

Multiple alignments as a tool in protein studies and engineering

Comparative bioinformatic analysis of functionally diverse protein families can be used to:

- study structure-function relationship in proteins;
- predict key residues to be mutated in order to produce more stable and functionally diverse proteins and enzymes;
- discover and characterize novel binding sites in protein structures.

Mustguseal

Multiple Structure-Guided Sequence Alignment

- a bioinformatic **protocol** to build large alignments of functionally diverse protein families;
- a **platform of five integrated servers** to provide a user-friendly web-based interface to the Mustguseal protocol and sister methods to further study the obtained multiple alignments;
- Mustguseal can be used to build a large alignment of the *selected protein families* or superimpose a diverse set of proteins representing a *superfamily*.

Mustguseal: the Aim

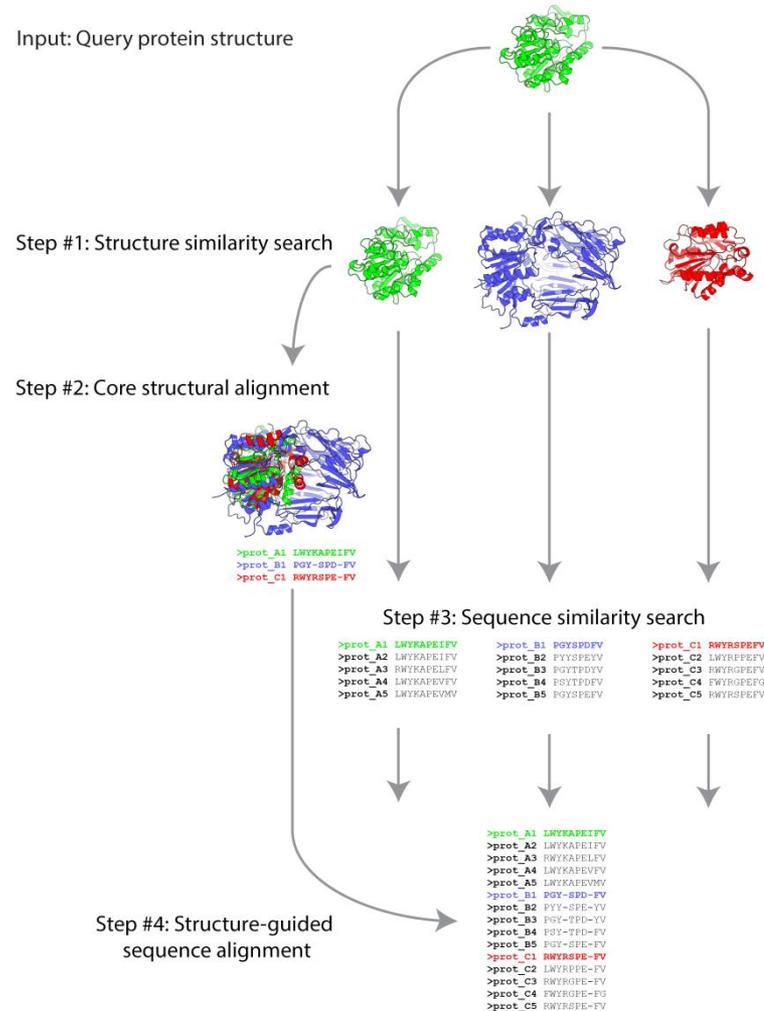
- to construct large alignments of functionally diverse protein families based on all available information about their structures and sequences in public databases;
- to automate complex bioinformatic procedures;
- to provide a freely-available user-friendly platform on the Internet for daily use in the laboratory practice.

Mustguseal: the Approach

- **structure similarity search** is implemented to collect remote evolutionary relatives, which are expected to represent different protein families;
- **sequence similarity search** is implemented to collect close evolutionary relatives - members of the corresponding families;
- a combination of **structure and sequence alignment** procedures is then implemented to build the final multiple alignment;
- multiple alignments of **thousands** of protein sequences and structures can be automatically constructed using this public web-server.

Mustguseal: the Input

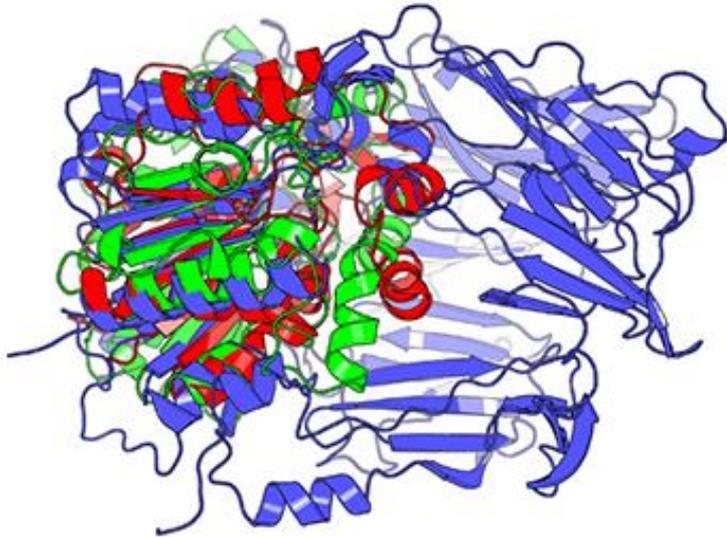
Mode 1: Submit a query protein



<https://biokinet.belozersky.msu.ru/mustguseal>

Mustguseal: the Input

User-defined core structural alignment



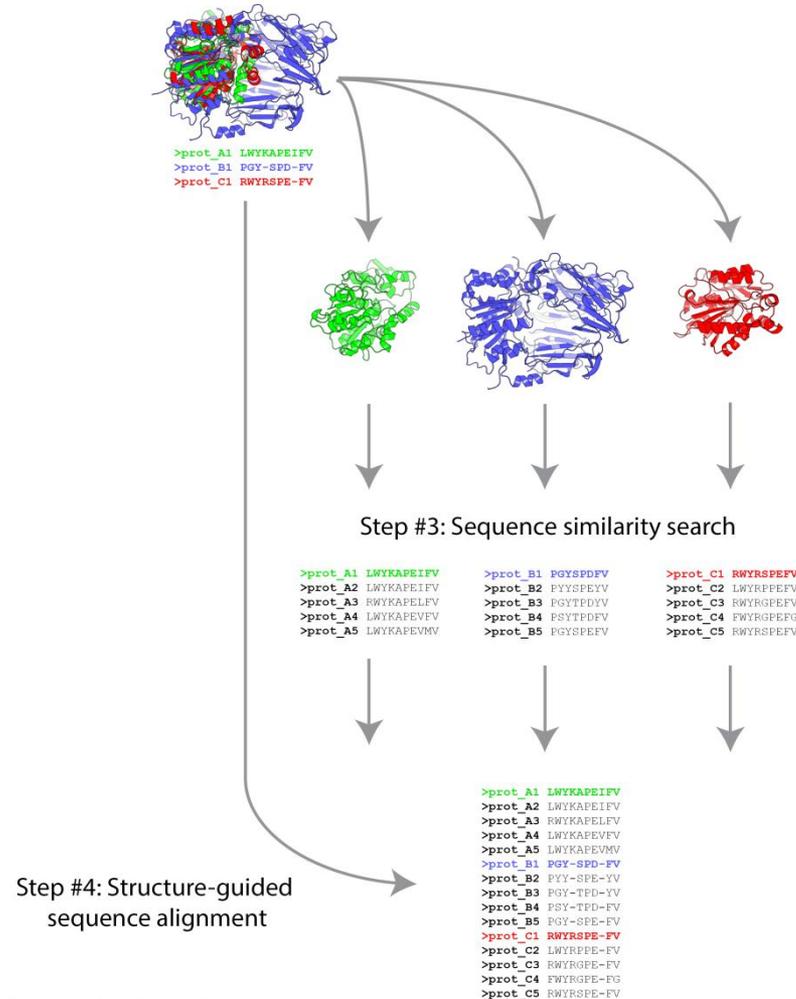
```
>prot_A1 LWYKAPEIFV  
>prot_B1 PGY-SPD-FV  
>prot_C1 RWYRSPE-FV
```

- Mustguseal can be used to build an alignment of the *selected protein families* or superimpose a large collection of proteins representing a *superfamily*;
- The scope of the final alignment is defined by the **diversity of representative proteins in the core structural alignment** which can be created on-site or submitted by the user;
- A user-defined 3D-alignment can be built from a selected set of protein structures on a local computer or a supercomputer and then submitted to the Mustguseal server in Mode 2.

