SeeSAR Beginner's Guide
Version 10

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.

2ZFF - Extract your ligand

Hetero groups

	Name	Estimated affinity			
		pM	nM	µM	mM
○	Do not extract a ligand				
○	53U_H_2001				

1.

2.

Data

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)

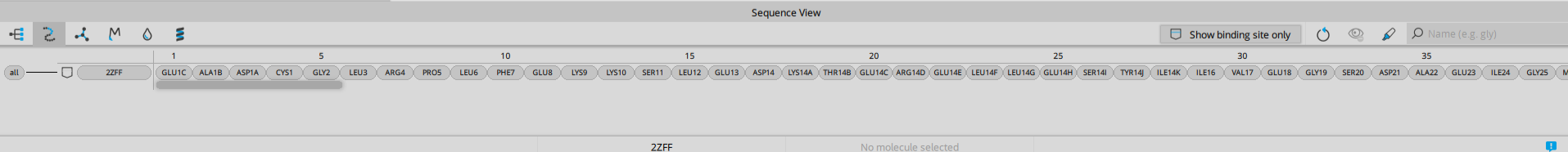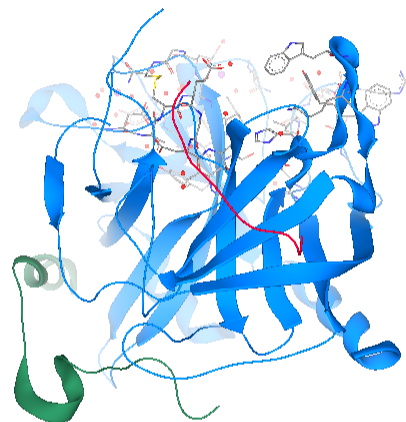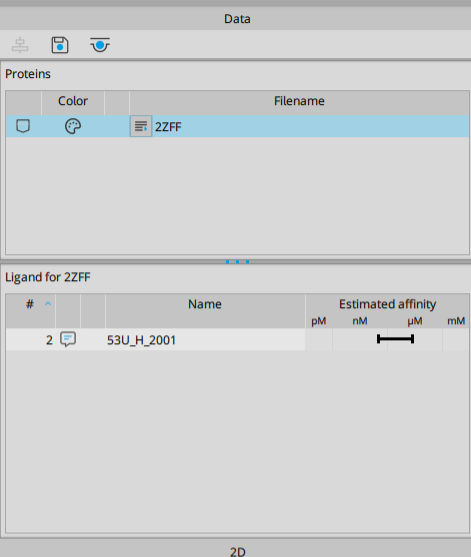| all | 2ZFF | GLU1C | ALA1B | ASP1A | CYS1 | GLY2 | LEU3 | ARG4 | PRO5 | LEU6 | PHE7 | GLU8 | LYS9 | LYS10 | SER11 | LEU12 | GLU13 | ASP14 | LYS14A | THR14B | GLU14C | ARG14D | GLU14E | LEU14F | LEU14G | GLU14H | SER14I | TYR14J | ILE14K | ILE16 | VAL17 | GLU18 | GLY19 | SER20 | ASP21 | ALA22 | GLU23 | ILE24 | GLY25 |

1   5   10   15   20   25   30   35

2ZFF   No molecule selected

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

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

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

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!

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.

In the Proteins mode you can load and superpose proteins.

Proteins

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

Analyzer

The Binding Site mode sets the reference pocket.

