

CCP5 Summer School

July 2023

**Practical sessions: Structure-based and ligand-based design
of C5 inhibitors with BioSolveIT suite**

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Contents

- Molecular docking and virtual screening
- Introduction to SeeSAR
- Application 1: lead optimisation
- Application 2: core expansion
- Application 3: core replacement/scaffold-hopping
- Application 4: fast generation and evaluation of analogues
- Application 5: Virtual screening of massive virtual libraries with InfiniSee (Scaffold Hopper) and SeeSAR

Molecular docking: two main tasks

- **Sampling** of ligand conformational space and pose generation (geometry)
- **Scoring** protein-ligand complexes (energetics)

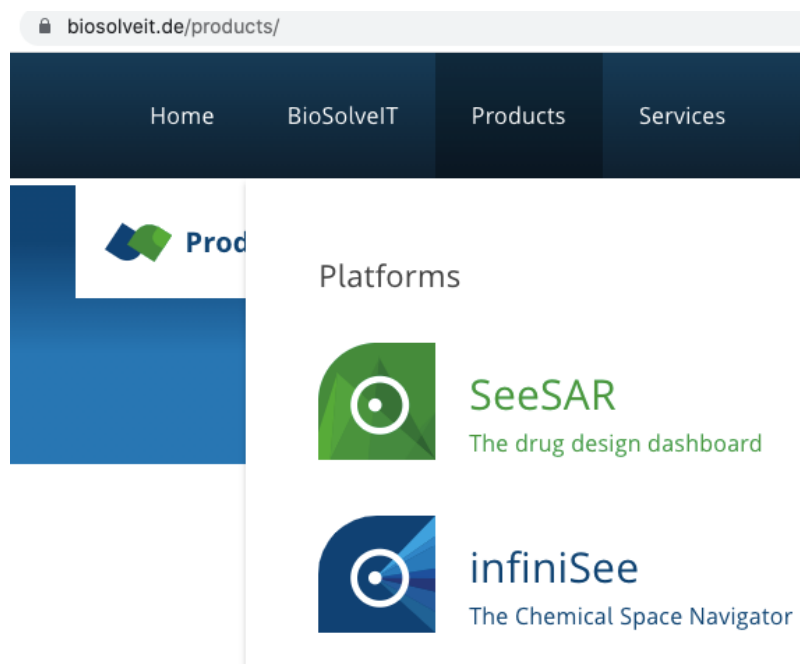
Molecular docking: to-do list

Problem: a pair of molecules represented by their 3D coordinates

- Decide whether the molecules will form a complex;
- Determine the binding affinity (free binding energy);
- Predict the 3D structure of the complex (binding mode);
- Deduce function (agonist/antagonist)*;

Installing SeeSAR and InfiniSee

- Platform-independent
- Quick to install
- Temporary license key (works for both) provided



SeeSAR 13.0.1

Download SeeSAR for your operating system ([System requirements](#)):



Windows

exe (113.6 MB)



macOS

dmg (201 MB)



Linux

tar.gz (206.1 MB)

rpm (158 MB)

deb (131.2 MB)

Introduction to SeeSAR

Tasks 1 and 2

- Import your target structure to SeeSAR

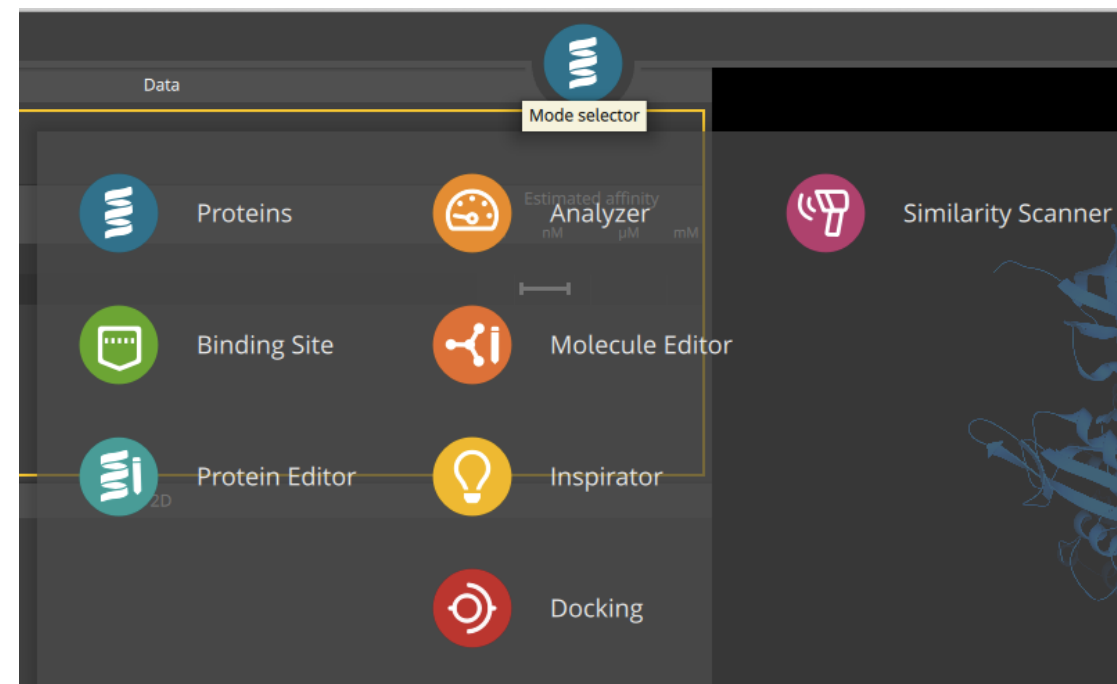
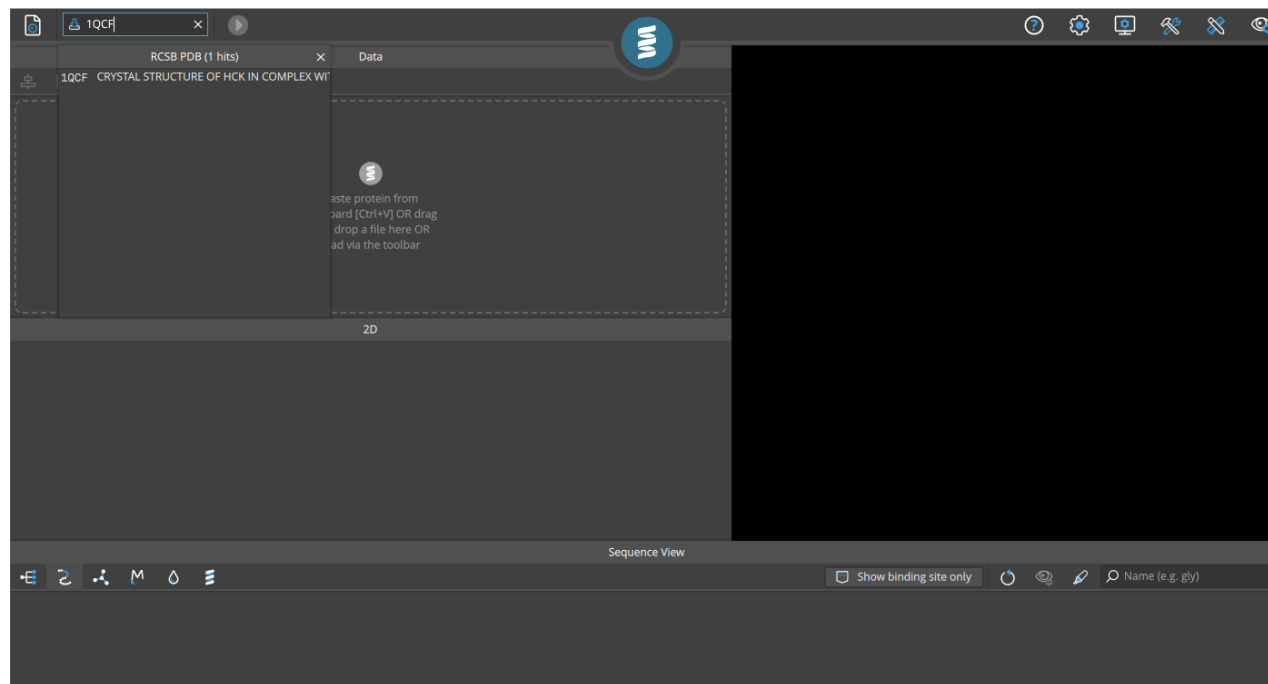
You can either import directly from RCSB PDB Data Bank, or read your local file

