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DOUBLE NOBEL PRIZE WINNER: FREDERICK SANGER – THE FATHER OF GENOMICS

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This paper aims to outline briefly the main stages of Frederick Sanger's scientific activity – the only person to have won two Nobel Prizes in Chemistry (1958, 1980). His work on the structure of proteins, especially that of insulin, and the determination of base sequences in nucleic acids made an immense impact on the development of biochemistry and especially on the development of a new scientific field – molecular biology. His methods for determining the primary structure of proteins and nucleic acids helped biochemists and molecular biologists to determine the structure of many proteins and nucleic acids and laid the basis for genetic engineering.

K e y w o r d s: Frederick Sanger, Nobel Prize, insulin, proteins, nucleic acids, Sanger's method of DNA sequencing.

The Nobel Prize is considered the highest honor in the scientific community. Reflecting the highest achievements one can attain in society, it has become more than just a prize – it provides worldwide recognition, respect, and prestige. It is a great honor to deliver a Nobel lecture, and even a greater honor to deliver it twice. Between 1901 and 2020, 934 Laureates and 28 organizations have been awarded the Nobel Prize. Four scientists obtained a Nobel Prize twice – Marie Skłodowska Curie (Physics 1903, Chemistry 1911), Linus Pauling (Chemistry 1954, Peace 1962), John Bardeen (Physics 1956, Physics 1972), and Frederick Sanger (Chemistry 1958, Chemistry 1980) [1]. Frederick Sanger and Linus Pauling's scientific creativity was directly related to the central focus of generating new Life Science knowledge [2]. This paper we devote to Frederick Sanger and his scientific legacy.

Frederick Sanger was born on August 13, 1918, in the village of Rendcomb (Gloucestershire, England). He was the middle child in the family of Frederick Sanger, a country medical doctor, and his wife Cicely Sanger (née Crewdson), the daughter of a wealthy cotton manufacturer. Cicely and Frederick Sanger had three children: Theodore was born in 1917, Frederick – in 1918, and Mary – in 1923.

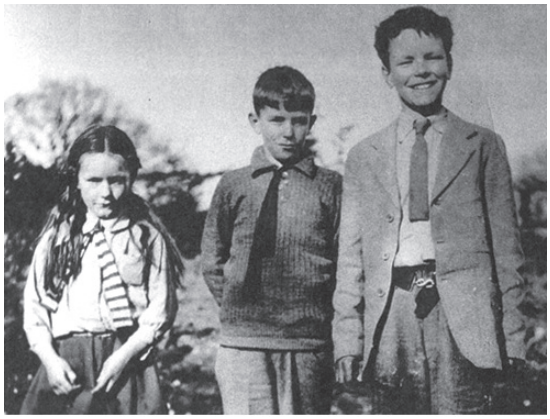
All children were brought up as Quakers as their father was a devoted religious man active in the Society of Friends [5]. The children were taught such values as truth and hard work that defined Frederick's personality. Even when he no longer followed Quaker belief, these personal traits remained. When Frederick was 5 years old, his family moved in Tanworth-in-Arden, which was closer to Birmingham. His early schooling was taken care by a Quaker governess. At the age of nine, he went to the Downs School – a Quaker boarding school. In 1932, Frederick moved as a boarder to Bryanston, Dorset.

The liberal education there had a huge impact on Frederick. Being introduced to the joy of

Frederick Sanger [3]

science and having a good rapport with his biology master *Frazer Hoyland* and chemistry master *Henry Geoffrey Ordish*, he was involved in biology and chemistry clubs [6]. Doing well at school, he passed the School Certificate exams on seven subjects that qualified him for entry to Cambridge University. In 1936, he was accepted by St. John's College. Frederick's parents had been hoping their son would follow his father's footsteps by studying medicine. However, a young student abandoned the idea as he believed that science would provide him a more suitable lifestyle and would not be so time-consuming.

