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HISTORY AND BIOGRAPHY

Louis Nico Marie Duysens (March 15, 1921–September 8, 2015): a leading biophysicist of the 20th century

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Abstract Louis Nico Marie (L. N. M.) Duijsens (Duysens) was one of the giants in the biophysics of photosynthesis. His PhD thesis "Transfer of Excitation Energy in Photosynthesis" (Duysens, 1952) is a classic; he introduced light-induced absorption difference spectroscopy to photosynthesis research and proved the existence of reaction centers, introducing advanced methods from physics to understand biological processes. Further, it is his 1959–1961 seminal work, with Jan Amesz, that provided evidence for the existence of the series scheme for the two light reactions in oxygenic photosynthesis. In one word, he was one of the master biophysicists of the 20th century—who provided direct measurements on many key intermediates, and made us understand the intricacies of photosynthesis with a simplicity that no one else ever did. We present here our personal perspective of the scientist that Lou Duysens was. For an earlier perspective, see van Grondelle and van Gorkom (Photosynth Res 120: 3–7, 2014).

Keywords Reaction Centers · Two light reactions · Two photosystems · Primary photochemistry · Excitation energy transfer · Electron carriers

Introduction

We consider it highly appropriate to begin this Tribute to Lou, as we called him, with what was published as a Dedication to him in a book on chlorophyll a fluorescence, edited by Papageorgiou and Govindjee (2004):

> During his scientific life, Louis (Lou) Nico Marie Duysens quietly contributed some of the most seminal ideas in photosynthesis, with still central influence over our thinking today. His approach was a perfect blend of deep theoretical understanding and experimental innovation. Typical of Duysens' early work was his insightful application of Förster theory to light harvesting by the accessory pigment beds, which firmly established the physics underlying the function of the photosynthetic unit. Using his own state-of-the-art absorption spectrophotometer, he discovered the spectroscopic signature of oxidation and reduction of the photochemical reaction center in bacteria. Later, adapting this technique to plant (algal) systems, he discovered the alternating effects of green and red light on the redox state of cytochrome f in red algae, to firmly establish the now familiar model of Photosystem I and Photosystem II acting in series. These major contributions exemplified Lou Duysens' philosophy towards technical developments. Technology never drove nor limited his research, but was harnessed to address specific scientific questions and hypotheses.

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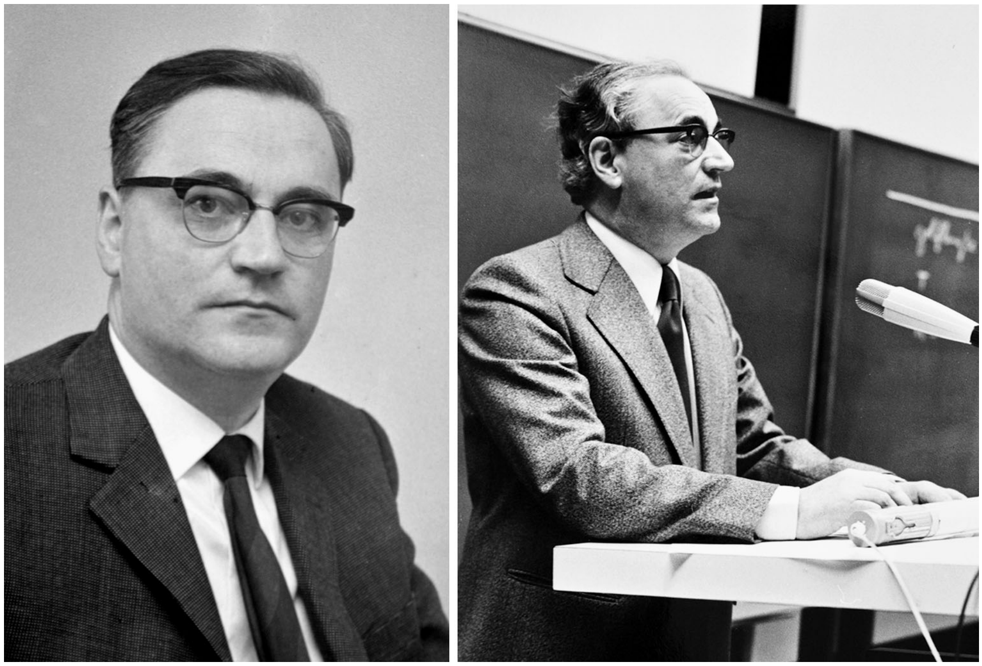


Fig. 1 *Left* A portrait of Lou Duysens, 1963. *Right* Lou at the lectern giving a speech at the opening of Huygens Lab, 1976

With his easy understanding of photochemical and photophysical principles, Duysens pioneered the use of Chlorophyll fluorescence as a powerful probe of photosynthetic function, in all classes of photosynthetic organisms, to discover many of the essential events of primary energy conversion in photosynthesis: excitation energy transfer in the light-harvesting antenna and charge separation in the reaction center.

