

PREDICTING THE EFFECT OF MUTATIONS ON A GENOME-WIDE SCALE

by

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# **Abstract**

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# Chapter 1

## Introduction

Advances in DNA sequencing technology have drastically lowered the cost and improved the accuracy of high-throughput sequencing [1]. This has made exome and whole genome sequencing a viable and cost-effective tool in both the laboratory, where it permits the study of individual cells and cell populations at an unprecedented level of detail [2], and in the clinic, where it can assist in the diagnosis and treatment of pediatric diseases [3] and in the design of targeted therapies against cancer [4]. However, while there has been enormous growth in the amount of genomic data that is generated and the number of sequence variants that are discovered, interpreting this data to produce meaningful and actionable results remains a challenge.

*In vivo* and *in vitro* experimental techniques remain the gold standard for elucidating the effect of DNA sequence variants. However, evaluating experimentally the effect of all discovered variants is not feasible, both in terms of time and resources that would be required. Computational techniques have been developed to predict the effect of different variants and to prioritize them for experimental validation. Those techniques can be loosely divided into three categories: sequence-based tools, structure-based tools, and tools that combine both sequential and structural information.

Sequence-based tools rely on some form of a conservation score, describing the frequency with which a particular nucleotide or amino acid is found at the given position in domain-, protein- or genome-level alignments, in order to make their prediction [5, 6, 7, 8, 9, 10, 11]. Due to their speed and scalability, sequence-based tools are the de-facto standard for annotating newly discovered variants. However, they remain limited in their accuracy and the type of information that they can provide [12]. In particular, they only predict whether or not a particular mutation is likely to be deleterious, and provide no information as to *why* that mutation may be deleterious. This makes it difficult to act upon those predictions, for example by designing drugs that would curtail the effect of disease-causing mutations or would take advantage of mutations found in cancer.

Structure-based tools predict the effect of mutations on protein structure and / or function using features describing the three-dimensional structure of the protein. They range from accurate but computationally expensive alchemical free energy calculations, which involve modelling the structural transition from wildtype to mutant proteins and using different integration techniques to calculate the energy of the transition [13], to quicker but more approximate techniques, which use semi-empirical or statistical potentials and assume that the backbone of the protein remains fixed [14, 15, 16, 17]. In theory, structure-based tools should be able to offer more insight into the effect of missense mutations

than sequence-based tools, since the effect is caused by changes in protein structure and function and not by changes in DNA sequence. However, since existing structure-based tools require manual setup and a crystal structure of the protein being mutated, there has not been a systematic, genome-wide comparison of the performance of sequence- and structure-based tools in the analysis of mutations.

Several tools have been developed that attempt to combine sequence- and structure-based information in order to make more accurate predictions about the deleteriousness [18] and the structural impact [19, 20, 21] of mutations. These tools generally are “meta-predictors” which integrate the results of several sequence- and structure-based tools using machine learning algorithms trained on an appropriate dataset. ELASPIC, developed by Niklas Berliner *et al.*, is a particularly interesting example, because, while trained using homology models instead of crystal structures, it still reports state of the art accuracy in predicting the effect of mutations on protein stability and protein-protein interaction affinity [20]. With the growth in the number of crystal structures deposited in the Protein Data Bank [22], it is now possible to create homology models of proteins and protein-protein interactions with high coverage of an entire proteome [23]. This suggests that ELASPIC could be extended to work on a genome-wide scale, offering a way to examine the contribution that structural information could make to our analysis and interpretation of mutations.

The aim of this project was to extend ELASPIC so that it could predict the effect of mutations on protein stability and protein-protein interaction affinity on a genome-wide scale. In Chapter 2, we describe modifications that had to be made to ELASPIC and the underlying pipelines in order to make the genome-wide analysis of mutations possible. We also discuss the implementation of the ELASPIC web service, which allows ELASPIC to be run through a webserver in a scalable way. In Chapter 3, we describe the performance of ELASPIC on the training, validation and test datasets, and compare its performance to other sequence- and structure-based tools. In Chapter 4, we discuss the results of this work and propose several directions for future study.

## Chapter 2

# Implementation

### 2.1 Profs

ELASPIC uses a domain-based approach for creating homology models of query proteins, and therefore requires access to accurate domain definitions. The most widely-used source of protein domain definitions is Pfam [24]. However, since Pfam domains definitions are based entirely on protein sequence, they correlate poorly with the structural fold of the protein. Using Pfam domain definitions when making homology models tends to produce unstable models of fragmented and / or truncated domains, and this would compromise our subsequent analysis of the structural impact of mutations.

In order to improve the structural accuracy of Pfam domains, Andres Felipe Giraldo Forero developed a pipeline that uses structural alignments and a set of heuristics to modify Pfam domain definitions and make them better aligned with the tertiary structure of the protein, as defined by CATH [25]. He named this pipeline Profs, for Protein families. A schematic of this pipeline is presented in Figure 2.1, and an R package implementing the pipeline is available at <https://bitbucket.org/afgiraldofo/profs>. Profs domains have an advantage over Pfam domains in that they have been corrected and expanded to match the structural fold of the protein. They have an advantage over CATH domains in that they are backed by large, manually-seeded alignments, and can be easily detected in any protein sequence using Pfam HMMs.

We used Andres' pipeline to annotate with Profs domains all proteins in the UniProt database. The resulting table of Profs domain definitions is available for download from the ELASPIC website ( <http://elaspic.kimlab.org/static/download/>) and is included in the ELASPIC database (see **domain** and **domain\_contact** tables in Figure 2.6 and Table 2.1). The following sections describe the procedure used to generate tables of Profs domain definitions and Profs domain-domain interactions that are used by ELASPIC.

#### 2.1.1 Domains

We used Profs domain definitions, which had been calculated for all proteins in the PDB, to find Profs domains, and structural templates for those domains, for all proteins in UniProt (step 6 in Figure 2.1). To do this, we followed the same procedure that was used by the authors of Profs to annotate structures in the PDB that have Pfam domains but no CATH domains [26].

We started with Pfam domain definitions for all known protein sequences, which we download from



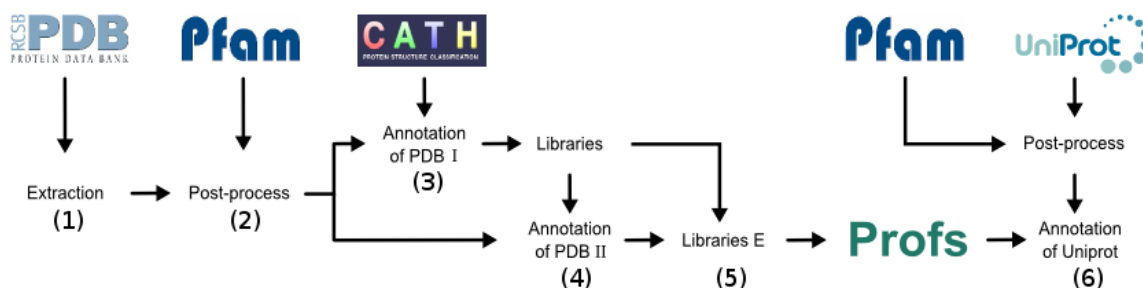


Figure 2.1: Flowchart illustrating the steps in the Profs pipeline (courtesy of Andres Felipe Giraldo Forero). **(1)** All structures in the PDB are parsed to extract protein sequences, and *hmmsearch* is ran to find Pfam domains in those sequences. **(2)** Pfam domains of proteins in the PDB are processed in order to join and / or remove overlapping and repeating domains. **(3)** Pfam domain definitions are altered in order to make them compatible with CATH definitions, for structures that have been annotated by CATH. **(4)** Pfam domain definitions are altered in order to make them compatible with CATH definitions, for structures that have not been annotated by CATH. This is done by performing pairwise alignments with structures that do have CATH annotations. **(5)** Libraries of Profs domain definitions, and Profs domain-domain interactions, are generated for all proteins in the PDB. **(6)** Libraries of Profs domain definitions, and Profs domain-domain interactions, are generated for all proteins in Uniprot.

the SIMAP website [27]. We mapped those protein sequences to Uniprot using the MD5 hash of each sequence, and we joined or removed overlapping and repeating domains using a mapping table supplied with the Profs R package. Next, we tried to find a Profs structural template for each Pfam domain by running *blastp* against libraries of Profs domains, which also are included in the Profs R package. If a suitable template was found, we proceeded to do iterative global alignments using Muscle [28] while expanding domain boundaries of the Pfam domains to match domain boundaries of the Profs templates. If two Pfam domains were expanded to occupy the same region in the protein, that region was divided in half and attached to the preceding and the succeeding domains.

The results of this analysis are stored in the **uniprot\_domain** and the **uniprot\_domain\_template** tables in the ELASPIC database (Figure 2.6). The **uniprot\_domain** table contains all Pfam domains and supradomains that are obtained after removing repeating and overlapping domains, as outlined above. The *pdbfam\_name* column contains the name of the Profs domain. The *alignment\_def* column contains either the original Pfam domain definitions or, in the case of supradomains, the merged domain definitions of multiple Pfam domains. The **uniprot\_domain\_template** table contains information describing the alignment of the Pfam domain or supradomain with the corresponding Profs structural template, for domains for which a suitable Profs template could be found. The *cath\_id* column identifies the Profs structural template that was selected, and the *domain\_def* column contains the corrected and expanded domain definitions.

### 2.1.2 Comparison with Pfam and Gene3D

In order to ascertain the validity of Profs domain definitions, we compared Profs, Pfam and Gene3D in terms of sequence coverage (Figure 2.2) and domain size (Figure 2.3).

We downloaded Pfam and Gene3D domain definitions for all human proteins from SIMAP [27], and we calculated Profs domain definitions following the pipeline described above. The analysis was

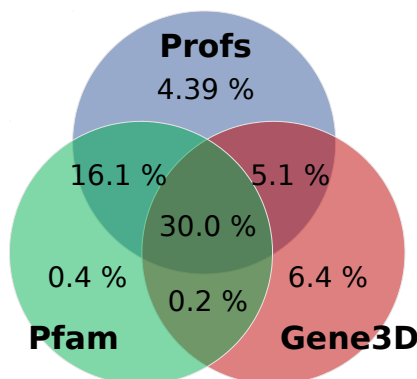


Figure 2.2: Venn diagram showing the overlap in domain definitions between Profs, Pfam, and Gene3D. Values represent the fraction of amino acids, of all human proteins in UniProt, which are covered by the particular domain or domains. A total of 18,828 human proteins and 10,868,810 amino acids were considered, after excluding proteins which had no predicted domains by any method. Profs has the highest coverage, with 55.7 % of amino acids being annotated by a Prof domain.

restricted to 18,828 human proteins from UniProt which are annotated with at least one Profs, Pfam or Gene3D domain.

In order to compare sequence coverage, we looked at the fraction of all protein sequences which are covered by each domain type (Figure 2.2). Overall, Profs has the highest sequence coverage, with 55.7 % of 10,868,810 amino acids in 18,828 proteins residing inside a Profs domain. Profs annotates  $\approx 9\%$  more amino acids than Pfam and  $\approx 14\%$  more amino acids than Gene3D, although the relatively low coverage by Gene3D is expected, as it can only detect domains which are represented in the PDB.

