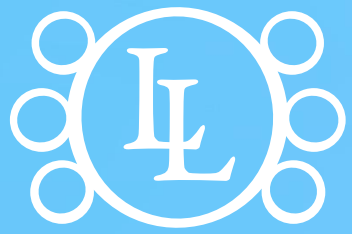

LOSCHMIDT
LABORATORIES

PROTEIN ENGINEERING

7. Rational and semi-rational design

Loschmidt Laboratories
Department of Experimental Biology
Masaryk University, Brno

Outline

- Protein engineering approaches
- Semi-rational design
  - identification of hot-spots
  - evaluation of hot-spots
  - selection of substitutions
  - design of library
  - mutagenesis and screening
- Rational design
  - molecular modeling

Outline

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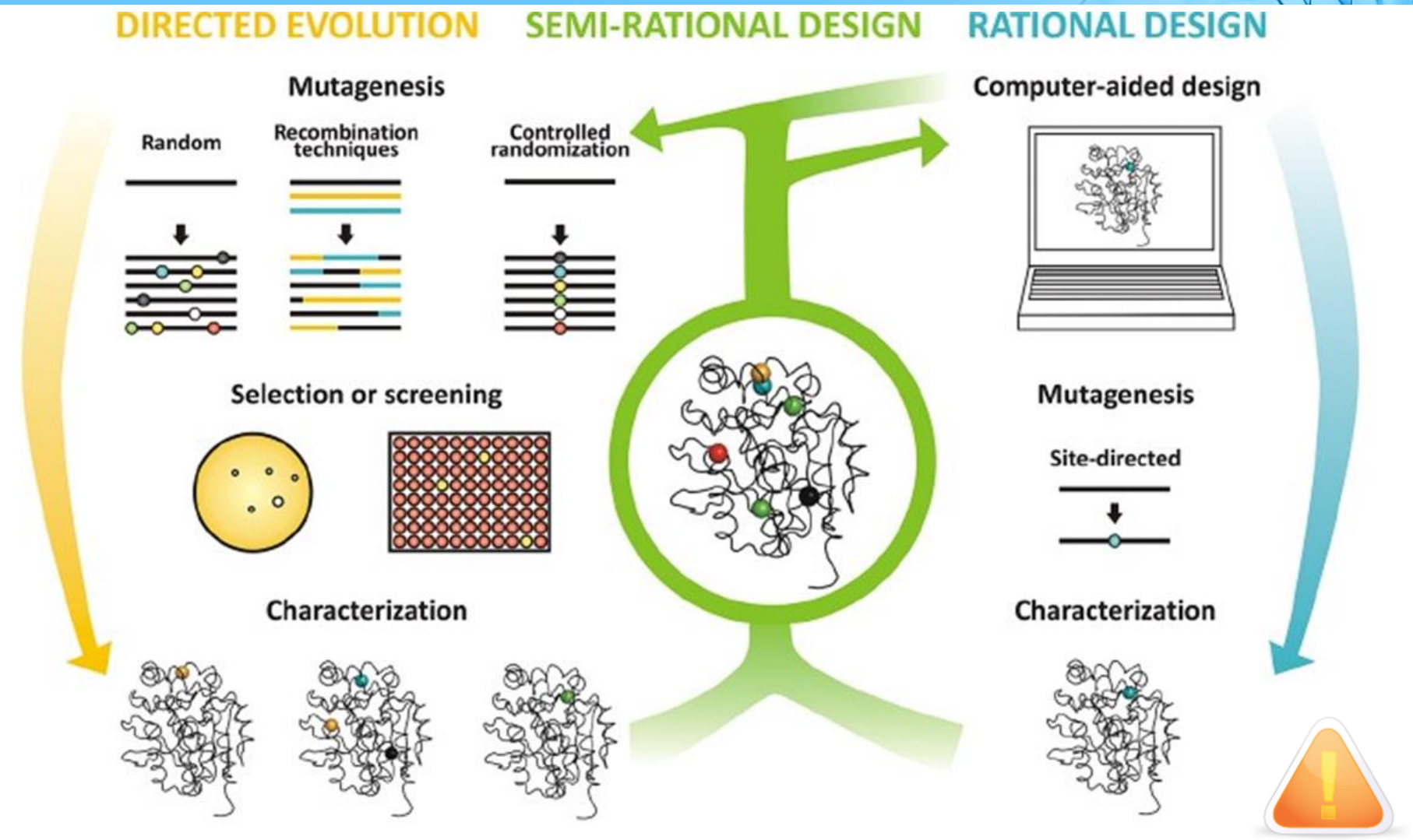
  - molecular modeling

Protein engineering

- altering protein structure to improve its properties
- three main approaches
  - rational design
  - directed evolution
  - semi-rational design

Protein engineering approaches



Protein engineering approaches

	Rational design	Directed evolution	Semi-rational design
high-throughput screening/selection	not essential	essential	advantageous but not essential
structural and/or functional information	both essential	neither essential	either is sufficient
sequence space exploration	low	high, random	moderate, targeted
probability to obtain synergistic mutations	moderate	low	high

Structural information

- **worldwide Protein Data Bank (wwPDB)**
  - http://www.wwpdb.org/
  - central repository of ~220,000 experimental macromolecular structures (April 2024)
- **RCSB PDB**
  - https://www.rcsb.org
- **PDBe**
  - https://www.ebi.ac.uk/pdbe
- **PDBj**
  - https://pdbj.org

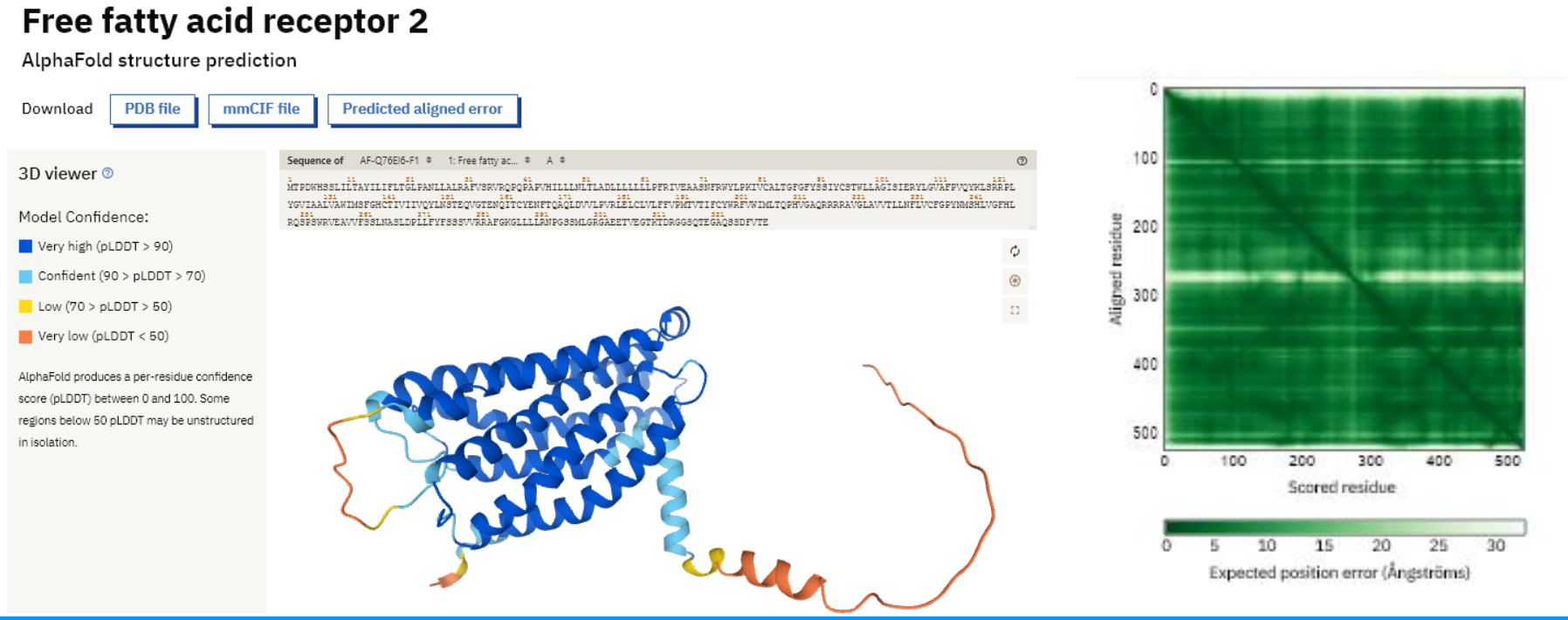
Structural prediction

- **Alpha Fold 2**
  - Galaxy: https://usegalaxy.eu/?tool_id=alphafold, Colab: https://colab.research.google.com/github/deepmind/alphafold/blob/main/notebooks/AlphaFold.ipynb
  - structure prediction directly from sequence using deep learning, evolutionary information (MSA), and structure optimization
  - Multimer mode – lower accuracy
  - Not precise in sidechain orientations prediction (not appropriate for protein-ligand interaction - molecular docking)
  - Rare folds, alternative conformations, and co-factors not predicted

Structure prediction

Alpha Fold database

- https://alphafold.ebi.ac.uk
- Database of protein structures predicted by Alpha Fold 2
- Over 200 million sequences modeled (available also in UniProt)



- Protein engineering approaches
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Semi-rational design

- combine advantages of rational and random approaches
- selection of promising target sites (hot-spots) → mutagenesis → creation of small "smart" libraries
- based on knowledge of protein structure and function
- ☺ high-throughput screening usually not needed
- ☺ increased chance of obtaining variants with desired properties
- ☹ certain knowledge of protein structure-function relationships is still required, ☺ but not that much

- Protein engineering approaches
- Semi-rational design
  - identification of hot-spots
  - evaluation of hot-spots
  - selection of substitutions
  - design of library
  - mutagenesis and screening
- Rational design
  - molecular modeling

Identification of hot-spots

- hot-spots for engineering catalytic properties
- hot-spots for engineering thermostability

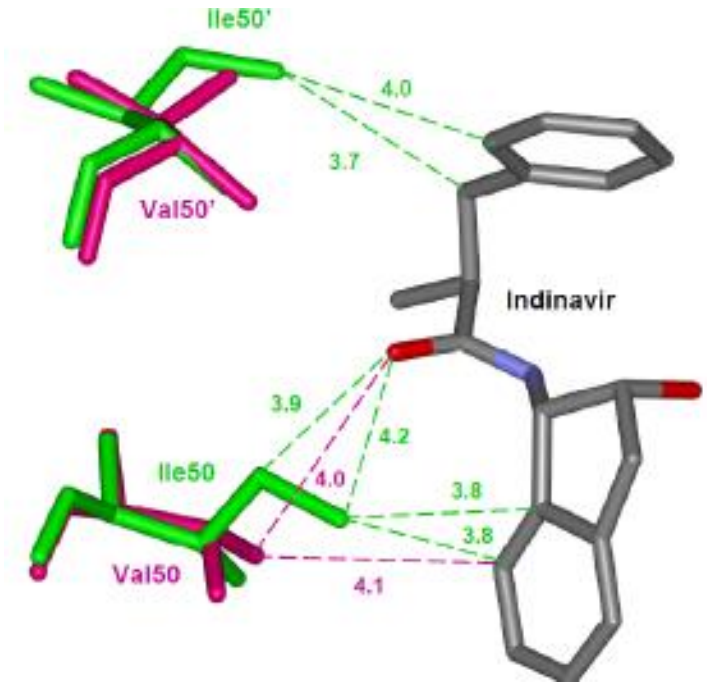
Hot-spots for engineering catalytic properties

- residues mediating substrate binding, transition-state stabilization or product release → mutations can improve or disrupt binding, catalysis or ligand transport
  - residues involved in protein-ligand interactions
  - residues located in binding pockets
  - residues located in access tunnels

→ these residues also include catalytic or other essential residues which generally should not be mutated!

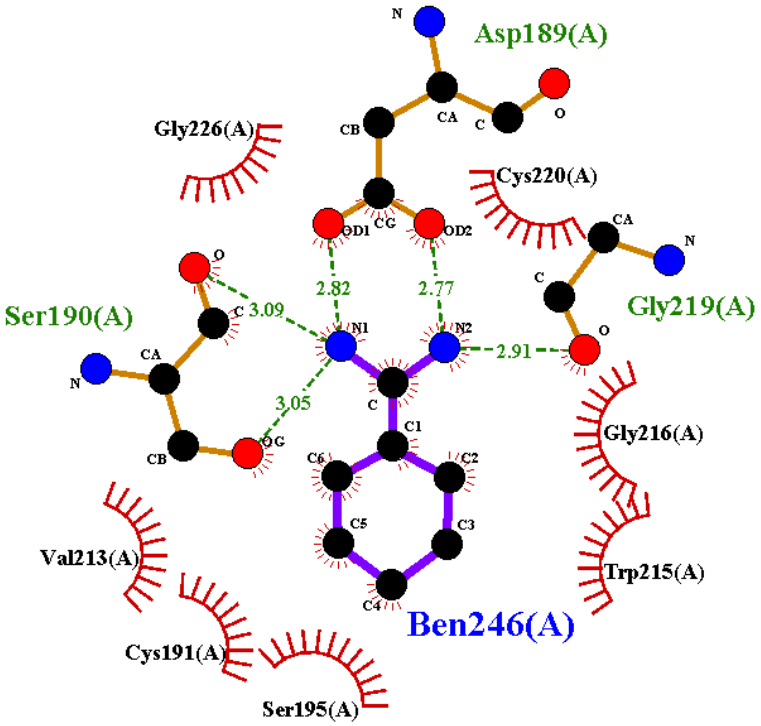
Analysis of protein-ligand interactions

- requires 3D structure of protein-ligand complex
  - experimental structure (wwPDB, PDBbind)
  - theoretical model (molecular docking)

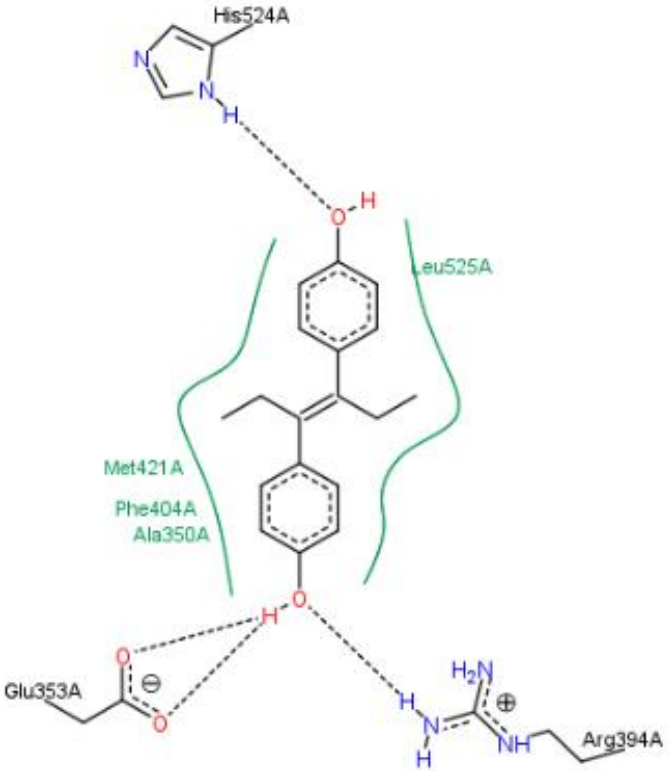


Analysis of protein-ligand interactions

- schematic diagrams of protein-ligand interactions



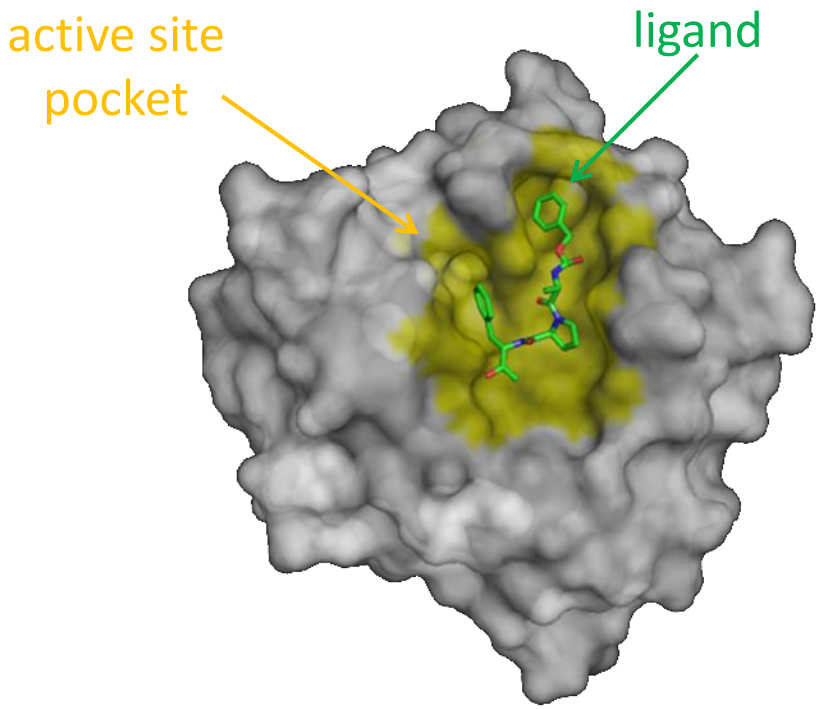
LigPlot, LigPlot+



PoseView

Analysis of binding pockets

- binding and active sites of enzymes are often associated with structural pockets and cavities



