Computational(rational) protein engineering

Presented by:

Ladan Mafakher

Pharmaceutical biotechnology of Pasteur institute of medical sciences

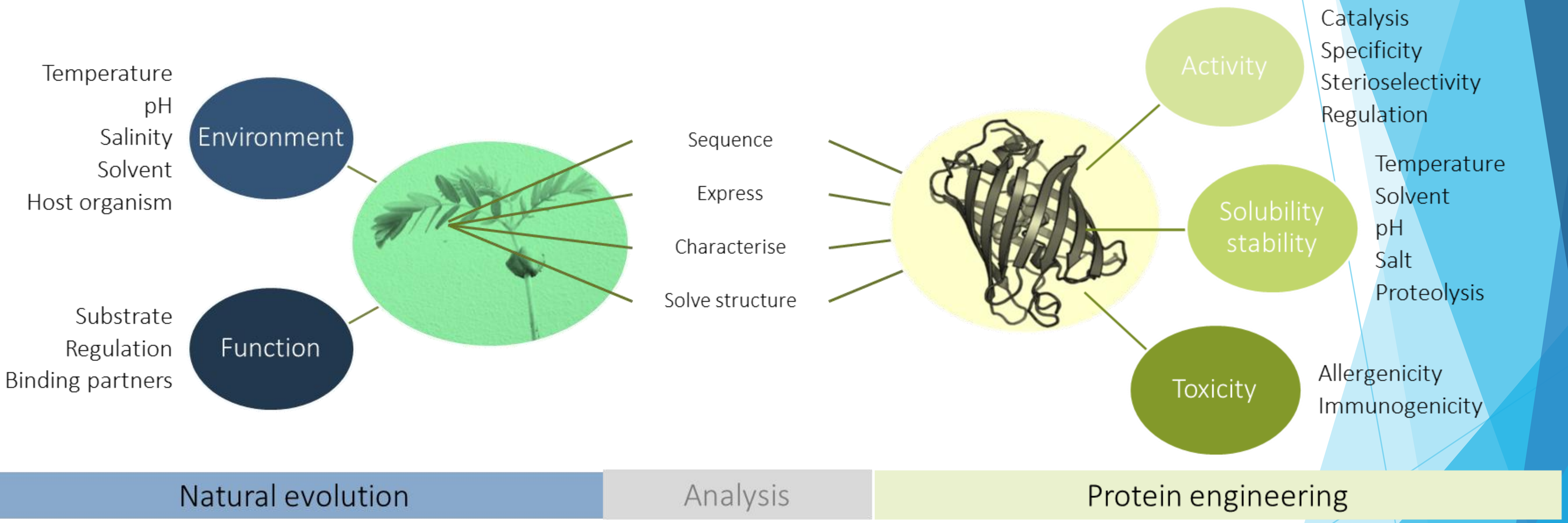
Thalassemia & Hemoglobinopathy Research center, Health research institute, Ahvaz Jundishapur

Ladan.mafakher@gmail.com

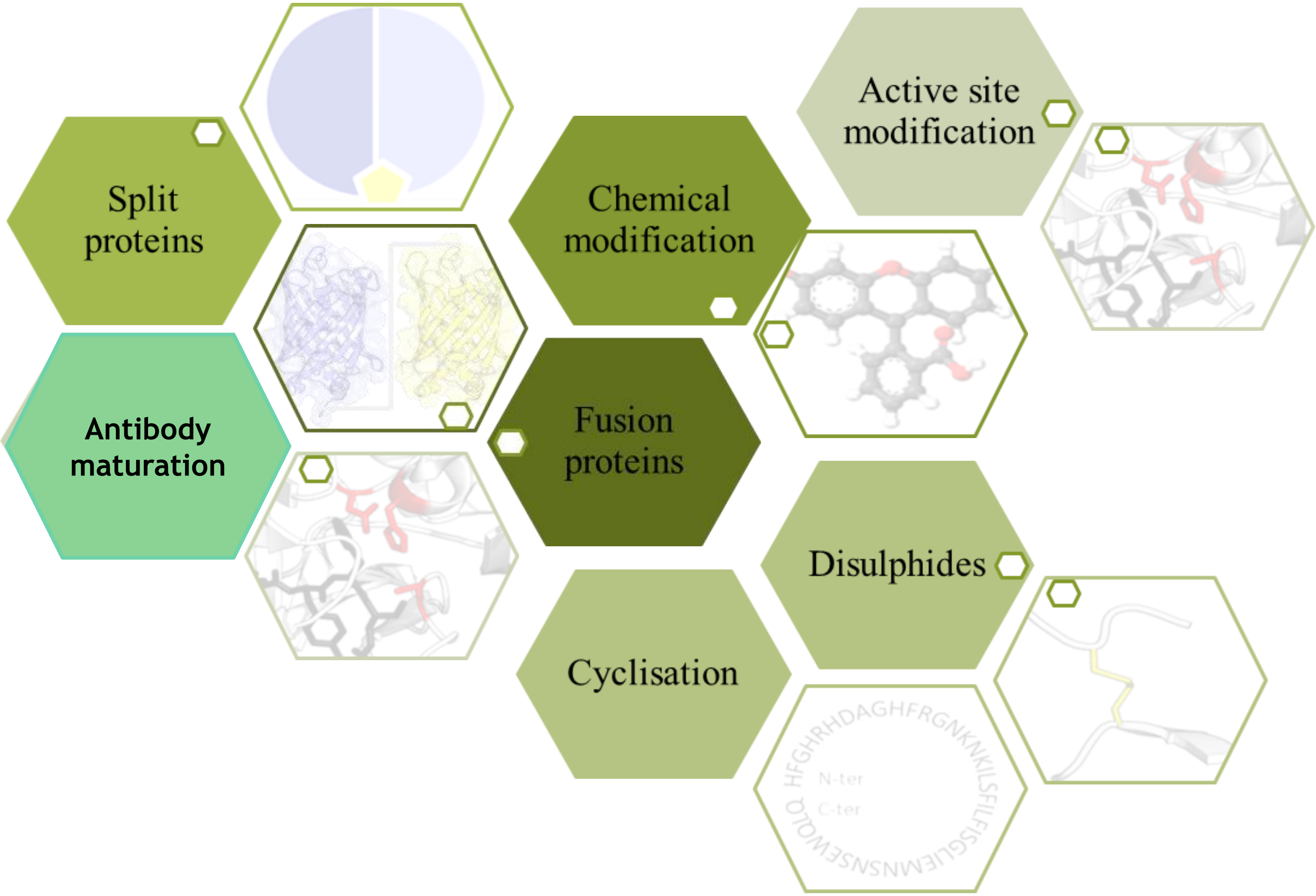
What is protein engineering?

- Proteins are molecular devices, in the nanometer scale, where biological function is exerted.
- Proteins are the building blocks of all cells.
- Nature has sampled only ~10^{12} different possible proteins, while a 100 amino acid protein has 20^{100} potential sequence variations.
- protein engineering is to identify specific changes in the amino acid sequence and to alter such sequence for desired functional properties.
- construction of new proteins or enzymes with novel or desired functions, through the modification of amino acid sequences using recombinant DNA technology.
- The sizes and three dimensional conformations of protein molecules are also manipulated by protein engineering.

Aims of protein engineering



Topics



Split proteins
Chemical modification
Active site modification
Antibody maturation
Fusion proteins
Disulphides
Cyclisation

Available online at www.sciencedirect.com
ScienceDirect
Advanced Drug Delivery Reviews 60 (2008) 59–68
Advanced DRUG DELIVERY Reviews
www.elsevier.com/locate/addr

PEG-uricase in the management of treatment-resistant gout and hyperuricemia ☆

Merry R. Sherman [a,*], Mark G.P. Saifer [a], Fernando Perez-Ruiz [b]

[a] *Mountain View Pharmaceuticals, Inc., 3475-S Edison Way, Menlo Park, CA 94025, USA*
[b] *Sección de Reumatologia, Hospital De Cruces, Pza Cruces, Barakaldo 48903, Spain*

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Abstract

Hyperuricemia results from an imbalance between the rates of production and excretion of uric acid. Longstanding hyperuricemia can lead to gout, which is characterized by the deposition of monosodium urate monohydrate crystals in the joints and periarticular structures. Because such deposits are resolved very slowly by lowering plasma urate with available drugs or other measures, the symptoms of gout may become chronic. Persistent hyperuricemia may also increase the risk of renal and cardiovascular diseases. Unlike most mammals, humans lack the enzyme uricase (urate oxidase) that catalyzes the oxidation of uric acid to a more soluble product. This review describes the development of a poly(ethylene glycol) (PEG) conjugate of recombinant porcine-like uricase with which a substantial and persistent reduction of plasma urate concentrations has been demonstrated in a Phase 2 clinical trial. Two ongoing Phase 3 clinical trials include systematic assessments of gout symptoms, tophus resolution and quality of life, in addition to the primary endpoint of reduced plasma urate concentration.

Keywords: Poly(ethylene glycol); Urate oxidase; Therapeutic enzyme; Gout; Hyperuricemia

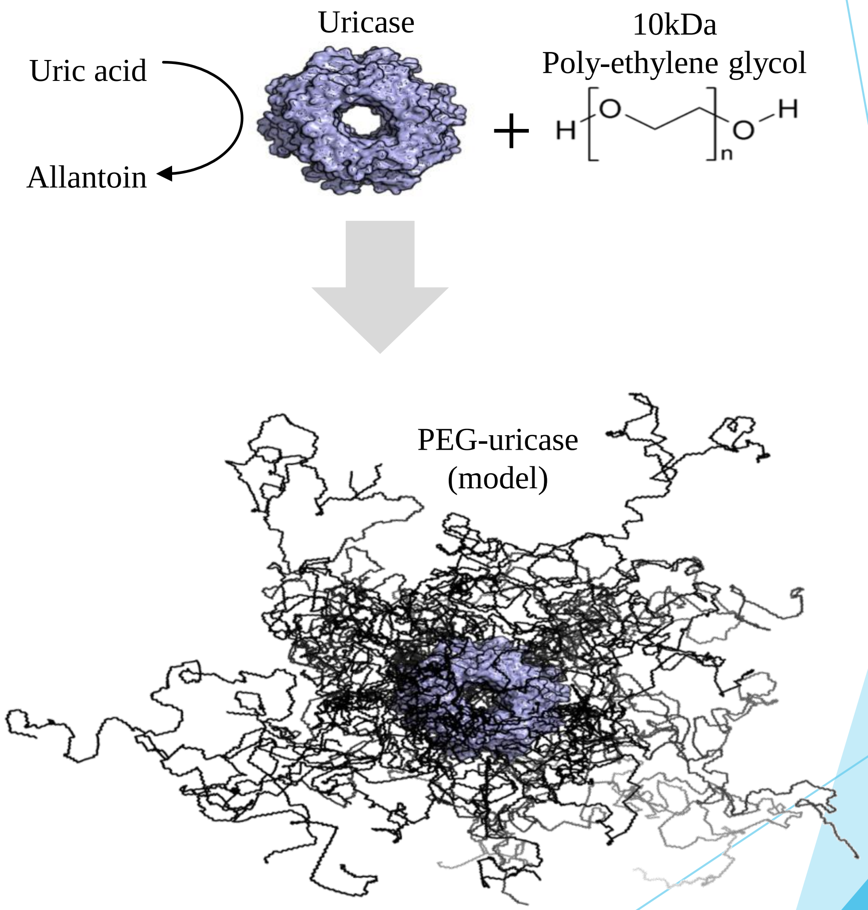
Uricase

- Engineering

- Optimise uricase as gout treatment
- Reduce immunogenicity
- Increase serum half-life
- Attached PEG polymers
  - Lysine coupling
- Optimised PEG number and length
  - Maximise improvements
  - Avoid destabilisation or activity reduction

- Outcome

- Optimal PEG number and length
  - 10kDA polymers
  - 9 polymers per subunit of the tetramer
- 1000x reduced antigenicity
  - Also improved solubility at neutral pH
  - Also increased serum half-life



Oral Treatment With an Engineered Uricase, ALLN-346, Reduces Hyperuricemia, and Uricosuria in Urate Oxidase-Deficient Mice

Kateryna Pierzynowska [1,2,3], Aditi Deshpande [4], Nadiia Mosiichuk [5], Robert Terkeltaub [6], Paulina Szczurek [7], Eduardo Salido [8], Stefan Pierzynowski [2,3,9] and Danica Grujic [4]**

[1] Department of Animal Physiology, Kielanowski Institute of Animal Nutrition and Physiology Polish Academy of Sciences, Jablonna, Poland, [2] Department of Biology, Lund University, Lund, Sweden, [3] SGP+Group, Trelleborg, Sweden, [4] Allena Pharmaceuticals, Newton, MA, United States, [5] Department of Biochemistry and Biotechnology, Vasyl Stefanyk Precarpathian National University, Ivano-Frankivsk, Ukraine, [6] VA Medical Center, University of California, San Diego, La Jolla, CA, United States, [7] Department of Animal Nutrition and Feed Sciences, National Research Institute of Animal Production, Balice, Poland, [8] Hospital Universitario de Canarias, Universidad La Laguna & Center for Rare Diseases (CIBERER), Tenerife, Spain, [9] Department of Biology, Institute Rural Medicine, Lublin, Poland

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Hideki Yokoi, Kyoto University Hospital, Japan

***Correspondence:**
Kateryna Pierzynowska
katerina.goncharova@biol.lu.se
Danica Grujic
dgrujic@allenapharma.com

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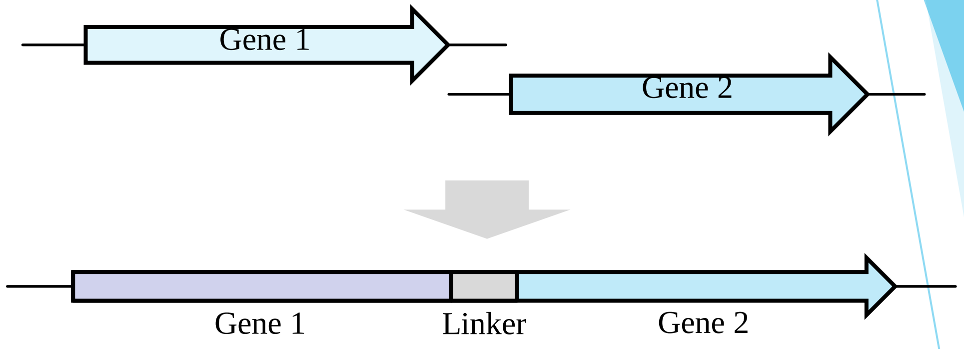
Limitations in efficacy and/or tolerance of currently available urate-lowering therapies (ULTs), such as oral xanthine oxidase inhibitors, uricosurics, and intravenous uricase agents contribute to the development of refractory gout. Renal excretion is the major route of uric acid elimination, but the intestinal tract plays an increasingly recognized role in urate homeostasis, particularly in chronic kidney disease (CKD) in which the renal elimination of urate is impaired. We targeted intestinal degradation of urate *in vivo* with ALLN-346, an orally administered, engineered urate oxidase, optimized for proteolytic stability, and activity in the gut. We tested ALLN-346 in uricase/urate oxidase deficient mice (URKO mice) with severe hyperuricemia, hyperuricosuria, and uric acid crystalline obstructive nephropathy. A total of 55 male and female URKO mice were used in the two consecutive studies. These seminal, proof-of-concept studies aimed to explore both short- (7-day) and long-term (19-day) effects of ALLN-346 on the reduction of plasma and urine urate. In both the 7- and 19-day studies, ALLN-346 oral therapy resulted in the normalization of urine uric acid excretion and a significant reduction of hyperuricemia by 44 and 28% when therapy was given with food over 24 h or was limited for up to 6 h, respectively. Fractional excretion of uric acid (FEUA) was normalized with ALLN-346 therapy. Oral enzyme therapy with engineered urate oxidase (ALLN-346) designed to degrade urate in the intestinal tract has the potential to reduce hyperuricemia and the renal burden of filtered urate in patients with hyperuricemia and gout with and without CKD.

