CCP5 Summer School

July 2023

Simulations of biomolecules: design of small molecule complement C5 inhibitors

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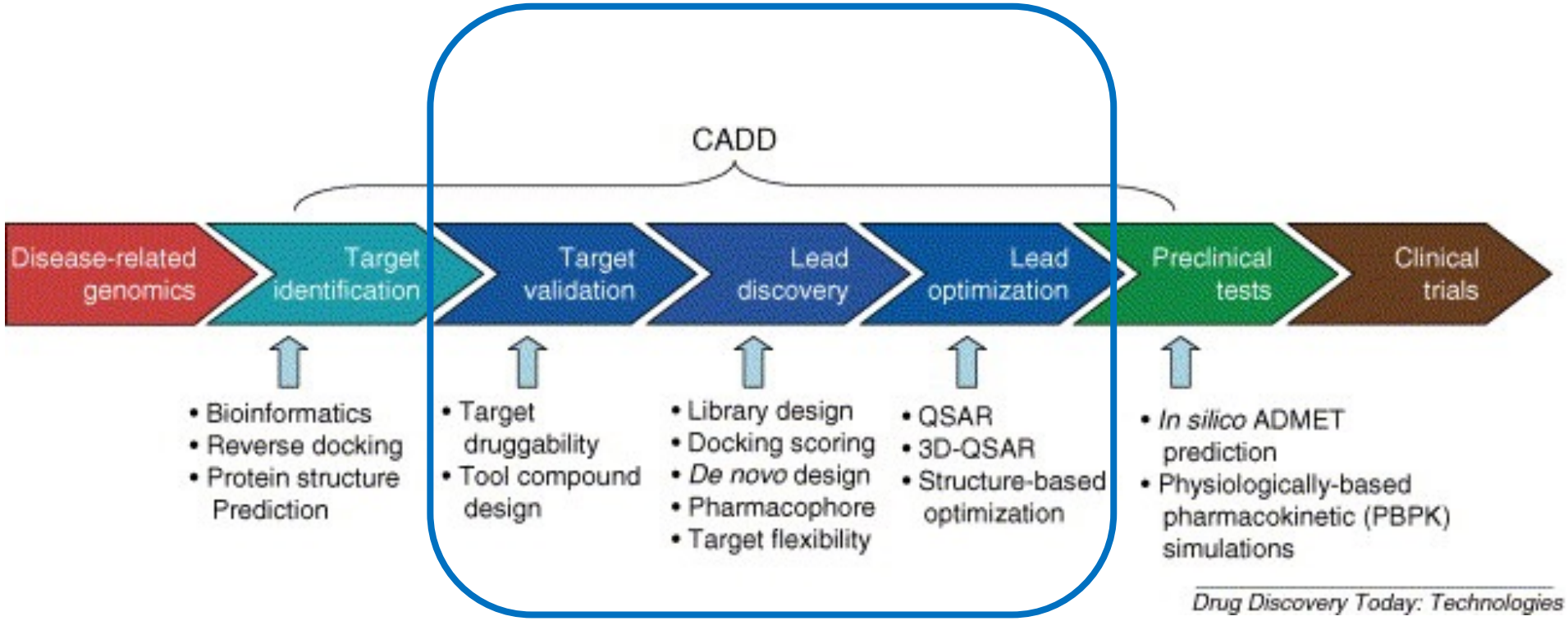
CADD pipeline



Molecular docking

The process of predicting the stable 3D geometry
of an interacting pair of molecules - a ***binding mode/pose***
Energy-based docking relies on molecular mechanical force fields (energy minimisation, sampling, scoring function)

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

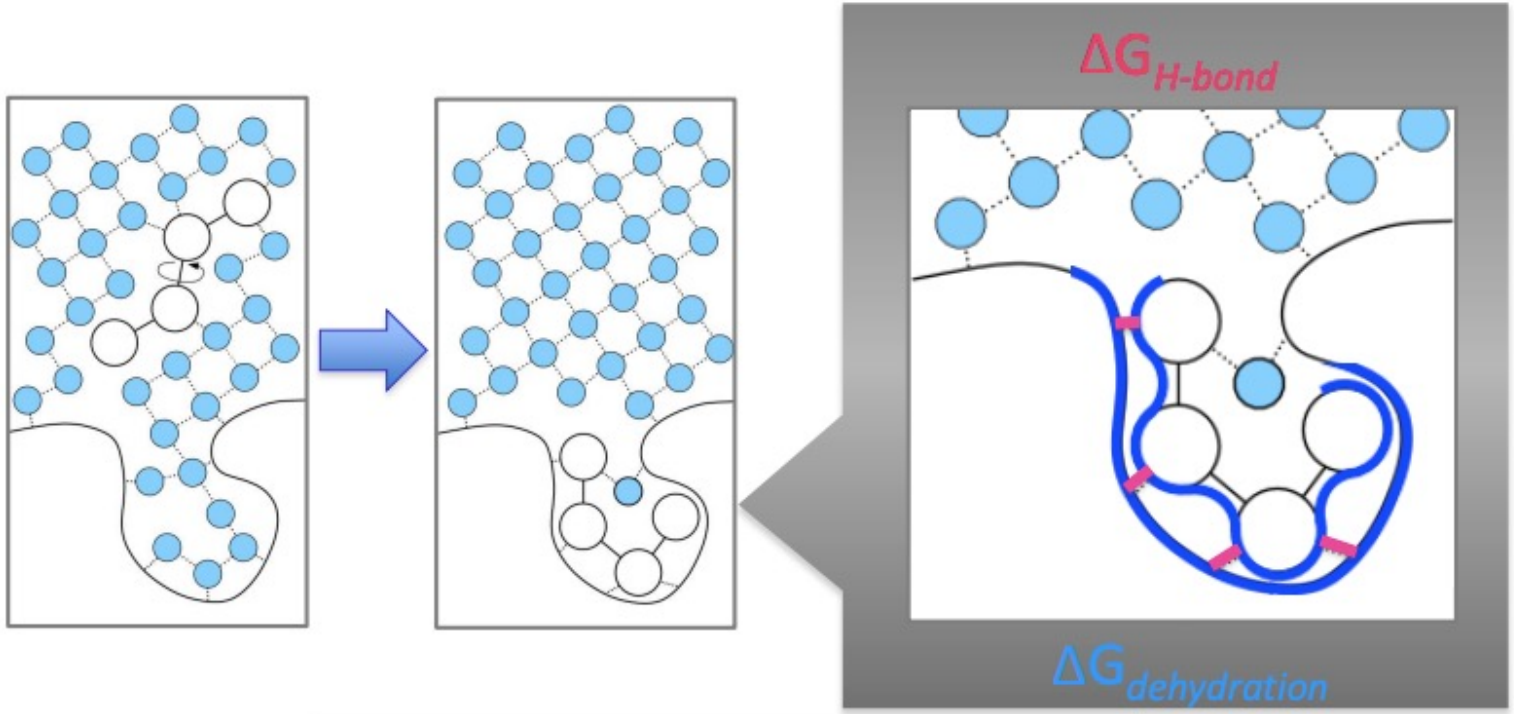
- Decide whether the molecules will form a complex;
- Determine the binding affinity (free binding energy);
- Predict the 3D structure of the complex (binding mode);
- Deduce function (agonist/antagonist);

