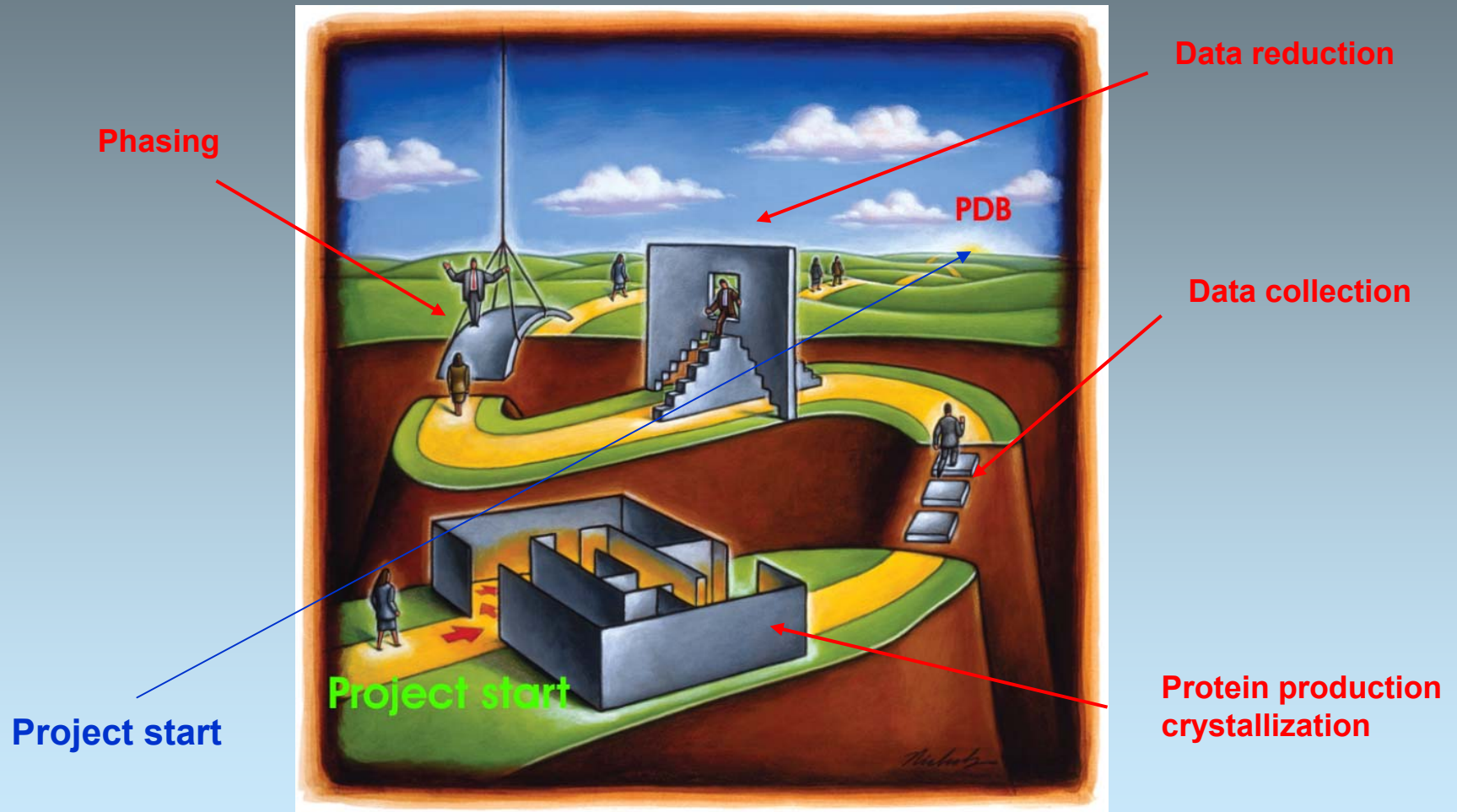


HKL-3000

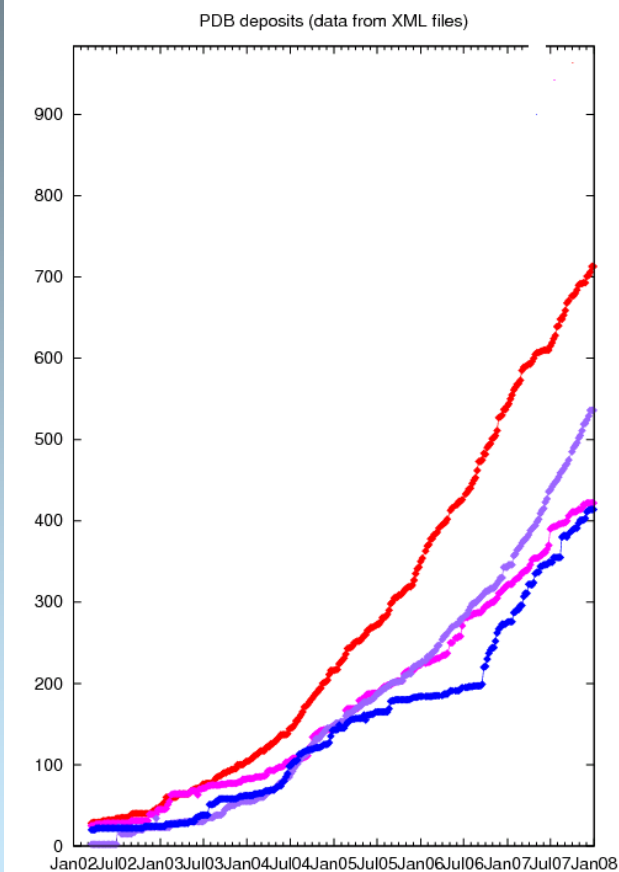
Toward the future of protein crystallography

Long and winding road (with traps)



SG dynamics

PSI Centers: March 2002-January 2008



In every PSI Large Center

Roughly
new structure every 48 hours



Current situation

W0308

Teaching Elves to Collect Data: An Analysis of the Last Million Diffraction Images from ALS 8.3.1.
James Holton, Physical Biosciences, Lawrence Berkeley National Laboratory, Berkeley, CA 94720.

Most X-ray data sets collected at synchrotron sources do not produce usable results. An analysis of data collected in 2003 at the ALS beamline 8.3.1 shows that 2346 datasets were collected and 41 structures were deposited in the PDB. Although it is understandable that not every dataset leads to a published structure, it is troubling that ~98% of them do not. This large gap between collected data and useful results is not unique to 8.3.1. The 28 operating American PX beamlines collect ~100,000 datasets/year. This suggests that a great deal of improvement in scientific productivity can be attained if the reasons for failed projects are better understood.

57 datasets/deposit !!!!

Worldwide < 40 hours/deposit

~ 40-150 crystals/structure

Priceless crystals



Star of Africa



Crystallography lab

How to define high throughput at the beamline ?

Integration

HKL-3000 is a pipeline component

Pepdb

expression & purification

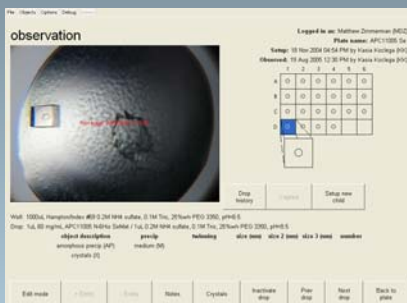
PepDB @ UNIVERSITY of VIRGINIA

Expression: 24 records found, page 1 of 2

No.	Project	Macromolecule	expression_id	clone_id	date_expressed	expression_level	inhibitory_level
1	TEST		112	2	01/19/05	5	5
2	APC11091	APC11091	111	1	02/09/05	3	1
3	APC4973	APC4973	110	4	03/06/04	3	3
4	APC4542	APC4542	109	4	03/06/04	3	3
5	APC4251	APC4252	108	4	03/06/04	3	3
6	APC4973	APC4973	107	4	03/06/04	3	3
7	APC4542	APC4542	106	4	03/06/04	3	3
8	APC4973	APC4973	105	4	03/06/04	3	3
9	APC4973	APC4973	104	4	12/11/03	3	3
10	APC4252	APC4252	103	4	12/11/03	3	3
11	APC11091	APC11091	102	4	12/11/03	4	3
12	APC11091	APC11091	101	4	12/11/03	4	3
13	APC11091	APC11091	100	4	07/04/03	4	3

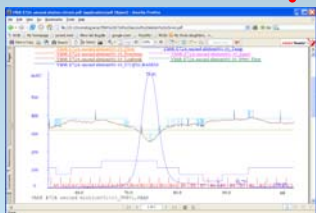
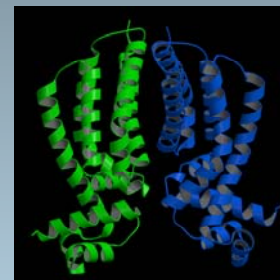
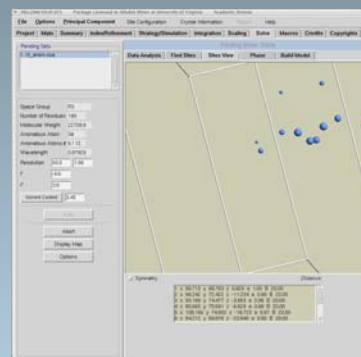
Xtaldb

crystallization



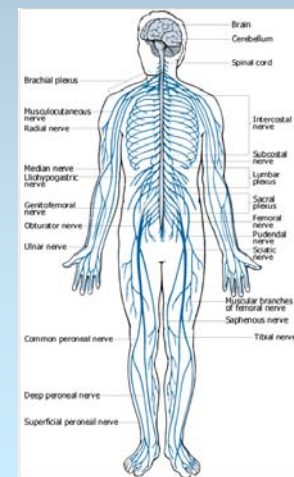
HKL-3000

Data collection,
diffraction & structure solution



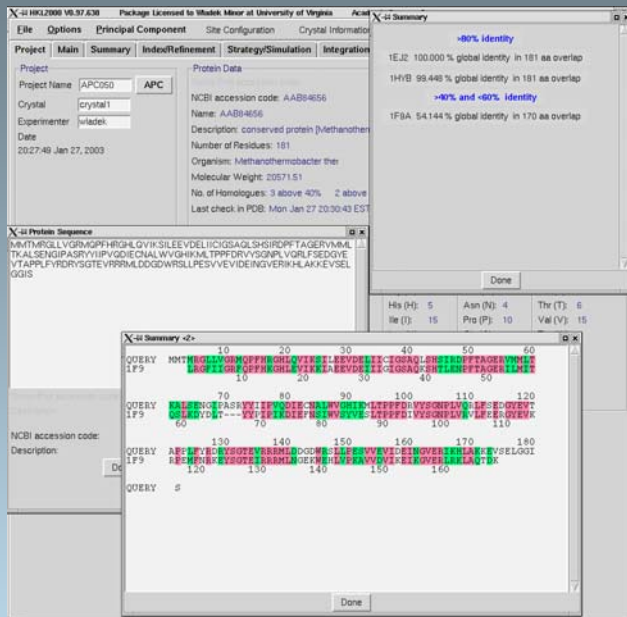
Wetlab
chemicals & solutions

Other systems provide additional
contextual up- and downstream
information

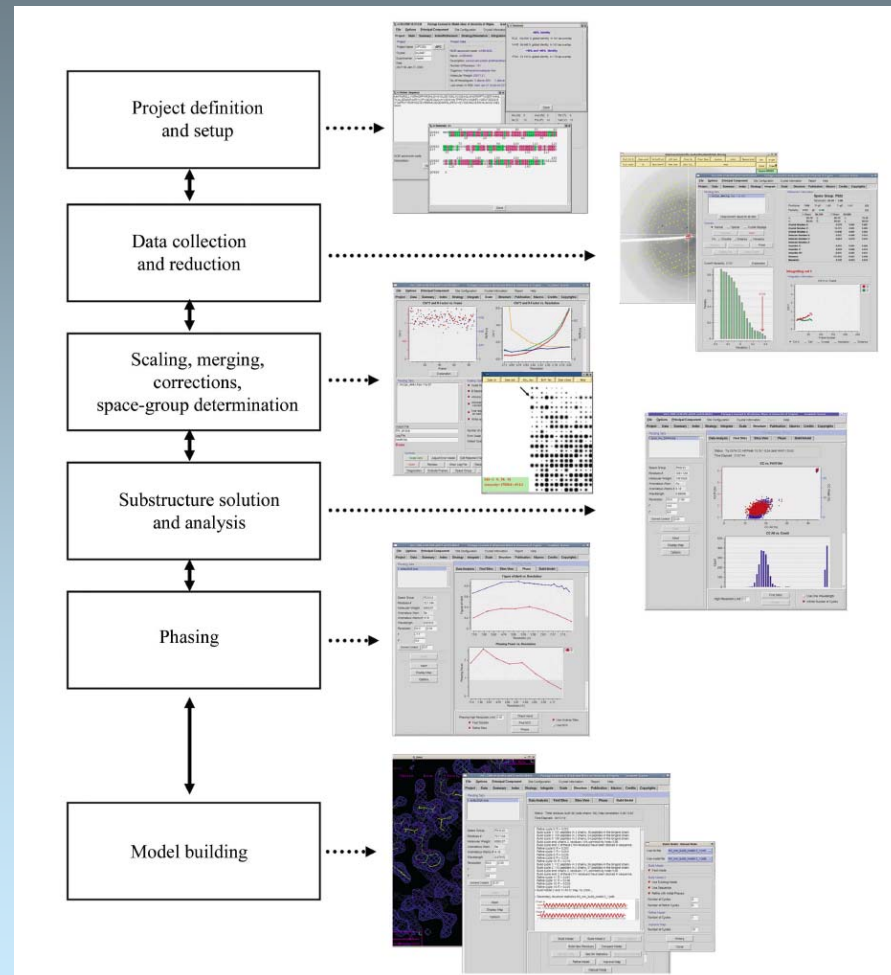


Project

Check against DB during data collection

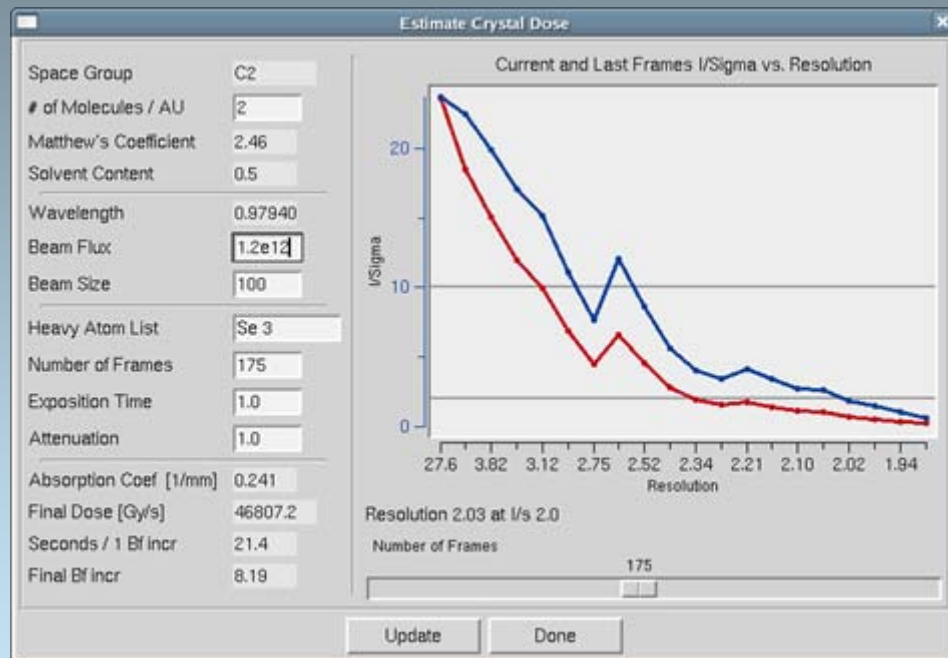
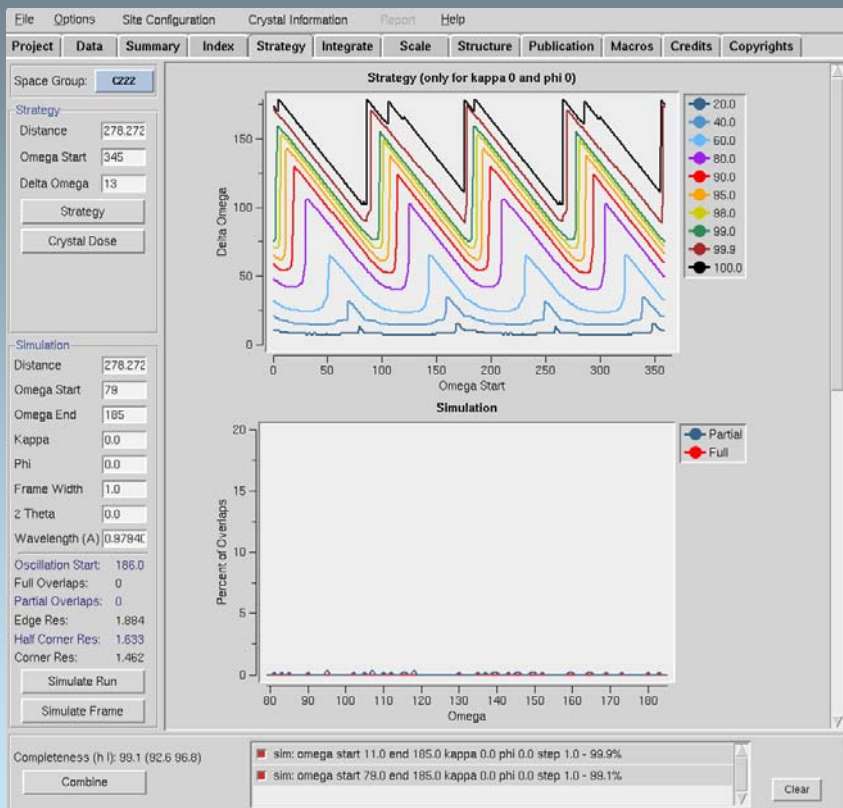


