Data article

SoluProtMut\textsuperscript{DB}: A manually curated database of protein solubility changes upon mutations

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\begin{abstract}
Protein solubility is an attractive engineering target primarily due to its relation to yields in protein production and manufacturing. Moreover, better knowledge of the mutational effects on protein solubility could connect several serious human diseases with protein aggregation. However, we have limited understanding of the protein structural determinants of solubility, and the available data have mostly been scattered in the literature. Here, we present SoluProtMut\textsuperscript{DB} – the first database containing data on protein solubility changes upon mutations. Our database accommodates 33000 measurements of 17000 protein variants in 103 different proteins. The database can serve as an essential source of information for the researchers designing improved protein variants or those developing machine learning tools to predict the effects of mutations on solubility. The database comprises all the previously published solubility datasets and thousands of new data points from recent publications, including deep mutational scanning experiments. Moreover, it features many available experimental conditions known to affect protein solubility. The datasets have been manually curated with substantial corrections, improving suitability for machine learning applications. The database is available at loschmidt.chem.muni.cz/soluprotmutdb. © 2022 The Author(s). Published by Elsevier B.V. on behalf of Research Network of Computational and Structural Biotechnology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
\end{abstract}

1. Introduction

Protein mutational databases accumulate results from experiments examining how mutations introduced to a protein affect a selected property. Several such databases have arisen recently, including FireProt\textsuperscript{DB} [1] for the protein stability data for single-point mutants, the MPTherm [2] database for membrane protein thermodynamics, or D3DistalMutation [3] for enzyme activity. However, there has not been any mutational solubility database yet despite solubility being a basic characteristic of any globular protein. Moreover, high solubility is essential for high-dosing protein therapeutics or for efficient protein production [4,5]. The lowered solubility of a body protein due to a mutation may also cause a disease [6]. And neither too low nor too high solubility is required for successful structure determination of a protein in the crystalline form.

Prediction of solubility change upon mutation is thus an important problem. Several predictors for this task were developed, usually using mutational solubility data sets for training collected independently from the literature [7–10]. While these attempts showed great promise, the training datasets were rather limited in the number of entries and their annotations. These limitations provide a possible explanation as to why recent studies comparing the predictors revealed significant room for improvement, as the latest predictors did not exceed the correct prediction ratio of 70% [10,11].

The data available in the solubility datasets come mostly from small-scale experiments. These often search for a solubilizing mutation to a particular protein in order to enhance its insufficient solubility. A small-scale experiment measures only a small number of mutants and only one direction of solubility change is often observed among all of them. Another drawback is that these experiments may be incomparable due to the different conditions under which they were conducted. Most typically, a variant of an electrophoresis assay...
and protein staining is used to assess protein solubility through mass separation, e.g., the SDS–PAGE assay. Other, less frequent methods include Western blotting, where the soluble fraction of protein of interest is separated and marked via antigen binding.

In contrast, high-throughput experiments provide many results from a single run. Apart from the clear advantage of obtaining a large amount of data at once, they allow a more precise comparison thanks to the elimination of setup differences. High-throughput methods typically measure solubility indirectly through another property, e.g., fluorescence, which can be achieved in an automated manner more easily. For instance, in recent studies by Whitehead’s group [12,13], fluorescence-activated cell sorting (FACS) was used to select solubilizing mutations out of almost all possible single-point variants. While such a strategy is usually applied to one protein at a time, it has the potential to provide the sufficient data abundance for modern data-hungry machine learning (ML) methods [14].

Here we present a database incorporating solubility data from several sources (Fig. 1); (i) curated data from OptSolMut [7], CamSol [8], A3D [9] and PON-Sol [10] datasets, (ii) recently conducted deep mutational scanning (DMS) of solubility at Whitehead’s research group [12,13], (iii) our own literature search for solubility experiments, and (iv) data from high-throughput experiments currently conducted in our laboratories.