Binding Site

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

Molecule Editor

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

Protein Editor

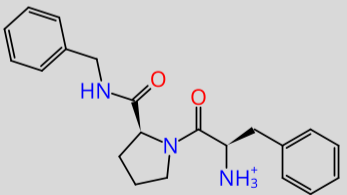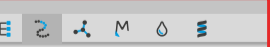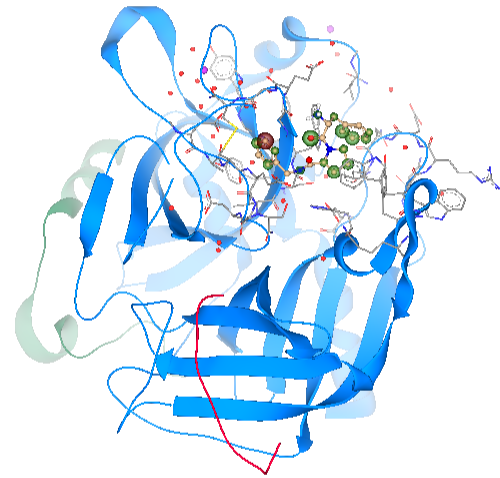The Inspirator mode helps you to generate new ideas.

Inspirator

Docking

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

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

The view controls let you toggle on/off:

Individual amino acids

ligands

metals

waters

backbone

complete proteins

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).

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.

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

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

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

Name (e.g. gly)

| all | 2ZFF | 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
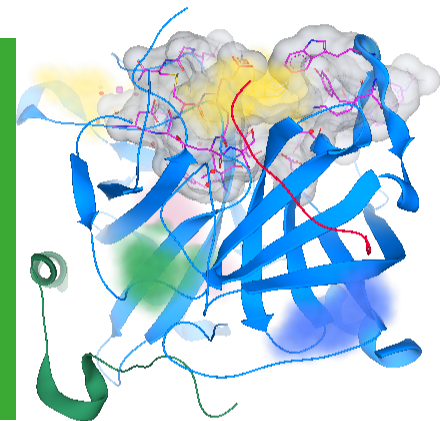
Here you can search for unoccupied binding pockets.

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.)

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

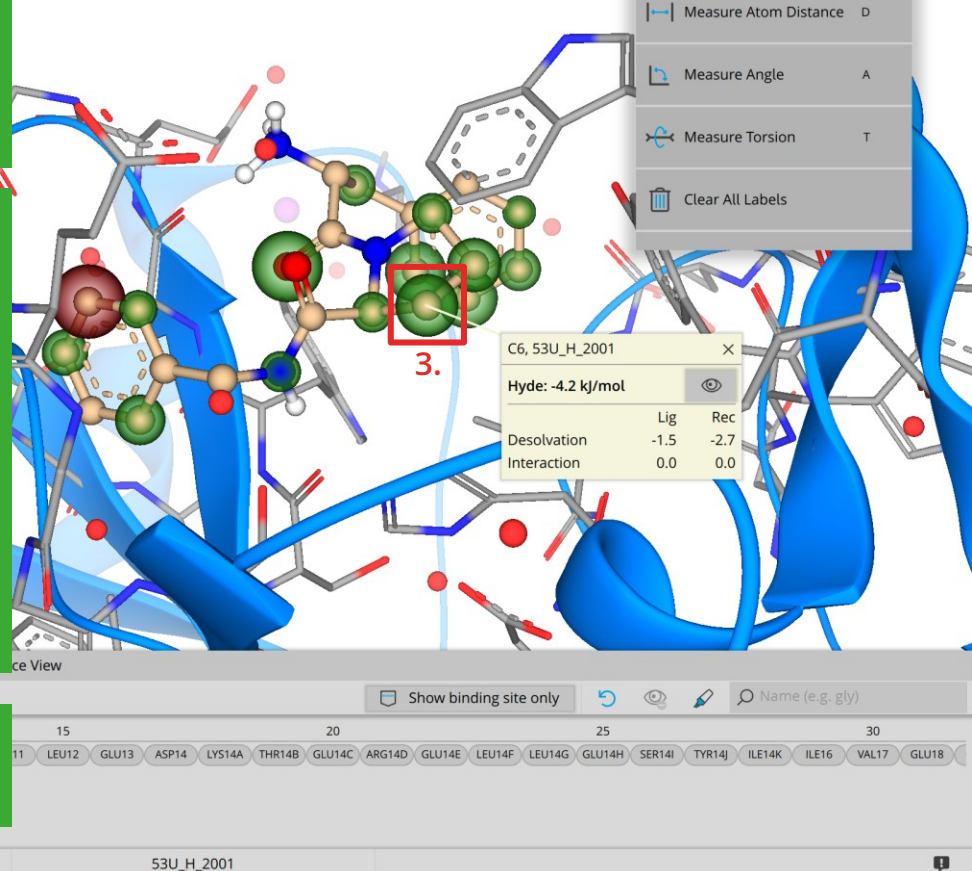| Pocket ID	Number of residues
Pocket 1	56
Pocket 2	19
Pocket 3	16
Pocket 4	12

