Joana Pereira
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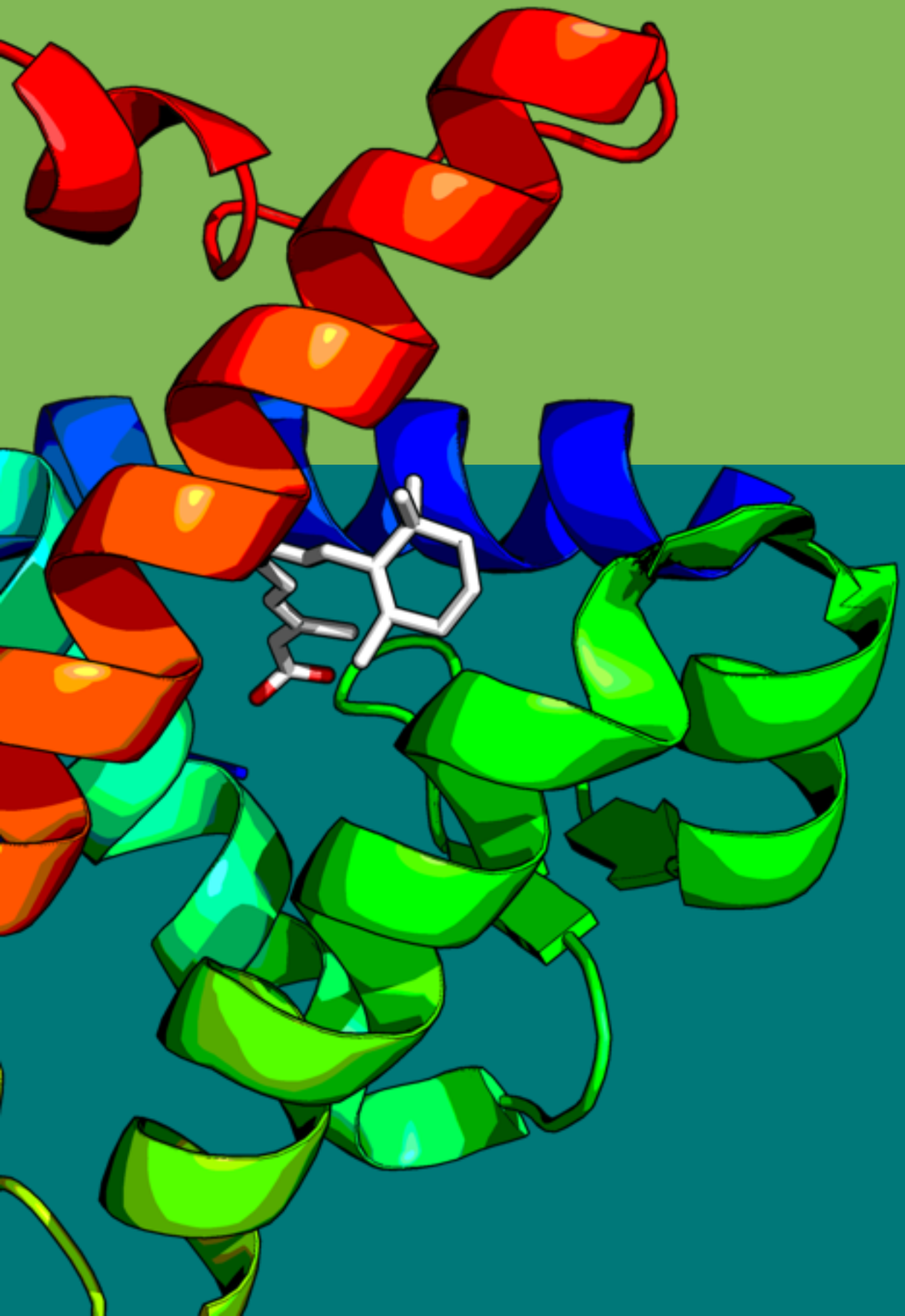


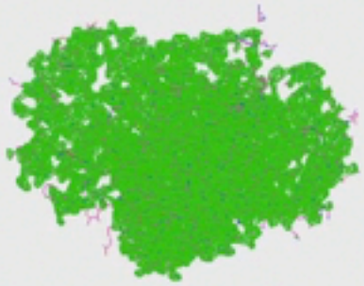
Small molecules
How to identify and build them
(with ARP/wARP)

EMBL 40 YEARS | 1974–2014

7.4 ARP wARP

The task at hand

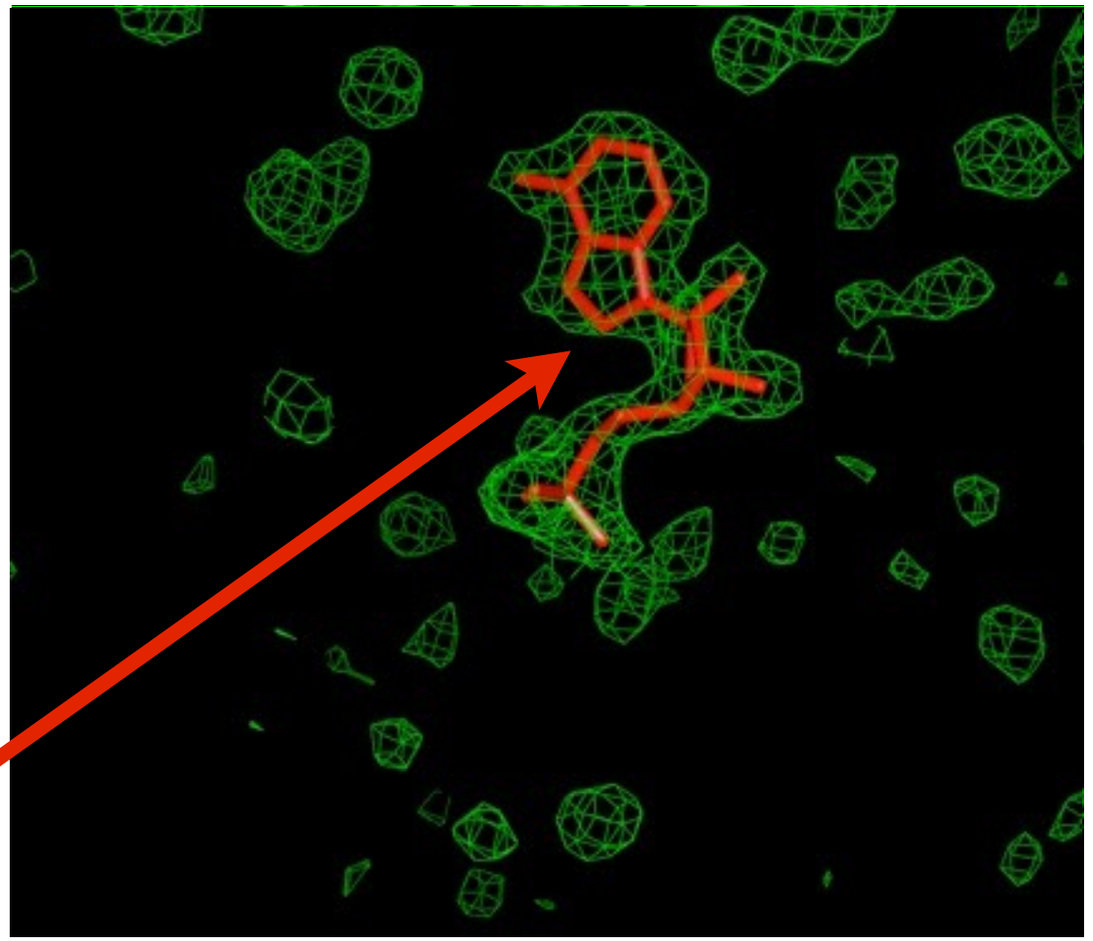
To find ligand density and build it!

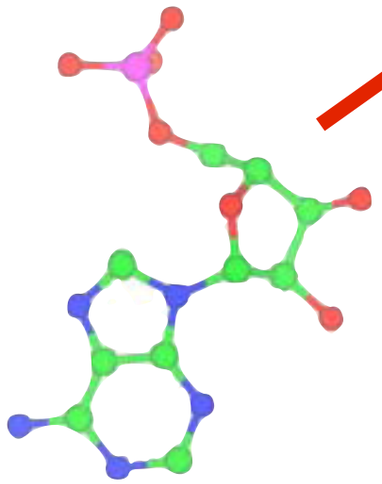


Fitting a ligand

We have:

- electron density map (good resolution)
- + protein model
  - shows **excess density** not accounted for by protein
- **difference density map**
  - to fit the appropriate ligand into

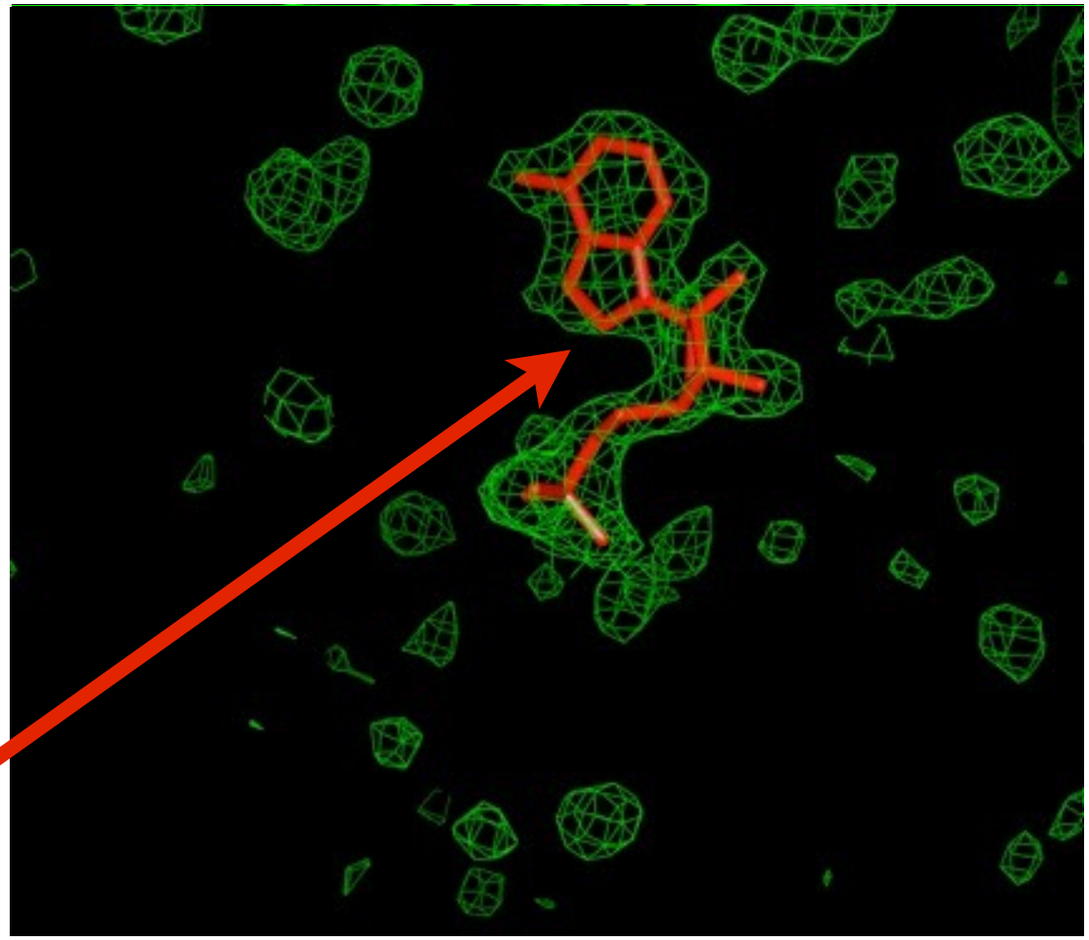


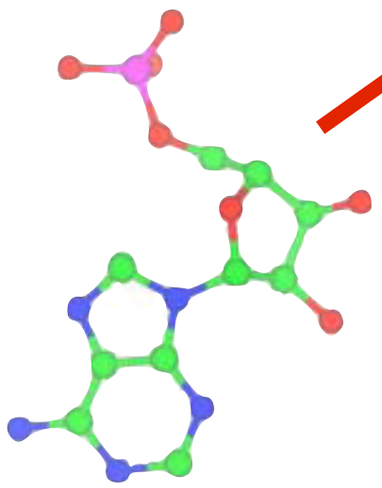


Fitting a ligand

Challenges:

- different **resolutions** and data quality
- different ligand **complexity / topology**
- **partial disorder** of a ligand
- different ligands at the same site?





