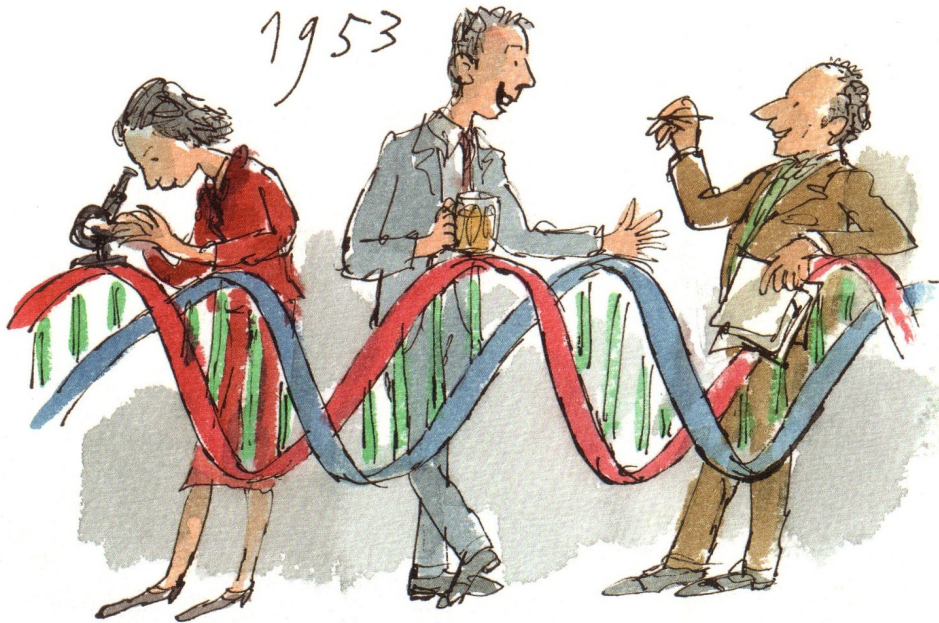
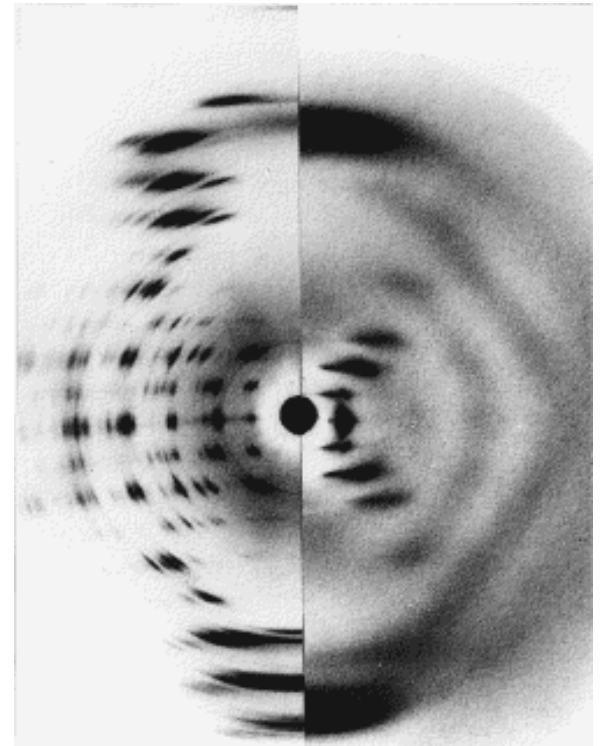


DNA: Franklin, Crick & Watson
1953

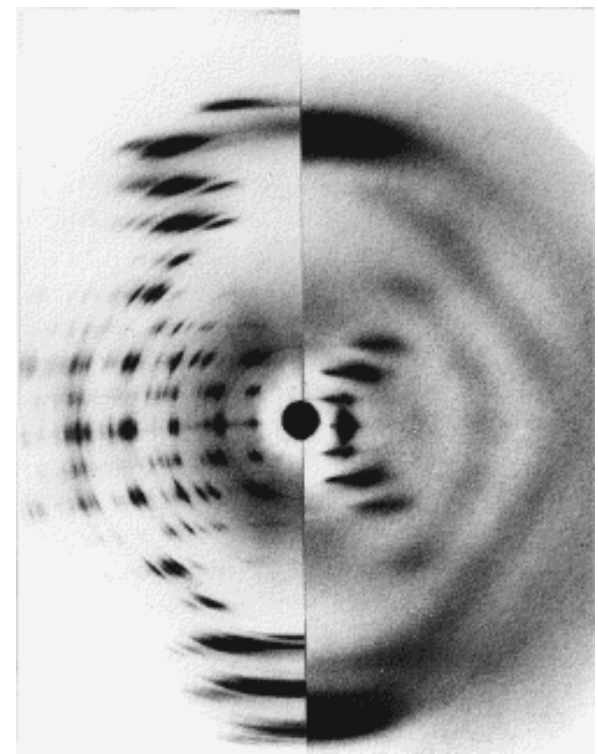


Lecture:
Close encounters of the atomic kind:
Rosalind Franklin's legacy and the
origins of molecular structural biology.



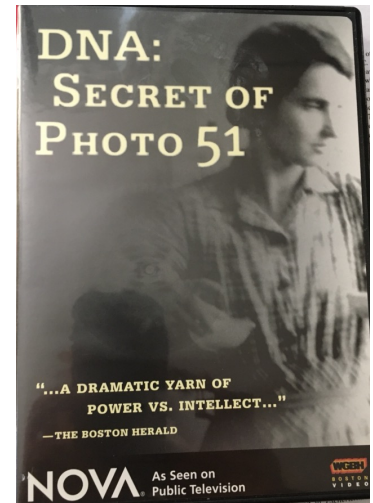
Historical and scientific lecture reviewing the legacy and contributions of
Rosalind Franklin to the early achievements of structural molecular biology.
HWC October 4, 2023 at 1:00 pm.

=====
Celerino Abad-Zapatero, Ph. D. PSCI Department. Affiliate to the Institute of
Tuberculosis Research and the Center for Biomolecular Sciences.



Close encounters of the atomic kind: Crystals and X-rays.

*The Origins of Molecular Biology and
the Legacy of Rosalind E. Franklin*



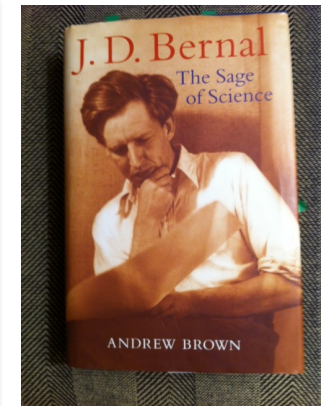
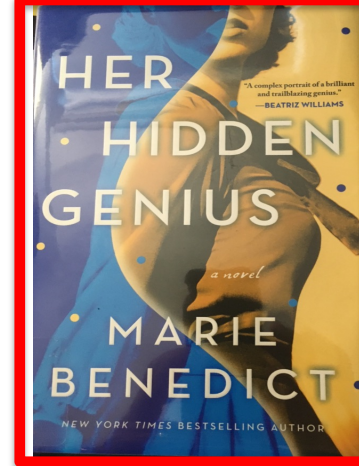
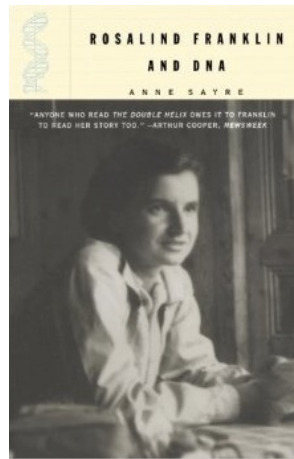
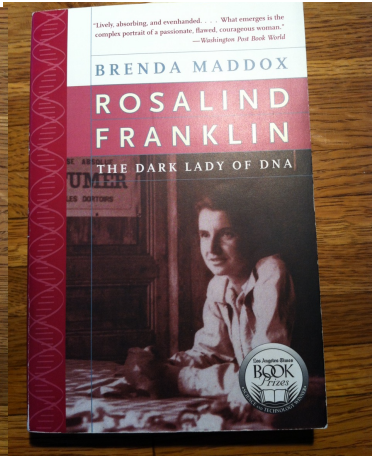
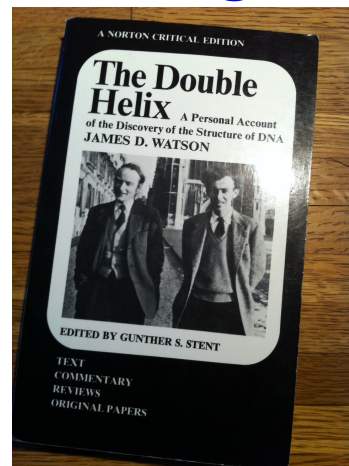
Scientific and Historical seminar

HW College, Lecture , October 4, 2023

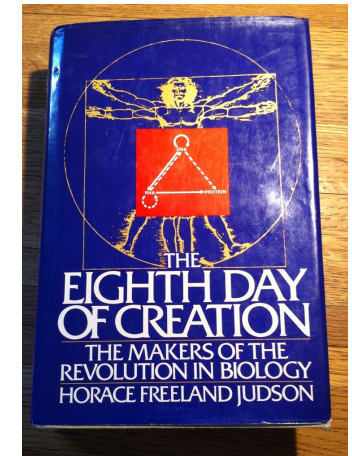
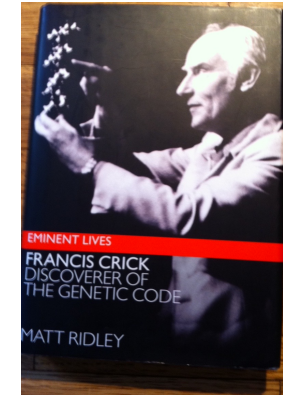
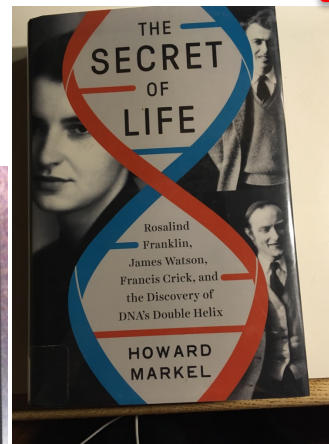
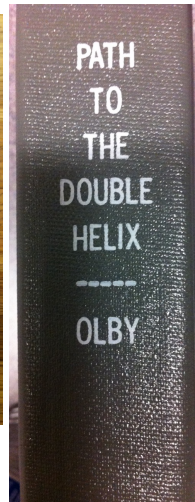
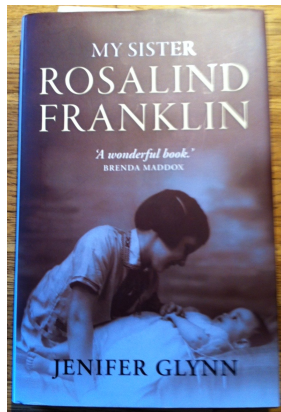
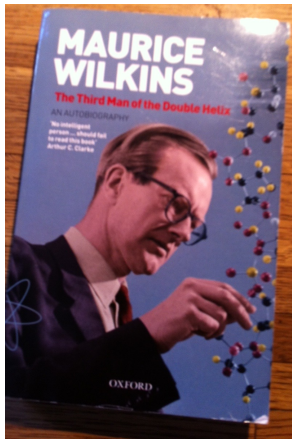
Presented by Cele Abad-Zapatero,

Research Professor. Department of Pharmaceutical Sciences.

Background:



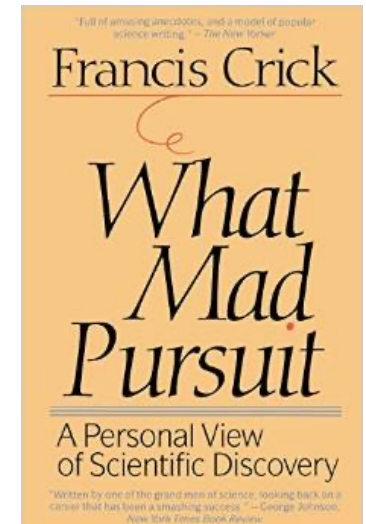
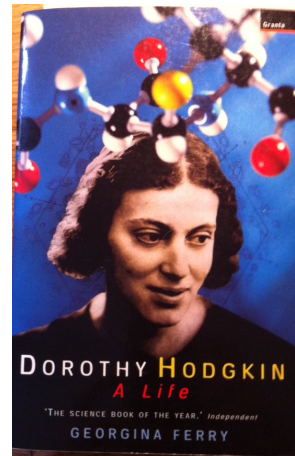
Fall 2022



Rosalind Franklin and the Double Helix

Although she made essential contributions toward elucidating the structure of DNA, Rosalind Franklin is known to many only as seen through the distorting lens of James Watson's book, *The Double Helix*.

L. O. Elkin. Physics Today (2003).



Rosalind E. Franklin (1920-1958). A premier structural biologist whose research played a critical role in the determination of the structure of DNA and the helical viruses, particularly TMV. These milestones paved the way to structural biology.

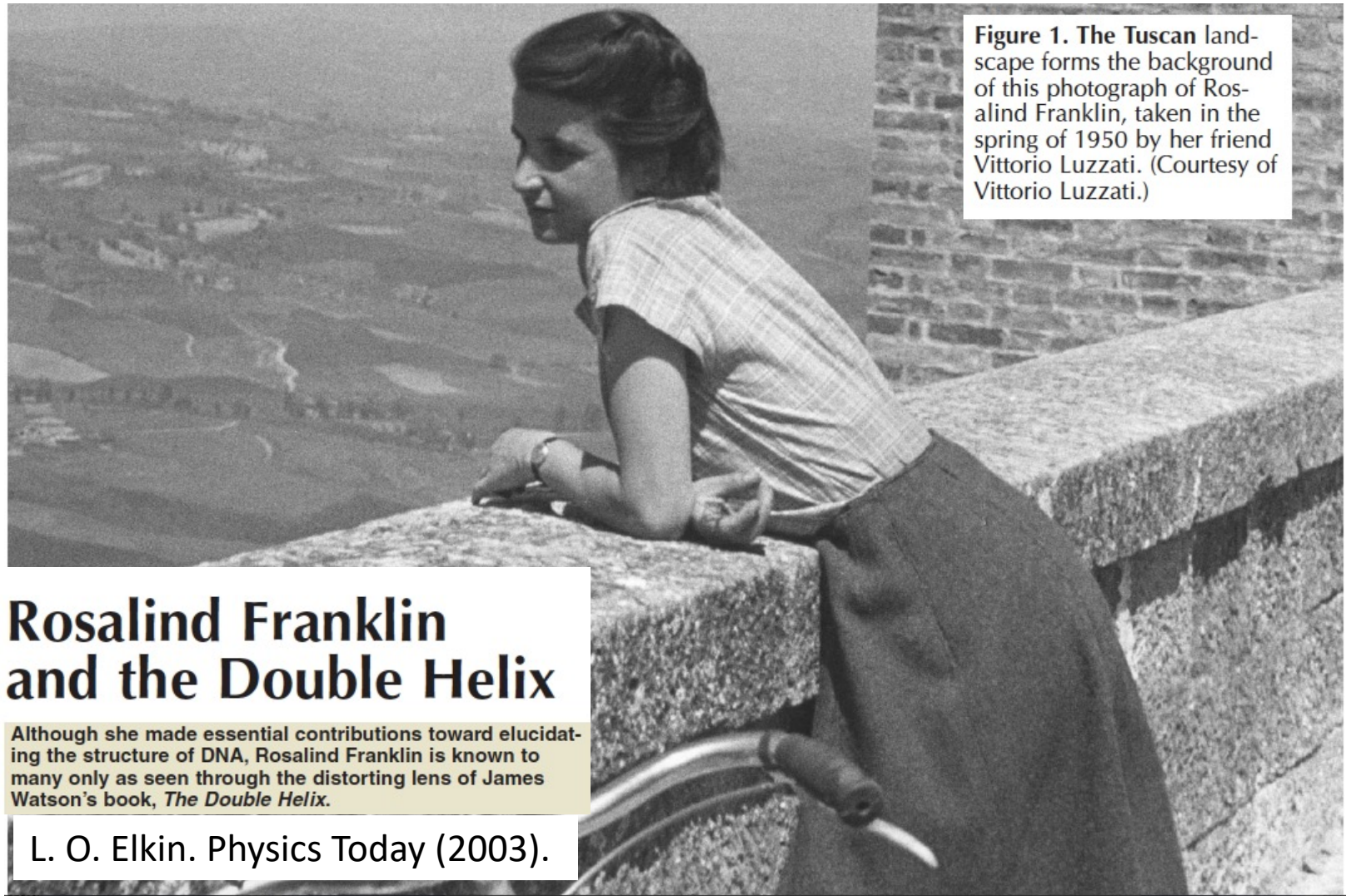
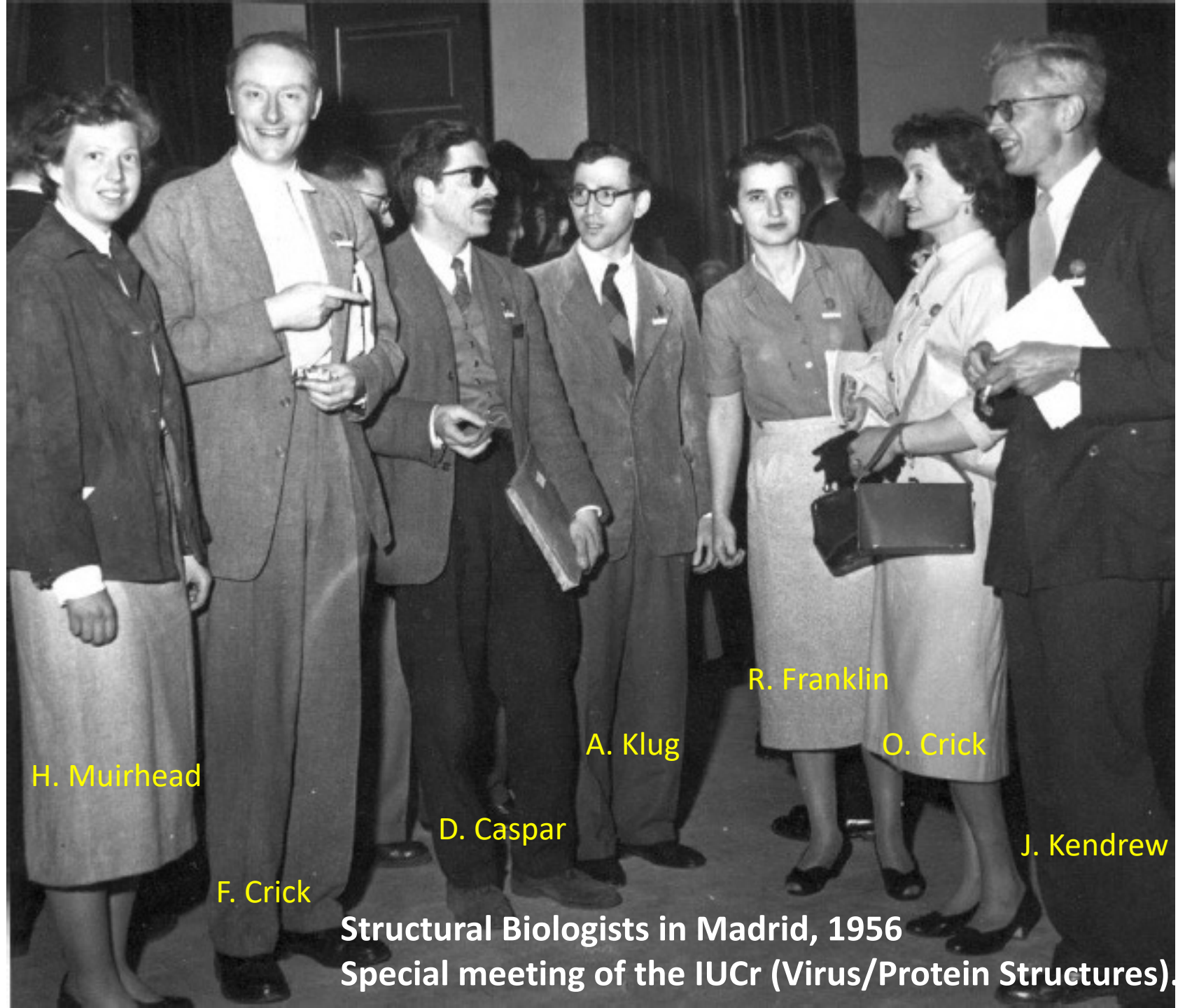


