生体分子構造解析学特論

シンクロトロン光研究センター
渡邉 信久

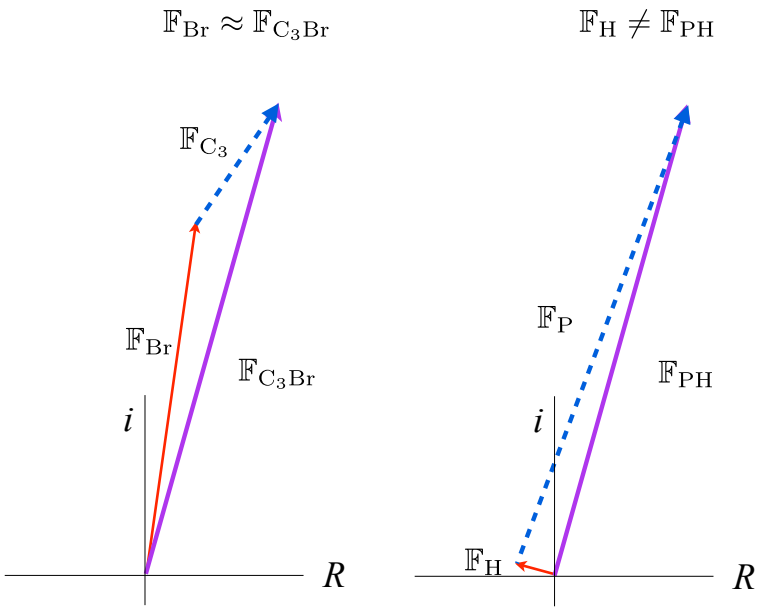
第5回の2

講義スケジュール

- 1：混沌の時代から繊維写真の時代
- 2：サイクロール説
- 3：二次構造の解明
- 4：DNAの構造
- 5：結晶構造解析法の発展
- 6：高分解能構造解析の始まり

for example

myoglobin	Hg
molecular weight: 17,184	
1,200 non-hydrogen atoms	only 80 electrons
more than 9,000 electrons	



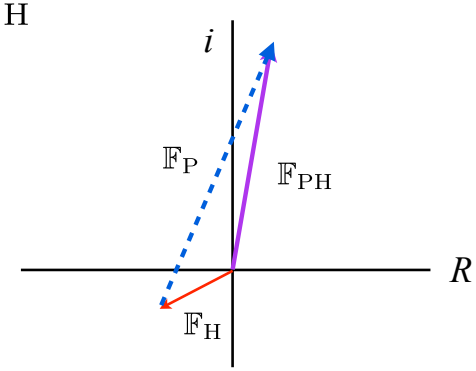
MIR method

how multiple isomorphous replacement works

Perutzがhemoglobinで先行させたが,
実際の解析はmyoglobinが先に…

relationship of the 3 vectors

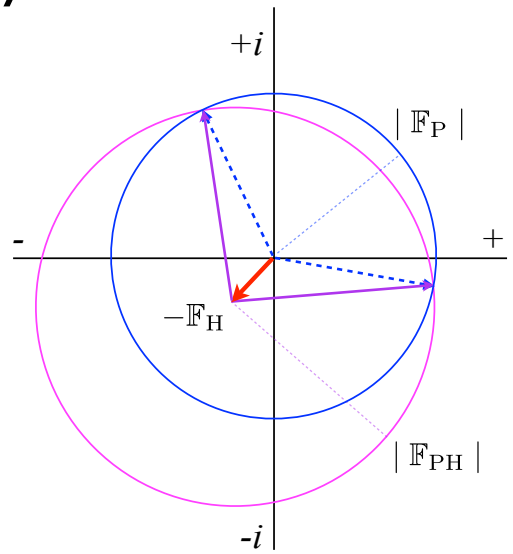
$$\mathbb{F}_{PH} = \mathbb{F}_P + \mathbb{F}_H$$



