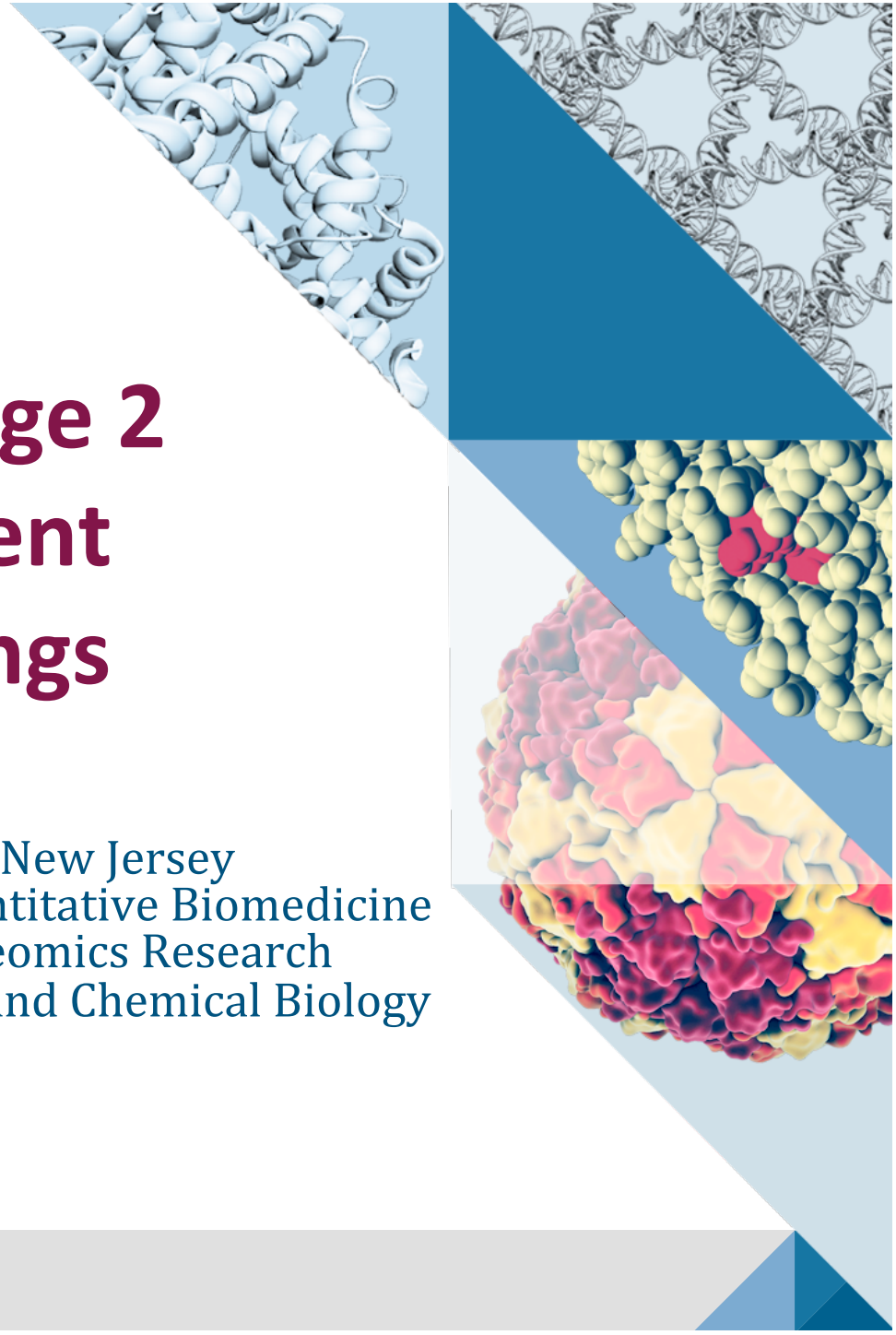
RCSB PDB
PROTEIN DATA BANK



D3R Grand Challenge 2 Structure Refinement Experience/Learnings

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Distinguished Professor, Chemistry and Chemical Biology

