

The Story of the Scientific Discovery of Plastocyanin Dimorphism

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By

Mitko Ivanov Dimitrov

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ABBREVIATIONS USED FOR THE AMINO ACIDS AND THE IMINO ACID PROLINE

Glycine	Gly	or	G
Alanine	Ala	or	A
Valine	Val	or	V
Leucine	Leu	or	L
Isoleucine	Ile	or	I
Lysine	Lys	or	K
Aspartic acid	Asp	or	D
Glutamic acid	Glu	or	E
Serine	Ser	or	S
Threonine	Thr	or	T
Cysteine	Cys	or	C
Methionine	Met	or	M
Arginine	Arg	or	R
Phenylalanine	Phe	or	F
Tyrosine	Tyr	or	Y
Tryptophan	Trp	or	W
Histidine	His	or	H
Asparagine	Asn	or	N
Glutamine	Gln	or	Q
Proline	Pro	or	P

SOME OTHER ABBREVIATIONS

DNA	– Deoxyribonucleic acid
RNA	– Ribonucleic acid
HTI	– Chemical-Metallurgical Technological Institute (Sofia), now HMTU
HTC	– High Testimonial Committee
RLSBP	– Research Laboratory of Spectrometry and Biological Physics
ASUSSR	– Academy of Sciences of USSR
Archipelago GULAG	– a system of concentration camps in USSR during Stalin's dictatorship
PCP	– Petro-Chemical Plant
DEAE-cellulose	– Diethylaminoethyl cellulose
DCIP	– 2,6-dichlorophenolindophenol
ATP	– Adenosintriphosphate
UV/Vis	– Absorption spectroscopy in the ultraviolet and visible spectral areas
EPR	– Electron paramagnetic resonance
MW	– Molecular weight (Relative molecular mass)
Da	– Dalton (Atomic unit for mass)
kDa	– Kilodalton (1000 Daltons)
Å	– Angstrom, $1 \text{ \AA} = 10^{-10} \text{ m} = 0,1 \text{ nm}$
AIC	– an agro-industrial complex
PAAG	– Polyacrylamide gel
SDS	– Sodium dodecyl sulfate
HPLC	– High performance liquid chromatography
IPPh	– Institute of Plant Physiology
IMB	– Institute of Molecular Biology
IEF	– Iso-electric focusing
CNRS	– National Center of Research Investigations, France
FRET	– Fluorescence resonance energy transfer
IBPhBMI	– Institute of Biophysics and Biomedical Engineering
BASA	– Bulgarian Academy of Sciences and Arts

INTRODUCTION

The book “The Story of One Scientific Discovery” written by Acad. Prof. DSc. Mitko I. Dimitrov – Bulgarian Academy of Sciences and Arts (BASA) appears to be a sincere and touching revelation about the discovery of **Plastocyanin Dimorphism**, as it also tells about the people who have brought to fruition that very discovery while presenting the general atmosphere and methods of work across the institutes of the Bulgarian Academy of Sciences (BAS) during the end of the last and the beginning of the current centuries. Photosynthesis is the only most important process on Earth in which the Sun energy is directly used for a carbon dioxide fixation, and from there for the synthesis of the whole biosphere. This second-to-none process consists of **two phases** – the first one is the phase of the thylakoid reactions (known as “**a light phase**”) because it takes place in the thylakoid membranes of the chloroplast grana, the second phase is the one of carbonic reactions, known as “**a dark phase**” because it takes place in the absence of light and it runs in the inner space of the chloroplasts (stroma). During the light phase the Sun energy gets captured by the chlorophyll of the pigment-protein antennae complexes, it is then transferred to the reaction centers of photosystem II and photosystem I, thus being used for a synthesis of high energetic compounds, e. g. ATP, and also for a reduction potential (NADPH). During the dark phase, a fixation of the carbon dioxide from the environment into highly molecular organic compounds (saccharine compounds) takes place. The phase of the carbon reactions had been deciphered long ago and for that discovery the American biochemist Melvin Calvin won a Nobel Prize in 1961. Despite the epochal discovery of water oxidation (“splitting”) mechanism and the generating of molecular atmospheric oxygen, the first, the light phase of the photosynthesis is still partly clarified. The light quanta, getting onto the chlorophyll molecules are absorbed by them and it results in a transition of electrons on a higher energy level. The excited electrons return to their basic state as they pass through a cascade of intermediate compounds-carriers (redox-systems) and the released energy along with a parallel flow of hydrogen cations remains in the form of high energy compounds and a reducing potential that are used during the dark phase for biosynthesis of organic compounds.

One of the key molecules in the cascade of the electron transport from

the excited chlorophyll to the final electron acceptor is plastocyanin. Plastocyanin represents a copper-containing complex protein that functions in the thylakoid lumen and performs the role of a redox-component in the photosynthetic electron chain directly before the complex of Photosystem I. It is present in some cyanobacteria species, as well as in many green algae, probably in all higher plants, and its structure and function are being broadly studied. For that reason, the discovery of plastocyanin being present in the higher plants in a dimorphic form was to a great extent unexpected and caused a well-founded interest. This discovery allows for a number of facts to be interpreted, likewise new hypotheses about the mechanisms of the light phase of the photosynthesis to be formulated. The discovery of the plastocyanin dimorphism has been realized by the author of this book Acad. Prof. DSc. Mitko Dimitrov and the late Anthony Donchev, his close friend and collaborator. Obviously, this very discovery appears to be an original contribution to the world of science.