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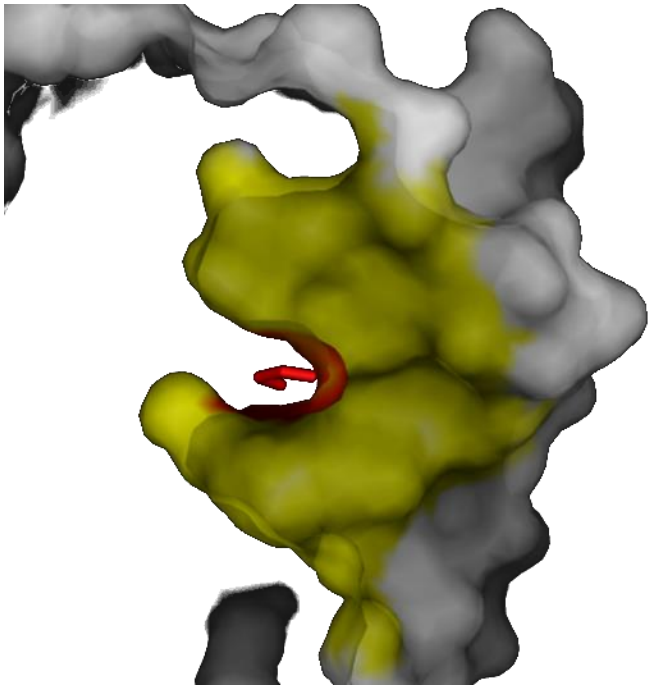

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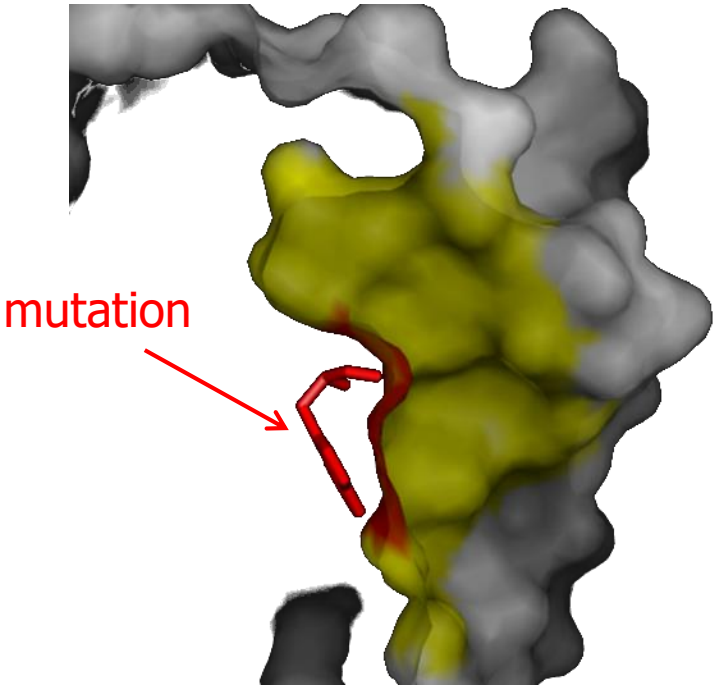

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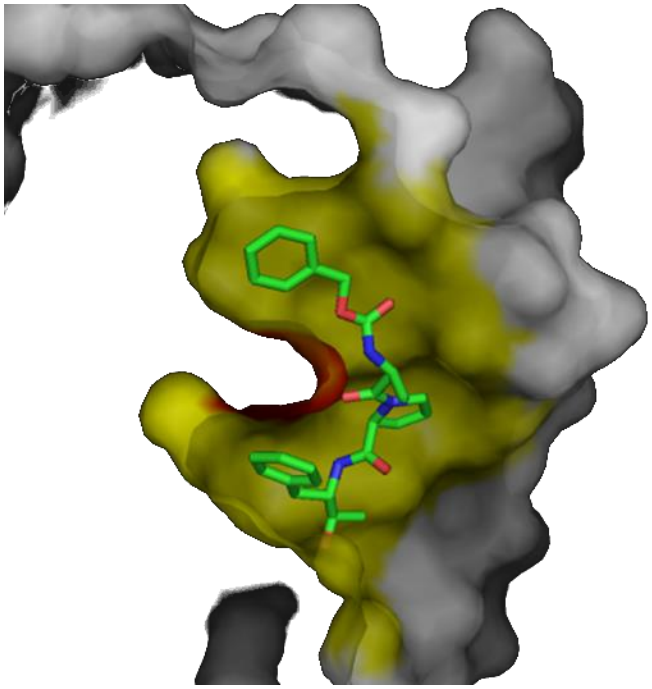

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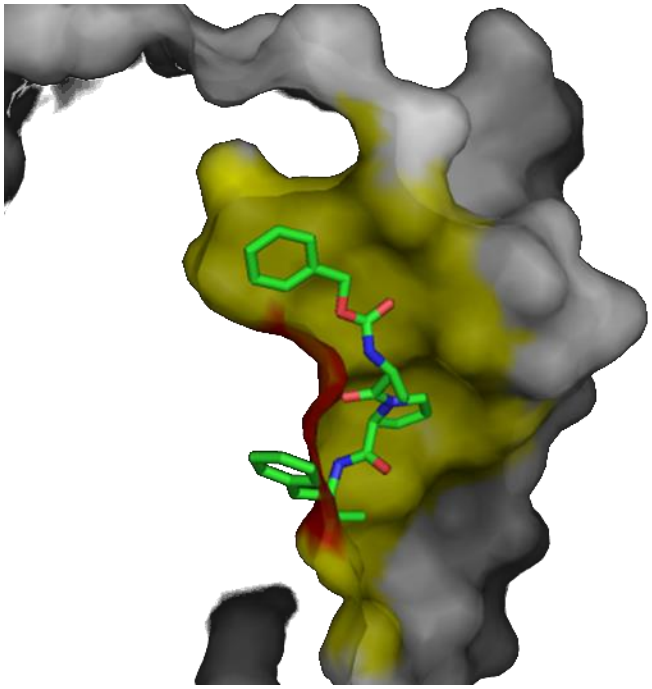
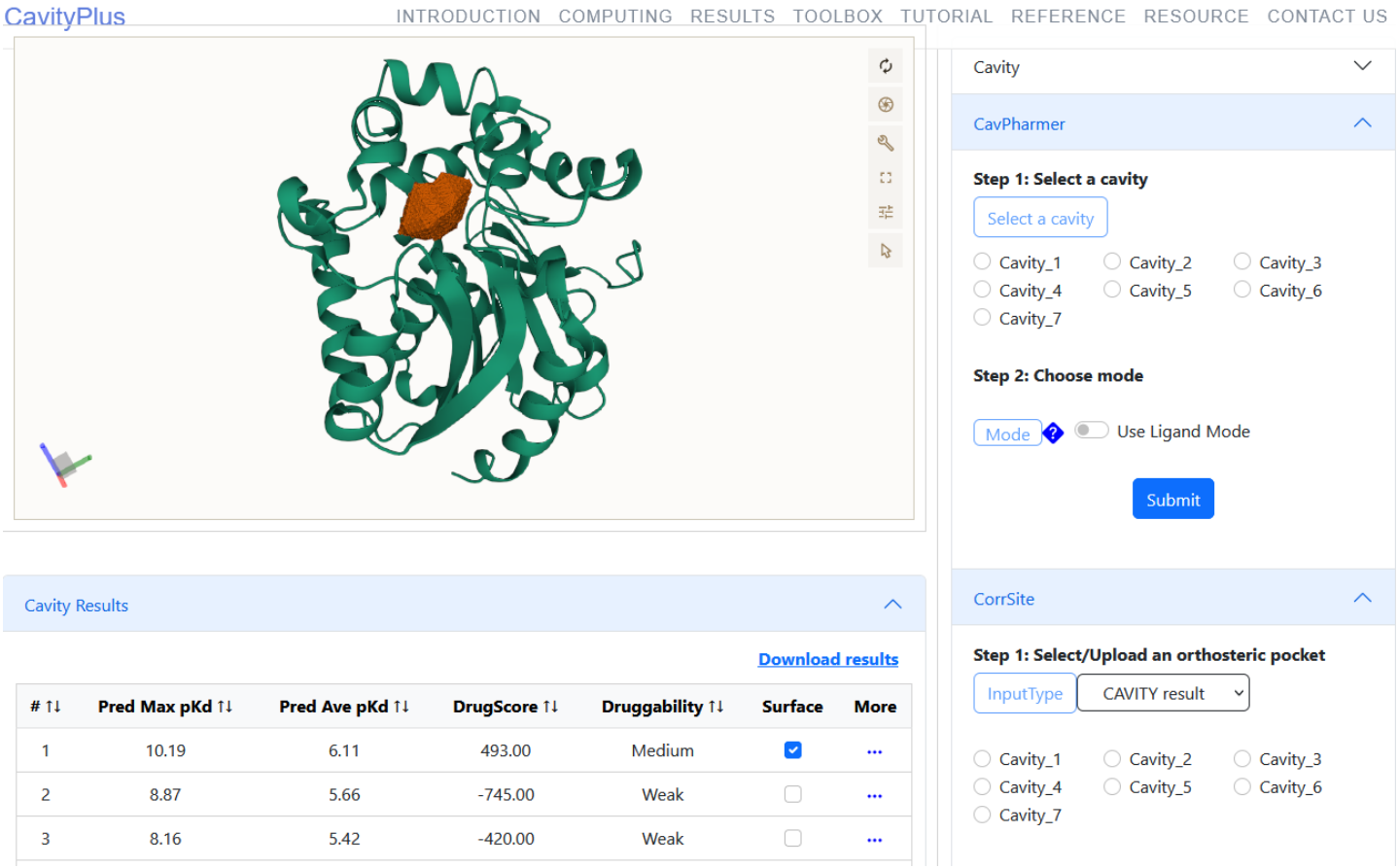


Analysis of binding pockets

- binding and active sites of enzymes are often associated with structural pockets and cavities
  - most amino acid residues located in these pockets may come into contact with the ligands during the catalytic cycle
  - → one can accurately predict which residues may interact with the ligand even without precise knowledge of ligand orientation in the active site
- requires 3D structure of protein
- software for detection of pockets
  - CASTp, fPocket, CavityPlus, etc.

Analysis of binding pockets

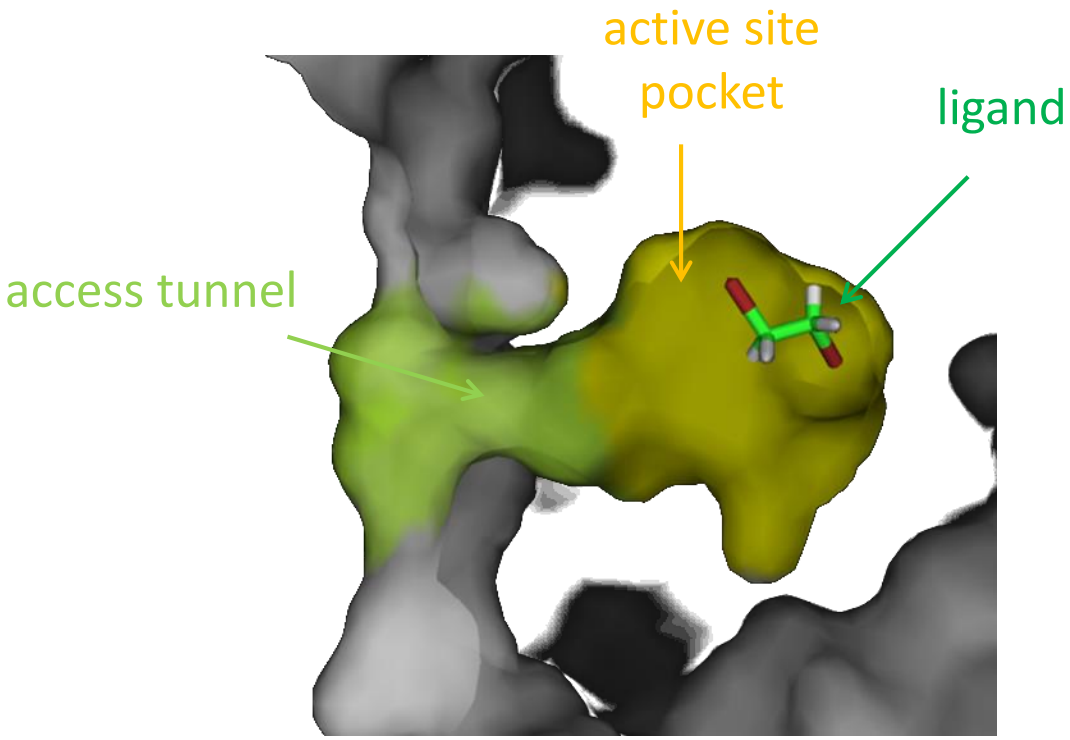
- detailed characterization of all pockets in the structure



CavityPlus

Analysis of access tunnels

- buried binding or active sites are connected with bulk solvent by access tunnels



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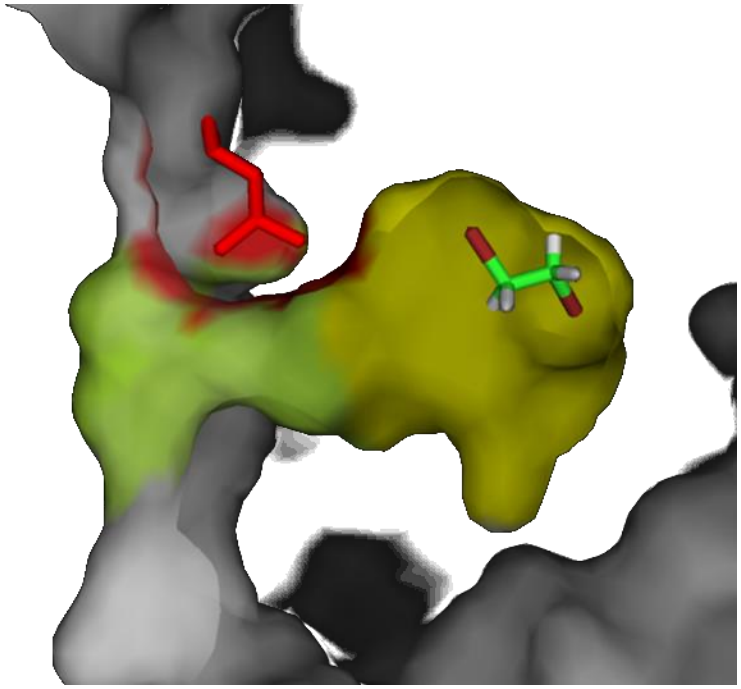