Figure 1. The Tuscan landscape forms the background of this photograph of Rosalind Franklin, taken in the spring of 1950 by her friend Vittorio Luzzati. (Courtesy of Vittorio Luzzati.)

Rosalind Franklin and the Double Helix

Although she made essential contributions toward elucidating the structure of DNA, Rosalind Franklin is known to many only as seen through the distorting lens of James Watson's book, *The Double Helix*.

L. O. Elkin. *Physics Today* (2003).



H. Muirhead

F. Crick

D. Caspar

A. Klug

R. Franklin

O. Crick

J. Kendrew

Structural Biologists in Madrid, 1956
Special meeting of the IUCr (Virus/Protein Structures).

Close encounters of the atomic kind: Crystals and X-rays



Crystals:

Known since antiquity (quartz)

Objects of wonder: precious
(gemstones, wealth, power)

High symmetry

Unusual refraction/reflection of light

Unknown internal structure

Mineralogist would study crystals



X-rays



Discovered in 1895 (W. Roentgen)

Penetrated organic matter revealing
bones, soft/hard tissues

Unknown physical nature:

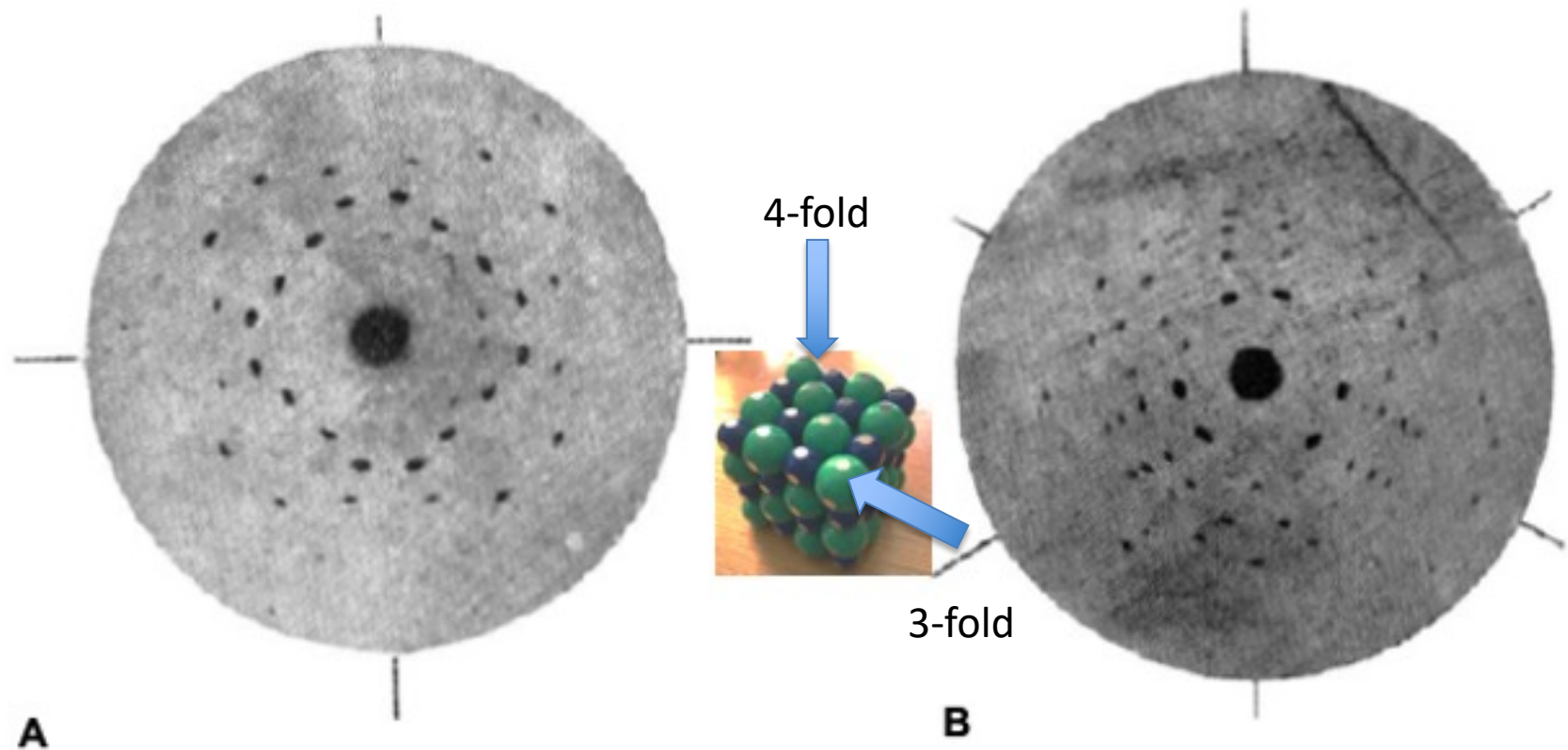
Waves or Particles?

Could they be reflected/refracted?

Could they be 'diffracted'?

➤ How and when did the encounter take place?

The 'encounter' between crystals and X-rays (1912): The diffraction pattern

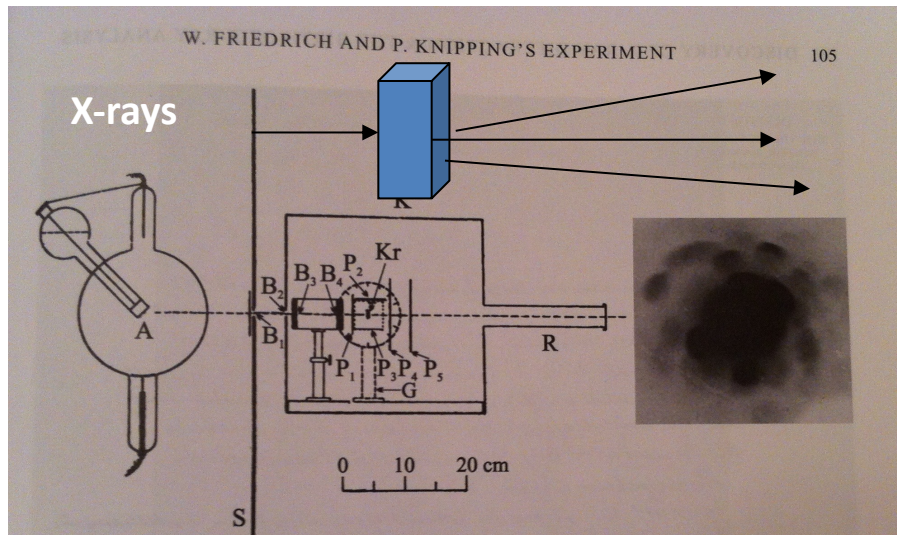


The experiment of Friedrich, Knipping and Von Laue (1912) confirmed, at the same time, the wave nature of the X-rays and the regular lattice organization of crystals

First diffraction patterns of crystals of the cubic mineral zinc blende. A) along the 4-fold axis of symmetry; B) along the 3-fold axis. Inset, the atomic structure of NaCl (common salt) inferred from the diffraction patterns studied by the Braggs in 1914. Green: Cl⁻, Blue: Na⁺.

The meeting of X-rays and Crystals (1912-1914)

(A 'Magic' encounter)



WH & WL
Bragg (1914)

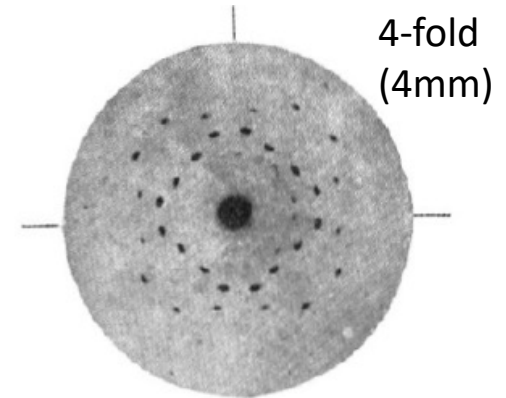


Figure 3. Laue photograph of zinc blende along the four-fold axis (von Laue, Friedrich and Knipping 1912). From Ewald (1962).

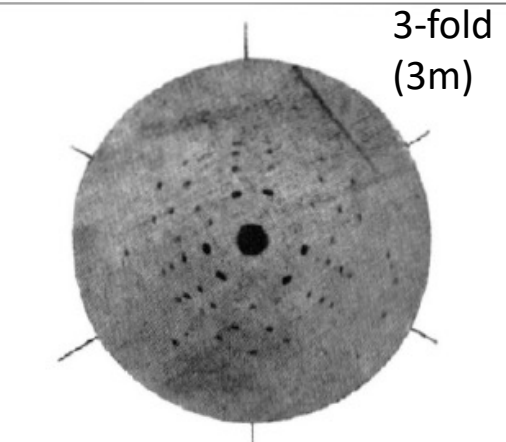
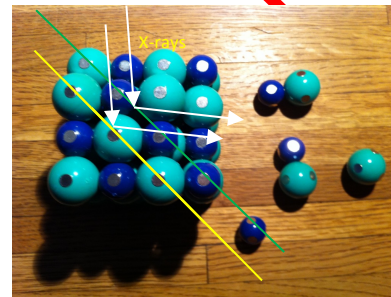
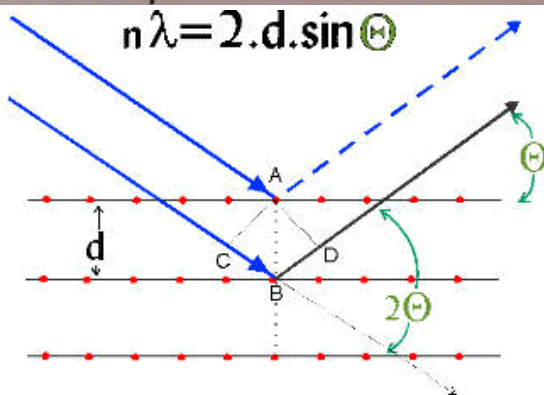


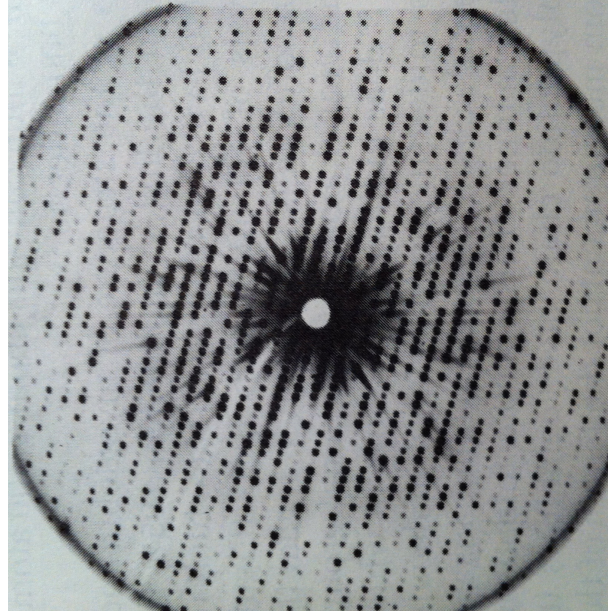
Figure 4. Laue photograph of zinc blende along the three-fold axis (von Laue, Friedrich and Knipping 1912). From Ewald (1962).



Interpretation

What is 'diffraction'?

- It is not 'Reflection' nor 'Refraction': well-know with light rays.
- Does it only happen between crystals and X-rays?
- Why is it so important and rare?
- Why do we care?
 - ❖ From the diffraction pattern we can infer the 3D structure of the molecules in the crystal.



**Diffraction of
hemoglobin crystal.**

**Observe regularly
spaced spots related
to the regular lattice
spacings of the crystal.**

Diffraction only occurs under very special conditions, but it does show in our daily lives, just be alert (!).



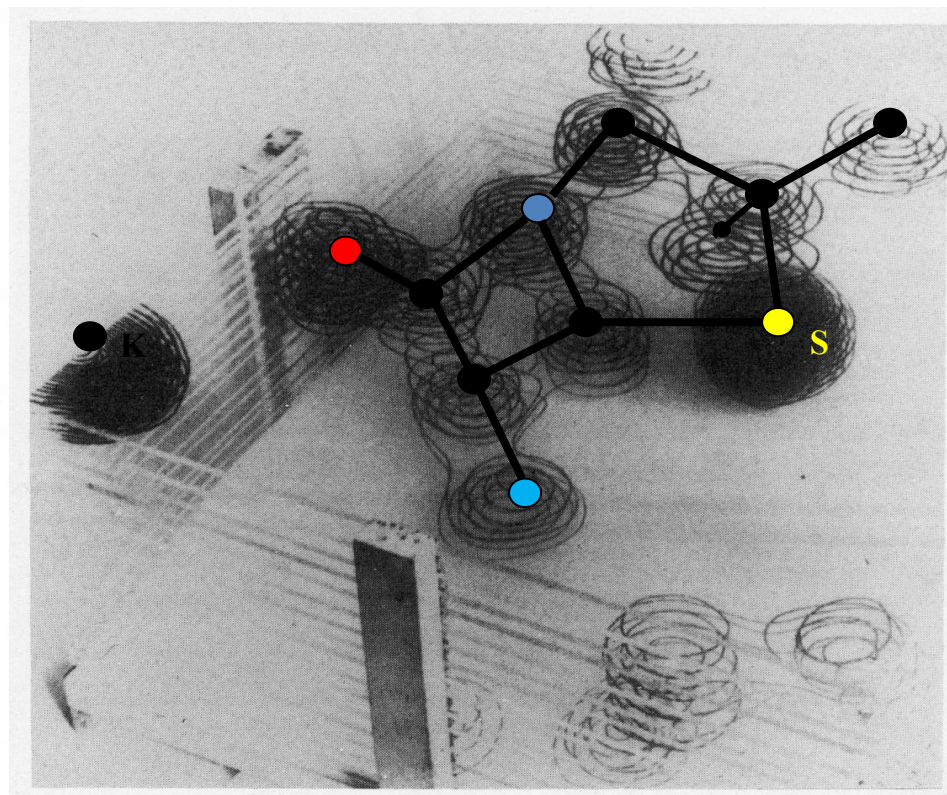
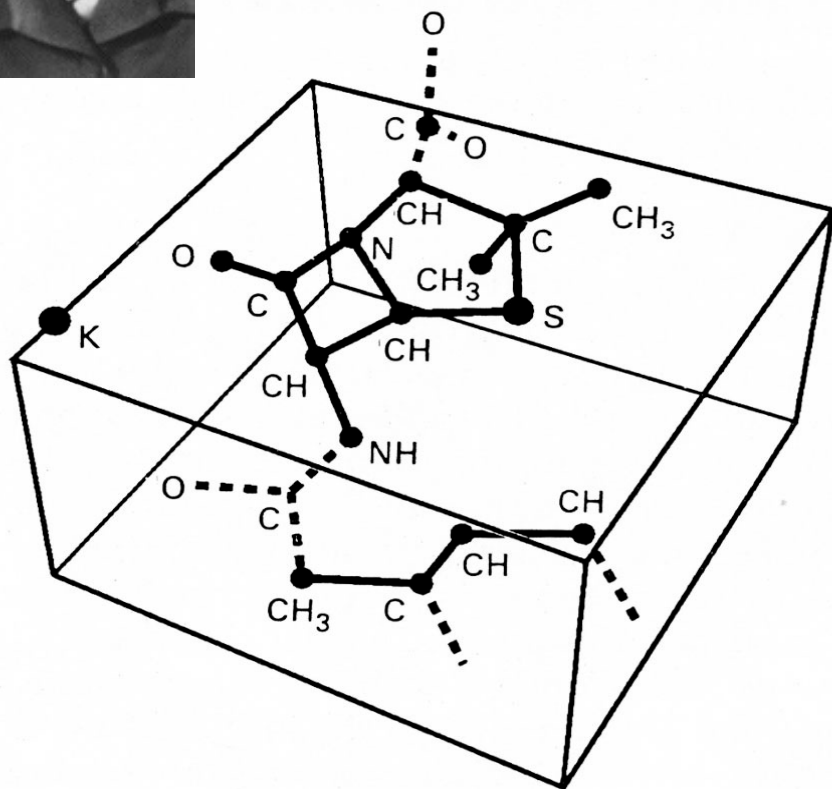
Diffraction occurs when the dimensions of the object are 'commensurate' (i. e. same order of magnitude) as the wavelength of the radiation used to illuminate the object.



