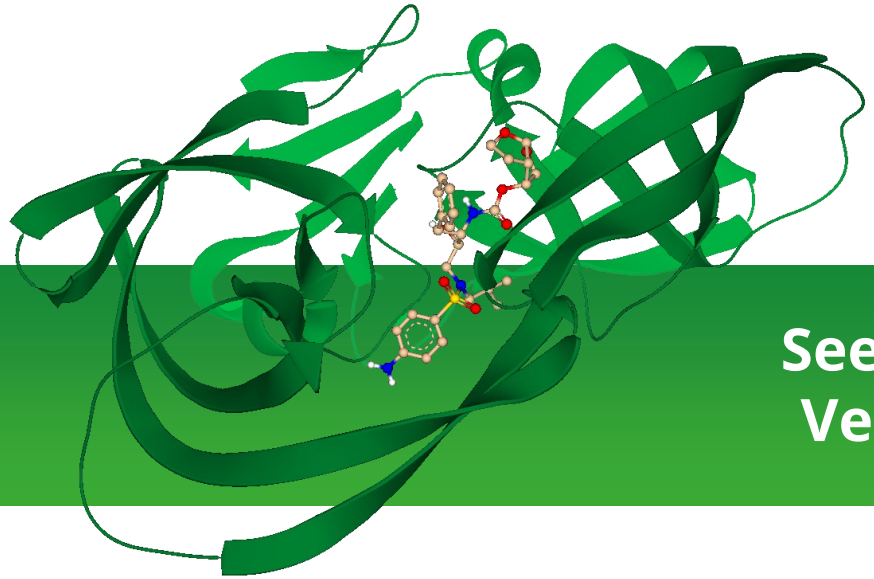
SeeSAR

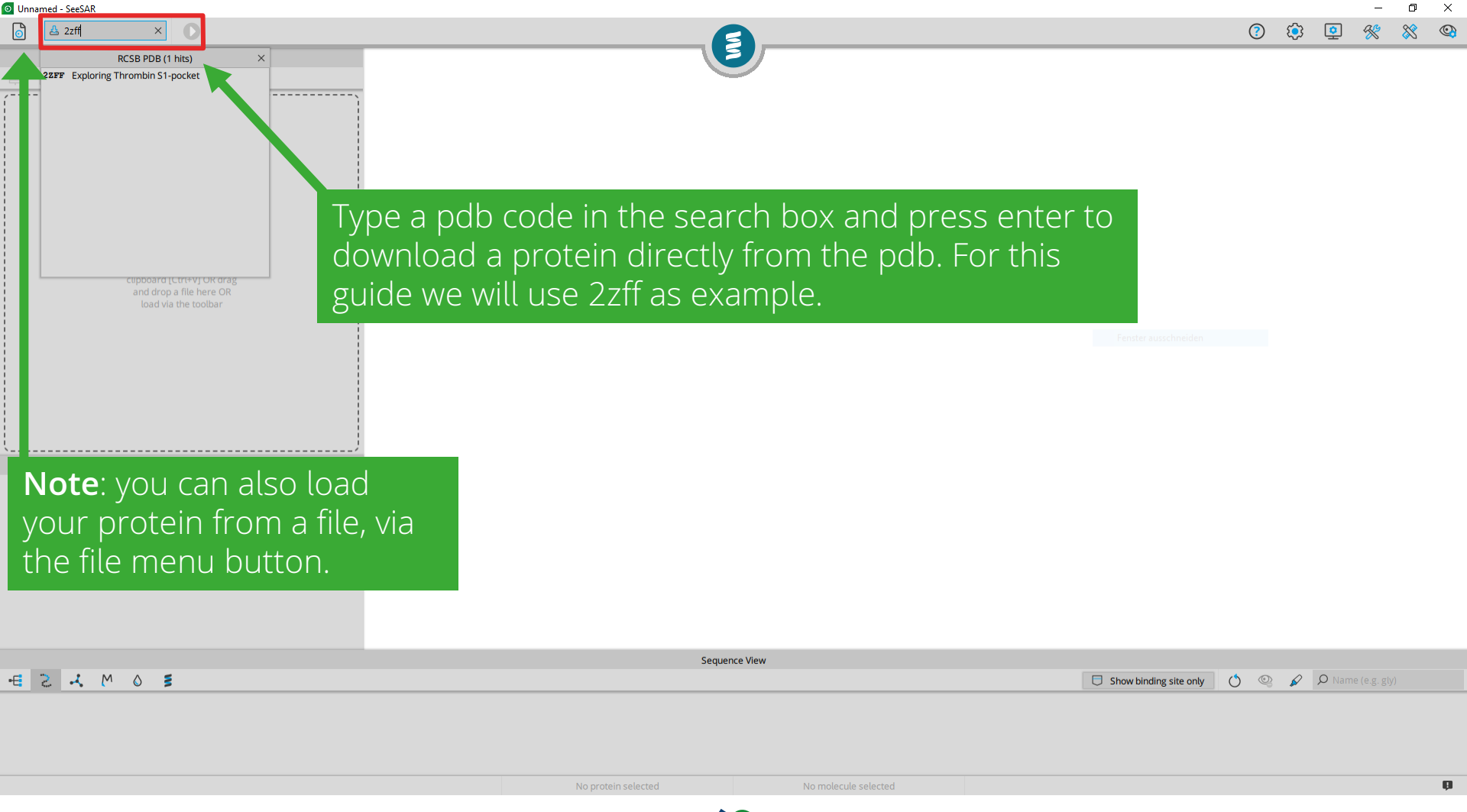
BioSolveIT
expect actives!

