Web-based Tools for Computational Enzyme Design

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Supplementary Information

Supplementary Table S1. List of web-based tools for enzyme discovery, found in PubMed, published within 2018-2020 and ordered by their relevance to enzyme engineering.

Webserver	URL *Description*	Reference	Year
Phylo-PFP	http://kiharalab.org/phylo_pfp.php *Functional annotation of sequences based on PSI-BLAST and phylogeny; maximum 10 sequences at a time*	Jain A, Kihara D. **Phylo-PFP: improved automated protein function prediction using phylogenetic distance of distantly related sequences.** *Bioinformatics*. 2019; 35(5):753-759. doi: 10.1093/bioinformatics/bty704	2019
HECNet	http://hecnet.cbrlab.org/ *Prediction of EC number from the amino acid sequence*	Memon SA, Khan KA, Naveed H. **HECNet: a hierarchical approach to enzyme function classification using a siamese triplet network.** *Bioinformatics*. 2020; 25:btaa536. doi: 10.1093/bioinformatics/btaa536	2020
EnzymeMiner	https://loschmidt.chemi.muni.cz/enzymeminer/ *Prediction of soluble protein sequences putatively performing an enzyme activity*	Hon J, Borko S, Stourac J, Prokop Z, Zendulka J, Bednar D, Martinek T, Damborsky J. **EnzymeMiner: automated mining of soluble enzymes with diverse structures, catalytic properties and stabilities.** *Nucleic Acids Res.* 2020; 48(W1):W104-W109. doi: 10.1093/nar/gkaa372	2020
ANASTASIA	Not applicable *Installable Galaxy framework for metagenomic analysis.*	Koutsandreas T, Ladoukakis E, Pilalis E, Zarafeta D, Kolisis FN, Skretas G, Chatziioannou AA. **ANASTASIA: An automated metagenomic analysis pipeline for novel enzyme discovery exploiting next generation sequencing data.** *Front Genet*. 2019; 10:469. doi: 10.3389/fgene.2019.00469	2019
PSSMSearch	http://slim.ucd.ie/pssmsearch/ *Rapid statistical modeling, visualization, discovery and annotation of protein motif specificity determinants*	Krystkowiak I, Manguy J, Davey NE. **PSSMSearch: a server for modeling, visualization, proteome-wide discovery and annotation of protein motif specificity determinants.** *Nucleic Acids Res.* 2018; 46(W1):W235-W241. doi: 10.1093/nar/gky426	2018
dbCAN2	http://cys.bios.niu.edu/dbCAN2 *Mining of carbohydrate active enzymes*	Zhang H, Yohe T, Huang L, Entwistle S, Wu P, Yang Z, Busk PK, Xu Y, Yin Y. **dbCAN2: a meta server for automated carbohydrate-active enzyme annotation.** *Nucleic Acids Res.* 2018; 46(W1):W95-W101. doi: 10.1093/nar/gky418	2018
antiSMASH	https://antismash.secondarymetabolites.org/ *Genomic mining from bacterial/fungal/plant biosynthetic gene clusters*	Blin K, Kim HU, Medema MH, Weber T. **Recent development of antiSMASH and other computational approaches to mine secondary metabolite biosynthetic gene clusters.** *Brief Bioinform.* 2019; 20(4):1103-1113. doi: 10.1093/bib/bbx146	2019
GSP4PDB	https://structuralbio.utalca.cl/gsp4pdb/ *Search of protein-ligand structural motifs in the PDB*	Angles R, Arenas-Salinas M, Garcia R, Reyes-Suarez JA, Pohl E. **GSP4PDB: a web tool to visualize, search and explore protein-ligand structural patterns.** *BMC Bioinformatics*. 2020; 21(Suppl 2):85. doi: 10.1186/s12859-020-3352-x	
LIBRA-WA	http://biochimica3.bio.uniroma3.it/LIBRAWA/ *Identify the biologically relevant ligand/ligand-binding site and performs ligand clustering*	Toti D, Viet Hung L, Tortosa V, Brandi V, Polticelli F. **LIBRA-WA: a web application for ligand binding site detection and protein function recognition.** *Bioinformatics*. 2018; 34(5):878-880. doi: 10.1093/bioinformatics/btx715	2018
SCLpred-EMS	http://distilldeep.ucd.ie/SCLpred2/ *Prediction of subcellular location of a protein*	Kaleel M, Zheng Y, Chen J, Feng X, Simpson JC, Pollastri G, Mooney C. **SCLpred-EMS: subcellular localization prediction of endomembrane system and secretory pathway proteins by Deep N-to-1**	2020

