Automated structure refinement with *phenix.refine*

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Crystallographic structure refinement

- **Today's choices for refinement programs**
  - SHELX
  - REFMAC
  - CNS
  - BUSTER-TNT
  - MOPRO
  - phenix.refine

- **Focus of next slides is:**

  phenix.refine: a highly-automated state-of-the-art structure refinement program which is part of PHENIX package

  Development mainly at Lawrence Berkeley National Lab (USA):

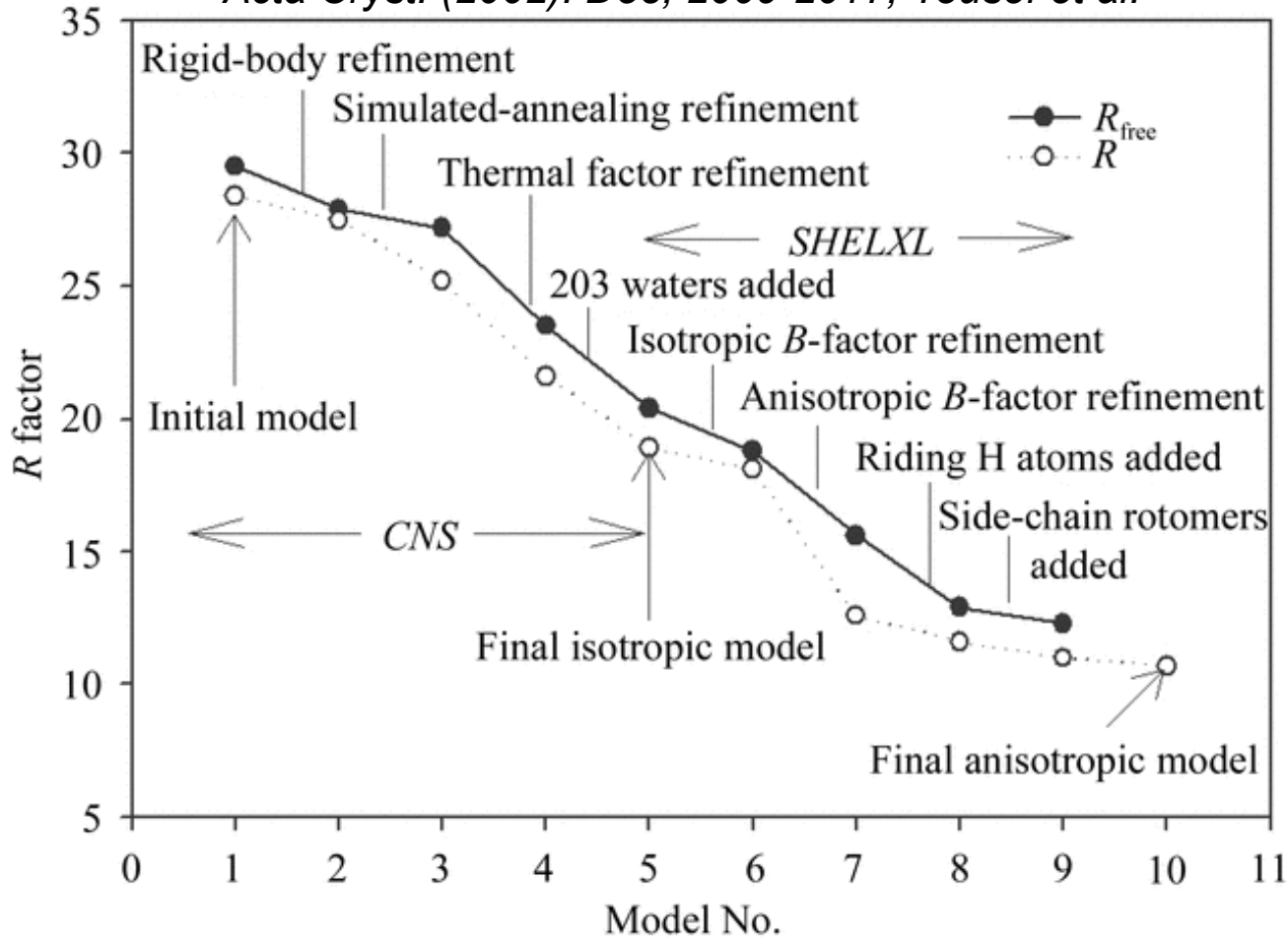
  Paul Adams, Pavel Afonine, Nathaniel Echols, Ralf Grosse-Kunstleve, Jeff Headd, Nigel Moriarty, Peter Zwart

  + *valuable contribution by many others (*Marat Mustyakimov*, Sasha Urzhumtsev, Vladimir Lunin, …)*

Automation of structure refinement

- What used to be in the past … and often still the case nowadays

Acta Cryst. (2002). D58, 2009-2017, Yousef et al.



- Clearly, the modern software should do all these steps automatically

- This is one of the goals of phenix.refine

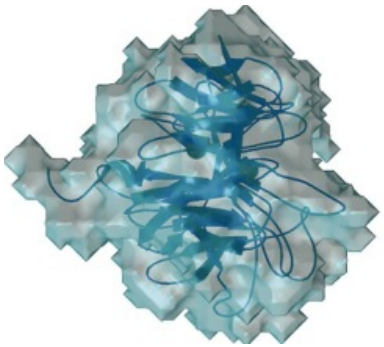
Automation of structure refinement

Round	Action taken	Program	Resolution (Å)	R (%)	R_{free} (%)
1	Simulated annealing	P	30–1.5	24.83	27.28
2	Isotropic, add solvent	P	10–1.1	15.17	16.80
3	Same atoms, isotropic	S	10–1.1	14.75	16.83
4	All-atom anisotropic	S	10–1.1	11.59	14.52
5	Add disorders (add Leu129)	S	10–1.1	10.93	14.00
6	Change resolution	S	8–1.0	11.22	13.75
7	Isotropic, water	P	8–0.65	16.78	17.24
8	Same atoms, isotropic	S	8–0.65	16.56	17.53
9	Anisotropic, add disorders	S	8–0.65	10.75	11.86
10	Isotropic, water	P	30–0.65	16.95	17.55
11	Same atoms, isotropic	S	30–0.65	16.33	17.24
12	Anisotropic, add disorders	S	30–0.65	10.71	11.69
13	Minor adjustments	S	30–0.65	10.10	11.12
14	Riding hydrogens added	S	30–0.65	9.16	10.04
15	Minor adjustments	S	30–0.65	9.00	9.95
16	Add flexible loop	S	30–0.65	8.71	9.63
17	Weighting changed	S	30–0.65	8.65	9.62
18	Restraints removed	S	30–0.65	8.48	9.59
19	Water occupancies refined	S	30–0.65	8.39	9.52
20	Free R removed	S	30–0.65	8.39	—

Wang *et al., Acta Cryst. (2007). D63, 1254-1268*

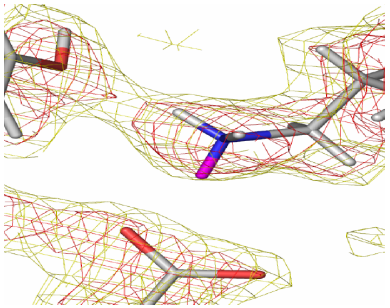
phenix.refine: single program for a very broad range of resolutions

Low



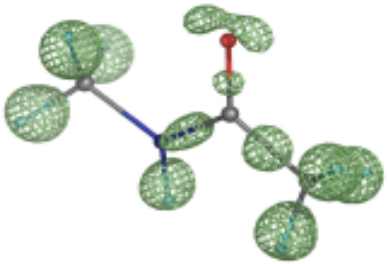
- Group ADP refinement
- Rigid body refinement
- Torsion Angle dynamics
- Reference model
- Ramachandran plot restraints
- Secondary structure restraints

Medium and High



- Restrained/constrained refinement of individual parameters
- Automatic water update

Subatomic

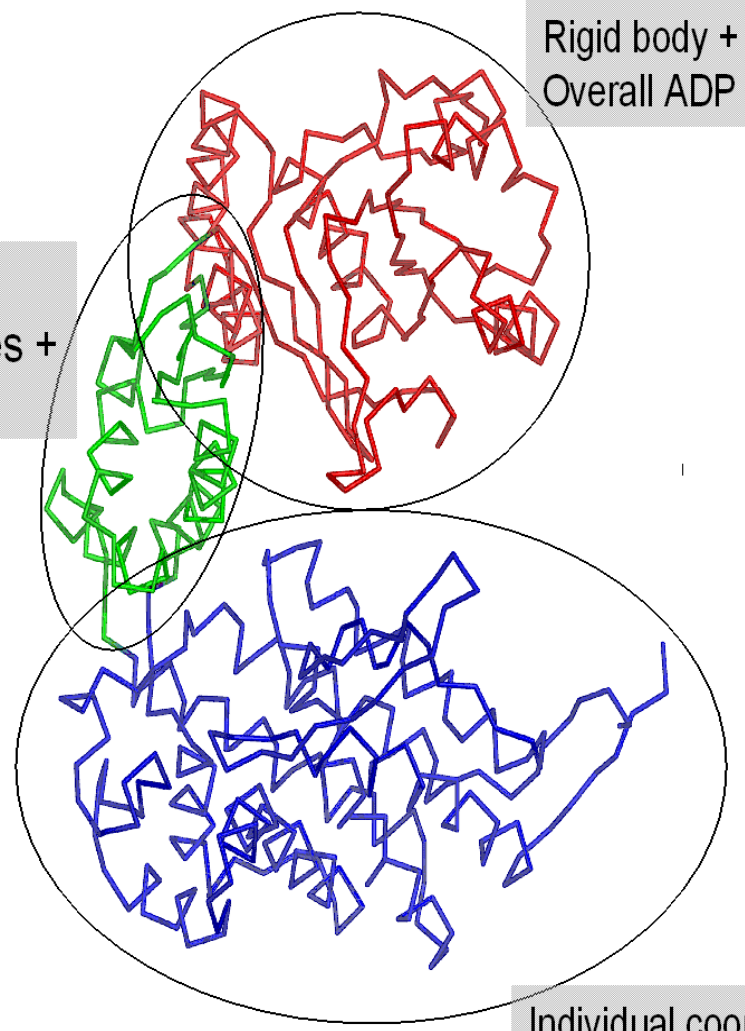


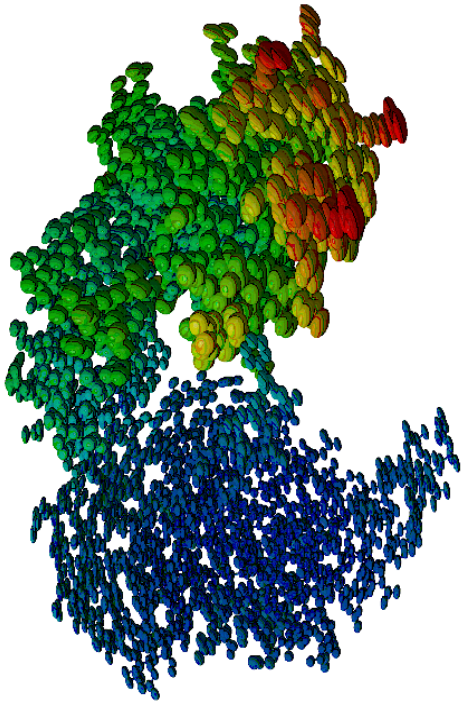
- Bond density model
- Unrestrained refinement
- FFT or direct
- Explicit hydrogens

- Automatic NCS restraints
- Simulated Annealing
- Automatic side chain rotamer fixing
- Occupancies (individual, group, automatic constrains for alternative conformations)
- Various targets: LS, ML, MLHL,…
- Dual (real/reciprocal) space refinement

- TLS refinement with automated TLS groups identification
- Use hydrogens at any resolution
- Refinement with twinned data
- X-ray, Neutron, joint X-ray + Neutron

Refine any part of a model with any strategy: *all in one run*





+ Automatic water picking

+ Simulated Annealing

+ Add and use hydrogens

Running phenix.refine

- Designed to be very easy to use
- Several ways of running:
  - command line version:

```
phenix.refine model.pdb data.hkl [parameters]
```

    - Can be highly customized (more than 300 parameters available to change)
  - can be called from (a Python) script allowing to run it within different contexts
  - GUI version

Refinement flowchart

PDB model,
Any data format
(CNS, Shelx, MTZ, …)

Input data and model processing

Refinement strategy selection

Bulk-solvent, Anisotropic scaling, Twinning parameters refinement

Ordered solvent (add / remove)

Target weights calculation

Coordinate refinement (real- and reciprocal space) (rigid body, individual) (minimization or Simulated Annealing)

ADP refinement
(TLS, group, individual iso / aniso)

Occupancy refinement (individual, group)

Output: Refined model, various maps, structure factors, complete statistics, ready for deposition PDB file

Repeated several times

Files for COOT, O, PyMol

✓ *Input data: can be intensities: French&Wilson method is used to convert Iobs to Fobs – no need to run Truncate*

Automatic Water Picking

✓ Water is updated (add/remove/refine) automatically as part of refinement run:

- No need to do it as a separate step using external tools

Input data and model processing

Refinement strategy selection

Bulk-solvent, Anisotropic scaling, Twinning parameters refinement

Ordered solvent (water picking)

Target weights calculation

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(rigid body, individual) (minimization or SA)

ADP refinement
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Occupancy refinement (individual, group)

Output: Refined model, various maps, structure factors, complete statistics, ready for deposition PDB file

- **remove** "bad" water:
  - 2mFo-DFc (peak height)
  - distances
  - map CC (2mFo-DFc, Fc)
  - B-factors and anisotropy
  - occupancy
- **add** new:
  - mFo-DFc,
  - distances
- **pre-refine** water parameters

