*REFMAC***5**

Roberto A. Steiner

Structural Biology Laboratory
University of York
United Kingdom

Aim of this talk/seminar

Enable new users to get started with *REFMAC*5

How this talk will be carried out

This talk will be mainly live.

Handouts are just a reference for home use.

GENERAL

What is *REFMAC*5?

***REFMAC*5 is a program for the refinement of macromolecular structures. It is distributed as part of the CCP4 suite** (http://www.ccp4.ac.uk/download.php http://www.ysbl.york.ac.uk/~garib/refmac/latest_refmac.html).

Some points about the program:

It is strongly based on ML and Bayesian statistics *[Murshudov, G.N. & al. (1997), Refinement of macromolecular structures by the maximum-likelihood method, Acta Cryst. D53, 240-255]*

It is highly optimised

It is easy to use (CCP4*i*)

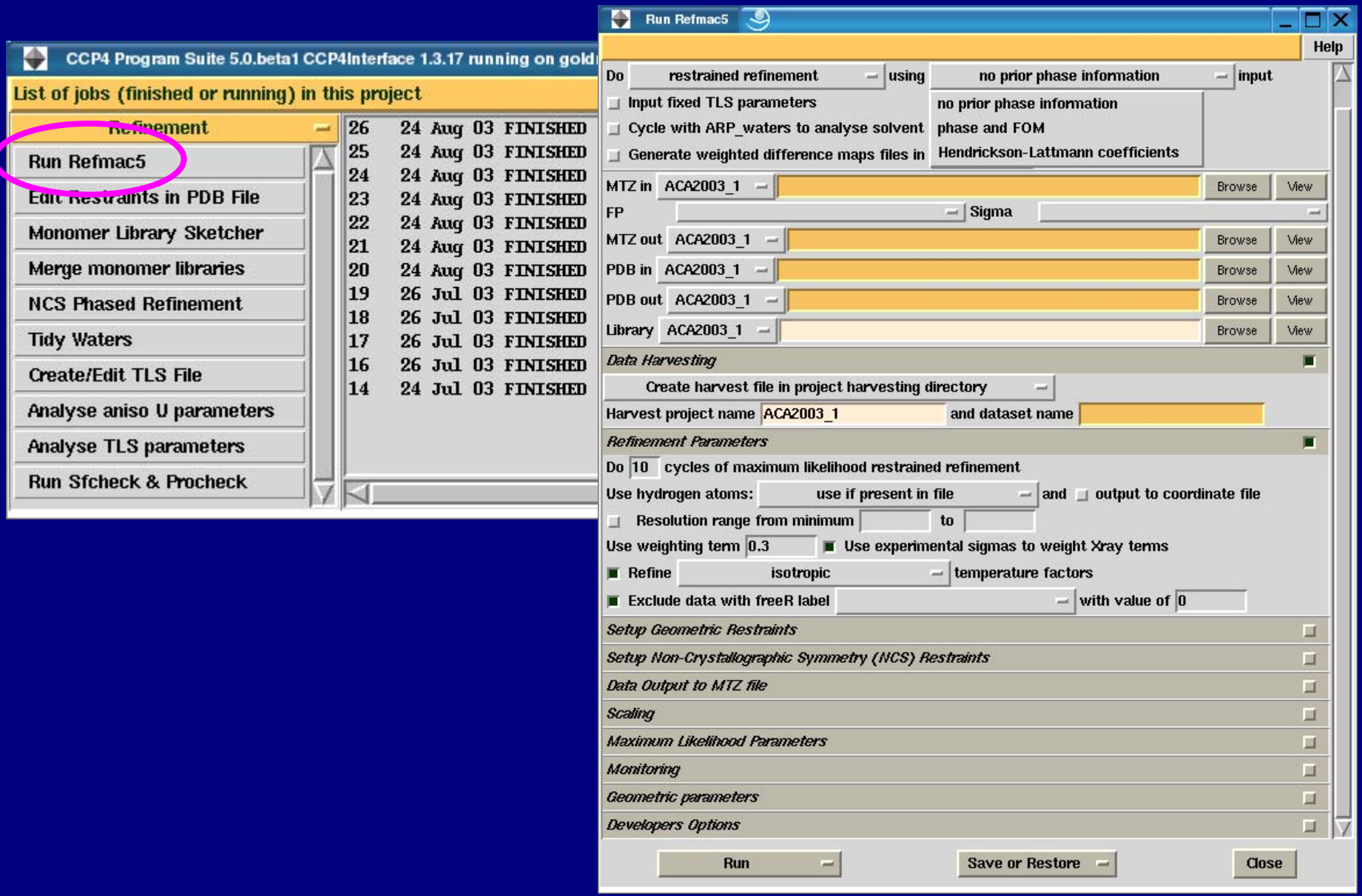
It has an extensive built-in dictionary

It allows various tasks (model idealisation, rigid-body refinement, phased and non-phased restrained and unrestrained refinement)

It allows a flexible model parameterisation (iso-,aniso-, mixed-ADPs, TLS, bulk solvent)

It exploits a good minimisation algorithm

CCP4*i*


CCP4 Program Suite 5.0.beta1 CCP4Interface 1.3.17 running on gold
List of jobs (finished or running) in this project
Refinement
Run Refmac5
Edit Restraints in PDB File
Monomer Library Sketcher
Merge monomer libraries
NCS Phased Refinement
Tidy Waters
Create/Edit TLS File
Analyse aniso U parameters
Analyse TLS parameters
Run Sfcheck & Procheck
26 24 Aug 03 FINISHED
25 24 Aug 03 FINISHED
24 24 Aug 03 FINISHED
23 24 Aug 03 FINISHED
22 24 Aug 03 FINISHED
21 24 Aug 03 FINISHED
20 24 Aug 03 FINISHED
19 26 Jul 03 FINISHED
18 26 Jul 03 FINISHED
17 26 Jul 03 FINISHED
16 26 Jul 03 FINISHED
14 24 Jul 03 FINISHED
Run Refmac5
Help
Do restrained refinement using no prior phase information input
Input fixed TLS parameters
Cycle with ARP_waters to analyse solvent
Generate weighted difference maps files in
no prior phase information
phase and FOM
Hendrickson-Lattmann coefficients
MTZ in ACA2003_1 Browse View
FP Sigma
MTZ out ACA2003_1 Browse View
PDB in ACA2003_1 Browse View
PDB out ACA2003_1 Browse View
Library ACA2003_1 Browse View
Data Harvesting
Create harvest file in project harvesting directory
Harvest project name ACA2003_1 and dataset name
Refinement Parameters
Do 10 cycles of maximum likelihood restrained refinement
Use hydrogen atoms: use if present in file and output to coordinate file
Resolution range from minimum to
Use weighting term 0.3 Use experimental sigmas to weight Xray terms
Refine isotropic temperature factors
Exclude data with freeR label with value of 0
Setup Geometric Restraints
Setup Non-Crystallographic Symmetry (NCS) Restraints
Data Output to MTZ file
Scaling
Maximum Likelihood Parameters
Monitoring
Geometric parameters
Developers Options
Run
Save or Restore
Close

