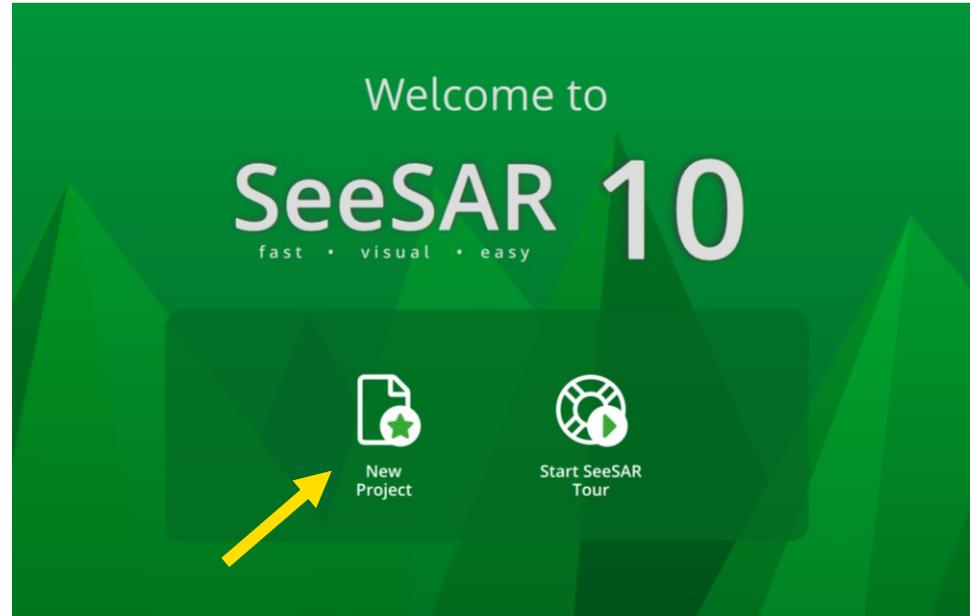


SeeSAR Beginner's Guide

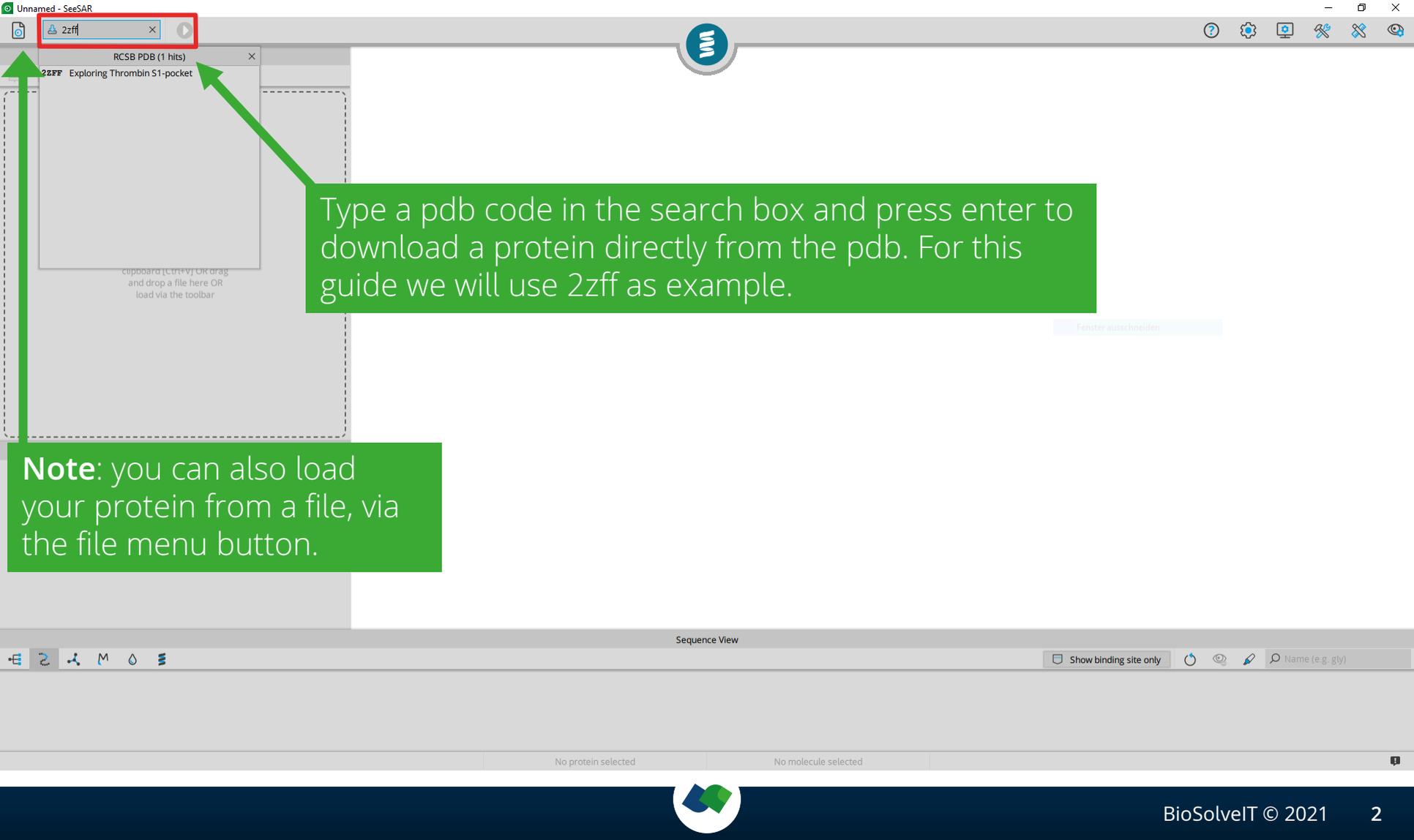
Version 10

1. Basics



To begin, let's start with a new project.





Type a pdb code in the search box and press enter to download a protein directly from the pdb. For this guide we will use 2zff as example.

Note: you can also load your protein from a file, via the file menu button.

Data

ZZFF - Extract your ligand

Hetero groups

	Name	Estimated affinity			
		pM	nM	µM	mM
<input type="radio"/>	Do not extract a ligand				
<input type="radio"/>	53U_H_2001				

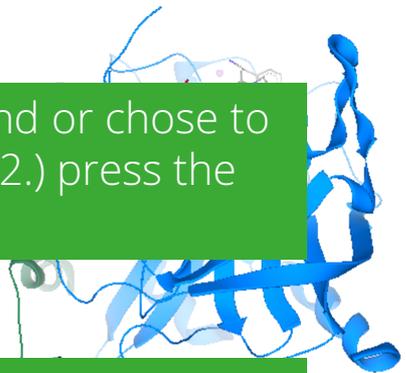
1.

2D

The protein is loaded and all molecules, buffers, co-factors etc will be listed.

Please (1.) select the ligand or chose to not extract a ligand and (2.) press the "Apply" button.

Note: if you are not sure what name contains which molecule, click on the name and have a look at the 2D structure below.



Sequence View

Show binding site only

Name (e.g. gly)

all

1 5 10 15 20 25 30 35

GLU1C ALA1B ASP1A CYS1 GLY2 LEU3 ARG4 PRO5 LEU6 PHE7 GLU8 LYS9 LYS10 SER11 LEU12 GLU13 ASP14 LYS14A THR14B GLU14C ARG14D GLU14E LEU14F LEU14G GLU14H SER14I TYR14J ILE14K ILE16 VAL17 GLU18 GLY19 SER20 ASP21 ALA22 GLU23 ILE24 GLY25

