NZIC Chemistry IN NEW ZEALAND

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New Zealand Institute of Chemistry

supporting chemical sciences

January News

Comment from the President

It is with great pleasure that I assume the 2010 Presidency of the NZIC from John Spencer. On behalf of the Institute I would like to thank John for his time and efforts. This is also an opportunity to thank the people behind the scenes; Honorary General Secretary, Richard Rendle, who does a great job keeping the Institute running smoothly; Colin Freeman, Treasurer, for his astute financial management; Brian Halton, Editor of *Chemistry in New Zealand* and Peter Hodder, Assistant Editor and Editor of *Chemistry Education in New Zealand*.

Past Presidents have commented on the need to raise the profile of chemistry and to educate the public about the positive impact that chemistry has had and will continue to have on our society. This is an important goal for the NZIC. We have a great opportunity to address these issues with the International Year of Chemistry in 2011. To make the most of IYC 2011 we will need a well coordinated programme of events that engage the public interest and demonstrate the benefits of chemistry to society. 2010 is the time to organise these events! The Council is asking each Branch to plan their events and Council will take a co-ordinating role, especially with nationwide activities.

One of the positive initiatives for science from our new National Government has been the appointment of Professor Sir Peter Gluckman to the role of Chief Scientist. This is significant for the NZIC because the Chief Scientist role represents a conduit between chemists working at the coalface and the decision makers in Wellington. One thing on my to-do list as President is to meet with Sir Peter on behalf of the NZIC and to tell him about all the talented chemists that we have working in NZ. I'll keep you updated with progress on that.

On the other hand, it is disappointing to see that the Government has chosen not to invest in science and technology, or tertiary education, at a time when our Australian counterparts (and American to a lesser extent) are receiving an almost unprecedented boost in funding. In the University system, the old paradigm of bums-on-seats has been replaced with the capped-funding model and it will be interesting to see how the various tertiary institutions respond to this strange new world. Having experienced the CRI system for a couple of years it is pleasing to see that at least a certain proportion of funding for the CRIs has been given some rigidity, and the increase in the Marsden Fund is a welcome boost that has been long overdue.

I am looking forward to visiting the Branches and discussing some of these issues with you all.

All the best for a successful and productive year,

Mark Waterland
President

About the President

Mark completed his BSc(Hons) and PhD studies (in 1998), studying the spectroscopy and excited-states of rhenium and copper polypyridyl complexes, under the supervision of Prof Keith Gordon at the University of Otago. Following postdoctoral study with Prof Anne Kelley (nee Myers) at the University of Rochester, he followed the Myers group to Kansas State University as a FRST NZ Science and Technology Fellow. At KSU, he carried out ultrafast spectroscopic studies of ruthenium solar cell sensitizer dyes with Prof David Kelley. After a brief period teaching at Southwest Missouri State University he returned to this country in 2001 as a staff member with the Materials Technology group at IRL in Lower Hutt. He took up his current position at Massey University in 2003 and he is presently a Senior Lecturer in the Institute of Fundamental Sciences. Some of his research interests are described in an article in this issue of *Chemistry in New Zealand*. He has served as Branch Chair, Student Liaison and Council Delegate for the Manawatu Branch before assuming the role of 2nd Vice-President in 2008.

RSNZ Awards & Fellowships

Congratulation go to Prof *Peter* ***Steel*** (Canterbury University) who has been awarded the **2009 Hector Medal** for the advancement of chemical sciences through his world-renowned work in the field of metallosupramolecular chemistry leading to potential applications in medicine and nanotechnology. Dr *Richard* ***Garland*** (Managing Director, NZ Pharmaceuticals Ltd.) received the **2009 Thomson Medal** for his outstanding leadership in the development and application of science and technology to New Zealand business development. The awards were announced at the Sciences Dinner in Auckland on Nov. 18.

Prof Peter Steel receiving the 2009 Hector Medal (photo with permission from RSNZ)

In addition, Massey's Palmerston North scientists, Prof *Tim* ***Brown*** (Applied Health Sciences) and Dr *David* ***Shillington*** (Fundamental Sciences – Chemistry) have collected RSNZ Certificates of Excellence for promoting science to the public through their columns in the *Manawatu Standard*. They provide easily digestible comment on scientific advances and topical science issues keeping it real. On alternate Mondays, Dr Shillington writes ***Analyse This***, while Dr Brown pens ***Analyse That***. Each week, they try to address the hottest scientific topic in an interesting way, to try to get facts across in a clear and uncomplicated manner, without jargon. Furthermore, Dr *Andreas* ***Hermann***, of Peter Schwerdtfeger's Research Group at Massey-Albany, took out the 2009 **Hatherton Award** for the best paper published from a PhD study in physical, earth or mathematical and information sciences at a NZ university.

Chemists elected to Fellowship of RSNZ at the Fellows AGM on November 11 last were:

Prof *David* ***Williams*** - University of Auckland. Peter is a leader among the international electrochemistry community with his most notable work being about the pitting corrosion of stainless steels and the successful commercialization of gas sensor devices.

Dr *Philip* ***Boyd*** - NIWA/University of Otago. Philip is internationally recognized for his work in the field of oceanography and the productivity of the global ocean.

Professor *Timothy* ***Burstein*** - a graduate of the University of Auckland who works in the field of electrochemistry and corrosion science at the University of Cambridge (UK), was elected to Honorary Fellowship.

NZAS Awards

Prof *Ian* ***Shaw*** (Canterbury University) was been awarded the Science Communicator Award for 2009. It was presented by the Minister for Research, Science and Technology, Hon Dr Wayne Mapp on Thursday November 12 in Wellington.

Society of Chemical Engineers in New Zealand

The members of the Society of Chemical Engineers in NZ (SCENZ) have voted to become the NZ Branch of IChemE (Institution of Chemical Engineers).

The decision was made at a SCENZ special general meeting last month and the Chair of the Board of SCENZ–IChemE in NZ Dr *Max* ***Kennedy*** says the move will benefit chemical engineers in this country. The member vote provides the Board the mandate to develop IChemE in NZ and to enhance the networks and profile of chemical and process engineering here.

IChemE Chief Executive, Dr *David* ***Brown*** says: *IChemE is 100% committed both to the support of the NZ chemical and process engineering community and to contribute to the general promotion of the engineering profession in conjunction with the leading national bodies such as IPENZ. This new branch is very important for IChemE to be able to effectively promote chemical and process engineering careers and the profession in NZ and to connect its chemical engineers with their global colleagues*.

NZ IChemE members will benefit from a programme of continuing professional development, technical meetings and events, and enhancing networks across the country that is to include the NZIC.

NZIC NEWS

The Officers of the Institute elected for the 2010 year at the November AGM in Wellington are:

President:

Dr *Mark Waterland* (Massey University, Palmerston North)

1st Vice-President:

Dr *Gordon Rewcastle* (Auckland University)

2nd Vice-President:

Appointment pending

Hon. Gen. Secretary:

Mr *Richard Rendle* (Christchurch)

Treasurer:

Dr *Colin Freeman* (Canterbury University)

The **2010 Branch Chairpersons**, elected at the various Branch AGMs, are:

Auckland: Dr *David* ***Salter***, a Senior Tutor in the Chemistry Department at Auckland University for some 12 years. He is heavily involved both with Stage I teaching, and the training and development of secondary school chemistry teachers.

Waikato: *Marisa* ***Till*** has been re-elected as Chairperson of the Branch.

Manawatu: Dr *Ghislaine* ***Cousins***, a research chemist at NZ Pharmaceuticals and a VUW graduate.

Wellington: Dr *Peter* ***Hodder***, who recently received a special award from the Exscite Trust for his work popularising science in the Waikato region (see below), has been re-elected for a second term of office.

Canterbury: Dr *Michael* ***Edmonds,*** the Applied Sciences and Allied Health programme manager at Christchurch Polytechnic Institute of Technology, who teaches analytical, biological and natural products chemistry.

Otago: A/Prof *Julian* ***Eaton-Rye*** of the Otago University Biochemistry Department, whose interests lie in photosynthesis.

NZIC MEMBERSHIP MATTERS

We welcome to the Institute the following as new members:

MNZIC

Timothy ***Babbage,*** Consultants Auckland

Christopher ***Williams,*** Auckland

Robert ***Stainthorpe,*** Canterbury University

Dr *David* ***Winter,*** Canterbury University

Dr *Donald* ***Law,*** Massey University

Dr *Christopher* ***McAdam,*** Otago University

Dr *Shelley* ***Wilson,*** Wintec, Waikato

Dr *Miruna* ***Petcu,*** Wintec, Waikato

Dr *Daryl* ***Crimmins,*** Wellington

Student Members

Mr *Chris* ***Hawes,*** University of Canterbury Canterbury

Mr *Antony* ***Parnell,*** University of Waikato

Miss *Briar* ***Naysmith,*** University of Auckland

Mr *Ashley* ***Easter,*** University of Waikato

Mr *Nathaniel* ***Alcorn,*** Victoria University

Miss *Siobhan* ***Bradley,*** Victoria University

Mr *Christopher* ***Blackford***, Victoria University

International Year of Chemistry 2011

International Year of CHEMISTRY 2011

2011 has been designated International Year of Chemistry by UNESCO in partnership with IUPAC. NZIC Council is investigating ways of using this focus to promote Chemistry and has initiated some ideas, one of which, a national schools crystal growing competition, is planned.

Prizes

The ***Easterfield Medal*** was presented to Dr *Richard* ***Tilley*** by NZIC President Professor *John* **Spencer** at the RSNZ Awards dinner in Auckland on 18 November.

Dr. Richard Tilley receiving the 2009 Easterfield Prize from NZIC President Prof John Spencer (Photo with permission from RSNZ)

The ***Fonterra Award for Industrial and Applied Chemistry*** was presented at the December Wellington Branch meeting by Dr *Justin* ***Bendall*** to Dr *Owen* ***Catchpole*** (*in abstentia)*. The ***Maurice Wilkins Award for Academic Research*** was presented to Professor *Sally* ***Brooker*** by the Director of The Maurice Wilkins Centre, A/Prof *Rod* ***Dunbar***, at an Otago Branch gathering in Dunedin. The ***ABA Books Denis Hogan Award for Chemical Education*** was presented to *Ian* ***Torrie*** by NZIC President Professor *John* ***Spencer*** during his Presidential visit to the Auckland Branch

BRANCH NEWS

AUCKLAND

The Branch Annual dinner was held in September at the Four Seasons restaurant, staffed by students in training at AUT University. They provided professional service for an enjoyable evening for Auckland region chemists. The Auckland annual Branch AGM was held in late October and featured an address by Prof *John* ***Spencer*** (NZIC President) on *Hydrogen-the cinderella of chemistry?* The meeting also voted in the new committee for 2010, with Dr *David* ***Salter*** replacing A/Prof *Jadranka* ***Travas-Sejdic*** as Branch Chairperson.

Massey University – Albany

Prof *Peter* ***Schwerdtfeger*** and Dr *Matthias* ***Lein*** (Centre for Theoretical Chemistry and Physics) were each awarded Massey University Research Medals – Peter the Individual and Matthais the Early Career one. The awards were presented by the Chief Science Advisor to the Prime Minister Sir *Peter* ***Gluckman*** at a ceremony on September 30 last.

As noted above, *Andreas* ***Hermann*** (Peter Schwerdtfeger's group) won the 2009 RSNZ Hatherton Award. Andreas finished his thesis earlier in 2009 and has since taken up a postdoctoral position with Dr *Shaun* ***Hendy*** (IRL) and Prof *David* ***Williams*** (Auckland University).

University of Auckland

The Chemistry Department has a number of achievements and successes to report at the end of 2009. These include Prof *Margaret* ***Brimble***'s appointment as a Titular Member of the IUPAC Organic and Biomolecular Chemistry Division. A recent article from Margaret's group entitled *Wine lactone and its analogues by a Diels–Alder approach* featured on the cover of the *European Journal of Organic Chemistry*. Prof *David* ***Williams*** was elected FRSNZ in recognition of his leading international role in electrochemistry (see above). Recent appointments made to the Department have been Drs *Duncan* ***McGillivray*** and *Jonathan* ***Sperry*** to lectureships in Physical and Medicinal Chemistry, respectively. Dr *Joanne* ***Wojnar*** joined Prof *Margaret* ***Brimble***'s group in October last, having spent a year as a postdoctoral fellow at the University of Chicago.

Among the students, *Brendan* ***Harvey*** received a Top Achiever Doctoral Scholarship from the TEC, and will work with A/Prof *James* ***Wright*** in developing catalysts for green chemistry. Several students featured in the Faculty of Science annual postgraduate poster competition, with *merit awards* given to *Cosmin* ***Laslau***, *Jacqueiline* ***MacCallum***, *Kathrin* ***Stahler***, *Karthik* ***Kannappan*** and *Marsilea* ***Booth***, and *high distinctions* to *Zoe* ***Wilson*** and *Raoul* ***Peltier***, while *Mandy* ***Herbst*** gained second place for her poster on *Factors influencing the aroma stabil-*

ity of NZ Sauvignon Blanc from over 70 Faculty entries.

In December, the Department hosted visitors from the University of Tokushima (Japan) with which it has joint projects across a range of topics in the materials science and sensors area. Presentations were made by A/Profs ***Yasuzawa*** (*Real time glucose monitoring*), ***Murai*** (*Properties of functional materials*), and ***Yabutani*** (*Carbonization of platinum nanoparticles*).

The November-December period saw a number of review meetings with researchers from other Departments and Centres throughout NZ. Mid-November saw the MacDiarmid Institute *student and postdoc symposium* at the University. Presentations were centred around potential commercial and financial gains from nanotechnology. In the following week, wine chemists joined with yeast scientists and viticulturalists for the annual review of the Sauvignon Blanc research programme. Dr *Laura* ***Nicolau***'s work on the aroma chemistry of NZ Sauvignon Blanc is allowing researchers across the programme to focus their efforts on specific aroma chemicals, while the loss of passionfruit aroma typically seen in these wines was linked to rapid hydrolysis losses of acetates such as 3-mercaptohexanol acetate. A demonstration from the group of A/Prof *Paul* ***Kilmartin*** showed that refrigerated storage can extend the shelf-life of the Sauvingon Blanc wine three-fold compared to room temperature storage. In early December around 60 researchers assembled for the two-day 6th annual *Polymer Electronics Research Centre Symposium* (director A/Prof *Jadranka* ***Travas-Sejdic***) in conjunction with the 1st symposium for the *Hybrid Plastics Programme* (director Prof *Ralph* ***Cooney***). Invited speakers gave presentations on the latest developments in *Electrospotting for biochips* (Dr *Pascal* ***Mailley*** from CEA, Grenoble), *Density functional theory calculations* (Dr *Johannes* ***Reynisson*** of the Chemistry Department), through to *New plastics processes and materials* (*Steve* ***Wilson***, Managing Director, Talbots Plastics, Christchurch); and students and postdoctorals presented on a wide variety of conducting polymer, microfabrication and nanotechnology research projects.

Recent visitors who presented seminars have included Dr *Fei* ***Liu*** (Macquarie University) on *Multidentate organocatalysis in asymmetric synthesis*; Prof *Marcus* ***Jager*** (University of California) on *Biomolecular structure, dynamics and interactions from single molecule fluorescence spectroscopy;* Dr *Justin* ***Hodgkiss*** (VUW) on *Illuminating the operation of organic solar Ccells using laser spectroscopy*; Prof *Daniel* ***Kost*** **(**Ben Gurion Universityl) on *Penta- and hexacoordinate silicon compounds*; Professor *Masayuki* ***Satake*** (University of Tokyo), on *Structures and activities of polyether compounds produced by marine dinoflagellate*; and Dr *Andreas* ***Klamt*** (University of Regensburg) on *COSMO-RS, the bridge from quantum chemistry to fluid phasetThermodynamics and physiological partitioning*.

CANTERBURY

The Branch congratulates Profs *Peter* ***Steel*** and *Ian* ***Shaw*** on their RSNZ and NZAS awards, respectively (see above). Prizes from the Branch were awarded to the top chemistry related exhibits at the Canterbury–Westland Science Fair held on September 13 last as per: Best Year 7 Exhibit – *Mild steel dissolving in sulfuric acid* by *Matthew* ***Hay*** (Cobham Intermediate); Best Year 8 Exhibit – *Arsenic in water* by *Prakriti* ***Panthi*** (Kirkwood Intermediate); Best Year 9^+ Exhibit – *Experimental rocketry* by *Joseph* ***Stretch*** (St Bede's College).

On October 1, Prof *Thorri* ***Gunnlaugsson*** presented a talk entitled *Catch me if you can: the detection of ions and molecules using colorimetric and luminescent sensors* to a good turnout of NZIC members. Prof Gunnlaugsson was an Erskine visitor to the University of Canterbury during September and October. The annual ***Trivia and Truffles*** quiz took place in mid-October. Over wine, juice and copious quantities of truffles, teams puzzled over chemistry related questions such as: *Which company produces the men's cologne* ***Element***, and *What chemical might have killed Schrodinger's cat?* (Answers: Hugo Boss and HCN). The music round produced by *Marie* ***Squire*** proved very challenging, while the *Who am I* round sorted out those who knew the famous chemists.

The Presidential Address was delivered to the Branch on Nov. 16, with Prof *John* ***Spencer*** speaking to some thirty members. A discussion of where the institute is heading drew some suggestions from the audience, and was followed by John's research talk on *Hydrogen – the Cinderella of Chemistry?*

CPIT

On the 24th of September, 24 teams from over 12 Canterbury schools took part in the CPIT/NZIC Year 11 chemistry competition that tested the students' chemistry knowledge and practical skills, as well as their teamwork and time management skills. The top team on the evening was from Middleton Grange, with Burnside High School and Avonside Girl's High, 2nd and 3rd, respectively. The school chemistry and science competitions at CPIT are organised by Dr *David* ***Hawke*** and are thoroughly enjoyed by all of those who attend.

On 19th November, 15 teams from 8 South Canterbury schools take part in the CPIT Year 10 science competition held, this year in Timaru. This competition tested students' chemistry laboratory skills as well as their knowledge of chemistry, biology and physics. The winning team was from Geraldine High School, followed by teams from Waitaki Boys' High and Timaru Girls' High.

The 25th of November saw 24 teams from 15 Canterbury schools took part in the CPIT/CSTA (Canterbury Science Teachers' Association) Year 10 science competition at CPIT. Similar in format to the Timaru competition, the top team came from Rangi Ruru Girls' School, with Christchurch Boys' High and Cashmere High 2nd and 3rd, respectively.

The 2nd of December saw over 400 Year 10 students visit CPIT to investigate the various career opportunities. About 40 of these students visited the Applied Science Laboratory to partake in a forensic chemistry exercise – using luminol to detect blood traces, chemical tests to determine the presence of drugs, and paper chromatography to look at different pen inks.

University of Canterbury

A very successful Chemistry and Biology Ball was held on the last Saturday of September.

Recent Erskine visitors to Chemistry have included Profs *Mick* ***Collins*** from the Research School of Chemistry at the ANU and *Thorri* ***Gunnlaugsson*** from Trinity College (Dublin). Prof Collins' research interests are chemical reaction dynamics and quantum chemistry while Prof Gunnlaugssons' include organic and inorganic supramolecular chemistry and he was kind enough to deliver a Branch lecture (above).

Other visitors have included Dr *Emma* ***Veale***, also from Trinity College, who did some work with *Chris* ***Hawes*** and *Paul* ***Kruger*** and presented a talk on *Colorimetric and luminescent sensing of anions and biological systems*. Prof *Dmitry* ***Murzin*** from Åbo Akademi University (Turku, Finland) presented an interesting talk to the department on *Catalytic transformations for production of biofuels, specialty chemicals and pharmaceuticals from wood* in early October. Prof *Daniel* ***Krost*** (Ben Gurion University, Israel) and Dr *Duncan* ***McGillivray*** (Lecturer and AINSE Research Fellow, Auckland University) gave talks on *Penta- and hexaco-ordinate silicon compounds: a remarkably flexible molecular system* and *Proteins at the edge: Biomembrane – protein interactions through neutron reflextometry* in early November, while on November 30, Dr *James* ***Gardiner*** (Melbourne University) presented *To beta or not to beta: synthesis, structures, & biological investigations of β-peptides*. James previously completed his PhD with Prof *Andrew* ***Abell***, and was part of Marsden funded research looking at *β*-peptides with Dr *Michael* ***Edmonds***. On December 1, A/Prof *Masayuki* ***Satake*** (School of Science - Chemistry, University of Tokyo) presented a talk on *Marine polyether compounds: Structures, activities and origins*.

A/Prof *Owen* ***Curnow*** has been awarded Bright Idea funding to work on the development of a new class of ionic liquids. Prof *Peter* ***Steel*** is part of a multinational team that has been awarded an Australian Research Council Discovery Grant to look at *Spin switching in nanoporous, nanomolecular and multifunctional hybrid systems*. A/Profs *Paul* ***Kruger*** and *Antony* ***Fairbanks*** received Marsden funding for projects involving the development of *Spin-crossover driven molecular switches and oligosaccharide synthesis* and *Protein engineering to access homogeneous defined glycoproteins for therapeutic use and biological study*, respectively. Paul's project involves the development of spin-crossover driven molecular switches while Antony is using oligosaccharide synthesis and protein engineering to access homogeneous defined glycoproteins for therapeutic use and biological study.

MANAWATU

An NZIC movie night was held on October 22 in the MUSA lounge area. Shown first was a documentary about Alan MacDiarmid entitled *Super plastics man* which talked about his life and the significant contributions he made with regards to conductive organic polymers. Second to screen was a visually appealing demonstration lecture presented by Dr *Peter* ***Wothers*** of Cambridge University under the title *The chemistry of light.*

Dr *Mark* ***Waterland***, the 2010 NZIC President, received the 2009 IFS teaching award in chemistry. The awards are given in recognition of outstanding achievement in the delivery of high-quality, research-led education. We welcome back *David* ***Lun*** to the Institute as a Technical Officer working for *Shane* ***Telfer*** and *Simon* ***Hall***, providing technical support to research projects funded by the MacDiarmid Institute.

In September, NZ Pharmaceuticals completed the purchase of Dextra Laboratories, which was a UK subsidiary of the drug development company Summit Pic. Dextra, based at the Science and Technology Centre at the University of Reading. They is a specialist carbohydrate chemistry and analytical services business with a strong process development and GMP manufacturing capability. As well as a custom synthesis service, Dextra provides a catalogue range of over 1000 carbohydrate building blocks and combinatorial libraries. This significant investment will enable NZP to expand into new markets and offer a more diversified range of products.

It was the turn of Massey University to host the 18th annual Massey/Victoria chemistry postgraduate student seminar day on November 12 last. There was a wide range of interesting topics discussed, from *characterization of complex carbohydrates from bacteria, purification studies of industrial waste waters* and *Phenolics and condensed tannis from Botswanan forage plants and their anthelmintic effects*, just to name a few.

Rachel ***White*** was awarded a Rutherford Foundation *Outstanding Student Poster Presentation Award* at the 2009 NZ Postgraduate Conference. She is studying for a PhD in Chemistry supervised by Dr. *Dave* ***Harding*** and Dr *Paul* ***Plieger***. *Leonardo* ***Negron*** graduated at the November ceremonies with his PhD in Chemistry. His thesis was entitled *Synthetic targets as mechanistic probes for the key biosynthetic enzyme, dehydroquinate synthase*.

Dr *Masayuki* ***Satake*** (Department of Chemistry - Science, University of Tokyo) gave his talk entitled *Structures and activities of polyether compounds produced by marine dinoflagellate* when he visited in mid-December.

OTAGO

The Branch events include the September visit to OU's *W. D. Trotter Anatomy Museum*, whilst October saw a busload of chemists and family members travelling to the Macraes Gold Mine for a very informative tour about gold extraction and processing techniques. In November, the Branch held its AGM at which the NZIC President, *John* ***Spencer***, gave a seminar. The 2010 Branch Committee comprises *Julian* ***Eaton-Rye*** (Chair), *James* ***Crowley*** (Secretary), *Guy* ***Jameson*** (Treasurer), *Kimberly* ***Hageman*** (Branch Editor), *David* ***Warren*** (Chemical Education Group Representative), *David* ***McMorran*** (Student Liason), and *Scott* ***Cameron*** and *Matthew* ***Cowan*** (Student Representatives).

University Chemistry Department

Keith ***Hunter***, current Head of Department, has been appointed Pro-Vice Chancellor (Sciences). *Jim*

McQuillan has been promoted to Professor. Congratulations to Keith and Jim!

The Department welcomes its newest lecturer, *Carla* ***Meledandri*** who obtained her BSc (Chemistry) from Penn State University in 2001. She then worked as a Research Associate at the Walter Reed Army Institute of Research, where her work involved the investigation of membrane lipid and protein interactions with novel cryprotecting agents and the analysis of membrane thermodynamics. She then completed a PhD at Dublin City University in 2008 under the supervision of Dermot Brougham, involving the synthesis and NMR characterization of membrane-bound nanoparticles and nanoparticle assemblies for biomedical applications. In 2009, as a Postdoctoral Researcher in Brougham's group, she worked on the development of novel magnetic nanoparticle clusters for applications in magnetic resonance imaging and drug delivery. She plans to direct her research at Otago toward the design and preparation of new, multifunctional nanoscale materials for bioanalysis and targeted drug therapy.

