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4回

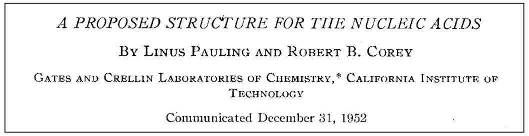
講義スケジュール

- 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

---

key paper

triple helix


A PROPOSED STRUCTURE FOR THE NUCLEIC ACIDS

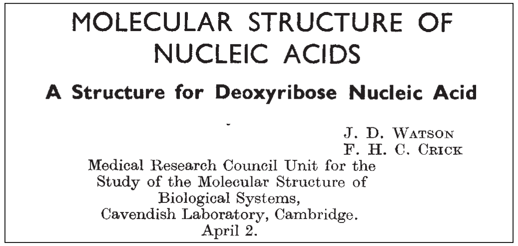
By Linus Pauling and Robert B. Corey

Gates and Crellin Laboratories of Chemistry,* California Institute of Technology

Communicated December 31, 1952

PNAS (1953) 39, 84-97

double helix


MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

J. D. Watson
F. H. C. Crick
Medical Research Council Unit for the
Study of the Molecular Structure of
Biological Systems,
Cavendish Laboratory, Cambridge.
April 2.

Nature (1953) 171, 737-738

(*Nature* (1953) 171, 737-741)

