ProBiS Web Server

2012

User's Guide

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Background

Binding sites are often conserved in evolution of proteins. ProBiS can detect such conserved binding sites regions on a protein structure given as query. ProBiS performs a local, surface oriented structural comparison of a query structure to a database of non-redundant protein structures (nr-PDB), and finds proteins that are locally similar to the query protein. Then it calculates degrees of structural conservation for query amino acid residues, which measure frequency of occurrence of a particular residue in the local structural alignments that were found. The degrees of structural conservation are represented as colors on the query protein from blue (unconserved) to red (conserved). In contrast to most structural alignment algorithms, ProBiS can detect similar binding sites, even when proteins are of different folds.

Software Requirement

ProBiS web server requires Java due to the use of Jmol applet. You can get Java at http://java.com. ProBiS has been shown to work on the following platforms.

Linux - Ubuntu

Sun Java (Version 6 Update 30)

- Firefox
- Chrome
- Opera

OpenJDK (IcedTea Version 1.1.1)

- Firefox

Windows 7

Sun Java (Version 6 Update 30)

- Firefox
- Chrome
- Opera
- Safari (version 5.1)
- Internet Explorer (version 8)

New User Interface Features

Submit a Binding Site as a Query

The new Jmol based tool for selection of binding sites, which is available on the *Detection of Structurally Similar Binding Sites* and *Pairwise Local Structural Alignment* input pages, now allows one to easily define a binding site, and submit it as a query to the ProBiS web server. This focuses the search for structural similarities to the interesting part of a protein.

Change the Default Comparison Database

The *Detection of Structurally Similar Binding Sites* page now provides the option to change the default Comparison Database, the non-redundant PDB, for a user-provided list of protein chains. A search for similar binding sites can thus be narrowed to a subset of the PDB, e.g., proteins of the same fold as query, or even to complete PDB of currently ~180 thousand chains.

Tabs

The *ProBiS output page* has a new streamlined tab layout, which allows better organization of the larger content.

Similar Proteins Tab

The *ProBiS output page* now supports a tabular view of the similar proteins, to aid in structural and functional annotation of the query protein. Relevant information, i.e., links to the Pfam, SCOP, UniProt, or ProBiS databases, are displayed for each similar protein, which also allows identification of unexpected binding sites similarities across protein folds. In addition, the table can be downloaded in Column Separated Values (CSV) format, which is supported in popular software, e.g., Excel.

Structural Alignments Tab

The structural alignments of proteins can now be downloaded in the CLUSTAL format as multiple sequence alignments.

Search Tool

The table of similar proteins on the *ProBiS output page* has an integrated keyword search tool. For example, all similar proteins with the same Pfam accession number can be identified.

Details Tab

The *ProBiS output page* also provides detailed alignment view for each pairwise local structural alignment. This view allows examination of correspondences between the aligned residues in tabular format, which aids in the detection of mutations in binding sites. A pairwise alignment can be loaded into the Jmol to view it in 3D, or can be downloaded as PDB or XML file.

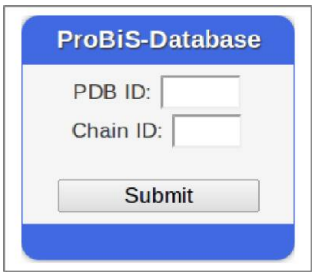
Selection Tab

The selection tab on the *ProBiS output page* shows a list of molecules that are in the Jmol viewer, and enables hiding/showing the molecules.

Progress Indicator

A prominent progress indicator allows precise tracking of the status of a ProBiS job. It is colored like a semaphore, i.e., red - job was cancelled, yellow - job is waiting in queue, and green - the calculation is in progress.

ProBiS-Database Widget



The ProBiS-Database Widget provides access to local structural similarity profiles available in the ProBiS-Database. It can be included in any web page by adding a single line of code.

Jmol Help

Some key tips on how the user can manipulate the 3D protein model in Jmol are presented in Figure 1.

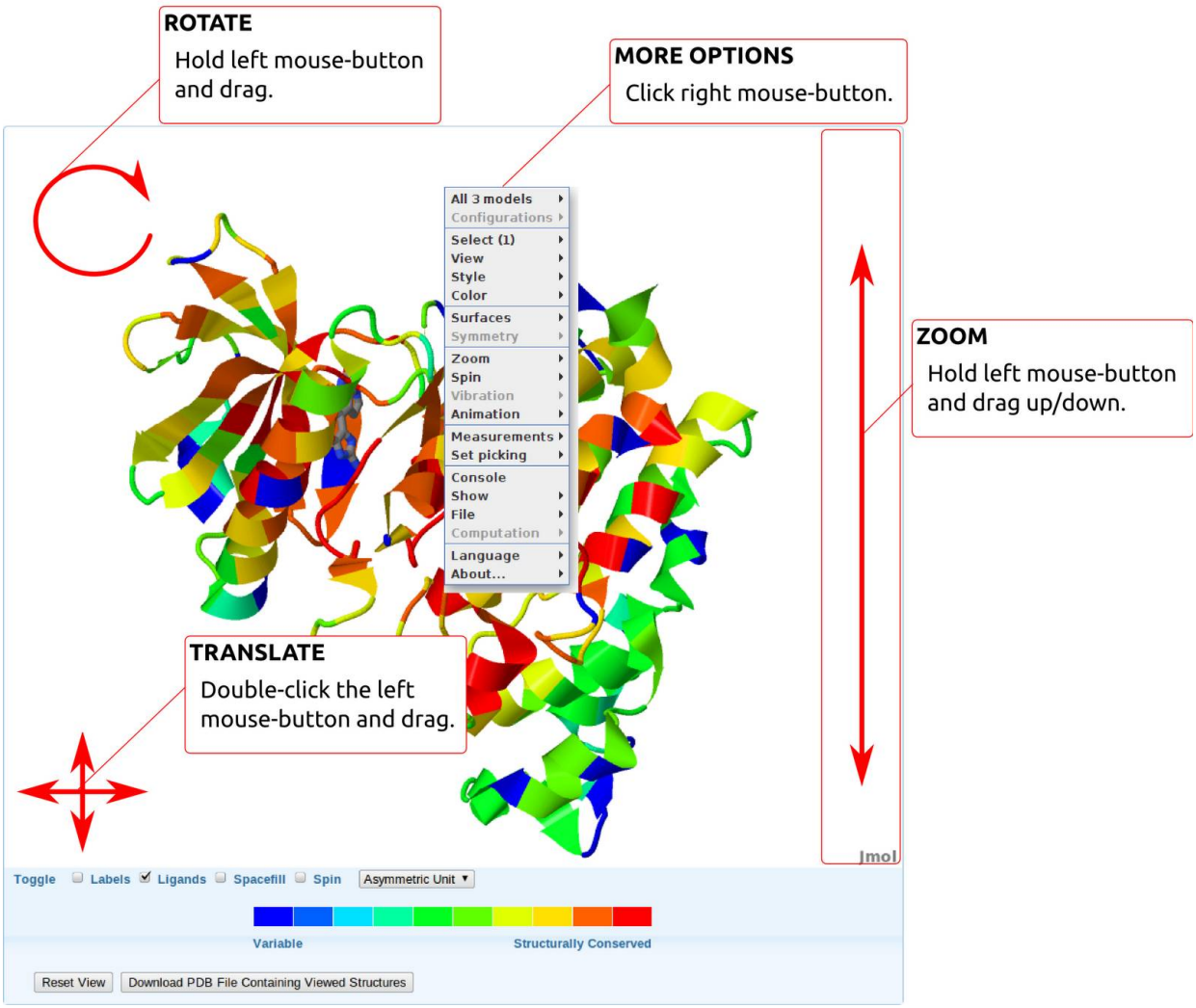


Figure 1. Mouse operations in Jmol.

Protein Binding Sites Tools

ProBiS web server is used for detection of structurally similar binding sites in PDB and local pairwise alignment of protein structures. The main page of the ProBiS is shown in Figure 2.

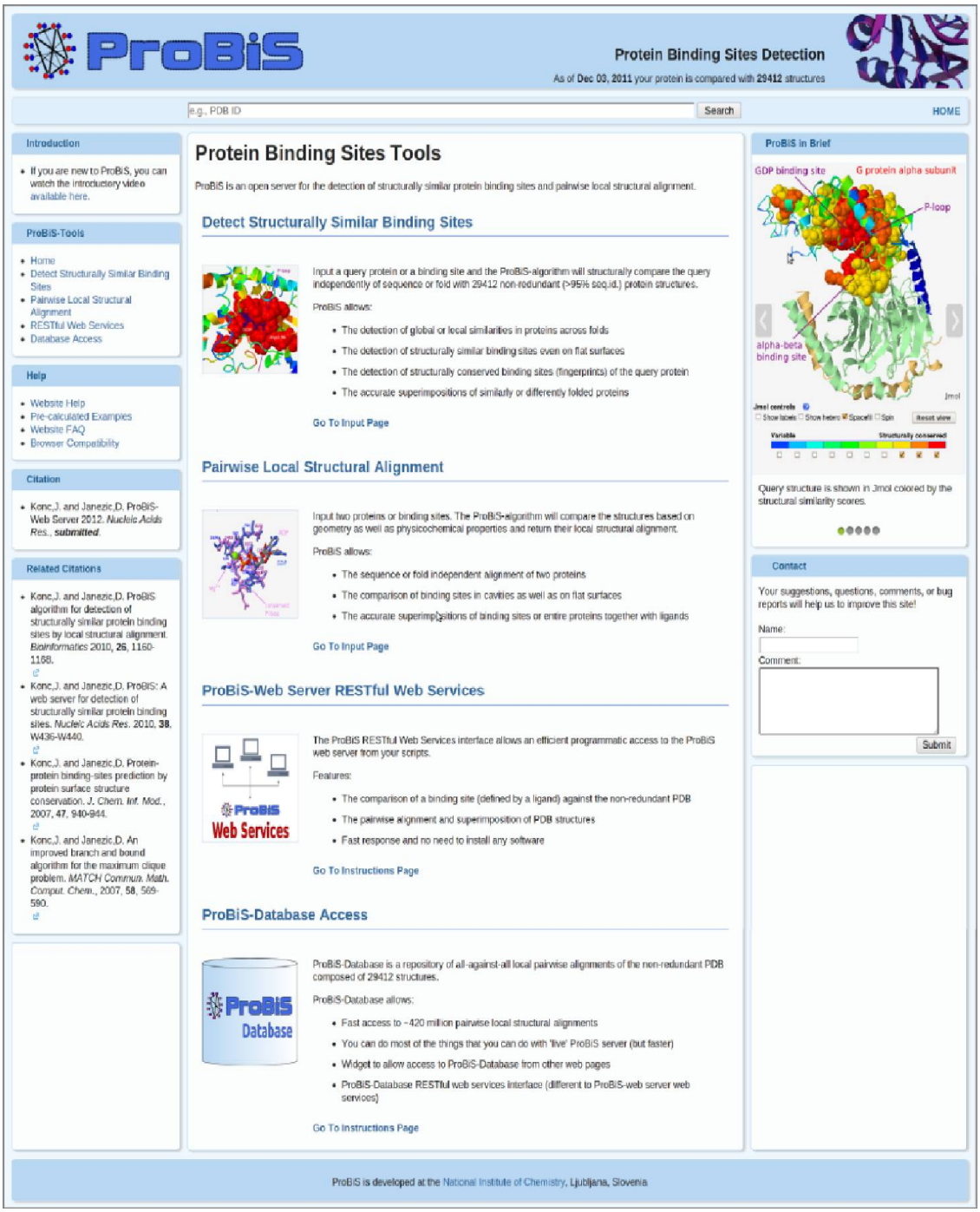


Figure 2. The ProBiS web server main page provides access to the Protein Binding Sites Tools.

