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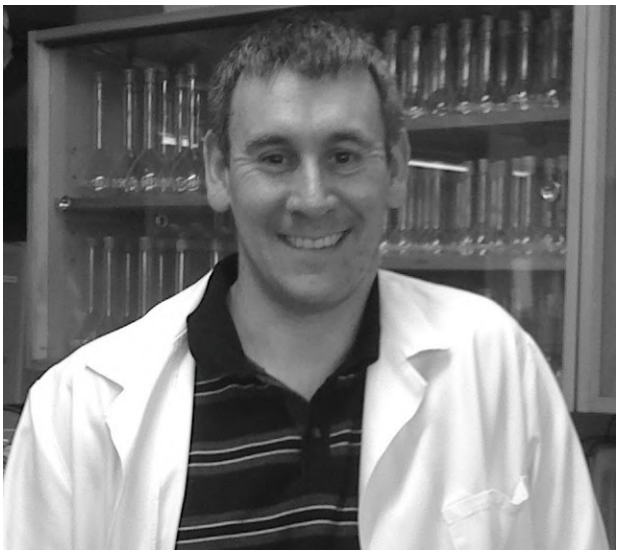
Front cover: Photo by Matt Walters, School of Biological Sciences, University of Canterbury

Comment from the President

It has been a busy few months since the last edition of *Chemistry in New Zealand*. On the local front, I have been busy in Canterbury with events to encourage students to study chemistry and science. Last week saw more than 60 secondary school students invading the labs at CPIT to compete in our annual Year 12 chemistry competition. I get a great buzz out of watching the student teams work together using wet chemistry to identify unknown organic compounds, as well as extracting key information from journal articles. And this weekend saw the Canterbury Career Expo where hundreds of local teenagers came to find out the various career and training options around New Zealand. I always look forward to talking to students who want to know what career options there are that can fulfil their interest in chemistry. I just hope I don't bewilder them too much once I have finished listing the many career and tertiary options available to them! I find it quite frustrating sometimes that many students only see chemistry as a stepping stone to health sciences or engineering, and are not aware of the wider opportunities for study involving chemistry. This is why at the ChemEd conference this year I will be giving a talk which reminds teachers about the wide range of careers available to someone who excels in, or simply enjoys, chemistry.

On a national level, we have seen the government reveal an increase in science funding, including an almost doubling of the funding originally set aside for the National Science Challenges. My colleague at sciblogs, Peter Griffin, provides a concise summary of the increased funding (http://sciblogs.co.nz/griffins-gadgets/2013/05/16/budget-2013-whats-in-it-for-science/). While it still remains to be seen how funding is distributed amongst the National Science Challenges, virtually all 12 challenges have some relevance to chemistry, reflecting that chemistry is indeed, the central science. Whether it is contributing to challenges involving high value nutrition, aging well, primary sector production, or our southern oceans, chemists will have roles to play in these areas. Furthermore, the addition of a 13th challenge focusing on science and society will focus on the promotion of science literacy within schools and across the wider community. The benefits of improving science literacy in New Zealand cannot be underestimated. This challenge will also provide opportunities in the area of educational research, something I know will interest our members who are already active in this area.



I'd also like to thank Shimadzu for agreeing to sponsor our Industrial and Applied Chemistry prize for the next five years. We have some excellent work going on in New Zealand in the area of industrial and applied chemistry, so it is fantastic to be able to continue to recognise this work through this award. And thank you to our on-going sponsors: ABA books, the Maurice Wilkins Centre and the Royal Society of New Zealand.

I'd also like to remind members that this year we have a national NZIC conference. Members of the Wellington Branch have been working hard to prepare for the conference, and have already confirmed some excellent speakers. Preliminary information on the conference can be found at *http://nzic.org.nz/conferences.html* I hope to see many of you there.

Michael Edmonds
NZIC President

New Zealand Institute of Chemistry

supporting chemical sciences

July News

AUCKLAND

The first Chemistry Pub Quiz of the NZIC Auckland Branch was held at the end of April. Over sixty people attended, making up fifteen teams. The winners were the 'Willy Wonka and the Oompa Loompas' consisting of Dr *Pooja* ***Yadava***, Dr *Ali* ***Hosseini***, Dr *Michel* ***Nieuwoudt*** and Dr *Peter* ***Swedlund***. This event was organised by *John* ***Arabshahi*** (Quiz Master), *Charles* ***Kong***, Dr *Viji* ***Sarojini*** and Dr *Jóhannes* ***Reynisson***. The Quiz was great fun, a ringing success and we plan to make it an annual event.

Chemistry Quiz winner the 'Willy Wonka and the Oompa Loompas'.

The NZIC Presidential talk for 2013, entitled *Chemistry – an Antidote to Pseudoscientific Thinking?*, was given by Dr *Michael* ***Edmonds***, Department of Applied Sciences & Allied Health, Christchurch Polytechnic Institute of Technology.

School of Chemical Sciences, University of Auckland

The School of Chemical Sciences now has a new Head. We welcome Distinguished Professor *Margaret* ***Brimble*** in her new role. Also, as the 2012 Rutherford Medal recipient, our new head presented the lecture *Mastering molecular chess to mine Nature's medicine chest* around the country in May. In this lecture, she showed how natural products derived from either micro-organisms that live in extreme environments, or algal blooms, can be harnessed to develop anticancer, antibacterial and antiviral drugs and drugs to treat neurodegenerative diseases.

Congratulations to Dr *Cosmin* ***Laslau*** who was one of the five PhD students from The University of Auckland awarded the prestigious Vice-Chancellor's Prize for the Best Doctoral Thesis in 2012. Cosmin's thesis is titled *Novel Fabrication and Characterization Methods for Conducting Polymer Nanostructures and Microstructures*. The research was supervised by A/Prof. *Jadranka* ***Travas-Sejdic*** and Prof. *David* ***Williams***.

In March the MSc class of 1963 came back to the School for their 50th anniversary reunion. Several of the staff that taught them were also present; among them were former Heads of Department *George* ***Clark*** and *Brian* ***Davis***, as well as *Graham* ***Wright*** and *John* ***Packer***. The group of 33 were impressed with the new undergraduate laboratories and many of the new research facilities. Thanks to Prof. *Laurie* ***Melton*** for organizing this wonderful event.

Congratulations to the BioMEMS Group who made the cover of the February issue of the journal *Cytometry A*. This signifies the recent innovations that the group has achieved in the fields of non-invasive and real-time probing of tumour cell death using Lab-on-a-Chip flow cytometry in conjunction with inert fluorescence probes (Akagi, J. *et al*. *Cytometry* Part A **2013**; *83*(2), 227-34).

Many visitors gave excellent talks over the past few months. Prof. *Chris* ***Orvig*** from the University of British Columbia, Vancouver Canada gave a seminar on *Inorganic Medicinal Chemistry*. Professor *Suzanne A.* ***Blum***, Chemistry Department University of California, Irvine presented her seminar, *Microscopy for Synthetic Chemists and Dual-Metal Catalysis with Gold*. A MacDiarmid seminar, *Ultraviolet Vision – New Frontiers in Health and Technology*, was given by Dr *Martin* ***Allen***, Dept. of Electrical and Computer Engineering, University of Canterbury, and Dr *Scott* ***McInode*** of the Department of Chemistry, University of Victoria, BC, Canada presented his seminar, *Mass Spectrometry-led Catalyst Discovery*, to the School. Dr *Annie K. Powell* of the Institute of Inorganic Chemistry, Karlsruhe Institute of Technology, Germany gave a seminar on *Incorporation of Highly Anisotropic Ions into Coordination Clusters in the Search for Enhanced Single Molecule Magnets*. Prof. *Muthupandian* ***Ashokkumar*** of the School of Chemistry, University of Melbourne, Australia gave his talk *Sound and Bubbles: Fundamentals and Applications of Acoustic Cavitation* to the School.

The MSc class of 1963 with their lecturers at the Coral Hall.

The New Zealand Institute for Advanced Study, Massey University Auckland

Massey University in Auckland finally decided to start a Chemistry Major from 2014. The new chemistry laboratories have already been built and a number of new chemistry positions will be advertised. The University is currently seeking a lecturer in synthetic organic chemistry to start in Albany. Massey in Albany will also receive major funding in capital equipment, with a new Bruker NMR coming in 2014.

A recent research highlight has been a collaboration between Lyon (*F.* ***Calvo***), Heidelberg (*M.* ***Wormit***) and Massey University's Research Centre for Theoretical Chemistry and Physics (CTCP) (*E.* ***Pahl*** and *P.* ***Schwerdtfeger***), which has resulted in the solution of a long- standing problem of why mercury is liquid at room temperature, for which the paper is in press in *Angewandte Chemie*. In addition, *Anastasia* ***Borschevsky*** from CTCP has just published a paper in *Nature* on the ionisation potential of the radioactive element astatine, and a paper by *John* ***Harrison*** is one of the Top 20 Most Read Manuscripts in the *Journal of Chemical Physics*. *Joshua* ***Bodyfelt*** has arrived here from the United States to take up a research officer position within CTCP. His field of expertise is in theoretical and computational physics, and he will be responsible for the system administration of our CTCP high-performance computer cluster.

CANTERBURY

Inaugural Ferrier Lecture

The Ferrier lectureship was established in honour of Emeritus Professor *Robert J.* ***Ferrier*****,** an academic at Victoria University's Department of Chemistry from 1971-1997. Robin is one of New Zealand's most prominent chemists and a leader in the field of carbohydrate chemistry. The Ferrier Lecture was given by Prof. *Vern* ***Schramm*** of Albert Einstein College of Medicine, New York to the NZIC Canterbury branch gathering on 6 March. The lecture was entitled *Enzymatic Transition States and Drug Design*.

Prof. Schramm's transition state theory proposes that chemically stable mimics of individual enzymatic transition states will be the most powerful inhibitors known. The approach has the potential to create transition state analogues as powerful inhibitors of enzymes. Experimental isotope effects and computational methods are used to establish the nature of enzymatic transition states and design transition state analogues. The implications of Professor Schramm's work are frankly astonishing. With examples that included drugs designed to treat conditions such as gout, malaria, human cancers and quorum sensing molecules, he showed how drugs designed to mimic the transition states of key enzymes and their substrates rather than the substrates themselves were, in many instances, vastly more effective inhibitors. The implications for medicine are profound.

On 30 April NZIC President, Dr *Michael* ***Edmonds****,* Department of Applied Science and Allied Health, Christchurch Polytechnic Institute of Technology, gave a highly entertaining and informative presentation at the University of Canterbury entitled *Chemistry – an Antidote to Pseudoscientific Thinking?* In his introduction Michael stated, "As chemists it is easy to take for granted our understanding of the physical world around us. For others, however, an ignorance of basic chemistry can put them at risk from relying on homeopathic remedies to treat malaria to spending thousands of dollars on bogus health therapies.

"Over the past five years I have spent time pushing back against pseudoscientific beliefs and absurd health therapies through articles, blog posts and via the Advertising Standards Authority. In this presentation I will describe some of these 'engagements' – the stupid, the funny, the downright scary. I will also discuss why it is important to challenge such beliefs, and suggest key tactics for doing this successfully."

Michael defined pseudoscience as 'pretend' science: a collection of beliefs presented as 'scientific' in spite of a lack of plausibility and supporting evidence. Examples of pseudoscience cited included homeopathy, detox therapies, miracle mineral solutions and climate change arguments. The consequences of pseudoscientific thinking are not necessarily benign, frequently resulting in pain, suffering and death, and costing time and money. When confronted with dubious claims about a product or treatment there is the option of complaining to the Advertising Standards Authority; complaints can be registered online. A successful complaint can result in the individual or company offering a product or service being forced to modify their claims or have their websites temporarily shut down. Michael subjected homeopathy to a particularly detailed vivisection, which included an amusing video that can be found at: https://www.youtube.com/watch?v=HMGIbOGu8q0

The presentation included a list of observations indicative of, and tactics employed in pseudoscientific claims: too good to be true, simplistic and scientifically implausible, anecdotes and 'cherry-picking', misuse of scientific terminology, telling outright lies, use of dubious experts, attacking legitimate science, and conspiracy claims.

Not content to merely detect pseudoscience, Michael also offered his "Five rules" for engaging pseudoscientists and their dupes in debate: 1, Listen and ask questions; 2, Know what you are talking about; 3, Use precise, simple, neutral language; 4, Remember you are dealing with a person; 5, Remain calm and rational. Michael makes frequent appearances on Sciblogs (*http://sciblogs.co.nz/*).

CPIT

On Thursday 16 May, CPIT held its annual Year 12 Chemistry competition. Twenty two teams of three from schools around Christchurch competed in the one hour competition, working to identify unknown organic compounds using a range of chemical tests, as well as analysing several analytical chemistry papers to answer questions about environmental contaminants.

The results of the competition were as follows: 1st with 136/150, St Andrew's College (Tom, Nick, Michael); 2nd with 129/150, Rangi Ruru Girls' School (Hanseul, Amy, Madison); 3rd

The CPIT annual Year 12 Chemistry Competition

with 124/150, Burnside High School (Ryan, Tom, Dingcheng); 4th equal with 123/150, Papanui High School (John, Florjan, Chettha) and Christ's College (Jeremy, Boris, Oliver).

University of Canterbury

Visitors

Dr *Markus* ***Rex*** arrived at the Department in March for a three month stay. Markus is based at the Alfred Wegener Institute for Polar and Marine research in Potsdam (near Berlin) in Germany teaching at the Free University in Berlin and at the University of Bremen. His research focuses on several aspects of the role of the stratosphere in the climate system and for climate change, with a particular focus on ozone/climate interactions. Markus adds, "Together with my group, I have developed strategies for quantifying the anthopogenic signal in polar ozone loss based on concerted measurement campaigns involving dozens of stations and hundreds of balloon launches in the Arctic and Antarctic and we are developing models for chemical processes in the atmosphere from the local scale to the global scale."

Dr *Claire* ***Vallance***, from the University of Oxford, is also visiting. Claire will be teaching a CHEM400 course on astrochemistry and a CHEM100 course on kinetics. At Oxford Claire worked on reaction dynamics, applications of velocity-map and spatial-map imaging to mass spectrometry, and the development of laser spectroscopy techniques for microfluidics and chemical sensing applications.

Professor Len Linroy is currently undertaking the second part of his Erskine Fellowship in the Department accompanied by his wife Fay. He obtained his PhD (1968) and DSc (1986) from the University of New South Wales. Len is an Emeritus Professor at Sydney University and holds Honorary/Guest Professorships at East China University of Science and technology, Guizhou Normal University (China), Gyeongsang National University (Korea), and is a conjoint Professor at James Cook University. He is a fellow of the Australian Academy of Science and a Senior Member of Robinson College, Cambridge (with life tenure). He has received a number of awards for excellence in research: the Burrows (1991), Smith (1995); Olle (2001); and Leighton (2008) medals/awards of the RACI, the AINSE Gold medal for research (1995), an Australian Government Centenary Medal (2003); Craig Medal (Australian Academy of Science, 2009) and the Centenary medal (RSC, UK, 2009). He is the author or co-author of approximately 360 research papers and patents and two monographs. Len continues as a full-time researcher based in Sydney; however, in recent times he has had the good fortune to spend the majority of each year overseas, undertaking research collaborations in several countries in Europe and Asia.

Awards and appointments

Dr *Solomon* ***Wasseyehum Kelemu*** completed his PhD on Wednesday 3 April 2013, with a seminar to the Department in the morning and an oral examination in the afternoon. Solomon is thought to be the Department's first PhD graduate from Ethiopia.

Sarah ***Masters*** was recently awarded a teaching Development Grant of $3,800 to develop a communication exercise between scientists and art students, recognising that communication of your field of expertise to non-experts is a vital skill. This collaborative project between the Department of Chemistry, the School of Fine Arts and the Department of Art History and Theory enabled students to 'communicate a phenomenon' by creating a tangible piece of art that communicates a scientific concept. The staff involved in the exercise were Sara Masters, *Richard* ***Hartshorn*** (Chemistry), Barbara Garrie and Rosie Ibbotson (Art History and Theory) and Louise Palmer (Fine Arts). The artworks were presented in a public exhibition at Ilam Campus Gallery, curated by students from the Postgraduate Diploma in Art Curatorship, who also produced an exhibition catalogue. The exhibition ran from 30 May to 14 June.

Sandra ***Atkinson*** (Master's Group) has been selected to participate in the 63rd Nobel Laureate Meeting, to be held from 30 June to 5 July, in Linda (Germany). She is one of 625 undergraduate and postgraduate students selected from 78 countries to participate in the Lindau Nobel Laureate Meetings. Thirty Nobel laureates will meet outstanding young researchers from all over the world in Lindau to exchange knowledge and ideas, to share their enthusiasm for science and to establish new contracts. The scientific programme, dedicated to the Nobel Prize discipline of chemistry, will comprise lectures, discussion sessions, master classes and panel discussions. Among the main topics are Green Chemistry as well as biochemical processes and structures. Sandra will create a blog during the meeting, which will be put onto the Department of Chemistry webpage.

MANAWATU

Landcare Research, Palmerston North

Benny ***Theng*** will be giving a pre-conference short course on the clay-polymer interaction in association with the forthcoming XV International Clay Conference in Rio de Janeiro, Brazil (7−11 July, 2013). The website www.15ICC.org has more details. The course is based on his book *Formation and Properties of Clay-Polymer Complexes*, 2nd edition published 12 months ago by Elsevier (Amsterdam). In return, the Organizing Committee of the conference will cover the cost of Benny's airfares, registration, and accommodation.

Massey University, Institute of Fundamental Sciences

In January *Vyacheslav* ***Filichev*** was invited to spent one week in Australia visiting Assoc. Prof. *Tracy M.* ***Bryan*** (Children's Medical Research Institute, Sydney, Australia) and attend a one-day symposium on *Molecular Interactions in Telomeres: Clues to Cell Immortality and Cancer* held at the University of Wollongong (24 January 2013). Vyacheslav presented a keynote lecture entitled *Organic Chromophores in the Structure of DNA: G-quadruplexes and DNA Triplexes* at the symposium and participated in the discussions devoted to the development of the G-quadruplex network in Australasia.

In February while waiting for his PhD defence *Osman* ***Doluca***, a PhD student in the group of Vyacheslav Filichev, took up a lecturer position at the International Burch University in Sarajevo (Bosnia) where he will continue working on modification of DNA molecules.

Pat ***Edwards*** spent three weeks working in Madrid (Spain) in the NMR facility led by Prof. *Carlos* ***Gonzalez*** at the Insituto de Quimica Fisica 'Rocasalano', and performed numerous NMR experiments on chemically modified DNAs obtained in the lab of Vyacheslav Filichev. This trip was supported by the International Mobility Fund (Royal Society of New Zealand) awarded to Vyacheslav and Pat last year.

Ashley ***Way*** has completed her Master's degree under the supervision of *Mark* ***Waterland***, and has since joined the Synthodics group as a research technician.

Recent talks at Massey have included one presented by *Jerome* ***Pelloux***, who spoke about the roles of PME/PMEI-mediated changes in pectins during plant development. Nobel laureate Professor *Peter* ***Doherty*** gave a seminar as part of the graduation ceremonies in May. After successfully defending her PhD, *Gaile* ***Dombroski*** gave a talk on her project, which involved enhancing sensitivity in the analysis of small biomolecules by surface plasmon resonance. In addition to these talks, this year's chemistry honours and masters students presented their research proposals. These students were *Josh* ***Blazek***, who is working with *Paul* ***Plieger***; *Amy* ***Toms***, who will be working with Vyacheslav Filichev; *Yongdong* ***Su***, under the supervision of *Gareth* ***Rowlands*** and Vyacheslav Filichev; *Ben* ***Munro***, under the supervision of *Pat* ***Edwards*** and *Bill* ***Williams***; and *Karla* ***Dunn*** under the supervision of *Trevor* ***Kitson*** and Mark Waterland.

OTAGO

University of Otago, Department of Chemistry

Current and past colleagues of *Jim* ***Simpson*** gathered at the University Staff Club for a special reception to mark Jim's retirement from the Department of Chemistry in April. Both *Brian* ***Robinson*** and *Lyall* ***Hanton*** spoke about Jim's significant contribution to the Department of Chemistry and the University since his arrival in 1969, and reflected on the many changes seen over those years.

Don Eigler, formerly of IBM and 2010 Kavli Nanoscience Prize Laureate, visited in March-April as John and Jean Davis Fellow at the University. In his public seminar *Life Among the Atoms – An Expedition to the Small Frontier*, Don described the science behind his famous 1989 demonstration of the ability to manipulate individual atoms with atomic scale precision using scanning tunneling microscopy. A small symposium was held on 5 April where some of the research in the Department of Chemistry was presented to Don, who later spoke himself on *Spin Excitation Spectroscopy – A Tool Set for Atomic-Scale Spin Systems*.

The following week the Department hosted Dame Carol Robinson from the University of Oxford. During her visit, Dame Carol met with staff and students in a range of fora, then presented a public seminar *Finding the Right Balance: From Rare Gases to Rotary Motors* where she outlined a career working in mass spectrometry and her observations as a woman working in science.

Rajni ***Wilson*** from Brookers Bunch has handed in the hard bound copies of her PhD thesis, and so is now all ready to graduate.

Rajni Wilson with the copies of her hard-bound PhD thesis ready to be handed in. [Photo: Sally Brooker]

Elaine ***Burgess*** and *Nigel* ***Perry*** of the Plant and Food Research Unit presented on *The Science of Curry* to a full house at the Little India Restaurant, Dunedin, in early March. The event was to raise funds for the International Science Festival, run in Dunedin every two years. Nigel talked about the chemistry of garlic and onions (including the rapid analyses of tearless onions) and the pain principle capsaicin in chillies.

WAIKATO

NZIC sponsored a Branch visit by Professor Vern Schramm of the Department of Biochemistry, Albert Einstein College of Medicine, New York. His most enjoyable talk *Enzymatic Transition States and Drug Design* was followed by a pizza lunch

for current and prospective members of the Waikato Branch.

Professor Vern Schramm after his talk at the Waikato Branch meeting

University of Waikato

Open Day at the University of Waikato was held on Friday 17 May. *Bill* ***Henderson*** and graduate students *Sophie* ***Sim*** and *Jane* ***Spenceley*** from the Chemistry Department did displays of liquid nitrogen and fluorescent dyes and Adam Hartland gave Chemistry's mini lecture *Chemistry across the science disciplines: How chemists can go anywhere*.

Bill Henderson has just returned from four months study leave at Durham University, hosted by Professor Paul Low. Undeterred by significant amounts of snow, he also managed to squeeze in trips to London and Birmingham.

Among the new Master's students this year, Megan Wyllie is looking at applications of hydroxymethylphosphines with Bill Henderson; Chris Lockley is examining Welsh bryozoans with *Michèle* ***Prinsep***; and Alice Wang is working with Michèle Prinsep and Ryan Martinus on modes of action of bioactive marine natural products.

Waikato alumnus, Associate Professor Scott McIndoe of the University of Victoria, British Columbia, Canada visited the department recently and spoke on *Mass Spectrometry-led Catalyst Discovery*.

The undergraduate (Chemistry major) student prizes for 2012 were presented recently. Congratulations to the winners, who were: Orica Chemnet prize for First Year Chemistry, Lewis Dean and Melissa Oosterwijk; NZIC JE Allan prize for Second Year Chemistry, Cameron Crombie and Danielle van der Heijden; Dow AgroSciences prize for Third Year Chemistry, Charlotte Bradley.

WELLINGTON

The April meeting was held at Callaghan Innovation and starred *Gary* ***Evans*** of the Carbohydrate Chemistry Team. After pizza to stave off hunger pangs, 20 Branch members enjoyed hearing the story of the development of the immucillins – pico molar inhibitors of purine nucleoside phosphorylases. These transition state inhibitors were envisaged by Vern Scramm (Albert Einstein College of Medicine) and brought to reality by the skilled chemists at IRL. The development of these compounds is into the third generation, with therapeutic activity against many T-cell diseases. Gary described aspects of the synthesis of the compounds, as well as providing insights into the rationale of transition state inhibitor design, scale-up synthesis and protection of intellectual property. After his talk, we were taken on a guided tour of the carbohydrate chemistry research labs and the associated c-GMP facility, Glycosyn.

Waikato Chemistry undergraduate prize winners for 2012 with Waikato branch chairperson, Michael Mucalo. From left to right Lewis Dean, Charlotte Bradley, Michael Mucalo, Melissa Oosterwijk, Cameron Crombie and Danielle van der Heijden.

The Garage Project is a local Wellington microbrewery located in Wellington's Aro valley that provided the location for the Branch meeting on a cold, wet and blustery May evening. The 'Project' began in a garage as a home-brewing collective and then, in 2011, moved to an old petrol station on Aro Sreet. Head brewer Peter Gillespie has brought his more than ten years of brewing experience (gained in England and Australia) to the local operation, which is run in collaboration with Jos Ruffell and Ian Gillespie. Peter and Jos gave a summary of beer brewing, the setting up of the Garage Project, and their beer philosophy that was followed by a guided tour of the brewery. This was accompanied by tastings of five beers including Hāpi Daze blonde ale, named after the Māori word for hops, via several pale ales and a stout through to the Lords Cockswain's Courage, a porter aged in bourbon barrels to give a distinctive and unique flavour suitable for a night-cap and just perfect for the weather conditions. Further information can be obtained from: http://garageproject.co.nz.

Callaghan Innovation

Dr *Jeff* ***Tallon*** is to be presented with a Victoria University Distinguished Alumnus award at a ceremony and dinner on 31 July.

Malaghan Institute

Senior Malaghan Institute cancer researcher *Mike* ***Berridge*** has been awarded $150,000 from the NZ

Health Research Council to develop technology that will allow scientists to manipulate the genomes of mitochondria. If successful, this research will significantly advance understanding of the role of mitrochondrial mutations in disease.

In May, Dr *Bridget* ***Stocker*** took up a position as Senior Lecturer in Chemistry in the School of Chemical and Physical Sciences (SCPS), but will remain affiliated with Malaghan Institute as a Senior Research Associate.

Victoria University – SCPS

As noted above, Dr *Bridget* ***Stocker*** took up a senior lectureship in chemistry on 1 May and Prof. *John* ***Spencer*** left on sabbatical leave for two months in mid-May. He is based at the University of York, collaborating with Prof. Duncan Bruce. Dr *Suzanne* ***Boniface*** was awarded a VUW Teaching Excellence Award in March for her commitment to teaching Chemistry at the 100-level.

Peter ***Moore***, a recent MSc graduate from *Joanne* ***Harvey***'s group, has secured an APA Scholarship for his PhD to be undertaken at University of Queensland with A/Prof Craig Williams, while *Claire* ***Turner*** has submitted her MSc thesis. Dr *Jonathan* ***Singh***, a former PhD student in the ***Northcote*** group, is now working in the School as an SPCS-Magritek postdoctoral fellow and *Helen* ***Wolmer***, who gained her MSc degree last year, has returned to the group for PhD study.

There were a number of visitors to the School in late April and early May. Dr *Ruth* ***Knibbe*** (Callaghan Innovation) spoke on *Electron Microscopy Characterization of Functional Materials for Energy Applications* and met with colleagues at the end of April. She is employed at the new government organisation as an electron microscopist and works within the Superconductivity and Energy Group. Dr *Jonathan* ***Halpert*** (University of Cambridge) gave his seminar in very early May on *Semiconductor Nanocrystals as the Active Material in Optoelectronic Devices: Exciton Dynamics in a $CuInS_2$ Nanocrystal Solar Cell*. He provided a description of this unique material set for producing optoelectronic devices, including LEDs and solar cells. In particular, he explained that copper indium sulfide (CIS)-based solar cells are expected to replace rare or toxic thin film technologies using cadmium or lead. However, colloidally synthesized CIS nanocrystal-based solar cells produced using moderate temperatures (<300 ^{o}C) have only been shown to be ~1.5% efficient. In contrast, charge and exciton processes in CIS NC-based solar cells using an ITO/CIS/CdS/Al device structure have efficiencies up to 1.60% PCE, open circuit voltage 470 mV, short-circuit current 6.9 mA/cm^2, fill factor 52% and external quantum efficiency (EQE) of 20%. He has been using ultrafast near-IR and IR transient absorption spectroscopy methods to look at exciton lifetimes, charge separation and charge extraction in functioning devices. He argued that, ultimately, a better understanding of these devices should lead to improvements in efficiency and should facilitate future advances in materials design. *Lin* ***Du*** (University of Copenhagen) met staff and gave his seminar on *Understanding our atmosphere at the molecular level by vibrational spectroscopy*, when he showed that atmospheric aerosols have profound impacts on the Earth-atmosphere system, influencing the weather, climate, atmospheric chemistry and air quality, ecosystem, and public health. He pointed out that current understanding of the nucleation and growth of atmospheric clusters is still inadequate even though H-bonding interaction has been recognized to play a significant role in formation of the smallest clusters in the atmosphere. By using vibrational spectroscopy, hydrogen bonded clusters have been identified and measured, and relevant methods for obtaining the thermochemistry data of the clusters have been applied.