- Assign the binding site

The process is automated: user defines the site either by existing ligand (docked or experimental), or by unoccupied pockets

For the covalent docking, you need to prepare your site (target residue) – or to start with the ligand covalently bound

Getting started



The binding site definition in SeeSAR (1)

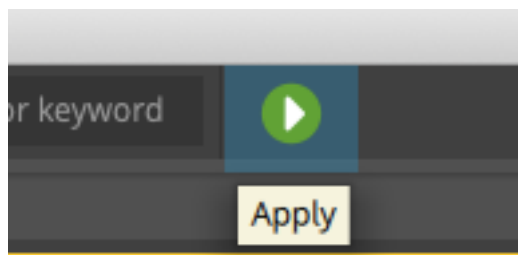
PDB code: 1QCF

1.

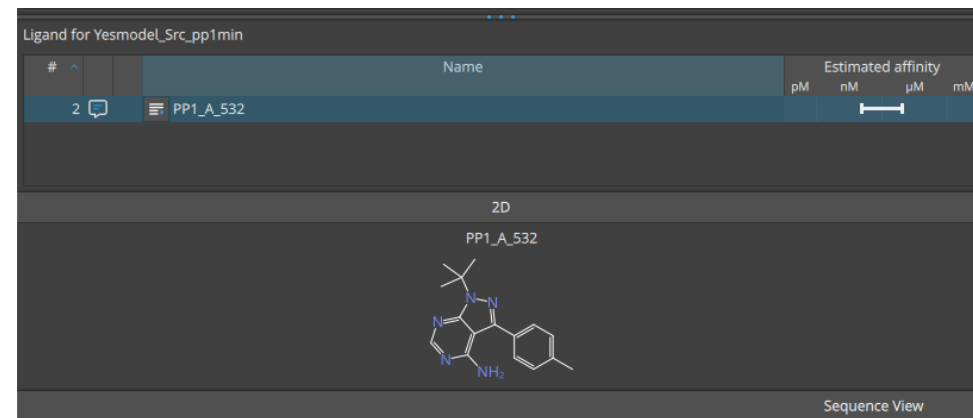
You must tick here



2.



3. The outcome



The binding site definition in SeeSAR (2)

- Using unoccupied pockets



Show/hide unoccupied pockets

Data

1QCF - define your binding site

Choose residues to form the binding site. Hint: First select molecules loaded in the table below and/or calculate unoccupied pockets to help you add residues

Click show pockets in the toolbar OR paste molecule from clipboard [Ctrl+V] OR drag and drop a file here OR load via the toolbar

1QCF - define your binding site

Choose residues to form the binding site. Hint: First select molecules loaded in the table below and/or calculate unoccupied pockets to help you add residues

Molecules

Unoccupied pockets

Pocket ID	# Residues	DoGSiteScore	# Donors	# Acceptors	Hydrophobicity	Surface	Volume
1	40	0.52	27	30	0.69	633.96	771.98
2	33	0.41	24	29	0.64	557.28	832.68
3	31	0.35	17	22	0.67	427.32	541.51
4	18	0.28	7	13	0.69	254.16	397.66
5	16	0.24	6	9	0.75	153.36	122.69
6	10	0.15	6	9	0.70	129.24	183.82

2D

Lead optimisation

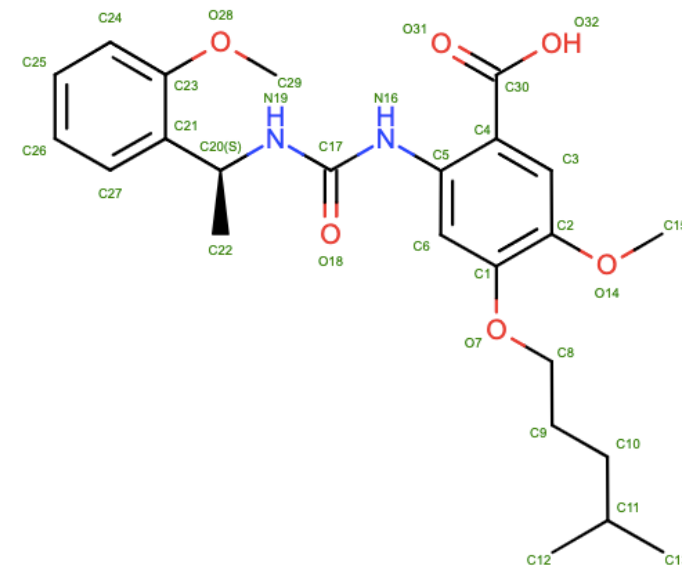
Complement C5 inhibitor

PDB code (source): 8AYH

Use PDB file provided: C5_H1H_CCP5.pdb

CryoEM structure (3.35 Å resolution) of human complement C5 in complex with small molecule inhibitor (H1H) and CVF