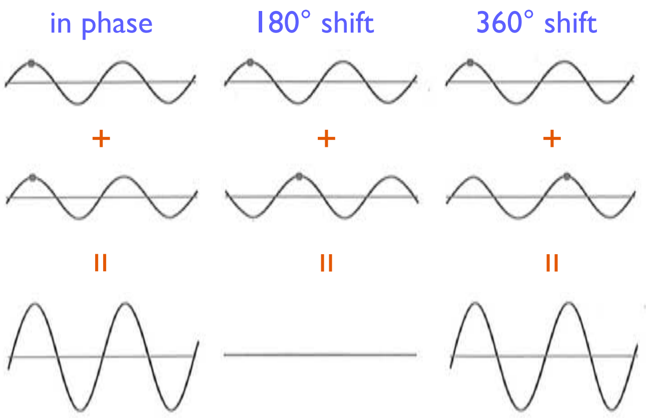
Crystallography without Mathematics

- 逆空間
- Fourier 変換
- Convolution

Crystallography without Mathematics

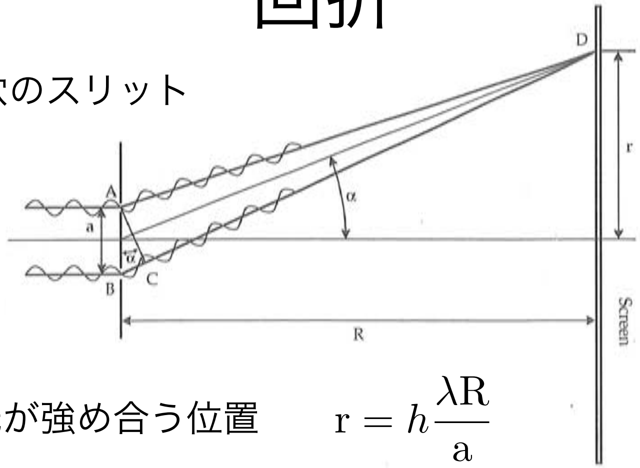
- 逆空間
- Fourier 変換
- Convolution

光(波)の重ね合わせと位相の関係



回折

2つ穴のスリット

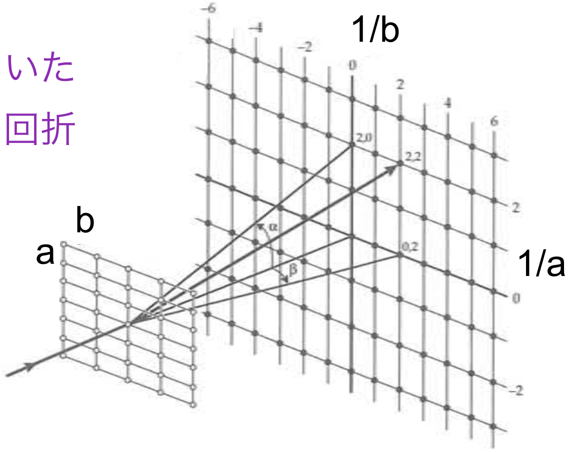


光が強め合う位置 $r = h\dfrac{\lambda R}{a}$

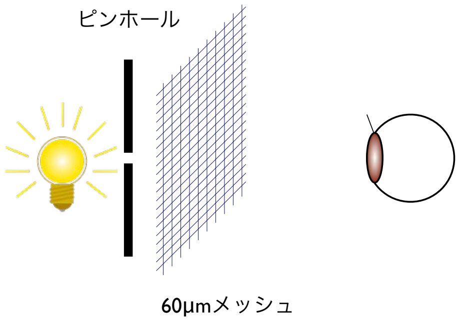
a と r は反比例

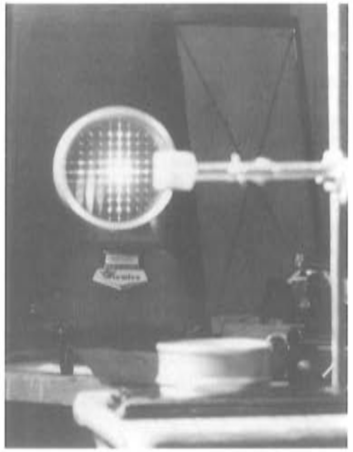
2次元の場合

穴の開いた
格子の回折



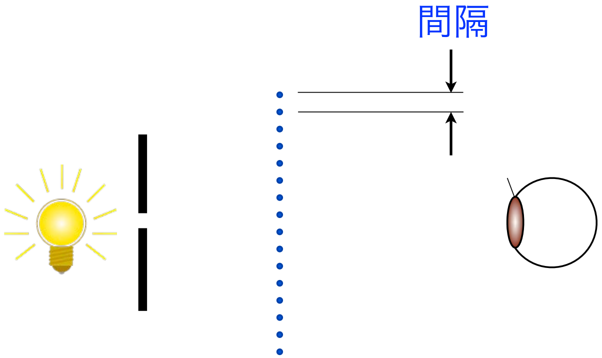
2次元の場合の「例」





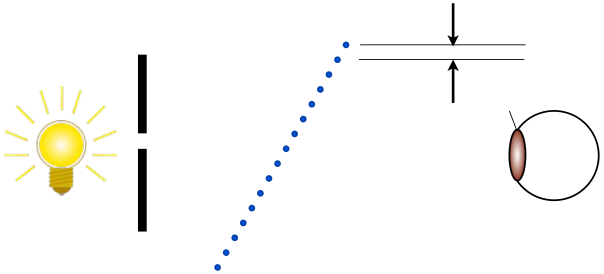
“reciprocal space”

逆空間の概念



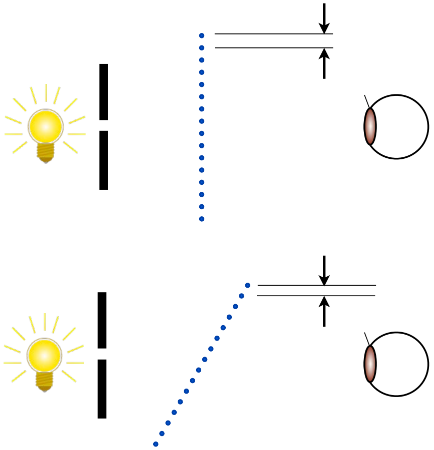
“reciprocal space”

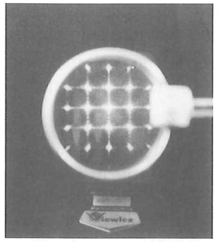
逆空間の概念



“reciprocal space”

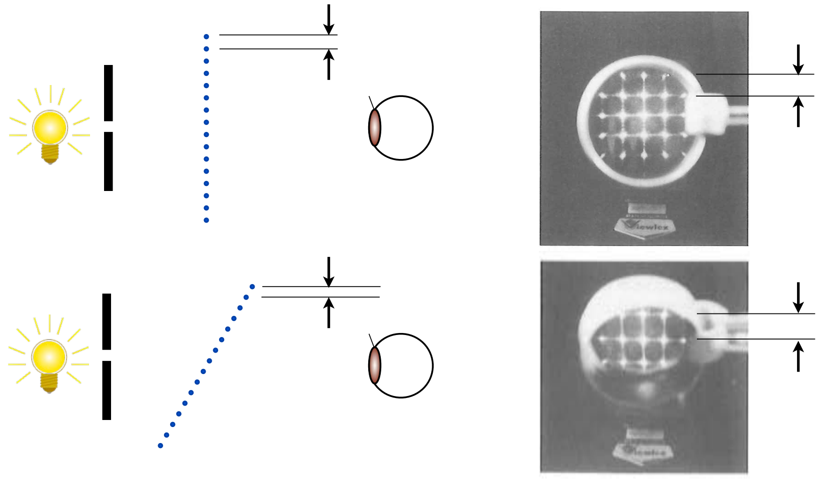
逆空間の概念



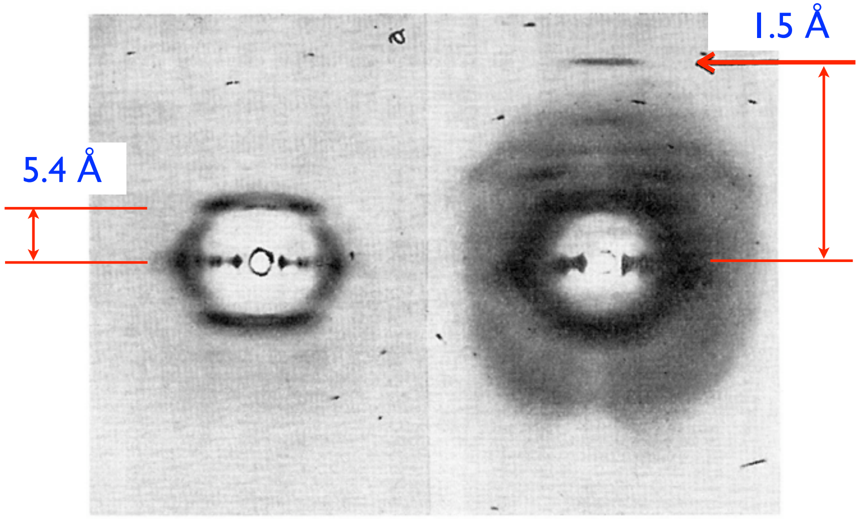


“reciprocal space”

逆空間の概念



Perutzの検証実験



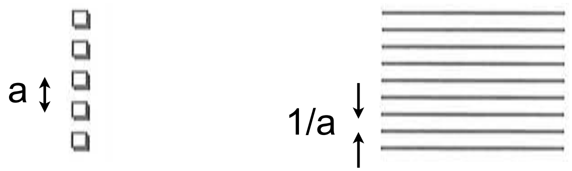
Crystallography without Mathematics

- 逆空間
- Fourier 変換
- Convolution

回折パターン(Fourier変換)