Prof *Ken* ***MacKenzie***'s group has recently expanded with the enrolment of *Mohammad* ***Alzeer***, a PhD student from Jordan. His research topic is the development of new inorganic polymers with catalytic functionality. The group is hosting for the next six months *Naprarath* ***Waijean***, a PhD student from King Mongut's University, Bangkok, who is working on the use of water treatment waste to produce materials which selectively adsorb heavy metals from industrial waters. *Siti Noor Md* ***Hairi***, a new Master's student who has just joined the group from Malaysia, will be working on a related ecological research project, seeking to utilise the toxic red mud waste from alumina processing to produce useful building products. The group has said goodbye to Dr *Amirabbas* ***Nourbakhsh***, who was a visiting researcher from Isfahan, Iran.

PhD students *Travis* ***Ancelet*** (Weatherburn/Davy) and *Brad* ***Anderson*** (Spencer) graduated with their PhD degrees at the May ceremony. Travis is employed at GNS Science and Brad is continuing with some further research. *Dave* ***Herman***, a PhD student with *Richard* ***Tilley***, has successfully completed his studies and is continuing to work in the Tilley group as a postdoctoral fellow.

Prof *Jim* ***Johnston*** and most of his group attended the mid-May Micro-Tech conference organized by the Nano-Science and Technology Institute and held in Washington DC from 12 May. It incorporated the TechConnect meeting.

Precise synthesis of conducting polymers by DNA-directed self-assembly

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Key words: DNA; Polyaniline, Templated polymer, Programmed synthesis

Introduction

Because of its ability to spontaneously self-assemble into predictable structures, DNA is viewed as a useful tool for the "bottom-up" preparation of molecular scaffolds useful for the construction of nanoscale materials.[1,2] In this regard, unmodified DNA oligomers have been used to form elaborate two- and three-dimensional nano-objects in which the DNA plays an exclusively structural role.[3,4] We have been exploring the possibility of developing a dual role for DNA in the construction of nanoscale materials in which the DNA provides a structural scaffold and is modified to contain functional elements.[5-12] In this approach, the DNA maintains its self-organizing properties and, in addition, contains functional groups oriented so that their subsequent reaction confers unique properties on the resulting assembly. In particular, we have modified selected cytosines in DNA oligomers by the covalent attachment of monomers of conducting polymers. Subsequent oxidative reaction of DNA assemblies containing these modified cytosines generates a polymer covalently attached to the DNA scaffold (which we term a "conjoined polymer"). This approach maintains the structure predicted by Watson-Crick base pairing and also confers the properties of the conducting polymer on the assembly. For example, DNA oligomers modified with aniline (ANi) or 2,5-bis(2-thienyl)pyrrole (SNS) monomers can be converted to conducting polymers upon treatment with horseradish peroxidase (HRP) and H_2O_2.[6,8] These assemblies might function as active elements in a nanometre scale self-assembled device where the conducting polymer functions as a molecular wire.

We have developed two strategies to facilitate the construction of DNA-based nano-assemblies with conjoined conducting polymers.[10,12] In the first, which we call the "duplex strategy", shown in Figs. 1 and 8, complementary DNA oligomers are prepared in which either one or both of the single strands that comprise the duplex is modified to contain appropriately spaced monomers so that their subsequent reaction generates a continuous conducting polymer. The polymer follows the major groove of the duplex DNA and may be considered operationally to be a third strand in the normal DNA. If the monomers are attached to both strands of the duplex, then polymerization crosslinks the two strands resulting in a permanent or "fixed" assembly that does not readily denature. In the second strategy, which we call "encoded module", shown in Fig. 12, a single strand DNA oligomer ("module"), comprised of a central region containing covalently linked monomer units (SNS or ANi) and "recognition" sequences flanking the central region on its 5′- and 3′-sides, self-assembles with other modules that contain complementary recognition sequences. The recognition sequences are coded to assemble uniquely, enabling the spontaneous formation of cyclic and linear arrays having ordered blocks of monomers. Treatment of these arrays with HRP/H_2O_2 generates the corresponding DNA-conjoined copolymers with precisely programmed sequences and well-defined structures.

In this article, we summarize our application of the duplex strategy and the encoded module strategy for the precise synthesis of conducting polymers conjoined to DNA assemblies.

Duplex Strategy

Polyaniline

The DNA oligomers shown in Fig. 1 contain cytosines in which the 4-amino group is replaced by an N-(2-aminoethyl)aniline (X).[7] The remaining proton of the cytosine 4-amino group participates in hydrogen bonding with its paired guanine. When the modified oligomer assembles into a duplex with its complementary strand, the aniline groups extend into the major groove.

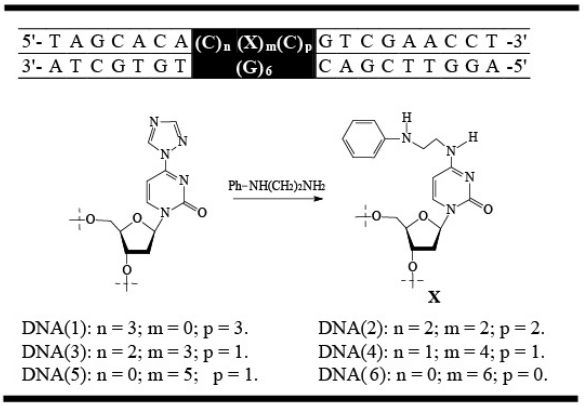


Fig. 1. Structures of aniline-linked DNA oligomers Reprinted with permission. Copyright (2006) American Chemical Society (see reference 7).

Each of the oligomers shown in Fig. 1 contains an embedded $(G)_6$ segment with a variable number of complementary C or X nucleobases. The incorporation of aniline-containing nucleotides X results in the destabilisation of the duplexes as is revealed by a decrease in their melting temperature (T_m). Reaction of these aniline-containing duplexes with HRP/H_2O_2 results in the formation of polyaniline.

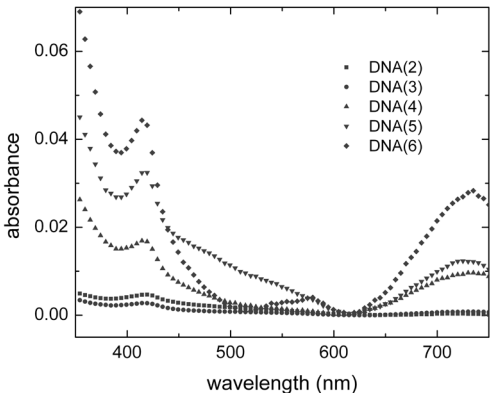


Fig. 2. Absorption spectra of polyaniline (PANi) oligomers formed by treatment of DNA(2) through DNA(6) with H_2O_2 and HRP. The DNA structures are defined in Fig. 1 Reprinted with permission. Copyright (2006) American Chemical Society (see reference 7).

This is apparent from inspection of their absorption spectra, shown in Fig. 2. Polyanilines exist in oxidation states which are traditionally referred to as leuco, emeraldine and pernigraniline (Fig. 3), that have characteristic spectroscopic properties.[13] The absorption spectrum of the PANi oligomer formed from the aniline-containing oligomers indicates that they correspond to the conducting "pseudo-proton doped" emeraldine form.

Polymerization of ANi groups to form polyaniline occurs with significant geometric change that causes structural distortion of the conjoined DNA duplex. Computational analysis of the conjoined PANi-DNA structure (Fig. 4) shows that the nucleobase positions are distorted and the Watson-Crick hydrogen bonds of the duplex are broken in the region of the PANi. The "rise", the distance between adjacent base pairs, and pitch, the twist angle

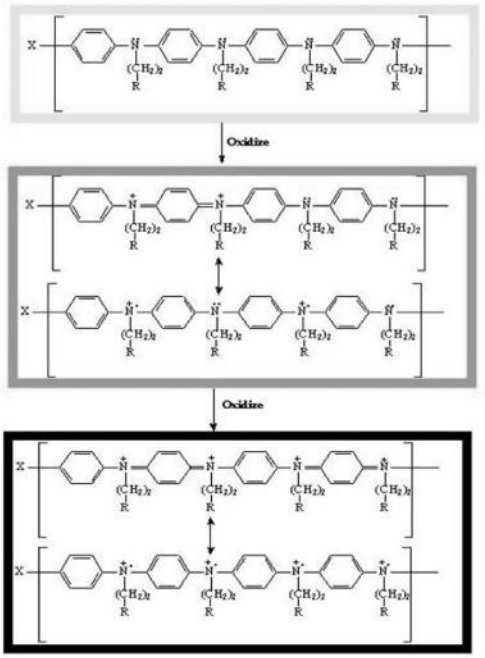


Fig. 3. Three oxidation states of polyanilines. In these cases, R corresponds to the cytosine amino group of the conjoined DNA. Upper: the leuco oxidation state; middle: the emeraldine oxidation state; lower: the pernigraniline oxidation state of polyaniline Reprinted with permission. Copyright (2008) American Chemical Society (see reference 6).

between adjacent base pairs, of DNA and the conjoined conducting polymer must match in order to form stable, undistorted assemblies. This is not the case for DNA and PANi. Figure 4 shows a model for an oligomer of six aniline monomers attached to adjacent cytosines on one strand of DNA. The PANi resides in the major groove of the duplex, but the DNA is highly distorted. In fact, if this assembly contains more than six ANi monomers, the conjoined polymer is not stable and denatures into single strands at room temperature. We sought other monomers that would form conjoined polymers whose rise and twist are commensurate with that of DNA.

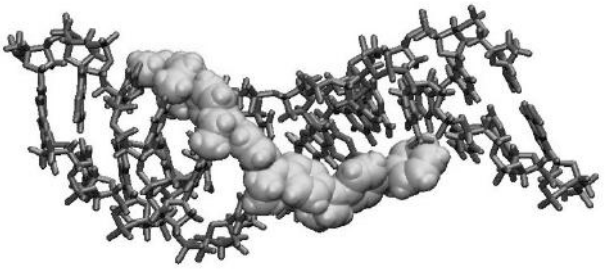


Fig. 4. A structural model showing six aniline groups bonded head-to-tail conjoined to a DNA oligomer Reprinted with permission. Copyright (2006) American Chemical Society (see reference 6).

Conjoined Poly-SNS

Polythiophene and its derivatives are among the most extensively studied conducting polymers.[14] They are relatively easy to synthesize and they exhibit a wide range of readily adaptable properties. Moreover, oligothiophenes are described as forming good molecular wires characterized by having β values equal to 0.1 $Å^{-1}$.[15] Monomers comprised exclusively of thiophenes cannot be easily linked to DNA, but their analogues, the 2,5-bis(2-thienyl) pyrroles (SNS), contain nitrogen atoms suitable for linking. Such monomers have been prepared previously, their electrochemistry and polymerization mechanism studied, and their electrochromic properties described.[16] We assessed computationally the ability of these SNS monomers to form commensurate, conjoined polymers with DNA.

In duplex DNA modified to contain SNS monomers, one strand (the "complementary" strand) comprises a repeating sequence of adenine and guanine nucleotides, while the second strand (the "modified" strand) comprises thymines, which are opposite the adenines of the complementary strand, and a cytosine or an SNS-linked cytosine ("X") opposite the guanines of the complementary strand, i.e, 3′-$(TX)_n$T-5′. Computational modeling of these structures suggests that SNS monomers will line up head-to-tail in the major groove of DNA (Fig. 5). We anticipated that the polymerization of linked SNS monomers by reaction with HRP/H_2O_2 will generate conjoined conducting polymers with a rise and twist very close to that of B-form DNA.

We prepared and examined a series of SNS-linked duplex oligomers (Fig. 6). They exhibit single, reversible melting transitions lower than those of the unmodified duplex,

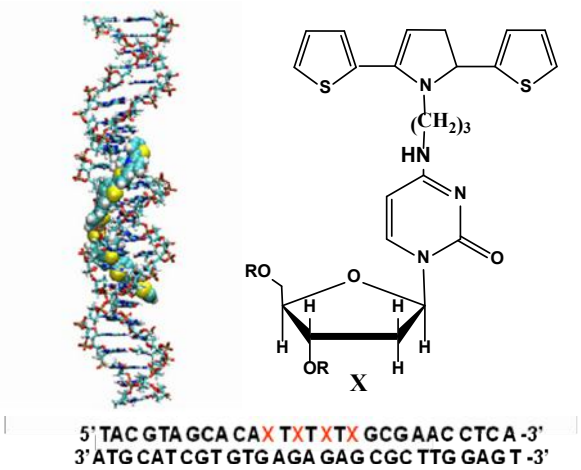
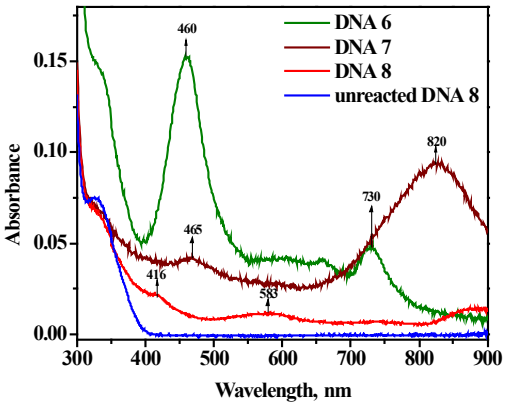


Fig. 5. A computer generated structural model for conjoined duplex DNA-SNS oligomer comprised of four SNS monomer units (symbolized as X) placed on alternating nucleobases of the modified strand in the DNA major groove. Reprinted with permission. Copyright (2010) American Chemical Society (see reference 8).

which shows that replacement of cytosines with the SNS-modified nucleotides reduces the stability of the duplex. However, the T_m for each of these modified duplexes is well above room temperature. Circular dichroism (CD) spectroscopy confirms formation of heteroduplex DNA from the SNS-modified oligomers and their complementary strands. The CD spectra indicate that the global structures of these modified duplexes are similar to that of B-form DNA.

Unique optical absorption spectra are characteristic features of conducting polymers.[13] The reaction of the SNS-linked duplexes with HRP/H_2O_2 results in the rapid appearance of new absorption bands that are characteristic of polythiophene-like conducting polymers, see Fig. 6. The optical spectra of the products formed from this reaction are characteristic of polarons and bipolarons $(SNS\text{-}SNS)_n^{+\cdot}$ in the conducting polymer. Confirmation of bond formation between SNS monomers linked to the DNA comes from ligation experiments using [^{32}P]-labeled DNA. In this experiment, intramolecular bond formation between adjacent SNS monomers, required for the generation of the conducting polymer, ligates two DNA oligomers. This is easily discerned by denaturing polyacrylamide gel electrophoresis (PAGE) analysis. Further evidence for the regiochemistry of bond formation between the thiophene groups of the SNS monomers linked to duplex DNA comes from experiments with 5,5'-dimethyl-substituted SNS. In this case, no ligation occurs, but an optical absorption spectrum characteristic of the dimethyl-SNS radical cation is observed. This shows that bond formation between the SNS monomers aligned in the major groove proceeds, as expected, by coupling between the 5- and 5'-positions of adjacent monomers.

The capability of SNS monomers to form DNA-conjoined conducting polymers was explored by the reaction of HRP/H_2O_2 with a DNA duplex that has 15-consecutive monomers.[8] This reaction yields a clear green solution with a strong absorption band at 630 nm and a band



DNA(**5**)	5' TGA GGT TCG CGA GAG AGT GTG CTA CGT A 3' 3' ACT CCA AGC GCT CTC TCA CAC GAT GCA T5'
DNA(**6**)	5' TGA GGT TCG CGA GAG AGT GTG CTA CGT A 3' 3' ACT CCA AGC G**X**T CTC TCA CAC GAT GCA T5'
DNA(**7**)	5' TGA GGT TCG CGA GAG AGT GTG CTA CGT A 3' 3' ACT CCA AGC G**X**T **X**TC TCA CAC GAT GCA T5'
DNA(**8**)	5' TGA GGT TCG CGA GAG AGT GTG CTA CGT A 3' 3' ACT CCA AGC G**X**T **X**T**X** T**X**A CAC GAT GCA T5'

Fig. 6. Optical absorption spectra observed from the reaction of SNS-containing duplex DNA oligomers with HRP/H_2O_2 in buffer solution at pH 4.5. The structures of the duplex DNA oligomers is shown, "X" has the same meaning as in Fig. 3. Reprinted with permission. Copyright (2010) American Chemical Society (see reference 8).

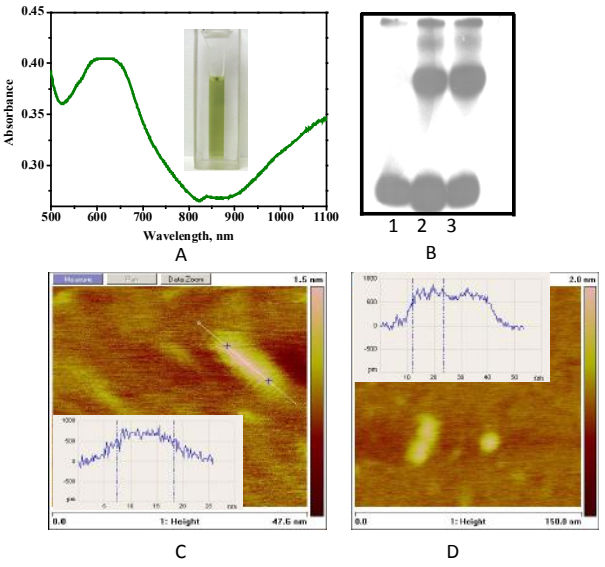


Fig. 7. (**A**): Optical absorption spectrum resulting from the reaction of duplex DNA containing 15 SNS monomers with HRP/H_2O_2. (**B**) PAGE of the reaction of DNA with HRP/H_2O_2. Lane 1 is the control before reaction, lane 2 is the result from the reaction of the SNS-containing single strand, lane 3 is the result from reaction of the duplex. AFM images. (**C**): AFM image recorded before reaction with HRP/H_2O_2. The inset shows the height dimension. (**D**): AFM image recorded after reaction with HRP/H_2O_2 Reprinted with permission. Copyright (2010) American Chemical Society (see reference 8).

in the near IR region with a maximum beyond 1100 nm, as shown in Fig. 7A, which indicates that the HOMO-LUMO band gap for the conducting polymer is *ca.* 1 eV. PAGE analysis of this material (Fig. 7B) shows a conjoined $(SNS)_{30}$ dimer. This structure was studied by AFM

on a treated mica surface. The image of the conjoined polymer indicates an apparent length of *ca*. 11 nm, which is within the experimental uncertainty of the expected length of the B-form DNA duplex 30-mer formed between the two conjoined $(SNS)_{15}$ oligomers.

Crosslinking DNA with SNS

SNS monomers can be used to crosslink duplex DNA oligomers so that they remain intact under conditions that would denature normal DNA.[5] In these oligomers, the SNS monomers of modified DNA are attached to both strands of the duplex and are arrayed in the major groove in an ordered zipper-like fashion (Fig. 8). The reaction of a duplex comprised of complementary oligomers of modified DNA with HRP/H_2O_2 results in conversion of the SNS monomer array to a linear polymer closing the zipper and crosslinking the duplex.

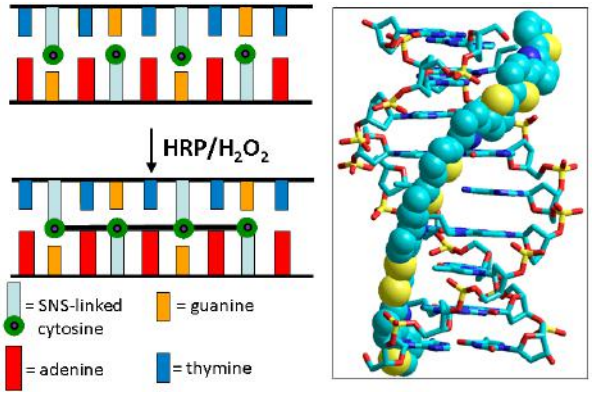


Fig. 8. DNA-templated crosslinking polymerization of SNS monomers covalently linked to cytosines on each of the two strands of a DNA duplex. The image on the right is the simulated 3D structure of $(SNS)_4$ formed from the reaction of 5′-AGTXAGTXC-3′/3′-TXAGTXAGG-5′ in which X represents the SNS-linked cytosines. Reprinted with permission. Copyright (2012) American Chemical Society (see reference 11)

The UV-Vis spectra of modified DNA duplexes in which both strands carry SNS monomers are shown in Fig. 9. The absorption spectrum of the SNS monomer exhibits a maximum at 332 ± 2 nm The absorption of DNA that contains one SNS monomer shows a peak at 348 nm, a red shift of 16 nm compared with that of the SNS monomer. As the number of SNS units positioned in the major groove increases from one to six, the SNS absorption maximum shifts to shorter wavelength because of π-stacking of the SNS groups in the DNA major groove.

The reaction of HRP/H_2O_2 with DNA modified so that both strands of the duplex contain covalently linked SNS monomers results in efficient polymerization to give polymers with unique optical absorption spectra. Bond formation between the SNS monomers results in crosslinking of the duplex, which prevents its denaturation into single strands. This conclusion is supported by analysis of the melting behavior of the duplex oligomers after reaction. The DNA-templated crosslinking reaction was analyzed by denaturing PAGE. Under the conditions of this experiment normal duplex DNA is denatured and the two strands migrate separately. The analysis shows that

DNA(1): 5′-GGA CTG CCT GAC TGA CCG ACC-3′
DNA(2): 3′-CCT GAC GGA CTG ACT GGC TGG-5′
DNA(3): 5′-GGA CTG CCT GAX TGA CCG ACC-3′
DNA(4): 3′-CCT GAC GGA XTG ACT GGC TGG-5′
DNA(5): 3′-CCT GAC GGA XTG AXT GGC TGG-5′
DNA(6): 5′-TGA CAC GCT XTGTXTG TGA CCG ACG-3′
DNA(7): 3′-ACT GTG CGA GAXAGAX ACT GGC TGC-5′
DNA(8): 5′-TGA CAC GCT XTGTXTGTXTG TGA CCG ACG-3′
DNA(9): 3′-ACT GTG CGA GAXAGAXAGAX ACT GGC TGC-5′

X=

DNA	(1,2)	(3,2)	(3,4)	(3,5)	(6,7)	(8,9)
X_n	0	1	2	3	4	6
T_m (°C)	71.4	67.2	63.3	60.1	63.0	63.6

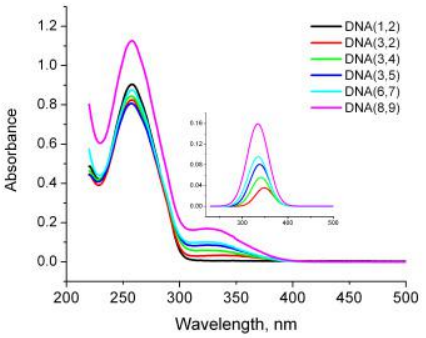


Fig. 9. UV-Vis spectra of SNS linked DNA duplexes in buffer solution. The number of SNS monomers varies from 0 to 6. Inset: The spectra of the SNS groups have been mathematically deconvoluted from the DNA absorption bands. Reprinted with permission. Copyright (2012) American Chemical Society (see reference 11).

with only one SNS monomer, the DNA is not crosslinked. However, when there are at least two adjacent SNS monomers on complementary strands, the reaction with HRP/H_2O_2 results in crosslinking.

Copolymers of SNS and ANi

Hybridization of two complementary DNA oligomers, one modified with SNS monomers and the other with ANi monomers, form duplexes with both types of monomers aligned in the major groove.[12] The monomers may be arranged in blocks such as $(SNS)_n$ and $(ANi)_m$ or interdigitated $(SNS/ANi)_n$, as shown in Fig. 10. The formation of these duplexes was confirmed by T_m measurements and by CD spectroscopy, which show that these SNS and ANi-linked oligomers form stable duplexes under conditions required for polymerization of the monomers by reaction with HRP/H_2O_2. The CD spectra have peaks at that are typical of B-form DNA. At high concentration, there is an induced negative CD band corresponding to the absorption of the SNS monomers, suggesting that the monomers form an ordered array with chirality induced by the right-handed helix of the DNA.

The reactions of these DNA oligomers with HRP/H_2O_2 result in the crosslinking of the two DNA strands. UV-Vis-NIR spectroscopy reveals spectra of the copolymers in their polaron or bipolaron oxidation states characteristic of conducting polymers. Analysis of the reaction product by denaturing PAGE shows strongly retarded bands well separated from the single strands, indicating bond formation between the SNS and ANi monomers has crosslinked the oligomer. Clearly, self-assembly of modified DNA containing SNS and ANi monomers enables their polymerisation and the specific synthesis of alternating copoly-

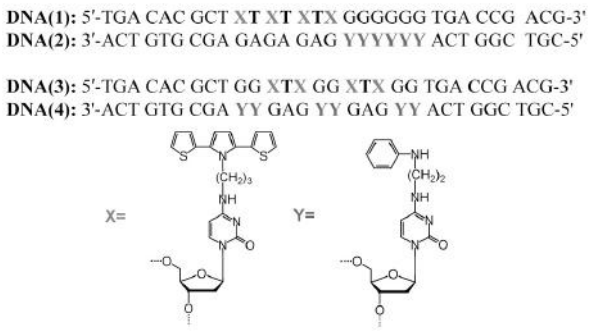


Fig. 10. Structures of the SNS- and ANi- containing duplex oligomers Reprinted with permission. Copyright (2013) American Chemical Society (see reference 12).

mer with the sequence $(ANi)_2(SNS)_2(ANi)_2(SNS)_2(ANi)_2$ from the interdigitated array or the block copolymer with sequence $(SNS)_4(ANi)_6$.

Encoded Module Strategy

Poly SNS

Encoded modules of DNA enable the programmed polymerization of SNS and ANi monomers into a variety of linear and closed-cycle arrays of conducting polymers. [10] A typical DNA module is comprised of a single DNA strand having six covalently linked SNS monomer units separated by thymines, $(XT)_6$, placed between flanking "recognition sequences", see Fig. 11. The recognition sequences are designed to uniquely hybridize with oligonucleotides containing complementary sequences by Watson-Crick self assembly. For example, as shown in Fig. 11, the last 12 bases on the 3'-side of DNA(1) (designated *b*) are complementary to the first 12 bases on the 5'-side of DNA(2) (designated *b*'). Similarly, the sequences *a*/*a*' and *c*/*c*' are also complementary.

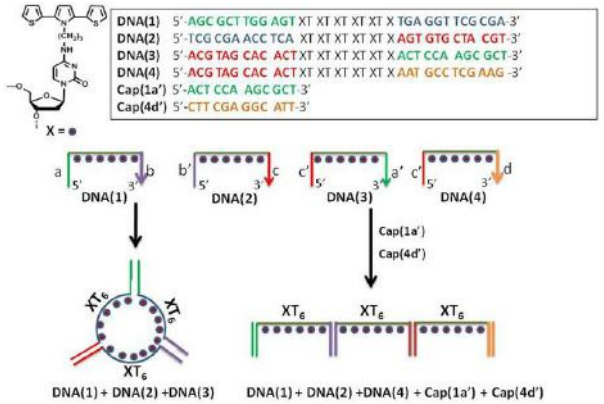


Fig. 11. The structures of the DNA oligomers used in this work and a schematic representation of the programmed assembly of these modules into cyclic and linear arrays containing ordered DNA-linked SNS monomers Reprinted with permission. Copyright (2012) American Chemical Society (see reference 10).

Thus, when the three oligomers DNA(1), DNA(2) and DNA(3) of Fig. 11 are combined in buffer solution, they are expected to self-assemble into a closed cycle array with the adjacent SNS-modified segments held in place by three double stranded DNA "arms". In contrast, the self-assembly of oligomers DNA(1), DNA(2) and DNA(4) is expected to form a linear array. In this array the recognition sequences *a* and *d* are "capped" by the complementary 12-mer oligomers Cap(1*a*') and Cap(4*d*'). Clearly, encoded DNA modules may be combined to form a variety of cyclic and linear arrays.

Hybridization of encoded DNA modules to form linear and cyclic assemblies was confirmed by melting temperature experiments and by gel electrophoresis. The T_m of the cyclic array of three modules shows a striking increase indicative of cooperative stabilization. Non-denaturing PAGE analysis shows that the cyclic and linear DNA arrays migrate as appropriate for their increased size (Fig. 12). Arrays comprised of three, four and five modules are readily formed. These structures contain 18, 24 and 30 ordered SNS monomers, respectively. Treatment of these assemblies with HRP/H_2O_2 results in covalent bond formation between the SNS monomers. This reaction ligates the component DNA modules and forms rings of conducting polymers. For example, the product of reaction of the three-armed assembly with HRP/H_2O_2 shows a single melting transition at 80.5 °C, an increase in T_m of 16.3 °C compared with the unreacted assembly, confirming ligation of the modules.

