Biophysical reviews ‘meet the editor series’—Jeremy R. H. Tame

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As an editor of *Biophysical Reviews*, I have been asked to provide readers with a short description of my career in science. The opinions expressed are entirely personal.

I was born not very far from London, in the county of Surrey, the old Anglo-Saxon *Suth-rig*, or ‘Southern Kingdom’ in modern English. I knew I was a scientist from the age of about seven. It was the habit of taking things apart to see what was inside mainly, although later my interests turned towards making loud explosions with a variety of homemade fireworks. For my eighth birthday, my parents bought me a chemistry set, and for my 11th birthday a large bottle of concentrated sulphuric acid. My experiences with practical chemistry and amateur ballistics helped me through the Cambridge entrance exam, so that by the tender age of 17 I found myself enrolled in the Natural Sciences programme (or Tripos in Cambridge-speak). While my focus was initially on chemistry, the Cambridge system requiring first-year students to study diverse topics introduced me to geology and cell biology. That year, 1983, saw the first publication of the now legendary Alberts et al. text-book on molecular biology, a subject almost entirely new to me. The supervision system at Cambridge means that students in pairs visit a specialist supervisor for each course, once a week for hour-long discussions of the lecture material. By complete luck, my supervisor in *Biology of Cells* happened to be Tim Hunt, then a fellow of my college (Clare), and in the middle of the research that would bring him a Nobel prize almost two decades later. Tim was a huge influence and inspiration, leading me to take biochemistry in my final year. One of the lecturers was Max Perutz, who had retired a few years earlier as Director of the Laboratory of Molecular Biology (LMB) at the edge of town. Perutz had won his own Nobel prize in 1962, for solving the structure of haemoglobin (Hb) by X-ray crystallography, alongside Watson and Crick

who worked in his laboratory on their model of DNA. At the end of Perutz’s last lecture of the course on protein structure, I went to speak to him overcome with excitement and burst out ‘Max, can I work for you?!’ He was surprised but amused by an unknown 20-year-old addressing him by his first name off the bat, but sufficiently impressed by my enthusiasm to offer me an interview. He introduced me to Kiyoshi Nagai, (Fig. 1) who was then working on making Hb in bacteria, and creating artificial mutants. Afterwards he promised me a place to study for a PhD at the LMB if my degree proved acceptable. It did, and my daily bicycle commute stretched to four miles down Hills Road everyday to the far end of the known Universe.

While Perutz kept his promise, it turned out that his ideas for funding me had fallen through. His ‘photocopying expenses’ sustained me for about six months while a more permanent solution could be found. By pulling a few strings he eventually made me a more regular student, with a stipend of 2500 pounds a year. It was the time of the Big Bang in London, the massive deregulation that pulled into the banking system thousands of graduates who could count to ten, for starting salaries ten times my income. But I did not envy them in the slightest, and with all my financial worries out of the way could focus on doing some real science. Within a few months though, I became convinced that one of Perutz’s pet ideas was wrong. He had published a paper in 1983 on the working of crocodile Hb, suggesting that its unique bicarbonate ion sensitivity was due to bicarbonate binding at the site where di-phosphoglycerate (DPG) binds human Hb (Perutz 1983). My own study of the protein sequences suggested a totally different site with three adjacent positively charged residues, and I took Perutz to his large wire model of the protein to explain my idea. He was not patient with me. He had a lot of letters after his name, including OM,¹ and I was not yet 21. He left with the words ‘Nonsense. Absolute nonsense’. A description was written in the last chapter of my thesis though, just in case.

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¹The British monarch awards the Order of Merit to up to 24 living individuals, who have distinguished themselves by their achievements and public service.

Fig. 1 The author with Kiyoshi Nagai in 2017

Being very young, I never appreciated the people around me in Cambridge at the time. In my first years at Clare the Dean was Arthur Peacocke, a renowned theologian who had made early progress towards the structure of DNA using analytical ultracentrifugation (AUC). I ended up buying Peacocke's book on the osmotic pressure of biological macromolecules years after he died, instead of simply knocking on his door when he was alive. He was replaced as Dean by Rowan Williams, a polyglot who went on to become Archbishop of Canterbury. I simply took it for granted that these people were around, and went to tea everyday in the canteen with Nobel prize winners. Fred Sanger had two. My X-ray crystallography teachers included some of the old masters like Max Perutz himself, and Giulio 'Judd' Fermi, son of the great Enrico. It just felt normal at the time, I was so oblivious of my extraordinary luck.

