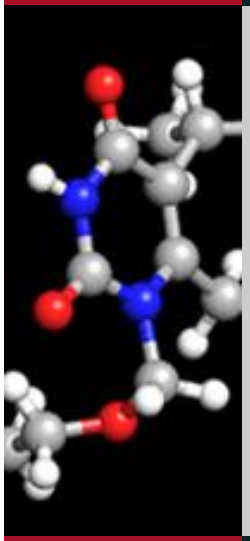

Ligand placement in PrimeX protein crystal structure refinement

Jean-Christophe Mozziconacci
Application Scientist
jcmozzic@schrodinger.com

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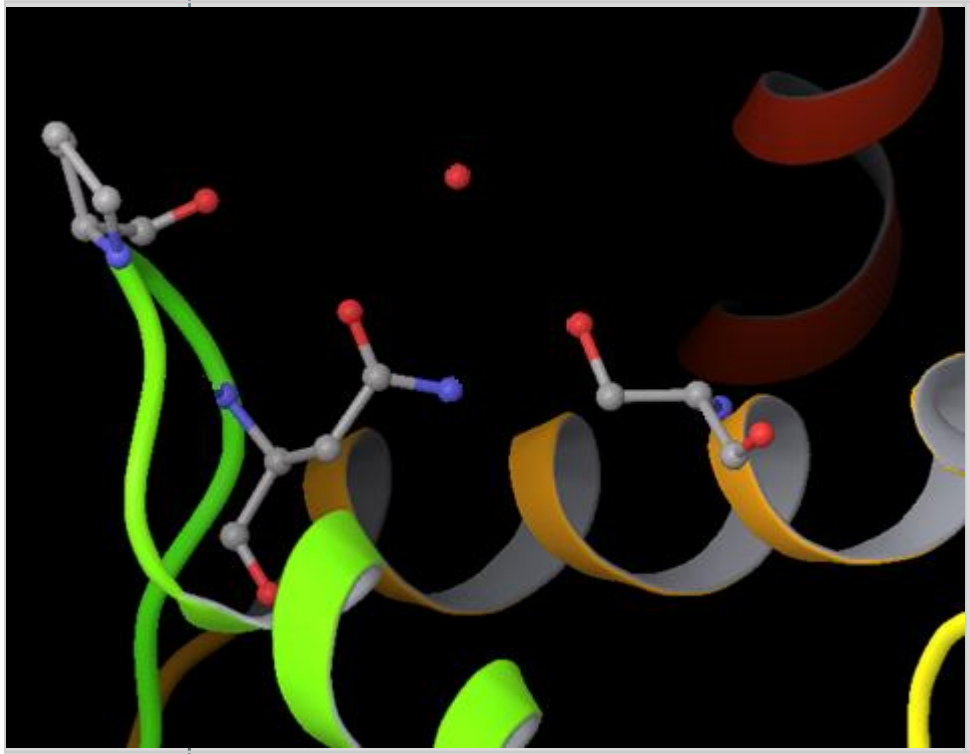
PrimeX – Protein Crystal Structure Refinement

- Ligand placement based on Glide
- Ligand placement, refinement and docking are related: refinement details matter.
- Complete graphics and refinement package with innovative ligand placement and loop building tools
- Reciprocal-space refinement includes simulated annealing
- OPLS-AA used for geometric restraints in refinement, producing structures ready for use in docking
- Refines molecular replacement solutions to completion

Errors in X-ray structures are common…

Deposited in PDB
(hydrogens added)

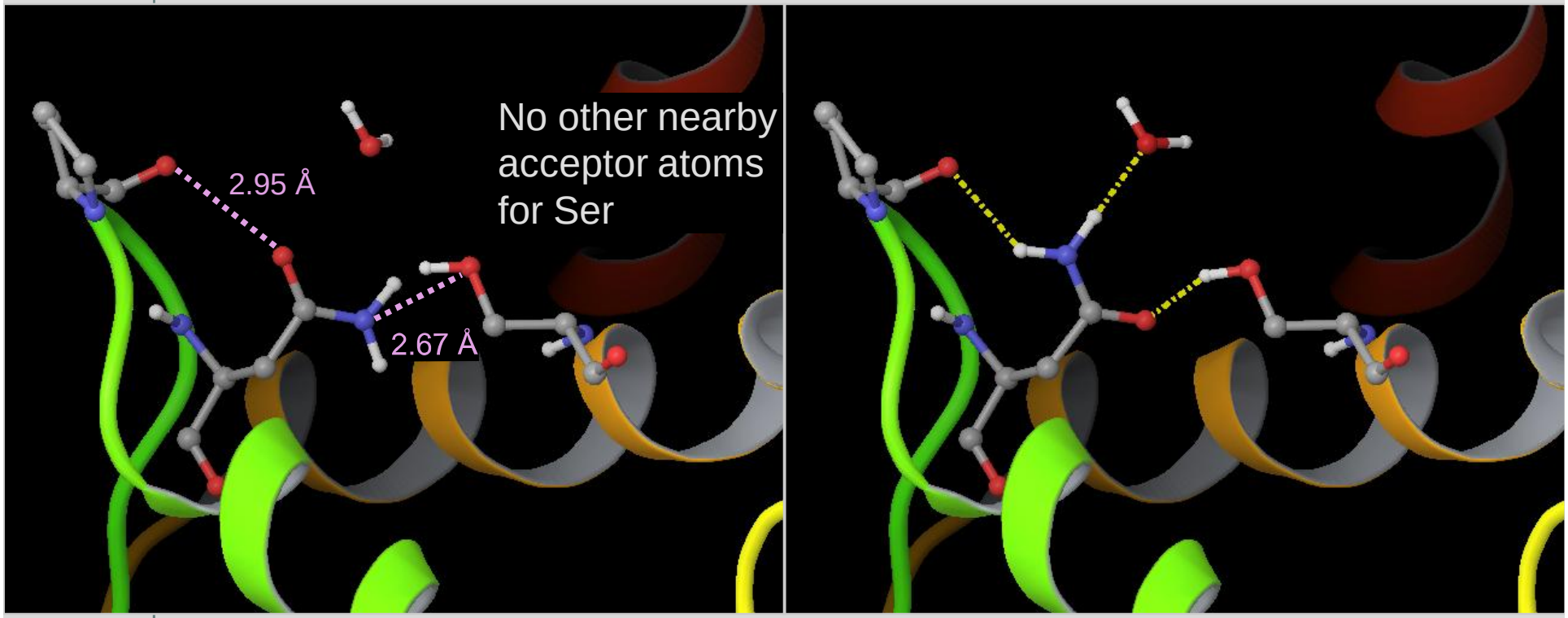


1XOZ (Resolution = 1.37 Å)

