Case Studies in Molecular Replacement

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Overview

- Information about target protein
- Tools for model editing
- Examples

Tools for Model Finding

- Related PDB entries known from literature
  - Know as much about your target as possible

- Data base search based on sequence and/or structural homology/alignment
  - http://toolkit.tuebingen.mpg.de/hhpred
  - http://ekhidna.biocenter.helsinki.fi/dali_server
  - http://ffas.ljcrf.edu/ffas-cgi/cgi/ffas.pl

Tools for Model Preparation

- Coot
  - Superpositioning of multiple models and eliminate non-aligning parts to create a core
- Sequence alignment
  - Sequence alignment between sequence of unknown structure and sequence of search model
  - http://www.ebi.ac.uk/Tools/msa/clustalw2
  - http://probcons.stanford.edu/
  - http://mafft.cbrc.jp/alignment/software/
  - http://www.ebi.ac.uk/Tools/msa/tcoffee/
- Chainsaw
  - Creating merged model based on target sequence and search model details
  - Creating of poly-Ala models
  - Within CCP4

Examples for Molecular Replacement

SGC

- Intermediate resolution
  - Muscle specific human β-enolase (PDB-ID: 2XSX)
- High resolution
  - PH domain of human AKT3 kinase (PDB-ID: 2X18)
- Low homology
  - PH domain of human Actin-binding protein anillin ANLN (PDB-ID: 2Y7B)
- Low resolution
  - Rotating transmembrane ring of F_oF_1 ATPsynthase from spinach chloroplasts (PDB-ID: 2W5J)
- NMR model
  - Thioredoxin domain of human TXNL2 (PDB-ID: 2WZ9)
- Twinning
  - Macro domain of human core histone H2A (PDB-ID: 2XD7)

Intermediate resolution PDB-ID 2XSX

SGC

- Muscle specific human β-enolase
- One of three isoforms of enolase
- Location: cytoplasm
- Function: involved in glycolysis
- Enzymatic reaction:

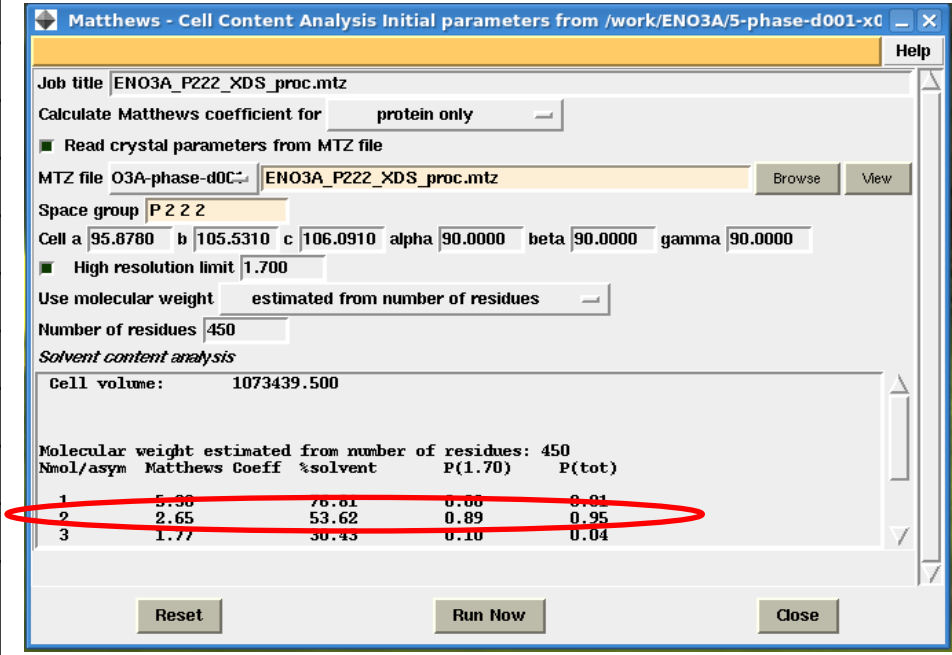
  2-phospho-D-glycerate = phosphoenolpyruvate + H_2O
- Co-factor: Mg^{2+}
- Biologically active form: dimer
- Number of residues: 434
- Molecular weight: 46.9 kDa
- UniProt-ID: P13929

Intermediate resolution PDB-ID 2XSX

SGC

- Processing results (XDS, Scala)

	Overall	Inner Shell	Outer Shell
Low resolution limit	29.49	29.49	1.79
High resolution limit	1.70	5.38	1.70
Rmerge	0.093	0.036	0.716
Rmerge in top intensity bin	0.036	-	-
Rmeas (within I+/I-)	0.099	0.039	0.759
Rmeas (all I+ & I-)	0.099	0.039	0.759
Rpim (within I+/I-)	0.033	0.013	0.250
Rpim (all I+ & I-)	0.033	0.013	0.250
Fractional partial bias	0.000	0.000	0.000
Total number of observations	996168	32548	143502
Total number of unique	114102	3820	16176
Mean ((I)/sd(I))	15.7	39.1	3.1
Completeness	96.4	95.0	94.6
Multiplicity	8.7	8.5	8.9
Average unit cell	95.88	105.53	106.09
	90.0	90.0	90.0
Space group	P 2 2 2		
Maximum resolution	1.70 Å		

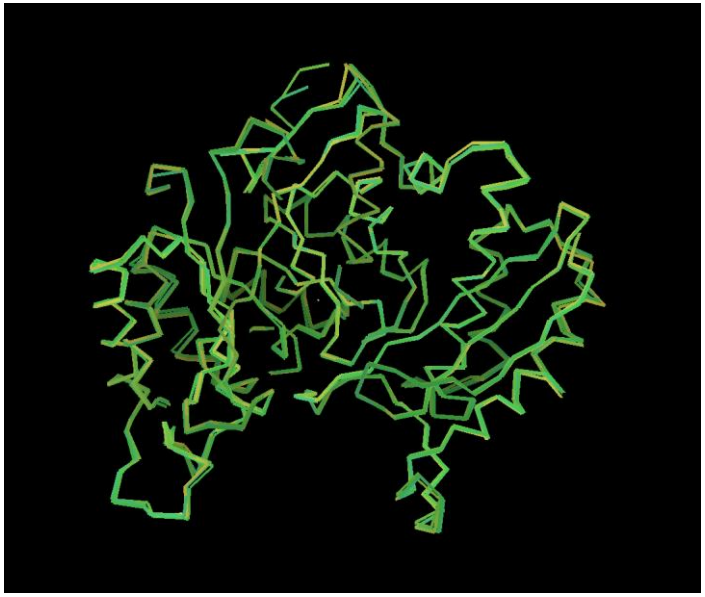


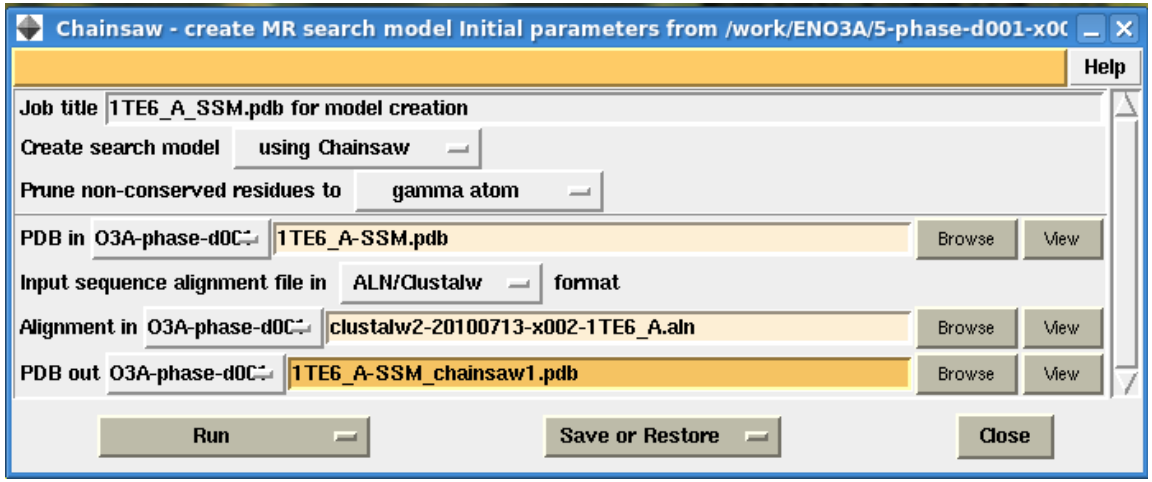
Intermediate resolution PDB-ID 2XSX

SGC

Model preparation

- Multiple homologues structures
  e.g. 1TE6 (82%), 2AKM (82%), 2AKZ (82%), 2PSN (83%), 3B97 (83%)
- Reduce model PDB files to one chain (e.g. chain A); delete water molecules and ligands
- Use Coot for superpositioning
- Sequence alignment between model and target
- Run Chainsaw with edited model and sequence alignment; reduces side chains to last common atom between model and target sequence





Intermediate resolution PDB-ID 2XSX

SGC

Phasing

- Using Phaser
- Using search models as prepared above
- Using data as resulting from Scala

Phaser result

SpaceGroup of Solution: P 21 21 21

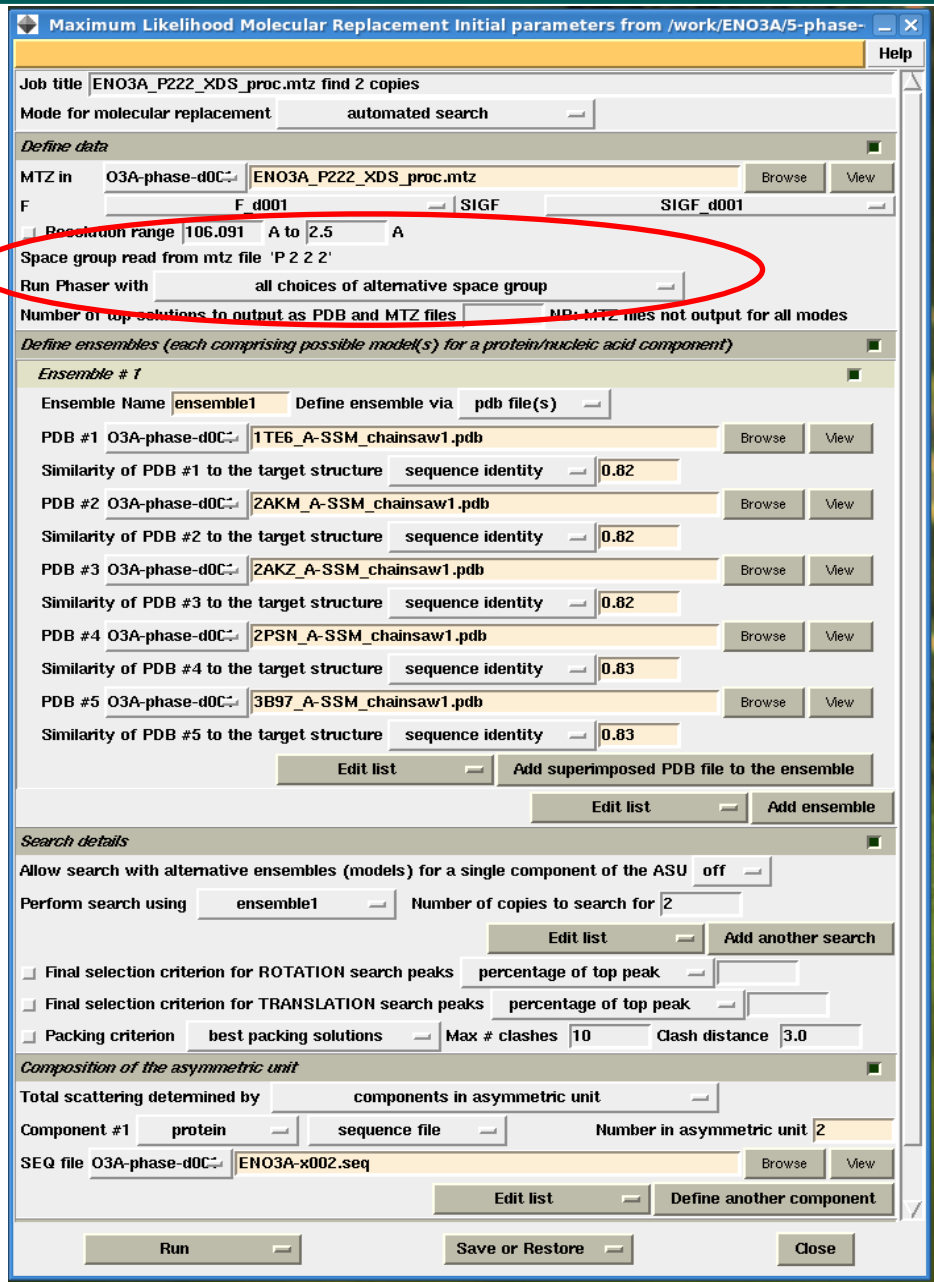
SINGLE solution

Solution written to PDB file:
/work/ENO3A/5-phase-d001-x002-mv/ENO3A-phase-d001_7.1.pdb
Solution written to MTZ file:
/work/ENO3A/5-phase-d001-x002-mv/ENO3A-phase-d001_7.1.mtz
Solution log-likelihood gain: 3623.52
Solution annotation (history):

