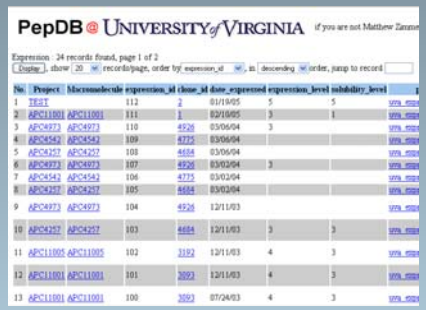
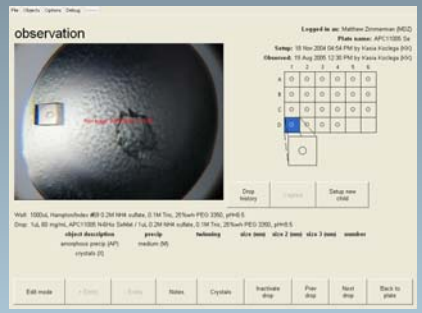
Integration
HKL-3000 is a pipeline component

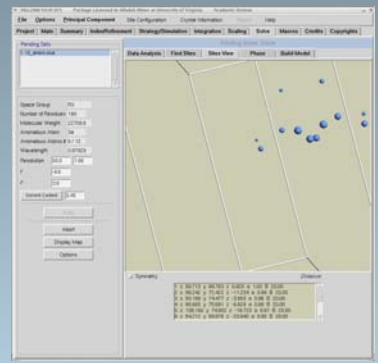
Pepdb
expression & purification

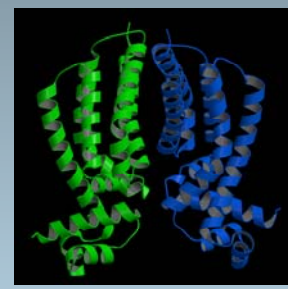
Xtaldb
crystallization

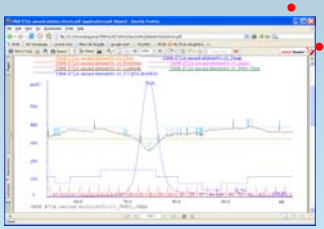
HKL-3000
Data collection,
diffraction & structure solution

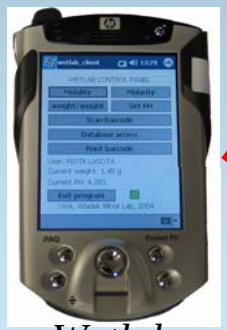








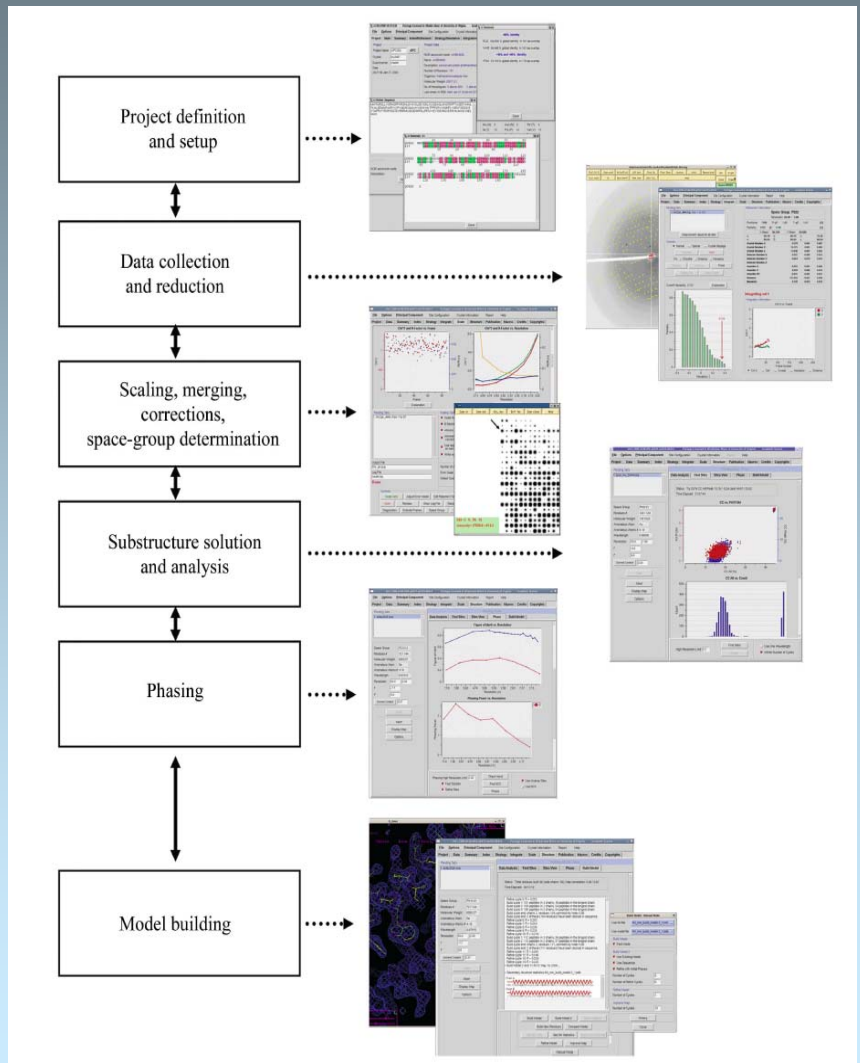




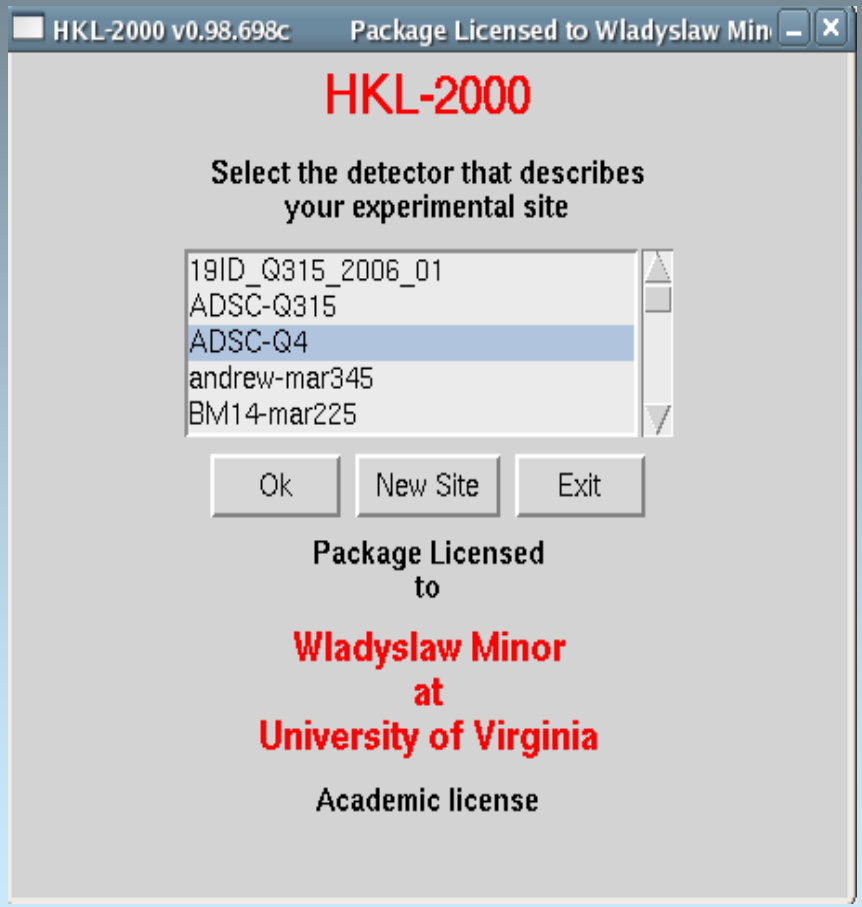
Wetlab
chemicals & solutions

Other systems provide additional contextual up- and downstream information

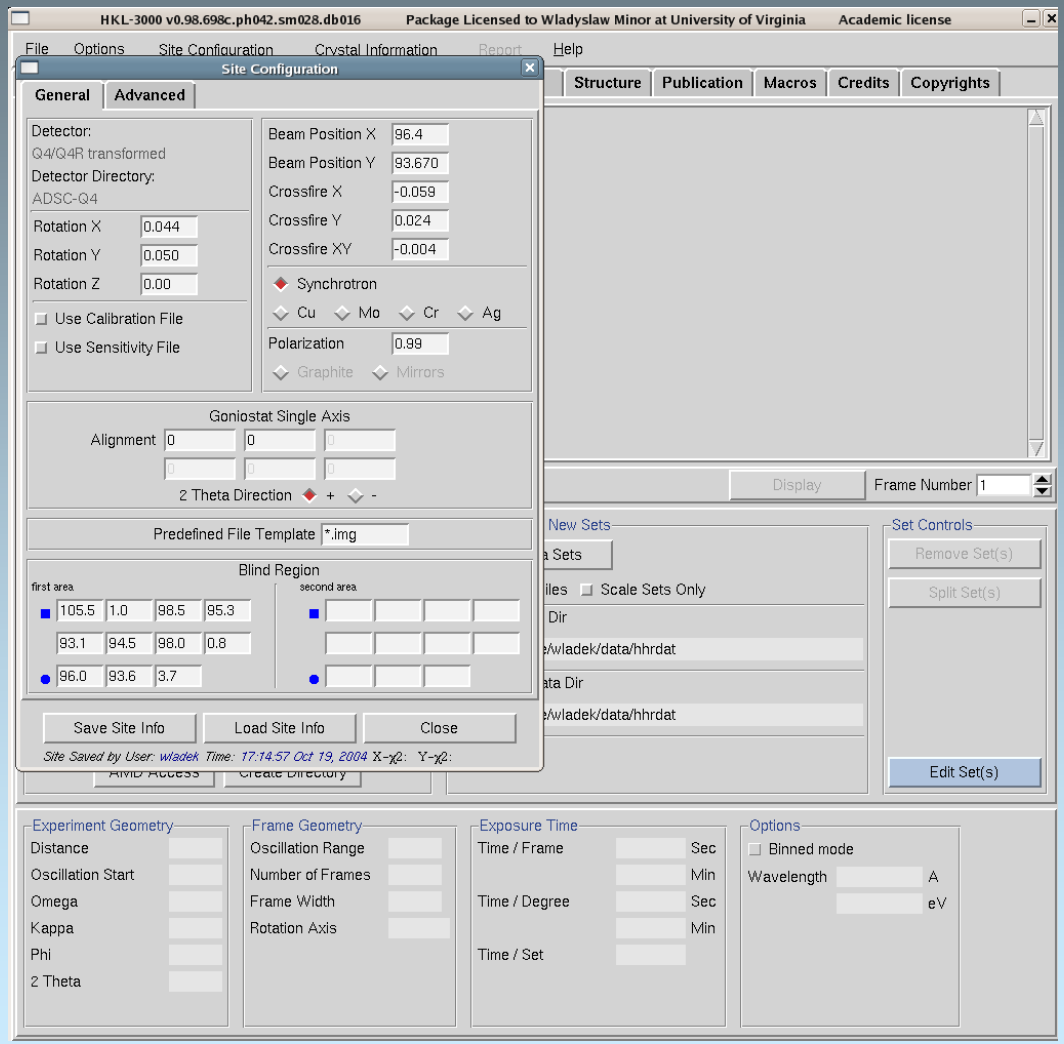
HKL-3000


Project definition and setup
Data collection and reduction
Scaling, merging, corrections, space-group determination
Substructure solution and analysis
Phasing
Model building