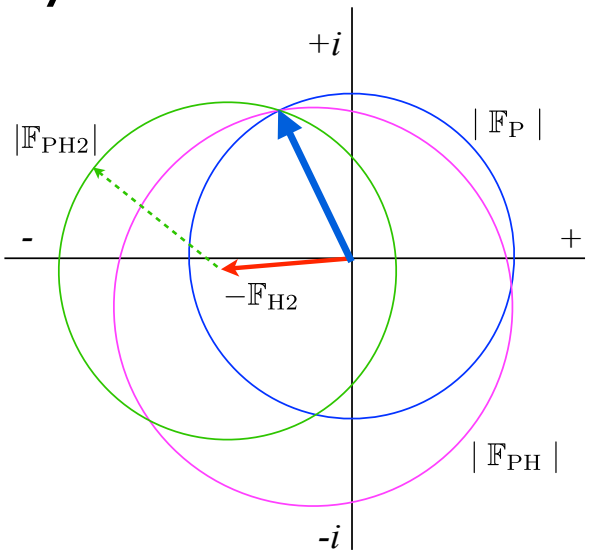
single heavy atom derivative

$$\mathbb{F}_{PH} = \mathbb{F}_P + \mathbb{F}_H$$

$$-\mathbb{F}_H + \mathbb{F}_{PH} = \mathbb{F}_P$$



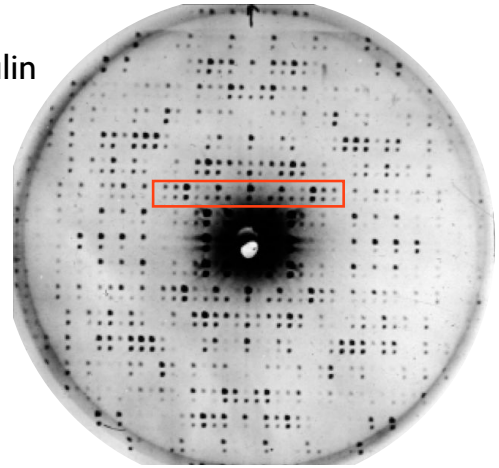
two heavy atom derivatives



$|\mathbb{F}_P|$ and $|\mathbb{F}_{PH}|$

example:
bovine β-lactoglobulin

native
and
Hg derivative



Taylor, GL., *Acta Cryst.* D66, 325-338 (2010)

$|\mathbb{F}_P|$ and $|\mathbb{F}_{PH}|$

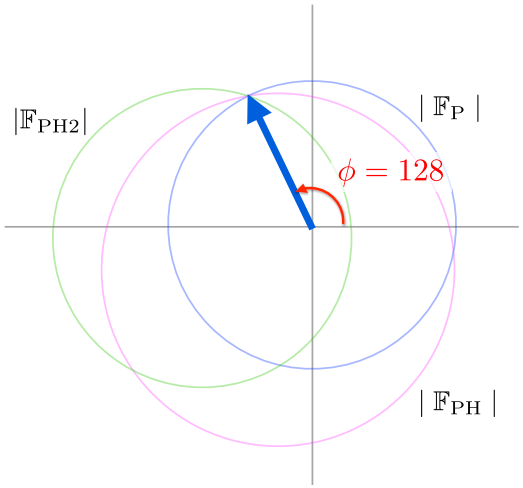
example:
bovine β-lactoglobulin

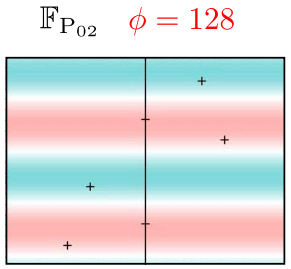
native
and
Hg derivative

$$\mathbb{F}_H = \mathbb{F}_{PH} - \mathbb{F}_P$$

Taylor, GL., *Acta Cryst.* D66, 325-338 (2010)

位相が決まるということ…





the Hemoglobin Saga

key paper

Proc. Roy. Soc. London (1947) A191, 83-132

An X-ray study of horse methaemoglobin. I

By Joy Boyes-Watson, Edna Davidson and M. F. Perutz

Cavendish Laboratory and Molteno Institute, University of Cambridge

(*Communicated by Sir Lawrence Bragg, F.R.S.—Received 3 February* 1947)

II: 1948
III, IV, V, VI: 1954
VII: 1958
VIII: 1961
IX: 1962

p.83

Horse methaemoglobin crystallizes in the monoclinic space group $C2$ with two molecules of weight 66,700 per unit cell. In addition, the wet crystals contain liquid of crystallization which fills 52·4% of the unit cell volume. Deliberate variations in the amount and composition of the liquid of crystallization, and the study of the effects of such variations on the X-ray diffraction pattern, form the basis of the entire analysis.