SeeSAR

SeeSAR

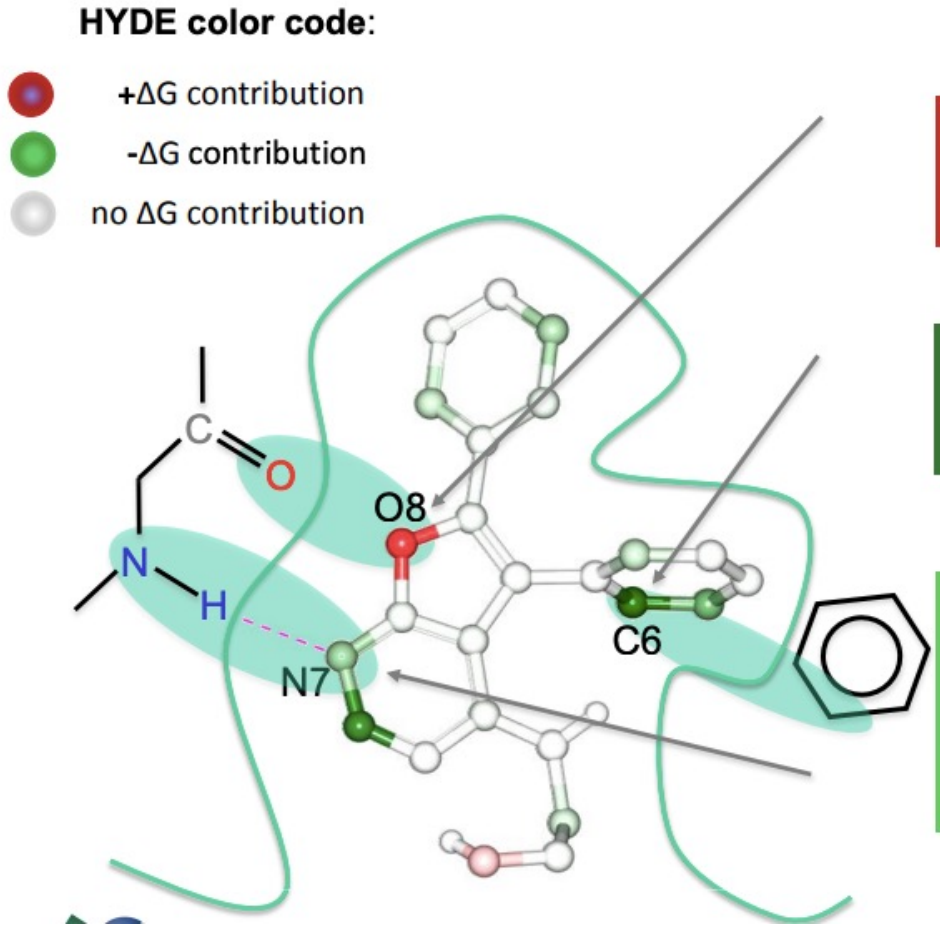
- Platform-independent
- Easy to use (for basic functionalities) – developed for bench chemists
- Decent scoring function (interactions + solvation term used)
- Minimum prep and requirement for e.g. format parsing
- Rapid core expansion, core replacement and analogue search enabled
- Handles multiple file formats
- Covalent docking enabled
- Integrated with other tools, e.g. InfiniSee (chemical space search), RDKit, KNIME

HYDE scoring function: the concept



$$\Delta G^{i}_{\mathrm{HYDE}} = \sum_{atom\ i} \Delta G^{i}_{dehydration} + \Delta G^{i}_{H-bond}$$

HYDE – visual affinities



receptor carbonyl oxygen	8.2 kJ/mol
ligand aromatic oxygen	2.4 kJ/mol
total desolvation cost	**10.6 kJ/mol**

receptor aromatic carbons	-5.2 kJ/mol
ligand aromatic carbon	-2.0 kJ/mol
total desolvation gain	**-7.2 kJ/mol**

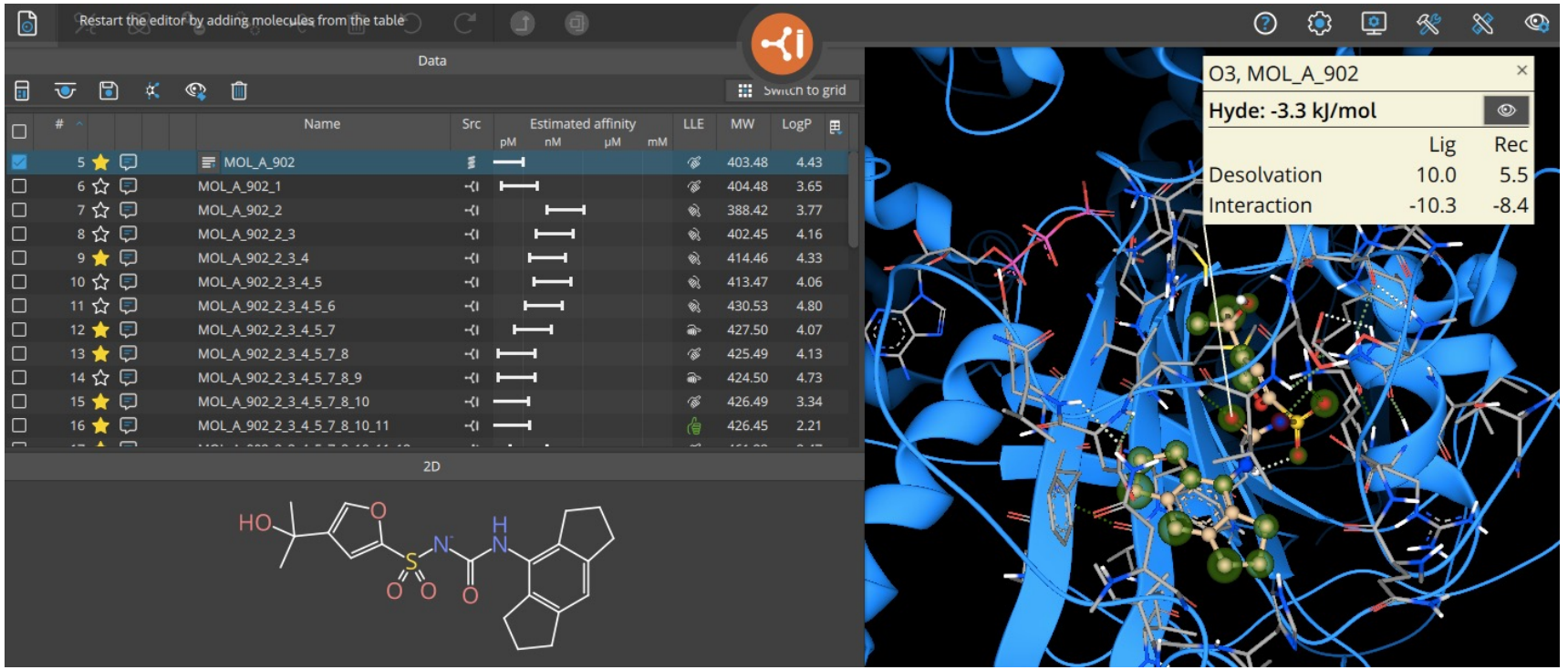
receptor amide N dehydrat	6.3 kJ/mol
interaction energy	-7.4 kJ/mol
ligand aromatic N dehydrat	6.4 kJ/mol
interaction energy	-7.5 kJ/mol
total H-bond energy	**-2.2 kJ/mol**

