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20. Neutron Structural Biology

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Abstract

Neutron structural biology will be one of the most important fields in the life sciences which will interest human beings in the 21st century because neutrons can provide not only the position of hydrogen atoms in biological macromolecules but also the dynamic molecular motion of hydrogen atoms and water molecules. However, there are only a few examples experimentally determined at present because of the lack of neutron source intensity. Next generation neutron source scheduled in JAERI (Performance of which is 100 times better than that of JRR-3M) opens the life science of the 21st century.

1. Introduction

It is said that universe began with so-called big bang about 15 billion years ago and the first living cell was born about 3 and half billion years ago. Evolution after evolution, at present tremendous number of living cells exist on the earth. For example, number of kinds of human proteins is about 100 thousand. In the latter half of 20th century, three dimensional structure determination of biological macromolecules such as proteins and DNA by X-ray single crystal structure analysis brings many of the mysteries of life sciences to light. The structural information contributes the understanding of the physiological function of proteins and DNA and this field is called as structural biology.

Structural biology is one of the most important fields in the life sciences, which will interest human beings in the 21st century. At the same time it clearly suggests that hydrogen atoms and water molecules around proteins and DNA play a very important role in many physiological functions. However, since it is very hard for X-rays to determine the position of hydrogen atoms in protein molecules, detailed discussion of protonation and hydration can be only speculated upon so far.

On the other hand, neutrons can provide not only the position of hydrogen atoms in biological macromolecules but also information

about the dynamic molecular motion of hydrogen atoms and water molecules. Since the physiological functions accompany microscopic motion of atoms at active sites, neutron inelastic scattering is expected to be an experimental clues to clarify physiological functions from a microscopic view point. In principle, these have been well identified, but there are only a few examples experimentally determined at present. The biggest obstacle is the lack of neutron source intensity.

The next generation neutron source scheduled in JAERI, the performance of which is 100 times better than that of JRR-3M, will be expected to provide the breakthrough in the 21st century life science. A few prospective examples will be demonstrated.

2. Neutron scattering for structural biology

Neutron has following distinctive features for investigation of structural biology:

1) Neutron can identify hydrogen in a protein,
2) neutron can distinguish deuterium from hydrogen,
3) neutron can observe dynamics of atoms and molecules, and
4) neutron does not give irradiation damage to protein.

In order to apply these features to structural biology, single crystal neutron diffractometry, small angle neutron scattering and inelastic neutron scattering experiments are carried out.

Figure 1 shows the correlation between the neutron experiments and titles on structural biology investigated.

3. Structure of biological macromolecules complex

Main processes how the protein synthesis information flows in a cell are illustrated in figure 2. Extra-cellular information is received by membrane protein locating in cell membrane. This becomes a trigger of the following processes. The DNA information is transferred to RNA by DNA binding protein, and RNA which has the information of DNA is passed to a protein synthesis factory, ribosome where protein is synthesized according to the DNA information. In the processes plenty of proteins in the complex form take part in, such as membrane protein which is a complex of protein and lipid, and DNA binding protein which is a complex of DNA and protein, and ribosome which is a complex of various kinds of protein and RNA.

Small angle neutron scattering (SANS) experiment is very effective to determine the complex structure of biological macromolecules by the use of contrast variation (CV) method. The contrast is defined as the difference between the mean neutron scattering length density of the solute molecule, ρ_m and the one of

the solvent water, ρ_s. Since ρ_s is changed with the D_2O/H_2O ratio in the solvent water, consequently the contrast for SANS is varied with the different D_2O/H_2O ratio. This is called as the CV method.

Why is CV method effective to the structural investigation of the complex? Let me have an example of the DNA-protein complex. ρ_s of D_2O and H_2O are 0.06404 x 10^{-12} cmÅ^{-3} and -000562 x 10^{-12} cmÅ^{-3}, respectively, and ρ_m of protein and DNA are about 0.02 x 10^{-12} cmÅ^{-3} and 0.04 x 10^{-12} cmÅ^{-3}, respectively. When ρ_s of D_2O/H_2O solvent becomes equal to ρ_m of protein, neutron can see only DNA, and the structure of DNA in the complex can be determined.