RFZ=18.6 TFZ=28.0 PAK=0 LLG=739 Molecule 1

RFZ=15.7 TFZ=34.5 PAK=1 LLG=2364 Molecule 2

LLG=3624

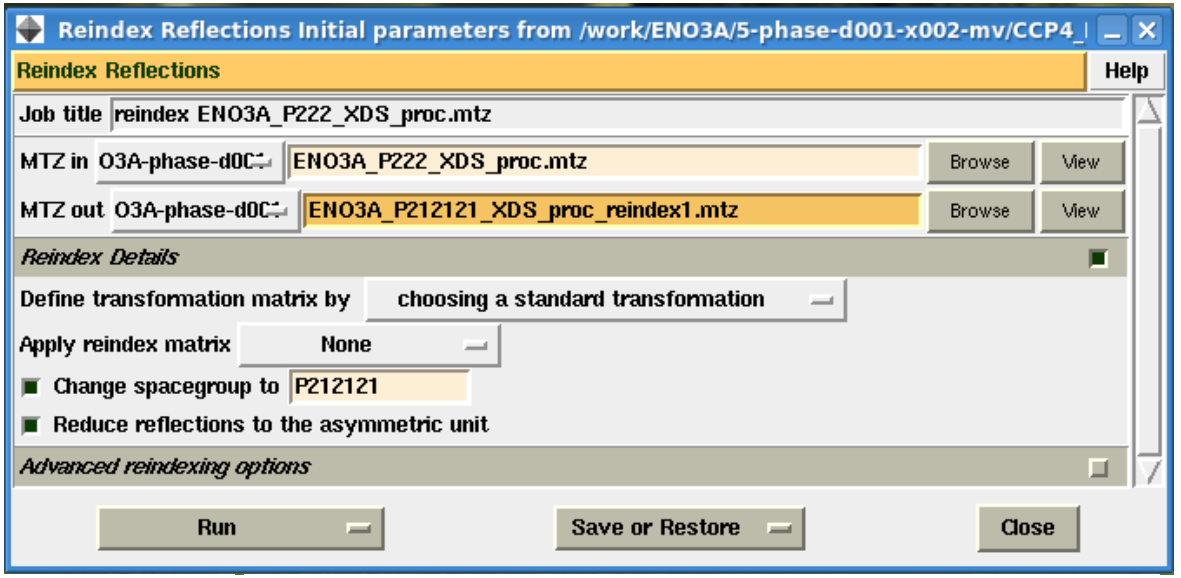


Intermediate resolution PDB-ID 2XSX

SGC

Change space group

- Using Reindex from CCP4
- Input: Scala MTZ file




```
* Cell Dimensions : (obsolete - refer to dataset cell dimensions above)
  95.8780  105.5310  106.0910   90.0000   90.0000   90.0000
*  Resolution Range :
   0.00009     0.34602     (     106.091 -      1.700 A )
* Sort Order :
     1     2     3     0     0
* Space group = 'P 2 2 2' (number     16)
```

MTZDMP before changing

```
* Cell Dimensions : (obsolete - refer to dataset cell dimensions above)
  95.8780  105.5310  106.0910   90.0000   90.0000   90.0000
*  Resolution Range :
   0.00009     0.34602     (     106.091 -      1.700 A )
* Sort Order :
     0     0     0     0     0
* Space group = 'P 21 21 21' (number     19)
```

MTZDMP after changing

Examples for Molecular Replacement

SGC

- Intermediate resolution
  - Muscle specific human β-enolase (PDB-ID: 2XSX)
- High resolution
  - PH domain of human AKT3 kinase (PDB-ID: 2X18)
- Low homology
  - PH domain of human Actin-binding protein anillin ANLN (PDB-ID: 2Y7B)
- Low resolution
  - Rotating transmembrane ring of F_oF_1 ATPsynthase from spinach chloroplasts (PDB-ID: 2W5J)
- NMR model
  - Thioredoxin domain of human TXNL2 (PDB-ID: 2WZ9)
- Twinning
  - Macro domain of human core histone H2A (PDB-ID: 2XD7)

High resolution PDB-ID 2X18

- PH domain of human AKT3 kinase
- AKT3 = RAC-gamma serine/threonine-protein kinase
- Location: cytoplasm and membrane associated
- Function: regulating cell survival
- Enzymatic reaction (full length):

  ATP + a protein = ADP + a phosphoprotein
- Co-factor (PH domain): -
- Biologically active form (PH domain): -
- Number of residues (PH domain): 103
- Molecular weight (PH domain): 12.3 kDa
- UniProt-ID: Q9Y243

High resolution PDB-ID 2X18

SGC

- Processing results (HKL2000, Scalepack)

	Overall	Inner Shell	Outer Shell
Low resolution limit	50.00	50.00	1.64
High resolution limit	1.58	3.40	1.58
Chi**2	1.287	1.387	0.798
Rmerge	0.050	0.032	0.250
Total number of observations	126450	12875	11431
Total number of unique			
Mean ((I)/sd(I))	24.3	37.0	3.4
Completeness	95.8	97.5	86.5
Multiplicity	2.6	2.6	2.6
Average unit cell	62.46	62.39	71.40
	111.48	102.73	94.37
Space group	P 1		
Maximum resolution	1.58 Å		

Cell volume: 249998.031

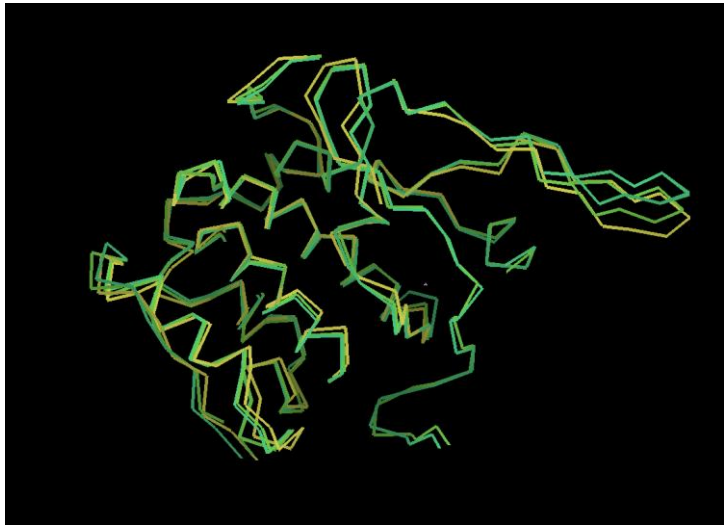
For estimated molecular weight 12760.

Nmol/asym	Matthews Coeff	%solvent	P(1.43)	P(tot)
1	19.59	93.73	0.00	0.00
2	9.80	87.45	0.00	0.00
3	6.53	81.18	0.00	0.00
4	4.90	74.90	0.00	0.00
5	3.92	68.63	0.00	0.02
6	3.27	62.36	0.01	0.07
7	2.80	56.08	0.07	0.18
8	2.45	49.81	0.23	0.31
9	2.18	43.53	0.38	0.29
10	1.96	37.26	0.25	0.11
11	1.78	30.99	0.04	0.01
12	1.63	24.71	0.00	0.00
13	1.51	18.44	0.00	0.00
14	1.40	12.16	0.00	0.00
15	1.31	5.89	0.00	0.00

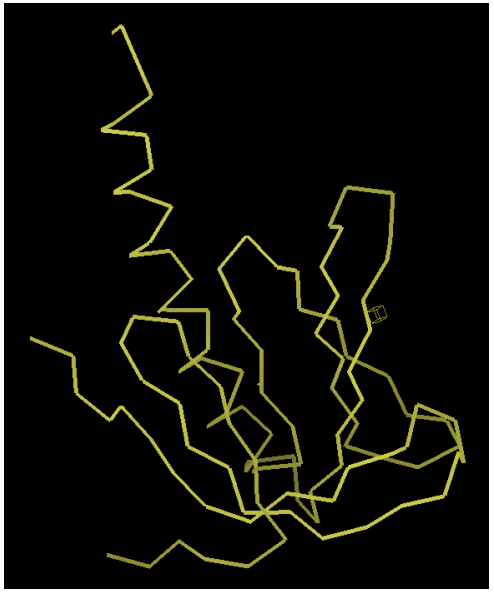
High resolution PDB-ID 2X18

Model preparation

- Multiple homologues structures
  e.g. 1MRY (84%), 1O6K (83%), 1GZ0 (86%), 3CQW (85%), 1UNP (82%)
- Reduce model PDB files to one chain (e.g. chain A); delete water molecules and ligands
- Use Coot for superpositioning
- Sequence alignment between model and target
- Run Chainsaw with edited model and sequence alignment; reduces side chains to last common atom between model and target sequence



Core of superpositioned search models of full length homologues



PH domain as in 1UNP

High resolution PDB-ID 2X18

SGC

Phasing

- Using Phaser
- One search model was picked randomly
- Data was further truncated and Rfree flag was added