ZZFF No molecule selected



Data

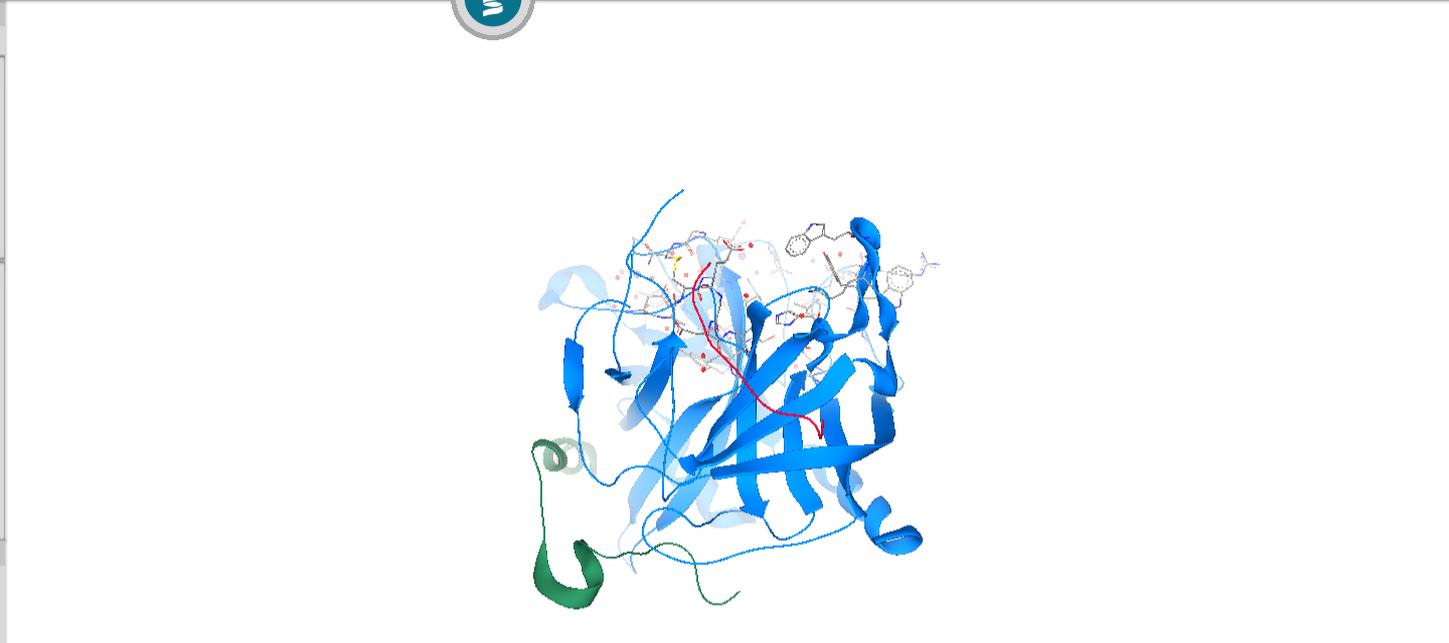
Proteins

Color	Filename
	ZZFF

Ligand for ZZFF

#	Name	Estimated affinity		
		pM	nM	mM
2	53U_H_2001			

2D



After ligand selection, all residues within a 6.5 Angstrom radius around it are automatically selected and presented in the model.

Sequence View

Show binding site only

Name (e.g. gly)

all

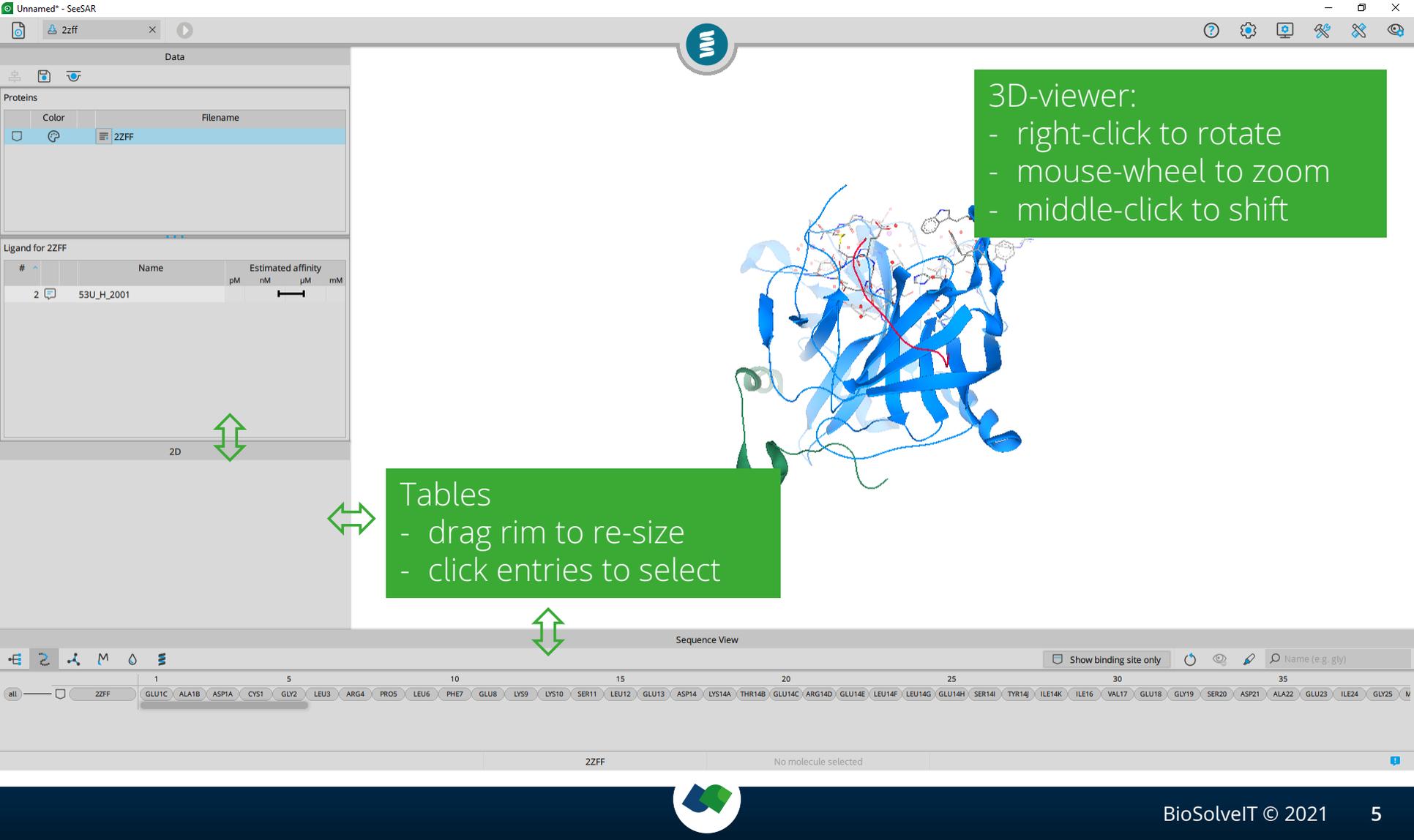
ZZFF

1	5	10	15	20	25	30	35																														
GLU1C	ALA1B	ASP1A	CYS1	GLY2	LEU3	ARG4	PRO5	LEU6	PHI7	GLU8	LYS9	LYS10	SER11	LEU12	GLU13	ASP14	LYS14A	THR14B	GLU14C	ARG14D	GLU14E	LEU14F	LEU14G	GLU14H	SER14I	TYR14J	ILE14K	ILE16	VAL17	GLU18	GLY19	SER20	ASP21	ALA22	GLU23	ILE24	GLY25

ZZFF

No molecule selected





3D-viewer:
- right-click to rotate
- mouse-wheel to zoom
- middle-click to shift

Tables
- drag rim to re-size
- click entries to select

#	Name	Estimated affinity
		pM nM μM mM
2	53U_H_2001	

Sequence View

all	ZZFF	1	5	10	15	20	25	30	35																														
		GLU1C	ALA1B	ASP1A	CYS1	GLY2	LEU3	ARG4	PRO5	LEU6	PHI7	GLU8	LYS9	LYS10	SER11	LEU12	GLU13	ASP14	LYS14A	THR14B	GLU14C	ARG14D	GLU14E	LEU14F	LEU14G	GLU14H	SER14I	TYR14J	ILE14K	ILE16	VAL17	GLU18	GLY19	SER20	ASP21	ALA22	GLU23	ILE24	GLY25

ZZFF

No molecule selected



Unnamed* - SeeSAR

2zff

Data

Proteins

Color	Filename
	ZZFF

Ligand for 2ZFF

#	Name	Estimated affinity			
		pM	nM	μM	mM
2	53U_H_2001				

2D

Adjust background color

Change the table layout

Switch between dark and light theme

Adjust label size

Switch to color blindness mode

If you want to customize the layout of SeeSAR, click on the 'appearance' button in the top right toolbar. For this guide will use the light one, but please feel free to use whatever you prefer!