SeeSAR: a working example

Ligand moves (optimisation after every modification), protein fixed: single conformation

Exhaustive conformational search on ligands prior to docking

Scoring function (HYDE) gives contributions from intrinsic interactions and desolvation



MCC950 bound to NLRP3 inflammasome

Predicted affinity range: High pM to low nM

Ki (SPR and MST): Low nM (8 – 24 nM)

Limitations and some 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)

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: fragments, diversity sets, target-focused, custom

Fragment libraries: Enamine

Fragment Collection

MiniFrag Library

sp^3 Rich Fragments

Single Pharmacophore

Carboxylic Acid Fragments

Fluorinated Fragments

Covalent Fragments

Warhead Subsets

Cysteine focused Covalent Fragments

Serine focused Covalent Fragments

Lysine focused Covalent Fragments

Acrylamides

Enamine Essential Fragment Library

- 320 fragments
- Universal tool for initial screen of novel targets
- Designed in collaboration with research group at University of Cambridge
- All fragments have been tested for water solubility and chemical stability in buffer solution
- Increased hit probability: the structures are based on frequently reported fragment hits and scaffolds derived from experimentally determined structures of protein-ligand complexes

Diversity libraries

DIVERSITY LIBRARIES

10 240 compounds

50 240 compounds

Hit Locator Library

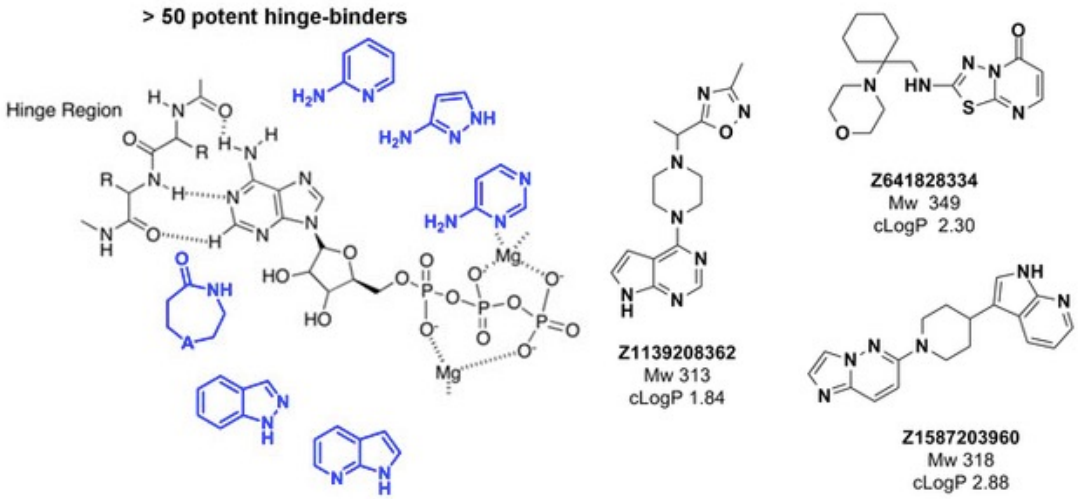
Phenotypic Screening Library

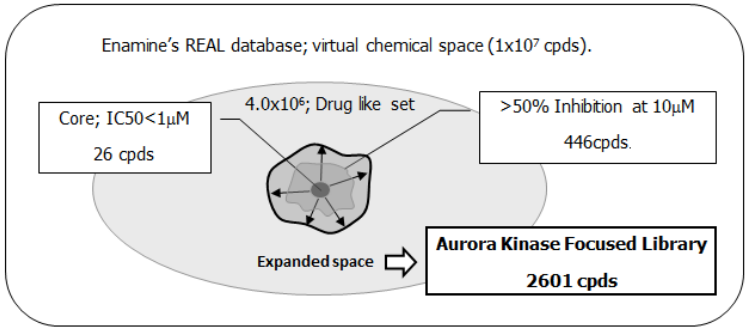
Covalent Screening Library

Target-focused virtual libraries

Kinase libraries:

- Hinge binders (18,000 molecules)
- Aurora A targeted set (2,600 molecules)
- Synthetically feasible
- Comply with drug-like rules
- Evaluated *in vitro*





Building your own set: InfiniSee

- Developed from REAL Space Navigator;
- REAL: 21 bln make-on-demand compounds;
- The current version (Artemis) has a choice of several massive libraries (REAL, CHEMriya, GalaXi, eXplore, KnowledgeSpace)

