Cheiron School BL Practice of BL38B1 (Protein Crystallography)

Title:

Data Collection and S-SAD Phasing of Protein Crystals

Staff:

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Abstract:

Sulfur SAD (S-SAD) phasing has been got attention because it does not need heavy atom derivative and is expected to improve the throughput of structure determination. However, accurate measurement of diffraction intensity is crucial for the success of S-SAD because of the small anomalous signal of sulfur atom. In this exercise, participants will collect diffraction data from a protein crystal and determine the structure by using S-SAD phasing.

Contents:

1. Brief Introduction of BL38B1

Energy range 6 ~ 17.5 keV

Energy resolution $\Delta E/E = 10^{-4}$

Spot size horizontally 87 μ m, vertically 142 μ m (@ 12.4 keV ($\lambda = 1.0$ Å)) at sample position.

Fig 1. BL38B1. (Left) Inside of experimental hutch. (Right) Outside. Operators desks and computers are located.

1.1. Optical Components

Simple optics which consists of SPring-8 standard double crystal monochrometor



and 2-dimensional focusing bend cylindrical mirror is adopted.

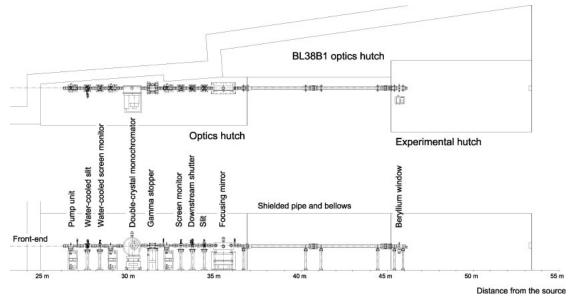


Fig. 2: Schematic View of Beamline

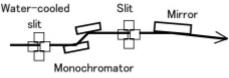


Fig. 3: Outline of Optics

1.2. Experimental Station

Rapid data collection system for protein crystallography is installed at BL38B1. Combination of the coaxial-microscope to observe crystal along x-ray beam path and the goniometer having motor driven x, y and z stage make centering of crystal very easy. Rapid readout CCD detector with a detective area of 315×315 mm² enables efficient data collection with minimum dead time and high resolution data collection even when using long wavelength.

Data collection, including measurement of XAFS spectrum and automatic MAD (Multi-wavelength Anomalous Diffraction) data collection, is performed through controlling software BSS (Beamline Scheduling Software). Since BSS can control all of the instruments required for data collection, switch of detector and change of camera length, wavelength are automatically performed at the beginning of measurement. Therefore, what beamline users have to do after crystal mounting and centering is to input measurement conditions to BSS.



Fig. 4. Diffi



Fig.5. Magnified view around the goniometer coaxial-microscope with a zoom lens enables easy crystal centering

1.3. Specification of beamline equipments
 * CCD detector (ADSC/ Quantum315r)
 Detector area : 315 × 315 mm²

Pixel size : $51 \times 51 \ \mu m^2$ (unbin) No. of pixels : 6144×6144 Camera length : $75 \ \sim \ 950 \ mm$

- * Horizontal axis goniometer having motor-driven x, y, z stage
- * Cryostat : temperature control range 80 \sim 350 K
- * Gas-flow type ionization chamber
- * Si PIN photodiode detector for fluorescence measurement
- * Multi-channel analyzer of fluorescence measurement
- * Control Software BSS
- * Sample Automated Changer



Fig. 6: Sample Auto Change SPACE

- 2. Optical Alignment and Adjustment
- 2.1. Control Software BSS

The software of BSS (Beamline scheduling software) can deal with all of the beamline operation as follows: 1) Control the optical and experimental components as monochromator, goniometer, and detector. 2) Data collection of crystal diffraction, X-ray fluorescence etc.