Guy ***Jameson*** was awarded $810,000 over three years from the Marsden Fund for his project *Iron's role in the enzyme cysteine dioxygenase: mechanism and biological relevance*. *Kimberly* ***Hageman*** was awarded $238,000 for upcoming involvement in the six-year FRST project, *Protecting NZ from pesticide exposure* led by Dr Andrew Hewitt at Lincoln Ventures Limited. *Philip* ***Boyd*** was elected FRSNZ for his work in the field of oceanography and the productivity of the global ocean (see above). *Allan* ***Blackman*** was interviewed about *Chemophobia* on National Radio (Nights with Bryan Crump) in October as part of a week of programming devoted to Chemistry. The mp3 is downloadable from *http://podcast.radionz.co.nz/ngts/ngts-20091019-1920-Chemophobia-048.mp3*

Barrie ***Peake*** and *Kimberly* ***Hageman*** attended an Environmental Science & Research workshop in October on *Developing a NZ strategy for emerging contaminant issues*. Barrie gave a presentation with Rhiannon Braund (NZ National Pharmacy School) on *Pharmaceuticals – disposal practices and environmental aspects in NZ*. *Christina* ***McGraw*** attended a two-week ocean acidification short course at the US Woods Hole Oceanographic Institute. The course (hosted by The US Ocean Carbon and Biogeochemistry Project Office and the European Project on Ocean Acidification) brought together postdoctorals and faculty from multiple sub-disciplines of biological and chemical oceanography in order to discuss future needs and best practices in this rapidly expanding field.

Three members of *Barrie* ***Peake***'s research group successfully completed their PhD studies in 2009: *Steve* ***Rusak*** for work on *Temporal variations and ecological effects of hydrogen peroxide in seawater*, *Shailini* ***Ashoka*** for *Trace metal distribution in ling (Genypterus blacodes) for provenance identification*, and *Amir* ***Hamidian*** for *Cd in the marine environment.*

Two Plant & Food Research group visitors recently gave seminars to the Department. *Rikard* ***Unelius*** (Kalmar University, Sweden), a regular visitor-to Plant & Food Research (Lincoln), gave a talk on *Vegetables as biocatalysts in stereoselective synthesis of insect semiochemicals*. *Matt* ***Miller*** of Plant & Food (Nelson) expounded on *The good oil on omega 3*, pointing out the need to supply omega 3 without depleting or destroying the marine ecosystem. *John* ***van Klink*** attended the 5th International Workshop on Anthocyanins in Nagoya where he presented a poster involving the collaboration with *Kelly* ***Kilpin*** and *Allan* ***Blackman*** entitled *Colourful cornflower chemistry: deciphering the details of supramolecular degradation kinetics*. It described the mechanisms for blue colour development and degradation.

WAIKATO

University of Waikato

The annual ChemQuest Competition was held by the Chemistry Department in October. A total of 61 teams from the greater Waikato region and Bay of Plenty participated – a few more than we were expecting or had catered for. As usual, this was a fun-filled evening for students studying NCEA Level 2 Chemistry. Alongside traditional general knowledge questions, there were also demonstrations to watch, smells to sniff and identify, and music to listen to - all with a chemistry theme. Balloons exploding in fire, chemical reactions that luminesced and £20 (GBP) notes that do not burn kept secondary students enthralled as they competed for medals and prize money.

The team *Two Blondes and a Brain* from St. Paul's Collegiate School, Hamilton took home the James and Wells Trophy, first place medals and $150 cash. Second prize also went to a St. Paul's team - *Stacked As*, third prize to *Fish and Rice* from Fraser High School, fourth prize to *Team Wolft* from St. John's College, and fifth prize to *Dumb, Dumber and David* from St. Paul's.

The winning team, *Two Blondes and a Brain* (L-to-R): Tim Prestage, Sam Hogg, Megan Cowley), St. Paul's Collegiate School (Hamilton) with (far L) Martin Lovell (Hill Laboratories) and (far R) David Macaskill (James & Wells)

Prizes were generously sponsored by James & Wells Intellectual Property and Hill Laboratories, as well as Waikato's School of Science and Engineering. Question masters were Richard Coll, *Michèle* ***Prinsep*** and *Bill* ***Henderson***, with *Brian* ***Nicholson*** the chief judge, assisted by Pat Gread, Jo Lane, *Bevan* ***Jarman***, Amu Upreti, *Nick* ***Lloyd*** and *Lyndsay* ***Main***. Others thanked for their participation in running the event are Merilyn Manley-Harris, Annie Barker, Graham Saunders, John Little, Steve Cameron, Amu Upreti, Wendy Jackson, Jenny Stockdill, *Jolene* ***Brown***, and *Jonathan* ***Puddick***.

Brian Nicholson and Bill Henderson were finalists in the science educator/communicator category of the Kudos awards, the Hamilton science excellence awards. Their nomination in this

category was recognition of the many outreach activities that both undertake with school students. On the awards night they treated the audience to a number of entertaining demonstrations. The well deserved winner of the category for his excellent work in developing problem-based learning in teams with secondary school students was Paul Lowe, a science teacher from Morrinsville College. Another very popular winner was the Lifetime Achievement Winner, Rex Munday of AgResearch, for his work developing the zinc bolus to treat facial eczema in animals.

Recent seminars from visitors to the Department include *Faecal sterols as chemical indicators of human and animal pollution in waterways* from Dr Peter Brooks (University of the Sunshine Coast), *Wine oxidation chemistry – focus on NZ Sauvignon Blanc* from A/Prof *Paul* ***Kilmartin*** (Auckland) and *Darwinian chemistry and the origin of life* from Dr *Andy* ***Pratt*** (Canterbury).

WELLINGTON

The Branch congratulates Drs *Richard* ***Tilley*** and *Owen* ***Catchpole*** on their Easterfield and Fonterra awards, respectively (above and below). The September meeting took the form of a site visit to IRL's Supercritical Fluid Technologies facility on the Petone campus, limited to twenty members. Dr *Owen* ***Catchpole***, the 2009 Fonterra Applied Chemistry research medallist, introduced the working of the facility and the principles behind supercritical processing and extraction, and then led members around the operation that included SuperEx. The meeting was followed by an informal get together dinner in Jackson Street, Petone, one of the Wellington area's more renowned dining sites.

The Branch AGM was held on 21 October. Re-elected were: *Peter* ***Hodder*** (Chairperson), *Joanne* ***Harvey*** (Secretary), *Suzanne* ***Boniface*** (Treasurer) and *Brian* ***Halton*** (Branch Editor). They will be joined on the Committee by a further eight Branch members. Alison Curtis was appointed as the Branch's Financial Reviewer. After the AGM, *Ashton* ***Partridge*** (Massey University, Palmerston North) spoke on *Applied nanotechnology - future diagnostics and high efficiency photovoltaics*. He focussed on developments in the application of nanoparticles in the sensing of biological materials that fall into four catalogues according to their configurations and assay uses. These include quantitation tags, substrates, signal transducers, and functional nanoparticles. The research at Massey focuses on the application of gold nanoparticles to enhance the signal of a Surface Plasmon Resonance sensor. Applications range from the detection of femptomolar concentrations of steriods in humans and cattle, to the detection of shellfish toxins in waterways. On the subject of photovoltaics (PV) Ashton described the major challenge in meeting the growing demand for energy, and securing an affordable energy solution that does not compromise our environmental responsibilities. Considerable research efforts around the world have focused on the development of the PV chemistry, with a growing number of commercial solutions available. Research at Massey to some extent focuses on the development of new OPV materials, however there is a major focus on supporting NZ industry to develop an all-plastic roofing product which incorporates any PV chemistry, and controls the way in which light is absorbed and utilised within the cell.

November saw the Branch host the NZIC AGM and, apparently, reach a quorum with 30 members attending. The likely reason for this was the presentation of the ***2009 Fonterra Prize for Industrial and Applied Chemstry*** by Dr *Justin* ***Bendall*** to Dr *Owen* ***Catchpole*** (*in abstentia)* and Fellowship certificates *Gary* ***Evans*** and *Peter* ***Hodder***. Peter also received a special award, an elegant carved sculpture, from the Hamilton-based ***Exscite Trust*** with which he has been associated for many years leading the move to a permanent science exhibition area in the Waikato Museum. The lecture that followed was given by *Emily* ***Parker*** (Canterbury University) entitled: *Probing reaction mechanisms and evolutionary relationships in a family of crucial biosynthetic aldolases* where she described her outstanding studies of KDO8P and DAH7P (3-deoxy-D-*manno*-octulosonate 8-phosphate and 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthases, respectively) which, in part, earned her the NZIC Easterfield Medal in 2005.

Victoria University

A/Prof *Peter* ***Northcote***'s research has gained further international attention with his *Journal of Organic Chemistry* paper on the isolation, structure, total synthesis, and bioactivity of Peloruside B being given *Featured Article* status by the American Chemical Society. It represents a study by his group in collaboration with that of *Arun* ***Gosh*** at Purdue University and *C-X* ***Xu*** at the US National Cancer Institute in Maryland. A/Prof *Kate* ***McGrath*** has been awarded a Research Excellence Award and Dr *Mattie* ***Timmer*** an Early Career Research Award by VUW.

Visitors to the School have included Dr *Mark A.* ***Le Gros*** (Assoc-Director, National Center for X-Ray Tomography, Lawrence Berkeley National Laboratory US) who spoke on *The application of sub-cellular soft X-ray tomography to biomedical research and fundamental cell biology* and Prof *Thomas* ***Nann*** (Nanoscience – Chemistry, East Anglia) whose seminar was entitled *Synthesis of InP quantum dots, upconverting nanoparticles and their application in energy conversion*; both visited in late September. A/Prof *Masayuki* ***Satake*** (School of Science – Chemistry, University of Tokyo) presented a talk on *Marine polyether compounds: structures, activities and origins* in early December.

Emma ***Dangerfield***, a PhD student in the ***Timmer-Stocker*** group and the inaugural *CiNZ Communicator of the Year* awardee, has been awarded a Victoria University postgraduate research excellence award for her work on the development of novel methodology for the synthesis of aza-sugars. *John* ***Beal*** of the *Richard* ***Tilley*** group has successfully defended his PhD studies, while *Almas* ***Zayya*** (Prof *John* ***Spencer***) has submitted her PhD thesis on molecular clamps.

What Are These Things Called MOFs?

Shane G. Telfer

MacDiarmid Institute for Advanced Materials and Nanotechnology, Institute of Fundamental Sciences, Massey University, Palmerston North (e-mail: *s.telfer@massey.ac.nz*)

What are MOFs?

Metal-organic frameworks (MOFs) are crystalline networks of organic ligands held together by nodes comprising metal ions or clusters. The prototype is MOF-5 [Zn_4O(benzene-1,4-dicarboxylate)$_3$], which was first reported[1] by Omar Yaghi's group in 1999. The structure of this material, as determined by X-ray crystallography, is presented in Fig. 1. MOF-5 is an infinite cubic framework in which Zn_4O clusters link benzene-1,4-dicarboxylate struts. It is remarkable for its aesthetic beauty, ease of synthesis, thermal stability, and the fact that it is mostly fresh air - about 60% of its volume is accessible to guests. The report of MOF-5 made a significant splash − the original paper has been cited 1400 times in the ten years since its publication − and it can be seen as the genesis of a new field of research. This paper aims to provide an overview of the development of MOF-5 and the direction that the field has taken in the ten years since this landmark publication. It is certainly neither comprehensive nor balanced, being distinctly biased towards MOFs derived from zinc(II) and carboxylate ligands.

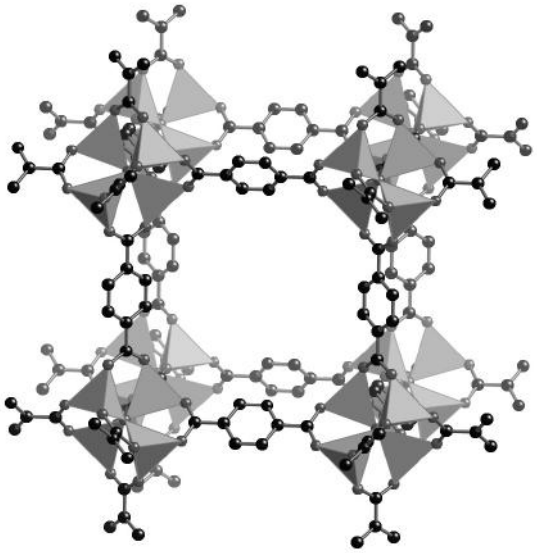

Fig. 1. The structure of MOF-5.

What Came Before MOF-5?

The ability of certain combinations of metal ions and ligands to from polymeric structures was noted by John Bailar Jr. in a 1964 review.[2] At this time, it was known that many simple inorganic compounds such as palladium(II) chloride and nickel(II) cyanide exist as polymers in the solid state (Fig. 2). The key feature of the ligands of these compounds is their ability to bridge two metal centres, which allows the polymeric structure to propagate. Another example is Prussian blue, a mixed valence iron(II)/iron(III) three-dimensional network with cyanide bridging ligands, which has been used as a pigment since the early 1700s. Bailar coined the term *co-ordination polymer* to describe this class of compound, although he commented that their properties (plasticity, elasticity *etc*.) do not correlate closely with organic polymers owing to the shortness of the bridging ligands and the rigidity of the co-ordination sphere of the metal ions.

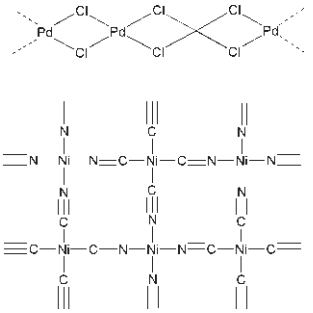

Fig. 2. The solid state structures of palladium(II) chloride and nickel(II) cyanide.

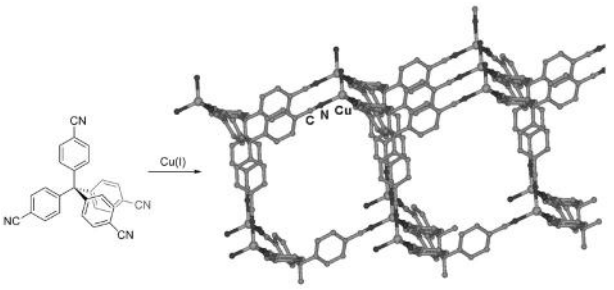

Fig. 3. Formation of a crystalline co-ordination polymer from copper(I) cations and tetracyanotetraphenylmethane. The BF_4^- anions have been omitted for clarity.

Although there were subsequent scattered reports of polymers held together by coordinative bonds, these materials tended to be rather ill-defined and poorly characterised. Richard Robson at the University of Melbourne put the field on a firmer footing by elucidating the network topologies that could result from the combination of rigid, divergent ligands with the preferred stereochemistry of various metal ions. In 1990, in collaboration with Bernard Hoskins, Robson reported[3] the X-ray crystal structure of Cu[tetracyanotetraphenylmethane]BF_4. The combination of tetrahedral copper(I) ions and the tetrahedrally disposed pyridyl donor groups generates a cationic diamondoid network (Fig. 3).

The real breakthrough of this report was the fact that the networks were crystalline and not *miserable, ill-defined, amorphous precipitates, difficult or impossible to characterise*[4] as had seemed much more likely. This approach overcame the *crystallization problem* that was actually later enunciated by Roald Hoffmann:[5]

> *Organic chemists are masterful at exercising control in zero dimensions. . . .One subculture of organic chemists has learned to exercise control in one dimension.*

These are polymer chemists, the chain builders... But in two or three dimensions, it's a synthetic wasteland. The methodology for exercising control so that one can make unstable but persistent extended structures on demand is nearly absent. Or to put it in a positive way—this is a certain growth point of the chemistry of the future.

The early days of this pioneering research were recently given a personal account by Robson.[4] He recounts that his ideas were seeded by the task of constructing ball-and-stick models of inorganic crystal structures such as sodium chloride, zinc blende, and rutile for classroom demonstrations. Robson pondered whether the simple anions that bridged the metals in these structures could be replaced by rigid molecular units with ligating groups fixed in suitable orientations. This became the guiding principle for the crystal engineering of co-ordination polymers, *i.e*. the pre-meditated rational construction of specific networks.

A surge of papers was published in the early 1990s on the crystal engineering of co-ordination polymers of polytopic pyridyl or nitrile ligands. The popularity of these donor groups stemmed from their simple monodentate nature, that they co-ordinate to a range of metal ions, and the predictability of the orientation of the M−L bond with respect to the ligand backbone (Fig. 4).

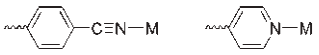

Fig. 4. M−L bond vectors for nitrile and pyridyl donor groups.

Network topologies could often be predicted in advance, or at least rationalised with hindsight, by considering the orientation of the ligating groups and the co-ordination geometry of the metal centre. For example, the reaction of 1,3,5-tris(4-ethynylbenzonitrile)benzene with silver(I) trifluormethanesulfonate (AgOTf) produces a two-dimensional network composed of ligands and trigonal planar metal ions (Fig. 5).

Conceptually, the above co-ordination polymer can be deconstructed to $Ag(CN)_3$ units that are linked by an extended trisubstituted benzene core (Fig. 5). It was this insight that led to the design of MOF-5, which can be viewed as an infinite, *polymerized* analogue of well known discrete zinc carboxylate clusters such as $[Zn_4O(acetate)_6]$ and $[Zn_4O(benzoate)_6]$. The route from the zero-dimensionality of these discrete clusters to the 3-dimensional network is provided by replacing the simple carboxylate ligands by a rigid ligand with two *divergent* carboxylic acid groups (benzene-1,4-dicarboxylic acid). The core Zn_4O cluster is the same in both structures; it is capped by the acetate ligands in $[Zn_4O(acetate)_6]$ but *articulated* into a 3-dimensional network by benzene-1,4-dicarboxylate (Fig. 6).

A candid insight into the genesis of MOF-5 was recently published by Michael O'Keeffe.[6] Initially, many people were sceptical of the ability of these kinds of materials to maintain their integrity upon removal of the solvent, *i.e.* to display permanent porosity. This is true of structures that rely on weak metal−ligand interactions, but the strength of the Zn-O bonds in MOF-5 means that crystallinity is maintained even after heating in air at 300 °C for 24 h. Gas sorp-

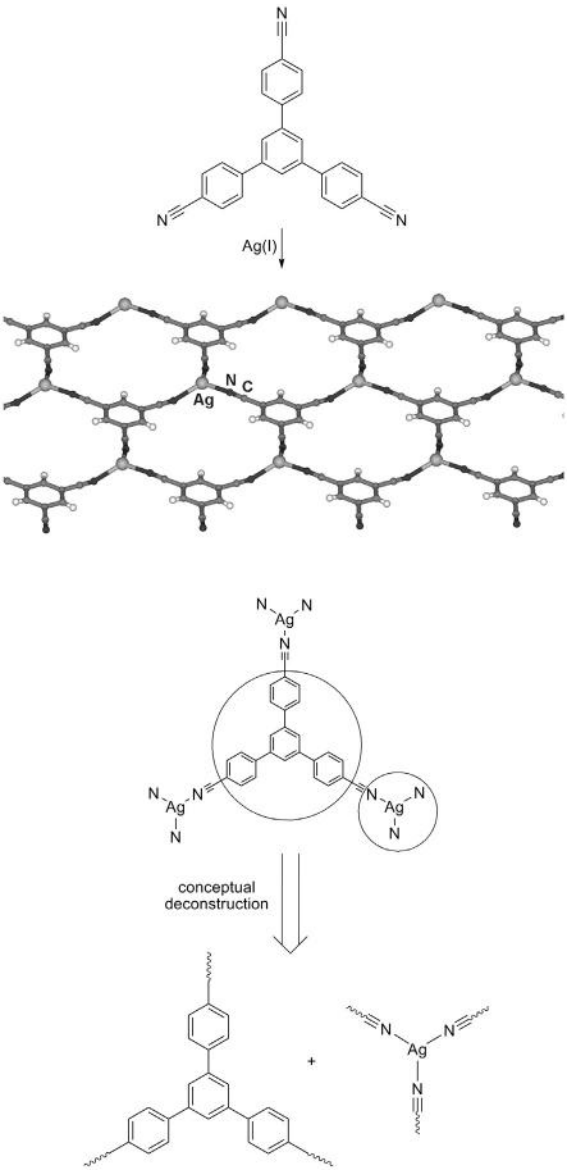

Fig. 5. Formation of a crystalline co-ordination polymer from Ag(I) and 1,3,5-tris(4-ethynylbenzonitrile)benzene). Triflate counter anions have been omitted for clarity.

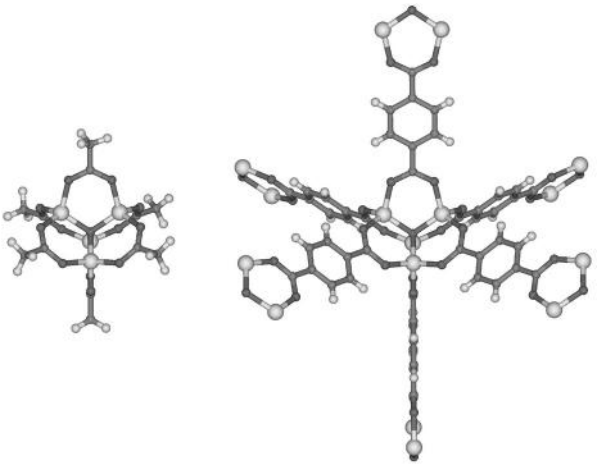

Fig. 6. Replacement of the capping acetate ligands in $[Zn_4O(acetate)_6]$ (left) by a ligand with two divergent carboxylic acid groups generates an infinite 3-dimensional network (MOF-5, right).

tion isotherms indicated significant porosity and allowed an estimate of the surface area of 2900 m^2/g. These key features of MOF-5 set it apart from previously known co-ordination polymers.

A second prominent milestone in MOF research was a 2002 *Science* report from Yaghi's group,[7] which has subsequently been cited 1360 times and describes the elaboration of the benzene-1,4-dicarboxylic acid ligand. In all cases, networks with the same cubic topology were observed (Fig. 7).

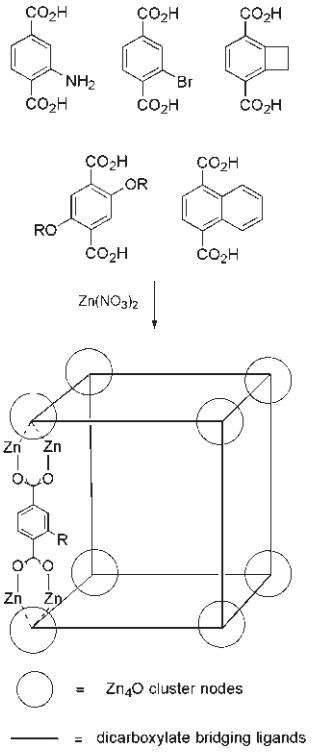

Fig. 7. Replacing benzene-1,4-dicarboyclic acid by more elaborate ligands generates functionalized frameworks with the same cubic topology as MOF-5.

MOFs and Co-ordination Polymers: What's in a name?

There exists some snobbery on the part of some MOF chemists who pride themselves on pre-designing specific networks using more exotic, customized ligands, and who, as a result, tend to look down upon many co-ordination polymers as the products of blind *shake and bake* experiments using ligands from the Aldrich catalogue. The term *MOF* is certainly a trendier brand name for your newly synthesized (and about-to-be-published) material, but some of those in the MOF camp believe that the term is being used where co-ordination polymer would be more appropriate. This has generated some spirited discussion in the literature (see below). While some researchers are happy to use the terms interchangeably, a consensus on appropriate use of the terms is beginning to emerge.

The term *metal-organic framework* was first introduced by Yaghi in 1995 to describe the newly synthesized compound $[Cu(4,4'\text{-bipyridine})_{1.5}](NO_3)$, which forms a stable and porous 3-D network structure.[8] Yaghi has since argued that the MOF moniker should be reserved for materials that, in addition to certain structural attributes, display certain properties such as robustness and linking units that are available for modification by organic synthesis.[9] More recently, he has proposed that the formal bond valence of the metal-ligand bond is also an important criterion.[10] Bond valence is a concept widely employed by solid state inorganic chemists, and it was proposed that metal-ligand bonds in MOFs should have a formal bond valence of around ½, which corresponds to a high bond energy, *ca*. 350 kJ/mol. In contrast, a typical co-ordination polymer such as $[Zn(N,N'\text{-bis(4-pyridyl)urea})_2]ClO_4$ contains Zn-N bonds, which have a formal bond valence of around zero and are therefore rather weaker.

There are several pitfalls in these arguments, not the least of which is the fact that they would disqualify the Cu(I)-bipyridine structure reported in the paper where the term was coined! The concept of formal bond valence is used by solid state inorganic chemists but is potentially quite unfamiliar to many of those working in the field of MOFs. Furthermore, there are many MOFs that contain mixtures of ligands with, for example, both pyridyl and carboxylate donors.

The view that MOF-5 and Prussian Blue are categorically different does have validity, however, and a distinction between MOFs and co-ordination polymers is sensible. Biradha *et al*. have recently suggested how this distinction may be made based on structural features alone.[11] They propose that *the term MOF is very much appropriate to use for three-dimensional networks, it is inappropriate to use for extended one-dimensional or two-dimensional networks*. This builds on accepted terminology in solid state chemistry where the term framework distinguishes a 3-D from a 2-D network (layer). In addition to having a 3-D structure, MOFs are categorically different from co-ordination polymers in that the framework should have potential porosity, *i.e.* potentially be accessible to incoming guests. The adjective *potential* is important here as it means that the porosity can be inferred from the structure of a material without the need for experimental verification by sorption experiments, which are often problematic from a practical perspective owing to framework collapse upon the removal of occluded solvent, *etc*.

How Are MOFs made?

