KIYOSHI NAGAI

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Kiyoshi Nagai pioneered research in several areas of structural and molecular biology in an innovative and imaginative style. His initial work was on haemoglobin, the oxygen carrier in blood. He devised new methods to express haemoglobin in bacteria, thereby enabling specific changes to be made in the amino acid sequence to test theories about the structure, function and evolution of the molecule. Later his focus changed to the structure of RNA–protein complexes. His major project became the elucidation of the structure of the spliceosome, the complex molecular machine that removes the intervening sequences from newly synthesized messenger RNA to create the correct template for translation into protein. Elucidation of this centrally important process required multiple techniques in molecular biology and structure determination. This memoir describes his scientific achievements and personal manner, which was marked by a passion to understand the workings of nature. He was an exemplary figure and mentor with a compassionate demeanour who continues to encourage and inspire multiple generations of scientists.

A PHD PROJECT, AND THE START OF A LIFETIME OF RESEARCH

Allostery, ‘… the second secret of life’ (Monod 1977).

Kiyoshi grew up in Wakayama in the prefecture next to his birthplace, Osaka. His father worked at Wakayama Medical College as a professor, and their home was near Wakayama.

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Castle, which could be seen from the house. After graduating from high school, Kiyoshi studied at Osaka University, obtaining a degree in biophysics and a master’s degree for research work with Professor Hideki Morimoto on haemoglobin, the oxygen carrier in blood. Having found the environment of Hideki’s laboratory supportive and stimulating, Kiyoshi chose to continue in that group and pursue a PhD project to investigate the mechanism of cooperativity in mammalian haemoglobin and to understand how the protein’s affinity for molecular oxygen increases as more oxygen binds.

At the time Kiyoshi commenced his PhD thesis studies in 1974, the emerging paradigm to explain cooperative behaviour in biological macromolecules was an allosteric model. Monod, Wyman and Changeux proposed that the changes in the ligand affinity or activity of physically separated active sites arose from a shifting equilibrium between two conformational states that place different structural constraints on the protein (Monod et al. 1965). When Kiyoshi started his PhD work, the abstraction of ‘conformational states’ had become more tangible in the form of the low-resolution crystal structures of the two quaternary states of haemoglobin, solved by Max Perutz FRS and colleagues at the Medical Research Council Laboratory of Molecular Biology (MRC LMB) in Cambridge, UK (Perutz 1972). These were among the very first protein structures elucidated. They addressed the long-standing puzzle of whether proteins adopt defined folds but also revealed that the three-dimensional shape of proteins can be more deeply conserved than the amino acid sequence.

Beyond these fundamental discoveries in protein structure, Max had sought to explain the molecular origins of cooperativity at the level of stereochemistry. Haemoglobin assembles as a heterotetramer comprising two alpha and two beta subunits (figure 1). These subunits share a high degree of sequence similarity, and Max observed two distinct arrangements of these subunits in the ligand-bound and ligand-free states. From his crystal structures of those two quaternary states, together with the available high resolution crystallographic data of iron–haem compounds, Max proposed that the globin fold in the deoxy-quaternary state restrained the movement of the haem iron into the porphyrin plane. The planar geometry is required to accept the axial oxygen ligand, and other changes are required for the oxygen molecule itself to be accommodated into the restricted space of the haem pocket (Perutz 1972). Accordingly, the oxy- and deoxy-quaternary states were referred to as R and T for ‘relaxed’ and ‘tense’, respectively, with the R state having a higher oxygen affinity than the T state. Max’s model proposed that the protein structure in the T state places greater forces restricting the iron movement into the haem plane to reduce the oxygen affinity in the T state. The restraining force was envisaged to place a possible strain on the proximal histidine-haem Fe bond. In the absence of oxygen, the T–R equilibrium strongly favours the T state, but as oxygen is added the equilibrium shifts toward the R state. With each oxygen bound by haemoglobin, the equilibrium shifts the quaternary state more toward the R state. Cooperativity then results, since each bound oxygen increases the haemoglobin affinity for the next oxygen molecule. The final molecules of oxygen bind to unoccupied sites in the R state with high affinity. Max also proposed that allosteric modulators, such as metabolites, binding at sites away from the haem pocket can alter the ligand binding affinity, since they can modulate structural changes. Specifically, allosteric modulators can bind to the inter-subunit interface (e.g. α1β2 interface) to control the quaternary transition, which in turn would modulate the oxygen ligand affinity needed to deliver oxygen to respiring tissues.

The concept for Kiyoshi’s PhD project was to find evidence for the role of the protein in modulating the oxygen affinity of the haem from analysis of mutants and haemoglobin
Figure 1. Experimental oxygen binding curves for normal and mutant haemoglobin studied by Kiyoshi and colleagues. The vertical axis is the percentage occupancy of oxygen binding sites at the haems, and the horizontal axis is the logarithm of the partial pressure of molecular oxygen. Haemoglobin titusville has a single amino acid substitution in the alpha subunit $\alpha_{94}$ Asp $\rightarrow$ Asn, corresponding to a single non-hydrogen atomic substitution from oxygen to nitrogen, with a large impact to decrease oxygen binding affinity (seen in the rightward shift of the binding curve). The model shows the structures of the haemoglobin tetramer, composed of two alpha and two beta subunits, in the R (high affinity) and T (low affinity) quaternary states. From (1)*, used with permission from Elsevier. (Online version in colour.)

reconstituted with haem derivatives. Hideki recognized the talent and potential of his new student, and only a few months into his PhD had arranged for Kiyoshi to make a short visit to Cambridge to work with Max Perutz and John Kilmartin (FRS 2002) to measure oxygen binding curves of mutant haemoglobins. A few months after he and his fiancée, Yoshiko Majima, married in Osaka, Kiyoshi set off for Cambridge and was joined two months later by Yoshiko.