Data

2D

1.

2.

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.

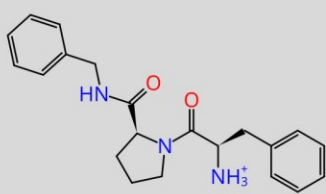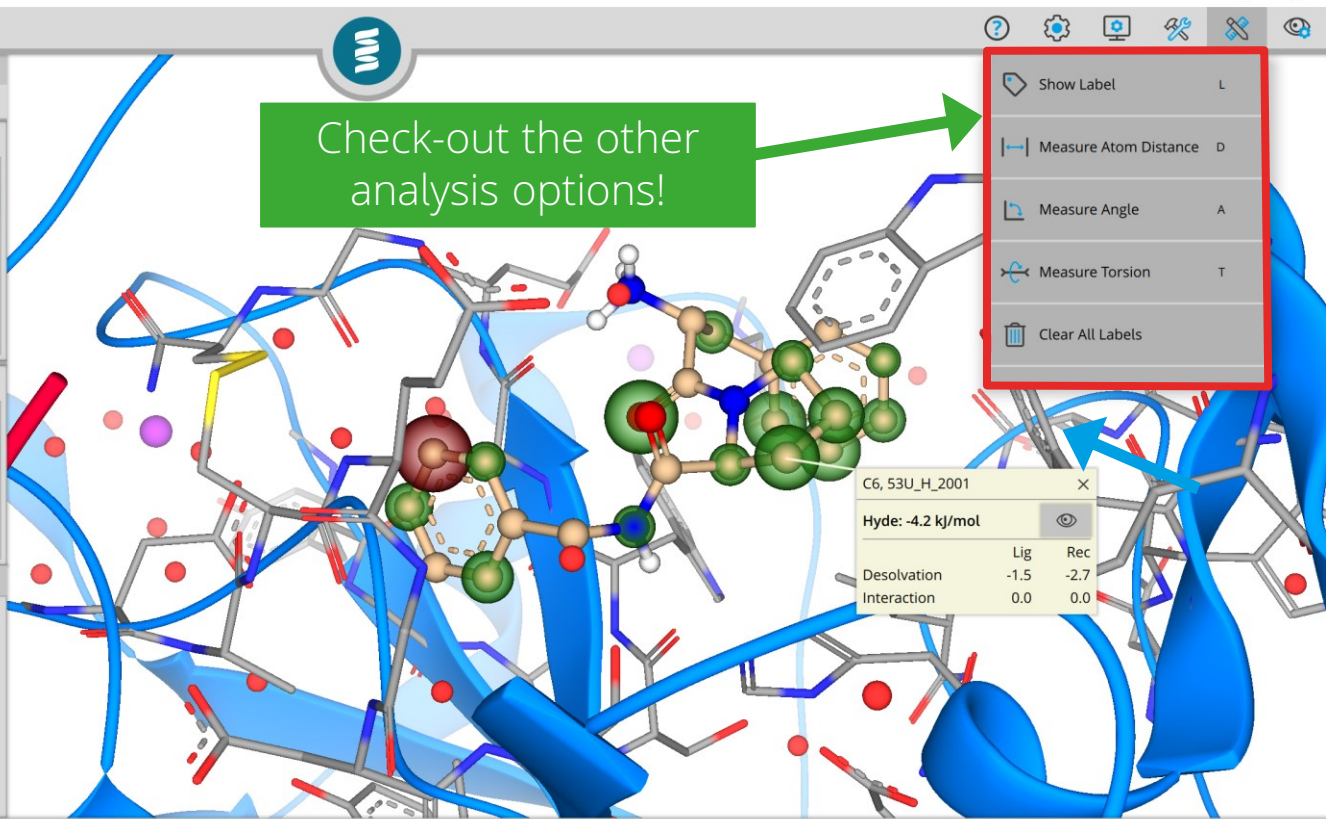**Note:** You can use the shortcut key 'L' + left click to label your atoms.