Scattering Object
A

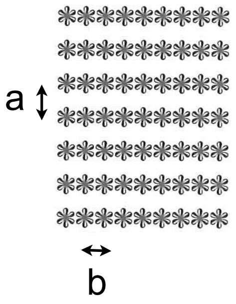
Diffraction Pattern
T(A)



回折パターン(Fourier変換)

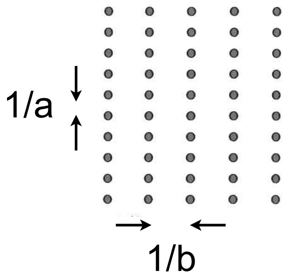
Scattering Object

A



Diffraction Pattern

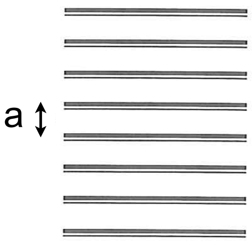
T(A)



回折パターン(Fourier変換)

Scattering Object

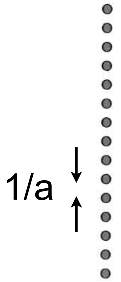
A



b = 0

Diffraction Pattern

T(A)

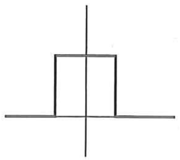


1/b = ∞

回折パターン(Fourier変換)

Scattering Object

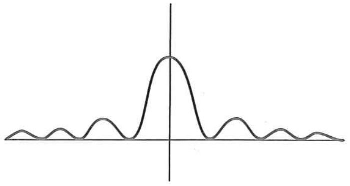
A



step function

Diffraction Pattern

T(A)

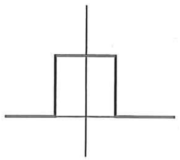


central peak flanked by dying ripples

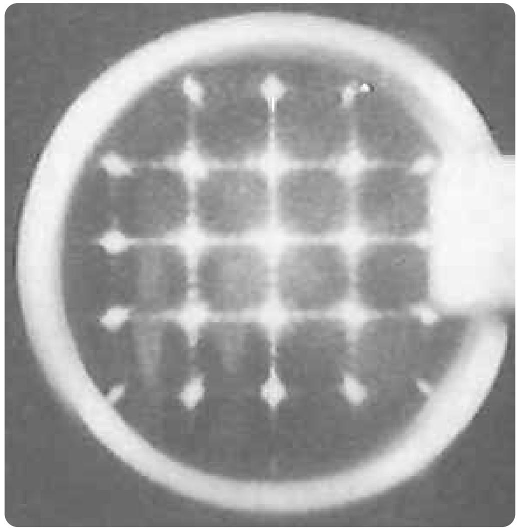
回折パターン(Fourier変換)

Scattering Object

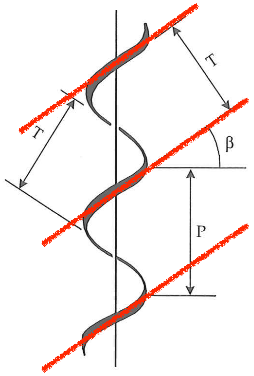
A

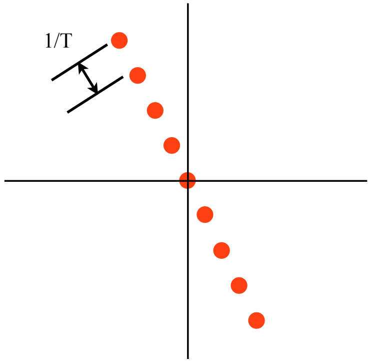


step function

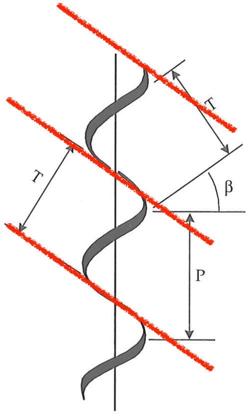


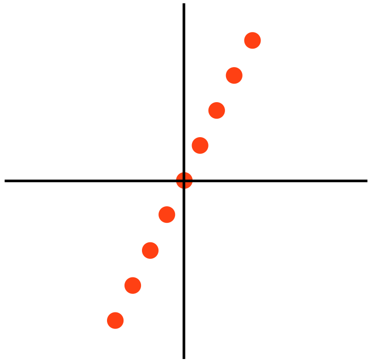
ら旋の回折パターン(Fourier変換)



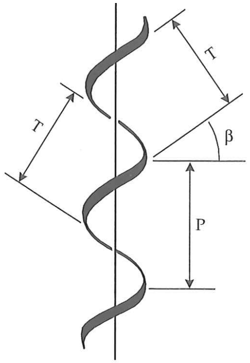


ら旋の回折パターン(Fourier変換)

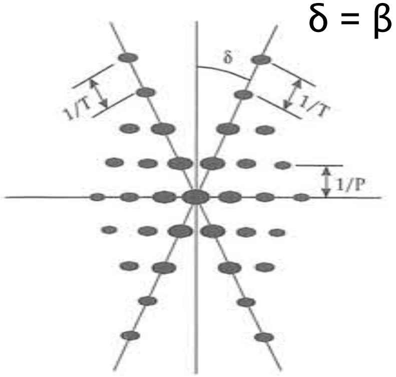




ら旋の回折パターン(Fourier変換)



