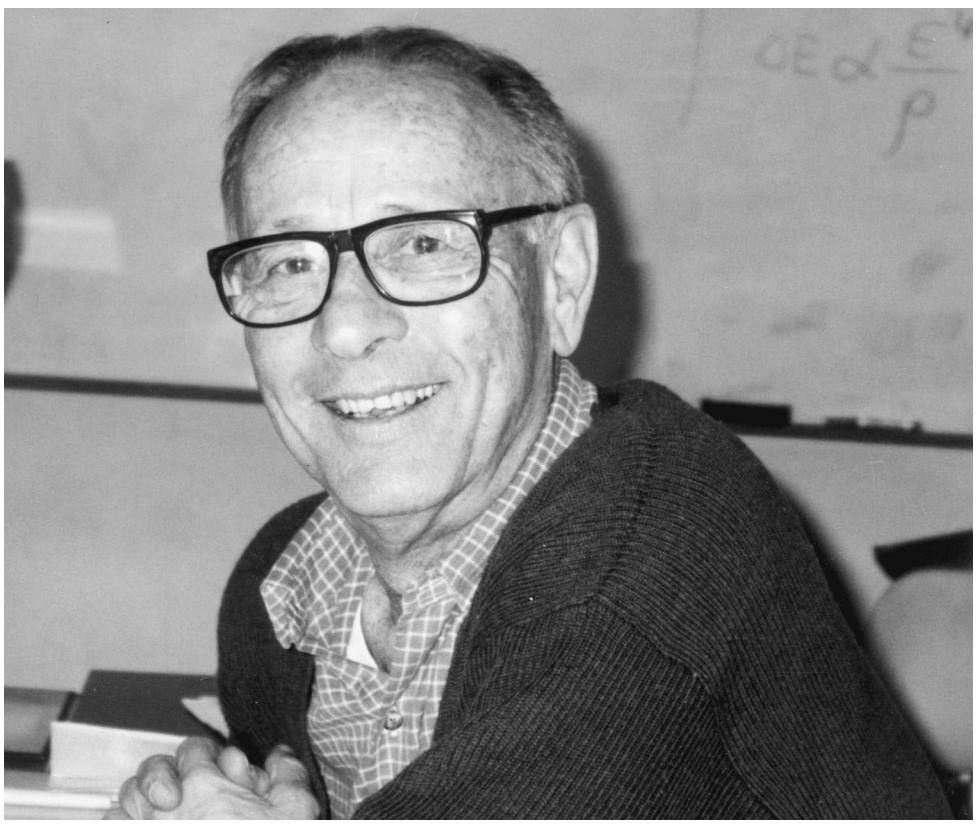

George Feher (Photograph by R.A. Icaacson)

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Personal perspective/Minireview

Three decades of research in bacterial photosynthesis and the road leading to it: A personal account *

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Abstract

In this minireview I present a very personal account of my life and research in bacterial photosynthesis. It is divided into two parts. The first part is autobiographical and narrates the events that led me to change fields from electrical engineering to physics and ultimately to biophysics. The second part describes the work on the primary processes in bacterial photosynthesis carried out with numerous collaborators in our laboratory over the past ~ 30 years.

Govindjee has asked me for some time to write an account of my life and research for his historical corner. I was reluctant. On the one hand were my inhibitions to write about personal matters; isn't it presumptuous to assume that my trials and tribulations and experiences will be of interest to other people? On the other hand to present a factual, dry account of my research would make dull reading. As I was contemplating these issues, Mel Okamura asked me to give the traditional last (retrospective) lecture at the Gordon Conference on Photosynthesis this August (1997). The reaction from the audience was positive and so I decided to use the talk as a basis for the personal perspective.

I have divided the main presentation into two parts. The first part is a brief biographical sketch, corresponding to '..... the Road Leading to it' in the title. I have followed Govindjee's admonition to write a 'totally personal' account. The second half deals with the work on bacterial photosynthesis performed in our laboratory during the past $\sim$ 30 years. This is also a highly personal account biased toward our interests and our activities and therefore, should not be taken as a comprehensive historical overview of the field of bacterial photosynthesis. Consequently, many important results of other groups have not been included. This also explains why there are more references to our own work than to those of others.

My life before photosynthesis: 1924–1968

Czechoslovakia: 1924–1941

It would be nice to start the story by saying that since my early childhood in Czechoslovakia I dreamt of unraveling the mysteries of how bacteria convert light into chemical energy. But that was not the case; I had more mundane interests. They were mechanical and electrical devices and toys. One of my favorite occupations, to the consternation of my mother, was to take apart all the clocks and watches in our household. Most of the time, but not always, I was able to put them back together in working condition. At age 14 I became an avid radio ham and joined forces with a good friend, Tom Hornak to try to understand electrical circuits and to build what now would be called

* Invited and edited by Govindjee.

electronic devices. Tom persevered in these endeavors, became a well known electronic expert in Czechoslovakia, and after Dubček's fall in 1968 escaped to the US and made a distinguished career for himself at Hewlett Packard. After 60 years we are still the best of friends. In parallel with my interest in electronics I developed another hobby – growing crystals, an interest and fascination that stayed with me to this day. I had tens of dishes under my bed which nobody was allowed to touch and which I remember caused some friction in the household. I had lots of time to pursue these hobbies. In 1938 at the age of 14 I was expelled as a Jew from school. Slovakia had broken off from Czechoslovakia and had nominally become an independent state. In reality it was a puppet state of Nazi Germany. The atmosphere in Nazi Slovakia was grim; it was a time of persecution and fear, a time to look for a way out.

Palestine: 1941–1946

In the middle of the war, in 1941, at age 17 I escaped with a bunch of other kids through Hungary, Rumania,Turkey and Lebanon to Israel, at that time Palestine, a British mandate. The British were waiting at the Lebanese border and promptly arrested and interned us. A shocking welcome after having barely succeeded in escaping from hell. After a brief internment we were released and joined a kibbutz (communal settlement) for which we had prepared ourselves ideologically and emotionally during the previous three years. Alas, for me and a few others of our group, the kibbutz fell short of our expectation, and perhaps we fell short of their expectations. I wanted something more stimulating than to work from sunrise to sunset in the fields. So after a year and a half I left for Haifa where my sister lived and where a technical University, the Technion was located. I started to work as a radio repairman while taking some technical courses in the trade school adjacent to the Technion. One of my teachers was Franz Ollendorff, an internationally recognized authority in electrical engineering who was also a professor at the Technion. He offered me a position as his laboratory assistant which I enthusiastically accepted. One of the first challenges that Ollendorff confronted me with was to build an oscilloscope. It is difficult to imagine nowadays that in 1943 there was not a single oscilloscope in the Technion or probably in all of Palestine. From captured German and Italian equipment I was able to salvage a cathode ray tube plus other components and design and construct an oscilloscope. Being a patriot I had the time axis running from right to left in accord with Hebrew writing. Ollendorff was delighted and when Haim Weizmann (a scientist and one of the great Zionists, who later became the first president of Israel) visited the Technion, he was shown the 'Hebrew oscilloscope'. He enthusiastically shook my hand and I felt that I was in Heaven (which in Israel is closer to earth than here).

In addition to repairing radios and being Ollendorff's lab assistant I also worked for the Hagana (a Jewish underground organization) as an electronics expert. Curiously, some of the work was only recently declassified and appeared in a long article in an Israeli newspaper in 1992 (Abramovich 1992). It dealt with tapping into the direct telephone line between the British High Commissioner in Jerusalem and 10 Downing Street in London and building an unscrambling device to make the conversations intelligible. I also invented with a friend, Hanan Myer, a secret optical signaling device which is now part of the 'Signals' exhibition at the Reuben Fleet Science Center in San Diego. I experienced strange feelings recently when I met John Kendrew at the Board of Governor's meeting at the Weizmann Institute in Rehovot and he told me that he was stationed as a British officer in Palestine at about the same time that I performed these 'jobs'.

Unfortunately, none of my above activities made up for the lack of a high school education and I could not get accepted into the Technion which I very much wanted. In 1944 Ollendorff arranged for me to take a special entrance examination (just a formality, he said) in English, Math, Physics, History and the Bible (Old Testament). I passed everything but the Bible exam. All of Ollendorff's pleading with the administration did no good. As rector Kaplanski and his admission committee declared: 'a Jewish engineer has to know the Bible'. I was devastated at that time, although some incidents played out decades later seem to me now funny. One of them occurred in 1975. I was invited to give a set of lectures in connection with the opening of the Solid State Institute at the Technion. After my last talk an old man with a cane hobbled to the podium, congratulated me on my research and delivery, and remarked that part of the credit for my career should be given to him. 'Excuse me, Sir, would you be so kind to refresh my memory as to when we have met before'; I asked politely. 'We have never met, but I was a member of the admission committee in 1944 that refused your entrance to the Technion'. Another incident occurred more recently. In 1994 Rachel Nechushtai kindly arranged a 70th birthday party for

me at the Hebrew University of Jerusalem. Among the many people who crossed my life the past 60 years and who gave brief speeches there appeared an elderly lady whom I did not recognize. Her speech was brief: 'You don't know me, but I am the daughter of Mr. Kaplanski and came to apologize for my father's actions 50 years ago'. And when I got an honorary degree from the Hebrew University of Jerusalem in 1994 I couldn't resist to point out how much easier it was to obtain that degree than to get accepted to the Technion. Concerning the lack of a high school education, I wonder in retrospect whether it actually didn't have a beneficial effect. The educational system in Slovakia was very formal and rigid and it might easily have suppressed any creativity and imagination.

Back to 1944. There was nothing left for me but to try to get accepted at a University abroad that had less strict admission requirements (certainly the Bible would not be a required subject). Professor Ollendorff was not only in favor of the idea but promised that a US organization called 'The Friends of the Technion' would support my studies in the USA. So I applied to 50 universities in the US; only 2 were willing to accept me as a special student: Harvard and the University of California in Berkeley. For financial reasons I opted for Berkeley. There was still the financial problem of the passage to the US, which was by no means trivial, considering the poor economic situation in Palestine at that time. My previous hobby helped me out. Let me explain this briefly since it bears some resemblance to my activity some 40 years later.

I remembered the beautiful Rochelle salt crystals that grew under my bed in Slovakia. These crystals are piezoelectric. That is, when pressure is applied a voltage is induced. They can therefore be used for microphones and phonograph pickups. During the war none of these items could be imported to Palestine and there was a dire need to have them for public address systems to entertain Allied troops stationed in Palestine. I set up a small production line to manufacture piezoelectric devices, mostly microphones which I sold to the entertainment establishments (a photograph of these devices is shown in Figure 1). One would think that with 20 or 30 night clubs in Palestine the market would soon have been saturated but nature came to my aid. Rochelle salt is not the ideal material for Mediterranean weather. It does not tolerate high temperatures. So after every desert wind (Hamsin) most microphones were wiped out, the market became desaturated and orders for new microphones came in. Lest you think that this was unethical, I hasten to add

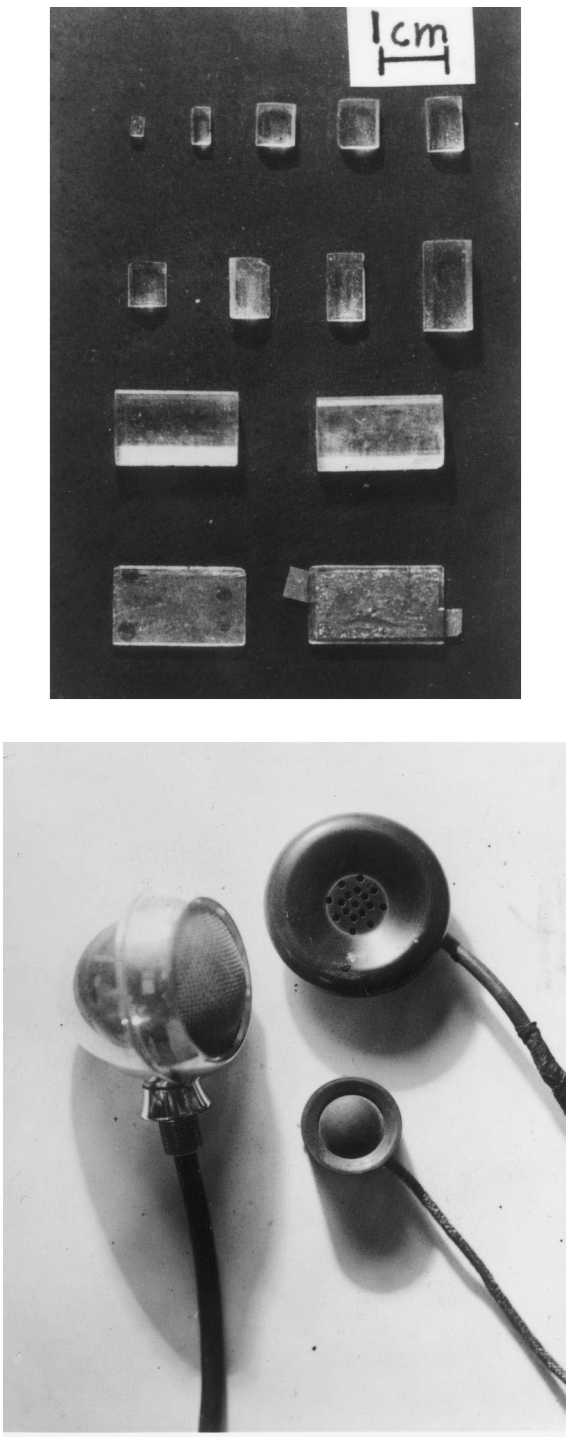


Figure 1. Top: Piezoelectric crystals of Rochelle salt. Lowest row shows cut wafers with electrodes attached. Bottom: Piezoelectric devices (microphone, earphone, stethoscope) Haifa, Fall, 1944.

that buyers were duly warned of the finite lifetime. By 1946 I had accumulated enough money to embark on the trip to the US.

University of California, Berkeley: 1947–1954

After spending a few months in Europe I arrived in the US in December 1946. My first stop in New York was a visit to the 'Friends of the Technion'. They received me very nicely and asked me all about the well being of that marvelous person, Professor Ollendorff. But when I related to them his message, i.e. that they support my studies, the roof caved in. 'Young man, you have it all wrong; we're here to collect money, not to dispense it.' I was, of course, very disappointed, but not discouraged. As I would put it now, the situation looked hopeless but not serious. I proceeded by train to the West Coast with some money in my pocket which hopefully would modestly carry me over for one semester.

I arrived in Berkeley in mid December with 2 weeks left before the start of the new semester in January. I looked for a job in radio shops, but was unsuccessful. Then I saw on the bulletin board that the Radiation Laboratory was looking for an electronic technician. I hiked up the hill, applied, and was interviewed by no less an authority than the great Luis Alvarez. He asked me to design an amplifier with certain specifications which I did on the spot. He seemed impressed, said I was well suited for the job and that I would hear from him. And hear I did after a few days; an irate Alvarez shouting at me for having wasted his time. The job was classified and required an immigration and not a student visa. I felt hurt by his treatment. Years later I took an excellent course from Alvarez but his past behavior towards me still irked me. I heard through the grapevine that Alvarez was a compulsive problem solver. I bought one of those wooden chinese puzzles that by proper manipulation can be taken apart. I put a drop of glue into it and left it on the table before his lecture. As I had anticipated, he eyed it, asked to whom it belonged and when nobody answered, pocketed it. I heard from the graduate students that he was in a bad mood for days. In retrospect, it probably wasn't a nice thing to do, but at the time it seemed like a good way to try to even the score.

January 1947 arrived and with it the long awaited semester. I was very excited to get started after an ~ 8 year hiatus in my formal education. I was enrolled in engineering physics for reasons that I need to explain. I really wanted to study biophysics, being influenced by a little book by Schrodinger (1944) 'What is Life', that I had read while still in Israel, and which I found very stimulating and exciting. But there were a couple of problems. UC Berkeley did not have an undergraduate program in biophysics, and the Jewish Agency in Palestine was unwilling to grant a visa to study such 'useless' fields. Not even physics was condoned. It had to be something more practical like engineering, so I chose the nearest field, engineering physics. The first semester I soaked it all in like a sponge. I took math, physics, chemistry, astronomy, engineering drawing and physiology, 24 units in all. This was above the allowed limit but I got permission to do it.

How did I make ends meet? I had a little money to cover tuition and rent for one semester, but there wasn't much left for food. I roomed with an equally poor Israeli, Aaron Gibor, who majored in biology. We took Physiology 1A together, a huge class with laboratory sessions in which we dissected frogs. After class we collected them and boiled the legs for dinner, a delicacy in France, we kept telling ourselves. Fortunately toward the end of the semester we worked on rabbits so our diet improved. Somehow, we managed to get through the semester. When summer came around I got a fruit picking job in the San Joaquin valley. I thought that my kibbutz experience would come in handy. But I was in for a surprise. The pace of the Mexican fruit pickers was breathtaking (literally). Whereas in the kibbutz we discussed ideological issues, like the plight of the suffering proletariat, while picking fruit at a relatively leisure pace, here I encountered the suffering proletariat not having time to discuss anything, trying to pick as much fruit as possible since we were paid by the box. It was hard work. The money I made in the summer, together with working for room and board (washing dishes and cleaning rooms) carried me through the fall semester of 1947. Thereafter I became a 'reader', which meant correcting homework papers and exams at $1.00/hour in all courses in which I had received a good grade. In my senior year I worked part time as an electronic technician at the Microwave Laboratory in the Electrical Engineering department. There I met Mel Klein who, as you know, also wound up working in photosynthesis. In 1950 I graduated with a BS in Engineering Physics. I had married the year before, we were expecting a baby and I started to work full time as an engineer. I took some graduate courses in Electrical Engineering on the side and fortunately my work (on thermionic emission) turned out to be accepted as a

thesis topic. I received an MS degree in Electrical Engineering in 1951.

By now, being married to an American citizen I had an equivalent of a green card and was therefore free to switch to physics. I joined the solid state group of A. Kip and C. Kittel. They had a program in Electron Paramagnetic Resonance (EPR) which was a relatively new field and they needed somebody with an engineering background to design and build EPR spectrometers. My thesis project was electron spin resonance of conduction electrons in metals. But I still had biophysics on my mind and so on Sundays I came to the lab and put miscellaneous biological materials like leaves and blood into the microwave cavity. Lo and behold, when the leaves were illuminated a signal appeared. This, incidentally was before the publication of the pioneering paper by Commoner et al. (1954) on EPR in biological systems. I also found a signal in blood at g = 6, but all hell broke loose when one Sunday C. Kittel came in and found me doing these 'frivolous' experiments instead of focusing on my thesis project. He threatened to throw me out if he caught me at it again. In addition he pooh-poohed the signal at g = 6 which he was convinced must be an artifact; every fool knows that angular momentum is quenched and the g values cannot deviate far from g = 2. It was later that Ingram's group published their work on hemoglobin (Bennett et al. 1955) and that Griffith (1956) explained the origin of the g = 6 signal. This experience reinforced my belief not to be awed by authority. I also wonder now whether these frustrating early experiments had anything to do with me decades later working on heme proteins and photosynthesis.