Sequence View

Show binding site only

Name (e.g. gly)

all

2ZFF

1 5 10 15 20 25 30

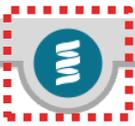
GLU1C ALA1B ASP1A CYS1 GLY2 LEU3 ARG4 PRO5 LEU6 PHE7 GLU8 LYS9 LYS10 SER11 LEU12 GLU13 ASP14 LYS14A THR14B GLU14C ARG14D GLU14E LEU14F LEU14G GLU14H SER14I TYR14J ILE14K VAL17 LEU18

2ZFF

53U_H_2001

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Data

Proteins

Color	Filename
	2ZFF

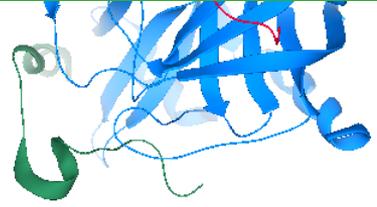
Ligand for 2ZFF

#	Name	Estimated affinity			
		pM	nM	μM	mM
2	53U_H_2001		— —		

2D

C1CCN(C1)C(=O)N(Cc2ccccc2)C(=O)C(C)CN

Note that you are in the **Protein mode**. The mode switch button shows in which mode you are and allows you to change the mode as well. Hover over it so see your options.



Sequence View

Show binding site only

Name (e.g. gly)

all	2ZFF	1	5	10	15	20	25	30																								
		GLU1C	ALA1B	ASP1A	CYS1	GLY2	LEU3	ARG4	PRO5	LEU6	PHE7	GLU8	LYS9	LYS10	SER11	LEU12	GLU13	ASP14	LYS14A	THR14B	GLU14C	ARG14D	GLU14E	LEU14F	LEU14G	GLU14H	SER14I	TYR14J	ILE14K	ILE16	VAL17	GLU18

2ZFF 53U_H_2001





In the Proteins mode you can load and superpose proteins.



Proteins



Analyzer

The Analyzer mode is for filtering molecule sets, hit triaging etc.

The Binding Site mode sets the reference pocket.



Binding Site



Molecule Editor

The Molecule Editor mode is for designing new molecules in 3D.

The Protein Editor mode is for editing side chains, deleting waters or buffers, or search for similar binding sites.



Protein Editor



Inspirator

The Inspirator mode helps you to generate new ideas.



Docking

In the Docking mode you can generate poses for new molecules.



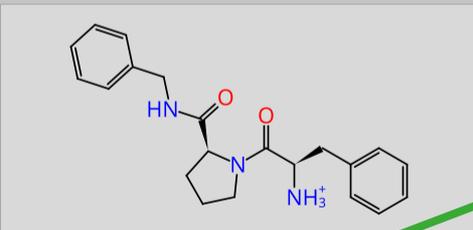
Proteins

Color	Filename
	2ZFF

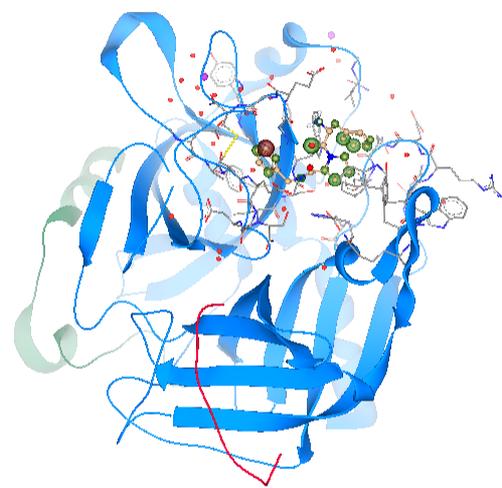
Ligand for 2ZFF

#	Name	Estimated affinity
		pM nM μ M mM
2	53U_H_2001	

2D



As the 3D view can easily get busy, let's customize the visualization.



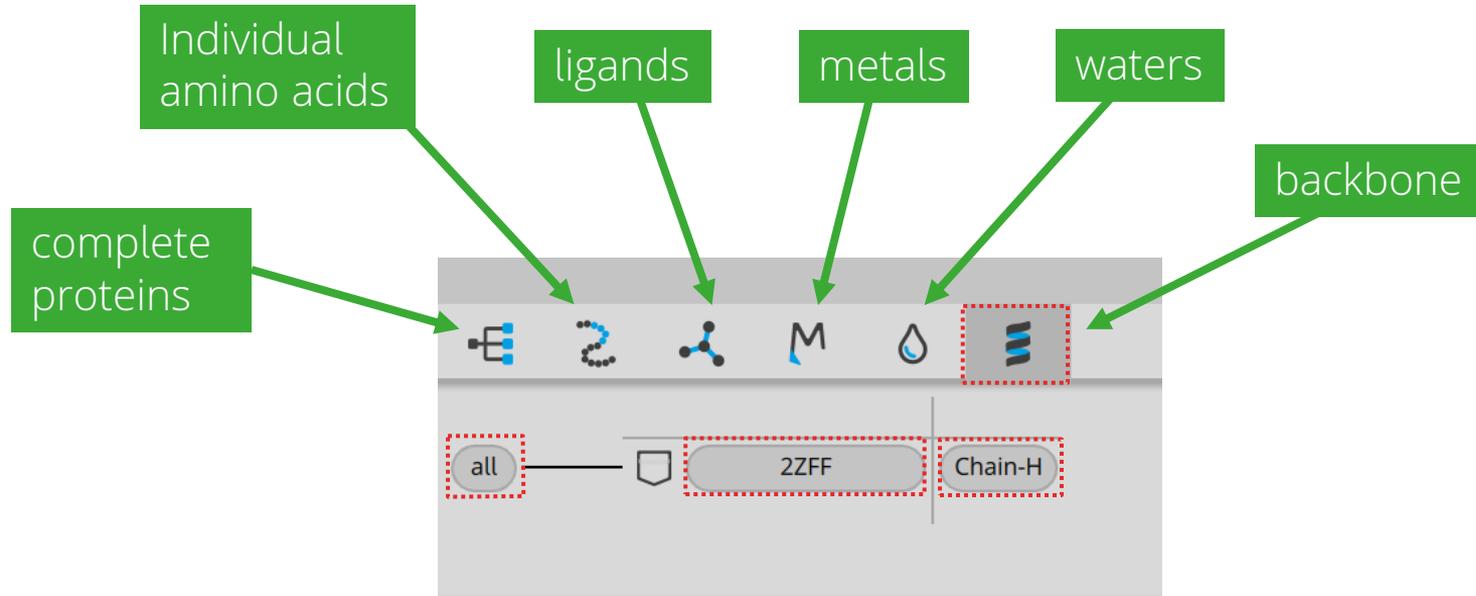
Sequence View

all Z2FF GLU1C ALA1B ASP1A CYS1 GLY2 LEU3 ARG4 PRO5 LEU6 PHE7 GLU8 LYS9 LYS10 SER11 LEU12 GLU13 ASP14 LYS14A THR14B GLU14C ARG14D GLU14E LEU14F LEU14G GLU14H SER14I TYR14J ILE14K ILE16 VAL17 GLU18 GLY19 SER20 ASP21 ALA22 GLU23 ILE24 GLY25 M

Z2FF 53U_H_2001



The view controls let you toggle on/off:



Let's hide the secondary structure, by clicking on the "Chain-H" button in the backbone tab. Upon clicking it turns grey (deactivated)

Note: all buttons are clickable, so that you can hide **all** parts of one protein in one click (useful with several proteins).



Data

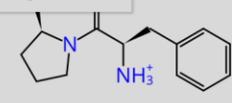
Proteins

Color	Filename
	ZZFF

Ligand for ZZFF

#	Name	Estimated affinity			
		pM	nM	μM	mM
2					

- Calculate estimated affinity
- Add to Binding Site mode**
- Add to Analyzer
- Add to Molecule Editor
- Add to Inspirator
- Add to Docking mode



If you want to add or remove individual amino acids after the automatic selection of residues for the binding site, right click your ligand and add it to Binding Site mode.

