1. EASE-MM

Protein engineering and characterisation of non-synonymous single nucleotide variants (SNVs) require accurate prediction of protein stability changes (ΔΔGu) induced by single amino acid substitutions. Here, we have developed a new prediction method called Evolutionary, Amino acid, and Structural Encodings with Multiple Models (EASE-MM), which comprises five specialised support vector machine (SVM) models and makes the final prediction from a consensus of two models selected based on the predicted secondary structure and accessible surface area of the mutated residue. The new method is applicable to single-domain monomeric proteins and can predict ΔΔGu with a protein sequence and mutation as the only inputs. EASE-MM yielded a Pearson correlation coefficient of 0.53–0.59 in 10-fold cross-validation and independent testing and was able to outperform other sequence-based methods. When compared to structure-based energy functions, EASE-MM achieved a comparable or better performance. The application to a large dataset of human germline non-synonymous SNVs showed that the disease-causing variants tend to be associated with larger magnitudes of ΔΔGu predicted with EASE-MM. The EASE-MM web-server is available at http://sparks-lab.org/server/ease.

2. pStab

We present a web-server for rapid prediction of changes in protein stabilities over a range of temperatures and experimental conditions upon single- or multiple-point substitutions of charged residues. Potential mutants are identified by a charge-shuffling procedure while the stability changes (i.e. an unfolding curve) are predicted employing an ensemble-based statistical-mechanical model. We expect this server to be a simple yet detailed tool for engineering stabilities, identifying electrostatically frustrated residues, generating local stability maps and in constructing fitness landscapes.

3. PopMusic

PoPMuSiC-2.1 is a web server that predicts the thermodynamic stability changes caused by single site mutations in proteins, using a linear combination of statistical potentials whose coefficients depend on the solvent accessibility of the mutated residue. PoPMuSiC presents good prediction performances (correlation coefficient of 0.8 between predicted and measured stability changes, in cross validation, after exclusion of 10% outliers). It is moreover very fast, allowing the prediction of the stability changes resulting from all possible mutations in a medium size protein in less than a minute. This unique functionality is user-friendly implemented in PoPMuSiC and is particularly easy to exploit. Another new functionality of our server concerns the estimation of the optimality of each amino acid in the sequence, with respect to the stability of the structure. It may be used to detect structural weaknesses, i.e. clusters of non-optimal residues, which represent particularly interesting sites for introducing targeted mutations. This sequence optimality data is also expected to have significant implications in the prediction and the analysis of particular structural or functional protein regions. To illustrate the interest of this new functionality, we apply it to a dataset of known catalytic sites, and show that a much larger than average concentration of structural weaknesses is detected, quantifying how these sites have been optimized for function rather than stability.

4. MuStab

In this study, a new machine learning method has been developed for sequence feature-based prediction of protein stability changes upon amino acid substitutions. Support vector machines were trained with data from experimental studies on the free energy change of protein stability upon mutations. To construct accurate classifiers, twenty sequence features were examined for input vector encoding. It was shown that classifier performance varied significantly by using different sequence features. The most accurate classifier in this study was constructed using a combination of six sequence features. This classifier achieved an overall accuracy of 84.59% with 70.29% sensitivity and 90.98% specificity.

Relevant sequence features can be used to accurately predict protein stability changes upon amino acid substitutions. Predictive results at this level of accuracy may provide useful information to distinguish between deleterious and tolerant alterations in disease candidate genes. To make the classifier accessible to the genetics research community, we have developed a new web server, called MuStab (http://bioinfo.ggc.org/mustab/).

5. NeEmo

Here we propose NeEMO, a tool for the evaluation of stability changes using an effective representation of proteins based on residue interaction networks (RINs). RINs are used to extract useful features describing interactions of the mutant amino acid with its structural environment. Benchmarking shows NeEMO to be very effective, allowing reliable predictions in different parts of the protein such as b-strands and buried residues. Validation on a previously published independent dataset shows that NeEMO has a Pearson correlation coefficient of 0.77 and a standard error of 1 Kcal/mol, outperforming nine recent methods. The NeEMO web server can be freely accessed from URL: http://protein.bio.unipd.it/neemo/.