IC₅₀: 0.1 - 5 nM, from 3 assays



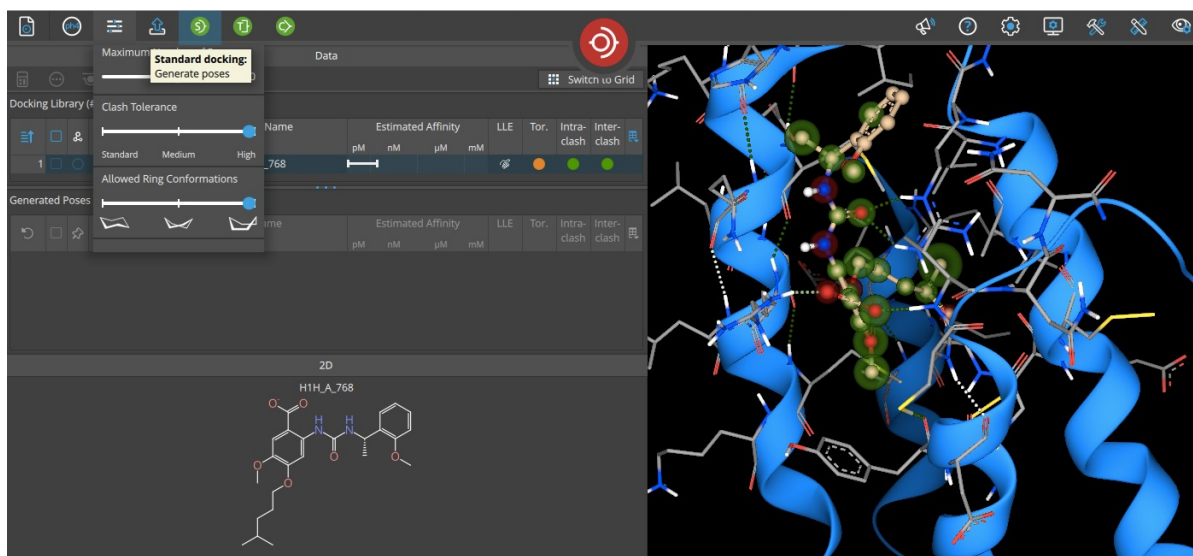
Complement C5 inhibitor

- **Task 3:** open the target-inhibitor complex in SeeSAR, indicate the binding site by the inhibitor (H1H), and bring it to the Molecule Editor mode
- Once you are in the Editor mode, you can manually modify/grow your compound
- **Before you start modifying:** what is the affinity range calculated? How does this compare to the experimental binding affinity?
- What you may try (a good practice): **molecular docking**
- Bring your compound to the **Docking mode**

Complement C5 inhibitor: docking

Task 4: Use standard (noncovalent) docking, 50 poses, high clash tolerance and maximum allowed ring conformations

First you generate the poses, then you select them (select all) and rank them by calculating their binding affinities



2D
H1H_A_768

COc1ccc(cc1)C(=O)N[C@@H](C)C(=O)Nc2cc(OC)c3cc(OC)cc3c2C(=O)O

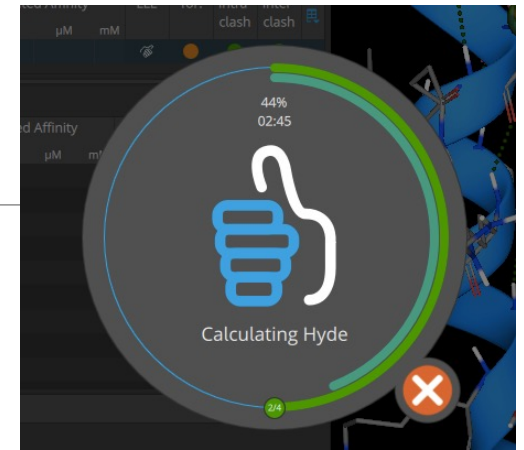
Generated Poses (# 50)		Checked (# 50)					
		Name	Estimated Affinity	LLE	Tor.	Intra-clash	Inter-clash
			pM	nM	μM	mM	
1	<input checked="" type="checkbox"/>	H1H_A_768_1_001					
2	<input checked="" type="checkbox"/>	H1H_A_768_1_002					
3	<input checked="" type="checkbox"/>	H1H_A_768_1_003					
4	<input checked="" type="checkbox"/>	H1H_A_768_1_004					
5	<input checked="" type="checkbox"/>	H1H_A_768_1_005					
6	<input checked="" type="checkbox"/>	H1H_A_768_1_006					
7	<input checked="" type="checkbox"/>	H1H_A_768_1_007					
8	<input checked="" type="checkbox"/>	H1H_A_768_1_008					
9	<input checked="" type="checkbox"/>	H1H_A_768_1_009					

2D
H1H_A_768

COc1ccc(cc1)C(=O)N[C@@H](C)C(=O)Nc2cc(OC)c3cc(OC)cc3c2C(=O)O

Complement C5 inhibitor

Task 5: Sort the poses from the best to the worst and bring the best one back to the Editor. Compare it with the starting structure.
What do you see?



Complement C5 inhibitor

Task 6: Try 5-10 modifications to improve the binding affinity (5-10 minutes).

Do not forget to save the project file every now and then.

Complement C5 inhibitor

Task 7: Bring an “external” molecule to SeeSAR (Editor mode) and dock it to C5.