Research at Bell Telephone Labs with a stint at Columbia University: 1954–1960

During my last year in Berkeley I decided to apply for an industrial job rather than seek an academic position. There were two main reasons underlying that decision. One was the academic rat-race that I witnessed in Berkeley. The stress on the Assistant Professors to make tenure seemed awesome. People like W. Knight, C. Jeffries, A. Kip, all to become well known scientists, seemed to be fighting for their academic life. I reasoned that by going to a good industrial research lab, I had the opportunity to devote all my energies to research without the distractions of academia. If my research should turn out well, there might be a chance to skip the fight and step into a tenured position. If, on the other hand, my research was not sufficiently successful for such a transition, I could stay on in industry. The second reason was more subtle. I still intended to return to Israel, in which case the industrial experience would be important, since there were no academic research jobs available in Israel at the time. In addition, the research situation in Israel might change and industry would be a good place to wait it out.

So, after receiving my PhD in physics I joined the research group at Bell Telephone Labs in Murray Hill, NJ. I had been interviewed by Bill Shockley of transistor fame but by the time I arrived, Shockley had left. That turned out to be lucky, as Shockley's motto was: 'You can work on any problem of your choice, but if my ideas are better than yours, you damn well will work on mine.' And they usually were better. So in the absence of Shockley I was given complete freedom. I chose to continue to work on EPR: this time in semiconductors. Since this work is unrelated to biology, I'll not describe it here. An account of the development of ENDOR, performed during this period, has appeared elsewhere (Feher 1998a). But I would like to mention two projects. In one, crystallization again played a role. The first solid state MASER (the precursor of the LASER), built in collaboration with Derek Scovil (Feher and Scovil 1957; Scovil et al. 1957) used a single crystal of gadolinium ethyl sulfate doped with cerium that we grew from an aqueous solution. Not a great feat, to be sure, but my past experience did come in handy. The second was a brief foray into a biophysics problem. K.S. Cole, a well known neurophysiologist from NIH, gave a lecture at Bell Labs in 1956 describing nerve conduction. He described the unresolved problem of whether the sodium flux is made up of individual ions or whether they pass in clusters. I was at the time concerned with noise problems and suggested to Cole that one could, in principle, resolve this question by measuring the quantal nature of the fluctuations in the current, i.e. the noise amplitude should be proportional to the square root of the number of charged entities crossing the membrane. Cole invited me to spend a week in his laboratory at NIH where I re-acquainted myself with an old friend, the frog sartorius muscle, which had contributed to my diet a decade earlier. The experiments were inconclusive; they floundered because we did not have a sufficiently noise free amplifier. I was in the middle of developing ENDOR and the solid state MASER and therefore did not pursue this problem further. Later I learned about the elegant experiments of B. Katz and others involving essentially the same idea of applying quan-

tal analyses to neurotransmission (reviewed in Katz 1966).

In 1958, Felix Bloch, the co-inventor of NMR offered me a tenured position at Stanford. It was a tempting offer but after much thought I refused in view of my plan to return to Israel. Bloch was initially incredulous that I preferred Bell Labs over Stanford, but after I explained to him my personal reasons, he understood and told me the following story: In the 1940's his conscience started to bother him. As a Jew he should contribute to the building of a nation in Palestine instead of sitting comfortably in the USA. These feelings became stronger with time until he could no longer sleep at night. At this point his wife intervened: 'Felix, we can't go on like this. Go and see your friend Albert in Princeton; he is supposed to be a wise man, discuss your problems with him.' So Bloch went to see Einstein and told him of his problem. 'But Felix, I have solved this problem a long time ago. I am first a scientist and second a Jew', said the great man. 'Thank you, Albert' replied Bloch and from then on the problem never bothered him again. Well, unfortunately Einstein's solution didn't work for me and I never really made peace with not living in Israel. Perhaps if I had heard it first hand .....? If, if, if. If I were a numerologist and believed in the Kabalah, my disquiet would be even heightened: Take for instance the number 137. It is the value of the reciprocal of the fine structure constant, a dimensionless number, probably the most important one in physics. And what is Psalm #137?: 'If I forget thee Jerusalem, may my right hand forget its cunning.....'. And is it an accident that chlorophyll has 137 atoms? And what is the numerical value of the word[1] Kabalah (קבלה)? ק=100; ב=2; ל=30; ה=5, which adds up to 137. But I am being carried away. Let me get back on track.

In 1959 another opportunity arose. The physics department at Columbia University was thinking of starting a program in Solid State physics. Furthermore, Charlie Townes, the co-inventor of the MASER and LASER was leaving and somebody had to take over his students. Having worked both on Masers and in Solid State physics, I was approached to apply for the position. I.I. Rabi, the strong man at Columbia, was opposed to a program in Solid State physics. So, when I gave a colloquium at Columbia, he heckled me throughout, probably in the hope of seeing me fall apart, as I heard a few of my predecessors did. But I wasn't concerned. There wasn't much at stake; I had a good job with the Bell Telephone Co. It also helped

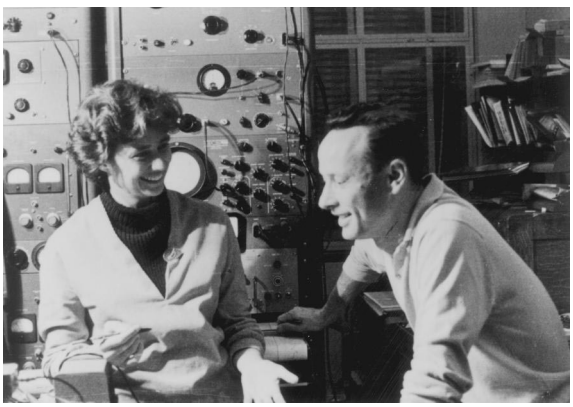


Figure 2. Columbia University graduate student, Elsa Rosenvasser (now Elsa Feher), with me at Bell Labs, Fall, 1959.

that throughout the colloquium I kept before my eyes a scene from my graduate student days in Berkeley. There, a famous and difficult person, E.O. Lawrence, the inventor of the cyclotron, once entered a lab and saw a young man with his feet on his desk munching a sandwich. To Lawrence, such behavior in the Sacred Halls of Science was intolerable. He quietly closed the door to give the man time to shape up but when he opened the door again, the man hadn't changed his position or activity. Lawrence got furious, red in the face and started to shout at the man. At this point, we graduate students emerged to witness the spectacle. After a while the chairman, R.T. Birge was able to calm Lawrence down. Upon which the young man calmly said: 'Sir, I don't know who you are, I work for the Telephone Company.' About 25 years later I had dinner with Rabi in La Jolla. I told him this story. He wasn't amused at all, but his wife was.

At any rate, I got the job; a joint appointment between Bell Labs and Columbia University for the 1959/1960 academic year with the possibility of making it permanent. I inherited 10 graduate students of Townes, with his admonition to pay special attention to a young student from Argentina, Elsa Rosenvasser. I not only followed instructions, I went overboard. I paid exclusive attention to Elsa, essentially neglecting the rest (see Figure 2). As you may have guessed by now, Elsa became my wife.

There actually was one occasion where I had to intervene with one of the graduate student. His officemates complained to me that he made constant noises eating matzes (the crisp, unleavened bread primarily eaten at Passover) and complaining how hard physics is. He was also the only student that I had ever seen reading the Wall Street Journal. His name was Arno

Penzias. If we had to vote for the person least likely to succeed in physics, I believe that Penzias would have easily won. Well, as it turned out, we were wrong. Penzias shared the Nobel Prize with R. W. Wilson in 1978 for measuring the cosmic radiation background noise and from it determining the temperature of outer space. When Elsa and I heard the news of the Nobel Prize, we immediately sent him 2 packages of matzes to go with the champagne. He was very gracious about it and sent us a T-shirt with his famous result, T = 3.5 °K, imprinted.

At Columbia I collaborated with Alan Sachs and R. Prepost on the creation of muonium in silicon (Feher et al. 1960). I used this opportunity to see how the high energy half of the physics community lives. Working at an accelerator with a large supporting group and a grueling schedule did not appeal to me. Twenty five years later I had the same reaction doing EXAFS experiments on reaction centers at the Stanford Linear Accelerator (Eisenberger et al. 1982).

Columbia was an interesting experience. However, it was not a long term solution. The joint appointment with Bell Labs made it a neither here nor there situation. In addition, commuting was a pain and I didn't like living in New York and, for that matter, I was not enamored with Murray Hill, New Jersey either. At that point another opportunity presented itself.

UCSD; mostly solid state physics research, with attempts to switch to biophysics: 1960–1967

In 1960 Roger Revelle, Director of the Scripps Institute of Oceanography at La Jolla, a fascinating man, a combination of charismatic visionary and con man convinced the Regents of the University of California to establish a new campus in San Diego (La Jolla). He came to Bell Labs to recruit solid state physicists and invited three of us to come separately for a visit to La Jolla. He promised us that UCSD[2] would remain a graduate school with a light teaching load and emphasis on research. He also showed me a beautiful lot with an ocean view that I could get if I came (we later found out that he showed the same lot to all of us). It sounded exciting to be in on the ground floor in building a new campus, where one's ideas can still make a difference. We were all very much taken by it. My colleagues at Columbia thought I was crazy to forego a professorship at Columbia (which was offered to me at the end of the year) and move to La Jolla. They and others thought that it would be impossible to build a first rate University in an idyllic playground like La Jolla. They were, of course, wrong. Scientists carry their compulsions, neuroses and ..... talents with them and are, to first order, unaffected by the environment.

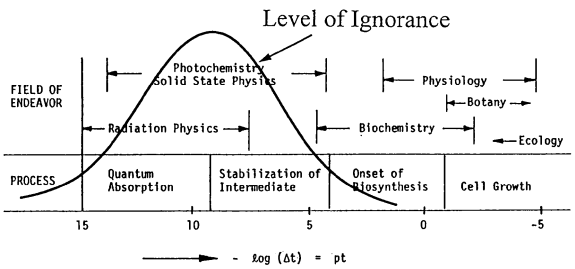


Figure 3. 'pt' diagram showing the various processes and fields of endeavor in photosynthesis. Δt is the time interval for a process to occur. At a seminar that Kamen gave to our group in 1964, the level of ignorance peaked at our field – Solid State Physics (modified from Kamen 1963).

I accepted Revelle's offer with the following proviso: I will come to La Jolla to help establish an experimental solid state research program and after having accomplished that, I'll be free to pursue my interest in biophysics. Well, it sounds easier than it was in practice. Being surrounded by physicists I was likely to come up with 'one more good idea' in physics before switching fields: nuclear polarization (Clark and Feher 1963), cooling by adiabatic depolarization of electric dipoles (Shepherd and Feher 1965) and electron paraelectric resonance (Feher et al. 1966) were some of these. Martin Kamen who joined the chemistry department of UCSD around 1963 tried his best to get me interested in photosynthesis. I remember a seminar that he gave to our solid state group in which he presented his famous pt-diagram (Kamen 1989) showing the various processes and fields of endeavor as a function of the log of the time interval for a process to take place (Figure 3). The level of ignorance peaked around our field, solid state physics. I later learned that he shifted the peak depending on the group that he was addressing. The breadth and interdisciplinary aspect of the field definitely appealed to me. Kamen also brought the physical chemist David Mauzerall as a visitor from the Rockefeller University to breach the gap between our disciplines. With David we did a few EPR experiments on porphyrin as a model compound for chlorophyll (Mauzerall and Feher 1964). But serious involvement with photosynthesis remained at the time remote.

In 1962 I had an interesting interaction with Albert Szent Gyorgyi, the discoverer of vitamin C and for over half a century a colorful personality in science. I had heard about him since childhood because, dur-

ing World War I, he was the youngest professor in Bratislava, the town in which I was born. I had just finished reading one of his books whose main theme was the nature of ordered water around a biological structure (Szent Györgyi 1957). He claimed that water is ordered over distances of several microns (> 10^4 Å). As a solid state physicist I had a hard time believing that H_2O would be ordered over $\sim > 10^4$ layers. I did a quick, 'Friday afternoon' experiment to disprove Szent Györgyi's contention. By measuring the frequency response of the dielectric constant of a water-soaked millipore filter with 100 Å pore sizes I showed that H_2O is not ordered even at a distance of 100 Å. Just about that time I received a letter from him out of the blue, inviting me and my wife to spend a weekend at his home in Woods Hole, MA to discuss EPR, his newest interest. I thought that this would be a good opportunity to raise the question of the 'ordered' H_2O. Besides, I also wanted to meet my childhood hero about whom my mother used to tell bedtime stories. One of them described how Szent Györgyi after the establishment of Czechoslovakia in 1918, fearing for his life, built a raft, took his microscope and floated down the Danube to Budapest. And so we happily accepted the invitation. Szent Györgyi was a charming host, a marvelous raconteur but totally unreceptive when I tried to broach the subject on my mind. Finally, after 2 days of unreceptiveness, I dropped all diplomacy on the way to the airport and told him that the proposition in his book was wrong; H_2O is not ordered over several microns. He looked at me quizzically and nonchalantly replied: 'Oh, did I say several microns? I am so bad with numbers, don't pay any attention to them.' What an anti-climax and blow to the image of my childhood hero! At any rate you can't become a saint (and certainly not a biophysicist) by proving a famous man a sinner.

In 1964 I went to Cold Spring Harbor, Long Island, to take the phage and bacterial genetics course which had been started by Max Delbruck close to two decades earlier. It was a great experience. However, I did not follow it up by establishing a program in that field. A good part of the next year my wife and I spent in Buenos Aires to set up an EPR group in solid state physics. My wife, a native of Buenos Aires, had studied at Columbia University with the aid of an Argentinian fellowship, and felt that she wanted to give something in return by contributing to Argentinian science. So I slipped back into physics and we established a nice EPR group there. Unfortunately, a short time after we left, one of the periodic coups took place, the group was dissolved, and we ended up finding positions for the members of the group. So in the end, instead of helping Argentinian science, we facilitated the brain drain. One of the members of the group, Rafael Calvo, came to UCSD, obtained a PhD with us and we continue to collaborate to this day (e.g. Calvo et al. 1990).

A critical sabbatical year at MIT, a summer at Woods Hole and one at Cold Spring Harbor: 1967–1968

By 1967 it had become clear to me that if I seriously wanted to switch fields I needed to leave, at least temporarily, the physics department and to immerse myself in a more biological environment. The opportunity came with an invitation from Cyrus Levinthal at MIT to spend a year as a visiting professor in the biology department there. Before going to MIT I spent the summer at Wood's Hole taking the Physiology course. One of the sections that I took was on Bacterial Photosynthesis given by Rod Clayton (see Clayton 1988). Having been primed by Martin Kamen, I immersed myself in it for a few weeks and I liked it. But I didn't want to commit myself to this topic before exploring other areas during my sabbatical year at MIT.

MIT was quite an experience: the mad hustle and bustle compared to UCSD, and the people in obvious quest for fame and glory left little time for a visiting 'greenhorn'. There was one exception, Lisa Steiner, an immunologist whose course I took and with whom I became and still am, a good friend and colleague. She taught me a lot of biology and protein chemistry, and we started a very fruitful collaboration that I shall describe shortly. But before that I want to mention my slow realization of the differences in research approaches pursued by physicists and biologists. This is perhaps best illustrated by an encounter with Salvador Luria at MIT.

> 'Hi, George, how nice to see you here. What are you doing?' he asked
> 'I am sitting in several biology and biochemistry courses', I answered.
> 'Yes, but what are you doing?'.
> 'Well, I am going to seminars and talking to people about their work.'
> 'Yes, but what are you doing?' he insisted.
> 'I am also getting some experience in the laboratory of Lisa Steiner.'
> 'Yes, but what are you doing?'

So it finally dawned on me: biology is a 'doer's' field; you have got to run centrifuges and gels and not

'waste your time' in deep thoughts. I was reminded of that episode when I later came across the pronouncement of the 18th century British anatomist John Hunter: 'Why think? Why not try the experiment?' On the other extreme is the statement by the 20th century physicist/astronomer Sir Arthur Eddington: 'Do not put too much weight on experimental results until they are confirmed by theory.' I clearly don't subscribe to either extreme but favor planned experiments, guided but not biased by prevailing theories.

The encounter with Luria sensitized me to the difficulties that some physics graduate students have when entering the more empirical field of biochemistry and biology. An example might be the running of a chromatography column. After they disappear for weeks to read everything that there is to read about chromatography columns and finally run one, their question is likely to be 'is the elution profile a Gaussian or a Lorentzian?'. One more thought on the differences between physics and biology: Physics is *difficult but simple* (i.e. when you have mastered, often with great difficulty, the basic principles of quantum mechanics, Newton and Maxwell's equations, everything follows logically). Whereas biology is *easy but complex* (i.e. no difficult concepts but a great number of facts).

After the year at MIT I spent the summer of 1968 at Cold Spring Harbor, this time taking the Phycomyces course with Max Delbruck. Although Delbruck was an exciting person he did not convince me that phycomyces was the system of choice to work on. Instead, I had by now made the decision to work on the primary processes in bacterial photosynthesis. This decision was based on several factors. I liked the seeming simplicity of the bacterial system, the multidisciplinary nature of the field, the small number of people working in it and the possibility of using a technique that we were familiar with (EPR) in solving some basic questions concerning the primary reactants.

Research in bacterial photosysnthesis: 1968–present

Isolation, purification and preliminary characterization of the reaction center; identification of the primary donor and acceptors

I returned to UCSD in the fall of 1968, ready finally to make the commitment to switch to biophysics with bacterial photosynthesis as the main project. Since our lab had some expertise in electron paramagnetic resonance (EPR) spectroscopy, it was natural for us to attack the problem of the identification of the primary reactants, each of them having an unpaired electron and therefore, in principle, amenable to EPR spectroscopy. In addition the problem had the appeal of being very basic. In fact, it struck me as a scientific incongruity that after $\sim$ 200 years of research in photosynthesis the main actors participating in the light-induced charge separation had not been identified. So together with Jim McElroy, a brave physics graduate student, and Dave Mauzerall, who spent a sabbatical year and several summers at UCSD, we embarked on identifying the primary donor.

We obtained an inoculum of the carotenoidless mutant, R-26[3], of *Rb. sphaeroides* from Rod Clayton, grew them up and followed Reed and Clayton's (1968) triton procedure to isolate a photosynthetic unit that had a molecular weight of $\sim 10^6$. When the preparation was illuminated it gave a strong EPR signal at cryogenic ($\sim 80\,^{\circ}$K) temperatures as had been observed previously in whole cells by Sogo et al. (1959). To identify the EPR signal we used two independent approaches (McElroy et al. 1969, 1974). In the first, we compared the kinetics of the light-induced EPR signal and optical absorbance changes at cryogenic temperatures. They were identical (Figure 4) showing that the free radical and the species undergoing light-induced optical change are the same. Similar conclusions were reached by Loach and Sekura (1967) and Bolton et al. (1969). The optical changes, first observed by Duysens (1952), had been associated with the oxidation of a specialized bacteriochlorophyll called P_{870}, and were implicated in the primary process (Parson 1968).

In the second approach we compared the EPR parameters of the photo-induced signal with those of chemically oxidized bacteriochlorophyll ($BChl^+$) *in vitro*. The electronic g-values were identical (g = 2.0026) but the linewidth observed in $BChl^+$ was $\sim$40% larger than in the photosynthetic unit (McElroy et al. 1967, 1972). From these 2 sets of experiments we concluded that the primary donor is a specialized bacteriochlorophyll. The linewidth difference, with some misgivings to be described later, was attributed to different environments of $BChl^+$ *in vitro* and in the photosynthetic tissue.