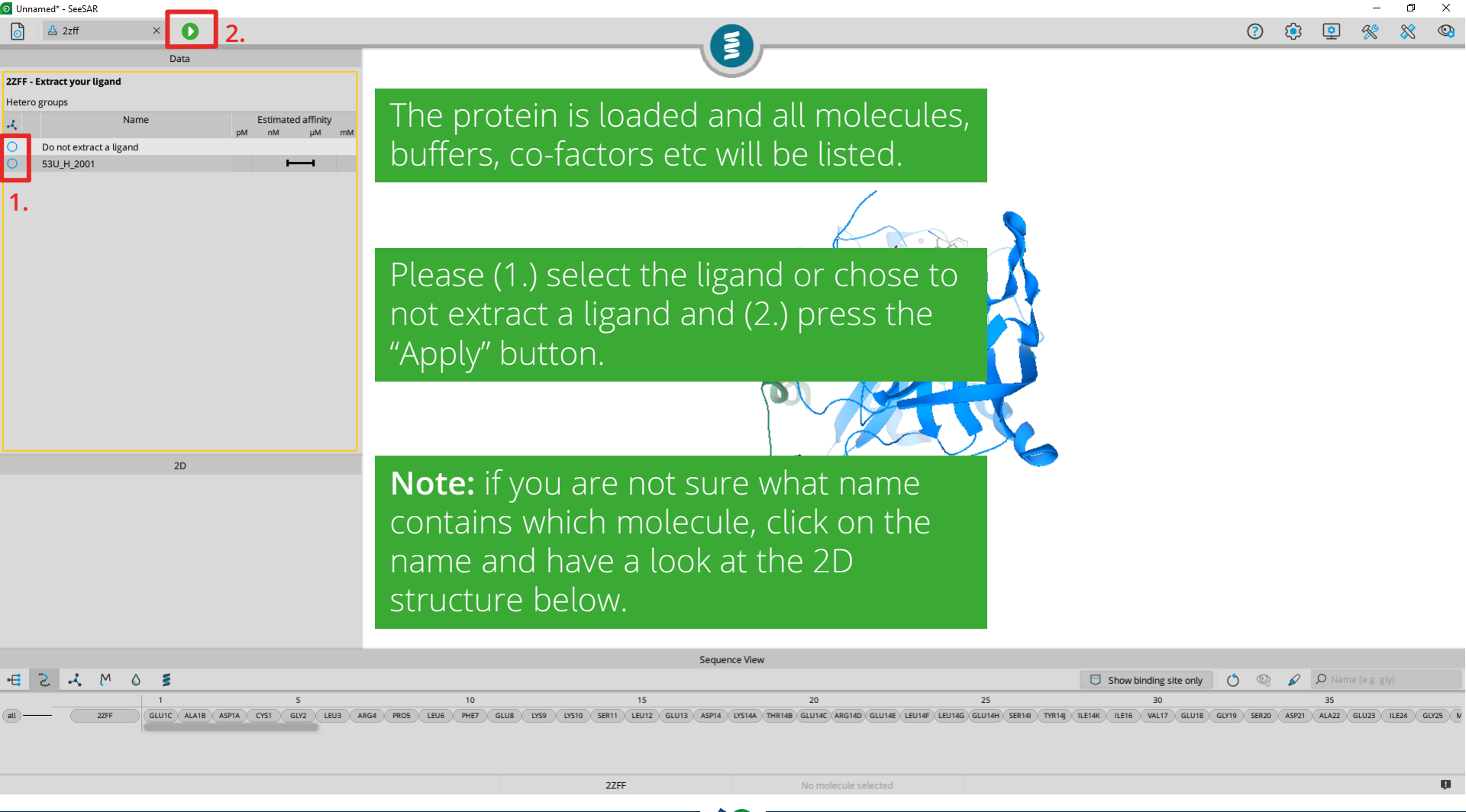


SeeSAR Beginner's Guide
Version 11 - Hephaestus

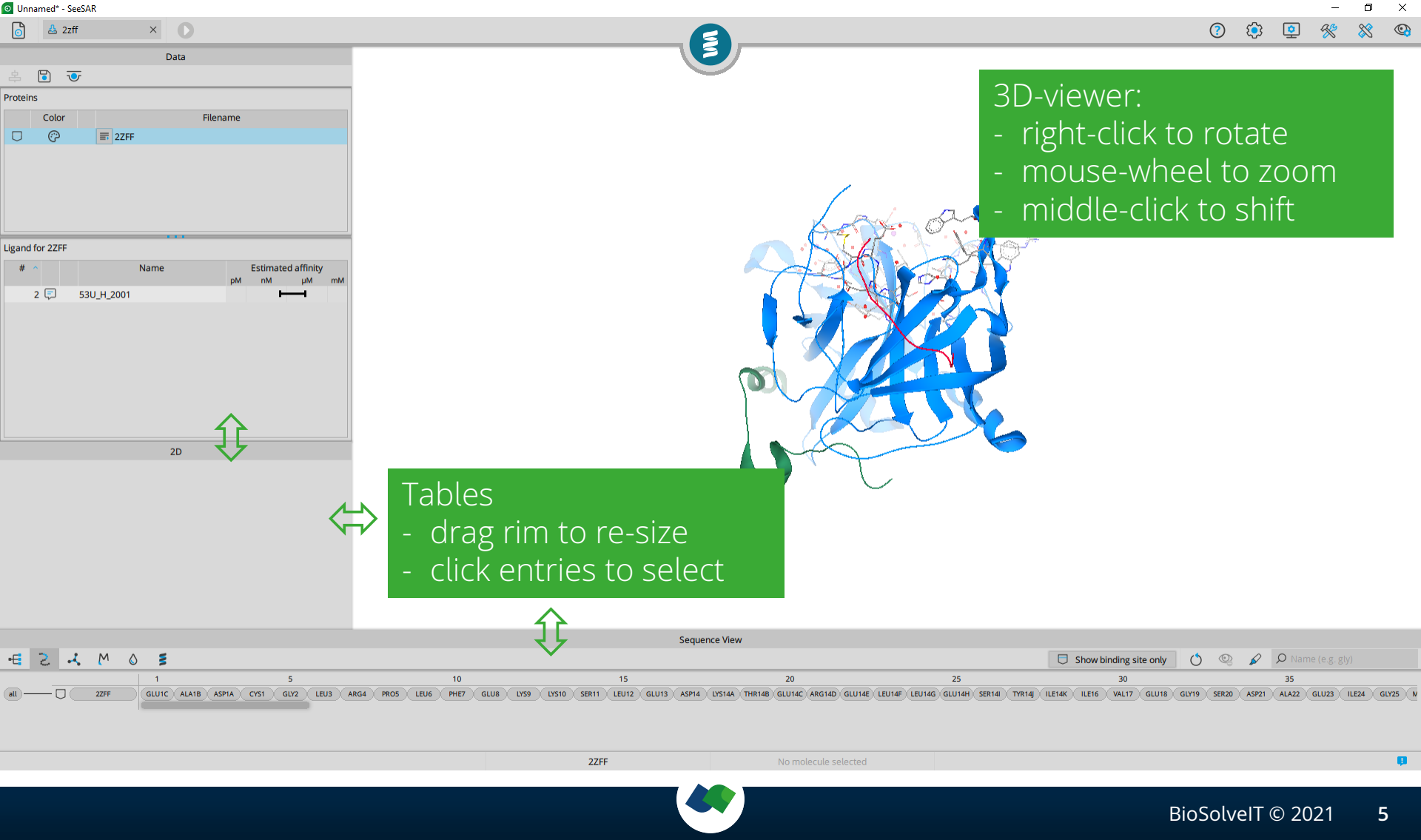
1. Basics

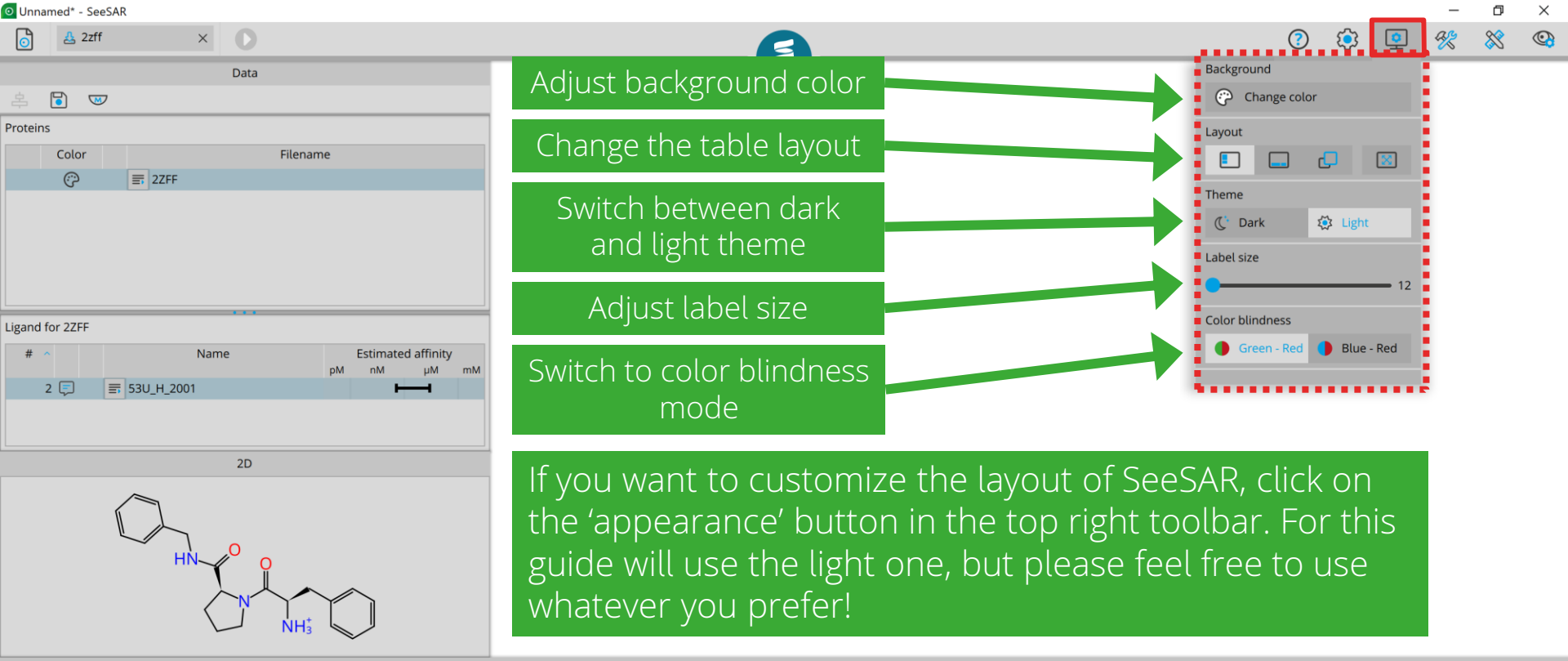
To begin, let's start with a new project.


Unnamed - SeeSAR
2zff
RCSB PDB (1 hits)
2ZFF Exploring Thrombin S1-pocket
clipboard [Ctrl+V] OR drag and drop a file here OR load via the toolbar
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.
Sequence View
Show binding site only
Name (e.g. gly)
No protein selected
No molecule selected

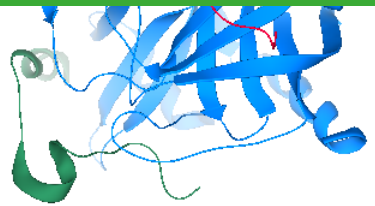

Unnamed* - SeeSAR
2zff
2.
Data
2ZFF - Extract your ligand
Hetero groups
Name
Estimated affinity
pM nM µM mM
Do not extract a ligand
53U_H_2001
1.
2D
The protein is loaded and all molecules, buffers, co-factors etc will be listed.
Please (1.) select the ligand or chose to not extract a ligand and (2.) 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)
2ZFF
No molecule selected

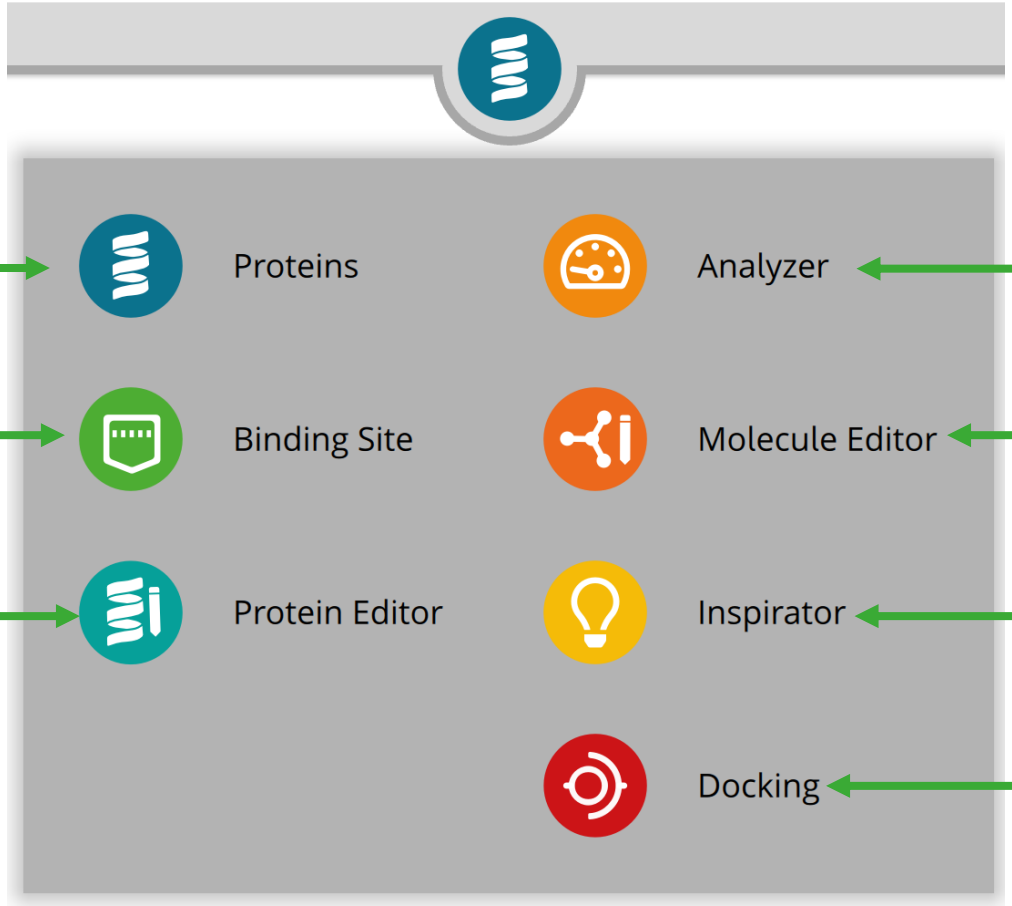

Unnamed* - SeeSAR
2zff
Data
Proteins
Color
Filename
2ZFF
Ligand for 2ZFF
#
Name
Estimated affinity
pM
nM
µM
mM
2
53U_H_2001
2D
After ligand selection, all residues within a 6.5 Angstrom radius around it are automatically selected and presented in the model.
Sequence View
Show binding site only
Name (e.g. gly)
all
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
2ZFF
No molecule selected


Unnamed* - SeeSAR
2zff
Data
Proteins
Color
Filename
2ZFF
Ligand for 2ZFF
#
Name
Estimated affinity
pM
nM
µM
mM
2
53U_H_2001
2D
3D-viewer:
- right-click to rotate
- mouse-wheel to zoom
- middle-click to shift
Tables
- drag rim to re-size
- click entries to select
Sequence View
Show binding site only
Name (e.g. gly)
2ZFF
No molecule selected


Unnamed* - SeeSAR
2zff
Data
Proteins
Color
Filename
2ZFF
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
Background
Change color
Layout
Theme
Dark
Light
Label size
12
Color blindness
Green - Red
Blue - Red
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
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

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.

The Binding Site mode sets the reference pocket.