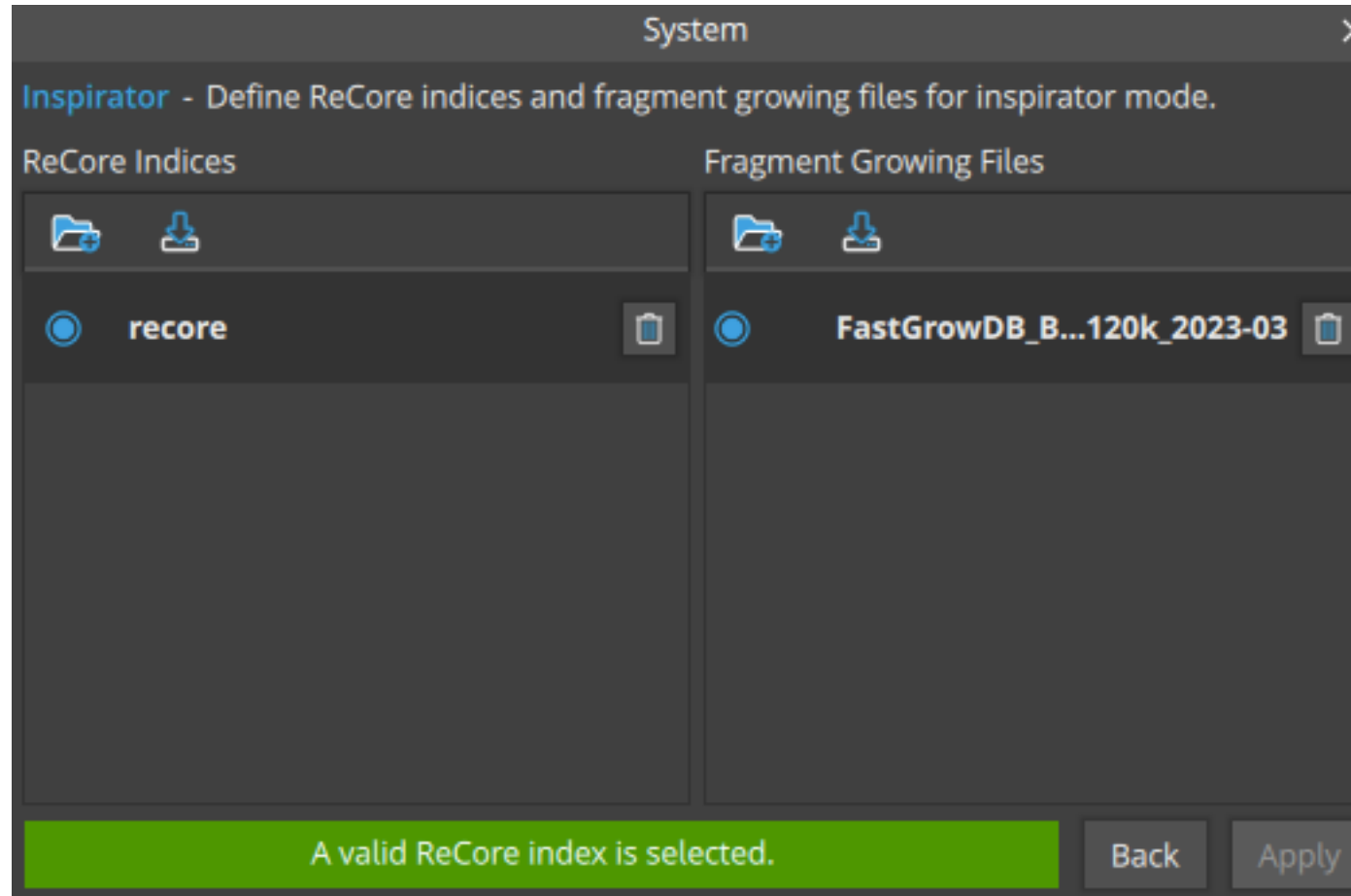
(I will let you to figure out how)

Remember: SeeSAR can deal with a lot of file formats – including SMILES strings.

Good practice: after scoring and ranking, delete the worst/clashed poses to avoid massive files

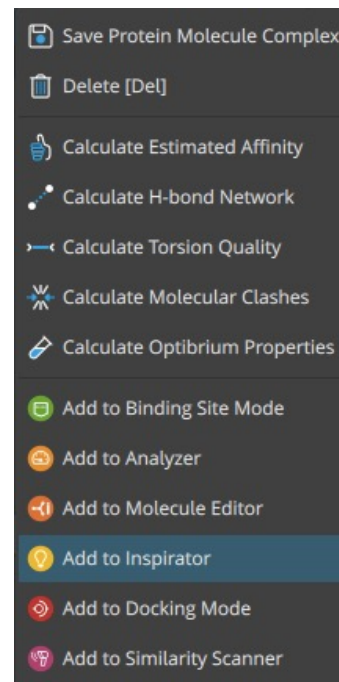
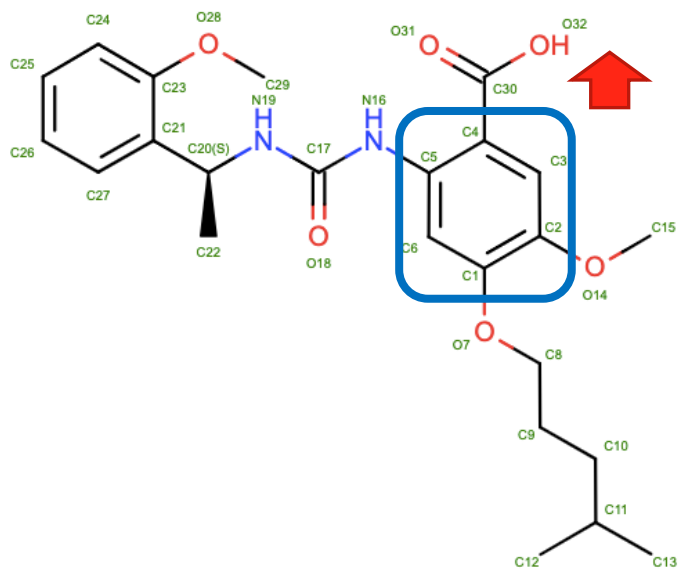
Core expansion in SeeSAR