Crystallographic refinement

Given

Set experimental values $\{|F|_{\underline{h}o}, \sigma_{\underline{h}o}\}$

Theoretical model M $(x_1, y_1, z_1, B_1, \ldots)$

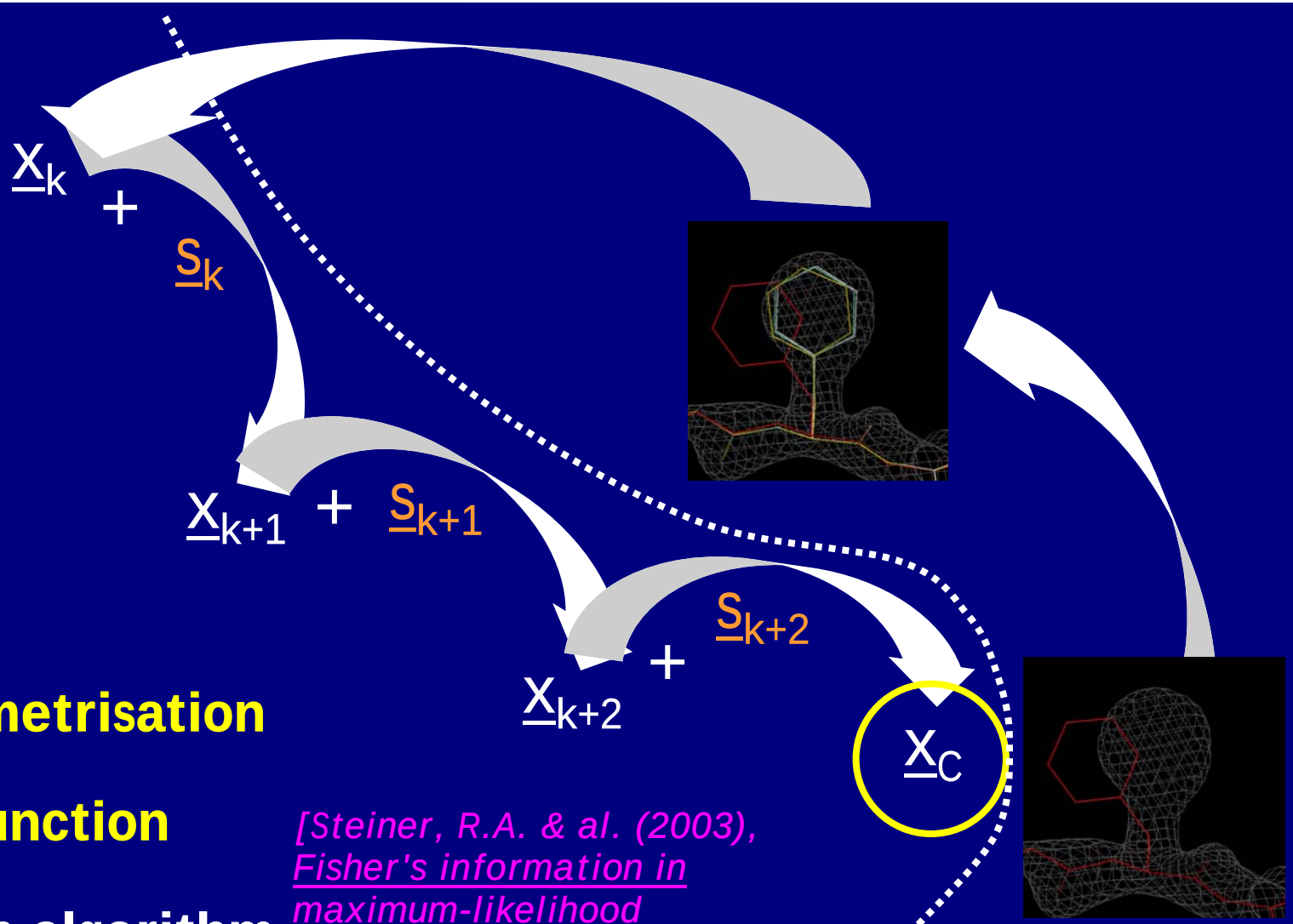
Initial values $\{x_{1I}, y_{1I}, z_{1I}, B_{1I}, \ldots\} \equiv \underline{x}_I \xrightarrow{P} F_{\underline{h}cI}$

Find

$\{x_{1B}, y_{1B}, z_{1B}, B_{1B}, \ldots\} \equiv \underline{x}_B \xrightarrow{P} F_{\underline{h}cB}$ which give the best fit to the data

The accuracy of $\{x_{1B}, y_{1B}, z_{1B}, B_{1B}, \ldots\} \equiv \underline{x}_B$

Model fitting



Model parametrisation

Objective function

Minimisation algorithm

Prior knowledge

[Steiner, R.A. & al. (2003), Fisher's information in maximum-likelihood macromolecular crystallographic refinement, Acta Cryst. D59, 2114-2124]

Objective function and Bayesian approach

$$f = \sum_{\underline{h}} W(\underline{h})(|F_o| - |F_c|)^2 +$$

Least-squares crystallographic function

$$\sum_{b} W(b)(Q_o - Q_c)^2$$

Least-squares restraints function

The best model is the one which has the highest probability given a set of observations and a certain prior knowledge.

Bayes' theorem

$$P(M;O) = P(M)P(O;M)/P(O)$$

Maximum likelihood residual (posterior)

$$P(M;O) = P(M)\frac{P(O;M)}{P(O)} = P(M)L(O;M)$$
$$L(O;M)$$

$$\max P(M;O) \Leftrightarrow \min -\log P(M;O) =$$
$$\min [-\log P(M) - \log L(O;M)]$$

[Bricogne, G. & al. (1997), Methods in Enzymology. 276]
[Murshudov, G.N. & al. (1997), Refinement of macromolecular structures by the maximum-likelihood method, Acta Cryst. D53, 240-255]

