

Emerging Areas of Biological Crystallography
APS, Argonne - July 26-28, 2004

Using the weak scatterer substructures

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Lysozyme - Phillips *et al.* 1960s

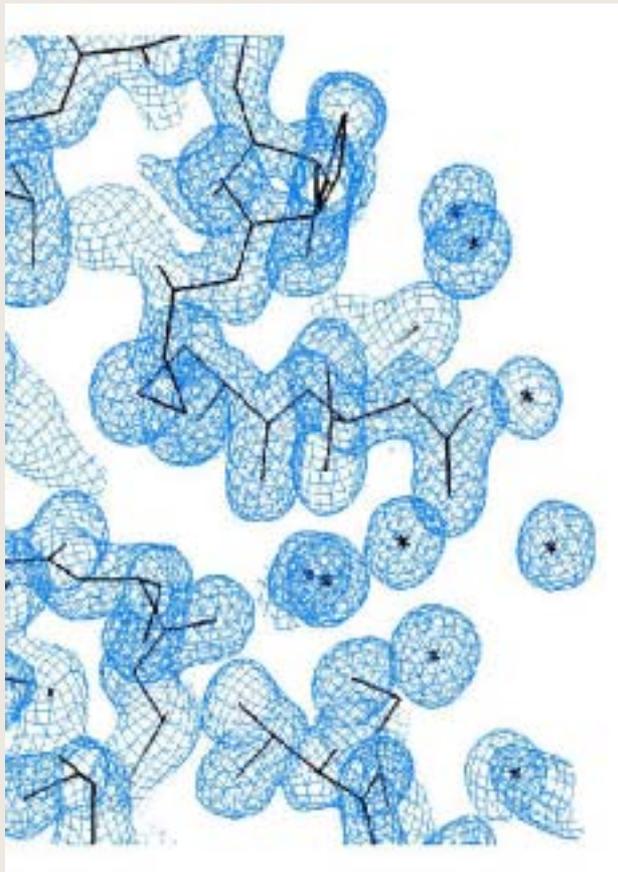
In the original structure of lysozyme
(crystallized from 1 M NaCl)

Phillips *et al.* identified one Cl^- site
from its chemical context

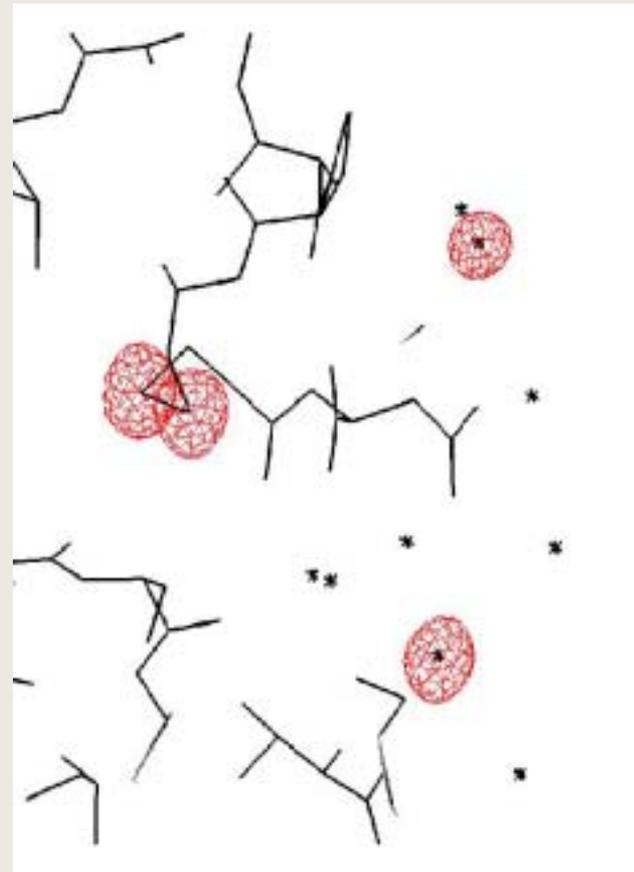
Lysozyme - from 1 M NaCl

8 anomalous solvent sites

$2F_o - F_c$ map



ΔF_{anom} map



Anomalous scattering properties of halides

Cl - at $\lambda = 1.54 \text{ \AA}$ $f'' = 0.7$

I - at $\lambda = 1.54 \text{ \AA}$ $f'' = 6.8$

Br - at $\lambda = 0.92 \text{ \AA}$ absorption edge
suitable for MAD

Indeed, halides have been used for phasing:

I - iodination of Tyr with KI_3 or N-I-succinimide

Br - MAD on DNA with Br-Uracil

Cl - e.g. vancomycin

RNase A - soaked in 1 M NaBr

ΔF^\pm difference Fourier map

10 σ

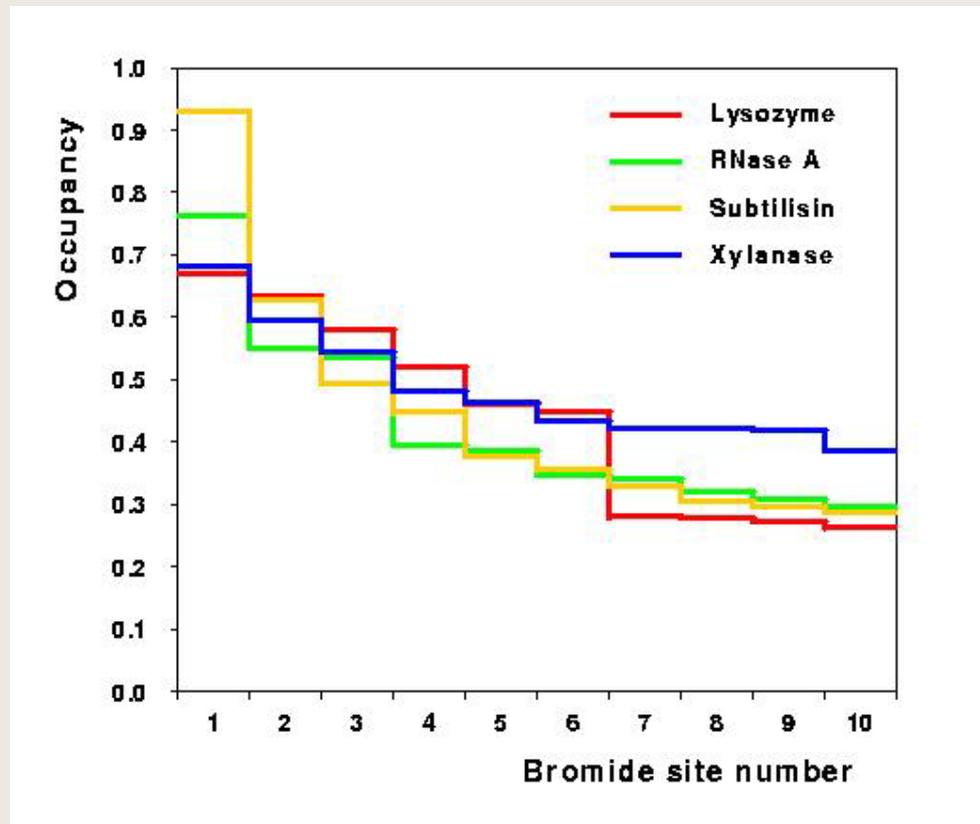
5 σ

3 σ



Halide sites occupancies

Br occupancies refined with SHELXL



remaining occupancy taken up by H₂O

Soaking

diffusion is rapid

10-15 seconds is enough

concentration: 1.0 - 0.2 M

solutions: PEG, MPD, various salts

salts may be in part

exchanged for NaBr, KI

concentrated LiBr, NaBr are

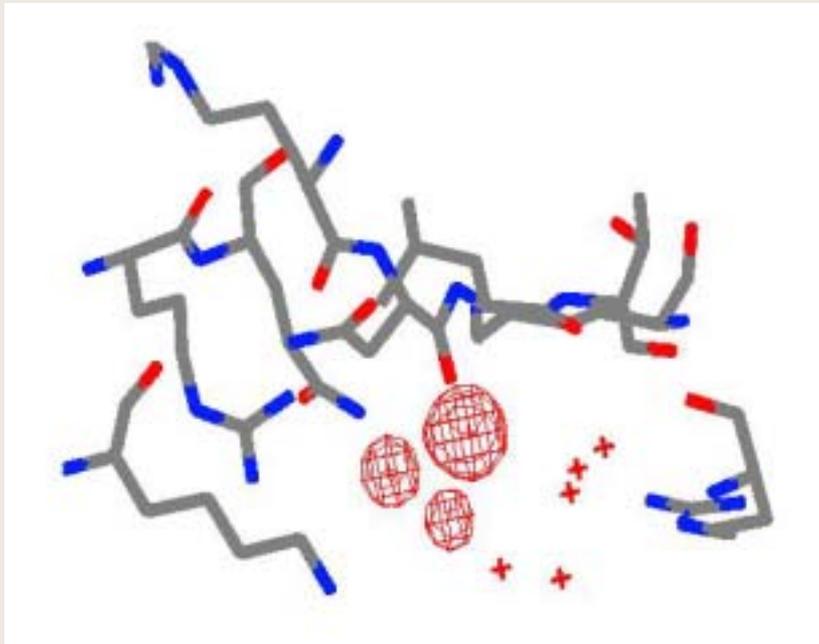
cryoprotecting

Properties of halides (Br^- , I^-)

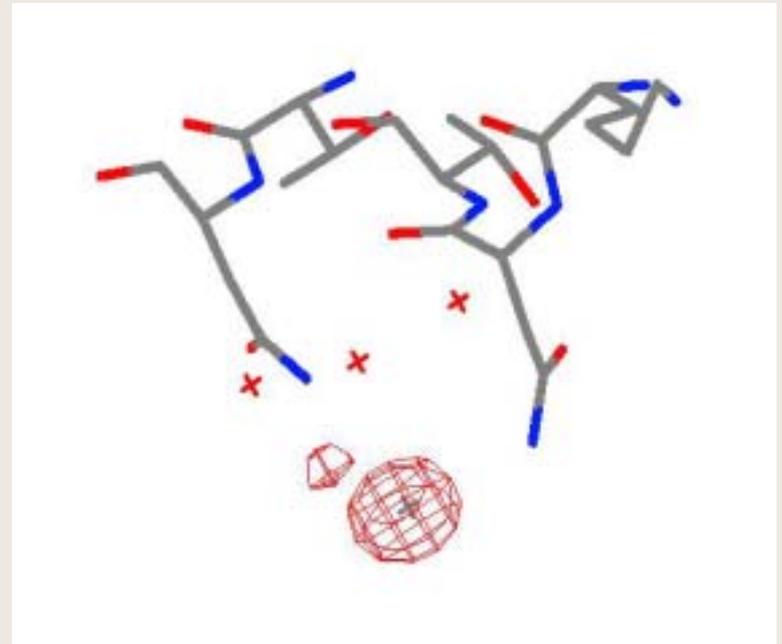
- small, monoatomic
- soft, polarizable ions
- form H-bonds and hydrophobic interactions
- no chemical reaction necessary
- no specific geometry of coordination
- rapid diffusion through crystal solvent channels
- share partially occupied sites with water

