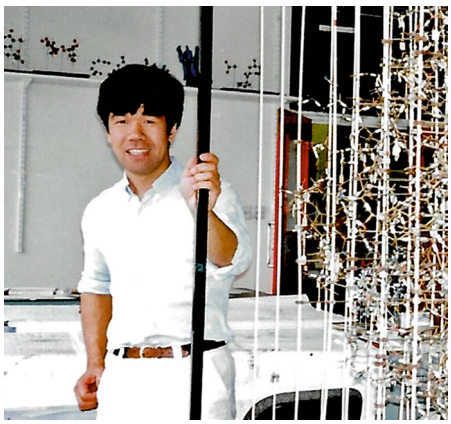
# Kiyoshi Nagai (1949–2019)

Nearly 40 years ago in Cambridge, UK, the visionary scientist Max Perutz received a letter from Kiyoshi Nagai, a young assistant professor in Nara, Japan, thanking him for a marvelous and fruitful visit to the Medical Research Council Laboratory of Molecular Biology (LMB). Kiyoshi's nostalgic letter reflected on the 18 highly productive months he had spent at the LMB studying cooperativity in mammalian hemoglobin as part of his PhD studies with Hideki Morimoto at Osaka University. The letter ended with a polite comment that he would be delighted to return to the Cambridge laboratory if ever an opportunity arose. This last sentence in particular proved to be scientifically eventful.

At the time he received the letter, Max had been wondering how allosteric modulation evolved in hemoglobins and had formulated testable hypotheses for the origins of the allostery based on his crystal structures of hemoglobin, which accounted for the protein's hallmark cooperativity. The tests involved site-directed mutagenesis and generation of recombinant protein. This might seem a straightforward endeavor today, but four decades ago there were virtually no procedures for engineering and expressing proteins, yet alone a multi-subunit protein assembly. The protocols and reagents required development almost entirely from scratch. Max had an eye for talent and immediately wrote back to Kiyoshi, inviting him to return to Cambridge and explore methods for making and characterizing the first-ever recombinant mutant hemoglobins. Kiyoshi gave up a secure lecturing post to undertake a risky project with the temporary support of a two-year fellowship and returned to Cambridge with his young family in 1981 to work again with his beloved mentor.

Through imagination, hard work, intelligence and inspired late-night discussions with Hans Thogersen and other colleagues, Kiyoshi invented methods to overexpress human hemoglobin subunits in bacteria as fusion proteins. The key to success was the liberation of hemoglobin from the fusion with a sequence-specific endopeptidase. The material was then reconstituted from a denatured state to fold with heme. This successfully produced recombinant hemoglobins with directed mutations that could be used in functional assays with allosteric modulators and for X-ray crystal structure analyses to visualize their impact on stereochemistry. In subsequent studies, Kiyoshi used this method to understand the molecular origins of divergent allosteric effects in hemoglobins of different species. The methodology revolutionized hemoglobin studies and was adopted internationally with Kiyoshi's help.



Kiyoshi next to Max's original wire model of the hemoglobin structure. LMB model room, ca. 1990. Credit: MRC Laboratory of Molecular Biology

When Kiyoshi returned from a trip to Paris, where he measured oxygen-binding curves for the first-ever mutant hemoglobins, he seemed so happy that everyone in the lab assumed that he had results that proved Max's hypothesis. In fact, Max's proposal for the origins of pH response in hemoglobin was not borne out by these early mutagenesis data. Kiyoshi's happiness was in testing Max's hypothesis properly; and, much to his credit, Max accepted the robust results readily, and he and Kiyoshi developed better models to explain the origins of the allosteric effects. It turned out that even a simple switch like an enhanced response to protons required many amino acid substitutions, which illustrated how complex and contingent the pathway of the evolution of protein function is.

Kiyoshi was soon given a group leader position at the LMB on the strength of his talent and accomplishments. He remained there for the rest of his life. Never afraid to try new things, Kiyoshi launched another risky endeavor: to determine how RNA is recognized within complex ribonucleoprotein assemblies. His inspired insight was to go beyond equilibrium interactions to understand how substrate recognition, specificity and assembly occur in highly dynamic ribonucleoprotein complexes. No more challenging dynamic process may be imagined than eukaryotic pre-messenger RNA (pre-mRNA) splicing, which was discovered by Phil Sharp and Rich Roberts and shown a few years later by Joan Steitz to involve small nuclear ribonucleoprotein particles (snRNPs). Kiyoshi's interest was to structurally characterize those snRNPs, and he was joined in this long-term endeavor by one of us (C.O.). Together, they worked doggedly for decades to prepare stable recombinant subassemblies of the eukaryotic intron splicing machinery.