Kiyoshi found the atmosphere at the MRC LMB exciting. He encountered a laboratory culture that encouraged original thinking and critical discussion to identify and experimentally approach fundamentally important problems. At the LMB he met numerous scientists working on topics at the forefront of molecular biology, including Fred Sanger FRS, Aaron Klug FRS, Francis Crick FRS and Sydney Brenner FRS. Kiyoshi enjoyed the interactions and opportunity to share experimental strategies or ponder over and trouble-shoot puzzling results. These were frequently centred around the canteen, where routine morning coffee breaks, lunchtimes and afternoon tea provided opportunities for spontaneous meetings. Participating in these daily events initiated for Kiyoshi a habit that he sustained happily with life-long enthusiasm.

During his stay in Cambridge, Kiyoshi collaborated with the clinical haematologist Herman Lehmann FRS, who worked in the nearby Department of Clinical Biochemistry, and together they characterized naturally occurring haemoglobin variants identified in patients (figure 1).

* Numbers in this form refer to the bibliography at the end of the text.
While the analyses showed the potential of studying mutations to gain insight into mechanism and evolution, it also had the frustrating limitation of relying on rare natural variants discovered through abnormal phenotype. It must have occurred to Kiyoshi that it would be very powerful if somehow mutations could be generated at chosen sites to test models of allostery.

Although the visit to Cambridge was only a comparatively short period of 18 months, it was highly productive. He produced a single-author paper (2), which was quite an achievement for a PhD student just starting out, and there was also a momentous event for Yoshiko and Kiyoshi: their son Ken was born in 1975. The young Nagai family left Cambridge to return to Japan, where Kiyoshi continued his work on haemoglobin and completed his doctorate in 1977. In a brief post-doctoral period in Osaka, Kiyoshi applied spectroscopic methods such as electron paramagnetic resonance to look for spectroscopic signatures of possible strain on the proximal histidine-haem Fe bond in the T state.

In Osaka, Yoshiko and Kiyoshi’s daughter Yuko was born in 1978. That year, Kiyoshi accepted a position as assistant professor in the Physiology Department of Nara Medical College. Although the appointment had a heavy teaching commitment, Kiyoshi was able to continue work on haemoglobin and collaborated with Teizo Kitagawa, who used resonance Raman spectroscopy to examine the tension in the haem Fe-proximal histidine N bond. They reasoned that changes in the Fe-N stretching frequency detected by resonance Raman spectroscopy should reflect the altered vibrational mode of the molecular bond overlap in the R versus the T state. To identify the iron proximal histidine (Fe-N) signal, Kiyoshi substituted isotopically enriched iron in the haem to increase its mass. The resultant alteration in stretching frequency enabled the assignment of the Fe-N signal in the resonance Raman spectra. In this way, a change in the frequency would reflect the Fe-N bond tension and would allow comparison between the T and the R states. Using such hybrid haemoglobins, Kiyoshi showed that the stretching in the T state is greater in the alpha subunit than the beta subunit (3).

In a complementary effort, Kiyoshi made a three-month visit to the University of California (UC) Davis where he collaborated with Gerd La Mar and Thomas Jue and used paramagnetic nuclear magnetic resonance (NMR) to characterize the haem iron electronic structure in the R and T states. His work there showed that the proton resonances of the proximal histidine N_H and in the \( \alpha_1\beta_2 \) interface changed in a manner consistent with the protein structural changes predicted by Perutz’s model (4, 5).

**AN INVITATION FROM MAX PERUTZ**

In 1980 Kiyoshi sent Max a nostalgic letter to express his appreciation for the fruitful and stimulating visit to Cambridge and concluded with a polite comment that he would be delighted to return if a future opportunity arose. This letter proved to be propitious for Kiyoshi, both personally and scientifically.

At the time he received the letter, Max had been reflecting on the evolution of allostERIC modulation in haemoglobin and had formulated hypotheses that could be tested by site-directed mutagenesis and preparation of recombinant proteins. Max wrote back to Kiyoshi immediately, inviting him to return to Cambridge to explore methods for making and characterizing the first-ever recombinant, mutant haemoglobins. This might seem a straightforward endeavour today, but in 1980 there were virtually no procedures for engineering and expressing individual proteins, let alone for protein complexes like
haemoglobin. The protocols and reagents for the envisaged project would have to be developed almost entirely from scratch. Keen to accept the challenge, Kiyoshi took leave from his secure lecturing post in Nara to undertake a risky project with the temporary support of a two-year fellowship. He returned to Cambridge with his young family in 1981 to work again with his inspiring mentor Max.