The protocol of a modelling task

What we know at the beginning will determine the protocol:

Ligand identity \ Binding site	Known	Unknown
Known	X	
Unknown		

1 density cluster

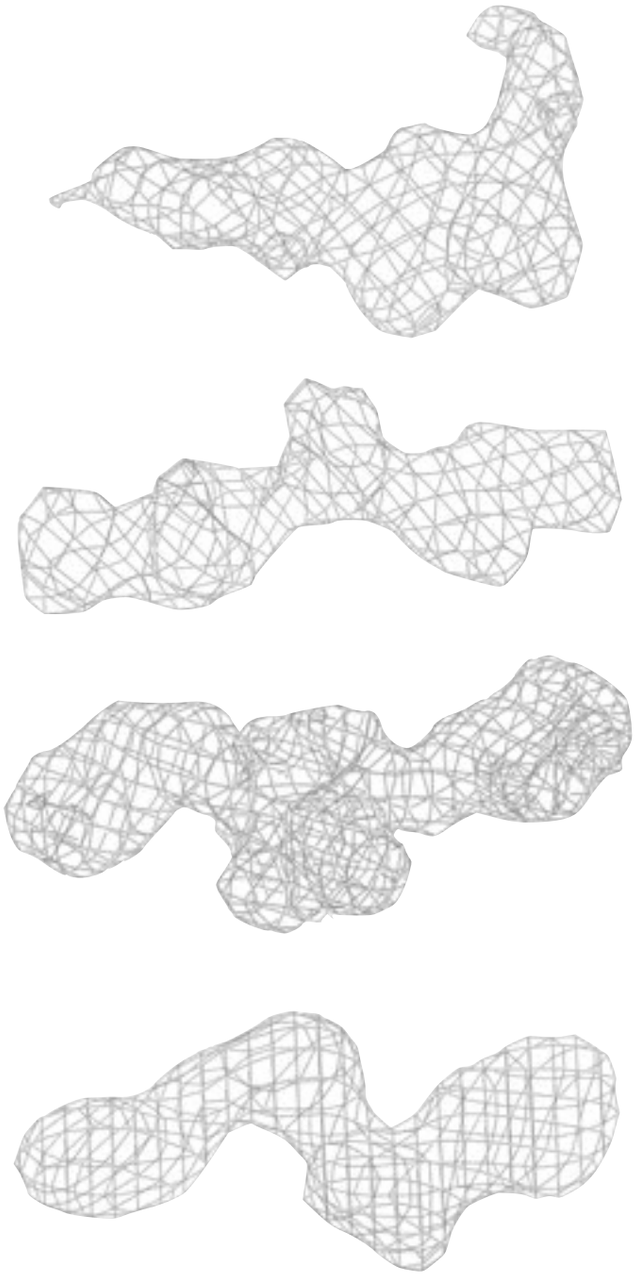
1 ligand

The protocol of a modelling task

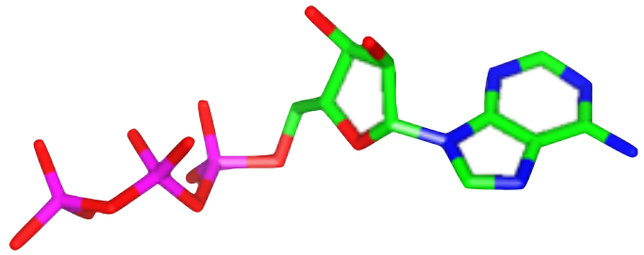
What we know at the beginning will determine the protocol:

Ligand identity \ Binding site	Known	Unknown
Known		X
Unknown		

N density clusters



1 ligand



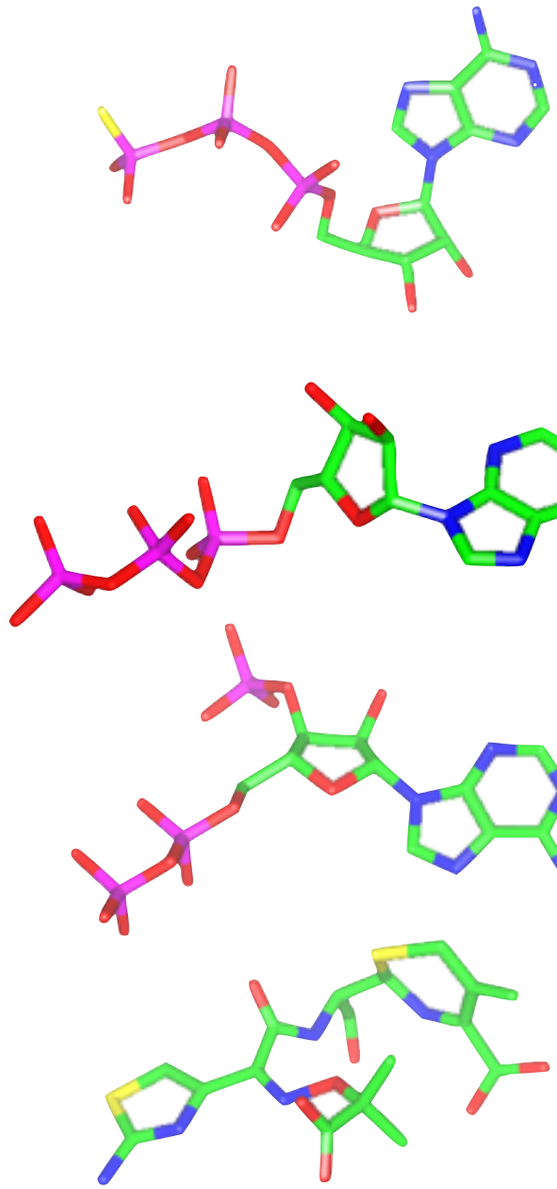
The protocol of a modelling task

What we know at the beginning will determine the protocol:

Ligand identity \ Binding site	Known	Unknown
Known		
Unknown	X	

1 density cluster

N ligand candidates

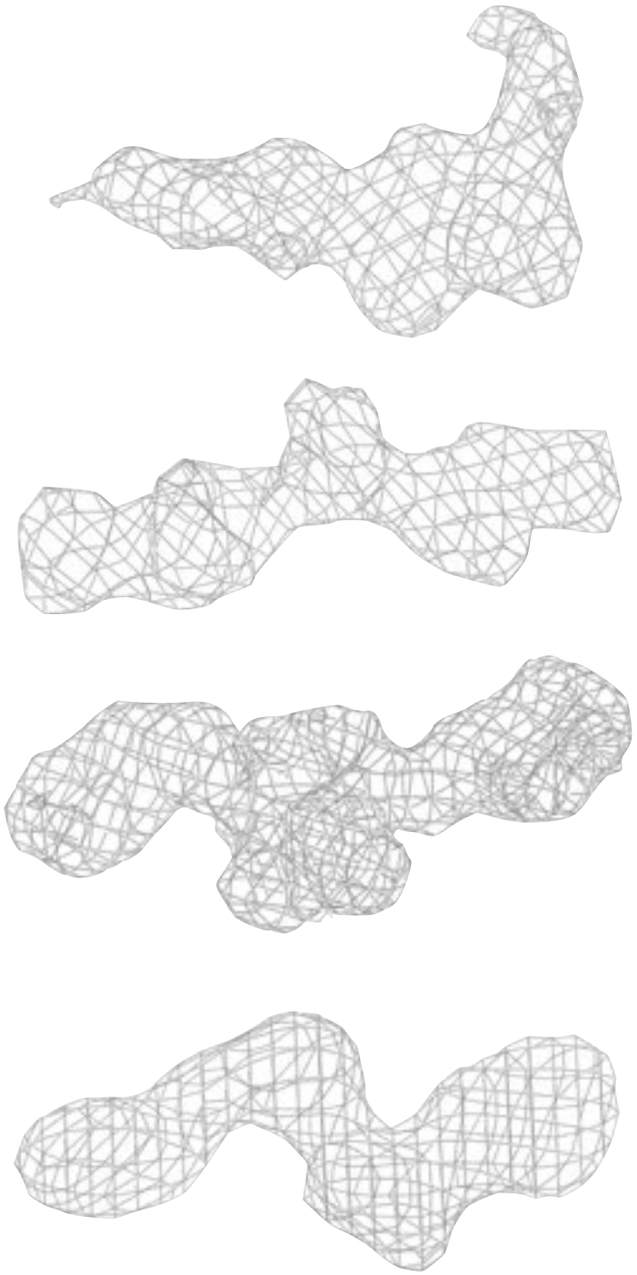


The protocol of a modelling task

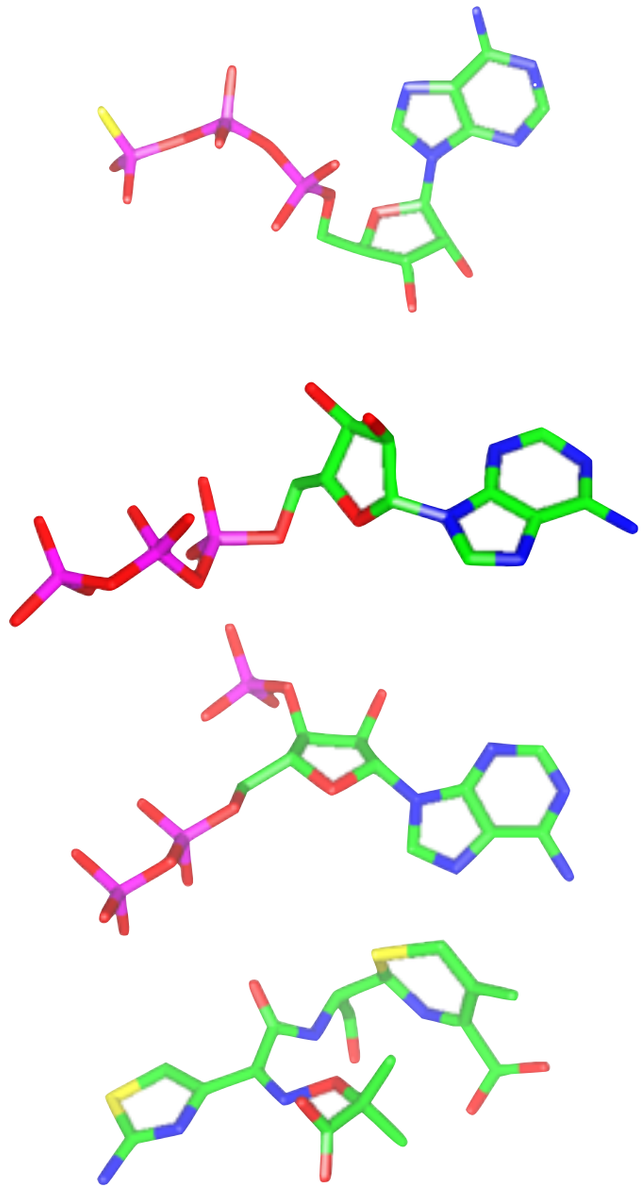
What we know at the beginning will determine the protocol:

Ligand identity \ Binding site	Known	Unknown
Known		
Unknown		X

N density clusters



N ligand candidates



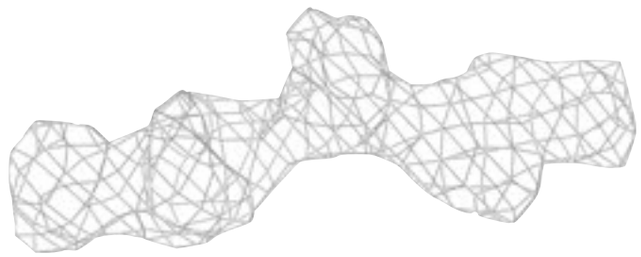
Researcher's needs #1

I am trying to solve a structure of a protein with some inhibitor. **I want to know how I can put in my inhibitor in the density map of the data I got.** I can see some density in the active site where the inhibitor should be…

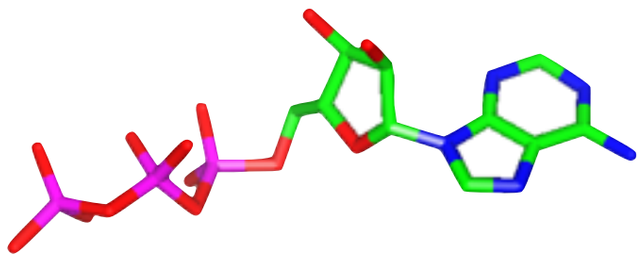
I am not sure of how to do it.

Which case is this?

I density cluster

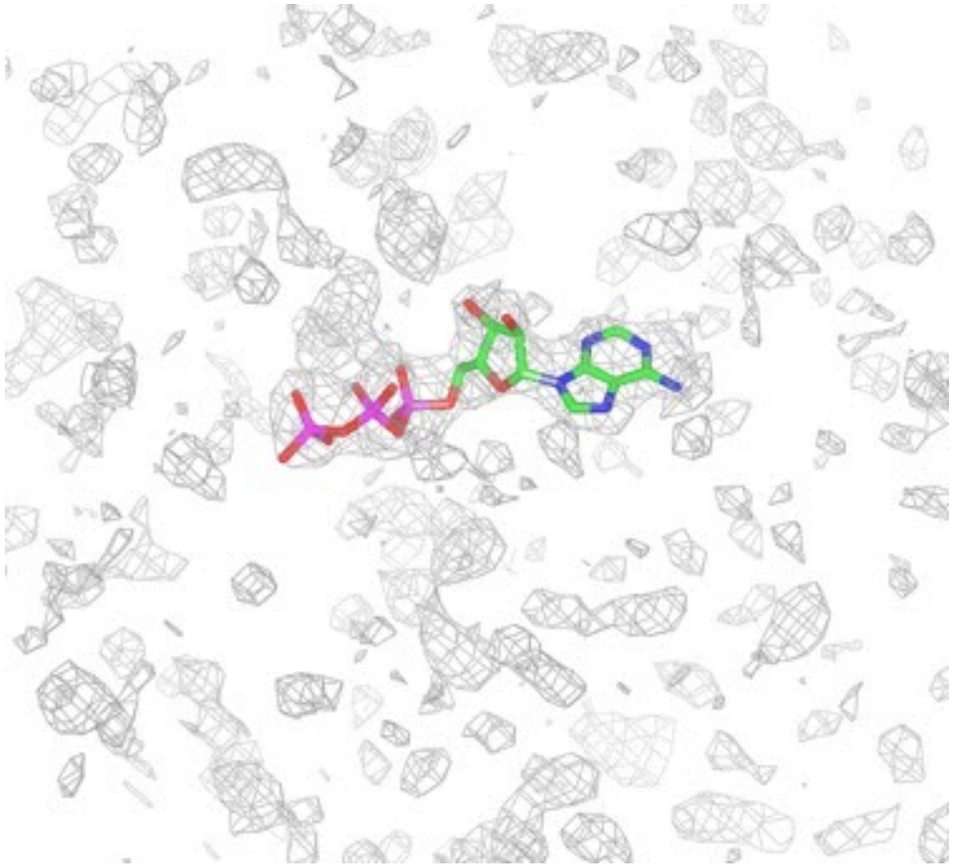


I ligand



Ligand fitting with ARP/wARP

Organised as a **pipeline** of core modules for **specific sub-tasks** following an intuitive approach



Prepare

- Identify ligand **binding site** and /or **ligand**
- Sparse density map and **generate ligand topology**

Construct

- Construct **ensemble** of ligand models in plausible conformation to **fit sparsed density**

Refine

- Refine ligand coordinates to satisfy **geometric** constraints and **maximise** fit to density
- Choose **best model**