Halide sites

near
positively charged
Arg and Lys

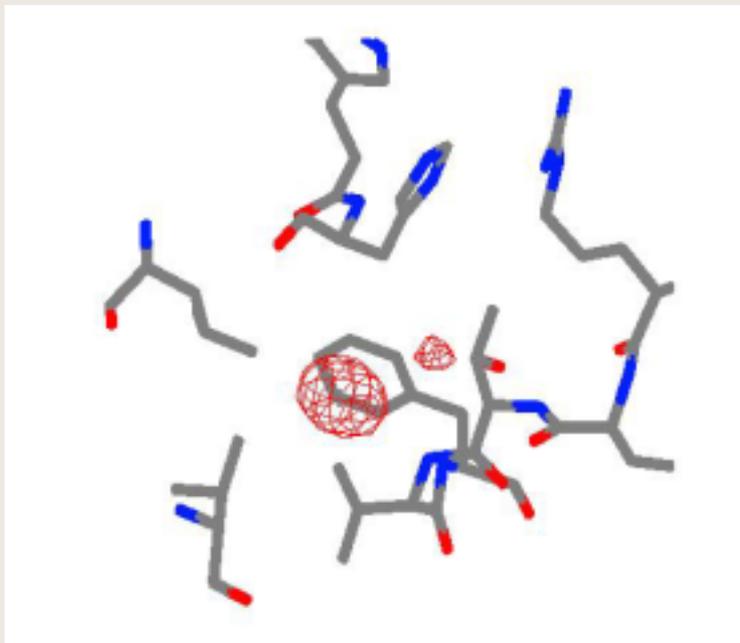


near
NH groups from
main chain, Asn or Gln

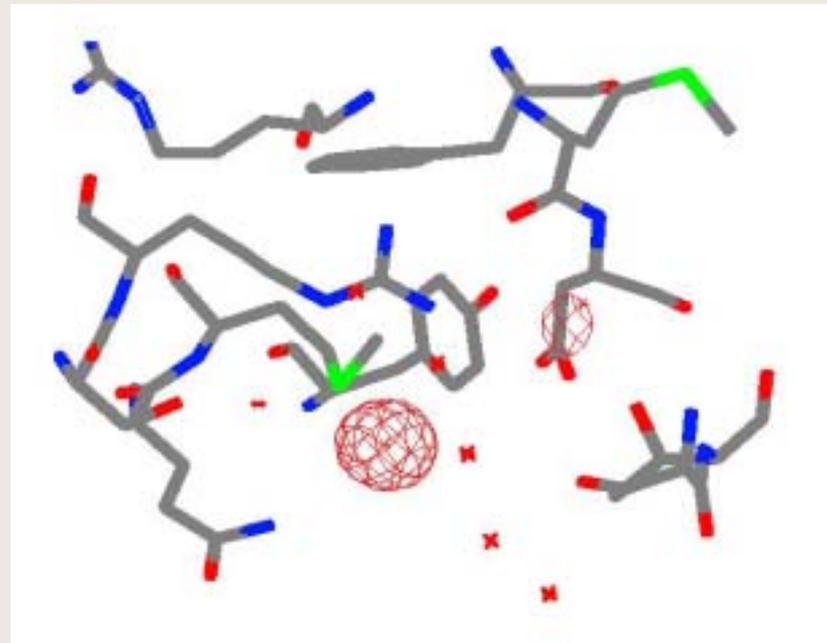


Halide sites

near
hydrophobic and
aromatic rings



near
all of these
functions, mixed

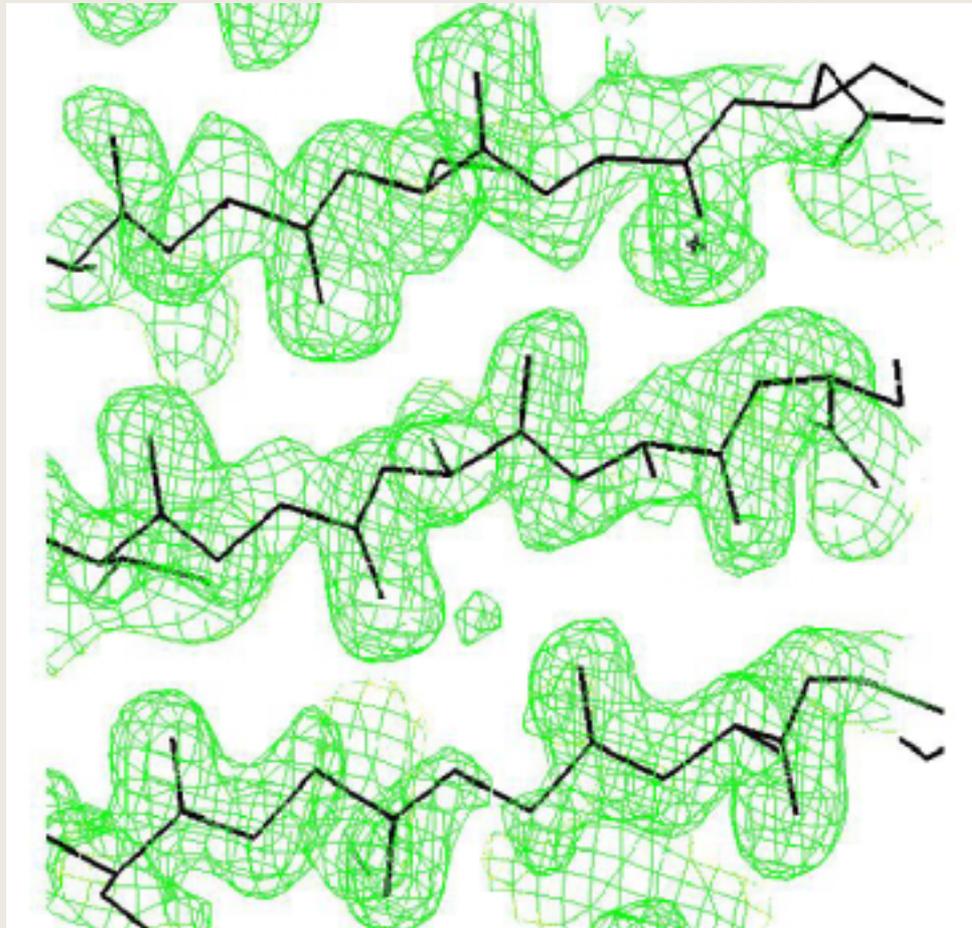


Number of halide sites

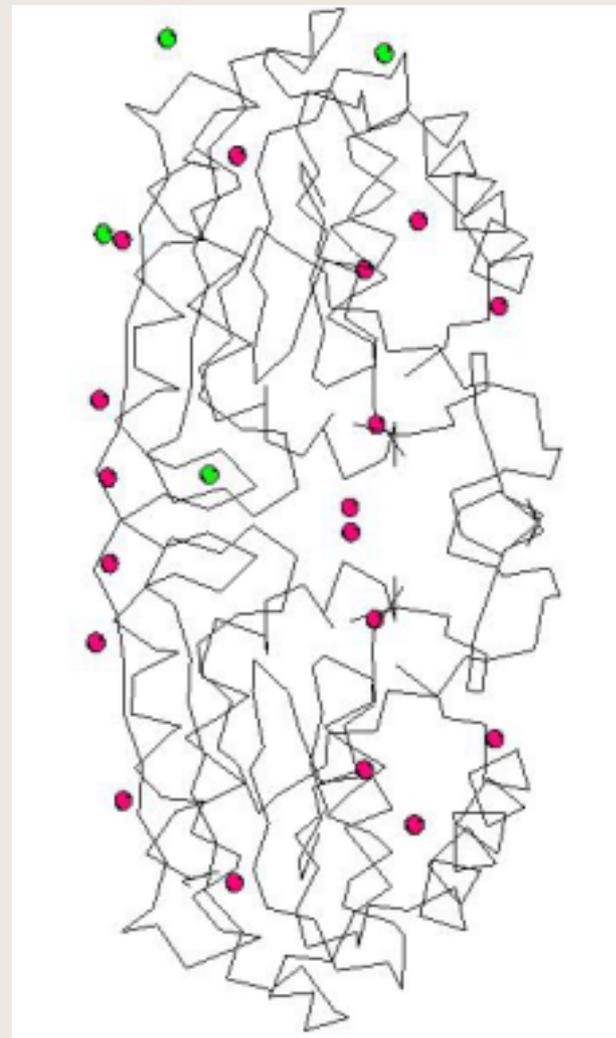
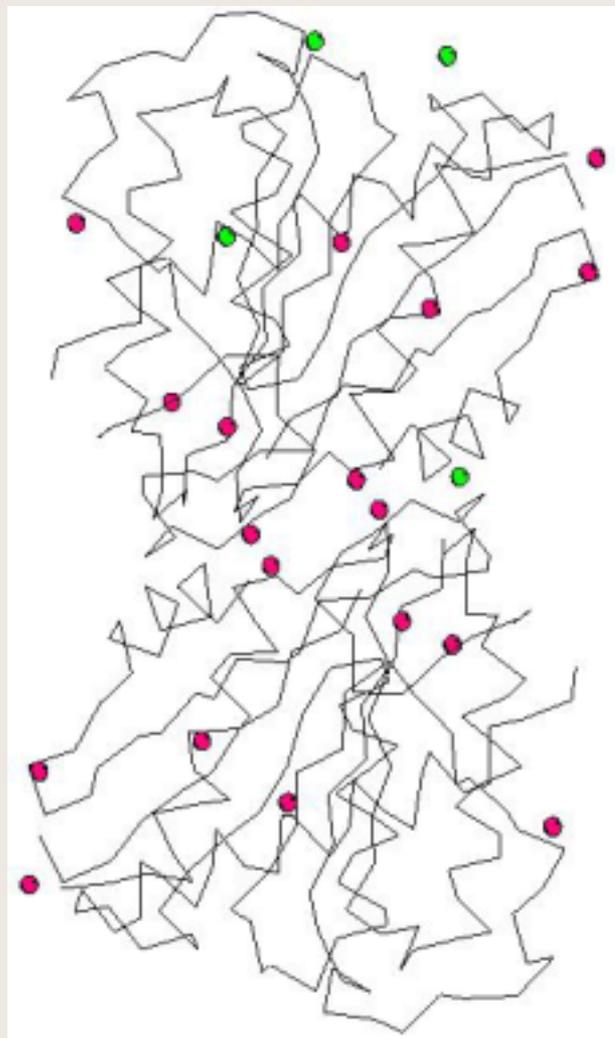
In contrast to SeMet
the number of sites is not known
site occupancies diminish gradually