The composition of the liquid of crystallization can be varied by allowing heavy ions to diffuse into the crystals. This increases the scattering contribution of the liquid relative to that of the protein molecules and renders it possible to distinguish the one from the other. The method is analogous to that of isomorphous replacement commonly used in X-ray analysis. It yielded valuable information on the shape and character of the haemoglobin molecules and also led to the determination of the phase angles of certain reflexions.

hemoglobin crystal
contains 52% liquid

p.90

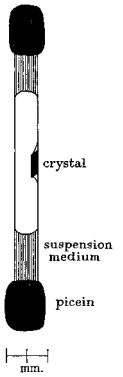
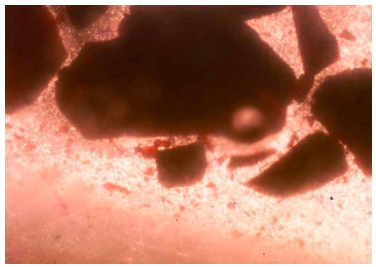
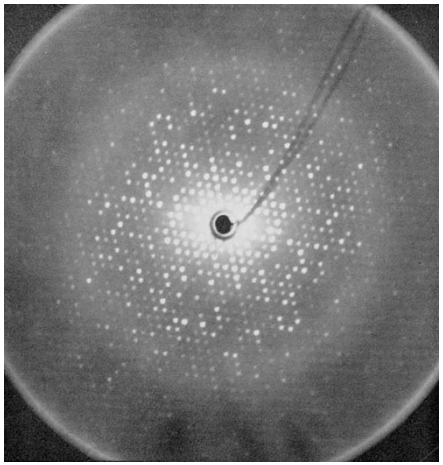


Figure 1. Wet protein crystal mounted for X-ray diffraction work.

Haemoglobin crystals and diffraction photo





(@1960's)

http://www.wellcomecollection.org/

p.123

crystal packing of hemoglobin

hemoglobin crystal
contains 52% liquid

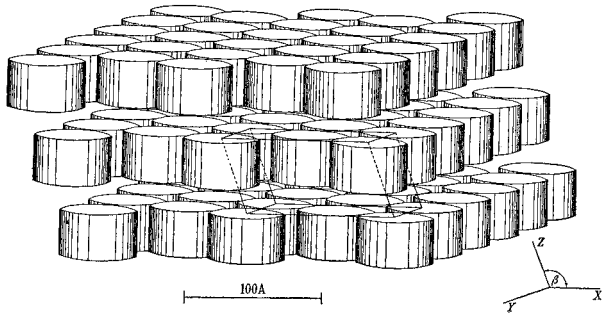


Figure 13. Packing of haemoglobin molecules in the crystal structure, showing layers of close-packed molecules separated by liquid. One unit cell is shown in the foreground on the right.

key paper にしなかったが…

Proc. Roy. Soc. London (1949) A195, 474-499

An X-ray study of horse methaemoglobin. II

By M. F. Perutz

Cavendish Laboratory and Molteno Institute, University of Cambridge

(*Communicated by Sir Lawrence Bragg, F.R.S.—Received* 8 *June* 1948—*Read* 16 *December* 1948)

p.475

2. Experimental

(*a*) *Preparation of relative intensity data*

The diffraction pattern of wet haemoglobin crystals extends to a spacing of 2·5 Å, but indexing was not carried beyond 2·8 Å spacing, since only isolated weak reflexions occurred beyond this range. Even so the limiting sphere contained 62,700 reciprocal lattice points which symmetry reduces to 7840 reflexions relevant for analysis. The photographing, indexing, measuring, correcting and correlating of some 7000 reflexions was a task whose length and tediousness it will be better not to describe. In the indexing and measuring the writer was helped by Miss Joy Boyes-Watson and Dr Edna Davidson, who each did one-third of the work.

p.489

Arguing purely from considerations of packing there should be twenty such chains in the haemoglobin molecule. Porter & Sanger (1948), on the other hand, have shown the horse haemoglobin molecule to contain only six terminal α-amino groups. Hence the twenty chains cannot be independent, but must be combined into six bigger chains folded backwards and forwards through the molecule in long zigzags. Alternatively, there might be six open chains together with a number of closed rings.

p.490

"hat box" model...