		Convolutional Neural Networks. *Bioinformatics*. 2020; 36(11):3343-3349. doi: 10.1093/bioinformatics/btaa156	
PVPred-SCM	http://camt.pythonanywhere.com/PVPred-SCM *Specific to the prediction of phage virion proteins*	Charoenkwan P, Kanthawong S, Schaduangrat N, Yana J, Shoombuatong W. **PVPred-SCM: improved prediction and analysis of phage virion proteins using a scoring card method.** *Cells*. 2020; 9(2):353. doi: 10.3390/cells9020353	2020
iFeature	http://iFeature.erc.monash.edu/ *Feature extraction, clustering, selection and dimensionality reduction for training and building predictors*	Chen Z, Zhao P, Li F, Leier A, Marquez-Lago TT, Wang Y, Webb GI, Smith AI, Daly RJ, Chou KC, Song J. **iFeature: a Python package and web server for features extraction and selection from protein and peptide sequences.** *Bioinformatics*. 2018; 34(14):2499-2502. doi: 10.1093/bioinformatics/bty140	2018
Cry Processor	https://lab7.arriam.ru/tools/cry_processor *Specific to 3d-Cry toxins*	Shikov AE, Malovichko YV, Skitchenko RK, Nizhnikov AA, Antonets KS. **No more tears: mining sequencing data for novel Bt Cry Toxins with CryProcessor.** *Toxins*. 2020; 12(3):204. doi: 10.3390/toxins12030204	2020
FiRES	http://fires.ifc.unam.mx *Identification of structural element repeats; only applicable as a filter if searching for proteins with structural element repeats*	Alvarez-Carreno C, Coello G, Arciniega M. **FiRES: a computational method for the de novo identification of internal structure similarity in proteins.** *Proteins*. 2020; 88(9):1169-1179. doi: 10.1002/prot.25886	2020
PeNGaRoo	http://pengaroo.erc.monash.edu/ *Machine learning approach to predict a special non-classic class of bacterial secreted proteins*	Zhang Y, Yu S, Xie R, Li J, Leier A, Marquez-Lago TT, Akutsu T, Smith AI, Ge Z, Wang J, Lithgow T, Song J. **PeNGaRoo, a combined gradient boosting and ensemble learning framework for predicting non-classical secreted proteins.** *Bioinformatics*. 2020; 36(3):704-712. doi: 10.1093/bioinformatics/btz629	2020
AcrFinder	http://bcb.unl.edu/AcrFinder *Genome mining of anti-CRISPR proteins*	Yi H, Huang L, Yang B, Gomez J, Zhang H, Yin Y. **AcrFinder: genome mining anti-CRISPR operons in prokaryotes and their viruses.** *Nucleic Acids Res*. 2020; 48(W1):W358-W365. doi: 10.1093/nar/gkaa351	2020
AcRanker	http://acranker.pythonanywhere.com/ *Genome mining of anti-CRISPR proteins*	Eitzinger S, Asif A, Watters KE, Iavarone AT, Knott GJ, Doudna JA, Minhas FUAA. **Machine learning predicts new anti-CRISPR proteins.** *Nucleic Acids Res.* 2020; 48(9):4698-4708. doi: 10.1093/nar/gkaa219	2020
Bio2Rxn	http://design.rxnfinder.org/bio2rxn/ *Prediction of enzymatic function from sequence*	Zhang T, Tian Y, Yuan L, Chen F, Ren A, Hu QN. **Bio2Rxn: sequence-based enzymatic reaction predictions by a consensus strategy.** *Bioinformatics*. 2020; 36(11):3600-3601. doi: 10.1093/bioinformatics/btaa135	2020

Supplementary Table S2. List of web-based tools for engineering the solubility of proteins, found in PubMed, published within 2018-2020 and ordered by their relevance to enzyme engineering.