DICTIONARY

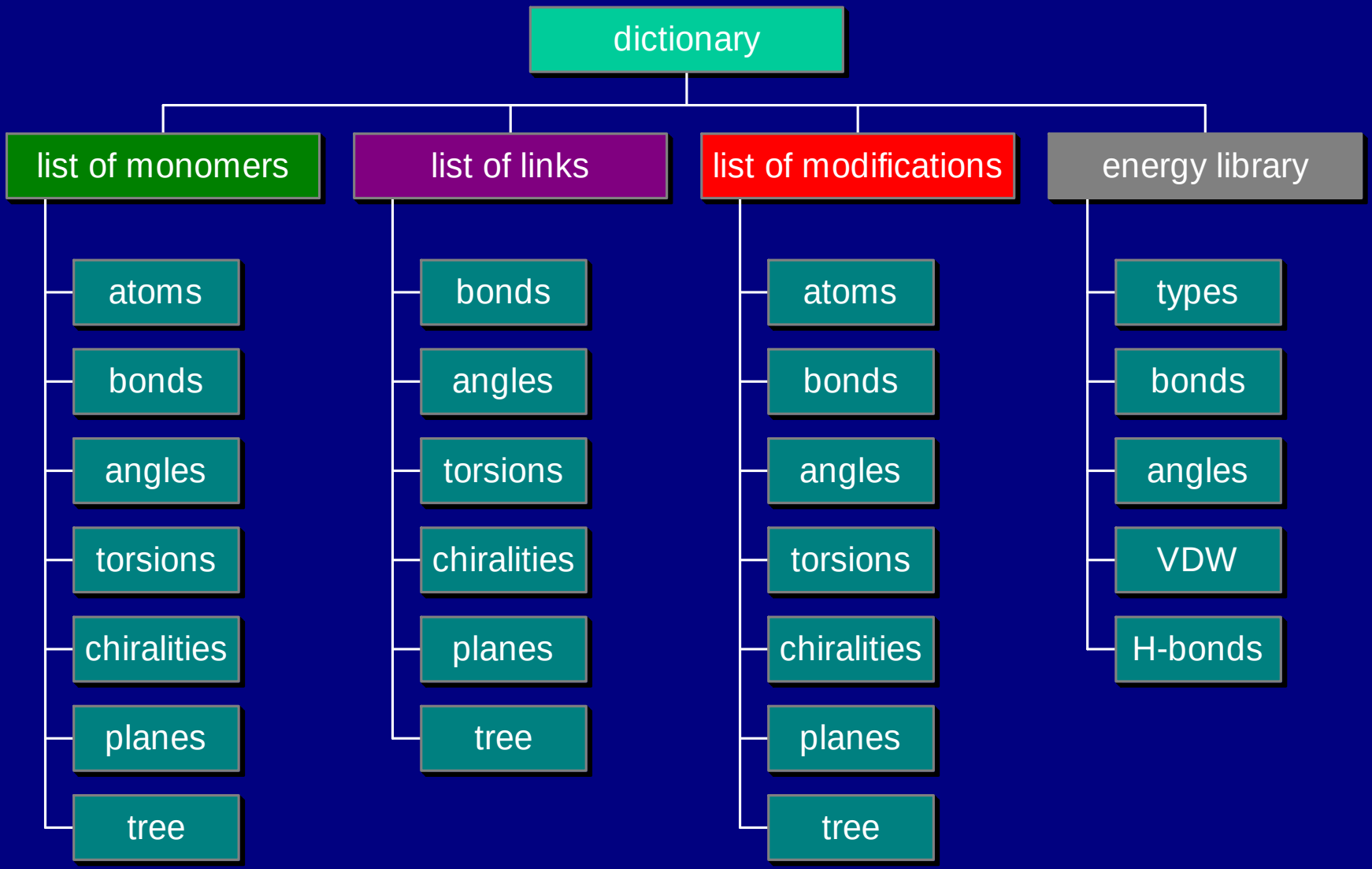
Dictionary

The use of prior knowledge requires its organised storage.

$CCP4/html/mon_lib.html

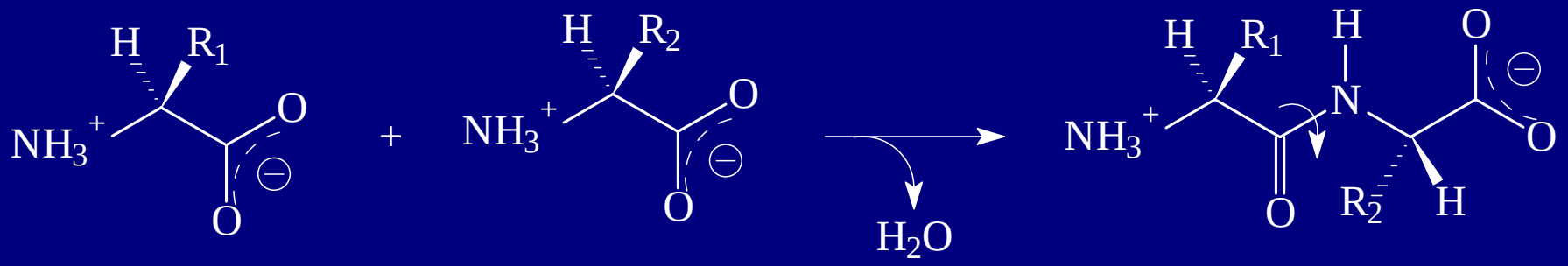
http://www.ysbl.york.ac.uk/~alexei/dictionary.html

Organization of dictionary

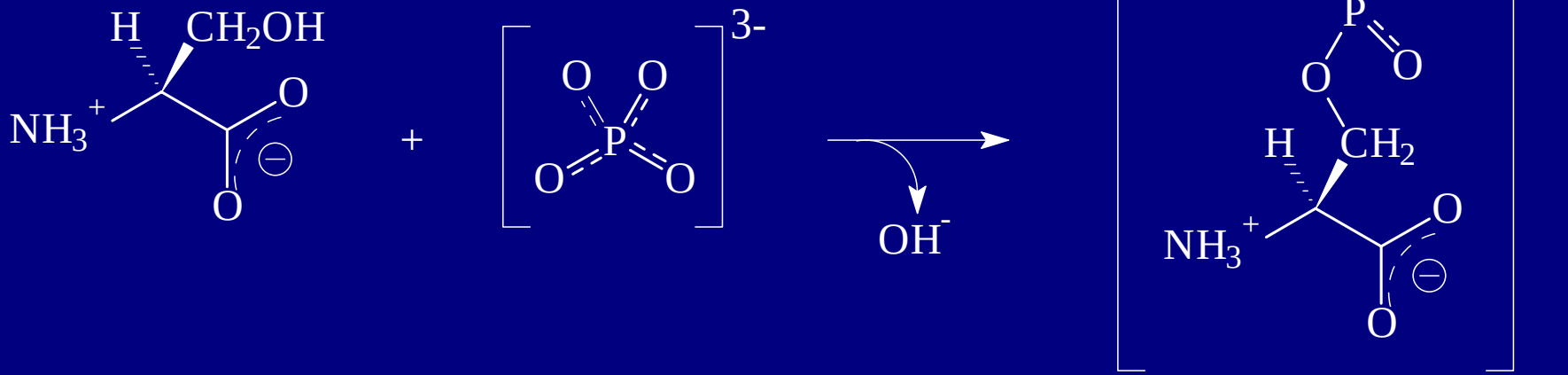


Links and Modifications

LINK



MODIFICATION



Monomer library

$CCP4/lib/data/monomers/

ener_lib.cif	**definition of atom types**
mon_lib_list.html	**info**
0/,1/,…a/,b/,…	**definition of various monomers**

Description of monomers

In the files:

a/A##.cif

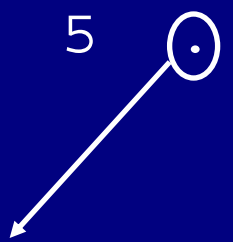
Monomers are described by the following catagories:

```
_chem_comp
_chem_comp_atom
_chem_comp_bond
_chem_comp_angle
_chem_comp_tor
_chem_comp_chir
_chem_comp_plane_atom
```