In order to compare domain size, we looked at the average number of domains per protein for each of the three methods (Figure 2.3). Profs has more proteins with only one domain per protein, while Pfam and Gene3D have more proteins with two or more domains per protein. This is consistent with Profs trying to join fragmented and repeating domains into consistent structural units. Gene3D does not detect domains in many proteins with Profs and Pfam domains, likely because those domains have not been crystallized.

The result of this analysis shows that, at least for human proteins, Profs achieves higher sequence coverage using fewer domains per protein than either Pfam or Gene3D. This makes Profs well-suited for the ELASPIC pipeline.

### 2.1.3 Domain interactions

We also created a table of domain-domain interactions for proteins that are known to interact and for which a homology model of the interaction can be created. We started by creating a comprehensive list of protein-protein interactions (PPIs), by taking the union of all PPIs listed in the HIPPIE database [29] and in the datasets hosted by the Harvard Center for Cancer Systems Biology (CCSB) [30]. The overlap in the PPIs obtained from each source is presented in Figure 2.4. We filtered those PPIs to select pairs of proteins where each protein has at least one domain with a structural template. This information is stored in the `uniprot_domain_pair` table in the ELASPIC database. For each of those

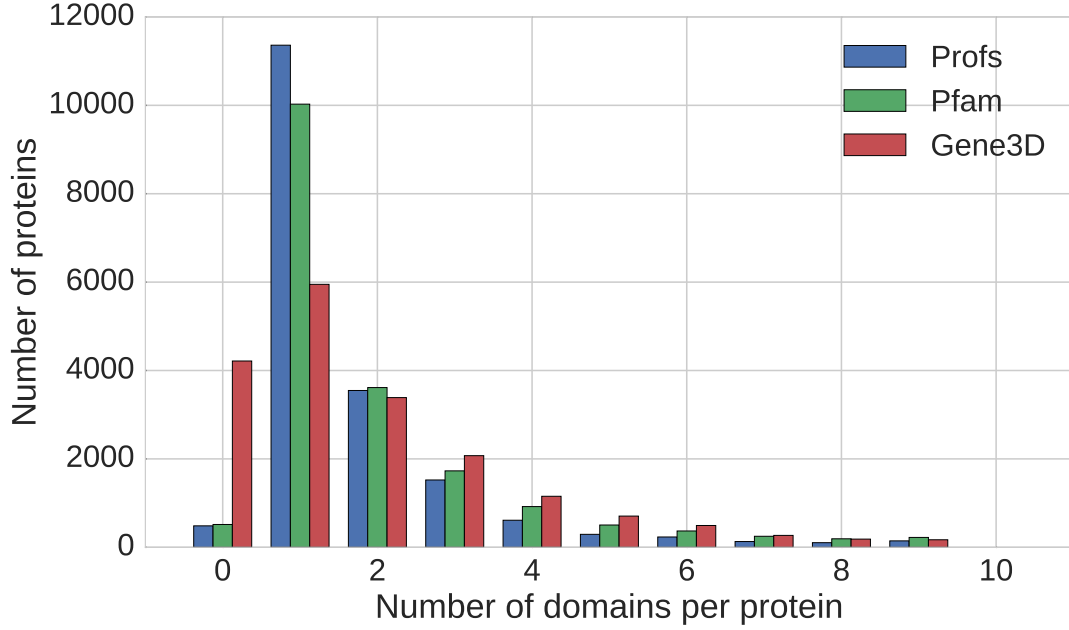


Figure 2.3: Average number of Profs, Pfam and Gene3D domains per protein, for all human proteins containing at least one domains. Profs tends to have fewer domains per protein then either Pfam or Gene3D, even though Profs domains have higher sequence coverage (see Figure 2.2). Gene3D lacks domain annotation for many proteins which contain at least one Pfam and Profs domain.

domains, we perform a Blast search of the domain sequence against a library of Profs domains in the PDB (the **domain** table in Figure 2.6), and we selected only those templates that occur in the same crystal structure in both proteins and that interact according to the **domain\_contact** table. In order to select the best template for the interaction, we calculate a quality score for each of the two domains using Equation 2.1, and chose the template with the highest geometric mean of the two scores (Equation 2.2).

$$alignment\_score = 0.95 \cdot seq\_identity \cdot coverage + 0.05 \cdot coverage \quad (2.1)$$

$$combined\_alignment\_score = \sqrt{alignment\_score\_1 \cdot alignment\_score\_2} \quad (2.2)$$

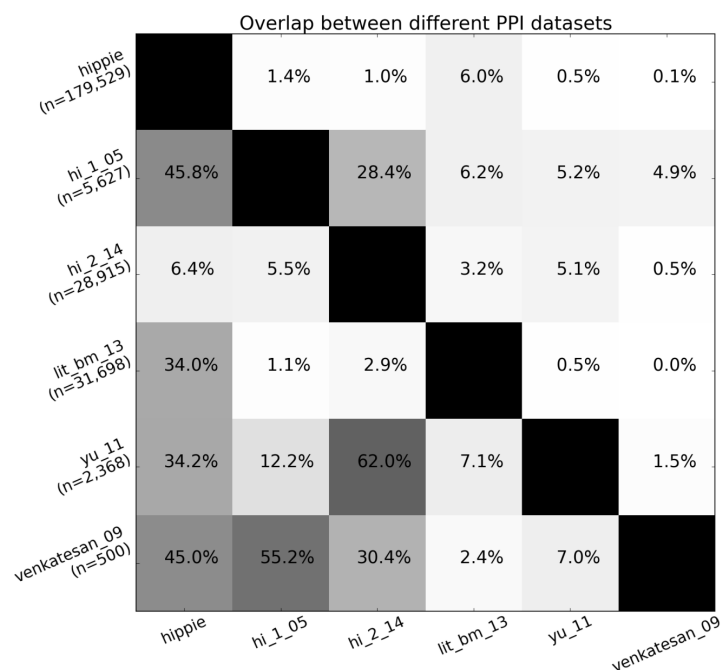


Figure 2.4: Overlap in protein-protein interaction (PPI) databases. The shade and value of each square denotes the percentage of PPIs in the database named on the y-axis that are also found in the database named on the x-axis. **hippie** is a meta-database, which integrates PPIs from many different sources [29]. **hi\_1\_05** contains PPIs discovered through a proteome-wide yeast two-hybrid experiment conducted by Rual *et al.* [31]. **hi\_2\_14** contains PPIs discovered through a proteome-wide yeast two-hybrid experiment conducted by Rolland *et al.* [30]. **lit\_bm\_13** contains PPIs obtained from the literature and supported by multiple pieces of evidence [30]. **yu\_11** contains PPIs obtained using “stitch-seq”, which combines PCR stitching with next-generation sequencing [32]. **venkatesan\_09** corresponds to high-quality binary interactions found in repeat yeast two-hybrid assays conducted by Venkatesan *et al.* [33]. The **hippie** database was downloaded from the Hippie website: <http://cbdm-01.zdv.uni-mainz.de/~mschaefer/hippie/>. All other datasets were downloaded from the Harvard Center for Cancer Systems Biology: [http://interactome.dfci.harvard.edu/H\\_sapiens/](http://interactome.dfci.harvard.edu/H_sapiens/).

## 2.2 ELASPIC

The ELASPIC project was started by Niklas Berliner and others in 2014 [20]. ELASPIC uses Modeller [34] to construct homology models of domains and domain-domain interactions, FoldX to optimize those model and to introduce mutations [35], and the gradient boosting regressor algorithm [20], implemented in scikit-learn [36], to combine FoldX energy scores with sequence-based and other features and predict the energetic impact of a mutation on the stability of a single domain or the affinity between two domains.

Since the original publication, ELASPIC was updated in many ways in order to make it easy to configure and run on different systems, and to make it possible to run ELASPIC on a distributed cluster as a backend to the ELASPIC webserver. Instead of using SIFT, as discussed in the original publication, we switched to using Provean, as it proved to be much easier to configure and install, and gave an option of precalculating supporting sets which could be used to calculate the Provean score for other mutations in the same protein in seconds. We switched to using MSMS instead of NACCESS for calculating solvent-accessible surface area of residues because MSMS was able to handle better misformed PDB files. We created two libraries for running *selaspic*

An overview of the updated ELASPIC pipeline is presented in Figure 2.5. ELASPIC includes a Python library for construction sequence alignments, constructing Provean supporting sets and computing the Provean score, constructing homology models, running FoldX, and predicting the  $\Delta\Delta G$  of the mutation. It also includes a “Standalone Pipeline” (Figure 2.5 right) and a “Database Pipeline” (Figure 2.5 left), which wrap the ELASPIC library and provide command line options for mutating local PDB files and proteins in the ELASPIC database.

The source code for the ELASPIC pipeline is hosted on GitHub (<https://github.com/kimlaborg/elaspic>), ELASPIC documentation is hosted on ReadtheDocs (<http://elaspic.readthedocs.io/>), and precalculated data can be downloaded from the ELASPIC website (<http://elaspic.kimlab.org/static/download/>).

### 2.2.1 Standalone pipeline

The standalone pipeline works without downloading and installing a local copy of the ELASPIC and PDB databases, but requires a PDB structure to be provided for every mutation. The output of the pipeline is saved as JSON files inside the *.elaspic* subfolder created in the working directory. The general overview of the local pipeline is presented on the right side of Figure 2.5.

### 2.2.2 Database pipeline

The database pipeline allows mutations to be performed on a proteome-wide scale, without having to specify a structural template for each protein. This pipeline requires a local installation of a relational database containing ELASPIC domain definitions and templates, as well as a local copy of the BLAST and PDB databases.