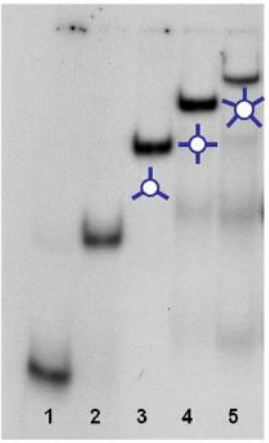


Fig. 12. Autoradiography of non-denaturing PAGE analysis of the cyclic DNA assemblies. Lane 1, DNA(1) only; lane 2, DNA(1,2); lane 3, DNA(1,2,3) cyclic trimer; lane 4, DNA cyclic tetramer; lane 5, DNA cyclic pentamer. Figure 11 shows the structures of the DNA modules. Reprinted with permission. Copyright (2012) American Chemical Society (see reference 10).

The UV-Vis-NIR absorption spectra of these assemblies also change characteristically upon treatment with HRP/H_2O_2. Before reaction they exhibit absorptions typical of DNA and of the SNS monomer. After reaction with HRP/H_2O_2, the absorption spectrum shifts, revealing visible and near IR bands characteristic of conducting polymers. These conducting polymer bands shift red as the number of monomer units in the assembly is increased, which demonstrates that each of the monomers is incorporated into the polymer. Denaturing PAGE analysis shows that the reaction of the assembled modules with HRP/H_2O_2 causes them to become irreversibly ligated by covalent bond formation between the SNS monomers.

AFM images that corroborate the structure of the polymerized cyclic assembly formed by treatment of three-armed assembly with HRP/H_2O_2 are shown in Fig. 13.

The expected diameter of these structures, including the three duplex DNA arms, is *ca*. 10 nm and the expected height is ca. 1.0-2.0 nm. The structures observed by AFM are highly uniform with an average diameter of 12.7 ± 1.9 nm and height of 0.8 ± 0.2 nm, which is typical of that observed for duplex DNA by AFM.[17]

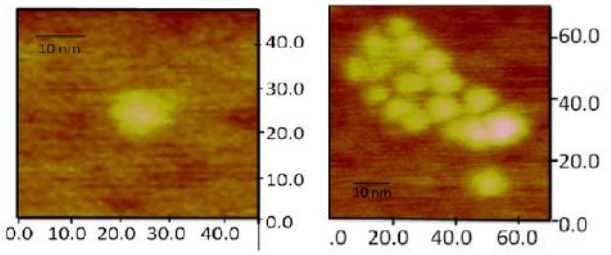


Fig. 13. AFM imaging of cyclic assembly after polymerization with HRP/H_2O_2. Reprinted with permission. Copyright (2012) American Chemical Society (see reference 10)

The modular assembly process enables the simple synthesis of nanometer scale objects comprising a DNA scaffold linked to and supporting a linear or cyclic conducting polymer. This approach enables the preparation of a cyclic conducting polymer arrays with precise control of the synthetic process.

Copolymers of SNS and ANi

Copolymers of SNS and ANi with pre-programmed sequences and precisely controlled molecular architectures can be readily prepared by the encoded module strategy.[12] Single strand modules that comprise a central region containing covalently linked SNS or ANi monomer units can be assembled into cyclic arrays having ordered blocks of SNS and ANi monomers, as shown in Fig. 14. Alternatively, the monomers may be assembled into the linear array in which the "dangling" single strand recognition sequences of the terminal modules are capped by complementary single strand oligomers. Treatment of these cyclic or linear arrays of monomers with HRP/H_2O_2 generates the corresponding DNA-conjoined block copolymers, with unique sequences determined by assembly of the DNA modules.

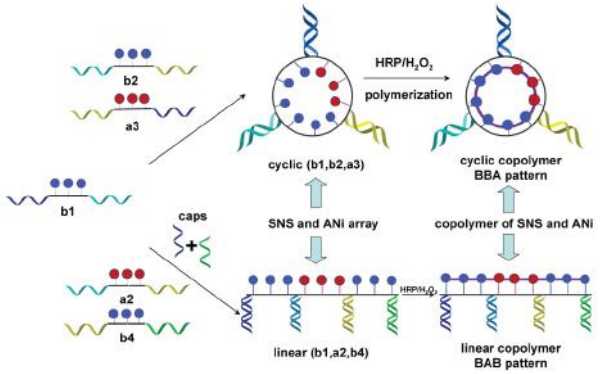


Fig. 14. Illustration of the "encoded modules" strategy for DNA-templated alignment of SNS and ANi monomers in cyclic and linear arrays. The single-strand DNA recognition sequences are shown as color-coded wavy lines that indicate their affinity with complementary modules and ability to form duplex segments by Watson/Crick recognition. Red spheres represent SNS and blue spheres represent ANi monomers, respectively. In the copolymer patterns BBA and BAB, 'A' represents an $(SNS)_6$ block and 'B' represents an $(ANi)_5$ block Reprinted with permission. Copyright (2013) American Chemical Society (see reference 12).

Because of designed sequence similarity, when appropriate ANi-linked DNA modules are mixed with corresponding SNS-linked modules the self-assembly process forms an array having a specific structure and a specific monomer order. For example, three modules can be assembled into a cyclic array containing ten ANi monomers and six SNS monomers having a cyclic BBA pattern where B stands for an $(ANi)_5$ block and A stands for an $(SNS)_6$ block. The encoded modules may also be used to assemble linear arrays of monomers. For example, modules comprised of ten ANi monomers and six SNS monomers can be assembled in a linear BAB pattern, as in Fig. 14. The reaction of cyclic and linear modular assemblies containing SNS and ANi monomers with HRP/H_2O_2 was investigated by means of melting temperature, denaturing PAGE, and UV-Vis-NIR spectroscopy. The findings show the formation of cyclic copolymers with programmed sequences of SNS and ANi monomers in high yield.

The optical properties of the cyclic copolymers were assessed by UV-Vis-NIR spectroscopy. After reaction with HRP/H_2O_2, the absorptions of the copolymers are characteristic of conducting polymers and are dependent upon the monomer pattern. The cyclic copolymer with the BAA pattern having composition $(ANi)_5(SNS)_{12}$ exhibits strong bands not observed in the spectra of cyclic $(ANi)_{15}$ or cyclic $(SNS)_{18}$. Significantly, DNA-conjoined cyclic $(ANi)_5(SNS)_{12}$ copolymers from cyclic arrays with ABA and BAA monomer patterns yield identical products, as they should, but the corresponding linear copolymers with ABA and BBA patterns have different spectra, reflecting their structural uniqueness.

One of the principal goals of synthetic polymer chemistry is to develop methods to construct sequence-controlled polymers similar to those observed in natural macromolecules such as proteins and nucleic acids. Both the duplex and the encoded modular strategy enable the precise synthesis of conducting copolymers conjoined to DNA that have structures defined by the sequence of nucleobases.

Conclusions

DNA is a powerful building block for the programmed "bottom-up" assembly of the supermolecular scaffolds needed for the construction of nanoscale materials and devices. The findings described here show that SNS and ANi monomers, appropriately spaced and covalently linked to DNA oligomers, self-assemble into designed structures and react intramolecularly to form conjoined conducting polymers and copolymers. The conjoined polymers that result have the optical properties expected for polarons or bipolarons in the conducting polymer. The lengths and the chemical and dimensional properties of the polymers formed in this process are determined primarily by the DNA constructs used as their templates. The capability to precisely synthesize conducing polymers of designed composition and length attached to DNA that retains its self assembling properties provides an ability to create functional assemblies that might play a role in

molecular electronic devices. Such work is continuing in our laboratory.

Acknowledgment

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References

1. Stulz, E. *Chem.-Eur. J.* **2012**, *18*, 4456.
2. Wilner, O. I.; Willner, I. *Chemical Reviews* **2012**, *112*, 2528.
3. Jones, M. R.; Osberg, K. D.; Macfarlane, R. J.; Langille, M. R.; Mirkin, C. A. *Chem. Rev.* **2011**, *111*, 3736.
4. McLaughlin, C. K.; Hamblin, G. D.; Sleiman, H. F. *Chemical Society Reviews* **2011**, *40*, 5647.
5. Chen, W.; Schuster, G. B. *Org. Biomol. Chem.* **2013**, *11*, 35.
6. Datta, B.; Schuster, G. B. *J. Am. Chem. Soc.* **2008**, *130*, 2965.
7. Datta, B.; Schuster, G. B.; McCook, A.; Harvey, S. C.; Zakrzewska, K. *J. Am. Chem. Soc.* **2006**, *128*, 14428.
8. Chen, W.; Guler, G.; Kuruvilla, E.; Schuster, G. B.; Chiu, H. C.; Riedo, E. *Macromolecules* **2010**, *43*, 4032.
9. Chen, Y.; Cheng, W. L. *Wiley Interdiscip. Rev.-Nanomed. Nanobiotechnol.* **2012**, *4*, 587.
10. Chen, W.; Schuster, G. B. *J. Am. Chem. Soc.* **2012**, *134*, 840.
11. Ma, Z. J.; Chen, W.; Schuster, G. B. *Chem. Mater.* **2012**, *24*, 3916.
12. Chen, W.; Schuster, G. B. *J. Am. Chem. Soc.* **2013**, *135*, 4438.
13. Bredas, J. L.; Street, G. B. *Accounts Chem. Res.* **1985**, *18*, 309.
14. Yamamoto, T. *NPG Asia Materials* **2010**, *2*, 54.
15. Yamada, R.; Kumazawa, H.; Noutoshi, T.; Tanaka, S.; Tada, H. *Nano Letters* **2008**, *8*, 1237.
16. Tarkuc, S.; Ak, M.; Onurhan, E.; Toppare, L. *Journal of Macromolecular Science Part a-Pure and Applied Chemistry* **2008**, *45*, 164.
17. Klinov, D., Dwir, B., Kapon, E., Borovok, N., Molotsky, T., Kotlyar, A. *Nanothechnology* **2007**, *18*, 225102.

Conference Calendar

5th Asia-Pacific NMR Symposium (APNMR5)

27-30 October 2013, Brisbane Convention and Exhibition Centre (BCEC), Brisbane, Australia

APNMR5 will be a forum for showcasing the exciting developments in all aspects of magnetic resonance in the Asia-Pacific region. The conference will include sessions on solution NMR, solid-state NMR, NMR imaging, NMR theory and methods, and EPR. The meeting will include both oral and poster sessions and will provide significant opportunities for presentations by young investigators.

Abstract submission deadline 1st August 2013

See: http://apnmr2013.org/

ISE Satellite Student Regional Symposium on Electrochemistry & 19th Australian and New Zealand Electrochemistry Symposium (19ANZES)

25-25 November 2013, CSIRO, Clayton, Melbourne, Victoria, Australia

The meeting will be a forum for the presentation and discussion of research on all aspects of electrochemistry.

The symposium will feature lectures by two renowned international experts and the Electrochemistry Division's 2013 Bond medallist:

Prof. David E. Williams (University of Auckland)

Prof. Julie V. Macpherson (University of Warwick, UK)

The A.M. Bond Medallist is to be announced.

Abstract deadline: 1 September 2013

See: www.raci.org.au/events/event/19th-australasian-electrochemistry-symposium

2013 NZIC Conference

1-5 December 2013, Victoria University of Wellington, Rutherford House, Pipitea Campus

Plenary Speakers include:

Pieter Dorrestein (Skaggs Institute/UCSD, Analytical Chemistry);

Ben Davis (Oxford; Organic Chemistry);

Jim Watkins (U. Massachusetts; Materials/Industrial Chemistry);

Tina Overton (Hull; Education);

Jeff Tallon (Callaghan Innovation, Physical Chemistry); and

Philip Power (University of California, Davis).

For venue information see: *www.victoria.ac.nz/vicvenues/venues/index.aspx*

A tentative booking has been made for delegate accommodation with Victoria's Te Puni Village. See: *www.tepunivillage.co.nz*

12th International Conference on Frontiers of Polymers and Advanced Materials

8-13 December 2013, University of Auckland

The 12th ICFPAM will bring together chemists, materials scientists and industry representatives for a major biennial conference series that is coming to New Zealand for the first time. Registrations are now open, and abstract submissions for oral and poster presentations may be made until the end of June.

Plenary speakers include:

Professor Jean M. J. Fréchet — Vice President for Research at King Abdullah University of Science and Technology;

Professor Paul S. Weiss — Fred Kavli Chair in Nanosystems Sciences at UCLA;

Professor Paras N. Prasad — SUNY Distinguished Professor at University at Buffalo, The State University of New York;

Professor Dan Shechtman — Nobel Laureate in Chemistry in 2011;

Professor Takuzo Aida — Director of the Riken Advanced Science Institute and Professor at the University of Tokyo; and

Professor Krzysztof Matyjaszewski of Carnegie Mellon University — who received the Herman F. Mark Award and Wolf Prize in 2011.

Themes include:

From Synthesis to Advanced Materials

Nano-scale and nano-structured materials and devices

Biomaterials and biopolymers

Organic based photonics and electronics materials

Composites and hybrid materials

See: http://www.icfpam2013.com/

Article

Qualitative testing of global QSAR models: The hERG K^+ ion pump.

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Key words: Known Drug Space, Benchmarking, Computer Aided Drug Design, Marketed Drugs

Introduction

With the rising costs of drug development, there is increased attention on reducing the failure rate of drug candidates in clinical trials.[1,2] One approach is to develop *in silico* methods to predict various biological processes which can cause adverse effects. An interesting example for such a predictive approach is the modelling of hERG (human ether-a-go-go related gene), a potassium ion channel in the heart. This is largely motivated by the realisation that drugs causing sudden cardiac death are linked to the inhibition of the hERG channel.[1] The blocking of this channel can result in a delayed rectification of the resting current of the heart; on an electrocardiograph (ECG) this is observed as a prolonged QT interval, commonly referred to as long Q - T syndrome (LQTS).[3] From 2000 to 2005 at least five drugs were withdrawn from the market owing to sudden cardiac death, and other drugs required a 'black box' warning.[1] The main problem that caused many of these drugs to be withdrawn was that the occurrence of LQTS was rare and therefore difficult to detect in the small cohorts of patients in the phase I and II clinical trials.[1,4] The structure of the drugs withdrawn is shown in Fig. 1.

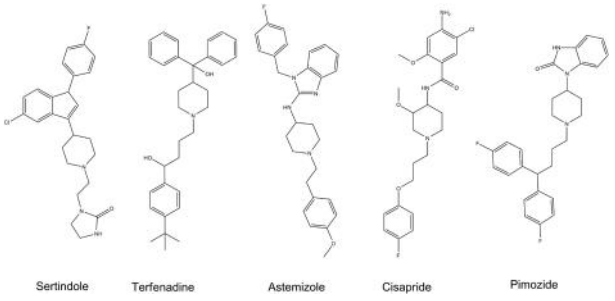

Fig . 1. The structure of the withdrawn drugs owing to cardiovascular issues. Sertindole and pimozide are antipsychotics, terfenadine and astemizole are antihistamines, and cisapride is indicated for gasprokinetic symptoms.

Development of structural models of hERG has been attempted by various research groups using isolated bacterial potassium channels.[5-7] These and other studies have led to the commercialisation of a number of predictive algorithms, including QSAR (Quantitative Structure Activity Relationship) models.[5] When the structures of the withdrawn drugs are considered, many similarities are apparent, i.e., a piperdine moiety is in the centre of an elongated molecule with lipophilic aryl ring systems on each end. Such similarities should aid the development of robust QSAR models. Many QSAR models work well within families of structures, but perform poorly when used to predict relationships for structures with a greater chemical diversity, i.e., for compounds with different structural motifs than found in the training set.[8] In order to introduce predictive tools into drug discovery programmes, and scientific work in general, their predictive power must be rigorously verified. The application of untested algorithms can damage the outcome of the projects because decisions are made that are based on unreliable or even simply wrong data. Unfortunately, the uncritical use of predictive algorithms is not unknown in the field of molecular modelling, degrading its reputation.[9]

An interesting way to test predictive algorithms developed for the use in drug discovery is to calculate the biological effect for a collection of drug molecules in clinical use, i.e., qualitatively rather than quantitatively.[10,11] The use of marketed drugs to explore the nature of known drug space was first introduced in the 1990s to identify frameworks and side chains unique to clinically approved small molecules.[12,13] Using known drugs is a simple yet a robust approach to gauge the reliability of predictive algorithms. In this work we apply this methodology to test an hERG affinity QSAR model to explore its applicability and compare it to more commonly applied quantitative testing.

Methodology

A list of 962 organic small drugs in clinical use was obtained from DrugBank,[14] representing a large portion of known chemical space. In addition, 76 experimental values for hERG inhibitors were collected from the literature. [1,3,4,6,15] All of the compounds were structurally optimised using the MM3 force field[16] in the Scigress 7.7.0.47 software suite.[17] The program QikProp 3.2[18] was used to generate the predicted hERG binding affinity Log IC_{50} values.

Results

All predicted Log IC_{50} values for effectiveness of the 962 organic small drugs are shown in Fig. 2. According to the developers of QikProp, predicted Log IC_{50} values below -5 indicate a strong interaction between the drugs and the hERG ion pump. These compounds are therefore considered to be of concern.

From Fig. 2 a large proportion (377 drugs, 39%) of the predicted values are under the suggested limit of log -5. If the predictions were indeed accurate, surely the incidence of LQTS would be drastically increased for the people on these medications, resulting in a high incidence rate of torsades de pointes (polymorphic ventricular tachycardia) and sudden death. This clearly shows that more work is required to refine the QSAR model, since a large cohort of known drugs is deemed unsuitable for drug discovery programmes. However, the predicted Log IC_{50} values for

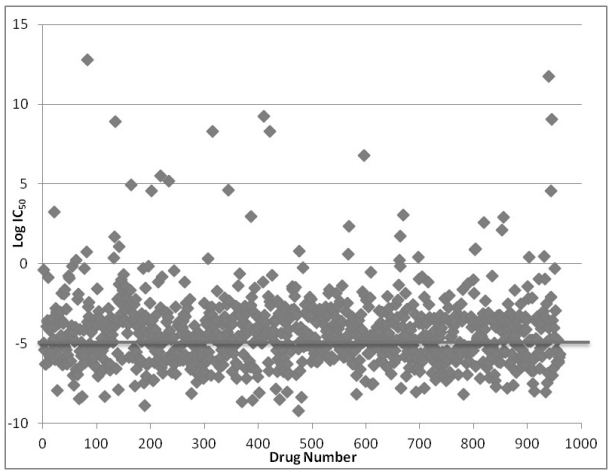

Fig. 2. The calculated Log IC_{50} values for hERG binding for a collection of drugs used in the clinic (n = 962). A Log IC_{50} value below -5 is considered to be of concern.

the withdrawn drugs shown in Figure 1 were all under the -5 limit suggesting potential cardiovascular toxicity. The experimental and calculated log IC_{50} values for astemzole were -9.1 and -8.4; for sertindole, -8.5 and -7.0; and for pimozide, -7.7 and -8.1. Also, when only cardiovascular drugs are considered, the average predicted Log IC_{50} value is -4.3 ± 2.4., with about half (36 / 82, 44%) of these cardiovascular drugs having a predicted Log IC_{50} lower than -5. Inherently cardiovascular drugs need to reach the heart to exert their efficacy. In general, the pharmacokinetics of the drugs must be taken into consideration, as it determines the concentration reaching the heart and therefore the hERG channel.

In a similar study the Log IC_{50} for hERG affinity was calculated for 465 orally bioavailable drugs on the market using the QikProp software.[10] Two thirds (66%) of those were predicted to be under the -5 limit, which is a somewhat a larger percentage than reported here. However, in this study the drugs used have various routes of administration, which can explain the difference in the results.[10]

The classical way of correlating experimental and theoretical data is to plot the two data sets against each other. A collection of 76 experimental hERG IC_{50} values were compiled from the literature.[1,3,4,6,15] These values were plotted against their predicted counterparts and the results are shown in Fig. 3.

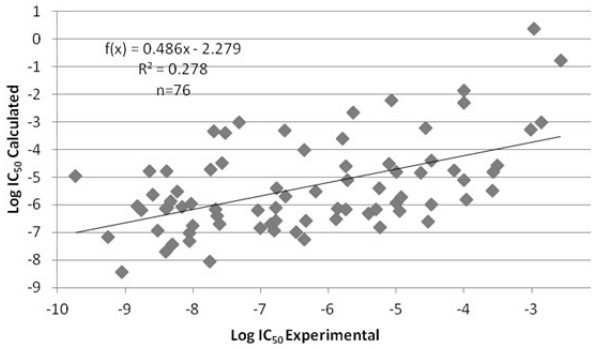

Fig. 3. The experimental determined Log IC_{50} values for hERG inhibition plotted against their predicted counterparts for 76 organic compounds. The best-fit line, *f(x)* = Ax + B, is shown.

A weak linear trend is seen in Fig. 3, reflected in a low Pearson's correlation coefficient (R^2) of 0.278. Furthermore, the slope and *y*-axis intercept are far from their ideal values of A = 1 and B = 0, respectively.

In Fig. 4 the structures of four antiarrhythmic drugs and a known hERG inhibitor, E-4031, are shown together with their calculated and experimental log IC_{50} values. It is clear that the algorithm gives good correlation with experimental data for these inhibitors with R^2 = 0.740 and a fitted line of *f(x)* = 0.683x – 0.2175 (fig. not shown).

This contrasts with the poor linear correlation of in Fig. 3. It is possible that the QSAR model is well parameterised for the structures shown in Figure 4 and therefore performs well. However, it must also be considered that their experimental data from the literature could have been used to build the QSAR model under investigation and therefore renders the comparison invalid. Without the knowledge of the training set's content it is impossible to establish whether the QSAR model is preforming well. Indeed, the qualitative test shown in Figure 2 supports the notion that the model is incomplete, demonstrating the usefulness of qualitative testing.

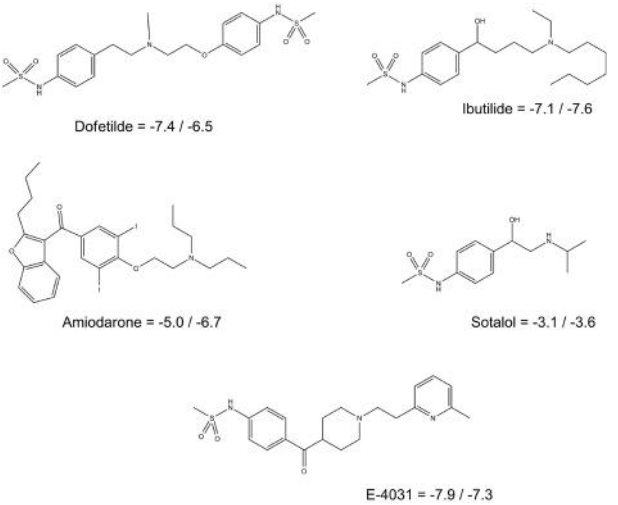

Fig. 4. The structure of four antiarrhythmic drugs and E-4031, a known hERG inhibitor. The calculated and predicted hERG log IC_{50} values are given (predicted / experimental).

Experimental Data

The patch clamp assay is the primary method used to measure hERG IC_{50} values based on the HEK-293 (human embryonic kidney) or CHO-k1 (Chinese hamster ovary) cell lines.[5,15,19,20] This assay derives the binding of a specific drug by the reduced electric current of the ion channels.[21,22] It is known from the literature that considerable inconsistency – an order of magnitude is not uncommon – is reported for the experimentally derived hERG Log IC_{50} values between laboratories.[5,23] One reason for this discrepancy is the different levels of the hERG channel expressed in the cells used.[23] Interestingly, the concentration of the hERG channels in the membranes of the model cells is unknown. Furthermore, these experiments are sensitive to temperature, e.g., for the drug sotalol an increase of 13°C resulted in a change of the log IC_{50} value from -3.1 to -3.6.[23] A prerequisite for building and testing quality QSAR models is to have access to robust experi-

mental datasets, i.e., the model can only be as good as the data used. An additional consideration for model builders is that the calculated physiochemical properties used for QSARs also have theoretical errors.[10] Indeed the calculations made by QikProp are based on the entire molecule's topological and calculated physiochemical properties.[15]

Conclusions

Qualitative testing of global QSAR models is clearly useful. First, it is simple and quick to run calculations on a collection of marketed drugs. Second, it sidesteps the problem of collating a consistent dataset of experimental data for benchmarking, which is often not available in the public domain. Finally, using known drug space truly tests the global prediction power of the algorithm under investigation owing to the vast chemical diversity found in marketed drugs.[24] We believe that the methodology presented here is a valuable tool for molecular modellers to test the quality of the theoretical models they employ.

References

1. Aronov, M. A. *Drug Discovery Today* **2005**, *10*, 149-155.
2. Csermely, P.; Korcsmaros, T.; Kiss, H.J.M.; London, G.; Nussinov, R., *Pharmacol. Therapeut.* **2013**, *138*, 333-408.
3. Cavalli, A.; Poluzzi, E.; De Ponti F.; Recanatini, M. *J. Med. Chem.* **2002**, *45*, 3844-3853.
4. Fenichel, R. R.; Malik, M.; Antzelevitch, C.; Sanguinetti, M.; Roden, D. M.; Priori, S. G.; Ruskin, J. N.; Lipicky, R. J.; Cantilena, L. R., *J. Cardio. Electrophys.* **2004**, *15*, 475-495.
5. Seierstad, M.; Agrafiotis, D. K. *Chem. Biol. & Drug Design* **2006**, *67*, 284-296.
6. Mitcheson, J. S.; Chen, J.; Lin, M.; Culberson, C.; Sanguinetti, M. C., *Proc. Nat. Acad. Sci.* **2000**, *97*, 12329-12333.
7. Aronov, A. M., *Curr. Opin. Drug Dis. & Devel.* **2008**, *11*, 128-140.
8. Leach, A. R.; Gillet, V. J. *An Introduction to Chemoinformatics* Dordrecht, Springer, 2007, Chapter 5, pp. 75-97.
9. Höltje, H.; Sippl, W.; Rognan, D.; Folkers, G. *Molecular Modeling: Basic Principles and Applications* 2nd Edition, Weinheim, Wiley-VCH, 2003, Chapter 1.2, pp. 3-4.
10. Ioakimidis, L.; Thoukydidis, L.; Naeem, S.; Mirza; A.; Reynisson, J., *QSAR Comb. Sci.* **2008**, *27*, 445-456.
11. Mirza, A.; Desai, R.; Reynisson, J., *Eur. J. Med.Chem.* **2009**, *44*, 5006-5011.
12. Bemis, G. W.; Murcko, M. A., *J. Med. Chem.* **1996**, *39*, 2887-2893.
13. Bemis, G. W.; Murcko, M. A., *J. Med. Chem.* **1999**, *42*, 5095-5099.
14. Wishart, D. S.; Knox, C.; Guo, A. C.; Cheng, D.; Shrivastava, S.; Tzur, D.; Gautam, B.; Hassanali, M., *Nucl. Acids Res.* **2008**, *36*, D901-D906.
15. Song, M.; Clark, M., *J. Chem. Inf. Model.* **2005**, *46*, 392-400.
16. Lii, J. H.; Allinger, N. L., *J. Am. Chem. Soc.* **1989**, *111*, 8576-8582.
17. Scigress Explorer Ultra Version 7.7.0.47. (2000-2007) Fujitsu Limted.
18. QikProp 3.2 (2009). Schrödinger, New York.
19. Axerio-Cilies, P.; Castañeda, I. P.; Mirza, A.; Reynisson, J., *Eur. J. Med. Chem.* **2009**, *44*, 1128-1134.
20. Hamill, O. P.; Marty, A.; Neher, E.; Sakmann, B.; Sigworth, F. J., *Pflugers Arch.* **1981**, *391*, 85-100.
21. Snyders, J.; Knoth, K. M.; Roberds, S. L.; Tamkun, M. M., *Mol. Pharm.* **1992**, *41*, 322-330.
22. Rampe, D.; Wible, B.; Fedida, D.; Dage, R. C.; Brown, A. M., *Mol. Pharm.* **1993**, *44*, 642-648.
23. Kirsch, G. E.; Trepakova, E. S.; Brimecombe, J. C.; Sidach, S. S.; Erickson, H. D.; Kochan, M. C.; Shyjka, L. M.; Lacerda, A. E.; Brown, A. M., *J. Pharmacol. Toxicol. Meth.* **2004**, *50*, 93-101.
24. Bade, R.; Chan, H.F.; Reynisson, J., *Eur. J. Med. Chem.* **2010**, *45*, 5646-5652.