Sequence View

Show binding site only

Name (e.g. gly)

all	ZZFF	1	5	10	15	20	25	30																								
		GLU1C	ALA1B	ASP1A	CYS1	GLY2	LEU3	ARG4	PRO5	LEU6	PHE7	GLU8	LYS9	LYS10	SER11	LEU12	GLU13	ASP14	LYS14A	THR14B	GLU14C	ARG14D	GLU14E	LEU14F	LEU14G	GLU14H	SER14I	TYR14J	ILE14K	ILE16	VAL17	GLU18

2ZFF 53U_H_2001





You are now in the **Binding Site mode.**

Data

2ZFF - define your binding site

30 residues are currently selected for the binding site
You can modify the binding site selection, or **confirm with the green button above**

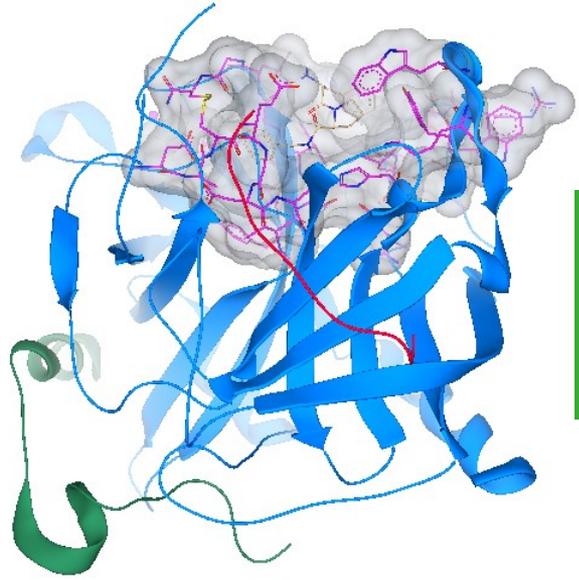
Molecules

Name	Number of residues
53U_H_2001	30

Unoccupied pockets

Pocket ID	Number of residues

2D



Residues already included in the binding site are highlighted in pink.

Sequence View

Show binding site only

Name (e.g. gly)

all	2ZFF	1	5	10	15	20	25	30																								
		GLU1C	ALA1B	ASP1A	CYS1	GLY2	LEU3	ARG4	PRO5	LEU6	PHE7	GLU8	LYS9	LYS10	SER11	LEU12	GLU13	ASP14	LYS14A	THR14B	GLU14C	ARG14D	GLU14E	LEU14F	LEU14G	GLU14H	SER14I	TYR14J	ILE14K	ILE16	VAL17	GLU18

2ZFF No molecule selected



Here you can search for unoccupied binding pockets.



Data

2ZFF - define your binding site
30 residues are currently selected for the binding site
You can modify the binding site selection, or **confirm with the green button above**

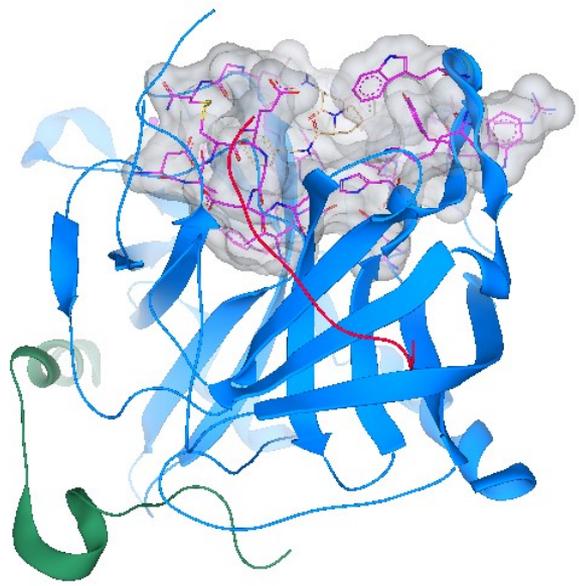
Molecules

Name	Number of residues
53U_H_2001	30

Unoccupied pockets

Pocket ID	Number of residues

2D



Sequence View

Show binding site only

all	2ZFF	1	5	10	15	20	25	30																								
		GLU1C	ALA1B	ASP1A	CYS1	GLY2	LEU3	ARG4	PRO5	LEU6	PHE7	GLU8	LYS9	LYS10	SER11	LEU12	GLU13	ASP14	LYS14A	THR14B	GLU14C	ARG14D	GLU14E	LEU14F	LEU14G	GLU14H	SER14I	TYR14J	ILE14K	ILE16	VAL17	GLU18

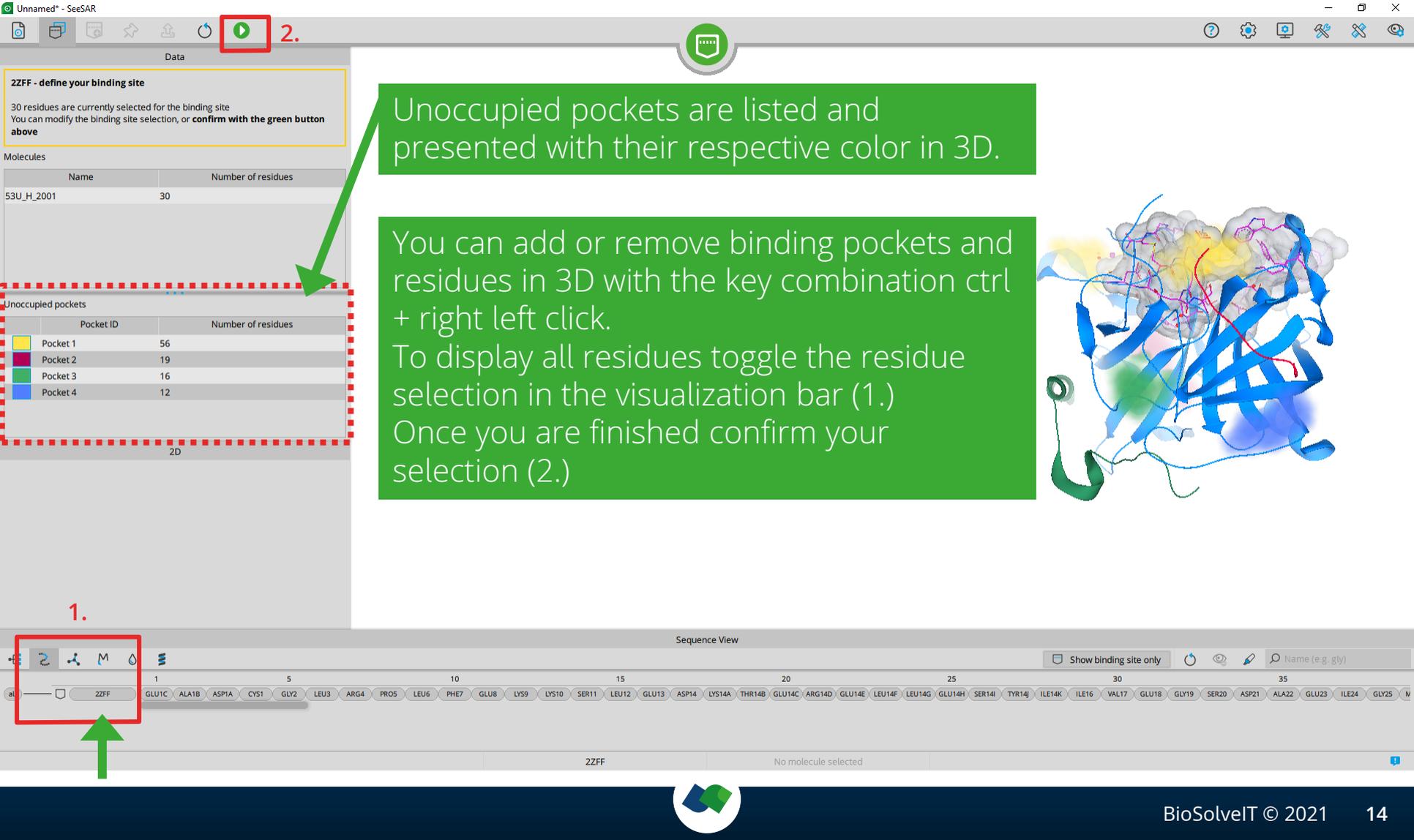
2ZFF

No molecule selected



Unoccupied pockets are listed and presented with their respective color in 3D.

You can add or remove binding pockets and residues in 3D with the key combination ctrl + right left click. To display all residues toggle the residue selection in the visualization bar (1.) Once you are finished confirm your selection (2.)

