Nick Dixon.

On Being at the Right Place at the Right Time

We used to think success in research depends as much on luck as hard work, and those who worked hardest were the luckiest… Nick Dixon wonders if this is still true.

The University of Wollongong (UoW) sponsors an annual series of lectures in its Research Showcase Series, and the lectures are often accompanied by biographical articles in the local newspaper. Last year, the *Illawarra Mercury* asked me about the linearity of my career and my 'Eureka moments'. This led me to reflect on how fortunate I am, so often to have been at the right place at the right time.

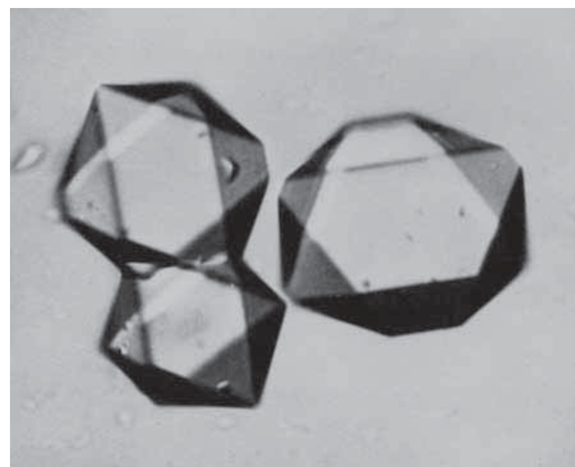
At UQ, 1973

My first Eureka moment came very early in my career, and the publication this year of the structure of jack bean urease (*J. Mol. Biol.* **400**, 274), the first enzyme to have been crystallised (85 years ago!), makes it timely to reflect on it. I did my PhD studies in the mid-70s on this venerable enzyme under the supervision of Burt Zerner and Bob Blakeley in the Department of Biochemistry at the University of Queensland (UQ). Burt had returned to UQ in 1963 after postdoctoral positions with Frank Westheimer at Harvard and Myron Bender at Northwestern, where he had done some of the very best classical enzymology on the chemical mechanisms of acetoacetate decarboxylase and chymotrypsin. One of the enzymes he chose to work on at UQ was urease, the first enzyme shown to be a protein by James B. Sumner in 1926 (*J. Biol. Chem.* **69**, 435). Work in the intervening years had shown urease to be one of the most efficient enzymes known, in spite of working on an extremely stable substrate, so understanding its mechanism promised to teach new chemistry about how enzymes work. The non-enzymatic decomposition of urea to ammonium cyanate was very well understood, but Blakely and Zerner were the first to show that the enzymatic reaction was different; urea was hydrolysed to ammonium carbamate as the first product.

A Nickel Metalloenzyme

I had begun to work on urease as an undergraduate, and in my Honours year in 1973, I set out to titrate its active sites using radiolabelled inhibitors. Urease was purified from jack bean meal, recognised by Sumner to be an unusually rich source of the enzyme. We isolated crystalline urease essentially as he described, extracting it with warm 30% acetone, filtering the extract and cooling it for a couple of days in the cold room. The octahedral crystals were collected by centrifugation, and then resolubilised by extracting the pellet repeatedly with a neutral buffer. A modern step, gel filtration, yielded the highly purified enzyme. I managed to extract urease with a high and more reproducible yield. Bob (Blakeley) asked me how I did this, and I told him I just kept extracting the pellets until the green colour disappeared. Being colour-blind, he couldn't have known that urease was pale green! I went on to show that the colour changed when the enzyme was treated with inhibitors and that the rate was the same as for loss of activity. The colour clearly had something to do with the enzyme's active site.

I set one of my first goals in my PhD to find out why urease was green. Students and staff in the Zerner group in those years included many who have gone on to distinguished careers (John de Jersey, Susan Hamilton, Hugh Campbell, Michael Berndt, Peter Riddles, and others). We were all encouraged to take ownership of our projects, and to work hard to answer questions incisively.



Crystals of urease (circa 1976).