Detect Structurally Similar Binding Sites

To start using this tool the user should follow instructions described in Figure 3.

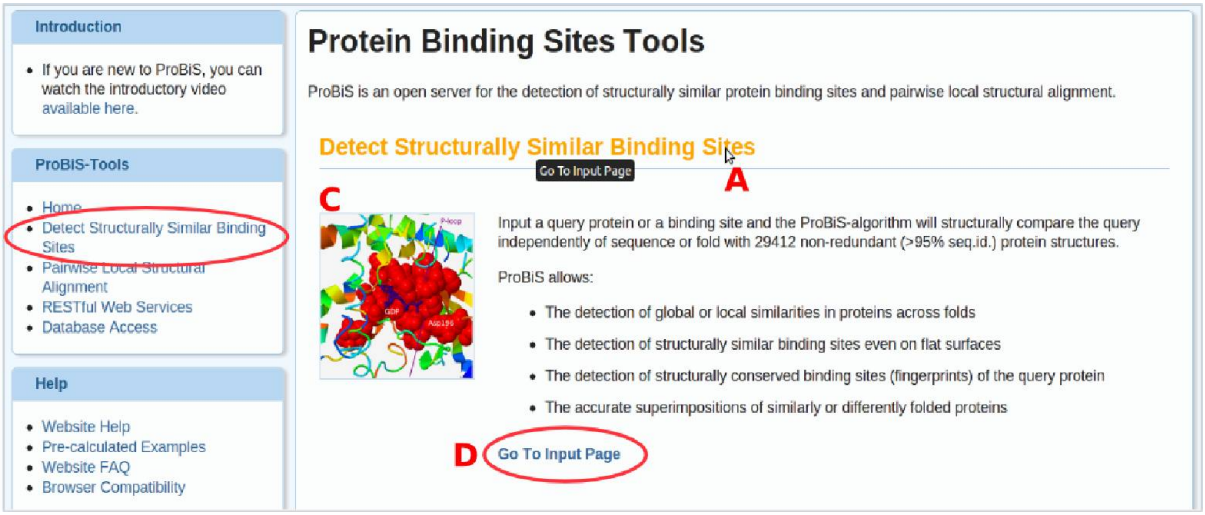


Figure 3. ProBiS main allows access to the input page: (A) by clicking Detect Structurally Similar Binding Sites, (B) by clicking a link located in the bar on the left-hand side of the ProBiS main page, (C) by clicking the picture or (D) by clicking Go To Input Page.

Input

Simple Query

The form in Figure 4 allows the comparison of a query protein against the non-redundant PDB proteins.

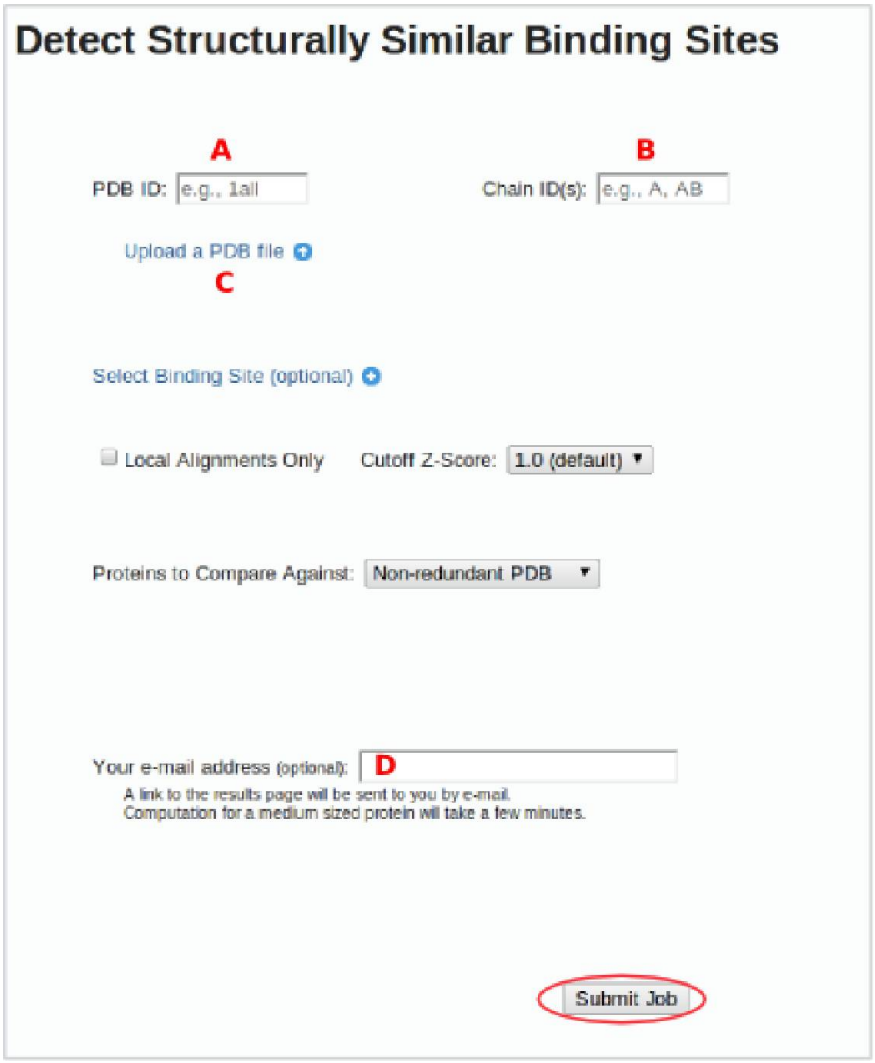


Figure 4. Simple query. Enter (A) PDB ID; (B) Chain ID; (C) alternatively to (A) upload a PDB file (D) optional email address. Then click the Submit Job button.

Precalculated Results

Alternatively, the user can get instant results from the ProBiS-Database. If the user enters a PDB ID and chain ID, ProBiS will display a pop-up window, presented in Figure 5, which has a link to precalculated results (see also ProBiS-Database Access).

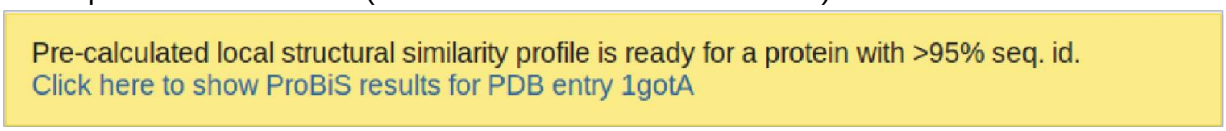


Figure 5. Pop-up window that allows to obtain pre-calculated results from the ProBiS-Database.

Advanced Query

Figure 6 shows, how to submit a binding site (or any other site) as a query, or compare the query protein against a custom list of proteins.

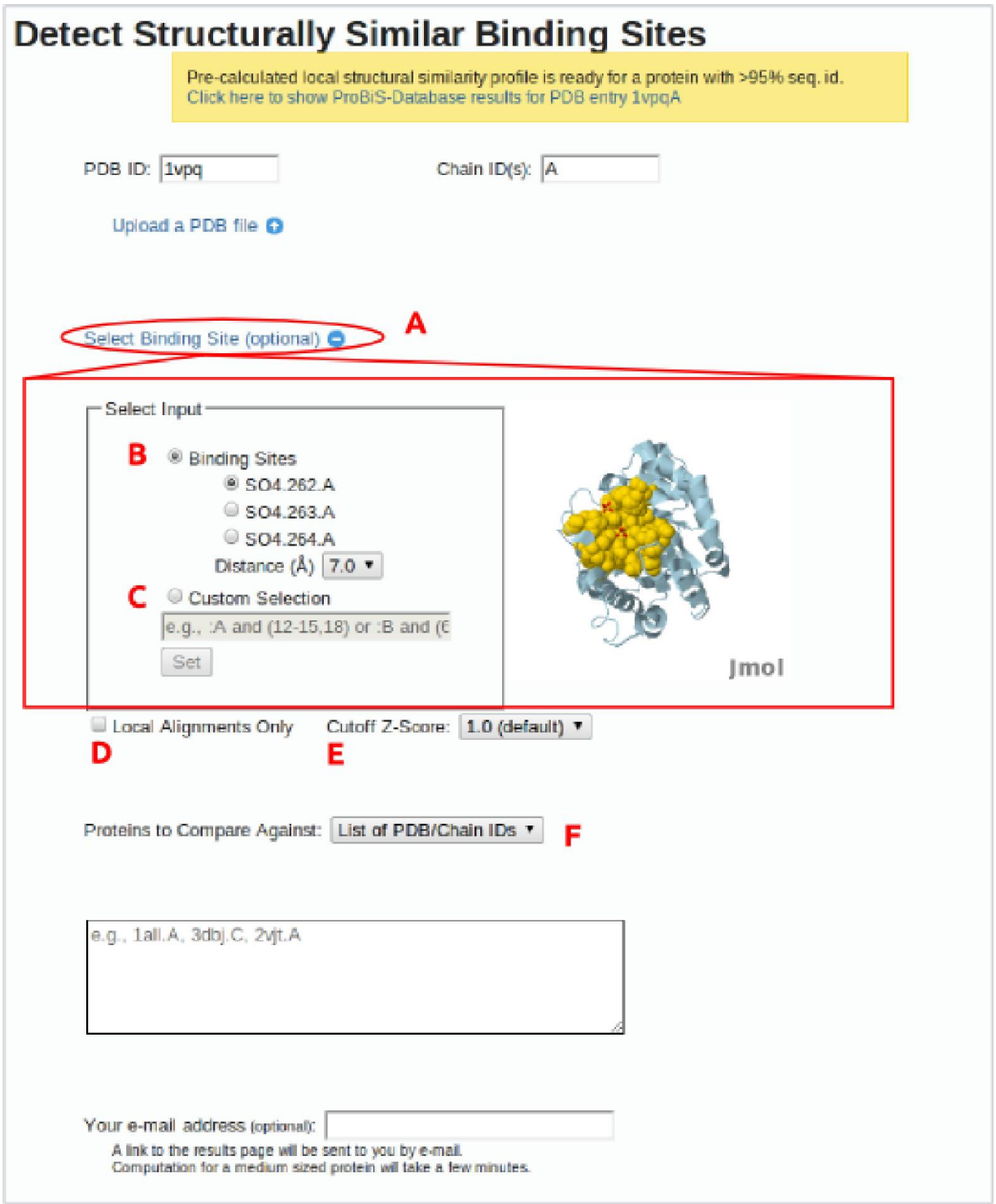


Figure 6. Advanced query. Clicking on (A) Select Binding Site link will open the form (red rectangle), where you can define part of the surface on the query protein that will be taken as input. Input can be

(B) a binding site, which you select by a ligand (e.g., SO4.262.A) and distance: all residues in a radius of 3.0 Å within SO4 ion will be considered as a query binding site; (C) a custom selection of residues given by chain identifier and residue numbers, e.g., ":A and (12-15,18)". (To select residues numbered 12,13,14,15,18 on chain A and 64,67,89,90 on chain B of a query protein, enter *:A and (12-15,18) or :B and (64,67,89-90)*.) The selected surface region appears as yellow spheres in the Jmol viewer. (D) If enabled, the alignments will only be searched for in the selected surface region and the local alignments found will not be extended along compared proteins' backbones. (E) Regulated filtering of the alignments. Lower Z-Score means that more alignments will be displayed among results. (F) The drop-down list enables to enter a custom list of proteins against which to compare the query protein, e.g., 1all.A, 3dbj.C, 2vjt.A. Default is to compare against the non-redundant PDB.