Grants and awards

Prime Minister's Science prizes

Entries are open for the 2013 Prizes. There will be five prizes awarded with a combined value of $1 million. The categories are:

Science Prize, $500,000

To an individual or team which has made a transformative discovery or achievement in science that has had a significant impact on New Zealand or internationally

MacDiarmid Emerging Scientist Prize, $200,000

To an outstanding emerging scientist undertaking a PhD or within five years of the date of the award of a PhD

Science Teacher Prize, $150,000

To a science teacher for outstanding achievement in teaching science

Science Media Communication Prize, $100,000

To a practising scientist who is an effective communicator, to provide them with an opportunity to further develop their knowledge and capability in science media communication

Future Scientist Prize, $50,000

Awarded to a secondary school student for outstanding achievement in carrying out a practical and innovative research or technology project.

Applications are due by 17 July 2013

See: www.pmscienceprizes.org.nz

Primary Science and Endeavour Teacher Fellowships

Applications are now open for Primary Science and Endeavour Teacher Fellowships for terms one and two 2014.

Primary Science Teacher Fellowships

This is a two-term Fellowship designed to create curriculum leaders in science for the primary sector.

Endeavour Teacher Fellowships

This is a two-term Fellowship open to all fully registered teachers who have taught in the sciences, mathematics or technology areas for five or more years.

Applications are due by Tuesday 20 August 2013

See: http://www.royalsociety.org.nz/media/p-murphy-home.jpg

BayerBoost Scholarship Opportunities for Year 13 students

Funded by Bayer New Zealand and administered by the Royal Society of New Zealand, BayerBoost provides scholarships for secondary school students to undertake environmental research projects during their summer break.

As well as obtaining funding, scholarship recipients receive guidance from a host organisation to carry out their environmental study.

Applications close on Friday 20 September at 5.00pm

See: http://www.bayerboost.co.nz/

Peptides: From Emil Fischer to Psa

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Key words: Peptides, Solid Phase Peptide Synthesis, Therapuetic Potential, Antimicrobial Petpides, Psa, Fire Blight

Introduction

Peptides represent a ubiquitous class of small molecular weight biomolecules with immense biological potential. Peptides are formed from their building block amino acids by dehydration. Early functional peptides were short and made up of very few amino acids such as Gly, Ala, Val and Asp produced in prebiotic synthesis and meteorites.[1] The biosynthetic origin of natural peptides involves either the post-translational modification of protein precursors or the direct assembly from amino acid building blocks using synthetases (in lower organisms). The latter process allows the incorporation of unusual amino acids not recognised by the genetic code. Peptides are attractive candidates for fundamental research as well as for specific applications. Designed peptides serve as models to understand the intricate process of protein structure, folding, function and biochemical mechanisms of several pathological conditions. Novel peptide structures can be designed from first principles or as analogues of naturally occurring counterparts for targeted applications. Peptide chemists often use structural modifications to create novel molecules suitable for the desired application. Unfortunately, peptides have been in the news recently for the wrong reasons too! (see http://www.nzherald.co.nz/sport/news/article.cfm?c_id=4&objectid=10880545). This article provides a brief history of the chemical synthesis of peptides, followed by the common structural modifications employed in peptide chemistry with emphasis on non-protein amino acids. A brief mention of the antimicrobial peptide research in the author's laboratory at the University of Auckland is made towards the end, with concluding remarks on the opportunities and challenges offered by these fascinating molecules.

Chemical Synthesis – an historical perspective

The beginning of chemical peptide synthesis dates back to Emil Fischer's synthesis of the dipeptide glycyl-glycine in 1901 by partial hydrolysis of the diketopiperazine of glycine in the laboratory.[2] A year later, at the 14th meeting of the German Natural Scientists and Physicians, Fischer coined the term 'peptide', derived from the Greek word pepsis (digestion). The lack of reversible amino protecting groups hampered Fischer's efforts at progressing peptide chemistry any further, until the introduction of the amino protecting group, benzyloxycarbonyl (Cbz) by Leonidas Zervas in 1932.[3] The synthesis of the nonapeptide hormone oxytocin (Fig. 1) by du Vigneaud in 1953 was a major milestone in the history of peptide synthesis, for which he received the Nobel prize in Chemistry in 1955.[4] However, du Vigneaud's method of solution phase synthesis was tedious because of the necessity of isolating and purifying intermediate fragments. The limitations of solution phase methods for the synthesis of longer sequences, led to the invention of the Solid Phase Peptide Synthesis (SPPS) strategy which revolutionized the field of peptide chemistry.[5] The synthesis of complex peptides and small proteins such as Ribonucleases A and S were achieved by the application of this methodology.[6] The SPPS strategy has undergone significant improvements since then, particularly with the introduction of the base labile amino protecting group – fluorenylmethoxy carbonyl, Fmoc, by Louis Carpino in 1972.[7] Development of new solid supports (resin) used in the SPPS strategy has been another major contribution that has led to the growth in peptide synthesis.[8] Technological advancements also led to the introduction of high-tech automated peptide synthesizers, making the synthesis of long polypeptides a routine job for peptide chemists. The advent of High Performance Liquid Chromatography (HPLC)[9] added yet another dimension to the field by simplifying the difficult task of analysing and purifying the synthetic peptides produced by SPPS.

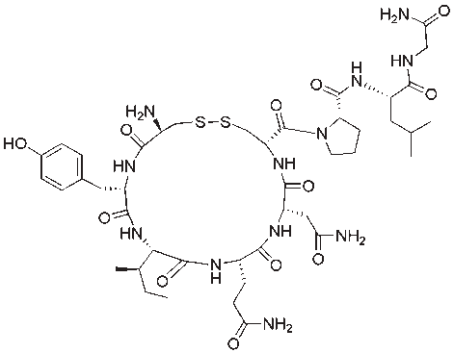

Fig. 1. Chemical structure of the nonapeptide hormone oxytocin

Even though success has been mostly confined to syntheses on a research scale, the large scale production of therapeutic peptides has gathered momentum during the past decade as evidenced by the multi-kilogram synthesis of the anti-HIV peptide Fuzeon by Roche to combat the latest medical challenge faced by mankind – the HIV virus.[10] This was achieved through a hybrid solid and solution phase synthesis method.[11] Another major breakthrough in peptide synthesis was the development of Native Chemical Ligation by the Kent Laboratory which permits the chemoselective ligation of unprotected peptide fragments, drastically expanding the size of synthetic proteins achievable by chemical means.[12]

Bulk Peptide Production – opportunities and challenges

It is important to develop a highly economical and reproducible process (synthesis and purification) for a manufacturing scale operation. Additionally, storage, trans-

portation and waste-management need to be carefully managed when going from laboratory scale to the production scenario. Up-scaling can lead to the accumulation of common by-products from coupling reagents (urea, phosphotriamides, etc.) necessitating a critical evaluation of the bench-scale process before scaling up. Despite the high level of environmental and personal protection provided by modern chemical laboratories, the adverse effect of chemicals (carbodiimides, HBTU, HOBt etc., see Fig. 2) can cause problems in bulk production. Increased alertness to safety precautions and use of appropriate protective equipment are necessary to deal with these issues. As in any drug development scenario, strict regulatory requirements need to be met in the development of peptides for clinical use on a commercial scale. Any changes in process or reagents are required to be part of the Investigational New Drug (IND) application (for phase I clinical trials) and the New Drug Application (NDA) submitted to the Food and Drug Administration (FDA) on completion of the subsequent phases of clinical trials.

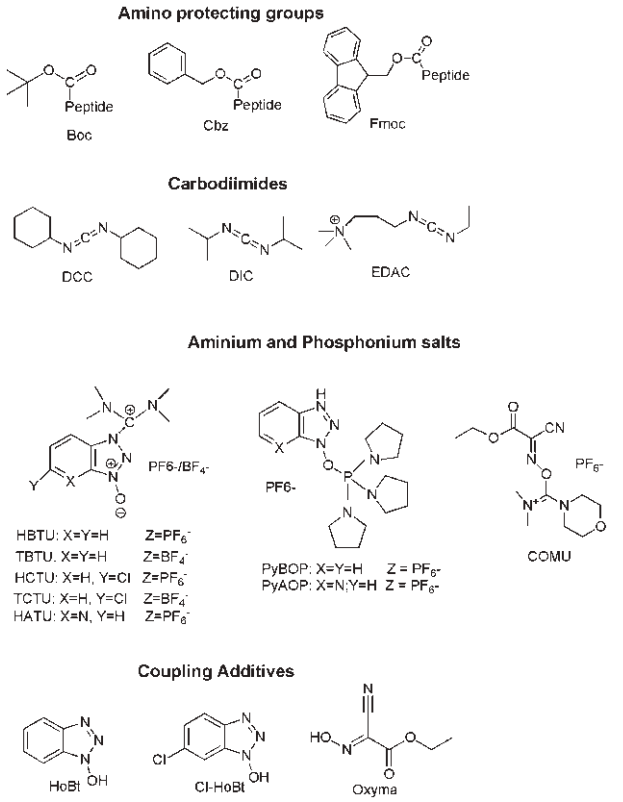

Fig. 2. Chemical structures of three amino protecting groups Boc, Cbz and Fmoc, common coupling reagents and additives used in peptide synthesis.

However, technological advancements in SPPS and HPLC enable the use of identical processes for all phases of drug development from discovery through to clinical trials and commercial production. The dramatic increase in the number of new peptide Active Pharmaceutical Ingredients (API) approved during the last decade (the majority of which have been made through chemical synthesis) and hundreds of new peptides currently under clinical development (facilitated by the success of the human genome project)[13] is evidence of the potential of chemical peptide synthesis as the method of choice for development of peptides for clinical research.

Therapeutic Potential

Naturally occurring peptides perform a wide range of functions as hormones, enzyme inhibitors, neurotransmitters, growth promoters and immunomodulators, which make them ideal targets in drug design. As potential therapeutics, peptides have increased specificity (over small molecules) and the advantage of smaller size (in comparison to antibodies). However, inherent conformational flexibility, rapid renal clearance, lack of *in vivo* stability, difficulty in crossing biological membranes and cost of syntheses are obstacles that have hampered their development as main stream pharmaceuticals in the past. The only notable exceptions to these are the peptide hormones insulin and oxytocin. The therapeutic potential of peptide drugs makes it interesting to solve these problems. The aim of structure-based drug design is to combine the biological and structural properties to create novel compounds with enhanced bioactivity. The ever expanding arsenal of peptide chemistry[14] allows the structure of natural peptides to be modified to generate conformationally restricted analogues for pharmacological applications. The following section briefly reviews the basics of polypeptide chain conformation and the methods used by peptide chemists to generate novel peptide structures with enhanced biological activity.

Chemical Modification of Peptide Structure

Polypeptide chain conformations are dictated by two degrees of torsional freedom about N-C^a (ϕ) and C^a-C' (ψ) (Fig. 3). The torsional angle about C' to N is either restricted to 180° (trans) or 0° (cis), with the trans conformation being the predominant one in nature. The net effect is that the backbone conformations adopted by peptides are a consequence of the f and y torsional angles at individual amino acid residues. Seminal work by Ramachandran and colleagues in the 1960s established that stereochemically allowed backbone conformations in peptides and proteins are determined by van der Waals criteria that prohibit the approach of non-bonded atoms beyond the sum of their van der Waal's radii.[15] Their calculations established that the energetically preferred backbone conformations for a peptide are the α-helix, the β-sheet and the β-turn, the most common secondary structural elements in proteins. Slight variations of these secondary structures do exist in nature (Table 1). These secondary structural elements organise to form supersecondary structures – the helix-turn-helix motif, the β-hairpin, four-helix bundle etc., which are not discussed further here.

Peptides can be restricted to the required conformation in order to achieve increased binding specificity to the target and enhanced resistance to proteolysis by the introduction of constraints that provide specific structural and stereochemical properties.[16] These include backbone cyclizations,[17] use of novel scaffolds,[18,19] use of metal ions,[20] incorporation of non-protein amino acids,[21,22] branching the peptide chain (dendrimers),[23] use of peptidomimetics,[18,24] peptide bond surrogates,[25] and peptoids.[26] Peptide structures being non-palindromic in nature, sequence reversal and inversion of chirality in synthetic mimetics (the retro-inverso approach) represent another powerful method for modifying native peptides to overcome their

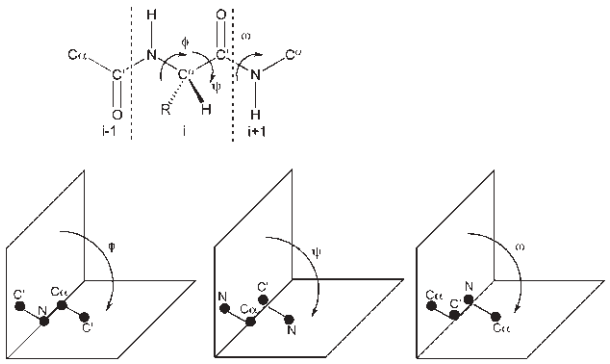

Fig. 3. Definition of torsional angles in peptides (redrawn from reference 16)

inherent drawbacks.[27] Figs. 4 and 5 show the structures of representative non-protein amino acids and some of the structural modifications mentioned above.

A wide range of possibilities exist for modification of peptide structure at the amino acid level (Fig. 4). These include the use of mono alkylation at N or C^{a}atoms, dehydro amino acids, homologs of a amino acids (β, γ, etc.) D-amino acids, and $C^{a,a}$-dialkylated amino acids. Interest in $C^{a,a}$-dialkylated amino acids was stimulated by the widespread occurrence of the parent amino acid in this category, i.e., α-amino isobutyric acid (Aib) in peptaibol antibiotics.[28] Alkylation at the α carbon dramatically restricts the torsion angles ϕ and ψ for $C^{a,a}$-dialkylated amino acids.[22] A wealth of information exists in the literature on structural and biological studies of peptides incorporating Aib and its higher homologues with linear and cycloalkyl side chains.[29,30] Theoretical calculations have indicated that Aib (and its higher homologues with cycloalkyl side chains) favour β-turns in short peptides and helices in longer peptides, whereas the dialkyl glycines with linear side chains (except Aib) favour extended conformations.[31] Substitution at the β-carbon generates a new chiral centre, resulting in diastereomers. N-alkylation reduces the energy barrier for rotation about the N-alkylated peptide bond, affecting the cis-trans ratio. Additionally, the ability of the peptide NH to act as a hydrogen bond donor is eliminated because of alkylation at the nitrogen atom. A similar situation exists in the case of peptoids. α,-β-dehydro amino acids restrict backbone conformations in peptides and promote β-turns in short peptides and helices in longer peptides. Our laboratory is engaged in the use of α,α-dialkyl glycines and other non-protein amino acids in biologically active peptides.

Antimicrobial Peptides

The wide ranging applications of peptides include pharmaceuticals, cryoprotectants, bioadhesives, biomaterials, biosensors, catalysts and cosmetics, to name a few. Of these, antimicrobial peptides have gained particular attention as human therapeutics as well as for plant protection.[32] Antimicrobial peptides (AMPs) are small molecules made up of fewer than 50 amino acids; they possess a net positive charge and are amphipathic in nature. This cationic nature and amphipathicity play important roles in their mechanism of action. In particular, the cationic nature of AMPs makes them selective to bacterial (microbial) cell membranes with a high density of anionic phospholipids. The mechanism of action of AMPs is currently thought to involve disruption of the bacterial membrane through a combination of electrostatic and hydrophobic interactions (Fig. 6) that eventually leads to cell lysis.[33] Unlike conventional antibiotics, such as streptomycin, development of resistance to AMPs is thought to be highly

Table 1. Characteristics of various helical structures, β-sheet and types I and I' β-turns in proteins or polypeptides*

Structure	φ (deg)	ψ (deg)	n	r	h (Å)	t (deg)
Right handed α-helix	-57	-47	3.6	13	1.5	100
3_{10}-heix	-60	-30	3	10	2.00	120
Left handed α-helix	+57	+47	3.6	13	1.5	100
π-helix	-57.06	-69.6	4.4	16	1.15	81.8
δ-helix	-98	-80	4.3	14	1.23	-85.4
ω-helix	-64.04	-55.06	4	13		90
α_{11}-helix	-93	-18	3.6	13	1.5	100
γ-helix	-84.64	-91.44	5.1	17	0.98	70
2.2_7-helix	-78.1	59.2	2.17	7	2.75	
2_7-helix	-74.9	69.5				
Collagen-helix	-51	153				
β -sheet	ϕ = -139	ψ = +135				
Type 1 β -turn	ϕ2 = -60 ϕ3 = -90	ψ2 = -30 ψ3 = 0				
Type 1'β -turn	ϕ2 = 60 ϕ3 = 90	ψ2 = 30 ψ3 = 0				

Biochim. Biophys. Acta.* **1971, *229*, 1-17 (IUPAC-IUB system of nomenclature)

ϕ and ψ are the backbone torsion angles; n = number of residues per turn; r = number of atoms in the hydrogen bonded ring; h = unit height and t = unit twist

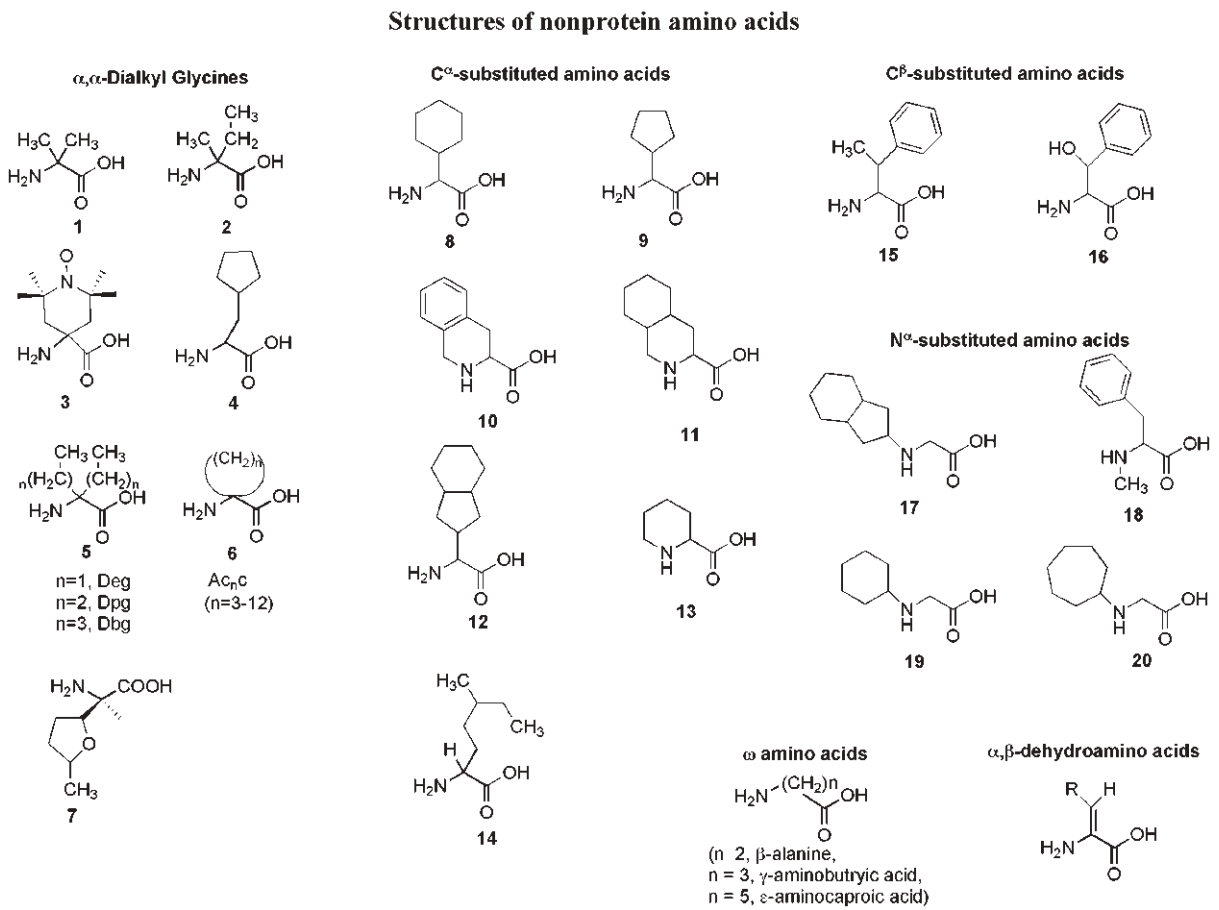

Fig. 4. Structures of some representative non-protein amino acids: **1**, α-amino isobutyric acid; **2**, isovaline; **3,** 2,2,6,6-tetramethylpiperidin-1-oxyl-4-amino-4-carboxylic acid, (TOAC); **4,** amino-3-cyclopentyl-propanoic acid; **5**, α,α-dialkyl glycines; **6**, 1-amino-cycloalkane-carboxylic acids; **7**, furanomycin; **8**, cyclohexylglycine; **9**, cyclopentylglycine; **10**, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; **11**, octahydroindole-2-carboxylic acid; **12**, α(2-indanyl)glycine; **13**, pipecolic acid; **14**, 2-amino-4-methylhexanoic acid; **15**, α-amino-β-phenylbutyric acid; **16**, β-phenylserine; **17**, N-(2-indanyl)glycine; **18**, N-methyl-phenylalanine; **19**, N-cyclohexylglycine; **20**, N-cycloheptylglycine. Also shown are the structures of ω amino acids and α,β-dehydroamino acids.

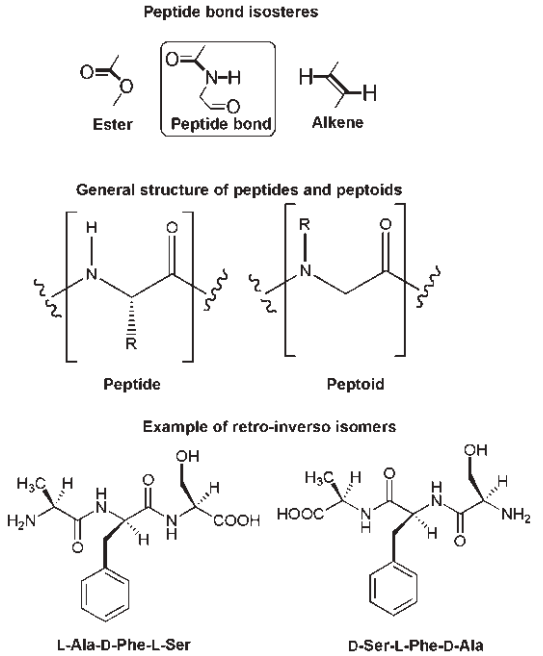

Fig. 5. Some of the common peptide bond modifications

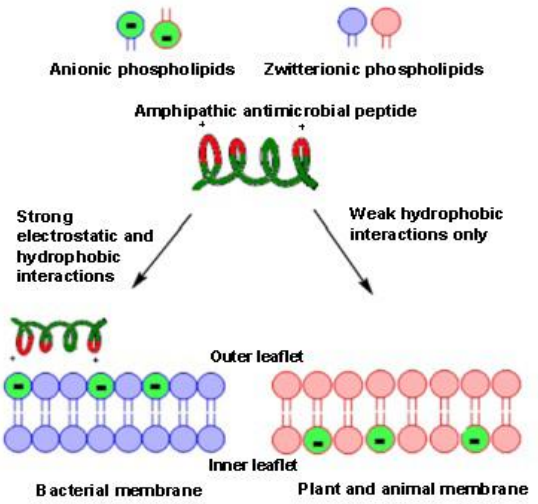

Fig. 6. A theoretical amphipathic α-helical antimicrobial peptide showing selectivity for the bacterial plasma membrane over those of plants and animals. AMP: Red = cationic, green = hydrophobic.

improbable, mainly because of their unique mechanism of antibacterial action. Since the main target of AMPs is the microbial membrane, bacteria (or other microbes) would have to significantly alter the structure of their membrane to gain resistance to AMPs, which is highly unlikely. Our research group has been involved in the development of antimicrobial peptides against two economically relevant phytopathogens in New Zealand – *Erwinia amylovora* (Ea) that causes the fire blight of rosaceous plants and *Pseudomonas syringae pv. actinidiae* (Psa) that causes the bacterial canker of kiwi fruit.

Peptides for Psa Control

Pseudomonas syringae pv. *actinidiae* (Psa) is a Gram negative bacterium that causes the bacterial canker of both green (*Actinidia deliciosa*) and yellow (*Actinidia chinensis*) fleshed kiwifruit. Recent outbreaks of Psa, particularly in New Zealand and Italy, have been devastating to the kiwi fruit industry. Fig. 7 shows some of the common symptoms of Psa infected kiwifruit plants. Globally coordinated disease control strategies are being developed to contain the pandemic and minimise economic loss to growers. These include strict orchard hygiene practices, breeding resistant varieties, scheduled spraying of bactericidal compounds, use of elicitors that activate the plant's immune system, and the use of biological control options as well as reliable detection methods.[34] Bactericidal agents like copper compounds and antibiotics like streptomycin, which are the most common chemical control options used in plant disease control, have the drawbacks of resistance development, phytotoxicity and regulatory issues. Lack of satisfactory chemical control options for Psa and the virulent nature of the New Zealand strain of Psa, viz., Psa-LV,[34,35] led us to investigate the potential of novel antimicrobial peptides for Psa control.

Fig. 7. Typical Psa disease symptoms: leaf spots (left), and collapsed fruits (right)

Three different types of synthetic antimicrobial peptides were investigated for their ability to inhibit Psa. The first of these (Table 2: Peptide 1) are hybrid versions of cecropin (the first known insect antibacterial peptide found in the haemolymph of the north American native silk moth)[36] and melittin (a 26 amino acid peptide found in the venom of the honey bee).[37] We designed novel sequences based on cecropin-melitin hybrid peptides (CM hybrid) by introducing additional aromatic residues near the N-terminus to facilitate better interaction with the bacterial membrane (hydrophobic contact). Amphipathic character was induced in the peptide sequence through the proper positioning of hydrophobic and hydrophilic amino acids (Fig. 8).

The second type (Table 2: Peptide 2) investigated for Psa control were hexapeptides that potentially bind Holliday junctions, preventing the resolution of dimeric chromosome structures, ultimately inhibiting cell division.[38] Analogues of a naturally occurring cyclic lipopeptide (Table 2: Peptide 3, 3a) were also investigated. Lead peptides showed potency against Psa strain 1602. These peptides were also tested against the fire blight pathogen (Table 2).

Scanning electron microscopy (SEM) revealed distinct morphological changes to bacterial cells treated with the different peptides, leading to blistering of the membrane and cell lysis. Mechanistic investigations indicated clear

Table 2. Antibacterial activity of peptides

Compound*	MIC (μg/mL)	
	Psa	Ea
Streptomycin	1-2.5	1-2.5
NFA	1-2.5	1-2.5
Peptide 1 (CM hybrid)	5-10	5-10
NFA-Peptide 1	10-15	15-25
Hexapeptide 2 (amide)	5-10	5-10
Hexapeptide 2 (acid)	No activity	No activity
NFA-Hexapeptide 2 (acid)	2.5-5	2.5-5
Peptide 3 (linear lipopeptide)	1-2.5	2.5-5
Peptide 3a cyclic lipopeptide)	5-10	15-25

***Compound key:** Peptide 1: Cecropin-melitin hybrid peptide and its NFA conjugated version. Peptide 2: Holliday junction binding Hexapeptide; and its NFA conjugated version; Peptide 3: Linear lipopeptide; Peptide 3a: Cyclic lipopeptide

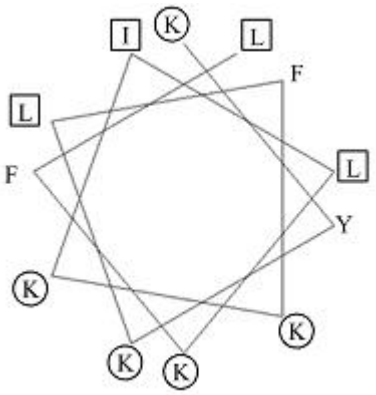

Fig. 8. An α-helical wheel diagram to depict the amphipathic nature of the cecropin-melittin hybrid peptide (modified from images generated using an online tool provided by the European Molecular Biology Open Software Suite)

differences in the mode of action between the peptides. Obvious membrane disruption was observed in CM hybrids, whereas the hexapeptides did not show signs of significant membrane disruption (Fig. 9). Our observations indirectly support the reported antibacterial mechanism of these peptides as Holliday junction binders which does not involve membrane disruption.