Program start – site file



Site file


HKL-3000 v0.98.698c.ph042.sm028.db016
Package Licensed to Wladyslaw Minor at University of Virginia
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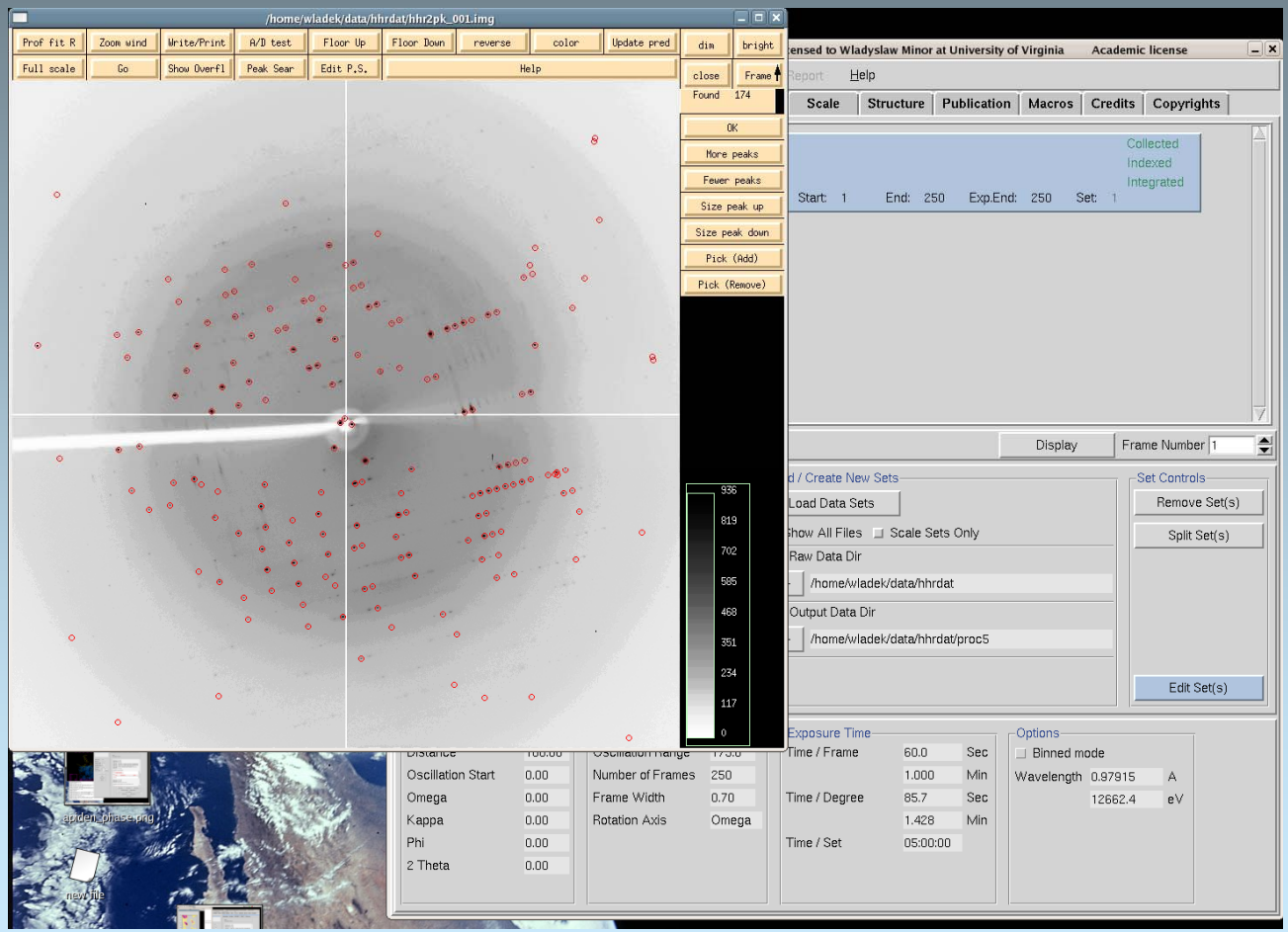
File
Options
Site Configuration
Crystal Information
Report
Help
Site Configuration
General
Advanced
Detector:
Q4/Q4R transformed
Detector Directory:
ADSC-Q4
Rotation X 0.044
Rotation Y 0.050
Rotation Z 0.00
Use Calibration File
Use Sensitivity File
Beam Position X 96.4
Beam Position Y 93.670
Crossfire X -0.059
Crossfire Y 0.024
Crossfire XY -0.004
Synchrotron
Cu Mo Cr Ag
Polarization 0.99
Graphite Mirrors
Goniostat Single Axis
Alignment
2 Theta Direction + -
Predefined File Template *.img
Blind Region
first area
second area
105.5 1.0 98.5 95.3
93.1 94.5 98.0 0.8
96.0 93.6 3.7
Save Site Info
Load Site Info
Close
Site Saved by User: wladek Time: 17:14:57 Oct 19, 2004 X-χ2: Y-χ2:
Structure
Publication
Macros
Credits
Copyrights
Display
Frame Number 1
New Sets
Scale Sets Only
Dir
/wladek/data/hhrdat
/wladek/data/hhrdat
Set Controls
Remove Set(s)
Split Set(s)
Edit Set(s)
Experiment Geometry
Distance
Oscillation Start
Omega
Kappa
Phi
2 Theta
Frame Geometry
Oscillation Range
Number of Frames
Frame Width
Rotation Axis
Exposure Time
Time / Frame
Time / Degree
Time / Set
Sec
Min
Options
Binned mode
Wavelength
A
eV

Image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Prof fit R
Zoom wind
Write/Print
A/D test
Floor Up
Floor Down
reverse
color
Update pred
dim
bright
Full scale
Go
Show Overfl
Peak Sear
Edit P.S.
Help
close
Frame
Found 174
OK
More peaks
Fewer peaks
Size peak up
Size peak down
Pick (Add)
Pick (Remove)
936
819
702
585
468
351
234
117
0
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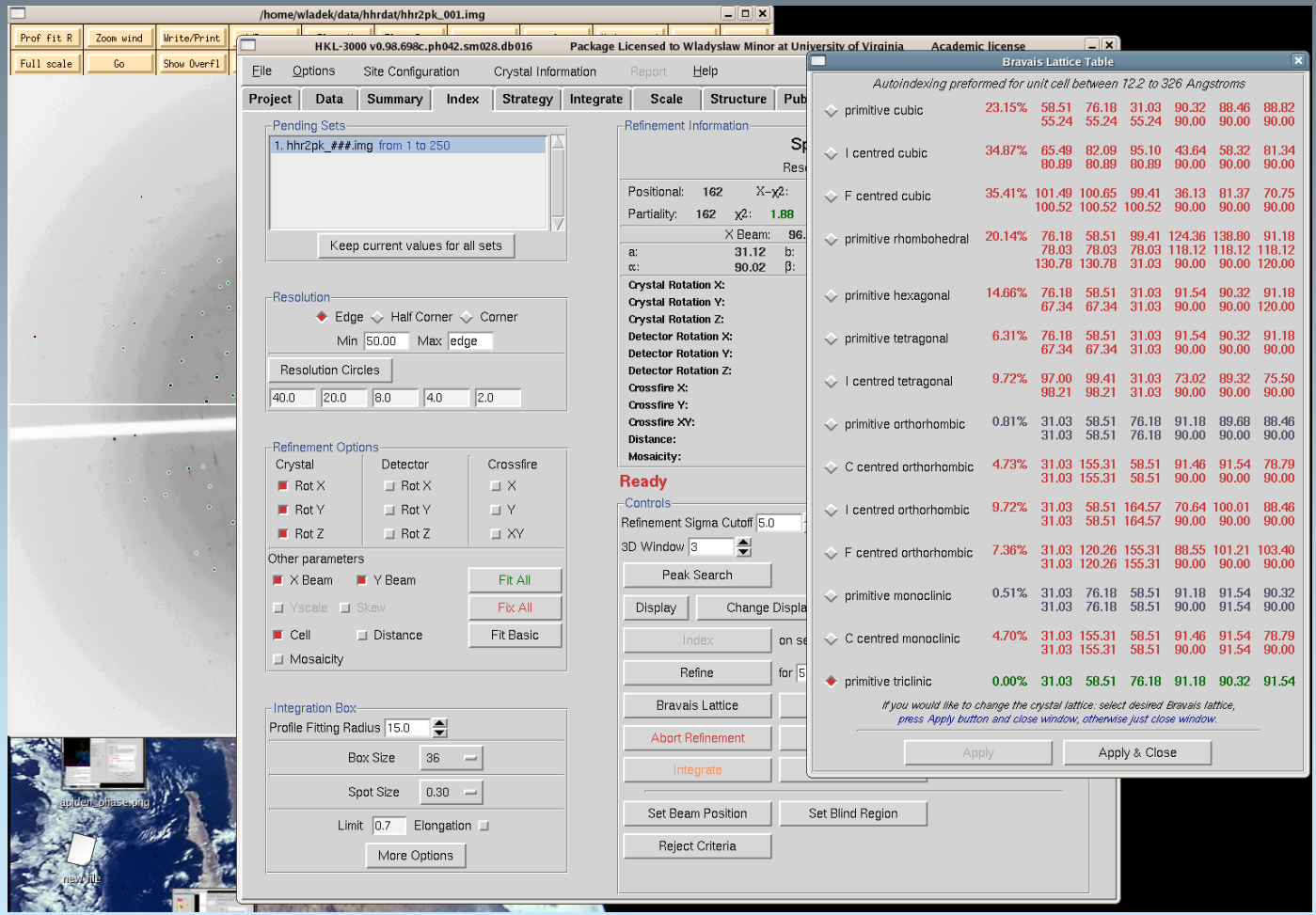
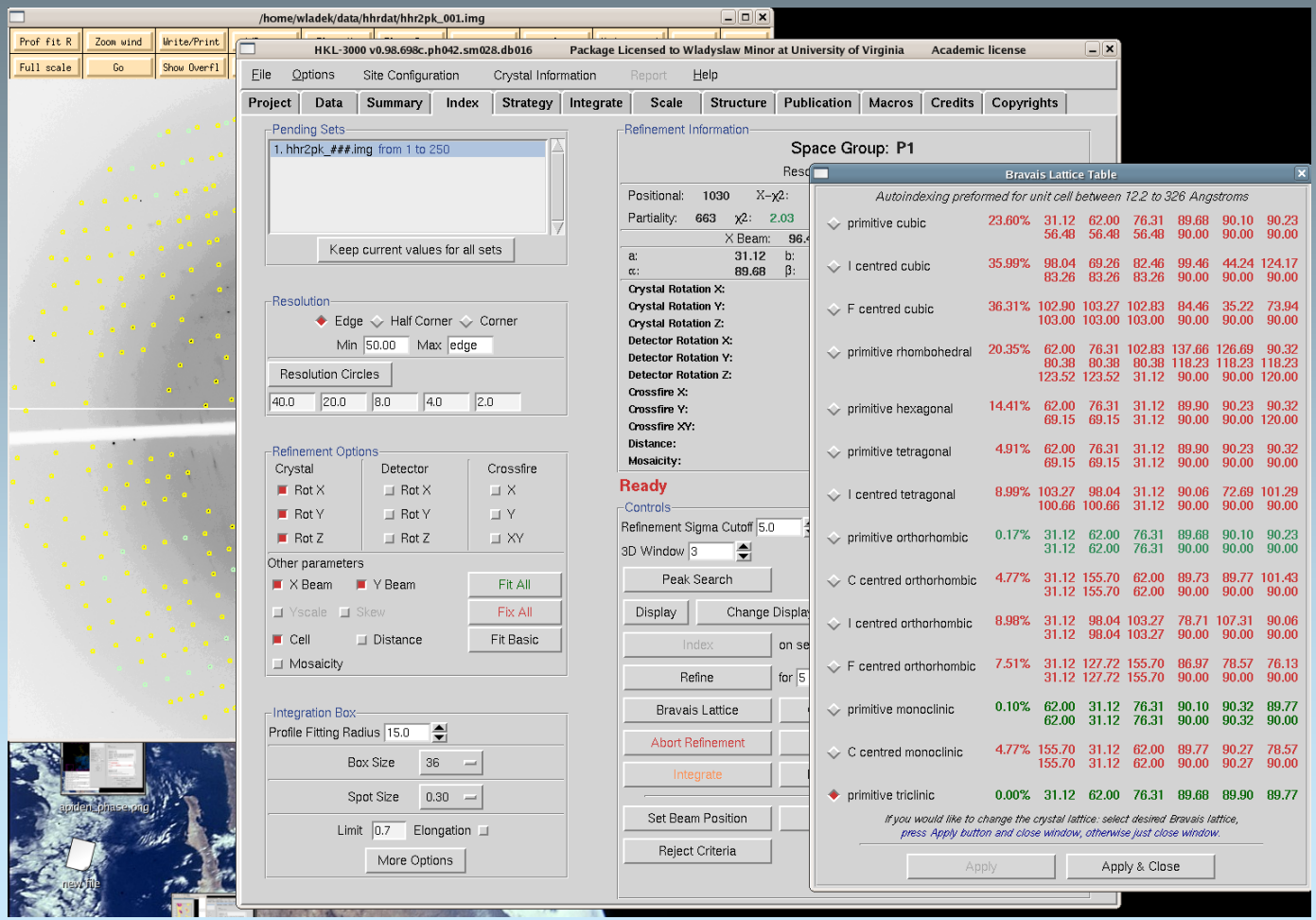
Academic license
Report
Help
Scale
Structure
Publication
Macros
Credits
Copyrights
Collected
Indexed
Integrated
Start: 1
End: 250
Exp.End: 250
Set: 1
Display
Frame Number 1
d / Create New Sets
Load Data Sets
how All Files
Scale Sets Only
Raw Data Dir
/home/wladek/data/hhrdat
Output Data Dir
/home/wladek/data/hhrdat/proc5
Set Controls
Remove Set(s)
Split Set(s)
Edit Set(s)
Oscillation Start 0.00
Omega 0.00
Kappa 0.00
Phi 0.00
2 Theta 0.00
Number of Frames 250
Frame Width 0.70
Rotation Axis Omega
Exposure Time
Time / Frame 60.0 Sec
1.000 Min
Time / Degree 85.7 Sec
1.428 Min
Time / Set 05:00:00
Options
Binned mode
Wavelength 0.97915 A
12662.4 eV

