David T. Yue: In Memoriam

David Tuckchow Yue, a renowned biophysicist who dedicated his life to the study of voltage-gated calcium (Ca_V) channels, passed away suddenly on December 23, 2014 from cardiac arrest. David was a passionate, dedicated, and brilliant scientist who pursued fundamental mechanistic understanding of Ca_V channel regulation with quantitative rigor, child-like wonder, and remarkable creativity. To those who knew him (both scientists and non-scientists alike), David's tremendous enthusiasm for science and the passion he brought to seeking truth was both infectious and inspirational. David's deep appreciation of the process of scientific discovery is aptly captured by a quote from a 2006 essay he wrote entitled "The Privilege of Discovery": "Every so often, the veil of confusing experimental results is parted, and something deep and beautiful about how biological life works is revealed. It is as if a syllable that God spoke becomes suddenly audible. The thrill of unearthing such 'God speak' is one of the special rewards of my profession."

David T. Yue

David was born in Midland, Michigan on February 13th, 1957 to parents Alfred and Virginia Yue, whom David credited for imbuing him with a "passion for discovery" and teaching him the "virtue of scholarship." Growing up in California, David's love for science was evident at an early age; he would often rescue old, discarded equipment and bring it back to life. In fact, David even became a licensed ham radio operator at the tender age of 11. David attended Harvard University where he studied biochemistry, graduating magna cum laude in 1979. From there he went to the Johns Hopkins School of Medicine (JHUSOM), where he would spend the rest of his illustrious career. David received his MD and PhD from Johns Hopkins in 1987 under the instruction of Kiichi Sagawa, whom David credited with teaching him to "search wherever the scent of truth appeared." During this period, David dreamed of "melding engineering and molecular-level biology," an endeavor encouraged by his mentor. With this goal in mind, David spent time in W. Gil Wier's lab at the University of Maryland, where he first experienced "the challenge and excitement of intracellular calcium." It was in this setting that David began his love affair with calcium, first studying the interrelation between intracellular calcium and muscle contraction in the heart. He excelled in these pursuits, receiving the David Israel Macht Memorial Research Award in 1987 (high honor for distinguished graduate research from the JHUSOM). David stayed on at Johns Hopkins, crediting Dr. Richard Johns, then Chair of Biomedical Engineering at JHUSOM, for luring him away from a cardiac fellowship by offering him an Assistant Professor position.

From the outset, David focused his new lab on mechanistic understanding of the regulation of Ca_V channels. These are integral membrane proteins which play a

pivotal role in the biology of excitable cells by converting electrical signals into Ca^{2+} influx. The focus on Ca_V channels resonated with David's early love of electronics, as he often spoke about the importance of transistors and pointed out that in biology, the transistor is the Ca_V channel. L-type ($Ca_V1.2$) Ca^{2+} channels, which are prevalent in heart cells, undergo a negative feedback regulation whereby their permeant ion, Ca^{2+}, reduces further Ca^{2+} influx through the channel. This phenomenon, first described by Brehm and Eckert in 1978, and referred to as Ca^{2+}-dependent inactivation (or CDI), became a launching pad for a line of inquiry into how Ca^{2+} regulates Ca_V channels that endured in David's laboratory for 25 years. With his first graduate student, John Imredy, David used in-depth quantitative analyses of single $Ca_V1.2$ channel recordings in heart cells to gain unprecedented insights into the microscopic signature of CDI (Imredy and Yue, 1992, 1994; Yue et al., 1990). Following the cloning of Ca_V channel subunits, David began to apply molecular biology tools to his studies on mechanisms of Ca_V channel regulation. Using chimeric channel analyses and site-directed mutagenesis, his group identified the carboxy-tail of $Ca_V1.2$ as harboring critical determinants of CDI (de Leon et al., 1995; Peterson et al., 2000).

