Important Note

So far SeeSAR is limited to handling 50,000 (50k) molecules and respective docking poses to ensure a sophisticated user experience. Translated covalent compound libraries may exceed this limit and thus require a prefiltering to be handled satisfactorily. The procedures are explained in this guide.

If you need any help or support please do not hesitate to contact us:

support@biosolveit.de
1. Basics

Welcome to the exciting world of covalent docking in SeeSAR!

BioSolveIT has translated supplier libraries featuring a broad range of covalent warheads into a convenient ready-to-dock format for SeeSAR.

We offer two kinds of covalent libraries:

- **Teaser set (10k molecules)**
  Ready to be used in SeeSAR to evaluate covalent docking at your target.

- **Translated supplier sets**
  Require prefiltering before covalent docking.
Covalent docking

To perform covalent docking you need two things prepared:

A **covalent target**
see → 2. Attachment Site

Covalent molecules
see → 3. Covalent libraries
2. Attachment Site

Upon loading your protein structure and defining your ligand, SeeSAR will inform you if a ligand is covalently binding at your structure. In this case the attachment point will be recognized and kept with the binding site definition by selecting the covalent ligand.

If your structure lacks a covalently-bound ligand you need to prepare the attachment site.
You can define a covalent attachment site by transferring your protein to the Protein Editor Mode.
By clicking on a residue you select it as the residue you want to modify.
Select your attachment point (hydrogen) which will be the linker atom to your ligand. It will be highlighted in purple.
Change the atom type to linker by either selecting it in the ‘Change element’ dialog or by pressing the hotkey ‘R’.

\[
\text{R} \quad \text{O} \quad \text{R} \\
\text{R} \quad \text{NH} \quad \text{SH}
\]
The cysteine residue has now been prepared for covalent docking. Save the edited protein and transfer it back to the Protein Mode to redefine your binding site on the edited protein for docking.
Selection of covalent binding residue

**In the Docking Mode:**
If several covalent attachment dummies are present in your structure you can freely decide which one of them shall be docked at by clicking on the dummy tubus. The active residue is highlighted in purple.
3. Covalent libraries

Every translated covalent library sd-file contains information on:
- vendor
- warhead functionality
- target residue
- (sublibrary)

You can use this information to filter for covalent functionalities you are interested in or for compounds likely to target a specific residue.

We recommend to use KNIME to assess and filter compound libraries containing more than 50k members.
Prefilter your library in SeeSAR

Go to the **Analyzer Mode** to filter your compounds for a specific residue or warhead.
Click on the funnel icon to open the filter window.

Scroll down to select a property of interest to filter for.
Filter for target residues

Type the 3-letter-code of your target residue (and press the ‘Apply filters’ button if it is green to activate your selection).

Residues you can filter for:
- **Cys** – cysteine
- **Glu** – glutamic acid
- **Lys** – lysine
- **Ser** – serine
- **Thr** – threonine
- **Tyr** – tyrosine
Likewise you can filter for a warhead of your choice.

possible warheads

- Aldehyde
- Carbamate
- Maleimide
- Alkynyl
- Cyanamide
- Nitrile
- Alkynylyl
- Diazerine
- Nitroalkane
- Allylamide
- Disulfide
- Oxetane
- Allylester
- Epoxide
- Propargylamine
- Arylator
- Imidazole
- Pyrazole
- Azaridine
- Ketoalkynylyl
- Sulfonylallyl
- Azido
- Ketoamide
- Sulfonylfluoride
- β-aminoketone
- Ketoalogen
- Thiol
- Boronate
- Lactam
- Urea
To proceed with covalent docking transfer the compounds to the docking mode.

1. Check all
2. Uncheck all
3. Add molecules to Docking mode
4. Transfer your selection to Docking Mode
4. Covalent Docking

A greyed out docking button can have different reasons:

1. No covalent binding residue has been defined
2. No covalent compound is present in the docking mode
3. The resulting docking poses would exceed the limit of 50,000 entries → Number of molecules in the docking library x maximum number of poses during docking
Adjust the number of poses

1. Go to the pose generator parameters
2. Adjust the maximum number of poses till the covalent docking button is green.
3. Start the covalent docking with your selected parameters.
After docking and HYDE assessment:
Rank your compounds by clicking on the ‘Estimated affinity’ column till the arrow points upwards (^).
The compounds are now ranked with the highest (best) score on the top and the lowest (worst) score on the bottom of the table.

Now it is up to you to decide which binding modes are of interest. Go through your generated poses and visually inspect the results.