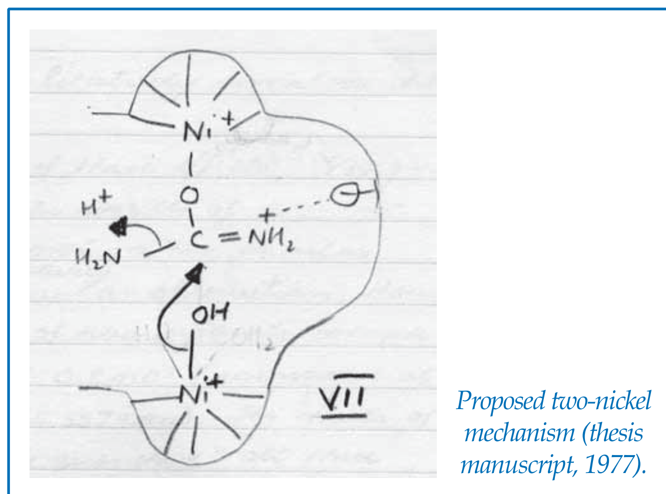
An obvious hypothesis was that urease contained a prosthetic group; it was not really a pure protein as Sumner had proposed. Perhaps it contained a metal ion. Some old literature taught us that it could be irreversibly inactivated by EDTA at low pH, so I abused precious urease samples for various times with EDTA, dialysed them back to neutral pH, removed the copious precipitates of protein I had destroyed, and assayed the supernatants for protein and enzymatic activity. We sent the samples off to the office of the Queensland Government Analyst for metal ion analysis by atomic absorption spectrometry, with a prioritised list

of metal ions – iron, copper, manganese, cobalt, etc. – and Burt randomly renumbered my samples to make collusion impossible. Nickel was on the list, near the bottom, to be analysed only if sufficient sample remained.

Burt took a short break to visit his brother in Goondiwindi, and ended up being stuck in a flood. Meteorological records show this was probably in November 1974. While he was away, the analyst called – he had found insignificant amounts of our high priority metals, but since he had the nickel lamp already on the instrument, he looked for nickel anyway. I was momentarily annoyed at his having wasted precious materials, but he assured me that my samples contained high and variable amounts of nickel. I couldn't interpret the data without Burt's secret key, but when we eventually sat together and could work out nickel/protein ratios and their relationship to activity, the data showed direct dependence of specific enzymatic activity on nickel content, with two nickel atoms per active site in the fully active enzyme. Eureka! In 1975, we reported urease to be the first discovered nickel metalloenzyme in a letter to the Editor of the *Journal of the American Chemical Society* that continues even now to be highly cited. I sometimes wonder how much more effort it would have been if the analyst had followed our instructions!

Binuclear Metalloenzymes

As usual in science, new discoveries provoke more questions than they answer. I went on to probe the mechanism of urease with the chemical tools available to us, and eventually proposed a novel mechanism that involved both metal ions in the chemistry – this was the first enzyme mechanism that used a binuclear metallocentre, where one metal activates the substrate while the other provides a nucleophilic hydroxide. This turned out not only to be essentially how urease works, but also how binuclear metallocentres work in many other hydrolases (including many nucleases and protein phosphatases), and also in RNA and DNA polymerases. Subsequent crystal structures of bacterial ureases coupled with mechanistic studies show it is at least as devious as Burt Zerner had intuitively believed – once urea is bound between the two nickel ions buried in a deep cavity at the active site, it is destabilised by binding to the nickel ions and really has no choice but to be hydrolysed by attack of a single water molecule coordinated to one of them.



Proposed two-nickel mechanism (thesis manuscript, 1977).