The GUIs of the software is as follows:

| ▼ Beamline | Scheduling Software [Last modified at 19:30, Sep/6/2006 (W | Ved)]running by ueno@Linux |
|---|--|---|
| Fil <u>e</u> Settings Tools Database H <u>e</u> lp | | |
| Schedule Centering XAFS Current Status | Devices Sample Changer | |
| Centering mode and tools Start auto-centering Click-on centering Back Light Evacuate Devices | Crystal Rotation Current spindle angle: 0.0 [deg] + Side view - Side view Reverse Manual rotation 45 * [deg] Abs Rel Crystal Translation Up 10 * [um] Left Right Down | Operator tools Save sample image Finish centering Beam size circle 1 • Set Update Snap shot X-ray image Expose 1.00 $\stackrel{<}{\Rightarrow}$ sec. \checkmark Invoke viewer Start |
| | [| Start Kill Stop BSS Dump Exit |
| | Message Console | |
| System Message Job Message Error Message | - | |
| (bss's pid = 1999, videosrv's pid = ????) Inserting back light, st shutter will be forced to close, 2 Close st-shutter 2006/10/25 [Wed] 11:44:42 success 2006/10/25 [Wed] 11:44:42 | 006/10/25 [Wed] 11:44:42 | |
| MODE : Manual, OPTION : Simulation, STATUS : Cr | vstal Evaluation | |

Fig. 7: BSS Crystal Centering Tab

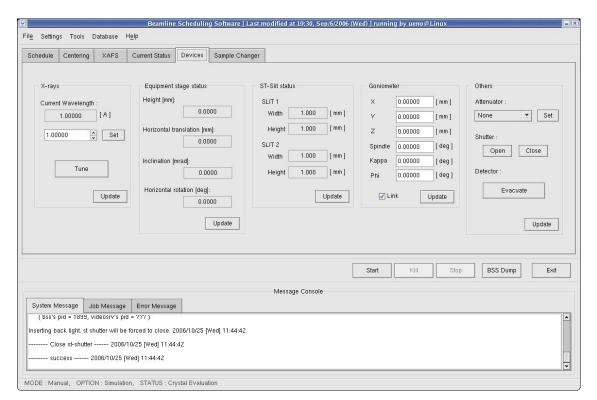


Fig. 8: BSS Device Control Tab

- 2.2. Operations of Beam Alignment
- 2.2.1. Confirm and define the rotational center of the goniometer head at the window of the sample monitor software using a sample mount Lithographic loop. If the position is not located at the center of the display, the position should be aligned by the micrometer of the sample monitor CCD camera. The cross of the center position can also be aligned by the software definition of the video capture software.
- 2.2.2. BSS Run Option is set to "Admin" mode.
- 2.2.3. Set the spindle axis to 0 degree.
- 2.2.4. Put the fluorescent plate on the goniometer head.
- 2.2.5. Focus the microscope on the surface of the fluorescent plate by moving stl_gonio_1_x axis from Axis Tools window of BSS.
- 2.2.6. Turn off the sample illuminator.
- 2.2.7. Set wavelength 1 Å and open X-ray shutter.
- 2.2.8. Record the X-ray intensity at 1A (Electric current of ionization chamber).
- 2.2.9. Display the monochromator alignment GUI on BL-Work Station. Record the position of $\Delta \theta_1$ axis.
- 2.2.10. The size of two slits (ST1/ST2) is set to 3 mm by Axis Tools.
- 2.2.11. Tune $\Delta \theta_1$ to maximize intensity by using the Energy tab of Axis Tools. Record the I₀ value,

the position of the beam, and the shift of $\Delta \theta_1$.

- 2.2.12. Beam position should be aligned to the rotational center of the goniometer by the translating the experimental stage. In Z axis direction (height), beam position should be placed on the spindle axis. In Y axis direction (horizontal), the position should be the central cross of the video monitor.
- 2.2.13. Change the slit aperture size to the original values. The aperture size of ST1 and ST2 are 0.5/0.3mm and 0.4/0.4mm, respectively. Record the I₀ value.
- 2.2.14. Tune $\Delta \theta_1$ again.
- 2.2.15. BSS Run Option is set to "None" mode.
- 3. Data Collection and Phasing of Insulin Crystals
- 3.1. Theoretical background of SAD phasing
- 3.1.1. Anomalous effect

Thomson scattering occurs the following scheme: 1) X-ray photon strikes a free electron, 2) the electromagnetic field of the incident oscillates the electron. 3) the oscillated electron scatters X-ray with the same wavelength as the incident X-ray.