Initial efforts to express human haemoglobin subunits in *Escherichia coli* yielded poor results, and Kiyoshi suspected that this might be due to poor translational initiation. To overcome this, he generated expression constructs in which the human beta-globin sequence was fused to a short segment of the highly expressed cII protein from bacteriophage lambda. This indeed succeeded to produce large amounts of the fusion protein, and the next hurdle became how to liberate the globin from the phage protein fusion. Through inspired late-night discussions, Kiyoshi and Hans Thøgersen invented novel methods to liberate the haemoglobin from the fusion using an endopeptidase that cleaves at a defined peptide sequence (coagulation factor Xa) (6, 11).

The liberated beta-globin was then reconstituted from a denatured state to co-fold with haem and alpha-globin, and the procedure successfully generated recombinant haemoglobins with directed mutations. These recombinant haemoglobins opened new and exciting opportunities to explore the relationship between structure and function. Kiyoshi’s success in preparing and handling recalcitrant materials by a combination of ingenuity, patience and persistence was to be a recurring theme that underpinned many of his later achievements.

When Kiyoshi returned from a trip to Paris, where he could finally measure the oxygen binding curves for the first ever mutant haemoglobin, he seemed so happy that everyone in the lab assumed that the experiment had been successful. In fact, Max’s hypothesis for the origins for pH response in haemoglobin was not supported by these early mutagenesis data. Kiyoshi’s joy arose from a chance to test rigorously Max’s hypothesis and from discovering an unexpected result. Having the initial hypothesis refuted, Max and Kiyoshi together vigorously pursued alternative hypotheses and developed better models to explain the pH effect. It turned out that even a simple molecular switch, such as a strong coupling between oxygen binding and proton concentration, required many amino acid substitutions, which illustrated how complex and contingent is the pathway of the evolution of protein function.

Kiyoshi’s work on haemoglobin represented a landmark achievement, because it introduced for the first time an effective method to produce sufficient eukaryotic proteins in bacteria to support structural biology experiments. His research demonstrated that the potential for functional and structural studies was limitless. Samples could be made in sufficient abundance to measure oxygen binding curves and to determine X-ray crystal structures so that experiments could precisely investigate the impact of mutations at the level of stereochemistry and relate these to protein function. Kiyoshi later made the complete recombinant human globin, with both alpha and beta chains synthesized in *E. coli*, which opened avenues to mutate residues anywhere in the protein. Taking a large risk in pursuing a daunting scientific challenge, Kiyoshi had revolutionized haemoglobin studies.

**Appointment as group leader at the MRC LMB**

Encouraged by Max Perutz and Sydney Brenner, Kiyoshi applied for a group leader position at the LMB, and the appointment was awarded in 1984. He received tenure in 1987 on the strength of his research accomplishments and scientific vision. Kiyoshi remained in the
structural studies division at the LMB for the rest of his life, and he never took sabbatical leave to work elsewhere.

Starting his own group, Kiyoshi expanded his studies on the molecular origins of divergent allosteric effects in haemoglobins of different species. Allosteric modulators were explored, such as bicarbonate and metabolites that confer species-specific adaptions, for instance the capacity of some birds to fly at high altitude or for fish to tolerate ocean depths. Many scientists came to work and train with Kiyoshi to explore haemoglobin structure and mechanism, including Noboru Komiyama, Jeremy Tame, Gentaro Miyazaki, Beatrice Vallone, Jean-Paul Renaud and Tim Jessen. Daniel Shih and Hiroshi Imai were experts in accurately measuring oxygen binding curves, and they spent many months in Cambridge working with Kiyoshi, studying the impact of mutations in the ligand binding pockets. Efforts to engineer or transplant allosteric effects from one species into another explored tricky issues in molecular evolution, including the much-debated role of neutral mutations (17). The research programme tested the hypothesis that most amino acid replacements between haemoglobins of different species are functionally neutral, while regulatory functions evolved by a few replacements in key positions. Substitutions of residues in the haem pocket provided evidence for their roles in modulating oxygen binding in the alpha and beta subunits (7–10, 16) (Giardina et al. 1992).

Kiyoshi and his colleagues also explored engineering haemoglobin to serve as a blood substitute for transfusion. One challenge is that the oxygen affinity of haemoglobin is too high to efficiently unload oxygen to the tissues without the allosteric modulator 2,3-diphosphoglycerate. Further engineering was used to transplant novel allosteric effects into haemoglobin to improve its potential utility for transfusion (17). He and his group collaborated with an industrial laboratory to produce tandemly fused alpha-globin, which prevents subunit dissociation (18). The project was successful from the standpoint of engineering a complex function, although the costs for sample preparation prevented this approach from being commercially viable.

The methods to overexpress and purify proteins developed by Kiyoshi and his group were also applied in numerous other projects. Kiyoshi worked with several colleagues at the MRC LMB to apply his skills in protein expression and purification. With John Kendrick-Jones, he studied myosins to determine metal binding sites and demonstrate their role in the regulation of muscular contraction. Aaron Klug and colleagues had recently discovered the zinc finger motif, and Kiyoshi worked with him and Daniela Rhodes (FRS 2007) to prepare samples for structural and functional characterization (12, 14).

In 1988, Kiyoshi was joined by Chris Oubridge, a recent BSc graduate in microbiology from the University of Bristol. Chris would go on to complete his PhD with Kiyoshi and to help drive the group’s transition to study dynamic RNA machines. Following the completion of his PhD studies, Chris considered opportunities to move elsewhere but found the environment and personal chemistry in Nagai’s lab conducive to his style of science. He would remain in the group for more than 30 years, and as a close colleague and friend who contributed to the collegiate and creative atmosphere of the Nagai group.