The database goes beyond the basic reporting of introduced mutations and their effects on protein solubility. We performed an extensive manual curation of each entry based on the original publications. We also keep track of the experimental setup wherever possible as it has a major influence on the experimental outcome [17]. This setup has two main components: expression-related conditions (how the protein was produced) and assay-related conditions (how the solubility was measured). For instance, the expression conditions include host cells, the temperature, and induction times used. Assays differ mainly in the physical property used to measure solubility change. Finally, the data are annotated with dataset memberships, links to UniProt [15] and its annotations, and HotSpot Wizard [16] features per sequence or structure as depicted in Fig. 1.

While the database will serve as a valuable source of insights for protein engineers, structural biologists, or biochemists, we have made our database convenient for the broad ML and data science communities as well, e.g., to facilitate using the deposited data in the development and testing of predictive models. All the aforementioned experimental conditions and annotations are utilizable as features. We also performed a systematization of reported changes and created a flexible Export Wizard. The systematization deals with the verbally-assessed changes – these are discrete and inexact values with no scale specified by the authors. Export Wizard allows exporting the filtered data and converting the values to the desired classes to be used in a target model.

With the advent of high-throughput screening methods, we may see a flood of mutational solubility data published, and SoluProtMut[18] should serve as a central depository for this type of data. A centralized and regularly updated depository for mutational solubility data will facilitate the in silico engineering of protein solubility, which is critical in biopharmacy, biotechnology, or structural biology. The depository will also be useful for data scientists, ML engineers, protein engineers and medical doctors.

2. Materials and methods

2.1. Data from small-scale experiments

The cornerstones of SoluProtMut[18] are four mutational solubility datasets, published between 2010 and 2017, which we merged together: OptSolMut [7], CamSol [8], A3D [9], and PON-Sol [10]. Every datapoint in each of these datasets represents a mutated variant of a particular protein, where the protein is specified either by its sequence or Protein Data Bank ID (PDB ID) and labeled according to the effect on the protein solubility. While none of the datasets is fully contained in another, they do overlap significantly. Therefore, we ensured that each datapoint is contained in the final database only once and assigned to all the datasets it appears in. We also added new data from the updated PON-Sol dataset [11]. Furthermore, as all these datasets only comprise the solubility data from publications before 2017, we conducted a data search in more recent literature and added new results.

We carried out manual validation and curation of the datasets against the source publications as the data are not in a machine-readable format in most of the source publications. We found and resolved a substantial number of discrepancies of the following types by correction or removal of the affected datapoints: reports of changes in properties with no clear relation to solubility; measurements which are not present in the source publication; wrong values; wrong positions or residues of substitutions.

During the manual processing of the publications, we additionally extracted the data that do not appear in the published datasets. These include reported experimental conditions, such as measurement assay, host organism and strain, temperature, pH, and concentration method used; originally reported numerical changes in solubility; and even more than hundred instances of measured protein variants that were left unnoticed by the authors of the datasets. We also distinguish the types of solubility the continuous values referred to: the soluble fraction, soluble concentration, or total concentration.

During the validation, we assigned a UniProt accession number (UniProt AC) of an original variant to every datapoint and renumbered the mutated positions with respect to the UniProt sequence. This was necessary as the proteins in the datasets are only assigned with PDB IDs or protein/gene names, which are, however, less reliable, stable, or not unique in comparison to UniProt ACs in the long term. In the case of PDBs, one structure can refer to several proteins, and a single protein typically has multiple relevant PDBs with new and refined structures of proteins appearing over time.

2.2. Deep mutational scanning data

The eminent source is the data collected at Whitehead’s research group – the first use of DMS for solubility screening. The group measured the soluble expression of the levoglucosan kinase, TEM-1 β-lactamase, and pyrrolidine ketide synthase variants in E. coli or yeast assays [12,13]. Their DMS approach consisted of three steps. The first step was comprehensive saturation mutagenesis across the entire protein, which yielded a cell library of all possible single-point mutants. The second step was the selection of cells with soluble protein. And the third step was deep sequencing – measuring the frequencies of the variants before and after the selection procedure of the second step by sampling and sequencing them. The authors explored two selection procedures: Tat-export and FACS. In the former, soluble protein provided antibiotic resistance and was required for cell survival. In the latter, the fluorescence change upon binding with a fluorescence-enabled antibody or a green-fluorescence-protein (GFP) tag was exploited as the proxy to protein solubility, and then the cells with higher solubility were sorted out using FACS. The enrichment ratio for each variant was calculated based on the number of reads before and after the selection, normalized, and reported as the score for the effect of the mutations on protein solubility.

