MOSBRI
Molecular-Scale Biophysics
Research Infrastructure

Protein stabilisation: Design, Experiments and Assessment (ProteSta)

ALS1 (BIFI-LACRIMA) 3rd-7thJuly 2023, Zaragoza, Spain

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

DAY4 (6 July, 2023) 9:00/13:00

Computational tools for protein stabilization

Javier Sancho

- Rosetta
- FoldX
- PoPMusic
- Protposer: a different approach

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Rosetta

Rosetta Commons

The hub for Rosetta modeling software

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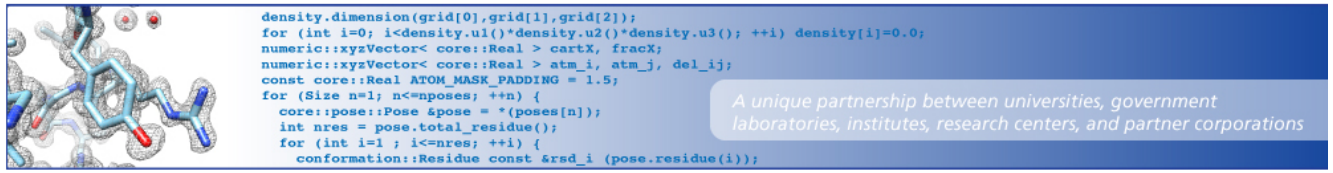

Documentation & Support

- Overview of Rosetta
- Forum
- User Guide
- Demos
- Education

Home » Documentation & Support

Overview of the Rosetta Software Suite

Rosetta is a suite of software libraries for macromolecular modeling. The diverse functionality of the libraries may be used by the end user in different ways:

- A set of premade applications define protocols that can be used to perform a specific task.
- A set of frameworks (PyRosetta, RosettaScripts) allow creation of your own protocols for new use cases.
- Various web servers, notably ROSIE, are set up and lend their computing power to provide applications that may be used remotely, without having to install anything on your computer.

A detailed list of protocols, frameworks and applications is available in the User Guide.

Use cases

Rosetta is best described by the science that our community, as well as other communities, have used it for. It is extendable and adaptable if you need it to be.

Protocols

Rosetta attempts to provide a flexible library of functionality to accomplish a diverse set of biomolecular modeling tasks. The basic tasks and operations that the libraries define, are combined together as algorithms which we call **protocols**, each of which use Rosetta's flexible molecular modeling library to accomplish specific modeling tasks. These protocols can either be used as self contained units, or they can be chained together to accomplish more complex tasks, either by successive use of different applications or by combining the protocols within the generalizable frameworks.

Rosetta algorithms are able to accomplish prediction, design and analysis on a diverse set of bio-molecular systems, including

Below is a partial list of protocols. For the full list, please advise the User Guide.

RosettaAbinitio	De novo protein structure prediction.
RosettaDesign	Identifies low free energy sequences for target protein backbones.
Rosetta Design PyMol Plugin	PyMOL plugin A user-friendly interface for submitting Protein Design simulations using RosettaDesign.
RosettaDock	Predicts the structure of a protein-protein complex from the individual structures of the monomer components.
RosettaAntibody*	Predicts antibody Fv region structures. See PubMed 24519881 and 19062174.
RosettaFragments	Generates fragment libraries for use by Rosetta ab initio in building protein structures.
RosettaNMR	Incorporates NMR data into the basic Rosetta protocol to accelerate the process of NMR structure prediction
RosettaDNA	For the design of proteins that interact with specified DNA sequences.
RosettaRNA	Fragment assembly of RNA.
RosettaLigand	Small molecule - protein docking
RosettaSymmetry	Enforcing symmetry in Rosetta
RosettaEnzdes	Enzyme design
RosettaMembrane	Membrane protein ab initio modeling
RosettaDDG*	Estimating the impact of sequence changes on protein stability
RosettaScripts	An xml-based scripting language for control of modeling trajectories. Supports all major Rosetta functionalities
RosettaSnugDock*	Enables docking an antibody Fv region to an antigen and allows backbone flexibility in the paratope.
RosettaMultigraft	Performs matching, backbone, and side chain grafting of functional motifs onto scaffold proteins.
Rosetta FlexPepDock	Peptide-protein docking
Rosetta ERRASER	Remodeling RNA crystallographic models with electron density constraint.
RosettaVIP	Stabilize a protein by identifying and filling voids
RosettaMatdes	Materials design

