

# 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  
渡邊 信久

第3回

1

## 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



Space-filling model of the alpha-helix. 1951.  
<http://osulibrary.oregonstate.edu/>

2

## 構造解析への期待

Astbury :

immense. Exact analyses of the proteins, though always laborious, need no longer be the thankless tasks they have been. Every possible reliable observation now is urgently needed and must sooner or later be fitted into the puzzle. Above all, complete analyses of single proteins are necessary. . . .

Pauling :

It has not yet been possible to make a complete determination with X-rays of the positions of the atoms in any protein crystal; and the great complexity of proteins makes it unlikely that a complete structure determination for a protein will ever be made by X-ray methods alone.<sup>7</sup> Never-

3

## Bernalの決意

J.D.Bernal がタンパク質結晶構造解析の研究グループを組織



1933年：D.C.Hodgikin

ペプシン、インシュリン



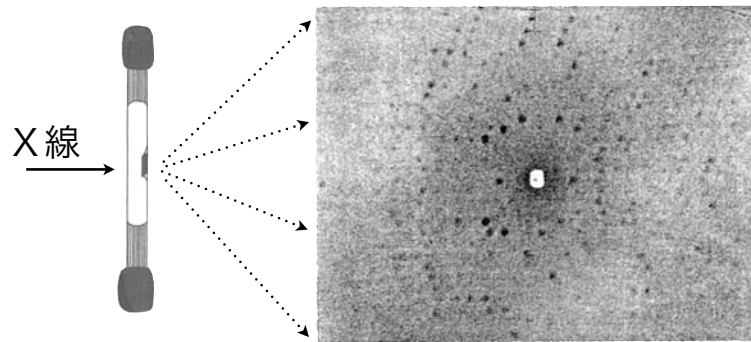
1936年：M. Perutz

ヘモグロビン



4

# タンパク質の「結晶」の登場

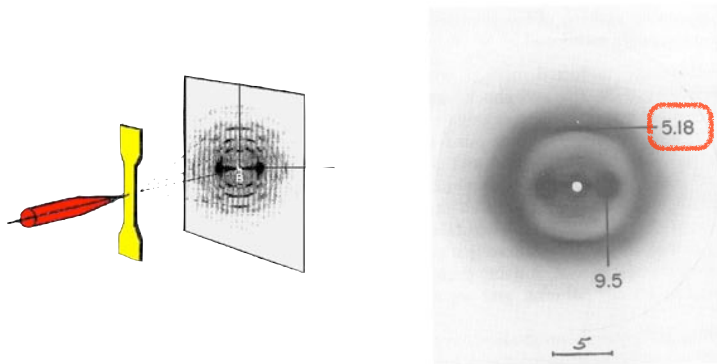


ヘモグロビンの結晶のX線回折写真  
(1937年 ペルーツらが撮影)

Astburyの繊維写真に

# 残されていた課題

# α pattern



ケラチン繊維

# key paper

ヘリカルモデルの提案

Polypeptide chain configurations in crystalline proteins  
BY SIR LAWRENCE BRAGG, F.R.S., J. C. KENDREW AND M. F. PERUTZ  
Cavendish Laboratory, University of Cambridge  
(Received 31 March 1950)

[Proc. Roy. Soc. London \(1950\) A203, 321-357](#)

α-helix の提案

THE STRUCTURE OF PROTEINS: TWO HYDROGEN-BONDED HELICAL CONFIGURATIONS OF THE POLYPEPTIDE CHAIN  
BY LINUS PAULING, ROBERT B. COREY, AND H. R. BRANSON\*  
GATES AND CRELLIN LABORATORIES OF CHEMISTRY,  
CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA†  
Communicated February 28, 1951

「紙」と手による論考

[Proc. Natl. Acad. Sci. USA \(1951\) 37, 205-211](#)

α-helix の実験的検証

No. 4261 June 30, 1951 NATURE 1053  
NEW X-RAY EVIDENCE ON THE CONFIGURATION OF POLYPEPTIDE CHAINS  
M. F. PERUTZ  
Cavendish Laboratory,  
University of Cambridge.