At ANU, 1978

I became fascinated by the ways metal ions promote enzymatic reactions. Although I had studied physical and organic chemistry, inorganic chemistry was largely a mystery to me. For the urease mechanism, I had drawn heavily for chemical precedent on the work of Alan Sargeson at the Research School of Chemistry, ANU. Alan (along with his colleague David Buckingham) had the insight that chemistry organised about substitution-inert cobalt(III) centres could be used to model how other metals promote enzymatic reactions. So I joined the Sargeson group to learn coordination chemistry (1978–81). I especially wanted to demonstrate hydrolysis of urea at a binuclear Co(III) centre, but urease was rather special, so modelling it this way was never going to work. Nevertheless, we did manage to develop new methods to make Co(III) and Rh(III)-urea complexes and studied their very interesting coordination chemistry and reactivity. I had worked hard and had been lucky, and had by this stage published a long series of first author papers. This apparently made me quite competitive to win fellowship support that would keep me in useful work for some years to come.

The ABS Annual Conference, 1979

Burt Zerner presented our work on urease as his Lemberg Lecture at the ABS (now ASBMB) meeting in Canberra in 1979. Another highlight of that meeting was the plenary lecture by Arthur Kornberg, who described his new model for the complex series of events that occur during DNA synthesis at replication forks in *E. coli*. The model was derived from studies of the *in vitro* replication of the single-stranded DNA of bacteriophages M13, G4 and ϕX174 by purified host-derived protein reagents.

At Stanford, 1981

After hearing Kornberg's lecture, and with strong encouragement from Zerner, I sought a postdoctoral position in Arthur's lab, and accepted Fulbright and C.J. Martin Fellowships to join him at Stanford. Thus began my work on bacterial DNA replication that has continued for almost 30 years. Kornberg's interest was to show which proteins act when and where in the replication process, and he confessed to me much later that he would not have started that work if he had known how complex an undertaking it would be.

A few weeks before I arrived with my young family at Stanford in July 1981, Bob Fuller had made a major breakthrough, managing to convince a crude cell extract to copy the double-stranded DNA of plasmids that contained *oriC*, the chromosomal origin of replication. I worked closely with Bob and Jon Kaguni to identify additional protein factors required for faithful initiation at *oriC* and subsequent coupled leading- and lagging-strand DNA replication at true replication forks. We would meet together with Arthur every morning to map out the assays to be done each day, and who would do which of them. This complemented other work in the group by Peter Burgers and Mike O'Donnell on the constitution and mechanism of the multisubunit chromosomal replicase, DNA polymerase III, and led to many lifelong friendships as we each went on to establish

our separate research groups working on complementary aspects of what has turned out to be a beautiful and perfectly orchestrated, but extraordinarily complex process.

At JCSMR, ANU, 1983

Although I was at Stanford during the exciting period of discovery and early development of recombinant DNA techniques, I had not actually ever cloned a gene or made a plasmid to direct overproduction of a protein when I returned in late 1983 as a QEII Fellow to the John Curtin School of Medical Research at ANU. There, I formed affiliations with the groups led by Frank Gibson, Graeme Cox and Harry Rosenberg. While I continued to search for new proteins required for *oriC*-dependent DNA replication, I also learned skills in molecular and classical bacterial genetics from them that enabled me to set ambitious goals.

I saw the opportunity to use the *E. coli* DNA replication complex (or replisome) as a model system for study of dynamic multiprotein assemblies, and wanted to focus my research on the mechanisms of the individual proteins and the chemistry that underpins the interactions among them and with DNA templates and nucleotide cofactors. That meant we needed ready access to more than 30 different proteins, many of which were expressed from yet unidentified genes and were purified in minute amounts from wild-type *E. coli* extracts. But how to find a position that would give me the time and resources needed to develop such an ambitious project?

