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## Refinement: The BUSTER perspective

Clemens Vonrhein  
Global Phasing Ltd.

DLS/CCP4 2025

# MX & CryoEM - Complimentary methods

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total number of PDB entries = 241922 (Sep 2025)

|                       |                  |
|-----------------------|------------------|
| X-Ray crystallography | = 197707 (81.7%) |
| Cryo-EM               | = 28918 (12.0%)  |
| Electron diffraction  | = 273 (0.1%)     |
| NMR                   | = 14421 (6.0%)   |

total number of PDB entries with the concept of "resolution" (X-Ray, cryo-EM and ED) = 226898

| Resolution | X-Ray  |                 |        | cryo-EM |                |        | ED      |               |        |         |
|------------|--------|-----------------|--------|---------|----------------|--------|---------|---------------|--------|---------|
|            | #PDB   | #PDB            | %total | %method | #PDB           | %total | %method | #PDB          | %total | %method |
| - 4.0      | 7212   | 1304            | 18.1   | 0.7     | 5882           | 81.6   | 20.3    | 26            | 0.4    | 9.5     |
| 4.0 - 3.0  | 29339  | 13940           | 47.5   | 7.1     | 15363          | 52.4   | 53.2    | 36            | 0.1    | 13.2    |
| 3.0 - 2.5  | 38694  | 32709           | 84.5   | 16.5    | 5945           | 15.4   | 20.6    | 40            | 0.1    | 14.7    |
| 2.5 - 2.0  | 61089  | 59534           | 97.5   | 30.1    | 1510           | 2.5    | 5.2     | 45            | 0.1    | 16.5    |
| 2.0 - 1.5  | 68286  | 68055           | 99.7   | 34.4    | 195            | 0.3    | 0.7     | 36            | 0.1    | 13.2    |
| 1.5 - 1.0  | 21090  | 21011           | 99.6   | 10.6    | 11             | 0.05   | 0.04    | 68            | 0.3    | 24.9    |
| 1.0 -      | 1173   | 1153            | 98.3   | 0.6     | 0              | 0.0    | 0.0     | 20            | 1.7    | 7.3     |
| Total      | 226883 | 197706 (=87.1%) |        |         | 28906 (=12.7%) |        |         | 271 (=0.001%) |        |         |

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**Snapshot of current state of depositions!**

total number of PDB entries with the concept of "resolution" (X-Ray, cryo-EM and ED) = 226898

**62% of**

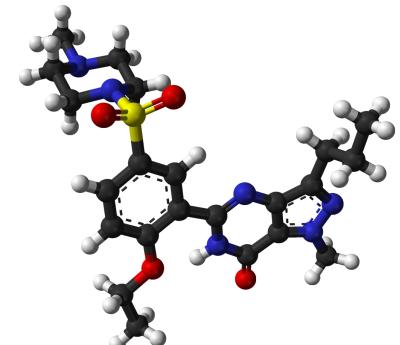
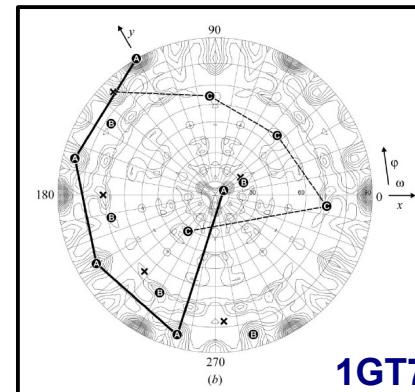
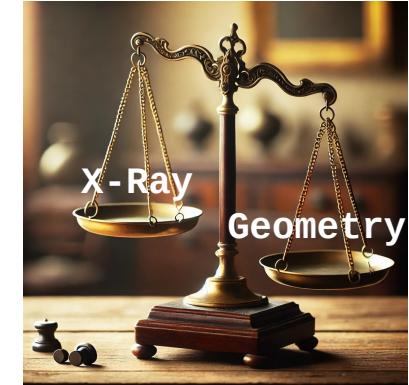
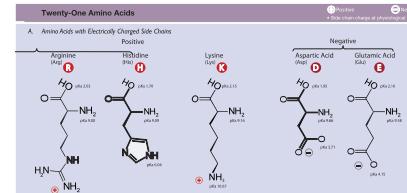
**full PDB**

| Resolution | #PDB   | X-Ray           |        |         | cryo-EM        |        |         | ED            |        |         |
|------------|--------|-----------------|--------|---------|----------------|--------|---------|---------------|--------|---------|
|            |        | #PDB            | %total | %method | #PDB           | %total | %method | #PDB          | %total | %method |
| - 4.0      | 7212   | 1304            | 18.1   | 0.7     | 5882           | 81.6   | 20.3    | 26            | 0.4    | 9.5     |
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| Total      | 226883 | 197706 (=87.1%) |        |         | 28906 (=12.7%) |        |         | 271 (=0.001%) |        |         |

In X-Ray crystallography (MX) we are looking for detailed chemical information with high accuracy.

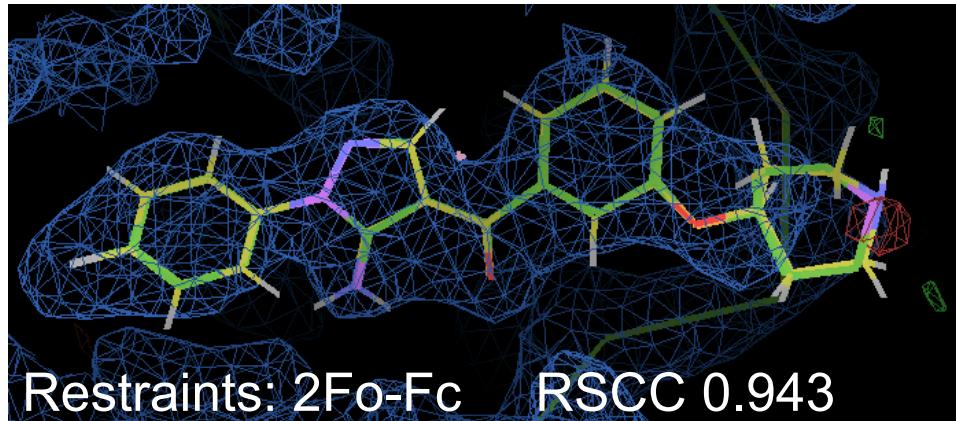
# Refinement - a balancing act

- Combines prior knowledge and observed (X-Ray) data
- Chemistry (bonds, angles, ...)
  - Protein (Engh & Huber)
  - Compound/Ligand
  - ...
- Similarity
  - Within the crystal (NCS)
  - To other structures (“targeting”)
- Occupancy, disorder (alternate conformations), ...



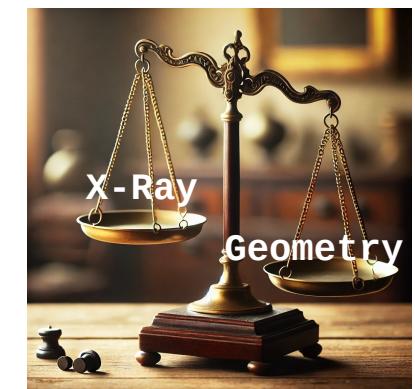
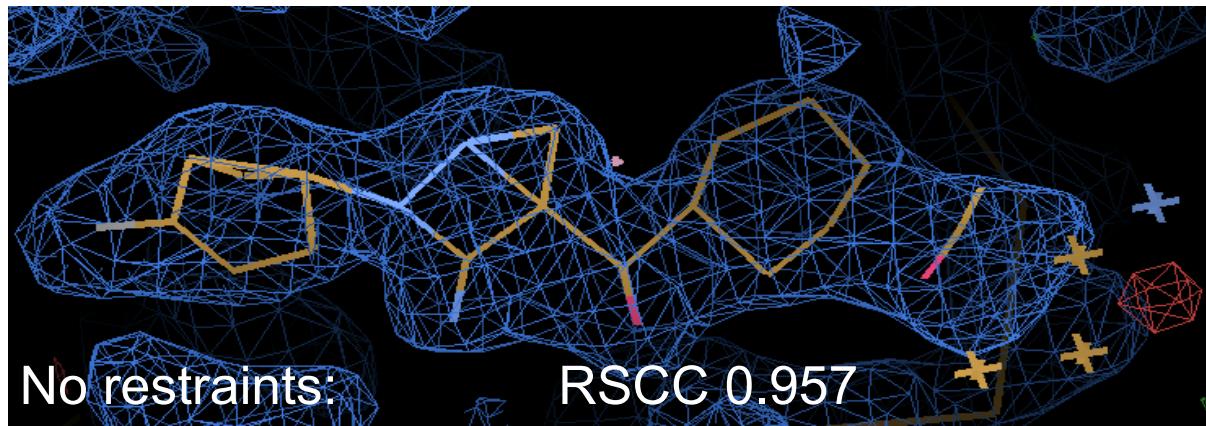
# Prior knowledge is essential

## 2bal kinase 2.1Å resolution.



RSCC is a poor validation criteria here: it tells us that ligand model and map agree, but not if we have a good ligand model that also follows prior chemical knowledge

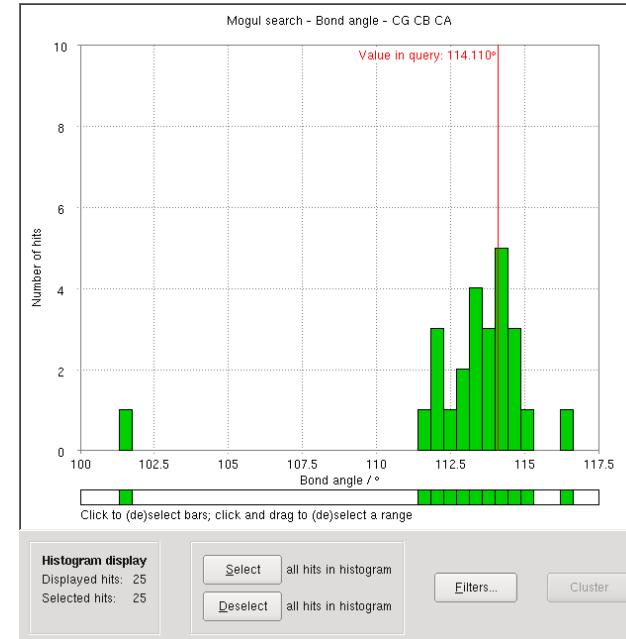
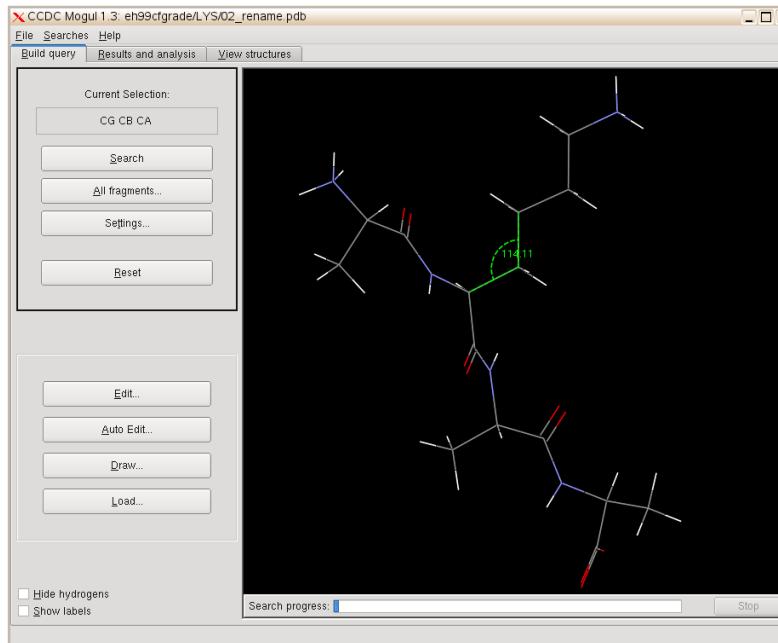
→ pick your quality criteria carefully!



RSCC = real-space correlation coefficient

# Ligand dictionaries based on CSD grade.globalphasing.org

- ❑ Grade (2011): use CCDC mogul program to survey CSD



- ❑ Use CSD as **source of information for restraints** (not only in validation)
- ❑ Also: AceDRG (Long et al, 2017: COD), eLBOW (Moriarty et al, 2011: CSD)

**Grade2 (rewrite, July 2021):**

<https://www.globalphasing.com/buster/manual/grade2/manual/>

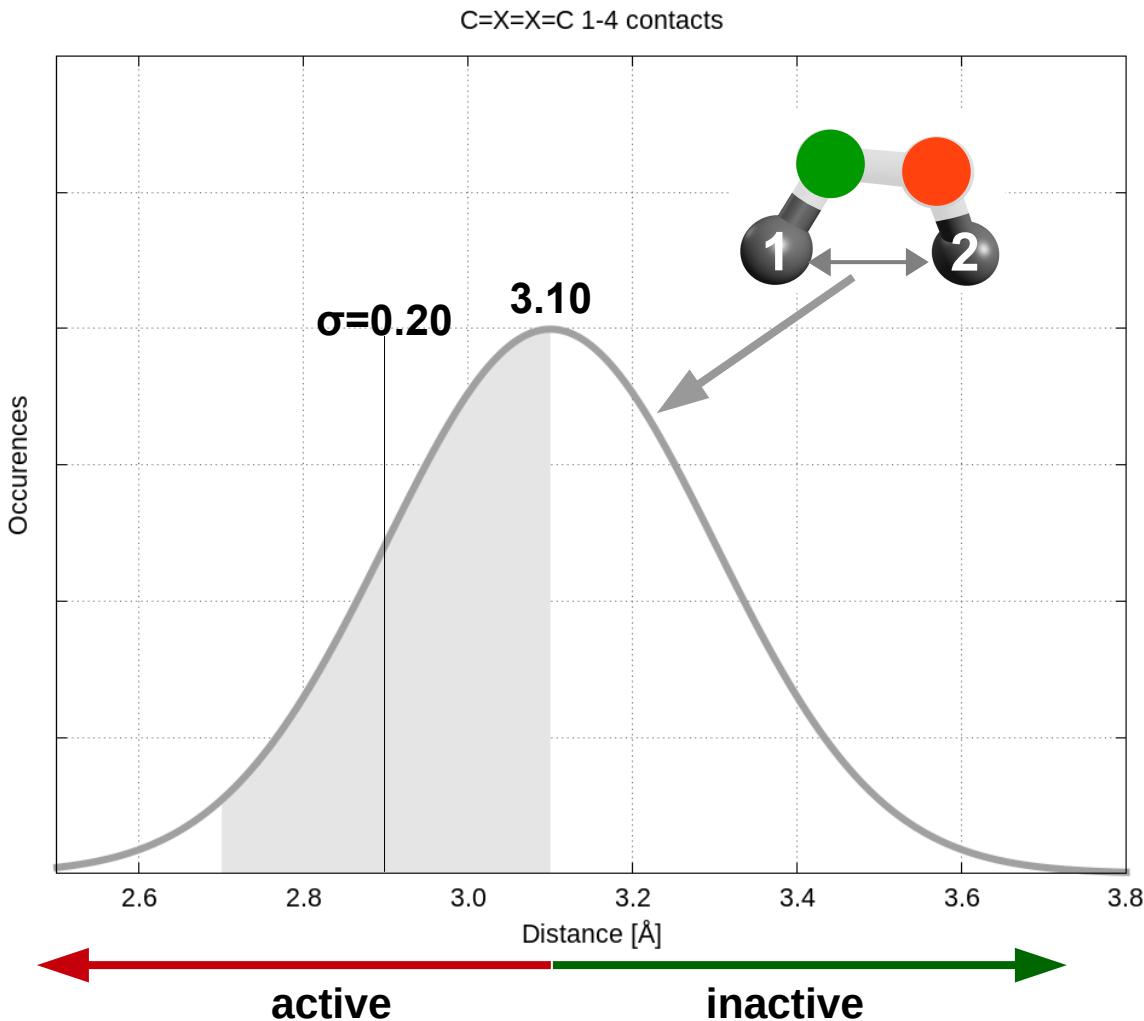
# Restraints - not just for ligands

- ❑ Standard geometry restraints for bonded atoms
  - BOND (1-2)
  - ANGLE (1-3)
  - TORSION (1-4)
  - CHIRAL
  - PLANE
- ❑ Non-bonded atoms
  - anti-bumping restraints
- ❑ B-factor restraints
  - bonded atoms
  - isotropic/anisotropic B-factor
- ❑ Similarity restraints
  - NCS
  - targeting
- ❑ Conformational restraints
  - *Ramachandran*
  - *rotamer*



That balancing act can become tricky  
... or sometimes dangerously biased  
towards a particular restraint type.