The general overview of the database pipeline is presented on the left side of Figure 2.5. A user runs the ELASPIC pipeline specifying the Uniprot ID of the protein being mutated, and one or more mutations affecting that protein. At each decision node, the pipeline queries the database to check whether or not the required information has been previously calculated. If the required data has not been calculated, the pipeline calculates it on the fly and stores the results in the database for later

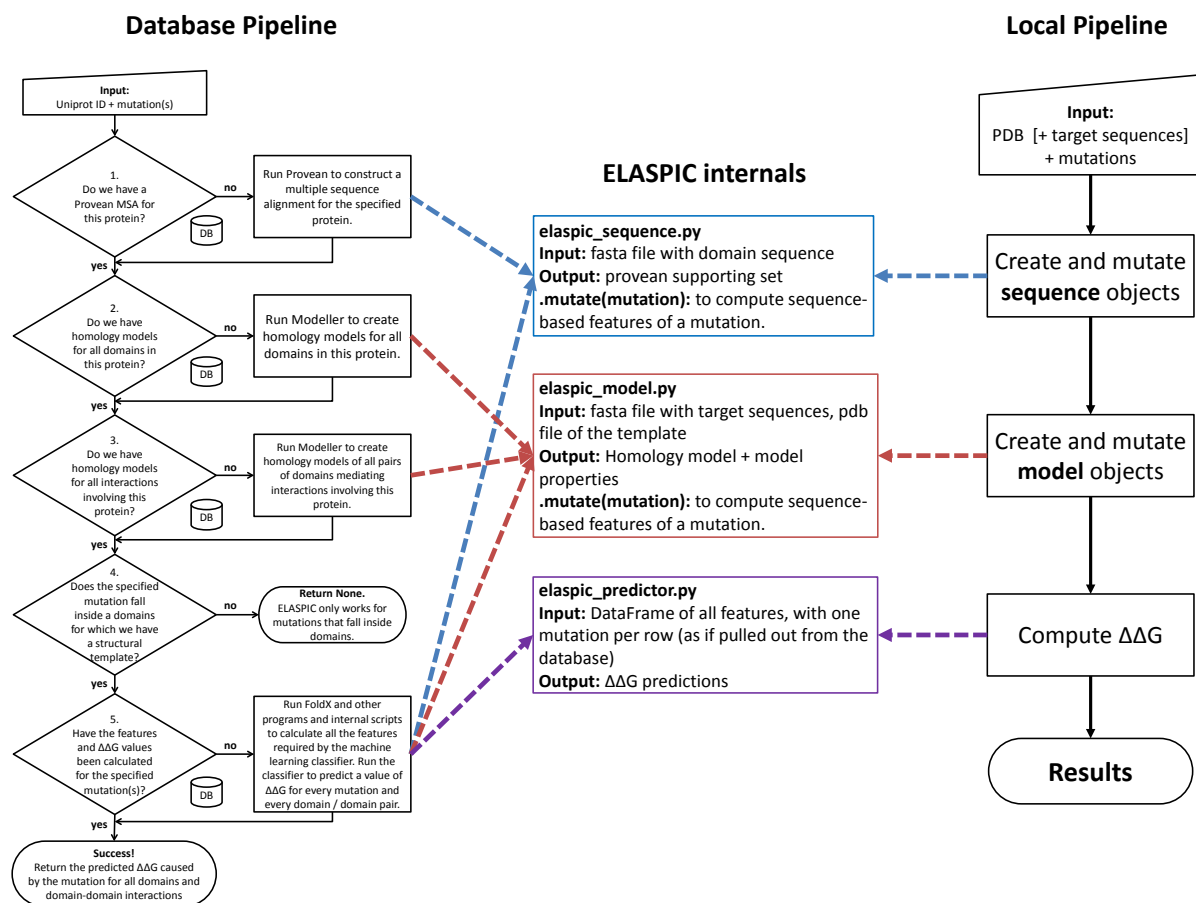


Figure 2.5: Overview of the ELASPIC pipeline. **Database Pipeline:** A user runs the ELASPIC pipeline by specifying the UniProt identifier of the protein being mutated, and one or more mutations affecting that protein. At each decision node, the pipeline queries the database (Figure 2.6) to check whether or not the required information has been calculated previously. If the required data has not been calculated, the pipeline calculates it on the fly and stores the results in the database for later retrieval. The pipeline proceeds until homology models of all domains in the protein, and all domain-domain interactions involving the protein, have been calculated, and the  $\Delta\Delta G$  has been predicted for every specified mutation. **Local Pipeline:** A user runs the ELASPIC pipeline by specifying a PDB file with the structure of the protein that they wish to mutate and one or more mutations, or by specifying a FASTA file with the sequence of the protein that they wish to mutate, a PDB file with the structural template to be used for homology modelling and one or more mutations. ELASPIC runs Proveal to calculate the supporting set, runs MODELLER to make the homology model, and runs FoldX to compute structural features describing the wildtype and mutant residues. Results are stored in a local *.elaspic* folder, and the Proveal supporting set and homology models are *not* recalculated if the user decides to run more mutations.

retrieval. The pipeline proceeds until homology models of all domains in the protein, and all domain-domain interactions involving the protein, have been calculated, and the  $\Delta\Delta G$  has been predicted for every specified mutation.

Results of the database pipeline are stored in the ELASPIC database. An overview of the ELASPIC database schema is presented in Figure 2.6, and a description of each database table is provided in Table 2.1.

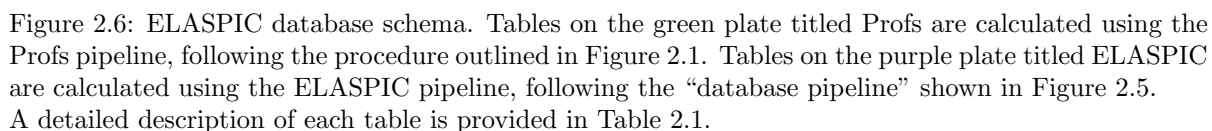


Table 2.1: Description of the tables in the ELASPIC database schema (Figure 2.6).

Table name	Table description
<b>domain</b>	Contains Profs domain definitions for all proteins in the PDB.
<b>domain_contact</b>	Contains information about interactions between Profs domains in the PDB. Only interactions that are predicted to be real by NOXclass [37] are included in this table.
<b>uniprot_sequence</b>	Contains protein sequences for all proteins that are annotated with Profs domains in the <b>uniprot_domain</b> table. This table is constructed by downloading and parsing <i>uniprot_sprot_fasta.gz</i> , <i>uniprot_trembl_fasta.gz</i> and <i>homo_sapiens_variation.txt</i> files from Uniprot.
<b>provean</b>	Contains information about Provean [11] supporting set files. The construction of a supporting set is the longest part of running Provean. Thus, in order to speed up the evaluation of mutations, the supporting set is precalculated and stored for every protein.
<b>uniprot_domain</b>	Contains Profs domain definitions for proteins in the <b>uniprot_sequence</b> table. This table is obtained by downloading Pfam domain definitions for all known proteins from SIMAP [27], and mapping those proteins to Uniprot using the MD5 hash of each sequence. Overlapping and repeating domains are either merged or deleted, as described in [26].
<b>uniprot_domain_template</b>	Contains structural templates for domains in the <b>uniprot_domain</b> table. The <i>domain_def</i> column contains expanded and corrected domain definitions for every domain.
<b>uniprot_domain_model</b>	Contains information about the homology models that were created using structural templates in the <b>uniprot_domain_template</b> table.
<b>uniprot_domain_mutation</b>	Contains information about the structural impact of core mutations, calculated by introducing those mutations into homology models listed in the <b>uniprot_domain_model</b> table. The <i>ddg</i> column contains the predicted change in the Gibbs free energy of protein folding.
<b>uniprot_domain_pair</b>	Contains pairs of domains that are likely to mediate the interaction between pairs of proteins listed in Hippie [29] and Rolland <i>et al.</i> [30].
<b>uniprot_domain_pair_template</b>	Contains structural templates for domain pairs in the <b>uniprot_domain_pair</b> table.
<b>uniprot_domain_pair_model</b>	Contains information about homology models that were created using structural templates in the <b>uniprot_domain_pair</b> table.
<b>uniprot_domain_pair</b>	Contains information about the structural impact of interface mutations, calculated by introducing those mutations into homology models listed in the <b>uniprot_domain_pair_model</b> table. The <i>ddg</i> column contains the predicted change in the Gibbs free energy of protein-protein binding.



### 2.2.3 Jobsubmitter

In order to make ELASPIC accessible to a wider scientific audience, Daniel Witvliet created the ELASPIC webserver, which allows users to run ELASPIC for their protein and mutation of interest and to analyze interactively ELASPIC results [26].

One limitation of the webserver was that it spawned ELASPIC jobs on the same virtual machine as the webserver. This meant that only a few mutations could be analyzed at a time, and that the webserver could stall when running mutations in a protein lacking a precalculated Provean supporting set, since constructing a Provean supporting set could require more RAM than the virtual machine had available. In order to make the webserver scale to thousands of mutations, we decided to restructure the job execution backend to run ELASPIC on the local Sun Grid Engine (SGE) cluster. However, this design introduced several challenges.

First, since users can run multiple mutations affecting the same protein, we had to make sure that the Provean supporting sets and homology models are calculated first, before jobs for individual mutations are submitted to the cluster. Otherwise, each mutation would initiate the calculation of a Provean supporting set, which can require more than 5 GB of memory, and a homology model, which can take more than 30 minutes to complete. This would lead to many unnecessary jobs, drastically lowering our throughput, and could lead to inconsistent results, since different jobs can generate different supporting sets and homology models, even for the same protein, due to the inherent randomness of those tasks.

Second, jobs running on a SGE cluster can die unexpectedly, if, for example, they exceed allocated resources, or if the node on which they are executing experiences a hardware failure. In most cases, jobs do not get an opportunity to send an error message before they are terminated. Therefore, we had to keep track of all running jobs, and resubmit jobs that do not finish successfully.

Third, in order to send a “Job Complete” email once all mutations submitted by a particular user have been evaluated, we had to keep track of the relationship between mutations and users that submitted those mutations.

One possible way to address those design requirements would be to use an asynchronous task queue, such as Celery. However, since different tasks inside the queue do not have a shared memory state, each task would have to periodically execute a *qstat* command on the SGE master node in order to monitor the status of the submitted jobs. Since we could have thousands of mutations running on the cluster at the same time, this would not be a scalable solution.

An alternative approach, which we used for the final design, was to create an independent web service which would submit ELASPIC jobs to the SGE cluster and would monitor their progress. We called this web service the ELASPIC “jobsubmitter”. It was implemented using the *aiohttp* library, which leverages the *asyncio* event loop and improved support for asynchronous programming present in Python 3.5 (Figure 2.7a).

Once the jobsubmitter receives a *GET* or *POST* request containing a set of mutations, information concerning those mutations is distributed into the following queues:

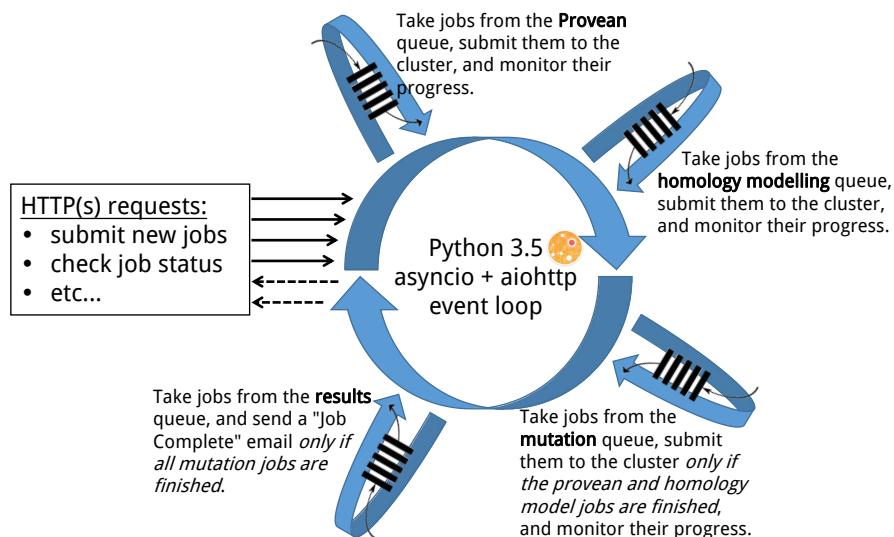
- A “Provean” queue, which contains proteins for which a Provean supporting set has not been calculated.
- A “homology model” queue, which contains proteins for which a homology model has not been calculated.
- A “mutation” queue, which contains individual mutations.

