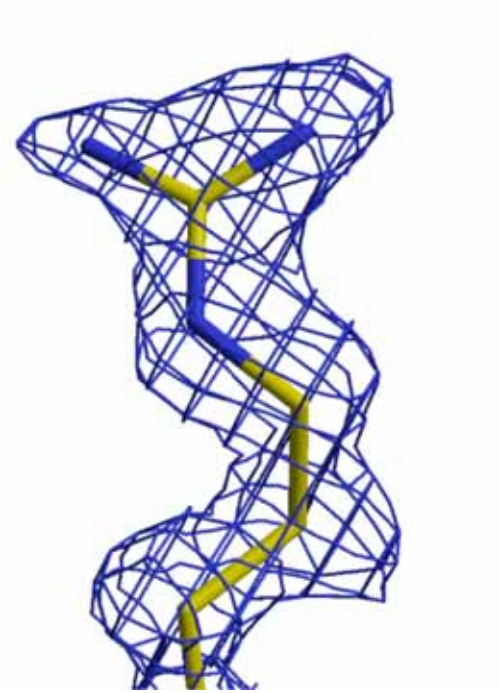
Protein Refinement at Atomic Resolution

Thomas R. Schneider • EMBL Hamburg
thomas.schneider@embl-hamburg.de

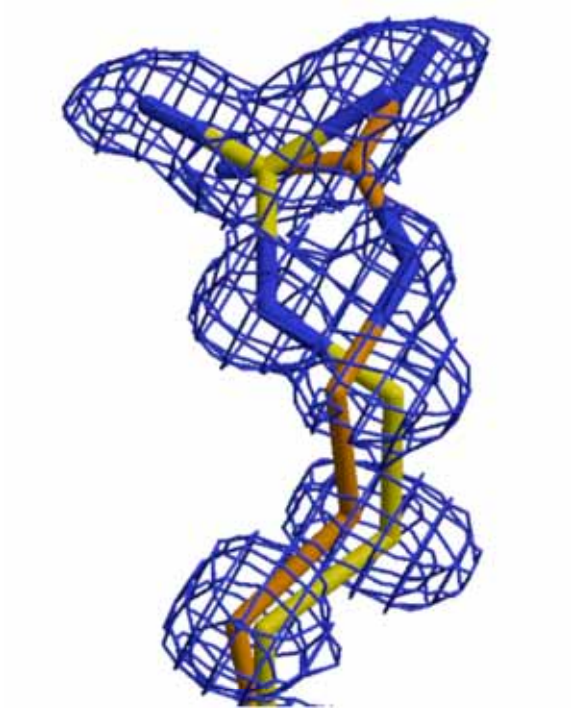
SHELX-Workshop ACA Meeting
Salt Lake City • 21-Jul-2007