Size matters: screening ultra-large libraries

Ultra-large library docking for discovering new chemotypes

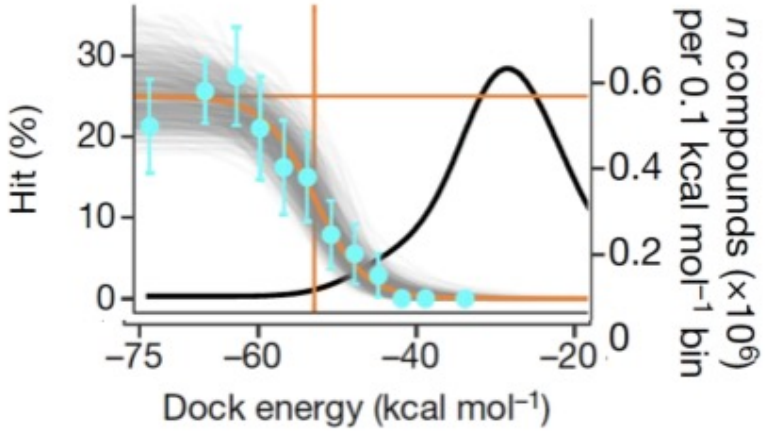
Jiankun Lyu[1,2,10], Sheng Wang[3,4,10], Trent E. Balius[1,10], Isha Singh[1,10], Anat Levit[5], Yurii S. Moroz[5,6], Matthew J. O'Meara[1], Tao Che[4], Enkhjargal Algaa[1], Kateryna Tolmachova[7], Andrey A. Tolmachev[7], Brian K. Shoichet[1]*, Bryan L. Roth[4,8,9]* & John J. Irwin[1]*

Nature. 2019; 566(7743): 224–229.

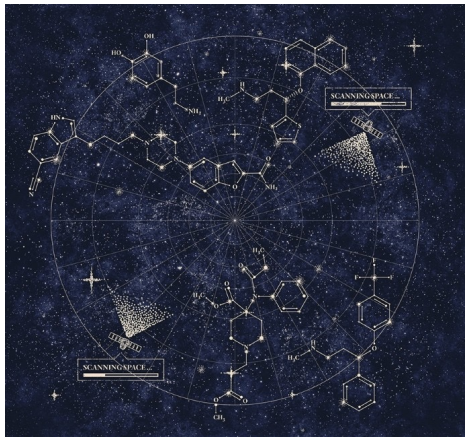
RESEARCH HIGHLIGHT | 05 January 2022

Screening ultra-large virtual libraries

Synthesis-on-demand compounds were chosen from 12 different docking score bins (cyan)

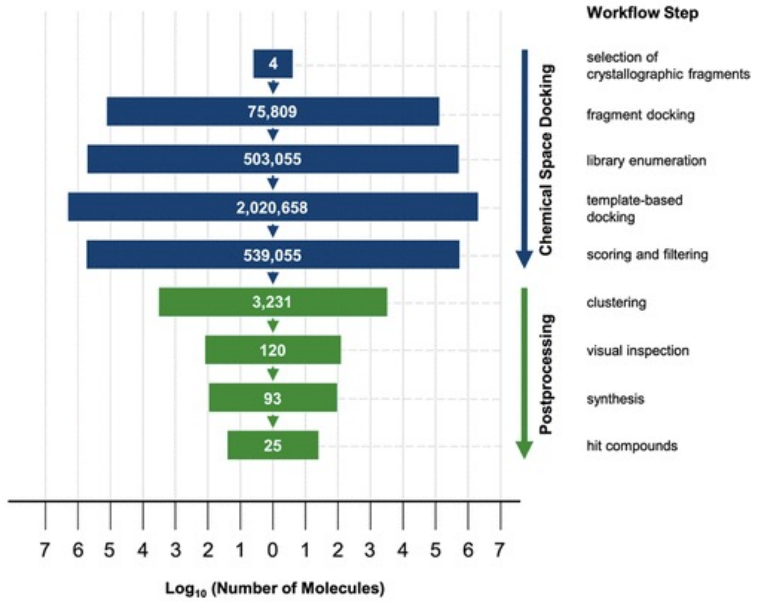


A group of new (no precedent) AmpC inhibitors has been identified

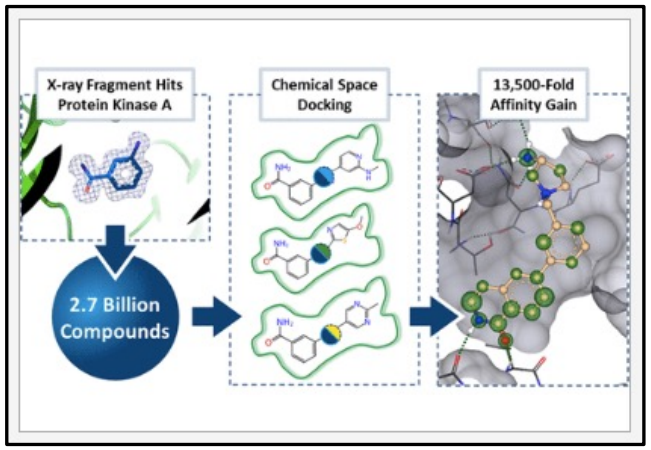


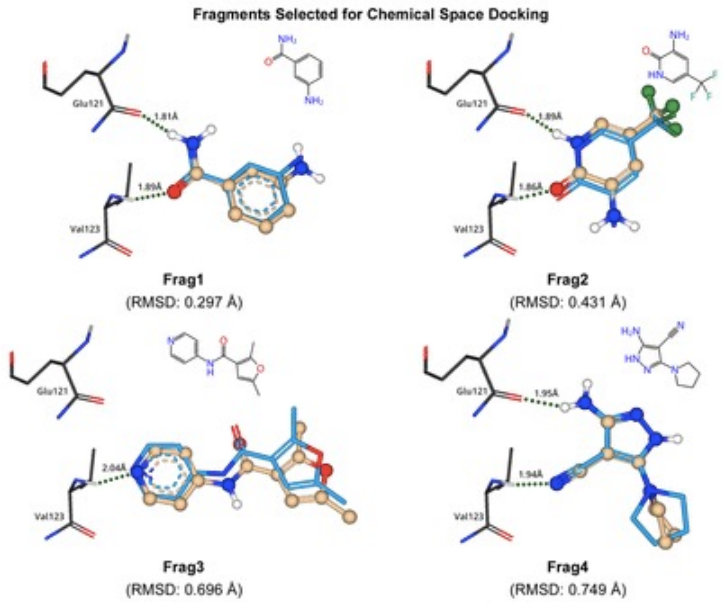
Message: we should aim to explore as much of the chemical space as possible

Screening of large chemical spaces: “crystals first”



Start: 4 PKA-fragment complexes (X-ray)