HKL-2000
SHELXD, SHELXE
CCP4
SOLVE, RESOLVE
ARP/WARP
O, COOT, CCP4

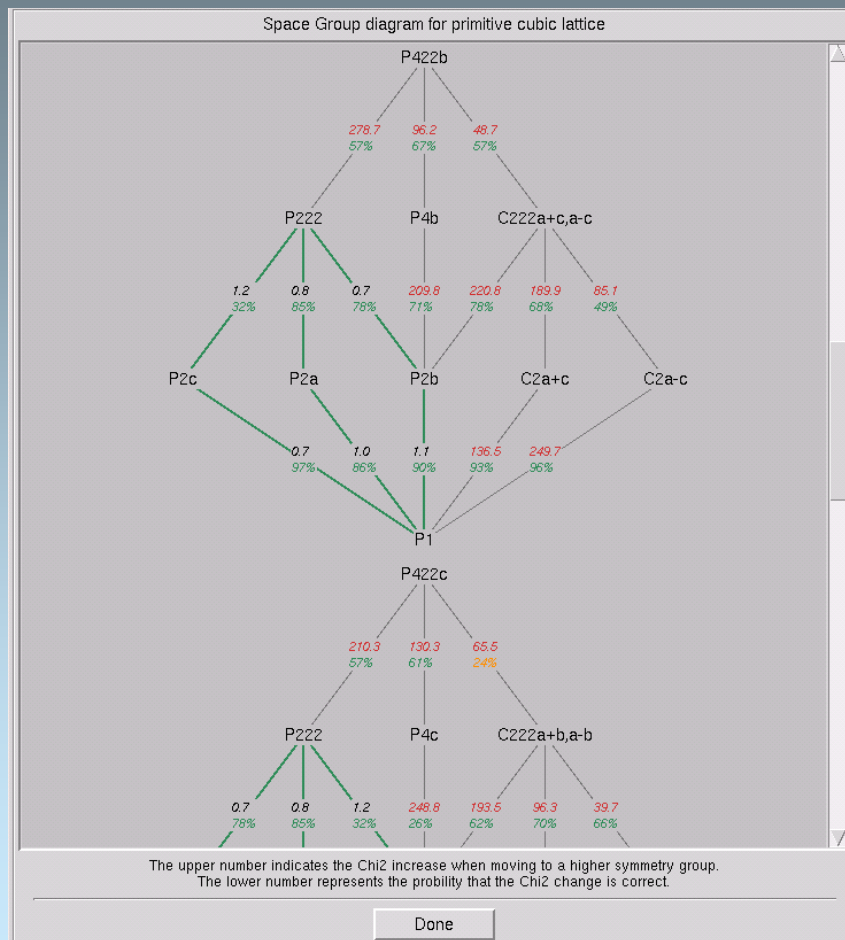


Completeness – overlaps

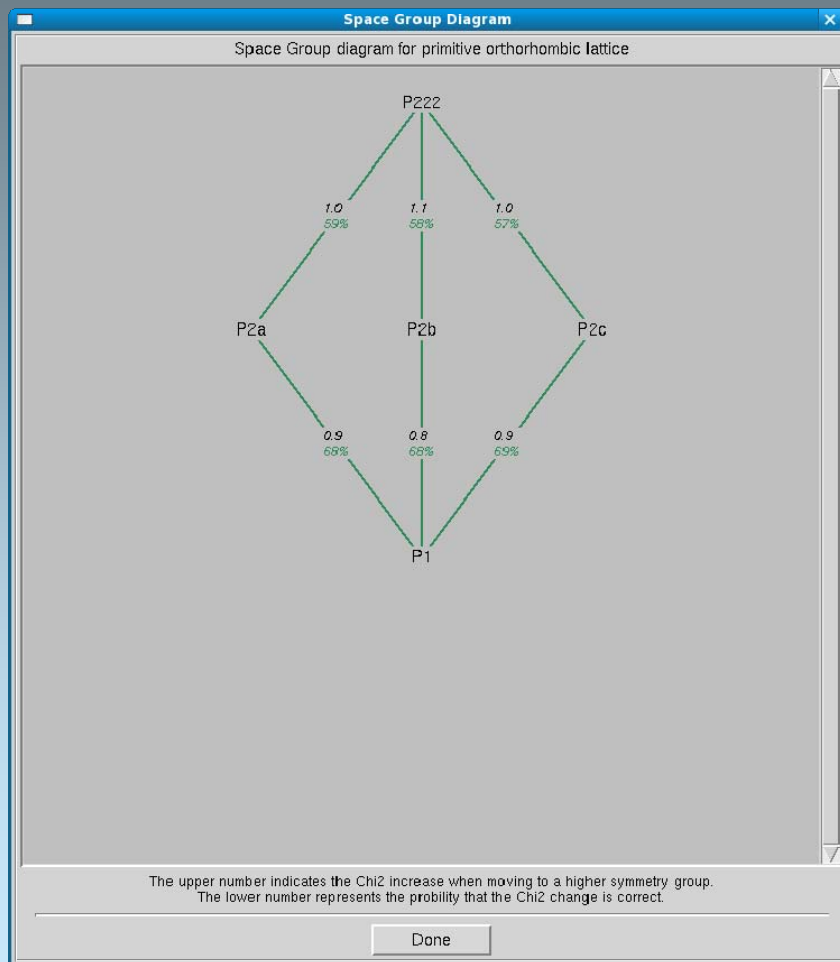
Multi-crystal strategy



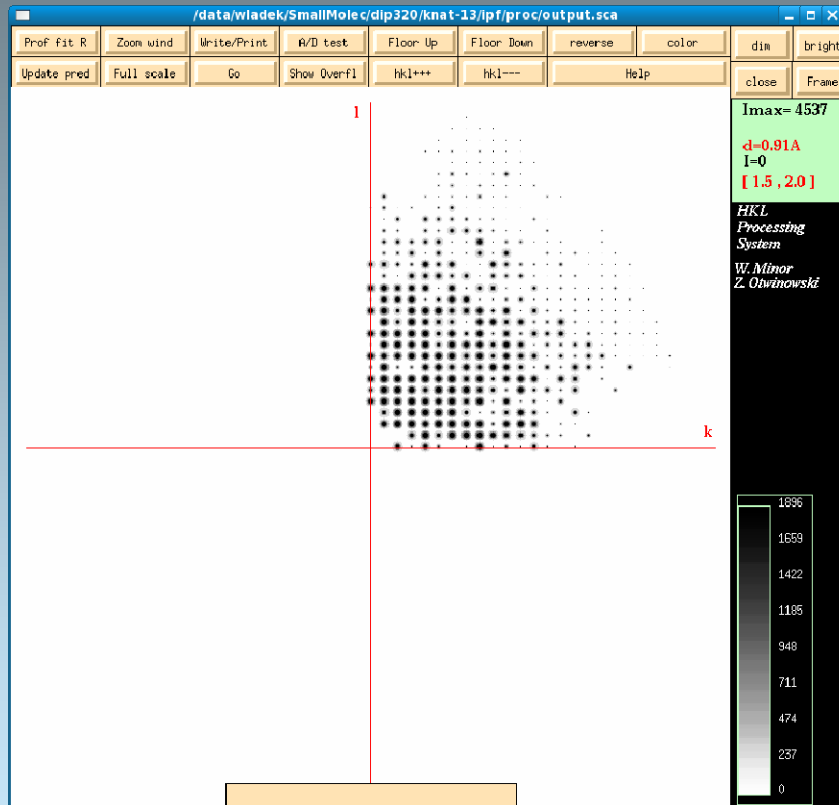
Space Group Determination



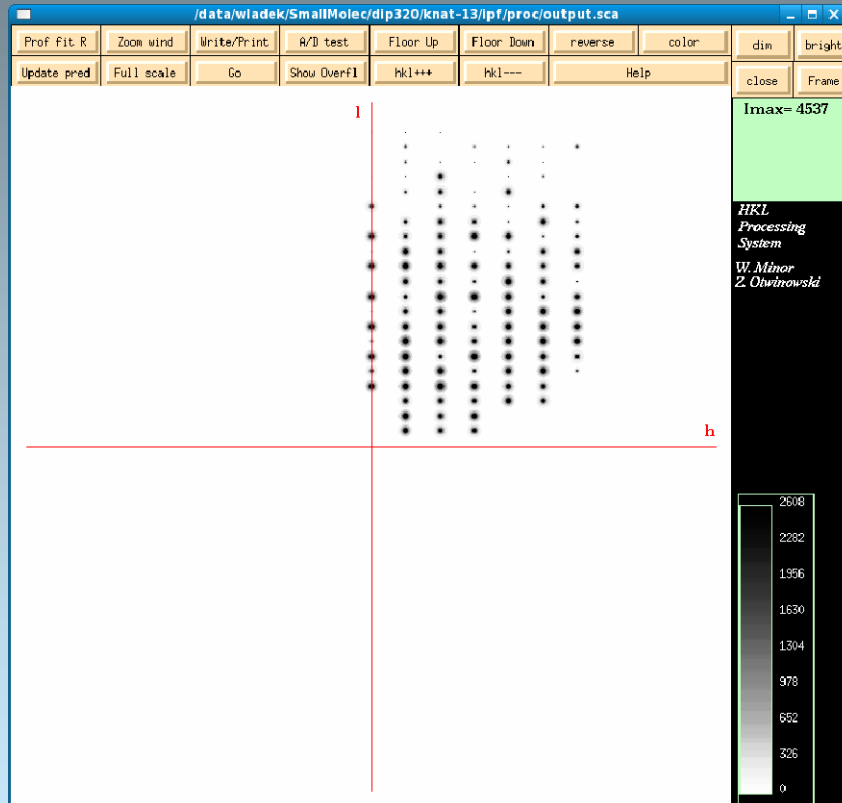
Reliable Space Group Determination



P2?2?2?



P2?2?2?



Scaling

HKL-3000 v0.98.698c.ph042.sm028.db016 Package Licensed to Wladyslaw Minor at University of Virginia Academic license

File Options Site Configuration Crystal Information Report Help

Project Data Summary Index Strategy Integrate Scale Structure Publication Macros Credits Copyrights

Explanation

Chi² and R-Factor vs. Frame



Chi² and R-Factor vs. Resolution



Pending Sets

1. hhr2pk_###.img from 1 to 250

Scaling Options

<input checked="" type="checkbox"/> Scale Restrain	0.01	<input type="checkbox"/> Fix B
<input checked="" type="checkbox"/> B Restrain	0.1	<input checked="" type="checkbox"/> Anomalous
<input checked="" type="checkbox"/> Absorp Restrain	0.05	<input type="checkbox"/> Scale Anomalous
<input checked="" type="checkbox"/> Absorption Correction		<input checked="" type="checkbox"/> Ignore Overloads
<input checked="" type="checkbox"/> Use rejections on next run		<input type="checkbox"/> Direction Cosines
<input checked="" type="checkbox"/> Write rejection file		<input type="checkbox"/> Fit Goniostat

Space Group: **P21212**

Global Refinement

- Non-Slipping Crystal Perfect Goniostat
- Non-Slipping Crystal Imperfect Goniostat
- Small Slippage Imperfect Goniostat
- Custom Postrefinement
- No Postrefinement

Number of Cycles 10

Scalepack Auto

Output File: output.sca

Log File: scale.log

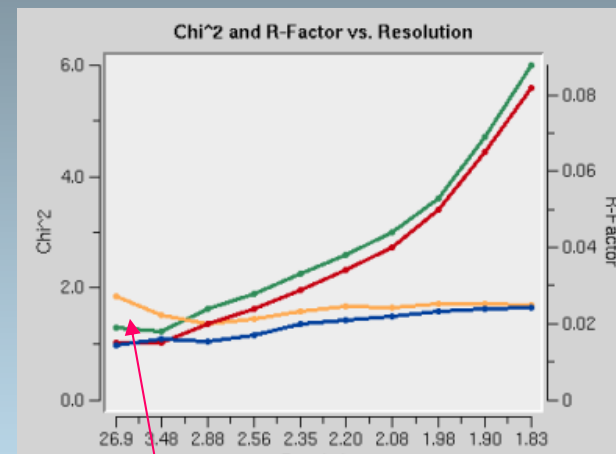
Done

Controls

Scale Sets Adjust Error Model Edit Rejection File Show Redundancies Display Frame 126 Load Output File

Abort Reindex Show Log File Reciprocal Lattice Reprocess Delete Reject File

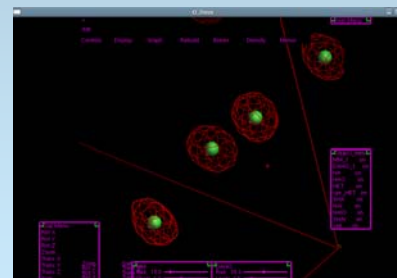
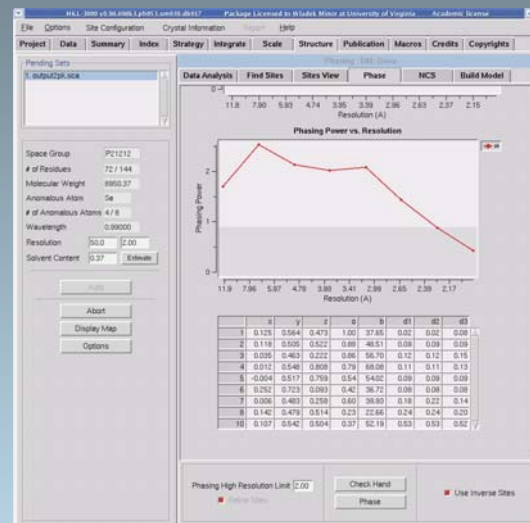
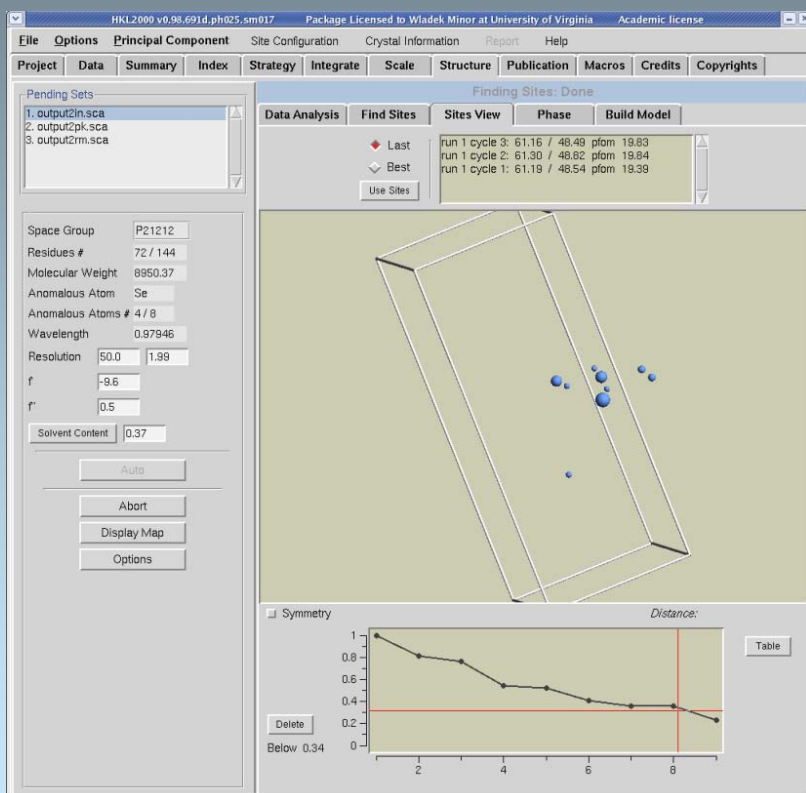
Diagnostics Exclude Frames Space Group Laue Group



Signal from Sulfur

Substructure solution

- Data management in HKL-3000 - substructure solution



NCS handling

HKL-3000 v0.98.698k3.ph053.sm030.db017 Package Licensed to Wladek Minor at University of Virginia Academic license

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Project Data Summary Index Strategy Integrate Scale Structure Publication Macros Credits Copyrights

Phasing - DM: Done

Pending Sets
1. output2pk.sca

Space Group P21212
of Residues 72 / 144
Molecular Weight 8950.37
Anomalous Atom Se
of Anomalous Atoms 4 / 8
Wavelength 0.99000
Resolution 50.0 2.00
Solvent Content 0.37 Estimate

Auto
Abort
Display Map
Options

Data Analysis Find Sites Sites View Phase NCS Build Model

of NCS Copies Found: 2 # of Sites Accepted: 6 # of Sites Rejected: 1
Phase Improvement NCS cc: 0.23 / 0.71 Time Elapsed: 00:00:39

Figure of Merit vs. Resolution
NCS cc

```
> Find NCS start 15:22:33 Dec 21, 2007 ...
List of NCS related sites:
  1      -22.77  -4.83  14.56  -0.38  -0.06  0.47
  3      -28.24   2.79   6.83  -0.47   0.04  0.22
  2      -23.20  -0.38  16.07  -0.38   0.00  0.52
  4  1    0.70   30.03   3.39  -5.38   0.49   0.05  -0.19
  8  3    0.55   23.66   2.93 -15.71   0.39   0.04  -0.50
  5  2    0.51   29.82  -0.82  -7.50   0.50  -0.02  -0.24
List of sites not fitting NCS:
  6      -15.06 -16.82   2.86  -0.25  -0.22  0.09
# of NCS Copies Found: 2
# of Sites Accepted: 6 # of Sites Rejected: 1
> Find NCS stop 15:22:36 Dec 21, 2007

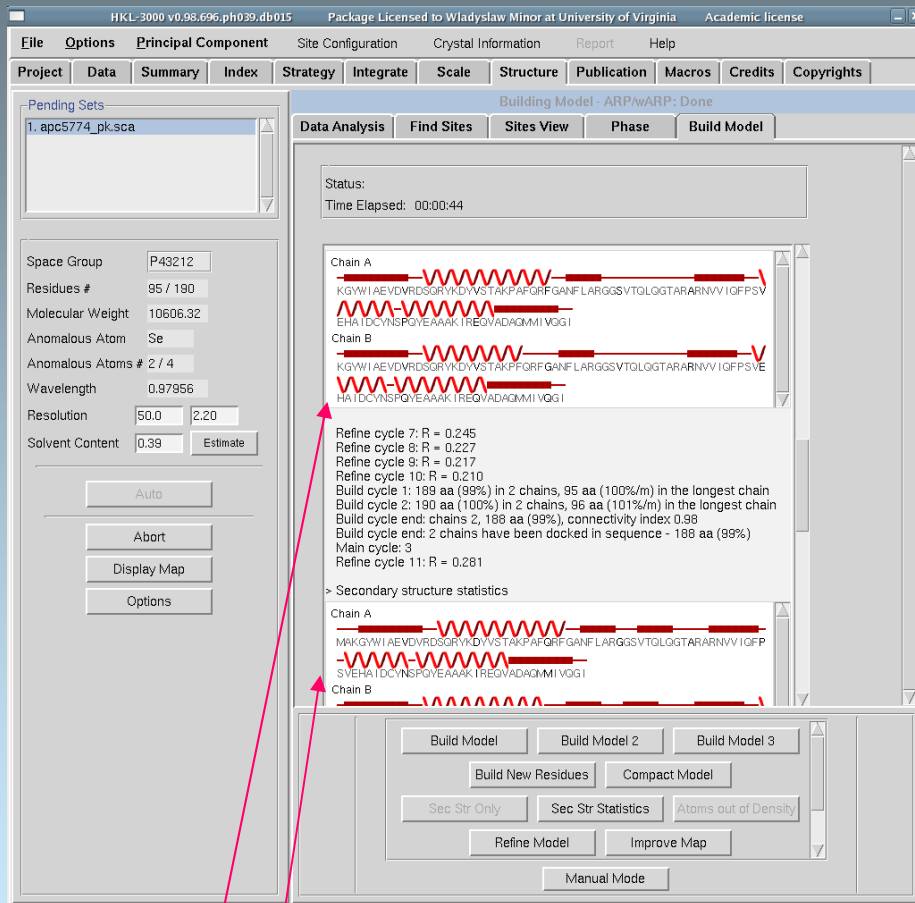
> Improve phases start 15:24:14 Dec 21, 2007 ...
NCS correlations between related density regions: 0.23 / 0.07
NCS correlations between related density regions: 0.23 / 0.71
> Improve phases stop 15:24:53 Dec 21, 2007 ...
```

NCS Input Sites

	x	y	z	a	b	include
1	0.125	0.564	0.473	1.00	37.60	<input checked="" type="checkbox"/>
2	0.118	0.505	0.522	0.88	48.50	<input checked="" type="checkbox"/>
3	0.035	0.463	0.222	0.86	56.70	<input checked="" type="checkbox"/>
4	0.012	0.548	0.808	0.79	68.10	<input checked="" type="checkbox"/>
5	-0.004	0.517	0.759	0.54	54.00	<input checked="" type="checkbox"/>

of NCS copies 2 Find NCS Improve Phases Use Inverse Sites
Advanced Mode

Model building and preliminary refinement (10 minutes, 190aa)



Building Model - ARPwARP: Done

Status:
Time Elapsed: 00:00:44

Chain A
 KGVW I AEVDVDRDSGRYKDYVS TAKP AFORF GANF LARGGSVTLOGTARARNVV IGFPSV
 EHA I DCYNSPQVEAAAK IREQVADAGMMI VGG I

Chain B
 KGVW I AEVDVDRDSGRYKDYVS TAKP AFORF GANF LARGGSVTLOGTARARNVV IGFPSVE
 HA I DCYNSPQVEAAAK IREQVADAGMMI VGG I

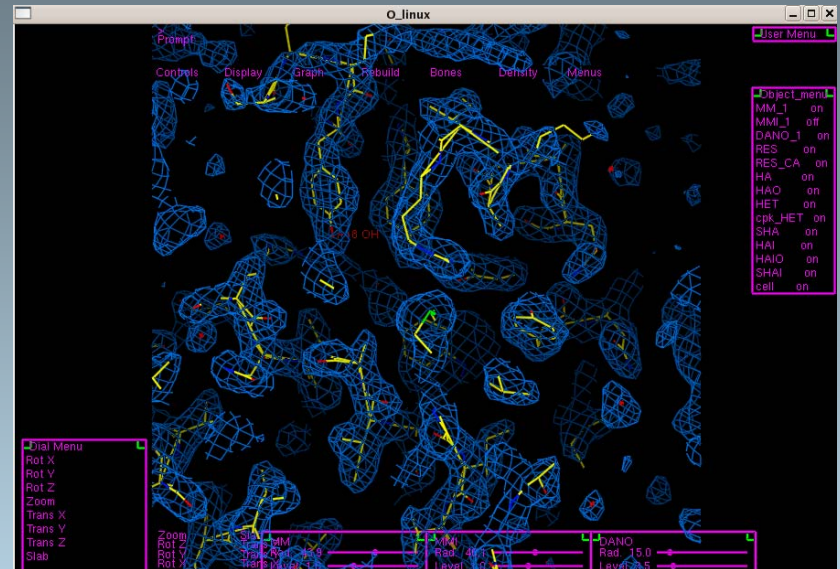
Refine cycle 7: R = 0.245
 Refine cycle 8: R = 0.227
 Refine cycle 9: R = 0.217
 Refine cycle 10: R = 0.210
 Build cycle 1: 189 aa (98%) in 2 chains, 95 aa (100%/m) in the longest chain
 Build cycle 2: 190 aa (100%) in 2 chains, 96 aa (101%/m) in the longest chain
 Build cycle end: chains 2, 188 aa (99%), connectivity index 0.98
 Build cycle end: 2 chains have been docked in sequence - 188 aa (98%)
 Main cycle: 3
 Refine cycle 11: R = 0.281