Resolution and Electron Density I



Arg79 at 1.8 Å

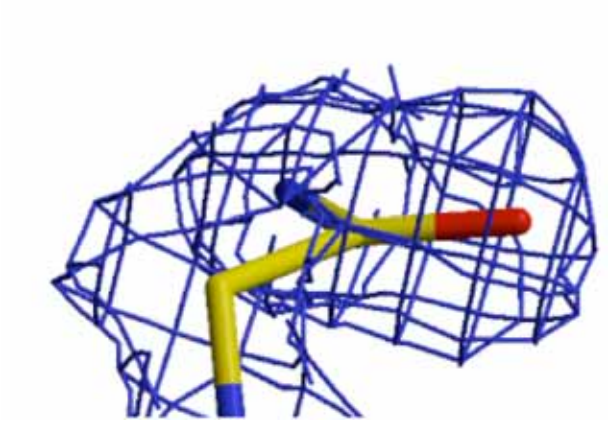
7 x 4 = 28 par



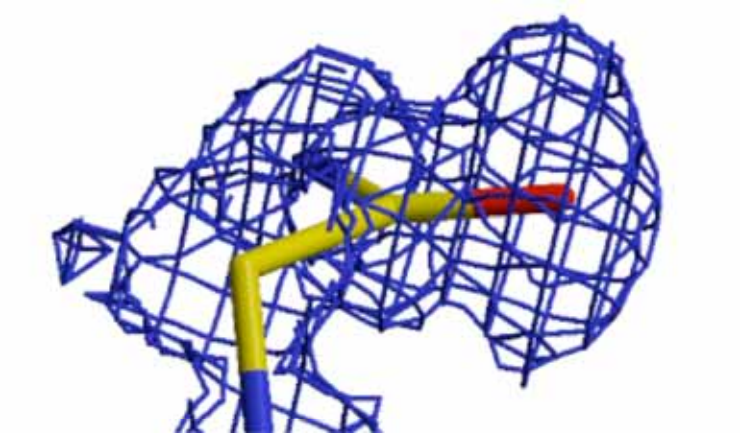
Arg79 at 0.9 Å

14 x 4 = 56 par

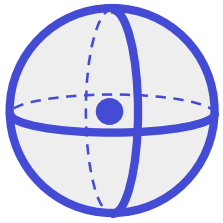
Resolution and Electron Density II



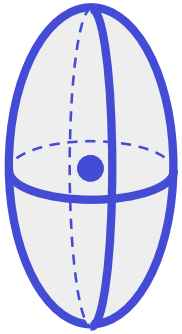
O_{Phe71} at 1.8 Å



O_{Phe71} at 0.9 Å



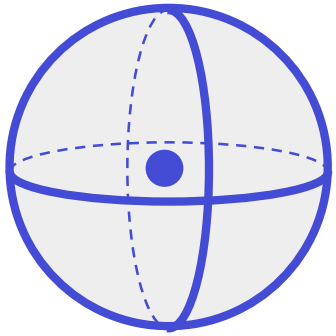
3 + 1 = 4



3 + 6 = 9

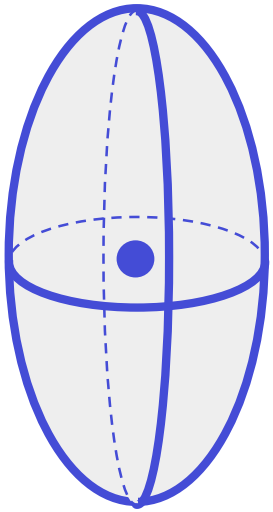
B Factors and Multiple Sites

isotropic



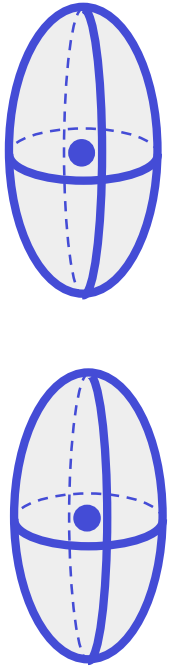
3 + 1 = 4

anisotropic



3 + 6 = 9

multiple sites



(3+6) + (3+6) + 1 = 19

Observables and Parameters

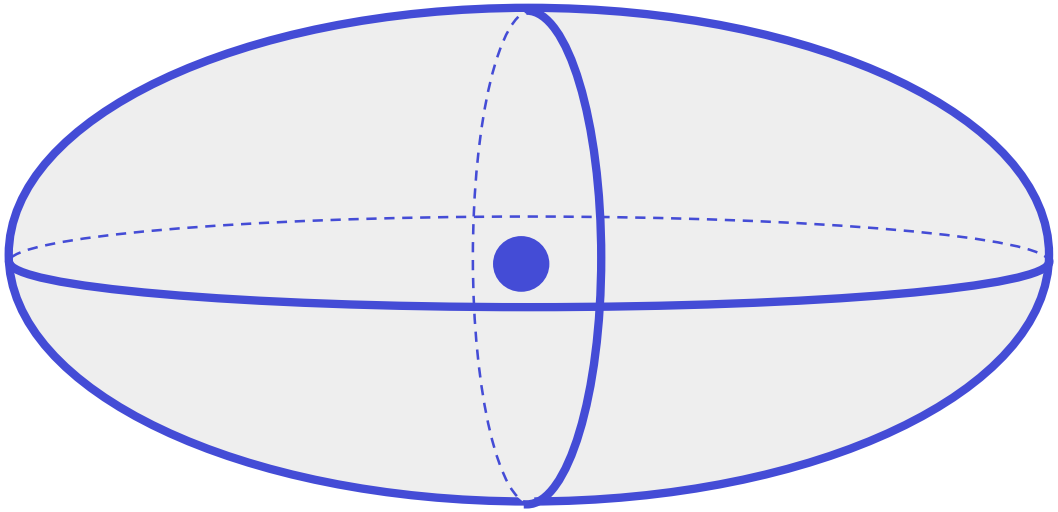
res	#r/a	parameters	d:p
3.0	2	x y z	0.6:1
2.5	4	x y z B?	1:1
2.0	8	x y z B	2:1
1.5	20	x y z B	5:1
1.1	50	x y z U_{11} U_{22} U_{33} U_{12} U_{13} U_{23}	5:1
0.9	90	x y z U_{11} U_{22} U_{33} U_{12} U_{13} U_{23}	10:1

Refinement at Atomic Resolution

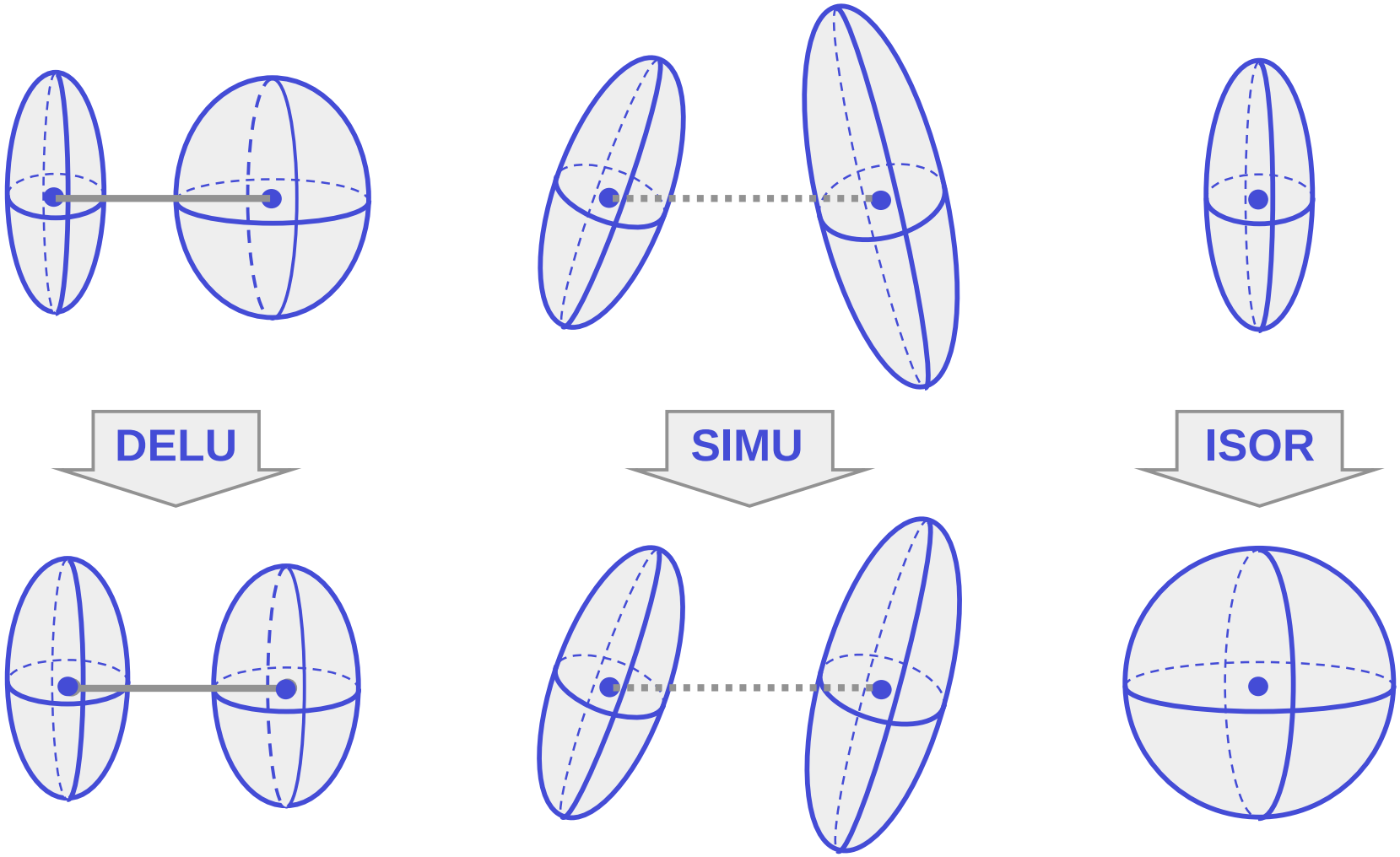
- rigid body, STIR
- some building and refinement at 1.5 Å
- preliminary water structure
- inclusion of data to max. resolution
- anisotropic B-factors
- disorder
- more water
- hydrogens
- matrix inversion