Output

ProBiS output page is shown in Figure 7.

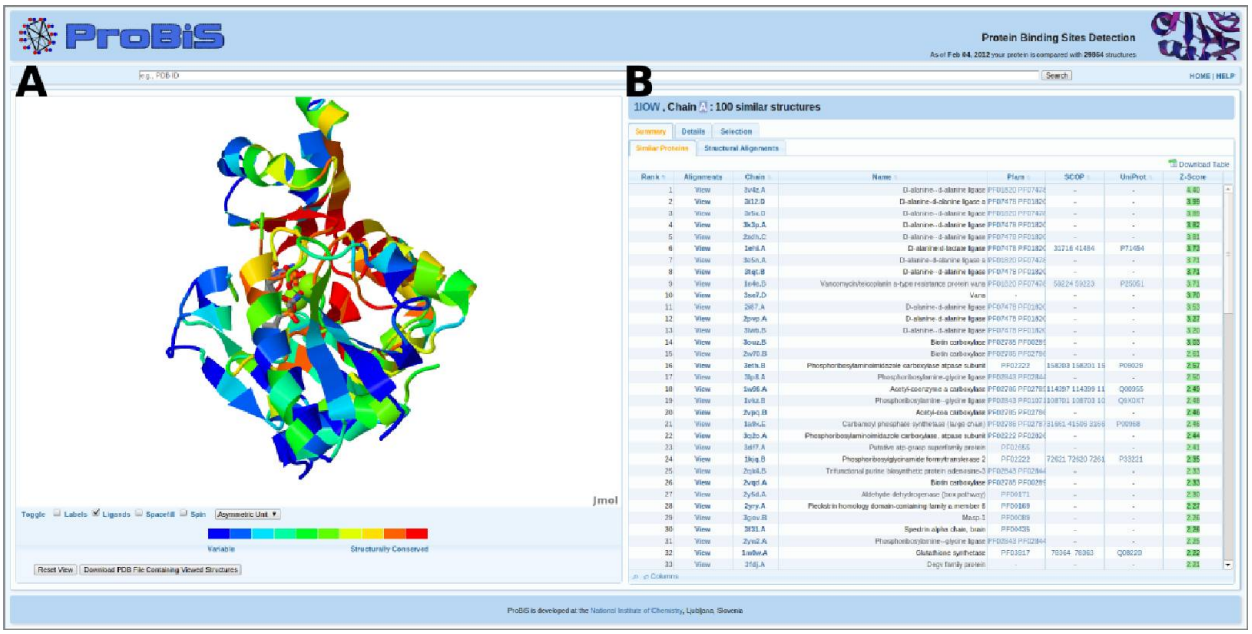


Figure 7. ProBiS output page: (A) 3D query protein colored by degrees of structural conservation from blue (unconserved) to red (structurally conserved) in Jmol viewer; (B) table of similar proteins.

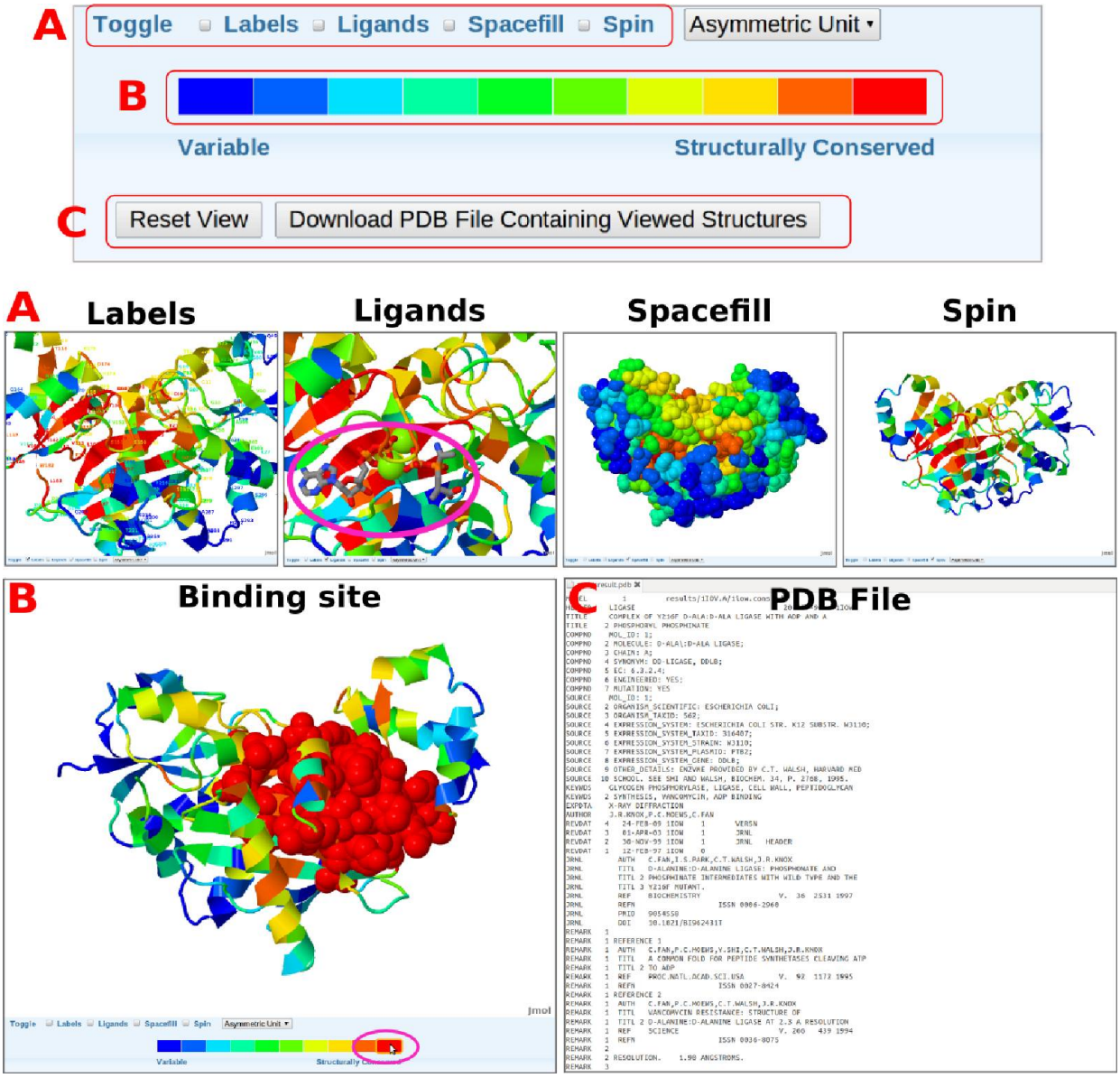
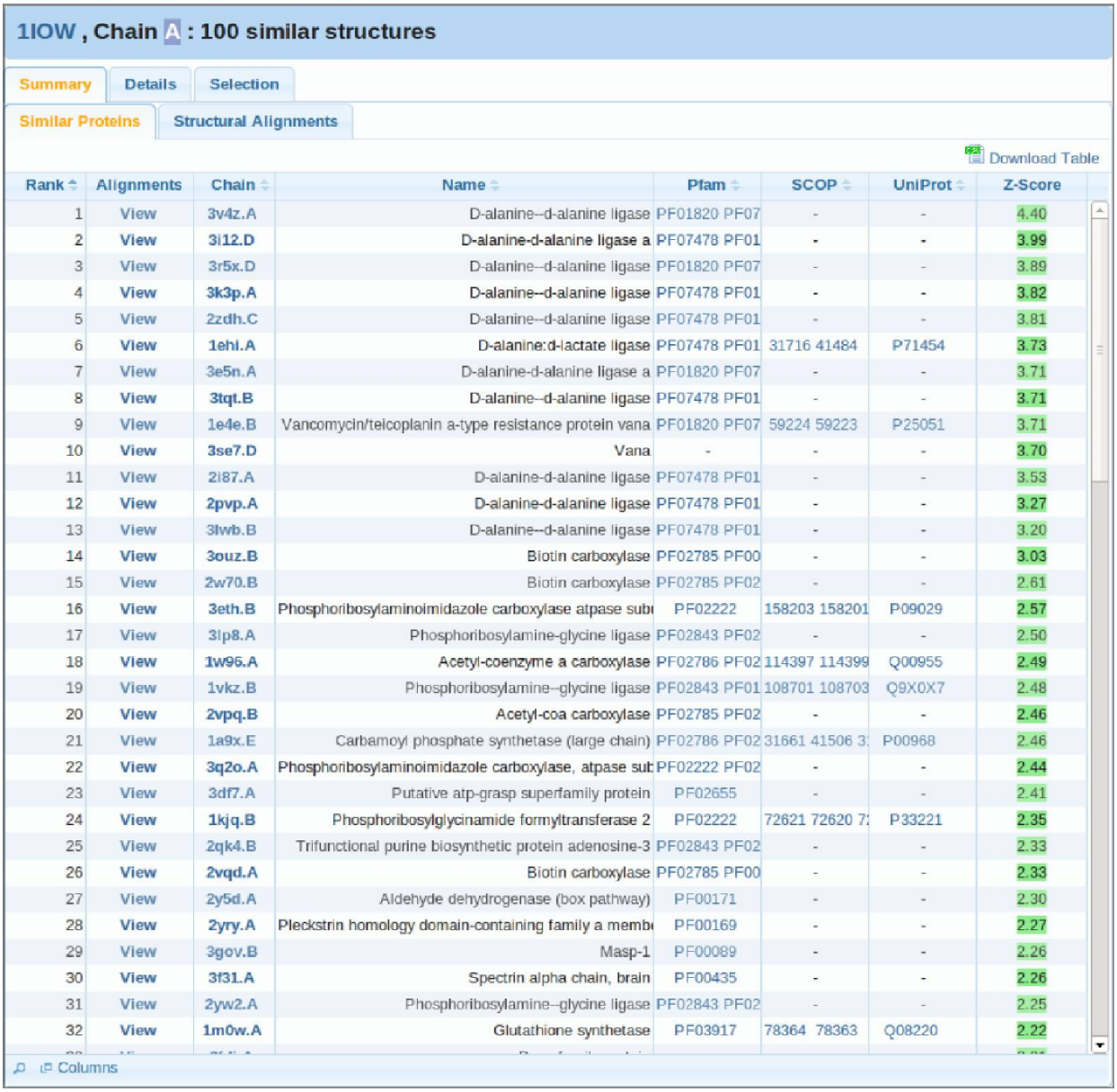


Figure 8. Jmol options. (A) Toggle options; (B) Click on a color, shows residues of this color as spheres in the 3D query protein model. Each color represents a conservation grade from 0 to 9. (C) The downloaded PDB file of query protein will have conservation grades from 0.0 to 0.9 in beta factor fields.