1.

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   👁

	Lig	Rec
Desolvation	-1.5	-2.7
Interaction	0.0	0.0

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:

add atoms or rings

change charge

adjust torsion

remove atoms

store the new molecule to the table after a template-based docking

change atom type

change bond type

ReCore parts of molecule

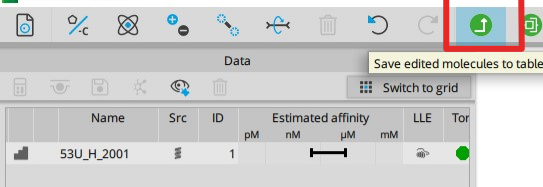undo/ redo

store the current molecule to the table

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,
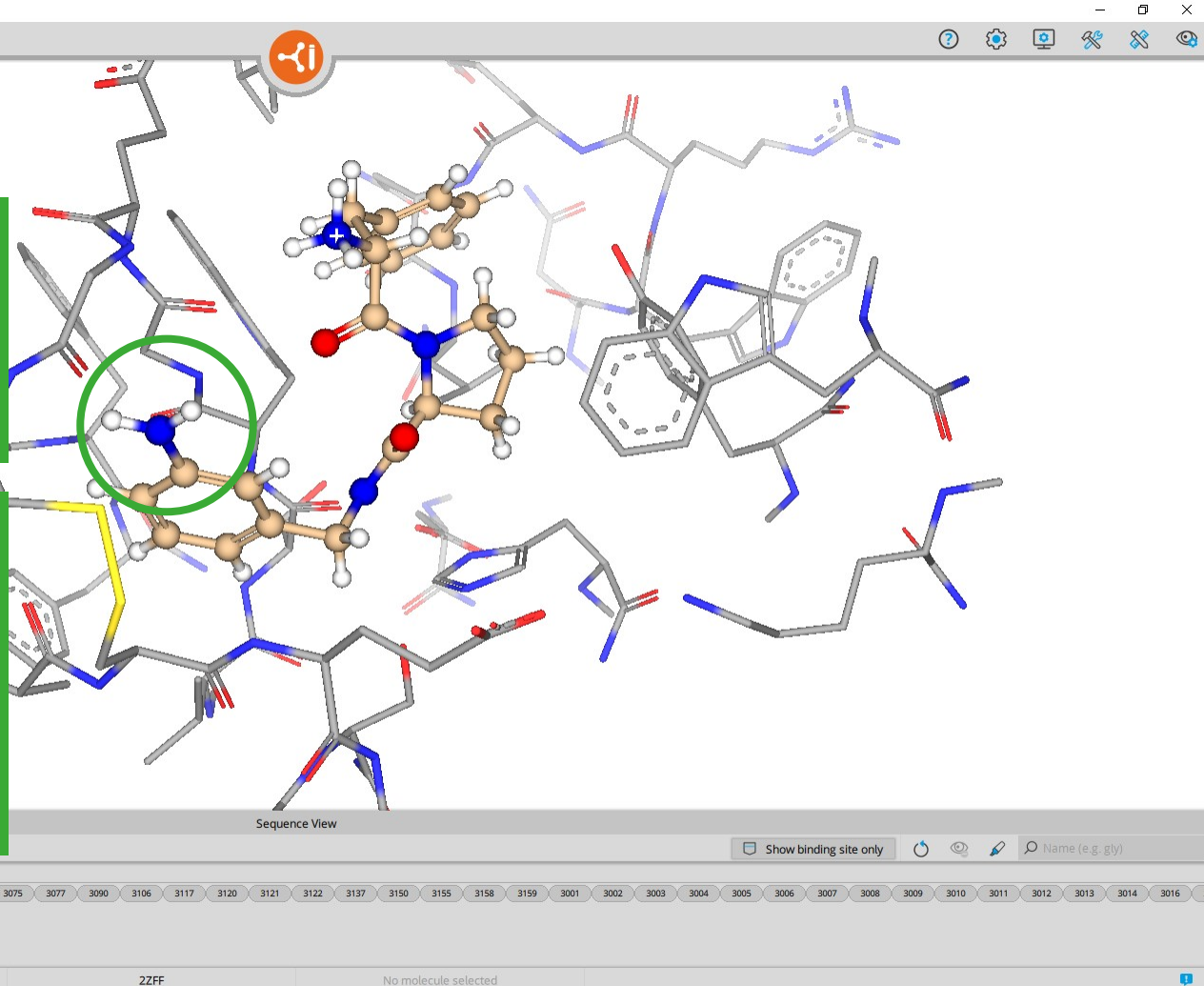or select an atom and type the element (C, N, O, …).