Webserver	URL *Description*	Reference	Year
SOLart	http://babylone.ulb.ac.be/SOLART/ *Random Forest solubility predictor based on aggregated potentials of 20 different structural- and sequence-based protein features; the tool predicts the likelihood of the protein being soluble, ranging from 0 to 130%*	Hou Q, Kwasigroch JM, Rooman M, Pucci F. ***SOLart: a structure-based method to predict protein solubility and aggregation.*** *Bioinformatics*. 2020; 36(5):1445-1452. doi: 10.1093/bioinformatics/btz773	2020
AggreRATE-Pred	http://www.iitm.ac.in/bioinfo/aggrerate-pred/ *Predicts the effects of mutations in aggregation rate*	Rawat P, Prabakaran R, Kumar S, Gromiha MM. ***AggreRATE-Pred: a mathematical model for the prediction of change in aggregation rate upon point mutation.*** *Bioinformatics*. 2020; 36(5):1439-1444. doi: 10.1093/bioinformatics/btz764	2020
Aggrescan3D 2.0	http://biocomp.chem.uw.edu.pl/A3D2/ *Calculates a per-residue aggregation score and predicts the aggregation effects upon mutation*	Kuriata A, Iglesias V, Pujols J, Kurcinski M, Kmiecik S, Ventura S. ***Aggrescan3D (A3D) 2.0: prediction and engineering of protein solubility.*** *Nucleic Acids Res*. 2019; 47(W1):W300-W307. doi: 10.1093/nar/gkz321	2019
FungiPAD	http://chemyang.ccnu.edu.cn/ccb/database/FungiPAD/ *Predicts physico-chemical properties on fungicidals*	Hebditch M, Warwicker J. ***Web-based display of protein surface and pH-dependent properties for assessing the developability of biotherapeutics.*** *Sci Rep.* 2019; 9(1):1969. doi: 10.1038/s41598-018-36950-8	2019
EnzymeMiner	https://loschmidt.chemi.muni.cz/enzymeminer/ *Tool for mining novel enzymes that provides with a prediction of solubility based on SoluProt; provides annotations and graphical representation for prioritization of sequences for experimental testing*	Hon J, Borko S, Stourac J, Prokop Z, Zendulka J, Bednar D, Martinek T, Damborsky J. ***EnzymeMiner: automated mining of soluble enzymes with diverse structures, catalytic properties and stabilities.*** *Nucleic Acids Res*. 2020; 48(W1):W104-W109. doi: 10.1093/nar/gkaa372	2020
SoluProt	https://loschmidt.chemi.muni.cz/soluprot/ *To predict the solubility of an input protein sequence in Escherichia coli based on the manually filtered TargetTrack database.*	Hon, J., Marusiak, M., Martinek, T., Zendulka J., Bednar, D., Damborsky, J. ***SoluProt: prediction of soluble expression from protein sequence.*** *Bioinformatics* 2020*;* accepted.	2020
Solubility-Weighted Index	https://tisigner.com/sodope *Given a nucleotide sequence input, predicts the solubility of its protein product and gives better length alternatives to optimize solubility; the prediction is based on residue averaged assigned normalized B-factors*	Bhandari BK, Gardner PP, Lim CS. ***Solubility-Weighted Index: fast and accurate prediction of protein solubility.*** *Bioinformatics*. 2020; btaa578. doi: 10.1093/bioinformatics/btaa578	2020
AMYCO	http://bioinf.uab.cat/amyco/ *Predicts the effects of mutations in the prion-like aggregation behavior of proteins*	Iglesias V, Conchillo-Sole O, Batlle C, Ventura S. ***AMYCO: evaluation of mutational impact on prion-like proteins aggregation propensity.*** *BMC Bioinformatics*. 2019; 20(1):24. doi: 10.1186/s12859-019-2601-3	2019
fDETECT	http://biomine.cs.vcu.edu/servers/fDETECT/ *Prediction of the expected success of protein production, purification, crystallization, and structure determination*	Meng F, Wang C, Kurgan L. ***fDETECT webserver: fast predictor of propensity for protein production, purification, and crystallization.*** *BMC Bioinformatics*. 2018; 18(1):580. doi: 10.1186/s12859-017-1995-z	2018
VarQ	http://varq.qb.fcen.uba.ar/	Radusky L, Modenutti C, Delgado J, Bustamante JP, Vishnopolska S, Kiel C, Serrano L, Marti M, Turjanski A. ***VarQ: a tool for the structural and***	2018

	Predicts a plethora of properties and effects of mutations on a 3D protein structure using the FoldX force field	***functional analysis of human protein variants.*** *Front Genet.* 2018; 9:620. doi: 10.3389/fgene.2018.00620	
MISCAST	http://miscast.broadinstitute.org/ *MISCAST is an interactive and user-friendly web server to visualize and analyze precompiled missense variants in human protein sequence and structural space. Does not provide with solubility prediction at the protein level (detects enrichment in certain amino acid classes)*	Iqbal S, Hoksza D, Perez-Palma E, May P, Jespersen JB, Ahmed SS, Rifat ZT, Heyne HO, Rahman MS, Cottrell JR, Wagner FF, Daly MJ, Campbell AJ, Lal D. ***MISCAST: MIssense variant to protein StruCture Analysis web SuiTe.*** *Nucleic Acids Res*. 2020; 48(W1):W132-W139. doi: 10.1093/nar/gkaa361	2020
ImmunomeBrowser	http://tools.iedb.org/immunomebrowser/ *Aggregation and visualization of heterogeneous immunological data*	Dhanda SK, Vita R, Ha B, Grifoni A, Peters B, Sette A. ***ImmunomeBrowser: a tool to aggregate and visualize complex and heterogeneous epitopes in reference proteins.*** *Bioinformatics*. 2018; 34(22):3931-3933. doi: 10.1093/bioinformatics/bty463	2018

Supplementary Table S3. List of web-based tools for engineering the activity and selectivity of enzymes, found in PubMed, published within 2018-2020 and ordered by their relevance to enzyme engineering.