Over many years, in his laboratory in Leiden (The Netherlands), a multitude of state-of-the art fluorescence methods was created, driven by the ideas of Lou Duysens. Fundamental concepts that followed from these experiments include the carotenoid to (bacterio-) chlorophyll energy transfer in plant and bacterial light harvesting systems, the efficient energy transfer among a large number of quasi-identical chlorophylls to reach the small population of reaction centers, the 'Lake model' of photosynthetic unit function, the control of the fluorescence yield of Photosystem II by the redox state of the quencher Q (now known as Q_A), and chlorophyll excitation quenching by carotenoids. These concepts underlie the veritable industry of fluorescence-based, non-invasive methods now used to study photosynthesis in vivo and to analyze plant productivity, from the lab bench to satellite monitoring.

Lou's understanding of physical principles is immense and so facile that his colleagues and competitors struggled to keep up with him. His seemingly simple analysis of the thermodynamic limits of photosynthetic energy conversion sparked a literature that lasted several decades, with each incremental advance simply rediscovering what he had said at the outset. Indeed, this is still an area that few fully comprehend.

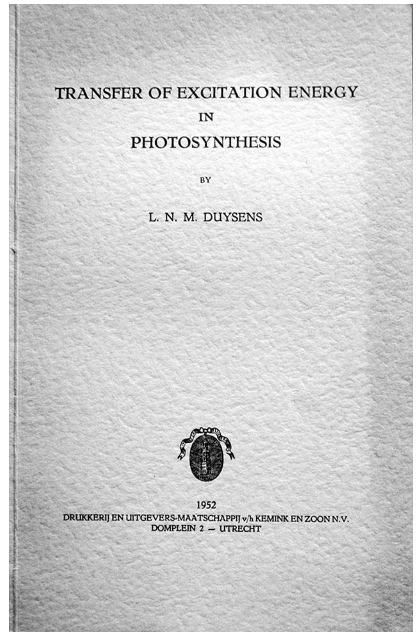
Since Lou's retirement, many ultrafast spectroscopic techniques have emerged, revealing intricate details of function in the photosynthetic apparatus, which can be well understood in terms of the fantastic atomic resolution structures of photosynthetic pigment-protein structures that are now available. In this new era of understanding, it is amazing to see how the concepts, developed by Duysens even 50 years ago, have survived as solid foundations of our current models of photosynthetic activity.

Further, van Grondelle and van Gorkom (2014) have eloquently described Lou's scientific contributions, and, in our opinion, correctly captured the history by stating "We would never be where we are now without his insights and experimental skills". Figure 1 shows two portraits of Lou Duysens in the 1970s.

Early life

Louis Nico Marie Duijsens was born on March 15, 1921 in Heerlen, a little town in the southern part of the Netherlands. He was the first child of Louis Winand Duijsens, a

Fig. 2 Lou (the boy on the *left*) at the age of seven with his parents and his sister and brother in 1928. *Source* Archives of Duysens's family



TRANSFER OF EXCITATION ENERGY
IN
PHOTOSYNTHESIS
BY
L. N. M. DUYSENS

1952
DRUKKERIJ EN UITGEVERS-MAATSCHAPPIJ v/h KEMINK EN ZOON N.V.
DOMPLEIN 2 – UTRECHT

Fig. 3 The cover page of the PhD thesis of Lou Duysens (1952)

civil servant at the municipality, and Marie Eickenboom. Figure 2 shows his photograph with his parents, his younger brother Frans and his little sister Ria. Lou, as he was called, lived for many years in the town of his birth.

From his early childhood onward, Lou was interested in science, performing chemical experiments while he was still in the secondary school in his hometown. Although his family was not much inclined into academics, Lou decided to start his academic training in Physics (& Math) at the University of Utrecht. In 1947, he passed the final examination and received the degree of doctorandus (Drs), Latin for "he who still has to become a doctor". In the educational system in the Netherlands, this indicated, until the 1980s, the completion of a 6–7 year academic training. Somewhere during this time, Lou started to write his name a bit different from the official spelling: Instead of Duijsens, he wrote Duysens, as has been known by most of us.

