User's Manual

Contents

MANORAA user's Manual

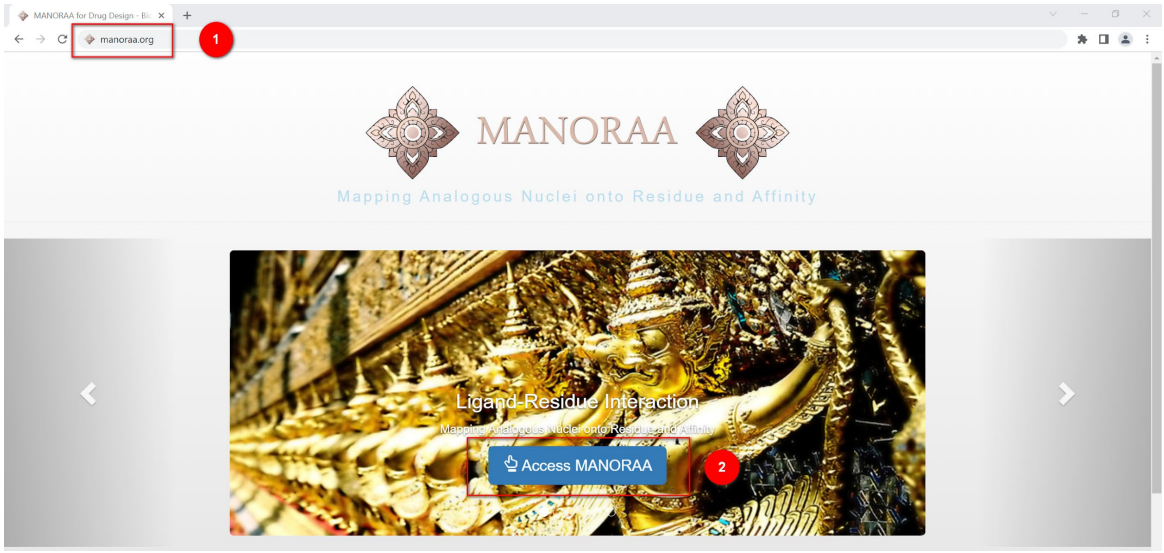
MANORAA is a website to assist structure-based drug design. The webservice is developed by the Integrative Computational BioScience (ICBS) Center, Mahidol University. Its database consisted of 116,864 PDB structures and 22,506 ligands. The users can download the output in the form of CSV file. There is also support for API and 3D visualization of protein structure from this webservice.

1. MANORAA webserver access

MANORAA system is freely accessible without user's registration. The users can access by entering https://manoraa.org at the web browser search panel.

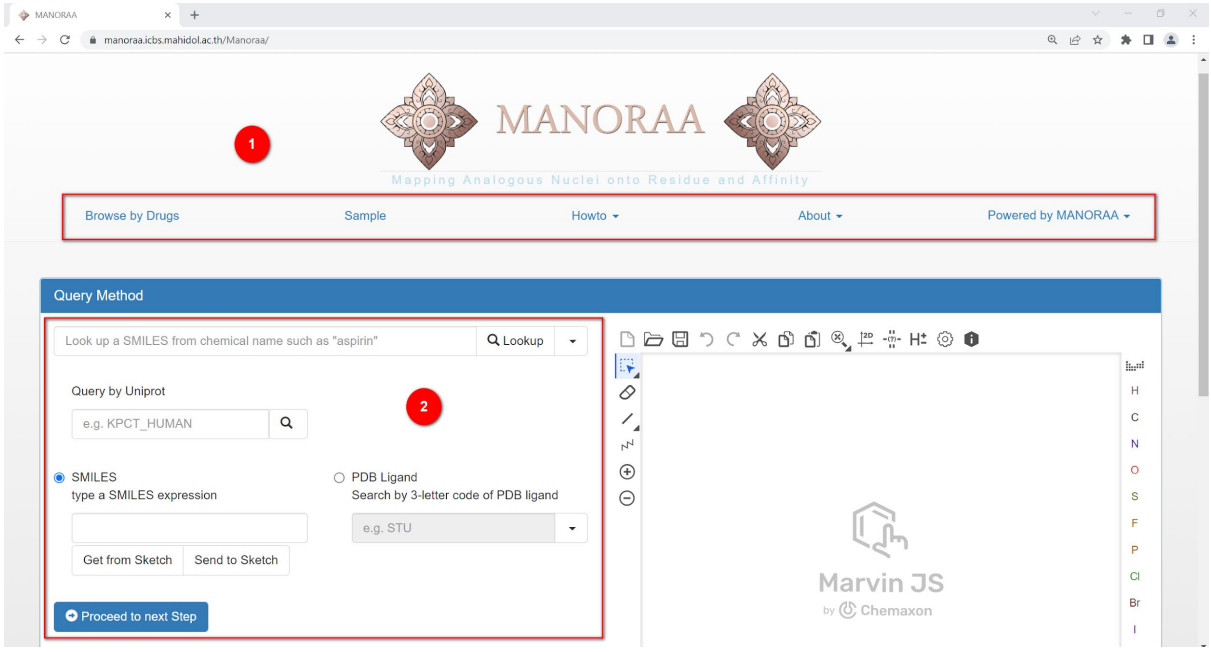
(1) Open Google Chrome or Mozilla Firefox and type the URL https://manoraa.org on the URL panel

(2) After reaching the webservice, click on "Access MANORAA" to start the service.



2. Menu Bars to access functions

On the MANORAA's website, there is this main searching panel as shown below.



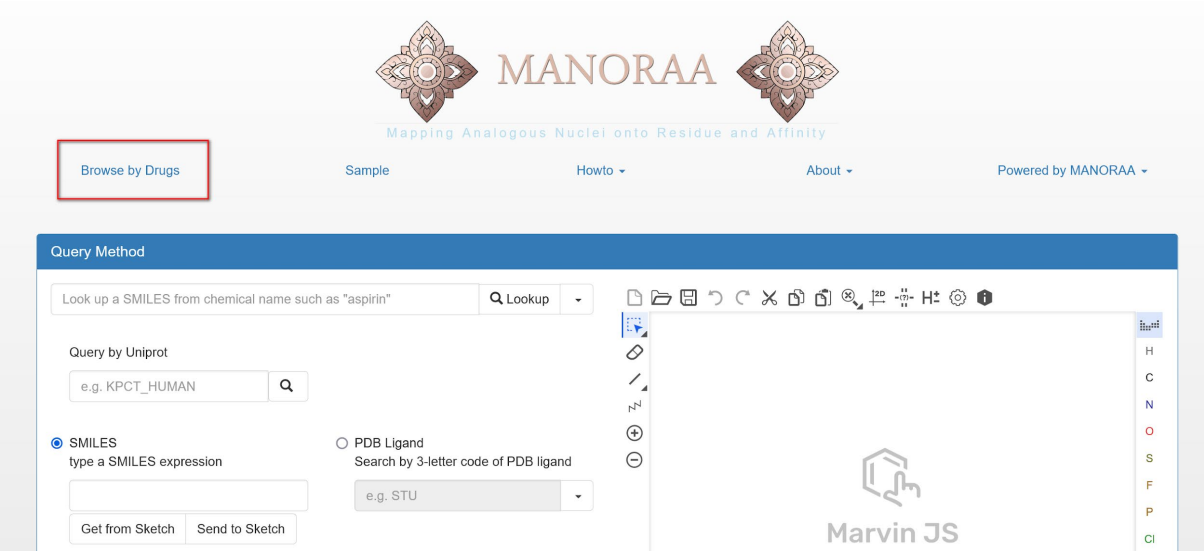
The top panel (1) consists of 5 menu bars as follows.

- Browse by Drugs: The lists of access to DrugBank's items on WRITE
- Sample: An example of Staurosporine (STU), a workable test case across every function
- Howto: Video clips describing how to use MANORAA
- About: Published scientific papers about MANORAA
- Powered by MANORAA: Other servers that relates to drug design using MANORAA backend & algorithms

The second panel (2) allows desired chemical input to get start on using MANORAA

3. MANORAA Drug Browser

User can click on the menu “Browse by Drugs” to reach all the drug compounds in the system



The panel below is shown when clicking on Browse by Drugs

The components of the “Browse by Drugs” page are as follows

(1) Drug Tabs – There are 2 tabs, the evidenced-based drug on “Drug Browser” and the Traditional Chinese Medicine (TCM) Drug Ingredients.