Webserver	**URL** ***Description***	**Reference**	**Year**
CaverDock	https://loschmidt.chemi.muni.cz/caverweb/ *To calculate trajectory and interaction energy of a ligand travelling through a protein tunnel; available over the more complete webserver CaverWeb*	Vavra O, Filipovic J, Plhak J, Bednar D, Marques SM, Brezovsky J, Stourac J, Matyska L, Damborsky J. **CaverDock: a molecular docking-based tool to analyse ligand transport through protein tunnels and channels.** *Bioinformatics*. 2019; 35(23):4986-4993. doi: 10.1093/bioinformatics/btz386	2019
FuncLib	http://FuncLib.weizmann.ac.il *For redesigning the active site residues based on conservation analysis and Rosetta calculations*	Khersonsky O, Lipsh R, Avizemer Z, Ashani Y, Goldsmith M, Leader H, Dym O, Rogotner S, Trudeau DL, Prilusky J, Amengual-Rigo P, Guallar V, Tawfik DS, Fleishman SJ. **Automated design of efficient and functionally diverse enzyme repertoires.** *Mol Cell*. 2018; 72(1):178-186.e5. doi: 10.1016/j.molcel.2018.08.033	2018
DaReUS-Loop	http://bioserv.rpbs.univ-paris-diderot.fr/services/DaReUS-Loop/ *For (re-)modeling of loops in a protein structure or homology models*	Karami Y, Rey J, Postic G, Murail S, Tuffery P, de Vries SJ. **DaReUS-Loop: a web server to model multiple loops in homology models.** *Nucleic Acids Res*. 2019; 47(W1):W423-W428. doi: 10.1093/nar/gkz403	2019
P2K	https://p2k.uwaterloo.ca *For prediction of protein interacting residues. Based on machine learning*	Wong AKC, Sze-To HY, Johanning GL. **Pattern to knowledge: deep knowledge-directed machine learning for residue-residue interaction prediction.** *Sci Rep.* 2018; 8(1):14841. doi: 10.1038/s41598-018-32834-z	2018
DeepBindPoc	http://cbblab.siat.ac.cn/DeepBindPoc/ *To identify and rank ligand-binding pockets in proteins. Based on deep learning*	Zhang H, Saravanan KM, Lin J, Liao L, Ng JT, Zhou J, Wei Y. **DeepBindPoc: a deep learning method to rank ligand binding pockets using molecular vector representation.** *PeerJ*. 2020; 8:e8864. doi: 10.7717/peerj.8864	2020
nAPOLI	http://bioinfo.dcc.ufmg.br/napoli/	Fassio AV, Santos LH, Silveira SA, Ferreira RS, de Melo-Minardi RC. **nAPOLI: a graph-based strategy to**	2019