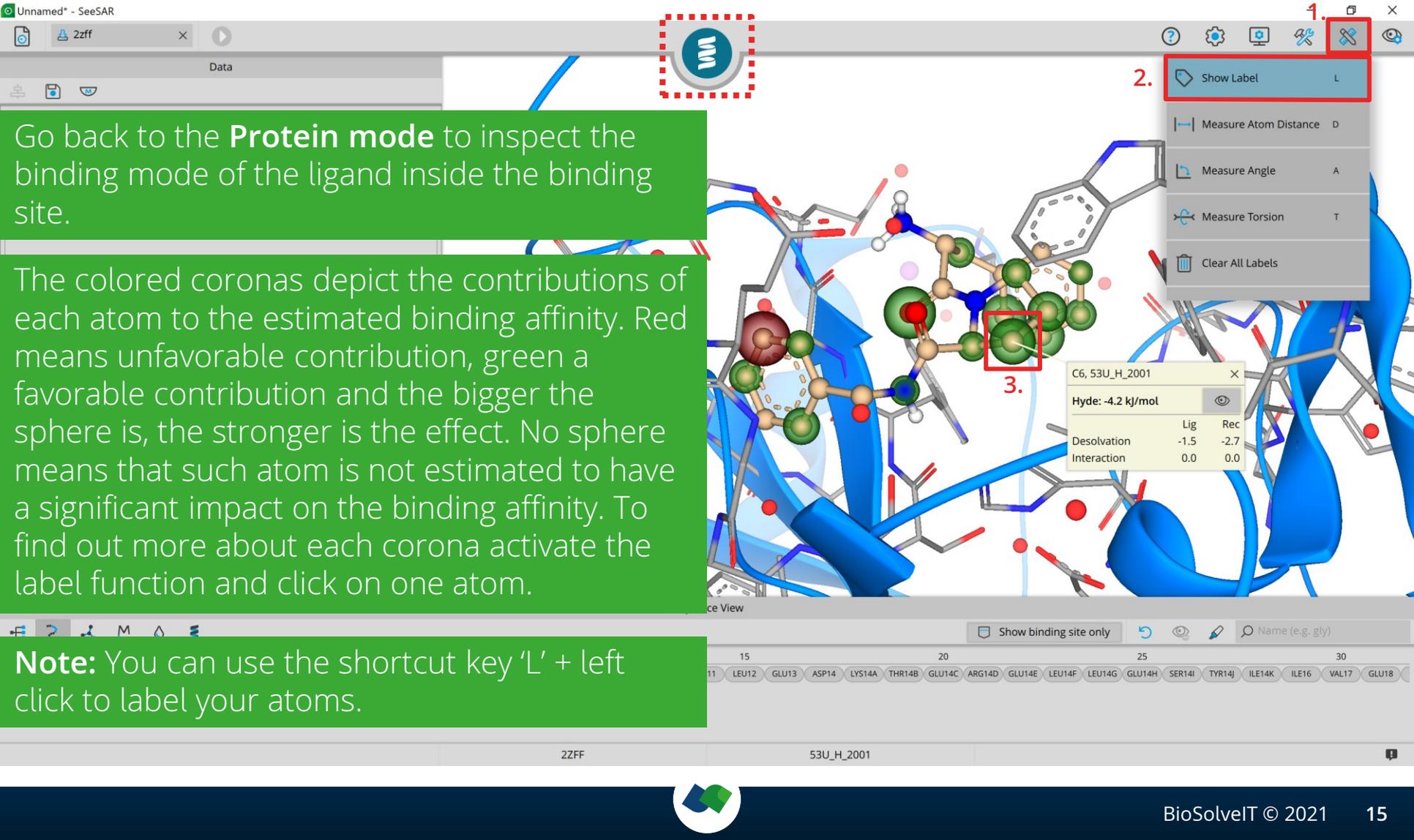


The screenshot displays the SeeSAR software interface. At the top, a toolbar contains a green play button icon labeled '2.'. Below it, a 'Data' panel shows a table for 'Molecules' with one entry: '53U_H_2001' with 30 residues. A red dashed box highlights the 'Unoccupied pockets' table, which lists four pockets with their respective colors and residue counts. A 3D visualization of a protein structure is shown on the right, with different regions highlighted in yellow, red, green, and blue. At the bottom, the 'Sequence View' panel shows a protein sequence with a red box around the 'ZZFF' residue, labeled '1.'. A green arrow points from the '1.' label to the 'ZZFF' residue in the sequence view.

Unoccupied pockets		
	Pocket ID	Number of residues
Yellow	Pocket 1	56
Red	Pocket 2	19
Green	Pocket 3	16
Blue	Pocket 4	12

Molecules	
Name	Number of residues
53U_H_2001	30

Sequence View	
Residue	Residue
1	35
GLU1C	ALA1B
ASP1A	CYS1
GLY2	LEU3
ARG4	PRO5
LEU6	PHI7
GLU8	LYS9
LYS10	SER11
LEU12	GLU13
ASP14	LYS14A
THR14B	GLU14C
ARG14D	GLU14E
LEU14F	LEU14G
GLU14H	SER14I
TYR14J	ILE14K
ILE16	VAL17
GLU18	GLY19
SER20	ASP21
ALA22	GLU23
ILE24	GLY25



Go back to the **Protein mode** to inspect the binding mode of the ligand inside the binding site.

The colored coronas depict the contributions of each atom to the estimated binding affinity. Red means unfavorable contribution, green a favorable contribution and the bigger the sphere is, the stronger is the effect. No sphere means that such atom is not estimated to have a significant impact on the binding affinity. To find out more about each corona activate the label function and click on one atom.

Note: You can use the shortcut key 'L' + left click to label your atoms.

- 2. Show Label L
- Measure Atom Distance D
- Measure Angle A
- Measure Torsion T
- Clear All Labels

3.

C6, 53U_H_2001		
Hyde:	-4.2	kJ/mol
Desolvation	Lig	Rec
Interaction	-1.5	-2.7
	0.0	0.0

Show binding site only

Name (e.g. gly)

15 20 25 30

11 LEU12 GLU13 ASP14 LYS14A THR14B GLU14C ARG14D GLU14E LEU14F LEU14G GLU14H SER14I TYR14J ILE14K ILE16 VAL17 GLU18

2ZFF 53U_H_2001



Unamed* - SeeSAR

2zff

Data

Proteins

Color	Filename
	ZZFF

Ligand for ZZFF

#	Name	Estimated affinity
		pM nM μ M mM
2	53U_H_2001	

2D

C1CCN(C1)C(=O)NCC2=CC=CC=C2

Sequence View

1. Show binding site only

Name (e.g. gly)

1	5	10	15	20	25	30
GLU1C	ALA1B	ASP1A	CYS1	GLY2	LEU3	ARG4
PRO5	LEU6	PHE7	GLU8	LYS9	LYS10	SER11
LEU12	GLU13	ASP14	LYS14A	THR14B	GLU14C	ARG14D
GLU14E	LEU14F	LEU14G	GLU14H	SER14I	TYR14J	ILE14K
ILE16	VAL17	GLU18				

2ZFF 53U_H_2001

Check-out the other analysis options!

- Show Label L
- Measure Atom Distance D
- Measure Angle A
- Measure Torsion T
- Clear All Labels

C6, 53U_H_2001

Hyde: -4.2 kJ/mol

	Lig	Rec
Desolvation	-1.5	-2.7
Interaction	0.0	0.0

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The screenshot displays the SeeSAR software interface. On the left, a sidebar contains a 'Proteins' table with one entry 'ZZFF'. Below it is a 'Ligand for ZZFF' table with one entry. A context menu is open over this entry, listing several actions, with 'Add to Molecule Editor' highlighted in red. The main window shows a 3D molecular model of a protein (grey sticks) with a ligand (green spheres) bound to it. A green text box is overlaid on the model, providing instructions. At the bottom, there is a 'Sequence View' section with a search bar and a 'Show whole protein' button. The status bar at the very bottom shows '2ZFF' and '53U_H_2001'.