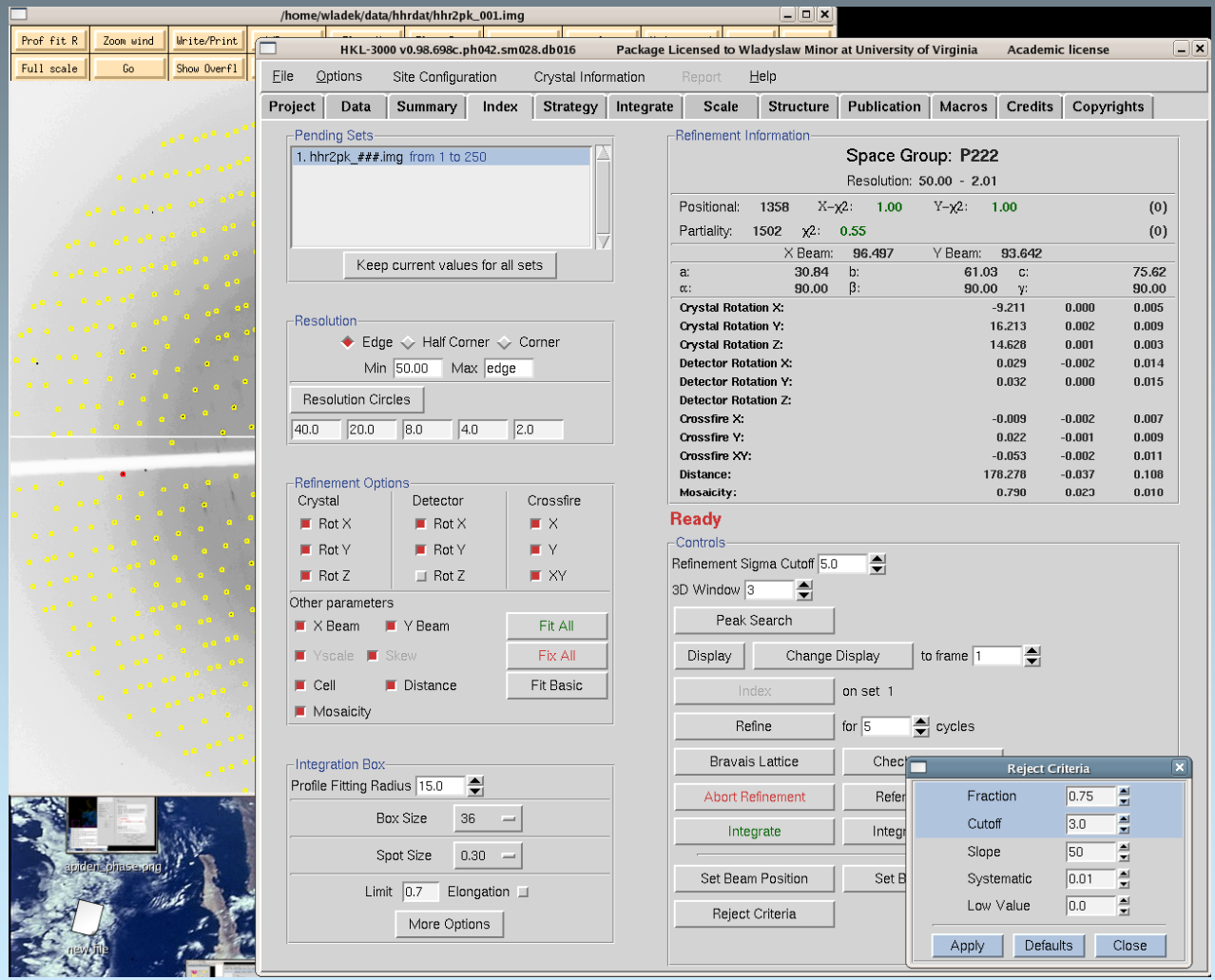
Indexing


/home/wladek/data/hhrdat/hhr2pk_001.img
Prof fit R
Zoom wind
Write/Print
Full scale
Go
Show Overfl
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
Pending Sets
1. hhr2pk_###.img from 1 to 250
Keep current values for all sets
Resolution
Edge
Half Corner
Corner
Min 50.00
Max edge
Resolution Circles
40.0
20.0
8.0
4.0
2.0
Refinement Options
Crystal
Rot X
Rot Y
Rot Z
Detector
Rot X
Rot Y
Rot Z
Crossfire
X
Y
XY
Other parameters
X Beam
Y Beam
Yscale
Skew
Cell
Distance
Mosaicity
Fit All
Fix All
Fit Basic
Integration Box
Profile Fitting Radius 15.0
Box Size 36
Spot Size 0.30
Limit 0.7
Elongation
More Options
Refinement Information
Positional: 162
Partiality: 162
X Beam: 96.
a: 31.12
α: 90.02
Crystal Rotation X:
Crystal Rotation Y:
Crystal Rotation Z:
Detector Rotation X:
Detector Rotation Y:
Detector Rotation Z:
Crossfire X:
Crossfire Y:
Crossfire XY:
Distance:
Mosaicity:
Ready
Controls
Refinement Sigma Cutoff 5.0
3D Window 3
Peak Search
Display
Index
Refine
Bravais Lattice
Abort Refinement
Integrate
Set Beam Position
Set Blind Region
Reject Criteria
Bravais Lattice Table
Autoindexing preformed for unit cell between 12.2 to 326 Angstroms
primitive cubic 23.15% 58.51 76.18 31.03 90.32 88.46 88.82 55.24 55.24 55.24 90.00 90.00 90.00
I centred cubic 34.87% 65.49 82.09 95.10 43.64 58.32 81.34 80.89 80.89 80.89 90.00 90.00 90.00
F centred cubic 35.41% 101.49 100.65 99.41 36.13 81.37 70.75 100.52 100.52 100.52 90.00 90.00 90.00
primitive rhombohedral 20.14% 76.18 58.51 99.41 124.36 138.80 91.18 78.03 78.03 78.03 118.12 118.12 118.12 130.78 130.78 31.03 90.00 90.00 120.00
primitive hexagonal 14.66% 76.18 58.51 31.03 91.54 90.32 91.18 67.34 67.34 31.03 90.00 90.00 120.00
primitive tetragonal 6.31% 76.18 58.51 31.03 91.54 90.32 91.18 67.34 67.34 31.03 90.00 90.00 90.00
I centred tetragonal 9.72% 97.00 99.41 31.03 73.02 89.32 75.50 98.21 98.21 31.03 90.00 90.00 90.00
primitive orthorhombic 0.81% 31.03 58.51 76.18 91.18 89.68 88.46 31.03 58.51 76.18 90.00 90.00 90.00
C centred orthorhombic 4.73% 31.03 155.31 58.51 91.46 91.54 78.79 31.03 155.31 58.51 90.00 90.00 90.00
I centred orthorhombic 9.72% 31.03 58.51 164.57 70.64 100.01 88.46 31.03 58.51 164.57 90.00 90.00 90.00
F centred orthorhombic 7.36% 31.03 120.26 155.31 88.55 101.21 103.40 31.03 120.26 155.31 90.00 90.00 90.00
primitive monoclinic 0.51% 31.03 76.18 58.51 91.18 91.54 90.32 31.03 76.18 58.51 90.00 91.54 90.00
C centred monoclinic 4.70% 31.03 155.31 58.51 91.46 91.54 78.79 31.03 155.31 58.51 90.00 91.54 90.00
primitive triclinic 0.00% 31.03 58.51 76.18 91.18 90.32 91.54
If you would like to change the crystal lattice: select desired Bravais lattice, press Apply button and close window, otherwise just close window.
Apply
Apply & Close
apidel_phase.png
new file

Bravais lattice



Rejections


/home/wladek/data/hhrdat/hhr2pk_001.img
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HKL-3000 v0.98.698c.ph042.sm028.db016
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Half Corner
Corner
Min 50.00
Max edge
Resolution Circles
40.0
20.0
8.0
4.0
2.0
Refinement Options
Crystal
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Rot Z
Detector
Rot X
Rot Y
Rot Z
Crossfire
X
Y
XY
Other parameters
X Beam
Y Beam
Yscale
Skew
Cell
Distance
Mosaicity
Fit All
Fix All
Fit Basic
Integration Box
Profile Fitting Radius 15.0
Box Size 36
Spot Size 0.30
Limit 0.7
Elongation
More Options
Refinement Information
Space Group: P222
Resolution: 50.00 - 2.01
Positional: 1358 X-χ2: 1.00 Y-χ2: 1.00 (0)
Partiality: 1502 χ2: 0.55 (0)
X Beam: 96.497 Y Beam: 93.642
a: 30.84 b: 61.03 c: 75.62
α: 90.00 β: 90.00 γ: 90.00
Crystal Rotation X: -9.211 0.000 0.005
Crystal Rotation Y: 16.213 0.002 0.009
Crystal Rotation Z: 14.628 0.001 0.003
Detector Rotation X: 0.029 -0.002 0.014
Detector Rotation Y: 0.032 0.000 0.015
Detector Rotation Z:
Crossfire X: -0.009 -0.002 0.007
Crossfire Y: 0.022 -0.001 0.009
Crossfire XY: -0.053 -0.002 0.011
Distance: 178.278 -0.037 0.108
Mosaicity: 0.790 0.023 0.010
Ready
Controls
Refinement Sigma Cutoff 5.0
3D Window 3
Peak Search
Display
Change Display
to frame 1
Index
on set 1
Refine
for 5
cycles
Bravais Lattice
Abort Refinement
Integrate
Set Beam Position
Reject Criteria
Reject Criteria
Fraction 0.75
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Slope 50
Systematic 0.01
Low Value 0.0
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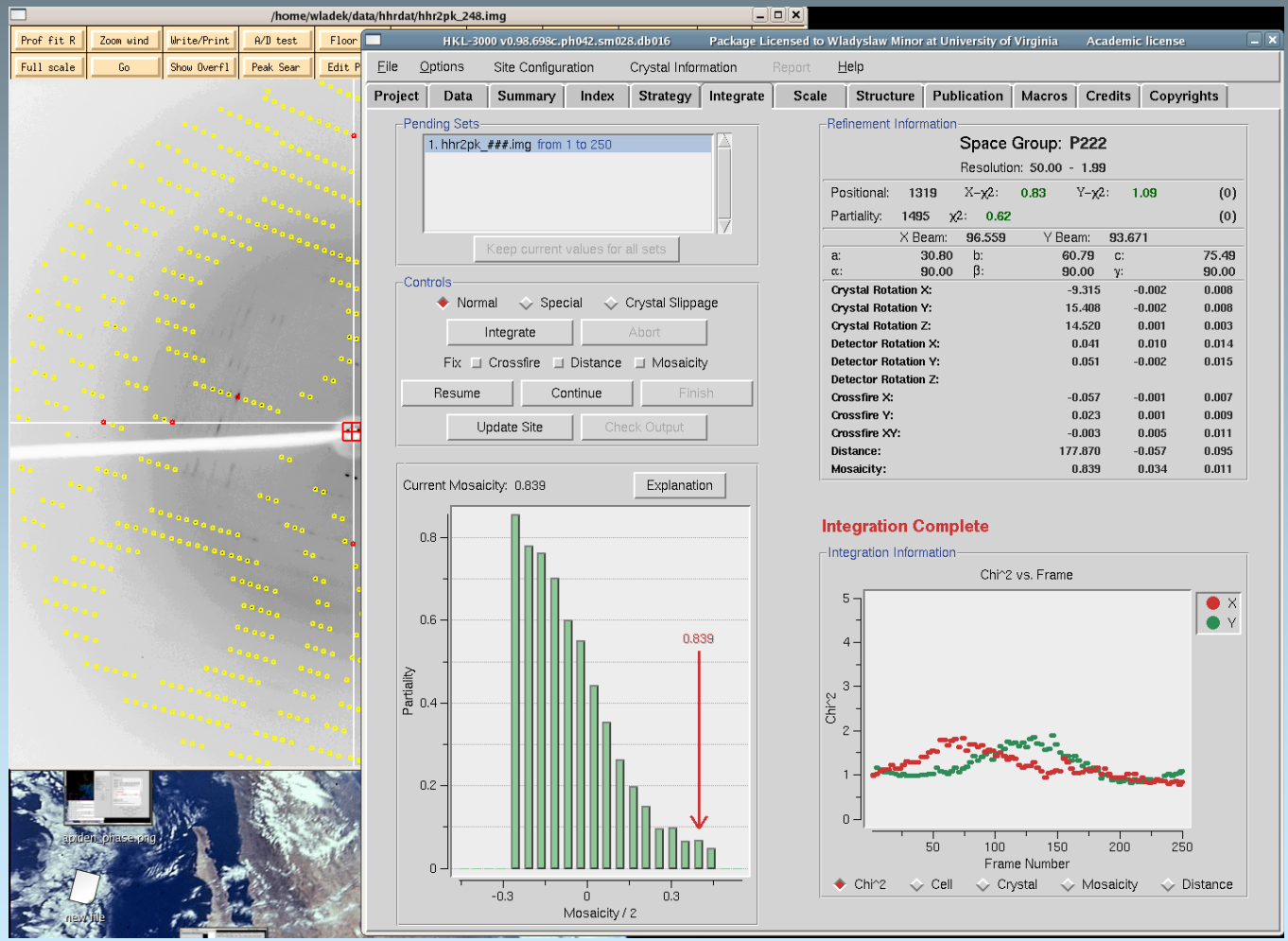
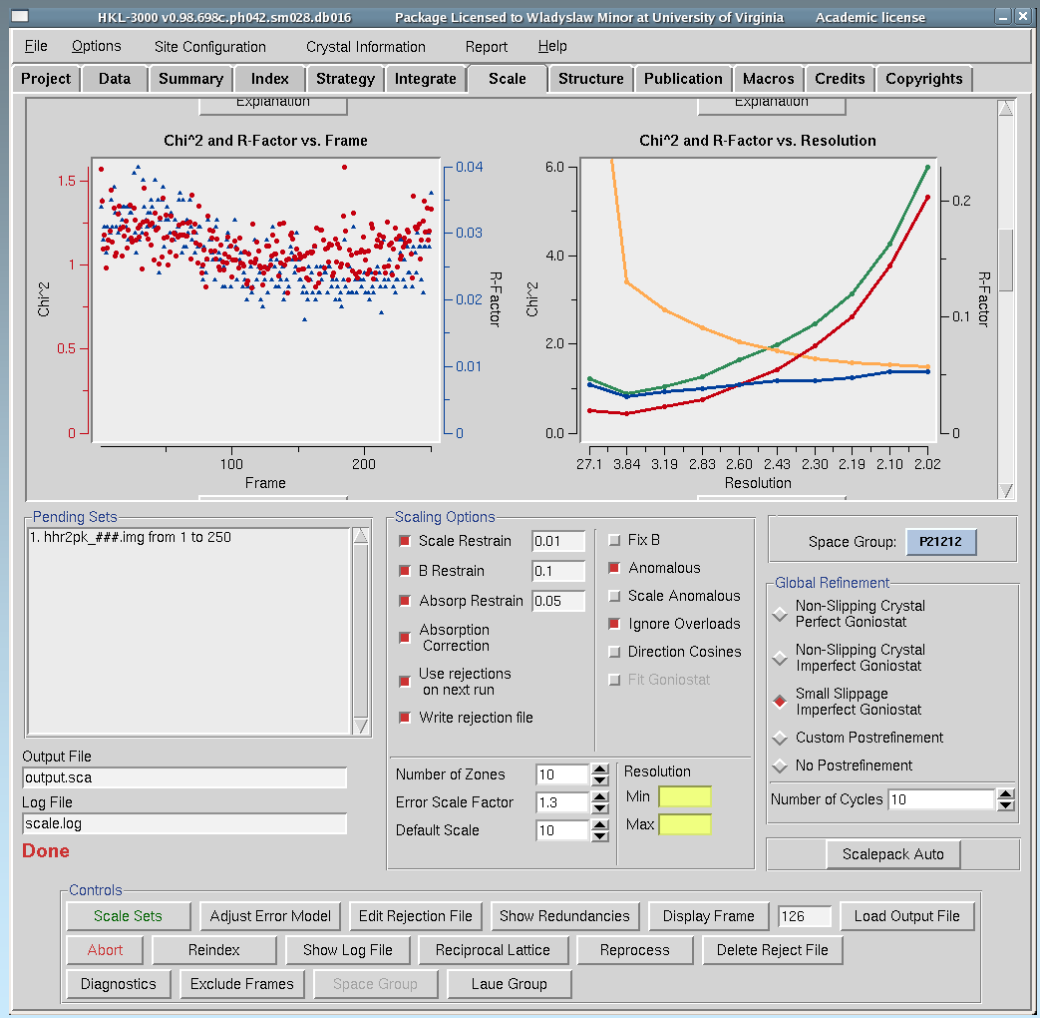
Defaults
Close
apiden_phase.png
new file