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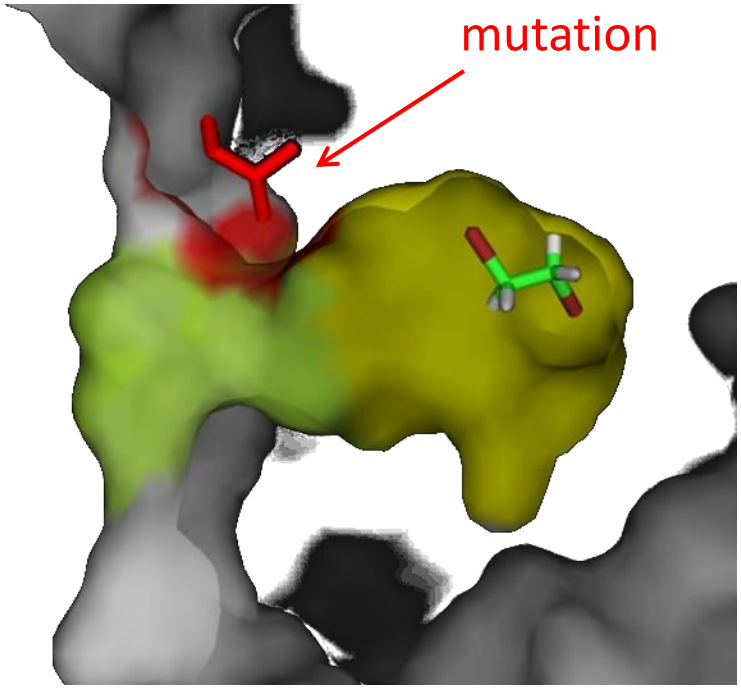

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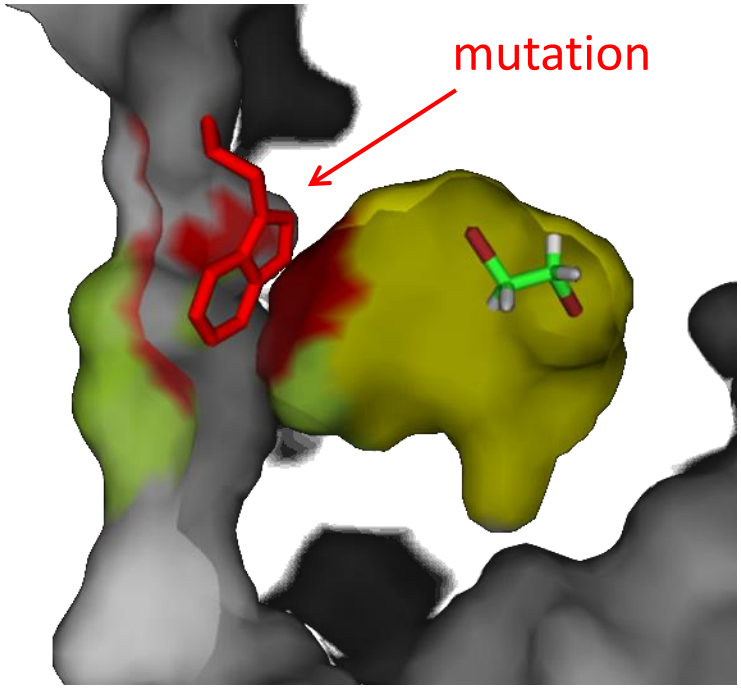
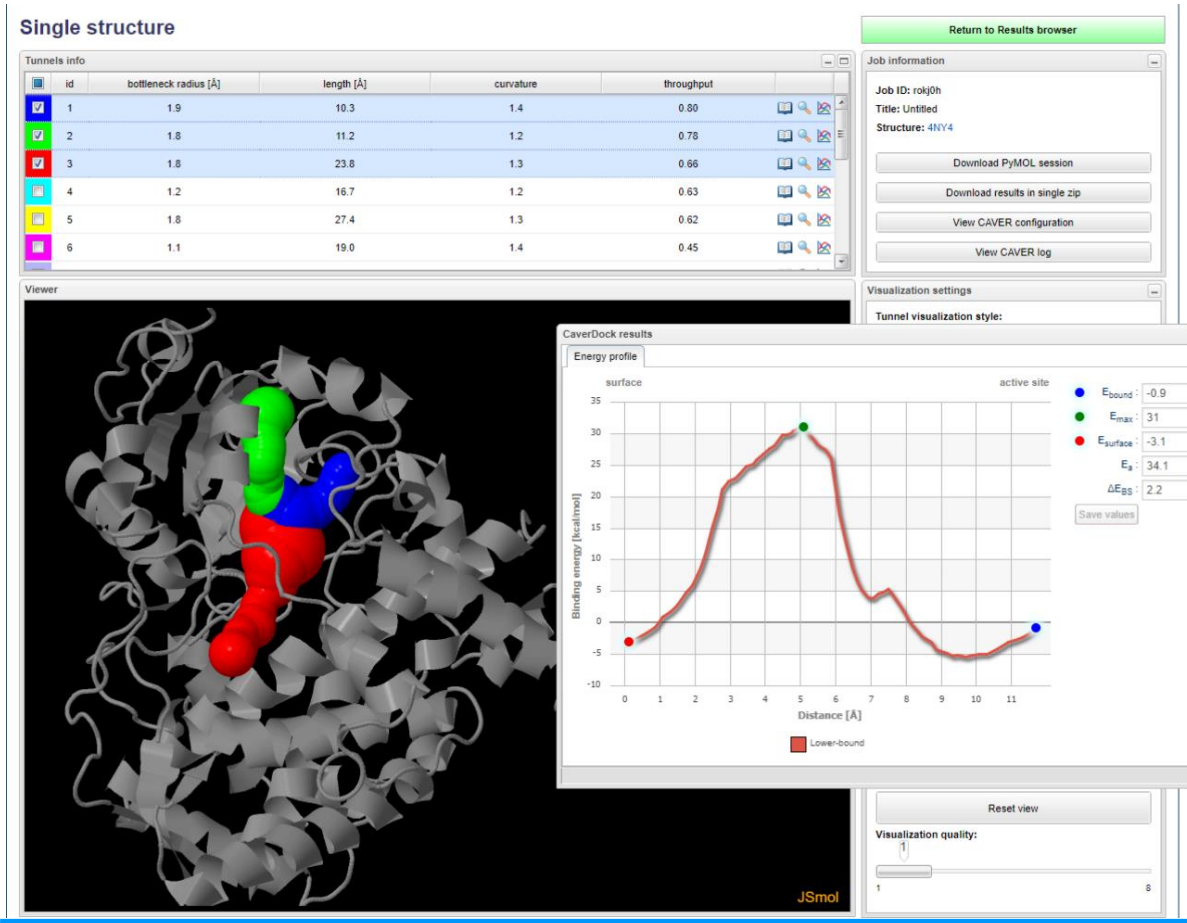


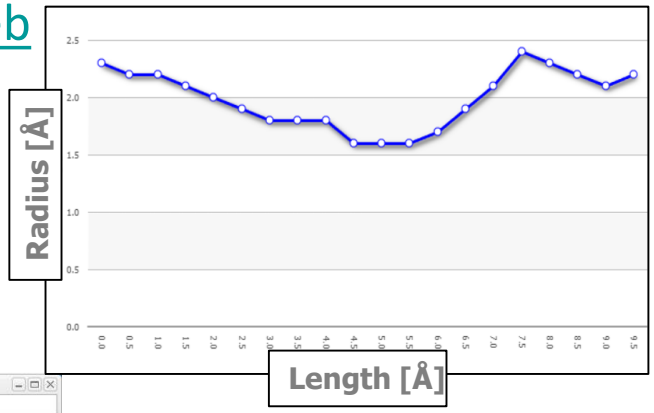
Analysis of access tunnels

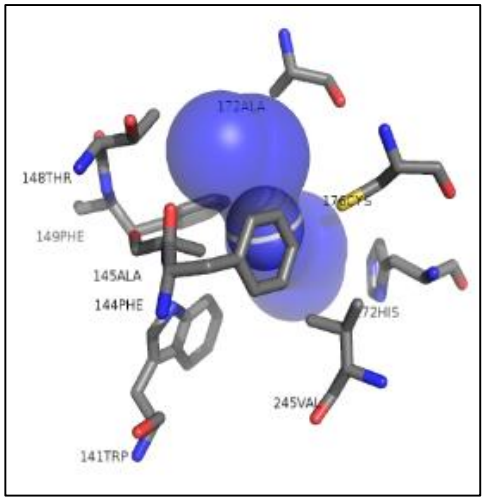
- buried binding or active sites are connected with bulk solvent by access tunnels
  - adjusted to permit transport of specific molecules
  - mutations can speed-up or hinder transport of molecules as well as allow transport of other molecules
- requires 3D structure of protein
- software for detection of tunnels
  - Caver, Mole, HOLE, PoreWalker

Analysis of access tunnels

- Caver Web
  - https://loschmidt.chemi.muni.cz/caverweb







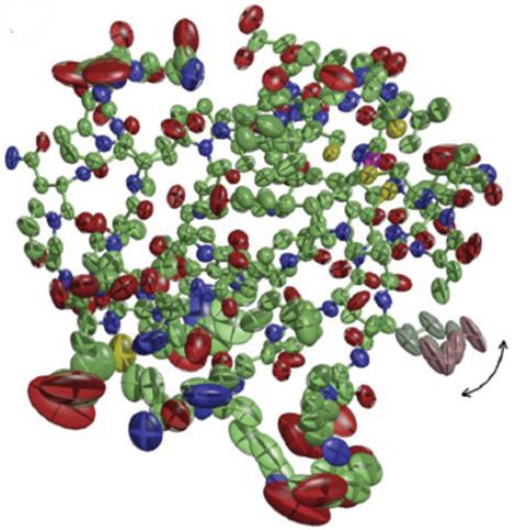
Hot-spots for engineering thermostability

- highly flexible residues – introduction of rigidifying mutations
- residues located in access tunnels

→ these residues may also include catalytic or other essential residues which generally should not be mutated!

Identification of highly flexible residues

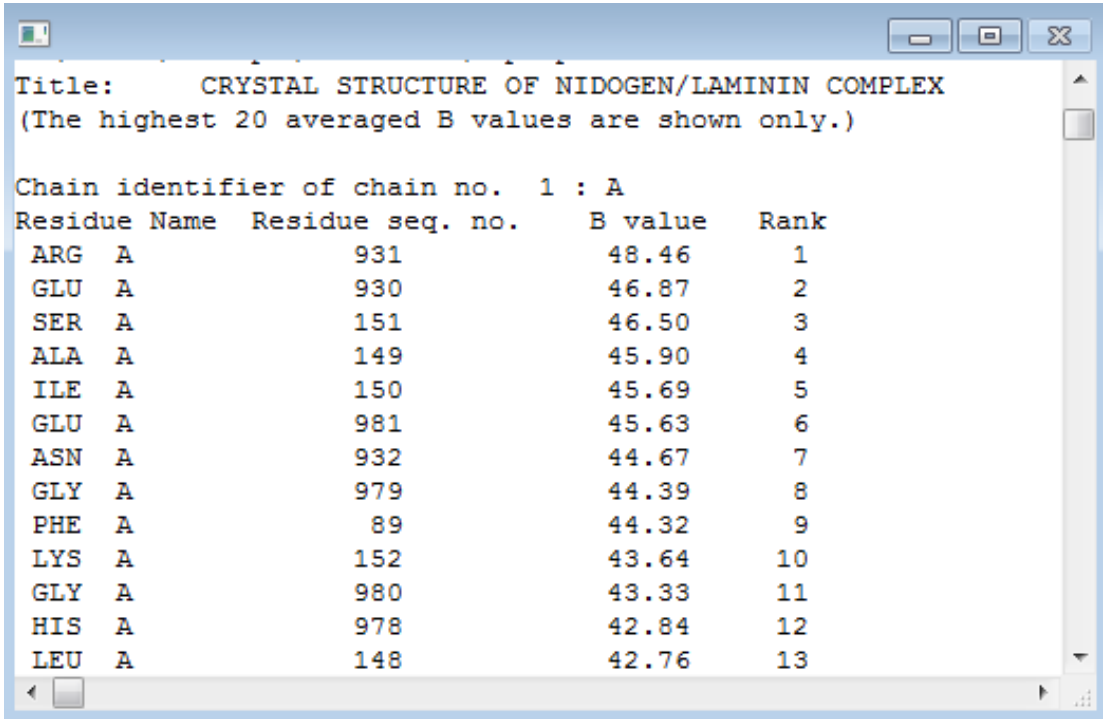
- prediction based on crystallographic B-factors
  - reflect the degree of thermal motion, and thus the flexibility of individual residues



- requires 3D structure of protein
  - experimental structure determined by X-ray crystallography (wwPDB)

Identification of highly flexible residues

- average B-factor of each residue in the target protein



Title: CRYSTAL STRUCTURE OF NIDOGEN/LAMININ COMPLEX
(The highest 20 averaged B values are shown only.)