In his book, Acad. Prof. DSc. M. Dimitrov chronologically unfolds the way and phases of discovering the second form of plastocyanin (plastocyanin *b*, PS*b*), initially in a poplar tree and then in parsley, tobacco, female fern, and moss. A special share is given to the confirmation of the fact that only in water-photosynthesizing organisms like blue-green and green algae does plastocyanin exist in a single form. This fact shows that the two forms of this important photosynthetic protein originated during the process when plants were leaving water for land, merely with the clear purpose of more-actively-performing-photosynthesis under the influence of a direct Sunlight ensured.

It should be mentioned that the author is not content only by simply analyzing the concrete chemical approaches in his valuable experimental work – isolation, purification and sequence of the PC*b* forms of different by their complexity and character plant species, but he also tries to get some logical explanation of the cardinal question that naturally crops up – why was it necessary for nature to create another plastocyanin protein molecule? Based on literature data of the contemporary transcriptomic and proteomic analysis of *Arabidopsis thaliana* (a discovery of two individual genes for each of the two isoforms, as well as of their mutant forms), Dimitrov's results can be interpreted in favour and in confirmation of the idea that the origin of the second plastocyanin form has appeared to ensure a normal electron transfer from plastocyanin to the first acceptor protein of electrons in Photosystem I – the Ps*a*F protein, known to be a weak oxidizer, i. e. it takes away electrons from the reduced form of plastocyanin with difficulty.

Nevertheless, not only a story of one scientific discovery has been told in the book of Acad. Prof. DSc. M. Dimitrov, but also a story of comradery amidst colleague-scientists is shared. In an extraordinarily pleasant narrative style and in somewhat accessible language, the lives of all scientists at the “Biophysics of Proteins” department are followed and presented, whose story in fact is somehow relevant to the experience of most scientists at different labs across the world. The readers are to join in the events, as they may eventually turn into virtual participants in the endless experiments held, and while they may become accomplices of betrayals or otherwise celebrate success, readers may witness either the despairs or intense fervor that accompany us in our daily routines of research workers in investigating animated nature. Our readership might experience, while advancing on with the revelations, the dedication of many scientists to a single cause upon which they steadfastly pursue a scientific breakthrough. In this respect, the book of Acad. Prof. DSc. M. Dimitrov goes beyond the description of a scientific discovery and represents a chronological account of events in the lives of many scholars as it covers the process of various scientific investigations all over the world. For that reason, this book may be of interest not only to the narrow specialists who explore protein structures or photosynthesis, but also to all our colleagues, especially to the younger ones simply taking their first steps into the difficult but exciting world of science.

29.11.2012 Member Correspondent, Prof. Georgi Rousev, DSc.
Sofia Prof. Dr. Evgeni Ananiev

PREFACE



Acad. Prof. DSc. Mitko I. Dimitrov

I have never had the intention of writing this book. I was thinking that the articles wherein we had the present scientific result published should be sufficient to give enough substance to the idea about plastocyanin dimorphism as a sort of innate principle of the higher plants. The chemical analysis impartially registered the availability of two plastocyanin isoforms in the photosynthetic chain of evolutionary different plant species. The two protein molecules exhibited intriguing

differences at all levels of the structural organization, thus inferred a possible physiological motive. The meaning of this motive tempted my imagination by the amusing perspective of new experimental adventures and new ideas cropping up. Sadly, at that time I lost Tony. Darkness swallowed forever my best collaborator and loyal friend whom with I went through our exciting twenty-year journey having been in pursuit of the two blue plastocyanin molecules, and that loss marked my life further with the sign of an unending grief. His untimely death categorically obliged me (when describing events and the way we literally survived together in chemistry labs, at home and elsewhere in the world) to preserve an eternally living memory of him – a thing he undoubtedly would deserve, had he been around.

The events preceding the present scientific achievement began in the distant year of 1959 when Dr. Sakae Katoh discovered the plastocyanin molecule under fortunate circumstances. Next, some detailed investigations of the unknown chemical entity were realized yet to come to the eternal, still inevitable question: “Why has nature by means of its evolution created plastocyanin; what was nature’s physiological motive?”. After a series of changing events, Dr. Katoh localized the correct site of plastocyanin in the photosynthetic chain, thus getting the true answer: Photosynthesis is a process which includes two reactions of light! This is

the next triumph of a scientific truth against ultra-conservatism, thus having immediately sent into the historical archive of science the wrong concept about a photosynthesis with three reactions of light.

However, neither Dr. Katoh, nor our colleagues across the world who have shown interest in the new molecule have ever assumed that in the higher plants plastocyanin may appear to be a mixture of two structurally different isoforms. Anyway, whims of fortune proved for the subsequent events to be somewhat analogical to the above mentioned, as the several small drops of the potassium ferricyanide oxidizer that we added to the total plastocyanin mixture turned out to be our lucky chance. On the white background of the chromatographic column, the oxidized blue fraction split into two sub-fractions that we specified *PCa* and *PCb*. *PCa* happened to be a type of plastocyanin investigated in different aspects, whereas *PCb* was an unknown plastocyanin substance. Our lengthy investigations showed that this plastocyanin dimorphism is a rare peculiarity of all higher plants. And again, the inevitable and inexorable question about the physiological significance of this natural phenomenon remained for us to answer. Many years had gone by until we reached the conclusion that *PCa* and *PCb* realize different electron transport functions in the higher plants, “serving” the cyclic and the linear electron flows of the popular Z-scheme of the photosynthetic chain, respectively. It seemed as if this dimorphic physiological motive appeared to have been the long awaited criterion suggesting the validity of the Z-scheme in the higher plants. In contrast to Dr. Katoh’s case, in our case, the other one – the alternative scheme of autonomous-cum-synchronous-parallel-functioning-in-a-tandem 2 photo-systems still have the chance to survive, if its probable existence in lower plant species happens to be proven.