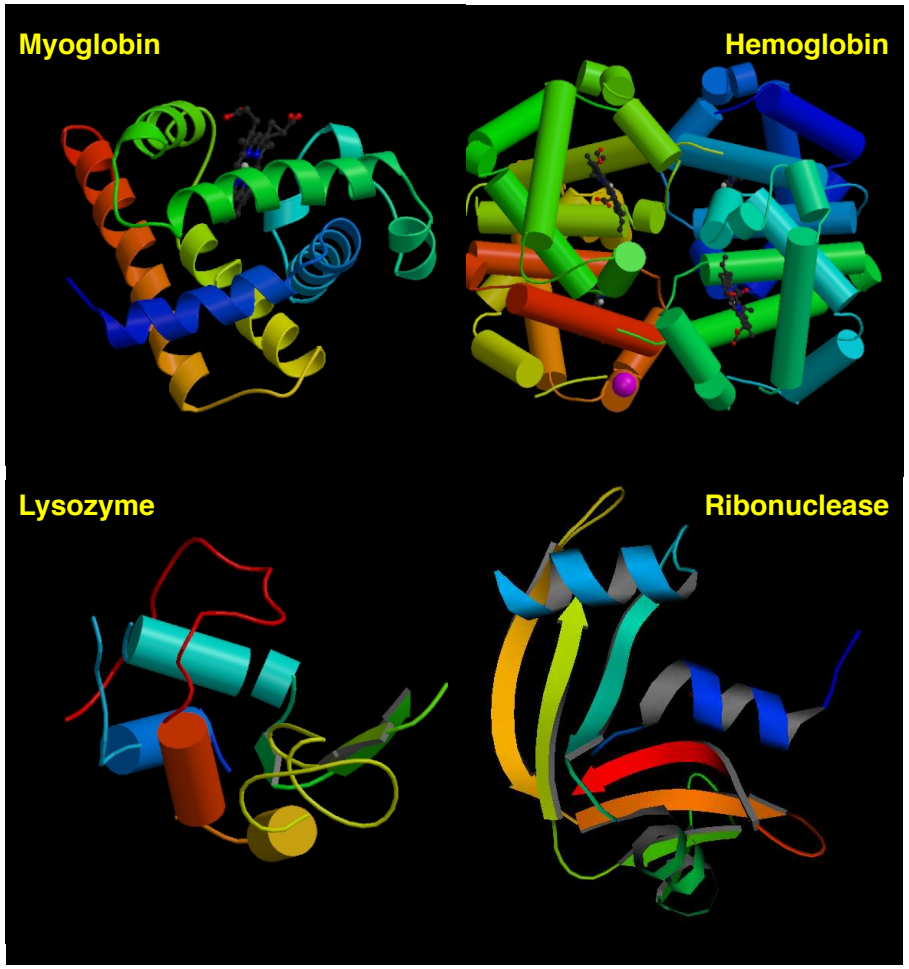
D3R Webinar Meeting
March 27th 2017

Outline

- Protein Data Bank (RCSB PDB, rcsb.org)
- RCSB PDB Group Deposition System
- D3R Structure Refinement Protocol
- Experience/Learnings
- Acknowledgements

Protein Data Bank Archive

- PDB 1st Open Access digital data resource in all of biology
- Founded 1971with 7 X-ray structures
- Today, single global archive
- Managed by wwPDB Partnership(RCSB PDB, PDBe, PDBj, BMRB)