Cambridge made me. Or rather Kiyoshi Nagai made me. His revolutionary work was aimed not just at further understanding how the Hb molecule takes up and releases oxygen, but towards making artificial blood substitutes, still a very important field in medicine. My PhD research extended his work to make mutations in the α chain as well as the β chain, and carried out all sorts of analyses of the products, showing the α and β subunits behave quite differently (Imai et al. 1991; Olson et al. 1988). Kiyoshi had a group of two at the time — me and him. We worked closely together at the bench, and he taught me how to handle DNA and proteins, and the tricks of dealing with haem proteins like Hb. My work included showing how some birds have evolved Hb with higher oxygen affinity to allow them to fly over the Andes or the Himalayas. The changes to the molecule are tiny, but the effects on the organism are enormously important (Weber et al. 1985). My PhD viva took place a few days before my 24th birthday, shortly after the wall fell in Berlin.

Initially my thoughts were to stay at the LMB to study crystallography further, as I was still very much an amateur at that stage, but there was also by this time a need to travel abroad. Seven years in Cambridge had done me very well, but there was more to explore outside. I was interested in languages and applied to several positions in Europe. Sadly the responses were largely all of the same kind — we'd love to have an experimentalist who can make pure proteins for us and crystallise them, and then you can watch while we solve the structures! At the time, many crystallography groups were entirely dependent on someone else providing suitable samples, but it didn't look much fun to become someone's technician. In the end, my best offer came from York, where the group of Guy Dodson was also dabbling in Hb. Guy was an external examiner for my PhD, and thought someone with my skill-set might be an asset.

Guy was a professor in the Chemistry department, where he founded and ran the York Structural Biology Laboratory, having worked in Oxford with Dorothy Hodgkin. Guy's wife Eleanor remains to this day a leading light in the CCP4 project, which creates open software for solving X-ray structures. My project was the bacterial protein OppA, which had been cloned almost by accident by a group in the Biology Department at York. The protein has the remarkable property of binding tightly to all sorts of peptides between two and four residues long, with very little regard to sequence, which remained completely unexplained without a molecular model of its structure. The YSBL had obtained a crystal some three years before, and collected a data-set which remained unsolved.

My training with Kiyoshi really paid off. The OppA being made in York was not very pure, and no attention was paid to whether or not it had any ligand bound. Using a long wash in acidic buffer to remove peptides, and testing samples were uniform by iso-electric focussing, proved key to reliable crystallisation. By adding back synthesised tripeptides, pure protein-peptide complexes could be made for crystallisation trials. This next problem also proved hard — OppA by this time had a reputation as a sticky protein in every sense. Crystallisations were in those days carried out entirely by hand, and my days were filled with micro-pipetting. I started playing with additives, and worked my way through the periodic table to see if it would help. With the addition of uranium — bingo! Beautiful crystals appeared that diffracted to atomic resolution. OppA is about 57 kDa in size, and the resolution of 1.2 Å was a world record at the time for such a large protein. As a bonus, the uranium ions were holding the new crystal lattice together, so that the structure could be solved using multi-wavelength anomalous diffraction (MAD). The X-ray detectors of the time (film in the early 1990s) were very poor by today's standards, and the weak anomalous signal was often lost, but uranium has very large anomalous scattering that can be