Mustguseal: the Input

Mode 2: Submit a core structural alignment

Input: Core structural alignment

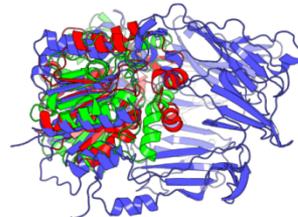


<https://biokinet.belozersky.msu.ru/mustguseal>

Mustguseal: the Input

Mode 3: Submit a core structural alignment and results of sequence similarity search

Input: Core structural alignment



```
>prot_A1 LWYKAPEIFV  
>prot_B1 PGY-SPD-FV  
>prot_C1 RWYRSPEFV
```

Input: Sequence similarity search results

```
>prot_A1 LWYKAPEIFV  
>prot_A2 LWYKAPEIFV  
>prot_A3 RWYKAPELFV  
>prot_A4 LWYKAPEFV  
>prot_A5 LWYKAPEVMV
```

```
>prot_B1 PGYSPDFV  
>prot_B2 PYYSPPEYV  
>prot_B3 PGYTPDYV  
>prot_B4 PSYTPDFV  
>prot_B5 PGYSPEFV
```

```
>prot_C1 RWYRSPEFV  
>prot_C2 LWYRPPEFV  
>prot_C3 RWYRGPEFV  
>prot_C4 FWYRGPEFG  
>prot_C5 RWYRSPEFV
```



Step #4: Structure-guided
sequence alignment

```
>prot_A1 LWYKAPEIFV  
>prot_A2 LWYKAPEIFV  
>prot_A3 RWYKAPELFV  
>prot_A4 LWYKAPEFV  
>prot_A5 LWYKAPEVMV  
>prot_B1 PGY-SPD-FV  
>prot_B2 PYY-SPE-YV  
>prot_B3 PGY-TPD-YV  
>prot_B4 PSY-TPD-FV  
>prot_B5 PGY-SPE-FV  
>prot_C1 RWYRSPE-FV  
>prot_C2 LWYRPPE-FV  
>prot_C3 RWYRGPE-FV  
>prot_C4 FWYRGPE-FG  
>prot_C5 RWYRSPE-FV
```

<https://biokinet.belozersky.msu.ru/mustguseal>

User may alter the results of sequence similarity search (i.e. choose different proteins or change the way sequences are being superimposed within each group) and submit all building blocks of the alignment as new task in Mode 3

Mustguseal: the Output

- **Download section**

Download the final alignment and supplementary output to your computer;

- **Analysis section**

View basic alignment statistics and use interactive on-line tools for sequence and structure analysis.

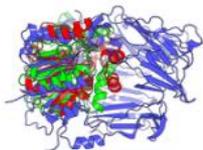
Mustguseal: the Output Download section

Download section

Primary output

Download the final alignment	FINAL_A-a14ow8b47q5e49.tar.gz	124 KB	<pre> >prot_A1 LWYKAPLIFV >prot_A2 LWYKAPLIFV >prot_A3 FWYKAPLIFV >prot_A4 LWYKAPLIFV >prot_A5 LWYKAPLIFV >prot_B1 PGY-SPD-FV >prot_B2 PYY-SPE-YV >prot_B3 PGY-TPD-YV >prot_B4 PSY-TPD-FV >prot_B5 DGY-SPE-FV >prot_C1 RWYRQPE-FV >prot_C2 LWYRQPE-FV >prot_C3 RWYRQPE-FV >prot_C4 FWYRQPE-FV >prot_C5 RWYRQPE-FV </pre>	Download
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Supplementary output

Download the core structural alignment	strcore_A-a14ow8b47q5e49.tar.gz	4.0 MB	 <pre> >prot_A1 LWYKAPLIFV >prot_B1 PGY-SPD-FV >prot_C1 RWYRQPE-FV </pre>	Download
Download structure similarity search results	strsearch_A-a14ow8b47q5e49.tar.gz	11.4 MB		Download
Download sequence similarity search results	seqsearch_A-a14ow8b47q5e49.tar.gz	36.9 MB	<pre> >prot_A1 LWYKAPLIFV >prot_A2 LWYKAPLIFV >prot_A3 LWYKAPLIFV >prot_A4 LWYKAPLIFV >prot_A5 LWYKAPLIFV >prot_B1 PGY-SPD-FV >prot_B2 PYY-SPE-YV >prot_B3 PGY-TPD-YV >prot_B4 PSY-TPD-FV >prot_B5 DGY-SPE-FV >prot_C1 RWYRQPE-FV >prot_C2 LWYRQPE-FV >prot_C3 RWYRQPE-FV >prot_C4 FWYRQPE-FV >prot_C5 RWYRQPE-FV </pre>	Download

Mustguseal: the Output

Analysis section: Basic alignment statistics

Basic alignment statistics

General alignment statistics:

Total number of proteins in the final alignment: 5211

Total number of columns in the final alignment: 3831

Protein length statistics:

Protein length average: 311 aa

Protein length minimum: 223 aa

Protein length maximum: 463 aa

Alignment coverage statistics:

Number of columns with at most 0% of gaps:	13	(4% of the average protein length)
Number of columns with at most 5% of gaps:	230	(74% of the average protein length)
Number of columns with at most 30% of gaps:	253	(81% of the average protein length)
Number of columns with at most 50% of gaps:	275	(88% of the average protein length)

Column conservation statistics:

Number of columns with conservation index at least 100% :	0	(0% of the average protein length)
Number of columns with conservation index at least 95% :	3	(1% of the average protein length)
Number of columns with conservation index at least 75% :	14	(5% of the average protein length)
Number of columns with conservation index at least 50% :	69	(22% of the average protein length)

The conservation index for each column is calculated as the occurrence of the most frequent amino acid

Mustguseal: the Output

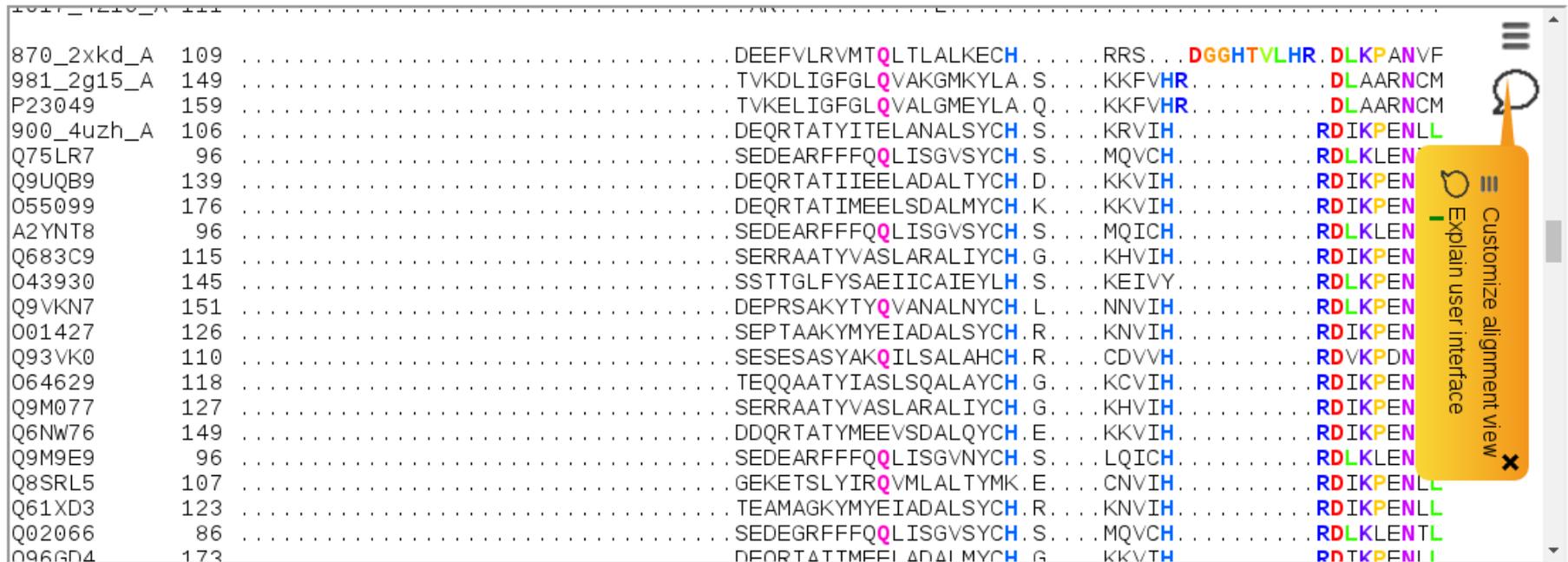
Analysis section: Sequence analysis of the Final Alignment

