Over nearly five decades, David Christopher Gadsby pioneered biophysical research that advanced our mechanistic understanding of ion-transporting proteins in biological membranes. His passion for hands-on do-it-yourself electrophysiology, his depth of analytical rigor, and his idiosyncratic scientific aesthetic expanded the edge of discovery in two areas: the electrical character of the Na\(^+\) pump, and the molecular workings of ‘cystic fibrosis transmembrane regulator’ (CFTR), the chloride ion channel whose mutations cause cystic fibrosis. His approach was flavoured by an appreciation for common underlying features between these ostensibly distinct types of membrane-transport systems. While David’s focus was first on the basic molecular biophysics of a problem, he was always attuned to implications of his discoveries for human health. Based in New York at The Rockefeller University throughout his independent scientific career, and at the Marine Biological Laboratory in Woods Hole, Massachusetts, as a squid-season research-scientist, he was proficient in wrestling with problems spanning a wide swath of membrane biology: from determinants of the cardiac electrical waveform, to microsecond-timescale ionic currents in squid axons, to details of structure–mechanism relations in membrane pump and ion-channel proteins. He wore his eminence lightly and never distanced himself from the laboratory, where he often performed experiments with his own hands right up to his retirement. His reserved scientific personality, which demanded equally from his colleagues and himself immaculate data, unclouded logic, and substantive pertinence to the issues at hand, contrasted with his palpable joy in a good experiment and in his sea-loving life outside the lab.
The ideal scientist thinks like a poet, works like a bookkeeper, and . . . writes like a journalist.

E. O. Wilson

EARLY LIFE AND EDUCATION

David Christopher Gadsby was born on 26 March 1947 in Penarth, Wales, the second of three siblings. His parents, Christopher Gadsby and Esther Evans (always known as Betty) met when both were employed in the Civil Service in Bath. They had been sent there from their London offices, he as a draftsman in the Ministry of Works, she as a typist. One day in August, 1939, she was ordered to pack up her typewriter without knowing where she was going, becoming a ‘hush-hush girl’ as the Admiralty prepared for a move to Bath with the war looming. After marrying Chris in 1941, Betty was obliged to quit her job according to the rules at that time. She joined the Red Cross while Chris served in the RAF as a pilot and navigator in Burma and Thailand. He rejoined the Civil Service in Cardiff in 1946.

The family’s house was a short bike ride to the beach on the Severn Estuary, and from his earliest childhood, David profoundly bonded with the sea. His father often took the children fishing and would bring them to the salt-water public pool for a swim before school. David's family nickname (still heard in adulthood) was ‘Eyes-on-the-ground’ because he noticed everything—from pound notes blowing on the sand to collectable marine detritus tumbling in the shallows; his sister recalls how, emerging from the water after a collecting-bout, he would seem to be wearing grey socks as a result of coal-dust coming down the river from the mines upstream.

When David was 12, his father was offered a transfer to Cambridge, and he moved the family mainly for the educational environment there. David was enrolled in a public school, the Cambridgeshire High School for Boys (now the Hills Road Sixth Form College) over his strenuous objections against attending a ‘fancy school’—an insult added to the injury of leaving his seaside paradise. Later acknowledging that his education there was excellent, he also evinced a budding skepticism towards institutional self-regard by noting that his high-school friends, many of whom expected to go on to Oxbridge, were no cleverer than his mates at the local grammar school back in Penarth, where it was almost unheard of to even aspire to those preening spires of higher education.

The move to Cambridge was life-changing. Drawn towards biology by a favourite high-school teacher, David considered a medical career. A friend’s father, a large-animal surgeon, would invite him into the operating room to watch procedures on race-horses close up, and a veterinarian cousin occasionally asked him to help out in his clinic with pets brought in for treatment. He was fascinated by these experiences but eventually rejected a future in medicine because he found himself often impatient with the worried pet-owners and imagined that this attitude would carry over to sick patients. Scientific research appeared as an attractive vocation during his high school years; among his school and family friends were the children of three FRS biological scientists, Alec Bangham, Alan Hodgkin, and Richard Keynes. He was deeply impressed by how these ‘dads’ went off in the morning eagerly as if to play rather than work—a novel idea to him. Bangham explicitly encouraged him to consider a career in science, and Keynes offered him a gap-year position at the Babraham Institute.

David entered Trinity College, Cambridge, after a year as a research assistant at Babraham, taking a BA in Physiology and Biophysics in 1969. Cambridge, at that time a venue
of rich ferment in the physiology of membrane transport, nourished David’s growing interest in electrophysiology and ion pumps through lectures from Richard Adrian, Peter Baker, Ian Glynn, Alan Hodgkin, Richard Keynes, and many visiting scientists. Combining these research areas, upon graduation he moved to University College London (UCL) for postgraduate studies in the lab of Rolf Niedergerke. There, he examined the contribution of the Na\(^+\) pump to the electrical behaviour of frog skeletal muscle, showing that the pump current’s hyperpolarizing influence on muscle contractility was more nuanced than the view of the day had it (1)*. During this five-year period, which required him to master demanding electrophysiological manoeuvres under the eyes of a famously perfectionist supervisor, David locked in his natural tendencies towards cunning design, deft implementation, and rigorous analysis of tricky experiments. (For example, in preparing to accurately tare quartz pipettes before filling them with 10 nL samples weighed to microgram accuracy, David would wash his hands thoroughly in acetone to remove any grease, in those days before latex lab-gloves.) One day, Paul Cranefield, a senior cardiac electrophysiologist at Rockefeller University, visited London to deliver a research seminar on Na\(^+\) pump currents in mammalian heart cells. Though Cranefield’s conclusions largely overlapped with the UCL results in frog muscle, they

* Numbers in this form refer to the bibliography at the end of the text.
differed in some respects, a circumstance that evoked tough questions from David. In the long
discussion following, Cranefield invited the young upstart to come to his lab in New York to
sort out their differences, expressing this invitation in the spirit: ‘Well, if you’re so smart, why
don’t you join my lab?’