The evidence of the genetic status of *PCa* and *PCb* by Kiselbach et al. confirmed my strong belief about how real and universal plastocyanin dimorphism is. Moreover, the indicated by Pessaresi and Abdel-Gany preventive functions of *PCb* gave me a reason to assume that this phenomenon keeps other unknowns awaiting their further research. I am sharing all this having great hope that the visible perspective of the plastocyanin dimorphism being subject to a new study may intrigue the young generation and may push them into a new course of a true realization, for I believe in the typical-of-young-people adventurous spirit, self-confidence and optimism. On the other hand, I greatly desire for the new truths of this scientific achievement to be acknowledged once and forever their Bulgarian origin.

In this book I presented the ideas, the people, and I described us following the meandering way towards the discovery of plastocyanin

dimorphism – characteristic of the photosynthetic chain of the higher plants. Herein, apart from the compulsory scientific sections, some paragraphs of a mere narrative writing are present. They reflect the-inevitable-to-the-existing-of-each-ordinary-creature incidental occurrences of great emotional intensity which to some extent had their own effect on the rhythm of our twenty-year research marathon. The absence of Tony had undoubtedly troubled me deeply at recalling details about certain occurrences which the distance of time tenaciously strived to remove from my burdened memory. However, I consider that in my consciousness the main events around this very discovery still remain quite vivid and I attempted to share them correctly whilst unfolding in their right chronology. Hence, the present book uncovers my personal recollection for the authenticity of which I fully accept moral and ethical responsibility.

I would like to ask the valuable reader to pardon my style of writing for I have widely used professional terms while revealing the scientific events, which to some extent will most probably perplex the general readership. I have made our discovery known, bearing all the good intentions for the next generation of enthusiasts, have they decided to follow our steps to refer to the present book as a useful methodical tool. At the same time, I tell the sad truth about what a supreme effort and compromises a Bulgarian scientist has to make once he dares keep abreast with the leading scientific teams in the world.

I am very much obliged to Prof. DSc. Milka Sechenska when she entered timely the world of my scientific animations, as also with her perspicacious editorial notes she helped me produce my book in its present form. I have always looked up to her noble scientific erudition and greatly admired her unique literary expression.

I am sincerely grateful to my one and only teacher of literature from the secondary school, Ms. Yonka Yanakieva. Thanks to her, I had cultivated some good taste for the literary genre and I am happy that fifty years after my graduation from First High School St. St. “Kiril and Metody”, in Veliko Tarnovo, Ms. Yanakieva made the first corrections of the current book (the author refers to the Bulgarian edition) by applying the grace of her literary talent.

I am, also, especially grateful to Ms Antoaneta Dimitrova – a pretty charming, and yet creatively inclined associate of mine. Apart from having become my accomplice in narrating all the events linked to the lengthy process of the *Plastocyanin Dimorphism* scientific discovery she has made an improvement on the entire story in her peculiar but sensitive manner.

Each and every story has its own end. The end of Plastocyanin dimorphism, however, came up suddenly and untimely. My quite surprising superannuation followed by discarding my plastocyanin subject matter (it was outright ended), the dissolution of my work team and the mere act of giving away my laboratory to another colleague discontinued too soon one promising research campaign. For such an absurd situation the French soothingly say: “C’est la vie!” (Such is the life). The planned-in-advance topics of this scientific program concerning the interaction of PCa/PCb with their natural redox partners, the visualization of their associations, the *in vivo* data in favour of the Z-scheme, and the reconstruction of the plastocyanin as part of the photosynthetic chain in terms of the dimorphic character of the plastocyanin molecule, as well as Tony’s great wish to take part in these analyses, and furthermore having been deeply frustrated by his untimely death, all of it later put me to stand up before the alternative of a new beginning, for which the pragmatic German well-meaningly have a saying: “Jeder Anfang ist schwer!” (Every beginning is hard!). But nevertheless, this beginning proved to be an easy one, because a selected society of élite Bulgarian scientists and talented creators of different areas of art founded (lead by their most noble intentions) the Bulgarian Academy of Science and Arts (BASA) in 2004 and cordially invited me into their league. I’ll always be much obliged to the President of BASA Acad. Prof. Dr. Grigor Velev and to my boldly courageous referrals: Acad. Prof. Dr. Miladin Apostolov and Acad. Prof. Dr. Margarita Kamenova for having had me introduced to this society, thus enabled me to endure the hardship of my scientific enthusiasm, as it also helped me accomplish the process I started with my fellow-colleague Tony, but sadly, we couldn’t finish together. These following reminiscences I dedicate to the sacred memory of my late friend Tony.

The author

THE BEGINNING

I dedicate this book to my friend Tony

1

My juvenile imagination bears a mythical notion about the society of scientists being a congregation of scholar-paragons who share a common purpose and look at the same direction, the aim of making scientific discoveries. The phenomenal achievements of Einstein, Bohr, Watson, Crick, Mendeleev, etc. have dramatically excited the growing-up generations possessing the typical for young people adventurous spirit, self-confidence and optimism. The gravitation force of science irresistibly attracts many of those who have succeeded to conquer Alma mater. The chance of success in science, however, calls upon those to whom idealism, romance and diligence are constant values of their human character. Trying to be one of them, on 21 September 1967 I stepped inside the heart of the “temple of science” – BAS, since research work had always been the prime dream of my early youth. It is impossible to forget that date, for date of birth is the last thing one may forget. I was exactly 25 years old, I believed in the code of honour, I lifted weights of 150 kg, and I thought the Moon was made of green cheese. This illusion would later bring me quite a lot of troubles and problems. Nevertheless, right there I was in front of the department of “Natural Organic Substances” inside the Institute of Organic Chemistry – BAS with the naive self-confidence of having gained enough amount of professional experience which should guarantee me a somewhat sudden and easy success. My first director of studies, Assistant Prof. Dr. Radoslav Vlahov, welcomed me with a sobering phrase that things learned at University were good, but the real professional growth was to begin today, and it would end on the last day of my career. The startling to me nature of these words, then, made me ponder over things. It also made me take the wrong decision – I went home exactly at the end of my first working day. Dr. Vlahov did not hesitate to make a remark that an early going home wouldn’t guarantee a future success, so on the next day I went home at 21:00 sharp, simply having it turned into a habit even until today. Particularly, I was not prejudiced about taking the first steps of my career. It was the time of the