# Contact restraints for 1-4 connected atoms - V1



only first and last atoms of 1-4 (torsion) contact taken into account

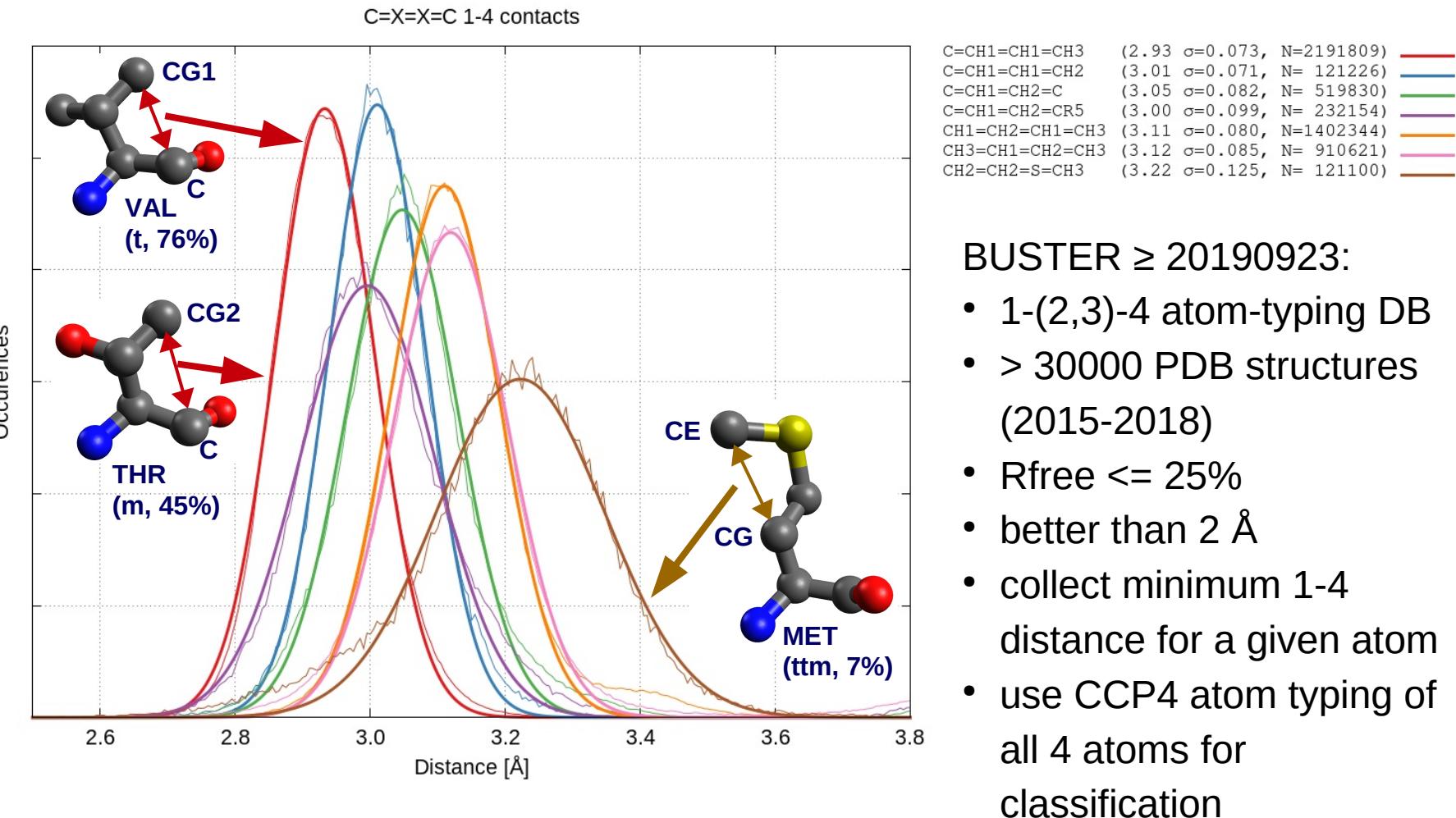
before Sep 2019:

$$vdW_1 + vdW_2 + Dinc_1 + Dinc_2$$

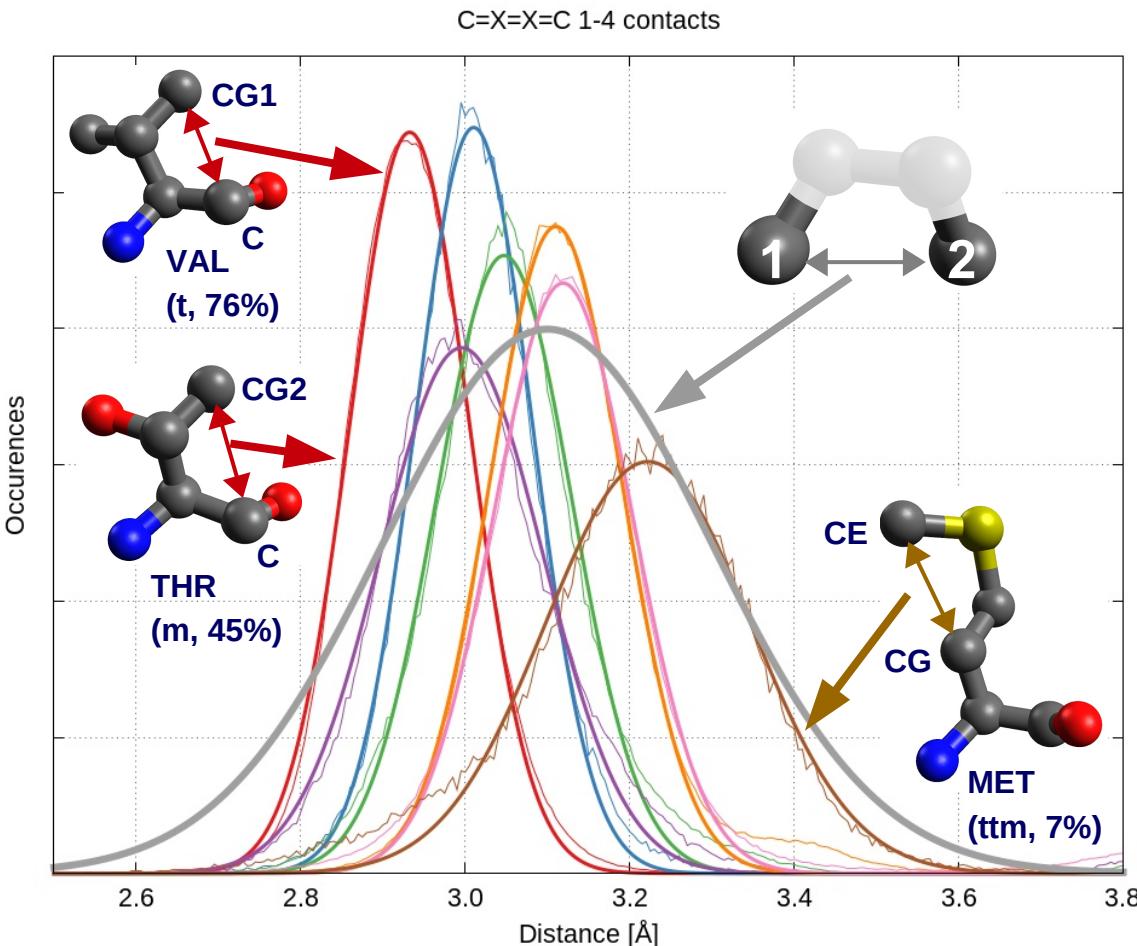
| Element | Dinc  |
|---------|-------|
| C       | -0.15 |
| N       | -0.10 |
| O       | -0.10 |
| X       | -0.15 |

fixed sigma (0.20)

## Contact restraints V2



# Contact restraints V1 vs V2



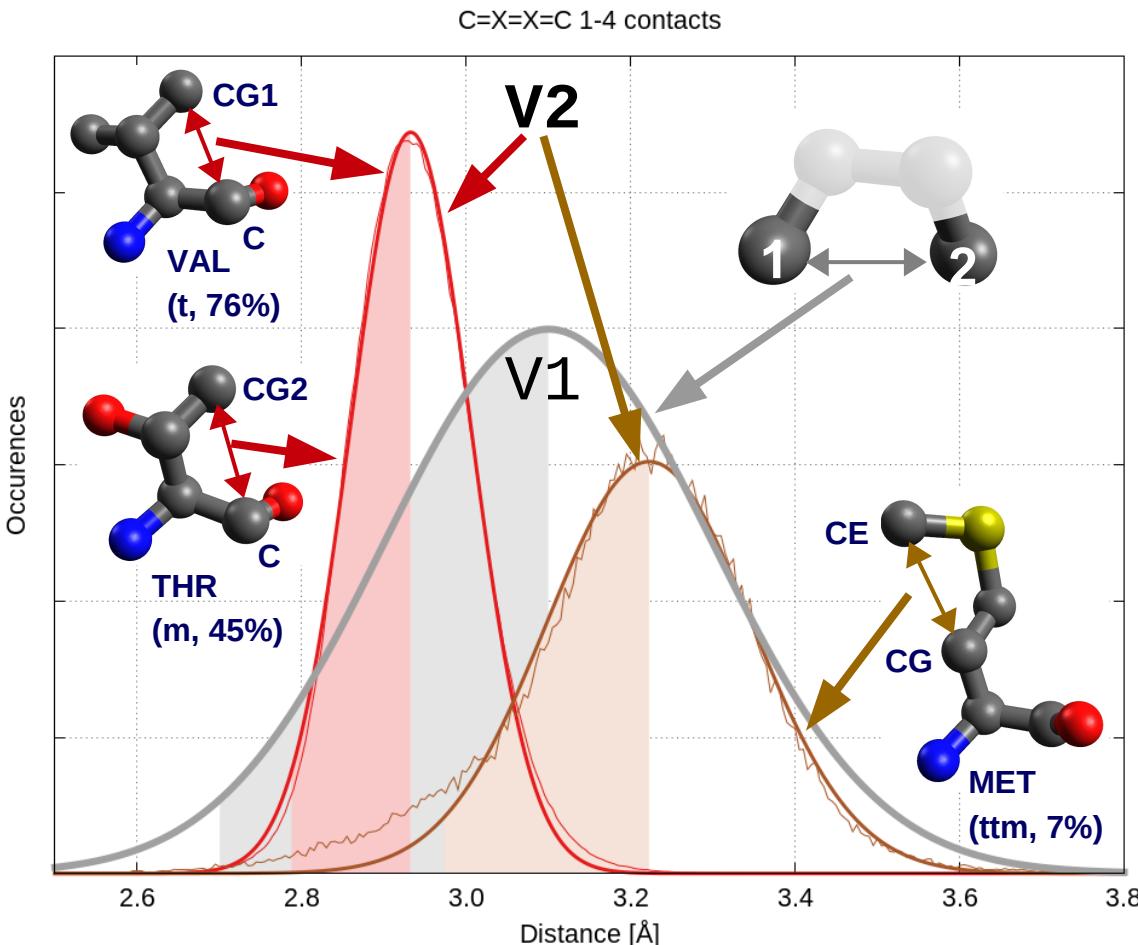
$\text{C}=\text{CH}_1=\text{CH}_1=\text{CH}_3$  (2.93  $\sigma=0.073$ ,  $N=2191809$ )  
 $\text{C}=\text{CH}_1=\text{CH}_1=\text{CH}_2$  (3.01  $\sigma=0.071$ ,  $N= 121226$ )  
 $\text{C}=\text{CH}_1=\text{CH}_2=\text{C}$  (3.05  $\sigma=0.082$ ,  $N= 519830$ )  
 $\text{C}=\text{CH}_1=\text{CH}_2=\text{CR}_5$  (3.00  $\sigma=0.099$ ,  $N= 232154$ )  
 $\text{CH}_1=\text{CH}_2=\text{CH}_1=\text{CH}_3$  (3.11  $\sigma=0.080$ ,  $N=1402344$ )  
 $\text{CH}_3=\text{CH}_1=\text{CH}_2=\text{CH}_3$  (3.12  $\sigma=0.085$ ,  $N= 910621$ )  
 $\text{CH}_2=\text{CH}_2=\text{S}=\text{CH}_3$  (3.22  $\sigma=0.125$ ,  $N= 121100$ )  
 $\text{C}=\text{X}=\text{X}=\text{C}$  (3.10  $\sigma=0.200$ )

before Sep 2019:

$$\text{vdW}_1 + \text{vdW}_2 + \text{Dinc}_1 + \text{Dinc}_2$$

| <u>Element</u> | Dinc  |
|----------------|-------|
| C              | -0.15 |
| N              | -0.10 |
| O              | -0.10 |
| X              | -0.15 |

# Contact restraints V1 vs V2



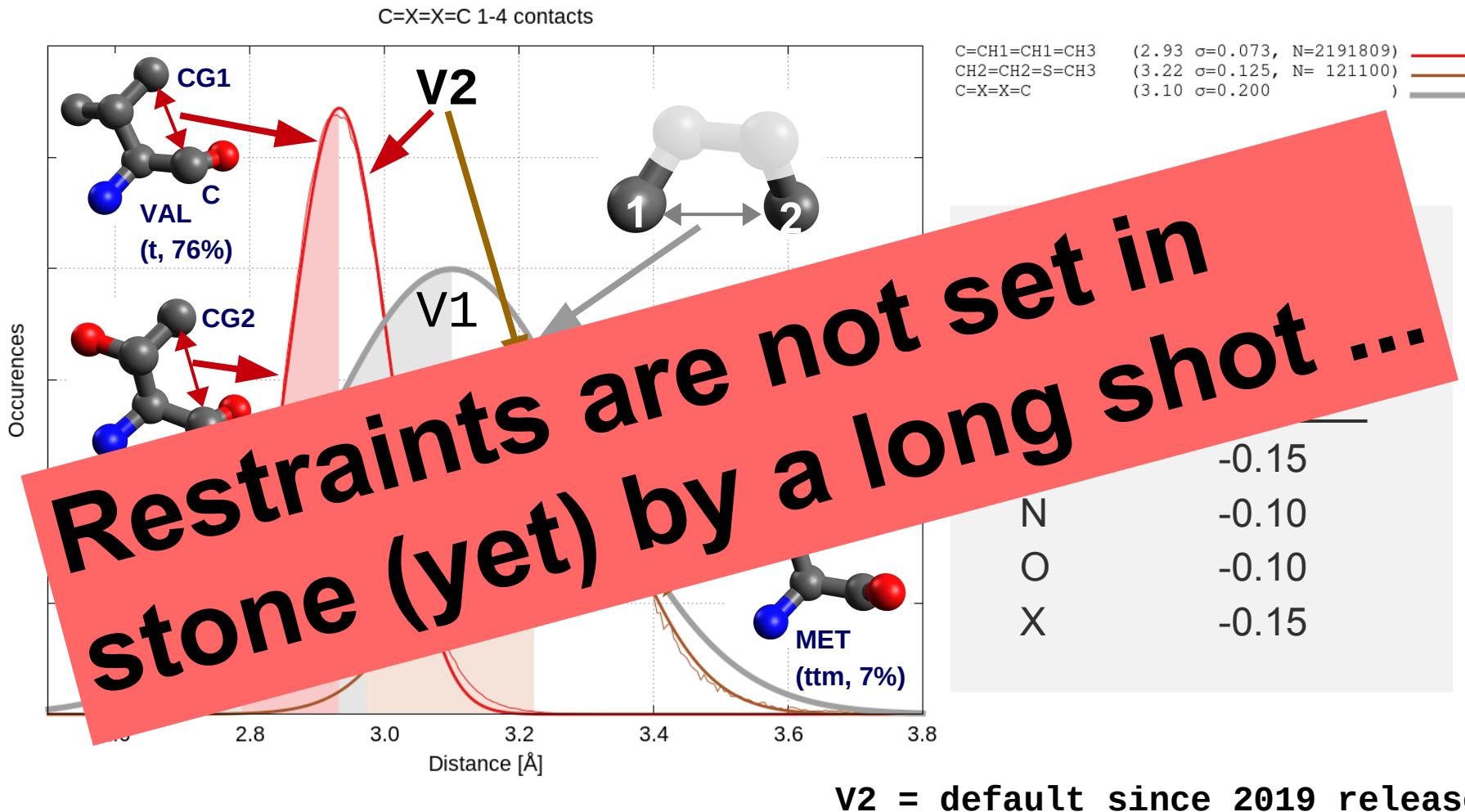
before Sep 2019:

$$\text{vdW}_1 + \text{vdW}_2 + \text{Dinc}_1 + \text{Dinc}_2$$

| Element | Dinc  |
|---------|-------|
| C       | -0.15 |
| N       | -0.10 |
| O       | -0.10 |
| X       | -0.15 |

V2 = default since 2019 release

## Contact restraints V1 vs V2



# Some notes about rotamers and outliers

- ❑ During validation, **MolProbity** rotamer analysis often used as a **quality criterion** for model
- ❑ Outliers are considered problematic and highly unlikely (the stated goal is <1% outliers)

6D0E (deposited, 1.95 Å)

|     |   |    |   |
|-----|---|----|---|
| 2%  | A | 10 | LEU:1.00:0.4:280.2:36.0:::Allowed:mp                |
| 2%  | A | 19 | LEU:1.00:0.4:273.1:36.4:::Allowed:mp                |
| 2%  | A | 24 | LEU:1.00:0.4:260.6:43.2:::Allowed:mp                |
| 2%  | A | 45 | LYS:1.00:0.5:199.5:130.2:301.8:173.9:Allowed:ttmt   |
|     | A | 50 | ILE:1.00:0.0:249.5:205.1::: <b>OUTLIER:OUTLIER</b>  |
| 20% | A | 82 | AVAL:0.33:2.1:311.2::: <b>Favored:m</b>             |
|     | A | 97 | LEU:1.00:0.0:235.3:15.2::: <b>OUTLIER:OUTLIER</b>   |
| 59% | B | 23 | LEU:1.00:0.4:247.2:198.3:::Allowed:mt               |
| ?   | B | 41 | ARG:1.00:0.4:142.9:262.7:191.0:181.3:Allowed:tmt170 |
| 2%  | B | 45 | LYS:1.00:0.3:203.2:129.9:301.1:170.3:Allowed:ttmt   |
| 8%  | B | 50 | ILE:1.00:0.4:215.5:155.0:::Allowed:tt               |
| 20% | B | 82 | VAL:1.00:1.2:312.5:::Allowed:m                      |
|     | B | 97 | LEU:1.00:0.0:236.7:13.8::: <b>OUTLIER:OUTLIER</b>   |