**ELECTRICAL SIGNATURES OF THE Na\(^+\) PUMP—THE FORMATIVE YEARS**

Thus, in 1975 David moved to New York, a few years in advance of a British brain-
drain provoked by Margaret Thatcher’s devastating cuts to basic science funding. Still
formally a graduate student at UCL, he now switched his investigation of Na\(^+\) pump
currents from skeletal muscle to cardiac cells, bringing to Cranefield’s lab his enhanced
electrophysiological capabilities (fast perfusion, millisecond temperature jumps, single-fibre
dissection and mounting, among other tricks). The sudden move also necessitated a hurried
wedding so that his Belgian/English girlfriend, Patricia Ault, could obtain a residence visa;
throughout their lives together, David and Patricia would toast anniversaries of what they
playfully called their *mariage de convenance*. Patricia became a science journalist, including
a stint as senior editor at *Discover* magazine. David would remain at Rockefeller for the rest
of his life.

The Na\(^+\) pump, otherwise known as the Na\(^+\)/K\(^+\)-ATPase, is a membrane-embedded
enzyme essential for cellular function in metazoans. It moves Na\(^+\) outward and K\(^+\) inward
across plasma membranes against concentration gradients using the energy of ATP hydrolysis
to maintain the electrolyte homeostasis necessary for a panoply of physiological processes.
As such, it is fundamentally essential for cell survival. Since its discovery (Skou 1957) it
had been studied mainly by characterizing fluxes of radiolabelled \(^{22}\)Na\(^+\) and \(^{42}\)K\(^+\) across
membranes of various cellular preparations. These are quite nasty isotopes to work with,
and such fluxes, though technically straightforward to measure, cannot be followed in
real-time assays. In contrast, electrical recording of transmembrane ionic currents has the
advantage of continuous-time observation, although it requires specialized equipment and
expert experimental ‘hands’, and the pump-current must somehow be isolated away from
pervasive background currents—typically much larger in magnitude—arising from other
membrane processes. In his graduate-student years, David had developed ways to record
clean, reliable pump currents, and he continued to improve these measurements at Rockefeller.
After receiving his PhD from UCL in 1978, he was appointed Assistant Professor in
Cranefield’s ‘sphere of influence’, Rockefeller being organized without academic departments
and in those days retaining the very best elements of feudalism.

In his first single-author paper (2), David applied his improved tools to a detailed kinetic
analysis of the Na\(^+\) pump in voltage-clamped cardiac fibres. He blocked the pump current by
removing extracellular K\(^+\), thus allowing Na\(^+\) to leak into the cell for a specified time; then,
upon rapid re-exposure to K\(^+\), the pump, stimulated by the resulting increased intracellular
Na\(^+\) concentration, announced itself clearly. By varying the time of zero-K\(^+\) exposure,
he established a quantitative kinetic K\(^+\)-activation curve for the pump (while cleaning up
an erroneous result from another lab) and showed that the 3Na\(^+\)/2K\(^+\) stoichiometry is a
fundamental parameter independent of the ion concentrations. This paper, which established
David’s signature approach of ‘quantitative electrophysiological enzymology’, launched a
35-year parade of increasingly detailed studies of ion movements into, out of, and through the Na\(^+\) pump.

The year 1980 was an *annus mirabilis* for electrophysiology, as it witnessed the introduction of gigaseal patch-recording (Hamill *et al.* 1981). This breakthrough, whereby a tiny area of cell membrane is sealed to the end of a fine glass microelectrode, hugely expanded the range of cell-types accessible to electrical observation and enabled manoeuvres by which a membrane patch could be completely pulled away from the cell in a flowing stream in either an outside-out or inside-out orientation. Though this method is mainly used to record the large currents carried by ion channels, David quickly mastered it to attack Na\(^+\) pump currents, orders of magnitude smaller. To expand his repertoire, he travelled to Akinori Noma’s lab in Japan to learn how to perfuse both intracellular and extracellular sides of a cardiac myocyte, an exceedingly challenging technique that opened a range of otherwise impossible experiments particularly suited for the pump, whose activity is controlled by extracellular K\(^+\) and intracellular Na\(^+\) and ATP.

