

## 11. Examples of Macromolecular Refinement

The following extracts from the file *6rxn.ins* (provided together with *6rxn.hkl* as an example) illustrate a number of points. The structure was determined by Stenkamp, Sieker & Jensen, (1990) who have kindly given permission for it to be used in this way. As usual in *.ins* files, comments may be included as REM instructions or after exclamation marks. The resolution of 1.5Å does not quite justify refinement of all non-hydrogen atoms anisotropically ('ANIS' before the first atom would specify this), but the iron and sulfur atoms should be made anisotropic as shown below. Note that it would be better to flag the  $R_{\text{free}}$  reflections randomly using SHELXPRO rather than just using every twelfth reflection.

```
TITL Rubredoxin in P1 (from 6RXN in PDB)
CELL 1.54178 24.920 17.790 19.720 101.00 83.40 104.50 ! Lambda & cell
ZERR      1 0.025 0.018 0.020 0.05 0.05 0.05 ! Z & cell esds
LATT -1 ! Space group P1
SFAC C H N O S FE ! Scattering factor types and
UNIT 224 498 55 136 6 1 ! unit-cell contents

DEFS 0.02 0.2 0.01 0.05 ! Global default restraint esds

CGLS 10 -12 ! 10 Conjugate gradient cycles, every 12th reflection
SHEL 999 0.1 ! for R(free), all other data used for refinement
FMAP 2 ! Difference Fourier
PLAN 200 2.3 ! Peaksearch and identification of potential waters

LIST 6 ! Output phased reflection file to generate maps etc.
WPDB ! Write PDB output file
HTAB ! Output analysis of hydrogen bonds (requires H-atoms !)

DELU $C_* $N_* $O_* $S_* ! Rigid bond restraints - ignored for iso.

SIMU 0.1 $C_* $N_* $O_* $S_* ! Similar U restraints - iso. or anis.
! Esd should be changed to ca. 0.05 if whole structure is anis.

ISOR 0.1 O_201 > LAST ! Approximate isotropic restraints for waters;
! ignored for isotropic

ANIS_* FE SD SG ! Make iron and all sulfur atoms anisotropic

CONN 0 O_201 > LAST ! Don't include water in connectivity array and
BUMP ! generate antibumping restraints automatically

SWAT ! Diffuse water model

REM HOPE ! Anisotropic scaling not included

MERG 4 ! Remove MERG 4 if Friedel opposites should not be merged

MORE 1 ! MORE 0 for minimum, 2 or 3 for more output for diagnostics
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REM Special restraints etc. specific to this structure follow:

```
REM HFIX 43 C1_1      !
DFIX C1_1 N_1 1.329  ! O=C(H)- (formyl) on N-terminus
DFIX C1_1 O1_1 1.231 ! incorporated into residue 1
DANG N_1 O1_1 2.250  !
DANG C1_1 CA_1 2.435 !

DFIX_52 C OT1 C OT2 1.249      !
DANG_52 CA OT1 CA OT2 2.379    ! Ionized carboxyl at C-terminus
DANG_52 OT1 OT2 2.194          !

SADI_54 0.04 FE SG_6 FE SG_9 FE SG_39 FE SG_42 ! Equal but unknown Fe-
S
SADI_54 0.08 FE CB_6 FE CB_9 FE CB_39 FE CB_42 ! distances around Fe

REM HFIX 83 SG_38 SG_138 ! -SH for remaining cysteine (disordered)

DFIX C_18 N_26 1.329          ! Patch break in numbering - residues
DANG O_18 N_26 2.250          ! 18 and 26 are bonded but there is a
DANG CA_18 N_26 2.425         ! gap in numbering for compatibility
DANG C_18 CA_26 2.435         ! with other rubredoxins that have an
FLAT 0.3 O_18 CA_18 N_26 C_18 CA_26 ! extra loop
RTAB Omeg CA_18 C_18 N_26 CA_26      !
RTAB Phi C_18 N_26 CA_26 C_26        !
RTAB Psi N_18 CA_18 C_18 N_26       !

REM DFIX from CSD and R.A.Engl & R.Huber, Acta Cryst. A47 (1991) 392.
REM Remove 'REM ' before HFIX to activate H-atom generation

REM HFIX_ALA 43 N
REM HFIX_ALA 13 CA
REM HFIX_ALA 33 CB

REM HFIX_ASN 43 N
REM HFIX_ASN 13 CA
REM HFIX_ASN 23 CB
REM HFIX_ASN 93 ND2

REM HFIX_ASP 43 N
REM HFIX_ASP 13 CA
REM HFIX_ASP 23 CB

... etc ...