Some details in protein refinement are easily overlooked

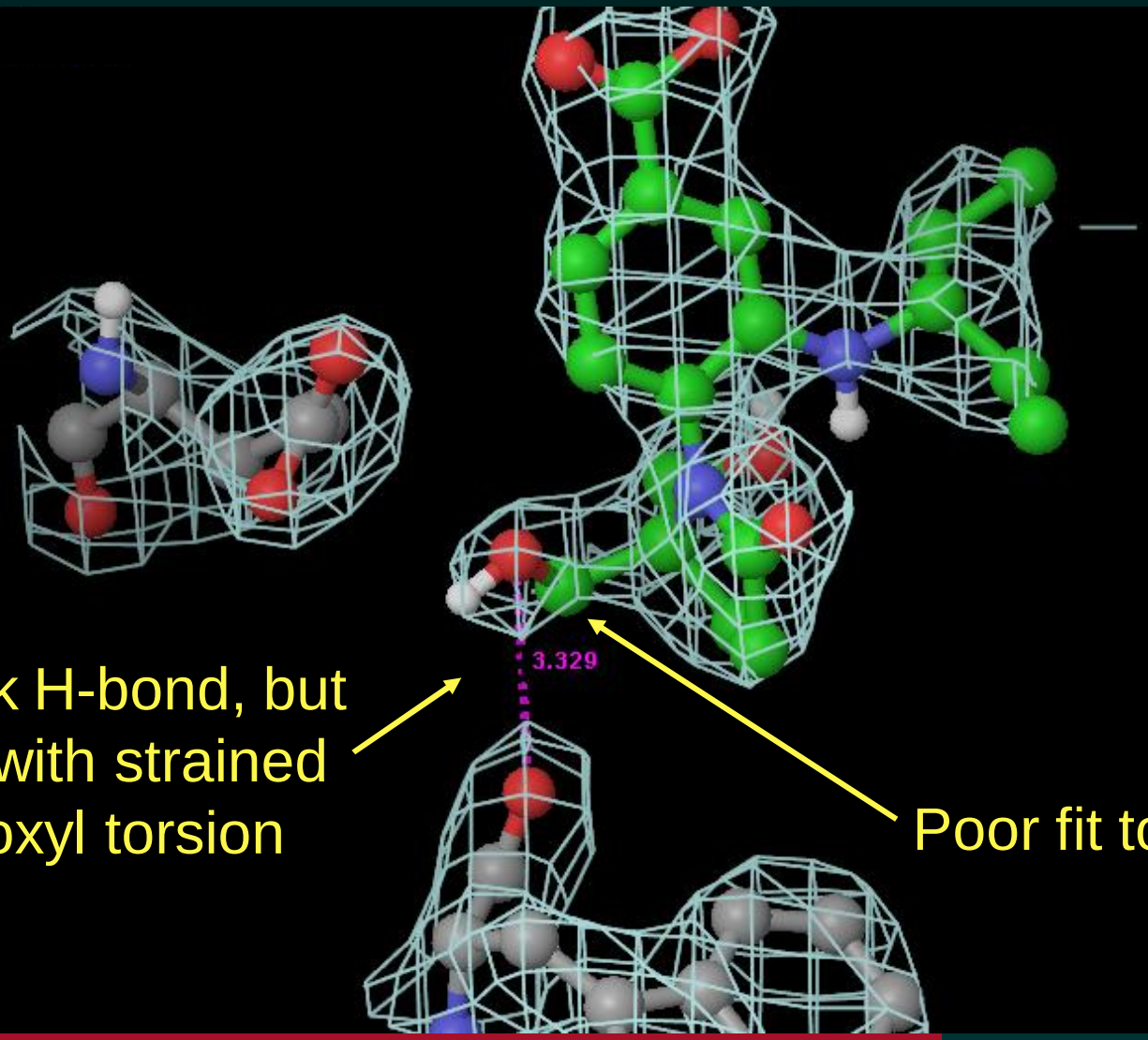
Deposited in PDB (hydrogens added)

Refined by PrimeX

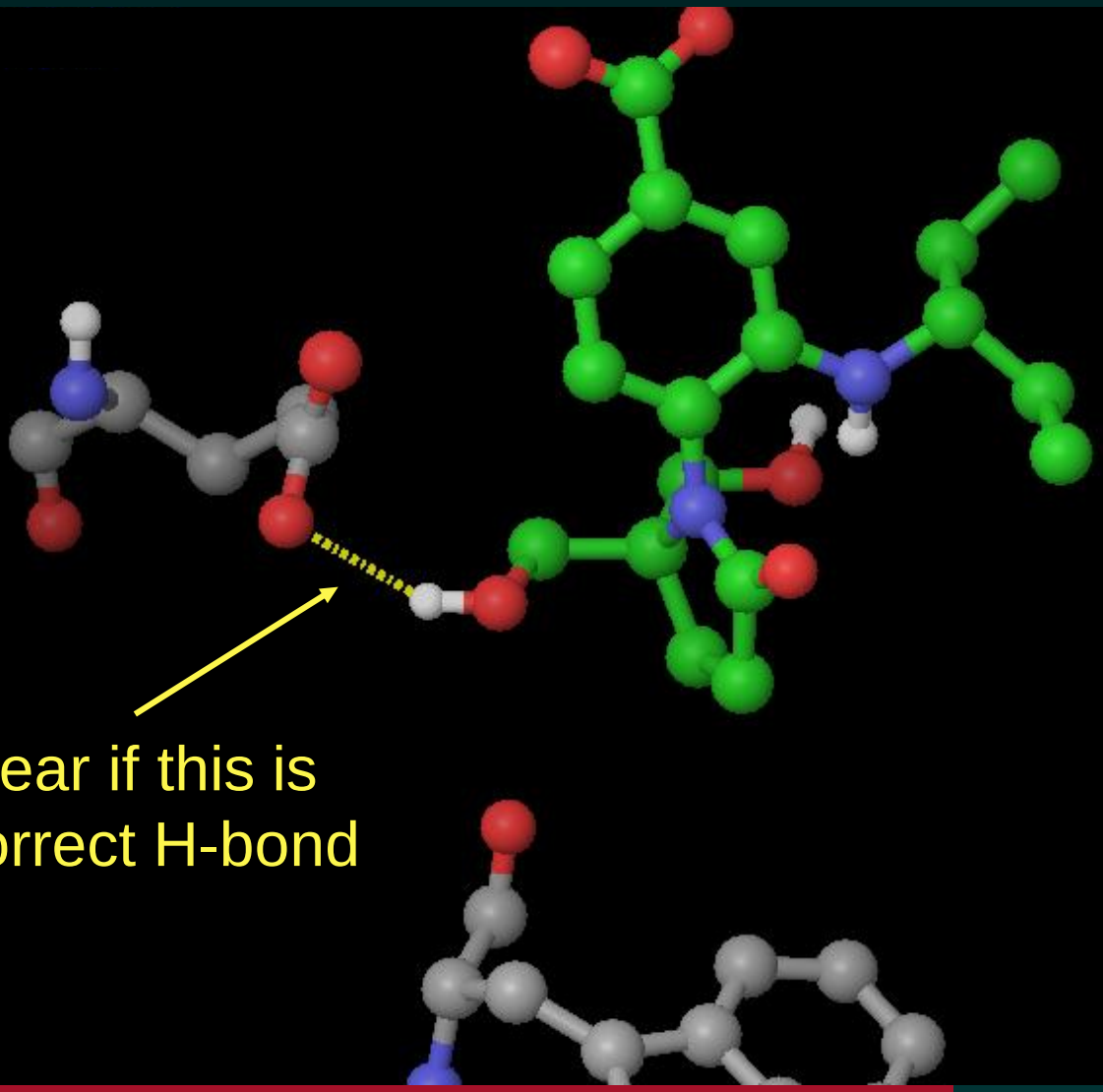


1XOZ (Resolution = 1.37 Å)