NeEMO offers an innovative and reliable tool for the annotation of amino acid changes. A key contribution are RINs, which can be used for modeling proteins and their interactions effectively. Interestingly, the approach is very general, and can motivate the development of a new family of RIN-based protein structure analyzers. NeEMO may suggest innovative strategies for bioinformatics tools beyond protein stability prediction.

6. AUTO-MUTE

The AUTO-MUTE 2.0 stand-alone software package includes a collection of programs for predicting functional changes to proteins upon single residue substitutions, developed by combining structure-based features with trained statistical learning models. Three of the predictors evaluate changes to protein stability upon mutation, each complementing a distinct experimental approach. Two additional classifiers are available, one for predicting activity changes due to residue replacements and the other for determining the disease potential of mutations associated with nonsynonymous single nucleotide polymorphisms (nsSNPs) in human proteins. These five command-line driven tools, as well as all the supporting programs, complement those that run our AUTO-MUTE web-based server. Nevertheless, all the codes have been rewritten and substantially altered for the new portable software, and they incorporate several new features based on user feedback. Included among these upgrades is the ability to perform three highly requested tasks: to run “big data” batch jobs; to generate predictions using modified protein data bank (PDB) structures, and unpublished personal models prepared using standard PDB file formatting; and to utilize NMR structure files that contain multiple models.

7. ProMaya

Accurate prediction of protein stability is important for understanding the molecular underpinnings of diseases and for the design of new proteins. We introduce a novel approach for the prediction of changes in protein stability that arise from a single-site amino acid substitution; the approach uses available data on mutations occurring in the same position and in other positions. Our algorithm, named Pro-Maya (Protein Mutant stAbilitY Analyzer), combines a collaborative filtering baseline model, Random Forests regression and a diverse set of features. Pro-Maya predicts the stability free energy difference of mutant versus wild type, denoted as G.

We evaluated our algorithm extensively using cross-validation on two previously utilized datasets of single amino acid mutations and a (third) validation set. The results indicate that using known G values of mutations at the query position improves the accuracy of G predictions for other mutations in that position. The accuracy of our predictions in such cases significantly surpasses that of similar methods, achieving, e.g. a Pearson’s correlation coefficient of 0.79 and a root mean square error of 0.96 on the validation set. Because Pro-Maya uses a diverse set of features, including predictions using two other methods, it also performs slightly better than other methods in the absence of additional experimental data on the query positions.

8. mCSM

Mutations play fundamental roles in evolution by introducing diversity into genomes. Missense mutations in structural genes may become either selectively advantageous or disadvantageous to the organism by affecting protein stability and/or interfering with interactions between partners. Thus, the ability to predict the impact of mutations on protein stability and interactions is of significant value, particularly in understanding the effects of Mendelian and somatic mutations on the progression of disease. Here, we propose a novel approach to the study of missense mutations, called mCSM, which relies on graph-based signatures. These encode distance patterns between atoms and are used to represent the protein residue environment and to train predictive models. To understand the roles of mutations in disease, we have evaluated their impacts not only on protein stability but also on protein–protein and protein–nucleic acid interactions.

We show that mCSM performs as well as or better than other methods that are used widely. The mCSM signatures were successfully used in different tasks demonstrating that the impact of a mutation can be correlated with the atomic-distance patterns surrounding an amino acid residue. We showed that mCSM can predict stability changes of a wide range of mutations occurring in the tumour suppressor protein p53, demonstrating the applicability of the proposed method in a challenging disease scenario.