MIC analysis and SEM studies using peptides conjugated to small molecule drugs also allowed us to evaluate the ability of the peptides to transport such drug molecules across the bacterial membrane. The effect on MIC and Psa cell morphology after exposure to the peptides conjugated to 5-nitro-2-furaldehyde, the core toxic moiety in several antibacterial nitrofurans used in human and veterinary therapeutics, was analysed (Table 2 and Fig. 9). Comparison of Fig. 9(c) and (d) shows similar morphological changes to Psa cells treated with the hexapeptide and its NFA conjugated hexapeptide, distinctly different from the membrane disruption observed on treatment with the CM hybrid [Fig 9(b)]. The hexapeptides (peptide 2; Table 2) showed clear characteristics of a 'peptide shuttle', as reflected in the MIC assays and cell morphology analysis (Table 2, Fig. 9). The NFA conjugated peptide 2 acid showed higher potency than the unconjugated

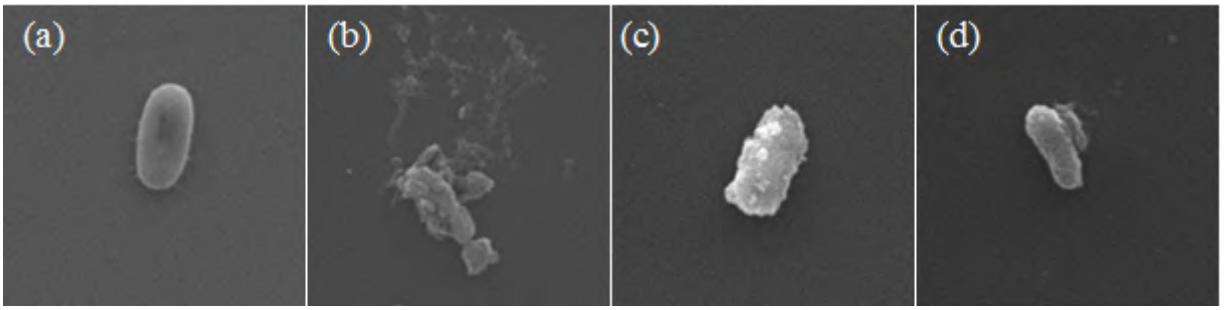

Fig. 9. SEM images of Psa cells showing representative morphology induced by (b) cecropin-mellitin peptides (c) NFA (d) NFA-hexapeptide conjugate, relative to a control (a)

peptide acid (no activity) or NFA itself, indicating that the increased potency arises from NFA. It also points to the ability of the peptide acid to target and shuttle small molecules like NFA across the membrane. This reinforces the potential of short non-membrane active peptides to be developed as receptor-targeted therapeutics – a new generation approach – which is attracting interest, particularly in anticancer therapy.[39] NFA conjugated CM hybrid did not show increased potency over the unconjugated analogue.

The lipopeptide 3 and its analogues showed moderate to good activity against Psa and Ea, with the most potent analogue showing activity at 1 mg/mL against Psa. Strong sequential NH-NH nuclear Overhauser effects (blue cross peaks labelled as 1/2, 2/3, etc. in Fig. 10) indicated that this peptide adopts a reasonably good helical structure.

Peptides for Fire Blight Control – Biofilm Inhibition

Biofilms are matrix-embedded microorganisms that grow on biotic and abiotic surfaces and are resistant to antimicrobial agents.[40] Microbial biofilms are ubiquitous in natural, industrial and clinical settings. *Erwinia amylovora*, the causative agent of fire blight, has been reported to form antibiotic resistant biofilms.[41] In an attempt to find compounds that inhibit Ea biofilms, we screened several compounds (antibiotics, peptides, amino acids etc.) against biofilms of *E. amylovora* strain 1501 (streptomycin sensitive) and Str4Ea (streptomycin resistant strain).[29,42] Selected compounds were investigated for their ability to disperse Ea biofilms. Representative fluorescence images of the Ea 1501 biofilms after "48 hour" incubation in the absence and presence of the compounds are shown in Fig. 11. Comparison of the control biofilm image (A) with that of the treated ones (B and C; 1 mg/ml) show clear dispersion of Ea1501 biofilms; indicating complete inhibition of biofilm formation in Ea1501 by the compounds. Fig.11 (F-H) shows the effect of the same compounds on the streptomycin resistant strain of Ea. As would have been expected, streptomycin did not show a noticeable effect in dispersing the Str4Ea biofilms (F vs G), as was evident by the presence of a large number of live cells (green spots). Even though the streptomycin treated Str4Ea biofilms showed changes in the overall architecture, the presence of several green live cells indicates that these are indeed resistant to streptomycin. In contrast, no live cells were present in the peptide treated sample, confirming its ability to inhibit biofilms formed by the streptomycin resistant strain of *E. amylovora* (Fig. 11, H).

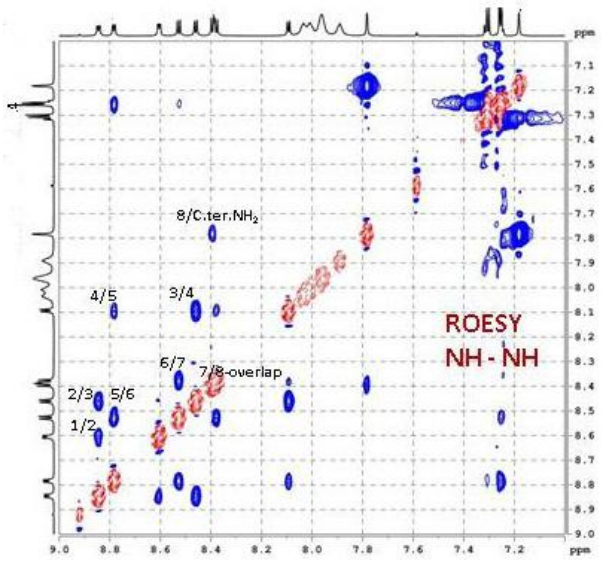

Fig. 10. Sequential NH-NH NOEs observed in peptide 3

These two case studies provide examples of AMPs with potential as chemical control options for economically relevant bacterial diseases prevalent in the NZ horticultural sector. Our group is also involved in the development of novel antimicrobial peptides against *Mycobacterium tuberculosis*,[43] and other MDR human pathogens (*S. aureus*, *P. aeruginosa*, *Klebsiella pneumoniae*, *E.coli* etc.) and diseases (diabetes and osteoarthritis), bioactive peptides for applications in food industry[44] and nutrition, as well as the design of peptide supersecondary structures, e.g., helix-turn-helix motif, to understand the fundamentals of protein structure and function.[45]

Concluding Remarks

Since the discovery of peptides more than one hundred years ago by Emil Fischer, peptide science has evolved considerably, aided by pioneering discoveries and developments in the field. New synthetic procedures, novel coupling reagents and the possibility of automation have enabled modern-day peptide chemists to develop novel peptides for various applications as a routine job. New peptides discovered and developed will provide solutions for the current unmet medical and pharmaceutical needs.

My journey in peptide science starting from my alma mater, the Indian Institute of Science, Bangalore, to my current research on peptides at the University of Auckland has been reviewed in this paper – including the history of peptide synthesis, the therapeutic potential of peptides,

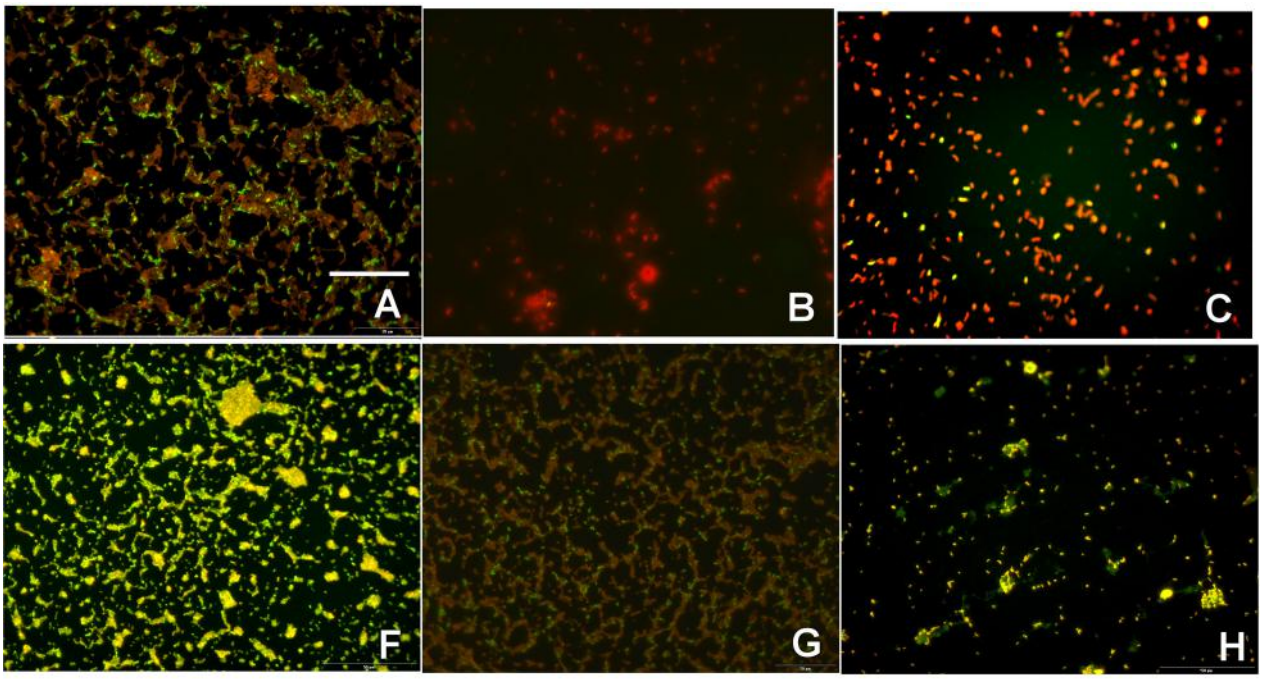

Fig. 11. Representative fluorescence images of *E. amylovora* 1501 (A-C) and Str4Ea (F-H) biofilms, after 48-hour incubation in the absence (A and F) and presence of test compound (C and H) or streptomycin (B and G). Bar 20 μm.[29]

basics of peptide conformation, structural modifications, properties and mechanism of action of antimicrobial peptides. This article is by no means an exhaustive review of the area and readers are directed to the various review and research articles cited here for more information.

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References

1 van der Gulik, P.; Massar, S.; Gilis, D.; Buhrman, H.; Rooman, M. *J. Theor. Biol.* **2009**, *261*, 531-539.

2 Fischer, E. *Ber. Dtsch.Chem. Ges.* **1907**, *40*, 1754-1767; Fisher, E. F., E. *Ber. Dtsch. Chem. Ges.* **1901**, *34*, 2868-2877.

3 Bergmann, M.; Zervas, L. *Ber. Dtsch. Chem. Ges.* **1932**, *65*, 1192-1201.

4 Duvigneaud, V.; Ressler, C.; Swan, J. M.; Roberts, C. W.; Katsoyannis, P. G.; Gordon, S. *J. Am. Chem. S*oc. **1953**, *75*, 4879-4880; Gordon, S.; Duvigneaud, V. *P. Soc. Exp. Biol. Med.* **1953**, *84*, 723-725.

5 Merrifield, R. B. *J. Am. Chem. S*oc. **1963**, *85*, 2149-2154.

6 Hirschman, R.; Nutt, R. F.; Veber, D. F.; Vitali, R. A.; Varga, S. L.; Jacob, T. A.; Holly, F. W.; Denkewal. Rg *J. Am. Chem. S*oc. **1969**, *91*, 507-508; Gutte, B.; Merrifield, R.B. *J. Am. Chem. S*oc. **1969**, *91*, 501-502.

7 Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404-3409.

8 Meldal, M. *Method Enzymol.* **1997**, *289*, 83-104; Martin, F. G.; Albericio, F. *Chim Oggi* **2008**, *26*, 29-34; Jensen, K. J.; Alsina, J.; Songster, M. F.; Vagner, J.; Albericio, F.; Barany, G. *J. Am. Chem.* Soc. **1998**, *120*, 5441-5452; Garcia-Martin, F.; Quintanar-Audelo, M.; Garcia-Ramos, Y.; Cruz, L. J.; Gravel, C.; Furic, R.; Cote, S.; Tulla-Puche, J.; Albericio, F. *J. Comb. Chem.* **2006**, *8*, 213-220; Garcia-Martin, F.; Bayo-Puxan, N.; Cruz, L. J.; Bohling, J. C.; Albericio, F. *Qsar Comb. Sci.* **2007**, *26*, 1027-1035.

9 Horvath, C.; Lipsky, S. R. *J. Chromatogr. Sci.* **1969**, *7*, 109-116.

10 Bray, B. L. *Nat. Rev. Drug Discov.* **2003**, *2*, 587-593.

11 Riniker, B.; Florsheimer, A.; Fretz, H.; Sieber, P.; Kamber, B. *Tetrahedron* **1993**, *49*, 9307-9320.

12 Dawson, P. E.; Muir, T. W.; Clarklewis, I.; Kent, S. B. H. *Science* **1994**, *266*, 776-779.

13 Lander, E. S.; Consortium, I. H. G. S.; Linton, L. M.; Birren, B.; Nusbaum, C.*et al. Nature* **2001**, *409*, 860-921.

14 Coin, I.; Beyermann, M.; Bienert, M. *Nat. Protoc.* **2007**, *2*, 3247-3256; Bruckdorfer, T.; Marder, O.; Albericio, F. *Curr. Pharm. Biotechno.* **2004**, *5*, 29-43.

15 Ramachandran, G. N.; Ramakrishnan, C.; Sasisekharan, V. *J. Mol. Biol.* **1963**, *7*, 95-99; Ramachan.G, N.; Sasisekharan, V. *Advances in Protein Chemistry* **1968**, *23*, 1283-1438.

16 Hruby, V. J. *Nat. Rev. Drug Discov.* **2002**, *1*, 847-858.

17 Gilon, C.; Halle, D.; Chorev, M.; Selinger, Z.; Byk, G. *Biopolymers* **1991**, *31*, 745-750; Gilon, C.; Halle, D.; Chorev, M.; Selinger, Z.; Goldshmith, R.; Byk, G. *Peptides 1990* **1991**, 404-406; Hruby, V. J. *Life Sci.* **1982**, *31*, 189-199; Kessler, H. *Angew. Chem. Int. Ed.* **1982**, *21*, 512-523.

18 Hirschmann, R. *Angew. Chem. Int. Ed.* **1991**, *30*, 1278-1301.

19 Gante, J. *Angew. Chem. Int. Ed.* **1994**, *33*, 1699-1720.

20 Ghadiri, M. R.; Choi, C. *J. Am. Chem.* Soc. **1990**, *112*, 1630-1632; Ghadiri, M. R.; Fernholz, A. K. *J. Am. Chem.* Soc. **1990**, *112*, 9633-9635.

21 Mazaleyrat, J. P.; Goubard, Y.; Azzini, M. V.; Wakselman, M.; Peggion, C.; Formaggio, F.; Toniolo, C. *Eur. J. Org. Chem.* **2002**, 1232-1247; Crisma, M.; Peggion, C.; Formaggio, F.; Kaptein, B.; Broxterman, Q. B.; Kamphuis, J.; Toniolo, C. *Peptides for the New Millennium* **2000**, 270-271; Formaggio, F.; Crisma, M.; Toniolo, C.; Tchertanov, L.; Guilhem, J.; Mazaleyrat, J. P.; Gaucher, A.; Wakselman, M. *Tetrahedron* **2000**, *56*, 8721-8734; Reissmann, S.;

Imhof, D. *Curr. Med. Chem.* **2004**, *11*, 2823-2844; Mahalakshmi, R., Balaram, P. In *D-amino acids: A new frontier in amino acid and preotein research - Practical methods and protocols* (Konno R, Bruckner H, D'Aniello A, Fisher G, Fujii N, Homma H.) Nova Publishers **2006**, 415-430.

22 Mahalakshmi, R. and Balaram, P. *Methods in Molecular Biology* **2006**, *340*, 71-94.

23 Bracci, L.; Falciani, C.; Lelli, B.; Lozzi, L.; Runci, Y.; Pini, A.; De Montis, M. G.; Tagliamonte, A.; Neri, P. *J. Biol. Chem.* **2003**, *278*, 46590-46595; Tam, J. P. *Methods in Enzymology* **1989**, *168*, 7-15; Tam, J. P. *J. Immunol. Methods* **1996**, *196*, 17-32; Tam, J. P.; Zavala, F. *J. Immunol. Methods* **1989**, *124*, 53-61.

24 Hirschmann, R.; Sprengeler, P. A.; Kawasaki, T.; Leahy, J. W.; Shakespeare, W. C.; Smith, A. B. *Tetrahedron* **1993**, *49*, 3665-3676; Marshall, G. R.; Nikiforovich, G.; Kaczmarek, K.; Plucinska, K.; Tafi, A.; Cornille, F.; Slomczynska, U.; Li, K. M.; Zhang, W. J. *J Cell Biochem.* **1993**, 203-203; Marshall, G. R. *Tetrahedron* **1993**, *49*, 3547-3558; Smith, A. B.; Akaishi, R.; Jones, D. R.; Keenan, T. P.; Guzman, M. C.; Holcomb, R. C.; Sprengeler, P. A.; Wood, J. L.; Hirschmann, R.; Holloway, M. K. *Biopolymers* **1995**, *37*, 29-53; Hruby, V. J. *Abstr. Pap. Am. Chem. S* **1995**, *209*, 135-MEDI; Tomasini, C.; Castellucci, N. *Chem. Soc. Rev.* **2013**, *42*, 156-172.

25 Rodriguez, M.; Heitz, A.; Martinez, J. *Int. J. Pept. Prot. Res.* **1992**, *39*, 273-277; Choudhary, A.; Raines, R. T. *Chembiochem.* **2011**, *12*, 1801-1807.

26 Park, M.; Wetzler, M.; Jardetzky, T. S.; Barron, A. E. *Plos. One* **2013**, *8*; Zuckermann, R. N. *Biopolymers* **2011**, *96*, 545-555.

27 Briand, J. P.; Benkirane, N.; Guichard, G.; Newman, J. F. E.; vanRegenmortel, M. H. V.; Brown, F.; Muller, S. *P. Natl. Acad. Sci. USA* **1997**, *94*, 12545-12550; Chorev, M.; Goodman, M. *Trends Biotechnol.* **1995**, *13*, 438-445.

28 Aravinda, S.; Shamala, N.; Balaram, P. *Chem. Biodivers* **2008**, *5*, 1238-1262; Degenkolb, T.; Berg, A.; Gams, W.; Schlegel, B.; Grafe, U. *J. Pept. Sci.* **2003**, *9*, 666-678.

29 Dezoysa, G. H.; Washington, V.; Lewis, G. L.; Sarojini, V. *Plant Pathology* (in press) **2013**.

30 Vijayalakshmi, S.; Rao, R. B.; Karle, I. L.; Balaram, P. *Biopolymers* **2000**, *53*, 84-98; Datta, S.; Rathore, R. N. S.; Vijayalakshmi, S.; Vasudev, P. G.; Rao, R. B.; Balaram, P.; Shamala, N. *J. Pept. Sci.* **2004**, *10*, 160-172; Karle, I. L.; Rao, R. B.; Prasad, S.; Kaul, R.; Balaram, P. *J. Am. Chem.* Soc. **1994**, *116*, 10355-10361; Balaram, P. *J Pept Res* **1999**, *54*, 195-199; Toniolo, C.; Benedetti, E. *Isi Atlas-Biochem* **1988**, *1*, 225-230; Nagaraj, R.; Shamala, N.; Balaram, P. *J. Am. Chem.* Soc. **1979**, *101*, 16-20; Burgess, A. W.; Leach, S. J. *Biopolymers* **1973**, *12*, 2599-2605; Marshall, G. R.; Bosshard, H. E. *Circ Res* **1972**, *30/31*, 143-150.

31 Benedetti, E.; Toniolo, C.; Hardy, P.; Barone, V.; Bavoso, A.; Diblasio, B.; Grimaldi, P.; Lelj, F.; Pavone, V.; Pedone, C.; Bonora, G. M.; Lingham, I. *J. Am. Chem.* Soc. **1984**, *106*, 8146-8152; Bonora, G. M.; Toniolo, C.; Diblasio, B.; Pavone, V.; Pedone, C.; Benedetti, E.; Lingham, I.; Hardy, P. *J. Am. Chem.* Soc. **1984**, *106*, 8152-8156; Paul, P. K. C.; Sukumar, M.; Bardi, R.; Piazzesi, A. M.; Valle, G.; Toniolo, C.; Balaram, P. *J. Am. Chem.* Soc. **1986**, *108*, 6363-6370.

32 Zasloff, M. *New Engl J Med* **2002**, *347*, 1199-1200; Montesinos, E. *Fems Microbiol. Lett.* **2007**, *270*, 1-11.

33 Zasloff, M. *Nature* **2002**, *415*, 389-395; Epand, R. M.; Vogel, H. J. *Bba Biomembranes* **1999**, *1462*, 11-28.

34 Scortichini, M.; Marcelletti, S.; Ferrante, P.; Petriccione, M.; Firrao, G. *Mol. Plant Pathol.* **2012**, *13*, 631-640.

35 Chapman, J. R.; Taylor, R. K.; Weir, B. S.; Romberg, M. K.; Vanneste, J. L.; Luck, J.; Alexander, B. J. R. *Phytopathology* **2012**, *102*, 1034-1044.

36 Hultmark, D.; Steiner, H.; Rasmuson, T.; Boman, H. G. *Eur. J. Biochem.* **1980**, *106*, 7-16.

37 Haberman.E; Jentsch, J. *H-S Z Physiol. Chem.* **1967**, *348*, 37-50.

38 Gunderson, C. W.; Segall, A. M. *Mol. Microbiol.* **2006**, *59*, 1129-1148; Gunderson, C. W.; Boldt, J. L.; Authement, R. N.; Segall, A. M. *J. Bacteriol.* **2009**, *191*, 2169-2176.

39 Sun, L. C.; Coy, D. H. *Curr. Drug Deliv.* **2011**, *8*, 2-10; Sun, L. C.; Coy, D. H. *Drug Future* **2008**, *33*, 217-223.

40 Parsek, M. R.; Singh, P. K. *Annu. Rev. Microbiol.* **2003**, *57*, 677-701.

41 Koczan, J. M.; McGrath, M. J.; Zhao, Y. F.; Sundin, G. W. *Phytopathology* **2009**, *99*, 1237-1244.

42 Sarojini, V. *Acs. Sym. Ser.* **2012**, *1095*, 397-414.

43 Amso, Z.; Miller, C.; O'Toole, R.; Sarojini, V. *J. Pept. Sci.* **2012**, *18*, S33-S33.

44 Kong, C.; Evans, C.; Sarojini, V. *J. Pept. Sci.* **2012**, *18*, S160-S160.

45 Rydberg, J., Baltzer, L. Sarojini, V. *J. Pept. Sci.* (in print) **2013**.

Article

Solid-state NMR of polyanilines with different morphologies

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Introduction

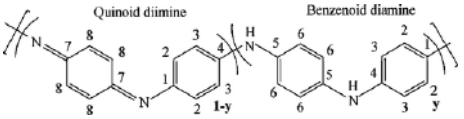
Electrically conducting polymers (CPs) constitute a unique group of materials, offering the possibility of controlled electrical conductivity combined with the processing characteristics, low production cost and stability of properties associated with polymers.[1,2] Polyaniline (PANI) is probably one of the most interesting CPs owing to its reasonably high conductivity (100 S/cm) upon an acid doping, good processability, ability to form various nanostructures and good environmental stability.[3] It is the only electro-organic polymer whose conductivity can be controlled in two independent ways: (a) controlling the degree of protonation, and (b) adjusting the degree of oxidation.[4] It is well known that the level of protonation can change PANI from an insulator (10^{-10} S/cm) to a semiconductor (100 S/cm).[3] Therefore, the wide range of its related electrical, electrochemical and optical properties, together with its good environmental stability, make PANI attractive for possible applications in a variety of fields such as antimicrobial agents, energy storage, catalysts, antistatic coating, sensors, nanotechnology.[1]

One of the most disadvantageous characteristics of conducting PANI is its insolubility in common organic solvents. Therefore, methods for the structural characterisation of various forms of PANI are limited and it is of paramount importance to have a technique available which can give structural information about the material in its native form. Solid-state NMR (SSNMR) is a non-destructive method. Because the technique can be used on amorphous as well as on crystalline samples, it is not surprising that it has been widely used in the study of amorphous PANI materials. Comparative advantages of SSNMR over other analytical techniques are its capability to investigate different materials using multinuclear and multidimensional experiments (2D or 3D). Besides structural data, SSNMR can give information about molecular dynamics and mobility of various structural units. In this article we will show the usefulness and limitations of SSNMR to detect subtle structural differences in PANI samples with different morphologies in conjunction with FTIR and UV-Vis spectroscopy.

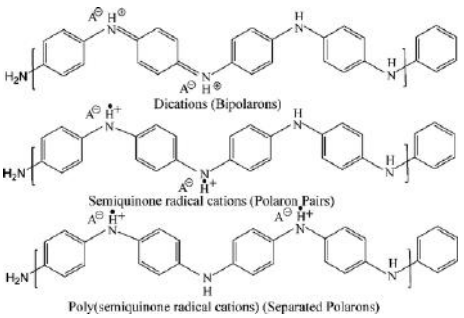
Cross-polarization (CP) is a double resonance solid-state NMR technique designed to overcome two common problems in the NMR of solid samples: low sensitivity and the long time needed to acquire spectra. The sensitivity is significantly improved by magnetization transfer from abundant *I* (usually hydrogen) to low abundant spins *S* (usually ^{13}C, ^{15}N, etc). On the other hand, experimental time is extensively shortened owing to the fact that the CP experiment repetition time is determined by the *I* spin-lattice relaxation time (T_1). The resolution in solid-state NMR spectra is considerably improved by introducing MAS (magic angle spinning) where the sample is rotated at the specific angle (magic angle, 54.7°) which reduces or completely removes direct dipolar coupling and chemical shift anisotropy.

SSNMR in Structural Investigations of PANIs – an Overview

Hjertberg *et al*. reported a ^{13}C CPMAS (cross-polarization magic angle spinning) investigation of PANI,[5] confirming the benzenoid-quinoid alternating structure (Scheme 1).[6]



Scheme 1. The chemical structure of PANI, where y = 1 is Leucoemeraldine Base (LEB), y = 0.5 is Emeraldine Base (EB), y = 0 is Pernigraniline base (PNB). Reprinted with permission from publishers of ref. 6.



Scheme 2. Various protonation states of the emeraldine salt form of PANI. Reprinted with permission from publishers of ref. 7.

Satfstrom *et al*. compared modified neglect of diatomic overlap (MNDO) calculations with NMR spectra.[8] They showed that the structure of PANI is unchanged upon protonation (Scheme 2) and that the positive charge introduced by the proton is delocalized in the polymer. Kaplan *et al*. investigated the structure of PANI in emeraldine (EB) and leucoemeraldine (LEB) form (Scheme 1), determining that the emeraldine base consists primarily of a well-defined microstructure of alternating benzenoid diamine and quinoid diimine units,[9] although their results showed that PANI was slightly overoxidised ($y < 0.5$, Scheme 1). Menardo *et al*. used ^{13}C NMR studies to confirm that reduced PANI only consists of benzenoid rings and amine nitrogens (Scheme 1).[10]

In one of the first papers related to ^{15}N CPMAS of PANI, Wehrle *et al*. have proposed this method as promising and useful for structural investigations of ^{15}N labelled PANI and other heterocyclic polymers which have nitrogen.[11] Stein *et al*. performed variable contact time experiments to reveal the structure of PANI at various temperatures.[12] They assigned resonances in the PANI spectrum based on different cross-relaxation behaviour of different carbons depending on their distance from the protons. Also, they observed changes in the spectra upon crossing the insulating-conduction transition which are in line with the motion of bipolarons in the polymer chain. In their paper Kaplan e*t al*. applied ^{13}C and ^{2}H SSNMR measurements to reveal the structure and dynamics of different forms of PANI.[13] Richter *et al.* used ^{15}N CPMAS spectroscopy for structural investigations of LEB and EB forms of PANI, [14] confirming that EB polymer exists as an alternating copolymer of oxidised and reduced units. Stein *et al*. presented a ^{13}C two-dimensional chemical exchange NMR experiment that gave information about the local structure and the ratio of freely moving rings to rigid rings.[15] This experiment also confirmed inhomogeneous broadening of the CPMAS lines in PANI spectrum. Adams *et al.* prepared ^{15}N enriched PANI samples with different molecular weights.[16,17] They compared GPC (gel permeation chromatography) results with NMR data obtained by end group analysis. Kolbert *et al*. investigated the metallic nature of highly conducting PANI by using relaxation measurements at variable temperatures along with two-dimensional spin exchange experiments,[18] which showed heterogeneity in the sample over a distance of at least 30 Å. They observed Korringa relaxation in PANI, which is strong evidence for a metallic state in highly conducting PANI. Espe *et al*. explored anhydrous ^{15}N labelled PANI powders as a function of HF doping by ^{13}C, ^{15}N, and ^{19}F SSNMR.[19] They revealed the presence of three different types of charged environments. Also, they showed that the entire crystalline region (pseudo-metallic and conducting) and amorphous regions within 50 Å of the crystalline boundary have been unobservable with SSNMR owing to the efects of paramagnetic delocalized electrons.