With these capabilities in hand, David was poised to examine finer details of the pump’s operation, specifically the ion movements between solution and their binding sites within the protein (3), a problem that had never been attacked before. The trick here was to prevent the pump from turning over fully by removing all K\(^+\) while maintaining Na\(^+\) on both sides of the membrane, so as to study the ‘half-cycle’ of Na\(^+\) ions binding to the pump from one side of the membrane and dissociating to the other side in the reversible process of ‘Na\(^+\)–Na\(^+\)’ exchange’. As expected, zero-K\(^+\) conditions eliminated steady-state pump current. Then, a strong, intracellular-positive voltage pulse elicited a transient outward current, as intracellular Na\(^+\) ions were electrically pushed from solution into their binding sites deep within the protein. This transient current required intracellular ATP, as expected for the Na\(^+\)–bound form of the pump, and was abolished by specific inhibitors. The kinetic results implied that the observed charge movement reflects the key conformational transition of the ‘E\(_1\)–P–Na\(_3\)’ phosphorylated form of the enzyme, whereby three bound Na\(^+\) ions initially in equilibrium exclusively with intracellular solution become occluded within the protein and then subsequently gain access to extracellular solution in the ‘E\(_2\)–P–Na\(_3\)’ form. Quantitative analysis of the voltage dependence of these transients showed that most of this charge movement arises from the release of the Na\(^+\) ions to the extracellular side—a key observation that eventually led to a structural glimpse of the protein. David also used the results to quantify the density of pumps on the plasma membrane from the absolute value of net charge movement during the transient.

**Squid summers in Woods Hole**

Having discovered these ‘ion-displacement currents’ in cardiac muscle, David pursued a quantitatively enhanced analysis of the pump by switching to a higher-resolution system: the classical squid axon preparation, wherein Na\(^+\) pump currents had been studied since the early 1970s at the Marine Biological Laboratory (MBL), Woods Hole, Massachusetts (De Weer & Geduldig 1973). For the next 25 years, a ‘dream team’ of self-assembling Na\(^+\) pump biophysicists would travel to the MBL for summer work to collaboratively exploit the superb sensitivity, time resolution, and intracellular dialysis capability offered by this specialized experimental system: Paul De Weer from Washington University, Robert ‘Bob’ Rakowski...
from Chicago Medical School, Miguel Holmgren from the National Institutes of Health (NIH), Francisco ‘Pancho’ Bezanilla from the University of California at Los Angeles, and David, who owned a cottage in Woods Hole. Reminiscences from some of these colleagues can be found in the online supplementary information. The team would set up their equipment in early spring, when large squid with unusually large axons began to show up in Cape Cod waters, and they would collect data through June, when these squid were replaced by less useful, smaller specimens (as well as by a tsunami of students taking summer courses at the MBL). Tasks were clearly defined. Paul would dissect out the axon; David and Miguel would mount it in the recording chamber and set up the intracellular dialysis system; Bob and Pancho would optimize electronics and acquisition programs to the task at hand and run the experiment, which David and Miguel would then analyse. One or two experiments might be achieved on a ‘good’ day. The remaining weeks of summer were devoted to further data analysis, writing up results . . . and fishing. Bob Rakowski tragically died in 2008, depriving David of a close friend, revered colleague, and expert fishing-buddy. David acquired his own boat the next summer, christening it *Pathfinder*, and, punctilious at home just as in the lab, kept in his cottage a –60°C freezer hooked up to a generator for insurance against power-outages in Cape Cod storms, so as to enjoy fresh bluefish and striped bass filets through the winter (figure 2).

Over the years, as these scientists refined their techniques and pushed time-resolution, the ion-displacement currents progressively brought to light new inferences about conformational changes in the millisecond time domain (3). Eventually they also revealed microsecond-timescale processes producing signals roughly 1000-fold smaller than the background that had to be subtracted to unearth them (9, 17). These turned out to report on the ion movements and protein gyrations essential for transmembrane Na⁺ pumping. With continually improving methods, three distinct, sequential Na⁺ dissociation (‘de-occlusion’) events came into focus, one for each Na⁺ ion leaving its site to extracellular solution in a strictly ordered process—a level of microscopic, dynamic detail unparalleled in any other membrane-transport protein.

One unusual improvement that the team introduced is worth a digression. In 2005, while visiting his parents in Chile, Miguel Holmgren noticed that Humboldt squid (*Dosidicus gigas*) was being sold in food-markets. This was remarkable to him, since this large squid species of the Pacific Ocean (up to 2 m in length and 40 kg in weight) with exceedingly large-diameter axons (>1 mm) had disappeared from Chilean waters in the 1970s, leading to the shut-down of the Montemar laboratory, the only marine lab in the Southern Hemisphere devoted to squid-axon neurobiology. Located on a beach near Valparaiso, this vibrant, productive lab had spawned a school of talented South American biophysicists, many of whom left Chile in 1974 (along with the squid) on what were ruefully called ‘Pinochet Fellowships’, and it had hosted many international scientists chasing squid axons during Northern Hemisphere winters (Bezanilla 2018). Now, 30 years later, the big squid were back, their huge axons offering improved signal-to-noise characteristics. The following December, Holmgren and Bezanilla returned to Montemar to try to record pump currents from these axons. The lab was a horrible mess, having suffered the ravages of time, neglect, dust, ocean floods, and cable-chewing rodents, but the pair managed to spruce things up, bring in electric power, and obtain high-quality preliminary data. An NIH grant to renovate the Montemar lab soon followed, and David joined the group for subsequent winter data-collection trips (figure 3). (His love of the sea propelled him to accompany the fishermen in open boats in the dark at 3 a.m. to catch squid; he’d deliver them to the lab by 7 a.m., and after a quick nap would join the team for the day’s work.) The technical advantages of the Humboldt squid allowed the group to delve more
Figure 2. (a) Piloting *Pathfinder*; (b) *Fishproud* (Credit: Patricia Gadsby). (Online version in colour.)
deeply into pump mechanism: to quantify the thermodynamic landscape of the multiple states of Na$^+$ occupancy and conformations of the pump, and to detect the K$^+$ current transients of the ‘return’ half-reaction of the pump’s full turnover cycle (16).

