LOSCHMIDT LABORATORIES

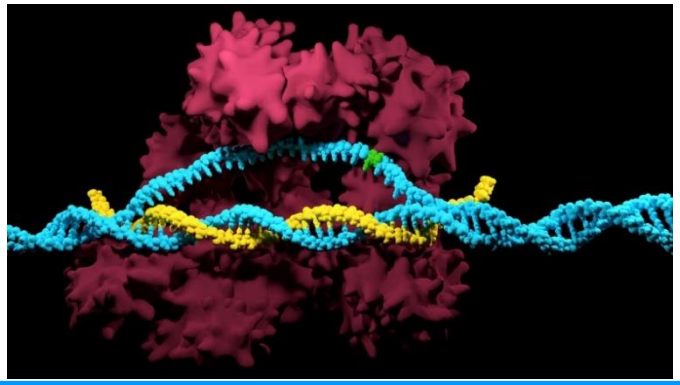
Engineering of protein structures

Outline

- Overview of mutations
- Databases of mutations
- Missense mutations
- Prediction of mutational effects
- Rational design of proteins

Overview of mutations

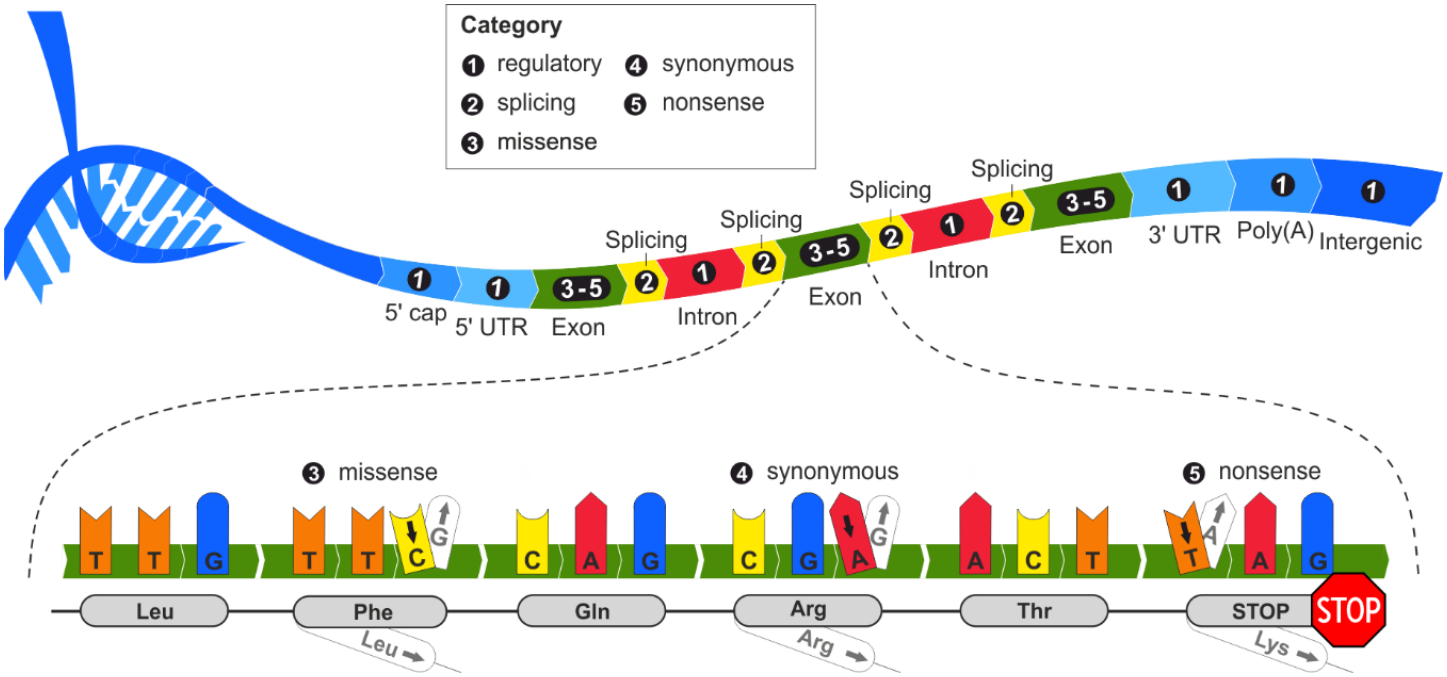
- **Mutations in DNA or RNA may occur**
  - Errors in DNA replication during cell division
  - Exposure to mutagens (physical or chemical agents)
  - Viral infections
  - …Or scientist intervention ☺
- **Can be harmful or not**



Overview of mutations

Location in the DNA

- Non-coding region –> affect gene expression (transcriptional regulation, mRNA stability, translation rates, location, etc.)
- Coding region –> may affect protein sequence



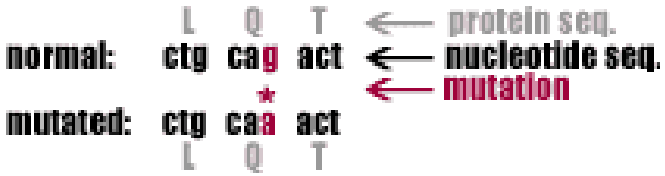
Overview of mutations

- **Types**
  - **Point mutations** – a single nucleotide is changed in DNA (or RNA)
    - Substitutions
      - Single nucleotide polymorphism (SNP – pronounced "*snip*")
      - Genetic variation; occurs in > 1 % of population
      - About 10,000,000 in the human genome
    - Insertions or deletions
      - Codons have triple nature (3 nucleotides → 1 amino acid)
      - Potential for frameshift (change in the grouping of codons, resulting in a different translation)
      - Can be very deleterious
  - **Other types** (duplications, translocations, inversions, etc.)

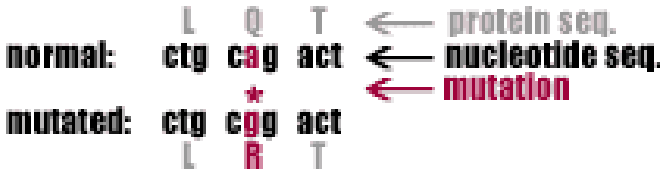
Point mutations at protein level

Types of point mutations

- Silent (synonymous SNP) – no effect on protein sequence



- Missense (non-synonymous SNP) – substitution of amino acid



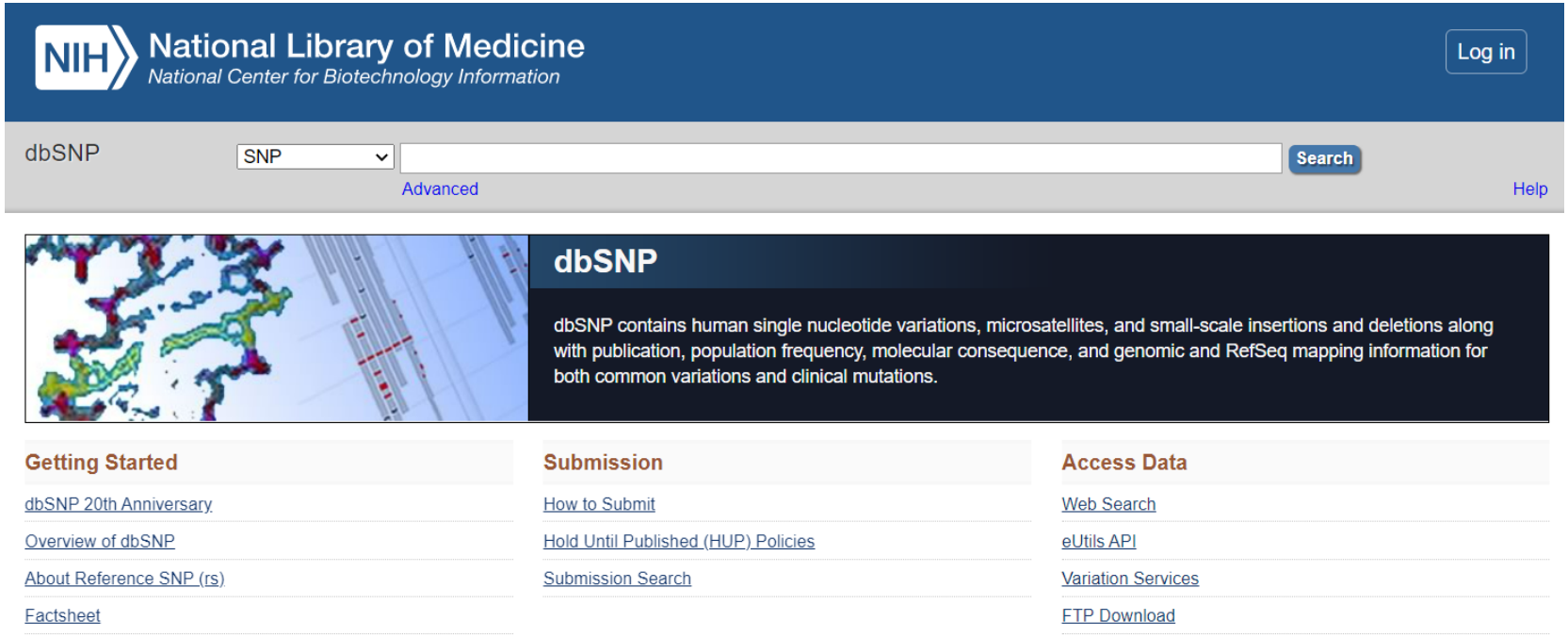
- Nonsense – introduction of a stop codon -> protein truncation

Databases of mutations

- **Human Genome Variation Society**
  - http://www.hgvs.org
  - Lists all the available databases of human mutations
- **Central mutation databases (>20)**
  - Substitutions in all genes
  - Variability in protein sequences
  - Data mainly from literature
- **Locus-specific databases (about 700)**
  - Substitutions in specific genes
  - Typically manually annotated

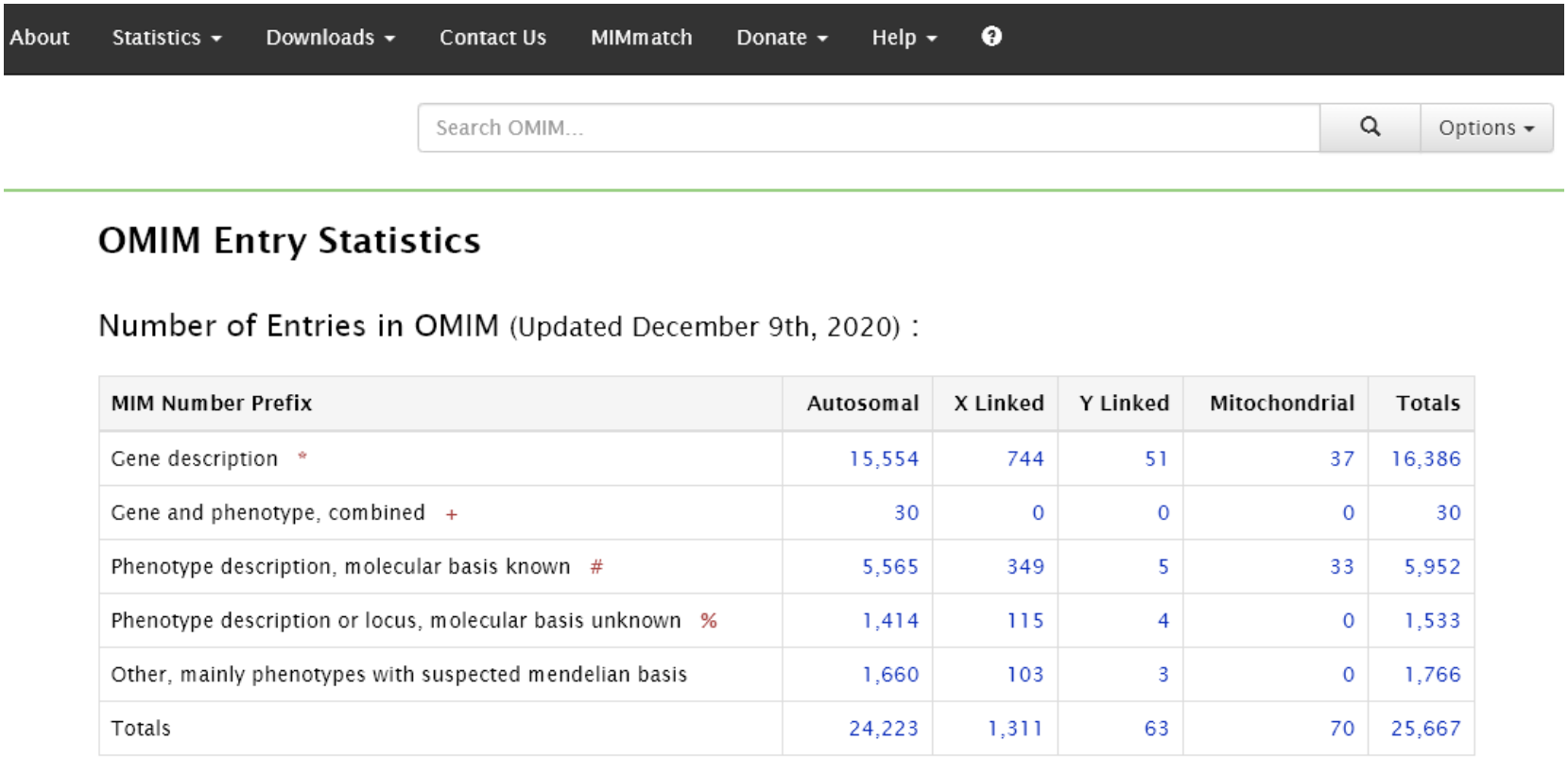
Central mutation databases

- **Database of Single Nucleotide Polymorphisms - dbSNP**
  - http://www.ncbi.nlm.nih.gov/SNP/
  - Repository for both SNP and short deletion and insertion
  - For human genome