MOF-5 can be prepared by several different methods. In the original 1999 paper, crystallization was achieved by the diffusion of triethylamine into a solution of zinc(II) nitrate, benzene-1,4-dicarboxylic acid, and hydrogen peroxide in DMF (N,N'-dimethylformamide).[1] The hydrogen peroxide was the source of the O^{2-} of the Zn_4O nodes, while the amine acted as the requisite base for deprotonation of the carboxylic acids. Control of the rate of this latter step by the slow diffusion of vapours of amine limits crystal nucleation and allows large crystals to grow at the expense of microcrystalline or amorphous material. The formation of large (> *ca*. 20 micron), high quality single crystals (Fig. 8) is a key target, as these are generally amenable to structural determination by single crystal X-ray diffraction.

A more general and convenient route to zinc(II)-carboxylate MOFs has since been developed. This involves the

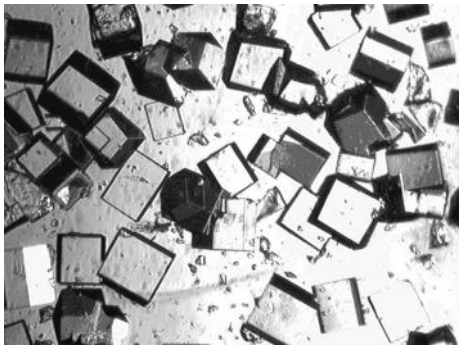

Fig. 8. A photograph of MOF crystals (size *ca*. 0.2 x 0.2 x 0.2 mm) prepared in our laboratory.

reaction of hydrated zinc(II) nitrate with aromatic carboxylic acids in DEF (N,N'-diethylformamide) under solvothermal conditions.[12] This procedure reliably produces large single crystals of MOFs involving a wide variety of ligands.[7] Under these conditions, the base required to deprotonate the carboxylic acids is generated by the slow hydrolysis of the solvent. Initially this produces diethylamine and formic acid, although the latter is thought subsequently to decompose to hydrogen and carbon dioxide.[13] The source of the central O^{2-} ion in this case is probably water. It has been shown that the nitrate anion *can* serve as the source of this oxide,[14] although it is not clear to what extent this occurs under standard conditions. Furthermore, MOFs can be synthesized at room temperature by replacing the zinc(II) nitrate by zinc(II) acetate. Zinc(II) oxide can also be used as a precursor, which parallels the synthesis of discrete complexes such as $[Zn_4O(OAc)_6]$,[15] in which case the origin of the O^{2-} ion is obviously beyond doubt.

In light of the potential applications outlined below, BASF has recently started the production of MOFs on an industrial scale.[16] Several interesting challenges had to be met regarding their bulk synthesis and processing. For example, although $Zn(NO_3)_2$ is typically used as a precursor to Zn-based MOFs, this generates high nitrate concentrations, which pose a safety hazard. An electrochemical method was therefore developed, which relies on bulk sacrificial metal electrodes which are oxidised in the presence of dissolved ligands. Owing to the high amount of occluded solvent in these materials, filtration and activation of the materials is a tedious operation even on a laboratory scale.

What Are MOFs Good For?

Upon guest removal, MOFs can often support a vacuum owing to the strength of the bonding interactions that hold them together. MOFs with pore sizes of up to 30 Å and surface areas of 5000 m^2/g have been reported.[17] They are stable in dry air up to temperatures at which ligand combustion occurs (~400 °C). As they comprise mostly fresh air by volume, they can have densities lower than 0.21 g/cm^3, the lowest yet known for crystalline materials. This combination of key properties is not seen in any other class of molecular materials. It is noteworthy that the application of co-ordination polymers as molecular sieves and heterogeneous catalysts was anticipated by Robson in his 1990 paper.[3]

The performance and utility of MOFs in the applications outlined below can be compared with their zeolite cousins. Zeolites have higher thermal stabilities and are generally less susceptible to hydrolysis than MOFs, which means they are more suited to high temperature applications such as the cracking of hydrocarbons. Advantages of MOFs include:

- MOFs do not have walls to impede the diffusion of guest molecules, unlike zeolites.
- Most MOFs are neutral, negating the need for charge-balancing counterions in the pores.
- The solvent itself acts as the main template in MOF synthesis, obviating the requirement for added templates such as amines and quaternary ammonium salts.
- Functional groups can be introduced into the structure via the ligands. These often line the MOF channels and are accessible to incoming guests.
- MOF pores are lined with both hydrophobic (ligand backbone) and hydrophilic (metal cluster) constituents which may enhance their sorption properties.
- MOFs can have greater surface areas (1000-4000 m^2/g) than zeolites (typically ~700 m^2/g).

Undoubtedly, the most sought-after technological application of MOFs, which draws on these properties, is gas storage.[18] Relatively non-polar gases such as hydrogen, carbon dioxide and methane can be absorbed by MOFs in significant quantities. Vessels that are pre-filled with MOFs can thus enhance gas storage capacity in a given volume or store an equivalent amount of gas at a lower pressure.[19] This is highlighted in Fig. 9 for the storage of hydrogen in various MOFs. Efforts to further improve the hydrogen storage capacity of MOFs are motivated by the US Department of Energy which has set targets for its on-board storage on hydrogen-powered vehicles of 6.0 wt% (45 g/L) for 2010 and 9.0 wt% (81 g/L) for 2015 under ambient conditions. In practice, the latter target is anticipated to allow vehicles to store around 10 kg of hydrogen, which will allow them to travel about 500 km. The best MOFs are approaching these targets at 77 K, however significant sorption has not yet been observed at room temperature.

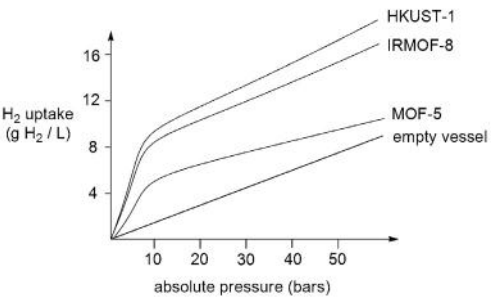

Fig. 9. Comparison of the hydrogen storage capacity of empty and MOF-filled vessels. IRMOF-8 and HKUST-1 are other MOF materials.

A derivative of MOF-5, which has a cyclobutyl group appended to the benzene ring (IRMOF-6), was found to have an exceptionally high affinity for methane.[7] The

amount of methane contained in a volume of 155 cm^3 at standard temperature and pressure can be taken up by 1 cm^3 of this material at a pressure of 36 atm (3.6 MPa). This is 70 % of the amount stored in the same volume in liquefied methane, which requires a much higher pressure (>200 atm). The reduction in the pressure required to store methane makes MOF-filled cylinders potentially both safer and more cost-effective. Although some carbon adsorbents have a similar capacity, it can be anticipated that even higher gas uptake can be achieved by optimization of the MOF structure.

One notable feature of MOFs is that the organic struts bear various functional groups, which can be tailored using the toolbox of synthetic organic chemistry prior to incorporation in the MOF. These often line the pores and channels in the resultant frameworks and thus are accessible to incoming guests. Additionally, vacant co-ordination sites on the framework metal atoms may also bind and activate substrates. Applications of MOFs as sensors and heterogeneous catalysts can thus be envisaged, and this aspect of MOF chemistry is witnessing a flurry of activity.

Representative examples of MOF catalysis include the finding that HKUST-1,[20] a MOF derived from copper(II) and benzene-1,3,5-tricarboxylic acid, is capable of catalyzing the cyanosilylation of carbonyl compounds such as benzaldehyde and acetone. The catalysis mechanism relies on the Lewis acid activation of the carbonyl group by open copper co-ordination sites.[21] In a departure from carboxylic acid ligands, Volkmer's group have reported that a cobalt(II)-BPB MOF [BPB = 1,4-bis(4'-pyrazolyl)benzene] can be oxidised by *t*-butyl hydroperoxide to give the corresponding cobalt(III) framework. This material can subsequently catalyze the conversion of cyclohexene to *t*-butyl-2-cyclohexenyl peroxide.[22] Researchers at BASF have carried out MOF-catalysed reaction on bulk scales to determine their suitability for industrial processes. For example, the conversion of propyne to methoxypropene at 250 °C was achieved using a copper(II)-benzene-1,4-dicarboxylate MOF (MOF-2) catalyst.[19]

What's Hot in MOF Research?

One aspect of MOF chemistry that has surged recently is their post-synthetic modification.[23] The potential feasibility of modifying open co-ordination polymers by reactions with external reagents was first identified by Robson as a method of tailoring the chemical and physical properties of these structures. This approach also allows the incorporation of functional groups that would otherwise perturb formation of the solid sate network, *e.g* by binding to the metal ions in preference to the designated donor groups. However, it is only in the past couple of years that the investigation of this phenomenon has moved on from simple guest exchange processes to the covalent derivatization of organic MOF components. The major impetus has come from Seth Cohen's group who, in 2007, reported on the derivatization of IRMOF-3, [Zn_4O(2-aminobenzene-1,4-dicarboxylate)] with various anhydrides (Fig. 10a).[24] Reactions with isocyanates have also been pursued. These produce urea groups that are of interest for their potential anion binding and organocatalytic properties. Other creative post-synthetic modification reactions on MOFs have included the reaction of MOF-5 with $M(CO)_6$ complexes to generate *piano stool* complexes in which the aromatic MOF strut functions as an η6 π-donor ligand (Fig. 10b),[25] and the low temperature reaction of acetaldehyde with the amino group of 1-aminotriphenylene encapsulated in co-ordination polymer composed of zinc(II) iodide and 2,4,6-tris(4-pyridyl)-1,3,5-triazine.[26] This latter reaction allowed the structure of the carbinolamine intermediate of a classic Schiff base condensation reaction to be observed by X-ray crystallography for the first time (Fig. 10c).

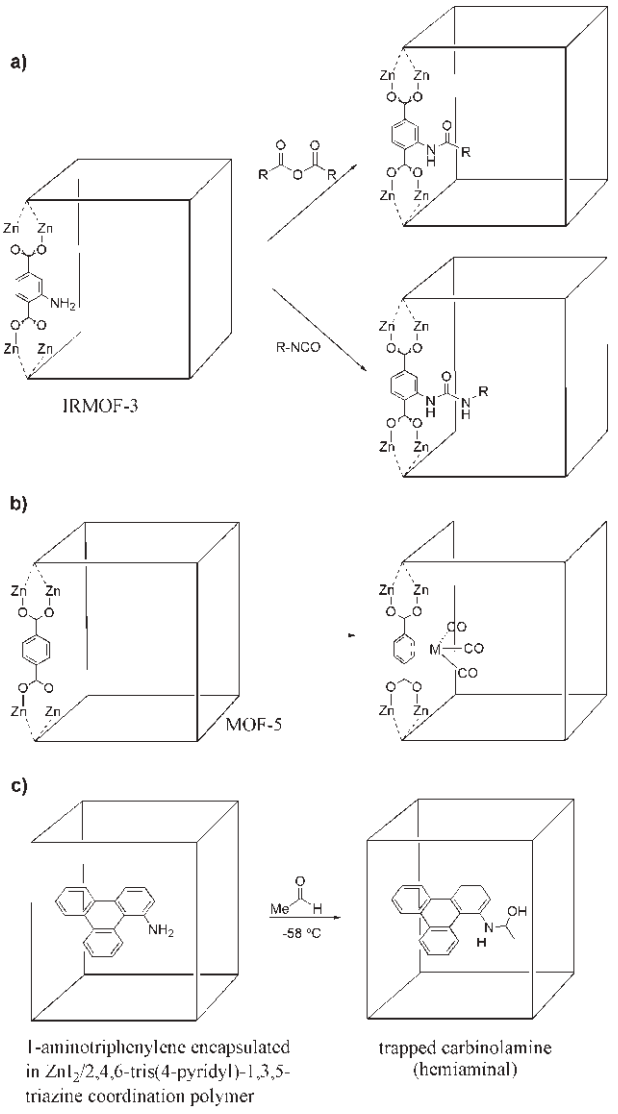

Fig. 10. Examples of the post-synthetic modification of MOFs.

Another area that has recently flourished is the growth of thin films of MOFs on surfaces.[27] The fabrication of porous MOF films on solid supports is a pressing challenge, as this MOF morphology is most likely to find application in devices such as sensors and selective membranes. Ideally, the resulting films should be continuous, defect-free, and crystalline, with a well-defined orientation of their pores with respect to the surface. The production of free-standing MOF membranes by the subsequent detachment of the film from the surface can also be envisaged. Research to date has mainly focussed on the growth of MOF-5 and HKUST-1 ([Cu_3(1,3,5-benzenetricarboxyl-

ate)$_2$]) on supports such as alumina, silica, and self-assembled monolayers (SAMs) on gold.[28] Although this field is in its infancy, notable progress has already been made. For example, Lai *et al*. have prepared a continuous membrane of intergrown MOF-5 crystals on α-alumina by immersing the substrate in a solution containing the precursor components at elevated temperatures.[29]

In an intriguing recent development that draws on the high porosity of MOFs, Matzger *et al*. have discovered that microporous co-ordination polymers can remove organosulfur compounds from diesel fuel.[30] The process is efficient and highly selective and the high loading capacity of certain MOFs means that practical applications are realistic. The same group has also taken advantage of the porosity of MOF-5 and HKUST-1 in exploring their use as stationary phases in liquid chromatography and gel permeation chromatography.[31] A combination of molecular sieving and adsorption effects operate to efficiently separate variously substituted aromatic hydrocarbons.

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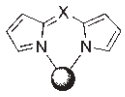
Controlling Molecular Excitons with Coordination Chemistry

Mark R. Waterland, Shane G. Telfer and Tracey M. McLean*

MacDiarmid Institute for Advanced Materials and Nanotechnology, Institute of Fundamental Sciences, Massey University, Private Bag 11 222, Palmerston North (e-mail: *T.M.McLean@massey.ac.nz*)

Introduction

The conjugated π system of dipyrrins illustrated by **1** is analogous to that of porphyrins and endows dipyrrin and dipyrrinato complexes with interesting and useful light absorption, light emission,[1] and optical properties.[2] BF_2 complexes of dipyrrin ligands (or BODIPYs) have been well studied.[3]



1, dipyrrin ligand, X = CR or N

A defining feature of dipyrrins is that they possess a large transition dipole moment across the pyrrolic rings. When two or more dipyrrin units come into close proximity, *e.g.* by coordinating to a metal centre, the transition dipole moments interact strongly, owing to the strength and close spatial proximity of the chromophores (see Fig. 1). This leads to new electronic states that are delocalized across the dipyrrin units,[2,4] described as molecular excitons.

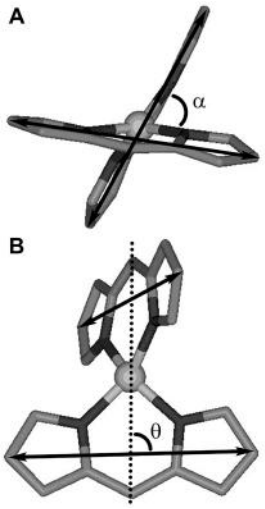
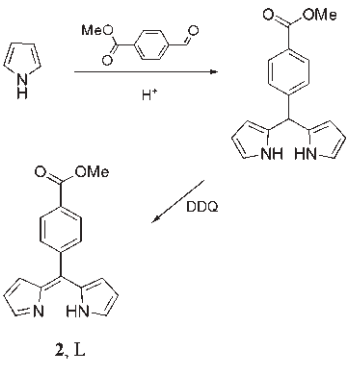


Fig. 1. Tetrahedral [ML_2] with the transition dipoles shown with double headed arrows; α is the dihedral angle with respect to a vector that connects the transition dipoles (A). The line of molecular centres, shown with the broken vertical line, makes an angle θ with the transition dipole, which is 90° for all [ML_2] dipyrrin complexes (B).

Synthesis of Dipyrrins and Azadipyrrins

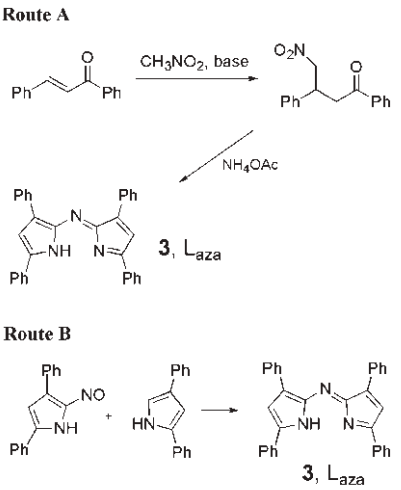
meso-Substituted dipyrrins **2** are easily accessible from arylaldehydes via an acid-catalyzed condensation with pyrrole, followed by oxidation (Scheme 1).[1,5,6]

There are two general methods for preparing azadipyrrins (Scheme 2) of which the first involves a Michael addition across an *α,β*-unsaturated ketone with nitromethane, followed by a reaction with an ammonia source, such as ammonium acetate or carbamate (Route A).[7] Route B involves the condensation of a diarylpyrrole bearing a nitroso group at the 5-position with a second molecule of pyrrole.[8]



2, L

Scheme 1. Classic synthesis of dipyrrins.



Scheme 2. The two general syntheses of azadipyrrins.

Dipyrrin Complexes

Dipyrrins chelate to metal ions in a monoanionic fashion following deprotonation and are known to form homoleptic and heteroleptic complexes with a variety of metal ions.[1] Preparation of these complexes often involves a reaction with the appropriate metal acetate in an alcoholic solvent. If the complex is neutral then the complex can be conveniently isolated by filtration and purified by recrystallization. Other metal salts are also commonly used in conjunction with a base. Various functional groups may be incorporated on the periphery of complexes of dipyrrinato ligands by substitution on the aryl and/or pyrrole rings. On kinetically inert complexes, these functional groups can be interconverted using standard synthetic methodologies.[9]

The mutual orientation of two or more dipyrrin ligands in a ML_2 or ML_3 complex is governed largely by the coordination geometry preferred by the metal ion. For example the coordination geometry of d^6 Co(III) is octahedral (see Fig. 2A). Four coordinate CoL_2 dipyrrin complexes are

reported to oxidise to the octahedral CoL_3 complex in air.[9] A further example is the coordination geometry of a PdL_2 complex (see Fig. 2B).[10] The coordination geometry of d^8 Pd(II) is strictly square planar. However, to accommodate the preferred coordination geometry of Pd the two dipyrrin ligands cannot remain coplanar due to the steric interactions between the α hydrogen atoms. As a consequence the ligands cannot get away from the PdN_4 plane and the bispyrrolic core of the ligand contains significant curvature.[10] The coordination geometry of d^9 Cu(II) can be manipulated by substituents in the α positions. In the case of $Cu(L_{aza})_2$ the coordination geometry is distorted tetrahedral to accommodate the bulky phenyl substituents (Fig. 2C).[4]

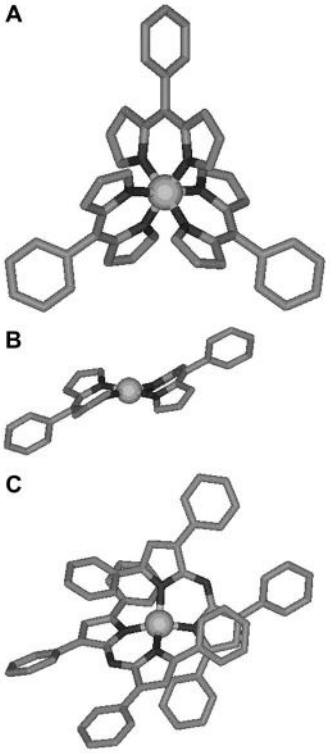


Fig. 2. Coordination geometries of A - CoL_3 (ref. 9), B - PdL, (ref. 10), and C - $Cu(Laza)_2$ (ref. 4).

Interactions between π–π* excited-states of dipyrrin ligands (see below) lead to large electronic energy level shifts which are reflected in the electronic absorption spectra. Chelation of the dipyrrin unit to a metal centre also gives rise to shifts in the absorption spectra of dipyrrins. Fig. 3 shows electronic absorption spectra of a free dipyrrin ligand and a single dipyrrin ligand coordinated to a palladium centre. In the free ligand, protonation of only one pyrrole ring introduces an asymmetry that reduces the degree of delocalization across the pyrrole rings. Upon coordination (and deprotonation), the pyrrole rings become equivalent, and delocalization reduces the gap between the ground and π–π* electronic state. This results in a red-shift and substantial increase in the oscillator strength. Similar effects are observed in weak acid solution where protonation of the second pyrrole rings also removes the asymmetry.[11] The strong σ-donor properties of the dipyrrin also contribute to the red-shift.

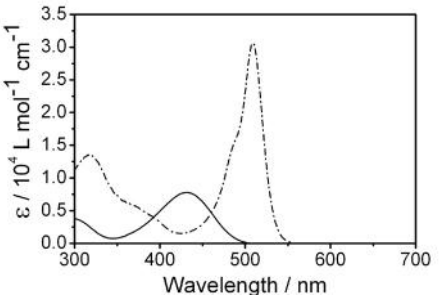


Fig. 3. Electronic absorption spectra of free dipyrrin ligand (L) and $[PdLdppe]^+$ where dppe = 1,2-bis(diphenylphosphino)etha ne.

Exciton Interactions with Two States

Exciton effects are well-established in molecular aggregates[12,13] and composite molecules[14] such as the light-harvesting complexes in green plants and some bacteria.[15] They may be observed if there are sufficiently strong interactions between electronic states. Consider the system shown in Fig. 2 which shows a tetrahedral complex: dipolar coupling interactions between the π–π* states of each ligand result in a resonance splitting of the excited state energy levels which are degenerate or near-degenerate in the absence of interactions. The excited-state coupling leads to a new set of electronic states (the excitonic states), and this results in strong spectral shifts or splitting of the absorption bands in absorption spectroscopy[14] or as positive and negative bands in circular dichroism spectroscopy.

The exciton concept originates in solid-state physics (and semiconductor materials in particular) where it describes a *correlated* electron-hole pair that results from excitation of an electron across the band gap between the valence and conduction bands.[16] The hole describes the absence of an electron in the valence band. In molecular systems, generation of an excited-state arises from promotion of an electron into an unoccupied orbital and the creation of a hole in the originally occupied orbital. The exciton concept is useful in chemistry because it focuses attention on the dynamics that result from correlation of the photo-excited electron in the previously unoccupied orbital and the hole in the occupied orbitals.

A simple state interaction theory accounts for the electronic structure of the interacting system, using the framework of perturbation theory.[14] The nature of the perturbation is dipolar coupling between the transition dipole moments of the unperturbed states. A necessary assumption is that the perturbation is sufficiently strong to generate new electronic states and these new states can be accurately described using the unperturbed (diabatic) states as a basis. For the case of interacting dipyrrin units, the diabatic states in this theory are the π–π* excited-states of the individual dipyrrin chromophores. Fig. 4 illustrates the simple case of only two interacting units. If the transition dipoles are parallel (Fig. 4A), then for one of the new excitonic states, **E″**, there will be a Coulombic interaction resulting in an energy lowering; and in the other, **E′**, an energy rise relative to the original energy **E**. Furthermore, the transition moment is given by the vector sum of the individual

transition dipole moments of the ligand component of the complex. Therefore, transitions from the ground state (**G**) to **E′** are forbidden while transitions from the **G** to **E″** are allowed. The physical consequence of this is that the dipole-allowed electronic transition for the system will be *blue shifted* with respect to the uncoupled π–π* dipyrrin states. If the transition dipoles are co-linear (see Fig. 4B), a similar splitting is observed but the vector coupling of the transition dipole moments leads to transitions from the **G** to **E′** being allowed while transitions from **G** to **E″** are forbidden. In this case the physical consequence is that the dipole-allowed electronic transition for the system will be *red shifted* with respect to the uncoupled π–π* dipyrrin states.

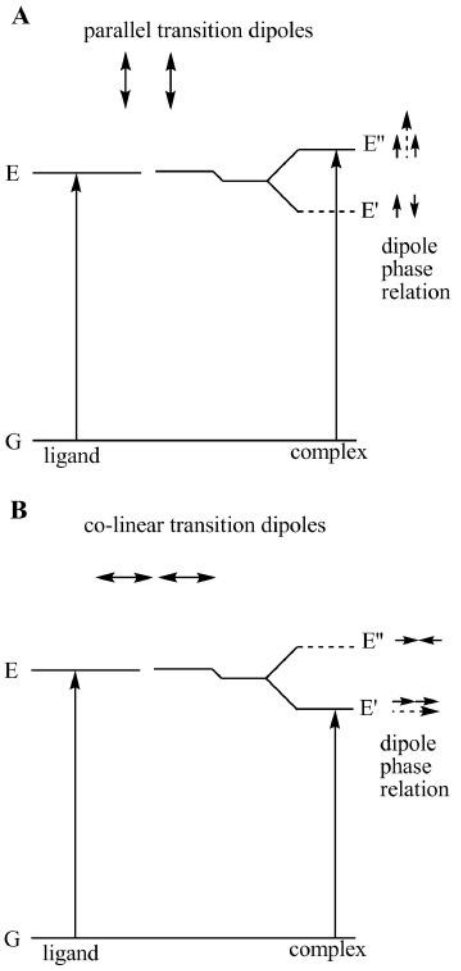


Fig. 4. Exciton energy diagram for a complex with components with A) parallel transition dipoles and B) co-linear transition dipoles.