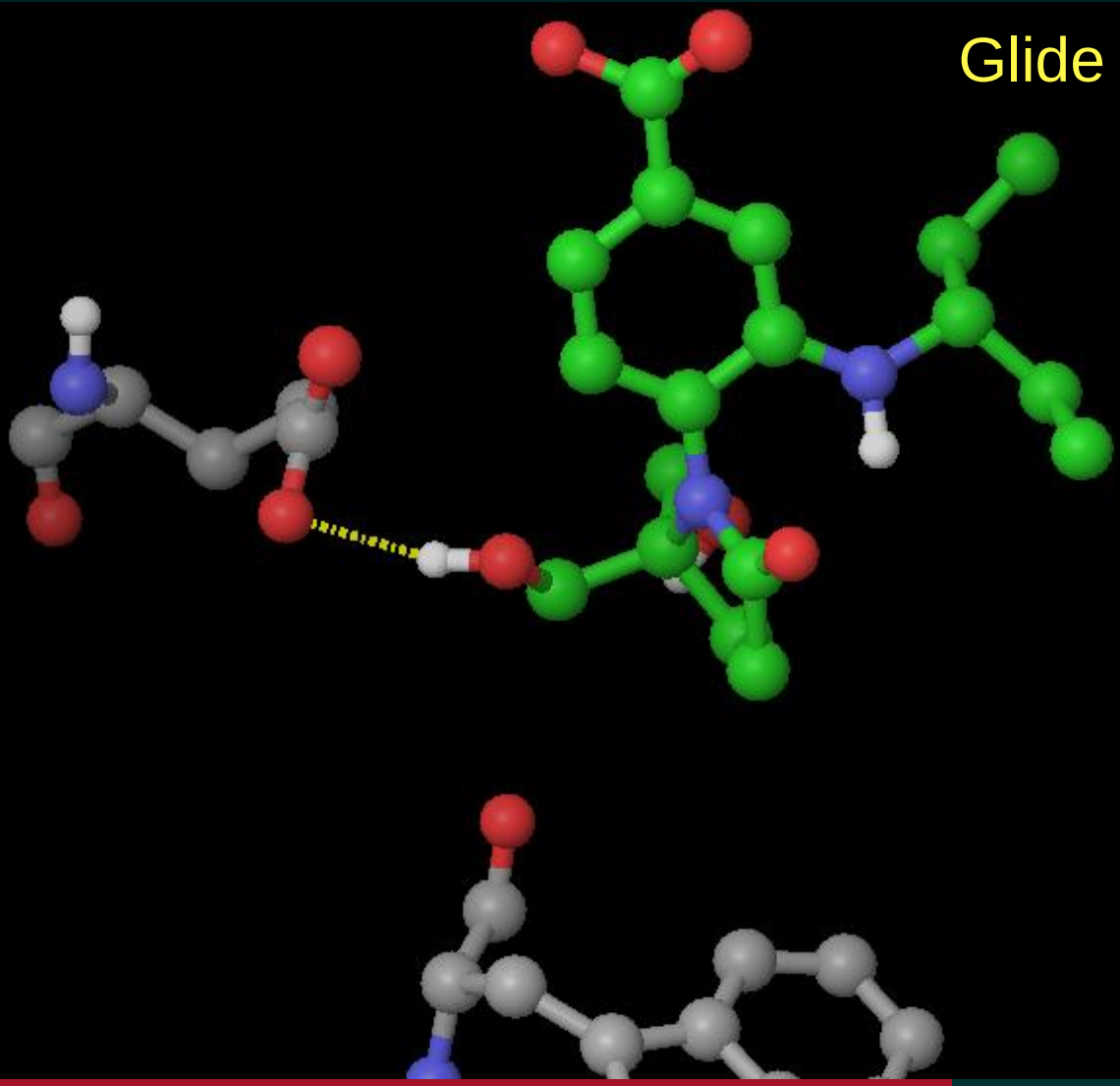
PDB structure of 1b9v (neuraminidase)



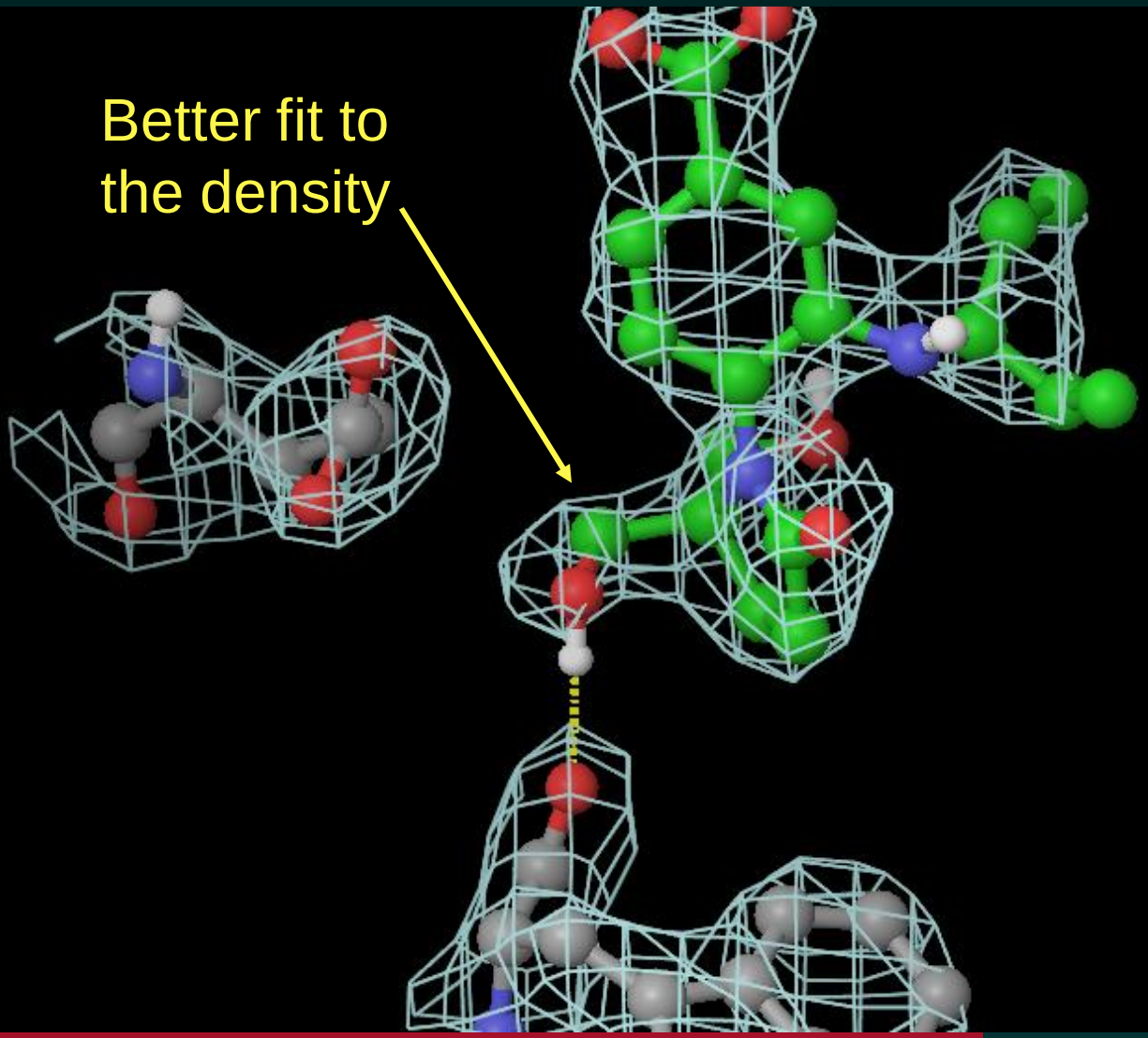
Structure prepared for docking (restrained minimization)