Central mutation databases

- **Online Mendelian Inheritance in Man – OMIM**
  - http://omim.org/
  - Comprehensive database of human genes and genetic phenotypes



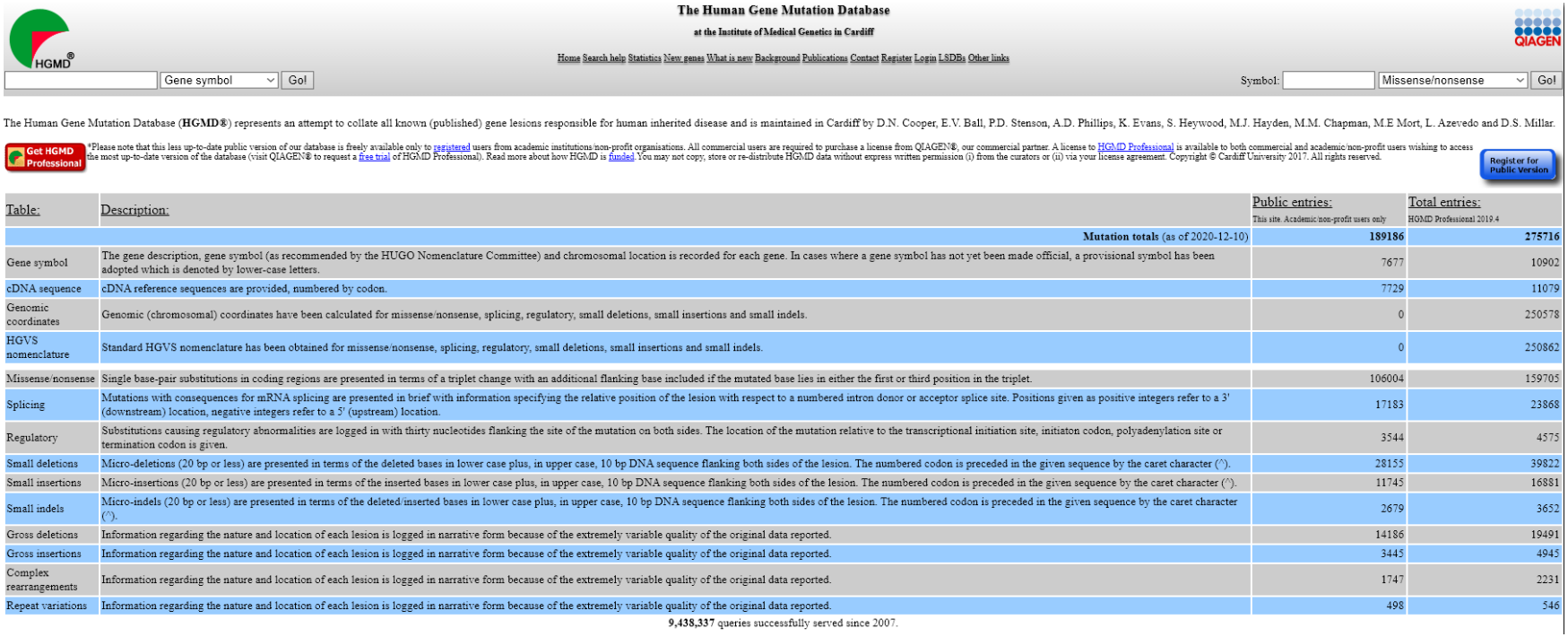
OMIM Entry Statistics

Number of Entries in OMIM (Updated December 9th, 2020) :

MIM Number Prefix	Autosomal	X Linked	Y Linked	Mitochondrial	Totals
Gene description *	15,554	744	51	37	16,386
Gene and phenotype, combined +	30	0	0	0	30
Phenotype description, molecular basis known #	5,565	349	5	33	5,952
Phenotype description or locus, molecular basis unknown %	1,414	115	4	0	1,533
Other, mainly phenotypes with suspected mendelian basis	1,660	103	3	0	1,766
Totals	24,223	1,311	63	70	25,667

Central mutation databases

- **Human Gene Mutation Database - HGMD**
  - http://www.hgmd.cf.ac.uk/ac/index.php
  - Comprehensive collection of mutations in nuclear genes that underlie or are associated with human inherited disease



The Human Gene Mutation Database

at the Institute of Medical Genetics in Cardiff

Home Search help Statistics New genes What is new Background Publications Contact Register Login LSDBs Other links

Gene symbol Go! Symbol: Missense/nonsense Go!

The Human Gene Mutation Database (**HGMD®**) represents an attempt to collate all known (published) gene lesions responsible for human inherited disease and is maintained in Cardiff by D.N. Cooper, E.V. Ball, P.D. Stenson, A.D. Phillips, K. Evans, S. Heywood, M.J. Hayden, M.M. Chapman, M.E Mort, L. Azevedo and D.S. Millar.

Get HGMD Professional

*Please note that this less up-to-date public version of our database is freely available only to registered users from academic institutions/non-profit organisations. All commercial users are required to purchase a license from QIAGEN®, our commercial partner. A license to HGMD Professional is available to both commercial and academic/non-profit users wishing to access the most up-to-date version of the database (visit QIAGEN® to request a free trial of HGMD Professional). Read more about how HGMD is funded. You may not copy, store or re-distribute HGMD data without express written permission (i) from the curators or (ii) via your license agreement. Copyright © Cardiff University 2017. All rights reserved.

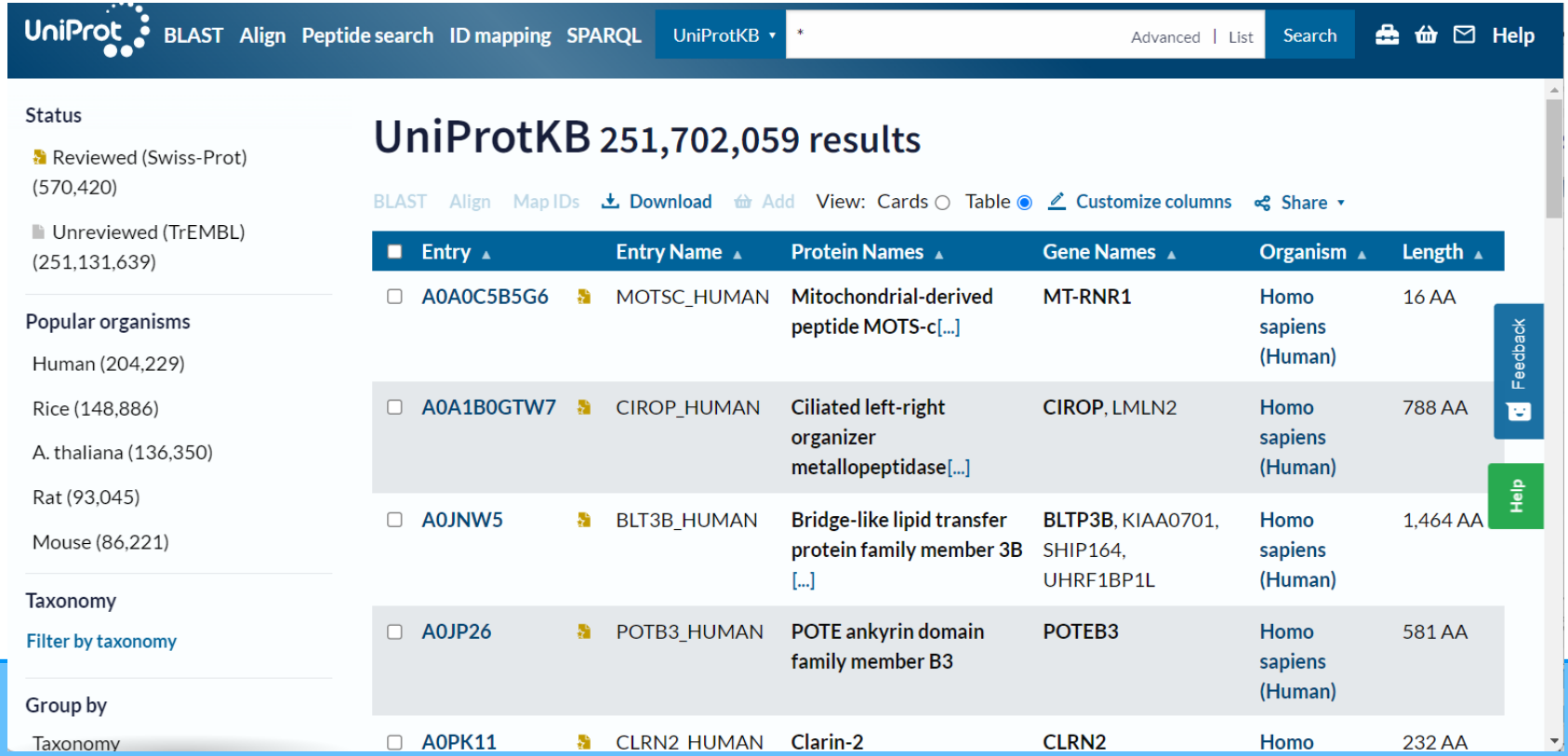
Register for Public Version

Table:	Description:	Public entries: This site. Academic/non-profit users only	Total entries: HGMD Professional 2019.4
	Mutation totals (as of 2020-12-10)	**189186**	**275716**
Gene symbol	The gene description, gene symbol (as recommended by the HUGO Nomenclature Committee) and chromosomal location is recorded for each gene. In cases where a gene symbol has not yet been made official, a provisional symbol has been adopted which is denoted by lower-case letters.	7677	10902
cDNA sequence	cDNA reference sequences are provided, numbered by codon.	7729	11079
Genomic coordinates	Genomic (chromosomal) coordinates have been calculated for missense/nonsense, splicing, regulatory, small deletions, small insertions and small indels.	0	250578
HGVS nomenclature	Standard HGVS nomenclature has been obtained for missense/nonsense, splicing, regulatory, small deletions, small insertions and small indels.	0	250862
Missense/nonsense	Single base-pair substitutions in coding regions are presented in terms of a triplet change with an additional flanking base included if the mutated base lies in either the first or third position in the triplet.	106004	159705
Splicing	Mutations with consequences for mRNA splicing are presented in brief with information specifying the relative position of the lesion with respect to a numbered intron donor or acceptor splice site. Positions given as positive integers refer to a 3' (downstream) location, negative integers refer to a 5' (upstream) location.	17183	23868
Regulatory	Substitutions causing regulatory abnormalities are logged in with thirty nucleotides flanking the site of the mutation on both sides. The location of the mutation relative to the transcriptional initiation site, initiaton codon, polyadenylation site or termination codon is given.	3544	4575
Small deletions	Micro-deletions (20 bp or less) are presented in terms of the deleted bases in lower case plus, in upper case, 10 bp DNA sequence flanking both sides of the lesion. The numbered codon is preceded in the given sequence by the caret character (^).	28155	39822
Small insertions	Micro-insertions (20 bp or less) are presented in terms of the inserted bases in lower case plus, in upper case, 10 bp DNA sequence flanking both sides of the lesion. The numbered codon is preceded in the given sequence by the caret character (^).	11745	16881
Small indels	Micro-indels (20 bp or less) are presented in terms of the deleted/inserted bases in lower case plus, in upper case, 10 bp DNA sequence flanking both sides of the lesion. The numbered codon is preceded in the given sequence by the caret character (^).	2679	3652
Gross deletions	Information regarding the nature and location of each lesion is logged in narrative form because of the extremely variable quality of the original data reported.	14186	19491
Gross insertions	Information regarding the nature and location of each lesion is logged in narrative form because of the extremely variable quality of the original data reported.	3445	4945
Complex rearrangements	Information regarding the nature and location of each lesion is logged in narrative form because of the extremely variable quality of the original data reported.	1747	2231
Repeat variations	Information regarding the nature and location of each lesion is logged in narrative form because of the extremely variable quality of the original data reported.	498	546

9,438,337 queries successfully served since 2007.