Although the positional resolution determined by SANS is about 10Å, requirement of the experiment never decreases since there are plenty of complexes which are not crystallized.

4. Hydrogen and hydrates of biological macromolecules

It is easily understood that hydrogen atoms and hydration water molecules play an important role in most of physiological functions (ex. hydrolysis, dehydrogenation, oxidation-reduction, phosphorylation, dephosphorylation and so on) since half of the constituents of biological macromolecules are hydrogen atoms and life cannot live without water. Hydrogen atoms and hydration water molecules play an important role to fold and stabilize a protein into a globular conformation. There are hydrophilic and hydrophobic amino acids which form the protein. The hydrophilic amino acid side chains tend together on the outside of the protein, where they can interact with water; the hydrophobic amino acid chains are buried on the inside to form a hydrophobic core that is hidden from water. However, since there are few reports to define the accurate position of hydrogen atoms of protein and its hydration water molecules, the quantitative discussion on the folding and protein stability could not be carried out so far.

The X-ray diffraction of single crystals has supplied knowledge on the atomic structure of protein, viruses, t-RNA and DNA. Since the structure-function relationship of protein is dominated by the behavior of hydrogen atoms, it is important to know their positions. However, it is difficult for X-ray crystallography to provide structural information of hydrogen atoms. On the other hand, neutron diffraction provides an experimental method of directly locating hydrogen atoms. To date, there are relatively few examples of neutron crystallography in biology since it takes considerable time to collect a sufficient number of Bragg reflections due, in past, to the low flux of neutrons illuminating the sample.

A large area detector system can be constructed using an imaging plate (IP), which is now routinely used in X-ray protein crystallography. The X-ray IP can be converted into a neutron detector when a neutron converter, such as ^{10}B, ^{6}Li or Gd, is combined with the IP. We have successfully developed the neutron IP (NIP), by mixing the neutron converter, ^{6}Li or Gd with a photostimulated luminescence (PSL) material on a flexible plastic support.[1)]

Neutron quasi-Laue diffraction data (2Å resolution) from tetragonal hen egg-white lysozyme were collected in ten days with NIP.[2)]

Figure 3 shows the one of raw data and was demonstrated as the cover image of the Journal, Nature Structural Biology as shown in Figure 4.

Figure 5 shows the three-dimensional arrangement of the lysozyme molecule with the 157 bound water molecules and 696 hydrogen and 264 deuterium atoms determined in this study.

The identification of hydrogen atoms on the residues Glu35 and Asp 52 agrees with the proposed hydrolysis mechanism of the sugar by the lysozyme molecule. The enzyme reaction activity is maximal at pH 5 when the carboxylate group of Glu35 is protonated, and it is this protein that is transferred to the oxygen atom on the substrate (sugar) bound during the hydrolysis process. During the reaction, Asp52 remains in the dissociated state. The lysozyme crystal in this study was grown at pH7.0, and it is known that lysozyme is less active at pH7.0. Figures 6 (a) and (b) show the $2|F_o| - |F_c|$ map around the carboxylate groups of Glu35 and Asp52, respectively. Around the carboxylate oxygen atoms labeled E35OE1 and E35OE2 and around the carboxylate oxygen atoms labeled D52OD1 and D52OD2 there is no indication of hydrogen (deuterium) atoms, and this de-protonation of the catalytic site residues explains lysozyme reduced activity at pH7.0. Since these results are consistent with the model for lysozyme's activity, it would be instructive to compare the results of triclinic lysozyme crystallized at pH 4.2.