Some of the very first structures in the PDB

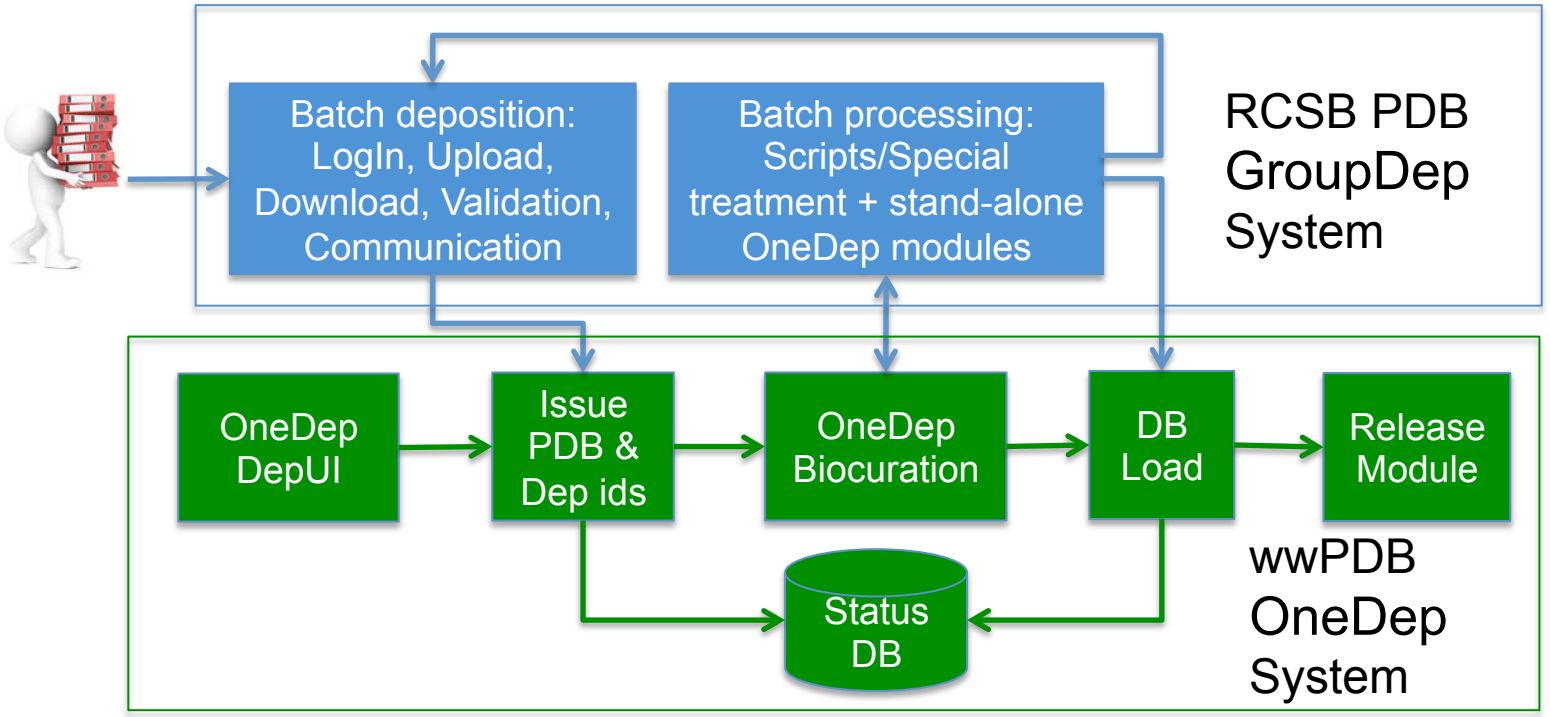
PDB Facts and Figures

- >128,000 Structures Archived since 1971
- ~11,500 New Structures Deposited/Year
- ~30,000 Depositors Worldwide
- >1 Million Unique Users Worldwide/Year
- >550 Million Data Files Downloaded/Year
- ~1.5 Million Data Files Downloaded/Day
- US PDB Headquartered at Rutgers/UCSD
- ~70% of Global PDB Usage through Rutgers/UCSD

RCSB PDB Group Deposition System

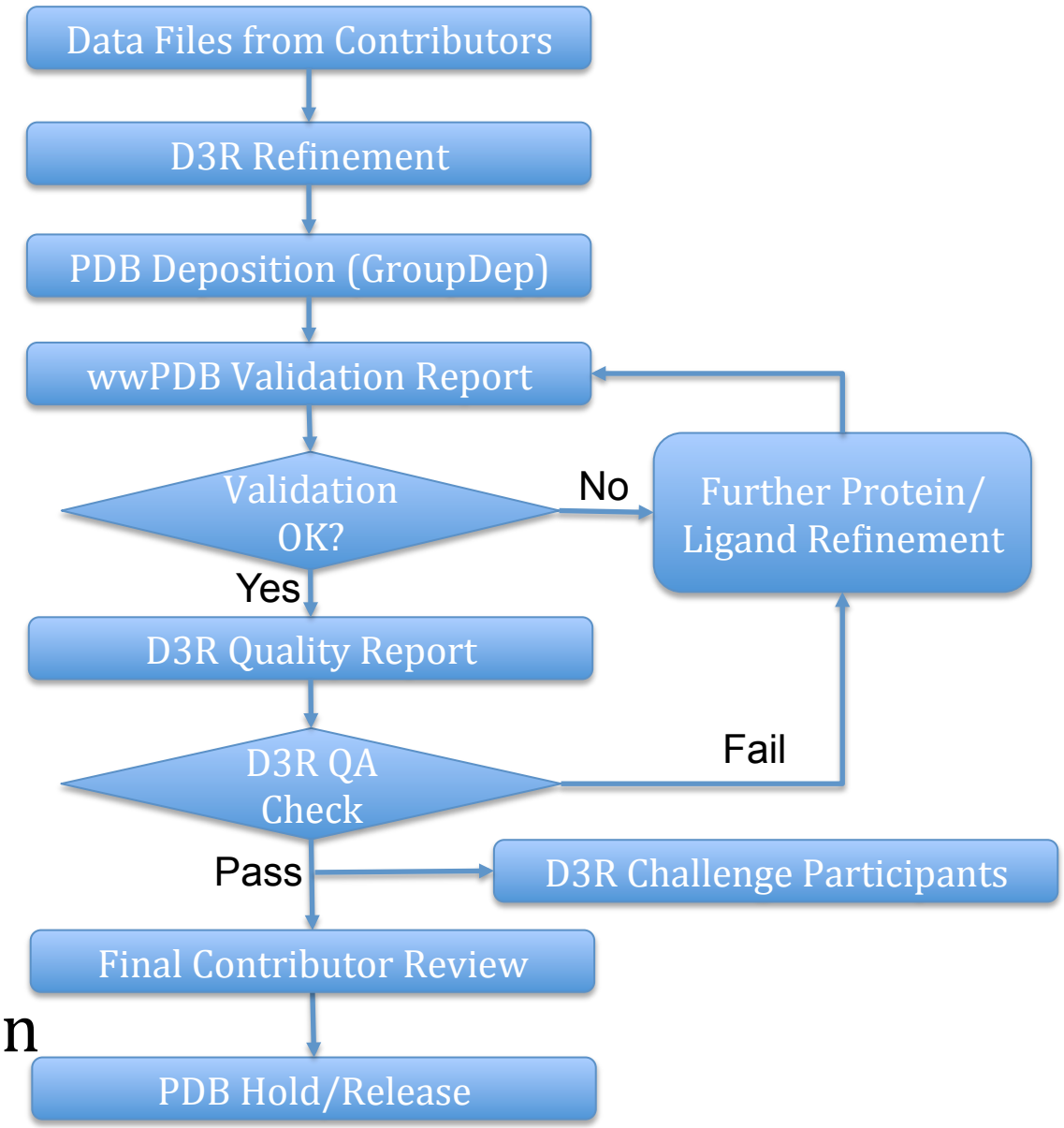
https://deposit-group.rcsb.rutgers.edu/groupdeposit/

