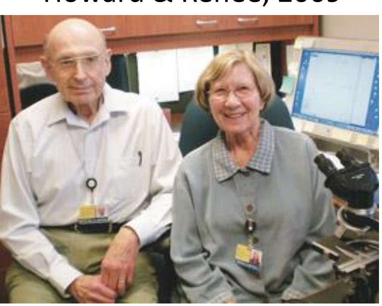
Protein Chemistry and Biosynthesis

Dintzis - 1961

Howard

1928-2024

Howard & Renée, 2009



1

Letter from Matthew Meselson to Salvador Luria (18/january/1958)

(the Meselson-Stahl paper was sent to PNAS on may/14/1958)

File : Meselson

CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

18. January '58

Dear Lu,

Please pardon this long delay in answering your letter. I've been traveling since before Christmas. Last week I visited Washington University and was quite impressed with the present performance of Kornberg's system. Howard Schactman has been there and has found that the synthesized DNA resembles the primer in regard to sedimentation constant and(more significantly) intrinsic viscosity. hen sonicated primer is used, the product DNA seems to be shortened accordingly although this last result is preliminery according to Howard. The system makes 10x increase in DNA over primer, but if T.P. is used as primer the transforming activity goes neither up nor down. However, if any one of the nucleotide triphosphates is omited, the transforming activity is nearly completely eliminated in the experimental time period. They blame the inability to make net increase of T.P. on nucleases known to be present and are accordingly seting about the preparation of really pure enzyme from several hundred pounds of Coli! The system puts in deoxy-UTP as well as thymine TP and deoxy-inosine TP for GTP in accord with WC pairing expectations. Ribonucleotides won't go in. The base composition of DNA made with Coli primer resembles Coli DNA while T2 primer makes T2-like product DNA. The system seems to be well enough established to justify using heavy nitrogen DNA for primer and looking for half-heavy molecules in CsCl gradient. I'll make heavy T4 DNA for them and perhaps centrifuge it too if they don't prefer to do it there.

HMC?

We have not done any new centrifuging(not even to repeat the transfer experiment) for two reasons. First, we're all out of CsCl

CALIFORNIA INSTITUTE OF TECHNOLOGY
PASADENA, CALIFORNIA

and our old supplier has vanished. (He does not answer letters and his 'phone in Phila. has been disconnected.) We have contracted with a local firm to make it for us but they won't have any for perhaps another month. The second difficulty Frank and I have had is that we have almost no time on the centrifuge schedules. We had tied up a machine for almost a year and now Dintzis, Vinograd, and Sinsheimer quite understandably want to get on with their own work. Our long equilibrium runs require a machine of our own. Accordingly Max has very quickly arranged for us to buy one to be kept in the phage group. It will be here in about a month. When we're going again, we'd be glad and interested to run P1 and the antigenicity mutants too. I hope we could be ready within two months..we will be if there are no snags in setting up the machine.

To make runs yourself, choose a CsCl solution of density equal to that of the phage under the assumption that the density of the phage is the arithmetic average of the protein(1.3) and the DNA(1.70) densities weighted according to the per cent DNA in a phage. Buffer at pH 7 with 0.01 M phosphate. In the first runs put in enough phage so the OD at 260A of the starting solution is about 0.4. With this much phage you will see which way they move incase the band is off scale. You can tell how much to shift the density of the starting solution to put the band in the middle with the aid of the approximate relation density gradient = $8x10^{-10}$ w^2r with w in radians per second and r, the distance in cm from the center of rotation. I suggest a speed of 30-40 thousand rpm for exploratory runs. The CsCl equilibrium takes about 7 hours and with molecules as big as P1 two or three hours more will be enough to get quite near equilibrium at the above speeds. At lower speeds, the CsCl equilibrium takes still 7 hours but the macromolecules band much more slowly. The rate of band formation goes as log w^2 over w^1. The ratio of separation between two bands to their half-width is speed independent so resolution is the same at any speed. Good luck,

Matt

https://profiles.nlm.nih.gov/ps/access/QLBBGS.pdf

2

Letter from Matthew Meselson to Salvador Luria (18/january/1958)

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even to repeat the transfer experiment) for
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Mini Biografia de Howard M. Dintzis

Dintzis, Biochem. *Mol. Biol. Ed.* **34:**242-246, 2006

1940s - UCLA - biochemistry classes with Bruce Merrifield (postgraduate student at the time) - NPW in 1948 (solid phase polypeptide synthesis).

1948 - graduate in chemistry – begins PhD in Biophysics at Harvard with Edwin Cohn (1892-1953) - fractionation and characterization of human plasma proteins.

1951 - attends seminar by Fred Sanger - sequence of insulin.

attends Max Perutz seminar - structure of hemoglobin molecule.

Talks with Perutz about mercury use to resolve hemoglobin structure - Perutz invites him for a post-doc.

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1952 – finishes PhD on the dielectric properties of serum mercaptalbumin solutions - supervisor Larry Oncley

1953 - Studies on electrostatic forces between protein molecules.

1954-1956 - Works with Max Perutz at the MRC (Cambridge, UK). In the building where Rutherford worked (1900). Building closed at 17:00 and reopened at 7:00. They could enter the building to change the diffraction plates

- he stood at a table next to an interesting fellow: Francis Crick.

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- They worked on solving structures using a computer only after 20:00 until one of the valves burned!

- Visits Faraday's laboratory at the Royal Institution at the invitation of Lawrence Bragg (then president, newly elected) and sees the instruments Faraday has built for his experiments.

- Receives an invitation from Linus Pauling to go to CalTech

1956 - despite the huge teaching load accepts Pauling’s invitation and goes to the US

6

Decides to study protein biosynthesis using radioactively labeled amino acids.

- Initially uses mouse pancreas and liver but gives up both systems for what he initially imagined.

- Uses Jerome Vinograd's laboratory in the Department of Biology for initial experiments.

Vinograd's laboratory resembles the MRC; dynamic with people always willing to discuss results from other laboratories.

Among the people attending Vinograd's laboratory was a post-graduate student: Mat Meselson.

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Dintzis meets Henry Borsook in the neighboring building where Borsook studies hematopoiesis in anemic rabbits -> source of reticulocytes.

When Dintzis explained to Borsook what he wanted to do, he was perplexed that Borsook did not accept that proteins were encoded in DNA

Borsook believed that proteins were copied from preexisting molecules by a molding process.

His research slowed down when Dintzis accepts an invitation to the Department of Biology at MIT

1958 - restarts experiments in a new environment with crucial advantages.

8

- Presence of Vernon Ingram -> fingerprinting
- Presence of Michael Naughton -> worked with Fred Sanger on paper chromatography of peptides.

- ^{3}H-Leucine with high specific activity becomes available

1960 - sends the manuscript to John Edsall and publishes it in PNAS.

1961 - moves again to Johns Hopkins with Michael Naughton

1970 - shows that globin biosynthesis begins with a methionine.

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HISTORY OF PROTEIN STRUCTURE

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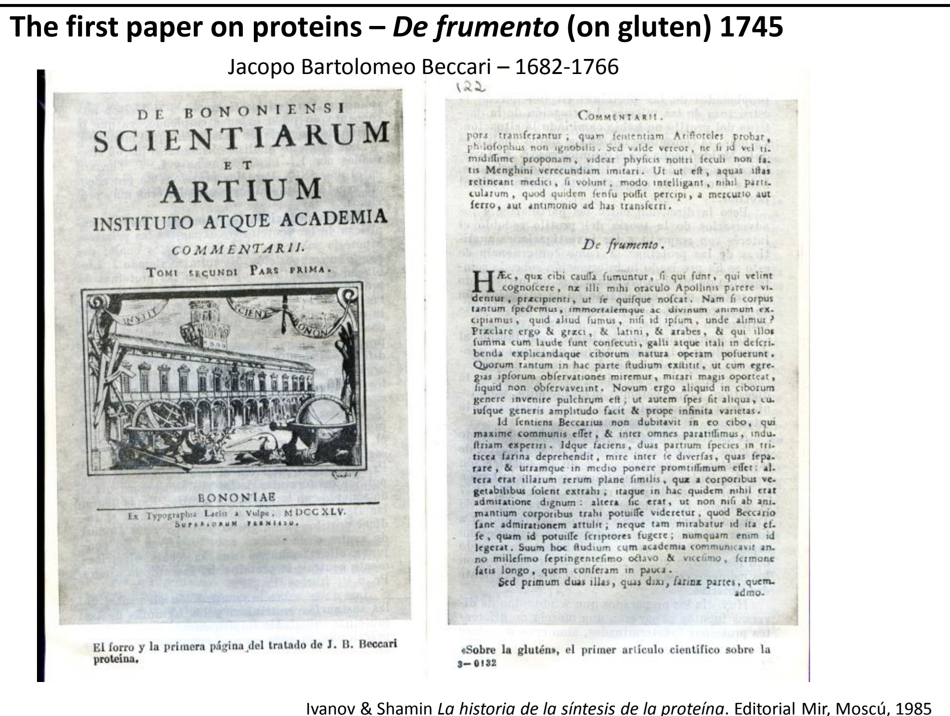

DE BONONIENSI
SCIENTIARUM
ET
ARTIUM
INSTITUTO ATQUE ACADEMIA
COMMENTARII.
TOMI SECUNDI PARS PRIMA.

BONONIAE
Ex Typographia Lælii a Vulpe. MDCCXLV.
SUPERIORUM PERMISSU.

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COMMENTARII.

poræ transferantur; quam ſententiam Ariſtoteles probat, philoſophus non ignobilis. Sed valde vereor, ne ſi id vel timidiſſime proponam, videar phyſicis noſtri ſeculi non ſatis Menghini verecundiam imitari. Ut ut eſt, aquas iſtas retineant medici, ſi volunt; modo intelligant, nihil particularum, quod quidem ſenſu poſſit percipi, a mercurio aut ferro, aut antimonio ad has transferri.

De frumento.

HÆc, quæ cibi cauſſa ſumuntur, ſi qui ſunt, qui velint cognoſcere, næ illi mihi oraculo Apollinis parere videntur, præcipienti, ut ſe quiſque noſcat. Nam ſi corpus tantum ſpectemus, immortalemque ac divinum animum excipiamus, quid aliud ſumus, niſi id ipſum, unde alimur? Præclare ergo & græci, & latini, & arabes, & qui illos ſumma cum laude ſunt conſecuti, galli atque itali in deſcribenda explicandaque ciborum natura operam poſuerunt. Quorum tantum in hac parte ſtudium extitit, ut cum egregias ipſorum obſervationes miremur, mirari magis oporteat, ſiquid non obſervaverint. Novum ergo aliquid in ciborum genere invenire pulchrum eſt; ut autem ſpes ſit aliqua, cujuſque generis amplitudo facit & prope infinita varietas.

Id ſentiens Beccarius non dubitavit in eo cibo, qui maxime communis eſſet, & inter omnes paratiſſimus, induſtriam experiri. Idque faciens, duas partium ſpecies in triticea farina deprehendit, mire inter ſe diverſas, quas ſeparare, & utramque in medio ponere promtiſſimum eſſet: altera erat illarum rerum plane ſimilis, quæ a corporibus vegetabilibus ſolent extrahi; itaque in hac quidem nihil erat admiratione dignum: altera ſic erat, ut non niſi ab animantium corporibus trahi potuiſſe videretur, quod Beccario ſane admirationem attulit; neque tam mirabatur id ita eſſe, quam id potuiſſe ſcriptores fugere; numquam enim id legerat. Suum hoc ſtudium cum academia communicavit anno milleſimo ſeptingenteſimo octavo & viceſimo, ſermone ſatis longo, quem conferam in pauca.

Sed primum duas illas, quas dixi, farinæ partes, quemadmo.

El forro y la primera página del tratado de J. B. Beccari proteína.