Unnamed* - SeeSAR

2zff

Data

Proteins

Color	Filename
	ZZFF

Ligand for ZZFF

#	Name	Estimated affinity			
		pM	nM	μM	mM
2					

- Calculate estimated affinity
- Add to Binding Site mode
- Add to Analyzer
- Add to Molecule Editor**
- Add to Inspirator
- Add to Docking mode

HN NH₃⁺

Sequence View

Show whole protein

Name (e.g. gly)

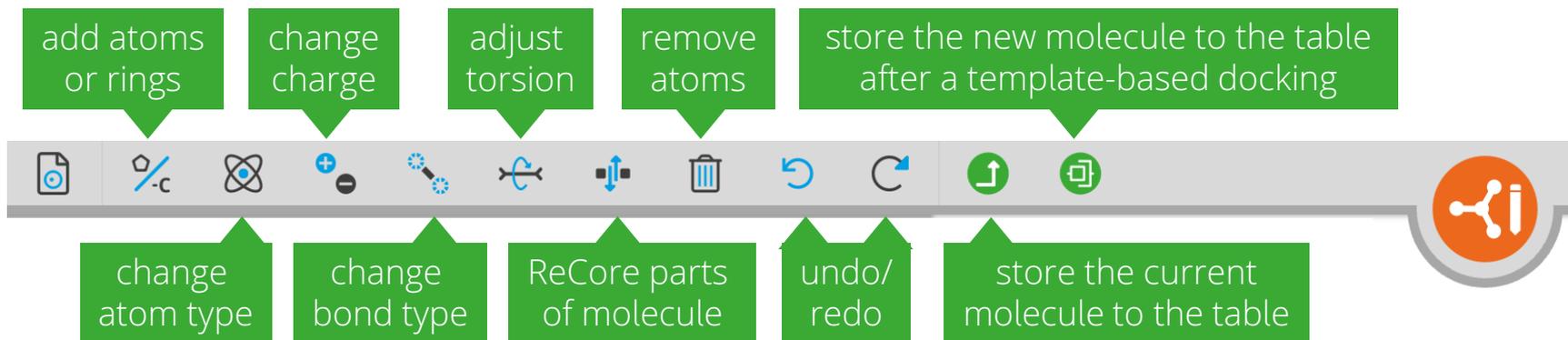
all 2ZFF Chain-H

2ZFF 53U_H_2001

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Add to Molecule Editor is accessible with a right-click on the table entry. This copies the molecule into the mode **and** automatically switches to that mode.

The editor-menu will appear on the top left. There you can:



To edit a molecule ALWAYS:

1. **select** (atoms or bonds)
2. **modify** (using the function of choice from above)

Note that many editor functions have shortcut-keys.
E.g. select a bond and type 1, 2 or, 3 on the keyboard,
or select an atom and type the element (C, N, O, ...).





Data

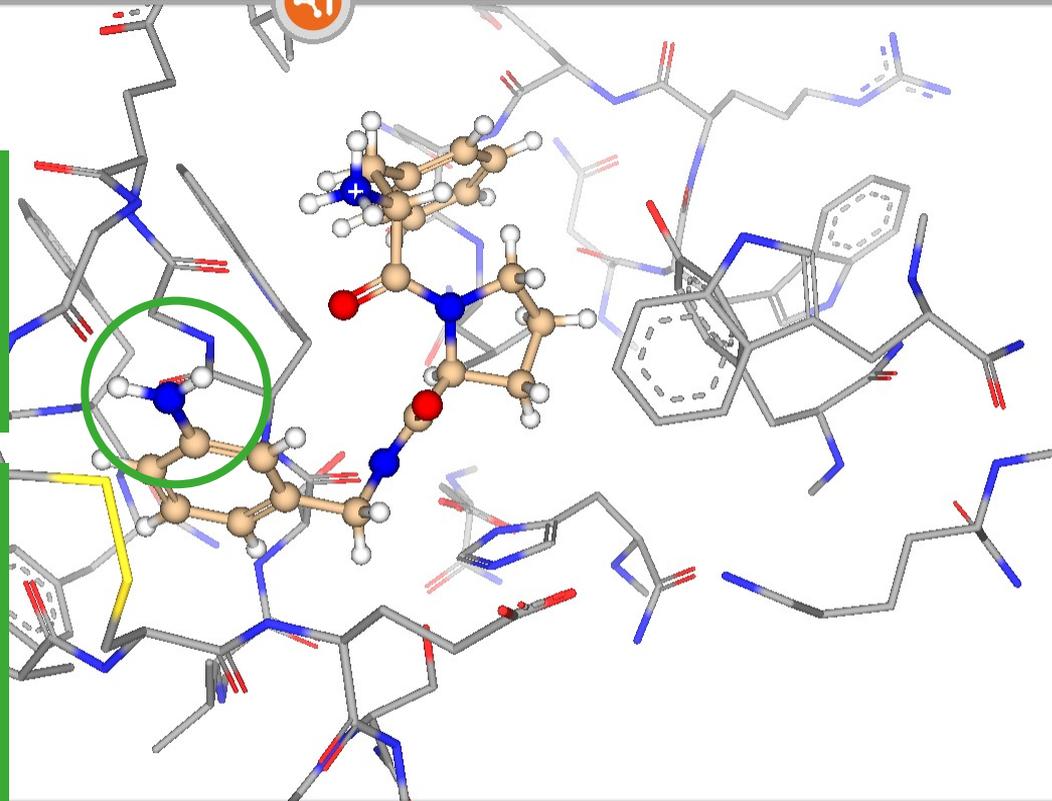
Save edited molecules to table [Ctrl+E]

Switch to grid

Name	Src	ID	Estimated affinity	LLE	Tor
			nM	μ M	mM
53U_H_2001		1			

As an exercise, we add an amino group to the ring by selecting the Hydrogen in meta-position and changing its element type to "N".

Note: During editing you see all Hydrogens but no estimated affinity and no coronas. To see them, 1st add the edited ligand to the table (with the green button) and 2nd select the new entry in the table!



Sequence View

Show binding site only

Name (e.g. gly)

all 2ZFF 3015 3019 3020 3021 3031 3046 3053 3055 3059 3063 3075 3077 3090 3106 3117 3120 3121 3122 3137 3150 3155 3158 3159 3001 3002 3003 3004 3005 3006 3007 3008 3009 3010 3011 3012 3013 3014 3016

2ZFF

No molecule selected



The screenshot displays the SeeSAR software interface. At the top, a toolbar contains various icons, with a red box highlighting the 'Resume' button (a play icon). On the left, a 'Data' table lists molecule entries. The main area shows a 3D molecular model of a protein with a specific molecule highlighted in green. A red box highlights a 'Resume' button (a play icon) with a tooltip that reads 'Molecule/protein editing is now paused. Click here to resume.' At the bottom, a sequence viewer shows the protein sequence with residue numbers from 3015 to 3016.

Name	Src	ID	Estimated affinity	LLE	Tor
53U_H_2001		1			●
53U...01_8	-	8			●

Sequence View

Show binding site only

Name (e.g. gly)

all

2ZFF

3015 3019 3020 3021 3031 3046 3053 3055 3059 3063 3075 3077 3090 3106 3117 3120 3121 3122 3137 3150 3155 3158 3159 3001 3002 3003 3004 3005 3006 3007 3008 3009 3010 3011 3012 3013 3014 3016