5. Dynamics of biological macromolecules

It is expected that physiological function correlates strongly to dynamics of biological macromolecules. Figure 7 shows temperature dependence of the isotropically averaged mean-square displacements of myoglobin from the neutron experiments.[3)] For temperatures below 200 K the mean-square displacement increases linearly with temperature, in accord with harmonic models for the internal dynamics. Above 200 K there is a transition above which the mean-square displacement increases

more rapidly with temperature. The nonlinear behavior implies anharmonicity in the potential-energy surface. And above 200 K, physiological reaction occurs and below the temperature it ceases.

Dynamics of biological macromolecules are theoretically treated by normal mode analysis or molecular dynamics which can provide the dynamic structure factor which is observed by neutron inelastic scattering experiments. Figure 8 shows (a) displacements of atoms in a low-frequency vibration according to a normal mode analysis of BPTI (bovine pancreatic trypsin inhibitor). Root-mean-square displacements of atoms, calculated at room temperature, are shown by vectors magnified 20 times.[4] (b) Time-of-flight spectra from BPTI in powder (solid line) and in solution (dotted line).[5] (c)The neutron-derived frequency distribution from the BPTI powder experiment (circles), and the vibrational frequency distribution from the molecular dynamics of BPTI in solution (solid line) and in powder (dotted line).[6]

6. JAERI project and neutron structural biology

At present, because of the lack of neutron flux, it is compelled that it takes a long time to collect a full data set and/or it is necessary to prepare a lot amount of specimen although both are not preferable especially on biological material. Increment of neutron flux gives benefit not only to diminish the above troubles but also to enable new experiments.

Figure 9 shows what kind of protein can be measured in a neutron single crystal diffractometry in accordance with increments of neutron incident flux. Neutron diffraction intensity is proportional to the crystal volume and square of inverse of the cell volume. Line(a) indicates that a protein sample, crystal and cell volume of which is above the line(a) can be measured using a diffractometer equipped a normal position sensitive detector installed in 20-60 MW size reactor. If NIP is equipped on the diffractometer, a sample, crystal and cell volume of which is above the line(b) can be measured since NIP enables data collection efficiency 10 times better. If JAERI project is realized, a protein sample above the line(c) can be measured. The cell volume of most of the important protein is less than 10^6 Å^3 (thick dotted line) and the crystal volume which X-ray crystallography needs is larger than 0.003 mm^3 (thick dotted line). Figure shows that neutron source obtained by JAERI project covers most of the important proteins to be investigated.

Up to now, neutron structural biology was expected as a complementary of X-ray structural biology, but it has failed to meet the expectation. JAERI project will surely open the new

generation in neutron structural biology and consequently contribute to life science.