	For the analysis of protein-ligand interactions detecting important conserved interacting residues	**detect and visualize conserved protein-ligand interactions in large-scale.** *IEEE/ACM Trans Comput Biol Bioinform*. 2019; 17(4):1317-1328. doi: 10.1109/TCBB.2019.2892099.	
MOLEonline	https://mole.upol.cz *To calculate protein tunnel and channels and analyze the tunnel residues and their properties*	Pravda L, Sehnal D, Tousek D, Navratilova V, Bazgier V, Berka K, Svobodova Varekova R, Koca J, Otyepka M. **MOLEonline: a web-based tool for analyzing channels, tunnels and pores (2018 update).** *Nucleic Acids Res*. 2018; 46(W1):W368-W373. doi: 10.1093/nar/gky309	2018
visualCMAT	https://biokinet.belozersky.msu.ru/visualcmat *Visual tool to detect and interpret correlated mutations*	Suplatov D, Sharapova Y, Timonina D, Kopylov K, Å vedas V. **The visualCMAT: A web-server to select and interpret correlated mutations/co-evolving residues in protein families.** *J Bioinform Comput Biol.* 2018; 16(2):1840005. doi: 10.1142/S021972001840005X	2018
I-LBR	https://jun-csbio.github.io/I-LBR *Binding residues prediction: based on sequence using support vector machine algorithm*	Hu J, Rao L, Fan X, Zhang G. **Identification of ligand-binding residues using protein sequence profile alignment and query-specific support vector machine model.** *Anal Biochem.* 2020; 604:113799. doi: 10.1016/j.ab.2020.113799	2020
SkeleDock	https://playmolecule.org/SkeleDock/ *Ligand docking: scaffold docking using the structure of a protein-ligand complex as a template to model the binding mode of a chemically similar system*	Varela-Rial A, Majewski M, Cuzzolin A, MartÃnez-Rosell G, De Fabritiis G. **SkeleDock: a web application for scaffold docking in PlayMolecule.** *J Chem Inf Model.* 2020; 60(6):2673-2677. doi: 10.1021/acs.jcim.0c00143	2020
Web-ARM	www.web-arm.org *Building the models of rhodopsins based on QM/MM Models. Specific to rhodopsins*	Pedraza-Gonzalez L, Marin MDC, Jorge AN, Ruck TD, Yang X, Valentini A, Olivucci M, De Vico L. **Web-ARM: a web-based interface for the automatic construction of QM/MM models of rhodopsins.** *J Chem Inf Model*. 2020; 60(3):1481-1493. doi: 10.1021/acs.jcim.9b00615	2020
en_DCNNMoRF	http://vivace.bi.a.u-tokyo.ac.jp:8008/fang/en_MoRFs.php *Predicts functional regions of intrinsically disordered proteins; based on sequence and neural networks*	Fang C, Moriwaki Y, Tian A, Li C, Shimizu K. **Identifying short disorder-to-order binding regions in disordered proteins with a deep convolutional neural network method.** *J Bioinform Comput Biol*. 2019; 17(1):1950004. doi: 10.1142/S0219720019500045	2019
PrankWeb	http://prankweb.cz/ *Prediction of ligand binding pockets over the entire protein, based on machine learning*	Jendele L, Krivak R, Skoda P, Novotny M, Hoksza D. **PrankWeb: a web server for ligand binding site prediction and visualization.** *Nucleic Acids Res*. 2019; 47(W1):W345-W349. doi: 10.1093/nar/gkz424	2019
DMRpred	http://biomine.cs.vcu.edu/servers/DMRpred/ *Predicts the propensity of the amino acids being in a disordered region; based on protein sequence*	Meng F, Kurgan L. **High-throughput prediction of disordered moonlighting regions in protein sequences.** *Proteins*. 2018; 86(10):1097-1110. doi: 10.1002/prot.25590	2018
AlloFinder	http://mdl.shsmu.edu.cn/ALF/ *Identification of allosteric sites and allosteric docking of ligands*	Huang M, Song K, Liu X, Lu S, Shen Q, Wang R, Gao J, Hong Y, Li Q, Ni D, Xu J, Chen G, Zhang J. **AlloFinder: a strategy for allosteric modulator discovery and allosterome analyses.** *Nucleic Acids Res.* 2018; 46(W1):W451-W458. doi: 10.1093/nar/gky374	2018
ProtDCal-Suite	https://protdcal.zmb.uni-due.de *Protein's descriptor calculation based on structure or sequence can be effectively used for machine learning analyses using proper attribute selection and modeling techniques*	Romero-Molina S, Ruiz-Blanco YB, Green JR, Sanchez-Garcia E. **ProtDCal-Suite: a web server for the numerical codification and functional analysis of proteins.** *Protein Sci*. 2019; 28(9):1734-1743. doi: 10.1002/pro.3673	2019

CB-Dock	http://cao.labshare.cn/cb-dock/ *New docking server that pre-calculates possible docking sites according to surface geometry and then implements Autodock Vina for docking the molecule to those docking sites*	Liu Y, Grimm M, Dai WT, Hou MC, Xiao ZX, Cao Y. **CB-Dock: a web server for cavity detection-guided protein-ligand blind docking.** *Acta Pharmacol Sin*. 2020; 41(1):138-144. doi: 10.1038/s41401-019-0228-6	2020
PPD	http://weilab.math.msu.edu/PPD/ *To detect protein pockets using a novel algorithm*	Zhao R, Cang Z, Tong Y, Wei GW. **Protein pocket detection via convex hull surface evolution and associated Reeb graph.** *Bioinformatics*. 2018; 34(17):i830-i837. doi: 10.1093/bioinformatics/bty598	2018

Supplementary Table S4. List of web-based tools for engineering the stability of proteins, found in PubMed, published within 2018-2020 and ordered by their relevance to enzyme engineering.

