IN MEMORIAM

A tribute to Dr Frederick Sachs

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Frederick Sachs, PhD, a world-renowned biophysicist and a State University of New York (SUNY) Distinguished Professor of Physiology and Biophysics at the Jacobs School of Medicine and Biomedical Sciences at the University at Buffalo, died on 27 December 2023. This article presents a brief history of Dr Sachs's scientific career, which is closely related to the focus of this special issue on cardiac mechano-electric crosstalk (Emig et al., 2024) by virtue of his major contributions to our understanding of mechano-electric signalling in the heart and other tissues. In addition, this special issue has come primarily out of research presented at the latest triennial Cardiac Mechano-Electric Coupling and Arrhythmias meeting (https://www.uniklinik-freiburg.de/experimental-cardiovascular-medicine/mec-2022.html), which is a meeting Dr Sachs frequently attended, contributed to, and at which he enjoyed the comradery of fellow researchers of biomechanics.

Dr Sachs began his research career in cell electrophysiology as the leading edge of the ion channel recordings wave occurred, with the advent of the patch clamp in the early 1970s. In 1969, he earned his PhD in physiology from the State University of New York Upstate Medical University under Dr Alex Bortoff (Sachs, 1969, 1976), where he first honed his electrophysiology skills by measuring cable properties of linearly arrayed cultured heart cells. By cutting grooves in the agar on which the cells grew, he pioneered the use of mechanical substrate cues to guide tissue structure formation in a dish. With this new expertise, he joined Dr Harold Lecar's laboratory at the US National Institutes of Health (NIH), and in 1973 showed that noise analysis of muscle whole-cell currents could be used to estimate the unitary conductance of ACh receptor channel openings. These findings prompted him to visit the laboratory of Nobel Prize winners Drs Bert Sakmann and Erwin Neher in the mid-1970s as they were developing the patch-clamp method to record unitary channel currents from membrane gigaseals formed in glass electrodes. Dr Sachs brought this novel technology with him to his first faculty appointment in the Department of Pharmacology at the State University of New York at Buffalo, where he used it to begin studying single channels in muscle cells.

Dr Sachs was fascinated with the gigaseal and spent considerable time studying how to improve seal resistance to achieve lower-noise recordings in the late 1970s to early 1980s. He designed the first programable horizontal electrode puller for fabricating complicated glass pipette tips and worked with multiple glass compositions and coatings, seeking the lowest noise and most stable patches. Using these techniques in 1983, he and Dr Anthony Auerbach were able to show that ion channels had more than one open state by identifying sub-conductance states in ACh receptor channel open events. He incorporated early mathematical models of ion channel behaviour, developed by Dr David Colquhoun of University College London, into the first automated analysis software facilitating rapid determination of single-channel kinetic parameters used to understand protein gating conformational transitions.

In 1984, Dr Sachs made the seminal discovery, reported in *The Journal of Physiology* (Guharay & Sachs, 1984), of mechanically sensitive ion channels, which would define his career and is, arguably, his greatest contribution to science. Through his dogged attempts to improve patch recordings, he and Dr Falguni Guharay observed channel openings that were activated when applying negative pressure to the pipette to

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increase seal resistance. Although mechanically activated whole-cell currents were identified by Dr James Hudspeth in sensory organs, such as inner ear hair cells, Dr Sachs's identification of unitary channel conductances in heart cells demonstrated to the world that, in addition to electrically and chemically activated channels, 'non-sensory' cells also possess receptors that sense when it is mechanically stretched. This opened a new field of research into stretch-activated ion channels [SACs; now more accurately called mechanosensitive channels (MSCs)] in many different cell types. The field expanded rapidly, with studies by Sachs and many others. Three of these early studies by Dr Sachs were published in *The Journal of Physiology* (Guharay & Sachs, 1985; Yang & Sachs, 1990). However, owing to the novelty of the patch-clamp method and the high susceptibility to current leakage when the seal was mechanically stretched, scepticism arose among many in this new community of ion channel researchers about whether MSCs were simply an artefact of patch manipulation. This prompted Dr Sachs to define the electrical and mechanical properties of the patch further. From the 1990s to the early 2000s, he published studies that made major contributions to our understanding of patch membrane–cytoskeleton composite structure, the adhesion energy of the glass–membrane seal and the relationship between the kinetics of the membrane mechanical motion and MSC gating.