* marked applications might not be available in the latest version of Rosetta

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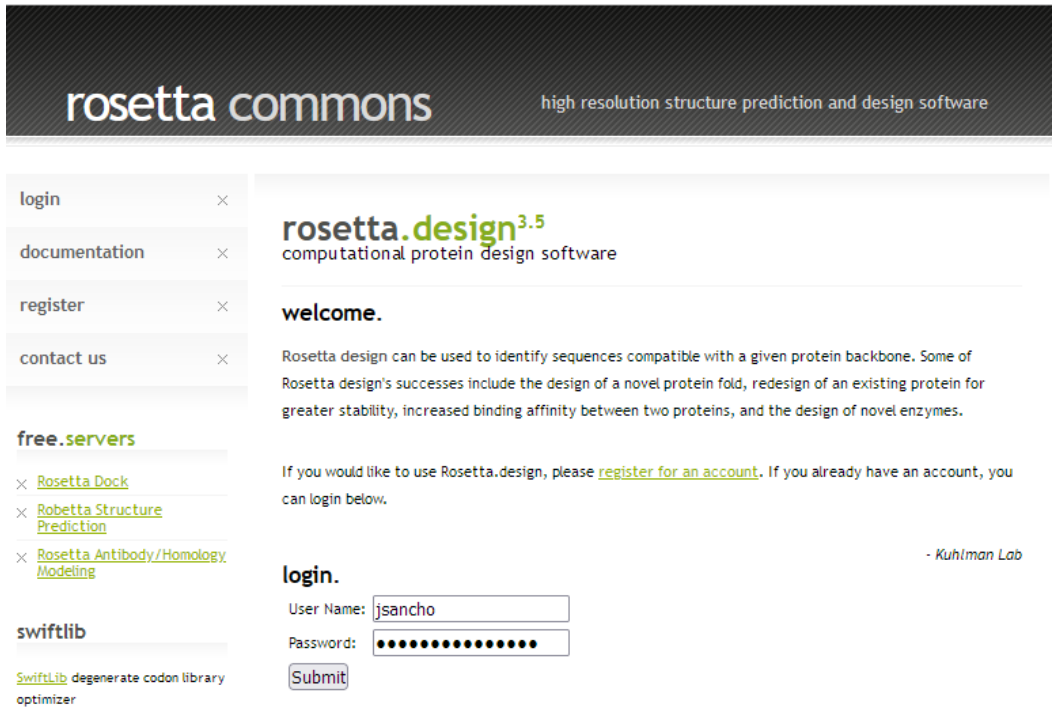
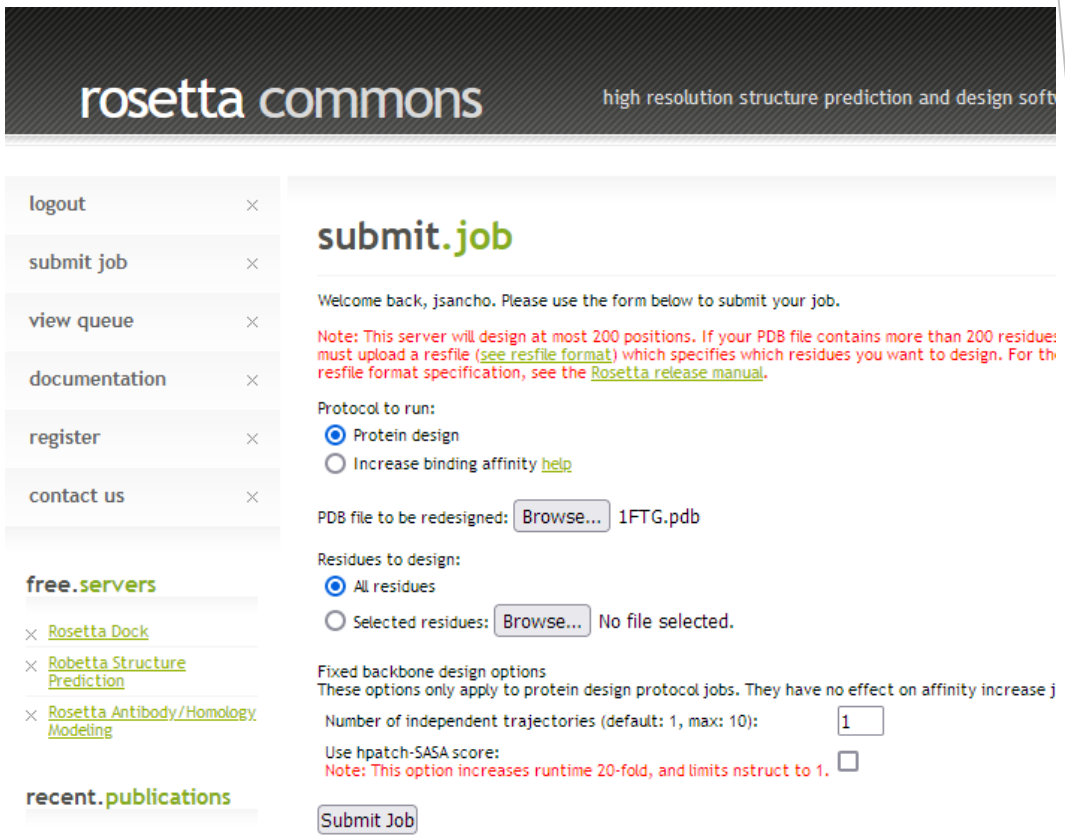
Rosetta





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Rosetta

Score Function

Score functions in Rosetta are weighted sums of energy terms, some of which represent physical forces like electrostatics and van der Waals' interactions, while others represent statistical terms like the probability of finding the torsion angles in Ramachandran space. Below is a list of the energy terms used in the *ref2015* score function:

fa_atr	Lennard-Jones attractive between atoms in different residues
fa_rep	Lennard-Jones repulsive between atoms in different residues
fa_sol	Lazaridis-Karplus solvation energy
fa_intra_sol_xover4	Intra-residue Lazaridis-Karplus solvation energy
lk_ball_wtd	Asymmetric solvation energy
fa_intra_rep	Lennard-Jones repulsive between atoms in the same residue
fa_elec	Coulombic electrostatic potential with a distance-dependent dielectric
pro_close	Proline ring closure energy and energy of psi angle of preceding residue
hbond_sr_bb	Backbone-backbone hbonds close in primary sequence
hbond_lr_bb	Backbone-backbone hbonds distant in primary sequence
hbond_bb_sc	Sidechain-backbone hydrogen bond energy
hbond_sc	Sidechain-sidechain hydrogen bond energy
dslf_fa13	Disulfide geometry potential
rama_prepro	Ramachandran preferences (with separate lookup tables for pre-proline positions and other positions)
omega	Omega dihedral in the backbone. A Harmonic constraint on planarity with standard deviation of ~6 deg.
p_aa_pp	Probability of amino acid, given torsion values for phi and psi
fa_dun	Internal energy of sidechain rotamers as derived from Dunbrack's statistics
yhh_planarity	A special torsional potential to keep the tyrosine hydroxyl in the plane of the aromatic ring
ref	Reference energy for each amino acid. Balances internal energy of amino acid terms. Plays role in design.
METHOD_WEIGHTS	Not an energy term itself, but the parameters for each amino acid used by the ref energy term.

Further description of energy terms can be found here.

The weights associated with the *ref2015* score function are:

```
METHOD_WEIGHTS ref 0.773742 0.443793 -1.63002 -1.96094 0.61937 0.173326 0.388298 1.0806 -0.358574 0.761128 0.249477 -1.19118 -0.250485 -
fa_atr 1
fa_rep 0.55
fa_sol 0.9375
fa_intra_rep 0.005
fa_elec 0.875
pro_close 1.25
hbond_sr_bb 1.17
hbond_lr_bb 1.17
hbond_bb_sc 1.17
hbond_sc 1.1
dslf_fa13 1.25
rama 0.25
omega 0.625
fa_dun 0.7
p_aa_pp 0.4
yhh_planarity 0.625
ref 1
```

Comparing Rosetta Scores to Real-Life Energies

While much of the energy function in Rosetta is physics-based, it also has certain statistical terms to favor structures that look like known protein structures (as nature often conserves protein folds).

While a lower scoring structure is more likely to be closer to the native structure, the scores do not have a direct conversion to physical energy units like kcal/mol. Instead we represent them in *Rosetta Energy Units (REU)*.

