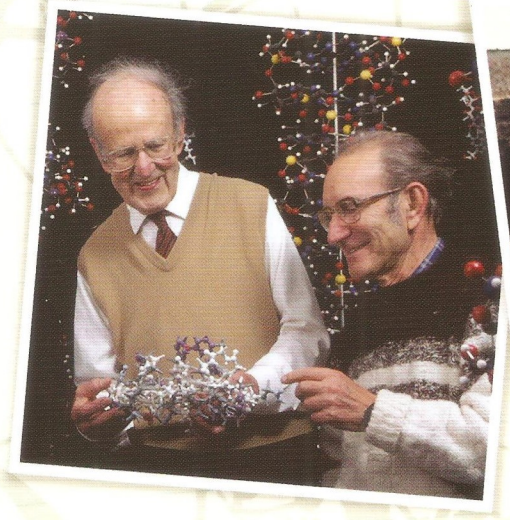
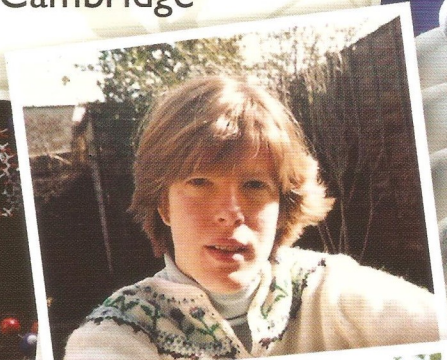
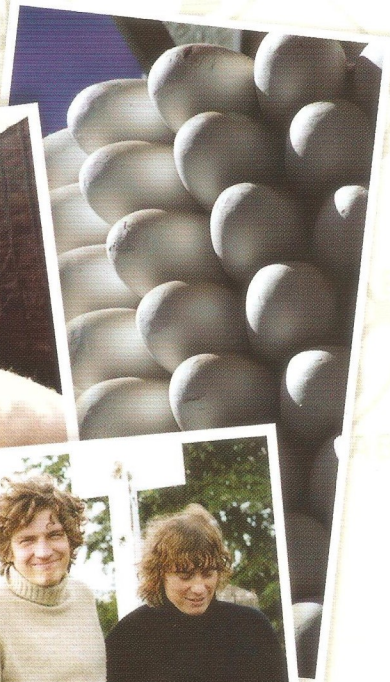
Memories and Consequences

Visiting Scientists at the MRC Laboratory of Molecular Biology, Cambridge







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Hugh Huxley

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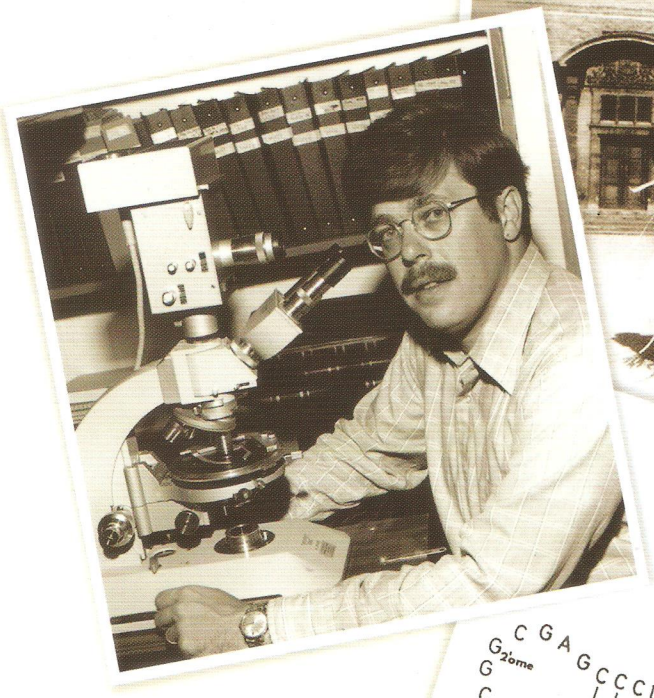