Major contributions of L. N. M. Duysens

PhD research

On October 1, 1946, Lou was appointed on a 'half time assistantship' at the Biophysical Research Group, a joint research activity of the University of Utrecht and Delft Technical University. This was made possible by the continuation, after World War II (in the 1940s) of a grant from the Rockefeller Foundation. Bessel Kok was amongst others already working in the same group since 1942 (Myers 1987). In those days, a "doctorandus" appointed at an academic research group would generally work his first few years to prepare a PhD thesis. Bessel Kok published and defended his thesis (Kok 1948a, also published as Kok 1948b) and Lou published his 4 years later (Duysens 1952).

Lou's thesis (Fig. 3) marks an important moment in photosynthesis research, since it clearly showed the strength of applying methods from physics to understand processes in biological systems. This classical thesis, an important part of which was published a year earlier (Duysens 1951), included many discoveries and insight into the process of photosynthesis: (1) discovery that a very few chlorophyll *a* molecules undergo light-induced absorption changes; the idea that these are "reaction centers" was born; for these molecules, he used a prefix "P" (for pigment); the concept of an antenna (of $\sim$200 chlorophyll *a* molecules) serving one reaction center was there; (2) existence of two types of chlorophyll *a*, one strongly fluorescent when excited by phycobilins in the red algae, and another weakly fluorescent when directly excited; (3) systematic and clear information about the efficiency and mechanism of excitation energy transfer from various accessory pigments (phycobilins; fucoxanthol; and chlorophyll *b*) to chlorophyll *a* (in algae) and to bacteriochlorophyll (in photosynthetic bacteria). As an example, chlorophyll *b* transferred excitation energy to chlorophyll a with close to 100 % efficiency. Figure 4 shows Lou receiving the doctoral degree from Professor J. M. W. Milatz (1910–2000), "promotor" of his thesis, from the University of Utrecht.

Postdoc in the USA and return to the Netherlands

After passing his thesis "cum laude" in Utrecht, Lou went on a two-year research trip to the USA. He spent 1 year with C. Stacy French at the Carnegie Institute of Science, at Stanford. (For a biography of French, see Govindjee and Fork 2006). There he built an absorption difference spectrophotometer and discovered cytochrome oxidation in *Rhodospirillum rubrum* (Duysens 1954a). In *Chlorella* it seemed to happen as well, but the difference spectrum was dominated by other contributions: the 515 nm absorbance change (Duysens 1954b). French allowed him to take the home-made parts of the apparatus when he moved to Eugene Rabinowitch's lab at the University of Illinois in Urbana-Champaign (Fig. 5) for another year of postdoc research. There he worked with *Porphyridium cruentum*, which did not show the 515 nm change, but allowed an unambiguous demonstration of cytochrome oxidation (Duysens 1955; see Duysens 1989a; and a quote from it in "Appendix 1", showing Lou's fair-mindedness to those who preceded him).

Upon his return from the USA, Lou spent two more highly productive years at the Biophysical Research Group in Utrecht: (1) He found that the P800 bandshift is not part of the absorption difference spectrum of the photochemically active bacteriochlorophyll (Duysens et al. 1956), thus removing the only remaining doubt about the reaction center concept. It is this paper that he reprinted and discussed again (Duysens 1989b). (2) Lou solved the "sieve effect" puzzle (Duysens 1956a), a phenomenon studied extensively by Eugene Rabinowitch (see discussion in Rabinowitch (1951, 1956); also see Das et al. 1967). (3) He

Fig. 4 Lou Duysens, receiving his PhD diploma cum laude, after successfully defending his thesis in 1952, Utrecht, The Netherlands

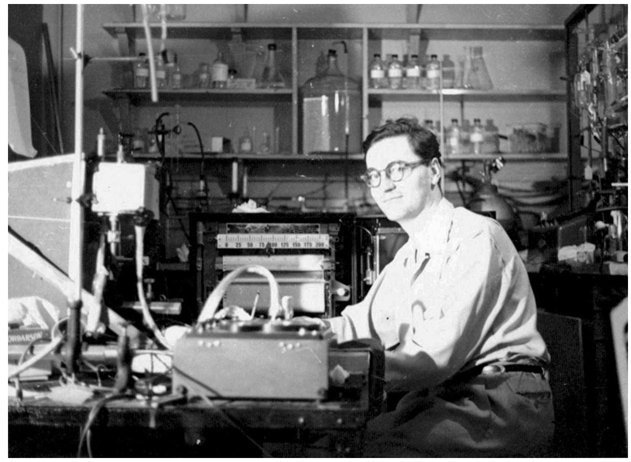


Fig. 5 Lou Duysens in the laboratory of Eugene I. Rabinowitch (156 Natural History Building, University of Illinois at Urbana-Champaign), 1954

wrote an elegant and thorough review on excitation energy transformation in photosynthesis, in which 105 of the 123 references were no more than 5 years old (Duysens 1956b). *Things were really happening in those days!* (4) Lou developed a fluorescence method to measure NAD (P) reduction (with the help of an undergraduate chemistry student who was thereby motivated for a scientific career, and who came to Leiden in 1958 for a PhD study with Lou, and stayed with him forever: This was Jan Amesz (see e.g., Duysens and Amesz 1957, 1959; Amesz and Duysens 1962).