Central mutation databases

- **UniProtKB/Swiss-Prot**
  - http://www.uniprot.org/UniProtKB/
  - High-quality manually annotated protein entries with partial lists of known sequence variants



Locus-specific databases

- For information on gene-specific databases

ATP-binding cassette, sub-family D (ALD), member 1 300371	X-linked Adrenoleukodystrophy Database http://www.x-ald.nl	Ronald R.J.A. Wanders Lab. of Genetic Metabolic Diseases Academic Medical Ctr. Amsterdam, The Netherlands.
ABO ABO blood group (transferase A, alpha 1-3-N-acetylgalactosaminyltransferase; transferase B, alpha 1-3-galactosyltransferase) 110300	Blood Group Antigen Mutation Database http://www.ncbi.nlm.nih.gov/gv/mhc/xslcgi.cgi?cmd=bgmut/home	Olga O. Blumenfeld Department of Biochemistry, Santosh Patnaik, Department of Cell Biology, Albert Einstein College of Medicine New York, NY. U.S.A
ACAD8 acyl-CoA dehydrogenase family, member 8 604773	Innsbruck Metabolic Diseases Pages http://lovd.i-med.ac.at/home.php?select_db=ACAD8	Barbara Lanthaler, Stefanie Kalb and Martina Witsch-Baumgartner
ACADM acyl-CoA dehydrogenase, C-4 to C-12 straight chain 607008	CCHMC - Human Genetics Mutation Database https://research.cchmc.org/LOVD/home.php?select_db=ACADM	Ammar Husami, Brian Richardson, Edita Freeman, Kerry Shooner, Thedia Jacobs and Theru A. Sivakumaran
ACADSB acyl-CoA dehydrogenase, short/branched chain 600301	Innsbruck Metabolic Diseases Pages http://lovd.i-med.ac.at/home.php?select_db=ACADSB	Barbara Lanthaler, Stefanie Kalb and Martina Witsch-Baumgartner
ACADVL acyl-CoA dehydrogenase, very long chain 609575	CCHMC - Human Genetics Mutation Database https://research.cchmc.org/LOVD/home.php?select_db=ACADVL	Ammar Husami, Brian Richardson, Edita Freeman, Kerry Shooner, Thedia Jacobs and Theru A. Sivakumaran
ACE2 angiotensin I converting enzyme (peptidyl-dipeptidase A) 2 300335	ACE2 database at LOVD http://www.LOVD.nl/ACE2	Johan T. den Dunnen Leiden Univ. Med Centre *(acting), Curator vacancy*
ACHE acetylcholinesterase (Yt blood group) 100740	Blood Group Antigen Mutation Database http://www.ncbi.nlm.nih.gov/gv/mhc/xslcgi.cgi?cmd=bgmut/home	Olga O. Blumenfeld Department of Biochemistry, Santosh Patnaik, Department of Cell Biology, Albert Einstein College of Medicine New York, NY. U.S.A
ACOT9 acyl-CoA thioesterase 9	ACOT9 database at LOVD http://www.LOVD.nl/ACOT9	Johan T. den Dunnen Leiden Univ. Med Centre *(acting), Curator vacancy*
ACSL4 acyl-CoA synthetase long-chain family member 4 300157	ACSL4 database at LOVD http://www.LOVD.nl/ACSL4	Johan T. den Dunnen Leiden Univ. Med Centre *(acting), Curator vacancy*
ACTA1 actin, alpha 1, skeletal muscle 102610	Laing Laboratory Skeletal muscle alpha-actin (ACTA1) http://acta1.waimr.uwa.edu.au/home.php?select_db=ACTA1	Nigel Laing and Kristen Nowak

Missense mutations

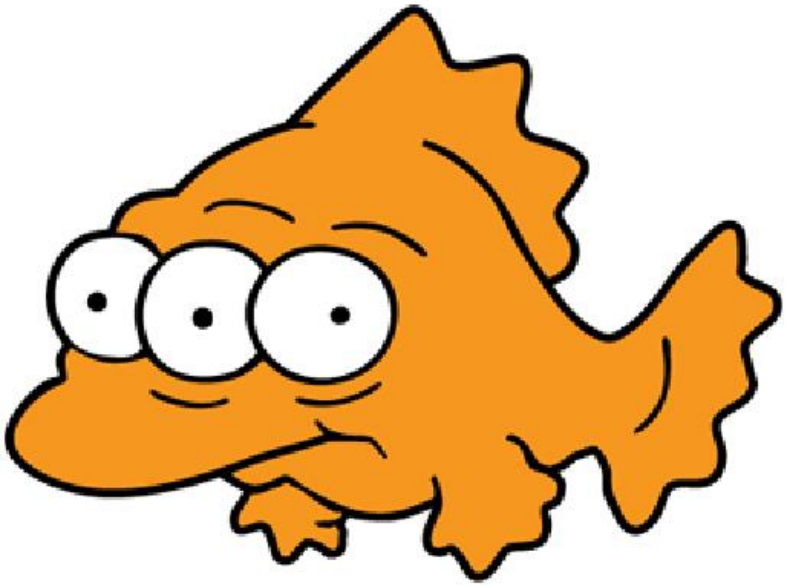
- What are they?...
- How can they affect proteins?

Missense mutations

- Mutations affecting structure
  - Stability & folding
  - Aggregation
- Mutations affecting function
  - Binding & catalysis
  - Transport processes
  - Protein dynamics
  - Protein localization

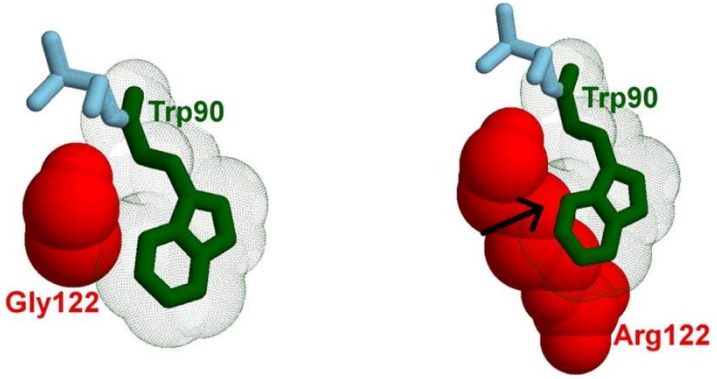
Mutations affecting structure

- **Major <u>pathogenic</u> consequences of missense mutation**
  - Compromised folding – the protein has modified folds or presents more unfolded states
  - Decreased stability – the lifetime of the protein is decreased
  - Increased aggregation

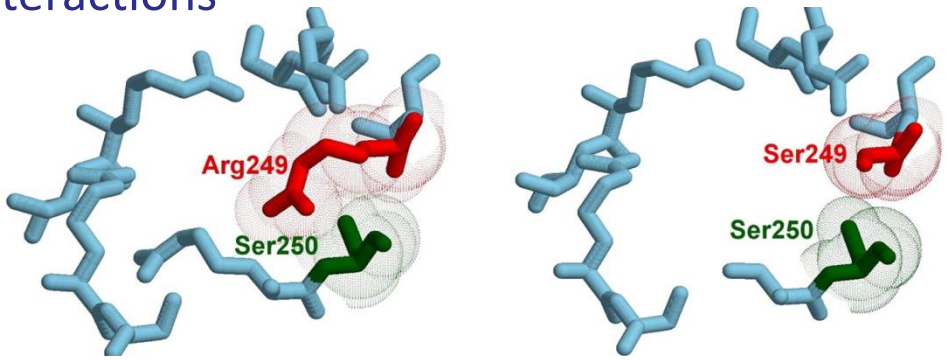


Mutations affecting structure

- **Molecular basis of mutations affecting folding & stability**
  - Introduced clashes – common for small to large mutations in buried residues



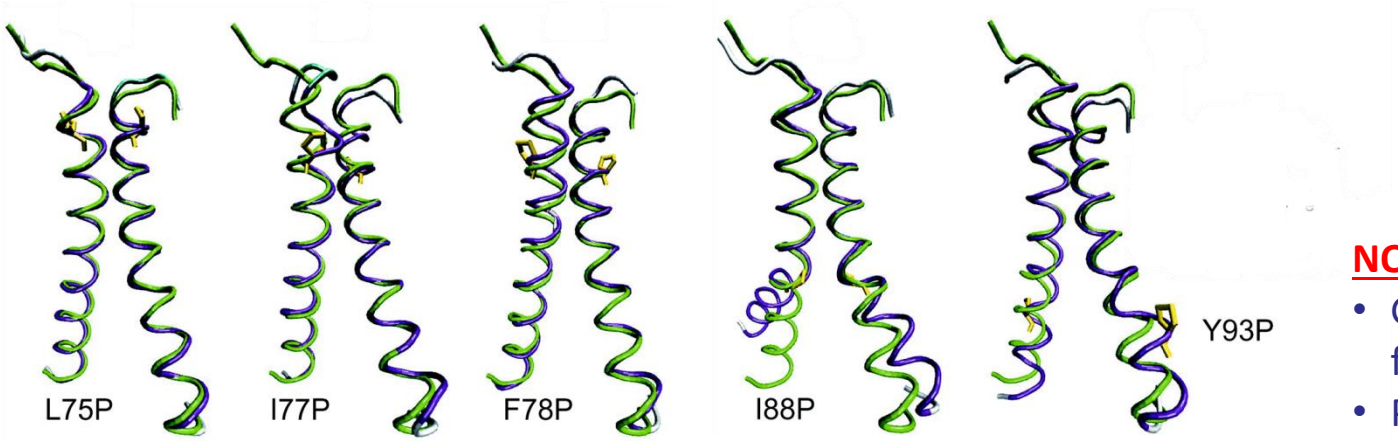
  - Loss of interactions – most pronounced effects related to H-bonds, salt bridges and aromatic interactions



Mutations affecting structure

Molecular basis of mutations affecting folding & stability

- Altered conformation of protein backbone – mutations concerning residues with specific backbone angles (especially glycine and proline)

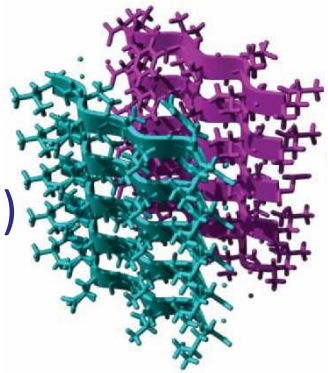


NOTE:
- Glycine – the most flexible amino acid
- Proline – the most rigid

- Changes in charge/hydrophobicity
  - Introducing hydrophilic/charged residue into the protein core
  - Introducing hydrophobic residue onto the protein surface

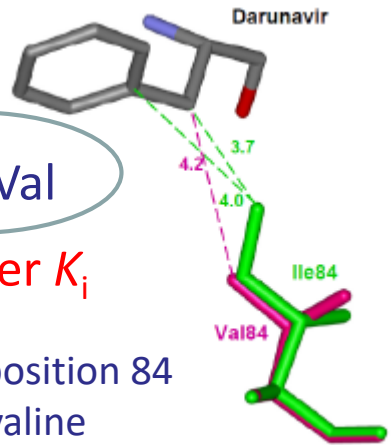
Mutations affecting structure

- **Mutations can reduce solubility or increase aggregation**
  - Alterations on the surface residues may affects the solubility (ex: reduction of charge)
  - Hydrophobic mutations can increase protein aggregation
  - Aggregating proteins usually have high level of β-structures
- **Aggregation modulated by short specific sequences**
  - Aggregation-prone regions (APRs) are sequences of 5-15 hydrophobic residues
  - They tend to stack and form amyloid fibrils (cross-β spines)
  - Some mutations can increase the propensity to form such amyloid structures