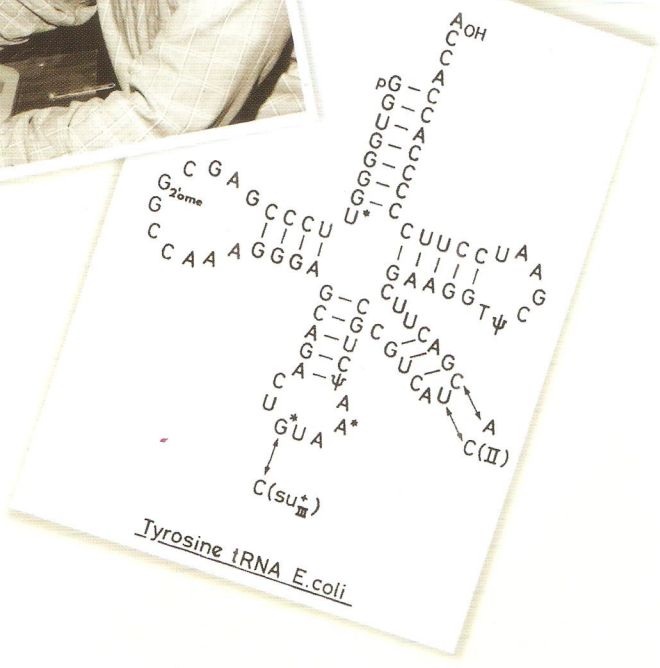

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MRC/LMB

This book is a collection of 41 essays by some of the many visiting scientists who came to the MRC Laboratory of Molecular Biology in Cambridge, England during the period 1957–1986. This was a time of rapid development of the subject. It built on the demonstration by the founder members of the lab that high-resolution structures for crystalline proteins could be obtained from X-ray diffraction data; that the double-helical structure of DNA could provide the basis for heredity and replication; that methods could be developed to sequence nucleic acids as well as proteins; and that a combination of X-ray diffraction and electron microscopy could provide a powerful technique for elucidating the structure and function of larger molecular assemblies.

The laboratory was remarkably successful in advancing the field of molecular biology (thirteen of its members have shared nine Nobel prizes), and it attracted many visiting scientists, predominantly but not exclusively from the United States, who wanted to work in this promising field. They contributed significantly to the spirit and success of the lab, and six of the early visitors have won Nobel prizes for their subsequent work.

These essays provide detailed, first-hand accounts of some of the important scientific problems which were being tackled, and may indicate what features of the laboratory environment seemed particularly favourable for successful experimental work.

MRC/LMB UK

HUGH HUXLEY was the first research student to join the MRC Unit for the Study of the Molecular Structure of Biological Systems, in the Cavendish Laboratory, Cambridge in 1948, with John Kendrew as his supervisor. He soon decided to work on muscle structure, because it seemed to offer more opportunity for adventure than did crystalline proteins at that time. He was able to obtain low-angle X-ray diffraction diagrams from live muscles which indicated the presence of a double hexagonal array of actin and myosin filaments several hundred Ångstroms apart, and suggested that they must interact via crossbridges to produce contraction. During post-doctoral work at MIT in 1953–54, he and Jean Hanson proposed the sliding filament model of muscle contraction based on their phase-contrast and electron microscope observations (simultaneously with A.F. Huxley (no relation) and R. Niedergerke). He carried out further e/m work in Bernard Katz's Biophysics Department at University College London in the later 1950s, which confirmed this model.

He rejoined the MRC Group as one of the founding members of the Laboratory of Molecular Biology when it opened on the Addenbrooke's site in 1962, and pioneered the use of X-ray synchrotron radiation to obtain millisecond time resolution of structural changes in muscle during contraction.

After retirement from the MRC, he continued work on muscle at the Rosenstiel Center at Brandeis University, first as Director and now as Emeritus Professor.