[Nature \(1951\) 167, 1053-1054](#)

# @ Cambridge



Braggのチーム



Perutz



Kendrew

## key paper

Bragg, Kendrew & Perutz, *Proc. Roy. Soc. London* (1950)  
A203, 321-357

Polypeptide chain configurations in crystalline proteins

BY SIR LAWRENCE BRAGG, F.R.S., J. C. KENDREW AND M. F. PERUTZ  
*Cavendish Laboratory, University of Cambridge*

(Received 31 March 1950)

結晶学者の論文...

p.322-3

## 2. PREVIOUS SPECULATIONS ABOUT THE CONFIGURATION OF THE POLYPEPTIDE CHAIN

Astbury and his co-workers in their pioneer investigations have made an exhaustive study of the fibrous proteins such as the keratin of hair and wool. Their most important result, in the present connexion, is their inference that the marked  $5.1 \text{ \AA}$  repeat along the fibre axis which is shown prominently by X-ray photographs of  $\alpha$ -keratin and its analogues corresponds to an element of folded chain containing three amino-acid residues. Briefly, Astbury (private communication, 1949) summarizes the evidence as follows:

$5.1 \text{ \AA}$

Astburyの $\alpha$ -keratin

$\beta$ -keratin

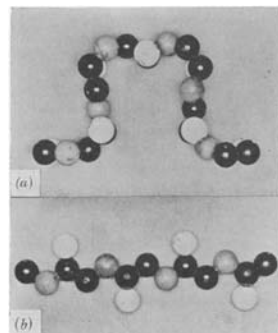


FIGURE 2. Chain configurations proposed by Astbury (1949) for (a)  $\alpha$ -keratin, (b)  $\beta$ -keratin.

## 蛋白質が全部同じ「二次構造」を持つことはあたりまえ!

p.328

## 4. CLASSIFICATION OF CHAIN STRUCTURES

In this and the following sections we attempt to survey systematically all those types of folded polypeptide chain configurations which satisfy certain conditions, established by experiment or plausible on general grounds.

It cannot be assumed as certain that the polypeptide chain has the same configuration in all crystalline proteins, or that a similar configuration occurs in fibrous proteins such as  $\alpha$ -keratin. It is, however, not unreasonable to expect that haemoglobin and myoglobin contain chains of the same type, because these proteins appear to be closely related in several ways; and furthermore, the repeat distance, the inter-chain distance, and the number of residues per repeat, are similar in these two proteins to the corresponding features of  $\alpha$ -keratin. It will therefore be assumed as a working hypothesis that the chain configurations in large classes of proteins resemble one another closely, while bearing in mind that this hypothesis is based on slender evidence and may have to be abandoned when further experimental data are available.

In our survey of chain configurations

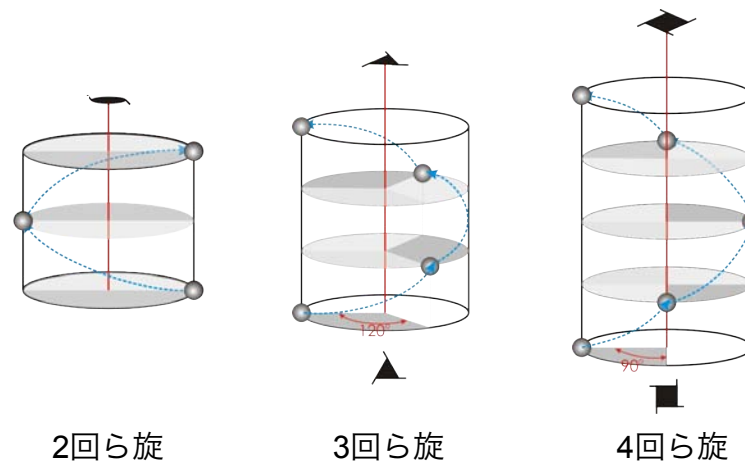
we have adopted the following conditions:

- (a) *Interatomic distances and bond angles*
- (b) *Optical configuration of amino-acids*     *laevo only*
- (c) *Symmetry of the chain*                     *have screw axis*
- (d) *The role of hydrogen bonding*             *S<sub>R</sub>*

結晶学者の失敗...