Rosetta Scoring tutorial

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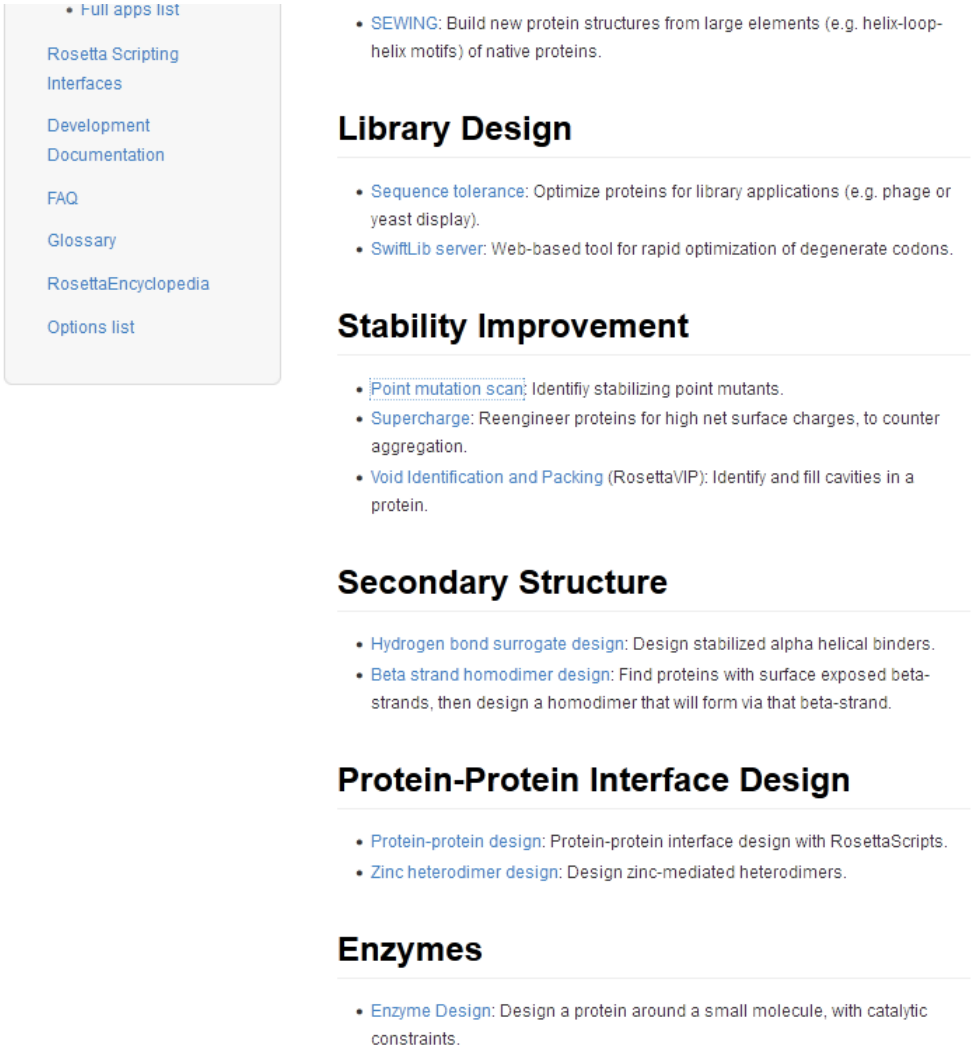
Rosetta



Point Mutation scan

Expected Outputs

The only output from the protocol, by default, is the log file for the run. The log file will contain the predicted ddG and difference in average total energy for all mutants found to be stabilizing. If the option "-output_mutant_structures" was specified, a PDB file for each of the mutants found to be stabilizing will also be output. The PDB files will be named with the input file + a string representing the mutant. For example, the mutant histidine-1-glycine on chain A for structure 1l2y_renameH.pdb will be output to a file named 1l2y_renameH.A-H1G.pdb.

Limitations

Mutants predicted to be stabilizing by the pmut scan protocol may not be stabilizing, and mutants which are stabilizing may be missed. The reasons for these outcomes are varied. The Rosetta energy function is imperfect. Thus, certain energetics which Rosetta does not account for can make a mutant predicted to be stabilizing actually destabilizing. Additionally, to model the mutations, the protocol assumes a fixed backbone conformation. Studies have shown that better results can be achieved using protocols that allow backbone flexibility. Refining the protocol based on the results of experimental characterization of predictions will be necessary to improve prediction accuracy.

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https://github.com/ELELAB/RosettaDDGPrediction

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TOOLS FOR PROTEIN SCIENCE

THE PROTEIN SOCIETY WILEY

RosettaDDGPrediction for high-throughput mutational scans: From stability to binding

Valentina Sora[1,2] | Adrian Otamendi Laspiur[2] | Kristine Degn[2] | Matteo Arnaudi[1,2] | Mattia Utichi[1,2] | Ludovica Beltrame[1,2] | Dayana De Menezes[2] | Matteo Orlandi[2] | Ulrik Kristoffer Stoltze[3,4,5] | Olga Rigina[2] | Peter Wad Sackett[2] | Karin Wadt[3,5] | Kjeld Schmiegelow[4,5] | Matteo Tiberti[1] | Elena Papaleo[1,2]

[1]Cancer Structural Biology, Danish Cancer Society Research Center, Copenhagen, Denmark
[2]Cancer Systems Biology, Section for Bioinformatics, Department of Health and Technology, Technical University of Denmark, Lyngby, Denmark
[3]Department of Clinical Genetics, Copenhagen University Hospital Rigshospitalet, Copenhagen, Denmark
[4]Department of Pediatrics and Adolescent Medicine, University Hospital Rigshospitalet, Copenhagen, Denmark
[5]Institute of Clinical Medicine, Faculty of Medicine, University of Copenhagen, Copenhagen, Denmark

Correspondence
Elena Papaleo, Cancer Structural Biology, Danish Cancer Society Research Center, 2100 Copenhagen, Denmark.
Email: elpap@dtu.dk; elenap@cancer.dk

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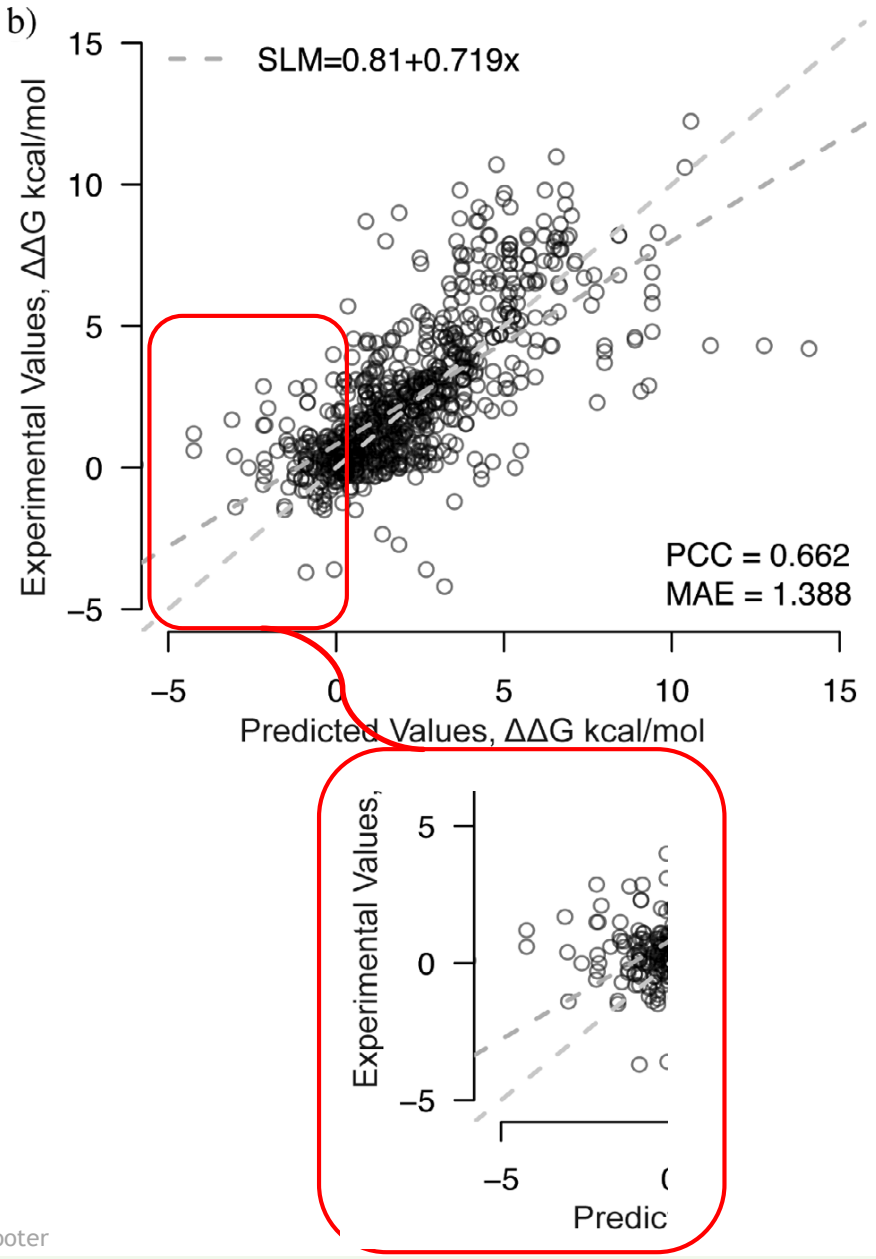
Review Editor: Nir Ben-Tal