«Sobre la gluténs», el primer artículo científico sobre la
3—0132

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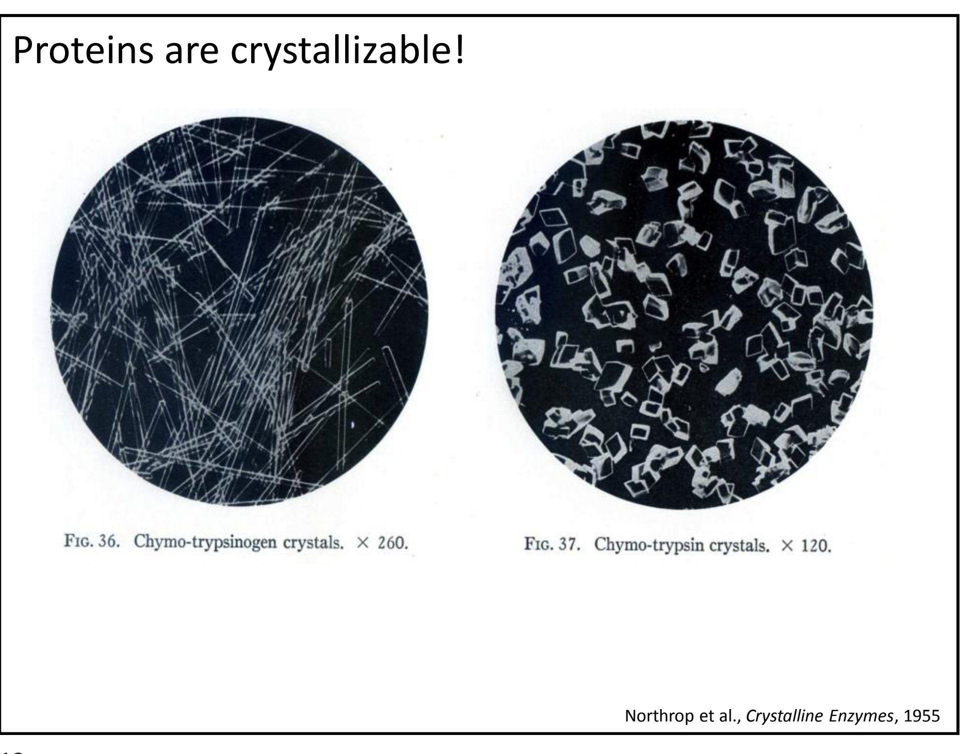

FIG. 36. Chymo-trypsinogen crystals. × 260.

FIG. 37. Chymo-trypsin crystals. × 120.

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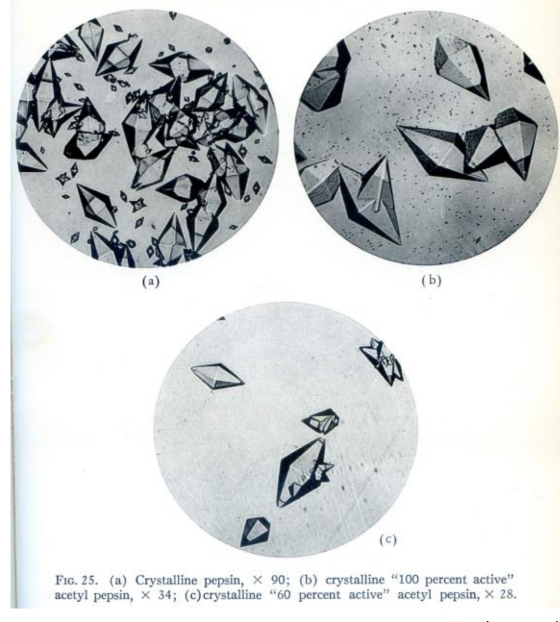
Proteins are crystallizable!



Northrop et al., *Crystalline Enzymes*, 1955

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Dorothy Maud Wrinch (Rosario, AR 12/set/1894 – 11/fev/1976)

In 1929 she was the first woman to receive a DSc from Oxford University

Mathematician and theoretical biochemist

She was secretary to Bertrand Russell and organized the publication of the Tractatus of Wittgenstein

She proposed the cycle theory to explain the structure of proteins

Together with chemist Irving Langmuir proposed the hydrophobic effect

She influenced members of Theoretical Biology Club as J.D. Bernal and Joseph Needham

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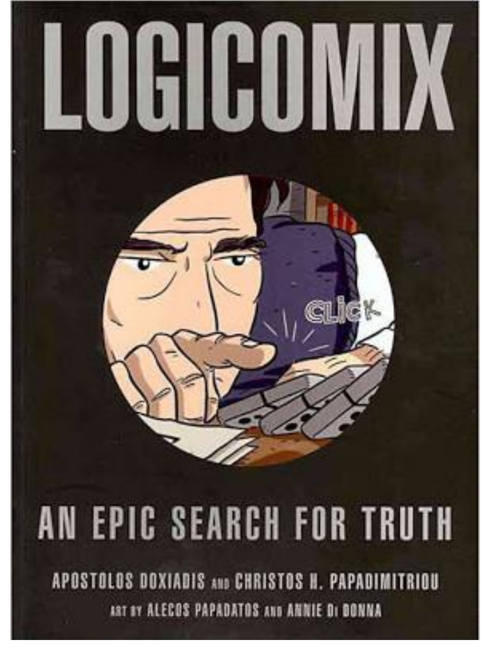

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972 NATURE JUNE 5, 1937

The Cyclol Theory and the 'Globular' Proteins*

By Dr. D. M. Wrinch

Wrinch, *Nature* **139**, 972-973, 1937

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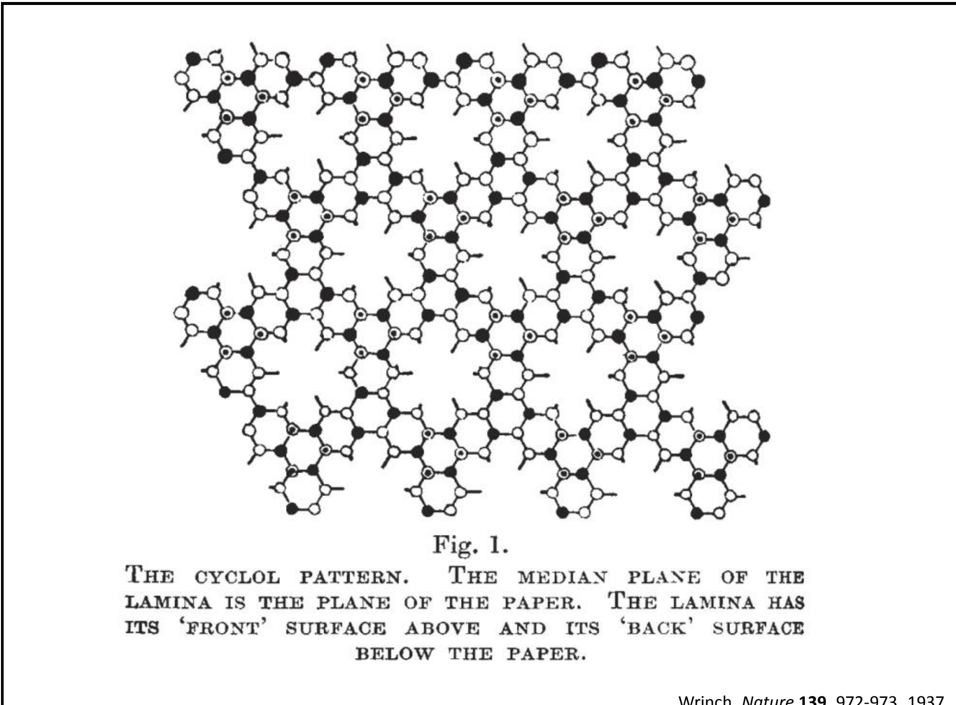

Fig. 1.
THE CYCLOL PATTERN. THE MEDIAN PLANE OF THE LAMINA IS THE PLANE OF THE PAPER. THE LAMINA HAS ITS 'FRONT' SURFACE ABOVE AND ITS 'BACK' SURFACE BELOW THE PAPER.

Wrinch, *Nature* **139**, 972-973, 1937

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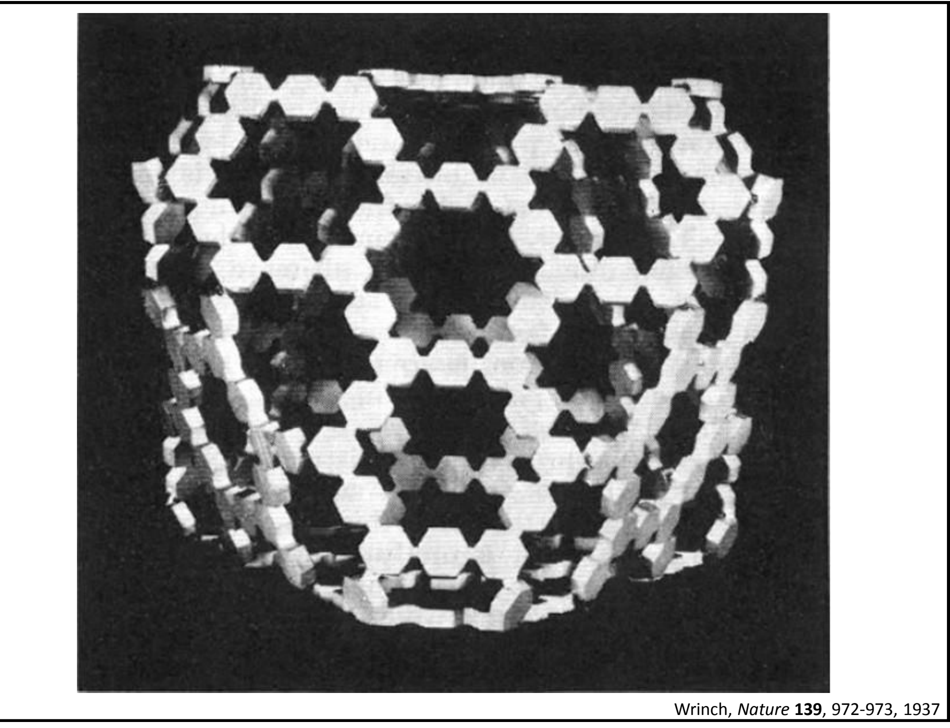

Wrinch, *Nature* **139**, 972-973, 1937

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The reaction of the "cyclol"

C^{α} — C=O, HN — C^{α} + C^{α} — C=O, HN — C^{α}

Thermodynamically unfavorable because it eliminates the stabilization by resonance of the peptide bond

Therefore Linus Pauling did not accept the proposed model, despite its intrinsic beauty

https://en.wikipedia.org/wiki/Cyclol

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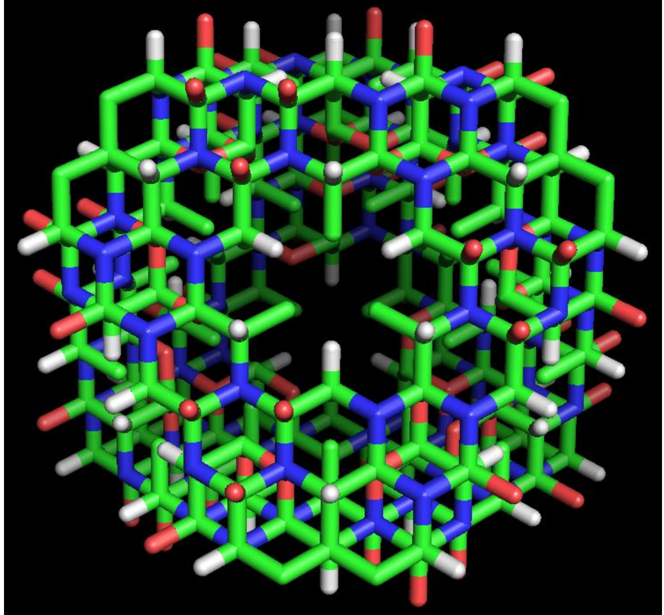
The cyclol in a modern version



https://en.wikipedia.org/wiki/Cyclol

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THE STRUCTURE OF PROTEINS

By IRVING LANGMUIR

Address delivered 20 December 1938

Irving Langmuir (1881-1957) – NPW 1932

Melhorou a bomba de difusão para atingir alto vácuo

Sugeriu o uso de argônio na lâmpada de filamento de tungstênio e mostrou que o filamento enrolado tinha maior durabilidade

Langmuir, *Proc. Phys. Soc.* **51(4)**:592-612, 1939

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§ 9. CONCLUSION

structures. These cage molecules explain in one simple scheme the existence of megamolecules of definite molecular weights capable of highly specific reactions, of crystallizing, and of forming monolayers of very great insolubility. The agreement between the properties of the globular proteins and the cyclol structures proposed for them is indeed so striking that it gives an adequate justification for the cyclol theory, especially in view of the fact that this great variety of independent facts are on this theory seen to be logical consequences of one simple postulate.