Constructing the ligand

- To **address the different ligand sizes** and complexities encountered
  - to increase the **robustness** of the software

- We use 2 separate construction methods:

Label swapping on the sparsed grid

Metropolis search in conformation space

Constructing the ligand: graph search

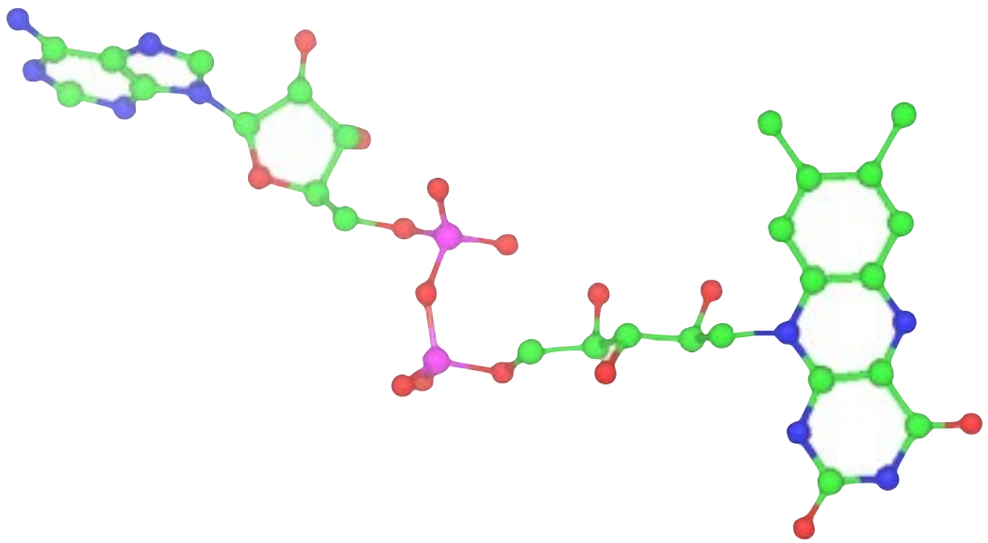
- Uses the **sparse grid representation** of the electron density at the chosen **binding site** & **topology of known ligand**

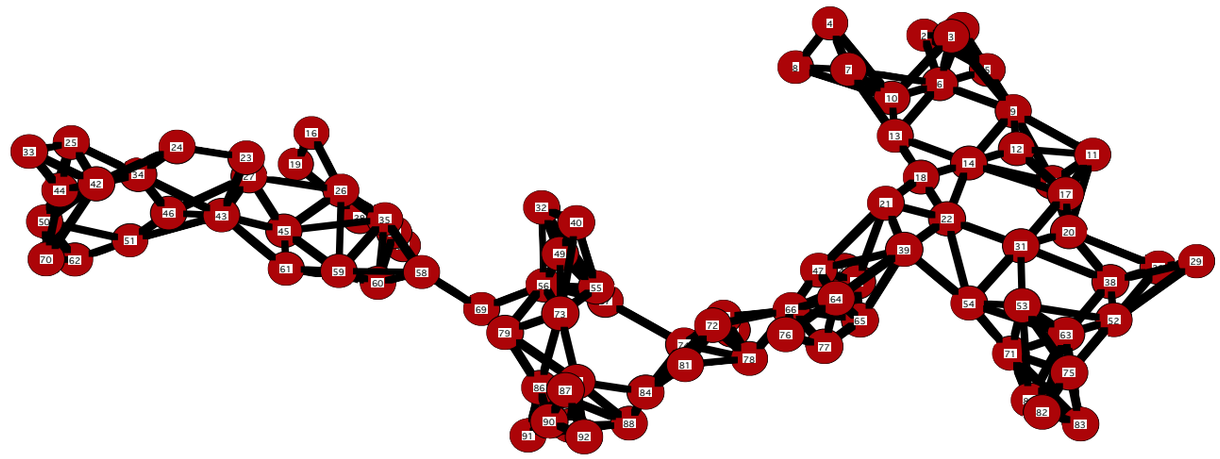
Knowledge about ligand

- Connectivity
- Distances
- Angles
- Chirality

Knowledge about grid

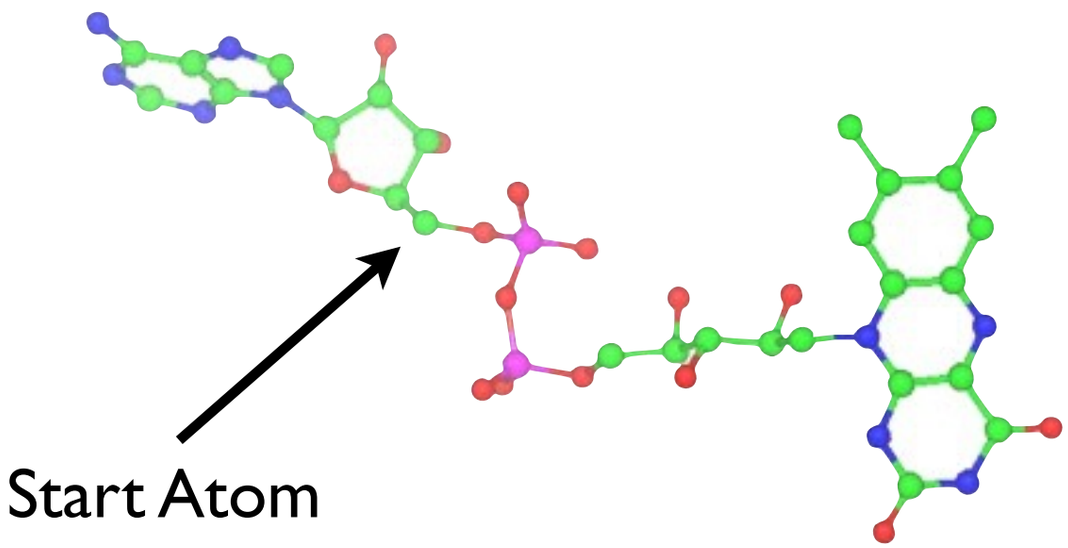
- Connectivity
- Distances
- Density
- *NO IDENTITIES*

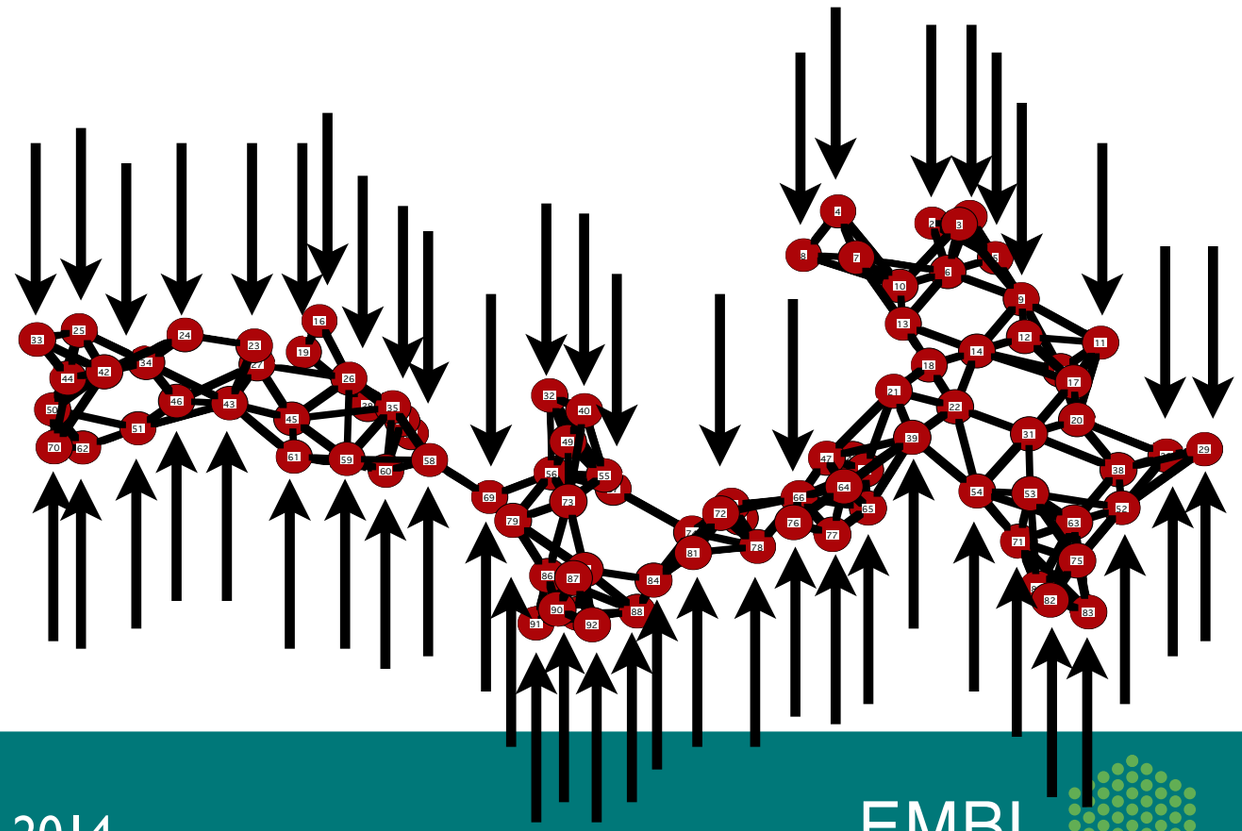




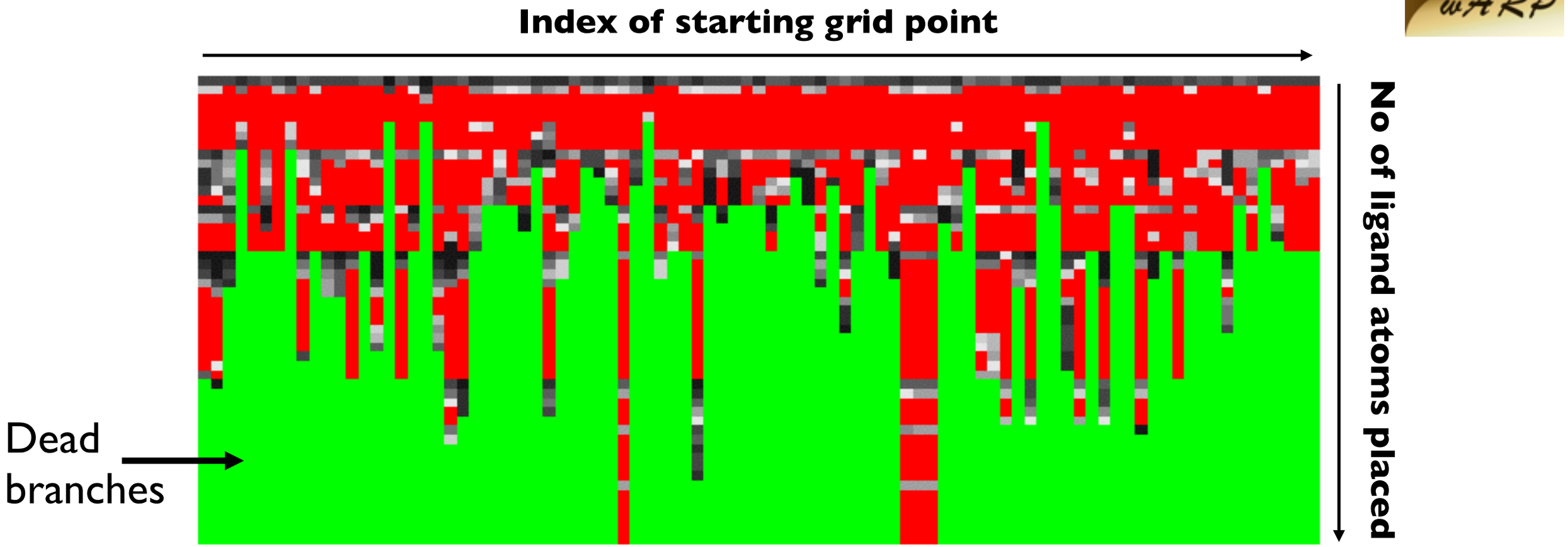
Constructing the ligand: graph search

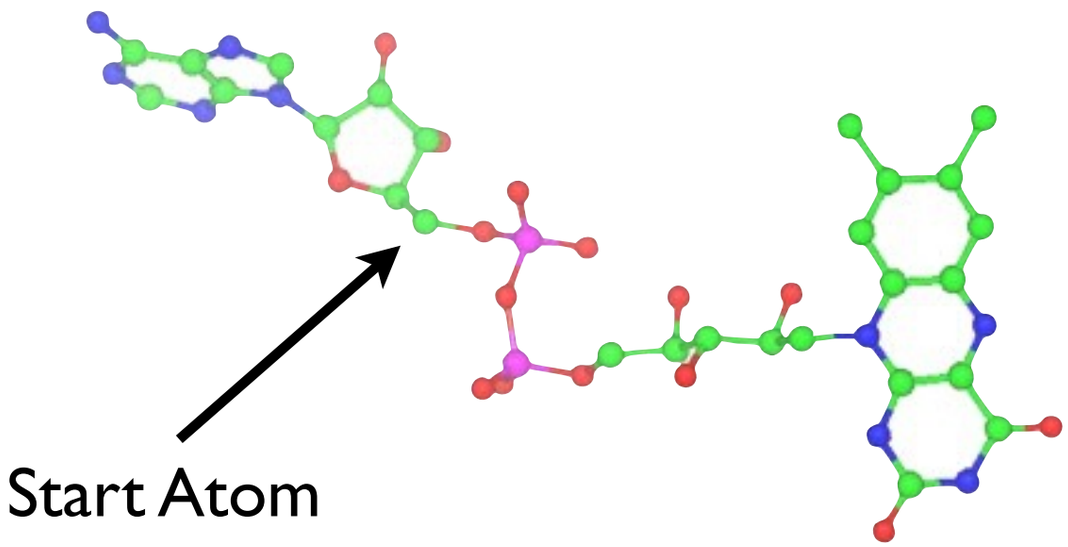
- Label swapping
  - Ligand is **expanded** on the sparse density, **preserving connectivities**
  - Every 'dummy atom' of the sparse grid is tried as a **start point**
  - An **exhaustive graph search** is performed
  - Models are scored by their **fit to density** and expected **stereochemical features**