Webserver	**URL** ***Description***	**Reference**	**Year**
mCSM-membrane	http://biosig.unimelb.edu.au/mcsm_membrane *Prediction the stability or pathogenic effects of mutation on membrane protein stability and the likelihood of them being disease-associated*	Pires DEV, Rodrigues CHM, Ascher DB. ***mCSM-membrane: predicting the effects of mutations on transmembrane proteins.*** *Nucleic Acids Res*. 2020; 48(W1):W147-W153. doi: 10.1093/nar/gkaa416	2020
pStab	http://pbl.biotech.iitm.ac.in/pStab *Engineering of protein stability through mutations involving charged residues; a statistical mechanical model is then employed to predict the unfolding curves for selected mutants as a function of temperature*	Gopi S, Devanshu D, Krishna P, Naganathan AN. ***pStab: prediction of stable mutants, unfolding curves, stability maps and protein electrostatic frustration.*** *Bioinformatics*. 2018; 34(5):875-877. doi: 10.1093/bioinformatics/btx697	2018
Proteus	http://proteus.dcc.ufmg.br *A new algorithm for finding mutation pairs to enhance protein stability*	Barroso JRMS, Mariano D, Dias SR, Rocha REO, Santos LH, Nagem RAP, de Melo-Minardi RC. ***Proteus: an algorithm for proposing stabilizing mutation pairs based on interactions observed in known protein 3D structures.*** *BMC Bioinformatics*. 2020; 21(1):275. doi: 10.1186/s12859-020-03575-6	2020
FireProt-ASR	http://loschmidt.chemi.muni.cz/fireprotasr/ *Performs fully automated Ancestral Sequence Reconstruction to infer primordial more stable proteins; the results are provided in easy-to-use graphical format*	Musil, M., Khan, R. T., Beier, A., Stourac, J., Konegger, H., Damborsky, J., Bednar, D. ***FireProt^{ASR}: a web server for fully automated ancestral sequence reconstruction.*** *Briefings Bioinf.* 2020; in press.	2020
ProTSPoM	http://cosmos.iitkgp.ac.in/ProTSPoM/ *To estimate ΔΔG upon single-point mutations based on a machine learning framework*	Banerjee A, Mitra P. ***Estimating the effect of single-point mutations on protein thermodynamic stability and analyzing the mutation landscape of the p53 protein.*** *J Chem Inf Model.* 2020; 60(6):3315-3323. doi: 10.1021/acs.jcim.0c00256	2020
Yosshi	https://biokinet.belozersky.msu.ru/yosshi *To select hot-spots for introducing disulfide bonds which naturally occur in some proteins, based on multiple-sequence alignments*	Suplatov D, Timonina D, Sharapova Y, Svedas V. ***Yosshi: a web-server for disulfide engineering by bioinformatic analysis of diverse protein families.*** Nucleic Acids Res. 2019; 47(W1):W308-W314. doi: 10.1093/nar/gkz385	2019
TKSA-MC	http://tksamc.df.ibilce.unesp.br *For rational mutation via optimizing the protein charge interactions; presents the electrostatic free energy contribution of each polar-charged residue which contribute to destabilizing the protein native state*	Contessoto VG, de Oliveira VM, Fernandes BR, Slade GG, Leite VBP. ***TKSA-MC: a web server for rational mutation through the optimization of protein charge interactions.*** *Proteins*. 2018; 86(11):1184-1188. doi: 10.1002/prot.25599	2018

SSbondPre	http://liulab.csrc.ac.cn/ssbondpre *Predicts cysteine mutation to enhance protein structural stability based on neural networks*	Gao X, Dong X, Li X, Liu Z, Liu H. ***Prediction of disulfide bond engineering sites using a machine learning method.*** *Sci Rep*. 2020; 10(1):10330. doi: 10.1038/s41598-020-67230-z	2020
DenseCPD	http://protein.org.cn/densecpd.html *Predicts the probabilities of 20 natural amino acids for each residue in a protein structure, considering the three-dimensional density distribution of protein backbone*	Qi Y, Zhang JZH. ***DenseCPD: improving the accuracy of neural-network-based computational protein sequence design with DenseNet.*** *J Chem Inf Model*. 2020; 60(3):1245-1252. doi: 10.1021/acs.jcim.0c00043.	2020
PastML	https://pastml.pasteur.fr/ *Visualization of ancestral characteristics of the tree, but not sequences; the algorithm takes a sequence-agnostic tree as input and an annotation metafile*	Ishikawa SA, Zhukova A, Iwasaki W, Gascuel O. ***A fast likelihood method to reconstruct and visualize ancestral scenarios.*** *Mol Biol Evol.* 2019; 36(9):2069-2085. doi: 10.1093/molbev/msz131	2019

Supplementary Table S5. List of web-based tools for engineering protein dynamics, found in PubMed, published within 2018-2020 and ordered by their relevance to enzyme engineering.