Phaser result

SpaceGroup of Solution: P 1

There were 7 solutions

Pdb and Mtz files have been output for 1 of these solutions

Solution #1 written to PDB file: /work/AKT3A/8-phase-mv/AKT3_phase_12.1.pdb

Solution #1 written to MTZ file: /work/AKT3A/8-phase-mv/AKT3_phase_12.1.mtz

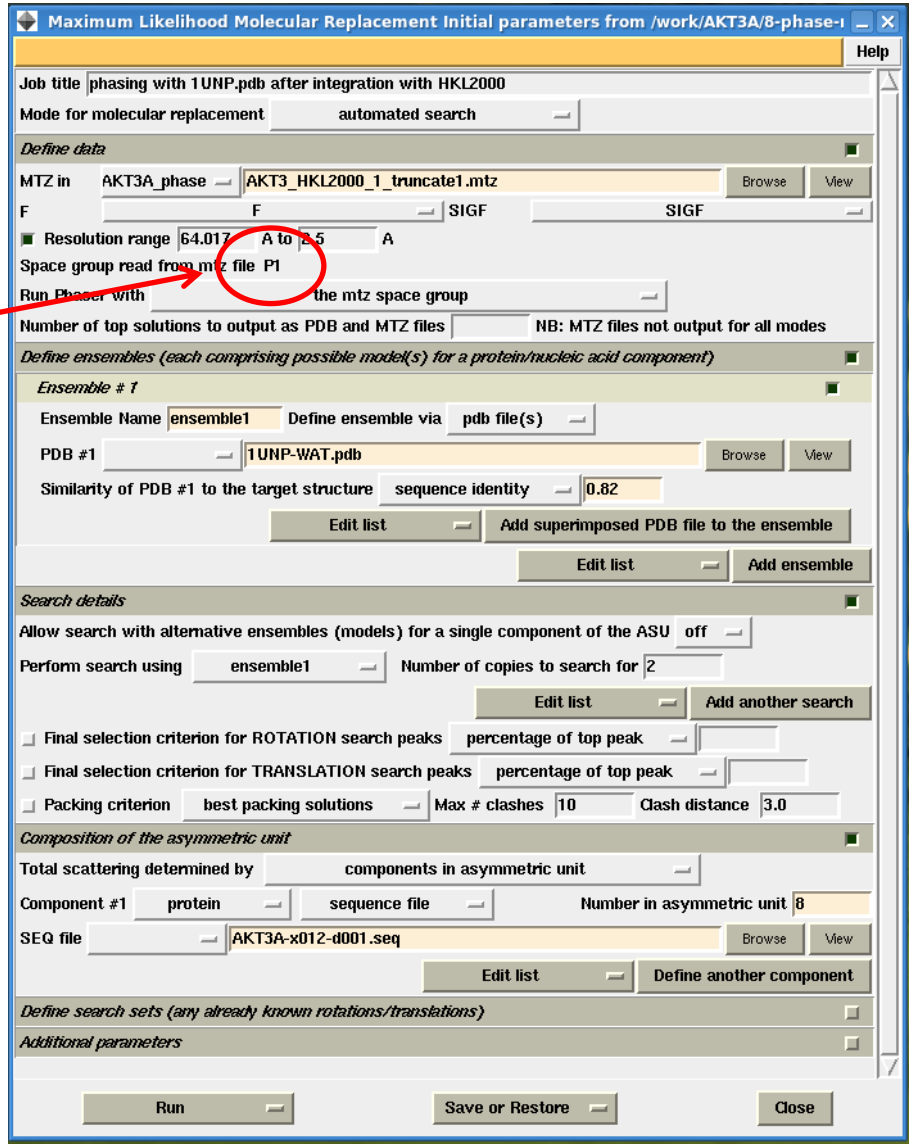
Solution #1 log-likelihood gain: 302.771

Solution #1 annotation (history):

RFZ=10.2 TFZ=100.0 PAK=0 LLG=89 Molecule 1

RFZ=9.0 TFZ=5.8 PAK=0 LLG=158 Molecule 2

LLG=303

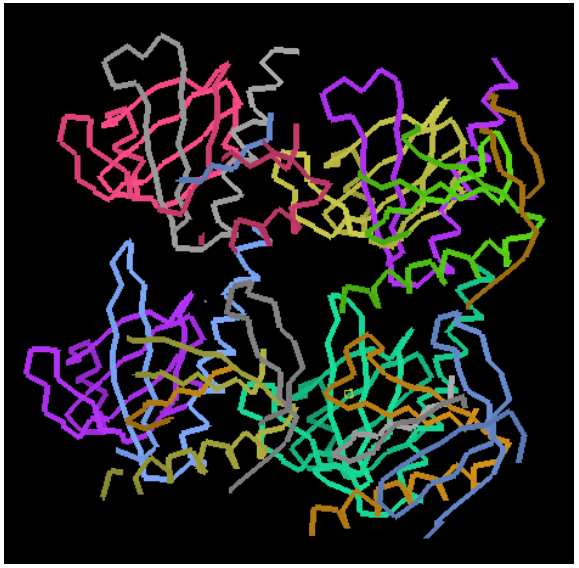


High resolution PDB-ID 2X18

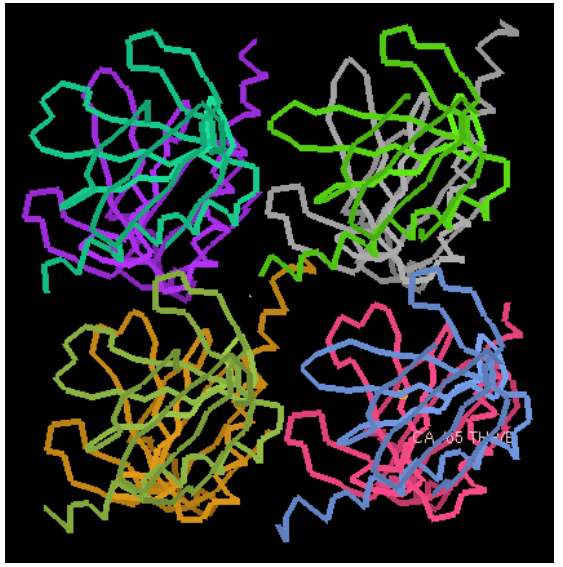
SGC

Phasing results and autobuilding

Initial phases



1st round of autobuild
with Buccaneer



2nd round of autobuild
with Buccaneer

Examples for Molecular Replacement

SGC

- Intermediate resolution
  - Muscle specific human β-enolase (PDB-ID: 2XSX)
- High resolution
  - PH domain of human AKT3 kinase (PDB-ID: 2X18)
- Low homology
  - PH domain of human Actin-binding protein anillin ANLN (PDB-ID: 2Y7B)
- Low resolution
  - Rotating transmembrane ring of F_oF_1 ATPsynthase from spinach chloroplasts (PDB-ID: 2W5J)
- NMR model
  - Thioredoxin domain of human TXNL2 (PDB-ID: 2WZ9)
- Twinning
  - Macro domain of human core histone H2A (PDB-ID: 2XD7)

Low homology PDB-ID 2Y7B

- PH domain of human Actin-binding protein anillin ANLN
- Actin-binding protein anillin
- Location: nucleus and cytoplasm
- Function: Required for cytokinesis; interacts with cytosceleton
- Enzymatic reaction:

  -
- Co-factor: -
- Biologically active form: -
- Number of residues (PH domain): 125
- Molecular weight (PH domain): 14.8 kDa
- UniProt-ID: Q9NQW6

Low homology PDB-ID 2Y7B

SGC

- Processing results (XDS, Scala)

	Overall	Inner Shell	Outer Shell
Low resolution limit	18.94	18.94	2.0
High resolution limit	1.90	6.01	1.9
Rmerge	0.136	0.037	0.962
Rmerge in top intensity bin	0.037	-	-
Rmeas (within I+/I-)	0.153	0.043	1.077
Rmeas (all I+ & I-)	0.153	0.043	1.077
Rpim (within I+/I-)	0.069	0.021	0.478
Rpim (all I+ & I-)	0.069	0.021	0.478
Fractional partial bias	0.000	0.000	0.000
Total number of observations	50794	1595	7380
Total number of unique	10888	391	1550
Mean ((I)/sd(I))	9.2	24.0	2.0
Completeness	99.6	96.3	99.8
Multiplicity	4.7	4.1	4.8
Average unit cell	34.30	59.42	64.72
	90.0	90.0	90.0
Space group	P 21 21 21		
Maximum resolution	1.90 Å		

Cell volume: 132210.984

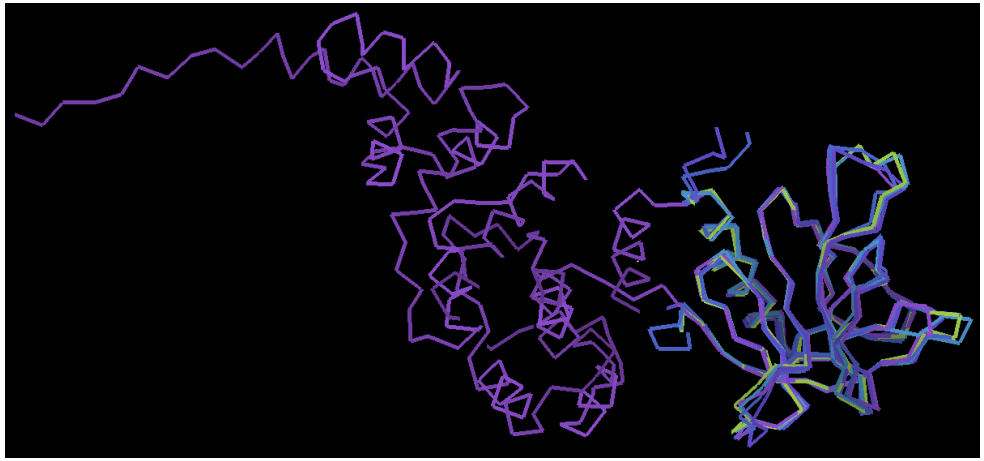
For estimated molecular weight 16313.

Nmol/asym	Matthews Coeff	%solvent	P(1.94)	P(tot)
1	2.03	39.33	1.00	1.00

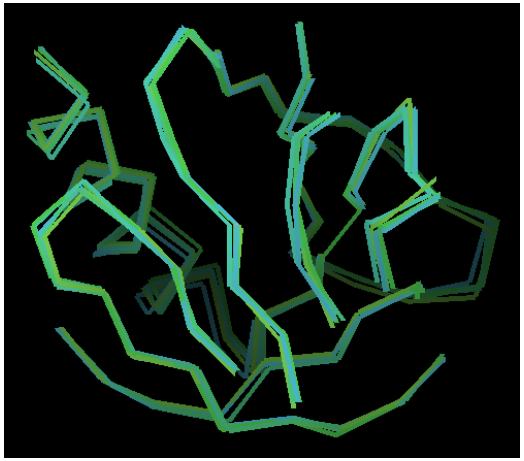
Low homology PDB-ID 2Y7B

Model preparation

- Single homologues structure in multiple conformations
  e.g. 1U29 (20%), 1FHW (20%), 1U27 (20%), 1U2B (18%), 2R0D (15%)
- Reduce model PDB files to one chain (e.g. chain A); delete water molecules and ligands
- Use Coot to eliminate flexible protein parts
- Sequence alignment between model and target
- Run Chainsaw with edited model and sequence alignment; reduces side chains to last common atom between model and target sequence



Superpositioned search models



Core of superpositioned search models

Low homology PDB-ID 2Y7B

SGC

Phasing

- Using Phaser
- Using search models as prepared above
- Using data as resulting from Scala

Phaser result

SpaceGroup of Solution: P 21 21 21

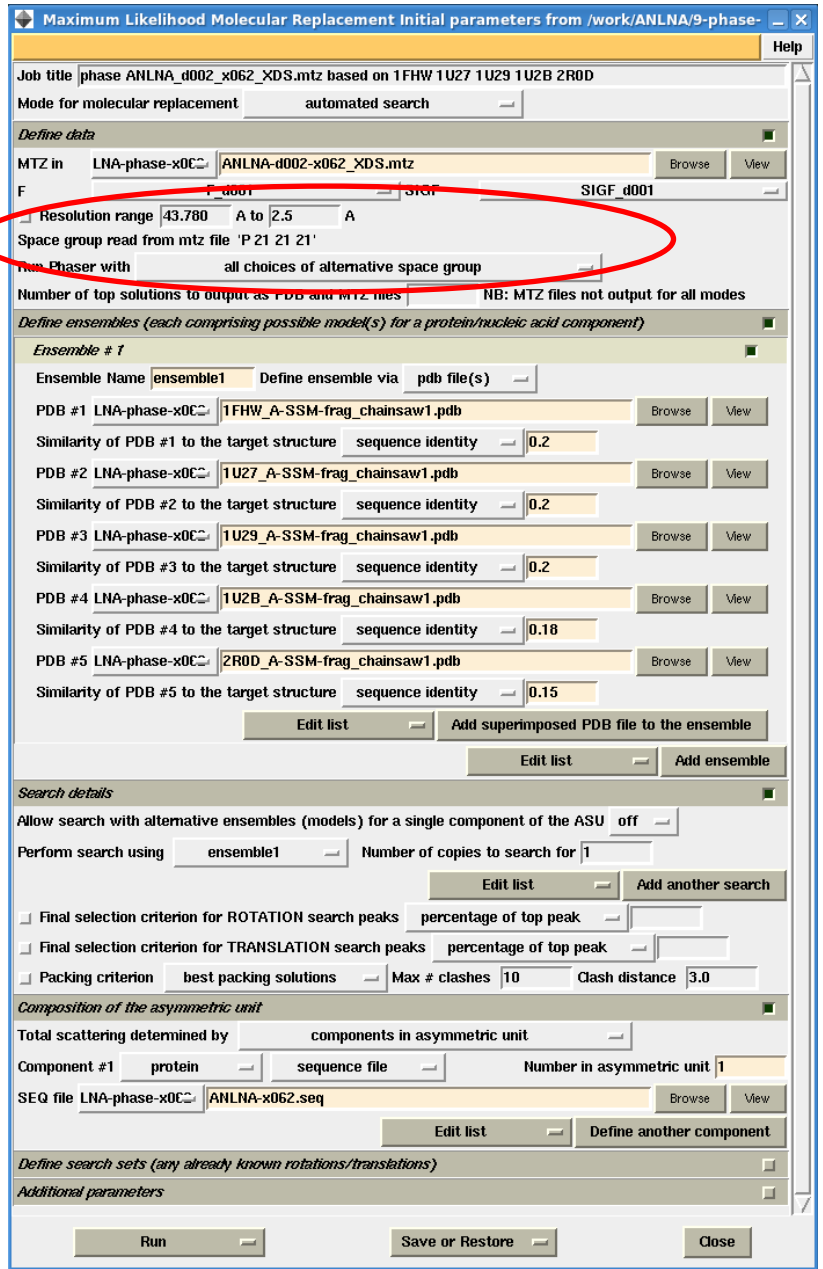
SINGLE solution

Solution written to PDB file:
/work/ANLNA/9-phase-d001-x062-mv/ANLNA-phase-x062_8.1.pdb
Solution written to MTZ file:
/work/ANLNA/9-phase-d001-x062-mv/ANLNA-phase-x062_8.1.mtz
Solution log-likelihood gain: 21.6602
Solution annotation (history):