Chance only favors the prepared mind' (Louis Pasteur). **More:** <https://www.caz.crystaledges.org>



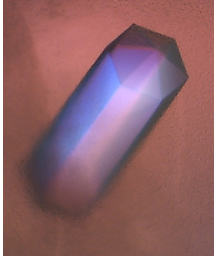
Crystallography: Achievement of the first half of the 20th century



Structure of penicillin (the wonder drug!) as derived from the crystallographic maps of D. Hodgkin (1945)

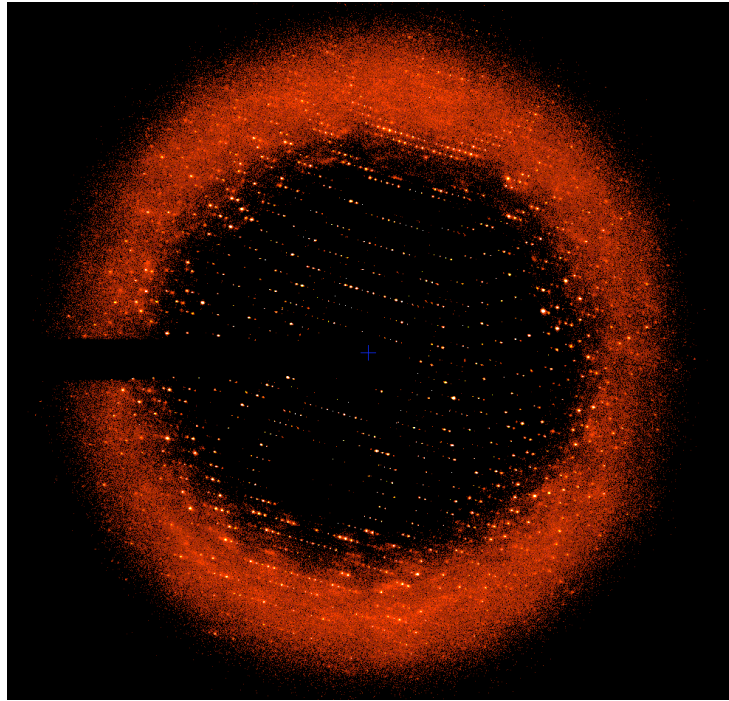
Practical Aspects of Crystallography

X
rays

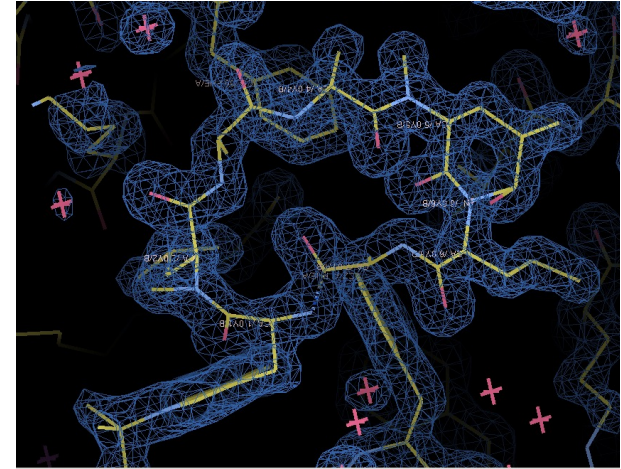


FT

1. Grow
crystals

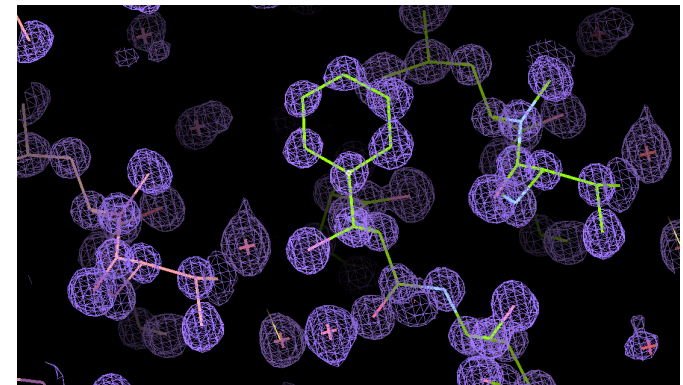


FT⁻¹



3. Calculate FT⁻¹ to obtain 'electron density' in the crystal. **1.40 Å**

4. Interpret the electron density in terms of **Chemistry (!)**: atoms, amino acids, benzene rings, etc



FT: *Fourier Transform*

Combines:

- Biochemistry
- Physics
- Mathematics
- Chemistry
- Biology

2. Collect diffraction data:
Several 100-thousands of reflections typical; millions for viruses: measure $|F(hkl)|$.

0.83 Å resolution

For those who do not believe in atoms!

Events related to the origins of Structural/Molecular Biology I

- ✓ I. W.M. Stanley, *Science* 1935. 'Crystallization' of TMV 'proteinaceous' particles.
- ✓ II. Bernal & Crowfoot insight on the handling of protein crystals (1934) (vs. W. Astbury).
- ✓ III. Oriented gel diffraction patterns of TMV: Bernal, Fankuchen (1941). Not crystals!
- ✓ IV. Structure of the polypeptide chain: the uncharted territory (1950's).
 - The blunder of Bragg, Kendrew, Perutz.
 - Pauling's contribution: α -helix, β -sheet:
 - Motifs of protein structure 'unveiled'.

I. Isolation of a crystalline protein possessing the properties of tobacco-mosaic virus (TMV).

W.M Stanley's Publication, *Science* (1935) 81 (2113) pgs. 644-645.

Isolation of a crystalline protein
possessing the properties of
tobacco-mosaic virus

has been obtained. Tobacco-mosaic virus is regarded as an autocatalytic protein which, for the present, may be assumed to require the presence of living cells for multiplication.

mosaic virus. *Science*, Vol. 81, No. 2113, pages 644-645.

A CRYSTALLINE MATERIAL, WHICH HAS the properties of tobacco-mosaic virus, has been isolated from the juice of Turkish tobacco plants infected with this virus. The crystalline material

10 successive crystallizations. In a fractional crystallization experiment the activity of the first small portion of crystals to come out of solution was the same as the activity of the mother

Wendell Meredith Stanley



Born	August 16, 1904 Ridgeville, Indiana, USA
Died	June 15, 1971 (aged 66) Salamanca, Spain
Nationality	United States
Fields	Chemistry
Institutions	Rockefeller Institute University of California, Berkeley
Alma mater	Earlham College
Notable awards	Nobel Prize in Chemistry (1946) Willard Gibbs Award (1947) Franklin Medal (1948)

I. What did Stanley actually 'crystallize'?

Wendell Meredith Stanley



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Fields	Chemistry
Institutions	Rockefeller Institute University of California, Berkeley
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Notable awards	Nobel Prize in Chemistry (1946) Willard Gibbs Award (1947) Franklin Medal (1948)

- Sample was not pure 'protein'. It contained about 6% of nucleic acid (0.5% phosphate) material that Stanley failed to recognize.
- The 'crystals' were only sheaves of virus particles roughly aligned along the long 3000 Å axis of the particle. They were not 'crystals' by any means. (Bernal & Fankuchen, 1941).

II. Bernal's insight to maintain the crystalline order in protein crystals (Crowfoot & Bernal, Nature, 1934)

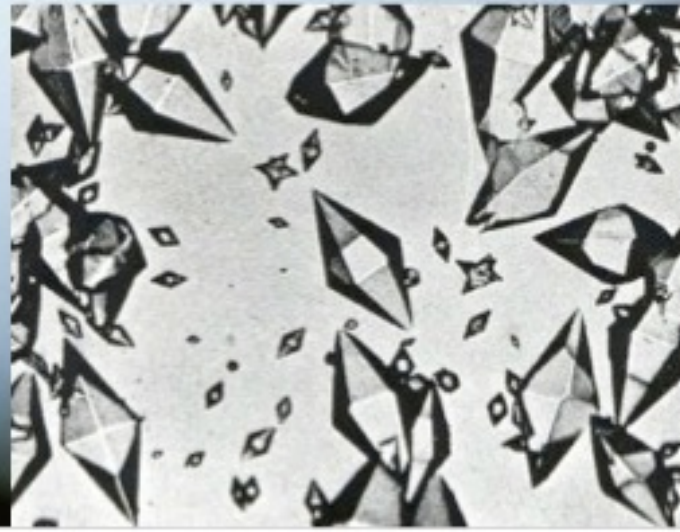
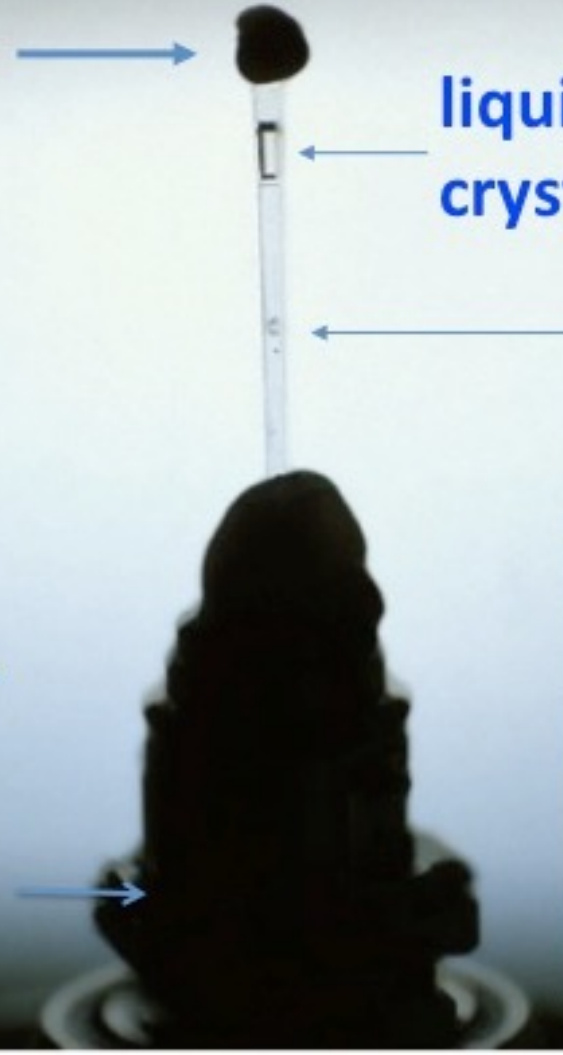
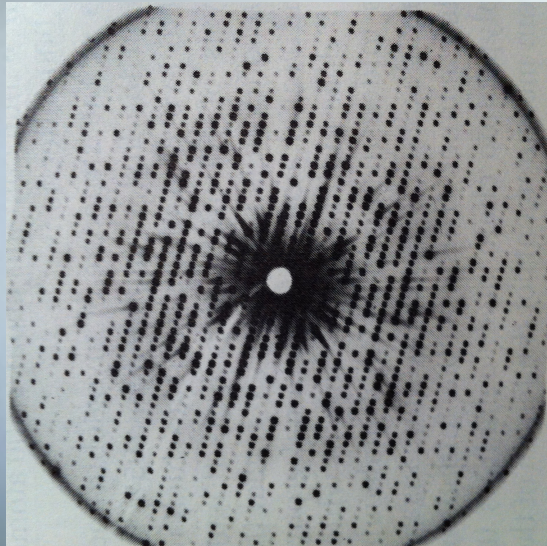
Diffraction pattern

wax

liquid column to maintain
crystal moist

crystal

mounting head or
goniometer head



III. Bernal, Fankuchen, Bawden, Pirie, Wyckoff (1936-1941).

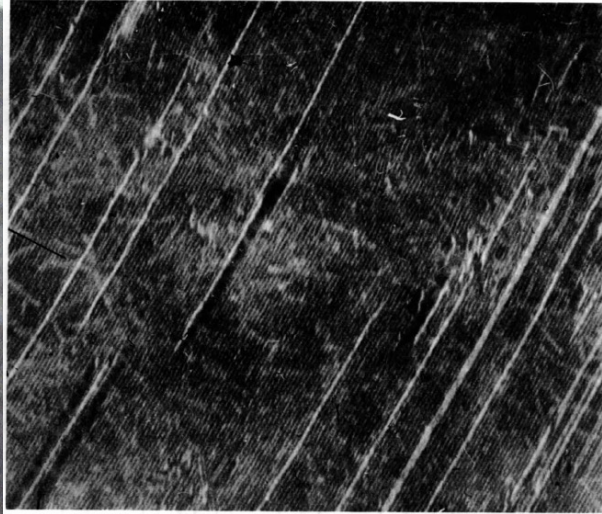
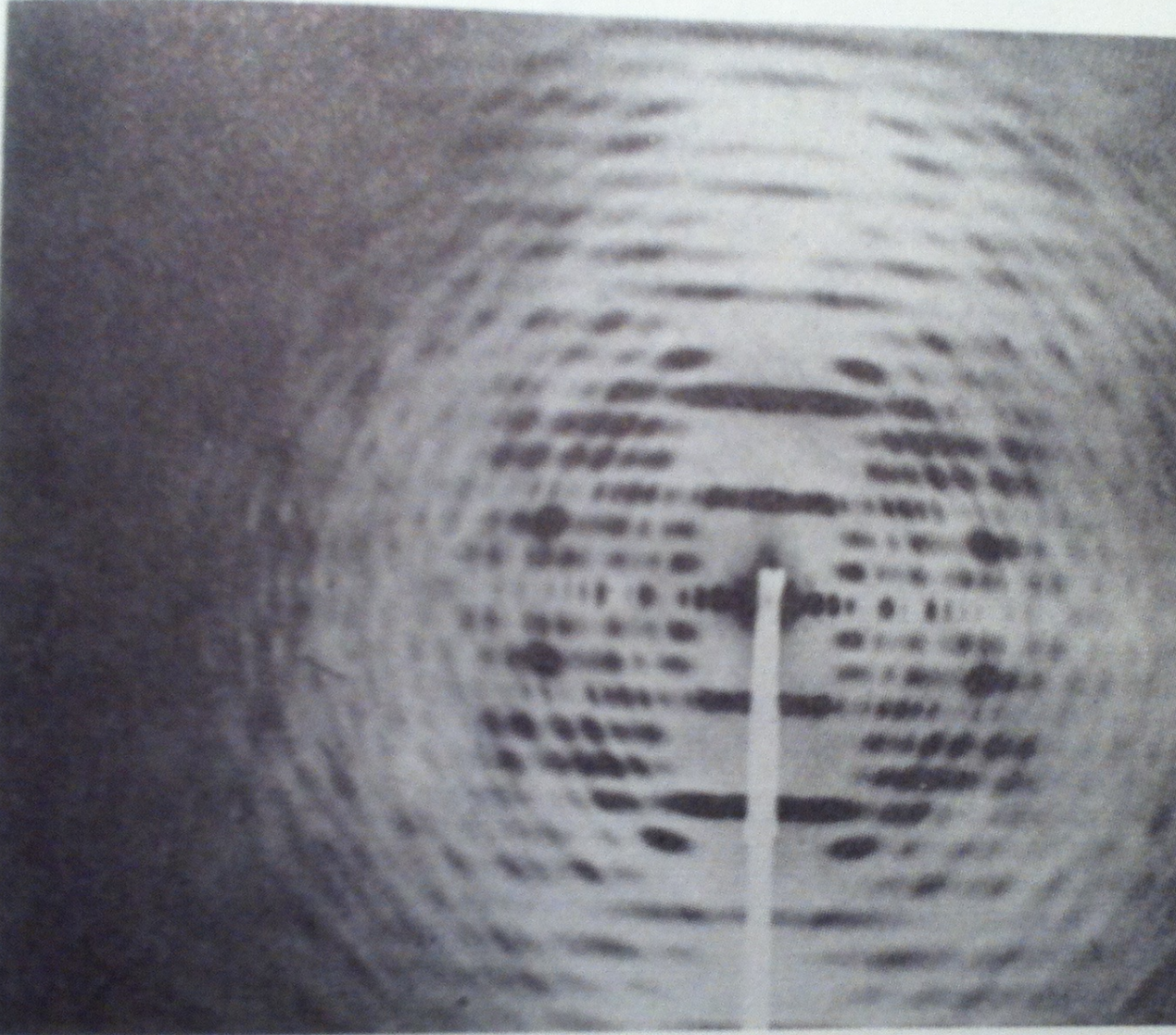


Fig. 2. A 'pseudo-replica' of the top surface of a dried tobacco mosaic virus protein gel showing the molecules in a strictly parallel array. The molecular rods have diameters of 150 Å. Magnification, 50,000.