To make these continuous scores comparable with the discrete values reported in the other literature, we binned them into 5 levels according to the threshold of 0.15, suggested by the authors (that is +10% on a linear scale) to label enhancing mutations, and a threshold of +50% for significantly enhancing mutations. Symmet-
rically, we used $-10\%$ and $-33.3\%$ to label slightly and significantly deteriorating mutations, respectively. The remaining data-points were binned into the neutral class. During this process, we also omitted the scores of nonsense mutations and those having statistically insignificant enrichment values due to the low number of reads.

### 2.3. In-house data

In addition to the published literature, the database contains the data from medium-throughput experiments on haloalkane dehalogenase, recently conducted by our research group [18]. Our assay, validated by comparison with SDS–PAGE on multiple proteins, measures solubility through fluorescence activity introduced by the split-GFP approach. The mutant library was created with error-prone PCR, and randomly selected mutants were measured and sequenced. Measuring was conducted in replicates, and the mutants with statistically insignificant results were discarded. This resulted in 22 datapoints available in the database.

### 2.4. Systematization of values

By analyzing the literature, we identified five patterns appearing in solubility experiments for a mutation effect assessment. We systematized these patterns into reporting systems as per Table 1 to make the reported changes comparable even when they come from different publications and are described in different terms. These differences are partially due to the use of various assays as their precision varies, and sometimes the effect was not quantifiable. In other cases, incomplete information was published. For example, in experiments aiming to solubilize a particular protein, only verbal assessment is often reported for mutants not improving solubility.

We distinguish the orientation (positive, negative, or neutral) of an effect and, whenever applicable, also its significance (slight or significant). Altogether, up to five discrete values are defined: significantly/slightly deteriorating, neutral, and slightly/significantly enhancing. This system suggests different resolutions in different experiments, e.g., a value from the 5-value system should be more precise than from the 3-value system. Hence, if one mutation is enhancing in the 3-value system and another is slightly enhancing in the 5-value system, we can assume the former to be at least as enhancing as the latter, and possibly substantially more.

### 2.5. Annotations

In addition to the data extracted from the literature, we annotated proteins on sequence and structure levels. As all the sequences were mapped to UniProt through their accession numbers, we extracted protein names, species of origin, InterPro families, and Enzyme Commission numbers from there. We also manually linked proteins with their structures in PDB. We prioritized the X-ray crystallographic structures with the highest resolution, without ligands or mutations. The assigned structures were then used as an input to HotSpot Wizard (HSW) [19] to obtain additional sequence and structural features.

HSW sequence features come from multiple sequence alignment of homologous sequences. HSW obtains these sequences by a BLAST search [20] against the UniRef90 database [21] and clusters them using the UCLUST algorithm [22] with a 90% sequence identity. Sorted by the coverage of the BLAST query, the top 200 cluster-representing sequences are selected and subsequently aligned using Clustal Omega [23]. The resulting alignment is then employed (i) to estimate the conservation score for each position...
using the Jensen-Shannon divergence [24], (ii) to identify correlated positions using the consensus prediction of several tools integrated with HSW, and (iii) to identify potential back-to-consensus mutations, i.e., the positions in the multiple sequence alignment where an amino acid in the query sequence differs from the majority of amino acids at conserved positions.

Apart from sequence features, the following structural features are included: (i) the protein secondary structure calculated by DSSP [25], (ii) the accessible surface area calculated with the Shrame and Rupley algorithm [26], (iii) average B-factors for protein residues [27], (iv) protein pockets identified by the fpocket tool [28], and (v) protein tunnels and their bottlenecks calculated by CAVER [29]. Only the tunnels connected with catalytic pockets are stored in the database. The structural features are mapped back onto UniProt sequences using the SIFTS database [30].

