library. The resulting large-scale mutagenesis datasets have a distinct advantage over traditionally used variant effect predictor training datasets such as HumDiv/HumVar, HGMD, and the Protein Mutant Database. Traditional datasets contain a large number of proteins, each with a median of four to six variant effect measurements. A large-scale mutagenesis dataset contains deep and unbiased information, capturing the effects of most variants at every position in a protein. We hypothesize that large-scale mutagenesis datasets contain informative and generalizable patterns that can be used to predict variant effects in disparate proteins.

Here, we use the molecular effects of 21,026 variants of eight proteins, determined through deep mutational scans, to train Envision, a decision tree ensemble-based quantitative variant effect predictor. Envision uses a stochastic gradient boosting learning algorithm, which excels at analyzing nonlinear interactions between features and has performed well in a myriad of regression tasks (Friedman, 2002). To maximize Envision’s generalizability, proteins in the Envision training set have disparate structures and functions, and are drawn from diverse organisms. We demonstrate the generality of Envision’s predictions by iteratively training models that exclude a single-protein dataset and then comparing the resulting model’s predictions with the observed variant effects for the excluded protein. We also assess performance using independent variant effect data that were not generated by deep mutational scanning but included in Envision’s training. Envision’s predictions are generally more accurate than other state-of-the-art predictors. Envision’s prediction accuracy is also consistent across different amino acids, unlike other predictors that perform well on some amino acids and poorly on others. We precomputed Envision predictions for all possible single amino acid variants of proteins in the human, mouse, fruit fly, zebrafish, yeast, and yeast proteomes. We provide a web-based tool allowing users to visualize and explore predicted protein sequence-function maps, which can be used to prioritize variants. Envision is available at https://envision.gs.washington.edu.

RESULTS
Data Collection and Curation
We collected previously published, large-scale mutagenesis datasets with quantitative measures of variant effect on protein function. Exploratory analysis led to the following inclusion criteria: (1) the experiment must have measured single amino acid variant effects, rather than averaging across different genetic backgrounds; (2) the experiment must have been on a natural protein instead of a designed protein; and (3) the experiment must have quantitated effects for at least ~50% of all possible variants of the mutagenized region. Ultimately, deep mutational scans of ten proteins from 12 studies comprising 28,545 single amino acid variant effects met these criteria (Figure 1A and Table S1). Variant coverage ranged from ~50% for the Ube4b domain of murine E3 ligase to 100% for the immunoglobulin G-binding domain of influenza protein G and the PDZ domain of human PSD-95 (Figure 1B). Variant coverage depended on experimental details such as the protocol used for library generation (e.g., doped oligomer [Matteucci and Heyneker, 1983] versus site saturation mutagenesis [Jain and Varadarajan, 2014]), the number of clones generated, and the sequencing depth. The proteins in the dataset were distinct, coming from different organisms, having different structures, and having functions ranging from catalysis to peptide binding (Table S1). To make datasets comparable, we normalized variant effect scores in each dataset such that variants that were more active than wild-type had a variant effect score greater than 1, wild-type-like variants had a score of 1, and variants that were less active than wild-type had a score less than 1 (Figures S1 and 1C).

Next, we annotated each variant with 27 biological, structural, and physicochemical features. The biological features captured evolutionary constraints using both site-specific and co-varying conservation metrics. The structural features include local density and solvent accessibility, while the physicochemical features describe properties of amino acids, such as polarity and size. Physicochemical and biological features were available for nearly all variants, but structural features were not (Figure 1D and Table S2).

Predicting Quantitative Variant Effects
We first tested whether a stochastic gradient boosting regression algorithm could model the relationships between our 27 features and quantitative variant effect scores for each protein individually. To train each single-protein model, we tuned hyperparameters, such as the number of decision trees in the ensemble and tree depth, using 10-fold cross-validation. After hyperparameter tuning we reserved 20% of mutations for testing, allowing us to estimate the generality of each model to unseen variants. Nine of the 12 models performed well (median Pearson’s R = 0.83, Spearman’s ρ = 0.80, Figure 2A), while three, the BRCA1 RING domain BARD binding, BRCA1 RING domain E3 activity, and E4B ubiquitin ligase models, performed poorly (median R = 0.22, ρ = 0.35).