Sequence analysis of the Final Alignment

This sub-section implements the Strap application to provide you with a tool for the on-site analysis and annotation of your alignment. Allow some time for loading of the content and then follow the popup hints. The alignment is initially displayed using default settings and can be modified with the graphical user interfaces. In particular, you can change the color scheme, zoom and wrapping options by pressing the button in the upper right corner of the screen and then pressing the "Toolbar" icon. Please note that Strap removes all gaps before the first amino acid and after the last amino acid of each protein sequence in the alignment. Interactivity is implemented in HTML5, a language native to web browsers, therefore no plugins nor Java are required. For additional information and troubleshooting please see the *Strap homepage*.

Full screen

Press **Full screen** to enter the full screen mode



The screenshot displays a sequence alignment in a web browser. The alignment consists of 20 rows of protein sequences, each with a residue number on the left. The sequences are color-coded by amino acid type. A toolbar is visible in the top right corner, featuring a hamburger menu icon and a speech bubble icon. A yellow popup menu is open, listing options: 'Customize alignment view', 'Explain user interface', and a close button (X).

870_2xkd_A	109	DEEFVLRVMTQLTLALKECH	RRS	DGGHTVLHR	DLKPANVF
981_2g15_A	149	TVKDLIGFGLQVAKGMKYLA	KKFVHR		DLAARNCM
P23049	159	TVKELIGFGLQVALGMEYLA	KKFVHR		DLAARNCM
900_4uzh_A	106	DEQRTATYITELANALSYCH	KRVIH		RDIKPENLL
Q75LR7	96	SEDEARFFFQQLISGVSYCH	MQVCH		RDLKLEN
Q9UQB9	139	DEQRTATIEELADALTYCH	KKVIH		RDIKPEN
O55099	176	DEQRTATIMEELSDALMYCH	KKVIH		RDIKPEN
A2YNT8	96	SEDEARFFFQQLISGVSYCH	MQICH		RDLKLEN
Q683C9	115	SERRAATYVASLARALIYCH	KHVIH		RDIKPEN
O43930	145	SSTTGLFYSAEICAIEYLH	KEIVY		RDLKLEN
Q9VKN7	151	DEPRSAKYTYQVANALNYCH	NNVIH		RDLKLEN
O01427	126	SEPTAAKMYEADALSCH	KNVIH		RDIKPEN
Q93VK0	110	SESESASYAKQILSALAHCH	CDVVH		RDVKPDN
O64629	118	TEQQAATYIASLSQALAYCH	KCVIH		RDIKPEN
Q9M077	127	SERRAATYVASLARALIYCH	KHVIH		RDIKPEN
Q6NW76	149	DDQRTATYMEEVSDALQYCH	KKVIH		RDIKPEN
Q9M9E9	96	SEDEARFFFQQLISGVNYCH	LQICH		RDLKLEN
Q8SRL5	107	GEKETSLYIRQVMLALTYMK	CNVIH		RDIKPENLL
Q61XD3	123	TEAMAGKMYEADALSCH	KNVIH		RDIKPENLL
Q02066	86	SEDEGRFFFQQLISGVSYCH	MQVCH		RDLKLENTL
O96GD4	173	DEQRTATIMEELADALMYCH	KKVTH		RDIKPENLL

Mustguseal: the Output

Analysis section: Structure-based annotation of the Final Alignment

Structure-based annotation of the Final Alignment

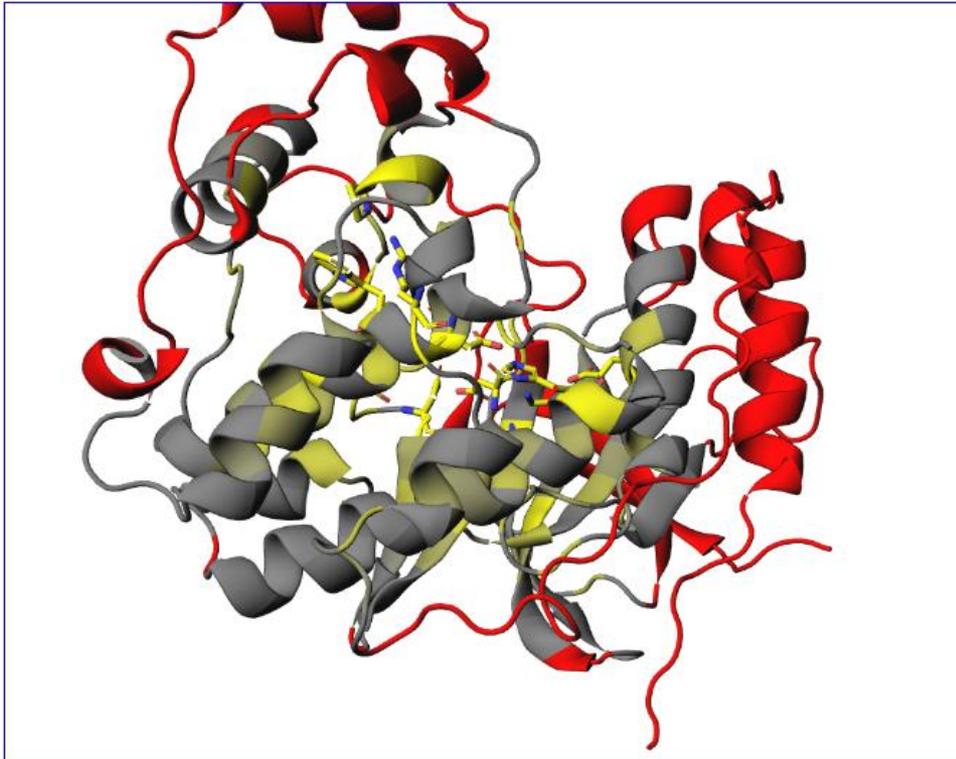
This sub-section implements the JSMol application to provide you with a tool for the structure-based analysis of your alignment. Each representative protein structure, which was used to build the core structural alignment, has been annotated according to the final alignment. See the legend below the 3D-viewer. Choose a protein from the dropdown menu and allow some time for loading of the content. The first protein in the list is shown by default. Left-click-and-hold and then move your mouse to rotate the structure, scroll mouse wheel to zoom in and out, right-click for more options. Interactivity is implemented in HTML5, a language native to web browsers, therefore no plugins nor Java are required. For additional information and troubleshooting please see the [JSMol homepage](#).

Select a protein from the dropdown menu:

0_1p38_A

Choose a protein from the dropdown menu and allow some time for loading of the content.