Configuring Inspirator mode

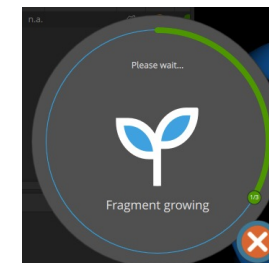


Task 8: Core expansion in SeeSAR

- We will select a central core of H1H for the expansion
- Bring H1H compound from the Editor to the Inspirator mode



Inspirator



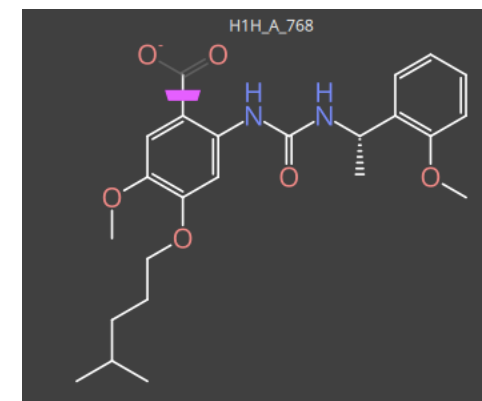
Toolbar: [Icons for file, pH, list, upload, zoom, link, grow (active), wave, lightbulb]

Growing: requires a binding site and one selected single bond on one molecule

Switch to Grid

Molecules (# 1)

		Name	Estimated Affinity				Deviation	Fragment Origin	LLE	Tor.	Inti cla
			pM	nM	μM	mM					
1	[Icons]	H1H_A_768	[Bar]				n.a.		[Icon]	[Orange]	[Green]



		Name	Estimated Affinity				Deviation	Fragment Origin	LLE	Tor.	Inti cla
			pM	nM	μM	mM					
1	[Icons]	H1H_A_768	[Bar]				n.a.		[Icon]	[Orange]	[Green]
2	[Icons]	H1H_A_...1324_5					0.00	CSSB00...011324			
3	[Icons]	H1H_A_...6515_5					0.00	CSSB00...186515			
4	[Icons]	H1H_A_...7244_5					0.00	CSSB00...057244			
5	[Icons]	H1H_A_...1346_5					0.00	CSSB00...741346			
6	[Icons]	H1H_A_...0718_5					0.00	CSSB00...560718			
7	[Icons]	H1H_A_...9724_5					0.00	CSSB00...729724			
8	[Icons]	H1H_A_...9698_5					0.00	CSSB00...989698			
9	[Icons]	H1H_A_...4871_5					0.00	CSSB00...724871			
10	[Icons]	H1H_A_...2727_5					0.00	CSSB00...582727			
11	[Icons]	H1H_A_...0883_5					0.00	CSSB00...560883			

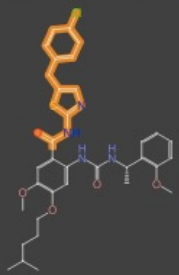
		Name	Estimated Affinity				Deviation	Fragment Origin	LLE	Tor.	Inti cla
			pM	nM	μM	mM					
1	[Icons]	H1H_A_...6515_5	[Bar]				0.00	CSSB00...186515			
2	[Icons]	H1H_A_768	[Bar]				n.a.		[Icon]	[Orange]	[Green]
3	[Icons]	H1H_A_...1324_5	[Bar]				0.00	CSSB00...011324			
4	[Icons]	H1H_A_...4871_5	[Bar]				0.00	CSSB00...724871			
5	[Icons]	H1H_A_...0883_5	[Bar]				0.00	CSSB00...560883			
6	[Icons]	H1H_A_...9698_5	[Bar]				0.00	CSSB00...989698			
7	[Icons]	H1H_A_...0718_5	[Bar]				0.00	CSSB00...560718			
8	[Icons]	H1H_A_...1346_5	[Bar]				0.00	CSSB00...741346			
9	[Icons]	H1H_A_...9724_5	[Bar]				0.00	CSSB00...729724			
10	[Icons]	H1H_A_...7244_5	[Bar]				0.00	CSSB00...057244			
11	[Icons]	H1H_A_...2727_5	[Bar]				0.00	CSSB00...582727			

Inspirator: example results

							Name	Estimated Affinity				Deviation	Fragment Origin	LLE	Tor.	Inti
								pM	nM	μM	mM					
1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_...15_5	—				0.00	CSSB00...186515			
2	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_768	—				n.a.				
3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...1324_5	—				0.00	CSSB00...011324			
4	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...4871_5	—				0.00	CSSB00...724871			
5	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...0883_5	—				0.00	CSSB00...560883			
6	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...9698_5	—				0.00	CSSB00...989698			
7	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...0718_5	—				0.00	CSSB00...560718			
8	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...1346_5	—				0.00	CSSB00...741346			
9	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...9724_5	—				0.00	CSSB00...729724			
10	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...7244_5	—				0.00	CSSB00...057244			

2D

H1H_A_768_CSSB00000186515_5

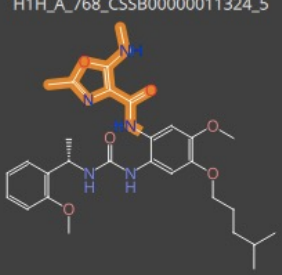


Chemical structure of H1H_A_768_CSSB00000186515_5, showing a complex molecule with a central orange ring system and various side chains.

Molecules (# 11) Checked (# 11)																
							Name	Estimated Affinity				Deviation	Fragment Origin	LLE	Tor.	Inti
								pM	nM	μM	mM					
1	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...6515_5	—				0.00	CSSB00...186515			
2	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_768	—				n.a.				
3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_...24_5	—				0.00	CSSB00...011324			
4	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...4871_5	—				0.00	CSSB00...724871			
5	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...0883_5	—				0.00	CSSB00...560883			
6	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...9698_5	—				0.00	CSSB00...989698			
7	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...0718_5	—				0.00	CSSB00...560718			
8	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...1346_5	—				0.00	CSSB00...741346			
9	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...9724_5	—				0.00	CSSB00...729724			
10	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H1H_A_...7244_5	—				0.00	CSSB00...057244			

2D

H1H_A_768_CSSB00000011324_5



Chemical structure of H1H_A_768_CSSB00000011324_5, showing a complex molecule with a central orange ring system and various side chains.