Mutations affecting function

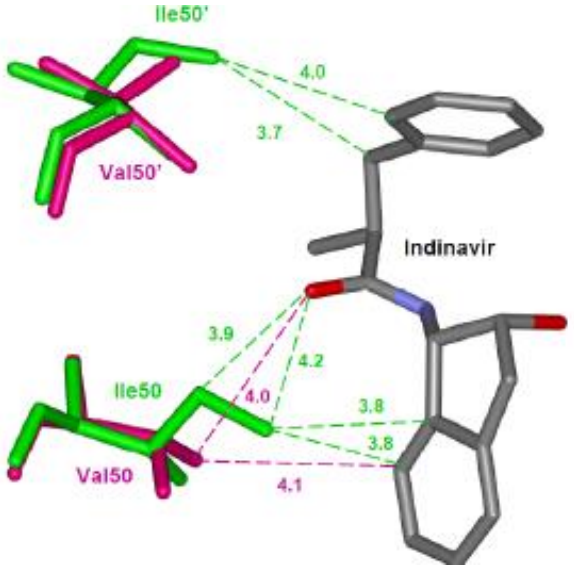
- **Effect on binding and catalysis**
  - Binding sites are tuned to bind specific molecules and stabilize transition states
  - Mutations can improve or disrupt the binding and catalysis
- Example – drug-resistance of HIV-1 protease mutants
  - Loss of interactions with inhibitors



Ile84Val

3x higher K_i

Means: isoleucine in position 84 was mutated to valine

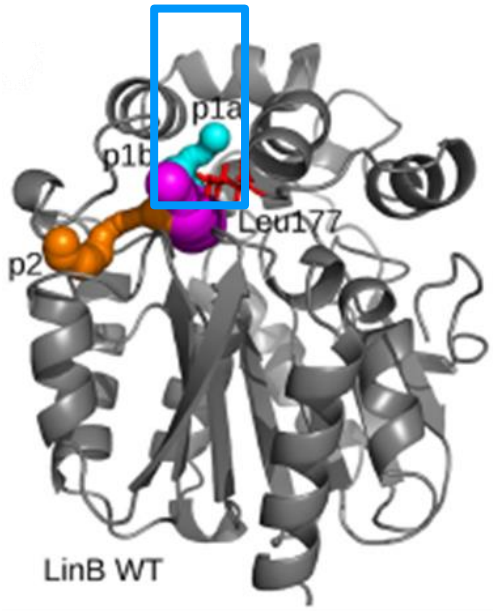


Ile50Val

37x higher K_i

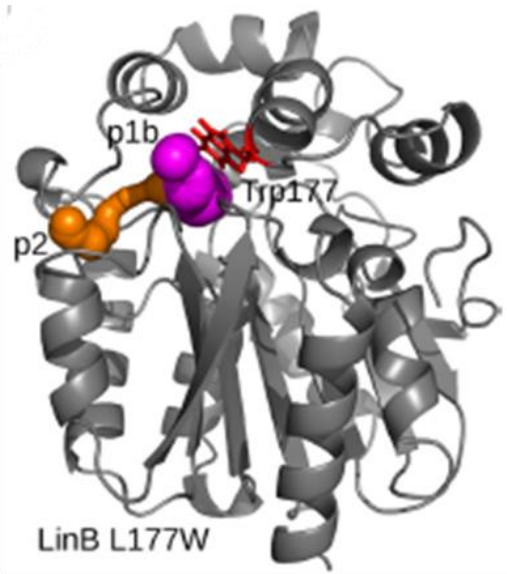
Mutations affecting function

- **Effect on ligand transport**
  - Pathways are adjusted to permit transport of specific molecules
  - Mutations can speed-up or disrupt their transport or allow the transport of different molecules



Leu177Trp => tunnel becomes almost *closed*

release of products 500x slower



Mutations affecting function

- **Effect on protein dynamics**
  - Dynamics enables proteins to adapt to their binding partners and interchanging between conformations
  - Mutations can:
    - Make regions more rigid (targeting hinge or very mobile regions, ex.: loops) -> reduced adaptability
    - Increase flexibility of rigid regions (targeting residues with many contacts in mobile elements) -> increased adaptability
  - These change may affect activity, specificity or even recognition

Mutations affecting function

- **Effect on protein localization**
  - After translation, the protein must be translocated to the appropriate cellular compartment
  - Translocation can be regulated by short sequences (Signal Peptides) on the N-terminus, by Translocation Complexes, Chaperones, etc.
  - Mutations can disrupt or alter the signal, or complex formation -> protein fails to be transported to the correct subcellular location
    - Missing protein -> inactive reaction pathways or unregulated signaling cascades
    - Mislocalized protein -> active in the wrong cellular compartment, causing harmful effects

Prediction of mutational effects

- Identification of mutable residues
- Prediction of the effects on structure
- Prediction of pathogenicity

Identification of mutable residues

- What is it?

Identification of mutable residues

- The effect of mutations on the protein can be predicted directly from the role of the modified residue

- **Mutation of evolutionary conserved residues**
  - Residues important for protein function or stability tend to be highly conserved over evolution
  - Mutation of highly conserved residues -> often lead to destabilization or loss of function
  - Mutation of highly variable residues -> often neutral

Identification of mutable residues

- **Mutations affecting stability & folding**
  - Mutation of residues with many contacts or with favorable interaction energy -> often destabilizing or compromise folding
  - Mutation of residues in protein core -> often destabilizing
    - Small residue to large -> steric clashes
    - Large to small -> loss of contacts (creation of a void)
    - Polar to non-polar -> loss of H-bond
    - Neutral to charged -> introduction of isolated charge
  - Mutation of residues on protein surface (often neutral)
    - Polar to hydrophobic -> desolvation penalty (destabilizing)
  - Mutation involving proline or glycine -> altered conformation

Identification of mutable residues

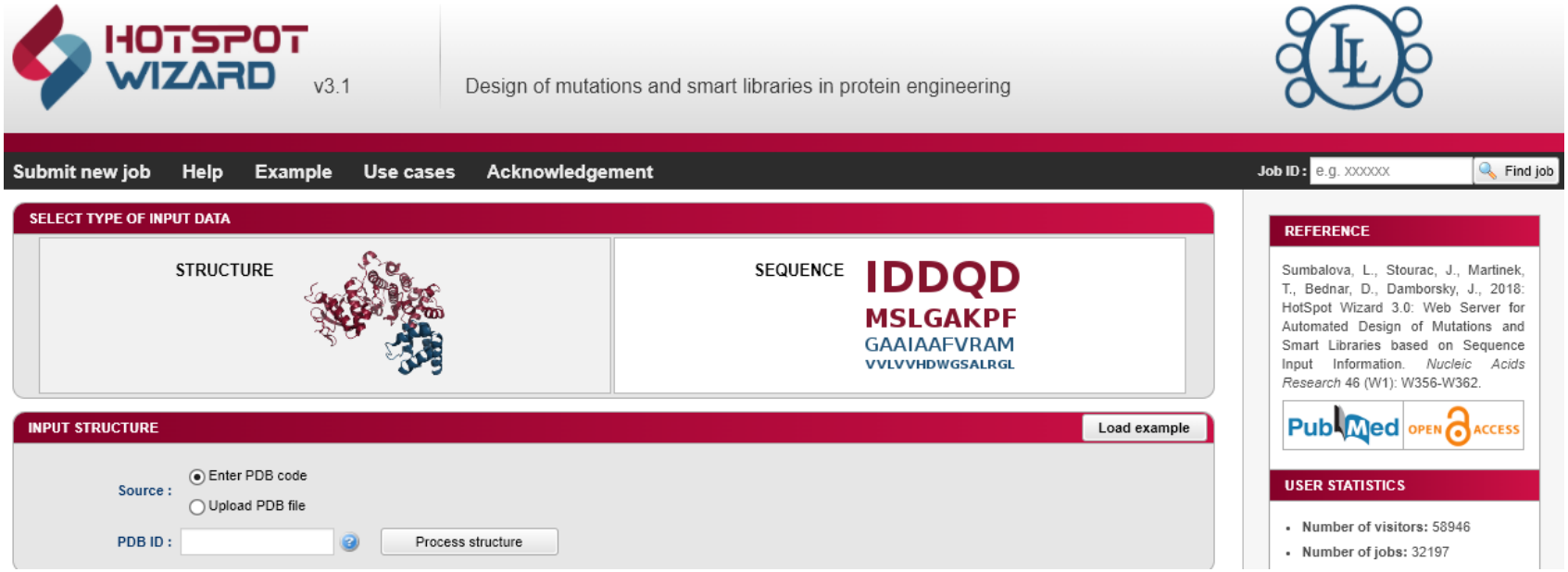
- **Mutations affecting function**
  - Mutation of residues in binding or active sites -> modify binding or catalysis
  - Mutation of residues in transport pathways -> modify transport
  - Mutation of hinge or mobile residues, residues on loops with many contacts -> modify flexibility
  - Mutation of residues directing protein localization -> mislocalization of proteins

Identification of mutable residues

- **Tools for annotating (identifying) the role of residues**
  - Individual tools for specific analysis
    - Evolutionary conservation – ex:. ConSurf, …
    - Residue contacts – ex: Contact Map Web Viewer, …
    - Residue interactions – ex: Protein Interaction Calculator, …
    - Accessible surface area – ex: AsaView, Naccess, …
    - Binding sites – ex: CASTp, metaPocket 2.0, meta-PPISP, …
    - Transport pathways – ex: CAVER 3.0, POREWALKER, …
    - Protein dynamics – ex: NMA, molecular dynamics, …
    - Protein localization – ex: SignalP, TargetP, Phobius, TMHMM, …

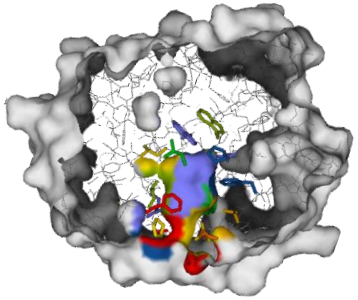
Identification of mutable residues

- **HotSpot Wizard – meta-server combining several tools**
  - http://loschmidt.chemi.muni.cz/hotspotwizard/
  - Homology modelling, MSA, conservation, correlation, pockets and tunnels detection, docking, stability prediction, design of smart library

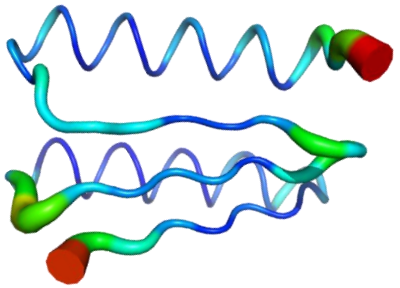


Identification of mutable residues

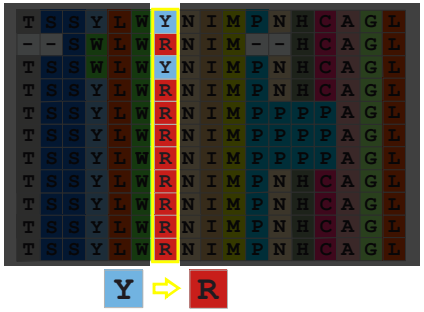
Functional hot-spots



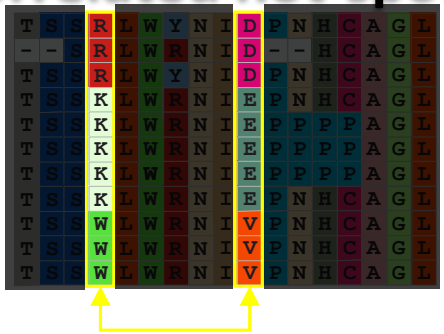
Stability hot-spots (flexibility)