9. ELASPIC

ELASPIC is a novel ensemble machine-learning approach that predicts the effects of mutations on protein folding and protein–protein interactions. Here, we present the ELASPIC webserver, which makes the ELASPIC pipeline available through a fast and intuitive interface. The webserver can be used to evaluate the effect of mutations on any protein in the Uniprot database, and allows all predicted results, including modeled wild-type and mutated structures, to be managed and viewed online and downloaded if needed. It is backed by a database which contains improved structural domain definitions, and a list of curated domain–domain interactions for all known proteins, as well as homology models of domains and domain–domain interactions for the human proteome. Homology models for proteins of other organisms are calculated on the fly, and mutations are evaluated within minutes once the homology model is available.

10. STRUM

Mutations in human genome are mainly through single nucleotide polymorphism, some of which can affect stability and function of proteins, causing human diseases. Several methods have been proposed to predict the effect of mutations on protein stability; but most require features from experimental structure. Given the fast progress in protein structure prediction, this work explores the possibility to improve the mutation-induced stability change prediction using low-resolution structure modeling.

We developed a new method (STRUM) for predicting stability change caused by single-point mutations. Starting from wild-type sequences, 3D models are constructed by the iterative threading assembly refinement (I-TASSER) simulations, where physics- and knowledge-based energy functions are derived on the I-TASSER models and used to train STRUM models through gradient boosting regression. STRUM was assessed by 5-fold cross validation on 3421 experimentally determined mutations from 150 proteins. The Pearson correlation coefficient (PCC) between predicted and measured changes of Gibbs free-energy gap, DDG, upon mutation reaches 0.79 with a root-mean-square error 1.2 kcal/mol in the mutation-based cross-validations. The PCC reduces if separating training and test mutations from non-homologous proteins, which reflects inherent correlations in the current mutation sample. Nevertheless, the results significantly outperform other state-of-the-art methods, including those built on experimental protein structures. Detailed analyses show that the most sensitive features in STRUM are the physics-based energy terms on I-TASSER models and the conservation scores from multiple-threading template alignments. However, the DDG prediction accuracy has only a marginal dependence on the accuracy of protein structure models as long as the global fold is correct. These data demonstrate the feasibility to use low-resolution structure modeling for high-accuracy stability change prediction upon point mutations.

11. MuPro

Accurate prediction of protein stability changes resulting from single amino acid mutations is important for understanding protein structures and designing new proteins. We use support vector machines to predict protein stability changes for single amino acid mutations leveraging both sequence and structural information. We evaluate our approach using cross-validation methods on a large dataset of single amino acid mutations. When only the sign of the stability changes is considered, the predictive method achieves 84% accuracy—a significant improvement over previously published results. Moreover, the experimental results show that the prediction accuracy obtained using sequence alone is close to the accuracy obtained using tertiary structure information. Because our method can accurately predict protein stability changes using primary sequence information only, it is applicable to many situations where the tertiary structure is unknown, overcoming a major limitation of previous methods which require tertiary information. The web server for predictions of protein stability changes upon mutations (MUpro), software, and datasets are available at http://www.igb.uci.edu/servers/servers.html. Proteins 2006;

12. I-Mutant2.0

I-Mutant2.0 is a support vector machine (SVM)-based tool for the automatic prediction of protein stability changes upon single point mutations. I-Mutant2.0 predictions are performed starting either from the protein structure or, more importantly, from the protein sequence. This latter task, to the best of our knowledge, is exploited for the first time. The method was trained and tested on a data set derived from ProTherm, which is presently the most comprehensive available database of thermodynamic experimental data of free energy changes of protein stability upon mutation under different conditions. I-Mutant2.0 can be used both as a classifier for predicting the sign of the protein stability change upon mutation and as a regression estimator for predicting the related DDG values. Acting as a classifier, I-Mutant2.0 correctly predicts (with a cross-validation procedure) 80% or 77% of the data set, depending on the usage of structural or sequence information, respectively. When predicting DDG values associated with mutations, the correlation of predicted with expected/experimental values is 0.71 (with a standard error of 1.30 kcal/mol) and 0.62 (with a standard error of 1.45 kcal/mol) when structural or sequence information are respectively adopted. Our web interface allows the selection of a predictive mode that depends on the availability of the protein structure and/or sequence. In this latter case, the web server requires only pasting of a protein sequence in a raw format. We therefore introduce I-Mutant2.0 as a unique and valuable helper for protein design, even when the protein structure is not yet known with atomic resolution. Availability: http://gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant2.0/I-Mutant2.0.cgi.