RFZ=3.5 TFZ=6.2 PAK=0 LLG=22

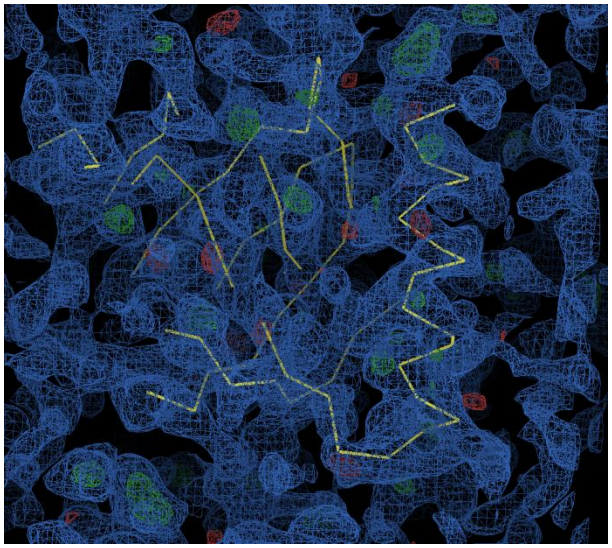
Molecule 1

LLG=22



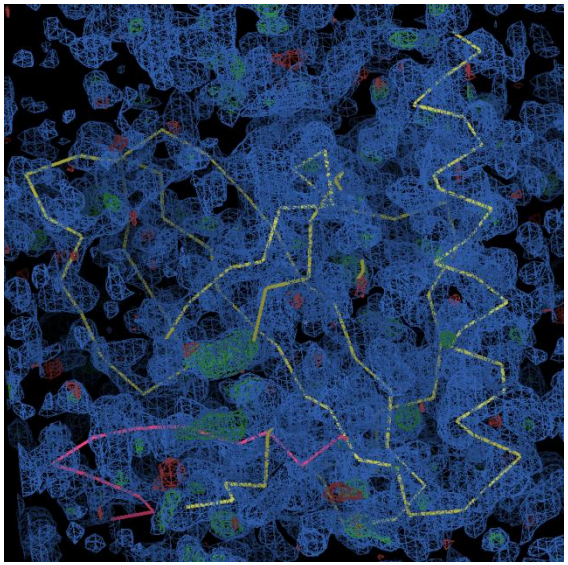
Low homology PDB-ID 2Y7B

Results



After phasing

Ncyc	Rfact	Rfree	FOM	-LL	-LLfree	rmsBOND	zBOND	rmsANGL	zANGL	rmsCHIRAL
$$										
0	0.5281	0.5462	0.344	51639.	2595.4	0.0310	1.084	1.921	1.070	0.111
1	0.4966	0.5533	0.358	51086.	2577.5	0.0345	1.563	1.613	0.803	0.119
2	0.4804	0.5389	0.397	50883.	2571.6	0.0236	1.037	1.470	0.678	0.096
3	0.4740	0.5203	0.421	50496.	2553.0	0.0131	0.595	1.331	0.608	0.096
4	0.4649	0.5133	0.448	50599.	2561.3	0.0125	0.561	1.402	0.642	0.098
5	0.4586	0.5102	0.470	50424.	2555.4	0.0129	0.578	1.495	0.681	0.102
6	0.4537	0.5081	0.484	50358.	2554.7	0.0136	0.617	1.580	0.732	0.104
7	0.4508	0.5043	0.489	50145.	2545.4	0.0146	0.657	1.645	0.770	0.109
8	0.4479	0.5024	0.489	50093.	2544.0	0.0139	0.624	1.697	0.775	0.111
9	0.4462	0.4999	0.493	50051.	2542.9	0.0138	0.616	1.713	0.754	0.111
10	0.4441	0.5010	0.496	50169.	2549.7	0.0138	0.622	1.761	0.765	0.112



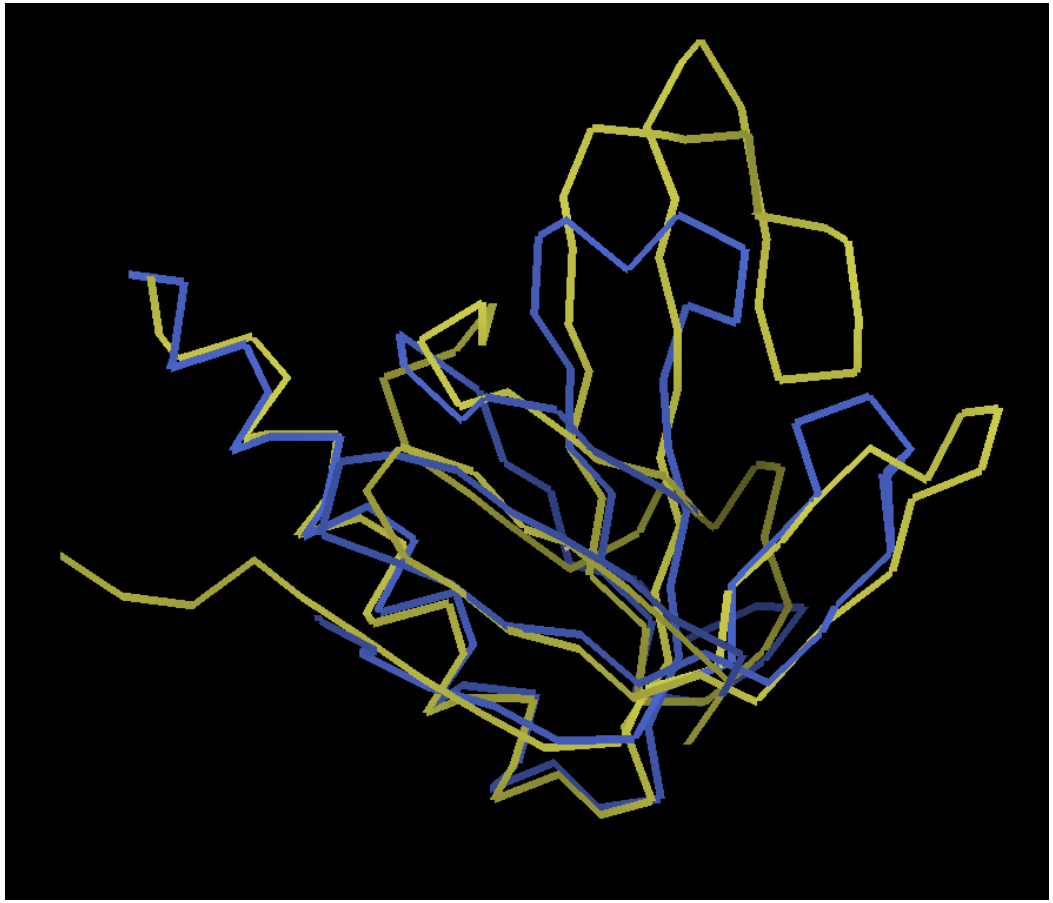
Some rounds of autobuilding and manual editing

Ncyc	Rfact	Rfree	FOM	-LL	-LLfree	rmsBOND	zBOND	rmsANGL	zANGL	rmsCHIRAL
$$										
0	0.3663	0.3868	0.739	33811.	2638.0	0.0273	1.694	2.393	1.223	0.121
1	0.3158	0.3571	0.757	31368.	2595.3	0.0251	1.094	2.058	0.985	0.129
2	0.2949	0.3439	0.761	29929.	2567.2	0.0225	0.975	2.010	0.954	0.132
3	0.2876	0.3407	0.761	29487.	2557.6	0.0226	0.962	2.019	0.960	0.131
4	0.2853	0.3420	0.760	29323.	2554.6	0.0224	0.952	2.020	0.961	0.132
5	0.2844	0.3450	0.759	29255.	2553.4	0.0225	0.948	2.021	0.961	0.133
6	0.2842	0.3469	0.758	29222.	2553.4	0.0225	0.946	2.025	0.962	0.133
7	0.2840	0.3483	0.758	29200.	2553.8	0.0226	0.950	2.030	0.964	0.133
8	0.2837	0.3481	0.758	29188.	2554.1	0.0227	0.950	2.038	0.967	0.134
9	0.2833	0.3484	0.758	29174.	2554.4	0.0228	0.958	2.044	0.970	0.134
10	0.2830	0.3481	0.758	29174.	2554.8	0.0229	0.965	2.050	0.973	0.134

Comparison PH domains

Comparison of PH domains from AKT3 and ANLN

- Although both identified as PH domains differences are big enough to show low homology for MR
- Yellow → ANLN
- Blue → AKT3



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- Low homology
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- Low resolution
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- NMR model
  - Thioredoxin domain of human TXNL2 (PDB-ID: 2WZ9)
- Twinning
  - Macro domain of human core histone H2A (PDB-ID: 2XD7)

Low resolution PDB-ID 2W5J

- Rotating transmembrane ring of chloroplast F_oF_1 ATP synthase from spinach chloroplasts
- F-type ATPase subunit c
- Location: chloroplast thylacoid membrane
- Function: transport of protons across the membrane and thereby fuelling ATP production
- Enzymatic reaction:

  -
- Co-factor: -
- Biologically active form: 14mer
- Number of residues (monomer): 81
- Molecular weight (14mer): 112 kDa
- UniProt-ID: P69447

Low resolution PDB-ID 2W5J

SGC

- Processing results (Mosflm, Scala)

	Overall	Inner Shell	Outer Shell
Low resolution limit	28.28	28.28	4.0
High resolution limit	3.80	12.02	3.8
Rmerge	0.085	0.065	0.201
Rmeas (within I+/I-)	0.081	0.039	0.248
Rmeas (all I+ & I-)	0.081	0.039	0.248
Total number of observations	132804		
Total number of unique	11213		
Mean ((I)/sd(I))	10.4	18.8	4.2
Completeness	92.2	71.6	92.2
Multiplicity	2.6	2.4	2.5
Average unit cell	128.60	89.99	124.89
	90.0	104.69	90.0
Space group	C2		
Maximum resolution	3.80 Å		

Cell volume: 1398161.375

For estimated molecular weight 9000.