Integration



Scaling

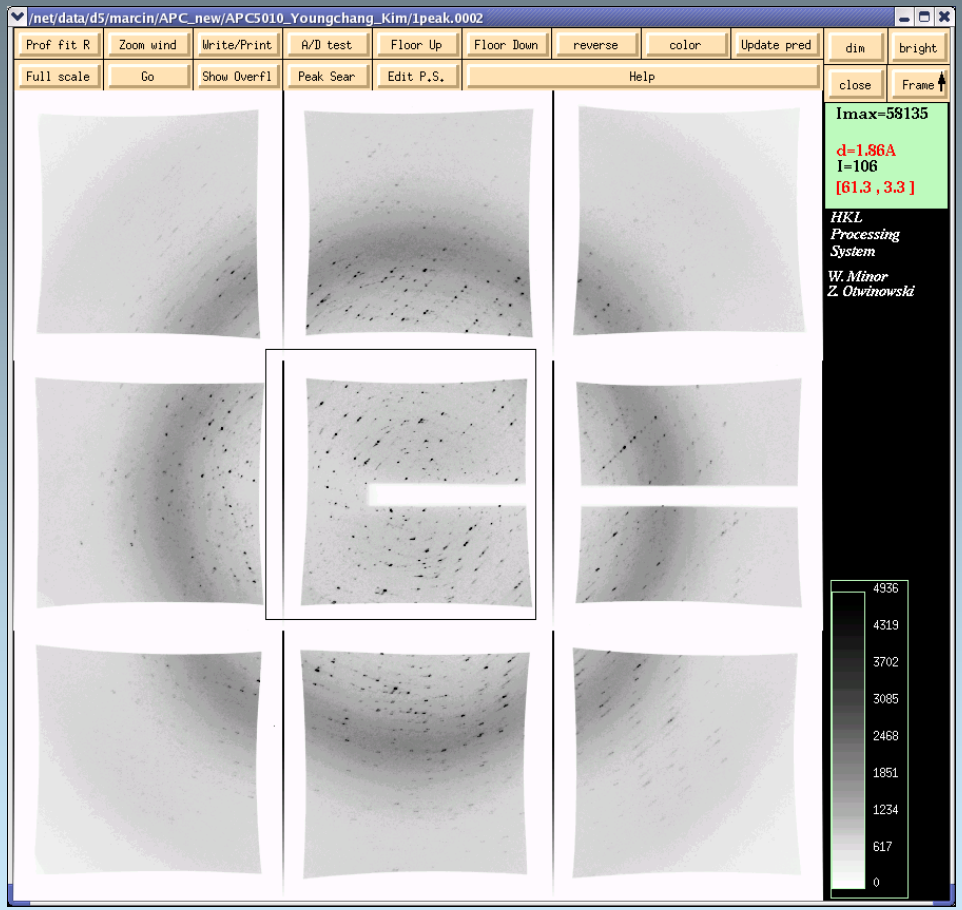

HKL-3000 v0.98.698c.ph042.sm028.db016 Package Licensed to Wladyslaw Minor at University of Virginia Academic license
File Options Site Configuration Crystal Information Report Help
Project Data Summary Index Strategy Integrate Scale Structure Publication Macros Credits Copyrights
Explanation
Chi^2 and R-Factor vs. Frame
Chi^2
R-Factor
Frame
Explanation
Chi^2 and R-Factor vs. Resolution
Resolution
Pending Sets
1. hhr2pk_###.img from 1 to 250
Scaling Options
Scale Restrain 0.01
B Restrain 0.1
Absorp Restrain 0.05
Absorption Correction
Use rejections on next run
Write rejection file
Fix B
Anomalous
Scale Anomalous
Ignore Overloads
Direction Cosines
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
Output File
output.sca
Log File
scale.log
Done
Number of Zones 10
Error Scale Factor 1.3
Default Scale 10
Resolution
Min
Max
Scalepack Auto
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

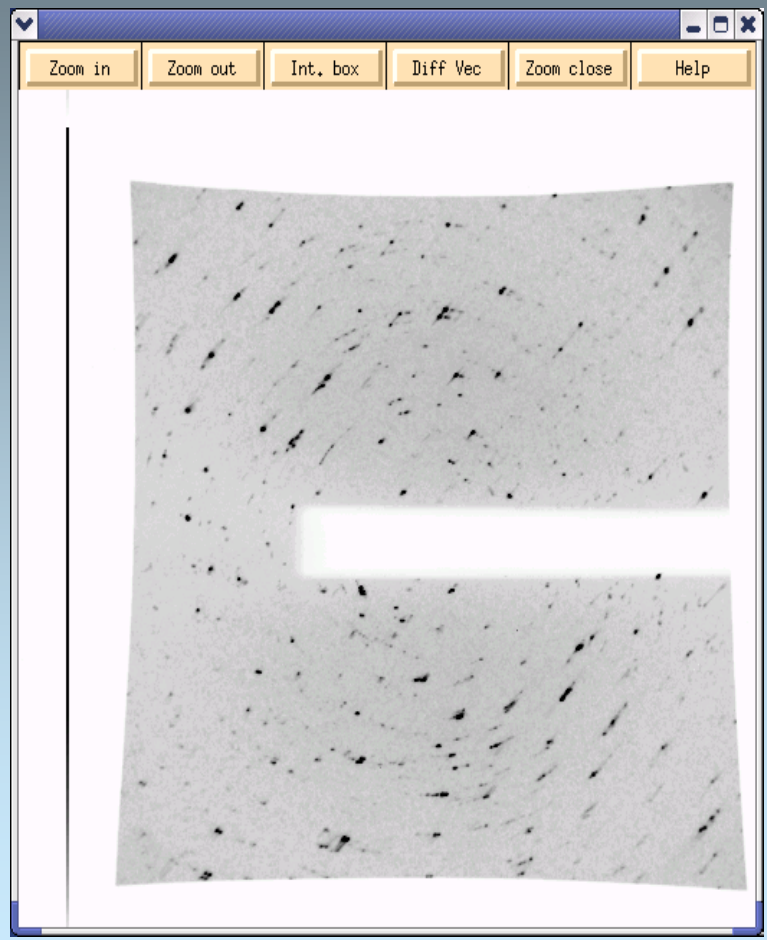
Integrated system – HKL-3000 current status

- **HKL-2000, DENZO, SCALEPACK**
- ***SHELXD, SHELXE***
- **MLPHARE, CYCLMLPHARE**
- ***DM, REFMAC***
- ***SOLVE, RESOLVE***
- ***ARP/WARP***
- **STATBUILD**
- ***O, COOT,CCP4***

Interactive development:
Integrated approach shows how the choice in one step influences subsequent steps.

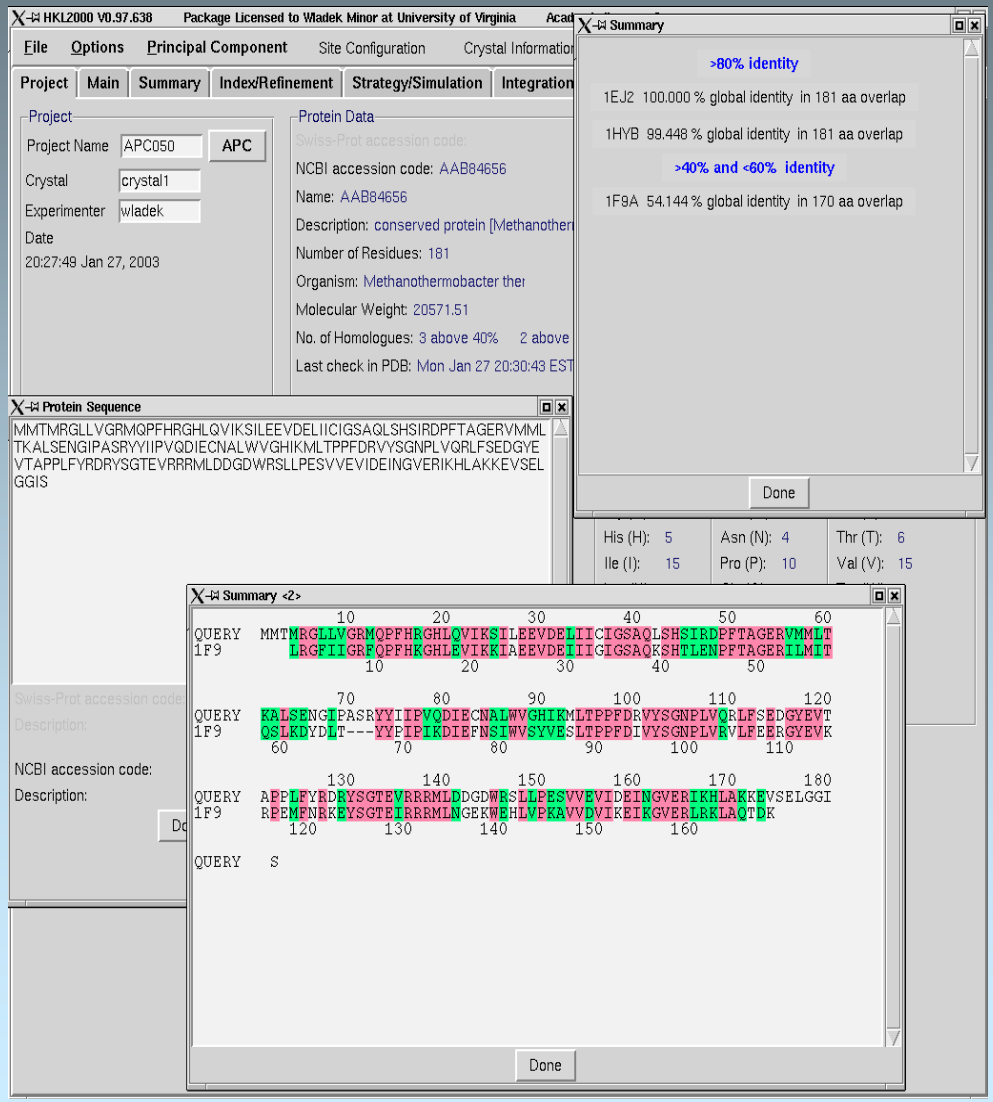
APC 5010 (1xvi)