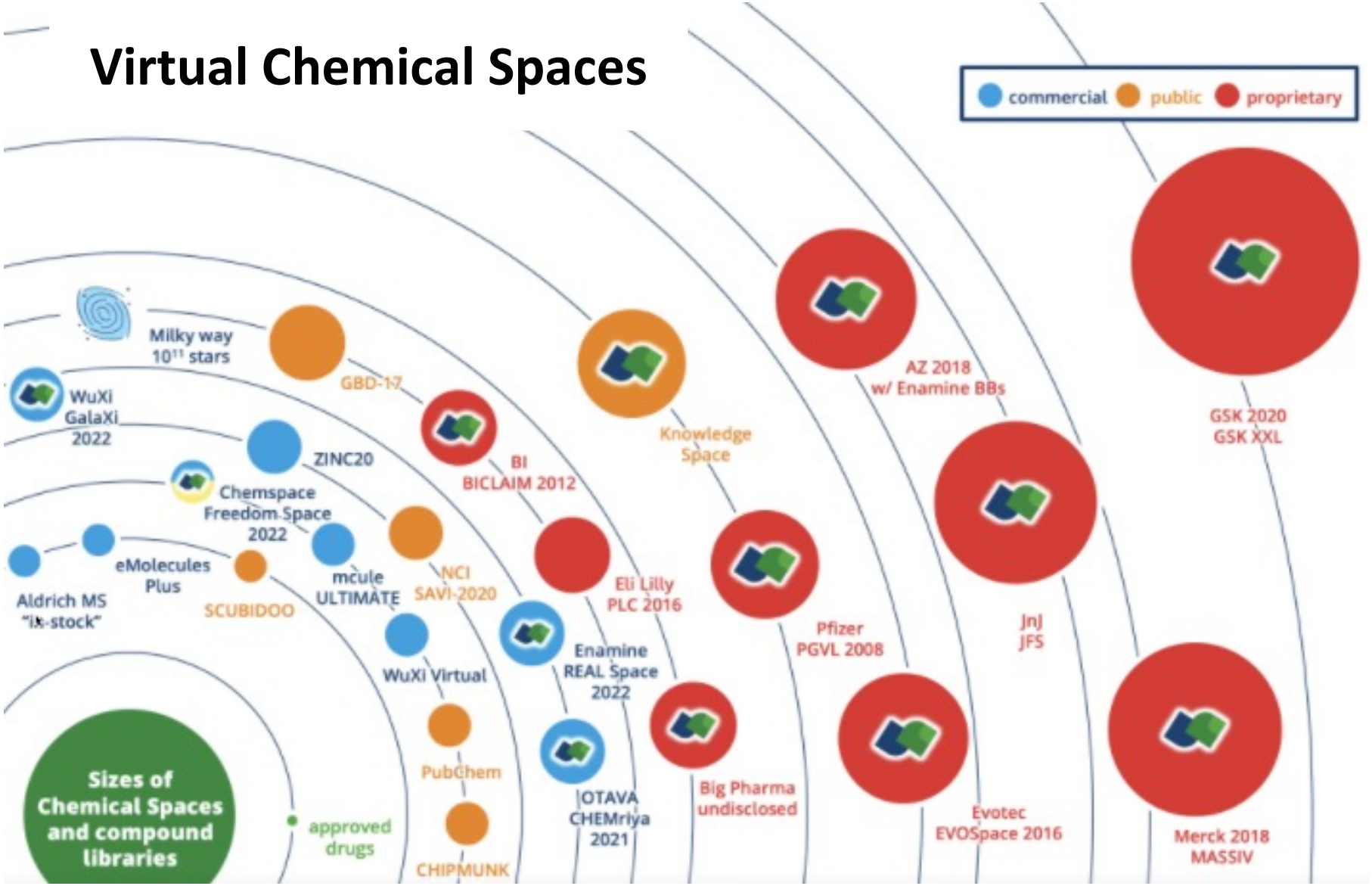


- Template-based docking using Enamine REAL space (20 bn)
- 93 molecules out of 106 selected compounds synthesized
- 40 compounds were active in at least one validation assay
- Most active follow-up having a 13,500-fold gain in affinity

Crystal structures for 6 of the most promising binders were obtained, verifying the predicted binding modes

Müller *et al.*, JCIM 2022

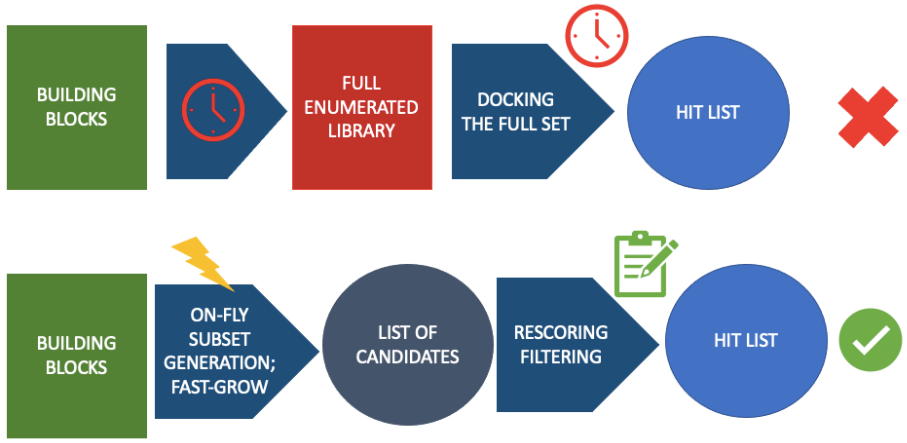
Virtual Chemical Spaces



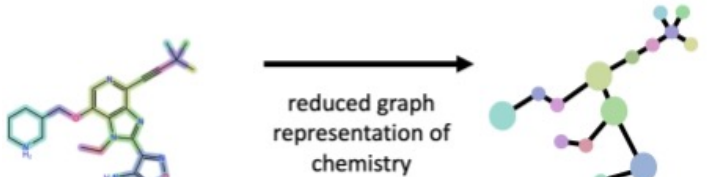
The ”combinatorial challenge”

- Enumerating the GSK space would take 400 Yottabytes (400,000000000000 GB)
- Estimated to take ~11 trillion years to download using high-power broadband
- Storage of all products of a library of such size is unfeasible
- **Way out:** storing building blocks and reaction rules allowing on-fly generation of products