Nmol/asym	Matthews Coeff	%solvent	P(3.80)	P(tot)
1	38.84	96.83	0.00	0.00
2	19.42	93.67	0.00	0.00
3	12.95	90.50	0.00	0.00
4	9.71	87.34	0.00	0.00
5	7.77	84.17	0.00	0.00
6	6.47	81.01	0.00	0.00
7	5.55	77.84	0.00	0.00
8	4.85	74.68	0.00	0.00
9	4.32	71.51	0.01	0.00
10	3.88	68.35	0.01	0.01
11	3.53	65.18	0.03	0.02
12	3.24	62.02	0.05	0.04
13	2.99	58.85	0.08	0.06
14	2.77	55.69	0.11	0.09
15	2.59	52.52	0.14	0.13
16	2.43	49.36	0.16	0.16
17	2.28	46.19	0.15	0.17
18	2.16	43.03	0.12	0.14
19	2.04	39.86	0.07	0.10
20	1.94	36.70	0.03	0.05
21	1.85	33.53	0.01	0.02
22	1.77	30.37	0.00	0.00
23	1.69	27.20	0.00	0.00
24	1.62	24.04	0.00	0.00
25	1.55	20.87	0.00	0.00
26	1.49	17.71	0.00	0.00
27	1.44	14.54	0.00	0.00
28	1.39	11.38	0.00	0.00
29	1.34	8.21	0.00	0.00
30	1.29	5.05	0.00	0.00
31	1.25	1.88	0.00	0.00

Note: There is no preferred Matthews coefficient

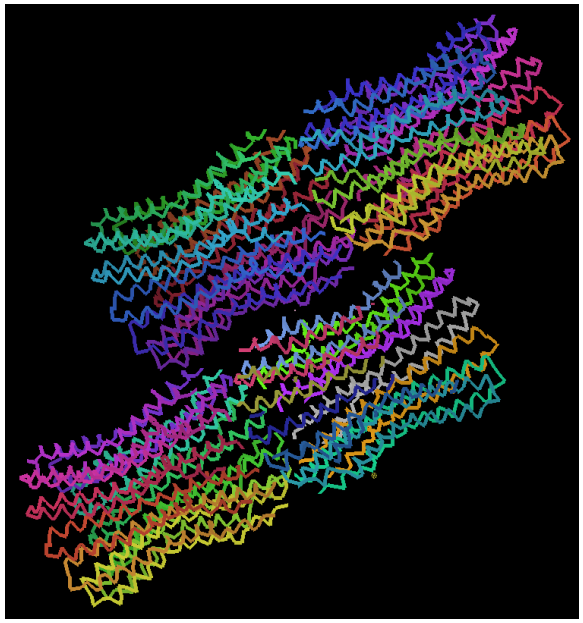
But: Number of 14 monomers known from biochemical experiments and electron microscopy results

Low resolution PDB-ID 2W5J

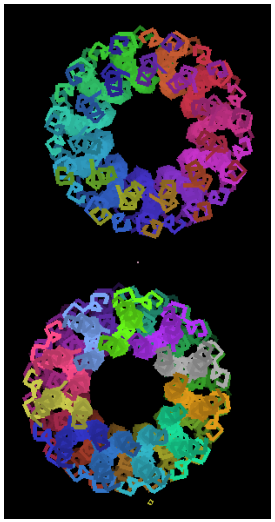
SGC

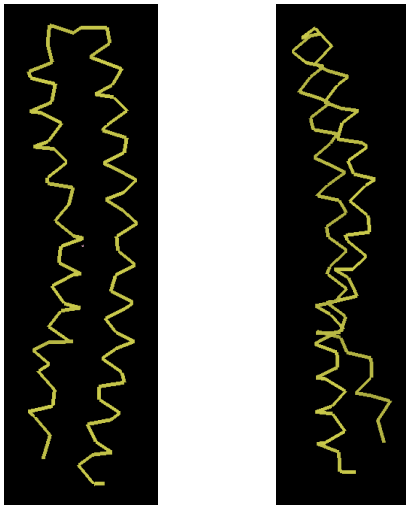
Model preparation

- Multiple homologues structures
  e.g. 1YCE (56%)
- Reduce model PDB files to one chain (e.g. chain A); delete water molecules and ligands
- Sequence alignment between model and target
- Run Chainsaw with edited model and sequence alignment; reduces side chains to last common atom between model and target sequence



Closest homologue





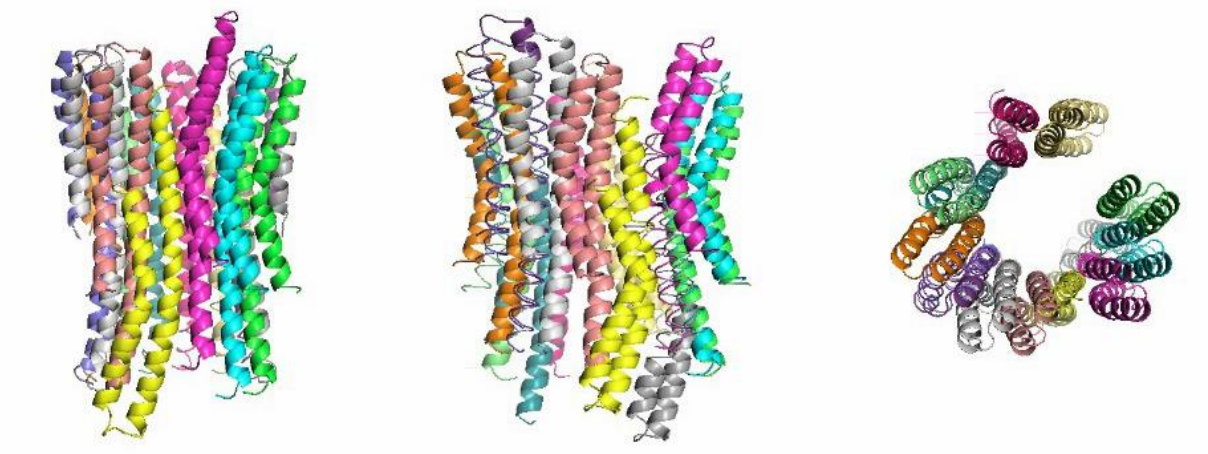
Isolated single chain

Low resolution PDB-ID 2W5J

SGC

Phasing

- Using Phaser and single chain as search model
- Searching for 14 chains

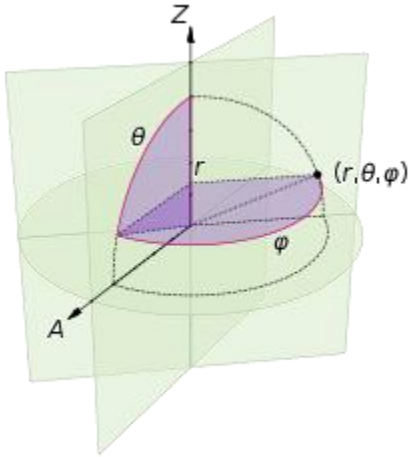


Low resolution PDB-ID 2W5J

Phasing

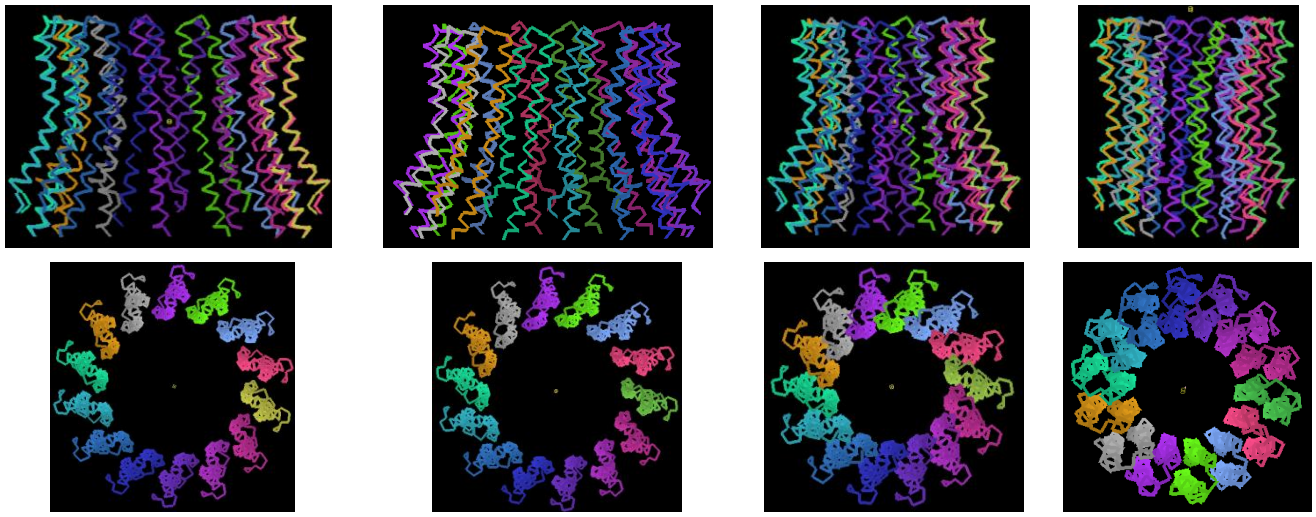
- Molrep and scripting

Step 1



- Monomer at origin
- Rotate by angle *φ*
- Rotate by angle θ
- Rotate around *r*

Step 2



d = 62 Å | d = 59 Å | d = 43 Å | d = 43 Å

- Monomer in new orientation
- Copy monomer
- Combine to 14mer ring
- Vary radius/diameter of ring

Low resolution PDB-ID 2W5J

SGC

Phasing

- Molrep and scripting

Step 3

Run Molrep from command line with default settings

Contrast = 2.63 solution

After stick correction:
Move closer to origin
I_sym_operator : 1
new position(frac): 0.179 0.000 0.214

Sol_
S_ Nmon RF TF theta phi chi tx ty tz TFcnt wRfac Scor
S__ 1 2 1 180.00 0.00 0.43 0.179 0.000 0.214 4.35 0.404 0.869
--- convert "molrep.crd" to "molrep.pdb" ---

Contrast values for solutions:
3.0 → definitely solution
2.0 – 3.0 → solution
1.5 – 2.0 → maybe solution

Step 4

Rigid body refinement followed by restrained refinement of solution with Refmac5 with default settings and using 14fold NCS

decrease increase

Ncyc	Rfact	Rfree	FOM	-LL	-LLfree	rmsBOND	zBOND	rmsANGL	zANGL	rmsCHIRAL
$$										
0	0.4932	0.4620	0.432	71684.	3688.5	0.0213	0.963	1.758	0.891	0.094
1	0.4362	0.4480	0.461	70870.	3688.3	0.0304	1.504	1.787	0.967	0.106
2	0.4133	0.4404	0.482	70589.	3680.2	0.0152	0.723	1.074	0.531	0.053
3	0.4006	0.4364	0.495	70388.	3675.5	0.0094	0.444	0.912	0.415	0.042
4	0.3919	0.4320	0.509	70216.	3668.9	0.0082	0.379	0.916	0.416	0.042
5	0.3856	0.4275	0.517	70060.	3661.5	0.0076	0.343	0.922	0.420	0.043
6	0.3749	0.4257	0.525	69889.	3662.6	0.0091	0.402	1.030	0.465	0.048
7	0.3648	0.4231	0.529	69784.	3667.9	0.0104	0.466	1.151	0.517	0.055
8	0.3610	0.4213	0.535	69657.	3665.7	0.0101	0.451	1.162	0.522	0.055
9	0.3570	0.4238	0.535	69590.	3666.5	0.0103	0.460	1.185	0.532	0.058
10	0.3485	0.4244	0.539	69491.	3672.1	0.0125	0.552	1.346	0.604	0.064
11	0.3459	0.4261	0.540	69516.	3676.8	0.0122	0.542	1.353	0.607	0.064
12	0.3440	0.4251	0.540	69483.	3676.9	0.0122	0.539	1.367	0.611	0.064
13	0.3424	0.4251	0.540	69491.	3679.1	0.0121	0.534	1.367	0.611	0.064
14	0.3413	0.4228	0.542	69472.	3678.6	0.0121	0.535	1.373	0.613	0.064
15	0.3402	0.4230	0.541	69501.	3682.2	0.0122	0.537	1.373	0.611	0.064
16	0.3394	0.4198	0.546	69462.	3679.9	0.0122	0.538	1.378	0.614	0.064