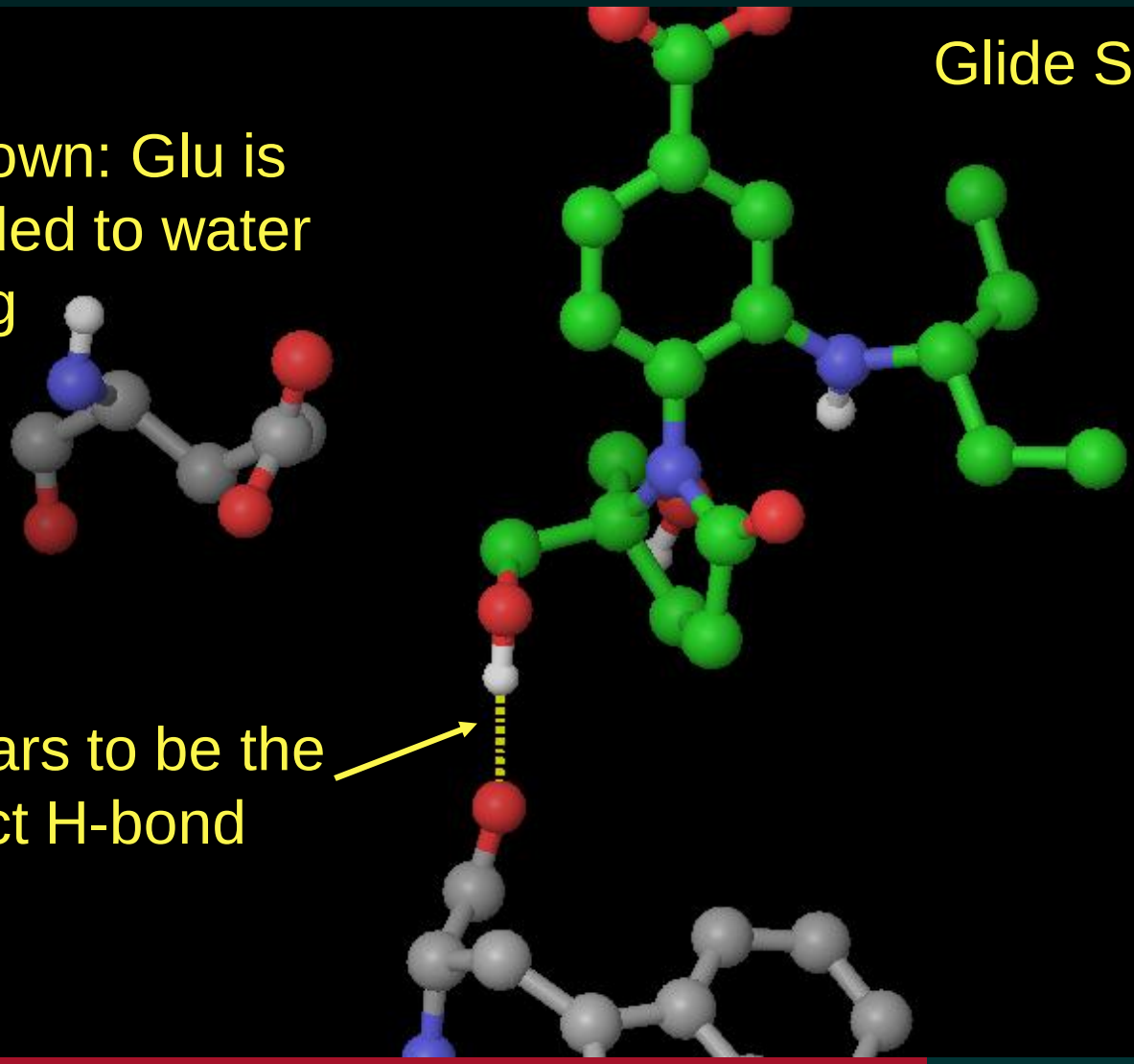
Dock with Glide XP



Place ligand into density with PrimeX followed by reciprocal space minimization (with solvation)



