1. What is TLS?
2. TLS refinement in Refmac5
3. Outputs of TLS refinement
4. More on choice of TLS groups
Displacements of atoms in the crystal

- Experiment measures time- and space-averaged structure
- Atoms have thermal motion and static disorder
- In addition to mean atomic positions, mean square atomic displacements from mean position (static and dynamic) are an important part of the model of a protein.
Most probable locations represented by thermal ellipsoids

anisotropic

$$\Rightarrow U = \begin{pmatrix} 0.3252 & 0.0373 & 0.0214 \\ 0.0373 & 0.4834 & 0.0618 \\ 0.0214 & 0.0618 & 0.2816 \end{pmatrix}$$

ANISOU 7 SD MET 1 3252 4834 2816 373 214 618

isotropic

$$\Rightarrow B = 8\pi^2 \frac{(0.3252 + 0.4834 + 0.2816)}{3}$$

ATOM 7 SD MET 1 23.171 26.299 8.707 1.00 28.69
TLS refinement: Aims

- Atomic displacements are likely anisotropic, but rarely have luxury of refining individual anisotropic $U$s. Instead have to use isotropic $B$s.

- TLS parameterisation allows an intermediate description

\[
\begin{align*}
T &= \text{translation} \\
L &= \text{libration} \\
S &= \text{screw-motion}
\end{align*}
\]

*anisotropy without many parameters*!!
Rigid body model

Atoms $r_1$, $r_2$ ... belong to rigid bodies.
Motion of atoms *partly* due to motion of rigid bodies.
Rigid body motion

General displacement of atom (position \( r \) w.r.t. origin O) in rigid body:

\[ u = t + D \cdot r \]

For small libration \( \lambda \):

\[ u \approx t + \lambda \times r \]
TLS parameters

• Corresponding dyad:
  \[ uu = tt + t\lambda \times r - r \times \lambda t - r \times \lambda \lambda \times r \]

• Average over dynamic motion and static disorder \(\Rightarrow\) anisotropic displacement parameter (ADP):
  \[ U_{TLS} \equiv \langle uu \rangle = T + S^T \times r - r \times S - r \times L \times r \]

• \(T, L\) and \(S\) describe mean square translation and libration of rigid body and their correlation.

• \(T \Rightarrow 6\) parameters, \(L \Rightarrow 6\) parameters, \(S \Rightarrow 8\) parameters (trace of \(S\) is undetermined)

N.B. rigid body model \(\Rightarrow\) more general motion (bananas).

Here we look at implied ADPs.
Rigid body motion (TLS) $\Leftrightarrow$ Atomic motion (U)

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

• Given refined atomic U’s, fit TLS parameters
  - analysis

• Use TLS as refinement parameters

  TLS $\Rightarrow$ U’s $\Rightarrow$ structure factor
  - refinement
TLS in refinement

- TLS parameters are contribution to displacement parameters of model
- Can specify 1 or more TLS groups to describe contents of asymmetric unit (or part thereof)
- $6 + 6 + 8 = 20$ parameters per group (irrespective of number of atoms in the group)

- Number of extra refinement parameters depends on how many groups used!
At what resolution can I use TLS?

Any! Resolution only affects level of detail:

- Resolution < **1.2 Å** - full anisotropic refinement
- Resolution ~ **1.5 Å** - marginal for full anisotropic refinement. But can do detailed TLS, e.g. Howlin *et al*, Ribonuclease A, **1.45 Å**, 45 side chain groups; Harris *et al*, papain, **1.6Å**, 69 side chain groups.
- Resolution **1.5 Å** - **2.5 Å** ⇒ model molecules/domains rather than side chains.
- T.Sandalova *et al* - thioredoxin reductase at **3.0 Å** - TLS group for each of 6 monomers in asu

In fact, rigid-body assumption works better at low resolution.
Gert Vriend’s group recent refinement of 17000 structures – TLS helped in all but a handful of cases.
Implementation in REFMAC

Stage 1:
- refine scaling parameters + TLS parameters
- other parameters fixed

Stage 2:
- traditional restrained refinement
- TLS parameters fixed
- B factor refinement refines “residual” B factors.
Parameter choices

**TLS groups:**
- Refmac defaults to 1 TLS group / protein chain
- Group includes waters in contact with chain
- *Or* can explicitly define TLS groups via TLSIN file
  - see Create / Edit TLS File task

**TLS parameters:**
- initialised to zero
- can use previous values (TLSOUT → TLSIN) if model has not changed too much (but easy to start from zero)

**B factors:**
- set B values to constant value (precise value irrelevant) - allows TLS parameters to describe coarse-grained features
- can keep residual Bs from earlier cycle
Re-running Refmac

1. If major re-building or changes in model - start again from zero TLS parameters. Ensures realistic set of TLS parameters.