Webserver	**URL** ***Description***	**Reference**	**Year**
DynaMut	http://biosig.unimelb.edu.au/dynamut/ *To assess changes in stability and flexibility upon mutation*	Rodrigues CH, Pires DE, Ascher DB. **DynaMut: predicting the impact of mutations on protein conformation, flexibility and stability.** *Nucleic Acids Res.* 2018; 46(W1):W350-W355. doi: 10.1093/nar/gky300	2018
DynaMut2	http://biosig.unimelb.edu.au/dynamut2 *To assess changes in stability and flexibility upon mutation*	Rodrigues CHM, Pires DEV, Ascher DB. **DynaMut2: assessing changes in stability and flexibility upon single and multiple point missense mutations.** *Protein Sci*. 2020. doi: 10.1002/pro.3942	2020
CABS-flex 2.0	http://biocomp.chem.uw.edu.pl/CABSflex2 *To evaluate the flexibility of the input protein structure*	Kuriata A, Gierut AM, Oleniecki T, Ciemny MP, Kolinski A, Kurcinski M, Kmiecik S. **CABS-flex 2.0: a web server for fast simulations of flexibility of protein structures.** *Nucleic Acids Res.* 2018; 46(W1):W338-W343. doi: 10.1093/nar/gky356	2018
ProSNEx	http://prosnex-tool.com *For the construction and analysis of Protein Structure Networks (PSNs) alongside amino acid flexibility, sequence conservation and annotation features*	Aydinkal RM, Sercinoglu O, Ozbek P. **ProSNEx: a web-based application for exploration and analysis of protein structures using network formalism.** *Nucleic Acids Res*. 2019; 47(W1):W471-W476. doi: 10.1093/nar/gkz390	2019
AlloSigMA	http://allosigma.bii.a-star.edu.sg *To evaluate the allosteric effects of ligand binding or mutations*	Tan ZW, Guarnera E, Tee WV, Berezovsky IN. **AlloSigMA 2: paving the way to designing allosteric effectors and to exploring allosteric effects of mutations.** *Nucleic Acids Res*. 2020; 48(W1):W116-W124. doi: 10.1093/nar/gkaa338	2020
CoNSEnsX+	http://consensx.itk.ppke.hu *To analyze structural ensembles generated to represent the internal dynamics of proteins from NMR data*	Dudola D, Kovacs B, Gaspari Z. **Evaluation and selection of dynamic protein structural ensembles with CoNSEnsX+.** *Methods Mol Biol.* 2020; 2112:241-254. doi: 10.1007/978-1-0716-0270-6_16	2020
LARMD	http://chemyang.ccnu.edu.cn/ccb/server/LARMD/	Yang JF, Wang F, Chen YZ, Hao GF, Yang GF. **LARMD: integration of bioinformatic resources to profile ligand-driven protein dynamics with a**	2019

	Runs classical and steered molecular dynamics and Normal Mode Analysis to compute ligand-binding trajectory and interaction energies	**case on the activation of estrogen receptor.** *Brief Bioinform*. 2019; bbz141. doi: 10.1093/bib/bbz141	
NAPS	http://bioinf.iiit.ac.in/NAPS/ *Network analysis on molecular dynamics simulations to analyze conformational changes, dynamic cross-correlations and binding pockets*	Chakrabarty B, Naganathan V, Garg K, Agarwal Y, Parekh N. **NAPS update: network analysis of molecular dynamics data and protein-nucleic acid complexes.** *Nucleic Acids Res*. 2019; 47(W1):W462-W470. doi: 10.1093/nar/gkz399	2019
webPSN v2.0	http://webpsn.hpc.unimore.it *Protein Structure Network and Elastic Network Model analysis to infer fingerprints of structural communication in biomacromolecules*	Felline A, Seeber M, Fanelli F. **webPSN v2.0: a webserver to infer fingerprints of structural communication in biomacromolecules.** *Nucleic Acids Res.* 2020; 48(W1):W94-W103. doi: 10.1093/nar/gkaa397	2020
MERMAID	http://molsim.sci.univr.it/mermaid/ *Prepare, run and analyze coarse-grained MD of membrane proteins*	Damre M, Marchetto A, Giorgetti A. **MERMAID: dedicated web server to prepare and run coarse-grained membrane protein dynamics.** *Nucleic Acids Res*. 2019; 47(W1):W456-W461. doi: 10.1093/nar/gkz416	2019
DynDom	*http://dyndom.cmp.uea.ac.uk/dyndom/* *To determine domains, hinge axes and hinge bending residues in proteins where two conformations are available*	Veevers R, Hayward S. **Methodological improvements for the analysis of domain movements in large biomolecular complexes**. *Biophys Physicobiol. 2019; 16:328-336. doi: 10.2142/biophysico.16.0_328*	2019
D3Pockets	http://www.d3pharma.com/D3Pocket/index.php *To explore the dynamic properties of the protein pocket based on either molecular dynamics simulation trajectories or conformational ensembles*	Chen Z, Zhang X, Peng C, Wang J, Xu Z, Chen K, Shi J, Zhu W. **D3Pockets: a method and web server for systematic analysis of protein pocket dynamics.** *J Chem Inf Model*. 2019; 59(8):3353-3358. doi: 10.1021/acs.jcim.9b00332.	2019
Hingeseek	http://hingeseek.cmp.uea.ac.uk *To identify residues in a protein sequence that may be part of a hinge-bending region using FASTA sequence using machine learning*	Veevers R, Cawley G, Hayward S. **Investigation of sequence features of hinge-bending regions in proteins with domain movements using kernel logistic regression.** *BMC Bioinformatics*. 2020; 21(1):137. doi: 10.1186/s12859-020-3464-3	2020
Ohm	https://dokhlab.med.psu.edu/ohm/ *Server for: (1) prediction of allosteric sites, (2) identification of allosteric pathways, (3) identification of critical residues in allosteric pathways, and (4) prediction of allosteric correlations between pairs of residues*	Wang J, Jain A, McDonald LR, Gambogi C, Lee AL, Dokholyan NV. **Mapping allosteric communications within individual proteins.** *Nat Commun*. 2020; 11(1):3862. doi: 10.1038/s41467-020-17618-2	2020
GalaxyRefine2	http://galaxy.seoklab.org/refine2 *Structural refinement that performs short molecular dynamics relaxations after repeated side chain repacking perturbations; limited to 300 residues*	Lee GR, Won J, Heo L, Seok C. **GalaxyRefine2: simultaneous refinement of inaccurate local regions and overall protein structure.** *Nucleic Acids Res.* 2019; 47(W1):W451-W455. doi: 10.1093/nar/gkz288.	2019
CHARMM-GUI DEER	http://www.charmm-gui.org/input/deerre *To prepare restrained ensemble molecular dynamics (reMD) simulations*	Qi Y, Lee J, Cheng X, Shen R, Islam SM, Roux B, Im W. **CHARMM-GUI DEER facilitator for spin-pair distance distribution calculations and preparation of restrained-ensemble molecular dynamics simulations.** *J Comput Chem*. 2020; 41(5):415-420. doi: 10.1002/jcc.26032	2020