Thioesterase 1.8 Å map

2 x 28 kDa per asym. unit, 22 (8+8+6) Br



Thioesterase Br⁻ sites



Halide cryo-soaking

Halide cryo-soaking method

is very easy and quick

(whether it works or not...)

suitable for high-throughput projects

Sulfur – weak anomalous scatterer

Cl - at $\lambda = 1.54 \text{ \AA}$ $f'' = 0.7$

S - at $\lambda = 1.54 \text{ \AA}$ $f'' = 0.56$

$\lambda = 1.74 \text{ \AA}$ $f'' = 0.70$

Enough to phase 12 kDa protein on 1 SS bridge

when $\langle \Delta F / F \rangle = 0.6 \%$ (Wang, 1985)

At low resolution SS bridge acts as superatom

since $(2 * 0.7)^2 > 2 * (0.7)^2$

Xylanase data

β -barrel, 302 residues with 5 sulfurs (one S-S)

collected @ X9A, processed with HKL2000

$P2_1$, $a = 41.2$, $b = 67.2$, $c = 50.9$ Å, $\beta = 113.5^\circ$

1.65 Å resolution, $\lambda = 1.74$ Å,

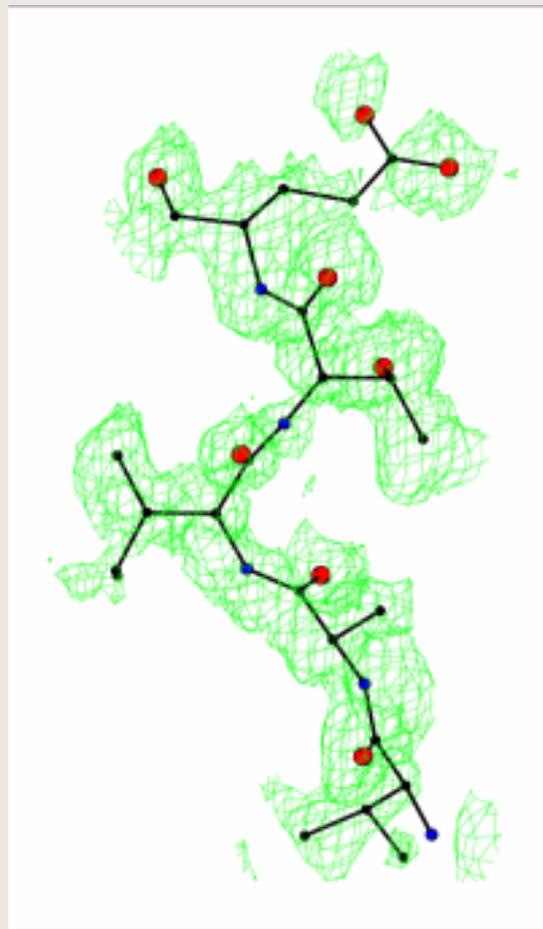
$R_{\text{merge}} = 4.5$ %, multiplicity = 12.1, $f''(\text{S}) = 0.70$

5 sulfurs in 2300 atoms gives $\langle \Delta F \rangle / \langle F \rangle = 0.70$ %

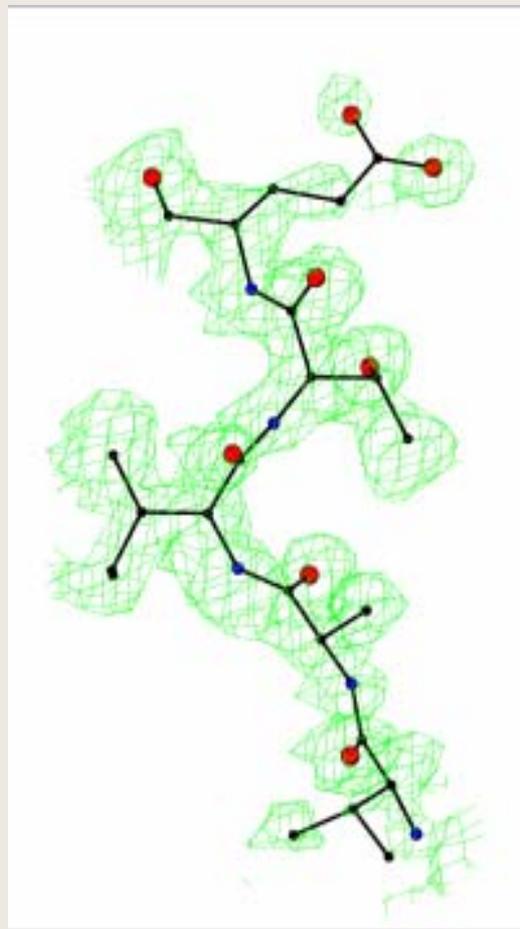
clear (100/100 trials) SHELXD solution for 5 sulfurs