exploited using synchrotron radiation. Getting synchrotron time was almost impossible though (Diamond was not yet out of the planning stage). The synchrotron at Daresbury had little interest in anomalous scattering, and the beam-line it had built was very sub-optimal because it was also intended for Laue diffraction, a faddish idea that never gained much traction. It took over two years to get proper beam-time for the OppA experiment, but in the meanwhile some very limited data-sets were collected using different radiation wavelengths with the help of Ian Glover at Keele University, and we showed that phasing this kind of data is perfectly possible using standard CCP4 software, treating anomalous data as a special case of standard multiple isomorphous replacement (MIR). Other groups were trying to write dedicated programmes to do the same thing. In the end, Venki Ramakrishnan (who later became Nobel Laureate and President of the Royal Society) used our MAD-as-MIR method to publish a protein structure before we did. He did acknowledge Eleanor and myself, but it was a blow.² As a consolation, we published the structure of OppA in *Science* soon afterwards (Tame et al. 1994), which led to me being awarded a Royal Society University Research Fellowship. Two graduate students (Tom Davies and Sara Sleight) joined the project, and we solved OppA structures with all sorts of ligands throughout the late 1990s. Using calorimetry we tried to compare the models with the thermodynamics of peptide binding (Davies et al. 1999). Isothermal titration calorimetry was still in its early days, so the data attracted tremendous interest from drug design groups (Wang and Wade 2002), and it was a thrill to see the work discussed at many meetings. Tom moved on to Astex to carry on with structure-based drug design, while Sara went to the Royal Society of Chemistry. The hands-on work with thermodynamics led me to pen notes on entropy, which Springer-Nature published in book form a few years ago (Tame 2019).

Around this time I also became a visiting professor in Rome to help Beatrice Vallone at the Department of Biochemical Sciences of the University La Sapienza with crystallographic work. Rome was an absolute Mecca for globin research for many years, Eraldo Antonini and Maurizio Brunori writing the then definitive book on the subject in 1971. I had met Beatrice earlier in Cambridge when she visited Kiyoshi, and my fellowship in York allowed me the freedom to visit her in Rome, some of the happiest days of my life. I continued to publish on Hb with Kiyoshi, producing several papers on its evolution, for example showing that the entire D-helix can be removed from the β chain and re-introduced into the α chain

(which doesn't have one), leaving a functional molecule (Komiyama et al. 1991).

By this time I had met my partner Chris (then a PhD student at YSBL), and bought a house. York is a lovely city and many people settle down there, but it didn't happen for me. There were tensions and disappointments. At one point a PhD student in the Physics Department publicly presented research he had conducted using my unpublished data that had been passed to his supervisor behind my back. Guy very much fostered the attitude that it is easier to ask forgiveness than permission, and in the end it was simply time for me to move on.

Kiyoshi was well aware of my unhappiness and arranged for me to move to the far outskirts of Kyoto, close to Nara, where Keiichi Namba was running the Protonic Nanomachine Project, researching the motor that drives the bacterial flagellum. Chris found an independent position at a nearby institute to work on biological means of carbon dioxide capture. Keiichi granted me an enormous salary, and there were plenty of chances to explore Nara and Kyoto in my convertible British racing green MX-5. Chris had long blonde hair that she let blow in the breeze on sunny days when we sped along with the roof down. Quite a sight in rural Japan, before the hordes of foreign tourists had appeared! They were good days, and I relive them often.

Left to my own devices, I re-solved the structure of OppA ab initio by direct methods using the atomic resolution data (Tame 2000), and feel sure this is still some sort of record; it certainly was at the time. Gerard Bricogne would no doubt dispute that a complex with uranium ions is really a case of 'direct methods', but, quibbles of terminology aside, it worked.

Within a few months of arriving in Kyoto in mid-1999, a phone call came from Yokohama City University. A new campus was being established, and it was planned to have a new protein crystallography group. I was asked to head it, and accepted. It really was an amazing stroke of luck, with a big helping hand from Kiyoshi. There were not many foreign professors in Japan then, and certainly none under 35. I was very lucky to be joined in my group by Sam-Yong Park and Satoru Unzai, all of us students of Kiyoshi or his PhD supervisor, Hideki Morimoto. In April 2001 our new building opened in Tsurumi. We had two state-of-the-art X-ray generators, three state-of-the-art R-Axis IV detectors, a top-of-the-range analytical ultracentrifuge, and not a single test-tube or pipette. The budget had been arranged before my appointment, and there was seemingly no way to exchange an X-ray detector for other essential items. Over time, we seem to have acquired enough.

