

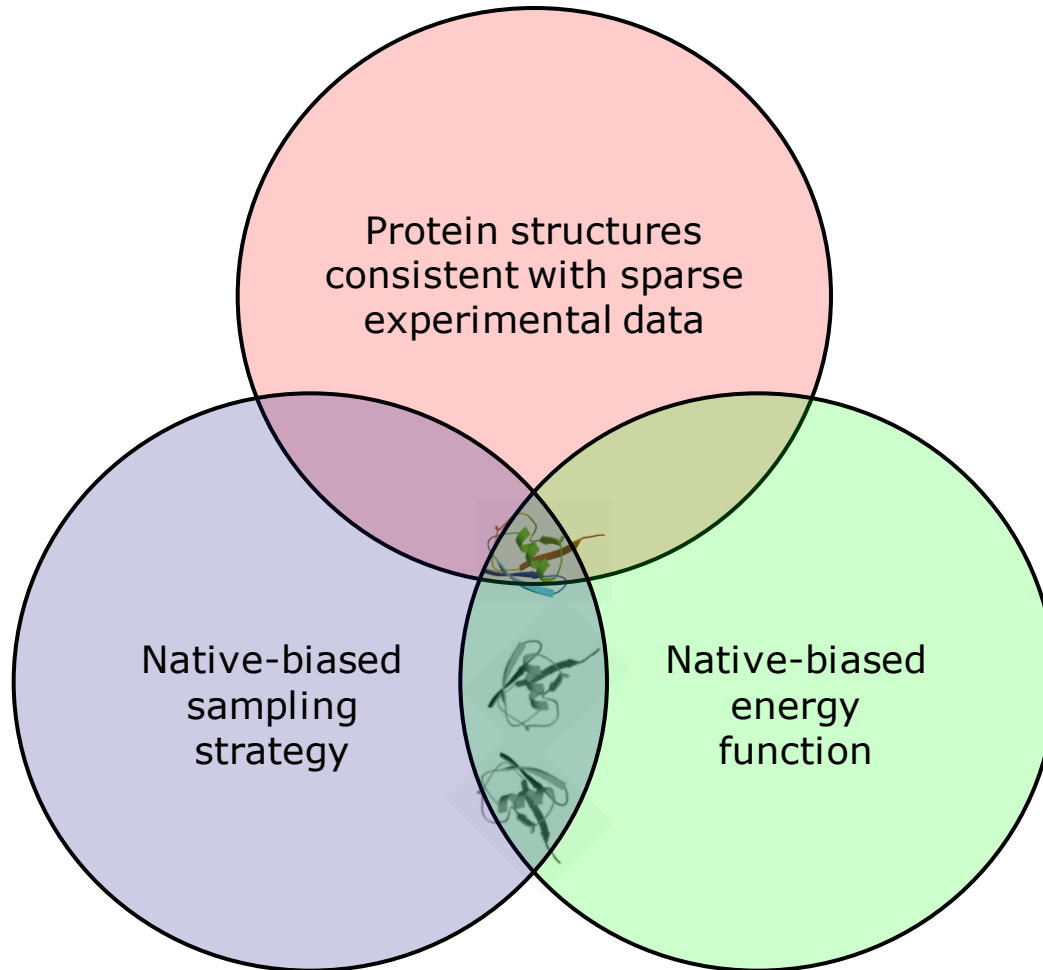
Using experimental data in Rosetta structure prediction

Clara T. Schoeder

Virtual RosettaWorkshop August 2020

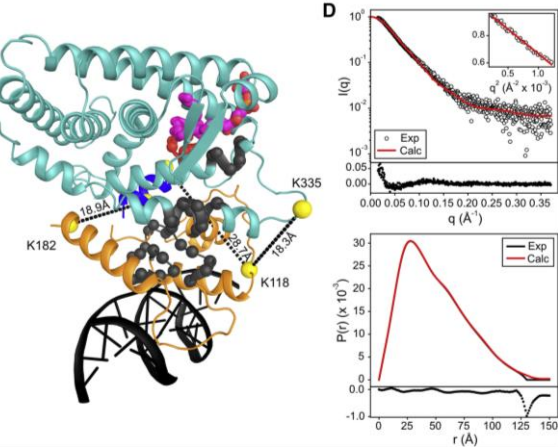
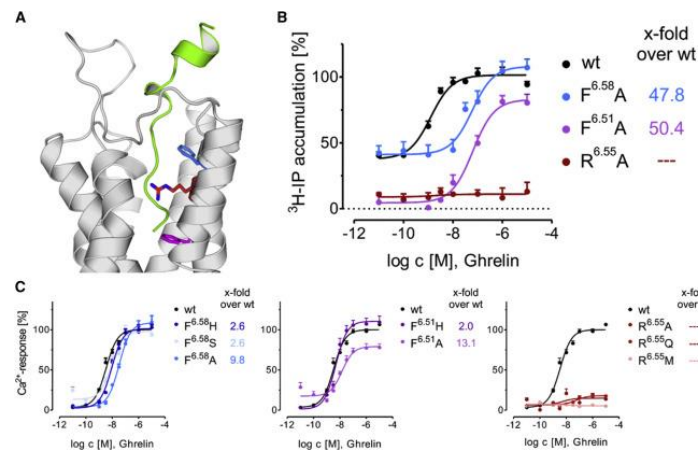


Combine strengths to build accurate models from limited data

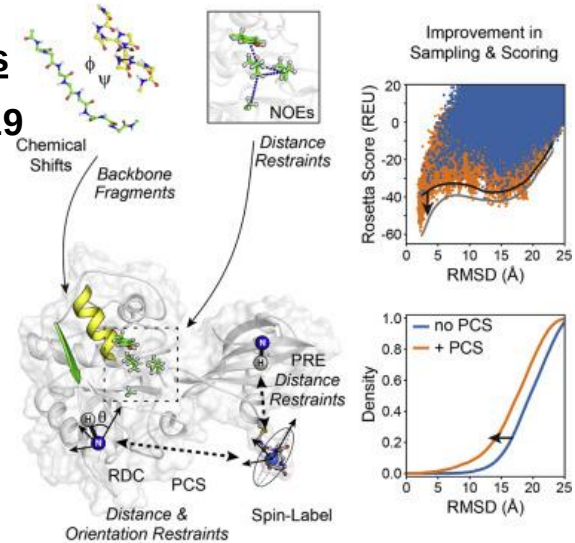
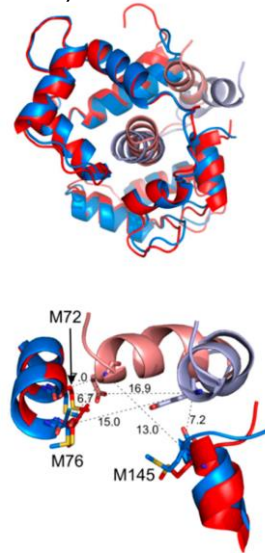


Complete
conformational
space



Seacrist, C. *et al.* *Structure*, 2020Bender, B. *et al. Structure*, 2019

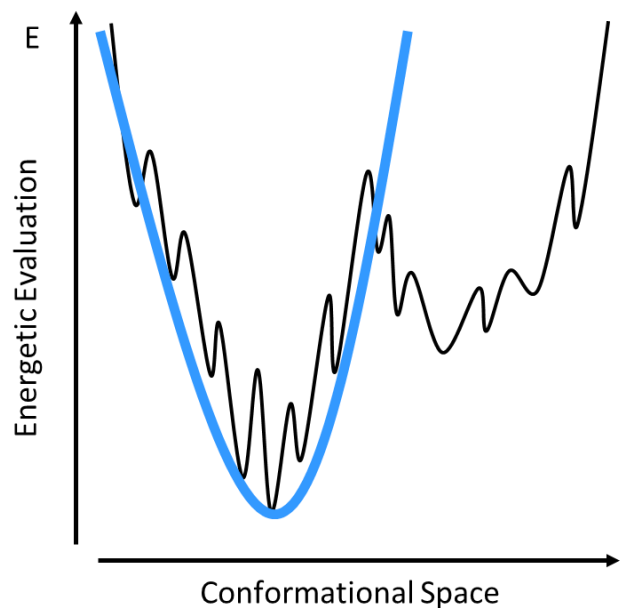
Kuenze, G. *et al. Structure*, **2019**

Chou, H. *et al.* *Structure* **2020**

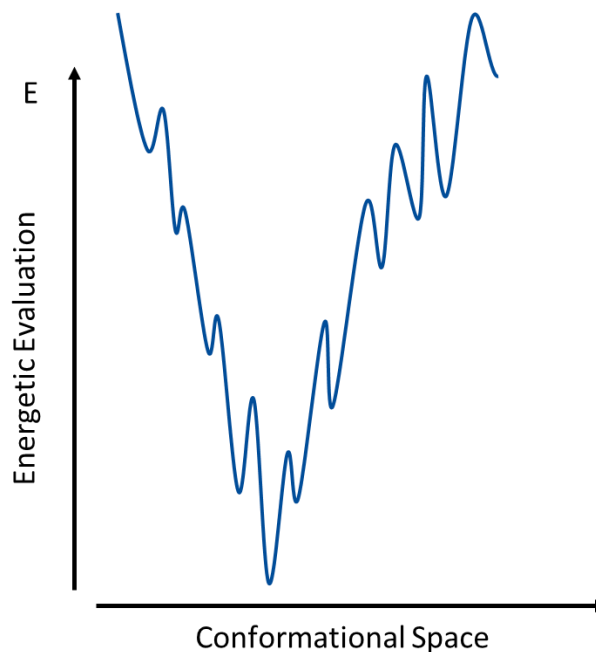
Rosetta Constraints alter the energy function