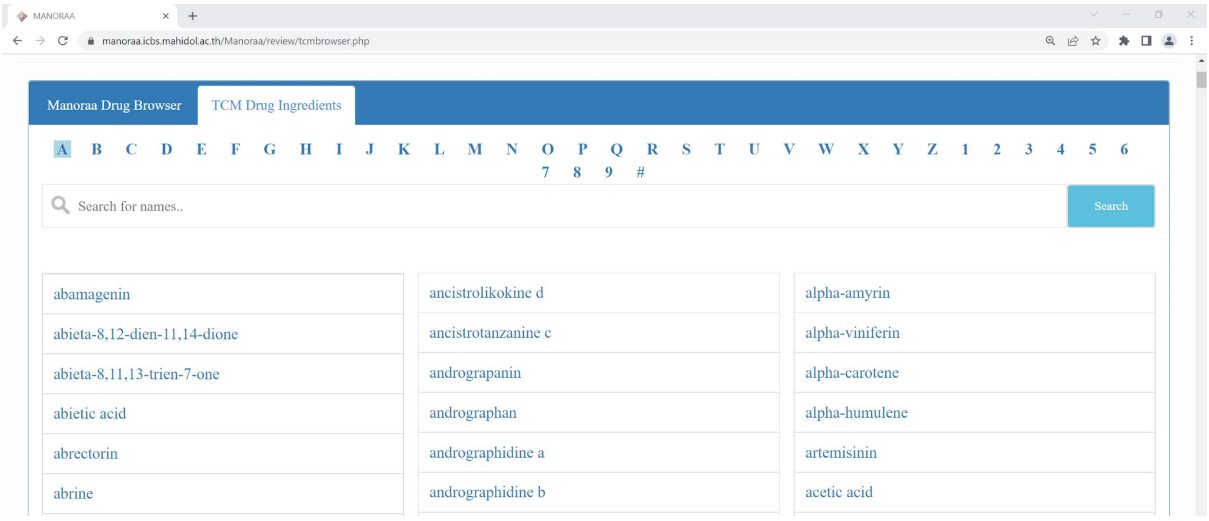
(2) Alphabetical Index – Users can click on the letters to reach the initials of the drug names

(3) Search Bar – The search panels with recommendations to the input alphabets

(4) The list of output entries – The output of several drugs retrieved from the ligands/drug input query

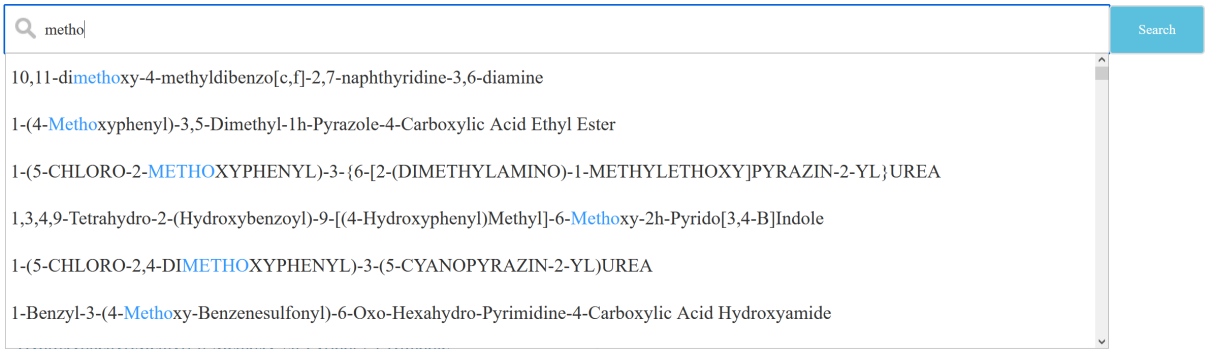
Drug Browser Tabs

The drug browser tabs are divided into evidence-based drug browser and Traditional Chinese Medicine (TCM) drug ingredients. Examples below from TCM, are listed by alphabet A.



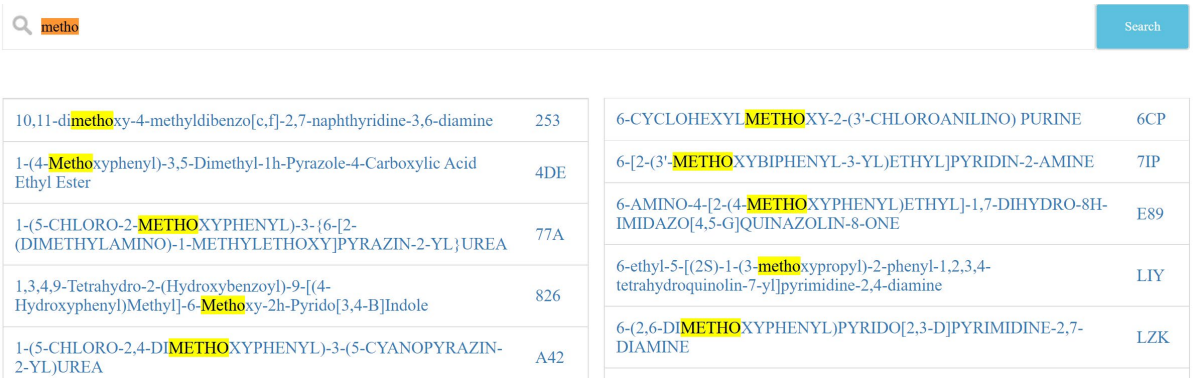
Search Bar

The search bar is used for guessing the input query. The users can either match similar words, or search the exact term and then click on search to find the drug name.



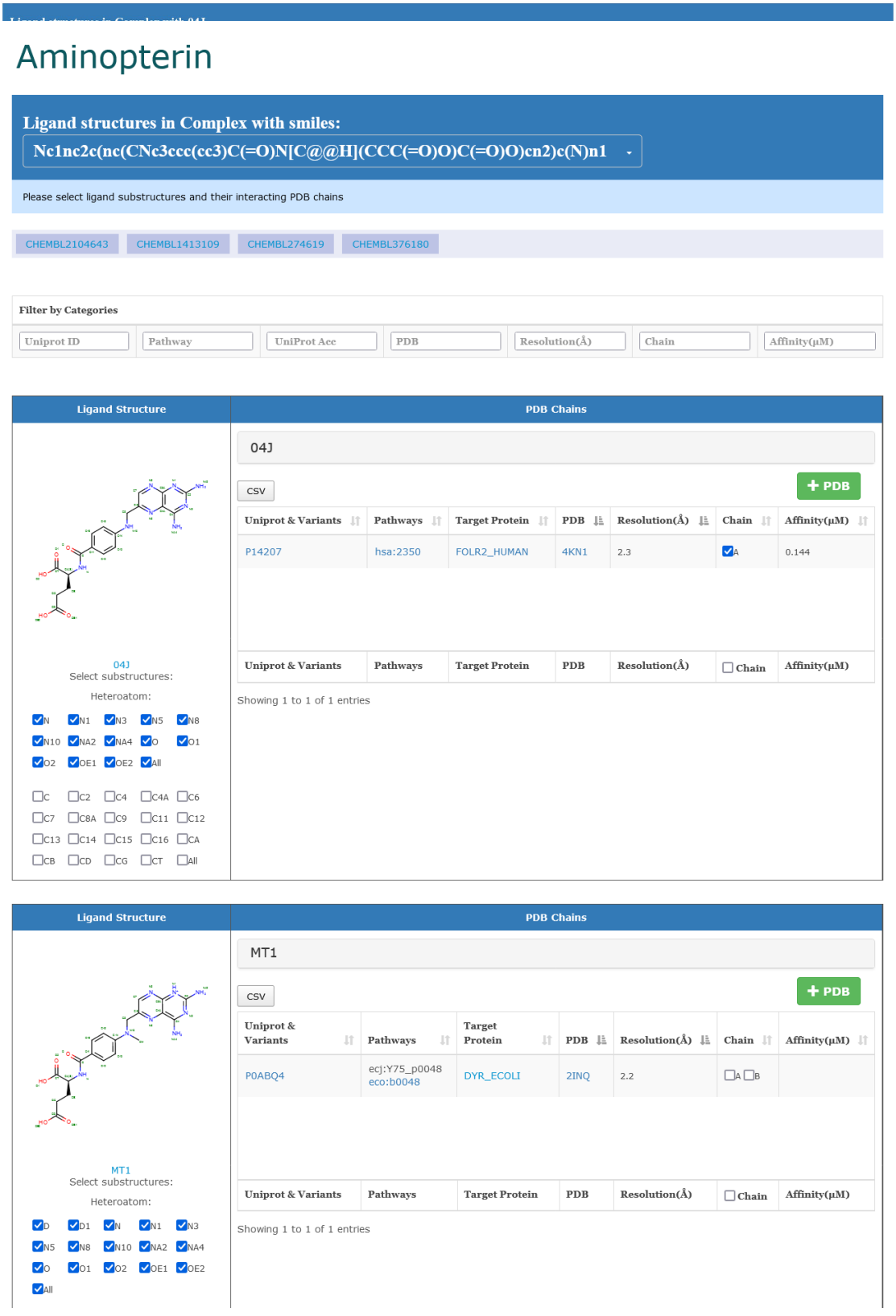
The listed entry

This is the list of output entries to show all the drug names that matches with the input queries.