**Structure and function of the pre-mRNA splicing machinery**

When he became a group leader, Kiyoshi was strongly encouraged to pursue a fresh problem. His colleagues appreciated his tenacity in tackling a problem, and encouraged him to direct
this gift to new problems that were all his. Continuing his pursuit of important and challenging topics, Kiyoshi launched a highly ambitious research programme in the early 1990s to determine how RNA is recognized within complex ribonucleoprotein assemblies. His inspired insight was to go beyond equilibrium interactions to understand how recognition, specificity and assembly occur in highly dynamic ribonucleoprotein complexes.

One of the projects Kiyoshi started was on the signal recognition particle, which is the highly conserved RNA–protein complex involved in translocation of secretory proteins across membranes. He and his colleagues explored the signal recognition particle assembly, including induced structural changes in the RNA, and recognition of the signal peptide required for trans-membrane protein trafficking (25, 27). But the work that Kiyoshi and colleagues developed furthest was the subject of eukaryotic pre-messenger RNA (pre-mRNA) splicing.

The process involves the removal of non-coding regions (introns) from newly synthesized pre-mRNAs to leave just the fused coding exons as a mature mRNA to be translated into protein. This transformation of mRNA was discovered by Phil Sharp (ForMemRS 2011) and Rich Roberts (FRS 1995) and was suggested a few years later by Joan Steitz (ForMemRS 2014) to involve small nuclear ribonucleoprotein particles (snRNPs, each consisting of a highly conserved snRNA component and multiple proteins). It became clear from biochemical work during the 1980s that a set of these snRNPs (so-called U1, U2, U4, U5 and U6 snRNPs) assembles together with the pre-mRNA substrate and other splicing factors to form a large and dynamic ribonucleoprotein machine, named the spliceosome. This complex RNP machine recognizes the introns in pre-mRNAs and catalyses the chemistry of RNA splicing, which occurs in two steps: ‘branching’ and ‘exon ligation’. These trans-esterification reactions remove the introns and splice together the protein-coding exons to produce mRNAs for translation, releasing the excised non-coding introns in a branched or ‘lariat’ conformation. Kiyoshi sought to characterize the spliceosomal snRNPs structurally, and he was joined in this long-term endeavour by Chris Oubridge. Together they worked tirelessly for many years to prepare stable recombinant subassemblies of the eukaryotic intron splicing machinery for structural and biochemical analyses. In one of the first key advances, Kiyoshi and his team elucidated the X-ray crystal structure of the RNA recognition motif (RRM) domain of the A protein component of the U1 snRNP (13). Their work illuminated how this ancient motif recognizes structural features in RNA throughout all domains of life. Soon following this important advance was the structure of the U1 A protein RNA-binding domain in a complex with its cognate RNA hairpin from the U1 snRNA, revealing the basis of sequence-specific RNA recognition by this family of RNA-binding proteins (19).

The Nagai laboratory succeeded in making successively larger and more complex assemblies that were ever more challenging to prepare and required a continuous series of innovations. The complexes were reconstituted in vitro from proteins overexpressed in E. coli and co-crystallized with RNA made either by chemical synthesis or by in vitro transcription. One approach to prepare biochemically synthesized RNA which was developed by Steve Price was to prepare fusions with ribozymes that would self-cleave to liberate the target RNA. The first complexes included the human U1A/RNA complex, the U2B′/A′/RNA complex, Sm proteins as subassemblies lacking RNA and then all seven Sm proteins with RNA (19, 23, 24, 28). The structures revealed how specificity of interactions is achieved through networks of hydrogen bonding between RNA bases and protein side chains, mediated also by water molecules. Conformational changes such as helix reorientation indicated binding
is accompanied by mutually induced fit of nucleic acid and protein (13, 15, 19, 20, 22). Using NMR of the U1A protein domain and cognate RNA, the C-terminus of the protein was demonstrated to be involved in binding RNA (21).

One of the major advances made by Chris Oubridge, Kiyoshi and their collaborators involved reconstituting the human U1 snRNP from 10 recombinant components and determining the crystal structure of the assembled RNA–protein complex. This proved to be a difficult challenge, but after intensive effort over several years they produced the first structure of the functional core of a spliceosomal snRNP, revealing important insights into the assembly of the U1 snRNP complex and its biological role in recognition of intron 5′ splice sites (26). Based on this structure, Kiyoshi and his colleagues then designed two sub-structures of the human U1 snRNP and determined their crystal structures at high resolution, revealing that selection of 5′ splice sites by U1 snRNP is achieved predominantly through base-pairing with the U1 snRNA, while the U1C protein fine-tunes this interaction (31).

Kiyoshi’s group made further important contributions to our understanding of spliceosome structure and function by determining the crystal structures of the Prp8 and Brr2 proteins, which are large, highly conserved components of the U5 snRNP particle. Prp8 interacts with key positions in the U5 and U6 snRNAs and the pre-mRNA substrate, so is located at the heart of the spliceosome and closely associated with the spliceosome’s catalytic RNA core. The Prp8 crystal structure revealed a conserved electropositive cavity proposed to accommodate the RNA-based active site of the spliceosome and also uncovered a deep evolutionary relationship between the spliceosome and distant relatives from the ancient RNA world (30). Brr2 is an ATPase that interacts with Prp8 and is responsible for a crucial RNA duplex unwinding step during spliceosome activation. The structure of Brr2 in complex with a domain of Prp8 provided important clues about its RNA-unwinding mechanism and new insights into the process of U5 snRNP assembly (29).