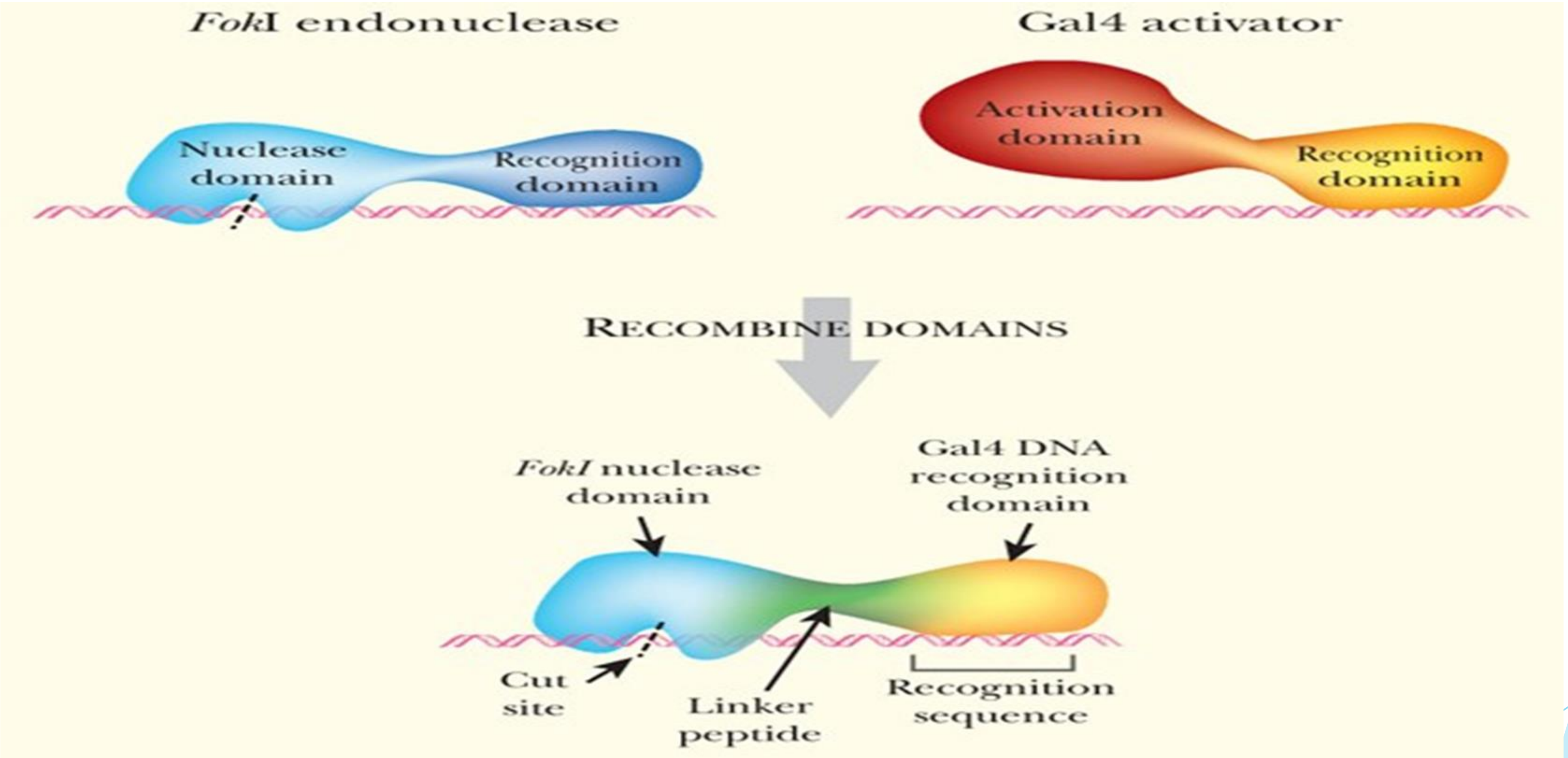
Keywords: gout, CKD, urolithiasis, urate-lowering therapy, ABCG2

Fusion proteins

- Creation
- Remove stop codon of first gene
- Ligate genes together in frame
- Include linker codons
- Aims
- Combine the properties of the components
- E.g. Addition of antibody Fc fragment to proteins increases their serum half-life
- Co-localise the components
- E.g. Set of enzymes that work in a reaction pathway
- Considerations
- Linker length and flexibility
- Ability for proteins rotate relative to each other
- Distance between protein components
- Protease resilience
- Ability for domains to fold



Fusion proteins



Research Article

Computational Design of a DNA- and Fc-Binding Fusion Protein

Jonas Winkler,[1] Giuliano Armano,[2] J. Nikolaj Dybowski,[1] Oliver Kuhn,[1] Filippo Ledda,[2] and Dominik Heider[1]

[1] *Department of Bioinformatics, Center for Medical Biotechnology, University of Duisburg-Essen, Universitaetsstraβe 1-5, 45117 Essen, Germany*
[2] *Department of Electrical and Electronic Engineering, University of Cagliari, Piazza d'Armi, 09123 Cagliari, Italy*

Correspondence should be addressed to Dominik Heider, dominik.heider@uni-due.de

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Computational design of novel proteins with well-defined functions is an ongoing topic in computational biology. In this work, we generated and optimized a new synthetic fusion protein using an evolutionary approach. The optimization was guided by directed evolution based on hydrophobicity scores, molecular weight, and secondary structure predictions. Several methods were used to refine the models built from the resulting sequences. We have successfully combined two unrelated naturally occurring binding sites, the immunoglobin Fc-binding site of the Z domain and the DNA-binding motif of MyoD bHLH, into a novel stable protein.

JBC RESEARCH ARTICLE

Rational affinity maturation of anti-amyloid antibodies with high conformational and sequence specificity

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Alec A. Desai[1,2], Matthew D. Smith[1,2], Yulei Zhang[1,2], Emily K. Makowski[2,3], Julia E. Gerson[4], Edward Ionescu[1,2], Charles G. Starr[1,2,3], Jennifer M. Zupancic[1,2], Shannon J. Moore[5,6], Alexandra B. Sutter[4,7], Magdalena I. Ivanova[4,7], Geoffrey G. Murphy[5,6], Henry L. Paulson[4,5,8], and Peter M. Tessier[1,2,3,5,9,*]

From the [1]Department of Chemical Engineering, [2]Biointerfaces Institute, [3]Department of Pharmaceutical Sciences, [4]Department of Neurology, [5]Protein Folding Disease Initiative, [6]Department of Molecular & Integrative Physiology, [7]Biophysics Program, [8]Michigan Alzheimer's Disease Center, [9]Department of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan, USA

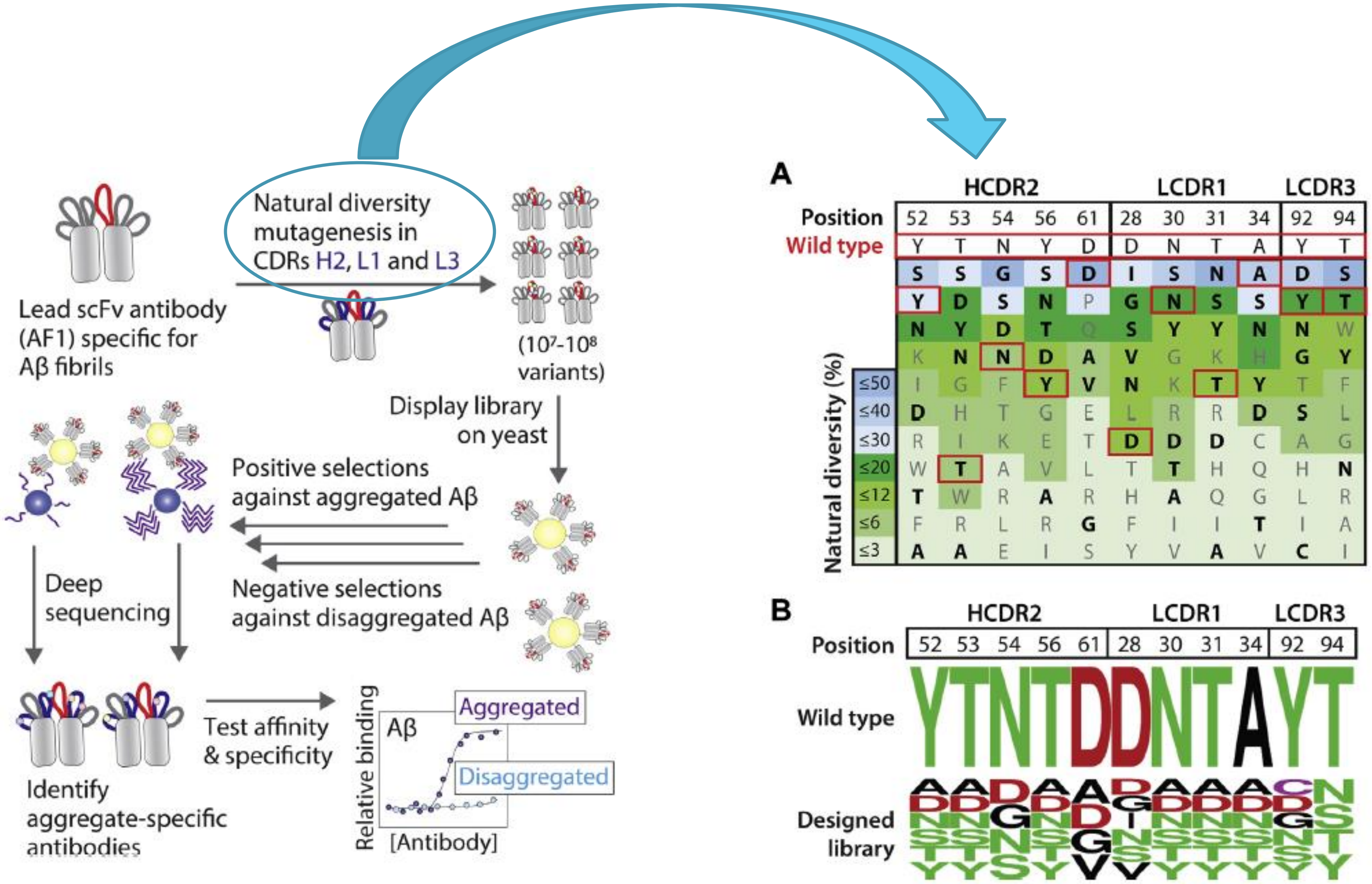
Edited by Paul Fraser

The aggregation of amyloidogenic polypeptides is strongly linked to several neurodegenerative disorders, including Alzheimer's and Parkinson's diseases. Conformational antibodies that selectively recognize protein aggregates are leading therapeutic agents for selectively neutralizing toxic aggregates, diagnostic and imaging agents for detecting disease, and biomedical reagents for elucidating disease mechanisms. Despite their importance, it is challenging to generate high-quality conformational antibodies in a systematic and site-specific manner due to the properties of protein aggregates (hydrophobic, multivalent, and heterogeneous) and limitations of immunization (uncontrolled antigen presentation and immunodominant epitopes). Toward addressing these challenges, we have developed a systematic directed evolution procedure for affinity maturing antibodies against Alzheimer's Aβ fibrils and selecting variants with strict conformational and

Of the many human disorders facing our society today, neurodegenerative diseases such as Alzheimer's and Parkinson's diseases are arguably the most menacing and least treatable (1, 2). These diseases – which are linked to the formation of toxic prefibrillar oligomers and amyloid fibrils – are particularly concerning because their frequency of occurrence is linked to age and, thus, the number of cases is expected to increase as life expectancy increases in the coming years due to significant advances in treating other human disorders such as cancer and heart diseases.

Conformational antibodies specific for different conformers of amyloid-forming proteins are important for detecting, disrupting, and reversing toxic protein aggregation (3, 4). Several previous reports have demonstrated creative methods for using immunization (4–12), autoantibody screening (5, 13–22), directed evolution (23–26), and rational design methods

Affinity maturation



Article

Machine learning-aided engineering of hydrolases for PET depolymerization

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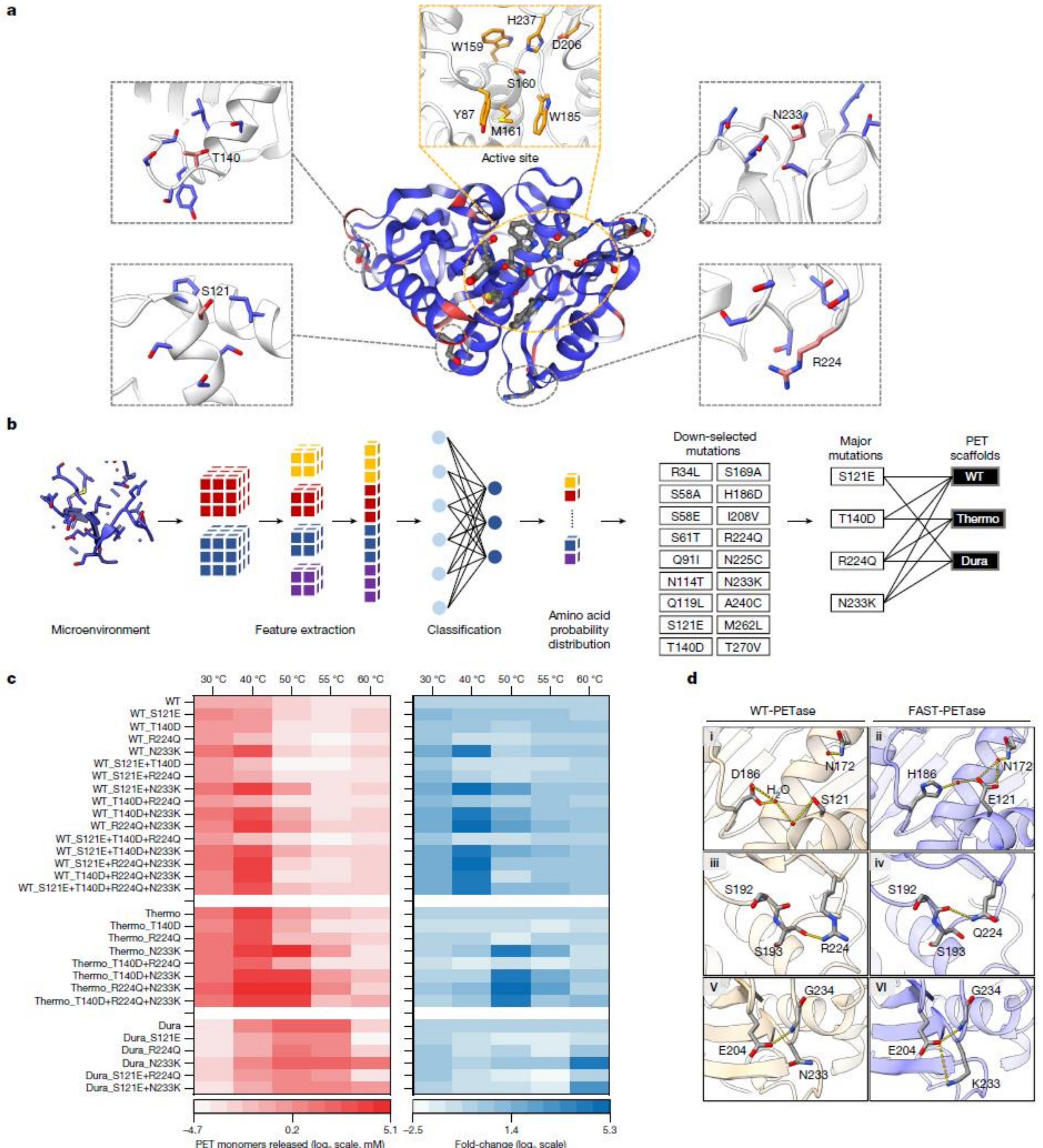
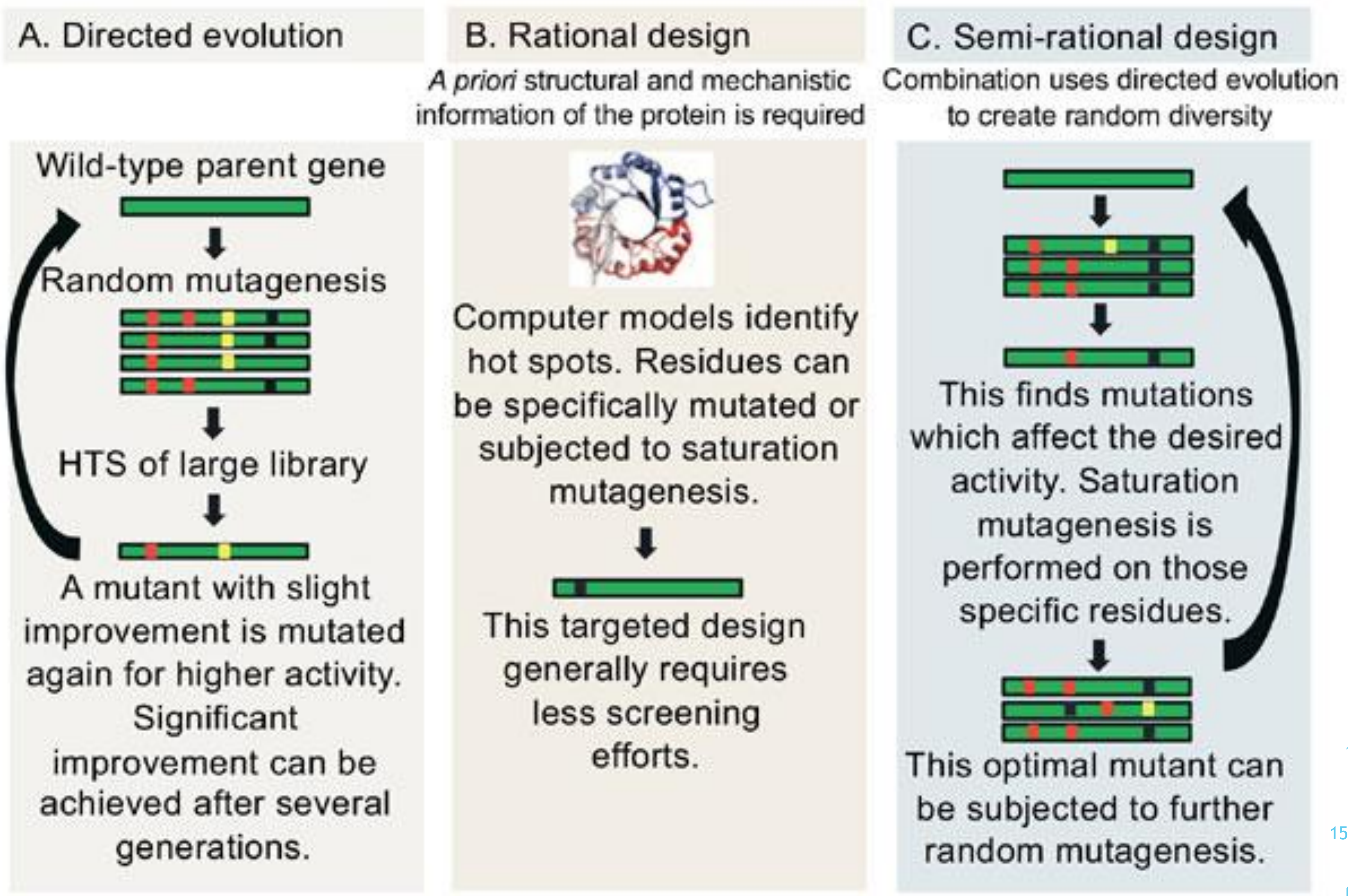
Check for updates