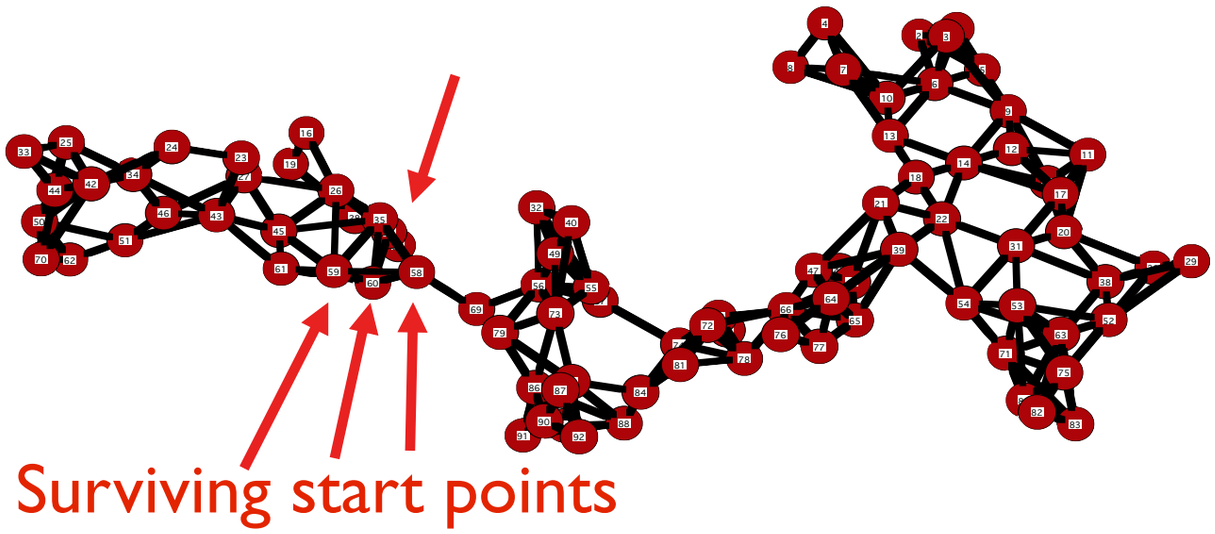




Constructing the ligand: graph search



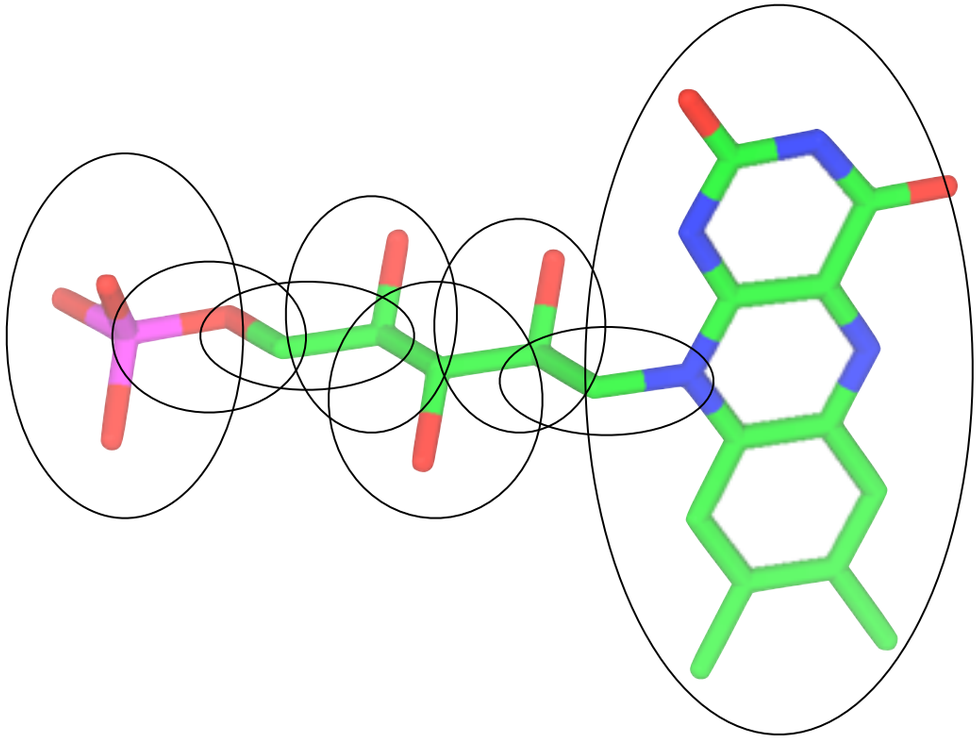




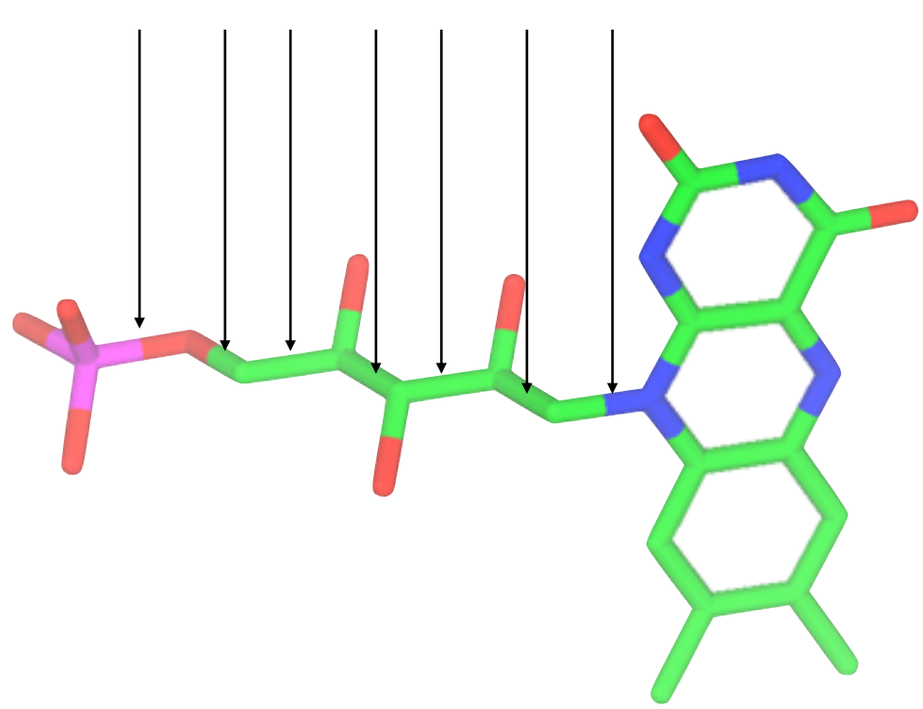
Constructing the ligand: metropolis search

- Perform a **random walk** in parameter space biased towards the **optimum of a score function**
  - Parameter space: **position, orientation** and **conformation**
  - Score function: **'pseudo' map correlation**
  - Advantage: **less degrees of freedom**

Rigid groups

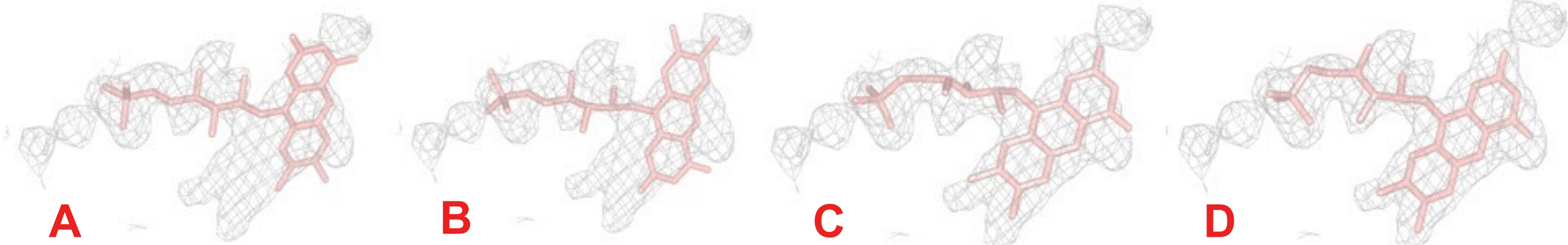


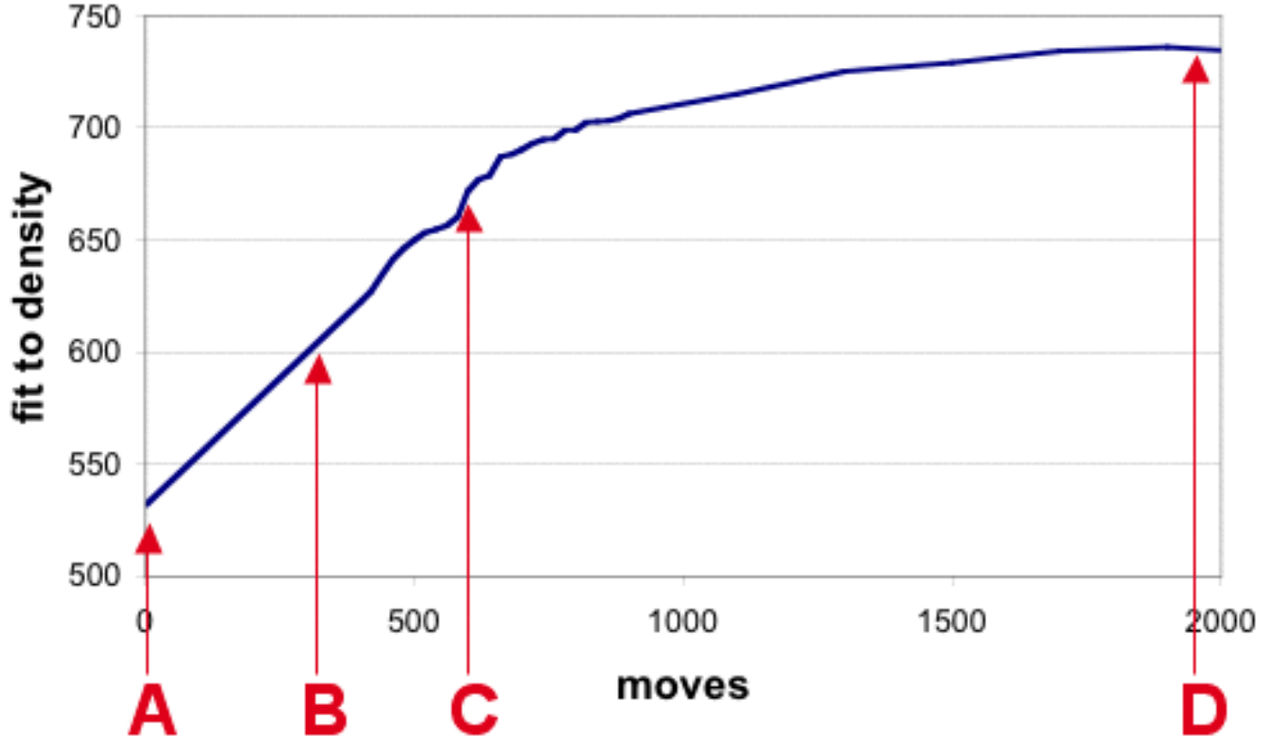
Rotatable bonds

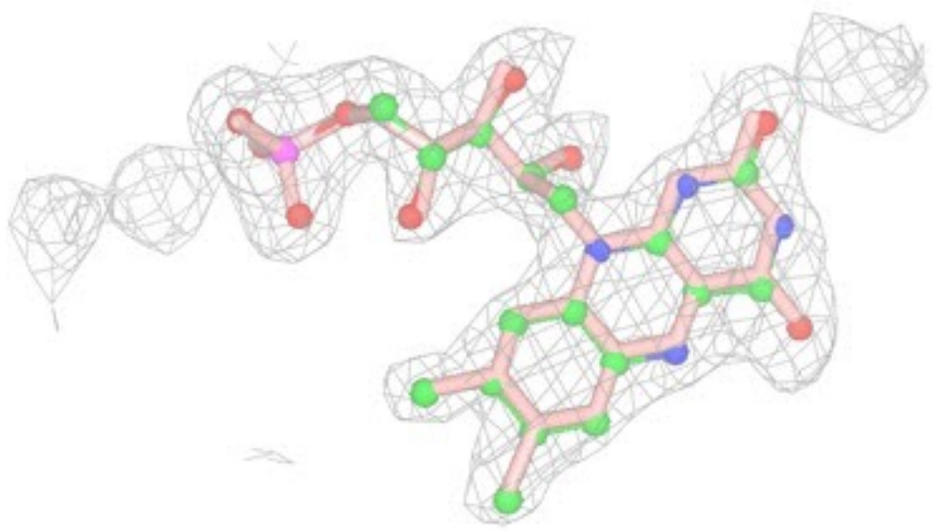


Constructing the ligand: metropolis search

- Evolution of a model during a metropolis optimisation







FMN bound to 1jqv at 2.1Å: 0.23Å rmsd.