After clicking on the names, the output will be retrieved by matching the SMILES string with PDB's 3-letter codes. On the other hand, the three letter codes on the side are the exact matches of the compound name and also the quickest way to retrieve the tabulated output results.

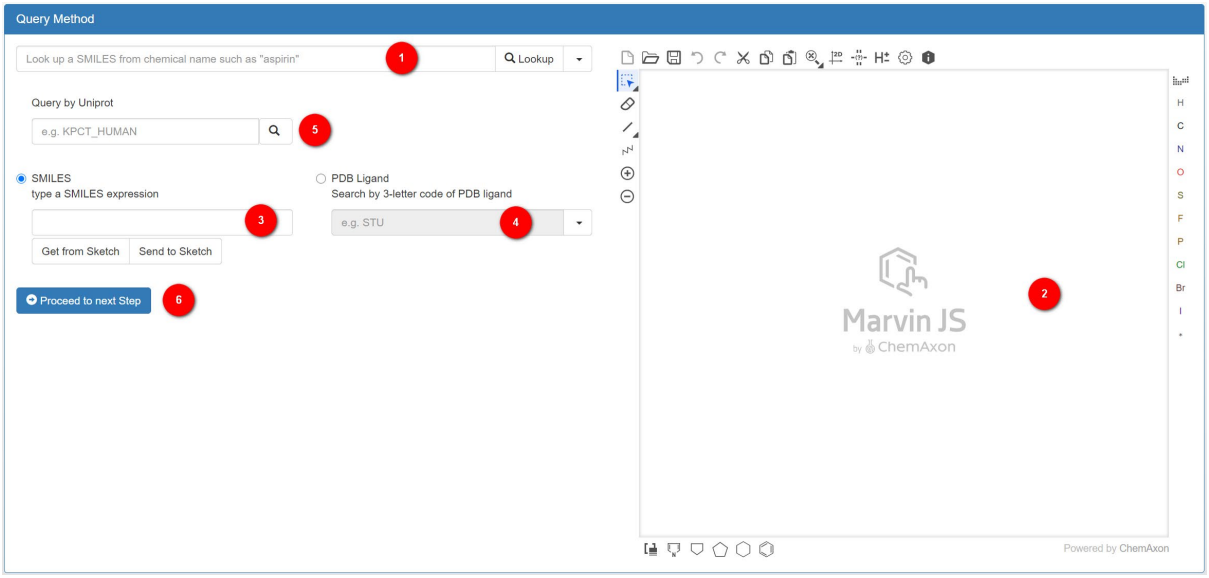
Aminopterin



Either way, once clicking on the item corresponds to a particular ligand, the system will take you to the PDBs that are found in complex with the input ligands. If any compound has some text matches by the chemical names, multiple PDB 3-letter ligand codes will appear for further analysis.

4. Query Methods

After clicking access to MANORAA, the input panels will appear in the main page as follows.



The data to be submitted to the next step can be in 2 entry forms, either by the SMILES string or by the ligand code. The query form will facilitate the user to submit a proper input format for MANORAA is various forms.

(1) Chemical name: This input box will allow human readable words that match the drug name and transform it into a SMILES string and also the 2D sketch chemical sketch on the right panel.

(2) Sketch: This tool permits the users to draw any chemical compounds or its fragments which can be translated into the SMILES string by the “Get from Sketch” button.

(3) SMILES: This box allows the user to enter the whole SMILES string to match with the chemical compound database

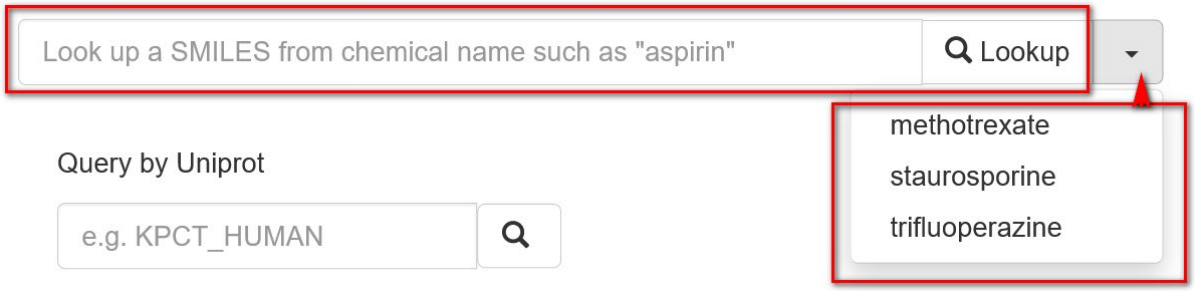
(4) PDB Ligand: This box allows input ligand in the form of PDB’s ligand 3-letter code

(5) Query by Uniprot: This box allows searching of the protein’s UNIPROT code.

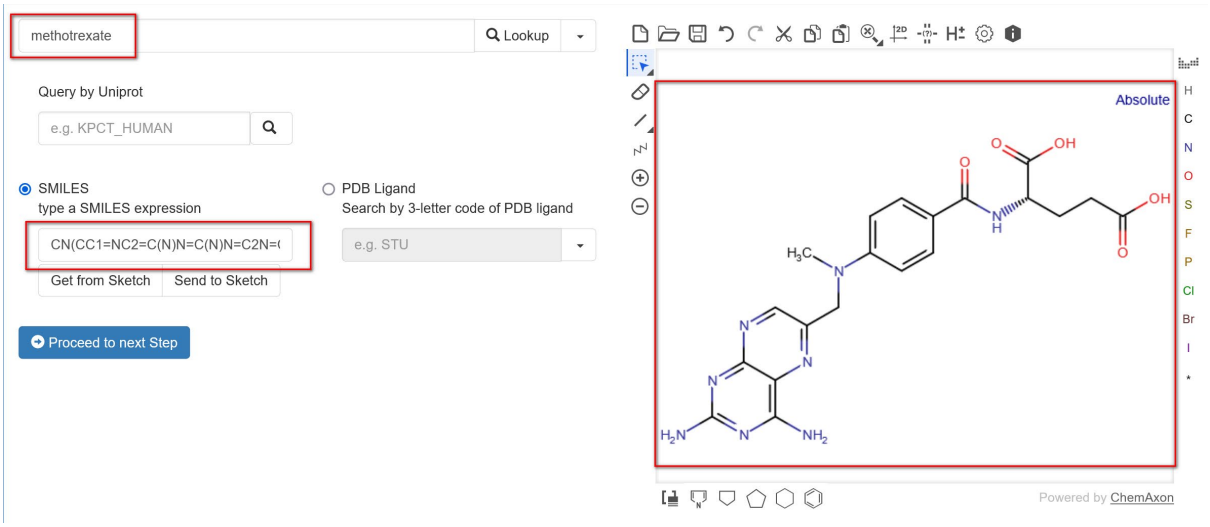
(6) From selecting the option above, then the user can click "Proceed to next step".

Entering input with Chemical name

Users can search by entering information in the search box and then tap on the Lookup symbol. The system will display SMILES string information that users just search for. As example, user can select from a list of drug names as shown in the Dropdowns.