*S*: screw symmetry  
*R*: # of atoms in the H-bond ring

### screw axes

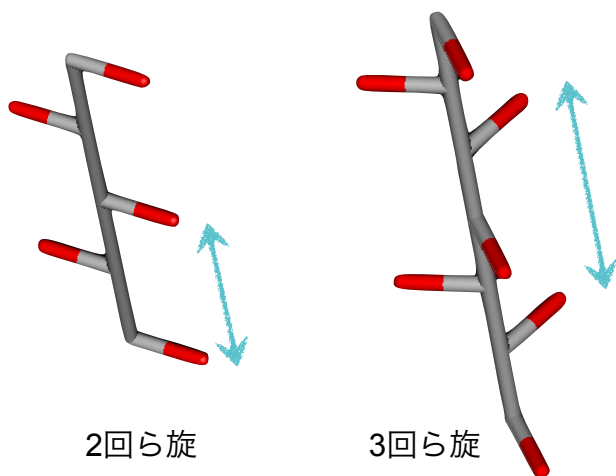


2回ら旋

3回ら旋

4回ら旋

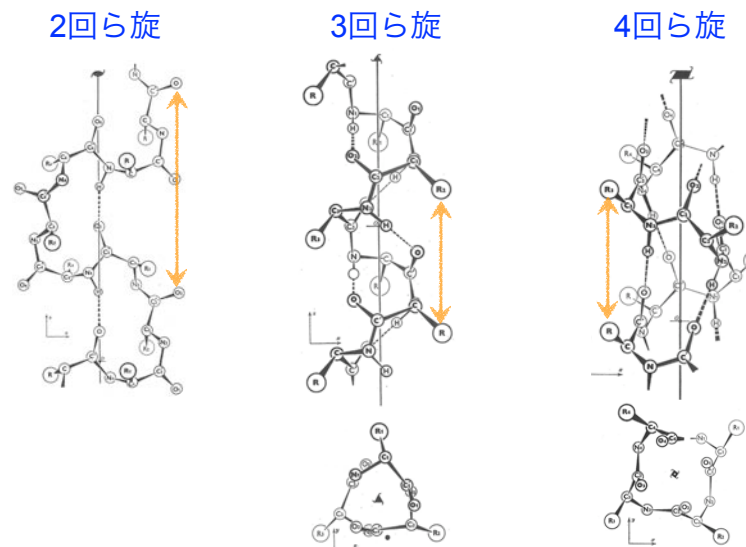
### screw axes



2回ら旋

3回ら旋

### screw model



2回ら旋

3回ら旋

4回ら旋

# screw model

$S_R$

TABLE 1					
screw axis of symmetry	$S$ no. of atoms in ring	$R$ repeat distances (Å)	no. of residues per repeat	illustrations (figure no.)	comments
twofold	7a	5-5-6	2	5	structure proposed by Huggins (1943)
	7b	5-5-6	2	6	structure proposed by Zahn (1947) and Ambrose <i>et al.</i> (1949); readily folds in pairs; see §7, 8
	8	4-6-4-8	2	7	structure proposed by Huggins (1943); only one configuration possible; repeat distance too short
	13	10-2	6	8	structure proposed by Astbury & Bell (1941); see §7, 8
	14	10-2	6	9	see §7, 8
threefold	7	7-5	3	—	repeat distance too short
	8	5-4	3	10	hydrogen bonds mutually perpendicular; see §7
	10	5-2	3	11	structure proposed by Taylor (1941) and Huggins (1943); hydrogen bonds oriented nearly parallel to the chain direction; see §8
	11	—	—	—	no possible structures
	13	—	—	—	
14	—	—	—		
fourfold	7	—	—	—	no possible structures
	8	—	—	—	
	10	—	—	—	
	11	5-4	4	—	rings somewhat strained; similar to 4 <sub>13</sub> ;
	13	5-6	4	12	a possible structure
14 or greater	—	—	—	—	no possible structures
fivefold and higher symmetries	—	—	—	—	all such structures contain more than four amino-acid residues per repeat unit