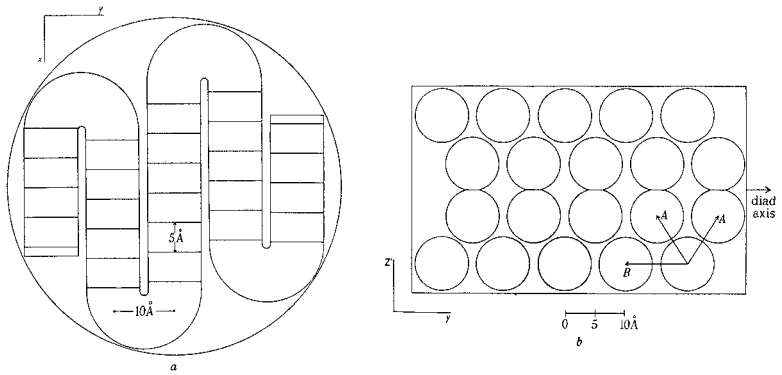


Figure 23. Idealized picture of type of haemoglobin structure compatible with the Patterson synthesis. (*a*) shows a basal section through the cylindrical molecule with one polypeptide chain folded in a plane and a pattern repeating at intervals of 5 Å along the chain direction. As nothing is known about the geometry of this pattern its existence is indicated merely by a series of lines normal to the chain length. (*b*) represents a vertical section through the cylinder normal to *X* with the chains seen end-on.

Dr. Frankendrew's Monster

Myoglobin structure solved by the MIR method using Ag, Hg & Au derivatives

key paper

Nature (1958) 181, 662-666

662 NATURE March 8, 1958 Vol. 181

A THREE-DIMENSIONAL MODEL OF THE MYOGLOBIN MOLECULE OBTAINED BY X-RAY ANALYSIS

By Drs. J. C. KENDREW, G. BODO, H. M. DINTZIS, R. G. PARRISH and H. WYCKOFF
Medical Research Council Unit for Molecular Biology, Cavendish Laboratory, Cambridge
AND
D. C. PHILLIPS
Davy Faraday Laboratory, The Royal Institution, London

21

p.662

examples. Secondary structure has been assigned in broad outline to a number of fibrous proteins such as silk, keratin and collagen; but we are ignorant of the nature of the secondary structure of any globular protein. True, there is suggestive evidence, though as yet no proof, that α-helices occur in globular proteins, to an extent which is difficult to gauge quantitatively in any particular case. The *tertiary*

The present article describes the application, at low resolution, of the isomorphous replacement method in three dimensions to type *A* crystals of sperm whale myoglobin[5]. The result is a three-dimensional Fourier, or electron-density, map of the unit cell, which for the first time reveals the general nature of the tertiary structure of a protein molecule.

22

p.662-663

Isomorphous Replacement in Myoglobin

globin could not be employed. Eventually, we were able to attach several heavy atoms to the myoglobin molecule at different specific sites by crystallizing it with a variety of heavy ions chosen because they might be expected, on general chemical grounds, to possess affinity for protein side-chains. X-ray, rather than chemical, methods were used to determine whether combination had taken place, and, if so, whether the ligand was situated predominantly at a single site on the surface of the molecule. Among others, the following ligands were found to combine in a way suitable for the present purpose: (i) potassium mercuri-iodide and auri-iodide; (ii) silver nitrate, potassium auri-chloride; (iii) *p*-chloromercuri-benzene sulphonate; (iv) mercury diammine ($Hg(NH_3)^{2+}$, prepared by dissolving mercuric oxide in hot strong ammonium sulphate), *p*-chloro-aniline; (v) *p*-iodo-phenylhydroxylamine. Each group of ligands combined specifically at a particular site, five distinct sites being found in all. The substituted

Hg Au Ag

23

p.663

Methods of X-ray Analysis

the accuracy of phase determination. In the present stage of the analysis the most urgent objective was an electron-density map detailed enough to show the general layout of the molecule—in other words, its tertiary structure. If the α-helix, or something like it, forms the basis of the structure, we need only work to a resolution sufficient to show up a helical chain as a rod of high electron density. For this purpose we require only reflexions with spacings greater than about 6 A.; in all there are some 400 of these, of which about 100 are *h*0*l*'s already investigated in the two-dimensional study. The Fourier synthesis described here is computed from these 400 reflexions only, and is in consequence blurred; besides this, it is distorted by an unknown amount of experimental error, believed to be small but at the moment difficult to estimate. Thus while the general features of the synthesis are undoubtedly correct, there may be some spurious detail which will require correction at a later stage.