→ Tutorial (P1-Lysozyme to 1.1 Å) on the SHELX-Server:
shelx.uni-ac.gwdg.de / Chapter in the book

Anisotropic Displacement Parameters



Restraints on ADP's

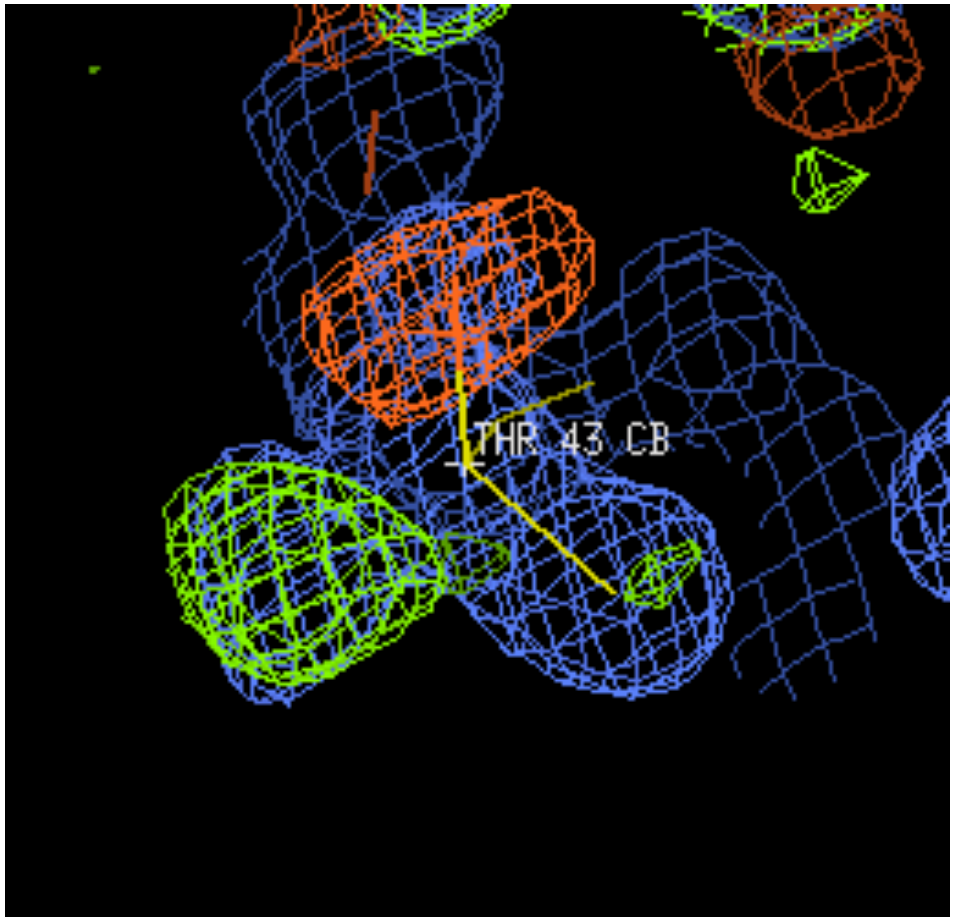


Switching on anisotropic B-factors

- Add an ANIS-card
- Activate ADP-restraints: DELU, SIMU, ISOR

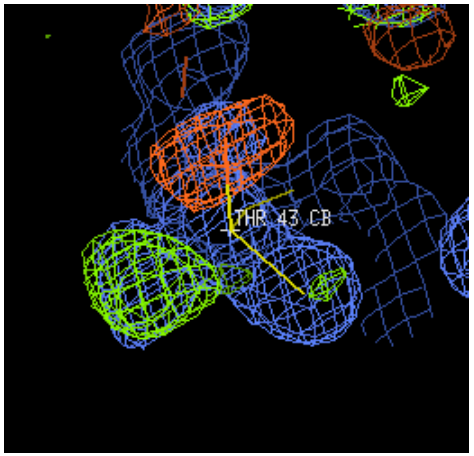
- R_{free} should go down by > 1.0 % upong making all non-hydrogen atoms anisotropic
- only a few *** NON POSITIVE DEFINITE *** messages
- Anisotropic treatment can be limited to certain atoms (e.g. sulphurs / irons)

Discrete Disorder



P1-Lysozyme - Thr43

Disorder: How to find places

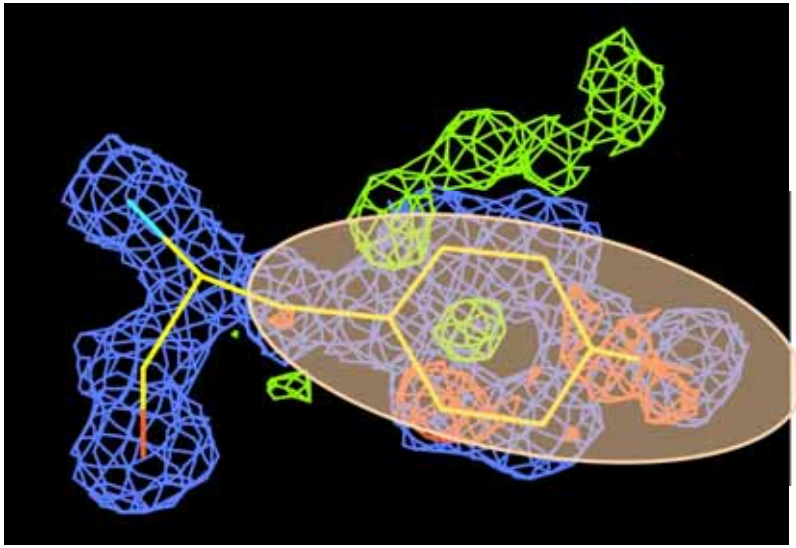


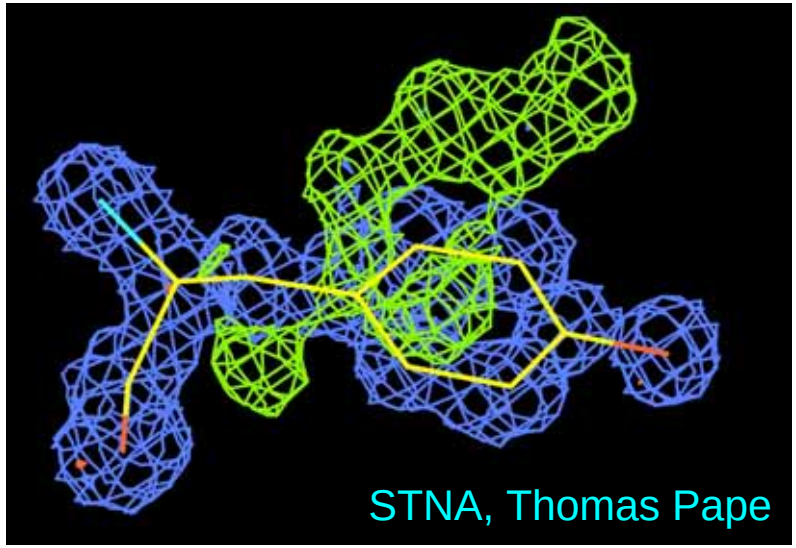
- High B-values
- negative F_o-F_c density close to atoms
- Disagreeable restraints: SIMU / DELU