- Supports Batch Deposition/Validation of Related Structures
- Originally developed for D3R Blind Challenges
- To date, >1300 structures from Roche, Merck Serono, BMS, SGC, etc., with 10s-100s of structures/group

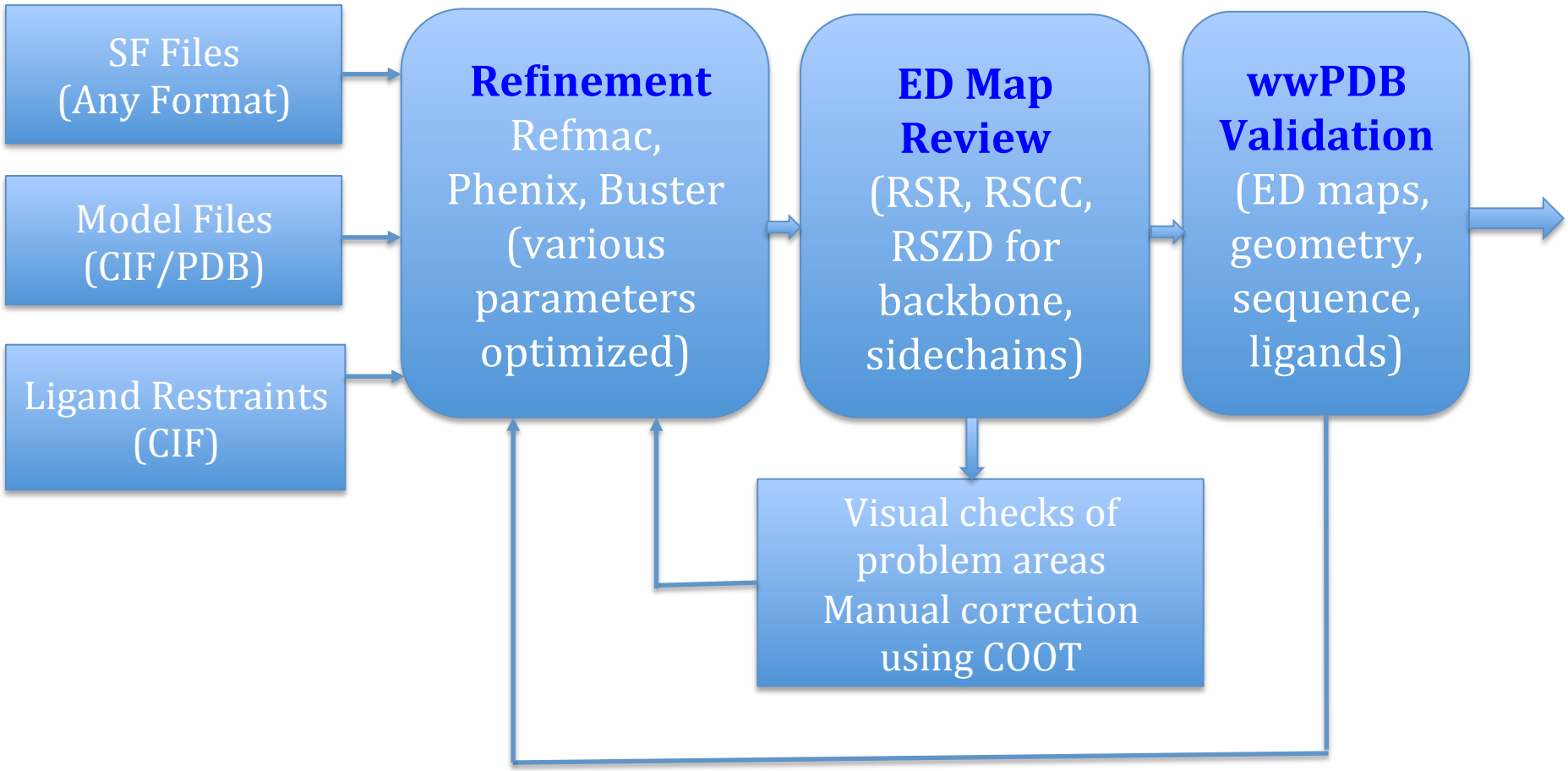


D3R Protocol

- D3R completes refinements
- GroupDep→PDB
- PDB Validates all refined structures
- Contributors and D3R co-author final PDB deposition



D3R Structure Refinement Protocol



Roche FXR Data Set Characteristics

- 36 Co-crystal/1 Apo Structures
  - Resolution Limits: 2.6-1.7Å
  - Asymmetric Unit MWs: 26-110kDa
  - 3 Point Groups/5 Space Groups

- Ligand MWs: 219-564 Da
- Three Ligands: Br atoms
- Four Ligands: Alternative Conformations
- All Ligands: Buried in Binding Pocket