> Secondary structure statistics

Chain A
 MAKGVW I AEVDVDRDSGRYKDYVS TAKP AFORF GANF LARGGSVTLOGTARARNVV IGFPS
 SVEHA I DCYNSPQVEAAAK IREQVADAGMMI VGG I

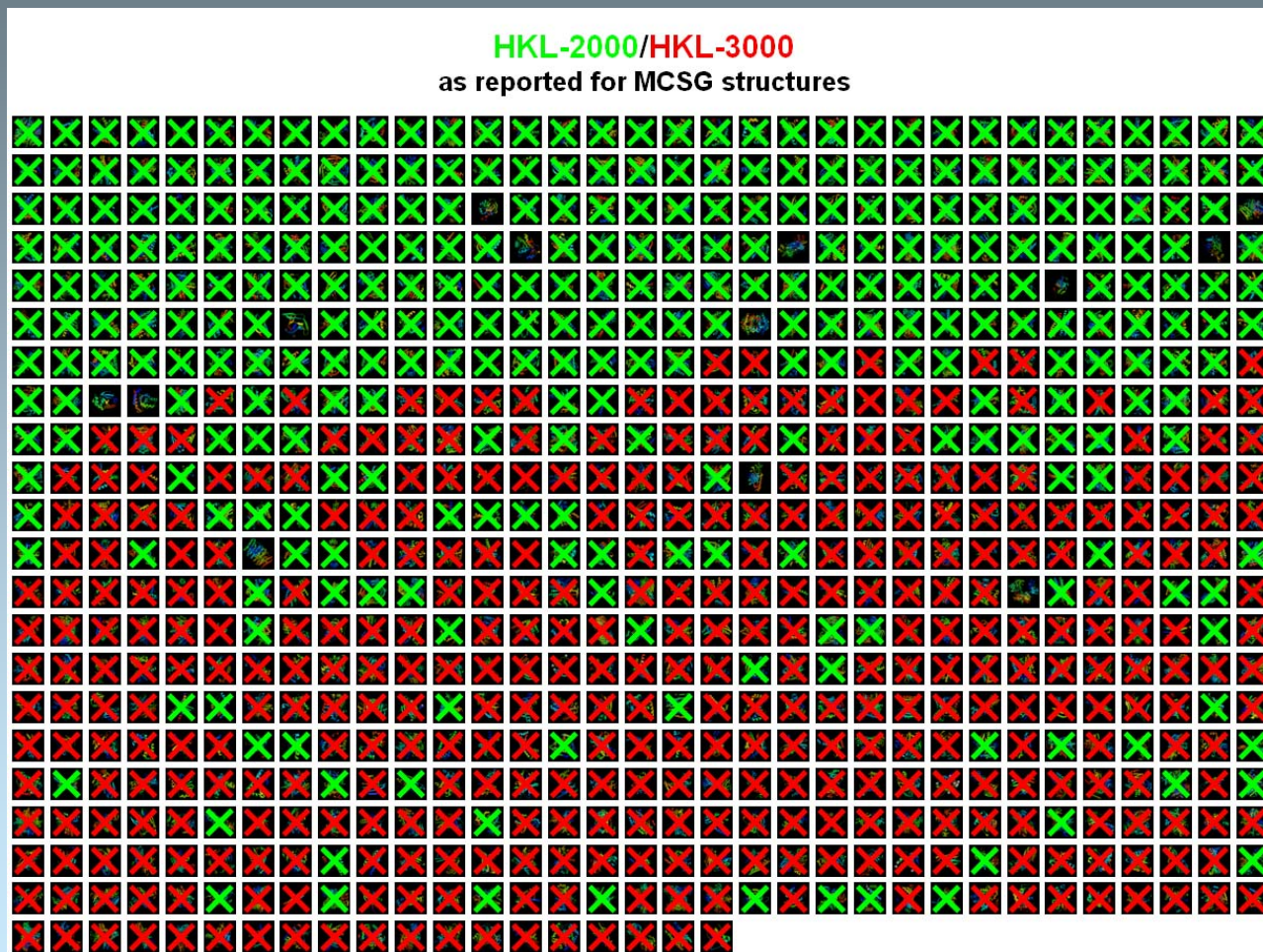
Chain B
 KGVW I AEVDVDRDSGRYKDYVS TAKP AFORF GANF LARGGSVTLOGTARARNVV IGFPSVE

Build Model Build Model 2 Build Model 3
 Build New Residues Compact Model
 Sec Str Only Sec Str Statistics Atoms out of Density
 Refine Model Improve Map
 Manual Mode



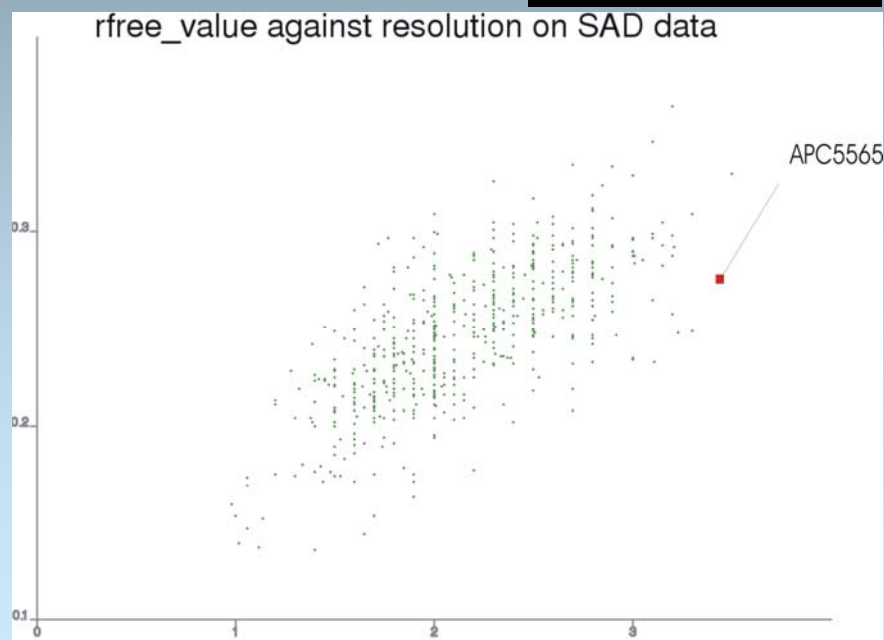
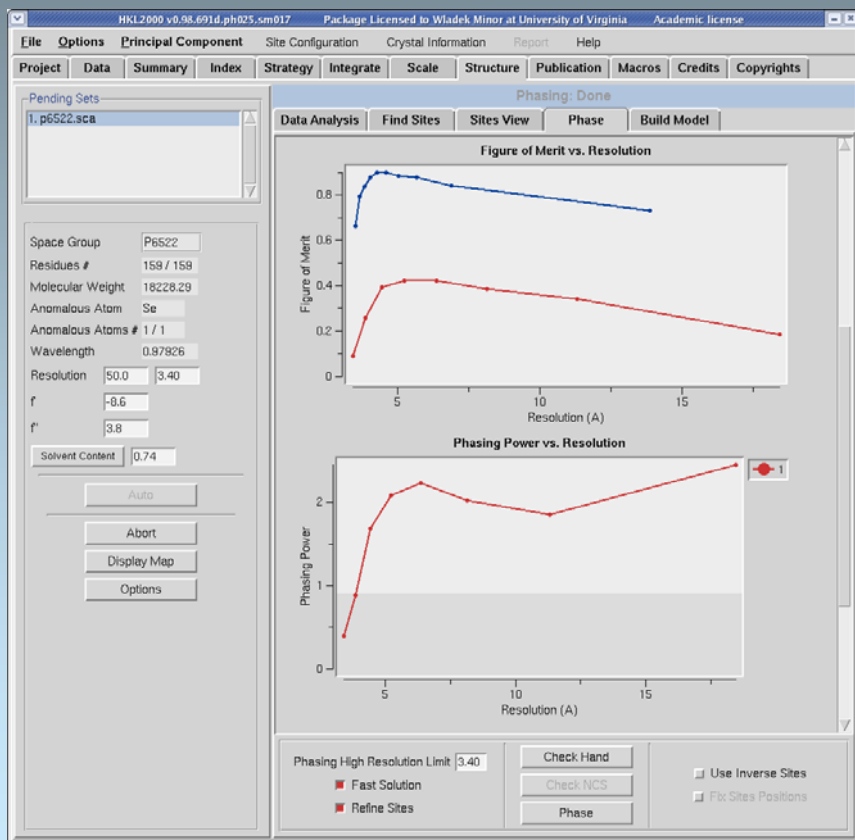
Secondary structure after each cycle

MCSG 712 [HKL-3000]

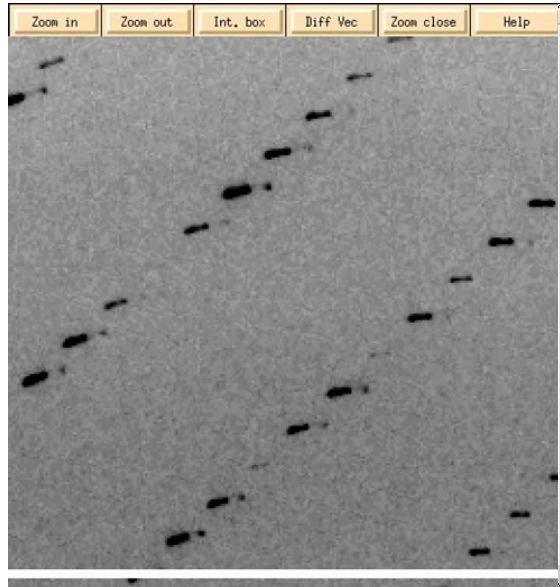


Low resolution SAD phasing difficult structure – no

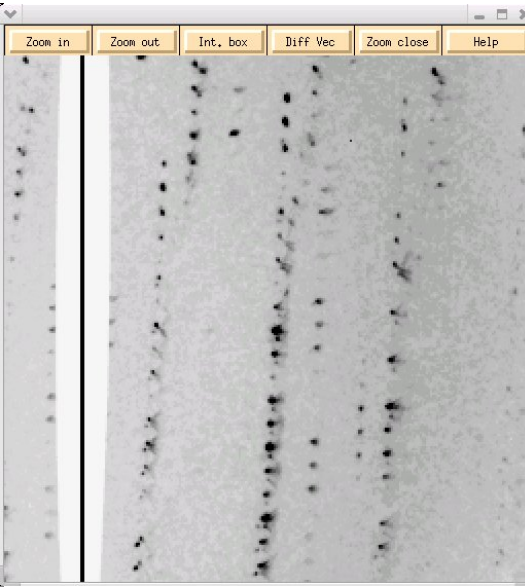
1ze0



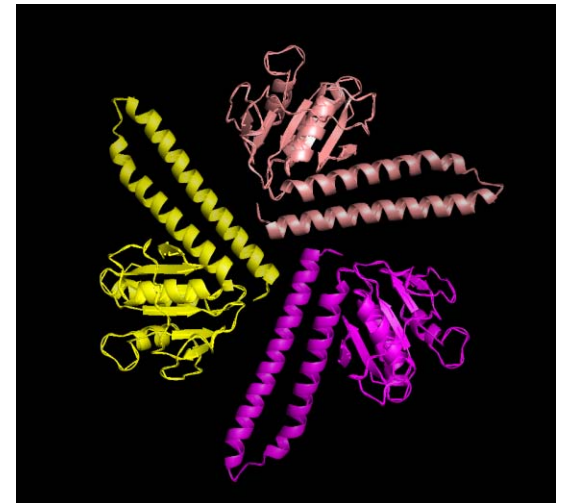
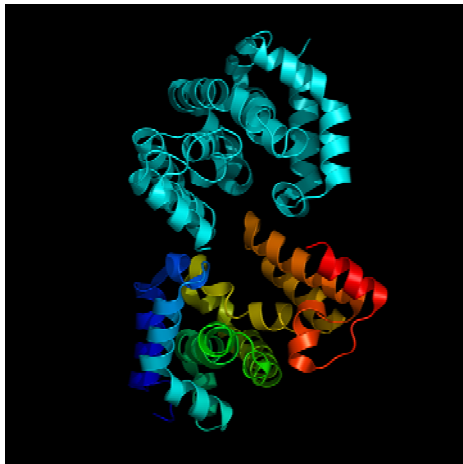
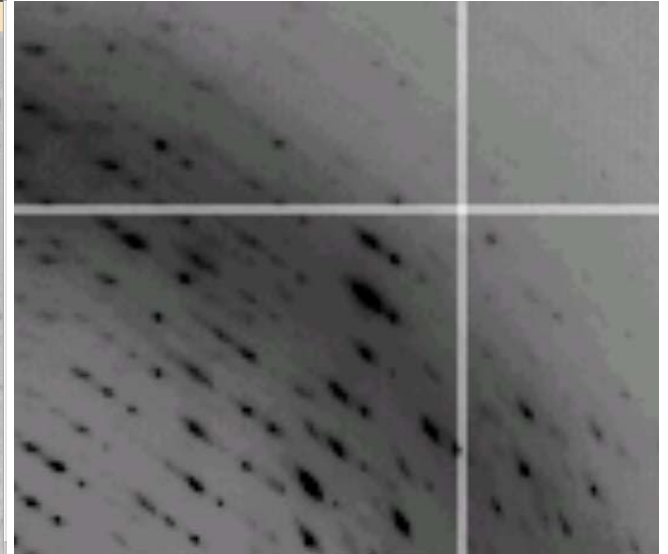
APC5905



APC24929

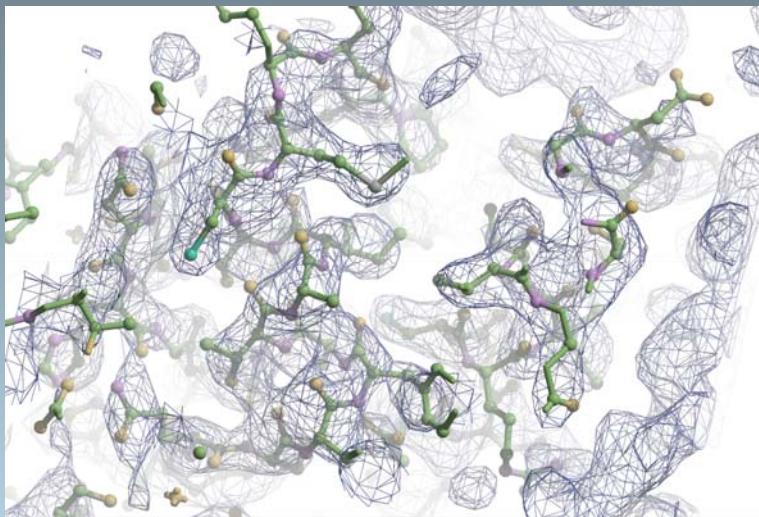


APC25179

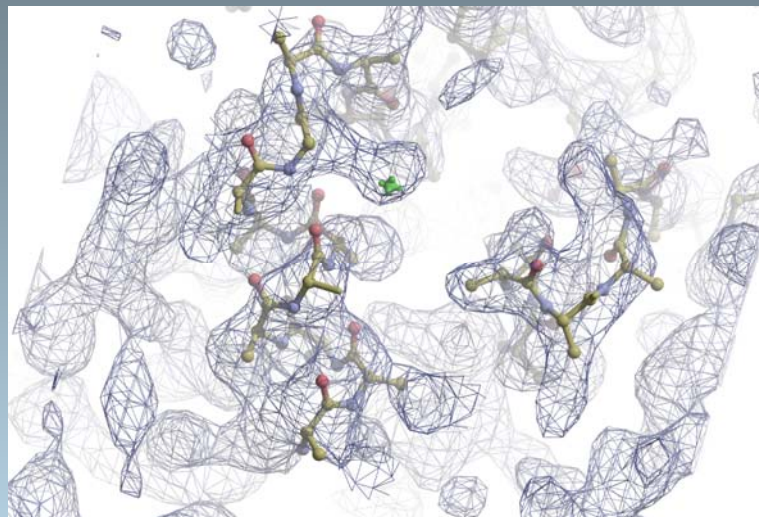


Structures solved in wrong target

APC81521 (ChangSoo)



Final model with right target
APC81521



Auto-build (resolve) model with wrong target
APC81501

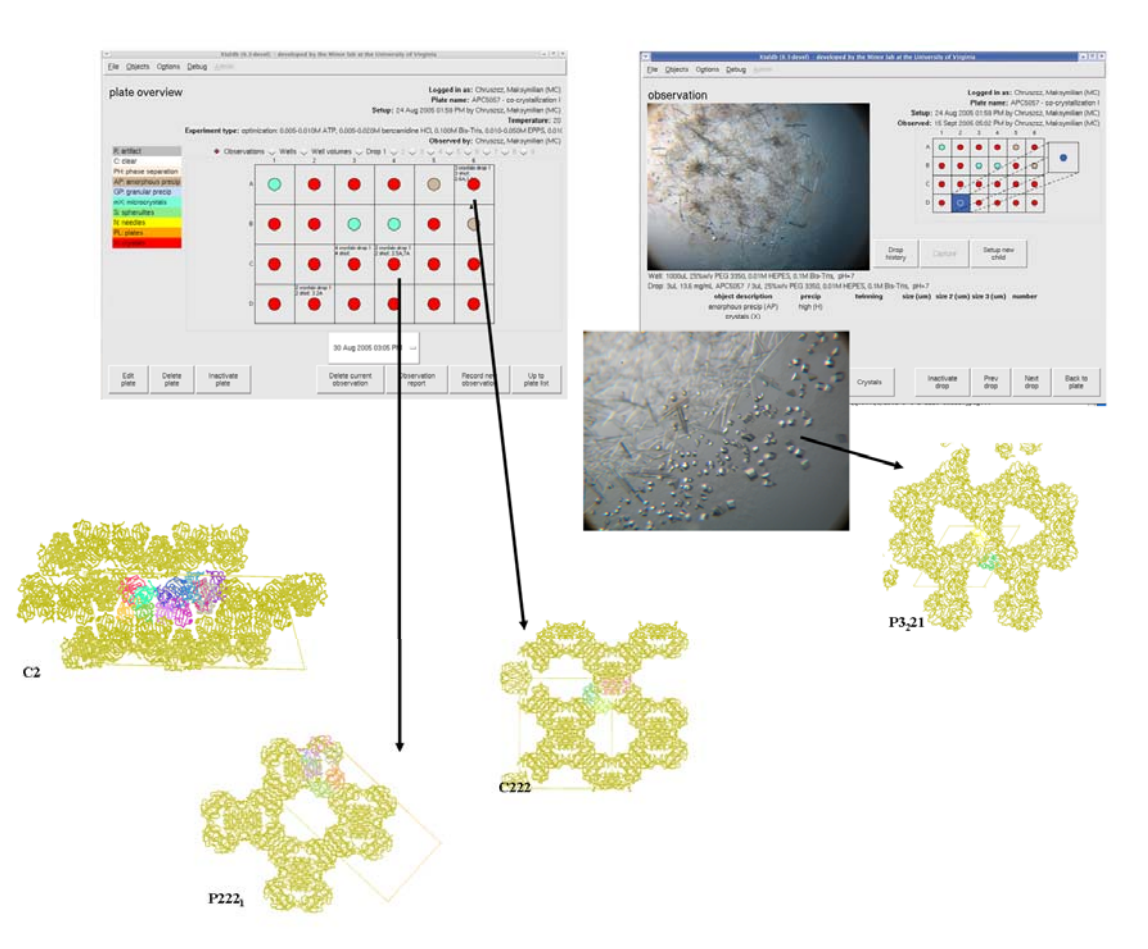
To figure out the right target at the resolution of 2.5Å

1. Manually build helices and stands;
2. Submit this model to dali to find out several similar structures in PDB;
3. Search targetDB(MCSG) using the sequence of these structures to find out the right target.

Twinning



Xtaldb – crystallization database



The image displays the Xtaldb software interface, which is used for managing crystallization data. It is divided into two main sections: 'plate overview' and 'observation'.

