

PROTEUM R

SAS Phasing

at University of Georgia, Athens, GA.

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EXPERIMENTAL SYSTEM

Bruker AXS PROTEUM R including SMART 6000 CCD detector with three-axis goniometer mounted on Rigaku RU300 0.3 x 3 filament 50/90 and Osmic "blues" CMF-12-38Cu6.

Highly redundant data was collected using the Osmic "blues" for maximum intensity and the SMART 6000 from cubic insulin.

The data was 27-fold redundant in two passes.

The CCD detector was straight on, and resolution at the edge was 2.1 Å. 3.5 Å data was used for phasing. SOLVE was used to phase from the highly redundant data set with excellent anomalous signal. The data was collected using SMART, processed using new Bruker AXS software, PROTEUM, and then scaled using the Bruker AXS software, ProScale.

SOLVE was able to phase and solve the positions for all disulfides in the Insulin molecule. The data were checked with the Patterson maps and the map from SOLVE was instantly interpretable as insulin.

TECHNICAL DATA

- Zinc-free insulin Cubic I 2₁3 and cell is 78 Å.
- Data extended to 2.1 Å in corners of frame and collected straight on at 2-theta zero.
- 161,301 reflections total, 122,122 observed, and 131 rejected in ProScale.
- Integrated with PROTEUM software and scaled with ProScale, both from Bruker AXS.
- Rsym after ProScale was 3.91%.
- At the edge of data shell, the Rsym was 4.53% and I/sigma was 14.9 at highest resolution shell.
- At 3.5 Å, where the data was truncated, Rsym was 3.4% and I/sigma was 21.7.
- Redundancy 27 fold.
- The phasing was solved using the standard SOLVE package with 3.5 Å data.

Success of this method is attributed to the very sensitive CCD detector and the high redundancy of the data.

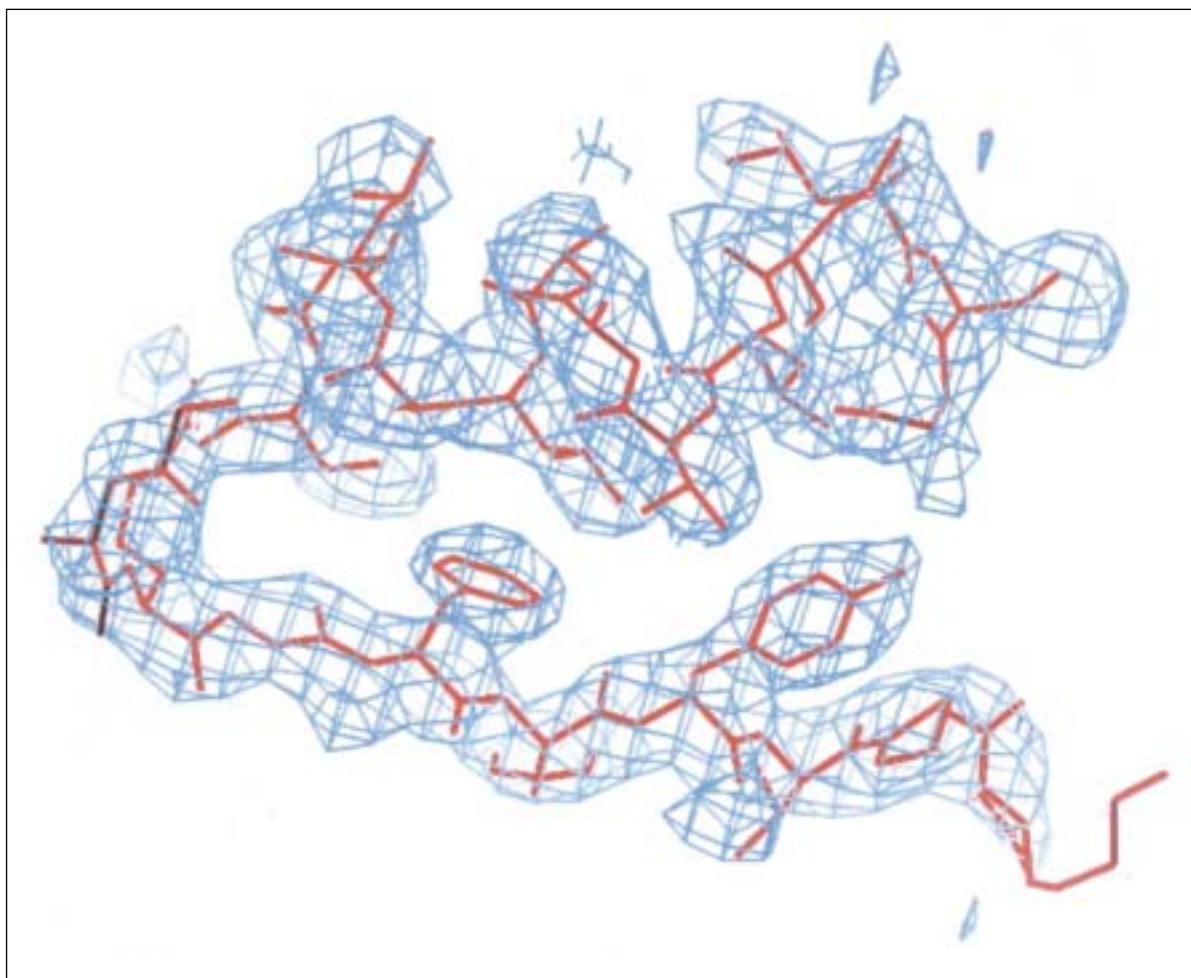


Fig. 1: SAS phasing of zinc-free bovine insulin by B. -C. Wang, Z.-J. Liu, John Rose, Gary Newton, Lei Wang, University of Georgia. Structure solved from lab PROTEUM R data.

ISAS electron density map calculated from Bovine insulin (5.7 kDa) I 2₁3 crystal form at 3.0 Å resolution. The phases were obtained from 3 sulfur super-atoms that were found by SOLVE. The X-ray diffraction data was collected from a single crystal (0.2 x 0.2 x 0.15 mm) on a Bruker SMART 6000 CCD detector using 5 kW Cu K α .

X-rays. The data set was processed using the Bruker AXS PROTEUM software. 122122 reflections were observed and only 131 reflections were rejected. There are 4196 unique reflections to 2.1 Å. The R-merge for this data set is 3.91%.

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