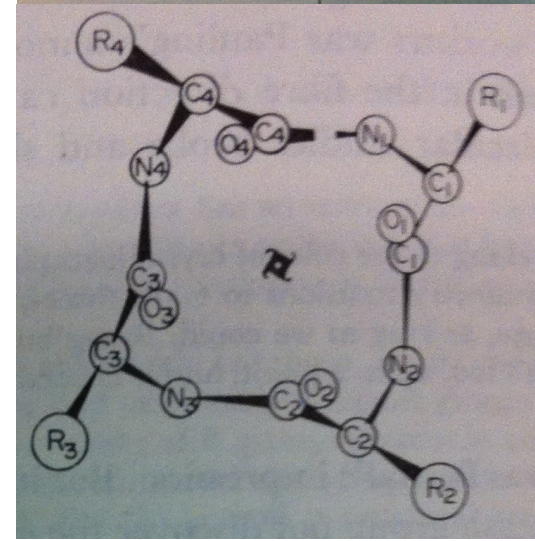
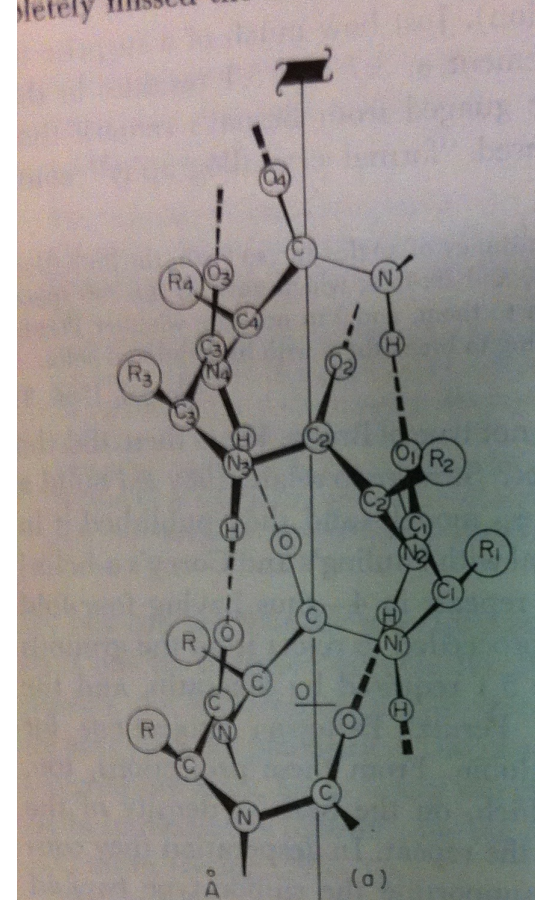
Demonstrated that Stanley's 'crystals' were just sheaves of TMV particles 3000 Å long and 150 Å thick. Developed superior protocols to produce ordered gels producing diffraction patterns indicating **complex helical symmetry** (Bernal & Fankuchen, 1941).

IV. Structure of Proteins I:

Bragg, Kendrew, Perutz's blunder (1950)

Helical fold of the polypeptide chain:
but.....

- ✓ Driven by W.T. Astbury 5.1 Å reflection/helical pitch (raise/turn)
- ✓ Retained an 'integer number of residues/turn' helix, biased by crystallographic symmetry.
- ✓ Did not preserve the right stereo-chemical parameters of peptide bond, i. e. bond planarity.



Pauling's insight into the folding of the polypeptide chain

(Pauling, Corey, 8 papers in PNAS, Spring 1951).

Chapter 8 Protein conformation



β -SHEET: parallel & antiparallel

The two most prominent regular motifs of protein structure

Events related to the origins of Structural/Molecular Biology II

- ✓ The analytical 'holy grail': the theory of helical diffraction (1952). Cochran, Crick, Vand, Acta Cryst. 5, 581 and A.R. Stokes (unpublished).
- ✓ Pioneering work of W. S. Astbury
- ✓ Studies on the structure of Sodium thymonucleate (DNA). King's College London. Wilkins, Gosling, **Franklin**.
- ✓ **R.E. Franklin critical contributions: A vs. B forms.**
- ✓ Watson and Crick's DNA structure and the supporting evidence provided by Franklin, Gosling, Wilkins.
(*Nature* papers accompanying, Watson & Crick's, 1953).

The analytical 'holy grail': the theory of helical diffraction (1952). Cochran, Crick, Vand, Acta Cryst. 5, 581

COC 4152

Acta Cryst. (1952). 5, 581

The Structure of Synthetic Polypeptides. I. The Transform of Atoms on a Helix

By W. COCHRAN

Crystallographic Laboratory, Cavendish Laboratory, Cambridge, England

F. H. C. CRICK

*The Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems,
The Cavendish Laboratory, Cambridge, England*

AND V. VAND*

Chemistry Department, The University, Glasgow W. 2, Scotland

(Received 16 February 1952)

The formulæ are given for the Fourier transforms of a number of helical structures; namely, a thin helical wire, a set of identical atoms spaced at regular intervals on a helix, and the general case of a group of atoms repeated by the operation of a non-integer screw. General predictions are made concerning the intensities of the X-ray diffraction pattern of the synthetic polypeptide poly- γ -methyl-L-glutamate, assuming that its structure is based on the α -helix suggested by Pauling & Corey.

1. Introduction

The following calculations were undertaken because of current interest in the structures of certain synthetic polypeptides. The preliminary X-ray data for these polypeptides have been described by Bamford, Hanby & Happey (1951) and their infra-red behaviour by Ambrose & Elliott (1951). Pauling & Corey (1951)

have interpreted the structures of the two polypeptides which have so far given the best X-ray diffraction pictures, namely poly- γ -methyl-L-glutamate and poly- γ -benzyl-L-glutamate, in terms of the α -helix described by Pauling, Corey & Branson (1951). In this structure the residues repeat along the helix with a spacing of about 1.5 Å in the chain direction, and Perutz (1951) has found that a strong meridional reflexion of spacing 1.5 Å is given by poly- γ -benzyl-

* Imperial Chemical Industries Fellow.

Fourier Transform of a continuous helix is given by a family of integer Bessel Functions

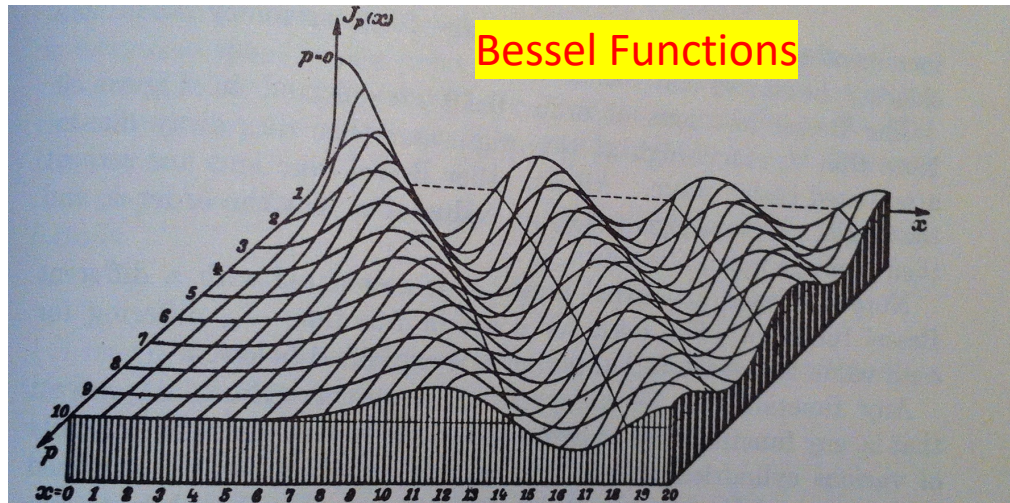


Fig. 30. The Bessel function $J_p(x)$ as functions of p and x . Note that J_0 is large at the origin but that all other orders are zero at the origin. At large values of p the first maximum value of the Bessel function $J_p(x)$ occurs at a value of x close to p . After the first maximum all the Bessel functions oscillate like an attenuated

16.7 The diffraction pattern of an infinite continuous helix

According to equation (16.18), the Fourier transform of an infinite continuous helix may be represented in reciprocal space in terms of cylindrical polar coordinates (R, ψ, ξ) , subject to the condition restricting the values of the coordinate ξ to

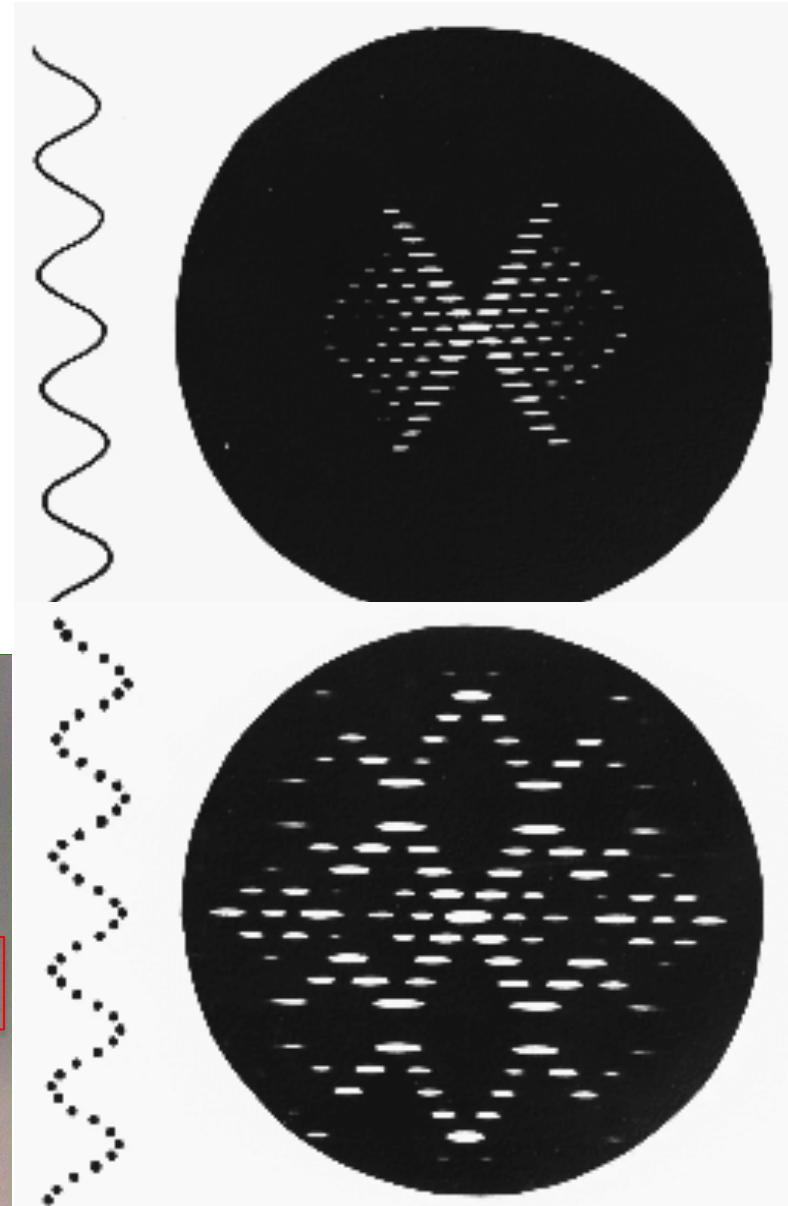
$$\xi = \frac{n}{P} \quad \text{for all integral } n.$$

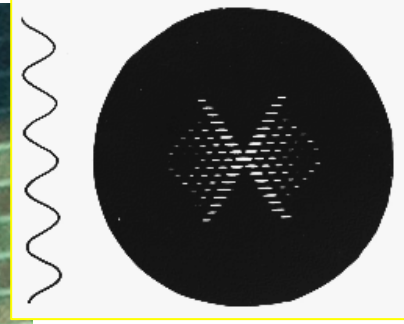
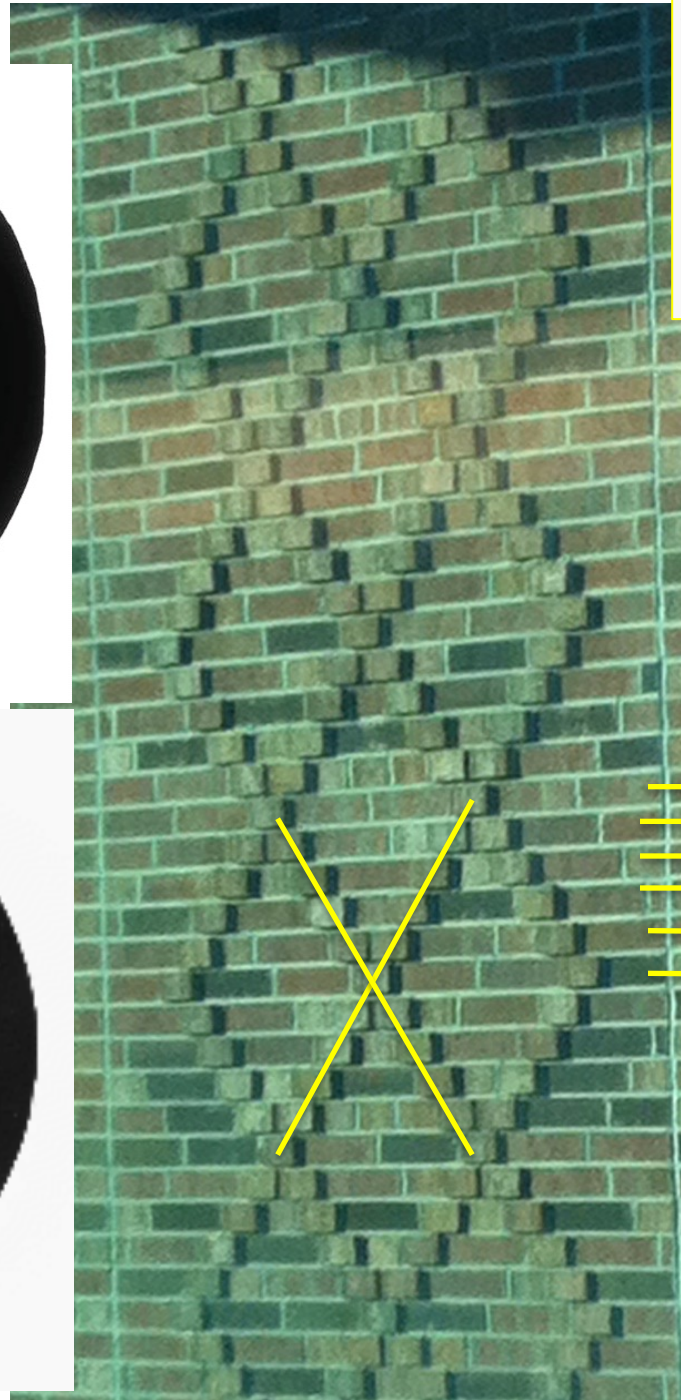
We then have

$$T[h_\infty(r, \theta, z)] = \sum_n F\left(R, \psi, \frac{n}{P}\right) = \sum_n r_0 e^{in(\psi + \pi/2 - \theta_0)} J_n(2\pi R r_0) \quad (16.18)$$

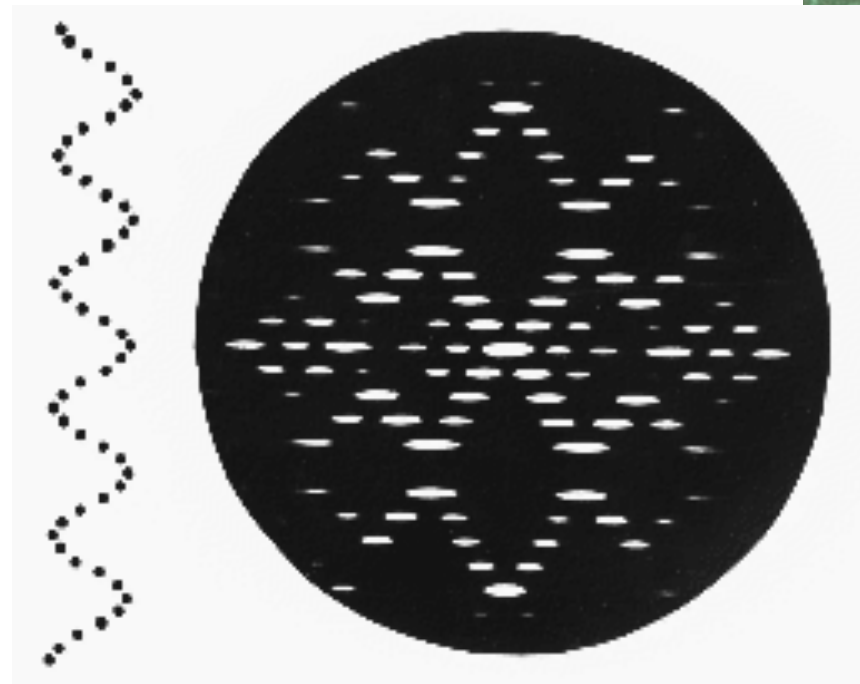
In any experiment, the observed quantity is intensity, corresponding to the squares of the magnitude of the Fourier transform which, for any given value of n is given by

$$\left| F\left(R, \psi, \frac{n}{P}\right) \right|^2 = r_0^2 [J_n(2\pi R r_0)]^2 \quad (16.19)$$





Diffraction pattern shows a series of layer lines with maxima corresponding to $J_n(2\pi R r_o)$ (Bessel Functions)