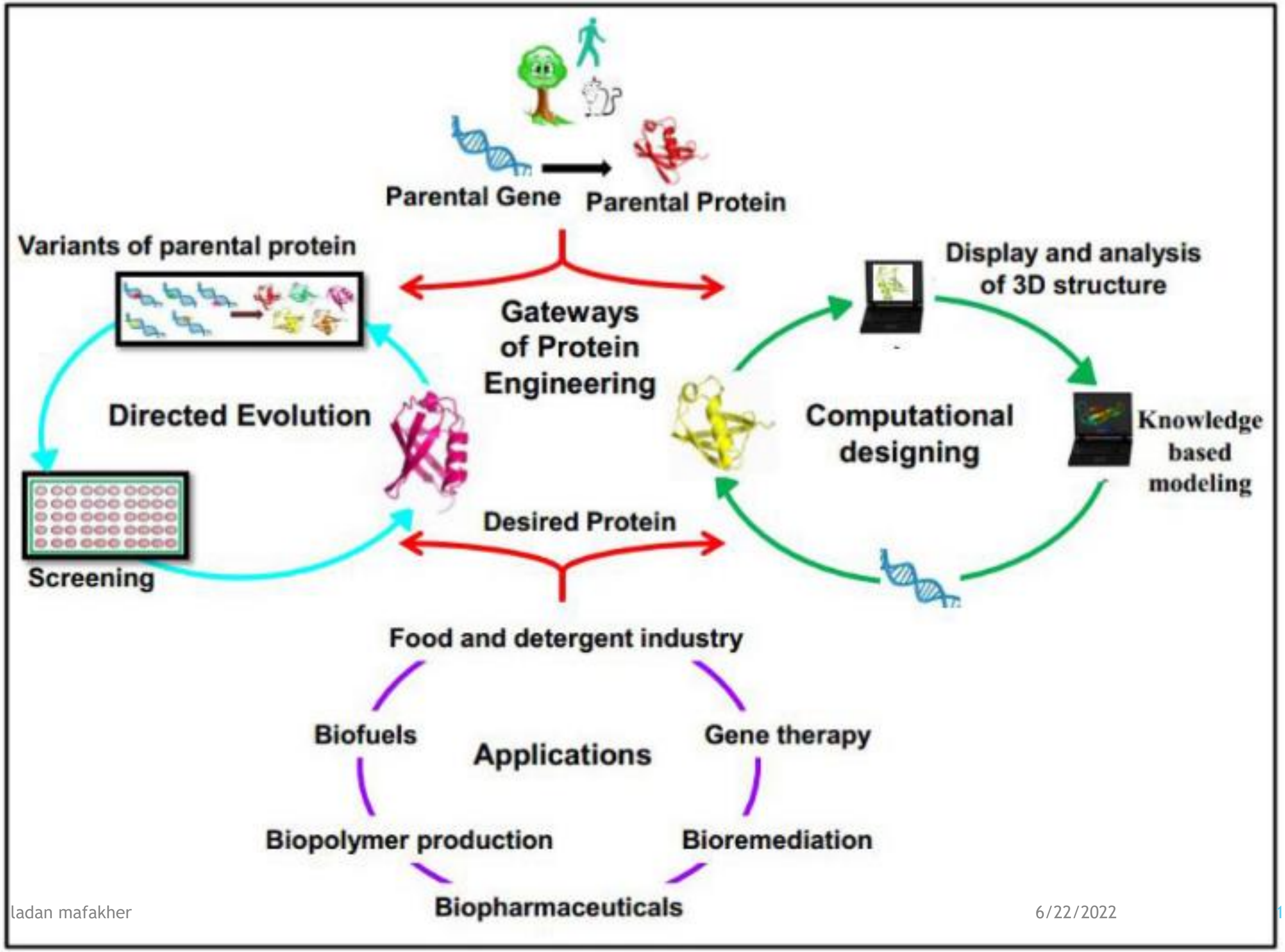
Hongyuan Lu[1], Daniel J. Diaz[2], Natalie J. Czarnecki[1], Congzhi Zhu[1], Wantae Kim[1], Raghav Shroff[3,4], Daniel J. Acosta[3], Bradley R. Alexander[3], Hannah O. Cole[1,3], Yan Zhang[3], Nathaniel A. Lynd[1], Andrew D. Ellington[3] & Hal S. Alper[1]✉

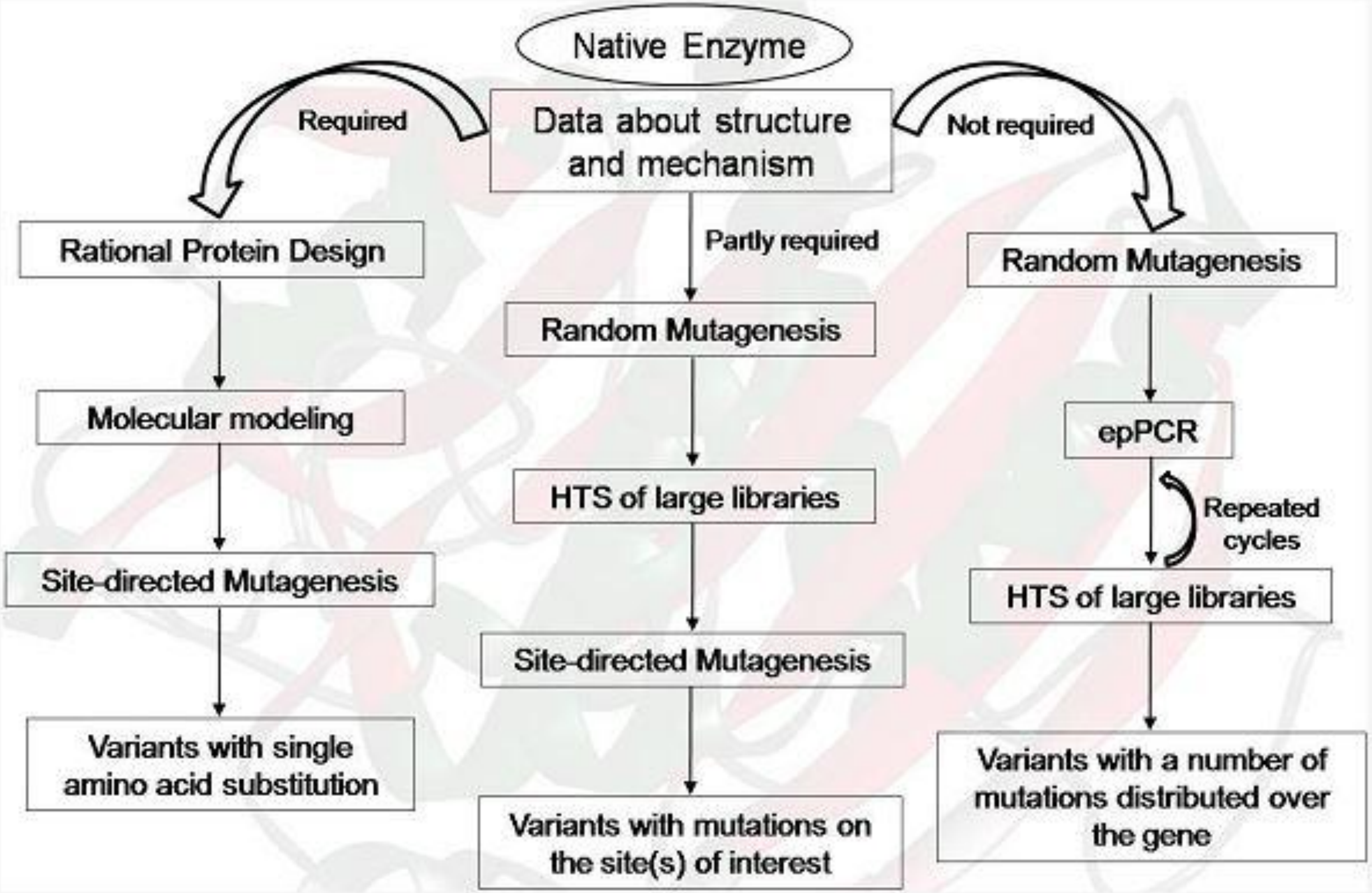
Plastic waste poses an ecological challenge[1–3] and enzymatic degradation offers one, potentially green and scalable, route for polyesters waste recycling[4]. Poly(ethylene terephthalate) (PET) accounts for 12% of global solid waste[5], and a circular carbon economy for PET is theoretically attainable through rapid enzymatic depolymerization followed by repolymerization or conversion/valorization into other products[6–10]. Application of PET hydrolases, however, has been hampered by their lack of robustness to pH and temperature ranges, slow reaction rates and inability to directly use untreated postconsumer plastics[11]. Here, we use a structure-based, machine learning algorithm to engineer a robust and active PET hydrolase. Our mutant and scaffold combination (FAST-PETase: functional, active, stable and tolerant PETase) contains five mutations compared to wild-type PETase (N233K/R224Q/S121E from prediction and D186H/R280A from scaffold) and shows superior PET-hydrolytic activity relative to both wild-type and engineered alternatives[12] between 30 and 50 °C and a range of pH levels. We demonstrate that untreated, postconsumer-PET from 51 different thermoformed products can all be almost completely degraded by FAST-PETase in 1 week. FAST-PETase can also depolymerize untreated, amorphous portions of a commercial water bottle and an entire thermally pretreated water bottle at 50 °C. Finally, we demonstrate a closed-loop PET recycling process by using FAST-PETase and resynthesizing PET from the recovered monomers. Collectively, our results demonstrate a viable route for enzymatic plastic recycling at the industrial scale.


a
H237
W159
D206
S160
Y87
W185
M161
Active site
T140
N233
S121
R224
b
Microenvironment
Feature extraction
Classification
Amino acid probability distribution
Down-selected mutations
R34L S169A
S58A H186D
S58E I208V
S61T R224Q
Q91I N225C
N114T N233K
Q119L A240C
S121E M262L
T140D T270V
Major mutations
S121E
T140D
R224Q
N233K
PET scaffolds
WT
Thermo
Dura
c
30 °C 40 °C 50 °C 55 °C 60 °C
WT
WT_S121E
WT_T140D
WT_R224Q
WT_N233K
WT_S121E+T140D
WT_S121E+R224Q
WT_S121E+N233K
WT_T140D+R224Q
WT_T140D+N233K
WT_R224Q+N233K
WT_S121E+T140D+R224Q
WT_S121E+T140D+N233K
WT_S121E+R224Q+N233K
WT_T140D+R224Q+N233K
WT_S121E+T140D+R224Q+N233K
Thermo
Thermo_T140D
Thermo_R224Q
Thermo_N233K
Thermo_T140D+R224Q
Thermo_T140D+N233K
Thermo_R224Q+N233K
Thermo_T140D+R224Q+N233K
Dura
Dura_S121E
Dura_R224Q
Dura_N233K
Dura_S121E+R224Q
Dura_S121E+N233K
−4.7 0.2 5.1
PET monomers released (log. scale, mM)
−2.5 1.4 5.3
Fold-change (log. scale)
d
WT-PETase
FAST-PETase
i
D186
H_2O
N172
S121
ii
H186
N172
E121
iii
S192
S193
R224
iv
S192
Q224
S193
v
G234
E204
N233
vi
G234
E204
K233

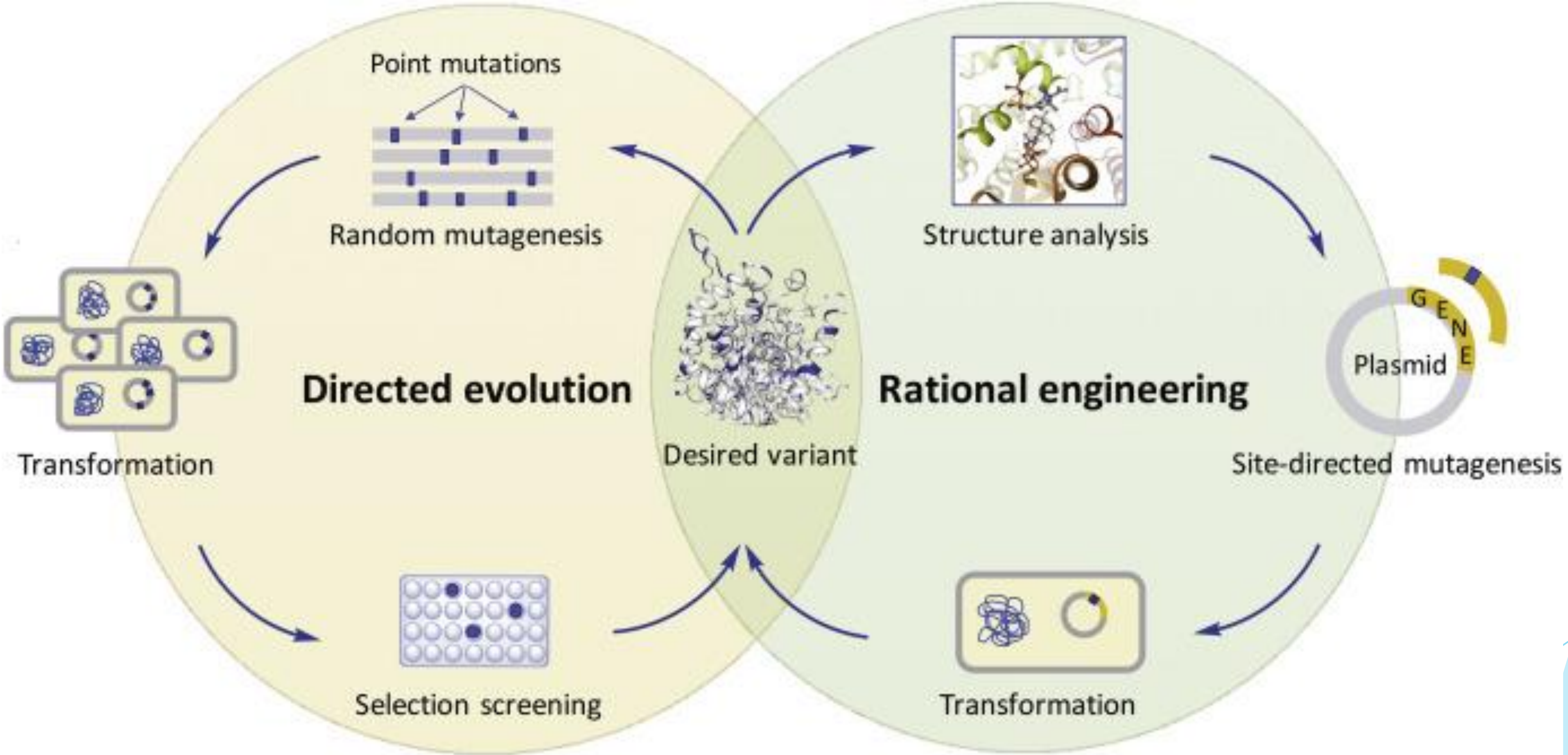
Protein engineering strategy




Parental Gene
Parental Protein
Variants of parental protein
Gateways
of Protein
Engineering
Display and analysis
of 3D structure
Directed Evolution
Computational
designing
Knowledge
based
modeling
Screening
Desired Protein
Food and detergent industry
Biofuels
Applications
Gene therapy
Biopolymer production
Bioremediation
Biopharmaceuticals