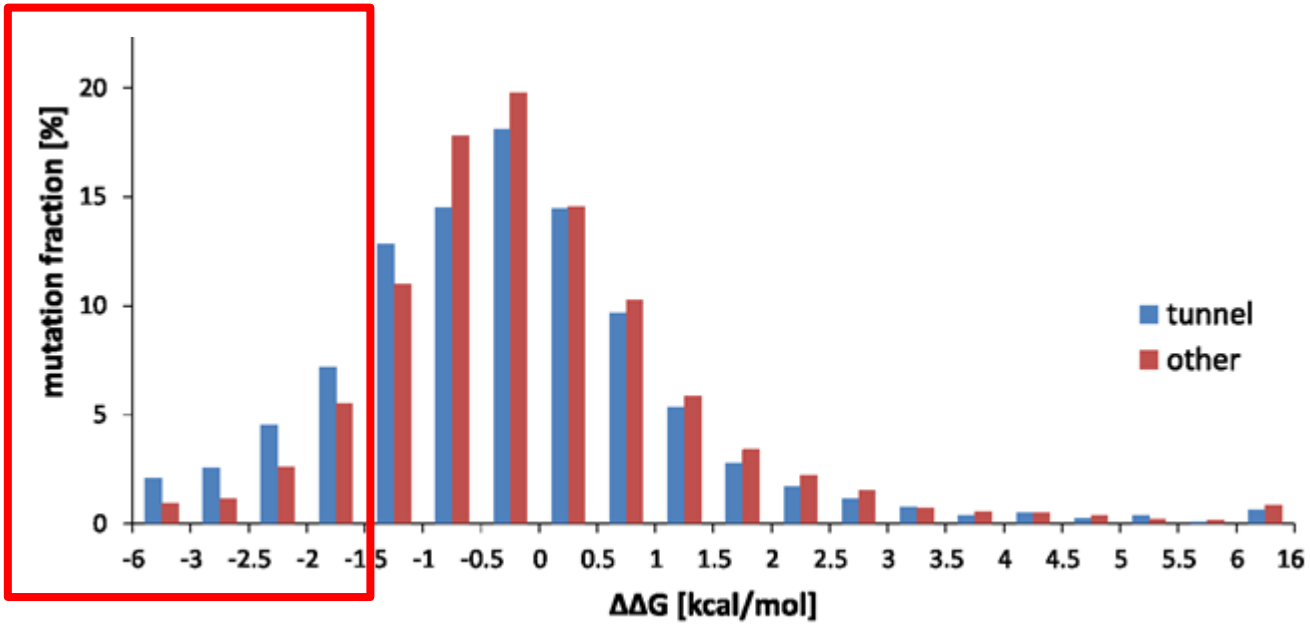
Chain identifier of chain no. 1 : A

Residue Name		Residue seq. no.	B value	Rank
ARG	A	931	48.46	1
GLU	A	930	46.87	2
SER	A	151	46.50	3
ALA	A	149	45.90	4
ILE	A	150	45.69	5
GLU	A	981	45.63	6
ASN	A	932	44.67	7
GLY	A	979	44.39	8
PHE	A	89	44.32	9
LYS	A	152	43.64	10
GLY	A	980	43.33	11
HIS	A	978	42.84	12
LEU	A	148	42.76	13

B-FITTER

Analysis of access tunnels

- saturation mutagenesis in tunnel residues has 2× better chance to significantly improve stability than mutagenesis in other protein regions (based on computational predictions)



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- Semi-rational design
  - identification of hot-spots
  - evaluation of hot-spots
  - selection of substitutions
  - design of library
  - mutagenesis and screening
- Rational design
  - molecular modeling

Evaluation of hot-spots

- hot-spots identified by computational tools can be further evaluated to prevent replacing indispensable amino acid residues and to prioritize the hot-spots (i.e., order the hot-spots based on their suitability for mutagenesis)

- analysis of evolutionary conservation
- prediction of effects of mutations on protein stability or function

Analysis of evolutionary conservation

- residues essential for maintaining structural or functional properties of a protein tend to be conserved during evolution
  - conserved residues are generally not recommended as suitable targets for mutagenesis - their replacement often leads to the loss of protein function
  - mutagenesis targeting highly mutable positions provides a significantly higher proportion of viable variants than random mutagenesis
  - targeting moderately or highly variable positions, which are expected to be tolerant to a wide range of substitutions, represents a good approach for producing efficient smart libraries (i.e., libraries with a high proportion of correctly folded and active variants)

Analysis of evolutionary conservation

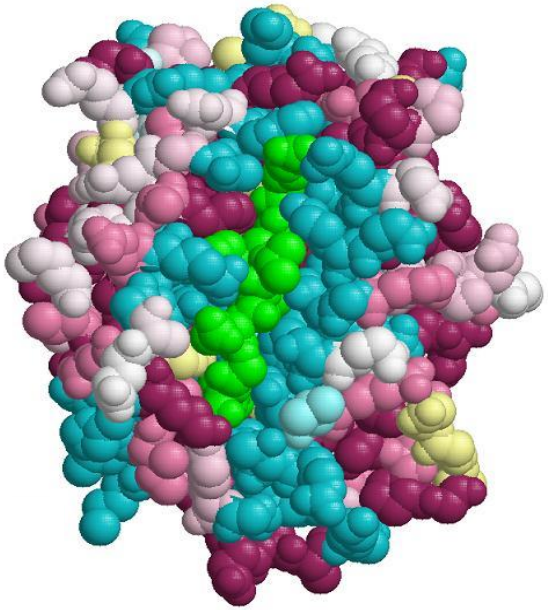
- residue conservation can be derived from a multiple alignment of a set of related proteins (3D structure not required)

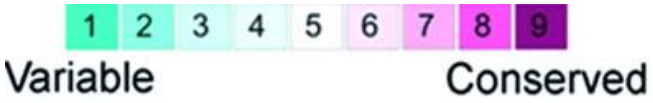
Analysis of evolutionary conservation

- residue conservation can be derived from a multiple alignment of a set of related proteins (3D structure not required)

Analysis of evolutionary conservation

- evolutionary conservation of individual positions in protein mapped on protein 3D structure





ConSurf

Prediction of mutation effects

- computational tools for the prediction of effect of amino acid substitutions on protein stability or protein function
  - *in silico* site-saturation mutagenesis of identified hot-spots – check if mutations at a given site are likely to be tolerated
  - many highly destabilizing/deleterious mutations predicted for a certain position – given site is not a very good target for mutagenesis

  - sites with only a few highly destabilizing /deleterious mutations predicted can still represent promising hot-spots (the amino acids with potentially destabilizing/deleterious effects can be discarded from the library by the appropriate selection of degenerate codons)

Prediction of mutation effects

- effects on protein stability – usually requires 3D structure of protein
  - experimental structure (wwPDB)
  - theoretical model (AlphaFold, homology modeling)

- effects on protein function – sequence information often sufficient

Prediction of mutation effects

- prediction of effect of substitutions on protein stability
  - Evaluation of the change of protein free energy upon mutation
  - Evaluation of contributions of individual interactions to total energy
  - Usually requires structural information
- software for prediction of effect of mutation on stability
  - Rosetta, FoldX, CUPSAT, ERIS

Prediction of mutation effects

- prediction of effect of substitutions on protein stability

Singlepoint stability results

Stabilizing mutations | Destabilizing mutations

Energy is in kcal/mol

chain	position	residue	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Lys
A	169	Phe	3.5	7.1	5.4	6.5	6.0	4.2	5.1	5.9	5.7	45.7	5.8
A	135	Ala	-	35.9	4.1	4.5	2.4	17.2	15.5	2.1	25.7	12.5	17.9
A	11	Lys	-0.2	2.3	0.1	1.7	2.0	0.2	1.3	1.3	-1.9	-1.1	-
A	264	Thr	-0.1	0.9	0.6	1.8	1.9	0.0	0.7	1.3	-0.7	-0.3	0.8
A	126	Gln	1.3	1.3	3.1	3.4	5.5	-	-0.5	3.3	1.4	28.7	1.6
A	259	Thr	-0.2	-0.3	-0.5	0.3	1.7	-1.1	-0.1	0.6	-0.4	4.9	-1.0

Export table to CSV

Evaluate multiple point stability

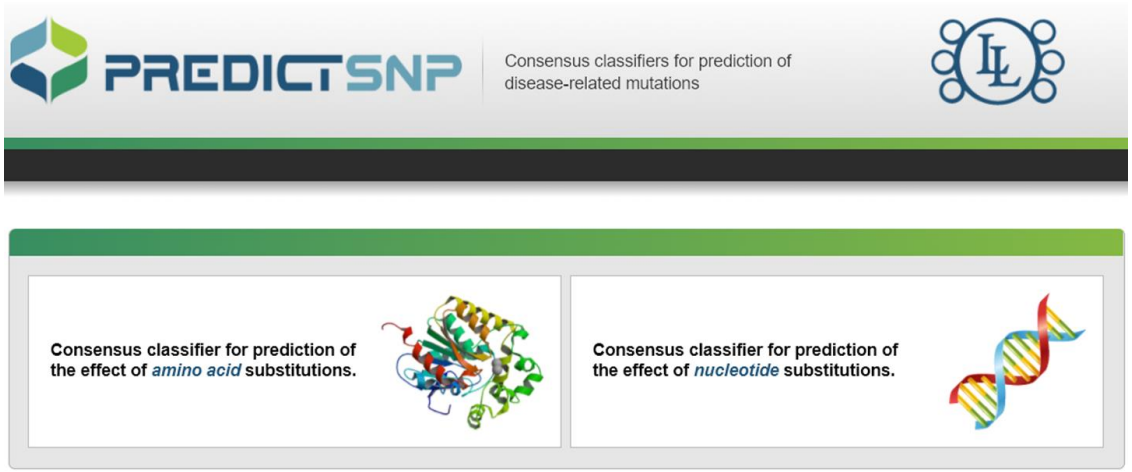
Codon usage : Escherichia coli K12

Generate report

Rosetta in HotSpot Wizard

Prediction of mutation effects

- prediction of effect of substitutions on protein function
  - Evaluation if a mutation would impair protein function
  - Hard to describe by physico-chemical properties > machine learning
  - Usually sequence based calculation
- software for prediction of effect of mutation on function
  - PredictSNP, SIFT, MAPP, PhD-SNP…



Prediction of mutation effects

- prediction of effect of substitutions on protein function

INPUT — Load example

Insert protein sequence in FASTA format :

```
>HBA_HUMAN
MVLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHFDLSHGSAQVKGHG
KKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFKLLSHCLLVTLAAHLPAEFTP
AVHASLDKFLASVSTVLTSKYR
```

Load

MUTATIONS — Manual input

Select positions:

1	M	V	L	S	P	A	D	K	T	N	V	K	A	A	W	G	K	V	G	A	H	A	G	E	Y	G	A	E	A	L	E	R	M	F	L	S	F	P	T	T
41	K	T	Y	F	P	H	F	D	L	S	H	G	S	A	Q	V	K	G	**H**	**G**	K	K	**V**	A	D	A	L	**T**	N	A	V	**A**	H	V	D	D	M	P	**N**	A
81	L	S	A	L	S	D	L	H	A	H	K	L	R	V	D	P	**V**	N	F	K	L	L	S	H	C	L	L	V	T	**L**	A	**A**	H	L	**P**	A	**E**	F	T	P
121	A	V	H	A	S	**L**	D	K	F	L	A	**S**	V	S	T	V	L	T	S	K	Y	R																			

Pos	Wild-type	Mutations	Clear
59	H	**Y** - Tyr	
60	G	**D** - Asp, **V** - Val	
63	V	**T** - Thr	
68	T	**V** - Val	
72	A	**E** - Glu, **V** - Val	

Clear all mutations

TOOLS FOR EVALUATION

Tool name	Time demands	Expected accuracy
PredictSNP	**32 min**	**73.4%**
MAPP	10 min	70.7%
PhD-SNP	32 min	71.5%
PolyPhen-1	15 min	68.1%
PolyPhen-2	15 min	69.2%
SIFT	15 min	70.3%
SNAP	30 min	67.6%

JOB INFORMATION

ID: vb17zolzbvrteajgmdyd5fvt3u7cg4nzsqf2z6logfjbgz3pht

RESULTS — neutral — deleterious — **XX %** expected accuracy — Expand all annotations

Annotation	Mutation	PredictSNP	MAPP	PhD-SNP	PolyPhen-1	PolyPhen-2	SIFT	SNAP
▶	**H59Y**	**87 %**	63 %	82 %	74 %	65 %	79 %	85 %
▶	**G60D**	**87 %**	88 %	68 %	59 %	55 %	79 %	85 %
▶	**G60V**	**87 %**	91 %	82 %	74 %	59 %	53 %	72 %
	V63T	**87 %**	76 %	61 %	74 %	63 %	79 %	62 %
	T68V	**71 %**	41 %	72 %	67 %	75 %	46 %	67 %
▶	**A72E**	**74 %**	70 %	58 %	67 %	87 %	65 %	77 %
▶	**A72V**	**60 %**	59 %	73 %	67 %	76 %	53 %	71 %
▶	**N79H**	**74 %**	72 %	55 %	67 %	87 %	46 %	67 %
▶	**V97W**	**61 %**	46 %	45 %	74 %	81 %	79 %	58 %
▶	**L110R**	**87 %**	88 %	88 %	74 %	81 %	79 %	62 %
	A112T	**83 %**	75 %	58 %	67 %	74 %	67 %	83 %
▶	**P115S**	**65 %**	72 %	59 %	67 %	75 %	46 %	77 %
▶	**E117A**	**68 %**	46 %	55 %	67 %	87 %	43 %	67 %

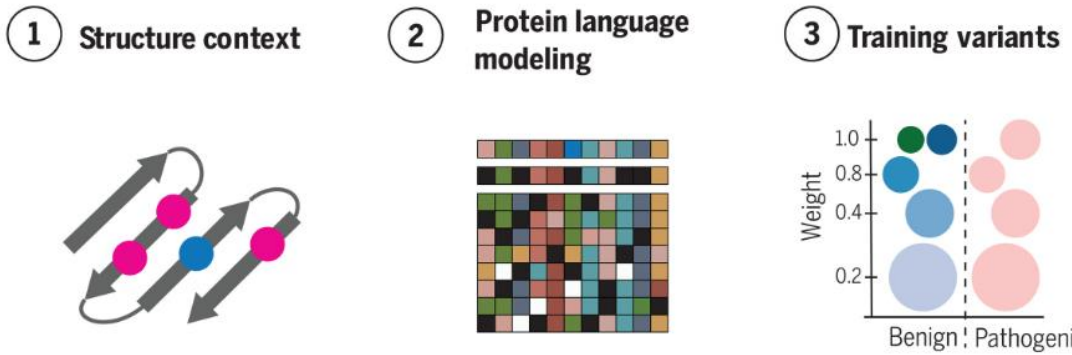
DOWNLOAD

Summary table | Raw results

PredictSNP

Prediction of mutation effects

- **AlphaMissense**
  - https://github.com/google-deepmind/alphamissense
  - deep learning predictor based on AlphaFold
  - analysis of human and primate missense mutations
  - trained on population frequency data and uses sequence and predicted structural context
  - all single–amino acid substitutions in the human proteome are provided



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  - mutagenesis and screening
- Rational design
  - molecular modeling

Selection of substitutions

- substitutions introduced using degenerate codons
  - e.g., NNK (N = A/T/G/C; K = T/G)

IUPAC Nucleotide Nomenclature Table

symbol	base	symbol	base
A	adenosine	M	A C (amino)
C	cytidine	S	G C (strong)
G	guanine	W	A T (weak)
T	thymidine	B	G T C
U	uridine	D	G A T
R	G A (purine)	H	A C T
Y	T C (pyrimidine)	V	G C A
K	G T (keto)	N	A G C T (any)

Selection of substitutions

- all possible substitutions - NNK or NNS degenerate codons
  - ☺ encode all 20 amino acids with the lowest redundancy and price (mixture of 32 codons)
  - ☹ redundancy is not completely eliminated (3× Arg, Leu, Ser, 2× Ala, Gly, Pro, Thr and Val)

Selection of substitutions

- all possible substitutions - NNK or NNS degenerate codons
- introduction of only selected substitutions using degenerate codons encoding reduced amino acid alphabets
  - ☹ do not encode all 20 amino acids
  - ☺ decreased library size → improved screening efficiency
  - NDT – balanced set of 12 amino acids (12 codons)

Selection of substitutions

- all possible substitutions - NNK or NNS degenerate codons
- introduction of only selected substitutions using degenerate codons encoding reduced amino acid alphabets

Table 1. Oversampling necessary for 95% coverage as a function of NNK and NDT codon degeneracy.

No.[a]	NNK		NDT	
	Codons	Transformants needed	Codons	Transformants needed
1	32	94	12	34
2	1028	3066	144	430
3	32768	98163	1728	5175
4	1048576	3141251	20736	62118
5	33554432	100520093	248832	745433
6	$>1.0\times10^{9}$	$>3.2\times10^{9}$	$>2.9\times10^{8}$	$>8.9\times10^{6}$
7	$>3.4\times10^{10}$	$>1.0\times10^{11}$	$>3.5\times10^{9}$	$>1.1\times10^{8}$
8	$>1.0\times10^{12}$	$>3.3\times10^{12}$	$>4.2\times10^{10}$	$>1.3\times10^{9}$
9	$>3.5\times10^{13}$	$>1.0\times10^{14}$	$>5.1\times10^{11}$	$>1.5\times10^{10}$
10	$>1.1\times10^{15}$	$>3.4\times10^{15}$	$>6.1\times10^{12}$	$>1.9\times10^{11}$

[a] Number of aa positions at one site.