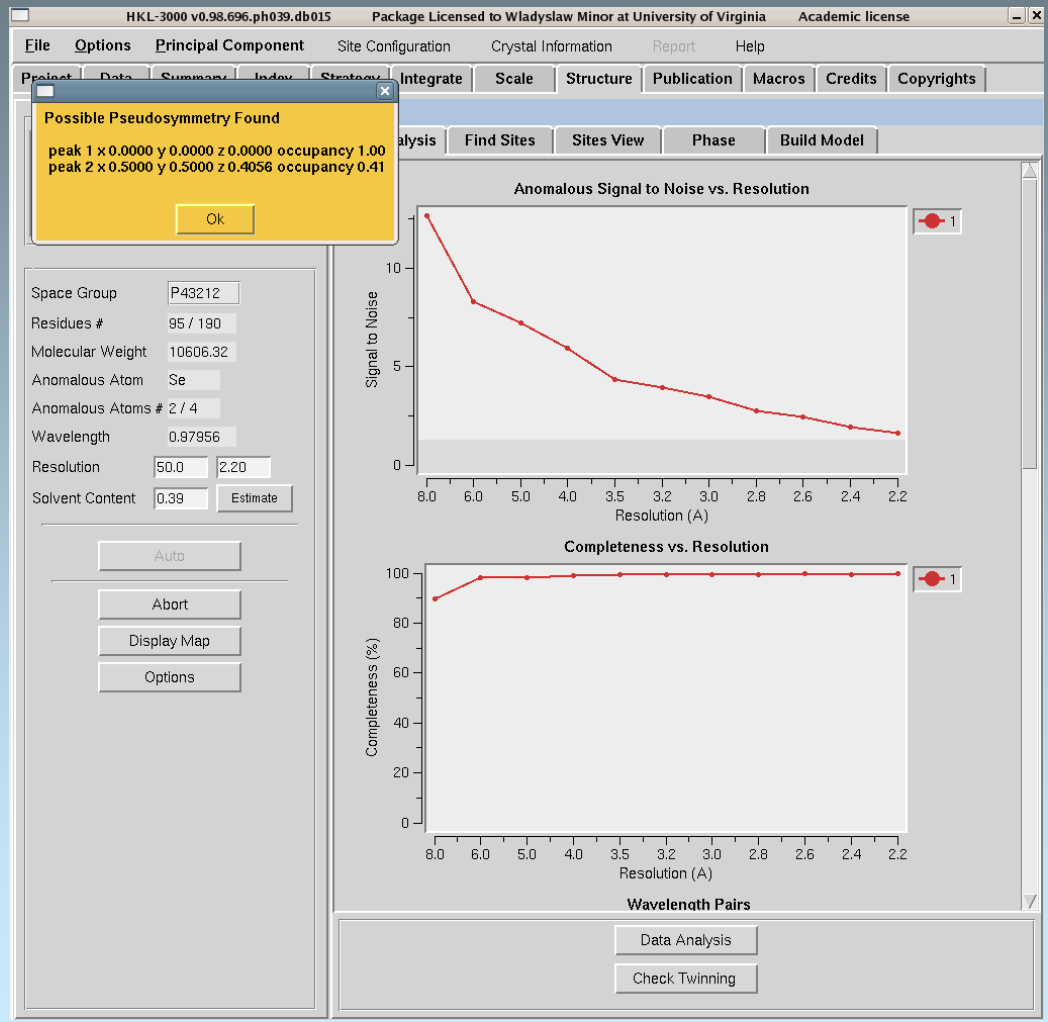


Project setup

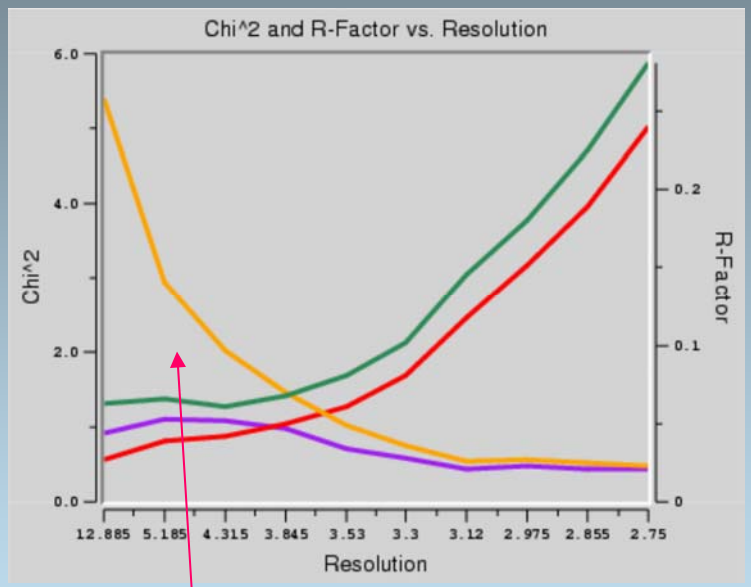
Check against PDB during data collection

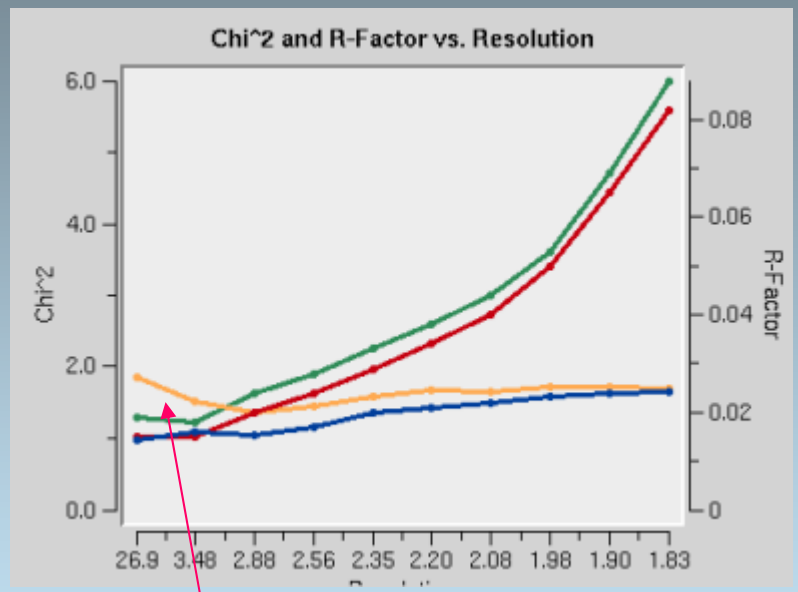


Possible Pseudo-translational Symmetry



Anomalous signal detection





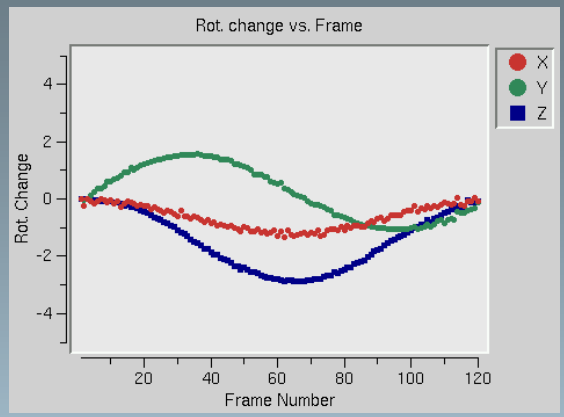
Signal from Se-Met

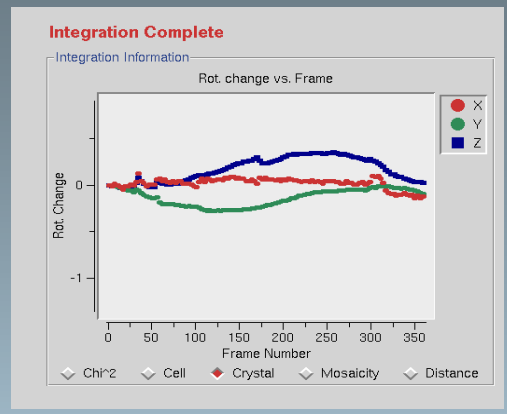
Signal from Sulfur

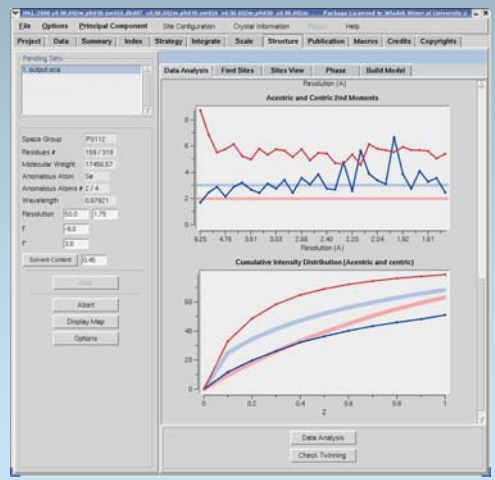
Feedback of possible hardware, sample or experiment problems

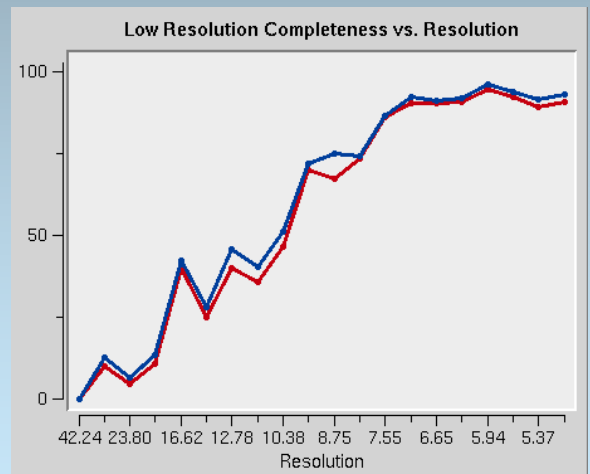
- Detector
- Goniostat
- Cooling

- Crystal decay
- Experiment
- Twinning
- …..