Building the Leiden biophysics group

In 1956, Lou was appointed as a "Lector" and in 1962 as a Professor in the Department of Physics of the University in Leiden to establish his own photosynthesis research group. In the 30 years until his retirement in 1986, Lou succeeded in building a unique research laboratory (van Grondelle and van Gorkom 2014).

Figures 6 shows two examples of the equipment built in- house in the 1970s: a dual wavelength absorption difference spectrometer, allowing simultaneous measurement of absorbance changes at two different wavelengths (Fig. 6) and a single beam spectrophotometer with simultaneous measurement of fluorescence. On these and similar experimental equipment many important discoveries were made over the course of Lou's professorship at Leiden University, resulting in important papers and theses (see a chronological list of Lou's PhD students; "Appendix 2"). Some of these became top scientists and discoverers in the field, including the late Jan Amesz, Wim Vredenberg, Hans van Gorkom and Rienk van Grondelle. Figure 7 (left) shows Lou's first doctoral student Jan Amesz. (For an

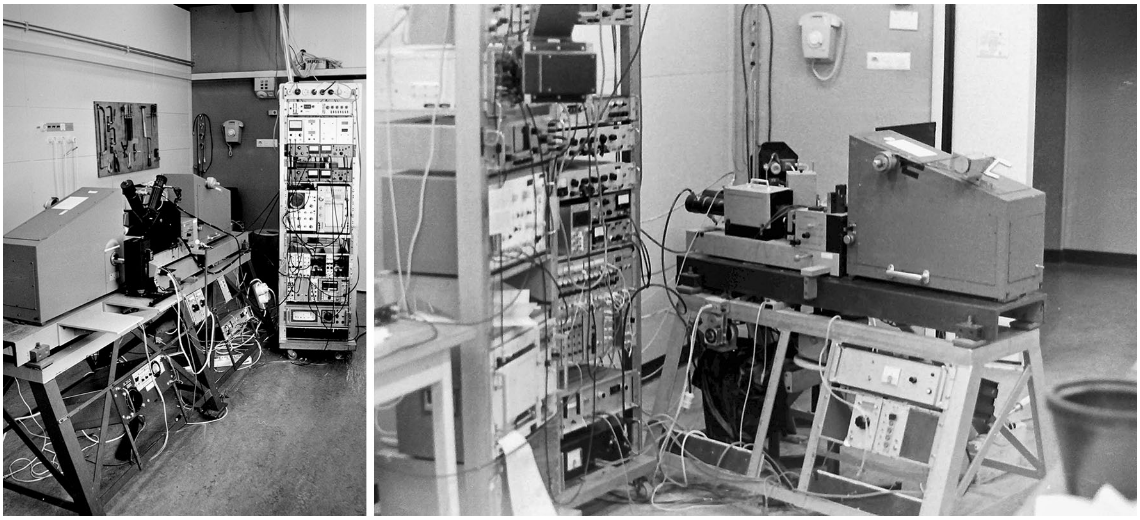


Fig. 6 *Left* Dual wavelength absorption difference spectrophotometer (labeled as ΔεII), Leiden. *Right* Simultaneous measurement of absorption changes and fluorescence, equipped with a timer controlled flow system to provide dark adapted samples (labeled as ΔεIII), Leiden

obituary of Amesz, see Hoff and Aartsma (2002); and for an obituary of Hoff, see Gast et al. (2002).)

Lou's idea of the ideal biophysics laboratory began to take shape only after the move in 1965 (at the time of the Woudschoten conference; Goedheer and Thomas 1966) from his single room in the Nieuwsteeg, next to the Kamerlingh Onnes laboratory in Leiden, to his own new 600 m^2 building at the Schelpenkade. Within 10 years, even this space had become too small, and the biophysics department, together with all other groups in experimental physics, moved to the newly built 12-floor Huygens Laboratory in 1975. We show here a 1974 picture of Jan Amesz playing table tennis with Lou Duysens (Fig. 7, right).