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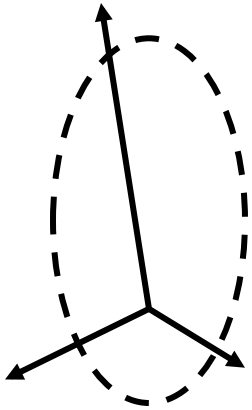
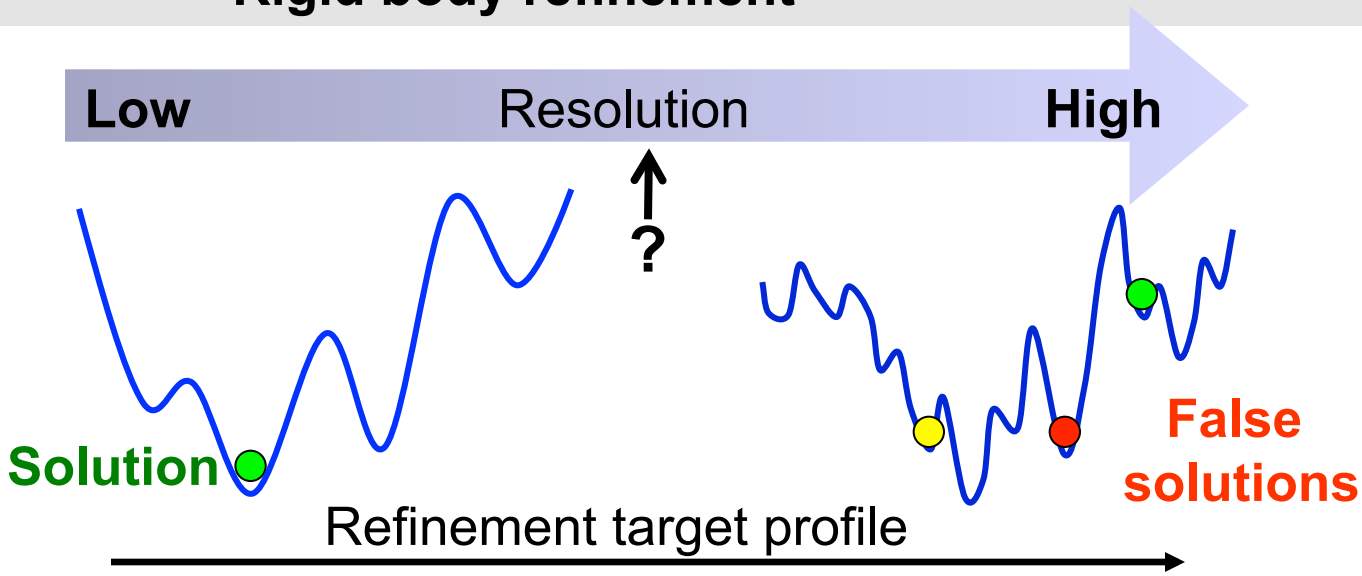
Rigid body refinement

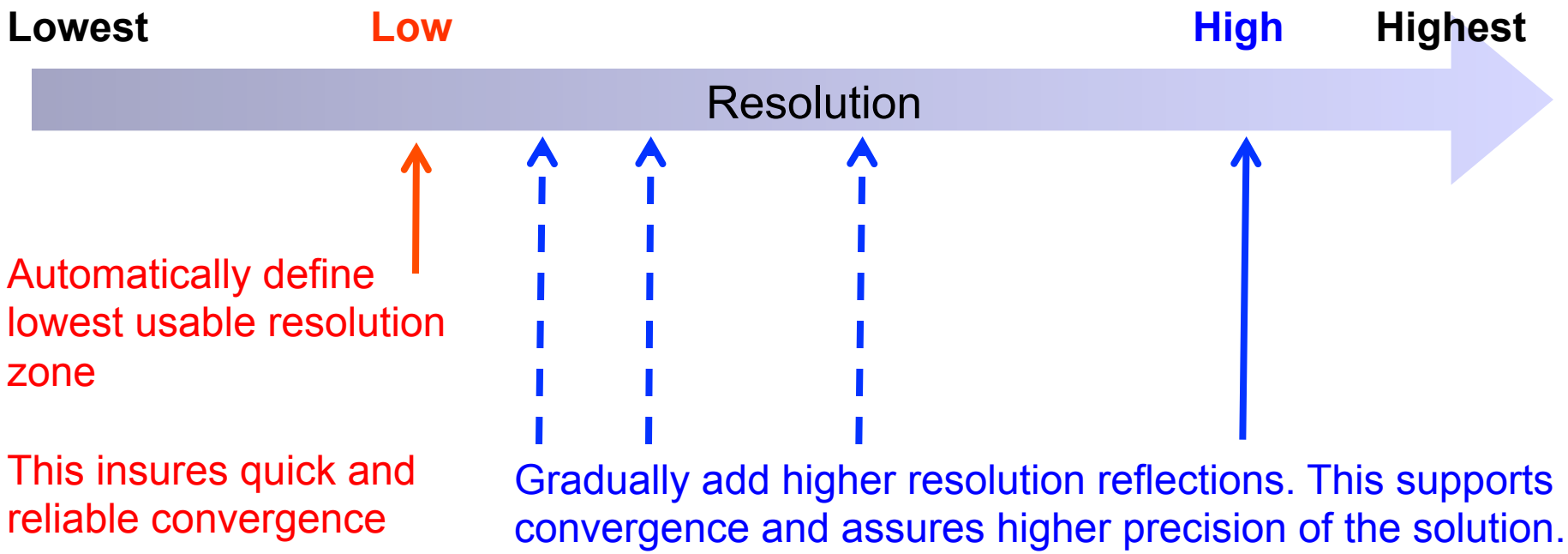




- **Rigid body refinement challenges:**
  - Need to use *low resolution* reflections to achieve a solution
    - Using too low resolution may not be good
    - Need to use higher resolution data to assure better solution
    - How to define low-high resolution border (3…4…6A)?
- **PHENIX MZ protocol** makes all these decisions automatically

Automatic multiple-zone rigid-body refinement with a large convergence radius.
P. V. Afonine, R. W. Grosse-Kunstleve, A. Urzhumtsev and P. D. Adams. J. Appl. Cryst. 42, 607-615 (2009)

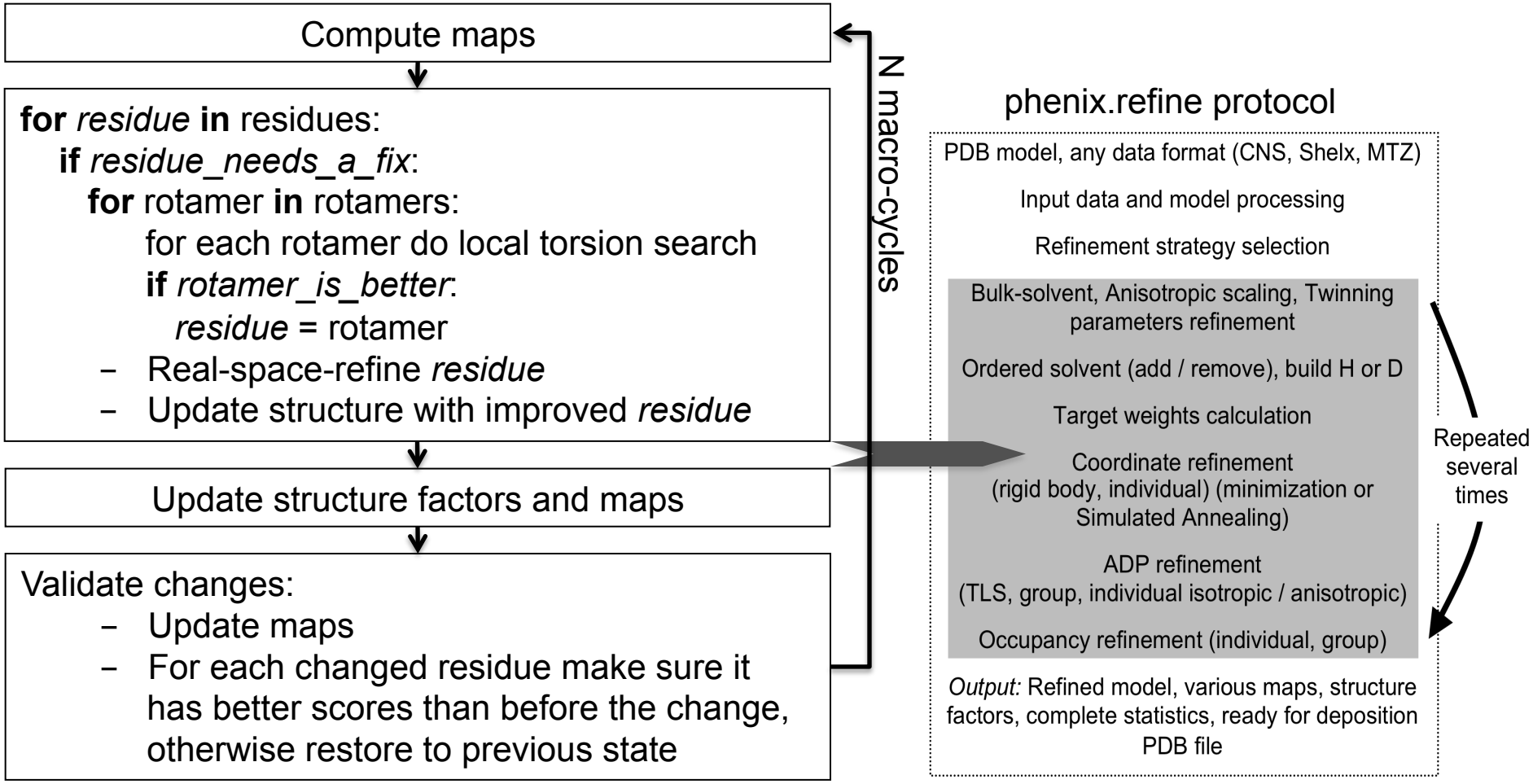
Automated Rigid Body Refinement in PHENIX (MZ protocol)



During rigid body refinement some large model movements are expected.
This invalidates the solvent mask, so the bulk-solvent model is updated at each step.

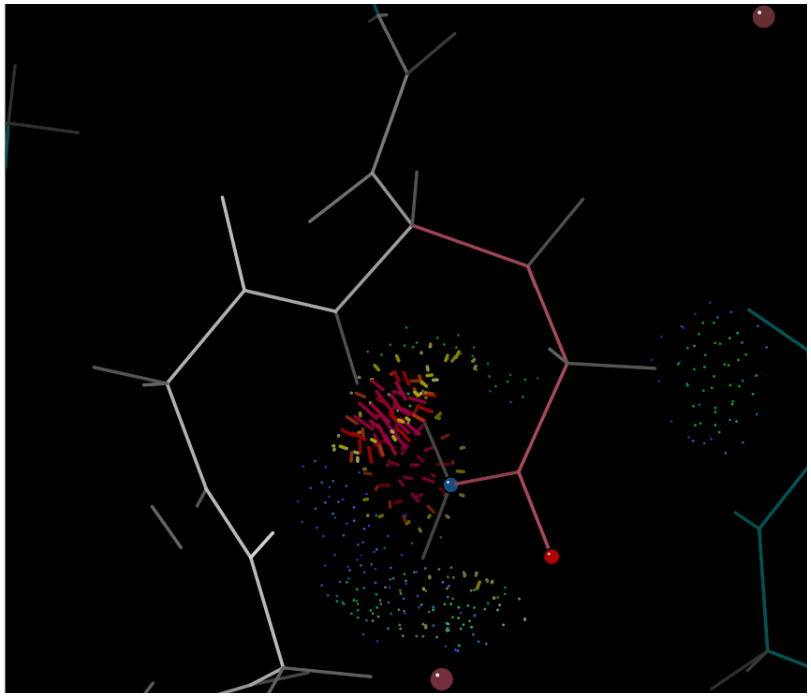
- All parameters used in the protocol are optimized to achieve the highest convergence radius at minimal runtime.
  - This is done by the grid search over ~100000 trial refinements using more than 100 different structures.

Local real-space refinement (fix_rotamers)

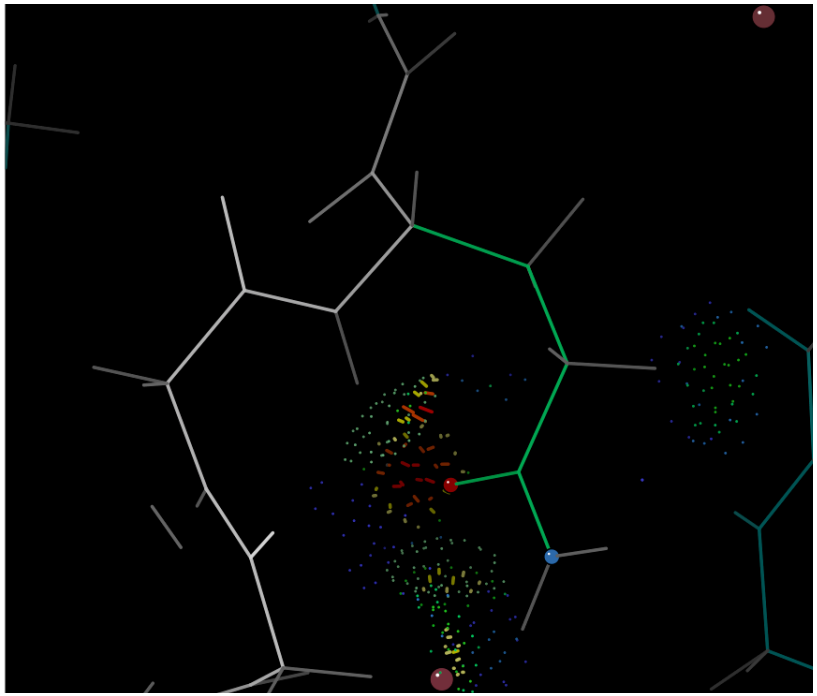


Automatic side chain flips to avoid bad clashes

- **phenix.refine** always applies side chain flips automatically (Asn/Gln/His)