Langmuir, *Proc. Phys. Soc.* **51(4)**:592-612, 1939

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§ 9. CONCLUSION

composition of proteins. The original idea of native proteins as long chain polymers of amino-acid residues, while consistent with the facts relating to the chemical composition of proteins in general, was not a necessary deduction from these facts. Moreover it is incompatible with the facts of protein crystallography, both classical and modern, with the phenomena of denaturation, with Svedberg's results which show that the native proteins have definite molecular weights, and with the high specificity of proteins discovered in studies in immunochemistry and enzyme chemistry. All these facts seem to demand a highly organized structure for the native proteins, and the assumption that the residues function as two-armed units leading to long-chain structures must be discarded. The cyclol hypothesis introduced

Langmuir, *Proc. Phys. Soc.* **51(4)**:592-612, 1939

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DISCUSSION

Dr D. Wrinch: I do not want to-night to go into any details regarding the theoretical aspects of protein structure, but rather to thank Dr Langmuir for the new light which he has thrown on the subject. I would also point out how critical it is for the future progress of our knowledge of protein structure—upon which of course the future of medicine also depends—that additional data should be obtained. It is perhaps hardly realized by workers in physics that we do not yet know the complete composition of any single globular protein. Data regarding the chemical composition of insulin are still very incomplete and make it impossible at present to locate the side chains. Such chemical data are urgently needed and can be obtained by the application of the established methods of chemical analysis. Dr Langmuir has stressed the great importance of applying to the proteins all the techniques of physics which are appropriate. Great progress in the isolation and crystallization of proteins now provides for physical investigations an almost unlimited wealth of material.

Langmuir, *Proc. Phys. Soc.* **51(4)**:592-612, 1939

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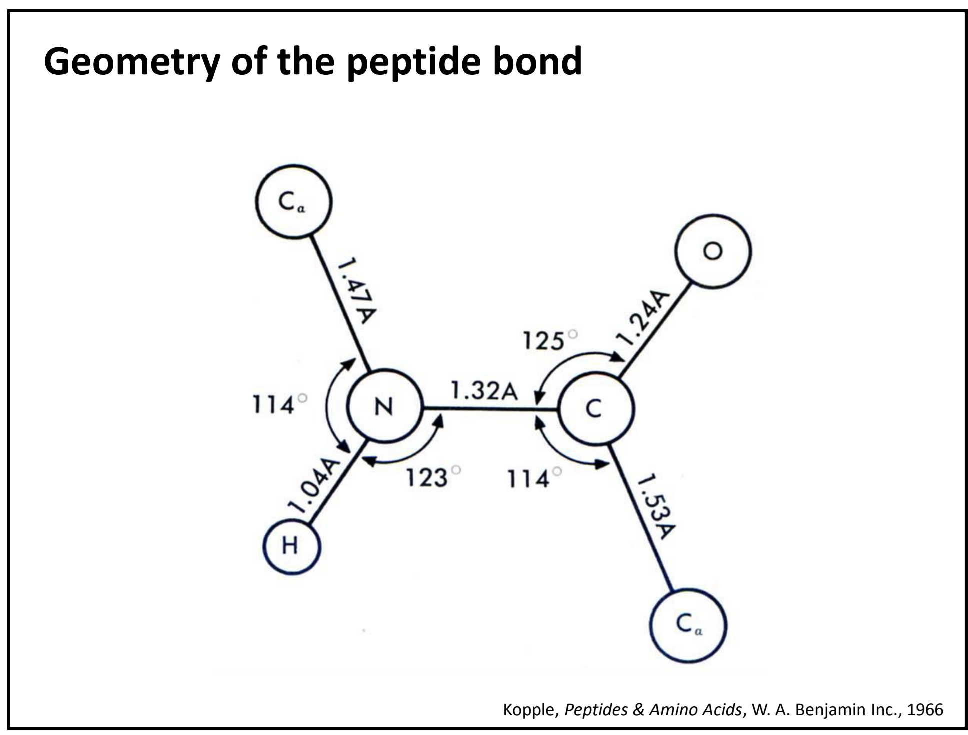

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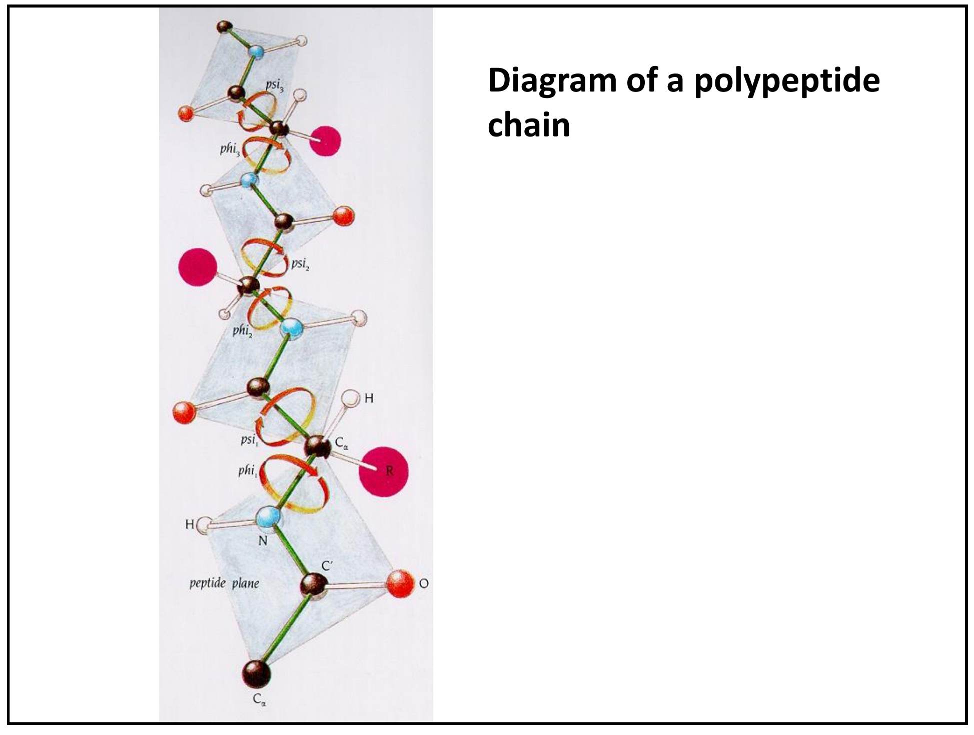

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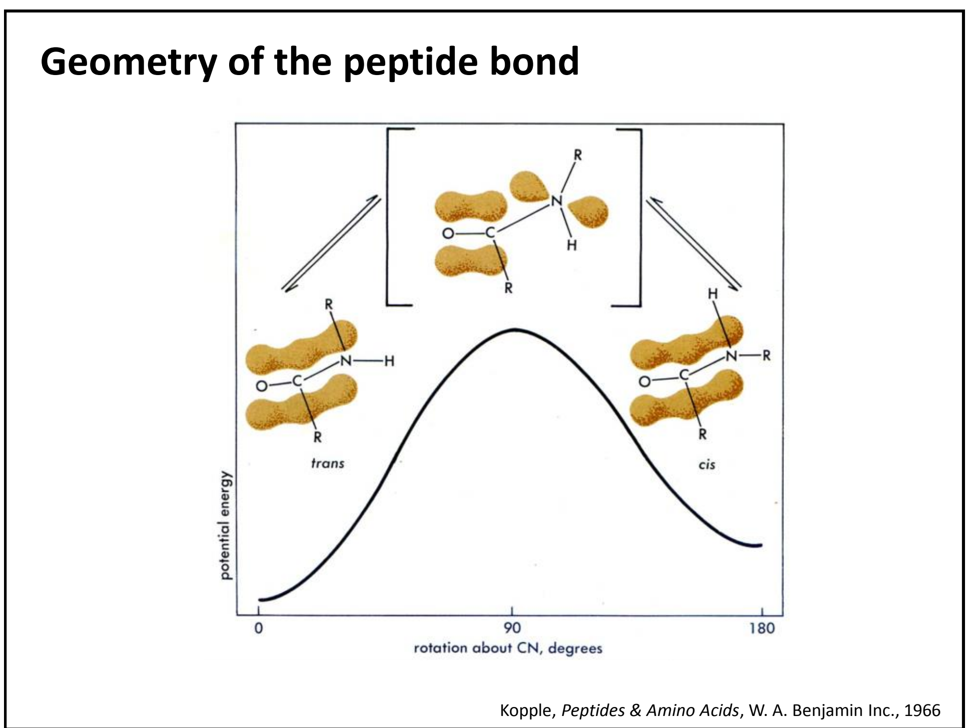

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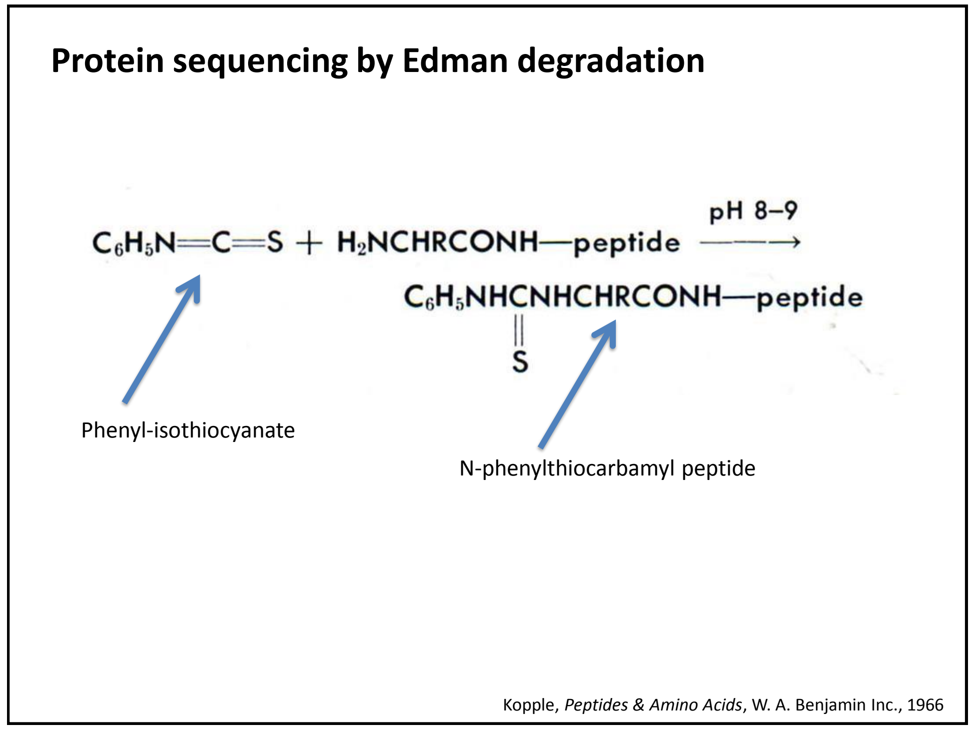

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Protein sequencing by Edman degradation

H^+

RCH—CO—NH—peptide

H—N S

C

NHC_6H_5

→

Thiazolone
(unstable)

RCH—CO

N S + H_2N — peptide
(4–4)

C

NHC_6H_5

X^-

RCH—CO

H^+ N S →

C

NHC_6H_5

[RCH—CO—X

HN N—H

C C_6H_5

S] →

RCH—CO

HN NC_6H_5

C

S (4–5)

Thiohydantoin
(soluble in organic solvents)

Kopple, *Peptides & Amino Acids*, W. A. Benjamin Inc., 1966

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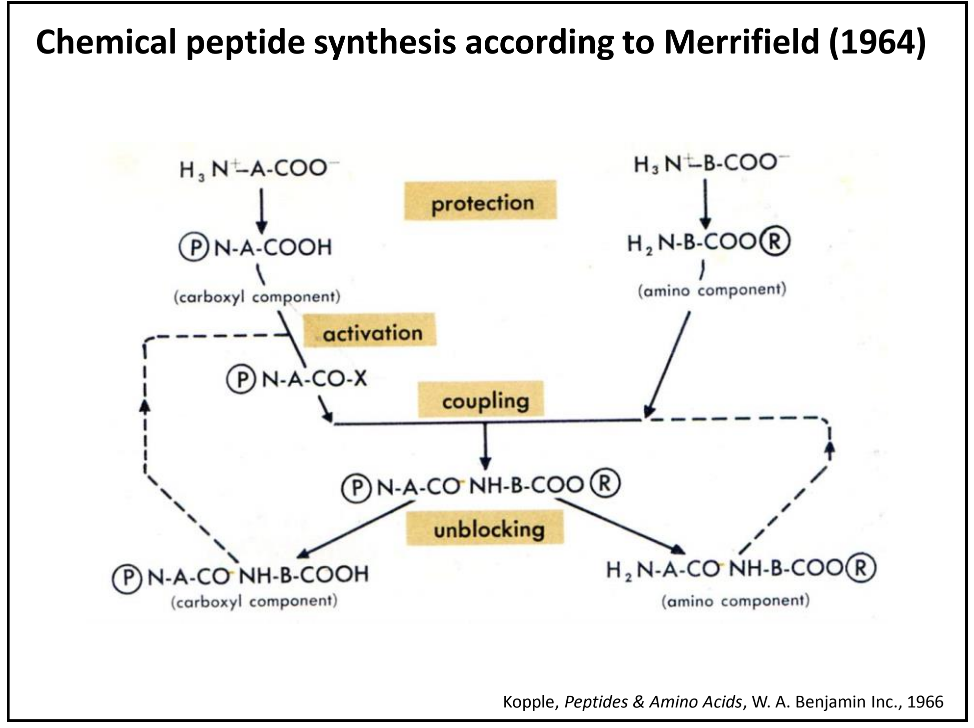
Chemical peptide synthesis according to Merrifield (1964)