Supplementary Table S6. List of web-based tools for multipurpose in enzyme engineering, found in PubMed, published within 2018-2020 and ordered by their relevance to enzyme engineering.

Webserver	URL *Description*	Reference	Year
Caver Web 1.0	https://loschmidt.chemi.muni.cz/caverweb *Easy-to-use tool for identification of tunnels and channels in proteins and analysis of ligand transport; provides visualization as well as energy profiles for transportation*	Stourac J, Vavra O, Kokkonen P, Filipovic J, Pinto G, Brezovsky J, Damborsky J, Bednar D. **Caver Web 1.0: identification of tunnels and channels in proteins and analysis of ligand transport.** *Nucleic Acids Res*. 2019; 47(W1):W414-W422. doi: 10.1093/nar/gkz378	2019
HotSpot Wizard 3.0	http://loschmidt.chemi.muni.cz/hotspotwizard *Server for automated identification of hotspots in semi-rational protein design to give improved protein stability, catalytic activity, substrate specificity and enantioselectivity; provides designs of point mutants as well as libraries for directed evolution*	Sumbalova L, Stourac J, Martinek T, Bednar D, Damborsky J. **HotSpot Wizard 3.0: web server for automated design of mutations and smart libraries based on sequence input information.** *Nucleic Acids Res.* 2018; 46(W1):W356-W362. doi: 10.1093/nar/gky417	2018
ROSIE	http://rosie.rosettacommons.org *Unified platform hosting several tools specialized in different aspects of molecular modeling of proteins and nucleic acids, based on Rosetta force-field and Rosetta protocols*	Moretti R, Lyskov S, Das R, Meiler J, Gray JJ. **Web-accessible molecular modeling with Rosetta: The Rosetta Online Server that Includes Everyone (ROSIE).** *Protein Sci.* 2018; 27(1):259-268. doi: 10.1002/pro.3313	2018
pPerturb:	http://pbl.biotech.iitm.ac.in/pPerturb *Quantification of the strength of an interaction network by employing perturbation; can predict the extent of destabilization of proteins arising from mutations*	Gopi S, Devanshu D, Rajasekaran N, Anantakrishnan S, Naganathan AN. **pPerturb: a server for predicting long-distance energetic couplings and mutation-induced stability changes in proteins via perturbations.** *ACS Omega*. 2020; 5(2):1142-1146. doi: 10.1021/acsomega.9b03371	2020
CavityPlus	http://www.pkumdl.cn/cavityplus Detection of binding sites on the surface and ranking them based on "ligandability" and druggability scores, pharmacophore features, detection of potential allosteric sites based on motion correlations	Xu Y, Wang S, Hu Q, Gao S, Ma X, Zhang W, Shen Y, Chen F, Lai L, Pei J. **CavityPlus: a web server for protein cavity detection with pharmacophore modelling, allosteric site identification and covalent ligand binding ability prediction.** *Nucleic Acids Res*. 2018; 46(W1):W374-W379. doi: 10.1093/nar/gky380	2018
ProteinsPlus	https://proteins.plus/ Multiple-task server for systematic analysis of protein-ligand interactions; platform integrating several tools	Schoning-Stierand K, Diedrich K, Fahrrolfes R, Flachsenberg F, Meyder A, Nittinger E, Steinegger R, Rarey M. **ProteinsPlus: interactive analysis of protein-ligand binding interfaces.** *Nucleic Acids Res*. 2020; 48(W1):W48-W53. doi: 10.1093/nar/gkaa235	2020
ezCADD	http://dxulab.org/software *User-friendly, web-based computer-aided drug design environment for non-experts to perform small-molecule docking, protein-protein docking, and binding pocket detection*	Tao A, Huang Y, Shinohara Y, Caylor ML, Pashikanti S, Xu D. **ezCADD: a rapid 2D/3D visualization-enabled web modeling environment for democratizing computer-aided drug design.** *J Chem Inf Model*. 2019; 59(1):18-24. doi: 10.1021/acs.jcim.8b00633	2019