Monomer library (_chem_comp)

```
loop_
_chem_comp.id
_chem_comp.three_letter_code
_chem_comp.name
_chem_comp.group
_chem_comp.number_atoms_all
_chem_comp.number_atoms_nh
_chem_comp.desc_level

ALA   ALA    'ALANINE '   L-peptide     10   5   .
```



Level of description
. = COMPLETE
M = MINIMAL

Monomer library (_chem_comp_atom)

```
loop_
_chem_comp_atom.comp_id
_chem_comp_atom.atom_id
_chem_comp_atom.type_symbol
_chem_comp_atom.type_energy
_chem_comp_atom.partial_charge
 ALA       N     N     NH1        -0.204
 ALA       H     H     HNH1        0.204
 ALA       CA    C     CH1         0.058
 ALA       HA    H     HCH1        0.046
 ALA       CB    C     CH3        -0.120
 ALA       HB1   H     HCH3        0.040
 ALA       HB2   H     HCH3        0.040
 ALA       HB3   H     HCH3        0.040
 ALA       C     C     C           0.318
 ALA       O     O     O          -0.422
```

Monomer library (_chem_comp_bond)

```
loop_
_chem_comp_bond.comp_id
_chem_comp_bond.atom_id_1
_chem_comp_bond.atom_id_2
_chem_comp_bond.type
_chem_comp_bond.value_dist
_chem_comp_bond.value_dist_esd
 ALA      N    H      single    0.860    0.020
 ALA      N    CA     single    1.458    0.019
 ALA      CA   HA     single    0.980    0.020
 ALA      CA   CB     single    1.521    0.033
 ALA      CB   HB1    single    0.960    0.020
 ALA      CB   HB2    single    0.960    0.020
 ALA      CB   HB3    single    0.960    0.020
 ALA      CA   C      single    1.525    0.021
 ALA      C    O      double    1.231    0.020
```

Monomer library (_chem_comp_chir)

```
loop_
_chem_comp_chir.comp_id
_chem_comp_chir.id
_chem_comp_chir.atom_id_centre
_chem_comp_chir.atom_id_1
_chem_comp_chir.atom_id_2
_chem_comp_chir.atom_id_3
_chem_comp_chir.volume_sign
ALA  chir_01  CA  N  CB  C    negativ
```

positiv, negativ, both, anomer

What happens when you run REFMAC5

You have a monomer for which there is a complete description

the program carries on and takes everything from the dictionary. Currently, there are about 1000 ligands with a complete description in the *REFMAC*5 library. Cis-peptides, S-S bridges, sugar-, DNA-, RNA-links are automatically recognised.

You have a monomer for which there is only a minimal description or no description

No description or minimal description

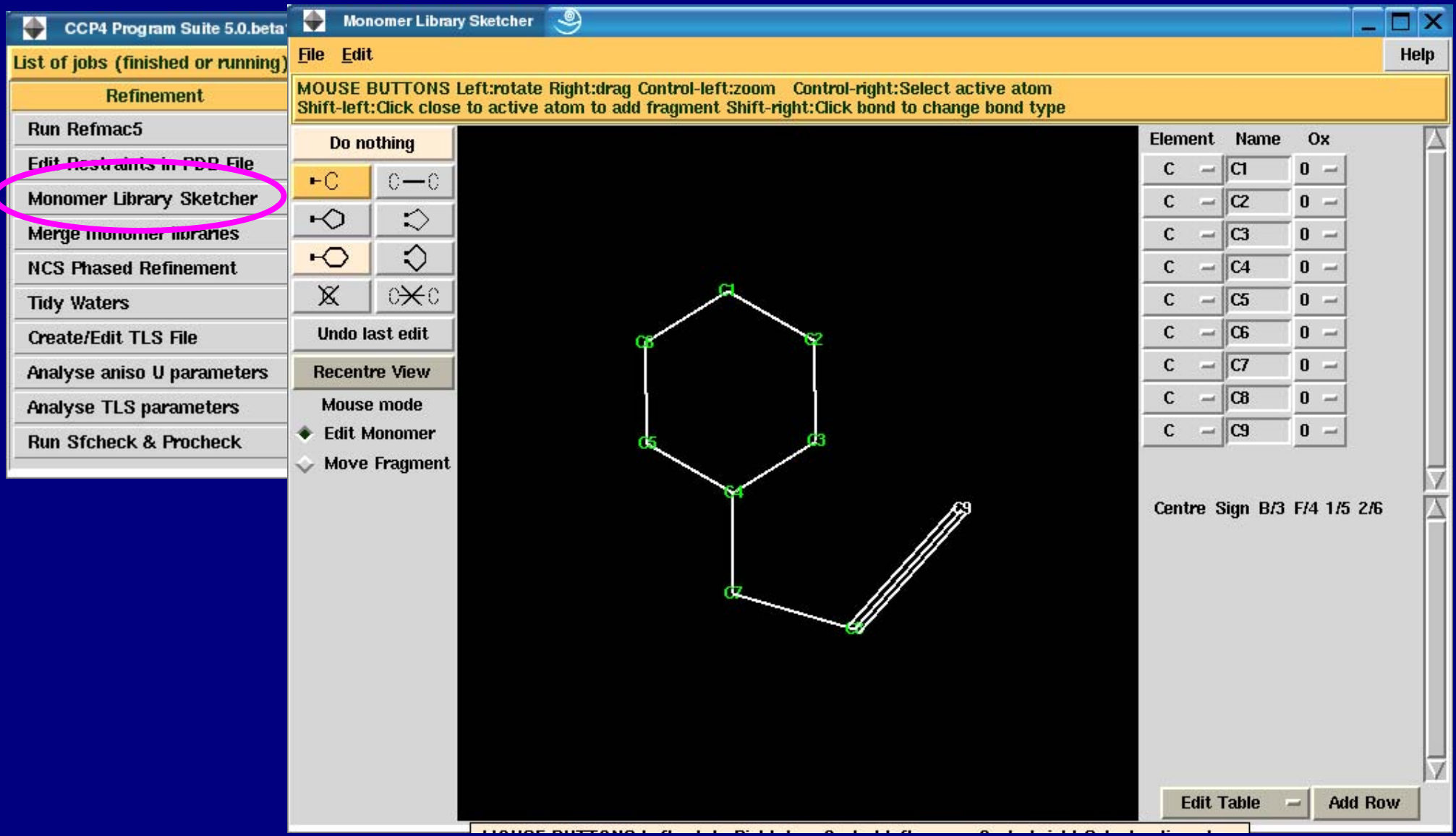
In the case you have monomer(s) in your coordinate file for which there is no description (or minimal description) *REFMAC***5 generates for you a complete library description (monomer.cif) and then it stops so you can check the result.**

If you are satisfied you can use monomer.cif for refinement. The description generated in this way is good only if your coordinates are good (CSD, EBI, any program that can do energy minimization).