Showing annotation based on protein 0_1p38_A:



The selected protein structures are annotated according to basic statistics of the final alignment:

- The most conserved residues are colored in yellow and shown as sticks;
- The gradient paint of the protein backbone corresponds to sequence conservation in the corresponding position (yellow – highly conserved, grey – variable);
- Red paint corresponds to positions that contain more than 5% of gaps in the final alignment.

Mustguseal: File Sharing

Your task #A-rmw94xafb83ta8 has been submitted.
Redirecting ...

- At the time of submission a new task is assigned a unique 16-symbol access code – **TaskID**;
- TaskID can be used to access results on the Mustguseal server at any time;
- TaskID can be sent to a colleague to share the results.

Mustguseal: File Sharing

Results for Task #A-vijeth5nwrb2f

Analysis

Log

Delete

Submit a new task

At any time the submission can be retracted by pressing the "**Cancel**" / "**Delete**" button at the top of every page - this will stop the task processing and delete the input data as well as any intermediate data and results that have been created.

Mustguseal: Security

Security and sharing

Warning! It seems that your connection is not secure. Switch to https now to encrypt the traffic between you and the server:
<https://zeus.cmm.msu.ru/>.

Allow access to task files by IP address:

No Yes

93.180.63.98

Allow access to task files by password:

No Yes

Mustguseal offers the following security features to protect your data:

- Data transfer to and from the server can be **encrypted** with a signed certificate using HTTPS protocol;
- **IP-based authentication** can be installed to restrict access to the input data and the results;
- **Password-based authentication** can be installed to restrict access to the input data and the results.

The Mustguseal publication

Suplatov D.A. et al. (2018) *Bioinformatics*,
[10.1093/bioinformatics/btx831](https://doi.org/10.1093/bioinformatics/btx831).

Supplementary Data are available [from the authors](#) or
at *Bioinformatics* online

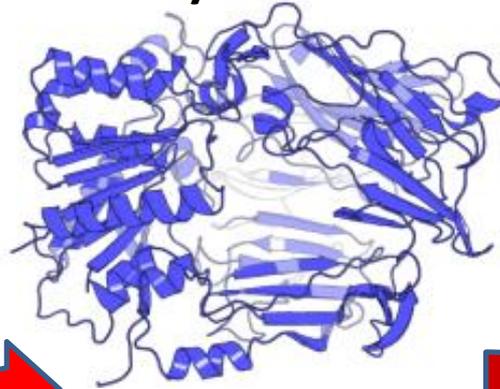
Please read the "Examples" section in the Supplementary Data file for a quick summary of the Mustguseal's capabilities. Please read "The Protocol", "The Input Modes", and "The Parameters" sections in the Supplementary Data file for an overview of the parameters selection.

Advanced tools to further study the Mustguseal alignment

The final alignment of protein families can be further submitted to sister services of Mustguseal to analyze **conserved, subfamily-specific** and **co-evolving** residues at studying a protein function and regulation, designing improved enzyme variants for practical application and selective modulators of enzyme functional properties.

The key concept

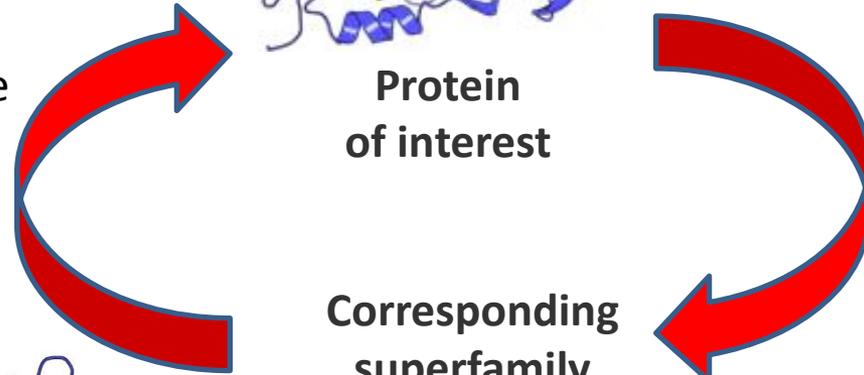
is to study the structure-function relationship of a particular protein by systematic bioinformatic analysis of the corresponding superfamily



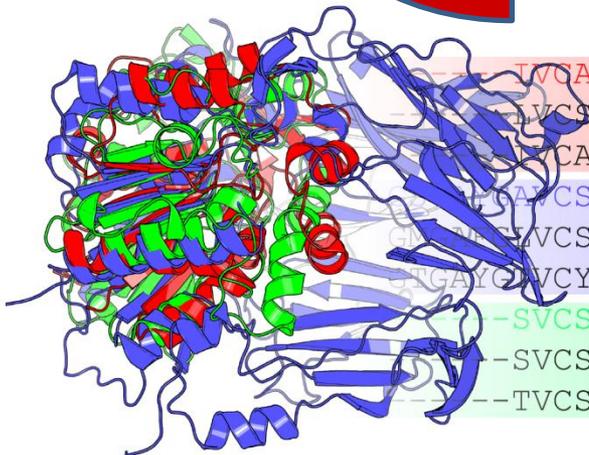
Protein
of interest

Collection and analysis
of all the available
sequence and structural
data corresponding to
the superfamily

Corresponding
superfamily



Conclusions regarding the
sequence/structure-
function relationship in
the particular protein



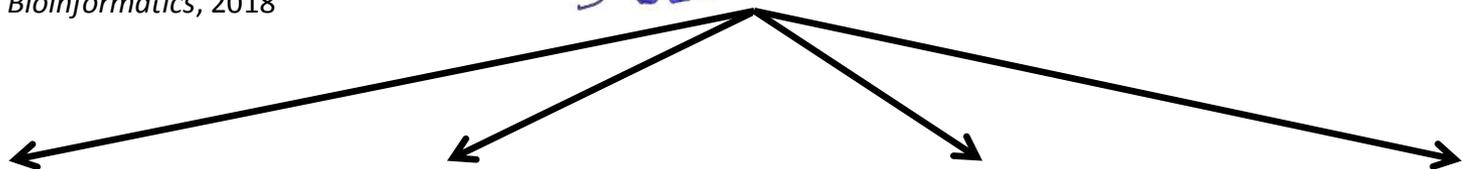
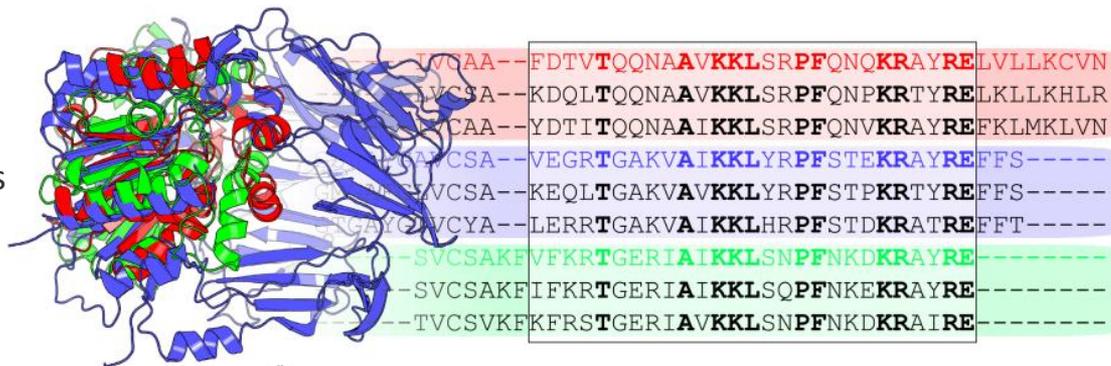
```
---IVGAA--FDTVTQQNAAVKKLSRPFQNQKRAYRELVLLKCVN
---IVCSA--KDQLTQQNAAVKKLSRPFQNPKRTYRELKLLKHLR
---IVCAA--YDTITQQNAAIKKLSRPFQNVKRAYREFKLMKLVN
---IVCSA--VEGRTGAKVAIKKLYRPFSTEKRAYREFFS-----
---IVCSA--KEQLTGAKVAVKKLYRPFSTPKKRTYREFFS-----
---IVCYA--LERRTGAKVAIKKLRPFSTDKRATREFFT-----
---SVCSAKEVFKRTGERIAIKKLSNPFNKDKRAYRE-----
---SVCSAKFIFKRTGERIAIKKLSQPFNKEKRAYRE-----
---TVCSVKFKFRSTGERIAVKKLSNPFNKDKRAIRE-----
```