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CHAPTER 38

Two and a Half Years at the LMB That Imprinted My Scientific Career (1980–83)

Raúl Padrón
Center of Structural Biology, IVIC, Caracas, Venezuela

In 1966, while still attending high school in Caracas, I joined Dr Karl Gaede's lab at the Biochemistry Department of the Venezuelan Institute for Scientific Research (IVIC). In his lab I was engaged in building a 2D-electrophoresis setup. One year later I started studying electrical engineering at the Central University of Venezuela, which was then closed for the next two years. In the meantime, I moved to the Biophysics and Biochemistry Centre joining first Dr Jorge Villegas' lab – while Dr John Nicholls visited him to work on recording signals from the neurons in the leech ganglia – and then to Dr Carlo Caputo's lab with the aim of understanding how the giant muscle fibres of barnacle were activated by using a calcium dye indicator. Once graduated I joined the lab of Dr Leonardo Mateu to study the structure and function of the nerve myelin sheath by low-angle X-ray diffraction and electron microscopy. I received my MSc in 1977 and my PhD in physiology and biophysics in February 1980, and joined IVIC as Associated Investigator.

While I was still wondering about a place for a postdoctoral stay, Dr Vittorio Luzzati (Dr Mateu's supervisor) visited the lab and suggested that I should write to Dr Aaron Klug at the MRC-LMB. He accepted me to work on a high resolution 3D-map of tobacco mosaic virus, starting in September 1980. IVIC awarded me the Vollmer Excellence Fellowship to support my stay in England. In the next few weeks I read several structural papers. One (*Journal of Molecular Biology*, 1967. 30, 383–434) captivated my attention and its author (Dr Hugh E. Huxley) happened to be at LMB. I realised after reading that paper how fascinating was the structure and function of muscle which involved force and movement. At that time Dr Humberto

Fernández-Morán visited the lab and he also mentioned Huxley's recent exciting synchrotron research, and suggested that I should work with him. I did feel brave enough to write again to Dr Klug telling him my great interest in working – instead – on muscle contraction. Dr Klug agreed without hesitation and put me in contact with Dr Huxley who approved my postdoctoral stay at his lab. With this gear change, I concentrated on muscle structure papers, reading all of Huxley's papers I could find at IVIC library; as well as attending an intensive English course. It was going to be my first trip (and flight) abroad. What awaited me on the other side of the Atlantic Ocean was not only a new country and people, but also an extraordinary cultural and scientific shock. Everything that happened after I landed in England was a surprise for me, including the adventure in muscle that was about to begin.

I arrived at Cambridge train station on 1 September 1980. I quickly learned that in this new country the way of life was very different: the first surprise was that people queued for a taxi. I had never in my life seen that. That was only the beginning of many cultural differences between Venezuela and England. Ms Jenny Brightwell kindly received me with the news that Dr Huxley was kind enough to let me stay in his house while he was away in Woods Hole. People at Cambridge were indeed very kind and helpful. I soon discovered that the English that I had learned in Caracas was not precisely British English, so for a few weeks I was slightly perplexed when talking to people. I went to Hills Road to visit the famous LMB without delay. I mentioned to someone in the building's entrance that I was there to work with Dr Huxley, and asked where I could find Dr Klug. They told me where Aaron's office was, joking that everybody was a doctor there. I introduced myself to Aaron, thanked him for the opportunity to work there and briefly mentioned the project I wanted to do with Hugh.