During this development, Lou, as well as Jan Amesz and Arnold Hoff, ran independent research projects that led to experiments instrumental for our present day understanding of the energy conversion process in photosynthesis. The

Fig. 7 *Left* Lou Duysens congratulating Jan Amesz after he passed his PhD defense in 1964, Leiden. *Right* Jan Amesz, wearing a tie, playing table tennis, with Lou Duysens in 1974, in the Leiden Lab. Photo by Hans van Gorkom

laboratory had hired highly qualified and innovative technical staff (mechanical engineers; electrical engineers; and computer programmers), to design and build the high precision optical equipment, the electronics with automatic timers, amplifiers and power supplies and one of the first minicomputers used in a research environment in the Netherlands (also see Pulles under *Personal Recollections*). Highly successful research papers in the area of biophysics of photosynthesis were published including those in collaboration between different members of the above groups, e.g., Rienk van Grondelle, Bart de Grooth, Kees Rijgersberg, Herman Kramer, Henk Rademaker, Herman Kingma, and Hans van Gorkom.

1956–1965: Biophysics at the Kamerlingh Onnes Laboratory in Leiden

By showing that the second law of thermodynamics applies to photosynthesis (Duysens 1958), Lou moved on to apply important laws of physics to biological processes. In our opinion, the first decade in Leiden was indeed thrilling—as mentioned earlier, the most important discovery by Lou was made, during this period: the experimental proof of the two light reactions, working in series, and sensitized by two different pigment systems (Duysens and Amesz 1962; also see Duysens 1964, 1966, 1989a). In another important paper, actually in a Proceedings volume from Japan (Duysens and Sweers 1963) the concept of the quencher "Q" (now Q_A) was proposed, based, partly on the observation that photosystem II (PSII) light increased chlorophyll *a* fluorescence yield and photosystem I (PS I) light decreased it. Lou suggested that PSII light removed a "quencher" (Q) and PSI light restored it; "Q" later became known as "Q_A" (see Govindjee's personal comments).

1966–1975: Lou's own laboratory at the Schelpenkade

In our opinion, this decade was quite different in one major way. Jan Amesz was tenured in 1968, after a few years as a postdoc with David Fork, and Lou had a large number of collaborators. They included many PhD students (see "Appendix 2") and international visiting scientists (including Govindjee, Warren Butler, Anastasios (Tasso) Melis, Joseph (Joe) Warden, Nigel (H.G.) Holmes, Colin Wraight, Secundino del Valle Tascon, and Ulrich Schreiber). We note that it was during this period that most of high-level detailed research on the primary and secondary steps of all the biophysical reactions on Photosystem I, Photosystem II, and bacterial photosystems was made (not presented here).

Lou realized that the study of charge separation and secondary electron transfer would benefit greatly if, in addition to all the optical spectroscopy, his lab would include facilities and expertise for EPR. He invited Joe Warden to set it up initially, and then Arnold Hoff was tenured to further develop the magnetic resonance approach of photosynthesis research (also see *Personal Recollections* by Govindjee).

Space had become a serious constraint again but that was solved when in 1975 the group moved to the new Huygens Laboratory. With Hans van Gorkom's tenure in 1976 the department now counted 4 group leaders, each with their own team of PhD students.

1976 to Lou's retirement: Biophysics at the Huygens Laboratory

During his final years at the Leiden University, Lou succeeded in developing and using a sophisticated picosecond absorption difference spectrophotometer with a picosecond time resolution. With this equipment, Lou and his coworkers could observe and measure primary reactions in a series of photosynthetically active membranes and fragments of photosynthetic organisms. These experiments confirmed and brilliantly extended his ideas, already developed in his thesis (van Grondelle and van Gorkom, 2014; Sonneveld et al. 1979, 1980; van Grondelle and Duysens 1980; Nuijs et al. 1986a, b; Bakker et al. 1983; Shuvalov et al. 1986; Mamedov et al. 2015).

It was during this period that both of us (Pulles and Govindjee) were there. Govindjee et al. (1976) showed that bicarbonate functions at the 2 electron gate on the acceptor side of Photosystem II (Velthuys and Amesz 1974). We end this section by citing van Grondelle and van Gorkom (2014) for contributions of Duysens during this period. We refer to a review of Duysens's own views during this period: see Duysens (1978).

Summary

We have mentioned above major contributions that Lou already made in his doctoral thesis in 1952. He established in the 1950s in Leiden, The Netherlands, one of the most important centers in the world for research on "Biophysics of Photosynthesis". It was here that in the 1960s the most convincing evidence for the series scheme of oxygenic photosynthesis was given. This antagonistic effect of light 1 and 2 really proved the existence of what we now call the "Z" scheme of photosynthesis for electron transport from water to NADP (see Duysens et al. 1961). Further, the nomenclature of "light reaction 1" & "light reaction 2", and "pigment system 1" & "pigment system 2" was born here (see Duysens (1989a) for his perspective). We emphasize that it is clearly the cornerstone evidence for the series scheme of photosynthesis, predicted in its bare bones by Rabinowitch (1945, 1956); see Hill and Bendall (1960), and a full discussion in Govindjee and Bjorn (2015).