These effects are well-known in the case of aggregated cyanine dyes where the parallel and co-linear arrangements are known as J- and H-aggregates respectively.[17] Signatures of J- and H- aggregation have also been observed in weakly interacting quantum dot systems, *e.g.* GaSe.[18] In the case of transition metal dipyrrin complexes, the specific requirements of the transition metal coordination geometry allow precise control over the exciton interaction and provide a route to a variety of new electronic states. In general, the transition dipole moments need not be coplanar and the dihedral angle (α) between ligand planes which contain the transition dipole may take any value from 0° to 90°. The relationship between the uncoupled π–π* states and the exciton states is shown in Fig. 5, which illustrates the general expression given by Eq. 1 below. Note that for α = 90° the exciton states are degenerate (but will have twice the intensity of a single π–π* transition).

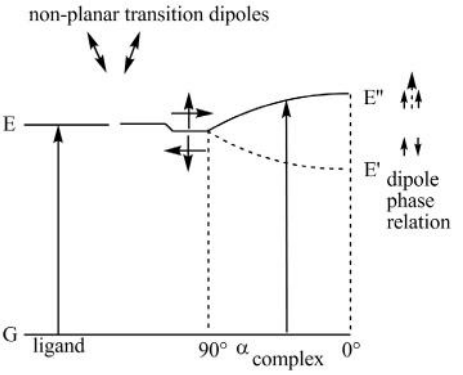


Fig. 5. Exciton energy diagram for a complex with ligand components with non-planar transition dipoles.

The exciton splitting energy is given by:

$$E'' - E' = \frac{2|\mathbf{M}|^2}{r_{ab}^3}(\cos\alpha - 3\cos^2\theta) \quad \text{.... (Eq. 1)}$$

where **M** is the transition dipole, r_{ab}^3 is the centre to centre distance between ligands a and b, α is the angle between the molecular planes of the ligands, and θ is the dihedral angle with respect to a vector that connects the transition dipoles (see Fig. 1).

The transition moments from the ground state to the exciton states **E′** and **E″** vary with the angle α. Equation 1 also explains why strong exciton interactions are rarely observed in the visible region for transition metal complexes. The difference in energy between the excitonic states depends on the square of the transition dipole moment (**M**), which is very large (ε ~ 50 000 mol/L/cm) for π–π* transitions in dipyrrins. Weak ligand field transitions do not have sufficient oscillator strength to generate substantial coupling between the electronic states. For moderate intensity metal-to-ligand charge-transfer transitions, strong coupling with fluctuations in the solvent bath destroys the coherence of the excitonic state and the individual MLCT chromophores behave as individual units.[19] For the dipyrrin complexes, the small separation between the units provided by the coordination geometry of the metal further enhances the exciton coupling due to the $1/r^3$ dependence.

The absorption spectra of a variety of dipyrrin species, including uncoordinated dipyrrin are shown in Fig. 6. The first of these (Fig. 6A) shows the large shift in the position and width of the absorption bands as exciton coupling effects increase in magnitude. Particularly striking is the copper complex of **3**, $Cu(L_{aza})_2$ (Fig. 6B) which, in particular, illustrates the potential of these materials as solar energy sensitizers, as efficient solar energy sensitizers should absorb a significant fraction of the solar spectrum. Biological systems adopt a similar strategy to extend the sensitizing properties of chlorophyll, for instance in the light-harvesting complexes of *Rhodobacter sp*.[20,21]

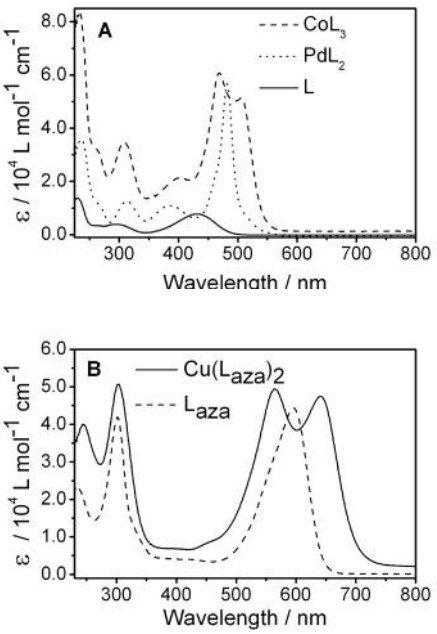


Fig. 6. Absorbance spectra of metallodipyrrin complexes of A - **2**, and B - L_{aza} (**3**).

Three (or more) π–π* States

The treatment of the coupling of two dipyrrin π–π* states to generate two excitonic states resembles a molecular orbital theory treatment of two-level systems.[22] In molecular orbital theory, group theory provides an efficient platform for extracting the essential features of a problem with minimal effort.[23] Group theoretical arguments can be applied to assemblies with three (or more) dipyrrin units. For instance, the case of a linear trimer of π–π* dipyrrin states is formally equivalent to the Huckel treatment of the π bonding in the allyl radical. For the Huckel treatment of the allyl radical it is assumed that only nearest neighbour interactions are non-zero and that these interactions are characterised by a single coupling, *J*. The resulting energy levels of the allyl radical are then $-\sqrt{2}J$, 0 and $+\sqrt{2}J$. For C_3H_3, the three-fold rotational axis generates energy levels -2*J* and *J*, with symmetries A + E.[23] For the excitonic states of dipyrrin complexes, it is now the π–π* states that play the role of the uncoupled levels. Coupling parameters (as determined by the symmetry of the problem) are required to complete the description of the system. The CoL_3 species possesses a three-fold rotational axis which, by analogy to the C_3H_3 system, immediately indicates that the excitonic states will be split into a single, non-degenerate level and two degenerate levels, *i.e.* A + E. The absorption spectrum of CoL_3 confirms this simple analysis with two peaks being observed at 469 nm and 505 nm, *cf.* Fig. 6A.

By analogy with the treatment of delocalized π systems, group theory arguments provide only a qualitative description of the number and types of energy levels. To obtain the energies of the excitonic states and the coupling strengths, the electronic absorption spectrum can be simulated using a time-dependent wavepacket model.[24,25] In this model $|\psi_g\rangle$ is the ground vibrational eigenstate, *i.e.* a Gaussian. The transition dipole moment operator, μ, induces a Franck-Condon transition into the excitonic state(s). By virtue of the Franck-Condon process, the vibrational wavefunction retains its Gaussian form and becomes a wavepacket in the excitonic state and executes dynamics according to the requirements of the propagator, $[-(i/\hbar)H_{ex}t]$ on the excitonic state potential surface. A time-dependent correlation function between the excited-state wavepacket and the ground-state wavefunction *records* the excited-state dynamics. The correlation function also contains all the spectral information about the electronic transition and this information is extracted in the form of the absorption cross-section by a Fourier Transform of the correlation function. The connection with the electronic structure of the excitonic states is made through the excitonic state Hamiltonian, H_{ex}. The CoL_3 system described above has three unperturbed π–π* states, each of which may be modelled as a simple displaced harmonic oscillator, x_i. This approach effectively replaces the large number of active vibrational modes in the Franck-Condon transition with one effective mode. Assuming only nearest neighbour interactions, H_{ex} is a 3 x 3 matrix of the form:

$$H_{ex} = \begin{bmatrix} H_1(x_1, x_2, x_3) & J_{12} & J_{13} \\ J_{21} & H_2(x_1, x_2, x_3) & J_{23} \\ J_{31} & J_{32} & H_3(x_1, x_2, x_3) \end{bmatrix}$$

Symmetry determines the form of the Hamiltonian, for instance, a linear trimer will have $J_{13} = J_{31} = 0$ (because the terminal states do not couple under the nearest neighbour approximation).[24] Simulation of the three-state CoL_3 presents some technical challenges;[26] however, a simulation of the two-state $Cu(L_{aza})_2$ system is shown in Fig. 7. For the purposes of the simulation, the single *effective mode* has a dimensionless displacement of 0.5, the energies of the perturbed states were 14 600 cm^{-1} and 16 800 cm_{-1} respectively.

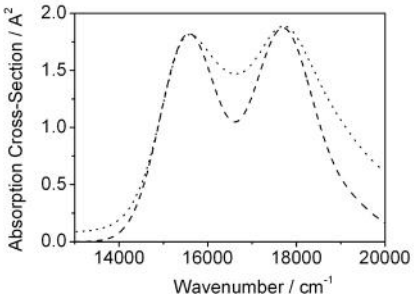


Fig. 7. Experimental (solid) and simulated (broken line) absorption spectrum of $Cu(L_{aza})_2$.

Summary

Metallodipyrrin complexes display a wide variety of exciton coupling effects that may enhance their utility in solar energy conversion and other optical applications. Their synthesis is relatively facile and simple quantum mechanical models explain their electronic structure. Beyond the basic spectroscopy and electronic structure lie interesting challenges in determining the dynamics in the excitonic states. Ultrafast spectroscopy and resonance Raman spectroscopy are currently being used by our group to investigate these aspects.

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A Saint with Feet of Clay

Bob Brockie

World of Science

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In the US, statues have been erected and schools, roads and bridges named in honour of Rachel Carson, the founding saint of the environmental movement. Carson's 1962 book *Silent Spring* opened our eyes to chemical pollutants and the damage they do to the environment, animals and people.

In 1962, the world had just had big scares over radioactive fallout and the damage caused by thalidomide. *Silent Spring* rode the anti-chemical, anti-science wave of the day and was touted as one of the most influential books of the 20th century. Rachel Carson's heart was in the right place. She wanted to save wildlife from death and degradation and for people to live healthy lives. She learned that DDT thinned eagles' eggshells and produced fewer chicks, and raised the spectre of DDT wiping out the United States' emblematic national bird. While writing *Silent Spring* she developed breast cancer and blamed her illness on DDT too.

Carson demonised DDT with passion, claiming it was a threat to life on Earth (though she failed to mention that DDT had eliminated malaria from Europe and America). She died of cancer in 1964 but an army of environmentalists and lobbyists took up her call and the use of DDT was banned worldwide. So far so good, but two of Carson's ideas have cast long shadows.

Banning DDT helped the eagles, but in tropical countries its banning was disastrous. In Sri Lanka, malaria cases rose from 17 to 520,000 and the disease increased by about 50% in Zanzibar and South Africa. Over the past 30 years, millions of malaria victims in the tropics have died because the World Health Organisation and the World Wildlife Fund have denied them DDT.

Endless surveys show that DDT has no connection with breast cancer or any other human illness, yet doctors and public health officers must battle well-heeled Western Greenies to have DDT restored to poor malaria-ravaged countries. Thank you, Rachel Carson.

NZ is in the grip of another Rachel Carson legacy. No matter that our foods are among the most chemical-free in the world, many Kiwis are convinced they are in imminent threat of being poisoned with pesticides. They're victims of her needless fear of chemicals, otherwise known as *paranoid chemophobia*. As a result, thousands, if not millions, of eco-chic Kiwis waste money on organic food to avoid ingesting harmless or non-existent chemicals.

The fearful should know that their bodies can and do detoxify small amounts of poison every day. There are 1000 chemicals in coffee. Given big enough doses, about half of them will cause cancer in rats. You drink more natural carcinogens in a cup of coffee than you're likely to get from pesticides in a year. Misconceptions about poisons, dose rates and risk force local and national authorities into needless enormous expense in removing trifling quantities of chemicals from the landscape. Despite their lethal reputation, DDT, dioxin, 245T or 1080 have never killed anybody.

Carson's book has also been voted one of the worst written in the 20th century.

Article

Unexpected Metabolites in Tobacco Genetically Modified to Accumulate Selenium

Adam J. Matich,[a] Nigel I. Joyce,[b] Marian J. McKenzie,[a] David A. Brummell,[a] Daryl D. Rowan.[a]

The New Zealand Institute for Plant and Food Research Limited, [a]Private Bag 11600, Palmerston North, [b]Private Bag 4704, Christchurch (e-mail: *daryl.rowan@plantandfood.co.nz*)

Selenium Accumulation by Plants

Most plants cannot tolerate high levels of selenium in the soil as the enzymes of the sulfur assimilation pathway do not distinguish between sulfur and selenium and consequently both are taken up by the plant.[1] Inorganic selenium is metabolised to selenocysteine and selenomethionine, the selenium analogues of cysteine and methionine, which are then incorporated into plant proteins causing defective secondary structure and reduced enzymatic activity, observed as stunting, necrotic lesions on the leaves, and reduced root growth.[2] Selenium non-accumulators are plants that cannot tolerate high-selenium soils, although in low-selenium soils they can discharge some selenium as dimethylselenide (MeSeMe).[1,2] In contrast, selenium accumulators such as garlic (*Allium sativum*) and *Brassica* species, *e.g.* broccoli, and hyperaccumulators, such as the two-grooved milk vetch (*Astragalus bisulcatus*), will tolerate high-selenium soils because they metabolise absorbed selenium so as to remove it from the pool of substrates that can be incorporated into proteins. The two-grooved milk vetch can accumulate several grams of selenium per kilogram of dry matter without showing signs of toxicity.

Selenium-accumulators use a selenocysteine methyltransferase (SMT) enzyme to remove selenocysteine from the cysteine pool by methylating it to methylselenocysteine (MeSeCys).[1,2] In young leaves of the two-grooved milk vetch, most of the selenium accumulates as MeSeCys, although some is transformed into γ-glutamyl-MeSeCys, or volatilized as dimethyl diselenide ($MeSe_2Me$).[2,3] The organoselenides produced by different types of plants are characteristic of their selenium accumulation status. Non-accumulators produce only MeSeMe, whereas in the two-grooved milk vetch $MeSe_2Me$ is the major volatile. Only in selenium accumulators, where selenium flow is diverted into MeSeCys, can $MeSe_2Me$ be produced, and its generation is therefore indicative of the presence of SMT activity. The ability to synthesise MeSeCys gives such plants an additional capacity to discharge a proportion of the accumulated selenium as volatiles.

The modification of selenium biochemistry in plants is of interest because of the essential role of selenium in human nutrition and health and the possible application of selenium volatilisation to the phytoremediation of selenium contaminated soils. Selenium is an essential micronutrient and may also play a role in cancer prevention as evidenced by the anti-carcinogenic activity of MeSeCys against animal cancer cell lines.[4,5] Although supplementation with selenium may help reduce the risk of cancer, the form in which the selenium is ingested is important. Rat cancer models fed with high-selenium broccoli showed an additional protection against cancer, when compared with control rats fed equivalent amounts of inorganic selenium and regular broccoli.[6,7] This is believed to be because these plants accumulate MeSeCys which is thought to have anti-cancer activity when converted to methylselenol in mammals.[8,9] Selenium-accumulators can also be used for phytoremediation of high-selenium soils,[10,11] in which case volatilisation of selenium may be more desirable than its storage, as selenium is dispersed from the local area as volatile compounds that are less toxic than those found in the soil.[10,12] This enables phytoremediation plantings to have a longer useful life, avoiding the need for harvesting and replanting to remove the accumulated selenium. Selenium volatilization from non-accumulators is enhanced by the expression of *SMT* or cystathione-γ-synthase transgenes in species used for phytoremediation.[12] Although $MeSe_2Me$ is the major organoselenium volatile produced by engineered selenium accumulators, MeSeMe is also produced in large quantities.[13,14]

A by-product of the genetic modification of plants may be the biosynthesis of new compounds, the discovery of which is aided by technological improvements to our ability to detect and measure multiple metabolites in complex biological samples. Re-direction of selenium metabolism and characterisation of the resulting compounds may shed light on presently unknown mechanisms and pathways for selenium assimilation in plants. We illustrate the process of metabolite identification using some new organoselenide semi-volatiles detected in tobacco genetically modified to accumulate selenium.

Organoselenides in Transgenic Tobacco Leaves

Two populations of transgenic tobacco plants were created: one constitutively over expressing an *Astragalus bisulcatus SMT* transgene, and the other constitutively over expressing both the *SMT* transgene and a broccoli *ATP sulfurylase* gene.[14]

LC-MS analysis with selective reaction monitoring (SRM) was used to selectively measure the selenium containing amino acids, MeSeCys and γ-glutamyl-MeSeCys, in the leaves after watering of the plants with sodium selenate for a period of 14 days (Fig. 1). Use of SRM allowed the sensitive quantitation of these amino acids by detection of specific fragment ions produced in the ion trap. The absolute concentration of γ-glutamyl-MeSeCys could not be determined owing to the absence of an authentic standard, but relative amounts of this metabolite were readily measured. In wild-type controls, neither MeSeCys nor MeCys was present much above the detection limit of 0.1 ng on column, but all transgenic lines over expressing *SMT* accumulated substantial amounts of MeSeCys: up to 5% of the plant's total accumulated selenium. MeSeCys accumulated to a greater extent in selenate-watered plants transformed

with both the *ATPS* and *SMT* transgenes. Concentrations ranged from 0.89 to 1.47 g/kg DW and constituted up to 10% of the plant's total accumulated selenium at concentrations around 10-fold higher than that of MeCys.[14]

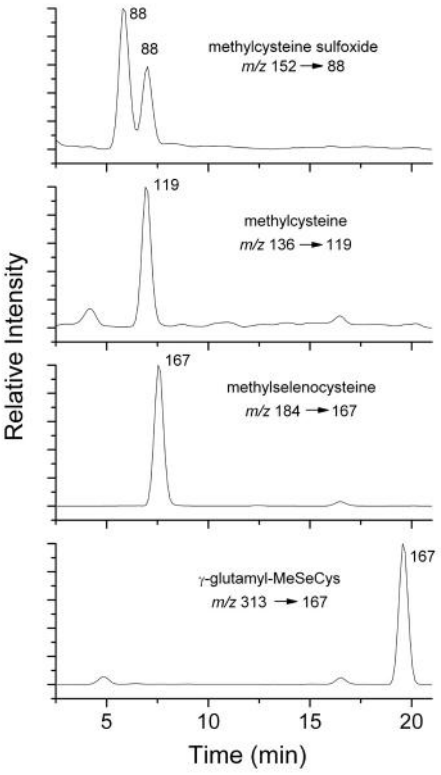


Fig. 1. Analysis of sulfur and selenium amino acids in transgenic tobacco leaf by ion trap LC-MS showing selected ion chromatograms of fragment ions selected for quantitation by selective reaction monitoring (SRM).

Organoselenides in the Headspace above Transgenic Tobacco Plants

Watering of transgenic plants with sodium selenate resulted in a distinctive off-odour of *cabbage* or *ocean* in the glasshouse, suggesting the production of volatile organoselenides. To identify these compounds, individual tobacco plants were enclosed in oven bags and sealed about the stem just above the level of the potting mix. The headspace in the bag was allowed to equilibrate for one hour prior to sampling with a Carboxen™-PDMS solid phase microextraction (SPME) fibre inserted through the wall of the oven bag and maintained in position overnight. Volatiles were then desorbed from the SPME fibres in the injection port of the gas chromatograph. GC-MS analysis showed the presence of MeSeMe, MeSeSMe, and $MeSe_2Me$.[14] These organoselenides were located in the GC-MS traces by their distinctive isotopic patterns and the mass deficiency of selenium-containing ions, and by using distinctive fragment ions to generate selective ion chromatograms (Fig. 2). The most useful of these fragment ions was *m/z* 92.9 ($CH^{80}Se^+$) found in all of the organoselenides.

The formation of MeSeMe from selenomethionine does not require SMT activity and so this compound was found in both the engineered and the wild-type plants. MeSeSMe and $MeSe_2Me$ were found only in the volatiles collected from transgenic plants fertilized with sodium selenate.[14] All transgenic plants produced substantial amounts of

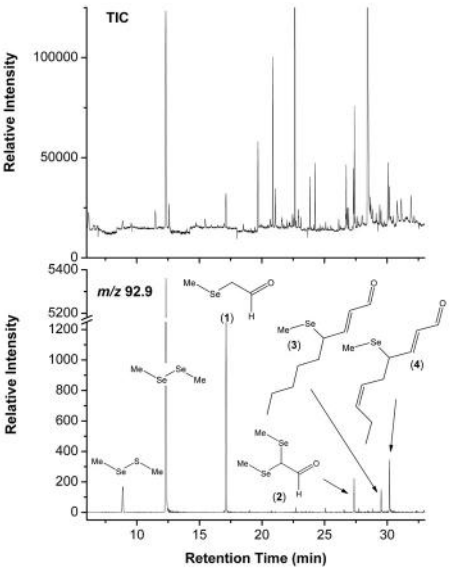


Fig. 2. Simplification of the complex metabolic profile of tobacco leaf extracts and detection of organoselenides achieved using *m/z* 92.9.

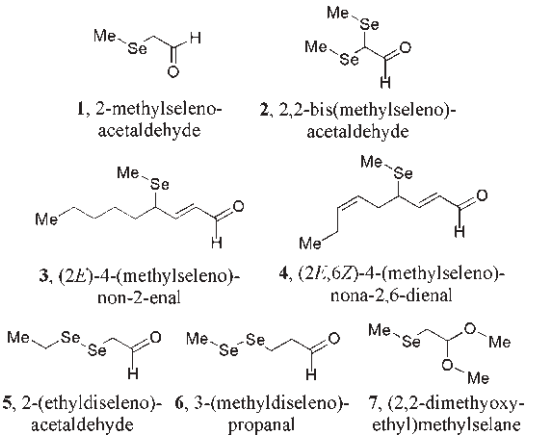
$MeSe_2Me$, which is formed from MeSeCys, indirectly confirming the presence of SMT activity in these plants. $MeSe_2Me$ is the volatile organoselenide compound most commonly reported in selenium accumulating organisms[13,15,16] and was found in the headspace at similar concentrations to MeSeMe. MeSeMe and $MeSe_2Me$ are commercially available, but MeSeSMe is not and so was identified by high resolution (accurate mass) EI-GCMS. Thus, MeSeSMe showed a molecular ion at *m/z* 141.9360 ($C_2H_6SeS^+$) and was identified by fragmentation analysis and comparison with the literature.[17] MeSeSMe may arise by disproportionation of $MeSe_2Me$ with MeS_2Me, traces of which were detected in some plants.

Semi-volatile Organoselenides in Leaf Extracts of Transgenic Tobacco Plants

Four new organoselenides (**1-4**, Chart 1) were identified in crude solvent extracts from the leaves of the transgenic tobacco plants (Fig. 2), based on the very distinctive isotope pattern and mass deficiency of selenium, and the seemingly ubiquitous fragment cluster at around *m/z* 92.9. As insufficient sample was available for isolation and NMR analysis, possible structures were deduced from the mass spectral data and candidate compounds were prepared by synthesis for GC-MS comparison with the compounds in the plant extracts.[18]

The first new metabolite, 2-(methylseleno)acetaldehyde (**1**, Chart 1) was the second most prevalent organoselenide in the solvent extracts (17.3 min, Fig. 2). The molecular ion for C_3H_6OSe was at *m/z* 137.9586, (137.9584 required), and a cluster of ions centred around *m/z* 108.9554 ($C_2H_5Se^+$, M^+-CHO) suggested that **1** was an aldehyde. Methylselenoacetate is one candidate compound, but the expected large base peak at *m/z* 43 was not observed (Fig. 3).[19] Aldehyde **1** seemed the most likely structure and the mass spectrum bore some similarity to that reported previously.[20] Synthesis of this compound confirmed that its GC retention time and mass spectrum (Fig. 3) matched that of the tobacco metabolite.

Chart 1. Orgaoselenides **1-4** identifed in leaves from transgenic tobacco plants, and synthetic compounds **5-7**



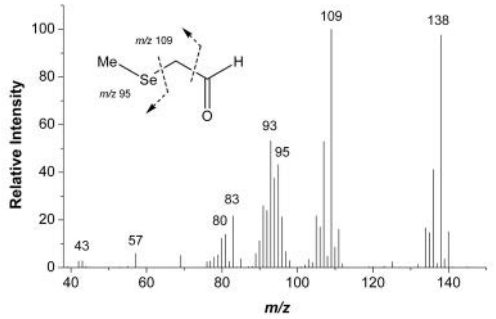
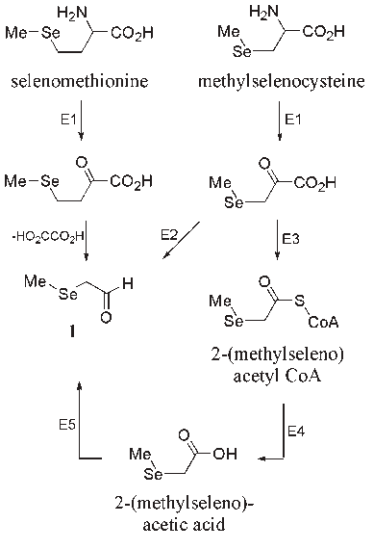


Fig. 3. Mass spectrum of 2-(methylseleno)acetaldehyde (**1**) found in leaves of transgenic tobacco plants treated with Na_2SeO_3 (redrawn from Matich *et al*.- see ref. 18).

Determination of the structure of **1** identifies a new pathway of selenium metabolism in tobacco that diverges from those responsible for the production of the well known organoselenides, MeSeMe and MeSeSeMe.[14] Aldehyde **1** may derive from either selenomethionine or MeSeCys, by mechanisms similar to those observed for the catabolism of branched-chain amino acids in bacteria and yeast, as shown in Scheme 1.



Scheme 1. Possible biosynthetic pathways for 2-(methylseleno)acetaldehyde (**1**) in transgenic tobacco leaves from selenomethionine (ref. 21) and MeSeCys. E1: amino acid amino transferase, E2: 2-oxoacid decarboxylase, *c.f.* pyruvate decarboxylase, E3: 2-oxoacid dehydrogenase. E4: acyl-CoA hydrolase (see ref. 22), E5, acyl-CoA reductases see (ref. 23).

