Roger Y. Tsien (1952–2016)

Roger Youchien Tsien, the founding pioneer of fluorescence imaging methods and Nobel Laureate in Chemistry in 2008, passed away on August 24. He was 64.

Roger was born in New York City on February 1, 1952. His early life and professional career prior to his professorship at University of California, San Diego are vividly described in an autobiography at nobelprize.org (https://www.nobelprize.org/nobel_prizes/chemistry/laureates/2008/tsien-bio.html). A talent for chemistry was evident from an early age, and he took full advantage of the encouragement—and occasional licensed reagent—from his father, an engineer, for increasingly complex and occasionally dangerous inorganic chemistry experiments in the basement and patio of their home in New Jersey. These youthful exploits included an ambitious, unsupervised multistep reaction sequence aimed at synthesizing aspirin. Looking back he said, "it was very good training in how to improvise equipment, plan and execute experiments, interpret confusing results, and decide how to do things better." Eventually, Roger's intellectual forays led him into the sparsely populated interface between major research fields, in which he decided to use the power of chemistry and optics to solve problems in biological visualization. This intrepid interdisciplinary spirit via technology development defined his work for the remainder of his career.

Roger was known for pioneering the development of novel calcium imaging techniques during the 1980s, and his group created a few first-generation calcium indicators (quin-2, fura-2, indo-1, fluo-3, and others), their membrane-permeable derivatives (acetoxymethyl [AM] ester forms) and caged calcium compounds. Using fura-2 and indo-1 as prototypes, he established methodologies for the quantitative measurement of intracellular calcium concentration based on ratiometric imaging. Moving beyond calcium, he worked alone or with colleagues to engineer and validate fluorescent indicators for cAMP, pH, and membrane voltage. Together, this multicolor toolbox for optical interrogation of molecular dynamics in live cells helped to unlock our understanding of many facets of intracellular signal dynamics. In addition to the technical development of fluorescence indicators, Roger's achievements extended further into the nascent field of microscopic optical systems and analytical software development. This work naturally inspired him and those around him to energetically pursue interdisciplinary studies in which he could devote his ideas and leadership to create new technologies that were useful to cross pollinate fields and catalyze new knowledge.

In launching new projects, Roger was prescient about how experimental biology should evolve. In most signal transduction diagrams of his time, arrows between molecules depicted enzymatic reactions and intermolecular interactions. While this notation has its merits and continues to be used heuristically, Roger realized that diagrams for the only accurate scientific understanding of signal transduction systems should contain three axes in space and a time base. In his generation, biochemistry was dominated by biochemical cell extraction and solubilization approaches in which electrophoresis was applied to lysates prepared after grinding millions of cells. These techniques continue to have practical value in research, but Roger knew that extreme observer effects would disrupt delicate molecular dynamics resulting in necessary approximations if not artifacts. In a visionary 1994 review article in *Chemical & Engineering News* entitled "Fluorescence Imaging Creates a Window on the Cell," Roger advocated real-time, three-dimensional, single-cell imaging techniques to accurately and comprehensively dissect molecular and cellular principles and to account for natural and sampling heterogeneity in biology.

Roger after the announcement of his winning of Nobel Chemistry Prize in 2008, credit of Erik Rodriguez.

During the early 1990s, based on these guiding principles, Roger expanded his research into the engineering of genetically encoded fluorescent probes, in which his wide interests drew him to GFP, one of nature's masterpieces. Using recombinant GFP cDNA, he and colleagues conducted an in-depth study on the mechanism of formation of the π-conjugation system and the physicochemical environment of the chromophore. Through random mutagenesis, they discovered spectral GFP variants, such as blue fluorescent protein (BFP), CFP, and a bright green-emitting variant (S65T), which later produced the most widely used variant, enhanced GFP (EGFP). At the same time, on the basis of GFP structural data, Roger rationally designed and generated yellow fluorescent protein (YFP). I was fortunate to participate in a project involving the atomic simulation of fluorescence profiles of GFP variants based on alternate π-electron behaviors of the chromophore. Discussion with Roger in front of the computer screen often filled me with a sense of awe: here was a person who could grasp, inside and out, the motivations and destinies of single electrons. The review on GFP Roger published in *Annual Review of Biochemistry* in 1998 is still regarded as a "bible" for researchers in the field and has been cited in a myriad of scientific articles. The scientific principles Roger discovered in his studies of GFP proved to be a foundational framework applicable to the growing number of newly discovered fluorescent proteins obtained from the genes of non-bioluminescent cnidarians. Performing directed evolution on DsRed, the prototype red fluorescent protein (RFP), the Tsien lab successfully generated a number of monomeric RFPs (fruit series), such as

Autograph of Roger with his left hand on May 28 2015 in La Jolla, credit of Atsushi Miyawaki.

mCherry. Driven by this genetic engineering ethos, the development of this array of fluorescent protein variants—what Roger called "molecular spies"—helped to usher post-genome biological research to even greater heights, empowering the field of super-resolution imaging and multi-color imaging and expanding research possibilities for genetic-based visualization techniques like fluorescence resonance energy transfer (FRET), fluorescence cross correlation spectroscopy (FCCS), and bimolecular fluorescence complementation (BiFC).