Kababya *et al*. characterised the product of a polymerisation procedure of aniline in aqueous medium in the presence of dodecylbenzenesulfonic acid (DBSA) as a dopant by various ^{15}N, ^{13}C and ^{7}Li SSNMR techniques – CPMAS, rotating frame relaxation measurements and REDOR (Rotational Echo Double Resonance).[20] Mathew *et al*. employed ^{15}N and ^{13}C CPMAS to investigate the effect of elevated temperature on the reactivity and structure of PANI.[21] They showed that heating at 175-190 ^{o}C caused cross-linking in the polymer which was completely converted to its fully reduced leucoemeraldine form. The same group investigated the structure and oxidation state of EB PANI in N-methylpyrrolidinone (NMP) solutions[22] which were heated to 120-190 ^{o}C by using ^{15}N and ^{13}C SSNMR. They confirmed the presence of cross-links in PANI films cast from heated NMP solutions. In their paper Goddard *et al*. investigated PANI and PANI/clay nanocomposite by using deuteron quadrupole echo and MAS NMR experiments.[23] They observed a Knight shift based on the appearance of an additional manifold of spinning sidebands shifted toward higher frequencies in the MAS spectrum of conductive PANI. The presence of Knight shift implied an active role of polarons in charge transport. Young *et al*. used ^{13}C CPMAS and the ^{31}P dipolar recoupling – DRAMA (dipolar recovery at the magic angle) experiment to reveal the distribution of tert-butylphosphonic acid (TBPA) in PANI at different doping levels.[24] The acid distribution was probed by measuring ^{31}P-^{31}P dipolar couplings with the DRAMA experiment, which provides information about the internuclear distances and the distribution of TBPA molecules in the doped PANI. Sahoo *et al.* investigated various forms of an enzymatically synthesised PANI (doped (as synthesised) conducting, dedoped base and redoped conducting) by ^{15}N and ^{13}C CPMAS spectroscopy.[25]

Zujovic *et al.* used ^{13}C and ^{15}N spectroscopy to explore the antioxidant properties of PANI.[26] In subsequent papers this group investigated nanostructured materials obtained in the oxidative polymerisation of aniline using SSNMR.[7,27-30]

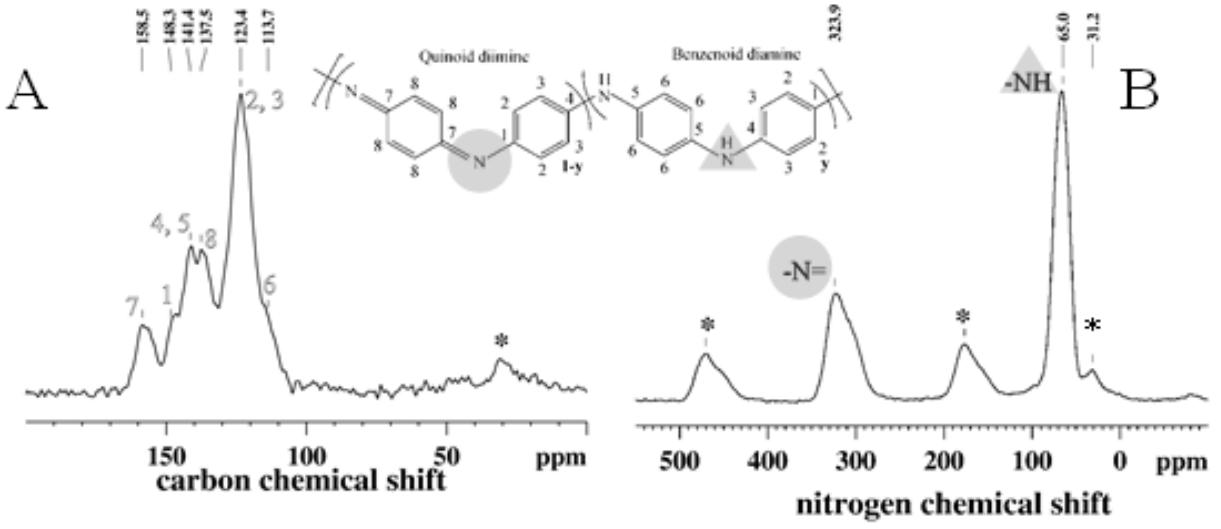


Fig. 1. ^{13}C (A) and ^{15}N (B) CPMAS NMR spectra of standard PANI. Experimental conditions are given in reference 26. The spinning sidebands are denoted by asterisks. Adapted with permission from publishers of ref. 26.

Carbon Spectra

^{13}C CPMAS spectra of PANI (Fig. 1A) usually consist of broad resonances which are partially overlapped, mostly attributed to a combination of several factors: compositional defects, a distribution of torsion angles between adjacent rings, variations in the sequencing of benzenoid and quinoid units, thermally induced molecular motions, the possibility of rotations or flips of benzenoid rings about their 1, 4 axes.[9] As shown in Fig. 1A, PANI has six broad and relatively well-defined resonances which are observed at *ca*. 114 (shoulder), 123, 137, 141, 148 and 158 ppm. The peak at 123 ppm and shoulder at 114 ppm are assigned to carbons C-2,3 and C-6, respectively (see Scheme in Fig. 1). The peaks at 137 ppm and 158 ppm originate from C-8 protonated and C-7 non-protonated carbon of the quinoid part of the PANI structure, respectively. The peaks at 141 ppm and 148 ppm are associated with C-4 and C-1 non-protonated carbon, respectively.

Nitrogen Spectra

^{15}N spectroscopy usually offers much better resolution and insight into possible structural changes. The spectrum (Fig. 1B) consists of only two very well resolved peaks from amine (ca. 65.0 ppm) and imine (323.9 ppm) nitrogens.[11] The sidebands (marked by asterisks in Fig. 1B) originate from the imine peak at 323.9 ppm. The peak from the end groups which should be at . 31 ppm is hidden by an imine sideband. The assignment of these peaks accords with the data published elsewhere.[11,16] The shoulder of the imine peak (303.2 ppm; see Fig. 1B) points to two different chemical environments for imine and amine groups. This implies that the sequence does not entirely consist of alternating benzenoid diamine and quinoid diimine units as would be expected from Scheme 1. Any redox process, i.e., change of the oxidation state of PANI, can be observed clearly from the change in the relative intensities of the peaks at 323.9 ppm and at 65.0 ppm.[26] The ratio of integrated intensities of amine and imine (including sidebands) resonances should be close to 1 (Scheme 1, EB PANI, y=0.5).

Nanostructured Materials Based on the Oxidative Polymerisation of Aniline

PANI is typically synthesised in the oxidative polymerisation of aniline, using a strong oxidising agent such as ammonium persulfate (APS) or potassium iodate (KIO_3) in a strongly acidic solution such as 1 M HCl.[1,2,31] Synthesised in this way, PANI exhibits a granular morphology, as shown in Fig. 2A. This typical form of PANI is known by its irregular agglomerates and high conductivity. However, one of the advantages of PANI as a CP is that under certain conditions it can form various supramolecular nanostructures – nanotubes (Fig. 2B), nanorods, nanofibres (Fig. 2C), nanospheres, etc.[32] The formation mechanisms and structural characteristics of these nanostructured PANI forms have been extensively studied and reported.[33-39] FTIR and UV-Vis spectroscopies have been commonly applied in these investigations.

FTIR spectroscopy

Figure 3 shows the FTIR spectra of PANI nanofibres[30] (A) and standard PANI (B).[6] All the samples show characteris-

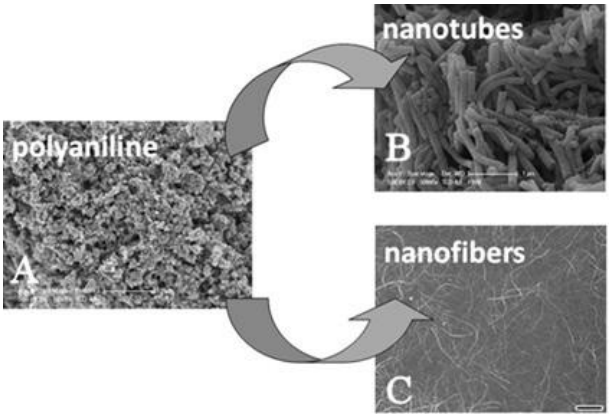


Fig. 2. SEM micrographs of standard PANI (A), nanotubes (B) and nanofibres (C). Experimental conditions are given in refs 27, 29, 30. Adapted with permission of the publishers of refs 7 and 30.

tic peaks at about 1580 cm^{-1} (C=C stretching mode of the quinoid rings),[6,40,41] and 1490 cm^{-1} (C=C stretching mode of benzenoid rings).[6,40,41] Bands at *ca.* 1290-1300 cm^{-1} are attributed to C-N stretching vibrations and C-H bending vibration of benzenoid rings.[6,40,41] For standard PANI, the strong band at *ca.* 1160 cm^{-1} (Fig. 3B) has been assigned to a C–H bending vibration of the quinoid rings.[41] Its wavenumber has been shown to be doping-level dependent, occurring at 1160 cm^{-1} in the intrinsic (undoped) structure and at 1140 cm^{-1} in the doped structure.[42] This band appears at 1140 cm^{-1} in the spectrum of PANI nanofibres (Fig. 3A). Both products in Fig. 3 had undergone a dedoping treatment, but the presence of the 1140 cm^{-1} band in the spectrum of the nanofibre product suggests that this treatment was not effective. Subsequent studies on other nanoscale PANI products suggest that these are not as easily dedoped as standard PANI.[43] A band at *ca.* 820 cm^{-1} can be attributed to C-H out of plane bending in 1,2-disubstituted ring structures (para-coupling).[42] Detailed comparison of the two spectra shows that they are very similar and that and both show features characteristic for fully polymerised PANI.

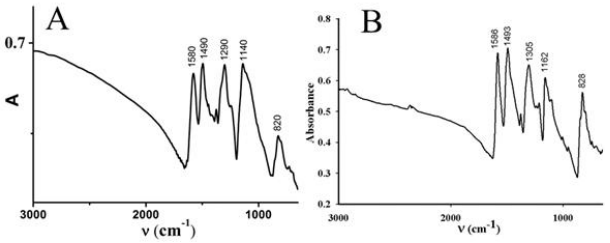


Fig. 3. FTIR spectra of PANI nanofibres (A) and standard PANI (B). Experimental conditions are given in references 6 and 30. Figure 3A is adapted with permission from the publishers of reference 30.

UV-Vis spectroscopy

UV-Vis spectra of nanofibrilar and standard PANI are shown in Fig. 4A and Fig. 4B, respectively. They exhibit similar features. The UV-Vis spectra have π-π* peaks at around 320 nm. The peak at around 600 nm can be assigned to the transition (n-π*) between the HOMO of the benzenoid ring (nonbonding nitrogen lone pair) and the LUMO (π*) of the quinoid ring.[44] Both peaks are charac-

teristic for the emeraldine base form of PANI (EB PANI), although the Q/B ratio indicates a slightly more reduced EB form.[44] The data obtained by UV-Vis data are in accordance with FTIR spectra and suggest that the structure consists of alternating quinoid and benzenoid segments. Therefore, both techniques imply that nanofibres and standard PANI have similar structures.

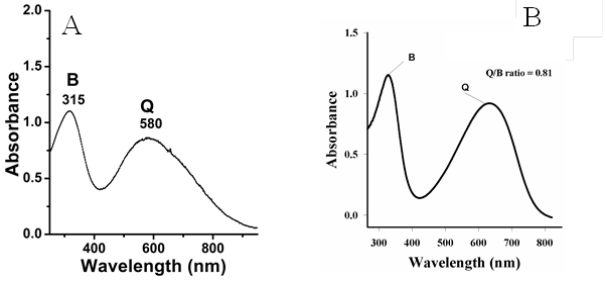


Fig. 4. UV-Vis spectra of PANI nanofibres (A) and standard PANI (B). Experimental conditions are given in references 6 and 30. Fig. 4A is adapted with permission from the publishers of ref. 30.

Inferences from SSNMR spectroscopy

^{13}C and ^{15}N SSNMR experiments were carried out to determine whether more subtle differences between these PANI samples could be revealed.

Figure 5 shows noticeable differences between the ^{13}C and ^{15}N CPMAS NMR spectra of standard PANI and nanofibres. The most obvious differences are the presence of the new peaks at 180.5 and 96.3 ppm in the carbon spectrum of nanofibres. The peak at 180.5 ppm probably originates from C=O carbons, not characteristic for the standard PANI spectra,[7,29] while the peak at 158.1 ppm originates from C=N carbons.[29] Therefore, both O and N could be preferred sites for hydrogen bonding. i.e., – C = O •••• H – N, or = N •••• H – N.[29] It has been suggested that hydrogen bonds are very important in forming the self-assembled supramolecular nanotube structures.[45] Also, there is an additional shoulder at 128.7 ppm in the spectrum of the nanofibres which implies a complex backbone structure or conformational changes owing to the formation of a nanofibrilar morphology. The ^{15}N spectra of PANI and the nanofibres are also different. The "standard" PANI spectrum shown in Fig. 5 exhibits peaks at 65.0 ppm and 323.9 ppm, from amine and imine nitrogen atoms respectively.[26] However, the spectrum of the nanofibrilar sample has different amine sites, with peaks at 71.1 and 91.2 ppm. This implies a complex molecular structure with several different –NH groups. This can probably be attributed to cross-linking which facilitates the formation of nanofibres.

The imine (I) to amine (A) ratio (the ratio of the corrected integrated areas for peaks at around 70, 90 and 320 ppm (and its related sidebands)) is *ca.* I/A = 0.3. However, the FT-IR, UV-Vis and ^{13}C CP spectra suggest the prevalence of head-to-tail coupling similar to standard PANI and a significantly higher value for the imine/amine ratio, probably closer to 1.0. To resolve this ambiguity, the direct polarization (DP) experiment, which does not involve cross-polarization from protons, was carried out. Although, the experimental time for DP measurements can be very long,

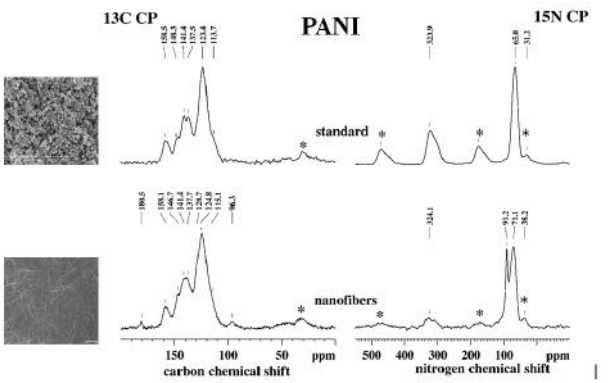


Fig. 5. SEM micrographs and ^{13}C and ^{15}N SSNMR spectra of standard PANI and nanofibres. Experimental conditions are given in refs 27 and 30. The spinning sidebands are denoted by asterisks. Adapted with permission from the publishers of refs 26 and 30.

this experiment does not suffer from the usual quantification problems that can result from CP.

There are differences between the DP and CP spectra. Most obvious is the much larger contribution of the imine resonance, since the peak at *ca.* 320 ppm is much more intense than its CP counterpart (see Fig. 5). The imine/amine ratio for the sample shown in Figure 6 is 0.82. This value is different from the data obtained using the CP technique, but closer to those obtained from FT-IR, UV-Vis and ^{13}C CP experiments. A possible reason for this could be a difference in the proton environments of the N atoms in the different kinds of material, e.g., protons are more remote from the N atoms in the nanofibres, or a difference in the proton dynamics, e.g., fast exchange of hydrogen bonded protons between =O and =N and H–N in the nanofibre sample. On the other hand, polarization transfer in the CP spectrum of standard PANI might be more efficient because this material possesses a featureless granular morphology in which grains are packed together very closely and randomly distributed.

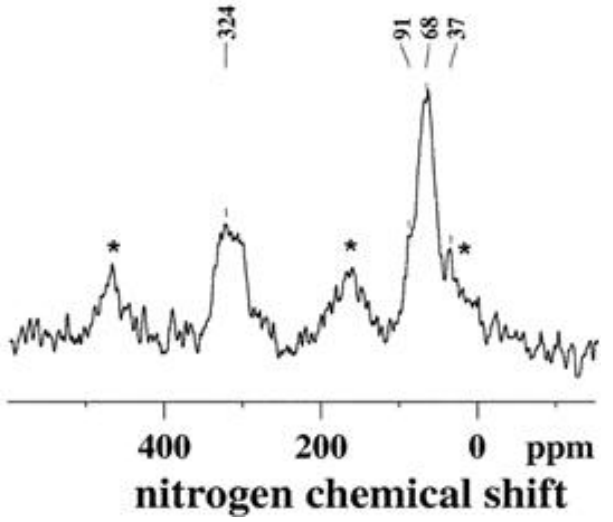


Fig. 6. ^{15}N direct polarization spectrum of PANI nanofibres. Experimental conditions are given in ref. 30. Adapted with permission from the publishers of ref. 30.

Conclusions

The SSNMR technique has been successfully applied to standard and nanofibre PANI products. The spectral features in the ^{13}C CPMAS spectra of nanofibres are similar to those of standard PANI, except for the presence of two peaks at 180.5 and 96.3 ppm. The ^{15}N NMR suggests the presence of strong hydrogen bonds between the imine nitrogen and a proton from the amine nitrogen. Furthermore, cross-linking which should be the basis for the formation of well-ordered structures (such as nanofibres) has been confirmed by the presence of the 96.3 ppm peak in the carbon spectrum. Although very useful, special care should be taken when the CPMAS SSNMR approach is used for nanostructured PANI materials. In other words, using only CPMAS to quantify different groups can be problematic. This is not the case for conventional PANI where the imine/amine ratio can be determined relatively accurately from ^{15}N CPMAS spectra.

References

1. Wallace, G. G.; Spinks, G. M.; Kane-Maguire, L. A. P. *Conductive Electroactive Polymers: Intelligent Materials Systems,* 2nd ed. 2002.
2. Skotheim, T. A.; Reynolds, J. R.; Editors *Handbook of Conducting Polymers, Third Edition. Conjugated Polymers Processing and Applications*, 2007.
3. Huang, W.-S.; Humphrey, B. D.; MacDiarmid, A. G. *J. Chem. Soc., Faraday Trans. 1* **1986**, *82*, 2385.
4. Ray, A.; Astarias, G. E.; Kershner, D. L.; Richter, A. F.; MacDiarmid, A. G.; Epstein, A. *Synth. Met.***1989**, *29*, E141.
5. Hjertberg, T.; Salaneck, W. R.; Lundstrom, I.; Somasiri, N. L. D.; MacDiarmid, A. G. *J. Polym. Sci., Polym. Lett. Ed.* **1985**, *23*, 503.
6. Gizdavic-Nikolaidis, M. R. **2005**. "Spectroscopic studies of chemically synthesized polyaniline and its ability to act as radical scavenger", PhD thesis, The University of Auckland. *http://hdl.handle.net/2292/2257*.
7. Zujovic, Z. D.; Zhang, L.; Bowmaker, G. A.; Kilmartin, P. A.; Travas-Sejdic, J. *Macromolecules* **2008**, *41*, 3125.
8. Stafström, S.; Sjögren, B.; Wennerström, O.; Hjertberg, T. *Synth. Met.***1986**, *16*, 31.
9. Kaplan, S.; Conwell, E. M.; Richter, A. F.; MacDiarmid, A. G. *J. Am. Chem. Soc.* **1988**, *110*, 7647.
10. Menardo, C.; Nechtschein, M.; Rousseau, A.; Travers, J. P.; Hany, P. *Synth. Met.* **1988**, *25*, 311.
11. Wehrle, B.; Limbach, H.-H.; Mortensen, J.; Heinze, J. *Angew. Chem.* **1989**, *101*, 1781.
12. Stein, P. C.; Hartzell, C. J.; Jorgensen, B. S.; Earl, W. L. *Synth. Met.* **1989**, *29*, E297.
13. Kaplan, S.; Conwell, E. M.; Richter, A. F.; MacDiarmid, A. G. *Synth. Met.* **1989**, *29*, E235.
14. Richter, A. F.; Ray, A.; Ramanathan, K. V.; Manohar, S. K.; Furst, G. T.; Opella, S. J.; MacDiarmid, A. G.; Epstein, A. J. *Synth. Met.* **1989**, *29*, E243.
15. Stein, P. C.; Earl, W. L.; Ray, A. *Synth. Met.* **1993**, *55*, 702.
16. Adams, P. N.; Monkman, A. P.; Apperley, D. C. *Synth. Met.* **1993**, *55*, 725.
17. Adams, P. N.; Laughlin, P. J.; Monkman, A. P.; Kenwright, A. M. *Polymer* **1996**, *37*, 3411.
18. Kolbert, A. C.; Caldarelli, S.; Thier, K. F.; Sariciftci, N. S.; Cao, Y.; Heeger, A. J. *Phys. Rev. B* **1995**, *51*, 1541.
19. Espe, M. P.; Mattes, B. R.; Schaefer, J. *Macromolecules* **1997**, *30*, 6307.
20. Kababya, S.; Appel, M.; Haba, Y.; Titelman, G. I.; Schmidt, A. *Macromolecules* **1999**, *32*, 5357.
21. Mathew, R.; Mattes, B. R.; Espe, M. P. *Synth. Met.* **2002**, *131*, 141.
22. Young, T. L.; Espe, M. P.; Yang, D.; Mattes, B. R. *Macromolecules* **2002**, *35*, 5565.
23. Goddard, Y. A.; Vold, R. L.; Hoatson, G. L. *Macromolecules* **2003**, *36*, 1162.
24. Young, T. L.; Cross, J. L.; Espe, M. P. *Macromolecules* **2003**, *36*, 5891.
25. Sahoo, S. K.; Nagarajan, R.; Roy, S.; Samuelson, L. A.; Kumar, J.; Cholli, A. L. *Macromolecules* **2004**, *37*, 4130.
26. Zujovic, Z. D.; Gizdavic-Nikolaidis, M. R.; Kilmartin, P. A.; Idriss, H.; Senanayake, S. D.; Bowmaker, G. A. *Polymer* **2006**, *47*, 1166.
27. Zujovic, Z. D.; Gizdavic-Nikolaidis, M.; Kilmartin, P. A.; Travas-Sejdic, J.; Cooney, R. P.; Bowmaker, G. A. *Appl. Magn. Reson.* **2005**, *28*, 123.
28. Zujovic, Z. D.; Bowmaker, G. A.; Tran, H. D.; Kaner, R. B. *Synth. Met.* **2009**, *159*, 710.
29. Zujovic, Z. D.; Laslau, C.; Bowmaker, G. A.; Kilmartin, P. A.; Webber, A. L.; Brown, S. P.; Travas-Sejdic, J. *Macromolecules* **2009**, *43*, 662.
30. Zujovic, Z. D.; Wang, Y.; Bowmaker, G. A.; Kaner, R. B. *Macromolecules* **2011**, *44*, 2735.
31. Stejskal, J.; Gilbert, R. G. *Pure Appl. Chem.***2002**, *74*, 857.
32. Ciric-Marjanovic, G. In *Nanostructured Conductive Polymers*; Eftekhari, A., Ed.; Wiley: London, 2010, p 19.
33. Konyushenko, E. N.; Stejskal, J.; Sedenkova, I.; Trchova, M.; Sapurina, I.; Cieslar, M.; Prokes, J. *Polym. Int.* **2006**, *55*, 31.
34. Huang, J.; Kaner, R. B. *Angew. Chem., Int. Ed.* **2004**, *43*, 5817.
35. Tran, H. D.; Wang, Y.; D'Arcy, J. M.; Kaner, R. B. *ACS Nano* **2008**, *2*, 1841.
36. Laslau, C.; Zujovic, Z.; Travas-Sejdic, J. *Prog. Polym. Sci.* **2010**, *35*, 1403.
37. Laslau, C.; Zujovic, Z. D.; Zhang, L.; Bowmaker, G. A.; Travas-Sejdic, J. *Chem. Mater.* **2009**, *21*, 954.
38. Zujovic, Z. D.; Laslau, C.; Bowmaker, G. A.; Kilmartin, P. A.; Webber, A. L.; Brown, S. P.; Travas-Sejdic, J. *Macromolecules* **2010**, *43*, 662.
39. Zujovic, Z. D.; Laslau, C.; Travas-Sejdic, J. *Chem. Asian J.* **2011**, *6*, 791.
40. Trchova, M.; Sedenkova, I.; Konyushenko, E. N.; Stejskal, J.; Holler, P.; Ciric-Marjanovic, G. *J. Phys. Chem. B* **2006**, *110*, 9461.
41. Boyer, M. I.; Quillard, S.; Rebourt, E.; Louarn, G.; Buisson, J. P.; Monkman, A.; Lefrant, S. *J. Phys. Chem. B* **1998**, *102*, 7382.
42. Tang, J.; Jing, X.; Wang, B.; Wang, F. *Synth. Met.* **1988**, *24*, 231.
43. Zujovic, D. Z; Nieuwoudt, M. K.; Bowmaker, G. A.; Kilmartin, P. A. "Detailed investigations of aniline oxidation products using NMR, FTIR, Raman and EPR spectroscopy", in preparation.
44. Yang, D.; Mattes, B. R. *Synth. Met.* **2002**, *129*, 249.
45. Lindoy, L. F.; Atkinson, I. M.; Editors *Self-Assembly in Supramolecular Systems*; Royal Society of Chemistry: Cambridge, 2000; Vol. 7.

Feature Series

Some Unremembered Chemists

A series of articles that explores the lives and work of selected chemists who have made a significant contribution to the advancement of the discipline, the profession and well-being of mankind, yet who are little remembered.

Sir Edward Frankland KCB, FRS, FCS (1825-1899)

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The name of Edward Frankland (Fig. 1) is recognised by many chemists, but his contribution to the discipline and profession is remembered by few. His name was commemorated by the Royal Society of Chemistry in 1984, initially with the Sir Edward Frankland Fellowship, and now the Frankland Award. It recognises study in organometallic chemistry – but what was it that Frankland did and why is he so recognised?

Fig. 1. Edward Frankland (out of copyright from *http://upload.wikimedia.org/wikipedia/commons/e/e9/Edward_Frankland.jpg*)

Edward Frankland was born near the small town of Garstang in Lancashire, England. The town lies between Preston and Lancaster and is east of Blackpool.[1,2] Edward was the son of Margaret (Peggy) Frankland who went into service with the wealthy Gorst family in Preston in 1824. She had an affair with the young heir, Edward Chaddock Gorst and, when discovered, the horrified family dispatched the pregnant Peggy with a handsome annuity, providing that the identity of the father was never disclosed. It was revealed only by 20th century historians. Peggy moved to Churchtown, in the Parish of Garstang, where her son Edward was born on 18th of January 1825. He was accorded her family name but given the first name of his father.

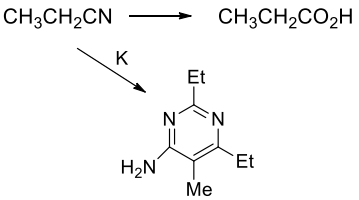
Edward Frankland's childhood has been described as ferocious as he and his mother were outcasts. Nonetheless, she ensured that Edward got an education using the annuity to meet the costs.[1] By the time he was seven years old he had been to seven schools where, it appears, he was routinely given beatings. It was only in his eighth school that he gained an element of stability as he stayed there until he was twelve.[3] This school, in Lancaster, was an enlightened one, as the pupils were encouraged to acquire first-hand knowledge of nature and even conduct simple experiments. From twelve to fifteen years of age the young Edward attended the Lancaster Free Grammar School, although this time is recorded in the Oxford Dictionary of National Biography as 'entirely unproductive'.[4] By then, Peggy had married William Helm, one of the first lodgers at the Lancaster guest house that she had run. At 15, Edward's wish was to enter the medical profession and so, on advice from others, his stepfather William Helm apprenticed him to a local druggist (pharmacist) by the name of Stephen Ross.