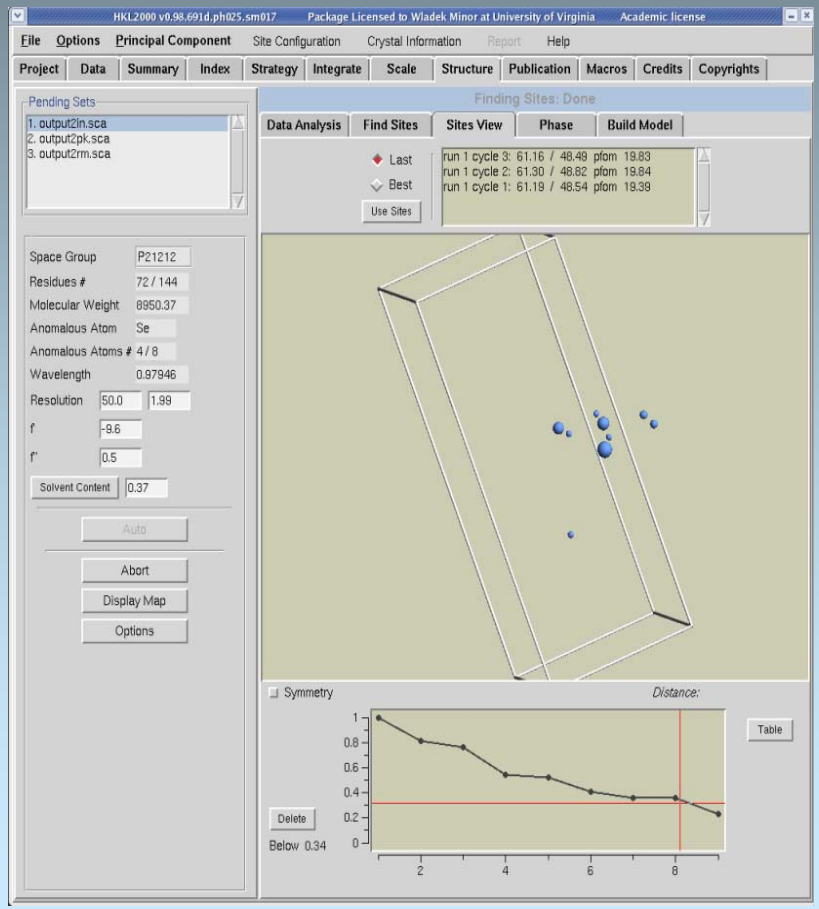


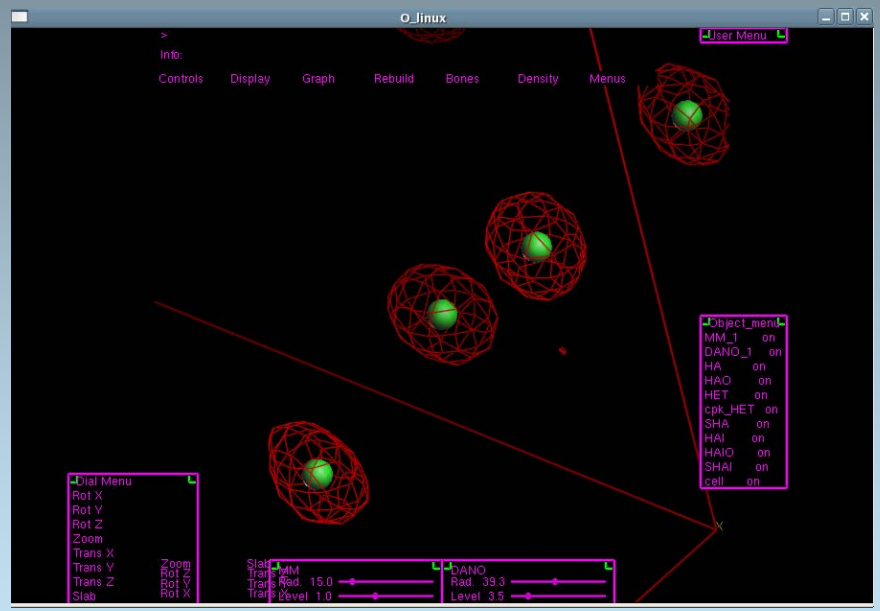




Substructure solution

- Data management in HKL-3000 - substructure solution



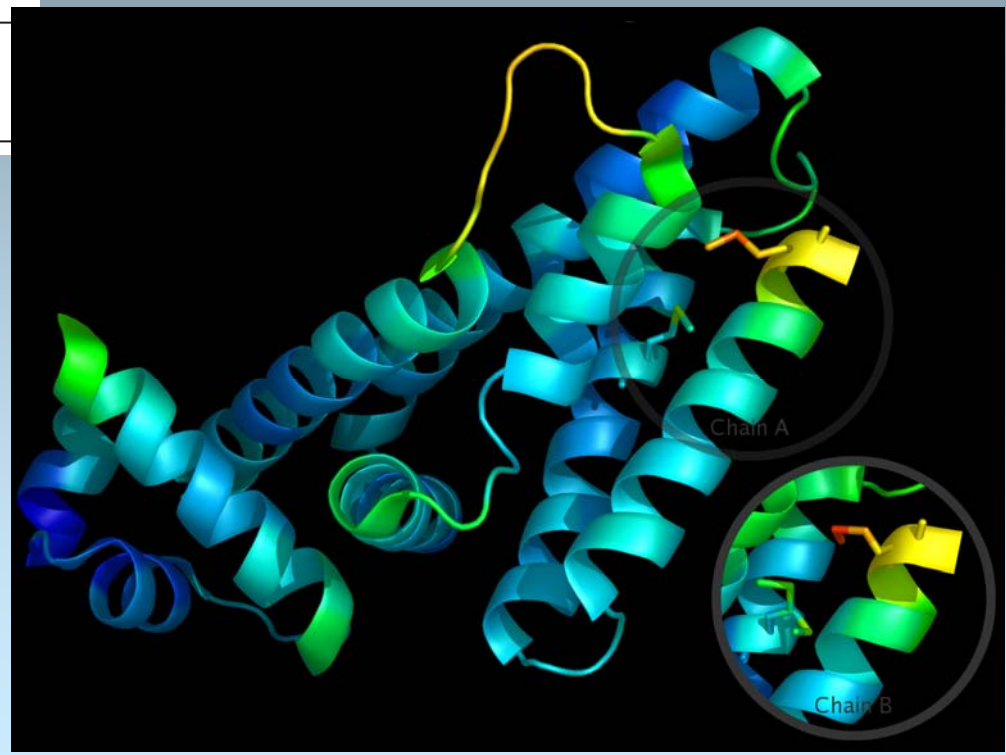


Double Conformation

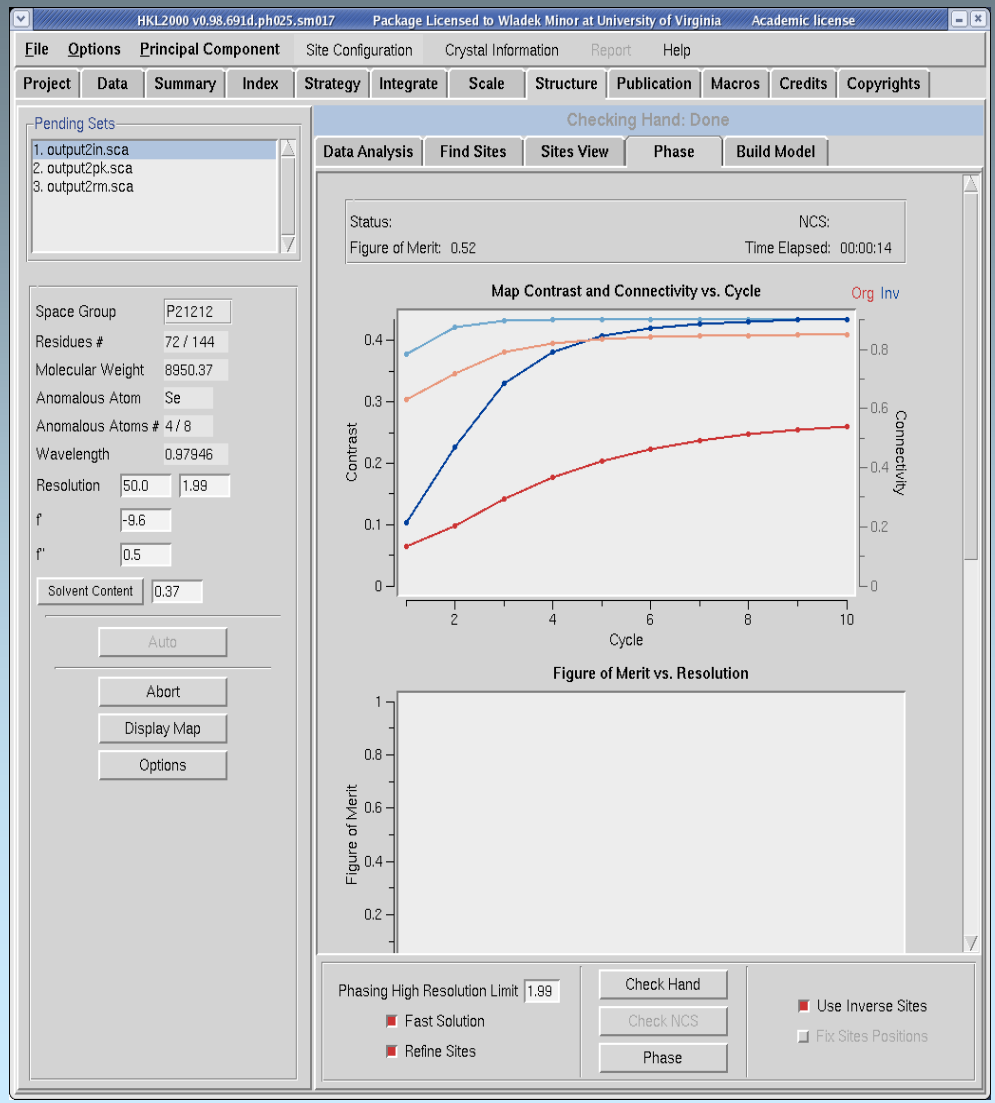
Experimental electron-density map correlation *versus* final model.

Each data set was phased with and without double sites. The correlation coefficient CC was calculated between the final refined model and the experimental density map obtained from density modification without NCS averaging.

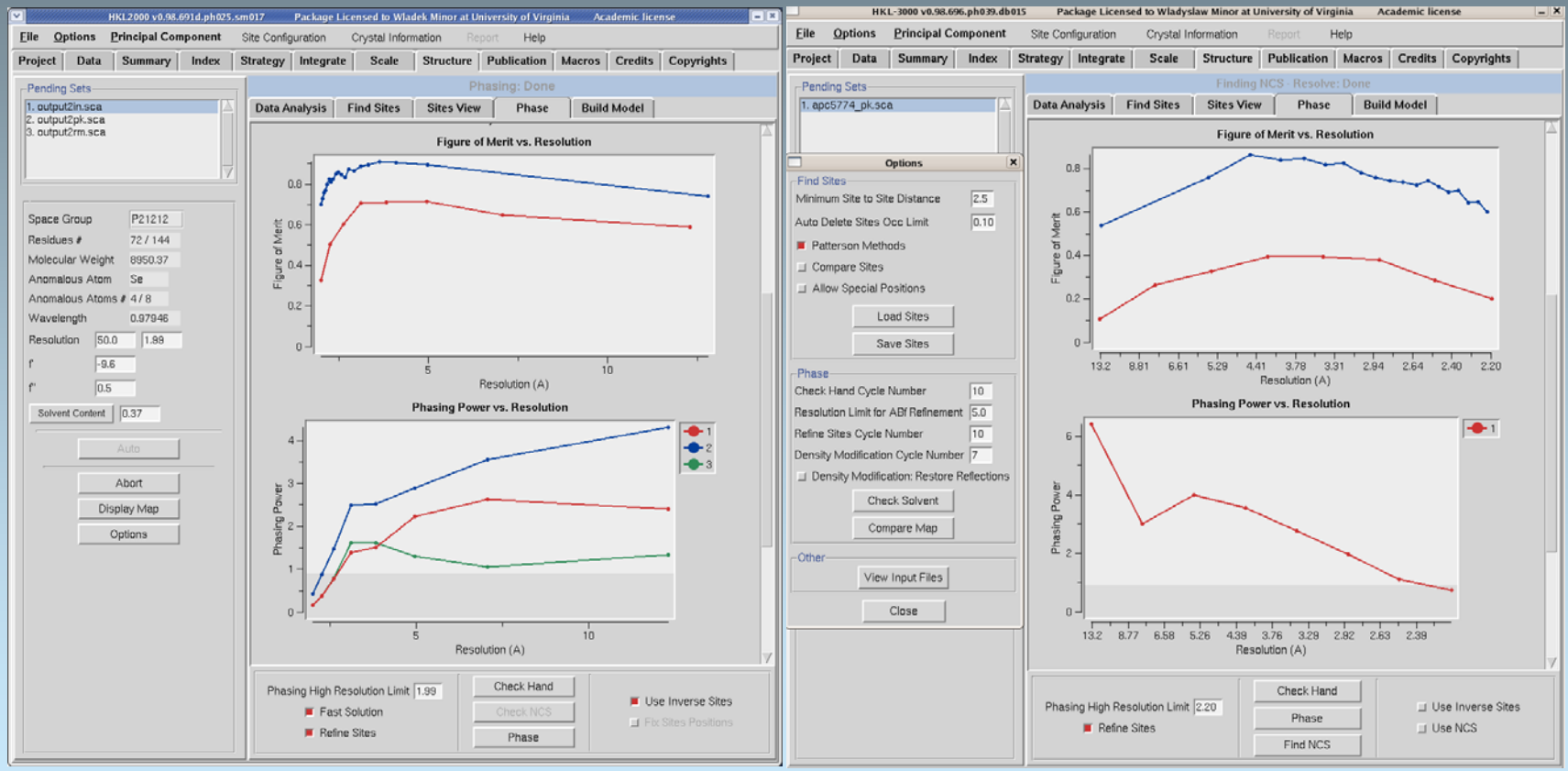
PDB code	Total No. of sites	No. of double sites	Solvent content (fraction)	Resolution (Å)	CC for double sites (main/side chain)	CC for single sites (main/side chain)
1wq6	5	3	0.37	2.0	0.76/0.67	0.70/0.61
2g3b	4	1	0.45	2.0	0.57/0.50	0.51/0.43
2g7u	16	4	0.53	2.3	0.81/0.64	0.80/0.63