Brick decorations at the Winnetka train station North Suburbs

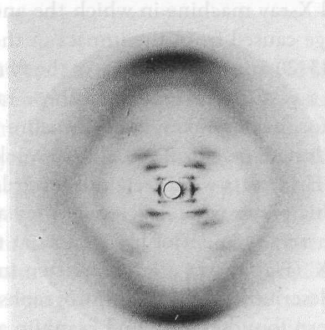
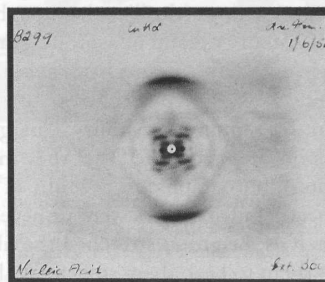
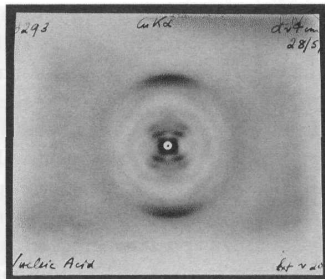
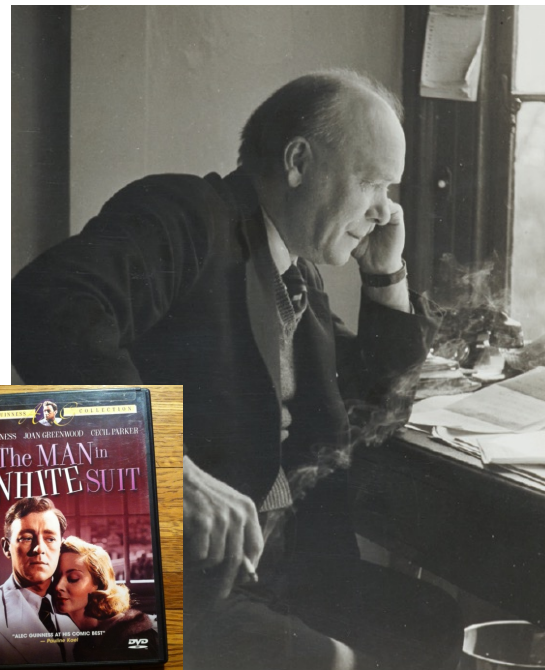
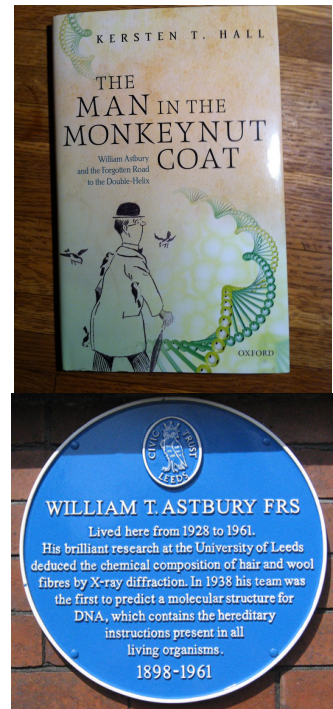


Figure 20 X-ray diffraction photographs taken by Elwyn Beighton in Astbury's laboratory of 'B'-form sodium thymonucleate on 28 May 1951 (a) and 1 June 1951 (b). A striking feature of both patterns is the same central black cross pattern seen in 'Photo 51' taken by Rosalind Franklin a year later (c). (a, b) Astbury Papers, MS419 Box C.7, University of Leeds Special Collections Brotherton Library. Reproduced with the kind permission of the University of Leeds, Brotherton Library Special Collections. (c) Reproduced with the kind permission of King's College Archive.



lography to unravel the changeable structures of proteins.



William T. Astbury (1898-1961).

Disciple of Bragg with J. D. Bernal.

Prof. of Structural Biology at Leeds next to textile industry of Northern England.

Worked with fiber related proteins (keratin) and biological materials (DNA) (see left).

Produced first diffraction patterns but failed to appreciate the importance of water/solvent in their structure. Helical diffraction theory not published yet.

Critical observations of R.E. Franklin as found and discussed by A. Klug in her notebooks. Summary of her work at King's College (London).

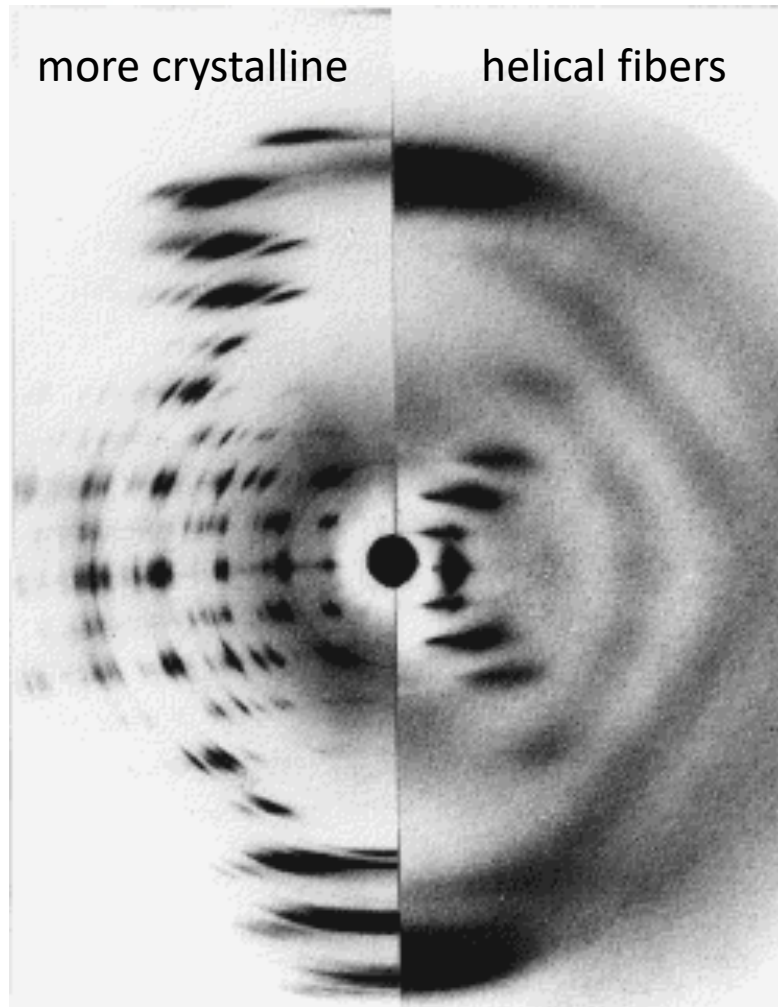
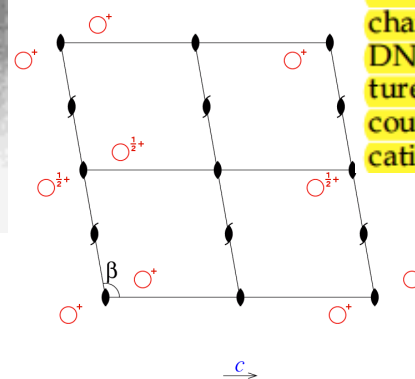
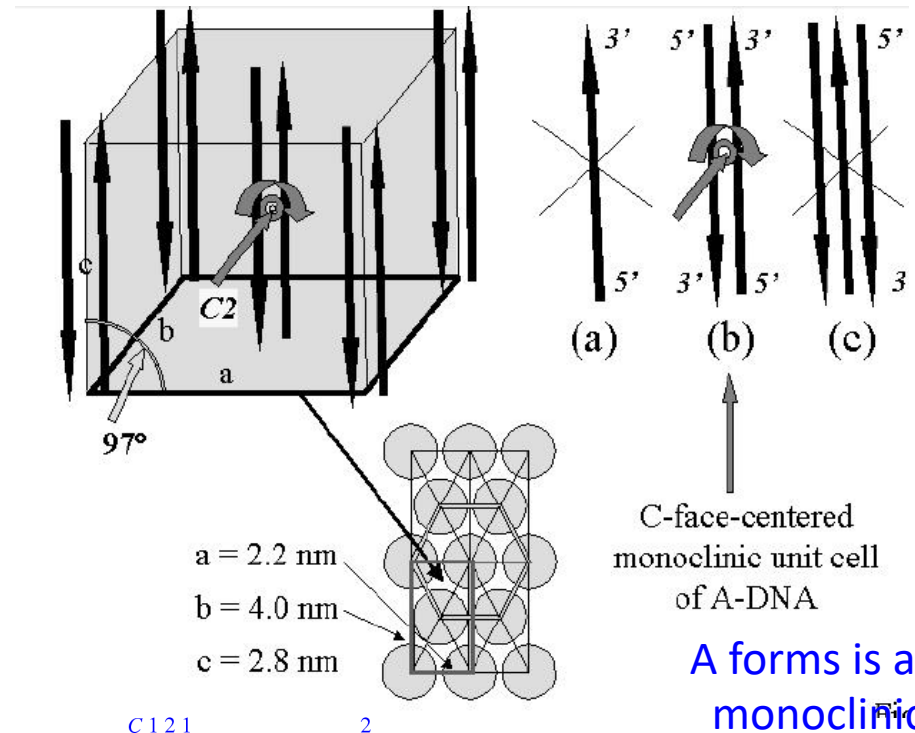


Figure 15. X-ray diffraction pattern of the A form of DNA (Franklin and Gosling, *Acta Crystallog.* 1953, 6, 673, Figure 1.

Figure 16. X-ray diffraction pattern of the B form DNA (Franklin and Gosling, *Acta Crystallog.* 1953, 6, 673, Figure 4; The B form pattern is that reproduced Franklin and Gosling *Nature* 25 April 1953, 171, 7 also known as photograph B51.



chains. (Franklin hardly ever reminisced about DNA in the years I worked with her on virus structure at Birkbeck College, but she once said that she could have kicked herself for missing the implications of the C2 symmetry).

Klug, A. J. *Mol. Biol.* (2004) 335, 3–26

Historical Perspective on the DNA Structure

Simulation of DNA fiber diffraction with laser and helical spring

How Rosalind Franklin Discovered the Helical Structure of DNA: Experiments in Diffraction

Gregory Braun, Dennis Tierney, and Heidrun Schmitzer, Xavier University, Cincinnati, OH

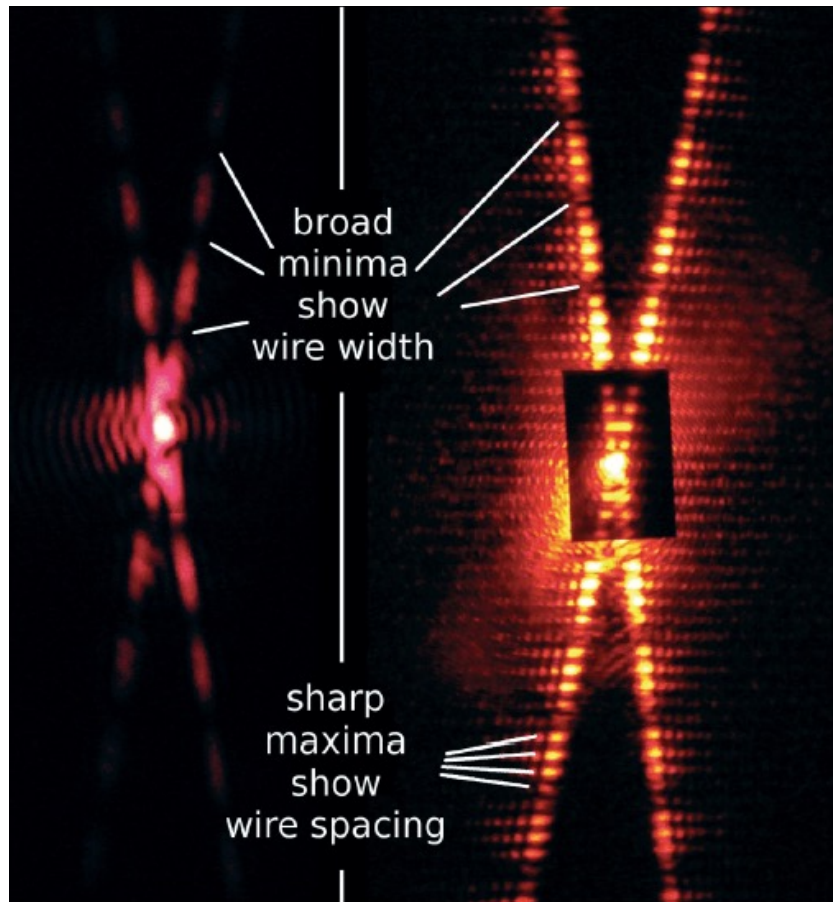
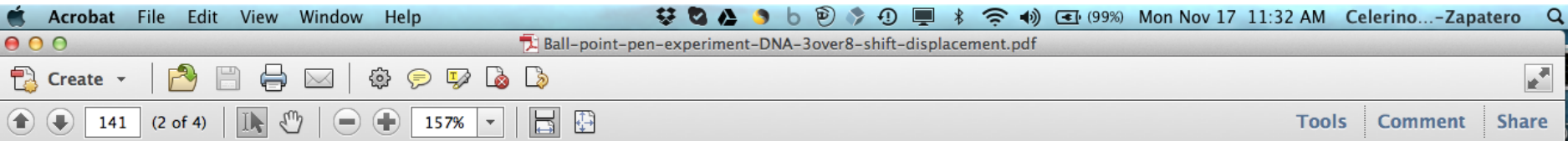
Rosalind Franklin, a chemical physicist (1920-1958), used x-ray diffraction to determine the structure of DNA. What exactly could she read out from her x-ray pattern, shown in Fig. 1?¹ In lecture notes dated November 1951, R. Franklin wrote the following: "The results suggest a helical structure (which must be very closely packed) containing 2, 3 or 4 co-axial nucleic acid chains per helical unit, and having the phosphate groups near the outside."² This was 16 months before J. D. Watson and F. Crick published their description of DNA, which was based on R. Franklin's x-ray photos. How they gained access to her x-ray photos is a fascinating tale of clashing personalities and male chauvinism.^{2,3}

In this paper we suggest four experiments that enable students to follow in the footsteps of Rosalind Franklin's dis-

Experiment 1: Why does the x-shaped pattern reveal the helical structure?

This is a variation on the single-slit experiment with readily available equipment, allowing the student to both see a diffraction pattern similar to Franklin's and to determine the pitch angle α of the helix, as in Fig. 2. If you take the spring from a typical retractable pen and place it on an overhead projector, you observe that the projection of a helix is a sine wave pattern (Fig. 2). We place a laser pointer⁶ or a diode laser (from PASCO) on one end of the lab table and direct the beam (diameter ~ 2 mm) through the side of the spring. (The distance between the laser and spring does not matter.) Holding a white card a couple of centimeters past the spring shows the projection is indeed a sinusoidal pattern and also

Diffraction is NOT ONLY for crystals and X-rays. Simulating Rosalind Franklin's X-ray work.



Substitution in the vertical column section of the diagram above 51 (Fig. 1) will render the correct pitch angle of DNA, which is listed in Table I.

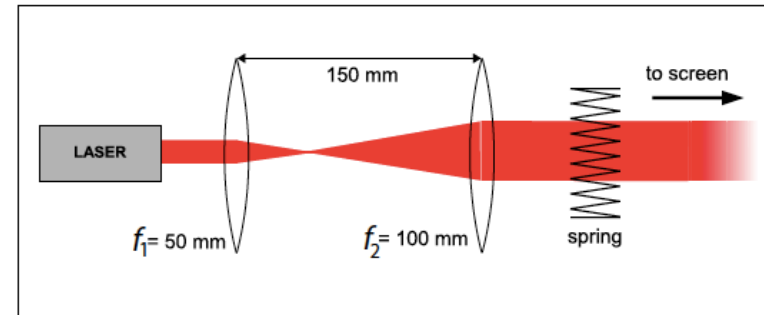


Fig. 3. We can expand the small laser beam by using two converging lenses, with the distance between them equal to the sum of their focal points. Increasing this distance results in converging light instead of a plane wave and shortens the distance needed to produce the far-field diffraction pattern.

wavelength of our sine function. A look at Fig. 3 reveals that the pitch P is connected to the distance d between multiple parallel wires (or equivalently multiple parallel slits) by:

$$P = \frac{d}{\cos \alpha}. \quad (2)$$

We see why we need to illuminate more than one pitch to deduce the distance d between wires from a multiple-wire diffraction pattern. The maxima in the diffraction pattern occur at angles $\theta_{\min}$ according to the equation⁷

$$d \sin \theta_{\max} = m \lambda. \quad (3)$$

There is a very 'tell-telling' absence in the B-DNA (photo 51) pattern that was correctly interpreted by R. E. Franklin.

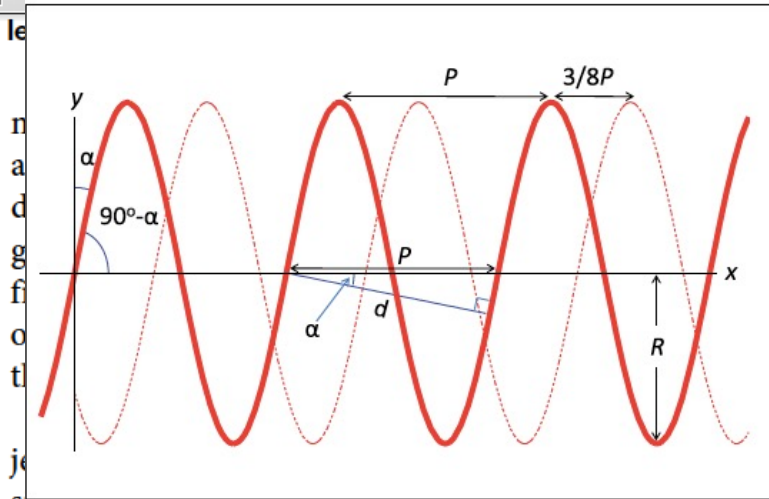
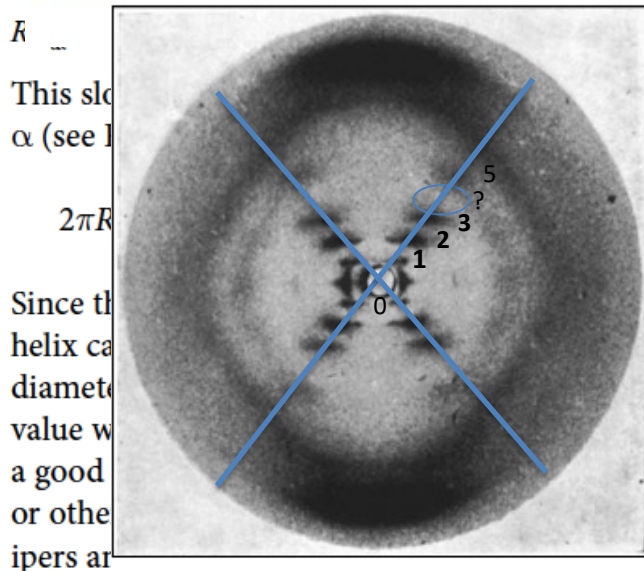


Fig. 5. The relationship among d , P , and α can be seen here, as well as that between α and the slope of the sine function.