Figure 8 shows photographs of Lou Duysens with a few top authorities on photosynthesis of his time: Bessel Kok (discoverer of the reaction center of photosystem I, P700 and of the Joliot-Kok clock of oxygen evolution; see Myers 1987); with Warren Butler (another leading biophysicist of our time; see Benson 1998); and with Eugene Rabinowitch (author of what was often called the "The Bible of Photosynthesis"; see Bannister 1972; Govindjee 2004; also see "Appendix 1").

Research in Lou's laboratory has covered experiments providing, in great depth, an almost complete understanding of the primary photochemistry of both anoxygenic as well as oxygenic photosynthesis. We have selected to cite here, almost at random, one paper each by some of Lou's coauthors: van Grondelle et al. (1976), Holmes et al. (1978), Rademaker et al. (1979), van Bochove et al. (1984), and Kingma et al. (1985).

Lou's wife Wil passed away long before he did; he is survived by his 3 children: Frank, Tom and Inge, and their families.

Some personal recollections

Govindjee

Long ago, upon my invitation, Duysens (1989a) wrote beautifully about his own work. It is indeed brilliant, but most importantly a real historical account of the two light reaction and two photosystem concept in photosynthesis. What impressed me most is that Lou recognized contributions of others, including mine in the most authentic manner. When Duysens and Sweers (1963) discussed the antagonistic effect of light 1 and light 2 on chlorophyll a fluorescence, he gave full credit to my work—that was just a quick brief observation, without much insight. However, Duysens and Sweers (1963) wrote "The first direct experimental suggestion of a different effect on fluorescence of two light beams of different wavelengths was obtained by Govindjee et al. (17[to be read as 1960])".

I would also like to mention two specific papers from the Leiden Lab: (1) Hoff et al. (1977a) since it dealt with a phenomenon of "magnetic field effects", a phenomenon that has interested me for a while (see Stacy et al. 1971; Hoff et al. 1977b); and (2) Sonneveld et al. (1979, 1980) on the use of chlorophyll a fluorescence since $P680^{+}$ had been shown to be a quencher (see Butler 1972; Shinkarev and Govindjee 1993; Shinkarev et al. 1997).

Lou was my hero since I began my PhD, in September of 1956, in Biophysics (then Physico-Chemical Biology)

Fig. 8 *Top* Lou Duysens with Bessel Kok, Rehovot, Israel, 1974. *Middle* With George Hoch and Warren Butler, date unknown. *Bottom* With Eugene Rabinowitch (a colored version of Figure II. 10 from Nickelsen and Govindjee 2011)

under Robert Emerson & Eugene Rabinowitch. Duysens's PhD thesis (Duysens 1952), and Rabinowitch's 3 volumes (1945, 1951, 1956), had served as my "Bible of Photosynthesis"; Duysens's thesis was read by me word by word; and I used this knowledge in my own research, and that of my PhD students, for years together: most of the early discoveries in my lab were indeed influenced by what Lou had to say (see Govindjee and Rabinowitch 1960; Govindjee et al. 1960). Lou told me that he had much praise for Rabinowitch's ideas, and he read very diligently all what Eugene had written. I went to Lou's Lab in 1976, for my 2nd sabbatical, and it was there that I was able to pinpoint that bicarbonate ion functions at the two-electron gate of Photosystem II, together with Tinus Pulles, my wife Rajni and Hans van Gorkom (see Govindjee Pulles et al. 1976; also see the main text, and a review by Shevela et al. 2012).

M.P.J.(Tinus) Pulles

With the passing away of Lou Duysens, one of the giants of the photosynthesis research community is no longer with us. His legacy, however, is broader than the field of photosynthesis. Although I left photosynthesis research in 1979, Lou has had an important influence on my scientific development and career. My 9 years (1971–1979) within his Leiden photosynthesis group has taught me that a scientific approach, using the methods and thinking developed and applied within the framework of physics, is instrumental in solving scientific problems in many scientific disciplines, including those of environmental sciences and climate change. Lou taught me and other PhD students in his group to be stubborn enough to doubt anything as long as it was not clearly proven to be correct and to never accept something only because an "expert" claims it to be true. It was due to innovative and then state of the art equipment in Lou's lab, I was able to, for the first time, observe absorbance changes in the near ultraviolet oscillating with flash number with periods of 2 and 4 (Pulles, et al. 1976). Together with Jan Amesz, Lou has made me the type of critical scientist I now am. I remember him with great gratitude.

