Local Structural Similarity Restraints (LSSR) enable exploitation of information from related structures and facilitate use of NCS

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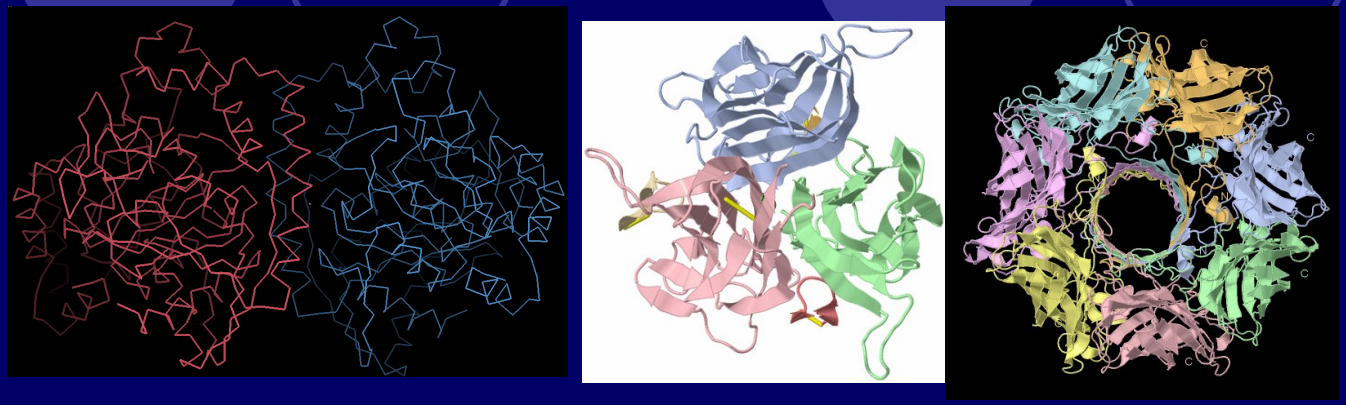
Presentation to ACA Meeting

Toronto 28th July 2009

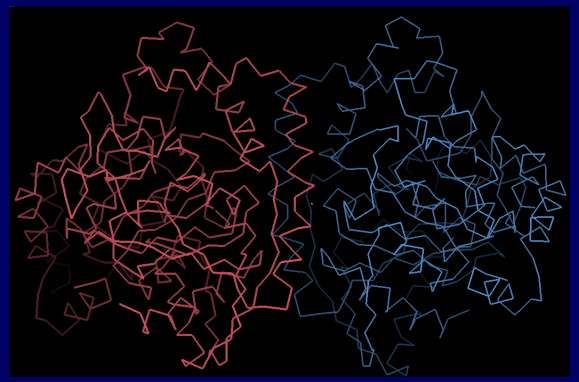
NCS in refinement

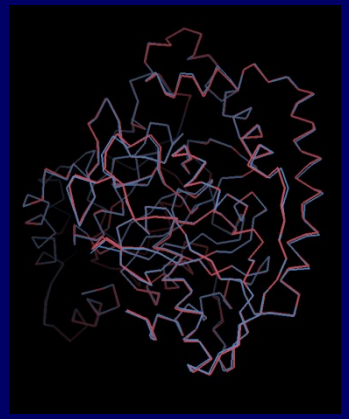


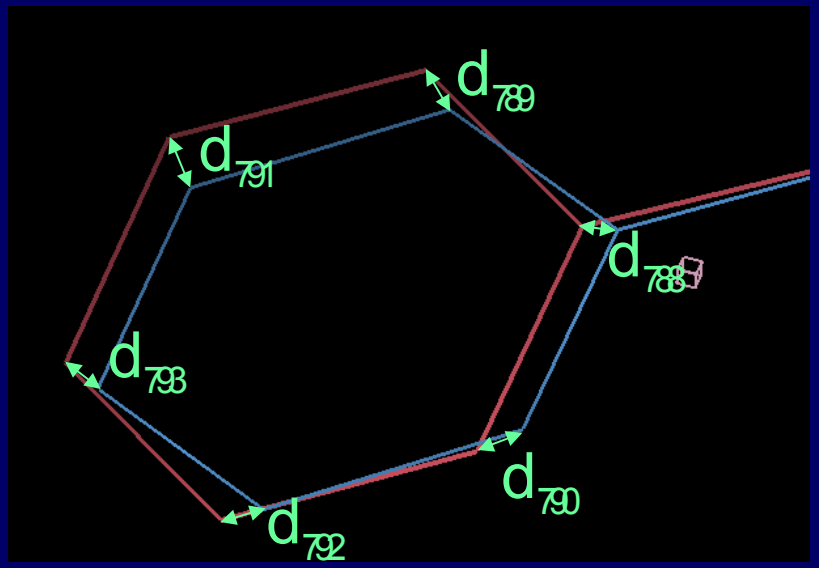
- NCS: Non Crystallographic Symmetry
- Have 2+ chains in asu that are similar
- Similarity not identity – but can be close
- very useful potentially can decrease parameter/data ratio by (up to) factor 2+
- NCS also superb for map averaging
- Ignore at your peril! G. Kleywegt *et al.*

Standard Approach to NCS in refinement: superposition-based rmsD restraints

- Here conserve similarity between two protein chains with a restraint function.
- Use least squares superposition:
  - Superposition chain B onto chain A
  - Harmonic restrain the distances d_i between each atom and its ncs partner – pulling them closer.
  - Restraints lower the rmsD of the superposition.