As evidence mounted in the early 1990s, the first molecular correlates for these channels started to emerge. The TRP, ASIC and K2P channel families were all shown to produce mechanosensitive currents, albeit most frequently in whole-cell current recordings, where the cytoskeleton is intact. The MSCs can be divided roughly into excitatory (cation selective/depolarizing) and inhibitory (K^+ selective/hyperpolarizing) types. Of these two channel types, the most commonly observed were the excitatory cation selective variety, which seemed to be expressed ubiquitously in every cell type tested. Throughout the 1990s and early 2000s, TRP channels were linked most commonly to the excitatory type of MSCs, but rarely were reported to produce mechanically activated single-channel currents in patches. In 2008, Dr Sachs, with his long-time colleague Dr Phillip Gottlieb, published evidence that exogenous TRP channel expression did not produce the commonly observed MSC currents in patches (Gottlieb et al., 2008), leading to continued concerns about the artefactual nature of cell patches and uncertainty regarding the molecular correlate for these channels. Others continued the search for MSC proteins, and in 2010 Dr Ardem Patapoutian described a new ion channel called Piezo that, when expressed exogenously in cells, robustly produced single-channel currents with properties of the most commonly occurring endogenous MSCs observed in cell patches.

This discovery launched a highly productive time between Drs Sachs and Gottlieb as they used this new gene to publish multiple papers together in the 2010s. By showing Piezo channel sensitivity to GsMTx4 inhibitor (see below), they demonstrated conclusively that these channels were the excitatory conductances that so many researchers had been studying in their patches for the previous decades. They identified amino acids crucial to the channel inactivation property and linked this to the pathogenesis of the rare blood disease xerocytosis, which causes red blood cell dehydration and haemolytic anaemia. They characterized Piezo channel selectivity, activation/inactivation gating kinetics and its sensitivity to pH, peptide inhibitors and cytoskeletal integrity. Their last study together was published in *Scientific Reports* in 2018, where they showed that Aβ peptide could inhibit Piezo channels at astoundingly low concentrations, probably through modulation of cytoskeletal/membrane tension, identifying the channel as a potential point of therapeutic intervention for neurological disorders. Sadly, Dr Gottlieb also died in 2023, 6 months before Dr Sachs.

In the mid-1990s, Dr Sachs, along with Drs Feng Qin and Anthony Auerbach of the Department of Physiology and Biophysics, began developing algorithms using Markov probability theory to create sophisticated models of the kinetic parameters describing single-channel gating behaviour (Qin et al., 1996, 1997). This led to the development of QuB single-channel analysis software, which was refined further in the 2000s with Dr Lauren Milescu, using maximum likelihood estimations to extract these kinetic parameters from macroscopic channel currents. This software was used around the world, and the group was awarded recurring multiyear NIH funding for continued software development and training of others in the use of these analysis methods. This modelling work is likely to have prompted Dr Sachs to investigate gating in other ion channels. From the late 1990s to the 2000s, he published important findings on P2X purinoceptor, K2P K^+ selective, MscL bacterial and ACh receptor ion channels.

It is fair to say that biophysics was Dr Sachs's greatest passion (rivalling even his love for playing the banjo and metal artwork). However, this new field of MSC signalling often turned his attention to the roles of these channels in normal cellular and tissue physiology and pathology. This passion was most apparent in his contributions to mechano-electric coupling in the heart. In 1991, he published the first observations of excitatory MSCs in adult cardiomyocytes (Bustamante et al., 1991), providing initial evidence for the theory that mechanically driven cationic currents in the heart might signal cardiac hypertrophy and its pathological consequences. Throughout the 1990s and 2000s, he published numerous studies describing how MSCs modulate the cardiac electrical activity and Ca^{2+} influx, in addition to their role in heart

disease, including atrial fibrillation, cardiac hypertrophy and cardiac arrest. The investigations were not limited to cardiac physiology. Realizing that every tissue has a mechanical component to normal and pathophysiology, he also investigated and made significant contributions to our understanding of mechanosignalling in endothelium, muscle and glia.