**Pump-to-channel metamorphosis**

Analysis of the voltage-dependent ion-displacement currents led David to a key structural inference: that the Na$^+$-saturated phosphorylated pump’s outward-facing conformation, E$_2$–P–Na$_3$, opens a narrow aqueous access pathway through which Na$^+$ ions in the centre of the protein dissociate to extracellular solution. This picture had already begun to emerge early...
in the squid work (6), when David explicitly argued that the ‘pump molecule incorporates a structure functionally analogous to an ion channel’. A decade later, he directly validated this idea in a series of beautifully designed experiments that exploited the immense power of single-channel recording to examine the pump (10). To understand these experiments, it is necessary to appreciate David’s favourite cartoon-view of pump proteins, wherein the ion-transport pathway is represented as a transmembrane pore capped by two ‘gates’, one on each side of the membrane. To work properly, the pump must regulate gate opening and closing to strictly forbid simultaneous opening of both gates; otherwise, a huge ion-leak would dissipate the gradients built up by ATP hydrolysis. The two main conformations of the Na\(^+\) pump—inward-facing, Na\(^+\)-binding E\(_1\) and outward-facing, K\(^+\)-binding E\(_2\)—would be cartooned with the intracellular gate open and extracellular closed, and vice versa, whereas in various intermediate, occluded states both gates would be closed.

It had been known for years that a highly lethal marine toxin, palytoxin, causes ion leaks in cell membranes, and that this effect is ‘somehow associated with’ the Na\(^+\) pump (Muramatsu et al. 1988). David established that palytoxin induces cation channels that can be naturally understood in terms of the pump cycle. He showed that the toxin uncouples the two gates such that both can sometimes open together to form a continuous transmembrane pathway through the protein. Millions of Na\(^+\) and K\(^+\) ions per second course through the toxin-modified pump, a throughput so fast—about five orders of magnitude higher than the normal Na\(^+\) pump turnover rate—as to produce readily observable single-channel behaviour. Channel opening was promoted by ATP-dependent phosphorylation in the presence of Na\(^+\), while Na\(^+\)-free K\(^+\) solution would shut the dephosphorylated channel, indicating that the protein, though its gates were discoordinated by toxin, was behaving in an expected way towards its physiological ligands. At this time, no Na\(^+\) pump crystal structure was known, but mutagenesis studies had suggested likely residues directly contributing to ion binding (Nielsen et al. 1998), and homology modelling based on Ca\(^{2+}\) pump structures had inferred the physical locations of the Na\(^+\) pump’s ion-binding sites (Ogawa & Toyoshima 2002). But the ion-access pathways connecting these deeply buried sites to bulk solution were unknown. Over the next few years, David’s lab, using mutagenesis, site-directed chemical modification, and single-channel recording of the toxin-channelized pump, developed an explicit structural proposal for how these pathways worm their way through the protein (12, 13). Five years later, crystal structures of the Na\(^+\) pump appeared (Nyblom et al. 2013; Kanai et al. 2013) that accurately mirrored this proposal based solely on functional behaviour of palytoxin channels.

**CFTR chloride channel**

Cystic fibrosis (CF) is the most common monogenic disease in populations of northern European ancestry. About 4% of UK residents are heterozygotic carriers of loss-of-function mutations in the ‘cystic fibrosis transmembrane regulator’ (CFTR) gene, which codes for a Cl\(^-\) ion channel. Symptoms appear in childhood, primarily as persistent lung infection, inflammation, and ultimately dysfunction; until recent successes in drug discovery, life expectancy of CF patients was on the order of 20 years. The physiological basis of the disease is impaired fluid secretion in epithelia due to low Cl\(^-\) permeability, leading to a thickened pulmonary mucus that prevents adequate clearing of environmental pathogens from the airways.
David fell into the CFTR field by serendipitous circumstances originating in the late 1970s via unrelated research in what was then termed ‘slow signal transduction’ in neurons. At that time a major puzzle in neurobiology was to understand how certain neurotransmitters—dopamine, acetylcholine, GABA, and others—in some contexts operate on timescales of seconds, far longer than the millisecond responses of classical synaptic transmission. It appeared that neurons could modulate their synaptic behaviours by somehow altering the ion channels that produce them, a subject attacked with electrophysiological and biochemical methods by labs such as Eric Kandel’s in New York, Irwin Levitan’s in Basel, Felix Strumwasser’s in Pasadena. In these labs and others, evidence accumulated that these slow-acting, long-lived electrical phenomena might reflect enzymatic modification of neuronal proteins by phosphorylation.

This idea—controversial well into the 1980s—attracted biochemically attuned neuroscientists wielding protein kinases as analytical tools. Prominent among these was Paul Greengard, whose lab at Yale studied phosphorylation of neuronal proteins through the 1970s. Moving to Rockefeller in 1983, Greengard brought with him postdoctoral associate Angus Nairn, a card-carrying biochemist expert in protein kinases. Greengard, always on the lookout for electrophysiologist collaborators to test his kinases in their systems and aware of David’s on-campus proximity, encouraged a long-term pairing of Angus’ nearly unique ability at the time to manipulate protein kinases and phosphatases with David’s fine tools of cardiac electrophysiology honed on the Na⁺ pump. The first harvest of this collaboration—and Nairn’s first project independent of Greengard—implicated protein kinase A (PKA) phosphorylation in activating an unexpectedly prominent modulatory Cl⁻ current in the cardiac myocytes routinely examined in David’s lab (4). Follow-up experiments (5) soon showed that this current resembled the phosphorylation-linked Cl⁻ channels previously known to be absent in epithelial cells of CF patients (Welsh & Liedtke 1986; Frizzell et al. 1986).