so called Planned Economy in Bulgaria. Then, our Institutes of Higher Education were throwing up, as if released off the production line, graduate “creatures” and all institutes were infested with specialists. So in this cocktail of capable and incapable ones dealing with science, high ambitions for career-making were churning inside, as all that was spontaneously triggered off by the criteria set for development of scientific cadres, which was actually based on the number of articles published and the impact factor. The non-standard approach – a real scientific result to be recognised as a scientific discovery – was missing as a criterion (and still is), and the lack of a solid financial and technical security had blocked the way to great success. And to cap it all, contrary to my desire to devote myself to fundamental science, I was assigned a topic of industrial applicability. It required frequent business trips to DIP “Bulgarian rose” – Plovdiv, for the next 20-litre container of sulfate turpentine by-product that would otherwise be readily thrown into Maritsa River guaranteeing its solid contamination. Such was the aim that by appropriate treatment of this unpleasantly smelling complex a mixture of pleasantly smelling substances be obtained. Undergoing a series of all the chemical reactions, a chlorination by aeration of the mixture with chlorine was involved – taking place in a bucket and in the presence of iron shavings. A single unreasonable leaning over the bucket, however, ensured my inhaling a little chlorine which appeared to have been enough to put me into a state of a tearing cough, as it also caused a lavish excretion from my mouth, nose and eyes. After about 15 minutes of tragicomic trance I succeeded in making a quick recovery, as it made me to never ever forget that Chemistry is great, yet rather dangerous a science that everyone should always regard with respect and veneration. Having experienced a not that optimistic beginning, this accidental event appeared to be a light in the tunnel, as it shattered the skepticism which was overpowering my mind about the sense of tolerance amongst colleagues, attitude of good-manners, and sympathy for a teammate.

1969 was slipping away when on a December day I was unexpectedly surprised by having been stipulated a deadline of only three days to synthesize and to isolate chlorine-sulfonic acid from the above mentioned mixture of 1.5 kg. I timorously argued the absurdness of this undertaking, objecting that after a potentially dangerous chlorination another slow rectification follows, then a crystallization of the mentioned acid takes place, but the imperative contra-argument of Dr. Vlahov that after three days there are three nights to follow, chilled my enthusiasm to demonstrate an opposing attitude, so I desperately made an attempt to bring to fruition the evident impossibility. I was not ready for an emergency situation, I did

not even have a written permission for a night-shift work, which would have eventually gotten me into a state of committing an offence at the workplace by simply breaking the rules. I naively thought that the heavy and dark window-curtains might hide the bedside lamp I decided to use because of its meagre light. Yet, a treacherous beam of light must have slipped through, for about midnight there was a knock on the door and in its doorframe stood alone the director of the institute. I had already developed some respect for Acad. Bogdan Kourtev, back then, due to my slow understanding of the rather complex world of organic molecular structures about which he was nobly trying to make us cognizant during our university studies, as that knowledge unfortunately, he would present to us in a low and barely audible voice. This time now his voice was faint again, and what on earth for I felt as if ants were crawling all over my back. Sternly, he asked me what the purpose of my midnight enthusiasm was. Of course, I shortly explained all, yet in a voice so subdued. Then an imperative phrase followed: "Come with me!" and off I went after him. It felt as if I was accompanying him to the end of the world. The academician, rather advanced in his years, was surprisingly conquering the steep staircase by taking three steps at-a-time. I was struggling to catch up with him despite my youth and my good physical conditioning, as if the muscles of my legs were not mine. I had the feeling of a strong sensation and I shivered in an anticipation of a grand adventure. Suddenly, all of it was over, as in a story with an unexpected end. The door of his office wide-opened and I found myself in front of the familiar huge table covered with the green cloth, where only a year ago during the exam on his scientific subject I thoughtfully speculated on the oddities of one complex organic molecule and in the end, though with some effort, I succeeded to persuade the academician on my broad competence. The academician rummaged through a locker and came back to me with a luxurious box of chocolates which he momentarily placed in my hands expressing his cordial wishes for my future success. Maybe the higher professional status of the academician was the reason why I never saw a smile on his face. This time once, however, the academician smiled at me – for the first and last time, and I shall always be thankful for he made me preserve the image of paragon-of-virtue a man whom I had just touched, because to all of us Academician Kourtev was one of the classical scholars of the Bulgarian organic chemistry.

The world remembered 1969 with an epochal heroism. The crew of Apolo-11 landed on the Moon. Neel Armstrong's airborne phrase: "That's one small step for man, one giant leap for mankind" was etched forever into the world history. The brilliance of this triumph a kind of pole-vaulted

the iron-curtain, as though it undoubtedly bedazzled our young generation. But nevertheless, mere common sense was to exclude any promising future related to similar feats under the cosmic contest, for small countries like Bulgaria were not to have their fair chance. As for me, I lost interest in digging into the sulfate turpentine being the right thing to do. Something had to be changed, and, the change came.