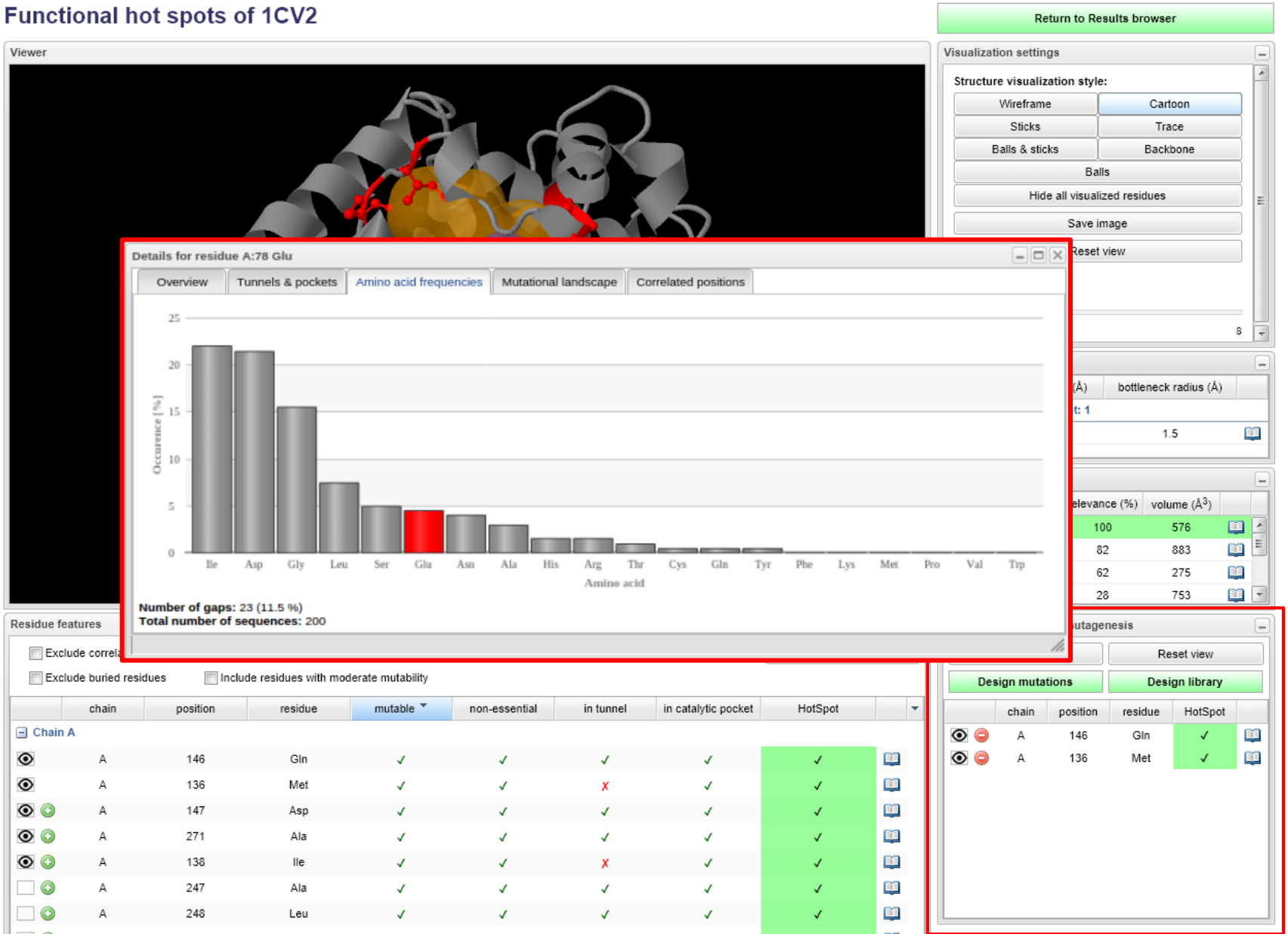
Stability hot-spots (evolution)



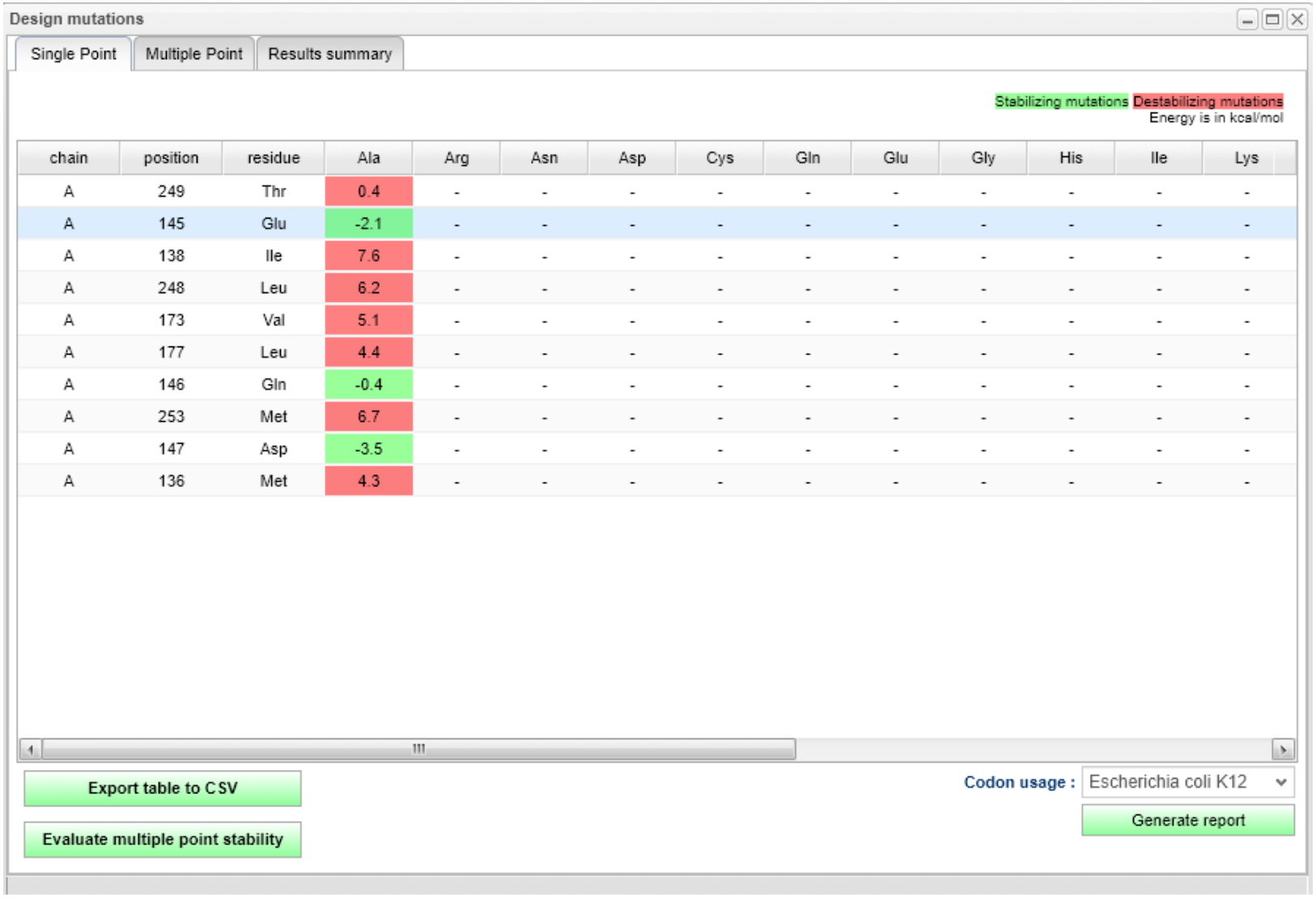
Correlated hot-spots



Identification of mutable residues



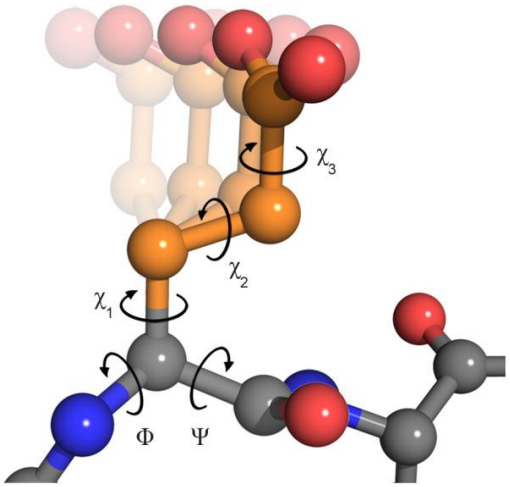
Identification of mutable residues



chain	position	residue	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Lys
A	249	Thr	0.4	-	-	-	-	-	-	-	-	-	-
A	145	Glu	-2.1	-	-	-	-	-	-	-	-	-	-
A	138	Ile	7.6	-	-	-	-	-	-	-	-	-	-
A	248	Leu	6.2	-	-	-	-	-	-	-	-	-	-
A	173	Val	5.1	-	-	-	-	-	-	-	-	-	-
A	177	Leu	4.4	-	-	-	-	-	-	-	-	-	-
A	146	Gln	-0.4	-	-	-	-	-	-	-	-	-	-
A	253	Met	6.7	-	-	-	-	-	-	-	-	-	-
A	147	Asp	-3.5	-	-	-	-	-	-	-	-	-	-
A	136	Met	4.3	-	-	-	-	-	-	-	-	-	-

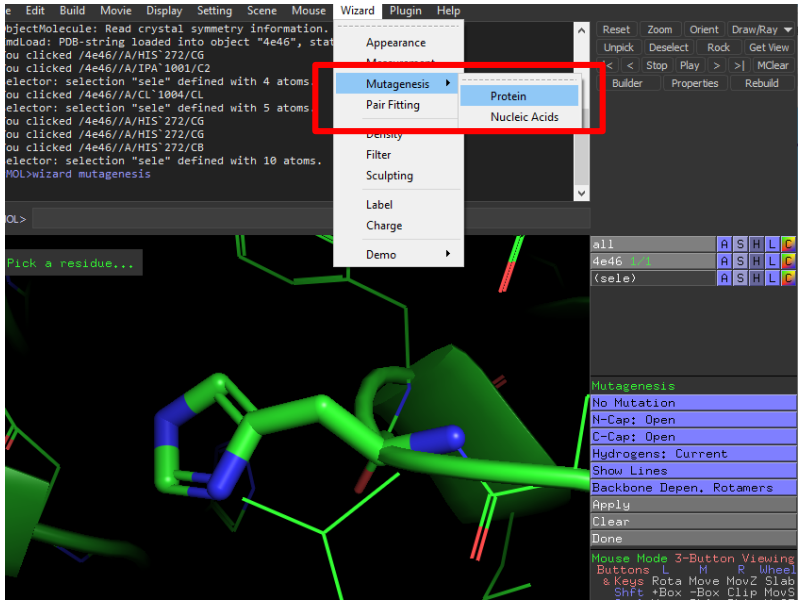
Prediction of effects on structure

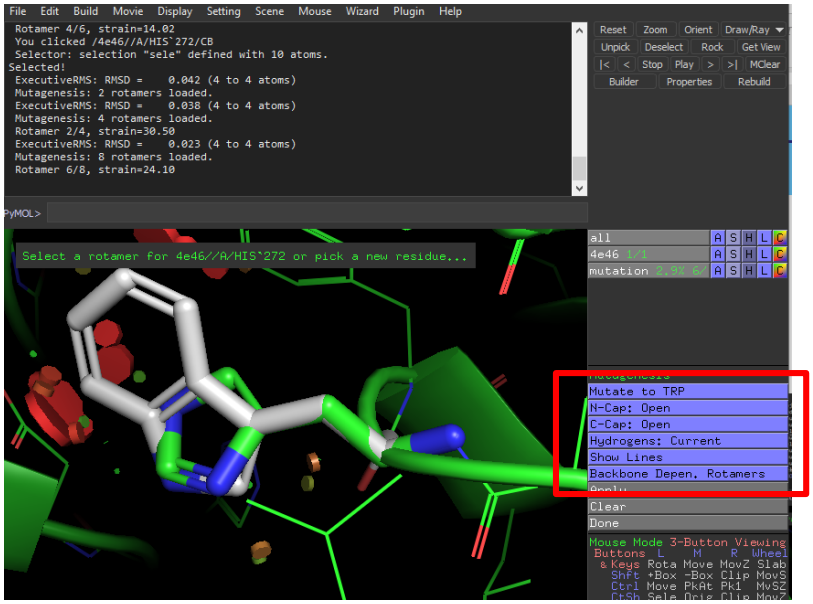
- **Prediction of mutant structures – general workflow**
  - Mutated residue and its surroundings represented by rotamers from rotamer library (conformations derived form X-ray structures)
  - The best set of rotamers selected by Monte Carlo approach
  - Optionally – energy minimization, backbone flexibility
  - Comparing structures of mutant and native protein -> assessment of the mutational effect ($\Delta\Delta G = \Delta G^{Mut} - \Delta G^{Native}$)
- Available tools
  - Geometric: PyMOL; WhatIF
  - Energy-based: FOLDX, Rosetta-ddG
  - Homology: Swiss Model, MODELLER, etc.