APPLICATION OF NEWLY DEVELOPED CRYO-EM TECHNIQUES

Despite exhaustive efforts, higher order complexes of the other snRNPs suitable for crystallization remained elusive. Fortunately, crucial contemporaneous advances in cryo-electron microscopy (cryo-EM), many of them pioneered at the MRC LMB, enabled the Nagai group to determine the cryo-EM structure of the yeast U4/U6/U5 tri-snRNP by single particle analysis. This RNP complex is a major pre-assembled component of the spliceosome, and the Nagai laboratory’s ground-breaking effort produced a structure at sufficient resolution to unambiguously assign the snRNAs and most protein components of the 1.5 MDa complex, in the first convincing demonstration of the power of this approach in the analysis of spliceosome structures (32, 34). This work provided new insights into the structure of the active site of the spliceosome and the molecular mechanism of spliceosome activation.

Kiyoshi’s group now took advantage of the knowledge gleaned from three previous decades of biochemical research into spliceosome dynamics to purify many of the key functional states of the spliceosome for structural analysis by cryo-EM. The biochemistry had shown that the spliceosome is assembled in a highly ordered manner and remodelled extensively by ATPases to form the active site, to enable catalysis of the branching and exon ligation reactions, and finally to release the spliced mRNA. Kiyoshi’s group first determined the structure of the yeast spliceosome stalled immediately after the branching
reaction (figure 2), providing new insight into recognition and positioning of the 5′ splice site and branchpoint at the active site and revealing how proteins stabilize the architecture of the spliceosome’s catalytic RNA core (33). The structure of the next complex in the pathway, after an ATPase-mediated remodelling step, showed how the branch was removed from the catalytic RNA core to create room for docking the 3′ exon at the catalytic centre in preparation for the exon ligation reaction (35). The cryo-EM structure of the post-catalytic spliceosome, immediately after exon ligation, resolved a long-standing mystery about the mechanism of 3′ splice site recognition: the 3′ splice site is recognized through non-Watson–Crick pairing with the 5′ splice site and the branchpoint (37).

The cryo-EM structures of yeast spliceosomes early in the assembly pathway yielded important new insights into the recognition of the 5′ splice site and branchpoint by the U1 and U2 snRNPs and the mechanism of spliceosome activation (36, 38). Kiyoshi’s group also broke new ground by determining the structures of human complexes early in the spliceosome assembly pathway, shedding new light on the role of the Brr2 ATPase and the detailed mechanism of spliceosome activation (39). The structure of the human post-catalytic spliceosome (just after exon ligation) showed that the mechanism of 3′ splice site recognition is conserved from yeast to human, and together with biochemical experiments revealed a role for the FAM32A splicing factor in the exon ligation reaction, suggesting unexpected complexity in the late stages of human mRNA synthesis (40).
Combined with the wealth of genetic and biochemical data accumulated over the last 40 years the spliceosome structures determined in Kiyoshi’s group and in several other laboratories across the world—notably those of Reinhard Lührmann and Yigong Shi—have made an enormous contribution to our current understanding of pre-mRNA splicing. The structures reveal how the active site of the spliceosome is formed, how the splice sites and branchpoint are recognized and positioned for catalysis, and how ATPases remodel the spliceosome to dock and undock reactants and products at the active site. The spliceosome project was moving rapidly toward his dream of elaborating the full spliceosome pathway when Kiyoshi learned that he was terminally ill with cancer and had little remaining time. He reacted to this news much like Max Perutz did when he was terminally ill: he was not deterred and kept working, right up to the end.

**Music, art, friendships and devoted colleagues**

Kiyoshi made extensive contributions to the scientific community. He served on the editorial board for the *Journal of Molecular Biology*, and he participated in numerous review panels. He was joint head of the Structural Studies Division (2000–2010) at the MRC LMB, recruiting personnel and formulating strategic decisions that ensured the future of the division. He was always a source of valuable advice for younger colleagues.

Aside from science, Kiyoshi also shared with others his passion for music, and he was often heard cheerfully whistling Mozart and Brahms tunes in the lab in the late evening, even when experiments were not going particularly well. He had fond memories of attending a Simon and Garfunkel concert in California with Thomas Jue when he visited UC Davis in the late 1970s. He attended classical music concerts in London and Cambridge, and wherever else his scientific trips might take him. Kiyoshi organized trips to London to hear the Academy of Saint Martin in the Fields, or the Borodin string quartet. He himself played the cello and enjoyed making music with friends and colleagues. Art was another of his passions, which he shared with many others—organizing visits to museums and iconic sites on the French coast at Étretat that had been captured by impressionist masterpieces. There were visits to Monet’s home in Giverny and to impressionist art galleries in Paris, combined with scientific meetings.

Kiyoshi frequently hosted social events at his home, where science was enriched by diverse culinary delights (figure 3). In the lab, there were spontaneous cheese and baguette parties with José Pagnier on her return trips from Paris. With Daniela Rhodes, cooking lessons involved preparation of zabaglione in beakers on lab hotplates.