Dock with Glide XP



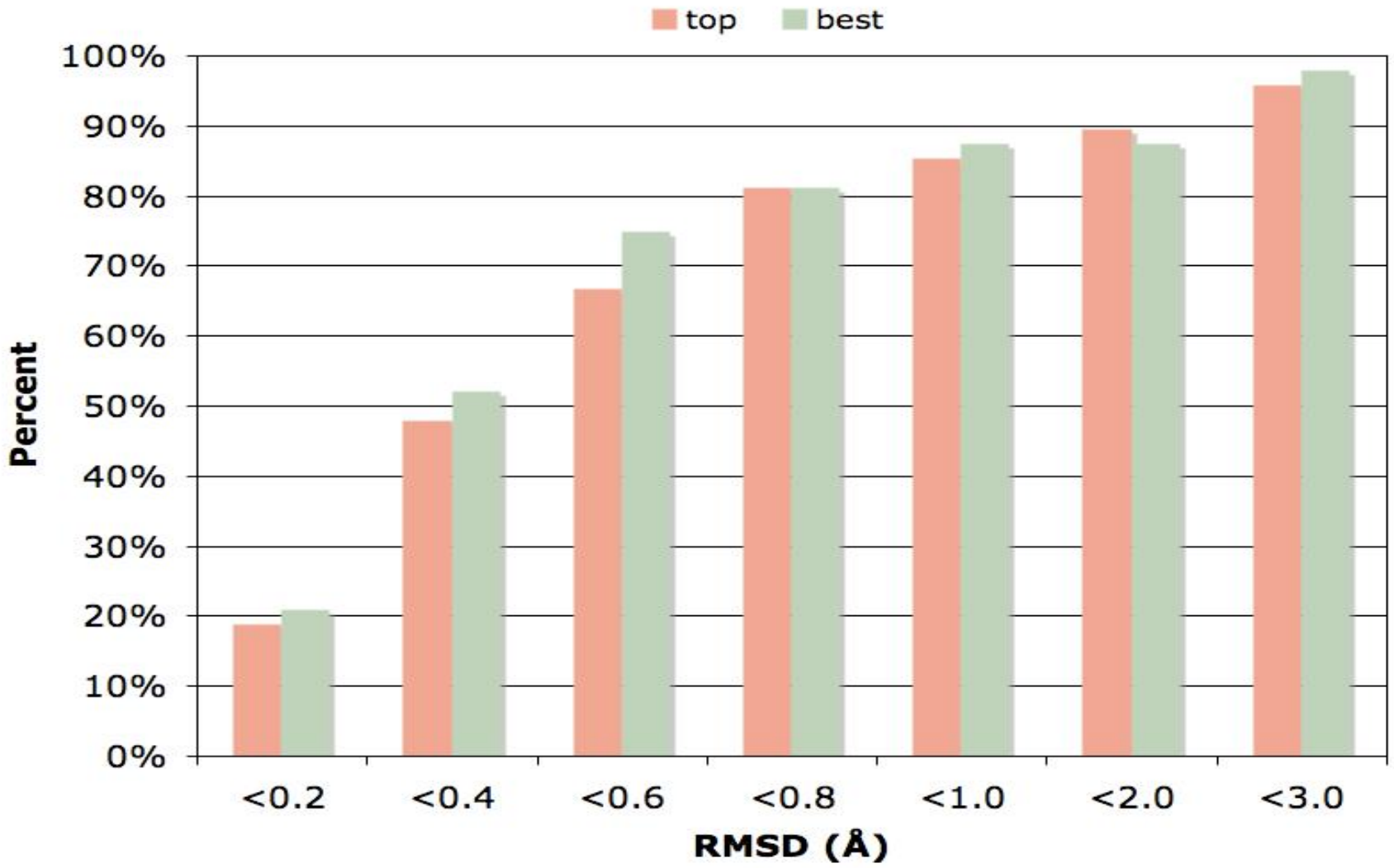
Ligand placement

- Ligand placement is based on the docking program Glide, and considers both chemical interactions and the electron density fit to find the correct ligand pose.
- This approach is especially useful in situations with electron density to cover only part of the ligand, ambiguously-shaped electron density, quasi-symmetric ligands, etc.
- Ligand can be automatically treated with Epik to choose the most likely protonation states and tautomers.
- Several ligands may each be evaluated at a single site.
- Optional post-placement real-space refinement is available.

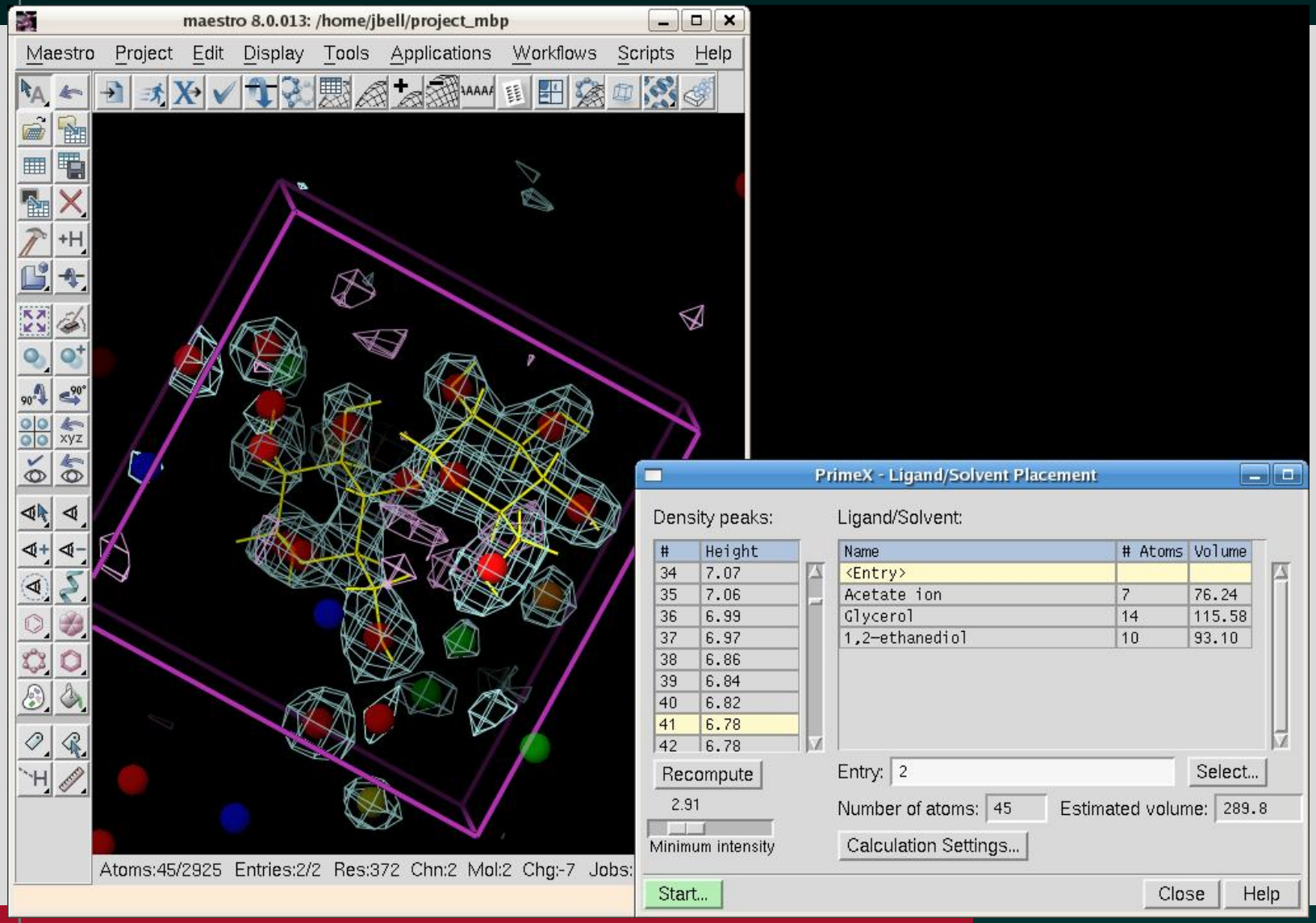
Validation

- Test cases designed to represent typical ligand placement situations
- 48 protein-ligand complexes selected (average resolution 2.14 Å, range 1.2 to 3.0 Å)
- No selection for quality of electron density
- Waters and other small molecules deleted
- Prepared with simulated annealing refinement followed by coordinate reciprocal-space minimization