At about the same time another graduate student, Mike Malley, worked with Dave Mauzerall on the Stark effect in porphyrins (Malley et al. 1968a) and the direct observation of the Zeeman effect of the excited state of porphyrins (Malley et al. 1968b). Although these problems were not directly related to photosyn-

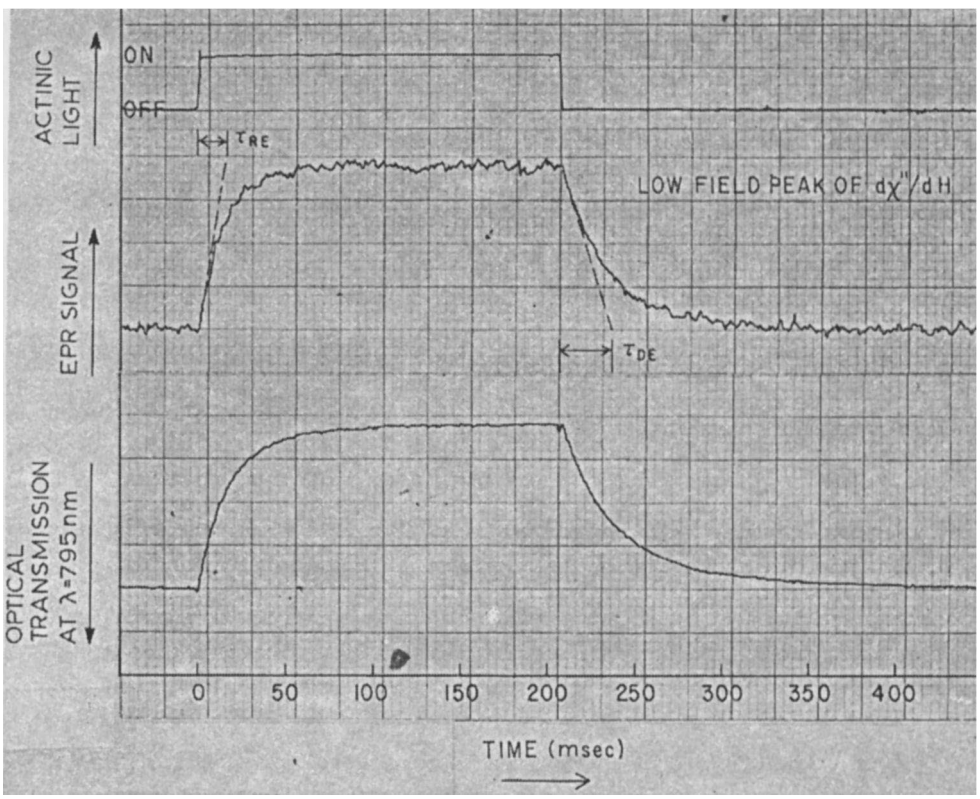


Figure 4. EPR and optical kinetics of RCs from *Rb. sphaeroides* at 77 °K. The rise and decay times are the same for both signals indicating that P870 and the free radical correspond to the same species. (Reprinted from McElroy et al. 1969 with permission from Elsevier Science.)

thesis, the experience with the Stark effect proved to be useful when we applied it ∼ 20 years later to reaction centers (Losche et al. 1987).

Parallel with these efforts we focused our attention on the further purification of the photosynthetic unit. The approach was entirely empirical. We knew that the critical step was the detergent solubilization. So we tried a variety of detergents, solubilized, ran centrifuges, optical spectra, column, gels, the whole bit. Luria would have been proud of me, and would have finally admitted me to the club of 'doers'. One detergent that we picked up from Bob Bartsch in Martin Kamen's lab, labeled LDAO (lauryl dimethyl amine oxide) gave spectacular results; the purified photosynthetic unit had an estimated molecular weight of $\sim 10^5$, i.e. about an order of magnitude smaller than the previous triton prep of Reed and Clayton (1968). About that time Rod Clayton passed through our lab, shared in our enthusiasm and took a bottle of LDAO with him. He and Wang confirmed our results (Clayton and Wang 1971). This marks the birth of the RC, essentially as we know it today.

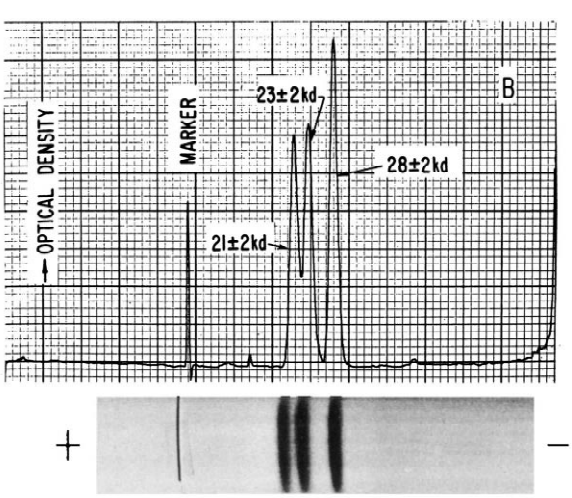


Figure 5. SDS-PAGE of reaction centers from *Rb. sphaeroides* R-26 showing the three subunits that were named L, M and H. (Reprinted from Feher 1971 with permission from Elsevier Science.)

There followed a busy time characterizing the RC. We sent samples of RCs to Lisa Steiner at MIT who determined the amino acid composition; she found it to be the most hydrophobic protein thus far reported; the molecular weight was estimated, the metal content was determined by atomic absorption; we had approximately one Fe/RC, and the subunit composition was obtained by SDS-PAGE electrophoresis as shown in Figure 5. There is some irony associated with the nomenclature of the subunits. We knew that the molecular weight determinations from the mobilities on SDS-PAGE were only applicable to water soluble proteins and were likely to be quantitatively wrong for the hydrophobic RC. We, therefore, did not want to call the subunits 21, 23 and 28 kilodaltons and named them L, M and H (for Light, Medium and Heavy). Unfortunately, as it turned out later, molecular weight determinations were even qualitatively off and H had the lowest molecular weight. But by that time, the nomenclature had already been accepted and it remains to this day.

The results on the isolation and characterization of the RC were presented at the International Conference on the Photosynthetic Unit, in Gatlinburg, Tennessee, in May 1970 (Feher 1971). I was excited about our accomplishment and expected a similar reaction from the audience. But to my surprise and disappointment a large fraction of the people in the audience were not only unenthusiastic but downright skeptical. They thought that something must be wrong; 'How can such a small unit with molecular weight of $\sim 10^5$ be responsible for that marvelous process of photosynthesis? Physicists, physicists, you must have lost something essential during your purifications.' Echos of Luria? Hadn't we still made it? No, it was definitely their problem.

At the Gatlinburg Conference we also reported on the observation of a very broad EPR signal that was ascribed to the primary acceptor. The large width precluded a free radical and Fe was implicated as being responsible for it. Incidentally, to observe such a broad signal, we developed with Roger Isaacson and Jim McElroy a new way of monitoring EPR signals using light or temperature modulation instead of the usual magnetic field modulation (Feher et al. 1969). More about that signal later.

In the fall of 1970, an important event occurred. Mel Okamura, a card-carrying biochemist joined our group as a postdoctoral fellow. It started an enjoyable and fruitful collaboration that now, 27 years later, is still going strong. Most of what I have to say about our work on photosynthesis from 1970 on was done in collaboration with Mel.

Back to the identification of the *primary acceptor*. As we were improving the signal-to-noise ratio of the broad EPR line reported at Gatlinburg, we heard from Paul Loach that he had observed a shift of the narrow light induced EPR line of P870 that he attributed to an underlying second narrow EPR line associated with the primary acceptor (Loach and Hall 1972). We exchanged samples, confirmed his findings and determined that his RCs were devoid of Fe^{2+}. His RCs had been rather harshly treated with 6M urea at pH 12, which presumably removed the iron. We proceeded to develop milder conditions for the removal of the Fe and performed EPR experiments at higher frequencies (35 GHz, rather than 9 GHz) to resolve the putative second line. The interfering EPR of the donor line was eliminated by reducing P_{870} with cytochrome *c*. To determine the chemical identity of the signal, we used again the 'model compound' approach that we had used earlier for the primary donor (Feher et al. 1972). The model compound that we chose was ubiquinone. Figure 6 shows that the EPR signal of the ubiquinone radical is the same as the acceptor signal X^- leaving little doubt that X^- is a ubiquinone-like structure. There still remained the uneasy feeling that by removing the Fe from native RCs an exogenous ubiquinone may have moved into the acceptor position, taking over the role of the Fe as an acceptor. What remained to be shown is that ubiquinone plays an *obligatory* role as the primary acceptor in native RCs.

Supporting evidence of the role of UQ was provided by Cogdell et al. (1974) who extracted UQ from RCs with organic solvents and observed an accompanying loss of photochemical activity However, organic solvents are known to denature RCs, making an accurate correlation between UQ content and activity difficult to determine. Mel Okamura developed in 1975 a gentle method of UQ removal and readdition of UQ or any other quinone, a procedure that is still widely used (Okamura et al. 1975). The conclusions of Mel's reconstitution experiments were that two quinones bind to the RC, one more tightly (called the primary quinone Q_A) and one less tightly (the secondary quinone Q_B). Perfect correlation was obtained between the Q_A content and photochemical activity, proving the obligatory role of Q_A. The Q_B was assigned the role of the secondary acceptor. The broad EPR line (Figure 7) was explained as being due to the electron on the quinone whose spin interacts magnetically with the large magnetic moment of

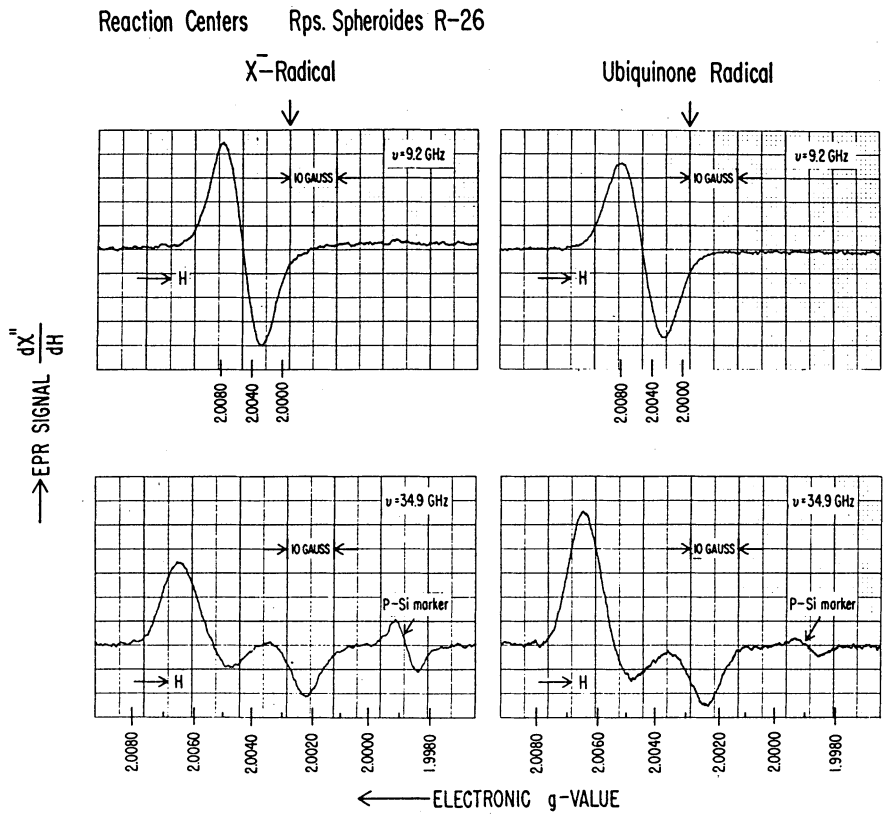


Figure 6. On the identification of the primary acceptor: Comparison of the light-induced EPR signal (X^-) from the electron acceptor in reaction centers of *Rhodopseudomonas sphaeroides* R-26 (left) with the ubiquinone radical (right) at 9.2 GHz and 34.9 GHz (T = 1.3 °K). (Reprinted from Feher et al. 1972 with permission from Elsevier Science.)

the Fe, forming an iron-quinone magnetic complex. It was hypothesized that the Fe plays no role in the primary photochemistry but facilitates electron transfer between Q_A^- and Q_B.

Subsequent to the identification of the acceptors a great deal of effort was put into the elucidation of the electronic structure of the Fe-quinone complex. This included Mossbauer spectroscopy in collaboration with Peter Debrunner's group (Boso et al. 1981), which established the valence of Fe to be 2; magnetic susceptibility (Butler et al. 1980); EPR spectroscopy (Butler et al. 1984); and EXAFS in collaboration with Peter Eisenberger (1982), which established the distances and nature of the Fe ligands. EXAFS results similar to ours were also obtained at about the same time by Bunker et al. (1982). A more detailed (historical) account leading to the identification of the acceptors and the characterization of the ferroquinone complex is given in Feher and Okamura (manuscript submitted).

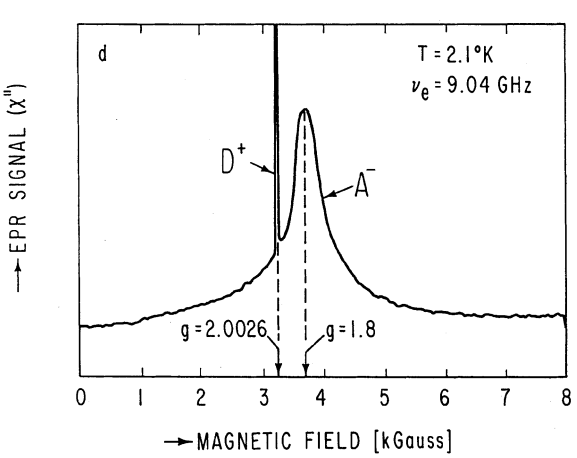


Figure 7. Low temperature light induced EPR signal of reaction centers from *Rb. sphaeroides* R-26 using light modulation. (Modified from Okamura et al. 1975.)

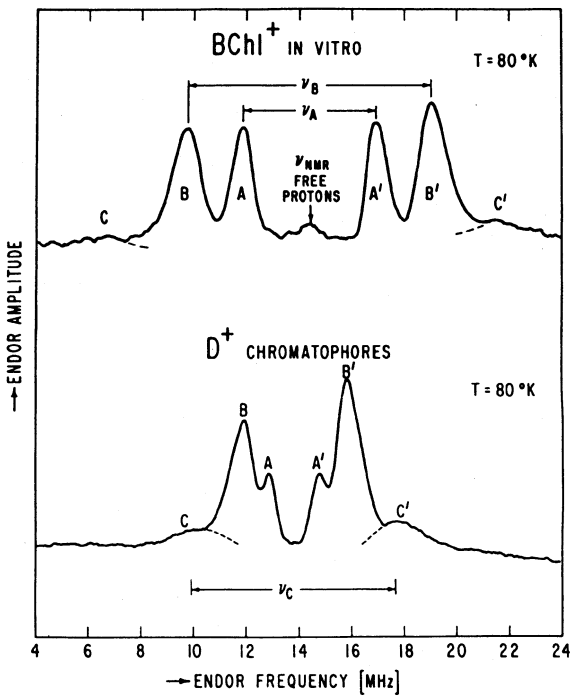


Figure 8. On the identification of the primary donor: Comparison of ENDOR spectra from $BChl^+$ in vitro (top) and the oxidized primary donor D^+ (bottom). (From G. Feher et al. 1975.)

Let me return now to the final identification of the *primary donor*. As mentioned before we associated it with a specialized bacteriochlorophyll. But there remained the nagging question of the 40% larger line width of the EPR signal of $BChl^+$ *in vitro*. We postulated a different magnetic environment, but were never quite happy with this explanation. In 1971 Jim Norris et al. (1971) pointed out that if the electron (or rather hole) is shared between two bacteriochlorophylls in the RC, the line width should narrow by $\sqrt{2}$ i.e. $\sim$ by 40%. In retrospect it amazes me that I didn't think of that since in my previous life at Bell Labs I had worked on EPR of donors in silicon, where in the limiting case of many interacting donors a very narrow line is observed (Feher 1959). At any rate, as the saying goes: 'I thought of it the minute I heard it' and enthusiastically embraced the idea of the bacteriochlorophyll dimer.

But to base the dimer hypothesis on a single number, i.e. the ratio of line widths, seemed a little shaky. A more definitive proof would be to show that the electron spends only half of the time on one BChl of the dimer, i.e. the square of its wavefunction, $|\Psi(r|^2$, should be half of that in the monomer of the model compound. The isotropic part of the hyperfine (hf) interaction of the unpaired electron with the various nuclei (e.g. protons) on the BChl ring is proportional to $|\Psi(r)|^2$. The electron-nuclear double resonance (ENDOR) technique allows one to measure the hf interactions. So with Arnold Hoff who at the time was a postdoctoral fellow in our lab and Roger Isaacson we measured and compared the hf interactions in the $BChl^+$ monomer and in the donor (Feher et al. 1975). The results (Figure 8) show that the hf couplings, i.e. the distances A, B and C between the ENDOR lines are on the average a factor of 2 smaller in the donor. Similar results were also obtained by Norris et al. (1975). Thus, the identification of the donor with a bacteriochlorophyll dimer was unequivocally established. A more detailed and historical account of the donor work can be found in my Bruker lecture (Feher 1992).

In addition to the two acceptors and the primary donor, there is a transient, intermediate acceptor, with whose identification we were not directly involved. Evidence of its existence came from the observation by Parson et al. (1975) of a short lived, transient state. Fajer et al. (1975) proposed that this intermediate acceptor, I, is bacteriopheophytin. Several groups were able to trap I^- at low temperature. Of particular interest to us was Tiede's (1976) observation of a doublet EPR signal in *Chromatium vinosum*, that was not observed in *Rb. sphaeroides*. With Mel Okamura we undertook a very detailed investigation of the spectroscopic and kinetic properties of I^- in *Rb. sphaeroides* (Okamura et al. (1979a). When we exchanged ubiquinone with menaquinone in the Q_A site, we observed the EPR doublet with a splitting 60 G. The doublet arises from the magnetic interactions between I^- and Q^- in the trapped I^- (Q^-Fe^{2+}) complex. From this interaction a distance between I^- and Q_A of $\sim$ 10 Å was deduced. From the splitting and the activation energy for the electron transfer $I^-Q^- \rightarrow IQ^{2-}$, we calculated the electron transfer rate using J. Hopfield's theory (Okamura et al. (1979b) for thermally activated electron tunneling. The good agreement between theory and experiment provided proof that electron tunneling is the mechanism of electron transfer (DeVault 1984).

Further characterization of the reaction center

While the primary reactants were being identified and characterized, we also investigated other properties of the RC. With Mel we isolated the three subunits, determined that their stoichiometry was 1:1:1 (Okamura et

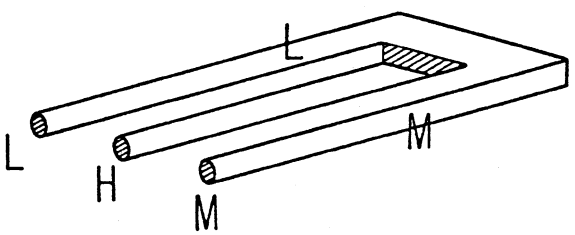


Figure 9. Cartoon representation of the question whether LM or LMH is the smallest active unit. It depends on one's point of view (i.e. the way one looks at it). (From Feher et al. 1984.)

al. 1974) and sent the isolated subunits to Lisa Steiner who determined the *amino acid composition* (Steiner et al. 1974). I look back with pleasure and nostalgia on the many hours spent with Mel cutting up gels to separate the L and M subunits. At the beginning the amino acid compositions were not very reproducible. This was traced to contaminations presumably arising from the shedding of cells from our faces as we leaned over to cut the gels. The problem was eliminated by using a protective plastic shield; we never found out whose dandruff was responsible for the erratic results. We also determined that the H-subunit, which could be removed by centrifugation in a sucrose gradient, was not required for photochemical activity, i.e. all the cofactors were associated with the LM complex. This gave rise to a debate (which I never found very fruitful) whether LM or LMH should be called the reaction center. After all, the reaction center is an operational concept. It refers to the minimum unit that can perform a specific function. If the function is charge stabilization on Q_A^-, then LM is the RC. If it involves electron transfer to Q_B^-, then LMH is the RC since the electron transfer is impaired in the LM complex. So it depends on the way one views the problem. To make this point, in the hope of terminating this discussion, I showed a known cartoon (Figure 9) at the VIth International Congress on Photosynthesis (Feher and Okamura 1984).