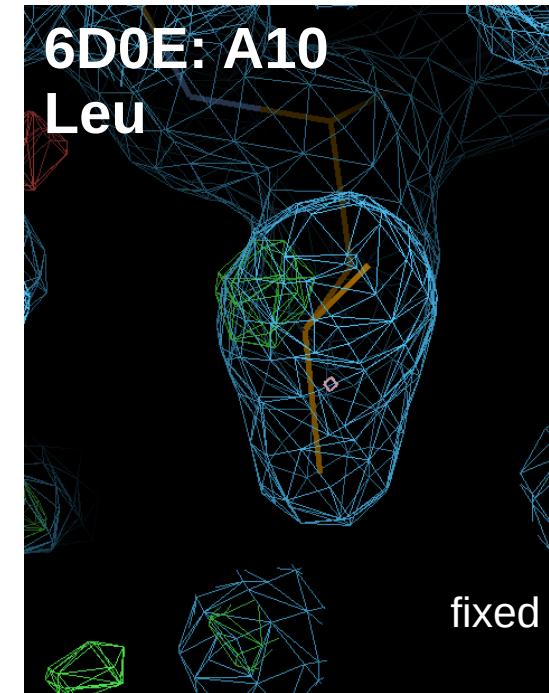
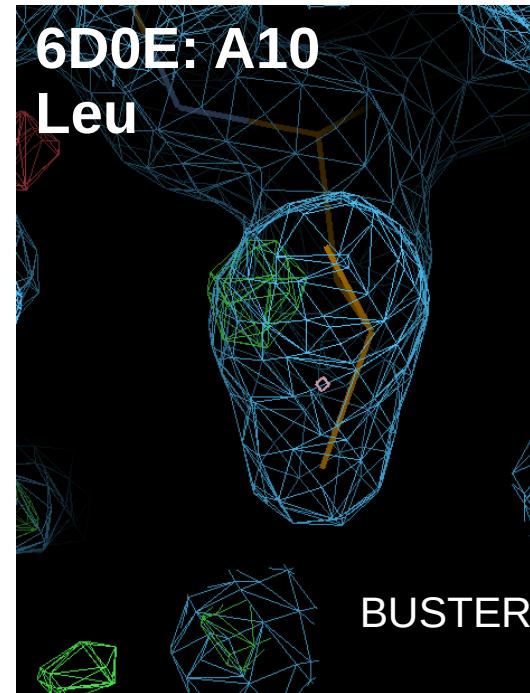
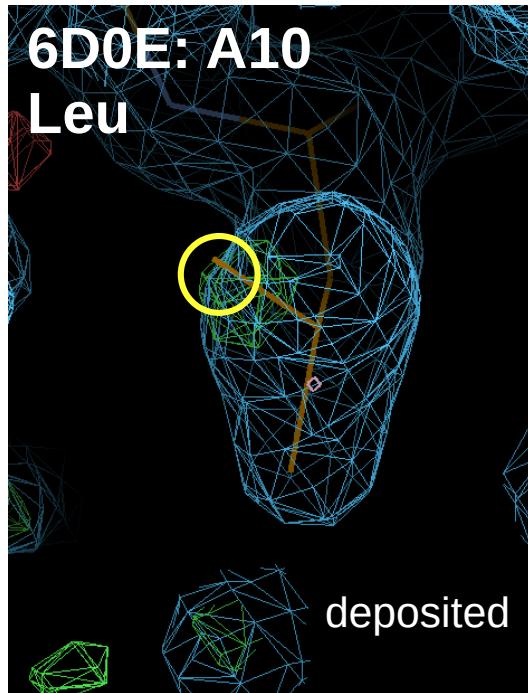
After BUSTER refinement

|   |    |  |
|---|----|--|
| A | 10 | LEU:1.00:0.0:259.1:21.3::: <b>OUTLIER:OUTLIER</b>            |
| A | 19 | LEU:1.00:0.1:309.3:303.1::: <b>OUTLIER:OUTLIER</b>           |
| A | 24 | LEU:1.00:0.2:297.4:296.7::: <b>OUTLIER:OUTLIER</b>           |
| A | 45 | LYS:1.00:0.1:197.6:122.2:308.4:165.6: <b>OUTLIER:OUTLIER</b> |
| A | 50 | ILE:1.00:0.0:239.0:174.6::: <b>OUTLIER:OUTLIER</b>           |
| A | 82 | AVAL:0.33:0.2:320.6::: <b>OUTLIER:OUTLIER</b>                |
| A | 97 | LEU:1.00:0.0:238.9:5.1::: <b>OUTLIER:OUTLIER</b>             |
| B | 23 | LEU:1.00:0.0:180.7:193.4::: <b>OUTLIER:OUTLIER</b>           |
| B | 41 | ARG:1.00:0.1:138.1:266.5:172.2:188.0: <b>OUTLIER:OUTLIER</b> |
| B | 45 | LYS:1.00:0.1:197.5:123.2:307.8:166.2: <b>OUTLIER:OUTLIER</b> |
| B | 50 | ILE:1.00:0.0:238.2:171.6::: <b>OUTLIER:OUTLIER</b>           |
| B | 82 | VAL:1.00:0.1:326.1::: <b>OUTLIER:OUTLIER</b>                 |
| B | 97 | LEU:1.00:0.0:238.8:5.0::: <b>OUTLIER:OUTLIER</b>             |

Unlikely – but not  
classified as “outlier”

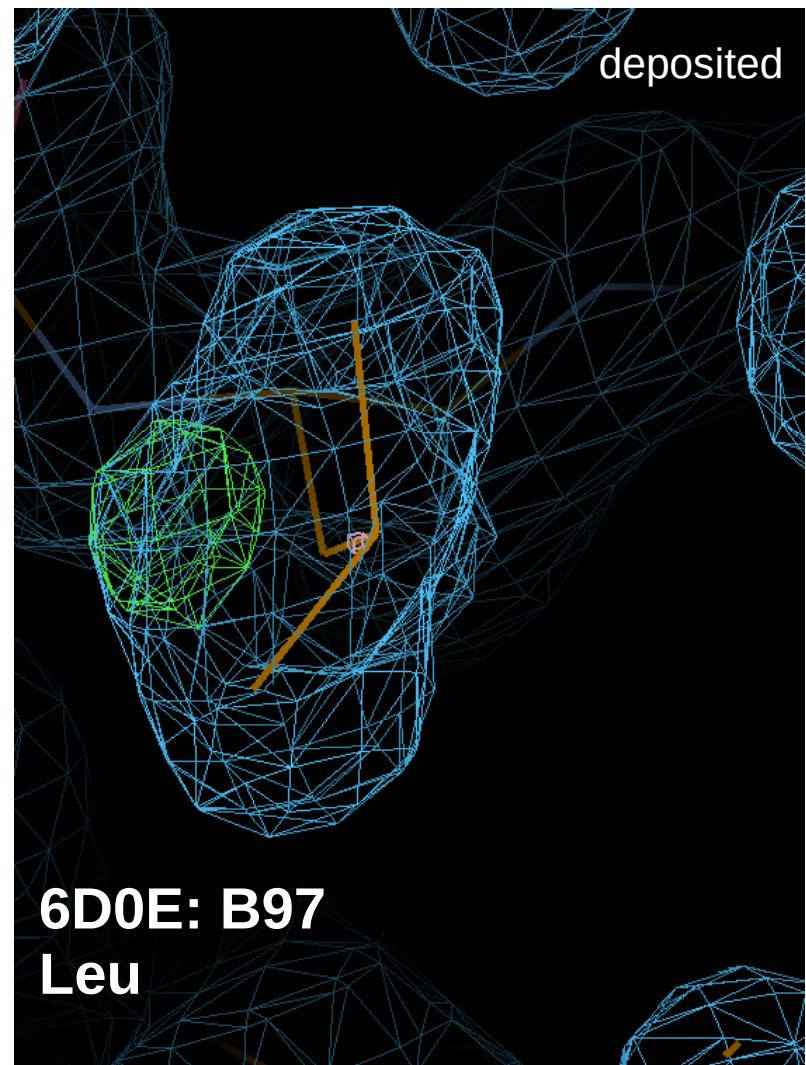
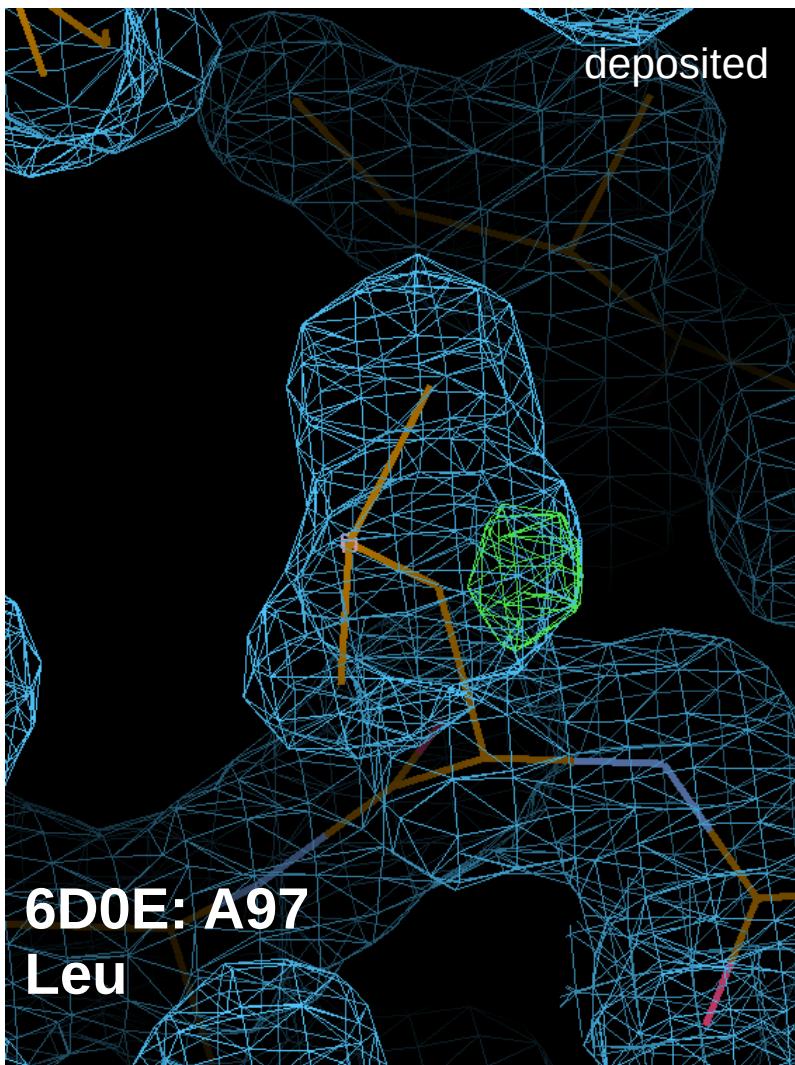
- ❑ Do we have a problem with rotamers in (BUSTER) refinements?

# Rotamer has to fit density (for X-Ray crystallographic models)

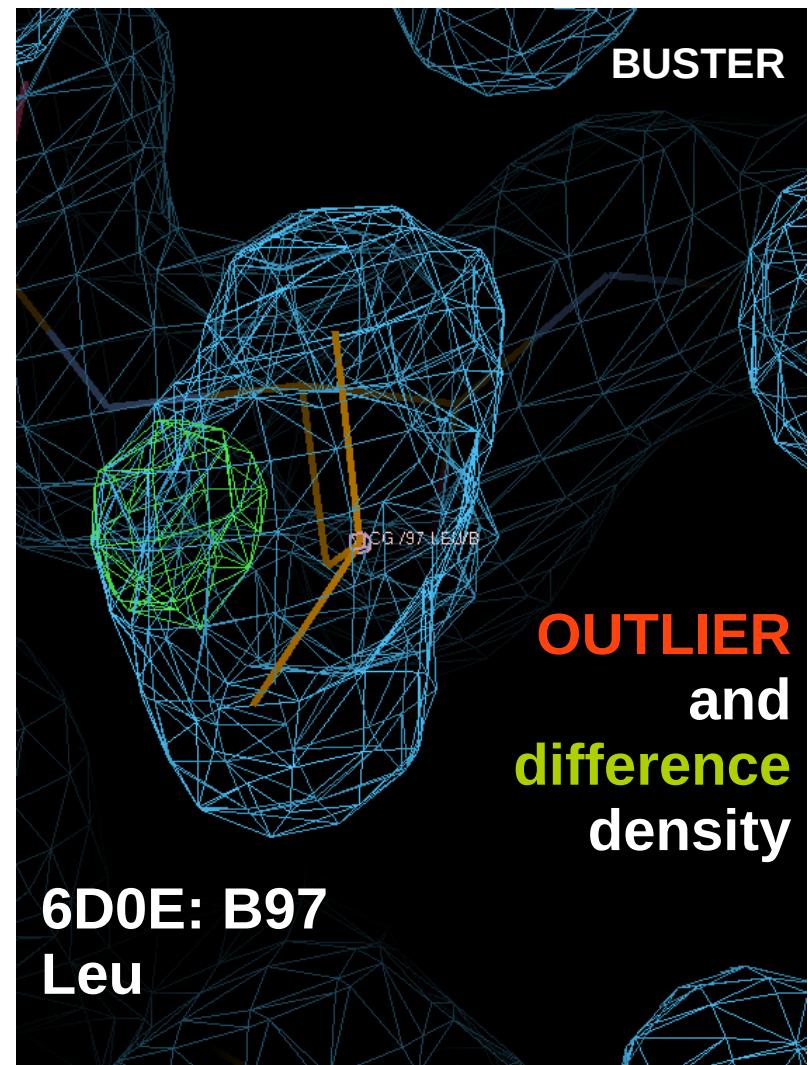
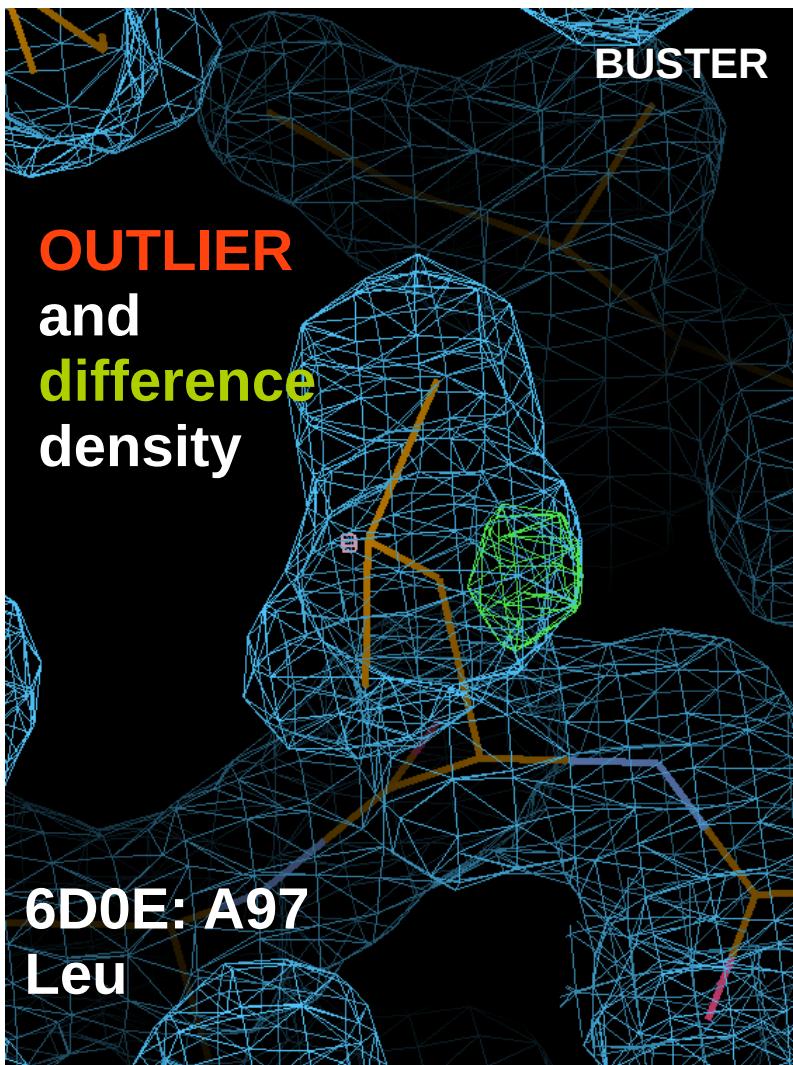


- small, but significant difference between mt (59%) and mp (2%)
- BUSTER refines model away from poor/wrong rotamer and gives clear indications:
  - positive difference density
  - rotamer now highlighted as outlier
- enforcing rotamer restraints can mask incorrect side-chain conformations

