Biophysical Reviews
https://doi.org/10.1007/s12551-024-01187-5

EDITORIAL

Biophysical Reviews' "Meet the Editors Series": a profile of Ronald Clarke

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Accepted: 18 March 2024

Abstract
This article of the continuing "*Biophysical Reviews* Meet the Editors Series" introduces Ronald Clarke, biophysical chemist, member of the *Biophysical Reviews* editorial board and current Secretary-General of the International Union of Pure and Applied Biophysics (IUPAB).

In January 2022, I was approached by Professor Tony Watts, President-Elect of IUPAB, to ask if I was willing to serve as IUPAB Secretary-General in place Prof. Juan Carmelo Gómez-Fernández, who unfortunately had to step down due to health reasons. In March 2022, my appointment was confirmed by IUPAB Council. At the same time, I joined the editorial team of *Biophysical Reviews*. In my home country of Australia, I was already very active in the biophysics community, having been a member of the Australian Society for Biophysics since 1999 and the society's treasurer from 2013 to 2019, a time which overlapped with Australia hosting the 18th IUPAB Congress in Brisbane in 2014.

My career in biophysical chemistry began in Adelaide, capital city of the state of South Australia and the city of my birth, in 1980, when I decided to complete the honours year of my Bachelor of Science degree in the laboratory of Dr. John Coates in the Department of Physical and Inorganic Chemistry. My project was an investigation of the kinetics and mechanism of inclusion complex formation of cyclodextrins. John Coates had emigrated to Australia in 1954 from the UK as a PhD student, when his then supervisor Professor Denis Oswald Jordan (referred to as "Doj" within the University) moved to Adelaide from the University of Nottingham to take up the professorship of physical chemistry. Although not widely credited, Jordan played an important part in the history of molecular biology. Together with his colleague Masson Gulland and PhD student Mike Creeth, in 1947, Jordan discovered the hydrogen-bonded base-pairing of the DNA structure (Creeth et al. 1947; Harding et al. 2018). This was five years before Watson and Crick's publication of their double-helical DNA structure (Watson and Crick 1953). Although not cited in Watson and Crick's *Nature* paper, Watson later acknowledged the influence that Jordan's work had on his thinking at the time in his book, *The Double Helix* (Watson 2012). Having narrowly missed a share of the 1953 Nobel Prize in Physiology or Medicine, one can only hope that Jordan appreciated a professorship at the University of Adelaide as a worthy consolation prize.

In Adelaide, Jordan supervised, apart from John Coates, a number of other PhD students who went on to build their own academic careers in biophysical chemistry, and who together established a tradition in the field in Australia. Another of Jordan's students, who began his PhD in Adelaide, was Donald Winzor, the PhD supervisor of *Biophysical Reviews* Editor-in-Chief Damien Hall (Hall 2023). Damien and myself are, thus, academic cousins so-to-speak. In my undergraduate years in Adelaide, 1977–1980, Jordan was still active and I recall having him as my lecturer in

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Published online: 08 April 2024

3rd year quantum chemistry, but after a successful honours year with John Coates, I decided to continue working on cyclodextrins with John for my PhD. At that time, rapid reaction kinetics was still a hot topic, with Manfred Eigen having been awarded the Nobel Prize in Chemistry in 1967 for the development of relaxation kinetic theory and rapid perturbation techniques, such as temperature-jump. At that time, early 1980s, as was common all around the world, the Department of Physical and Inorganic Chemistry still had its own mechanical, electrical and glass-blowing workshops, and John had his own Joule-heating temperature-jump instrument constructed for his laboratory. I still remember the sense of amazement, using this instrument, when, after discharging a capacitor charged to 25,000 V through my sample, in the blink of an eyelid a perfect exponential trace appeared on the oscilloscope screen. In those early days, we had no computers to help collect and analyse our data. I had to photograph the oscilloscope screen with a Polaroid camera, manually digitise the traces and calculate the relaxation time of each individual experiment. Apart from the joy of using the temperature-jump instrument and the pride of mastering its operation, I also found great satisfaction in the systems I was working on. Nowadays, cyclodextrins are very widely used for research purposes as well as for industrial applications, e.g. in the food and drug industries. But at that time, it was quite rare to come across a research paper studying cyclodextrin inclusion complex formation. A system that I found particularly intriguing was the complexation of the azo dye methyl orange by γ-cyclodextrin, a bucket-shaped molecule consisting of eight glucose units in a ring. I found that the γ-cyclodextrin cavity was large enough to simultaneously enclose two methyl orange molecules. The exciton interactions between the two dye molecules produced particularly large UV–vis spectral changes and temperature-jump traces with a very high signal-to-noise ratio. The biphasic concentration dependence of the relaxation times that I observed was also very unusual, with an increase followed by a decrease in reciprocal relaxation time with increasing cyclodextrin concentration. But after extensive reading of books by Bernasconi and Czerlinski on the theory of relaxation kinetics, I was able to develop a reaction mechanism and derive a corresponding mathematical equation that successfully described the behaviour I'd observed. As far as I'm aware, this was the first time that anyone had observed a 2:1 guest:host complex of the cyclodextrins. The satisfaction that I achieved in this early success (luck also no doubt played a big part) gave me the confidence and drive to continue with a scientific career in the field of biophysical chemistry.