Somehow I found myself in room 213. There, Roger Craig, a postdoc from Australia, knew in advance that a new postdoc from Venezuela was arriving. Roger briefly showed me the lab, and in the next few weeks educated me in brief about it, LMB customs, the Cambridge way of life and the weather. Coming from a tropical country, and not knowing what to expect there, soon I started asking him: 'Roger, is it the winter yet?' when the temperature dropped to 15°C! Two more people joined the room: Marcus Kress, a student from Germany who came to do his PhD with Hugh, and later Sengen Xu, a postdoc from China. That completed the capacity of the room of four desks, with four different nationalities, continents and languages. In retrospect, sharing rooms was one of the successful features of the LMB. After less than a week, Aaron pointed out to me another important key feature, when he politely mentioned to me that he noticed I was missing the coffee and tea times by working in the lab instead, stating seriously that going to the canteen was indeed *very* important. In fact, after this advice I started going to the canteen more frequently and finally every day, discovering for myself that, with the nice excuse of drinking coffee and tea, LMB people have the opportunity twice a day to discuss very informally – sometimes between different tables – ideas, current experiment results, failures and many other topics.

Hugh returned some weeks later from Woods Hole. He had pioneered the muscle field by discovering, with the late Jean Hanson (Roger's PhD supervisor), that the muscle sarcomere, the tiniest microscopic unit capable of shortening and producing mechanical force, was formed by two sets of filaments, the thick myosin-containing and the thin actin-containing filaments; he proposed that the myosin heads protrude from the backbone of the thick filament and cyclically interact with the thin filament, forming crossbridges and actively producing force while shortening the sarcomere. He also provided the first direct evidence for the tilting of these cross-bridges during force generation in muscle. We discussed the project I proposed to do in his lab – to use relaxed micro-dialysed single giant barnacle muscle fibres, studying them by X-ray diffraction to learn about their activation by calcium. As getting and keeping these bizarre animals could become a problem, Hugh suggested instead trying to 'freeze' biochemically the cross-bridge cycle by using AMPPNP, a non hydrolysable ATP analog. It sounded very interesting to me and I started reading and preparing to do the experiments (Figure 38.1, left) while Hugh showed me how to set up and align a Huxley-Holmes mirror-monochromator camera setup on the big wheel X-ray generator. It surprised me that muscle fibre X-ray diffraction patterns were recorded on film, as in Venezuela I had recorded the one-dimensional X-ray diffraction patterns from the myelin using an electronic linear position sensitive detector, which we learned to build while Andre Gabriel was visiting from the Luzzati lab. Learning directly from Hugh how to prepare glycerinated rabbit psoas muscle was indeed an interesting experience, especially the rabbit preparation and dissection. Luckily after a few weeks I managed to get some good rabbit psoas rigor diffraction patterns. I still remember clearly the day when I developed and fixed in the dark room my first muscle fibre X-ray diffraction film: the room was dark, and someone else was there; I asked this person to help me in developing my exposed film as it was my first one. This person kindly helped me, and when I asked for reassurance that he really knew what he was doing, the person replied that he knew quite well. Once the light was on I discovered the person was Max Perutz!

Another feature of LMB was the frequent visitors to the lab. One day Ken Holmes visited the lab and Hugh mentioned my recent rigor X-ray diffraction patterns. Ken was kind enough to assess the quality of my initial rigor patterns as very good, especially one, boosting me to keep working hard on it. In fact the AMPPNP project kept me very busy until the end of 1982 when I completed it. I accompanied Hugh, Wasi Faruqi, Marcus Kress and Bob Simmons on one of their visits to the DESY synchrotron in Hamburg. I clearly remember the moment in which a reflection started to change in the screen and Hugh's excitement was so extreme that I worried he might have a heart attack. Wasi Faruqi helped me with setting and using his position sensitive detectors for recording the meridian and equatorial X-ray reflections of the AMPPNP-treated muscles. Tony Woollard was always there helping to maintain the big wheel generator I was using and ensuring that it continued to work well during the long exposure runs. Another lively LMB moment showed the importance of experimental data for a scientist; one morning there was a fire in the building, and

Hugh literally jumped out with a stack of computer tapes, computer listings and films, refusing to risk losing them in the fire. Later I had the valuable opportunity to record some synchrotron data for my project in the Daresbury synchrotron (in July 1982), joining a trip there with Hugh and Marcus.