Kopple, *Peptides & Amino Acids*, W. A. Benjamin Inc., 1966

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A SPECIFIC CHEMICAL DIFFERENCE BETWEEN THE GLOBINS OF NORMAL HUMAN AND SICKLE-CELL ANÆMIA HÆMOGLOBIN

By Dr. V. M. INGRAM

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

Ingram, *Nature* **178:**792-794, 1956

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Ingram, *Nature* **178:**792-794, 1956

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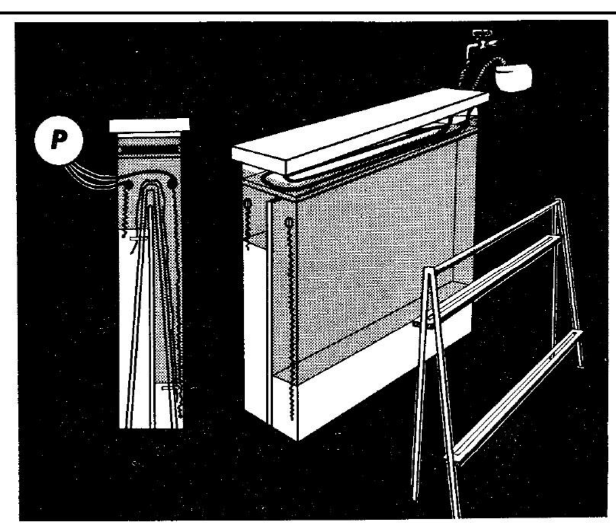

FIG. 1. A Lucite tank 20 inches high, 25 inches wide, and 6 inches deep is divided into two watertight compartments by a center partition 21 inches high. Platinum electrodes, connecting to the power supply (*P*), are immersed in buffer (clear) on either side of the partition, the circuit being completed by the chromatogram which is hung over the rack. The entire paper is immersed in Varsol (stippled) which is cooled by tap water flowing through stainless steel coils. The Lucite rack used to support the paper is illustrated to the right.

Peptide Separation by Two-dimensional Chromatography and Electrophoresis

Katz et al., *JBC* **234:**2897-2900, 1959.

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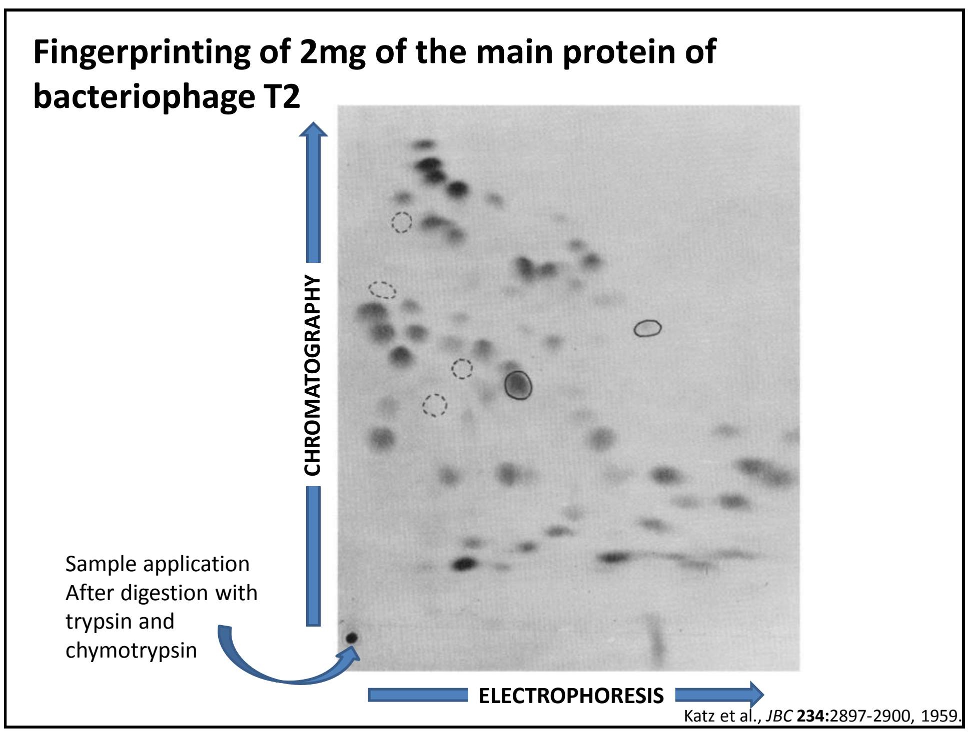

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PROTEIN BIOSYNTHESIS

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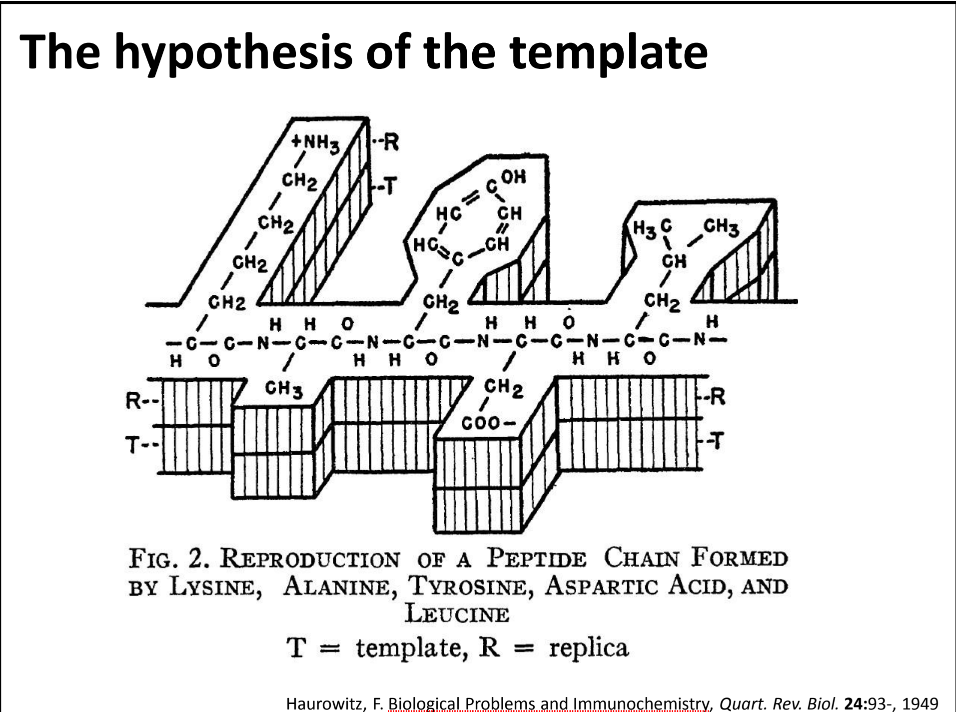

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Two hypothesis to explain the biosynthesis of proteins:

(1) Through the reversion of protease hydrolytic reaction:

$$HOOC-CH(R_1)-NH-C(=O)-CH(R_2)-NH_2 \underset{-H_2O}{\overset{+H_2O}{\rightleftharpoons}} HOOC-CH(R_1)-NH_2 + HOOC-CH(R_2)-NH_2$$

"Proteins are hydrolyzed by enzymes and therefore they must also be synthesized by the enzyme which caused hydrolysis, since catalysts must accelerate the reaction rate equally in both directions"

Northrop, Kunitz & Herriott – Crystalline Enzymes – 2nd edition revised - 1955

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Two hypothesis to explain the biosynthesis of proteins:

(2) There is energy expenditure in the biosynthesis:

"The acyl phosphate of fatty acids might then condense with glycerol to form the fats. **The acyl phosphate of amino acids likewise might condense with the amino groups of other amino acids to form the proteins.**"

$$H_2N-R_2-C(=O)-O-PO_3H_2 + H_2N-R_2-C(=O)-O-PO_3H_2 \rightarrow H_2O_3P-O-C(=O)-CH(R_1)-NH-C(=O)-CH(R_2)-NH_2 + H_3PO_4$$

Fritz Lipmann, Metabolic Generation and Utilization of Phosphate Bond Energy in: *Adv. Enzymol. Related Areas of Molecular Biology* **vol. 1**, pp. 99-162, 1941

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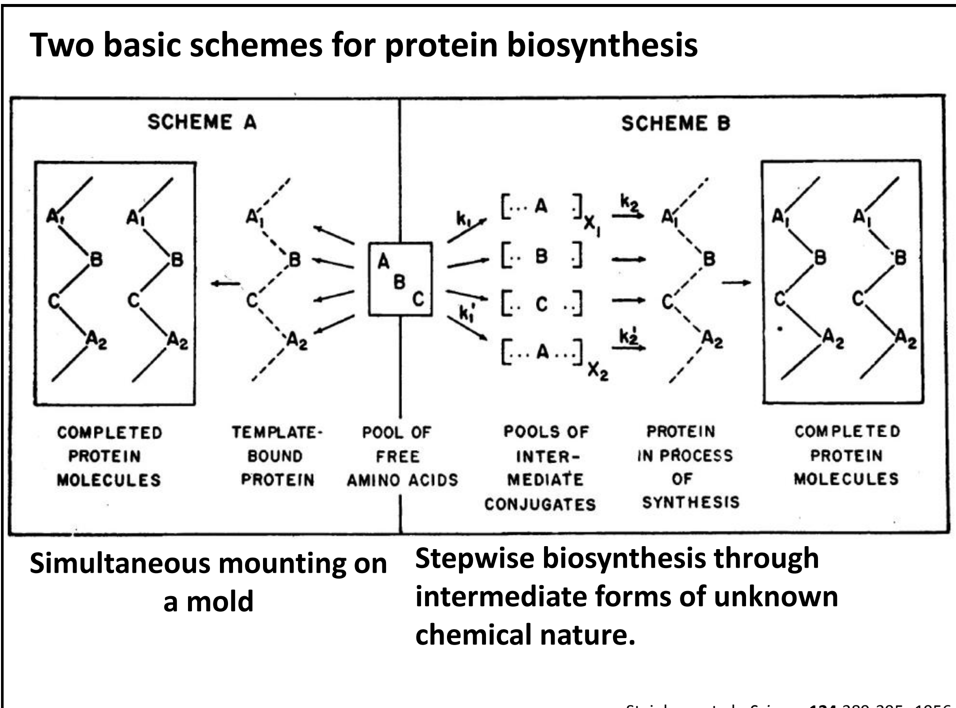
Two basic schemes for protein biosynthesis



Simultaneous mounting on a mold

Stepwise biosynthesis through intermediate forms of unknown chemical nature.

Steinberg et al., *Science* **124:**389-395, 1956

39

Monod (*BBActa* **16:**99, 1955) & Spiegelman (*J. Bacteriol.* **68:**419, 1954) have clearly shown that, under certain conditions, protein synthesis can proceed with no significant contribution from preexisting proteins or readily demonstrable peptides. …under the conditions used, more that 97 percent and possibly all of the precursor material for protein synthesis consists of free amino acids.