References

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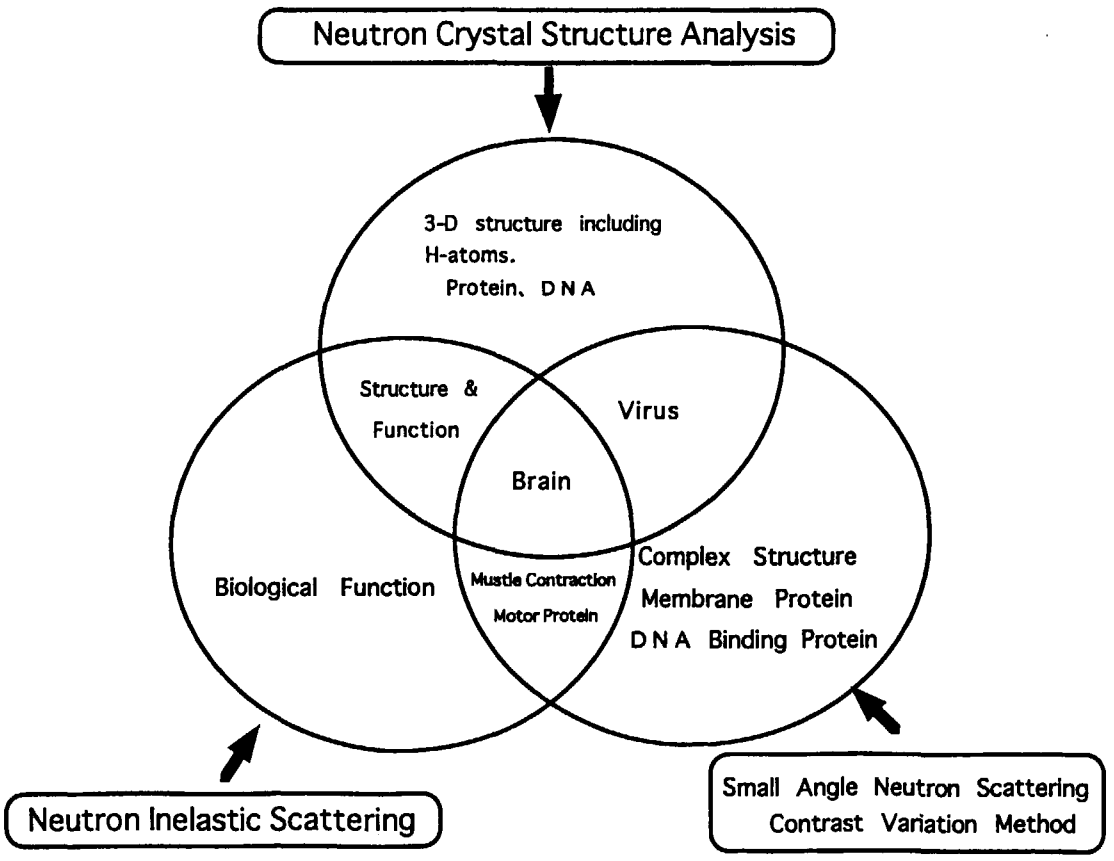


Figure 1 The correlation between the neutron experiments and titles on structural biology investigated.

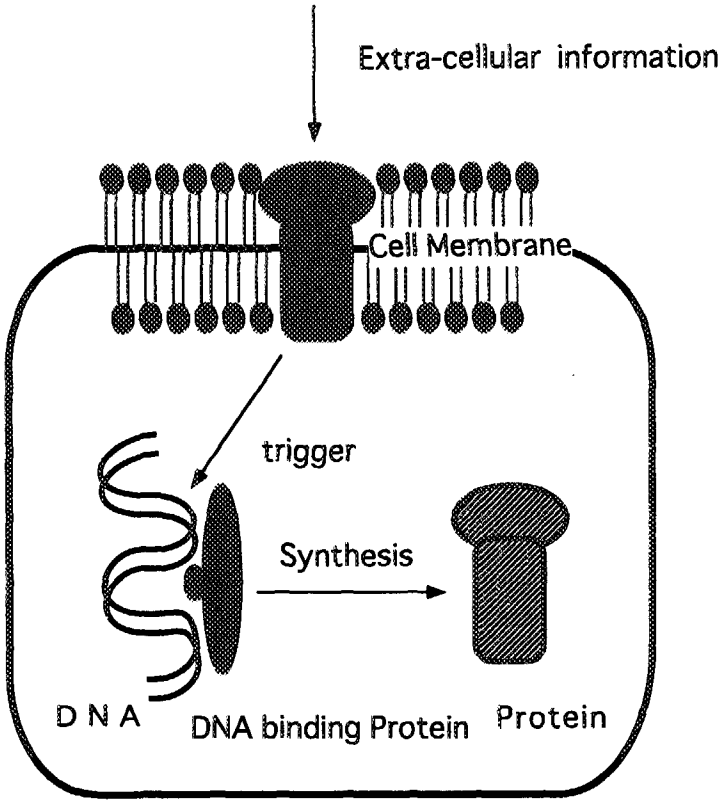


Figure 2 Main processes how the protein synthesis information flows in a cell.