Final result: 9000 moves + refinement

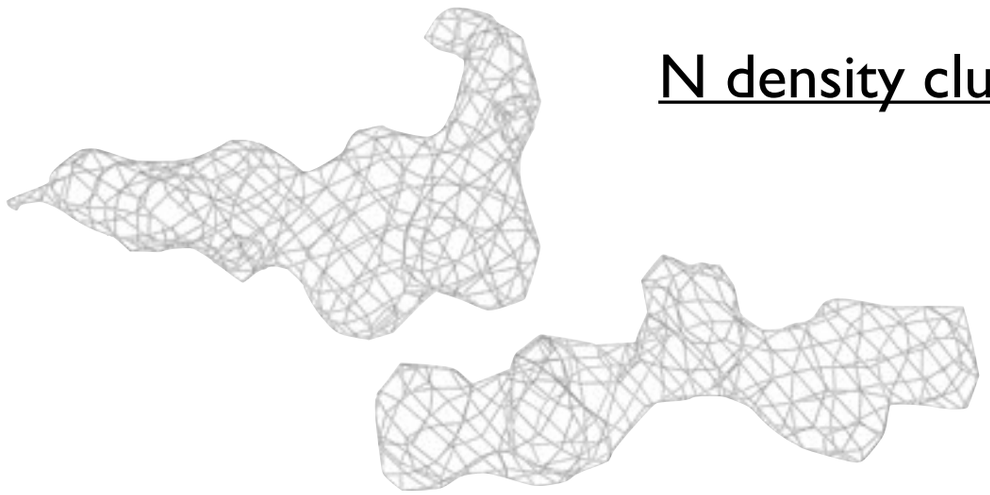
Researcher's needs #2

Is there a simple way to determine whether ligand is bound or not by comparing the diffraction patterns between ligand-free (structure known) and ligand-soaked protein crystals? I would like to solve the ligand bound protein structure, but before I do so, **I have to find out if the ligand is actually bound and if so, where**.

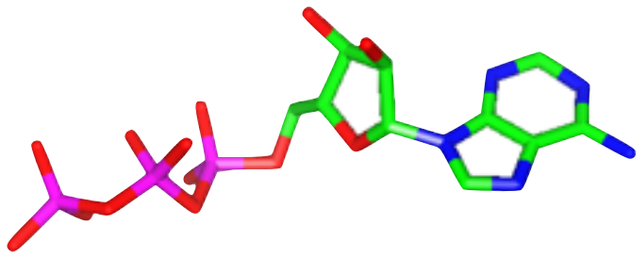
Thank you very much!

Which case is this?

N density clusters

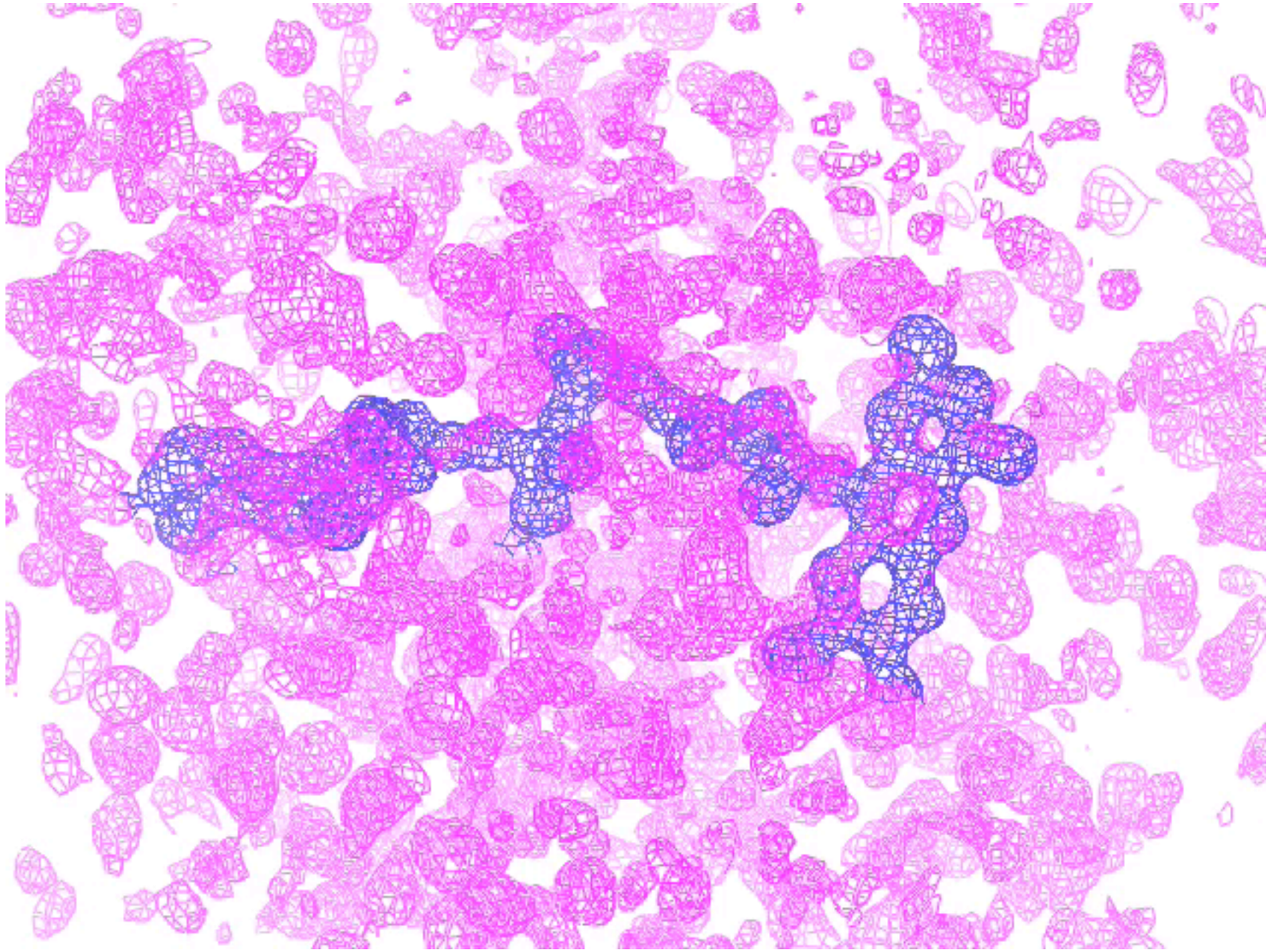


I ligand



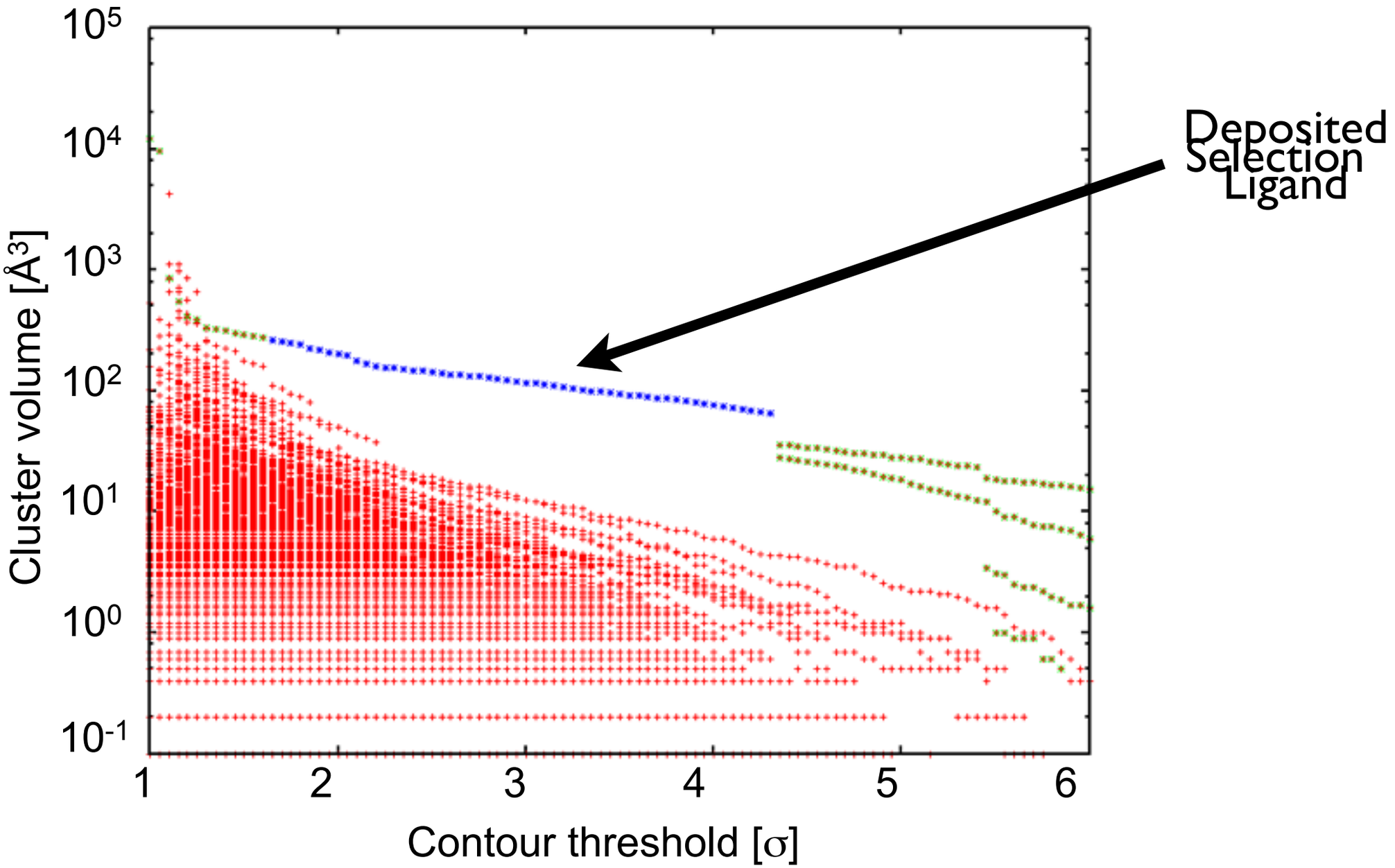
Automatic binding site identification

- The difference density map from **low to high** contour threshold



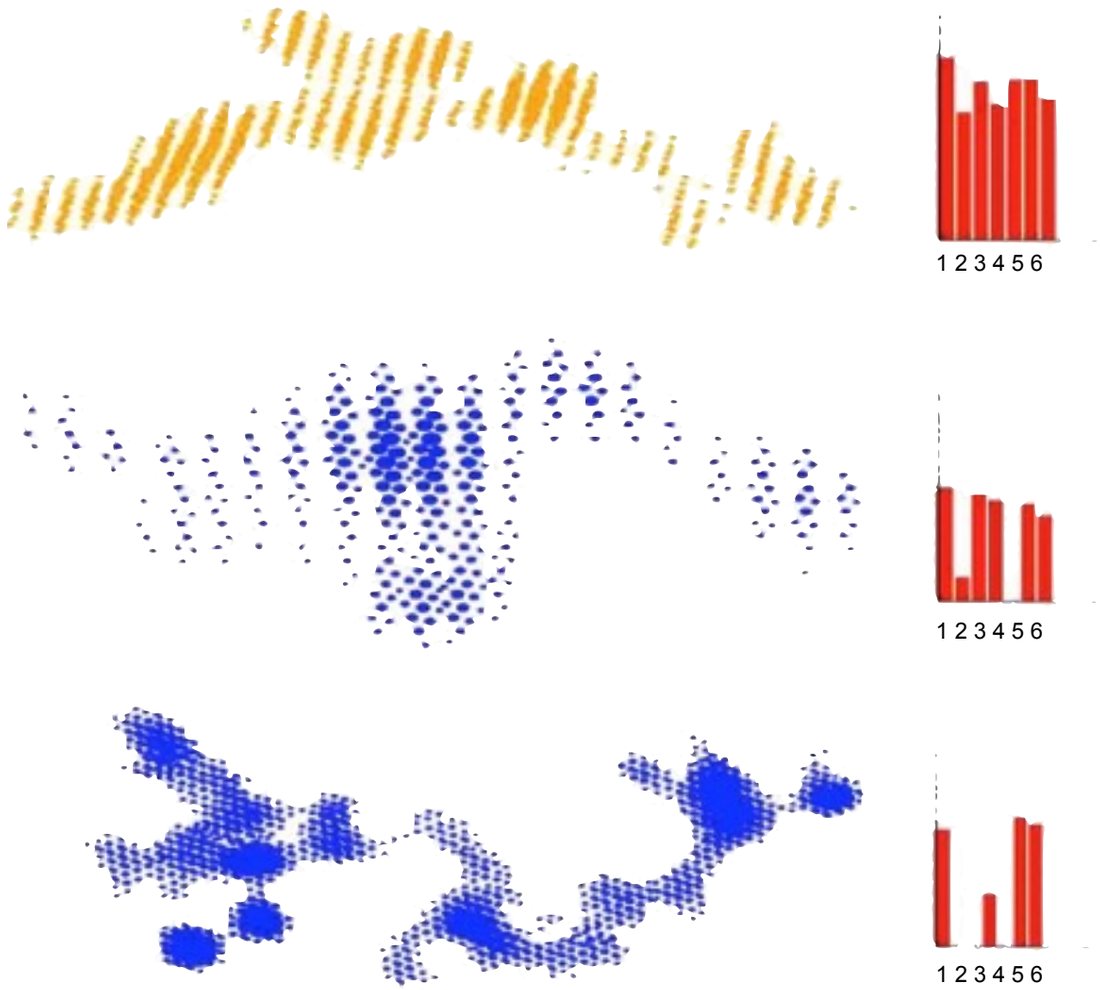
Automatic binding site identification

- Capturing the dependence of the difference density map on changing the contour thresholds: ***the fragmentation tree***

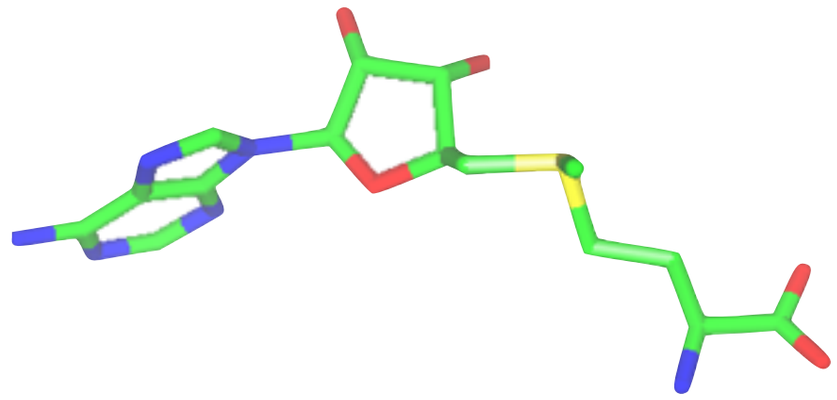


Shape features to measure similarity

An electron density map calculated from the ligand to be fit is compared to each potential density cluster:



Find the matching cluster for this ligand!