During David’s early work on these channels, the gene coding for the Cl⁻ channel defective in cystic fibrosis was identified and named (Riordan et al. 1989). This important advance opened the way for heterologous expression and genetic manipulation of CFTR. It also produced a big surprise: the channel’s predicted amino acid sequence, which showed it to belong to a widespread family of ATP-driven pumps, the ‘ABC transporters’. No ion channel was known in this vast family, whose members move solutes such as nutrients, metabolites, and drugs across cell membranes against concentration gradients at the expense of ATP hydrolysis. All ABC transporters contain a pair of homologous nucleotide-binding domains (N-terminal NBD1, C-terminal NBD2 in CFTR) where the hydrolysis reaction occurs. Moreover, a sequence unique to CFTR positioned between the NBDs was seen to contain multiple consensus sites for PKA phosphorylation; this ‘R-domain’ immediately attracted attention as the likely culprit responsible for PKA activation. With inside-out membrane ‘macro-patches’ exposed to a switchable flowing stream, the Gadsby lab carried out a definitive single-channel study showing that activation of CFTR requires two separate and distinct ATP-dependent processes: a few minutes of phosphorylation on the R-domain by PKA, followed by prompt, reversible opening by ATP that does not require the continued presence of the protein kinase (5).

This inside-out patch system was of key importance because it enabled single CFTR channels to be manipulated with fast solution changes and hence to be analysed by the kinetics of nucleotide-dependent ‘gating’—electrophysiological jargon synonymous with the biochemically familiar phrase ‘the process of switching between non-conducting (closed)
and conducting (open) conformations’. The central question of how ATP opens the pre-
phosphorylated channel raised a binary controversy in the field: does ATP work simply by
reversible binding, as with well-known ligand-activated ion channels, or does hydrolysis of the
nucleotide somehow drive channel gating? Early on, David’s group came down unequivocally
on the side of the latter idea with a blunderbuss of biochemical manoeuvres showing that
whereas ATP must bind for the channel to initially open, inhibiting its subsequent hydrolysis
prevents closing (7, 8). The next decade witnessed his group’s single-channel enzymology
experiments of ever-increasing sophistication (figure 4), as well as the introduction of
heterologous expression and CFTR mutagenesis guided by known structures of other ABC
transporters (11). As the details steadily sharpened with each new publication, it became
clear to the field that channel opening and closing requires a pump-like cycle of nucleotide
binding, hydrolysis, and product release, although controversy on this point continued for
over ten years (Aleksandrov et al. 2007) before fading away. A picture emerged of a channel
endowed with a pump’s molecular personality: the closed channel with its intracellular NBDs
separated from each other binds two Mg$^{2+}$-ATP molecules as the NBDs come together and
dimerize, burying the nucleotides and opening the pore at its extracellular end in a clothes-peg
movement, followed by hydrolysis of the ATP at NBD2 (NBD1 being essentially non-
catalytic) to form a second, distinguishable open state which is unable to close until ADP
and phosphate are released upon re-separation of the NBDs.

Thus, David established CFTR gating as a nonequilibrium process, rationalizing an
early observation that single CFTR channels seemed to violate microscopic reversibility
(Gunderson & Kopito 1995). By 2010 his pursuit of the gating mechanism had allowed him
to infer structural details of NBD movements coupled to pore conformational changes, as well as to quantify the thermodynamics of NBD interactions during dimerization and separation (11, 15). David’s last paper (19), a massive, capstone review of CFTR mechanisms, was written at a time when he could look back over the long trajectory of his CFTR work shortly after Jue Chen’s lab at Rockefeller had produced the first atomic-resolution structures of the channel (Zhang & Chen 2016; Liu et al. 2019). David had the good fortune in his final two years to act as next-door cheerleader for Jue and as her electrophysiological collaborator (18). With Jue as a close colleague, David watched structural flesh growing on the mechanistic skeleton of CFTR that his work had done so much to assemble: the location of the Cl⁻ ion pathway, the NBD movements coupled to gating, intimations of how the dephosphorylated R-domain physically blocks the NBDs from dimerizing and how it gets out of the way upon phosphorylation, and the binding-region for the new CF drugs, which have recently and so dramatically improved the lives of patients. Jue recalls:

After I moved to Rockefeller in 2014, David and I quickly became close colleagues, drawn together by our shared passion for CFTR. His generosity in sharing his knowledge was instrumental to my understanding of CFTR’s function. David would get excited by even the smallest progress we made on the structure front. To me, David was a friend, a resource, and an inspiration. As our understanding of CFTR as a molecular machine in action grows deeper, I miss him more than ever.
At first glance, David Gadsby’s research appears to consist of two separate, independent, long-term projects on the mechanisms of completely different types of membrane transport proteins: the Na\(^+\) pump and the CFTR Cl\(^-\) channel. Indeed, from a thermodynamic viewpoint these two systems are stark opposites, since the pump uses a free energy source to move ions thermodynamically uphill, whereas CFTR, like all ion channels, dissipates ion gradients by allowing the Cl\(^-\) ion to diffuse downhill through a pore. However, David’s approach, which he emphasized throughout his writings, reveals a deep connection between channels and transporters. With a view of a pump always in mind as a channel with two tightly coordinated gates, David showed how to understand the Na\(^+\) pump by analysing it as a channel using palytoxin; and he showed how to understand the CFTR channel by analysing it as an ATP-driven pump that in evolution had lost one of its two gates. This insight—the ‘blurred boundary between channels and transporters’ highlighted in a Royal Society symposium of that title that David helped organize (14)—added generality and further depth to the two systems in his experimental hands.