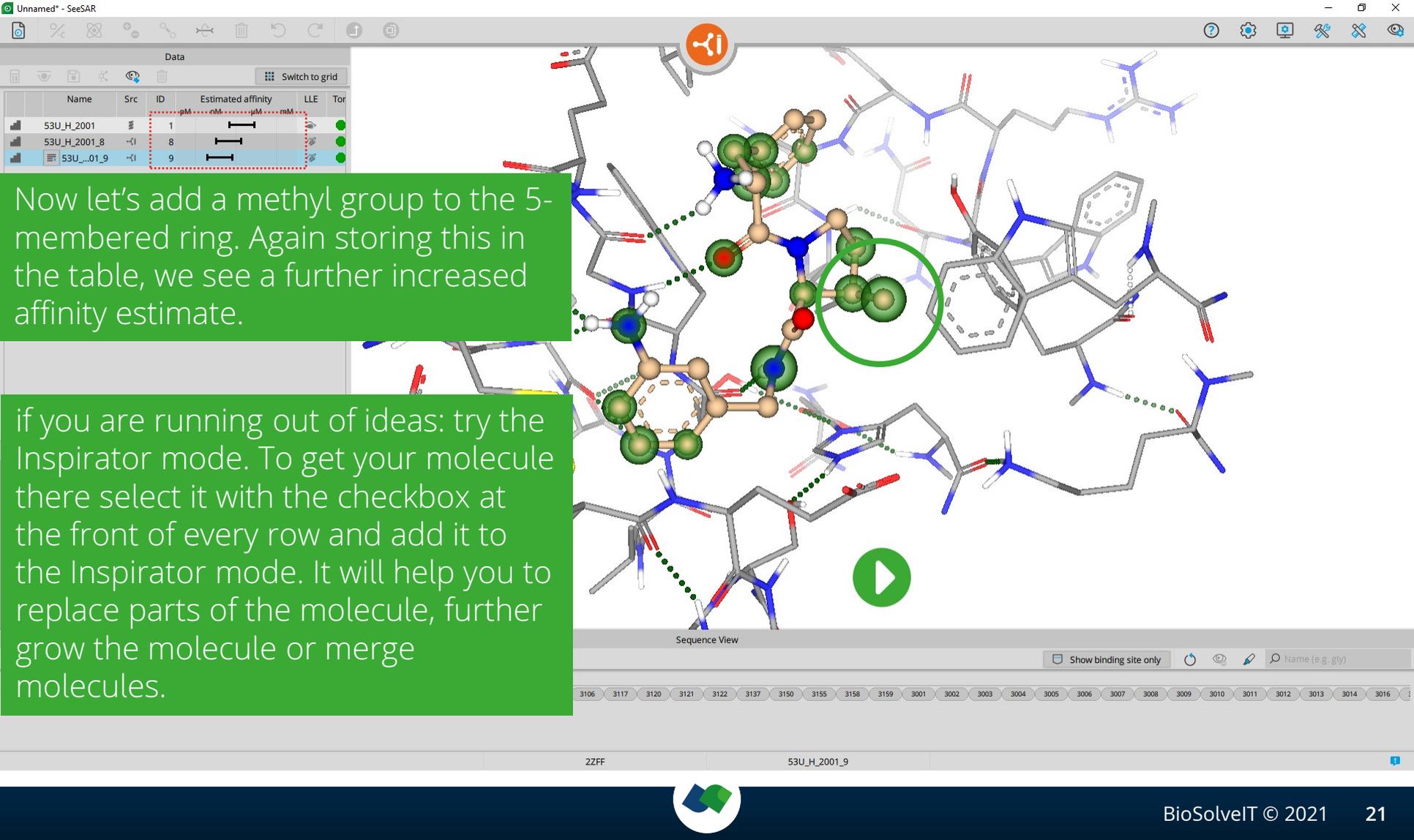
2ZFF

53U_H_2001_8

If you click on the molecule entry you see the estimated affinity and related coronas, but only polar Hydrogens. The editor menu is locked now.

To continue editing, click on the 'Resume' button in the center!





Now let's add a methyl group to the 5-membered ring. Again storing this in the table, we see a further increased affinity estimate.

if you are running out of ideas: try the Inspirator mode. To get your molecule there select it with the checkbox at the front of every row and add it to the Inspirator mode. It will help you to replace parts of the molecule, further grow the molecule or merge molecules.

Name	Src	ID	Estimated affinity	LLE	Tor
53U_H_2001	█	1	μM	█	█
53U_H_2001_8	-	8	nM	█	█
53U...01_9	-	9	μM	█	█

Sequence View

Show binding site only

Name (e.g. gly)

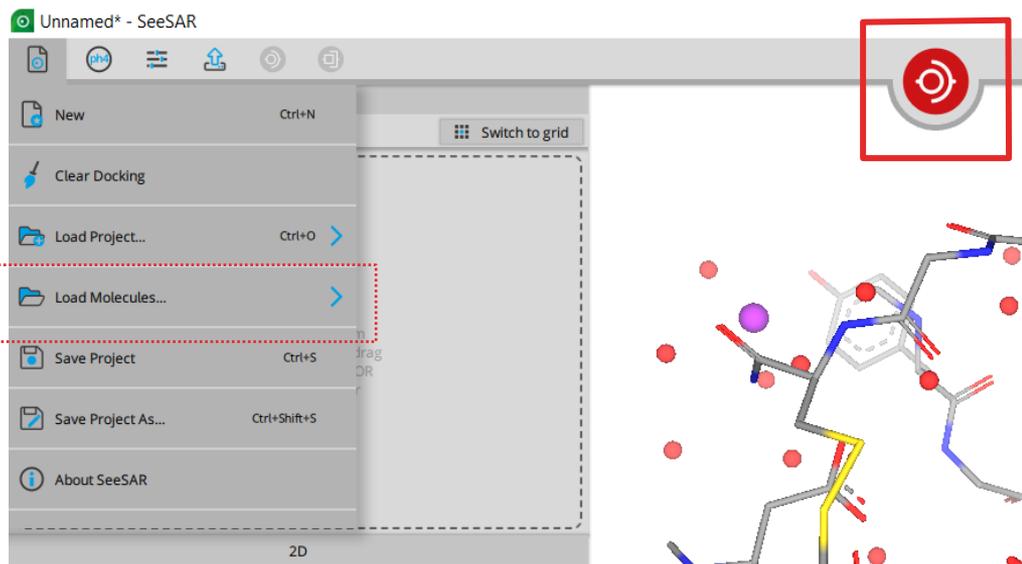
3106 3117 3120 3121 3122 3137 3150 3155 3158 3159 3001 3002 3003 3004 3005 3006 3007 3008 3009 3010 3011 3012 3013 3014 3016

2ZFF 53U_H_2001_9



2. Adding own molecules

If you want to add your own molecules to a SeeSAR-session: use e.g. your favorite drawing tool and save the molecules as sdf-, smiles-, or mol2-file. Switch to the **Docking mode** in SeeSAR and add your molecules via the load button or copy/paste them to the input library field.

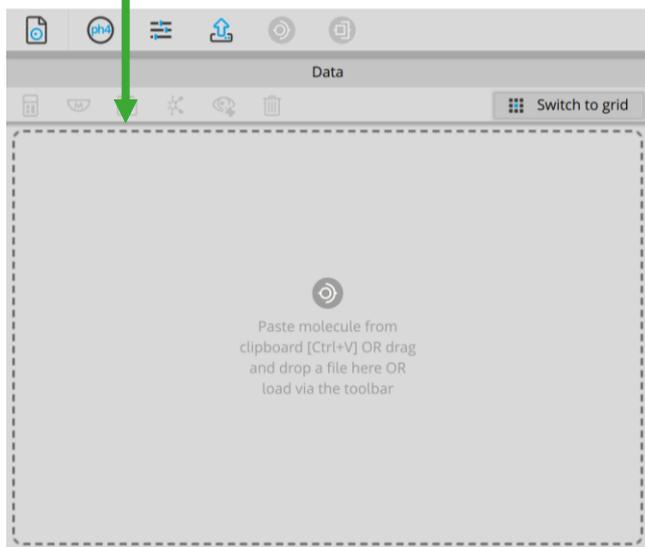


Loaded molecules may not yet be placed in the binding site (the information icon tells you upon mouse-over). If your molecules were docked using another program you can load them straight into the Analyzer mode.



Alternatively, copy/paste (ctrl + c/ctrl + v) your molecules (as smiles or sdf) here.

For example, copy the three molecules on the right in there, and change their names:



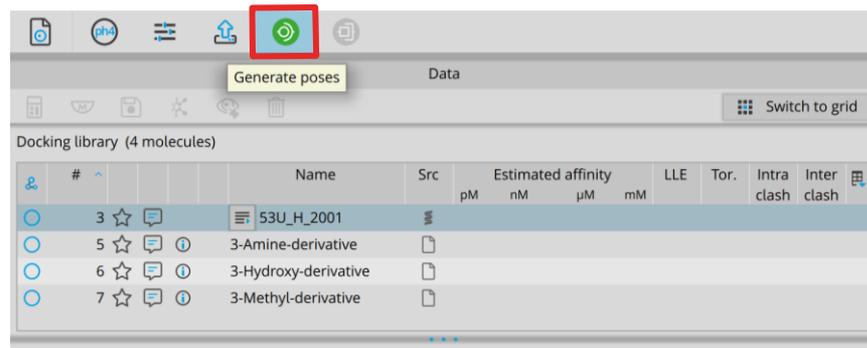
3-Amine-derivative:



3-Hydroxy-derivative:



3-Methyl-derivative:



Now you can start the docking by pressing the **Generate Poses** button!