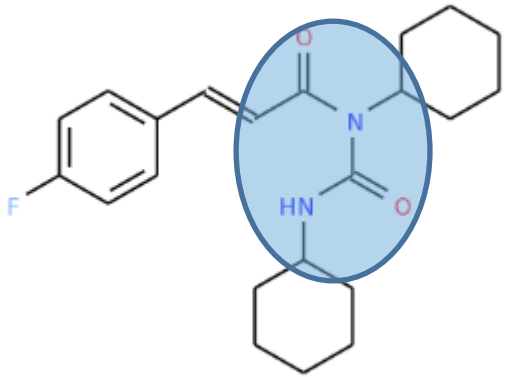
Protein Structure Quality Improved

	Initial Range	Final Range	Average Change
Rfree (%)	23.0-35.0	22.0-31.0	-1.0 (better)
Rwork (%)	17.0-30.0	18.0-25.0	<1.0 (no change)
Rfree – Rwork (%)	4.0-11.0	3.0-10.0	-1.0 (better)
Clash Score	4.38-40.41	0.51-6.90	-15.16 (better)
Ramachandran Outliers (%)	0.0-2.2	0.0-1.3	-0.3 (better)
Sidechain Rotamer Outliers (%)	0.5-24.5	0.5-9.9	-7.9 (better)
% RSRZ Outliers	0.0-29.5	0.0-22.1	-2.2 (better)
Bonds RMSZ	0.3-0.7	0.4-0.9	+0.2 (worse)
Angles RMSZ	0.5-0.8	0.5-0.9	+0.1 (worse)
Overall Quality (%ile of archive, see Shao et al. 2017)	0% - 70%	5% - 80%	+ 25%(better)

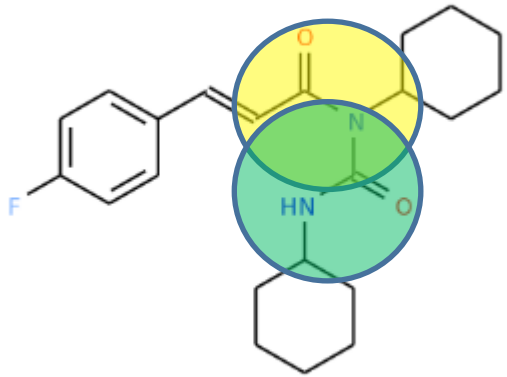
Ligand Structure Quality Improved

	Initial	Final	Average Change
RSR (%)	8.0 – 21.0	7.0 – 19.0	-1.0 (better)
RSCC (%)	82.0 – 98.0	79.0 – 98.0	+1.0 (better)
Bond Lengths Outliers	0 – 17	0 - 10	-3 (better)
Bond Angles Outliers	3 – 24	0 – 7	-7 (better)
Clashes	0-8	0-4	-1 (better)
Bonds RMSZ	0.8 – 2.9	0.5 - 2.9	-0.5 (better)
Angles RMSZ	1.1 - 3.1	0.5 - 2.0	-0.7 (better)
RSZD+(absolute)	0.0 - 2.8	0.0 - 3.4	+0.2 (balanced)
RSZD-(absolute)	0.0 - 4.2	0.0 - 3.3	-0.2 (balanced)