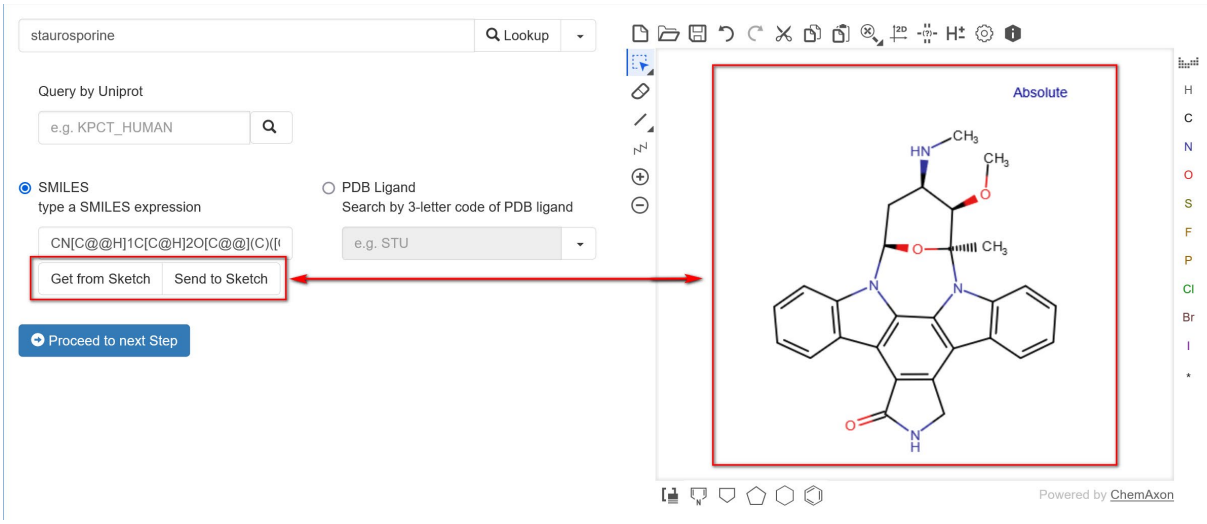


When the user presses search by drug name, the system will automatically search and put its SMILES data into the input box below and as well as a chemical sketch on the panel Sketch on the right hand side.



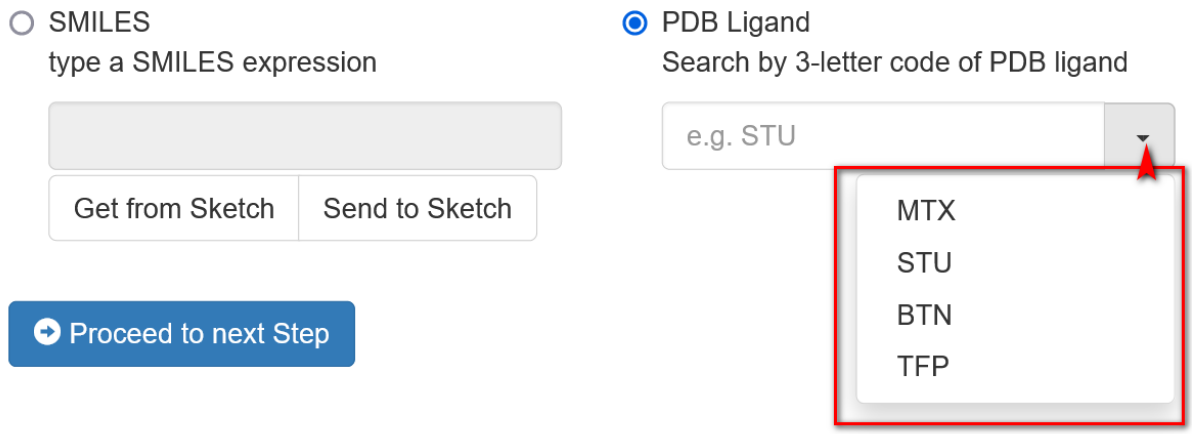
Entering input with Sketch and SMILES

Users can input data in the form of a SMILES string directly to be sent to the next step. Alternatively, users can draw a chemical sketch and convert it to SMILES. The SMILES input box and the Sketch panel can convert the data and send them back and forth with the "Get from Sketch" and "Send to Sketch" buttons.



Entering input with PDB Ligand

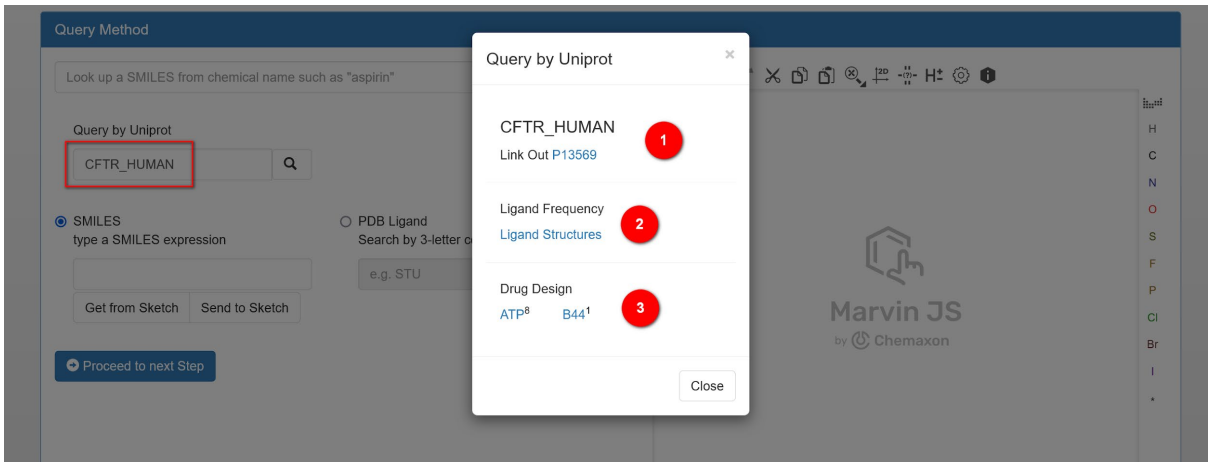
Users can enter the PDB Ligand 3-letter code directly into the input field to proceed.



As shown in the figure above, these are examples that can be chosen from a list of provided ligand three-letter codes.

Searching with the Query by UniProt function

Query by UniProt will search for proteins by Uniprot ID and will show a pop-up as follows.



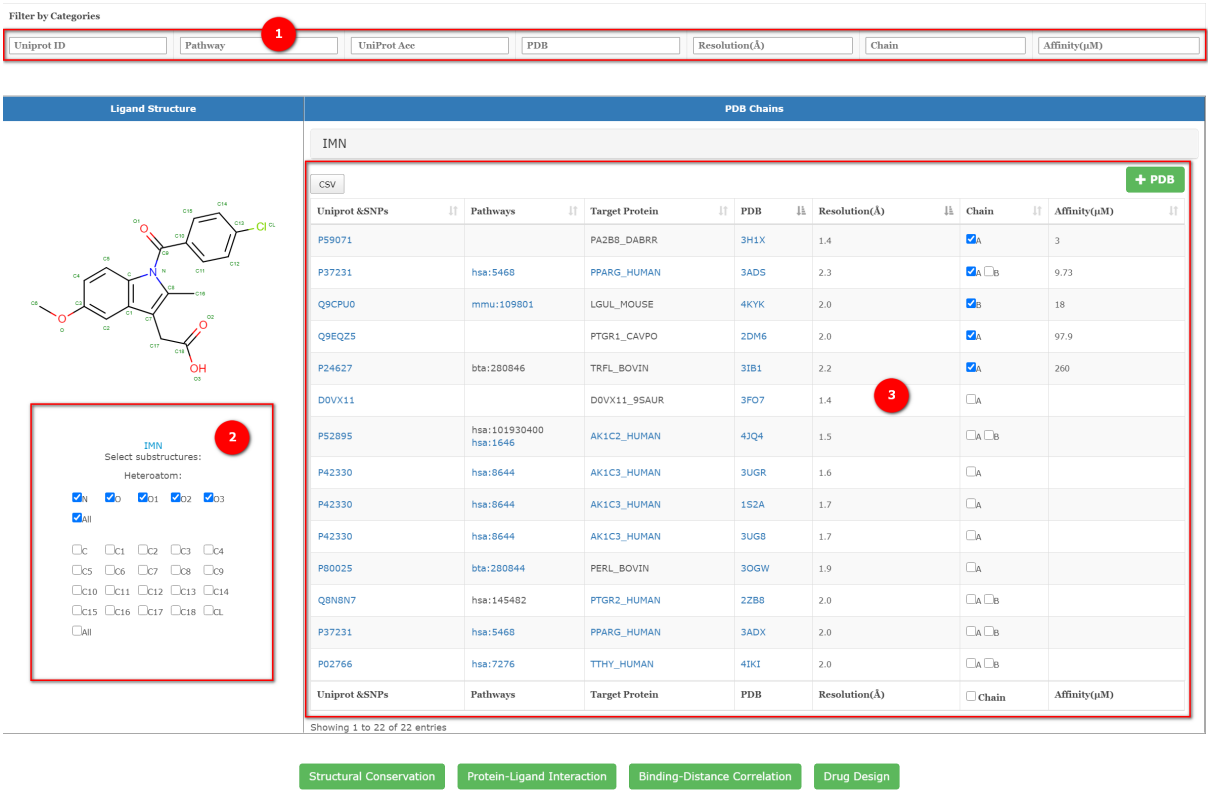
Pop-up components are as follows:

(1) Link Out is a function to bring up the link to https://www.uniprot.org by the UniProt ID that the user just searched.