Bad



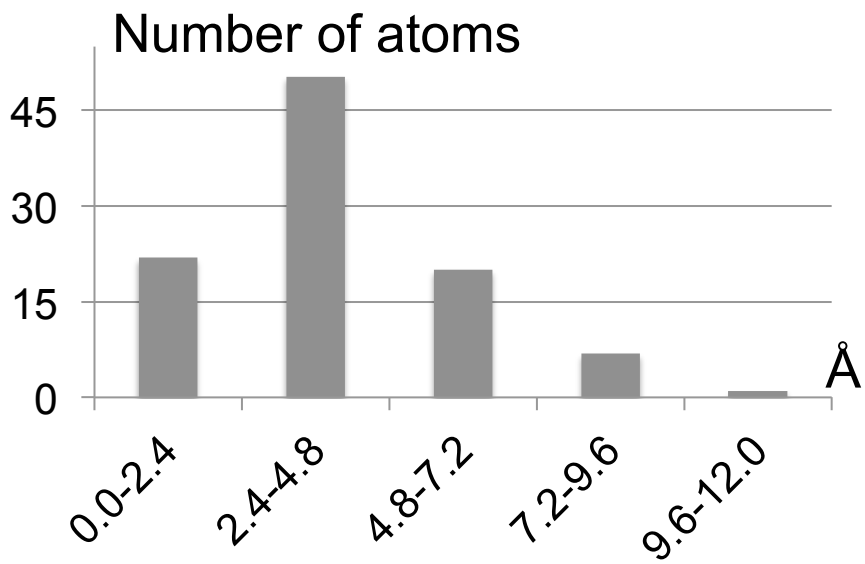
Good

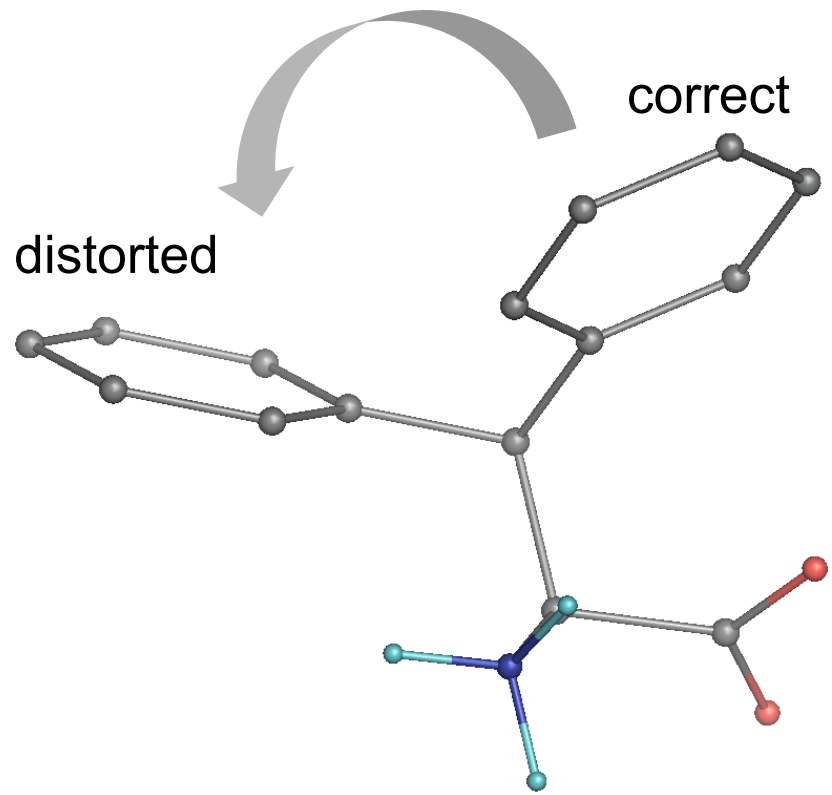
Test refinements: distorted models

- **Distorted models** (150 randomly picked from PDB structures at resolutions from 1.5 to 3Å):

  1. Remove water
  2. For each residue select the most distant rotamer
  3. Quick geometry regularization to remove bad clashes

- **Side chain distortions**:



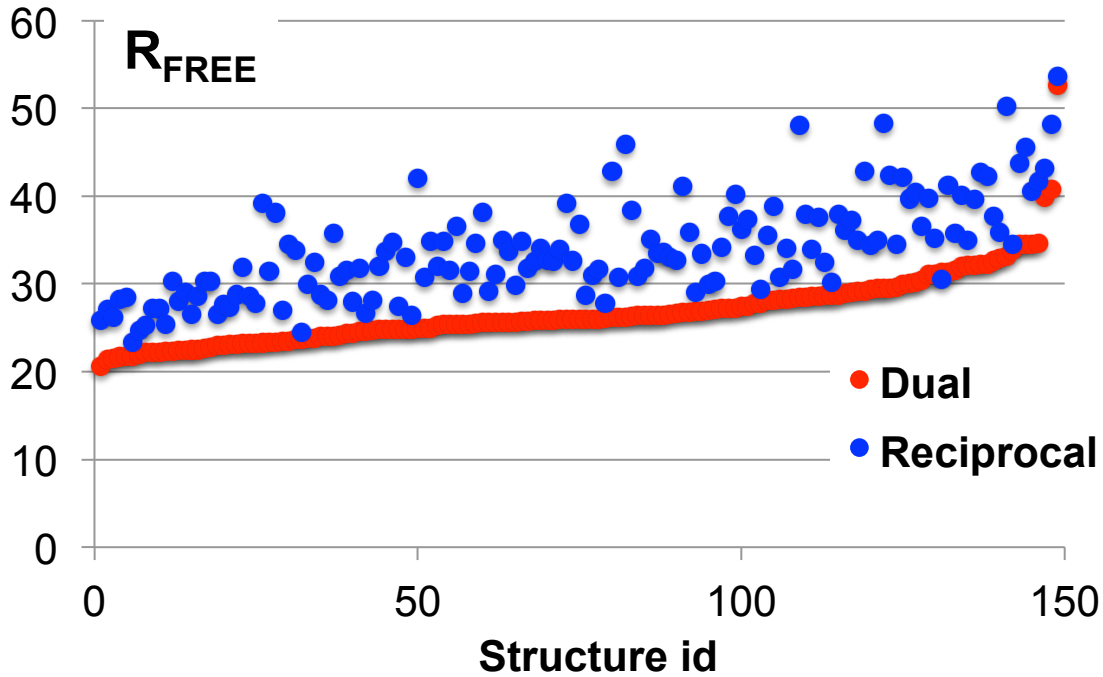


- **Main chain distortions**: rmsd~0.4 Å

Dual-space refinement: example

- **150 randomly picked from PDB structures at resolutions from 1.5 to 3Å**
  - Structures severely distorted:
    - > remove water
    - > each side-chain switched to a different rotamer
    - > geometry regularization

- **R_{FREE} after Reciprocal and Dual space refinement (sorted by R_{FREE} Dual)**



Reference Model Selections

Same chain ID:

```
refinement.reference_model.reference_group {
     reference = chain A
     selection = chain A
}
```

Different chain ID:

```
refinement.reference_model.reference_group {
     reference = chain A
     selection = chain B
}
```

Same chain ID, same residue range:

```
refinement.reference_model.reference_group {
     reference = chain A and resseq 2:119
     selection = chain A and resseq 2:119
}
```

Same chain ID, different residue range:

```
refinement.reference_model.reference_group {
     reference = chain A and resseq 130:134
     selection = chain A and resseq 120:124
}
```

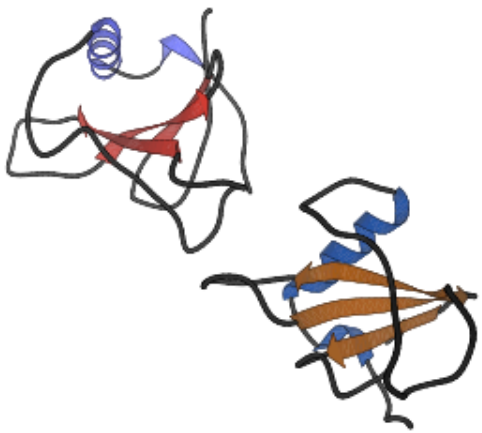
reference model refinement

MolProbity statistics after 5 macrocycles of *phenix.refine*

	Validation Criteria	1GTX, no ref.	1OHV	1GTX, 1OHV ref.	Target Value
All-Atom Contacts	Clashscore, all atoms:	**24.5**	**7.98**	**13.54**	
	Clashscore percentile	**89th**	**97th**	**97th**	
Protein Geometry	Poor rotamers:	**12.31%**	**2.30%**	**4.63%**	< 1%
	Rama outliers:	**0.65%**	**0.22%**	**0.27%**	< 0.2%
	Rama favored:	**92.88%**	**97.06%**	**96.14%**	> 98%
	Cβ dev. > 0.25Å:	**3**	**0**	**3**	0
	MolProbity score:	**3.16**	**1.87**	**2.41**	
	MP score percentile	**64th**	**94th**	**96th**	
	Res w/ bad bonds:	**0.00%**	**0.00%**	**0.00%**	0%
	Res w/ bad angles:	**0.38%**	**0.00%**	**0.43%**	< 0.1%
Residual	R-work	**0.1546**		**0.1586**	
	R-free	**0.2379**		**0.2186**	

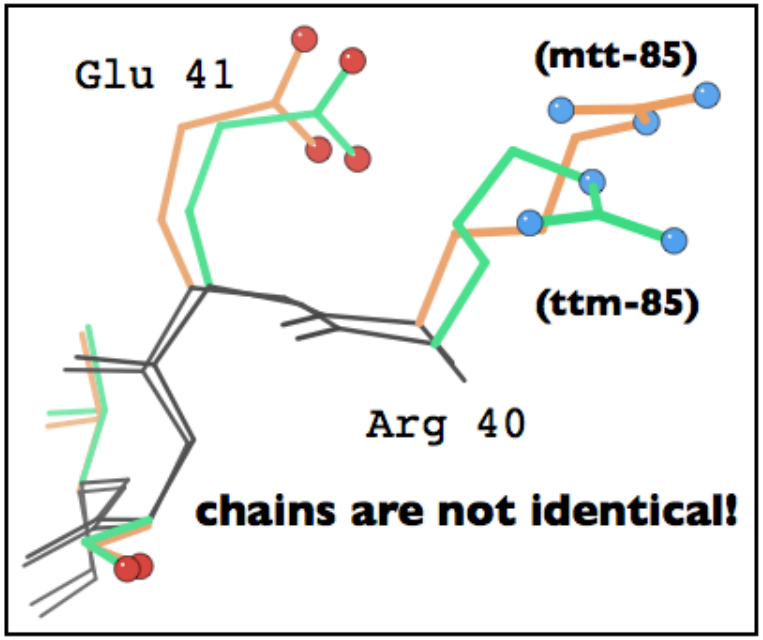
Extending Reference Model Restraints to NCS

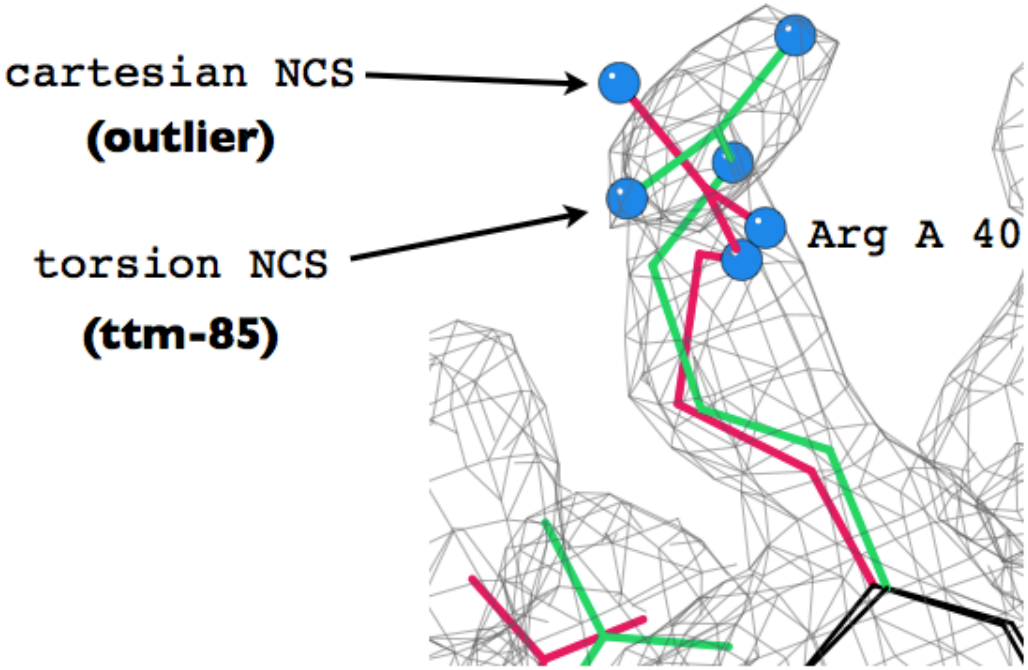
Rnase S - 2.5Å



5 macrocycles of *phenix.refine*

	R	R-free	clashscore	rotamer outliers
Default + fix_rotamers	0.1899	0.2557	7.98	1.83%
Cart. NCS + fix_rotamers	0.2106	0.2705	8.67	7.93%
Torsion NCS + fix_rotamers	**0.1890**	**0.2389**	**6.59**	**1.22%**





Refinement flowchart

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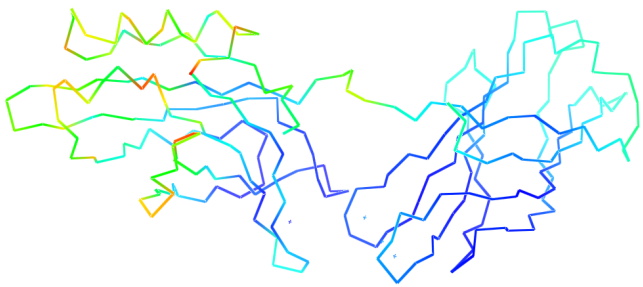
Repeated several times