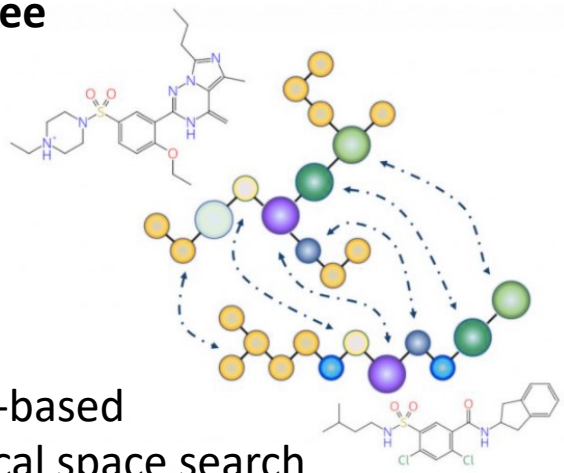
Virtual library	Size
GalaXi	10^9
Enamine REAL	10^{10}
eXplore	10^{12}
BiosolveIT KnowledgeSpace	10^{14}
GSK XXL	10^{26}



Three-stage workflow for ulvHTS



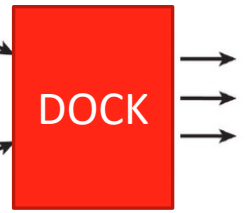
InfiniSee

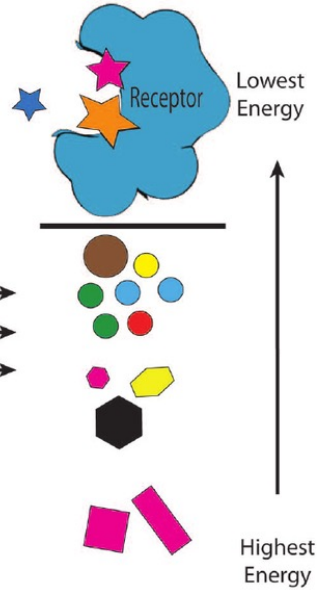


Ligand-based
Chemical space search
Finding “hidden” similarities

Primary

RockDock



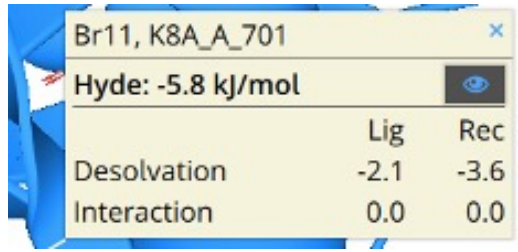


slurm
workload manager

Structure-based
Fast
Hits to be scored by more accurate methods

Secondary