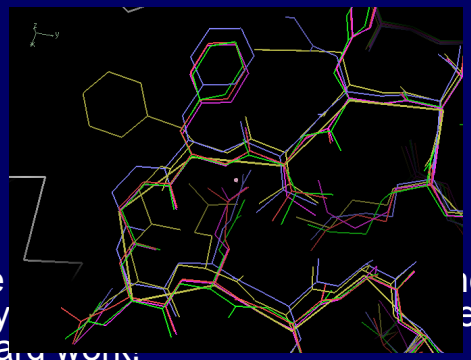




- Conventional approach used by almost everyone to date

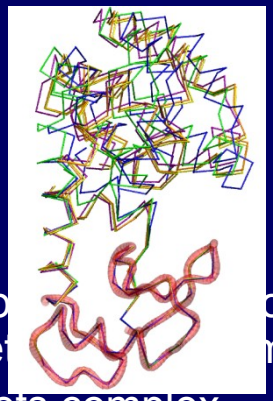
NCS violations and Domain motions make superposition-based restraints difficult to use

- NCS violations
  - Have parts so the with conformations that are distinct in different NCS copies.
  - For example you have a loop that from density has distinct conformation in 6 NCS copies – carefully build these in coot



  - Simple … hese as very … estroy your hard work.
- Was known as “Soft NCS”

- Domain motions
  - Domain motions between copies – here 4 chains with two domains and different hinge bend

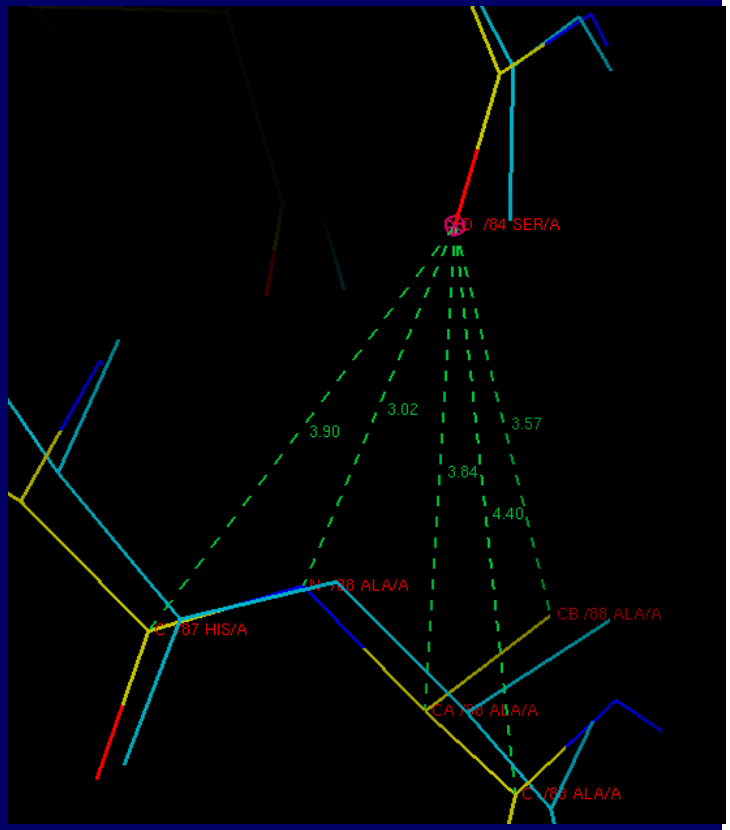


  - Sp… on in se… n an…
  - Gets complex

Alternative: Extend the SHELX approach

- RMS based restraints too laborious
- Avoid any idea of superposition instead lets look at the **local** environment of each atom.
- Consider local contact distances
- Find atoms within 5.5Å of each atom
- Exclude bond, angle and within plane contacts
- Include 1-4 contacts
- **Extends the SHELX 1-4 distance restraint approach to non-bonded**

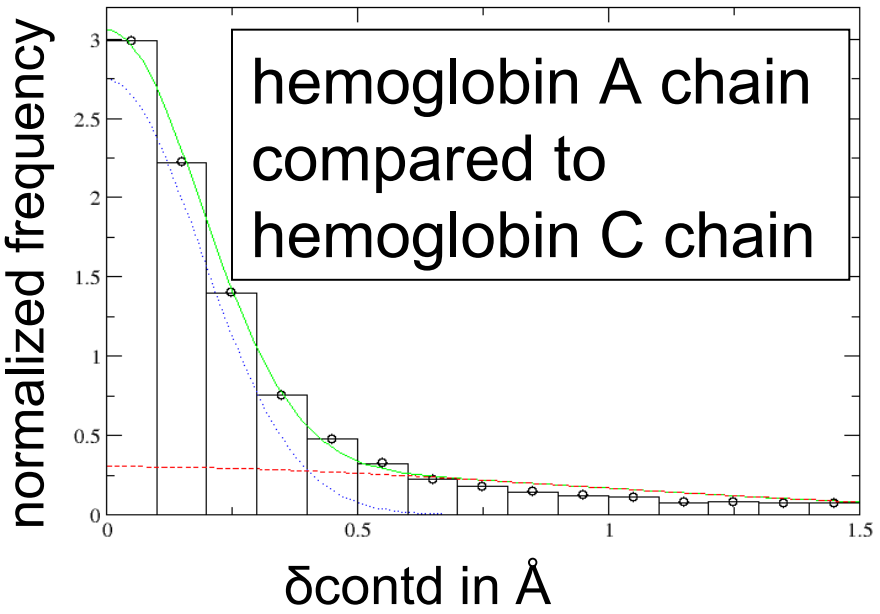
Consider interatomic distances between atoms which are close for two NCS pairs