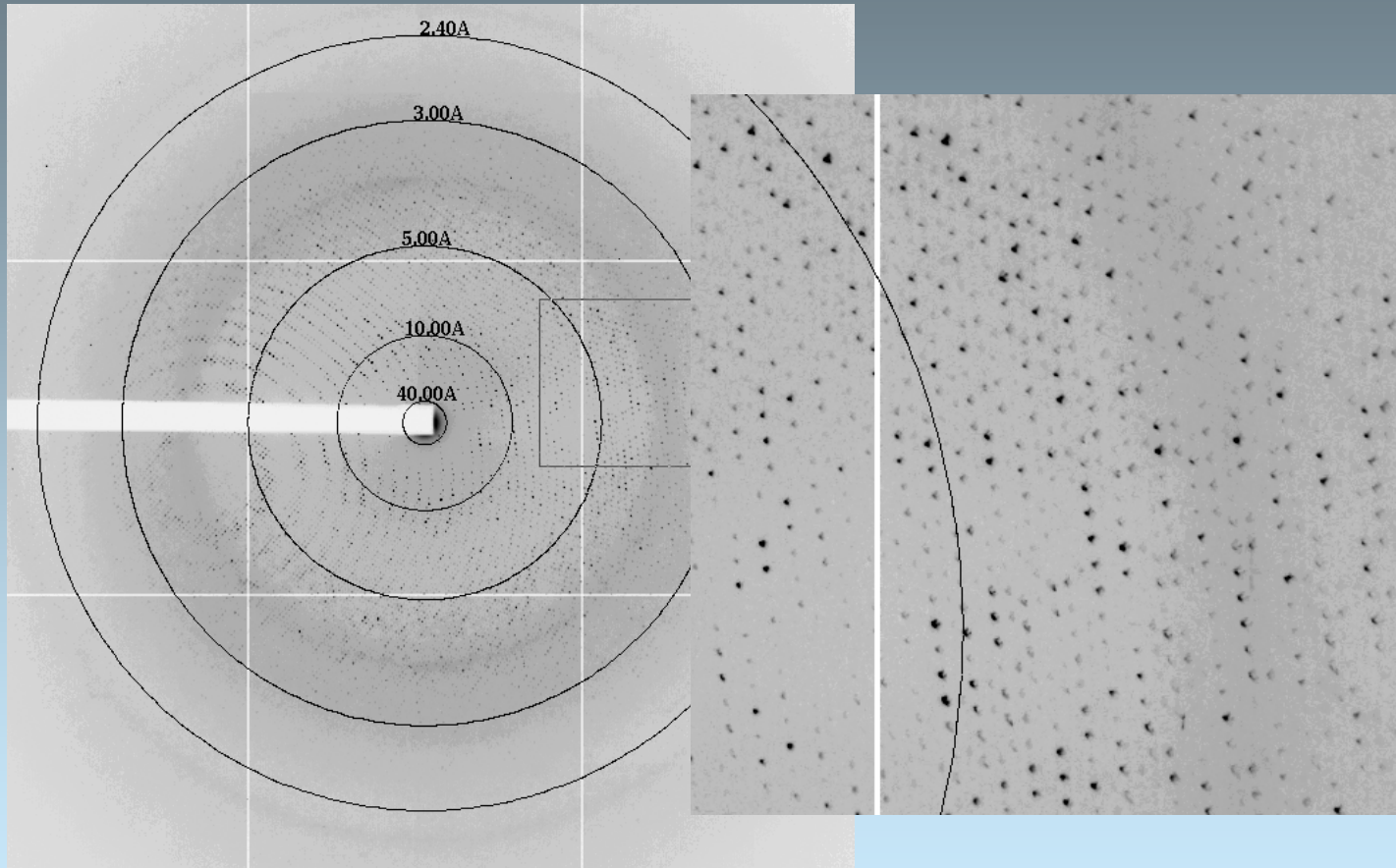
Plate Overview: This section shows a 4x8 grid of wells. A legend on the left indicates different well states: 'C' (blue), 'P' (red), 'O' (green), 'A' (yellow), 'S' (orange), 'I' (purple), and 'M' (brown). The 'Experiment type' is listed as 'optimization: 0.005-0.01M ATP, 0.005-0.02M benzamide HCl, 0.10M Bis-Tris, 0.10-0.20M DDM, 0.1M'. The 'Setup' date is '24 Aug 2005 01:58 PM' and the 'Observed' date is '30 Aug 2005 09:05 PM'.

Observation: This section shows a micrograph of a well. Below the micrograph, there is a table with columns for 'subject description', 'precip', 'solubility', 'size (um)', 'size 2 (um)', 'size 3 (um)', and 'number'. The 'subject description' is 'amorphous precip (AP) crystals (C)'. The 'precip' is 'high (S)'. The 'solubility' is 'high (S)'. The 'size (um)' is '10000'. The 'size 2 (um)' is '2000'. The 'size 3 (um)' is '1000'. The 'number' is '7'.

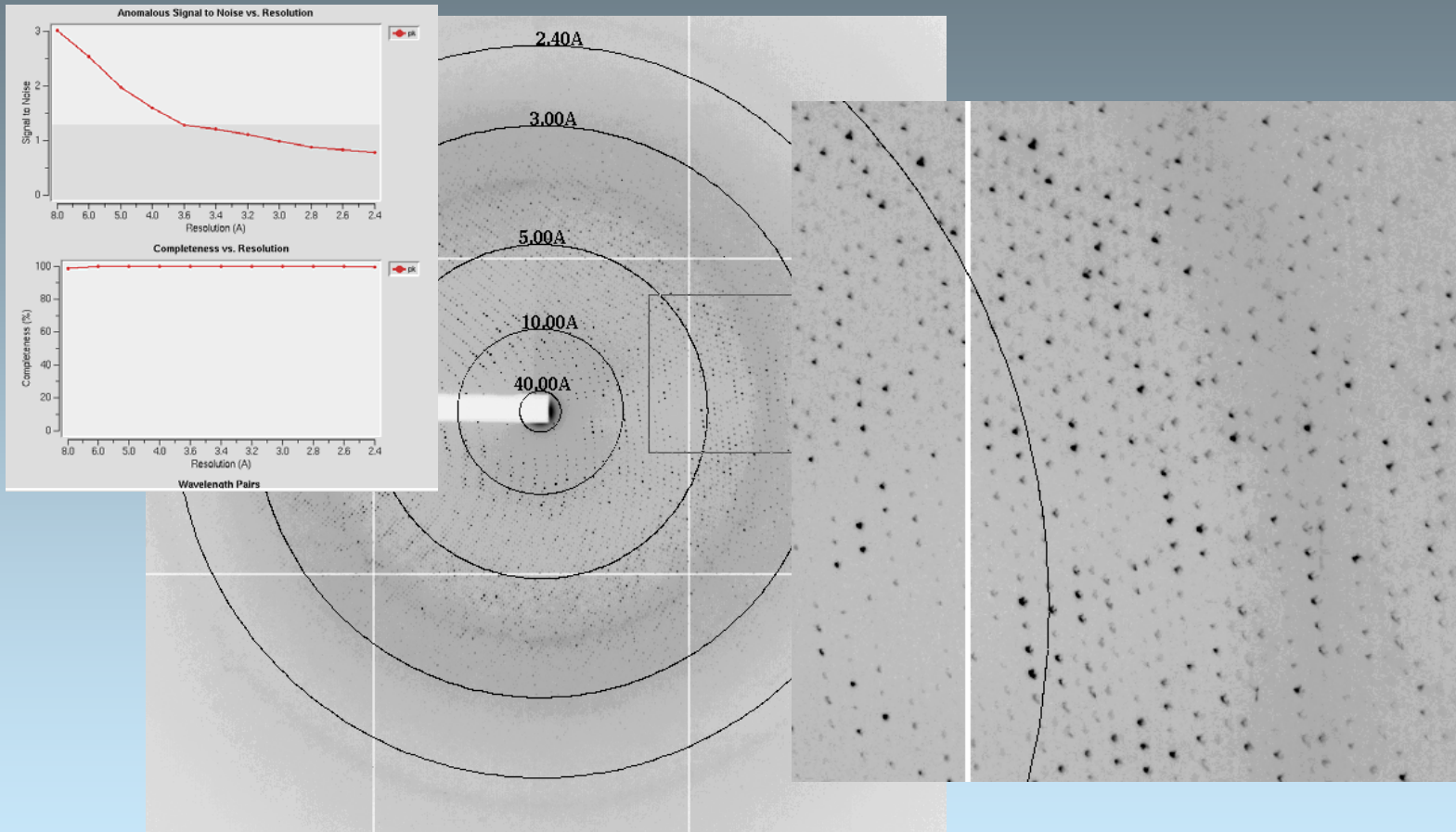
Arrows from the 'plate overview' and 'observation' sections point to four 3D molecular models:

- C2:** A large, complex, multi-colored structure.
- P222₁:** A smaller, more compact structure.
- C222:** A structure with a central core and three arms.
- P3₂1:** A structure with a central core and two arms.

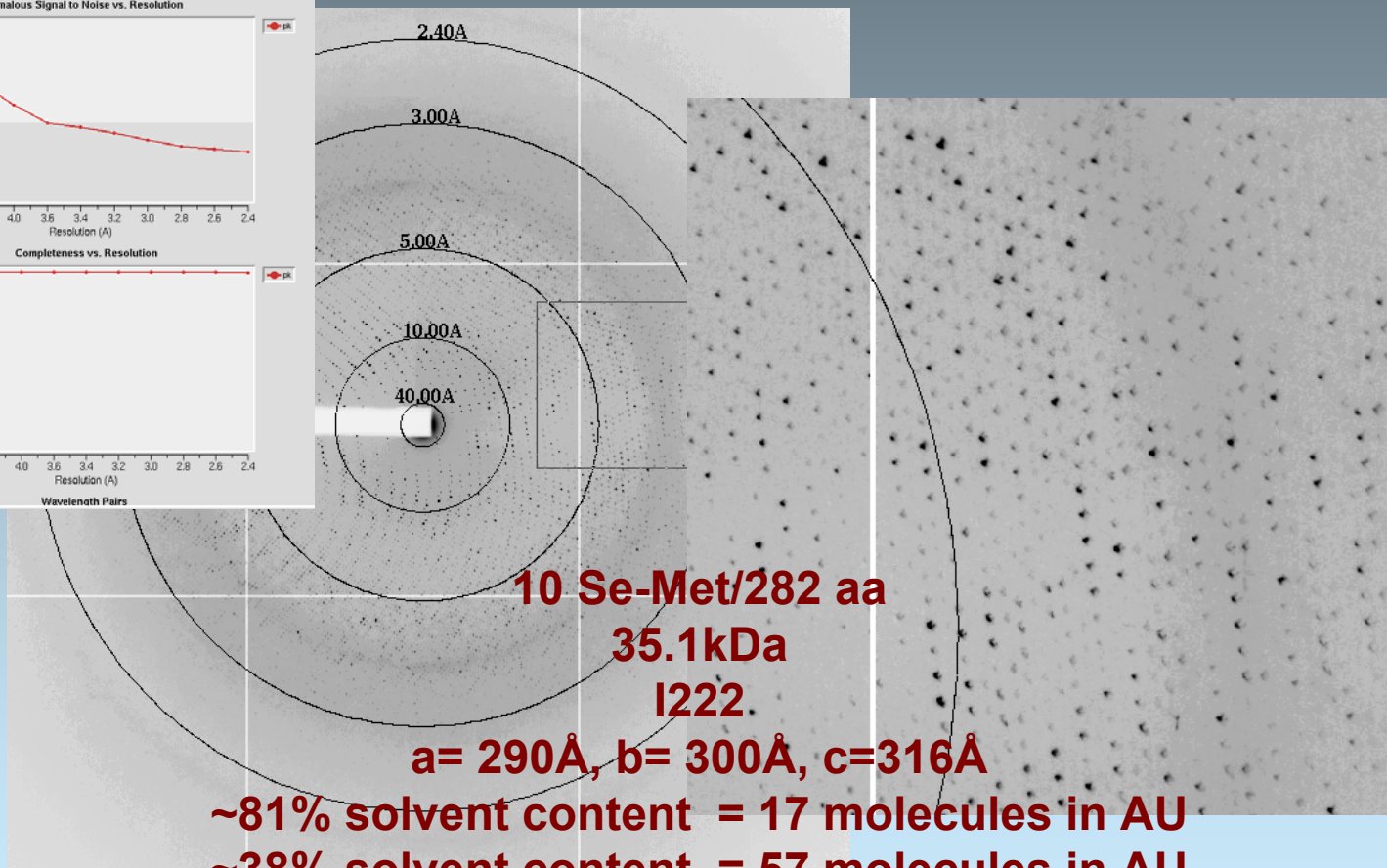
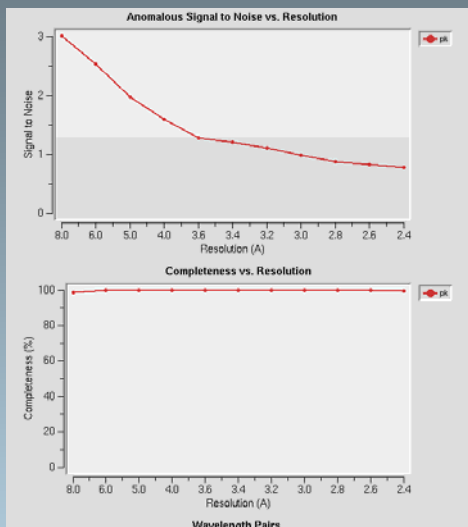
TM1086 (APC4579)



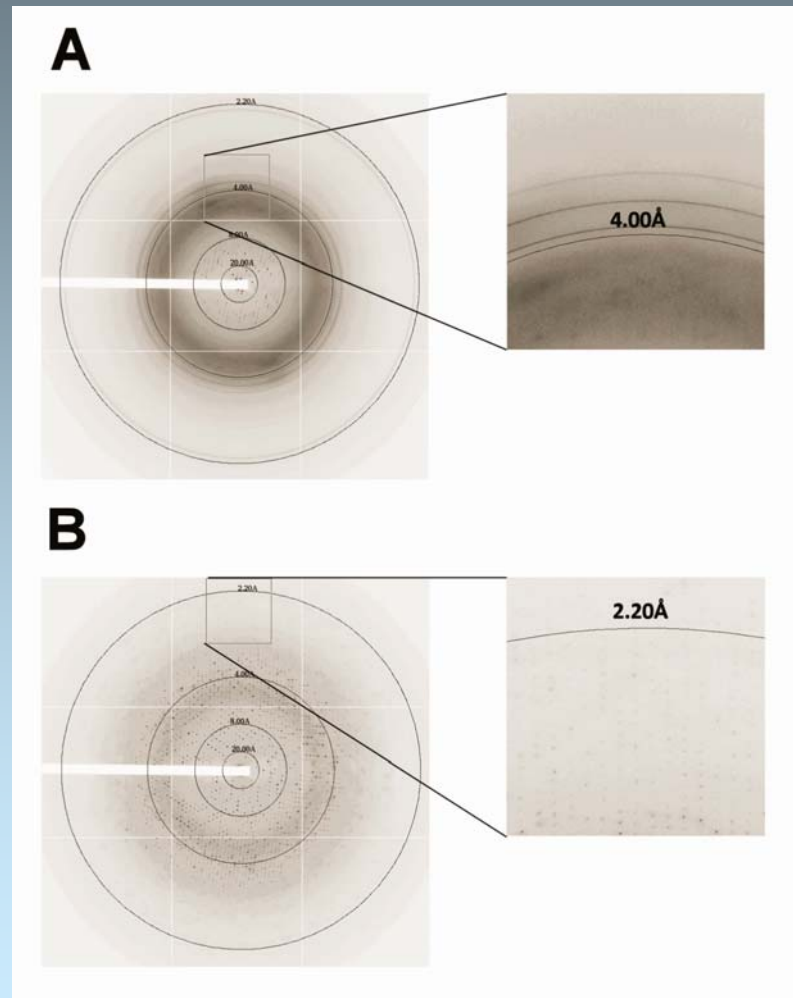
TM1086 (APC4579)



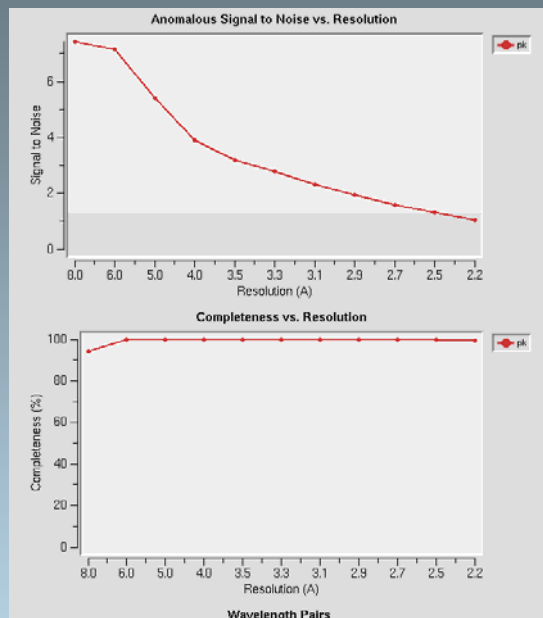
TM1086 (APC4579)



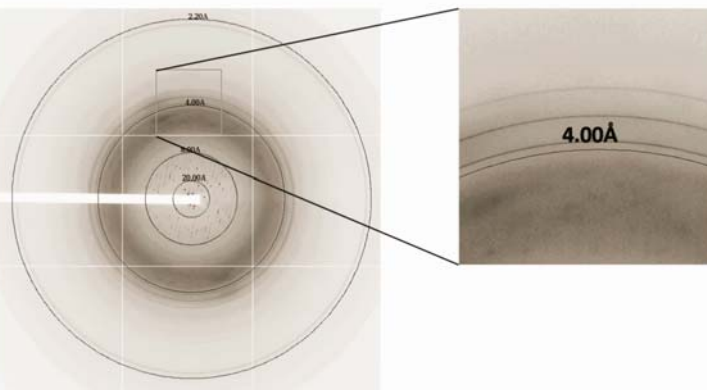
TM1086 (APC4579)



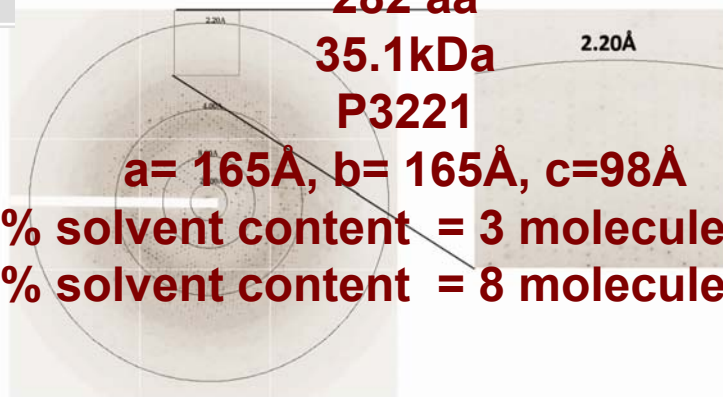
TM1086 (APC4579)