Rank	Alignments	Chain	Name	Pfam	SCOP	UniProt	Z-Score
1	View	3v4z.A	D-alanine--d-alanine ligase	PF01820 PF07	-	-	4.40
2	View	3i12.D	D-alanine-d-alanine ligase a	PF07478 PF01	-	-	3.99
3	View	3r5x.D	D-alanine--d-alanine ligase	PF01820 PF07	-	-	3.89
4	View	3k3p.A	D-alanine--d-alanine ligase	PF07478 PF01	-	-	3.82
5	View	2zdh.C	D-alanine--d-alanine ligase	PF07478 PF01	-	-	3.81
6	View	1ehi.A	D-alanine:d-lactate ligase	PF07478 PF01	31716 41484	P71454	3.73
7	View	3e5n.A	D-alanine-d-alanine ligase a	PF01820 PF07	-	-	3.71
8	View	3tqt.B	D-alanine--d-alanine ligase	PF07478 PF01	-	-	3.71
9	View	1e4e.B	Vancomycin/teicoplanin a-type resistance protein vana	PF01820 PF07	59224 59223	P25051	3.71
10	View	3se7.D	Vana	-	-	-	3.70
11	View	2i87.A	D-alanine-d-alanine ligase	PF07478 PF01	-	-	3.53
12	View	2pvp.A	D-alanine-d-alanine ligase	PF07478 PF01	-	-	3.27
13	View	3lwb.B	D-alanine--d-alanine ligase	PF07478 PF01	-	-	3.20
14	View	3ouz.B	Biotin carboxylase	PF02785 PF00	-	-	3.03
15	View	2w70.B	Biotin carboxylase	PF02785 PF02	-	-	2.61
16	View	3eth.B	Phosphoribosylaminoimidazole carboxylase atpase subu	PF02222	158203 158201	P09029	2.57
17	View	3lp8.A	Phosphoribosylamine-glycine ligase	PF02843 PF02	-	-	2.50
18	View	1w96.A	Acetyl-coenzyme a carboxylase	PF02786 PF02	114397 114399	Q00955	2.49
19	View	1vkz.B	Phosphoribosylamine--glycine ligase	PF02843 PF01	108701 108703	Q9X0X7	2.48
20	View	2vpq.B	Acetyl-coa carboxylase	PF02785 PF02	-	-	2.46
21	View	1a9x.E	Carbamoyl phosphate synthetase (large chain)	PF02786 PF02	31661 41506 3	P00968	2.46
22	View	3q2o.A	Phosphoribosylaminoimidazole carboxylase, atpase sut	PF02222 PF02	-	-	2.44
23	View	3df7.A	Putative atp-grasp superfamily protein	PF02655	-	-	2.41
24	View	1kjq.B	Phosphoribosylglycinamide formyltransferase 2	PF02222	72621 72620 7	P33221	2.35
25	View	2qk4.B	Trifunctional purine biosynthetic protein adenosine-3	PF02843 PF02	-	-	2.33
26	View	2vqd.A	Biotin carboxylase	PF02785 PF00	-	-	2.33
27	View	2y5d.A	Aldehyde dehydrogenase (box pathway)	PF00171	-	-	2.30
28	View	2yry.A	Pleckstrin homology domain-containing family a membe	PF00169	-	-	2.27
29	View	3gov.B	Masp-1	PF00089	-	-	2.26
30	View	3f31.A	Spectrin alpha chain, brain	PF00435	-	-	2.26
31	View	2yw2.A	Phosphoribosylamine--glycine ligase	PF02843 PF02	-	-	2.25
32	View	1m0w.A	Glutathione synthetase	PF03917	78364 78363	Q08220	2.22

Figure 9. Table of similar proteins. Columns specifications are: Alignments - click on View shows local structural superimposition of the query and the similar protein in Jmol viewer and opens the Details tab, Chain - click on a link opens a new web page with precalculated results for the clicked chain from ProBiS-Database, Name - protein name, Pfam, SCOP, UniProt - links to corresponding proteins annotation databases, Z-Score - indicate how many standard deviations each alignment differs from the mean, e.g., a pairwise alignment with Z-Score of 2.0 is in the top ~2% of all alignments.

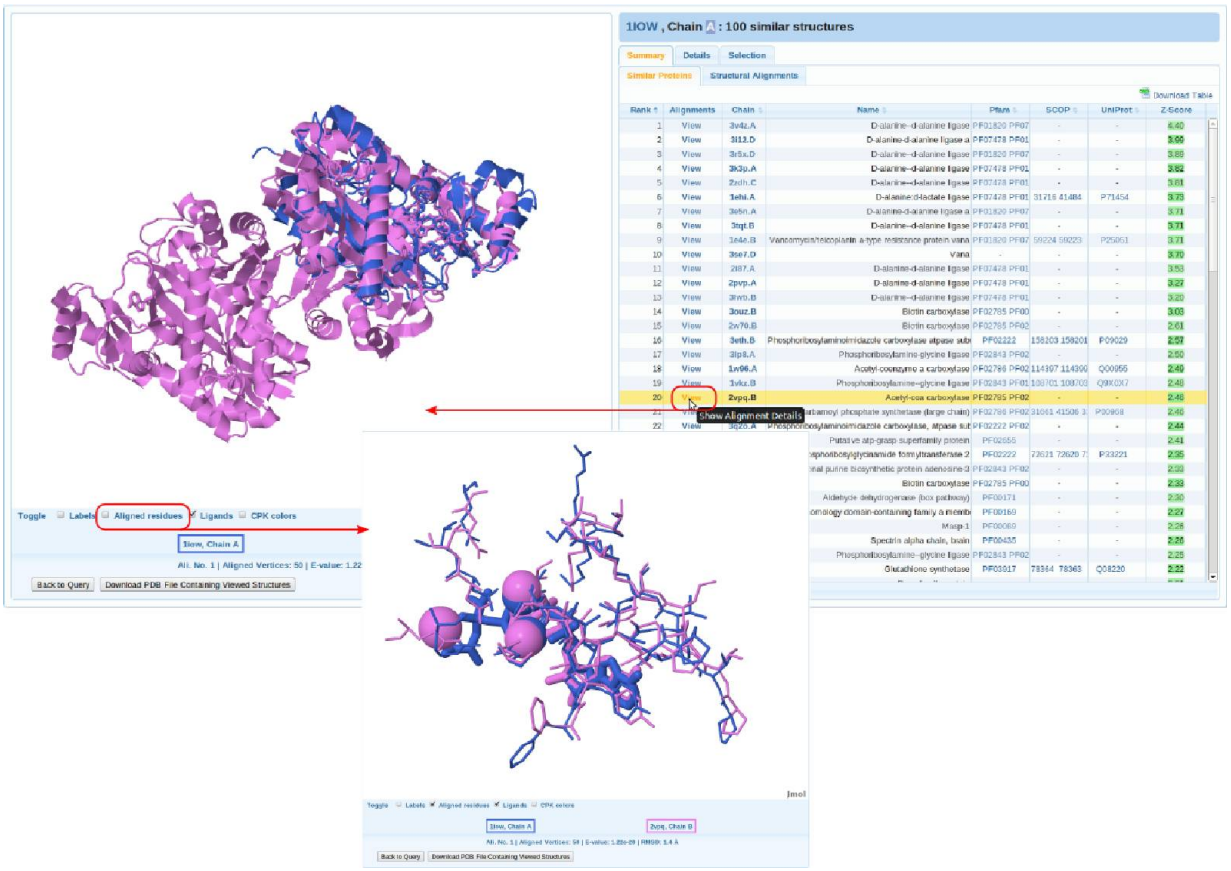


Figure 10. Click on a View link in the Alignments column loads the local structural alignment between query protein and similar protein into the Jmol viewer. To see only the aligned residues, click the respective checkbox under the Jmol window.

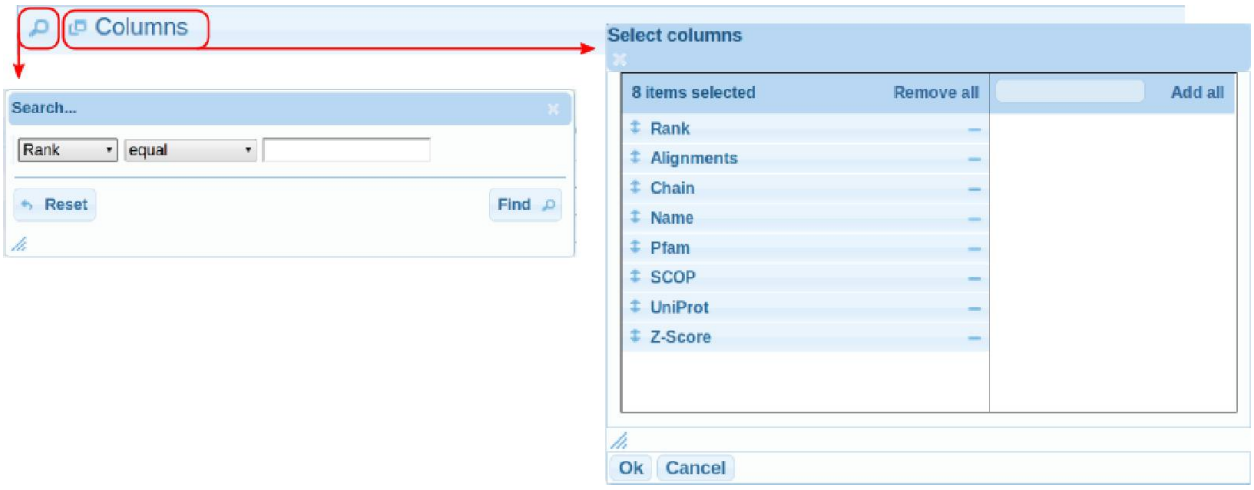


Figure 11. Search & Reorder columns tools. They are located below the table of similar proteins.