Inspirator

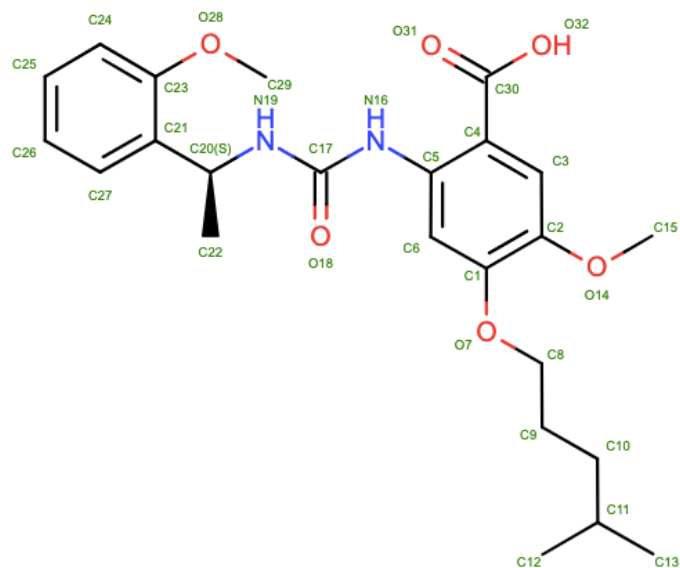
It may be useful to output the following:

- MW
- logP
- TPSA
- Torsional quality estimates
- Clashes

						Name	Estimated Affinity				Deviation	Fragment Origin	LLE	Tor.	Intra-clash	Inter-clash	MW	LogP	TPSA	
							pM	nM	μM	mM										
1	<input checked="" type="checkbox"/>						H1H_A_768_CSSB00000186515_5				0.00	CSSB00...186515					651.22	8.35	110.8	
2	<input checked="" type="checkbox"/>						H1H_A_768				n.a.						443.52	3.77	109.0	
3	<input checked="" type="checkbox"/>						H1H_A_768_CSSB00000011324_5				0.00	CSSB00...011324					553.66	5.99	136.0	
4	<input checked="" type="checkbox"/>						H1H_A_768_CSSB000000724871_5				0.00	CSSB00...724871					566.63	4.92	151.2	
5	<input checked="" type="checkbox"/>						H1H_A_768_CSSB00102560883_5				0.00	CSSB00...560883					666.67	5.87	144.5	
6	<input checked="" type="checkbox"/>						H1H_A_768_CSSB00009989698_5				0.00	CSSB00...989698					589.48	6.40	124.0	
7	<input checked="" type="checkbox"/>						H1H_A_768_CSSB00102560718_5				0.00	CSSB00...560718					684.66	6.00	144.5	
8	<input checked="" type="checkbox"/>						H1H_A_768_CSSB00010741346_5				0.00	CSSB00...741346					554.66	5.92	127.9	
9	<input checked="" type="checkbox"/>						H1H_A_768_CSSB000000729724_5				0.00	CSSB00...729724					569.48	4.40	138.4	
10	<input checked="" type="checkbox"/>						H1H_A_768_CSSB000000057244_5				0.00	CSSB00...057244					720.87	7.80	147.7	

Task 9: Core expansion in SeeSAR

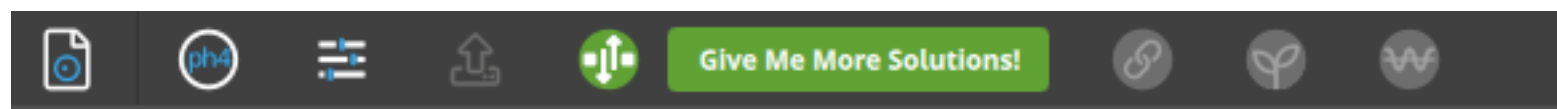
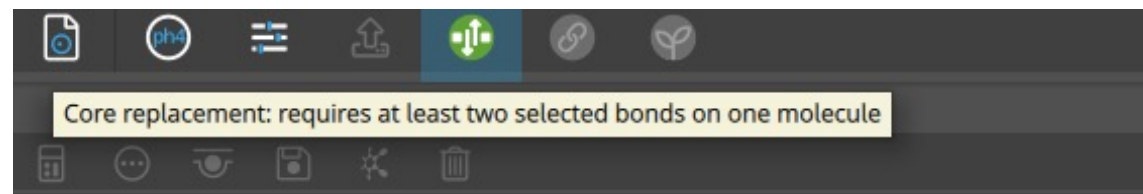
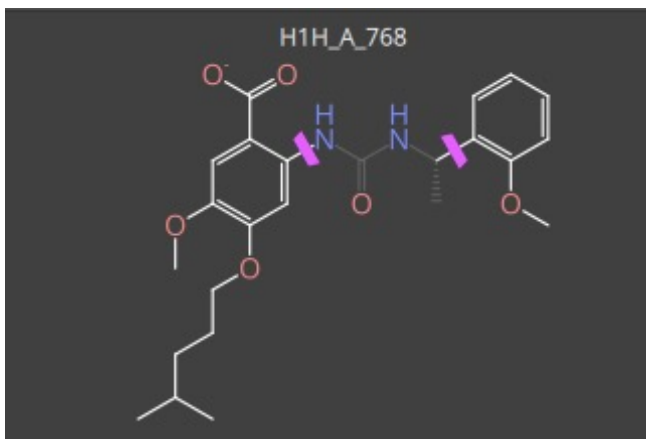
- Repeat the expansion using different direction of the growth
- You may start from H1H, of your “best” analogue from the previous growth iteration



Core replacement

Task 10: Core replacement in Inspirator

- Requires 2 bonds selected
- Does not cut through the ring
- May be done iteratively with core expansion/growth

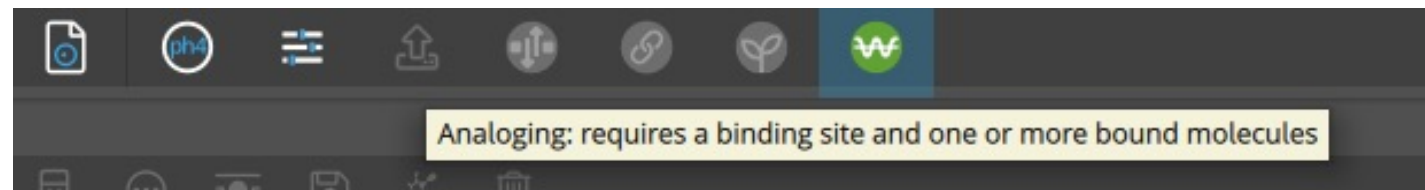


Generation of analogues

SEESAR VERSION 13+ ONLY

Task 11: Analogues in Inspirator

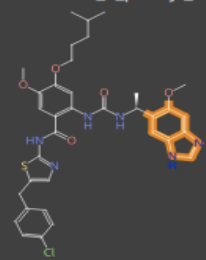
- Requires a binding site and at least one bound molecule