Open-access on-line platform for bioinformatic analysis in computational enzymology

Mustguseal

can automatically collect from public databases and align thousands of sequences and structures of proteins within a superfamily to produce a large structure-guided sequence alignment

Bioinformatics, 2018



Zebra

To identify and rank the subfamily-specific positions as determinants of functional diversity and binding specificity

J Biomol Struct Dyn., 2014

pocketZebra

To identify and rank binding sites in proteins by functional significance and select particular positions in the structure that are important for selective binding of substrates and ligands

Nucleic Acids Research, 2014

visualCMAT

To predict and visualize correlated mutations/co-evolving residues in protein structures as a mechanism of allosteric communication, and a source of compensatory mutations for rational design

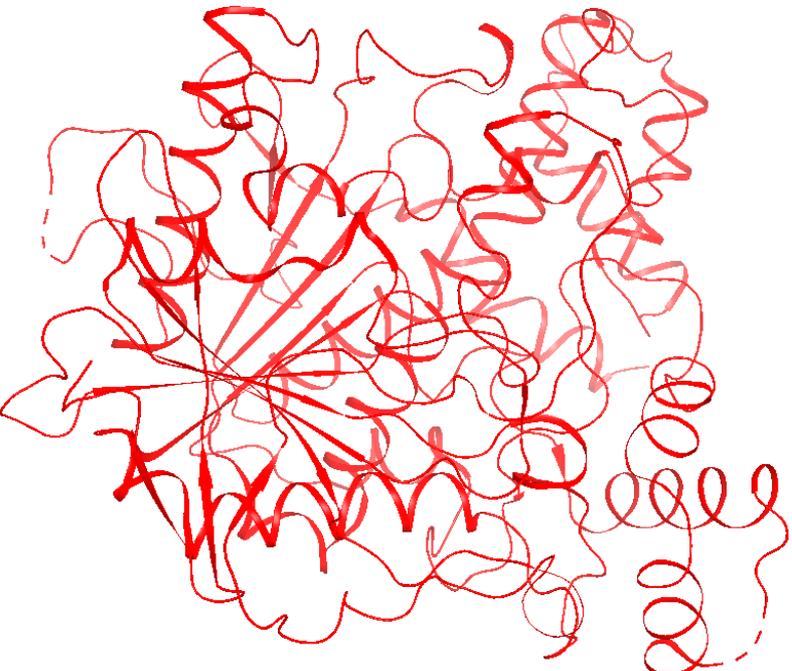
J Bioinform Comput Biol., 2018

Yosshi

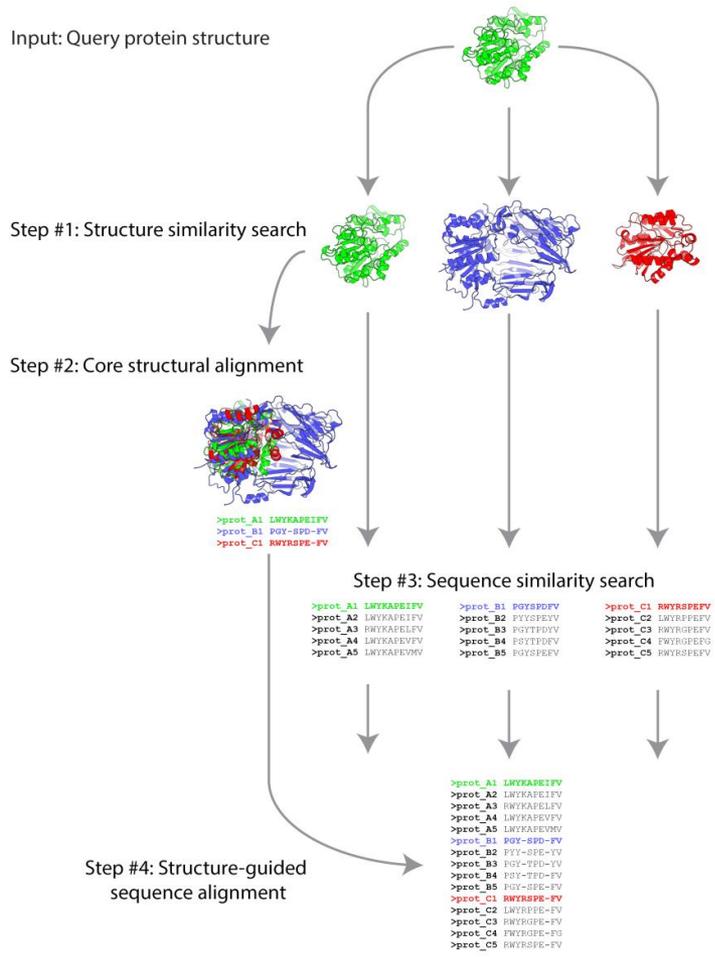
To systematically classify and study disulfide bonds in diverse protein families, and to assist at selecting hot-spots for disulfide engineering

Nucleic Acids Research, 2019

Automatic construction of a large structure-guided sequence alignment of your protein family by the Mustguseal



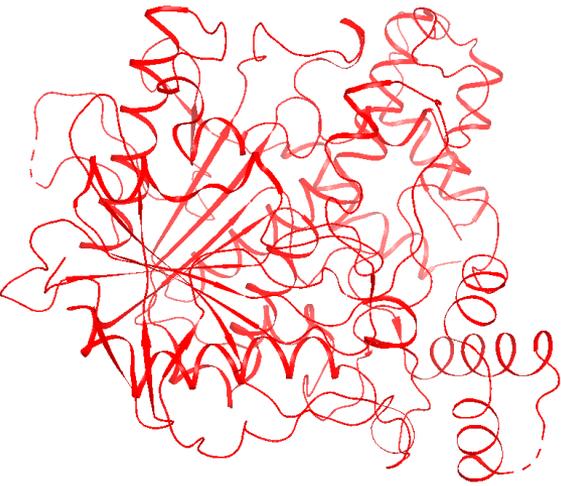
The input:
PDB structure of human acetylcholinesterase