Accuracy of ligand placement



PrimeX – Ligand placement



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Acknowledgment

- Schrödinger:
- Jeff Bell, Ramy Farid and developers
- QA
- Tech Support
- Applications Scientists

- Customers:
- Beta testers
- Requesting enhancements
- General feedbacks

Summary of PrimeX Features

- PrimeX performs refinement of protein crystal structures from initial model to finished coordinates
- Distinguishing features of PrimeX:
  - building of up to 40-residue loops, based on Prime protein structure modeling and guided by electron density fit
  - flexible placing of ligands and other small molecules into electron density using Glide docking technology
  - automatic parameter generation for ligands, other small molecules and modified residues
  - simulated annealing refinement based on the Impact molecular dynamics engine
  - conjugate gradient, truncated Newton and quasi-Newton (LBFGS) minimizers
  - restraints based on the advanced, well-validated OPLS-AA force field
  - execution of all operations through a logical user interface integrated into Maestro
  - step-by-step organization of refinement statistics in the Maestro Project Table
  - access to operations through command-line input as well as scripting with Python
  - easy analysis of protein structure geometry through interactive tables
  - optimized addition of hydrogens that is transparent to the user

PrimeX – Protein Crystal Structure Refinement

- Maestro
  - Integration
  - Graphical functions for interactive model building and refinement
  - Refinement statistics organized in Project Table
- Real-space density fitting
  - Loops and missing residues building for polypeptides of up to 40 residues
  - Side chains placement into density with cooperative optimization
  - Ligand and solvent molecules placement (atom typing and parameter generation)
  - Water peaks identified
- Reciprocal-space refinement
  - Least-squares and maximum likelihood targets
  - Rigid body refinement with multiple groups
  - Coordinate, B-factor and grouped occupancy refinement
  - Simulated annealing refinement
  - NCS restraints and geometric restraints based OPLS-AA force field

PrimeX – Protein Crystal Structure Refinement

- All-atom force field in refinement
  - Force field more accurately reflects molecular geometry
  - Hydrogens are intelligently added before refinement
  - Hydrogens can be optimized to better reflect chemical interactions
  - Final structures are already “prepared” for use in computational chemistry
- Full suite of structure analysis tools
  - Interactive Ramachandran plot
  - Geometric statistics in interactive tables
  - Density fit table for real-space R-factors
- Python scripting
- Alternate conformations and missing atoms supported
- data exchange with other programs
  - PDB and mae files
  - Reads cv and mtz phased reflection files
  - Imports and exports CNX/CNS and CCP4 maps

Loop building and Simulated annealing

- Loop building is based on the protein modeling program Prime. Loops up to 40 residues in length can be built. Tails of up to 20 residues in length can be built.
- Simulated annealing was developed in Prof. Ron Levy's lab and is based on the modeling program Impact.
- Evaluation: CNS simulated annealing refinement was performed on a set of protein structures derived from NMR results or from high temperature molecular dynamics. The best result from torsion and Cartesian dynamics is shown. PrimeX simulated annealing refinement was run on the same set of proteins using default parameters.

Simulated Annealing Refinement Test Results

PDB ID	Resolution	starting model		final model					
				PrimeX with solvation		PrimeX without solvation		CNS*	
		BBrms	Rfree	BBrms	Rfree	BBrms	Rfree	BBrms	Rfree
1g35	1.8	1.76	0.56	0.35	0.31	0.57	0.36	0.68	0.34
1a3s	2.8	1.62	0.53	0.62	0.29	0.91	0.30	1.12	0.30
1a3s	2.8	2.32	0.52	0.74	0.29	1.11	0.34	2.77	0.38
1jug	1.9	0.83	0.52	0.62	0.36	0.70	0.37	0.95	0.40
1c9w	2.4	1.87	0.58	0.55	0.39	0.58	0.38	0.30	0.33
1mpd	2.3	0.31	0.37	0.19	0.32	0.22	0.32	0.17	0.31
1myt	1.74	1.27	0.54	0.44	0.37	0.60	0.39	0.68	0.37
2h17	1.7	2.70	0.54	2.58	0.45	2.60	0.45	2.37	0.43
AVERAGE		1.59	0.52	0.76	0.35	0.91	0.36	1.13	0.36

* CNS Studies conduced at Rutgers in Ron Levy's lab

Simulated Annealing Refinement Test Results

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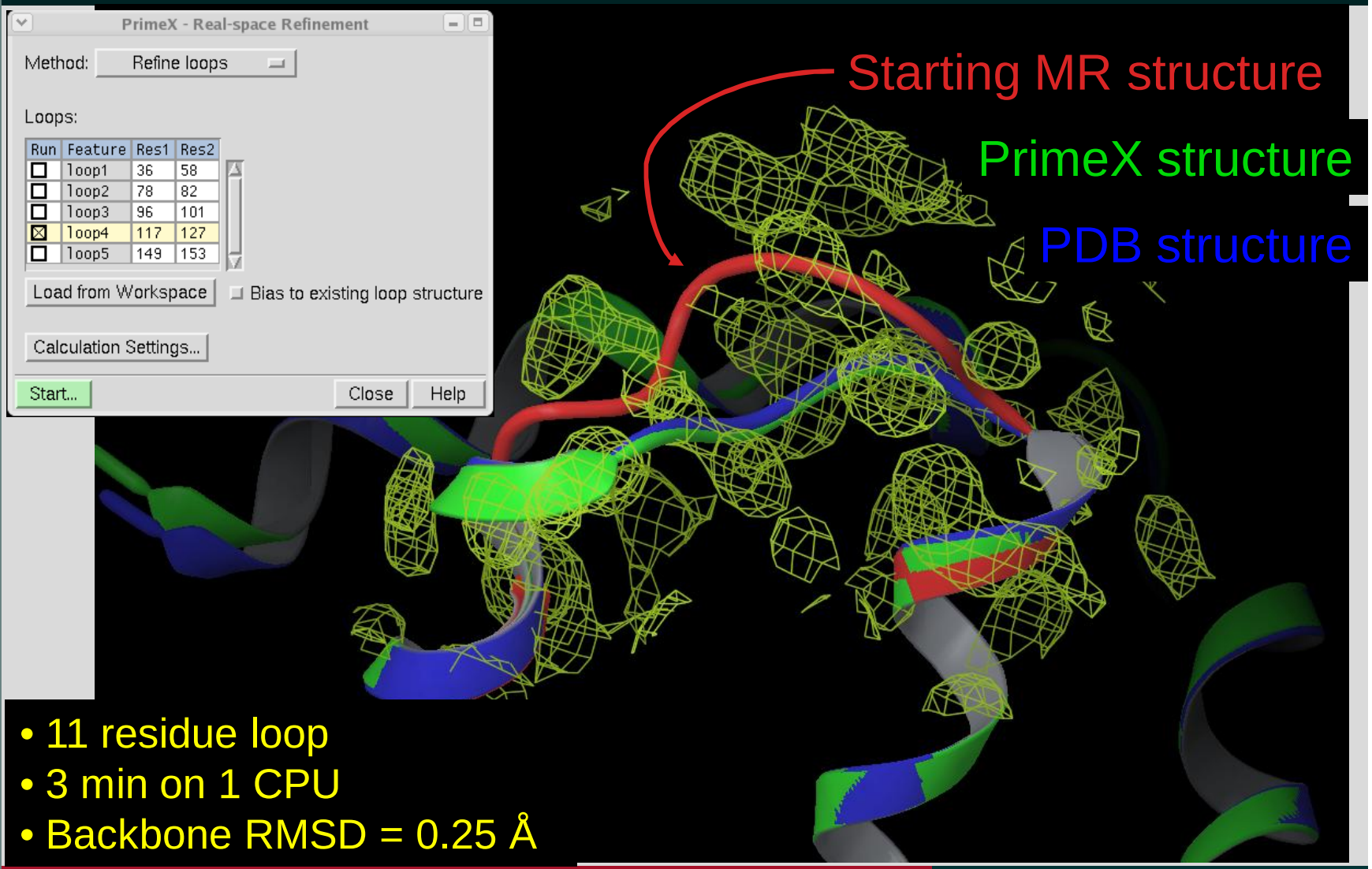
* CNS Studies conduced at Rutgers in Ron Levy's lab