Files for COOT, O, PyMol

ADP refinement: example

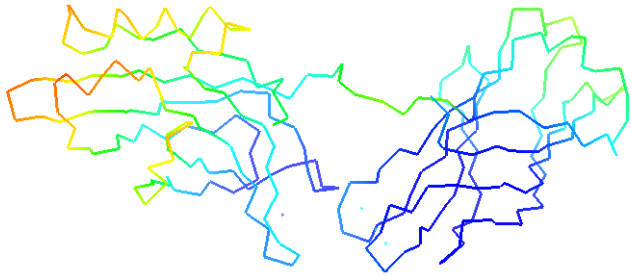
Synaptotagmin refinement at 3.2 Å



Original refinement (PDB code: 1DQV)

R-free = ***34*** *%*

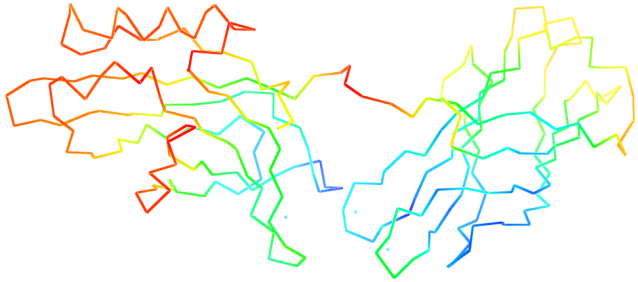
R = ***29*** *%*



PHENIX – Isotropic restrained ADP

R-free = ***28*** *%*

R = ***23*** *%*



PHENIX – TLS + Isotropic ADP

R-free = ***25*** *%*

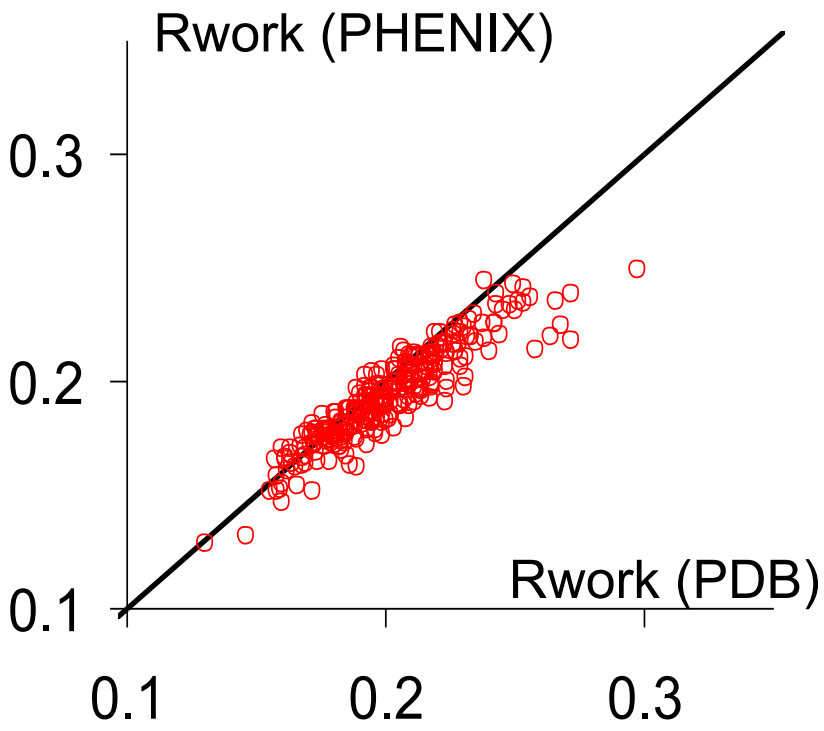
R = ***20*** *%*

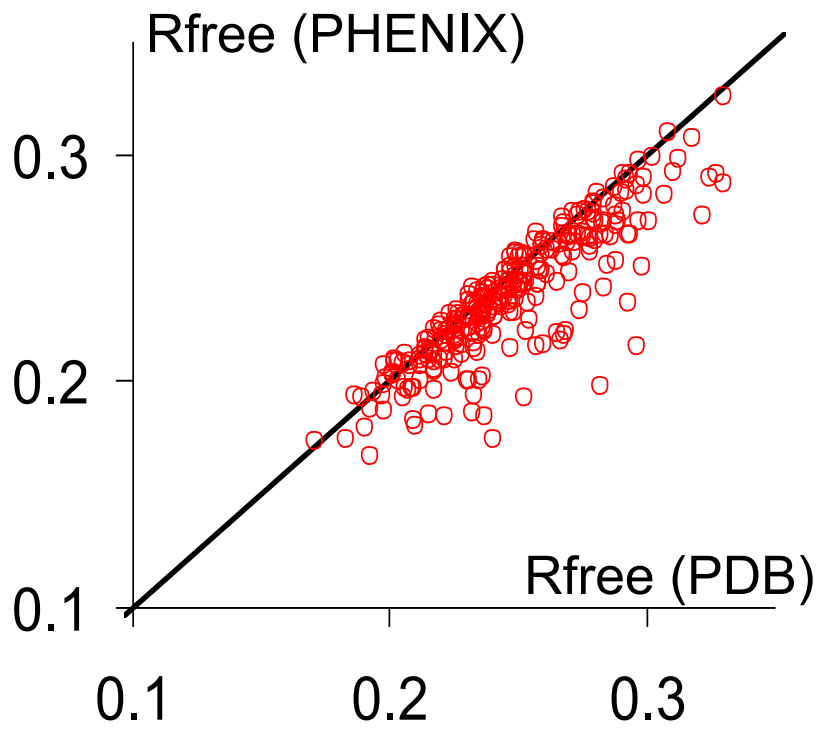
9% improvement in both Rwork and Rfree !

TLS groups determined automatically…

TLS refinement in PHENIX: robust and efficient

- Highly optimized algorithm based on systematic re-refinement of ~350 PDB models
- In most of cases *phenix.refine* produces better R-factors compared to published
- Don't crash or get "unstable"





ADP refinement: what goes into PDB

phenix.refine outputs TOTAL B-factor (iso- and anisotropic):

$$U_{TOTAL} = U_{ATOM} + U_{TLS} + U_{CRYST}$$

Isotropic equivalent

```
ATOM    1  CA  ALA  1    37.211  30.126  28.127  1.00 26.82        C
ANISOU  1  CA  ALA  1  3397    3397    3397    2634    2634    2634  C
```

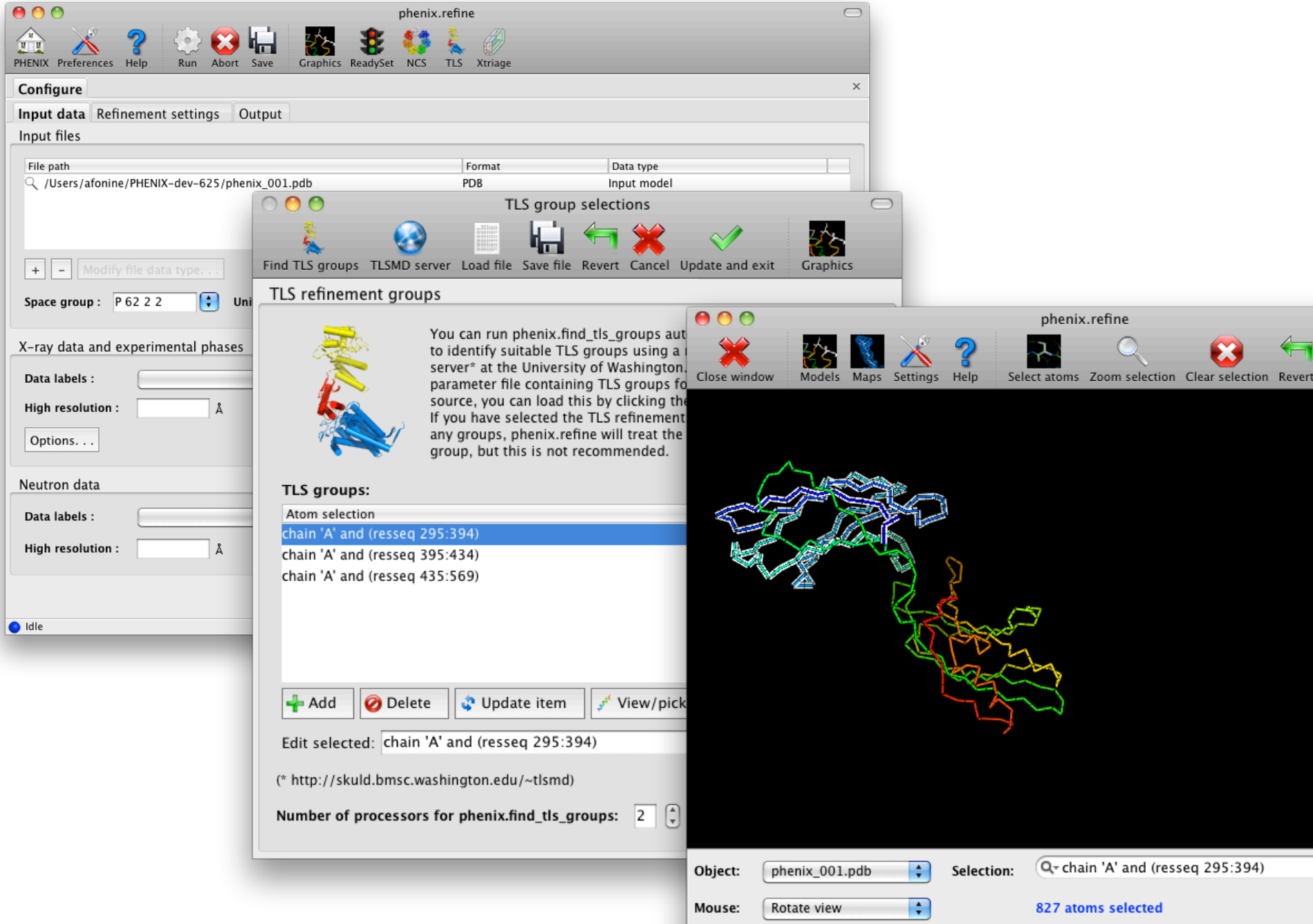
$$U_{TOTAL} = U_{ATOM} + U_{TLS} + U_{CRYST}$$

Stored in separate record in PDB file header

Atom records are self-consistent:

- ✓ Straightforward visualization (color by B-factors, or anisotropic ellipsoids)
- ✓ Straightforward computation of other statistics (R-factors, etc.) – no need to use external helper programs for any conversions.

TLS groups for refinement automatically (well, in three clicks!)

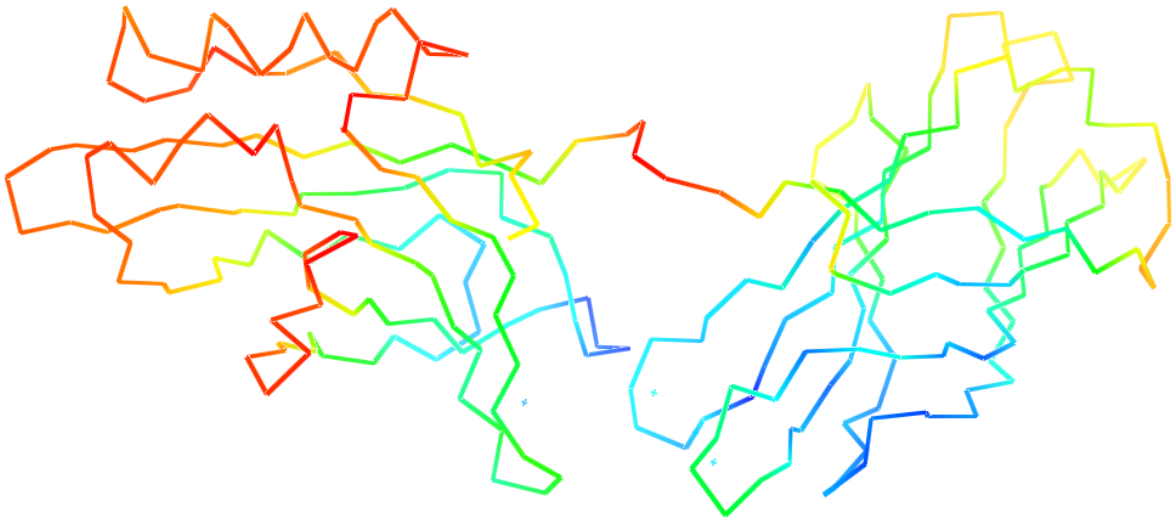


TLS

- Using TLS in refinement requires partitioning a model into TLS groups. This is typically done by
  - visual model inspection and deciding which domains may be considered as rigid
  - using TLSMD method

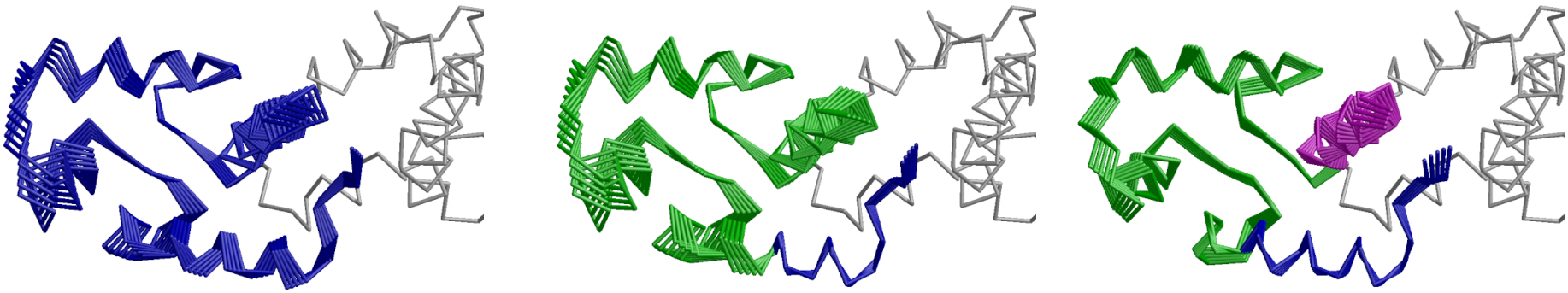
    Painter & Merritt. (2006). Acta Cryst. D62, 439-450

    Painter & Merritt. (2006). J. Appl. Cryst. 39, 109-111



TLS

- Split a model into 1, 2, 3, …, N contiguous segments.