The second new organoselenide **2** (retention time of 27.3 min, Fig. 2) contained two selenium atoms, as shown by the isotopic distribution (Fig. 4) and the high resolution mass spectrum of its molecular ion $C_4H_8OSe_2$ (*m/z* 231.8935). The fragment ions $C_3H_7Se_2^+$ (*m/z* 202.8878) and $CH_3Se_2^+$ (*m/z* 175) indicated M^+-CHO and of M^+-C_2H_4CHO, respectively, suggesting this metabolite was another aldehyde but also containing a Se-Se bond. However, the absence of *m/z* 160 (Se_2^+) put this in dispute. The fragment ion *m/z* 109 ($C_2H_5Se^+$) corresponds to M^+-$SeCH_2CHO$ implying that **2** contains an ethyl group. The candidate compounds 2-(ethyldiseleno)-acetaldehyde (**5**) and 3-(methyldiseleno)propanal (**6**) were synthesised, but their mass spectra did not match that of tobacco compound **2**. Most notably, the mass spectra of these compounds contained *m/z* 43 (CH_2CHO^+) and *m/z* 57 ($CH_2CH_2CHO^+$), respectively, and in particular the Se-Se^+ moiety (*m/z* 160), which were not present in **2** (Fig. 4).

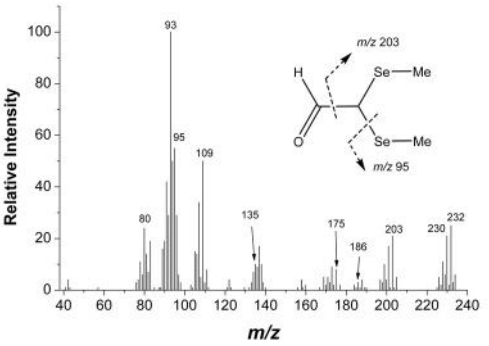
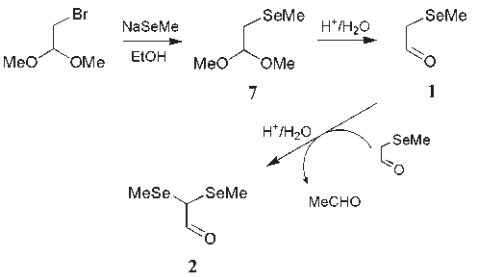


Fig. 4. Mass spectrum of 2,2-bis(methylseleno)acetaldehyde (**2**) found in leaves of transgenic tobacco plants treated with Na_2SeO_3 (redrawn from Matich *et al*. - see ref. 18).

Fortuitously, we noticed that a by-product from the synthesis of aldehyde **1** (Scheme 2) had the same GC retention time and mass spectrum as **2** and to 2,2-bis(methylseleno)-acetaldehyde **2**, a by-product previously reported from acid hydrolysis of the ethyl acetal analogue of **7**.[20] Therefore, the above mentioned ion at *m/z* 175 ($MeSe_2^+$) did not arise from a Se-Se bond and probably results from rearrangement of $(MeSe)_2CH^+$ (*m/z* 203) to $MeSeSe^+CHMe$ prior to fragmentation. *In planta*, aldehyde **2** would also seem to arise from acid-catalysed disproportionation of aldehyde **1** (Scheme 2).



Scheme 2. Synthesis of 2-(methylseleno)acetaldehyde (**1**) and its acid-catalysed conversion into diselenide **2** during hydrolysis of acetal **7**.

The third and fourth organoselenides **3** ($C_{10}H_{18}OSe$) and **4** ($C_{10}H_{16}OSe$) were found at their highest levels in leaves from doubly transformed plants. Their mass spectral fragmentation patterns below *m/z* 110 suggested these compounds differed by one double bond. In aldehyde **4**, *m/z* 163 ($C_5H_7SeO^+$) corresponded to M^+-C_5H_9 and *m/z* 135

($C_4H_7Se^+$) to the subsequent loss of CO (Fig. 5). Some of the ions clustered around *m/z* 135 also represented the loss of SeMe and MeSeH (*m/z* 137 and 136, respectively), to give a nine-carbon fragment. These fragmentations suggest that aldehyde **4** might have a 4-(methylseleno)-2,6-nonadienal structure. (2*E*,6*Z*)-4-(Methylseleno)nona-2,6-dienal was synthesised by adding the nonadienal to (2,2-dimethoxyethyl)(methyl)selane (**7**) prior to its acid hydrolysis, with the expectation that during the hydrolysis a reaction similar to that shown in Scheme 2 for the production of $(MeSe)_2CH_2CHO$ (**2**) would occur. Selective TOCSY NMR experiments confirmed that aldehyde **4** was in fact produced, and the mass spectrum (Fig. 5) and retention time of this synthetic compound matched to those of the tobacco compound. Aldehyde **3** was similarly synthesised from (2*E*)-nonenal and (**7**) and characterised as (2*E*)-4-(methylseleno)non-2-enal. Given the reactivity of aldehyde **1** with (2*E*)-nonenal and (2*E*,6*Z*)-nonadienal, under acid conditions *in vitro*, aldehydes **2-4** may well result from spontaneous chemical reaction in the plants or in the solvent extracts. Regardless, the presence of these compounds demonstrates the reactivity and potential of **1** to participate in interesting chemistry *in vivo*.

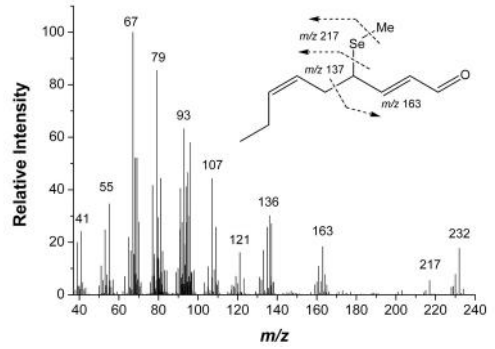


Fig. 5. Mass spectrum of (2*E*,6*Z*)-4-(methylseleno)nona-2,6-dienal (**4**) found in leaves of transgenic tobacco plants treated with Na_2SeO_3 (redrawn from Matich *et al.* – see ref. 18).

We have shown that a Solanaceous species (tobacco), lacking the sulfur secondary metabolism found in the *Brassicaceae*, can be converted from a selenium non-accumulator into a selenium accumulator by genetic modification. This work extends previous studies in *Arabidopsis* and Indian mustard and demonstrates that the trait of MeSeCys accumulation can be moved from the Se-hyperaccumulators to plants outside of the *Brassicaceae*. Transformation of tobacco into an accumulator of MeSeCys[14] resulted in the production of additional organoselenide metabolites, in particular aldehyde **1**, whose presence suggests the operation of a new pathway for selenium mobilisation in plants. The other new metabolites may arise by chemical reactions occurring *in planta* or in the plant extracts, but their identification demonstrates the usefulness of GC-MS metabolic profiling in assessing the chemical composition of genetically modified organisms.

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Article

Recent Advances in Labelling of DNA with Organic Chromophores

Vyacheslav V. Filichev

Institute of Fundamental Sciences – Chemistry, Massey University, Private Bag 11222, Palmerston North (e-mail: *v.filichev@massey.ac.nz*)

Introduction

Remarkable changes in the viscosity and sedimentation of complexes of DNA with acridine derivatives suggested to Lerman that intercalation was responsible.[1] Later, intercalative interactions were investigated by using various spectrophotometric methods including X-ray diffraction. Intercalators are typically described as a class of molecules that reversibly bind in the space between two adjacent base pairs of DNA. Traditionally, intercalators are considered as free ligands. If a large aromatic chromophore intercalates into DNA, then unwinding of the DNA double helix occurs, increasing both the flexibility of the DNA and the distance between nucleic acid bases.

Covalent attachment of intercalators to nucleic acids is a useful tool in chemical biology and attachment of fluorophores to either end of a DNA strand is a well-established procedure. Attachment of fluorescent tags via a tether to DNA bases at non-hydrogen bonding sites, *e.g.* the 5-carbon of C, T or U and the 7-position of 7-deazapurines, allows labelling of many sites in a DNA helix. These techniques found their application in genetic analysis, like DNA sequencing,[2] real-time PCR (the polymerase chain reaction),[3] visualization of chromosomes or DNA/RNA in FISH (fluorescence *in situ* hybridization).[4] Typical linkers for fluorophore attachment consist of up to a dozen bonds, providing flexibility but also creating an uncertainty in the location and orientation of the chromophore. In contrast, novel methodologies for development of new types of nucleic acids architectures rely on the predictable positioning of labels in the structure.[5] In that regard, nucleic acids as a helical self-assembled structure provide a clear scaffold, which can be used to develop functional π systems where the correct orientation of each component is vital for the optical properties of the entire system. In the last decade, in particular after sequencing the human genome, the number and variety of intercalating moieties that could be inserted covalently into DNA has increased dramatically, mostly driven by a demand for developing probes that could detect specific nucleic acid sequences. This article presents recent advances in nucleic acid architectures using an example of a logical development of pyrene insertions. Pyrene is one of the most extensively studied chromophores in DNA/RNA structures. Initially, pyrene and other organic chromophores were largely used as *molecular caps*. Later they found use opposite abasic sites, as a counter base, as artificial hydrophobic pairs and, very recently, as helically arranged π-π stacking arrays. Organic chromophores can be introduced into nucleic acid structures via modification of nucleotides, *i.e.* attachments to the base, sugar or phosphate, or via entirely non-nucleosidic linkers. By such means a chromophore can be placed in the interior of nucleic bases or in the grooves of the nucleic acid complexes.

DNA Structures: Duplex, Triplex and G-Quadruplex

Hydrogen bonding and π-π stacking between nucleic bases, electrostatic repulsion between negatively charged phosphates and hydration are responsible for the stability of nucleic acids. Depending on its sequence and media, a nucleic acid can accommodate numerous secondary structures, such as the classical B-type helix, the A-form or left-handed Z (zigzag) form. Alternative (non-Watson-Crick) Hoogsteen and reverse Hoogsteen base-pairing can give rise to high-order DNA and RNA structures that include triplexes and quadruplexes (Fig. 1).

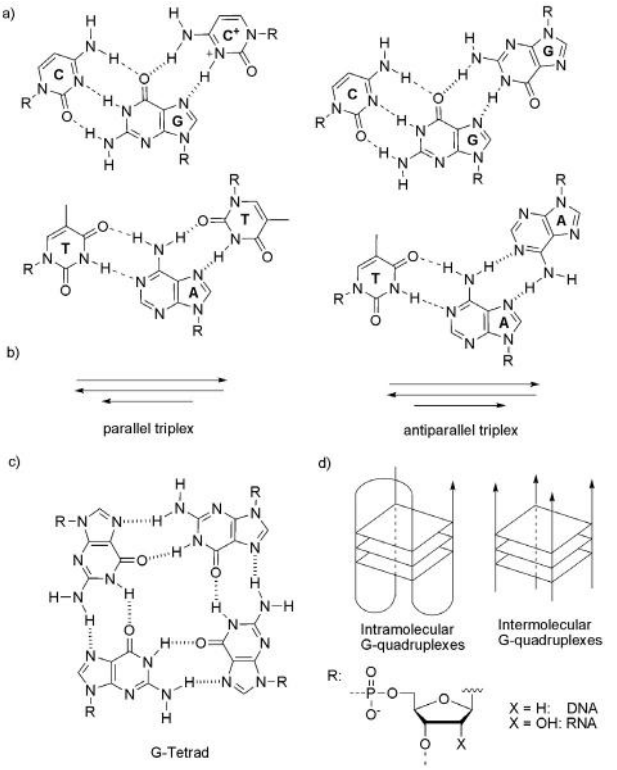

Fig. 1. Structures and secondary structures of DNA/RNA. Parallel and antiparallel triple helices, a): H-bonding formation, Watson-Crick base-pairing are represented by dashed bonds and Hoogsteen or reversed-Hoogsteen are represented by hashed bonds; b): representation of the strand orientation in triplex. G-quadruplexes; c): H-bond formation in G-tetrad; d): intra- and intermolecular G-quadruplexes.

Triple helices are formed when a single-stranded triplex-forming oligonucleotide (TFO) binds in the major groove of double-stranded DNA (dsDNA).[6] A homopyrimidine TFO can bind in a parallel fashion to the homopurine strand of dsDNA (Fig. 1). A nucleobase T binds to a nucleobase A of the duplex, but cytosine in the TFO must be protonated at the N-3 atom to form Hoogsteen base-pairing with dsDNA. This makes formation of parallel triplexes at neutral pH problematic. A homopurine third

strand can also bind to a homopurine-homopyrimidine duplex using reversed Hoogsteen patterns. In this triplex, a nucleobase A binds to a T-A base pair and a G to a C-G pair. Here, the homopurine TFO is antiparallel to the homopurine strand of the original dsDNA. GT-rich TFOs can also form parallel and antiparallel triplexes depending on the sequence.[7] G-Rich sequences can fold into stable G-quadruplexes, especially in the presence of K^+ (Fig. 1c, 1d).[8] The topology and molecularity of G-quadruplexes are determined by several factors including variations in strand polarity, loop geometry, the presence of metal ions, *etc*.[9] The extraordinary stability of G-quadruplexes has some unfortunate outcomes for G-rich sequences: TFO probes designed to form triplexes but possessing stretches of guanines do not bind to duplexes at all because of the probes' self-aggregation.[10]

Introduction of additional moieties to DNA has an impact on the thermodynamic stability of DNA complexes. When UV/VIS, fluorescence or CD spectrometers are fitted with a Peltier temperature programmer, melting of DNA complexes or thermal stability studies can be undertaken. The melting temperature (T_m, °C) is usually defined as the maximum of the first derivative of the curves obtained by measuring absorbance at 260 nm or CD against increasing temperature. Control experiments should be performed at other wavelengths also, depending on the DNA structure and the absorbance/fluorescence region of the intercalator. Luminescence is a widely explored feature of intercalators. The fluorescent sensitivity of some fluorophores depends highly on changes in the microenvironment. For example, the fluorescence of pyrene is quenched in low-polarity environments and increased in high-polarity environments. By inserting such intercalating units as reporter groups, one can gain a deeper understanding of fundamental features of nucleic acids, for example, folding and unfolding mechanisms, local dynamics within DNA helices, polymorphism in DNA, the type of forces involved in formation of different secondary structures, and so forth.

Excitation (λ_{ex}) and emission wavelengths (λ_{em}) are important descriptive values for each chromophore. Usually, organic chromophores exist in an excited state for a finite time (typically 1–10 ns). The difference in energy or wavelength, represented by ($h\nu_{ex} - h\nu_{em}$), is called the Stokes shift and is fundamental to the sensitivity of fluorescence techniques because it allows emission photons to be detected against an unpolluted background, isolated from excitation photons. The fluorescence quantum yield (Φ_F) is defined as the ratio of the number of photons emitted by fluorescence at a certain wavelength to the number of photons absorbed at this wavelength. In cases where there are multiple insertions of chromophores, particularly if two fluorescent molecules are positioned and overlapped in close proximity to each other (*ca*. 3.4 Å), formation of excimers or exciplexes can be observed. During *exci*ted di*mer* (excimer) formation, which can be obtained in saturated solutions of a free ligand, one molecule is in an electronically excited state and the other is in an electronic ground state. In contrast, an *exci*ted du*plex* (exciplex) is a bimolecular complex of two different chromophores.

Molecular Caps and DNA Interstrand Stacking Interactions

Pyrene's size (220 Å^2) allows it to occupy the area usually covered by two natural purine:pyrimidine base pairs (269 Å^2).[11] It is an ideal lid for canonical base pairs[12] because its size cannot fully cover purine:purine base pairs, whereas pyrimidine:pyrimidine pairs are too small to accommodate it. However, the choice of linker is crucial. This can be demonstrated by insertion of pyrene at the 5′- or 3′-terminus of oligonucleotides (Fig. 2). Introduction of such molecular caps can lead to an increased thermal stability of the resulting complexes with ssDNA/RNA that can exceed 10 °C per modification.[13] The thermodynamic stabilization gained is a result of the stacking pyrene with the nearest nucleic bases.[14] Additionally, the dangling-end residue acts as a hydrophobic cap and restricts bulk water access to the terminal base pair. This makes these terminal base pairs energetically comparable to the corresponding internal base pairs.[15] In the case of flexible linkers, such as butyric acid, pyrene can adopt a number of different conformations, whereas in structures such as 2-deoxyribofuranose (**P**) or pyrrolidine (**azaP**) the pyrene moiety remains in a more rigidly defined position on the top or the bottom of the helix. The defined position leads to slightly higher thermal stability and hyperchromicity, as it has been shown for **azaP** in comparison with 1-pyrenylbutyric acid.[12] These properties have already been used in the design of probes that combine enhanced affinity and base pair fidelity. An unmodified DNA probe can barely discriminate the perfectly matched and terminal mismatched DNAs (ΔT_m = –0.3 – +1.7 °C). However, when pyrene pyrrolidine **azaP** (n=0) as a 5′-end cap was combined with anthraquinone derivative (**AQ**) as a 3′-end cap,[16] the prepared dodecamer DNA gave a melting point decrease for a double-terminal mismatch of ΔT_m = +6.2 – 7.4 °C. Similarly, a doubly capped matched DNA duplex showed increased thermal stability in comparison with an unmodified one (ΔT_m = +11.1 °C).[12]

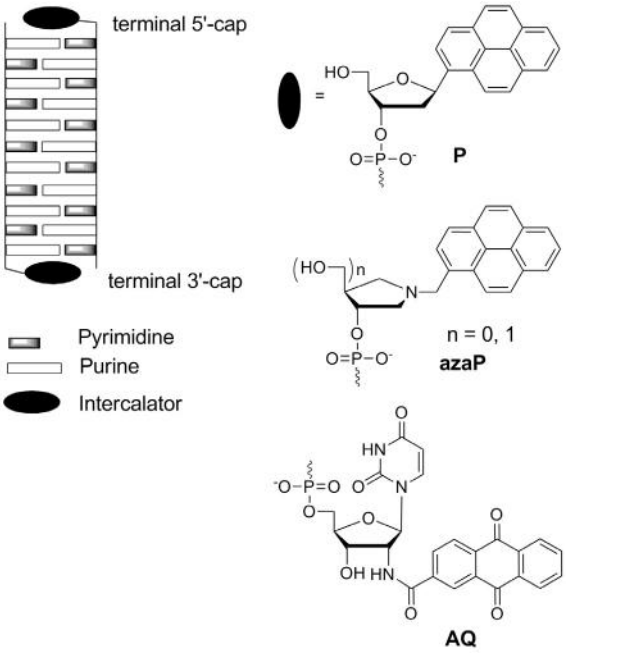

Fig. 2. Molecular caps - chromophores covalently attached to the 3'- or 5'-end of the duplex.

Insertion of an intercalating moiety in the core of the DNA duplex can be performed in three ways: opposite the natural base (Fig. 3), opposite an abasic site (Fig. 4), and as a bulge (Fig. 5). In contrast to free intercalating ligands, the covalently attached chromophores as base substituents do not have to unwind the DNA for intercalation. Hydrophobic base analogues do not form pairs with the natural bases and these combinations are usually strongly destabilized. This is an outcome of the absence of hydrogen bonding formation and imperfect steric fit within the DNA duplex.[17] Also, if the size of intercalator is too large the counter nucleobase may be flipped out of the helix.

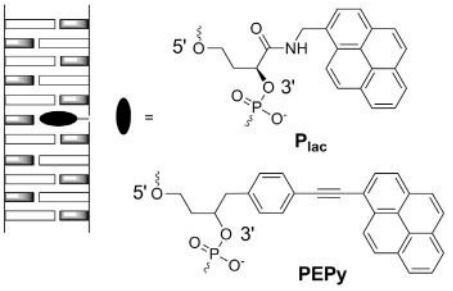

Fig. 3. Chromophores in the middle of a DNA duplex opposite the natural base.

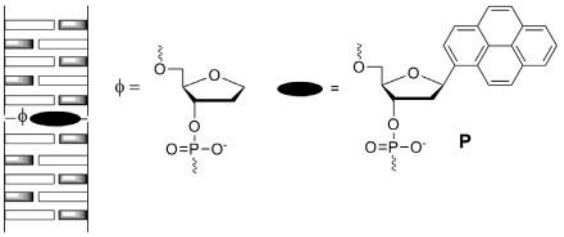

Fig. 4. Chromophores in the middle of a DNA duplex opposite an abasic site.

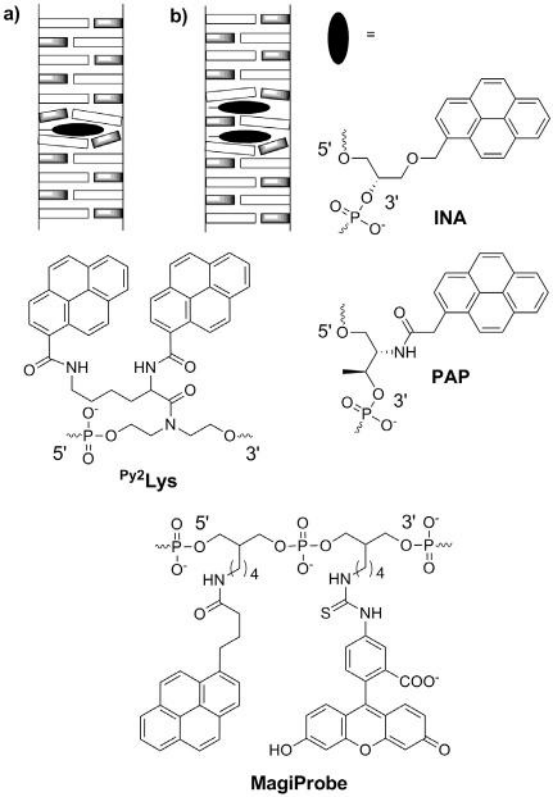

Fig. 5. Fluorescent probes designed using the principle of bulge insertions; a): a bulge insertion; b): chromophore insertions as next-nearest neighbours.

Generally, DNA duplexes can more easily accommodate non-native structures incorporated close to the ends than in the middle of the helix. Thus, incorporation of $\mathbf{P_{lac}}$ in the midst of the sequence led to much more pronounced destabilization (ΔT_m = –6.3 °C) than for insertions close to the 3′- or 5′-ends (ΔT_m = –2.7 °C).[18] 1-(Phenylethynyl)pyrene (**PEPy**) has been placed in a DNA duplex using a 1,3-butanediol linker instead of the nucleobase and a slight destabilization of the duplex was observed (ΔT_m = –1.4 °C).[19] Spectroscopic properties of **PEPy** analogues are beneficial compared with those of the initial pyrene. Nowadays, standard genetic analysis platforms are primarily designed to detect fluorescein at 520 nm,[3] which unfortunately eliminates many potentially useful labels based on the pyrene moiety (λ_{ex} ~330–340 nm; λ_{em} ~400–410 nm). Moreover, biomolecules are also excited upon irradiation at the same wavelength as pyrene.[20] For **PEPy** there is a bathochromic shift in the absorption spectra (373 nm *vs* 343 nm for pyrene) and fluorescence maxima are shifted to longer wavelength (400–410 nm *vs* 380 nm for the first peak in a monomer fluorescence and 500–510 nm, compared with 480 nm for excimer fluorescence).[19-21] This is also accompanied by high fluorescence quantum yields in buffer solutions even in the presence of oxygen. A further shift in fluorescence maxima to longer wavelengths was observed upon introduction of the second and the third 1-phenylethynyl residues in the structure of pyrene (positions 1, 6 and 8).[20,22] Insertion of two **PEPy** units opposite the variable nucleotide sites has been successfully used in the design of fluorescence probes for detection of single polymorphisms in the gene fragment of 23S rRNA *Helicobacter pylori* showing remarkable sensitivity for the presence of a T or of a non-T base opposite to the **PEPy** pair.[23]

If the DNA base opposite is missing a so-called *abasic site* (Fig. 4), some aromatic molecules can actually fit within the DNA duplex and restore the π-π DNA duplex base stack. A good example of this is pyrene C-nucleoside **P** (Fig. 2).[11] The 5′-triphosphate of **P** was incorporated into DNA using a Klenow fragment opposite an abasic site with a selectivity and efficiency which were greater than those for the natural DNA triphosphates. The termination of the DNA biosynthesis was detected after **P** incorporation,[24] which makes this fluorescent pseudo-nucleoside useful for distinguishing abasic mutations. Insertion of **P** opposite an abasic site was more energetically favourable than the use of natural bases. The stabilization of the duplex was detected in a ΔT_m range of 18 – 23 °C in comparison with native duplexes having an internal abasic site.[11]

Incorporation of organic chromophores as a bulge (Fig. 5) was used in the development of DNA probes for various applications. Chromophores actually intercalate between two adjacent base pairs in a way similar to free ligands. In general, acyclic linkers that can be more easily accommodated in the helix are used for bulged constructions.

Discriminative binding to ssDNA over ssRNA having identical sequences was observed for intercalating nucleic acids (**INA**) having a bulged pyrene moiety connected via a glycerol linkage in the middle of the DNA sequence ($\Delta T_{m\ \mathbf{INA}/DNA}$ = +3.0 °C, $\Delta T_{m\ \mathbf{INA}/RNA}$ = –4.4 °C per modifica-

tion).[25] Two factors contribute to the discrimination upon binding to DNA and RNA: distortion of the nucleic acid backbone by short ethyleneglycol linker and stabilization of the **INA**/DNA structure by intercalation. Interestingly, using pyrene connected via a flexible linker to form a bulge was energetically more favorable than the use of a nucleobase (ΔT_m = –12.8 °C) and the ethylene glycol linker on its own (ΔT_m = –8.2 °C),[25] the latter being a clear indication of intercalation. When two **INA** monomers were placed as next-nearest neighbours (Fig. 5b) a strong excimer fluorescence at 480 nm (λ_{ex} =340 nm) was observed for the probe alone.[26] The excimer band was completely quenched upon formation of a fully complementary **INA**/DNA duplex. If there was a mismatch between **INA** residues, there was no or little quenching of the excimer band. A deletion polymorphism was effectively detected using two **PAP** monomers as next-nearest neighbours. No excimer fluorescence was detected for the perfectly matched duplex, whereas a deletion of a single-base opposite **PAP** monomers resulted in the formation of the pyrene excimer band.[27] A **^{Py2}Lys** backbone with two 1-pyrenyl residues was designed to report insertion mutations.[28] The fluorescence intensities for the probe alone and for the perfectly matched duplexes with **^{Py2}Lys** as a bulge were very weak (Φ_F = 0.001-0.007). If an extra base was presented in the target opposite the **^{Py2}Lys** insertion, a strong fluorescence peak at 495 nm appeared (Φ_F = 0.088).