These were the glory days of structural genomics, and the Tsurumi campus, shared with Riken, housed the world's largest collection of NMR machines dedicated to protein structure determination. As crystallographers we

²The MAD-as-MIR method was written up first in the informal CCP4 newsletter in 1993 (No.29).

didn't quite fit in, but there was plenty of money for solving structures. We looked at targets for new antibiotics, influenza proteins, and of course Hb. Throughout my time in Yokohama I have hugely benefitted from long-standing collaborations with old friends from York, David Roper (now in Warwick) and David Scott (who first taught me to run the AUC, and is now based in Nottingham). Their support and unfailing humour has been invaluable.

A real highlight for me was the structures of the autotransporter Hbp (also called Tsh), one of a large family of pathogenic proteins causing enormous medical costs. Jonathan Heddle, a post-doc from England, joined us in 2001, and helped solve the structure of the Hbp passenger domain which is released by the bacteria (Otto et al. 2005). After four rejections by well-known journals, the paper has now been cited almost 200 times. The late British chess grandmaster Tony Miles once famously reviewed a book of chess stratagems in two words, the first of them being 'utter', and I sometimes wonder if he has been brought back to life from time to time to referee my manuscripts.

We went on to solve the structure of the membrane domain, through which the passenger passes, showing it has a novel proteolytic mechanism, the last to be discovered of the seven known (Tajima et al. 2010). Others claimed credit for the discovery in 2011, but only by citing our 2010 paper, titled *A novel intein-like autoproteolytic mechanism in autotransporter proteins!* This work resulted from the PhD thesis of Nami Tajima, who moved on to Cold Spring Harbor and now heads her own group in Ohio.

In York I shared an office with Fred Antson, who had solved the structure of a ring-shaped protein called TRAP, with 11 identical subunits. It was an interesting model, and we discussed several mutations that might help solve the problem of how TRAP might interact with a much smaller trimeric factor called anti-TRAP. Fred and colleagues went on to publish a model for this in 2005, when Jonathan started making TRAP tandem mutants to see what structures we could make with the protein by linking two subunits into one polypeptide chain. He soon had crystals and X-ray data showing that instead of the wild-type 11-mer we had a 12-mer ring, which we described in a paper called *Rounding up* (Heddle et al. 2006). This was of course an ideal protein to crystallise with anti-TRAP (Watanabe et al. 2009), and led to the beautiful Star of David structure shown in Fig. 2. Each anti-TRAP trimer binds to two adjacent TRAP subunits, so that exactly six anti-TRAP molecules can fit perfectly around 12-mer TRAP. With the wild-type 11-mer TRAP, there is only space for five anti-TRAP molecules. The AUC really came into its own with this project, giving us accurate molecular weights of the complex as more anti-TRAP was added. By titrating the two proteins, we could see clearly that 12-mer TRAP has a higher binding capacity than 11-mer TRAP, which ruled out other models of the complex. In

Fig. 2 The crystal structure of the TRAP-anti-TRAP complex. Six copies of the anti-TRAP trimer (blue) are found around the edge of the TRAP ring built from 12 TRAP domains (various colours). Each copy of anti-TRAP interacts with two adjacent TRAP domains, with one anti-TRAP subunit facing away from the TRAP ring. An earlier model from York proposed an interaction via the TRAP residues facing the central hole. Figure taken from Watanabe et al. (2009), with permission

recent years AUC has become rather neglected, but perhaps if Arthur Peacocke had been alive, he would have liked our paper. Not quite in the class of showing GC pairs have more hydrogen bonds than AT pairs in DNA duplexes, but still. It was another manuscript that Tony Miles got to review, but YSBL called to congratulate us when it was finally published.

