Paul F. Cranefield Award to Rikard Blunck

The late Paul F. Cranefield, MD, PhD, was the editor of *The Journal of General Physiology* for 30 years, from 1966 to 1995. During his editorship, Dr. Cranefield worked tirelessly to advance the mission of the Journal: to promote and publish original research of the highest quality that elucidates basic biological, chemical, or physical mechanisms of broad physiological significance, and provides insight into fundamental mechanisms that govern biological function at all levels.

When Dr. Cranefield stepped down as editor, the Council of the Society of General Physiologists created the Paul F. Cranefield Award to recognize his enduring contributions to the Journal and the Society, and to carry on his vision of excellence. The award is given to a young, independent investigator who in the preceding year published an article of exceptional quality in the Journal. The award is given at the Annual Meeting and Symposium of the Society in Woods Hole, MA.

In 2011, the leadership of the Society selected Rikard Blunck of the University of Montreal for the Cranefield Award. Dr. Blunck received a bachelors/masters degree in physics in 1996 from the Christian-Albrechts University of Kiel, Germany, for research on the Na^+ block of plant (*Chara coralina*) K_V channels under Professor Ulf-Peter Hansen (Hansen et al., 1997). He then completed the doctorate in physics at the Christian-Albrechts University under Professor Ulrich Seydel investigating the effect of bacterial lipopolysaccharides (LPS) on the gating behavior of BK channels in human macrophages, demonstrating that immunologically agonistic LPS activate BKs (Blunck et al., 2001; Seydel et al., 2001). Dr. Blunck went on to do postdoctoral research under Professor Francisco Bezanilla at UCLA, where he was involved in the designing of innovative experimental approaches for understanding conformational changes of ion channels. Using voltage-clamp fluorimetry, he investigated structure–function relations of NaChBac channels (Richardson et al., 2006), developed, together with fellow postdoc Baron Chanda, a genetically encoded fluorescent voltage sensor to optically record action potentials (Chanda et al., 2005), and, in a collaboration with the Perozo group, investigated the gating behavior of KcsA channels with fluorescence lifetime methods, establishing that the helical bundle crossing in these channels remains open during inactivation, whereas the selectivity filter closes (Blunck et al., 2006). Dr. Blunck initiated the work on single-channel fluorescence of K^+ channels during his postdoctoral training, which he continued when he was appointed to assistant professor of physics at the University of Montreal in 2006. In collaboration with Dr. Bezanilla, he followed the gating of single KcsA channels in supported lipid bilayer using fluorescence spectroscopy (Blunck et al., 2008).

He also developed chips that allow voltage-clamp fluorimetry of proteins in planar lipid bilayer, which paved the way for his recent work on pore-forming toxins. In his laboratory, moreover, the gating of K_V channels continues to be investigated but with an emphasis on the electromechanical coupling between voltage sensors and pore domain (Batulan et al., 2010; Haddad and Blunck, 2011). Dr. Blunck was promoted to the rank of associate professor this past summer.

The Cranefield Award was given to Dr. Blunck for work in his laboratory in Montreal characterizing the molecular mechanism of pore formation of a toxin, Cry1Aa, produced by the bacterium *Bacillus thuringiensis* (Groulx et al., 2010). In addition to the general importance of understanding how such toxins kill cells, Cry toxins are important because members of this family are being introduced into genetically modified crops as alternatives to chemical pesticides for protection against insect pests. The crystal structures for several the Cry toxins of *B. thuringiensis*, including Cry1Aa (Grochulski et al., 1995), have been solved. The toxin is an ~65-kD core protein with a three-domain structure; domain I, comprising 7 α helices, has been shown responsible for pore formation, whereas domains II and III are responsible for receptor binding. Based on atomic force microscopy data, the pore has been hypothesized to be a tetramer (Vié et al., 2001). The Cry toxins must necessarily

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undergo a major refolding process upon encountering the host cell membrane, but the details of the pore formation had not been established. Groulx et al. (2010) developed a novel FRET-based approach to study this refolding process in planar lipid bilayers. Site-directed mutagenesis was used to place cysteine residues at critical positions in the toxin, and these were then labeled with an organic thiol-reactive fluorophore (tetramethylrhodamine-maleimide or fluorscein maleimide). The labeled residues constituted the fluorscent donor in the experiments, and one or both of two FRET acceptors were used: dipicrylamine and oxonol, amphipathic negatively charged compounds that partition into the membrane. In response to a voltage pulse applied across the membrane, the acceptors move electrophoretically through the bilayer to position themselves apposite to the phospholipid head groups as determined by the pulse polarity, although the two acceptors move with a 600-fold difference in speed. For example, in response to a hyperpolarizing voltage step (trans side of membrane negatively polarized), the acceptors move to the outer leaflet of the membrane. Control experiments with the amphipathic fluorescent donor di-8-ANEPPS, which intercalates into the cis leaflet of the bilayer between the head groups, established the ability of the assay to determine the membrane sidedness of a donor. Combining these FRET tools with electrical recording of currents produced as the toxin channels form, Blunck and coworkers mapped the approximate location in the bilayer of key residues of domain I and established that the α3-α4 hairpin translocates through the membrane during pore formation. Moreover, with the "double-FRET" method, in which both acceptors are used simultaneously, they indeed were able to resolve the existence of populations of toxins on both sides of the membrane that normally conceal each others' signals. The investigation of Groulx et al. (2010) is the first report of the fluorescent monitoring of the conformational changes of a protein accompanying the formation of an ion-conducting pore. The methods developed also should have much broader application to membrane protein biology, not only in bilayers but also in native cells.

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