Ligand Geometrical Restraints

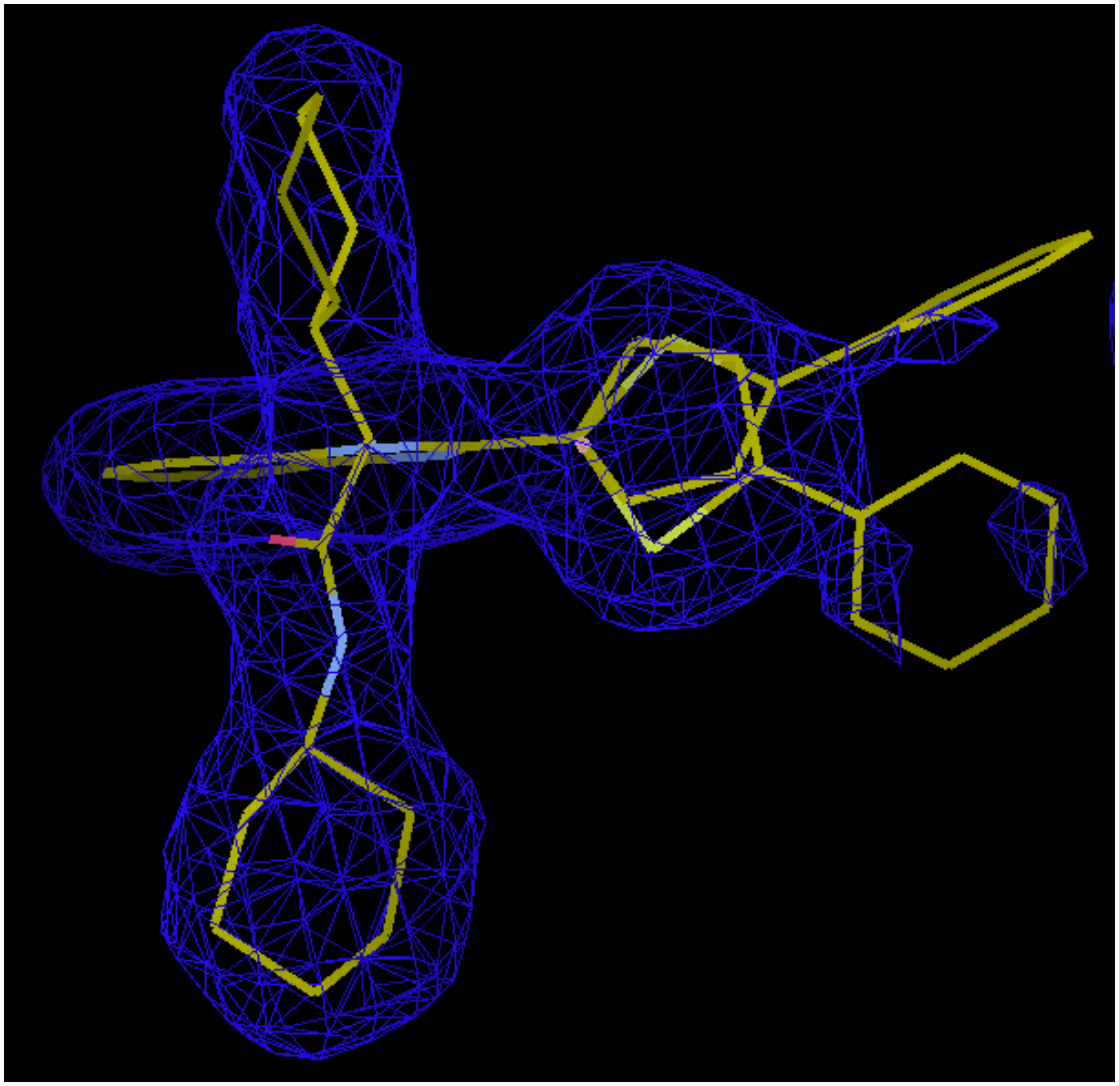


Roche 1ymrc



- Generated ligand restraints with
  - AM1 and RM1 QM optimizers
  - +/- Mogul values
  - Varied planarity settings
- Best results with AM1 + Mogul and tighter planarity, and manual setting of some restraints as needed

Experience/Learnings: Partial Disorder



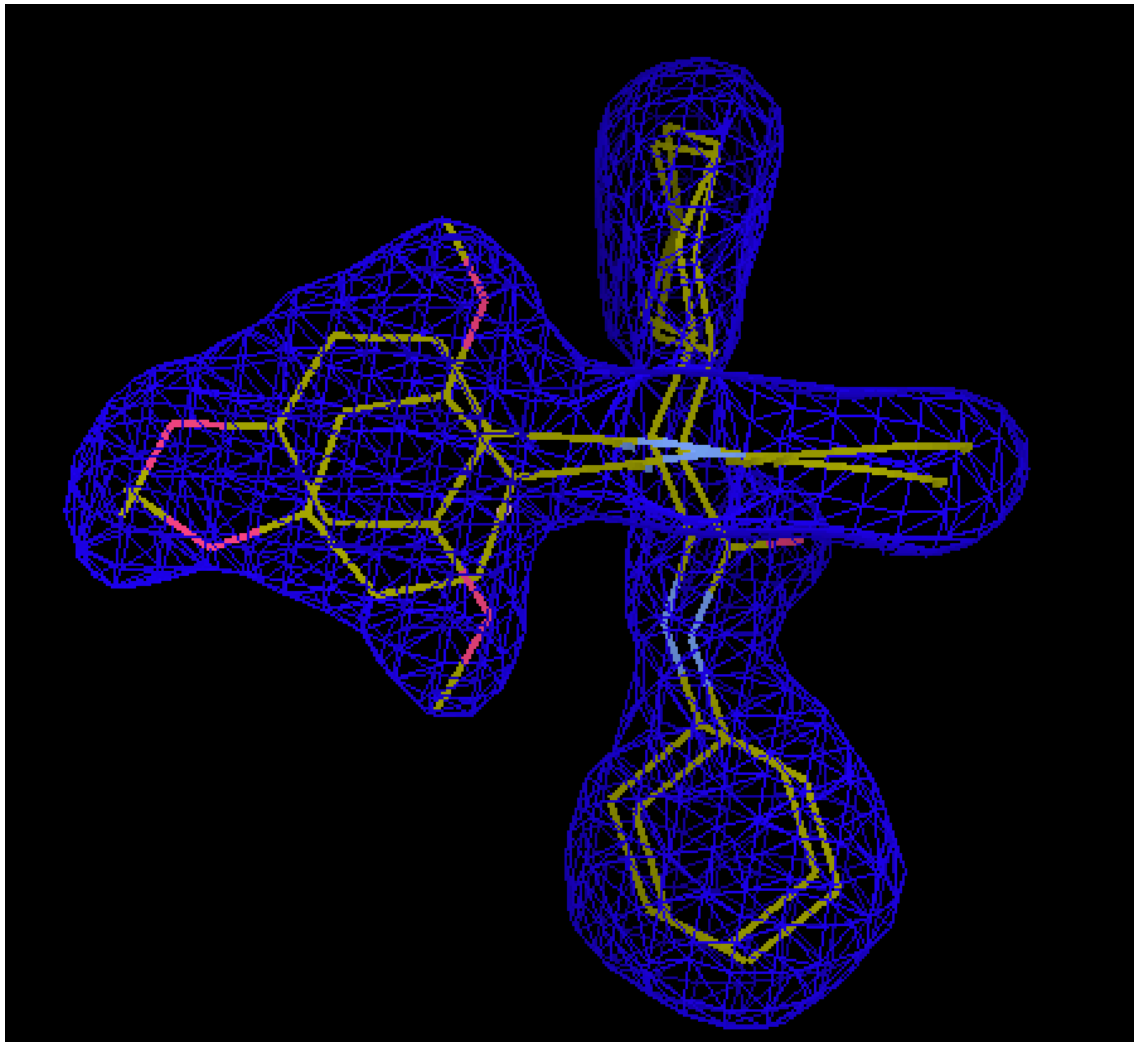
Partial disorder alternative conformers
(1dqzc, 2.0Å)