Xylanase maps

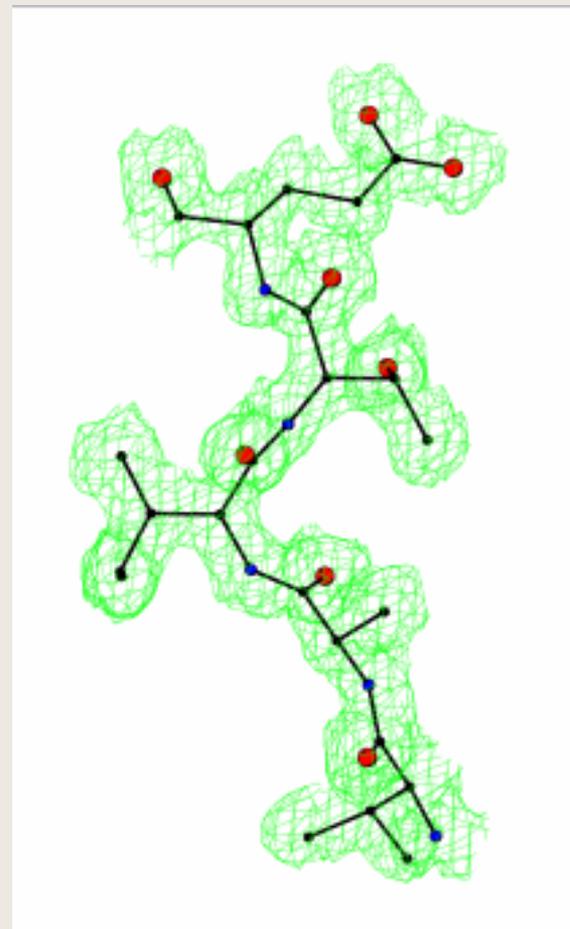
SHELXE map



wARP free map



warpNtrace map



Thaumatin data

P4₁2₁2 , 207 residues

8 S-S bridges, 1 Met

Data collected at X9B / NSLS

0.98 Å wavelength, 1.5 Å resolution

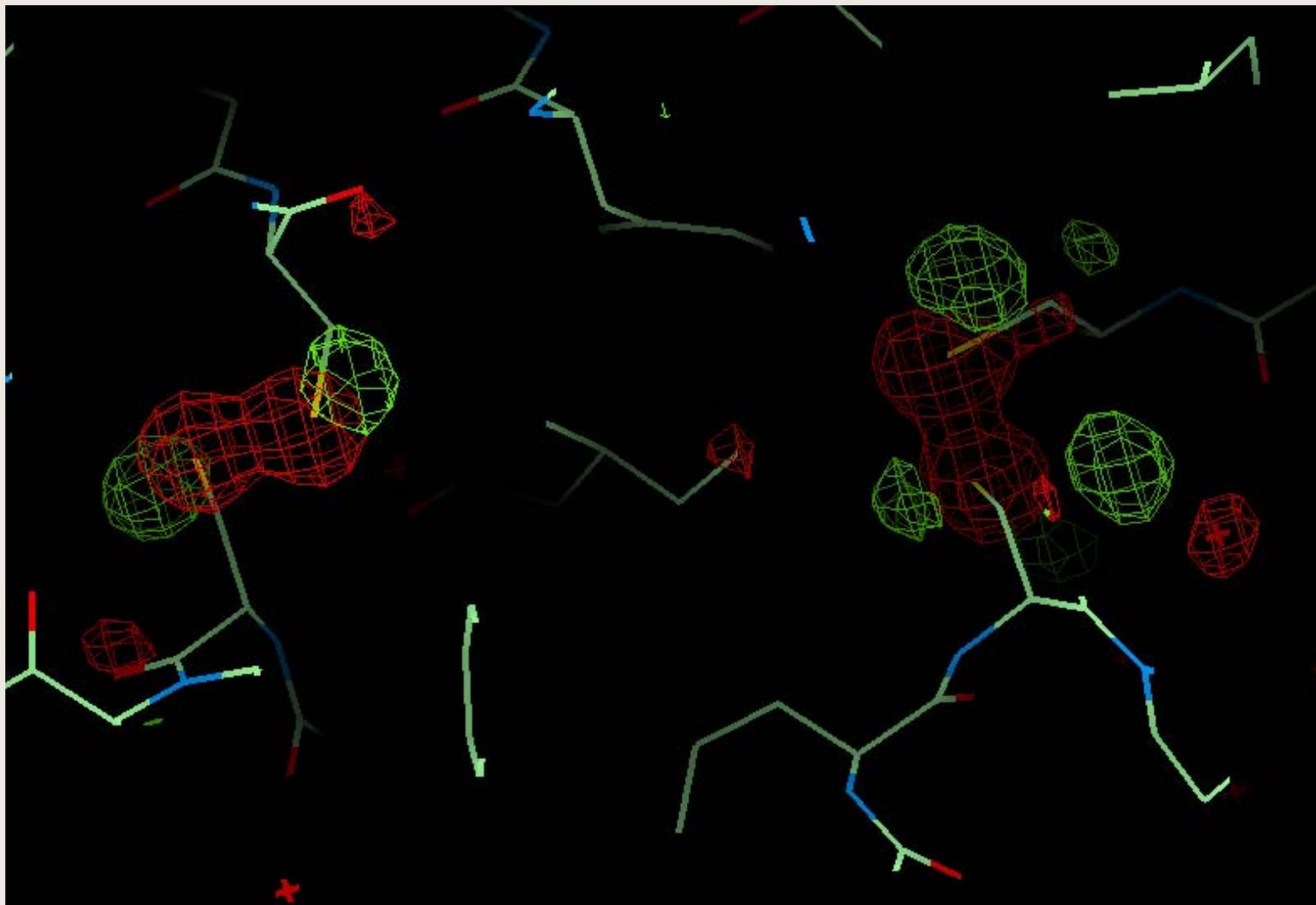
20 data sets, 90° each

scaled separately

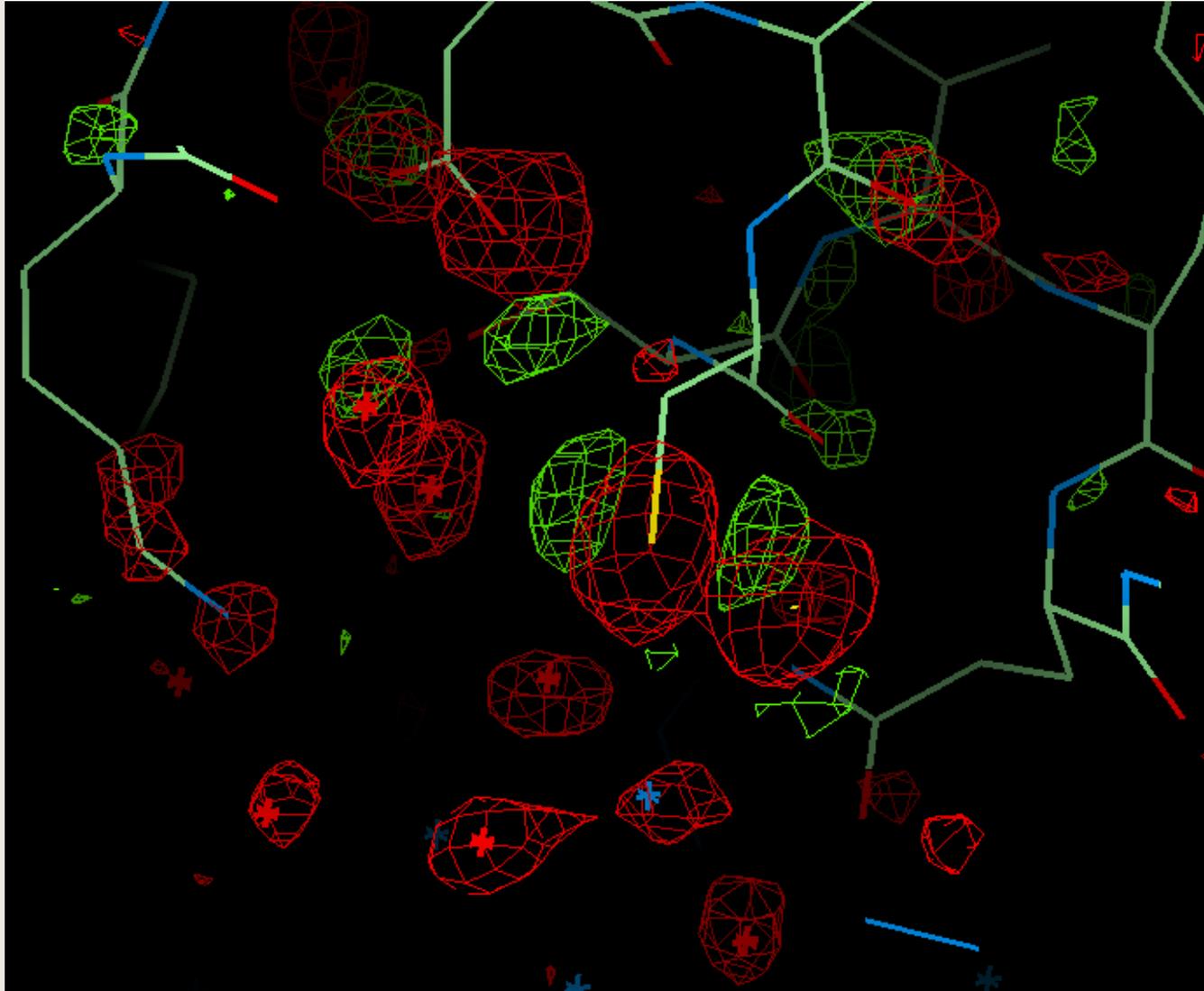
Thaumatococcus intensities

	1 0 37	1 1 25	2 2 13	3 0 62	8 1 22	8 1 31	8 2 16
1	360.6	1152.8	939.0	2197.5	1584.9	1202.3	548.1
2	452.5	1025.3	935.8	2144.5	1581.1	1112.9	587.1
3	561.0	922.9	965.5	2052.9	1634.9	1003.3	694.5
4	677.1	796.3	959.6	1974.7	1559.7	867.3	716.9
5	788.8	731.1	1077.1	1888.0	1605.4	797.0	842.0
6	871.2	694.1	1059.3	1834.5	1618.0	747.0	913.9
7	993.8	644.3	1119.4	1844.7	1653.0	722.6	979.9