2

I remember 1970. It was the beginning of days when women started wearing miniskirts, men keeping their hair long, and the “The Double Helix” was released. Written in an improbable literary style, that book of James Watson categorically persuaded the reader that 20th century had another unrivalled triumph – the discovery of the DNA-structure: the carrier of the genetic information. This discovery had utterly changed the biological science, it inspired a series of scientific investigations and rejected forever Lisenko’s retrograde concept. The philosophical interpretation of progress as a motion ahead along a spiral, found its symbol in the double-helix DNA-structure as the winding ladder responsible for the essence of life. The book is with somewhat a narcotic effect and I found myself to be among those people to whom having entered the enticing world of biological sciences it became an unconquerable desire for me to pursue. Not that the organic chemistry is an area that has not being exhausted yet, rather the organic-metallic compounds of Academician Dimitar Ivanov have remained forever in the aureole of all higher scientific achievements, whereas both organic the analysis and synthesis still inspire with their possibilities for the search and formation of new material substances. Nevertheless, the great mystery of life locked in a single living cell irresistibly entices and tempts, for I had made a decision to turn my back to the sulfate-turpentine perspective once and for all in favour of biochemistry. I had to find a suitable place under the Sun and it appeared to be the Central Laboratory of Biochemistry (CLBC) of BAS. I remember well that in 1970 it was not easy at all for me to apply and fight for a post-graduate work. The highly swollen population of students at most institutes resulted in approving of a reduced number of entries, thus leading to shortlisting several candidates per competition, whereupon formulating irrational rules for selection of cadres ensued, i. e. the so called “brother-in-law” syndrome (the notorious nepotism), which drastically reduced the optimism of most common mortals. I personally felt the impact, since I had personally nominated myself a post-graduate-student candidate and I applied at both HTI and the

Sofia University St. "Kliment Ochridsky". They did welcome me politely, as in the same manner did they politely send me off by hinting to me not to waste my time, because contests were advertised to favour particular individuals. My feverish search took me to CLBC where, as I had intuitively assumed, the first two tempting competitive themes: one on matters of DNA and the other on synthesis of medicinal substances with anti-tumor effect had been already "booked". The third theme, however, was surprisingly vacant. It was formulated: "Cell bioenergetics influenced by different pathologic states", under Assoc. Prof. DSc. Racho Rachev. The lack of other candidates raised some suspicion within, telling me that something was not as it should be. I could understand the truth later when I would experience unasked-for a burden. Now, however, the long coveted aim for a successful performance at the examination threw me into a feverish preparation and I began to fill my brains with all sorts of truths on ATP being a source of energy to both human and plant organisms, as well as I started digesting all about the Krebs' cycle plus the conjugated with it complex chain of respiration, and the inherent three chains of oxidative phosphorylation which unharness themselves into different levels during thyrotoxicosis. Having the desire to impress the exam-panel I took the liberty, during the written examination, to present the so called multistage reactions as a series of complex electron transitions. My overzealousness, however, didn't much delight the chairman of the jury Prof. Toncho Radev, who had got used to looking at all these processes in the light of classical biological notions. So that, on the following day during the oral examination, whereat I again frivolously and imprudently continued to perform the organic chemistry scenario, he criticized me severely on the hard-to-be-regarded metaphysical notions of mine and he was trying persistently to convince me that if I wanted to venture into the field of biology I had to learn to look into all its problems through the eyes of a biologist and not using the approach of an organic-chemist. Ultimately, the professor deducted a whole half-a-point from the overall grade mark, yet what remained of it (5.50) was more than enough for me to be accepted into the Bulgarian Biology society. What awaited before me was a quick experimental start, yet again my new leader hurried to chill down my enthusiasm with the news that he was to go to the USSR for a year, in order to write a book. I ought not to feel much anxious because my forthcoming compulsory examinations would make up for his absence. The exams came, then ended, and I eventually received the disappointing news that Dr. Rachev extended his stay in USSR for another year. Thus, I dared act over his head in order to give an attempt on my first experiments and within a-month-time I wasn't facing any problems at all until the

period of assimilation of the methodical approaches had been going perfectly well when the managing elite issued equally a-logical-cum-an-illogical verbal order upon me to cease all my experimental trials. The logic of that interdiction was based on the formal reason that I did not have an-approved-of post-graduate theme by the Scientific Council, whereas the illogical aspect was based on the nipped-in-the-bud enthusiasm and the accumulated waste of time (a dear asset, as it never comes back). In order to get out of that unexpected state of weightlessness I took to some intense reading of various literature and some entry-terms like: viruses, bacteria, DNA, RNA, proteins, nuclei, mitochondrion, microsomes, so that a lot more biochemical terms began to relentlessly engage my consciousness. However, one month before the returning of the chief, a shocking offer from a high-standing authority of CLBC disturbed the idyll of my actual status, but I was foolish enough to frivolously decline the invitation. They said I could be given a new post-graduate student theme related to RNA, as the outcome might as well be granted publishing in elite scientific journals, I also could be provided untroubled dissertational defence, long-lasting specialization beyond the iron-curtain, in short, all perks and benefits of the scientific world, except for the Nobel Prize!? I was aware that Dr. Rachev didn't share the traditional policy and tendencies of the institute biochemical elite and in the long-standing intrigue I saw clearly for me one, not quite optimistic a perspective. Only now I understood the reason behind the lack of rival candidates for post-graduate students in the competitive 1970. The demoralization that seized me, however, imperceptibly sank into the busyness of the initiated experimental activity and again my future looked rather promising. In 1976, I already knew that the iodine ion successfully realizes thyroxin-mimetic effect on the synthesis of protein in an animal cell and that the radioactively-measured-by-its-iodine-atoms hormone preferably iodine-ates the non-histone proteins of the cell nucleus with a presumption of some influence on the gene expression. The path to verifying these facts, however, wasn't covered with roses. Back then, Acad. Rumen Tsanev who was being in-the-best-of-his-condition alongside his scientific team were chasing science through hard work and strenuous effort, every working day till 11 p.m. As for me, I couldn't afford the luxury to get a hold of the expensive technique. I had to work at night all-year-round, during 1973. Over one weekend I had so much zeal exploring, that I overstayed in the hot isotope laboratory, as I spent three days and three nights inside. It was on the last night that I went fast asleep inside the last midnight trolley-bus going back home. At the terminal, that man – Mr. Know-it-all driver – shook me deservingly and with an outright conviction stated his temporary diagnosis when he

announced me a well-indulged-at-some-tavern bon vivant. That was that.