2.6. Database structure

Measurement results of differential solubility experiments are at the core of our database. Each result is linked to a protein variant defined by a particular protein and a set of substitutions in its sequence. The effect of any protein variant on solubility contains a difference in the measured property compared to the original protein variant, both measured under the same experimental setup. This setup includes the host cell, assay, or temperature used and is linked to the corresponding results. The corresponding protein is identified by UniProt AC, and the mutated positions are based on the UniProt indexing. Each result has its alphanumerical accession code, which is meant to be stable, searchable, and therefore citable. In addition, each result may be linked to one or more published datasets.

3. Results

The basic statistics summarizing the content of the database are given in Table 2. The total number of datapoints consists of (i) merged 764 (610 unique) datapoints from the previously published datasets, (ii) Whitehead’s DMS data – accounting for 32,081 of the datapoints, (iii) 279 new measurements from the literature and (iv) 22 measurements carried out in-house.

The data reveal that a random mutation likely has a desolubilizing effect, as shown in the mutational effect distribution in Fig. 2. Only 18% of mutants increase solubility and just one third of them significantly. This is confirmed when the distribution is plotted per protein (Fig. 3). The three most frequent proteins from small-scale experiments, on the other hand, display a strong distribution bias compared to the DMS data and the ‘Other’ category alike. The exact ratio is protein-dependent.

While the database size is several orders of magnitude larger than the sizes of the prior datasets, the results from the high-throughput experiments from Whitehead’s group dominate the deposited data. The exhaustiveness of Whitehead’s data provides the database with great variability in mutated positions and in combinations of substituted and target amino-acid pairs (Fig. 4) but is limited to only three proteins. The protein variability of the database is provided by the rest of the data - Fig. 5 contrasts the entry counts for these three proteins with the remaining ones.

We kept the FAIR principles (Findable, Accessible, Interoperable, Reusable) [31] in mind during the database development. In addition to making the data accessible and searchable online (see the section 3.1) and exportable in a machine-readable format (see the section 3.2), we also assigned a unique accession code (SPMDB AC) to each entry of a measurement result. The accession code is an identifier that is stable in time and can be used for searching or linking. Our database crosslinks SPMDB AC with UniProt, PDB, and InterPro databases.
developed Export Wizard for user-friendly exporting a currently browsed subset of the database, e.g., defined by the active search filter, as a tabular dataset in the CSV format. This functionality is specifically aimed at data scientists and machine learning developers to allow them to analyze or use the data with minimum processing effort. Optionally, additional filtering/labeling and data augmentation may be applied before data export.

The filtering also allows selecting only the results measured in continuous values, suitable for a regression analysis and modeling. The alternative is the labeling that adapts the data to a specific model according to the number of bins distinguished by effects on solubility: after selecting a model from Table 1, each exported datapoint is assigned a label from that system. If a reported effect is not present in the selected system, it is either converted to a partially compatible label or dropped. The process may be adjusted by selecting one of the abundance, reliability, or compromise modes.

The first option converts as many values as possible; the second option leaves out all incompatible values; and the third option compromises on the significance, i.e., all converted labels are marked defensively as a slight change. Users can display the active conversion table by clicking ‘See details’. The user interface for this step is shown in Fig. A.6.

Finally, in the case of ML-dataset creation, users may want to use the data-augmentation (data-symmetrization) function, which adds the reverse mutations to the dataset, i.e., datapoints with substituted and target residues swapped and inverse solubility effects. This will resolve the likely problem of the imbalance between the counts of deteriorating and enhancing mutations (Fig. 2), which has often been reported to decrease the performance of predictors for other mutational data types [34–36].

4. Discussion

SoluProtMutDB is the first mutational database of solubility data and is ready to serve as a central depository for data from mutagenesis experiments targeting protein solubility. To date, our database contains almost 33 000 experimental results of solubility effects upon mutations, thereby representing an essential digital resource for this type of data. The database comprises the previously published datasets and new data from the more recent literature. We have improved the reliability of these datasets by manual curation and overlap checks. We examined over a hundred original publications from which the data were gathered, including a few studies that produced hundreds to thousands of datapoints each, thanks to the use of such high-throughput experimental techniques as FACS. Lastly, we deposited the solubility data measured in our group. We will maintain the database, add new data, and continue with the curation process.