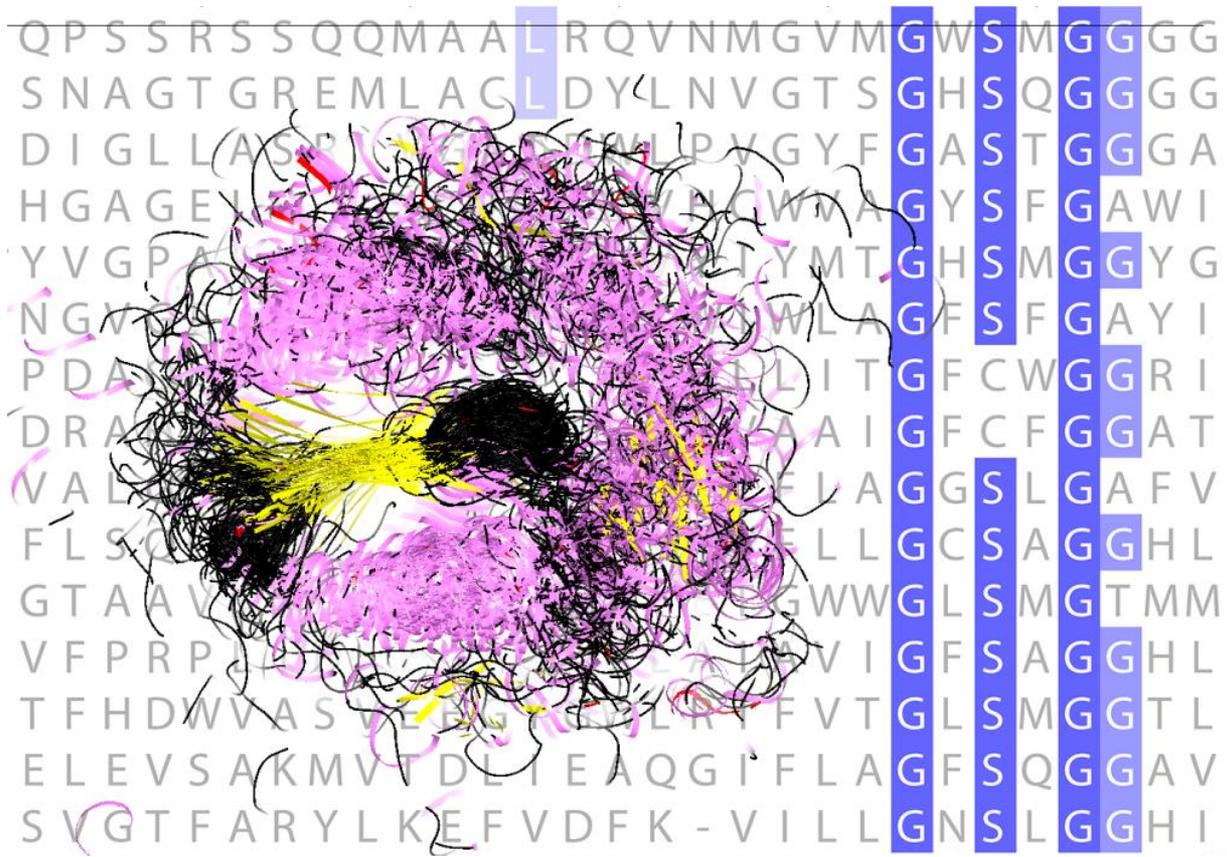
<https://biokinet.belozersky.msu.ru/mustguseal>

The process:
Automatic collection and alignment of all the available protein sequences and structures from public databases

Automatic construction of a large structure-guided sequence alignment of your protein family by the Mustguseal



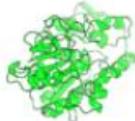
The input:
PDB structure of human acetylcholinesterase



```
QPSSRS SQQMAALRQVNMGMVMSMGGGG
SNAGTGREMLAGLDYLVNGTSGHSQGGGG
DIGLLAS...ALPYGYFGASTGGGA
HGAGE...WAGYSFGAWI
YVGP...TYMTGHSMSGGYG
NGV...WLAGFSFGAYI
PDA...LLITGFCWGGRI
DRA...AAIGFCFGGAT
VAL...ELAGGS LGAFV
FLSC...ELLGCSAGGHL
GTAAV...GWGLSMGTMM
VFPRP...EVIGFSAGGHL
TFHDWVAS...LRLRFVTGLSMGGTL
ELEVS AKMVTDLIEAQGIFLAGFSQGGAV
SVGTFARYLKEFVDFK-VILLGNSLGGHI
```

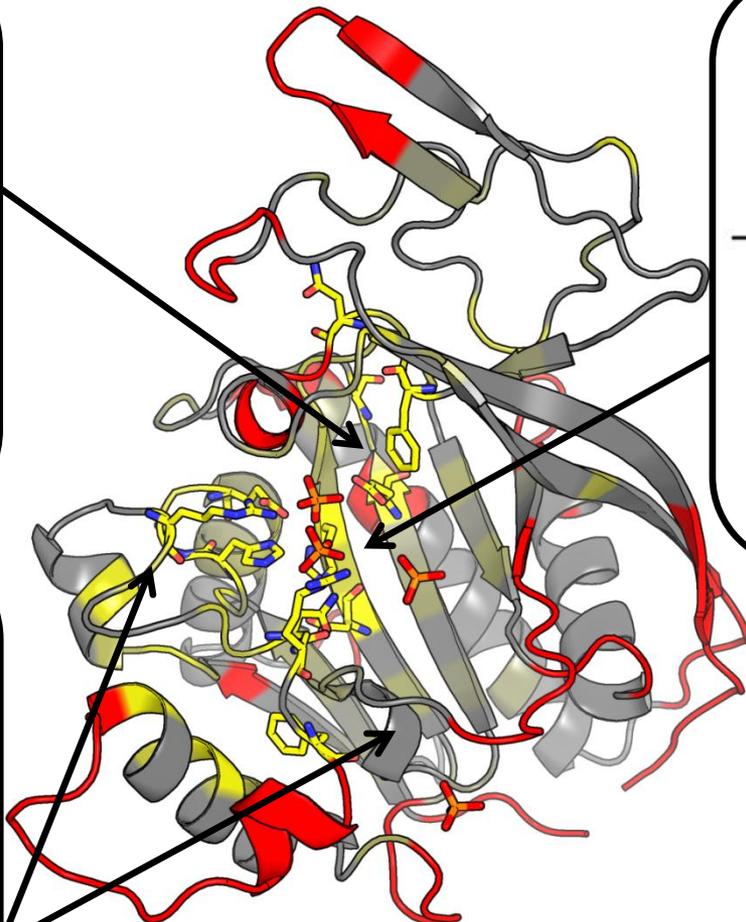
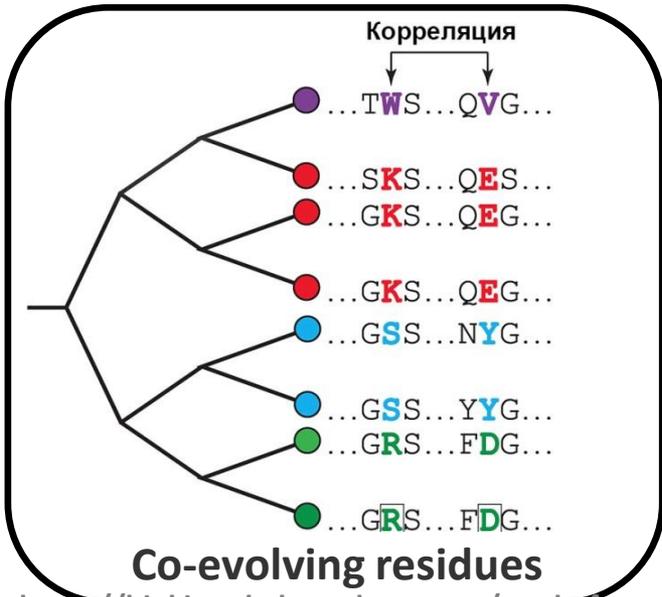
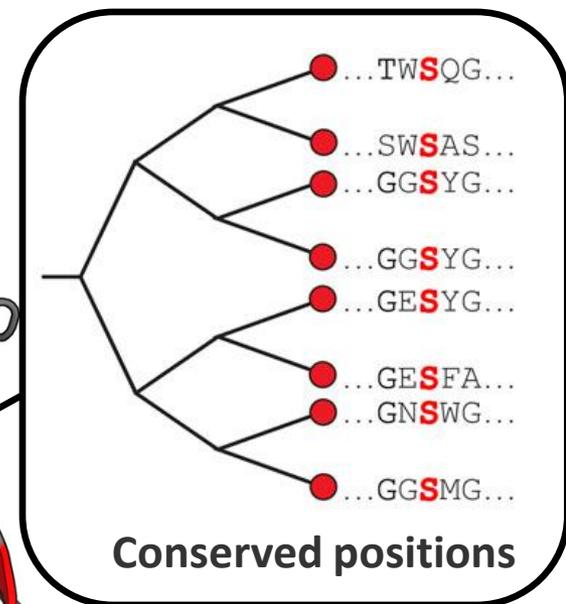
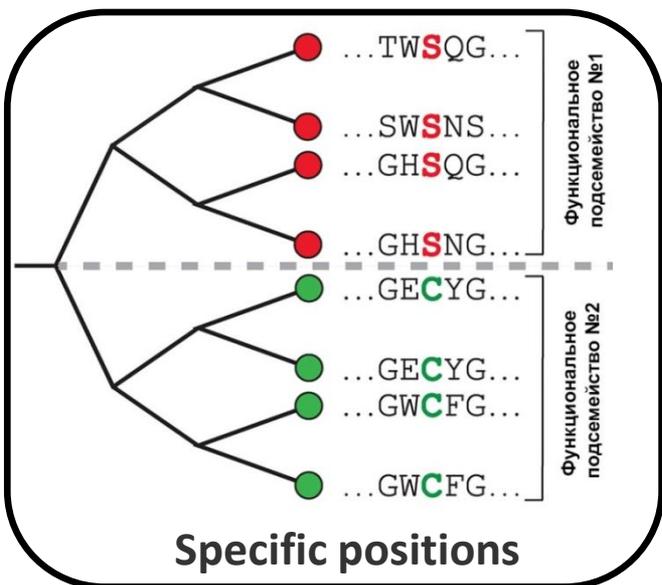
The output:
Structure guided-sequence alignment of human acetylcholinesterase and its homologs from the α/β -hydrolase superfamily