Experimental noise cannot account for these models’ poor performance, since the correlation of model predictions with the training and testing data is much lower than the correlation between replicate experiments (Table S1). We hypothesized
that poor performance arose because correlations between the features and variant effect scores were low (Figure S2). Low correlation might occur because the assays did not test every function of these proteins. For instance, BRCA1 RING domain variants were assayed for E3 ligase activity and BARD binding. However, BRCA1 has many functions and interacts with >25 other proteins (Deng and Brodie, 2000; Kerrien et al., 2012). Another possibility is that these two datasets were missing some structural features. However, the YAP65 WW domain dataset, missing the same features, resulted in an accurate model. Thus, we could not identify the cause of poor performance in the BRCA1 RING domain and E4B ubiquitin ligase models. We excluded these three datasets from subsequent analyses.

For most proteins, our feature set and learning procedure generated accurate models of variant effect. Beyond validating our approach, these single-protein models enabled us to complete each large-scale mutagenesis dataset by predicting missing variant effect scores (Table S3). For example, we used the Pab1 model ($R = 0.86$; $r = 0.79$) to predict $20\%$ of scores that were missing, completing the Pab1 dataset (Figure 2B).

Next, we trained a global model with the 21,026 empirically derived variant effect scores in the nine large-scale mutagenesis datasets. We tuned hyperparameters using a leave-one-protein-out (LOPO) approach designed to avoid protein-specific overtraining (Figure S3 and Table S4). Once hyperparameters were tuned, we trained Envision with all available data, except for a random $5\%$ of variant effect scores that we withheld for testing and to assess overfitting. Training and testing data root-mean-squared errors were similar at each model training iteration, indicating that the model is not overfitted to the training data (Figure S4). Envision predicted the training data well ($R = 0.79$, $r = 0.78$; Figure 3A).
and our results confirm that they should not be used to do so. and SIFT were not designed to predict variant effect magnitude, scores (R 0.46 versus 0.03; effects. The WW domain LOPO model also outperformed SIFT also not expected to capture the magnitude of variant molecular
tions and scores for human proteins. SIFT scores represent the scaled probability of a missense variant being tolerated, and are

To evaluate Envision’s performance, we employed a jack-knife LOPO approach. Here, we repeated the training procedure described above, leaving one protein completely out of the hyperparameter tuning and model training process. We then used the resulting model to predict variant effect scores for the left-out protein and determined performance. We repeated this procedure for all nine proteins. Variant effect scores for left-out proteins were predicted with Pearson’s R ranging from 0.38 to 0.69 and Spearman’s \( r \) ranging from 0.30 to 0.74 (Figures 3B and S5). To determine the effect of our variant effect score normalization scheme on model training and performance, we compared LOPO models trained using either normalized or non-normalized variant effect scores. Models trained using normalized data predicted variant effect scores for the left-out protein better than models trained using non-normalized data (median R = 0.56 versus 0.39, median \( r \) = 0.51 versus 0.35; Figure S6). This result highlights the utility of our normalization scheme.

Next, we compared our LOPO models’ performance with that of other predictors. PolyPhen2 is trained to predict the categorical clinical effect of variants, but also generates a numerical score. This score is the naive Bayes posterior probability that a variant is damaging, and, although quantitative, it is not designed to predict the magnitude of a variant’s molecular effect. As expected, for the only human protein in our dataset, YAP65 WW domain, our LOPO model outperformed PolyPhen2 when predicting WW domain variant effect scores (\( R = 0.46 \) versus 0.17; \( r = 0.36 \) versus 0.19). Like PolyPhen2, SIFT also generates categorical predictions and scores for human proteins. SIFT scores represent the scaled probability of a missense variant being tolerated, and are also not expected to capture the magnitude of variant molecular effects. The WW domain LOPO model also outperformed SIFT scores (\( R = 0.46 \) versus 0.03; \( r = 0.36 \) versus 0.04). PolyPhen2 and SIFT were not designed to predict variant effect magnitude, and our results confirm that they should not be used to do so.

Assessing Envision’s Performance

To evaluate Envision’s performance, we employed a jack-knife LOPO approach. Here, we repeated the training procedure described above, leaving one protein completely out of the hyperparameter tuning and model training process. We then used the resulting model to predict variant effect scores for the left-out protein and determined performance. We repeated this procedure for all nine proteins. Variant effect scores for left-out proteins were predicted with Pearson’s R ranging from 0.38 to 0.69 and Spearman’s \( r \) ranging from 0.30 to 0.74 (Figures 3B and S5). To determine the effect of our variant effect score normalization scheme on model training and performance, we compared LOPO models trained using either normalized or non-normalized variant effect scores. Models trained using normalized data predicted variant effect scores for the left-out protein better than models trained using non-normalized data (median R = 0.56 versus 0.39, median \( r \) = 0.51 versus 0.35; Figure S6). This result highlights the utility of our normalization scheme.