A



B



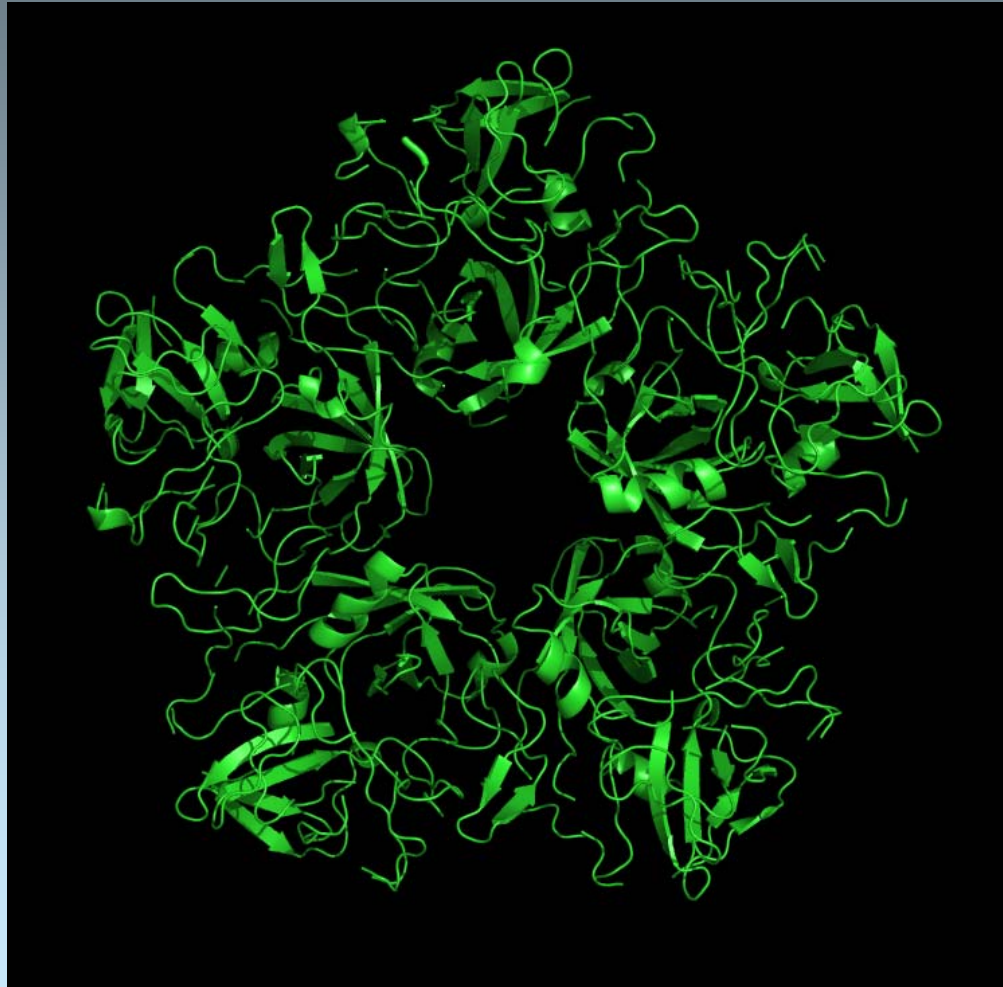
282 aa
35.1kDa
P3221

a= 165Å, b= 165Å, c=98Å

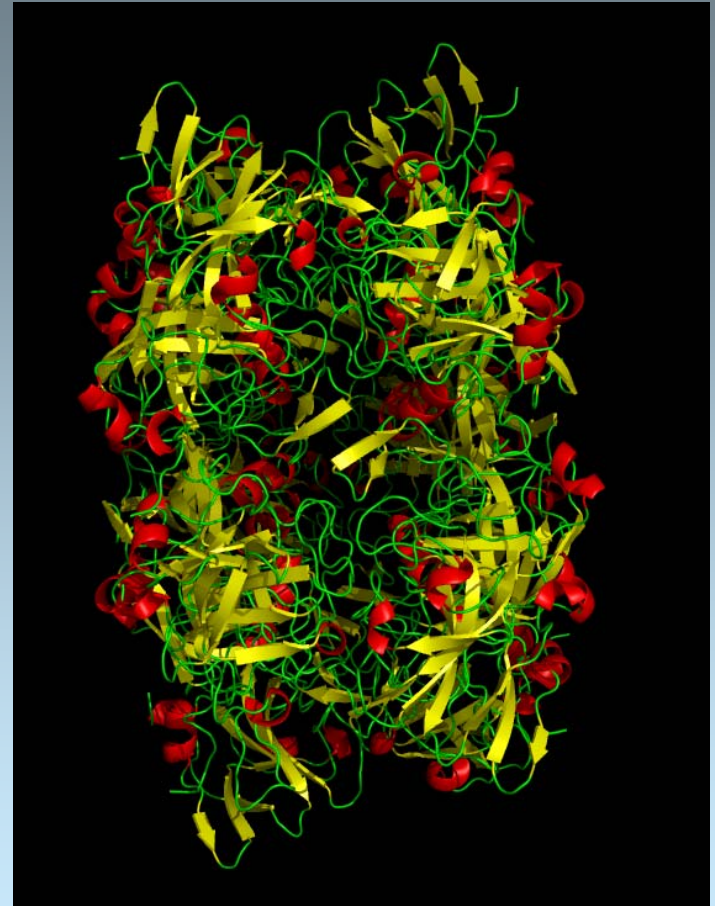
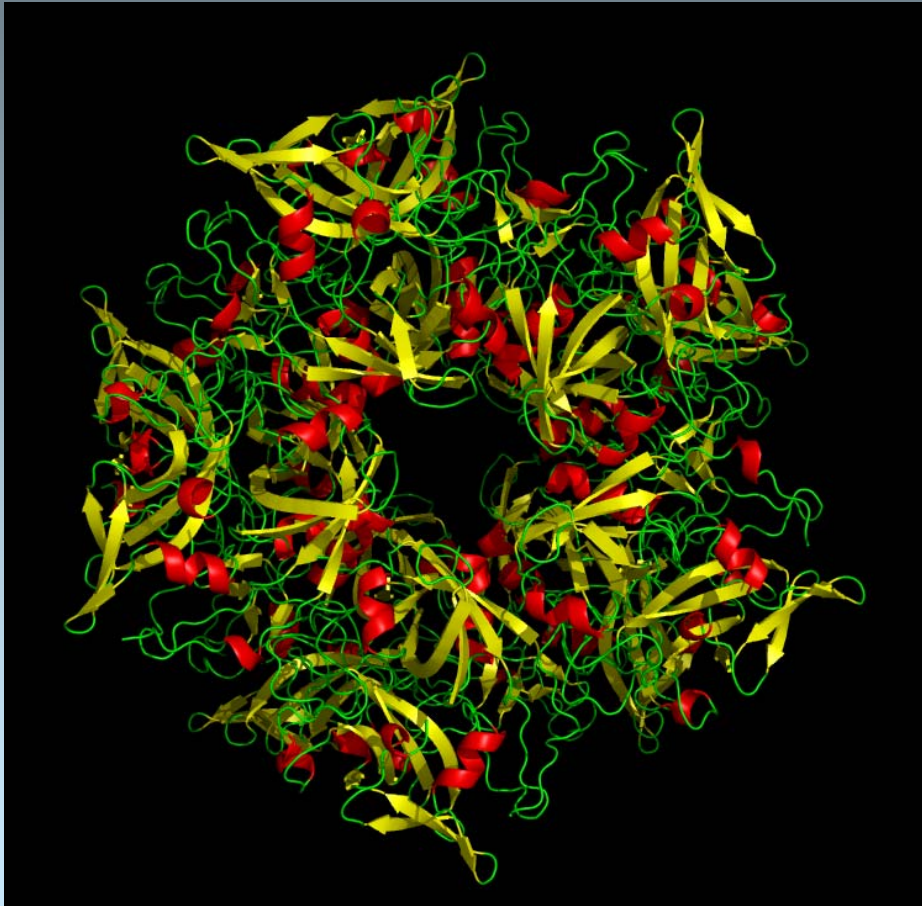
~78% solvent content = 3 molecules in AU

~40% solvent content = 8 molecules in AU

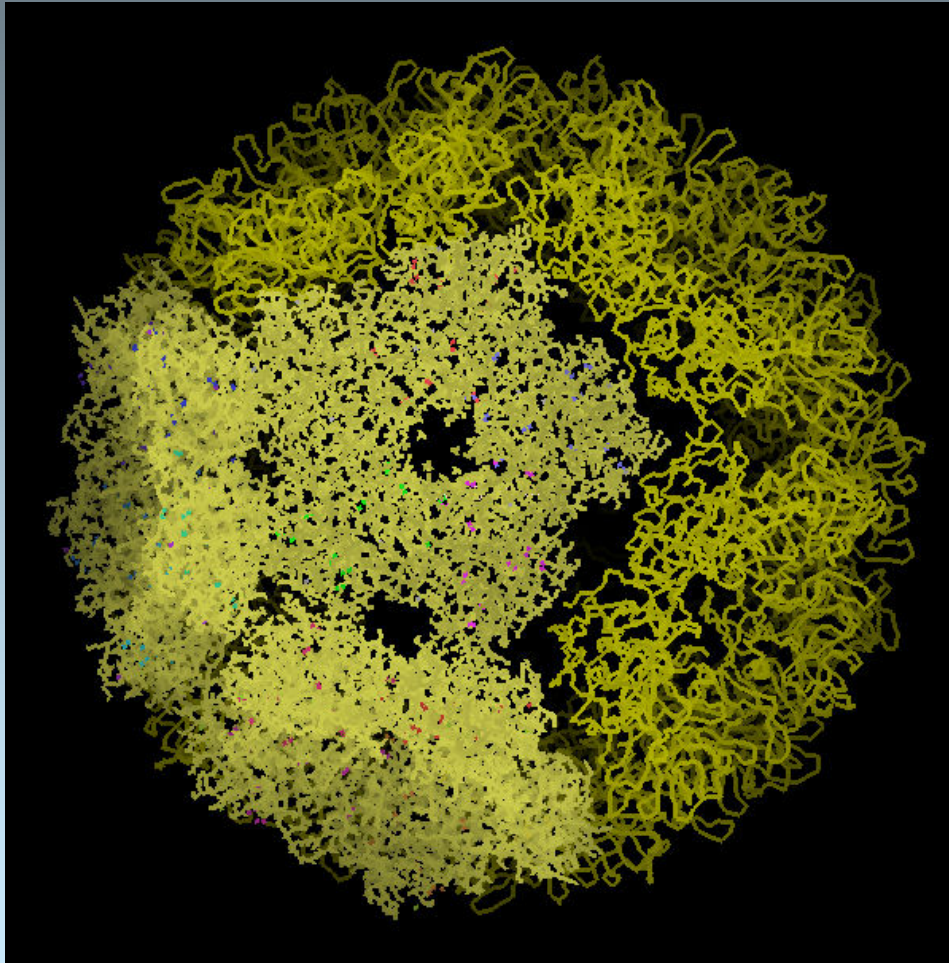
TM1086 (APC4579)



TM1086 (APC4579)

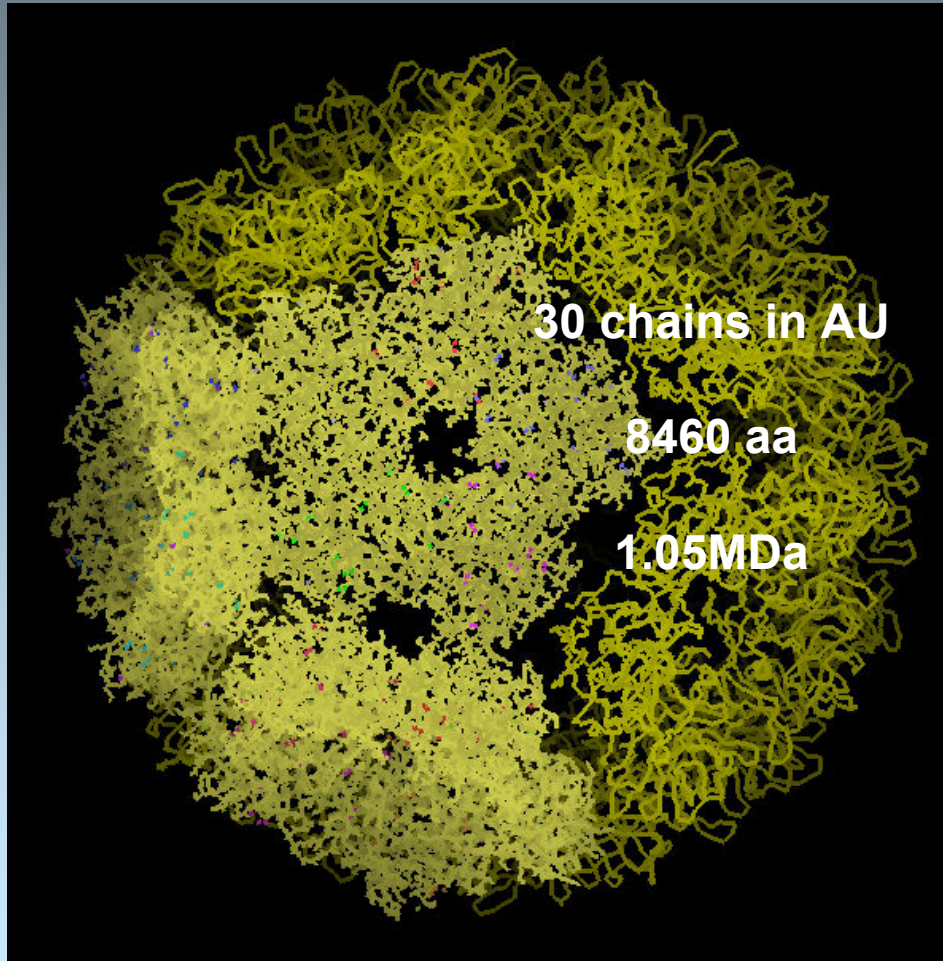


I222 crystal form



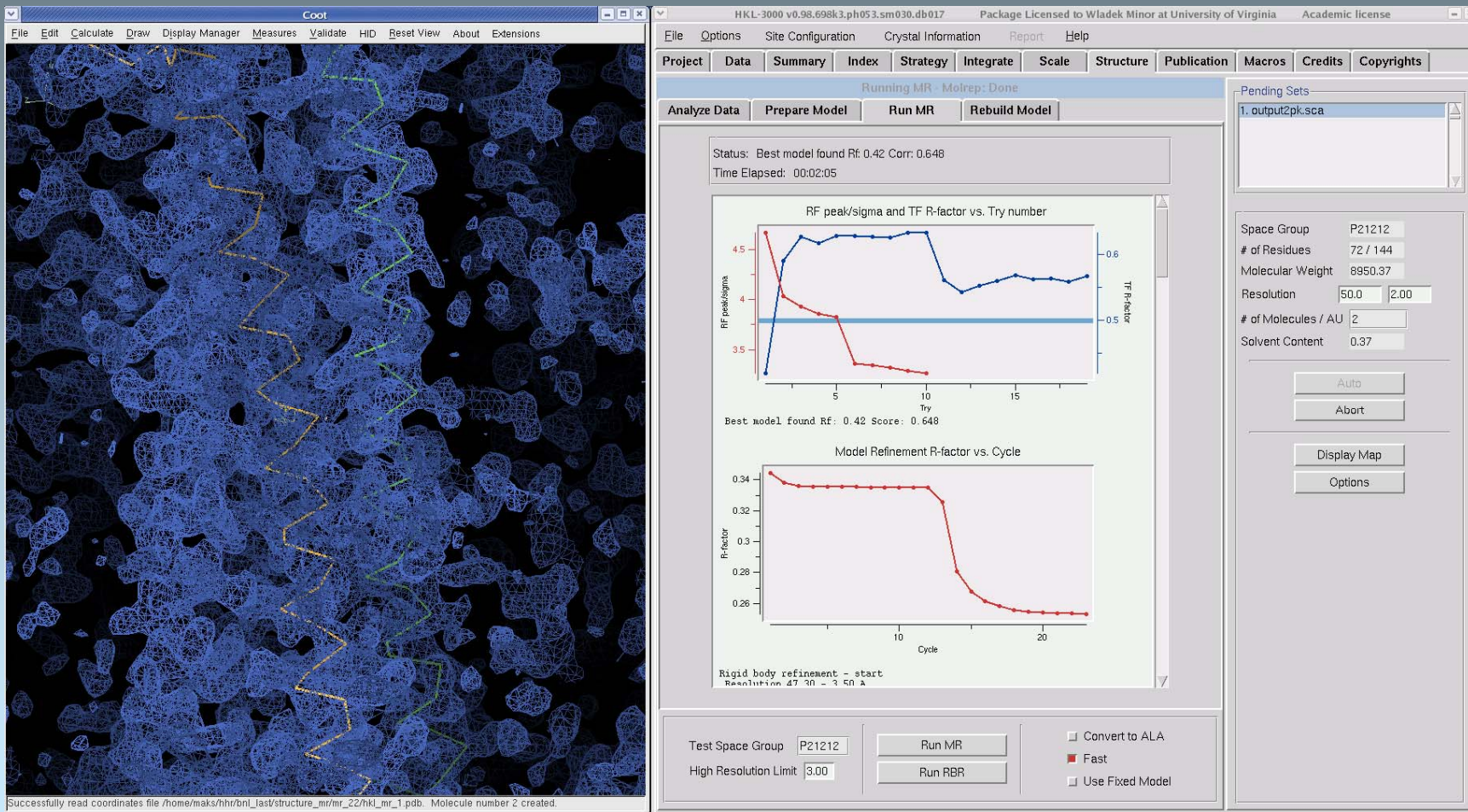
~210 Å

I222 crystal form



~210 Å

Molecular Replacement



The screenshot displays the Coot software interface for molecular replacement. The main window shows a 3D model of a protein structure in blue mesh, with a yellow zig-zag line indicating the path of the molecule. The status bar at the bottom reads: "Successfully read coordinates file /home/maks/hhr/bnl_last/structure_mr/mr_22/hkl_mr_1.pdb: Molecule number 2 created."

The right-hand panel shows the "Running MR - Molrep: Done" status. The "Status" section indicates: "Best model found Rf: 0.42 Corr: 0.648" and "Time Elapsed: 00:02:05". Below this are two plots:

RF peak/sigma and TF R-factor vs. Try number

Try	RF peak/sigma	TF R-factor
1	4.5	0.45
2	4.0	0.55
3	3.8	0.55
4	3.7	0.55
5	3.6	0.55
6	3.5	0.55
7	3.4	0.55
8	3.3	0.55
9	3.3	0.55
10	3.3	0.55
11	3.3	0.55
12	3.3	0.55
13	3.3	0.55
14	3.3	0.55
15	3.3	0.55
16	3.3	0.55
17	3.3	0.55
18	3.3	0.55
19	3.3	0.55
20	3.3	0.55

Best model found Rf: 0.42 Score: 0.648

Model Refinement R-factor vs. Cycle

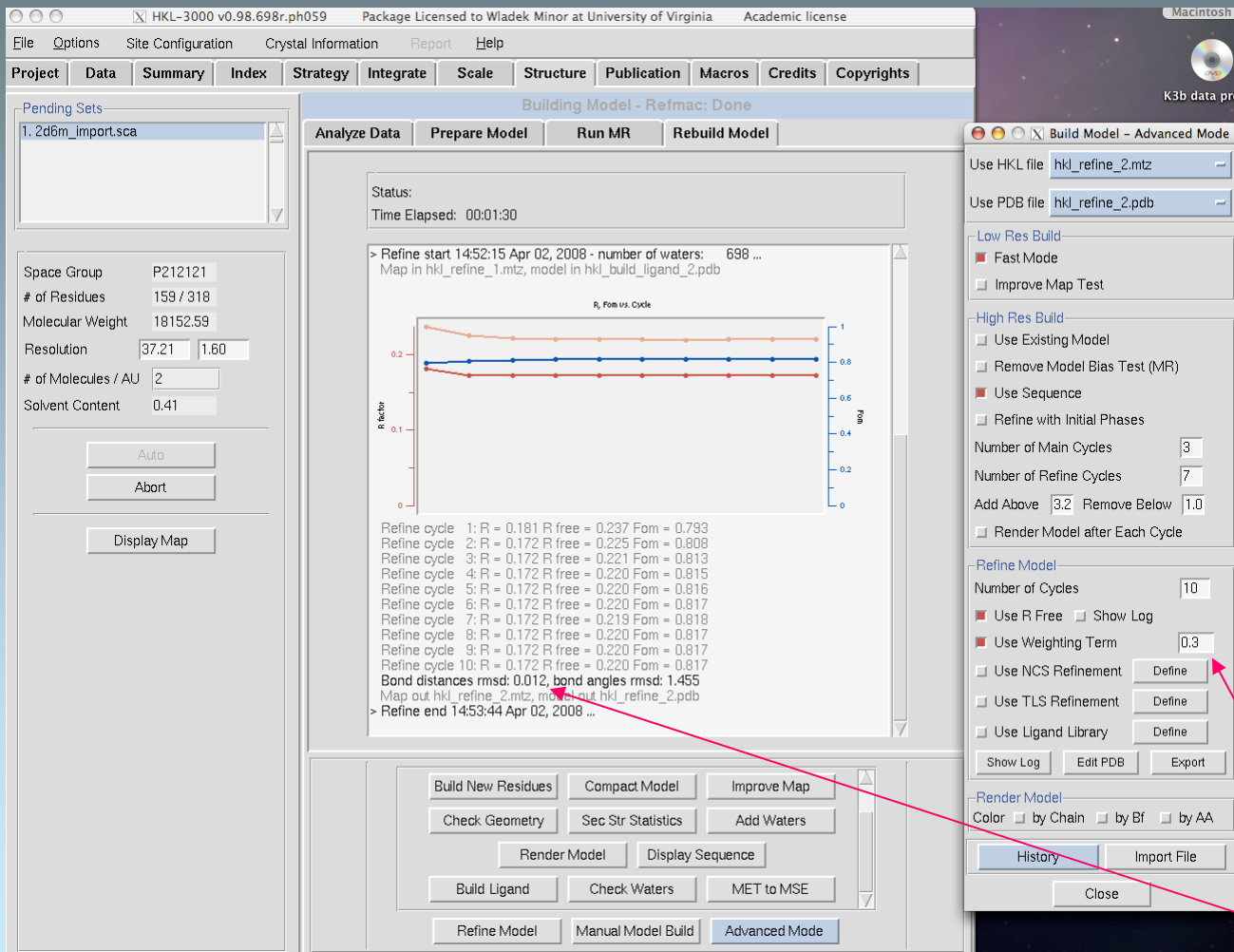
Cycle	R-factor
1	0.34
2	0.33
3	0.33
4	0.33
5	0.33
6	0.33
7	0.33
8	0.33
9	0.33
10	0.33
11	0.33
12	0.33
13	0.28
14	0.27
15	0.26
16	0.26
17	0.26
18	0.26
19	0.26
20	0.26

Rigid body refinement - start
Resolution 47.30 - 3.50 Å

The right-hand panel also includes a "Pending Sets" list with "1. output2pk.sca". Below this are fields for "Space Group" (P21212), "# of Residues" (72 / 144), "Molecular Weight" (8950.37), "Resolution" (50.0 / 2.00), "# of Molecules / AU" (2), and "Solvent Content" (0.37). Buttons for "Auto", "Abort", "Display Map", and "Options" are present.

At the bottom of the right-hand panel, there are controls for "Test Space Group" (P21212), "High Resolution Limit" (3.00), and checkboxes for "Convert to ALA", "Fast", and "Use Fixed Model". Buttons for "Run MR" and "Run RBR" are also visible.

Fast refinement (full control)



The screenshot displays the HKL-3000 software interface for X-ray crystallography. The main window shows the 'Building Model - Refmac: Done' status. A graph titled 'R_i, Fom vs. Cycle' plots the R factor and Fom over 10 refinement cycles. The R factor (left y-axis, 0 to 0.2) and Fom (right y-axis, 0 to 1) both decrease and stabilize around cycle 5. Below the graph, a table lists the statistics for each cycle.

Refine cycle	R	R free	Fom
1	0.181	0.237	0.793
2	0.172	0.225	0.808
3	0.172	0.221	0.813
4	0.172	0.220	0.815
5	0.172	0.220	0.816
6	0.172	0.220	0.817
7	0.172	0.219	0.818
8	0.172	0.220	0.817
9	0.172	0.220	0.817
10	0.172	0.220	0.817

The 'Advanced Mode' dialog box is open on the right, showing settings for 'Low Res Build', 'High Res Build', 'Refine Model', and 'Render Model'. The 'Use Weighting Term' checkbox is checked, and the 'Weighting scheme' is set to 0.3. A red arrow points to this field.