In Cambridge for Part I of the Natural Sciences Tripos, Frederick Sanger took chemistry, physics, and math and biochemistry as half subjects [6]. Explaining his choice of biochemistry, terra incognita for him at that time, Sanger wrote: "*When I arrived at the University of Cambridge as an undergraduate, I had to decide which three scientific subjects I should take. I had chosen two-and-a-half subjects and was looking through the list of "half" subjects when I noticed one I had not heard of before: "Biochemistry, supervisor Ernest Baldwin". The idea that biology could be explained in terms of chemistry seemed an exciting one, and this was amply confirmed when I met the enthusiastic Dr. Baldwin*" [7].



Mary, Frederick and Theodor Sanger [4]

Ernest Baldwin was a member of the Biochemistry Department of the University of Cambridge established by an English biochemist *Frederick Gowland Hopkins* – the Nobel Prize winner in Physiology or Medicine 1929 for the discovery of vitamins [8]. Hopkins had the enthusiastic support of his younger colleagues. *Joseph* and *Dorothy Needhams* dubbed him the Fundator et Primus Abbas of biochemistry in England [9]. In 1961 during a Symposium on Biochemistry and Nutrition to celebrate the centenary of the birth of Frederick Gowland Hopkins, Joseph Needham stated: "*our meeting today symbolizes the feeling of discipleship which we all have for Frederick Gowland Hopkins, essentially the founder of modern biochemistry in our country*" [10]. When Frederick Gowland Hopkins retired, Joseph Needham moved out of biochemistry to focus on his other scope of interest – Chinese civilization and science. The Department of Biochemistry headed by Hopkins became an interesting and exciting place for Frederick Sanger. As he expected, it was here where he had a chance to acquire the necessary fundamental knowledge on living matter for solving problems in the field of medicine.

During his second year at the University, Frederick dropped physics and chose to study physiology instead, keeping his studies in chemistry, biochemistry, and math, which he was not happy with. He liked biochemistry most and it became the subject for Part II of the Tripos [5, 6].

In 1936, Frederick Sanger joined the Cambridge Scientists' Anti-War Group – a left-wing pacifist group founded in 1932 to campaign against militarism. Joseph Needham – one of his lecturers in biochemistry – was also a member of the Group. Sanger's religious background to a large extent shaped his pacifist views. Here he met his future wife *Joan Margaret Howe*. She was an economics student at Newnham College. They married in 1940 and remained married until her death in 2012. They had three children – Robin (1943), Peter (1946), and Sally Joan (1960). Frederick Sanger ascribed his wife

a key role in his successful career, saying: "*Although not a scientist herself she has contributed more to my work than anyone else by providing a peaceful and happy home*" [11].

In 1939, he graduated in biochemistry from St. John's College, Cambridge. Earning a First on his biochemistry exam, he was surprised. Being a modest person, Sanger wrote: "*I was not academically brilliant. I never won scholarships and would probably not have been able to attend Cambridge University if my parents had not been fairly rich…*" [7]. During World War II, a tribunal granted Sanger conscientious objector status and he became an orderly at a military hospital near Bristol. Frederick went to Cambridge to become a research student. As far as he did not need university financial support, he gained admission [5]. He started his PhD in October 1940 under the supervision of *Norman Wingate* (*Bill*) *Pirie*. He aimed to investigate whether edible protein could be obtained from grass. However, Pirie left the department, and *Albert Neuberger* was assigned as Frederick's new mentor. Sanger changed his thesis title and worked on the topic "*The metabolism of the amino acid lysine in the animal body*" [12]. Describing the beginning of his scientific journey, Sanger stated: "*I regard Albert as my main teacher. The most important thing he taught me, both by instruction and by example, was how to do research. I shall always be grateful for his kindness and patience. He also had an extremely wide knowledge of biochemistry, which I admired and used but could never emulate*" [7].

Frederick Sanger received his PhD in 1943. The emphasis on chemistry in Sanger's thesis was to be the mainstay of his future scientific project. After receiving his doctorate, Sanger joined a research group led by *Albert Charles Chibnall*, who replaced Hopkins as a professor of biochemistry at Cambridge. A research group led by him at the time was studying the chemistry of proteins including the structure of insulin.