24

p.662

The Three-dimensional Fourier Synthesis

The synthesis was computed in 70 min. on the EDSAC Mark I electronic computer at Cambridge (as a check, parts of the computation were repeated on DEUCE at the National Physical Laboratory). It is in the form of sixteen sections perpendicular to y and spaced nearly 2 A. apart; these must be piled on top of one another to represent the electron density throughout the cell, containing two myoglobin molecules together with associated mother liquor (which amounts to nearly half the whole). Unfortunately, the synthesis cannot be so represented within the two-dimensional pages of a journal; furthermore, if the sections are displayed side by side, they give no useful idea of the structure they represent. The examples reproduced in Fig. 1 illustrate some of the more striking features.

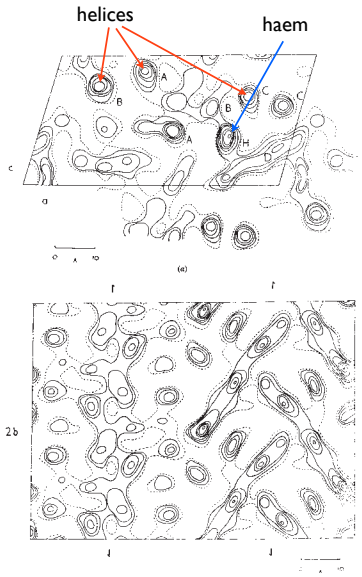
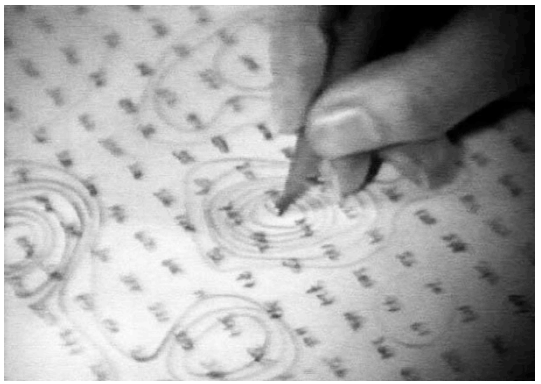


Fig. 1. (*a*) Section of three-dimensional Fourier synthesis of type *A* myoglobin at $y = -1/8$ *b*. *A*–*D*, polypeptide chains; *H*, hæm group. (*b*) Section parallel to $[20\bar{1}]$ at $x = 0$, showing polypeptide chain *A* (on the right)

25

Fourier drawing, @1957



http://www.wellcomecollection.org/

26

plate 8

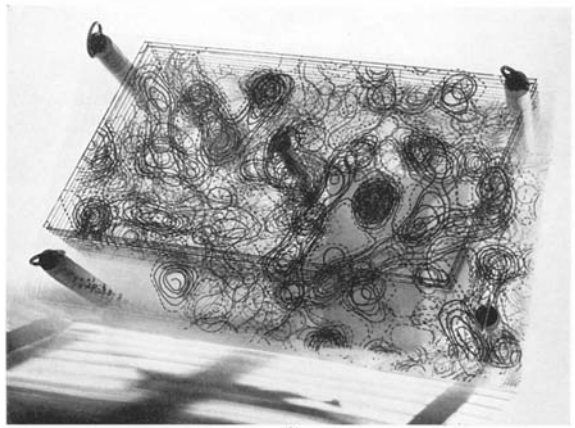


FIGURE 19. Photographs of the three-dimensional Fourier synthesis of myoglobin, constructed from the sections illustrated in figure 18.