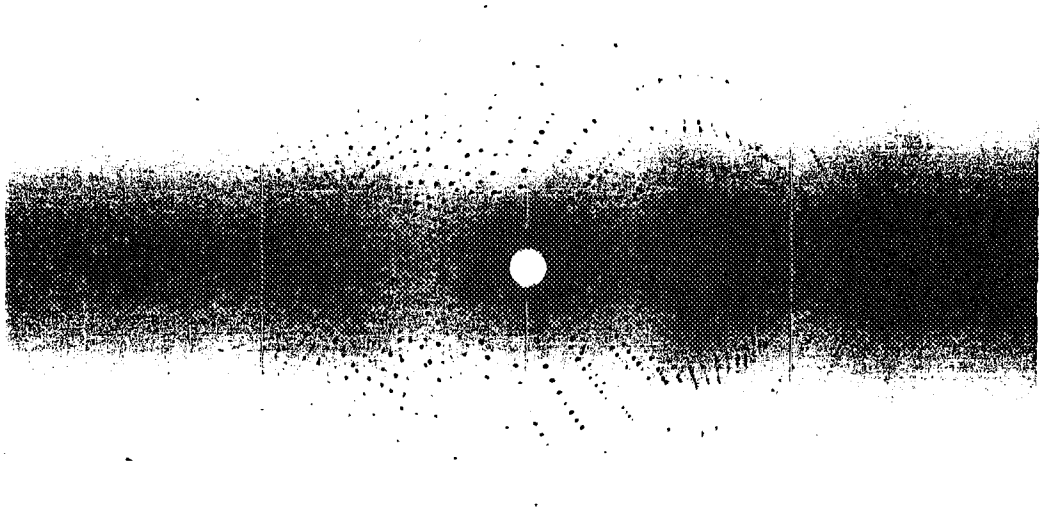


Figure 3 Raw data recorded on the NIP.

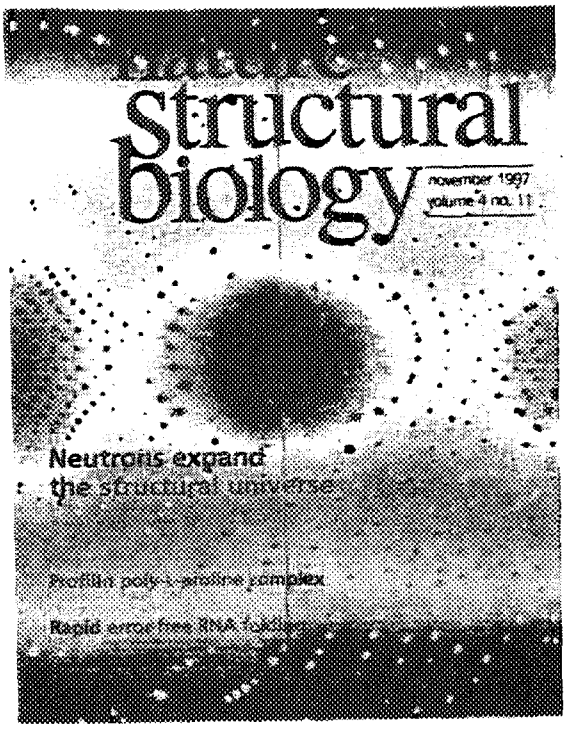


Figure 4 Raw data shown as the cover image of the Journal, Nature Structural Biology.