Additional information from the interface:

- Space Group: P212121
- # of Residues: 159 / 318
- Molecular Weight: 18152.59
- Resolution: 37.21 / 1.60
- # of Molecules / AU: 2
- Solvent Content: 0.41
- Bond distances rmsd: 0.012, bond angles rmsd: 1.455

Unidentified densities ?

BMC Structural Biology 2006, 6:27

http://www.biomedcentral.com/1472-6807/6/27

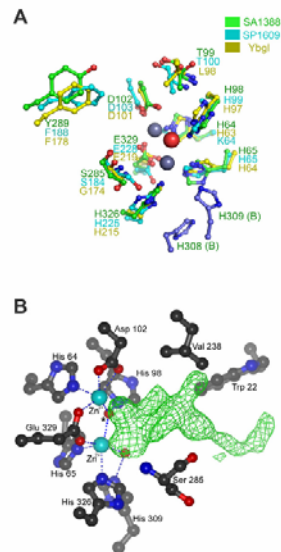


Figure 5
The putative active site of SA1388. (A) Conserved residues at the putative active sites of SA1388 (green), SP1609 (cyan) and *E. coli* ybgI (yellow). The two zinc ions of SA1388 and the bridging water molecule are shown as spheres and the equivalent residues in the three structures are labelled in their respective colors. The two histidine residues of SA1388 that are contributed by the adjacent subunit are colored in navy blue. (B) Electron density of the endogenous ligand (Fo-Fc map contoured at 3.0 σ) that is directly ligated to the active site zinc ions in SA1388. Residues interacting with the two zinc atoms are shown and the metal coordinating interactions are indicated by dotted lines. The bridging water molecule between the two Zn atoms is indicated by an asterisk (*).

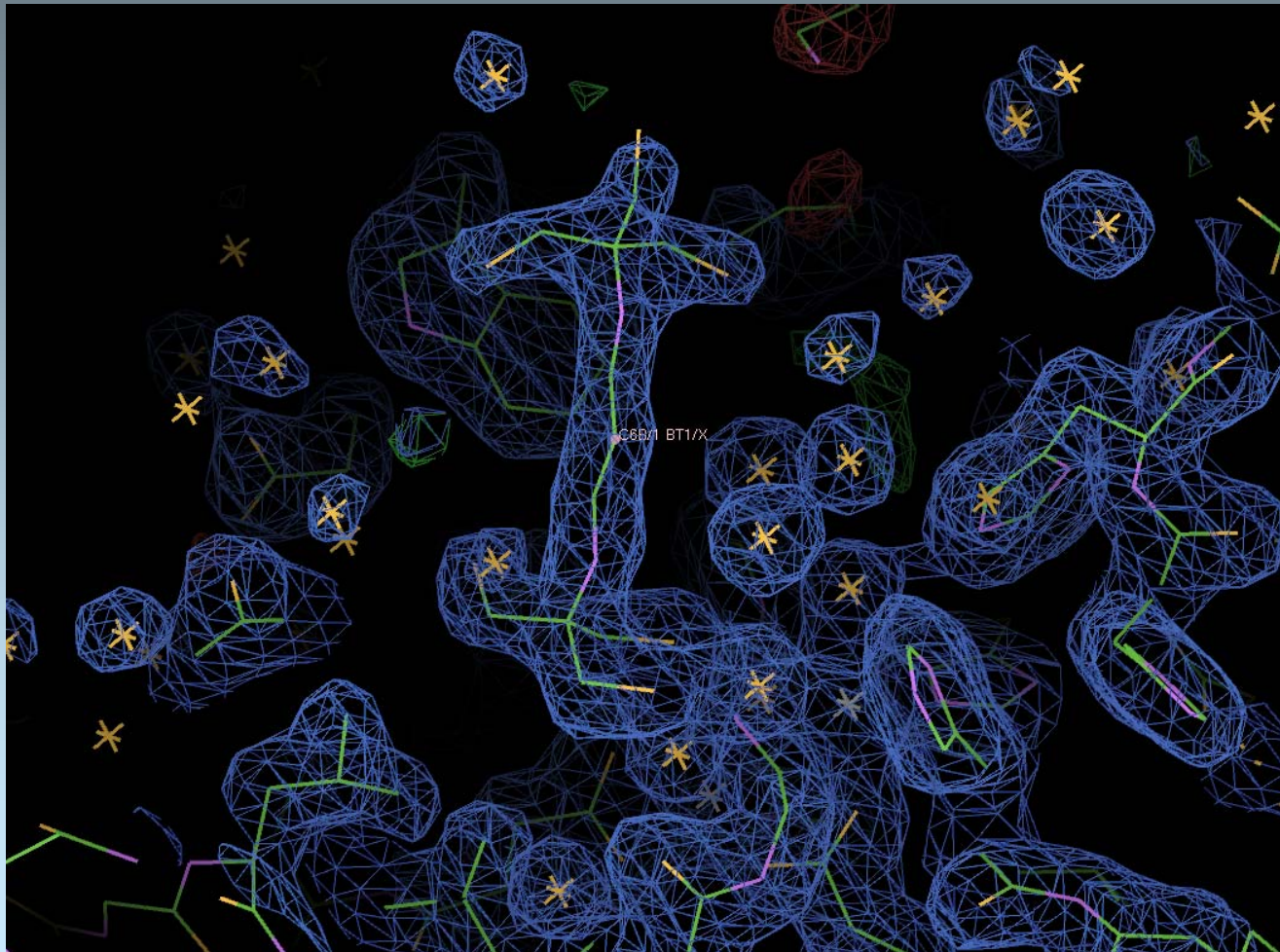
cryoprotection and therefore conclude that the observed ligand must in fact be an endogenously bound ligand that was co-purified after recombinant overexpression. The contours of the difference electron density map (Figure 5B) after final refinement suggests the presence of a head group directly ligated to the metal ions and a mostly aliphatic tail that has few specific polar interactions with the surrounding protein residues (Figure 5B). Two aromatic residues, a tyrosine (Y289, shown in Figure 5A) and a tryptophan (W22) lie in close proximity to the bound ligand and may be involved in ligand binding.

Discussion

Although homologs of both PII and NIF3 proteins are found ubiquitously in all three kingdoms of life, the function of SA1388 and its homologs that contain both PII-like and NIF3-like domains remain completely unknown. The fusion of the two proteins in one peptide chain indicates a functional coupling of these two proteins. The structural features revealed in the present study, such as the cage-like hexameric toroid structure with its NIF3 domains as walls and the two PII-like domain trimers as lids, the dinuclear metal site, and the intrinsically bound ligand, may provide certain clues of its potential function. The primary role of the nitrogen regulatory PII proteins is to integrate various intracellular carbon and nitrogen signals by regulating enzymes involved in nitrogen assimilation [10]. PII exerts its regulatory effects by undergoing different post translational modifications, such as uridylation [24] and phosphorylation [25] by various modifying enzymes in response to the primary cellular nitrogen signal glutamine. Structures of several PII and PII-like proteins have been solved including glbB (from *E. coli*, *T. thermophilus*, *Synecoccus* and *Herbaspirillum*), GlnA (*E. coli*), glnK (*E. coli*), HisG (*E. coli* and *M. tuberculosis*), CutA1 (human, rat and *T. maritima*), and a hypothetical protein from COG1993. Effectors of PII include transcription factors [10,11], signalling proteins e.g., histidine kinases [26], and metabolic enzymes like glutamine synthase [27]. Apart from its primary signalling modulator glutamine, PII proteins are known to bind a range of small molecule effectors such as ATP, UMP, and 2-ketoglutarate, which affects its function antagonistically to glutamine (reviewed in [10]). It is highly likely that the trimeric PII domain of SA1388 also plays a ligand induced signalling role and probably regulates the function of the NIF3-like domains.

Unlike PII domains, the NIF3-like domains have only recently begun to be structurally characterized, and their structure-function relationship remains sketchy. NIF3-like proteins are ubiquitously conserved from bacteria to higher eukaryotes [28]. They have been defined in uniprot database as the uncharacterized protein family UPP0135 that has 64 homologs [29]. Several homologs of this fam-

Unidentified densities



Fast refinement

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File Options Site Configuration Crystal Information Report Help

Project Data Summary Index Strategy Integrate Scale Structure Publication Macros Credits Copyrights

Pending Sets
1. 2ukd_import.sca

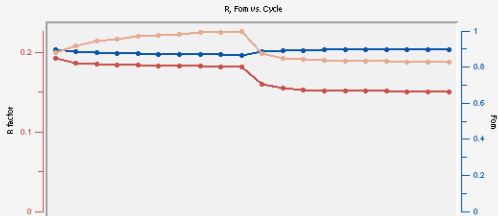
Space Group P41212
of Residues 194 / 388
Molecular Weight 21943.73
Resolution 37.21 | 2.20
of Molecules / AU 2
Solvent Content 0.30

Auto
Abort
Display Map

Building Model - Refmac: Done
Analyze Data Prepare Model Run MR Rebuild Model

Status:
Time Elapsed: 00:00:46

> Refine start 15:43:51 Apr 02, 2008 - number of waters: 111 ...
Map in hkl_import.mtz, model in hkl_import.pdb

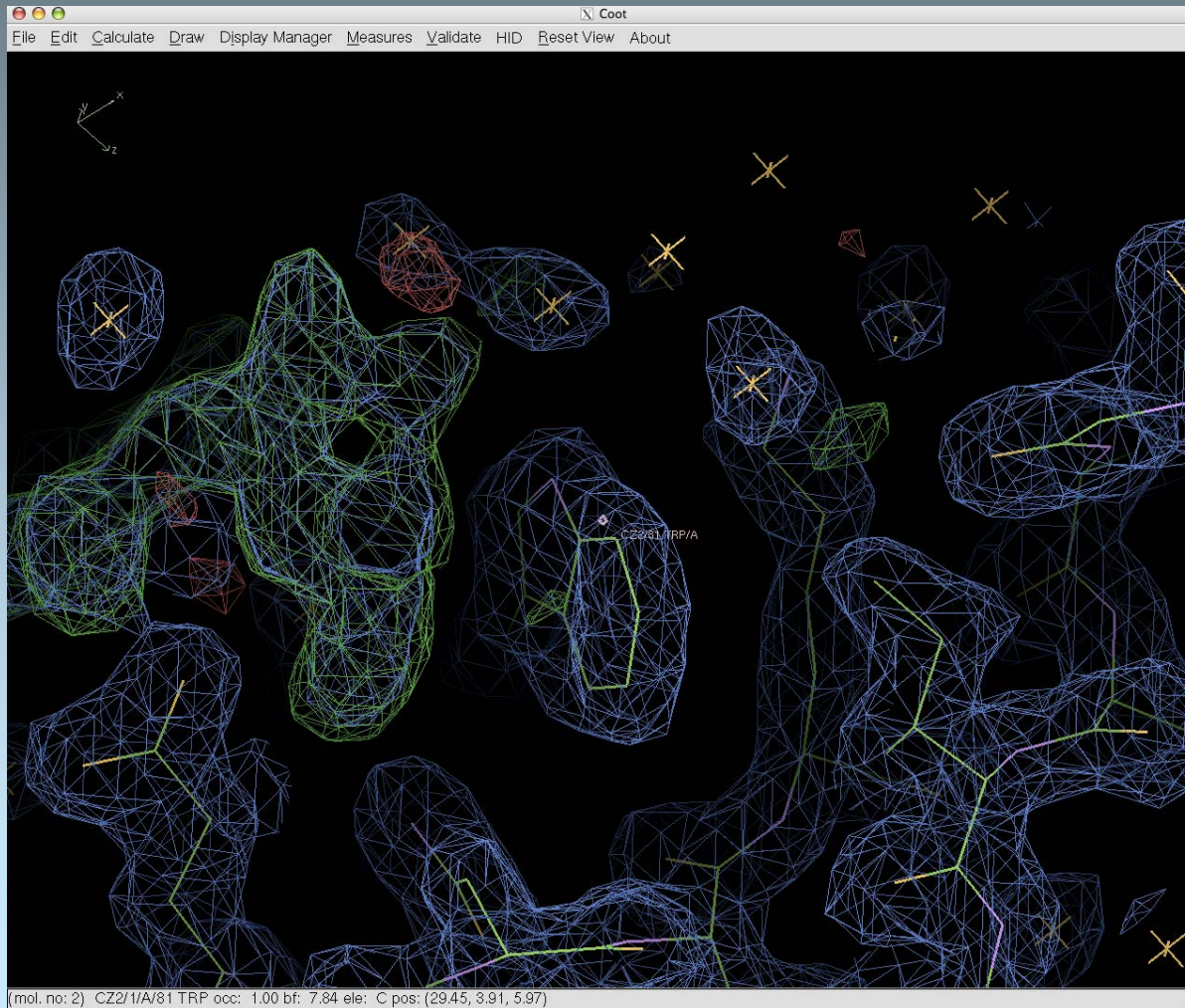


Refine cycle 1: R = 0.193 R free = 0.200 Fom = 0.895
Refine cycle 2: R = 0.187 R free = 0.208 Fom = 0.887
Refine cycle 3: R = 0.185 R free = 0.214 Fom = 0.881
Refine cycle 4: R = 0.184 R free = 0.217 Fom = 0.877
Refine cycle 5: R = 0.184 R free = 0.220 Fom = 0.875
Refine cycle 6: R = 0.183 R free = 0.222 Fom = 0.872
Refine cycle 7: R = 0.183 R free = 0.223 Fom = 0.871
Refine cycle 8: R = 0.183 R free = 0.225 Fom = 0.869
Refine cycle 9: R = 0.182 R free = 0.225 Fom = 0.868
Refine cycle 10: R = 0.182 R free = 0.227 Fom = 0.867

Bond distances rmsd: 0.019, bond angles rmsd: 1.758
Map out hkl_refine_1.mtz, model out hkl_refine_1.pdb
> Refine end 15:44:38 Apr 02, 2008 ...

Build New Residues Compact Model Improve Map
Check Geometry Sec Str Statistics Add Waters
Render Model Display Sequence
Build Ligand Check Waters MET to MSE
Refine Model Manual Model Build Advanced Mode

Unidentified density



Integration

HKL-3000 is a pipeline component

Pepdb

expression & purification

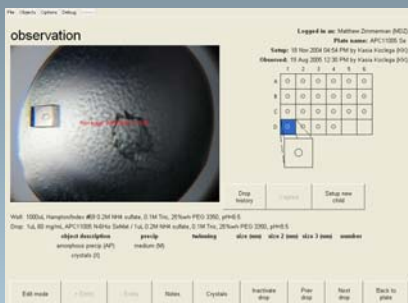
PepDB @ UNIVERSITY of VIRGINIA

Expression: 24 records found, page 1 of 2

No.	Project	Macromolecule	expression_id	clone_id	date_exp	expression_level	inhibitory_level
1	TEST		112	2	01/19/05	5	5
2	APC11091	APC11091	111	1	02/09/05	3	1
3	APC4973	APC4973	110	4	03/06/04	3	1
4	APC4542	APC4542	109	4	03/06/04	3	1
5	APC4251	APC4252	108	4	03/06/04	3	1
6	APC4973	APC4973	107	4	03/06/04	3	1
7	APC4542	APC4542	106	4	03/06/04	3	1
8	APC4973	APC4973	105	4	03/06/04	3	1
9	APC4973	APC4973	104	4	12/11/03	3	1
10	APC4252	APC4252	103	4	12/11/03	3	1
11	APC11091	APC11091	102	4	12/11/03	4	3
12	APC11091	APC11091	101	4	12/11/03	4	3
13	APC11091	APC11091	100	4	07/04/03	4	3

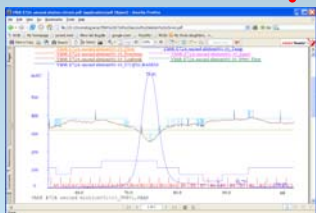
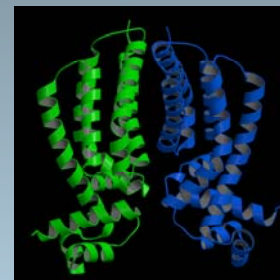
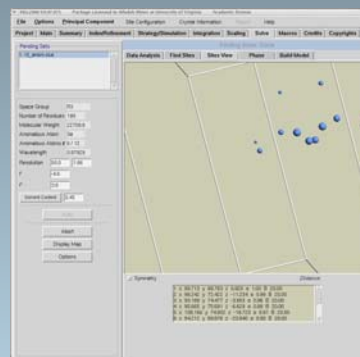
Xtaldb

crystallization



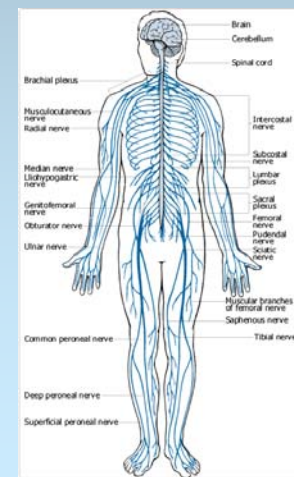
HKL-3000

Data collection,
diffraction & structure solution



Wetlab
chemicals & solutions

Other systems provide additional
contextual up- and downstream
information




Ligand libraries

Build Ligand

Use Standard Library
 Use Custom Library
 Use File

3D View



List of Ligands

code	name	type	# of atoms
ACE	acetyl	polymer	3
FOR	Formyl	polymer	2
BOC	TERT-BUTYLOXYCARBONYL GROUP	polymer	7
IVA	Isovaleric_acid	polymer	6
DFO	2,2-difluoro-3-hydroxatone	polymer	14
NME	N-methylamide	polymer	2
STA	STATINE	polymer	11
ETA	ethanolamine	polymer	4
TFA	TRIFLUOROACETYL GROUP	polymer	6
ANI	4-TRIFLUOROMETHYLANILINE	polymer	11
MPR	BETA-MERCAPTOPROPIONATE	polymer	5
DAM	N-METHYL-ALPHA-BETA-DEHYDROALANINE	polymer	6
ACB	2-AMINO-3-CARBONYLBUTANOIC ACID	polymer	9
ADD	2,6,8-TRIMETHYL-3-AMINO-9-BENZYL-9-M	polymer	23
CXM	N-CARBOXYMETHIONINE	polymer	11
DIP	DIPENTYLAMINE	polymer	11
BAL	BETA-ALANINE	polymer	5
MAN-b-D	beta_D_mannose	D-pyranose	12
NAG-b-D	beta_D_N-acetyl-Glucose	D-pyranose	15
SIA	sialic-acid	L-saccharid	21

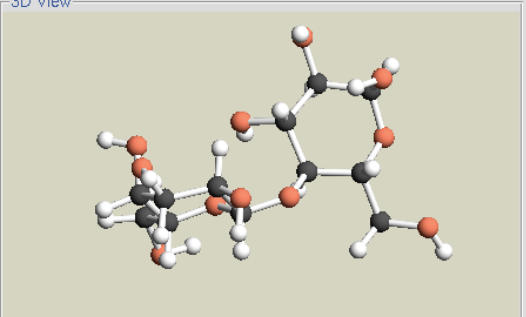
Ligand Id:

Done Cancel

Build Ligand

Use Standard Library
 Use Custom Library
 Use File

3D View



List of Ligands

code	name	type	# of atoms
BT1	BIS-TRIS PROPANE	non-polymer	19
NTS	NAPHTHALENE TRISULFONATE	non-polymer	22
ADP	ADENOSINE-5'-DIPHOSPHATE	non-polymer	27
LAT	LACTOSE	D-saccharid	23

Ligand Id:

Done Cancel

Automatic ligand building and refinement

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Building Model - Refmac

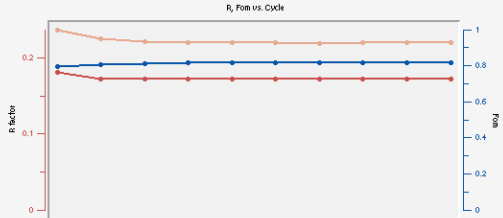
Analyze Data Prepare Model Run MR Rebuild Model

Status:
Time Elapsed: 00:01:21

> Build ligand start 14:34:57 Apr 02, 2008 ...
Cycle 1 - ligand build
Cycle 2 - ligand build
> Build ligand end 14:35:40 Apr 02, 2008

> Build ligand start 14:36:44 Apr 02, 2008 ...
Cycle 1 - ligand build
Cycle 2 - ligand build
> Build ligand end 14:37:30 Apr 02, 2008

> Refine start 14:52:15 Apr 02, 2008 - number of waters: 698 ...
Map in hkl_refine_1.mtz, model in hkl_build_ligand_2.pdb



Refine cycle	R	R free	Fom
1	0.181	0.237	0.793
2	0.172	0.225	0.808
3	0.172	0.221	0.813
4	0.172	0.220	0.815