Theoretical world example

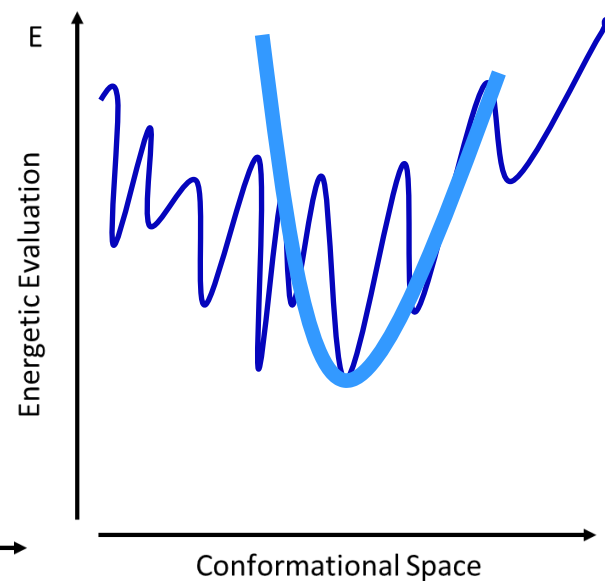
Unconstrained



Constrained



Real world example



Rosetta supports different types of constraints as also different combinations of them

AtomPair NE2 13 V3 32 HARMONIC 0.0 0.2

Angle CD2 13 NE2 13 ZN 32 HARMONIC 2.09 0.35

Dihedral CG 13 CD2 13 NE2 13 ZN 32 CIRCULARHARMONIC 3.14 0.35

Constraint type definitions

Scoring penalties function



Rosetta supports different types of constraints as also different combinations of them

AtomPair NE2 13 V3 32 HARMONIC 0.0 0.2

Angle CD2 13 NE2 13 ZN 32 HARMONIC 2.09 0.35

Dihedral CG 13 CD2 13 NE2 13 ZN 32 CIRCULARHARMONIC 3.14 0.35

Constraint type definitions

- Distance (AtomPair)
 - Angle
 - Dihedral
- Coordinate
- SiteConstraint
- MultiConstraint
- AmbiguousConstraint
- KofNConstraint

Scoring penalties function

- Harmonic
- CircularHarmonic
- Flat-bottomed Harmonic
 - Gaussian
 - Sigmoid
 - Bounded



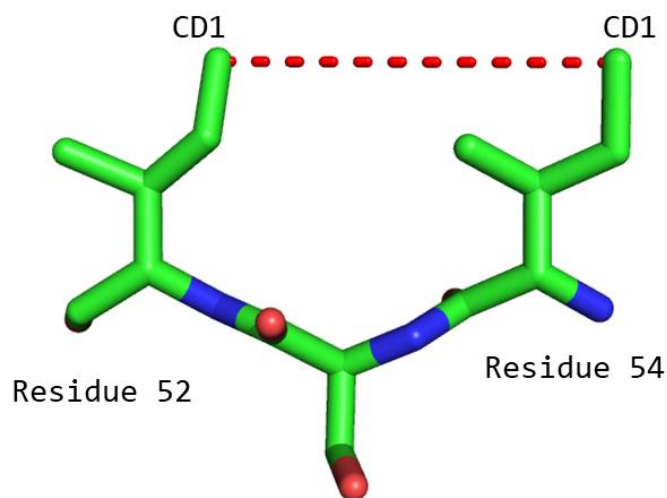
Using distance constraints

Definition

AtomPair	Atom1_Name	Atom1_ResNum	Atom2_Name	Atom2_ResNum	Func_Type	Func_Def
----------	------------	--------------	------------	--------------	-----------	----------

Example

AtomPair	CD1	52	CD1	54	HARMONIC	6.0 0.2
----------	-----	----	-----	----	----------	---------



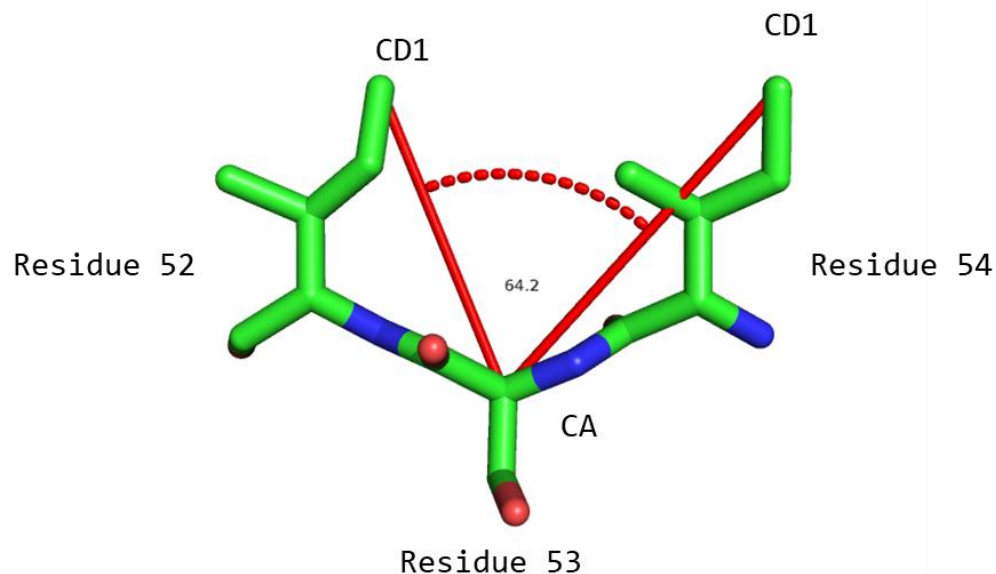
Using angle constraints

Definition

Angle	Atom1_Name	Atom1_ResNum	Atom2_Name	Atom2_ResNum	Atom3_Name	Atom3_ResNum	Func_Type	Func_Def
-------	------------	--------------	------------	--------------	------------	--------------	-----------	----------

Example

Angle	CD1	52	CA	53	CD1	54	HARMONIC	60.0 0.2
-------	-----	----	----	----	-----	----	----------	----------



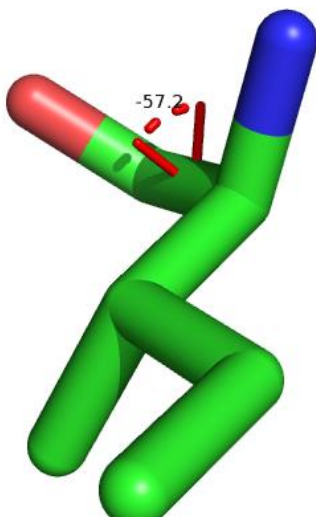
Using dihedral constraints

Definition

```
Dihedral Atom1_Name Atom1_ResNum Atom2_Name Atom2_ResNum  
Atom3_Name Atom3_ResNum Atom4_Name Atom4_ResNum Func_Type  
Func_Def
```

Example

```
Dihedral N 52 CA 52 C 52 O 52 HARMONIC 0.0 0.2
```



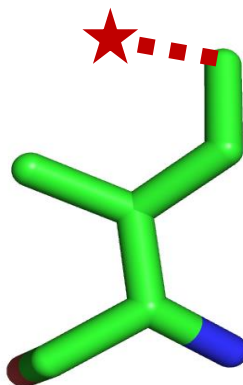
Using coordinate constraints

Definition

```
CoordinateConstraint Atom1_Name Atom1_ResNum[Atom1_ChainID]  
Atom2_Name Atom2_ResNum[Atom2_ChainID] Atom1_target_X_coordinate  
Atom1_target_Y_coordinate Atom1_target_Z_coordinate Func_Type  
Func_Def
```

Example

```
CoordinateConstraint CA 1 CD1 54 X Y Z HARMONIC 0.0 0.2
```



Rosetta can work with multiple constraint definitions at once

```
MultiConstraint  
Constraint_Type1 Constraint_Def1  
[Constraint_Type2 Constraint_Def2  
...]  
END
```

Sum of all constraints

```
AmbiguousConstraint  
Constraint_Type1 Constraint_Def1  
[Constraint_Type2 Constraint_Def2  
...]  
END
```

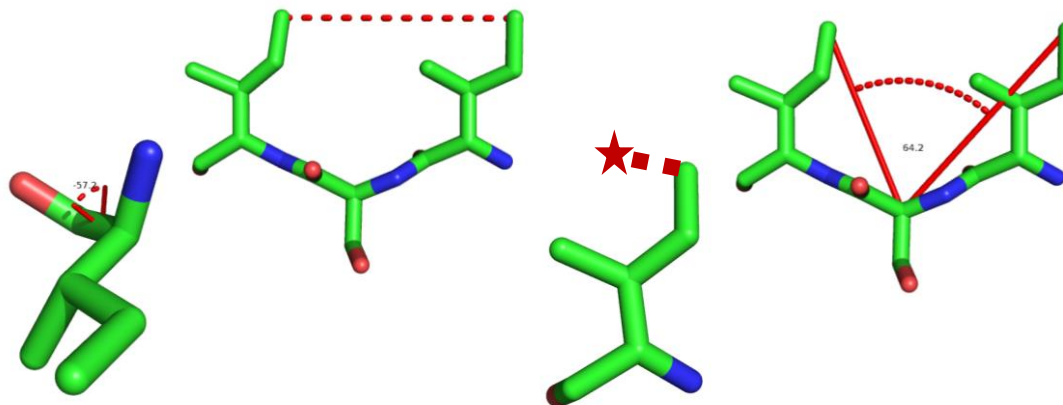
Only the best satisfied (lowest energy)
constraint is considered

```
KofNConstraint k  
Constraint_Type1 Constraint_Def1  
[Constraint_Type2 Constraint_Def2  
...]  
END
```

Sum of k lowest energy constraints



Rosetta can work with multiple constraint definitions at once



```
KofNConstraint 2
```

```
AtomPair CD1 52 CD1 54 HARMONIC 6.0 0.2
```

```
Angle CD1 52 CA 53 CD1 54 HARMONIC 60.0 0.2
```

```
Dihedral N 52 CA 52 C 52 O 52 HARMONIC 0.0 0.2
```