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SNAP2, EVmutation, and Evolutionary Action were developed to predict variant effect magnitude (Hecht et al., 2015; Hopf et al., 2017; Katsonis and Lichtarge, 2014). Evolutionary Action scores could not be obtained by batch query, preventing us from including them in our analysis. SNAP2 predicted variant effect scores much better than PolyPhen2 or SIFT, but not as well as our LOPO models, which outperformed SNAP2 on seven of nine datasets (median R = 0.56 versus 0.44; Figure 3C). EVmutation predicts variant effect magnitude using either an epistatic or an independent conservation-based unsupervised statistical model. Our LOPO models outperformed EVmutation’s epistatic model on six out of nine datasets (median R = 0.56 versus 0.47) and EVmutation’s independent model on seven of nine (mean R = 0.56 versus 0.48; Figure 3C). An equivalent analysis using Spearman’s \( r \) revealed similar results (Figure S7). Across all datasets, our LOPO models’ predictions are 4%, 14%, and 21% more correlated with the observed variant effect scores than predictions from EVmutation-epistatic, EVmutation-independent, and SNAP2, respectively.

Next, we analyzed which factors led to our improved performance. Envision’s features are similar to those used by SNAP2 and PolyPhen2, so the improvement we observed is not likely due to feature choice. Instead, we hypothesized that our use of deep mutational scanning data and our cross-validation approach, designed to yield a generalizable model, are the two attributes that led to improved performance. The lack of a large database of quantitative variant effects measured by means other than deep mutational scanning made it impossible to evaluate the performance advantage conferred by using deep mutational scanning data. However, we quantified the impact of our cross-validation approach by comparing the performance of models trained using standard 10-fold cross-validation with models trained using our LOPO scheme. We found our LOPO approach improved performance by ~10%–20% over all protein datasets compared with 10-fold cross-validation (median R = 0.56 versus 0.45, \( r = 0.50 \) versus 0.45; Figure S8). We
Figure 3. Envision Outperforms Other Quantitative Variant Effect Predictors

(A) A hexagonal bin plot shows the correlation between predicted and observed variant effect scores for all the large-scale mutagenesis data used to train Envision (Pearson’s R = 0.79).

(B) To evaluate performance on data not used in training, we retrained models excluding each one of the nine proteins (see Figures S3 and S4 for cross-validation scheme and training performance). A radar plot shows the correlation (Pearson’s R) between predicted and observed variant effect scores when the indicated protein was left out (see Figure S5 for scatterplots).
suggest that our LOPO approach, designed to yield generalizable models, was especially important given that our training dataset contained relatively few proteins.

Our LOPO analysis demonstrated that Envision provided improved quantitative predictions of variant effects measured using deep mutational scanning. However, Envision’s performance advantage might have arisen because it learned deep mutational scanning-specific patterns in the data. To ensure that Envision was not overfitted to deep mutational scanning data, we obtained a TP53 tumor suppressor mutagenesis dataset whereby the effects of 2,312 variants on TP53 transactivation were measured individually using a fluorescent reporter (Kato et al., 2003). We predicted these TP53 variant effect scores using Envision, which was trained on all nine large-scale mutagenesis datasets. The TP53 data were never used, directly or indirectly, in the training procedure. Despite the fact that the TP53 dataset was not acquired using deep mutational scanning, Envision predicted the TP53 variant effect scores well (R = 0.58, p = 0.53; Figures 3C and 3D). Importantly, Envision outperformed SNAP2 (R = 0.53; p = 0.50), whose training dataset included the effects of ~400 human TP53 mutations, and EVmutation (epistatic R = 0.45, p = 0.49; independent R = 0.49, p = 0.52; Figure 3C). Thus, Envision learned patterns of the molecular effects of variants that do not depend on the measurement method.

Next, we sought to determine whether Envision performance depended on the identity of either the mutant or wild-type amino acid. We evaluated performance on the TP53 dataset to enable comparison with EVmutation and SNAP2. We found that Envision prediction performance did not depend much on the identity of the mutant amino acid (Figures 3E and S9A). However, EVmutation and SNAP2 showed large biases in performance. For instance, EVmutation predicted mutations to phenylalanine with high accuracy (R = 0.69, p = 0.70), but predicted mutations to leucine with low accuracy (R = 0.24, p = 0.33). SNAP2 performance was also biased in favor of mutations to tryptophan and methionine and against mutations to alanine. These biases were also apparent for the wild-type amino acid, where EVmutation predicted mutations from wild-type cysteine well (R = 0.82, p = 0.71) and wild-type aspartic acid poorly (R = 0.02, p = 0.05; Figure S9B). Consequently, in addition to greater overall accuracy, Envision performance was more consistent.