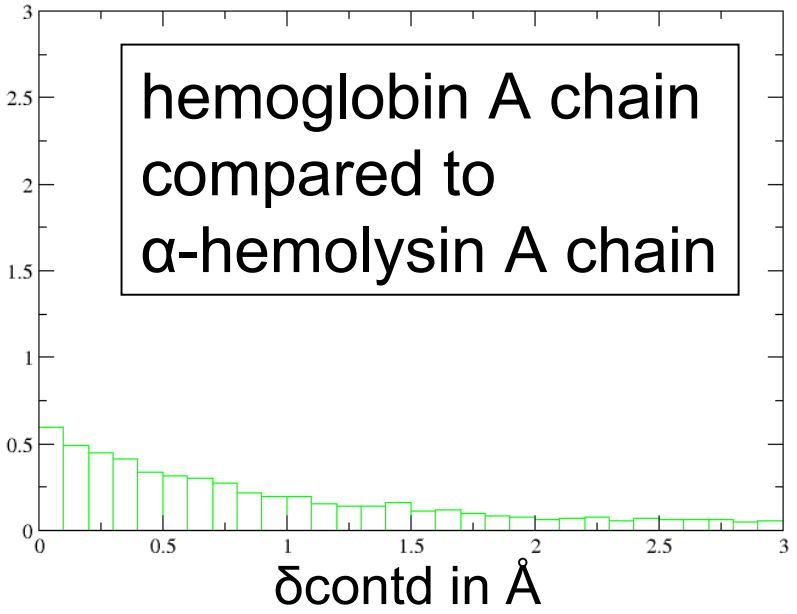


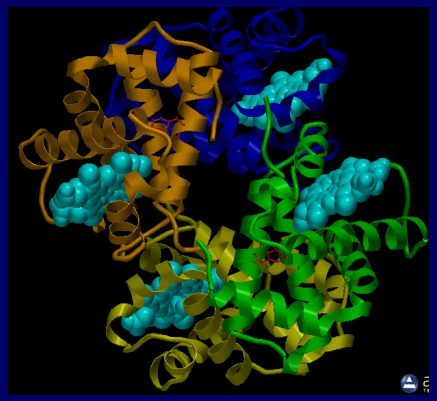
	87 C	88 N	88 CA	88 CB	88 C
84 O Chain A	3.90	3.02	3.84	3.57	4.40
84 O Chain C	4.01	3.07	3.80	3.34	4.62
d=|δ A-C|	0.11	0.02	0.04	0.23	0.22
84 C Chain A	5.05	4.24	5.03	4.60	5.53
84 C Chain C	4.96	4.19	5.01	4.54	5.73
d=|δ A-C|	0.09	0.05	0.02	0.06	0.20

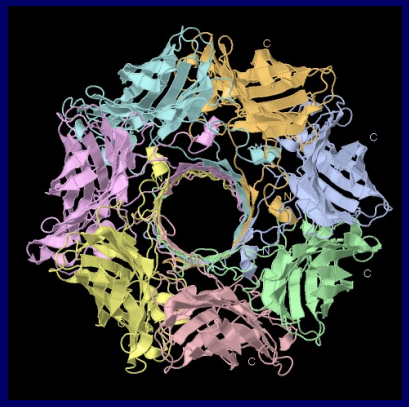
- Example main chain contacts for a helix in hemoglobin 1qpw
- Distances are closely related – and do not depend on superposition.