Chris Oubridge, a mainstay of Kiyoshi’s group at the LMB MRC for more than three decades, died on 25 August 2020 from cancer, less than a year after Kiyoshi. He and Kiyoshi were a highly productive team, and their approach to science created an atmosphere at the LMB that drew the very best from students and postdocs who passed through the group. The relationship between Kiyoshi and Chris was much richer than that of mentor/student, and they formed a scientific partnership that is rare in its productivity. The nurturing, open environment they cultivated allowed many initially daunted young people still struggling with their English skills to find their feet before taking off into their careers. Kiyoshi and Chris complemented each other in their rigour, tenacity and good humour in a field that is often unforgiving in its competition, beset by countless pitfalls and by scoops by other groups after years of work. They were both immensely proud of the group’s achievements and of those of everyone who
Kiyoshi Nagai

Figure 3. Photos of Kiyoshi. The upper panel is a picture of Kiyoshi and Chris Oubridge taken in the original building of the MRC LMB. The lower panel is from a dinner party at Kiyoshi’s home. Richard Henderson FRS is on the left of Kiyoshi, and Venki Ramakrishnan FRS on the right. The pictures were provided courtesy of Venki Ramakrishnan. (Online version in colour.)

passed through it. Chris was buried in the same woodlands cemetery, near his mentor and scientific partner.

Kiyoshi embodied intense creativity, intelligence, compassion, integrity and personal warmth. He had a clever and somewhat mischievous wit with an uncanny skill at making insightful comments with an economy of words. He was remarkably patient and thoughtful. Despite taking on a highly risky project at the beginning of his stay in Cambridge, he still found time to support, advise, encourage and directly help numerous people with experiments. He helped PhD students who were green, naive and even temperamental and obstinate, charming them with reason and good humour. He subsidized student travel to international laboratories with his own personal travel funds. He sometimes convinced conference organizers to allow his trainees to take his talk slot at major meetings, giving them an opportunity to take the spotlight. This generosity was in his genuine nature, and he was supportive throughout his career to colleagues and the many young scientists whom
Kiyoshi was a master in the practice of small kindnesses. On one occasion, dignitaries from the Japanese embassy in London visited the LMB, and Kiyoshi organized a lunch trip to a pub for visitors and some members of the staff. When Kiyoshi realized that the driver who took the group to the pub was waiting patiently in the car park, he invited him in to join the meal. When his colleague Giulio Fermi died, Kiyoshi collected Giulio’s published work and presented it to his widow Sara at her house, who very much appreciated the visit and the memory. He visited Gisela Perutz at home on several occasions after Max had passed away, to keep her company and bring her much appreciated flowers.

Kiyoshi radiated a remarkable and inspirational strength of spirit throughout his lifetime, and during the last stages of his illness he gave encouragement and peace of mind to the many colleagues, friends and family who were fortunate to spend a precious moment with him. Kiyoshi focused his remaining energy on writing a review on the spliceosome, spending precious time with friends and family, and providing career advice for his younger colleagues. He spent his last days receiving many visitors, whom he always greeted with a warmth and charm that belied the discomfort he must have been feeling. His scientific work was far from finished, but he took real joy in having seen it advancing rapidly and revealing so much of the astonishing workings of nature.

Kiyoshi passed away peacefully at Addenbrooke’s hospital, Cambridge, with Yoshiko and Yuko at his side. The memorial service was held at St Matthew’s church, where he was a parishioner and active member for many years, and where Yuko Nagai and Gavin Smith had married. In addition to his wife Yoshiko and daughter Yuko, Kiyoshi is survived by his son Ken and his sister Sachiko, who prepared the drawings of Kiyoshi included in this
memoir (figure 4), and by his adoring grandsons, Joshua and Zachary. His scientific legacy and personal influence continue in strength through the extensive network of friends and colleagues who, like his scientific visions, were nurtured carefully over the years.

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REFERENCES TO OTHER AUTHORS

Giardina, B. et al. 1992 Protein engineering in haemoglobin. Nature 355, 777–778. (doi:10.1038/355777c0)

Monod, J. 1977 Chance and necessity: essay on the natural philosophy of modern biology. London, UK: Penguin Books.

Monod, J., Wyman, J. & Changeux, J.-P. 1965 On the nature of allosteric transitions: a plausible model. J. Mol. Biol. 12, 88–118. (doi:10.1016/S0022-2836(65)80285-6)

Perutz, M. F. 1972 Nature of haem–haem interaction. Nature 237, 495–499. (doi:10.1038/237495a0)

BIBLIOGRAPHY

The following publications are those referred to directly in the text. A full bibliography is available as electronic supplementary material at https://doi.org/10.6084/m9.figshare.c.5893508.