Native Enzyme
Data about structure and mechanism
Required
Not required
Partly required
Rational Protein Design
Molecular modeling
Site-directed Mutagenesis
Variants with single amino acid substitution
Random Mutagenesis
HTS of large libraries
Site-directed Mutagenesis
Variants with mutations on the site(s) of interest
Random Mutagenesis
epPCR
Repeated cycles
HTS of large libraries
Variants with a number of mutations distributed over the gene

Advantage of rational protein engineering



Workflow of computational protein engineering

1
- In silico analysis and design

2
- Gene library construct

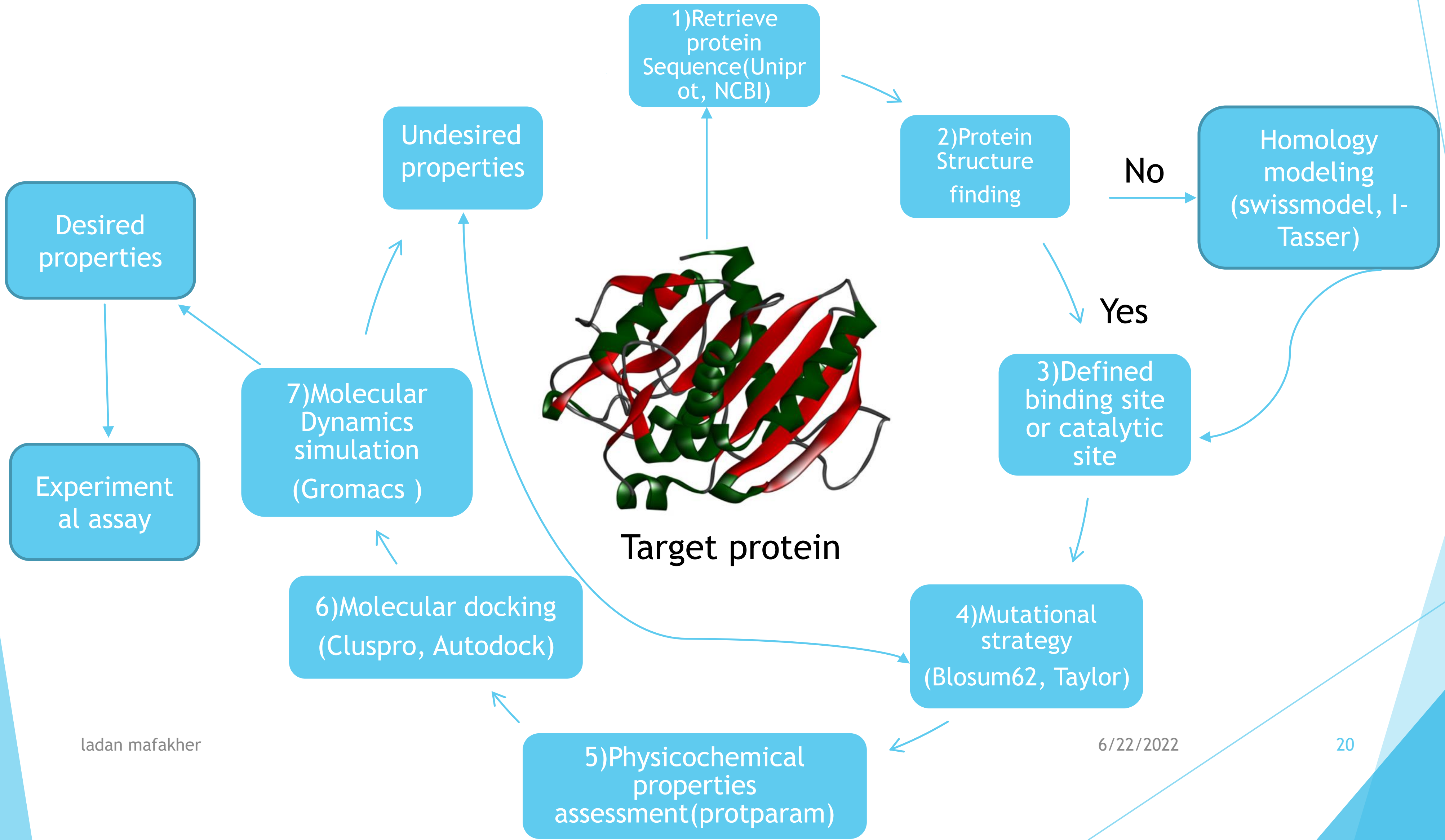
3
- Protein display/expression

4
- High throughput screening/selection

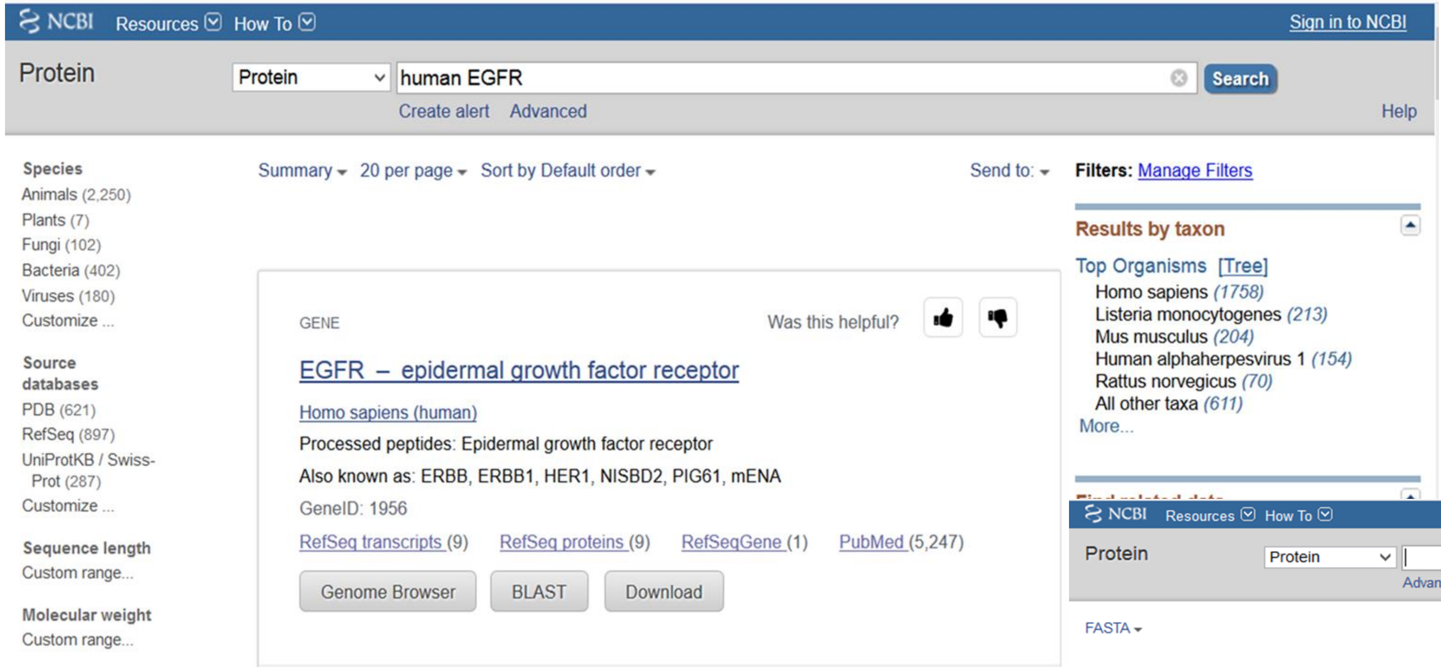
5
- Validation and characterization

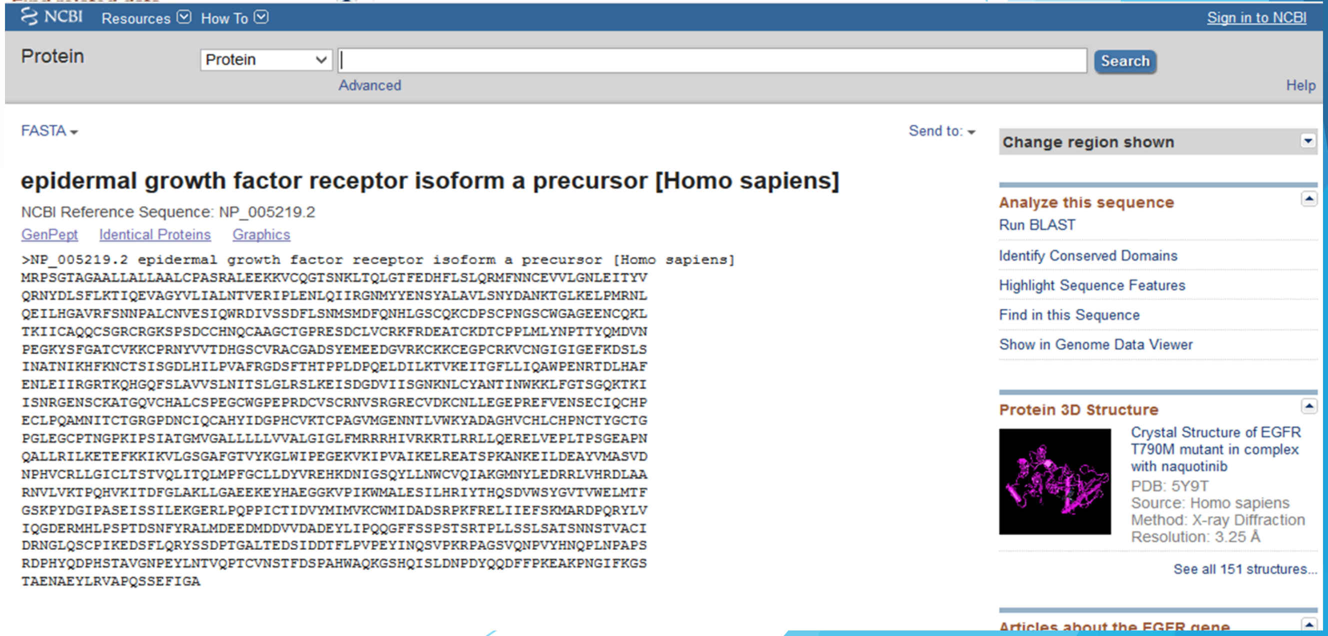
6
- Selection the most potential protein

Steps of rational protein engineering

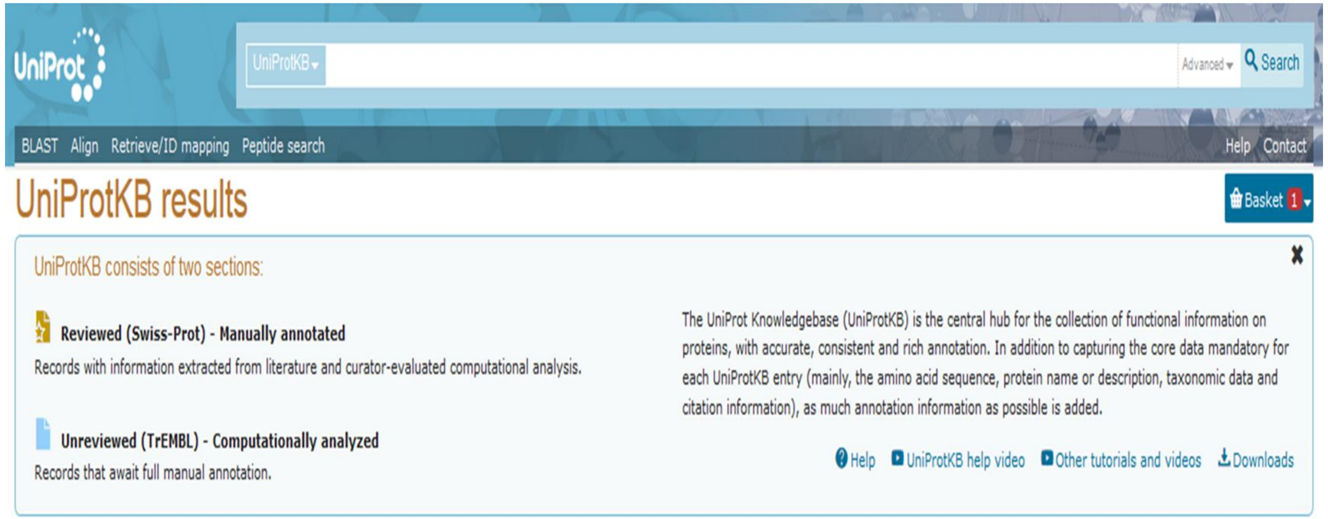


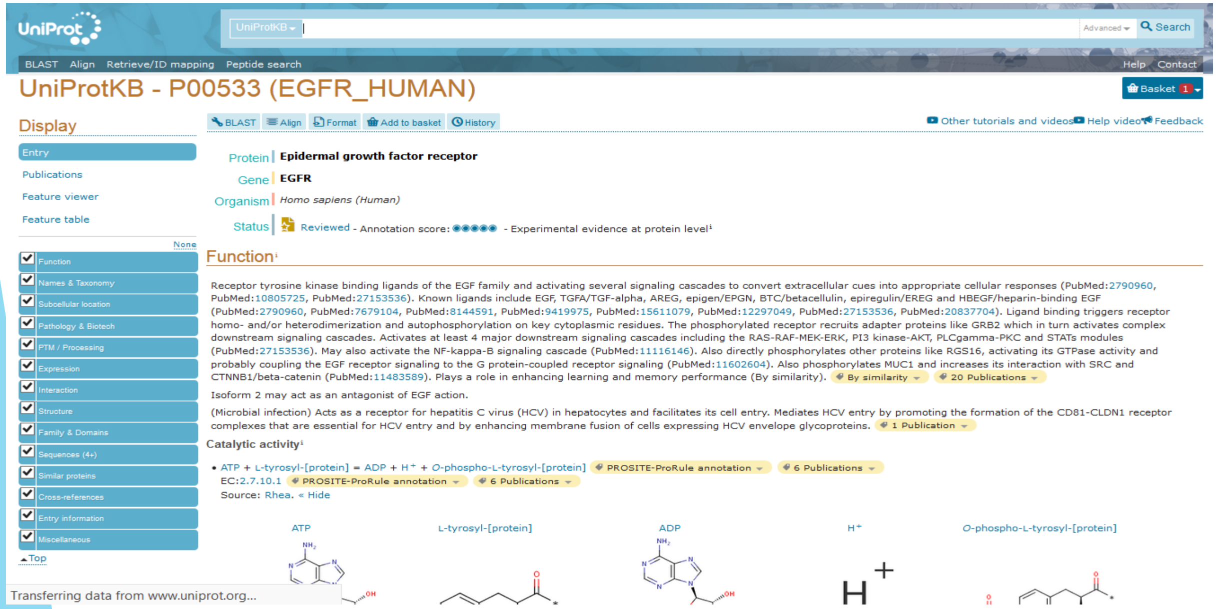
1)Retrieve protein(NCBI)
https://www.ncbi.nlm.nih.gov/protein