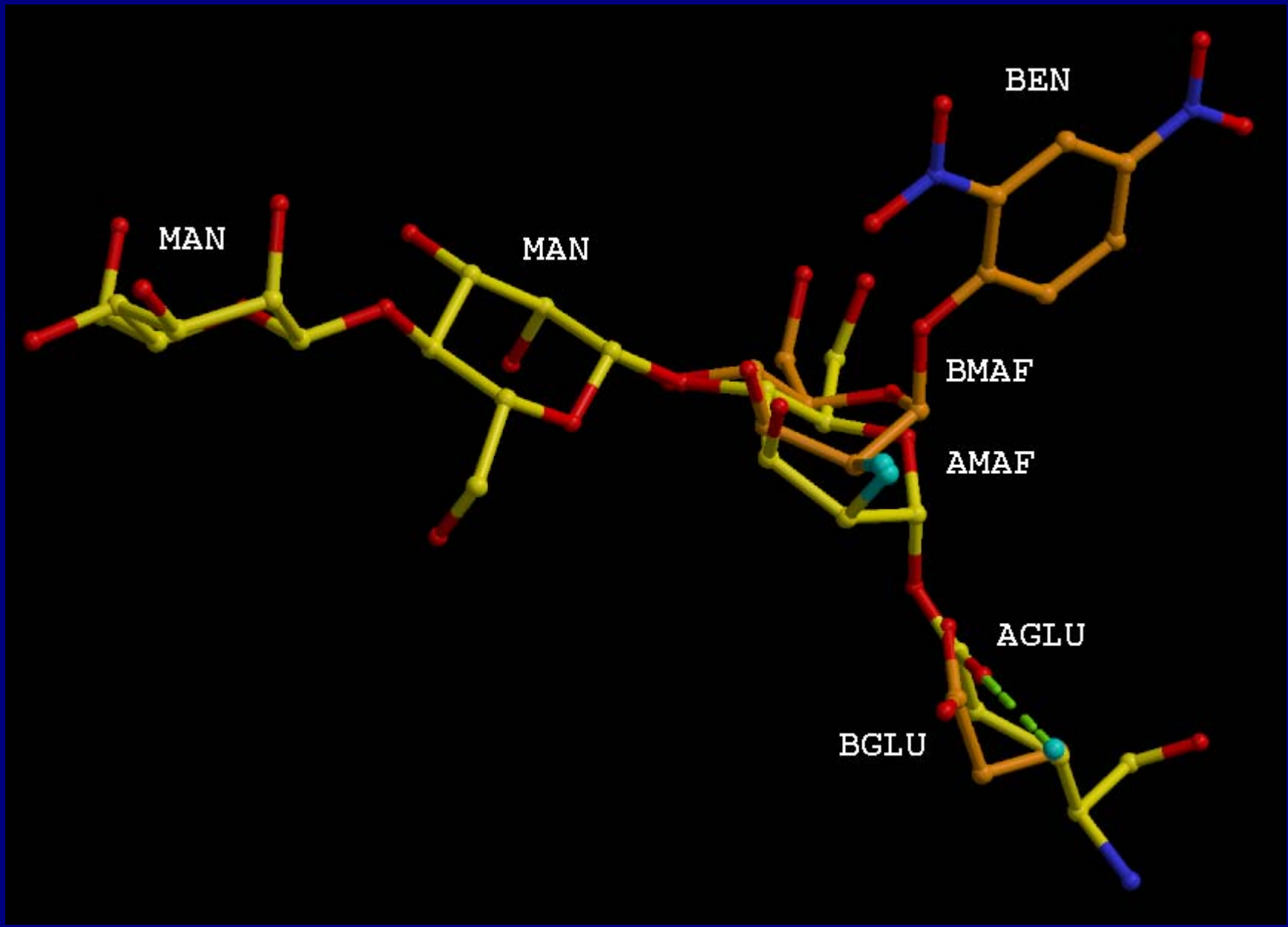
A more general approach for description generation requires the use of the graphical program SKETCHER from CCP4*i*. SKETCHER is a graphical interface to LIBCHECK which creates new monomer library descriptions
http://www.ysbl.york.ac.uk/~alexei/libcheck.html

Alternatively, you can use the PRODRG2 server
http://davapc1.bioch.dundee.ac.uk/programs/prodrg/prodrg.html

SKETCHER


CCP4 Program Suite 5.0.beta
List of jobs (finished or running)
Refinement
Run Refmac5
Monomer Library Sketcher
NCS Phased Refinement
Tidy Waters
Create/Edit TLS File
Analyse aniso U parameters
Analyse TLS parameters
Run Sfcheck & Procheck
Monomer Library Sketcher
File
Edit
Help
MOUSE BUTTONS Left:rotate Right:drag Control-left:zoom Control-right:Select active atom
Shift-left:Click close to active atom to add fragment Shift-right:Click bond to change bond type
Do nothing
Undo last edit
Recentre View
Mouse mode
Edit Monomer
Move Fragment
Element
Name
Ox
C1
C2
C3
C4
C5
C6
C7
C8
C9
Centre Sign B/3 F/4 1/5 2/6
Edit Table
Add Row

*REFMAC*5 can handle complex descriptions



Links and Modifications in practice

```
0         1         2         3         4         5         6         7
12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789
LINK         C6   BBEN B    1                 O1   BMAF S    2                  BEN-MAF
LINK         OE2  GLU A   67          1.895   ZN     ZN R    5                  GLU-ZN

LINK               GLY H 127                    GLY H 133                      gap

LINK               MAF S    2                   MAN S    3                     BETA1-4

SSBOND    1 CYS A  298     CYS A  298                          4555

MODRES      MAN S      3   MAN-b-D                                              RENAME
```

TLS

TLS

ADPs are an important component of a macromolecule
Proper parameterisation
Biological significance

Displacements are likely anisotropic, but rarely we have the luxury of refinining individual aniso-*U*. Instead iso-*U* are used.

TLS parameterisation allows an intermediate description
T = translation
L = libration
S = screw-motion