(*Facing p.* 93)

27

p.664

The Myoglobin Molecule

We are now in a position to study the tertiary structure of a single myoglobin molecule separated from its neighbours. Fig. 2 illustrates various views of a three-dimensional model constructed to show the regions of high electron density in the isolated molecule. Several points must be noticed. First,

p.665

Perhaps the most remarkable features of the molecule are its complexity and its lack of symmetry. The arrangement seems to be almost totally lacking in the kind of regularities which one instinctively anticipates, and it is more complicated than has been predicated by any theory of protein structure. Though the detailed principles of construction do not yet emerge, we may hope that they will do so at a later stage of the analysis. We are at present engaged in extending the resolution to 3 A., which should show us something of the secondary structure; we

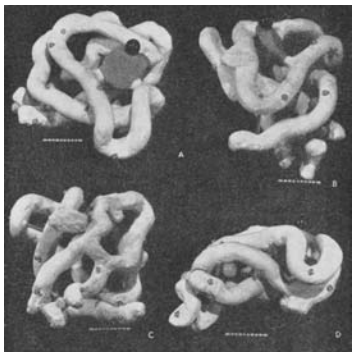
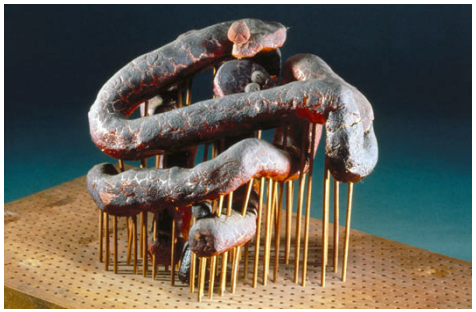


Fig. 2. Photographs of a model of the myoglobin molecule. Polypeptide chains are white; the grey disk is the hæm group. The three spheres show positions at which heavy atoms were attached to the molecule (black: Hg of *p*-chloro-mercuri-benzene-sulphonate; dark grey: Hg of mercury diammine; light grey: Au of auri-chloride). The marks on the scale are 1 A. apart

28

ミオグロビンの「ソーセージモデル」



http://www.sciencemuseum.org.uk/

フランケンドリュー・モンスター

key paper

Proc. Roy. Soc. London (1959) A253, 70-102

The crystal structure of myoglobin

V. A low-resolution three-dimensional Fourier synthesis of sperm-whale myoglobin crystals

By G. Bodo, H. M. Dintzis, J. C. Kendrew and H. W. Wyckoff

Medical Research Council Unit for Molecular Biology, Cavendish Laboratory, University of Cambridge

(*Communicated by Sir Lawrence Bragg, F.R.S.—Received 22 April* 1959)

p.83

We adopted the working hypothesis that a considerable proportion of the polypeptide chain has a configuration similar to the α-helix of Pauling, Corey & Branson (1951). There is no proof that this is the case, but there is some direct evidence of it in haemoglobin (Perutz 1951), as well as indirect evidence in myoglobin from studies of optical rotation (P. M. Doty, unpublished) and of rates of deuterium exchange (E. E. Benson & K. Linderstrøm-Lang, unpublished). At low resolution the α-helix would appear as a solid rod with axial electron density about 1·0 electrons/Å^3, embedded in a matrix of side chains of mean electron density about 0·3 electron/Å^3 (the mean overall electron density of the myoglobin molecule is about 0·4 electron/Å^3, and in type *A* crystals the electron density of the liquid regions has about the same value). Neighbouring α-helices would pack together with axial separations of 9 to 10 Å. We reached the conclusion that helices, if indeed they exist in myoglobin, would be clearly resolved if the Fourier synthesis included all terms having $d > 6$ Å; they should appear in such a synthesis as solid rods, since the region of reciprocal space being scanned includes only the first maximum of the Fourier transform of an α-helix. In fact there are about 400 reflexions having $d > 6$ Å, of which about 100 are *h*0*l* reflexions with real phases which had already been determined in the two-dimensional work.