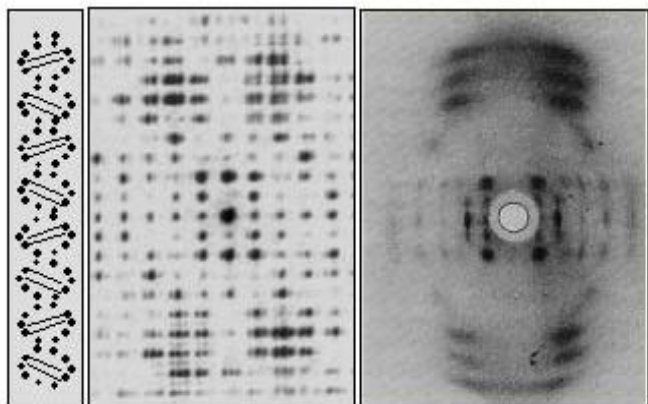


plement of
Photo 51
radius of the
measure the
compare this
action. This is
eter of DNA
ured with cal-
diffraction.

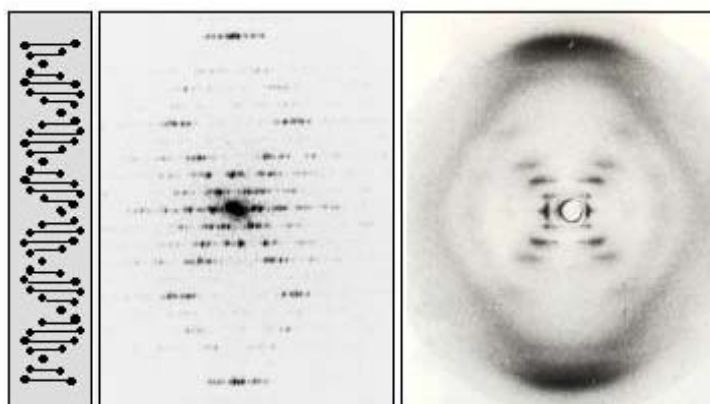
determine the angle, pitch, and radius of the DNA molecule. The zero-order and the first-order maxima had been blocked by a lead disk because they would have overexposed the film. So the order closest to the hole in the center of *Photo 51* is the second order. We used the second, third, and fifth order to calculate the pitch according to Eqs. (2) and (3). Rosalind Franklin correctly attributed the missing fourth order in the diffraction pattern to a second helix, the double helix (see Fig. 5), which is offset by $3/8$ of the helical pitch:¹ "The structural unit probably consists of two co-axial molecules which are not equally spaced along the fibre axis, ... If one molecule is displaced from the other by about three-eighths of the fibre-axis period, this would account for the absence of the fourth layer line maxima and the weakness of the sixth." The fourth maximum of the pattern due to one helix occurs precisely at the second-order minimum of the pattern due to the spacing between the helices, similarly to how bright spots from the double-slit pattern do not appear when they occur at a minimum of the single-slit pattern. The patterns from each helix are out of phase with each other at this point, and so no light appears. This can be verified by the student with the equation $n \lambda = d \sin \theta$, by showing that the $n = 4$ maximum of the full pitch pattern is at the same angle as the $n = 1.5$ minimum of the $3/8$ pitch pattern.

Table I gives an overview over our results for the helical spring of the retractable pen that we used and the DNA.

OPTICAL SIMULATIONS OF A & B FORMS



P11



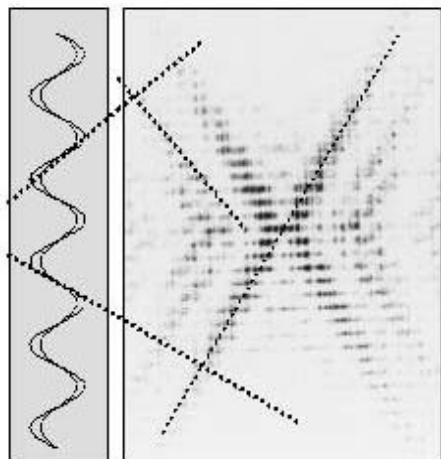
P12

OPTICAL SIMULATIONS OF INTER-CHAIN DISPLACEMENTS:

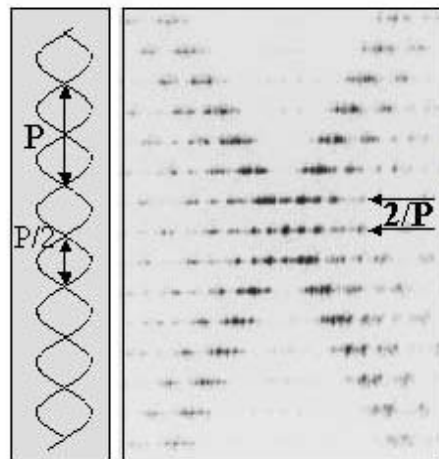
$$\Delta = P/2$$

$$\Delta = 3/8 P$$

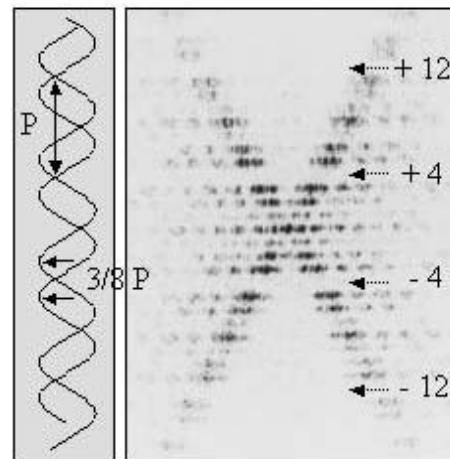
4TH Layer line
Reflection extinction



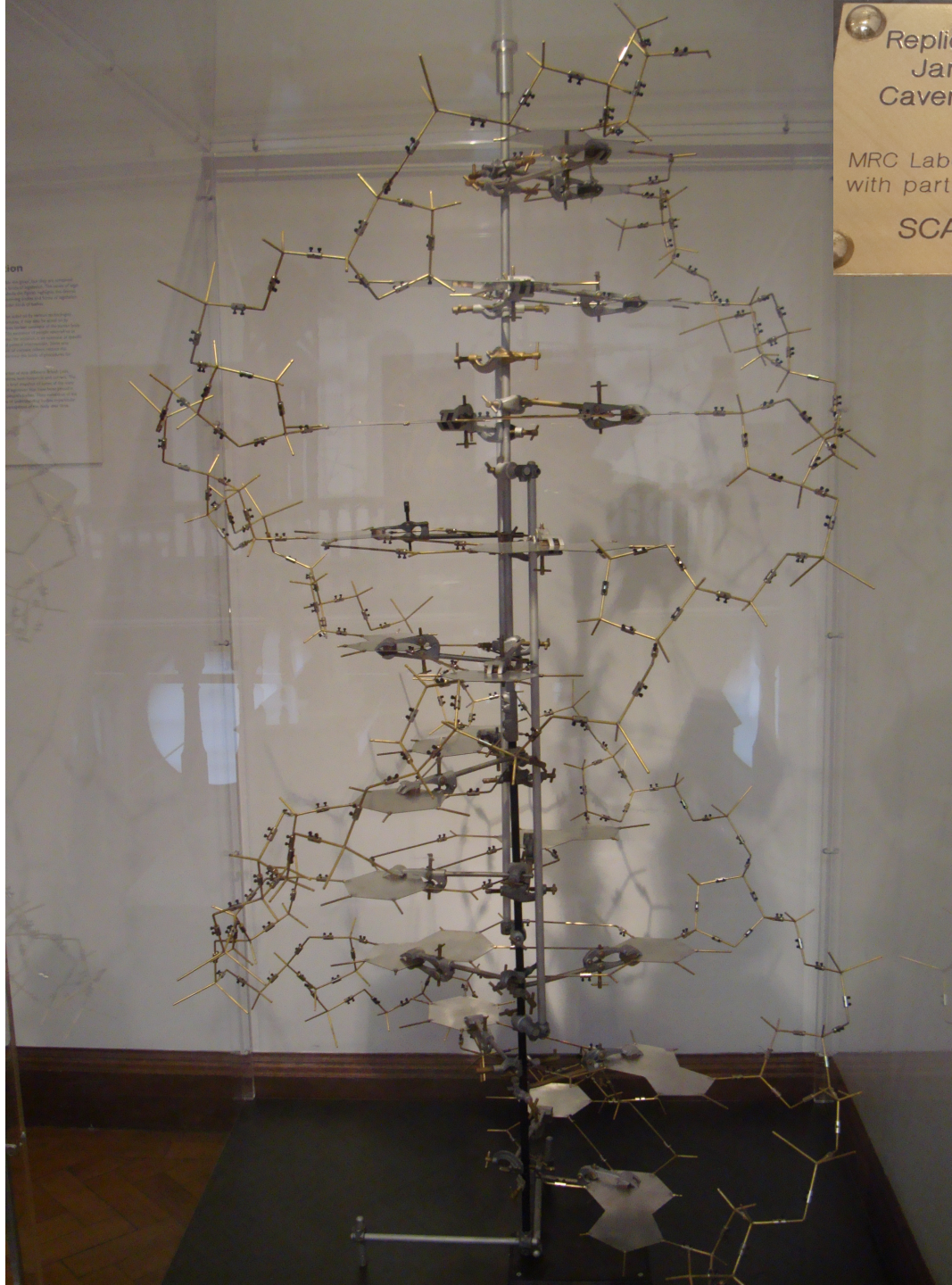
P7



P8



P9

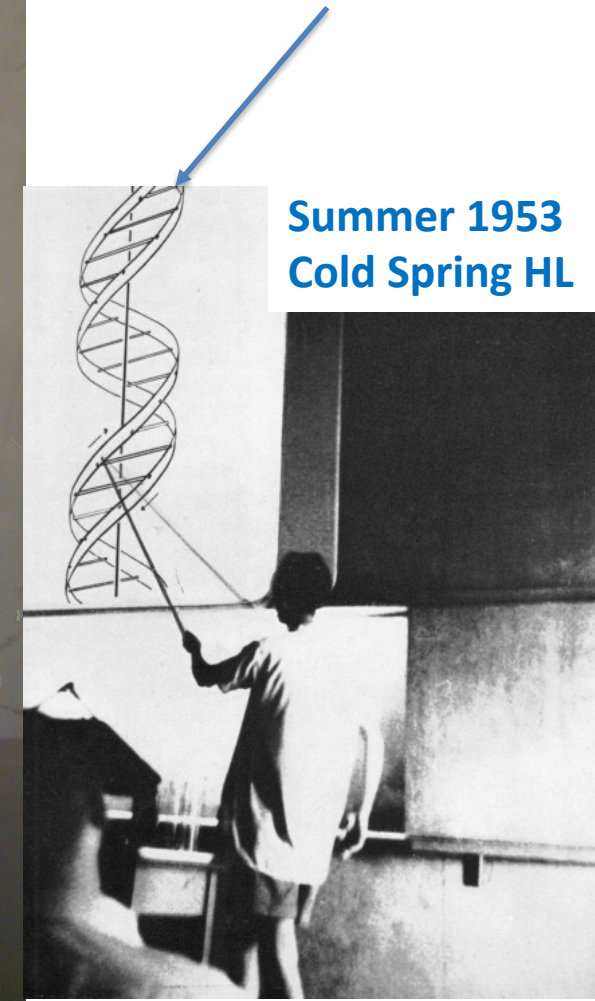


Replica of the structure of DNA built by
James Watson and Francis Crick,
Cavendish Laboratory, Cambridge, 1953.

Model constructed in the
MRC Laboratory of Molecular Biology workshop, 2002,
with parts from Cambridge Repetition Engineers Ltd.

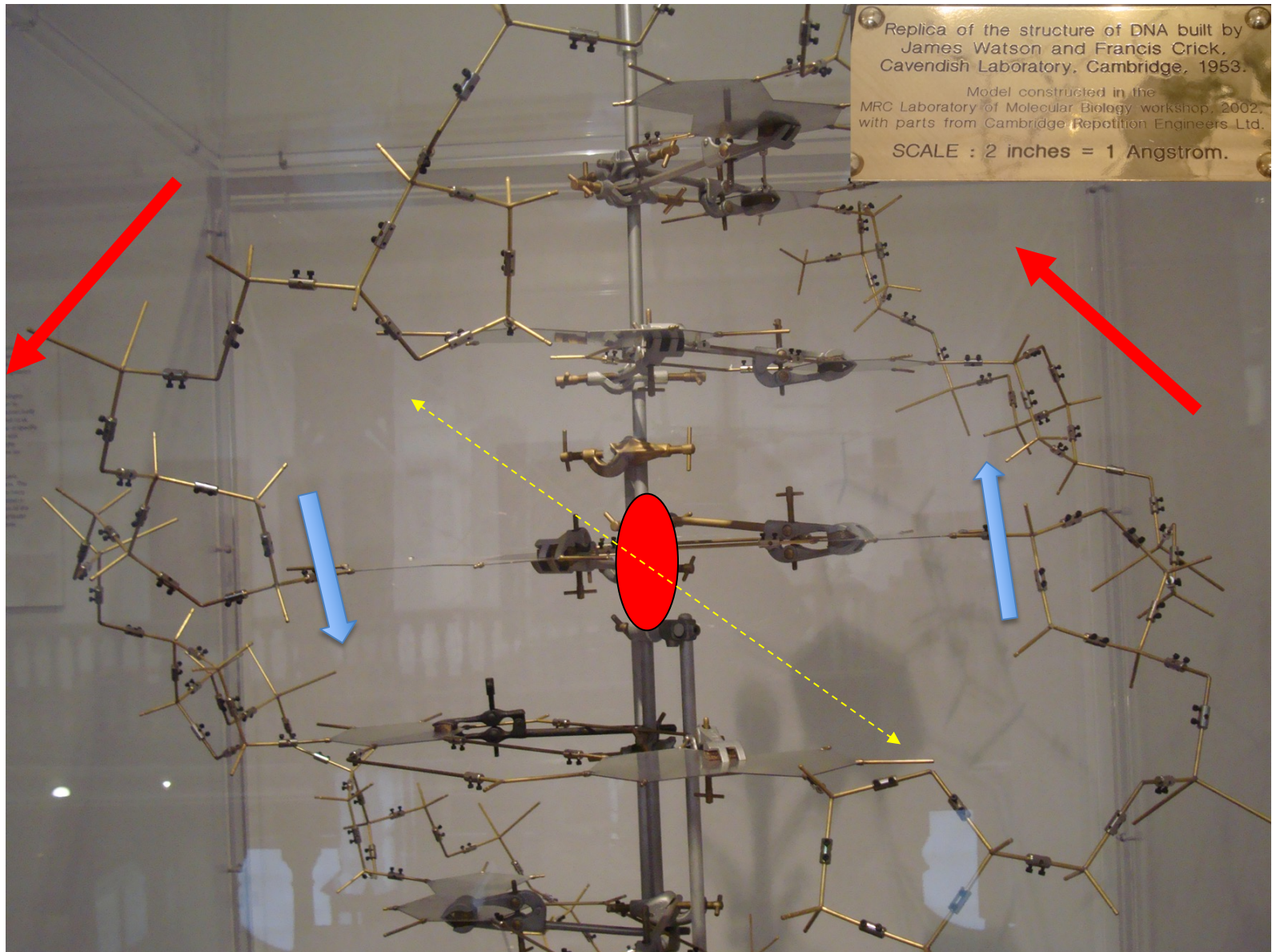
SCALE : 2 inches = 1 Angstrom.

Schematic representation
drawn by Odile Crick



Summer 1953
Cold Spring HL

Key feature of DNA structure is that the two chains are 'antiparallel'



What did/did not Rosalind Franklin know about the DNA structure that would have allowed her build the correct model?

(from her notebooks as revealed by her closest associate Aaron Klug)

Did know

Helical parameters
(pitch, angle, radius,
P-P distance)

Two chains

Relative displacement
of the chains (3/8 coaxial)

Phosphates outside

Did not know

Chains were antiparallel
(had the crystal data but
did not realize implications)

Base pairing G-C, A-T

After the Double Helix

Rosalind Franklin's Research on *Tobacco mosaic virus*

By Angela N. H. Creager and Gregory J. Morgan***

ABSTRACT

Rosalind Franklin is best known for her informative X-ray diffraction patterns of DNA that provided vital clues for James Watson and Francis Crick's double-stranded helical model. Her scientific career did not end when she left the DNA work at King's College, however. In 1953 Franklin moved to J. D. Bernal's crystallography laboratory at Birkbeck College, where she shifted her focus to the three-dimensional structure of viruses, obtaining diffraction patterns of *Tobacco mosaic virus* (TMV) of unprecedented detail and clarity. During the next five years, while making significant headway on the structural determination of TMV, Franklin maintained an active correspondence with both Watson and Crick, who were also studying aspects of virus structure. Developments in TMV research during the 1950s illustrate the connections in the emerging field of molecular biology between structural studies of nucleic acids and of proteins and viruses. They also reveal how the protagonists of the "race for the double helix" continued to interact personally and professionally during the years when Watson and Crick's model for the double-helical structure of DNA was debated and confirmed.