8	1086.5	617.7	1188.4	1785.6	1708.4	696.7	1006.7
9	1157.4	582.6	1255.6	1709.3	1755.2	660.2	1082.8
10	1155.2	568.2	1241.9	1701.3	1707.6	634.6	1041.7
11	1313.5	518.6	1349.4	1642.5	1768.3	585.3	1155.7
12	1434.1	476.7	1361.3	1586.8	1789.0	568.0	1187.5
13	1548.6	460.5	1467.1	1551.3	1865.0	526.7	1174.2
14	1671.7	429.4	1559.8	1477.3	1870.6	500.5	1297.2
15	1815.4	424.4	1605.4	1466.9	1950.6	524.8	1284.4
16	1873.3	397.5	1631.5	1423.2	1931.3	478.3	1329.3
17	2049.5	376.7	1738.3	1417.2	2035.2	480.0	1394.5
18	2185.5	329.6	1832.6	1332.9	2028.3	441.2	1446.5
19	2285.1	370.5	1927.2	1326.7	2054.3	414.4	1476.0
20	2365.7	311.3	2011.2	1287.9	2119.2	436.2	1537.2
21	2394.6	282.7	2011.6	1270.6	2131.1	425.7	1461.0
22	2403.8	298.7	2006.2	1226.3	2149.0	415.5	1506.5