13. FoldX

FoldX is an empirical force field that was developed for the rapid evaluation of the effect of mutations on the stability, folding and dynamics of proteins and nucleic acids. The core functionality of FoldX, namely the calculation of the free energy of a macromolecule based on its high-resolution 3D structure, is now publicly available through a web server at http://foldx.embl.de/. The current release allows the calculation of the stability of a protein, calculation of the positions of the protons and the prediction of water bridges, prediction of metal binding sites and the analysis of the free energy of complex formation. Alanine scanning, the systematic truncation of side chains to alanine, is also included. In addition, some reporting functions have been added, and it is now possible to print both the atomic interaction networks that constitute the protein, print the structural and energetic details of the interactions per atom or per residue, as well as generate a general quality report of the pdb structure. This core functionality will be further extended as more FoldX applications are developed.

14. Sride

Residues expected to play key roles in the stabilization of proteins [stabilizing residues (SRs)] are selected by combining several methods based mainly on the interactions of a given residue with its spatial, rather than its sequential neighborhood and by considering the evolutionary conservation of the residues. A residue is selected as a stabilizing residue if it has high surrounding hydrophobicity, high long-range order, high conservation score and if it belongs to a stabilization center. The definition of all these parameters and the thresholds used to identify the SRs are discussed in detail. The algorithm for identifying SRs was originally developed for TIM-barrel proteins [M. M. Gromiha, G. Pujadas, C. Magyar, S. Selvaraj, and I. Simon (2004), Proteins, 55, 316–329] and is now generalized for all proteins of known 3D structure. SRs could be applied in protein engineering and homology modeling and could also help to explain certain folds with significant stability. The SRide server is located at http://sride.enzim.hu.

15. CUPSAT

CUPSAT (Cologne University Protein Stability Analysis Tool) is a web tool to analyse and predict protein stability changes upon point mutations (single amino acid mutations). This program uses structural environment specific atom potentials and torsion angle potentials to predictDDG, the difference in free energy of unfolding between wild-type and mutant proteins. It requires the protein structure in Protein Data Bank format and the location of the residue to be mutated. The output consists information about mutation site, its structural features (solvent accessibility, secondary structure and torsion angles), and comprehensive information about changes in protein stability for 19 possible substitutions of a specific amino acid mutation. Additionally, it also analyses the ability of the mutated amino acids to adapt the observed torsion angles. Results were tested on 1538 mutations from thermal denaturation and 1603 mutations from chemical denaturation experiments. Several validation tests (split-sample, jack-knife and k-fold) were carried out to ensure the reliability, accuracy and transferability of the prediction method that gives .80% prediction accuracy for most of these validation tests. Thus, the program serves as a valuable tool for the analysis of protein design and stability. The tool is accessible from the link http://cupsat.uni-koeln.de.

16. DUET

Cancer genome and other sequencing initiatives are generating extensive data on non-synonymous single nucleotide polymorphisms (nsSNPs) in human and other genomes. In order to understand the impacts of nsSNPs on the structure and function of the proteome, as well as to guide protein engineering, accurate in silicomethodologies are required to study and predict their effects on protein stability. Despite the diversity of available computational methods in the literature, none has proven accurate and dependable on its own under all scenarios where mutation analysis is required. Here we present DUET, a web server for an integrated computational approach to study missense mutations in proteins. DUET consolidates two complementary approaches (mCSM and SDM) in a consensus prediction, obtained by combining the results of the separate methods in an optimized predictor using Support Vector Machines (SVM). We demonstrate that the proposed method improves overall accuracy of the predictions in comparison with either method individually and performs as well as or better than similar methods. The DUET web server is freely and openly available at http://structure.bioc.cam.ac.uk/duet.