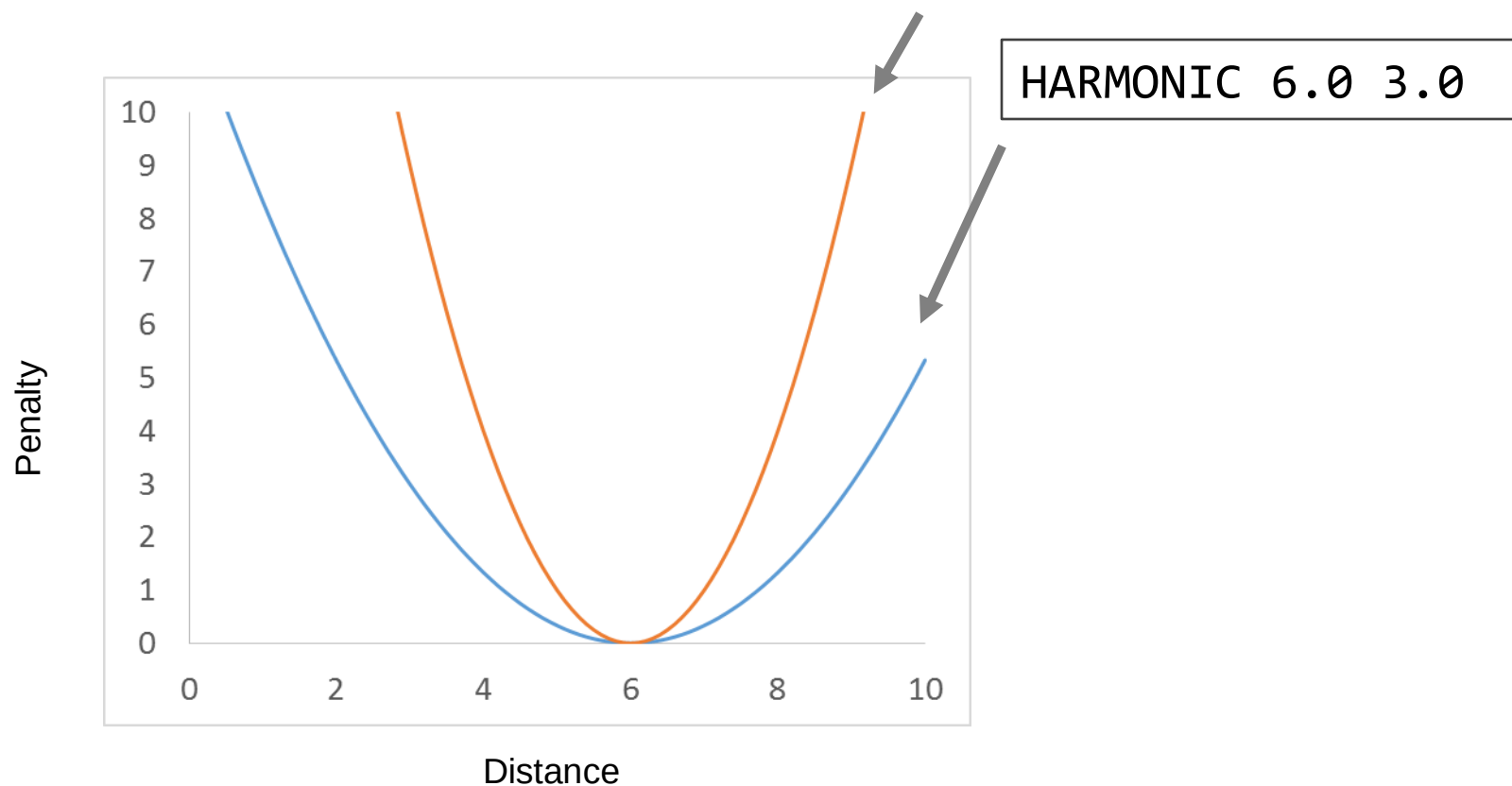
```
CoordinateConstraint CA 1 CD1 54 HARMONIC 0.0 0.2
```

```
END
```



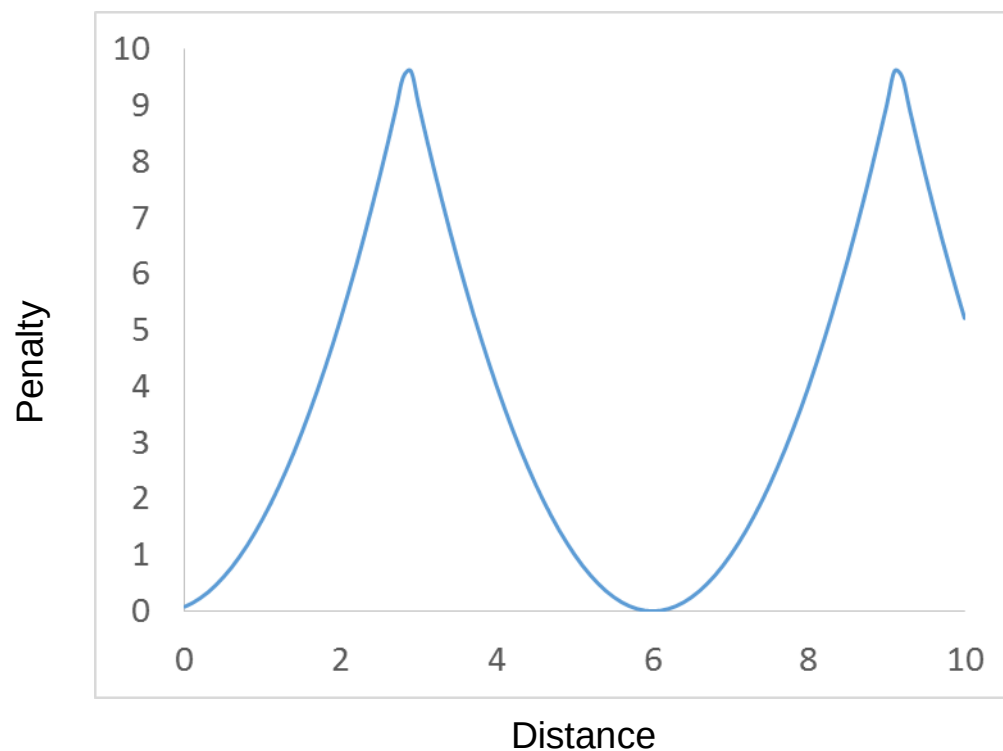
Scoring functions: Harmonic

```
AtomPair CD1 52 CD1 54 HARMONIC 6.0 1.0
```



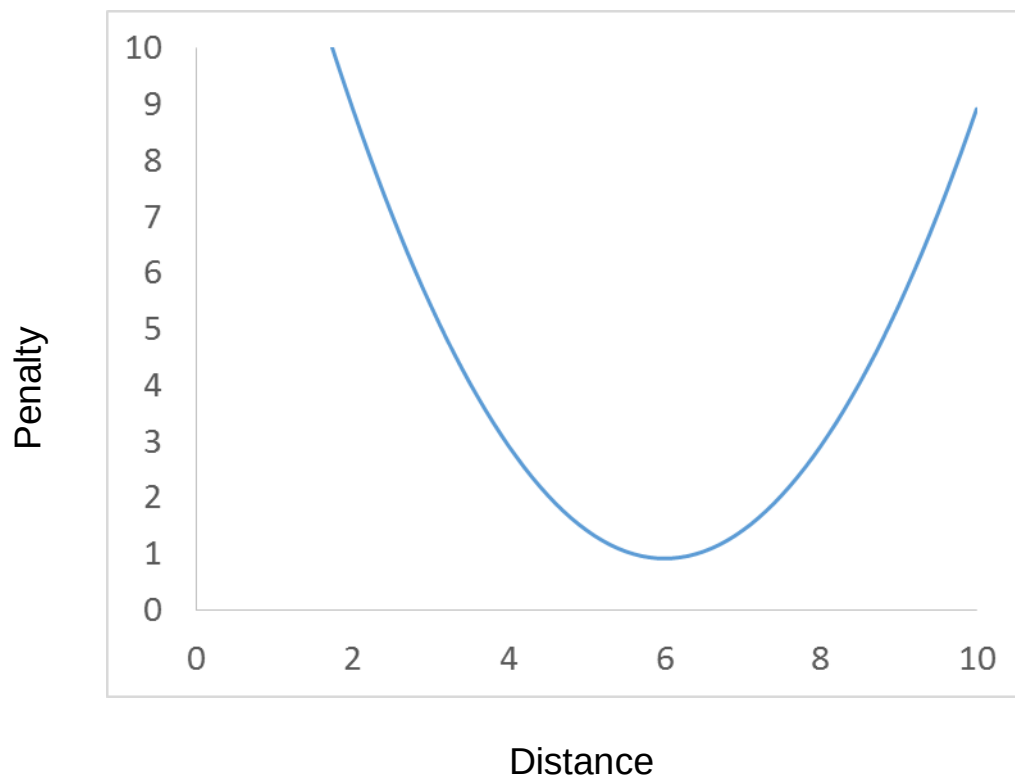
Scoring functions: CircularHarmonic

AtomPair	CD1	52	CD1	54	CIRCULARHARMONIC	6.0	1.0
----------	-----	----	-----	----	------------------	-----	-----



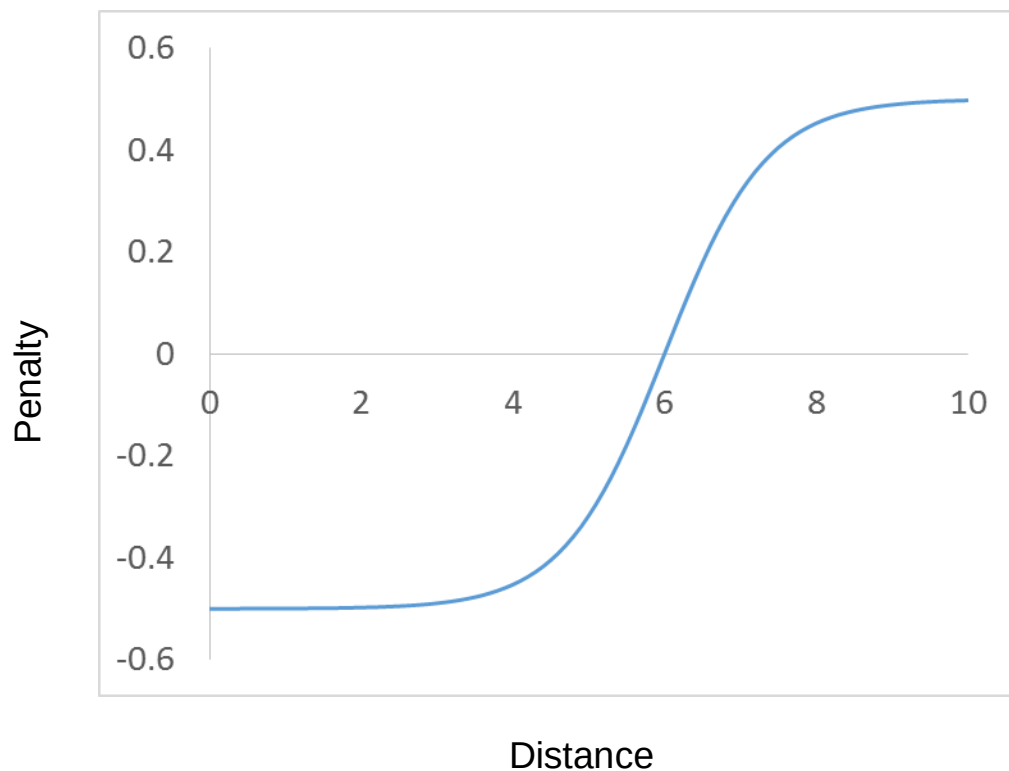
Scoring functions: Gaussian

```
AtomPair CD1 52 CD1 54 GAUSSIANFUNC 6.0 1.0 TAG
```