SeeSAR

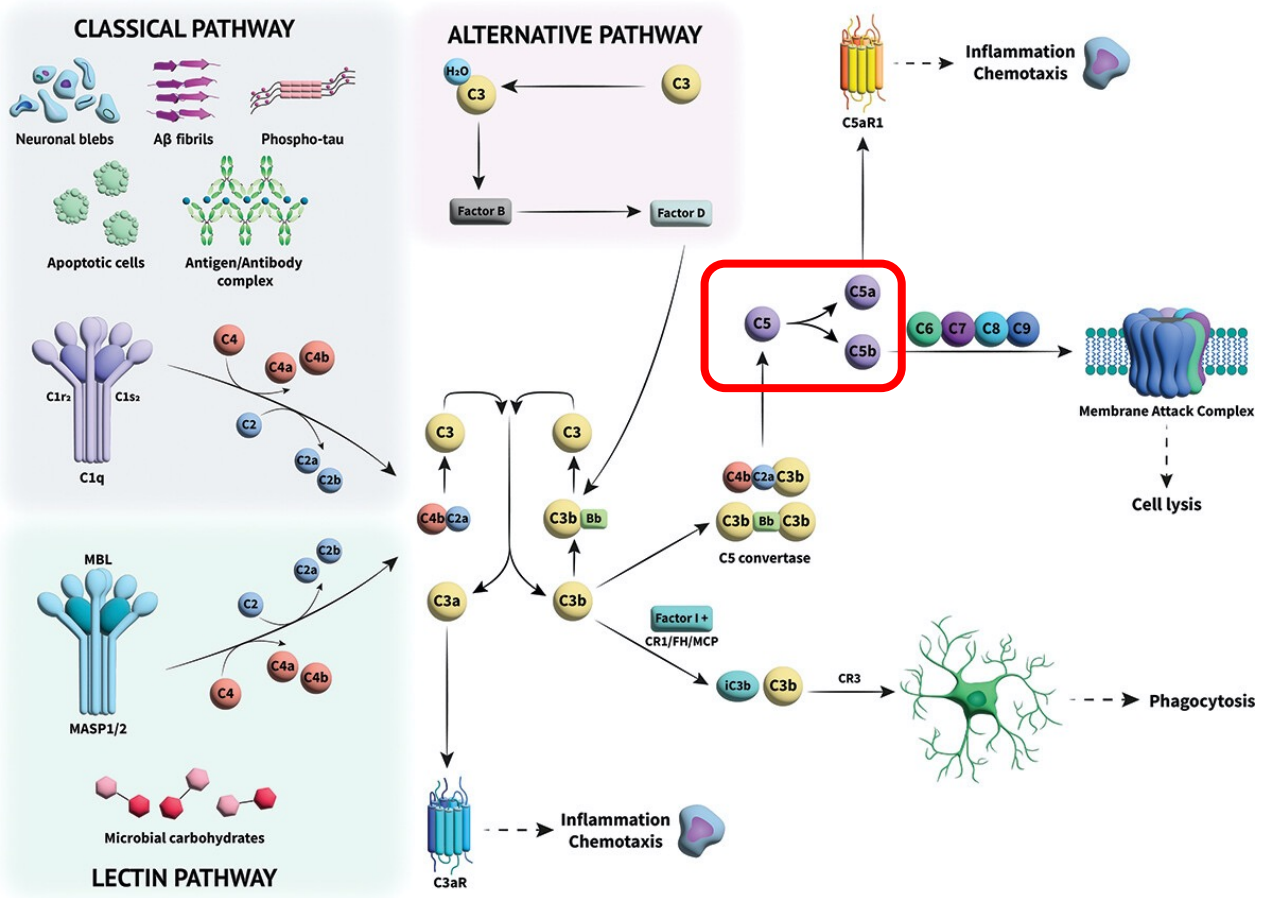


Structure-based
HYDE scoring function

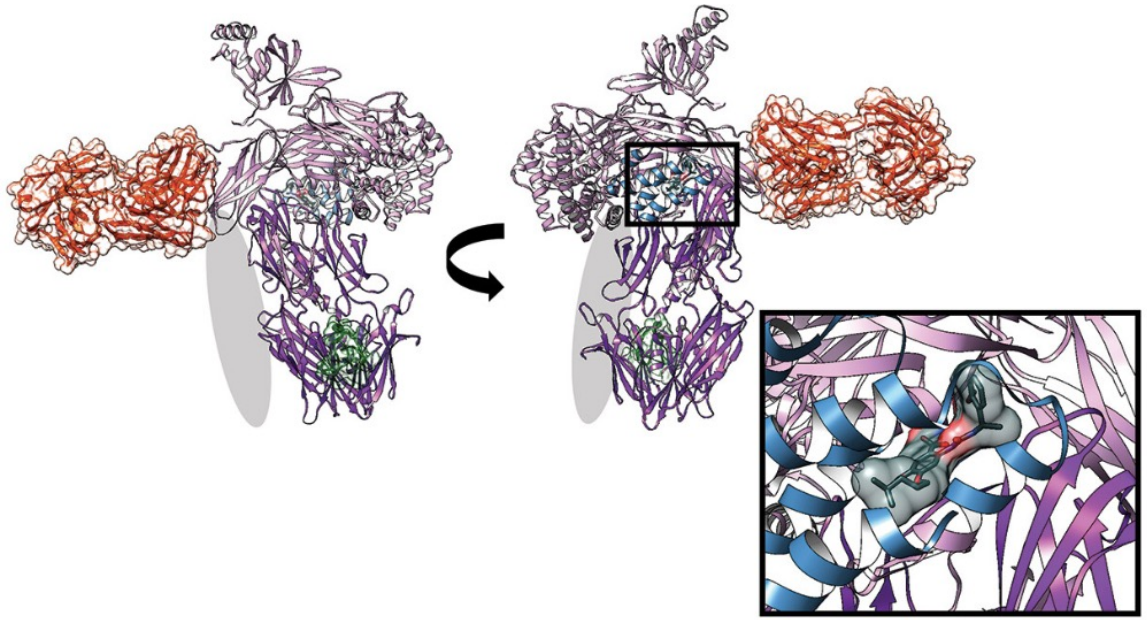
Case Study

COMPLEMENT C5

Complement pathway and C5

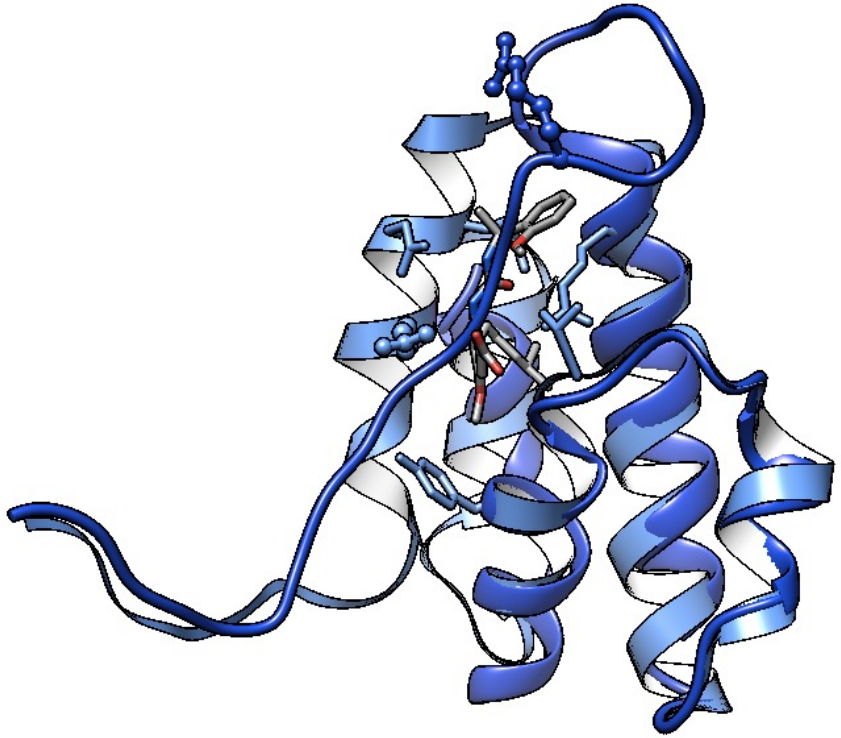


PDB: 8AYH



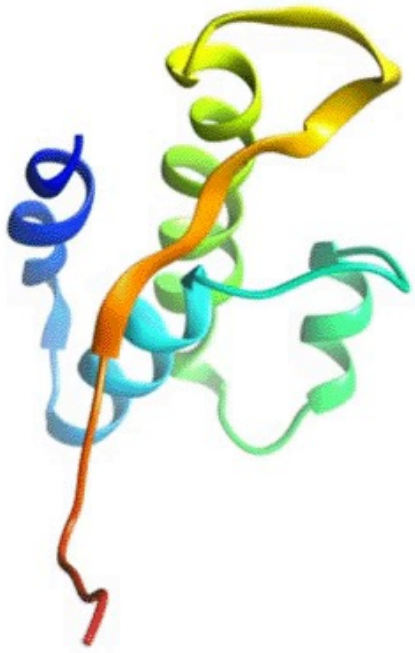
Stennett *et al*., 2023

Complement C5 inhibitors: MoA



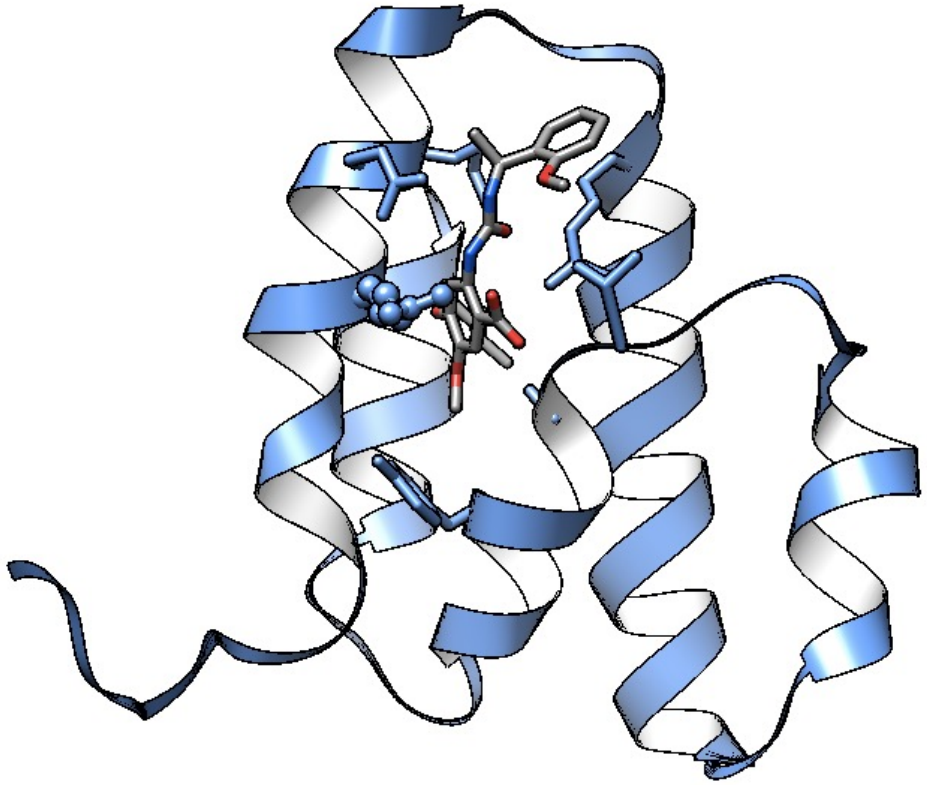
PDB: 3CU7

Ensemble (apo)



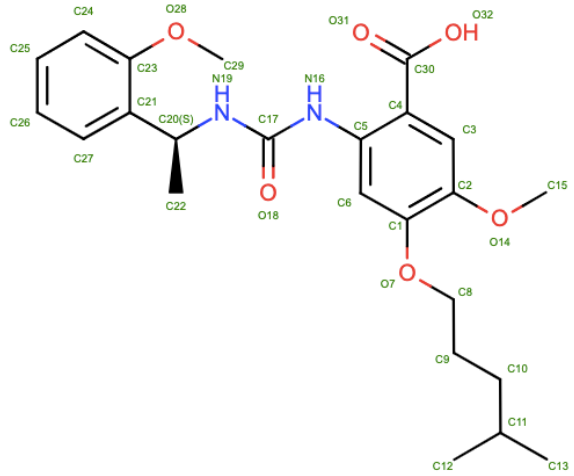
PDB: 8AYH

Complement C5 inhibitors



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