1) 1975 (With R. G. Schneider, R. J. Atkins, T. S. Hosty, G. Tomlin, R. Casey, H. Lehmann & P. A. Lorkin) Haemoglobin titusville: α94 Asp → Asn: a new haemoglobin with a lowered affinity for oxygen. Biochim. Biophys. Acta Prot. Struct. 400, 365–373. (doi:10.1016/0005-2795(75)90192-0)

2) 1977 The effect of ferric ligands on the oxygen affinity of the ferrous subunits in valency hybrid haemoglobins. J. Mol. Biol. 111, 41–53. (doi:10.1016/S0022-2836(77)80130-7)

3) 1980 (With T. Kitagawa) Differences in Fe(II)-N epsilon(His-F8) stretching frequencies between deoxyhemoglobins in the two alternative quaternary structures. Proc. Natl Acad. Sci. USA 77, 2033–2037. (doi:10.1073/pnas.77.4.2033)

4) 1982 (With G. N. La Mar, T. Jue & H. F. Bunn) Proton magnetic resonance investigation of the influence of quaternary structure on iron-histidine bonding in deoxyhemoglobins. Biochemistry 21, 842–847. (doi:10.1021/bi00534a005)

5) 1984 (With G. N. La Mar, T. Jue & B. M. Hoffman) Proton nuclear magnetic resonance investigation of the allosteric transition in ligated and unligated carp hemoglobin: evidence for structural heterogeneity in the heme pocket. J. Mol. Biol. 178, 929–939. (doi:10.1016/0022-2836(84)90320-6)

6) (With H. C. Thøgersen) Generation of β-globin by sequence-specific protolysis of a hybrid protein produced in Escherichia coli. Nature 309, 810–812. (doi:10.1038/309810a0)

7) 1985 (With M. F. Perutz & C. Poyart) Oxygen binding properties of human mutant hemoglobins synthesized by Escherichia coli. Proc. Natl Acad. Sci. USA 82, 7252–7255. (doi:10.1073/pnas.82.21.7252)

8) 1986 (With B. F. Luisi) Crystallographic analysis of mutant human haemoglobins made in Escherichia coli. Nature 320, 555–556. (doi:10.1038/320555a0)

9) (With F. C. Reinach & J. Kendrick-Jones) Site-directed mutagenesis of the regulatory light-chain Ca2+/Mg2+ binding site and its role in hybrid myosins. Nature 322, 80–83. (doi:10.1038/322080a0)
(10) 1987 (With B. Luisi, D. Shih, G. Miyazaki, K. Imai, C. Poyart & N.-T. Yu) Distal residues in the oxygen binding site of haemoglobin studied by protein engineering. *Nature* **329**, 858–860. (doi:10.1038/329858a0)

(11) 1988 (With H. C. Thøgersen) Synthesis and sequence-specific proteolysis of hybrid proteins produced in *Escherichia coli*. *Methods Enzymol.* **153**, 461–481. (doi:10.1016/0076-6879(87)53072-5)

(12) 1988 (With Y. Nakaseko, K. Nasmyth & D. Rhodes) Zinc-finger motifs expressed in *E. coli* and folded in *vitro* direct specific binding to DNA. *Nature* **332**, 284–286. (doi:10.1038/332284a0)

(13) 1990 (With Y. Nakaseko, K. Nasmyth & D. Rhodes) Zinc-finger motifs expressed in *E. coli* and folded in *vitro* direct specific binding to DNA. *Nature* **332**, 284–286. (doi:10.1038/332284a0)

(14) 1990 (With C. Oubridge, T. H. Jessen, J. Li & P. R. Evans) Crystal structure of the RNA-binding domain of the U1 small nuclear ribonucleoprotein A. *Nature* **348**, 515–520. (doi:10.1038/348515a0)

(15) 1991 (With C. Oubridge, T. H. Jessen, C. H. Teo & C. Pritchard) Identification of molecular contacts between the U1 A small nuclear ribonucleoprotein and U1 RNA. *EMBO J.* **10**, 3447–3456. (doi:10.1002/j.1460-2075.1991.tb04909.x)

(16) 1991 (With J. Tame, D. T.-B. Shih, J. Pagnier & G. Fermi) Functional role of the distal valine (E11) residue of α subunits in human haemoglobin. *J. Mol. Biol.* **218**, 761–767. (doi:10.1016/0022-2836(91)90264-7)

(17) 1991 (With N. H. Komiyama, D. T.-B. Shih, D. Looker & J. Tame) Was the loss of the D helix in α globin a functionally neutral mutation? *Nature* **352**, 349–351. (doi:10.1038/352349a0)

(18) 1992 (With D. Looker, D. Abbott-Brown, P. Cozart, S. Durfee, S. Hoffman, A. J. Mathews & G. L. Stetler) A human recombinant haemoglobin designed for use as a blood substitute. *Nature* **356**, 258–260. (doi:10.1038/356258a0)

(19) 1994 (With C. Oubridge, N. Ito, P. R. Evans & C.-H. Teo) Crystal structure at 1.92 Å resolution of the RNA-binding domain of the U1A spliceosomal protein complexed with an RNA hairpin. *Nature* **372**, 432–438. (doi:10.1038/372432a0)

(20) 1995 (With C. Oubridge, N. Ito, C.-H. Teo & I. Fearnley) Crystallisation of RNA-protein complexes II. The application of protein engineering for crystallisation of the U1A protein-RNA complex. *J. Mol. Biol.* **249**, 409–423. (doi:10.1006/jmbi.1995.0306)

(21) 1996 (With J. M. Avis, F. H.-T. Allain, P. W. A. Howe, G. Varani & D. Neuhaus) Solution structure of the N-terminal RNP domain of U1A protein: the role of C-terminal residues in structure stability and RNA binding. *J. Mol. Biol.* **257**, 398–411. (doi:10.1006/jmbi.1996.0171)