Build New Residues Compact Model Improve Map

Check Geometry Sec Str Statistics Add Waters

Render Model Display Sequence

Build Ligand Check Waters MET to MSE

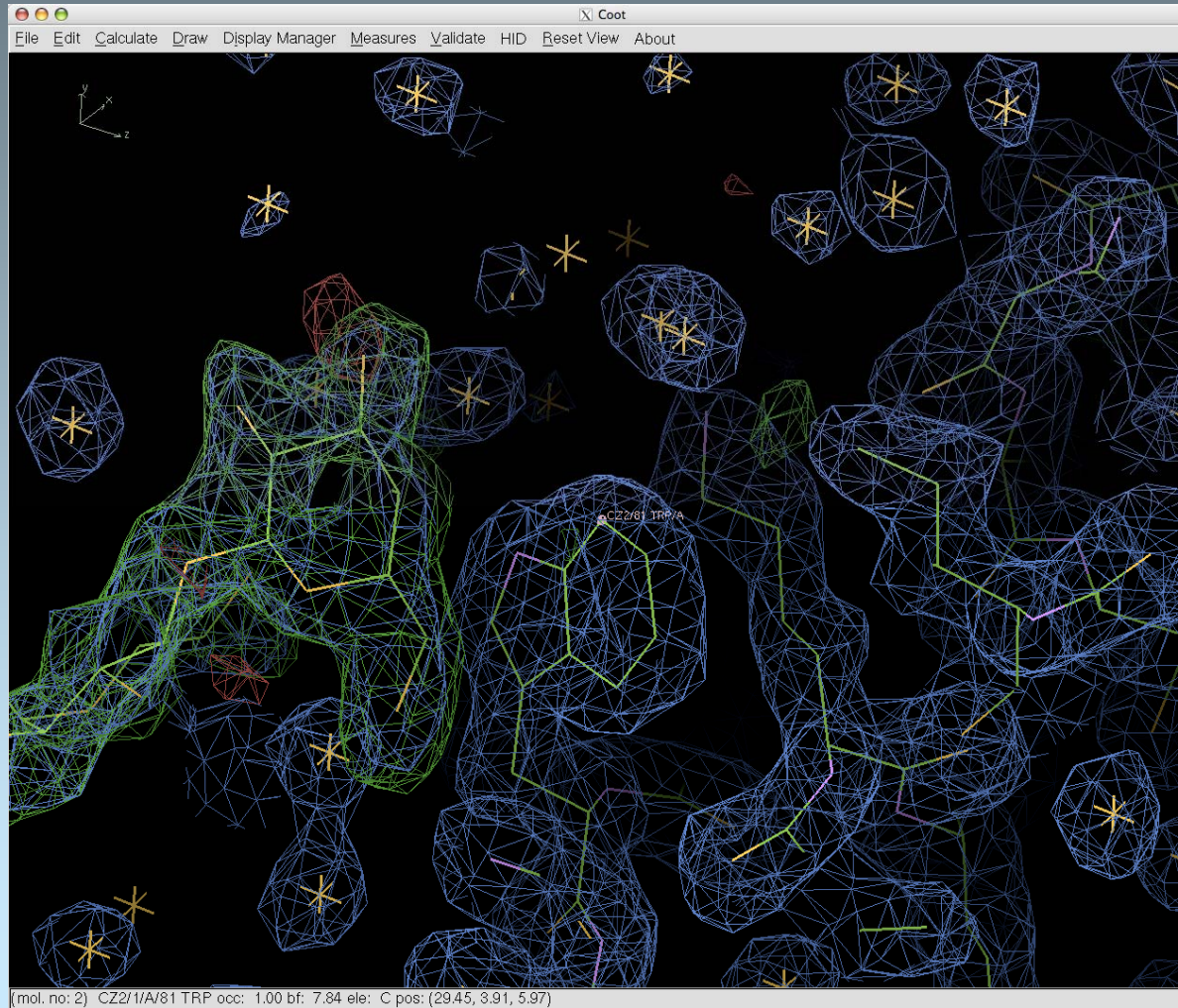
Refine Model Manual Model Build Advanced Mode

Pending Sets
1. 2d6m_import.sca

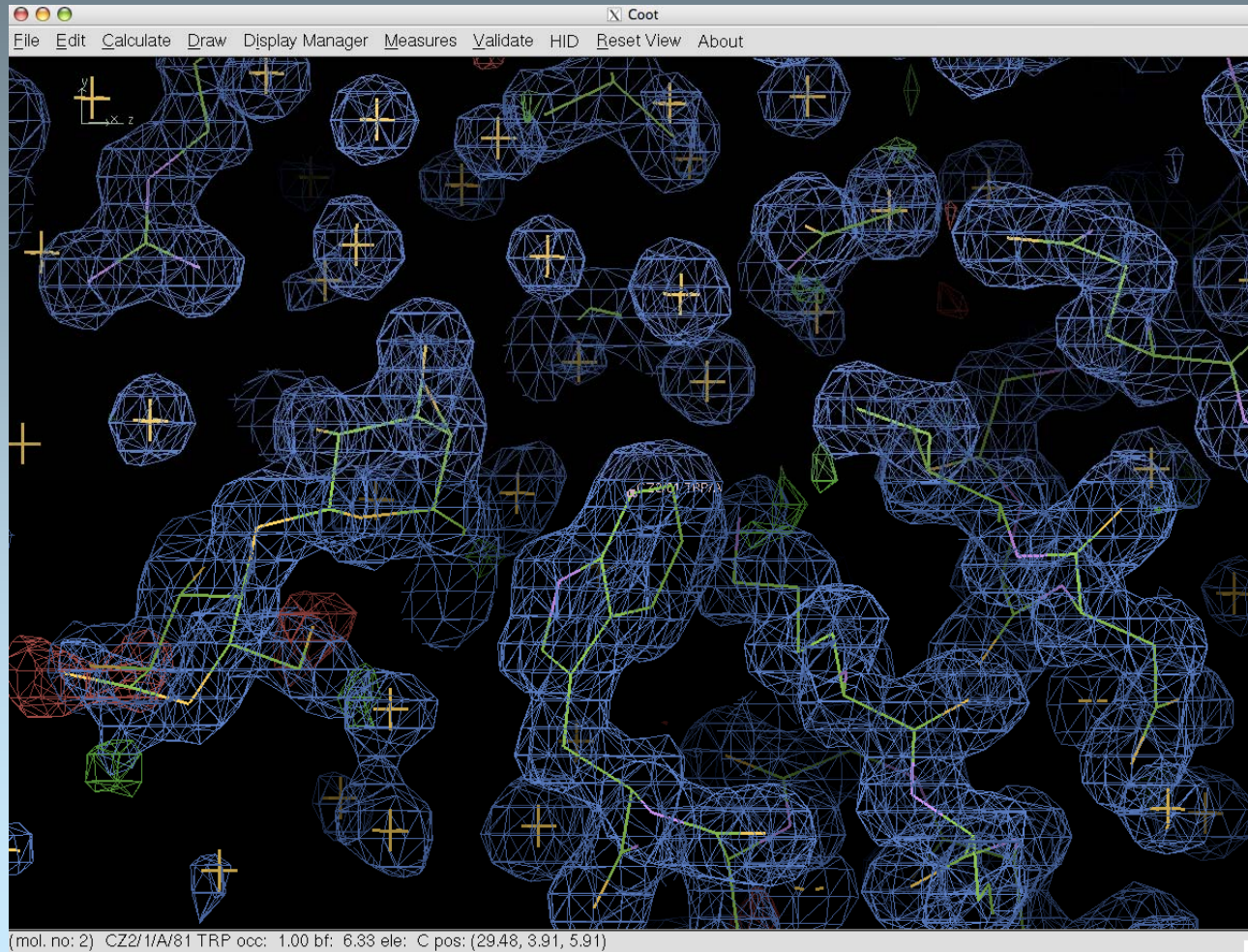
Space Group P212121
of Residues 159 / 318
Molecular Weight 18152.59
Resolution 37.21 1.60
of Molecules / AU 2
Solvent Content 0.41

Auto
Abort
Display Map

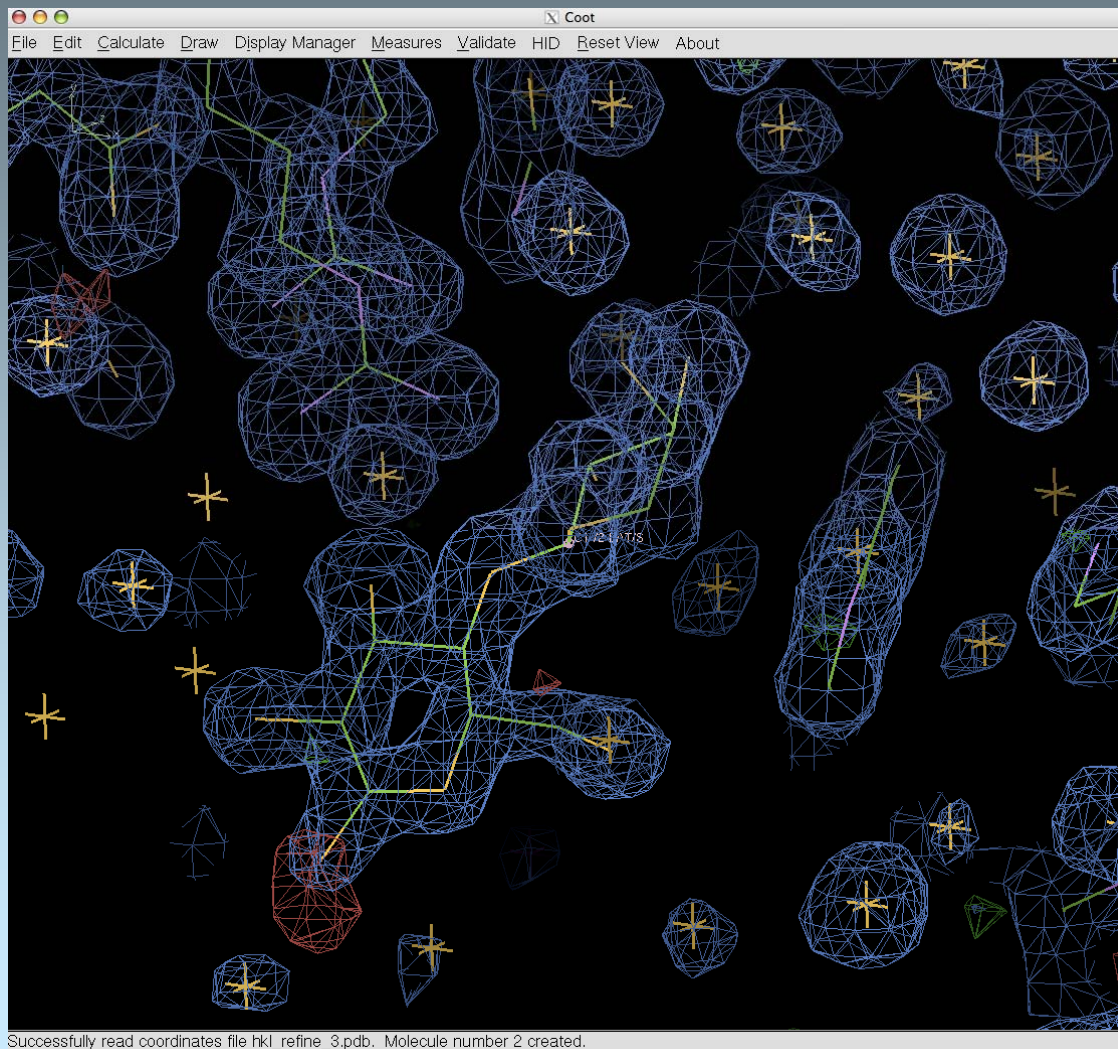
Unidentified densities



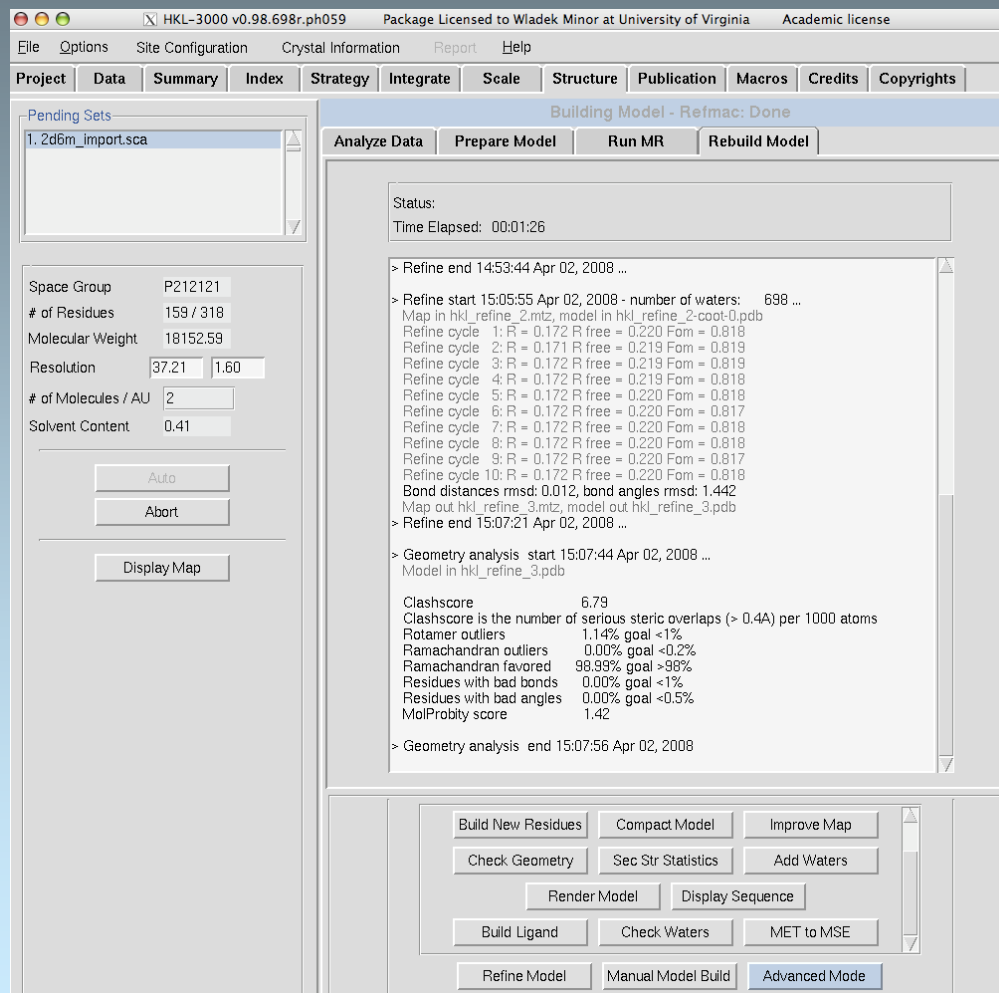
Automatic building



Fully refined ligand



Structure validation



The screenshot shows the HKL-3000 software interface. The window title is "HKL-3000 v0.98.698r.ph059 Package Licensed to Wladek Minor at University of Virginia Academic license". The menu bar includes "File", "Options", "Site Configuration", "Crystal Information", "Report", and "Help". The main menu includes "Project", "Data", "Summary", "Index", "Strategy", "Integrate", "Scale", "Structure", "Publication", "Macros", "Credits", and "Copyrights".

The "Building Model - Refmac: Done" window is active, showing the following status and results:

Status:
Time Elapsed: 00:01:26

> Refine end 14:53:44 Apr 02, 2008 ...

> Refine start 15:05:55 Apr 02, 2008 - number of waters: 698 ...
Map in hkl_refine_2.mtz, model in hkl_refine_2-coot-0.pdb
Refine cycle 1: R = 0.172 R free = 0.220 Fom = 0.818
Refine cycle 2: R = 0.171 R free = 0.219 Fom = 0.819
Refine cycle 3: R = 0.172 R free = 0.219 Fom = 0.819
Refine cycle 4: R = 0.172 R free = 0.219 Fom = 0.818
Refine cycle 5: R = 0.172 R free = 0.220 Fom = 0.818
Refine cycle 6: R = 0.172 R free = 0.220 Fom = 0.817
Refine cycle 7: R = 0.172 R free = 0.220 Fom = 0.818
Refine cycle 8: R = 0.172 R free = 0.220 Fom = 0.818
Refine cycle 9: R = 0.172 R free = 0.220 Fom = 0.817
Refine cycle 10: R = 0.172 R free = 0.220 Fom = 0.818
Bond distances rmsd: 0.012, bond angles rmsd: 1.442
Map out hkl_refine_3.mtz, model out hkl_refine_3.pdb

> Refine end 15:07:21 Apr 02, 2008 ...

> Geometry analysis start 15:07:44 Apr 02, 2008 ...
Model in hkl_refine_3.pdb

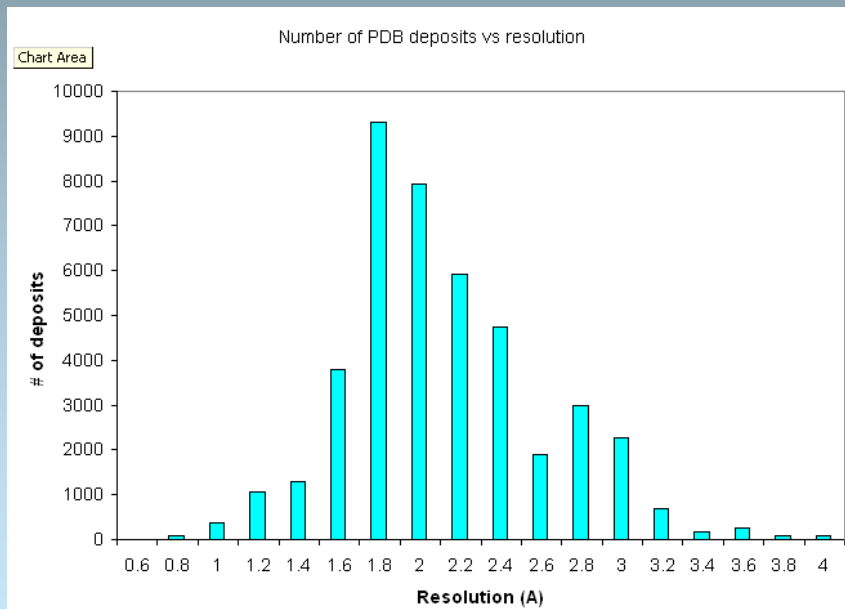
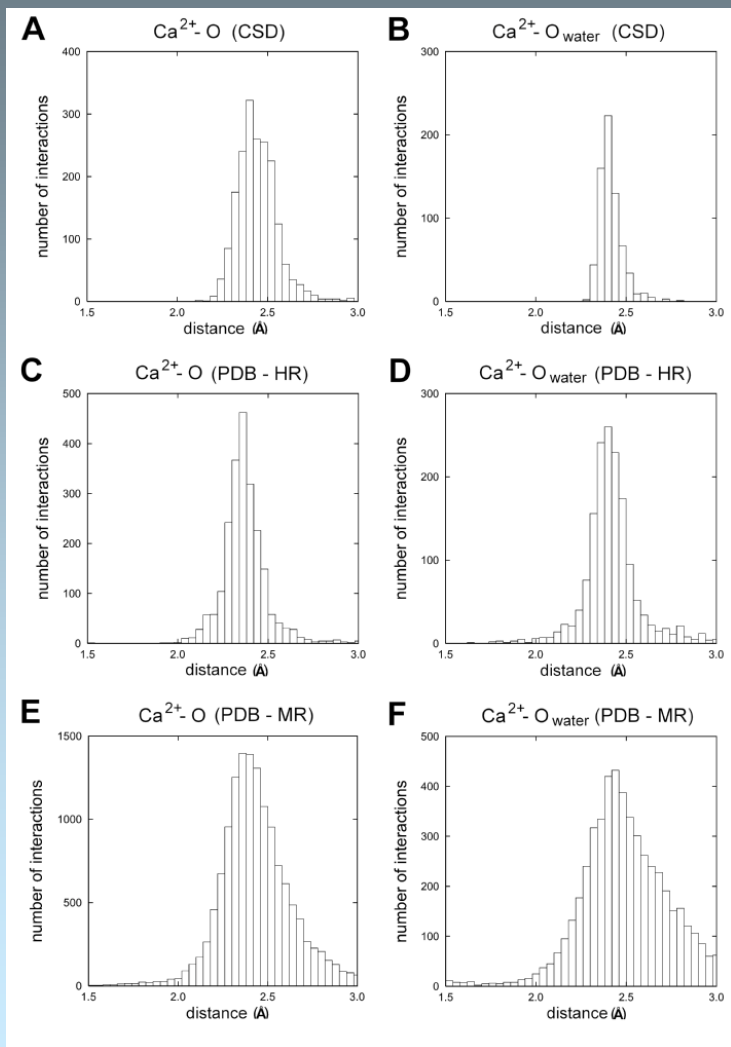
Clashscore	6.79
Clashscore is the number of serious steric overlaps (> 0.4A) per 1000 atoms	
Rotamer outliers	1.14% goal <1%
Ramachandran outliers	0.00% goal <0.2%
Ramachandran favored	98.99% goal >98%
Residues with bad bonds	0.00% goal <1%
Residues with bad angles	0.00% goal <0.5%
MolProbity score	1.42

> Geometry analysis end 15:07:56 Apr 02, 2008

The left sidebar shows "Pending Sets" with "1. 2d5m_import.sca". Below it, crystallographic parameters are listed: Space Group (P212121), # of Residues (159 / 318), Molecular Weight (18152.59), Resolution (37.21 / 1.60), # of Molecules / AU (2), and Solvent Content (0.41). Buttons for "Auto", "Abort", and "Display Map" are present.