Finally, we assessed the utility of Envision scores for clinical effect prediction, evaluating performance by constructing receiver-operating characteristic (ROC) curves using variants annotated as either pathogenic or benign in ClinVar. Envision predictions were better than random guessing (area under ROC curve [AUROC] = 0.72), but not as good as PolyPhen2, CADD, and SIFT (AUROC = 0.86, 0.85, and 0.84, respectively; Figure S10). This result is not surprising because Envision was not designed or optimized for this task, and because comparison of predictor performance on clinical data is difficult given that many predictors are trained on or optimized to predict these data (Grimm et al., 2015). Furthermore, the relationship between the magnitude of a variant’s molecular effect and disease phenotype is likely to be different for each disease-associated protein. For example, a weakly damaging variant in some proteins may be sufficient to cause disease, whereas only strongly damaging variants lead to disease in other proteins. Finally, we note that the rate at which training datasets grow in the coming years may be much greater for deep mutational scans than for clinical variants.

**Feature Importance and Future Improvements**

Features known to be predictive of variant effects, including solvent accessibility and evolutionary conservation, were the most highly represented in the Envision decision tree ensemble (Figure 4A and Table S5) (Kumar et al., 2009; Saunders and Baker, 2002). However, unlike for other feature-driven predictors (Adzhubei et al., 2010; Hecht et al., 2015), we found that the mutant amino acid identity was informative. This amino acid identity effect was largely driven by proline. Proline variants are generally disruptive of protein function and, indeed, proline variants were the most damaging substitutions in the large-scale mutagenesis datasets (proline mean effect score = 0.60 versus all-α-amino-acid mean = 0.81; paired t test, p << 0.001, n = 8; Figure S11). Envision predicted the effects of proline variants about as accurately as the effects of other variants (Figure S12). Thus, rather than simply predicting that all proline variants were strongly damaging, Envision predicted the degree to which proline variants maintain or disrupt function.

Structural and evolutionary features are important for Envision’s predictions but are not always available. Thus, we quantified predictive performance when these features were missing by masking them for each of the nine LOPO models when they predicted the left-out protein’s variant effect scores. As expected, models performed worse without structural features. For example, the β-lactamase LOPO model predicted β-lactamase variant effect scores 15% worse when structural features were masked (R = 0.69 versus 0.59; Figure S13). Similarly, the β-lactamase LOPO model predicted β-lactamase variant effect scores 13% worse when evolutionary features were masked (R = 0.69 versus 0.60). Across the nine LOPO models, we found that masking structural features degraded performance by 39% and masking evolutionary features degraded performance by 18%. Thus, we strongly encourage users to consider feature completeness when using Envision’s predictions. Feature information is available, along with predictions, on the Envision website. We note that all feature-driven predictors suffer when key features are unavailable.

Finally, we determined how the number of proteins in our training dataset affected Envision performance. We trained (C) We also compared the leave-one-protein-out (LOPO) models with SNAP2 (left panel), EVmutation-epistatic (middle panel), and EVmutation-independent (right panel). The log2 ratio of each LOPO model’s Pearson’s R to another predictor Pearson’s R on the left-out data is shown. Hashed bars on the right indicate relative performance on a set of 2,312 TP53 transactivation activity scores measured in a low-throughput assay and not used in training (see Figure S7 for raw comparison).

(D) A hexagonal bin plot shows the correlation between Envision predictions and TP53 activity scores (Pearson’s R = 0.58).

(E) A violin plot illustrates the distribution of Pearson’s correlation coefficients for variant effect scores and Envision, SNAP2, and EVmutation predictions for different mutant amino acids. The dashed horizontal line indicates the median Pearson’s correlation coefficients for each predictor (see Figures S9A and S9B for heatmap of correlations).
versions of Envision with different numbers of proteins and tested it on the left-out proteins. We found that model performance increased as more proteins were used in training, suggesting that accumulation of more data will improve Envision's predictive performance (Figure 4B).