During my stay at LMB I had the opportunity to attend many meetings, including the MRC-LMB Laboratory Symposium, six meetings in the UK, the 1981 Muscle Gordon Research Conference and the 1983 Alpbach Workshop on Muscle. The Laboratory Symposium was especially important for me. On another occasion, by mistake, I attended a meeting that I later discovered was intended only for the LMB permanent staff. To my surprise, in the meeting room there was a panel of Nobel Prize-winners leading an open and free discussion on – from what I understood – the future of the different divisions, labs and units at LMB. Quite a learning experience about science planning and revision for a visitor from other, more tropical latitudes! The fact that Aaron and Hugh were *joint* heads of the LMB Structural Studies Division was an unimaginable concept where I came from. The LMB building was busy all day and all night, including of course the weekends; it was like a scientific factory that could not stop production, driven by curiosity. Hugh's lab had periodic meetings of the Muscle and Cell Motility Discussion Group, known informally as the 'Muscle Club', to which I contributed a few times. Once, I told Roger that a speaker at these talks spoke very clear and understandable English for me. Roger explained why Murray Stewart's English was so familiar to me: I had learnt and practised my spoken English mainly with Roger, another Australian, and Murray was the second Australian I had heard in my life! I finished the AMPPNP project by the end of 1982 and we wrote a paper that was published later, in 1984 (*Journal of Muscle Research and Cell Motility* 1984. 5, 613–55), the same year as a paper in collaboration with Ed Egelman – who was a postdoc in the office opposite – on the actin molecule disposition on the native thin filament (*Nature* 1984. 307, 56–8). I still remember when I was in Jenny Brightwell's office, and a telex message came through announcing Aaron's 1982 Nobel Prize in Chemistry. The news spread around the building in minutes and in a very short time Michael Fuller (another key LMB figure) organised a celebration party in the canteen. At that party I discovered that people in England queue not only for taxis, but also for champagne cups! I vaguely remember hearing Michael say that champagne was always prepared in advance for the next Nobel Prize-winner. In fact, while writing these recollections I heard on the radio that John Gurdon had won the 2012 Nobel Prize for Medicine, and right after I arrived at the LMB Fred Sanger won his second (1980) Nobel Prize for Chemistry (by the way, the same year as the Venezuelan Baruj Benacerraf won the Nobel Prize for Physiology or Medicine). Another feature of the LMB was the rapidity with which Michael provided any biochemical you needed for an experiment, i.e. no grant writing! I was not aware of the concept of writing grants until I had the great opportunity to help Roger to prepare the final version of an application he was writing on a typewriter, during a very long day and night that finished the next day. Once or twice I came

close to staying forever in Cambridge, not working there but either frozen alive during the coldest winter in 50 years, or passing away after falling down when my bike slipped on ice – an unknown phenomenon for me – or a lorry smashed my bike on a roundabout, or drowning while punting on the Cam – fortunately quickly rescued by a young Briton. All of these memories would have been enough to fill the rest of the life of a young Venezuelan scientist, altering my whole future scientific career. However, another LMB feature changed it completely.

At the LMB we had complete intellectual freedom. Sharing the office with Roger proved to be an important turning point in my life as a scientist. Roger was working on viewing metal shadowed myosin molecules in the electron microscope to understand its function. We talked a lot every day about the experiments we were doing on our different projects, and many times were joined by Marcus with his results. We became great friends and spent a lot of time talking about muscle and many other things. We wondered if the structure of a thick filament – a big supramolecular arrangement – could ever be solved by structural techniques. Marcus was building a computer model of myosin heads on a thick filament using spheres to calculate its X-ray diffraction pattern. He wanted to use it to test John Haselgrove's proposal that the myosin heads were pointing in opposite directions along the thick filament axis. Roger and I were discussing the possibility of solving the structure of a thick filament by negative staining – a technique developed by Hugh 26 years earlier. At that time Roger was collaborating with Peter Vibert at Brandeis University doing electron microscopy of scallop thick filaments.