Selection of reduced amino acid alphabets

- introduction of amino acids exhibiting certain properties
  - VRK – 8 hydrophilic amino acids (12 codons)
  - NYC – 8 hydrophobic amino acids (8 codons)
  - KST – 4 small amino acids (4 codons)
  - ...

Selection of reduced amino acid alphabets

- introduction of amino acids exhibiting certain properties
- introduction of a balanced set of amino acids
  - NDT – balanced set of 12 amino acids (12 codons)

Selection of reduced amino acid alphabets

- introduction of amino acids exhibiting certain properties
- introduction of a balanced set of amino acids
- introduction of substitutions existing (at a given site) in known natural proteins
  - likely increasing the proportion of viable variants in the resulting library
  - can be obtained by analysis of multiple sequence alignment

Selection of reduced amino acid alphabets

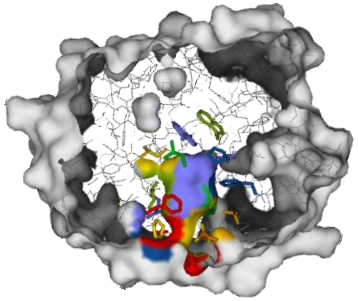
- introduction of amino acids exhibiting certain properties
- introduction of a balanced set of amino acids
- introduction of substitutions existing (at a given site) in known natural proteins
- discarding amino acids with potentially destabilizing/ deleterious effects
  - can be obtained by prediction of effects of mutations on protein stability or function

HotSpot Wizard

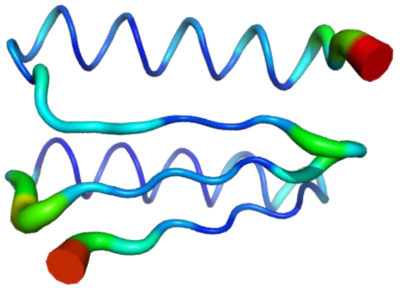
- meta-server combining several tools
  - automatic identification of hot-spots for engineering of enzyme catalytic properties
  - prioritization of hot-spots by their mutability
  - distribution of amino acids at individual positions
  - prediction of stability
  - molecular docking
  - design of smart libraries

HotSpot Wizard

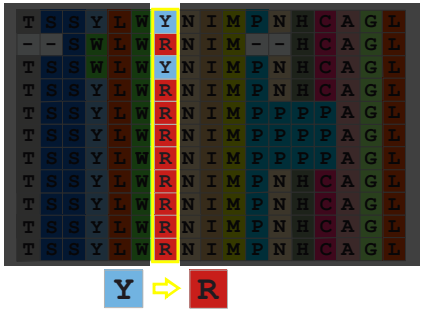
Functional hot-spots



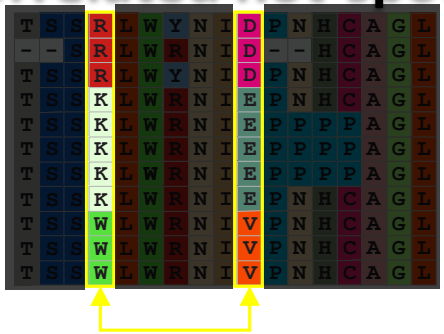
Stability hot-spots (flexibility)



Stability hot-spots (evolution)

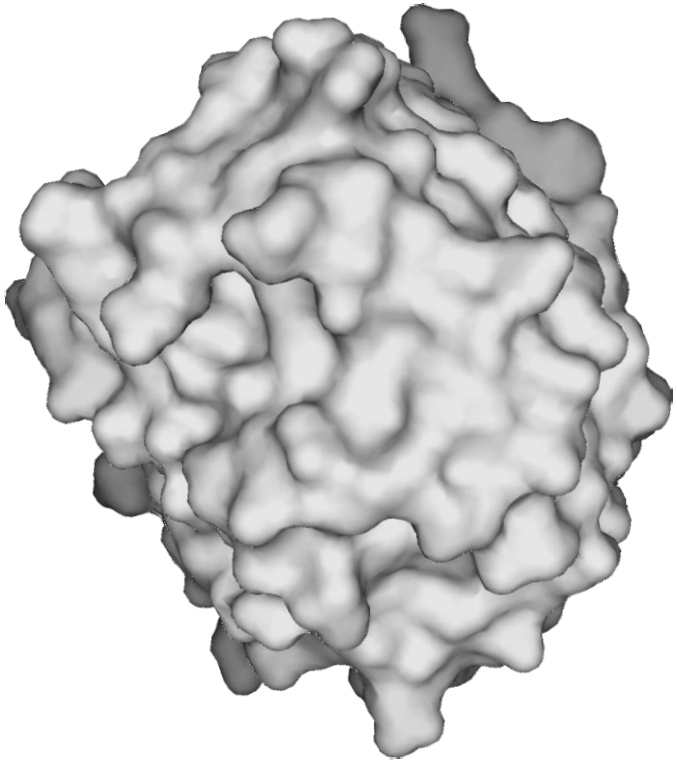


Correlated hot-spots



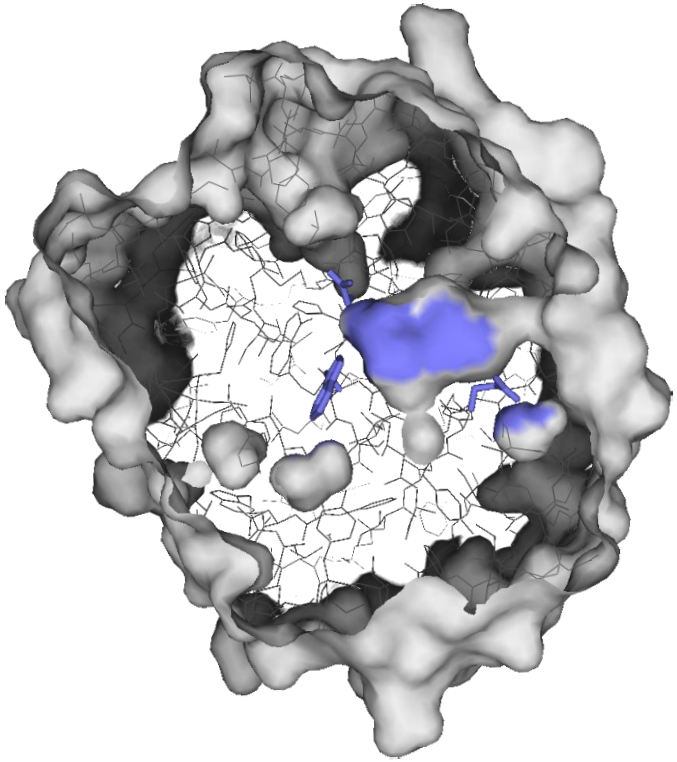
HotSpot Wizard

1. protein structure



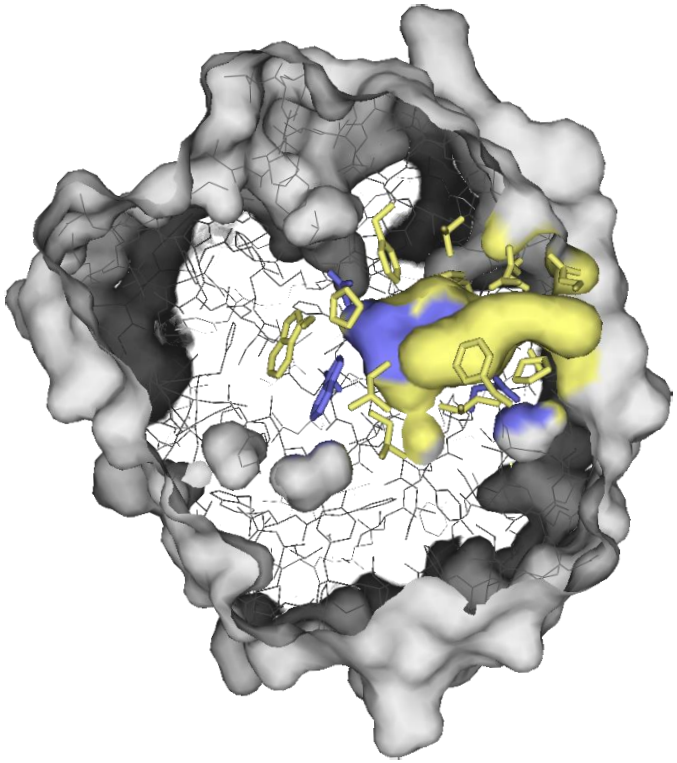
HotSpot Wizard

2. residues indispensable for protein function: catalytic and binding residues



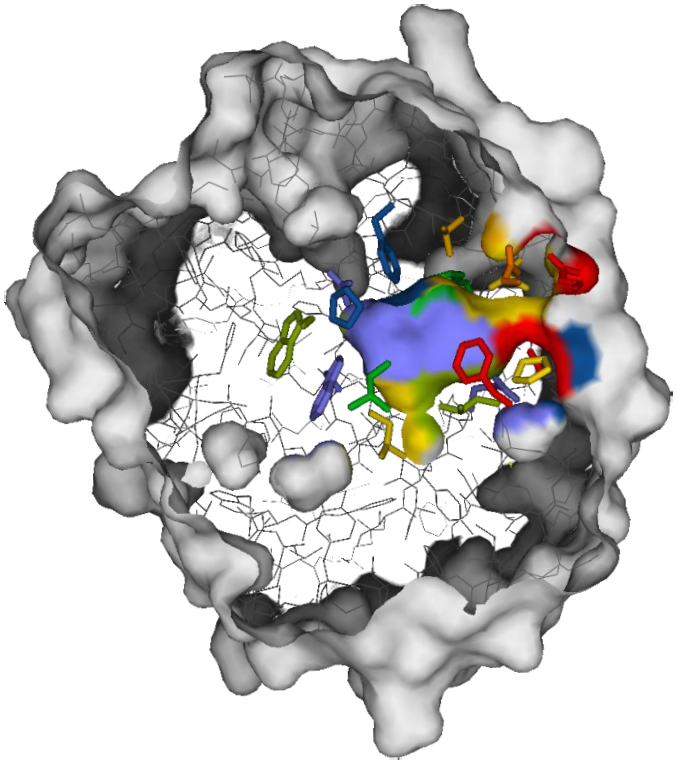
HotSpot Wizard

3. functional residues: active site pocket and tunnels



HotSpot Wizard

4. mutability of individual positions of protein

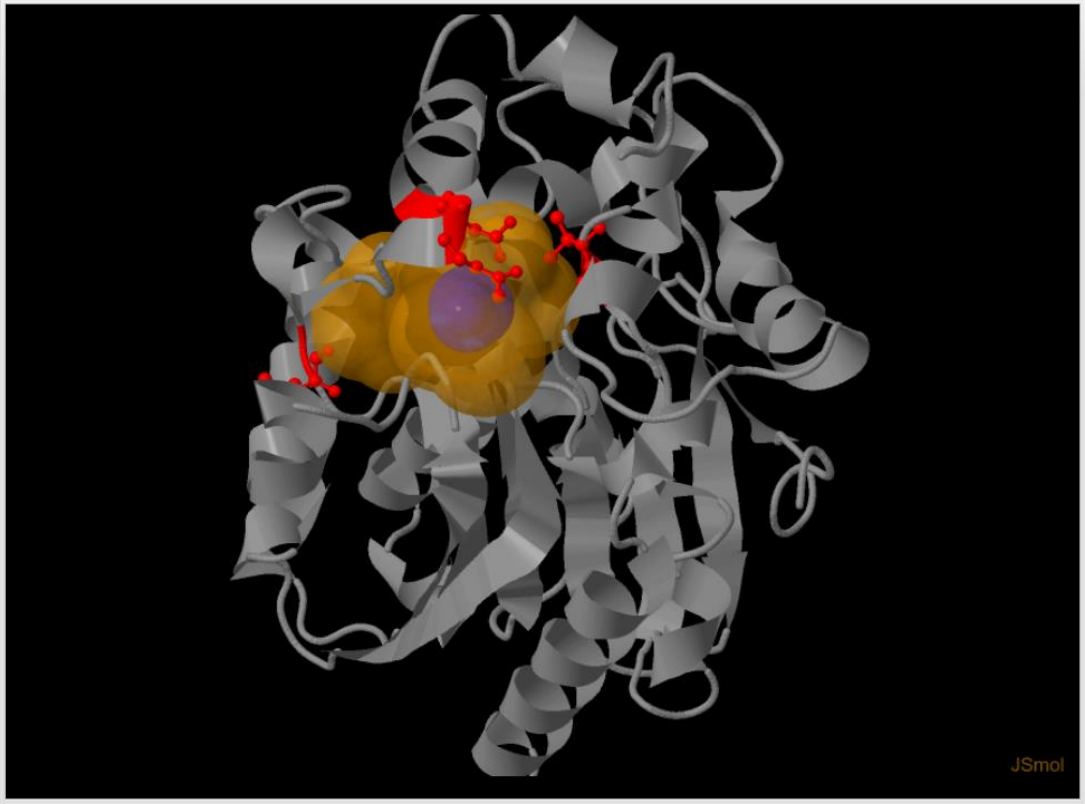


HotSpot Wizard

Functional hot spots of 1CV2

Return to Results browser

Viewer



Visualization settings

Structure visualization style:

Wireframe	Cartoon
Sticks	Trace
Balls & sticks	Backbone
Balls	

Hide all visualized residues

Save image

Reset view

Visualization quality: 1

1 — 8

Tunnels

id	length (Å)	bottleneck radius (Å)
Starting from pocket: 1		

Pockets

id	chain...	relevance (...	volume (Å^3)
1	A	100	514
2	A	82	907
3	A	62	245

Molecular docking

Run Docking

job id	ligand	pose
ztapvt	UDP-galactose	1
pxlsok	yperite	1
dymxo8	ligand5	1
vquhab	yperite	5
nkw1yt	yperite	1
x5yh9u	ligand10	3

Residue features

Exclude correlated positions | Exclude catalytic pockets | Exclude tunnels | Exclude α-helices and β-sheets | Show all residues

Exclude buried residues | Include residues with moderate mutability

chain	position	residue	mutable	non-essential	in tunnel	in catalytic pocket	HotSpot
Chain A							
A	136	Met	✓	✓	✗	✓	✓
A	146	Gln	✓	✓	✓	✓	✓
A	147	Asp	✓	✓	✓	✓	✓
A	173	Val	✓	✓	✓	✓	✓
A	249	Thr	✓	✓	✓	✗	✓
A	253	Met	✓	✓	✗	✓	✓