Abstract

Reliable prediction of free energy changes upon amino acid substitutions ($\Delta\Delta G$s) is crucial to investigate their impact on protein stability and protein–protein interaction. Advances in experimental mutational scans allow high-throughput studies thanks to multiplex techniques. On the other hand, genomics initiatives provide a large amount of data on disease-related variants that can benefit from analyses with structure-based methods. Therefore, the computational field should keep the same pace and provide new tools for fast and accurate high-throughput $\Delta\Delta G$ calculations. In this context, the Rosetta modeling suite implements effective approaches to predict folding/unfolding $\Delta\Delta G$s in a protein monomer upon amino acid substitutions and calculate the changes in binding free energy in protein complexes. However, their application can be challenging to users without extensive experience with Rosetta. Furthermore, Rosetta protocols for $\Delta\Delta G$ prediction are designed considering one variant at a time, making the setup of high-throughput screenings cumbersome. For these reasons, we devised RosettaDDGPrediction, a customizable Python wrapper designed to run free energy calculations on a set of amino acid substitutions using Rosetta protocols with little intervention from the user. Moreover, RosettaDDGPrediction assists with checking completed runs and aggregates raw data for multiple variants, as well as generates publication-

Valentina Sora and Adrian Otamendi Laspiur equally contributed to this study.



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Foldx

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STABILITY

Calculates the DG to fold the proteins from their unfolded state. The minimal configuration file for Stability is:

```
command=Stability
pdb=ST.pdb
```

It can be run from the command line:

```
FoldX --command=Stability --pdb=ST.pdb
```

FoldX uses output-file as a tag to label different outputs from different commands in batch runs. After running Stability you'll get one file to look at. Given output-file="TAG" the output file is:

TAG_ST.fxout -> calculates the different DG energy terms of folding the protein

If you don't set output-file, TAG will be the pdbId of the first pdb on the batch. Output file contains the Gibbs energy of protein folding decomposed into the different terms used by FOLD-X. This file will have different headers and then rows (one for each PDB run) with the energy decomposition in Kcal/mol, the different columns are described below in the table Energy Terms:

ENERGY TERMS

Title	energy_description
Pdb	Pdb file
Total Energy	This is the predicted overall stability of your protein
Backbone Hbond	This the contribution of backbone Hbonds
Sidechain Hbond	This the contribution of sidechain-sidechain and sidechain-backbone Hbonds
Van der Waals	Contribution of the VanderWaals
Electrostatics	Electrostatic interactions
Solvation Polar	Penalization for burying polar groups
Solvation Hydrophobic	Contribution of hydrophobic groups
Van der Waals clashes	Energy penalization due to VanderWaals' clashes (interresidue)
Entropy Side Chain	Entropy cost of fixing the side chain
Entropy Main Chain	Entropy cost of fixing the main chain
Sloop Entropy	ONLY FOR ADVANCED USERS
Mloop Entropy	ONLY FOR ADVANCED USERS
Cis Bond	Cost of having a cis peptide bond
Torsional Clash	VanderWaals' torsional clashes (intraresidue)
Backbone Clash	Backbone-backbone VanderWaals. These are not considered in the total
Helix Dipole	Electrostatic contribution of the helix dipole
Water Bridge	Contribution of water bridges
Disulfide	Contribution of disulfide bonds
Electrostatic Kon	Electrostatic interaction between molecules in the precomplex
Partial Covalent Bonds	Interactions with bound metals
Energy Ionisation	Contribution of ionisation energy
Entropy Complex	Entropy cost of forming a complex
Residue Number	Number of residues

https://github.com/ELELAB/mutatex

OXFORD

Briefings in Bioinformatics, 2022, 23(3), 1–16
https://doi.org/10.1093/bib/bbac074
Problem Solving Protocol

MutateX: an automated pipeline for *in silico* saturation mutagenesis of protein structures and structural ensembles

Matteo Tiberti, Thilde Terkelsen, Kristine Degn, Ludovica Beltrame, Tycho Canter Cremers, Isabelle da Piedade, Miriam Di Marco, Emiliano Maiani and Elena Papaleo

Corresponding authors: Elena Papaleo, Cancer Strucutral Biology, Danish Cancer Society Research Center, Copenhagen, Denmark and Cancer Systems Biology, Dep. Health and Technology, Technical University of Denmark, Lyngby. Tel.: 004535257311; E-mail: elenap@cancer.dk; elpap@dtu.dk; Matteo Tiberti, Cancer Structural Biology, Danish Cancer Society Research Center, Copenhagen 2100, Denmark. Tel.: 004535257307; E-mail: tiberti@cancer.dk

Abstract

Mutations, which result in amino acid substitutions, influence the stability of proteins and their binding to biomolecules. A molecular understanding of the effects of protein mutations is both of biotechnological and medical relevance. Empirical free energy functions that quickly estimate the free energy change upon mutation (ΔΔG) can be exploited for systematic screenings of proteins and protein complexes. *In silico* saturation mutagenesis can guide the design of new experiments or rationalize the consequences of known mutations. Often software such as FoldX, while fast and reliable, lack the necessary automation features to apply them in a high-throughput manner. We introduce MutateX, a software to automate the prediction of ΔΔGs associated with the systematic mutation of each residue within a protein, or protein complex to all other possible residue types, using the FoldX energy function. MutateX also supports ΔΔG calculations over protein ensembles, upon post-translational modifications and in multimeric assemblies. At the heart of MutateX lies an automated pipeline engine that handles input preparation, parallelization and outputs publication-ready figures. We illustrate the MutateX protocol applied to different case studies. The results of the high-throughput scan provided by our tools can help in different applications, such as the analysis of disease-associated mutations, to complement experimental deep mutational scans, or assist the design of variants for industrial applications. MutateX is a collection of Python tools that relies on open-source libraries. It is available free of charge under the GNU General Public License from https://github.com/ELELAB/mutatex.