The development and broad use of GFP technologies motivated researchers worldwide to further technical innovation in fluorescent protein labeling. In the latter phase of his career, Roger continued to spearhead new technological ventures, leading chemistry-equipped expeditions to the far frontiers of biological research. His lab developed proteins that became fluorescent upon incorporation of natural π-conjugated compounds (pigments) as chromophores. In the non-animal kingdoms, including plants and microorganisms, certain bilins (heme metabolites) are linked by covalent bonds to biliproteins, forming light-sensing phytochromes and light-harvesting phycobiliproteins. The Tsien lab conducted comprehensive genetic mutagenesis to achieve for the first time mammalian expression of near infrared fluorescent proteins from both of the two biliproteins: infrared fluorescent proteins (IFPs) from a bacterial phytochrome in 2007 and small ultra-red fluorescent proteins (smURFPs) from a cyanobacterial phycobiliprotein in 2016. His range of interests also extended to practical applications, and he and his colleagues developed a genetic tag that labels proteins of interest for electron microscopy and so-called activatable cell-penetrating peptides to illuminate tissue during tumor surgery.

I joined Roger's group in 1995 during the developmental phase of GFP with aspirations to explore FRET methods. At the time, there were only two available GFP variants suitable for a FRET donor and acceptor pair: BFP and EGFP. From their spectral overlap, I calculated that the R_0 (the distance at which the FRET efficiency is 50%) would be about 4 nm for this pair, an assumption that did not consider the relative spatial orientation between the two chromophores. At the time, there was no structural information on GFP to calculate orientation, a considerable bottleneck to achieving workable FRET. One day, Roger came over to my cluttered lab bench and gave me some math homework. "Let's assume that the 26 kD GFP is a spherical protein with a density of 1.3 mg/ml. And let's assume that the chromophore is positioned in the center. Now, you can calculate the proximity of the BFP and EGFP chromophores." Using the equation of $1.3 \times 4/3\pi r^3 = 26 \times 10^3 \times 1.661 \times 10^{-27}$, I calculated the radius of the imaginary GFP to be 2 nm. "Considering that the shortest inter-chromophore distance is equivalent to the R_0 (4 nm), we can't expect higher than a 50% FRET efficiency," I said to him, adding, "theoretically." Although we thought this estimate would make practical signal strength a challenge, we were not disappointed because we were certain that the physical principles guiding chromophore orientation as well as distance would allow us to rationally determine the best possible efficiency of the FRET from BFP to EGFP.

The question was, "How could we engineer an optimal FRET pair based on the genetically predicted structure of GFP?" When the crystallographic structure of GFP was resolved, it revealed a cylindrical β-barrel structure, 4.2 nm long and 2.4 nm in diameter, with the chromophore fixed inside. Since the average orientations of the two partner's transition dipoles should not occur on a time scale shorter than the transfer time, we found that it was possible to obtain very high FRET efficiencies depending on the relative angle between BFP and EGFP. Indeed, angle dependency is a very critical factor of GFP-based FRET technology and the race began to elicit larger and larger FRET dynamics by fusing BFP and EGFP (later CFP and YFP) to proteins and peptides that underwent large conformational changes upon binding to ions. Roger and I sometimes had conflicting opinions about these polypeptide fusion strategies, and we would exchange experimental data and notes with a variety of titles, "You win," "I win," and "The jury is still out." I loved confronting him during these passionate but good-natured intellectual discussions. Roger was very good at communicating with different researchers in his lab in accord with their intellectual manner. I think this is because he possessed a very wide range of knowledge and expertise, natural curiosity, and a respect for the personalities of those around him.

I worked in Roger's lab until 1998. On one occasion I was suffering from severe jet lag and, like a doctor on call, Roger prescribed for me a few homemade melatonin capsules with advice that commercial chemicals contain many detestable artificial additives. Likewise, he always asked us to check the quality of chemicals used for imaging experiments in the laboratory. His emphasis on chemical purity doubtless contributed to his lab's success in obtaining reproducible results. Similarly, he often warned his lab that the introduction of excessive amounts of any indicator for cellular activity into cells could cause problems suggesting that even the seemingly impartial process of labeling a sample for microscopic visualization must be viewed as a chemical reaction of the viewed object. We therefore always considered the potential impact of visualization methods on results and how to minimize or account for observer effects.

A stroke attacked Roger in March 2013. He struggled against the aftereffects, including paralysis in his dominant right arm/hand that forced him to perform daily tasks left-handed and robbed him of favorite pastimes such as piano playing. I imagine that the impairment would have given him more stress all the worse for his unaffected sharp mind. Last year, I had the chance to meet Roger in San Diego, and he told me about his dream to visualize the pattern of holes in the perineuronal net that he hypothesized may store very long-term memories. While doing so he showed his bright intellectual spirit was unquenched, which relieved

me. On my request, Roger casually used his left hand to write a warm message and sign it. The handwriting was so neat that, similar to our discussions decades earlier on π-electron behaviors in the chromophore, I was acutely aware of how he had marshalled his unquenchable intellectual strength and adaptability to succeed on his own terms in a new reality.

Roger's death was announced by University of California, San Diego on August 31. "Roger was an extraordinary man: kind, generous, gracious, and always the consummate scientist pushing the limits of his work to expand the possibilities of science. He was a rare talent we cannot replace," said UC San Diego Chancellor Pradeep Khosla. Added Roger's wife, Wendy, in an email to his friends and colleagues, "He was ahead of us all. He was ever the adventurer, the pathfinder, the free and soaring spirit. Courage, determination, creativity, and resourcefulness were hallmarks of his character. He accomplished much. He will not be forgotten." Immediately after the announcement, a large-scale e-mail circulated among current Tsien lab members, alumni, collaborators, and others in the international research community to share in the immense grief and acknowledge how lucky we were to have worked with him. We will miss the brilliance of his intellect and his warm company considerably from now on. Seeking a guiding star like Roger in our own lives and scientific explorations, we may cast our questions into the night sky, hoping for his advice: "How would you solve this technological problem"? On another world, he may perhaps be playing with pretty colorful substances innocently. If he should peer back to our side, Roger would see how his life continues to inspire us.

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