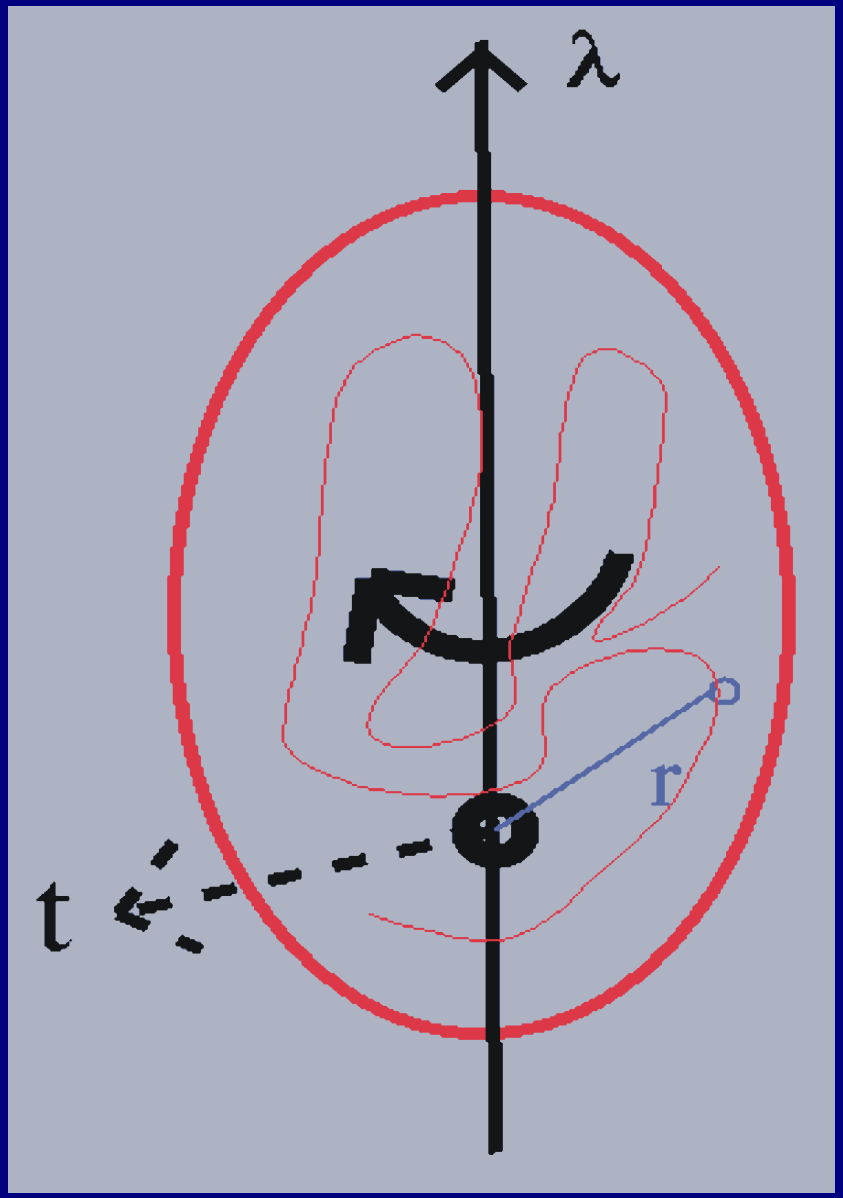
[Schomaker & Trueblood (1968) On the rigid-body motion of molecules in crystals Acta Cryst. B24, 63-76]
[Winn & al. (2001) Use of TLS parameters to model anisotropic displacements in macromolecular refinement Acta Cryst. D57, 122-133]

Decomposition of ADPs

$$U = U_{cryst} + U_{TLS} + U_{int} + U_{atom}$$

U_{cryst} : overall anisotropy of the crystal
U_{TLS} : TLS motions of pseudo-rigid bodies
U_{int} : collective torsional librations or internal normal modes
U_{atom} : individual atomic motions

Rigid-body motion



General displacement of a rigid-body point can be described as a rotation along an axis passing through a fixed point together with a translation of that fixed point.

$$\underline{u} = \underline{t} + D\underline{r}$$

for small librations

$$\underline{u} \approx \underline{t} + \underline{\lambda} \times \underline{r}$$

D = rotation matrix

$\underline{\lambda}$ = vector along the rotation axis of magnitude equal to the angle of rotation

TLS parameters

Dyad product:

$$\underline{uu}^T = \underline{tt}^T + \underline{t\lambda}^T \times \underline{r}^T - \underline{r} \times \underline{\lambda t}^T - \underline{r} \times \underline{\lambda\lambda}^T \times \underline{r}^T$$

ADPs are the time and space average

$$U_{TLS} = \langle uu^T \rangle = T + S^T \times \underline{r}^T - \underline{r} \times S - \underline{r} \times L \times \underline{r}^T$$

$T = \langle \underline{tt}^T \rangle$ **6 parameters, TRANSLATION**

$L = \langle \underline{\lambda\lambda}^T \rangle$ **6 parameters, LIBRATION**

$S = \langle \underline{\lambda t}^T \rangle$ **8 parameters, SCREW-ROTATION**

Use of TLS

analysis: **given inidividual aniso-ADPs fit TLS parameters**

[Harata, K. & Kanai, R., (2002) Crystallographic dissection of the thermal motion of protein-sugar complex, Proteins, 48, 53-62]

[Wilson, M.A. & Brunger, A.T.., (2000) The 1.0 Å crystal structure of Ca(2+)-bound calmodulin: an analysis of disorder and implications for functionally relevant plasticity, J. Mol. Biol. 301, 1237-1256]

[Harata, K. et al., (1999) Crystallographic evaluation of internal motion of human α-lactalbumin refined by full-matrix least-squares method, J. Mol. Biol., 26, 347-358]

refinement: **TLS as refinement parameters**

[Winn et al., (2003) Macromolecular TLS refinement in REFMAC at moderate resolutions Methods Enzymol., 374, 300-321]

[Papiz, M.Z. et al., (2003) The strcuture and thermal motion of the B800-850 LH2 complex from .....J. Mol. Biol.., 326, 1523-1538]

[Howlin et al., (1989) Segmented anisotropic refinement of bovine ribonuclease A by the application of the rigid-body TLS model, Acta Cryst., A45, 851-861]

Choice of TLS groups and resolution

Choice chains, domains, secondary structure, elements,…

Resolution not a big problem. There are only 20 more parameters per TLS group

Thioredoxin reductase 3.0 Å *[Sandalova, T. & al., (2001) 3D-structure of a mammalian thioredoxin reductase: implications for mechanism and evolution of a selenocysteine-dependent enzyme, PNAS., 98, 9533-9538]*