17. SAAFEC

Folding free energy is an important biophysical characteristic of proteins that reflects the overall stability of the 3D structure of macromolecules. Changes in the amino acid sequence, naturally occurring or made in vitro, may affect the stability of the corresponding protein and thus could be associated with disease. Several approaches that predict the changes of the folding free energy caused by mutations have been proposed, but there is no method that is clearly superior to the others. The optimal goal is not only to accurately predict the folding free energy changes, but also to characterize the structural changes induced by mutations and the physical nature of the predicted folding free energy changes. Here we report a new method to predict the Single Amino Acid Folding free Energy Changes (SAAFEC) based on a knowledge-modified Molecular Mechanics Poisson-Boltzmann (MM/PBSA) approach. The method is comprised of two main components: a MM/PBSA component and a set of knowledge based terms delivered from a statistical study of the biophysical characteristics of proteins. The predictor utilizes a multiple linear regression model with weighted coefficients of various terms optimized against a set of experimental data. The aforementioned approach yields a correlation coefficient of 0.65 when benchmarked against 983 cases from 42 proteins in the ProTherm database. Availability: the webserver can be accessed via http://compbio.clemson.edu/SAAFEC/.

18. iRDP

Engineering protein molecules with desired structure and biological functions has been an elusive goal. Development of industrially viable proteins with improved properties such as stability, catalytic activity and altered specificity by modifying the structure of an existing protein has widely been targeted through rational protein engineering. Although a range of factors contributing to thermal stability have been identified and widely researched, the in silico implementation of these as strategies directed towards enhancement of protein stability has not yet been explored extensively. A wide range of structural analysis tools is currently available for in silico protein engineering. However these tools concentrate on only a limited number of factors or individual protein structures, resulting in cumbersome and time-consuming analysis. The iRDP web server presented here provides a unified platform comprising of iCAPS, iStability and iMutants modules. Each module addresses different facets of effective rational engineering of proteins aiming towards enhanced stability. While iCAPS aids in selection of target protein based on factors contributing to structural stability, iStability uniquely offers in silico implementation of known thermostabilization strategies in proteins for identification and stability prediction of potential stabilizing mutation sites. iMutants aims to assess mutants based on changes in local interaction network and degree of residue conservation at the mutation sites. Each module was validated using an extensively diverse dataset. The server is freely accessible at http://irdp.ncl.res.in and has no login requirements.

19. Rosetta

The prediction of changes in protein stability and structure resulting from single amino acid substitutions is both a fundamental test of macromolecular modeling methodology and an important current problem as high throughput sequencing reveals sequence polymorphisms at an increasing rate. In principle, given the structure of a wild-type protein and a point mutation whose effects are to be predicted, an accurate method should recapitulate both the structural changes and the change in the folding-free energy. Here, we explore the performance of protocols which sample an increasing diversity of conformations. We find that surprisingly similar performances in predicting changes in stability are achieved using protocols that involve very different amounts of conformational sampling, provided that the resolution of the force field is matched to the resolution of the sampling method. Methods involving backbone sampling can in some cases closely recapitulate the structural changes accompanying mutations but not surprisingly tend to do more harm than good in cases where structural changes are negligible. Analysis of the outliers in the stability change calculations suggests areas needing particular improvement; these include the balance between desolvation and the formation of favorable buried polar interactions, and unfolded state modeling.