However, the electrons of atoms are bound tightly and its oscillation has a certain resonance frequency. Therefore, the scattering factor is depends on the incident wavelength. It can be expressed as the following formula.

$$f = f_o + f' + if''$$

In the case of sulfur atom, the wavelength dependent f' and f' terms are illustrated as following graphs.

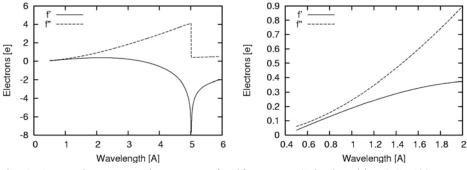


Fig. 9. Anomalous scattering terms of sulfur atom. Calculated by CCP4/Crossec.

3.1.2. Harker diagram and phase

Crystallographic F(h) values (structure factors) are given by Fourier transformation of

electron density $\rho(\mathbf{x})$. F(**h**) value is a complex and composed of absolute $|F(\mathbf{h})|$ and phase $a(\mathbf{h})$. Inverse Fourier transformation of F(**h**) gives electron density of crystal, however $|F(\mathbf{h})|$ is only obtained from diffraction experiments. The phase $\alpha(\mathbf{h})$ can be recovered from several experimental methods. Here we describe the scheme of SAD method.

Harker diagram (phase diagram) is a picture for understanding phase determination.

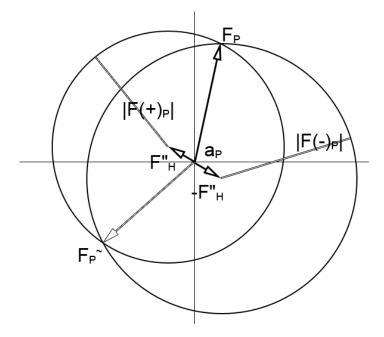


Fig 10: Harker diagram of SAD method.

Anomalous effect breaks down Friedel's Law where a reflection $F(\mathbf{h})$ and its centrosymmetric reflection $F(-\mathbf{h})$ have same value of $|\mathbf{F}|$. ΔF , the difference of $F(\mathbf{h})$ and $F(-\mathbf{h})$ is a good approximation of the structure factor amplitude of anomalous scatterers. Therefore, Patterson method or direct phasing can extract the position of the anomalous scatterers.

Once the positions of anomalous scatterers are determined, their structure factor ($\mathbf{F}^{"}_{H}$) is calculated. The vector of $\mathbf{F}^{"}_{H}$ and its negative can be drawn in the diagram. Next, we can draw two circles: one with radius of $|\mathbf{F}(+)_{P}|$ centered on the end of $\mathbf{F}^{"}_{H}$. another with radius $|\mathbf{F}(-)_{P}|$ centered on the end of $-\mathbf{F}^{"}_{H}$. We can see the two intersections F_{P} and F_{P}^{\sim} of the two circles. One of the two points is correct answer. Although the two possibilities cannot be distinguished without any information, we can obtain good phases by the treatment of electron density shape (density modification; solvent flattening etc.) in some cases.

3.2. Data Collection

3.2.1. Sample mounting on goniometer

1) Pick up a desired crystal by a cryoloop device: Firstly, a desired crystal is scooped with a cryoloop under a microscope. Protein crystal is mostly small and its typical dimension of $0.1 \sim 0.2$ mm. Protein crystal is obtained from water solution, therefore it is easy to dry up under atmospheric condition and also very fragile. Scooping process should be conducted rapidly.