- Compute residual for all segment partitions:

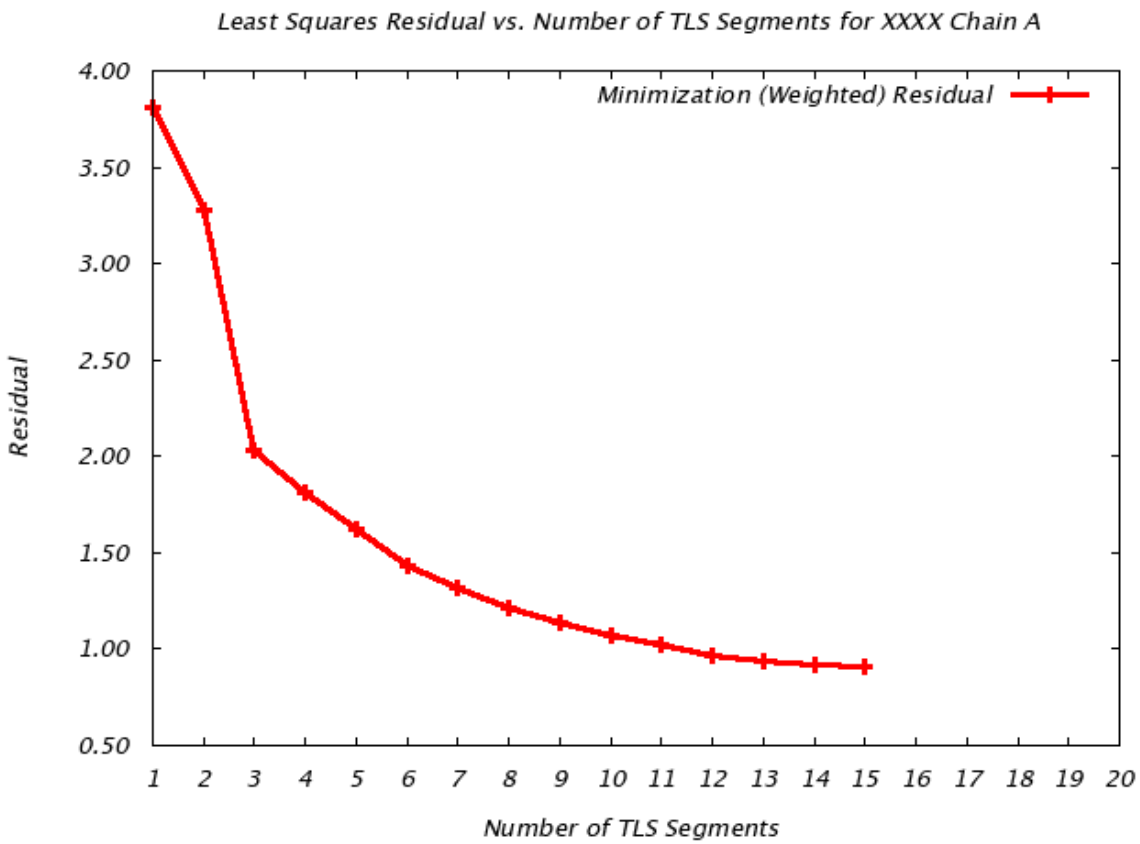
$$R = w \sum_{\text{atoms in segment}} W\left(\sum_{i=1}^{6}\left(U_{ATOM}^{i} - U_{TLS}^{i}\right)^{2}\right)$$

where TLS contribution of an individual atom participating in a TLS group:

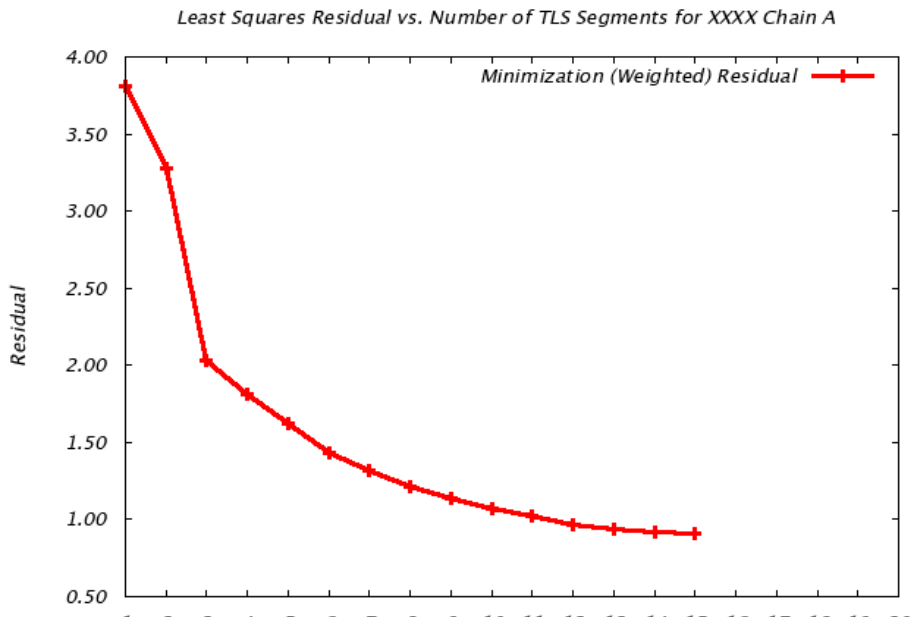
$$\mathbf{U}_{TLS} = \mathbf{T} + \mathbf{ALA}^{t} + \mathbf{AS} + \mathbf{S}^{t}\mathbf{A}^{t}$$

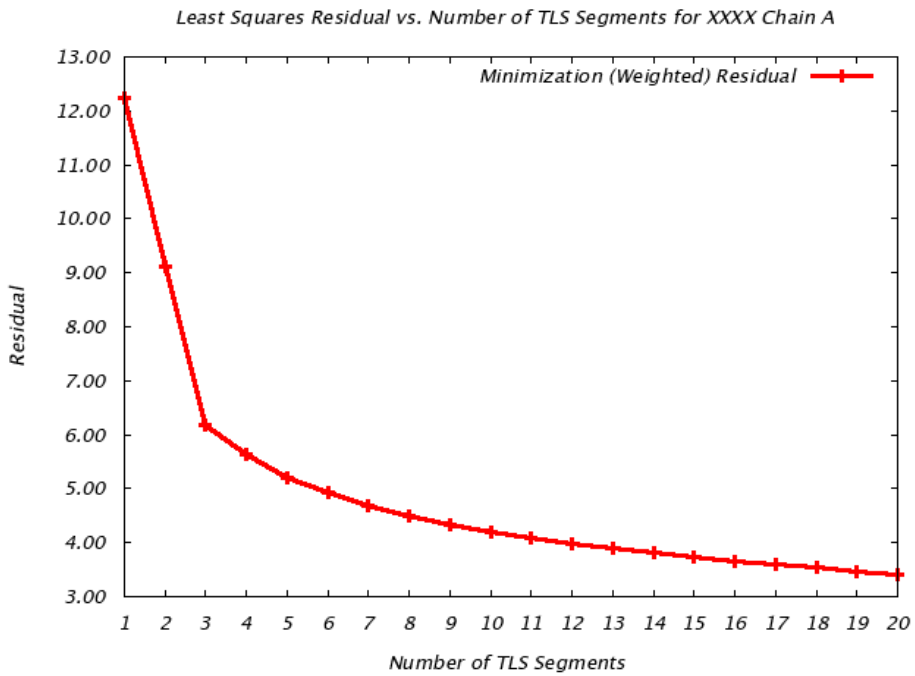
(20 TLS parameters per TLS group)

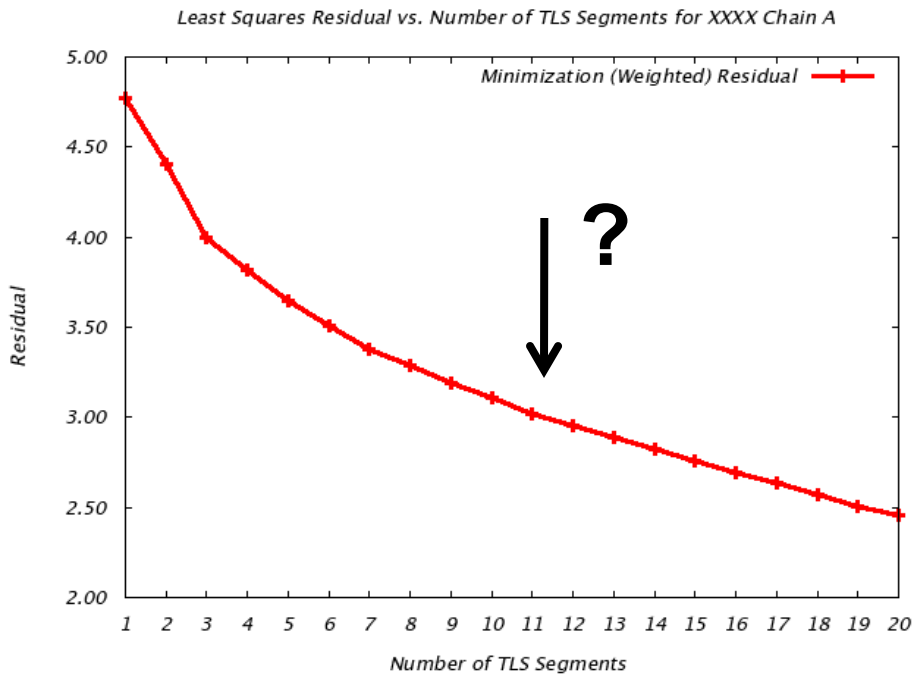
TLS


Least Squares Residual vs. Number of TLS Segments for XXXX Chain A
Minimization (Weighted) Residual
Residual
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Number of TLS Segments

TLS







How to pick up the *right* number of TLS groups?

One can try all 20 and see which one using in refinement results in best Rwork and Rfree

PHENIX approach to finding TLS groups

- **Goals:**
  - Have it as integrated part of PHENIX system:
    - No need to run external software or use web servers (=send your data somewhere, which your policy may even not allow you to do).
    - Use it interactively as part of refinement (update TLS group assignment as model improves during refinement).
    - Make it faster
  - Eliminate subjective decisions (procedure should give THE UNIQUE answer and not an array of possible choices leaving the room for subjective decisions).

PHENIX approach to finding TLS groups

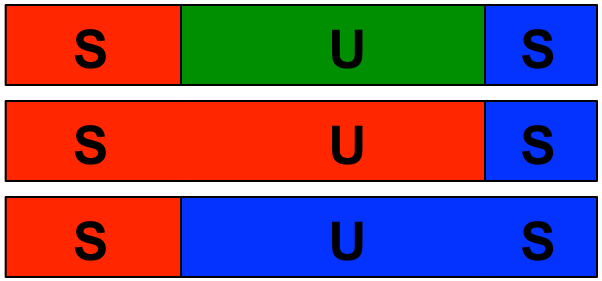
Step 1: For each chain find all secondary structure and unstructured elements

- *Number of elements defines maximum possible number of TLS groups*
- A secondary structure element can't be split into multiple TLS groups. Large unstructured elements, can be split into smaller pieces.

Chain

S – Secondary structure element
U – Unstructured stretch of residues (loop)

Step 2: Find all possible contiguous combinations

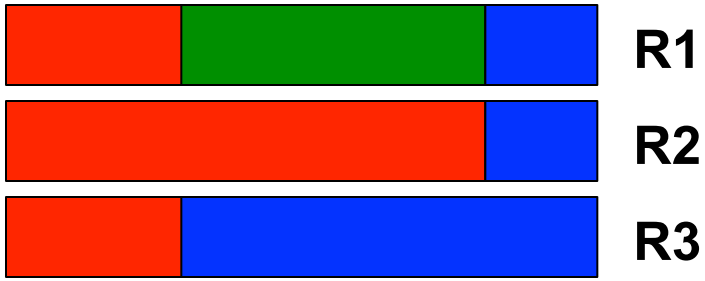


3 groups
2 groups
2 groups

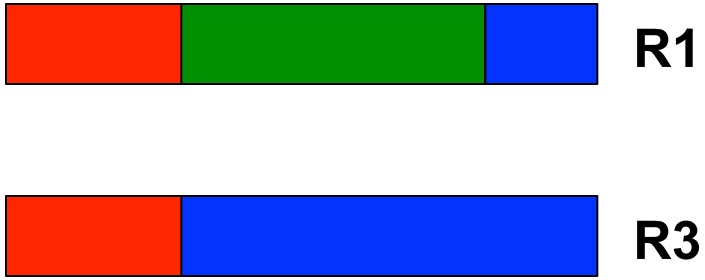
$N_{ELEMENTS} : N_{POSSIBLE\ PARTITIONS}$ 3:3, 4:7, 5:15, 6:31, …, 10:511, …

PHENIX approach to finding TLS groups

Step 3: For each partition fit TLS groups and compute the residual



Step 4: Find the best fit among the groups of equal number of partitions. In this example, if **R3**<**R2**:



Step 5: Find the best partition…

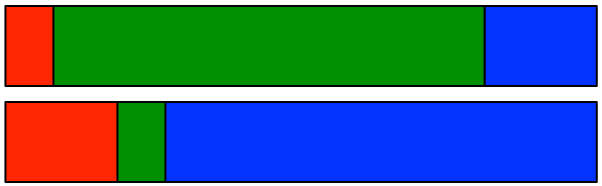
- Challenge: we can't directly compare **R1** and **R3** because they are computed using different number of TLS groups (different number of parameters)

PHENIX approach to finding TLS groups

<u>Step 5 (continued)</u>: Find the best partition…

- Randomly generate a pool of partitions for each candidate, fit TLS matrices compute, average residuals, and compute score:

R1



R11

R12

… many (20-50)

Average residuals: $\mathbf{R1_{AVERAGE}}$

Score = $(\mathbf{R1_{AVERAGE}} - \mathbf{R1})/(\mathbf{R1_{AVERAGE}} + \mathbf{R1})*100$

Do the same for the next candidate:

R3

<u>The final solution is the one that has the highest score.</u>

PHENIX approach to finding TLS groups: examples

GroEl structure (one chain):

No. of groups	Targets best	rand.pick	diff.	score
2	680.7	869.2	188.5	12.2
3	297.1	665.7	368.6	38.3
4	260.4	448.3	187.8	26.5
5	206.2	342.1	135.8	24.8
6	188.4	264.7	76.3	16.8
7	182.3	251.2	68.9	15.9
8	176.9	229.5	52.5	12.9
9	173.1	207.3	34.1	9.0
10	170.2	196.8	26.6	7.2
11	167.8	183.1	15.2	4.3
12	165.6	179.0	13.4	3.9
13	163.9	170.8	6.9	2.1

PHENIX approach to finding TLS groups: examples

GroEl:

phenix.refine refinement:

- Using TLSMD groups: R_{WORK} = 0.2044 R_{FREE} = 0.2454
- Using PHENIX determined TLS groups: R_{WORK} = 0.2054 R_{FREE} = 0.2448

Synaptotagmin:

phenix.refine refinement:

- Using TLSMD groups: R_{WORK} = 0.1967 R_{FREE} = 0.2546
- Using PHENIX determined TLS groups: R_{WORK} = 0.1970 R_{FREE} = 0.2599

1n0y:

phenix.refine refinement:

- Using TLSMD groups: R_{WORK} = 0.2093 R_{FREE} = 0.2287
- Using PHENIX determined TLS groups: R_{WORK} = 0.2088 R_{FREE} = 0.2274

1yqo:

phenix.refine refinement:

- Using TLSMD groups: R_{WORK} = 0.1580 R_{FREE} = 0.1943
- Using PHENIX determined TLS groups: R_{WORK} = 0.1584 R_{FREE} = 0.1955

Automatic TLS

- **Find optimal partition of a model into TLS groups:**

```
phenix.find_tls_groups model.pdb nproc=2
```

Examples:

GroEL structure (3668 residues, 26957 atoms, 7 chains:

135 seconds using 1 CPU

44 seconds using 10 CPUs

Analogous job using TLSMD server: 3630 seconds (plus lots of clicking to upload/download the files)

Lysozime structure:

9.5 seconds with one CPU

2.5 seconds using 10 CPUs

Automatic TLS

- **Why it is faster:**
  a) Use isotropic TLS model,
  b) Solve $R = w \sum_{\text{atoms in segment}} \left(U^i_{ATOM} - U^i_{TLS}\right)^2$ analytically (no minimizer used)

$$U_{TLS} = T_{iso} + \frac{1}{3}\left[L_{11}(y^2+z^2) + L_{22}(x^2+z^2) + L_{33}(x^2+y^2) - 2L_{12}xy - 2L_{13}xz - 2L_{23}yz + 2S_1z + 2S_2y + 2S_3x\right]$$

$$U_{TLS} = T_{iso} + L_{11}\frac{y^2+z^2}{3} + L_{22}\frac{x^2+z^2}{3} + L_{33}\frac{x^2+y^2}{3} - \frac{2}{3}xyL_{12} - \frac{2}{3}xzL_{13} - \frac{2}{3}yzL_{23} + \frac{2}{3}zS_1 + \frac{2}{3}yS_2 + \frac{2}{3}xS_3$$

$$X = \frac{y^2+z^2}{3};\ Y = \frac{x^2+z^2}{3};\ Z = \frac{x^2+y^2}{3};\ W = -\frac{2}{3}xy;\ V = -\frac{2}{3}xz;$$
$$T = -\frac{2}{3}yz;\ S = \frac{2}{3}z;\ R = \frac{2}{3}y;\ Q = \frac{2}{3}x$$

$$U_{TLS} = T_{iso} + XL_{11} + YL_{22} + ZL_{33} + WL_{12} + VL_{13} + TL_{23} + S\cdot S_1 + R\cdot S_2 + Q\,S_3$$

$$LS = \sum_{ATOMS}(U_{TLS} - U_{iso})^2;\quad LS = \sum_{ATOMS}\left(U_{TLS}^2 - 2U_{TLS}U_{iso} + U_{iso}^2\right)$$

$$LS = \sum_{ATOMS}\left(U_{TLS}^2 - 2U_{TLS}U_{iso}\right) + \sum_{ATOMS}U_{iso}^2$$

$$\frac{\partial LS}{\partial PAR} = \sum_{ATOMS}\left(2U_{TLS}\cdot\frac{\partial U_{TLS}}{\partial PAR} - 2U_{iso}\cdot\frac{\partial U_{TLS}}{\partial PAR}\right)$$

$$\frac{\partial LS}{\partial PAR} = 2\sum_{ATOMS}\left(U_{TLS}\frac{\partial U_{TLS}}{\partial PAR} - U_{iso}\cdot\frac{\partial U_{TLS}}{\partial PAR}\right) \quad (1)$$

$$\frac{\partial LS}{\partial PAR} = 2\sum_{ATOMS}\left([U_{TLS} - U_{iso}]\cdot\frac{\partial U_{TLS}}{\partial PAR}\right)$$

$$\frac{\partial LS}{\partial L_{11}} = 2\sum(U_{TLS} - U_{iso})\cdot X = 2\sum(U_{TLS}\cdot X - U_{iso}\cdot X) = 2\sum(U_{TLS}\cdot X) - \underbrace{2\sum U_{iso}\cdot X}_{P_X} = \boxed{2\sum(U_{TLS}\cdot X) + P_X} \quad (2)$$

$$\frac{\partial LS}{\partial L_{22}} = 2\sum(U_{TLS}\cdot Y) + P_Y$$

$$\frac{\partial LS}{\partial L_{33}} = 2\sum(U_{TLS}\cdot Z) + P_Z$$

$$\frac{\partial LS}{\partial L_{12}} = 2\sum(U_{TLS}\cdot W) + P_W$$

$$\frac{\partial LS}{\partial L_{13}} = 2\sum(U_{TLS}\cdot V) + P_V$$

$$\frac{\partial LS}{\partial L_{23}} = \sum(U_{TLS}\cdot T) + P_T$$

$$\frac{\partial LS}{\partial S_1} = \sum(U_{TLS}\cdot S) + P_S$$

$$\frac{\partial LS}{\partial S_2} = \sum(U_{TLS}\cdot R) + P_R$$

$$\frac{\partial LS}{\partial S_3} = \sum(U_{TLS}\cdot Q) + P_Q$$

$$\frac{\partial LS}{\partial T_{iso}} = 2\sum_{ATOMS}\left([U_{TLS} - U_{iso}]\cdot 1\right) = 2\sum_{ATOMS}(U_{TLS} - U_{iso})$$

$$U_{TLS}\cdot X = XT_{iso} + X^2L_{11} + XYL_{22} + XZL_{33} + XWL_{12} + XVL_{13} + XTL_{23} + XS\cdot S_1 + XR\cdot S_2 + XQ\,S_3$$
$$U_{TLS}\cdot Y = YT_{iso} + YXL_{11} + Y^2L_{22} + YZL_{33} + YWL_{12} + YVL_{13} + YTL_{23} + YS\cdot S_1 + YRS_2 + YQ\,S_3$$
$$U_{TLS}\cdot Z = ZT_{iso} + ZXL_{11} + ZYL_{22} + Z^2L_{33} + ZWL_{12} + ZVL_{13} + ZTL_{23} + ZS\cdot S_1 + ZRS_2 + ZQ\,S_3$$
$$U_{TLS}\cdot W = WT_{iso} + WXL_{11} + WYL_{22} + WZL_{33} + W^2L_{12} + WVL_{13} + WTL_{23} + WS\cdot S_1 + WR\cdot S_2 + WQ\cdot S_3$$
$$U_{TLS}\cdot V = VT_{iso} + VXL_{11} + VYL_{22} + VZL_{33} + VWL_{12} + V^2L_{13} + VTL_{23} + VS\cdot S_1 + VR\cdot S_2 + VQ\cdot S_3$$
$$U_{TLS}\cdot T = TT_{iso} + TXL_{11} + TYL_{22} + TZL_{33} + TWL_{12} + TVL_{13} + T^2L_{23} + TS\cdot S_1 + TR\cdot S_2 + TQ\cdot S_3$$
$$U_{TLS}\cdot S = ST_{iso} + SXL_{11} + SYL_{22} + SZL_{33} + SWL_{12} + SVL_{13} + STL_{23} + S^2\cdot S_1 + SR\cdot S_2 + SQ\cdot S_3$$

7 more pages …

Refinement flowchart

PDB model,
Any data format
(CNS, Shelx, MTZ, …)

Input data and model processing

Refinement strategy selection

Bulk-solvent, Anisotropic scaling, Twinning parameters refinement

Ordered solvent (add / remove)

Target weights calculation

Coordinate refinement (real- and reciprocal space) (rigid body, individual) (minimization or Simulated Annealing)

ADP refinement
(TLS, group, individual iso / aniso)

Occupancy refinement (individual, group)

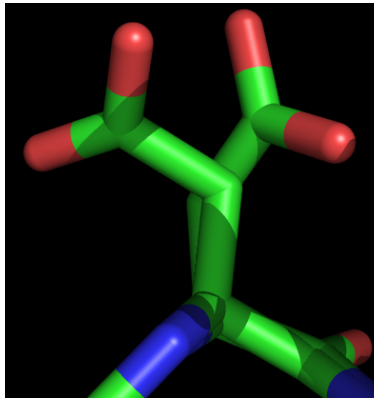
Output: Refined model, various maps, structure factors, complete statistics, ready for deposition PDB file

Repeated several times

Files for COOT, O, PyMol

Occupancy refinement

- Automatic constraints for occupancies of atoms in alternate locations



- Any user defined selections for individual and/or group occupancy refinement can be added on top of the automatic selection.

ATOM	1	N	AARG	A	192	-5.782	17.932	11.414	0.72	8.38	N
ATOM	2	CA	AARG	A	192	-6.979	17.425	10.929	0.72	10.12	C
ATOM	3	C	AARG	A	192	-6.762	16.088	10.271	0.72	7.90	C
ATOM	7	N	BARG	A	192	-11.719	17.007	9.061	0.28	9.89	N
ATOM	8	CA	BARG	A	192	-10.495	17.679	9.569	0.28	11.66	C
ATOM	9	C	BARG	A	192	-9.259	17.590	8.718	0.28	12.76	C
ATOM	549	AU		A	34	-23.064	7.146	-23.942	0.78	15.44	Au
ATOM	549	HA3	ARG	A	34	-23.064	7.146	-23.942	1.00	15.44	H
ATOM	550	H	AARG	A	34	-24.447	7.644	-21.715	0.15	8.34	H
ATOM	551	D	BARG	A	34	-24.447	7.644	-21.715	0.85	7.65	D
ATOM	552	N	ARG	A	35	-22.459	9.801	-22.791	1.00	8.54	N
ATOM	6	S	SO4		1	1.302	1.419	1.560	0.70	13.00	
ATOM	7	O1	SO4		1	1.497	1.295	0.118	0.70	11.00	
ATOM	8	O2	SO4		1	1.098	0.095	2.140	0.70	10.00	
ATOM	9	O3	SO4		1	2.481	2.037	2.159	0.70	14.00	
ATOM	10	O4	SO4		1	0.131	2.251	1.823	0.70	12.00	

Occupancy refinement – more examples

```
ATOM   3690  O2 AEDO C   1      23.106  -3.999  -8.239  0.58 15.69           O
ATOM   3691  C2 AEDO C   1      21.710  -4.102  -8.630  0.58 15.43           C
ATOM   3692  C1 AEDO C   1      20.965  -2.841  -8.282  0.58 16.78           C
ATOM   3693  O1 AEDO C   1      21.111  -2.587  -6.901  0.58 19.33           O
ATOM   3687  I  BIOD C   1      21.798  -3.596  -7.915  0.42 34.88           I
```

Refinement with twinned data

- Two steps to perform twin refinement:

  - run phenix.xtriage to get twin operator (twin law):

```
% phenix.xtriage data.mtz
```

  - run phenix.refine:

```
% phenix.refine model.pdb data.mtz twin_law="-h-k,k,-l"
```

- Taking twinning into account makes (big) difference:

Interleukin mutant (PDB code: 1I2h)

	R/R-free (%)
PHENIX (no twinning):	24.9 / 27.4
PHENIX (twin refinement):	15.3 / 19.2

Hydrogen atoms in refinement

- **Some facts about hydrogen atoms:**
  - H atoms are not visible in X-ray maps at “typical macromolecular” resolutions, that is ~1Å and lower. This is because:
    - H atom is a weak scatterer (much weaker than C, N or O atoms)
    - models contain too much noise so the H contribution is hidden in it. Ideally (nearly error free model) one would see H even at ~2Å resolution.
  - Some or most of H atoms can be seen in maps at ultra-high resolutions (~1Å and higher):
    - The resolution itself is not the sufficient condition to see H: the noise level should be low (small *R*-factor).
  - Hydrogen atoms constitute nearly 50% of the total atoms in protein structures. Typical example: Fab structure (PDB code: 1f8t): 3593 non-H atoms, 3269 H atoms.
  - Since H is a weak scatterer, it mostly contributes to the low resolution (and not to the high!). The reason why we see H atoms only in structures corresponding to high resolution data is because these structures are typically accurate enough and complete so the noise level is small (small *R*-factor).