Incidentally, at that conference a funny thing happened. I gave a plenary lecture in a large auditorium where I could hardly make out the faces of the people in the last row. I thought the lecture went over well, but soon found out that this feeling was not shared by all. After the lecture I went to the bar for a beer. Next to me sat a young woman who asked me what my background and current interests were. When I told her that I was a physicist she said: 'Oh, then you may have been one of the few who understood the plenary lecture'. Years later I met her at a Conference and she was still embarrassed. So Jeanne, if you should by any chance read this, please don't worry about the incidence.

Back to Science. The next aim was to obtain the amino acid sequence of the subunits. This proved to be difficult and I remember a few frustrating but interesting weeks spent in 1976 with Lisa Steiner in John Walker's lab at the MRC in Cambridge, UK, trying to work out the proper conditions for the liquid-phase sequencer. The isolated subunits were insoluble in aqueous solution and were difficult to purify in sufficient quantity. Moreover, the initial analyses of the M and H subunits resulted in very low yields. Lisa persisted with two postdocs, B. Gardlung and M. R. Sutton. After much hard work, the difficulties were overcome by modifying the preparation of the subunits and the sequencer conditions, and the sequence of the amino-terminal 25–28 residues of the three subunits was determined (Sutton et al. 1982). The amino-terminus of the H subunit was rich in hydrophobic residues, consistent with its being inserted into the membrane.

Although these results may seem modest considering the effort that went into this research, it proved crucial for the construction of oligonucleotide probes that were later used to identify the structural genes encoding the three polypeptides. That work was carried out by JoAnn Williams, a very talented graduate student in our lab with a 'green thumb' for recombinant DNA techniques. Mel Simon, a colleague from the UCSD biology department helped us get started with this methodology. JoAnn isolated the genes encoding for the M and L subunits and determined the sequence of each (Williams et al. 1983, 1984). The sequence showed 5 hydrophobic regions in each subunit that were postulated to form 5 transmembrane helices. The H-subunit was sequenced a couple of years later and showed only one hydrophobic section near the amino terminus as Sutton et al. had found earlier (Williams et al. 1986). Youvan et al. (1984) developed the genetic systems of a related bacterial species, *Rb. capsulatus* and determined the sequence of the RC subunits.

An exciting result of the sequence work was the homology that was found between the L & M subunits and the D_1 and D_2 polypeptides of photosystem II reaction centers from spinach (Williams et al. 1983; Youvan et al. 1984). These findings led to the conclusion that D_1 and D_2 constitute the core of the PS II RC, which was, therefore, postulated, to have a structure similar to that of the bacterial RC (Trebst, 1987; Michel and Deisenhofer 1988). Nanba and

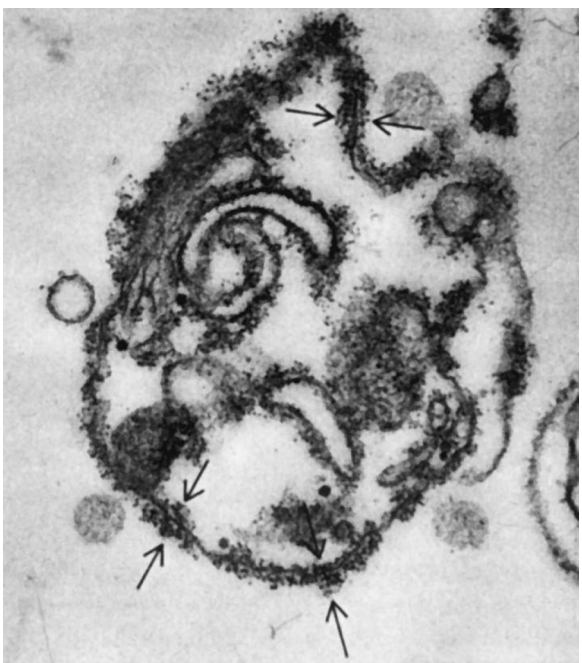


Figure 10. Electron micrograph of *R. sphaeroides* spheroplasts incubated with twice affinity-purified anti-M antibodies and immunoferritin labeled with goat anti-rabbit ferritin conjugate. Arrows correspond to regions where the membrane surface is clearly labeled on both sides. (Modified from Valkirs and Feher 1982.)

Satoh (1987) isolated a core complex of PS II that contained only D_1, D_2 and cyt *b*-559. This core complex was shown by EPR to be able to perform the primary charge separation between P680 and the intermediate acceptor (Okamura et al. 1987).

The *topography of the RC* in the bacterial membrane had been investigated by a variety of techniques in several laboratories. These studies showed that H is exposed on the cytoplasmic side of the membrane, but no clear consensus had been reached concerning the L & M subunits. Gunars Valkirs, a graduate student, addressed that problem by indirect immunoferritin labeling (Valkirs and Feher 1982). In this technique the photosynthetic membrane is first exposed to rabbit antibodies directed against the RC subunit and then to ferritin-conjugated goat antibodies that bind to rabbit IgG. Ferritin is an electron-dense molecule, thereby permitting localization of the binding site by direct electron-microscopic visualization. The result with anti-M antibodies is shown in Figure 10. The clear labeling (dark spots) of the membrane on both sides shows that M spans the membrane. All anti-H preparations labeled the cytoplasmic side and only one out of six labeled the periplasmic side. From this we concluded that H is asymmetrically oriented with respect to the membrane, i.e. with the bulk being on the cytoplasmic side. The anti-L antibodies labeled only the periplasmic surface of the membrane. However, in contrast to our conclusion reached about the H-subunit we said that '... absence of labeling is not meaningful since it may only demonstrate that no antibodies recognize a particular protein segment ...'. On re-reading the paper I was struck by this inconsistency in treating the similar results obtained for the H & L subunit. It shows the bias that we had in favor of the similar topology of the L & M subunits because of their similar amino acid composition. Although the conclusion turned out to be correct, the argument as we had presented it was flawed.

The *location of the Q_A binding site* was determined by Tim Marinetti, a post-doc in our lab in 1978–79. He introduced the photoaffinity label [^{3}H] 2-azidoanthraquinone into the Q_A site. When illuminated with UV light it photolyzed and became attached to the protein. Analysis of the photolyzed protein by SDS-PAGE revealed that the M-subunit was selectively labeled. This showed that the primary quinone site is located at or very close ($\sim$ 5 Å) to the M subunit (Marinetti et al. 1979). The location of the Q_B site could not be determined by photoaffinity labeling because of the more stringent structural requirement for the binding of the secondary quinone. Instead, an immunological approach was used. Antibodies against the subunits were tested for inhibition of the binding of quinone at the Q_B^-site. Anti-M was found to be most effective, from which we concluded that Q_B is also located on the M-subunit (Debus et al. 1982). This proved to be wrong. In retrospect, this was not a well-conceived experiment since antibodies are of the same size as RCs and, therefore, are not well suited to provide sufficient resolution to localize the binding site on the RC. Furthermore, antibodies bind to the surface and, therefore, provide at best a probe for the location of the isoprenoid chain that sticks out and not of the quinone head group which is buried inside the RC.

In addition to the reactants permanently bound to the RC in *Rb. sphaeroides*, there is the *secondary donor*, an exogenous cyt c_2 that docks transiently on the RC to reduce the oxidized primary donor $(BChl)_2^+$. The stoichiometry of binding had been investigated by several groups who reported different results. To settle this issue, Dan Rosen, a graduate student, undertook to investigate the number of binding sites and to determine the dissociation constant by equilibrium dialysis. He found one binding site with a dissociation constant

of $\sim 1\ \mu$M (Rosen et al. 1980). Rosen also attempted to localize the binding site by chemically crosslinking cyt c_2 to the RC. He found that it crosslinked to both the L & M subunits and concluded that the binding site is within 10 Å (the resolution given by the length of the crosslinks) of the L and M subunits (Rosen et al. 1983). Recent developments (to be discussed later) pin-point the binding site more precisely.

State of our knowledge of the structure and composition of the reaction center B.C. (Before Crystallization)

In the mid-1980s a landmark development occurred in the field of bacterial photosynthesis. That was the crystallization and the determination of the three-dimensional structure of the RC at atomic resolution by Johann Deisenhofer and Hartmut Michel in Robert Huber's lab (Deisenhofer et al. 1985). But before discussing this development, it may be instructive to summarize our knowledge of the structure and composition of the RC B.C. (Before Crystallization) or more precisely before the X-ray structure was determined in 1985.

1. The RC is composed of 3 subunits, L, M, H (Feher 1971; Feher et al. 1971).
2. L and M span the membrane, each having 5 transmembrane helices (Williams et al. 1983, 1984).
3. H predicted to be mostly on the cytoplasmic side with its amino terminal section inside the membrane (Valkirs and Feher 1982; Sutton et al. 1982).
4. The cofactors are: four BChl, two BPhe, 2Q-10, one Fe (Feher 1971; Straley et al. 1973).
5. All cofactors are associated with the LM subunit (Okamura et al. 1974).
6. The primary donor is a bacteriochlorophyll dimer $(BChl)_2$ (Norris et al. 1971, 1975; Feher et al. 1975).
7. The $(BChl)_2$ is located near the periplasmic side of the RC (see point 16).
8. The quinones are near the cytoplasmic side of the RC (follows, for instance from electrogenicity (Schonfeld et al. 1979) and point 7).
9. Q_A is associated with the M-subunit (Marinetti et al. 1979).
10. The cofactors are arranged in a line: $(BChl)_2$ BChl, BPhe, Q_A Fe Q_B (Okamura et al. 1982).
11. Fe is approximately equidistant from Q_A and Q_B (Butler et al. 1984).

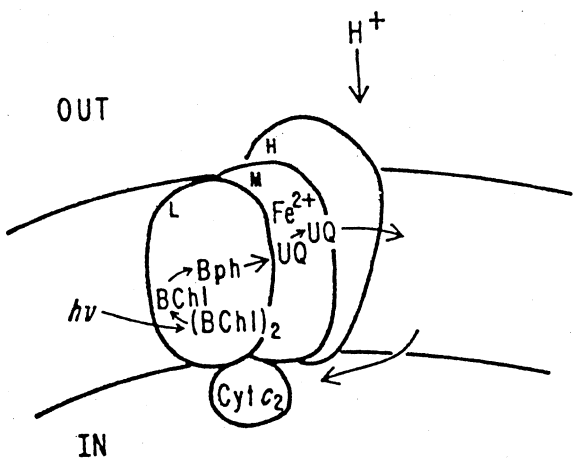


Figure 11. Schematic representation of a proposed structure of the RC from the purple bacterium (*Rp. sphaeroides*). The position of the bound Cyt c_2 (absent in purified RCs) is also indicated. The labeling 'in and out' refers to chromatophores, i.e. 'in' corresponds to the periplasmic side and 'out' to the cytoplasmic side of the plasma membrane. (From Okamura et al. 1982.)

12. Fe is 6-coordinated with nitrogens and oxygens as ligands (Bunker et al. 1982; Eisenberger et al. 1982).
13. The average ligand distance of the first Fe coordination shell is 2.10 ± 0.02 Å with a more distant shell at 4.14 ± 0.05 Å (Bunker et al. 1982; Eisenberger et al. 1982).
14. Quinones do not bind to Fe; likely ligands are histidines (Bunker et al. 1982; Eisenberger et al. 1982).
15. The distance between $BPhe^-$ and Q_A^- is ~ 10 Å (Peters et al. 1978; Okamura et al. 1979).
16. The secondary donor, cyt c_2, docks on the periplasmic side of L & M (Prince et al. 1975; Rosen et al. 1983).

All the above points were corroborated by the X-ray structure discussed in the next section. But before proceeding to it, I want to show a 'low resolution' structure that we proposed with Mel in a review article in Govindjee's book (Okamura et al. 1982) (Figure 11). This structure has a remarkable resemblance to the one determined by X-ray diffraction. What is conspicuously missing are the locations of one BChl and one BPhe.

Crystallization of the RC and determination of its structure by X-ray diffraction

A prerequisite for success in determining structures by X-ray diffraction is the availability of relatively large,

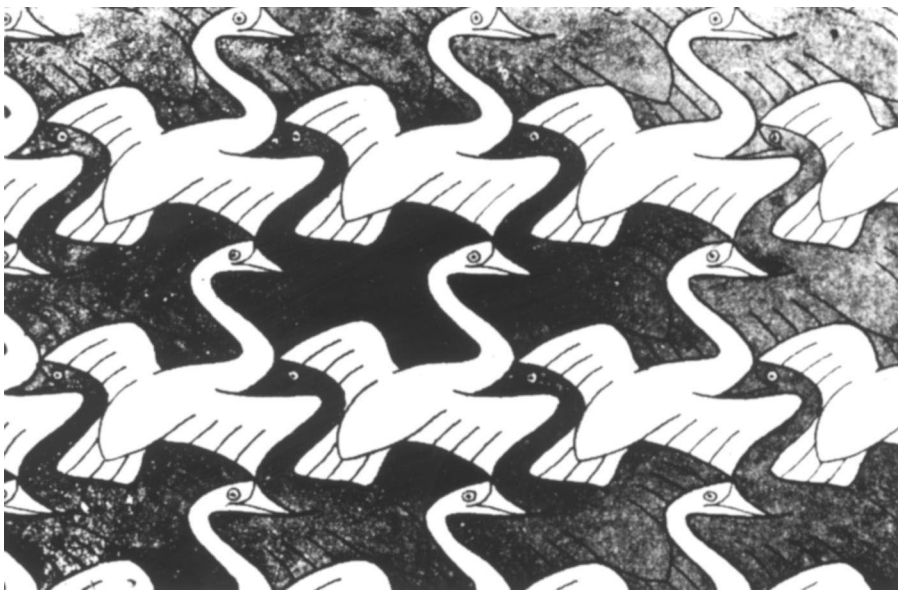
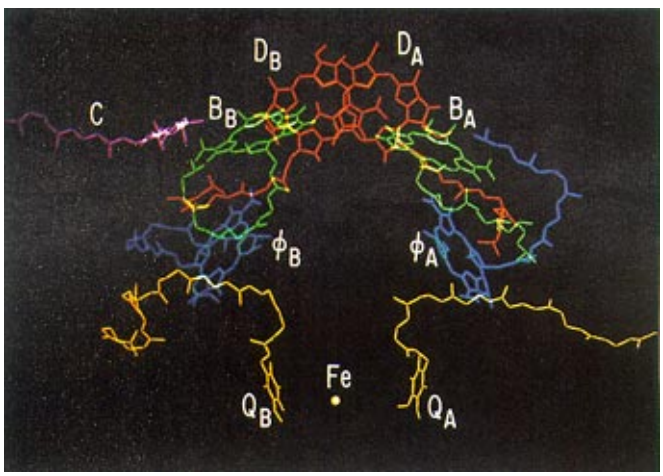


Figure 12. To keep our hope up we kept telling ourselves that if Escher can 'crystallize ducks', we should be able to crystallize integral membrane proteins. (From Feher 1988.)

well ordered single crystals. So the bottleneck is usually the crystallization step, which for macromolecules in the 1970s was more of an art than a science. We had started an active program in 1970 with postdocs Zvi Kam and, later, Steve Durbin to systematically investigate crystallization mechanisms of macromolecules (e.g. Kam et al. 1978; Durbin and Feher 1986). We chose the easily crystallizable, water-soluble protein lysozyme as a model compound hoping later to advance to the more complicated system of water-insoluble, hydrophobic proteins, i.e. the RC. The haphazard trial and error approach that was used for these proteins did not appeal to us (a known monkey on a physicist's back). Nevertheless, we occasionally (on Friday afternoons) abandoned our systematic approach and half-heartedly set up some crystallization dishes with RCs. We did this in spite of the prevailing belief in the 1970s that integral membrane proteins could not be crystallized because of the presence of randomly oriented detergent molecules that would interfere with the formation of an ordered array of identical units. To keep our hope up we had one of Escher's pictures showing an ordered array of ducks posted in our lab (Figure 12). An indication of the prevailing attitude was a reviewer's report of our 1978 grant in which he severely criticized us for being so naive as to pursue the futile effort to crystallize a membrane protein (for a verbatim excerpt of the review see G. Feher's 'Light reflections III', 1998b).

The 'dogma' of the noncrystallizability of membrane proteins was shattered in 1980 by the successful crystallization of bacteriorhodopsin (Michel and Oesterhelt 1980) and porin (Garavito and Rosenbush 1980), followed by H. Michel's success in crystallizing the RC from the photosynthetic bacterium *Rps. viridis* in 1982 (Michel 1982). Spurred by these successes, Jim Allen who joined us as a postdoc in 1982 put a concentrated effort in crystallizing the much better characterized RCs from *Rb. sphaeroides*. Success was relatively quick and in the National Lecture of the Biophysical Society in February 1983 I was able to present pictures of the crystals.

Following the crystallization, the structures of the cofactors for *Rps. viridis* were published by Deisenhofer et al. in 1984 and of the protein in 1985. In 1985 we joined forces with the crystallography group of Doug Rees at UCLA with whom we continue to have an enjoyable and fruitful collaboration. Jim Allen started out by providing the RC crystals but in the course of the work he became a crystallographer, getting heavily involved in structure determinations himself. Preliminary phases and structures were obtained by the molecular replacement method using the coordinates of the RC from *Rps. viridis* (Allen et al. 1986). Similar work was also carried out by Chang et al. (1986). We initially determined the structure of the RC from *Rb. sphaeroides* to a resolution of 2.8 Å (Allen et al. 1987). The resolution is being constantly



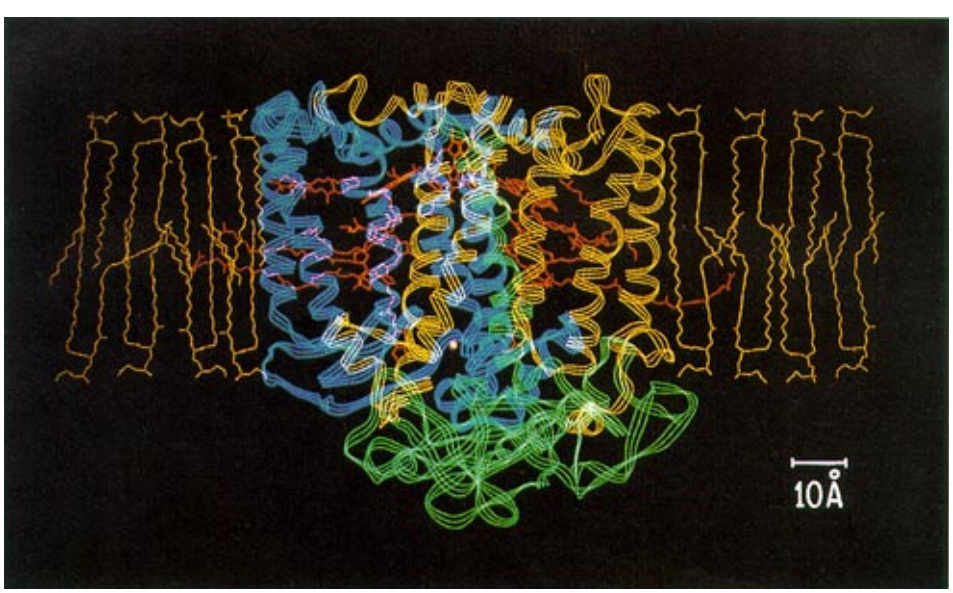


Figure 13. The structure of the reaction center from *Rb. sphaeroides* (wild type strain 2.4.1). (a) Cofactor structure. The symmetry axis is aligned vertically in the plane of the paper. (b) The structure of the reaction center from *Rb. sphaeroides* 2.4.1 and its position (modeled) in the lipid bilayer. For simplicity, only one major type of lipid, phosphatidylethanolamine, is shown. L-subunit: yellow; M-subunit: blue; H-subunit: green. Cofactors in red. The two-fold symmetry axis is in the plane of the paper joining the Fe (dot) near the cytoplasmic side (bottom) with the bacteriochlorophyll dimer near the periplasmic side (top). (From Feher et al. 1989.)

improved. Our most recent published structure, which I will discuss later, is at a resolution of 2.2 Å (Stowell et al. 1997).