Delta distance distribution has two parts – related peak and random background

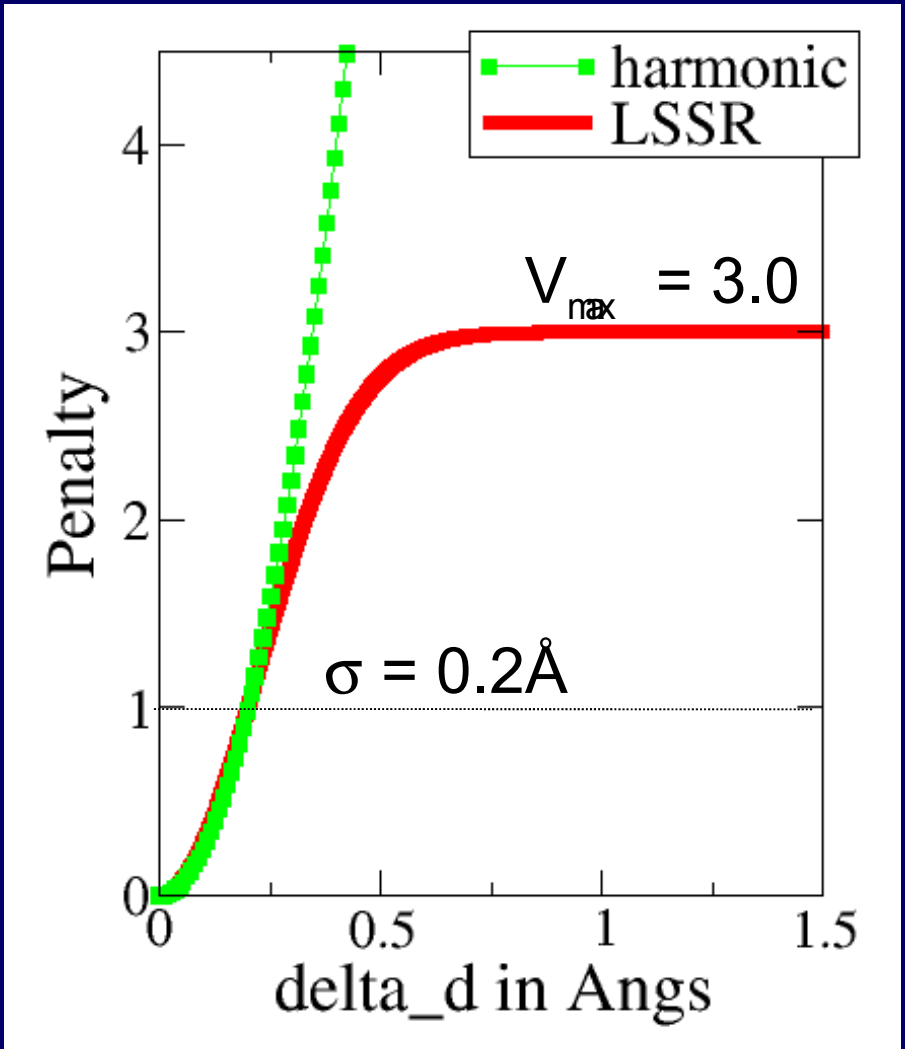








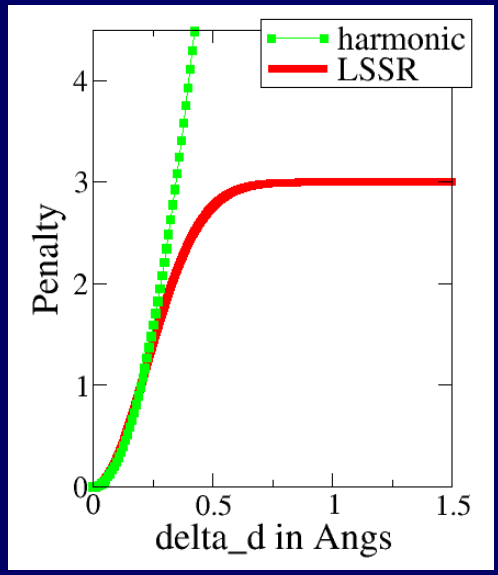
Use a function that plateaus instead of a harmonic term



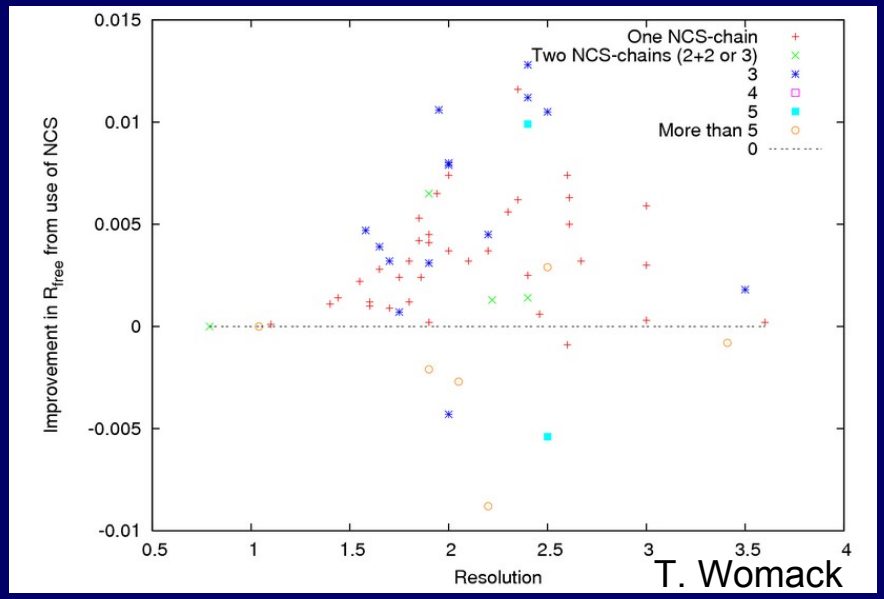
- Most similar previous approach:
- Sali: MODELLER probability density function

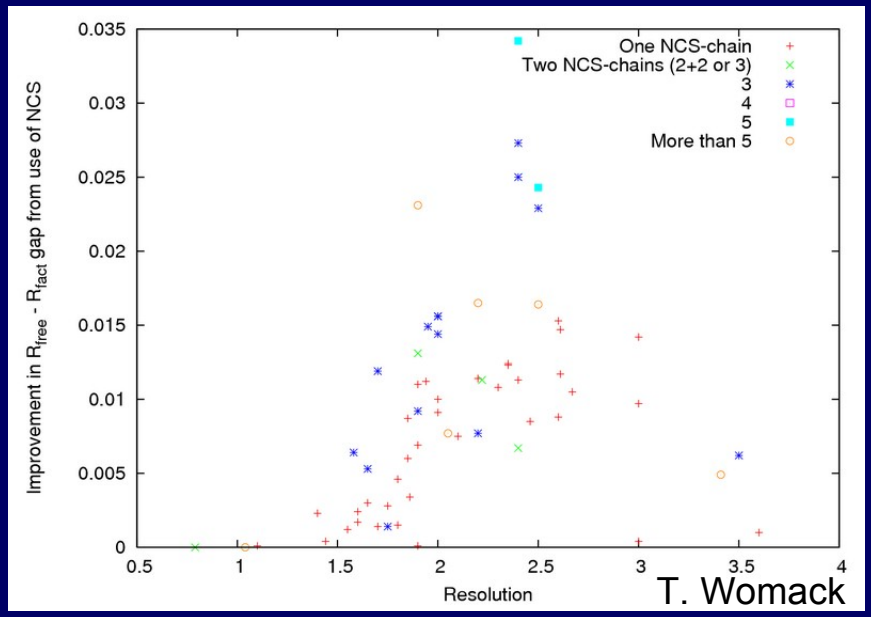
Local Structural Similarity Restraints (LSSR)

- Does not involve any superposition
- No restraint on absolute contact distances
- Only on difference in local contact distances
- Is much softer than superposition-based methods – violations entail only a fixed cost
- -autoncs method in BUSTER fully automated detection and application