δ = β



Crystallography without Mathematics

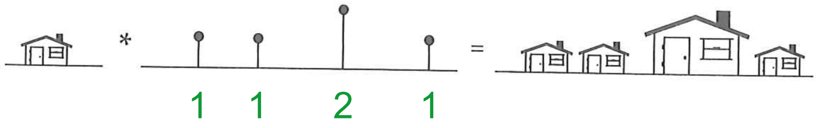
- 逆空間
- Fourier 変換
- Convolution

畳み込み積 A ＊ B

Convolution Product

一次元

A ＊ B = A ＊ B

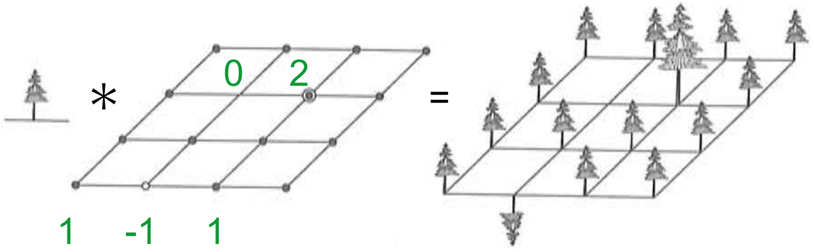


畳み込み積 A ＊ B

Convolution Product

二次元

A ＊ B = A ＊ B

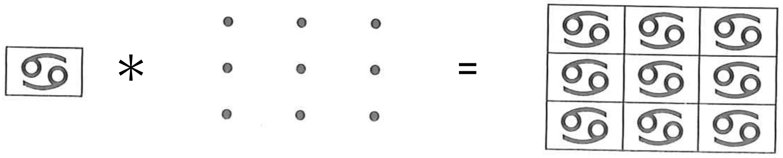


畳み込み積 A ＊ B

Convolution Product

「結晶」

A ＊ B = A ＊ B



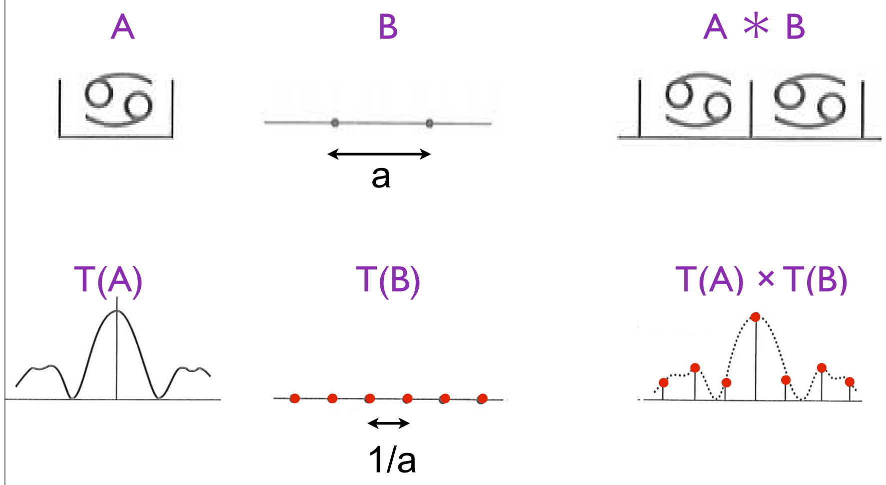
分子 格子 結晶

ConvolutionのFourier変換

$$T(A \ast B) = T(A) \times T(B)$$

$$T(A \times B) = T(A) \ast T(B)$$

T(A ∗ B) = T(A) × T(B)



A

B

A ∗ B

a

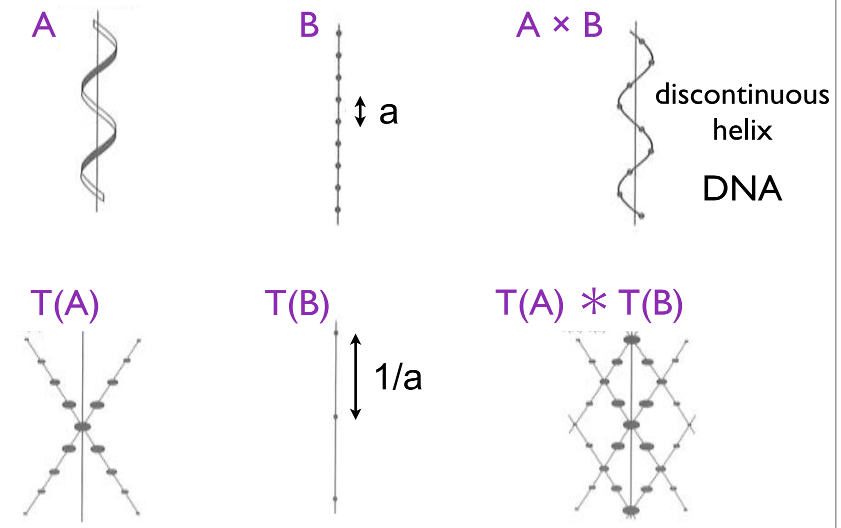
T(A)

T(B)

T(A) × T(B)

1/a

T(A × B) = T(A) ∗ T(B)