After completing my PhD, having studied German at high school and having a passion for the German language, I decided that I would do a postdoc in Germany. Even during my PhD, as I was writing my thesis, I read many German papers, e.g. published in *Annalen der Chemie* or *Chemische Berichte* from the first half of the twentieth century describing research into the structure of the cyclodextrins, and at the beginning of my thesis I included a quotation in German from Goethe's *Farbenlehre*. Initially, I was considering going to Göttingen to the Max-Planck-Institute of Biophysical Chemistry to the laboratory of Manfred Eigen. But after reading a review in *Angewandte Chemie* on the application of rapid reaction techniques to biological membrane systems, published by Peter Läuger of the University of Constance, I decided to apply to him. Herr Läuger quickly replied with a very kind letter, offering me a place in his laboratory, but recommending that I apply for a scholarship from the Alexander-von-Humboldt Foundation. I followed his advice, was successful, and in 1987 I moved from Australia to Germany. After a German course at the Goethe-Institute in Munich, I relocated to Constance and began working with voltage-sensitive dyes on the Na^+,K^+-ATPase. I still consider Peter Läuger as the major inspiration for my career and my most important mentor. He was a brilliant theoretician, but still with a strong connection to experiment. Sometimes when I was stumped and couldn't understand a result, I'd have a discussion with Herr Läuger and the next day he'd come with several pages of beautifully hand-written theory explaining my observations. It was a tragedy for the field of biophysics that he died still so young in a mountain-climbing accident in the Andes in 1990. The inspiration that I gained from working with him over those few years, however, made me decide that membrane biophysics was the field to which I wanted to dedicate the rest of my career.

For a successful scientific career, one needs a lot of luck and I certainly had that. One day when I was sitting at my desk in Constance, out of the blue I got a phone call from the UK. It was Professor Brian Robinson from the School of Chemical Sciences at the University of East Anglia in Norwich. Brian had been one of the examiners for my PhD thesis. He told me that he had some scholarship money from the Leverhulme Trust and he was looking for someone to give it to, so he thought he'd give me a try. Never in my life have I got a job more easily. So, in 1989, after a Christmas holiday back in Adelaide to visit my family, I moved to Norwich. There I continued my work in rapid reaction kinetics, this time using the stopped-flow technique, investigating the interaction with membranes and voltage-sensing mechanism of oxonol dyes. While I was there, a good friend of Brian's and another rapid reaction expert, Professor Josef Holzwarth, from the Fritz-Haber-Institute in Berlin came to visit. Josef was looking for postdocs to build up his group and he asked me whether I'd like to come to Berlin. Because of my passion for Germany and the great time I'd had in Constance, I jumped at the chance. So, in 1990, it was planned that I would come to work in Berlin. However, before moving to Berlin, the whole world changed.

I thought I was going to live in a divided city, but before I got there, first Hungary opened its border to Austria, and then on the 9th of November 1989, while I was in holiday in Adelaide, the Berlin Wall fell. For a time, Berlin was in complete chaos, and I didn't know if I was going to get my visa in time to get back, but Josef showed a lot of initiative in cutting through red tape and finally was able to get me the necessary paperwork that I could arrive in Berlin on time as planned in the winter of 1990. It was a great privilege to live the first five years after the fall of the wall in Berlin, to experience German reunification firsthand and to explore countries previously hidden behind the Iron Curtain, which I never thought would be possible to visit. Although I was a foreigner, at that time in Berlin, I was accepted as a citizen of Berlin, on the same footing as any German, just like in JFK's famous speech "Ich bin ein Berliner." Everyone was in the same boat. Nobody knew what was on the other side of where the Wall had been, and everyone was equally lost. Although I was an Australian, I was often asked by Germans for directions in those days.