We believe the database is of great value for data scientists and will help to understand the mechanisms controlling solubility. With this in mind, we also focused on the ML potential of the database by making our database friendly for the ML community: (i) we ensured the data are reliable; (ii) we systematized the solubility effects reported in the literature to be easily understood by the experts outside biology; and (iii) we created Export Wizard to facilitate adaptation of the data for ready-made ML models. As a result, we expect that the user-friendly web interface and the other steps taken will broaden the audience and user community. The data can now be analyzed or modeled, even without a deep understanding of the underlying technical or biological details.

Thanks to the new data published in recent years, the database is an order of magnitude larger than an average solubility dataset. This abundance comes from recent high-throughput experiments, generating a more realistic distribution of target amino acids and observed effects compared to the previous datasets owing to the possibility of covering all possible single-point mutants.
Specifically, the DMS experiments manifest their strength as they show no extreme per-protein deviation of the effect distribution (Fig. 3) from the overall distribution (Fig. 2), which is of particular importance for ML applications. The DMS data are highly representative as they lack a selection bias in introduced mutations (Fig. A.3). Moreover, the substituted amino acids in the database follow the distribution of amino acids seen in nature (Fig. A.2). In contrast, the selection bias is apparent in the small-scale experiments, even when all their data are merged (Fig. A.4). In terms of effect distribution, the DMS data display more desolubilizing mutations (Fig. A.5). And since the DMS data are measured indirectly and a systematic error of a measurement may be present, we suggest using non-DMS data for ML model evaluation.

In order not to miss any important factor possibly affecting solubility, we track many conditions of experiments. Yet, several factors known or suspected to influence protein expression or solubility are not stored explicitly in the current version of the database. Some of these factors are silent mutations, i.e., mutations on the nucleotide-sequence level that do not propagate into the amino-acid sequence but may strongly influence soluble expression, especially heterologous [37]. Another factor is the time of expression, often not reported clearly, e.g., due to a possible complexity of the assay. Timings of different steps of an experiment may influence soluble expression, for example, through expression rate or by providing a different time for molecular interactions (precipitation, aggregation) [38].

Finally, the database promotes the FAIR principles not only by making the solubility data more accessible but also by allowing negative reporting. Currently, many negative findings in solubility experiments remain unreported as they do not bring the desired outcome to the scientists. We encourage the deposition of negative solubility data in SoluProtMut DB to meet the obligations to publish results and reach FAIRness, often imposed by grant agencies. At the same time, these data are of considerable value for the field of ML, even to the extent comparable to that of positive results. Last but not least, non-reporting of negative findings may lead to repeating the same experiments and result in wasting human and material resources. Results of mutational solubility experiments can be sent to soluprot@sci.muni.cz to be deposited in the database.

**CRediT authorship contribution statement**

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ware, Visualization, Writing – original draft, Writing – review & editing. Jan Stourac: Writing – review & editing, Supervision. Milos Musil: Software, Writing – original draft. Jiri Damborsky: Writing – review & editing, Supervision. David Bednar: Writing – review & editing, Supervision. Stanislav Mazurenko: Data curation, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supportive information

Figs. A.1, A.2, A.3, A.4, A.5, A.6

Fig. A.1. A matrix showing the numbers of mutation occurrences in the database ‘from’ (rows) and ‘to’ (columns) specific amino acids. The \( \Sigma \) column and row represent the total numbers of mutations ‘from’ and ‘to’ given amino acids, respectively. The matrix is row-weighted – blue saturation corresponds to the relative abundance of the given ‘to’ amino acid in the corresponding row. This is to avoid accentuation of differences naturally caused by the uneven distribution of amino acids in natural sequences.

Fig. A.2. The histogram of the substituted amino acids in the database. The red bar shows the deviation from the natural distribution of amino acids, as in sequences of all kingdoms of life [39].

Fig. A.3. A row-weighted substitution matrix for Whitehead’s data. It shows some anomalies, such as visible under-representation of substitutions to methionine (M) or tryptophan (W).
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