Pyrene was used as a quencher of fluorescein in the MagiProbe design.[29] When hybridized to the perfectly matched sequence pyrene intercalates into the duplex, this results in the emission of fluorescence from fluorescein. No fluorescence signal was detected for the probe on its own or upon binding to the mismatched sequence close to the pyrene residue.

Bulged intercalators have also been introduced in DNA triplex technology (Fig. 6). We have recently developed an example of a triplex selective intercalator.[21] Bulge insertions of (*R*)-1-*O*-[4-(1-pyrenylethynyl)phenylmethyl]-glycerol (*t*wisted *i*ntercalating *n*ucleic *a*cids, **TINA**, Fig. 6) into the middle of a homopyrimidine strand led to high triplex thermal stability (ΔT_m = +19.0 °C per modification) and discrimination of Hoogsteen-type triplexes over Watson-Crick type duplexes. Under similar conditions the native TFO was unable to bind to the duplex. The 1-pyrenyl derivative of **TINA** was found to be the most effective among the tested modifications (acridine, naphthalene, *m*-phenylethynyl, 4-biphenyl, 2- or 4-pyrene) for binding to the Hoogsteen-type triplexes.[21,30] It is interesting to compare the ability of the rather similar structures **INA** (Fig. 5) and **TINA** (Fig. 6) to stabilize parallel triplexes. In contrast to **TINA**, pyrenemethylglycerol (**INA**) destabilized triplexes upon bulged insertions into identical sequences (ΔT_m = –5.0 °C).[31] These data highlight the importance of the proper intercalator, *e.g*. pyrene positioning in the interior of the dsDNA region of the triple helix. It is important to mention planarity of the intercalating unit is not an absolute requirement because bases are propeller-twisted to a varying degree within the triplets. Rigid linkers, such as single, double or triple C-C bonds, can connect two or three chromophores thus helping the entire intercalator to fit correctly within the base triplets.

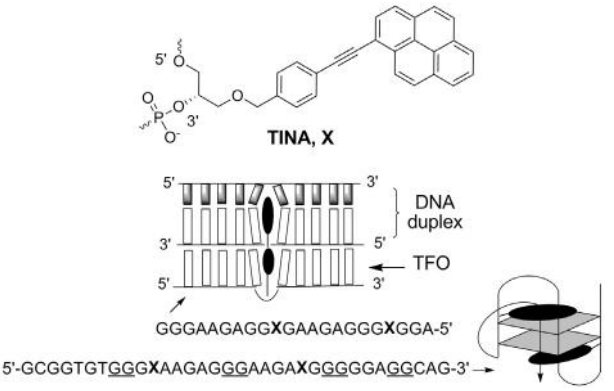

Fig. 6. TINA molecule and its use in the design of triplex-forming oligonucleotides and G-quadruplexes.

We recently investigated the ability of **TINA** to stabilize and destabilize G-quadruplexes. It is known that quadruplex DNA can be stabilized by free ligands such as cationic porphyrins,[32] trisubstituted acridines,[33] and others.[34] We assumed that the placement of pyrene units on the top or the bottom of the G-quadruplex structures would stabilize them via stacking interactions. On the other hand, insertions *between* adjacent guanines should, in principle, destabilize self-association of the G-rich sequences (Fig. 6). The latter can be useful for antiparallel TFOs. We covalently attached one or two **TINA**s in positions adjacent to the stretches of guanines in the quadruplex motif, mimicking the structure which is located in the promoter region of the human *KRAS* gene. A dramatic increase in the T_m (ΔT_m = +22 – 32 °C) and a strong antiproliferative effect in Panc-1 cells were observed.[35] In contrast, when **TINA** monomers were inserted in the middle of G-runs significant destabilization of G-quadruplex-like structures was observed. The final **TINA**-TFOs were able to bind to the DNA duplex in an antiparallel fashion even in the presence of potassium ions.[36]

Summarizing the effect of using aromatic substituents in the middle and at either end of the DNA sequence, we can conclude that organic chromophores can restore or enhance DNA stacking interactions by virtue of intercalation. In that regard not only the size of the hydrophobic molecule but also the proper coverage of the nucleic base surface and a linker are crucial in the molecules' design. It can be anticipated that future developments will be more focused on non-canonical base pairs and different DNA/RNA secondary structures such as cruciforms, hairpins and three- or four-way junctions.

From Single Chromophore Incorporation to the Extended Stacking Interactions within DNA Duplexes

Stacking interactions of organic chromophores in the middle of nucleic acid helices represent a promising approach for the design of DNA-based tools for nanobiotechnology. As could be expected, interstrand stacking interactions give rise to interesting and useful UV and fluorescence properties. Impressive changes in optical properties of organic chromophores were observed during formation of oligomers encompassing fluorescent *C*-nucleosides, the so-called fluorosides (Fig. 7).[37] Enhanced molar extinc-

tion coefficients and enhanced Stokes shifts up to 145 nm were observed for the oligomer with three pyrenyl derivatives (**3P**, Fig. 7). Using a set of four fluorosides, a combinatorial library was created with a broad array of emission colours from violet to orange. This diversity was achieved with a narrow range of UV excitation (340-380 nm). In a larger combinatorial library composed of eleven fluorosides in oligomers four units long (**4Ch**, Fig. 7), certain constructs exhibited large hypsochromic shifts in their emission spectra after exposure to UV light.[38]

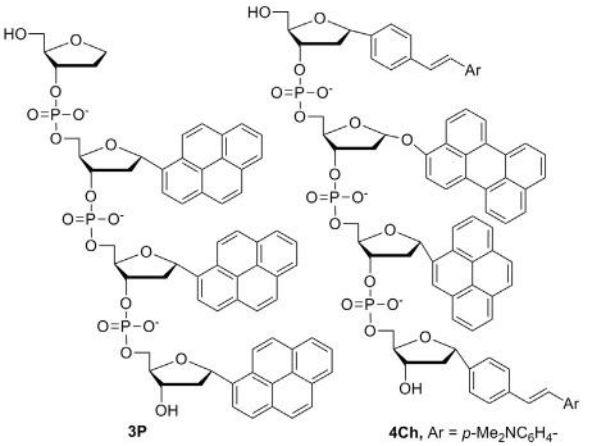

Fig. 7. Examples of polyfluorophores.

Insertion of pyrenyl fragments [**INA** (Fig. 5), $\mathbf{P_{1,8}}$, $\mathbf{P_{triazole1,8}}$ (Fig. 8)] opposite each other led to thermal destabilization in the range of ΔT_m from –0.2 to –3.0 °C per pair of intercalators when compared to wild-type DNA.[39,40] Overlapping of pyrene residues in the interior of a DNA duplex, which has been shown by NMR for **INA**,[39] led to formation of a strong excimer band. Depending on the structure the λ_{em} values for excimer varied from 480 nm for **INA**[41] to 493 nm for $\mathbf{P_{1,8}}$,[40] and to 520 nm for $\mathbf{P_{triazole1,8}}$[42] and $\mathbf{P_{triple1,8}}$[43] [λ_{ex} =340-350 nm (**INA**, $\mathbf{P_{1,8}}$, $\mathbf{P_{triazole1,8}}$) and 385 nm for $\mathbf{P_{triple1,8}}$]. In recent articles homopairs of 1,8-pyrene ($\mathbf{P_{1,8}}$) were placed one, two, three and even seven times in the DNA helix.[44,45] A blue shift in the excimer band from 515 nm to 504 nm was observed with increasing numbers of pyrene pairs, which was also accompanied by a decrease in thermal stability from 70.5 °C for wild-type duplex to 56.5°C for seven pairs of $\mathbf{P_{1,8}}$.[45]

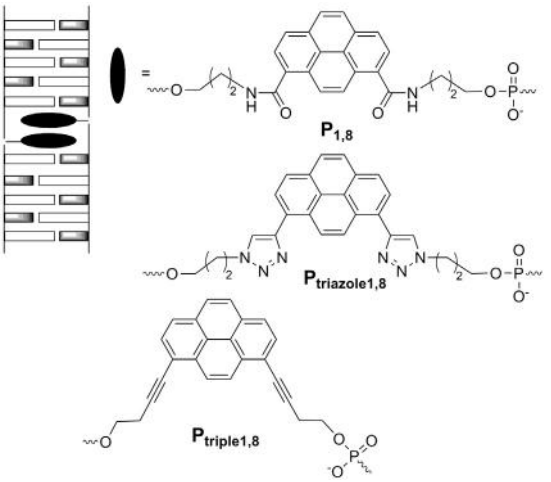

Fig. 8. Representation of a DNA duplex with hydrophobic aromatic moieties positioned opposite each other to form a pair.

Extensive research has been performed on the derivatization of nucleosides at the O2′-position. Depending on the linker length and its flexibility, it may be anticipated that a large chromophore spends a fraction of its time in the major/minor grooves and also partially moves between nucleobases (Fig. 9).

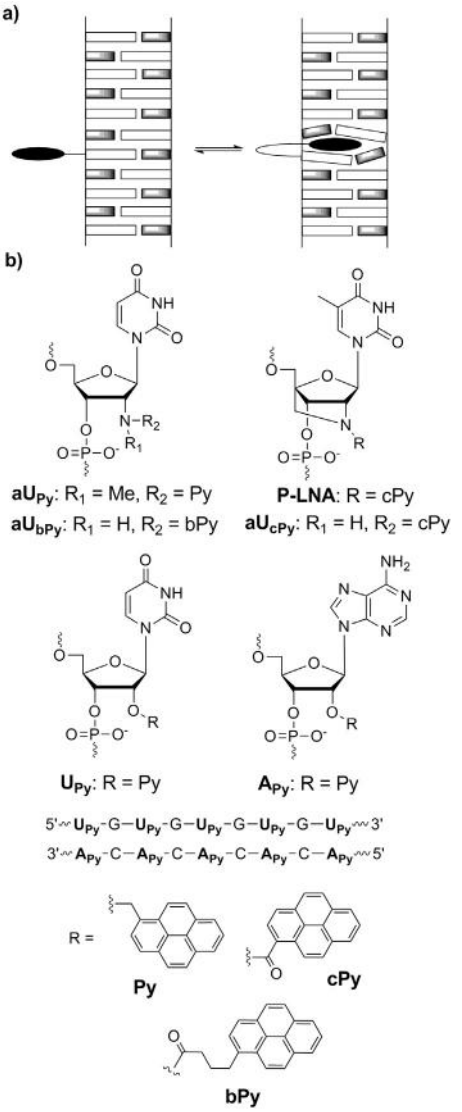

Fig. 9. a): Representation of equilibrium between the states when aromatic moiety positioned in DNA grooves (left) and when it intercalates (right); b): chromophores linked to the 2′-position of nucleotides.

It is believed that intercalators have a preference in binding to DNA rather than to RNA.[46] A substantial increase in the fluorescence signal was observed when probes possessing pyren-1-ylmethyl ($\mathbf{U_{Py}}$, Fig. 9) were bound to the complementary ssRNA, while marginal changes were observed for the complementary ssDNA.[47,48] Duplexes with complementary RNA were strongly destabilized relative to unmodified DNA/RNA duplexes. Such discrimination in fluorescence and thermal stability upon binding to complementary ssDNA and ssRNA is a result of different positioning of the chromophore in the interior of ssONs and duplexes. Pyrene fluorescence is quenched in ssONs and dsDNA because of interactions with neighbouring nucleobases, including intercalation.[49] However, pyrene attached to a duplex with RNAs is located outside of the duplex primarily in the minor groove, resulting in en-

hanced fluorescence. Sequential incorporation of 2-4 $\mathbf{U}_{Py}$ groups in the middle of RNA strands resulted in formation of an excimer band at 480 nm for the unhybridized probe (λ_{ex} = 350 nm).[50] A two- to ten-fold increase in the excimer fluorescence was observed upon formation of the RNA duplexes. The pyrene absorption band in the double-stranded form appeared at shorter wavelengths than that of the corresponding mono-pyrene labelled RNA duplex. A similar behaviour in fluorescence was observed for a pyrene zipper array along the RNA duplex by hybridization of two strands in which five $\mathbf{U}_{Py}$ groups formed a pair with five $\mathbf{A}_{Py}$ groups (Fig. 9).[51] CD spectra showed a considerable difference (a positive Cotton effect) for zipper arrays in the region of the pyrene absorption in comparison with the interrupted pyrene stack. These spectroscopic behaviours suggest that the pyrenes in the duplex form are located in the minor groove and are associated with each other as an H-aggregate that induces a hypsochromic shift of the absorption band. Disruption of the pyrene stack in the minor groove resulted in disappearance of the excimer band for duplexes.[50]

A different situation was observed for pyrene-modified N2′-uridines ($\mathbf{aU}_{Py}$ and $\mathbf{aU}_{bPy}$). Upon insertion of these analogues into DNA sequences a significant increase in T_m for the corresponding DNA duplexes, +15 and +3 °C, respectively, was observed, whereas duplexes with RNA were slightly destabilized.[52,53] Such a strong increase in T_m for pyrene analogues toward DNA is no doubt an intercalating effect. However, depending on the site of $\mathbf{aU}_{Py}$ insertion, probes showed different fluorescence properties. In some cases the monomer fluorescence of pyrene was quenched, in others only marginal changes were observed upon formation of complementary sequences. One possible explanation for these striking differences is that the pyrene moiety is only partially intercalating and is partially located in the minor groove. This situation was changed when a more rigid amide linkage was used. Thus, an increase in fluorescence was observed for DNAs having a single insertion of $\mathbf{aU}_{cPy}$ upon binding to complementary DNA.[52] Another valuable example in this series is 2′-amino-LNA molecules (**P-LNA**).[48] LNA or *l*ocked *n*ucleic *a*cids are known to induce large increases in thermal stability upon binding to RNA and DNA sequences, which is a result of the constrained 3′-*endo* configuration of the furanose ring.[54] During hybridization of DNA sequences comprising from one to three **P-LNA** monomers to either DNA or RNA complements, a large increase in thermal stability and fluorescence (up to 69-fold depending on the probe design) was detected. Molecular modeling of the resulting duplexes with **P-LNA** and $\mathbf{aU}_{cPy}$ suggested that the position of the pyrene is fixed in the minor groove.[48] Significant quenching of pyrene fluorescence in these single-stranded probes can be assigned to the interactions with nucleic bases.

Attachment of chromophores at non-hydrogen bonding positions of nucleic bases is a popular approach in the design of nucleic acids for analysis of singly matched/mismatched base pairs. Linker length and rigidity is critical in the design of these probes.

The fluorescence spectra of single stranded probe and mismatched duplexes possessing pyren-1-yl-modified 7-deazaadenine ($^{aPy}\mathbf{A}$, Fig. 10) showed strong fluorescence emission at 390 nm. The flexible propyl linker led to the pyrene intercalation into the duplex that resulted in the decreased fluorescence emission for the fully matched T: $^{aPy}\mathbf{A}$ duplex.[55] However, quenching of the fluorescence signal is not a desired property in the probe design, because a positive rather than a negative signal is required in major assays. In contrast to the $^{Py}\mathbf{A}$ molecule, use of the stiffer propargyl linker in the structures $^{Py}\mathbf{U}$, $^{Py}\mathbf{C}$ and C-C single and triple bonds ($^{P}\mathbf{U}$, $^{1EP}\mathbf{U}$, Fig. 10) led to strong fluorescence emission (λ_{em} = 397 nm for $^{Py}\mathbf{U}$ and $^{Py}\mathbf{C}$; λ_{em} = 455 nm for $^{1EP}\mathbf{U}$) with Φ_F values of 0.100-0.151 upon hybridization to the *matched* sequences, while a weak fluorescence was detected for the mismatched base pairs.[56] This strongly suggests that the position of the dye is fixed in the solvated major groove. Continuous stacking in the major groove of at least three pyrenes attached to uridine *via* single or triple C-C bonds (Fig. 10) led to drastic changes in UV/VIS and fluorescence spectra of fully matched duplexes relative to probes alone.[57,58] Fluorescent enhancement (*ca*. 22-fold) and a blue-shift in a maximum emission from 475 nm for ssDNA to 445 nm for the duplex were observed for five non-interrupted insertions of $^{P}\mathbf{Us}$ (Fig. 10) in the DNA strand. In contrast to that, a red-shift in emission spectrum was detected for five insertions of 1-ethynylpyrene uridine ($^{1EP}\mathbf{U}$, λ_{em} = 485-494 nm) in the duplex relative to the single insertion (λ_{em} = 445 nm).[57] For duplexes comprised of more than three $^{P}\mathbf{U}$ or $^{1EP}\mathbf{U}$ chromophores, distinct signals related to pyrene were observed in CD spectra that confirm a helical arrangement of pyrene in a major groove of DNA duplexes. This means that at least three chromophores in the intact DNA helix environment are required to obtain an ordered π-stacked array. Right-handed helical arrangement of PU and $^{1EP}\mathbf{U}$ chromophores could be destroyed by either thermal denaturation or by insertion of mismatches opposite to the pyrenes.

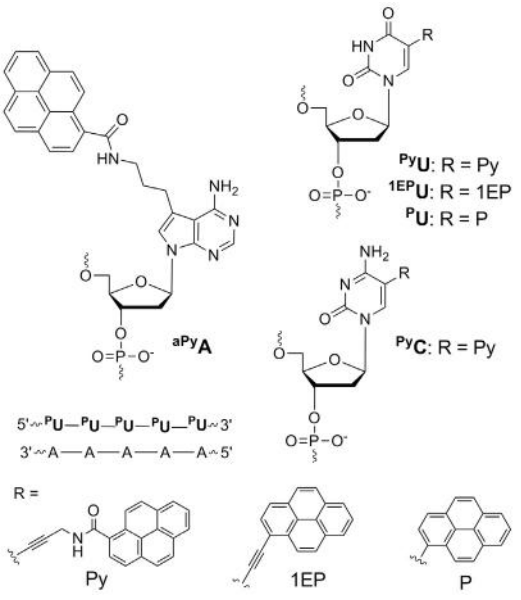

Fig. 10. Chromophores attached to nucleic bases at non-hydrogen bond forming sites.

The Future

Advances in the automated synthesis of DNA and post-synthetic oligonucleotide modifications led to the vigorous development of novel types of probes possessing organic chromophores. In the last decade there has been a considerable change in the design of probes. The predictable positioning of labels in the nucleic acid structure is vital to obtain the desired optical and binding properties. This goal is achieved by using short and rigid linkers that bring certainty in the chromophores' arrangements within the DNA/RNA helix. Those efforts described in this article lay a foundation for the generation of novel nucleic acid architectures which will have an enormous impact on the design of probes in molecular biology, biotechnology, and nanotechnology. Despite such an impressive development, a disadvantage is the use of aromatic molecules that are available on the market or easily modified synthetically. Their large size, photobleaching, quenching of fluorescence by surrounding nucleobases, formation of aggregates upon multiple insertions into DNA, overlapping of absorption or emission wavelengths with biomolecules are the main drawbacks of many organic chromophores. From that point of view, pyrene derivatives can be considered as extremely ineffective molecules. However, the efforts presented have already expanded our knowledge on stacking interactions within DNA and we have learned much about the properties of organic chromophores and their interactions with nucleic acid bases and themselves. It can be anticipated that this knowledge will be used in the construction of novel DNA architectures that will bring probe design to a conceptually new level. There is a considerable demand for novel chromophores with tunable wavelengths, high emission intensities and brightness, sensitivity to microenvironmental changes and insensitivity to surrounding nucleobases. For nanometre-sized materials availability of additional functional groups on organic chromophores would be of a high value for vigorous development of DNA entities as catalysts, nanomachines, nanorobotics, nanoelectronics, *etc*. In that regard, chemically modified pyrene, perylene, acridine and coumarine entities that have improved optical properties would be extremely valuable. Dramatic development of DNA-based approaches involving the use of nanoparticles and quantum dots in combination with fluorescent molecules can be expected.[59] This will expand areas of application and advance labelling techniques by taking the advantages of different classes of compounds. Post-synthetic labelling procedures, some of them recently highlighted,[60] will play a significant role in the synthesis, and more importantly, in the screening of DNA-conjugated organic chromophores. It will allow a rapid discovery of probes showing discriminative recognition of certain nucleic acid secondary structures with tunable luminescence properties. Moreover, it seems to pave the way for novel multichromophore architectures that will be of use in the development of photoactive materials and photonic nanodevices.

Acknowledgments

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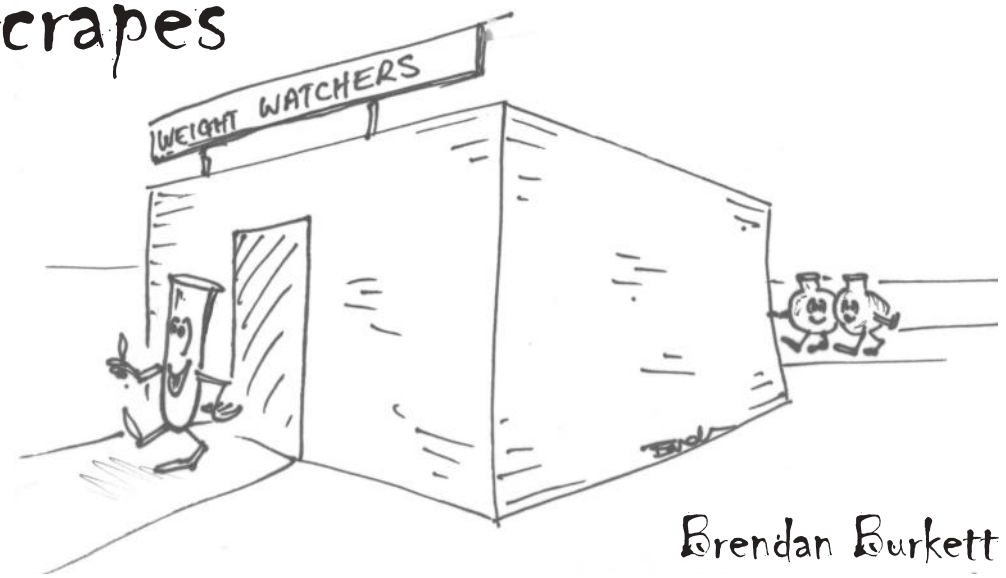
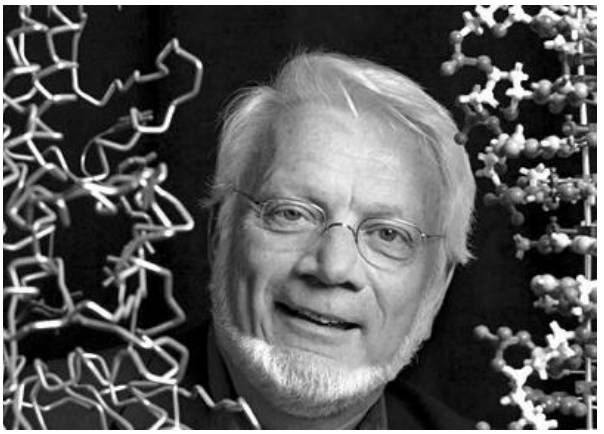


Brendan Burkett

Article

The 2009 Nobel Prize in Chemistry


L-R: Ada Yonath (courtesy of the Weizmann Institute - photographer Dan Porges), Thomas Seitz, and Venkatraman Ramakrishnan

The Royal Swedish Academy of Sciences awarded the 2009 Nobel Prize in Chemistry *for studies of the structure and function of the ribosome*. It went jointly to **Ada E. Yonath** (Weizmann Institute of Science, Israel), **Thomas A. Steitz** (Yale University, USA) and **Venkatraman Ramakrishnan** (MRC Laboratory of Molecular Biology Cambridge, UK),

The prize recognizes the work of these three scientists into life's core process - the ribosome's translation of DNA information into life and acknowledges their detailed mapping of the ribosome, the cell's own protein factory and one of the cell's most complex machines, at the atomic level. In essence, the ribosome reads the information in messenger RNA, and based upon that information, it produces protein.

Introduction

An understanding of the ribosome's innermost workings is important for a scientific understanding of life. This knowledge can be put to a practical and immediate use. Thus, many of today's antibiotics cure various diseases by blocking the function of bacterial ribosomes. Without functional ribosomes, bacteria cannot survive. This is why ribosomes are such an important target for new antibiotics.

The general theory of evolution, published by Charles Darwin in 1859, is based on the assumption that an organism's properties are hereditary and that, every now and then, random changes occur. Successful changes, which increase the chances of survival of the organism in question, are thus carried forward to future generations. When the scientific community had digested Darwin's thoughts, new questions arose, such as: *What exactly is being transferred over generations?; Where do the random changes occur?; How can they manifest themselves in a living organism?* The 2009 Nobel Prize in Chemistry is the third in a series of prizes that show how Darwin's theories actually function at the atomic level. Images, generated by X-ray techniques, show how the simple DNA code can manifest itself not only as hearing, feeling and taste, or muscles, bone and skin, but also as thoughts and speech. The trilogy of prizes began in 1962, when Francis Watson, James Crick, and New Zealander Maurice Wilkins were recognised for their elaboration of an atomic model of double-stranded DNA. The second prize in the trilogy was awarded Roger Kornberg in 2006 to for X-ray structures that explicate how information is copied to the messenger RNA molecule.