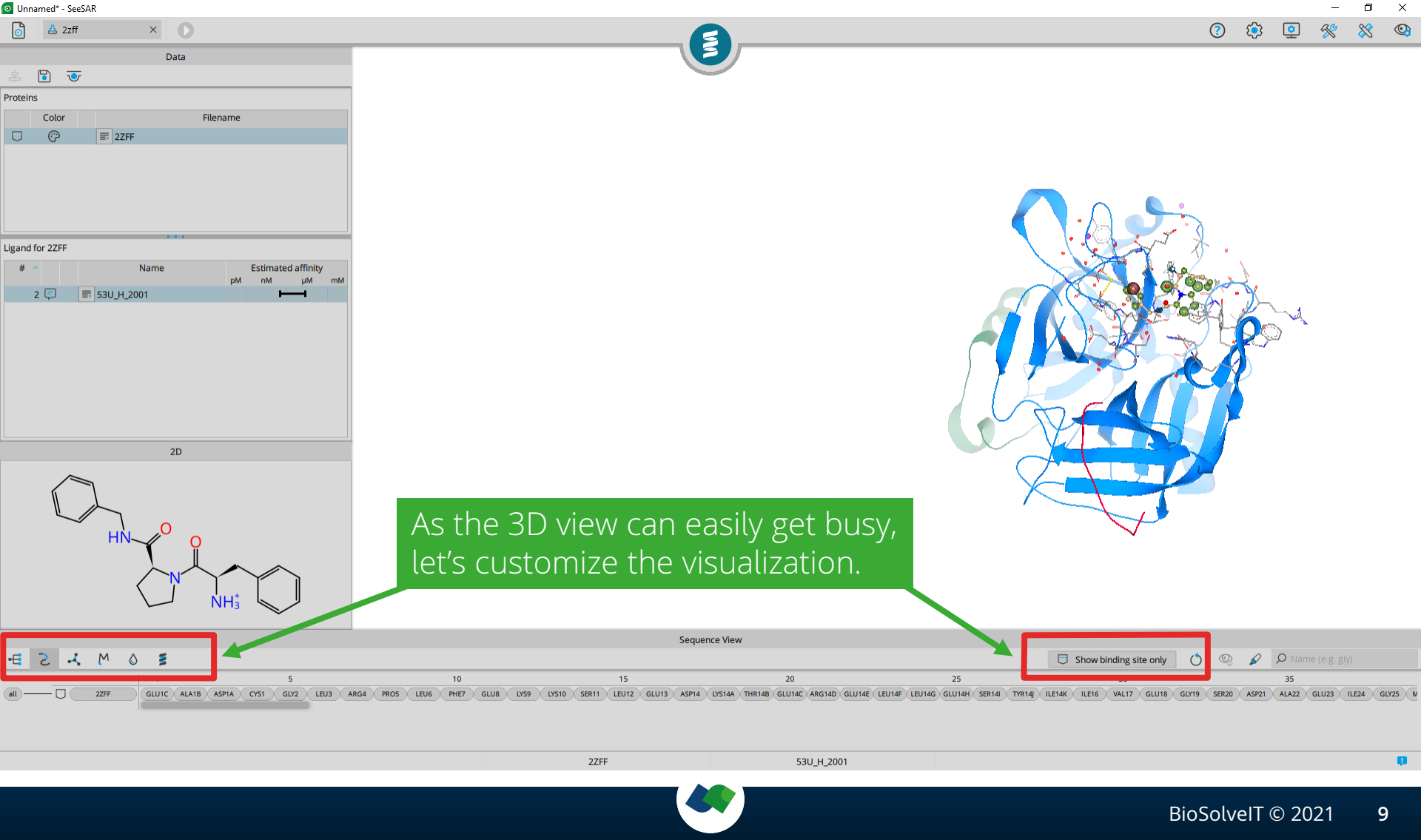
The Protein Editor mode is for editing side chains, deleting waters or buffers, or search for similar binding sites.

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

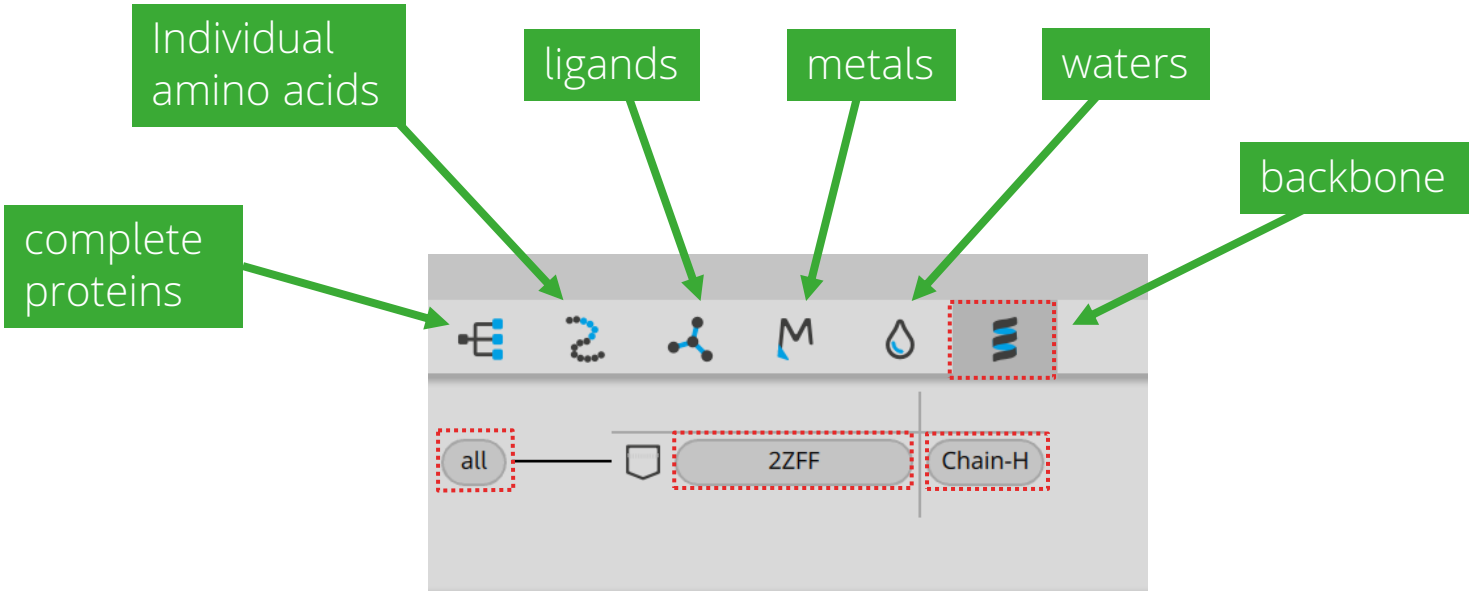
The Molecule Editor mode is for designing new molecules in 3D.

The Inspirator mode helps you to generate new ideas.

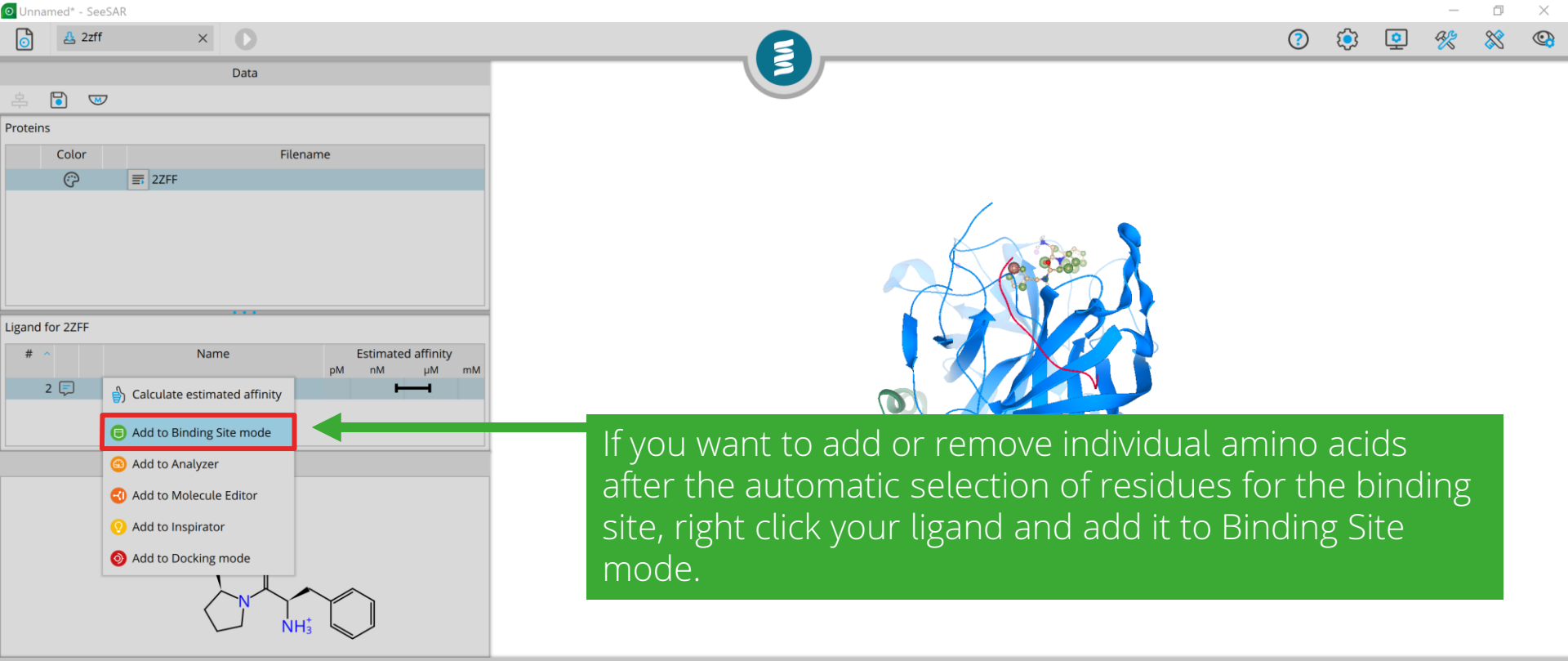
In the Docking mode you can generate poses for new molecules.

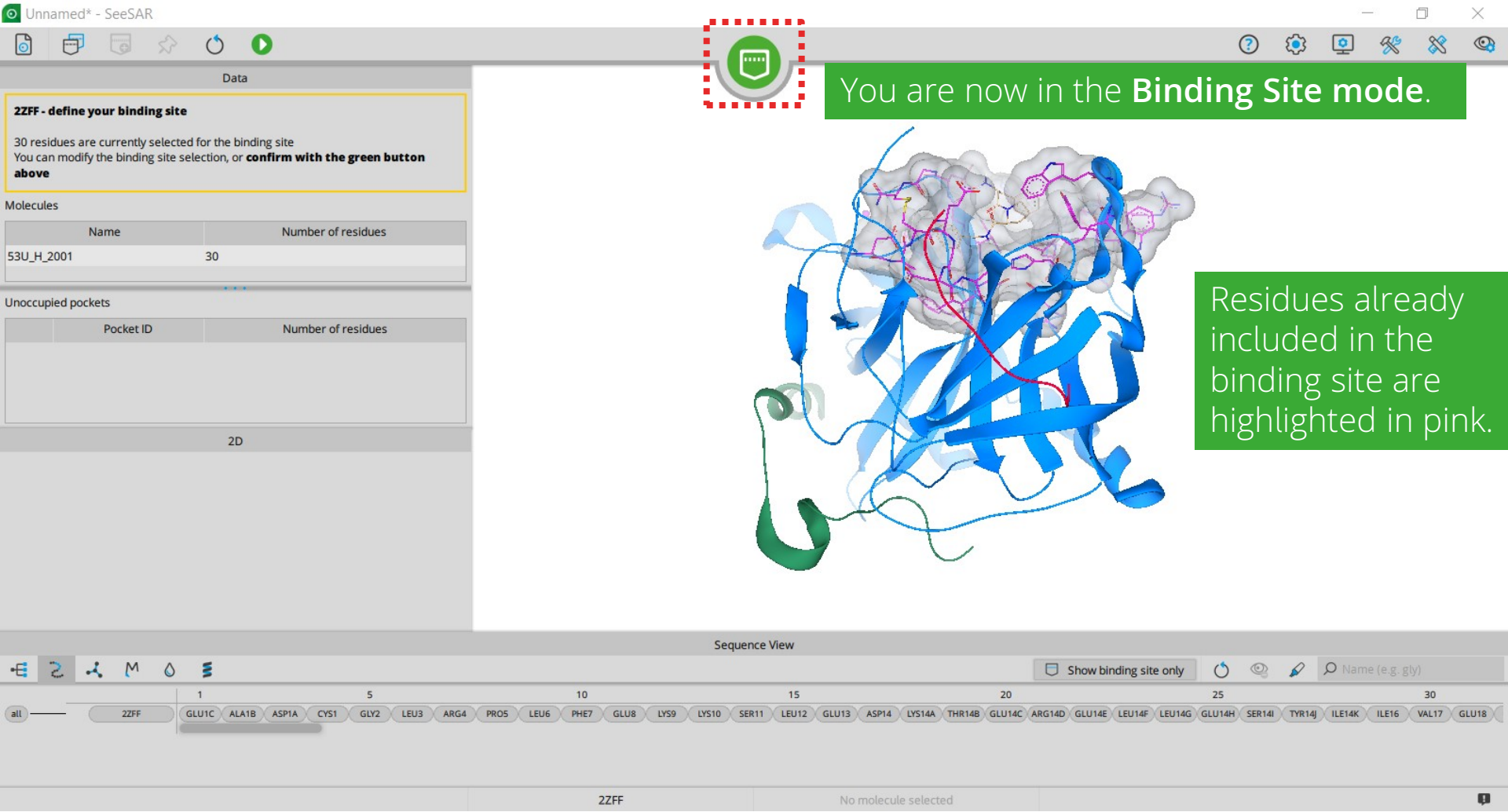

Unnamed* - SeeSAR
2zff
Data
Proteins
Color
Filename
2ZFF
Ligand for 2ZFF
#
Name
Estimated affinity
pM
nM
µM
mM
2
53U_H_2001
2D
HN
O
O
N
NH3+
As the 3D view can easily get busy,
let's customize the visualization.
Sequence View
Show binding site only
Name (e.g. gly)
all
2ZFF
GLU1C
ALA1B
ASP1A
CYS1
GLY2
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
2ZFF
53U_H_2001