The negative evidence that no intermediate compounds could be found by the rough survey methods used cannot be considered in any sense crucial evidence

Steinberg et al., *Science* **124:**389-395, 1956

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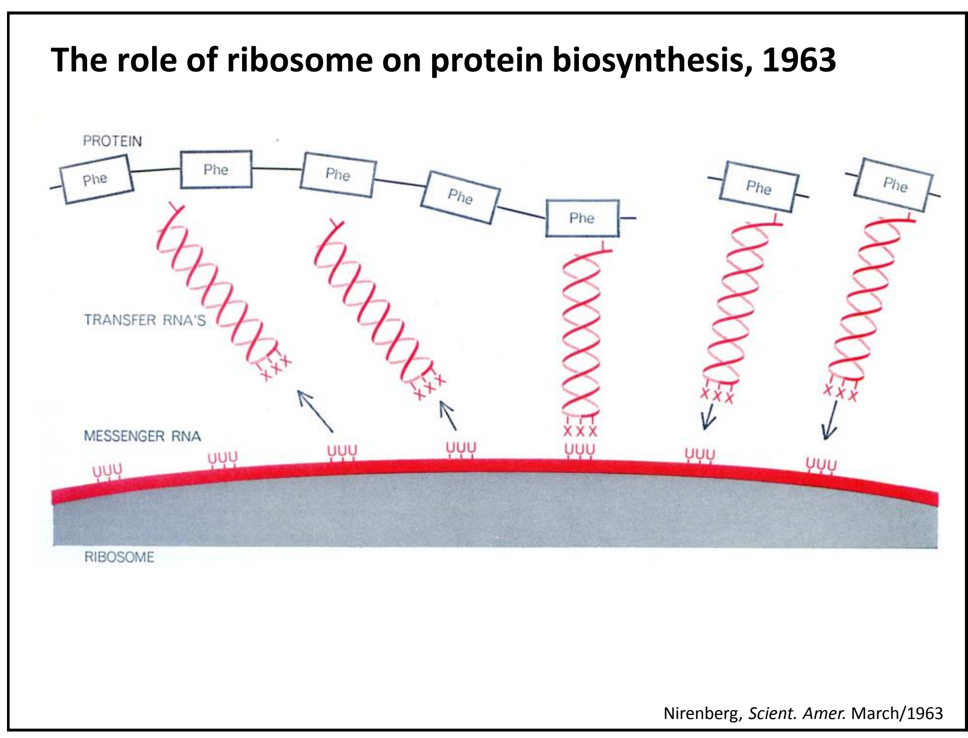
The role of ribosome on protein biosynthesis, 1963



Nirenberg, *Scient. Amer.* March/1963

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RADIOACTIVE PRECURSORS AND RADIOACTIVITY

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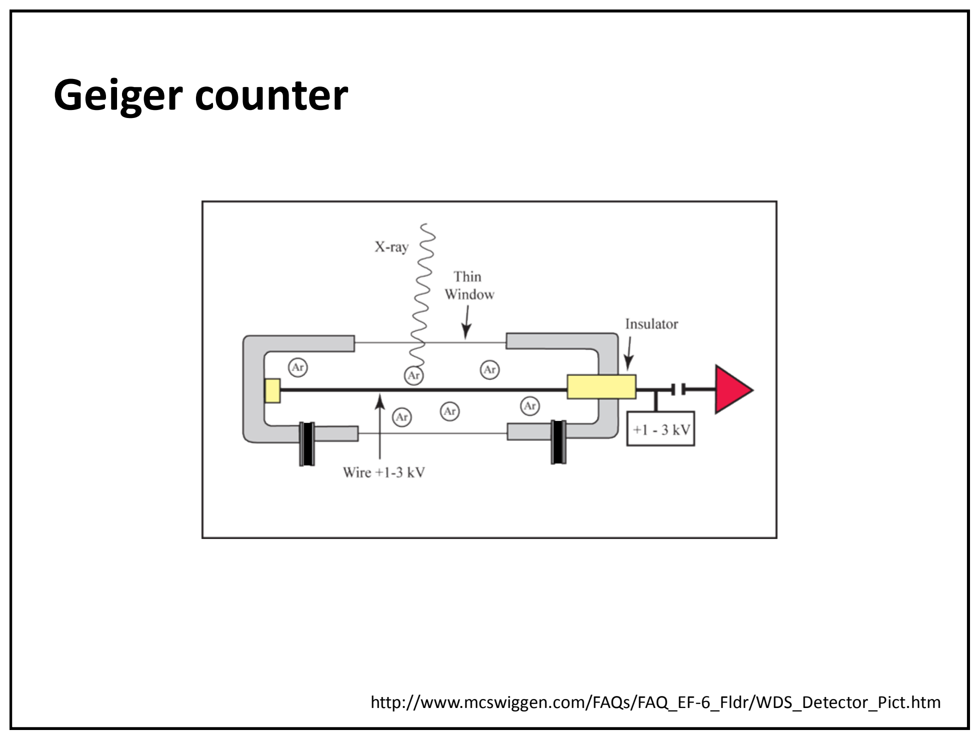

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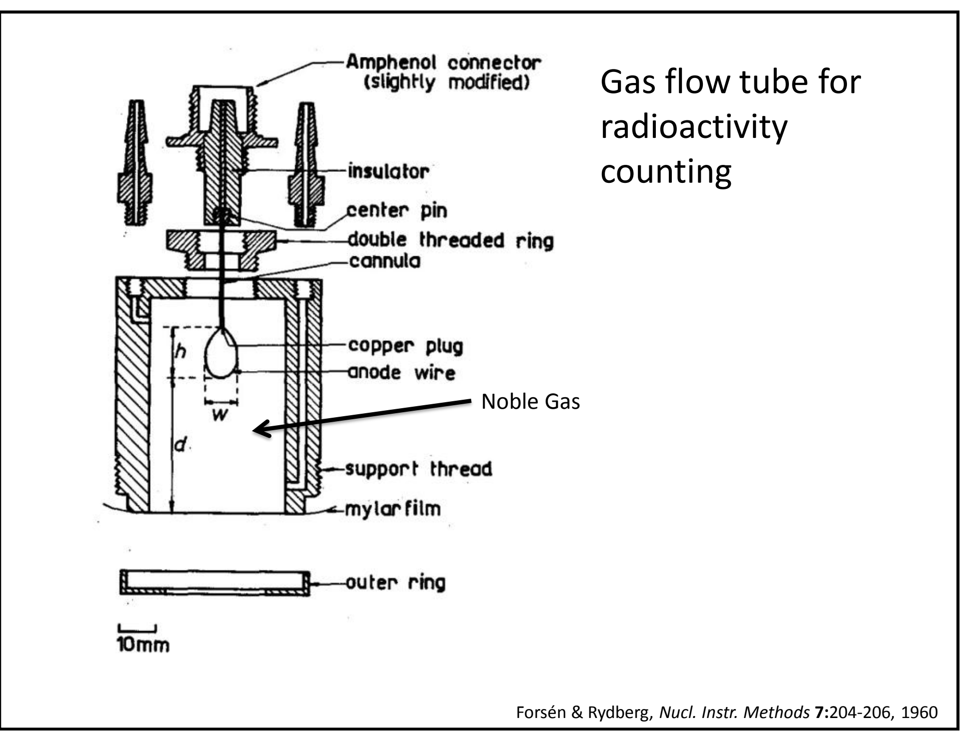

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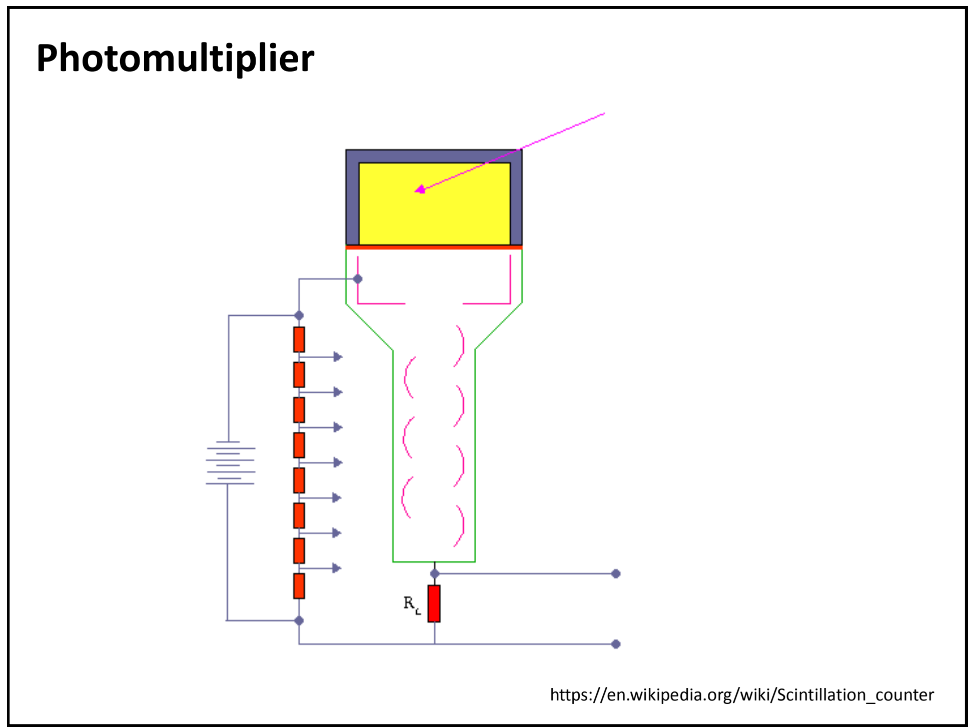

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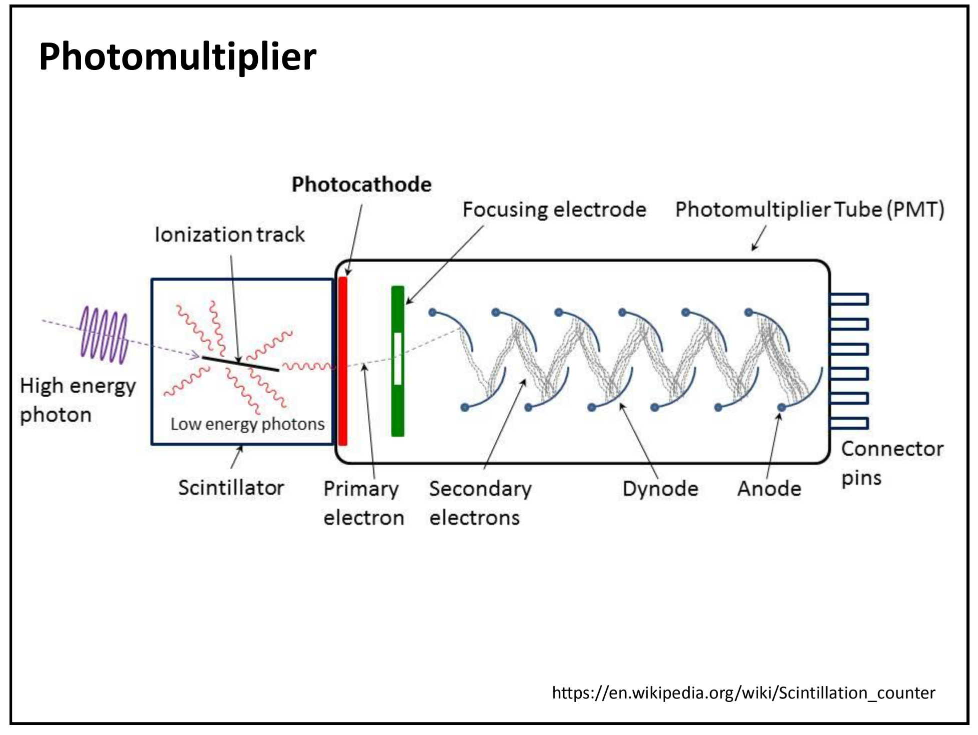

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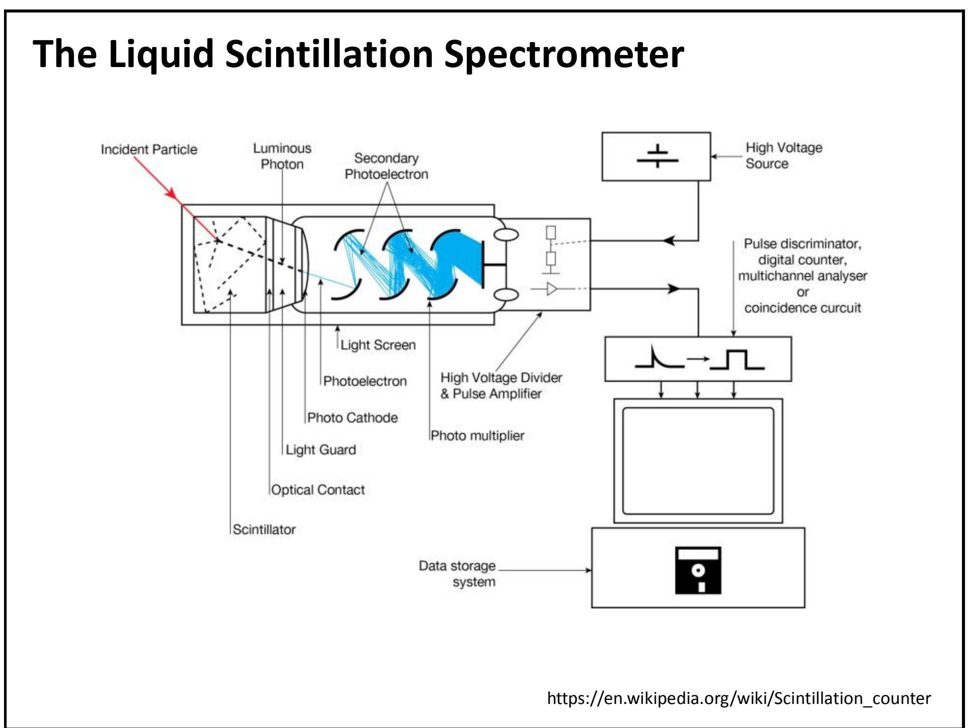