Thiophene alternative conformations

Cyclohexane disordered

Blue: 2|Fo|-|Fc|/1.0σ

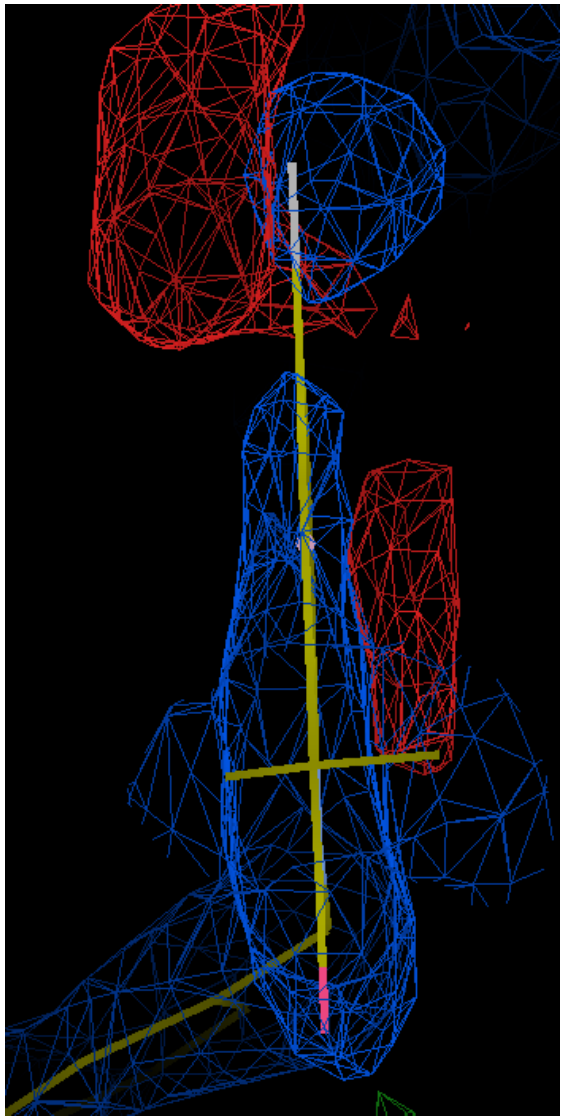
Experience/Learnings: Overall Disorder



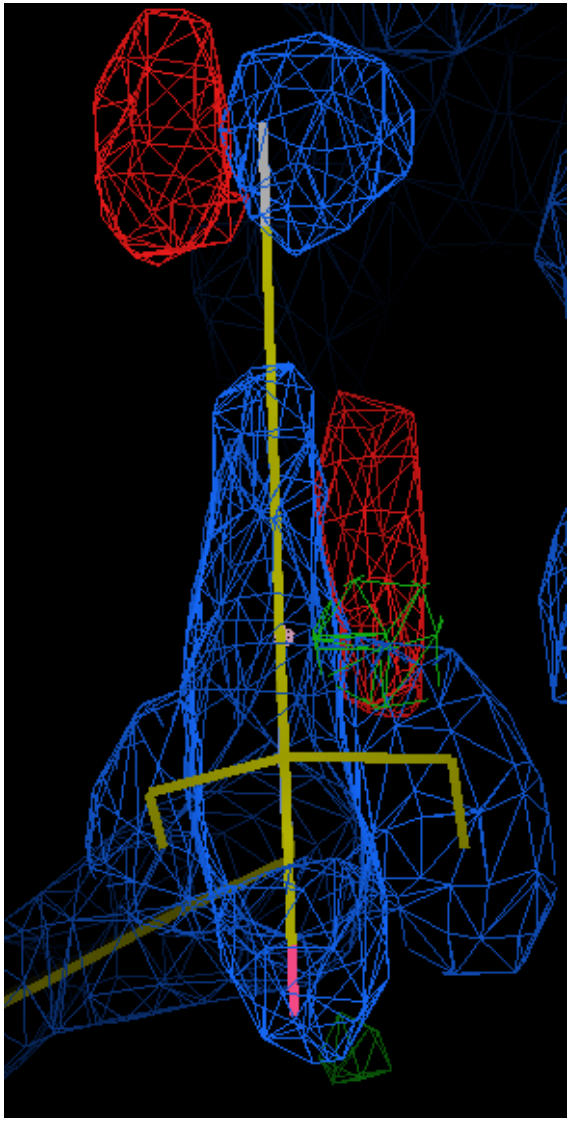
Entire ligand in two orientations
(1txca, 1.9Å)