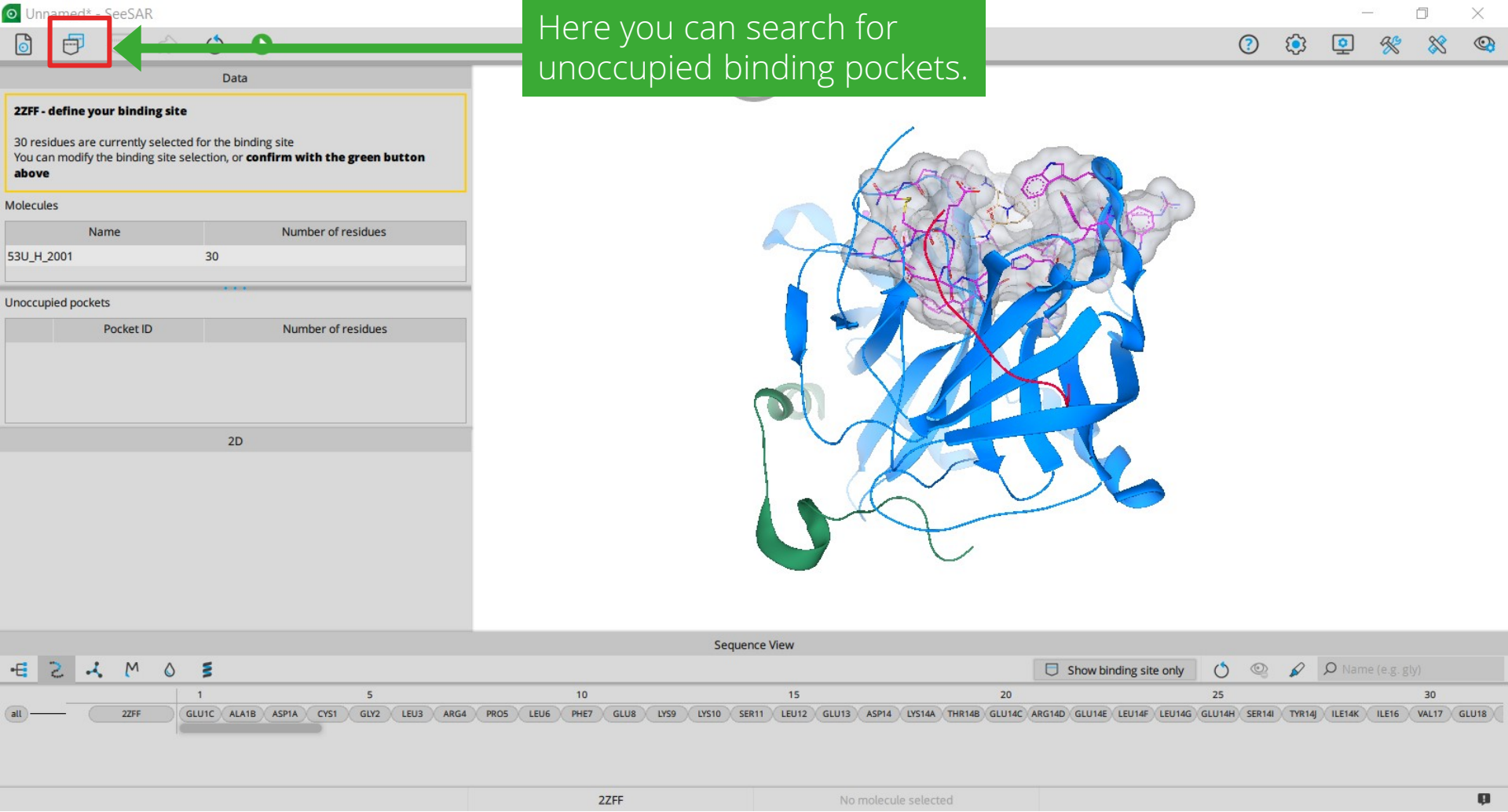
The view controls let you toggle on/off:



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).


Unnamed* - SeeSAR
2zff
Data
Proteins
Color
Filename
2ZFF
Ligand for 2ZFF
#
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
N
NH3+
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
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
53U_H_2001


Unnamed* - SeeSAR
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
You are now in the Binding Site mode.
Residues already included in the binding site are highlighted in pink.
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.
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)
2ZFF
No molecule selected

Unnamed* - SeeSAR

2.

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

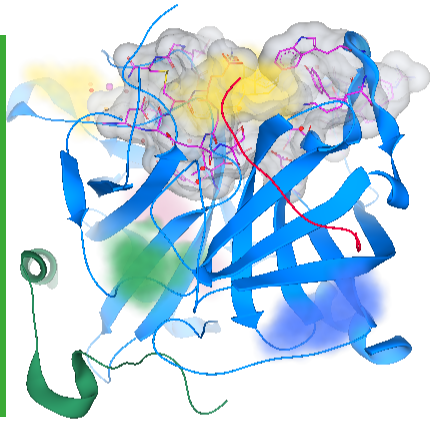
Unoccupied pockets

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

2D

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.)



1.

Sequence View

Show binding site only

Name (e.g. gly)