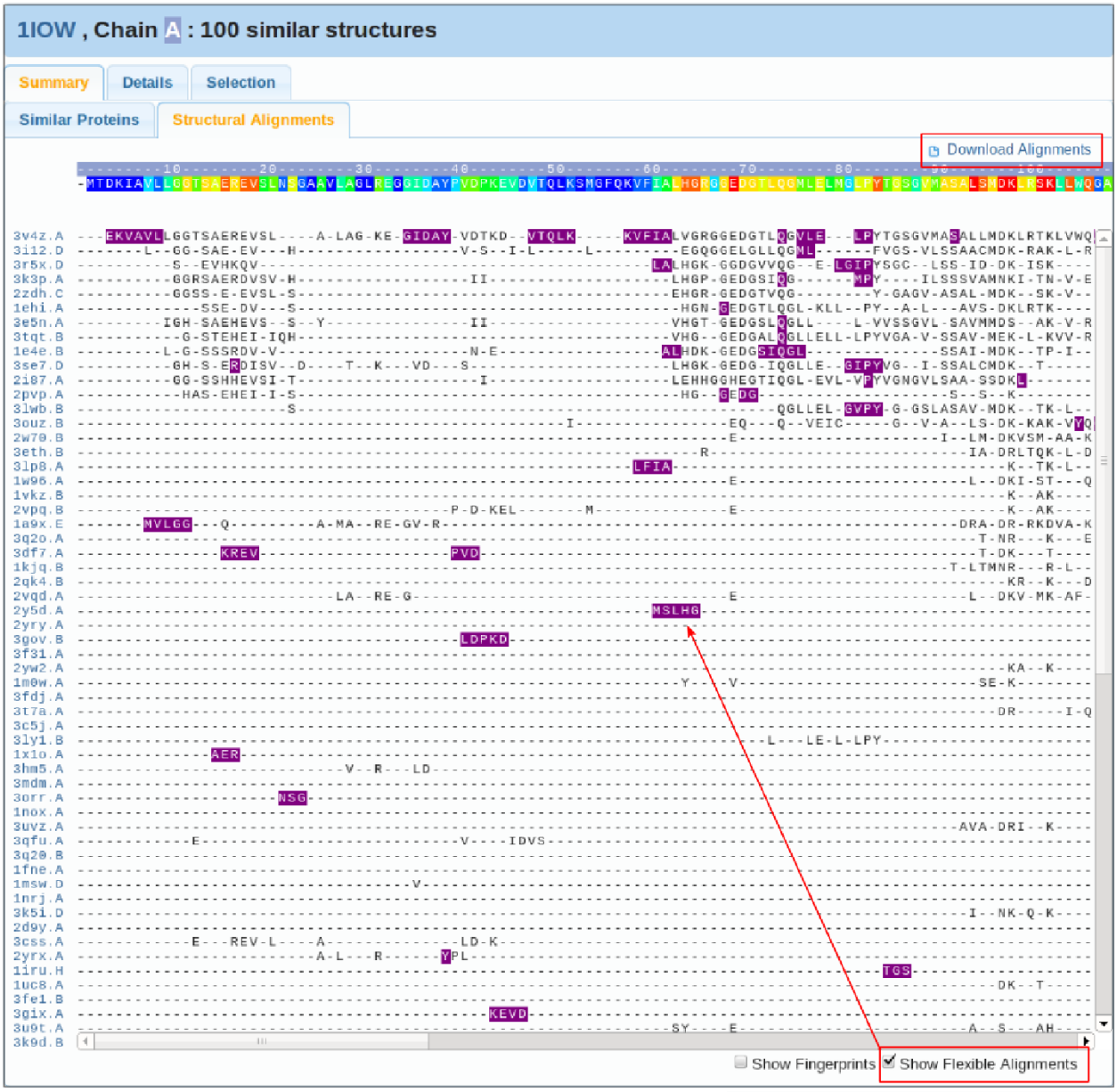


Figure 12. Structure-based sequence alignments. On the top is the query protein's sequence colored by degrees of structural conservation. The ruler above this sequence is colored by the Chain ID(s) of the query protein. Below are aligned amino acid residues of similar proteins. The flexible alignments, i.e., residues of similar proteins that cannot be aligned to a RMSD<2 A, are highlighted in purple. Click on aligned residues loads the alignment between the similar protein and the query protein into the Jmol viewer. Click on a PDB/Chain ID of an aligned protein structure, will open a new tab with the information about this structure in the RCSB Protein Data Bank. Click on the Download Alignments downloads a file with the aligned sequences in the CLUSTAL format.

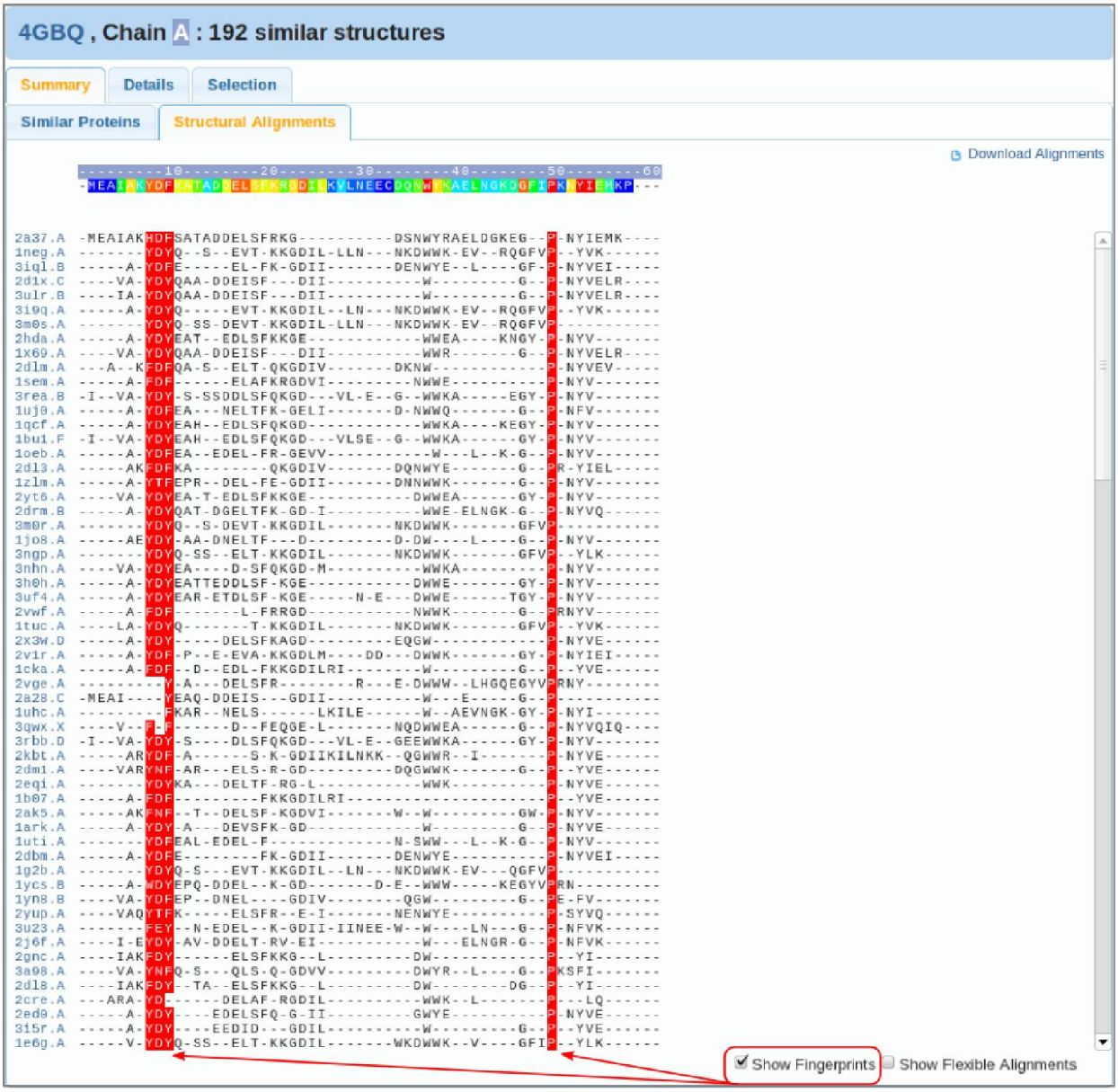


Figure 13. Fingerprint residues are highlighted as red vertical stripes, and often correspond to conserved active site residues. They are not always available, because they cannot always be determined, e.g., when there are no similar proteins with Z-Score>3.0.

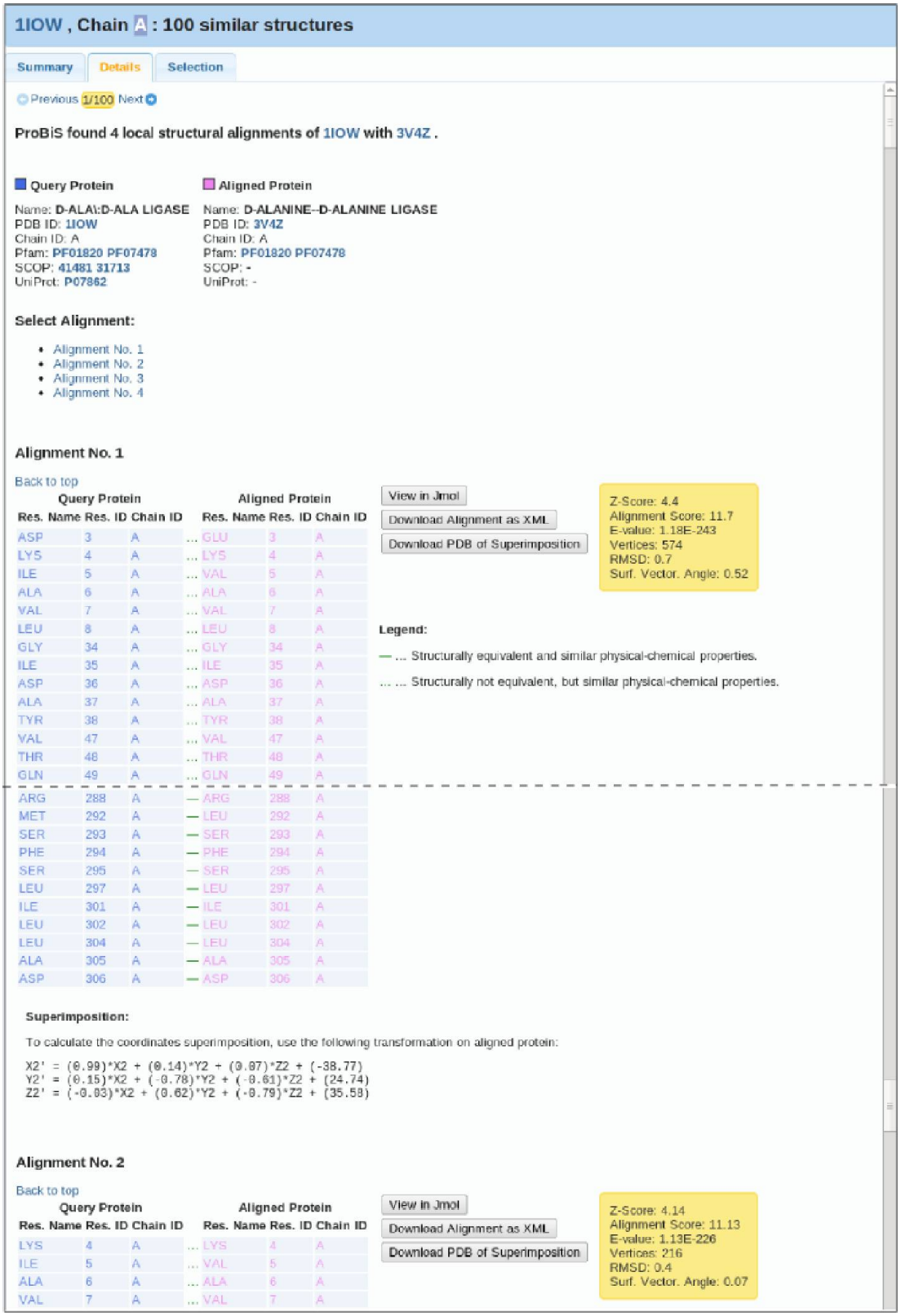


Figure 14. Detailed pairwise alignment. ProBiS found in this case four different alignments. Click on the Next at the top, opens the detailed alignments for the next similar protein.