1) Surface to volume ratio
2) Bounding box limits
3) Moments of inertia
4) Rotation match score
5) Eigenvalues
6) Distance histogram
7) Geodesic distance histogram

Decision is based on **feature vectors**

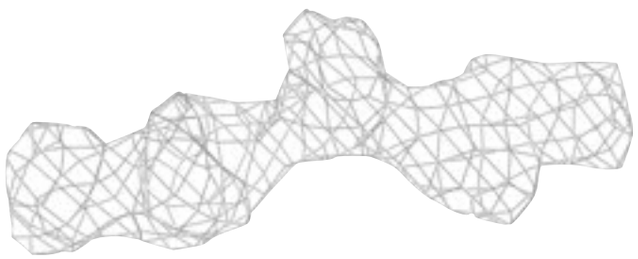
Researcher's needs #3

We are working on protein/inhibitor complex structure. However, we did find a strange density at the active site, it looks really like GSH, the natural co-enzyme of this protein. We tried to use very simple solution to get crystal then exclude the possibility of buffer molecules, but that density is always there.

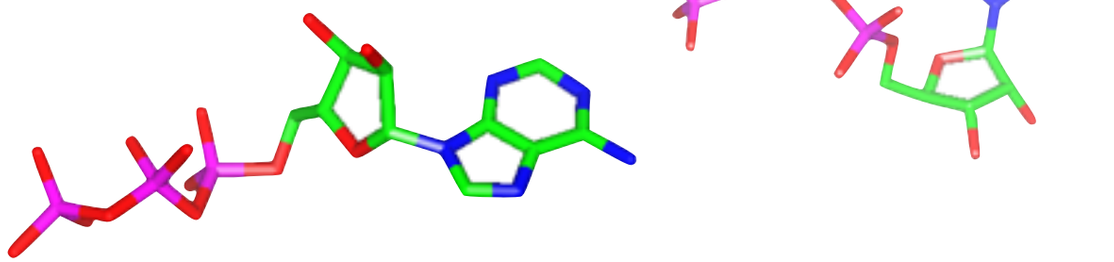
I want to identify this ligand. Are there any methods to do this work?

Which case is this?

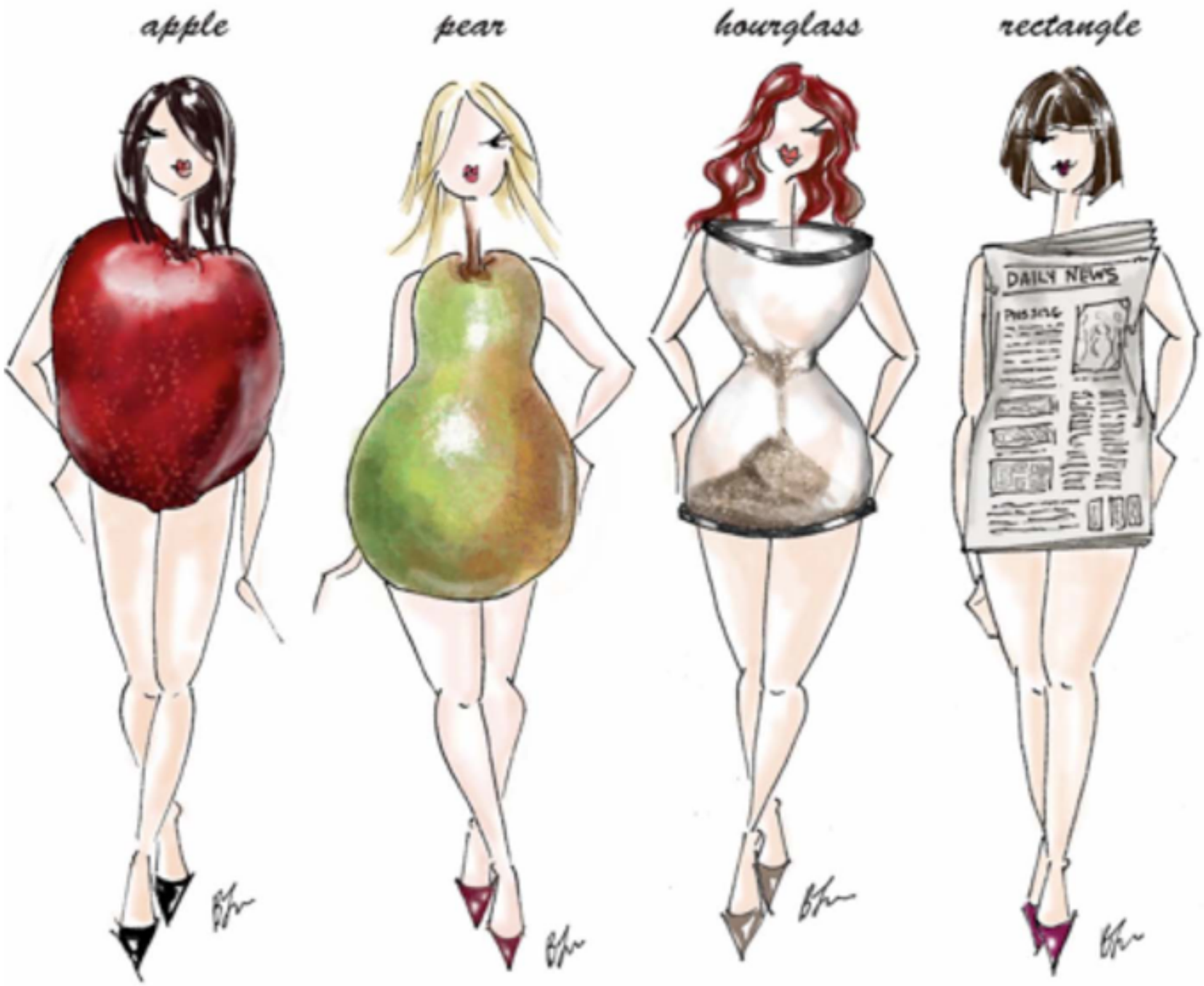
1 density cluster



N ligand candidates

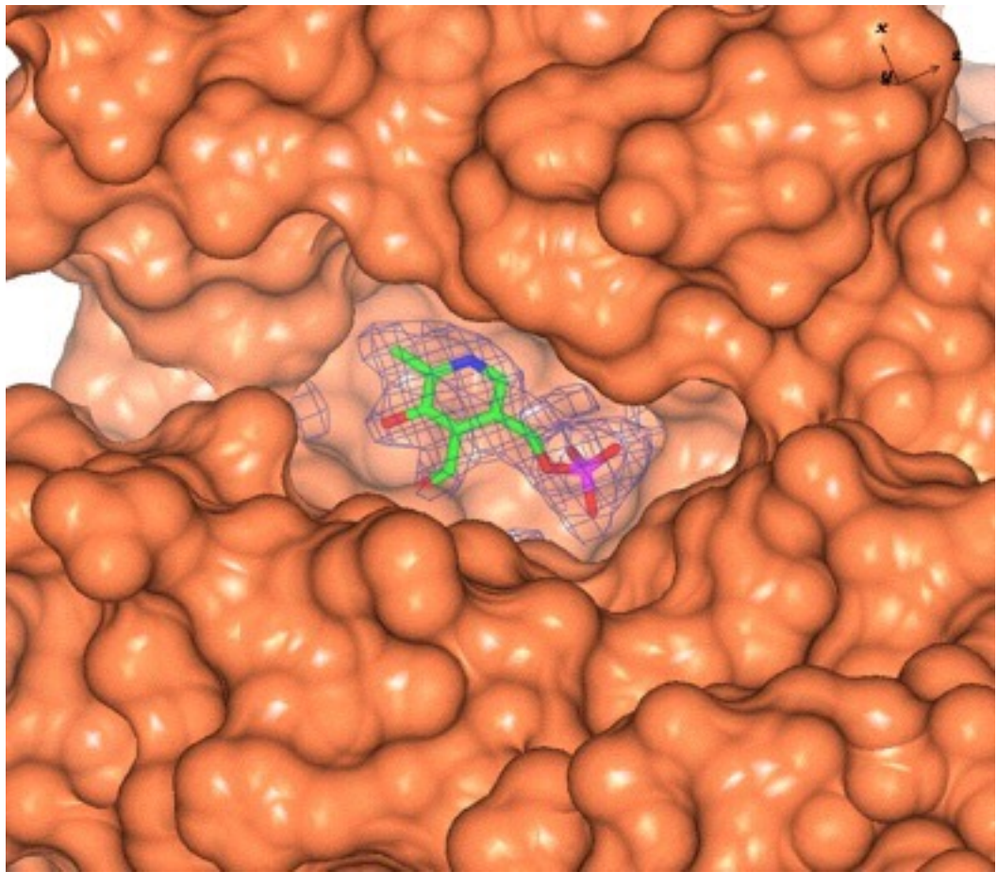


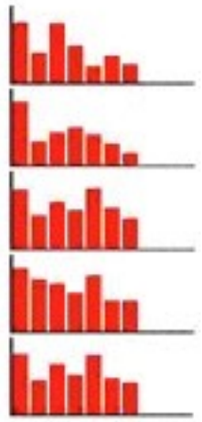
It's all in the shape, baby!



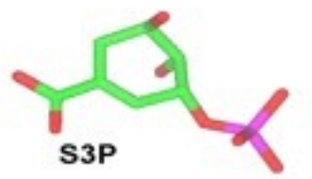
Shape features to measure similarity

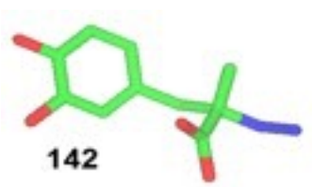
- To **assign a likelihood to a ligand** knowing the binding site

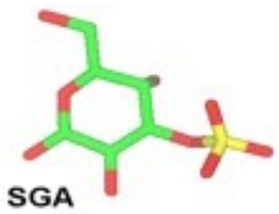




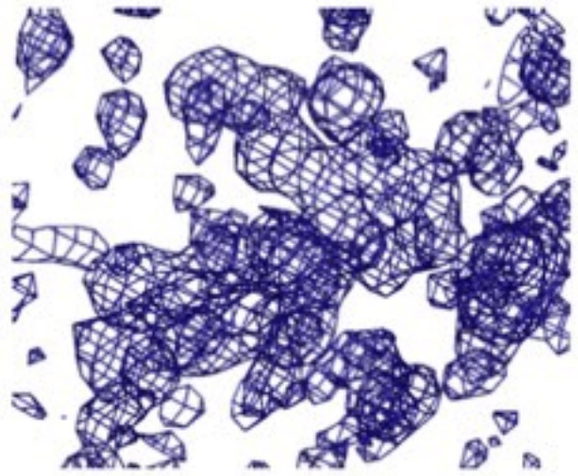
Final ligand ranking





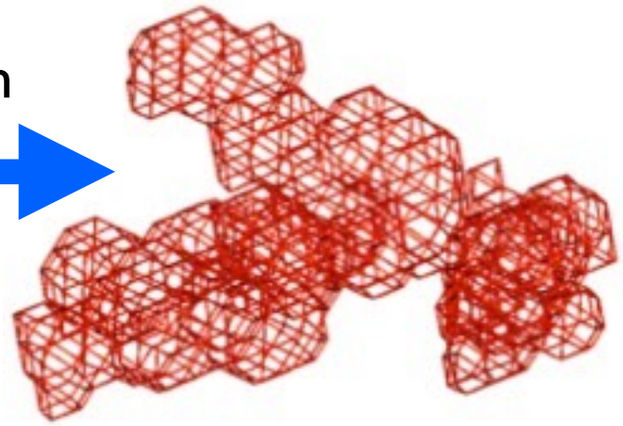


High throughput ligand identification



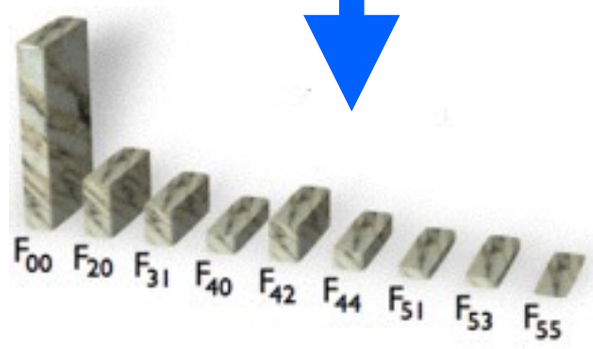
Density map (or otherwise surface of protein pocket)