- An “email” queue, which contains the set of mutations associated with each job.

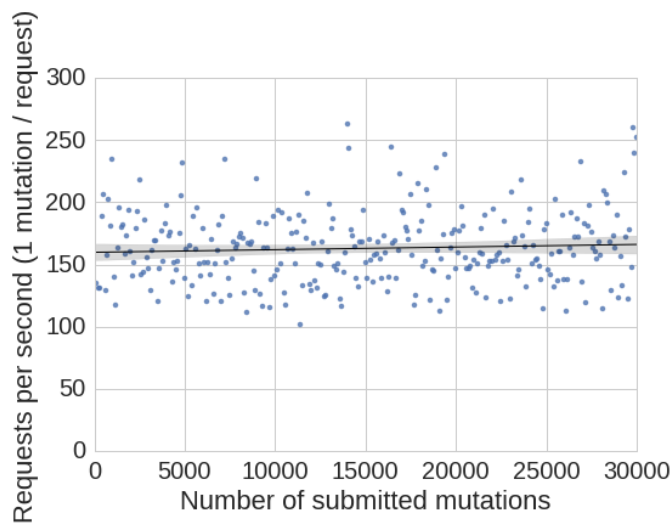
The information from those queues is then processed by the corresponding coroutines:

- For each protein in the “Provean” queue, a job is submitted to the SGE cluster, which calculates the Provean supporting set. If the Provean supporting set for the protein has already been calculated, the protein is taken of the “Provean” queue with no further action.
- For each protein in the “homology model” queue, a job is submitted to the SGE cluster, which calculates the homology model of the protein. If the homology model of the protein has already been calculated, the protein is taken of the “homology model” queue with no further action.
- For each mutation in the “mutation” queue, a job is submitted to the SGE cluster, which runs ELASPIC to calculate the  $\Delta\Delta G$  of the mutation. This happens *only if the Provean supporting set and homology model for the protein have already been calculated*.
- For each job in the “email” queue, a “Job Complete” email is sent to the specified email address once all mutations for the associated job have been completed.

The ELASPIC jobsubmitter is highly performant. It is able to handle over 150 requests per second, even with 30,000 mutations already being processed by the web service (Figure 2.7b).



(a) Overview of the ELASPIC “jobsubmitter” web service. The web service was implemented using Python 3.5 and the *aiohttp* library. It contains a central *asyncio* event loop, data structures holding information about the mutations being processed, and coroutines which submit jobs to the SGE cluster, monitor job progress, and perform other maintenance tasks.



(b) Plot showing the number of requests per second handled by the ELASPIC jobsubmitter as a function of the number of mutations that are already being processed.

Figure 2.7: Implementation (a) and performance (b) of the ELASPIC “jobsubmitter”.

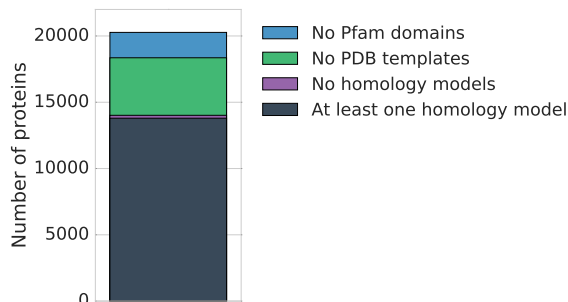
### 2.2.4 Precalculated data

In order to increase the speed with which the ELASPIC webserver generates results, we attempted to precalculate homology models and Provean supporting sets for all human proteins, and to precalculate mutations known to be involved in human disease.

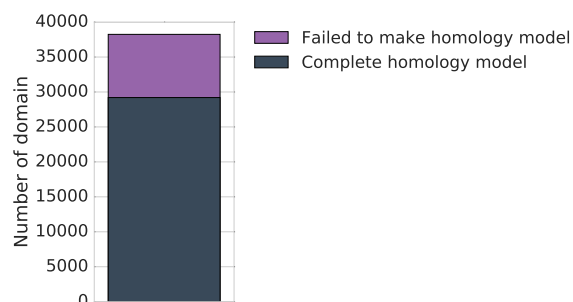
Out of 20,270 human proteins in the SwissProt database, 18,355 proteins have at least one Pfam domain, and 14,015 proteins have a Pfam domain for which we could find a structural template in the PDB (Figure 2.8a). We could create a homology model of at least one domain in 13,796 proteins, with the fraction of each protein covered by a homology model shown in Figure 2.8c. On the domain level, out of a total of 38,243 domains with a structural template, we could create a homology model for only 29,201 domains, or 76% (Figure 2.8b). The main reason for failing to calculate a homology model was low sequence identity between the domain being modelled and the structural template.

We also attempted to create a homology model for all protein pairs found in the HIPPIE [29] and CCSC [30] databases, keeping only the pairs where each protein has at least one domain with a homology model and where we could find a structural template of the protein-protein interaction. Out of a total of 19,964 such protein pairs, we calculated a homology models for 18,956, or 95 % (Figure 2.9).

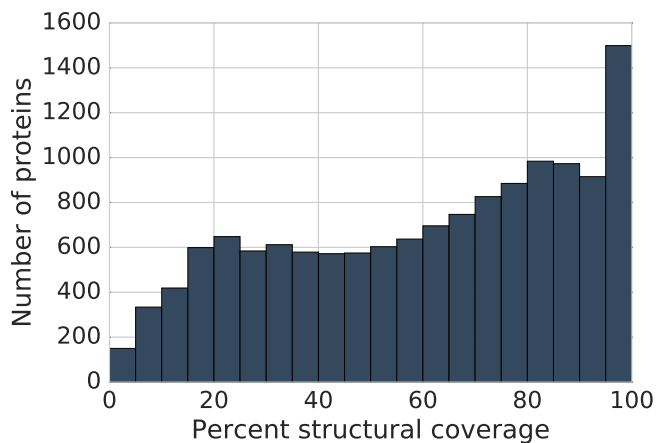
We successfully precalculated a Provean supporting set for all 14,015 human proteins with a Profs domain with a structural template. We also precalculated over 990,000 million mutations implicated in human diseases or found in human cancers, including nearly 600,000 mutations in different protein-protein interfaces.



(a) Diagram showing the number of *proteins* in the human SwissProt database that have no Pfam domains (blue), that have Pfam domains but no structural templates (green), that have Pfam domains and structural templates but no homology models (purple), and proteins with a homology model of at least one domain (grey).



(b) Diagram showing the number of *domains* in all proteins in the human SwissProt database for which we failed to create a homology model (purple) and for which we successfully created a homology model (grey). The most common reason for failing to create a homology model was low sequence identity between the Profs domain and the structural template.



(c) The percentage of protein sequence covered by Profs domains with homology models, for all proteins in the human SwissProt database that have a homology model of at least one domain.

Figure 2.8: Plots showing the number of proteins for which we could create a homology model (a), the number of domains for which we could create a homology model (b), and the structural coverage of proteins with at least one modelled domain (c). Plots were generated using all human proteins in the SwissProt database.

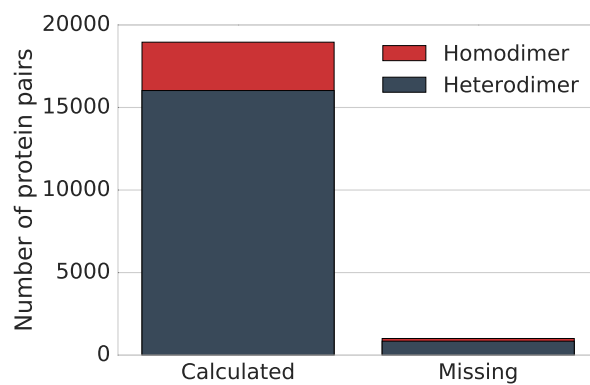


Figure 2.9: Number of homo-dimeric (red) and hetero-dimeric (grey) protein-protein interactions for which we created a homology model (left) and failed to create a homology model (right). In this figure, protein-protein interactions are defined as all pairs of proteins from the human SwissProt database that are found to interact according to one of the protein-protein interaction databases (see Figure 2.4) and that have at least one structural template of the interaction.

## Chapter 3

# Results

After making changes to the ELASPIC pipeline that are described in Section 2.2, we retrained ELASPIC core and interface predictors and validated them on new data. This involved curating high-quality training, validation and test datasets, selecting the best hyperparameters for the machine learning algorithm using grid-search, selecting the set of most informative features using feature elimination, and testing the final predictor on external datasets to compare our performance with competing methods.

The changes made to the ELASPIC pipeline and the large number of precalculated mutations associated with human disease.

### 3.1 Datasets

All datasets that we used to train, validate and test the predictors are described in Table 3.1. The dataset that we used to train the core predictor, labelled “Protherm” for simplicity, contains data from the Protherm database [38] combined with datasets curated by Kellogg *et al.* [39]. The dataset that we used to train the interface predictor, labelled “Skempi” for simplicity, contains data from the Skempi database [40] combined with the dataset curated by Kortemme and Baker [41].

We evaluated mutations in our training set using both the standalone and the database pipelines in order to make sure that both pipelines produce comparable results and that the resulting predictors perform equally well for both pipelines. The main difference between the standalone and database pipelines is that the standalone pipeline constructs the Proven supporting set using the sequence of the PDB chain that is being mutated, while the database pipeline constructs the Proven supporting set using the sequence of the entire protein containing the domain that is being mutated. For the database pipeline we attempted to construct several homology models ranging from low to high sequence identity for each mutation. We expected that including more proteins of lower sequence identity would improve the ability of the predictor to generalize to external datasets, since the training set is over-represented in proteins that have a crystal structure deposited in the PDB.

Previously, we had seen a low correlation between ELASPIC-predicted  $\Delta\Delta G$  and the deleteriousness of mutations. To address this, we split the Humsavar, ClinVar, and COSMIC datasets into validation and test subsets, and used the performance on the validation subset as part of the scoring function for selecting the optimal hyperparameters and number of features.

We made sure that no mutations in the test set appear in our training and validation sets (see

Table 3.1: Description of the datasets that were used in this study.