2.

1.

	#	Name	Src	Estimated affinity				LLE	Tor.	Intra clash	Inter clash
				pM	nM	μM	mM				
<input checked="" type="checkbox"/>	10	3-Meth..._1_01									
<input checked="" type="checkbox"/>	11	3-Meth..._1_02									
<input checked="" type="checkbox"/>	12	3-Meth..._1_03									
<input checked="" type="checkbox"/>	13	3-Meth..._1_04									
<input checked="" type="checkbox"/>	14	3-Meth..._1_05									
<input checked="" type="checkbox"/>	15	3-Meth..._1_06									
<input checked="" type="checkbox"/>	16	3-Meth..._1_07									

At most 10 poses per molecule are generated this way, as we have left the docking settings on default.

To get the estimated affinities, (1.) select all docking solutions with the checkmark in the first column and (2.) press the thumbs-up button under the calculator at the top of the table!

Note: You may restrict the HYDE-calculation to a pre-selected set of checked molecules.



Now the estimated affinities appear as a range on the logarithmic (!) scale.

Clicking on a column header sorts according to this value.

Generated poses

#	Name	Src	Estimated affinity	LLE	Tor.	Intra clash	Inter clash
			pM	nM	μM	mM	
27	3-Amine-derivat						
14	53U_H_2001_1_0						
31	3-Amine-derivat						
19	3-Methyl-derivat						
29	3-Amine-derivat						
33	3-Amine-derivat						
28	3-Amine-derivat						
20	3-Methyl-derivat						
30	3-Amine-derivat						
22	3-Methyl-derivat						
11	53U_H_2001_1_0						
21	3-Methyl-derivat						
23	3-Methyl-derivat						
24	3-Methyl-derivat						
43	3-Hydroxy-derivat						
46	3-Hydroxy-derivat						
10	53U_H_2001_1_0						
7	53U_H_2001_1_0						
8	53U_H_2001_1_0						
13	53U_H_2001_1_007						
12	53U_H_2001_1_006						

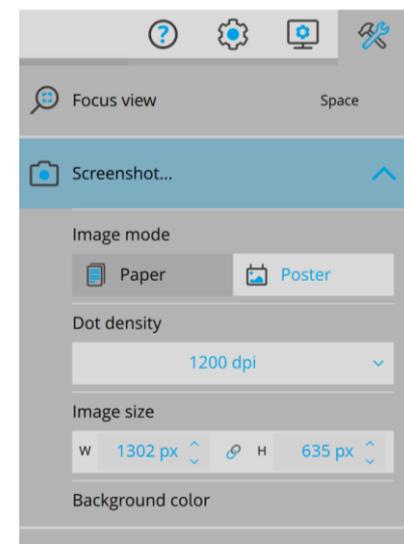
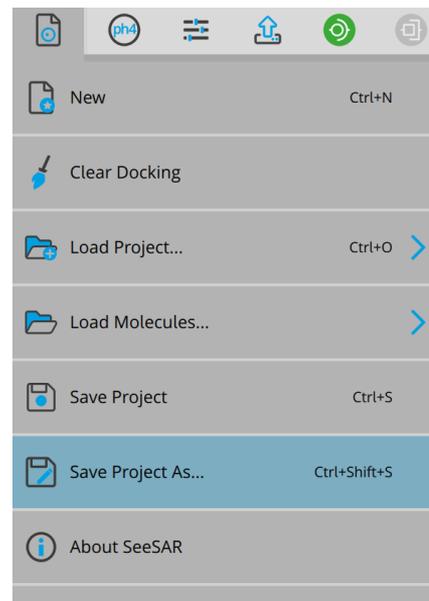
To inspect multiple poses in comparison, (1.) toggle the permanent visibility by marking a molecule as reference. Now it will appear in purple color and stay visible as you select other molecules.

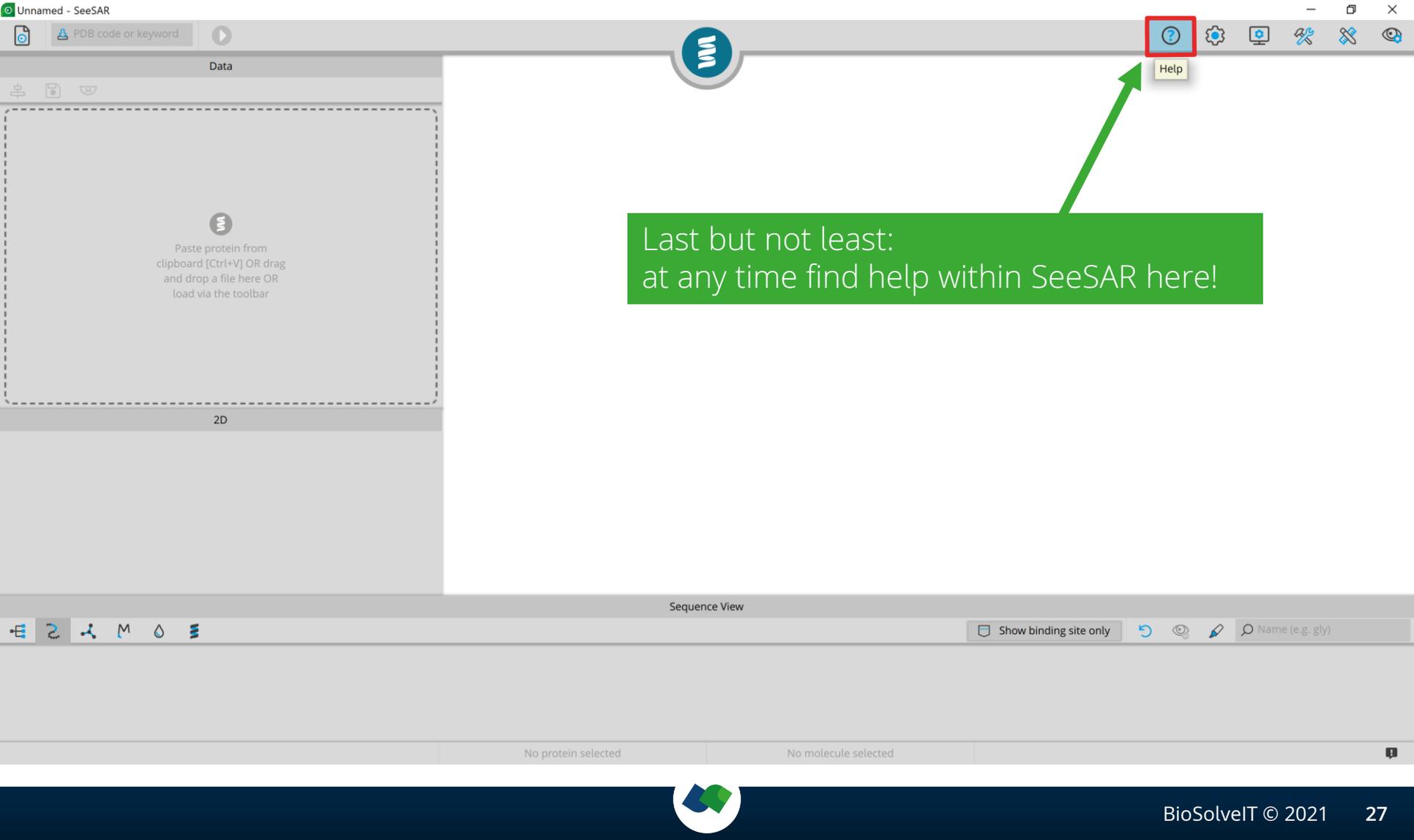
You can add more physico-chemical and ADME-properties as well as pharmacological parameters to your table with a click on the table button (2.).



By now you have a lot of interesting values and possibly many molecules. You may want to:

- apply some (pharmacophore) filters? Copy the molecules of interest to the Analyzer mode and use the filter panel on the right side of the table.
- grow your molecule? Add it to the Inspirator and use the growing functionality.
- generate pictures for a report or publication? You can do this under the 'utilities' button in the upper right toolbar.
- save your session, to continue working a different time, by clicking **Save Project As...**





Last but not least:
at any time find help within SeeSAR here!



Now we wish you happy SeeSAR-ing!