David B. Knaff

David, now deceased, had written to us: I'm so very sorry to hear this news. Although Jan Amesz was my day-to-day host during my 1983–1984 sabbatical in Leiden, Lou-in his capacity as Head of the [Biophysics] lab-was an incredibly warm and gracious host and, of course, his contributions to our understanding of photosynthesis were enormous.

Henk van der Wal

I first met Lou when as a student in Leiden I took his biophysics course. The lectures were in his own room at the Schelpenkade with only five or six students sitting around a table which was nice. I remember him as a very kind explainer. I was really fascinated by the phenomenon of random walks on 2D lattices and was thrilled that a little later I could use his explanation of the absorption flattening effect in a class on reactor physics. Much later I became aware that he himself had done crucial research on this flattening effect. I also became aware that I had been lucky because he didn't particularly like giving lectures and that this course had been a rare event. For Lou problems were never either too small or too big; from the stability of a light source to the problem of world peace, it could all be discussed as long as you stayed within the confines of what was physically possible. In 1977 he gave me the opportunity to work for a PhD thesis. He wanted me to disprove Mitchell's chemiosmotic hypothesis since he was convinced that in bacterial photosynthesis a direct pathway between the primary photosynthetic processes and ATP synthesis existed without intermediate of a bulk membrane potential. It is no secret that my efforts were in vain and he must have felt disappointed but in the end I was lucky, working in his laboratory if only because I met colleagues who became very good friends. I last saw Lou in 2006 during the celebration of the 50th anniversary of the beginning of his photosynthesis research in Leiden where he graciously enquired about my wife's research.

Rienk van Grondelle

My personal relation with Lou was not always easy. I was a student, in Amsterdam, of the 1968 generation, Lou was far, far away from this. When I entered the biophysics lab in 1973 it was still in the Schelpenkade in Leiden, I shared a working space with Jaap van Best, then using a Ruby laser, one pulse per minute, to measure luminescence from plant photosystems. When the pockels cell had to be cleaned we had to leave the building! There was still a big box of sand standing there to catch the bullets that were supposed to open a 'fast' shutter!!! Unbelievable. I was supposed to work on 'cytochromes' using a microsecond flash spectrophotometer. I will never forget the moment, say after a few months that I was in Leiden, that around 5 in the afternoon Lou entered this lab and started to discuss with me how to use a Tektronix storage oscilloscope. I lived in Amsterdam, I travelled every day between Amsterdam and Leiden and after 15 min I said 'sorry Lou, I have to go, my girlfriend is waiting for me', so I left. In retrospect, his world must have collapsed, but also in retrospect it gave me my independence in Leiden. In the final few years of my promotion and later as a post doc I could establish an amazing number of joint projects with students of Lou, with Jan [Amesz], with Arnold [Hoff], with Hans[van Gorkom]. Also my relation with Lou became better, at least we could discuss world-politics, albeit with little agreement. In 1982 I left Leiden to go to Amsterdam, a place where as compared with Leiden there was nothing, a CD-spectrophotometer, the late David Knaff knows! I built up a research group from scratch and only after 1986 Amsterdam became a center for fundamental photosynthesis research, the year that Lou decided to retire. I have always felt that I stood on the shoulders of Lou, Jan and Arnold.

Bruno Velthuys

L. N. M. Duysens has had a great impact on my life. Without his pioneering work in the Netherlands, I surely would never have strayed into the field of Biophysics of Photosynthesis, and as indirect consequence, I would not have worked in Baltimore for 8 years, and I would not have raised (up to kindergarten age) a daughter in France. Additionally, I would have missed meeting this uniquely gifted person on a quasi-daily basis for about 4–5 enjoyable years when, adjunct to (and hierarchal above) Jan Amesz, he was my teacher at the lab in Leiden where I stayed as a PhD-student. Hence, when I read about Lou's passing away, I was saddened. And I wished to help make sure that the American photosynthesis community also heard without much delay about this occurrence that leads to sorrow and eulogy. I am glad, Govindjee, to see that you in your usual energetic way have picked up on the sad news, and are publishing this Tribute… Please note that I left Leiden almost 40 years ago; after that I met Lou only a couple of times, but I have fond memories.

Wim J. Vredenberg

Lou Duysens was a brilliant, creative and inspiring scientist (see the personal part of my recent paper (Vredenberg 2015), where I discuss my work, on photochemical reactions in anoxygenic photosynthetic bacteria, done in collaboration with Lou Duysens (see Vredenberg and Duysens 1963, 1965; Vredenberg et al. 1965).