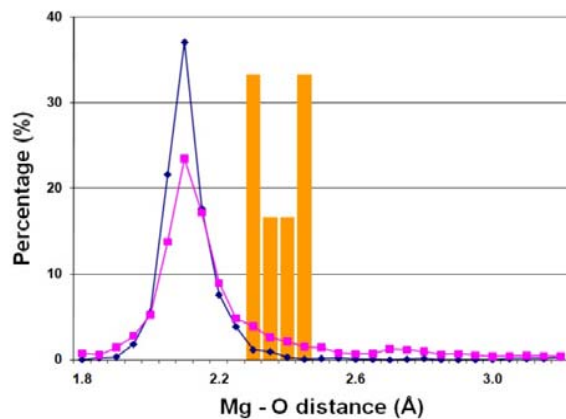
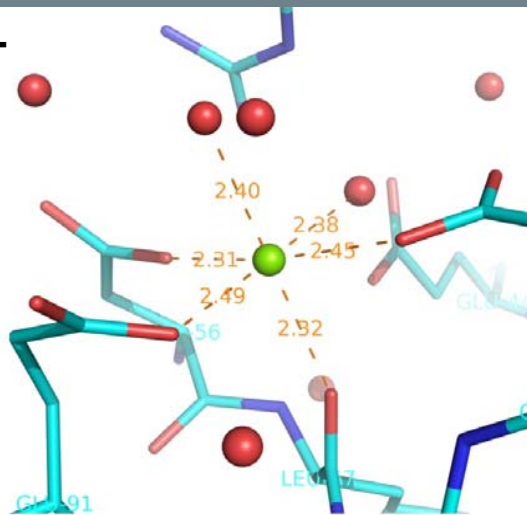
The bottom panel contains several buttons for model building and validation: "Build New Residues", "Compact Model", "Improve Map", "Check Geometry", "Sec Str Statistics", "Add Waters", "Render Model", "Display Sequence", "Build Ligand", "Check Waters", "MET to MSE", "Refine Model", "Manual Model Build", and "Advanced Mode".

Ca-O distances

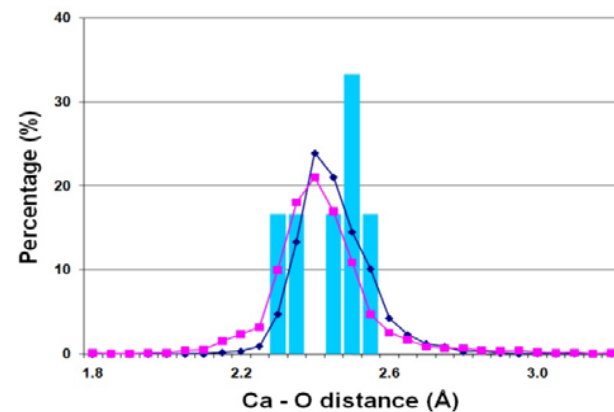
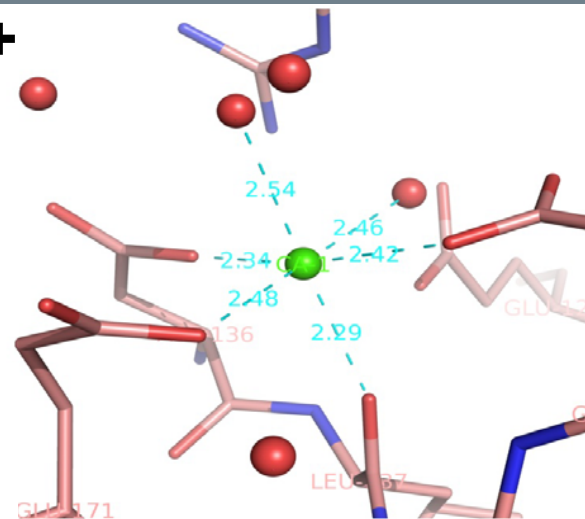


Metal ion in Der p 1

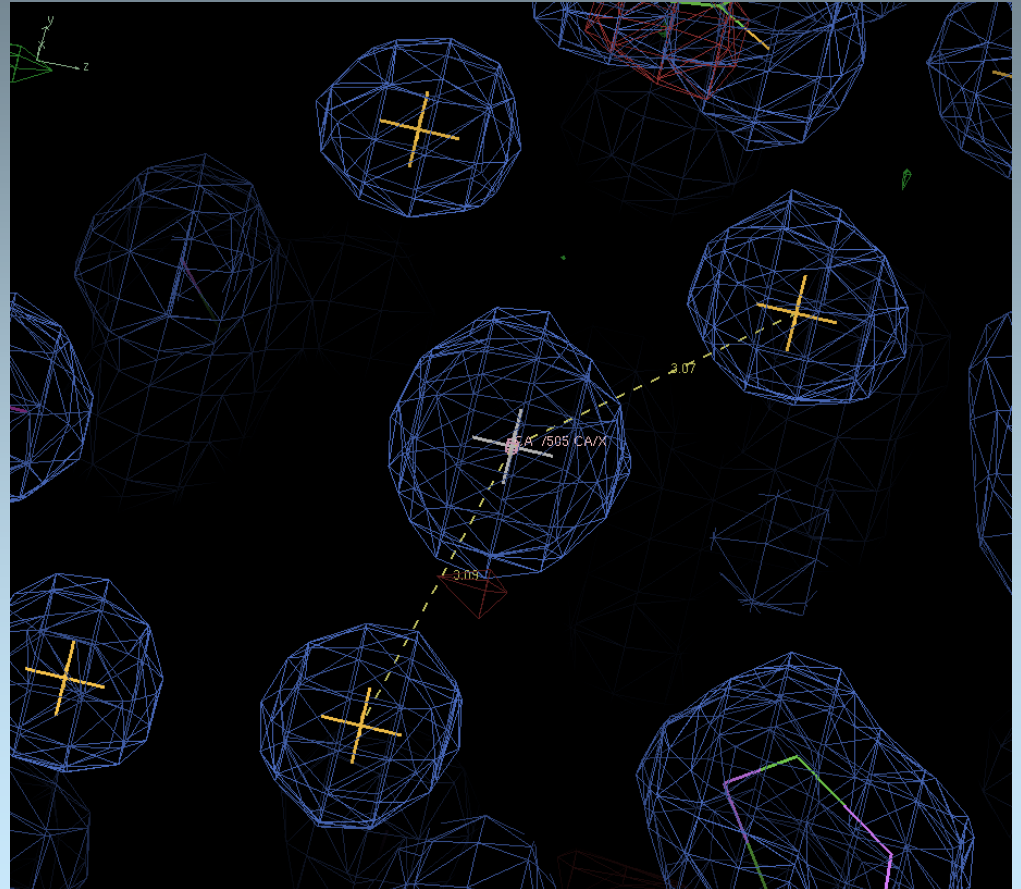
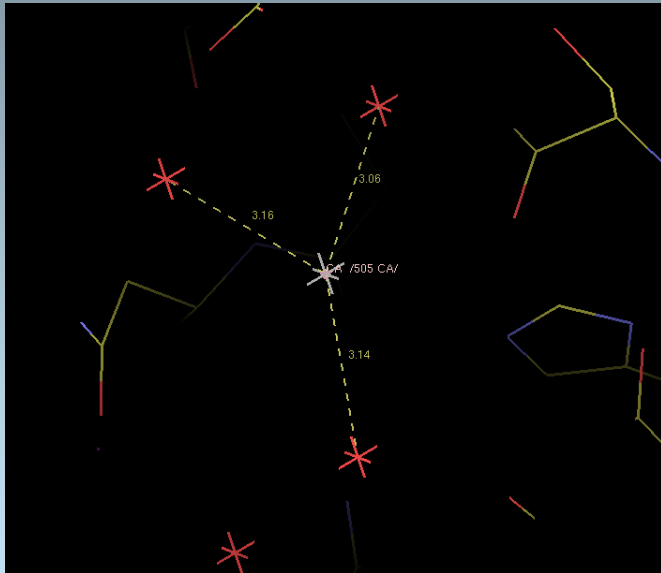
Mg^{2+}



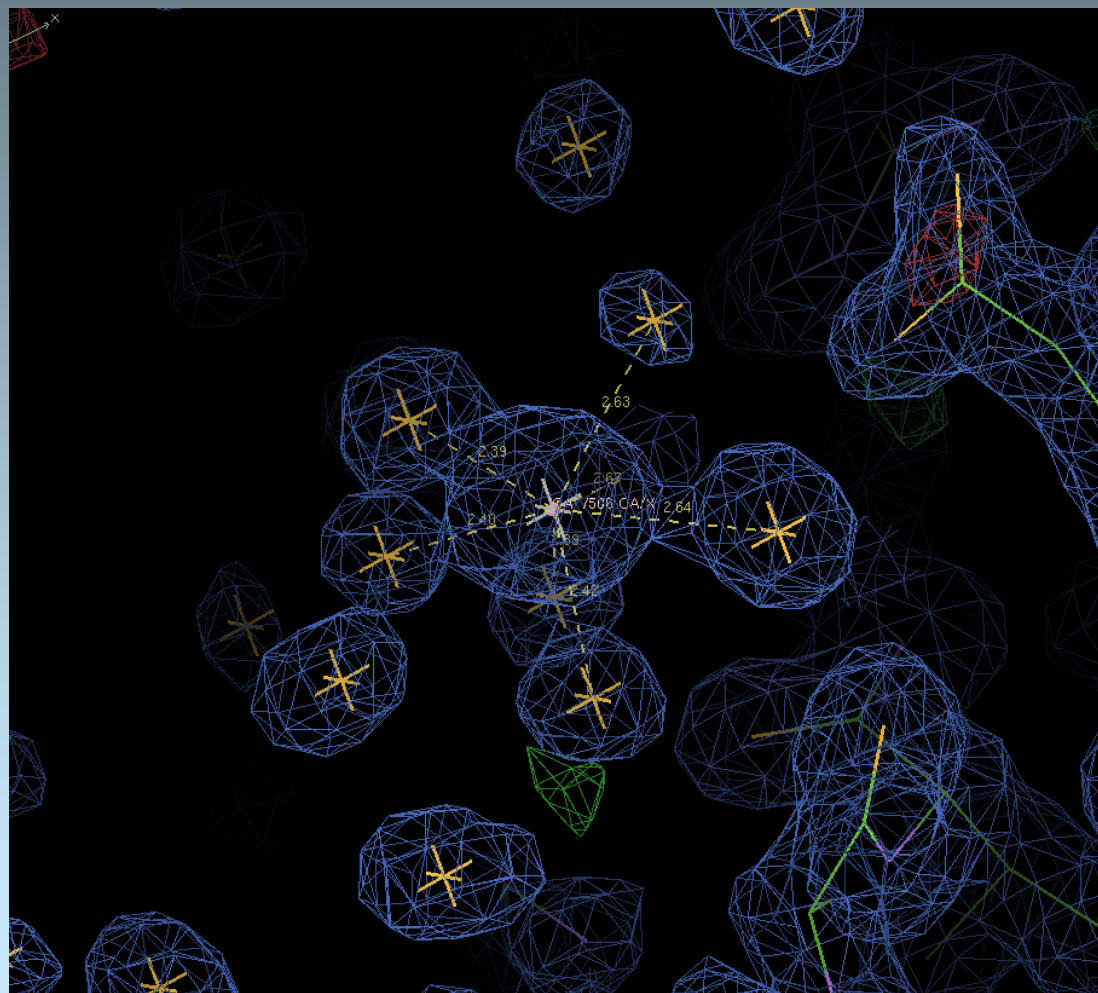
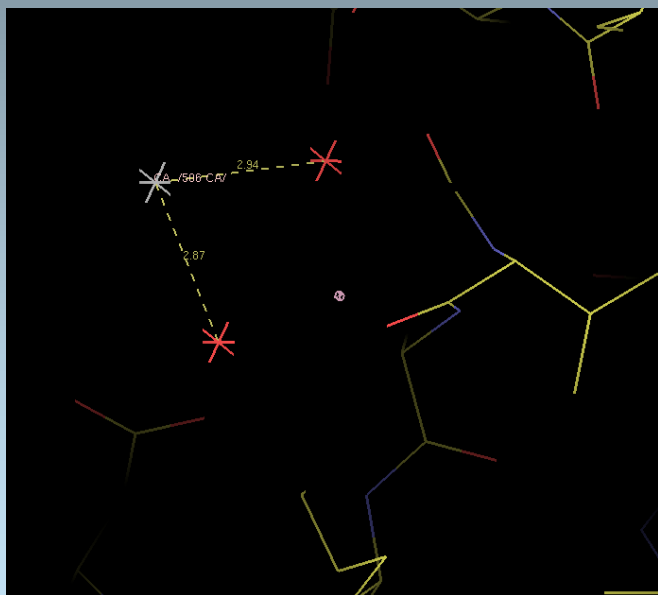
Ca^{2+}



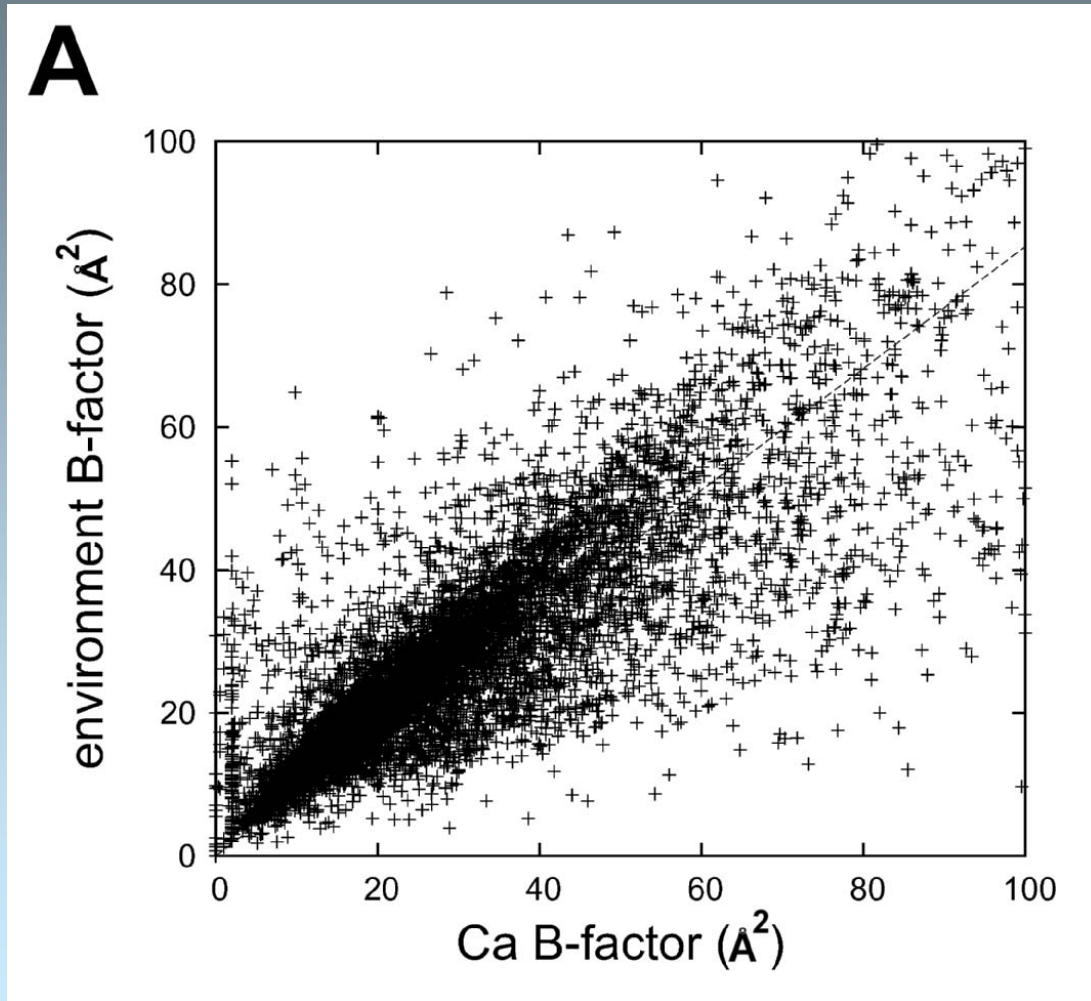
Incorrect assignments 1kyt



Correct assignment suboptimal refinement

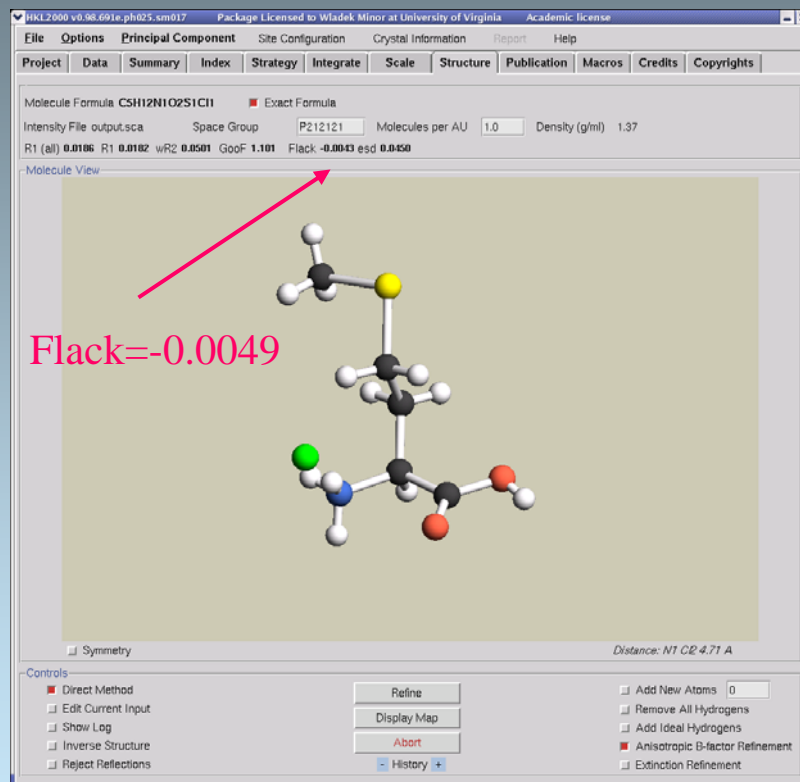
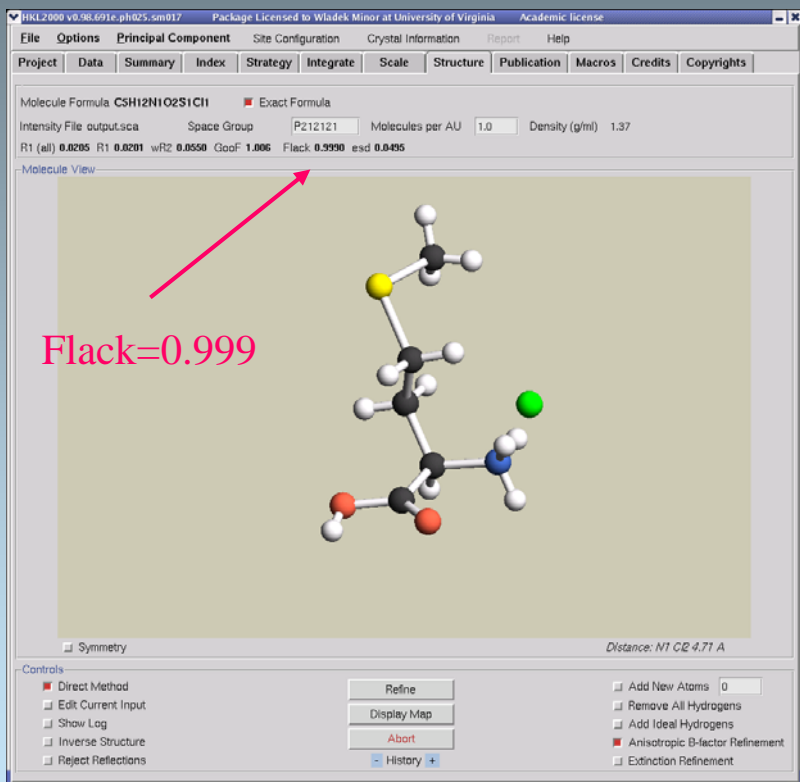


B factors



Absolute configuration and small molecule agents

HKL-3000SM



People involved

Wladek Minor

- Maks Chruszcz
- Marcin Cymborowski
- Matt Zimmerman
- Heping Zheng
- Marek Grabowski
- Haijun Qiu
- Olga Kirillova
- John Raynor

Zbyszek Otwinowski

- Dominika Borek
- Andrzej Kudlicki
- Roger Ljungberg

Andrzej Joachimiak

- Rongguang Zhan
- Marianne Cuff
- Chris Lazarski
- SBC staff

Zbyszek Dauter

Grants:

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DOE, NCI
HKL Research