Residues selected for mutagenesis

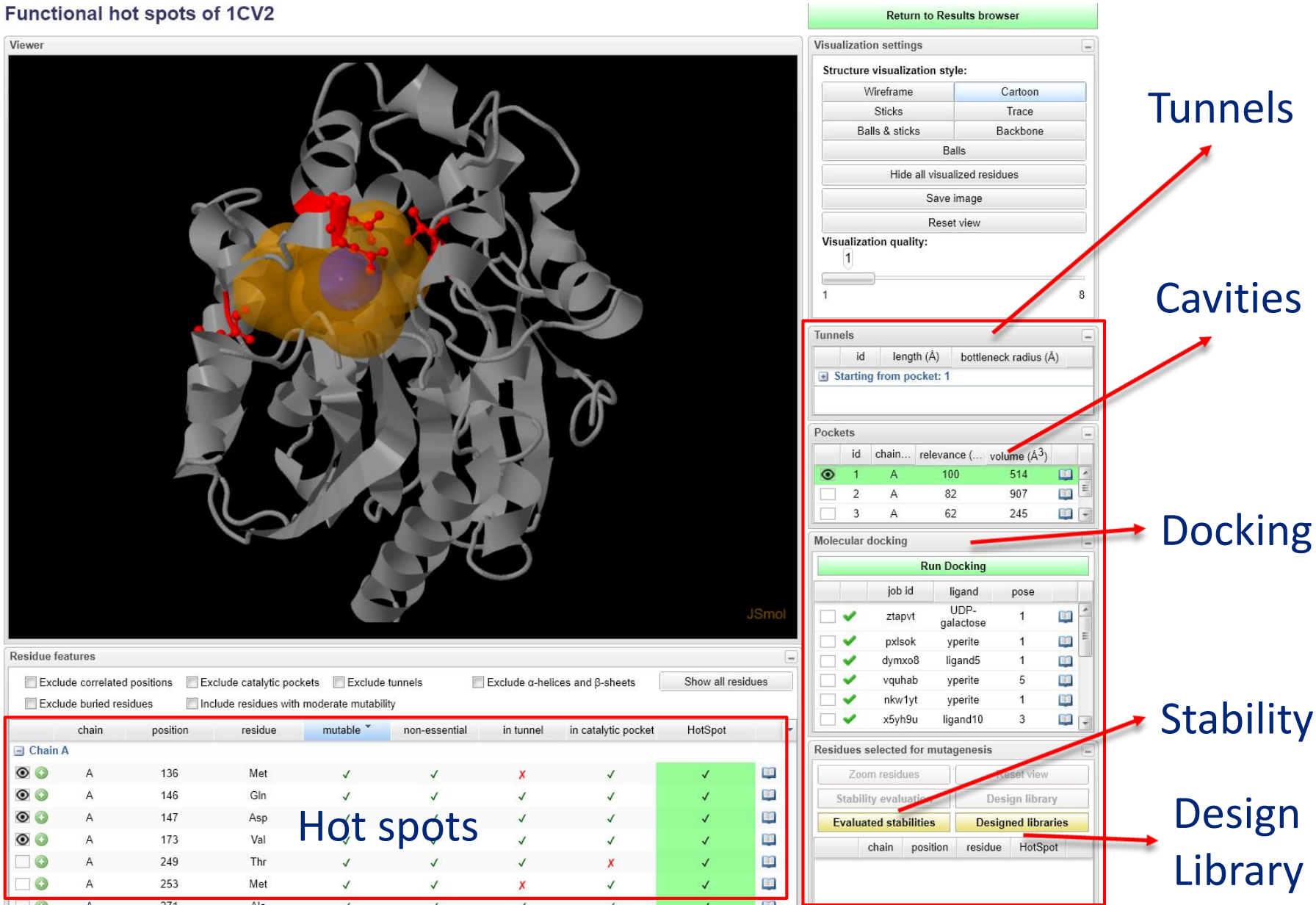
Zoom residues | Reset view

Stability evaluation | Design library

Evaluated stabilities | Designed libraries

chain	position	residue	HotSpot

HotSpot Wizard



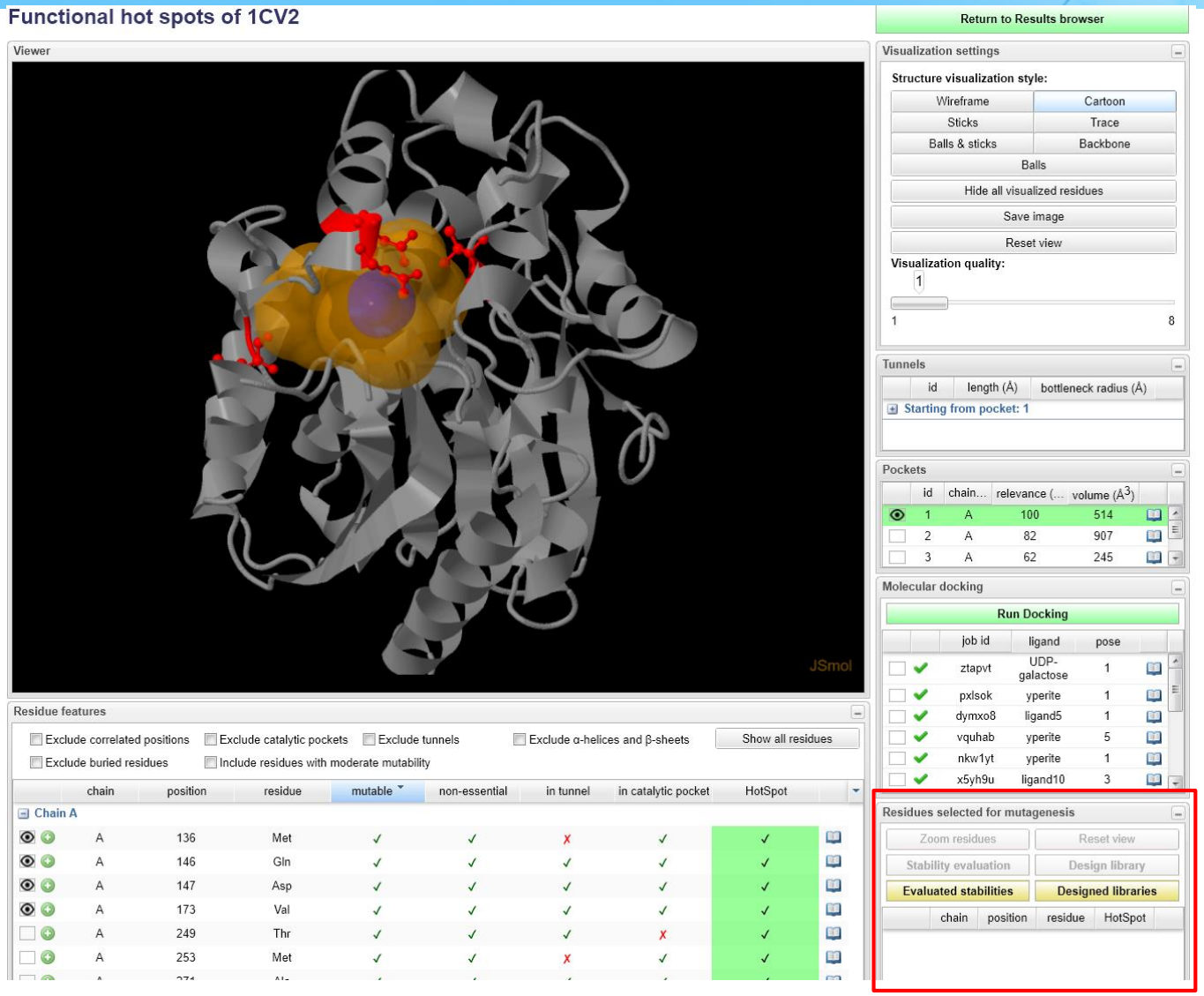
- Protein engineering approaches
- Semi-rational design
  - identification of hot-spots
  - evaluation of hot-spots
  - selection of substitutions
  - design of library
  - mutagenesis and screening
- Rational design
  - molecular modeling

Design of library

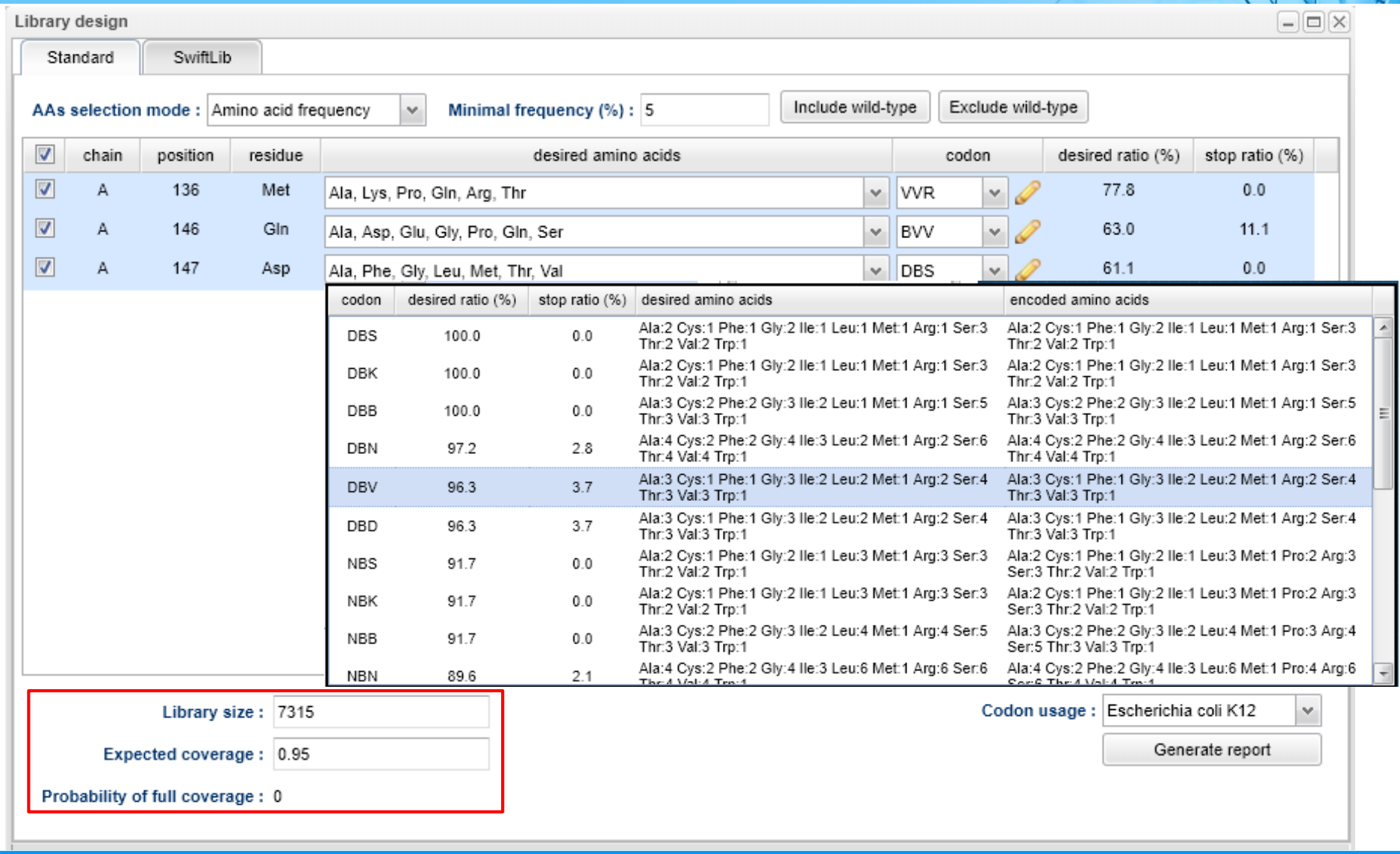
- decisions to be made after evaluation and prioritization of hot-spots:
  - how many and which positions to target?
  - should the positions be randomized simultaneously or separately?
  - should all or only a reduced set of amino acids be introduced at individual positions?

→ dramatic effect on the size of the resulting library

Design of library – HotSpot Wizard



Design of library – HotSpot Wizard



- Protein engineering approaches
- Semi-rational design
  - identification of hot-spots
  - evaluation of hot-spots
  - selection of substitutions
  - design of library
  - mutagenesis and screening
- Rational design
  - molecular modeling

Mutagenesis and screening

- saturation mutagenesis - next lecture ☺

- Protein engineering approaches
- Semi-rational design
  - identification of hot-spots
  - evaluation of hot-spots
  - selection of substitutions
  - design of library
  - mutagenesis and screening
- Rational design
  - molecular modeling→ design of mutations

Rational design

- site-specific changes on the target enzyme
- few amino-acid substitutions that are predicted to elicit desired improvements of enzyme function
- based on detailed knowledge of protein structure, function and catalytic mechanism
- ☺ relatively simple characterization of constructed variants
- ☹ complexity of protein structure-function relationships
- ☹ molecular modeling expertise usually required

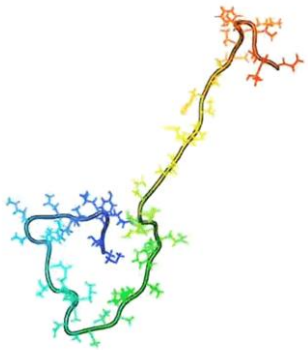
- Protein engineering approaches
- Semi-rational design
  - identification of hot-spots
  - evaluation of hot-spots
  - selection of substitutions
  - design of library
  - mutagenesis and screening
- Rational design
  - molecular modeling→ design of mutations

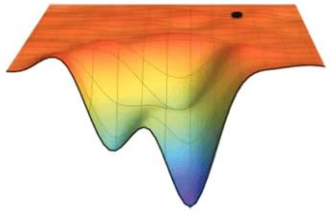
Molecular modeling

- "Theoretical or computational technique that provides insight into the behavior of molecular system."

  A. R. Leach

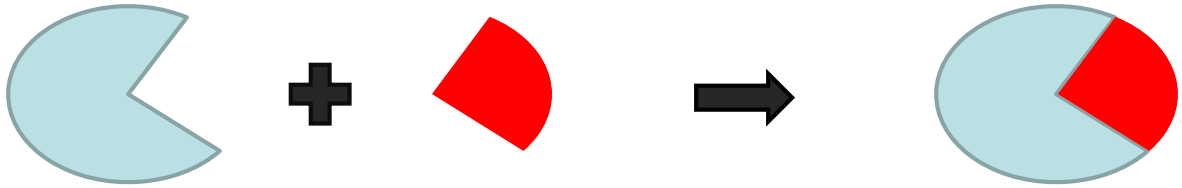
- **Ligand binding**
  - Molecular docking
- **Protein dynamics and transport of molecules**
  - Molecular dynamics
- **Reaction barriers and mechanisms**
  - Quantum chemistry or QM/MM
- **Protein design**
  - Molecular mechanics, machine learning