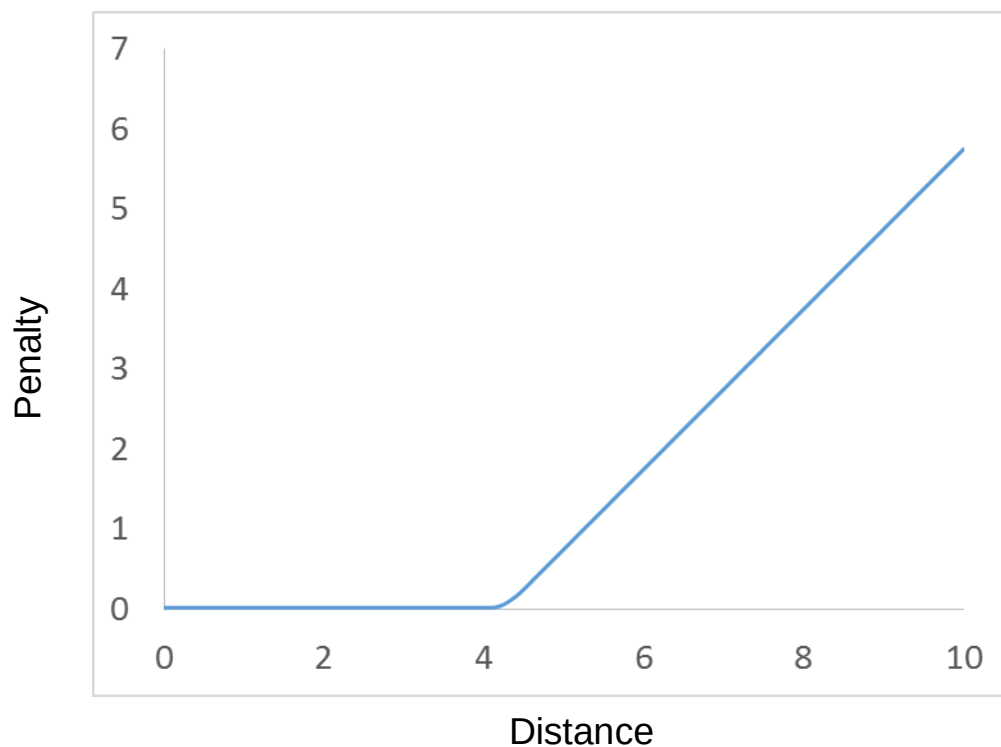
Scoring functions: Sigmoid

AtomPair	CD1	52	CD1	54	SIGMOID	6.0	5
----------	-----	----	-----	----	---------	-----	---



Scoring functions: Bounded

AtomPair	CD1	52	CD1	54	BOUNDED	0.0	4.0	1.0	0.5	TAG
----------	-----	----	-----	----	---------	-----	-----	-----	-----	-----



Applying constraints in Rosetta

- Constraints in the Pose

- Constraint file

```
Constraint_Type1 Constraint_Def1  
Constraint_Type2 Constraint_Def2  
...
```

- Constraints in the Scorefunction

- Weights file

- Constraint flags

```
-constraints:cst_file these_are_my.constraints  
-score:weights ref2015_cst.wts
```



Constraints alter your score which can be seen in the score file

```
<MOVERS>
  <ConstraintSetMover name="coordinate" add_constraints="true" cst_file="./constraint.cst"/>
  <Transform name="transform" chain="X" box_size="7.0" move_distance="0.2" angle="20" cycles="500" repeats="1"
temperature="5"/>
  <HighResDocker name="high_res_docker" cycles="6" repack_every_Nth="3" scorefxn="ligand_soft_rep"
movemap_builder="docking"/>
  <FinalMinimizer name="final" scorefxn="hard_rep" movemap_builder="final"/>
  <InterfaceScoreCalculator name="add_scores" chains="X" scorefxn="hard_rep" />
</MOVERS>
```

SEQUENCE:

SCORE:	total_score	Grid_score	Transform_accept_ratio	angle_constraint	atom_pair_constraint
SCORE:	-1332.090	-50.000	0.390	0.000	3.420
SCORE:	-1334.279	-52.000	0.312	0.000	0.000
SCORE:	-1301.160	-53.000	0.402	0.000	25.672
SCORE:	-1330.401	-53.000	0.406	0.000	0.573
SCORE:	-1345.552	-55.000	0.372	0.000	0.163
SCORE:	-1332.563	-54.000	0.330	0.000	0.673
SCORE:	-1332.901	-52.000	0.382	0.000	2.456
SCORE:	-1319.903	-51.000	0.382	0.000	5.502
SCORE:	-1334.278	-50.000	0.400	0.000	1.666
SCORE:	-1329.303	-52.000	0.362	0.000	0.409
SCORE:	-1322.152	-51.000	0.506	0.000	0.788
SCORE:	-1327.543	-55.000	0.456	0.000	3.339

if_X_angle_constraint	if_X_atom_pair_constraint	if_X_chainbreak	if_X_coordinate_constraint
0.000	-987698.178	0.000	0.000
0.000	-988266.962	0.000	0.000
0.000	-989998.391	0.000	0.000
0.000	-987789.008	0.000	0.000
0.000	-983002.402	0.000	0.000
0.000	-981014.417	0.000	0.000
0.000	-983893.538	0.000	0.000
0.000	-983545.562	0.000	0.000
0.000	-989936.265	0.000	0.000
0.000	-985873.833	0.000	0.000
0.000	-991691.778	0.000	0.000



And how to get rid of constraint scoring...

Constraints are added to the score table as additional terms that influence the per-residue and total pose score

```
<ClearConstraintsMover name="(&string)" />
```

```
<MOVERS>
  <ConstraintSetMover name="coordinate" add_constraints="true" cst_file="./constraint.cst"/>
  <Transform name="transform" chain="X" box_size="7.0" move_distance="0.2" angle="20" cycles="500" repeats="1"
temperature="5"/>
  <HighResDocker name="high_res_docker" cycles="6" repack_every_Nth="3" scorefxn="ligand_soft_rep"
movemap_builder="docking"/>
  <FinalMinimizer name="final" scorefxn="hard_rep" movemap_builder="final"/>
  <InterfaceScoreCalculator name="add_scores" chains="X" scorefxn="hard_rep" />
</MOVERS>
```