It seems that the apprenticeship was one of drudgery: Ross appears to have taught him little, requiring 70-hour weeks of bone-breaking labour, hauling, wrapping and grinding.[1] In his writings, Frankland speaks of his apprenticeship as wasted time, calling it, "six years' continuous hard labour, from which I derived no advantage whatever, except the facility of tying up parcels neatly".[5] Nonetheless, it is probable that he did gain some useful skills, not least the safe handling of chemicals. Despite the long working hours, Edward used his limited free time to borrow books from the mechanics' institute, attend the classes and conduct some experiments in the makeshift laboratory that the local doctors, Christopher Johnson and his son James, had made available there.[4] Apparently they evicted a tenant from a cottage to create the institute for local lads. Subsequently, Dr. James Johnson was instrumental in gaining Frankland a position at the end of his apprenticeship late in 1845 with Lyon Playfair, who had recently been appointed chemist in the Government Department of Woods and Forests in London. Frankland's progress there was so fast that, after six months, he was appointed as Playfair's lecture assistant for the additional post of Professor at the Putney College of Engineering that Playfair had taken. Apart from being assistant, Frankland took the course that Playfair gave and successfully completed the final examination, the only one he ever sat.[4]

One of Playfair's other assistants at that time was Herman Kolbe (Fig. 2), who had gained a PhD with Bunsen in Marburg and come to the belief that organic compounds

Fig. 2. Adolph W.H. Kolbe (out of copyright from *http://upload.wikimedia.org/wikipedia/commons http://commons.wikimedia.org/wiki/File:Adolph_Kolbe2.jpg*)

were comprised of identifiable groups of atoms which he termed *radicals* (then spelled 'radicles'). Frankland became caught up in this and, with Kolbe, showed that the hydrolysis of cyanoethane led to propionic acid, thus confirming the hypothesis. The results were communicated to the Chemical Society in April, 1847. The following month saw the pair go to Marburg where Frankland spent some time in Bunsen's laboratory attempting to prepare the ethyl radical by dropping cyanoethane onto potassium metal. In his words, "a very vigorous reaction often accompanied by fire and rapid gas evolution" took place.[6] The gaseous product was not the desired ethane but butane, which required the cyanide to be impure and contain some water. A side product was also obtained, isolated, purified, analyzed and named kyanethine. It was subsequently proved to be the pyrimidine **1**, a trimer of cyanoethane (Scheme 1).[6]



1, kyanethine - 2,6-diethyl-4-methylpyrimidinamine

Scheme 1

Frankland stayed for only three months in the Bunsen laboratory, as he was appointed a teacher at Queenswood School in King's Somborne, west of Winchester in Hampshire. He took up his post in September of 1847 and found that one of the other teachers was John Tyndall (Fig. 3), who became a prominent 19th century English physicist. The pair taught one of the first laboratory-based science courses in England, each teaching the other his own subject by rising at 4 am to exchange lessons before schoolwork began.[7] Again Frankland was drawn to research, and he continued his quest for the ethyl radical as he knew it. Initially, he tried to remove the oxygen atom from diethyl ether using both potassamide (potassium amide) and potassium metal, but was unsuccessful. He then directed his attention, not to cyanoethane again, but to the iodide since HI was known to easily be decomposed by potassium. After an explosion with potassium metal, he switched to zinc, heating the reagents in a sealed tube on 28 July 1848. Unfortunately he had no means of measuring or estimating the gaseous product formed in the sealed tube since his eudiometer he had had been broken (a eudiometer is similar to a graduated cylinder but closed at the top end with the bottom immersed in water or mercury thereby allowing a gas to be collected in the cylinder and its volume measured; see Fig. 4). Frankland left the tubes unopened, resigned his post leaving after a year to return to the Marburg laboratory, this time with Tyndall and the sealed tubes.

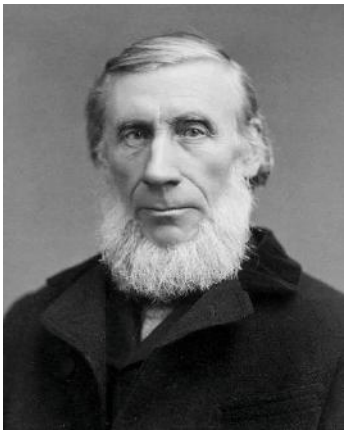


Fig. 3. John Tyndall mid-career (out of copyright from *http://upload.wikimedia.org/wikipedia/commons/b/bc/John_Tyndall_portrait_mid_career.jpg*

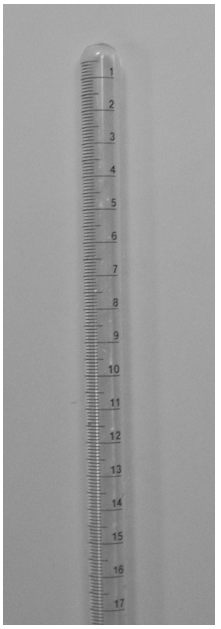


Fig. 4. The closed end of a eudiometer

His serious research started immediately during the summer of 1848. He studied the action of sodium on fatty acids, obtaining radicals such as 'methyl', which, in reality, was ethane. Opening one of the sealed tubes that he had brought from Hampshire under water in mid-February 1849 led to an evolution of gas that was apparently as spectacular as it was significant – he obtained ethyl (in reality butane)! His researches were successfully submitted for the PhD degree in July, 1849. However, during his last weeks with Bunsen (see Fig. 5) he noticed a liquid product in one of his unopened Queenswood tubes from the

reaction of ethyl iodide with zinc. This liquid was shown to contain zinc, but it was an organic compound and it contained the metal! It was diethyl zinc as we now know it. Organometallic chemistry was born with this July 1848 experiment.

$2EtI + Zn \longrightarrow Et_2Zn + I_2$;

$2MeI + Zn \longrightarrow Me_2Zn + I_2$; $Me_2Zn + 2H_2O \longrightarrow 2MeH + Zn(OH)_2$

On 12 July 1849 in Bunsen's laboratory he prepared dimethyl zinc by digesting methyl iodide with zinc. After discharging the gasses, he cut off the upper part of the tube so as to test the action of water on the solid residue. On adding water he observed,[8] "a greenish-blue flame several feet long shot out of the tube, causing great excitement amongst those present and diffusing an abominable odour through the laboratory".

Fig. 5. Robert Bunsen (out-of-copyright from http://upload.wikimedia.org/wikipedia/commons/2/28/Robert_Bunsen_02.jpg

Frankland moved from Marburg to Liebig's laboratory in Giessen for a few months then moved back to England early in 1850 as Professor of Practical Chemistry at Putney College.[9] He remained there for just one year, but it was a year full of research and significant output. During this time he examined the action of light on reactions between metals and alkyl halides, taking advantage of a flat part of the roof of his chemistry laboratory and the increasing daylight of the spring. The outcome was the formation of a range of organometallic compounds involving zinc, tin, mercury, etc., that firmly established the new branch of chemistry. At least as important were the deductions he made from his new data. He recorded regularity in the formulae of his compounds and came to recognise that each element had a limit to the number of radicals that could be attached to it; that each had a definite combining power. Thus, from his novel yet obscure compounds, Frankland discovered one of the most significant principles of chemistry, namely that known as valency, and he was the first to articulate the concept by terming it "combining power". The idea of valency laid the groundwork for Kekule's hypothesis of the structure of benzene and for Gerhardt's theory of types. The results, communicated to the Royal Society as his second paper on organometallic compounds and read at a meeting on 10 May 1852, were published subsequently,[10] securing his place in the documented history of the valency concept.[11]

On 2 January 1851 Frankland was appointed as the inaugural professor of chemistry at Owens College in Manchester, but before he left London he married Sophie Fick whom he had first met while with Bunsen in Marburg in 1847. The objective of the new Owens College was the provision of instruction and improvement to young men in such branches of learning and science as are usually taught in the English Universities; it was the beginning of the Victoria University of Manchester into which it evolved.[12] During his six and a half years at the college Frankland firstly designed the laboratories, established his own research using a new Mancunian technique of heating under pressure in an iron digester, and became consultant to a range of industries to a scale unprecedented among other universities. This led to Manchester's tradition in applied chemistry. His researches led to several new products and allowed for the synthesis of the zinc alkyls in large quantity. He was elected to the Fellowship of the Royal Society in 1853 and was awarded its Royal Medal in 1857.

Despite Frankland's reputation, Owens College was mismanaged and its students were of indifferent quality; it almost closed.[4,12] So it was, that in mid-1857, he accepted the position of lecturer in chemistry as St. Bartholomew's Hospital and moved to London with his wife and, by then, three children. While he had little opportunity for research there, he was able to use the better facilities of the Royal College of Chemistry in Oxford Street, headed by A. W. Hofmann. That college became the Royal School of Science in 1900, was the first part of Imperial College, and eventually became its Chemistry Department. Despite the Royal College facilities, Frankland took other positions that continued to make his life hectic, yet his research output surged.

Michael Faraday retired from the Royal Institution in 1861 and Frankland was appointed, initially on a temporary basis. Once tenured, he stayed there until 1868. However, from 1865 he acted as replacement for Hofmann at the Royal College while the latter was on three years' leave in Germany. Although this position became permanent in 1868, when Hofmann elected to remain in Germany, Frankland resigned from this post within the year.

The early part of Frankland's time in London saw him expand his interests by carrying out research in such diverse subjects as illuminating gases, explosives, atmospheric chemistry, and water quality. He also became involved with the Royal Society and the Chemical Society, serving on their Councils. He was a member of the influential X Club from its inception by Thomas Henry Huxley in 1864 until its closure in 1893. The X Club was a dining club of nine men who supported the theories of natural selection and academic liberalism in late 19th-century England.

During his time as replacement for Hofmann he was required also to assess the examination papers set by the Department of Science and Art, which led to him to publishing his *Lecture Notes for Chemical Students*.[13] Here, the atoms were represented by their letters and joined with 'bonds' (a term introduced in the book) and the concepts of valence were discussed. His involvement with educational matters led him to espouse the need for labo-

ratory training in chemistry for all students of the subject and, from 1869, he provided a lab course for interested teachers free of charge.

Back in London, Frankland's initial studies extended his organometallic work and, from 1859 with Duppa, he carried out the first studies on organoboron compounds that were published from 1860 and included the very reactive trialkylborons.[14] Not only was work on organomercury and phosphorus compounds reported, but in addition, Frankland and Duppa made significant contributions to synthesis of ethers, esters and dicarboxylic, hydroxy and unsaturated acids, revealing the structure and relationship between the compounds, all coming from the exploitation of their organometallics. To some, Edward Frankland (Fig. 6) is regarded as a founder of synthetic organic chemistry.

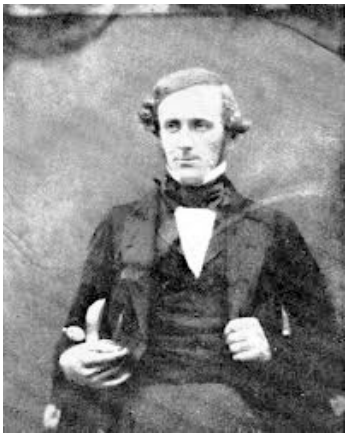


Fig. 6. Edward Frankland in mid-career (out-of-copyright from *http://en.wikipedia.org/wiki/File:Frankland_Edward_26.jpg*)

Despite all of the foregoing, Frankland's international reputation came just as much from work in applied chemistry. It was noted above that he had worked on water quality. In fact, it was during the summer of 1859 that he assisted Hofmann in reporting to the Metropolitan Board of Works in London on possible means of deodourizing sewage. At that time it was sent raw into the River Thames making it black and horribly offensive, causing much water-borne disease.[7] This involvement with water analysis and water purification gained momentum after he took over Hofmann's role at the Royal College. He found that he had inherited the role as analyst for the London water supply, and was appointed to the second royal commission on the pollution of rivers in 1868. This also provided him with a fully equipped government laboratory, which led to much valuable scientific information on sewage contamination, industrial effluent and water purification for domestic consumption over a six-year period. Within a very short time (with the assistance of H. E. Armstrong) he had developed a new analytical method for carbon and nitrogen in water. Later he suggested that previous sewage contamination could be detected from nitrate concentration. He pursued relentlessly the quest for safe drinking water, campaigning against supplies that failed to meet his standards. While this led to antagonism from many chemists employed by water companies, he became recognized as the world leader in the field. Increasingly, he was asked to analyze water samples to the extent that his consultancy demanded more and more of his time. He was analyzing water samples from numerous international clients. This work moved to a privately funded laboratory from about 1862 and continued well into his retirement. It has been said that he did not take kindly to the criticism that his analytical work took him away from his role at the Royal College, and that it was this that persuaded him into early retirement in 1885 aged 60 years.

The increasing recognition that Frankland attracted in the 1870s led to his reappointment to the Council of the Royal Society and the office of Vice-President, and then the 1871-73 President of the Chemical Society. His great concern for the education of chemists, the provision of laboratory programmes and the needs of the profession led him to campaign for professionalism in the discipline. So successful was he that in 1877 the Institute of Chemistry of Great Britain was founded, with a focus on the qualifications and professional status of chemists. Its aim was to ensure that consulting and analytical chemists were properly trained and qualified. It was the first of the professional organizations for scientists to be created, and Frankland served as its first President from 1877-1880. It is to this organization that the New Zealand Institute of Chemistry owes its origins, with the professional statutes and status that it accepted from its inauguration in1931 until its move to become a learned society from the early 1990s.

The 1901 supplement to the *Dictionary National Biography*[7] states that Frankland published sixty three papers alone, fifteen with B. F. Duppa, three with J. N. Lockyer (leading to Lockyer's recognition of helium in the sun's atmosphere), two with H. Kolbe (though a further one omitted his name), one with H. E. Armstrong, and ten with other chemists. Forty nine of these are recognised by SciFinder® though several of the earlier papers carry no year. The Royal Society archival journals carry forty papers by Frankland that date from 1850. However, the *Complete Dictionary of Scientific Biography*[15] states that Frankland published over 130 papers of which the *Royal Society Catalogue of Scientific Papers* (London 1867-1925) lists 107. Irrespective of the actual number, Edward Frankland made a major contribution to the subject of chemistry, to the nature of the profession, and to the well-being of mankind. In retirement (see Fig. 5) he received the Royal Society's Copley Medal (1894) and a KCB in the Queen Victoria's Diamond Jubilee Honours of 1897 for his water quality work.

Finally, it is worthy of note that both the Gorst and Frankland families have connections with New Zealand. The second legitimate son of Edward Chaddock Gorst (who changed his name Lowndes in order to inherit a considerable fortune from his childless great uncle), John Eldon – half-brother to Edward Frankland, decided to come to New Zealand following his father's death in order to help Bishop Selwyn (who he knew from St. John's College, Oxford) with his missionary work among the Māori. J. E. Gorst became Civil Commissioner in New Zealand from 1861-63 before returning to England where he had a distinguished political career for which he was knighted.[16]

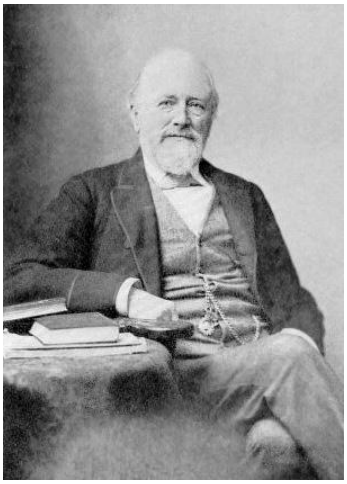


Fig. 7. Edward Frankland in 1894 (out of copyright from *http://upload.wikimedia.org/wikipedia/commons/0/08/Frankland_Edward_1894.jpg*)

The Frankland family are, however, more permanently connected in that, after attending London University, Edward's eldest son (Frederick William) came to New Zealand in 1875 for health reasons. He joined the Civil Service where he rose sequentially to the positions of Registrar of Friendly Societies, Government Actuary, and Government Insurance Commissioner.[17] He was married on 30 April 1879, at St. Paul's Church in Wellington (Old St. Paul's), to Miriam Symons, of Foxton, to where he returned in 1902 after an actuarial position in London and a period in New York. F. W. Frankland served on the Foxton Borough Council and unsuccessfully contested the Manawatu seat as a Liberal candidate in 1909.

References and Notes

1. Leinhard, J. H. *Engines of Our Ingenuity*, no. 2036; see: *www.uh.edu/engines/epi2036.htm* (accessed 25 Feb 2013).
2. See: *http://ghgraham.org/edwardfrankland1825.html* (accessed 25 Feb 2013).
3. McLeod, H. *Obituary, Edward Frankland, J. Chem Soc. Trans.,* **1905**, *87*, 565-618; see: *pubs.rsc.org/en/Content/ArticleLanding/1905/CT/ct9058700565* (accessed 28 Feb 2013).
4. Russell, C. A. *Oxford Dictionary of National Biography*; see: *www.oxforddnb.com/view/article/10083?docPos=1* (accessed 28 Feb 2013).
5. See: *http://deadscientistoftheweek.blogspot.co.nz/2010/01/edward-frankland.html* (accessed 28 Feb 2013).
6. *Bioactive Heterocyclic Compound Classes: Pharmaceuticals*, Dingers, J.; Lamberth, C, eds. Wiley-VCH, 2012, pp. 287-288.
7. Harthog, P. J. *Dictionary of National Biography 1901 Supplement*; see: *http://en.wikisource.org/wiki/Frankland,_Edward_(DNB01)* (accessed 7 Mar 2013).
8. Frankland, E. (1825 1899). *Experimental Researches in Pure, Applied, and Physical Chemistry*. John Van Voorst, Parternoster Row, London, 1877.
9. British History Online. See *www.british-history.ac.uk/report.aspx?compid=45294&strquery=Putney+college* (accessed 4 Mar 2013).
10. *Frankland, E. Phil. Trans. R. Soc. Lond*., ***1852***, *142*, 417-444; see also: Frankland, E. *On organometallic bodies: A discourse delivered to the members of the Chemical Society of London*, *Quart. J., Chem. Soc., Lond.,* **1861**, *13*, 177-235.
11. Russell, C. A. *The History of Valency*, Leicester University Press, 1971.
12. Hartog, P. *The Owens College, Manchester (founded 1851); a brief history of the college and description of its various departments*. Cornish, Manchester, 1900; see: *http://archive.org/details/owenscollegemanc00hartuoft* (accessed 5 Mar 2013).
13. Frankland, E. *Lecture Notes for Chemical Students: Embracing Mineral and Organic Chemistry*. John Van Voorst, Parternoster Row, London, 1866.
14. Frankland, E.; Duppa, B. *On boric ethide*, *Proc. R. Soc. Lond*., **1859**, *10*, 568-570; Frankland, E. *On a new series of compounds containing boron*, *Proc. R. Soc. Lond*., **1860**, *12*, 123-128, *Phil. Trans. R. Soc. Lond*., **1862**, *152*, 167-183 (Volume 10 of the Proceedings of the Royal Society is dated 1859, but the first paper cited was submitted in 1860).
15. *Complete Dictionary of Scientific Biography* (2008). See: *www.encyclopedia.com/topic/Edward_Frankland.aspx* (acessed 11 Mar 2013).
16. 'Gorst, John Eldon', in *Dictionary of New Zealand Biography*. See: *http://www.teara.govt.nz/en/biographies/1g15/gorst-john-eldon* and also: *www.teara.govt.nz/en/1966/gorst-sir-john-eldon* (accessed 7 Mar 2013).
17. 'Frankland, Frederick William', in *Dictionary of New Zealand Biography*. See: *www.teara.govt.nz/en/1966/frankland-frederick-william* (accessed 8 Mar 2013).

Indian patent decision highlights the bond between politics and patent law

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In a major ruling the Indian Supreme Court has rejected Novartis' appeal to gain patent protection for a novel beta crystalline form of its anti-cancer drug Glivec. The decision shines a fascinating light on the current principles of Indian patent law and their origins. It also illustrates the difficulties faced when trying to balance incentives for research with access to the commercial products of that research.

We examine the background of the case and grapple with the issues pitting patent versus patient in India and the developing world.

Development of the drug

Imatinib is the active ingredient in the anti-cancer drug Glivec. It consists of a derivative of N-phenyl-2-pyrimidineamine and was found to inhibit certain protein kinases, especially one called BCR-ABL. Research had shown that the presence of this protein kinase in the body caused chronic myeloid leukaemia (CML) as a result of continuous stimulation of cell-growth pathways and transformation of normal cells into ones that proliferate without restraint.

After an extensive screening programme, the inventors found that that imatinib killed cultured cells that required BCR-ABL activity to survive, but did not affect a cell line that depended on a different protein kinase, v-SRC. This was the holy grail: a compound that selectively blocked the disease causing protein kinase, but had no effect on other protein kinases thus avoiding wide ranging toxic side-effects.

As well as its use in treating CML through the inhibition of BCR-ABL, later research found that imatinib inhibited two

further protein kinases which cause other diseases. Following further human trials, the drug was found to be effective in treating patients with gastrointestinal stromal tumor (GIST) and hypereosinophilic syndrome (HES). In a 2009 estimate, 120000 CML patients and 28000 GIST patients were being treated with Glivec worldwide.[1] The researchers who developed imatinib were awarded the Lasker Clinical Medical Research Award in 2009 and the Japan Prize in 2012 for their work.

The imatinib patent

In 1994, patent applications were filed in over 30 countries worldwide (including New Zealand) but no application was filed in India. At the time, India only afforded patent protection to new chemical processes rather than new products. The patent specifications discussed the therapeutic benefits of the compound and contained claims covering a number of compounds that were believed to be effective inhibitors of BCR-ABL. One claim specifically covered the compound imatinib in free base form, i.e., the pure basic form of the amine, as opposed to its salt form, plus *pharmaceutically acceptable salts* of imatinib. Of 37 examples given in the patent specification, one related to the compound imatinib, but none specifically described how to prepare the mesylate salt of imatinib (which eventually became the major component of Glivec).

It may seem excessive to claim a large number of compounds when only one, i.e., imatinib, is eventually used. However, it should be considered that a patent application has to be filed before any human trials (and likely any animal trials) have been carried out to avoid compromising the novelty of the invention. There is a high rate of attrition for even the most promising lead compounds from the lab owing to the pharmacological and physicochemical constraints on new drugs.

Of all the salts of imatinib tested, imatinib mesylate (marketed under the name Glivec or Gleevec) was found to have appropriate properties for human administration and showed considerable promise in drug trials. In one study of the drug in more than 1000 chronic-phase patients it so outperformed the control interferon-based therapy that the researchers closed the trial and switched almost everyone to Glivec. Five years after diagnosis, overall survival of patients treated with Glivec was 89 percent compared with 60% for interferon-treated patients.[1] The patent on imatinib is due to expire in the USA on 4 January 2015 and expired in New Zealand on 31 March 2013.

The *beta* patent

In 1998, Novartis filed a further patent application to cover a new form of imatinib mesylate – the beta crystalline form of the compound. This form was found to have the following enhanced properties over the non-crystalline form:

i. more beneficial flow properties,
ii. better thermodynamic stability, and
iii. lower hygroscopicity.

According to the beta patent specification, these properties provided advantages for *processing and storing* the drug, i.e., more efficient production techniques and longer shelf life, when compared to the non-crystalline form. The beta patent has been granted in about 40 countries with the New Zealand patent due to expire in July 2018.

The Indian Supreme Court decision

In a refreshing departure from the typically staid, formulaic legal decisions, the Supreme Court's decision is not limited to consideration of solely legal matters. Reference is made to the moral and political background of the current law and the Court unashamedly refers in the introduction to a consideration of the need to strike a balance between promoting scientific research and development, and keeping private monopoly to a minimum.

The decision revolved around a challenge of the *beta* patent application by both generic pharma firm Natco Pharma and the charity Cancer Patients Aid Association. The application was rejected by the Supreme Court on the basis that it was unpatentable under section 3(d) of a 2005 amendment of the Indian Patents Act. Section 3(d) reads:

> *The mere discovery of a new form of a known substance which does not result in the enhancement of the known efficacy of that substance… [is not an invention within the meaning of the Act]*

A further explanation of this section states:

> *For the purposes of this clause, salts, esters, ethers, polymorphs, metabolites, pure form, particle size, isomers, mixtures of isomers, complexes, combinations and other derivatives of known substance shall be considered to be the same substance, unless they differ significantly in properties with regard to efficacy.*

The main question considered by the Court was therefore whether the advantageous properties of the beta crystalline form of imatinib mesylate were considered an *enhancement of the known efficacy* when compared to the *known substance*.

Novartis argued that the *known substance* was imatinib in free base form because this was what was specifically disclosed in the imatinib patent. However, the Court ruled that the salt imatinib mesylate was the *known substance*. This was on the basis of it being specifically mentioned in a January 1996 *Cancer Research* article[2] and assertions in an April 1998 Investigational New Drug Application[3] stating that the imatinib patent covered imatinib mesylate.

Internationally recognised definitions of *efficacy*[4,5] were considered by the Court and it was determined that efficacy meant therapeutic efficacy. Such efficacy was adjudged to be *known* from the extensive drug trials carried out. Therefore it fell to Novartis to establish that the *therapeutic efficacy* of the beta crystalline form of imatinib mesylate was enhanced when compared to the non-crystalline form.

While it was accepted by the Court that the enhanced properties of the *beta* form (better flow properties, better thermodynamic stability and lower hygroscopicity) may be beneficial for processing and storing the substance, these physical properties did not qualify as properties that indicated enhanced *therapeutic efficacy*.

Origins of the Indian position

Indian patent law clearly sets out (in section 3(d)) the position that new forms of known substances are unpatentable and this decision merely reinforces that position. Therefore the result of the case should be no surprise and similar decisions are to be expected in the future.

The text of the decision[6] itself provides a fascinating insight into the history and evolution of patent law in India. The latest step in this evolution occurred in 2005. Before this, India only allowed patents on new manufacturing processes; new chemical compounds were unpatentable. This led to the development of a strong generics sector which focused on circumventing Indian process patents filed from overseas to produce compounds with demonstrated therapeutic efficacy for supply to the Indian and developing world markets.

In 2005, to comply with obligations under the WTO TRIPS Agreement, India changed its patent law to allow patents to be granted for new chemical compounds. Members of the Indian Parliament expressed their concerns at the time the new law was being considered that it would unreasonably limit availability of medicines to the Indian population or to other developing countries owing to their inability to pay for patented medicines. To address these concerns, the 2005 law excluded from patentability new forms of known substances which do not result in the enhancement of the known efficacy of that substance.

The Indian position on patentability of new forms of known substances is at odds with every other major patent system which allows patents to be granted for non-obvious advancements in the form of known compounds. The policy rationale of most jurisdictions to allow such patents is that they encourage further development of known compounds to produce better methods of production and administration as well as safer and more convenient drugs.

The irony of the Indian position is that if it were widely adopted, research into novel forms would likely be curtailed owing to lack of commercial incentives, and the development of better drug forms would be limited. Even if such development were to be carried out, with no possibility of an exclusive market position, any advances would probably be kept as trade secrets rather than being disclosed as the patent process requires. Without the dissemination of knowledge, further innovation would likely be retarded and the knowledge concentrated in the hands of a few big players.

The role of the generics producers

India has made a name for itself as a large scale manufacturer of pharmaceuticals for export to other countries, both developing and developed. The generics industry has been assisted by the laws applied by the Indian Patent Office and Courts which are generally more favourable to the industry than in other major markets. One factor whom drives the government to support the industry is no doubt a desire to deliver affordable healthcare to India's 1.24 billion people, a third of which fall below the international poverty threshold of US$1.25 per day.[7] In 2006 a generic version of Glivec was available in India for $200 per month compared to a reported $2600[8] in some countries where Glivec was covered by a patent. This disparity has led to an understandable desire to limit patent monopolies on pharmaceuticals.

Exports of Indian generic drugs are relied on by countless developing countries. The international humanitarian organisation Médicins Sans Frontières (MSF) even heralded the Supreme Court decision as a 'major victory' for patient access to affordable medicines. However, generics from India are not only important to developing countries; they are also a major source of cut-price, high-grade pharmaceuticals for developed countries to access once a patent on the original drug has expired.

However, in the case of Glivec, freedom to manufacture a generic version of Glivec was never at stake as no patent was ever granted for the non-crystalline form in India. Even if the *beta* patent had been granted, this would only provide a monopoly for the beta crystalline form of the compound. A drug containing the free base form of imatinib or the non-crystalline form of imatinib mesylate would not infringe as long as there was no use of the beta form.

So why did the parties challenging the *beta* patent go to so much trouble and expense if there was never an issue of being able to make a therapeutically effective form of the drug? This question becomes even more pertinent when one considers that the non-crystalline form exhibits identical therapeutic efficacy so there would be no advantage in terms of improved patient outcome.