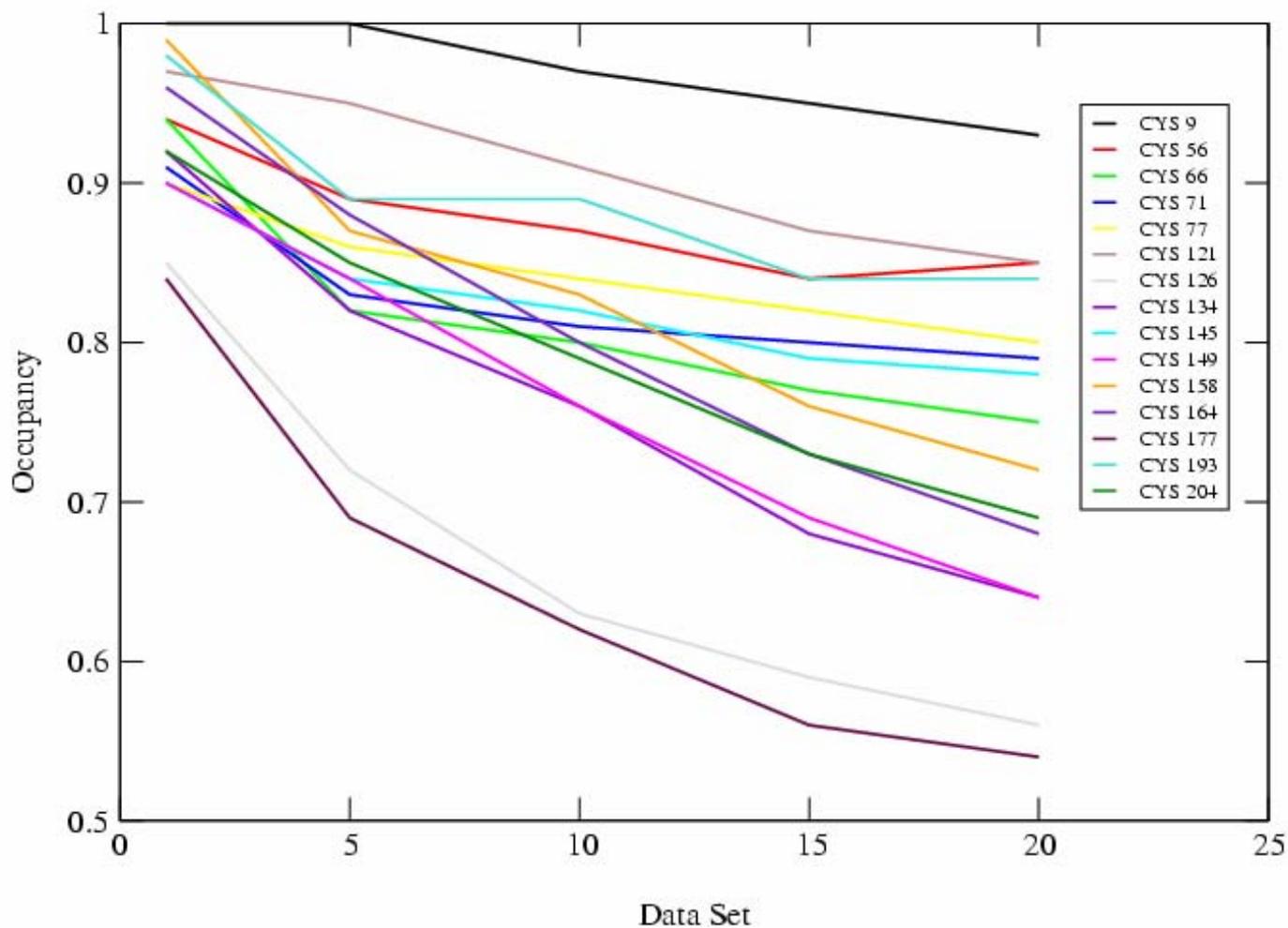
Thaumatococcus S-S bond breaking



Thaumatococcus decarboxylation

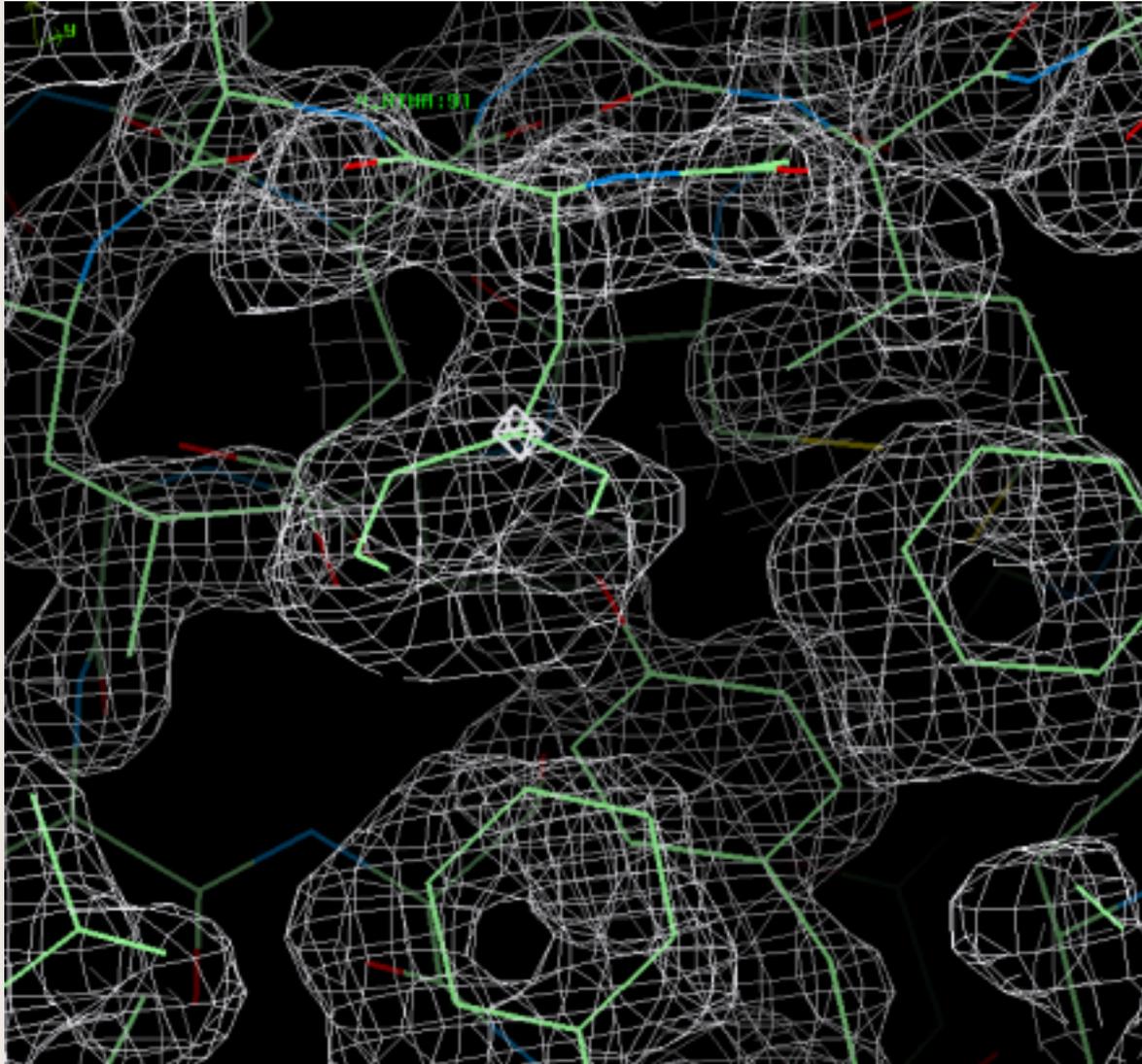


Sulfur occupancies from SHELXL



0.1 S =
1.6 el.

Thaumatococcus RIP map



phased on
differences

$$F_1 - F_{15}$$

SHELXD,
SHARP,
SOLOMON

Elastase + $\text{KAu}(\text{CN})_2$ data

$P2_12_12_1$, 240 residues

4 S-S bridges, 1 Met + 2 Au sites

Data collected at X9B / NSLS

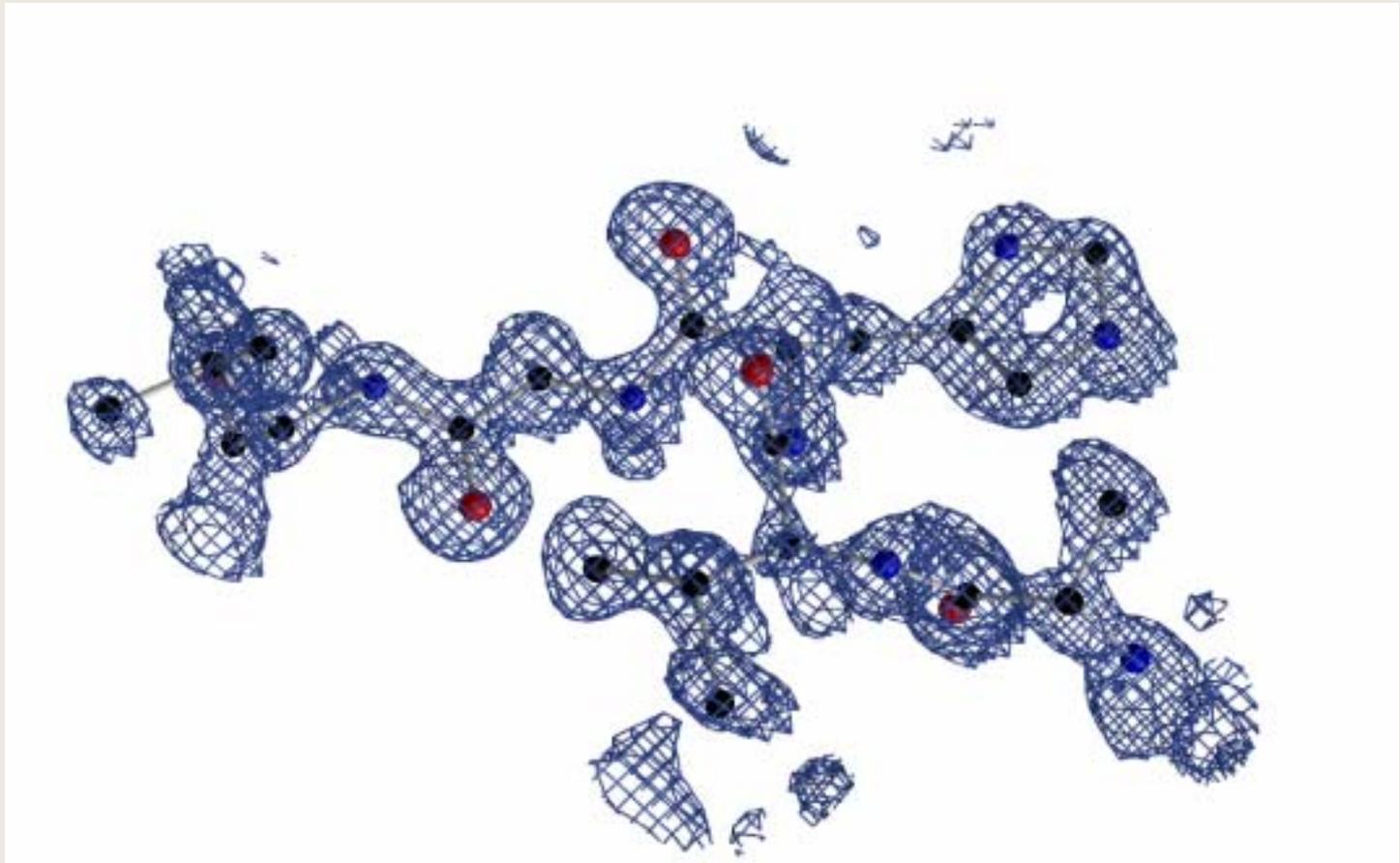
1.04 Å wavelength, 1.2 Å resolution

20 data sets, 100° each

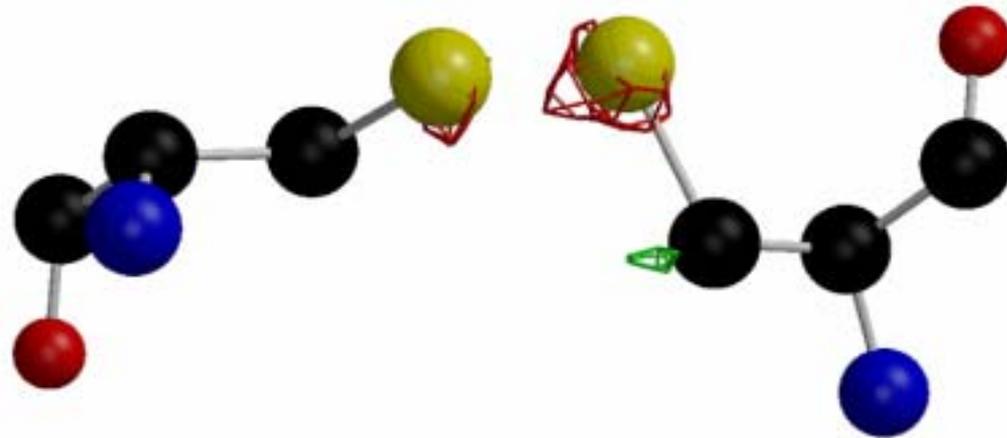
scaled separately

Elastase + $\text{KAu}(\text{CN})_2$

SAD map phased with BP3 – no DM