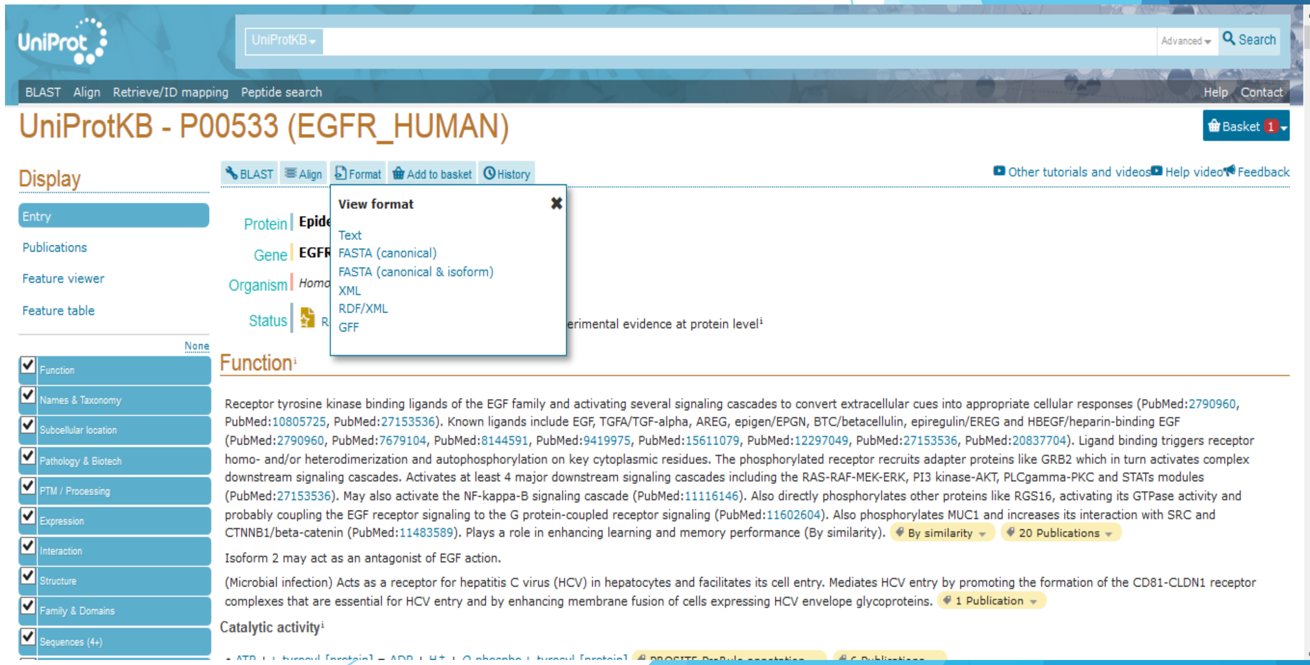




1)Retrieve protein(UNIPROT) https://www.uniprot.org/

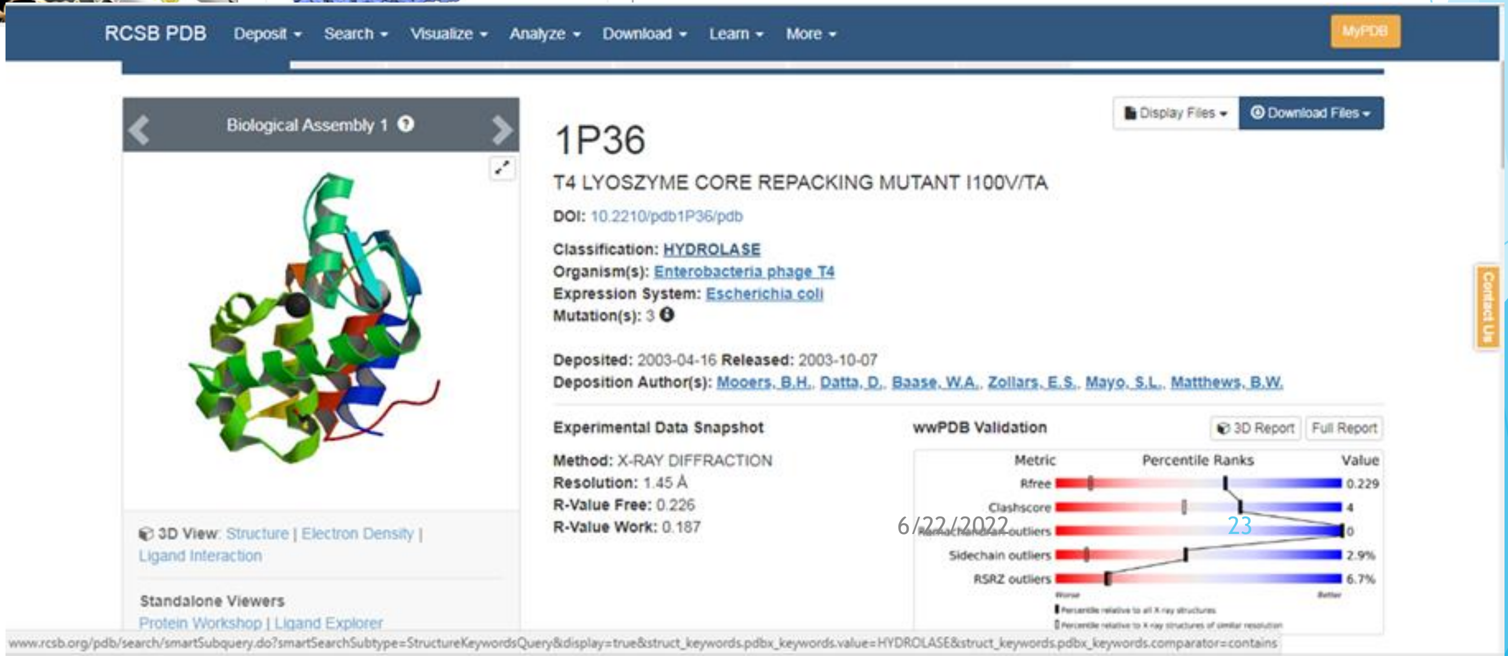




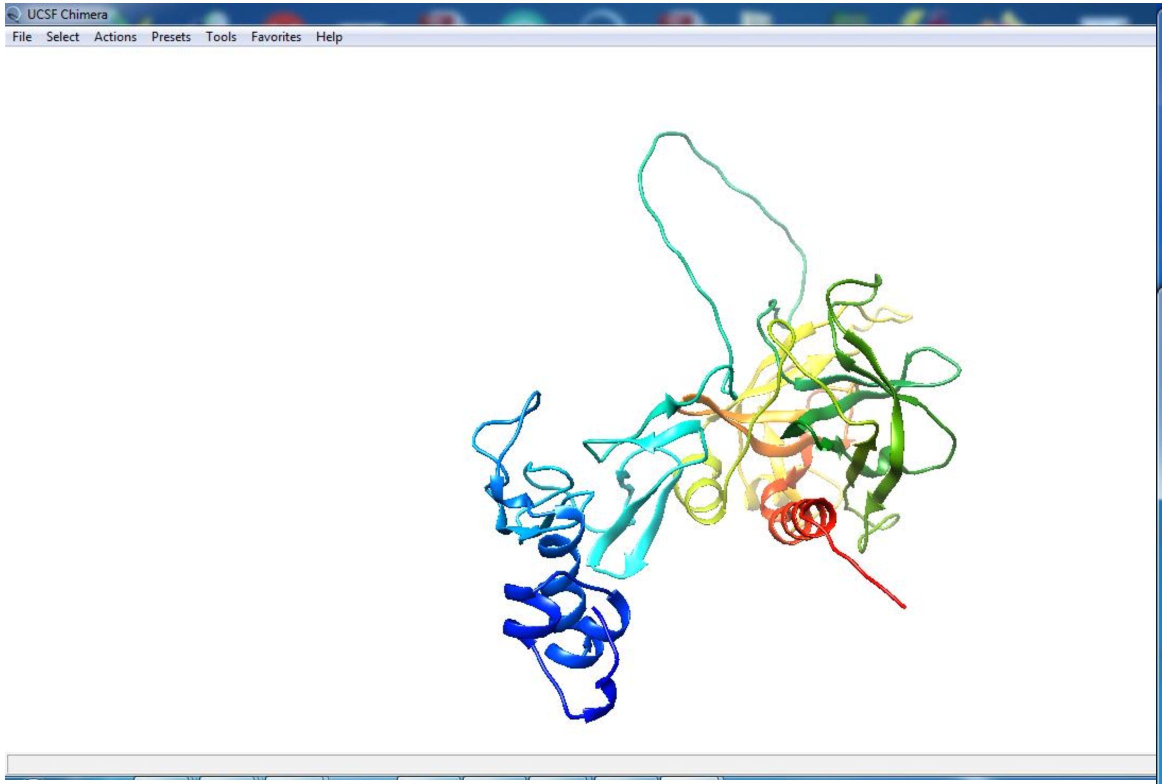


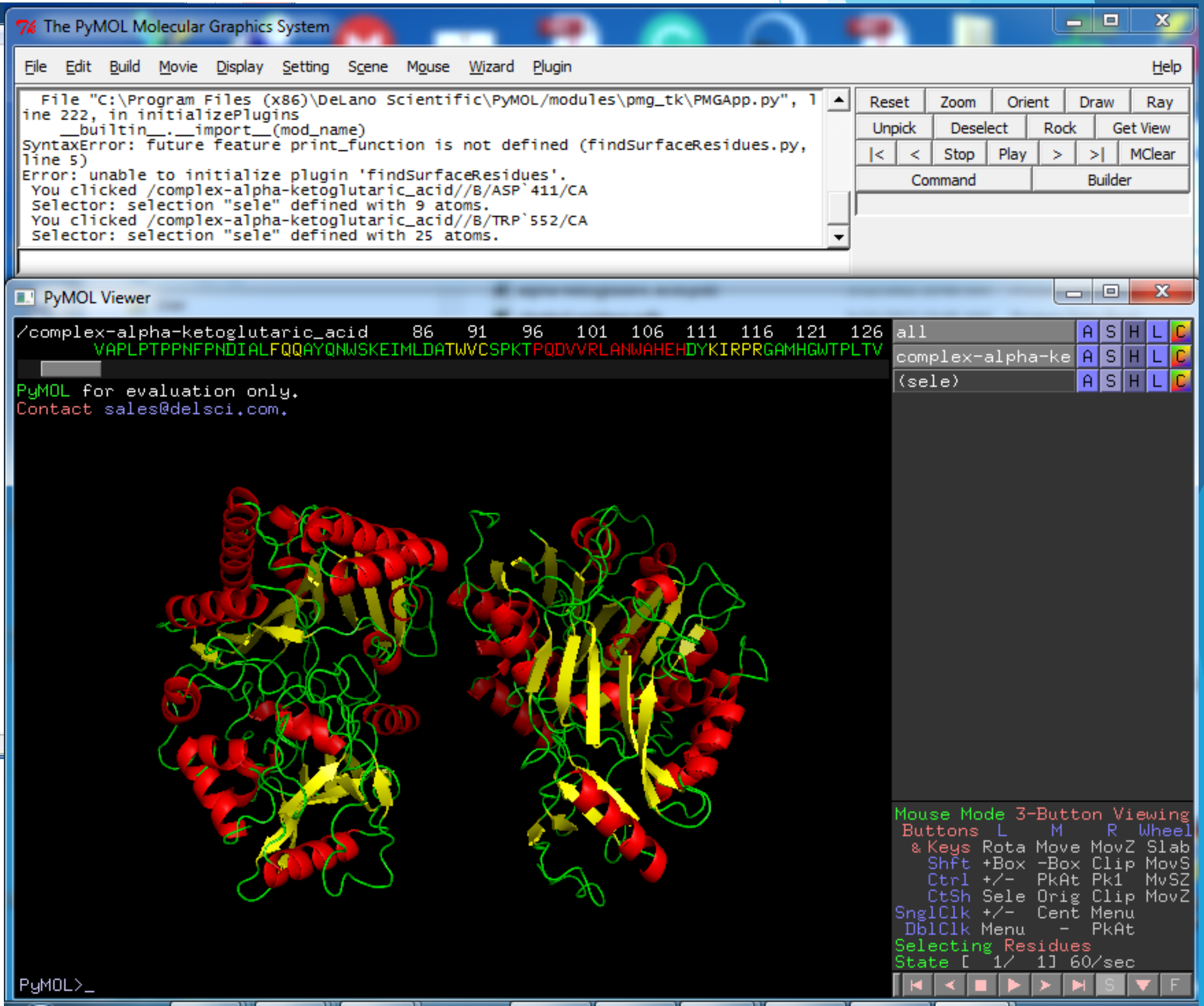
Protein Structure Databases

http://www.rcsb.org/pdb/

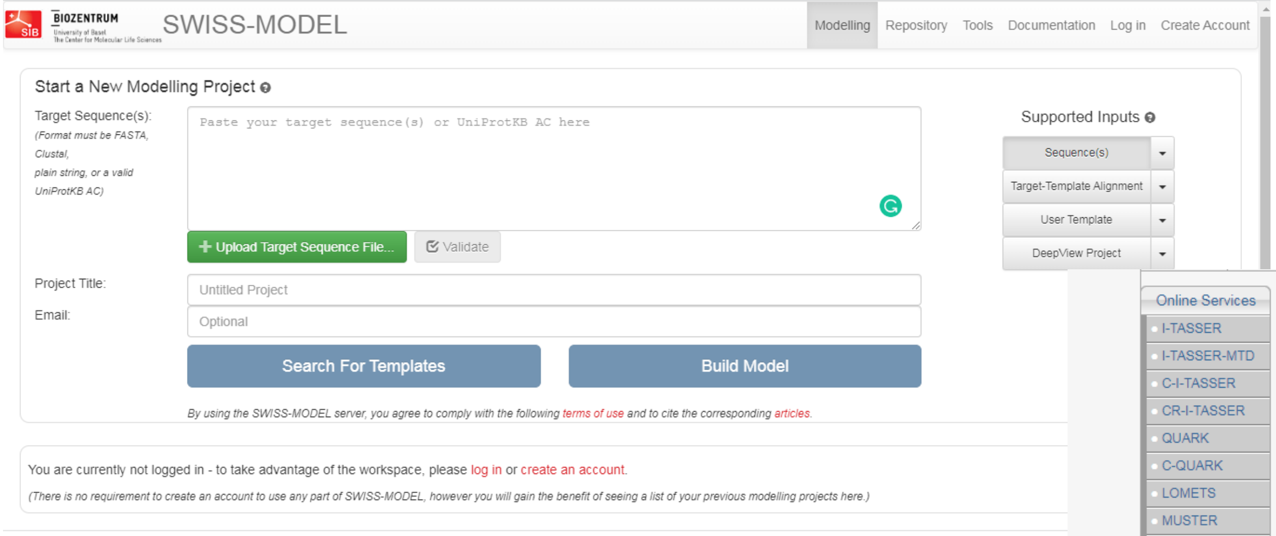


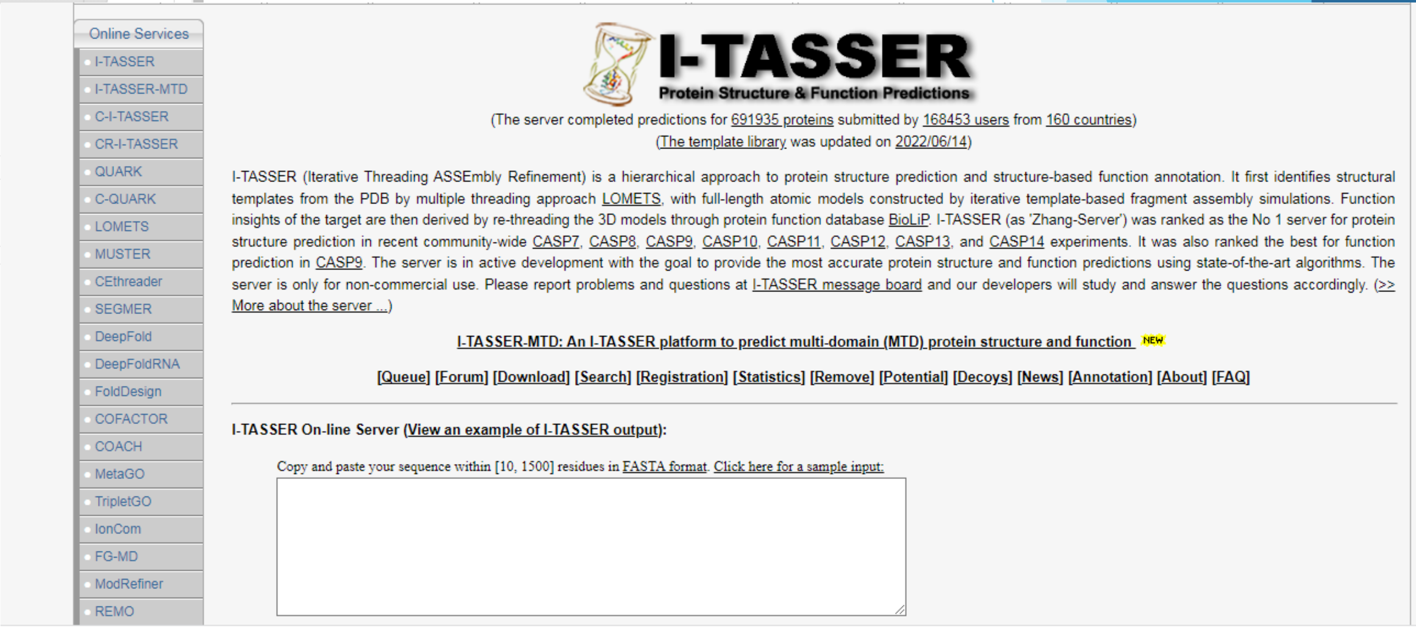
How to visualize tertiary protein structure Pymol(https://pymol.org/2/#opensource) , Chimera(https://www.cgl.ucsf.edu/chimera/download.html)