Availability of Envision Predictions
Envision predictions are available for proteins from seven commonly studied organisms: human (n = 20,130), mouse (n = 16,836), fruit fly (n = 3,375), clawed frog (n = 1,704), zebrafish (n = 2,982), worm (n = 3,802), and yeast (n = 8,322). We provide predictions for all 19 alternative amino acids at each position, with batch query and download options available. Along with predictions, features are also available for download.

DISCUSSION

We developed Envision, the first variant molecular effect predictor trained on large-scale mutagenesis data. Envision accurately predicts variant effects in large-scale mutagenesis data withheld from training as well as variant effects from low-throughput experiments. Overall, Envision outperforms other quantitative predictors such as SNAP2 and EVmutation in predicting experimentally measured molecular effects. In particular, the quality of Envision predictions is relatively uniform across different amino acid substitutions, whereas other predictors' accuracy is driven by high performance on some substitutions and poor performance on others. The promise of using large-scale mutagenesis data to develop variant effect predictors is highlighted by the fact that Envision was trained from deep mutational data on only nine proteins, but can outperform established methods that are trained using sparse mutational data on thousands of proteins. As more large-scale mutagenesis data become available, Envision will continue to improve.

Envision also has limitations. Envision’s predictions are provided as quantitative scores that range from ~0 to ~1, where scores less than 1 are damaging in comparison with wild-type. Envision can predict the scores of strongly damaging and wild-type-like mutations well, but predicts mutations of intermediate effect less well (Figure S5). Envision also relies on structural and evolutionary features that are not available for every protein, and predictive performance degraded when these features were missing. Thus, while Envision predictions are available for millions of variants, we recommend caution when key features are missing. The Envision web tool reveals missing features for each prediction.

To train Envision, we employed large-scale mutagenesis data from two types of deep mutational scans. One type is based on a generalized selection for protein function whereas the other is based on selection for a specific protein function. Specific selections could fail to capture the effect of variants on other functions of the protein, such as binding to a different substrate or catalysis. Envision was trained using data from both generalized and specific deep mutational scans and did not distinguish between them. Therefore, Envision predicts generalized variant effects and does not distinguish between specific molecular effects such as enzymatic activity for one substrate or another, or binding versus catalysis. Collection of more large-scale mutagenesis data for the specific molecular effects of variants may enable the development of predictors that capture these specific functional effects.

We anticipate that Envision will be useful for identifying candidate variants that tune protein activity levels. Envision’s predictions of molecular effect may also be useful when the relationship between protein function and disease is clear. Furthermore, Envision will continue to improve as new datasets become available.
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SUPPLEMENTAL INFORMATION
Supplemental Information includes 13 figures and 5 tables and can be found with this article online at https://doi.org/10.1016/j.cels.2017.11.003.

AUTHOR CONTRIBUTIONS
The project was conceived and designed by V.E.G., R.J.H., J.L., J.S., and D.M.F. V.E.G. and R.J.H. performed data analysis and generated figures. All authors wrote the manuscript.

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REFERENCES


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KEY RESOURCES TABLE

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<td><a href="https://marks.hms.harvard.edu/evmutation/">https://marks.hms.harvard.edu/evmutation/</a></td>
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<td><a href="https://github.com/FowlerLab/Envision2017">https://github.com/FowlerLab/Envision2017</a></td>
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<td>Protein G (IgG domain)</td>
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CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Douglas M. Fowler (dfowler@uw.edu).

METHOD DETAILS

Training Data Collection
Published large-scale mutagenesis datasets were used as training data if they met several criteria. First, we required that at least 50% of all possible single amino acids were substituted at each mutagenized position. Thus, alanine and proline scans did not qualify for this study. Second, we only accepted mutational scans of native proteins assayed for native biological function. Third, we excluded scans in which the complete variant sequence was unknown. We also removed variants with more than one mutation. In total, we accepted twelve datasets comprising ~30,000 missense mutations. These scans were performed on proteins from different organisms: human, mouse, rat, S. cerevisiae, and bacteria (Table S1).
Normalization
Each large-scale mutagenesis dataset was generated using a distinct experimental assay, which resulted in different variant effect score distributions. To enable meaningful comparison between datasets, we normalized them. For each dataset, every variant effect score was normalized to the wild type score and then log2 transformed. Next, we subtract the median effect of synonymous variants, if available. Synonymous variants were unavailable for the PSD95 (Pdz3 domain), Protein G (IgG domain), UBE4B (U-box domain) and BRCA1 datasets, so we instead subtracted 0 from each score in those datasets. Lastly, we divided each score by the negative median score of the bottom 1% of mutations of each dataset and added one. Our normalization scheme is expressed as $S_{\text{normalized}} = (S_{\text{reported}} - S_{\text{median synonymous}}) / (S_{\text{median bottom 1%}}) + 1$, where $S$ signifies score. This normalization scheme results in variants that are more active than wild type having scores of greater than one, wild type-like variants having scores of one, and damaging variants having scores of less than one.