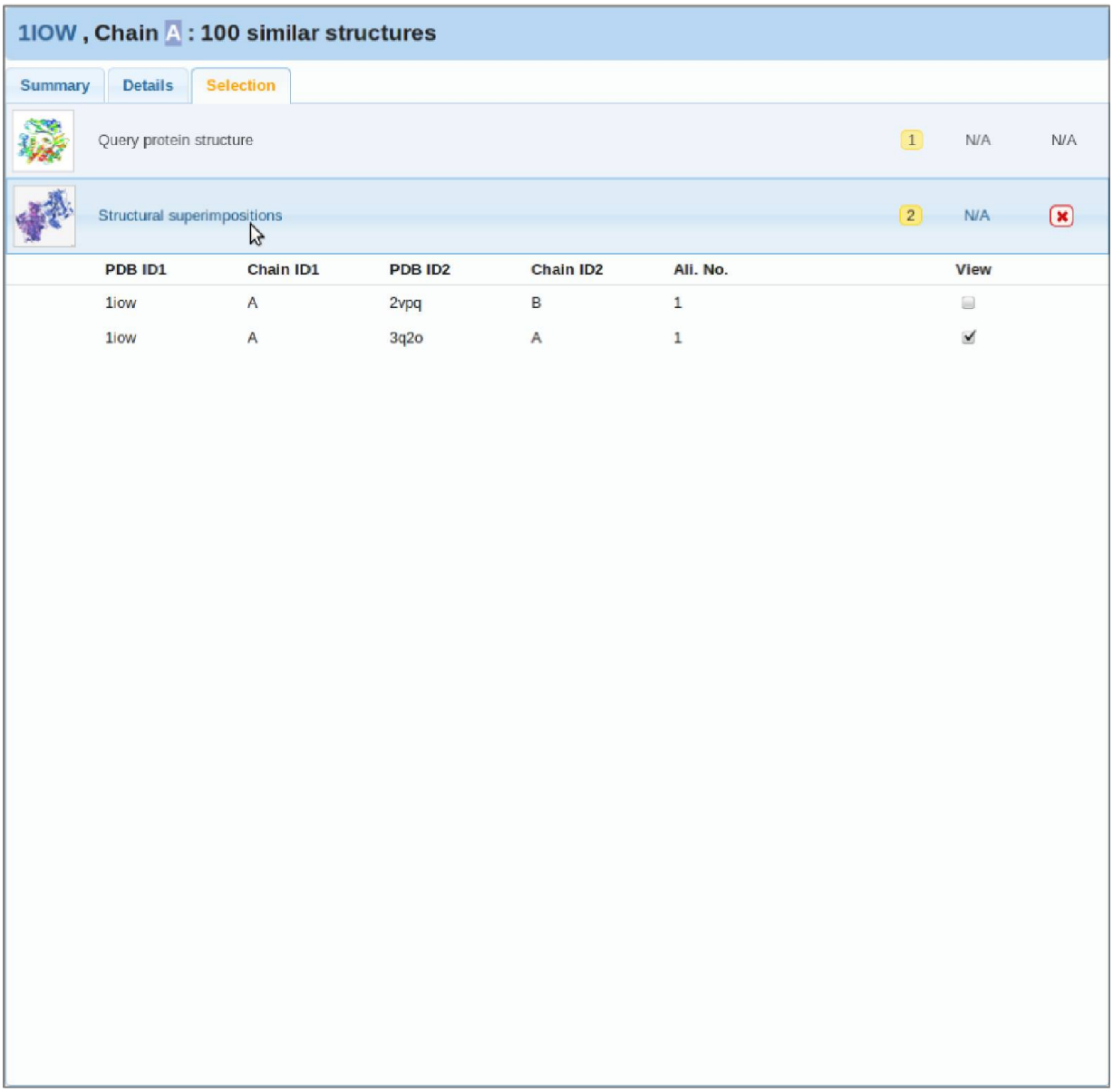


Figure 15. The Selection tab holds a list of structures that are loaded in Jmol viewer (but may be also hidden from view). Box with the Query protein structure is always present, and expands on click, showing the Asymmetric Units and Biological Units of the query protein. Box with the Structural superimpositions expands to show alignments that are already in the Jmol viewer. You can use the View checkbox to view or hide the alignments or query protein.

Pairwise Local Structural Alignment

The access to this tool from the ProBiS main page is shown in Figure 16.

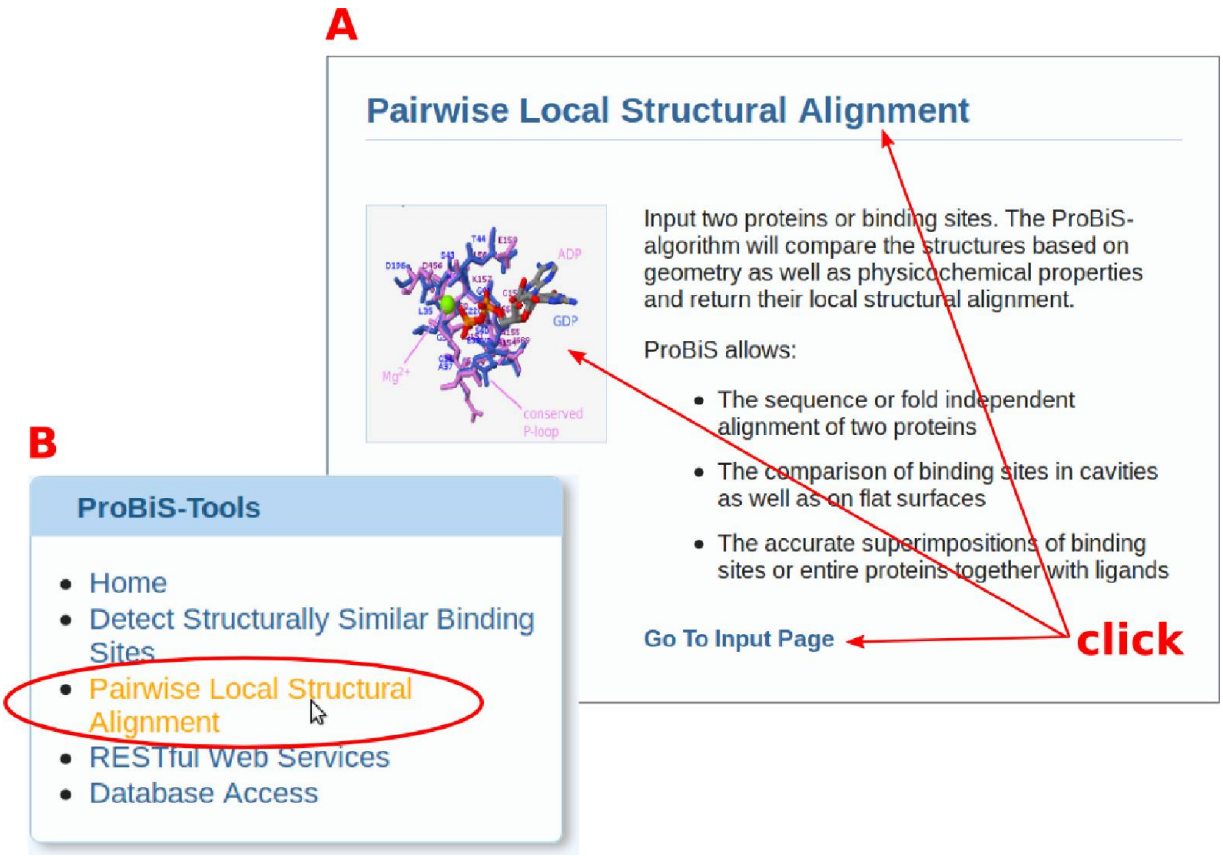


Figure 16. The access to the Pairwise Local Structural Alignment tool is from the ProBiS main page, as shown in panels (A) and (B).

Input

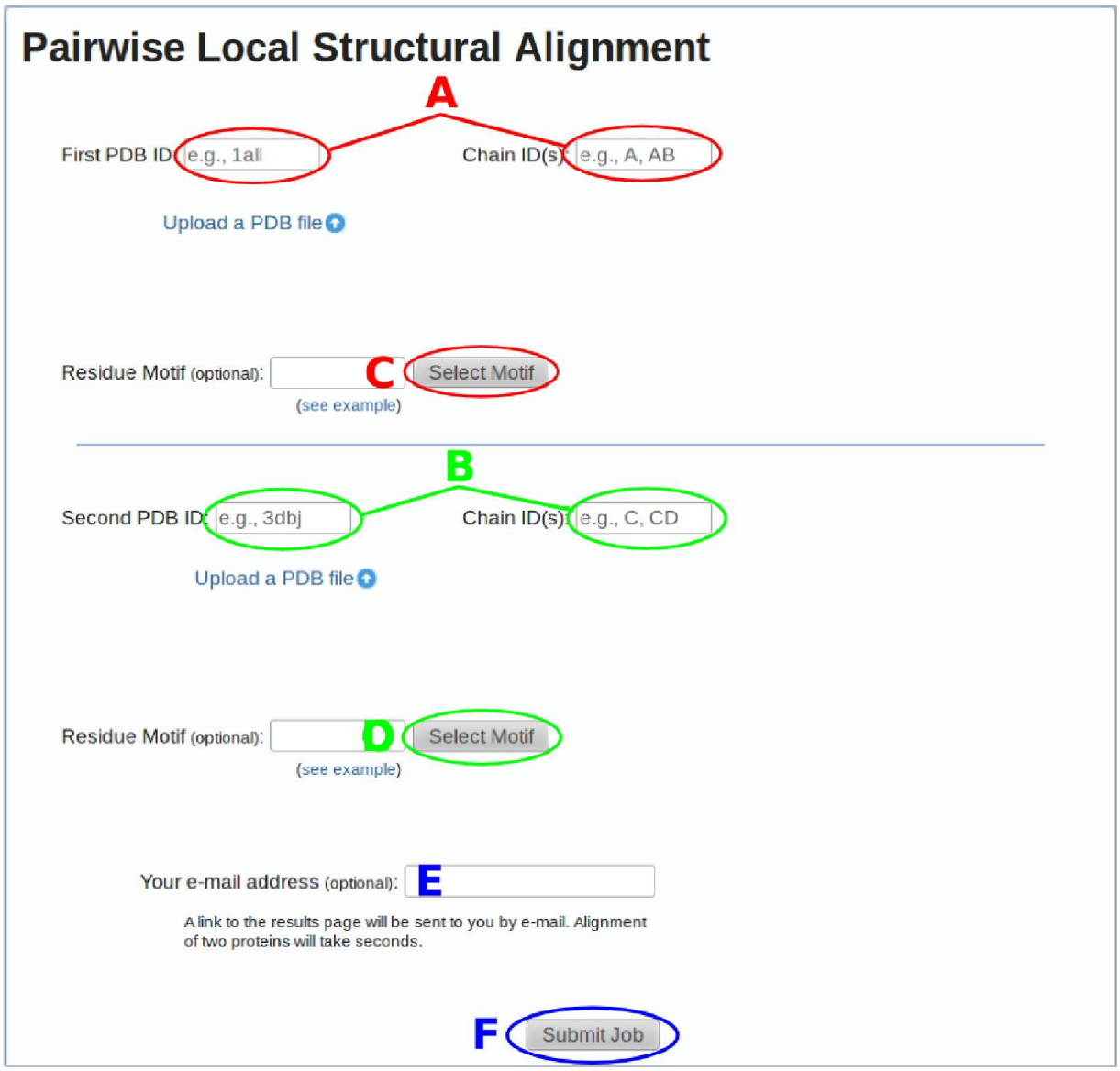


Figure 17. Pairwise Local Structural Alignment input page. To align a pair of complete proteins the user should only fill in (A) and (B) fields, (E) optionally provide email address, and (F) submit the query. To compare only selected structural motifs of one or both proteins, e.g., binding sites, the (C) and (D) buttons should be used in the same way as shown in Figure 6.