The ribosome translates genetic information into action by reading the information in messenger RNA and, based upon that information, produces protein in a process termed *translation*. It is during this translation process that the DNA/RNA language becomes protein language and life reaches its full complexity. The body contains tens of thousands of different proteins that control structure and function with astounding precision. Examples include haemoglobin (which transports oxygen from the lungs to the rest of the body), insulin, (which controls the sugar level in the blood), antibodies (that capture intruding viruses), and keratin (which builds hair and nails). Ribosomes exist in all cells in all living organisms, from bacteria to human beings. As no living creature can survive without ribosomes, they are the perfect targets for drugs. Many of today's antibiotics attack the ribosomes of bacteria, but leave those of humans alone. Thus, the knowledge that the 2009 Nobel Laureates have provided can be of substantial value for the development of new antibiotics.

The Structure and Function of the Ribosome

The Ribosome and the Central Dogma

The genetic information in living systems is stored in the genome sequences of their deoxyribonucleic acid (DNA) (Fig. 1). A large part of these sequences encode proteins

which carry out most of the functional tasks in all organisms still in existence. The DNA information is made available by *transcription* of the genes to messenger ribonucleic acids (mRNAs) that subsequently are *translated* into the various amino acid sequences of all the proteins of an organism. This is the central dogma[1] of molecular biology in its simplest form (Fig. 2).

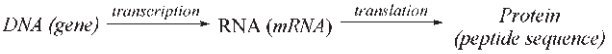


Fig. 2. The central dogma.

The genetic information in DNA is preserved by *replication* of the genome[2] carried out by DNA polymerase[3] so that each daughter cell can receive one genome copy at every cell division. In all organisms, transcription of DNA into mRNA is carried out by RNA polymerase,[4] and translation of mRNA is carried out by the ribosome. Each mRNA sequence consists of ribonucleotides with either one of four bases: **A** (adenine), **C** (cytosine), **G** (guanine) and **U** (uracil). Each amino acid is encoded by one or several triplets of bases known as codons, *e.g.* UUU or UUC for the amino acid phenylanine; termination of translation by the triplets UAG, UAA or UGA; and initiation of translation mainly by AUG, also encoding the amino acid methionine.[5] The mRNA sequence is decoded starting from an AUG codon, followed by a sequence of codons that specify the order of insertion of amino acids in the nascent protein. This is followed by a termination codon, which signals that the protein is ready for dissociation from the ribosome for subsequent folding into its functional state.

Components of the Ribosome

The bacterial (70S) ribosome consists of a small (30S) and a large (50S) subunit, with molecular weights of about 8 x 10^5 and 15 x 10^5 Da, respectively. The small subunit consists of about 20 different proteins and a sequence of ribosomal RNA (rRNA) containing about 1600 nucleotides. The large subunit consists of about 33 different proteins and rRNA sequences with about 2900 and 120 nucleotides each. Ribosomes from eukaryotes are larger and more complex than those from prokaryotes but, from everything we know, ribosomes from all three kingdoms of life function according to exactly the same principles. The ribosome has three binding sites for tRNA, the **A** (aminoacyl) site, the **P** (peptidyl) site and the **E** (exit) site, formed in the inter subunit interface.

Fig. 1. DNA carrying adenine (A), cytosine (C), guanine (G) and thymine (T). A connects to T and C to G. The genetic code is contained within the nucleotide sequences on each of the strands. Copyright The Royal Swedish Academy of Sciences and used with permission.

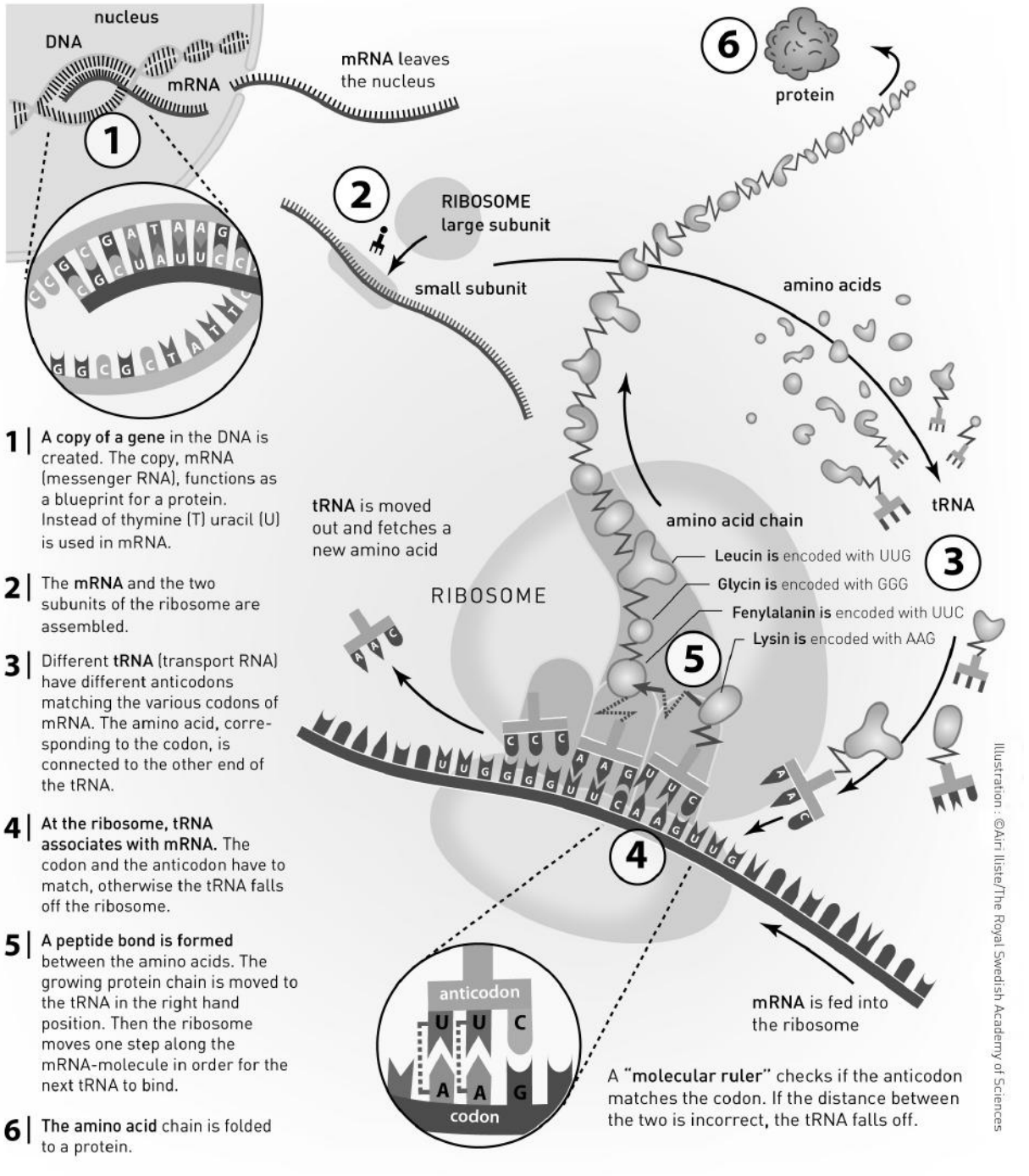


Fig. 3. From DNA to proteins. Copyright The Royal Swedish Academy of Sciences and used with permission.

Initiation of Protein Synthesis

Protein synthesis (Fig. 3) starts in bacteria when the mRNA binds to the ribosomal small subunit (Fig. 4). This event is followed by binding of the initiator tRNA, charged with formylated methione, to the P site in a reaction step greatly accelerated by three initiation factors IF1 - IF3. When mRNA and initiator tRNA are in place, the large subunit docks to the smaller pre-initiation complex in an initiation factor-aided reaction. Ribosomal ribosome is formed[6] with the mRNA in the correct reading frame, initiator tRNA in the P site, and the empty A site programmed with the first internal codon of the protein to be synthesized. The ribosome has now left the initiation phase and entered the peptide elongation phase that places a peptidyl-tRNA in the A site. The tRNA and nascent peptide chain are elongated by one amino acid and a deacylated tRNA is in the P site. This pre-translocation state proceeds through a major elongation step and the A site becomes programmed with the next codon to be read by an aminoacyl-tRNA.[7] Peptide elongation is repeated until a stop codon appears in the A site. Stop codons induce hydrolysis of the ester bond that links the finished protein chain with the P-site-bound tRNA. This leads to rapid release of the protein and its subsequent folding into the functional form.[8,9] Subsequently, the ribosome is recycled into a new round of initiation with a new mRNA.[10]

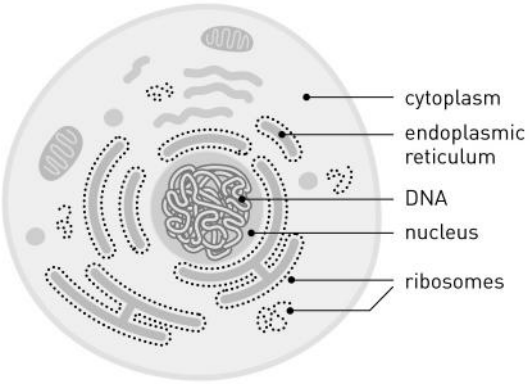


Fig. 4. Cross section of a cell. A ribosome is *ca*. 25 nm in size and a cell contains tens of thousands of them. Copyright The Royal Swedish Academy of Sciences and used with permission.

Cryo-electron microscopy (cryo-EM) has provided views of the ribosome bound to the ternary complex and the ribosome with peptidyl-tRNA in the A site and deacylated tRNAs in the P and E sites.[11]

Long Standing Mysteries in Ribosome Function

The ribosome catalyzes peptide bond formation and ester bond hydrolysis during termination. Problems exist relating to the delicate accuracy during protein elongation and termination. In the elongation phase an aminoacyl-tRNA cognate to an amino acid-encoding A-site codon (sense codon) must be selected efficiently and, at the same time, all near-cognate aminoacyl-tRNAs (or class-1 release factors) must be rejected with very high probability of avoiding amino acid substitution or premature termination errors. The first sort of errors would lead to reduced or altered activity of the synthesized proteins and the second to a greatly reduced ability of the ribosome to produce ready made proteins, *i.e*. to a large reduction of the ribosome's processivity.

The chemical mechanisms of the covalent reaction steps carried out by the ribosome remained mysterious, despite decades of intense work on the bacterial ribosome despite study. Just how tRNAs and class-1 release factors discriminate so precisely between their cognate and near-cognate codons in a ribosome-dependent manner was a question that remained unanswered. Finally, the way in which antibiotic drugs and ribosomal mutations tune the accuracy of codon reading up or down have remained obscure. The clarification of these and other central questions concerning normal ribosome function and how ribosome function is perturbed by the action of antibiotic drugs or mutations depended on the advent of crystal structures of ribosomal subunits at high resolution, the whole ribosome, and important functional complexes of the ribosome, its subunits and, finally, of the bacterial ribosome itself.

The Path to High Resolution Crystal Structures of Ribosomal Subunits

The Early Stage of Ribosome Crystallography

The ribosome, with its molecular weight of about 2.5 MDa is not only large but, unlike many virus particles, does not display symmetry properties that would facilitate crystallization and structure determination. As at 1980 it was not clear whether crystals of the ribosome diffracting to high resolution (~3 Å or less) would ever be found and, if such crystals did exist, whether the phase problem could be overcome and structures obtained. Thus, the report on 3-D crystals of the ribosomal large subunit from the thermophile bacterium *Geobacillus* (*G*.) *stearothermophilus* in 1980 by Ada Yonath and colleagues[12] marked a significant step forward. The first crystal structures of the large (50S) subunit to give crystallographic information were subsequently obtained by her group for *G. stearothermophilus*[13] and the archaeon *Haloarcola* (*H*.) *marismortui*[14] followed by crystals from the same organism diffracting to 6 Å.[15] Crystals of the 70S ribosome and its isolated small (30S) subunit for *Thermus* (*T*.) *thermophilus* were reported by Trakhanov *et al*.,[16] and for the 30S subunit from the same organism by Yonath and collaborators.[17] These early crystals diffracted only to about 10 Å and, in principle, could never lead to structures with a resolution allowing for construction of a detailed atomic model. Yonath's group[18] then showed that carefully prepared crystals from the large (50S) subunit from *H. marismortui* diffracted to 3 Å in 1991, another major step towards ribosome structures at high resolution. The ultimate success in this quest had to depend upon improved quality of ribosomal crystals obtained by, *e.g*. application of cryo-crystallography (to minimize radiation damage) as pioneered by Yonath and her collaborators. The introduction of charge-coupled device (CCD) area-detectors for precise and automated analysis of X-ray diffraction patterns and tuneable synchrotron radiation sources for optimal use of anomalous scattering for phase determination also made ribosome crystallography more feasible (Fig. 5).

Thus, Ada Yonath's work throughout the 1980s was instrumental in obtaining the robust and well diffracting ribosome crystals that eventually led to high resolution structures of the two ribosomal subunits. This took another ten years of work, with the main players including Thomas Steitz and his collaborators from Yale University, and Venkatraman Ramakrishnan with his collaborators at the MRC laboratory in Cambridge (UK).

The Ribosome and its Subunits at High Resolution

Despite Yonath's significant contributions to gaining a high resolution structure of the large (50S) ribosomal subunit from her crystals of *H. marismortui* (beyond 3 Å resolution)[12,14,17] it was Steitz and collaborators who solved the profoundly challenging phase problem of this structure. Moreover, since the phase problem had not been solved for the small (30S) subunit at that time, it required a decisive breakthrough in ribosomal crystallography. Initially, the Seitz group used a cryo-EM reconstruction of the ribosomal 50S subunit from Frank[19] along with multiple isomorphous replacement and anomalous scattering techniques; this gave a low resolution structure.[20] The reconstruction, at 9 Å resolution, displayed right-handed double helical density typical of A-form RNA. It demonstrated for the first time that the phase problem was tractable for ribosomal subunits and even the whole 70S

ribosome, and it implied that high resolution structures of ribosomal subunits - and the whole ribosome - were within reach. One year later, Steitz *et al*. reported[21] a mid-resolution structure (5 Å). Later the same year a 5.5 Å resolution structure of the 30S subunit (*T. thermophilus*) came from Ramakrishnan and collaborators[22] and then a 4.5 Å resolution structure of the *T. thermophilus* 30S subunit from Yonath.[23] In the same year, Noller's group reported the structure of the 70S ribosome from *T. thermophilus* at 7.8 Å resolution. It contained tRNAs in the ribosomal A, P and E sites and an mRNA in the track around the neck of the 30S subunit.[24] Neither one of these structures displayed resolution high enough to construct complete atomic models, but they did provide the necessary stepping stones on the path to the high resolution structures that rapidly followed.

In 2000, the Steitz group reported the 50S structure from *H. marismortui* at 2.4 Å resolution,[25] while Ramakrishnan reported the 30S structure from *T. thermophilus* at 3.0 Å,[26] and Yonath the structure from the same subunit at 3.3 Å resolution.[27] The two 30S structures were very similar, but with some differences at the atomic level. These discrepancies were removed by a subsequent structure reported from Yonath's laboratory at 3.2 Å resolution.[28] In 2001, she obtained a high resolution structure of the 50S subunit from the Gram positive bacterium *Deinococcus* (*D*.) *radiodurans*,[29] particularly suitable for studies of antibiotics targeting the bacterial ribosome.

In 2001, Noller and collaborators reported the crystal structure of the 70S ribosome from *T. thermophilus* at 5.5 Å resolution.[30] However, the structure of the whole 70S particle at high resolution (3.5 Å) was not obtained until 2005 from the work of Cate and collaborators for an empty ribosome from *Escherichia* (*E*.) *coli*.[31]

With the structures of the two ribosomal subunits at high resolution, it was clear that a radical change in the boundary conditions of ribosome research had occurred. One finding that initially gained considerable attention was that the peptidyl-transferase centre (where peptide bond formation is catalyzed) seemed to lack ribosomal protein components. In fact, there was no visible peptide chain within 18 Å of the identified peptidyl-transferase centre.[25] This was taken by many as the ultimate proof of previous suggestions that the ribosome is a ribozyme,[32] *i.e.* an enzyme deriving its catalytic power from RNA and not from protein. This result had been anticipated, not least by the support it gave to the view that the present biochemical world, in which proteins carry out the vast majority of biochemical functions, has been preceded by an *RNA world*, where RNA was not only an information carrier, but also performed the functional tasks.

The structures of the two ribosomal subunits rapidly provided a wealth of new insights in the structural folds of RNA and the binding properties of antibiotics. However, the answers to fundamental questions concerning the accuracy of tRNA selection during protein elongation, and the mechanism of peptidyl-transfer, required more crystallographic work in combination with biochemistry and computational approaches. Much of this has come from the work of Ramakrishnan and his collaborators who by using a range of 30S structures at high resolution have provided a simple and coherent explanation for a number of essential, but hitherto poorly understood, phenomena related to the accuracy of codon reading during mRNA translation.

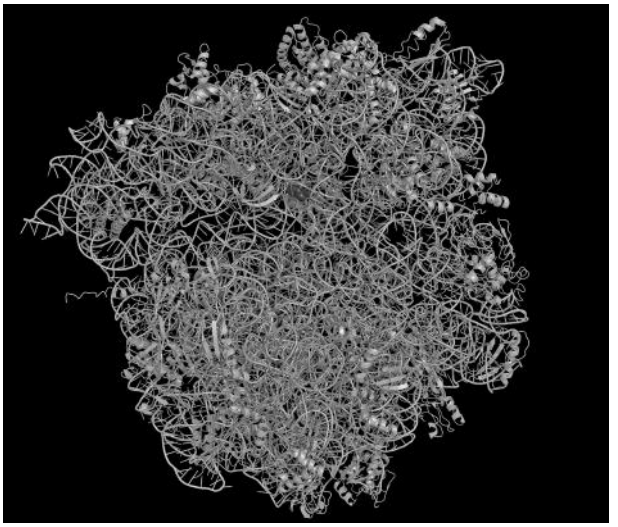


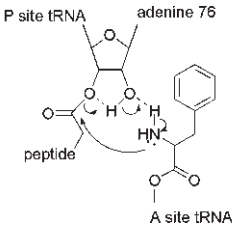
Fig. 5. X-ray structure of a bacterium ribosome. Copyright The Royal Swedish Academy of Sciences and used with permission.

Peptide Bond Formation and the 50S Subunit Structure

The high resolution structure of the 50S subunit from *H. marismortui*[25] was expected to solve the mechanistic principles of ribosome-catalyzed peptide bond formation by transfer of the nascent peptide from the P-site peptidyl-tRNA to the A-site aminoacyl-tRNA. The first decisive steps here were provided by the two structures of the 50S subunit.[25] Subsequently, Steitz and collaborators[33] reported on new 50S subunit structures with novel analogues and further refinements of previous structures. In this work, they emphasized that the peptidyl-transfer centre must carefully juxtapose the two substrates in peptide bond formation, but the mechanistic principles of catalysis remained elusive. Nonetheless, the crystal complexes reported by Seitz and his co-workers[25,33] clearly defined the structural *boundary conditions* for peptidyl-transfer, and demanded that any proposed mechanism needed to be compatible with the important features of these crystal structures.

In 2004, Wolfenden, Rodnina and collaborators made an important contribution to understanding this catalytic mechanism[34] and, in 2005, molecular computation methods (based on the previously published structures of the 50S subunits by Steitz and collaborators[25,33]) were used to formulate a mechanistic model for peptidyl-transfer.[35] Trobro and Åqvist predicted a network of hydrogen bonds, pre-organized in the ground state of the peptidyl-transfer reaction and persisting through the transition state of peptide-bond formation. This then explained why peptide bond formation on the ribosome is entropy- and not enthalpy-driven, as experimentally demonstrated. According to the proposed mechanism, the 2′-OH is part of a proton-shuttling pathway[36] that removes the excess

proton formed in the attack of the amino group of the A-site aminoacyl-tRNA on the ester bond of the P-site tRNA (Scheme 1).



Scheme 1. Peptide bond formation on the ribosome triggered by the α-amino group of aminoacyl-tRNA in the A site attacking the ester of the peptidyl-tRNA

In the same year, Steitz and collaborators provided a new series of complexes of the 50S subunit with improved resolution (~2.5 Å) of details in the peptidyl-transfer centre.[37] This was the crystallographic *tour de force* that validated the proton-shuttling role of the 2′-OH of A76 in the P-site bound peptidyl-tRNA along with the network of H-bonds involving 23S rRNA bases and water molecules.

Thus, it was the 50S subunit structures from Steitz and collaborators, with the 2005 *Molecular Cell* publication[37] as the jewel in the crown that clarified how ribosomes catalyze peptide bond formation.

Ribosomal Subunit Structures and Antibiotics

The years after WWII saw widespread use of antibiotics to treat bacterial infections, revolutionizing medicine and dramatically improving health globally. However, evolving antibiotic resistance among pathogens has heavily depleted the arsenal of effective antibiotic drugs.

The past few years have seen structure-based drug design (SBDD) come to the fore. It uses high resolution structures of drug targets and their resistance mutants, and has provided some novel drugs and scored some promising successes, *e.g.* in the quest against HIV-virus infections. The ribosome is the target for about a half of all antibacterial drugs to date. Moreover, availability of the high resolution structures of both of the ribosomal subunits has opened a large number of possibilities for SBDD of new and effective drugs in the race against resistance development among bacterial pathogens. For example, many different types of antibiotic drugs bind to the peptidyl-transfer centre of the large subunit of the bacterial ribosome, and the binding modes of a large number of antibiotic drugs to both subunits have now been revealed at high resolution. These serve as leads for the design of novel drugs yet to come.

The New Generation of Functional Crystal Complexes of the Ribosome

Termination of Protein Synthesis: Reading of mRNA Stop Codons by Proteins

The termination of protein synthesis (by hydrolysis of the ester bond connecting the finished protein to the tRNA in the P site) posed a number of fundamental questions that have remained unsolved for decades. Among these are: *How can the codon-reading proteins (RF1 and RF2) efficiently recognize the three stop (non-sense) codons and, at the same time and in the absence of proofreading precisely discriminate against premature termination at any of the 61 sense codons?; How do RF1 and RF2 induce ester bond hydrolysis in the P-site peptidyl-tRNA during termination?;* and in particular: *What role is played by the universally conserved GGQ motif in class-1 release factors?*

Neither low-resolution cryo-EM nor mid-resolution crystallography could answer these questions. Only with the report from Noller's group of the high resolution (3.1 Å) structure of the *T. thermophilus* ribosome in a termination complex with RF1 did the situation change.[38] Soon after, the Ramakrishnan and Noller groups reported[39] high resolution structures of the *T. thermophilus* ribosome in termination complex with RF2. These structures have led to a revision of previous suggestions that the RFs have anticodon-like peptide loops in analogy with tRNA anticodons that read the stop codons. They also provided the keys to quantitative, atomic level understanding of all aspects of stop codon reading and the involvement of the universally conserved GGQ-loop in inducing ester bond hydrolysis in the last peptidyl-tRNA during the synthesis of a protein.

The work continues and a high resolution (2.8 Å) structure of LepA has now been reported from the Steitz laboratory, clarifying aspects of the factor's reverse translocation activity.[40]

A high resolution (2.8 Å) crystal structure of the 70S ribosome from *T. Thermophilus* in pre-translocation state[41] has led to models of the tRNA and mRNA structures in the ribosome and how they interact with the ribosome. Even more recently, Ramakrishnan and collaborators reported high resolution crystal structures of two ribosomal complexes from *T. thermophilus*,[42] that showed extended peptide sequences of ribosomal proteins L27 and L16 of the 50S subunit stabilizing the CCA-ends of both tRNAs in the peptidyl-transfer reaction.

Conclusion

Ramakrishnan, Steitz and Yonath have made groundbreaking contributions to the crystallography of ribosomes by using high-resolution functional ribosome complexes to clarify long-standing and fundamental questions in protein synthesis. Their work has far-reaching implications for basic science and medicine.

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Compiled by Brian Halton, School of Chemical & Physical Sciences, Victoria University, PO Box 600, Wellington (e-mail: *brian.halton@vuw.ac.nz*) from material made available at the Nobel Foundation and The Royal Swedish Academy of Sciences web sites: *www.Nobel.se* and *http://kva.se*, respectively (accessed 20 Nov 2009). The Royal Swedish Academy is thanked for making the figures available.