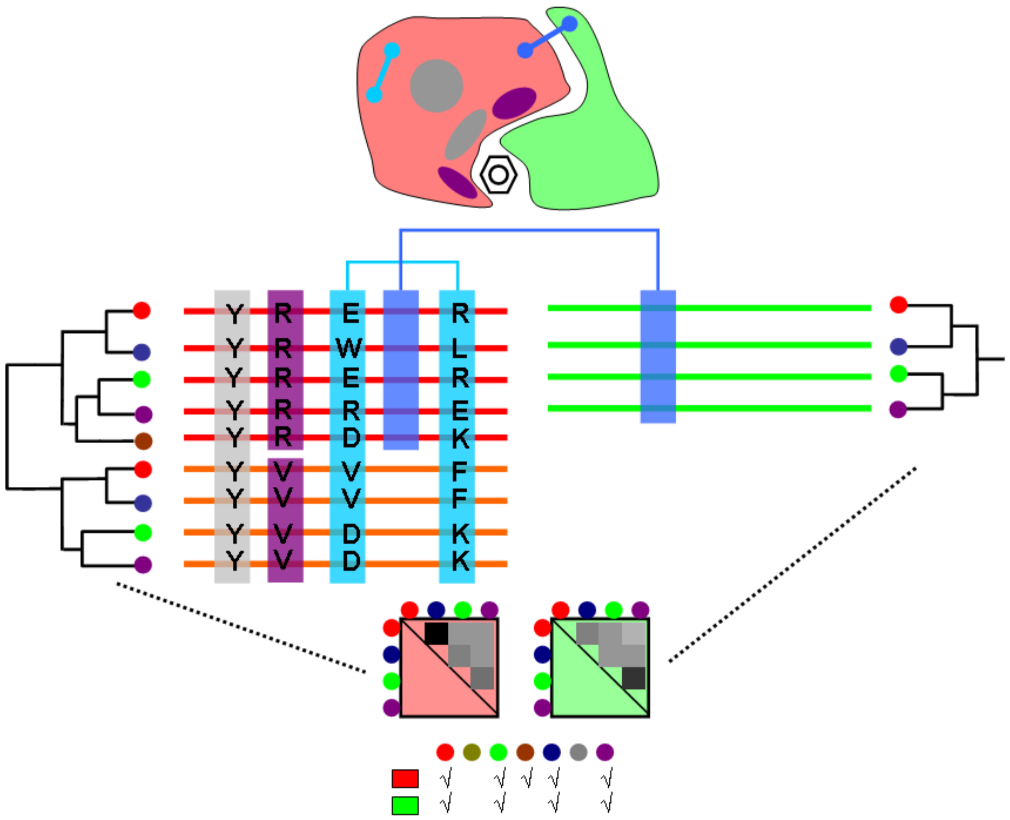


Homology modeling
swiss model(https://swissmodel.expasy.org/)
I-tasser(https://zhanggroup.org/I-TASSER/)





Mutational strategy



	C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W	
C	**9**																				**C**
S	-1	**4**																			**S**
T	-1	1	**5**																		**T**
P	-3	-1	-1	**7**																	**P**
A	0	1	0	-1	**4**																**A**
G	-3	0	-2	-2	0	**6**															**G**
N	-3	1	0	-2	-2	0	**6**														**N**
D	-3	0	-1	-1	-2	-1	1	**6**													**D**
E	-4	0	-1	-1	-1	-2	0	2	**5**												**E**
Q	-3	0	-1	-1	-1	-2	0	0	2	**5**											**Q**
H	-3	-1	-2	-2	-2	-2	1	-1	0	0	**8**										**H**
R	-3	-1	-1	-2	-1	-2	0	-2	0	1	0	**5**									**R**
K	-3	0	-1	-1	-1	-2	0	-1	1	1	-1	2	**5**								**K**
M	-1	-1	-1	-2	-1	-3	-2	-3	-2	0	-2	-1	-1	**5**							**M**
I	-1	-2	-1	-3	-1	-4	-3	-3	-3	-3	-3	-3	-3	1	**4**						**I**
L	-1	-2	-1	-3	-1	-4	-3	-4	-3	-2	-3	-2	-2	2	2	**4**					**L**
V	-1	-2	0	-2	0	-3	-3	-3	-2	-2	-3	-3	-2	1	3	1	**4**				**V**
F	-2	-2	-2	-4	-2	-3	-3	-3	-3	-3	-1	-3	-3	0	0	0	-1	**6**			**F**
Y	-2	-2	-2	-3	-2	-3	-2	-3	-2	-1	2	-2	-2	-1	-1	-1	-1	3	**7**		**Y**
W	-2	-3	-2	-4	-3	-2	-4	-4	-3	-2	-2	-3	-3	-1	-3	-2	-3	1	2	**11**	**W**
	C	**S**	**T**	**P**	**A**	**G**	**N**	**D**	**E**	**Q**	**H**	**R**	**K**	**M**	**I**	**L**	**V**	**F**	**Y**	**W**	

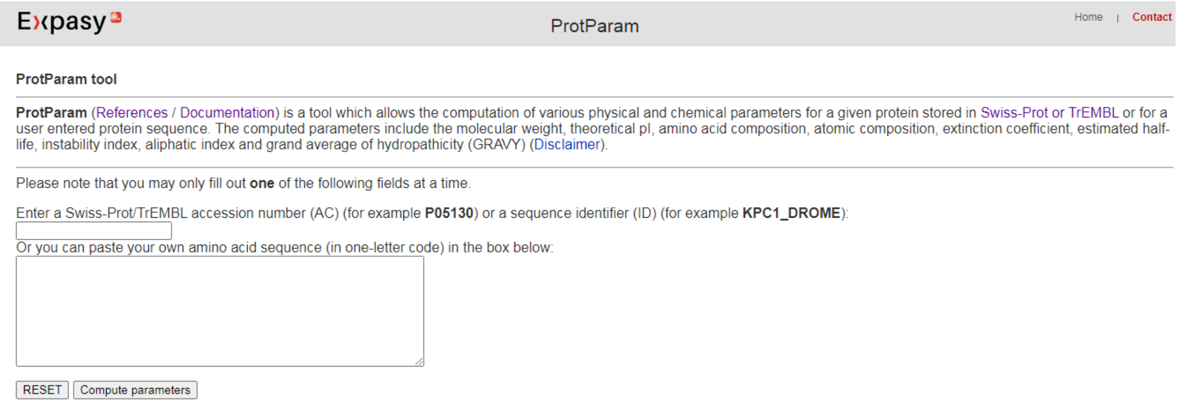
(a)

Target sequence	A	L	E	D	N
Functionally Identical or Related Enzymes (property A proteins)	H	L	E	P	L
Sequence-related Proteins (~A proteins)	L	L	P	D	L
Score	-1	0	6	-7	0

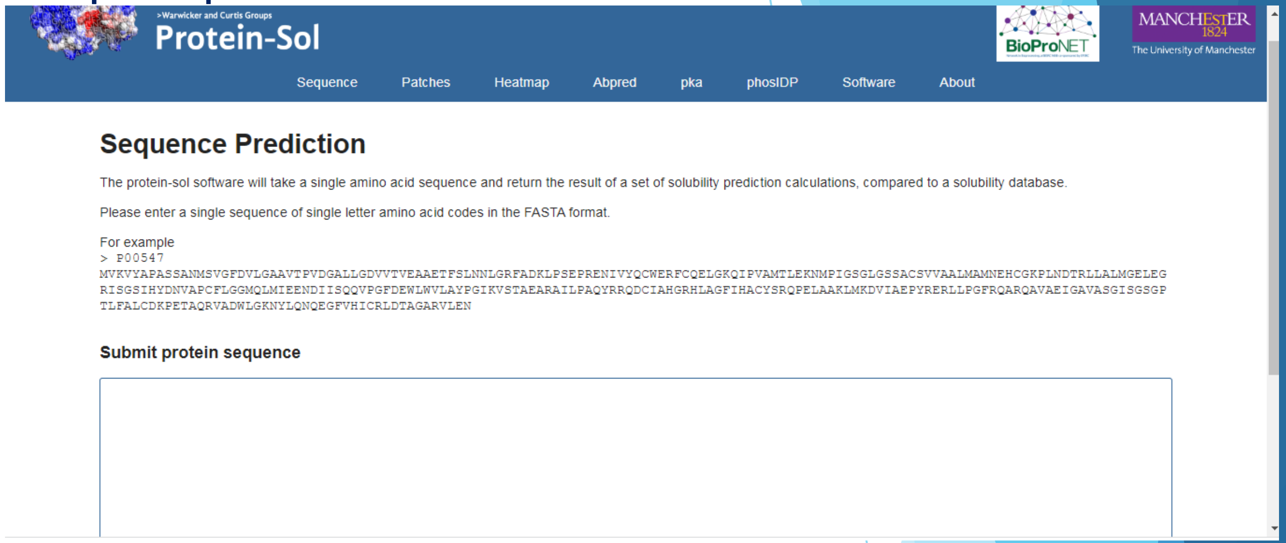
(b)