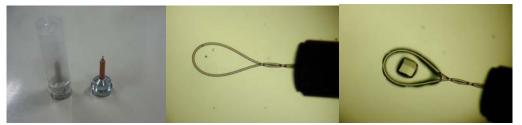


Fig. 10. Cryoloop. (Left) Cryoloop, (Middle) Nylon loop. The tip of the cryoloop. (Right) Cryoloop with a crystal.

2) Soaking the crystal into cryoprotectant: The scooped crystal is transferred into the solution containing cryoprotectant (typically it contains 30% glycerol) which prevents ice formation during sample cooling.

3) Picking the crystal again: To mount the crystal onto a goniometer head, the crystal is picked again. Since glycerol containing solutions are viscous, the excess of the solvent is sometime scooped onto the cryoloop. The excess causes unfavorable scattering which degrades the statistics of diffraction data. Since the anomalous signal for S-SAD phasing is weak, the solvent should be removed possibly.

4) Mounting and flush cooling the crystal: Immediately, the picked crystal should be put on the goniometer head with magnetic support. To cool the sample during X-ray irradiation and prevent the radiation damage of the sample, cold nitrogen gas stream is blowing at the crystal position. Before mounting the crystal, the blow is blocked out by the shutter system. And after mounting, the shutter should be immediately opened. This is 'flush cooling technique' for preventing ice formation in solvent and crystal.

3.2.2. Collection of diffraction images

1) Set up the data collection software: To take diffraction images, the program BSS is used at this beamline. Following two figures are the windows of data collection GUI.

| hedule Centeri | ing XAFS | Current Statu | us Devices | Sample Changer | | | | | | |
|---|---------------------------------------|-----------------------------------|------------------|----------------|----------------|-------|-------|--------|------------------|-------------------------|
| Centen | Ing AMPS | Current State | is Devices | Sample Changer | | | | | | |
| dit Menu | Job ID | Status | Mode | Crystal ID | Tray Position | From | To | step | Exp. Time | Wavelength |
| Edit / View | 1 | Waiting | Crystal Checl | k Unknown | Not Used | 0.00 | 1.00 | 1.00 | 4.00 | 1.00000 |
| Euit / View | 2 | Waiting | XAFS | Unknown | Not Used | 10000 | | 0.0001 | 1.0 | 0.97474> 0.98474 |
| Append | 3 | Waiting | Crystal Checl | k Unknown | Not Used | 0.00 | 1.00 | 1.00 | 4.00 | 1.00000 |
| mppelia | 4 | Waiting | Crystal Checl | k Unknown | Not Used | 0.00 | 1.00 | 1.00 | 4.00 | 1.00000 |
| Delete | 5 | Waiting | XAFS | Unknown | Not Used | 1000 | | 0.0001 | 1.0 | 0.97474> 0.98474 |
| Delete | 6 | Waiting | Crystal Checl | k Unknown | Not Used | 0.00 | 1.00 | 1.00 | 4.00 | 1.00000 |
| Up | 7 | Waiting | Single Wavelen | igth Unknown | Not Used | 0.00 | 90.00 | 1.00 | 4.00 | 1.00000 |
| | 9 | Waiting | Multiwaveleng | th Unknown | Not Used | 0.00 | 1.00 | 1.00 | 5.00, 4.00, 4.00 | 1.00000,1.02000,1.04000 |
| | | | | | | | | | | |
| All Clear | | | | | | | | | | |
| All Clear | | | | | | | | Start | Kill Sto | p BSS Dump Exit |
| All Clear | | | | | Message Cons | sole | | Start | Kill Sto | p BSS Dump Exit |
| All Clear | Job Messag | e Error Mess | age | | Message Cons | sole | | Start | Kill Sto | p BSS Dump Exit |
| | | | age | | Message Cons | sole | | Start | Kili Sta | p BSS Dump Exit |
| System Message (bss's pid = 185 | 19, videosrv's p | old = 777) | | 'ed] 11:44:42 | Message Cons | sole | | Start | Kill Sto | p BSS Dump Exit |
| system Message (bss's pid = 185 everting back light. | 39, videosrv's p st shutter will b | old = 777) be forced to close | e. 2006/10/25 [W | 'ed] 11:44:42 | Message Cons | sole | | Start | Kill Sto | p BSS Dump Exit |
| jystem Message | 39, videosrv's p st shutter will b | old = 777) be forced to close | e. 2006/10/25 [W | 'eq] 11:44:42 | — Message Cons | sole | | Start | Kili Sto | p BSS Dump Exit |