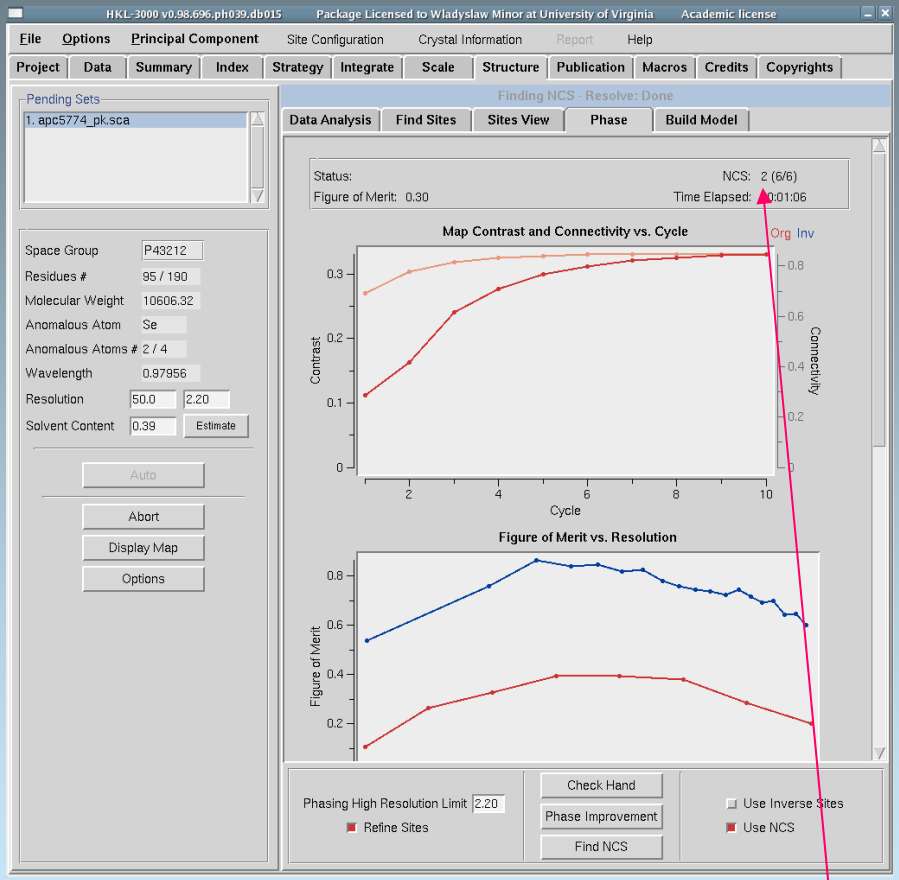
Substructure enantiomorph determination

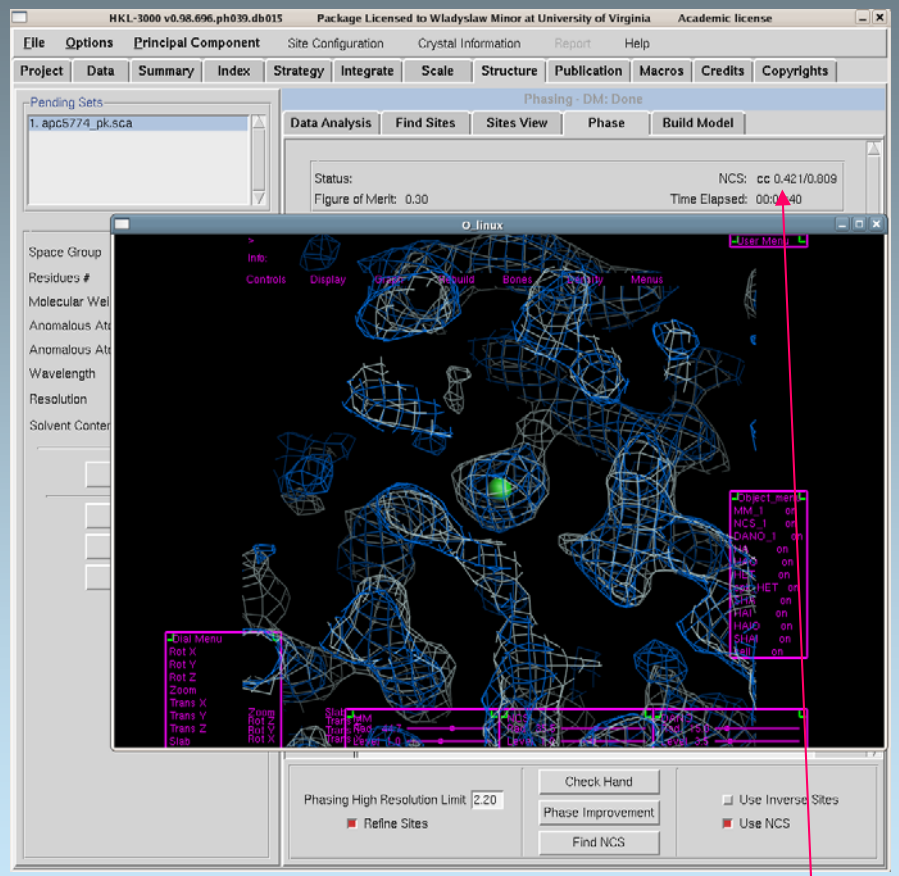


SAD or MAD phasing



NCS identification and use

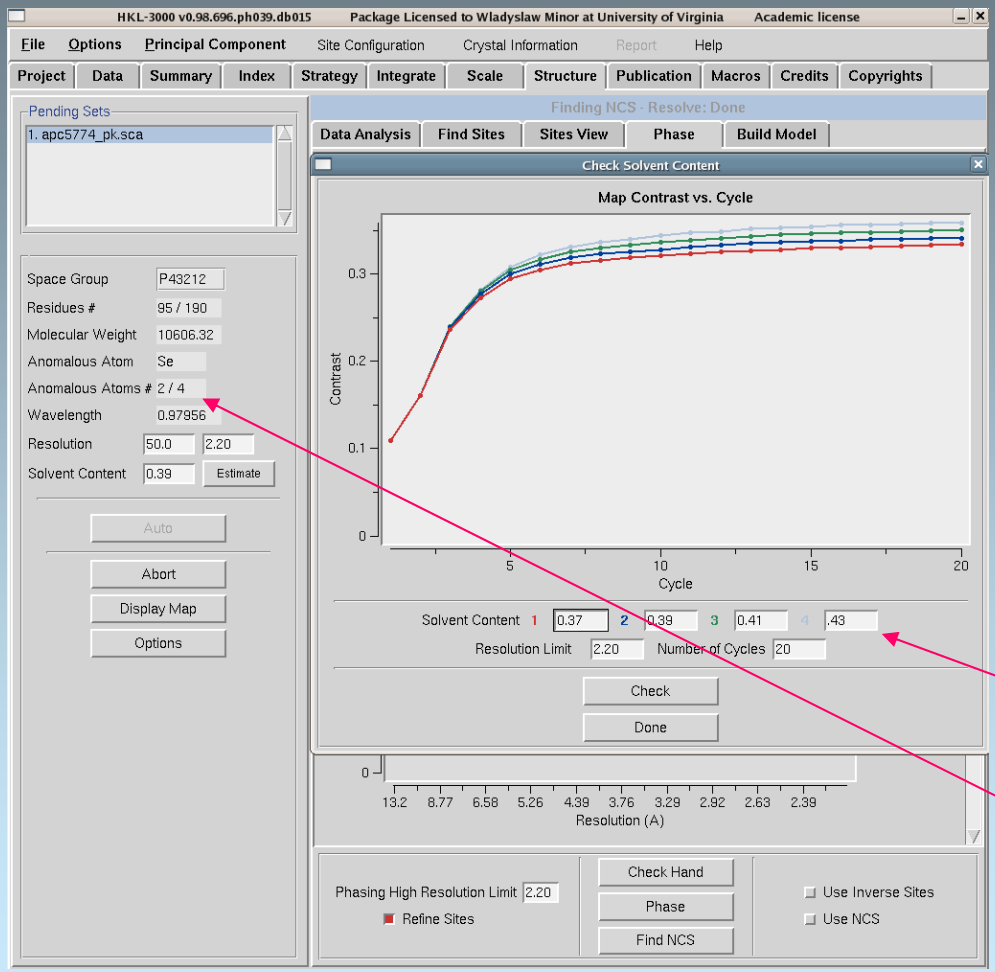


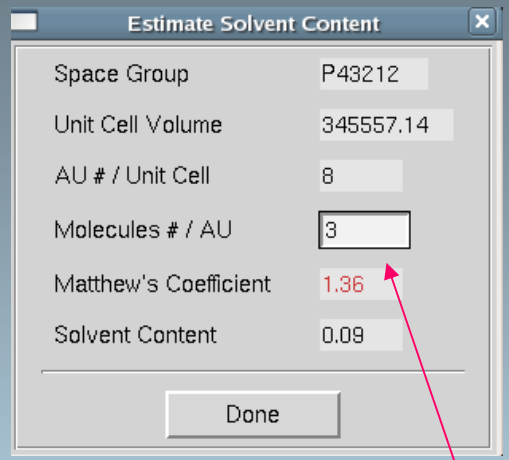


From sites

From map

Optimization of solvent content worthwhile but slow



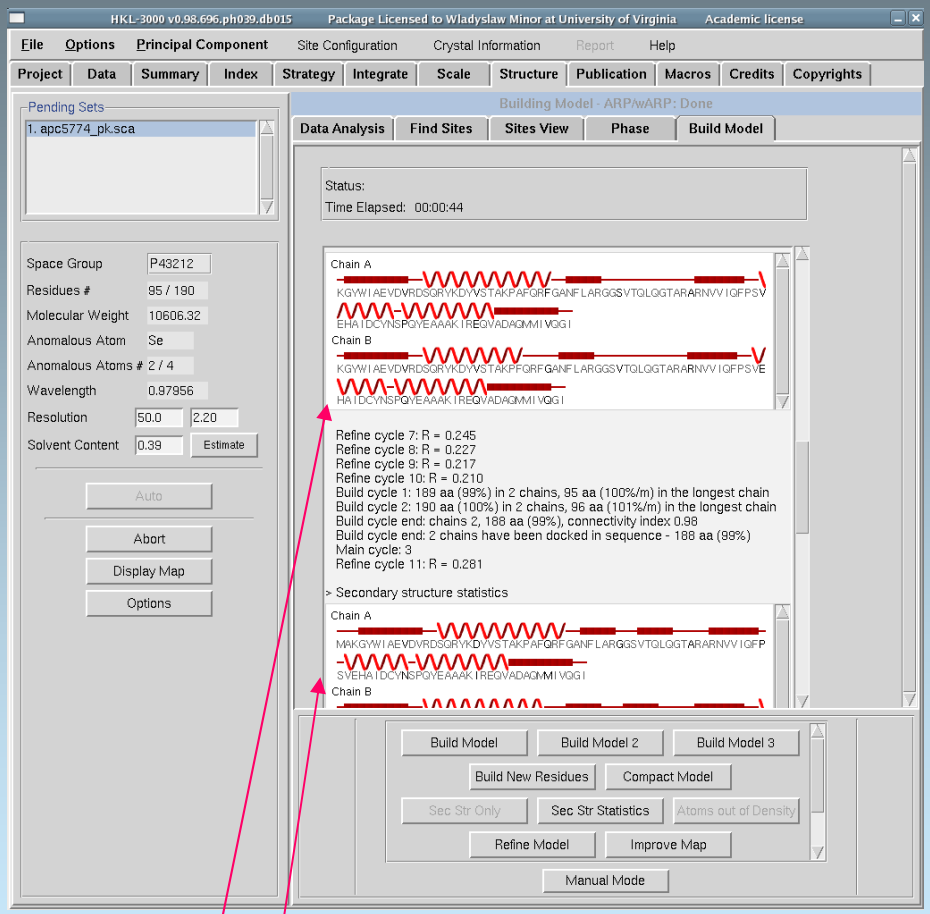


automatic

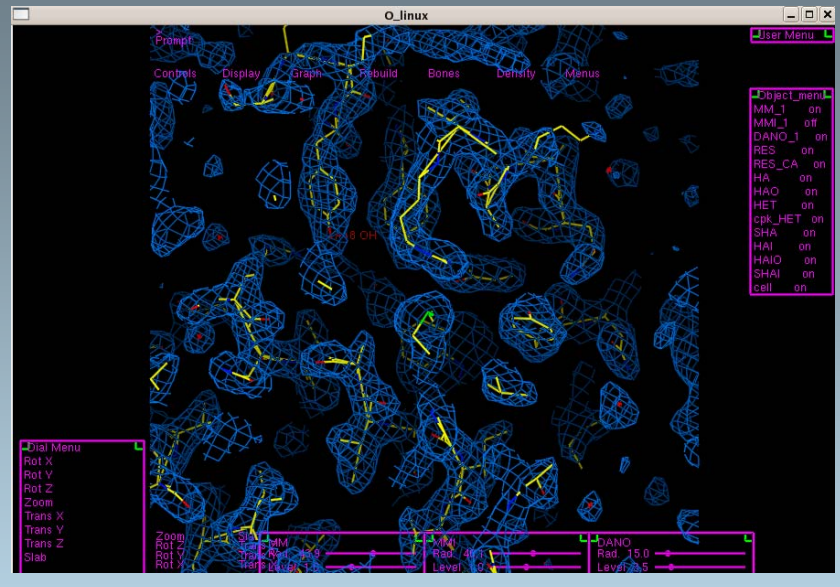
manual

optimization

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



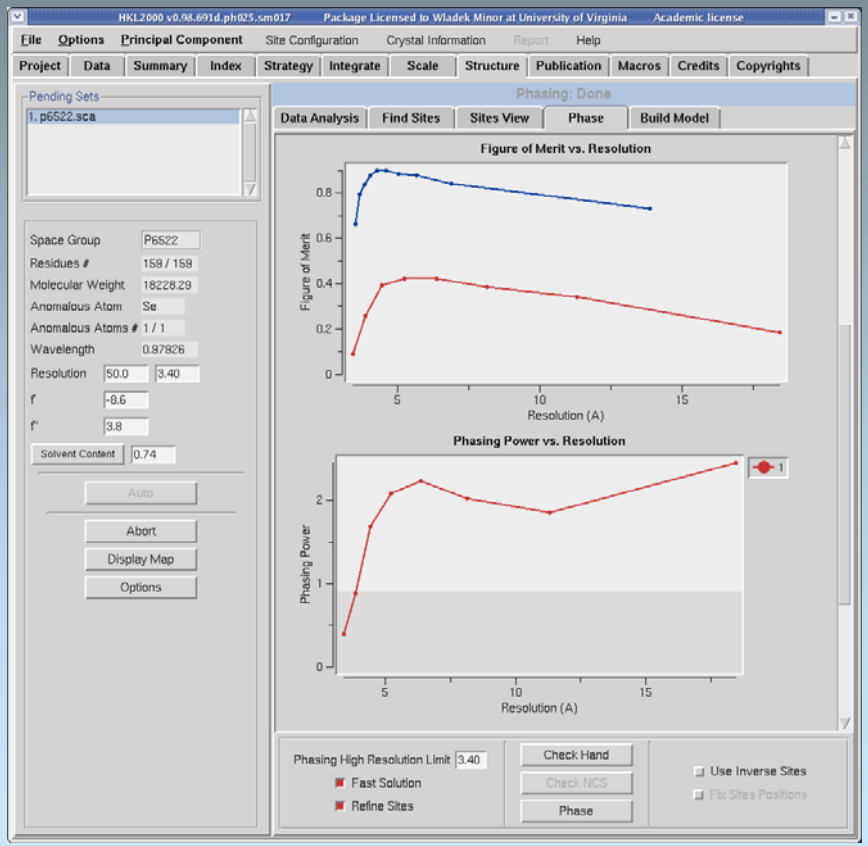
Secondary structure after each cycle

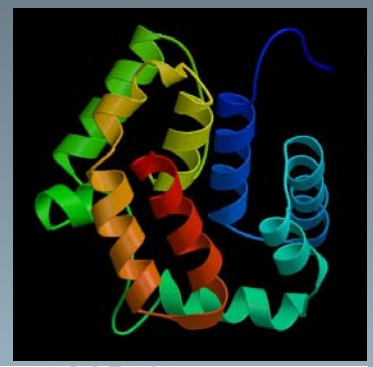


Unidentified density -> Xtaldb