PrimeX – Automated Loop Placement



- 11 residue loop
- 3 min on 1 CPU
- Backbone RMSD = 0.25 Å

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PrimeX - Loop Placement and Simulating Annealing

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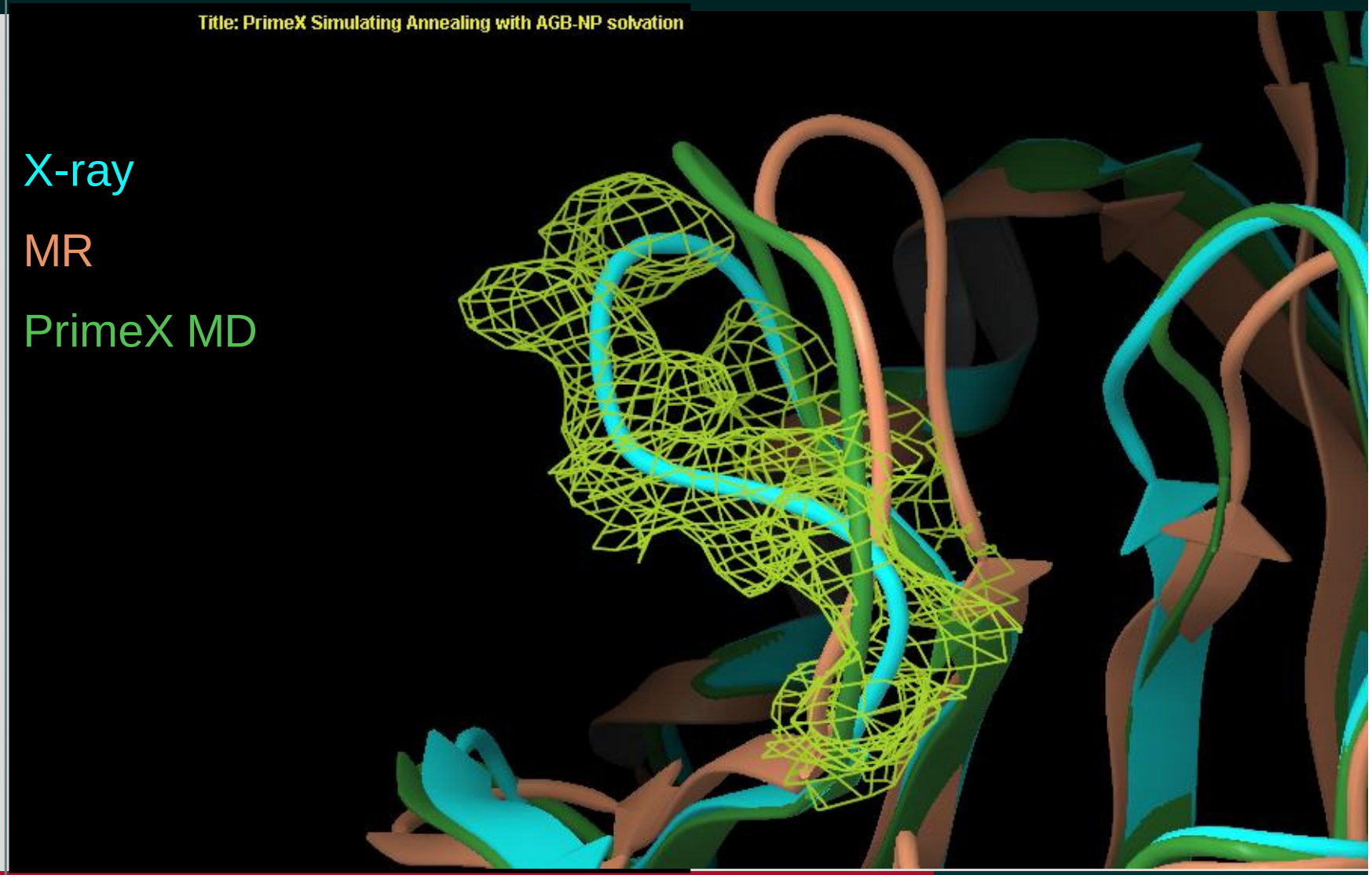
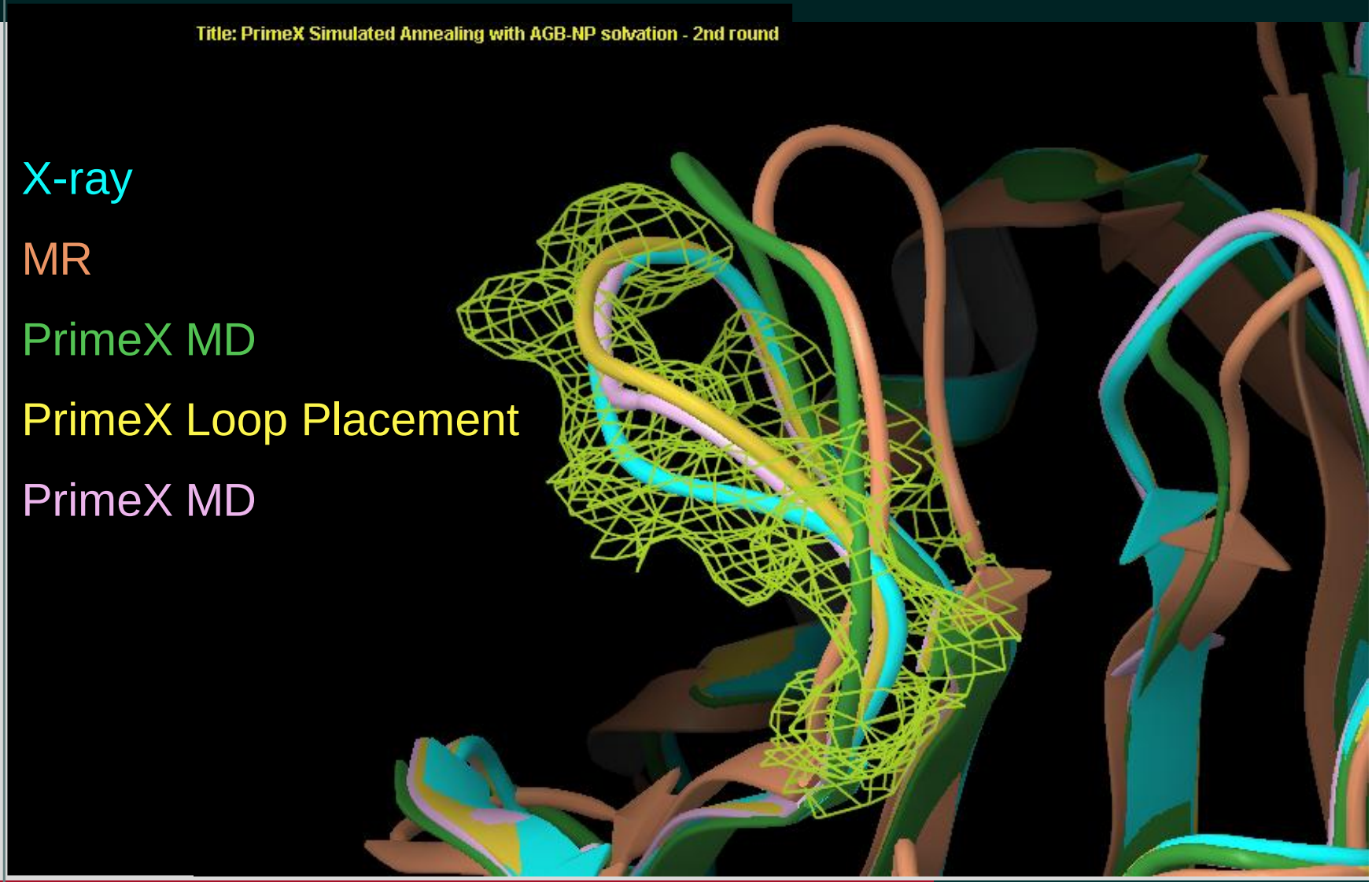
PrimeX - Loop Placement and Simulating Annealing