Fig. 11: Main window of BSS

| | Edit Schedule |
|---|---|
| Job ID | 1 |
| Crystal ID | Unknown |
| File name template | sample Start serial # 1 * |
| Data Directory | /image/test/ |
| Mode Collection | Detector XAFS Sample Changer Database Advanced |
| Scan condition from 0.0 | |
| Camera distance 150.0 × [m Wavelength [A] | Sampling interval m] 1 v [points] |
| #1: 1.00000 ÷ | #2: 1.02000 * #3: 1.04000 * #4: 1.06000 * |
| Exposure time [sec] | I |
| #1: 4.00 * | #2: 4.00 * #3: 4.00 * #4: 4.00 * |
| Number of Wavelen | gth MAD interval CCD 2 theta |
| 1 | 10 (points) 0.0 (deg) |
| | |
| | Apply X Close Show List |

Fig. 12: The window for editing data collection condition.

2) Centering the sample to beam position: Diffraction images are taken by oscillation

method. In the method, crystal rotates and oscillates with the spindle axis of goniometer. To keep the crystal position during the rotation against X-ray beam, the crystal is aligned to the center of the spindle axis, because X-ray beam is already aligned to the spindle axis. This operation can be achieved in the program BSS using video camera and monitor.

3) Closing experimental hutch and opening down stream shutter of the beamline.

4) Take a few images and check the diffraction: To estimate the quality of the mounted crystal, one or two images should be taken before taking data set.

5) Take a full data set: When the crystal passes through the quality check, the consecutive series of diffraction images are taken. In most cases, 180 images will be taken if you do not have any information of the crystal as space group, cell constant etc. In the case of SAD, highly redundant data collection is required, because it makes the statistics better.

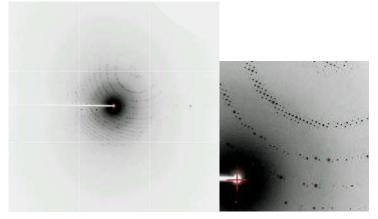


Fig. 13: A diffraction pattern of a protein crystal.

3.3. Data processing

Diffraction images are processed by a special computer program. Against the raw data of each image, the following process is performed: 1) the prediction of reflections (diffraction spots) through the determination of lattice constants and crystal orientation and the parameter-refinement of the camera-distance etc [indexing and prediction]. 2) the determination of reflection index and the estimation of its intensity [integration]

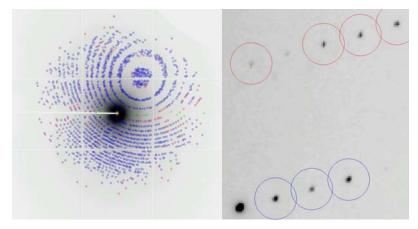


Fig: 14: Spot prediction

After obtaining the index and intensity of each reflection in all images, the intensity should be merged into reflections which are equivalent in crystallographic symmetry [scaling]. Finally we obtain F-data (containing *hkl*: Mirror index and F(hkl): structure factor amplitude). In the case of SAD, Friedel mates F(+) and F(-) should be recorded independently.