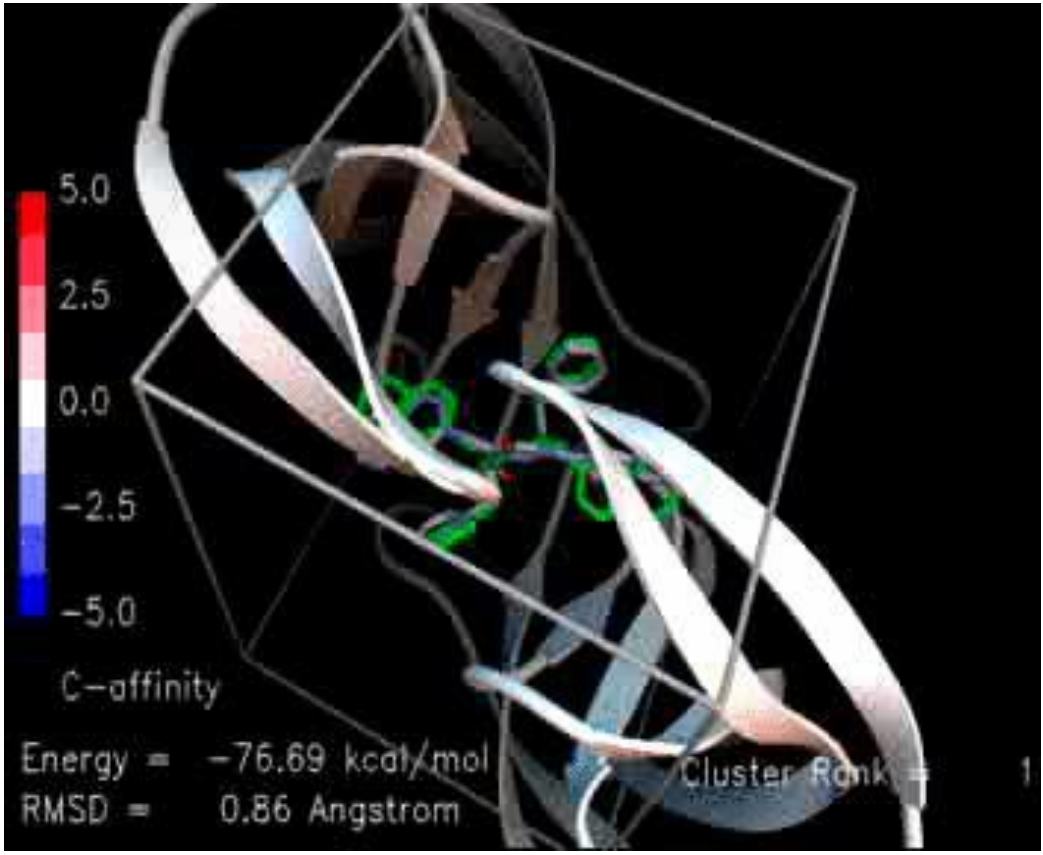
Molecular docking

- predicts structure of receptor (protein) – ligand complex



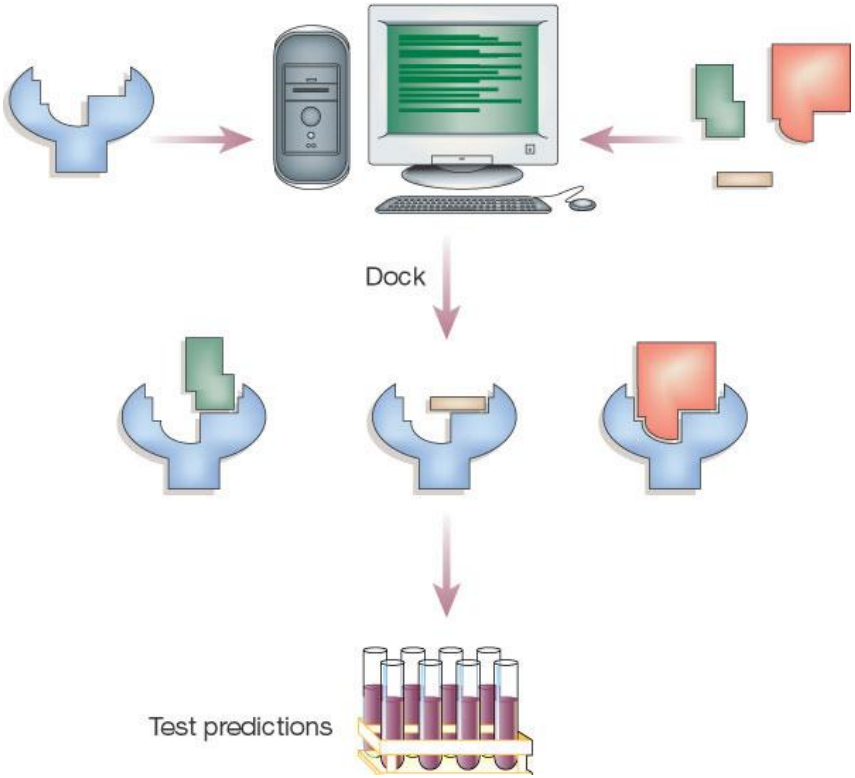
Molecular docking

- Two components procedure
  - searching – finding the conformation of ligand in the active site of the enzyme
  - scoring – evaluation of the binding free energy
- Docking software
  - Autodock, Vina, Gold, Medusa, Rosetta Dock…



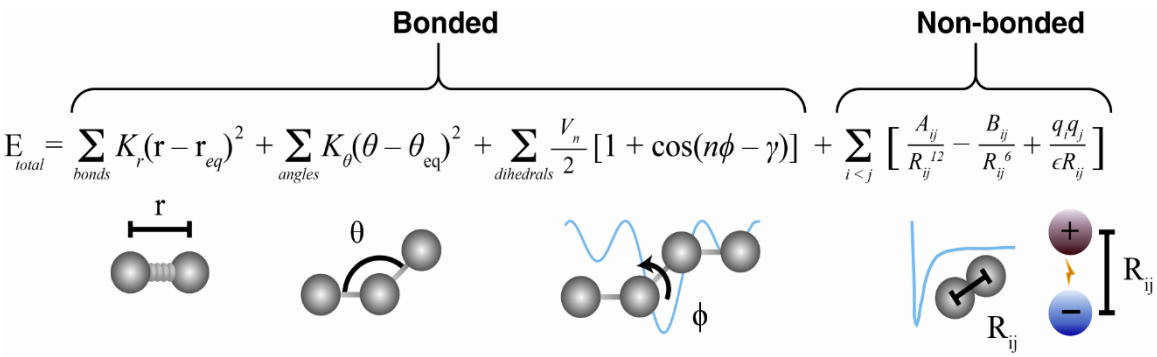
Molecular docking

- **Virtual screening**
  - database of compounds + protein structure > molecular docking > re-scoring > compound prioritization > experimental testing

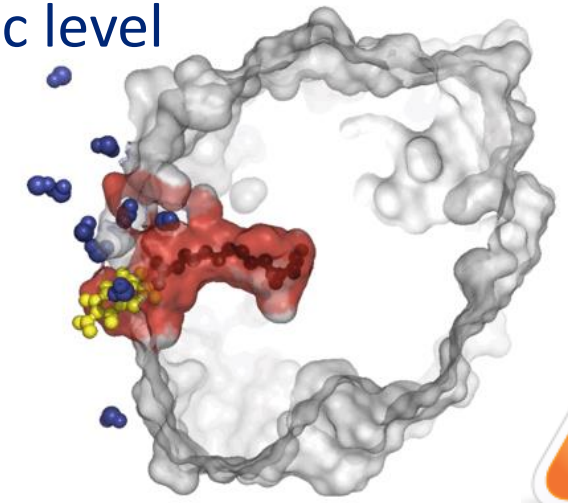


Molecular dynamics

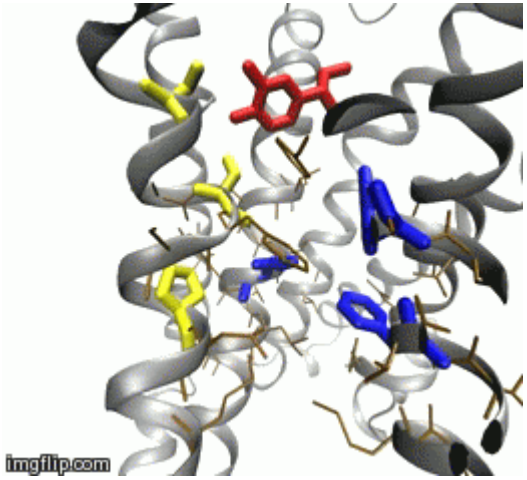
- **Principle**
  - physical description of interactions within the system (force field)
  - Newton's laws of motions
  - forces acting on all atoms due to all atoms
- Provides information on energetics, amplitudes, and time scales of local motions on the atomic level



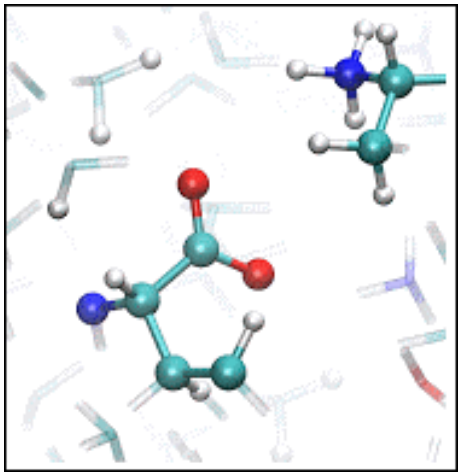
force field



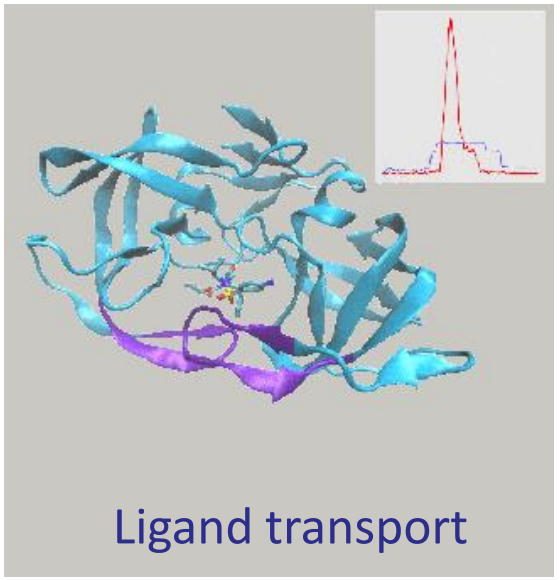
Molecular dynamics



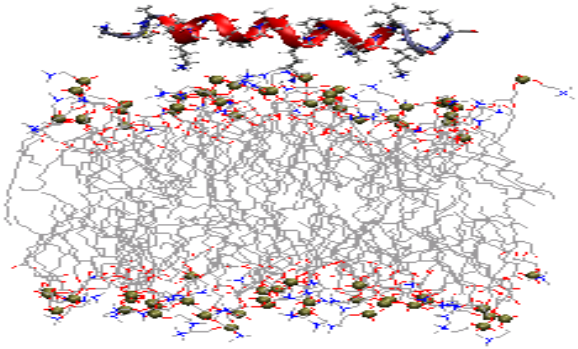
Analysis of interactions



Ligand conversion



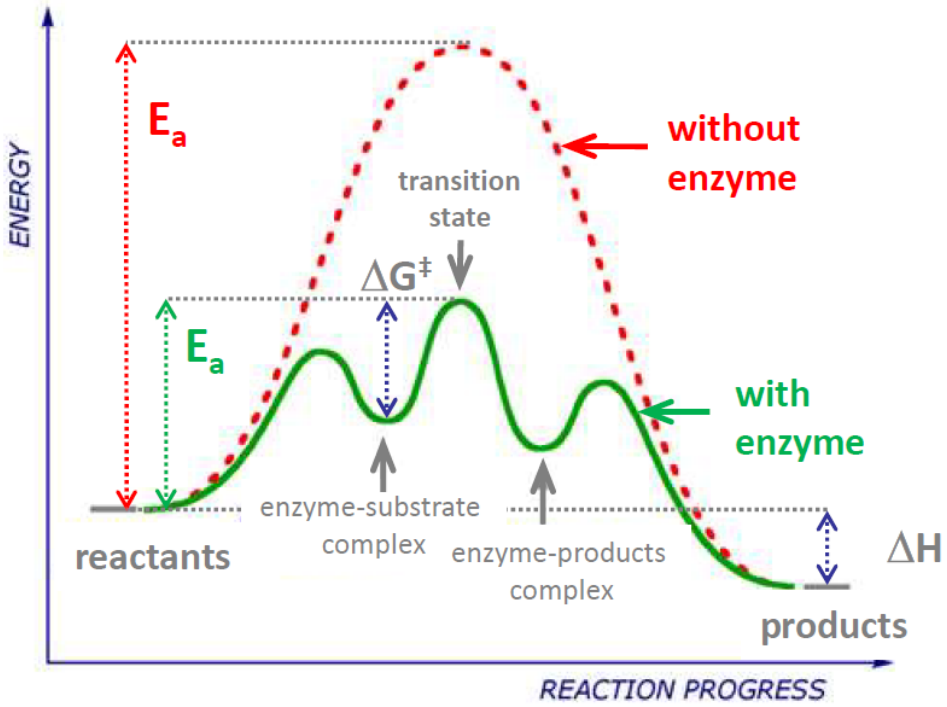
Ligand transport



Interaction with membrane

Quantum chemistry

- Modeling of reaction barriers
- Enzymes increase speed of chemical reactions by decreasing activation barrier



- Kinetic rate:

$$k = Ae^{\frac{-E_a}{RT}}$$

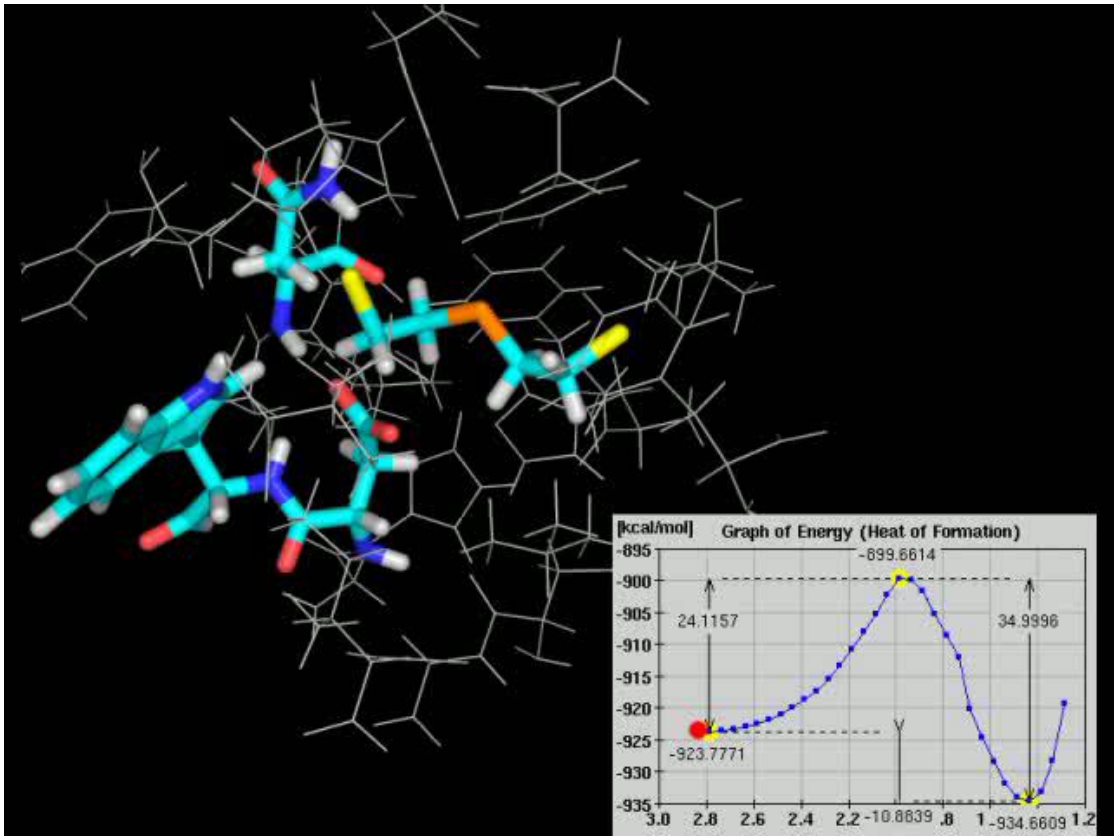
(Arrhenius equation)