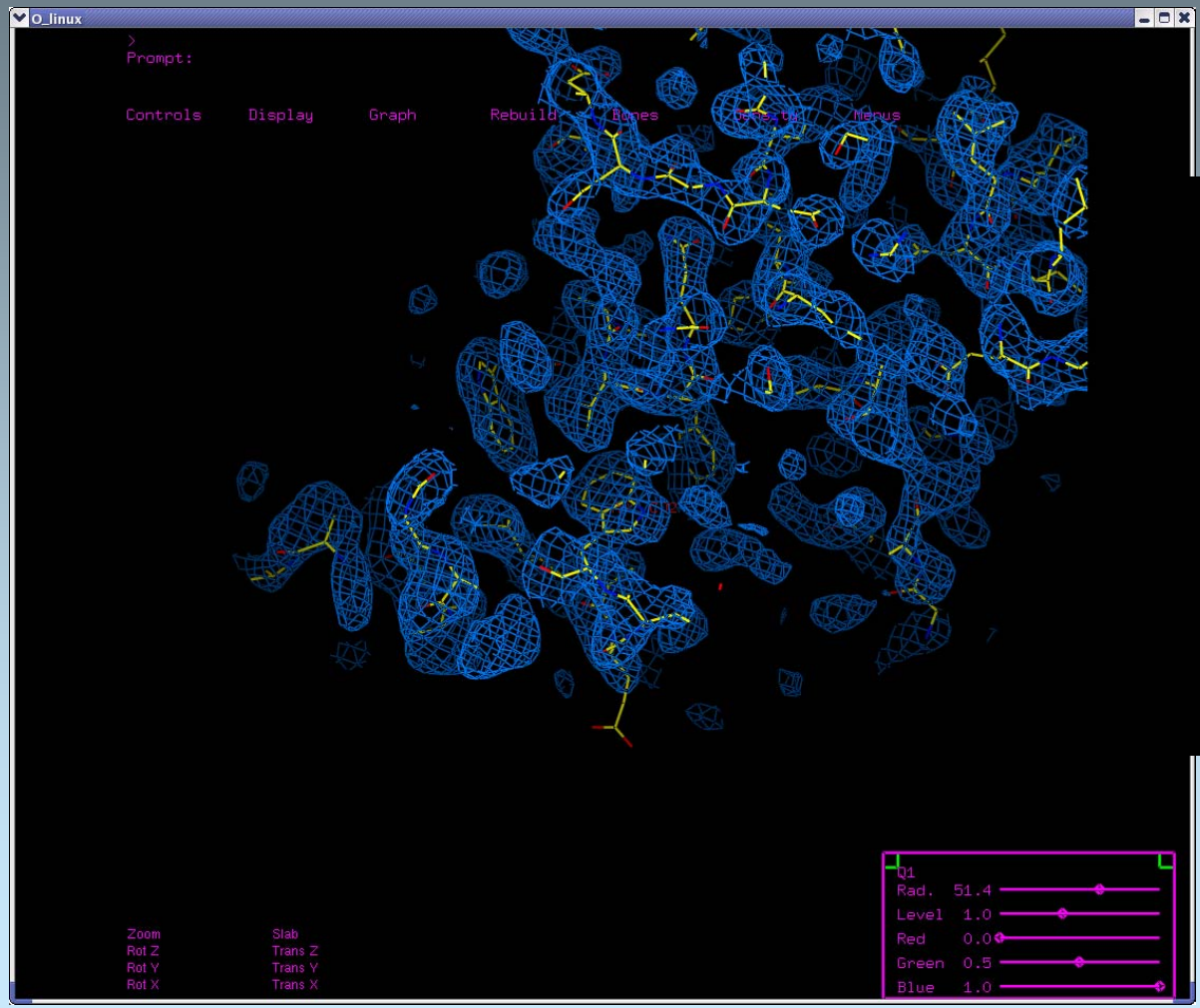
Low resolution SAD phasing difficult structure – no

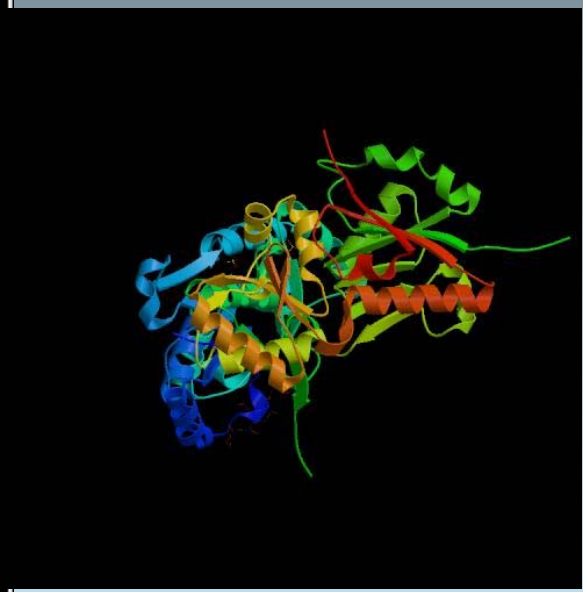
1ze0





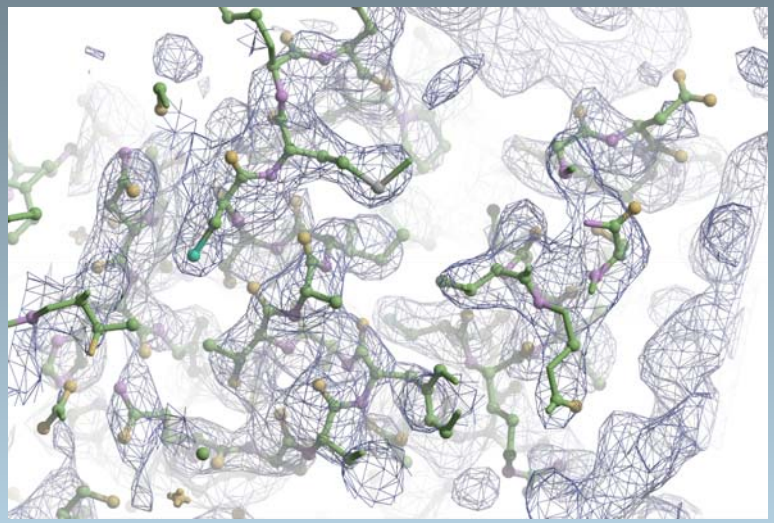
How well does it work ?
1xvi (Youngchang Kim)



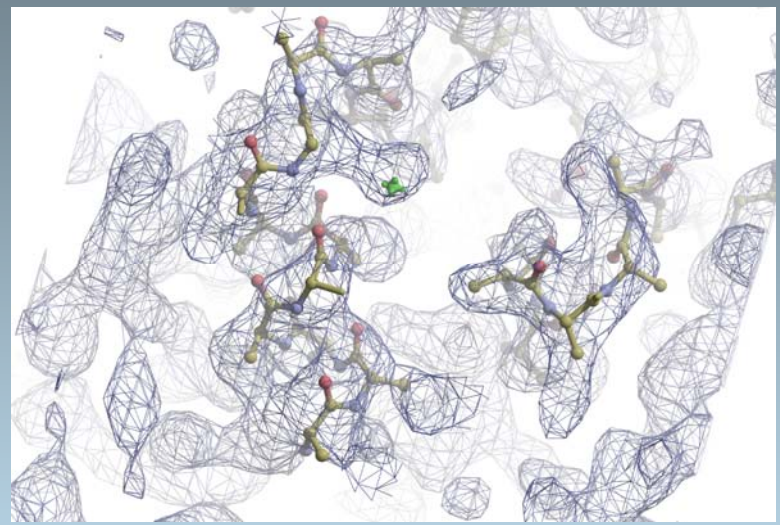


Structures solved in wrong target

APC81521 (ChangSoo)



Final model with right target
APC81521

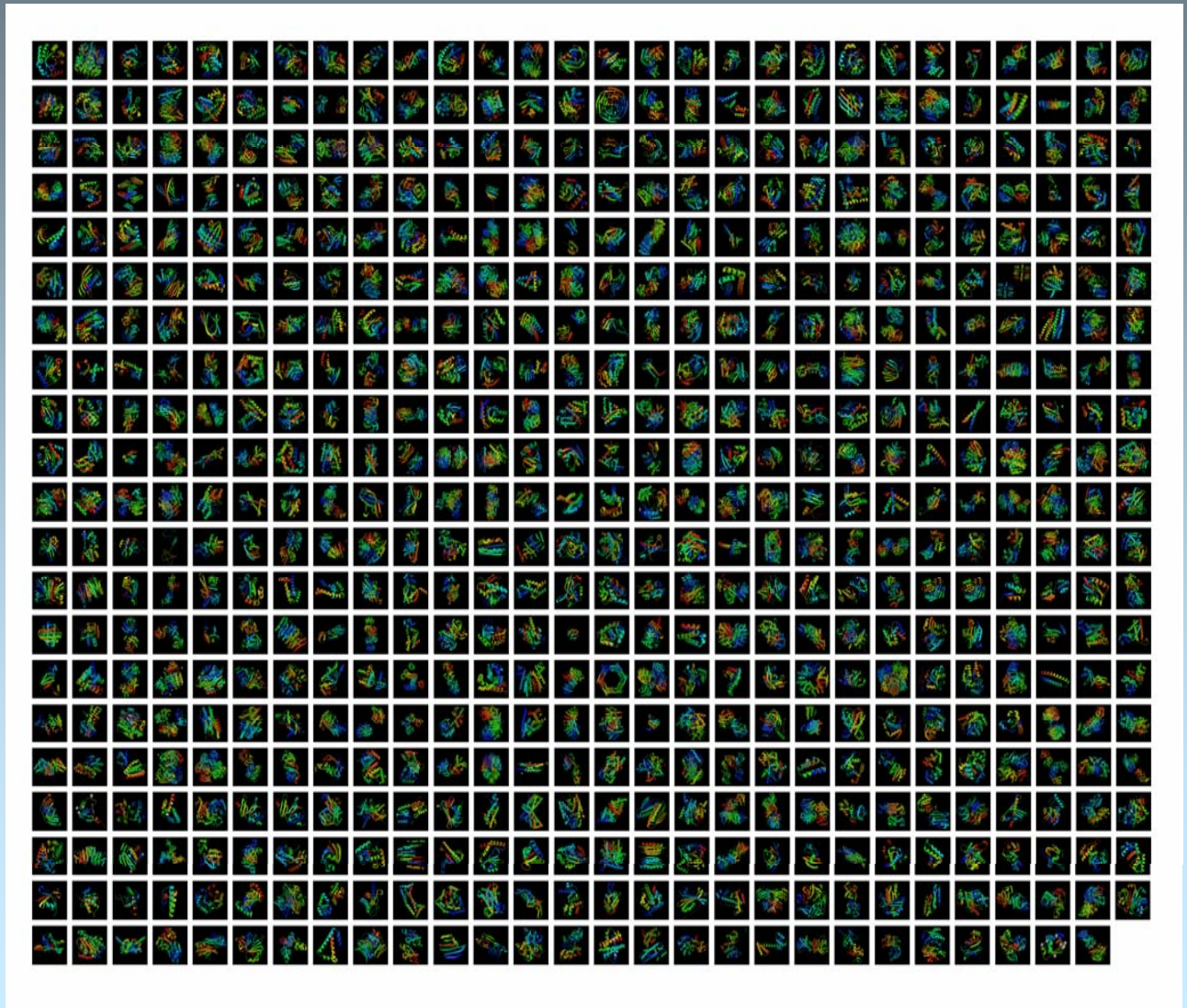
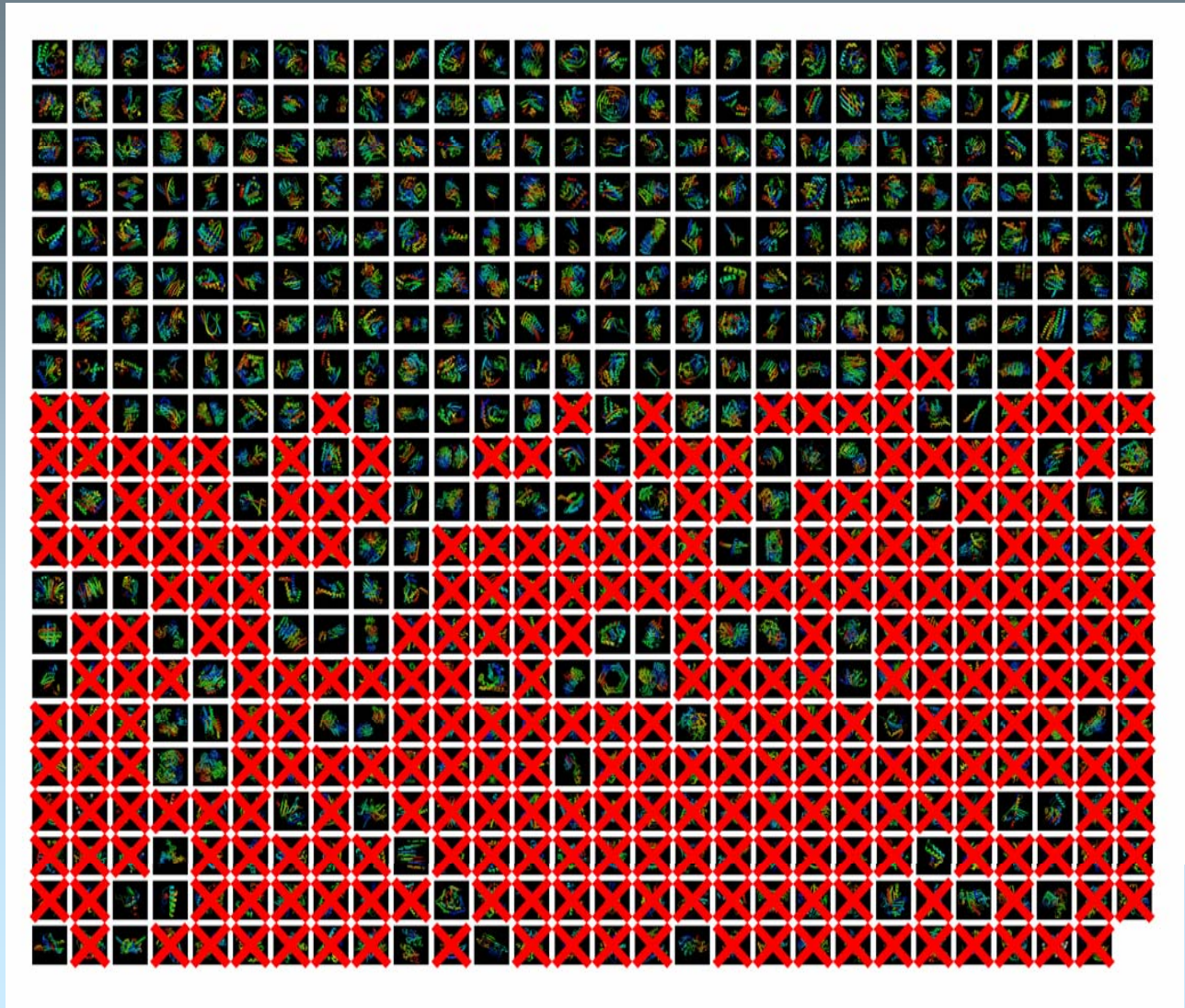


Auto-build (resolve) model with wrong target
APC81501

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

1. Manually build helixes 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.

MCSG 588



MCSG 588