Working with Chibnall, Sanger became engaged in the identification of free amino groups in insulin. That time was particularly successful for protein chemistry. New methods have been developed for the fractionation of biopolymers, and there was a real opportunity to determine the exact chemical structure of these fundamental components of living matter. Sanger's interest in chromatography methods developed by the British biochemists *Archer Martin* and *Richard Synge*, the 1952 Nobel laureates in chemistry [13], prompted him to begin work on determining protein structure.

Using insulin as a model for study, Sanger developed a new method for analyzing the structure of proteins and showed that the insulin molecule is composed of two peptide chains referred to as the A chain and B chain. They are linked together by two disulfide bonds, and in most species, the A chain consists of 21 amino acids and the B chain of 30 amino acids. Benefiting from the method of paper chromatography, Sanger identified unmodified amino acids [14, 15]. It took him years to definitively identify 51 amino acids in the molecule of this protein hormone [16].

F. Sanger worked out the sequence of 30-residue-long B chain together with the Austrian scientist *Hans Tuppy* and 21-residue-long A chain with *Ted Thompson*, a PhD student from Australia [17-21].

In 1955, Sanger completed his investigation on the insulin sequence, and his work later became the basis for the production of synthetic insulin and other hormones. From 1944 to 1951, Frederick Sanger held a Beit Memorial Fellowship for Medical Research and since 1951 he has been a member of the External Staff of the Medical Research Council.

In 1958, Frederick Sanger received the Nobel Prize in Chemistry "*for his work on the structure of proteins, especially that of insulin*" [3]. In his Nobel Lecture "The Chemistry of Insulin", Sanger stressed: "*The determination of the structure of insulin clearly opens up the way to similar studies on other proteins and already such studies are going on in a number of laboratories. These studies are aimed at determining the exact chemical structure of the many proteins that go to make up living matter and hence at understanding how these proteins perform their specific functions on which the processes of Life depend. One may also hope that studies on proteins may reveal changes that take place in disease and that our efforts may be of more practical use to humanity*" [22].

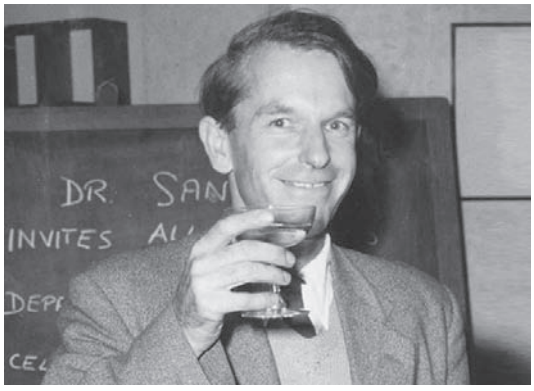
Frederick Sanger's discovery made it possible to "look inside" the protein molecule and thus opened a new era in the development of ***modern biochemistry – protein chemistry***.

Years after Nobel Prize award Sanger called "lean years". But moving to the new MRC Laboratory of Molecular Biology and joining forces with the group headed by *Max Perutz* marked a new period in Frederick's scientific life. One of three divisions was allotted to Sanger's group. Nucleic acids

that were not of much interest before came to the fore of Sanger's scientific research. According to Sanger, "*with people like Francis Crick around, it was difficult to ignore nucleic acids or to fail to realize the importance of sequencing them. An even more seminal influence was John Smith, who was the nucleic acid expert in the new laboratory and who was extremely helpful to me, so that I could turn to him for advice in this new field*" [7].