PrimeX - Loop Placement and Simulating Annealing

PrimeX - Loop Placement and Simulating Annealing

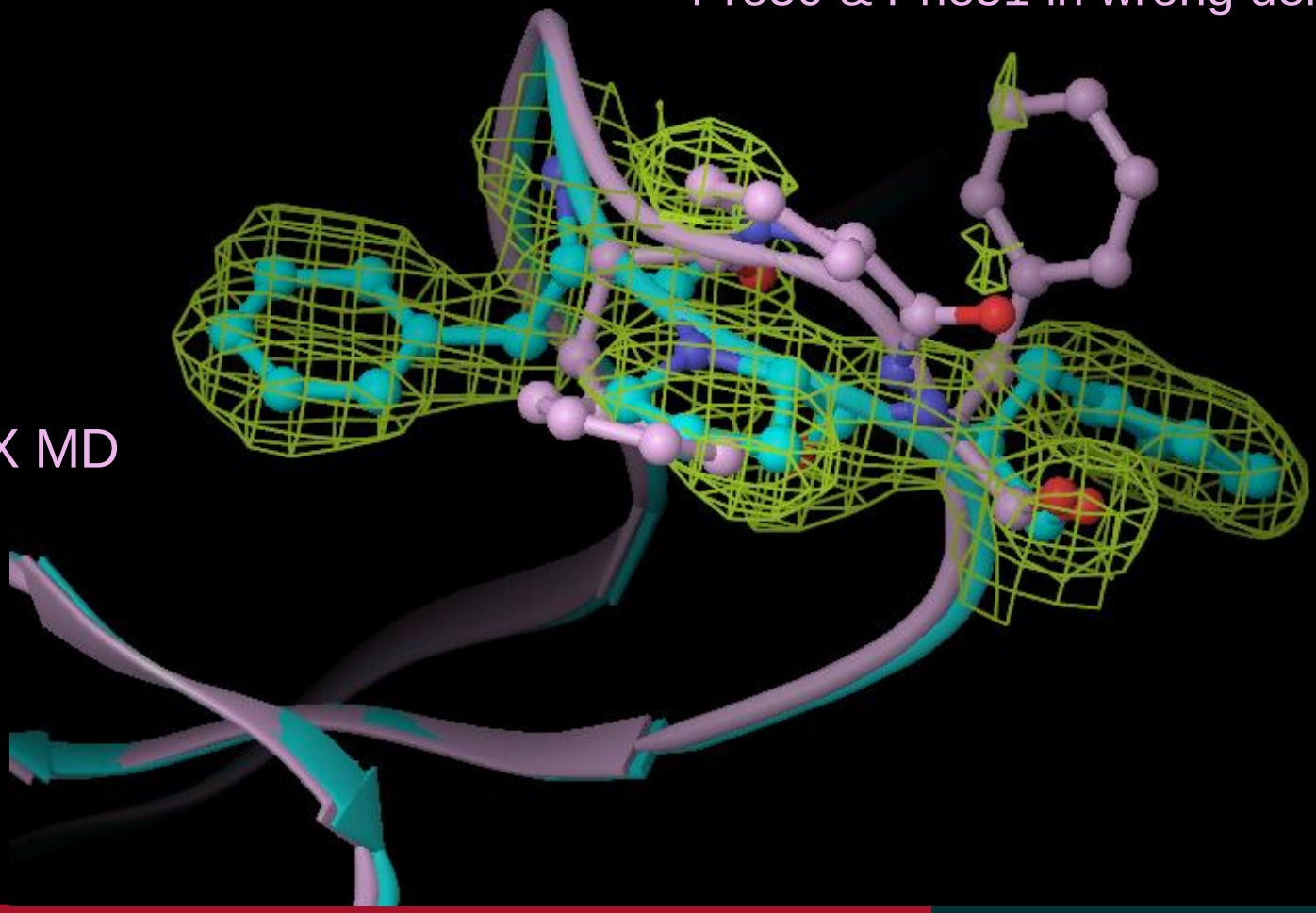


PrimeX - Loop Placement and Simulating Annealing

– Phe49 in Pro's density
– Pro50 & Phe51 in wrong density

X-ray

PrimeX MD



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PrimeX - Loop Placement and Simulating Annealing

X-ray

Real-time/real-space
refinement in Maestro

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