(2) Ligand Frequency is a screen to display ligand structures and PDB Chains according to the list of all Ligands for each UniProt ID. The button will call the Ligand Frequency function.

(3) Drug Design displays a list of all Ligands of the searched UniProt ID, as sorted by searched Ligand's ID counts in descending orders. Under the Drug Design function of the Ligand, the program will select its substructure automatically, and overlay them accordingly.

5. Selection of atoms and PDB Chains



After the users entering input in the form on the Query Method page, and then obtaining the desired SMILES string or Ligand code, user can press the "Proceed to next Step" button. It will take the user to the Ligand structure and PDB Chains page. This page will list all PDBs in the tabulated forms according to the entered ligand code. If the input data is a SMILES string, the system will search and list all matching ligands in each separated table on the screen.

The Ligand structure and PDB Chains page consists of three sections.

(1) Filtering Options are used to filter the columns in the table.

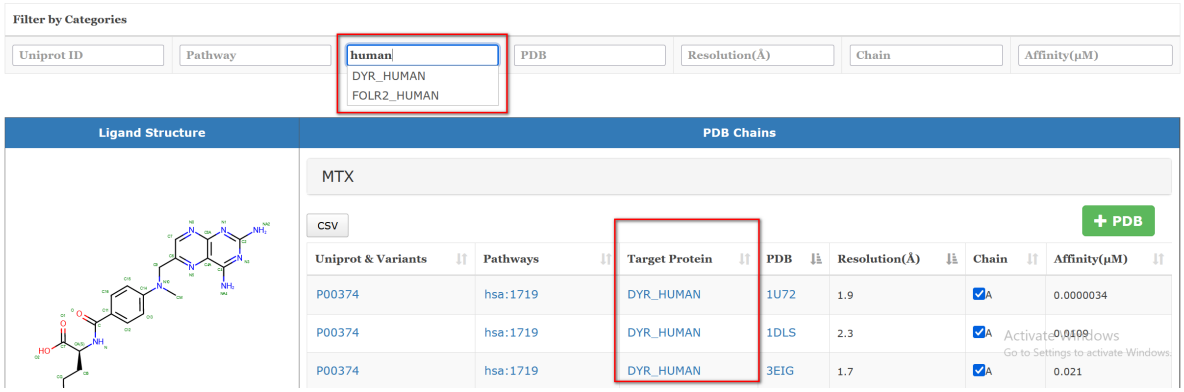
(2) Ligand substructures: The atom items can be selected in this panel in order to be used for the protein overlay.

(3) PDB Chains table: The PDB list are displayed. The user can select the PDB they want to overlay for the next structural analyses.

MANORAA System will automatically select Hetero-atoms and PDB with binding affinity as a default mode. The preliminary analysis can be done quickly by selecting all heteroatoms and selecting the first chain of the PDB that contains Binding Affinity value data under this default mode, and adjust the parameters later on after the users obtained enough information of what to look at from the first screen.

Filtering Options

Users can enter data into the desired filter fields. The system will display a list of recommended matches to select and filter in the display table.



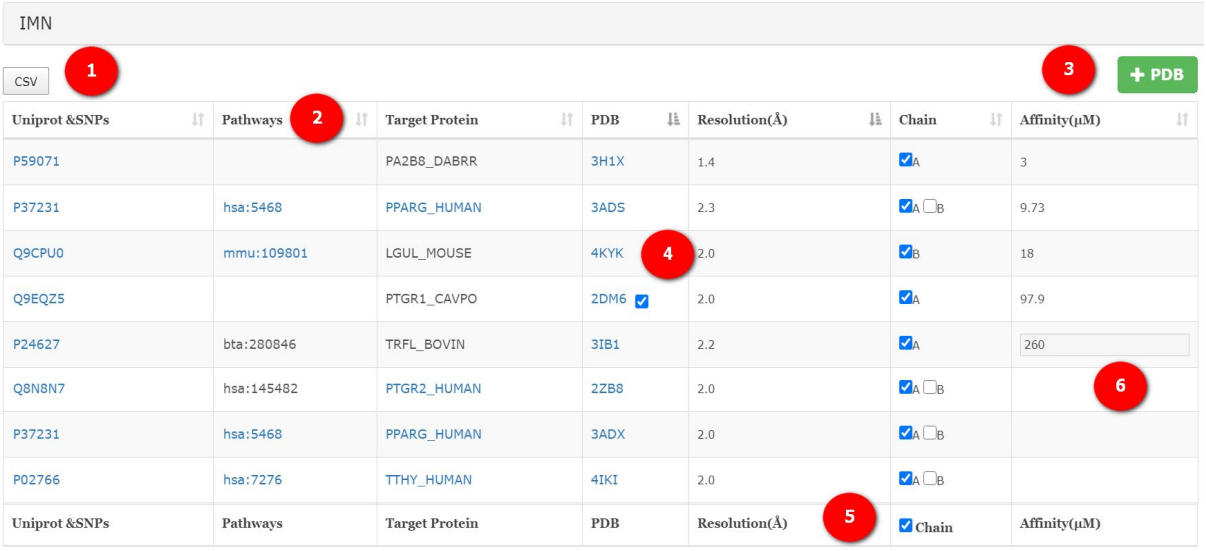
PDB Chains table

Data in the PDB Chains display table includes UniProt, Pathways, Target Protein, PDB, Resolution, protein chain, and binding affinity.

MANORAA matches PDB ID with various databases and provides a shortcut to link to the related websites such as:

https://www.uniprot.org/ https://www.genome.jp/

https://opentargets.org/ https://www.ebi.ac.uk/



The components of the PDB Chains display table are as follows:

(1) Save as CSV – Convert table data and download it as a comma-separated value (.csv) file.

(2) Sorting – pressing these arrows to sort data in each column.

(3) Upload – Button "+PDB" is used for uploading PDB to be used in various functions of the system.

(4) Template – Select the PDB to be used as the template for the protein overlay.

(5) Select All – this button can be pressed to select the first chain of every PDB.

(6) Affinity – The user can add or edit binding affinity value for a custom set of user input structural data.

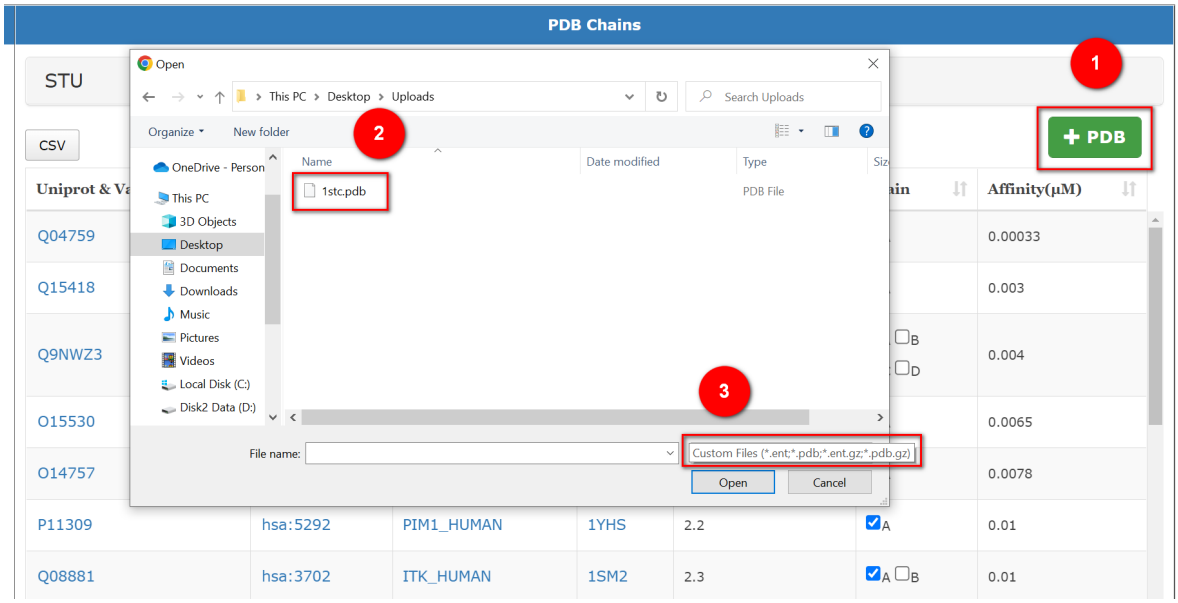
Save as CSV function

Sample data from the file MTX_pdb_chains.csv that are exported from the PDB Chains display table looks like below.

