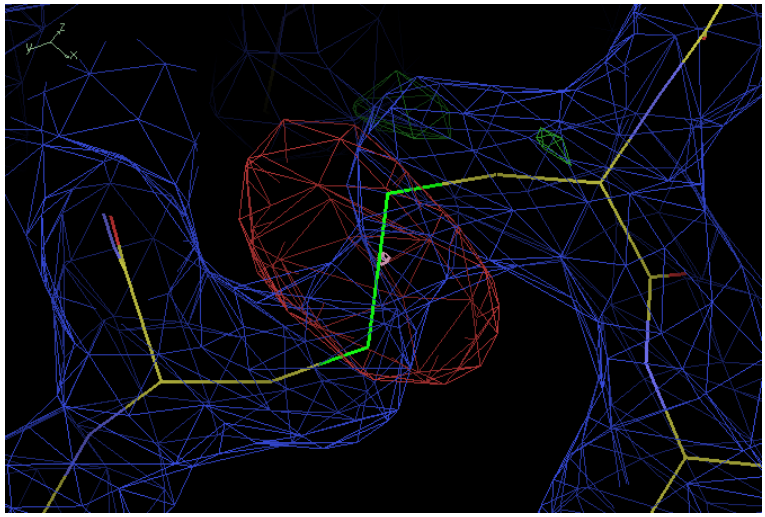
Handling in autobuster cys-cys alternate conformations where one form has disulfide bond but in the other this is broken.

The situation can arise because of radiation damage. We will use as an example 1osg a 3.0Å resolution structure "Complex between BAFF and a BR3 derived peptide presented in a beta-hairpin scaffold". The structure is composed of two BAFF trimers in the asymmetric unit. Each BAFF subunit binds a BR3-derived peptide that is cyclized by a disulfide bond. The BAFF protein has a single disulfide bond between residue 232 and 245. The structure provides a good example for NCS restraint development.

After refinement with autobuster (with NCS restraints handled by the new -autoncs option) the strongest difference map features are six strong negative peaks (from -8.2 to -5.6 sigma) centered on each of the disulfides in BAFF:



These peaks are lightly to arise because of radiation damage causing the partial reduction of the disulfide bond.

How can this be represented in an autoBUSTER refinement? A current limitation is that two alternate structures have to have the same bonding structure. There are two useful approaches:

Possibility A: remove all restraints across the disulfide
This can be achieved by editing out all the relevant SSBOND records from the input PDB file and including .Gelly file with cards:

```
EXCLUDE A|232 A|245
EXCLUDE B|232 B|245
EXCLUDE C|232 C|245
EXCLUDE D|232 D|245
EXCLUDE E|232 E|245
EXCLUDE F|232 F|245
```

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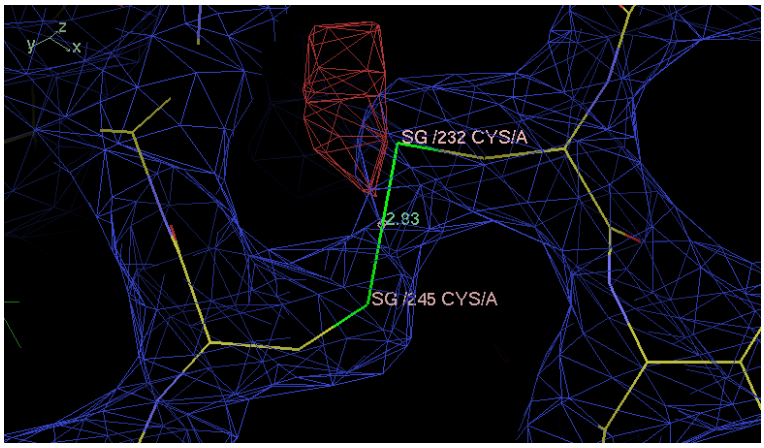
The cards will turn off any short contact between the two CYS residues across the disulfide. Refinement with this option results in a drop in Rwork and Rfree by 0.3% and a much healthier difference map:



The mixed state is represented by allowing the disulfide bond to lengthen, the six bonds all lengthen to between 2.80 and 2.86Å.