Name	Type	Description
<b>Protherm</b>	Train	Database of mutations-induced changes in the Gibbs free energy of protein folding ( $\Delta\Delta G_{core}$ ) [42].
<b>Skempi</b>	Train	Database of mutations-induced changes in the Gibbs free energy of protein-protein interactions ( $\Delta\Delta G_{interface}$ ) [40].
<b>Taipale</b>	Validation	Interaction between chaperones and wildtype or mutant proteins, quantified using the LUMIER assay [43].
<b>Taipale PPI</b>	Validation	Results of yeast two-hybrid experiments, measuring the presence or absence of protein-protein interactions for wild-type and mutant proteins [43].
<b>Taipale GPCA</b>	Validation	<i>Gaussia princeps</i> luciferase protein complementation assay, measuring the effect of mutations on protein affinity [43].
<b>Humsavar</b>	Validation & Test	Disease-causing mutations and polymorphisms obtained from the UniProt <i>humsavar.txt</i> file [44]. Mutations annotated with at least one disease were assigned a value of 1. Mutations annotated as “polymorphisms” were assigned a value of 0.
<b>ClinVar</b>	Validation & Test	Disease-causing mutations and polymorphisms obtained from ClinVar [45]. Mutations found in the ClinVar <i>clinvar_20160531.vcf</i> file were assigned a value of 1. Mutations found in the ClinVar <i>common_no_known_medical_impact_20160531.vcf</i> file were assigned a value of 0.
<b>COSMIC</b>	Validation & Test	Mutations found in cancer [46]. Mutations classified by FATHMM [10] as cancer drivers were assigned a value of 1. Mutations classified by FATHMM as cancer passengers were assigned a value of 0.
<b>SUMO Ligase</b>	Test	Mutations affecting the activity of SUMO ligase, measured using a cell viability assay [47].
<b>AB-Bind</b>	Test	Mutations explored in antibody affinity maturation experiments [48].
<b>Benedix</b>	Test	Mutations from alanine scanning of the TEM1 ( $\beta$ -lactamase) – BLIP ( $\beta$ -lactamase-inhibitor) interface [14].

Figures 3.1 and 3.2 for core and interface mutations, respectively). In the case of Humsavar, ClinVar and COSMIC datasets, we made sure that no *protein* in the test set appear in the training and validation sets.



Protherm (n = 4,374)	100.0	0.0	0.1	0.1	0.2	0.0	0.0	0.0	0.0	0.0
Taipale (n = 1,198)	0.1	100.0	68.9	58.4	9.9	0.0	0.0	0.0	0.0	0.0
Humsavar (Validation) (n = 18,623)	0.0	4.4	100.0	49.8	11.1	0.0	0.0	0.0	0.0	0.0
ClinVar (Validation) (n = 33,894)	0.0	2.1	27.4	100.0	11.0	0.0	0.0	0.0	0.0	0.0
COSMIC (Validation) (n = 174,627)	0.0	0.1	1.2	2.1	100.0	0.0	0.0	0.0	0.0	0.0
Humsavar (Test) (n = 10,511)	0.0	0.0	0.0	0.0	0.0	100.0	49.3	12.7	0.0	0.0
ClinVar (Test) (n = 24,897)	0.0	0.0	0.0	0.0	0.0	20.8	100.0	10.8	0.0	0.0
COSMIC (Test) (n = 156,871)	0.0	0.0	0.0	0.0	0.0	0.8	1.7	100.0	0.0	0.0
SUMO Ligase (n = 76)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	100.0	0.0
AB-Bind (n = 6)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
	Protherm	Taipale	Humsavar (Validation)	ClinVar (Validation)	COSMIC (Validation)	Humsavar (Test)	ClinVar (Test)	COSMIC (Test)	SUMO Ligase	AB-Bind

Figure 3.1: Overlap in core mutations between all the datasets used in this study. The shade and value inside each square denotes the percentage of mutations in the dataset named on the y-axis that are also found in the database named on the x-axis. Core mutations are defined as mutations that do not occur within 6 Å of a neighbouring chain in the provided PDB or protein-protein homology model. A description of each dataset can be found in Table 3.1.

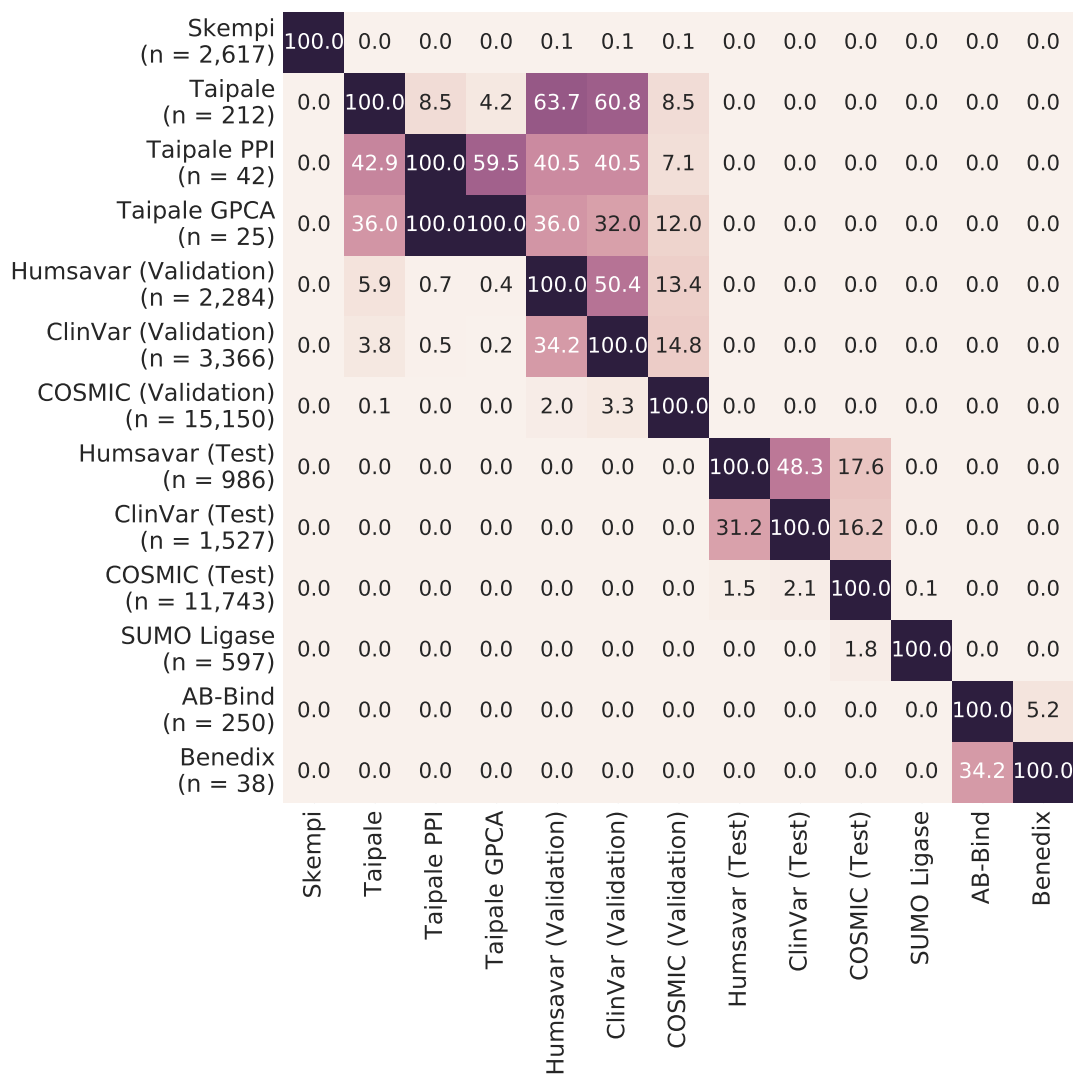


Figure 3.2: Overlap in interface mutations between all the datasets used in this study. The shade and value inside each square denotes the percentage of mutations in the dataset named on the y-axis that are also found in the database named on the x-axis. Interface mutations are defined as mutations that occur within 6 Å of a neighbouring chain in the provided PDB or protein-protein homology model. A description of each dataset can be found in Table 3.1.

## 3.2 Hyperparameter optimisation

ELASPIC uses the gradient boosting regressor (GBR) algorithm, implemented in scikit-learn [36], to combine over 70 different sequential and structural features into a score that corresponds to the Gibbs free energy change of protein folding or protein-protein interaction. The GBR algorithm was selected because it achieved higher performance than linear regression, support vector machine and random forest algorithms, in 20-fold cross-validation over the training set [20].

Since the ELASPIC pipeline received many bugfixes since the original publication, and was restructured to work as a backend to a webserver, we retrained GBR predictors using features generated by the updated pipeline. In order to select the best set of GBR hyperparameters, we performed exhaustive “grid-search”, where we measured the performance of the GBR algorithm for 3,600 different combinations of hyperparameters (Table 3.2). For each set of parameters, we computed the Spearman correlation between predicted and experimental  $\Delta\Delta G$  values for mutations in the training set, using 4-fold cross-validation. We also computed the Spearman correlation between predicted  $\Delta\Delta G$  values and the measured values for our “Validation” datasets (datasets in Table 3.1 annotated as “Validation”). In the case of the “Taipale” dataset, the experimental value was the difference in the average LUMIER score between the wildtype and mutant proteins and the corresponding chaperones. In the case of the “Taipale PPI” dataset, the experimental value was 1 if the mutation led to the loss of the interaction and 0 if it led to the gain of interaction or if it had no effect. In the case of the “Taipale GPCA” dataset, the experimental value was the difference in *Gaussia princeps* luminosity between wild-type and mutant proteins. In the case of “Humsavar”, “ClinVar” and “COSMIC” datasets, the experimental value was 1 if the mutation was classified as deleterious and 0 if it was classified as benign. While mutation deleteriousness and  $\Delta\Delta G$  are different metrics, it is expected that deleterious mutations, on average, should have a higher impact on protein structure than benign mutations. Therefore, accurate  $\Delta\Delta G$  predictions should have a higher correlation with the deleteriousness score, defined as 1 for deleterious mutations and 0 for benign mutations.

We used the combined scores  $CS_{core}$  (Equations 3.1) and  $CS_{interface}$  (Equation 3.2) to select the best set of hyperparameters for the core and interface predictors. The contribution of each dataset to the combined score was selected in an “ad-hoc” manner, assigning more weight to large datasets than to small datasets, and making sure that the performance on energy-based datasets, including training and Taipale datasets, had a bigger overall impact on the combined score than performance on a deleteriousness-based datasets, such as Humsavar, ClinVar and COSMIC. We used combined scores instead of cross-validation alone because we wanted to select predictors that not only perform well on the training set, but also generalize to external datasets. Since our training sets are limited and biased in the number and type of proteins and protein-protein interactions that they contain, the performance of the predictor in cross-validation may not be an accurate indicator of its performance in general. Since our validation datasets contain many more distinct proteins, protein-protein interactions and mutations than our training sets, they offer a helpful indication of how well predictors generalize to other proteins in the human genome.

$$CS_{core} = \frac{3 \cdot Cross\_validation + Humsavar + ClinVar + COSMIC + Taipale}{7} \quad (3.1)$$

$$CS_{interface} = \frac{3 \cdot Cross\_validation + Humsavar + ClinVar + COSMIC + \frac{Taipale\_PPI}{4} + \frac{Taipale\_GPCA}{4}}{6.5} \quad (3.2)$$

We plot the performance of core and interface predictors trained using different sets of hyperparameters and sorted according to the combined score in Figures 3.3 and 3.4. Cross-validation performance of the predictor is highly correlated with its performance on the validation datasets. However, selecting hyperparameters solely based on cross-validation performance would result in a predictor that substantially underperforms on the validation datasets.