When finally, the painstaking process was over I welcomed the end of my elaborate experimental work with relief as I was already seeing the outlines of my dissertation content, but I wasn't able to see that some purging rite from old times stigma for an old "sin" of mine was expecting me. My approbation defense (got failed on purpose), the official defense – turned out dramatic, yet successful and later the mysterious disappearance of my documents from HTC one day before the respective session looked like a punitive action that turned into a memory stored deep inside, which I would always wish to get rid of, yet it might never be able to escape me. At such real-life circumstances, it would be more than naive to hope for somewhat equal a status amongst the Biology elite of people where everyone was living with their steady sense of originality, rather uniqueness. So, I would not ever like to waste my energy in a search of some sensible explanation as to why these otherwise gifted and literate persons have cultivated within themselves a valuable system of shady rules, distorted moral and ethical norms, and moreover – lack of a sense of realism. You may be curious as to what happened with my mysteriously vanished documents, there, in HTC!? I will never forget my valiant reviewer Prof. Nicho Ivanov who anxiously phoned me in the late afternoon, one day before the session of HTC, about the issue concerning my strangely vanished documents. Although having been at low spirits of a doomed person I headed to the Agricultural Academy at once, but there in the respective offices, the invaluable cardboard box containing the documents was missing, and the door-keeper of the building unknowingly and indifferently was shrugging it off. I began getting around the floors and asking people at random, who of course knew nothing or whatsoever, and later while I was developing the opinion that I was looking for a needle in a haystack, armed with a broom and draped in a blue cotton-duck apron an aunty hurried past me on the way to her noble daily chores. And God only knows why something made me share with her my problem of that invaluable cardboard box. She said at once: "Over there, at the museum where the stuffed wolves are, something like a box has been gathering dust wallowing". It suddenly made me happy, and her specific dialect of the Sofia district tingled in my ears as a charming and tender refrain, still until today it resonates. I have never had any sort of intended dialogues with God, yet until today this fact brings to my mind thoughts about God's great justice, meaning that on whatever occasion when called upon, God restores the needed harmony in our troubled world. Do you believe in that sort of thing? As you wish!

I vividly remember my post-graduate internship through some frequent disputes with Dr. Rachev over the enforced by him experimental style. Having accomplished his studies at the so called worker's faculties, Dr. Rachev was naturally partial to certain specific aspects of the historical period of his days. The Stakhanov method of work based on the notion of a quantitative production was widespread at all levels of the social order then. My superior was firmly obsessed with that trend and his strong belief that 'when in a large quantity the results, if only 20% are accurate, it appears to be quite good an achievement' was throwing me into a big confusion and lots of doubts. Some rather painstaking and of-large-scale experiments had been planned, thus it predetermined to lead to a physical exhaustion and to a kind of natural lowering of the analytical precision. For a comparison, I may firmly state that even micro-quantities of a substance are sufficient enough for a successful chemical analysis. This vicious style was predicting mostly a defeat rather than a possible success. So, to prevent turning my dreams into a mirage I once again stood on the crossroad of fate asking the troublesome question: "Which way to go now?"

3

Assoc. Prof. Dr. Julia Vassileva came into my life with all her mightiness of possessing a respectable complex of managerial skills. I was astonished by the reactive speed at which she put together a contemporary and well-equipped laboratory with some intricate name RLSBP. Dr. Vassileva needed biochemists for the laboratory and I, the newly hatched Master of Science, found my place in her overpopulated team, losing, however, my Academic rank of Assist. Prof., Second degree. My rehabilitation through a new government contest came after nearly two years, whilst during that time I had the "privilege" to live the simple life of an expert chemist in a simple basement lodging. The new workplace was calling for new scientific themes, research was pressing upon us, and within a short period of time we were able to find out that in a specific animal tissue the associated-with-luteinizing-hormone receptors manifest prolonged life-cycle in correlation to prolonged adenylyl-cyclase activity. The analyses were gaining momentum as if a promising research perspective was surfacing this time, when in 1982 an event of a political type affected fatally Dr. Vassileva's professional status. Consequently, the personnel department underwent a revolutionary reorganization, but still possessing the democratic right to make their own choice on a new course of science experimenting, at any time they wanted to. I was in my early 40s! At such

age, the perspective for a new start in the field of science would hardly delight even the most positive optimist. It was so natural a feeling that some thoughts were beginning to germinate in my mind as they were ever more intrusively developing the mood of a defeated one. It also made me feel sorry for time earlier wasted, for some opportunities wasted, and for dreams that would never come true. It is illogical, though, for someone of common sense who has been chasing scientific dreams for over 15 years not to accept the fact that at least one last attempt should be made. Then and there, those eventful circumstances generously offered a fresh start! Frankly speaking, the study of bio-membranes as a main subject of the Institute of Bio-Physics (IBPh) wasn't charging me up with much enthusiasm, I was imagining that the generally accepted membrane-structure as a phospholipid bilayer had been exhausted a model, and it was barely worth searching for some undiscovered scientific truths, right there. It must have been a wrong notion though, most likely due to my being an amateur in this scientific area. I must admit that at that time I was deeply impressed by proteins, by their wide spreading in the biosphere and I was solely intrigued by their structural and functional variety, as all that gives sense to the idea that life is a form of existence of protein substances. Despite having been said by the prominent apologist of orthodox communism, Friedrich Engels, this phrase remains forevermore true with its unequivocal sense both in philosophical and biological aspect.