One reason may have been to simply uphold the principle that new forms of a known compound are unpatentable. While this would be laudable, a more likely reason is the potential commercial benefits to be had for the generics industry if they can exploit the best process to produce the drug with best storage characteristics. The lower labour costs in India and the minimal research and development costs would provide the generics producer with a competitive edge over the patent holder that would likely secure the entire Indian market for generic Glivec as well as servicing other countries where no patent exists. It would also place them in prime position to compete once the beta form of the drug went off-patent in developed countries. As such, there were clearly commercial reasons for the generics producers to mount a challenge to the grant of the patent.

A two-tier patent system

The decision examines the general principles that govern the global patent system and appears to arrive at the conclusion that what is best for the developed world is not necessarily best for the health and economy of India (and, by extension, the developing world). Essentially it affirms India's right to enact and apply patent laws in its national interest, as long as they comply with obligations under international trade agreements.

Effective enforcement of IP rights in the developed world is generally accepted to successfully result in promotion and dissemination of technology. However, this model breaks down when applied to countries with small or non-existent innovation ecosystems which also lack the funds to pay for patented products.

So should patent systems be allowed to vary depending on the industrial maturity of the country? If a country is less developed, poorer and has less capability to innovate, should higher thresholds of patentability should apply? While answers to these questions depend on your perspective, what is clear is that seeking to impose developed world IP laws on the developing world is unlikely to assist innovation or patients. These countries and the majority of their citizens simply cannot afford to pay developed world prices for patented medicines. In addition, pharma companies are often loath to cut prices for the legitimate concern that these products could find their way back to developed world markets as parallel imports.

While it may seem unfair that there could be a two-tier global IP system in which some countries enforce while others ignore IP rights, the economic reality is that without this enforcement, new drugs would not be developed, and if enforcement was ubiquitous, life-saving medicines would be inaccessible to millions more people in developing countries. The trick therefore is balancing the scales to encourage both development and access.

A two-tier system already exists to some degree but is manifested more by some countries lacking effective enforcement prospects or suitable disincentives for infringement. Effective enforcement of existing rights is certainly an issue in India and China and adds to the difficulties which multi-nationals have in doing business in these countries.

Global reactions

The US industry trade group Pharmaceutical Research and Manufacturers of America (PhRMA) said the decision reflected a deteriorating environment for innovation in India, "Protecting intellectual property is fundamental to the discovery of new medicines. To solve the real health challenges of India's patients, it is critically important that India promote a policy environment that supports continued research and development of new medicines". Novartis called the decision "a setback for patients that will hinder medical progress for diseases without effective treatment options [and] discourage future innovation in India".

So will this decision be a turning point for investment by the traditional pharma firms in India? Unlikely. Despite assertions that the decision will discourage innovation and investment, it would be a brave CEO to pass up the opportunity to gain a monopoly on their product in India; in doing so they would effectively cede control of manufacturing and distribution of the drug for a large part of the global market.

However, potential ramifications of the decision for India may materialise through other channels. The US Information Technology and Innovation Foundation (ITIF) – a US think tank – referred to the decision when lobbying the US Congress to increase trade tariffs on Indian imports. The ITIF said a response was needed to India "enacting regulations that harm American industry and jobs".

Patent versus patient

This decision by the Indian Supreme Court clearly reinforces the fact that Indian patent law has a threshold for patentability of chemistry-related inventions higher than most developed nations. However, the decision's significance should not be overstated; it only relates to the patentability of new forms of known compounds and does not prevent the protection of new chemical compounds (or even more highly efficacious forms of known compounds). It at least provides clarity on what is allowable in India.

Weighing intellectual property rights against access to medicines is a difficult balancing act with legitimate concerns by parties on both sides of the debate. However, tipping the scales too far in favour of one or the other will lead to either innovation or patients suffering.

If you have any queries regarding intellectual property related matters (including patents, trademarks, copyright or licensing), please contact: *tim.stirrup@baldwins.com* or Patent Proze, Baldwins Intellectual Property, PO Box 5999, Wellesley Street, Auckland.

Bibliography

1. http://www.laskerfoundation.org/awards/2009_c_description.htm
2. Buchdunger, Zimmermann and Mett, *et al.* (1996). Inhibition of the Abl Protein-Tyrosine Kinase in Vitro and in Vivo by a 2-Phenylaminopyrimidine Derivative. *Cancer Res.* **1996**; *56*, 100-104
3. IND# 55666
4. IUPAC describes efficacy as "the property that enables drugs to produce responses". When comparing the efficacy of two substances, efficacy describes "the relative intensity with which agonists vary in the response they produce even when they occupy the same number of receptors" - IUPAC Glossary of Terms used in Medicinal Chemistry, 1998 in CPAA Vol. 9, p. 7.
5. Expert witness Prof. Shamnad Basheer argued that safety or significantly reduced toxicity should also be taken into consideration to judge enhanced therapeutic efficacy although this was not relevant in deciding the present case.
6. http://judis.nic.in/supremecourt/imgs1.aspx?filename=40212
7. World Bank Poverty Data 2010 - http://povertydata.worldbank.org/poverty/country/IND
8. http://www.doctorswithoutborders.org/publications/article.cfm?id=5769

Katherine Hebditch and Tim Stirrup of Baldwins Intellectual Property in Auckland specialise in chemistry and biotechnology patents. Katherine obtained her PhD in organic chemistry from the University of Manchester in the UK in 2004. She is currently working towards registration as a patent attorney. Tim obtained his PhD in molecular biology from the University of Southampton in the UK in 2007. He is also working towards registration as a patent attorney.

Dates of Note

Rudolph A. Marcus, the Canadian-born American chemist and winner of the 1992 Nobel Prize for his work on the theory of electron-transfer reactions in chemical systems (Marcus theory), is 90 years of age on Ju1y 23. Also, on July 23, 125 years ago (1888), ***John Boyd Dunlop*** applied to patent the pneumatic tyre; and on the same day in 1903 the Ford Motor Company sold its first automobile in Detroit, the Ford Model A. In 1938, on July 24, Nescafé instant coffee was commercially introduced in Switzerland by the Nestlé Company. ***Charles Macintosh***, the Scottish chemist and inventor of rubberized waterproof clothing was born on July 25, 1843. Seeking uses for waste products from coal gasworks, he utilized naptha as a solvent in his 1823 method of waterproofing cloth by pressing together two rubberized layers; the name 'macintosh' remains associated with the raincoat made from such cloth. ***Paul Walden*** was the Russian-German chemist who, while teaching at Riga, discovered the inversion of malic acid, by which two varieties of malic acid could be formed. One rotated plane polarized light in a clockwise fashion, the other counter-clockwise. His name is immortalized in the 'Walden inversion'; he was born 150 years ago on July 26, 1863.

Friedrich Ernst Dorn was born on July 27, 1848. He was the German physicist who followed Madame Curie's discoveries with his discovery that radium not only emitted radiation, but released a colourless gas that was also itself radioactive. Initially called 'radium emanation' or 'niton', it was renamed 'radon' in 1923, and is the heaviest of the inert gases. On July 27, 1823, the effect of platinum as a catalyst was investigated by ***Johann Wolfgang Döbereiner***. He found that hydrogen combined with air in the presence of platinum powder to form water in a reaction so vigorous that the filter paper holding the powder was charred. Only later was the term 'catalysis' coined by Berzelius (1835). ***Otto Hahn***, the German chemist who, with radiochemist Fritz Strassmann, is credited with the discovery of nuclear fission, died on July 28, 1968. ***John Alexander Newlands*** was the British chemist credited with being the first to establish an order of elements by their atomic weights and to observe periodicity in their properties. Every eighth element has similar properties, hence he named the Law of Octaves on Feb 7, 1863; he died on July 29, 1898, the same date in 1953 as ***Richard Pearse***, the New Zealand inventor and aviation pioneer. On July 30, 1898, corn flakes were invented by ***William Kellogg***. ***Stephanie Kwolek***, the American chemist who invented Kevlar, has her 90th birthday on July 31.

Richard Kirwan, the Irish chemist whose *Elements of Mineralogy* (1784) was the first English systematic treatment of the subject, was born on August 1, 1733. ***Leopold Gmelin***, the German chemist who discovered potassium ferrocyanide (1822), devised a test named after him for bile pigments, researched the chemistry of digestion, and published his famous *Handbook of Chemistry* to comprehensively survey the subject, was born on August 2, 225 years ago. ***Benjamin Franklin Goodrich***, the American industrialist who founded the B.F. Goodrich Rubber Co., died on August 3, 125 years ago. August 4, 1693 is the reputed day that champagne was invented by ***Dom Perignon***. ***Jons Jacob Berzelius***, the Swedish chemist and one of the founders of modern chemistry especially noted for his determination of atomic weights and the development of modern chemical symbols, died on August 7, 1848. ***Cato Maximilian Guldberg***, the Norwegian chemist who, with his brother-in-law Peter Waage, formulated the law of mass action (1864), was born on August 11, 175 years ago (1836). ***Karl Ziegler***, the German chemist who shared the 1963 Nobel Prize for Chemistry (with Natta) for high polymer work, died on August 12, 1973. ***Frederick Sanger***, the English biochemist and twice the recipient of the Nobel Prize for Chemistry (1958 and 1980), was born on August 13, 1918. On the same day in 1913, the first true stainless steel, a steel alloy that contained 0.24% carbon and 12.8% chromium, was cast in Sheffield, England, and on the same day in 1903, the journal *Nature* reported that Ramsay and Soddy had shown that helium gas is produced by the radioactive decay of radium. ***Richard R. Ernst***, the Swiss researcher awarded the 1991 Nobel Prize for Chemistry for his contributions to the development of the methodology of high resolution NMR spectroscopy, has his 80th birthday on August 14. ***Frederic Joliot-Curie***, the French physical chemist and husband of Irène Joliot-Curie who were jointly awarded the 1935 Nobel Prize for chemistry, died on August 14, 1958. ***Frederic Stanley Kipping***, the British chemist who pioneered the chemistry of silicones, was born on August 16, 150 years ago, while ***John S. Pemberton***, the American pharmacist who invented Coca-Cola in 1885, died this day 125 years ago. Element 110 was formally named ***darmstadtium*** (Ds) on August 16, 2003. ***Walter Noddack***, the German chemist who, in collaboration with his wife Ida Tacke, discovered element 75 (in June 1925) and named it 'rhenium' after the river Rhiene, was born on August 17, 1893. Rhenium was the last stable element to be discovered.

Jean Servais Stas, the Belgian chemist noted for his accurate determinations of atomic weights, was born on August 21, 200 years ago. ***Willis R. Whitney***, the American chemist who founded the General Electric Company's research laboratory and known as the father of basic research in industry, was born on August 22, 1868. ***Robert F. Curl***, Jr., the American chemist who (with Smalley and Kroto) discovered the first fullerene in 1985, has his 80th birthday on August 23. ***Rudolf Clausius***, one of the founders of thermodynamics, died on August 24, 125 years ago. ***Antoine-Henri Becquerel***, the French physicist who discovered radioactivity and shared the 1903 Nobel Prize for Physics with Pierre and Marie Curie, died on August 25, 1908. ***Antoine-Laurent Lavoisier***, the father of modern chemistry, was born on August 26, 1743, the day in 1856 that ***William Henry Perkin*** applied for a British patent for his invention of aniline dye "producing a new colouring matter for dyeing with a lilac or purple colour stuffs of silk, cotton, wool or other materials". ***Eilhardt Mitscherlich***, the German chemist who promulgated the theory of isomorphism, died on August 28, 150 years ago. ***Christian Friedrich Schönbein***, the German-Swiss chemist who discovered and named ozone (1840) and was the first to describe guncotton (nitrocellulose), died on August 29, 1868. ***Sir George Porter***, noted for his study of very fast reactions, died on August 31, 2003.

Carl Freiherr (Baron) ***von Welsbach,*** the Austrian chemist, physicist and engineer who invented the gas mantle that becomes incandescent white-hot in a gas flame giving greatly increased light output by gas lamps, was born on September

1, 1858. On this same day in 1898, ***Ernest Rutherford*** coined the terms 'alpha' and 'beta' for two distinct types of radiation. ***Friedrich Wilhelm Ostwald***, the German chemist who almost single-handedly organized physical chemistry into a nearly independent branch of chemistry, was born on September 2, 1853. ***Stanford Moore***, the American biochemist who shared (with Anfinsen and Stein) the 1972 Nobel Prize for Chemistry for his understanding of the connection between chemical structure and catalytic activity of the active centre of ribonuclease molecule, was born 100 years ago on September 4. ***Aleksandr Butlerov***, the Russian chemist and one of the principal creators of the theory of chemical structure (1857–1861), was the first to incorporate double bonds into structural formulas. He was born on September 6, 1828, and has the crater Butlerov on the Moon named after him. Sir ***Derek H.R. Barton***, the British chemist and joint recipient (with Hassel of Norway) of the 1969 Nobel Prize for Chemistry for work that helped establish conformational analysis, was born on September 8, 1918. ***Viktor Meyer***, the German chemist who contributed greatly to knowledge of both organic and inorganic chemistry and invented an apparatus for determining vapour densities (and hence molecular weights) that is named after him, was born on the same day in 1848. ***Waldo Semon*** was the little remembered American chemical engineer who invented plasticized PVC; he was born on September 10, 1898. ***Felix Bloch***, the Swiss-born American physicist who shared (with independent discoverer, Purcell) the Nobel Prize for Physics in 1952 for developing NMR spectroscopy, died on September 10, 1983. ***Pierre Vernier***, the French mathematician who invented the vernier scale, died on September 14, 375 years ago (1638).

Guillaume François Rouelle, the French apothecary and chemist who first proposed the modern definition of salts and was the first to distinguish neutral, acid, and basic salts, was born on September 16, 1703. ***Friedrich Adolf Paneth*** was the Austrian-British chemist who improved methods to isolate and measure the minute amounts of helium (as little as 10^{-10} cm^3) slowly released by traces of radioactive elements in rocks. This enabled the determination of the age of rocks on earth and the age of meteorites. He died on September 17, 1958. ***Charles-Victor Mauguin*** was the French mineralogist and crystallographer and one of the first to make a systematic study of the silicate minerals. Using X-ray diffraction techniques, he determined the structure of a large number of micas, cinnabar, calomel and graphite, and devised the system of symbols for designating the symmetry properties of crystals adopted in 1935 as the international standard. He was born on September 19, 1878. ***David Marine***, the American pathologist whose substantial study of treatment of goitre with iodine led to the iodizing of table salt, was born on September 20, 125 years ago. It is also the day in 1848 when the first meeting of the American Association for the Advancement of Science was held in the library of the Academy of Natural Science in Philadelphia, Pennsylvania. ***Michael J.S. Dewar***, an early master of molecular orbital theory, was the Scottish organic chemist who was born in India on September 24, 1918. ***Hieronymus Theodor Richter***, the German mineralogist who in 1863 was a co-discoverer of the element indium, died on September 25, 1898. ***August Ferdinand Möbius***, of Möbius strip fame, died on September 26, 1868. ***Adolphe Wilhelm Hermann Kolbe***, the German chemist who accomplished the first generally accepted synthesis of an organic compound from inorganic materials, was born on September 27, 1818, the day in 1838 that ***Bernard Courtois***, the little known French chemist who discovered the element iodine, died. ***Rudolf Christian Karl Diesel***, the German engineer who invented the internal-combustion engine named after him, died on September 29, 1913. ***Johann Deisenhofer***, the German biochemist who (with Michel and Huber) won the Nobel Prize for Chemistry in 1988 for determining the three-dimensional structure of certain proteins that are essential to photosynthesis, has his 70th birthday on September 30.

Edwin Joseph Cohn, the American biochemist who helped develop the methods of cold ethanol blood fractionation (the separation of plasma proteins into fractions), died on October 1, 1953. ***Kenichi Fukui***, the Japanese chemist who shared the 1981 Nobel Prize for Chemistry with Hoffmann for the investigation of chemical reaction mechanisms and who introduced his frontier orbital theory of reactions in 1952, was born on October 4, 1918. ***Otto Warburg***, the German biochemist awarded the Nobel Prize for Physiology or Medicine in 1931 for his work on cellular respiration, was born on October 8, 1883. On October 11, 1938, ***R. Games Slayter*** and ***John H. Thomas*** patented glass wool and the machinery to make it. ***Vincenzo Dandol*** was the Italian pharmacist, natural scientist, writer and statesman, and innovator in both science and politics who championed the new scientific theories of Lavoisier. He also helped further democratic ideals in Italy, and was personally committed to the advancement of secondary education in general and to health care in particular. He was born on October 12, 1758. On October 14, 1863, 150 years ago, ***Alfred Nobel*** was granted his first patent, a Swedish one, for the preparation of nitroglycerin; he was born on October 21, 1833.

Evangelista Torricelli, the Italian physicist and mathematician who invented the barometer and whose work in geometry aided in the eventual development of integral calculus, was born on October 15, 1608. ***Carl Mosander*** was the Swedish chemist and mineralogist whose work revealed the existence of numerous rare-earth elements with closely similar chemical properties. He discovered lanthanum (La, in 1839), erbium (Er, in 1842) and terbium (Tb, in 1843) and died on October 15, 1858. ***Cyril Ponnamperuma***, the Ceylonese-American chemist and exobiologist, who was a leading authority on the chemical origins of life, was born on October 16, 1923. ***Samuel Guthrie*** was the American physician and chemist who independently discovered chloroform and invented the percussion priming powder for firearms prior to the use of flints. He experimented in a laboratory near his house and had a mill about a mile away for manufacturing large quantities of his powder and other explosives, including potassium chlorate and mercury fulminate. He made chloroform in 1831 by distilling $CaCl_2$ with alcohol in a copper vessel, prior to the independent discoveries of Soubeiran (France, 1831) and Liebig (Germany, 1832), and used it during amputation surgery in his hometown of Sackets Harbor, NY. He died on October 18, 1848. On October 20, 1983 the length of the metre was redefined as the distance that light travels in a vacuum in 1/299,792,458 of a second by the international body Conférence Générale des Poids et Mesures (GCPM) to give greater accuracy. Originally the metre was based on one ten-millionth of the distance from the North Pole to the equator.

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Article

Helping scientists redefine the kilogram

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The kilogram is the last unit of the International System of Units (SI) still based on an artefact - the international prototype of the kilogram (IPK). Mass comparisons with the IPK have indicated a mean drift in the official IPK copies of 0.5 µg/year. To achieve better stability, scientists have been working on a new definition of the kilogram based on a physical constant of nature, which should be reproducible regardless of geographical location.

Mettler-Toledo has recently developed a microgram load cell to help these scientists to establish the new definition of the kilogram. The new patented MonoBloc® high precision load cell helps to provide the reproducibility required to fulfill the acceptance criteria. The following paragraphs are from a press release from Mettler Toledo.[1]

Mettler Toledo has developed a new microgram load cell for use in an experiment aimed at establishing a new definition of the kilogram. The new load cell is a critical element in the Swiss based leg of this global challenge headed by the Swiss Federal Office of Metrology (METAS).

The IPK prototype, machined in 1878, is a cylinder of platinum-iridium alloy (Pt 90% - Ir 10% in mass) whose height (39 mm) is equal to its diameter. Six copies were designated as official copies and are kept in the same protective conditions as the international prototype. To assess the evolution of the official copies relative to the IPK, three comparisons have been carried out since 1880. The results of these comparisons have clearly put in evidence, a relative drift of this set of masses with respect to the IPK, evaluated at 0.5 µg/year.

Today, all modern mass measurements are directly related to the IPK. The relative uncertainty of each measurement is declared according to the comparison of a test weight used on the same weighing apparatus with the IPK. There is no currently accepted standard for defining the kilo in the same way as for other standards e.g., the metre is defined according to the distance light travels in a vacuum in a specified time period. The relative weight between the IPK and its copies is changing minutely over time and it is this which is driving scientists to redefine the kilo. METAS, in conjunction with other international scientific institutions, aims to complete this redefinition by 2015.

In a fifteen year project, METAS has been using a Watt balance in a two-phase experiment: weighing and moving. In the weighing phase, a macroscopic mass and a coil are suspended from a balance with the coil placed in a magnetic field. Using the principle of electromagnetic force restoration, the current needed to compensate for the addition of the weight is measured. In the moving phase, a voltage is induced in the same coil by moving it vertically within the same magnetic field. The voltage measured is directly related to the speed of the movement of the coil. By comparing the two experiments, an equation is derived in which the electrical power is related to the mechanical power, hence the name Watt balance. Collaborative partners in the project include the Federal Institute of Technology in Lausanne (EPFL) for the movement system, the European Organization for Nuclear Research (CERN) for the magnetic system and Mettler Toledo for their expertise in mass comparison.

The load cell needed to measure the residual force in the weighing phase is a critical and challenging component of the apparatus and must reach a 1 µg resolution for a total load just below 2 kg. To meet this requirement, a new microgram load cell was created by an interdisciplinary team from Mettler Toledo. The new patented MonoBloc® load cell is significantly lighter than the most accurate existing load cells and exceeds the requirements with a startling 0.3 µg resolution. This highly accurate load cell helps to provide the reproducibility required to fulfill the acceptance criteria.

Leading the Mettler Toledo project, Daniel Reber stated, "This is a really exciting development for us as a company and reinforces our reputation in weighing expertise. We owe a special thanks to the late Christophe Béguin whose dedication and ideas drove the design of the new load cell. It's a key part of the METAS experiment and they are confident about achieving their goal to redefine the kilo by 2015."

Reference

1. *http://www.abipressroom.com/pr_public/view_pr_web.cfm?prid=4860* (downloaded 3 May 2013)

NZIC Conference 2013 Update

The organisation of the 2013 December NZIC conference is progressing well. Six renowned scientists have accepted invitations to give plenary lectures. They are: Profs. ***Ben Davis*** (Oxford), ***Pieter Dorrestein*** (UC-SD), ***Tina Overton*** (Hull), ***Philip Power*** (UC-Davis), ***Jim Watkins*** (Massachusetts), and ***Jeff Tallon*** (Callaghan Innovation).

Ben Davis obtained both his BA (1993) and DPhil (1996) degrees in carbohydrate chemistry from the University of Oxford before spending two years as a postdoctoral fellow at the University of Toronto, exploring protein chemistry and biocatalysis. He returned to the UK in 1998 to take up a lectureship at the University of Durham, moving in 2001 to his current role at the Dyson Perrins Laboratory at Oxford. His group's research centres on chemical biology with an emphasis on carbohydrates and proteins. In particular, the group's interests encompass synthesis and methodology, inhibitor design, protein engineering, drug delivery, molecular modelling, molecular biology, and glycoscience. He has been the recipient of numerous awards, most recently the 2012 *Tetrahedron* Young Investigator Award. He is a member of the editorial board of several prestigious journals and a co-founder of two companies, Glycoform and Oxford Contrast.

Pieter C. Dorrestein is Associate Professor at the Skaggs School of Pharmacy and Pharmaceutical Sciences and Departments of Pharmacology, Chemistry and Biochemistry at the University of California, San Diego. He gained his BA in 1999 from Northern Arizona University followed by a PhD in Chemical Biology at Cornell in 2004. He was then an NRSA Fellow in Bioanalytical Chemistry at the University of Illinois before taking up his current role. Since 2006, he has been pioneering the development of mass spectrometry imaging methods to study the chemical ecological crosstalk between populations of organisms for agricultural, diagnostic and therapeutic applications. He has published over 110 articles and is the recipient of several awards, including the Beckman Foundation Young Investigators Award, ACACC award by Lilly in Analytical Chemistry (2008), the Pharmaceutical Research and Manufacturers of America Award (2008), and the Matt Suffness Award. He is a V-foundation Scholar, a Hearst foundation Scholar and holds an Exceptional, Unconventional Research Enabling Knowledge Acceleration grant from NIH. In addition, he is a technological advisor/consultant for INDICASET, Janssen, CUBIST and Sirenas Marine Discovery. At the present time his lab is funded by the NIH, the Keck foundation, Bruker, Janssen (J&J), Agraquest-BAYER and CUBIST.

Tina Overton is Professor of Chemistry Education at the University of Hull. She completed her first degree part-time whilst working in industry and in the National Health Service before completing a PhD and postdoctoral work in heterogeneous catalysis. She joined the Chemistry Department at Hull in 1992, first as a Teaching Fellow, progressing through the ranks to her current Chair. She has published widely on critical thinking, context and problem-based learning and their roles in developing conceptual understanding, cognitive skills and problem solving abilities. She has also produced widely adopted learning resources and has co-authored several textbooks in inorganic chemistry and on study skills. She was Director of the National Physical Sciences Centre of the UK Higher Education Academy that supports teaching and learning across chemistry, physics and astronomy. She has been awarded the Royal Society of Chemistry HE Teaching Award, Tertiary Education Award and Nyholm Prize, and is a National Teaching Fellow and Senior Fellow of the Higher Education Academy.

Philip Power received his BA degree from Trinity College (Dublin) in 1974 and a 1977 DPhil under Prof. Michael Lappert at Sussex. After postdoctoral studies at Stanford, he joined the Department of Chemistry at the University of California, Davis, in 1981, where he is currently a Distinguished Professor of Chemistry. His main interests lie in the synthesis of new main-group and transition-metal complexes. A particular focus is the use of sterically crowded ligands to stabilize species with new bonding types, low coordination numbers, and high reactivity. He has been the recipient of several awards including the 2005 Ludwig Mond Medal of the Royal Society of Chemistry, the 2005 F.A. Cotton Award in Synthetic Inorganic Chemistry of the American Chemical Society and, most recently, the 2012 ACS Award in Organometallic Chemistry. He was elected a Fellow of the Royal Society of London in 2005.

Jeff Tallon really needs no introduction to the New Zealand chemical community. He is a Principal Scientist at Callaghan Innovation and, until 2009, was concurrently Professor of Physics at VUW. He is known internationally for his research into oxide ceramic high-temperature superconductors (HTS) for applications across all sectors – health, transport, energy, and information technology amongst others. His discoveries have led to the establishment of HTS-110 Ltd., a New Zealand company developing high-level HTS products for the international market. He has been awarded the Rutherford Medal, is a Companion of the New Zealand Order of Merit (CNZM) and was presented with the inaugural Prime Minister's Science Prize (with Bob Buckley). He has been a Visiting Professor at Cambridge University, the Technical University of Denmark, the University of Paris, and EPFL, Lausanne, Switzerland.

Jim Watkins is a Professor of Polymer Science and Engineering, and Director of the Center for Hierarchical Manufacturing - a National Science Foundation Nanoscale Science and Engineering Center (NSEC) - at the University of Massachusetts, Amherst. He received both his BS and MS degrees in Chemical Engineering from the Johns Hopkins University and his PhD in Polymer Science and Engineering from the University of Massachusetts. He joined the Chemical Engineering faculty at UMass in 1996 and this was followed by a move to the Polymer Science and Engineering Faculty in 2005. He has been the recipient of a Camille Dreyfus Teacher-Scholar Award and a David and Lucile Packard Foundation Fellowship for Science and Engineering. He is also a Fellow of the American Physical Society.

Accommodation

Conference accommodation has been arranged with Te Puni Village (www.tepunivillage.co.nz) on the Victoria University Kelburn campus at a rate of $54 per night for a room and continental breakfast. Please note that the Kelburn campus is approximately 25 minutes by foot from the downtown conference venue at Rutherford House on the Pipitea campus. A range of accommodation options are available downtown literally across the road from Rutherford House, from backpackers dormitory rooms through to top international-level hotels. Delegates are more than welcome to make their own arrangements to stay closer to the venue than Te Puni. Please note, Rutherford House is found at the major transport hub of Wellington Railway Station and so staying outside of the city in the outlying suburbs and cities is also an entirely realistic option.

Futher information

Beyond the plenary lectures, the conference will include invited talks from many of New Zealand's top chemists at the universities and CRIs. These will be streamed into concurrent thematic sessions covering all the main branches of chemistry, including chemical education. Of course, the conference will include an afternoon of excursions for delegates to enjoy. Being "the coolest little capital", we have planned for various trips to highlight the Harbour Capital's unique opportunities combining land, sea and cultural activities. These will include a tour of Zealandia (*www.visitzealandia.com*), the only pest-free "island" sanctuary within a city, as well as the opportunity to visit the Weta Cave (*www.wetanz.com/cave/*), the museum dedicated to the thriving film and special effects industry based in Wellington that has helped put New Zealand tourism on the international stage.

Finally, a stunning location has been chosen for the conference dinner, which is a 10 min walk from Rutherford House along the Wellington waterfront, adjacent to Courtenay Place and the local evening entertainment zone. The dinner will, therefore, be a fitting and enjoyable way to end the conference on a high!

We look forward to seeing you in Wellington from December 1 to 5. Please visit the conference website.

www.chemistryconference.org.nz

for more information, where registration and abstract submission, etc., will be available in due course.