The first achievement of Kiyoshi and his team in this area was to elucidate the X-ray crystal structure of the RNA recognition motif (RRM) domain, illuminating how this ancient motif recognizes structural features in RNAs throughout all domains of life. In a piecemeal manner, he and his laboratory made larger and more complex assemblies that were ever more challenging and required a continuous series of innovations. The complexes were reconstituted in vitro from proteins overexpressed in *Escherichia coli* and cocrystallized with RNA made either by chemical synthesis or by in vitro transcription. These complexes included the human U1A–RNA complex, the U2B′′–U2A′–RNA complex, Sm proteins as subassemblies lacking RNA, and then all seven Sm proteins with RNA. The next big step was to combine the subcomplexes, and this led to the structural elucidation of minimal U1 snRNPs, which gave the first insights into 5′ splice site recognition. Kiyoshi's group also took on, successfully, the formidable protein crystal structures of spliceosomal proteins Prp8 and Brr2. Ultimately, despite exhaustive efforts, obtaining higher-order complexes of the other snRNPs that were suitable for crystallization remained elusive.

Starting with the work of Kelly Nguyen and Wojtek Galej, Kiyoshi's group began purifying complexes from native sources and moved from crystallography to electron cryo-microscopy using direct electron detectors. The early successful stages of this advance resulted in the first yeast tri-snRNP map in which assignment of the individual protein components became possible. This was quickly followed by the structure of the yeast C complex spliceosome, revealing how U2, U5 and U6 snRNPs assemble on a substrate directly after the first (branching) step of splicing. Imaginative ways of capturing splicing intermediates led to a series of landmark papers that revealed

in exquisite atomic detail the stepwise assembly and catalytic processes of the yeast and human spliceosomes. They explained how the many steps of pre-mRNA splicing are coordinated, particularly showing the importance of RNA helicases and the *trans*-acting splicing factors, which are not constitutive snRNP components. The work from his laboratory solved the puzzle of how the 3′ splice site is recognized for exon ligation in a remarkable example of ribo-recognition by the branched RNA structure formed in the first catalytic step. The efforts from Kiyoshi's lab and those of researchers in labs headed by Yigong Shi and Reinhard Lührmann were blossoming into a structural gallery of the intricate splicing process.

The spliceosome project was moving with tremendous momentum toward his dream of elaborating the full spliceosome pathway when Kiyoshi learned that he was terminally ill, with little remaining time. Kiyoshi reacted much like Max had when facing terminal illness: he was not deterred and kept working, right up to the end. He used his remaining energy and resources to write a review manuscript and to spend precious time with friends and family, as well as to provide career advice for his younger colleagues.

Aside from science, Kiyoshi also shared with others his love of classical music, and he was often heard cheerfully whistling Mozart and Brahms tunes in the lab late at night, even when experiments were not going well. He attended concerts in London and Cambridge, and wherever else his scientific trips might take him. Art was another of his passions, which he shared with many others — organizing visits to museums and to iconic impressionist

Playing cello at home, a recent photo. Credit: Sachiko Nagai

painting sites on the French coast. At home, he frequently hosted parties where science was enriched by culinary extravaganzas that included traditional Japanese food, Korean barbecue, freshly made Polish pierogi or spaghetti puttanesca (perhaps zabaglione made in beakers on lab hotplates should not be mentioned; do not try this in your laboratory).

Kiyoshi embodied intense creativity, intelligence, compassion, integrity and personal warmth. 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 numerous people, and provide direct help with experiments. He helped PhD students who were green, naive and even temperamental and obstinate, charming them with reason and good humor. He subsidized student travel to international laboratories with his own personal travel funds. This generosity was in his nature, and he was supportive throughout his career of colleagues and the many young scientists whom he mentored directly and indirectly.

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. His scientific work was far from finished, but he took real joy in seeing it advancing rapidly and revealing so much of the astonishing workings of nature. His scientific legacy and personal influence continue in strength through the extensive network of friends and colleagues that, like his scientific visions, were nurtured carefully over the years. He passed away gently and peacefully with his wife Yoshiko and daughter Yuko at his side. Kiyoshi's loving family includes his son Ken, sister Sachiko and his two adoring grandsons. ❐

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