FIRST YEARS IN MOSCOW

1

The fact that Assoc. Prof. Dr. Boris Atanassov had been invited to join in IBPh resulted in establishing the “Biophysics of Membrane Proteins” Dept. Dr. Atanassov graduated from the Moscow State University (MSU) and he belonged to a famous generation of Soviet capacities as regards protein chemistry, the generation of 1935 – Ovchinnikov, Egorov, Abdulaev, Kazakov, Grishin, Arutunian, etc. who had left their lasting marks in the world of biochemical science. As a scientist, Dr. Atanassov was active in BAS as a great expert on the nature of proteins and everybody’s opinion was that he possessed the real brain capable of containing somewhat enormous scientific information, hence he appeared to be the right leader of the newly created department. I have always thought that excessive burdening with various information may distance man far off from the possibility to focus on more concrete an aspect in one’s attempt to achieve a high scientific result. More important was the fact that somewhat reasonable for science a chance appeared, and it ought to be used. In my opinion, Dr. Atanassov happened to be the right man. The task assigned to me, to invent a quick and rational method for isolating leg-hemoglobin from soya bean tubers, was performed in the shortest time-span, therefore it was no wonder that the very fact moved my new chief, because he generously promised to lend me a hand for my specialization abroad, knowing it had been an old dream of mine. Those were the days of the so called “mature socialism” and my travel to the western world and my stay there would hardly be an idle walk. Years were quickly passing by, time was not to be wasted and I accepted the urgent variant to go to the USSR, with great pleasure. Thus, on June 1983 I set off on my journey aboard the international express Moscow-Sofia-Moscow to the Soviet capital bringing with me the inspiring list of names of those eminent Soviet scientists, as well as having a recommendation from Dr. Atanassov for a specialization, and also with the sensation that something in my life was going to change. Albeit the fact that I represented the traditional poverty of a Bulgarian scientist, I myself financed this private expedition as I had also made use of the generous invitation of Engineer Ognyan Stoichkov, who was then studying Foreign Trade in Moscow and whom with we had had our solid friendly terms

established since the English language courses we attended some time back. This energetic young man would later strengthen his position as a prosperous German businessman and whenever needed, he would gratuitously emerge in my life as a vitally important sponsor. Everyone in life knows many people, but has only a few friends. Ognyan will remain my friend for life. But nevertheless, he was there for me to welcome me in a warm way, as he also had the arduous task to help me get used to the busy and dynamic megalopolis of Moscow. And he handled successfully that task, because Moscow by its size and quite busy day-to-day lifestyle can confuse and discourage every ordinary man like me. I visited several institutes of ASUSSR in Moscow and Pushchino, I met personally eminent scientists like Volkenshtein, Mitin and Arutunian. Then two days before my departure I received the invaluable invitation for a specialization in the following year, 1984, personally signed by Jurij A. Ovchinnikov – his name, a legend in the Soviet bioorganic chemistry. On the following day after my returning to Bulgaria I showed some unacceptable naiveté by leaving neglectfully his invitation on my desk. Several minutes later, the invitation disappeared without leaving a single trace. Not a single one of all alerts, rather warnings that I submitted to head of department on my appeal for official investigation succeeded to threaten the ill-intentioned person or make them return the paper. Just now, I remembered another catchphrase by Konstantin Irechek, about the Bulgarian's psychology “what troubles me in this country is the marvelous delight all people bear and project when standing in the way of each other and moreover, when everybody is merely taking care of how to spoil the work of the other one”. I sadly concluded that a hundred years later this epigram would be woefully true. Fortunately, the outcome of this deplorable act was of no fatal consequence. After having sent a request letter to ASUSSR I soon received a duplicate of the invitation, by post. I had it kept in a safe place and began to wait impatiently the nearing of 1984.

2

Early morning, on 01st Oct 1984, I flew off to Moscow. I was carrying with me an impressive quantity of 10 gr of clean leg-hemoglobin *a* (*Lba*) from soya bean tubers, but that didn't fill me with great spirits because this protein had already been well investigated in structural and functional aspect. To shed a light on it, this protein has $MW \approx 15400$ Da, it possesses an active center with a ferrous-porphyrin ring and is found to be an important element of the nitrogen-fixation chain. *Lba* remains stable at liophylization that makes it easy to be kept in a storage and its well-known

primary structure makes it a convenient substance for the learning of various analytical procedures applied while determining protein amino acid sequences. This protein, however, would prove to be that lucky joker changing my fortune both in personal and in purely scientific plan because it brought forth a series of successful inter-connected events.

At the airport Sheremetievo-2 I was welcomed by my close friend Ognyan Stoichkov who had provided for me pretty comfortable transport on a nice limousine. It was a rather generous gesture, for in Moscow compared to Sofia the distances seem to equal cosmic measures. Shortly afterwards I was in the company of Dr. Tatyana Odintsova (available then and there to meet me personally, too), who charmed me not only by her jovial smile but also by handing me the shocking sum of 330 rubles which at that time appeared to be dear money. Once on the limo, we dashed off to the city center. Soon after we had neared the to-stop-tanks hedgehog spread used to mark the lane whereto the German army reached during the Patriotic war, I grasped the fact that Moscow could have been defeated without field-marshal Guderian's tanks, so long as one had come there as good-hearted and well-wishing a friend. Meanwhile, I was informed that my leg-hemoglobin (a protein studied in detail) had inspired not a single one of the dozens of these Soviet specialists who were to make a decision on my further specialization. During the heated debate that was going on, one after the other they all turned down my application for internship, except for one man – Prof. Egorov.