These forays into studying MSC contributions to pathology led Dr Sachs to consider searching for molecular modulators of the channel currents. In the late 1990s, Dr Sachs began seeking pharmaceutical company partners that could help fund the effort to investigate this novel therapeutic target and search for effectors. No established pharma companies showed interest owing to poor recognition of the role of mechanics in pathogenesis and the relatively new (and still disputed) existence of mechanosensitive channels. However, a small biopharma start-up from Utah, called NPS Pharmaceuticals, listened and saw the potential. Thus, in the late 1990s, with minimal funding, Dr Sachs and his long-term collaborator Dr Suchyna decided to start testing for compounds that could inhibit MSC activity using patches from cells in outside-out mode to allow access to the external membrane surface, where drugs would have immediate access to the channels. Dr Sachs had previously reported that elemental Gd^{3+} could inhibit these channels, but Gd^{3+} could not be used as a drug in humans. The funding was insufficient to purchase a large chemical library, and the patch assay was not high throughput. Based on earlier studies showing inhibition of MSCs using tarantula venom, Dr Sachs turned to nature's chemical library. In 2000, using binary fractionation of whole venom, they published the discovery of the most selective inhibitor of cationic excitatory MSCs, called GsMTx4 (Suchyna et al., 2000).

This peptide inhibitor became the basis of numerous studies investigating the functional role of excitatory MSCs in cellular and tissue physiology and to understand MSC gating properties before the discovery of Piezo channels. It soon became the gold standard for identifying MSC activity in disease models. In a pair of 2004 studies co-published in the journal *Nature* by Drs Sachs and Dr Robert Mckinnon, GsMTx4, and peptides like it, were shown to act through their hydrophobic membrane interactions to modulate channel gating (Suchyna et al., 2004) and not by directly binding to the channel, which was the accepted model for antagonist–receptor interactions at the time. By synthesizing a peptide enantiomer of GsMTx4 using all D- (right-handed) amino acids, his team showed that channel inhibition was unaffected, supporting the idea that these peptides act through the membrane, which was confirmed in later studies. In 2011, GsMTx4 was shown to inhibit Piezo channels, and in a 2023 *Nature* report, it was used to compact the enormous arms of Piezo channels, demonstrating that the arms function as local sensors of changes in membrane tension and transmitting this information to the gate (Mulhall et al., 2023).

He foresaw the potential utility of the D-enantiomer of GsMTx4 for drug development (D-peptides are resistant to enzymatic digestion), because for years it remained the only chemical inhibitor of MSCs. From his research into MSC contributions to heart disease and muscular dystrophy (Yeung et al., 2005), he co-founded a company in 2009 called Tonus Therapeutics with his friend Jeff Harvey to investigate GsMTx4 as a treatment for these diseases. The company gained funding for studies showing that GsMTx4-D could reduce infarct size in a mouse model of cardiac ischaemia, and it protected skeletal muscle in dystrophic mice from contraction damage and increased muscle mass after 6 weeks of treatment. GsMTx4 has been shown to impact the progression of many other diseases, including tumour growth, osteoarthritis, sickle cell anaemia, immune cell function in the lungs, neuropathic pain, cardiac hypertrophy, pulmonary hypertension and incontinence.

From his early patch-clamp work, Dr Sachs realized that the cell membrane mechanical properties were reliant on cytoskeletal support and other physical properties of the membrane. In the early 2000s, he started earnestly to consider ways to understand the mechanics of the membrane composite structure more clearly. He published studies on patch disruption with voltage, changing MSC sensitivity following cytoskeleton disintegration, and elevated MSC activity in diseases with a compromised cortical cytoskeleton like muscular dystrophy. He turned to another emerging technique, atomic force microscopy, to begin measuring membrane stiffness and monitoring how this stiffness changed in response to mechanical and electrical perturbations. He published studies on the membrane mechano-electric effect with his graduate student Kenneth Snyder, showing that voltage changes alone could stiffen the membrane. However, he quickly realized that most atomic force microscopy cantilevers at the time were good for working in air to provide exquisitely detailed images of hard surfaces, but were not suitable for rapid time-resolved measurements. This was especially true for measuring soft structures, such as cells, in a viscous water environment. Undeterred by the limitations of the current instrument technology, he guided his graduate student Arthur Beyder to design a conceptually new microfabricate torsion-sensitive atomic force microscopy cantilever with improved dynamics in water to allow visual inspection of very soft cells while scanning (Beyder & Sachs, 2006). To monitor the deflections of these new cantilevers while visualizing the cell, Dr Sachs and his long-term collaborator and engineer-extraordinaire, Dr Stephen Besch, developed a combined optics and laser-tracking system. With these new modifications, he was able to track changes in cell membrane stiffness during swelling