Together with Ichiro Yamashita from Panasonic, we made computer memory chips with TRAP (Heddle et al. 2007) and artificial protein nanotubes with record-breaking length-to-width ratio (Miranda et al. 2009). Jonathan left Yokohama to lead a group at Riken in Wako for five years, before establishing a laboratory in Krakow. His group continues to thrive, and some of their more recent work on large complexes built from TRAP was published by Nature in 2019 (Malay et al. 2019).³

The TRAP work led to new projects with protein design, mainly with Arnout Voet, who spent five years in Japan as a post-doc with Kam Zhang. Arnout now has his own group in Belgium, and we have published a number of novel protein structures, starting with Pizza, a β -propeller

³This paper is apparently the first Nature paper since the 1960s with a corresponding author working at a Polish institution.

protein built from six identical subdomains, or blades (Voet et al. 2014). Arnout and colleagues designed several more proteins with a little input from Yokohama, and we even managed to build the world's smallest reported crystal. Pizza can sandwich 19 cadmium and chloride ions into a nanocrystal of cadmium chloride, an unusual variation on the use of salts to crystallise proteins (Voet et al. 2015). Currently we are looking at other designs of small domains intended to fold into disc-like shapes with chosen internal cyclic symmetry (Mylemans et al. 2021). This work should help reveal any general structural requirements to build a circular protein oligomer. Is there really one rule to ring them all? Recently we were asked to write a review of this work on the evolution and artificial engineering of propeller proteins, which has just been published under the title *The Taming of the Screw* (Mylemans et al. 2020). Other work has included the redesign of lectins that show promise as diagnostics or even therapeutics for different cancers. Our collaborator Yasuhiro Ozeki in Yokohama has a penchant for extracting juices from all manner of marine invertebrates, and finding what lectins they include. Clinical applications are a long way off, but we have made some interesting observations along the way. The lectin SeviL from mussels, whose structure we published very recently, can enter and kill cells that display the right sugars at the surface. How useful it will be we do not yet know, but its cellular effects present an intriguing puzzle to solve.

And of course the cycle turns. After I left Cambridge, Noboru Komiyama in Kiyoshi's group showed that human Hb can indeed be turned into a form like crocodile Hb, with a small number of mutations. They included the ones I had suggested earlier, and comprehensively ruled out the Perutz model. Kiyoshi invited me to help write the paper that Nature published in 1995 (Komiyama et al. 1995), and we made headlines around the world. Over a decade later, with the help of Naoya Shibayama, Sam-Yong Park and I refined the structure of human Hb to atomic resolution, 1.25 Å, in the oxy, deoxy, and carbonmonoxy forms (Park et al. 2006). These models allowed us to finish off some of the chatter regarding the correctness of Perutz's models and analysis, a task I had set myself in York (Tame 1999). Perutz himself had sadly passed away in 2002, but after destroying one of his favourite ideas it was satisfying for me to defend his main achievements. Martin Karplus took up these models to help explain the effects of pH on Hb oxygen affinity, which was a wonderful compliment. Many fish Hbs outshine human Hb by far in sensitivity to pH though, and my group has also built on the work of Guido di Prisco and Lelio Mazzarella in Naples to put forward some of the firmest structural explanations for this eponymous Root effect (Yokoyama et al. 2004), through the PhD studies of Takeshi Yokoyama, now an assistant professor in Toyama University. With Kevin Campbell in Canada we also solved

the structure of woolly mammoth Hb — the first protein structure from an extinct species — showing that early elephants could adapt to the cold with a single mutation changing the heat profile of oxygen binding (Campbell et al. 2010). Human Hb holds on to oxygen very tightly in the cold, but Hb from Arctic animals shows a very small enthalpy change of binding. From evolution to medicine, Hb really is a wonderful testbed of a protein to work on, a very generous protein as the late Eraldo Antonini used to say. Gideon Davies in York used to rib me about my work on Hb, joking that I had shown why crocodiles don't fly over the Himalayas. What I and many others had done is help explain how crocodiles manage to stay underwater for hours. This unique evolutionary development is still being studied by the groups of Jay Storz and Angela Fago, who have recreated early Hbs from hundreds of millions of years ago. Perhaps in the future that work will help us increase oxygen transport efficiency in patients who need it — what a wonderful thought!

Kiyoshi Nagai passed away in 2019 from liver cancer, after an incredible career. His ground-breaking work into the mechanism of the spliceosome should surely have won him the Nobel prize too, had he lived longer. His right-hand man, Chris Oubridge, passed away less than a year later from the same disease at the age of only 54. It was a privilege to have known them both.