3.4. Phase Calculation by SAD

There are several program packages for this purpose. In this practice, HKL2MAP will be used. This program is a GUI software for SHELX crystallographic analysis package. This analysis is performed as following procedure: 1) SHELXC: the preparation of ΔF data from experimental F data. 2) SHELXD: finding the positions of sufur atoms as anomalous scatterers. 3) SHELXE: phase calculation and phase improvement by density modification. Finally, the calculated phases are checked by electron density maps using the program XtalView/Xfit. Its details will be introduced at the BL practice. Only several pictures are shown.

| hki2map Version 0.2 | 1 |
|--|---------------------------|
| File Tools Help | |
| Project name : Insulin_S-SAD | |
| SHELXC - prepare 🗄F or FA data from experiment 00:00:00 | |
| Prepare Fa data from SAD = experiment. | |
| Native in : Browse | |
| HA in : output sca. Browse | |
| Cell: a 77.938 b 77.938 c 77.938 alpha 90.000 beta 90.000 gamma 90.000 | |
| Space group name or number : 1213 confirmed : 📕 | |
| Native out : Insulin_S-SAD.hkl Browse | |
| Fa out : Insulin_S-SAD_fa.hkl Browse | |
| more options view graphics Run SHELXC | |
| | |
| SHELXD - find heavy atoms SHELXE - phasing and density modification | |
| Current status of data preparation, substructure solution and phasing : | |
| /SHELXC//SHELXD/SHELXE original/SHELXE inverted/ | |
| SYMM Y, Z, X SYMM -Y, 1/2+Z, 1/2-X SYMM 1/2-X 1/2-Z -X | |
| SYMM 1/2+Y, 1/2-Z, -X SYMM 1/2-Y, -Z, 1/2+X SFAC SE | |
| UNIT 768 SHEL 999 2.5 PATS | |
| PIND 8 MIND -1.5 -0.1 WINY 100 | |
| SEED 1 HKLF 3 | |
| END | |
| + SHELXC for Insulin_S-SAD finished at 16:29:15 on 04 Sep 2007 + | |
| | |
| | Fig. 15: hkl2map (Shelxc) |
| | (Surence) |
| hkl2map Version 0.2 | |
| hkl2map Version 0.2 | |
| | |
| File Tools Project name : Insulin_S-SAD SHELXC - prepare IF or FA data from experiment 00:00:00 | |
| File Tools Help Project name : [Insulin_S-SAD Insulin_S-SAD | |
| File Tools Project name : Insulin_S-SAD SHELXC - prepare IF or FA data from experiment 00:00:00 SHELXD - find heavy atoms CCmax : 35.62 Try : 64 / 100 00:00:4:46 Fa in : Insulin_S-SAD_fa.hkl | |
| File Tools Project name : Insulin_S-SAD Insulin_S-SAD Insulin_S-SAD SHELXC - prepare IF or FA data from experiment 00:00:00 SHELXD - find heavy atoms COmax : 35.62 Try : 64 / 100 | |
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| File Tools Holp Project name : Insulin_S-SAD SHELXC - prepare IF or FA data from experiment 00:00:00 SHELXO - find heavy atoms COmax : 35.62 Try : 64/100 00:04:46 Fa in : Insulin_S-SAD_fa.hkl Browse Ins in : Insulin_S-SAD_fa.hkl Browse Find 6 heavy atoms of type S Use data from 999 to 2.5 Å resolution. Allow sites on special positions? \vert yes IIO Imit number of tries to 100 . PDB out : Insulin_S-SAD_fa.pdb Browse Browse more options view graphics finish SHELXD SHELXE - phasing and density modification 7 SHELXE - phasing each density modification 7 SHELXC / SHELXC / SHELXE integenal / SHELXE interfee 7 SHEX 99 86 64 65 55 39 26 7 PSUM 27.02 PSUF 986 86 45 55 39 26 7 8 a 3 32 26 20 20 18 17 16 7 Try 62.18 Peaks 99 86 64 65 55 39 26 7 8 a 5 39 26 7 8 Try 63.18 Peaks 99 86 64 65 55 39 26 7 8 a 90, best PAITOM 9.00 9 PAT | |
| File Tools Help Project name : Insulin_S-SAD SHELXC - prepare EF or FA data from experiment 00:00:00 SHELXD - find heavy atoms CCmax : 35.62 Try : 64 / 100 SHELXD - find heavy atoms CCmax : 35.62 Try : 64 / 100 SHELXD - find heavy atoms of type S. Use data from 993 to 2.5 A resolution. Browse Browse Find 6 heavy atoms of type S. Use data from 993 to 2.5 A resolution. Allow sites on special positions? yes + 100 Dimit number of tries to 100 Limit number of tries to 100 . PDB out : Insulin_S-SAD_fa.pdb Browse more options view graphics finish SHELXD Prowse SHELXE - phasing and density modification // // // SHELXC_VSHELXD_VSHELXE original/SHELXE inverted // // // PSUM 27.02 PSMP Peaks: 105 86 53 42 41 38 33 32 26 20 20 18 17 16 // // Try 62.18 Peaks 99 86 64 55 53 92 66 // // // // PSUM 27.02 PSMP Peaks: 105 86 53 42 41 38 33 32 26 20 20 18 17 16 // <th>Fig. 16: hkl2map (Shelxd)</th> | Fig. 16: hkl2map (Shelxd) |