Although nucleic acids as experimental material was new for Frederick, his interest in the problem of determining the primary sequence of biomolecules – sequencing – remained unchanged. Turning attention to the nucleic acids, RNA and DNA, Frederick Sanger developed methods for determining small sequences in RNA. And later, he developed the "***dideoxy***" technique for DNA sequencing [25]. This was a relatively rapid method and was used to determine the DNA sequence of the bacteriophage φX 174 of 5375 nucleotides in 1977, of human mitochrondrial DNA (16,338 nucleotides) and of bacteriophage λ (48,500 nucleotides) [11]. This method is also referred as "***Sanger's method of DNA sequencing***". Sanger stated: "*I suppose the dideoxy method can be regarded as the climax of my research career and the fulfilment of an ambition that had gradually been forming as I became more and more involved in sequencing*" [7]. **Sanger's sequencing was effectively adopted in the Human Genome Project (2000) which decoded the three-billion-letters human genetic code.**

Sanger has repeatedly emphasized that this work owed much to his highly qualified collaborators. He had a high regard for *B. G. Barrell*, *A. R. Coulson*, and *G. G. Brownlee*, as well as for the



Frederick Sanger at his Nobel Prize celebration (1958) [23]

Frederick Sanger (left) with Sydney Brenner (center) and Max Perutz (right) at a social event at the MRC Laboratory of Molecular Biology in Cambridge [24]

students and postdoctoral fellows who had worked in the laboratory for several years.

In 1980, Frederick Sanger earned his second Nobel Prize in Chemistry jointly with *Paul Berg* and *Walter Gilbert*. One half was awarded to Paul Berg "*for his fundamental studies of the biochemistry of nucleic acids, with particular regard to recombinant-DNA*", the other half – jointly to Walter Gilbert and Frederick Sanger "*for their contributions concerning the determination of base sequences*

Frederick Sanger – the father of genomics looking at an autoradiogram of DNA sequence. MRC Laboratory of Molecular Biology [24]

in nucleic acids" [26]. An American physicist, biochemist, and molecular biologist Walter Gilbert and his student *Allan Maxam* independently devised a new technique for sequencing DNA in 1977 [27]. This amazing coincidence indicates that ideas are in the air [28, 29]. **The second Nobel Prize put Frederick Sanger in a select club of double Nobel Prize winners.**

Frederick Sanger retired shortly after this in 1983, saying he wanted to devote his remaining time to his family and hobbies. Explaining his decision to retire at the peak of his career, Sanger wrote: "*Unlike many scientists, I decided to retire and give up research when I reached the age of 65. This surprised my colleagues, and to some extent myself also. I had not thought about retirement until I suddenly realized that in a few years I would be 65 and would be entitled to stop work and do some of the things I had always wanted to do and had never had time for. The possibility seemed surprisingly attractive, especially as our work had reached a climax with the DNA sequencing method and I rather felt that to continue would be something of an anticlimax. The decision was I think a wise one – not only because I have greatly enjoyed the new life-style, but also because the aging process was not improving my performance in the laboratory and I think that if I had gone on working I would have found it frustrating and have felt guilty at occupying space that could have been available to a younger person. For more than 40 years I had had wonderful opportunities for research, and had been given the chance to fulfill some of my wildest dreams*" [7].

He spent the last decades of his life in tranquility cultivating his garden at his home in Swaffham Bulbeck, near Cambridge.

Frederick Sanger made an immense impact on modern biomedical science. In 1993, the Wellcome Trust and the MRC opened the **Sanger Centre** (now the **Wellcome Trust Sanger Institute**) near Cambridge, where a considerable part of the human genome was decoded with the technique Sanger developed [31]. Still now, Sanger sequencing remains the gold standard. *The Wellcome Trust Sanger Institute is regarded as one of the pioneer DNA sequencing centers of the Human Genome Project, including sequencing of other organisms.*

Frederick Sanger – the only person to have won two Nobel Prizes in Chemistry – died on November 19, 2013, at age of 95. His death was a great loss to the scientific community. Being described as one of the greatest scientists of any generation, Sanger remained a very modest person throughout his life. He turned down the offer of an honorary title of knighthood as he was against the idea of being addressed as "Sir". He stated that a knighthood made people different, and he did not want to be different [12].