20. Topologynet

Although deep learning approaches have had tremendous success in image, video and audio processing, computer vision, and speech recognition, their applications to three-dimensional (3D) biomolecular structural data sets have been hindered by the geometric and biological complexity. To address this problem we introduce the element-specific persistent homology (ESPH) method. ESPH represents 3D complex geometry by one-dimensional (1D) topological invariants and retains important biological information via a multichannel image-like representation. This representation reveals hidden structure-function relationships in biomolecules. We further integrate ESPH and deep convolutional neural networks to construct a multichannel topological neural network (TopologyNet) for the predictions of protein-ligand binding affinities and protein stability changes upon mutation. To overcome the deep learning limitations from small and noisy training sets, we propose a multi-task multichannel topological convolutional neural network (MM-TCNN). We demonstrate that TopologyNet outperforms the latest methods in the prediction of protein-ligand binding affinities, mutation induced globular protein folding free energy changes, and mutation induced membrane protein folding free energy changes. Availability: weilab.math.msu. edu/TDL/

21. PROTS-RF

The ability to improve protein thermostability via protein engineering is of great scientific interest and also has significant practical value. In this report we present PROTS-RF, a robust model based on the Random Forest algorithm capable of predicting thermostability changes induced by not only single-, but also double- or multiple-point mutations. The model is built using 41 features including evolutionary information, secondary structure, solvent accessibility and a set of fragment-based features. It achieves accuracies of 0.799,0.782, 0.787, and areas under receiver operating characteristic (ROC) curves of 0.873, 0.868 and 0.862 for single-, double- and multiple- point mutation datasets, respectively. Contrary to previous suggestions, our results clearly demonstrate that a robust predictive model trained for predicting single point mutation induced thermostability changes can be capable of predicting double and multiple point mutations. It also shows high levels of robustness in the tests using hypothetical reverse mutations. We demonstrate that testing datasets created based on physical principles can be highly useful for testing the robustness of predictive models.

22. MAESTRO

Point mutations can have a strong impact on protein stability. A change in stability may subsequently lead to dysfunction and finally cause diseases. Moreover, protein engineering approaches aim to deliberately modify protein properties, where stability is a major constraint. In order to support basic research and protein design tasks, several computational tools for predicting the change in stability upon mutations have been developed. Comparative studies have shown the usefulness but also limitations of such programs.

We aim to contribute a novel method for predicting changes in stability upon point mutation in proteins called MAESTRO. MAESTRO is structure based and distinguishes itself from similar approaches in the following points: (i) MAESTRO implements a multi-agent machine learning system. (ii) It also provides predicted free energy change (G) values and a corresponding prediction confidence estimation. (iii) It provides high throughput scanning for multi-point mutations where sites and types of mutation can be comprehensively controlled. (iv) Finally, the software provides a specific mode for the prediction of stabilizing disulfide bonds. The predictive power of MAESTRO for single point mutations and stabilizing disulfide bonds is comparable to similar methods.

MAESTRO is a versatile tool in the field of stability change prediction upon point mutations.

Executables for the Linux and Windows operating systems are freely available to non-commercial users from http://biwww.che.sbg.ac.at/MAESTRO.

23. INPS-MD

Protein function depends on its structural stability. The effects of single point variations on protein stability can elucidate the molecular mechanisms of human diseases and help in developing new drugs. Recently, we introduced INPS, a method suited to predict the effect of variations on protein stability from protein sequence and whose performance is competitive with the available state-of-the-art tools.

In this paper, we describe INPS-MD (Impact of Non synonymous variations on Protein Stability-Multi-Dimension), a web server for the prediction of protein stability changes upon single point variation from protein sequence and/or structure. Here, we complement INPS with a new predictor (INPS3D) that exploits features derived from protein 3D structure. INPS3D scores with Pearson's correlation to experimental ΔΔG values of 0.58 in cross validation and of 0.72 on a blind test set. The sequence-based INPS scores slightly lower than the structure-based INPS3D and both on the same blind test sets well compare with the state-of-the-art methods.