Output

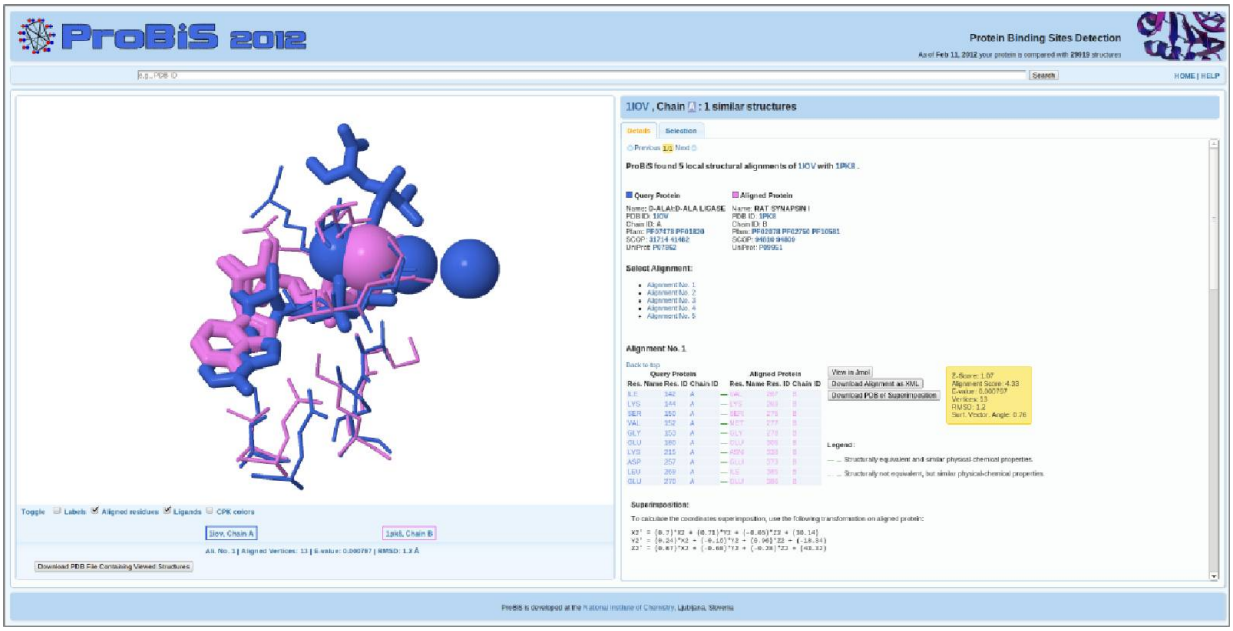


Figure 18. Pairwise Local Structural Alignment output page. This page shown only the structural alignment of the two inputted proteins.

ProBiS Web Server RESTful Web Services

The ProBiS web server features RESTful (Representational State Transfer) web services to make the binding site similarities and local pairwise alignments for any PDB protein structure easily accessible from your scripts. Full set of commands and useful examples are listed below.

Input

All commands below are working URLs, but in the text they do not appear as full URLs. To use the command "/rest/align?structure_id1=1all.A&structure_id2=3dbj.C", you should precede it with "http://probis.cmm.ki.si/update2012", e.g., the full command is "http://probis.cmm.ki.si/update2012/rest/align?structure_id1=1all.A&structure_id2=3dbj.C".

Pairwise Alignment of PDB Structures

Examples:

- Align two entire PDB structures: /rest/align?structure_id1=1all.A&structure_id2=3dbj.C
- Align two binding sites (defined as residues in 7 Å radius of ligands): /rest/align?structure_id1=1all.A&bsite1=CYC.175.A.7&structure_id2=3dbj.C&bsite2=CYC.201.C.7
- Align two entire PDB structures and return superimposition PDB file: /rest/align?structure_id1=1all.A&structure_id2=3dbj.C&return=pdb

Specification:

- **structure_id1, structure_id2** - Specifies the PDB/Chain ID(s) of PDB structures to compare (ex. 1all.A, 3dbj.C). For two chains use 1all.AB, 3dbj.CD.
- **bsite1, bsite2** (optional) - Specifies the binding sites to compare (ex. CYC.175.A.7, CYC.201.C.7). E.g., CYC - ligand name, 175 - ligand number, A - chain id, 7 - radius in Angstroms to select residues around ligand. If omitted, entire PDB structures are aligned
- **return** (optional) - Specifies the return type, which can be pdb, json, or xml. The default is xml.

Detect Similar Proteins or Binding Sites in the Non-redundant PDB

Caution: Examples in this section require all CPUs of the ProBiS-web server, and can load several minutes depending on the server work load.

Examples:

- Search the non-redundant PDB (nr-PDB) with a query protein: /rest/scan?structure_id=5cyt.R
- Search the nr-PDB with a binding site (defined as residues in a 5 Å radius of a ligand): /rest/scan?structure_id=5cyt.R&bsite=HEM.105.R.5
- Search the nr-PDB with a query protein and return alignments with Z-Score>2.0 in json format: /rest/scan?structure_id=5cyt.R&z_score=2.0&return=json

Specification:

- **structure_id** - Specifies the PDB/Chain ID(s) of the query PDB structure to be compared with all proteins in the nr-PDB (ex. 5cyt.R).
- **bsite** (optional) - Specifies the query binding site to search against nr-PDB (ex. HEM.105.R.5). E.g., HEM - ligand name, 105 - ligand number, R - chain id, 5 - radius in Angstroms to select residues around ligand. If omitted, entire PDB structure is considered as a query.
- **z_score** (optional) - Specifies the cutoff Z-Score; alignments with Z-Score>cutoff will be returned. The default is 1.0.
- **return** (optional) - Specifies the return type, which can be json or xml. The default is xml.

Perl Example Script

```
use strict;
use LWP::Simple qw( $ua );

# Make a request command (uncomment lines below if you want something else)
```

```
my $request = HTTP::Request->new( GET => 'http://probis.cmm.ki.si/update2012/rest/align?
structure_id1=1all.A&structure_id2=3dbj.C');
#my $request = HTTP::Request->new( GET => 'http://probis.cmm.ki.si/update2012/rest/align?
structure_id1=1all.A&bsite1=CYC.175.A.7&structure_id2=3dbj.C&bsite2=CYC.201.C.7');
#my $request = HTTP::Request->new( GET => 'http://probis.cmm.ki.si/update2012/rest/align?
structure_id1=1all.A&structure_id2=3dbj.C&return=pdb');
#my $request = HTTP::Request->new( GET => 'http://probis.cmm.ki.si/update2012/rest/scan?
structure_id=5cyt.R');
#my $request = HTTP::Request->new( GET => 'http://probis.cmm.ki.si/update2012/rest/scan?
structure_id=5cyt.R&bsite=HEM.105.R.5');
#my $request = HTTP::Request->new( GET => 'http://probis.cmm.ki.si/update2012/rest/scan?
structure_id=5cyt.R&z_score=2.0&return=json');

# Decide about the content type you want to get in return (default is XML) (applies to
get_alignments and get_representative; other two commands return "text/plain")
$request->header(Accept => "application/json");
#$request->content_type( 'application/xml' );

# Send the HTTP request
my $response = $ua->request( $request );

# Check to see if there is an error
unless( $response->is_success ) {
print "\n Error: ", $response->status_line, "\n";
}

# Output response
print "ProBiS returned:\n", $response->content;
```

Output

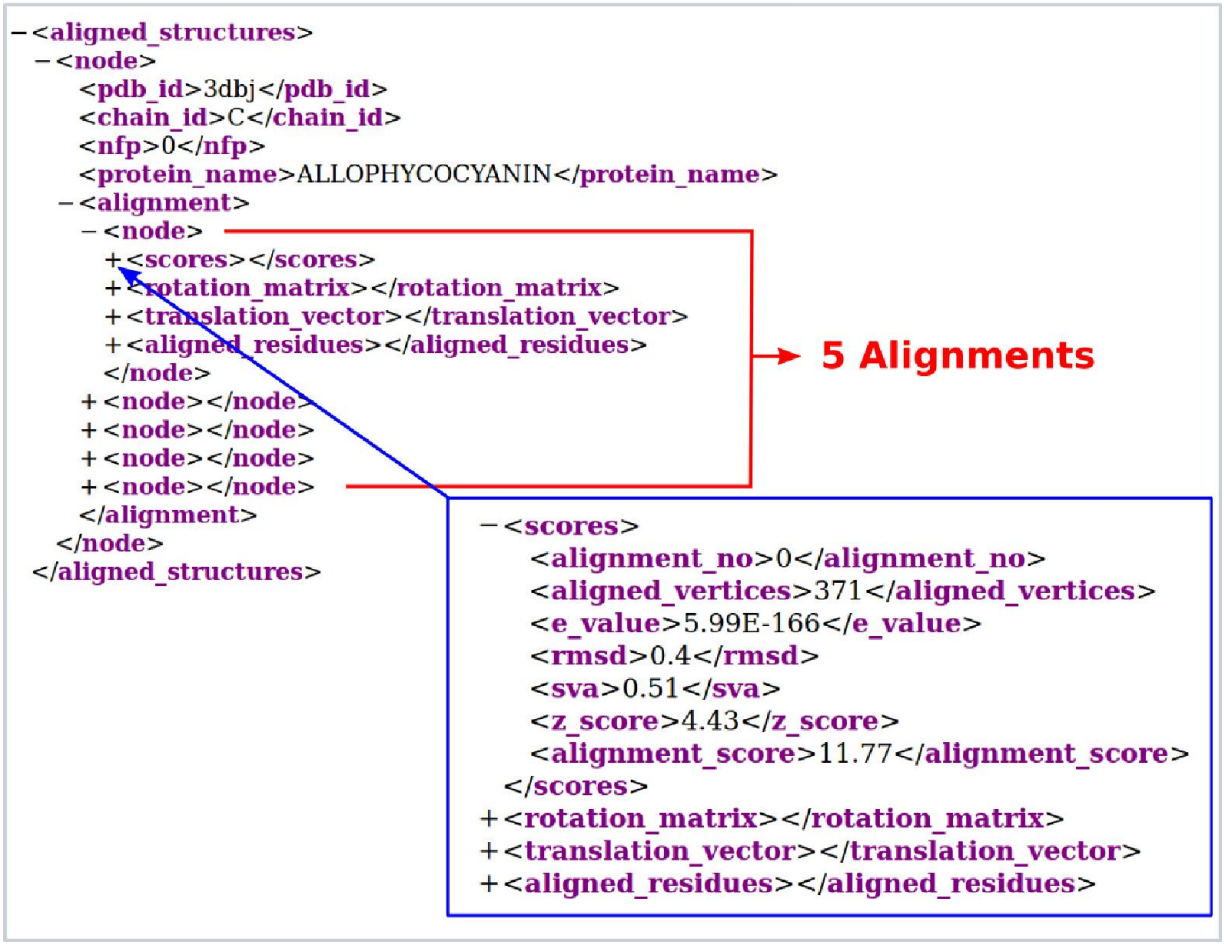


Figure 19. The web services output for alignment of two proteins 1all.A and 3dbj.C. The command is http://probis.cmm.ki.si/update2012/rest/align?structure_id1=1all.A&structure_id2=3dbj.C. Local structural alignments are in XML format. Alternatively, they can be returned in Json format**.**

ProBiS-Database Access

ProBiS-Database can be accessed through the pop-up window in the *Detect Structurally Similar Binding Sites* tool as shown in Figures 5 and 6. Here, we present the other means of access: through the search text box shown in Figure 20, through the ProBiS-Database widget in Figure 22, and from user scripts by ProBiS-Database web services.

Input

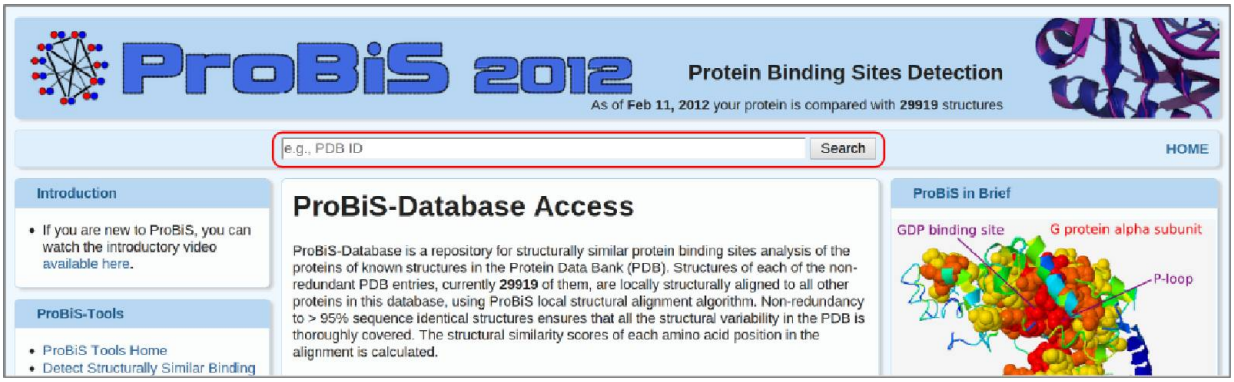


Figure 20. The ProBiS-Database access from the search text box, which is marked with red. This is the most common way of access, since it is available on all ProBiS web pages.