The structure of the RC has been shown so often that I am hesitant to present it again. But it is such a beautiful structure that I can't resist. Figure 13a shows the structure of the cofactors and Figure 13b the structure of the RC-protein with its modelled location in the membrane. The five transmembrane helices of the L & M subunits that have been predicted from the amino acid sequences are clearly discernible. Both the cofactors and the L & M subunits exhibit an approximate two-fold symmetry about a line joining the donor and the Fe. The electron transfer proceeds preferentially

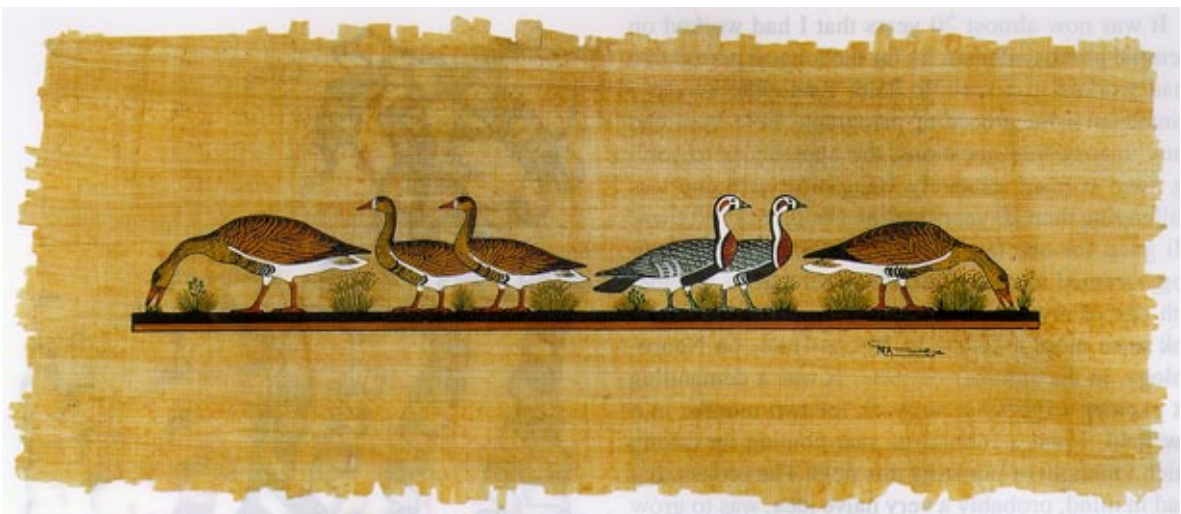


Figure 14. Ancient Egyptian painting. Old Kingdom, 4th Dynasty 2620 B.C.E. (Before Common Era). ∴ 4602 B.C. (Before Crystallization). Does the twofold symmetry remind you of anything? For story see text.

along the A-branch. This unidirectionality is at present not completely (i.e. quantitatively) understood. The two-fold symmetry endows the structure with an unusual beauty. In principle (and in retrospect) it could have been intuitively predicted by a far-sighted aestheticist – but, of course, it was not. The exciting and very satisfying aspect of the structure is that it corroborates all the predictions enumerated in the previous section. The prediction that Q_B is on the same subunit as Q_A was omitted from the list of predictions because of the criticism of this result discussed earlier. If this procedure sounds a bit arbitrary, think of it as a bad data point. But even if it is included, the batting ratio (16:1) is still impressive.

The two-fold symmetry brings to mind a funny incident. At the time that the structure of the RC became known I was traveling in Egypt, going down the Nile, visiting the various archeological sites. I was particularly fascinated by a beautiful ancient painting showing three ducks going to the right and three ducks to the left (Figure 14). It had the same two-fold pseudosymmetry as the RC. I explained my fascination to our guide who was an archeologist. He became very excited and came to the conclusion that the ancient Egyptians already must have understood photosynthesis. What worried me was that he intended to publish his conclusions in a Cairo newspaper. I finally dissuaded him by telling him about an incident that had occurred a few decades earlier. Egyptian archaeologists excavated a piece of copper wire in Egypt and concluded that the ancient Egyptians already communicated by wire telephony. This conclusion was duly published in their newspapers. A few days later the Jerusalem Post carried a small article to the effect that in all the excavations in Israel they had never found a copper wire. That proved that the ancient Hebrews already had wireless communication!

After the three-dimensional structure of the RC was published, there was a feeling in several quarters that the game was over; bacterial photosynthesis was understood. That in my opinion was a highly exaggerated point of view. The cream may have been skimmed of the top but there still remained a lot of unanswered questions. For example, the origin of the unidirectionality of electron transfer. Such a question could not have been asked before the structure was determined. Furthermore, now that one had the distances and orientations of the cofactors could one calculate the electron transfer rates? The structures showed that the secondary quinone is inside the RC in a hydrophobic region. How do protons get to it from the outside, aqueous, phase? And the role of the protein could now be systematically investigated using the structure of the RC as a guide to mutate specific residues. It should be kept in mind that the structures shown in Figure 13 were obtained when all the cofactors were neutral. Could one determine the putative structural changes accompanying charge separation? Some of these questions will be addressed in the next sections. But before getting to that, I would like to talk about an episode that almost terminated my involvement with photosynthesis.

It was now almost 20 years that I had worked on bacterial photosynthesis, by far the longest period that I had worked in a specific field. And although, as I pointed out in the preceding paragraph, there remained many unanswered questions, the approaches to solving them seemed relatively straight-forward and less challenging than 20 years earlier. I felt that at 60 I was still good for one last switch in my career. Neurobiology seemed to me an exciting field and so together with one of my graduate students, David Kleinfeld, I took once more a course in Woods Hole, on Neurobiology, in the summer of 1984. It was a demanding but exciting experience to work for two months in a new field. I had a sabbatical year coming up during which I thought of pursuing this field. The project that I had in mind, probably a very naive idea, was to grow nerve cells on a silicon chip, follow electrically their development, connections, etc. with the goal of determining the minimum unit that can 'learn', i.e. that has a memory. Sort of analogous to the RC of the brain. Bell Labs, known for its chip technology, offered me a position and in the Spring of 1985 I visited them to discuss the details of the sabbatical arrangement. On my return from Newark to Boston I suffered a heart attack on the plane. A miserable affair, to say the least. After the hospital stay and recovery I did not feel up to investing a few years in a new field and all previous plans were scrapped.[4] Bacterial photosynthesis was a familiar and comfortable field in which it was easy for me to direct students and postdocs. Therefore, I opted to stay with it. But the style of my research changed. I had always been involved in the actual lab work and in addition I always had a pet project on the side that was not necessarily connected with photosynthesis and on which I worked to a large extent myself, for instance fluctuation spectroscopy (Feher and Weissman 1973), a new method of determining the molecular weight of large DNA (Weissman et al. 1976), heme proteins (Feher et al. 1973), 1/f noise (Weissman et al. 1979), etc. These activities have to a large extent stopped. To be honest, I miss them. In supervising research rather than doing it oneself, something vital is lost. The best analogy that I can come up with is: 'kissing through a veil'. It is, of course, still fun and I am still enthusiastic about our research as I hope comes through in the next sections.

Structure of the RC-cyt $_2$ *complex*

In *Rps. viridis* the secondary donor that reduces D^+ is a tetraheme *c*-type cytochrome tightly bound to the polypeptide core of the RC. Its three-dimensional structure was determined as an integral part of the RC (Deisenhofer and Michel 1991). In contrast, in *Rb. sphaeroides*, the secondary donor is a water-soluble single heme cytochrome c_2, which reversibly binds to the RC. The three-dimensional structure of cyt c_2 from Rb. sphaeroides was determined by Axelrod et al. (1994).

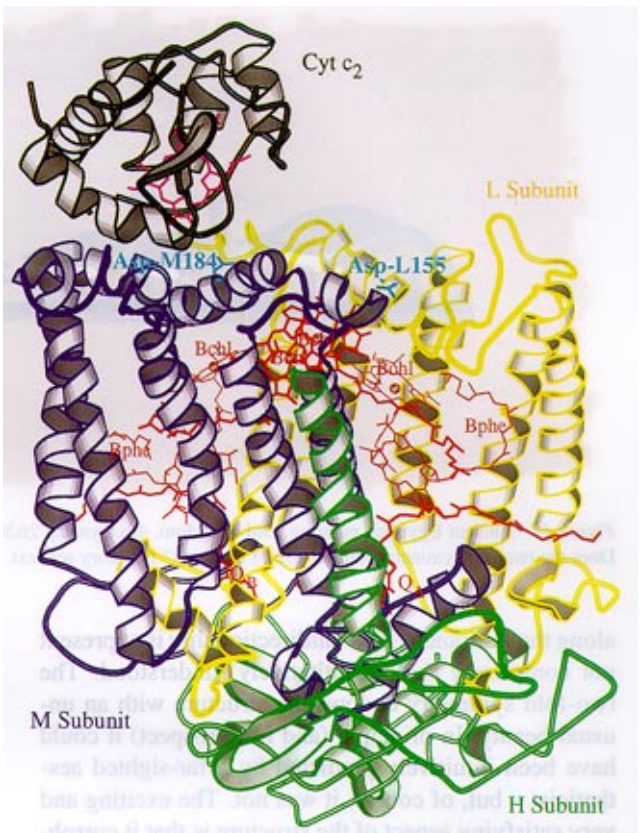


Figure 15. Preliminary structure of the RC-cyt c_2 complex in *Rb. sphaeroides*. Note the positions of Asp M(184) and Asp L(155) with respect to cyt c_2. A mutation of Asp M(184) is expected to have a larger effect on the dissociation constant, as was found experimentally to be the case. Color scheme of RC same as in Figure 13. (Modified from Adir et al. 1996.)

Several models for the structure of the transient RC-cyt c_2 complex had been proposed (Rosen et al. 1983; Allen et al. 1987; Tiede and Chang 1988; Drepper et al. 1997). They were all based on indirect experimental evidence and theoretical considerations. The most direct way to determine the structure of the bound complex is by X-ray diffraction analysis. This requires the co-crystallization of the complex, which was generally believed to be unattainable because of the transient nature of the association. Notwith-

standing these sentiments, Noam Adir, an enterprising Israeli post-doc who joined our group in 1990 undertook and succeeded after many trials to co-crystallize the complex[5]. The crystals diffracted to an initial resolution of 3.5 Å, which degraded during the data collection to 4.5 Å (Adir et al. 1996). Unfortunately, the occupancy of the cyt c_2 in the crystal (determined by optical spectroscopy and SDS-PAGE) was only 25%.

Knowing the structure of the RC, we were able to use the molecular replacement method to obtain the phases for solving the structure of the complex. However, because of the low cyt c_2 occupancy we were unable to trace the continuous polypeptide backbone of the cyt c_2. Patches of electron density near the M-side of the periplasmic surface of the RC were clearly discernible and attributed to cyt c_2. Into these patches we manually positioned the known structure of cyt c_2, maximizing the oppositely charged residues of the RC and cyt c_2. The structure of the RC-cyt c_2 complex obtained by the procedure is shown in Figure 15. It is similar, but differs in detail from the model proposed by Tiede and Chang (1988).

In view of the absence of a contiguous electron density map for cyt c_2, we used two additional pieces of evidence to corroborate the proposed structure of the complex (Adir 1996). In one, Paul Beroza calculated the lowest energies of different cyt c_2 docking models. Since the interaction between the RC and cyt c_2 is believed to be primarily electrostatic, it is likely that the most stable RC-cyt c_2 complex is in a minimum with respect to electrostatic interaction between the protein. The structure corresponding to the lowest energy agreed well with the proposed structure.

In the second approach, Scott Rongey explored the area of contact between the RC and the cyt c_2 by selectively mutating residues on the periplasmic side of the RC that were believed to be important to binding. Two Asp residues at positions L184 and M155 were changed to lysines. The dissociation constant increased $\sim$ 60 fold in the DK (M184)[6] mutant but only $\sim$ 10 fold in the DK (L155) mutant. In the proposed co-crystal structure, M184 is in contact with cyt c_2, whereas L155 is a distance away from the cyt c_2 binding site (see Figure 15). Thus, the much larger effect on the binding affinity of the DK (M184) mutation compared with the DK (L155) mutation is in agreement with the proposed co-crystal structure.

A question that needed to be addressed is whether the structure of the complex in the crystal is the same as that in solution. The fast component of the electron transfer rate between cyt c_2^{2+} and D^+ is expected to depend sensitively on the details of the structure. Consequently, we measured the kinetics of electron transfer in the co-crystal and found them to be the same (0.9 ± 0.1 μs) as in solution. This provides strong evidence that crystallization did not affect the structure of the complex.

In view of the preliminary nature of the proposed structure, we are continuing this project along several lines. Michelle Tetreault, a graduate student following Scott Rongey, has constructed several more site directed mutants and is exploring the contact of the RC with cyt c_2 in more detail. A second approach addresses the question of a higher occupancy of cyt c_2 in the co-crystal. Herb Axelrod has changed the crystallization conditions used by Adir (1996) and has obtained co-crystals with a $\sim$ 80% occupancy. In another approach, we have constructed two mutants, ND (M188) and QD(L264) that bind cyt c_2 4 and 20 times, respectively, stronger than wild type. We expect these to provide us with co-crystals that have essentially a 100% cyt c_2 occupancy. With these developments I am confident that a definitive structure of the RC-cyt c_2 complex will be forthcoming in the not too distant future.

Electron transfer reactions: Kinetics, mechanisms and light induced conformational changes

The initial electron transfer reactions, which occur on a pico-second time scale have been investigated by many groups, including P. L. Dutton, D. Holten & C. Kirmaier, M. E. Michel-Beyerle, W. W. Parson, C. Schenck, W. Zinth and others (for a recent review see Woodbury and Allen 1995). Our work has focused on the slower electron transfer reactions that lead to the stabilized ubisemiquinone and dihydroquinone. The reactions involving the first electron are described by Equation (1):

$$\begin{array}{ccc} DQ_A\,Q_B & \underset{k_{AD}}{\overset{h\upsilon}{\rightleftharpoons}} & D^+Q_A^-Q_B \underset{k_{BA}^{(1)}}{\overset{k_{AB}^{(1)}}{\rightleftharpoons}} D^+Q_AQ_B^- \\ \uparrow & \xleftarrow{\qquad\qquad k_{BD} \qquad\qquad} & \uparrow \end{array} \tag{1}$$

Let me start by focusing on the charge recombination kinetics k_{AD} and k_{BD}. David Kleinfeld, while still a graduate student in our lab, investigated in 1983 the kinetics and thermodynamics of the reactions shown in Equation (1) (Kleinfeld et al. 1984a). He showed that k_{BD} is not a direct recombination process but proceeds via the thermally activated state $D^+Q_A^-Q_B$. He

deduced that the ratio of the *direct* recombination rates $k_{AD}/k_{BD} \cong 100$. This at first sight seemed surprising since the distances Q_A – D and Q_B – D are within less than one Å the same and the two states $Q_A^- Q_B$ and $Q_A Q_B^-$ are close to being isoenergetic. This bothered us for several years until Andreas Labahn (1995), a postdoc in our lab, explained the puzzle a couple of years ago. He increased the energy difference between the Q_A^- Q_B and $Q_A Q_B^-$ states by substituting low potential quinones in the Q_A site. This eliminated the indirect pathway and made it possible to observe the direct recombination rate k_{BD}. Labahn confirmed the large ratio k_{AD}/k_{BD} obtained by Kleinfeld. By fitting the observed k_{BD} as a function of pH (i.e. the energy difference between the states $D^+Q_A Q_B^-$ and $DQ_A Q_B$) with the Marcus theory (Marcus 1993) for electron transfer, he deduced a reorganization energy, λ that was considerably larger than that associated with k_{AD} (1.1 eV vs. 0.6 eV, respectively). This is consistent with the more polar environment of Q_B and is believed to be the main contributor to the large ratio of k_{AD}/k_{BD}. How a difference in λ of ~ 0.5 eV changes k_{et} by two orders of magnitude is illustrated in Figure 16.

$$k_{ct} = \frac{2\pi |T_{AB}|^2}{\hbar (4\pi\lambda k_B T)^{1/2}} \exp\left\{ -\left[\frac{(\Delta G^0_{ct} + \lambda)^2}{4\lambda k_B T} \right] \right\}$$

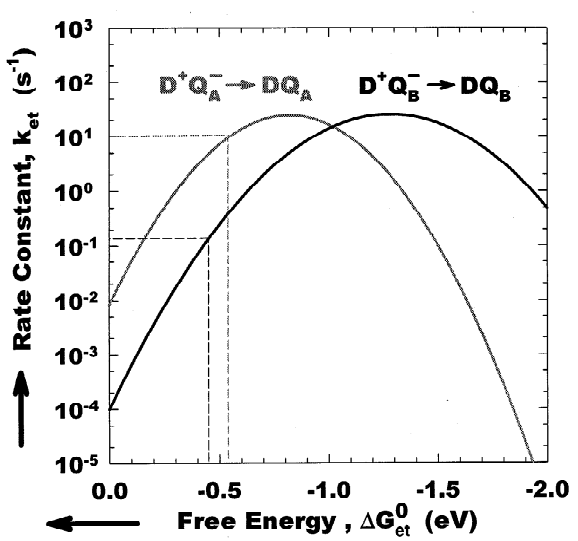


Figure 16. The difference in the recombination rates between $D^+Q_A^-$ and $D^+Q_B^-$ is due to the difference in reorganization energies which displaces the Marcus parabolas (see equation on top) for the two reactions by ~ 0.5 eV. The matrix elements $|T_{AB}|^2$ are 3.6×10^{-8} eV and 4.1×10^{-8} eV for k_{AD} and k_{BD}, respectively. The dotted line shows the position on the Marcus curve for the two reactions. (Modified from Allen et al. 1988.)