Application of autoBUSTER – autoncs to re refinement of 60 pdb structures

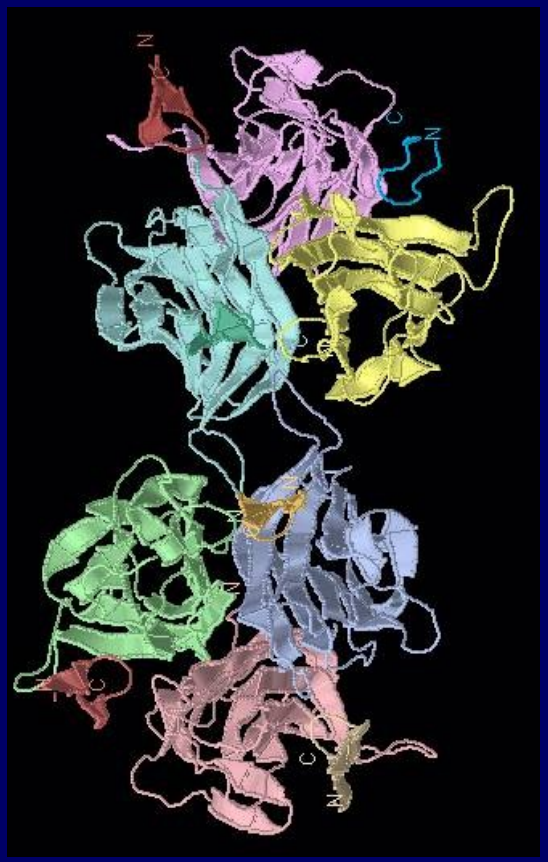




- Conclusion: as can be expected using NCS produces slightly better Rfree and markedly better Rfree-Rwork gap
- -autoncs circumvents the “to use or not to used” dilemma

tutorial example: 1osg

- complex between BAFF with the peptide bhpBR3
- 3.0Å resolution
- Two protein trimers each binds cyclic peptide
- Originally refined with refmac including (weak) rmsD NCS
- Tutorial wiki example *www.globalphasing.com/buster/wiki*