**David Gadsby: character and style**

In tributes from friends and colleagues, from long-past and recent collaborators, David’s integrity is repeatedly cited as a prominent element of his recognizable footprint on the scientific sands (figure 5). This striking uniformity from independent sources raises the question: what does ‘integrity’ actually mean? It’s a word often heard in appreciation of colleagues upon retirement, on round birthdays, at celebratory symposia, or in memorial essays like this. But it rolls off the tongue a bit too easily these days, I think. Has it become a facile signifier of general praise rather than a shout-out for the specific virtue linked to its proper meaning, a virtue separate from others we could also name in celebrating David’s life? Etymologically, the word denotes wholeness but also carries twin connotative underpinnings: of character uncorrupted by narrow self-interest and of attitude leaning towards truth perceived raw and towards strict objectivity in interpreting that perception. This underscores how David’s tributes apply the phrase ‘a man of integrity’ with a precision congruent to his conduct—at the rig producing new results, at the keyboard birthing a manuscript or on the speaker’s platform conveying a scientific story to an audience, at the editorial-board or study-section meeting evaluating the work of others. In such venues, David displayed an uncompromising wholeness—in always reaching for clean data, in applying severe self-criticism in interpretation, in judging collaborators and competitors equally with his signature rigor accompanied by generosity, open-mindedness, and fairness. His ‘wholeness’ also included an infectious sense of fun—on the tennis court, on Gordon Conference hikes, at oyster-cum-champagne lab celebrations, and at brush-burning weekends on Cape Cod to clean up after the winter storms. Paola Vergani reminisces that

David was first and foremost honest and hard-working. ‘Nothing worthwhile is easy’ was one of his most used quotes. In all he did he never cut corners. He was thoughtful, precise, and careful in every detail, but somehow he managed this without giving up joy and fun. On the contrary, from frigid fishing and oyster-harvesting outings, to multi-day experiments requiring the utmost precision, he sought out the physical and intellectual challenges with a quiet inner
happiness. He was genuinely interested in the people around him—from Nobel Laureate friends to PhD students standing in front of their first posters. He gave time and attention to each, listening intently, remembering previous conversations. He recognized each person’s hard-won achievements whatever their shape and size—from having the courage to tackle a difficult theoretical problem, to successfully training for a marathon, to mastering the art of filleting fish in exactly the right way.

David cared nothing about racking up a long list of publications. Holding himself to high standards, he consciously eschewed contributing ‘yet more noise’, as he put it, to the scientific literature, and he had little patience with colleagues who did so. Over a 48-year career, he published fewer than 75 research papers, a rather short list in his field. But these papers are widely appreciated to reflect a thoroughness that left no stone unturned, an unadorned lucidity in telling a scientific story, and a filleting-blade of pristine logic that would cut through the fog to the essence of the issues at hand. His colleague, Miguel Holmgren, remembers:

I learned from David something that perhaps not many people do in science. He told me ‘When doing an experiment, look at the data coming in and don’t think of anything else—not even the meaning of the data—because you never know when something comes in unexpected. No earbuds, no music, no thinking—just look at the data and see how it comes. You can think later.’

These ‘few’ papers had heft, materially expanding the edge of discovery in membrane biology and recalling Karl Friedrich Gauss’ famous self-assessment of his mathematical output: *pauca sed matura*. And while David’s publication list is short, the list of trainees in his wake, now productive in their own labs, is long. He was most proud of that legacy.

David could be quite hard-headed and impatient about doing things his own way, and he had a healthy skepticism towards authority-figures. Angus Nairn tells a story that illustrates these personality-elements working together outside the lab. About five years ago during the post-squid end of summer in Woods Hole, David took some visiting scientists on a fishing trip to Martha’s Vineyard, a lovely island a few miles across the water from the MBL. Racing his beloved *Pathfinder* at top speed through the waves on a direct diagonal towards a favourite fishing spot, he was stopped by two military-style craft bearing bow-mounted machine guns and manned by armed, uniformed officers. He’d entered a ‘temporary exclusion zone’ and was ordered to back up and follow a circuitous route to his destination. Puzzled and irritated but unfazed, he demanded to know what, exactly, he was being excluded from and if the zone was semicircular, rectangular, or triangular, as he intended to navigate as straight a path as possible to the fish. An officer replied that he should ‘just shut up and turn around’. But David kept on querying the officers and finally got them to explain that President Obama and his family were vacationing on the beach just ahead. He set out along the indirect route specified, but after a short while, frustrated with this inefficient path, turned the boat straight for the fishing-hole, whereupon a second pair of armed vessels stopped him. At that point, grumbling, he decided that it was wiser to follow directions, and eventually the scientists were casting for bluefish.