Physicochemical assessment analysis

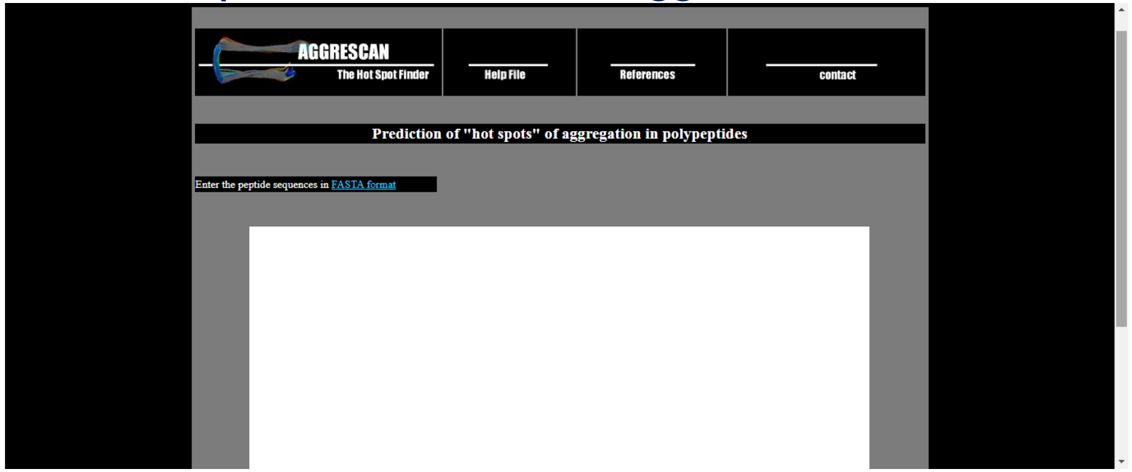
https://web.expasy.org/protparam/



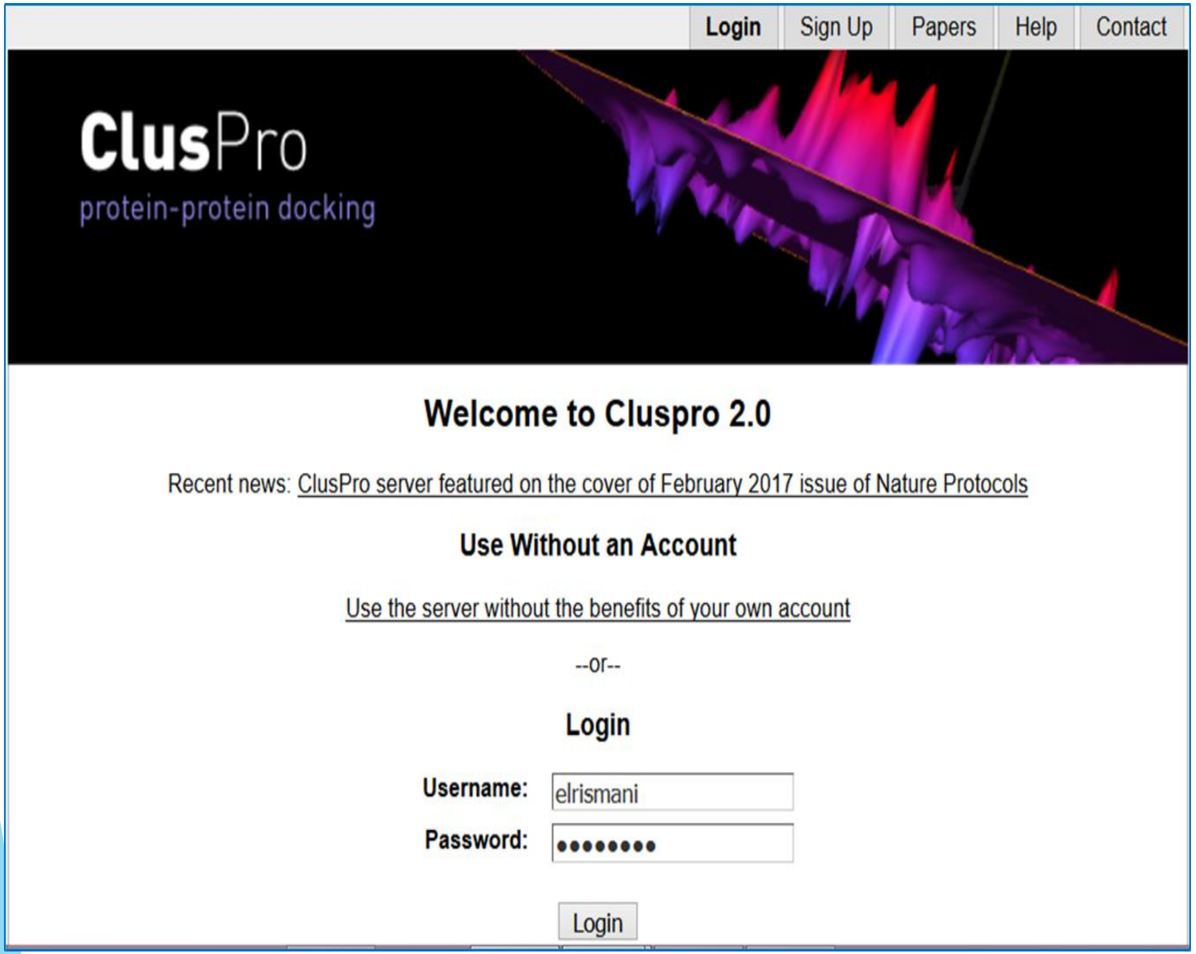
https://protein-sol.manchester.ac.uk/

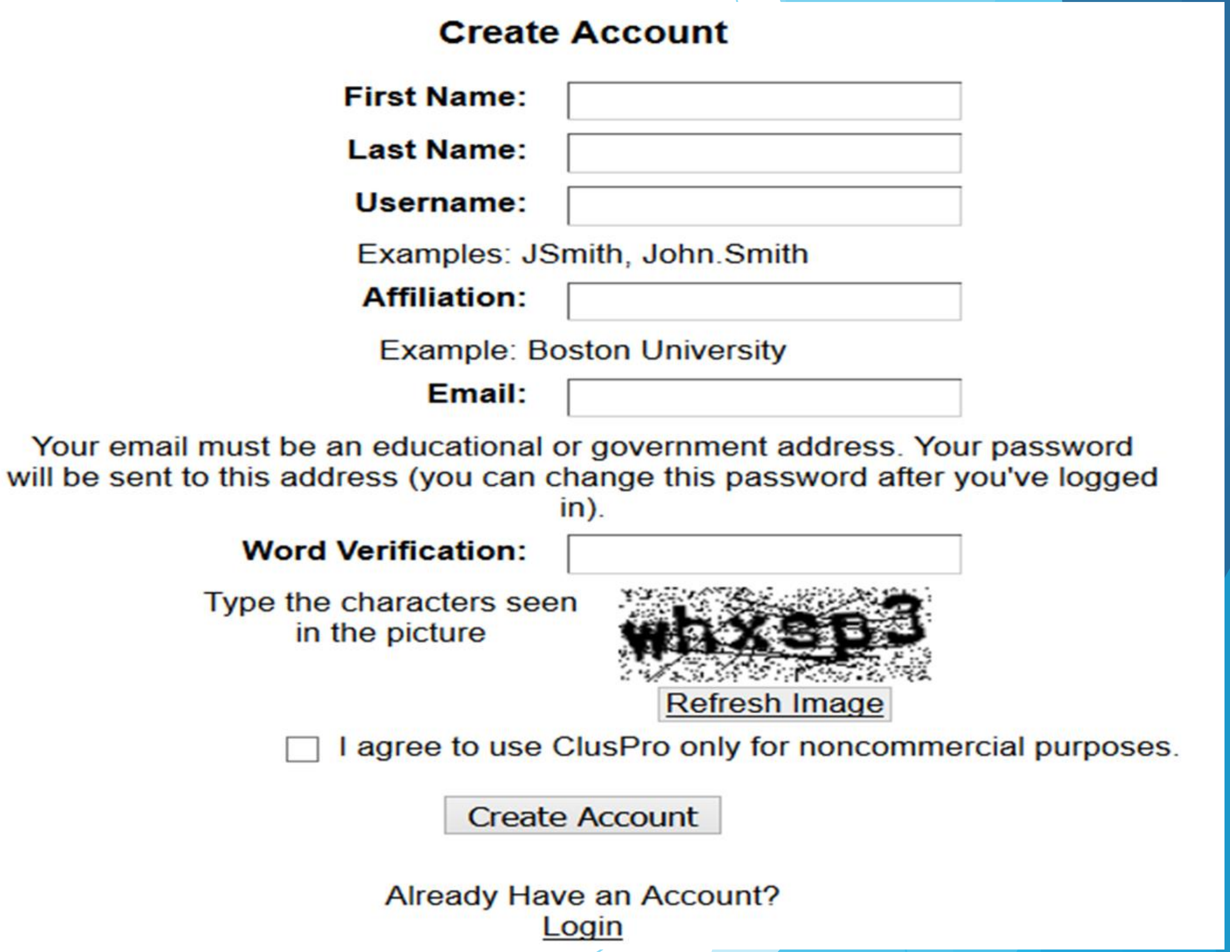


http://bioinf.uab.es/aggrescan/

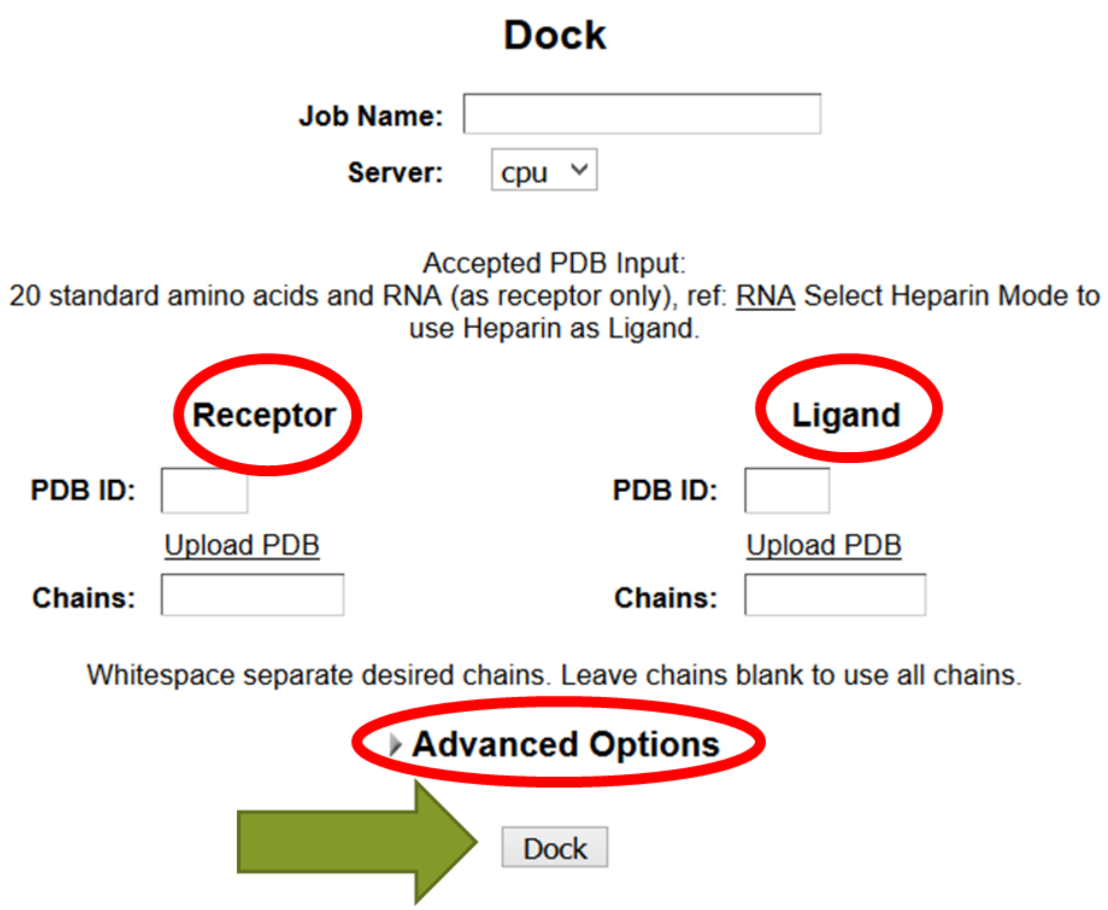


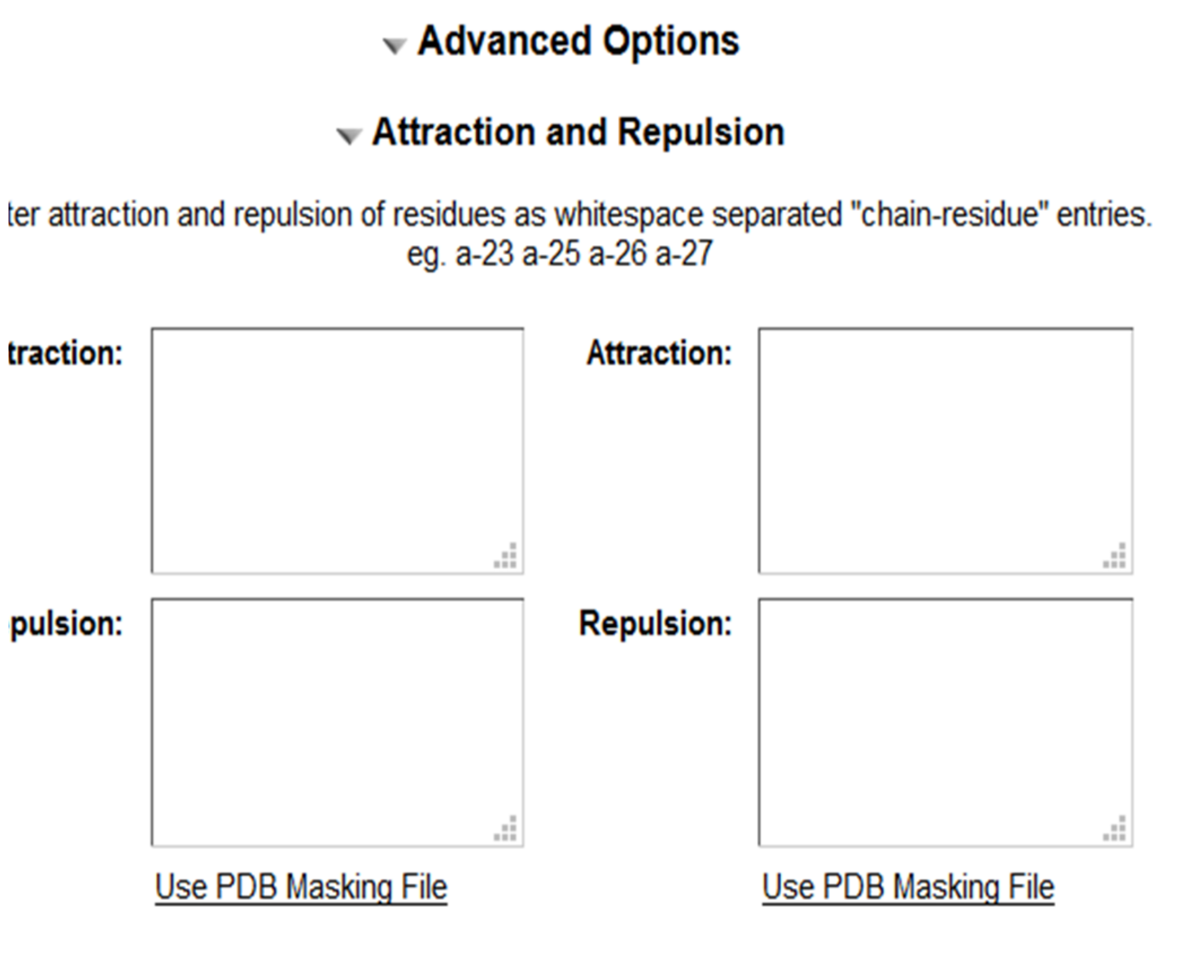
Molecular docking
Cluspro(https://cluspro.bu.edu/login.php)



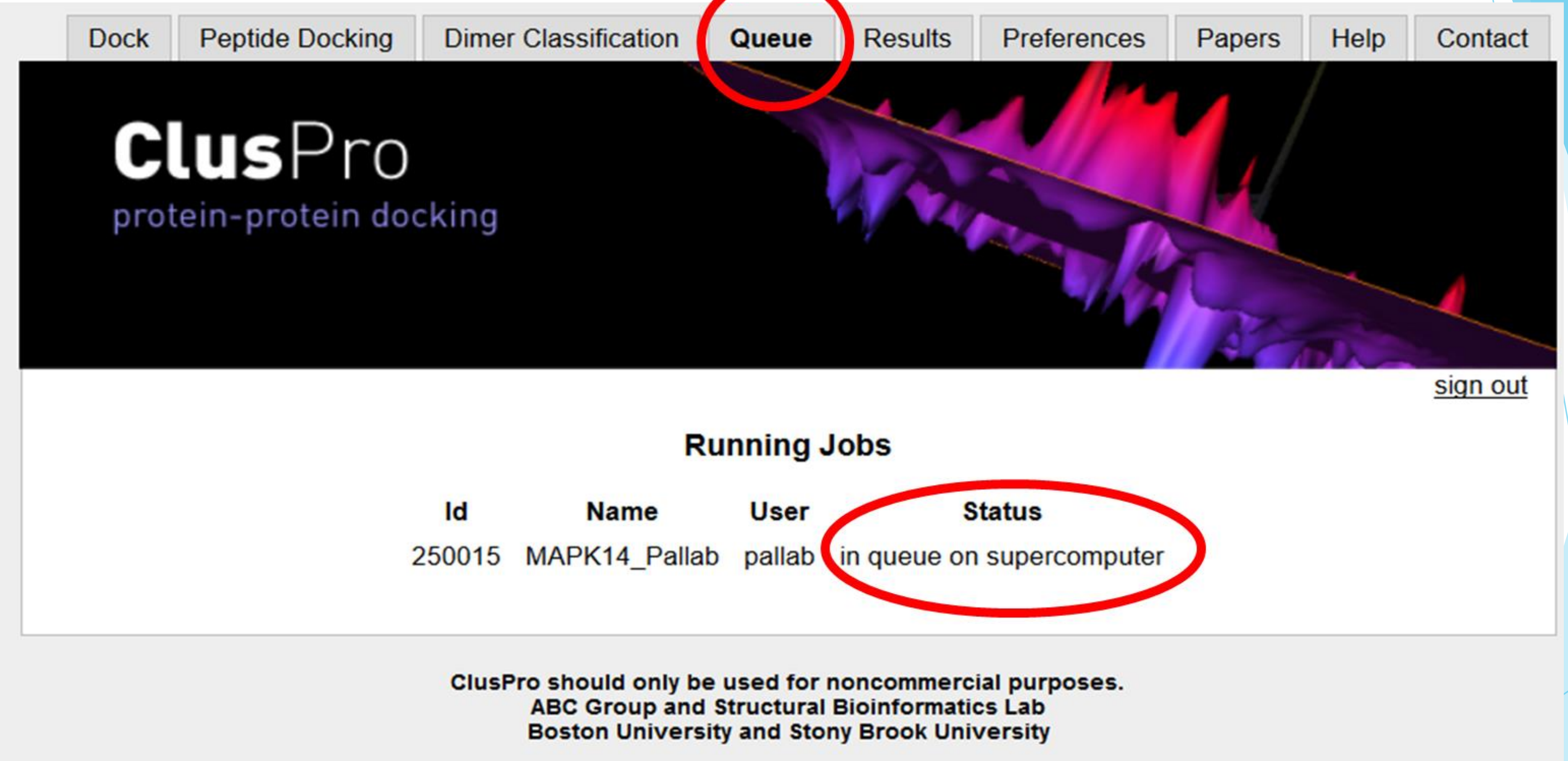


Molecular docking Cluspro





Molecular docking Cluspro



Molecular docking Cluspro

View Model Scores

Download all Models for all Coefficients

Balanced | Electrostatic-favored | Hydrophobic-favored | VdW+Elec

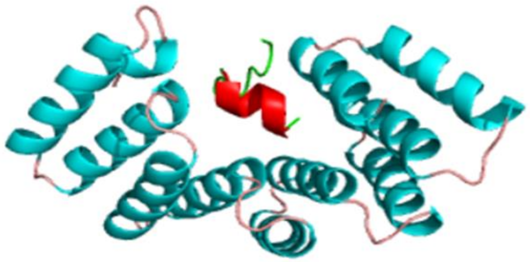
Display Models: 5

Download Displayed Models

If you use these models in a paper, please cite our papers

0

1



View Models

Balanced | Electrostatic-favored | Hydrophobic-favored | VdW+Elec

Download Model Scores for this Coefficient

Coefficient Weights

See *Kozakov et. al.* in Papers for a description of these terms

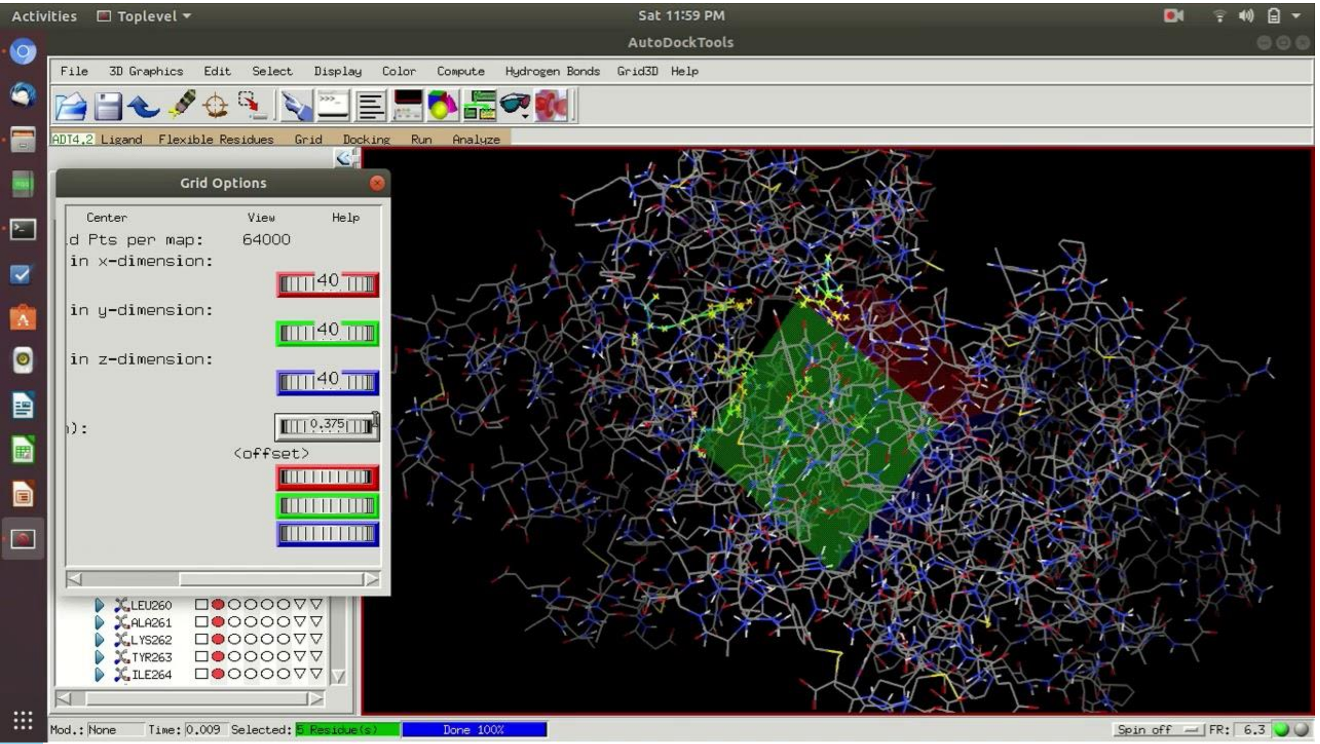
$$E = 0.40E_{rep} + -0.40E_{att} + 600E_{elec} + 1.00E_{DARS}$$