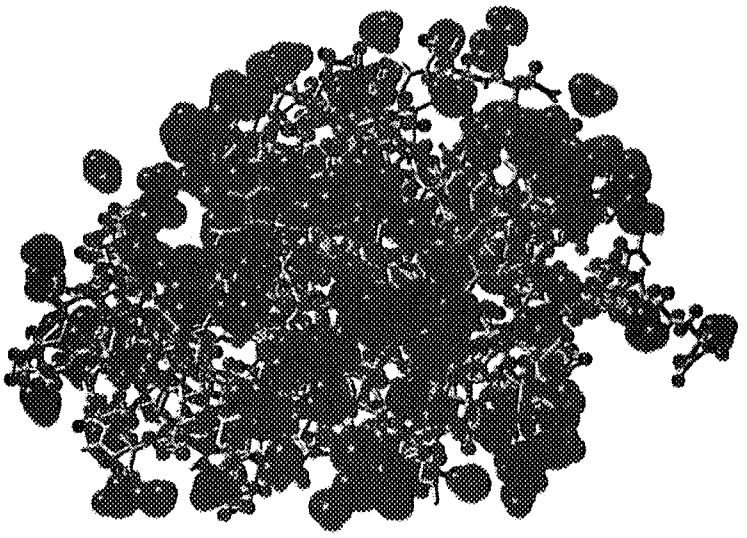
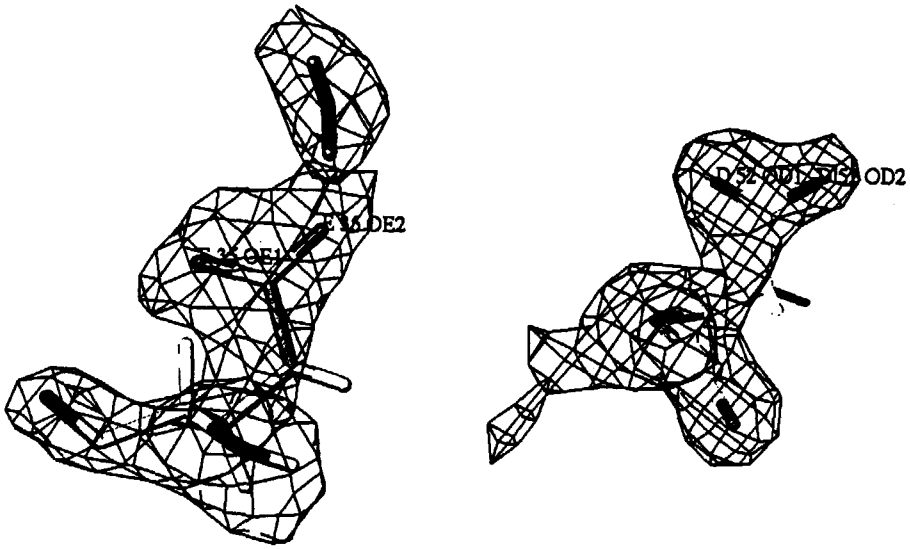


Figure 5 The three-dimensional arrangement of the lysozyme molecule with the 157 bound water molecules and 696 hydrogen and 264 deuterium atoms determined in this study.



(a) (b)

Figures 6 The $2|F_o| - |F_c|$ map around the carboxylate groups of (a) Glu35 and (b) Asp52, respectively.

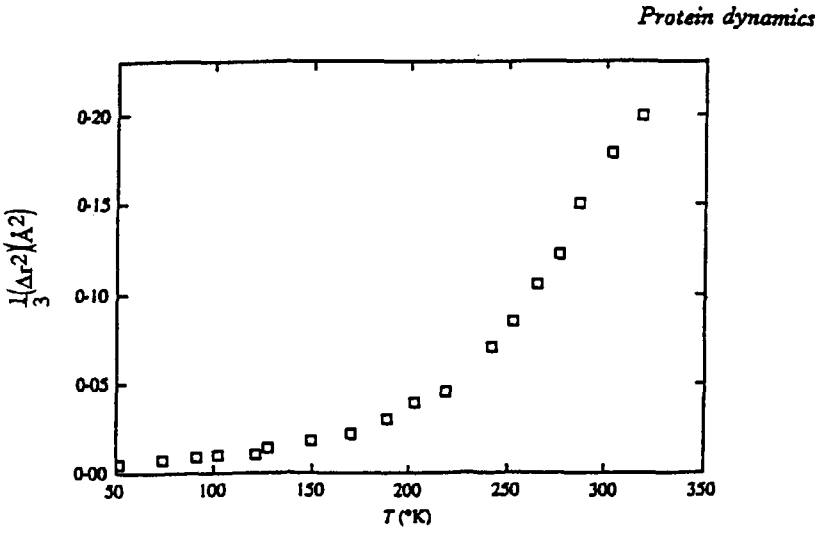
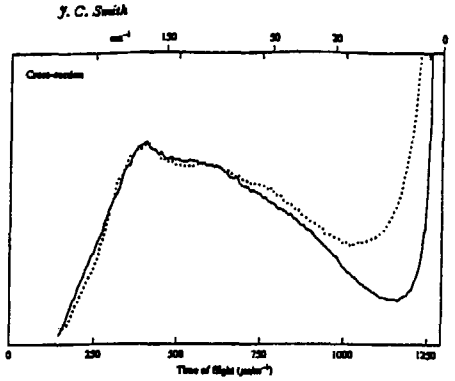