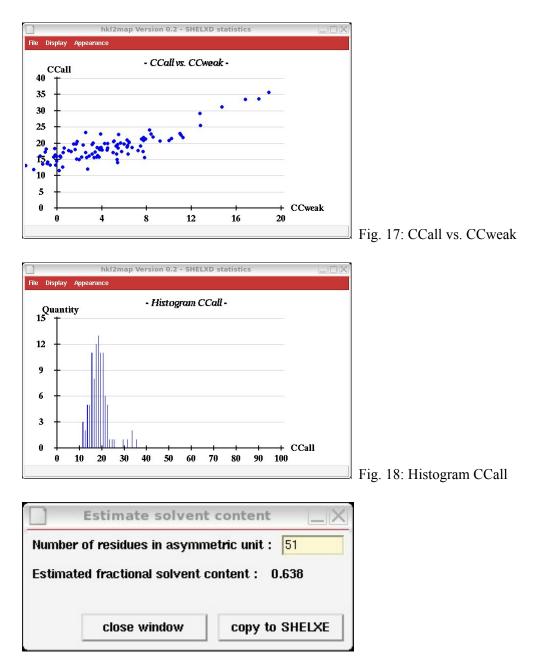
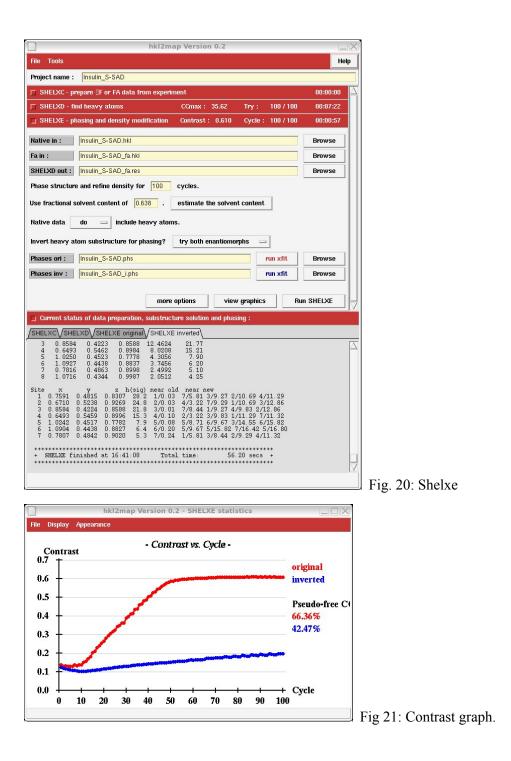


Fig. 19: Estimate Solvent Content. Insulin is composed of 51 amino acids



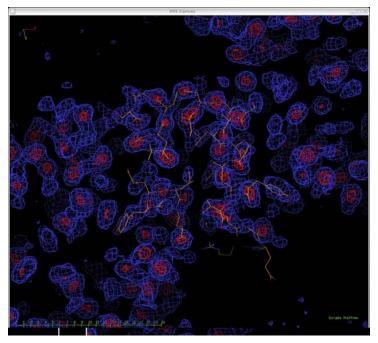


Fig. 22: Nicely determined phases. High contrast electron density map was obtained.