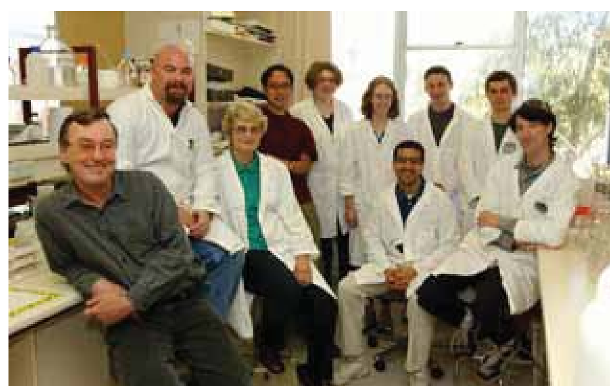
At RSC, ANU, 1986

The Research School of Chemistry (RSC) at ANU had undergone a recent review that recommended it establish new research groups, one of which should be in biological chemistry. I didn't really see myself as a chemist, but was encouraged to apply and was eventually successful. The appointment process was a protracted one, probably because 'biological chemistry' meant different things to different people, but in any case, they ended up with me as the first appointee to a biological chemistry position at an Australian school of chemistry. Part of my brief at RSC was to bring the chemists and biologists at ANU closer together. It was about 10 years before I eventually gave up trying to do this, but that's another story.

In the mid-80s, the RSC was a very fortunate place to lead a research group. We had very limited access to external grants, but were able to build productive research teams with competitive internal funding. I saw it as a unique opportunity, not available to others dependent on the uncertainties of ARC or NHMRC support, to build a program that would take some years (with few publications) to get off the ground. As well as expressing genes and purifying proteins, we also set about crystallising the enzymes for structure determination. Only later did we realise we needed to identify and trim off flexible regions, or to break them up into smaller domains, to succeed with structural biology. At the time, we thought of proteins as rigid structures, and only later came to realise that many proteins (or parts of them) only become structured as they function. We now at last have good structural models of every one of the replisomal components, though not yet of the whole assembly.

As we developed our capabilities and understanding of which proteins talked to which, and when, during the cycles within cycles of events that occur at replication forks, we also developed very strong interactions with technique-based collaborators who saw our protein interactions as very good ones for application and development of their often novel methods. Many of these collaborations are still active and productive, most notably those with Gottfried Otting (RSC) in protein NMR, David Ollis (RSC) and Aaron Oakley (now at UoW) in protein crystallography, Jenny Beck (UoW) in mass spectrometry of protein complexes, Thomas Huber (now at RSC) in computational methods, and Antoine van Oijen (Harvard, now Groningen) in single-molecule DNA replication techniques.

Funding of the Institute of Advanced Studies at ANU, at least in real terms, leveled off during the latter half of the '90s, at a time when I really wanted to exploit the strong research program we had worked so hard to develop. I was again very fortunate: from 2002, ANU gave up part of its budget to gain entry to ARC and NHMRC funding. The timing of this was perfect for us, and our continued success with applications to these agencies has enabled our work to prosper, along with that of our many collaborators.



The Dixon group at ANU in early 2002.
From left: Nick Dixon, Robert Wood, Penny Lilley, Kiyoshi Ozawa, Karin Loscha, Tanya Ronson, Samir Hamdan, Philip John, Pavel Prosselkov, Mark Mulcair. Robert, Tanya and Philip were ANU Summer Scholars.

To Wollongong, 2006

By 2006, I had spent pretty much all of my working life as an employee of ANU. It was time for a change. I was encouraged by the DVCR, Margaret Sheil, to join the University of Wollongong. My brief at UoW is once again to strengthen and promote research at the interface between the disciplines of chemistry and biology, and the greater involvement of undergraduate and Honours students in our research is adding a new dimension to what we do. My good fortune continues – I am especially grateful for the award of an ARC Australian Professorial Fellowship that enables me still to devote most of my time to research.

Although I an very grateful for the opportunities afforded me at ANU, I should note that considerable pressure comes with being judged only on research performance, and it can be difficult to balance resulting demands with others we meet in life. My solution is to work hard to target important

questions, and to nurture productive collaborations. In my generation, those who worked hardest were usually the luckiest. I am not certain this is generally true today. All of the early- and most of the mid-career researchers I know deserve more luck – we all need to continue to try to convince government to provide more resources to exploit your enormous talents.