6 TLS groups (1 for each of 6 monomers in asu)

Example GAPDH

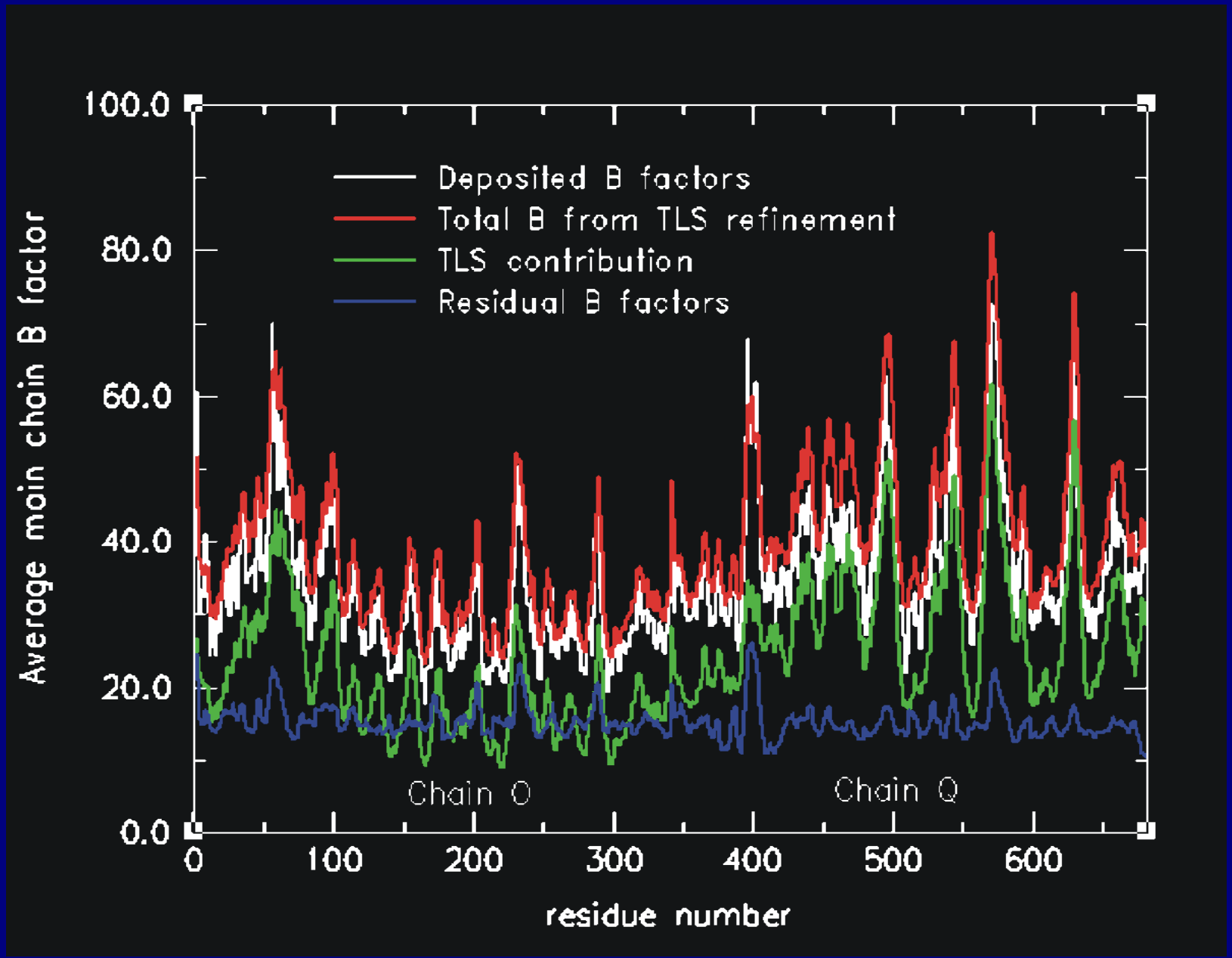
Glyceraldehyde-3-phosphate dehydrogenase from *Sulfolobus solfataricus* *[Isupov, M. & al. (1999), Crystal structure of the glyceraldehyde-3-phosphate dehydrogenase from Sulfolobus solfataricus, J. Mol. Biol., 291, 651-660].*

340 amino acids
2 molecules in asymmetric unit (O and Q)
each molecule has a NAD-binding and a catalytic domain
$P4_12_12$, data to 2.05Å

GAPDH before and after TLS

TLS	R	R_{free}
0	22.9	29.5
1	21.4	25.9
4	21.1	25.8

Contributions to equivalent isotropic *B*s

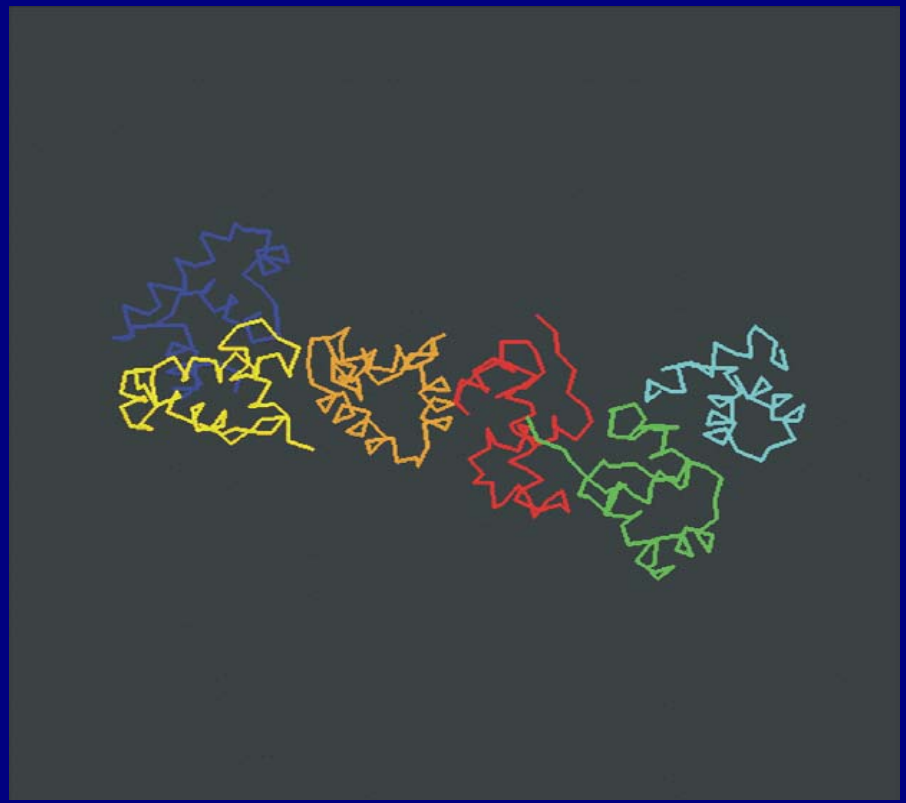


[Howlin, B. & al. (1993) TLSANL: TLS parameter-analysis program for segmented anisotropic refinement of macromolecular structures, J. Appl. Cryst. 26, 622-624]

Example GerE

Transcription regulator from *B. subtilis* *[Ducros, V.M. et al., (2001) Crystal structure of GerE, the ultimate transcriptional regulator of spore formation in Bacillus subtilis, J. Mol. Biol., 306, 759-771]*