Figure 7 Temperature dependence of the isotropically averaged mean-square displacements of myoglobin from the neutron experiments.

(a)



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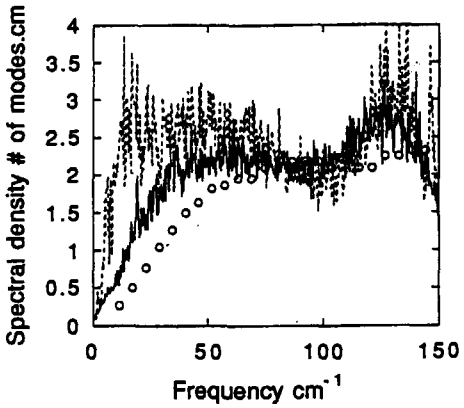

(c)

Figure 8 (a) Displacements of atoms in a low-frequency vibration according to a normal mode analysis of BPTI (bovine pancreatic trypsin inhibitor). Root-mean-square displacements of atoms, calculated at room temperature, are shown by vectors magnified 20 times. (b) Time-of-flight spectra from BPTI in powder (solid line) and in solution (dotted line). (c)The neutron-derived frequency distribution from the BPTI powder experiment (circles), and the vibrational frequency distribution from the molecular dynamics of BPTI in solution (solid line) and in powder (dotted line).

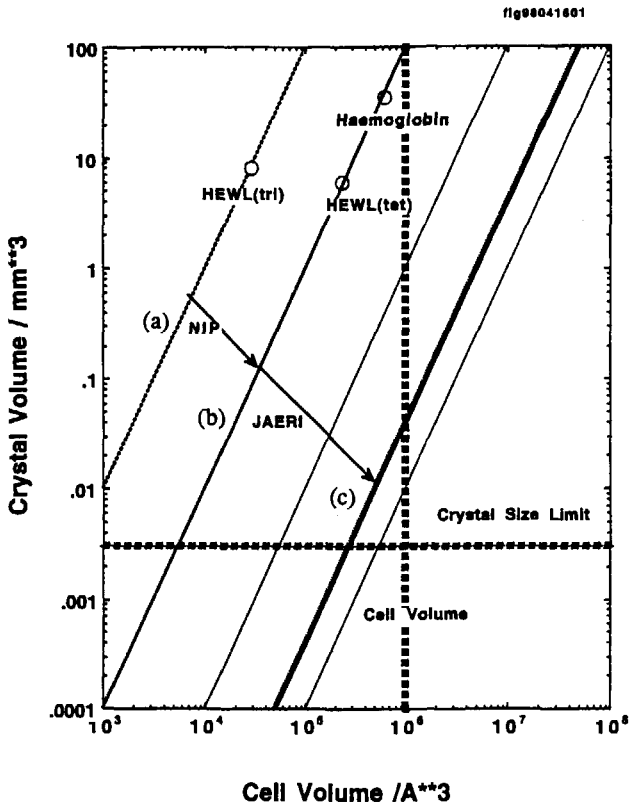

Figure 9 What kind of protein can be measured in a neutron single crystal diffractometry in accordance with increments of neutron incident flux.