						Name	Estimated Affinity				Deviation	Fragment Origin	LLE	Tor.	Intra-clash	Inter-clash
	✓	✕	☆	💬	🔥		pM	nM	µM	mM						
1	✓	✕	☆	💬	🔥	H1H_A...1_10_7						n.a. add_su...mide_1		●	●	●
2	✓	✕	☆	💬	🔥	H1H_...2_7						n.a. phenyl...idazole		●	●	●
3	✓	✕	☆	💬	🔥	H1H_A...F2_6_7						n.a. methyl-->_CHF2		●	●	●
4	✓	✕	☆	💬	🔥	H1H_A...le_3_7						n.a. phenyl...idazole		●	●	●
5	✓	✕	☆	💬	🔥	H1H_A...do_8_7						n.a. add_acetamido		●	●	●
6	✓	✕	☆	💬	🔥	H1H_A...xy_9_7						n.a. add_methoxy		●	●	●
7	✓	✕	☆	💬	🔥	H1H_A...le_4_7						n.a. phenyl...indole		●	●	●
8	✕	✕	☆	💬	🔥	H1H_A...6515_5						0.00 CSSB00...186515		●	●	●
9	✓	✕	☆	💬	🔥	H1H_A...2H_1_7						n.a. add_CH2-CO2H		●	●	●
10	✕	✕	☆	💬	🔥	H1H_A_768						n.a.		●	●	●

2D

H1H_A_768_CSSB00000186515_5_phenyl-->_benzimidazole_2_7



Virtual Screening

Virtual screening using SeeSAR and InfiniSee

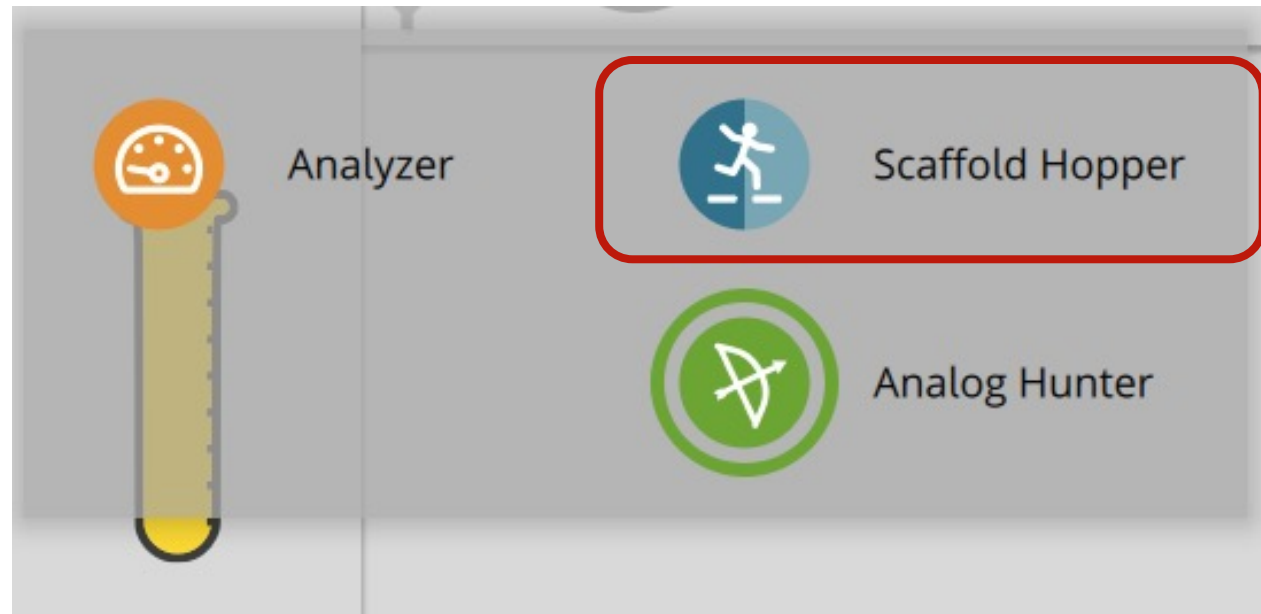
- Docking of a large virtual libraries (1,000+) of compounds
- Libraries: collections of small molecules for virtual screening
- Sources: open-access (e.g. ZINC, ChEMBL) or commercial (e.g. Enamine) virtual libraries
- Types of libraries commonly used: diversity sets, target-focused, custom

Building your own C5 focused library in InfiniSee

- You may use H1H as a query (“bait”)
- Load it into InfiniSee (Scaffold Hopper mode)
- Choose the chemical space
- You can pull thousands of synthetically-feasible analogues in minutes to create your own bespoke focused libraries (SDF/SMILES formats)
- Next, you can virtually screen these analogues