Hydrogen atoms in refinement

- phenix.refine: handling H atoms at ***any*** resolution:

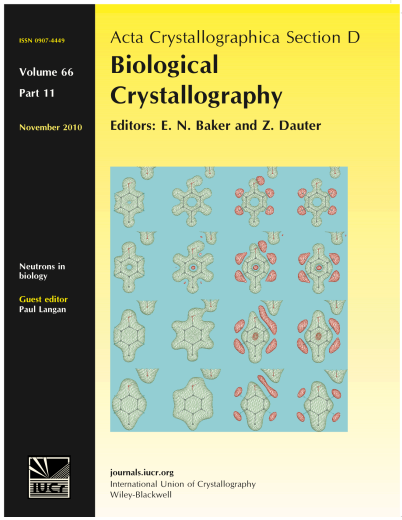
  - Riding model (low-high resolution)
  - Individual atoms (ultrahigh resolution or neutron data)
  - Account for scattering contribution or just use to improve the geometry

- Using the H atoms in refinement:

  - Improve R-factors
  - Improve model geometry (remove bad clashes)
  - Model residual density at high resolution or in neutron maps

- Example: automatic re-refinement of 1000 PDB models with and without H:

pdb	resolution	Rfree(no H) – Rfree(with H)
1akg	1.1	1.9
1byp	1.75	1.41
1dkp	2.3	0.93
1rgv	2.9	0.50



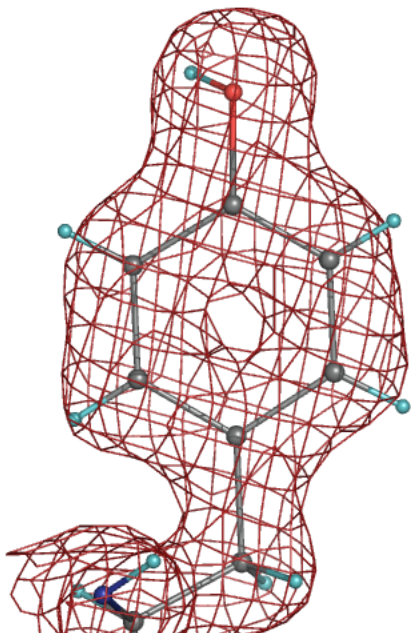
Review and latest developments:
Afonine, et al. (2010). Joint X-ray and neutron refinement with phenix.refine. Acta Cryst. D66, 1153-1163.

Refinement using X-ray and Neutron diffraction data

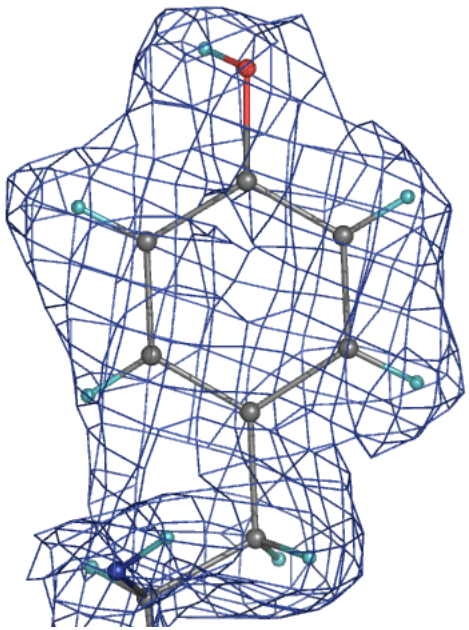
- Different techniques – different information (neutron maps show hydrogen atoms)

2mFo-DFc maps

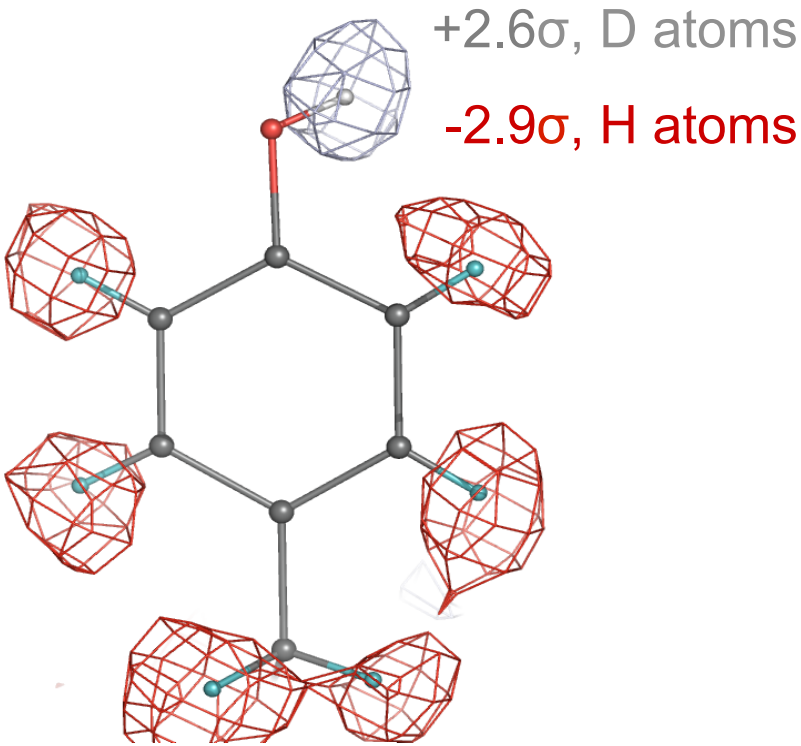
X-ray (1.8 Å)



Neutron (2.2 Å)



Fo-Fc, (H-, D-omit **neutron map**), **1.6 Å** resolution

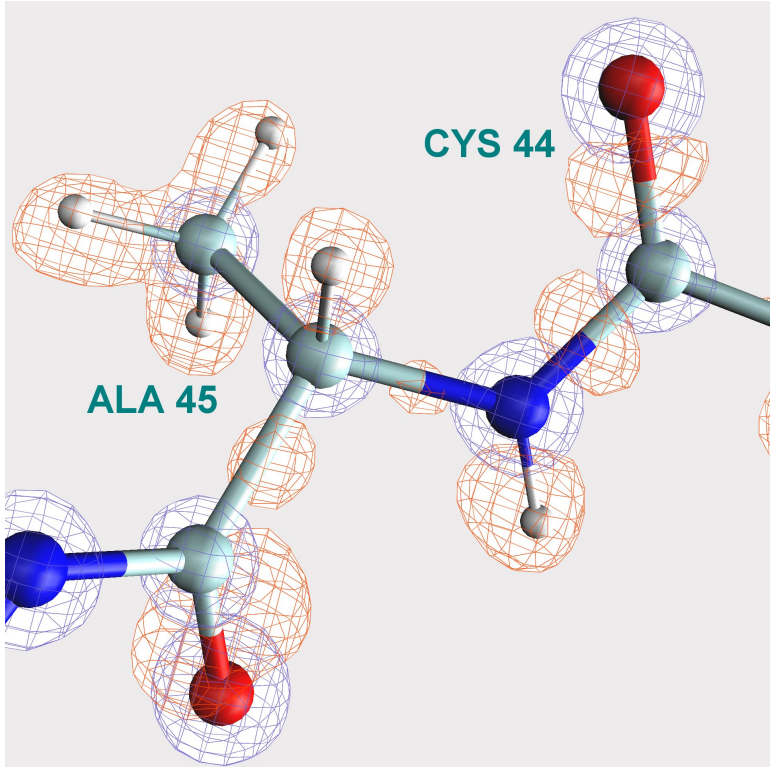


- *phenix.refine* can refine a structure against neutron data or both X-ray and neutron simultaneously

Refinement at subatomic resolution

~340 structures in PDB at resolution higher than 1.0 Å

Aldose Reductase (0.66 Å resolution)



Fo-Fc (orange) 2Fo-Fc (blue)

✓ phenix.refine has unique set of tools to correctly refine such structures

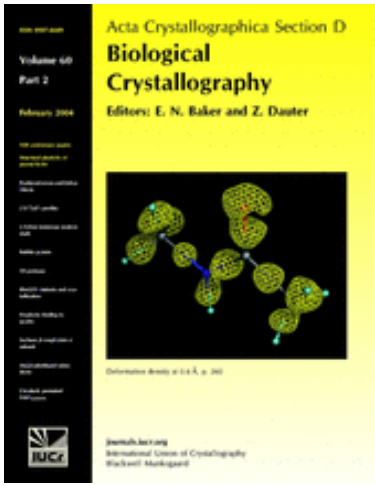
Modeling at subatomic resolution: IAS model

- Basics of IAS model:

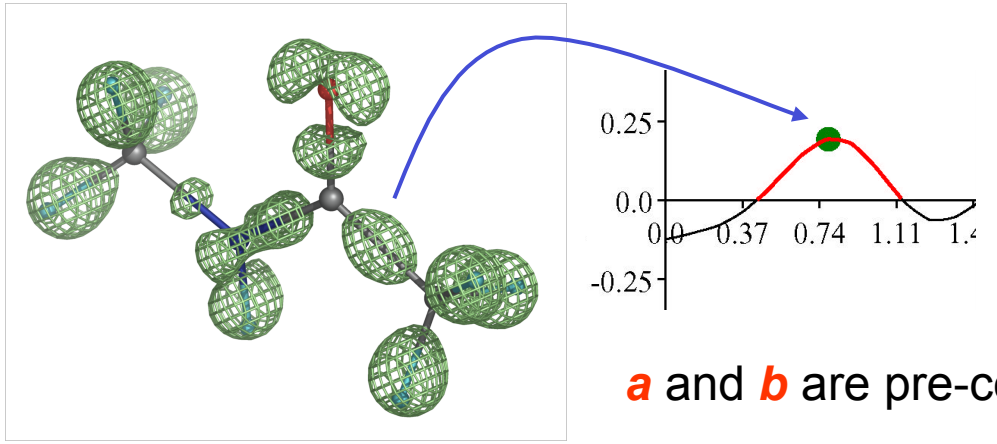
  Afonine et al, Acta Cryst. D60 (2004)

- First practical examples of implementation and use in PHENIX:

  Afonine et al, Acta Cryst. D63, 1194-1197 (2007)



IAS modeling in PHENIX



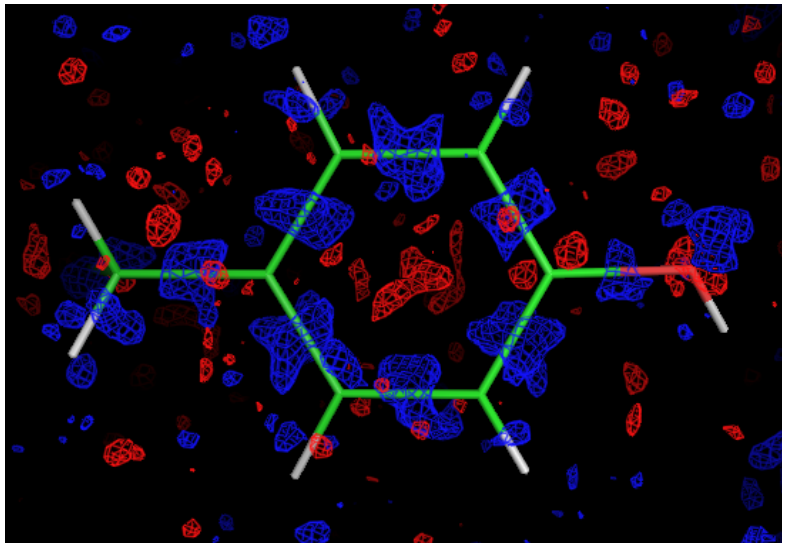
Simple Gaussian is good enough:

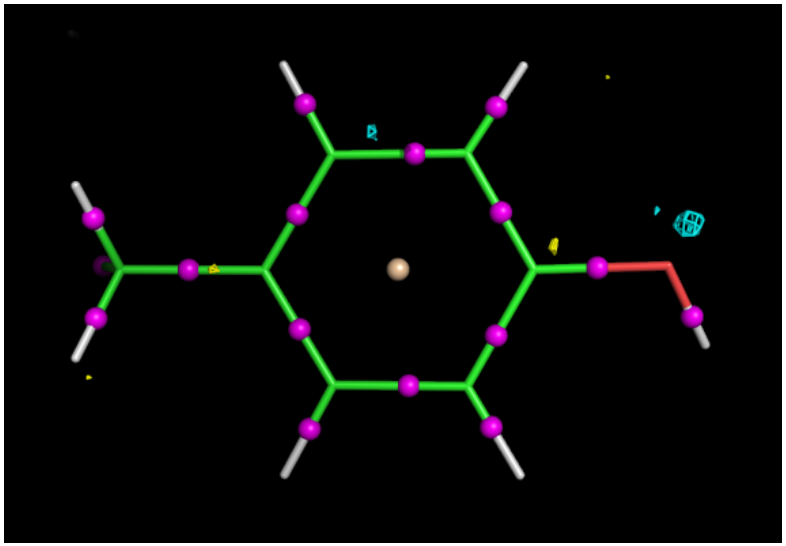
$$f_{bond\_scatterer}(\mathbf{s}) = \boldsymbol{a}\ \exp(\ \boldsymbol{b}\ \mathbf{s}^2)$$

a and ***b*** are pre-computed library for most bond types

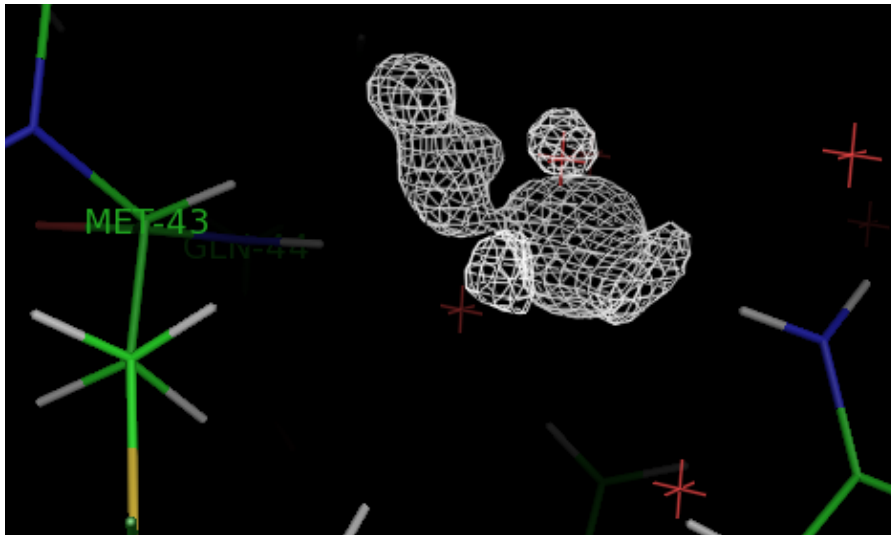
IAS modeling: benefits

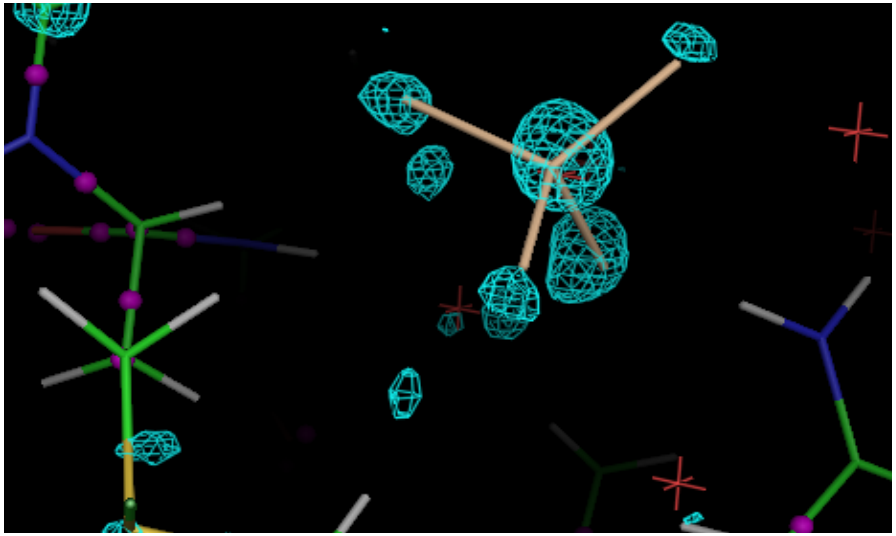
- Improve maps: reduce noise. Before (left) and after (right) adding of IAS.