This 'personal and professional' interaction was made possible by the fact that R.E. Franklin never knew (or even suspected) to what extent her diffraction data and results on the A, B DNA forms were given to Watson & Crick without her permission/approval

Events related to the origins of Structural/Molecular Biology III

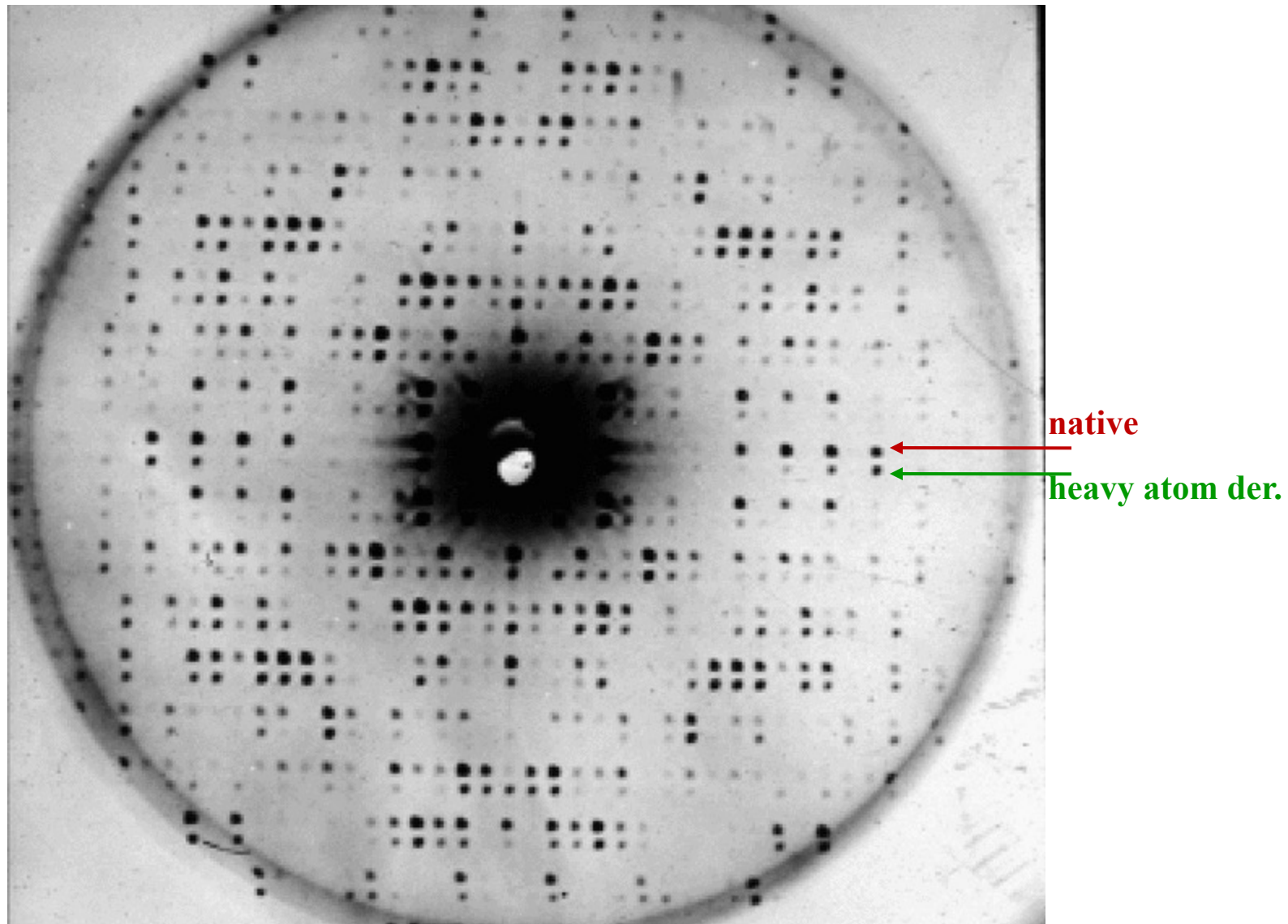
- ✓ Structural work on TMV: Watson*, Franklin, Klug, Holmes (Birkbeck College Group). Group leader.
 - ✓ Isomorphous Replacement Method (1954): Green, Ingram, Perutz.
 - ✓ Isomorphous Replacement applied to TMV (1957): Franklin & Holmes.
 - ✓ Structure of TMV (1958), Brussels exhibition.
 - ✓ Structure of the first proteins: myoglobin, hemoglobin (1959) Kendrew, Perutz.
- (Structural Biology/Molecular Biology established)

The Frontier of Structural/Molecular Biology: (1953-1959) Structure of TMV

worked during the day, and Franklin was impressed with their dedication. After the move to Birkbeck, she began her celebrated work with J. Desmond Bernal on RNA viruses like tobacco mosaic virus (TMV). She was a cautious scientist who began to trust her intuition more as she matured (see box 1 on page 45). She published 14 papers about viruses between 1955 and 1958, and completed the research for three others that colleague Aaron Klug submitted for publication after her death.

Stanley had accepted that the particles of TMV were 'nucleoproteins' and the critical question in understanding the physico-chemical processes of life was: How do DNA/RNA interact with proteins in TMV?

The breakthrough of Protein Crystallography: Isomorphous Replacement (MIR) 1953



Two protein crystal diffraction patterns superimposed, slightly out of vertical register. One is from a native protein crystal (red), the other (green) from a similar crystal soaked in a heavy atom solution. Note the intensity changes for certain reflections. The identical unit cell dimensions (separation) imply isomorphism.

Taylor (2003) *Acta Cryst.* **D59**, 1881

APPLICATION OF THE METHOD OF MULTIPLE ISOMORPHOUS REPLACEMENT TO TMV

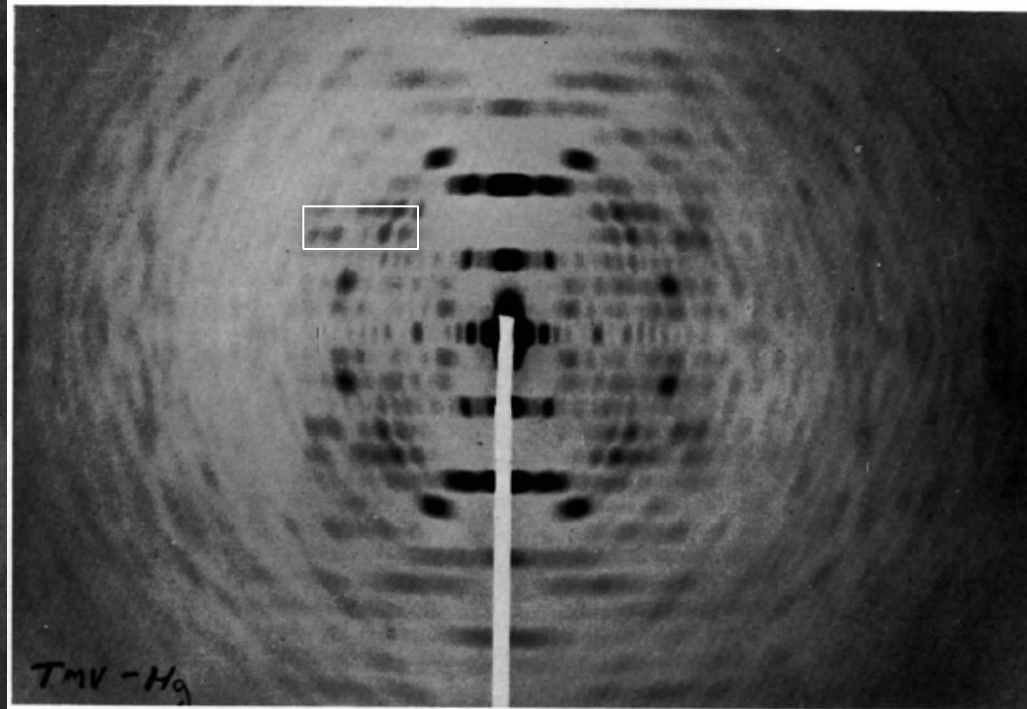
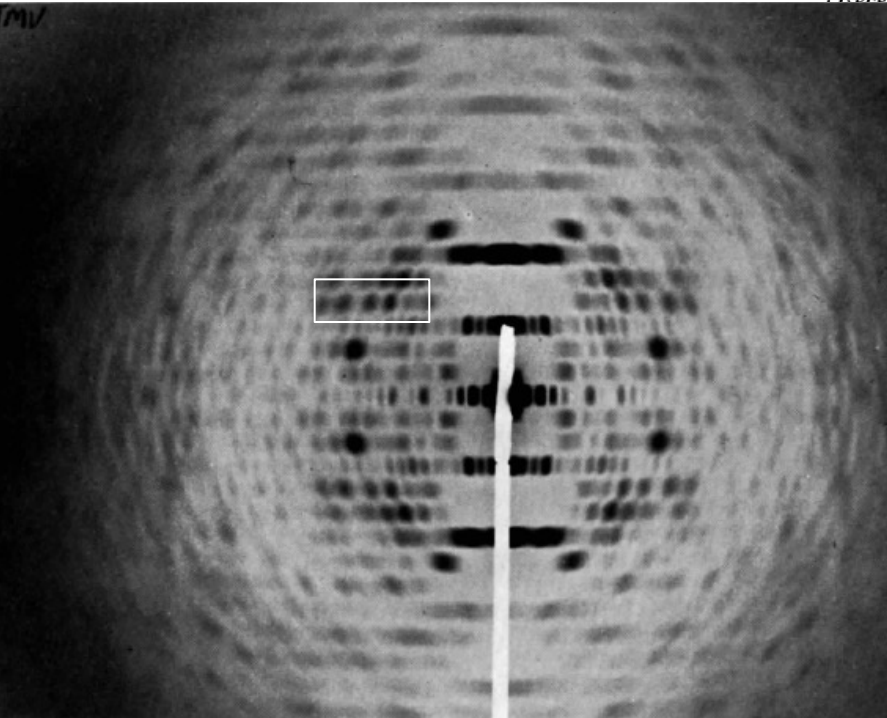
Acta Cryst. (1958). **11**, 213

Tobacco Mosaic Virus: Application of the Method of Isomorphous Replacement to the Determination of the Helical Parameters and Radial Density Distribution

BY ROSALIND E. FRANKLIN AND K. C. HOLMES

Birkbeck College Crystallography Laboratory (University of London), 21 Torrington Square, London W.C. 1, England

(Received 8 August 1957)



unit.

HELICAL PARAMETER: $J_n = (m u + l)/t$.
 u = number of subunits axial repeat.
 t = number of turns of helix per axial rep.
 P = Pitch of the helix. $u = 69 \text{ \AA}$.

Thus the TMV structure belongs to the simplest helical line-group, s , and contains 49 protein sub-units on 3 turns of the helix in the axial repeat period of $69 \text{ \AA}$. There are $16 \frac{1}{3}$ proteins chains/turn

RADIAL ELECTRON DENSITY DISTRIBUTION IN THE TMV PARTICLE AT 10 Å RESOLUTION

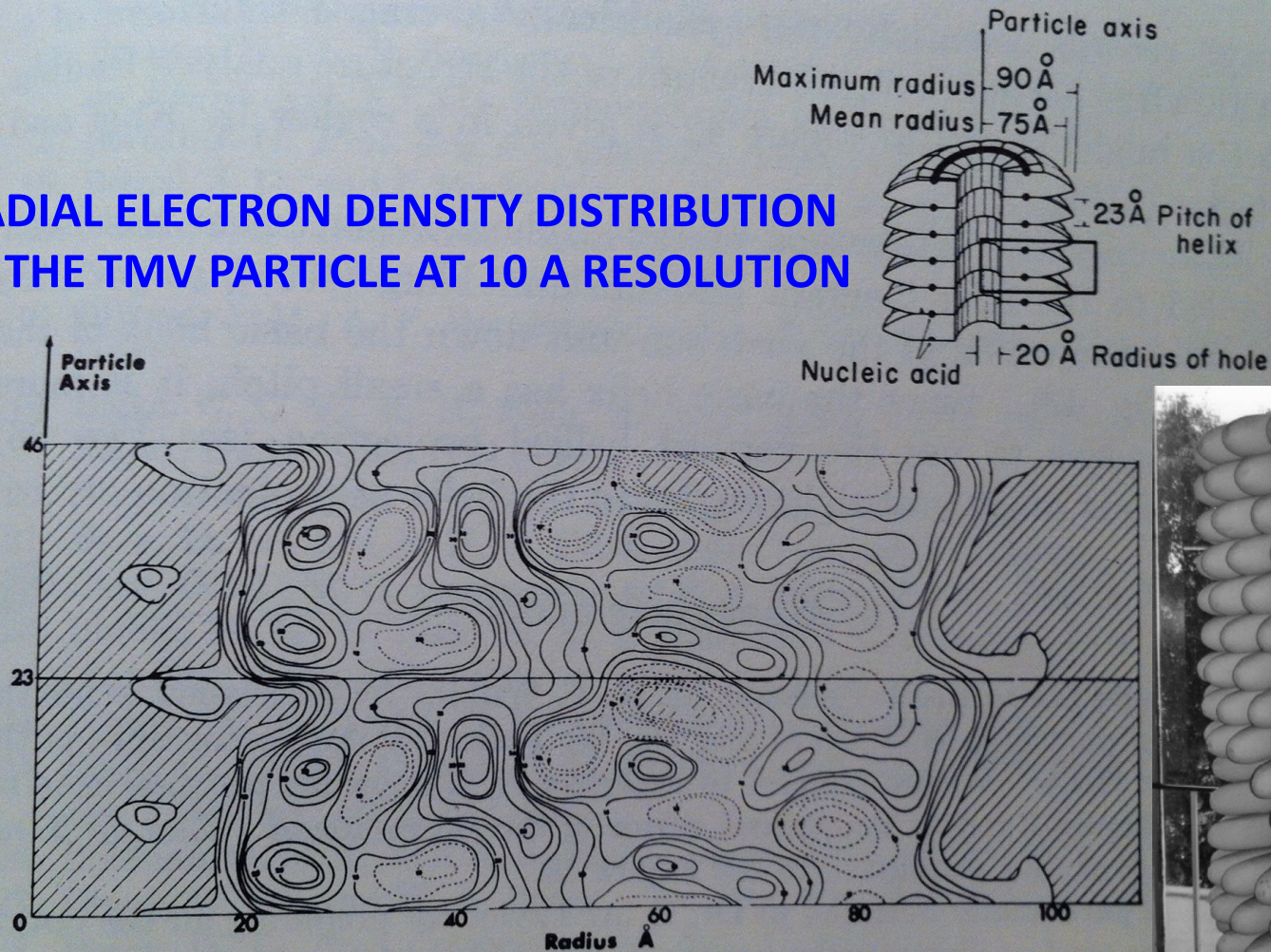


Fig. 43. A helical projection down the basic helix of TMV, at a resolution of 10 Å. Two repeats are shown corresponding to the area marked with a heavy line on the small upper diagram. On the electron density map the shaded areas represent a density of water. Density above this level is unshaded. Valleys in the electron density are shown with broken lines.

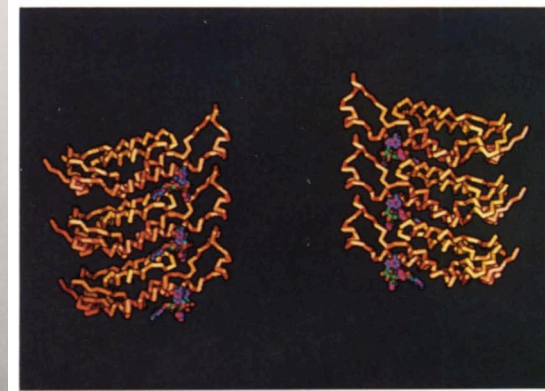
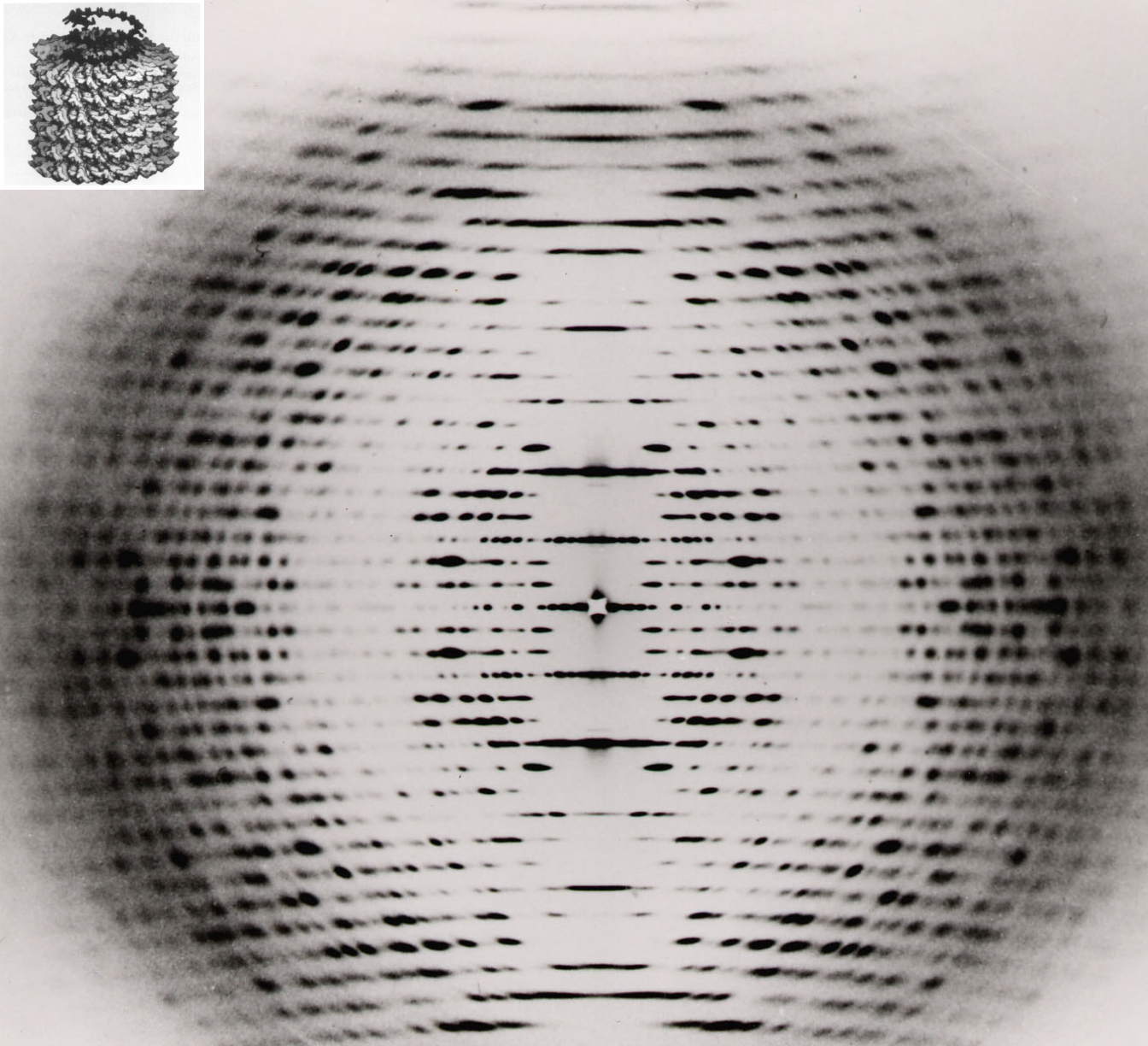
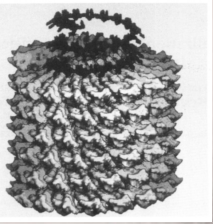


Fig. 6.