- positive F_o-F_c density → systematically check all peaks higher than 5σ

- 'may be split' messages
  'non positive definite' messages

Step 1: Adjust first conformation

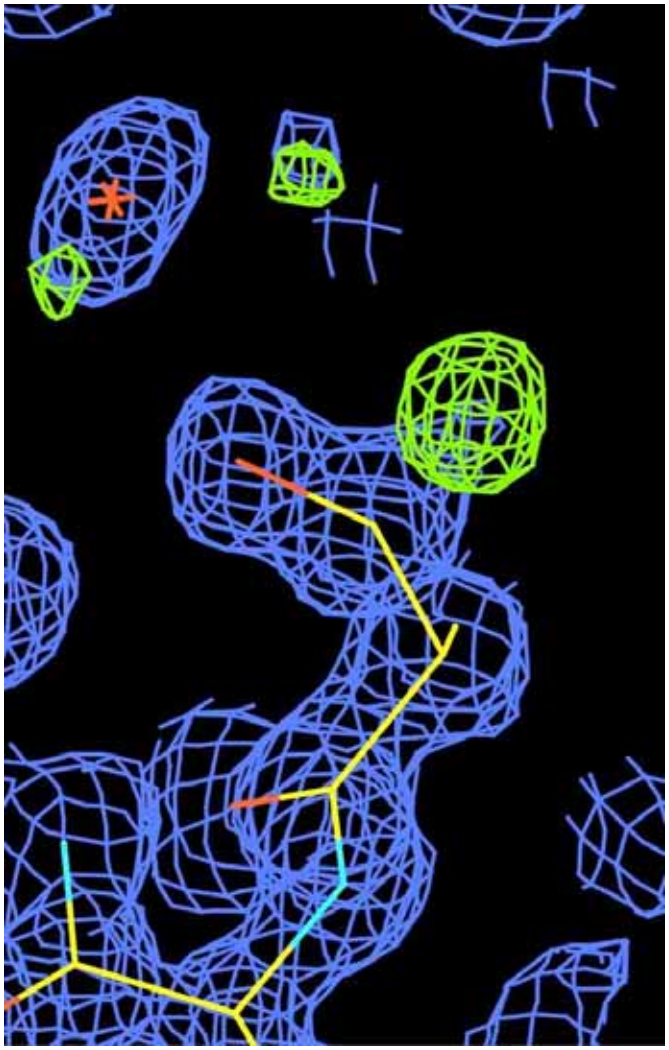


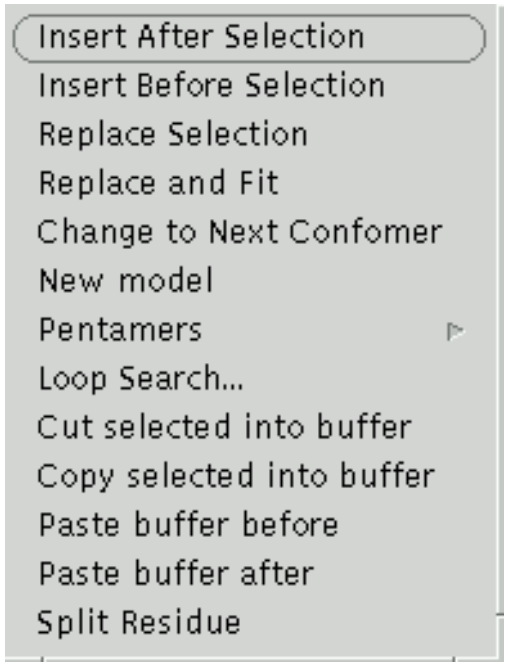



```
RESI   381    TYR
N    3   x y z 11.0 u11 u22 ..
CA   1   x y z 11.0 u11 u22 ..
CB   1   x y z 11.0 u11 u22 ..
CG   1   x y z 11.0 u11 u22 ..
..
OH   4   x y z 11.0 u11 u22 ..
C    1   x y z 11.0 u11 u22 ..
O    4   x y z 11.0 u11 u22 ..
```

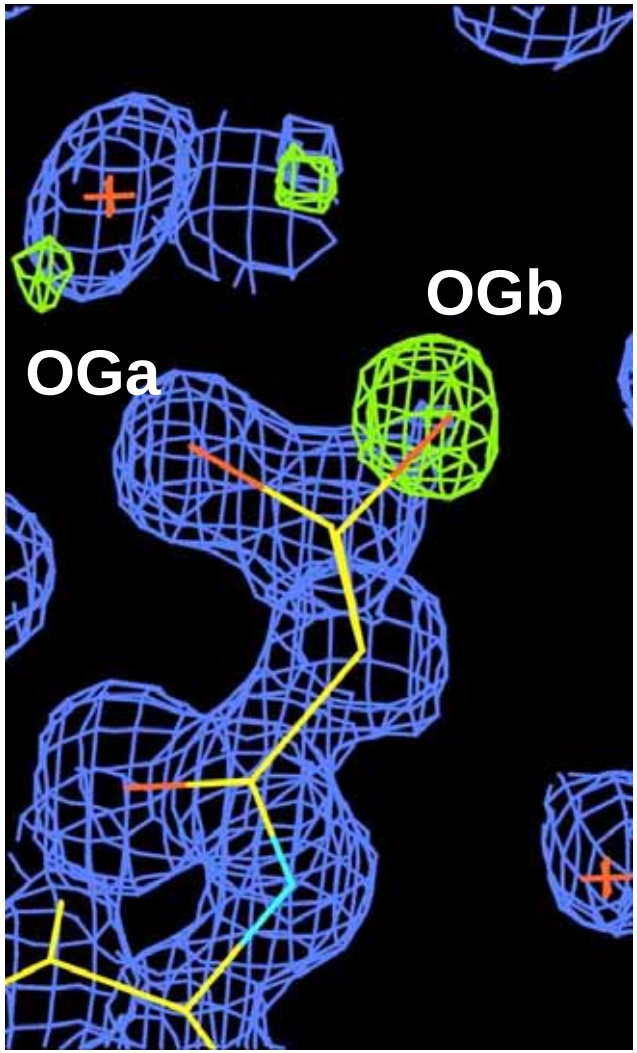
```
RESI   381    TYR
N     3   x y z 11.0 u11 u22 ..
CA    1   x y z 11.0 u11 u22 ..
PART 1 10.65
CB    1   x y z 11.0 0.1
CG    1   x y z 11.0 0.1
..
OH    4   x y z 11.0 0.1
PART 0
C       1   x y z 11.0 u11 u22 ..
```