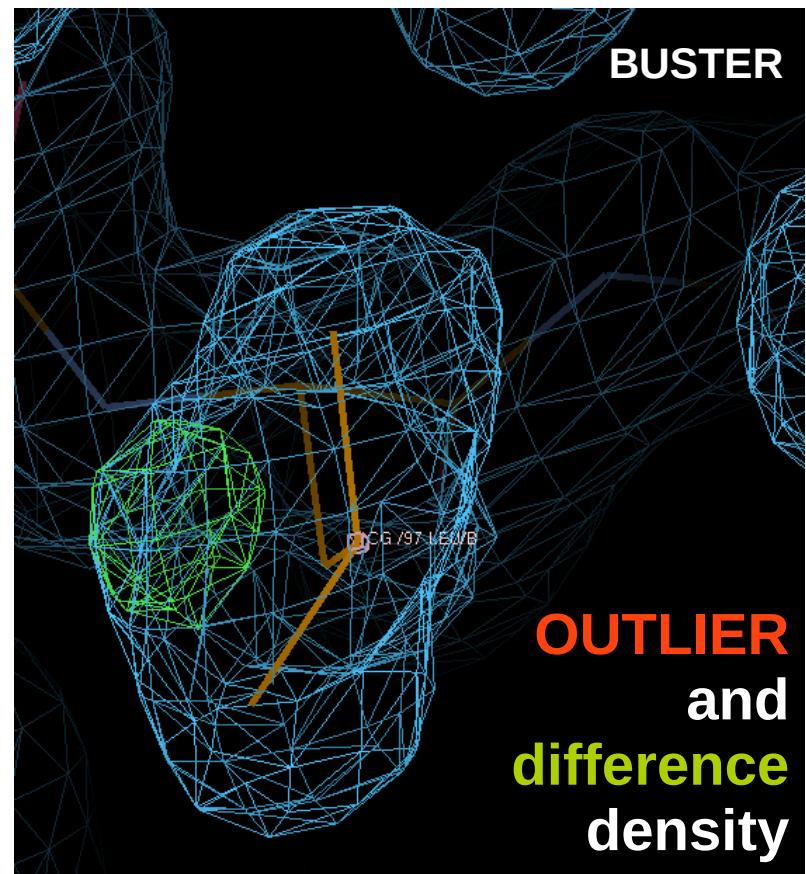
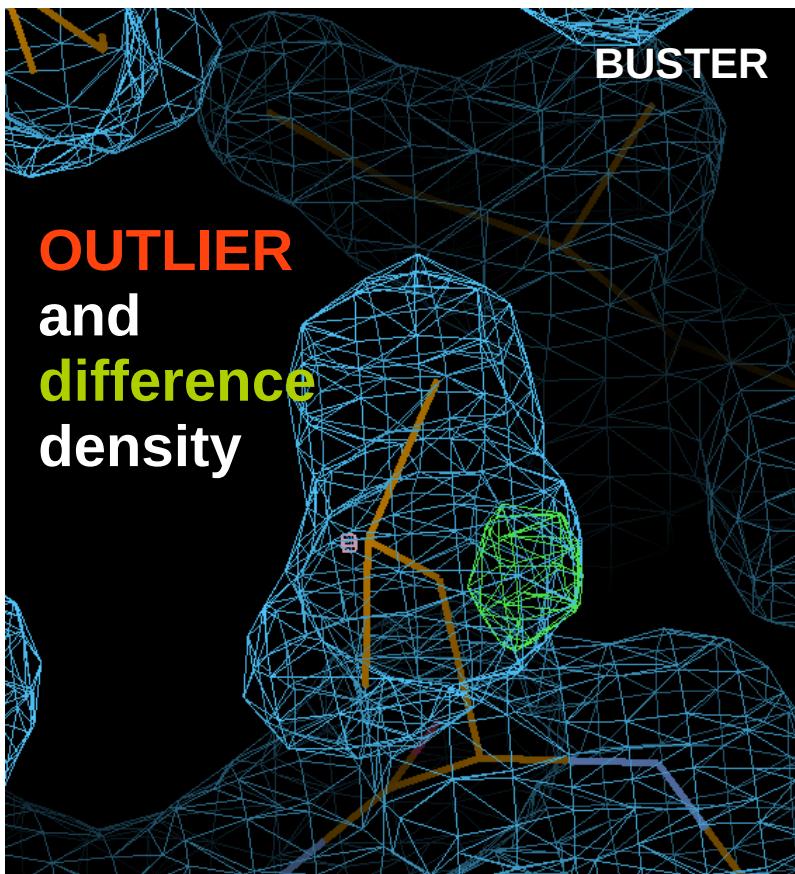
# Rotamer outliers are useful markers



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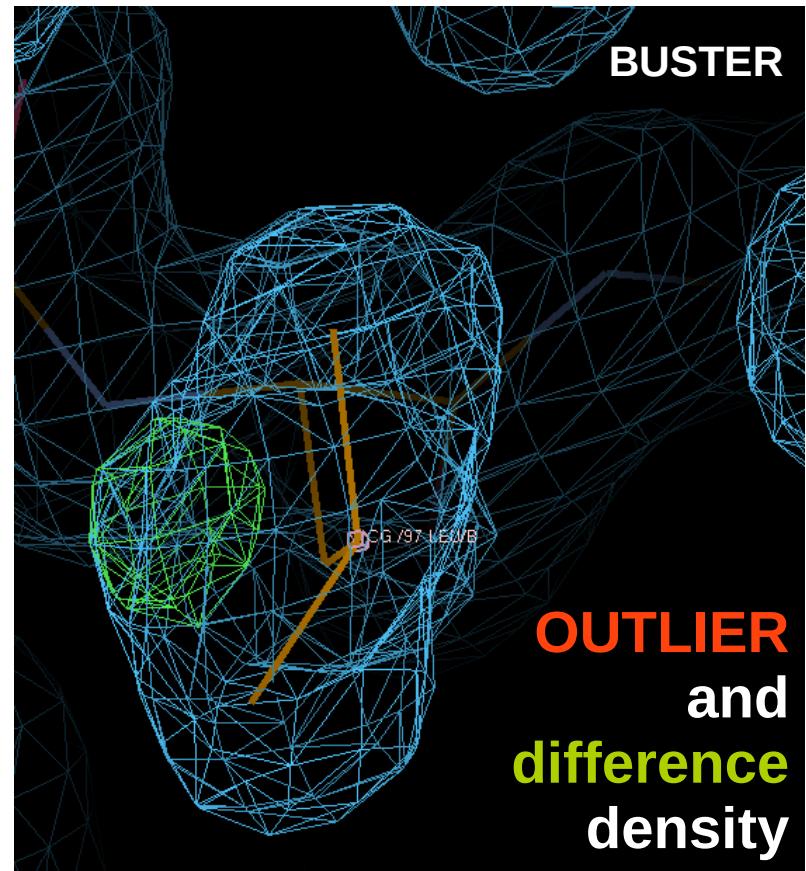


Do not use *too many* additional restraints to keep geometry (apparently) under control if underlying problem is incorrect atomic model - as visible in density!

# Rotamer outliers are useful markers



The big question: How much is *too many*?



Do not use *too many* additional restraints to keep geometry (apparently) under control if underlying problem is incorrect atomic model - as visible in density!

# BUSTER: Is there any ligand bound?

*Acta Cryst.* (2005). A61, C248

## Automated Structure Refinement for High-throughput Ligand Detection with BUSTER-TNT

Clemens Vonrhein, Gerard Bricogne, Global Phasing Ltd., Cambridge, UK. E-mail: vonrhein@GlobalPhasing.com

The use of crystallography for the discovery of lead compounds often involves a large number of experiments with different soaking or co-crystallization trials. The subsequent refinement and analysis of the resulting datasets can be time-consuming and tedious. Since the crystallographic parameters (resolution, space group, cell parameters) are quite often similar, this task is ideally suited for automation.

We present a method (**autoBUSTER**) that automates the refinement, solvent model update, ligand detection and analysis. Centered around the BUSTER-TNT program [1,2], it requires a minimal amount of user input. Although it can be used at any resolution and for any kind of macromolecular structure, it is tuned to the refinement of protein structures at better than 2.8 Å resolution.

The knowledge of any (possibly) bound ligand can be given (a) explicitly by supplying a PDB file of dummy atoms that describes the assumed binding site, or (b) by letting the system automatically analyze the residual density of difference Fourier maps. A unique feature of BUSTER-TNT is used, where the various masks describing the known fragment, the bulk solvent and the missing part can be given independently from each other. The results show that this can greatly enhance the capability of uniquely defining any bound ligand.

[1] Bricogne G., Irwin J., *Macromolecular Refinement: Proceedings of the CCP4 Study Weekend*, Warrington: Daresbury Laboratory, 1996, 85-92. [2] Blanc E., Roversi P., Vonrhein C., Flensburg C., Lea S. M., Bricogne G., *Acta Cryst.*, 2004, D60, 2210-2221.

- BUSTER's '**-L**' feature tries to take an **unbiased look** at data & model to decide **if, where and how** something **might** have bound.
- No prior knowledge (bias?) required/used.
- Used since 2003, with first academic release in 2009

Liebschner et al (2017): "Polder maps: improving OMIT maps by excluding bulk solvent.", *Acta D* 73, 148

# BUSTER: Is there any any ligand bound?

**GΦL LigandDetectionModes**

□ Favorite?  Search       

(You are *AnonymousGnome*)

Content:

- [Introduction](#)
- [Unknown location](#)
- [Known location](#)
- [Caveats](#)
- [Summary](#)

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## Introduction

Apart from the standard procedures for detecting ligands (difference Fourier maps), BUSTER has one particular feature that needs a bit further explanation - to explain what it can do, what it can't do and what potential bias it might introduce.

This feature is triggered by the `-L` and `-Lpdb` flags to the `refine` command:

```
% refine -p some.pdb -m some.mtz -L ...
- or -
% refine -p some.pdb -m some.mtz -Lpdb bindingsite.pdb ...
```

It will treat a certain region of the model differently during the last big cycle of refinement: that region will neither contain an atomic model nor a contribution from the bulk solvent. However, if there is some electron density present in this region, it should show up in difference ( $F_o - F_c$ ) maps as strong positive density. The interpretation, what this electron density might represent (atomic model, bulk solvent or a mixture) is up to the user.

Here we're going to explain the typical usage of this BUSTER feature, their assumptions and caveats.

---

## Unknown location

The least biased assumption is that there might be some ligand bound, but its location is unknown. In that case one would use the

- Unbiased towards “expected” binding (just because the crystal was soaked/co-crystallised with compound doesn’t mean it actually is bound)
- Unbiased towards binding site (there can be unexpected or new binding sites)
- Unbiased towards binding pose

[BUSTER wiki](#)  
[www.globalphasing.com/buster/wiki/](http://www.globalphasing.com/buster/wiki/)

# Difference Fourier maps - simple & useful

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- **Anomalous, F(+) - F(-):**
  - distinguish ion from solvent
  - help difficult sequencing (Cys/Met marker)
  - help placing of compound
  - automatically generated and analysed in BUSTER
- **F(early) - F(late):**
  - can show radiation damage effects
  - automatically prepared in autoPROC - then generated and analysed in BUSTER
- **F(obs-1) - F(obs-2):**
  - (Compound - Apo) to show compound

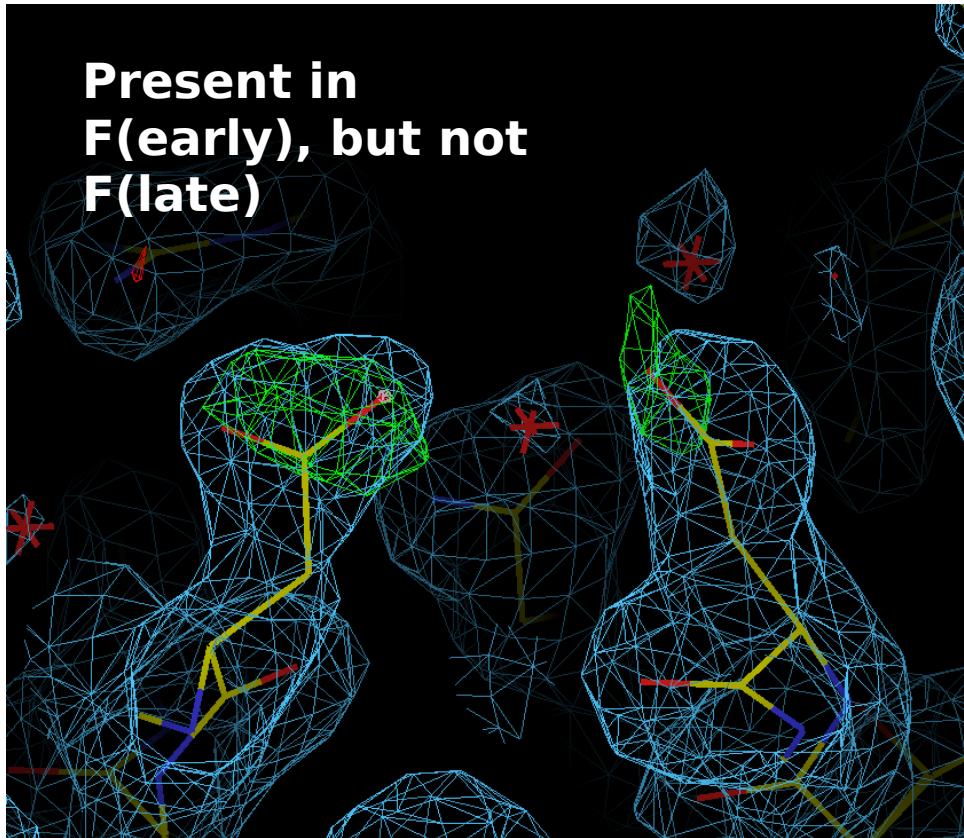
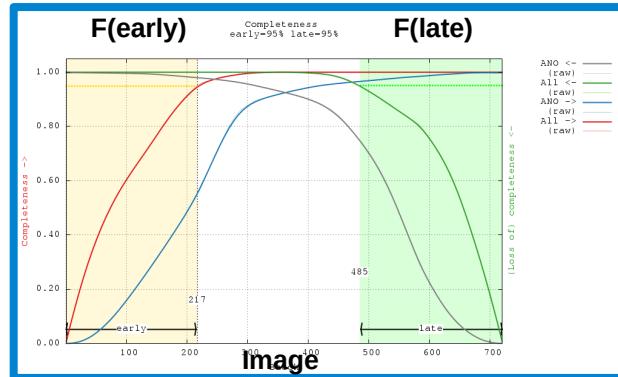
```
diff_fourier -h
```

# Difference Fourier maps: F(early)-F(late) detecting/describing radiation damage

Typical decarboxylation of ASP/GLU residues, damage on Cys, Met, Br, metals ...

Normal mFo-Dfc maps will show negative peaks.

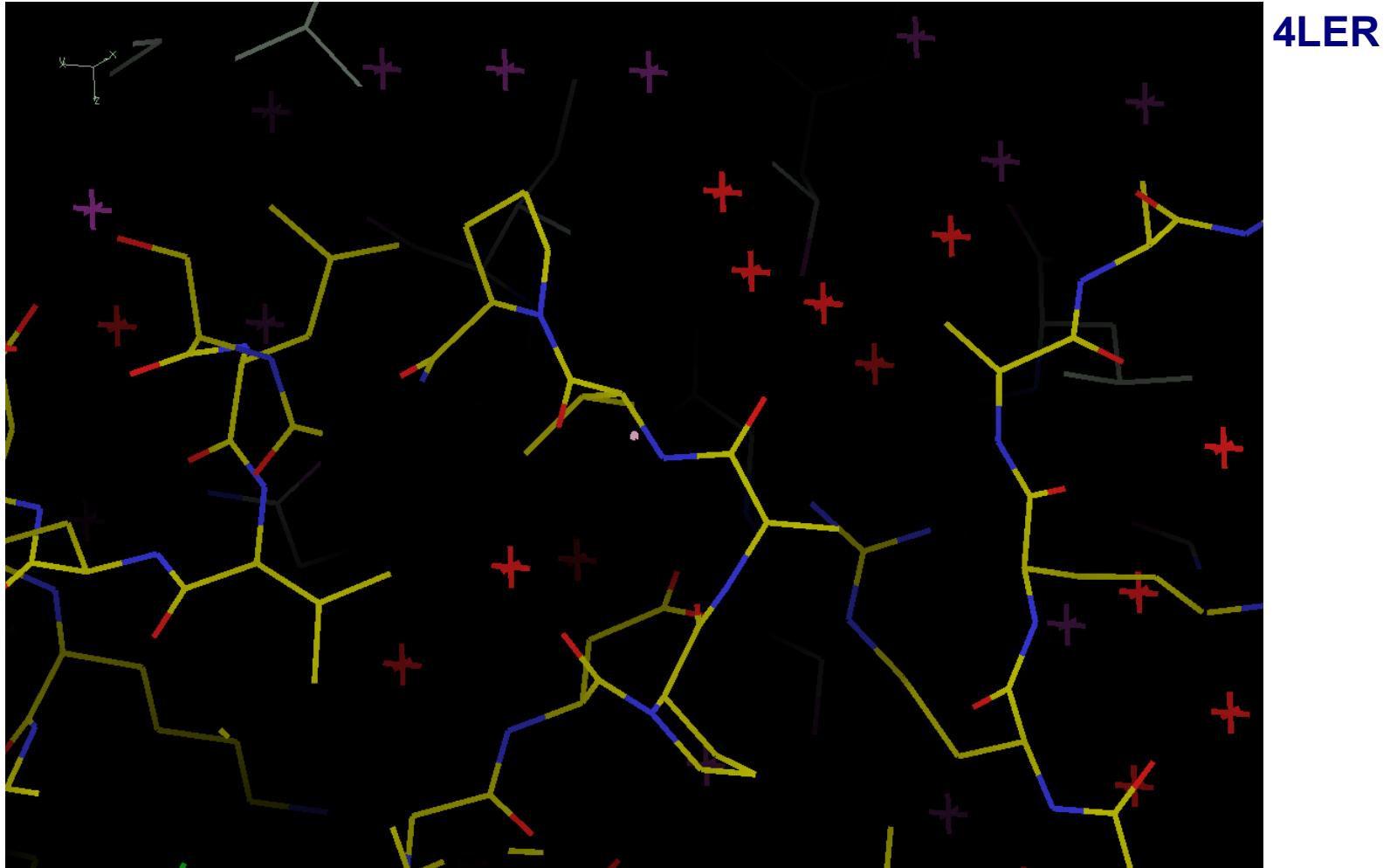
autoPROC:



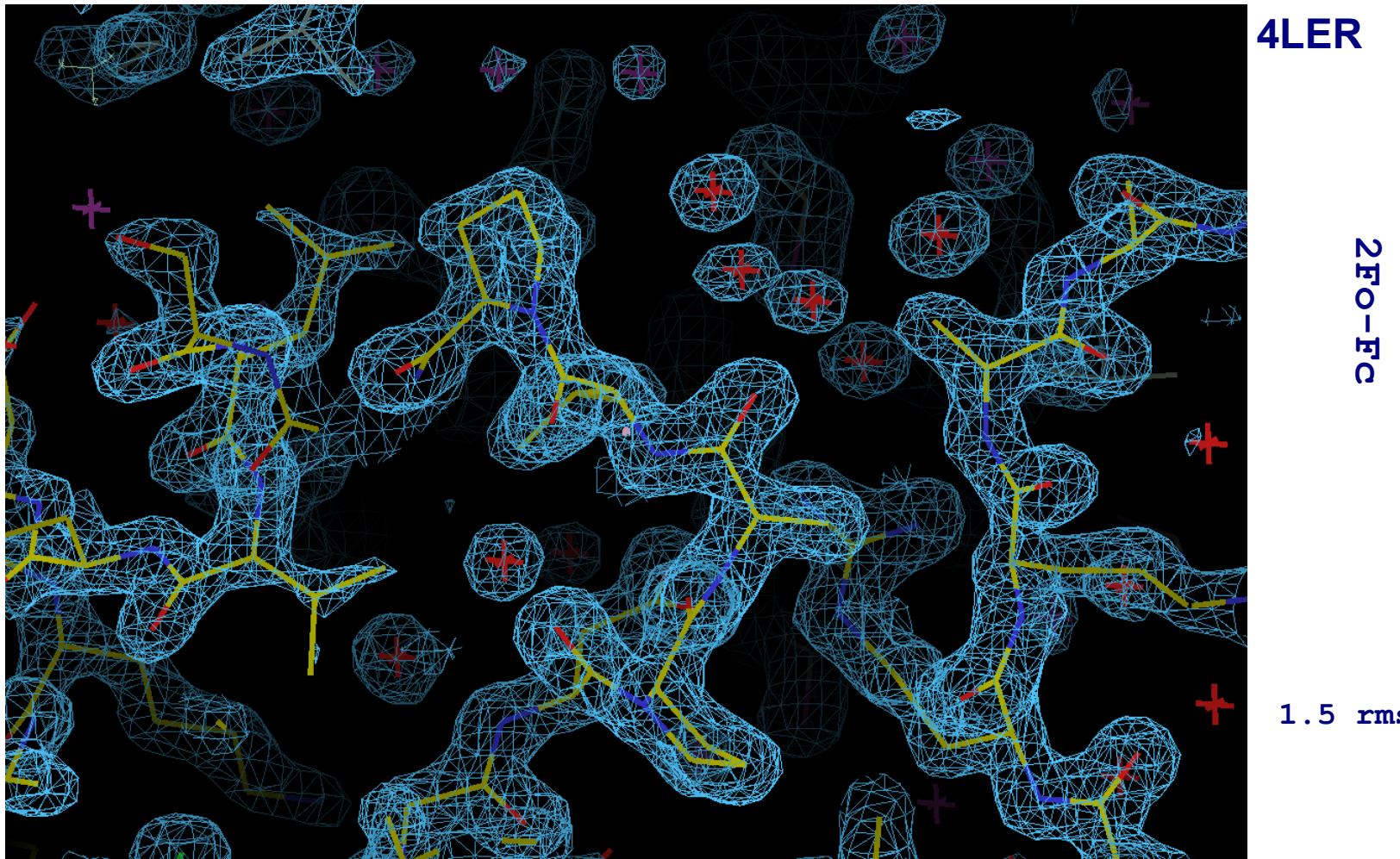
**low dose, high-multiplicity**

Vonrhein et al (2024). Acta Cryst. D, 80, 148-158.

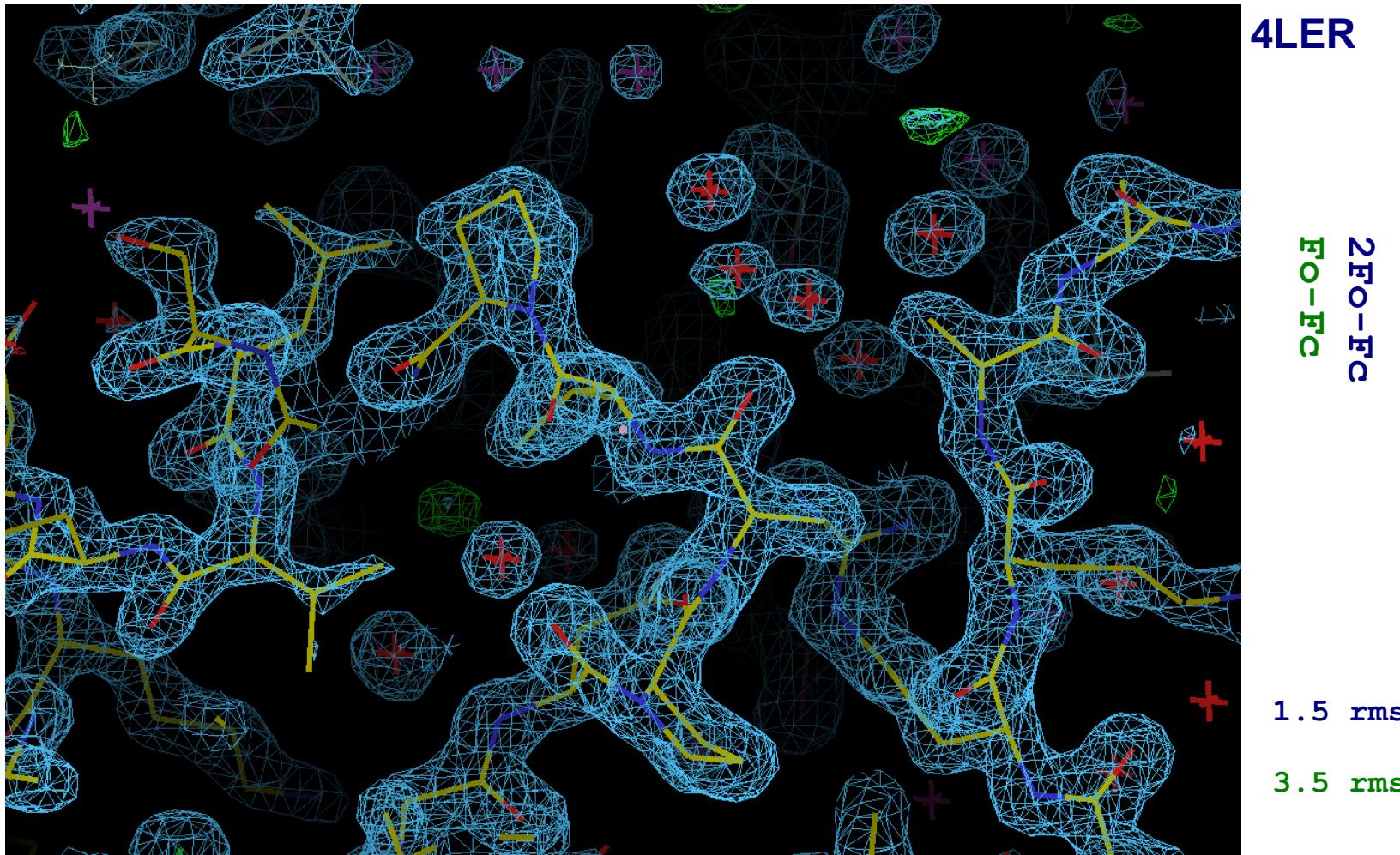
# F(early)-F(late): water in crystal contact



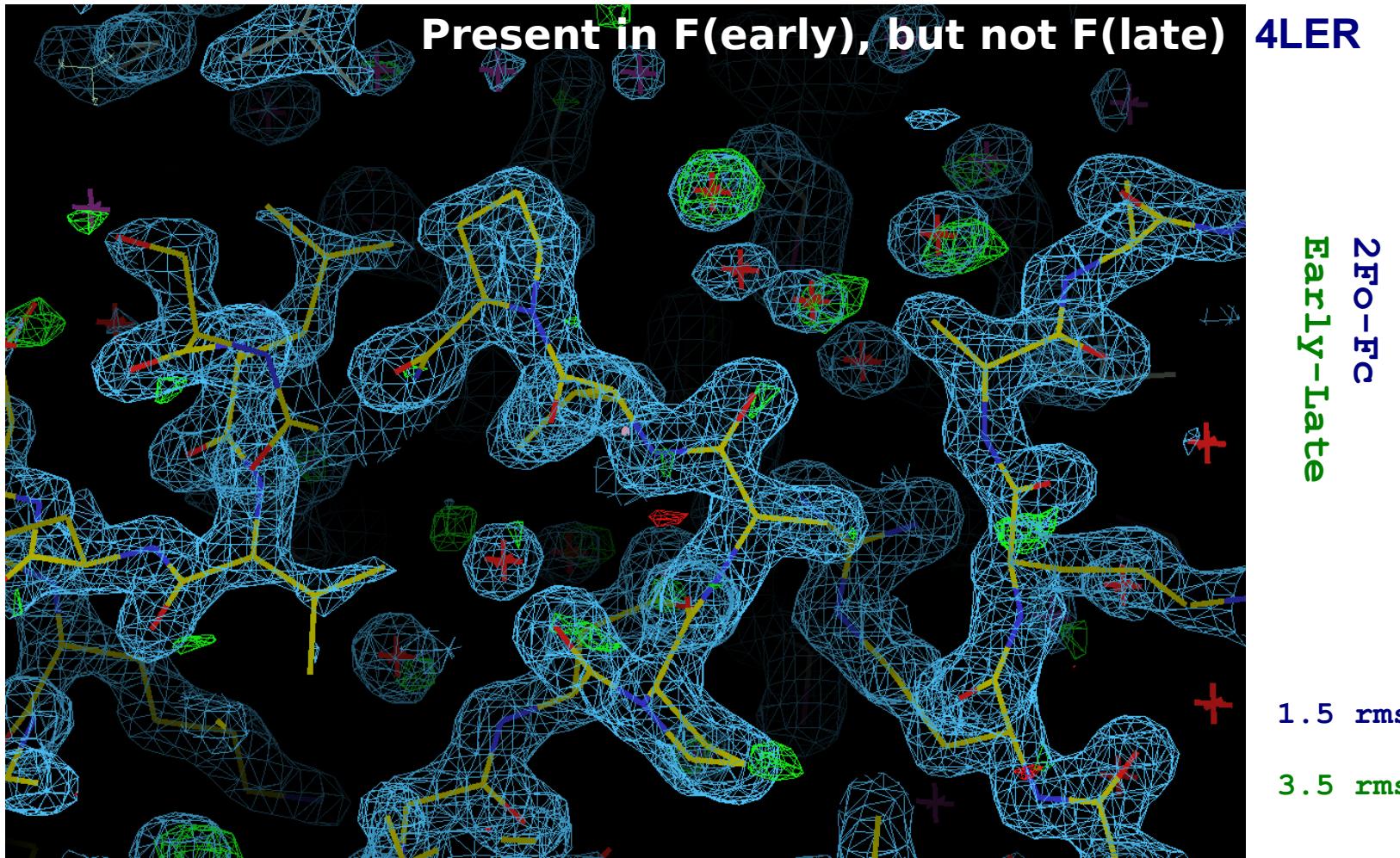
# F(early)-F(late): water in crystal contact



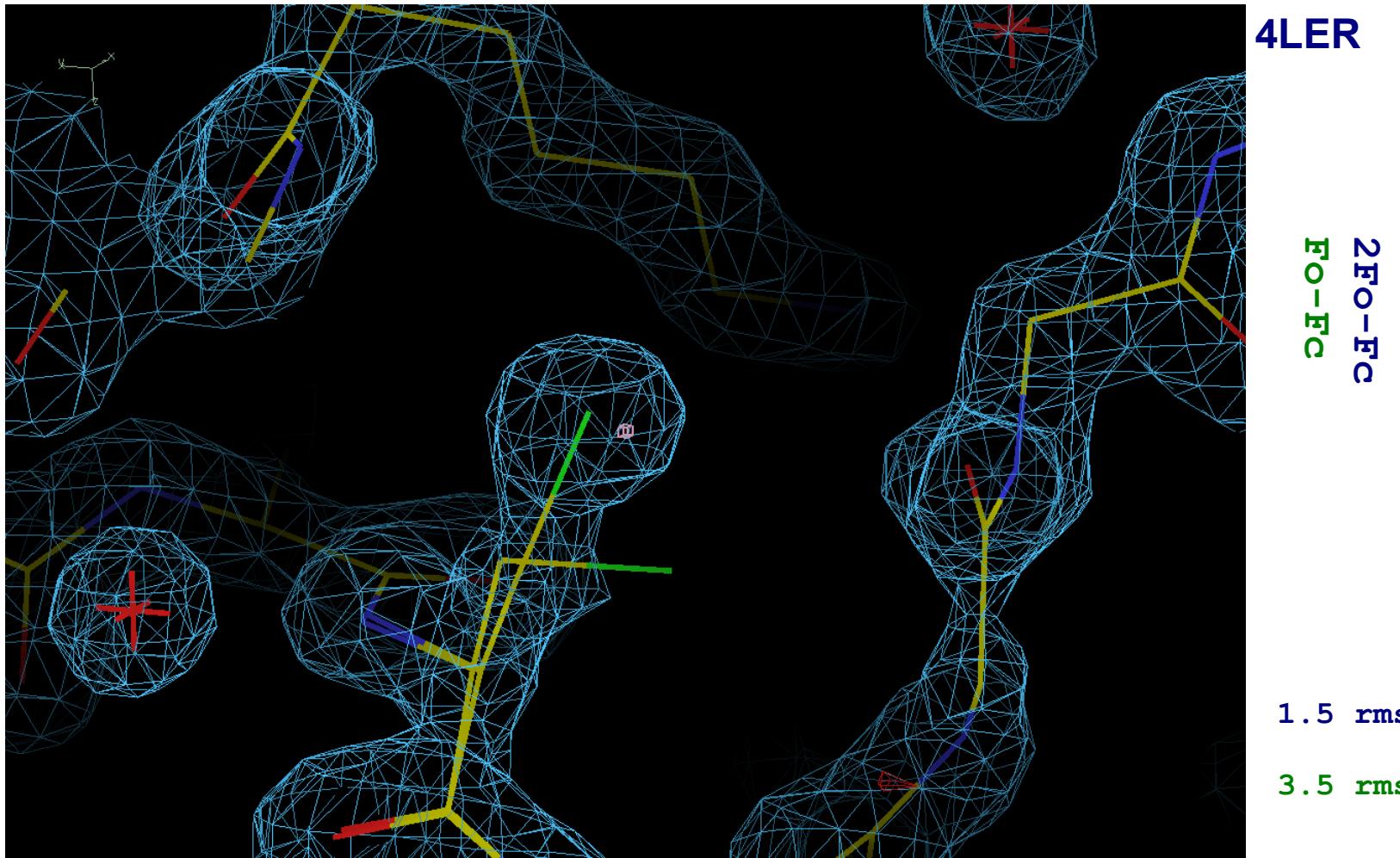
# F(early)-F(late): water in crystal contact