Roger was very keen that I should attend different muscle meetings. He pointed out that a very important one was the Muscle Gordon Research Conference to be held in Holderness School, Plymouth, by the end of June 1981. I applied, waited for a while and was accepted right before the meeting; luckily I was able to attend. Roger was right, this meeting was superb scientific experience for me. I had the opportunity to meet and talk to many famous muscle scientists. One, John Wray, was doing X-ray diffraction patterns of muscles from a variety of invertebrates. He told me that according to his X-ray diffraction patterns, the thick filaments from tarantula muscle were particularly well helically ordered, and advised me to view them in the electron microscope. After coming back to Cambridge, John visited the lab and gave me two beautiful tarantula X-ray diffraction patterns. Roger and I decided to pursue the new project together to visualise by negative staining isolated thick filaments from tarantula muscle with the hope – if they were indeed so well ordered – that we could even calculate a 3D reconstruction. Soon we made a short trip to a nearby town to buy a big tarantula in a pet shop. We did try the muscles from this tarantula, making a leg muscle homogenate one weekend in October 1981, and discovered that their thick filaments were indeed beautifully ordered. After that initial success we kept working on that topic – as well as our main projects – for the rest of 1981 and 1982. Hugh liked this unexpected digression from our original plans and encouraged us in pursuing it. We succeeded in getting low electron dose pictures in the electron microscope. I still remember one day when Aaron brought some visitors to the biochemistry room

where I was doing a dissection. He wanted to show them the electron microscopes. Brian Pope politely reminded him that the microscopes had been moved to another floor a few years before, something that Aaron acknowledged immediately as he had completely forgotten. We asked Tony Crowther for advice on image processing and after some time we started collaborating with him in learning how to calculate a 3D reconstruction from first principles. The first 3D map was obtained in March 1982. It is worth mentioning that the 3D reconstructions then were calculated in the computer room using the VAX-11/780 computer and that in 1982 the computer displays were only monochrome text monitors. Claudio Villa made some balsa wood stack models for visualising the 3D reconstruction and Tony taught me how to prepare a perspex stack 3D map. Ed wrote a clever computer program that enabled the surface representation of the tarantula thick filament to be displayed on the computer monitor (Figure 38.2, far left). The day that we brought our tiny tarantula 3D map balsa and perspex stack models to the model room to show them to Hugh and Aaron is still alive in my memory. I came back to Venezuela on 14 December 1982, returning to Cambridge a few weeks later to complete the 3D reconstruction, to work with Roger and Jake Kendrick-Jones on activated tarantula thick filaments and to attend the Alpbach Workshop on Muscle to present the tarantula 3D reconstruction (Figure 38.1, right; Figure 38.2, left two images). I finally returned to Venezuela at the end of March 1983. We completed the work and wrote the paper which was published later (*Journal of Molecular Biology*, 1985. 184, 429–39). By then it was clear to me how fortunate I was to have the opportunity to spend these years in Cambridge and the LMB working in the intellectually free and exciting environment of Hugh's lab, as well as collaborating with Roger on solving a scientific problem.

I frequently wrote notes on the key features of the LMB that I was discovering, hoping to implement them when I started my own lab in Venezuela. Not surprisingly, surrounded and fascinated by the high-quality science going on at the LMB, thoughts of returning to Venezuela faded every day. It was Carlo Caputo's visit to London, and a day spent together at the British Museum, that brought me back to reality. I returned to his lab at the Biophysics and Biochemistry Centre at IVIC. IVIC regulations did not allow me to start a new independent lab. Despite that, Carlo was kind enough to allow me to work independently, as well as actively supporting my research. Nelly Panté, Hernando Sosa and Lorenzo Alamo were some of the bright young students who joined my lab then. We were all very busy setting up low-angle X-ray diffraction and electron microscopy facilities, which enabled us to keep studying the structure and function of tarantula thick filaments. My collaboration with Roger during these initial years in Venezuela was very fruitful, with the opportunity for my students, collaborators and myself to visit his lab at the University of Massachusetts Medical School in Worcester very frequently. In 1988 Lorenzo visited the LMB to work with Tony Crowther on the 3D reconstruction of activated tarantula thick filaments; Nelly and Hernando went to Brandeis to work with Carolyn Cohen and Hugh. Later on, Jose Reinaldo Guerrero, Carlos Hidalgo, María Elena Zoghbi and Guidenn Sulbarán went to UMASS, Julio Ortiz