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!

Save edited molecules to table [Ctrl+E]

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!

Molecule/protein editing is now paused. Click here to resume.

Sequence View

Show binding site only

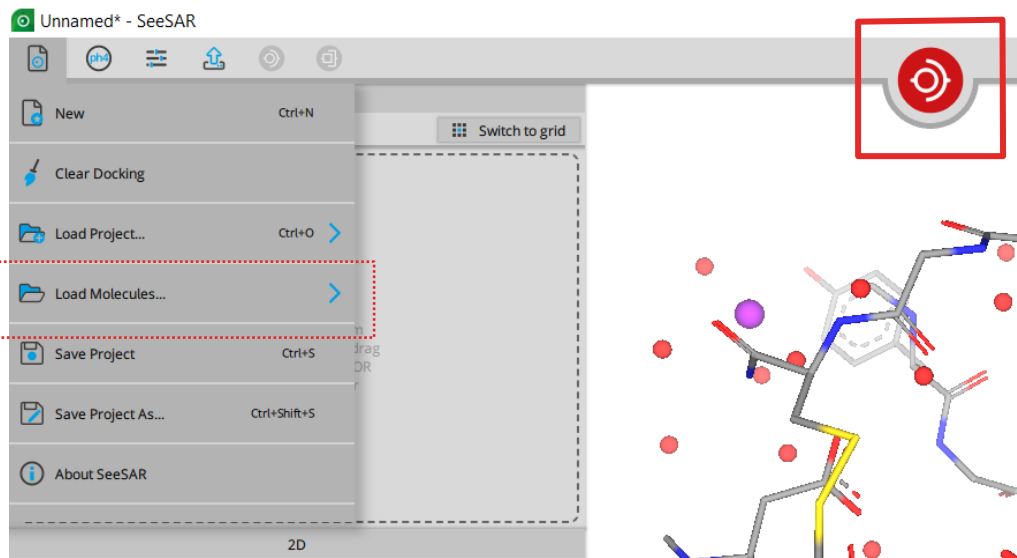| Name	Src	ID	Estimated affinity	LLE	Tor
53U_H_2001		1			●
53U_H_2001_8		8			●
53U_...01_9		9			●

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.