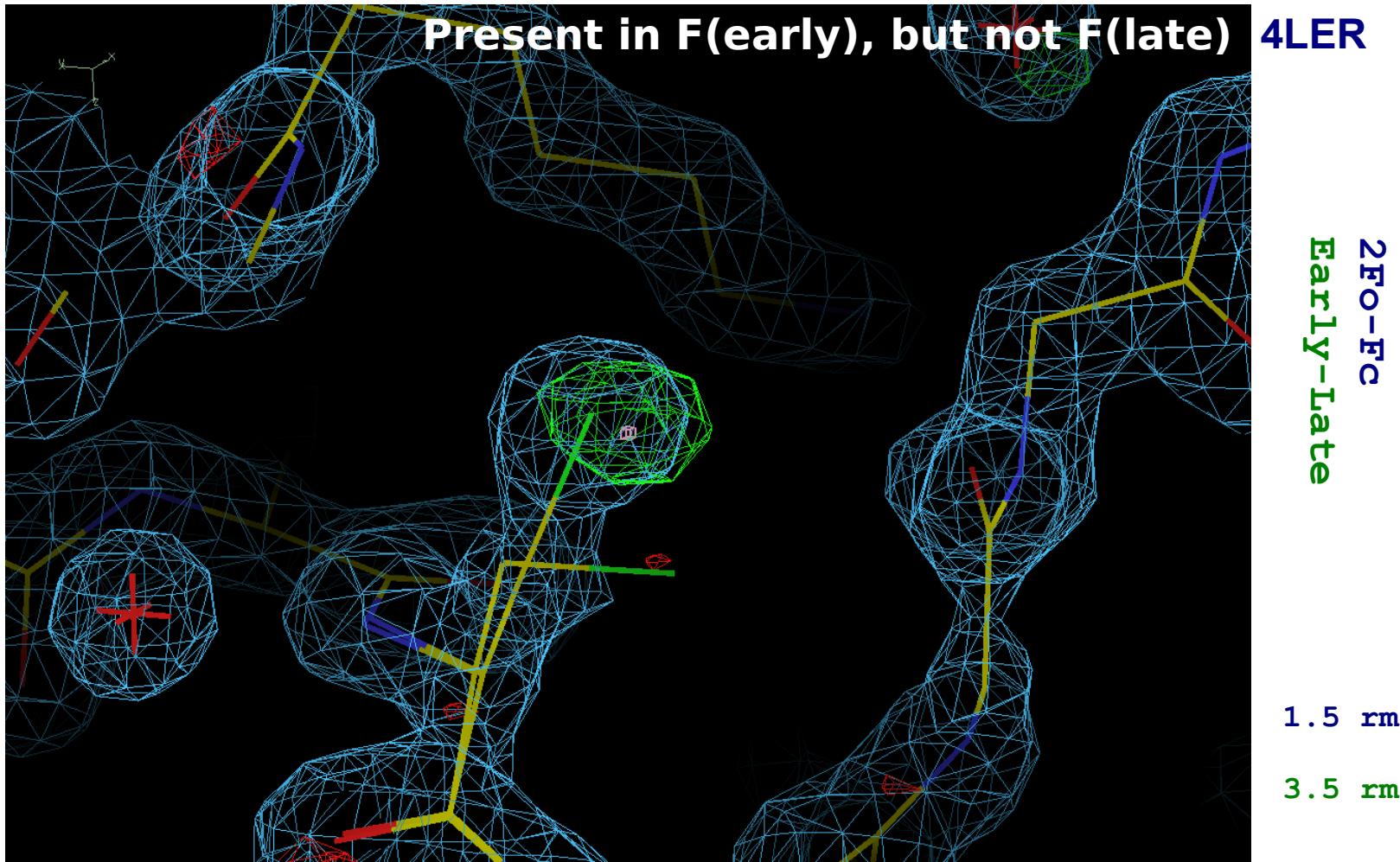
# F(early)-F(late): water in crystal contact



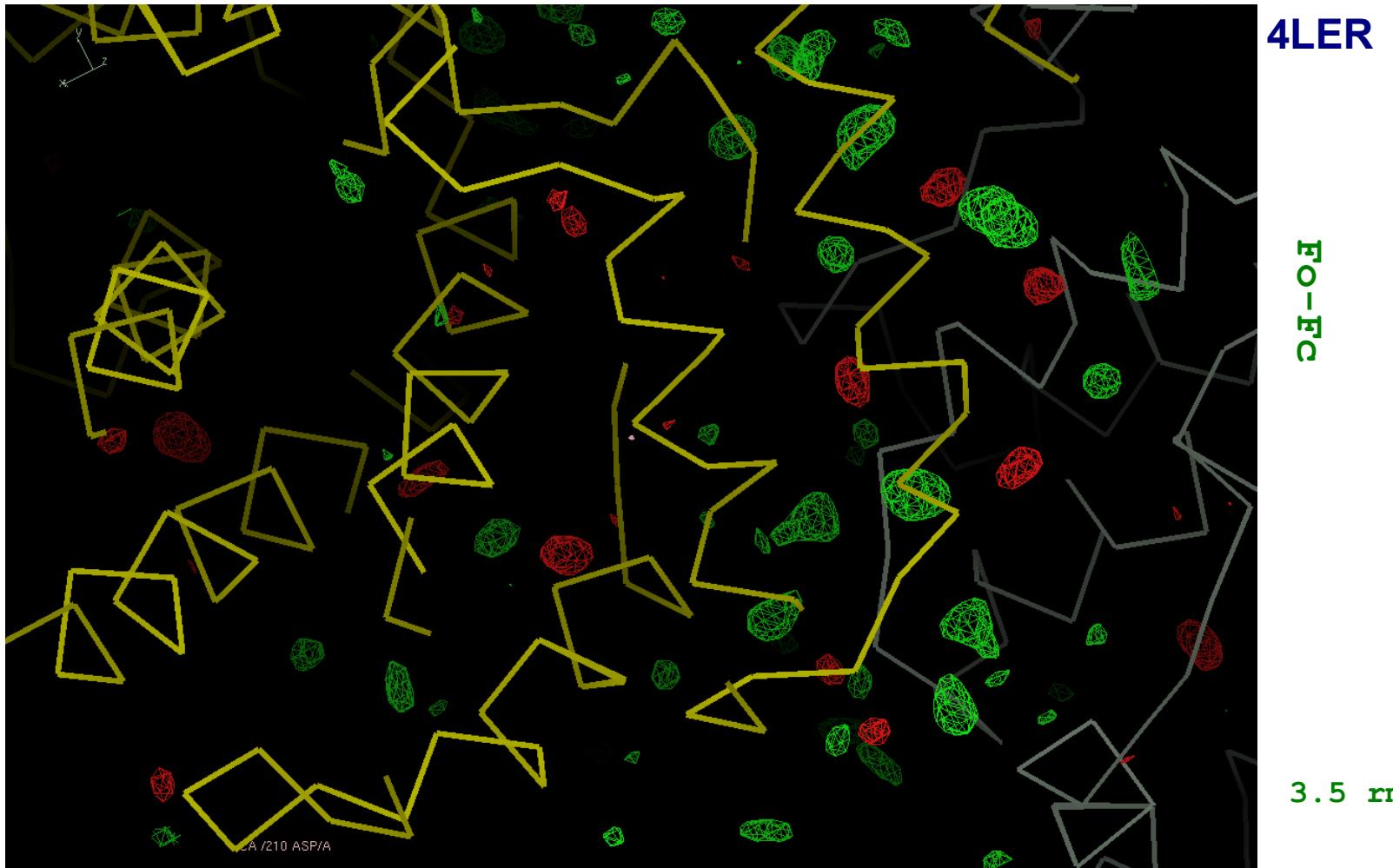
# F(early)-F(late): alternate conformation?



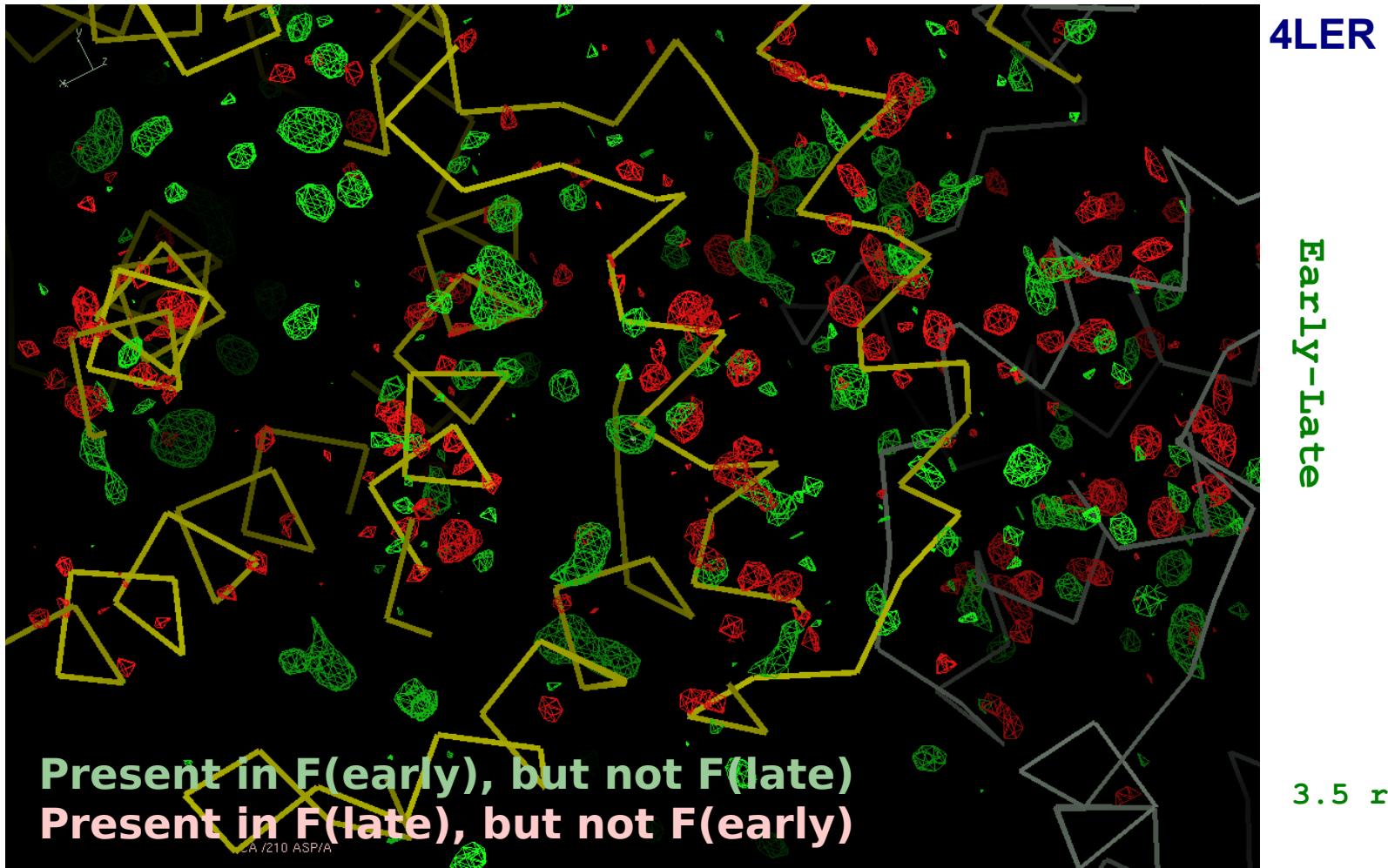
# F(early)-F(late): alternate conformation?



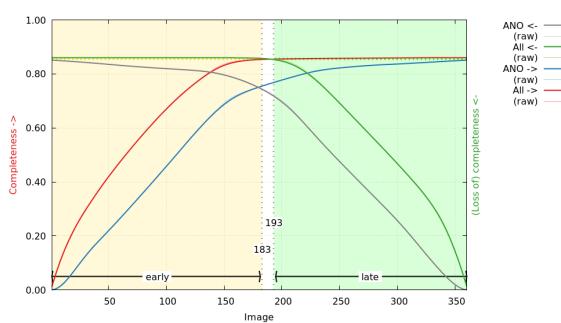
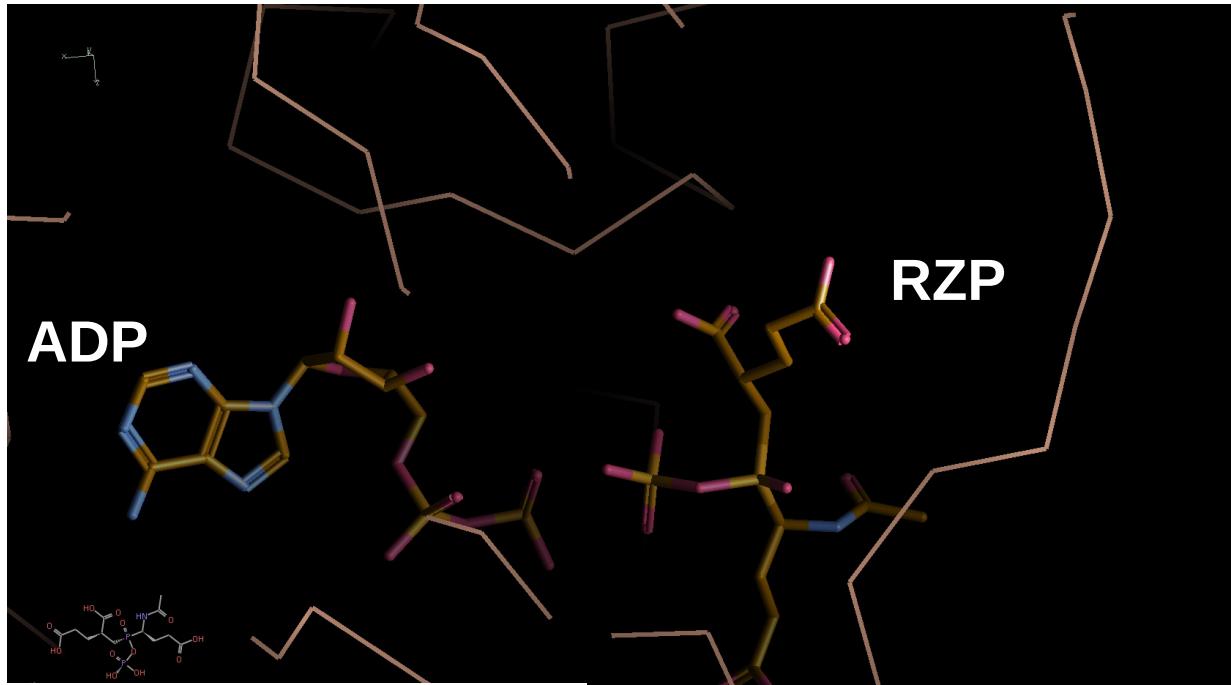
# F(early)-F(late): large-scale movements



# F(early)-F(late): large-scale movements



# Radiation damage - decarboxylation



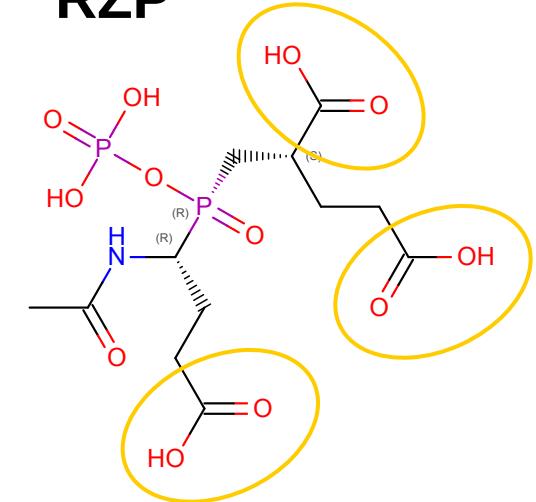
## 6VZU

Structural basis for polyglutamate chain initiation and elongation by TTLL family enzymes.

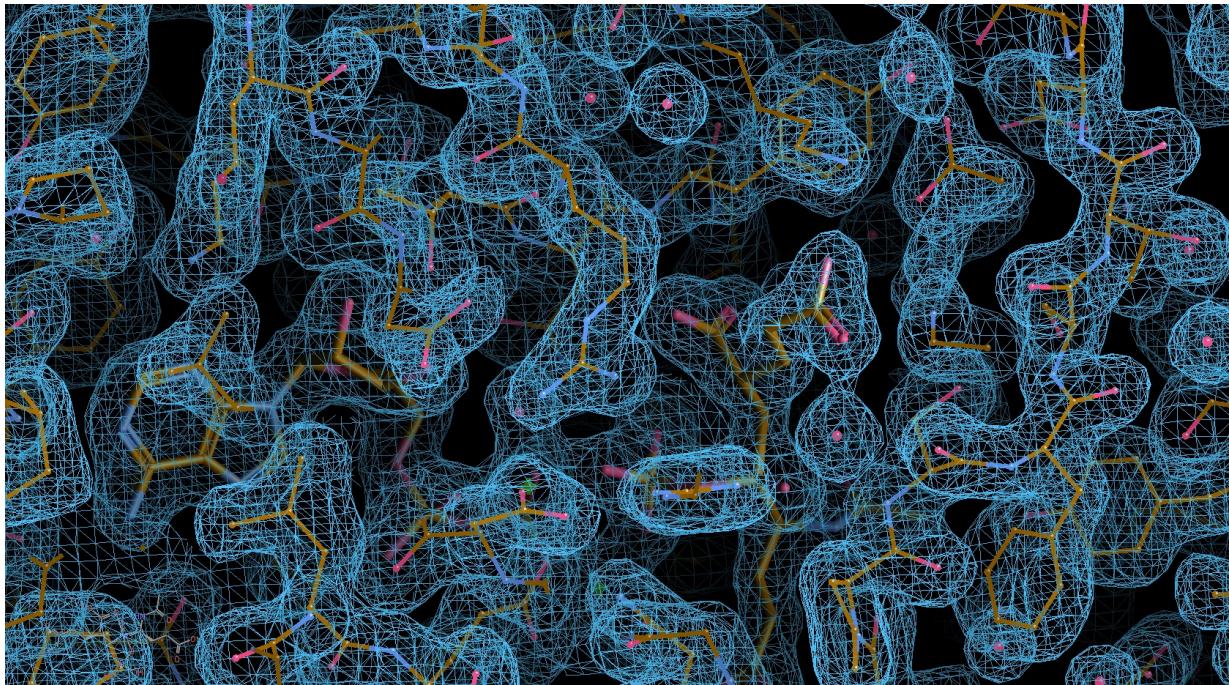
[Mahalingan, K.K., Keith Keenan, E., Strickland, M., Li, Y., Liu, Y., Ball, H.L., Tanner, M.E., Tjandra, N., Roll-Mecak, A.](#)