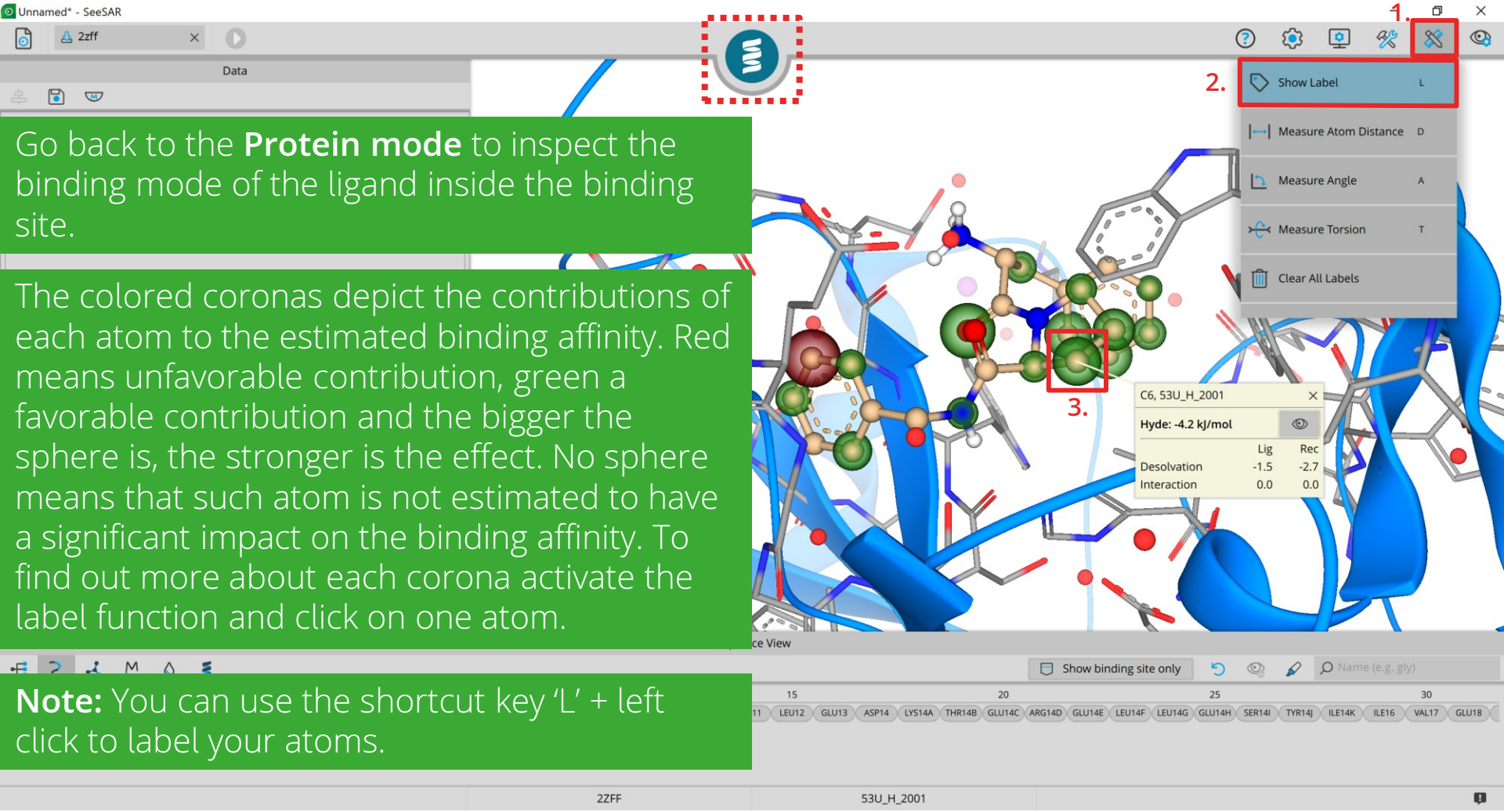
2ZFF

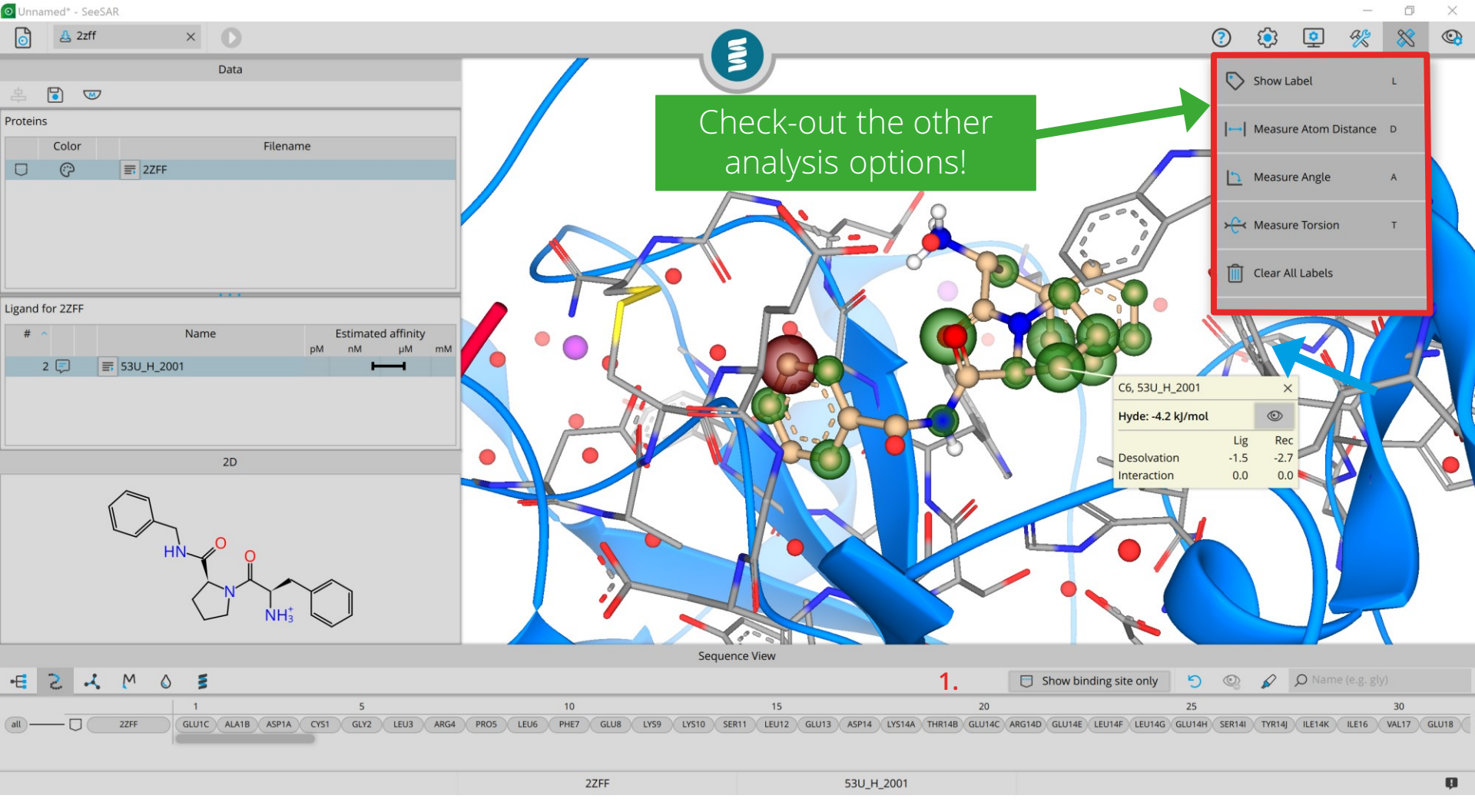
No molecule selected

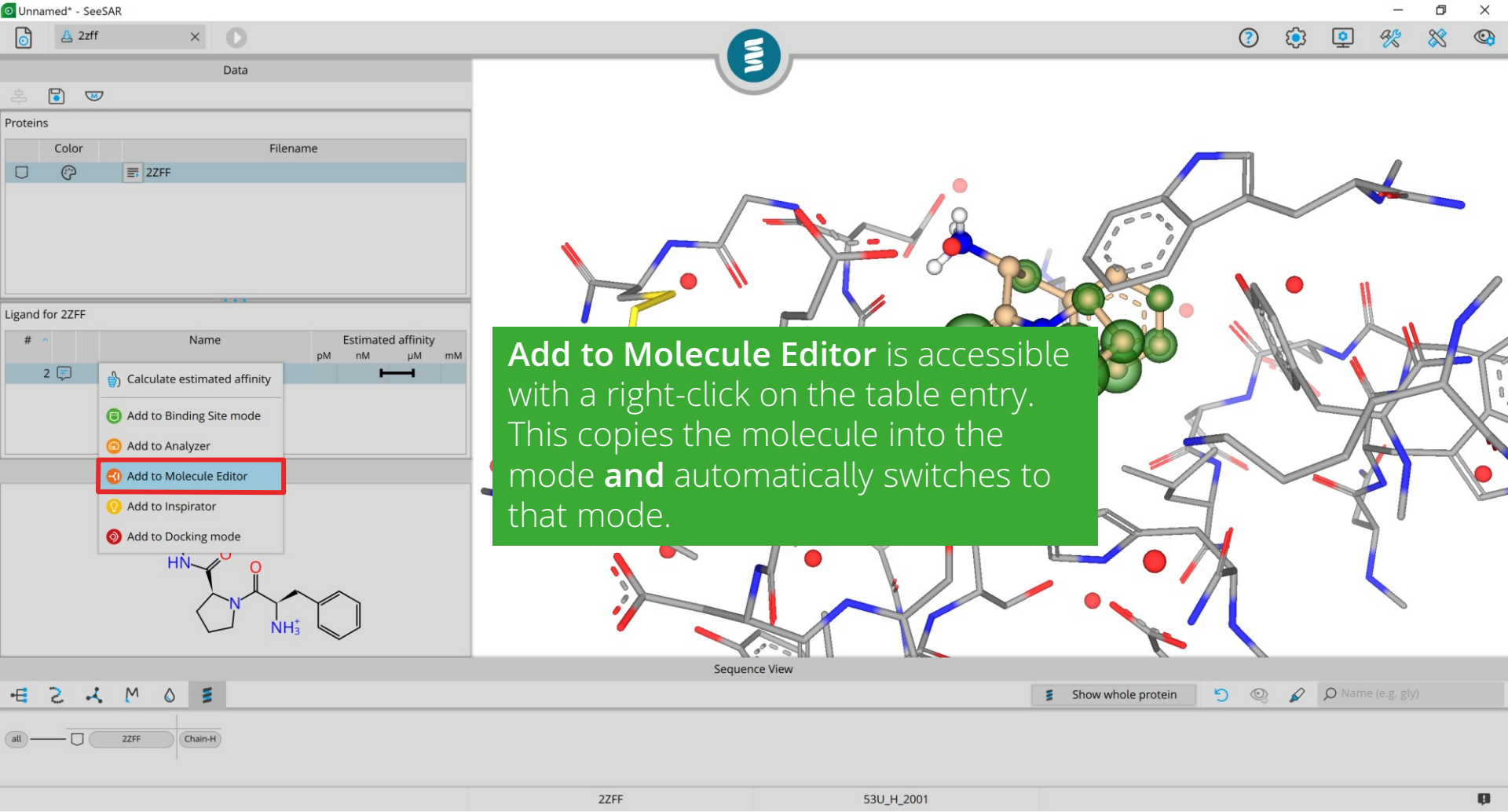
Go back to the **Protein mode** to inspect the binding mode of the ligand inside the binding site.

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.

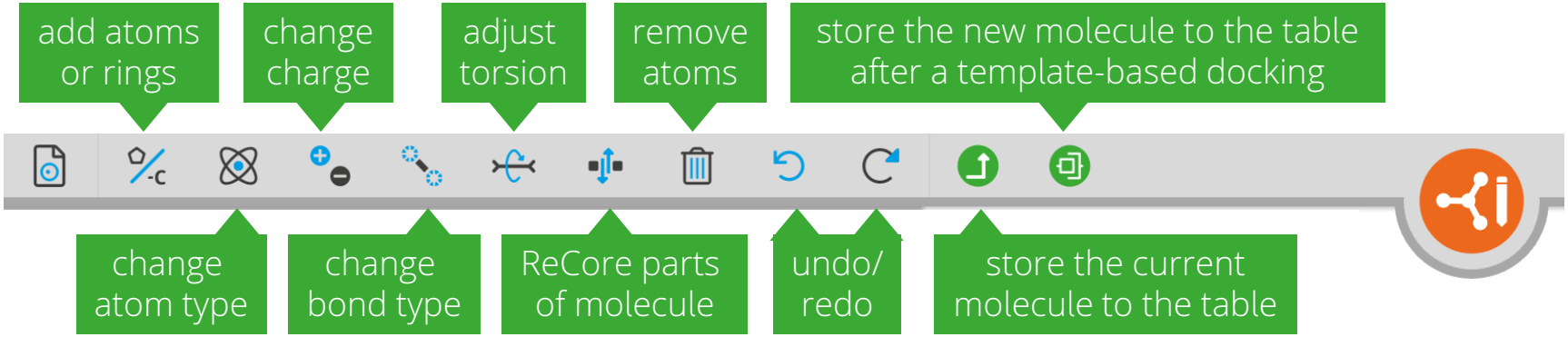
Note: You can use the shortcut key 'L' + left click to label your atoms.




Unnamed* - SeeSAR
2zff
Data
Proteins
Color
Filename
2ZFF
Ligand for 2ZFF
#
Name
Estimated affinity
pM
nM
µM
mM
2
53U_H_2001
2D
HN
O
O
N
NH3+
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
Sequence View
1.
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
53U_H_2001


Unnamed* - SeeSAR
2zff
Data
Proteins
Color
Filename
2ZFF
Ligand for 2ZFF
^
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
O
N
NH3+
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.
Sequence View
Show whole protein
Name (e.g. gly)
all
2ZFF
Chain-H
2ZFF
53U_H_2001

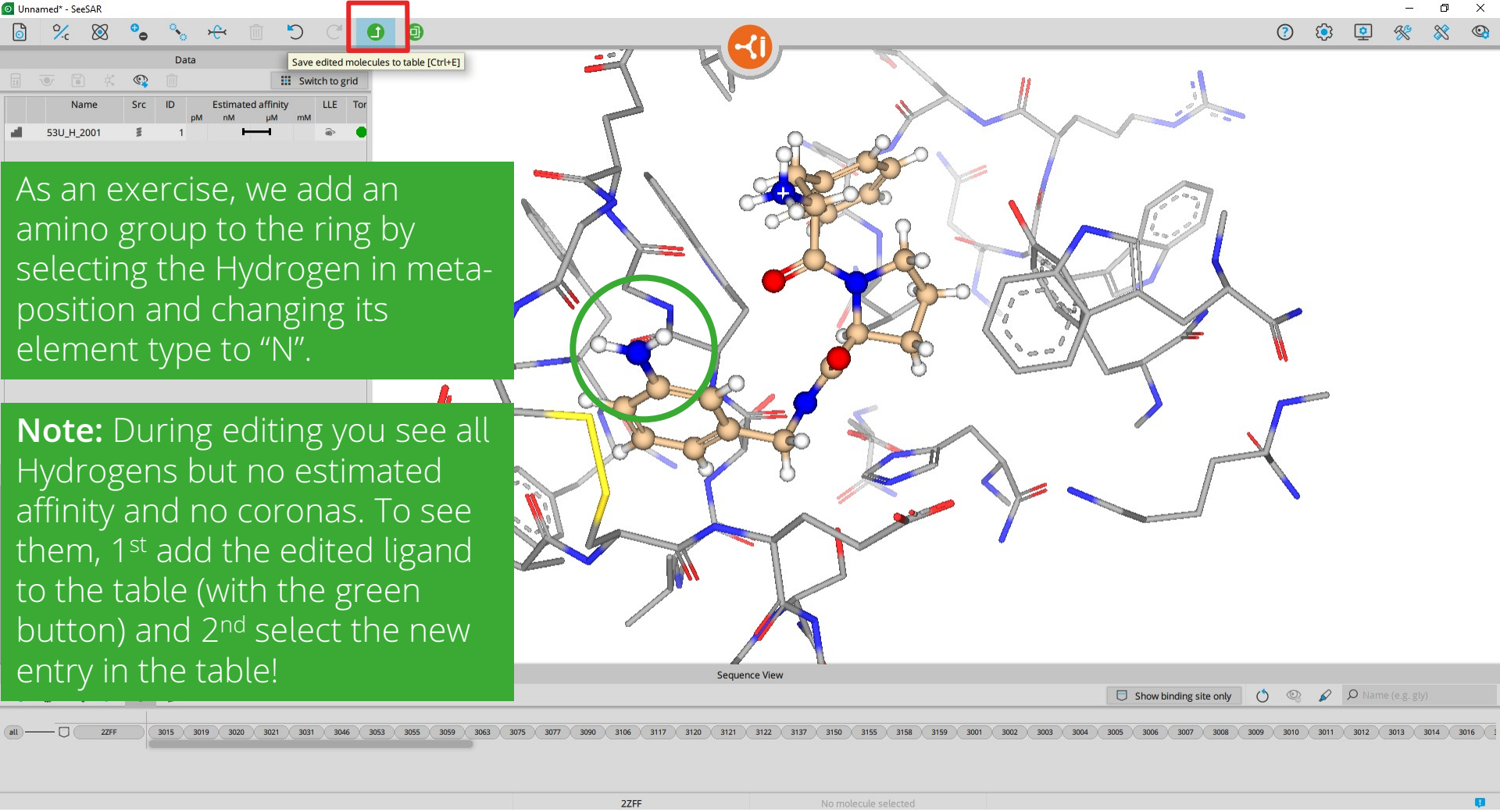
The editor-menu will appear on the top left. There you can:

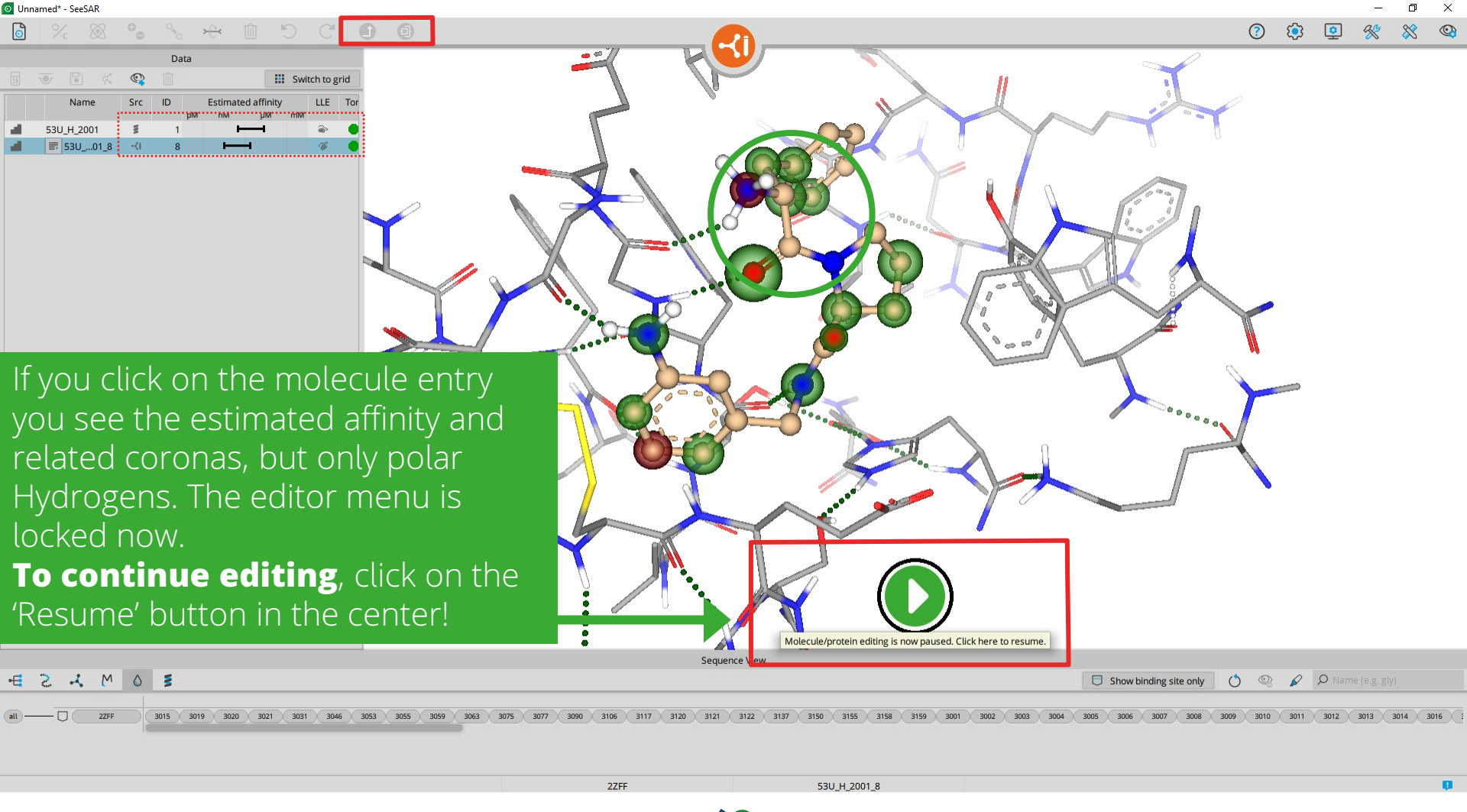


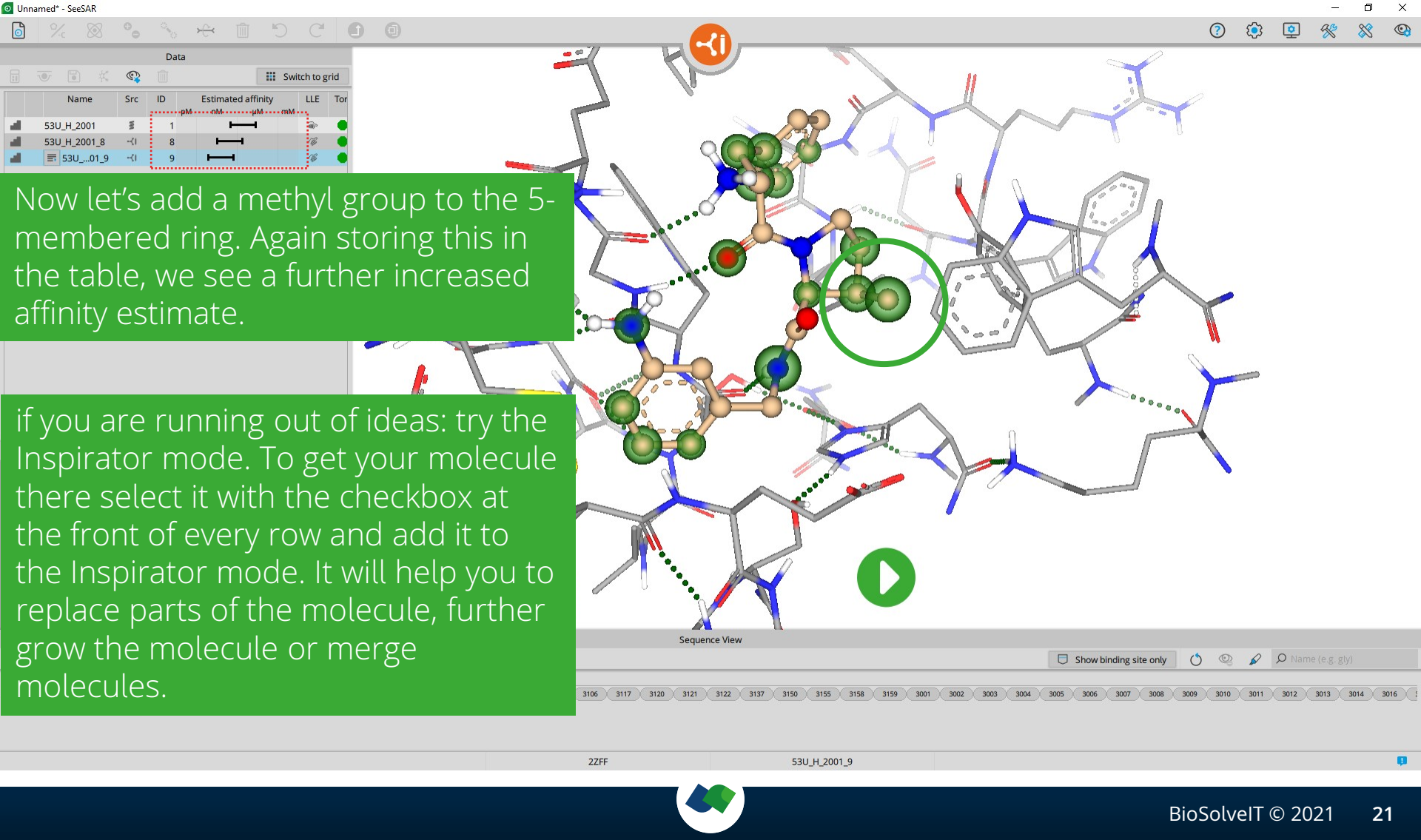
To edit a molecule ALWAYS:

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, ...).

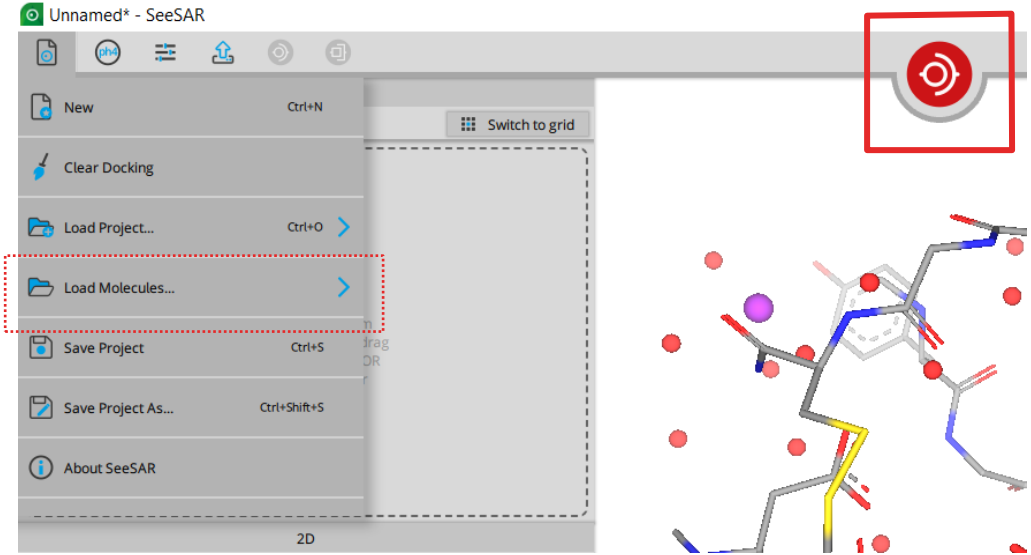

Unnamed* - SeeSAR
Save edited molecules to table [Ctrl+E]
Data
Switch to grid
Name
Src
ID
Estimated affinity
LLE
pM
nM
µM
mM
53U_H_2001
1
As an exercise, we add an amino group to the ring by selecting the Hydrogen in meta-position and changing its element type to “N”.
Note: During editing you see all 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)
2ZFF
No molecule selected


Unnamed* - SeeSAR
Data
Switch to grid
Name
Src
ID
Estimated affinity
LLE
Tor
pM
nM
µM
mM
53U_H_2001
53U_...01_8
1
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!
Molecule/protein editing is now paused. Click here to resume.
Sequence View
Show binding site only
Name (e.g. gly)
2ZFF
53U_H_2001_8