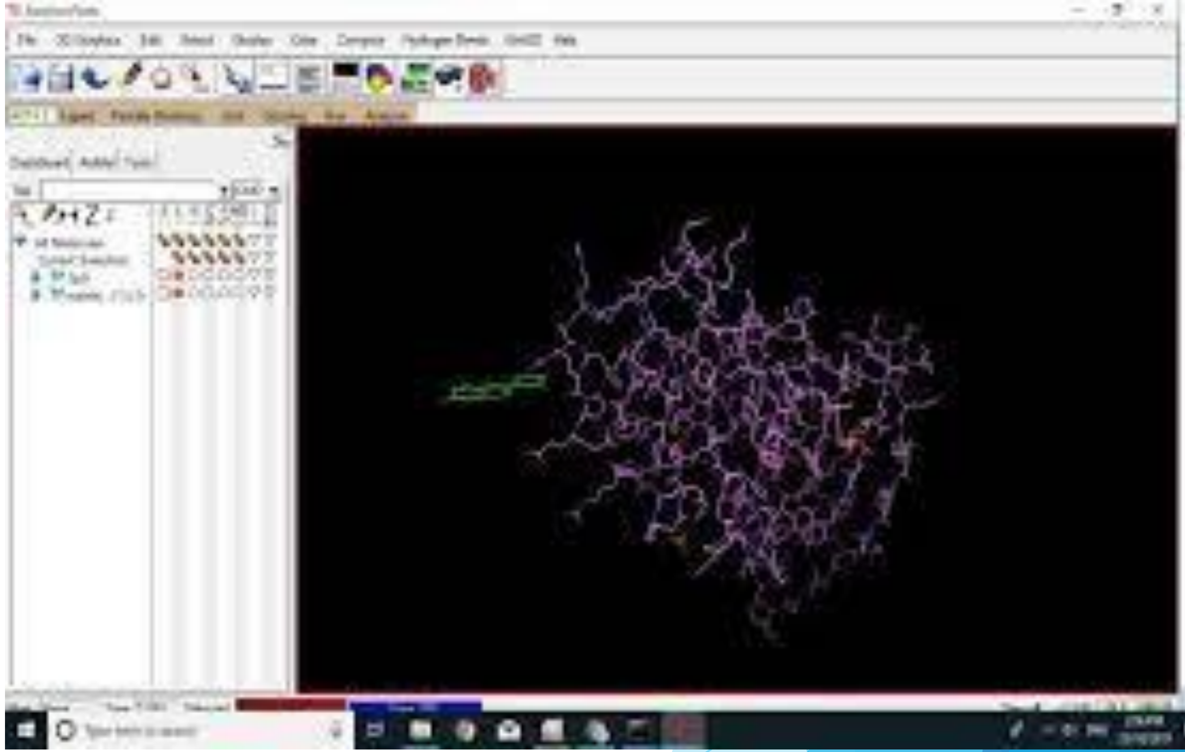
Cluster Scores

We strongly encourage you to read the FAQ related to these scores before using them.

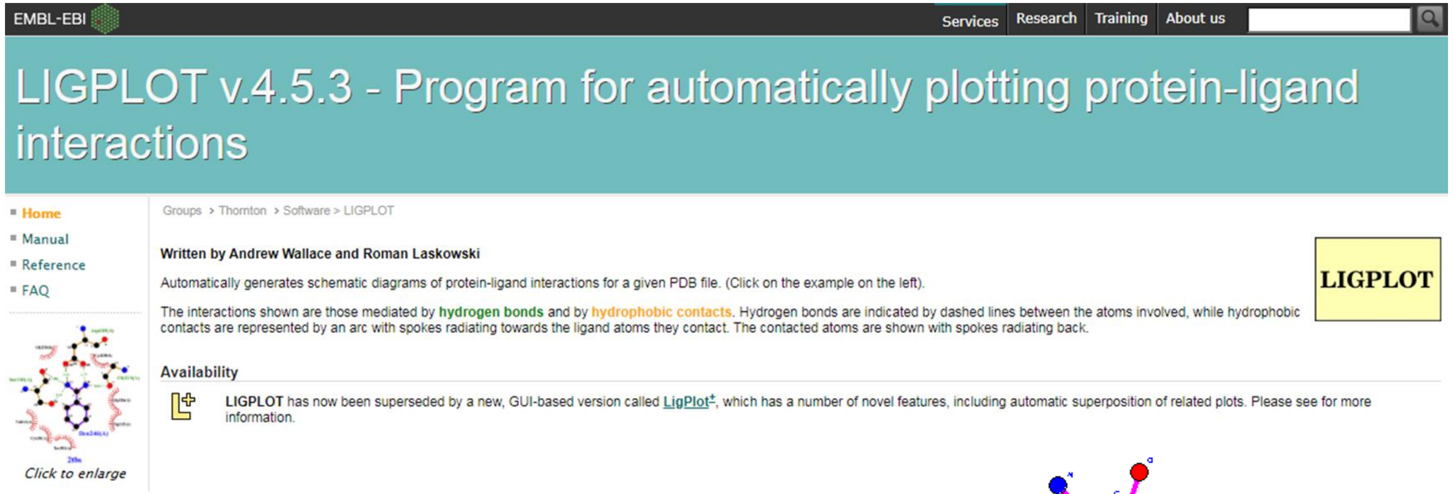
Cluster	Members	Representative	Weighted Score
0	433	Center	-847.2
		Lowest Energy	-956.6
1	322	Center	-853.3
		Lowest Energy	-959.0
2	140	Center	-842.2
		Lowest Energy	-915.7
3	53	Center	-844.7

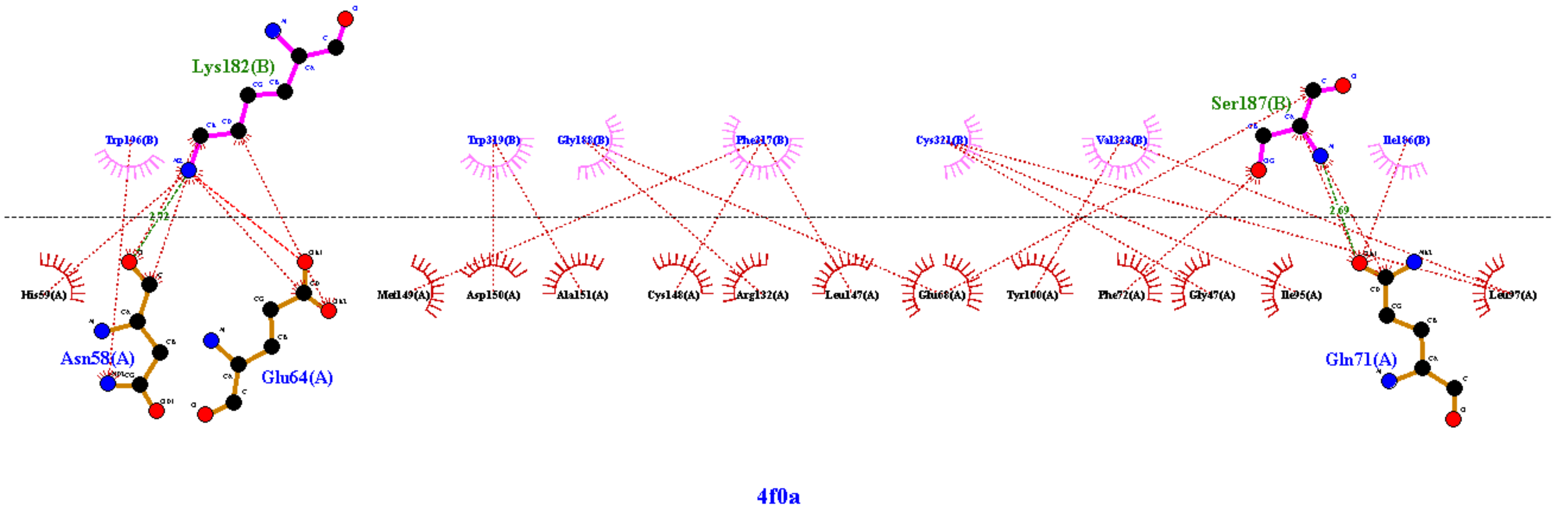
Molecular docking Autodock (https://autodock.scripps.edu/)



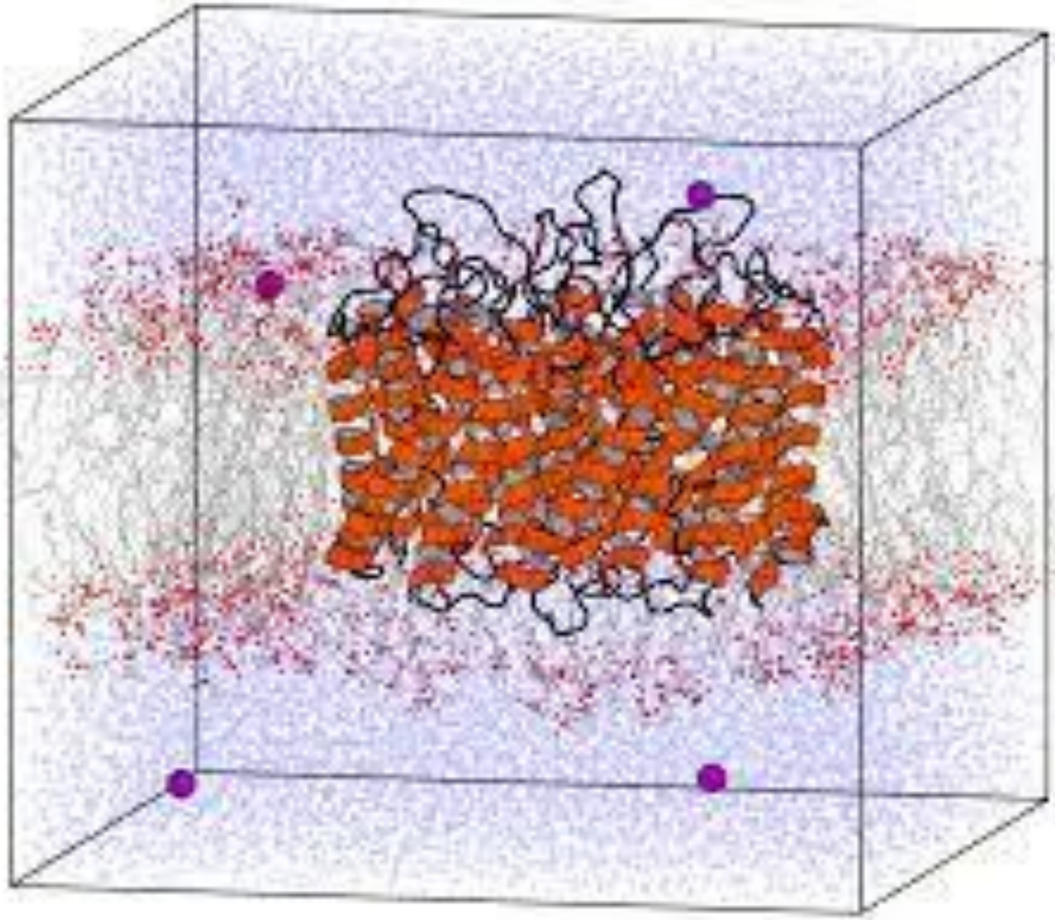


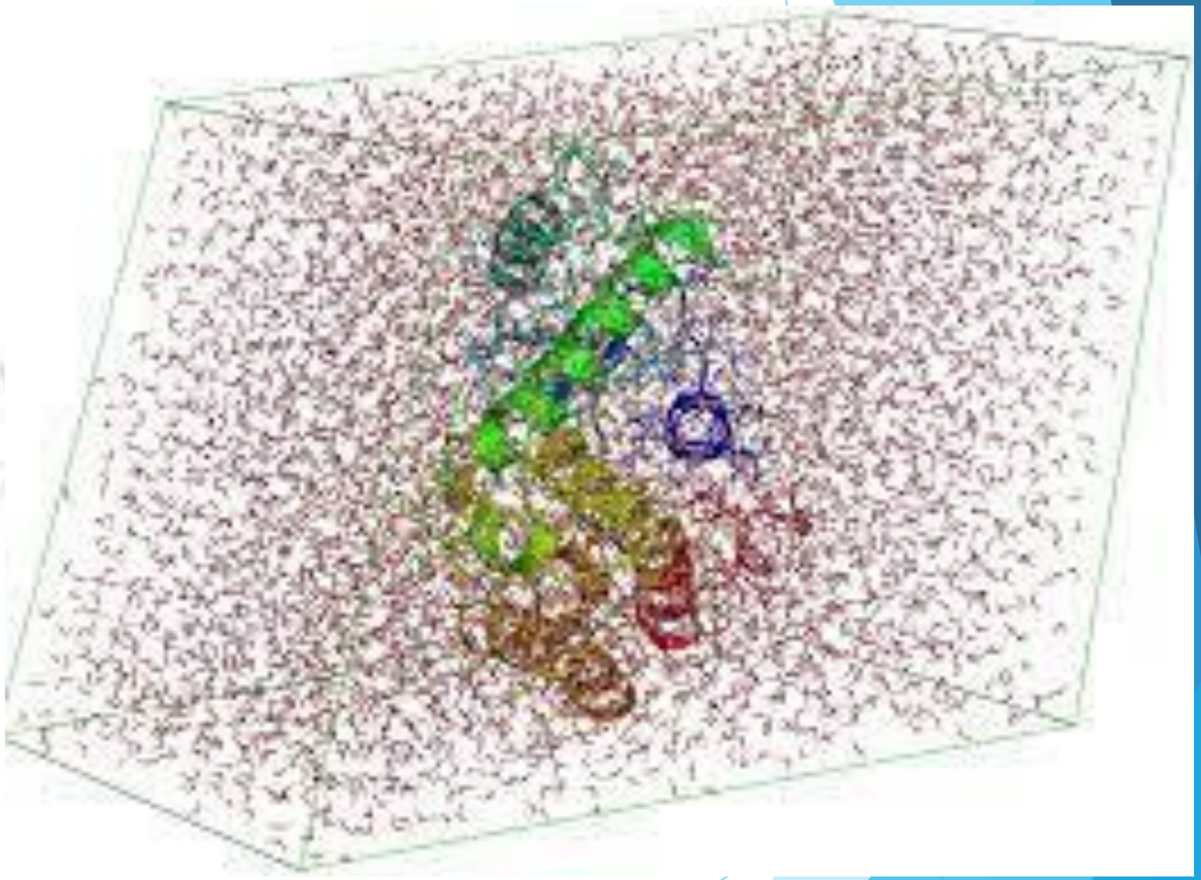
LigPLOT
https://www.ebi.ac.uk/thorntonsrv/software/LIGPLOT





Molecular dynamics simulation Gromacs(https://www.gromacs.org/)





Workflow of computational protein engineering

1. In silico analysis and design
2. Gene library construct
3. Protein display/expression
4. High throughput screening/selection
5. Validation and characterization
6. Selection the most potential protein