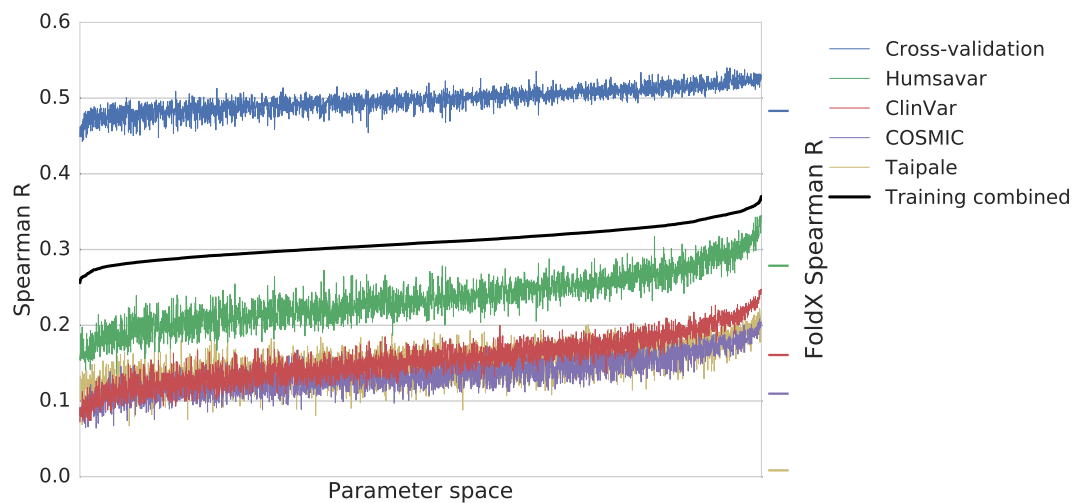


Figure 3.3: Core predictor hyperparameter optimization. The combined score (black line) was calculated using Equation 3.1.

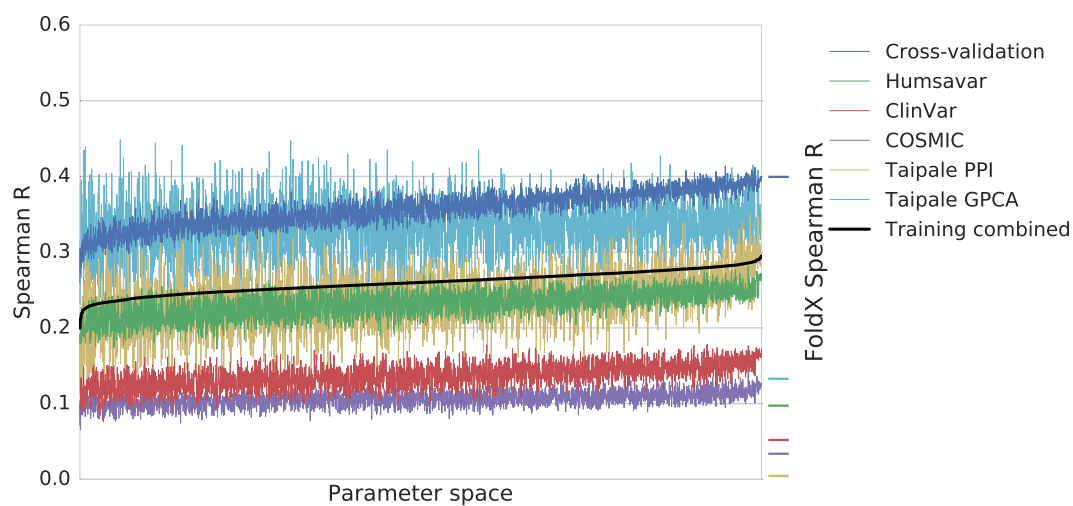


Figure 3.4: Interface predictor hyperparameter optimization. The combined score (black line) was calculated using Equation 3.2.

Table 3.2: Hyperparameter search space for tuning the gradient boosting regressor algorithm used in the core and interface predictors. An all-by-all combination of these hyperparameters was explored in order to find those that produce the best-performing predictors.

Parameter name	Parameter value
alpha	0.99, 0.95, 0.9, 0.8, 0.7, 0.5
learning_rate	0.1, 0.05, 0.02, 0.01
loss	huber
max_depth	10, 8, 6, 4
max_features	1.0, 0.8, 0.5, 0.3, 0.1,
min_samples_leaf	29, 21, 17, 13, 9, 5, 3
n_estimators	2000

Table 3.3: Hyperparameters selected for the core predictor.

Parameter name	Parameter value
alpha	0.5
learning_rate	0.01
loss	huber
max_depth	4
max_features	0.246
min_samples_leaf	17
n_estimators	2000

Table 3.4: Hyperparameters selected for the interface predictor.

Parameter name	Parameter value
alpha	0.9
learning_rate	0.01
loss	huber
max_depth	4
max_features	0.3
min_samples_leaf	13
n_estimators	2000

### 3.3 Feature elimination

After selecting the best set of hyperparameters for core and interface predictors, we performed feature elimination to evaluate the contribution of each feature to the overall accuracy of the predictor, and to select the sets of features that result in the most accurate predictions.

Feature elimination was performed using a recursive strategy, which involved:

- Leaving out each feature from the training set, one at a time.
- Training the predictor using all but the left out feature.
- Calculating the combined score ( $CS_{core}$  or  $CS_{interface}$ ) evaluating the performance of the predictor.
- Discarding the feature that, when left out, produced the predictor with the highest combined score.
- Repeating the process until only one feature remains.

Performances of the core and interface predictors at every step of feature elimination are shown in Figures 3.5 and 3.6. The sets of features that produced the best-performing predictors are described in Tables 3.5 and 3.6.

Most features play a surprisingly small role in the performance of the ELASPIC predictor. In fact, we can achieve near-optimal performance with both core and interface predictors by using only 6 features (displayed in bold in Tables 3.5 and 3.6). This suggests either that most features are not informative in predicting the energetic effect of mutations, or that the training set is too noisy for the contribution of those features to make a significant impact on the accuracy of the predictor.

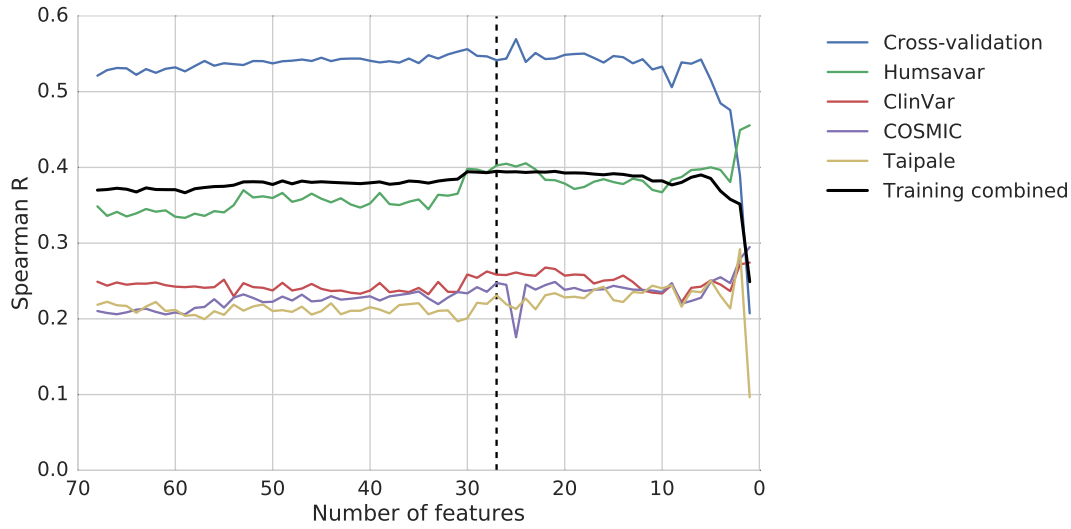


Figure 3.5: Performance of the core predictor at each step of feature elimination. The combined score (black line) was calculated using Equation 3.1. The set of features producing the predictor with the highest combined score are indicated by the vertical dashed line and are listed in Table 3.5.

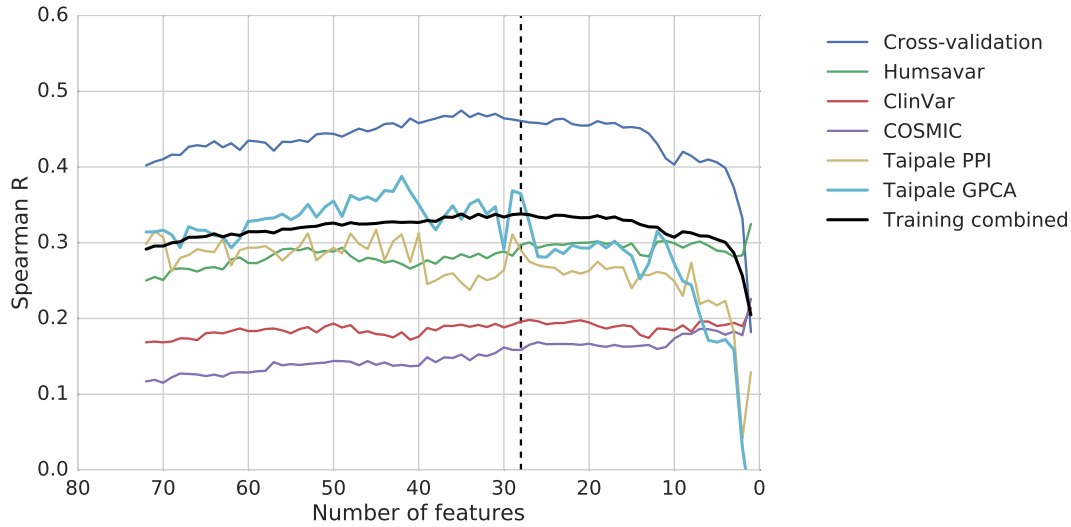


Figure 3.6: Performance of the interface predictor at each step of feature elimination. The combined score (black line) was calculated using Equation 3.2. The set of features producing the predictor with the highest combined score are indicated by the vertical dashed line and are listed in Table 3.6.



Table 3.5: Core predictor features. Rows in bold mark the 6 most important features. FoldX feature descriptions were taken from <http://foldxsuite.crg.eu/command/Stability>.