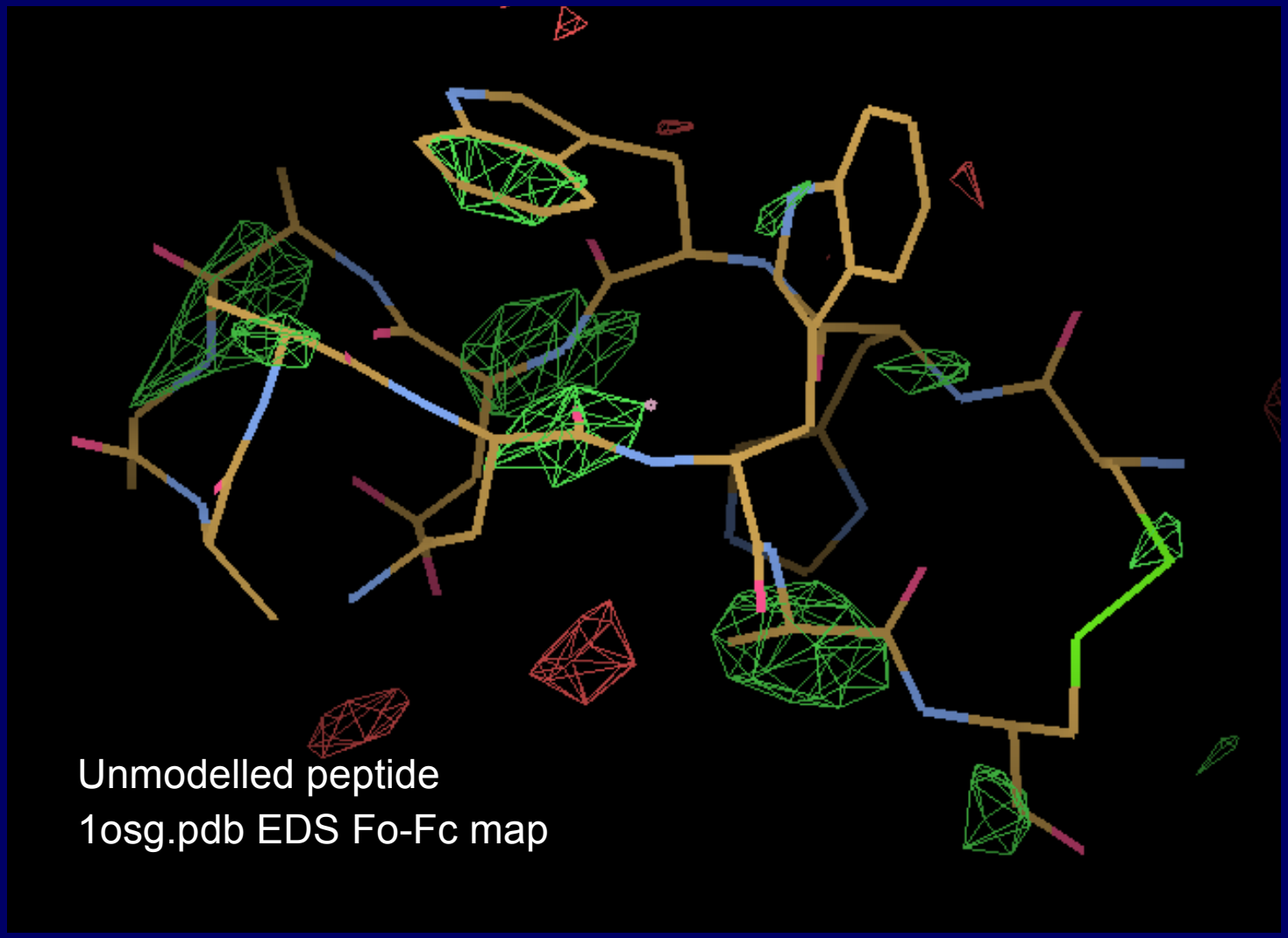


autoBUSTER refinement of 1osg

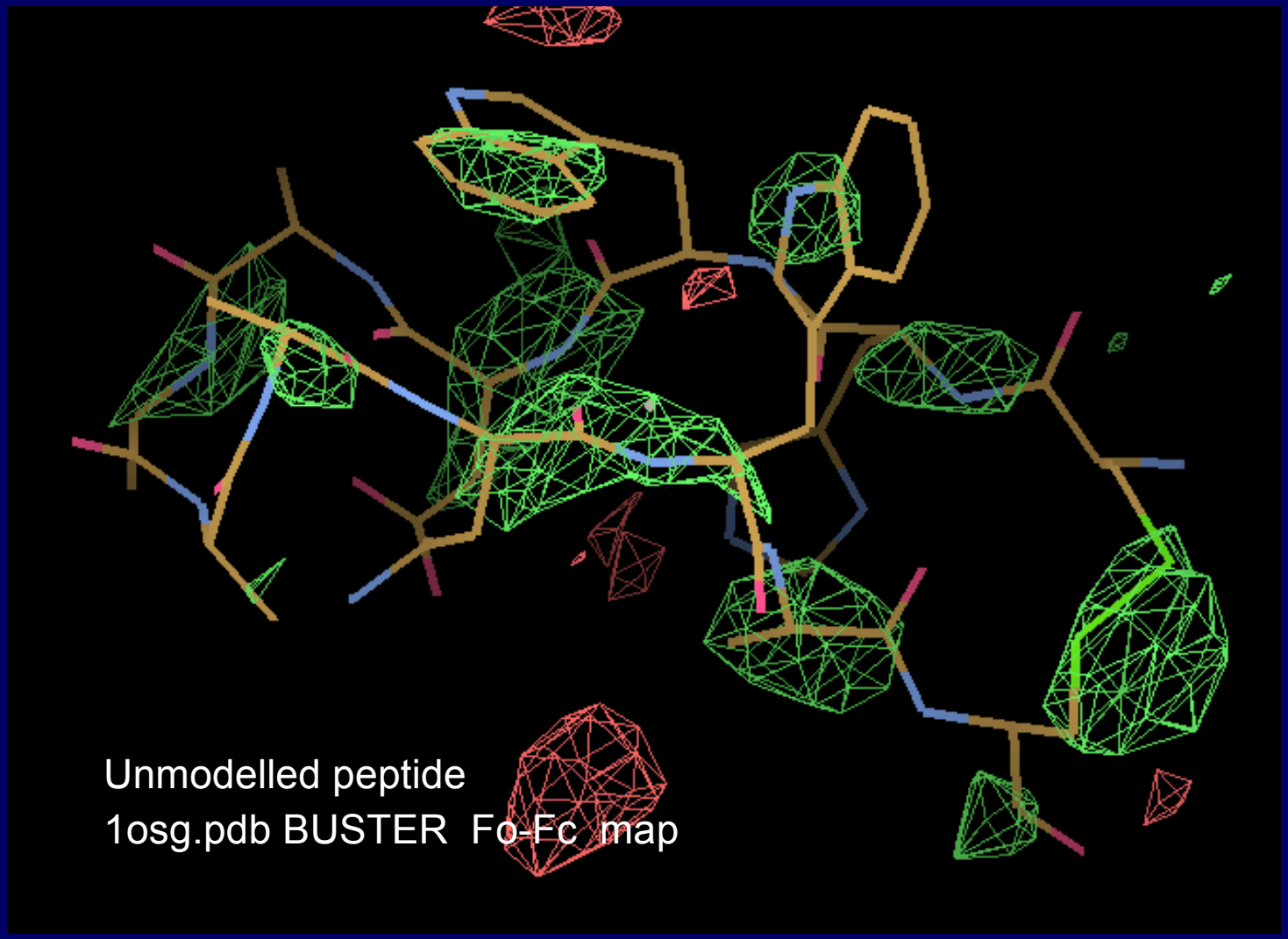
structure	BUSTER R_{work} R_{free}	Gap R_{free} –R_{work}	Molprobity Ramach. favored	Molprobity score
1osg pdb (refined B's)	0.185 0.243	5.8%	94.5%	2.81
autoBUSTER control no NCS	0.169 0.249	7.8%	95.4%	2.59
autoBUSTER - autoncs	0.181 0.223	4.3%	96.4%	2.23
autoBUSTER - autoncs TLS	0.169 0.211	4.1%	96.5%	2.23

- autoBUSTER with automated LSSR NCS results in 2% drop in Rfree, better gap and better geometry
- TLS produces further improvements.