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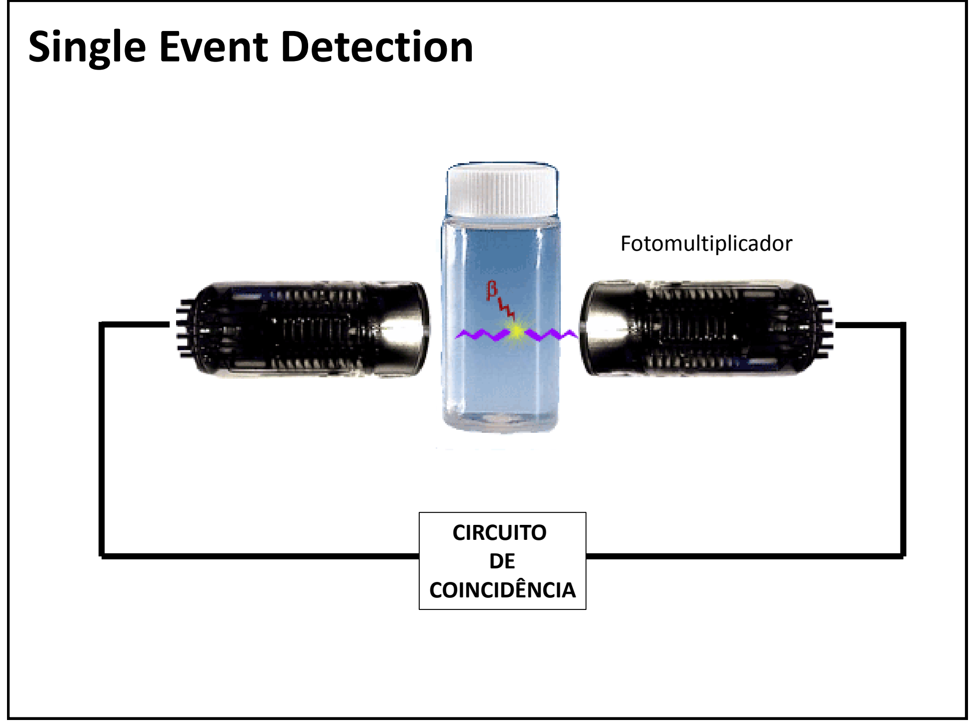

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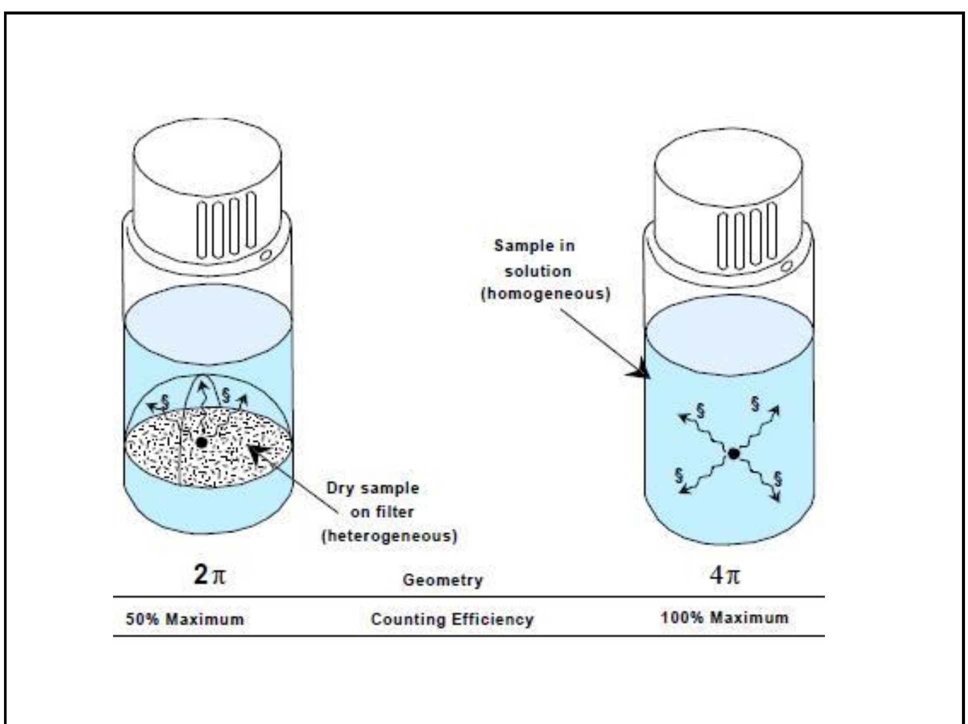

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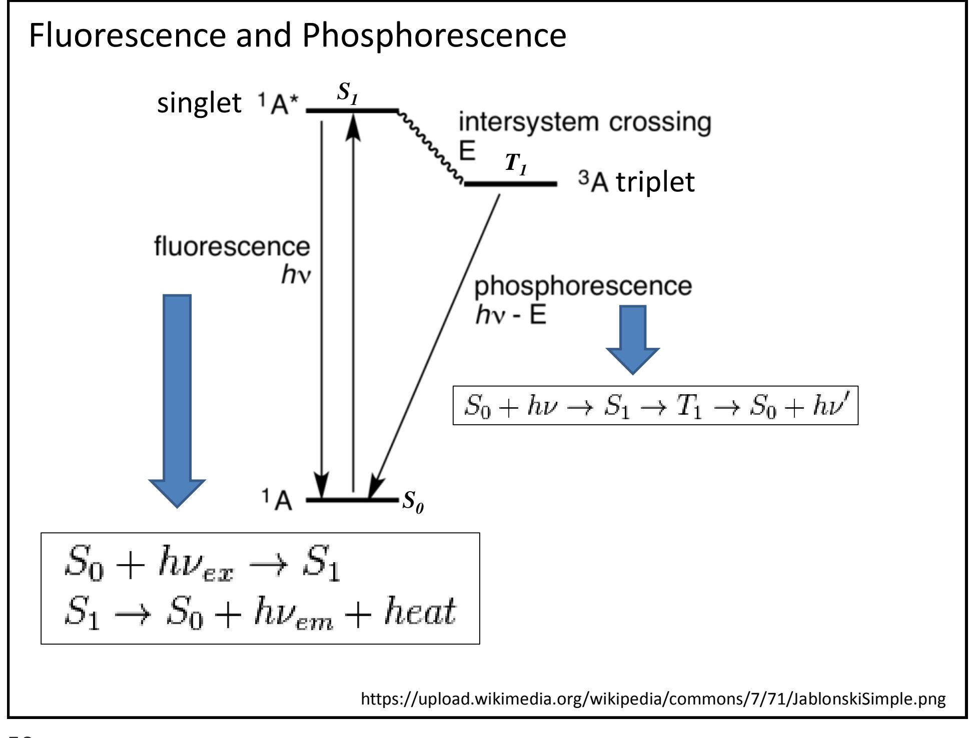

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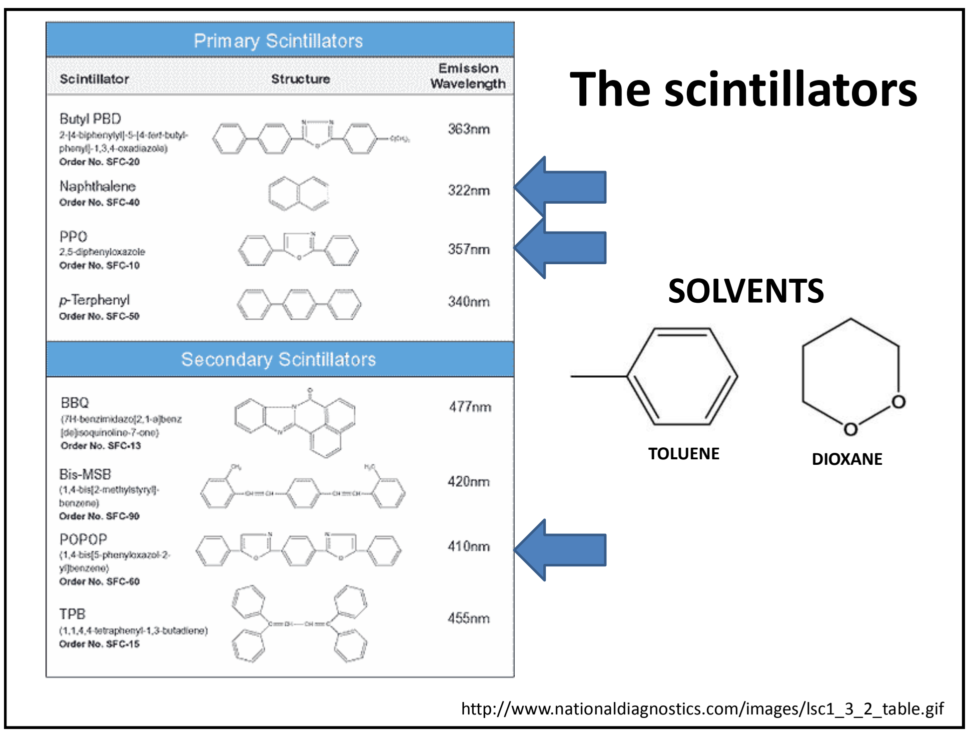

Primary Scintillators		
Scintillator	Structure	Emission Wavelength
Butyl PBD 2-[4-biphenylyl]-5-[4-tert-butyl-phenyl]-1,3,4-oxadiazole) **Order No. SFC-20**		363nm
Naphthalene **Order No. SFC-40**		322nm
PPO 2,5-diphenyloxazole **Order No. SFC-10**		357nm
p-Terphenyl **Order No. SFC-50**		340nm
Secondary Scintillators		
BBQ (7H-benzimidazo[2,1-a]benz[de]isoquinoline-7-one) **Order No. SFC-13**		477nm
Bis-MSB (1,4-bis[2-methylstyryl]-benzene) **Order No. SFC-90**		420nm
POPOP (1,4-bis[5-phenyloxazol-2-yl]benzene) **Order No. SFC-60**		410nm
TPB (1,1,4,4-tetraphenyl-1,3-butadiene) **Order No. SFC-15**		455nm

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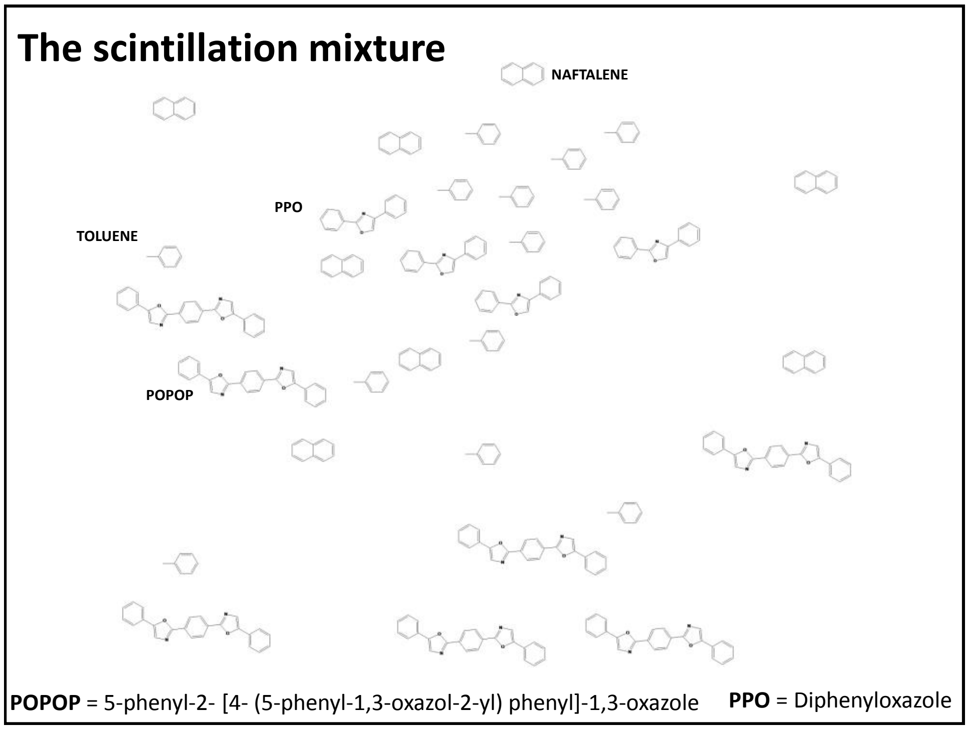