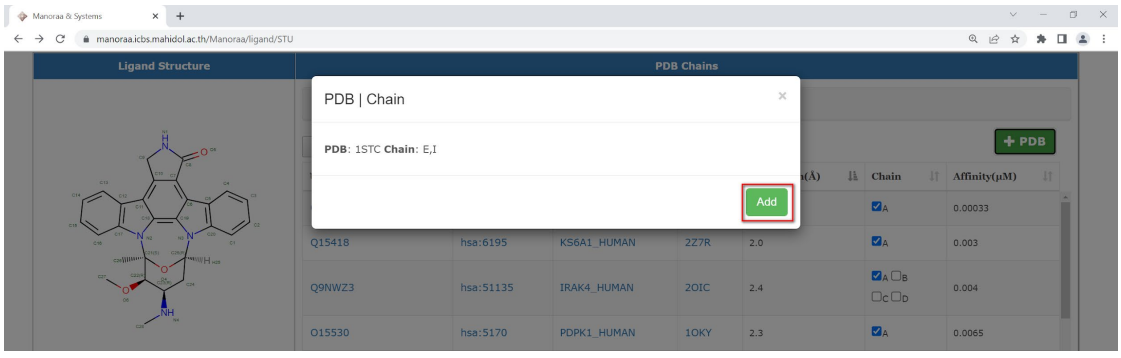
Uniprot & Variants	Pathways	Target Protein	PDB	Resolution(Å)	Chain	Affinity(μM)
P00374	hsa:1719 KEGG Pathway: hsa00670 One carbon pool by folate - Homo sapiens (human) hsa00790 Folate biosynthesis - Homo sapiens (human) hsa01100 Metabolic pathways - Homo sapiens (human) hsa01523 Antifolate resistance - Homo sapiens (human)	ANIMAL_HUMAN	1U72	1.9	A	0.0000034
P00374	hsa:1719 KEGG Pathway: hsa00670 One carbon pool by folate - Homo sapiens (human) hsa00790 Folate biosynthesis - Homo sapiens (human) hsa01100 Metabolic pathways - Homo sapiens (human) hsa01523 Antifolate resistance - Homo sapiens (human)	ANIMAL_HUMAN	1DLS	2.3	A	0.0109
P00374	hsa:1719 KEGG Pathway: hsa00670 One carbon pool by folate - Homo sapiens (human) hsa00790 Folate biosynthesis - Homo sapiens (human) hsa01100 Metabolic pathways - Homo sapiens (human) hsa01523 Antifolate resistance - Homo sapiens (human)	ANIMAL_HUMAN	3EIG	1.7	A	0.021
P14207	hsa:2350 KEGG Pathway: hsa01523 Antifolate resistance - Homo sapiens (human) hsa04144 Endocytosis - Homo sapiens (human)	FOLR2_HUMAN	4KN0	2.1	A	0.04

Upload function

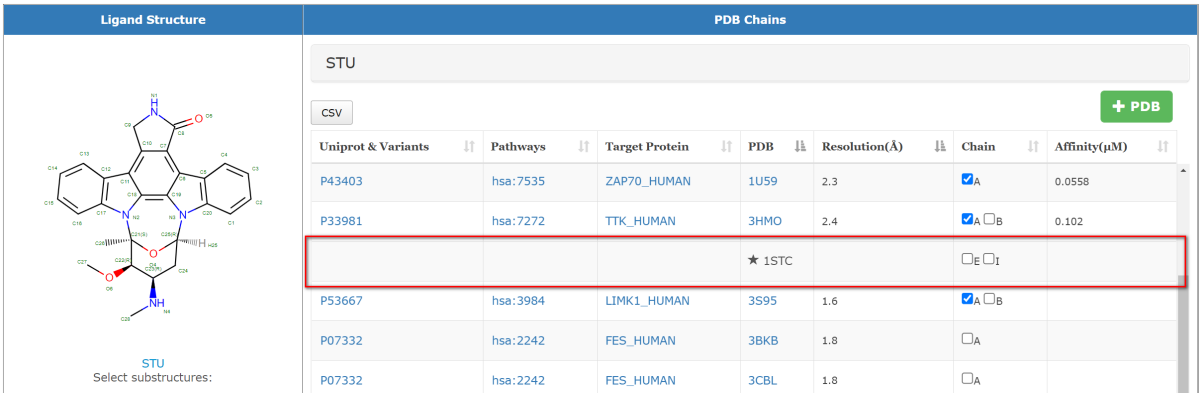
Users can upload the desired PDB file to be used with various functions in the system by pressing the "+PDB" button. Then users can select the desired file from their own computer and press “Open”. The structural data that can be uploaded are in the .ent, .pdb, . ent.gz and .pdb.gz file format with a maximum size of 25 MB.



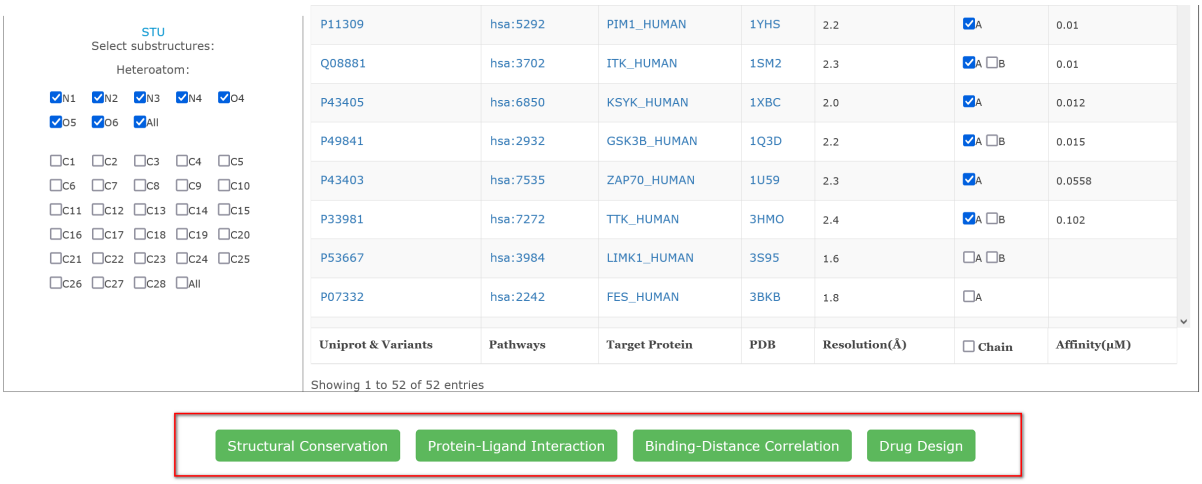
The screen will show the PDB and Chain which are retrieved from the imported data file. Once the structure file is validated, press the Add button to add it to the table.



The uploaded PDB data will be added to the table for selection.



After selecting the desired atoms and the PDB ID, the user can press any of these green buttons below the table in order to use the functions they want.