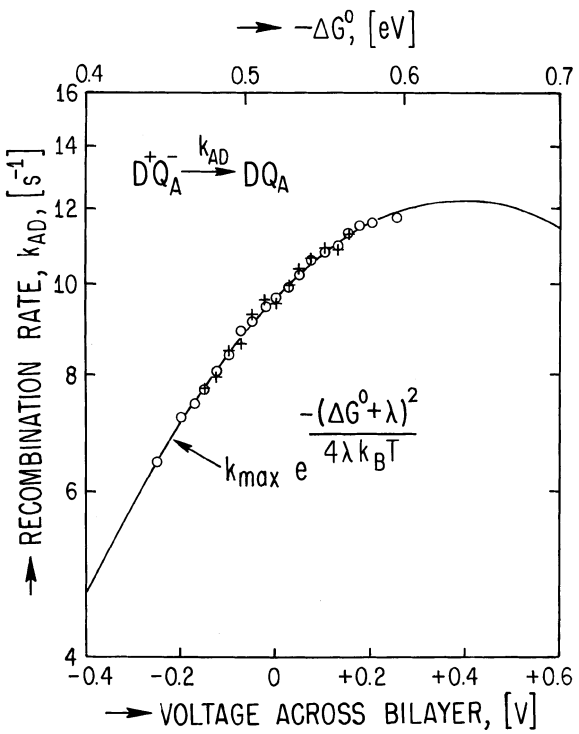


Figure 17. Recombination rate, k_{AD}, as a function of voltage across the bilayer in which reaction centers are incorporated. The solid line represents a theoretical fit (see equation on top of Figure 16) with ΔG^0 and λ as adjustable parameters. The difference in the scales of the upper and lower abscissa is due to the fraction of the applied voltage that occurs across D^+Q^-. (From Feher et al. 1988.)

It may be instructive to describe how λ for the k_{AD} process was obtained. To determine λ one needs to compare the energy dependence of k_{AD} with that predicted by the Marcus theory. Marilyn Gunner in Dutton's lab varied the energy of the charge separated state by substituting quinones with different redox potentials in the Q_A site (Gunner et al. 1986). Although this was a heroic piece of work, there lingered an uneasiness about the possibility that the substitution may alter other parameters, e.g. the structure in the vicinity of the quinone. To avoid this possibility we used an electric field to change the energies. RCs were incorporated into a lipid bilayer and a voltage was applied across the membrane. The recombinant rate k_{AD} vs. voltage was fitted with the Marcus theory as shown in Figure 17 using a value of $\lambda = 0.64 \pm 0.02$ eV (Feher et al. 1988). The error quoted represents the precision. The accuracy may be poorer due to the uncertainty of the scaling factor relating the voltage

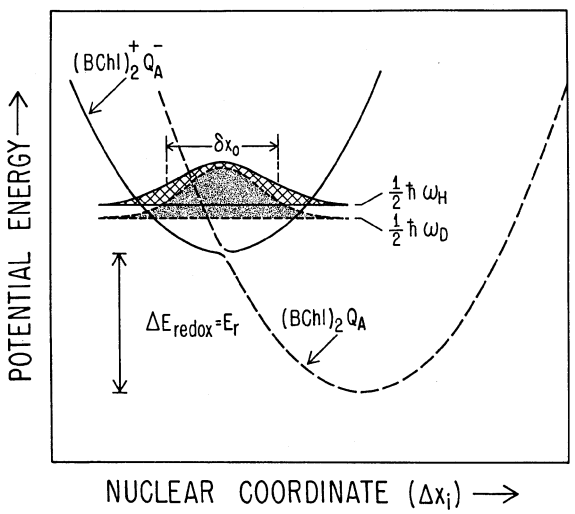


Figure 18. The potential energy vs. nuclear coordinate for the activationless charge recombination process $(BChl)_2^+ Q_A^- \rightarrow (BChl)_2 Q_A$. The reaction is assumed to take place from the ground vibrational state of the reactant manifold. The probability density distribution for the ground vibrational state is shown for the protonated (solid curve) and deuterated (dashed curve) species. The higher probability density of the deuterated species near the transition state predicts an increase in the electron transfer rate upon deuteration as has been experimentally observed. (Modified from Okamura and Feher 1986.)

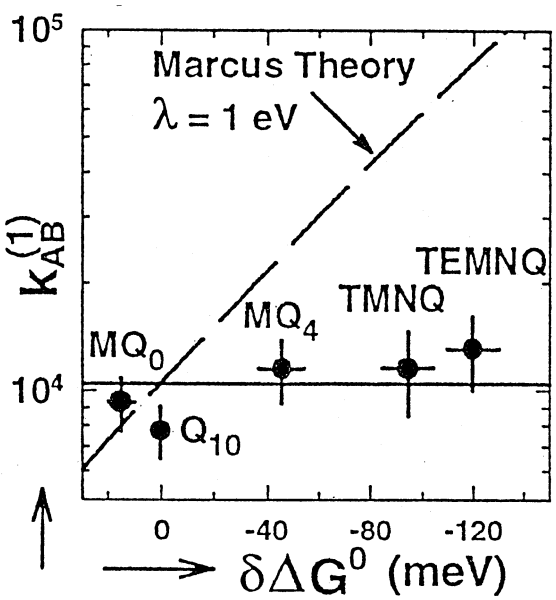


Figure 19. Change in $k_{AB}^{(1)}$ as a function of relative redox free energy for electron transfer. The independence of $k_{AB}^{(1)}$ on $\delta\Delta G^0$ shows that a process other than electron transfer is the rate limiting step of $k_{AB}^{(1)}$. MQ_o, menadione; Q_{10}, ubiquinone-10; MQ_4 menatetrenone; TMNQ, trimethylnaphtaquinone; TEMNQ, tetramethylnaphtaquinone. (From Graige et al. 1996a.)

across the membrane to the voltage across the D^+ - Q_A^- couple (see bottom and top abscissas in Figure 17). This probably accounts for the larger value of λ (0.82 eV) obtained recently by Allen et al. (1998) using a different technique. However, these authors obtained the same difference (0.5 eV) in the λ's for the two processes, k_{AD} and k_{BD} (for a more detailed discussion, see Allen et al. 1998).

The *mechanism of* k_{AD} had been inferred early on from the observed approximate temperature independence down to cryogenic temperatures (McElroy et al. 1974 and references therein). This activationless behavior suggested that the potential energy surface of the product state (DQ_A) intersects the energy surface of the reactant state ($D^+Q_A^-$) near its energy minimum, i.e. that the recombination process occurs close to the top of the Marcus parabola. This indeed has been shown to be approximately the case for k_{AD} as illustrated in Figures 16 and 17.

Theories of electron transfer require the coupling of electron transfer to vibrational motion of the molecules involved in the reaction (Hopfield 1974; Jortner 1976). To identify the vibrational modes that play a role in the electron transfer, we measured the isotope effect of k_{AD} (Okamura and Feher 1986). The two carbonyl oxygens of Q_B are hydrogen bonded to two protons. When they are exchanged with deuterons, the energy of the ground vibrational state from which electron transfer occurs is lowered (see Figure 18). As a consequence, the wave function is narrowed, its peak is higher resulting in a higher probability density at the transition state (at the bottom of the reactant state). This should result in an increase in k_{AD}. A simple theoretical model predicts a maximum increase in rate of 20%. We found a 5.7 ± 0.3% increase in k_{AD} upon deuteration, showing that the hydrogen bonded protons associated with Q_A provide a vibrational mode that is important in the electron transfer reaction.

Now to the electron transfer reaction from Q_A^- to Q_B (i.e. $k_{AB}^{(1)}$ in Equation 1). This reaction seems to be more complex than meets the eye. The main component of $1/k_{AB}^{(1)}$ is ∼ 100 μs with a minor 5 μs component found by Marilyn Gunner and co-workers (Li et al. 1998). The theoretically predicted time is 3 orders of magnitude faster than the 100 μs component (Page et al. 1996).

We believe that the 100 μs does not represent the intrinsic electron transfer time but is a characteristic time of another process that is a prerequisite for elec-

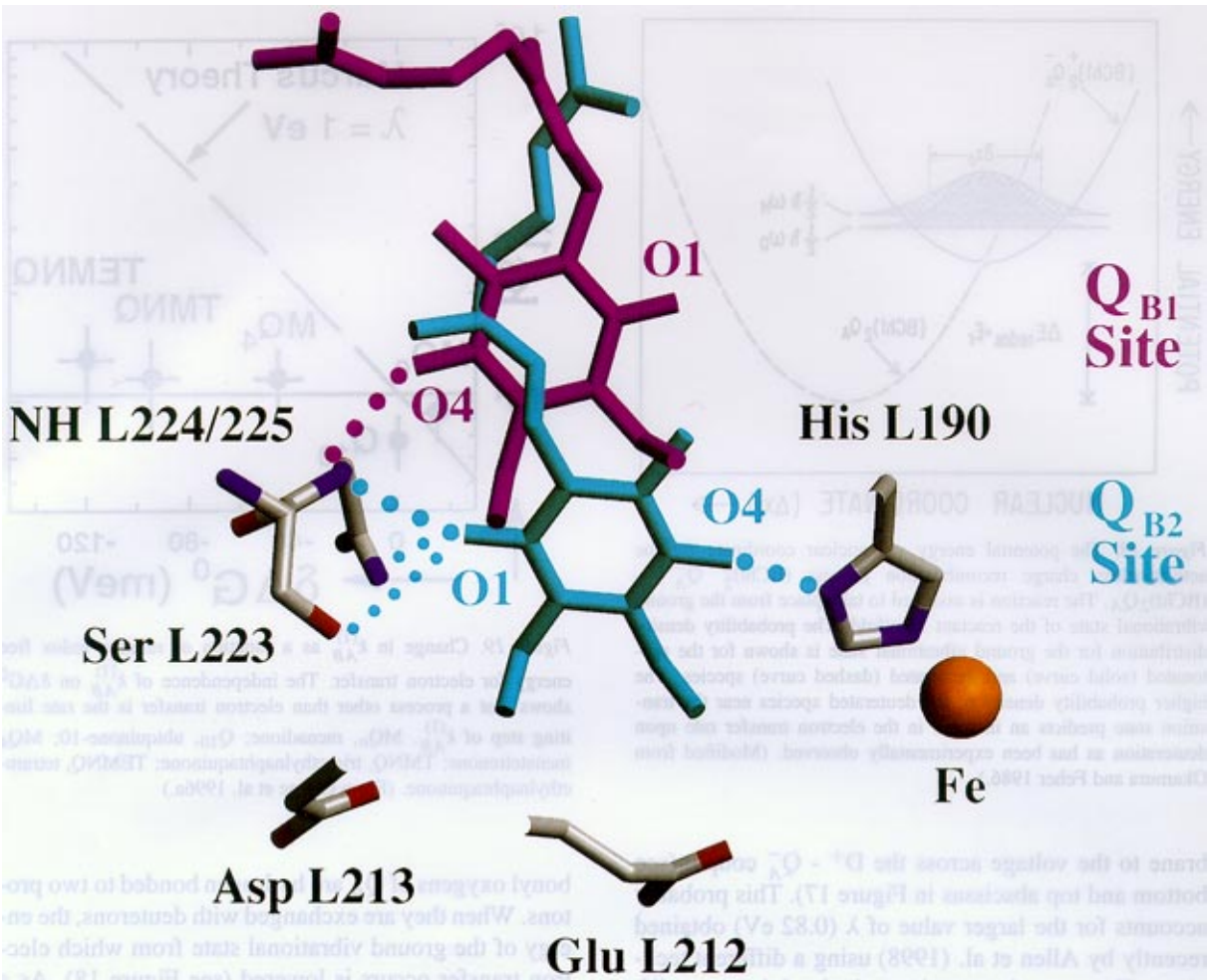


Figure 20. Comparison of the dark (DQ_AQ_B) and the light ($D^+Q_AQ_B^-$) RC structures in the Q_B binding pocket. The dark and light RC structures are colored red and blue, respectively. Hydrogen bonds are shown with dotted lines, where bond distances of less than and greater than 2.9 Å are shown by large and small dots, respectively. In the light RC structure, the ubiquinone has moved $\sim$ 4.5 Å and undergone a 180 degree propeller twist. Oxygen, nitrogen, and carbon atoms are colored red, blue, and gray, respectively. (Reprinted with permission from Stowell et al. 1997, ©1997 American Association for the Advancement of Science.)

tron transfer to take place. The evidence is provided by a powerful, general technique used by Mike Graige in our lab (Graige et al. 1996a). He substituted quinones with different redox potentials thereby changing the driving force for electron transfer. If the observed rate is due to electron transfer, its dependence on the free energy difference (driving force) should follow the Marcus theory. The 100 μs component of $k_{AB}^{(1)}$ was found to be independent of driving force (see Figure 19), which shows that a process other than electron transfer is responsible for the observed rate.

What is this other process? Perhaps it is a conformational change. This may sound like a cop-out; whenever we don't understand a process we seem to invoke a conformational change. But there are several independent experimental findings that point toward a structural change accompanying charge separation. A particularly drastic effect was observed by David Kleinfeld over a decade ago (Kleinfeld et al. 1984b). He compared electron transfer rates in RCs that were frozen under illumination with those frozen in the dark. He found that $k_{AB}^{(1)}$ was increased by several orders of magnitude when RCs were frozen under illumination, i.e. in the charge separated state, as compared to RCs frozen in the dark. The challenge was to determine the exact nature of these structural changes.

We recently addressed this challenge in collaboration with Doug Rees's group by comparing the X-ray structure of RCs in single crystals cooled to cryogenic ($\sim$ 90 °K) temperatures under illumination with the structure of RCs cooled to cryogenic temperatures in the dark (Stowell et al. 1997). The crystals diffracted at the low temperatures to a higher resolution (1.9 Å in the dark state) than previously reported RC crystals. The results of the X-ray diffraction analysis of the dark and light structures of the Q_B binding pocket are shown in Figure 20. The most striking observation in the light-adapted structure is a 4.5 Å movement of Q_B^- towards the cytoplasm with an accompanying 180 degree propeller twist about the isoprene tail.

The difference between the light and dark structure offers a simple explanation of Kleinfeld's observation that at low temperatures ($\sim$ 90 °K) the electron transfer from $Q_A^-Q_B$ to $Q_AQ_B^-$ is completely blocked in RCs cooled in the dark, whereas the electron transfer proceeds readily when RCs are frozen under illumination (Kleinfeld et al. 1984b). We postulate that ubiquinone can assume two positions; in one, electron transfer is inhibited, in the other it is not. In the dark adapted RCs at room temperature, the position Q_{B1} (Figure 20) is thermodynamically favored. In this position, the distance between the carbonyl O1 of Q_B and Nδ of His (L190) is large (7.2 Å), leaving the quinone disconnected from the direct pathway for electron transfer from Q_A^-. However, a small fraction (given by the Boltzman factor) of RCs exist in an activated state in which the quinone resides in the Q_{B2} position (Figure 20). In this position the ubiquinone is hydrogen bonded to His (L190) and electron transfer from Q_A^- can readily proceed. We propose that the movement of the quinone from the Q_{B1} to the Q_{B2} position is a necessary prerequisite for electron transfer from $Q_A^-Q_B$ to $Q_AQ_B^-$. In the light-adapted structure of the RC, the ubisemiquinone forms a strong hydrogen bond with His (L190), thereby favoring the Q_{B2} site. In this position, electron transfer can readily take place, as experimentally observed. The observed activation energy (Mancino et al. 1984; Kleinfeld et al. 1984) arises from the barrier between these two states.

So far I have discussed the kinetics and mechanisms involving the first electron transfer. The transfer of the second electron is coupled to proton transfer as represented by Equation (2):

$$Q_AQ_B^- \xrightarrow{h\nu} Q_A^-Q_B^- + H^+ \xrightarrow{k_{AB}^{(2)}} Q_A(Q_BH)^- + H^+ \rightarrow Q_AQ_BH_2 \quad (2)$$

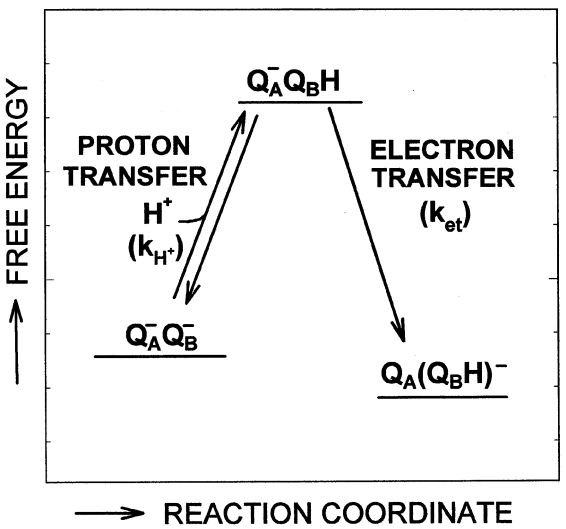


Figure 21. Energy levels of the states involved in the proton coupled second electron transfer reaction $Q_A^-Q_B^- + H^+ \rightarrow Q_A(Q_BH)^-$. Proton transfer is followed by rate limiting electron transfer. (Modified from Graige et al. 1996b.)

The rate constant $k_{AB}^{(2)}$ in *Rb. sphaeroides* is $\cong 10^3$ s^{-1} (Wraight 1979; Kleinfeld et al. 1985). It involves both an electron transfer and a proton uptake. The questions concerning the mechanism of $k_{AB}^{(2)}$ are: i) what is the temporal sequence of the two steps and ii) which of the two processes is rate limiting. For a two step process there are 4 possibilities. The proton uptake can precede or follow electron transfer and either of the two processes can be rate limiting. The difficulties in deciding between them is that in native RCs the two steps have not been kinetically resolved and also that the putative intermediate state has not been directly observed. Consequently, one has to resort to indirect methods. Mike Graige has again used the technique described earlier of substituting quinones with different redox potentials into the Q_A site and comparing the observed values of $k_{AB}^{(2)}$ with theoretical predictions for the 4 different cases (Graige et al. 1996b). I won't go into the details of his work and will quote only the conclusion. Proton transfer precedes electron transfer resulting in a protonated intermediate state. This process is fast and is followed by electron transfer which is the rate limiting step (Figure 21). The important question of how the proton gets to the Q_B will be discussed in a later section.

Up till now we have discussed the kinetics and mechanisms of some of the electron transfer processes. But do we understand them quantitatively? The test of our understanding lies in our ability to cal-

culate the rate constants from first principles. So far this has not been satisfactorily accomplished. There are two sets of data that need to be fed into the calculations. One is the *spatial structure* of and around the primary reactants; the other is the *electronic structure* of the reactants and the intervening medium.

The spatial structure has been experimentally solved at atomic resolution as described in a previous section. The electronic structure has been investigated by a variety of spectroscopic techniques. I shall focus on EPR/ENDOR, the technique that we had used to identify the primary reactants. This technique enables one to experimentally determine spin densities and the derived wave functions, the grist for the theoretical mill.

Electronic structure of the primary reactants

A prerequisite for utilizing the information obtained by ENDOR, which measures the hyperfine couplings (hfcs) of the unpaired electron with the various nuclei, is the identification of the hfcs with specific protons. This is the most difficult part of these experiments. It involves selective deuteration, comparison of the experimental results with molecular orbital calculations and a knowledge of the spatial structure. This has been by and large accomplished for the primary donor, the special bacteriochlorophyll dimer, D^+, but only partially for the acceptors, the semiquinones Q_A^- and Q_B^-. A detailed account of the results, including the historical aspects, has been presented for both D^+ (Feher 1992) and Q_A^-, Q_B^- (Feher and Okamura; Lubitz and Feher, manuscripts in preparation). I will restrict myself, therefore, to a discussion of some of the highlights.