Submit the final Mustguseal alignment for further analysis

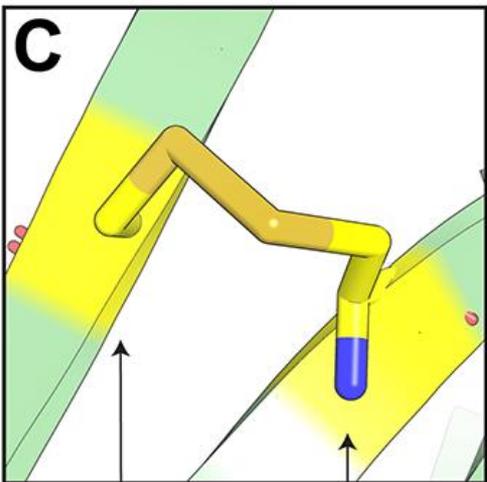
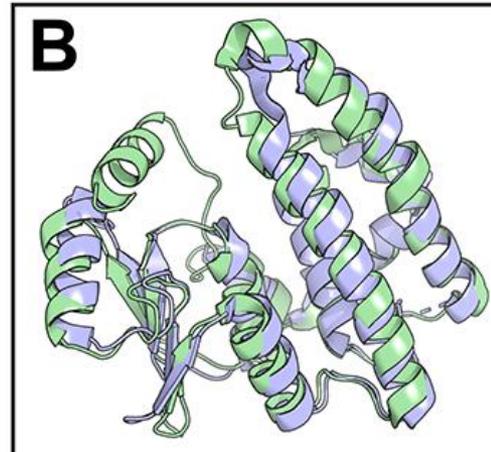
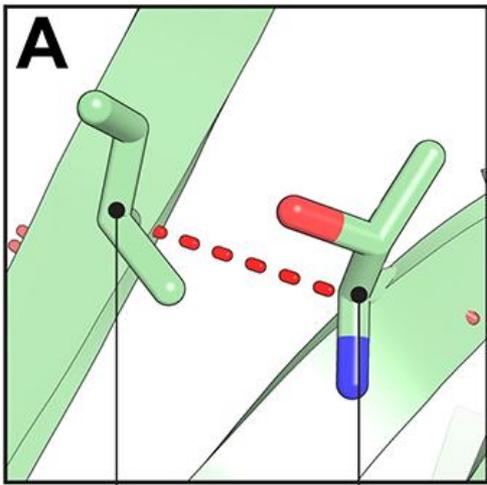
Submit the Final Mustguseal alignment	FINAL_A-ditxcb9jvnyuqz.fasta	12.0 MB	<pre>>prot_A1 LMYKAEELFV >prot_A2 LMYKAEELFV >prot_A3 RMYKAEELFV >prot_A4 LMYKAEELFV >prot_A5 LMYKAEELFV >prot_B1 NGE-SDE-FV >prot_B2 DYT-SDE-FV >prot_B3 DGT-TEC-FV >prot_B4 DGT-TEC-FV >prot_B5 DGT-SDE-FV >prot_C1 DMVGGG-FV >prot_C2 LMYRDEE-FV >prot_C3 DMVGGG-FV >prot_C4 DMVGGG-FV >prot_C5 DMVGGG-FV</pre>	Submit to Zebra
Submit the Final Mustguseal alignment and a PDB structure of the representative protein	FINAL_A-ditxcb9jvnyuqz.fasta	12.0 MB	<pre>>prot_A1 LMYKAEELFV >prot_A2 LMYKAEELFV >prot_A3 RMYKAEELFV >prot_A4 LMYKAEELFV >prot_A5 LMYKAEELFV >prot_B1 NGE-SDE-FV >prot_B2 DYT-SDE-FV >prot_B3 DGT-TEC-FV >prot_B4 DGT-TEC-FV >prot_B5 DGT-SDE-FV >prot_C1 DMVGGG-FV >prot_C2 LMYRDEE-FV >prot_C3 DMVGGG-FV >prot_C4 DMVGGG-FV >prot_C5 DMVGGG-FV</pre>	Submit to Zebra
	0_1u8f_O.pdb	166 KB		Submit to pocketZebra
				Submit to visualCMAT

- A new submission to Zebra, pocketZebra, visualCMAT, and Yosshi can be made directly from the Mustguseal Results page.

Annotation of the protein of interest according to the bioinformatic analysis of the superfamily



Systematically classify and study disulfide bonds in diverse protein families

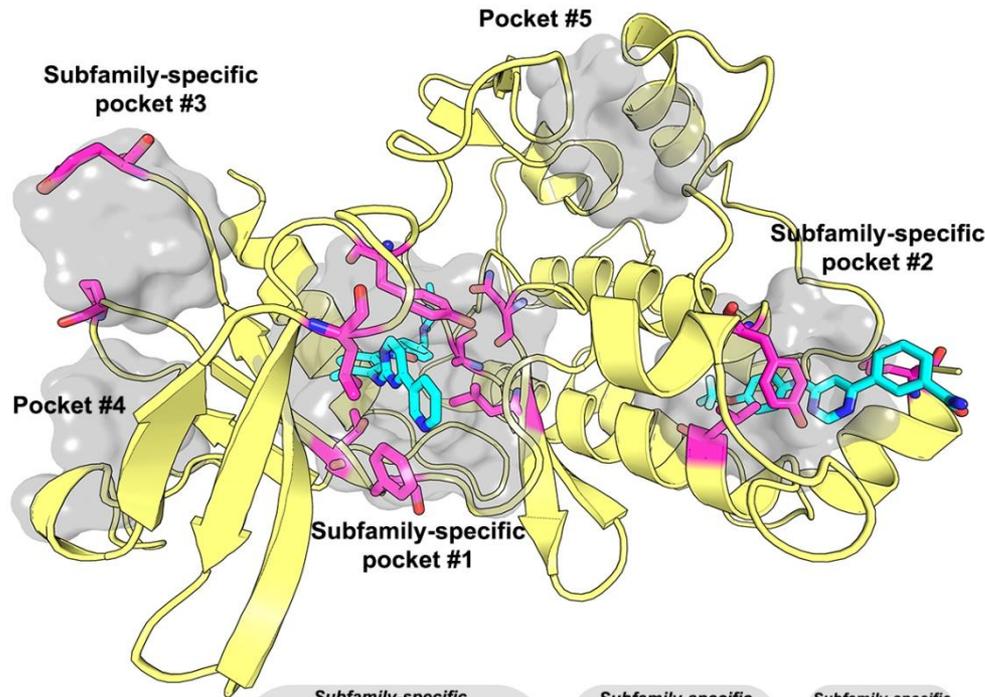


```

>PDB_A1 LE T KAPE I FV
>SEQ_A2 LE T KAPE I FV
>SEQ_A3 ID T KAPE L FV
>SEQ_A4 LE S KAPE V FV
>SEQ_A5 LD S KAPE V MV
>PDB_B1 TG I RSPD C FV
>SEQ_B2 TE I RSPE C YV
>SEQ_B3 TG C RTPD C YV
>SEQ_B4 SS C RTPD C FV
>SEQ_B5 SG C RSPE C FV
  