Possibility B: create alternate conformations and use utility distance restraints to make one disulfide bonded and the other reduced

Edit out all the relevant SSBOND records from the input PDB file and then create alternate positions A and B for each SG involved in the partial bond (each at half occupancy). Then use the following .Gelly file:

```
# turn off short contacts across disulfide
EXCLUDE A|232 A|245
EXCLUDE B|232 B|245
EXCLUDE C|232 C|245
EXCLUDE D|232 D|245
EXCLUDE E|232 E|245
EXCLUDE F|232 F|245

# alternate A should be forced to bond
NOTE BUSTER_DISTANCE =2.03 0.008 A|232:SG.A A|245:SG.A
NOTE BUSTER_DISTANCE =2.03 0.008 B|232:SG.A B|245:SG.A
NOTE BUSTER_DISTANCE =2.03 0.008 C|232:SG.A C|245:SG.A
NOTE BUSTER_DISTANCE =2.03 0.008 D|232:SG.A D|245:SG.A
NOTE BUSTER_DISTANCE =2.03 0.008 E|232:SG.A E|245:SG.A
NOTE BUSTER_DISTANCE =2.03 0.008 F|232:SG.A F|245:SG.A

# alternate B should be forced to above 3.5 angs short contact
NOTE BUSTER_DISTANCE -3.50 0.10 A|232:SG.B A|245:SG.B
NOTE BUSTER_DISTANCE -3.50 0.10 B|232:SG.B B|245:SG.B
NOTE BUSTER_DISTANCE -3.50 0.10 C|232:SG.B C|245:SG.B
NOTE BUSTER_DISTANCE -3.50 0.10 D|232:SG.B D|245:SG.B
NOTE BUSTER_DISTANCE -3.50 0.10 E|232:SG.B E|245:SG.B
NOTE BUSTER_DISTANCE -3.50 0.10 F|232:SG.B F|245:SG.B
```

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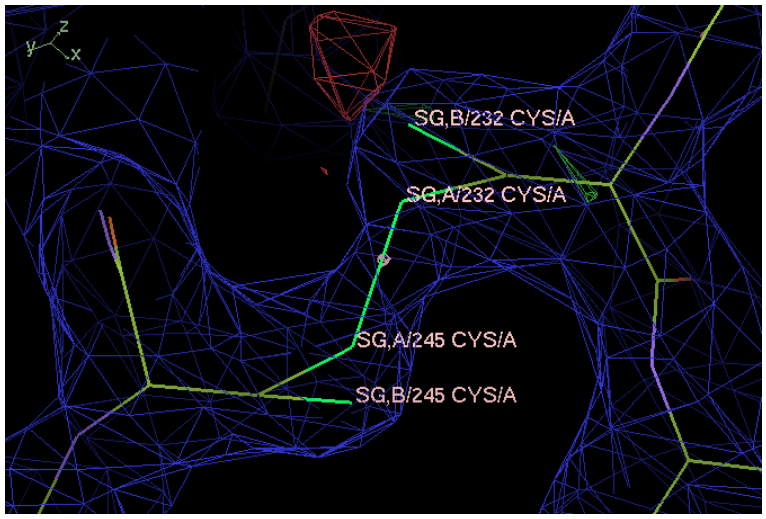
This results in a slightly greater 0.4% Rfree drop than possibility A and a rather clearer model:



Note that no restraints on the bond angle CB-SG.A-SG.A are imposed. In the next release of autobuster there will be “utility angle restraints” but in any case the geometry obtained is reasonable.

The resolution of data is not sufficient to decide whether the two alternates should be extended to the CB atom or beyond. A run where the occupancy of the two alternates was allowed to vary showed that 0.50 was a reasonable value.

The major difference map feature left in the structure is an extra copy of BR3-derived peptide bound at a crystal contact not modelled in the pdb entry. But that is another story.