In the original ENDOR experiments on D^+ in RCs only 3 sets of lines were observed (Figure 8). The reason for the absence of other lines associated with the rest of the protons is that we were dealing with a frozen sample in which the RCs are randomly oriented. Thus, hfcs that are highly anisotropic, i.e. that vary with the orientation of the molecular axis with respect to the external magnetic field, will give rise to broad, unobservable, lines. This problem can be overcome by working with single crystals, in which this source of broadening is eliminated. M. Huber, R.A. Isaacson and E. Lous in our lab, and in parallel, F. Lendzian, W. Lubitz, K. Möbius and M. Plato in Berlin have undertaken a detailed investigation of the ENDOR spectra of D^+ in single crystals of RCs (Lendzian et al. 1993).

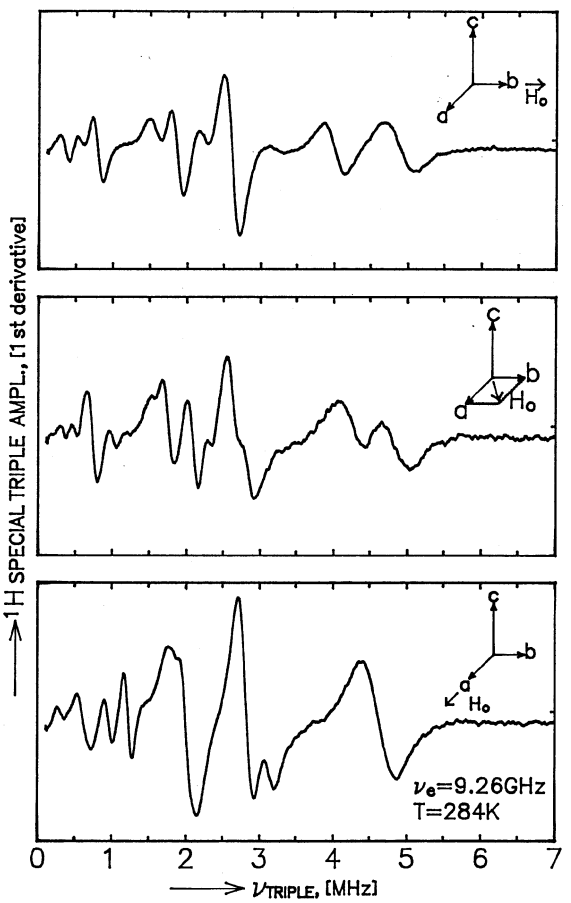


Figure 22. ENDOR/TRIPLE spectra of D^+ in single crystals of *Rb. sphaeroides* RC's with $\bar{H}_o \| b$ axis (top), $\bar{H}_o \| a$ axis (bottom) and with $\bar{H}_o$ 45° from both axes (center). Note the large number of lines compared to a frozen sample (Figure 8). (From Lous et al. 1990.)

A typical result of the ENDOR spectra of D^+ for three directions of H_o in the ab-plane of the crystal is shown in Figure 22. Note that these results were obtained with a technique that is a modification of the standard ENDOR method, the so-called special TRIPLE in which the observed transitions occur at one half of the hfc range. Figure 22 should therefore be compared with one half of Figure 8. The most striking feature is the larger number of ENDOR lines observed in Figure 22 as compared to Figure 8 and the dependence of the hfcs on the angle between the crystal axes and the magnetic field H_o. By systematically investigating this angular dependence, the values and principal axes of the hfcs tensors were obtained (Lendzian et al. 1993). It was found that the unpaired electron was unequally distributed over the two dimer halves, favoring D_L by 2:1. Rautter et al. (1995) have

shown that this ratio can be changed by changing the hydrogen bonding to the macrocycle of D^+ via site directed mutagenesis. A limiting case in which the unpaired electron resides entirely either on D_L or D_M is obtained in the so-called heterodimer in which the bacteriochlorophyll on either the L or the M half of the dimer is changed to a bacteriopheophytin by replacing His (L173) or His (M202) by lysine (Huber et al. 1996).

Let me now turn to the *quinone acceptors*. As was pointed out in a previous section the observed broad EPR signal (Figure 7) was attributed to a ferroquinone complex whose electronic structure was investigated by a variety of techniques (reviewed by Feher and Okamura, manuscript in preparation). However, the excessive width of the EPR line due to the magnetic moment of iron makes it difficult to perform ENDOR experiments to study the details of the electronic structure of the quinones. Consequently, the Fe^{2+} was replaced by diamagnetic Zn^{2+}. This was accomplished by using either the chemical techniques worked out by Rick Debus in our lab (Debus et al. 1986) or more recently by using the site directed mutant, HC (M266), constructed by JoAnn Williams et al. (Williams et al. 1991). This mutant incorporates Zn when grown in a Zn-rich/Fe-poor medium. The replacement of Fe^{2+} by Zn^{2+} reduces the EPR line width of the ferroquinone complex by approximately two orders of magnitude.

The ENDOR work on the quinones is an ongoing collaboration with Wolfgang Lubitz who came to our lab as a post-doc in 1983. The first ENDOR experiments were performed on Q_A^- in frozen solutions of Zn-substituted RCs (Lubitz et al. 1985; Feher et al. 1985). The one electron reduction was accomplished either chemially with dithionite or photochemically in the presence of cyt c_2^{2+}. The ENDOR spectrum of Q_A^- is shown in Figure 23. A striking feature is the large number of lines. Their origin can be assigned to three classes of protons: i) non-exchangeable protons on the quinone (i.e. methyl, methoxy and methylene; see inset in Figure 23); ii) exchangeable protons forming, for example, hydrogen bonds to the carbonyl oxygens and iii) protons associated with the protein in the vicinity of the quinone. Selective isotopic substitution was used to identify the ENDOR lines associated with the methyl, methylene and the exchangeable hydrogen bonded proteins. The hf lines due to the methoxy, the γ-protons of the isoprenoid chain and the proton associated with the protein have so far not been identified.

As discussed in connection with D^+, the hfcs are anisotropic. To obtain both the isotropic and

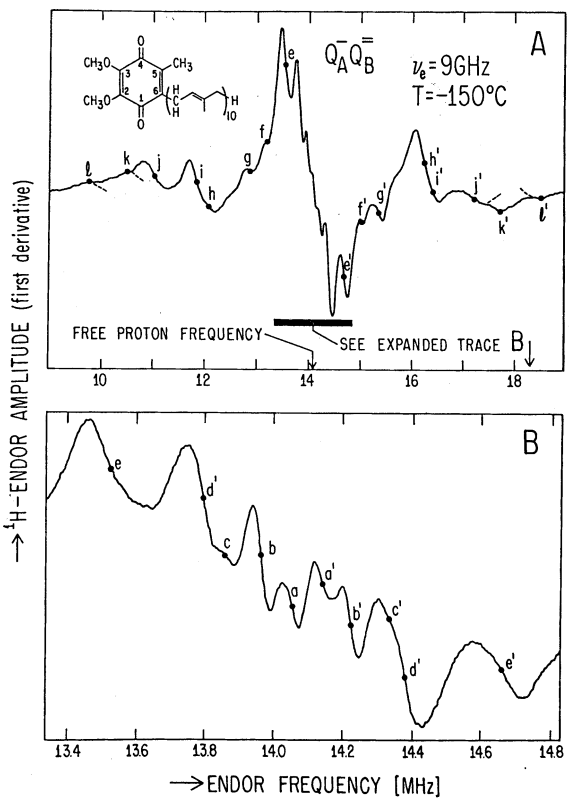


Figure 23. ^{1}H-ENDOR spectrum of Q_A^- (UQ-10) in Zn-substituted RCs from *Rb. sphaeroides* R-26. Lower trace shows expanded central region of upper trace. Inset in A shows the structure and numbering scheme of UQ-10. (From Feher et al. 1985.)

anisotropic part of the hfcs, the ENDOR spectra of Q_A^- were obtained in single crystals (Figure 24). The methyl and methylene protons are well resolved, whereas only one exchangeable proton is partially resolved in two of the principal crystallographic planes.

To reduce the secondary quinone, Q_B, in a single crystal is difficult and has so far not been accomplished. An alternate method to obtain the hfcs along specific directions is to use magnetic field selection. This method makes use of the g-anisotropy, e.g. when the magnetic field is set to the value corresponding to the resonance condition at g_z, only those RCs whose g-tensor points along the *z*-direction will give rise to ENDOR lines; i.e. the hfc along the g_z-axis will be measured. The results for both Q_A^- and Q_B^- are shown in Figure 25. The interesting feature of these spectra is that they are different for Q_A^- and Q_B^-, in spite of the fact that both quinones are UQ-10. The differences are due to the different environments of the two quinones, which are required for proper function of the RC. The environment influences the redox potentials giving rise

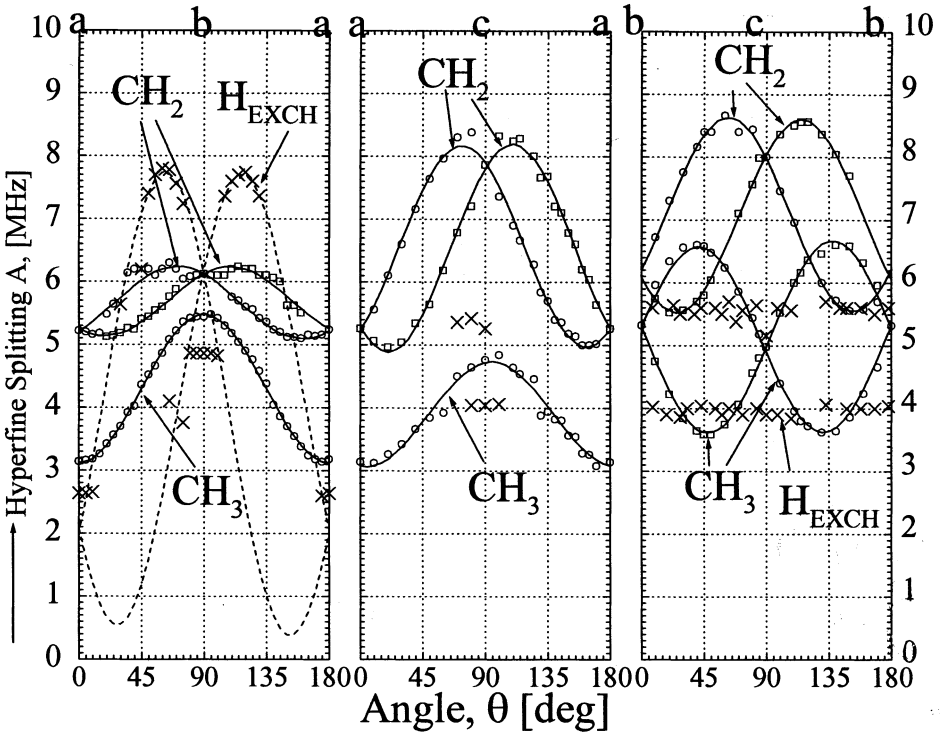


Figure 24. Angular variation of the Q_A^- hyperfine couplings, obtained from ENDOR, in the three principal crystallographic planes of Zn-substituted single crystals of reaction centers from *Rb. sphaeroides* HC (M266). ν_e = 9 GHz, T = 3 °C. (From Isaacson et al. 1995.)

to vectorial electron transfer from Q_A^- to Q_B and also accounts for the loose binding of Q_B, which must be able to leave the RC (as a quinol) to initiate the proton gradient. The quantitative details of these differences remain to be worked out.

EPR/ENDOR spectra provide not only information on the electronic structure of the co-factors but also information on cofactor-protein interaction (e.g. hydrogen bonds) and on the spatial structure. I shall give one example dealing with the hydrogen bonding of protons to the carbonyl oxygens of Q_A^- and Q_B^-. These are the exchangeable protons discussed before. From the measured hfcs that are assumed to be purely dipolar one can calculate the O-H bond lengths using the point dipole approximation. The resulting bond lengths are[7] (Feher et al. 1985):

r_1=1.55 Å	r_1 = 1.68 Å
$\mathbf{Q_A^-}$	$\mathbf{Q_B^-}$
r_2=1.71 Å	r_2=1.97 Å

The assignment of the bonds to specific oxygens was done by measuring the ^{13}C-hfcs in quinones that were labeled with ^{13}C at either the 1 or 4 position (see inset in Figure 23). The stronger bonds for both Q_A and Q_B are to the oxygens at position 4 (van den Brink et al. 1994; Isaacson et al. 1996). The asymmetry of the hydrogen bonding was also determined from the hfcs of preferentially ^{17}O-labeled quinones (Feher et al. 1985).

The hydrogen bond lengths cannot be obtained directly from the X-ray structure since hydrogens are not localized because of the weak scattering of X-rays by protons. However, the distances from the carbonyl oxygens to nearest nitrogens or oxygens can be obtained from the X-ray structure. Making the simplifying assumption of a linear H-bond, N–H–O and subtracting 1.0 Å for the N–H bond length, the hydrogen bond distances obtained from the X-ray structure (Brookhaven data bank, ID code: 1AIG) agree within $\sim$ 0.1 Å with the hydrogen bonds deduced from the ENDOR data (Equation (3)). This work is still in progress. For instance, by determining the complete hfc tensor of the exchangeable protons (see Figure 24), the direction of the hydrogen bonds can be obtained and the simplifying assumption of a linear H-bond can be lifted.

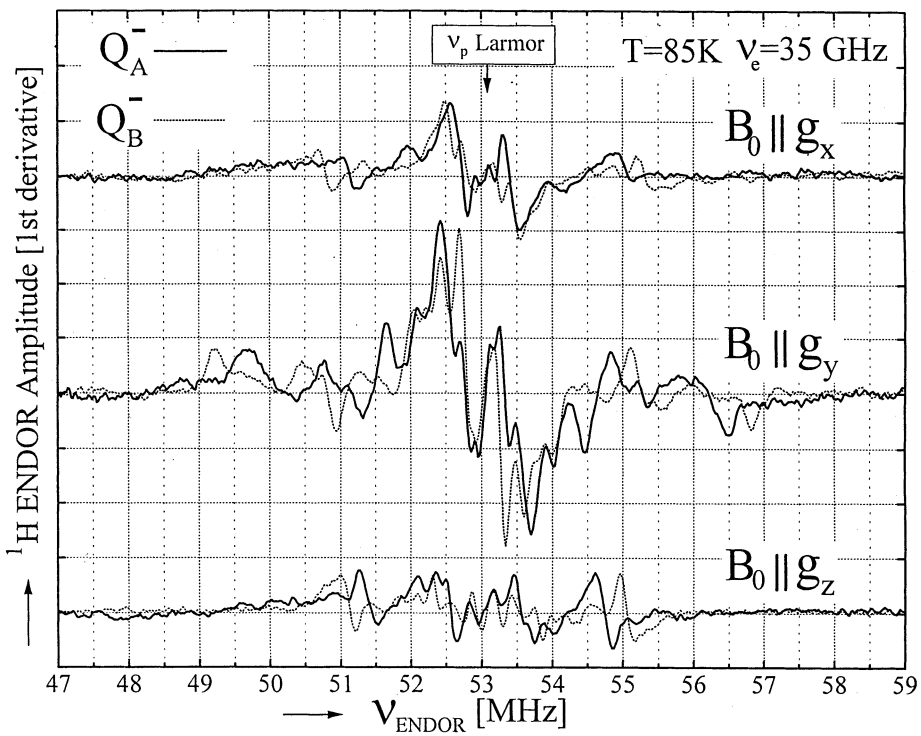


Figure 25. ENDOR spectra of Q_A^- and Q_B^- in Zn-substituted reaction centers from *Rb. sphaeroides* HC (M266). Magnetic field selection was used to obtain the spectra along the three principal directions of the g-tensor (R.A. Isaacson, W. Lubitz and G. Feher, 1997, unpublished results).

Protonation

A great deal of effort by many groups has gone into the elucidation of electron transfer reactions in RCs, with much less effort being devoted to the protonation of RCs. Yet, electrons are not directly involved in ATP formation. It is the protonation of Q_B and the subsequent release of Q_BH_2 (Equation (2) that initiates the formation of a proton gradient which is responsible for ATP formation. Thus, from a biochemical point of view protonation is the important process, while electron transfer can be viewed as a preliminary, albeit necessary step that prepares the RC for protonation. Consequently, we decided a decade ago to focus our attention on the protonation of RCs.

There are several problems associated with the protonation of RCs. One of them deals with the uptake of protons by the RC accompanying the one electron reduction of the quinones. When the quinones are singly reduced, they are not protonated directly as was first pointed out by C. Wraight (Wraight 1979). The observed proton uptake is due to shifts in the pK values of amino acid residues that interact with the quinones. The pH dependence of proton uptake upon formation of Q_A^- and Q_B^- was measured with pH sensitive dyes by Paul McPherson in our lab (McPherson et al. 1988) and by P. Maroti and C. Wraight (Maroti and Wraight 1988).

Knowing the structure of the RC one should be able to predict theoretically the proton uptake by calculating the titration curves of all the amino acids when the quinones are in their neutral and reduced states. This is a complicated problem because of the large number of amino acids and the electrostatic interaction between them. The problem was tackled by Paul Beroza who used a continuum electrostatic model and a Monte Carlo sampling method, which enabled him to take into account all the 172 titratable amino acids of the RC (Beroza et al. 1991). This represented a significant improvement over previous methods which were limited to calculating titration curves for at most 20–25 interacting residues (e.g. Bashford and Karplus 1990). Beroza's results were in fair agreement with the experimentally observed titration results. More recently, Gunner and Honig (1992), Beroza et al. (1995) and Lancaster et al. (1996) presented detailed calculations of the titration of residues in RCs.

The crucial protonation step occurs with the reduction of the semiquinone, Q_B^-, to the doubly reduced

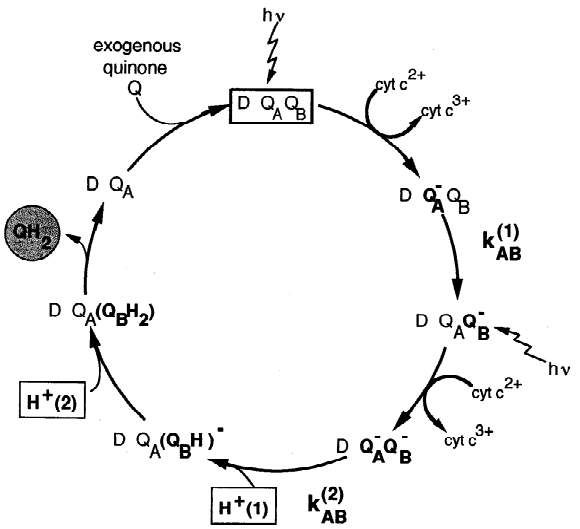


Figure 26. Photochemical cycle showing proton transfer coupled to electron transfer and the exchange of quinol by quinone. Cytochrome c_2 oxidation provides a convenient assay of the cycle. Time to complete a cycle is $\sim$ 1 ms. (Modified from Paddock et al. 1994.)