```

The [Yosshi](#) web-service can be used to systematically classify and study disulfide bonds in diverse protein families, and to assist at selecting hot-spots for disulfide engineering in the structure of your query protein. The "YOu web-server for S-S bond HarvestIng" is a new highly automated on-line tool for a systematic homology-driven analysis and engineering of disulfide bonds that can be easily used by a general biologist at a daily laboratory routine. The Yosshi facilitates a broader interpretation of disulfides not just as a factor of structural stability, but rather as a mechanism to implement diversity within a superfamily; [Suplatov D., et al. \(2019\) Nucl. Acids Res.](#)

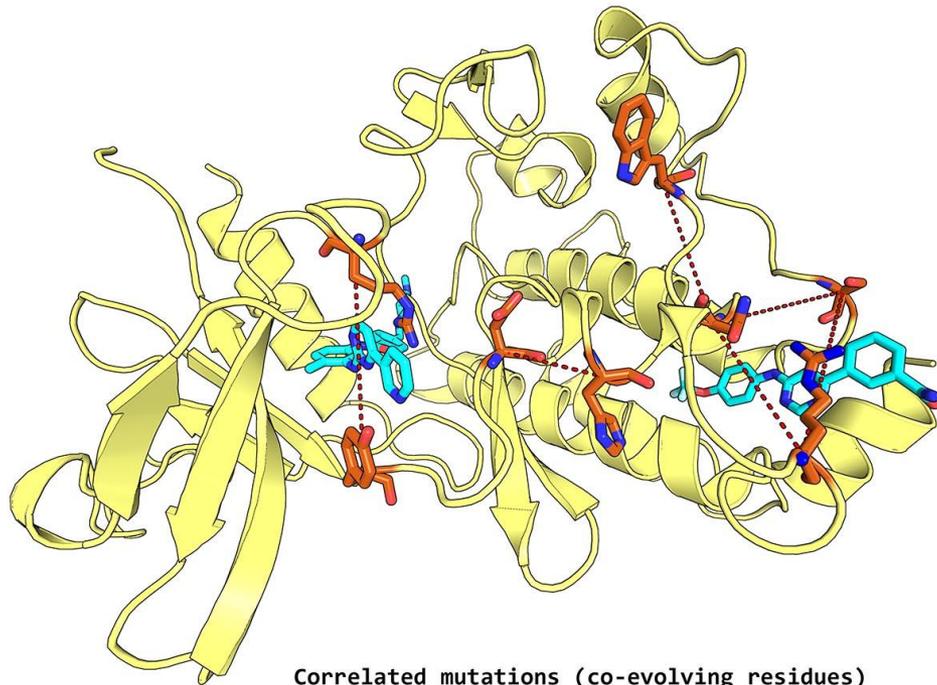
Identify and study the conserved and subfamily-specific positions



	Subfamily-specific pocket #1				Subfamily-specific pocket #2				Subfamily-specific pocket #3											
Representative PDB	...	R	LF	...	YL	...	TN	Q	...	HY	...	L	E	M	...	P	...	E	K	...
Functional subfamily 1	...	R	LF	...	YL	...	TN	N	...	QY	...	L	E	L	...	LP	...	E	R	...
	...	K	LF	...	YL	...	TN	N	...	EY	...	L	E	V	...	VP	...	E	R	...
	...	N	LF	...	YL	...	TN	D	...	HY	...	L	E	V	...	VP	...	E	K	...
Functional subfamily 2	...	R	HL	...	LD	...	GV	Q	...	NA	...	F	Q	V	...	VA	...	K	K	...
	...	K	HL	...	LD	...	GV	E	...	QA	...	F	Q	L	...	LA	...	K	R	...
	...	Q	HL	...	LD	...	GV	N	...	HA	...	F	Q	M	...	VA	...	K	R	...
...	L	HL	...	LD	...	GV	V	...	NA	...	F	Q	M	...	VA	...	K	K	...	

- The [Zebra](#) web-service can be used to identify conserved positions, which define properties common among functionally diverse protein families, as well as subfamily-specific positions responsible for functional diversity – to select hotspots for directed evolution or rational design experiments; [Suplatov D., et al. \(2014\) J.Biomol.Struct.Dyn.](#)
- The [pocketZebra](#) web-service can be used to identify and rank binding sites in proteins by functional significance and select particular positions in the structure that are important for selective binding of substrates/inhibitors/effectors; [Suplatov D., et al. \(2014\) Nucl. Acids Res.](#)

Predict and visualize the correlated mutations (co-evolving residues)



Representative PDB

Members of a protein superfamily

...	R	D	I	...	H	L	...	T	W	Q	...	H	N	...	R	...	S	M	...	L	Y	...	R	K	...
R	E	I	...	R	K	...	S	K	N	...	Q	...	A	...	S	...	R	L	...	L	F	...	F	R	...
...	K	E	I	...	R	L	...	T	W	N	...	E	N	...	S	...	R	V	...	V	Y	...	R	R	...
...	K	D	I	...	H	I	...	S	K	E	...	E	A	...	S	...	R	L	...	L	R	...	F	R	...
...	N	R	I	...	E	L	...	T	W	D	...	H	N	...	T	...	R	V	...	V	R	...	Y	K	...
...	R	D	I	...	H	V	...	S	V	Q	...	N	A	...	E	...	K	V	...	V	F	...	R	K	...
...	K	R	I	...	E	A	...	S	V	E	...	Q	A	...	E	...	K	L	...	L	W	...	R	R	...
...	Q	D	I	...	K	G	...	S	V	N	...	H	W	...	R	...	D	M	...	V	W	...	R	R	...
L	D	I	...	K	L	...	S	V	V	...	N	W	...	R	...	D	M	...	V	R	...	W	K	...	

<https://biokinet.belozersky.msu.ru/visualcmat>

- The [visualCMAT](#) web-service can be used to predict and visualize correlated mutations/co-evolving residues in protein structures. The visualCMAT can be used to understand the relationship between structure and function and identify co-evolution patterns in protein superfamilies, implemented at selecting hotspots and compensatory mutations for rational design and directed evolution experiments to produce novel enzymes with improved properties, and employed at studying the mechanism of selective ligand's binding and allosteric communication between topologically independent sites in protein structures; [Suplatov D., et al. \(2018\) J Bioinform Comput Biol.](#)

Structure-based sequence alignments of functionally diverse protein families as a tool in protein engineering and drug discovery

Implementation of Mustguseal, Zebra, pocketZebra, and visualCMAT in the laboratory practice can help at studying a protein function and regulation, designing improved enzyme variants for practical application and selective modulators of enzyme functional properties.

Suplatov, D., Kirilin, E., & Švedas, V. (2016). Bioinformatic Analysis of Protein Families to Select Function-Related Variable Positions. In *Understanding Enzymes: Function, Design, Engineering, and Analysis* (pp. 351-385) Ed. Allan Svendsen. Pan Stanford. [\[link\]](#)

Suplatov, D., Voevodin, V., & Švedas, V. (2015). Robust enzyme design: Bioinformatic tools for improved protein stability. *Biotechnology journal*, 10(3), 344-355. [\[link\]](#)

Suplatov, D., & Švedas, V. (2015). Study of functional and allosteric sites in protein superfamilies. *Acta Naturae*, 7(4), 27, 34-45. [\[link\]](#)

Contacts

- The Mustguseal title page
<https://biokinet.belozersky.msu.ru/mustguseal>
- Mustguseal Support
d.a.suplatov@belozersky.msu.ru
- Collaboration
vytas@belozersky.msu.ru
- Press to ask your question on-line

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