After 5 years working in Berlin in the department of future Nobel Prize-winner Gerhard Ertl, in 1995, I moved to the Max-Planck-Institute of Biophysics in Frankfurt to the Department of Biophysical Chemistry, led by Ernst Bamberg. Ernst Bamberg had been a PhD student of Peter Läuger's at the University of Constance and this connection no doubt helped me to secure the position in Frankfurt. In fact, before he died in his tragic accident, Peter Läuger wrote a glowing recommendation for me, which I was able to give to Herr Bamberg. In Frankfurt, I introduced the stopped-flow technique to the lab and embarked on a project, lasting many years, to resolve the kinetics and mechanism of the entire ion pumping cycle of the Na^+,K^+-ATPase. To resolve the individual reaction steps of the cycle, I used a fast voltage-sensitive fluorescent styrylpyridinium dye, RH421, which responds to changes in the local electric field in the membrane surrounding the protein (Kane et al. 1997, 1998). Dyes of this type had been introduced to the ion pump field ten years earlier (Klodos and Forbush 1988) by Irena Klodos of the University of Aarhus, where the discoverer of the Na^+,K^+-ATPase and 1997 Nobel Prize-Winner for Chemistry, Jens Christian Skou, was also working. In parallel with my work on the Na^+,K^+-ATPase, while I was in Frankfurt, I also worked on the use of styrylpyridinium dyes for the detection of the membrane dipole potential (Clarke and Kane 1997; Clarke 1997). For a biophysical chemist, working at the Max-Planck-Institute of Biophysics was like paradise. There I had virtually complete freedom in my research. The funding available to do research was seemingly limitless. Whatever experiment one imagined, money was never an issue. At the time, I probably didn't appreciate this, but now, having worked for 25 years in Australia at the University of Sydney under the Australian funding model, I certainly do.

In May 1999, I was appointed as a Lecturer in the Division of Physical and Theoretical Chemistry at the School of Chemistry, University of Sydney. In Sydney, in addition to my research, I'm now heavily involved in the teaching of undergraduate students. In teaching, particularly with large first year classes, I enjoy doing demonstrations and putting on a performance for the students. My favourite topic for teaching is quantum chemistry, although I've rarely applied it in my own research. The reason for my love of quantum is that it gives me the opportunity to play music for my students. Ever since I was at high school I've played French horn, and while I was living in Berlin, I was able to buy a double horn at a bargain price before the currency reform and the abolition of the East German mark. Music is the ideal way to teach quantum chemistry because the harmonics of a musical instrument are analogous to the energy levels of an electron within an atom. As soon as the wave nature of the electron was discovered by de Broglie, the electronic energy levels made sense. Standing waves, such as an electron bound to a nucleus or a sound wave resonating within the French horn, can only have discrete allowed frequencies. This can easily be demonstrated by blowing into the horn. On a natural horn, only the notes of a harmonic series can be played. The ability to play an entire chromatic scale only became possible with the invention of the valved instrument in the early nineteenth century.

In Sydney, I've continued my research into the biophysical chemistry of membranes and membrane proteins. I continued the project, started in Frankfurt, of resolving the kinetics of the Na^+,K^+-ATPase (Mares et al. 2014; Clarke et al. 2013), but in recent years I've turned my attention more to the probably more medically relevant question of the regulation of ion pumps. Research of which I'm most proud is the uncovering of the role of the intrinsically disordered N-termini of ion pumps in their regulation. In a recent review published in this journal (Clarke 2023), I summarised the evidence for an electrostatic switch mechanism, whereby an electrostatic interaction between the positively charged N-terminus and the negatively charged cytoplasmic surface of the membrane is switched on and off by the phosphorylation of conserved serine and tyrosine residues of the N-terminus by protein kinases.

I feel very privileged now to have the opportunity to serve the international biophysics community as Secretary-General of IUPAB. As you can see from this article, I have strong international connections. Apart from being a member of the Australian Society for Biophysics, I've been a member of the Biophysical Society since 1995 and a member of the Deutsche Gesellschaft für Biophysik also since the 1990s. In addition, I've spent time teaching physical or biophysical chemistry in Vietnam (Hanoi) and Israel (Beer-Sheva). Over the years, I've had research collaborations with scientists from many countries, including The Netherlands, Denmark, France, Italy, the USA, Japan, Argentina, Bangladesh, and The Sudan. I look

forward to continuing to serve the international biophysics community in my role with IUPAB and as an editorial board member of *Biophysical Reviews*, and this year, in particular, I look forward to the 21st IUPAB Congress in Kyoto in June.

Author contribution Not applicable.

Declarations

Ethics approval Not applicable.

Conflict of interest The authors declare no competing interests.

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