Step 2: Model second conformation

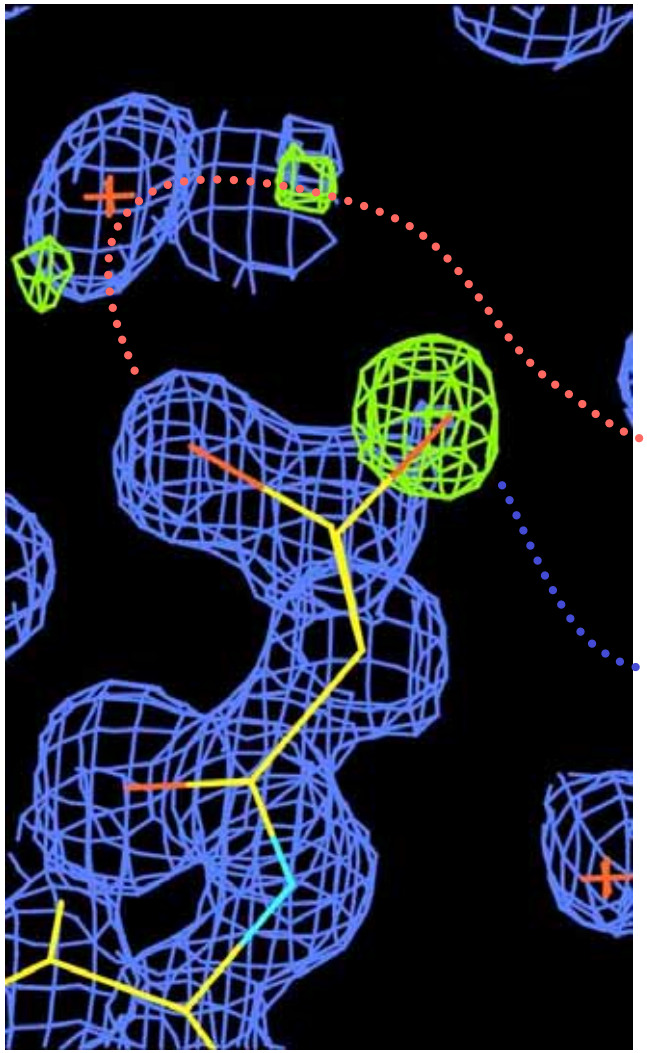




XtalView



Step 3: Refine double conformations



Occ.-Constraint

```
FVAR 0.765 0.65
..

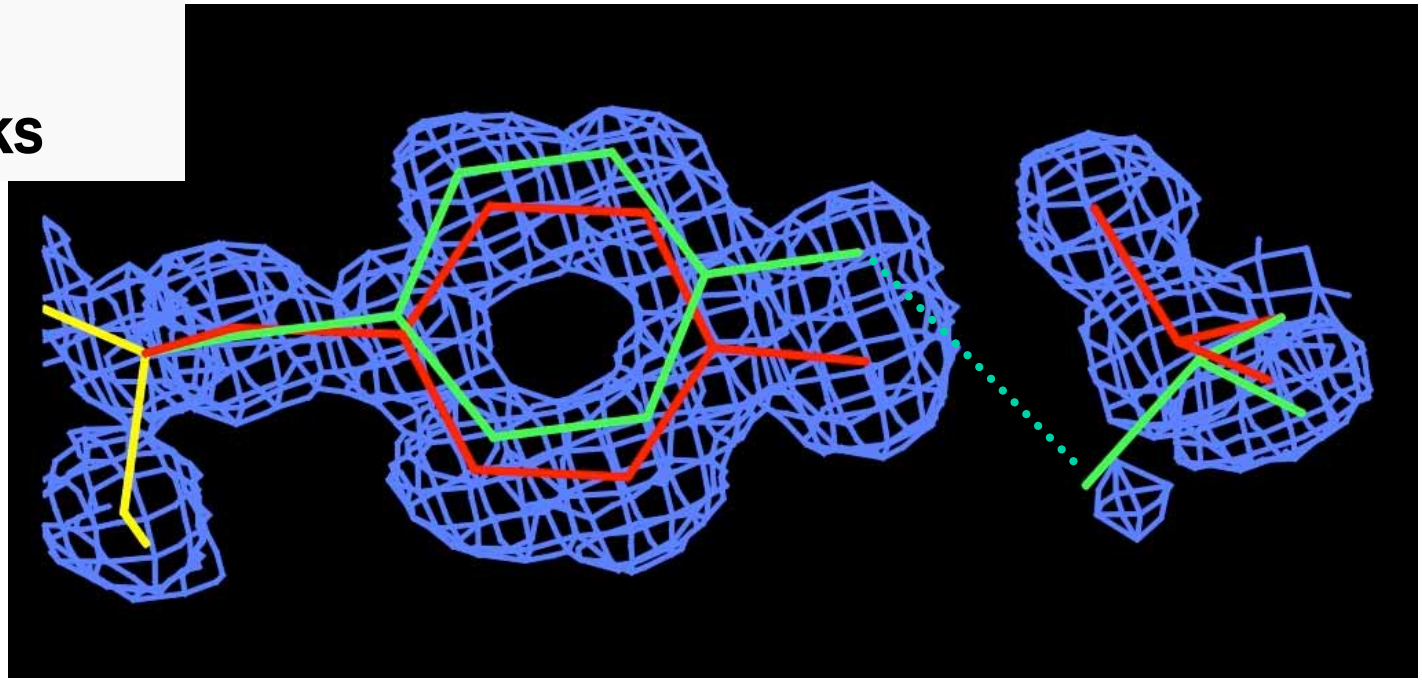

RESI  82 SER
N     3  x y z 11.0 u11 u22 ..
CA    1  x y z 11.0 u11 u22 ..

PART 1 21.0
CB    1  x y z 11.0 0.1
OG    4  x y z 11.0 0.1

PART 2 -21.0
CB    1  x y z 11.0 0.1
OG    4  x y z 11.0 0.1

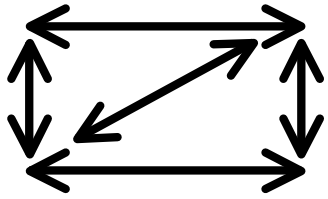
PART 0
C       1  x y z 11.0 u11 u22 ..
O       4  x y z 11.0 u11 u22 ..
```