Keywords: free energy, mutations, post-translational modifications, structural ensembles, binding, stability

Introduction

Advances in proteomics are now providing a massive amount of data on protein–protein interaction or post-translationally modified proteins [1–5] that benefit from structural studies to be rationalized [6, 7]. On the other hand, genomic initiatives allow the identification of missense mutations [8–10] in the coding region of genes that need to be understood at a structural and functional level [11–13]. Indeed, single amino acid substitutions (i.e. mutations) or post-translational modifications (PTMs) in proteins may alter structural stability and intermolecular interactions, impacting protein activity, function and cellular signaling. An accurate and systematic prediction of changes in stability and binding upon mutations or

Matteo Tiberti is a staff scientist at the Cancer Structural Biology group (Danish Cancer Society Research Center, DCRC, Copenhagen, Denmark) and his research focuses on software development for structural bioinformatics and annotations of cancer mutations.
Thilde Terkelsen worked as PhD student at the Cancer Structural Biology group at DCRC and she is now Data Scientist at the Center for Health and Data Science at the University of Copenhagen. At DCRC, she worked on the implementation of MutateX functions for downstream analyses and the analyses of -omics data.
Kristine Degn is a PhD student in the Cancer Systems Biology group (Department of Health and Technology, Technical University of Denmark, DTU, Lyngby, Denmark) and her research focuses on structure-based methods to characterize and classify variants found in cancer samples with impact on the protein products.
Ludovica Beltrame is an Erasmus exchange student at the Cancer Structural Biology group at DCRC. Her research is focusing on free energy calculations and molecular simulations to study the effect of mutations on protein structures.
Tycho Canter Cremers carried out his Master Studies in the Cancer Structural Biology group at DCRC, focusing on implementing new functions for MutateX and he is currently working as Bioinformatician at the Center of Medical Genetics, Antwerp.
Isabelle da Piedade worked as a Post-Doctoral Researcher at the Cancer Structural Biology Group at DCRC with a focus on analyses of genomics and transcriptomics data. She is currently working at Senior Bioinformatician at the Danish National Genome Center (Copenhagen, Denmark).
Miriam Di Marco worked as a pre-graduate fellow and master's student at the Cancer Structural Biology group at DCRC. She applied free energy calculations to characterize disease-related proteins and the impact of mutations on protein stability.
Emiliano Maiani worked as a Post-Doctoral Researcher and Senior Scientist at the Cancer Structural Biology group of DCRC. His main research focus in the group has been in the area of experimental structural and cellular studies of short linear motifs. He is currently an Adjunct Professor at UniCamillus-Saint Camillus International University of Health Sciences, Rome, Italy. His current research focuses on DNA damage response and autophagy.
Elena Papaleo is Associate Professor and leader of the Cancer Systems Biology group at DTU and group leader of the Cancer Structural Biology group at DCRC. Her research focuses on applying -omics bioinformatics and structural methods, accompanied by experimental validation with in vitro and cellular assays. These methodologies are used to unravel the effects of disease-related mutations and post-translational modifications on protein structures and to characterize protein–protein interactions mediated by disordered regions.
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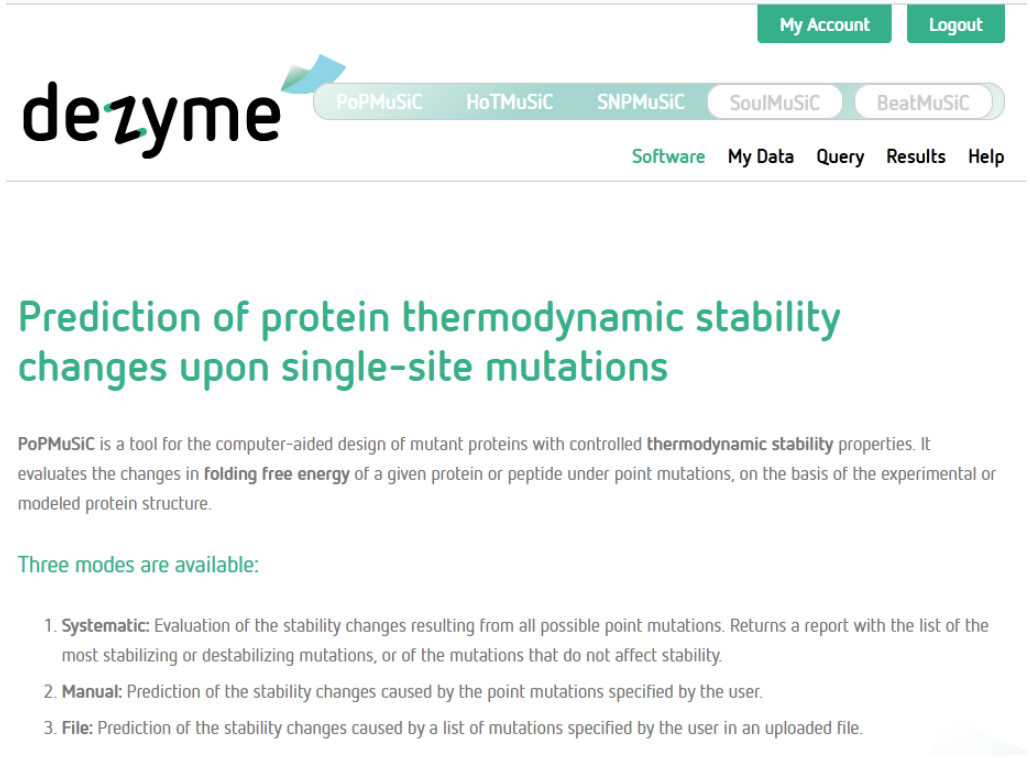
PopMusic