(2020) *Nat Struct Mol Biol* **27**: 802-813

## RZP



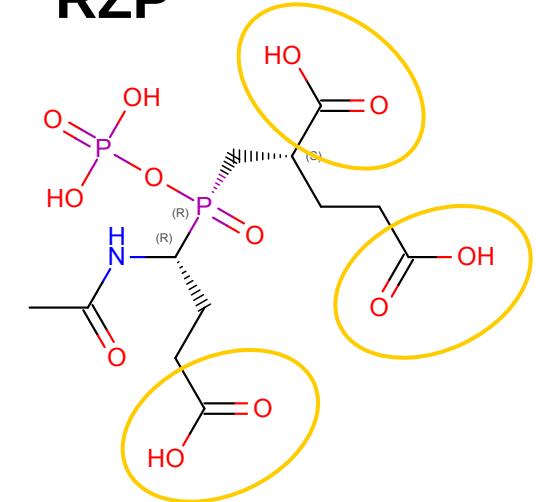
# Radiation damage - decarboxylation



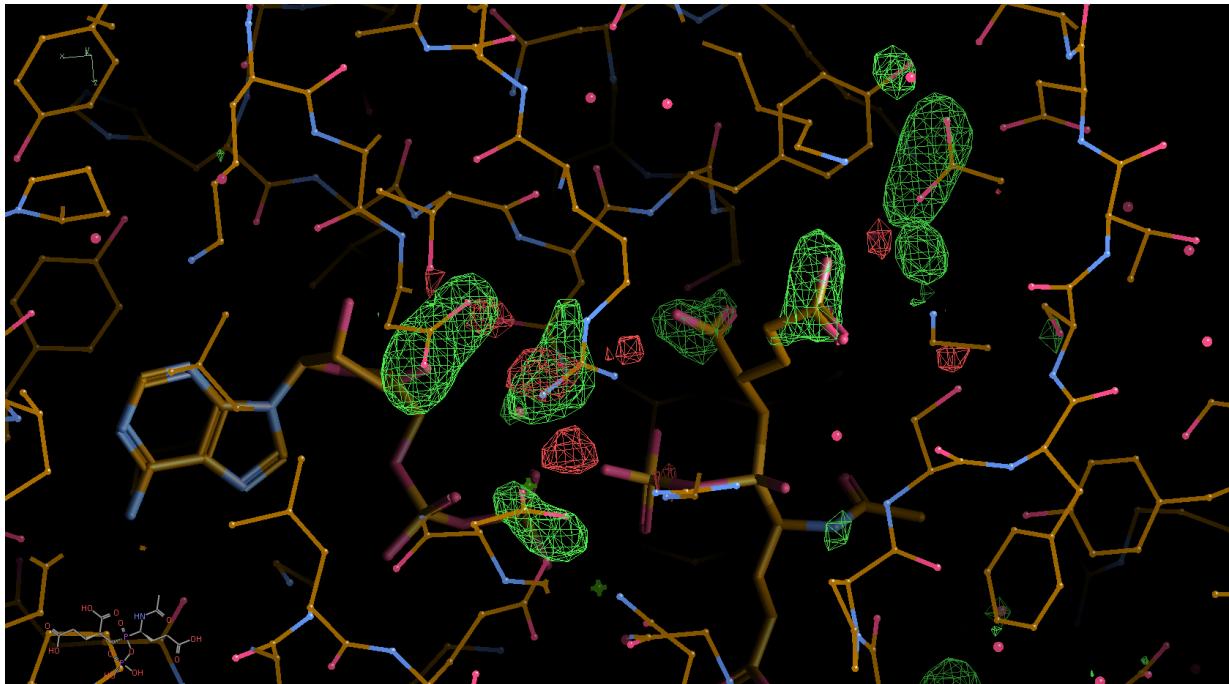
2mFo-DFc @ 1.0 rms, 2 Å  
resolution.

6VZU

RZP



# Radiation damage - decarboxylation

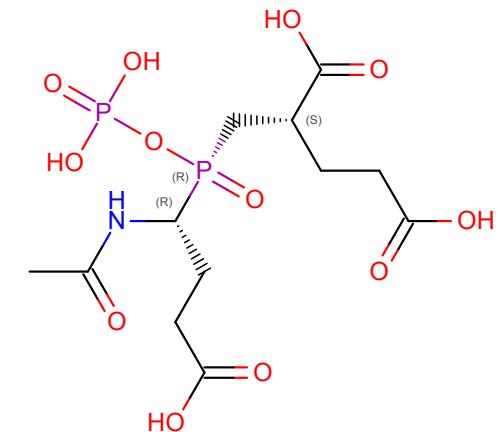


F(early)-F(late) @ 6.0 rms

Showing loss of density due to

- reduction in occupancy
- increase in temperature factor
- loss of atoms/groups
- movement of atoms/groups

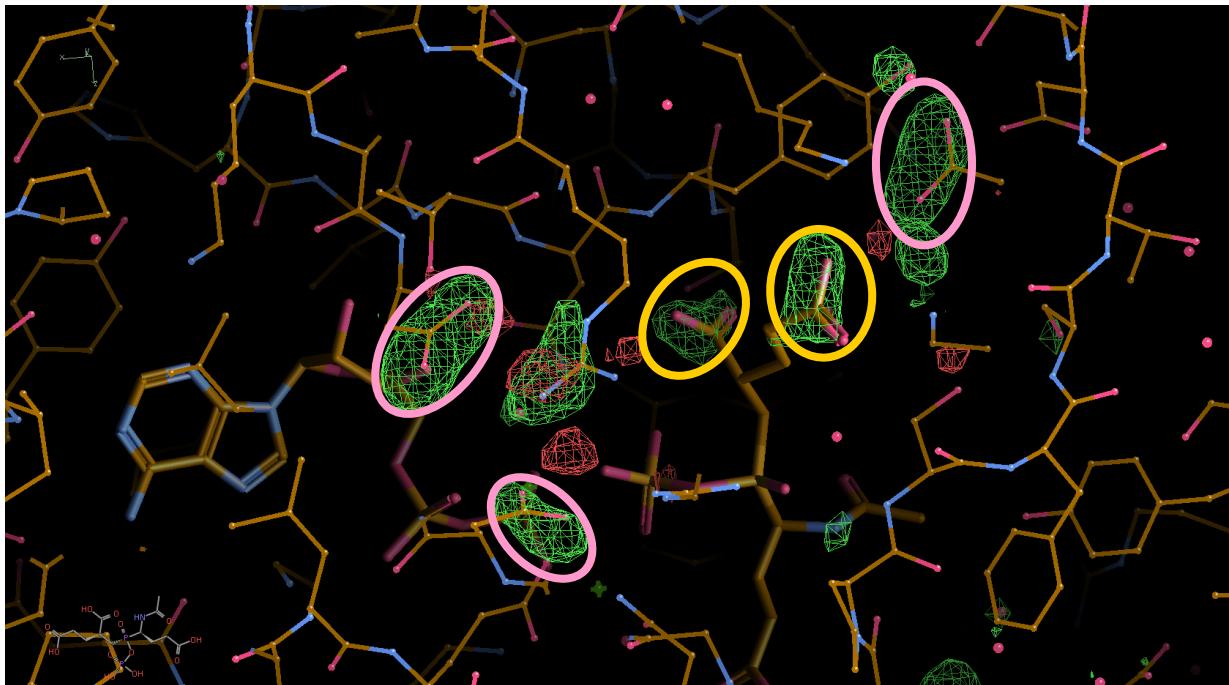
RZP



6VZU

- **early and late data** automatically generated by **autoPROC**
- **F(early)-F(late)** maps automatically generated and analysed by **BUSTER**

# Radiation damage - decarboxylation

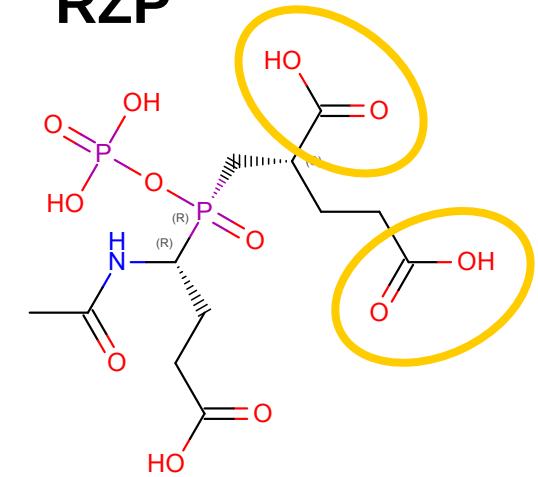


$\mathbf{F}(\text{early}) - \mathbf{F}(\text{late}) @ 6.0 \text{ rms}$

Showing loss of density due to

- reduction in occupancy
- increase in temperature factor
- loss of atoms/groups
- movement of atoms/groups

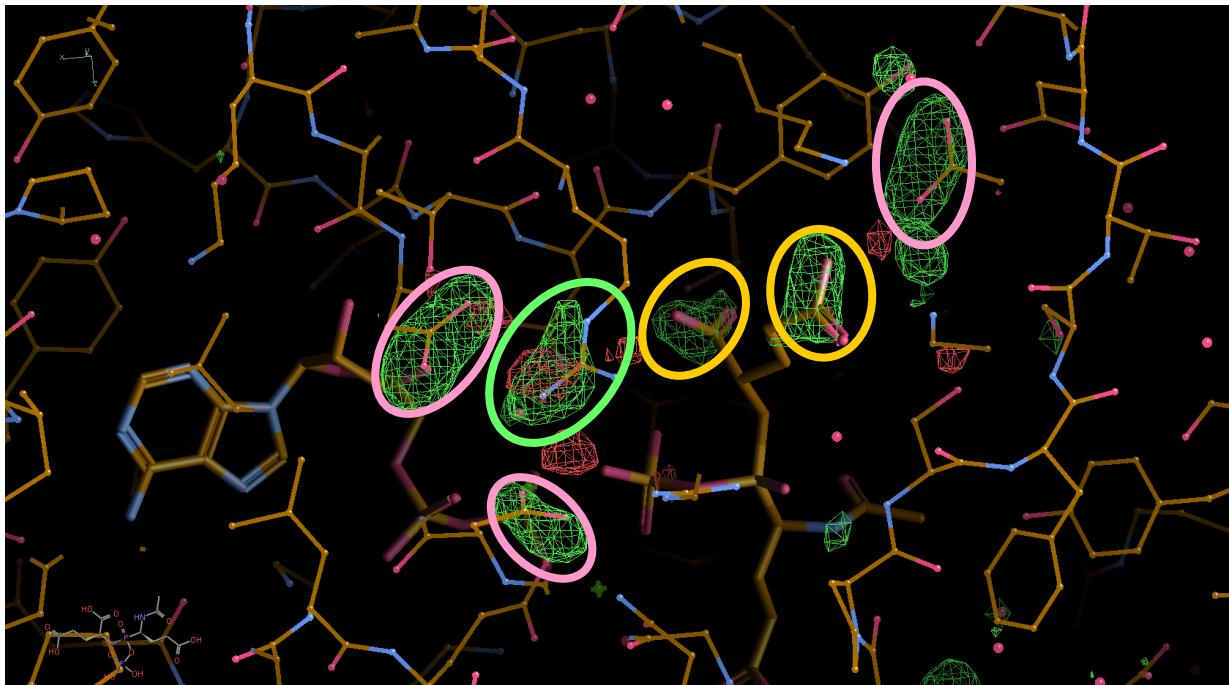
RZP



**6VZU**

de-carboxylation of ASP/GLU and compound

# Radiation damage - decarboxylation

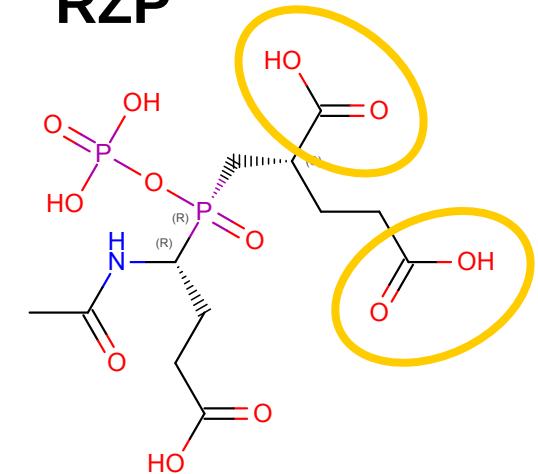


$\mathbf{F}(\text{early}) - \mathbf{F}(\text{late}) @ 6.0 \text{ rms}$

Showing loss of density due to

- reduction in occupancy
- increase in temperature factor
- loss of atoms/groups
- movement of atoms/groups

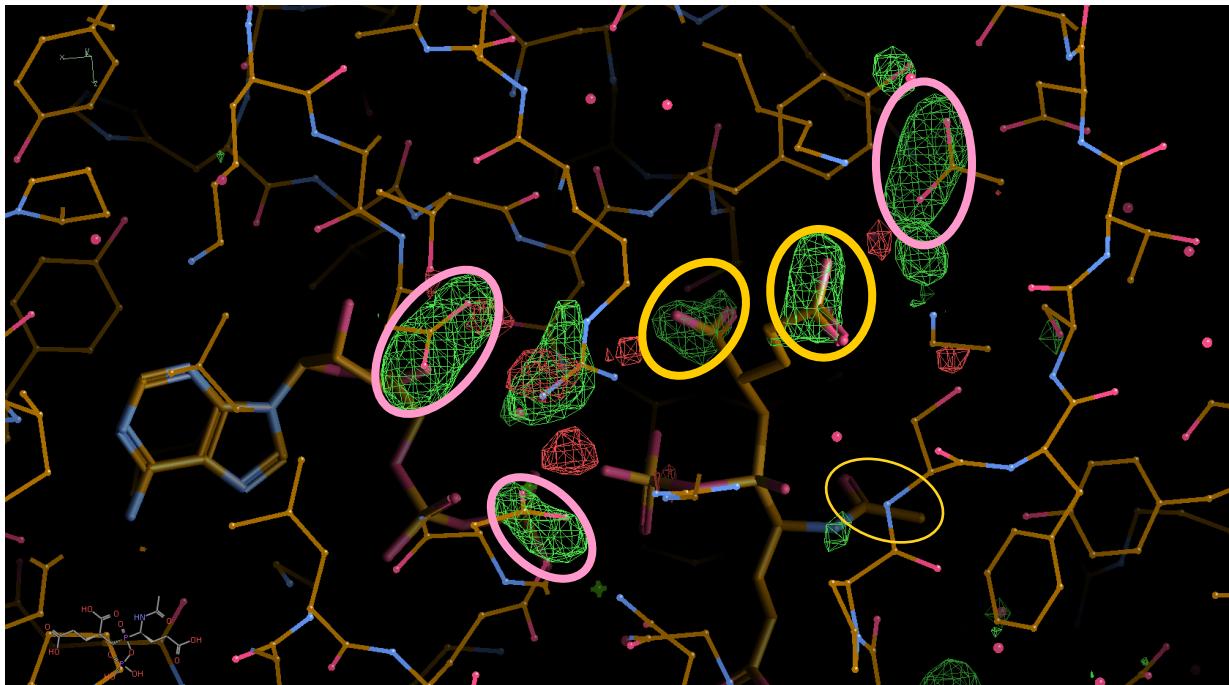
RZP



**6VZU**

de-carboxylation of ASP/GLU and compound

# Radiation damage - decarboxylation

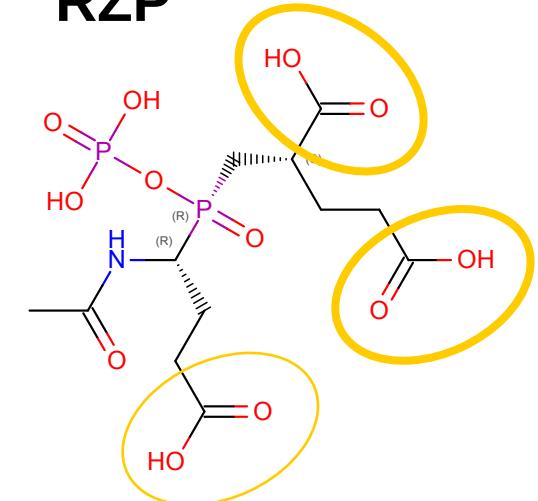


$\mathbf{F}(\text{early})-\mathbf{F}(\text{late}) @ 6.0 \text{ rms}$

Showing loss of density due to

- reduction in occupancy
- increase in temperature factor
- loss of atoms/groups
- movement of atoms/groups