Elastase S-S bond



Thaumatococcus + N-I -succinimide, data

P4₁2₁2 , 207 residues

8 S-S bridges, 1 Met, 8 Tyr - 11 I sites

Data collected at X9B / NSLS

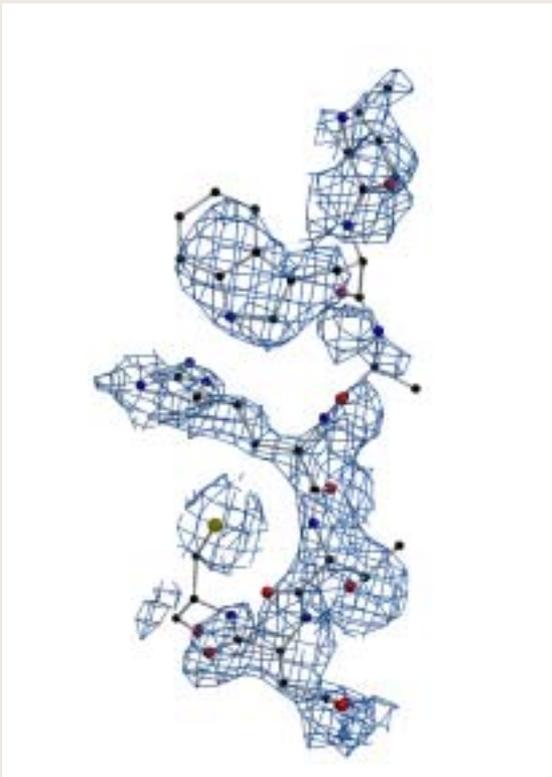
1.54 Å wavelength, 2.5 Å resolution

11 data sets, 60° each

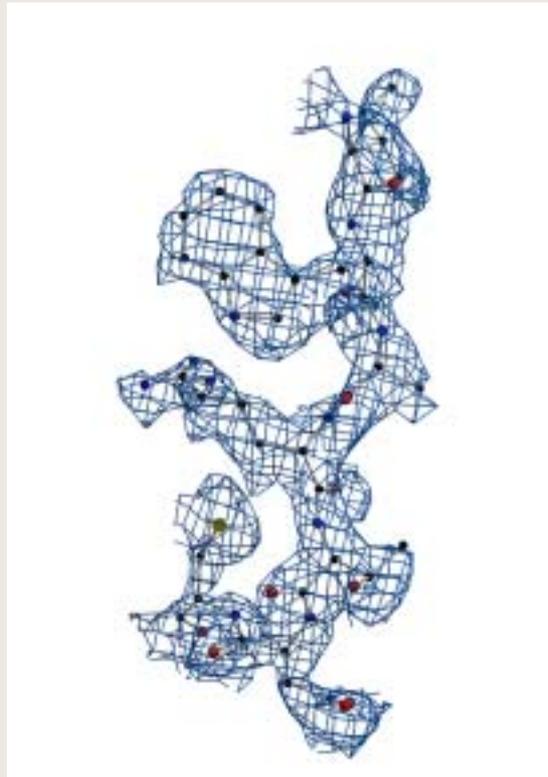
scaled separately

Thaumatococcus + N-I-succinimide, maps

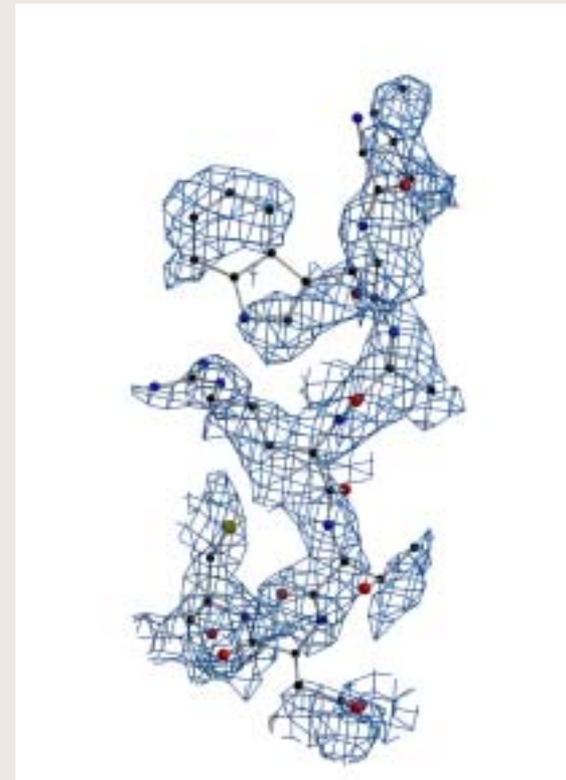
RIP map



RIPAS map



SAD map no DM



Conclusions

Weak scatterer sites located from
anomalous or
radiation damage signal
can be effectively used for phasing
by SAD, RIP or RIPAS
requires accurate data

thanks to powerful programs
and their developers