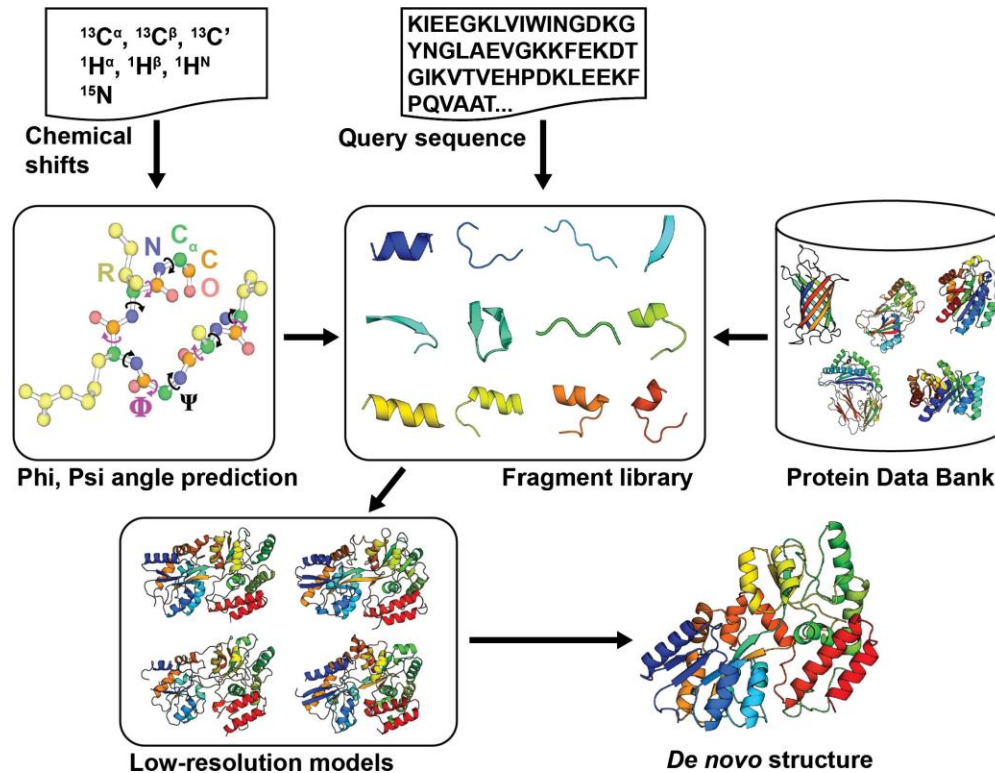


Some recent examples of using experimental guided structure prediction

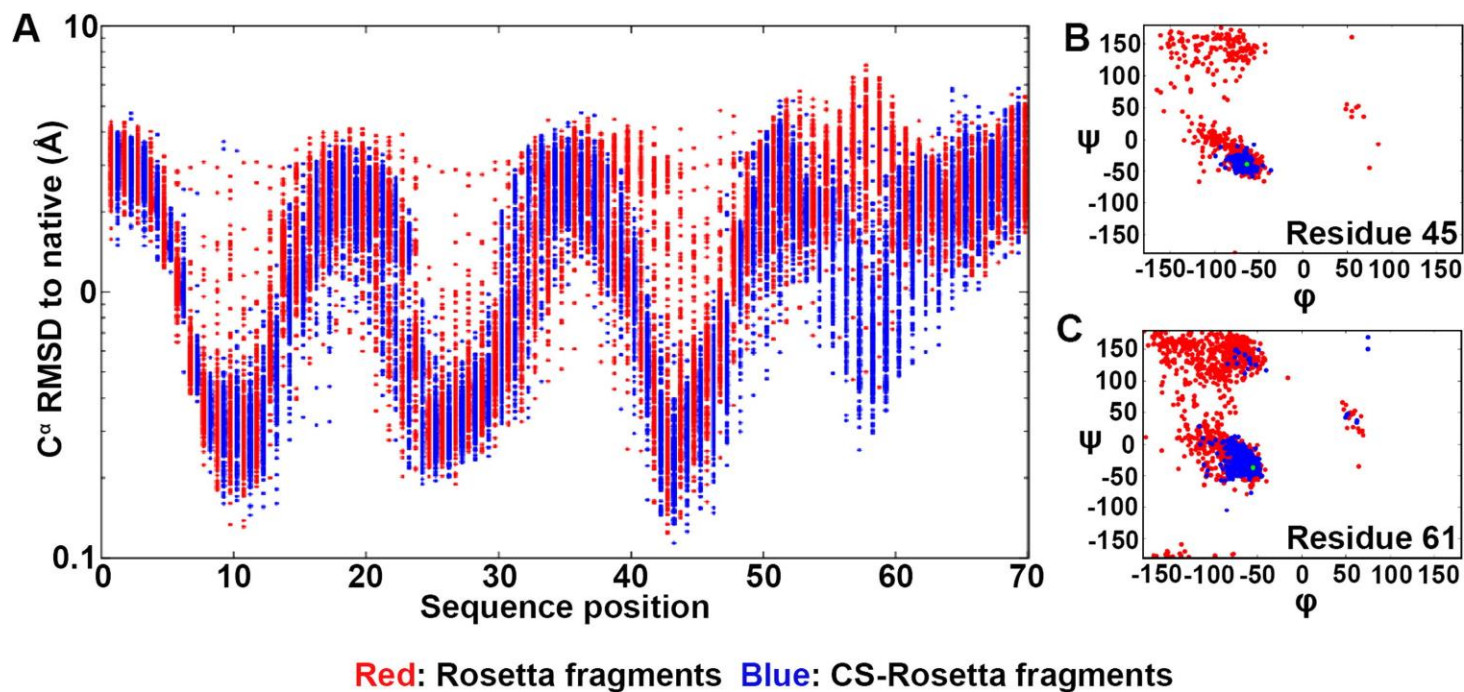
- Using NMR restraints
- Iterate NMR and mutagenesis to model ghrelin receptor interactions
- Using HDX-MS data to map antibody on antigens
- Modeling into cryo-EM densities
- Outlook into integrative structural biology



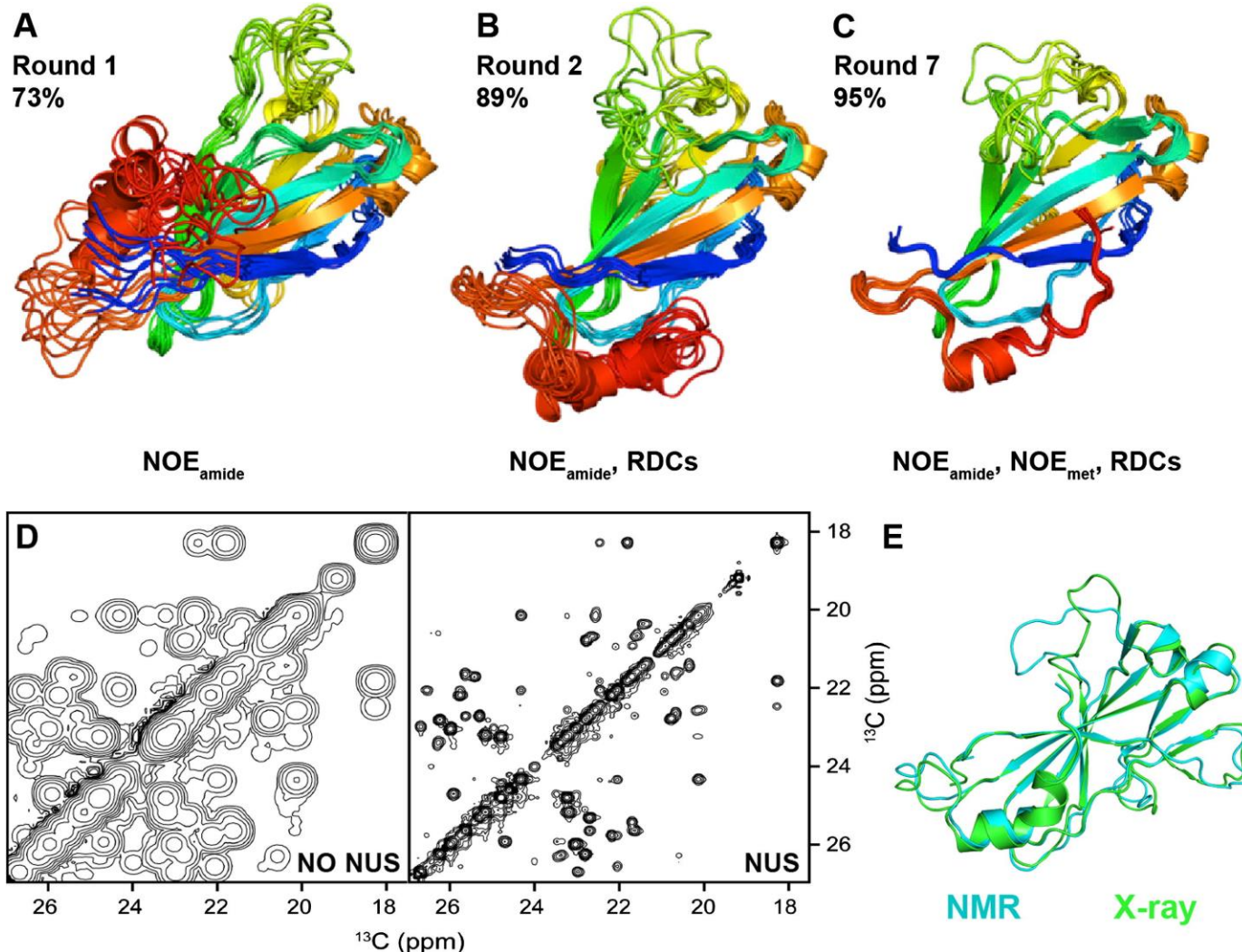
CS-Rosetta for ab-initio structure prediction guided from chemical shift data