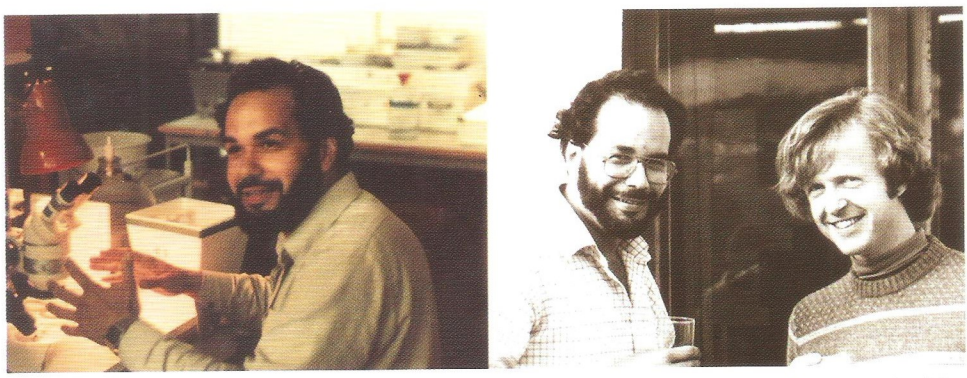

Figure 38.1. Raúl Padrón working at LMB in 1981 (left, photo by Roger Craig), and with Roger Craig (right, photo by Anne Anke Hennemann) attending the 1983 Alpbach Workshop on Muscle where they presented the first 3D reconstruction of negatively stained tarantula thick filaments (Figure 38.2, left and centre).

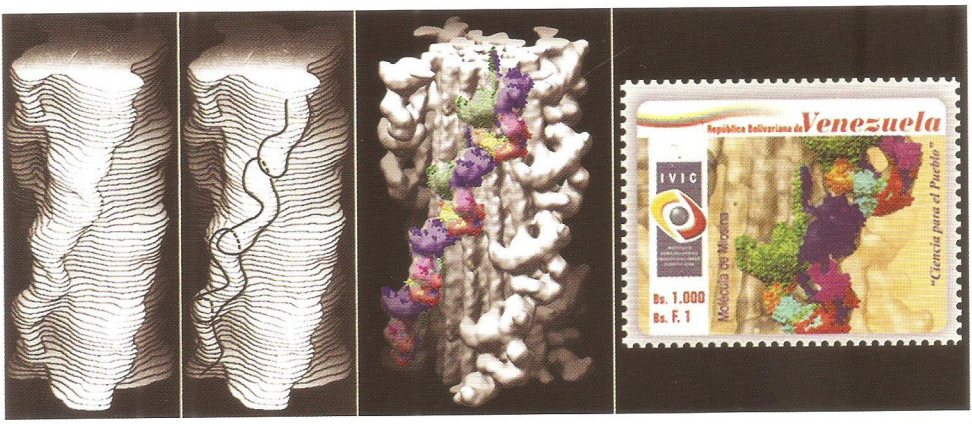

Figure 38.2. 3D reconstruction of negatively stained thick filaments of tarantula muscle plotted using a computer program written by Ed Egelman (far left: reproduced with permission from the *Journal of Molecular Biology*), with the putative arrangement of myosin heads superimposed (second left image). 3D reconstruction of frozen-hydrated tarantula thick filaments with the atomic models of four myosin interacting-heads motifs fitted along one helix (centre, photo by Lorenzo Alamo). The motif model appeared in a 2007 Venezuelan postage stamp (far right).