Step 4:
Build networks



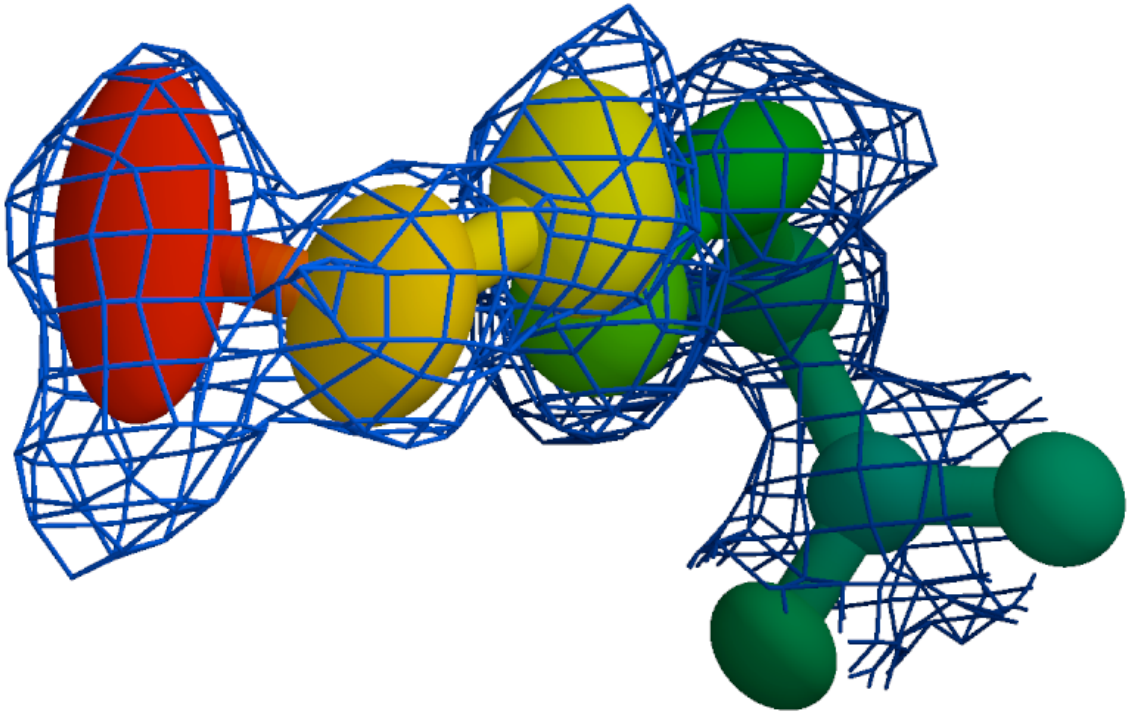
1 parameter describes the occupancies of 22 atoms !

```
RESI 233 TYR
..
PART 1 31.0
CB   ..
PART 2 -31.0
CB   ..
PART 0
..
```



```
RESI 123 THR
..
PART 1 31.0
CB   ..
PART 2 -31.0
CB   ..
PART 0
..
```