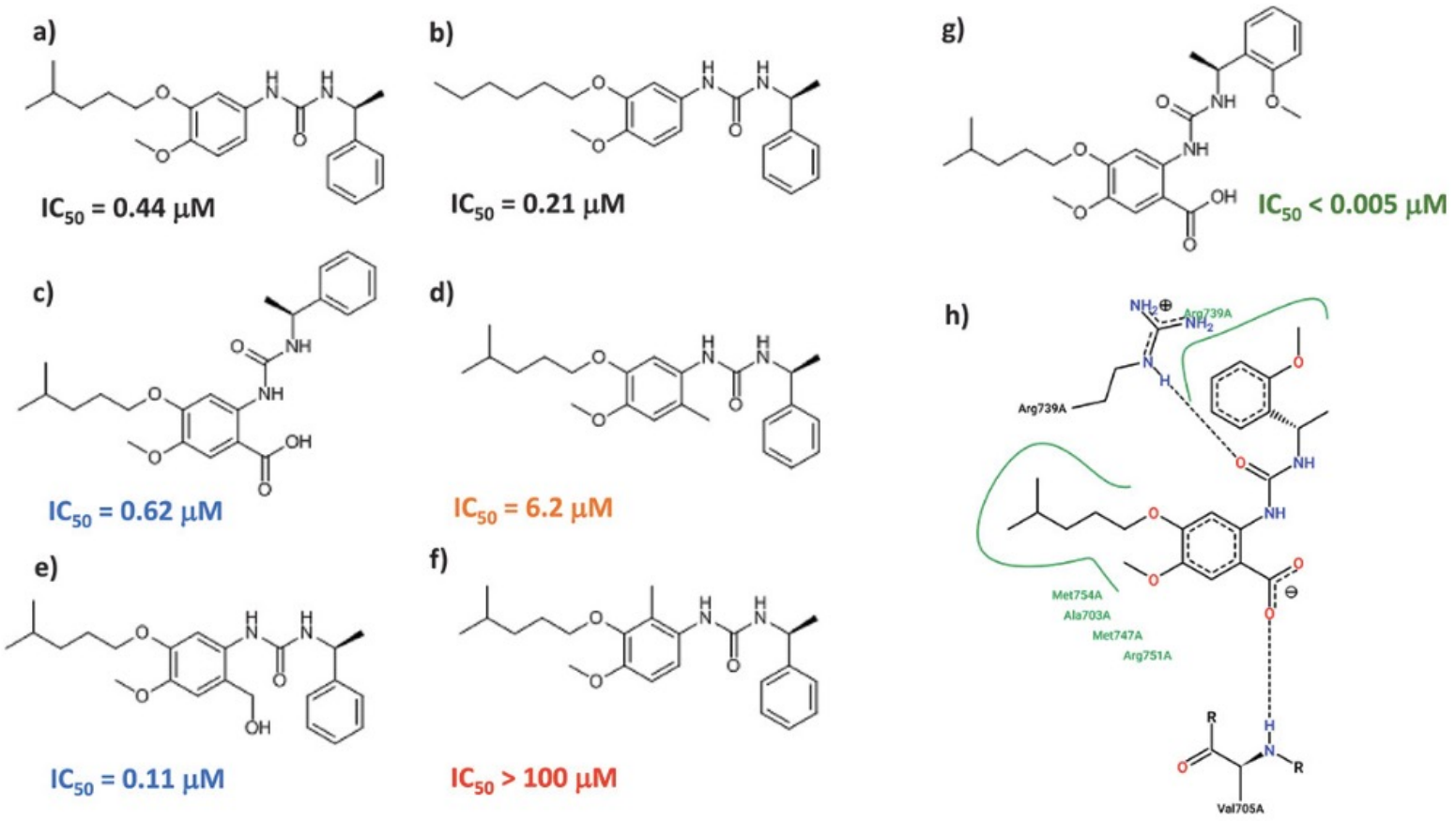
IC_{50}: 0.1 - 5 nM, from 3 assays



H1H

PDB: 8AYH

Complement C5 inhibitors



Stennett *et al*., 2023

Contents of practical sessions

- 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

Credits

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Adam Wollman

Wyatt Yue

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João de Souza (RxCelerate)

Jon Heal (RxCelerate)

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RxCelerate

BioSolveIT