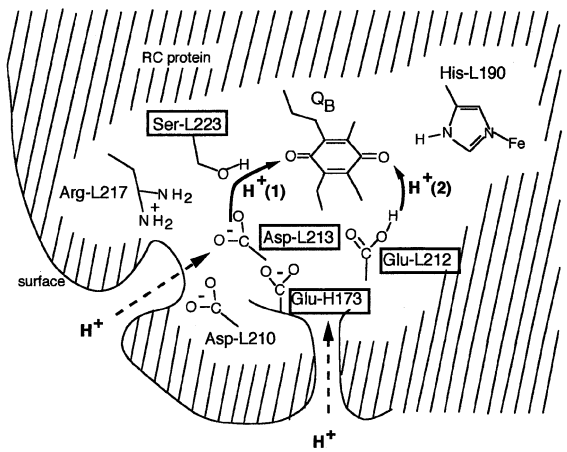


Figure 27. Schematic representation of the proposed pathway for proton transfer in RCs from *Rb. sphaeroides*. Boxed in residues have been shown by site directed mutagenesis to be important in proton transfer. Residues Asp L213, Ser L223 and Asp L213 are involved in the uptake of the first proton (solid line) and Asp L213 and Gln L212 in the uptake of the second proton (dashed line). For a more detailed picture of the pathways, including chains of watermolecules, see Figure 29. (Modified from Paddock et al. 1994.)

quinone which picks up two protons and leaves the RC as the dihydroquinone (quinol), Q_BH_2. This process is schematically illustrated in the cyclic electron transfer scheme shown in Figure 26. The uptake of the first proton, $H^+(1)$, is associated with the proton coupled electron transfer $k_{AB}^{(2)}$ discussed in a previous section. Following the uptake of the second proton, $H^+(2)$, the quinol Q_BH_2 leaves the RC and is replaced by an exogenous oxidized quinone Q. The stoichiometry of the proton uptake following the double reduction of Q_B was shown by McPherson et al. (1994) to be $2H^+$/2e- as expected. McPherson et al. (1990) also showed experimentally that Q_BH_2 leaves the RC as had been postulated by Wraight (1981). Thus, from a macroscopic point of view the protonation seems to be understood. The questions that remain to be answered are how the proton reaches Q_B, i.e. what is the proton path through the protein, what is the mechanism of proton transfer and what determines the rate of protonation.

The main problem with the protonation of Q_B is that it is buried inside the protein and, therefore, has no direct contact with the outside, aqueous phase, which is the ultimate source of all protons. The generally accepted view is that protons move from the aqueous phase to the interior of the protein along a chain of proton donor and acceptor groups. These groups could be either side chains of protonatable amino acids or water molecules. Several of the amino acids in the chain have been identified by site directed mutagenesis, which was guided by the known structure of the RC.

The first proton uptake mutant was constructed by Mark Paddock (Paddock et al. 1989). He changed the protonatable residue Glu L(212) to its non-protonatable homologue Gln. The cytochrome turnover rate in this mutant was slowed by a factor of $\geq$ 30 following the fast oxidation of **three** cytochromes. But the cycle in Figure 26 shows the oxidation of only two and not three cytochromes. The third cytochrome is due to a blockage of the uptake of the 2nd proton (see Figure 26). This prolongs the lifetime of the state $DQ_A(Q_BH^-)$, which can undergo an additional charge separation $D^+Q_A^-(Q_BH)^-$ with a concomitant oxidation of a third cyt c_2. Thus, Glu (L212) is in the pathway of the 2nd proton. Similar, Ser (L223) was shown by Paddock et al. (1990) to be important in the uptake of the 1st proton. Asp (L213) was also shown to be a crucial residue in proton transfer (Paddock et al. 1990; Takahashi and Wraight 1990; Rongey et al. 1991). Asp (L213) seems to be important in the pathway of both protons (discussed in Paddock et al. 1994). More recently, residue Glu (H173) was shown by Takahashi and Wraight (1995, 1996) and Rongey et al. (1995) to be also important for proton transfer. The proton transfer pathway to the two carbonyls of Q_B is schematically illustrated in Figure 27.

In addition to the protonatable residues in the vicinity of Q_B that were mentioned above, water is

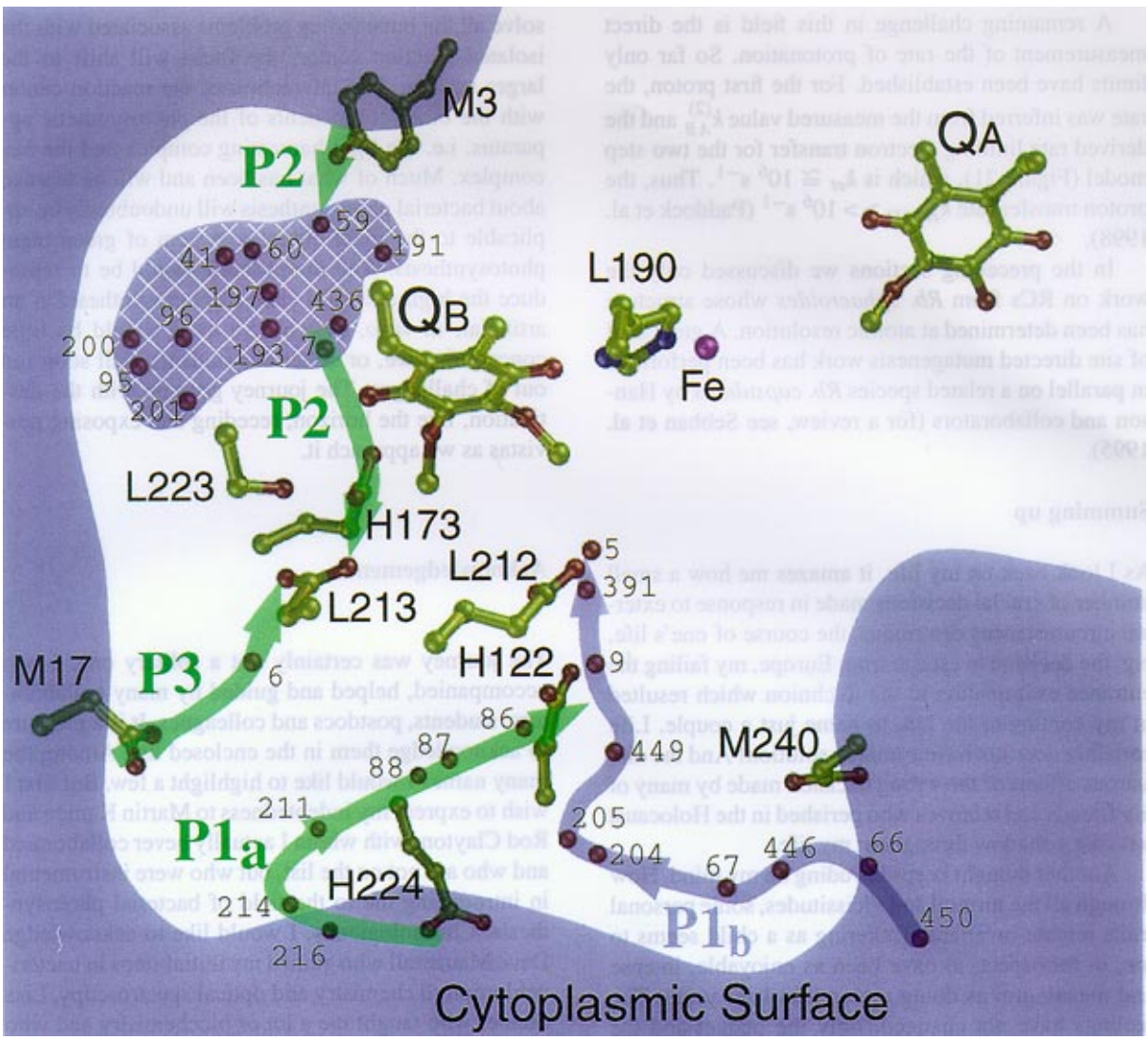


Figure 28. Three possible proton pathways (P1, P2, P3) deduced from the latest crystal structure of RCs from *Rb. sphaeroides* (Stowell et al. 1997). The pathways lead from the cytoplasmic surface to the 4 residues (Glu L212, Asp L213, Ser L223 and Asp H173), that were shown by site directed mutagenesis to be important in proton transfer. Crosshatched blue area shows a pool of ∼15 water molecules. The aquous cytoplasmic solution is shown in solid blue (From Abresch et al. 1998).

believed to play an important role in proton transfer. Water molecules are not easily observed by X-ray diffraction; the first structure in which water was observed was reported by Ermler et al. (1994). Many more waters were identified in the recent, high resolution structure, which was discussed earlier in connection with the light induced structural changes (Stowell et al. 1997). Several proton pathways that included these water molecules were delineated as shown in Figure 28 (Abresch et al. 1998). It should be noted that even before the waters were observed in the X-ray structure, their presence was inferred by Beroza et al. (1992) who modeled water molecules into the voids of the structure.

In addition to the importance of having a contiguous donor acceptor chain from the aqueous phase to Q_B, the electrostatic potential at Q_B also plays a crucial role. Mutations that make the potential more positive were shown to impede proton transfer (Paddock et al. 1994, 1997). Thus, the change of Asp (L213) to Asn results in two deleterious effects: i) the loss of a protonatable residue and ii) a change in the electrostatics (Paddock et al. 1998). The relative importance of these two effects remains to be determined.

A remaining challenge in this field is the direct measurement of the rate of protonation. So far only limits have been established. For the first proton, the rate was inferred from the measured value $k_{AB}^{(2)}$ and the derived rate limiting electron transfer for the two step model (Figure 21), which is $k_{et} \cong 10^6$ s^{-1}. Thus, the proton transfer rate $k_{H^+(1)} > > 10^6$ s^{-1} (Paddock et al. 1998).

In the preceding sections we discussed only the work on RCs from *Rb. sphaeroides* whose structure has been determined at atomic resolution. A great deal of site directed mutagenesis work has been performed in parallel on a related species *Rb. capsulatus* by Hanson and collaborators (for a review, see Sebban et al. 1995).

Summing up

As I look back on my life, it amazes me how a small number of crucial decisions made in response to external circumstances determines the course of one's life, e.g. the decision to escape from Europe, my failing the entrance examination to the Technion which resulted in my coming to the US, to name just a couple. Life certainly does not have a unique solution! And the disastrous effects of the wrong decision made by many of my friends and relatives who perished in the Holocaust has cast a shadow throughout my life.

Another thought keeps intruding on my mind. How through all the turmoil and vicissitudes, some personal traits remain invariant. Tinkering as a child seems to me, in retrospect, to have been as enjoyable, intense and meaningful as doing research in later years. The feelings have not changed, only the budget and the kinds of questions that are being asked. The amazing thing is that one is paid for what one likes to do and occasionally is even appreciated by the outside world. It would, of course, be nice and noble to say that I pursued research in photosynthesis because it addresses the important questions of an alternate energy source and food supply. But it wouldn't be true. I have no pretensions of being a do-gooder; I simply enjoy research and it fulfills an inner need. That it sometimes addresses a question of practical importance, and engenders support is fortuitous and lucky.

What about the question of the future of the field of bacterial photosynthesis? We certainly have come a long way in understanding bacterial photosynthesis, but we still have quite a way to go. I shall not attempt to enumerate the problems that remain to be solved and the loose ends that need to be tied up; they were mentioned throughout this article. And if we should solve all the outstanding problems associated with the isolated reaction center, the focus will shift to the larger system: The interaction of the reaction center with the other components of the photosynthetic apparatus, i.e. the light-harvesting complex and the bc_1 complex. Much of what has been and will be learned about bacterial photosynthesis will undoubtedly be applicable to the more complex system of green plant photosynthesis. And the ultimate would be to reproduce the high quantum yield of photosynthesis in an artificial, *in vitro*, system. So there should be little concern that we, or rather our students, will soon run out of challenges. The journey goes on with the destination, like the horizon, receding and exposing new vistas as we approach it.

Acknowledgements

The journey was certainly not a solitary one. I was accompanied, helped and guided by many collaborators: students, postdocs and colleagues. It is a pleasure to acknowledge them in the enclosed list. Among the many names I would like to highlight a few. But first I wish to express my indebtedness to Martin Kamen and Rod Clayton, with whom I actually never collaborated and who are not on the list, but who were instrumental in introducing me to the field of bacterial photosynthesis. Chronologically, I would like to acknowledge Dave Mauzerall who guided my initial steps in bacteriochlorophyll chemistry and optical spectroscopy, Lisa Steiner who taught me a lot of biochemistry and who has remained a valuable colleague, friend, constructive critic and sounding board, Mel Okamura whose contributions permeate all of the photosynthesis work described here and with whom it was a pleasure to work all these years, Roger Isaacson for his 36 years of expert and dedicated technical assistance and Ed Abresch for keeping our biochemical lab in working order and for providing our group with a continuous supply of reaction centers during the past $\sim$ 20 years, I am grateful to the NSF and the NIH for supporting our research. And finally and most importantly, I would like to thank my family: my wife, Elsa for her ongoing support and active interest in my work and our daughters for putting up with my long working hours and my frequent absences to attend scientific meetings.

Appendix

Collaborators on different aspects of bacterial photosynthesis (1968–present)

E. C. Abresch [tech]	Technical assistance
Ackerson [tech]	Technical assistance
N. Adir [post- doc]	RC-cyt c_2 complex, crystallization of PS II
J. P. Allen [post-doc &coll]	Crystallization & X ray structure determination
T. R. Arno [stnd.]	Effect of E-field on electron transfer
H. Axelrod [post-doc]	3D-structure by X-ray diffraction
K. A. Bagley [post-doc]	FTIR studies
P. Beroza [stnd. & post-doc]	Theory: Electrostatic energies in proteins
Y. Blatt [post-doc]	RCs in Monolayers
B. Boso (Univ. of Illinois) [stnd.]	Fe^{2+} structure by Mossbauer spectroscopy
J. M. Bruce (Univ. of Manchester) [coll]	Synthesis of quinones
P. Brzezinski [post-doc]	Electrogenic effects
W. F. Butler [stnd.]	Fe^{2+}-Structure
R. Calvo (Univ. Nacional, Santa Fe, Arg.) [coll]	Fe^{2+}-Structure-Theory
A. Chirino (Cal. Tech.) [stnd.]	3-D Structure by X-ray diffraction
P. G. Debrunner (Univ. of Ill.) [coll]	Fe^{2+}-Structure Mossbauer spectroscopy
R. J. Debus [stnd.]	Subunit reconstitution and characterization
H. Deisenhofer (Max Planck Inst.) [coll]	X-ray diffraction (molecular replacement)
S. Durbin [post-doc]	Crystallization mechanisms
P. Eisenberg (Bell Labs) [coll]	Fe^{2+}-Structure-EXAFS
D. R. Fredkin [coll]	Theory
A. Gopher [post-doc]	RCs in bilayers
M. Graige [stnd.]	Proton coupled electron transfer
A. J. Hoff [post-doc]	Identification of D_1
D. Holten (Washington U.) [coll]	Picosecond spectroscopy
M. Huber [post-doc]	Electronic structure by EPR
R. Huber (Max Planck Inst.) [coll]	X-ray diffraction (molecular replacement)
R. A. Isaacson [tech]	Technical assistance
A. Juth [tech]	Technical assistance
Z. Kam (Weizmann Institute) [post- doc]	Crystallization mechanisms
C. Kirmaier (Washington U.) [coll]	Picosecond spectroscopy
D. Kleinfeld [stnd. & post-doc]	Kinetics of e^- transfer
H. Komiya (UCLA) [stnd.]	3-D Structure by X-ray diffraction
A. Labahn [coll]	Electron transfer
F. Lendzian (Freie Univ., Berlin) [coll]	Electronic structure by ENDOR
M. Loesche [post-doc]	Stark effect in RCs
E. J. Lous [post-doc]	3-D and electronic structure
W. Lubitz (Freie Univ., Berlin) [post-doc & coll]	Electronic structure by ENDOR
J. D. McElroy [stnd.]	Identification of D_1, A_1
P. H. McPherson [stnd. & post-doc]	Protonation of RCs
T. M. McPhillips (Cal. Tech.) [stnd.]	3-D structure by X-ray diffraction
M. M. Malley [stnd.]	Picosecond spectroscopy
T. D. Marinetti [post-doc]	Q_A binding site
D. C. Mauzerall (Rockefeller U.) [coll]	BChl-chemistry
A. Messinger [stnd.]	Electrogenic effects
H. Michel (Max Planck Inst.) [coll]	X-ray diffraction (molecular replacement)
K. Mobius (Freie Univ., Berlin) [coll]	Electronic structure by ENDOR
M. Montal [coll]	RCs in bilayers
E. Moskowitz [stnd.]	Kinetics of e^- transfers
V. Nagarajan (Univ. of Wash.) [coll]	ΔG^0 from delayed fluorescence
R. Nechushtai (Univ. of Jerusalem) [coll]	Crystallization of PS II
M. Y. Okamura [coll]	All aspects
M. Paddock [stnd. & post-doc]	Herbicide resistant and site-directed mutants
W. W. Parson (Univ. of Wash.) [coll]	ΔG^- from delayed fluorescence
M. Plato (Freie Univ., Berlin) [coll]	Theory
D. C. Rees (Cal. Tech) [coll]	3-D Structure by X-ray diffraction
S. H. Rongey [stnd. & post-doc]	Site directed mutagenesis
D. Rosen [stnd.]	Isolation of subunits
K. Satoh (Okayama U.) [coll]	Photosystem II complex
C. C. Schenck (Univ. of Colorado) [coll]	Heterodimer mutants
M. Schönfeld [coll]	RCs in bilayers
M. E. Senft [stnd.]	Suppressor mutations
M. Simon [coll]	Sequencing of DNA
S. M. Soltis (Stanford, Syn. Rad. lab) [coll]	3-D structure by X-ray diffraction
L. A. Steiner (M.I.T.)	[coll] Protein chemistry, sequencing
M.H.B. Stowell (Cal. Tech.) [stnd.]	3-D structure by X-ray diffraction
M. R. Sutton (M.I.T.) [post-doc]	Sequencing
M. Tetreault [stnd.]	Cyt c_2 electron transfer and binding
M. Trotta [post-doc]	NMR studies
T. Turanchick [tech]	Technical assistance
G. E. Valkirs [stnd.]	Ferritin labeling
H. Ward [tech]	Technical assistance
J. C. Williams [stnd., post-doc & coll]	Sequencing & site directed mutagenesis
T. O. Yeates (UCLA) [stnd.]	3-D Structure by X-ray diffraction

Abbreviations

coll – collaborator; tech – technical assistance; stnd. – student.

Notes

1. In Hebrew there are no numerals. Every letter is assigned a numerical value.
2. Actually, the first year it was called UCLJ. It was changed in response to an offer by the San Diego municipality to give the University a substantial amount of land for expansion if the name is changed to UCSD.
3. My license plate is R-26 GF. When Rod Clayton saw it, he was a bit miffed and ‘threatened’ to put ENDOR on his license plate. I encouraged him to do so.

4. Incidentally, I am happy to report that David Kleinfeld did make the switch in 1984, became a neurobiologist and last year joined our department as a faculty member. One day I may start looking over his shoulder.
5. Adir also successfully crystallized PS II RCs from spinach. The crystals diffracted to a resolution of ~ 7 Å (Adir et al. 1992). In an abstract submitted to a Biophysical Society meeting (Adir et al. 1994) a better resolution was quoted. However, the diffraction turned out to be from a crystallized impurity (phycobilisome).
6. Nomenclature used: D (Asp) changed to K (Lys) at position M184.
7. These values differ in the third significant figure from the published ones (Feher et al. 1985) because of a numerical error. In view of the simplifying assumptions it is doubtful whether these values are reliable to three significant figures.

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