24. HotMusic

The accurate prediction of the impact of an amino acid substitution on the thermal stability of a protein is a central issue in protein science, and is of key relevance for the rational optimization of various bioprocesses that use enzymes in unusual conditions. Here we present one of the first computational tools to predict the change in melting temperature ΔTm upon point mutations, given the protein structure and, when available, the melting temperature Tm of the wild-type protein. The key ingredients of our model structure are standard and temperature-dependent statistical potentials, which are combined with the help of an artificial neural network. The model structure was chosen on the basis of a detailed thermodynamic analysis of the system. The parameters of the model were identified on a set of more than 1,600 mutations with experimentally measured ΔTm. The performance of our method was tested using a strict 5-fold cross-validation procedure, and was found to be significantly superior to that of competing methods. We obtained a root mean square deviation between predicted and experimental ΔTm values of 4.2°C that reduces to 2.9°C when ten percent outliers are removed. A webserver-based tool is freely available for non-commercial use at soft.dezyme.com.

25. ERIS

Just letter, no abstract

26. PROSS

Upon heterologous overexpression, many proteins misfold or aggregate, thus resulting in low functional yields. Human acetylcholinesterase (hAChE), an enzyme mediating synaptic transmission, is a typical case of a human protein that necessitates mammalian systems to obtain functional expression. We developed a computational strategy and designed an AChE variant bearing 51 mutations that improved core packing, surface polarity, and backbone rigidity. This variant expressed at 2,000-fold higher levels in E. coli compared to wild-type hAChE and exhibited 20 C higher thermostability with no change in enzymatic properties or in the active-site configuration as determined by crystallography. To demonstrate broad utility, we similarly designed four other human and bacterial proteins. Testing at most three designs per protein, we obtained enhanced stability and/or higher yields of soluble and active protein in E. coli. Our algorithm requires only a 3D structure and several dozen sequences of naturally occurring homologs, and is available at http://pross.weizmann.ac.il.

27. FireProt

There is a continuous interest in increasing proteins stability to enhance their usability in numerous biomedical and biotechnological applications. A number of in silico tools for the prediction of the effect of mutations on protein stability have been developed recently. However, only single-point mutations with a small effect on protein stability are typically predicted with the existing tools and have to be followed by laborious protein expression, purification, and characterization. Here, we present FireProt, a web server for the automated design of multiple-point thermostable mutant proteins that combines structural and evolutionary information in its calculation core. FireProt utilizes sixteen tools and three protein engineering strategies for making reliable protein designs. The server is complemented with interactive, easy-to-use interface that allows users to directly analyze and optionally modify designed thermostable mutants. FireProt is freely available at http://loschmidt.chemi.muni.cz/fireprot.

28. FRESCO

The ability to engineer enzymes and other proteins to any desired stability would have wide-ranging applications. Here, we demonstrate that computational design of a library with chemically diverse stabilizing mutations allows the engineering of drastically stabilized and fully functional variants of the mesostable enzyme limonene epoxide hydrolase. First, point mutations were selected if they significantly improved the predicted free energy of protein folding. Disulfide bonds were designed using sampling of backbone conformational space, which tripled the number of experimentally stabilizing disulfide bridges. Next, orthogonal in silico screening steps were used to remove chemically unreasonable mutations and mutations that are predicted to increase protein flexibility. The resulting library of 64 variants was experimentally screened, which revealed 21 (pairs of) stabilizing mutations located both in relatively rigid and in flexible areas of the enzyme. Finally, combining 10– 12 of these confirmed mutations resulted in multi-site mutants with an increase in apparent melting temperature from 50 to 8588 C, enhanced catalytic activity, preserved regioselectivity and a >250-fold longer half-life. The developed Framework for Rapid Enzyme Stabilization by Computational libraries (FRESCO) requires far less screening than conventional directed evolution.