The 30s of the 20th century marked an unprecedented progress of the Soviet economy. The potential of their colossal manpower created structures and facilities like Dneproges, Volga-Don and Belomor canals, aslo huge factories for the heavy industry, and the agriculture subordinated to the communal cooperative. Under the all-see eyes of an all-powerful dictator, the people of USSR turned one utopia into a steady dream. Prof. Tsezii Alexeevich Egorov was born in the middle of that excited decade (06.04.1935), thus probably his name was namesake to that interesting element (caesium) on the Mendeleev table. In the course of time, Prof. Egorov will justify completely his chemical name, as he will join the famous cohort of Soviet Chemistry scientists from the generation of year 1935 mentioned above, and he will excel them by his unique talent of an original technocrat. Just once, however, due to whims of fortune, this obvious superiority will play a practical joke on him, thus threatening to spoil his brilliant scientific career. Under the financial aegis of the Military and Industry complex, the Institute of Bioorganic Chemistry (IBOC) of ASUSSR was built in Moscow. This ambitious by its scale of planning edifice, yet imposing a building by size needed some avant-garde

technological equipment. This important and responsible job had been assigned to Prof. Egorov but it required both – to be well informed technically, whereat he is undoubtedly the most eligible one as it demands good knowledge of the financial affairs. The latter probably must have been the vulnerable part of this brilliant scientist, because one single and absurd financial mistake had put him into a dilemma to undergo a court-trial or to voluntarily move to the Institute of General Genetics (IGG) in Moscow. He preferred the second option. Soon after that I appeared into his world. Until today I don't know Prof. Egorov's motive to lend me a hand back then in those years, but it hardly was the not-too-enticing as an object of investigation leg-hemoglobin. It might have been some natural reaction for overcoming an undeserved transitory isolation, something about, considering my moral and ethical values, I would never dare ask him for I may dip my finger into a sore wound. The important thing was that due to the so called marketeer circumstances I entered into the scientific world of a high rank specialist in the field of protein chemistry, and in that respect I may say I had a streak of good luck. I still feel sorry that at that time I could not surprise him either with any original idea (such one I still did not have) or with any unknown protein substance (such one I had not discovered yet). This would occur after two years, but at that stage I knew nothing about it, neither did Egorov or anybody else. It was 1984, I was 42 years old and I was yet to learn methods of isolation, purification and analysis of proteins. The world of these super complex molecules in the whole biosphere is rather attractive and full of challenges, so even at that age it was worth beginning to explore their nature.

Usually, as a prime rule during experimenting a chemist follows a routine methodical procedure having his focus and attention engaged to the very end of it. Any distraction may lead him to the unfavourable chance of error-causing, thus making him start all over again, back to square one. Prof. Egorov seemed not to feel much troubled by the strict character of that rule. He liked, while working, to demonstrate and explain his temporary scientific obsession and he would encourage everyone who would poke his nose into his hands of "an officiant". Undertaking the role of a genial bore I succeeded to learn from him some fundamental methodical procedures innate to the traditional strategy on studying protein, within three months. In the classical protein analysis, it is compulsory to determine the N- and C-terminal amino acid residues, the total amino acid composition, the blocking of the reactive -SH groups, the breaking of the polypeptide chain to suitable peptides, the peptide mapping and the Edman's reaction. Respectively, the priorities of my specialization were the Dansyl method, treatment with carboxypeptidase, using some amino analyzing apparatuses, alkylation of -SH group by 4-

vinylpyridine (4-VP), treatment of a polypeptide chain by trypsin, chymotrypsin, glutamic protease, bromosuccinimide and cyanobromid, chromatographic separation of the obtained peptides and applying the Edman's degradation for determining the protein amino acid sequence (primary structure, sequence). One curious fact pushed me to go a little further into some professional details. A routine procedure supposes an isolation of S-containing proteins by means of their immobilization in alkaline conditions by formation of S-S bond on the adsorbent thiopropyl-sepharose 6B. By means of buffer elution the S-non-containing proteins are to be removed. After treatment with β -mercapto-ethanol, the S-S bond breaks and the S-containing proteins elute as a separate fraction. The method is convenient for putting into practice a detailed examination of the protein sequence, too. The breaking of the immobilized protein through a series of specific reagents and the appropriate arrangement of the abundant composition of the obtained peptides may facilitate and even determine the very sequence. In that regard, Prof. Egorov offered his own rational idea. Unfortunately, the appropriation of scientific ideas and their results is not an isolated occurrence in the scientific world, as only one year later would Prof. Egorov be able to accept a bitter disappointment – his personal results were published without his co-authorship, and even more without expressing any gratitude to him. Being an honest and reputable person both in life and science, Prof. Egorov became quite disheartened to be able to live through the consecutive series of many more acts of injustice and ingratitude that had befallen him.

At the sectional seminar held at IBPh in January 1985, I presented a detailed report about the work accomplished in Moscow. The positive comment from my chief and my colleagues certainly satisfied me, but that coveted uncertainty awaiting to be deciphered through the methodical skills acquired by me was somehow missing. It was soon going to come along with one boy with an expressive face, olive eyes and illustrative moustache, who was attentively listening to my scientific report. That was how in my life would come in and remain forever Tony.