Predictions for 1FTG by PopMusic

query_38154.pop

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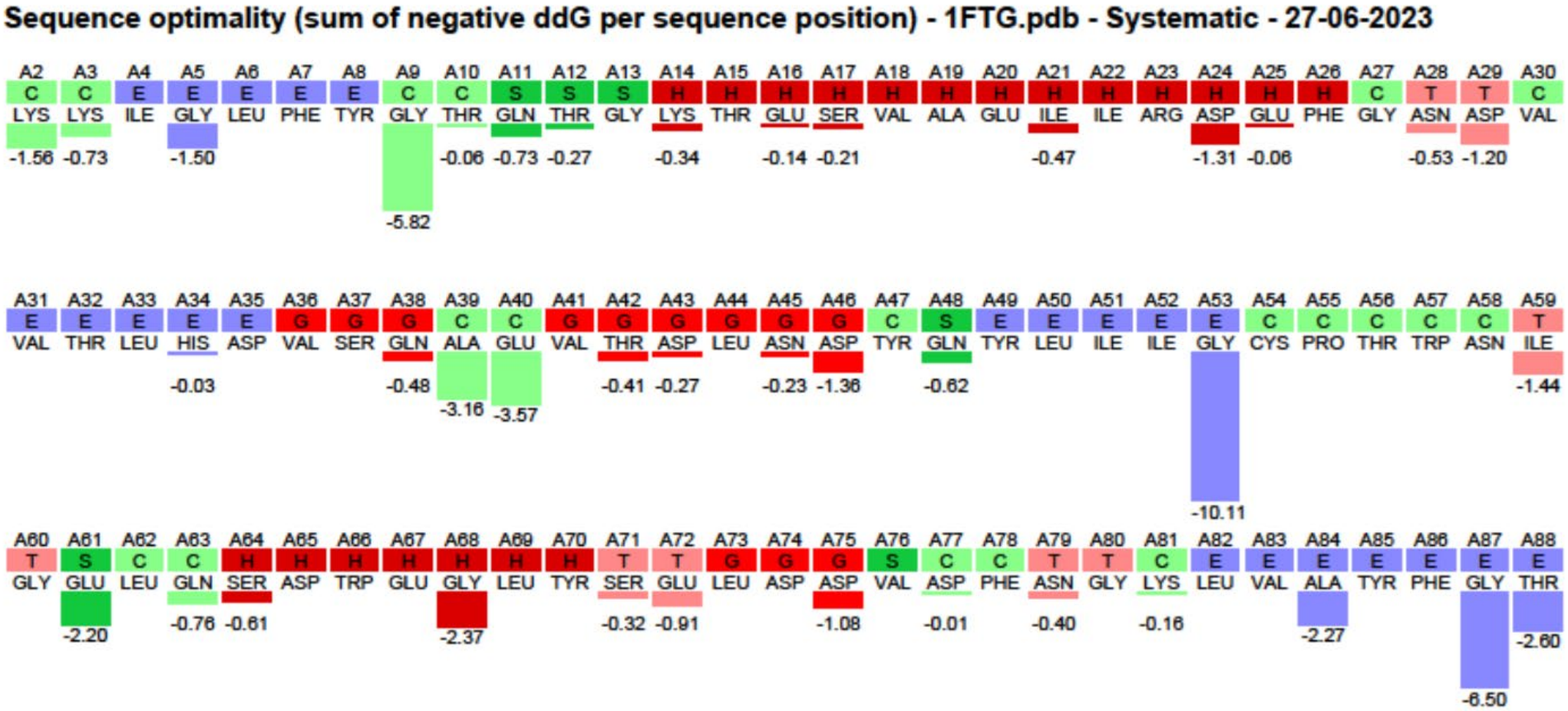
PopMusic



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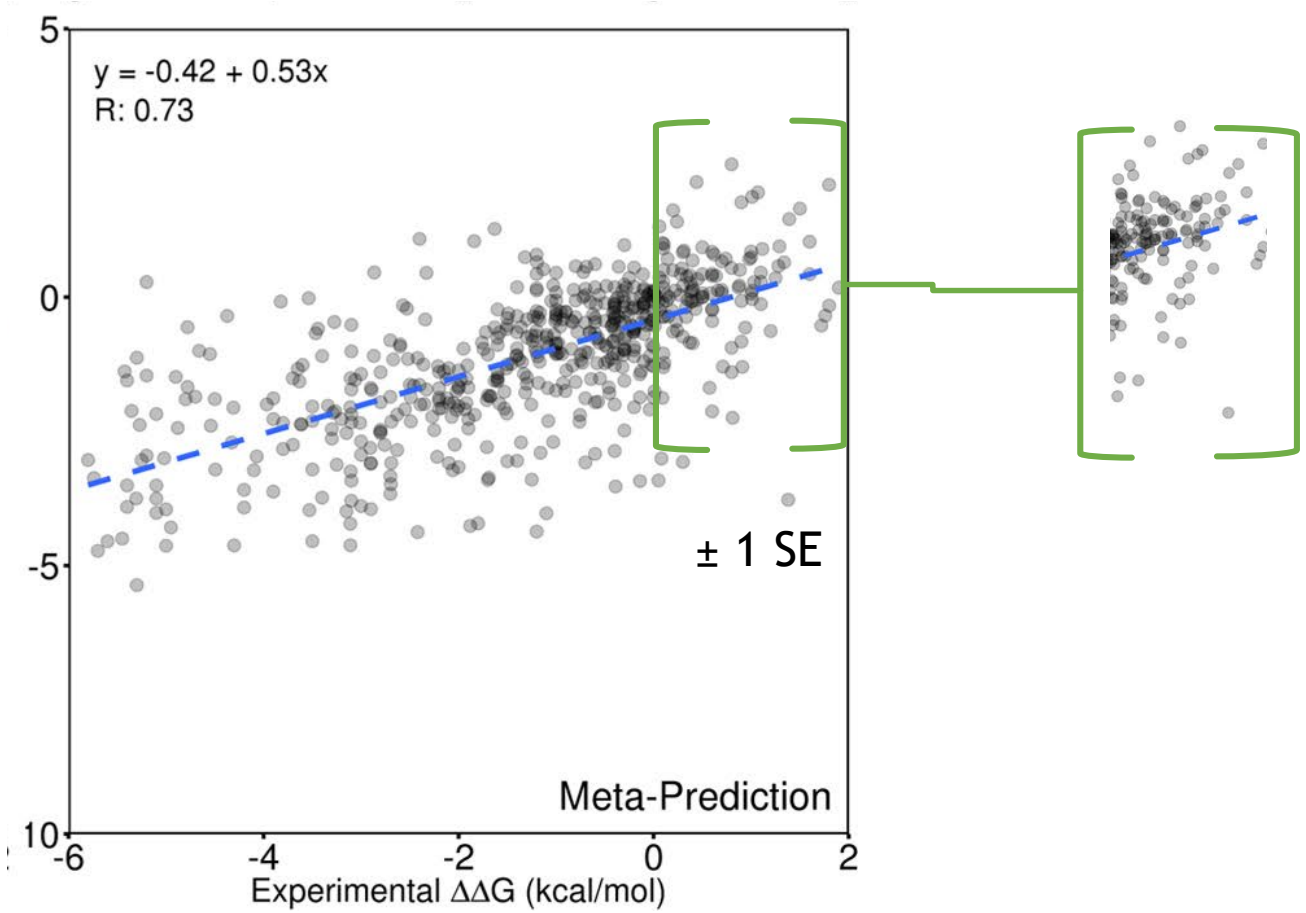
A common limitation of ΔΔG predictors



Tool	S.E.
	kcal/mol
EGAD	1.61
FoldX	1.78
Rosetta-ddG	2.34
CUPSAT	1.77
DFire	1.84
Hunter	1.89
MultiMutate	2.34
SDM	1.96
PoPMuSiC	1.32
IMutant3	1.52
MuPro	1.52
Meta-predictor	**1.29**

A. Broom, Z. Jacobi, K. Trainor, and E.M. Meiering
J. Biol. Chem. 292:14349–14361 (2017)

Protposer: a different approach

http://webapps.bifi.es/the-protposer

Computational and Structural Biotechnology Journal
Volume 20, 2022, Pages 2415-2433

Protposer: The web server that readily proposes protein stabilizing mutations with high PPV

Helena García-Cebollada [a, b, c], Alfonso López [a, b, c], Javier Sancho [a, b, c]