Awards and Honors

We end this Tribute to Lou Duysens by recognizing him as a major biophysicist of the twentieth century. Among the many honors he had over the years, we mention the 1964 Charles F. Kettering I Award for Excellence in Photosynthesis; for being the Foreign Associate of the National Academy of Science of USA; and for his membership of the KNAW (Koninklijke Nederlandse Akademie van Kunst en Wetenschappen: Royal Dutch Academy of Arts and Sciences). A special honor to Lou Duysens was a book "Current Topics in Photosynthesis" published in his honor at the time of his retirement in 1986 (Amesz et al. 1986). It included 25 papers by 70 authors, divided as follows: Antenna (4); Reaction Centers (6), Electron Transport (9); Membrane Structure and Ion Transport (3) Evolution (1); and Methods (2). It included several top authorities on photosynthesis who are no more with us: Jan Amesz; Gerry Babcock; Agnes Faludi-Daniel; Stacy French; Y. Fujita; Arnold Hoff; David Knaff; Alexander Krasnovsky; Jack Myers; Horst Witt; and Colin Wraight. In the "Introduction" of the book Stacy French wrote in this volume: "…….Prof. Louis N. M. Duysens, whose impending retirement is the occasion for this volume, has indeed more than fulfilled the hopes of the Utrecht Group's founders by his distinguished research career and productive leadership of the Biophysical laboratory at Leiden. Because Lou Duysens had been thoroughly trained in basic physics he was able to think clearly in mathematical terms about biological problems even as a graduate student. ……….The general principles of photochemical energy conversion have been elucidated by selection of the most suitable organism for each investigation. "As this volume will show Duysens' efforts have been outstandingly successful in this competitive field of worldwide interest."

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Appendix 1

Duysens (1989a) recognizing contributions of Eugene Rabinowitch, one of his postdoc advisors, the other being C. Stacy French:

When writing the present review article, I noted that Rabinowitch already in 1956, p. 1862, interpreted the light induced cytochrome f oxidation in *P. cruentum,* which I [Duysens] observed in his laboratory, in two possible ways. One was similar to that proposed by Hill and Bendall in 1960. He also stated: "The quantum requirement of the hydrogen transfer reaction as a whole would be (at least) 8, since two quanta will be needed to transfer each of the four required H atoms (or electrons), first from water to the cytochrome, and then from the cytochrome to the final acceptor." As a second possible hypothesis he mentions that of the cytochrome on a cyclic path between NADPH and oxygen or their precursors, both precursors being generated by a single photoreaction. *If the kinetics of cytochrome oxidation in P. cruentum had been measured between 1956 and 1960 both for excitation at 680 and 560 nm and Rabinowitch's discussion had not been overlooked, Rabinowitch would presumably have been recognized as the "inventor" of the two photochemical systems in series.*

Appendix 2

Doctoral students of Lou Duysens in the Biophysics Lab at Leiden were:

1964: Jan Amesz; 1965: Wim Vredenberg; 1971: Bert Kraan; 1973: Henk Otten; Jaap Haveman; Marcel Donze; 1976: Hans van Gorkom; 1977: Jaap van Best; Ger den Haan; 1978: Rienk van Grondelle; 1980: Henk Rademaker; 1981: Arie Sonneveld; 1983: Herman Kingma; 1986: Tom Nuijs; 1987: Marcel Vos; 1988: Henk van der Wal.

Appendix 3

List of ~70 authors who honored Lou Duysens by their research articles in "Current Topics in Photosynthesis", edited by Amesz et al. (1986) right after his retirement from Leiden University (those deceased are bolded).

Amesz, J; Araki S; Babcock, GT; Barber, J; Breton, J; Cleland, RE; Faludi-Daniel, A; Forster, V; French, CS; Fujita, Y; Gaul,DF; Gerken, S; Ghanotakis, DF; Golen, D; Gorter, PY; Gounaris, K; Govindjee; Hendrickson, DN; Hoff, AI; Joliot, A; Joliot, P; Junge,W; Kambara, T; Karapetyan, NY; Kiss, JG; Knaff, DB; Knox, R; Krasnovsky, AA; Kuwabara, T; Lavorel, J; Lous, EI; Mathis, P; Meiburg, RF; Melis, A; Meyer, B; Murata, N; Mustardy, LA; Myers, J; Neale, PI; Olson, JM; Overfield, RE; Padhye, S; Parson, WW; Pierson,BK; Saygin, O; Scherz, A; Schowanek, D; Schreiber, U; Setif, P; Shaw, EW; Shubin, VV; Slooten, L; Snel, JFH; Suzuki, K; Sybesma, C; Van der Wal, HN; Van Dorssen RJ; Van Gorkom, H; Van Grondelle R; Van Kooten, O; Vasmel, H; Vass, I;Vos, LI; Vredenberg, WJ; Walravens, N; Witt, HT; Won-Ki, C; Wraight CA; Wynn, RM; and Yocum, CF.

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