Examples for Molecular Replacement

SGC

- Intermediate resolution
  - Muscle specific human β-enolase (PDB-ID: 2XSX)
- High resolution
  - PH domain of human AKT3 kinase (PDB-ID: 2X18)
- Low homology
  - PH domain of human Actin-binding protein anillin ANLN (PDB-ID: 2Y7B)
- Low resolution
  - Rotating transmembrane ring of F_oF_1 ATPsynthase from spinach chloroplasts (PDB-ID: 2W5J)
- NMR model
  - Thioredoxin domain of human TXNL2 (PDB-ID: 2WZ9)
- Twinning
  - Macro domain of human core histone H2A (PDB-ID: 2XD7)

Using an NMR model PDB-ID 2WZ9

SGC

- Thioredoxin domain of human TXNL2
- Glutaredoxin-3, PKC-interacting cousin of thioredoxin
- Location: cytoplasm
- Function: critical negative regulator of cardiac hypertrophy and a positive inotropic regulator
- Enzymatic reaction (thioredoxin domain):

  does not posses any thioredoxin activity; no conserved catalytic motif
- Co-factor (full length): 2Fe-2S clusters
- Biologically active form (full length): Monomer and homodimer; the homodimer is probably linked by 2 2Fe-2S clusters
- Number of residues (thioredoxin domain): 116
- Molecular weight (thiordoxin domain): 12.6 kDa
- UniProt-ID: O76003

Using an NMR model PDB-ID 2WZ9

SGC

- Processing results (Mosflm, Scala)

	Overall	Inner Shell	Outer Shell
Low resolution limit	34.47	34.47	1.63
High resolution limit	1.55	4.90	1.55
Rmerge	0.091	0.023	0.581
Rmerge in top intensity bin	0.038	-	-
Rmeas (within I+/I-)	0.106	0.028	0.673
Rmeas (all I+ & I-)	0.106	0.028	0.673
Rpim (within I+/I-)	0.054	0.015	0.337
Rpim (all I+ & I-)	0.054	0.015	0.337
Fractional partial bias	-0.006	-0.034	0.049
Total number of observations	73276	2442	10641
Total number of unique	19534	703	2808
Mean ((I)/sd(I))	9.5	23.4	2.2
Completeness	99.8	99.5	99.6
Multiplicity	3.8	3.5	3.8
Average unit cell	42.63	52.06	58.60
	90.0	90.0	90.0
Space group	P 2 2 2		
Maximum resolution	1.55 Å		

Cell volume: 130052.023

For estimated molecular weight 14683.

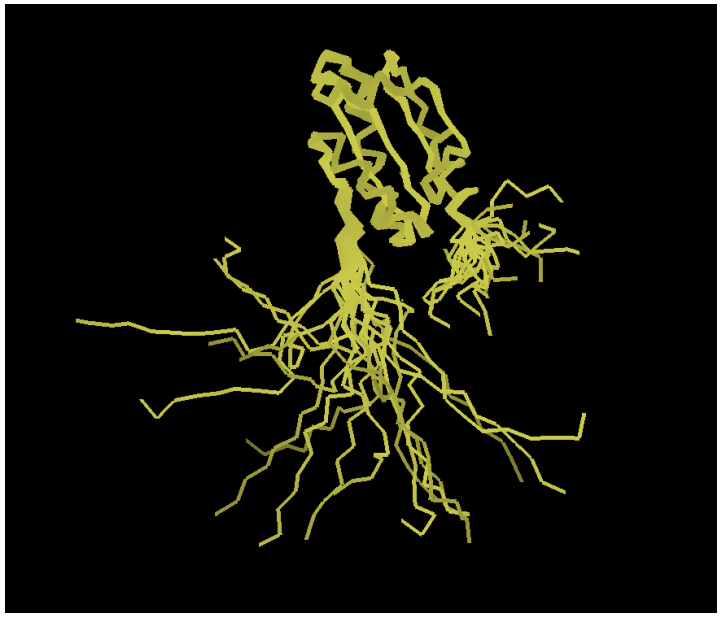
Nmol/asym	Matthews Coeff	%solvent	P(1.50)	P(tot)
1	2.21	44.49	1.00	1.00

Using an NMR model PDB-ID 2WZ9

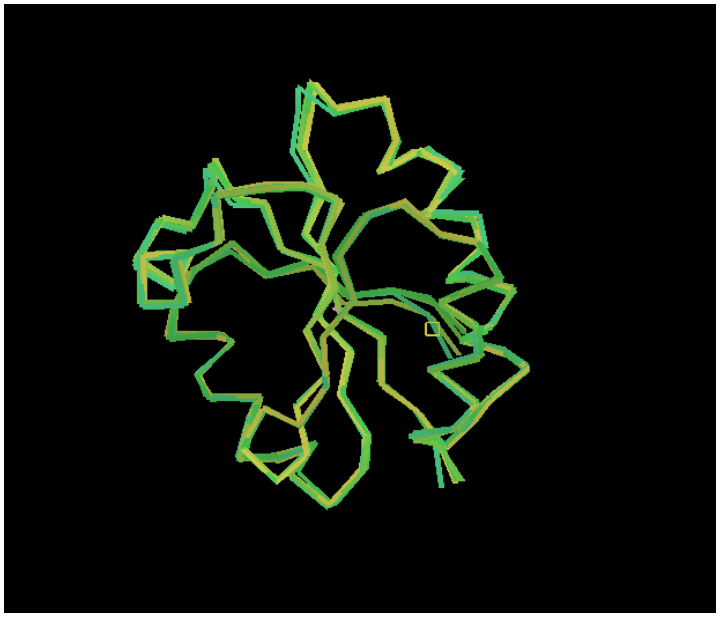
SGC

Model preparation

- Single homologues structure in multiple conformations
  e.g. 1DIY (90%)
- Reduce model PDB files to one chain (e.g. chain A); delete water molecules and ligands
- Use Coot to eliminate flexible protein parts
- Sequence alignment between model and target
- Run Chainsaw with edited model and sequence alignment; reduces side chains to last common atom between model and target sequence



Superpositioned search models



Core of superpositioned search models

Using an NMR model PDB-ID 2WZ9

SGC

Phasing

- Using Phaser
- Using search models as prepared above
- Using data as resulting from Scala

Phaser result

SpaceGroup of Solution: P 2 2 2

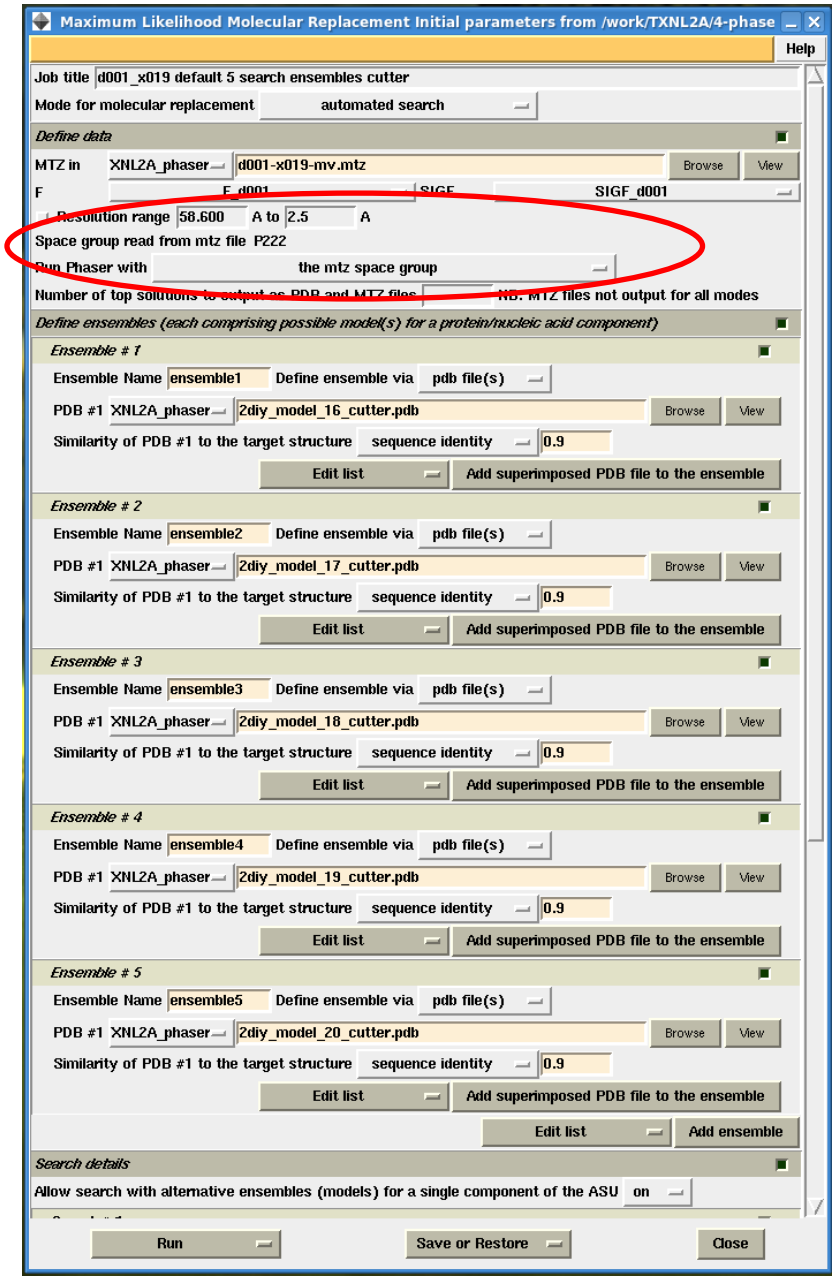
SINGLE solution

Solution written to PDB file:
/work/TXNL2A/4-phaser-d001-mv/TXNL2A_phaser_19.1.pdb
Solution written to MTZ file:
/work/TXNL2A/4-phaser-d001-mv/TXNL2A_phaser_19.1.mtz
Solution log-likelihood gain: -93.6145
Solution annotation (history):

RFZ=6.3 TFZ=3.8 PAK=2 LLG=-96

Molecule 1

LLG=-94



Using an NMR model PDB-ID 2WZ9

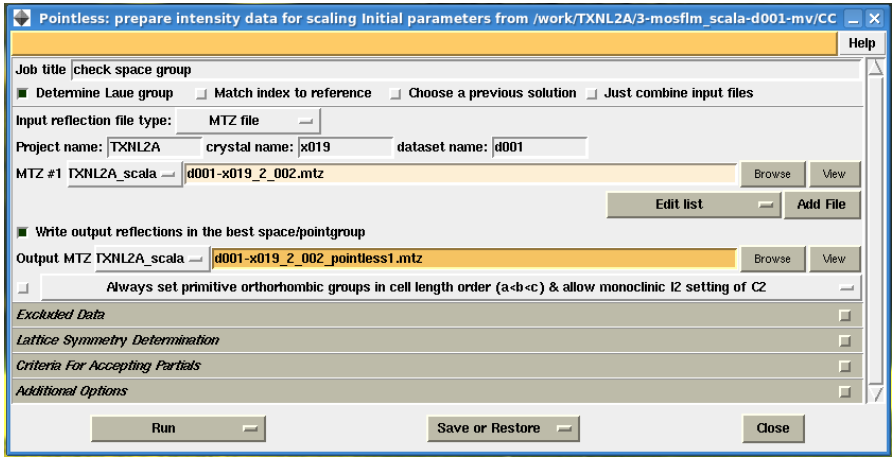
SGC

Changing space goup

- Use unmerged, unscaled data file to check on space with Pointless and get a new data file with appropriate symmetry