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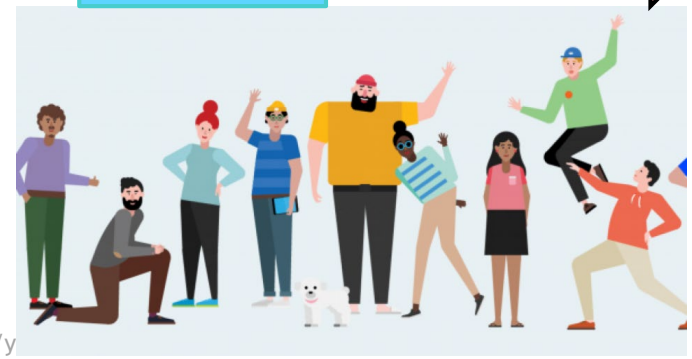
ΔΔG predictors

PDB file → User ideas → Proposed mutations → Energy calculation → Expected ΔΔG values for indicated mutations

PROTPOSER

PDB file → Mutation proposal → Proposed mutations → Evaluation of features → Evaluated mutations → Scoring → List of proposed mutations (with PPV)

Trained logistic regression classifier



Analysis of PDB file in search for wt residues that might represent opportunities for stabilization through their replacement by other residues

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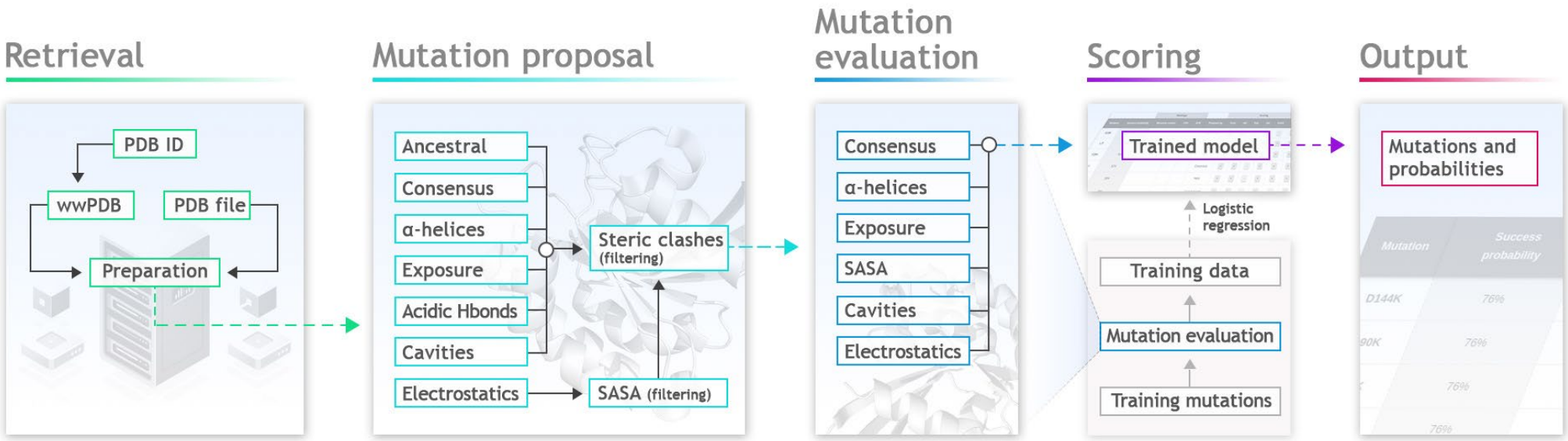
Protposer



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Testing and Precision or PPV(Positive Predictive Value)

Ex post test set

ED

Filtered ThermoMutDB database

916 single mutations in 9 proteins

Used as external validation

ED +

ED + 1FTG + 1PGA

1916 single mutations in 11 proteins

Used as external validation

$$PPV = \frac{TP}{TP + FP}$$

Fraction of mutations predicted to stabilize that do so experimentally

Protposer compared with other software

A real-case scenario: the user choses the 10 best mutations

Predictor	Positive Predictive Value
$Protposer_{Ok}$	78,6%
$Protposer_{classic}$	56,0%
$Protposer_{HM}$	42,9%
Protposer	36,1%
$PoPMuSiC_{sel}$	32,3%
$Rosetta_{sel}$	29,0%
$FoldX_{sel}$	22,1%