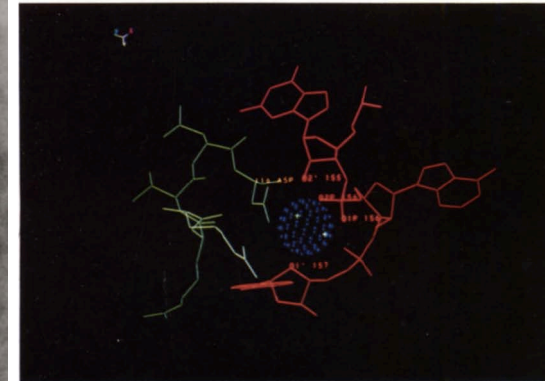
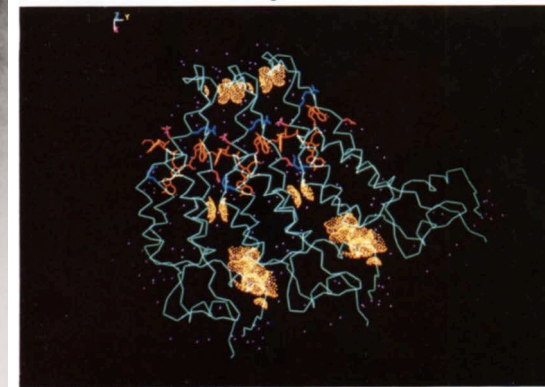


Fig. 10.



u = 49 subunits

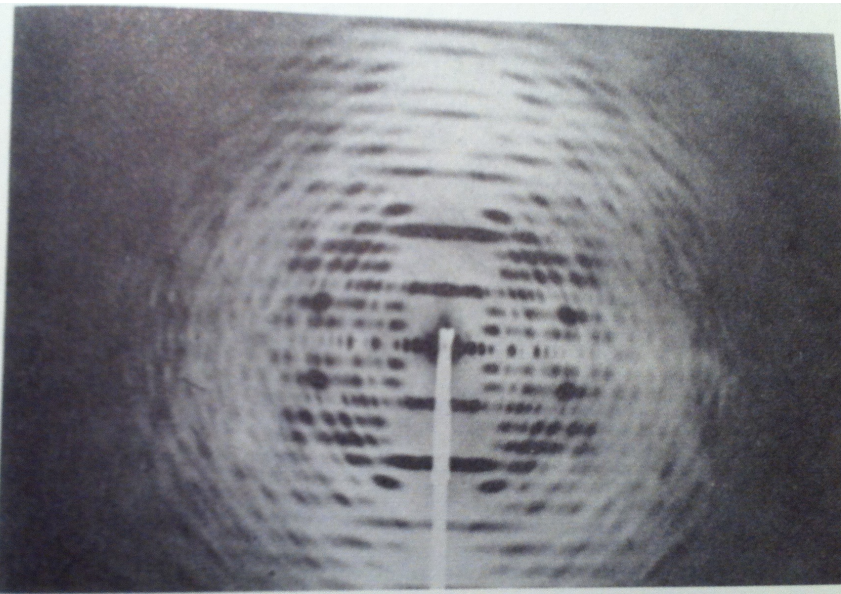
t = 3

P = 69 Å

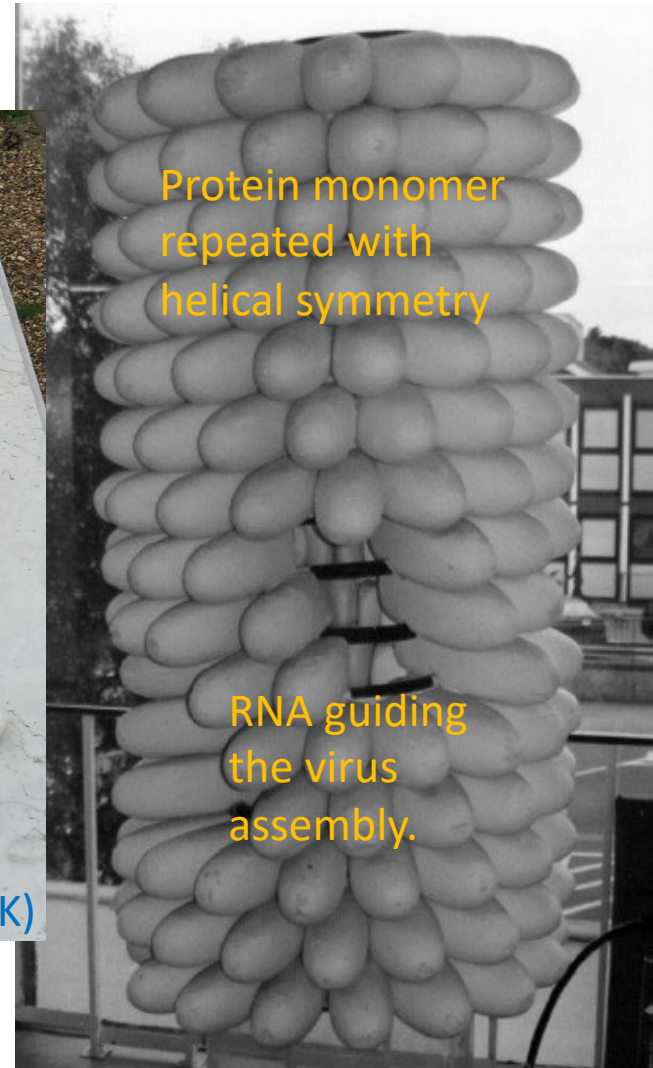
16 1/3 chains/turn

Namba, K., Patanayek, R. and Stubbs, G. (1989).
Visualization of protein-nucleic acid interactions in a virus.
J. Mol. Biol. 208, 307-325. (Structure at 2.9 Å resolution)

R.E. Franklin (1920-1958): her Legacy.



R.E. Franklin grave (UK)



Protein monomer
repeated with
helical symmetry

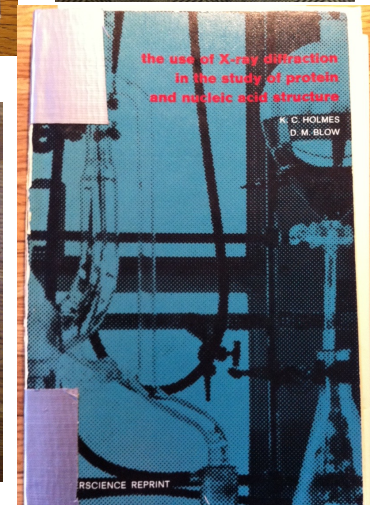
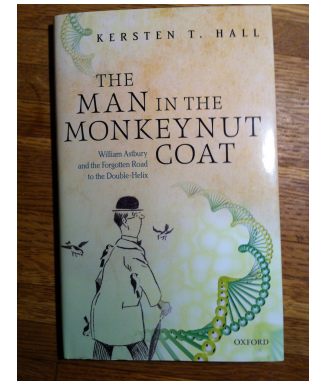
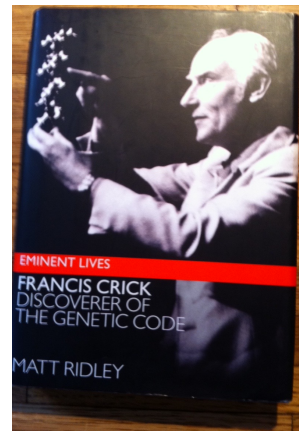
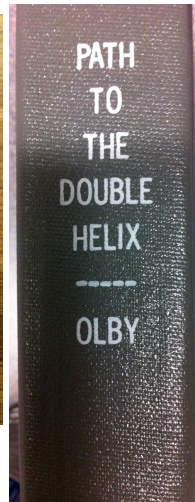
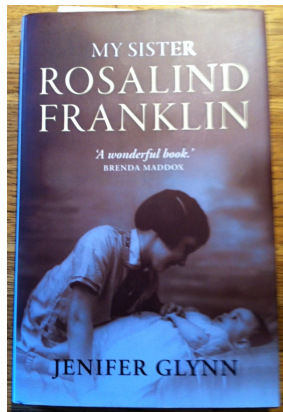
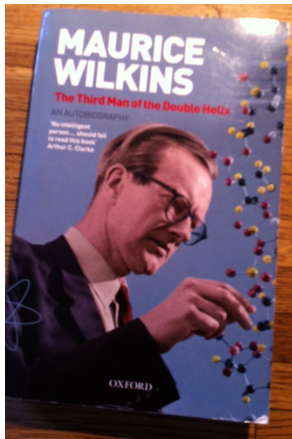
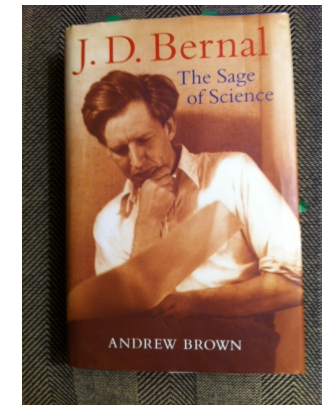
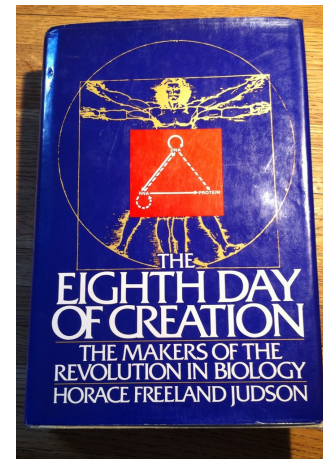
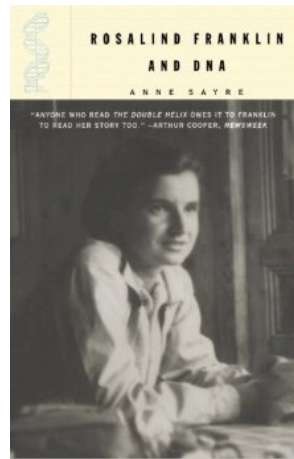
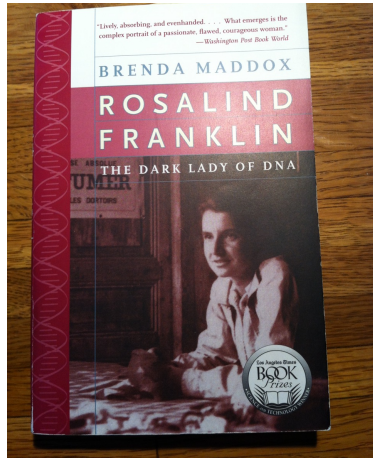
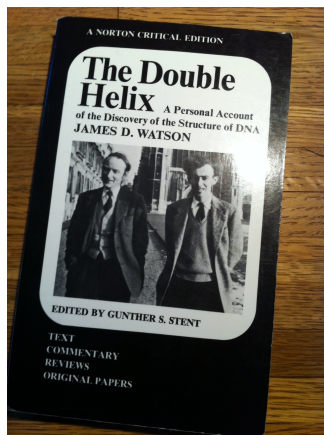
RNA guiding
the virus
assembly.

Gregory, J. and Holmes, K.C. (1965). Methods of preparing oriented tobacco mosaic virus gels for x-ray diffraction. J. Mol. Biol. 13, 769-801.

25TH JULY 1920 - 16TH APRIL 1958 / SCIENTIST
'HER RESEARCH AND DISCOVERIES ON VIRUSES
(AND DNA!) REMAIN OF LASTING BENEFIT
TO MANKIND.' (World Heritage Registry, 2017).

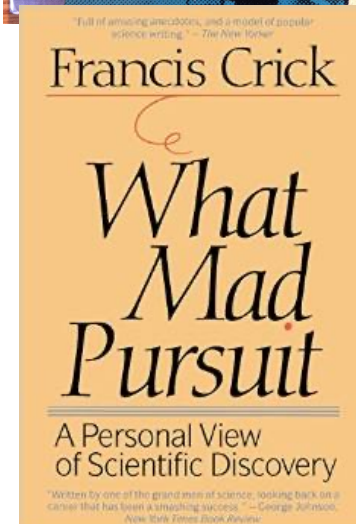
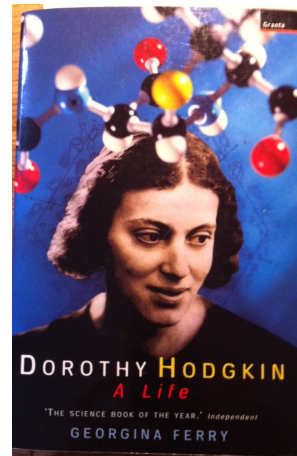
Model of TMV created by
R.E. Franklin using her latest
results and exhibited at
the 1958 World
Exposition in Brussels.
She had already passed away.

Further reading



Rosalind Franklin and the Double Helix

Although she made essential contributions toward elucidating the structure of DNA, Rosalind Franklin is known to many only as seen through the distorting lens of James Watson's book, *The Double Helix*.



L. O. Elkin. Physics Today (2003).

Thank you very much for your attention and attendance to this lecture.

Questions?

**If you can, read some of the books suggested.
I strongly recommend it!!**

Content of the seminar has been essentially posted as an essay at my website included as a pdf document:

[https://www. caz.crystaledges.org](https://www.caz.crystaledges.org) . There are many more things. (See 'News & Views' and 'Blog' tabs).

A more mathematical proof of the extinction of the l=4 layer line reflection.

The total scattering amplitude of the two identical coaxial helices has the form:

$$A(q)[1 + \exp(iq_z \Delta z)];$$

$A(q)$ is the amplitude of one single helix;

Δz is the axial distance between the two backbones.

$q_z = 2\pi/P$; reciprocal coordinate of the l layer line as a function of P (Pitch).

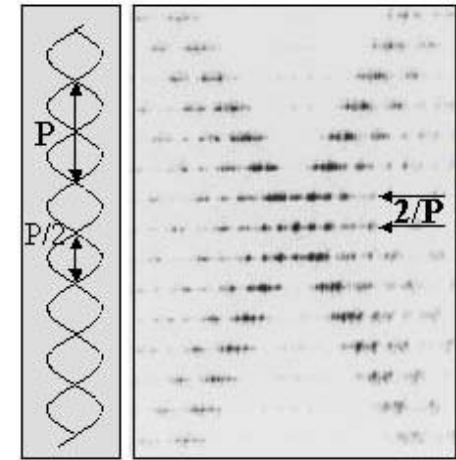
$$(*) \quad 1 + \exp(i\pi) = 1 + e^{i\pi} = 0;$$

‘The most beautiful formula of Mathematics’; combines five mathematical constants:

$$1, 0, e, i, \pi.$$

$$1 + \cos(\pi) + i \cdot \sin(\pi) = 1 - 1 + 0 = 0.$$

$$\Delta z = P/2$$



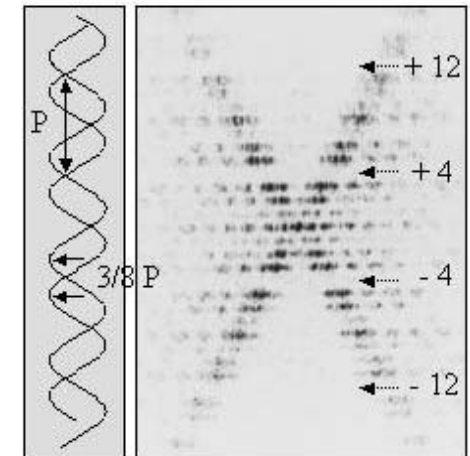
P8

$$A(q)[1 + \exp(i \cdot 2\pi l / P \cdot P/2)] =$$

$$A(q)[1 + \exp(i \cdot \pi l)] = 0 (*)$$

(all odd layer lines extinct)

$$\Delta z = 3 P/8$$



P9

$$A(q)[1 + \exp(i \cdot 2\pi l / P \cdot 3 \cdot P/8)] =$$

$$A(q)[1 + \exp(i \cdot \pi l \cdot 3/4)] = 0 (*)$$

(l=4, fourth layer line extinct)