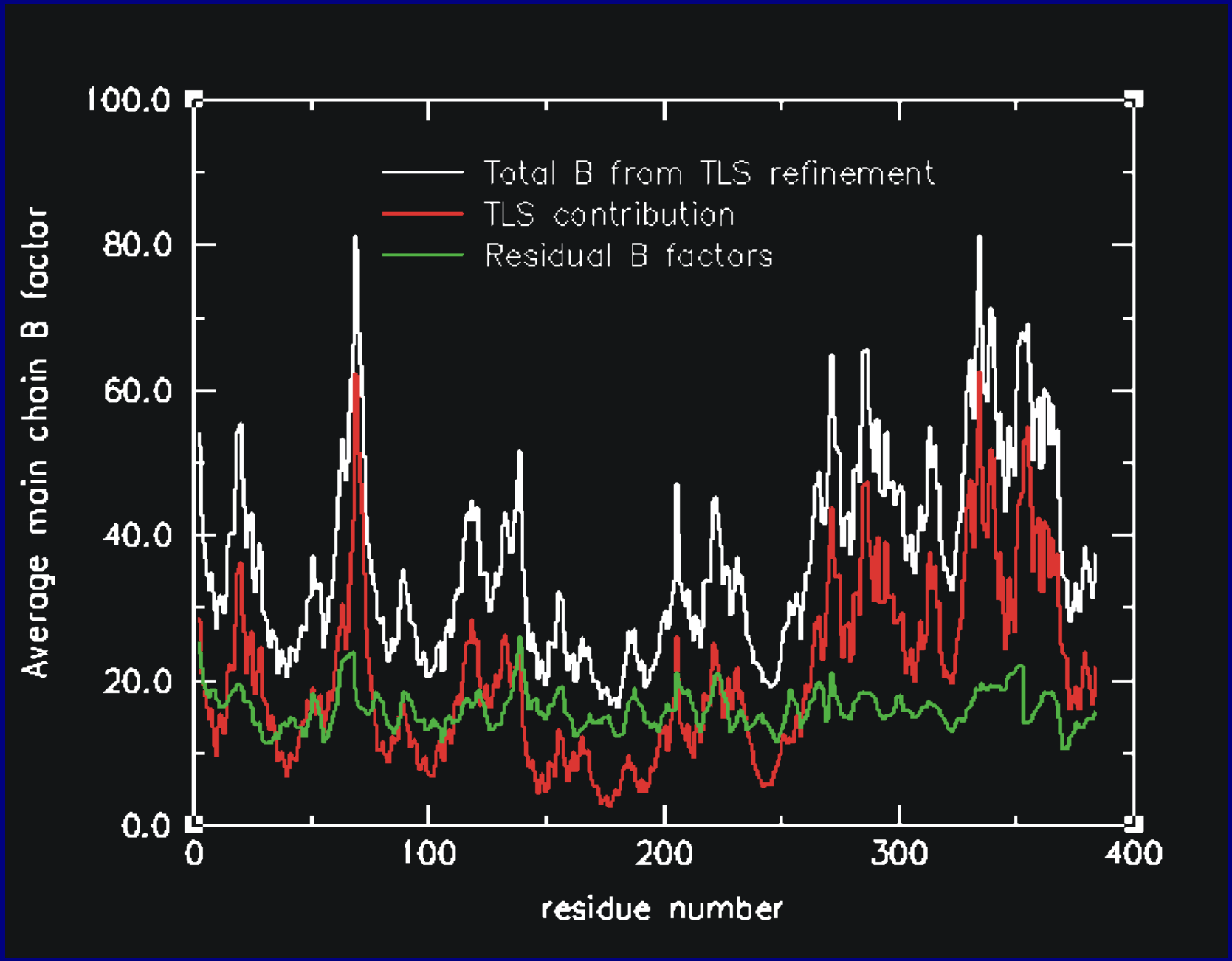
74 amino acids
6 chains A-F in asu
***C*2, data to 2.05Å**



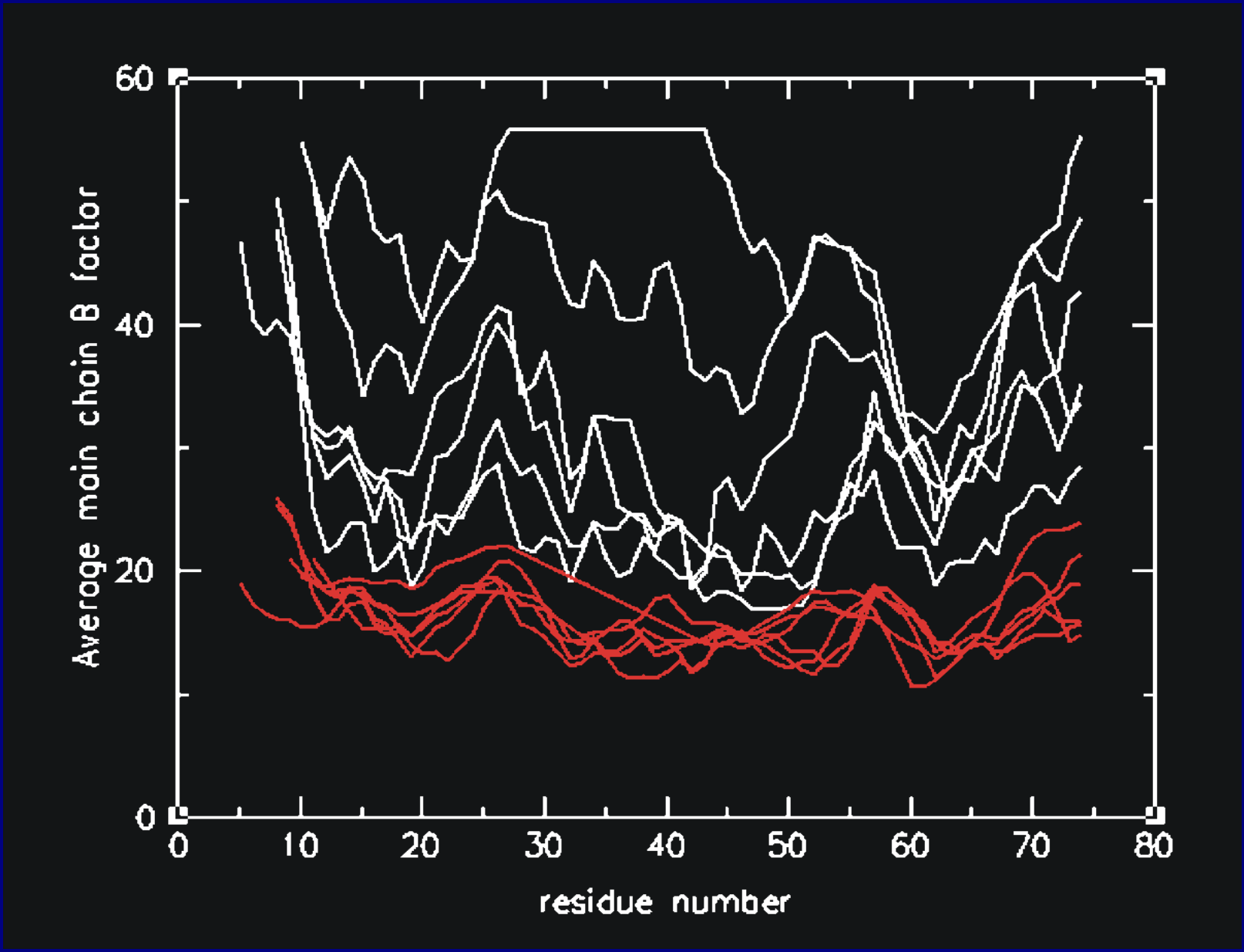
Refinement GerE

Model	TLS	NCS	R	R_{free}	cc_B
1	0	No	21.9	29.3	0.519
2	0	Yes	22.5	30.0	0.553
3	6	No	21.3	27.1	0.510
4	6	Yes	21.4	27.2	0.816

Contribution to equivalent isotropic *B*s



*B*s from NCS related chains



Summary TLS

TLS parameterisation allows to partly take into account anisotropic motions at modest resolution (> 3.5 Å)

TLS refinement might improve refinement statistics of several percent

TLS refinement in *REFMAC*5 is fast and therefore can be used routinely

Future

Routine determination of standard uncertainties

Refinement against intensities

Refinement using anomalous data

Bayesian refinement of twinned data

People

Garib N. Murshudov
Roberto A. Steiner
Alexei Vagin
Andey A. Lebedev
Fei Long
Martyn Winn
Liz Potterton
David Anderson
Dan Zhou

Financial support

The Wellcome Trust

CCP4

bbsrc
biotechnology and biological sciences research council