(22) 1996 (With L. Jovine, C. Oubridge & J. M. Avis) Two structurally different RNA molecules are bound by the spliceosomal protein U1A using the same recognition strategy. *Structure* **4**, 621–631. (doi:10.1016/S0969-2126(96)00066-4)

(23) 1998 (With S. R. Price & P. R. Evans) Crystal structure of the spliceosomal U2B′–U2A′ protein complex bound to a fragment of U2 small nuclear RNA. *Nature* **394**, 645–650. (doi:10.1038/292324)

(24) 1999 (With C. Kambach, S. Walke, R. Young, J. M. Avis, E. De La Fortelle & V. A. Raker) Crystal structures of two Sm protein complexes and their implications for the assembly of the spliceosomal snRNPs. *Cell* **96**, 375–387. (doi:10.1016/S0092-8674(00)80550-4)

(25) 2007 (With E. Menichelli, C. Isel & C. Oubridge) Protein-induced conformational changes of RNA during the assembly of human signal recognition particle. *J. Mol. Biol.* **367**, 187–203. (doi:10.1016/j.jmb.2006.12.056)

(26) 2009 (With D. A. Pomeranz Krummel, C. Oubridge, A. K. W. Leung & J. Li) Crystal structure of human spliceosomal U1 snRNP at 5.5 Å resolution. *Nature* **458**, 475–480. (doi:10.1038/nature07851)

(27) 2010 (With C. Y. Janda, J. Li, C. Oubridge, H. Hernández & C. V. Robinson) Recognition of a signal peptide by the signal recognition particle. *Nature* **465**, 507–510. (doi:10.1038/nature08870)

(28) 2011 (With A. K. W. Leung & J. Li) Structure of the spliceosomal U4 snRNP core domain and its implication for snRNP biogenesis. *Nature* **473**, 536–539. (doi:10.1038/nature09956)

(29) 2013 (With T. H. D. Nguyen, J. Li, W. P. Galej, H. Oshikane & A. J. Newman) Structural basis of Brt2-Prp8 interactions and implications for U5 snRNP biogenesis and the spliceosome active site. *Structure* **21**, 910–919. (doi:10.1016/j.str.2013.04.017)
Kiyoshi Nagai

(30) (With W. P. Galej, C. Oubridge & A. J. Newman) Crystal structure of Prp8 reveals active site cavity of the spliceosome. *Nature* **493**, 638–643. (doi:10.1038/nature11843)

(31) 2015 (With Y. Kondo, C. Oubridge & A.-M. M. van Roon) Crystal structure of human U1 snRNP, a small nuclear ribonucleoprotein particle, reveals the mechanism of 5′ splice site recognition. *eLife* **4**, e04986. (doi:10.7554/eLife.04986)

(32) (With T. H. D. Nguyen, W. P. Galej, X.-C. Bai, C. G. Savva, A. J. Newman & S. H. W. Scheres) The architecture of the spliceosomal U4/U6.U5 tri-snRNP. *Nature* **523**, 47–52. (doi:10.1038/nature14548)

(33) 2016 (With W. P. Galej, M. E. Wilkinson, S. M. Fica, C. Oubridge & A. J. Newman) Cryo-EM structure of the spliceosome immediately after branching. *Nature* **537**, 197–201. (doi:10.1038/nature19316)

(34) (With T. H. D. Nguyen, W. P. Galej, X.-C. Bai, C. Oubridge, A. J. Newman & S. H. W. Scheres) Cryo-EM structure of the yeast U4/U6.U5 tri-snRNP at 3.7 Å resolution. *Nature* **530**, 298–302. (doi:10.1038/nature16940)

(35) 2017 (With S. M. Fica, C. Oubridge, W. P. Galej, M. E. Wilkinson, X.-C. Bai & A. J. Newman) Structure of a spliceosome remodelled for exon ligation. *Nature* **542**, 377–380. (doi:10.1038/nature21078)

(36) (With C. Plaschka & P.-C. Lin) Structure of a pre-catalytic spliceosome. *Nature* **546**, 617–621. (doi:10.1038/nature22799)

(37) (With M. E. Wilkinson, S. M. Fica, W. P. Galej, C. M. Norman & A. J. Newman) Postcatalytic spliceosome structure reveals mechanism of 3′–splice site selection. *Science* **358**, 1283–1288. (doi:10.1126/science.aar3729)

(38) 2018 (With C. Plaschka, P.-C. Lin & C. Charenton) Prespliceosome structure provides insights into spliceosome assembly and regulation. *Nature* **559**, 419–422. (doi:10.1038/s41586-018-0323-8)

(39) 2019 (With C. Charenton & M. E. Wilkinson) Mechanism of 5′ splice site transfer for human spliceosome activation. *Science* **364**, 362–367.

(40) (With S. M. Fica, C. Oubridge, M. E. Wilkinson & A. J. Newman) A human postcatalytic spliceosome structure reveals essential roles of metazoan factors for exon ligation. *Science* **363**, 710–714. (doi:10.1126/science.aaw5569)

(41) 2020 (With M. E. Wilkinson & C. Charenton) RNA splicing by the spliceosome. *Annu. Rev. Biochem.* **89**, 359–388. (doi:10.1146/annurev-biochem-091719-064225)