Feature name	Feature source	Feature description
alignment_coverage	ELASPIC	Structural template alignment coverage.
alignment_identity	ELASPIC	Structural template sequence identity.
alignment_score	ELASPIC	Structural template quality (Equation 2.1).
backbone_hbond_change	FoldX	Backbone hydrogen bond energy.
backbone_hbond_wt	FoldX	This the contribution of backbone hydrogen bonds.
cis_bond_wt	FoldX	Cis peptide bond energy.
disulfide_wt	FoldX	Contribution of disulfide bonds.
electrostatic_kon_change	FoldX	Electrostatic interaction between molecules in the pre-complex.
<b>electrostatics_change</b>	<b>FoldX</b>	<b>Electrostatic interactions.</b>
entropy_mainchain_change	FoldX	Entropy cost of fixing the main chain.
helix_dipole_wt	FoldX	Electrostatic contribution of the helix dipole.
matrix_score	ELASPIC	BLOSUM62 matrix score.
pcv_hbond_change	ELASPIC	Hydrogen-oxygen contacts involving atoms of the mutated residue and atoms of the interacting chain.
pcv_hbond_self_change	ELASPIC	Hydrogen-oxygen contacts involving atoms of the mutated residue and atoms of the mutated chain.
pcv_salt_equal_change	ELASPIC	Charge repulsions between atoms of the mutated residue and atoms of the interacting chain.
pcv_salt_equal_self_wt	ELASPIC	Charge repulsions between atoms of the mutated residue and atoms of the mutated chain.
pcv_salt_equal_wt	ELASPIC	Charge repulsions between atoms of the mutated residue and atoms of the interacting chain.
pcv_salt_opposite_change	ELASPIC	Charge attractions between atoms of the mutated residue and atoms of the interacting chain.
pcv_vdw_self_change	ELASPIC	Carbon carbon contacts between atoms of the mutated residue and atoms of the mutated chain.
<b>provean_score</b>	<b>Provean</b>	<b>Sequence conservation score.</b>
sloop_entropy_wt	FoldX	Entropic cost according to the SLoop database of loop conformations.
<b>solvation_hydrophobic_change</b>	<b>FoldX</b>	<b>Contribution of hydrophobic groups.</b>
<b>solvation_polar_change</b>	<b>FoldX</b>	<b>Energetic penalty for burying polar groups.</b>
<b>solvent_accessibility_wt</b>	<b>MSMS</b>	<b>Solvent-accessible surface area of the mutated residue.</b>
torsional_clash_change	FoldX	Intra-residue van der Waals torsional clashes.
<b>van_der_waals_clashes_change</b>	<b>FoldX</b>	<b>Energy penalization due to van der Waals clashes (interresidue).</b>
water_bridge_wt	FoldX	Contribution of water bridges.

Table 3.6: Interface predictor features. Rows in bold mark the 6 most important features. FoldX feature descriptions were taken from <http://foldxsuite.crg.eu/command/AnalyseComplex>.

Feature name	Feature source	Feature description
alignment_score	ELASPIC	Alignment quality (Equation 2.2).
backbone.clash_change	FoldX	Backbone-backbone van der Waals energy.
backbone.clash_wt	FoldX	Backbone-backbone van der Waals energy.
backbone.hbond_change	FoldX	Backbone hydrogen bond energy.
cis_bond_wt	FoldX	Cis peptide bond energy.
electrostatic_kon_wt	FoldX	Electrostatic interaction between molecules in the pre-complex.
energy_ionisation_wt	FoldX	Ionization energy.
entropy_complex_change	FoldX	Entropic cost of forming a complex.
<b>entropy_sidechain_change</b>	<b>FoldX</b>	<b>Entropic cost of fixing the side chain.</b>
intraclashes_energy_2_change	FoldX	van der Waals clashes of residues at the interface of the complex with their own molecule (type 2).
<b>partial.covalent_bonds_wt</b>	<b>FoldX</b>	<b>Interactions with bound metals.</b>
pcv_hbond_self_change	ELASPIC	Hydrogen-oxygen contacts involving atoms of the mutated residue and atoms of the mutated chain.
pcv_hbond_wt	ELASPIC	Hydrogen-oxygen contacts involving atoms of the mutated residue and atoms of the interacting chain.
pcv_salt_equal_self_change	ELASPIC	Charge repulsions involving atoms of the mutated residue and atoms of the mutated chain.
pcv_salt_equal_wt	ELASPIC	Charge repulsions involving atoms of the mutated residue and atoms of the interacting chain.
pcv_salt_opposite_change	ELASPIC	Charge attractions involving atoms of the mutated residue and atoms of the interacting chain.
pcv_salt_opposite_self_change	ELASPIC	Charge attractions involving atoms of the mutated residue and atoms of the mutated chain.
pcv_salt_opposite_self_wt	ELASPIC	Charge attractions involving atoms of the mutated residue and atoms of the mutated chain.
pcv_vdw_self_change	ELASPIC	Carbon carbon contacts involving atoms of the mutated residue and atoms of the mutated chain.
<b>pcv_vdw_self_wt</b>	<b>ELASPIC</b>	<b>Carbon carbon contacts involving atoms of the mutated residue and atoms of the mutated chain.</b>
<b>pcv_vdw_wt</b>	<b>ELASPIC</b>	<b>Carbon carbon contacts involving atoms of the mutated residue and atoms of the interacting chain.</b>
<b>provean_score</b>	<b>Provean</b>	<b>Sequence conservation score.</b>
sloop_entropy_change	FoldX	Entropic cost according to the SLoop database of loop conformations.
solvation_hydrophobic_change	FoldX	Contribution of hydrophobic groups.
<b>solvation_polar_change</b>	<b>FoldX</b>	<b>Energetic penalty for burying polar groups.</b>
solvation_polar_wt	FoldX	Energetic penalty for burying polar groups.
torsional_clash_change	FoldX	Intra-residue van der Waals torsional clashes.
water_bridge_change	FoldX	Contribution of water bridges.

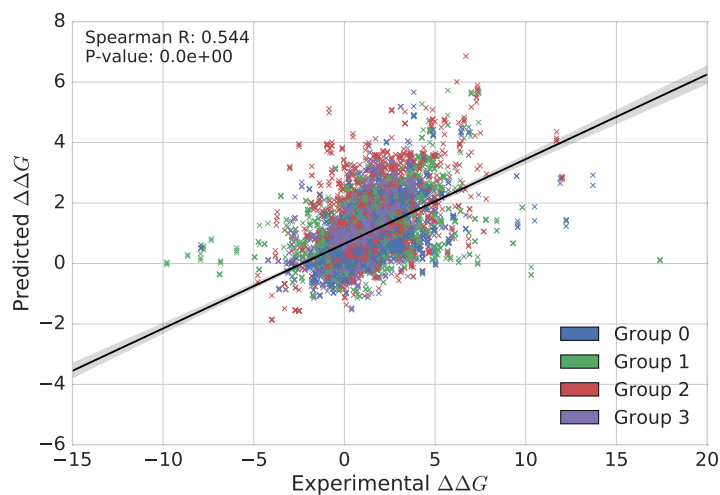
### 3.4 Validation

The final ELASPIC core and interface predictors were trained using gradient boosting regressor hyperparameters listed in Tables 3.3 and 3.5 and sets of features described in Tables 3.4 and 3.6. Performance of the resulting predictors on the training, validation and test datasets are presented in Figures 3.7 and 3.8, for core and interface predictors, respectively.

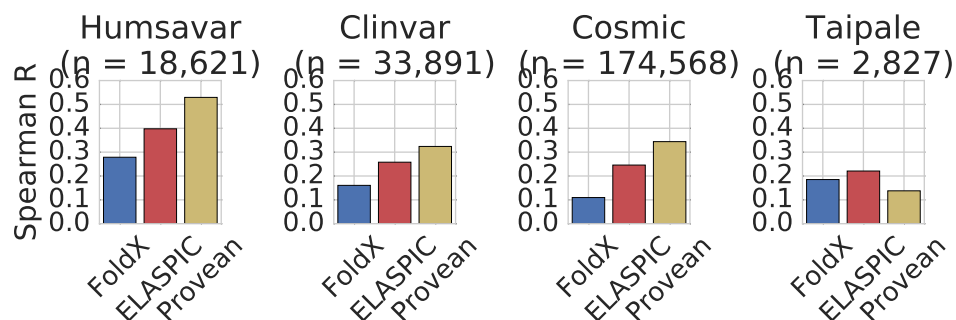
The ELASPIC core predictor outperforms FoldX and Provean on the Taipale dataset, which is the only validation dataset explicitly measuring the effect of mutations on protein stability rather than whether or not the mutation is associated with a disease (Figure 3.7b). It also outperforms FoldX and Provean on the core subsets of the SUMO and AB-Bind test datasets (Figure 3.7c). The core subsets of those datasets only contain mutations located more than 6 Å away from another chain in the PDB.

The ELASPIC interface predictor also outperforms FoldX and Provean on the Taipale GPCA dataset (Figure 3.8b). It performs marginally worse than Provean on the Taipale PPI dataset, but this is likely because the Taipale PPI dataset is mostly made up of deleterious mutations, which are predicted well by Protherm. The ELASPIC interface outperforms Protherm and FoldX on the SUMO Ligase and AB-Bind test datasets (Figure 3.8c) both alone and in combination with the core predictor. The ELASPIC interface predictor shows slightly lower performance than FoldX on the very small Benedix dataset.

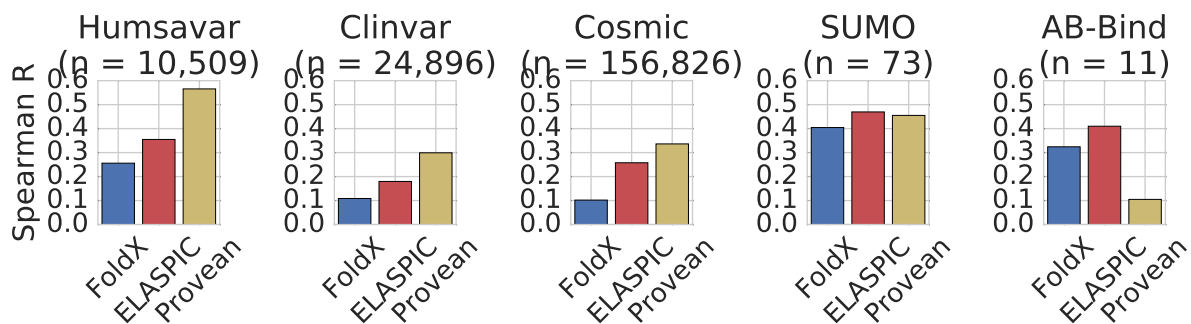
Both the core and interface predictors performs better than FoldX but worse than Provean on the validation and test subsets of the Humsavar, ClinVar and COSMIC (Figures 3.7c and 3.8c). The low performance of the ELASPIC compared to Provean was expected, since the ELASPIC core predictor attempts to model the effect of mutations on protein stability, and does not account for other reasons that may lead to a mutation being deleterious. For example, mutations could affect the active site or the signal sequence of a protein, which may prove to be highly deleterious to the organism but have only a marginal effect on protein stability.



(a) Performance of the ELASPIC core predictor on the training dataset, evaluated using four-fold cross-validation. Colours indicate different cross-validation bins.

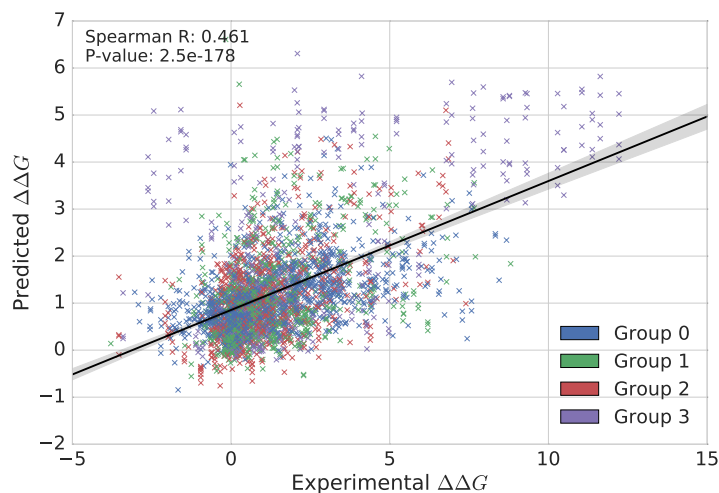


(b) Performance of the ELASPIC core predictor, FoldX and Provean on the validation datasets.