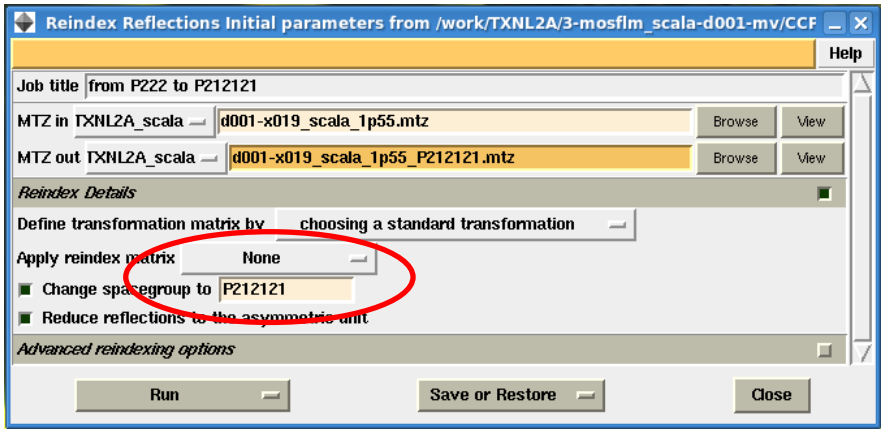
or

- Use Re-indexing to change space group




```
* Cell Dimensions : (obsolete - refer to dataset cell dimensions above)
  42.6300   52.0600   58.6000   90.0000   90.0000   90.0000
*  Resolution Range :
   0.00029    0.44443     (     58.600 -      1.500 A )
* Sort Order :
     1     2     3     0     0
* Space group = 'P222' (number     16)
```

Before changing




```
* Cell Dimensions : (obsolete - refer to dataset cell dimensions above)
  42.6300   52.0600   58.6000   90.0000   90.0000   90.0000
*  Resolution Range :
   0.00029    0.44443     (     58.600 -      1.500 A )
* Sort Order :
     0     0     0     0     0
* Space group = 'P 21 21 21' (number     19)
```

After changing

Using an NMR model PDB-ID 2WZ9

Phasing

- Using Phaser
- Using search models as prepared above
- Using data as resulting from Scala

Phaser result

SpaceGroup of Solution: P 21 21 21

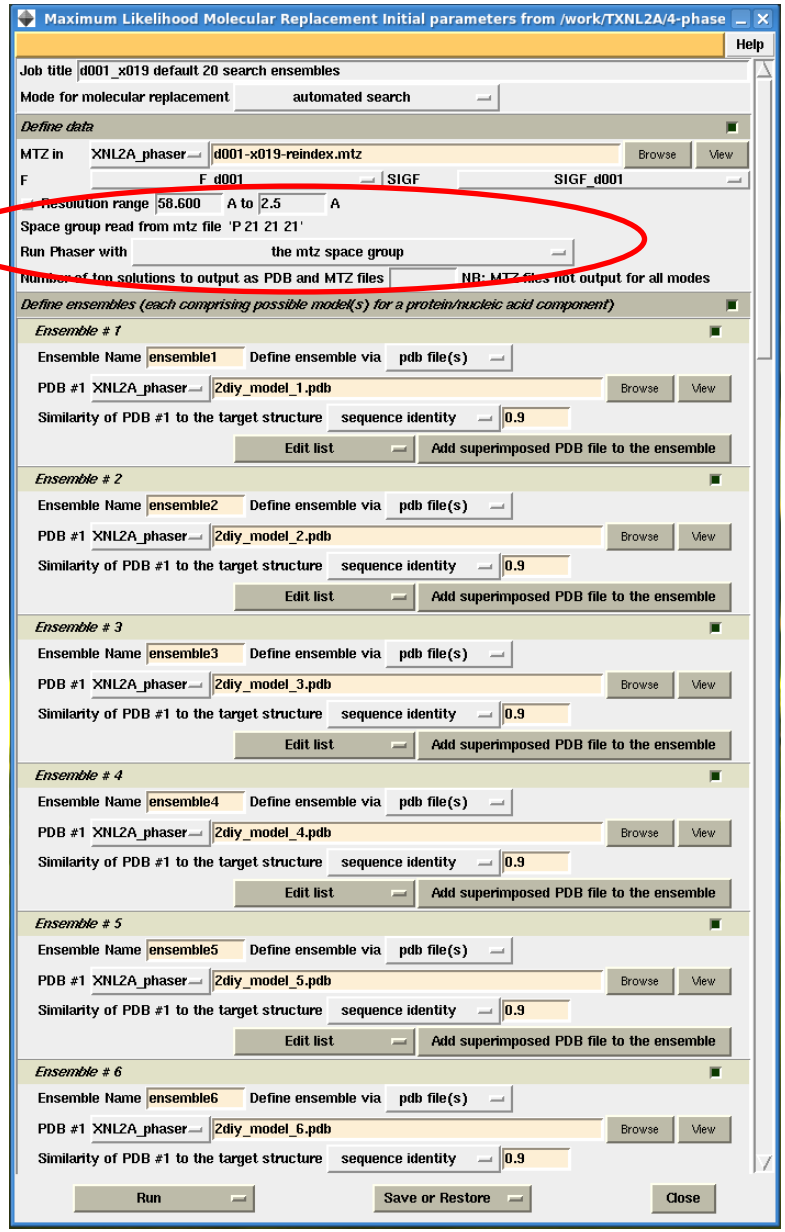
SINGLE solution

Solution written to PDB file:
/work/TXNL2A/4-phaser-d001-mv/TXNL2A_phaser_21.1.pdb
Solution written to MTZ file:
/work/TXNL2A/4-phaser-d001-mv/TXNL2A_phaser_21.1.mtz
Solution log-likelihood gain: 67.6119
Solution annotation (history):

RFZ=8.0 TFZ=11.1 PAK=0 LLG=36

Molecule 1

LLG=68



Examples for Molecular Replacement

SGC

- Intermediate resolution
  - Muscle specific human β-enolase (PDB-ID: 2XSX)
- High resolution
  - PH domain of human AKT3 kinase (PDB-ID: 2X18)
- Low homology
  - PH domain of human Actin-binding protein anillin ANLN (PDB-ID: 2Y7B)
- Low resolution
  - Rotating transmembrane ring of F_oF_1 ATPsynthase from spinach chloroplasts (PDB-ID: 2W5J)
- NMR model
  - Thioredoxin domain of human TXNL2 (PDB-ID: 2WZ9)
- Twinning
  - Macro domain of human core histone H2A (PDB-ID: 2XD7)

Twinned data set PDB-ID 2XD7

- Macro domain of human core histone H2A
- Location: nucleus
- Function: histone formation, DNA repair, DNA replication, chromosomal stability
- Enzymatic reaction:

  -
- Co-factor: -
- Biologically active form: assembled in histone
- Number of residues (macro domain): 187
- Molecular weight (macro domain): 20.2 kDa
- UniProt-ID: Q9P0M6

Twinned data set PDB-ID 2XD7

SGC

- Processing results (Mosflm, Scala) (before twinning was detected)

	Overall	Inner Shell	Outer Shell
Low resolution limit	41.85	41.85	2.31
High resolution limit	2.19	6.93	2.19
Rmerge	0.082	0.037	0.371
Rmerge in top intensity bin	0.049	-	-
Rmeas (within I+/I-)	0.093	0.042	0.416
Rmeas (all I+ & I-)	0.093	0.042	0.416
Rpim (within I+/I-)	0.043	0.019	0.185
Rpim (all I+ & I-)	0.043	0.019	0.185
Fractional partial bias	-0.064	-0.069	-0.015
Total number of observations	101359	3048	14575
Total number of unique	22223	696	3180
Mean ((I)/sd(I))	10.4	19.6	3.9
Completeness	99.0	90.2	99.0
Multiplicity	4.6	4.4	4.6
Average unit cell	57.04	61.58	242.51
	90.0	90.0	90.0
Space group	C 2 2 2		
Maximum resolution	2.19 Å		

Cell volume: 851822.000

For estimated molecular weight 22364.

Nmol/asym	Matthews Coeff	%solvent	P(1.50)	P(tot)
1	4.76	74.18	0.00	0.01
2	2.38	48.36	0.99	0.99
3	1.59	22.55	0.00	0.00

Pointless

No changes in space group were suggested

Phenix.Xtriage

Determining possible twin laws.

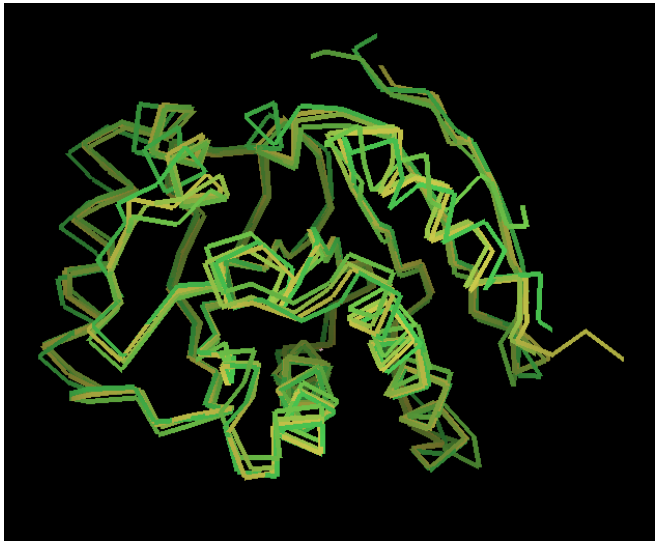
0 merohedral twin operators found
0 pseudo-merohedral twin operators found
In total, 0 twin operator were found

The results of the L-test indicate that the intensity statistics are significantly different than is expected from good to reasonable, untwinned data.
As there are no twin laws possible given the crystal symmetry, there could be
a number of reasons for the departure of the intensity statistics from normality.
Overmerging pseudo-symmetric or twinned data, intensity to amplitude conversion problems
as well as bad data quality might be possible reasons.
It could be worthwhile considering reprocessing the data.

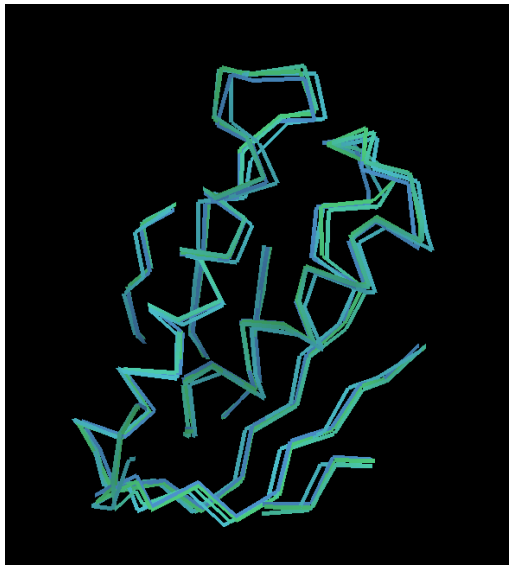
Twinned data set PDB-ID 2XD7

Model preparation

- Single homologues structure in multiple conformations
  e.g. 1YD9 (64%), 1ZR3 (62%), 1ZR5 (65%), 3IID (62%)
- Reduce model PDB files to one chain (e.g. chain A); delete water molecules and ligands
- Use Coot to eliminate flexible protein parts
- Sequence alignment between model and target
- Run Chainsaw with edited model and sequence alignment; reduces side chains to last common atom between model and target sequence



Superpositioned search models



Core of superpositioned search models

Twinned data set PDB-ID 2ZD7

SGC

Phasing

- Using Phaser
- Using search models as prepared above
- Using data as resulting from Scala