Variant Annotation
Mutations were annotated with three general types of descriptive annotations: evolutionary, biochemical and structural (Table S2). Several evolutionary features used in our model were obtained using the PolyPhen2 annotation pipeline (Sunyaev et al., 1999). We also derived a measure of average mutational covariance between a given position and all other positions in a multiple sequence alignment from EVfold (Hopf et al., 2017). To obtain structural information, we use DSSP (http://www.cmbi.ru.nl/dssp.html) (Kabsch and Sander, 1983) and PDB files from the Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) (Rose et al., 2011). Our biochemical annotations include measures of amino acid size, weight, volume, isoelectric point, and Grantham scores (Grantham, 1974).

Machine Learning
Stochastic gradient boosting is a method of machine learning that uses an ensemble of weak prediction models (e.g., decision trees) for classification or regression problems (Friedman, 2002). We constructed stochastic gradient boosting tree regression models using the GraphLab Create framework from Turi (https://turi.com/products/create/). Hyperparameters were optimized using a grid search. For each predictive model, we tuned six parameters in a stepwise fashion. First, we optimized for the number of decision trees in the ensemble. Next, we tuned the maximum depth of a decision tree and the minimum number of observations allowed in a terminal node of a tree. Then, we determined the value that the squared-loss must be reduced by in order to add an additional node to a tree. Finally, we identified the optimal proportion of variant effect scores and features used to train each tree. Once hyperparameters were tuned, we reduced the learning rate from 0.1 to 0.01 and increased the number of decision trees by five-fold. All tuned and trained models treat missing feature data as such, i.e., no imputing procedures were performed. Instead, during training, the algorithm uses variants with missing features to determine how feature missingness should handled by the model at each tree node (Chen and Guestrin, 2016).

Single Protein Models
To filter out datasets that are noisy or contain variant effects that cannot be explained by our evolutionary, structural or physicochemical features, we performed gradient boosting machine learning on a randomly selected 80% of variant effect scores from each protein dataset. This resulted in a model for each protein, which we used to predict the 20% of variant effect scores withheld from model training. Proteins whose specific models performed poorly on withheld data (Pearson’s R < 0.5) were excluded from the LOPO and global models.

Training Envision
Envision was trained using the same approach as our single protein models with an added leave-one-protein-out cross-validation procedure, where, at each round, a different protein was removed from the training set and used for validation (Figure S3). Thus, after each round of training, a model’s generality was tested on variant effect scores from a protein not used to train the model. This cross-validation procedure allowed us to test an array of hyperparameters to see which parameter sets yielded the most generalizable model. Here, model generality was determined by measuring the root mean squared error between model predictions and variant effect scores from a left-out protein. Once all hyperparameters were optimized (Table S4), we trained Envision with all available data except for a randomly selected 5% of which we excluded to evaluate model generality and ensure that the model was not overfitted. The resulting model was used to make all the Envision predictions available on our website.

Leave-one-protein-out (LOPO) Models
To estimate Envision’s performance on proteins not used in model training, we generated nine LOPO models. These models were trained using the same protocol as Envision, except that in each case a different protein was left completely out of the hyperparameter tuning and final model training procedures. These LOPO models were used to estimate Envision’s performance on proteins not included in the training set.

Downsampling Analysis
To evaluate the effect of additional training data on model performance, we trained models with 2, 4, 6 or 7 of the available nine protein datasets. Model training was performed as described in the Training Envision section above. Each model was used to predict
variant effects in proteins that were not used during the training phase. Confidence intervals were generated by repeated rounds of randomly selecting proteins to use in the training phase (n = 8).

QUANTIFICATION AND STATISTICAL ANALYSIS

The details of the statistical test we conduct, as well as definitions of center and correlation can be found in the main text. Criteria for inclusion of deep mutational scanning data sets are described in the Method Details section of the STAR Methods.

DATA AND SOFTWARE AVAILABILITY

All data and software in this study are freely available. The training data set and all code used to train the models and generate the figures presented in this manuscript are available at https://github.com/FowlerLab/Envision2017. Envision predictions, along with feature annotations, are available at https://envision.gs.washington.edu/.