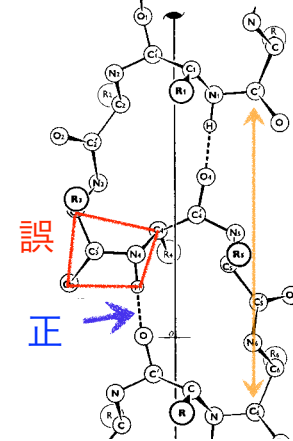
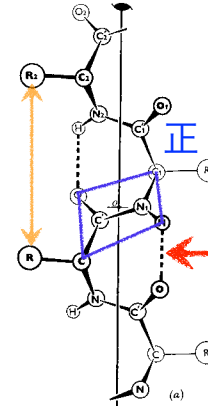
ペプチド平面, 水素結合の向きよりも...

screw の整数性を重視

$S_R$

2<sub>7</sub>

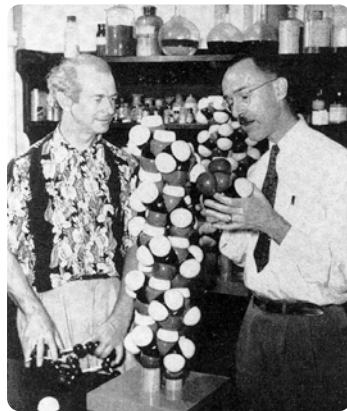
2<sub>14</sub>



@ Caltec

Pauling

(実は @ Oxford...)

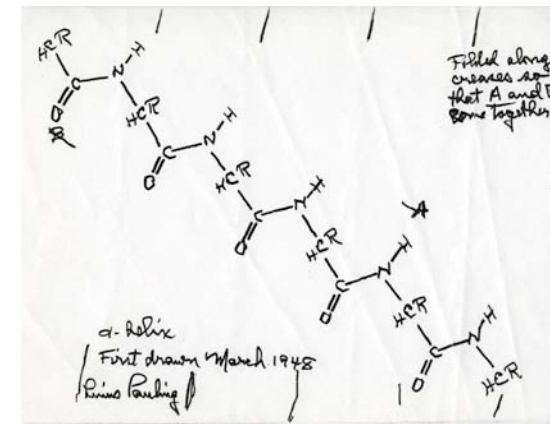


Pauling & Corey

結晶学者で  
なかった...

# Paulingの「紙」モデル

しかし、この後、3年間論文化せず...



Reconstruction of the alpha-helix paper model. Drawn and folded by Linus Pauling, 1982.

<http://paulingblog.files.wordpress.com/>

Proc. Natl. Acad. Sci. USA (1951) 37, 205-211

*THE STRUCTURE OF PROTEINS: TWO HYDROGEN-BONDED HELICAL CONFIGURATIONS OF THE POLYPEPTIDE CHAIN*

BY LINUS PAULING, ROBERT B. COREY, AND H. R. BRANSON\*

GATES AND CRELLIN LABORATORIES OF CHEMISTRY,  
CALIFORNIA INSTITUTE OF TECHNOLOGY, PASADENA, CALIFORNIA†

Communicated February 28, 1951

21

p.205

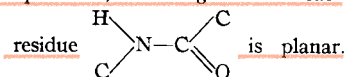
During the past fifteen years we have been attacking the problem of the structure of proteins in several ways. One of these ways is the complete and accurate determination of the crystal structure of amino acids, peptides, and other simple substances related to proteins, in order that information about interatomic distances, bond angles, and other configurational parameters might be obtained that would permit the reliable prediction of reasonable configurations for the polypeptide chain. We have now used this information to construct two reasonable hydrogen-bonded helical configurations for the polypeptide chain; we think that it is likely that these configurations constitute an important part of the structure of both fibrous and globular proteins, as well as of synthetic polypeptides. A letter announcing their discovery was published last year.<sup>1</sup>

22

p.206

## 2つの重要な「条件」

We assume that, because of the resonance of the double bond between the carbon-oxygen and carbon-nitrogen positions, the configuration of each