Chemical-shift data help pick better fragments

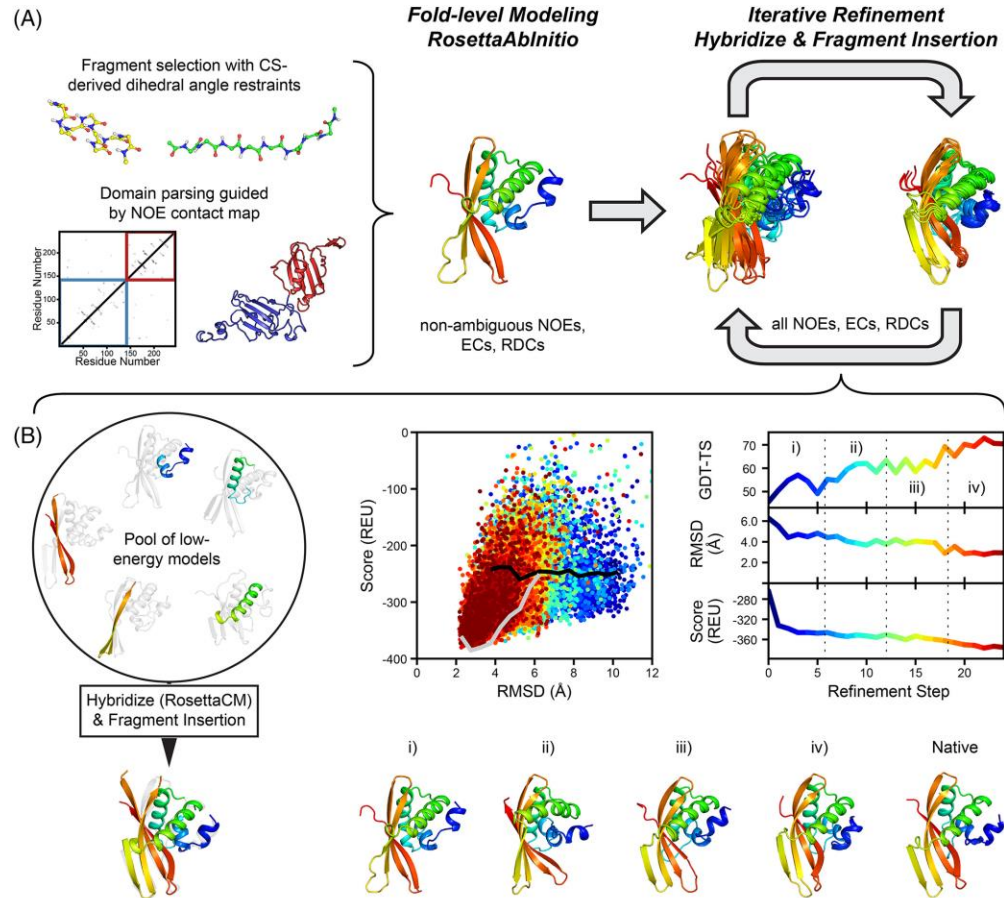


Improvement of structure prediction through incooperation of NMR restraints



Nerli, S. et al. *Prog. Nuc. Mag. Res. Spect.* **2018**

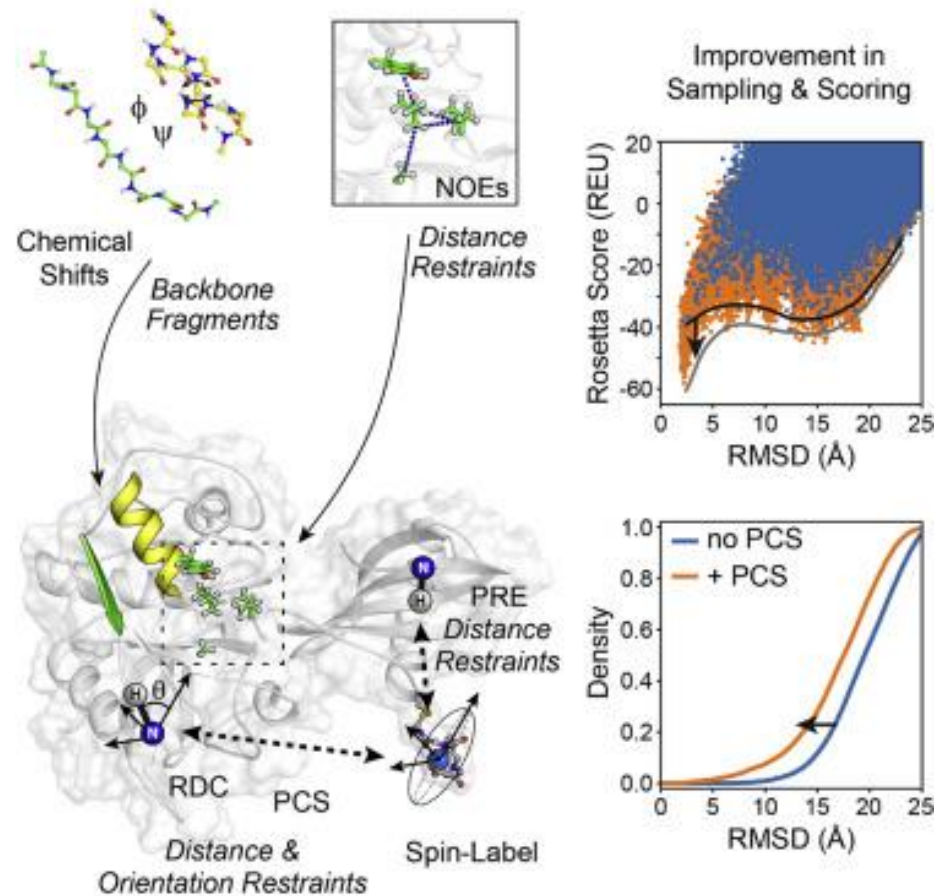
Using



Kuenze, G. *et al. Proteins*, 2019



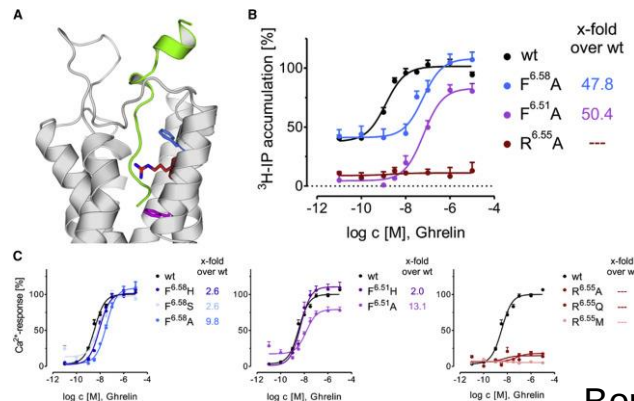
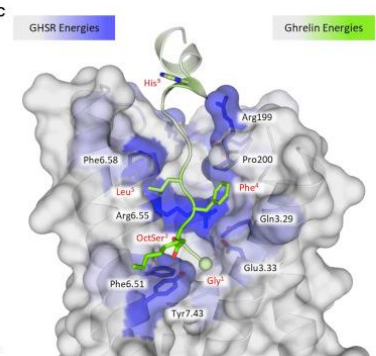
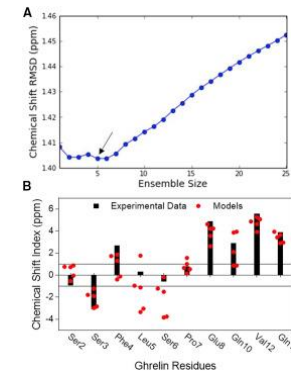
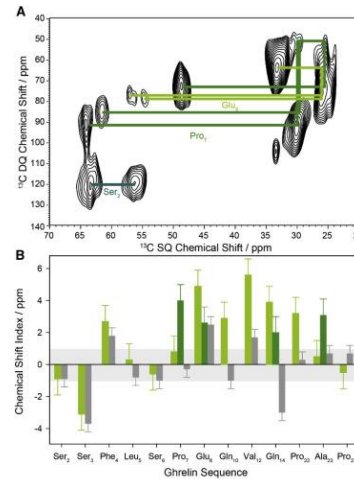
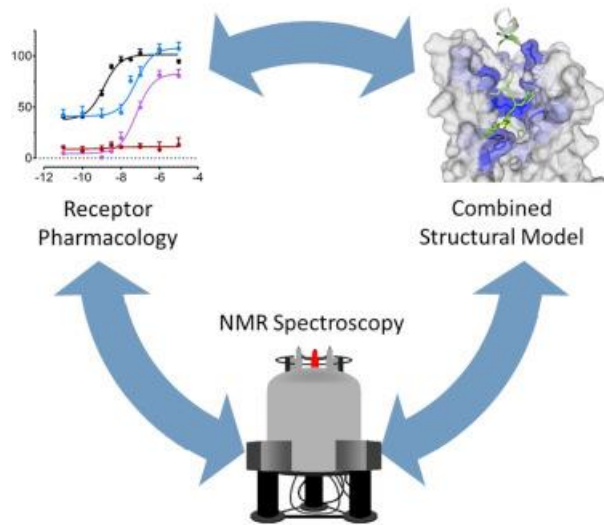
Using paramagnetic tagging for structure prediction



Kuenze, G. *et al. Structure*, 2019



Using NMR chemical shift data to map low affinity contacts for a peptide-GPCR

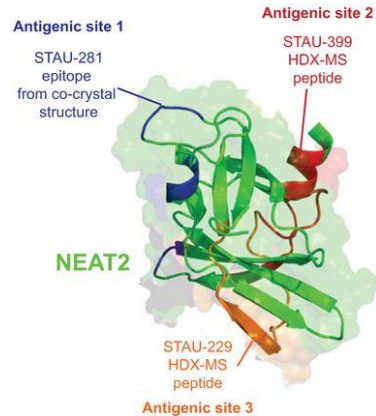


Bender, B. et al. *Structure*, 2019



Applying constraint-guided docking for epitope-mapping with HDX-MS data

a.



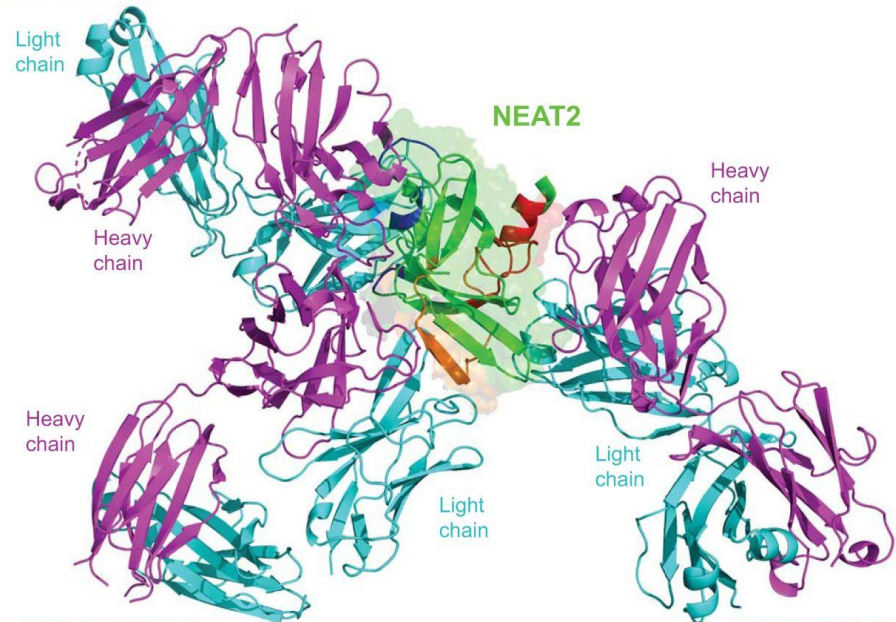
b.