REM HFIX_VAL 43 N
REM HFIX_VAL 13 CA CB
REM HFIX_VAL 33 CG1 CG2

REM Peptide standard torsion angles and restraints
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RTAB\_\* Omeg CA C N\_+ CA\_+  
RTAB\_\* Phi C\_- N CA C  
RTAB\_\* Psi N CA C N\_+  
RTAB\_\* Cvol CA

DFIX\_\* 1.329 C\_- N  
DANG\_\* 2.425 CA\_- N  
DANG\_\* 2.250 O\_- N  
DANG\_\* 2.435 C\_- CA

FLAT\_\* 0.3 O\_- CA\_- N C\_- CA

REM Standard amino-acid restraints etc.

CHIV\_ALA C  
CHIV\_ALA 2.477 CA

DFIX\_ALA 1.231 C O  
DFIX\_ALA 1.525 C CA  
DFIX\_ALA 1.521 CA CB  
DFIX\_ALA 1.458 N CA  
DANG\_ALA 2.462 C N  
DANG\_ALA 2.401 O CA  
DANG\_ALA 2.503 C CB  
DANG\_ALA 2.446 CB N

RTAB\_ASN Chi N CA CB CG

CHIV\_ASN C CG  
CHIV\_ASN 2.503 CA

DFIX\_ASN 1.231 C O CG OD1  
DFIX\_ASN 1.525 C CA  
DFIX\_ASN 1.458 N CA  
DFIX\_ASN 1.530 CA CB  
DFIX\_ASN 1.516 CB CG  
DFIX\_ASN 1.328 CG ND2  
DANG\_ASN 2.401 O CA  
DANG\_ASN 2.462 C N  
DANG\_ASN 2.455 CB N  
DANG\_ASN 2.504 C CB  
DANG\_ASN 2.534 CA CG  
DANG\_ASN 2.393 CB OD1  
DANG\_ASN 2.419 CB ND2  
DANG\_ASN 2.245 OD1 ND2

RTAB\_ASP Chi N CA CB CG

CHIV\_ASP C CG

CHIV\_ASP 2.503 CA  
DFIX\_ASP 1.231 C O  
DFIX\_ASP 1.525 C CA  
DFIX\_ASP 1.530 CA CB  
DFIX\_ASP 1.516 CB CG  
DFIX\_ASP 1.458 CA N  
DFIX\_ASP 1.249 CG OD1 CG OD2  
DANG\_ASP 2.401 O CA  
DANG\_ASP 2.462 C N  
DANG\_ASP 2.455 CB N  
DANG\_ASP 2.504 C CB  
DANG\_ASP 2.534 CA CG  
DANG\_ASP 2.379 CB OD1 CB OD2  
DANG\_ASP 2.194 OD1 OD2

RTAB\_CYS Chi N CA CB SG

CHIV\_CYS C  
CHIV\_CYS 2.503 CA

DFIX\_CYS 1.231 C O  
DFIX\_CYS 1.525 C CA  
DFIX\_CYS 1.458 N CA  
DFIX\_CYS 1.530 CA CB  
DFIX\_CYS 1.808 CB SG  
DANG\_CYS 2.401 O CA  
DANG\_CYS 2.504 C CB  
DANG\_CYS 2.455 CB N  
DANG\_CYS 2.462 C N  
DANG\_CYS 2.810 CA SG

... etc ...

RTAB\_VAL Chi N CA CB CG1  
RTAB\_VAL Chi N CA CB CG2

CHIV\_VAL C  
CHIV\_VAL 2.516 CA

DFIX\_VAL 1.231 C O  
DFIX\_VAL 1.458 N CA  
DFIX\_VAL 1.525 C CA  
DFIX\_VAL 1.540 CA CB  
DFIX\_VAL 1.521 CB CG2 CB CG1  
DANG\_VAL 2.401 O CA  
DANG\_VAL 2.462 C N  
DANG\_VAL 2.497 C CB  
DANG\_VAL 2.515 CA CG1 CA CG2  
DANG\_VAL 2.479 N CB

DANG\_VAL 2.504 CG1 CG2

WGHT 0.100000  
FVAR 1.00000 0.5 0.5 0.5 0.5

RESI 1 MET  
C1 1 -0.01633 0.35547 0.44703 11.00000 0.11817  
O1 4 0.01012 0.32681 0.48491 11.00000 0.17896  
  
N 3 0.00712 0.35446 0.37983 11.00000 0.11863  
CA 1 0.05947 0.33273 0.35391 11.00000 0.06229  
CB 1 0.07411 0.33732 0.27909 11.00000 0.15678  
CG 1 0.03196 0.28864 0.22872 11.00000 0.14569  
SD 5 0.04907 0.31846 0.14359 11.00000 0.23570  
CE 1 0.11380 0.29170 0.12261 11.00000 0.21476  
C 1 0.10634 0.38738 0.39766 11.00000 0.09178  
O 4 0.10329 0.45513 0.41972 11.00000 0.16480