Frederick Sanger at his second Nobel Prize ceremony [30]

Frederick Sanger has been recognized with numerous awards and honors, including Corday-Morgan Medal and Prize, Chemical Society (1951); Fellow of King's College, Cambridge Fellow of the Royal Society (1954), Foreign Honorary Member of the American Academy of Arts and Sciences (1958); Nobel Prize in Chemistry (1958); Honorary Member of the American Society of Biological Chemists (1961); Member of the Academy of Sciences of Ar-

The Wellcome Trust Sanger Institute [32]

gentina (1961); Member of the Academy of Science of Brazil (1961); Honorary Member of the Japanese Biochemical Society (1961); Corresponding Member of the Asociación Química of Argentina (1961); Member of the World Academy of Art and Science (1962); Commander of the Order of the British Empire (CBE) (1963); Alfred Benzon Prize, Denmark (1966); Honorary Fellow, National Institute of Sciences of India (1966); Foreign Associate of the US National Academy of Sciences (1967); Honorary DSc, Leicester University (1968); Royal Medal, Royal Society (1969); Honorary DSc, Oxford University (1970); Honorary DSc, Strasbourg University (1970); Sir Frederick Gowland Hopkins Memorial Medal, Biochemical Society (1971); Gairdner Foundation Annual Award, Canada (1971); William Bate Hardy Prize, Cambridge Philosophical Society (1976); Hanbury Memorial Medal, Pharmaceutical Society of Great Britain (1976); Fellow of the Royal Society of Edinburgh (1976); Copley Medal, Royal Society (1977); G. W. Wheland Medal, Chicago University (1978); Louisa Gross Horwitz Prize, Columbia University, New York (1979); Albert Lasker Award, New York (1979); Gairdner Foundation Annual Award, Canada (1979); Biochemical Analysis Prize, German Society of Clinical Chemists (1980); Nobel Prize in Chemistry (1980); Foreign Associate, French Academy of Sciences (1981); Companion of Honor (CH) (1981); Corresponding Member, Australian Academy of Sciences (1982); Dale Medal, Society for Endocrinology (1982); Honorary Fellow of King's College, Cambridge (1983); Gold Medal, Royal Society of Medicine (1983); Honorary ScD, University of Cambridge (1983); Honorary Member, Biochemical Society (1984); Order of Merit (OM) (1986); Association of Biomolecular Resource Facilities Award (1994); Honorary Fellow, St. John's College, Cambridge (2010); Fellow, American Association for Cancer Research Academy (2013) [16].

The DNA sequencing techniques Frederick Sanger and his colleagues developed during the 1970s are still being used today in genomics. His pioneering work defined genomics and provided the foundation for the way we explore genomes today, both at the Sanger Institute and worldwide.

Sanger's scientific discoveries have had a huge impact on the development of biochemistry and especially on the development of a new scientific field – molecular biology. His methods for determining the primary structure of proteins and nucleic acids helped biochemists and molecular biologists to determine the structure of many proteins and nucleic acids and laid the basis for genetic engineering.

ДВІЧІ ЛАУРЕАТ НОБЕЛІВСЬКОЇ ПРЕМІЇ: ФРЕДЕРІК СЕНГЕР – БАТЬКО ГЕНОМІКИ

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У статті представлено короткий огляд основних етапів життєвої та наукової діяльності Фредеріка Сенгера – єдиної людини, яка двічі отримала Нобелівську премію з хімії (1958, 1980). Його роботи з вивчення структури протеїнів, особливо інсуліну, та встановлення послідовностей основ у нуклеїнових кислотах справили величезний вплив на розвиток біохімії і, зокрема, на розвиток нової наукової галузі – молекулярної біології. Його методи визначення первинної структури біомакромолекул допомогли біохімікам і молекулярним біологам встановити структуру багатьох протеїнів і нуклеїнових кислот і заклали підвалини генної інженерії.

Ключові слова: Фредерік Сенгер, Нобелівська премія, інсулін, протеїни, нуклеїнові кислоти, метод секвенування ДНК Сенгера.

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