A major breakthrough was the discovery that calmodulin (CaM) was in fact the Ca^{2+} sensor for CDI of $Ca_V1.2$ channels (Peterson et al., 1999). The nature of the sensor for CDI of Ca_V channels had been a subject of intense study by several groups. For David, a seminal event leading to his discovery was a publication from the Adelman lab the prior year arguing that CaM was the Ca^{2+} sensor for Ca^{2+}-dependent activation of small conductance potassium channels (Xia et al., 1998). A key tool used in that study was a CaM mutated in each of its four EF-hand domains to yield a variant, CaM_{1234}, that no longer binds Ca^{2+}. Upon reading this paper, David wondered whether CaM might play a similar sensor role in CDI of $Ca_V1.2$ channels and obtained the constructs from the Adelman lab to test this conjecture. The first observation that co-expressing CaM_{1234} wiped out CDI of $Ca_V1.2$ channels was made by Carla DeMaria, then a postdoc in the lab. At the time of her discovery, David was a couple of floors downstairs teaching his graduate level ion channels class for which Jonathan Lederer was a guest lecturer that day. News of the discovery trickled down, and immediately after the class David rushed upstairs to join Carla at the rig to watch the results unfold, with Jon Lederer in tow. David's and the lab's excitement about the result and its implications were palpable and caused Jon to ask "is it like this every day in your lab"? While not quite every day, there were many such moments of discovery in the lab with David acting as the biggest cheerleader of all. In his own words, he would "get phosphorylated" whenever such new and provocative results arose in the lab. The discovery of CaM as the sensor for CDI ushered in a prolific period of productivity on a topic David referred to as "calmodulation of Ca_V channels."

With graduate student Mike Erickson, he refined FRET methods by developing a new three-cube approach to quantitatively determine the locus and binding affinity of CaM interaction with the carboxy-tail of Ca_V channels (Erickson et al., 2001, 2003). With Carla DeMaria and others, he discovered that CaM associated with P/Q-type ($Ca_V2.1$) channels bifurcated Ca^{2+} signals to produce opposing effects on the channel—Ca^{2+}-dependent facilitation (CDF) and CDI (DeMaria et al., 2001). Intriguingly, the two effects were independently mediated by the distinct lobes of CaM, with the C-lobe responding preferentially to the local Ca^{2+} from the host channel to initiate CDF, and the N-lobe attuned to the global Ca^{2+} signal arising from distant sources to control CDI (DeMaria et al., 2001). Subsequently, together with graduate students Ivy Dick and Mike Tadross, David discovered a sequence element that acts as a modular switch for spatial Ca^{2+} selectivity in CaM regulation of Ca_V channels (Dick et al., 2008) and provided revealing theoretical and experimental insights into the mechanism of CaM spatial selectivity in regulating Ca_V channels (Tadross et al., 2008). By combining a fused CaM strategy with polymer chain statistical theory, David, with postdoc Masayuki Mori, elucidated the functional stoichiometry of the CaM-channel interaction and discovered an unexpected "marked enrichment of local CaM, as if a 'school' of nearby CaMs were poised to enhance the transduction of local Ca^{2+} entry into diverse signaling pathways" (Mori et al., 2004). To gain deepened insights into how Ca^{2+}/CaM regulates Ca_V channels, David initiated what he referred to as the "Manhattan Project" with graduate students Manu Ben-Johny, Hojjat Bazzazi, and Phil Yang. The approach utilized an ambitious alanine scanning mutagenesis of the entire carboxy-tail of $Ca_V1.3$ channels and related the strength of channel modulation to the affinity of CaM/channel interactions by an individually transformed Langmuir anaylsis relation. The results led to the proposal of an elegant model in which Ca^{2+}/CaM dynamically shifts binding surfaces to initiate $Ca_V1.3$ regulation, implicating interaction sites outside the IQ region, thereby challenging the pre-existing IQ-centric dogma (Bazzazi et al., 2013; Ben Johny et al., 2013). David developed a further-deepened understanding of CDI, by considering the functional consequences of apoCaM binding (and its loss) for channel regulation. With postdoc Xiaodong Liu he discovered that a module present on the carboxy-tail of some Ca_V1 channels prevented CDI by acting as a competitive inhibitor, tuning the channel's ability to pre-associate with CaM (Liu et al., 2010). With postdoc Paul Adams, David arrived at a critical insight, which he eloquently expressed in a distinguished lecture he gave three weeks before his death—"channels bereft of CaM open with a low P_O and do not inactivate. Once armed with CaM, CDI is simply dissipation of the initial enhancement of opening by CaM. Simple, elegant signal bifurcation" (Adams et al., 2014). Noting the tantalizing similarity between the carboxy-tails of Ca_V and Na_V channels, David described an eerie similarity between the two in that both could undergo CDI with similar underlying structural determinants (Ben-Johny et al., 2014). Finally, David was deeply interested in the physiological and disease implications of his work. By overexpressing engineered CaMs in heart, his group discovered that eliminating CDI in cardiomyocytes