During the final days of a membrane biophysics meeting in Sicily in the spring of 2017, David mentioned to a few of his fellow conferees that he was feeling ‘a bit dodgy’, probably from food poisoning. But back in New York, an examination found an intestinal obstruction, and two malignant tumours were swiftly excised. Moreover, the cancer had metastasized to his liver, part of which was subsequently removed at New York’s premier cancer hospital, Memorial Sloan-Kettering. Despite this dire circumstance, David faced chemotherapy over the next year and a half with his characteristically insouciant optimism. He declared that his
worst complaint from the treatment was giving up beer, and even as he struggled to regain weight he could be found in the winter of 2018 raking oysters with Patricia in waist-high, icy waters off Woods Hole. During this time, he continued to engage with science in his work with Jue Chen at Rockefeller, as her breakthrough CFTR structures were emerging into the public eye. He’d made a remarkable comeback since his liver resection and felt that he was holding his own against his disease. But in March 2019, following corrective outpatient surgery, he developed the sepsis that killed him. Three months later, preceding an early-morning memorial gathering at the Marine Biological Lab, David’s family and friends guided *Pathfinder* through the roiling tidal waters off Woods Hole to embower his ashes in the sea.

**PERSONAL REMINISCENCES**

Additional reminiscences from his colleagues and friends can be found as online supplementary material entitled *David Gadsby—Personal Reminiscences*. These contributions are by: Miguel Holmgren, Nicolas Reyes, Paola Vergani, László Csanády, Jue Chen, Kouki Touhara and Roderick MacKinnon.

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**AUTHOR PROFILE**

*Christopher Miller*

Christopher Miller is Emeritus Professor of Biochemistry at Brandeis University, where he spent his entire academic career, 1976–2019. His research was concerned with the mechanisms of ion channels, particularly with the basis of ion selectivity, and with secondary active transporters. He developed methods for reconstituting ion channels from biological membranes into synthetic planar lipid bilayers, and he used this system to study their structure–mechanism relations, using a combination of electrophysiological recording, membrane biochemistry, and X-ray crystallography. Though he never formally collaborated with David Gadsby, the two first encountered each other during summers in Woods Hole, when Gadsby was chasing squid axons and Miller was running the MBL summer course in Neurobiology. Miller was deeply involved in the behaviour of CLC-type Cl⁻ channels at the time Gadsby began working on CFTR-type Cl⁻ channels, and so a natural scientific affinity developed that grew into a close friendship.
Biographical Memoirs

References to other authors

Aleksandrov, A. A., Aleksandrov, L. A. & Riordan, J. R. 2007 CFTR (ABCC7) is a hydrolyzable-ligand-gated channel. *Pflügers Arch.* 453, 693–702. (doi:10.1007/s00424-006-0140-z)

Bezanilla, F. 2018 Influences: The Cell Physiology Laboratory in Montemar, Chile. *J. Gen. Physiol.* 150, 1464–1468. (doi:10.1085/jgp.201812157)

De Weer, P. & Geduldig, D. 1973 Electrogenic sodium pump in squid giant axon. *Science* 179, 1326–1328. (doi:10.1126/science.179.4080.1326)

Frizzell, R. A., Rechkemmer, G. & Shoemaker, R. L. 1986 Altered regulation of airway epithelial cell chloride channels in cystic fibrosis. *Science* 233, 558–560. (doi:10.1126/science.2425436)

Gunderson, K. L. & Kopito, R. R. 1995 Conformational states of CFTR associated with channel gating: the role of ATP binding and hydrolysis. *Cell* 82, 231–239. (doi:10.1016/0092-8674(95)90310-0)

Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. 1981 Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* 391, 85–100. (doi:10.1007/bf00656997)

Kanai, R., Ogawa, H., Vilsen, B., Cornelius, F. & Toyoshima, C. 2013 Crystal structure of a Na⁺-bound Na⁺,K⁺-ATPase preceding the E1P state. *Nature* 502, 201–206. (doi:10.1038/nature12578)

Liu, F., Zhang, Z., Levit, A., Levring, J., Touhara, K. K., Shoichet, B. K. & Chen, J. 2019 Structural identification of a hotspot on CFTR for potentiation. *Science* 364, 1184–1188. (doi:10.1126/science.aaw7611)

Nyblom, M., Poulsen, H., Gourdon, P., Reinhard L., Andersson M., Lindahl E., et al. 2013 Crystal structure of Na⁺,K⁺-ATPase in the Na⁺-bound state. *Science* 342, 123–127. (doi:10.1126/science.1243352)

Ogawa, H. & Toyoshima, C. 2002 Homology modeling of the cation binding sites of Na⁺,K⁺-ATPase. *Proc. Natl Acad. Sci. USA* 99, 15977–15982. (doi:10.1073/pnas.202622299)

Riordan, J. R., Rommens, J. M., Kerem, B., Alon N., Rozmahel R., Grzelczak Z., et al. 1989 Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245, 1066–1073. (doi:10.1126/science.2475911)

Skou, J. C. 1957 The influence of some cations on an adenosine triphosphatase from peripheral nerves. *Biochim. Biophys. Acta* 23, 394–401. (doi:10.1016/0006-3002(57)90343-8)

Welsh, M. J. & Liedtke, C. M. 1986 Chloride and potassium channels in cystic fibrosis airway epithelia. *Nature* 322, 467–470. (doi:10.1038/322467a0)

Zhang, Z. & Chen, J. 2016 Atomic structure of the cystic fibrosis transmembrane conductance regulator. *Cell* 167, 1586–1597; e9. (doi:10.1016/j.cell.2016.11.014)

Bibliography

The following publications are those referred to directly in the text. A full bibliography is available as electronic supplementary material at https://doi.org/10.6084/m9.figshare.c.4888500.