So really I have been very, very lucky, in having the right parents, the right friends, the right supervisors, and of course, having Chris (who joined my group in Yokohama, where she is a mainstay in all aspects of the experimental work). But it's also been a rocky road at times, and a career in science is not something I wish for our children. My own eventual path through the minefield has depended completely on Max Perutz, Kiyoshi Nagai, Guy Dodson, Keiichi Namba, and of course Yokohama City University giving me a chance, and I am grateful to them all.

Even for those who make it past the pitfalls as a post-doc, science can be hard if you discover anything. Max Perutz had to fight very hard for years to have his allosteric mechanism accepted. He liked to say that 'In science, truth always wins', but unfortunately this rosy view pays no attention to kinetics, or the limited time-window for a young researcher to establish credit for his or her work. While extraordinary claims may require extraordinary evidence, the problem for science is error correction as much as error avoidance; errors can be very fruitful, as Erasmus pointed out centuries ago. What science has to be is *honest* and *open*. Open-mindedness is the most important quality in learning from experiment, as any good Bayesian will tell you, yet modern science has come to resemble a temple to scepticism and contempt for the maverick. Naomi Oreskes has written a very well-received book called *Why trust science?* on what she calls 'consensus science', which downplays the views

of the minority, and argues that consensus should give us confidence in the pronouncements of scientific authorities. As Voltaire so memorably expressed it long ago, however

Our wretched species is so made that those who walk on the well-trodden path always throw stones at those who are showing a new road.

Prof. Oreskes's thesis seems to me a thunderous manifesto for the stone-throwers. Memories of standing as a young stripling of no honours before the great Perutz have given me a different perspective. The history of science is replete with pioneers who were right, but ignored. John Waterston, for example, sent a paper to the Royal Society in 1845 suggesting gases were made of innumerable tiny particles continuously bouncing off each other. The Society reviewers felt it such rubbish that they never even bothered to respond; about fifty years later, after Waterston's death, his manuscript was discovered quite by accident, and found to have to been accurate in every point, at least fifteen years ahead of the field. Barbera McClintock is perhaps the outstanding example of a twentieth century scientist whose work was so revolutionary that she stopped publishing for fear of alienation. 'One must await the right time for conceptual change', she later commented. Consensus science is comfortable science, for those in high places. Science is not supposed to be comfortable, it is supposed to make us sit as little children before the facts, to look at the evidence, and sadly that fundamental core tenet has been widely abandoned in the age of 'debunking conspiracy theories'. 'Debunking' is a term widely used where 'dismissing' would accurately reflect the treatment of evidence in the process. Its use for political mud-slinging is regrettable, and its encroachment into science is disastrous.

I cannot conclude however, without giving proper thanks to my adopted country. As stable politically as it is unstable geologically, Japan is a unique, blessed haven of calm in the face of calamity. It was here in Yokohama that I met Damien Hall, then a refugee from the great Tohoku earthquake of March 2011. I could write an entire book on that single event — it's a strange feeling to point a Geiger counter at the sky and find plenty of counts. But it's an ill-wind, even a radioactive one, that blows no good, and Damien and I have been in touch ever since. At his invitation I have joined the Editorial Board of Biophysical Reviews, and it has been a very new learning experience. Being at the far edge of the world, my visitors are few. Some find it daunting, coming to an island where we drive on the left, drink a lot of tea, and adore gardens. Somehow I have fitted in. My children giggle at my Japanese, but my secretary has become fluent in my version of it. A number of post-docs have passed through, from the UK and Europe, most recently from Italy and Belgium. Jose Caaveiro, originally from Spain, moved on via Tokyo to become a professor in

Kyushu. With Ivan Korendovych of Syracuse University in New York, we have a wonderful manuscript in preparation on artificial enzymes. If anyone is interested in hearing the story, please drop in my office any time! I can show you round the marvellous marble palaces of science that we occupy in Tsurumi. Earthquake-proof as they are, somehow I still have fond memories of a long-demolished portakabin in York, with no heating or potable water, that shook so much no-one could walk around when we weighed things out, whose fire-exit door blew open in a high wind, whose fume-hood duct led to one of the post-doc offices, and where, a very long time ago, I became a biophysicist.

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