A

B

A × B

a

discontinuous helix

DNA

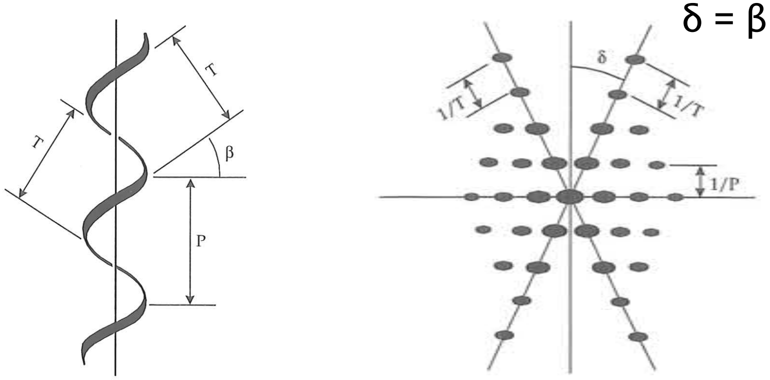
T(A)

T(B)

T(A) ∗ T(B)

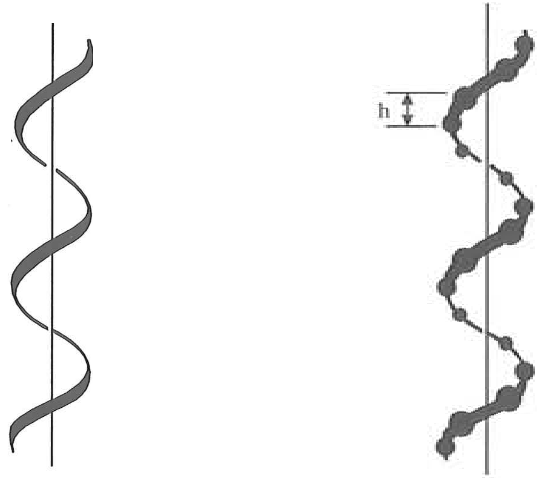
1/a

ら旋の回折パターン(Fourier変換)

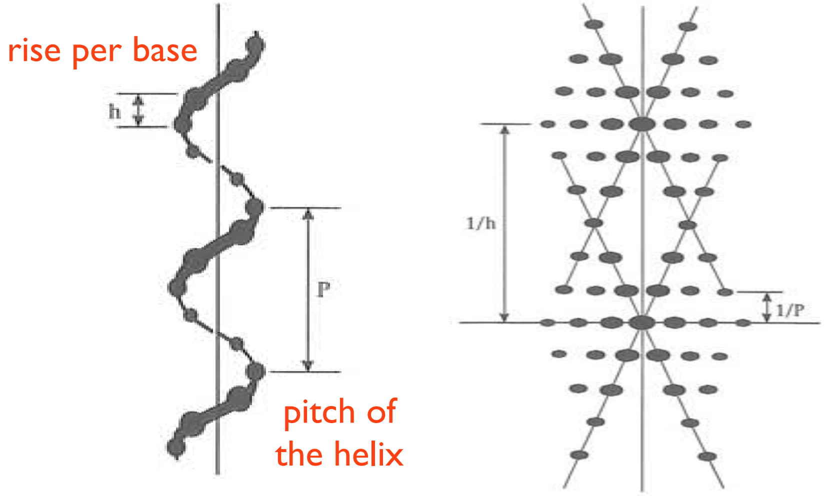


δ = β

DNAら旋



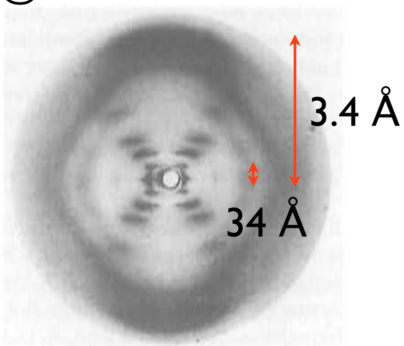
helixの回折パターン(Fourier変換)



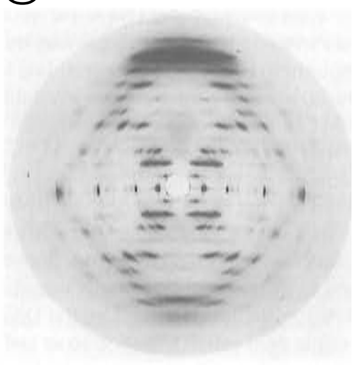
---

helixの回折パターン

@1953



@1981



P = 34 Å, h = 3.4 Å
⇒ 10 base pairs / turn

The Long Road

---

pre-histroy

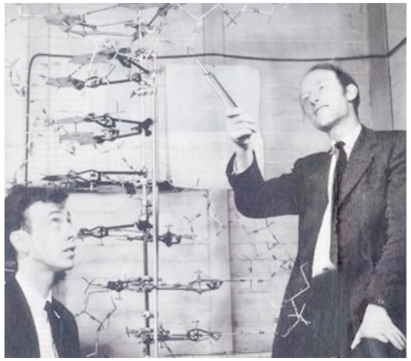
1860's	F Miesher	膿(うみ)とサケ精子から核酸を単離 リンを含むことを発見(蛋白質の一種と考えていた…)
1900's	A Kossel	リン酸，糖，塩基から成ることを決定
1920's	 tetranucleotide仮説	DNAとRNAの2種類ある DNA(動物)，RNA(植物)との説 -(ATCG)-(ATCG)-(ATCG)-
1944	O Avery	DNA injection (transform) 実験 DNAが遺伝情報を含む
1950	E Chargaff	A & T, G & C の「比が1」を発見 “Chargaff's ratio” “Pairing”については言及していない…(「理由」も…)
1952	Hershey & Chase	bactriophage infect E coli (only the DNA goes inside)