- Find new features: originally wrong water (left) replaced with SO4 ion (right) clearly suggested by improved map after adding IAS



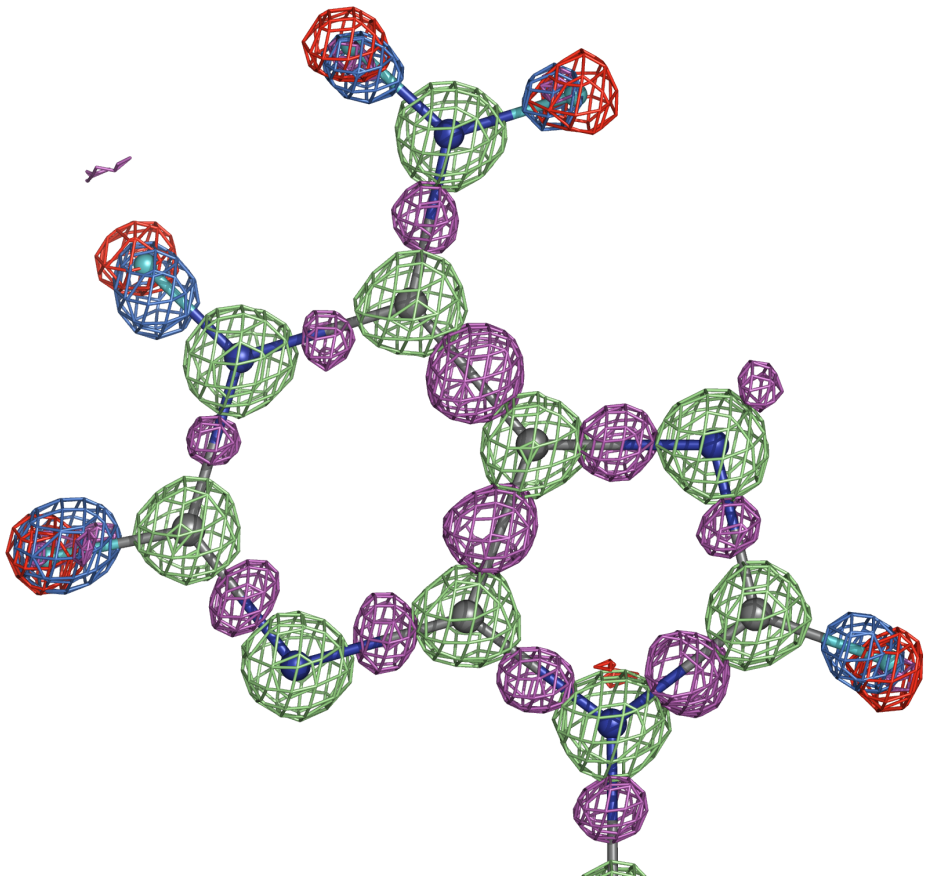


X-ray and Neutron Crystallography: Complimentary Methods

- Still complimentary even at subatomic resolution (NAD structure)

Neutron 2mFo-DFc map at 0.65 Å resolution, ±2.4σ, green (positive), red (negative)

X-ray mFo-DFc map at 0.6 Å resolution, blue: H omit, 5σ, magenta: 2.8σ all atoms included



Running phenix.refine (command line)

Model refinement

- Designed to be very easy to use

```
phenix.refine model.pdb data.hkl [parameters]
```

Some basic examples of running phenix.refine from the command line

- Refinement of individual coordinates, B-factors, and occupancies for some atoms:

```
phenix.refine model.pdb data.hkl
```

- Add water picking and Simulated Annealing to default run above:

```
phenix.refine model.pdb data.hkl simulated_annealing=true
ordered_solvent=true
```

- Refinement of individual coordinates and B-factors using neutron data:

```
phenix.refine model.pdb data.hkl
main.scattering_dictionary=neutron
```

- To see all parameters (a few hundreds):

```
phenix.refine --show_defaults=all
```

Running phenix.refine

```
% phenix.refine model.pdb data.hkl parameter_file
```

where **parameter_file** contains following lines:

```
refinement.main {
   high_resolution = 2.0
   low_resolution = 15.0
   simulated_annealing = True
   ordered_solvent = True
   number_of_macro_cycles = 5
}
refinement.refine.adp {
    tls = chain A
    tls = chain B
}
```

For typing enthusiasts, the equivalent command line run would be:

```
% phenix.refine model.pdb data.hkl xray_data.high_resolution=2
xray_data.low_resolution=15 simmulated_annealing=true
ordered_solvent=True adp.tls="chain A" adp.tls="chain B"
main.number_of_macro_cycles=5
```

Typical way of phenix.refine run from the command line

1. Get the file with all parameters:

```
% phenix.refine --show-defaults=all > parameter_file
```

2. Edit the file `parameter_file`:
   - Remove all parameters that you are not planning to change (make sure to have all { } matched)
   - Change the rest of parameters

3. Run phenix.refine as following:

```
% phenix.refine model.pdb data.hkl parameter_file
```

or (If `model.pdb` and `data.hkl` are included into `parameter_file` file)

```
% phenix.refine parameter_file
```

Useful tip: to compare the set of parameters in your `parameter_file` file against the set of all default parameters, type:

```
% phenix.refine --diff-params parameter_file
```

Some refinement runs require two steps: hydrogens and ligands

- When running: `% phenix.refine model.pdb data.hkl`

each item in model.pdb is matched against the CCP4 Monomer Library to extract the topology and parameters and to automatically build corresponding restraints.

- If model.pdb contains an item not available in CCP4 Monomer Library, e.g. a novel ligand, use ReadySet! program to generate topology and parameter definitions for refinement:

```
% phenix.ready_set model.pdb
```

This will produce the file LIG.cif and updated PDB file `model.updated.pdb` with all H atoms added which can be used for refinement:

```
% phenix.refine model.pdb data.hkl LIG.cif
```

Some refinement runs require two steps: twinning

- Two steps to perform twin refinement:

  - run *phenix.xtriage* to get twin operator (twin law):

```
% phenix.xtriage data.mtz
```

  - run *phenix.refine*:

```
% phenix.refine model.pdb data.mtz twin_law="-h-k,k,-l"
```

Model refinement - output

- Input command

```
phenix.refine model.pdb data.mtz [parameters]
```

- Output files

`model_refine_001.eff`	summary of all input parameters
`model_refine_001.geo`	summary of all restraints used
`model_refine_001.log`	complete information about refinement
`model_refine_001.pdb`	refined structure
`model_refine_001.mtz`	Fourier map coefficients, Fcalc, etc.
`model_refine_002.def`	parameters for the next run

If data file is not in MTZ format, or there are multiple data files at input (example: one with Fobs and the other one with free-R flags), then phenix.refine will combine them into one MTZ data file called: `model_data.mtz` and this file should be used in all subsequent runs.

MTZ phenix.refine

A run

```
phenix.refine model.pdb data.mtz
```

does not output anymore

```
model_001_map_coeffs.mtz
```

Instead, it always outputs a MTZ file

```
model_001.mtz
```

that contains:

MTZ phenix.refine

```
 Number of datasets: 4
  Dataset 1:
    Name: Original-experimental-data
(...)
    label           #valid  %valid  min     max type
    H                17129 100.00% 0.00   18.00 H: index h,k,l
    K                17129 100.00% 0.00   35.00 H: index h,k,l
    L                17129 100.00% 0.00   45.00 H: index h,k,l
    I-obs            16775  97.93% 0.00 1250.45 K: I
    SIGI-obs         16775  97.93% 0.00   46.36 M: standard deviation
  Dataset 2:
    Name: Experimental-data-used-in-refinement
(...)
    label                #valid %valid  min   max type
    F-obs-filtered        16464 96.12% 1.15 35.36 G: F
    SIGF-obs-filtered     16464 96.12% 0.05  2.96 L: standard deviation
  Dataset 3:
    Name: Model-structure-factors-(bulk-solvent-and-all-scales-included)
(...)
    label         #valid %valid     min    max type
    F-model(+)     16464 96.12%    0.00  83.91 G: F(+) or F(-)
    PHIF-model(+)  16464 96.12% -180.00 180.00 P: phase angle in degrees
    F-model(-)     14382 83.96%    0.02  91.93 G: F(+) or F(-)
    PHIF-model(-)  14382 83.96% -179.97 179.95 P: phase angle in degrees
  Dataset 4:
    Name: Fourier-map-coefficients
(...)
    label             #valid  %valid     min    max type
    2FOFCWT            17129 100.00%    0.00  43.21 F: amplitude
    PH2FOFCWT          17129 100.00% -180.00 180.00 P: phase angle in degrees
    2FOFCWT_no_fill    16657  97.24%    0.00  41.96 F: amplitude
    PH2FOFCWT_no_fill  16657  97.24% -180.00 180.00 P: phase angle in degrees
    FOFCWT             16657  97.24%    0.00  58.25 F: amplitude
    PHFOFCWT           16657  97.24% -180.00 180.00 P: phase angle in degrees
    ANOM               14189  82.84%    0.00   2.19 F: amplitude
    PANOM              14189  82.84% -180.00 179.96 P: phase angle in degrees
```

Example of a complex refinement run

- Do the following:
  - refine individual coordinates for all atoms using minimization and Simulated Annealing
  - refine coordinates of three rigid body groups:
    - chain A
    - chain B and chain C
    - chain D
  - individual anisotropic ADP for all Uranium atoms
  - individual isotropic ADP for all other atoms
  - three TLS groups:
    - atoms in residues from 1 to 300 of chain A and whole chain B
    - atoms from 301 to 500 in chain A
    - whole chain D
  - update water during refinement
  - use NCS in refinement
  - output everything into a files with prefix *test*

```
% phenix.refine model.pdb data.hkl parameters.eff
```

where **`parameters.eff`** contains following lines: see next slide…

Example of a complex parameter file

```
refinement {
  output {
    prefix = test
  }
  refine {
    strategy=*individual_sites individual_sites_real_space *rigid_body \
             *individual_adp group_adp *tls *occupancies group_anomalous
    sites {
      rigid_body = chain A
      rigid_body = chain B or chain C
      rigid_body = chain D
    }
    adp {
      individual {
        isotropic = not (element U)
        anisotropic = element U
      }
      tls = chain A and resseq 1:300 or chain B
      tls = chain A and resseq 301:500
      tls = chain D
    }}
  main {
    simulated_annealing = True
    ordered_solvent = True
    ncs = True
}}
```

Reporting bugs, problems, asking questions

- **Something didn't work as expected?... program crashed?... missing feature?...**

  Not Good: silently give up and run away looking for alternative software (or write your own program).

  Good: report us a problem, ask a question, request a feature (explain why it's good to have), ask for help.

- **Reporting a bug:**

  Not good: "Hi! PHENIX crashed, I don't know what to do."

  Good: "Hi! PHENIX crashed. Here are:
  1) PHENIX version;
  2) Command and parameters I used;
  3) Input and output files (at least logs)."

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Phenix

NEW Development release of PHENIX version 1.4 now available

*Python-based **H**ierarchical **EN**vironment for **I**ntegrated **X**tallography*

PHENIX is a new software suite for the automated determination of macromolecular structures using X-ray crystallography and other methods.

Citing PHENIX:
PHENIX: building new software for automated crystallographic structure determination P.D. Adams, R.W. Grosse-Kunstleve, L.-W. Hung, T.R. Ioerger, A.J. McCoy, N.W. Moriarty, R.J. Read, J.C. Sacchettini, N.K. Sauter and T.C. Terwilliger. *Acta Cryst.* D58, 1948-1954 (2002)

Download the latest development release (1.4-3) [First request download password]

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Using PHENIX (release 1.4-3): **Full Documentation** **PDF**

- Assessing data quality with phenix.xtriage
- Automated structure solution with AutoSol
- Automated molecular replacement with AutoMR
- Automated model building and rebuilding with AutoBuild
- Automated ligand fitting with LigandFit
- Structure refinement with phenix.refine
- Generation of ligand coordinates and restraints with elbow
- The PHENIX Graphical User Interface

Documentation for 1.3-final

The PHENIX system also includes SOLVE/RESOLVE, Phaser, Textal, the CCI Applications (phenix.xtriage, phenix.refine, elbow and many more), components from Molprobity, and the Computational Crystallography Toolbox in a Python framework.

Funding for PHENIX: Protein Structure Initiative (NIH General Medical Sciences)

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The PHENIX Industrial Consortium

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