Unnamed* - SeeSAR
Data
Switch to grid
Name
Src
ID
Estimated affinity
LLE
Tor
pM
nM
µM
mM
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)
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:

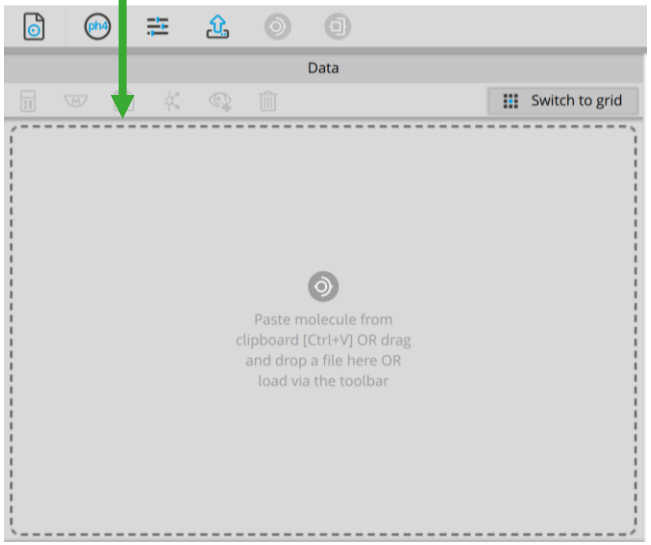
1) **N[C@H](CC1=CC=CC=C1)C(=O)N1CCC[C@H]1C(=O)NCC1=CC=CC(N)=C1**

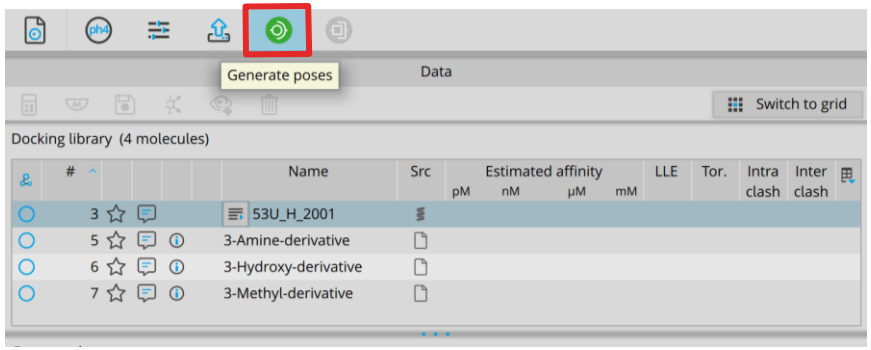
3-Hydroxy-derivative:

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

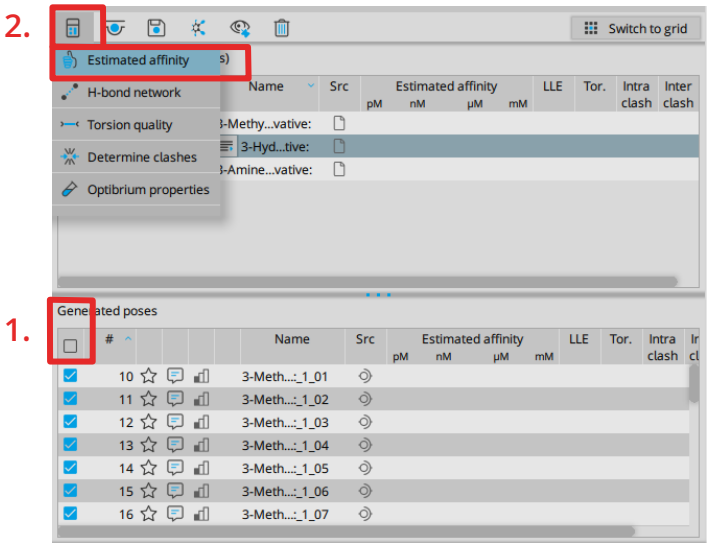
3-Methyl-derivative:

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!

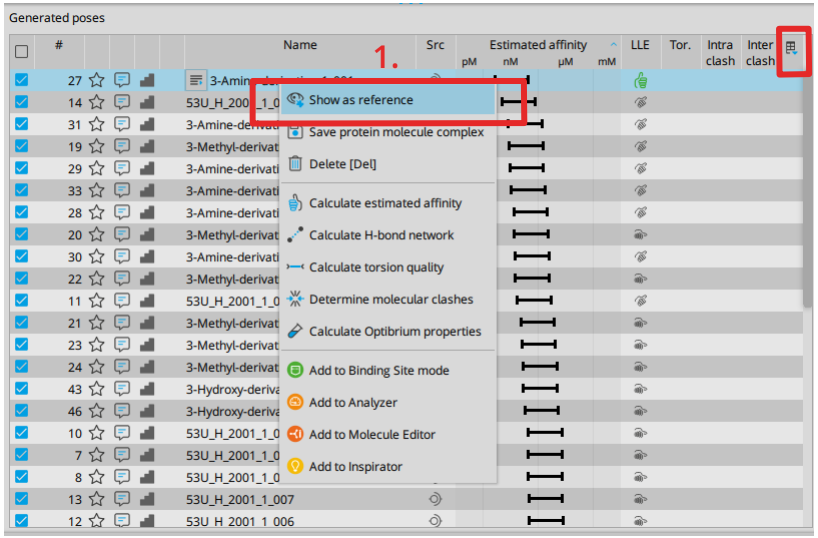


At most 10 poses per molecule are generate this way, as we have left the docking settings on default.
To get the estimated affinities, (1.) select all docking solutions with the checkmark in the first column and (2.) press the thumbs-up button under the calculator at the top of the table!

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.

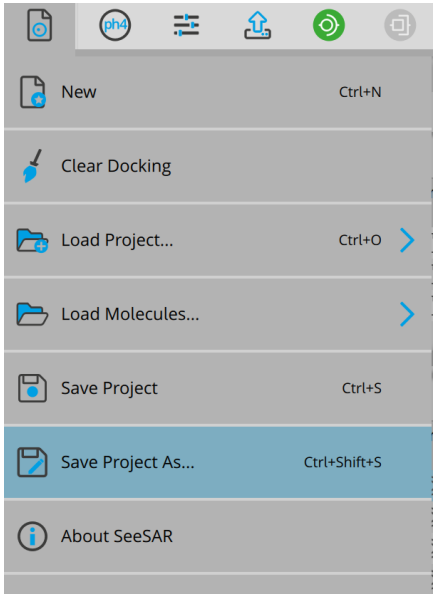


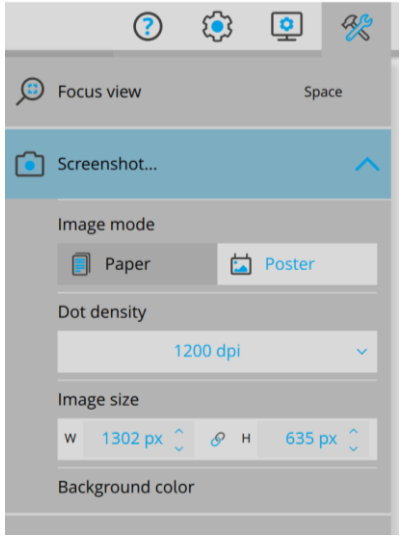
To inspect multiple poses in comparison, (1.) toggle the permanent visibility by marking a molecule as reference. Now it will appear in purple color and stay visible as you select other molecules.

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...**

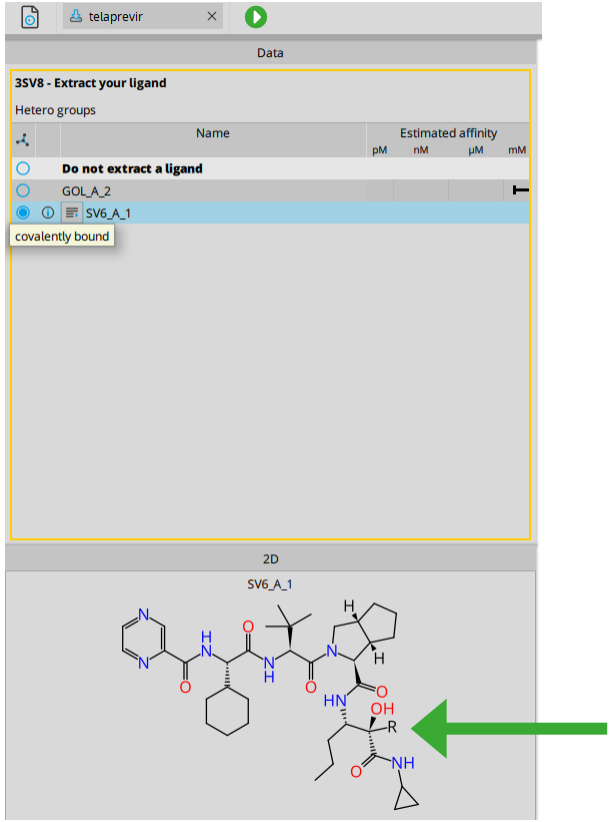




3. Covalent docking

You can perform covalent docking at any PDB protein structure. PDB files that contain a covalent ligand provide this information upon loading within the info icon.

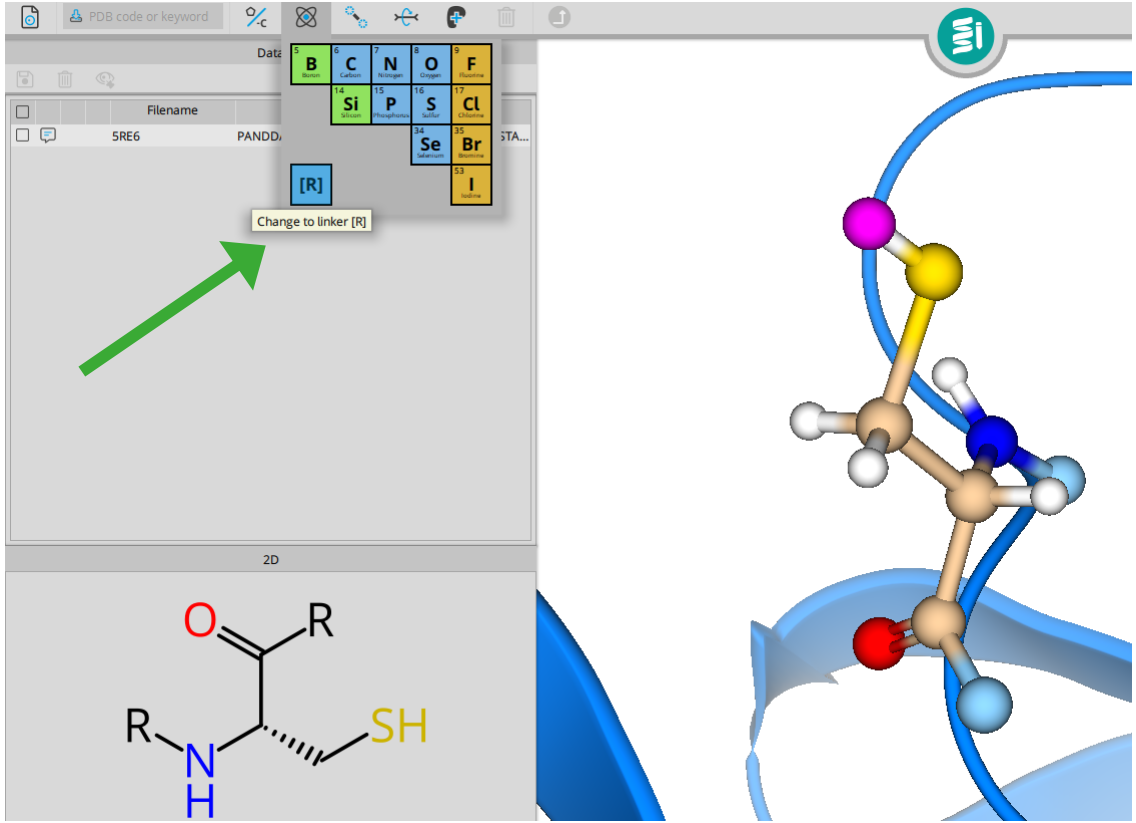
The linking point is represented as R in the 2D structure.