p.91

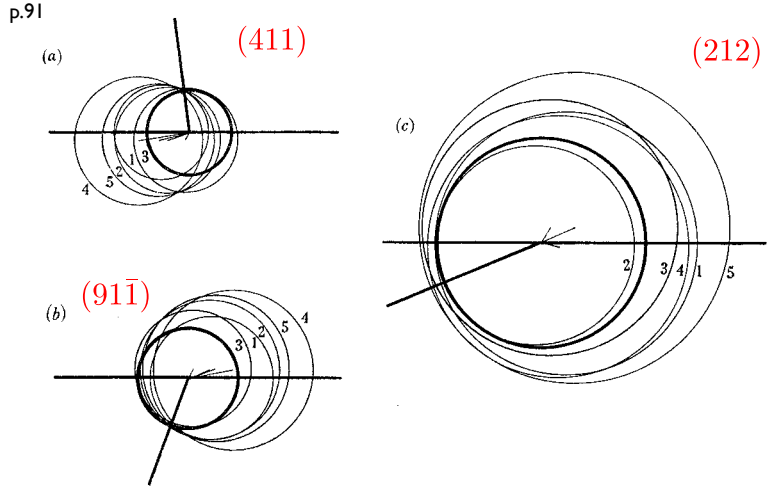


FIGURE 17. Examples of phase determination. The heavy circle represents the amplitude of the reflexion from unsubstituted protein, and the light circles those from the derivatives. 1, PCMBS; 2, $HgAm_2$; 3, Au; 4, PCMBS/$HgAm_2$; 5, PCMBS/Au. The short lines from the centres are the heavy-atom vectors; the heavy line indicates the phase angle eventually selected. (*a*) (411), (*b*) (91$\bar{1}$), (*c*) (212).

key paper にしなかったが…

Proc. Roy. Soc. London (1962) A265, 161-187

The structure of haemoglobin

IX. A three-dimensional Fourier synthesis at 5·5 Å resolution: description of the structure

BY ANN F. CULLIS, HILARY MUIRHEAD, M. F. PERUTZ, F.R.S. AND M. G. ROSSMANN

Medical Research Council Unit for Molecular Biology, Cavendish Laboratory, University of Cambridge

AND A. C. T. NORTH

Medical Research Council External Staff, Davy Faraday Laboratory, Royal Institution, London W. 1

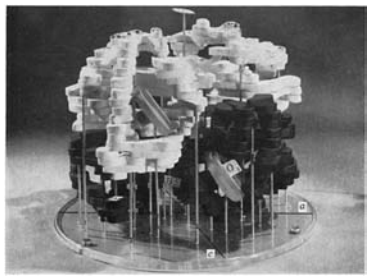


FIGURE 10. Complete haemoglobin model viewed normal to *a*. The haem groups are indicated by grey disks.

FIGURE 13. A view down the *b* axis. Note the proximity of the N- and C-terminal ends which could serve to form links between the two white chains.

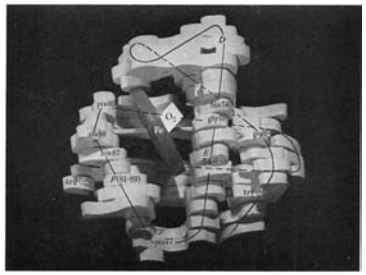
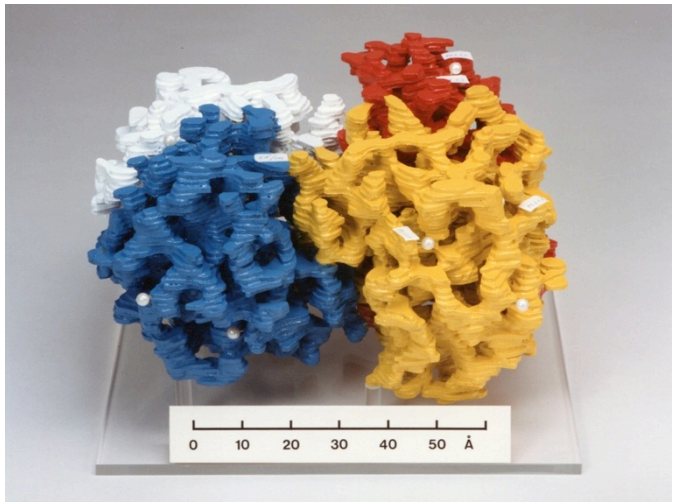


FIGURE 19

FIGURES 17 to 19. Three views of the white chain showing the probable positions of various residues in human haemoglobin.

1988年…



ω-amino acid : pyruvate aminotransferase