Blue: 2|Fo|-|Fc|/1.0σ

Experience/Learnings: Loss of Br



Br Occup=1.0



Br Occup=0.5

Ligand with Br
(1xxar, 1.8Å)

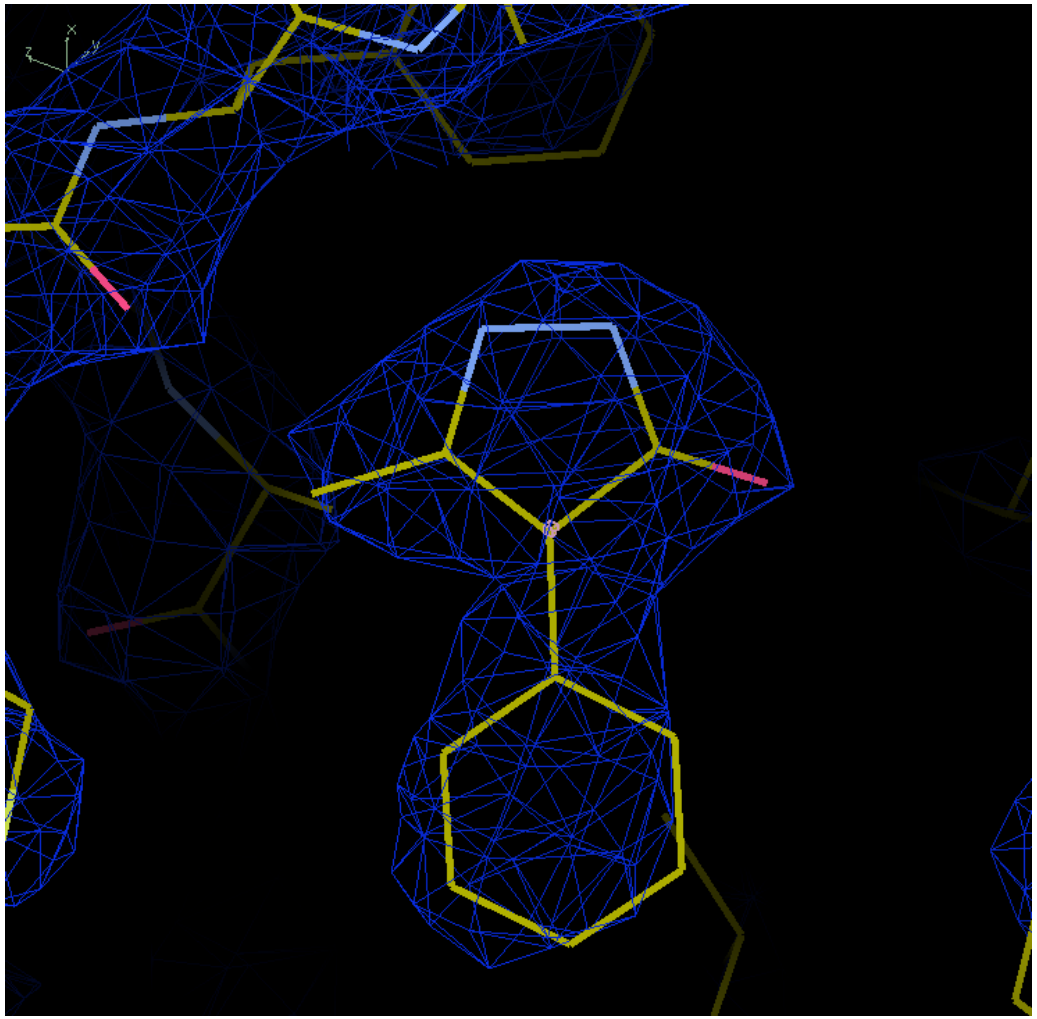
Br-C bond often
broken by X-ray beam

Crystal contains two
Ligand species (+/- Br)

Br Modeled with
reduced Occupancy

Blue: 2|Fo|-|Fc|/1.5σ
Red: -ve |Fo|-|Fc|/3.0σ
Green: +ve |Fo|-|Fc|/3.0σ

Experience/Learnings: Ambiguities



ED Map Ambiguity
(from different study)

Pseudo-symmetric ligands require inspection of binding environment

Wrong Orientation!

Blue: 2|Fo|-|Fc|/1.0σ

Acknowledgements

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