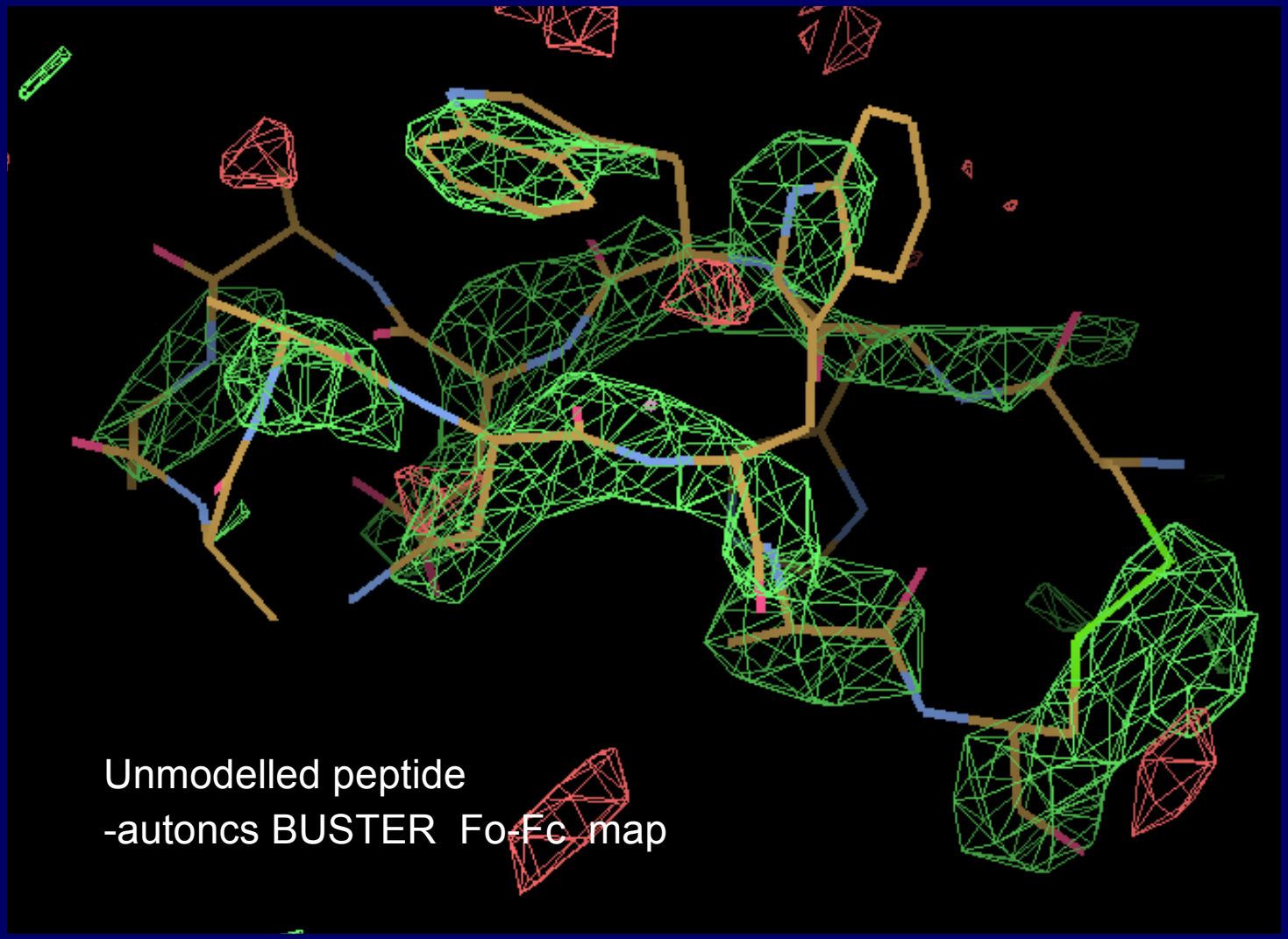
There is an extra peptide at a crystal contact: not clear from EDS