IsdB amino acid sequence, with epitopes indicated

```
241 HFNNKEEKYDYTLMEFAQPIYNSADKFKTEEDYKAEKLLAPYKKAKTLERQVYELNKIQD300
301 KLPEKLKAEYKKKLEDTKKALDEQVKSSTAU-399AITEFQNVQPTNEKMTDLQSTAU-229DTKYVVYESVENN359
360 ESMMDSTAU-281TFVKHPIKTGMLNGKKYMMVETTNDDYWKDFMSTAU-399VEGQSTAU-281RVRTISKDAKNNTRTIIF418
419 PYVEGKTLYDAIVKVHVKTIDYSTAU-281DGQYHVRIVDKAEFTKANTDKSNKKEQQDNSAKKEATP478
```

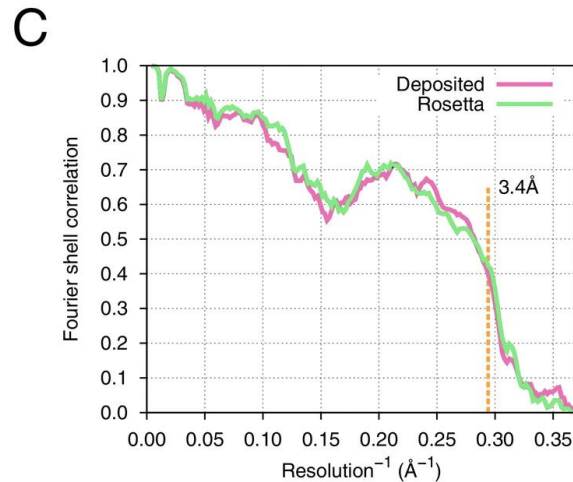
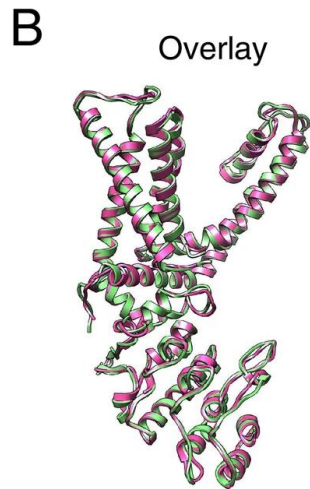
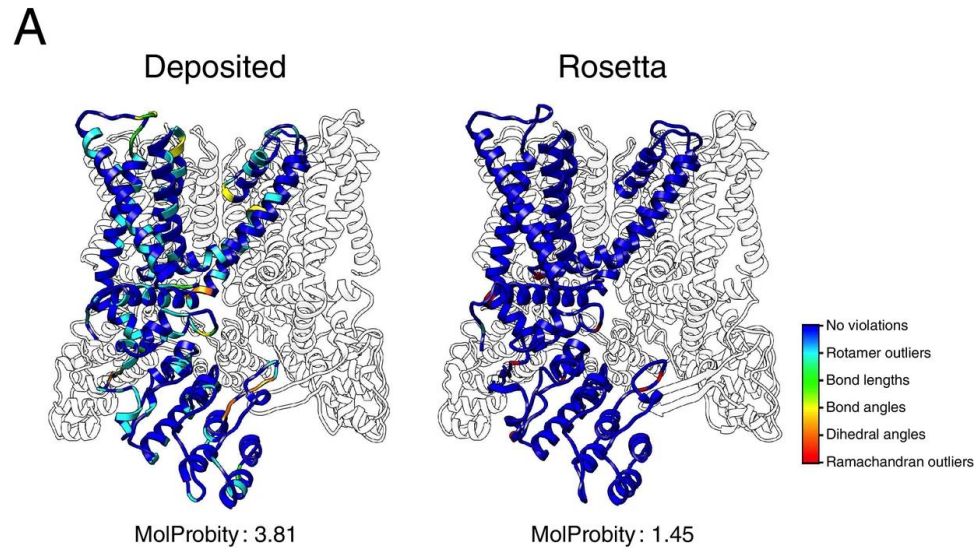
Constraint-guided docking

STAU-281 Fab



Bennett, M. *et al. mBio.* 2019

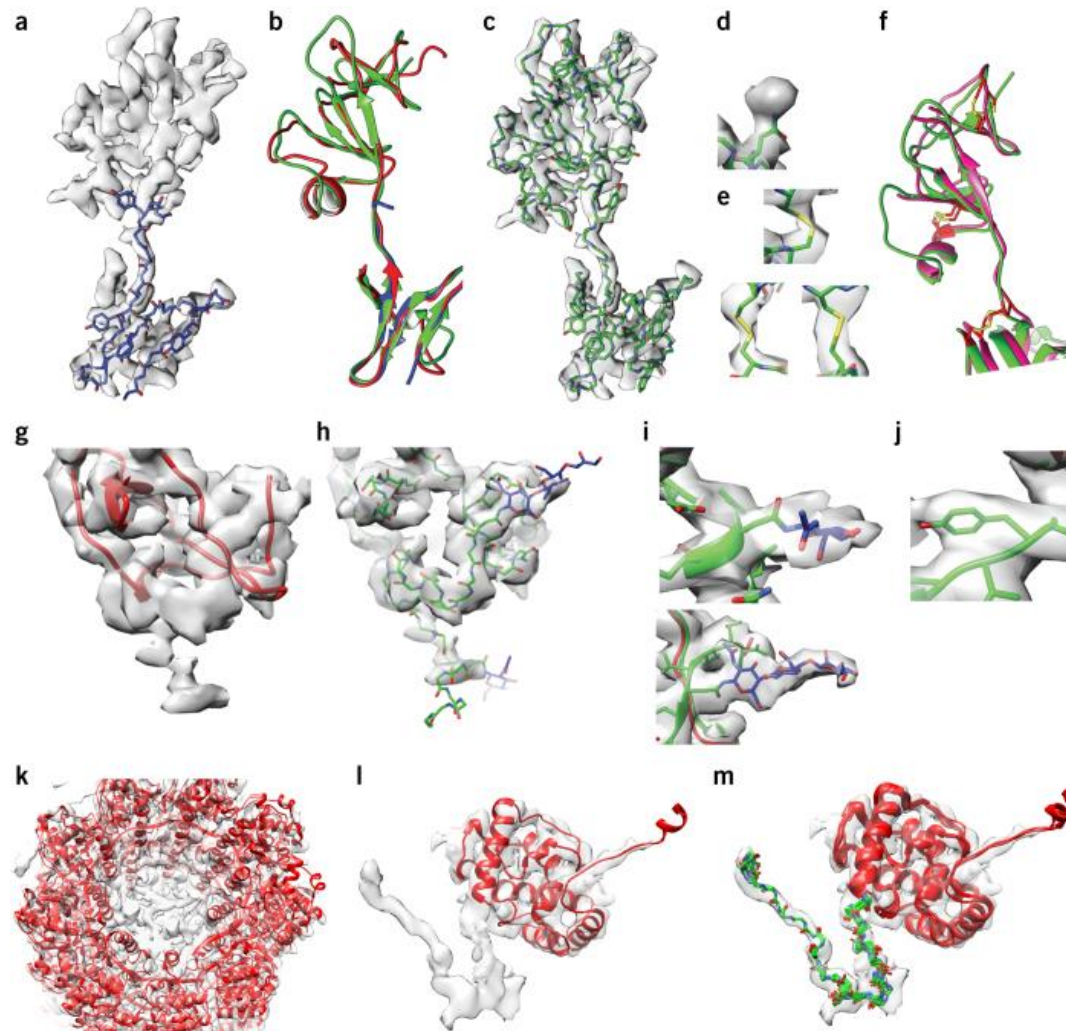
Rosetta refinement helps to improve cryo-EM density models



Wang, R. *et al. Elife.* 2016



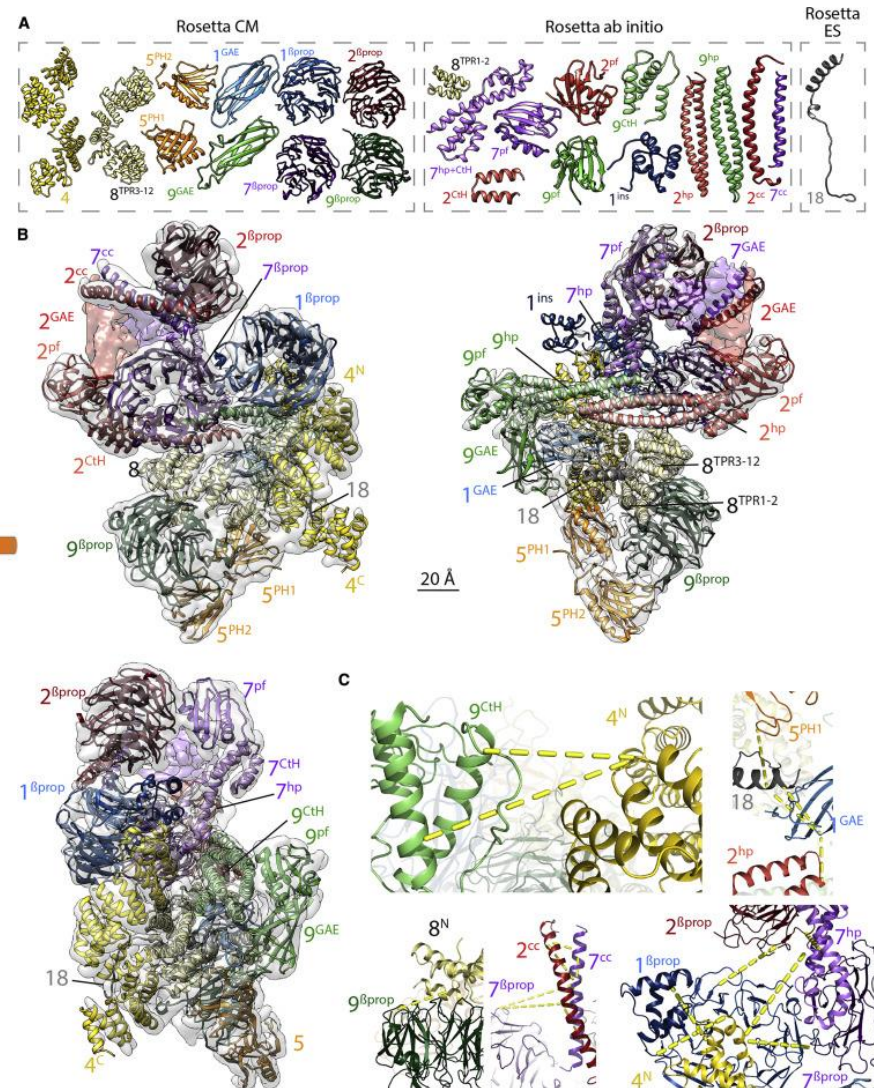
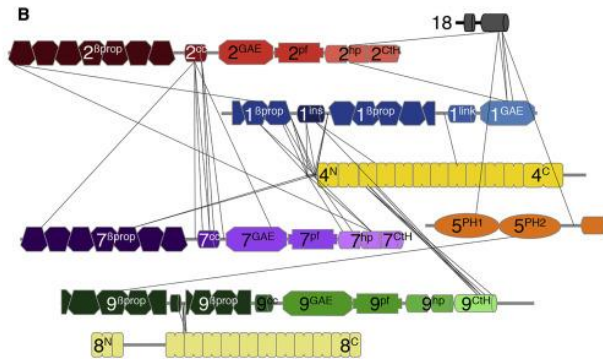
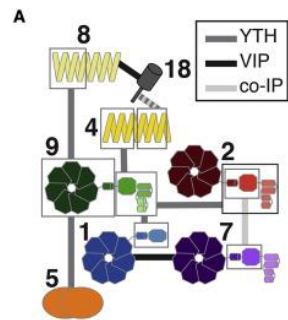
Modeling into low-density cryo-EM maps: RosettaES as automated de novo modeling