Discrete vs. Continous Disorder



0.2693	0.1741	0.1223	CD_83
0.2616	0.2331	0.1028	CE_83
0.5949	0.1497	0.0870	NZ_83

→ may be split into 0.4506 0.4862 0.5757 and 0.4366 0.4875 0.5754

0.1005	0.0776	0.0684	N_84
0.0855	0.0727	0.0688	CA_84

Neuramindase

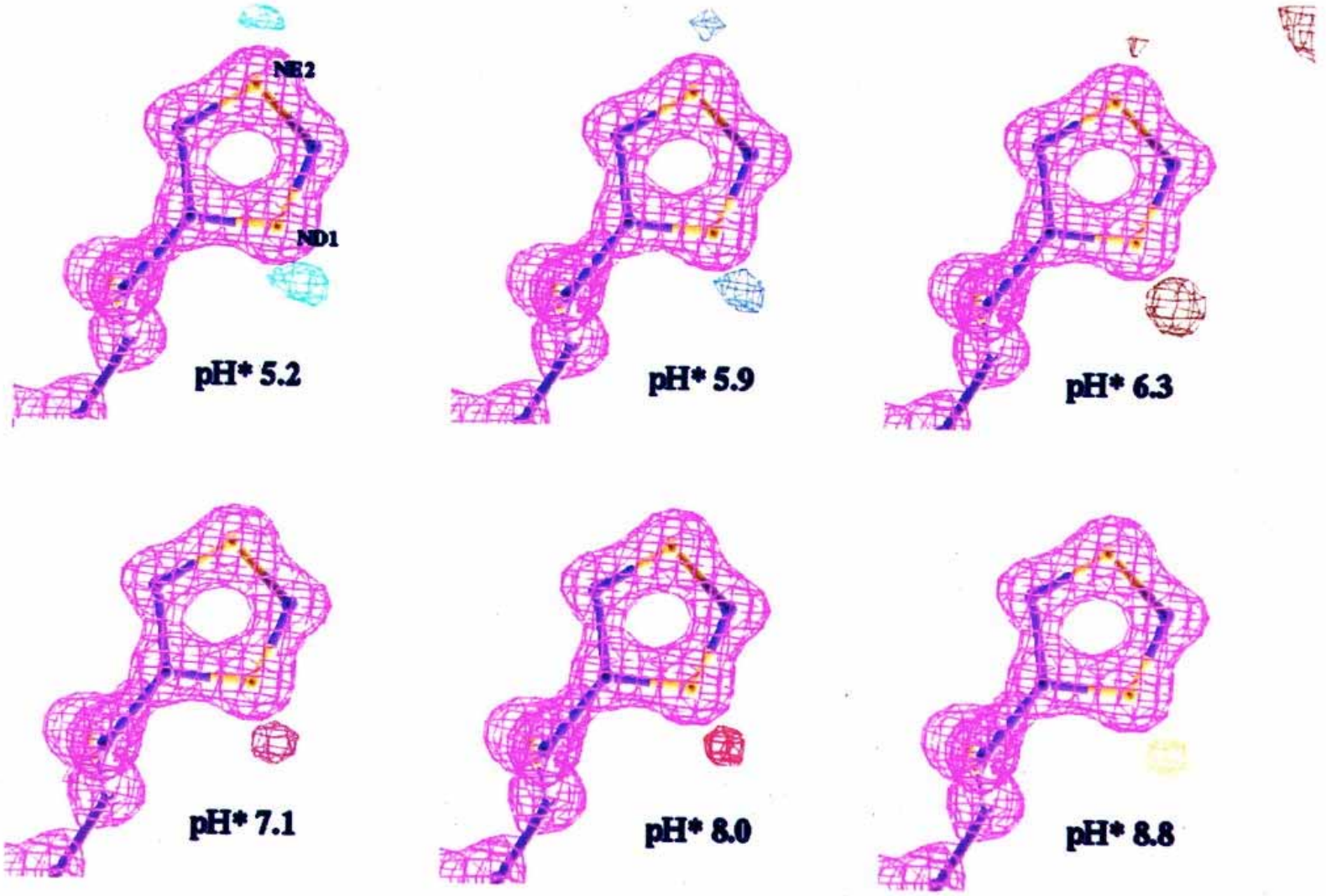
T. Pape - Univ. Göttingen

E. Garman - Univ. Oxford

P. Kuhn - SLS Stanford

Hydrogens

Berisio et al. (1999) J.Mol.Biol. 292:845-854



Hydrogens

- In principle one can include riding hydrogens at any resolution (no extra parameters)
- Hydrogens can be included late in refinement (computer time for structure factor calculation)
- It is advantageous to not include the interesting hydrogens (hydroxyl, His-imidazol) at any stage of the refinement (model bias)

An interesting situation

- A water molecule close to a His has a refined B-value of 2.1 Å²
- On top of the water molecule there is a +10σ peak in the Fo-Fc difference density
- The anomalous difference map shows a +7σ peak at the position of the water molecule

- If we refine a Cl-, the B-value refines to 7.1 Å² and the Fo-Fc difference map shows a -7σ peak.
- A Cl- with an occupancy fixed to 0.8 will end up with a B-value of 6.4 Å² and no difference density
- But then we have 20% vacuum ...

Two atoms in the same site

- Simultaneous refinement using different PARTs and constraining the sum of the occupancies to 1.0 never converged ...

- Refine only one parameter:

```
STIR 1.2 0.05
CLGS 20
FVAR[76] = 0.05

PART 1
CL    9    10.182964  9.898033 10.380161  10.1    761.00
PART 2
OW    4    10.182964  9.898033 10.380161  10.9    761.00
PART 0
```

Test different occupancies

occ(Cl-) [fract]	B(Cl-,O) [Å?]	Diff.Dens (Cl-) [σ(Fo-Fc)]	Other [σ(Fo-Fc)]
0.0	2.08	+11s	None
0.1	2.95	+9s	None
0.2	3.70	+7s	None
0.3	4.36	+5s	None
0.4	4.95	+2.5	+3s (two)
0.5	5.49	<2	+2.5s (two)
0.6	5.99	<1	+2.5 (one)
0.7	6.45	-3	+2.5(one)
0.8	6.89	-5	+2.5(one)
0.9	7.30	-6	+2.5(one)
1.0	7.69	-7	+2.5(one)