Segmentation



Projection/ normalisation

Calculation of shape descriptors

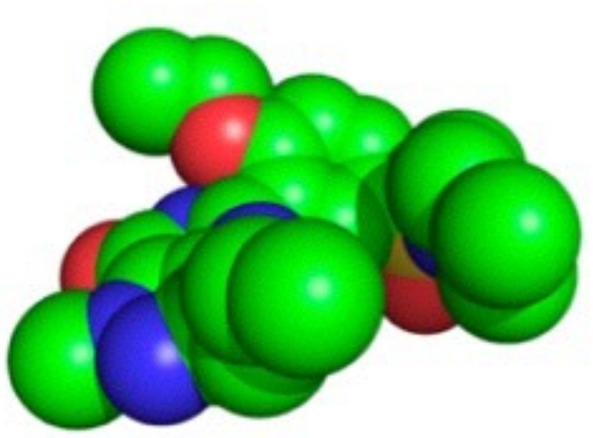


Comparison

Database

84 of the most common ligands in crystallography

Ranking



Ligand

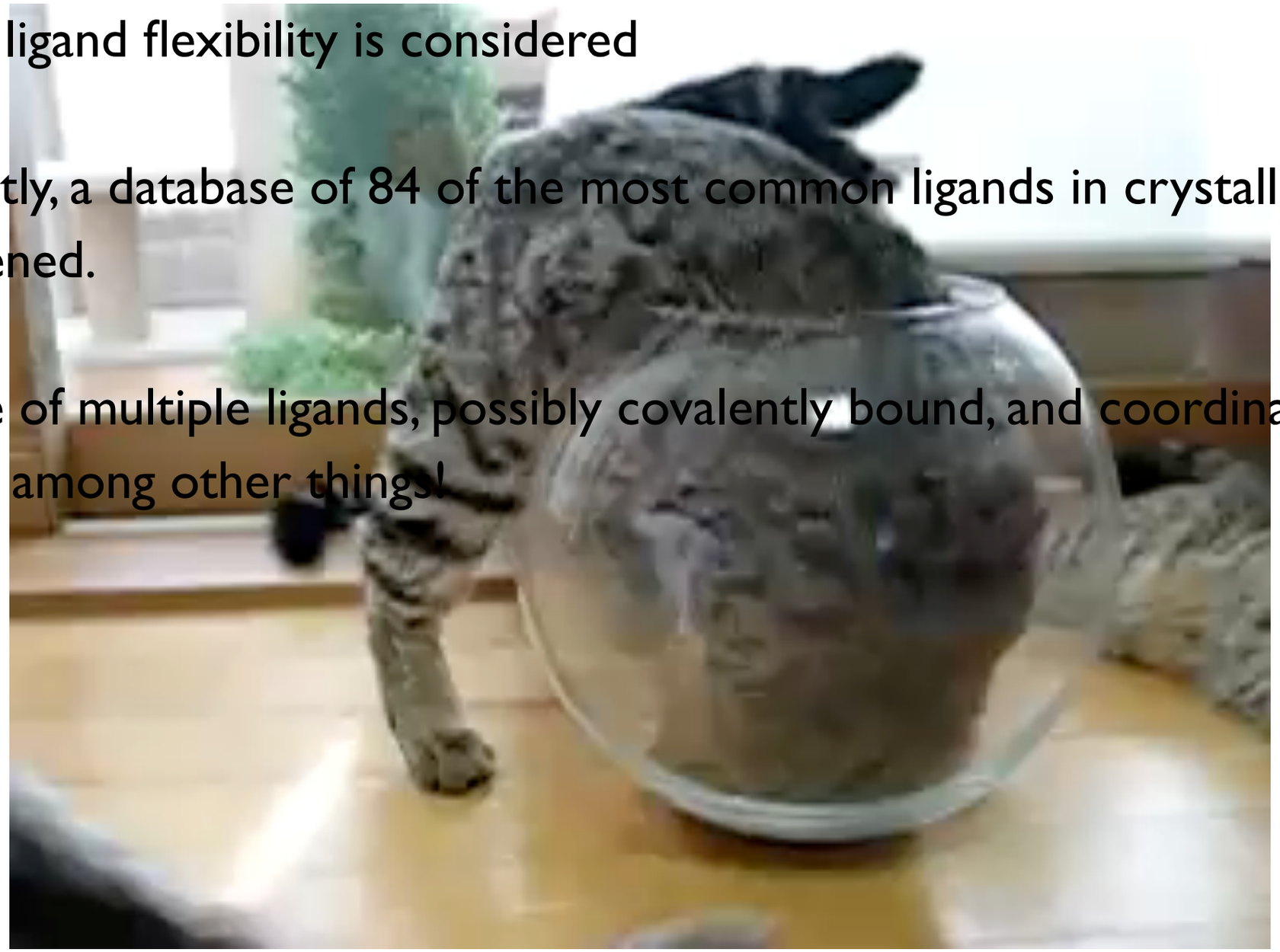
C. Carolan & V. Lamzin, Acta D (July 2014)

Ligand identification with ARP/wARP

- **<u>Flexibility - Ligand Conformations!</u>**
  - full ligand flexibility is considered

- Currently, a database of 84 of the most common ligands in crystallography is screened.

- Beware of multiple ligands, possibly covalently bound, and coordinated solvent among other things!



Researcher's needs #4

I have a ligand in the binding pocket and mediocre data. **The core of the ligand is well defined, however there are chains/tails of the ligand which are not**….The question here is how the chains/tails should be modelled (if at all)

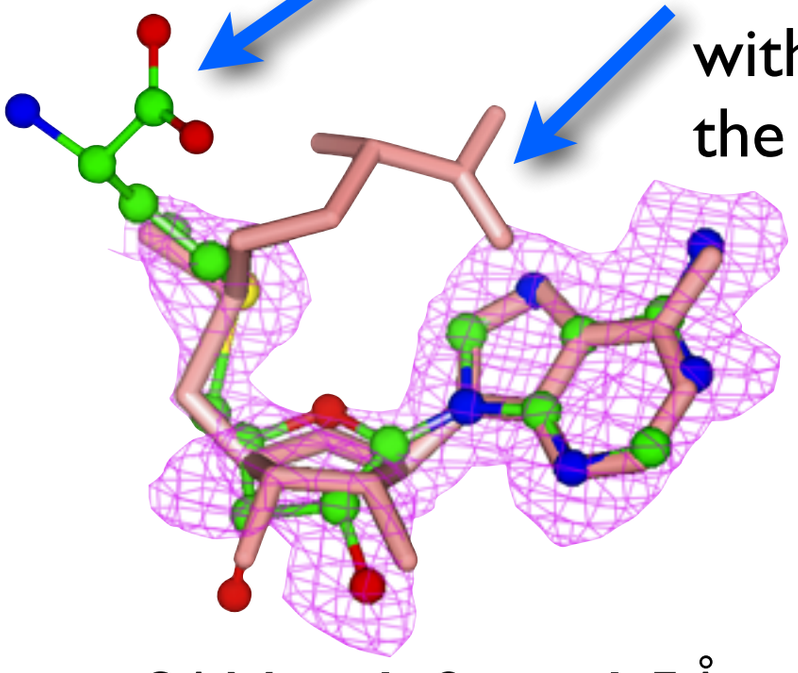
Which case is this?

None of the Above

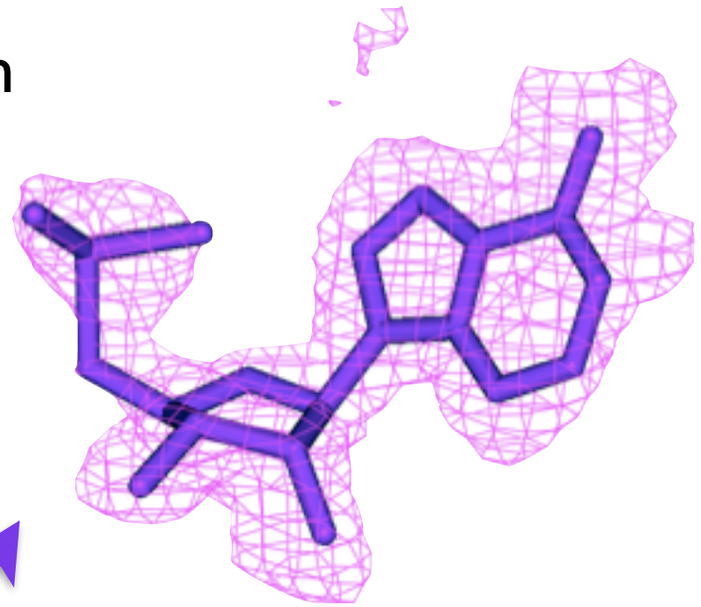
Partial Disorder, Partially Occupied Ligands

Deposited in PDB

Built automatically with ARP/wARP when the full ligand is given

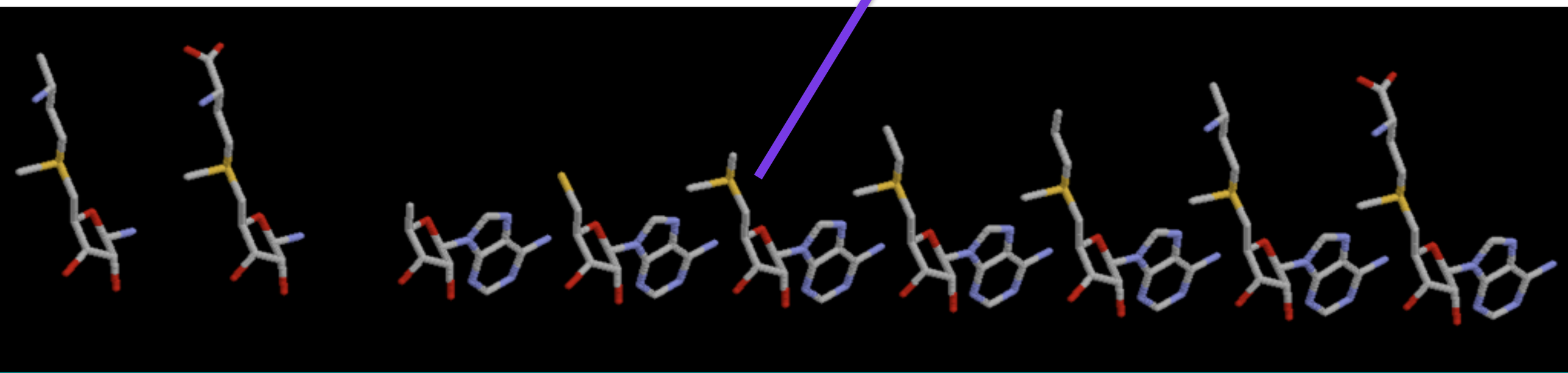


SAM in 1v2x at 1.5Å

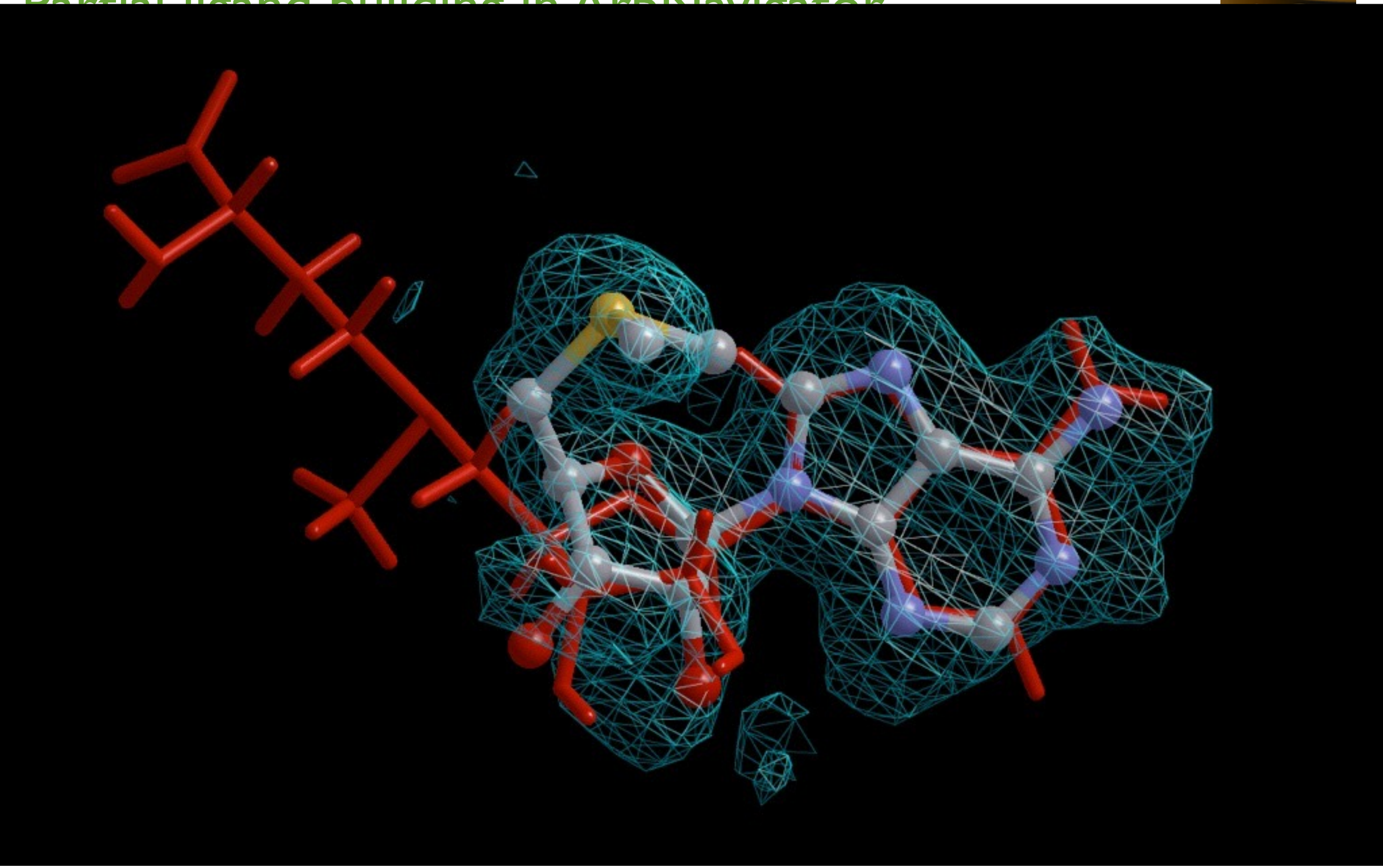


Compound chosen in cocktail case

Artificial Cocktail



Partial ligand building in ArpNavigator



Success rate

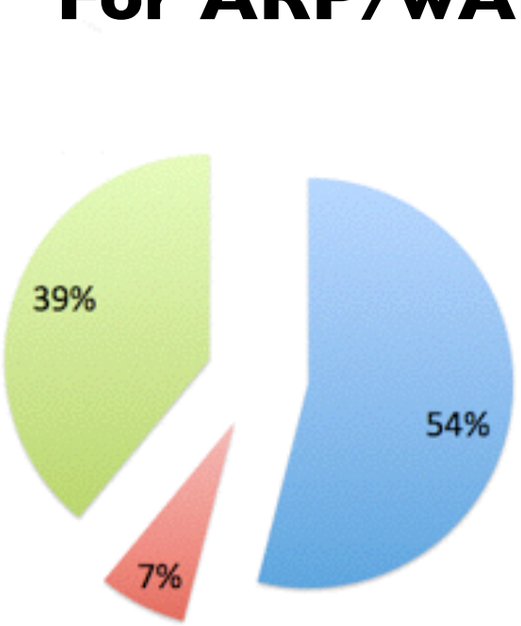
- Tested on > 20k PDB entries from EDS
  - X-ray resolution limits: 1.0 to 3.0 Å
  - Building the **largest fully occupied ligand**
  - Correctness criterion: r.m.s.d. < 1.0 Å