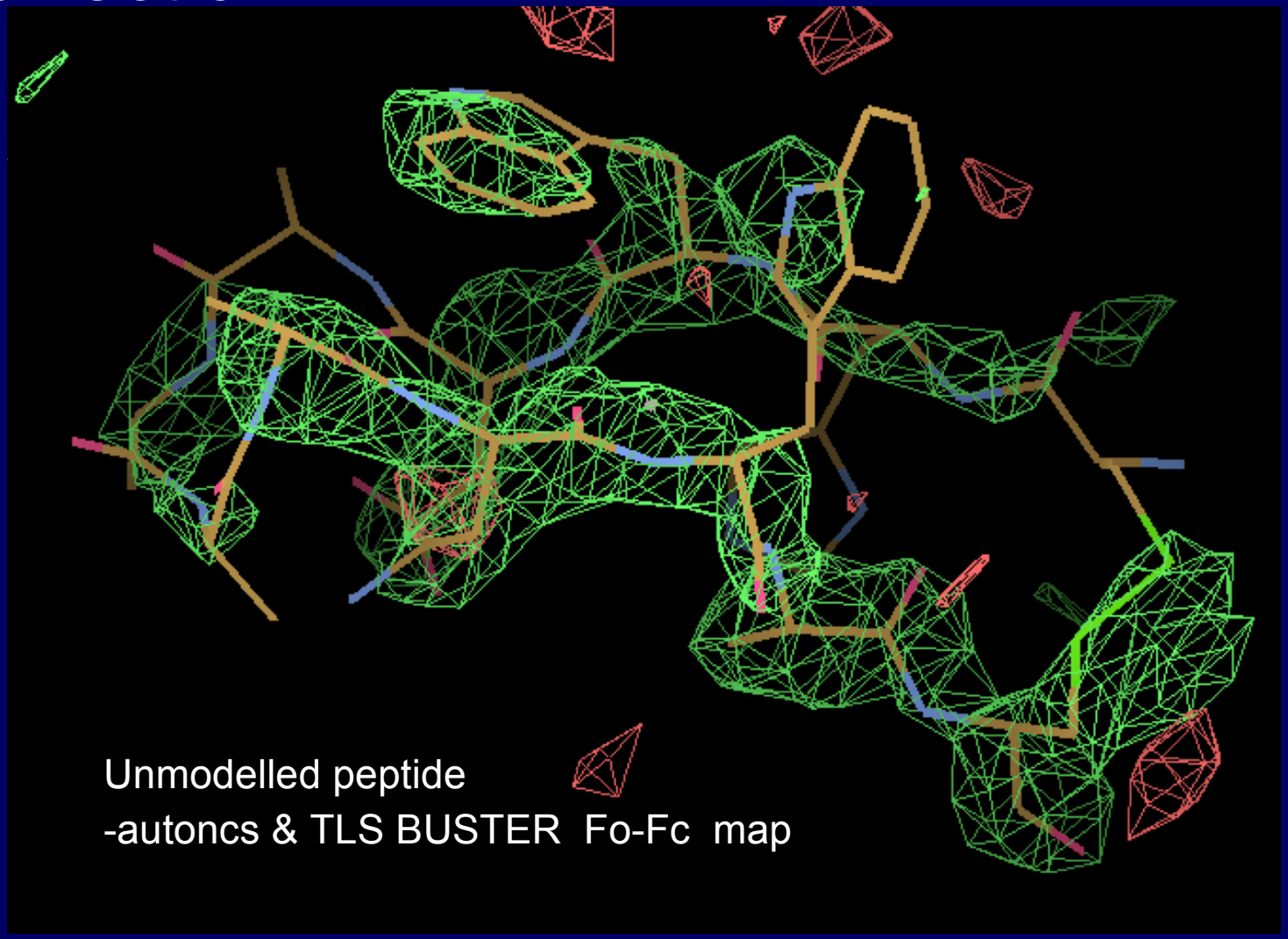
Extra peptide: initial BUSTER map



Extra peptide: -autoncs could just interpret



Extra peptide: clear from difference map with -autoncs & TLS despite bulk solvent correction



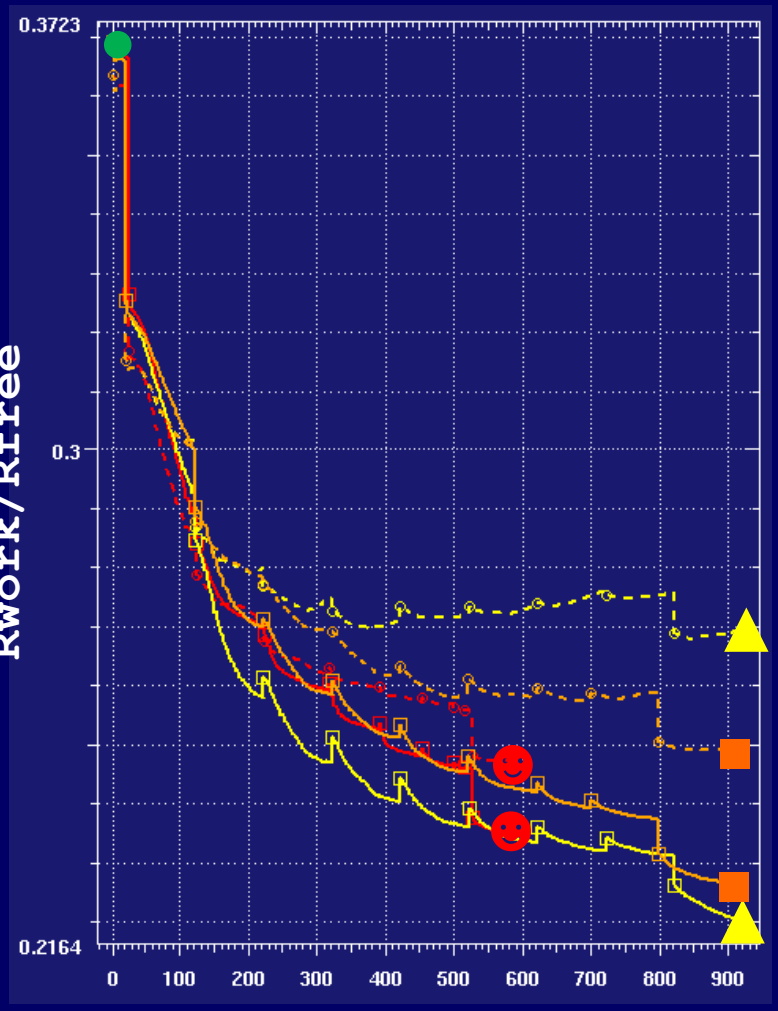
LSSR Target Restraints

- NCS restraints couple two chains within the structure being refined.
- But suppose you know the chain being refined is similar to a structure that has already been solved (the “target”).
- For example:
  - ligand complex with higher resolution apo
  - two crystal forms of the same protein
  - partial datasets from non-isomorphous crystals
  - following radiation damage
- Apply LSSR restraints to the fixed target structure supplied as pdb file

Exploiting similarity to existing structures: many approaches

- Rigid body decomposition – keep information by not allowing change
- Restraint approaches:
  - Brunger: XPLOR point restraints
  - Sali: Modeller pdf restraints (includes fixed cost violation)
  - BUSTER (2007) rmsD (NCS) restraints to a fixed external target structure
  - Levitt & Brunger: Deformable Elastic Network
  - BUSTER: LSSR to target structure: easy to use
  - refmac ....

LSSR Target example: glutamate receptors *2e4y* 3.40Å resolution MR solution from *2e4u* 2.35Å resolution



structure	Rwork/ Rfree	Ramach % core	molprob score
MR solution	0.354 0.351	91.7%	3.03
AB control no NCS no target	0.220 0.269	87.4%	3.22
AB -autoncs	0.227 0.249	91.0%	3.06
AB -target 2e4u -autoncs	0.235 0.247	92.2%	2.95

www.globalphasing.com/buster/wiki

Summary

- BUSTER
  - easy to use automated NCS *-autoncs*
  - target high resolution structure during refinement *–target highresol.pdb*
  - TLS
  - Can reveal marginal map details
  - Is available free to academics www.globalphasing.com/buster
  - Examples presented here on the wiki. www.globalphasing.com/buster/wiki/

Acknowledgements

- GΦL
  - Tom Womack, Clemens Vonrhein, Claus Flensburg, Wlodek Paciorek, Maria Brandl, Peter Keller, Gerard Bricogne
- BUSTER users
- Support:
  - Members of the Global Phasing Consortium,
  - Vizier Project FP6