There are 4 types of functions available to use:

- Structural Conservation button
- Protein-Ligand Interaction button
- Binding-Distance Correlation Button
- Drug Design button

6. Structural Conservation Function

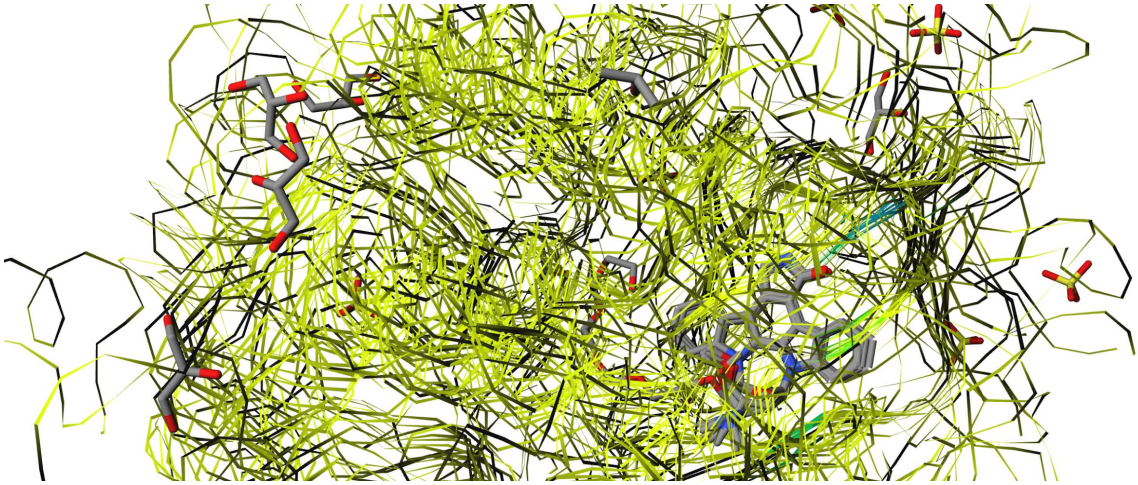
Jsmol protein superposition results are displayed in WebGL format. The selected ligand atom positions are overlaid and the points with the atoms deposited in the same position are displayed in blue. The gradient colour ranges from yellow to green and to blue as the number of atoms that are fallen into the bin increases. The most conserved entities are shown in blue.

The Structural Conservation display consists of 2 parts.

- WebGL/HTML5 – Press to toggle between WebGL and HTML5.
- 3D Panel – The section used to show the overlay of proteins.

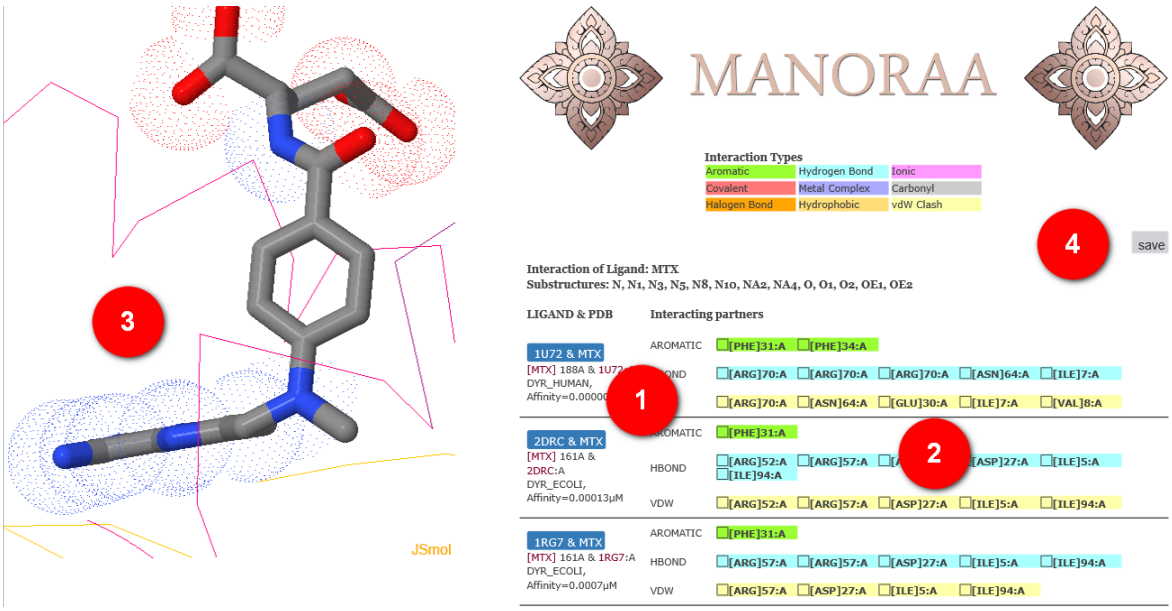
WebGL/HTML5

This button can render the overlay of the protein with either WebGL or JSmol in HTML5.



7. Protein-Ligand Interaction Function

The chosen atoms were used to calculate the protein-ligand interactions. The user can rank interactions that occurred between atoms and all the surrounding amino acid groups according to the binding affinity. For example, when the user sorts the binding affinities from lowest to highest, the numbers of hydrogen bonds can be found from the tightest bound to the loosely bound binding interactions.



The Protein-Ligand Interaction display consists of 4 sections.

(1) PDB ID & Ligand – Press to display the selected PDB and ligand.

(2) Residue – Select the Residue that you want to display.

(3) 3D Panel - This panel is used to display the results.

(4) Save – Press to save the screen as a .png image file.

8. Binding-Distance Correlation Function

It shows the amino acid position that is essential for drug binding, and the influential distances that affect the tightness of drug binding in the form of influential distance-binding equation. The distances are drawn on to the template structure with the anchoring point for distance measurement labeled at each end of the distance bar.

Multiple alignment and influential distance equation to correlate inter-residue distance with binding affinity.

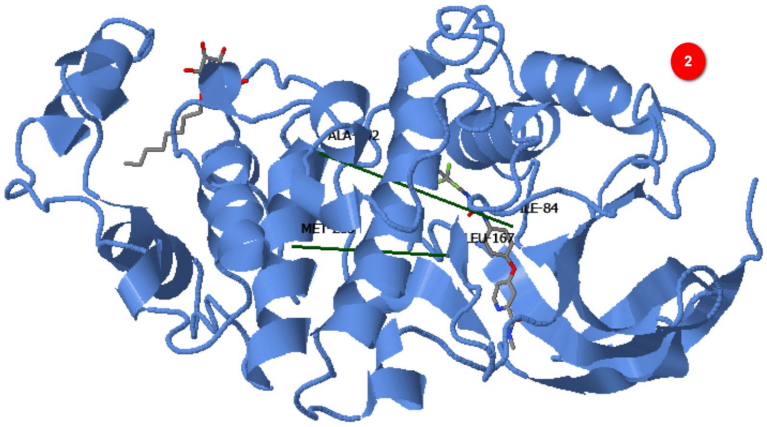
```
Ligand: BAX
Atom: F8, F9, F10, N12, N14, N26, N30, O15, O22, O32
Template: 4ASD
Structure:
      PDB: 4ASD Chain: A
      PDB: 3GCS Chain: A
      PDB: 3HEG Chain: A
      PDB: 3RGF Chain: A
```

PDB	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17	B18
3GCS	ALA-302	ARG-149	ARG-23	ASN-115	ASP-150	ASP-168	ASP-205	GLU-71	GLY-85	HIS-148	HIS-77	HIS-80	ILE-131	ILE-141	ILE-166	ILE-84	LEU-104	LEU-113
3HEG	ALA-302	ARG-149	ARG-23	ASN-115	ASP-150	ASP-168	ASP-205	GLU-71	GLY-85	HIS-148	HIS-77	HIS-80	ILE-131	ILE-141	ILE-166	ILE-84	LEU-104	LEU-113
3RGF	GLU-327	ARG-150	ARG-40	ILE-107	ASP-151	ASP-173	ASP-216	GLU-66	SER-80	HIS-149	ARG-71	HIS-75	LEU-136	LEU-142	ILE-171	ILE-79	LEU-95	LEU-104
*4ASD	SER-1154		ARG-1027		PHE-854	TYR-927	ASP-1028		ASP-1046		ASP-1087		GLU-885	ASN-900	HIS-1026		ILE-890	HIS-895

```
Influential Distance:
Log10Ki = -19.9127 +0.5207xD(B16,B1) +0.3484xD(B20,B27)
```

3RGF 3HEG 3GCS 4ASD (1)

3GCS



The Binding-Distance Correlation display screen consists of 2 parts.

(1) PDB ID – Choose the PDB you want to display.

(2) 3D Panel - This part is used to display the results.

9. Drug Design Function

It shows the position that is essential for drug binding, the distance that influences the tightness of drug binding and distance-binding affinity equation. The image of the protein overlay results can also be displayed according to the various selected options.