Triple Helix

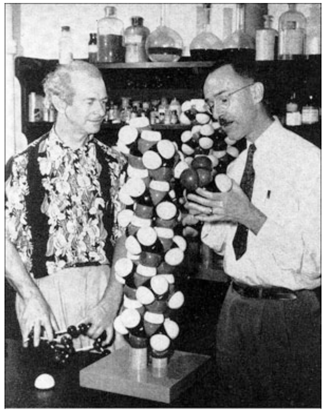
---

triple helix

- 1951: Watson & Crick
  - 外向きtriple helixのモデル
  - Wilkinsらに笑われ，Braggが怒る…
- 1951: Fraser @ Wilkins's lab
  - 内向きのtriple helixモデル (not published)
  - base間の水素結合を提唱
- 1953: Pauling & Corey



Watson & Crick

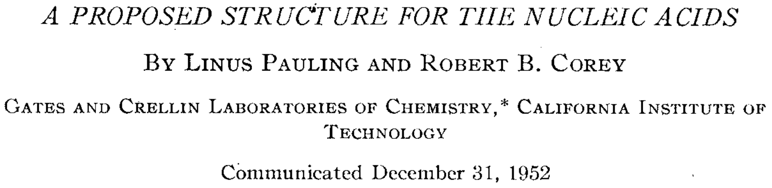


Pauling & Corey

---

key paper

PNAS (1953) 39, 84-97



A PROPOSED STRUCTURE FOR THE NUCLEIC ACIDS

By Linus Pauling and Robert B. Corey

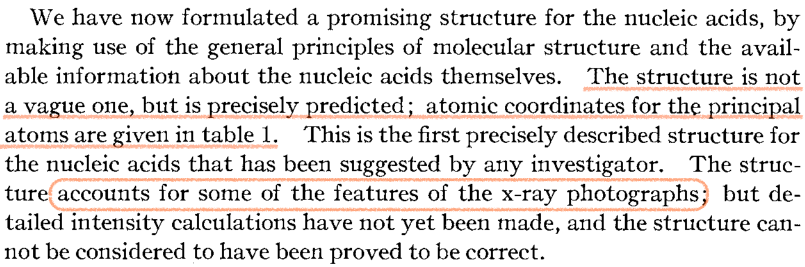
Gates and Crellin Laboratories of Chemistry,* California Institute of Technology

Communicated December 31, 1952

X線写真や実験データは無い

p.84

precisely predicetd...



We have now formulated a promising structure for the nucleic acids, by making use of the general principles of molecular structure and the available information about the nucleic acids themselves. The structure is not a vague one, but is precisely predicted; atomic coordinates for the principal atoms are given in table 1. This is the first precisely described structure for the nucleic acids that has been suggested by any investigator. The structure accounts for some of the features of the x-ray photographs; but detailed intensity calculations have not yet been made, and the structure cannot be considered to have been proved to be correct.

3つの問題点

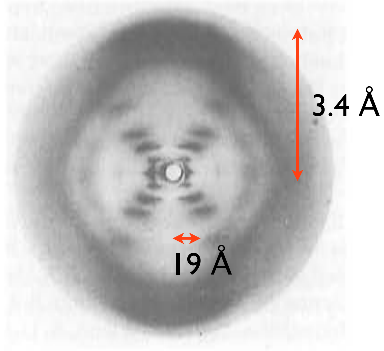
- 外向きhelix (内向き)
- triple helix (double helix)
- 8 fold (10 fold)

---

triple helix ?

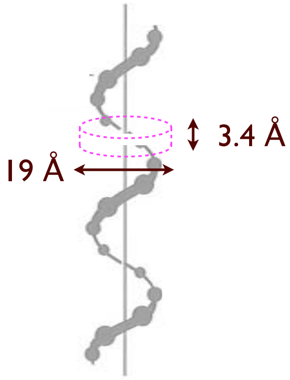
nucleotide / disk = 2.85 = 3

Franklin's photo



$\rho_{(density)}$ = 1.62 g/cm^3

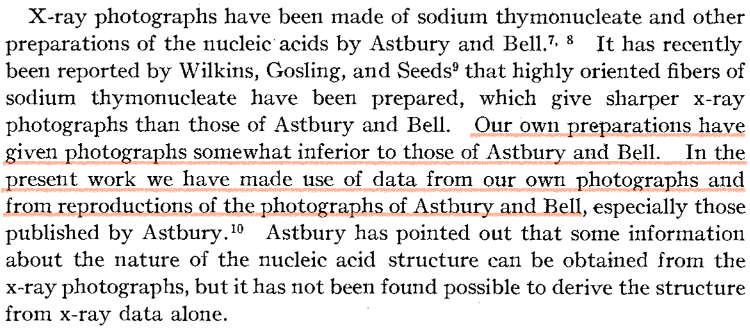
DNA helix



---

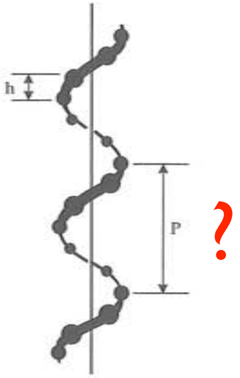
lack of access to DNA fiber diffraction pictures

p.85



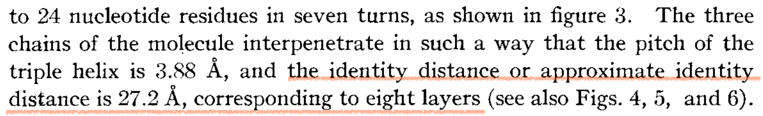
X-ray photographs have been made of sodium thymonucleate and other preparations of the nucleic acids by Astbury and Bell.[7, 8] It has recently been reported by Wilkins, Gosling, and Seeds[9] that highly oriented fibers of sodium thymonucleate have been prepared, which give sharper x-ray photographs than those of Astbury and Bell. Our own preparations have given photographs somewhat inferior to those of Astbury and Bell. In the present work we have made use of data from our own photographs and from reproductions of the photographs of Astbury and Bell, especially those published by Astbury.[10] Astbury has pointed out that some information about the nature of the nucleic acid structure can be obtained from the x-ray photographs, but it has not been found possible to derive the structure from x-ray data alone.