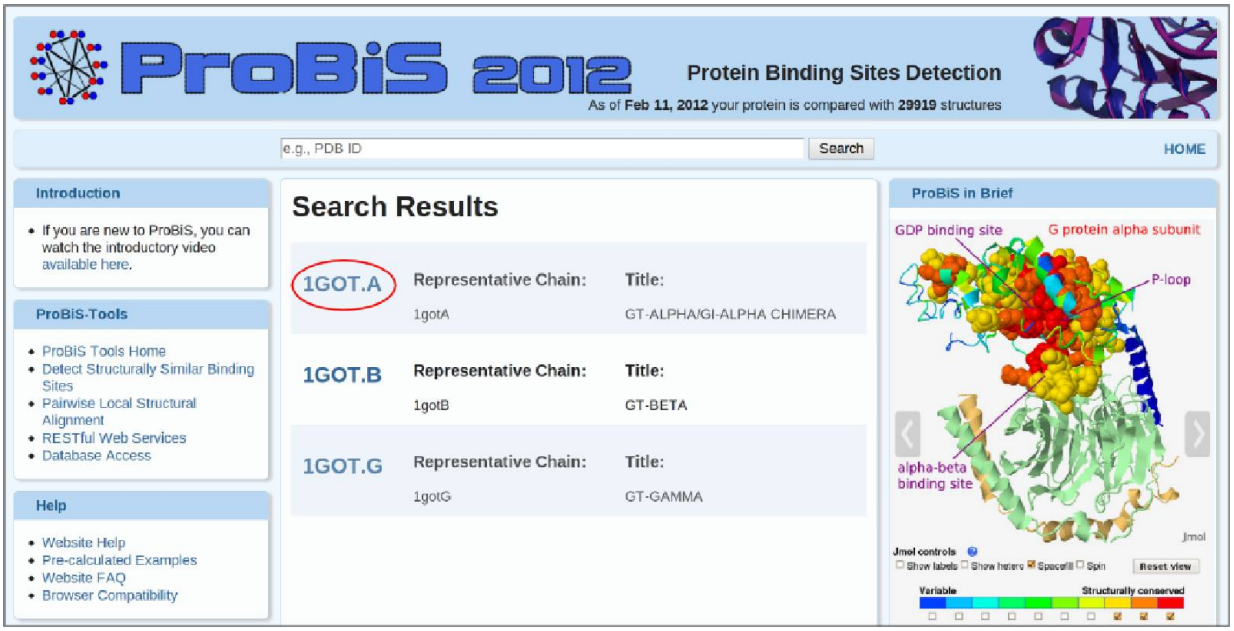


Figure 21. The search results for query protein “1got”. Three Chain IDs, A, B, and G are found in this protein. Click on the red encircled link opens precalculated results page for 1got.A.

The ProBiS-Database Widget

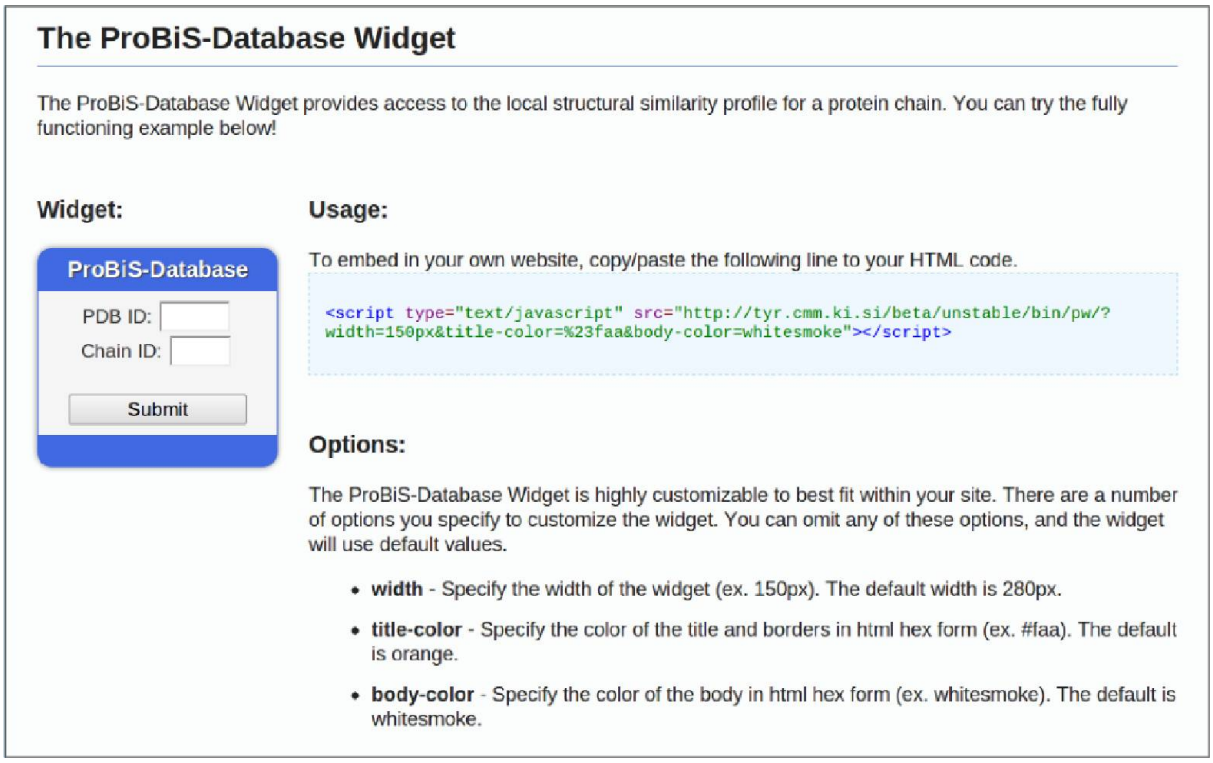


Figure 22. ProBiS-Database widget can be included in any web page to provide access to the ProBiS-Database.

The ProBiS-Database RESTful Web Service Interface

The ProBiS-Database features RESTful (REpresentational State Transfer) Web Services to make our data easily accessible from your scripts. ProBiS-Database contains data only for non-redundant PDB chains. This means that you have to use representative non-redundant PDB & Chain IDs as queries (see examples below).

Get Representative
Translate your PDB & Chain ID to > 95% sequence identical representative:
/rest/get_representative?structure_id=1ytf.A

Get Local Structural Alignments
Get local structural alignments with Z-Scores>2.0 for a representative: /rest/get_alignments?structure_id=1ytb.B&z_score=2.0

Get Superimposition
Get the PDB file of two superimposed representative structures: /rest/get_superimposition?structure_id1=1all.A&structure_id2=3nmd.B&alignment_no=1

Get Similarity Scores

Get representative with similarity scores in beta-factors at Z-Score>2.0:
/rest/get_beta_marked_PDB?structure_id=1all.A&z_score=2.0

Perl Example Script

```
use strict;
use LWP::Simple qw( $ua );

# Make a request command (uncomment lines below if you want something else)
my $request = HTTP::Request->new( GET =>
'http://probis.cmm.ki.si/update2012/rest/get_alignments?structure_id=1all.A&z_score=2.0');
#my $request = HTTP::Request->new( GET =>
'http://probis.cmm.ki.si/update2012/rest/get_representative?structure_id=1all.A');
#my $request = HTTP::Request->new( GET =>
'http://probis.cmm.ki.si/update2012/rest/get_beta_marked_PDB?
structure_id=1all.A&z_score=2.0');
#my $request = HTTP::Request->new( GET =>
'http://probis.cmm.ki.si/update2012/rest/get_superimposition?
structure_id1=1all.A&structure_id2=3nmd.B&alignment_no=1');

# Decide about the content type you want to get in return (default is XML) (applies to
get_alignments and get_representative; other two commands return "text/plain")
$request->header(Accept => "application/json");
#$request->content_type( 'application/xml' );

# Send the HTTP request
my $response = $ua->request( $request );

# Check to see if there is an error
unless( $response->is_success ) {
print "\n Error: ", $response->status_line, "\n";
}

# Output response
print "ProBiS-Database returned:\n", $response->content;
```

Output

Refer to figure 19.

FAQ

Can I upload a protein model?

Yes. A protein model in a standard PDB file format. The ATOM records must be listed as usual on the RCSB web page, i.e., for THR residue: N, CA, C, O, CB, OG1, CG2. If the modelling program that you used reordered the ATOM records in any other way, the results you will get, will be wrong.

Can I upload a PDB file with only backbone atoms Cα?

No. PDB file must be a full-atom representation of a protein. Hydrogens are ignored.

How is the protein surface defined?

The surface of a protein is defined by rolling a 1.4 Å radius sphere over its atoms. Cavities that have no connection to the outside are not considered, i.e., only the outer surface is used.

Why upload of a PDB file failed?

PDB models from various programs, such as CHARMM or MODELLER, could be in non standard formats. The ordering of ATOM records may be different, side chain ATOM records may be missing, ENDMDL records between different models in NMR structures may not be there - these are necessary, because ProBiS does not tolerate restarting of residue numbers. If the PDB file has no chain ID record then the whole structure will be taken as chain A - if there were to be two models in such file, not separated by ENDMDL records, then upload will fail.

Why are the results wrong for an uploaded PDB?

The ATOM records must be listed in the same order as in PDBs at the RCSB web page, i.e., for THR residue: N, CA, C, O, CB, OG1, CG2. If the modelling program that you used (such as MODELLER) reordered the ATOM records in any other way, the results you get will be wrong.

Can I use more than one Chain ID as query?

Yes. Up to 3 different chain IDs within the same PDB record can be considered, e.g., if you would like to input the complex of chains A and B, just write AB in the Chain ID(s) input box.

Can I upload an NMR structure?

You can upload an NMR structure as long as each model is in own MODEL/ENDMDL record.

Can I use a binding site as query?

Yes. Use the *Select binding site* button on the input page.

What happens with co-crystallized ligands in query protein structure?

Ligands in a PDB file, i.e., all HETATM records, are ignored.

Why ProBiS finds only a few similar proteins, when I know that there should be hundreds?

Similar proteins found by ProBiS are members of the non-redundant PDB which is a subset of the entire PDB. This means that each is a representative of a cluster of homologous proteins (>95 seq.id.) in the PDB. A cluster may have hundreds of members, e.g., as in protein kinases, but ProBiS will represent all these proteins by only one structure.

What is the non-redundant PDB?

The non-redundant PDB (nr-PDB) is obtained from the entire PDB by clustering the protein chains in the PDB. Clusters with >95% sequence identical proteins are generated. Then, a representative of each cluster is chosen, which is preferably an X-ray structure with lowest Resolution. These representatives constitute the nr-PDB. The nr-PDB is updated each week.