Frenz, B. *et al. Nat. Met.* 2017



Rosetta modeling and density fitting can be used to determine large complex structures



Tutorial set from the DiMaio lab:

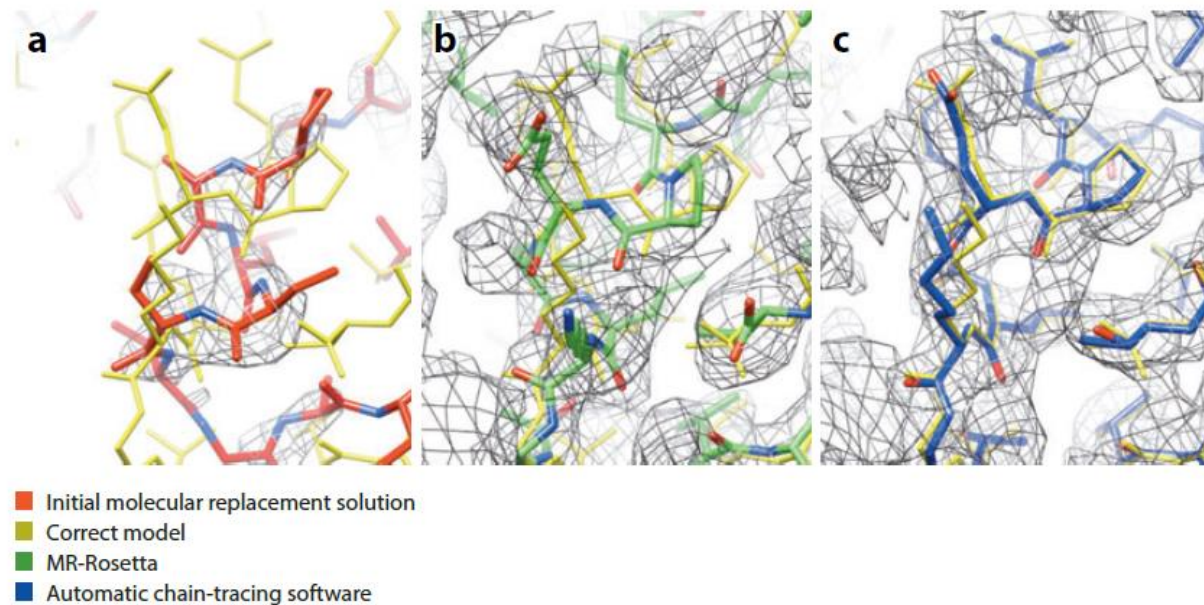
<https://dimaiolab.ipd.uw.edu/software/>

Chou, H. *et al. Structure* 2020

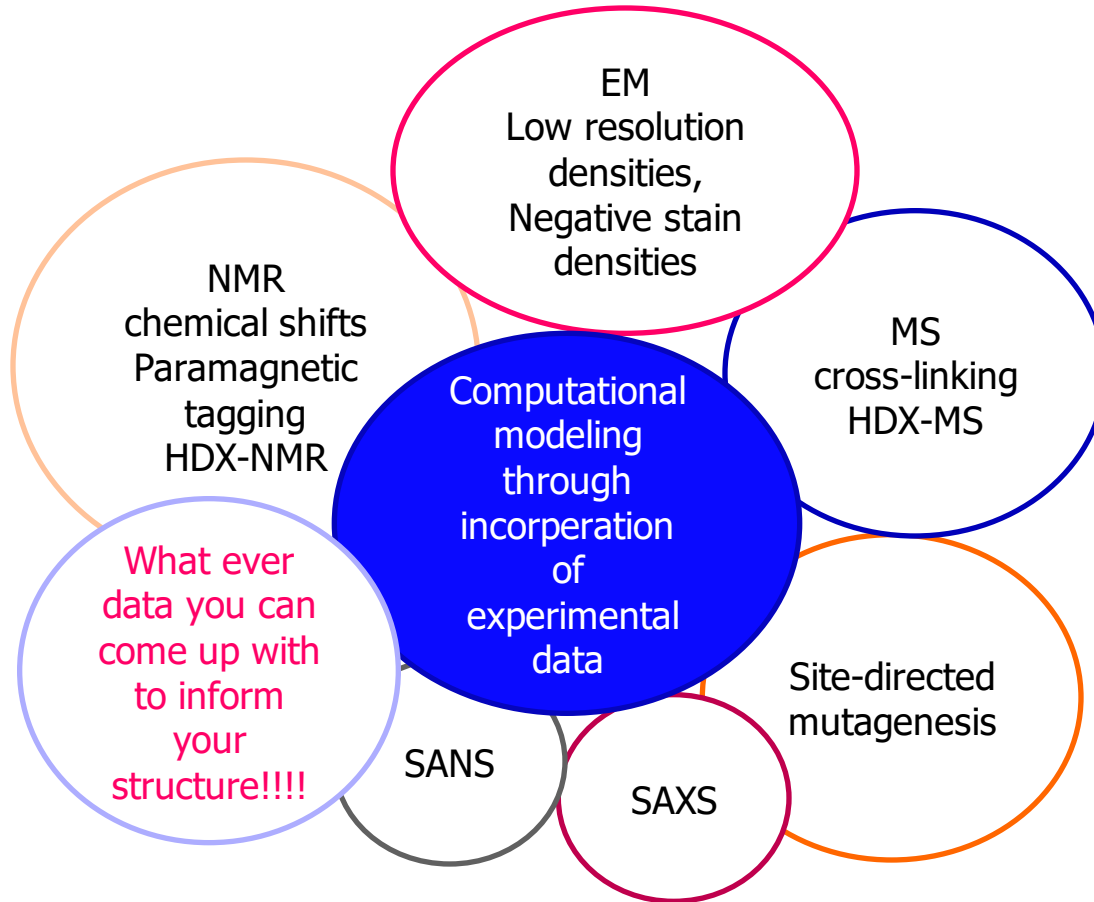


Phenix_rosetta as tool to refine low resolution densities

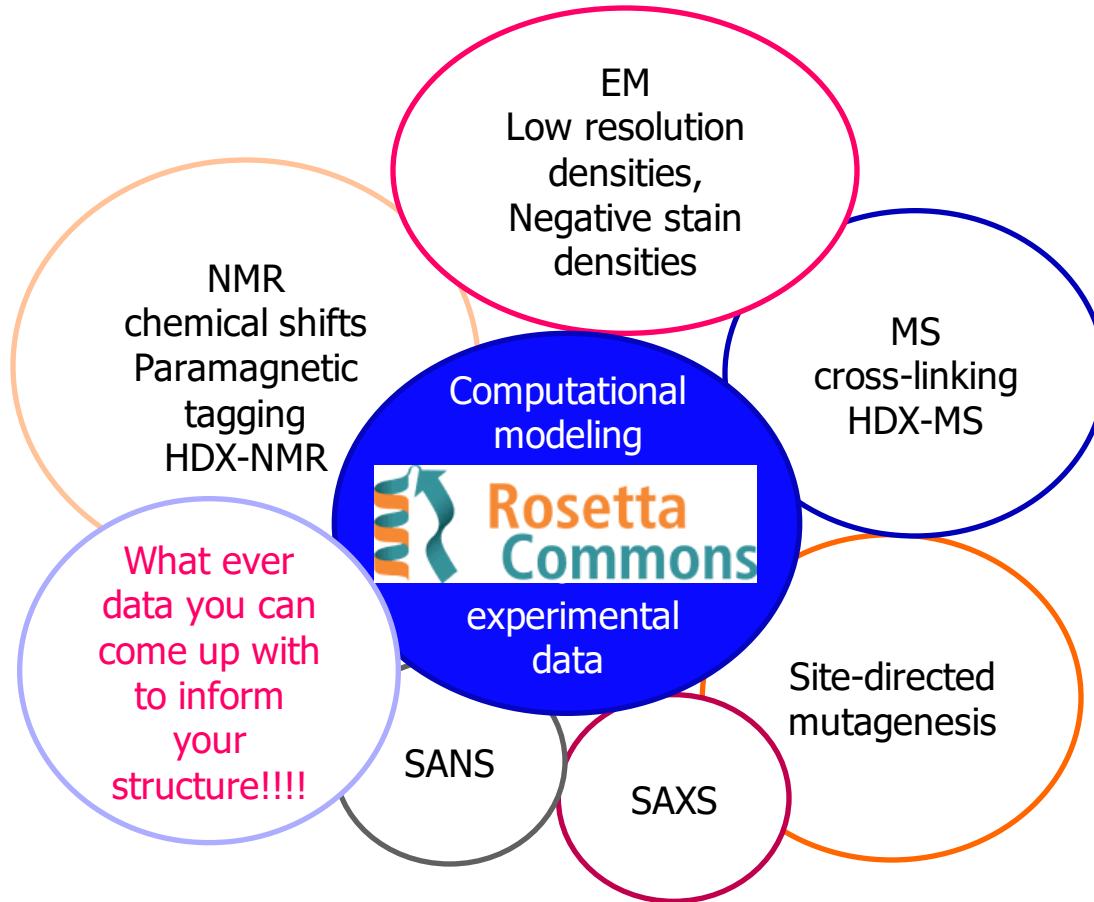
- Phenix, a common software package for model building in densities supports a Rosetta refinement option in Molecular replacement



An outlook into integrative structural biology



An outlook into integrative structural biology



In this tutorial

- We want to find out how we can apply constraints to restrict the sampling space in docking.
- We will gradually explore how docking changes when you start having constraints from experimental data
- As use case we take an antibody fragment from STAU-281 (Bennett, M. *et al.* **2019** *mBio*), which we will dock to its target, the NEAT2 domain of IsdB.