$n = P / h$



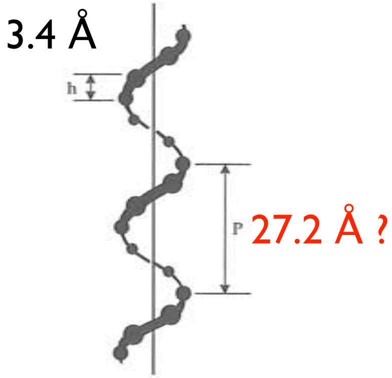
P=27.2Å ?

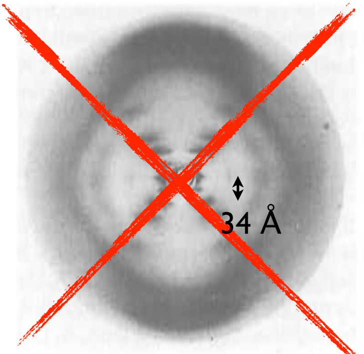
p.93



to 24 nucleotide residues in seven turns, as shown in figure 3. The three chains of the molecule interpenetrate in such a way that the pitch of the triple helix is 3.88 Å, and the identity distance or approximate identity distance is 27.2 Å, corresponding to eight layers (see also Figs. 4, 5, and 6).

$n = P / h = 8$?





Franklin's B-DNA photo

---

p.88

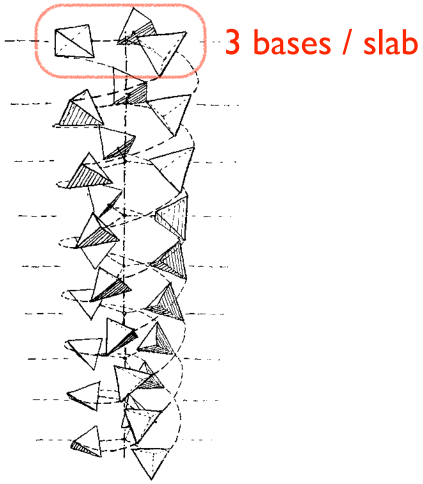


FIGURE 2

Figure 2 (*left*). A 24-residue 7-turn helix representing a single polynucleotide chain in the proposed structure for nucleic acid. The phosphate groups are represented by tetrahedra, and the ribofuranose groups by dashed arcs connecting them.

precisely predicetd...

p.91

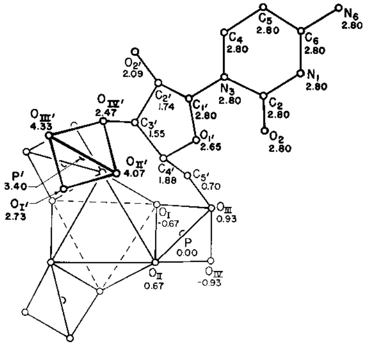


FIGURE 5

A plan of the nucleic acid structure, showing four of the phosphate groups, one ribofuranose group, and one pyrimidine group.

p.92

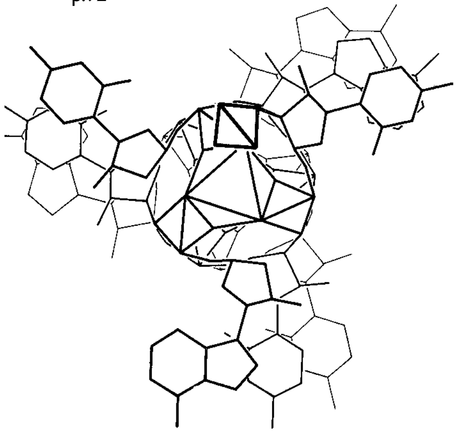
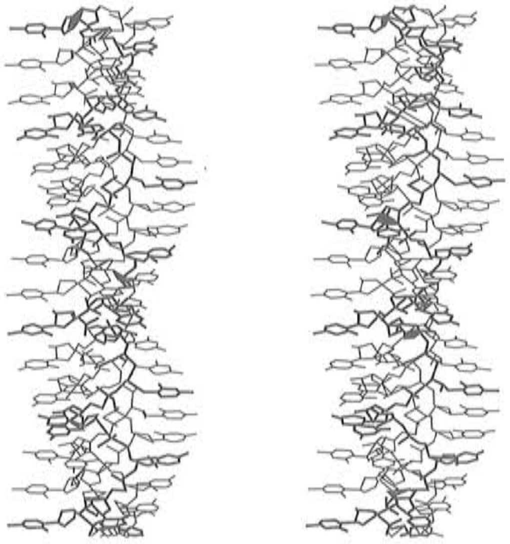


FIGURE 6

Plan of the nucleic acid structure, showing several nucleotide residues.