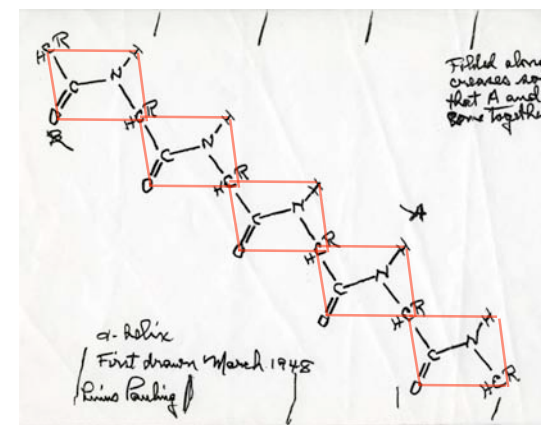


Laboratories. It is further assumed that each nitrogen atom forms a hydrogen bond with an oxygen atom of another residue, with the nitrogen-oxygen distance equal to 2.72 Å, and that the vector from the nitrogen atom to the hydrogen-bonded oxygen atom lies not more than 30° from the N—H direction. The energy of an N—H ··· O=C hydrogen bond is of the order

23

## Paulingの「紙」モデル

しかし、この後、3年間論文化せず...



Reconstruction of the alpha-helix paper model. Drawn and folded by Linus Pauling, 1982.

<http://paulingblog.files.wordpress.com/>

24

# 可能性は 3.7-residue か 5.1-residue helix

p.207

5.44Å  
(≠5.1Å)

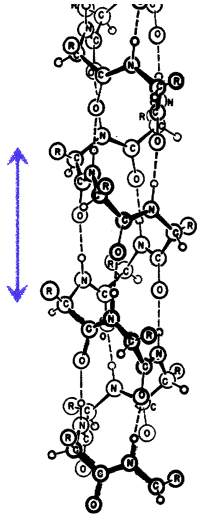


FIGURE 2  
The helix with 3.7 residues per turn.

p.208

*SR 3.613*

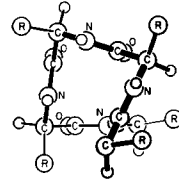
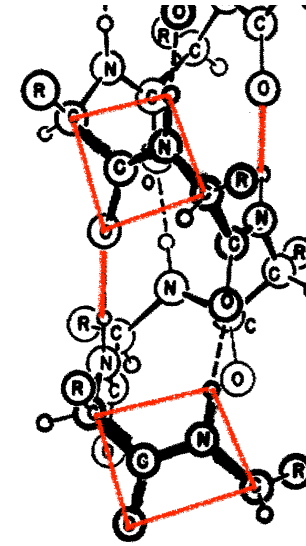


FIGURE 4  
Plan of the 3.7-residue helix.

*laevo only???*  
*left turn...*

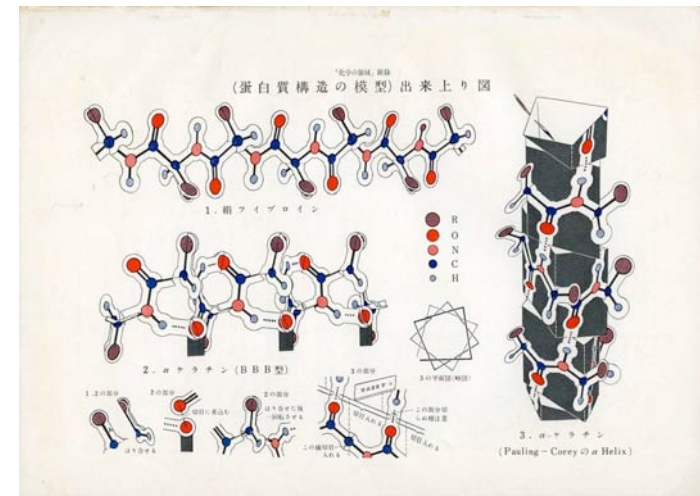


p.209-210

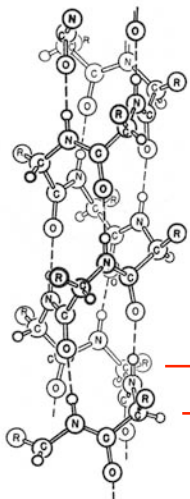
## Paulingの自信が観られる...