incorrectly identified binding site

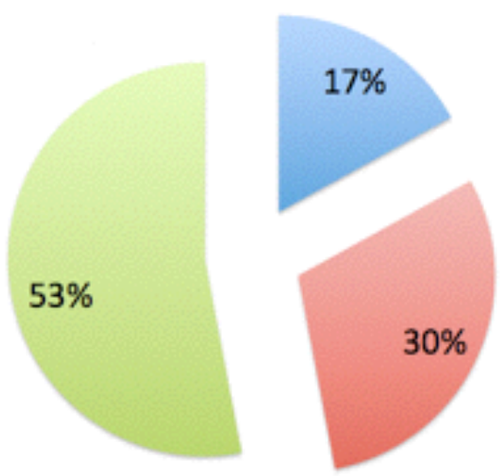
incorrectly built ligand at correct binding site

correctly built ligand

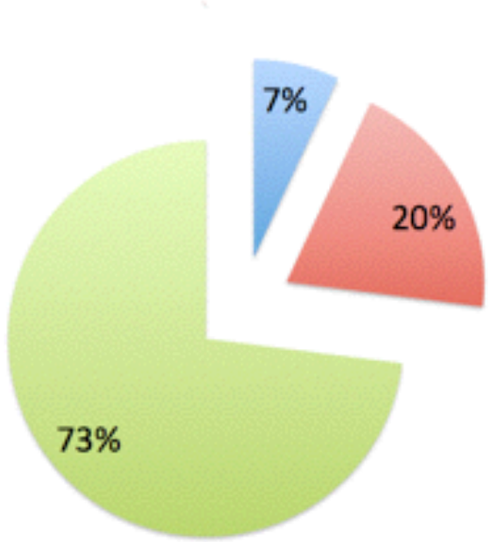
For ARP/wARP 7.2



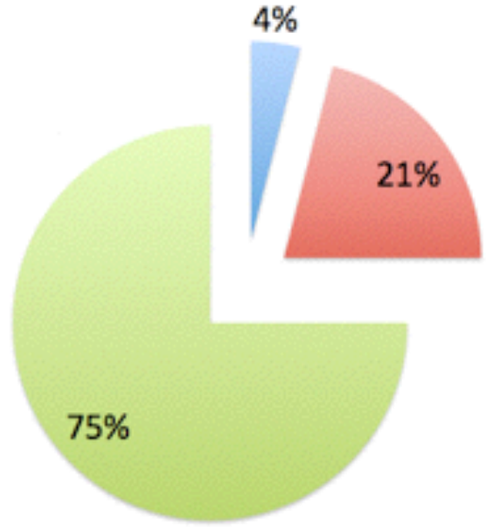
5…6 atoms,
any map corr.



7…100 atoms,
any map corr.



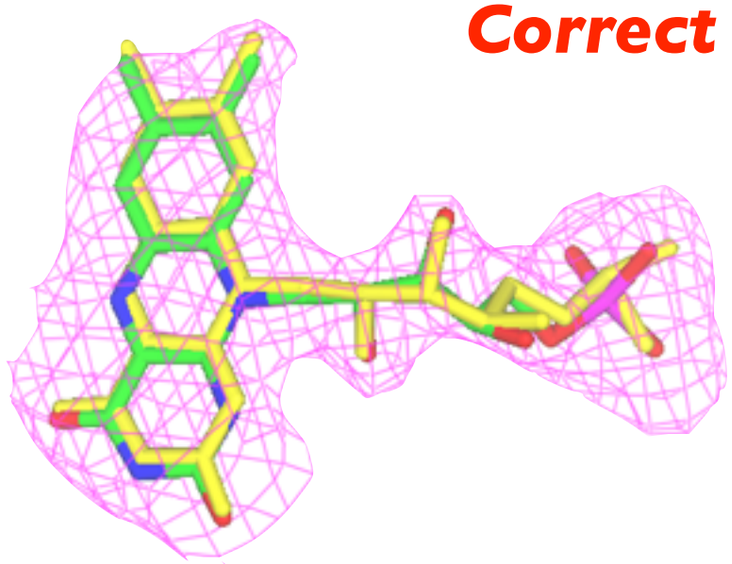
10…40 atoms,
map corr. > 80%



20…40 atoms,
map corr. > 80%

Success criteria: r.m.s.d < 1Å

Correct

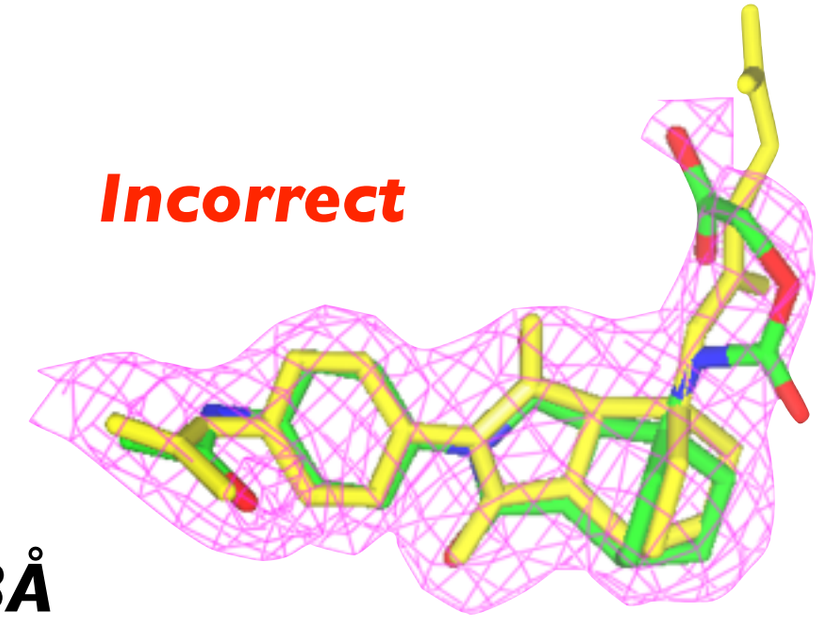


rmsd=0.4Å

Correct

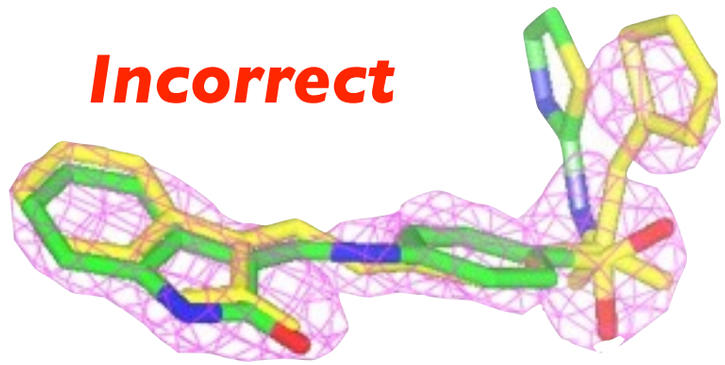
rmsd=1.0Å

Incorrect



rmsd=1.8Å

Incorrect

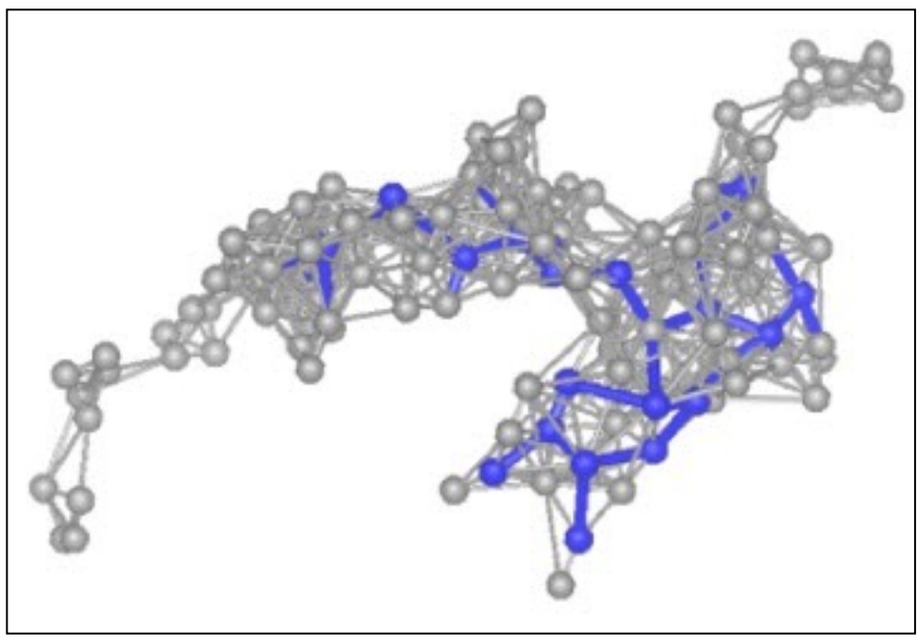


rmsd=1.5Å

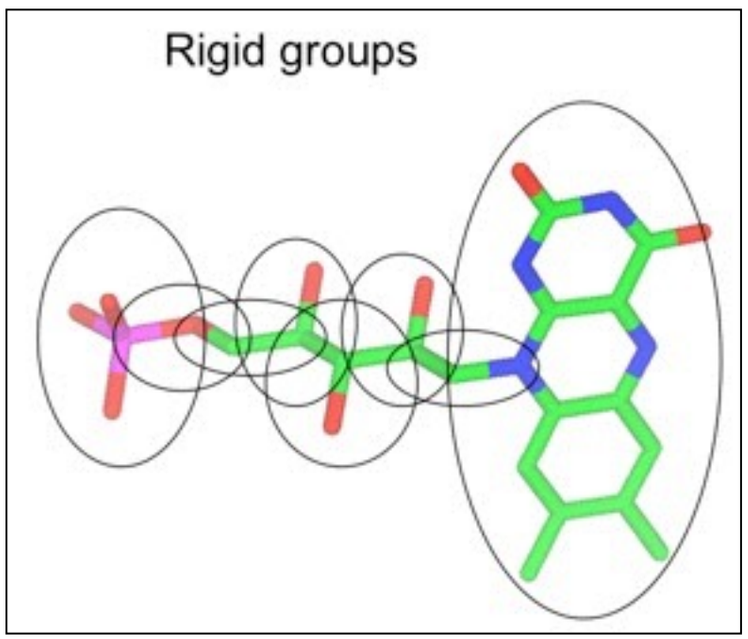
In yellow: the deposited ligand

Ligand building methods

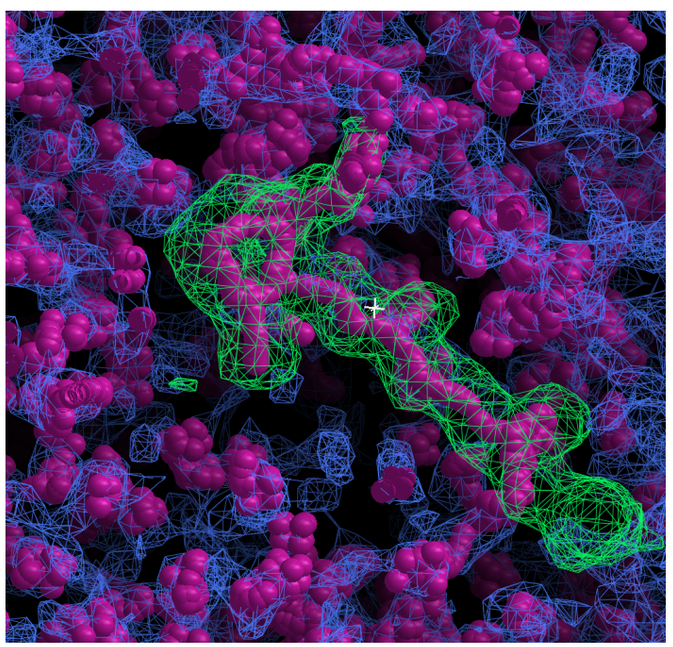
Sparse grids

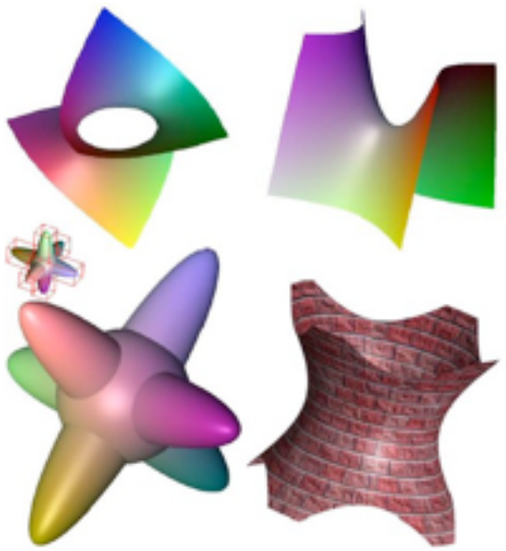


Conformational fit



Fine skeletons





- High-throughput ligand identification using shape descriptors

Building models which do NOT have repetitive motifs like peptides or nucleotides

The people - The power!

Collaborators

EMBL Hamburg: Gleb Bourenkov, Santosh Panjikar

MRC Laboratory, Cambridge : Garib Murshudov's group

Rutherford Appleton Laboratory: CCP4 team

Previous Members

Ciaran Carolan, Tim Wiegels

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