RESI 2 GLN  
N 3 0.14741 0.35678 0.40741 11.00000 0.08599  
CA 1 0.18940 0.39931 0.45565 11.00000 0.09291  
CB 1 0.22933 0.34643 0.45886 11.00000 0.13253  
CG 1 0.27354 0.38674 0.51173 11.00000 0.09866  
CD 1 0.24547 0.38838 0.58387 11.00000 0.05748  
OE1 4 0.22482 0.32772 0.60689 11.00000 0.16301  
NE2 3 0.24704 0.46053 0.62045 11.00000 0.10164  
C 1 0.22198 0.47895 0.43826 11.00000 0.08193  
O 4 0.25019 0.48377 0.38408 11.00000 0.10402

RESI 3 LYS  
N 3 0.21781 0.54034 0.48673 11.00000 0.07413  
CA 1 0.25088 0.62006 0.47934 11.00000 0.05181  
CB 1 0.21991 0.68311 0.51795 11.00000 0.09646  
CG 1 0.16130 0.66288 0.49255 11.00000 0.10455  
CD 1 0.12843 0.72146 0.52924 11.00000 0.22324  
CE 1 0.10532 0.70085 0.60053 11.00000 0.26354  
NZ 3 0.05943 0.74195 0.62796 11.00000 0.40338  
C 1 0.30678 0.63497 0.50917 11.00000 0.05714  
O 4 0.31462 0.59598 0.55179 11.00000 0.07986

... etc ...

RESI 12 GLU  
N 3 0.41413 1.09215 0.48246 11.00000 0.06790  
CA 1 0.37955 1.01183 0.48195 11.00000 0.05761  
PART 1  
CB 1 0.32666 1.01321 0.52971 21.00000 0.12219  
CG 1 0.29679 0.93111 0.54638 21.00000 0.15333  
CD 1 0.25357 0.93709 0.60700 21.00000 0.20272  
OE1 4 0.24346 1.00278 0.63210 21.00000 0.26315  
OE2 4 0.23012 0.87537 0.63031 21.00000 0.21375

PART	2					
CB	1	0.32549	1.01718	0.52772	-21.00000	0.12065
CG	1	0.27756	0.94582	0.50954	-21.00000	0.15928
CD	1	0.22547	0.95184	0.55635	-21.00000	0.20457
OE1	4	0.20774	0.90241	0.59575	-21.00000	0.22329
OE2	4	0.20259	1.00588	0.55325	-21.00000	0.31441
PART	0					
C	1	0.36477	0.97439	0.40859	11.00000	0.04768
O	4	0.34317	1.00861	0.37369	11.00000	0.06890

... etc ...

RESI	38	CYS				
N	3	0.77141	0.92674	0.00625	11.00000	0.10936
CA	1	0.78873	0.97402	0.07449	11.00000	0.13706
PART	1					
CB	1	0.83868	1.04271	0.05517	41.00000	0.11889
SG	5	0.89948	1.00271	0.02305	41.00000	0.18205
PART	2					
CB	1	0.84149	1.03666	0.06538	-41.00000	0.14933
SG	5	0.83686	1.10360	0.01026	-41.00000	0.17328
PART	0					
C	1	0.74143	1.01670	0.10383	11.00000	0.08401
O	4	0.70724	1.02319	0.06903	11.00000	0.10188

RESI	39	CYS				
N	3	0.74699	1.04547	0.17051	11.00000	0.08888
CA	1	0.70682	1.09027	0.20876	11.00000	0.06869
CB	1	0.72588	1.11964	0.28230	11.00000	0.04269
SG	5	0.67932	1.17560	0.33481	11.00000	0.08016
C	1	0.70922	1.16093	0.17333	11.00000	0.06208
O	4	0.75427	1.20325	0.15858	11.00000	0.07437

... etc ...

RESI	52	ALA				
N	3	0.33596	0.63469	0.69557	11.00000	0.04662
CA	1	0.30961	0.68882	0.74487	11.00000	0.08939
CB	1	0.34040	0.77357	0.74194	11.00000	0.13277
C	1	0.24852	0.67507	0.73435	11.00000	0.09032
OT1	4	0.22236	0.72170	0.77321	11.00000	0.11368
OT2	4	0.22682	0.61667	0.69191	11.00000	0.08341

RESI	54	FE				
FE	6	0.72017	1.22290	0.43784	11.00000	0.07929

REM Only the waters with high occupancies and low U's have been  
 REM retained, and all the occupancies have been reset to 1, with  
 REM a view to running the automatic water divining. Water  
 REM residue numbers have been changed to start at 201.

RESI 201	HOH					
O	4	0.13450	0.53192	0.60802	11.00000	0.13132
RESI 202	HOH					
O	4	0.84795	0.53873	0.69488	11.00000	0.15273
RESI 203	HOH					
O	4	0.27771	0.95750	0.25086	11.00000	0.11315
RESI 204	HOH					
O	4	0.37066	0.71872	0.90376	11.00000	0.10854

... etc ...

RESI 233	HOH					
O	4	0.27813	1.38725	0.25914	11.00000	0.10698

HKLF 3  
END