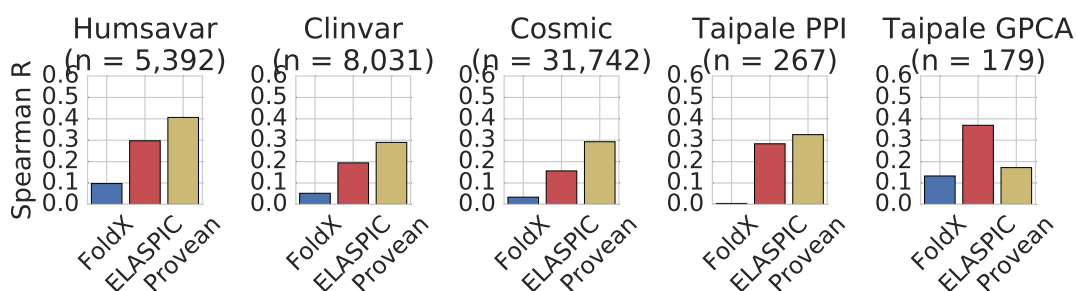


(c) Performance of the ELASPIC core predictor, FoldX and Provean on the test datasets. There is no overlap in mutations (or proteins for Humsavar, ClinVar and COSMIC) between the test datasets, and the training and validation datasets (see Figure 3.1).

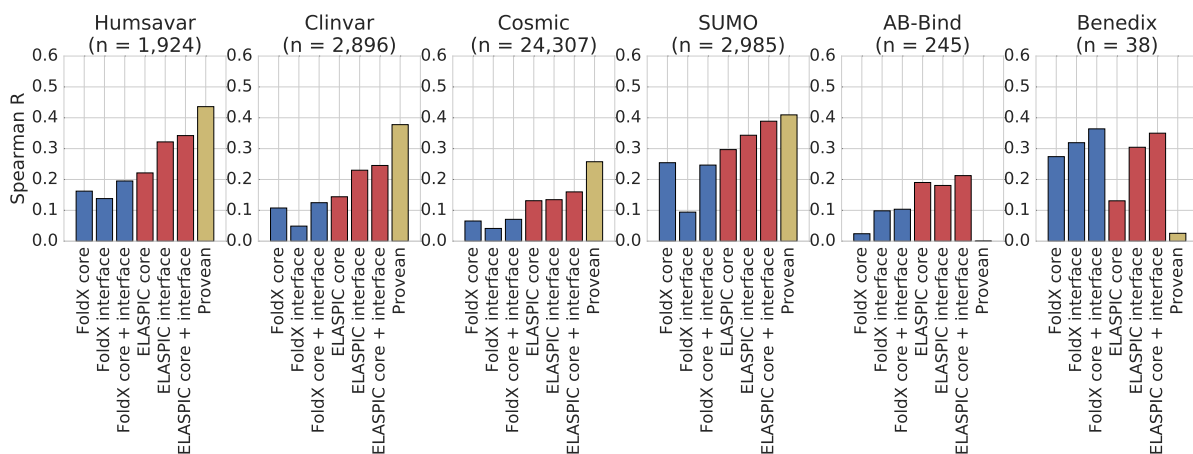
Figure 3.7: Performance of the ELASPIC core predictor on the training (a), validation (b) and test (c) datasets.



(a) Performance of the ELASPIC interface predictor on the training dataset, evaluated using four-fold cross-validation. Colours indicate different cross-validation bins.



(b) Performance of the ELASPIC interface predictor, FoldX and Provean on the validation datasets.



(c) Performance of ELASPIC, FoldX and Provean on the test datasets. Correlations are provided for core predictors, interface predictors, and the sum of core and interface predictors, for ELASPIC and FoldX. There is no overlap in mutations (or proteins for Humsavar, ClinVar and COSMIC) between the test datasets, and the training and validation datasets (see Figure 3.2).

Figure 3.8: Performance of the ELASPIC interface predictor on the training (a), validation (b) and test (c) datasets.

## Chapter 4

# Discussion

Two main factors limit the accuracy and the generalizability of ELASPIC. First, training datasets for both the core and interface predictors are limited in their accuracy and are biased in the types of mutations that they contain. For example,

The main goal of this work was to improve our ability to predict whether or not a mutations is going to be deleterious and predicting the phenotypic effect of mutations by evaluating the structural impact of mutations instead of looking solely at how conserved the particular is in different organisms and species.

In the set of features selected through feature elimination, there are both sequence-based features and structure-based features. The most important sequence-based feature is the Provean score.

Results of feature elimination support the view that electrostatics, van der Waals forces and entropy are the main forces determining the effect of mutations, as proposed by Benedix *et al.* in the Concoord/Poisson-Boltzmann surface area model (Equation 4.1).

$$\Delta G_{CC/PBSA} = \Delta G_{electrostatic} + \Delta G_{van\ der\ Waals} + \Delta G_{entropy} \quad (4.1)$$

Since the publication of the ELASPIC pipeline [20] and webserver [26], several other algorithms have been published which use a similar approach as ELASPIC to either .

### 4.1 Adding support for multiresidue mutations

ELASPIC can easily be extended to calculate the  $\Delta\Delta G$  for mutations involving multiple amino acids. The main problem that would have to be solved

The tricky part is that the number of features changes with the number of amino acids that are mutated. We could address this by treating a mutation affecting multiple amino acids as a set of single amino acid mutations. For example, we could use the following recursive strategy:

- Introduce each of the single amino acid mutations, one at a time.
- Select the single amino acid mutation with the most stabilizing effect.
- Repeat for the remaining mutations, using the structure containing the mutation selected in Step 2.

About one third on mutations in the Protherm and Skempi databases affect multiple amino acids. We could include those mutations in the training set by dividing them into single amino acid mutations and

assigning to them a  $\Delta\Delta G$  proportional to their contribution to the overall mutation score, as determined by the multiple amino acid substitution version of ELASPIC. This would require “bootstrapping” the ELASPIC predictor using single amino acid mutations, using the “bootstrapped” predictor to approximate the contribution of single amino acid mutations to the  $\Delta\Delta G$  affecting multiple amino acids, adding those mutations to the training set, and repeating.

In the case of the ELASPIC core predictor, we could create a dataset of multiple amino acid polymorphisms (MAAMs) from a thermophilic bacterium and its closest non-thermophilic relative (maybe such a database already exists?). Cross-validate ELASPIC making sure that we predict those MAAMs to be stabilizing. Incorporate those MAAMs into our training set, weighting them accordingly.

In the case of the ELASPIC interface predictor, we could construct a dataset from phage-display read counts, and cross-validate ELASPIC while keeping track of its performance on phage display counts. Could then recursively incorporate the phage display data into the training set, weighting it by how well the ELASPIC predictor does on those mutations, as determined through cross-validation.

It is likely that the performance of the ELASPIC predictor would be lower for mutations affecting multiple amino acids than for mutations affecting a single amino acid, as the former is more likely to induce changes in the conformation of the protein that are not modelled by ELASPIC. This drop in performance could in-part be ameliorated by including a backbone relaxation step between each mutation, using molecular dynamics [49], Rosetta Backrub [50], or other algorithms [51].

If the ELASPIC predictor can achieve reasonable results for mutations affecting multiple amino acids, it could be used “in reverse” to design protein domains with increased stability and protein interfaces with increased affinity.

## 4.2 Adding support for more interaction types

## 4.3 Multitask learning of mutation deleteriousness and energetic effects

In this work, we attempted to improve the generalizability of ELASPIC core and interface predictors by keeping track of their performance on mutation deleteriousness datasets throughout cross-validation and feature elimination (Figures 3.3, 3.4, 3.5 and 3.6). While this approach prevents us from selecting predictors that overfit the training set, it does not improve the accuracy of any individual predictor.

One way to improve overall accuracy of the predictors would be to leverage information contained in mutation deleteriousness datasets to discover better and more useful features. Mutation deleteriousness datasets are much larger than the  $\Delta\Delta G$  datasets, and they may allow sequential and structural features to “mix” in a more general environment, and produce combined features that are less noisy and better correlated with the actual effect of mutations.

We could learn those features by first training a boosted decision tree algorithm to predict mutation deleteriousness, and then use the output of those trees as input to a logistic regression model trained to predict mutation  $\Delta\Delta G$  (Figure 4.1). The resulting predictor should not only have better accuracy, but also have a better ability to extrapolate than the currently-used gradient boosted regressor algorithm, which never predicts values that are higher than the highest values observed in the training set.

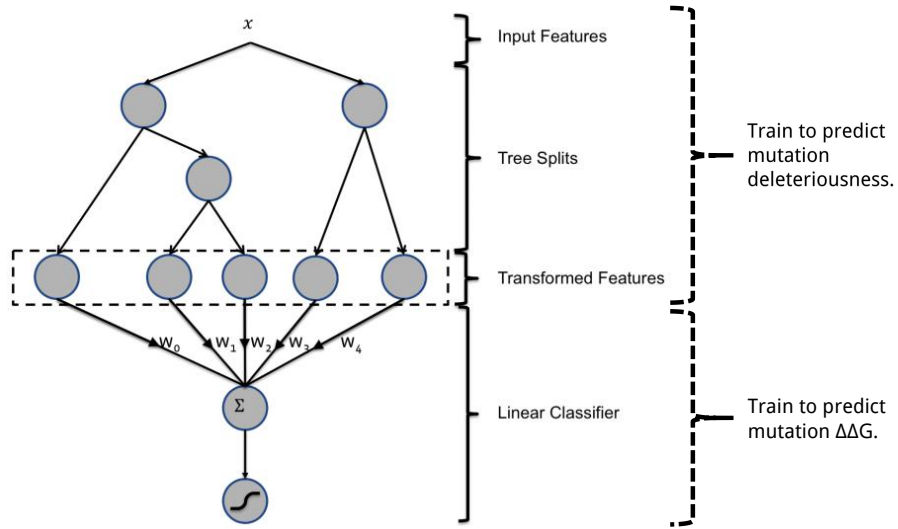


Figure 4.1: Multitask learning of mutation deleteriousness and  $\Delta\Delta G$ . The figure is adapted from He *et al.* [52], where it is used to describe an algorithm that couples boosted decision trees and linear regression to predict add click-through rate. Boosted decision trees are used to learn a feature “manifold” that is provided as input to the linear classifier, which in turn makes the final predictions.

We propose to use a similar design, but train boosted decision trees to predict mutation deleteriousness, and fit a linear regressor to predict mutation  $\Delta\Delta G$ . We anticipate that the large training set of benign and deleterious mutations would allow the boosted decision tree algorithm to learn useful and generalizable features.



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