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.
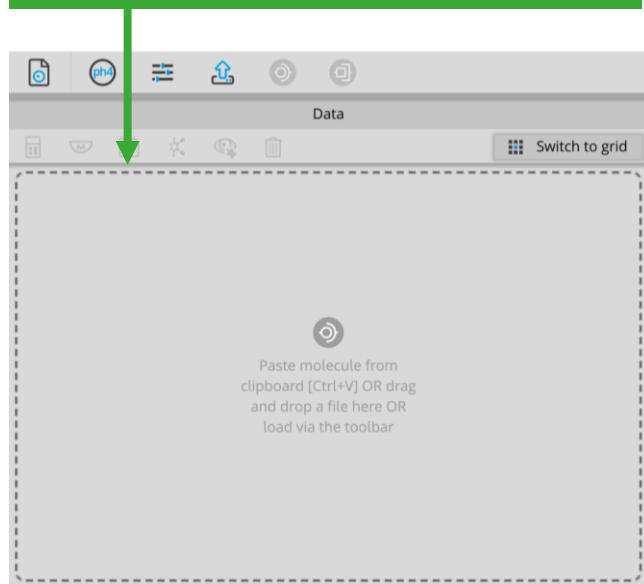

Loaded molecules may not yet be placed in the binding site (the information icon tells you upon mouse-over).
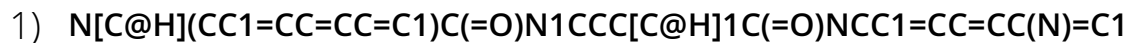If your molecules were docked using another program you can load them straight into the Analyzer mode.

Alternatively, copy/paste (ctrl + c/ctrl + v) your molecules (as smiles or sdf) here.
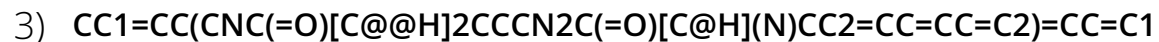For example, copy the three molecules on the right in there, and change their names:

3-Amine-derivative:

1) **N[C@H](CC1=CC=CC=C1)C(=O)N1CCC[C@H]1C(=O)NCC1=CC=CC(N)=C1**

3-Hydroxy-derivative:

2) **N[C@H](CC1=CC=CC=C1)C(=O)N1CCC[C@H]1C(=O)NCC1=CC=CC(O)=C1**

3-Methyl-derivative:

3) **CC1=CC(CNC(=O)[C@@H]2CCCN2C(=O)[C@H](N)CC2=CC=CC=C2)=CC=C1**

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

At most 10 poses per molecule are generate 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.

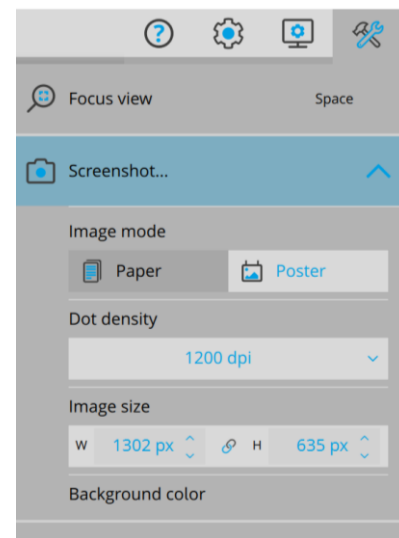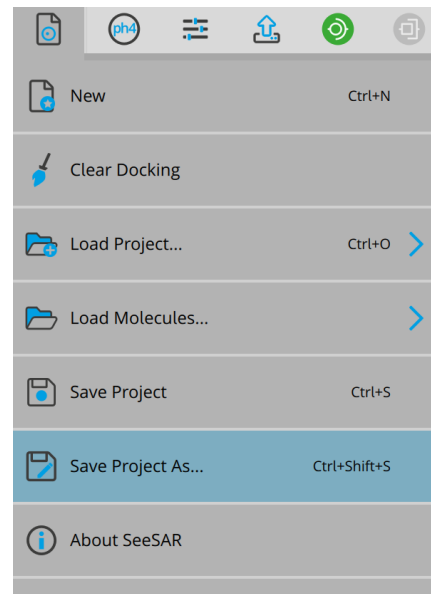Clicking on a column header sorts according to this value.

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!