RZP



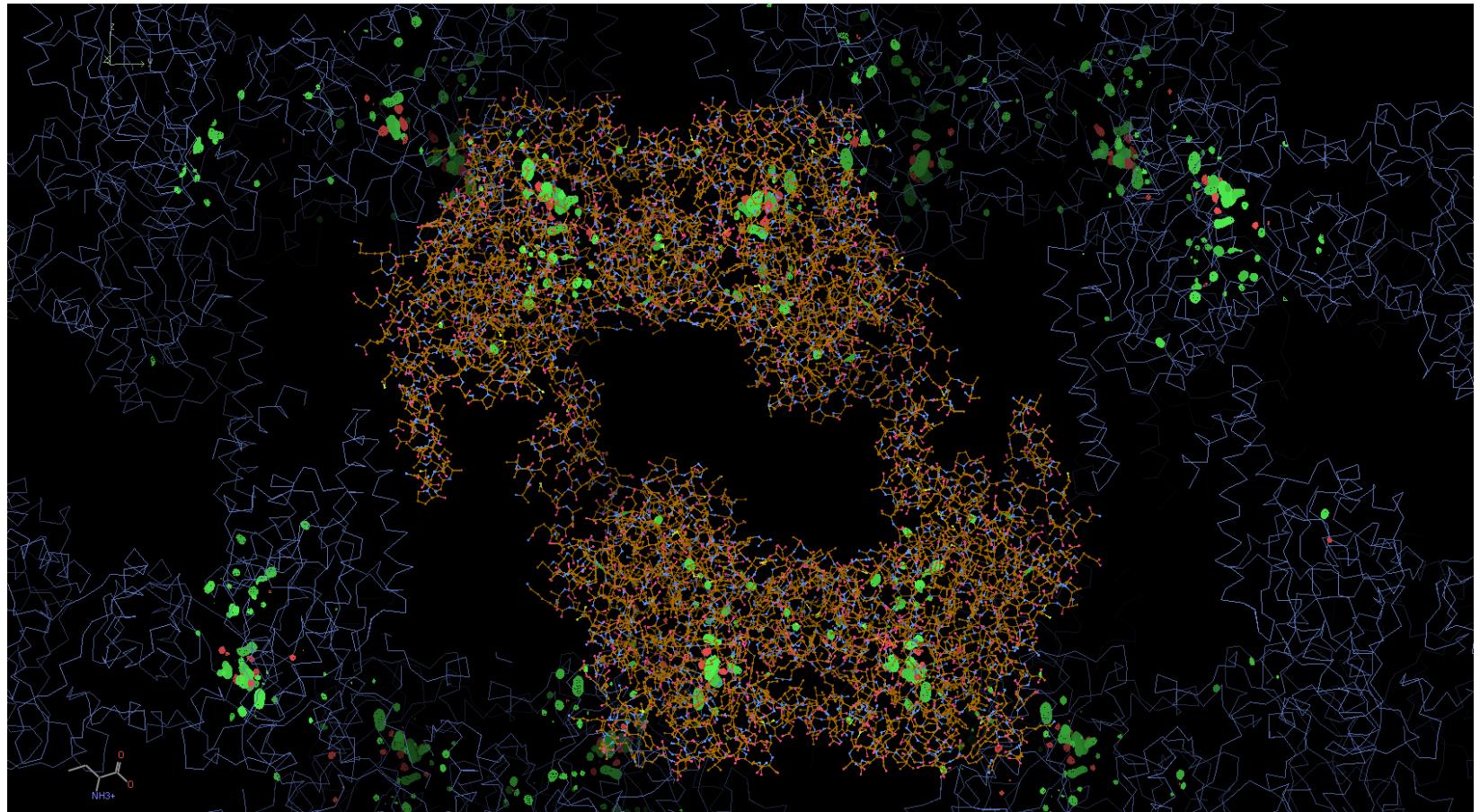
**6VZJ**

de-carboxylation of ASP/GLU and compound

not every carboxy group suffers radiation damage to the same extent

# Radiation damage - decarboxylation

6VZU

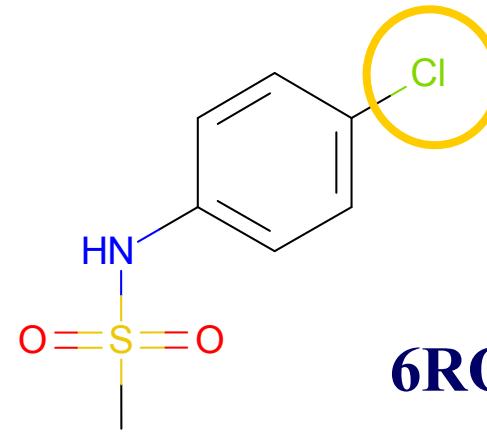
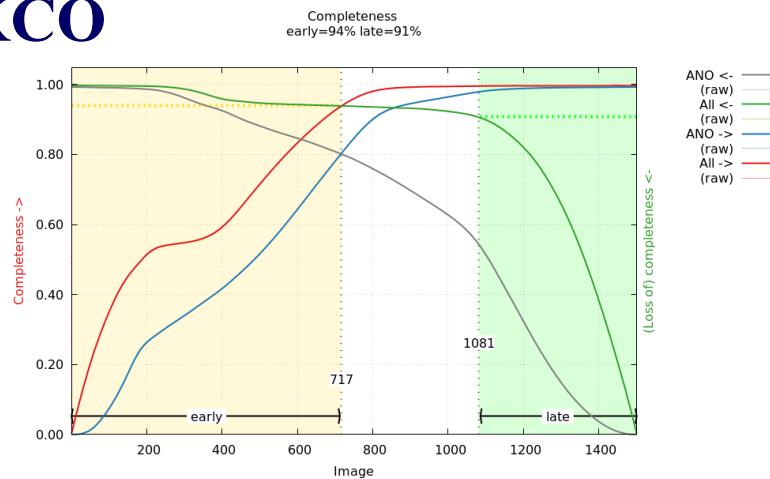


F(early)-F(late) @ 6.0 rms

showing hotspots and “connected” regions

# Radiation Damage - improved model parametrisation

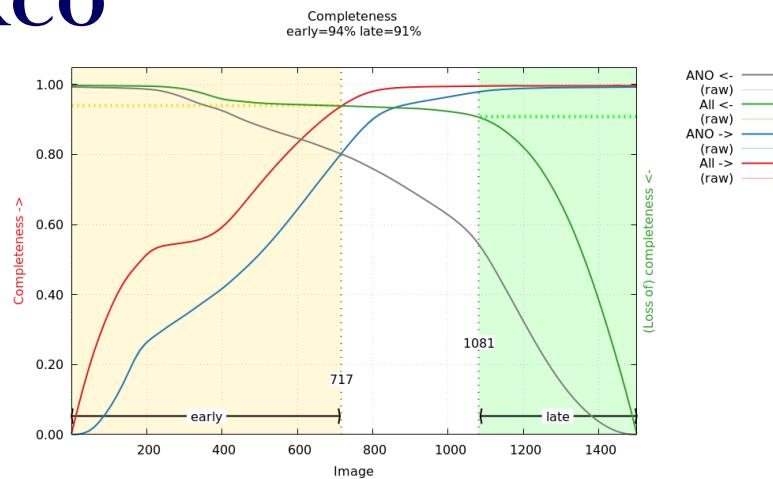
**5KCO**



**6RO**

# Radiation Damage - improved model parametrisation

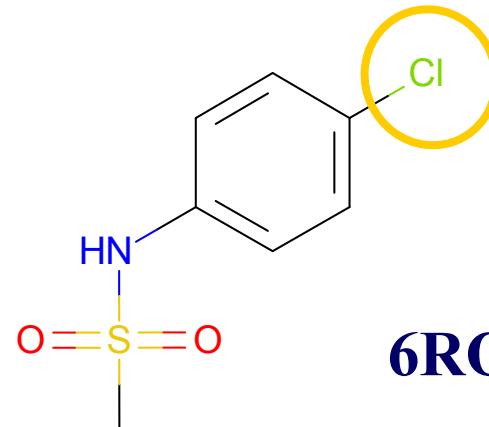
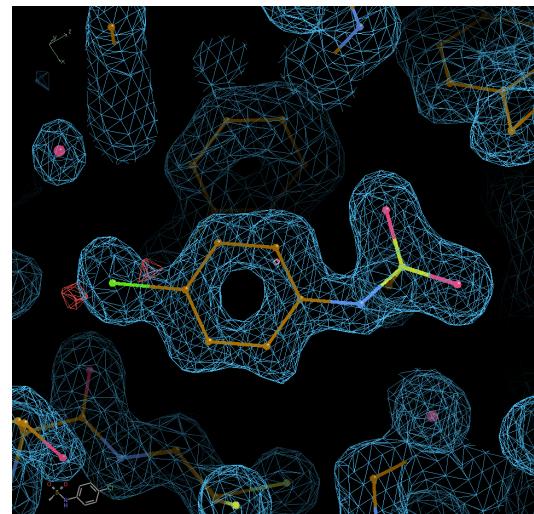
## 5KCO



2mFo-DFc @ 1.0  
rms

mFo-DFc @ 3.5  
rms

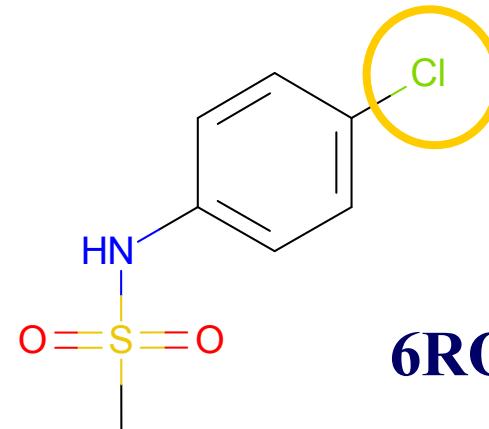
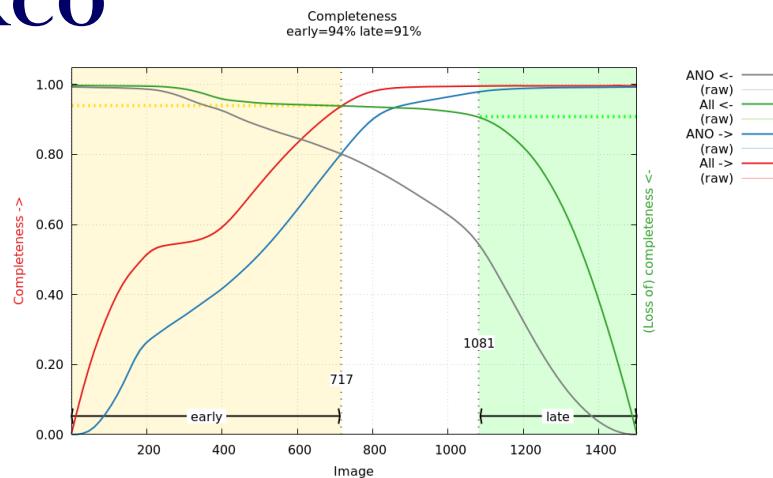
very  
clean



## 6RO

# Radiation Damage - improved model parametrisation

**5KCO**

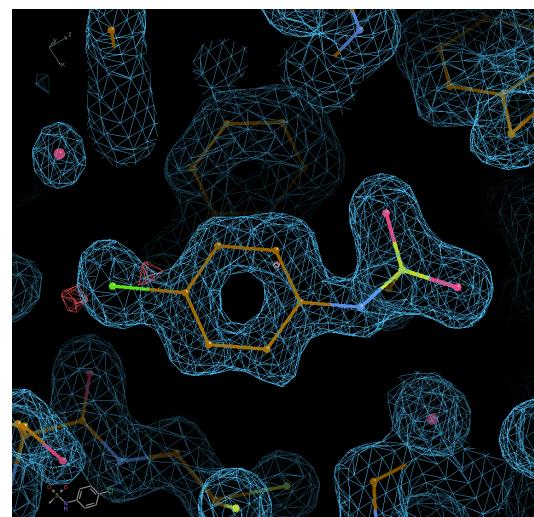


**6RO**

**2mFo-DFc @ 1.0 rms**

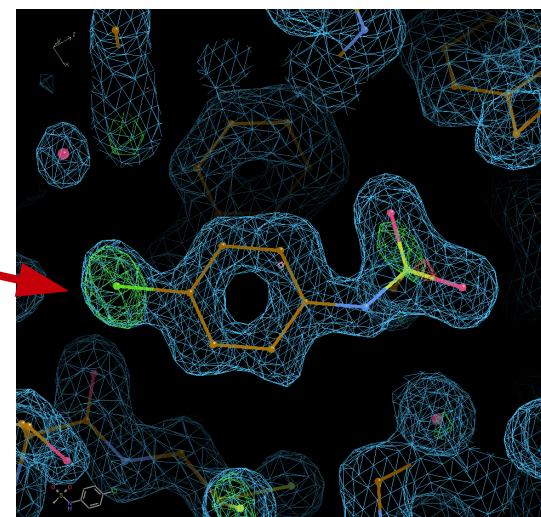
**mFo-DFc @ 3.5 rms**

**very clean** →



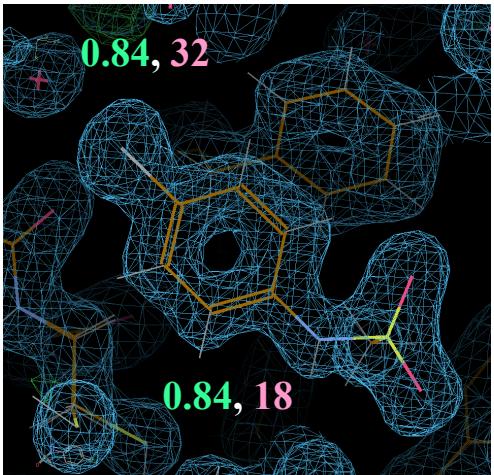
**F(early)-F(late) @ 4.0 rms**

**strong peak on chlorine** →

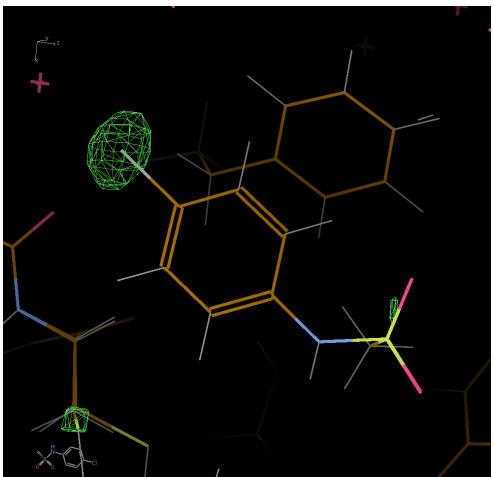


Re-refined  
2mFo-DFc @ 1.0 rms  
mFo-Dfc @ 3.5 rms

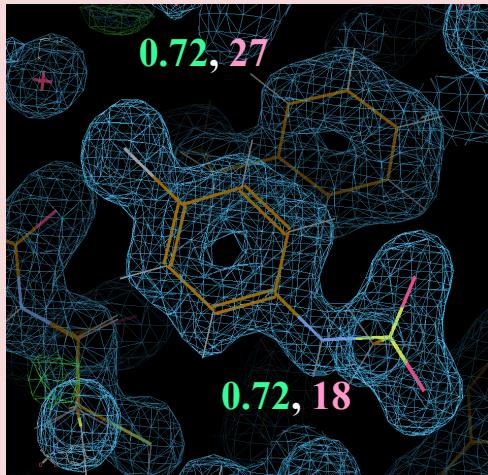
Deposited



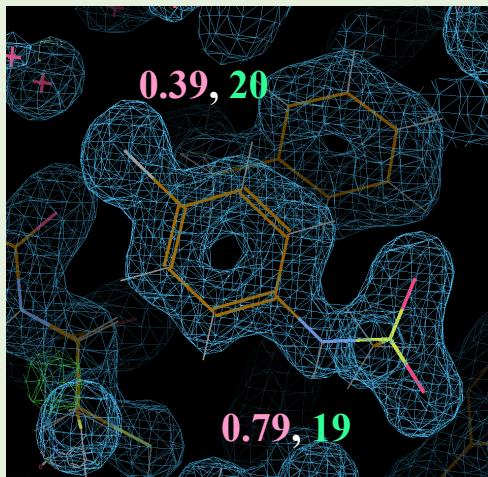
F(early)-F(late)  
@ 5.0 rms



Re-processed  
single OCC



Re-processed  
split OCC



- 
- ❑ Careful processing of data can be crucial (not everything is Lysozyme)
    - A good collection strategy (that adapts to crystal quality, SG, orientation, detector, beamline, goniostat, beam characteristics) will result in better data, better density, better understanding of optimal parametrisation and better models (as in “models fitting the experimental data and prior knowledge”)
  - ❑ Collect all prior knowledge
    - Geometric restraints (good ligand dictionaries)
    - Similarity restraints (NCS, targeting)
    - Occupancy refinement (beware of radiation damage!)
    - Correct formfactors (fluorescence scan)
    - Charges (`SupportPartialCharges=yes`)
  - ❑ Refine to convergence
    - Otherwise difference maps can be hard to interpret
    - And statistics like R/Rfree are (fairly) meaningless when used to compare models etc
  - ❑ Crystal and what happened to it during data collection is still (very) important at this stage
    - Radiation damage: F(early)-F(late) maps
    - Anisotropy (STARANISO)
  - ❑ Often the defaults (quick click) work well ... but to get the correct structure interpretation the **best/correct parametrisation and refinement is crucial**
-

# Acknowledgements

- ❑ Global Phasing, Cambridge (UK):
  - Gérard Bricogne, Leigh Carter, Claus Flensburg, Rasmus Fogh, Peter Keller, Wlodek Paciorek, Andrew Sharff, Oliver Smart, Ian Tickle
  - Thomas Womack, Eric Blanc, Gwyndaf Evans, Pietro Roversi, John Irwin, Eric de la Fortelle, Marc Schiltz
- ❑ CCDC (Cambridge Crystallographic Data Centre, Cambridge, UK)
- ❑ CCP4 (UK)
- ❑ Global Phasing Industrial Consortium members
- ❑ ... many, many users & collaborators

<https://www.globalphasing.com/>

<https://www.globalphasing.com/buster/wiki/> (Tutorials etc)