Prediction of effects on structure

- **PyMOL**
  - https://pymol.org/
  - Mutagenesis module
  - User can choose rotamers and visualize potential clashes
  - Very fast; fixed backbone; no mutational scoring





Prediction of effects on structure

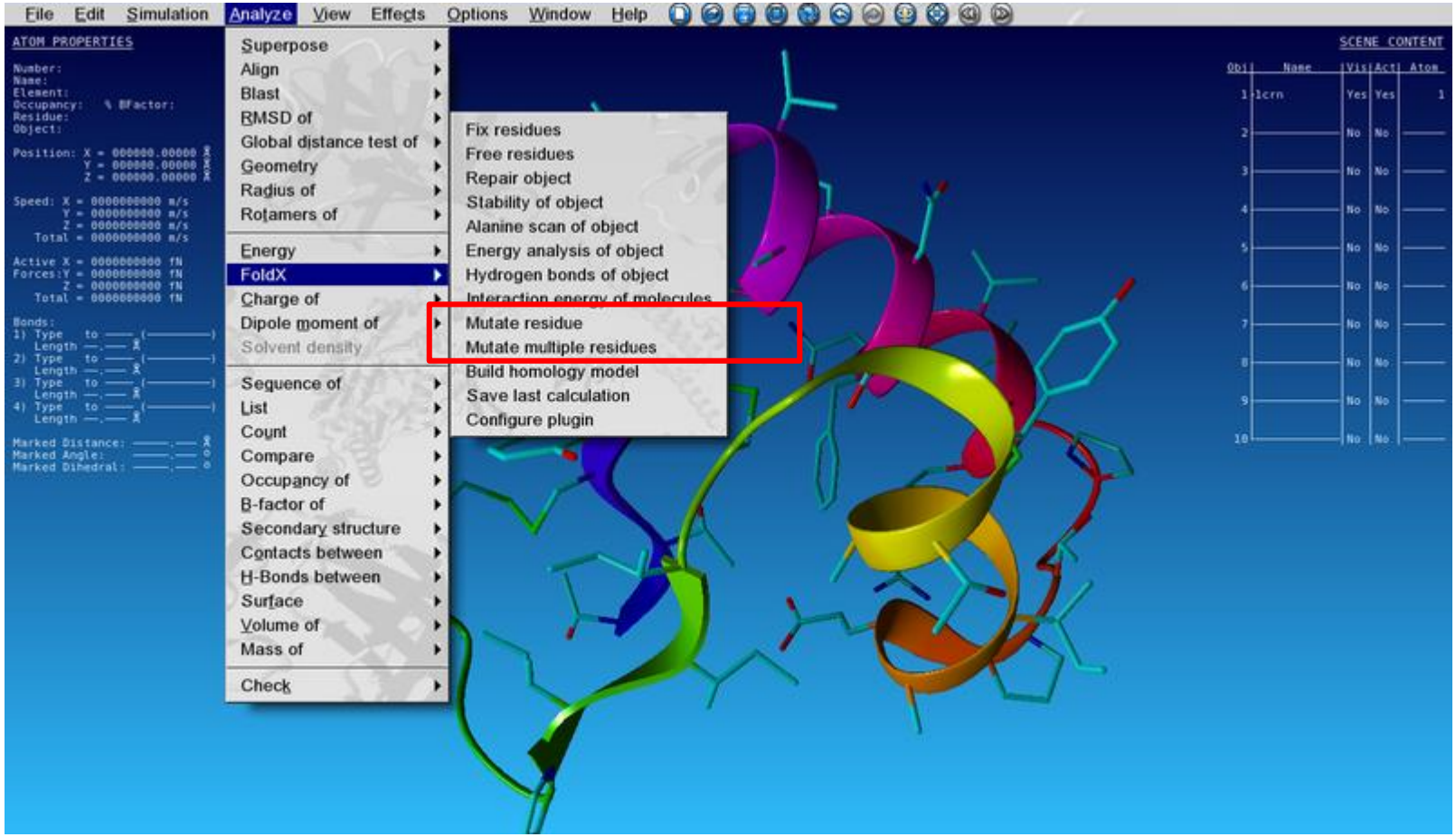
- **WHATIF**
  - https://swift.cmbi.umcn.nl/servers/html/index.html
  - Web server for multiple purpose including mutagenesis
  - Very fast
  - Fixed backbone conformation
  - Construction of single mutants only, or stabilizing Proline mutations
  - No scoring function

Prediction of effects on structure

- **FOLDX**
  - http://foldxsuite.crg.eu/
  - Stand alone, with plug-in to Yasara modeling tool
  - Fast (minutes)
  - Fixed backbone conformation
  - Construction of single or multiple mutants
  - Empirical scoring function for calculation of stability change (ΔΔG)

Prediction of effects on structure

- **FOLDX**



Prediction of effects on structure

- **Rosetta-ddG**
  - Under https://www.rosettacommons.org/
  - Stand alone with bash and python scripts available
  - Slow (hours-days)
  - Fixed or flexible backbone conformation
  - Construction of single or multiple mutants
  - Empirical force field for calculating structure and stability of wild-type and mutant
  - Construction of PDB and prediction of stability change (ΔΔG)

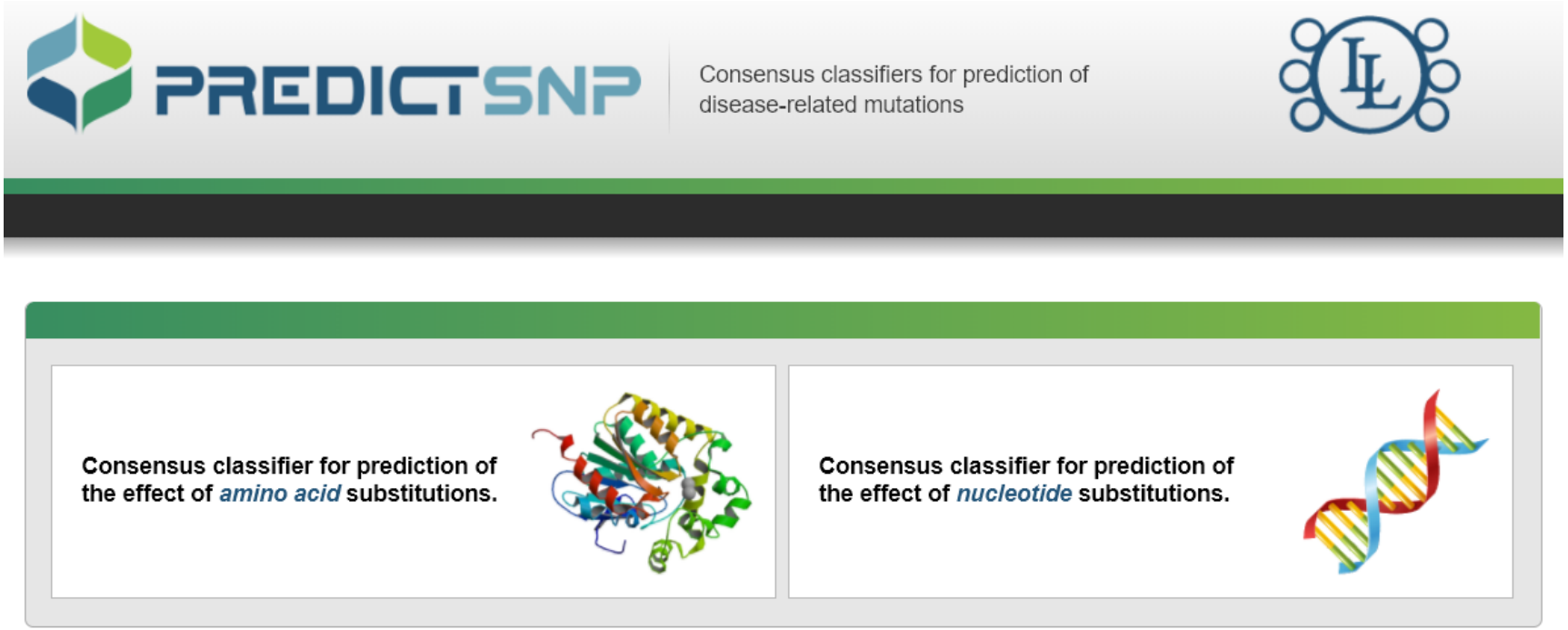
Prediction of pathogenicity

- **Prediction of impact of mutation on protein function**
  - Tools employ machine learning approaches
  - Trained on functional experimental data
  - Predictions can be based on sequence only
  - Qualitative results – i.e. deleterious versus neutral
  - Primarily intended for pathogenicity prediction (leading to disease)

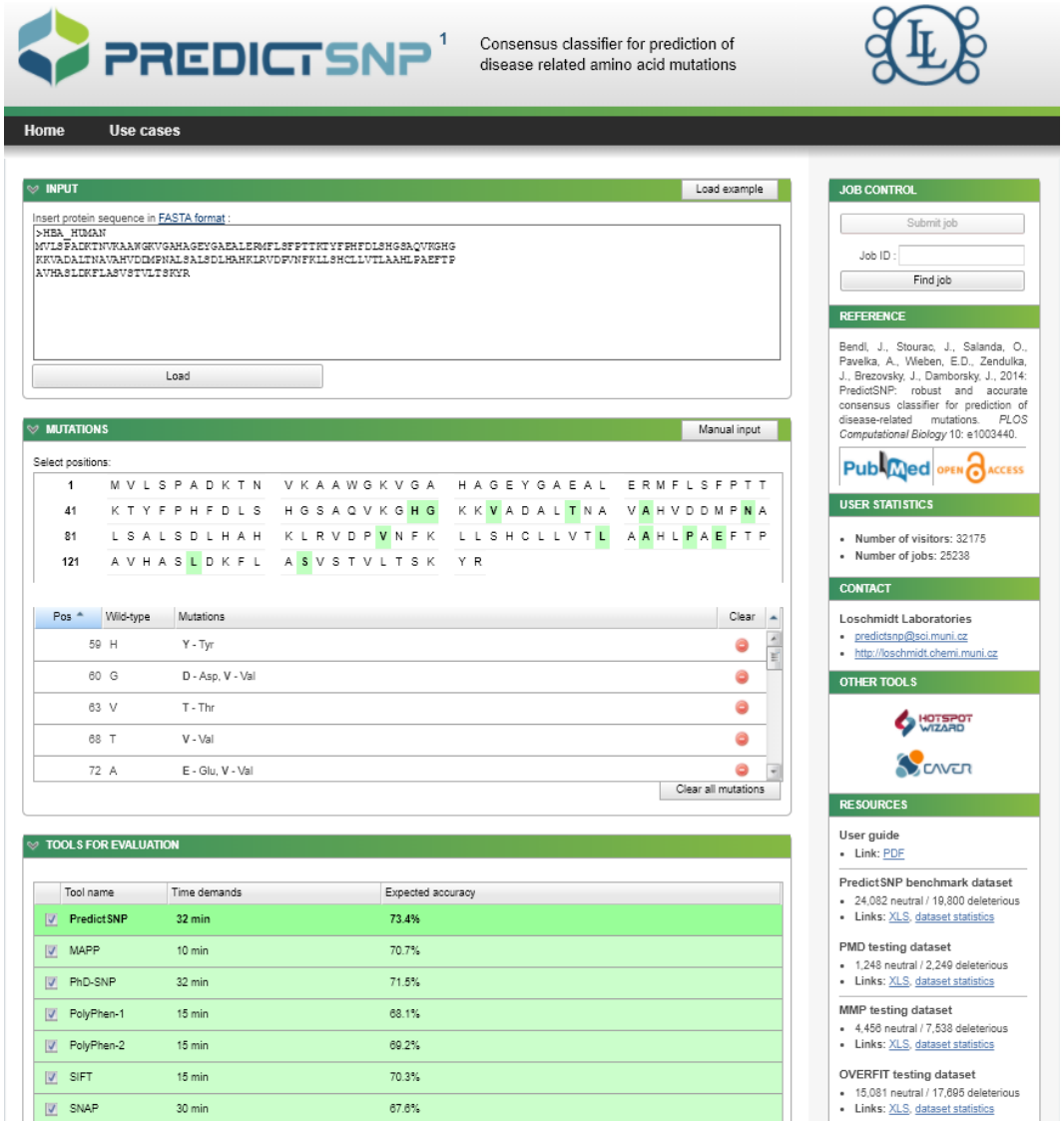
- **Available tools**
  - MutPred, SNAP, PhD-SNP, SIFT, MAPP …
  - PredictSNP – meta server combining many tools

Prediction of pathogenicity

- **PredictSNP:**
  - http://loschmidt.chemi.muni.cz/predictsnp/
  - Combines many tools for Protein or DNA assessment of SNPs



Prediction of pathogenicity



Prediction of pathogenicity

- **There are many more tools out there**

Method	Based on	Training set	Conservation analysis	Structural attributes	Annotations	Website
MutPred	RF	HGMD, Swiss-Prot	SIFT, Pfam, PSI-BLAST	Predicted attributes	–	http://mutpred.mutdb.org/
nsSNPAnalyzer	RF	Swiss-Prot	SIFT	Homologue mapping	–	http://snpanalyzer.uthsc.edu/
Panther	Alignment scores	–	Panther library, HMMs	–	–	http://www.pantherdb.org/tools/csnpScoreForm.jsp
PhD-SNP	SVM	Swiss-Prot	Sequence environment, sequence profiles	–	–	http://gpcr2.biocomp.unibo.it/cgi/predictors/PhD-SNP/PhD-SNP.cgi
PolyPhen	Empirical rules	–	PSIC profiles	Homologue mapping/predictions	Swiss-Prot	http://genetics.bwh.harvard.edu/pph/
PolyPhen2	Bayesian classification	Swiss-Prot, neutral pseudo-mutations	PSIC profiles	Homologue mapping/predictions	Pfam domain	http://genetics.bwh.harvard.edu/pph2/
SIFT	Alignment scores	–	MSAs	–	–	http://sift.jcvi.org/
SNAP	NN	PMD, neutral pseudo-mutations	PSIC profiles, Pfam, PSI-BLAST	Predictions	–	http://rostlab.org/services/snap/
SNPs&GO	SVM	Swiss-Prot	Sequence environment, sequence profiles, Panther	–	GO	http://snps-and-go.biocomp.unibo.it/snps-and-go/

Rational design of proteins

- Protein engineering: sometimes we can use mutagenesis to rationally design proteins according to our needs
- Properties that can be modified by mutagenesis
  - Such as?...