Pauling & Corey's triple helix



交差法ステレオ図

外向きhelixの「意味」

p.96

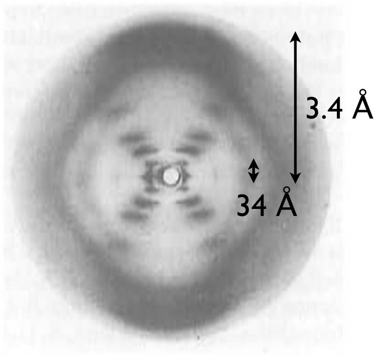
It is interesting to note that the purine and pyrimidine groups, on the periphery of the molecule, occupy positions such that their hydrogen-bond forming groups are directed radially. This would permit the nucleic acid molecule to interact vigorously with other molecules. Moreover, there is enough room in the region of each nitrogen base to permit the arbitrary choice of any one of the alternative groups; steric hindrance would not interfere with the arbitrary ordering of the residues. The proposed structure accordingly permits the maximum number of nucleic acids to be constructed, providing the possibility of high specificity. As Astbury has pointed out, the 3.4-Å x-ray reflection, indicating a similar distance along the axis of the molecule, is approximately the length per residue in a nearly extended polypeptide chain, and accordingly the nucleic acids are, with respect to this dimension, well suited to the ordering of amino-acid residues in a protein. The positions of the amino-acid residues might well be at the centers

Double Helix

わずか10週間後…

R Franklin...

“B-DNA” photo



⇒ 10 base pairs / turn

and crystallography...

DNA has *C*2 symmetry

⇓

anti-parallel

---

key paper

Nature (1953) 171, 737-738

(*Nature* (1953) 171, 737-741)

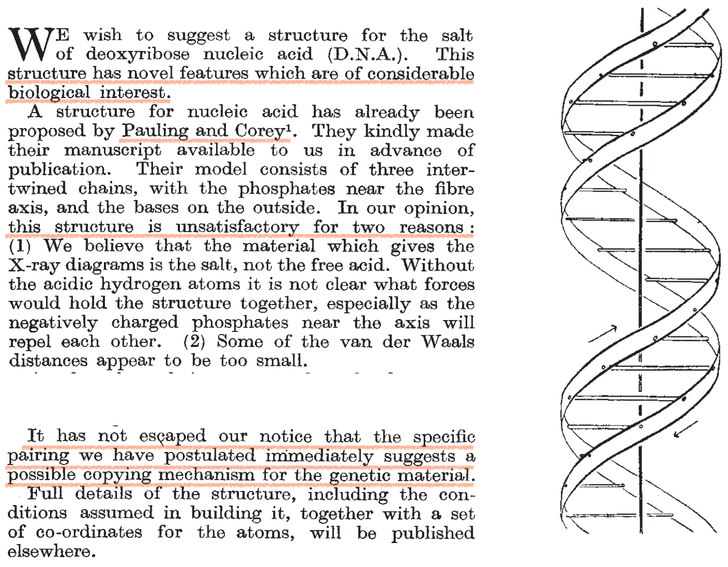
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

J. D. Watson
F. H. C. Crick
Medical Research Council Unit for the
Study of the Molecular Structure of
Biological Systems,
Cavendish Laboratory, Cambridge.
April 2.

---

p.737

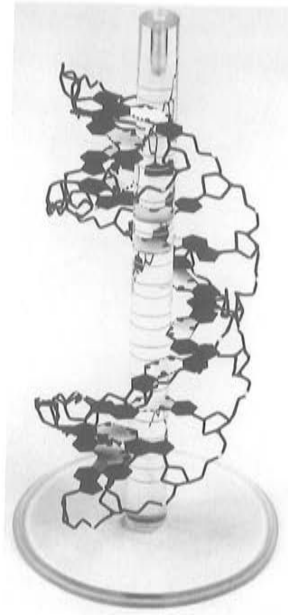


WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.



@1953
by T Broad

歴史の「もしも」

the House Un-American Activities Committee

By 1950, largely as a result of information sharing between the FBI, the Tenney Committee, the House Un-American Activities Committee (HUAC) and others, Linus Pauling's name was high on the lists of congressional investigatory bodies harboring an interest in U.S. communist subversion.

- snip -

His theories were also being challenged by several leading British scientists, forcing him to spend a great deal of his time addressing criticism. Indeed, he was so busy at the height of this work that he postponed a trip to Europe and turned down a visiting professorship at Harvard.

http://paulingblog.wordpress.com/

Denied Passport

"Linus Pauling, Crusading Scientist." 1977.
Produced for NOVA by Robert Richter/WGBH-Boston.

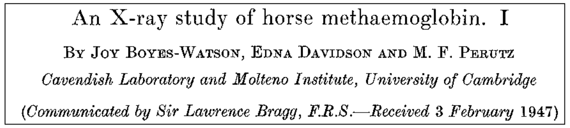
Denied Passport. (1:30)

http://osulibrary.oregonstate.edu/specialcollections/coll/pauling/dna/video/1977v.1-passport.html

---

key paper

hemoglobin sagaの
一報目



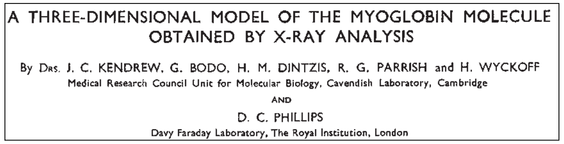
An X-ray study of horse methaemoglobin. I

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)

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

myoglobin の
3次元構造速報

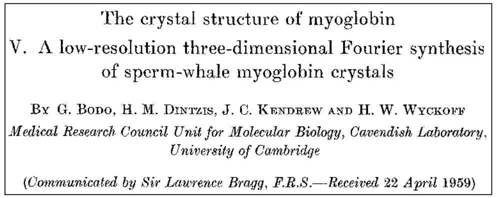


A THREE-DIMENSIONAL MODEL OF THE MYOGLOBIN MOLECULE OBTAINED BY X-RAY ANALYSIS

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

Nature (1958) 181, 662-666

myoglobin の
3次元構造解析



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)

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