Roger Y. Tsien

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Nobel Laureate Who Developed Colored Fluorescent Proteins

On August 24, 2016, Roger Tsien unexpectedly died during one of his weekly bike expeditions in Eugene, Oregon. Awarded the Nobel Prize in Chemistry in 2008 (together with Osamu Shimomura and Martin Chalfie), Tsien is credited with developing the wide palette of colored fluorescent proteins that are now an essential tool of much biological and medical research.

Roger Yonchien Tsien was born in New York in February 1, 1952, the youngest son in a family of gifted and illustrious engineers. He soon demonstrated a precocious gift for chemistry (and explosions) in the family basement with reagents supplied by his father. After winning the first prize in the Westinghouse Science Competition at the age of 15, he attended Harvard University and obtained a "double-major" degree in chemistry and physics, but with minimal organic chemistry. He then made a bold and fateful decision (partially guided by his elder brother, Dick) to obtain his PhD in physiology at the University of Cambridge (UK) in 1976. Given free rein to develop his own ideas by his supervisor, Richard Adrian, a noted muscle physiologist, and with a spare bench in the chemistry teaching laboratory and interaction with physiologists such as Tim Rink, he set forth on the path he was to continue along for his entire career.

Roger's thesis entitled *The Design and Use of Organic Chemical Tools in Cellular Physiology* includes the synthesis of dyes for studying membrane potentials ("voltage" dyes) and determining Ca^{2+} concentrations; these dyes he honed intermittently and tirelessly through many iterations and forms (including versions with fluorescent proteins) until his death. During a productive postdoctoral period in the same department he made two seminal inventions, namely the first fluorescent calcium sensor quin2, and the use of acetoxymethyl esters to load it into cells in a nondisruptive way. This combination permitted measurement of this important and dynamic second messenger (Ca^{2+}) in a multitude of small cell types, rather than just the few that could be microinjected with bioluminescent and colorimetric probes or pierced with selective microelectrodes.

Roger was frustrated on his return to academia in the USA because of the limited available offers, probably due to his then unusual multidisciplinary training, and he seriously considered an industrial position. However, in 1981, he settled on the Department of Physiology–Anatomy at the University of California, Berkeley. Unable to recruit chemistry graduate students, he resorted to hiring more senior chemists, and soon achieved great success with the development of fura-2 and then fluo-3. With the advent of digital ratiometric imaging, fura-2 was sufficiently bright and photostable to allow quantitative single-cell Ca^{2+} measurements, and the 1985 publication in *J. Biol. Chem.* is still among the journal's most highly cited articles. Although we were only a small group of four when I joined the laboratory in 1984, we collaborated with neighboring (and many outside) groups to make significant biological advances and Roger was soon promoted to full professor. Many scientists already expected that these developments would merit a Nobel Prize.

Not content with small-molecule probes, Roger (in collaboration with Susan Taylor at the University of California, San Diego (UCSD)) devised an ingenious but simple method for cellular imaging of the other major second messenger cAMP by using a change in fluorescent resonance energy transfer (FRET) upon the dissociation of the fluorescently labeled subunits of protein kinase A holoenzyme. But, again frustrated by the need for microinjection, he explored new methods for labeling specific proteins in living cells, aided by a move in 1989 to dynamic and rapidly growing UCSD and the freedom to explore far-fetched notions as a Howard Hughes Medical Institute Investigator. In the early 1990s, two alternative approaches were initiated, the first using the green fluorescent protein (GFP) as a fusion protein, and the second, a more chemical approach, the biarsenical–tetracysteine tag. Both approaches were ultimately successful, and have left a substantial legacy in biology and chemical biology, respectively. Initial progress was slow with GFP as the research group had no direct experience with molecular biology, but explored the chemistry and kinetics of fluorophore formation and their improvement through random and directed mutation. Different colored fluorescent mutants followed quickly, and were immediately applied by Atsushi Miyawaki to Ca^{2+} indicators in the form of FRET-based sensors that the cell generated itself, a key demonstration of the power and scope of this method. Further red-shifted colors have required new starting proteins; this research has steadily progressed to the infrared FPs recently being developed in Roger's laboratory, with the latest results published just before his death.

In 2003, Roger, in typical fashion, embarked on a major change in research focus upon losing his father to pancreatic cancer, and turned to chemically synthesized small-molecule detectors for tumors, with the goal of clinical approval before the end of his career. Masking the uptake of activatable cell-penetrating peptides (ACPPs) until cleaved and activated by upregulated proteases in

cancer cells, coupled to the usual FRET-based readout, allows a surgeon to more precisely resect tumors during surgery. Avelas (one of several companies cofounded by Roger) recently reported successful completion of phase 1 clinical trials with such an ACPP. More recently, he proposed and explored the hypothesis that very long-term memories in the brain are stored as holes in the perineuronal net, a sheathlike coat of the extracellular matrix around some neurons in the brain. Ironically, a debilitating stroke in 2013 affected his own prestigious and encyclopedic memory, but with typical drive and determination, he recovered his unique talent for simple chemical solutions to tackle complex biological problems.

Roger was an extremely well-known and admired scientist, a common sight at many conferences or seminars as he hurried between talks or appointments, avoiding eye contact, but with occasional grunts of acknowledgement until a potential source of information was spotted and cornered for his usual direct and insightful questioning. In the laboratory, he gave us encouragement and considerable freedom to explore ideas and strategies while pointing out their limitations and suggesting elegant solutions. In addition to his science, he was an avid runner (tricyclist after his stroke), accomplished pianist and music-lover, keen photographer, and was devoted to his wife, Wendy, who tirelessly supported him through his intense and too short life.

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