The Drug Design display screen consists of 2 parts.

(1) Option buttons on the left hand side, which can be chosen to display in different functions.

(2) 3D Panel - The part used to display the results.

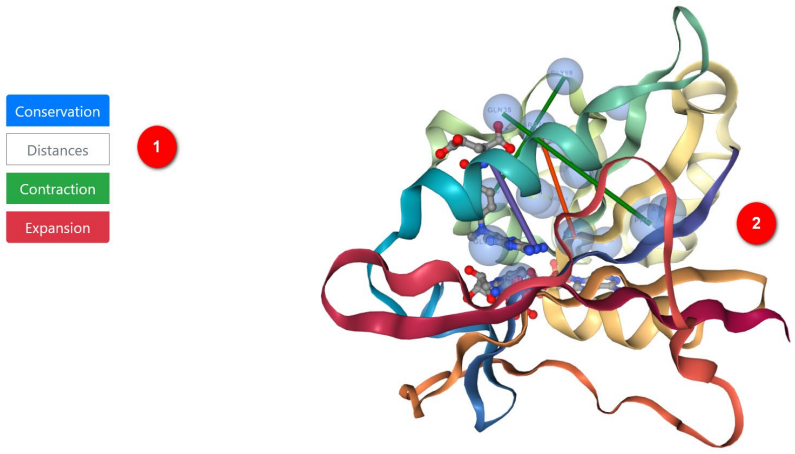
Position-specific interaction by highlighting the active site based on the percent conservation of the atomic position surrounding the ligand substructure complexes.

```
Ligand: MTX
Atom: N, N1, N3, N5, N8, N10, NA2, NA4, O, O1, O2, OE1, OE2
Template: 1U72
Structure:
      PDB: 1U72 Chain: A          PDB: 2QK8 Chain: A
      PDB: 1RG7 Chain: A          PDB: 3EIG Chain: A
      PDB: 3DFR Chain: A          PDB: 4KN0 Chain: A
      PDB: 1DLS Chain: A          PDB: 1U70 Chain: A
      PDB: 1DF7 Chain: A
```

PDB	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12	B13	B14	B15	B16	B17
1DF7	ARG-32	ARG-60	ARG-61	ASN-62	ASP-27	GLU-111	GLY-3	GLY-59	ILE-5	LEU-65	MET-42	PHE-31	PRO-51	THR-113	THR-35	VAL-64	VAL-78
1DLS	GLN-35	ARG-70	ILE-71	ASN-72	GLU-30	PHE-134	ASN-5	GLY-69	ILE-7	LEU-75	MET-52	PHE-34	ILE-60	THR-136	THR-38	VAL-74	SER-90
1RG7	LYS-32	ARG-57	LYS-58	ASN-59	ASP-27	TYR-111	SER-3	GLY-56	ILE-5	LEU-62	MET-42	PHE-31	ILE-50	THR-113	THR-35	ILE-61	VAL-75
1U70	GLN-35	ARG-70	ILE-71	ASN-72	GLU-30	PHE-134	ASN-5	ASP-69	ILE-7	LEU-75	MET-52	PHE-34	ILE-60	THR-136	THR-38	VAL-74	ALA-90
*1U72	GLN-35	ARG-70	ILE-71	ASN-72	GLU-30	PHE-134	ASN-5	GLY-69	ILE-7	LEU-75	MET-52	PHE-34	ILE-60	THR-136	THR-38	VAL-74	SER-90
2QK8	LYS-33	ARG-58	ARG-59	ASN-60	GLU-28	TYR-113	SER-4	GLY-57	MET-6	VAL-63	MET-43	PHE-96	ILE-51	THR-115	THR-36	ILE-62	ALA-77
3DFR	ARG-31	ARG-57	THR-58	ASN-59	ASP-26	LEU-114	ALA-2	GLU-56	LEU-4	LEU-62	VAL-41	PHE-30	PHE-49	THR-116	THR-34	VAL-61	VAL-76
3EIG	GLU-35	ARG-70	ILE-71	ASN-72	GLU-30	PHE-134	ASN-5	GLY-69	ILE-7	LEU-75	MET-52	PHE-34	ILE-60	THR-136	THR-38	VAL-74	SER-90
4KN0	ARG-152	LYS-147	CYS-146	HIS-82	ARG-122	TYR-191	LEU-182	PRO-164	LEU-186	LYS-91	TRP-80	TRP-187	ASN-77	PHE-123	ASN-149	ASP-81	LYS-85

```
Influential Distance:
Log10Ki = -4.5618 -0.5868xD(B2,B9) +0.3482xD(B6,B1) +0.1937xD(B8,B13)
```

1U72



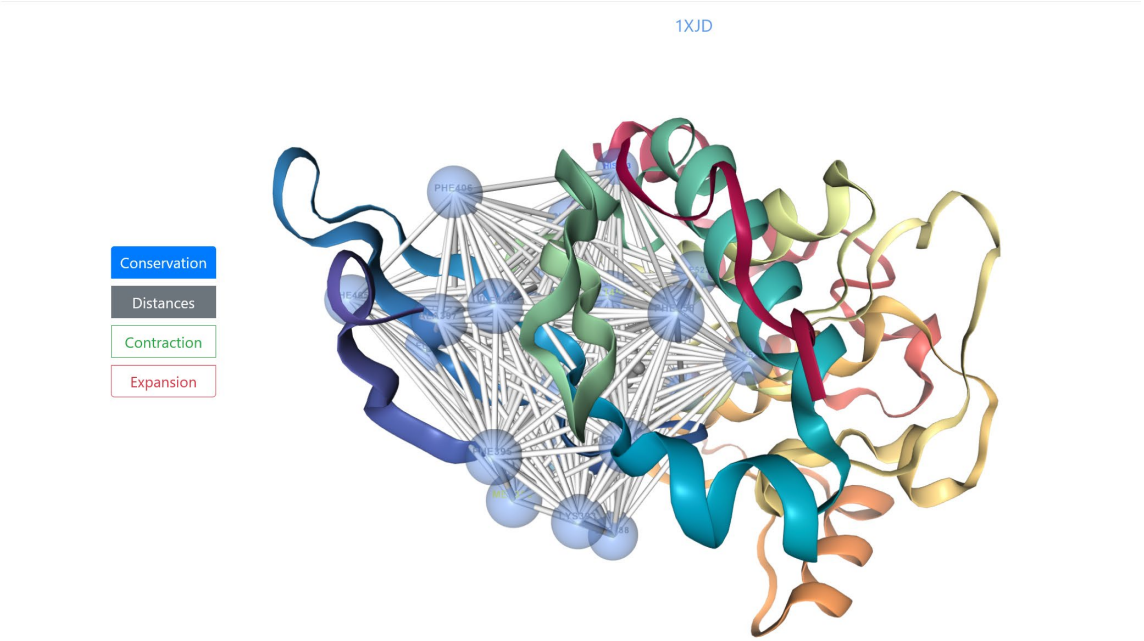
Options display format

Users can choose from 4 different display formats as follows:

- Conservation: returns position-specific positions that are Structurally Conserved Entities.
- Distances: shows all distances included in the calculation which are all positions that fall in the bin.

- Contraction: shows influential distances from the pocket with a preference for pocket contraction along this direction
- Expansion: shows influential distances from the pocket with a preference for pocket expansion for a better drug binding.

An example when displaying all distances with all atomic positions that are Structural Conservation Entities.



10. Web Services API

Servers: https://manoraa.icbs.mahidol.ac.th

Method	Path	Description	Type
GET	/Manoraa/api/ligand/{3-letter code}	Get information on Ligand Substructures and PDB Chains.	JSON

Example response:


```
[
  {
    "Ligand": "MTX",
    "PDB Chains": [
      {
        "Uniprot &SNPs": "O76290",
        "Pathways": [
          ""
        ],
        "Target Protein": "O76290_TRYBB",
        "PDB": "2C7V",
        "Resolution(Å)": 2.20000005,
        "Chain": [
          "A",
          "B",
          "C",
          "D"
        ],
        "Affinity(µM)": "0.000000152"
      },
      {
        "Uniprot &SNPs": "P00374",
        "Pathways": [
          "hsa:1719"
        ],
        "Target Protein": "DYR_HUMAN",
        "PDB": "1DLS",
        "Resolution(Å)": 2.29999995,
        "Chain": [
          "A"
        ],
        "Affinity(µM)": "0.0000000109"
      },
```