If no covalent linking point of the structure is defined, you can introduce it in the **Protein Editor Mode**.

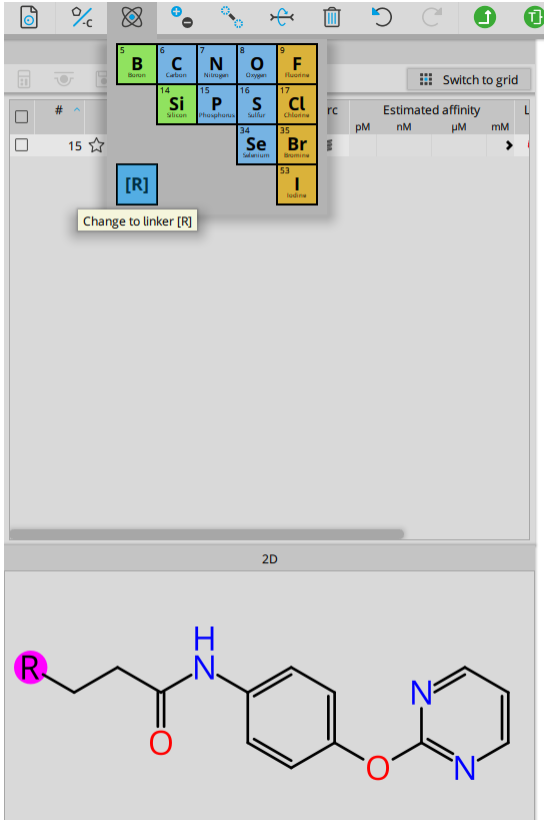
Select the atom of the target residue (e.g. cys, lys, ser) which will be replaced with the ligand.

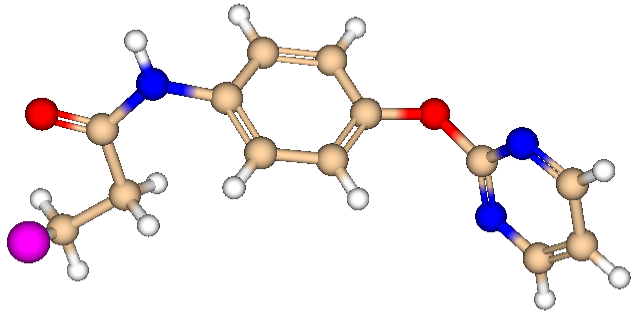
Export the modified protein back to the Protein Mode. Be sure, that your target residue is part of the binding site.

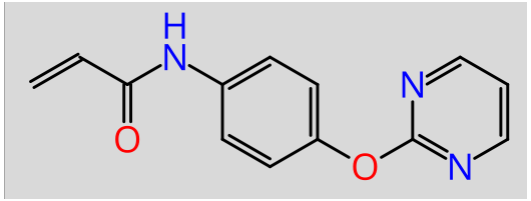


During covalent docking, your ligand is docked in it's bound state. Therefore, your structure has to include a linking point R and take into account any transformations that occur at the covalent warhead.

You can introduce a linker by selecting an atom and transform it into a linker.







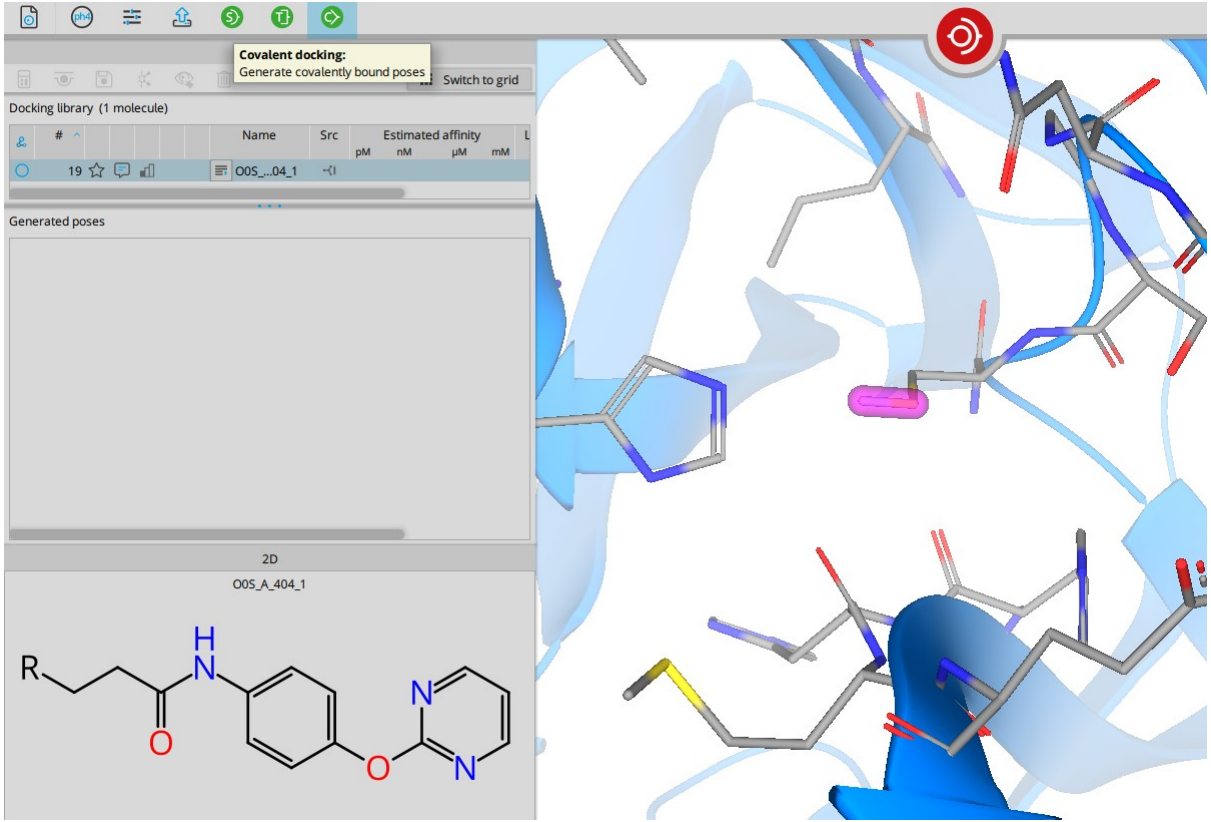
You can introduce a linker atom to your SMILES string with [R*]

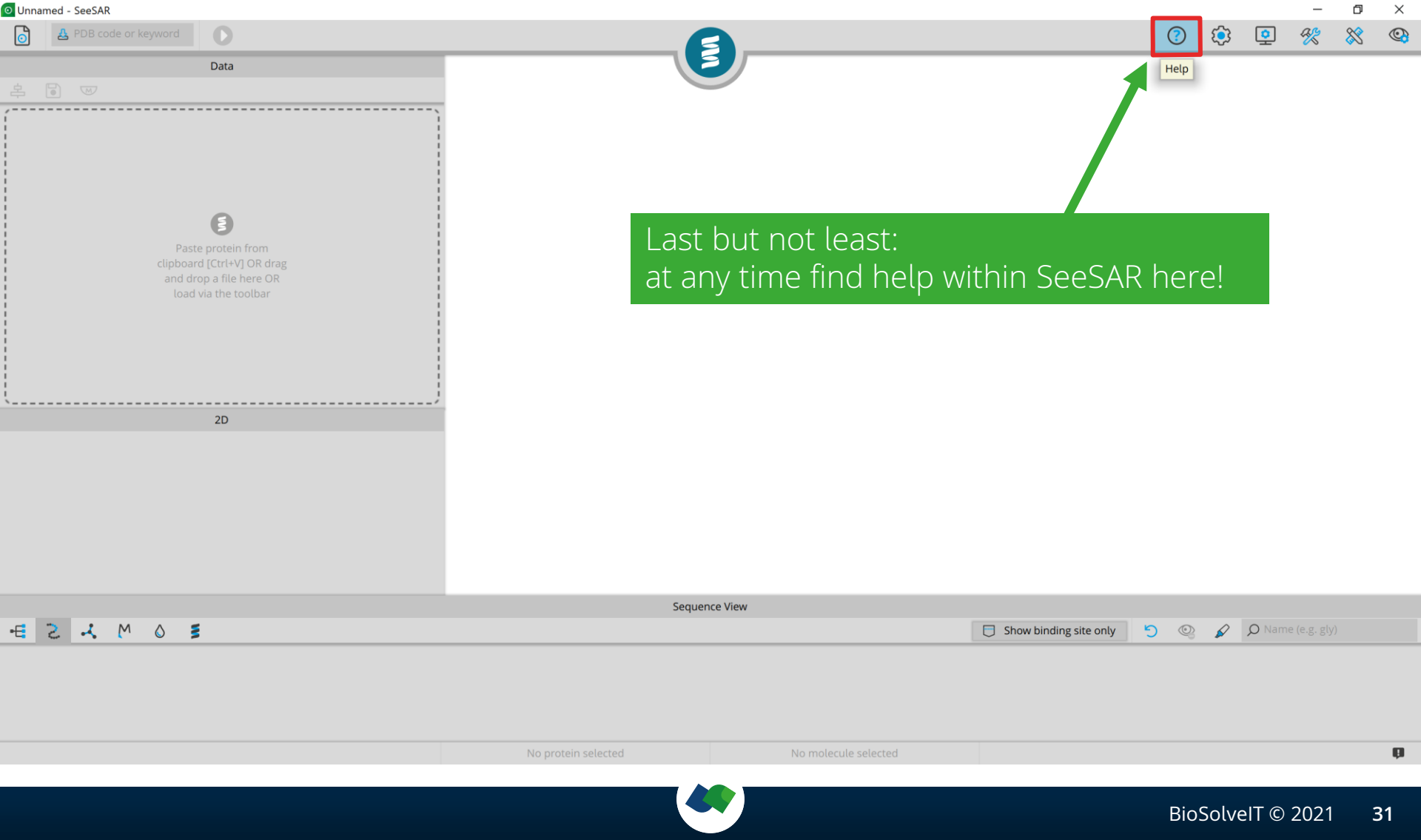
In this example we have transformed an acrylamide warhead to its bound form to prepare the ligand for covalent docking.

Once you have your target residue defined and your ligand prepared you can covalently dock in the **Docking Mode**.

You can adjust your docking setting similar to conventional docking.

If you have selected several residues as potential targets you can switch between them by clicking on the anchor point.




Unnamed - SeeSAR
PDB code or keyword
Help
Data
Paste protein from clipboard [Ctrl+V] OR drag and drop a file here OR load via the toolbar
2D
Last but not least:
at any time find help within SeeSAR here!
Sequence View
Show binding site only
Name (e.g. gly)
No protein selected
No molecule selected

Now we wish you happy SeeSAR-ing!