- lower E_a → higher k

⇔ faster reaction

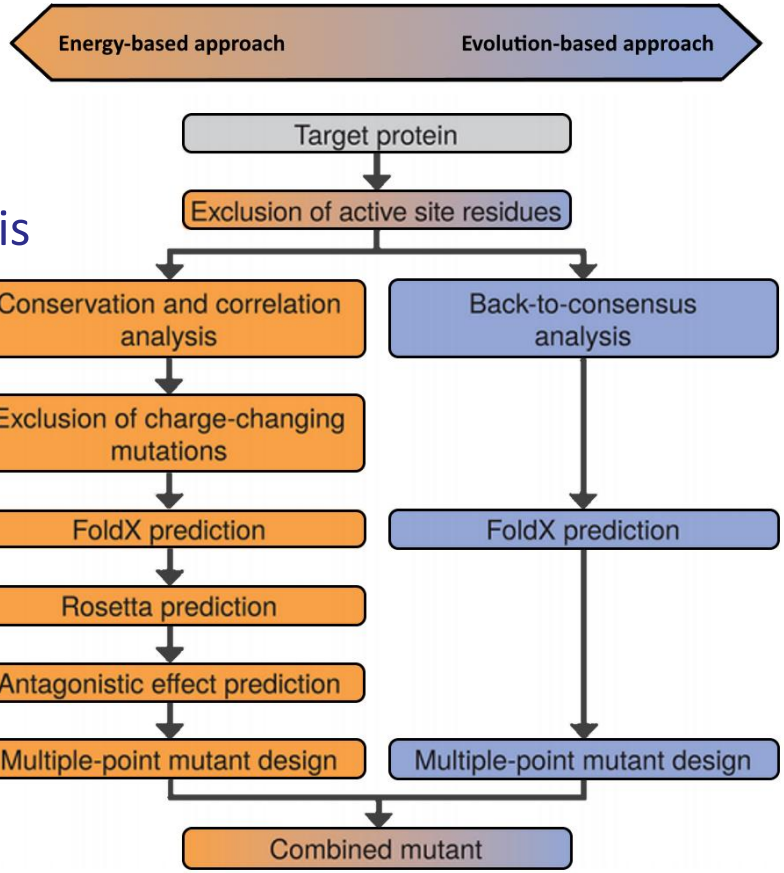
Quantum chemistry

- Using quantum mechanics to create or break bonds (usually hybrid quantum mechanics/ molecular mechanics simulation)



Design of stability

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



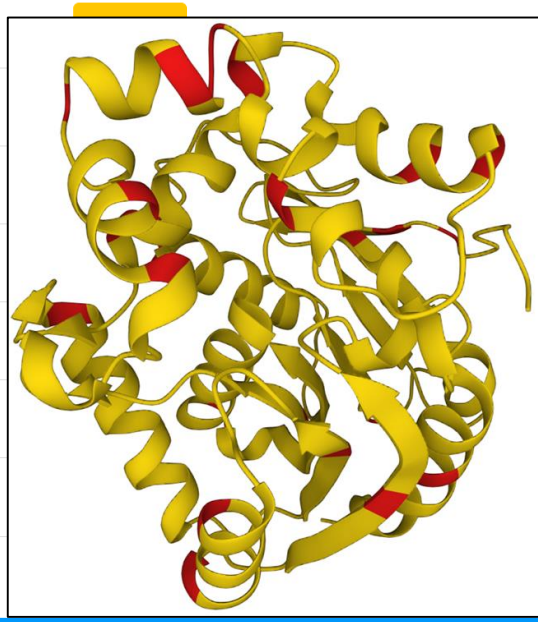
Design of stability

FireProt

Consensus	Energy low risk	Energy high risk	Comb. low risk	Comb. high risk	Ancestral design	All residues

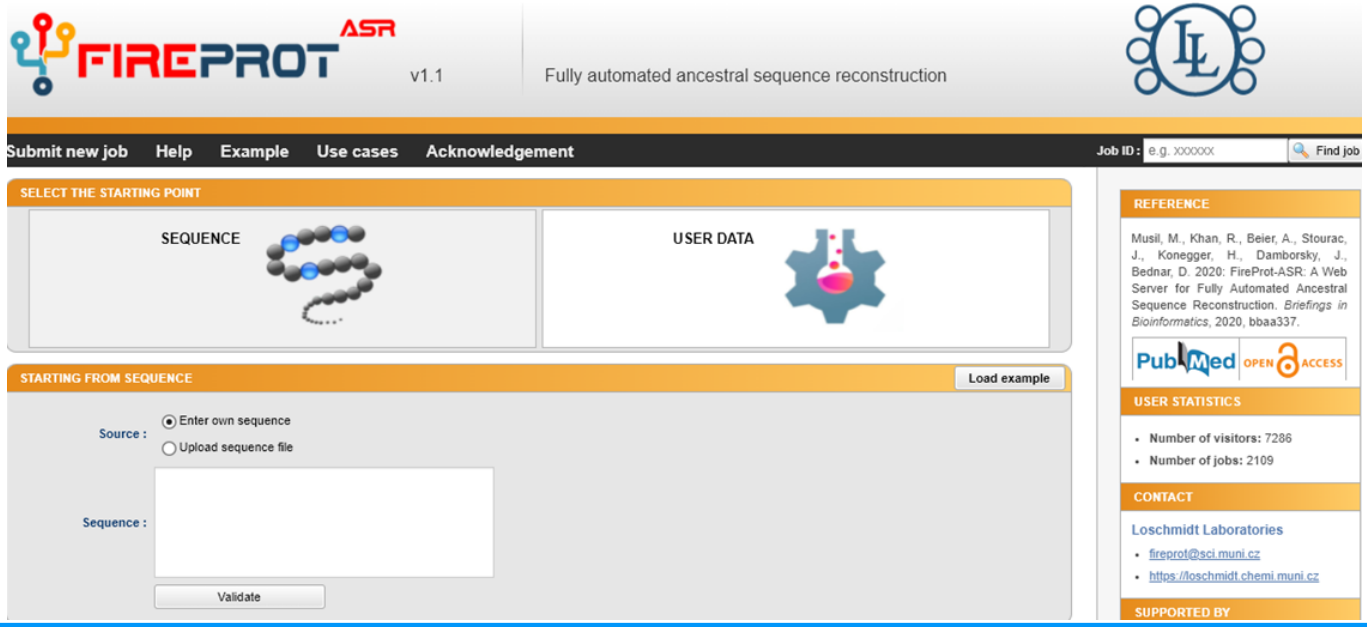
☑	Chain	Position	Ref	Alt	Conservation	Majority	Ratio	Foldx	Rosetta	Add to Design
☑	A	11	D	P	4	false	false	-1.40276	-3.423	+
☑	A	13	H	F	5	false	false	-1.21777	-2.16	+
☑	A	23	H	A	6	true	false	0.451804		+
☑	A	33	T	I	4	false	false	-1.55239	-2.294	
☑	A	80	F	R	5	true	true	-0.521484		
☑	A	82	D	W	1	false	false	-1.32168	-3.595	
☑	A	119	N	H	6	true	false	-1.14059		
☑	A	128	C	F	6	true	true	-1.13013		
☑	A	145	A	L	2	false	false	-2.8401	-2.366	
☑	A	148	T	M	2	false	false	-2.13847	-2.842	

Rows per page: 10 1-10 of 26



Design of stability

- **FireProt^{ASR}**
  - https://loschmidt.chemi.muni.cz/fireprotasr
  - ancestral sequence reconstruction
  - Analysis of protein evolution, sequence-based protein stabilization
  - Ancestrals are highly stable, have broad specificity and good yields



Design of stability

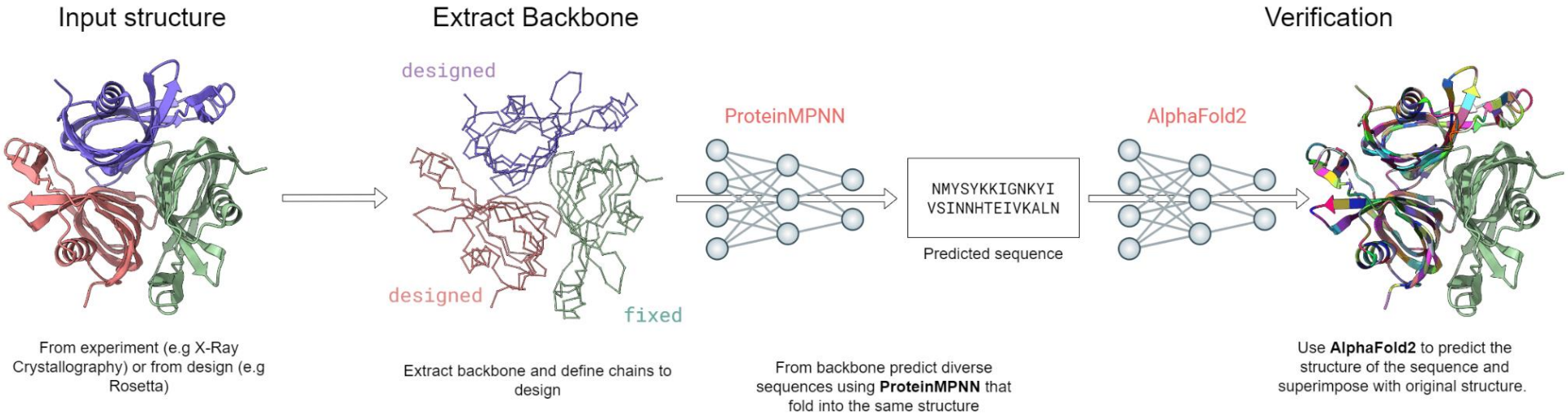
- **FireProt^{ASR}**

Design of solubility

- **ProteinMPNN**
  - https://huggingface.co/spaces/simonduerr/ProteinMPNN
  - deep learning model for protein optimization via mutations
  - takes structure on the input and provides optimized sequence

folding into the same backbone

  - good for improving yields and rescuing folding-compromised designs



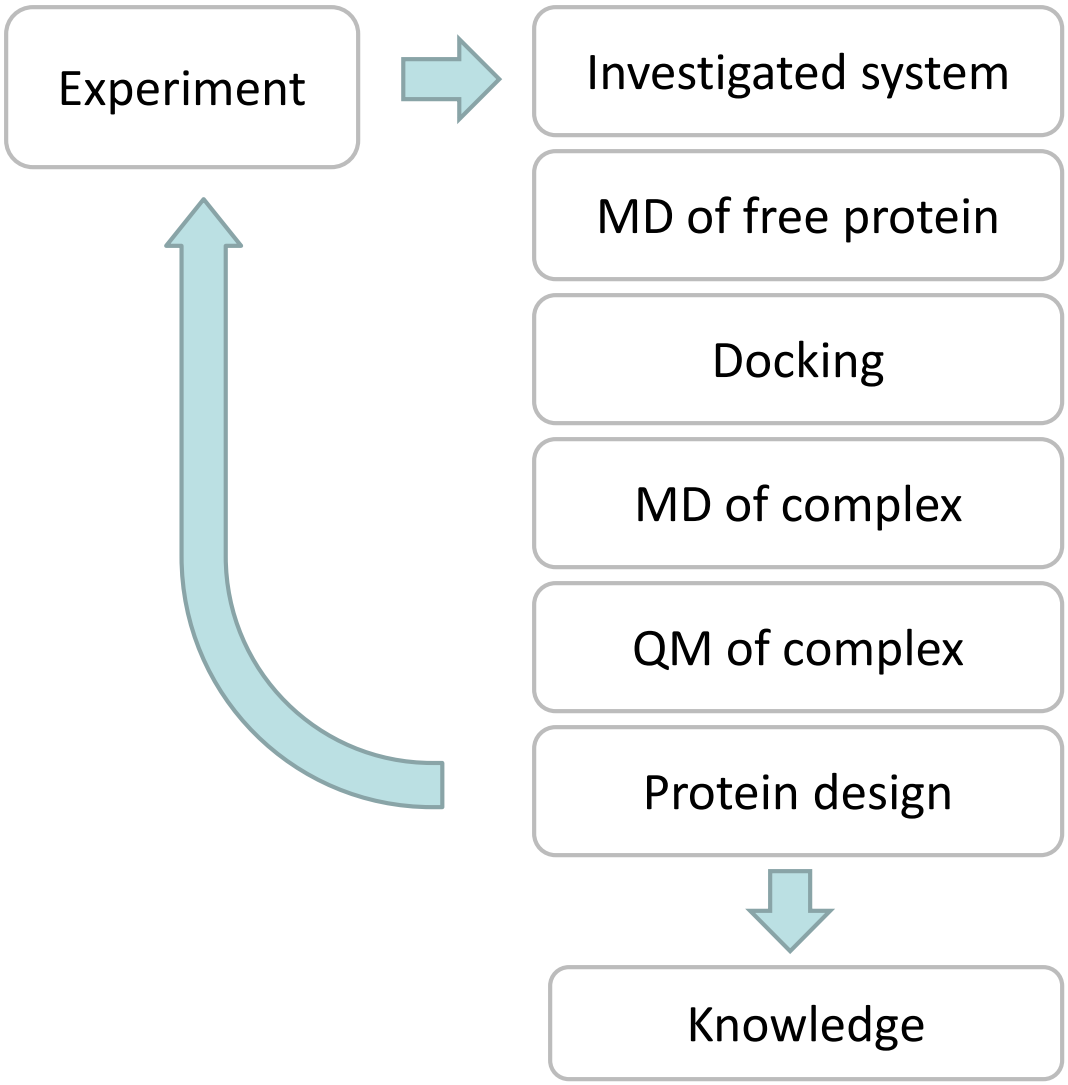
Design of protein-protein interactions

- AffiLib
  - https://affilib.weizmann.ac.il/bin/steps
  - RosettaDesign and evolution analysis to optimize macromolecular interface
  - mutations for improvement of the binding affinity
  - up to 50 multiple-point mutants for protein synthesis

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

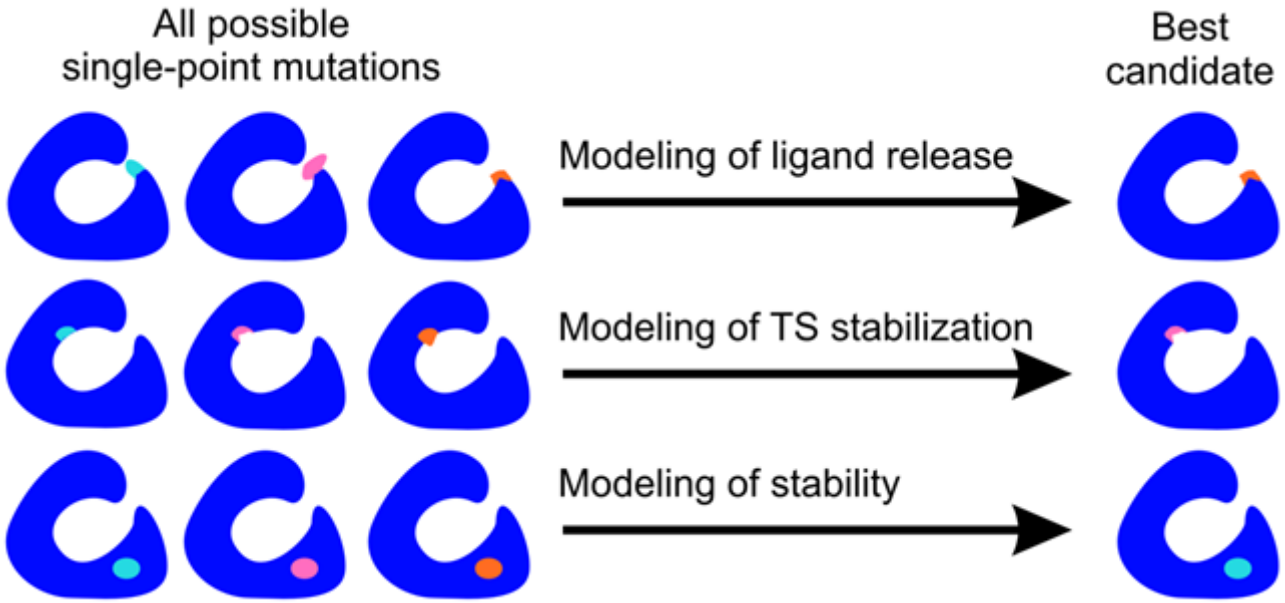
Reset Verify Proceed

Design of mutations



Design of mutations

- design of modified enzymes by *in silico* screening
  - study of effects of all relevant mutations
  - selection and combination of the best mutations



LOSCHMIDT LABORATORIES

PROTEIN ENGINEERING

8. Directed evolution

Loschmidt Laboratories
Department of Experimental Biology
Masaryk University, Brno