Scaffold Hopper in InfiniSee

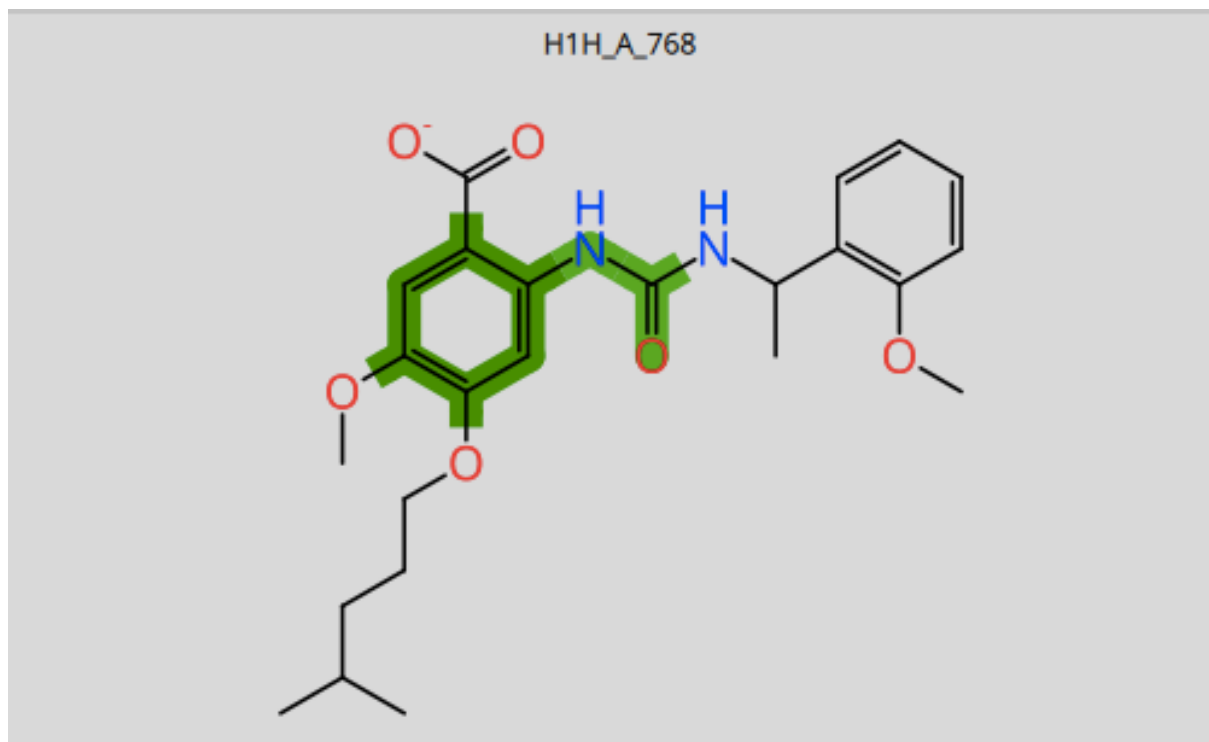


Configuring InfiniSee


Search - Load or download Chemical Spaces or library files for search.

Available online	Installed locally
 	
<div> 69 MB Freedom Space 5.1×10^9 ✓ 2023-06-12</div>	<div>Click to load Chemical Spaces from your local file system.</div> <p>OR</p> <div>Doubleclick to download Chemical Spaces from our website.</div>
<div> 560 MB REAL Space 3.6×10^{10} ✓ 2023-04-27</div>	
<div> 31 MB</div>	

Customise similarity features and results



Select Chemical Spaces and Libraries for Search

	Name	Type	Size
<input checked="" type="checkbox"/>	GalaXi		1.2×10^{10}

Maximum Number of Results

100

Target Similarity

1.00

Minimum Similarity

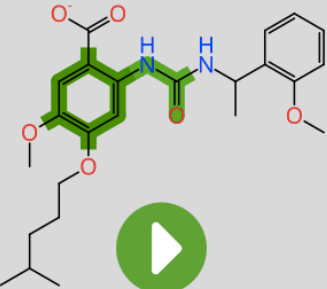
0.80

Total Diversity

1.00

InfiniSee/Scaffold Hopper: results

H1H_A_768



Result Summary:

- Query: H1H_A_768
- Found Molecules: 100
- From GalaXi_12bn_2023-03: 100

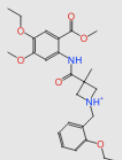
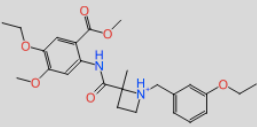
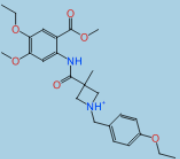
Used Parameters:

- Maximum Number of Results: 100
- Target Similarity: 1.00
- Minimum Similarity: 0.80
- Total Diversity: 1.00

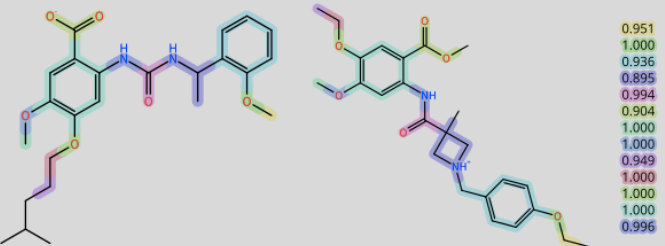
Search Session Info:

- ID:
- User: Agnieszka Bronowska
- Started: 13:57 2023-07-15
- Duration: 00:00:42
- InfiniSee Version: 5.0.0

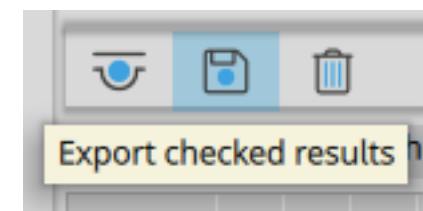
Molecules (# 100)

	Molecule	#	Similarity	Space	Name
97		97	0.926	GalaXi_12bn_2023-03	WXDL_...S0034
98		98	0.926	GalaXi_12bn_2023-03	WXDL_...S0520
99		99	0.926	GalaXi_12bn_2023-03	WXDL_...S0088

Matching



0.951
1.000
0.936
0.895
0.994
0.904
1.000
1.000
0.949
1.000
1.000
1.000
0.996



Chemical space search and virtual screening

Task 12: Using your “best” C5 inhibitor as a “bait” (query), find 100 compounds in GalaXi space.

Out of these 100, pick 10, save them as either SDF or SMILES, upload to SeeSAR and virtually screen them against C5.

Of all compounds virtually tested for C5 binding: select your favourite, export the complex as the PDB file, and you may follow it up using all-atom MD simulations.

Recap: limitations and known issues

- Protein is considered rigid: ideally, you should follow your calculations by running short MD simulations on the complexes and recalculating the binding affinities
- Results are very sensitive to even small changes in the conformation of the protein
- Binding affinities for certain groups are not reproduced well: hydrophobic effect tends to be overestimated, while highly polar groups are underestimated
- Every now and then, weird protonation states suggested (you can always manually adjust) and med-chem nonsense molecules suggested in core expansion in Inspirator
- Workflows are limited to small molecules
- Med-chem properties and/or synthetic feasibility of suggested analogues may be problematic
- Technical: 50,000 compounds/rows in SeeSAR GUI (you need to use KNIME to “downsize” very large data sets, or use non-GUI version)