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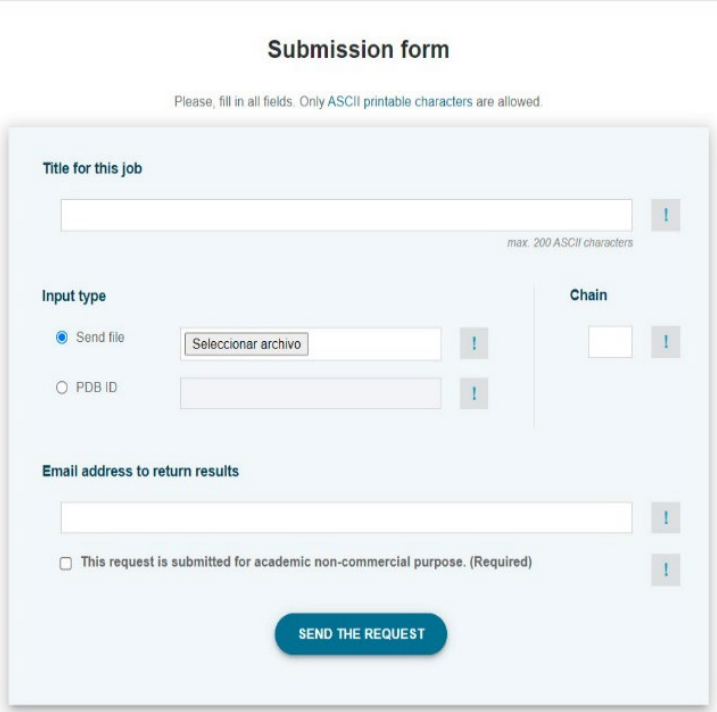
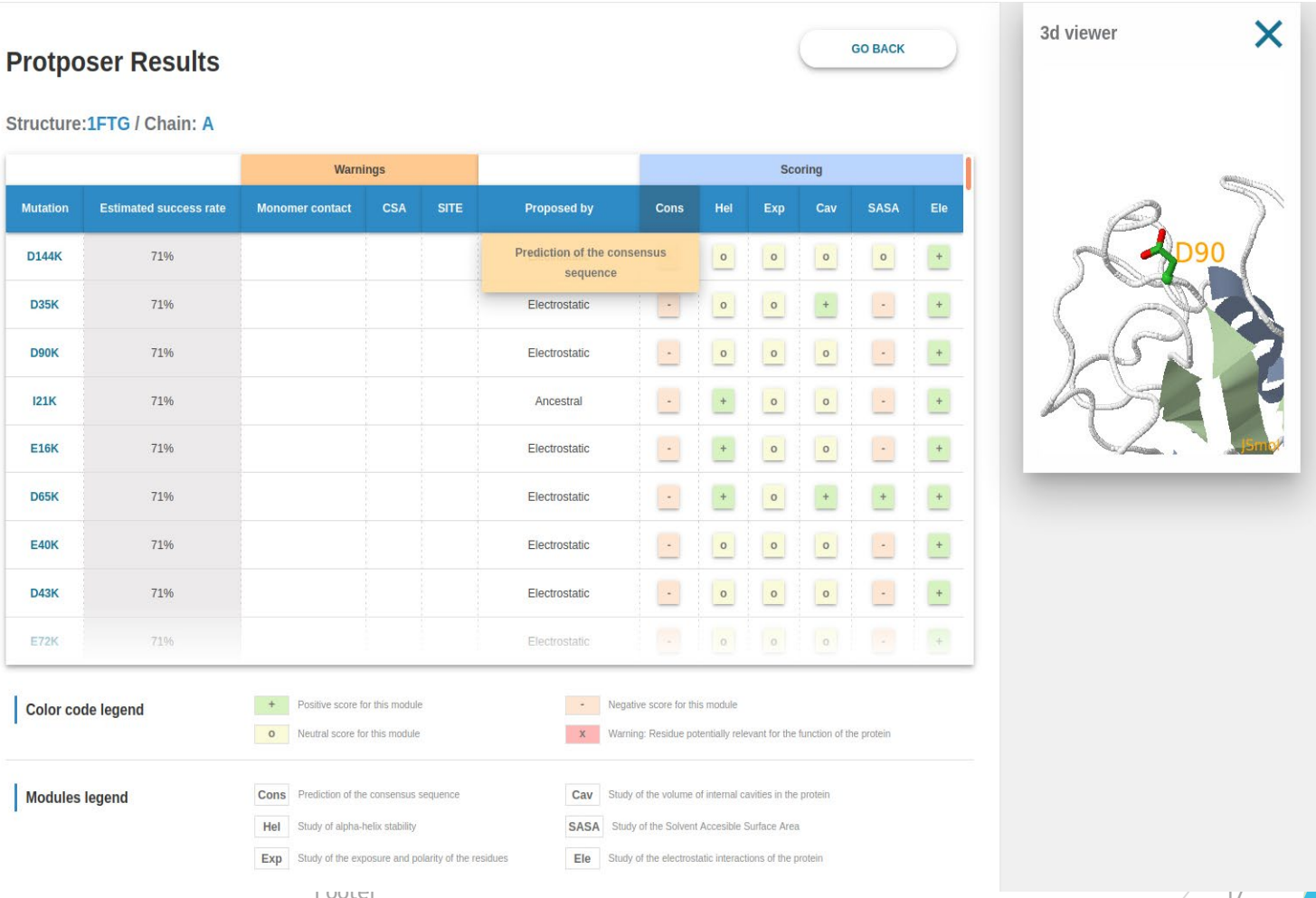
FoldX				Rosetta				PoPMuSiC				Protposer			
PDB	Mutation	ΔΔG	Exp. ΔΔG	PDB	Mutation	score	Exp. ΔΔG	PDB	Mutation	ΔΔG	Exp. ΔΔG	PDB	Mutation	score	Exp. ΔΔG
1ftg	Q63L	4,15	-0,01	1ftg	Q63L	-551,81	-0,01	1ftg	G87V	0,8	-0,13	1ftg	E40K	0,73	1,85
1ftg	Q99L	3,44	0,32	1ftg	Q99L	-551,05	0,32	1ftg	A142V	0,78	0,56	1ftg	E72K	0,72	1,24
1ftg	D43A	2,56	0	1ftg	W66F	-550,03	-0,71	1ftg	G68A	0,54	0,57	1ftg	D75K	0,71	0,92
1ftg	Q99A	1,73	1,63	1ftg	I59A	-549,41	1,05	1ftg	Q99L	0,37	0,32	1ftg	E61K	0,64	0,66
1ftg	T122S	1,6	-0,31	1ftg	E16Q	-549,33	1,37	1ftg	S71A	0,32	-0,69	1ftg	E20K	0,6	1,37
1ftg	V18I	1,25	0,86	1ftg	I52V	-549,28	-1,40	1ftg	G87L	0,28	-0,64	1ftg	D126K	0,61	0,86
1ftg	V117A	1,19	-1,74	1ftg	D150K	-548,93	-0,22	1ftg	A101V	0,24	-0,29	1ftg	D150K	0,59	-0,1
1ftg	S110A	1,13	-0,64	1ftg	G87A	-548,44	-0,05	1ftg	N128A	0,18	0,2	1ftg	I21A	0,56	0,45
1ftg	I59A	1	1,05	1ftg	E20K	-548,43	1,37	1ftg	Q63L	0,16	-0,01	1ftg	Q111A	0,53	-0,23
1ftg	V31A	0,94	-1,72	1ftg	K81M	-547,98	-1,52	1ftg	D96N	0,14	0,89	1ftg	Q99L	0,46	0,32
1ftg	WT	0	0	1ftg	WT	-549,06	0	1ftg	WT		0	1ftg	WT		0
Stabilizing if ΔΔG >0				Stabilizing if score more negative than WT				Stabilizing if ΔΔG >0				Stabilizing (probability given by the score)			

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101004806

Server input and output



Protposer results
ID: **696**
Your project: **apoflavodoxin**
PDB: **1ftg**
Chain: **A**
See results in the web



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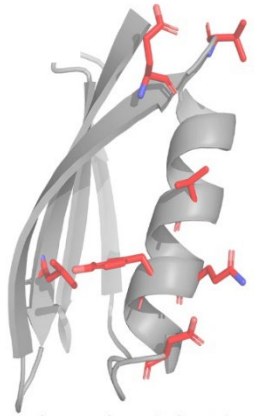
Two examples

1PGA



56 residues
B1 Immunoglobulin-binding domain of streptococcal protein G

7 proposed mutations (1 in Oκ)
7 with ΔΔG data (1 in Oκ)
- 3 clearly stabilizing (1 in Oκ)
- 1 moderately stabilizing (0 in Oκ)
- 2 slightly stabilizing (0 in Oκ)
- 1 destabilizing (0 in Oκ)

Average ΔΔG: 0.13 kcal/mol (0.97 in Oκ)
Accumulated ΔΔG: 0.92 kcal/mol (0.97 in Oκ)
Maximal ΔΔG: 2.69 kcal/mol (0.97 in Oκ)

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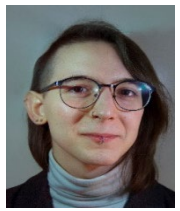
Protposer

- Very simple to use
- Great precision: up to 78% PPV in predicted mutations
- Free for academic use (no need to register so far)

http://webapps.bifi.es/the-protposer

Juan José Galano

Helena García



Alfonso López

ELSEVIER

Computational and Structural Biotechnology Journal

Volume 20, 2022, Pages 2415-2433

Protposer: The web server that readily proposes protein stabilizing mutations with high PPV

Helena García-Cebollada [a, b, c], Alfonso López [a, b, c], Javier Sancho [a, b, c]

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Protposer results
ID: **1948**
Your project: **Test**
PDB: **1ftg**
Chain: **A**
See results in the web

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