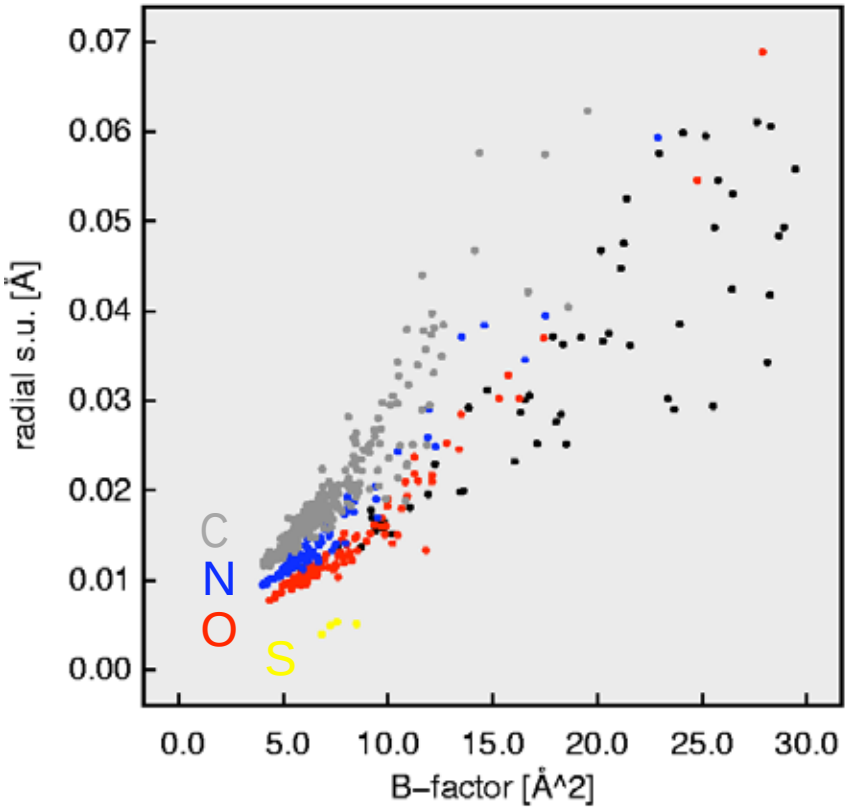


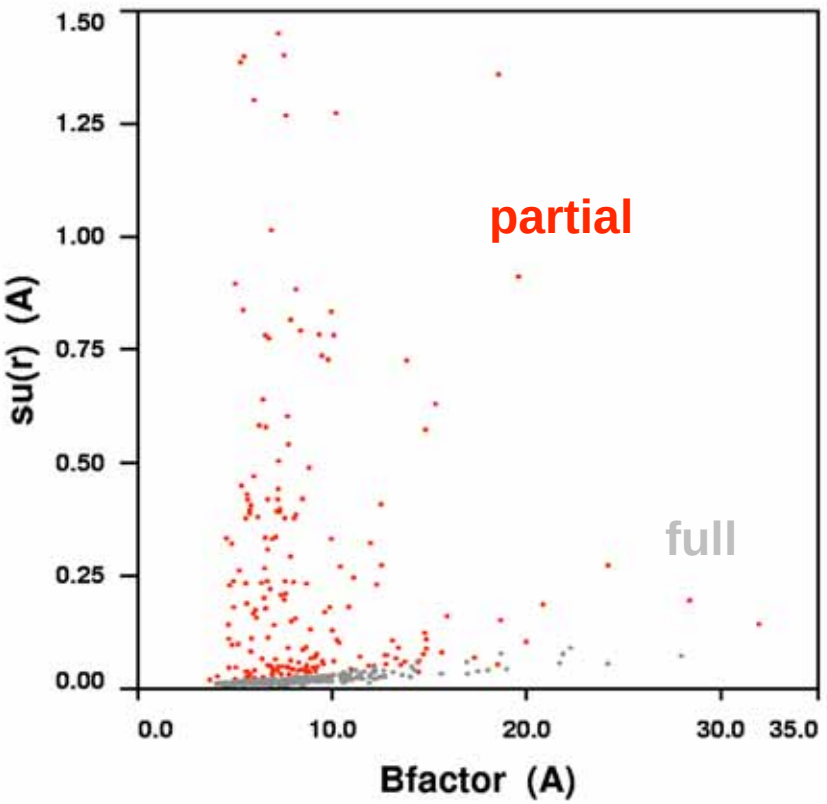
Determination of e.s.d.'s

- at the end of a refinement, the standard deviations of the refined parameters can be determined by inverting the normal matrix of the refinement.
- the recommended method is to:
  (1) remove all restraints
  (2) use a shift multiplier of 0.0
  (3) run one cycle of least-squares minimization

- Tendamistat: 74 residues, 7433 parameters against 41006 reflections 148 MB of memory in 33 min. on a 2.2GHz Linux-PC
- Aldose Reductase: 315 residues, 9166 out of 33158 parameters against 511265 reflections in 206 MB of memory (single precision) in 5.5 hrs on a 2.2 GHz Linux-PC

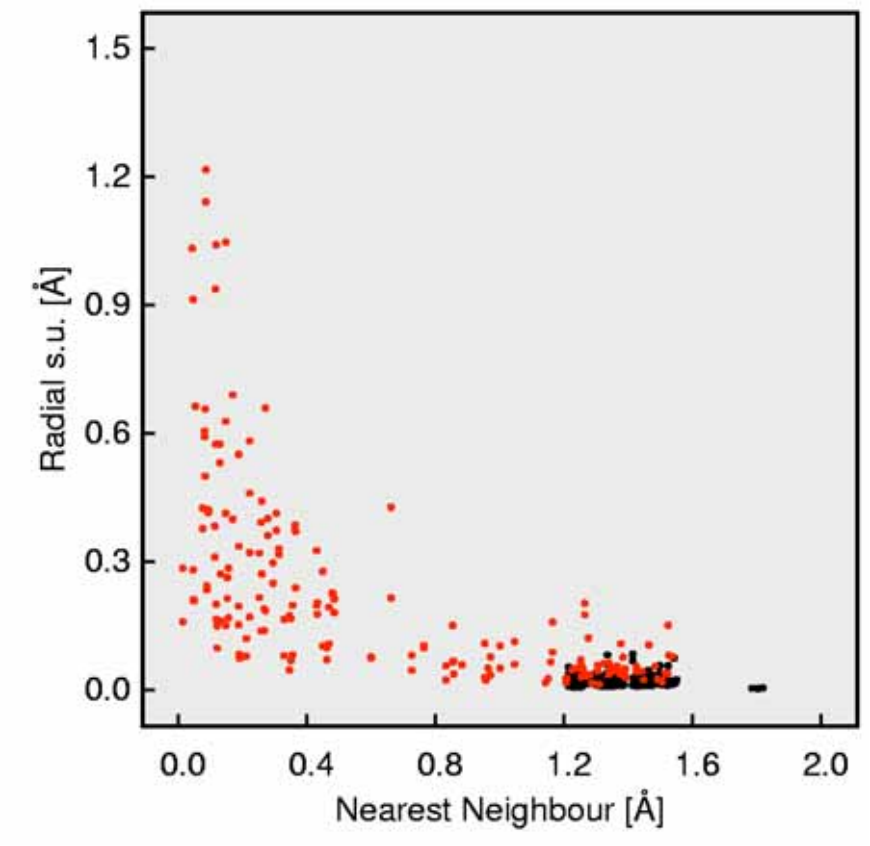
Radial s.u.'s for Tendamistat at 0.93 Å

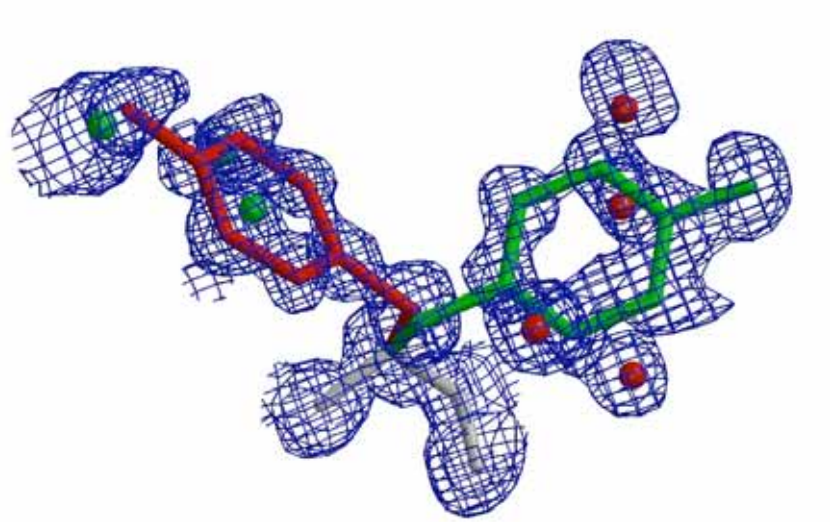




König et al. (2003)
Acta Cryst. D59:1737-1743

Radial s.u.'s for Tendamistat at 0.93 Å





König et al. (2003)
Acta Cryst. D59:1737-1743

The Complete Recipe for refinement of disordered sidechains

- Disagreeable Restraints and F_o-F_c maps

- Set occupancy of suspects to 10.65, and $U_{ij} \rightarrow U$ ⇨ refine
- Model 2nd conformations in F_o-F_c map at 2.5σ, put occ-constraints (PART 1/2, FVAR), activate restraints (automatic ...) ⇨ refine
- Build networks ⇨ refine
- Include partial waters (only if networked !) ⇨ refine