Rational design of proteins

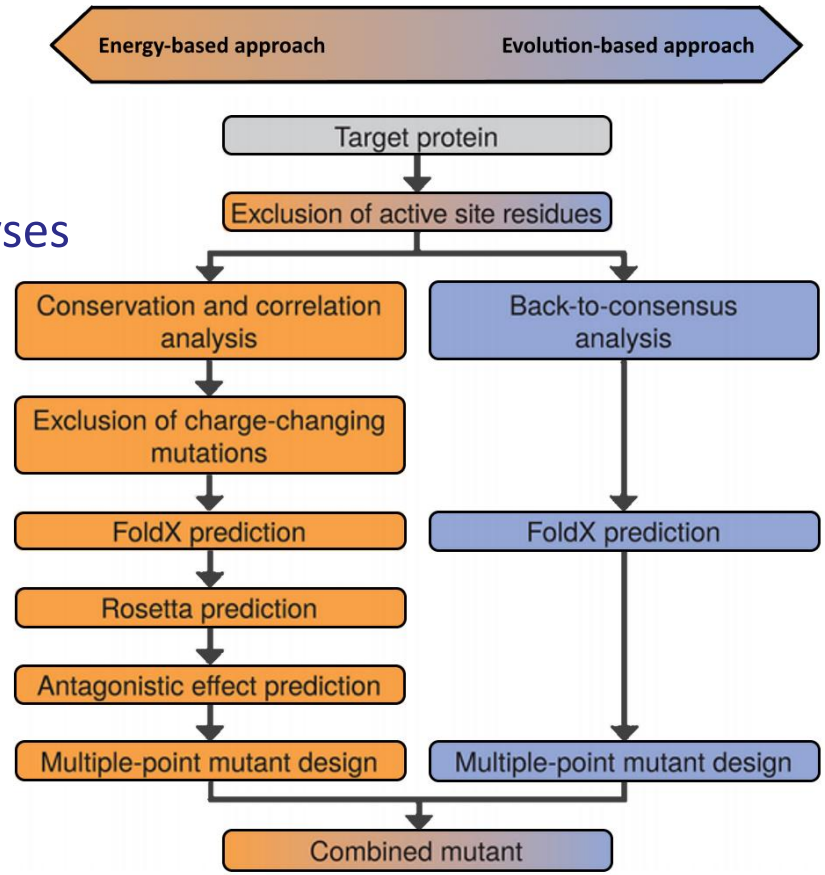
- Protein engineering: sometimes we can use mutagenesis to rationally design proteins according to our needs
- Properties that can be modified by mutagenesis
  - Stability
  - Function
    - Binging site (catalytic activity or substrate specificity)
    - Macromolecular interface
    - Molecular tunnels/channels
  - Solubility

Rational design: stability

- **Prediction of stability change upon mutation**
  - Structure of mutant protein may not be produced
  - Tools often employ
    - Empirical scoring functions
    - Evolutionary conservation analysis (ex: back-to-consensus)
    - Machine learning approaches
- Available tools
  - Energy-based: Rosetta-ddG, FOLDX ☑
  - Evolution-based: FireProt^{ASR}
  - Hybrid approaches: FireProt, PROSS

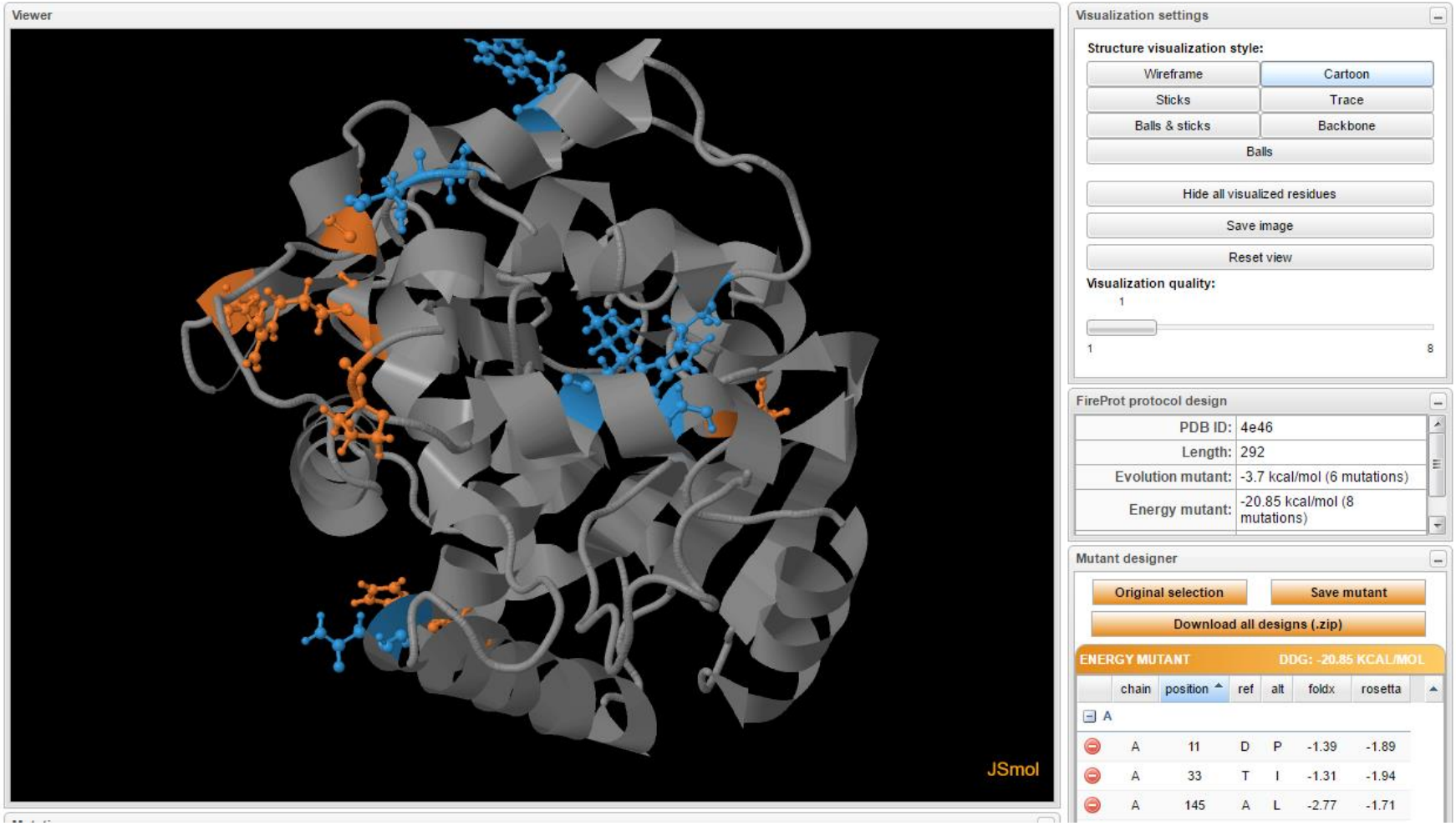
Rational design: stability

- **FireProt**
  - https://loschmidt.chemi.muni.cz/fireprotweb
  - *In silico* analysis of all possible mutations
  - Energy- and evolution-based analyses
  - Multiple-point mutants for gene synthesis



Rational design: stability

- **FireProt**



Rational design: stability

- **FireProt**

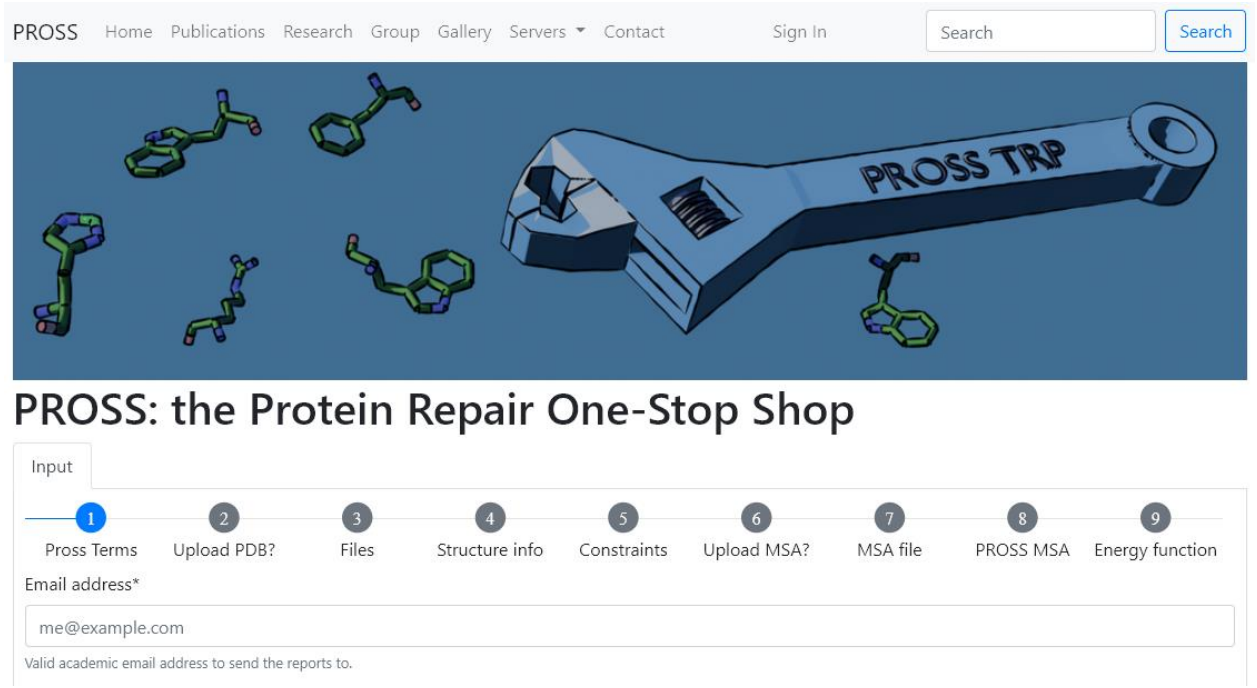
Mutations

Combined mutant | Energy mutant | Evolution mutant | Wild-type

Mutation info					Energy information			Evolution information		
visualize	chain	position	ref	alt	not conserved	not correlated	rosetta	mutable by majority	mutable by ratio	foldx
A										
👁	A	11	D	P	✓	✓	-1.89	✗	✗	-1.39
👁	A	20	E	S	✓	✓	-	✓	✓	0.08
👁	A	33	T	I	✓	✓	-1.94	✗	✗	-1.31
👁	A	119	N	H	✗	✓	-	✓	✗	-1
👁	A	145	A	L	✓	✓	-1.71	✗	✗	-2.77
👁	A	148	T	L	✓	✓	-2.15	✗	✗	-1.84
👁	A	155	A	P	✓	✓	-0.85	✓	✓	-1.1
👁	A	164	D	M	✓	✓	-1.85	✗	✗	-1.18
👁	A	176	C	W	✓	✓	-6.69	✗	✗	-1.76
👁	A	187	D	W	✓	✓	-2.81	✗	✗	-1.1
👁	A	198	D	S	✓	✓	-	✓	✗	-0.7
👁	A	200	E	R	✓	✓	-	✓	✗	-0.4
👁	A	217	N	W	✓	✓	-1.76	✓	✓	-1.38
👁	A	285	E	A	✓	✓	-	✓	✗	-0.38