tures involving intramolecular hydrogen bonds, and Bragg, Kendrew, and Perutz extended the discussion to include additional structures, and investigated the compatibility of the structures with x-ray diffraction data for hemoglobin and myoglobin. None of these authors proposed either our 3.7-residue helix or our 5.1-residue helix. On the other hand, we would eliminate, by our basic postulates, all of the structures proposed by them. The reason for the difference in results obtained by other investigators and by us through essentially similar arguments is that both Bragg and his collaborators and Huggins discussed in detail only helical structures with an integral number of residues per turn, and moreover assumed only a rough approximation to the requirements about interatomic distances, bond angles, and planarity of the conjugated amide group, as given by our investigations of simpler substances. We contend that these stereochemi-

## 「化学の領域」付録@1954



# @ Cambridge



“It was thunderstruck by Pauling and Corey’s paper.”

Perutz

一残基あたりの並進  
1.5Å

29

key paper

Nature (1951) 167, 1053-1054

No. 4261 June 30, 1951 NATURE 1053

NEW X-RAY EVIDENCE ON THE CONFIGURATION OF  
POLYPEPTIDE CHAINS

M. F. PERUTZ Cavendish Laboratory,  
University of Cambridge.

Paulingの論文の僅か2.5ヶ月後...

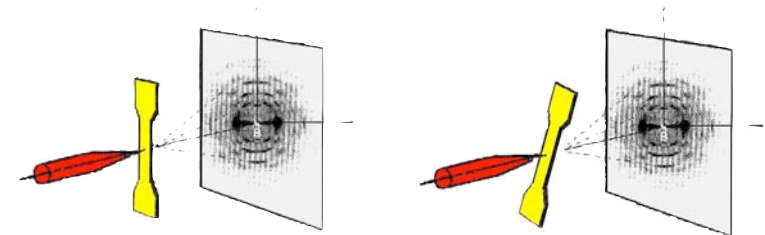
30

p.1053

stretched chain. Many different chain configurations have been proposed to account for the X-ray diffraction data<sup>1</sup>, the latest being those of Pauling, Corey and Branson<sup>2</sup>. Until now, however, the lack of any simple and decisive criterion in the X-ray diffraction pattern has made it difficult to test the validity of proposed models. This communication describes a new reflexion, not hitherto observed, which is given by the proteins mentioned above. The spacing at which this reflexion appears excludes all models except the 3.7 residue helix of Pauling, Corey and Branson, with which it is in perfect concord.

31

## Perutzの検証実験



Astburyの撮影法

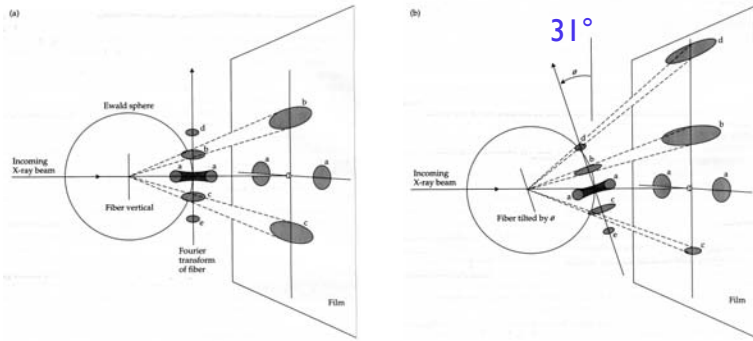
Perutzの実験

32



# Perutzの検証実験

の理屈...