(1) 1977 (With P. F. Cranefield) Two levels of resting potential in cardiac Purkinje fibers. *J. Gen. Physiol.* 70, 725–746. (doi:10.1085/jgp.70.6.725)

(2) 1980 Activation of electrogenic Na⁺/K⁺ exchange by extracellular K⁺ in canine cardiac Purkinje fibers. *Proc. Natl Acad. Sci. USA* 77, 4035–4039. (doi:10.1073/pnas.77.7.4035)

(3) 1986 (With M. Nakao) Voltage dependence of Na translocation by the Na/K pump. *Nature* 323, 628–630. (doi:10.1038/323628a0)

(4) 1989 (With A. Bahinski, A. C. Nairn & P. Greengard) Chloride conductance regulated by cyclic AMP-dependent protein kinase in cardiac myocytes. *Nature* 340, 718–721. (doi:10.1038/340718a0)
(5) 1992 (With G. Nagel, T. C. Hwang, K. L. Nastiuk, A. C. Nairn) The protein kinase A-regulated cardiac Cl\(^{-}\) channel resembles the cystic fibrosis transmembrane conductance regulator. Nature 360, 81–84. (doi:10.1038/360081a0)

(6) 1993 (With R. F. Rakowski & P. De Weer) Extracellular access to the Na,K pump: pathway similar to ion channel. Science 260, 100–103. (doi:10.1126/science.7682009)

(7) 1994 (With T. Baukrowitz, T. C. Hwang, A. C. Nairn) Coupling of CFTR Cl\(^{-}\) channel gating to an ATP hydrolysis cycle. Neuron 12, 473–482. (doi:10.1016/0896-6273(94)90206-2)

(8) (With T. C. Hwang, G. Nagel & A. C. Nairn) Regulation of the gating of cystic fibrosis transmembrane conductance regulator Cl\(^{-}\) channels by phosphorylation and ATP hydrolysis. Proc. Natl Acad. Sci. USA 91, 4698–4702. (doi:10.1073/pnas.91.11.4698)

(9) 2000 (With M. Holmgren, J. Wagg, F. Bezanilla, R. F. Rakowski, P. De Weer) Three distinct and sequential steps in the release of sodium ions by the Na\(^{+}/K\(^{+}\)-ATPase. Nature 403, 898–901. (doi:10.1038/35002599)

(10) 2003 (With P. Artigas) Na\(^{+}/K\(^{+}\)-pump ligands modulate gating of palytoxin-induced ion channels. Proc. Natl Acad. Sci. USA 100, 501–505. (doi:10.1073/pnas.0135849100)

(11) 2005 (With P. Vergani, S. W. Lockless & A. C. Nairn) CFTR channel opening by ATP-driven tight dimerization of its nucleotide-binding domains. Nature 433, 876–880. (doi:10.1038/nature03313)

(12) 2006 (With N. Reyes) Ion permeation through the Na\(^{+}/K\(^{+}\)-ATPase. Nature 443, 470–474.

(13) 2008 (With A. Takeuchi, N. Reyes & P. Artigas) The ion pathway through the opened Na\(^{+}/K\(^{+}\)-ATPase pump. Nature 456, 413–416. (doi:10.1038/nature07350)

(14) 2009 (With F. Ashcroft & C. Miller) Introduction. The blurred boundary between channels and transporters. Phil. Trans. R. Soc. Lond. B 364, 145–147. (doi:10.1098/rstb.2008.0245)

(15) 2010 (With L. Csándy & P. Vergani) Strict coupling between CFTR’s catalytic cycle and gating of its Cl\(^{-}\) ion pore revealed by distributions of open channel burst durations. Proc. Natl Acad. Sci. USA 107, 1241–1246. (doi:10.1016/j.bpj.2009.12.1748)

(16) 2011 (With J. P. Castillo, D. De Giorgis, D. Basilio, J. J. Rosenthal, R. Latorre, M. Holmgren, et al.) Energy landscape of the reactions governing the Na\(^{+}\) deeply occluded state of the Na\(^{+}/K\(^{+}\)-ATPase in the giant axon of the Humboldt squid. Proc. Natl Acad. Sci. USA 108, 20556–20561. (doi:10.1073/pnas.1116439108)

(17) 2012 (With F. Bezanilla, R. F. Rakowski, P. De Weer, M. Holmgren) The dynamic relationships between the three events that release individual Na\(^{+}\) ions from the Na\(^{+}/K\(^{+}\)-ATPase. Nat. Commun. 3, 669. (doi:10.1038/ncomms1673)

(18) 2017 (With F. Liu, Z. Zhang, L. Csándy, J. Chen) Molecular structure of the human CFTR ion channel. Cell 169, 85–95; e8. (doi:10.1016/j.cell.2017.02.024)

(19) 2019 (With L. Csándy & P. Vergani) Structure, gating, and regulation of the CFTR anion channel. Physiol. Rev. 99, 707–738. (doi:10.1152/physrev.00007.2018)