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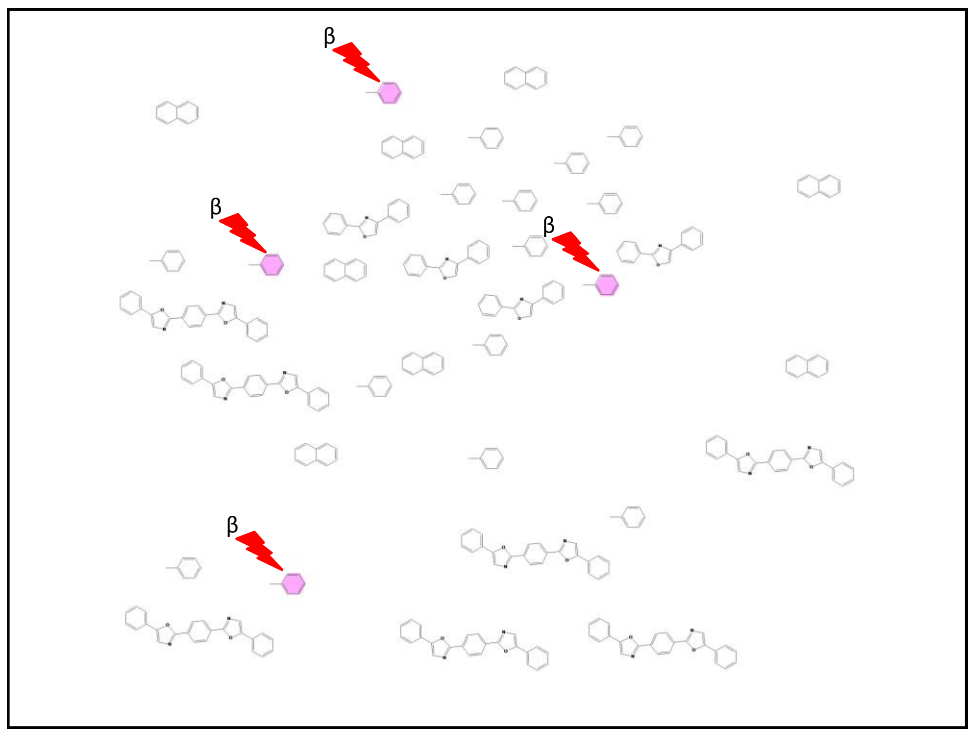

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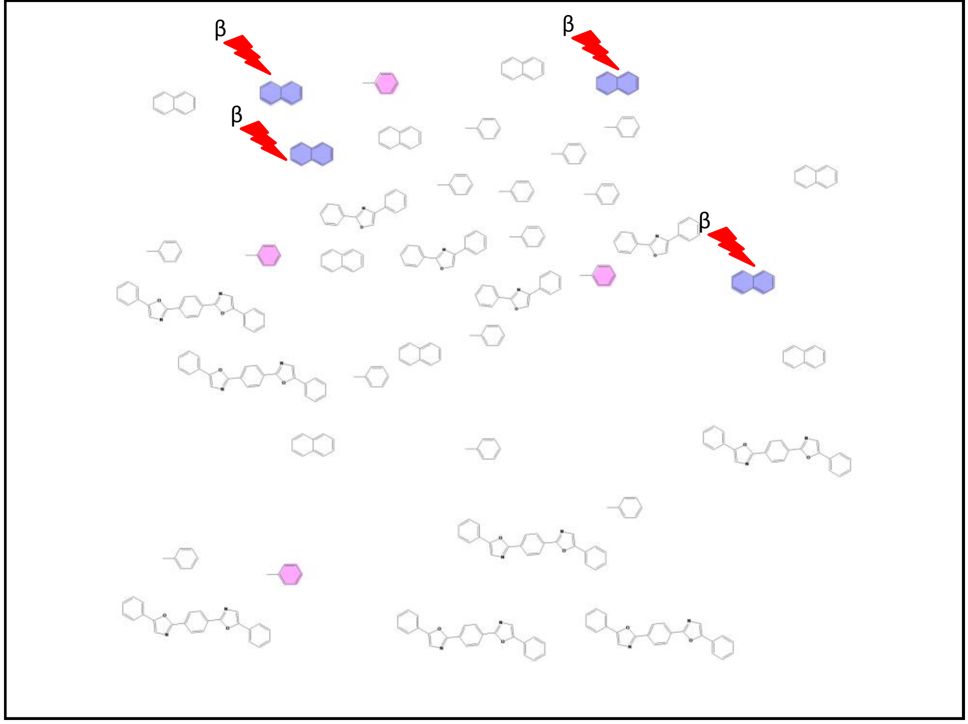

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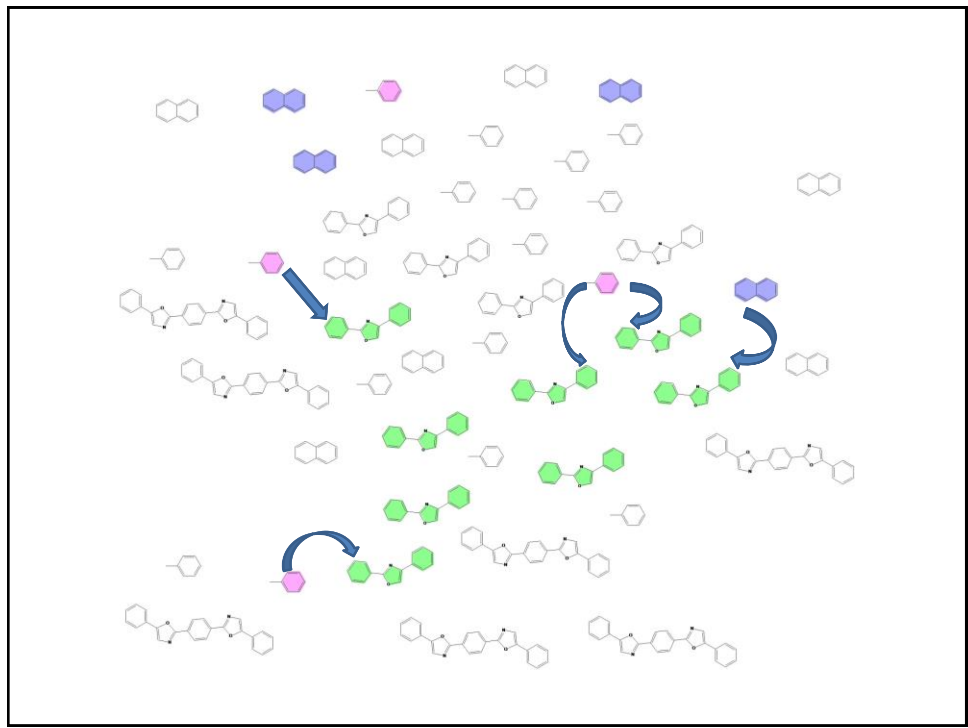

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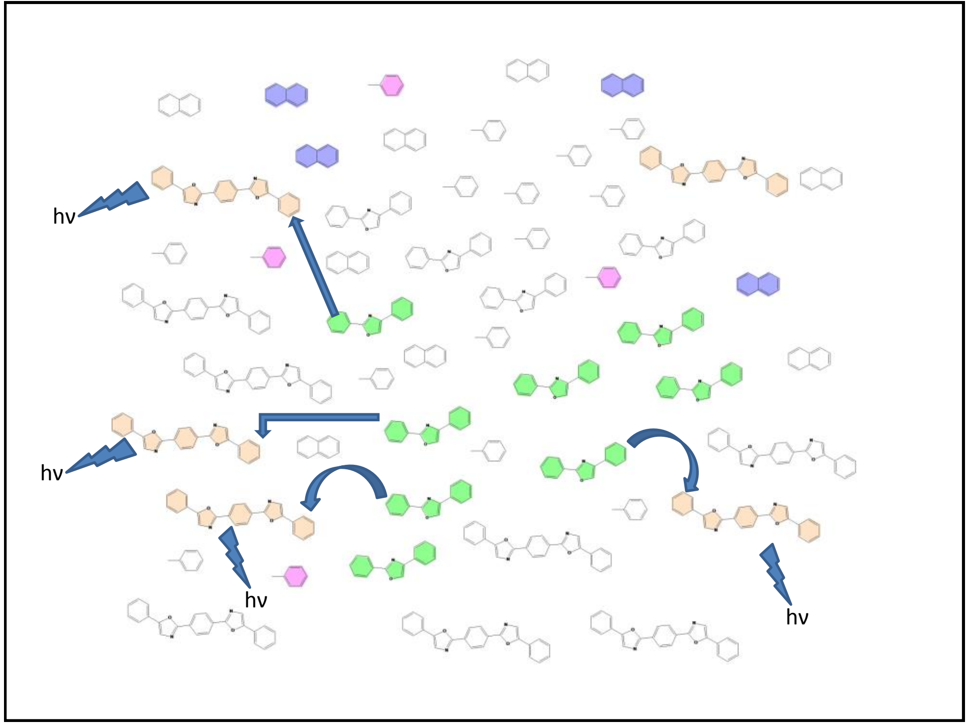

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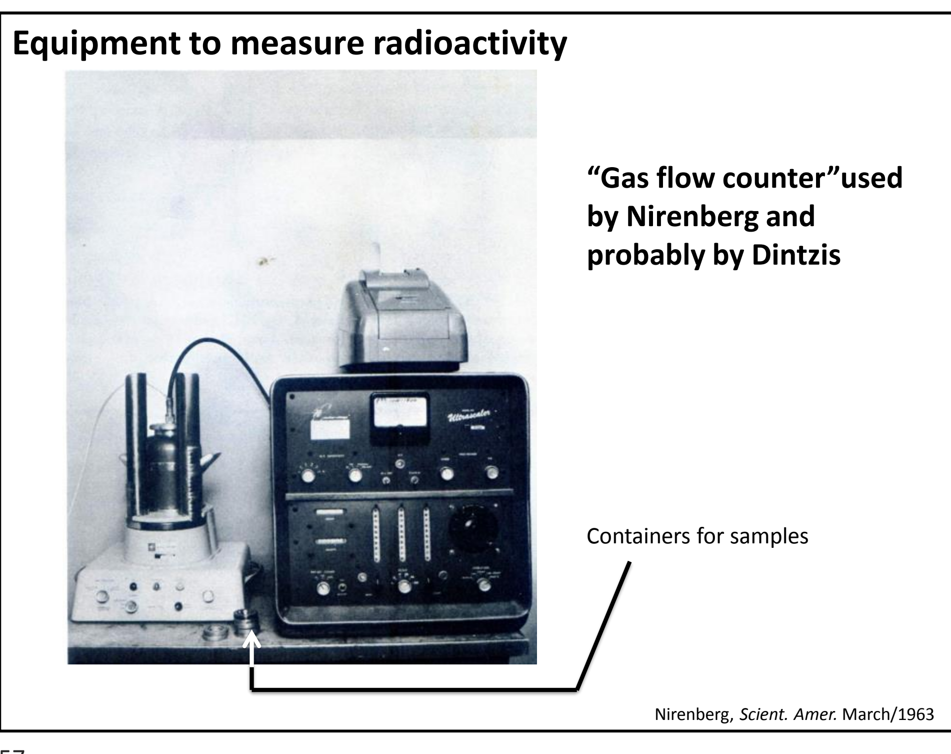