2. If minor re-building - TLSIN is TLSOUT from previous cycle

3. Can input fixed TLS parameters, and do restrained refinement only.
What to look for in output

- Usual refinement statistics.
- Check R_free and TLS parameters in log file for convergence.
- Check TLS parameters to see if any dominant displacements.
- Consider alternative choices of TLS groups

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Making sense of all those $B$ values

- TLS parameters $\rightarrow U_{TLS}$ for atoms in group
- $U_{TLS} \rightarrow B_{TLS}$ “equivalent isotropic $B$” (loses information on anisotropy)
  $B_{TLS}$ describes overall displacements of molecules or domains
- Also individually refined $B_{\text{residual}}$
  describes local displacements, and expected to be similar between molecules

$$B_{TOT} = B_{TLS} + B_{\text{res}}$$

But this ignores the anisotropy inherent in the TLS model !!
What does Refmac give you?

- **TLSOUT** containing refined TLS parameters
- **XYZOUT** containing:
  - TLS parameters in the header (REMARK 3)
  - $B_{\text{residual}}$ values in the ATOM lines

- Next version of Refmac will give:
  - TLSU cards containing $U_{\text{TLS}}$
  - ATOM containing $B_{\text{TOT}}$
Running TLSANL

Input (output from Refmac):
XYZIN: output coordinates from Refmac with residual B factors
TLSSIN: output TLS parameters from refmac

Output:
XYZOUT: ANISOU records including TLS and/or residual B contributions (ISOOUT keyword)
ATOM records containing TLS and/or residual B contributions (ISOOUT keyword)
AXES: file of principal axes in mmCIF format (for ccp4mg) or in molscript format

GAPDH: dimer as 1 TLS group

perpendicular to 2-fold

along 2-fold

orange = reduced translation, cyan = non-intersecting screw
11 a.a. (8% of TLS group)

30% probability level

Produced with Raster3D
GAPDH - TLS-derived aniso-U ellipsoids

Chain O
(2 groups)

Chain Q
(2 groups)

Figure produced by ccp4mg
GAPDH: $B_{TLS}$ and $B_{res}$
GAPDH: $B_{res}$ and NCS
Example - mannitol dehydrogenase

Hörer et al., J. Biol. Chem. 276, 27555 (2001)
1.5 Å data
3 tetramers in a.s.u.
TLS refinement with 1 group per monomer
Free-R 23.6% → 20.9%

<table>
<thead>
<tr>
<th>Tetramer</th>
<th>B’s before TLS</th>
<th>B’s after TLS</th>
<th>crystal contacts</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCD</td>
<td>27.1</td>
<td>13.3</td>
<td>38</td>
</tr>
<tr>
<td>EFGH</td>
<td>18.0</td>
<td>13.3</td>
<td>50</td>
</tr>
<tr>
<td>IJKL</td>
<td>18.6</td>
<td>13.3</td>
<td>49</td>
</tr>
</tbody>
</table>
Choice of TLS groups

- Refmac5 now defaults to one TLS group per subunit / segment

- Chemical knowledge, e.g. aromatic side groups of amino acids, secondary structure elements, domains, molecules

- Dynamic domains identified from multiple configurations, e.g. more than one crystal form (DYNDOM, ESCET), or from molecular dynamics simulations.


Choice of TLS groups (cont.)

- Best fit of TLS to ADPs of test structure, or rigid-body criterion applied to ADPs. Both implemented in CCP4 program ANISOANL.

- Fit TLS groups to refined isotropic B factors (or ADPs). TLSMD server finds best single group, then best split into 2 groups, etc.
TLS Motion Determination (TLSMD)

http://skuld.bmsc.washington.edu/~tlsmd

1. Partitions the protein chains into multiple segments that are modeled as rigid bodies undergoing TLS motion.
2. Generates all possible partitions up to a specified maximum number of TLS groups.
3. Each trial partition is scored by how well it predicts the observed Bs or Us

• Submit job to server.
• Returns statistics on different partitions
• Also XYZIN and TLSIN for Refmac5
• Animate Screw Displacement with JMol
TLS Motion Determination (TLSMD)

Fit to observed B factors

Fit improves as add in more groups

Choice of residue ranges for 3 groups
TLS Motion Determination (TLSMD)

calmodulin (1exr) 1.0Å

GAPDH (1b7g) 2.05Å

N.B. 3 groups combines red and pink into one

TLS groups 2-70, 71-90, 91-147

NAD-binding domain 1-137 & 303-340
catalytic domain 138-302
Large example - light harvesting complex

Complex is nonamer. Each monomer contains:

- $\alpha$ peptide
- $\beta$ peptide
- 2 x B850 bacteriochlorophyll
- 1 x B800 bacteriochlorophyll
- 2 x carotenoids

Crystallographic asu = 3 monomers
TLS models

a) 1 group for a.s.u. (20 parameters)
b) 1 group per NCS unit (3 x 20 pars)
c) 1 group per molecule (18 x 20 pars)
d) 3 groups per peptide
   + 1 group per pigment
   (total 30 x 20 parameters)
Another large example - GroEL

C. Chaudhry et al., JMB, 342, 229-245 (2004)

• TLS refinement of unliganded GroEL, GroEL-ATPγS complex, GroEL-GroES-ADP.AlF₆ complex, and GroEL-GroES-ADP complex.
• Best results with one TLS group per GroEL domain
  “indicating that the inclusion of relative domain displacements significantly improves the quality of the model”
• Show that binding of ligands and GroES causes large changes in dynamic properties
• Can correlate TLS results with changes between states of the machine.
GroES - 7 groups

GroEL - 42 groups

Figure produced by CCP4mg