Phaser result

SpaceGroup of Solution: C 2 2 21

SINGLE solution

Solution written to PDB file: /work/H2AFY2A/6-phase-mv/H2A_phase_2_4.1.pdb

Solution written to MTZ file: /work/H2AFY2A/6-phase-mv/H2A_phase_2_4.1.mtz

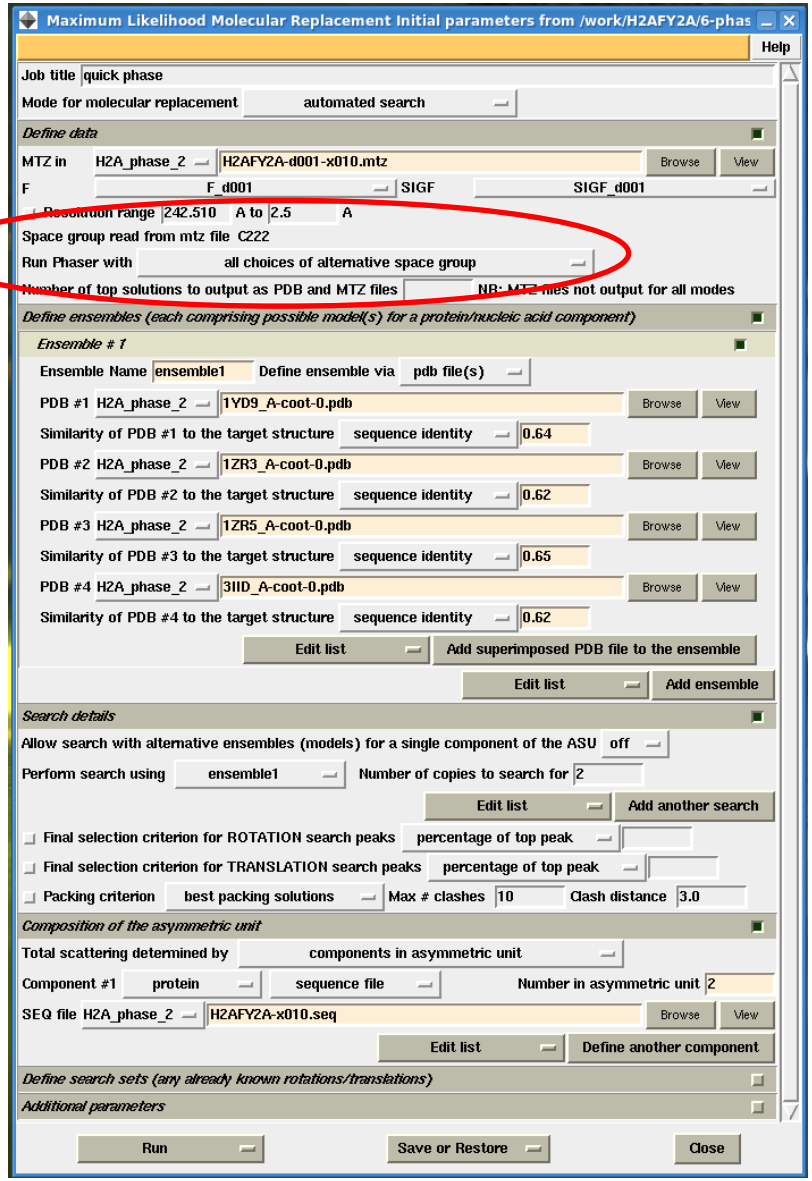
Solution log-likelihood gain: 648.297

Solution annotation (history):

RFZ=8.2 TFZ=13.8 PAK=0 LLG=181 Molecule 1

RFZ=8.0 TFZ=23.9 PAK=0 LLG=543 Molecule 2

LLG=648



Twinned data set PDB-ID 2ZD7

SGC

Refinement after phasing and changing space group

- Using Refmac5
- All settings are default

Refinement statistics:

Ncyc	Rfact	Rfree	FOM	-LL	-LLfree	rmsBOND	zBOND	rmsANGL	zANGL	rmsCHIRAL
0	0.4507	0.4454	0.546	127990.	6857.3	0.				
1	0.4362	0.4407	0.554	127316.	6838.6	0.				
2	0.4299	0.4391	0.559	127020.	6829.0	0.				
3	0.4257	0.4348	0.566	126892.	6826.0	0.				
4	0.4222	0.4307	0.568	126797.	6825.2	0.				
5	0.4199	0.4283	0.571	126731.	6825.2	0.				
6	0.4179	0.4257	0.573	126703.	6826.1	0.				
7	0.4164	0.4232	0.575	126684.	6826.9	0.				
8	0.4151	0.4224	0.577	126661.	6827.0	0.				
9	0.4141	0.4215	0.579	126654.	6827.7	0.				
10	0.4133	0.4205	0.581	126625.	6826.9	0.				

The results of the L-test indicate that the intensity statistics are significantly different than is expected from good to reasonable, untwinned data.
As there are no twin laws possible given the crystal symmetry, there could be
a number of reasons for the departure of the intensity statistics from normality.
Overmerging pseudo-symmetric or twinned data, intensity to amplitude conversion problems
as well as bad data quality might be possible reasons.
It could be worthwhile considering reprocessing the data.

Note:
Only marginal change in Rfactors and FOM

Twinned data set PDB-ID 2XD7

SGC

- Processing results (Mosflm, Scala) (assuming twinning)

	Overall	Inner Shell	Outer Shell
Low resolution limit	41.86	41.86	2.21
High resolution limit	2.10	6.64	2.10
Rmerge	0.070	0.029	0.372
Rmerge in top intensity bin	0.040	-	-
Rmeas (within I+/I-)	0.091	0.039	0.476
Rmeas (all I+ & I-)	0.091	0.039	0.476
Rpim (within I+/I-)	0.057	0.026	0.294
Rpim (all I+ & I-)	0.057	0.026	0.294
Fractional partial bias	-0.041	-0.062	-0.021
Total number of observations	109576	3184	12575
Total number of unique	45536	1454	5542
Mean ((I)/sd(I))	7.5	14.9	2.3
Completeness	92.7	88.8	77.7
Multiplicity	2.4	2.2	2.3
Average unit cell	57.03	61.64	242.23
	90.0	89.64	90.0
Space group	C 2		
Maximum resolution	2.10 Å		

Cell volume: 851502.000

For estimated molecular weight 22364.

Nmol/asym	Matthews Coeff	%solvent	P(1.50)	P(tot)
1	9.52	87.09	0.00	0.00
2	4.76	74.17	0.00	0.01
3	3.17	61.26	0.12	0.17
4	2.38	48.34	0.70	0.68
5	1.90	35.43	0.17	0.14
6	1.59	22.52	0.00	0.00
7	1.36	9.60	0.00	0.00

Phenix.Xtriage

The following twin laws have been found:

Type	Axis	R metric (%)	delta (le Page)	delta (Lebedev)	Twin law
PM	2-fold	0.278	0.360	0.005	-h,-k,l

M: Merohedral twin law
PM: Pseudomerohedral twin law

0 merohedral twin operators found
1 pseudo-merohedral twin operators found
In total, 1 twin operator were found

Twinned data set PDB-ID 2ZD7

Phasing

- Using Phaser
- Using search models as prepared above
- Using data as resulting from Scala

Phaser result

SpaceGroup of Solution: C 1 2 1

SINGLE solution

Solution written to PDB file: /work/H2AFY2A/9-phase-C2-mv/H2A_phaseC2_4.1.pdb

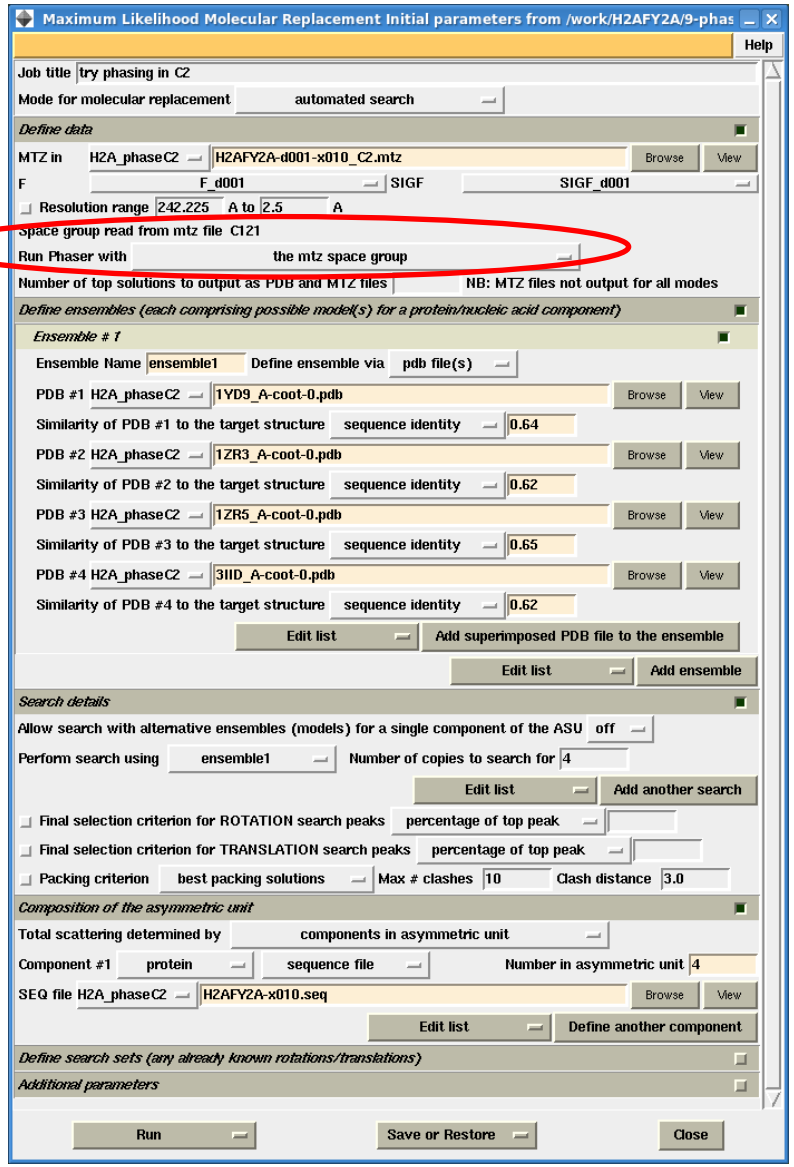
Solution written to MTZ file: /work/H2AFY2A/9-phase-C2-mv/H2A_phaseC2_4.1.mtz

Solution log-likelihood gain: 1101.92

Solution annotation (history):

RFZ=8.2 TFZ=4.9 PAK=0 LLG=89	Molecule 1
RFZ=8.2 TFZ=16.9 PAK=0 LLG=321	Molecule 2
RFZ=7.2 TFZ=19.1 PAK=0 LLG=585	Molecule 3
RFZ=7.2 TFZ=23.9 PAK=0 LLG=924	Molecule 4

LLG=1102



Summary – checking data quality

SGC

The following parameters are worth checking before setting up a phasing experiment:

- Check for R_{merge} overall and highest resolution shell
- Desired meanI/sigI in highest resolution shell >2; alter maximum resolution if necessary
- Desired completeness >95%
- Desired multiplicity >3
- Check for twinning
- Check space/point group
- Matthews coefficient → expected number of molecules to search for

Summary – checking model

The following parameters are worth checking before setting up a phasing experiment:

- Data base search to find closest homologues
- Prepare sequence alignments between model sequence and target sequence
- Delete all water molecules and ligands
- Use only one chain per homologue as search model
- Reduce molecules to a core and superpose models
- Based on sequence alignment create search models only having last common atom in side chains present or a polyAla model

Summary – Programs

SGC

The following programs have been used:

- Mosflm, XDS, HKL2000 → data processing
- Scala, Scalepack, Truncate/Ctruncate, Scalepack2MTZ → merging, scaling data and adding Rfree
- Matthews coefficient, Phenix_xtriage, Pointless, Reindex → check and edit/manipulate data
- Various data bases, Chainsaw, Coot → model preparation
- Phaser, Molrep → phasing
- Parrot, DM → density modification after phasing
- Coot, ARP/wARP, Buccaneer, Refmac, Phenix_refine, Buster_TNT, Tslanl various server and data bases→ building and refinement