led to ultra-long action potentials, revealing an unexpectedly prominent role of this phenomenon in regulating cardiac action potential duration (Alseikhan et al., 2002). This insight came full circle with the discovery that CaM mutations that cause long QT syndrome in patients diminish CDI of $Ca_V1.2$ channels and promote proarrhythmic behavior in cardiomyocytes (Limpitikul et al., 2014).

Beyond the work on Ca^{2+}-dependent regulation of Ca_V channels, David also made critical influential contributions in other arenas of Ca_V channel modulation including regulation by G-proteins, auxiliary subunits, and phosphorylation. Overall, his work spanned the gamut from biophysical analyses of single molecules to systems neuroscience and whole animal behavior (Issa et al., 2014). As a result of his substantial scientific discoveries David received many honors, including the Kenneth S. Cole award in 2011 for his contributions to the field of membrane biophysics. David accepted the award in his typical, humble style—wearing his signature black T-shirt and a brightly colored lanyard to hold his badge.

David believed in the importance of effective communication of scientific data and concepts, and he took this to an art form. He was well known for his eloquence in speech, expressive writing, and vivid figures. An important part of his legacy is his contribution as a mentor and an inspirational teacher. Over his career, dozens of trainees passed through his lab as undergraduate researchers, graduate students, postdocs, and research technicians. His mentorship style was hands-on and personal, and David forged deep bonds with his trainees by often laboring in the trenches with them. His excitement and passion for science was infectious, and working with him was inspirational. He gave his time freely, spending hours practicing and perfecting talks with students, and making sure that everyone in his lab knew how to make a perfect figure. It is no wonder he inspired such devotion among his many trainees. David took his teaching responsibilities seriously, and devoted a substantial amount of time to preparing his lectures. He was masterful at crafting a lecture that captivated and enthralled each student in the room, and his liberal use of humorous analogies was legendary. The JHU Gazette quoted one student, "he transforms his lectures into something like a thriller movie—you don't want to miss a second of it." Over his career, David taught literally thousands of undergraduate, graduate, and medical students, and he won a Johns Hopkins Excellence in Teaching Award in 2009.

One of David's sayings was "no victory in lab without victory at home." For all his long hours in the lab, David was a dedicated and present family man. He is survived by his wife, Nancy, and three exceptional sons, Michael, Jonathan, and Daniel, who were so often spoken of by their proud father. David's lab members watched his boys grow up through stories, photos, and even Facebook videos in the lab. David's family was a part of lab dinners and often welcomed the lab into their house—David had a whiteboard at home and used it to explain Baltimore Ravens football to the lab and math to his boys. The index cards in his pocket carefully recorded ideas for the lab as well as reminders for his family.

David was a man of deep faith who approached science as an act of worship, and strived to live his life in accord with the ideals of his faith. With his passing we have lost an outstanding scientist who was an inspirational mentor, a gifted teacher, and a valuable colleague to many. His spirit and the lessons he taught will live on in each of our hearts. David T. Yue will be deeply missed by all.

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