and the electromechanical effects on the patch membrane during voltage changes.

These experiments were fruitful but lacked the ability to monitor cytoskeletal changes non-invasively in the whole cell or over multiple cells simultaneously. Dr Sachs envisioned an optical stress-sensing probe to monitor cytoplasmic mechanics using the change in fluorescence resonance energy transfer (FRET) between a pair of fluorescent proteins attached by a flexible α-helix linker. In 2008, with his graduate student Fanjie Meng, he designed the first genetically encoded optical stress reporter, called stFRET, which could be grafted into the sequence of any cytoskeletal structural protein (Meng et al., 2008). When expressed exogenously in cells, force applied to the host protein would produce a strain on the encoded reporter, changing the distance between the encoded fluorophores and causing a change in the FRET ratio. By transient expression in fibrous cytoskeletal elements, such as spectrin, filamin and actinin, stFRET reported dynamic spatiotemporal strain in single cells. Impressively, it was also grafted into the extracellular matrix protein, collagen, and inserted in the *Caenorhabditis elegans* genome to create a transgenic worm, allowing visualization of mechanical stress in the skin of the whole animal. Optical force reporters have now become tools commonly used by many researchers to study changes in stress in cells and tissues. Over the next decade, Sachs and Meng created FRET pairs with many different linkers to improve the sensor function. This including an ingenious probe called cpstFRET that uses modified forms of fluorescence proteins linked by a short hinge (Meng & Sachs, 2012). When force is applied to the host protein containing cpstFRET, the strain causes the angle between the fluorophores to change instead of the distance. The advantages of the cpstFRET reporter are a higher FRET signal dynamic range and reduced length introduced into the host protein.

Dr Sachs was always developing new tools to facilitate the experiments that he envisioned. For decades, the main method of seal formation and stretching patches required a manometer and mouth suction, producing poorly defined stimuli making MSC analysis difficult. In 2002, Drs Sachs and Steven Besch developed a pressure clamp using a headstage with a piezo crystal valve to switch rapidly between positive and negative pressure (Besch et al., 2002). This is now a device commonly used by electrophysiologists and boasts 0−90% pressure changes of ∼1 ms and the ability to create complex pressure wave forms. The ability to apply square-wave pressure stimuli made analysis of MSC activation/inactivation kinetics possible. In 2005, with Dr Susan Hua, Dr Sachs developed a cell volume sensor by combining the pressure clamp with an optically translucent microfluidic device containing circuitry to measure conductance of the fluid-filled channel (Ateya et al., 2005). When cells were grown in the microfluidic channel, and switchable supply ports to the channel allowed rapid changes in osmolarity to be applied. Cell swelling was monitored by changes in the channel conductivity, providing high-temporal-resolution readout of cell volume changes while monitoring Ca^{2+} changes fluorescently.

The contributions by Dr Sachs to our understanding of mechanosensitive channels, cellular mechanics, mechanical signalling and their role in pathophysiology are incalculable. His conceptual ideas of force transmission through the bilayer and area expansion as a mode of channel gating have inspired many researchers to rethink the role of the membrane as a passive liquid-crystalline sea of lipids. The experimental methods and tools he invented have shaped the careers of researchers worldwide. This short description of Dr Sachs's scientific career cannot capture the full scope of how much his work has impacted our scientific understanding. But I hope this paints the picture of a person with immense curiosity, impeccable vision and an astounding drive to seek a different way. Godspeed Fred, and may the force be with you!

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