Astburyの撮影法

Perutzの実験

# Perutzの検証実験

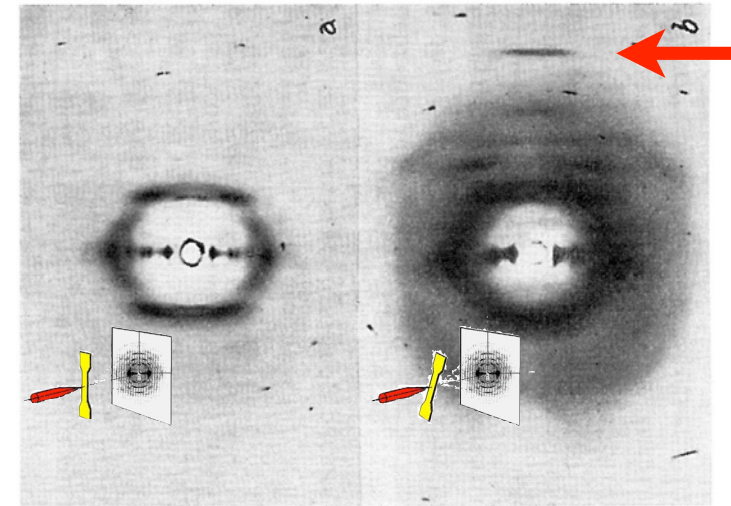
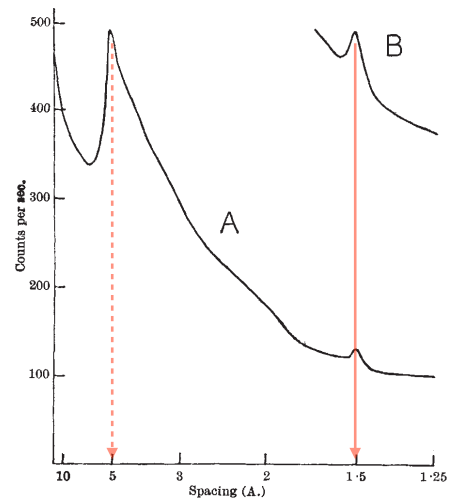
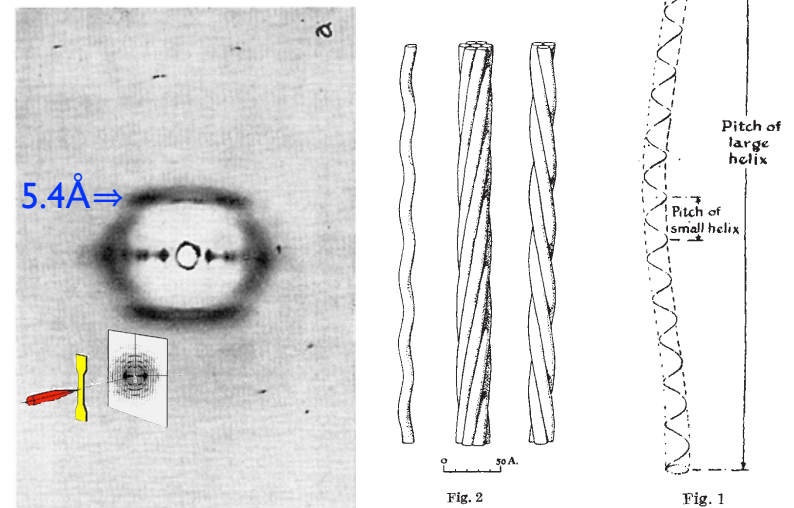


Fig. 2 is a Geiger counter spectrometer record of horse hair kindly placed at my disposal by Mr. Andrew Lang, which shows the relative intensities of the 5.1-A. and the 1.5-A. reflexions. When hair is stretched to the  $\beta$ -form the 1.5-A. reflexion vanishes.

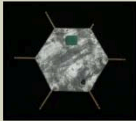


$5.4\text{\AA} \neq 5.1\text{\AA}$



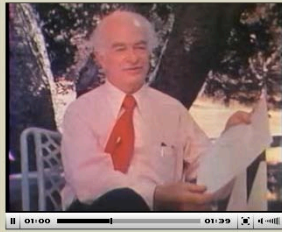
# Paulingの「紙」モデル

## All Documents and Media



"The DNA Story." 1973.  
Created by VSM Productions. Produced by Ronald  
Fouracre and Peter Shaw. Distributed by John Wiley &  
Sons, London, New York.

Pauling Discovers the Alpha-Helix. (2:40)



<http://osulibrary.oregonstate.edu/specialcollections/coll/pauling/dna/video/1973v.3-alpha.html>

37

# 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*)

38