to the EMBL at Heidelberg and Antonio Biassuto to Oxford, while Maristela Granados and Reicy Brito stayed at the lab. I was able to run my lab with the support of several grants from MDA, as well as some timely support from NSF, NIH and TWAS. Surprisingly in 1997 a miracle happened, when I was designated as an International Research Scholar of the Howard Hughes Medical Institute (HHMI). Their support – that lasted until 2011 – allowed me to start a new centre at IVIC that year: the Center of Structural Biology. The first HHMI grant triggered local support, and I was able to buy a Philips CM-120 cryo-electron microscope. I certainly brought to the new centre all the lessons I learned from the LMB; including, last but not least, the importance of keeping one's lab small.

During several years of work in close collaboration with Roger's lab, we finally found a way to visualise intact – frozen-hydrated – relaxed tarantula thick filaments in the cryo-electron microscope. To our dismay, achieving very nice low-electron dose electron micrographs of tarantula thick filaments was not enough to calculate their 3D reconstruction, as the Bessel separation program that Tony developed in 1982 and other approaches we tried did not work well with these very low contrast images. It was a completely different image processing approach, devised by Ed Egelman in 2000, that finally enabled the calculation of the frozen-hydrated 3D-reconstruction in mid-2004. Roger noticed that the arrangement of heads was very similar to the structure observed in 1999 in 2D crystals of myosin molecules by the lab of Ken Taylor, another MRC alumnus. In 2005 we published the atomic model of the tarantula thick filament in the native (unfixed, unstained, hydrated) state (*Nature* 2005. 436, 1195–99); four of the six coauthors (Roger, Ed, Lorenzo and myself) were MRC alumni. The 3D map revealed that in the relaxed state the two heads of each myosin molecule interact with each other (Figure 38.2, centre), surprisingly with an arrangement similar to the structure observed by Taylor's lab on the smooth muscle of a vertebrate. Our studies thus showed that this unusual, asymmetric head organisation, that we named the *myosin interacting-heads motif* (Figure 38.2, centre), was not an artifact of myosin isolation or crystallisation, but occurred in vivo. The conservation and uniqueness of this motif across the vast evolutionary distance between vertebrate smooth muscle and invertebrate striated muscle suggested that it was of fundamental functional importance for preserving the relaxed ('off') state in most or all muscles. Analysis of the interaction between the two heads has shown that one head ('free') physically blocks the actin-binding site of the other head ('blocked'); this interaction simultaneously blocks the ATPase site of the free head. This head-head interaction immediately suggested a simple mechanism to explain relaxation – by switching off the two heads in different ways. Subsequent studies have demonstrated the presence of this motif in non-muscle cells, and in smooth and striated muscles of other species, confirming its widespread occurrence and the importance of tarantula thick filaments as a model system for studying thick filament structure and function. In further analysis we discovered a new intermolecular interaction between adjacent motifs (*Journal of Molecular Biology*, 2008. 384, 780–97) that would further stabilise the off-state. Our analysis of the head organisation, together with motility assays, sequence analysis and mass spectrometry observations, suggested a molecular model for muscle activation in which heads are phosphorylated sequentially by protein kinase C and myosin light chain kinase in a way that can explain both force development and post-tetanic potentiation in striated muscle (*Journal of Molecular Biology*, 2011. 414, 44–61).

In retrospect I feel happy and satisfied that Roger and I were able to pursue a project we started as postdocs in Cambridge 32 years ago: revealing the atomic structure of Hugh's thick filaments. I am also happy to have successfully set up the new Center of Structural Biology at IVIC, the first structural biology centre in Latin America, in which I implemented the key lessons I learned at the LMB.