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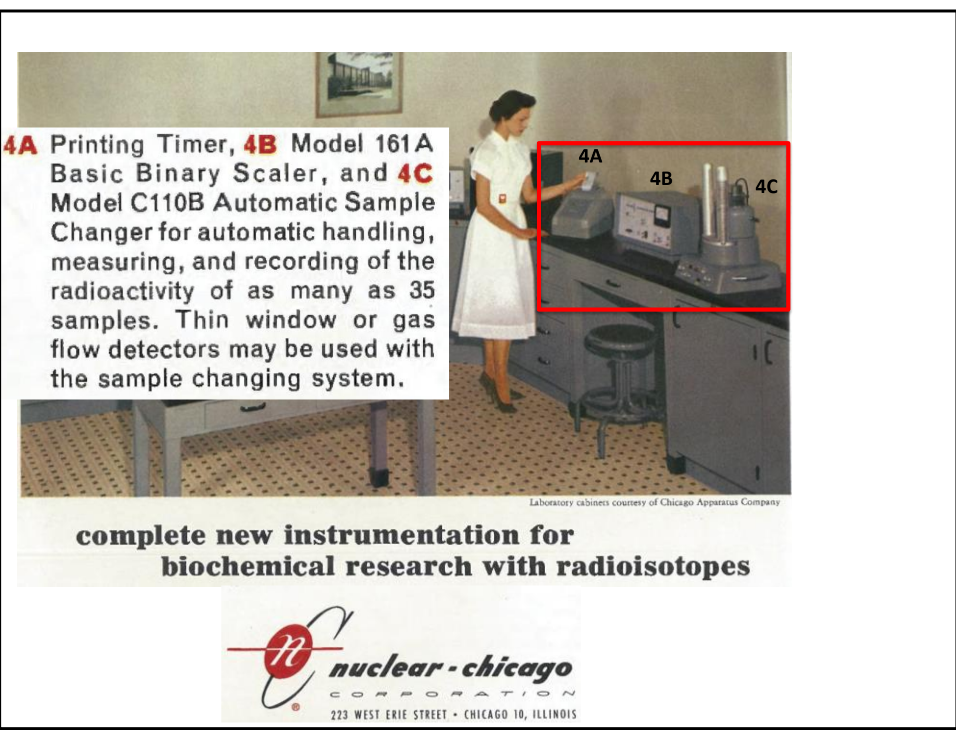

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SCIENTIFIC PRECURSORS

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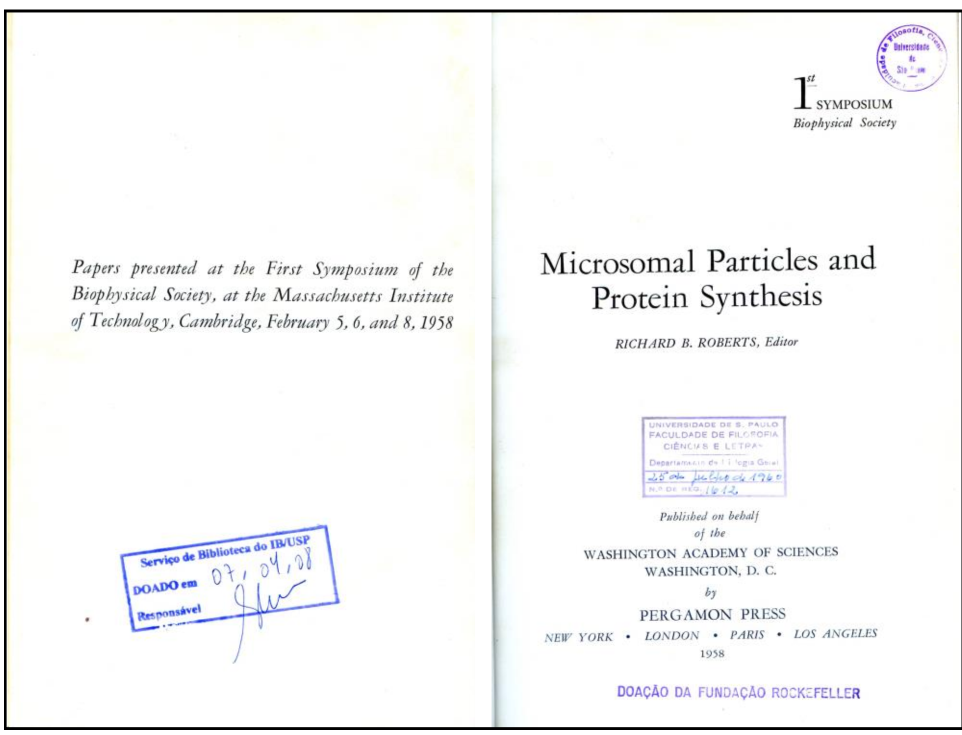

Papers presented at the First Symposium of the Biophysical Society, at the Massachusetts Institute of Technology, Cambridge, February 5, 6, and 8, 1958

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Microsomal Particles and Protein Synthesis

RICHARD B. ROBERTS, *Editor*

UNIVERSIDADE DE S. PAULO
FACULDADE DE FILOSOFIA
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Microsomal Structure and Hemoglobin Synthesis in the Rabbit Reticulocyte

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and Kerckhoff Laboratories of Biology
California Institute of Technology

A great deal of evidence has now accumulated which suggests very strongly that microsomal particles are somehow connected with the process of protein synthesis [1, 2, 3]. Because of their high content of ribonucleic acid which might act as a coding template, it has become fashionable to postulate that these particles are the actual sites of protein assembly from activated single amino acids. To date, however, no evidence has been put forth which could be called direct proof that such is indeed the case.

Nevertheless, the hypothesis is so attractive that it seems worth while to proceed on the assumption that it is true and to investigate the detailed structure of microsomal particles and the relation of this structure to the protein which the particles are supposedly synthesizing. Such a study would have as its object an understanding of the molecular nature of the microsomal particle, the molecular structure of the growing peptide chains of the protein being synthesized, and, if possible, the interrelation between the two.

To best carry out such a study, one would like to find a system consisting of free floating cells of a single type, actively engaged in the synthesis of predominantly a single type of protein molecule. Fortunately these desirable properties are exhibited by mammalian reticulocytes. Such cells can be made in quantity if rabbits are made anemic by daily injections of phenylhydrazine. After a week of such injections, reticulocytes (immature red cells) account for 80 to 90 per cent of the red cells present in the blood. These cells are actively producing hemoglobin and will continue to do so for many hours if suspended in the

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not very informative, and the measured leucine-arginine ratio, which lies almost exactly half way between microsomal protein and hemoglobin, is very ambiguous. These results are compatible with the assumption that the transient material in the microsome is largely pre-hemoglobin. More evidence is needed to confirm this assumption, however.

TABLE 2. Molar Ratios of Leucine to Various Amino Acids

	Microsomes		Hemoglobin	
	Total Composition	Label	Total Composition	Label
Histidine	3.7	2.2	1.8	1.9
Phenylalanine	2.5	2.1	2.3	2.0
Arginine	1.0	3.2	5.7	7.5

Boiling microsomes with 66 per cent ethanol extracted about 25 per cent of the radioactive material, whose free amino nitrogen increased greatly on refluxing with 6 *N* HCl, suggesting that this material is peptide in nature. The specific activity of the material was 5 to 10 times that of the unextracted microsomal protein. Both the extractable and nonextractable radioactive materials were transient; i.e., the counts were removed on incubating cells in nonlabeled amino acid for 15 minutes.

One would like to conclude from the above extraction data that the extracted material is richer than the whole microsome in growing peptide chains of short length, whereas the unextractable material represents growing peptide chains which are too long to dissolve in 66 per cent ethanol. The short chains would presumably represent the earliest stages of hemoglobin formation in the microsome. Further purification and characterization are necessary to prove this point.

SOME NUMEROLOGY AND CONCLUSIONS

The above data lead to some interesting results if the following assumptions (or approximations) are made: (1) hemoglobin is the only protein being made in rabbit reticulocytes; (2) all microsomal particles are equally active in synthesizing hemoglobin; (3) all the transient label in the microsome is pre-hemoglobin. The steady-state label level of the microsomal particle, the specific activity of leucine used, together with the facts that 12 per cent of the protein is leucine and one-half of the microsome is protein, lead to the conclusion that 0.05 per cent of the mass of the microsomal particle is pre-hemoglobin, i.e., growing peptide chain. Since the molecular weight of the microsomal particle is 4,000,000 as shown above, this means that the average weight of growing chain per particle is 2000. In a random population of growing chains the average weight might be expected to be about one-half of the finished chain weight. If all the growing chain per particle is in one piece, this leads to a value of 4000 for the finished weight of polypeptide chain made per microsomal particle, a value reasonably close to the weight of one-fourth of a hemoglobin molecule,

i.e., one polypeptide chain. If assumption 2 above is incorrect, and only a fraction of the microsomal particles is functional, the agreement is even better. From the rate of incorporation of label into finished hemoglobin molecules and the concentrations in the living cells of hemoglobin (15 per cent) and microsomes (0.5 per cent), one may calculate that to account for the production of new hemoglobin each microsomal particle must make one-quarter of a hemoglobin in 1.5 minutes.

SUMMARY

The above data lead to the picture of a microsomal particle as an almost spherical sponge-like structure of anhydrous molecular weight 4,000,000 and diameter 340 A. One-half of the mass is represented by ribonucleic acid which appears to be present as four strands of molecular weight 500,000. The half of the microsomal particle which is protein appears to be almost entirely (99.9 per cent) structural in nature; i.e., it is *not* transient protein precursor.

Woven into this sponge-like structure in some way is a very small amount (0.05 per cent by weight) of transient protein precursor. Taken together with the observed rate of hemoglobin production, this amount of precursor is compatible with the conclusion that one microsomal particle makes one polypeptide chain of hemoglobin in approximately 1 minute.

Contribution No. 2338

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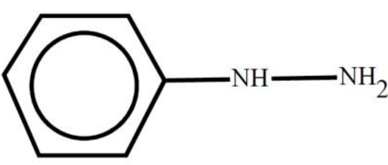
Mechanism of action of phenylhydrazine



Phenylhydrazine generates ROS (reactive oxygen species) that lead to modifications of intracellular erythrocyte proteins (hemoglobin, spectrin, etc.)

This alters the deformability of erythrocytes that are then destroyed in the spleen

Reduction in the number of erythrocytes (= anemia) leads to an increase in the production of cellular precursors in the bone marrow -> reticulocytes

Berger, *J. Appl. Biomed.* **5:**125-130, 2007 Pandey et al., *Amer. J. Phytomed. Clin. Therap.*, **2-3:**390-394, 2014

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Rabbit globin peptides obtained by trypsin digestion

α-GLOBIN

01. VLSPADK
02. TNIK
03. TAWEK
04. IGSHGGEYGAEAVER
05. MFLGFPTTK
06. TYFPHFDFTHGSEQIK
07. AHGK
08. K
09. VSEALTK
10. AVGHLDDLPGALSTLSDLHAHK
11. LR
12. VDPVNFK
13. LLSHCLLVTLANHHPSEFTPAVHASLDK
14. FLANVSTVLTSK
15. YR

β-GLOBIN

01. VHLSSEEK
02. SAVTALWGK
03. VNVEEVGGEALGR
04. LLVVYPWTQR
05. FFESFGDLSSAHAVMSNPK
06. VK
07. AHGK
08. K
09. VLAAFSEGLNHLDNLK
10. GTFAK
11. LSELHCDK
12. LHVDPENFR
13. LLGNVLVVVLSHHFGK
14. EFTPQVQAAYQK
15. VVAGVANALAHK
16. YH

Numerados em ordem crescente do N->C

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The Hemoglobins in Genetics and Evolution

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1963

30 STRUCTURE AND SYNTHESIS

time t_2 in Figure 2-6. The length of the pulse at time t_2 is short compared to the time which it takes to make the whole chain. After such a short time interval only a few of the peptide chains will be complete

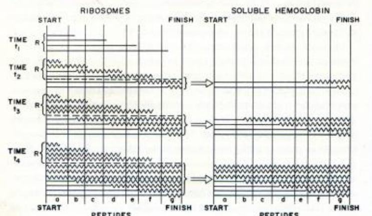

FIGURE 2-6. Model of sequential chain growth
The straight lines represent unlabeled peptide chain. The zigzag lines represent radioactively labeled peptide chain formed after the addition of radioactive amino acid at time t_1. The groups of peptides labeled *R* are those unfinished bits attached to the ribosome at each time; the rest, having reached the finish line, are assumed to be present in the soluble hemoglobin. In the ribosome at time t_2, the top two completely zigzag lines represent peptide chains formed completely from amino acids during the time interval between t_1 and t_2. The middle two lines represent chains which have grown during that time interval, but have not reached the finish line and are therefore still attached to the ribosomes. The bottom two chains represent those which have crossed the finish line, left the ribosomes and are to be found mixed with other molecules of soluble hemoglobin. The symbols a–g indicate the tryptic peptides to be expected. (Dintzis, 1961.)

enough to be released and therefore we will find amongst the soluble hemoglobin molecules some peptide chains which have that portion of the chain labeled which was synthesized last. If we have a means of separating and examining different portions of the peptide chain of the soluble hemoglobin and if we can locate the radioactivity in such portions of the peptide chain, then we can deduce which part of the chain

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