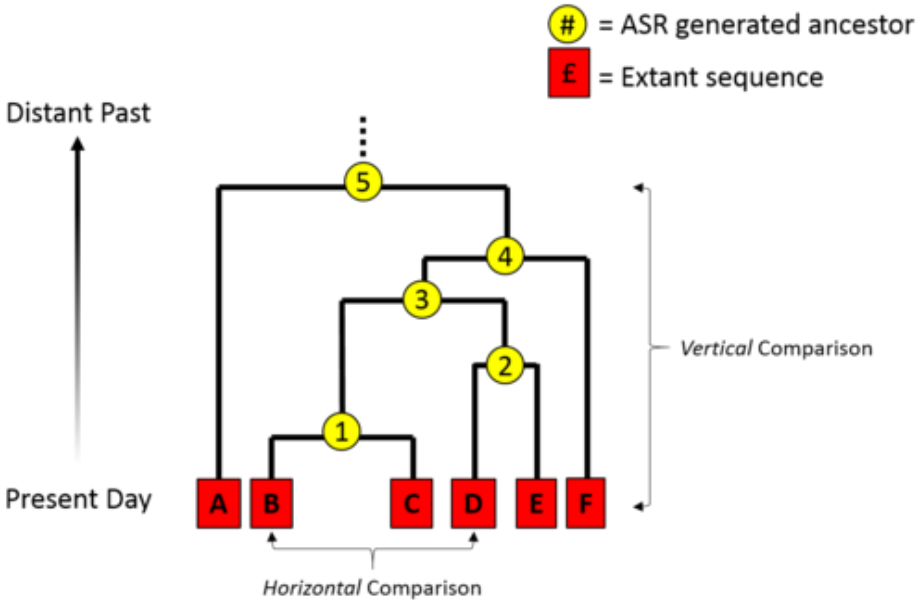
Rational design: stability

- **PROSS**
  - https://pross.weizmann.ac.il/step/pross-terms/
  - Combination of mutations “allowed” by conservation analysis and Rosetta calculations (energy)



Rational design: stability

- **FireProt^{ASR}**
  - https://loschmidt.chemi.muni.cz/fireprotasr
  - Ancestral sequence reconstruction (ASR)
  - Automated ancestral inference & phylogenetic tree
  - Useful to find stable ancestral enzymes



Rational design: stability

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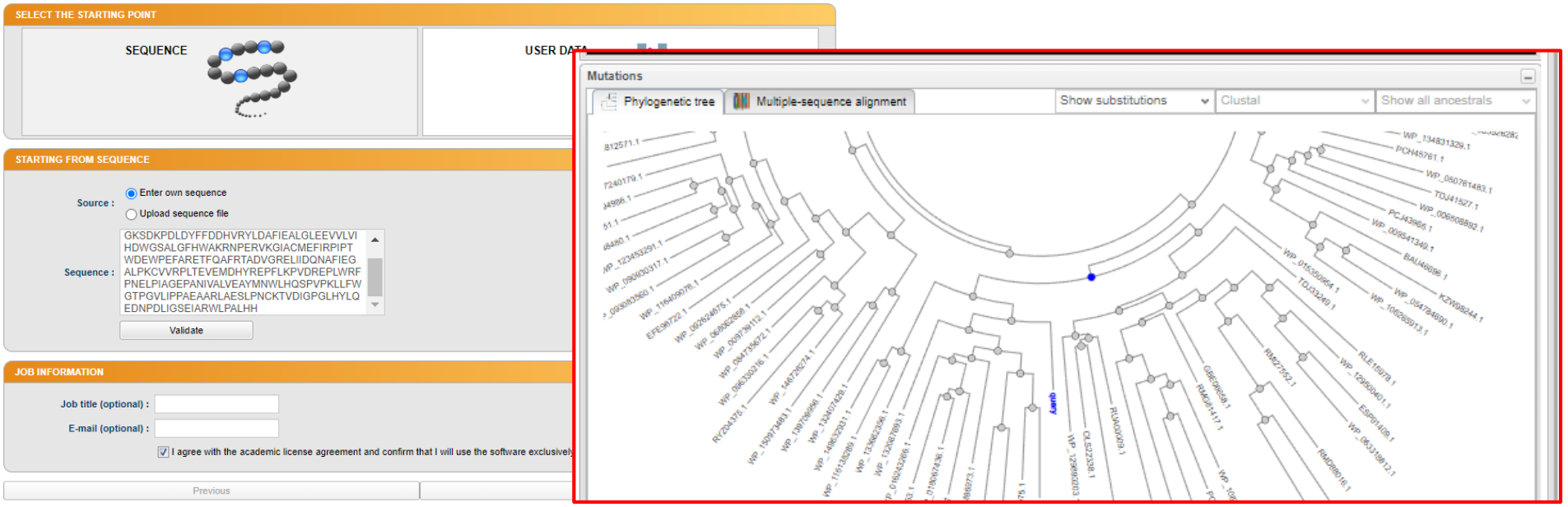
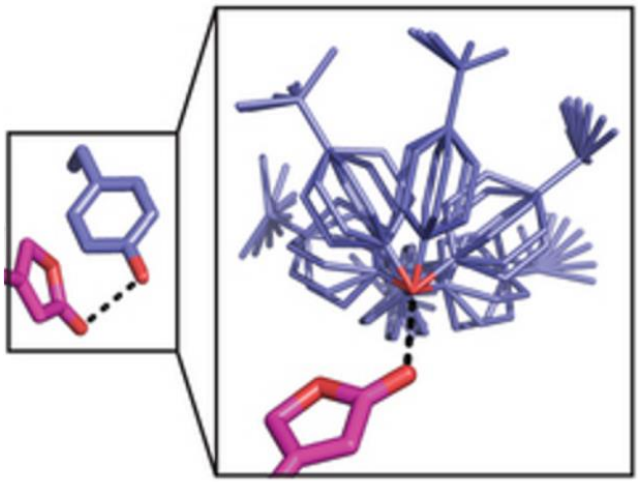


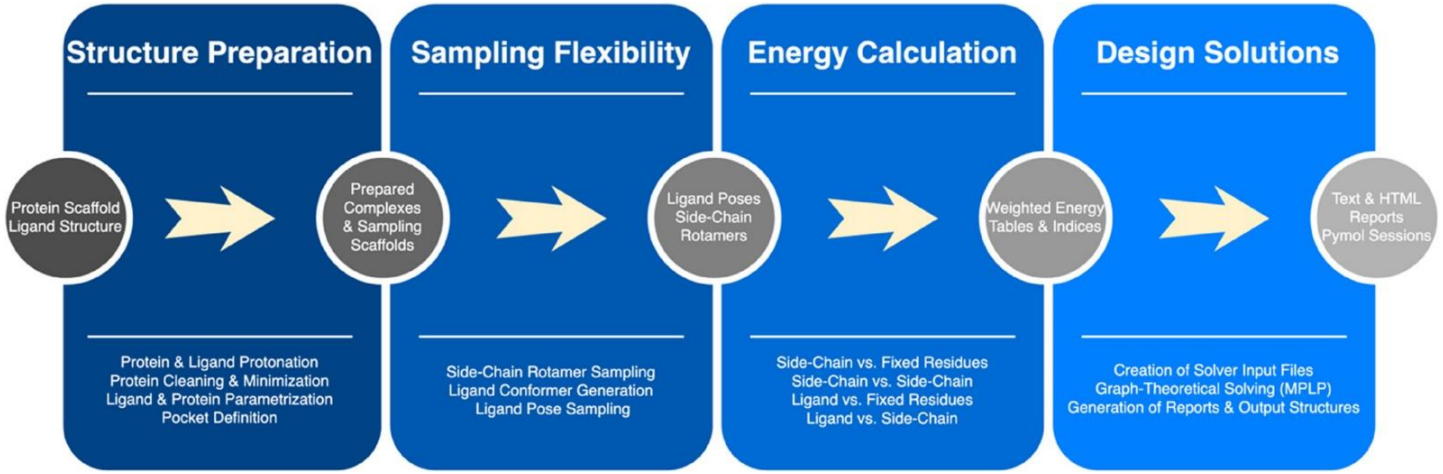
Rational design: function

- **RosettaDesign**
  - http://rosettadesign.med.unc.edu/
  - Monte Carlo sampling (random search) to predict minimum-energy structure of mutants
  - Predicts free energy changes upon mutations ($\Delta\Delta G$)
  - Helps design mutations to optimize the binding site and increase interactions with a ligand/substrate



Rational design: function

- **PocketOptimizer**
  - https://github.com/Hoecker-Lab/pocketoptimizer/
  - Aimed at maximizing the affinity of a binding site towards a ligand
  - Modular pipeline with different tools
    - Flexibility, docking, mutagenesis, energy calculation
    - Predicts global minimum-energy designs



Rational design: function

- **FuncLib**
  - https://funclib.weizmann.ac.il
  - To redesign and/or optimize binding site
  - Utilizes evolution (conservation) and Rosetta calculations (energy) to introduce multiple-point mutations to modify the properties of the binding site
  - Can be used to improve the binding affinity towards a ligand
  - Outputs up to 50 multiple-point mutants for protein synthesis

Rational design: function

- **FuncLib**

Parameter	Value
Minimal number of mutations per design	3
Maximal number of mutations per design	5
Minimal PSSM threshold	-1
ΔΔG	5.5
Sequence space	143A FY 144A P 151A FMY 177A LAGNST 211A ILMV 247A AGMSTVY 248A LIMV
Total number of designs in tolerated sequence space	3,313

Reset Verify Proceed

Rational design: function

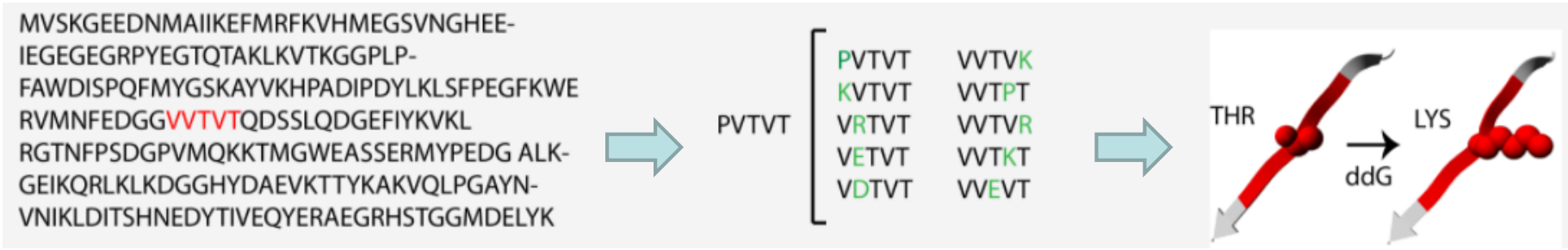
- **AffiLib**
  - https://affilib.weizmann.ac.il
  - To optimize protein-protein interface
  - Utilizes evolution (conservation) and Rosetta (energy) to introduce mutations and optimize macromolecular interface
  - Suggests mutations on the interface residues to improve the binding affinity
  - Outputs up to 50 multiple-point mutants for protein synthesis

Rational design: function

- **Mutation Cutoff Scanning Matrix (mCSM-PPI2)**
  - http://biosig.unimelb.edu.au/mcsm_ppi2/
  - To optimize protein-protein interface
  - Based on machine learning, evolutionary data and energy (FoldX)
  - Provides mutational $\Delta\Delta G$
  - Modes of calculations
    - Single mutation – single point mutations on interface
    - Mutation list – single mutations accordingly to a user
    - Alanine scanning (all interface residues are mutated to alanine)
    - Systematic – position saturation (all interface residues are mutated to all other 19 amino acids)

Rational design: solubility

- **Aggrescan3D; SoluProt** (see lecture 6 - Analysis of protein structures)
- **SolubiS**
  - https://solubis.switchlab.org/
  - To identify stabilizing mutations that reduce the aggregation tendency of a protein
  - 1) Identifies exposed APRs
  - 2) Introduces "gatekeeper" residues (P, R, K, D and E) into APRs
  - 3) Assesses the stability changes of mutations (ΔΔG)



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