Conference Calendar

International Conference On Nanoscience and Nanotechnology (ICONN 2010), Chennai, India, Asia, 24-26 February 2010

www.iconn2010.com/

Faraday Discussion 146: Wetting Dynamics of Hydrophobic and Structured Surfaces, Virginia, USA, 12-14 April 2010

www.rsc.org/ConferencesAndEvents/RSCConferences/FD146/index.asp

Third International Conference on Semiconductor Photochemistry, Strathclyde, United Kingdom, Europe, 12-16 April 2010

The aim of the conference is to cover recent developments in the area of semiconductor photochemistry.

www.sp3conference.com/

Frontiers of Chemistry: from Molecules to Systems: Celebrating 10 Years of ChemBioChem and ChemPhysChem, Paris, France, Europe, 21 May 2010

www.ldorganisation.com/produits.php?langue=english&cle_menus=1238915319&cle_data=1238740745

Faraday Discussion 147: Chemistry of the Planets, Saint Jacut de la Mer, Brittany, France, 14-16 June 2010

www.rsc.org/ConferencesAndEvents/RSCConferences/FD147/index.asp

Faraday Discussion 148: Spectroscopy, Theory and Mechanism in Bioinorganic Chemistry, University of Nottingham, United Kingdom, 5-7 July 2010

www.rsc.org/ConferencesAndEvents/RSCConferences/FD148/index.asp

The International Symposia on Advancing the Chemical Sciences (ISACS) is a significant new global symposia series organised by the RSC. During 2010, the first three symposia will be held on three continents, over three sequential weeks, focusing on distinct subject areas as follows:

Challenges in Organic Chemistry and Chemical Biology (ISACS1), San Francisco, USA, 6-9 July 2010

www.rsc.org/ConferencesAndEvents/ISACS/OrganicChemistryandChemicalBiology/index.asp

Challenges in Physical Chemistry and Nanoscience

(ISACS2), Budapest, Hungary, 13-16 July 2010

www.rsc.org/ConferencesAndEvents/ISACS/PhysicalChemistryandNanoscience/Index.asp

Challenges in Inorganic and Materials Chemistry (ISACS3), Hong Kong, China, 20-23 July 2010

www.rsc.org/ConferencesAndEvents/ISACS/InorganicandMaterialsChemistry/index.asp

43rd IUPAC World Polymer Congress, 'Macro2010',Glasgow UK, 11-16 July 2010

www.rsc.org/ConferencesAndEvents/RSCConferences/Macro2010/

ICCC39, 39th International Conference on Coordination Chemistry, Adelaide, Australia, 25-30 July 2010.

ICCC39 will encompass all aspects of coordination chemistry through plenary, keynote and section lectures and poster presentations.

http://iccc2010.eventplanners.com.au/

Analytical Research Forum 2010, Loughborough University, United Kingdom, 26-28 July 2010

www.rsc.org/ConferencesAndEvents/RSCConferences/ARF10/index.asp

Dalton Discussion 12: Catalytic C-H and C-X Bond Activation, Durham University, United Kingdom, 13-15 September 2010

www.rsc.org/ConferencesAndEvents/RSCConferences/DD12/index.asp

Grants and Scholarships

2010 Zonta Science Award Call for Applications

Applications are being called for the 2010 Zonta Science Award for women scientists. Applications must be received by 1 February 2010.

Wendy Saunders, email *w.saunders@gns.cri.nz*

Rutherford Foundation Freemasons Post Doctoral Fellowship 2010, University of Edinburgh

The 2010 award is for post-doctoral study at the University of Edinburgh, Scotland, in any area of ageing related research. The deadline for applications is Monday 22nd February 2010.

www.royalsociety.org.nz/Site/rutherford/postdoc.aspx

RSNZ International Conference Fund

It is funding to assist organisations and institutions to host major international conferences in New Zealand. No closing date for applications.

www.royalsociety.org.nz/site/funding/int_conf/

The Newton International Fellowship Scheme

The Scheme has been established to select the very best early stage post-doctoral researchers from all over the world and enable them to work at UK research institutions for a period of two years.

Deadline 8 February 2010

http://royalsociety.org/Newton-International-Fellowships/

RSNZ Travel Grants

This provides $1000 to assist students undertaking PhD degrees at New Zealand universities to attend their first overseas scientific conference (this includes summer schools or workshops).

Deadline: 1 March 2010

http://www.royalsociety.org.nz/Site/International/travel/

Conference Assistance Programme

Can provide assistance for bidding, in order to host an international conference in New Zealand. This can include financial feasibility study for conference, assistance with air travel, help in writing bid documents etc.

www.conventionsnz.com/default.aspx

RSNZ New Zealand-Japan Scientist Exchange Programmes

Postdoctoral Fellowship Programme

Provides opportunities for young postdoctoral researchers from New Zealand to conduct co-operative research with leading research groups in Japan.

www.royalsociety.org.nz/Site/international/jsps/

Marsden Fund

Preliminary proposals due 4 February 2010

http://marsden.rsnz.org/info/

Foundation of Research, Science and Technology

Have a number of on-demand schemes that provides funding to enable businesses to develop new research and development projects.

www.frst.govt.nz/investframe/process/ondemand

Te Tipu Putaiao Fellowships

For students completing masters, doctorate or postdoctoral work in a science, engineering or technology discipline. Funding depends on qualifications and ranges from $10,000 to $61,000 per annum.

http://www.frst.govt.nz/funding/students/TTP

Shirtcliffe Fellowship

This fellowship is to assist graduate students of outstanding ability and character to continue their studies. Fellowship is for up to 3 years at $5000 per annum.

www.nzvcc.ac.nz/scholarships/shirtcliffe

Vernon Willey Trust Fellowship

For research and education relating to the production, processing and marketing of wool, general development and improvement of the sheep and wool industry.

Deadline 15 March each year.

www.meatandwoolnz.com/main.cfm?id=337

Maori Education Trust Scholarships

The website lists 5 scholarships available one of $7000 and four of $5000 at postgraduate level.

Deadline 2 April 2010

www.maorieducation.org.nz/sch/post_grad.html

Chemistry Success Stories from the Marsden Fund 2009

The Marsden Fund supports research excellence in science, technology, engineering and mathematics, social sciences and the humanities. Its budget rose from $37.88 million to $46.88 million per year (exclusive of GST) in 2009.

A total of 934 preliminary proposals were submitted to the Marsden Fund 2009. Of these 675 were standard proposals and 259 were fast-start proposals. 215 applicants were invited to submit full proposals, which included 155 standard proposals and 60 fast-starts. 109 of these proposals were finally funded. This represents a success rate of 12%. These and other data are published on the Marsden website *http://marsden.rsnz.org/*

The Physical Sciences and Engineering (PSE) panel received 36 Fast-track pre-proposals and 9 of these were invited to the full proposal stage. In addition it received 105 standard pre-proposals and 20 of these reached full proposal stage.

39 Chemistry proposals were submitted to the preliminary round which represented 9 Fast-Start proposals and 30 Standard proposals. 12 Chemistry proposals were invited to full proposal stage and comprised 4 Fast-Start and 8 standard proposals. 4 standard contracts and 2 fast-track contracts were subsequently signed.

Thanks go to Dr Dean Peterson of RSNZ for help with the Chemistry proposal statistics. Congratulations and many thanks go to the researchers who provided information for this article.

Chemistry projects funded this year include the following:

1. Assoc. Prof. Paul E. Kruger, University of Canterbury.

www.chem.canterbury.ac.nz/people/kruger.shtml

Project title: *Spin crossover driven molecular switches triggered by external stimuli: toward spin-switching chemosensors*

The four iron centres in haemoglobin (Hb) in blood allow Hb to bind four molecules of oxygen and transport it around our body. Binding of oxygen is co-operative, as each iron centre communicates with one another. The basis of cooperation is the movement of the iron atom upon oxygen binding and results from a spin-crossover (SCO), *i.e.* the movement (switching) of electrons in the iron centre. This is a highly efficient and reversible function, and chemo-selective for oxygen.

Synthetic iron complexes can similarly take advantage of SCO to produce molecular switches. SCO may be induced by variation in temperature, pressure or light, or by the presence of other molecules. SCO in Fe(II) is accompanied by dramatic changes in colour, magnetism and structure, which signals the switching event and makes it suitable for device applications. Taking inspiration from Hb, in this Marsden Fund supported project we will synthesise molecular systems capable of binding guest molecules to invoke SCO to signal the binding event. The preparation of a switching device will result and we can envisage many potential applications for such a device in such diverse areas as molecule or ion detection, diagnostics, temperature or guest induced magnetic switches and in the realisation of multi-channel spin-switching chemosensors (see Fig. 1).

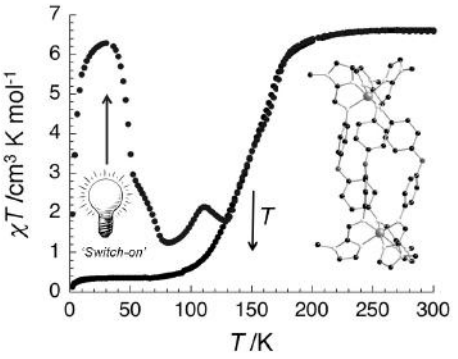


Fig 1. Molecular structure and (photo-)magnetic behaviour of $[Fe_2(L_O1)_3](ClO_4)_4$ with temperature.

2. Assoc. Prof. Antony Fairbanks, University of Canterbury.

www.chem.canterbury.ac.nz/people/fairbanks.shtml

Project title: *Glycosylation with endohexosaminidases:- access to defined homogenous glycoproteins*

Typically one may think that the biological roles of carbohydrates (sugars) are limited to acting either as sources of energy, *e.g.* glucose, or as structural materials, *e.g.* cellulose. However, much of the complexity of biological systems is actually achieved by the addition of carbohydrates to other biomolecules, such as proteins, peptides and lipids. The mechanisms by which sugars are added to proteins in living cells are complex, and many competing processes operate simultaneously, resulting in the formation of inseparable mixtures of different materials. This means that proteins with carbohydrates attached to them (glycoproteins) are unavailable in homogeneous form,

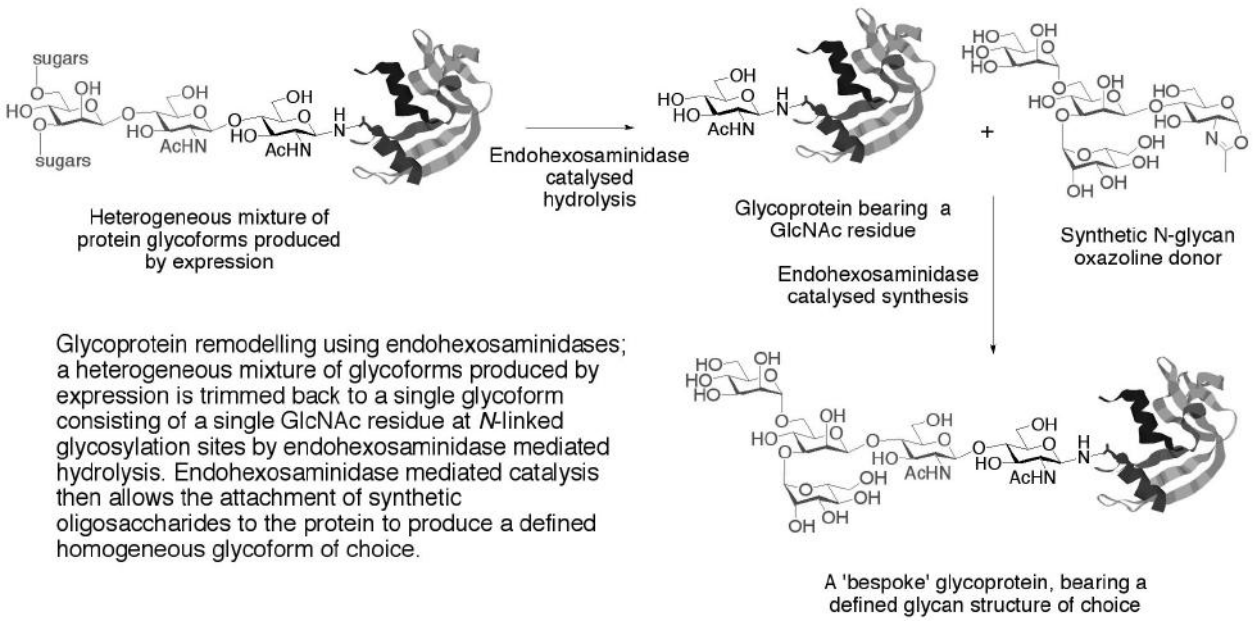


Fig. 2. Glycoprotein remodelling using endohexosaminidases.

impairing studies into the effects that attached sugars have on protein function. Furthermore current glycoprotein therapeutics, such as monoclonal antibodies (mAbs, *e.g.* Herceptin) or other therapeutic proteins, *e.g.* Erythropoietin, EPO, are necessarily administered as mixtures of compounds, a major proportion of which may have very low, or even undesired, biological activity. This project aims to develop synthetic methodology that will allow the production of bespoke homogenous glycoproteins. The approach combines the broad-reaching power of chemical synthesis with the exquisite selectivity achievable using biocatalysis. This enabling technology will facilitate the production of next generation glycoprotein therapeutics in defined homogeneous and optimised form (see Fig 2).

3. Dr Duncan McGillivray, University of Auckland and Prof Michael James, Australian Nuclear Science and Technology Organisation, Bragg Institute, AI

www.che.auckland.ac.nz/

Project title: *Probing the effects of oxidative stress on cellular membrane interactions*

Powerful methods of neutron scattering from model cell membranes will shed light on the effects of oxidative stress on membrane structure and function – especially membrane interactions with proteins and other important biological molecules – to help us understand how this damage leads to disease. Cell membranes are the structural boundaries of cells, and are also their primary gatekeepers. Membrane-protein based mechanisms regulate the transport of material into and out of the cell to maintain the chemical balance that is essential to life. They are also responsible for mediating cell interactions with functional molecules outside the cell.

When these complex and important biomembranes are exposed to oxidizing conditions, such as caused by radiation (including UV light) or free radicals, they can be stressed and damaged. This has been linked to many ageing-related problems, including cardiovascular, Parkinson's and Alzheimer's diseases. However, little is known about how the chemical changes in the membrane affect its function and bring about these failures. This research addresses this by looking at the behaviour of model membrane systems as they are stressed, and it will also help us understand how natural biological membranes respond to defend against these stresses (see Fig. 3).

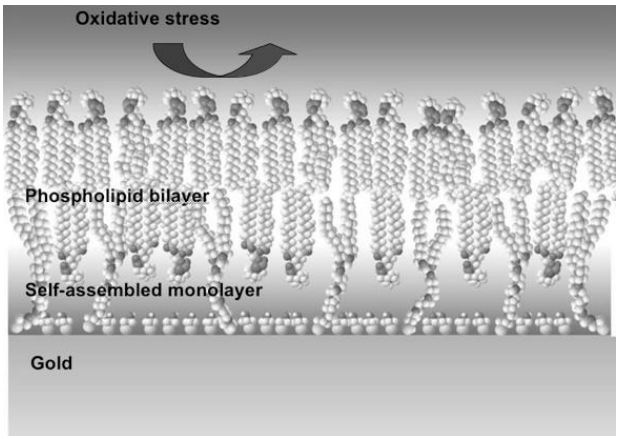


Fig. 3. Cartoon of a model cell-membrane self-assembled on a gold-coated substrate, which can be exposed to chemical oxidation while being characterised using neutron reflectometry to better understand structural changes caused by membrane oxidation.

Continued on page 44

EU dispute with India and Brazil ups stakes in generics saga

Tim Stirrup and Katherine Hebditch

Baldwins Intellectual Property, PO Box 5999, Wellesley St, Auckland
(email: *tim.stirrup@baldwins.com* or *katherine.hebditch@baldwins.com*)

In January 2008, a generic version of Cozaar™ (an antihypertensive drug) en route from India to Brazil was seized under EU customs regulations while in transit in the Netherlands. The seizure, carried out at the behest of the patent owners Merck and DuPont, has reignited the debate over the extent of intellectual property (IP) rights and the availability of patented medicines to developing countries.

Despite the absence of a patent in Brazil or India, Dutch officials deemed the storage of the patented drug on Dutch soil to be infringement of the Dutch patent. Although the seizure was later released and flown back to India by the generics producer (in this case Dr Reddy's Laboratories), the fallout from this and more recent EU seizures has led India and Brazil to beat a path to the door of World Trade Organisation (WTO) to file a complaint. If high-level diplomatic talks between the EU and India/Brazil fail to reach a settlement, it seems likely that the WTO will be left to decide on the extent of protection afforded by IP laws to goods in transit. The international interest (and in some quarters, outrage) in this case highlights the deeper rifts in respective attitudes towards IP between the so-called developed and developing countries.

Protecting your investment

Multinational pharmaceutical companies (collectively *Pharma*) argue that a robust IP enforcement strategy is essential to protect their investment in research and the extraordinary costs and time (on average USD802 million and 7.5 years) to take a drug to market.[1] They might ask why it is that generics companies are able to steal the fruits of their labour and sell them on as cut-price and, in some cases, sub-standard pharmaceuticals. It is argued that the practice exposes patients to potentially harmful counterfeit pharmaceuticals, and that cut-price generics deprive Pharma of revenue to develop new and improved medicines to treat future generations. Enforcing IP laws is therefore said to be an essential part of Pharma's strategy to ensure survival in a competitive global marketplace.

Protecting the poor

In the other corner of the ring are the generics manufacturers who argue that seizures such as those seen in the EU constitute anti-competitive practice on the part of *Big Pharma*; solely designed to sustain the market status quo and deny opportunities to companies from growing economies. Such practices are also argued to be to the detriment of millions of people in developing countries by denying them access to affordable medicines. The recent EU dispute drew protests from those in the Indian pharmaceutical industry who felt that the seizure was taking the scope of IP laws in Europe too far. Amar Lulla, the joint managing director of Cipla (one of India's largest pharmaceutical manufacturers), expressed his opposition to the seizure saying *The EU has to be resisted at every forum as this is an outrageous step to scuttle Indian exports. This is part of Big Pharma's multi-pronged strategy arising out of their desperate situation of scanty product pipelines*.[2]

The Patent System

Patent protection is designed to stimulate and incentivise innovation by guaranteeing the inventor a limited period of monopoly in exchange for publicly disclosing how to produce or work the invention. However, the scope of the monopoly only extends to the countries in which a patent has been granted. A global patent does not exist. Potential patent owners must therefore determine where to invest in a patent; a choice dictated by both economics and whether patent rights can be obtained and effectively enforced. These decisions can restrict access to the product from both a supply and affordability perspective, a situation more likely to occur in less developed countries where healthcare funding is limited or non-existent. In the countries where the patent has not been granted or has expired (usually after the twenty year term), generics manufacturers may legitimately take up the slack by manufacturing the drug and supplying it domestically and to other countries where a patent is not registered.

At the time the original Cozaar™ patent application was filed (1989), India did not allow patents on pharmaceutical compounds; only the manufacturing process could be protected. Reform came about as a result of a WTO deadline to adhere to the TRIPS agreement. From 2005, patent owners could obtain patents on the active compounds and could gain protection for novel compounds invented after 1995. Despite the reforms and significant improvements to the level of IP protection in India in recent years, India still lies second to bottom of the Global IP Index[3] (China sits at the bottom). This index assesses and compares how 24 major IP jurisdictions fare on obtaining, exploiting, enforcing and attacking particular types of IP.

Brazil (which lies one place ahead of India in the Global IP Index) has also had issues with providing robust IP protection to patent holders. While not overtly refusing to grant patents on active compounds, Brazilian patent law (in common with Indian and New Zealand patent law) contains compulsory licensing provisions that can be invoked if favourable terms are not offered by the patent holder. Potential enforcement of these provisions has been used as a bargaining tool by the Brazilian government to force patent owners to reduce prices and indications are that this trend will continue. Despite this, only one compulsory licence has been issued to date (for the antiretroviral drug efavirenz).

The decision by governments on how to legislate and apply their own IP law to benefit their economy has to be finely balanced with honouring commitments to international trade treaties (such as the TRIPS agreement). Other countries and organisations try to influence these decisions by employing incentives and penalties such as trade agreements and import tariffs. The recent reform in India reflects the shifting of this balance as the Indian economy moves from manufacturer towards an R&D led knowledge economy supported by strong IP protection.

The Ideas Pipeline

The practice regarding enforcement of IP rights is in a constant state of flux and depends on interpretation as well as statute. This fact is illustrated by a recent decision in the UK which allowed the transit of counterfeit goods; the court found that trademark infringement does not occur unless the counterfeit

goods were likely to be placed on the market in the transit country. The UK decision appears to be at odds with the Dutch practice although EU deliberations may lead to a more unified outlook.

The EU dispute is a minor piece in an immensely complex jigsaw to which this short article cannot do justice. The overwhelming reality is that many millions of people in developing countries lack access to even the most basic medicines and this is a situation that should not be overlooked by the more fortunate in developed countries. Generics manufacturers play an important role in providing competition in the global pharmaceutical economy. However, diluting the power that IP has to incentivise innovation in developed nations is not a sustainable solution. The developing world will only benefit from new medicines if the product pipeline from knowledge-based economies keeps flowing; patents and a return on investment are an essential part of that pipeline. The solution lies in government, Pharma and NGO backed initiatives and partnerships. These initiatives could include promotion of multi-tier pricing strategies by Pharma and the use of development funding to subsidise medicines and develop distribution infrastructure. The goal of providing affordable, high quality healthcare to the masses may then be a step closer.

A reminder: if you have any queries regarding intellectual property related matters (including patents, trademarks, copyright or licensing), please contact:

tim.stirrup@baldwins.com or
katherine.hebditch@baldwins.com

Patent Proze, Baldwins Intellectual Property, PO Box 5999, Wellesley Street, Auckland

References

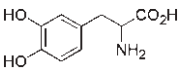
1 DiMasi, Hansen, Grabowski, *The price of innovation: new estimates of drug development costs*, *J. Health Economics*; **2003**, 22, 151-185.

2 *India Opposes Seizure Of Drugs By EU Even As Dr. Reddy's Labs Brings Its Goods Back. PharmAsia News* 29 Jan. 2009

3 Taylor Wessing Global IP Index 2009; see: *http://www.taylorwessing.com/ipindex/* (accessed 1 December 2009)

Dates of Note

On 14 Jan. 1970, L-dopa (3,4-dihydroxyphenylalanine) was reported to benefit about 5% of the patients in reversing the progress of Parkinson's disease. *William* ***Prout***, the English chemist and biochemist noted for his discoveries concerning digestion, metabolic chemistry, and atomic weights, was born 225 years ago on Jan. 15.



L-Dopa

Friedrich Wilhelm Georg ***Kohlrausch***, of conductivity measurement fame, died 100 years ago on Jan. 17, whilst Sir *Edward* ***Frankland***, the English chemist and one of the first investigators in the field of structural chemistry, was born 175 years ago on the 18th. *Adolf Friedrich Johann* ***Butenandt***, the German biochemist and co-winner (with Ruzicka) of the 1939 Nobel Prize for Chemistry for pioneering work on sex hormones, primarily the isolation of estrone, died 15 years ago on this day. He was forced by the Nazi government to refuse the prize, but subsequently able to accept the honour in 1949.

On Jan. 21 in 1970, the first Boeing 747 jet saw service as the Pan American Airways' J. F. Kennedy to London (Heathrow) flight. *André-Marie* ***Ampère*** was born on Jan. 22, 1775, while the 23rd marks 200 years since the birth of *Johann Wilhelm* ***Ritter*** who discovered the ultraviolet region of the spectrum. The 24th marks 60 years since the original microwave oven patent was issued to its inventor *Percy LeBaron* ***Spencer*** under the title *Method of Treating Foodstuffs*.

Edward ***Davy***, the physician, chemist, and inventor who devised the electromagnetic repeater for relaying telegraphic signals and invented an electrochemical telegraph, died 125 years ago on Jan. 26. He immigrated to Adelaide, South Australia, in 1838 where he indulged his interest, ultimately inventing the electromagnetic repeater which made wireless telegraphy possible. Jan. 27 marks 60 years since *Science* magazine announced the new antibiotic *terramycin*. Jan. 30 denotes 15 years since the announcement of the drug *hydroxyurea*, the first effective treatment for sickle-cell anaemia, and 220 years since the first lifeboat, specially built to rescue people from stormy seas - the *Original*, was first tested at sea by its English builder, *Henry* ***Greathead*** of South Shields.

Hans ***Jenny***, the Swiss agricultural chemist and pedologist, has his 111th anniversary on Feb. 7, the day three years ago that NZ Nobel Laureate *Alan* ***Macdiarmid*** died. *Jacques* ***Monod***, the French biochemist who (with Jacob) shared the 1965 Nobel Prize for Physiology or Medicine, was born 100 years ago on Feb. 9, whilst *Per Teodor* ***Cleve***, Swedish chemist and geologist who discovered the elements holmium and thulium, was born 170 years ago on Feb. 10.

Feb. 12 of 1935 saw US Patent 1,991,236 issued to *Robert Jemison* ***Van de Graaff*** for his *Electrostatic Generator*. It provided direct-current voltages much higher than the 700,000 V state-of-the-art at the time using other methods.

Sir *Julian* ***Huxley*** died 35 years ago on Feb. 14 and *Gottlieb Sigismund* ***Kirchhof***, the German-Russian chemist who applied the first controlled catalytic reaction to produce glucose, developed a method for refining vegetable oil, and experimented with brewing and fermentation, preceded him in 1833. *Robert John* ***Kane***, the Irish chemist who is remembered for his book, *The Industrial Resources of Ireland* (1844) died 120 years ago on Feb. 16 and *Henry* ***Cavendish***, who identified hydrogen as a separate gas, died 200 years ago on Feb. 24, 1810.

Feb. 26 marks 75 years since the feasibility of radar (*RA*dio *D*etection *A*nd *R*anging) was demonstrated to Air Ministry officials at Daventry, England, by *Robert* ***Watson-Watt***, a Scottish physicist, and nylon was discovered by Dr *Wallace H.* ***Carothers*** of DuPont 75 years ago on the 28th. The St. Gotthard Tunnel was completed, linking Switzerland and Italy on Feb. 29, 1880.

On 1 Mar 150 years ago (1860), the first meeting of the Chemical Society (London) was held: Dr. *W. A.* ***Miller*** (Vice-President) was in the Chair and *On New Zealand Iron Sand* was read by Mr. *E.* ***Riley***. The day is also the 99th anniversary of ***van't Hoff***'s death and, remarkably, only 40 years since the first direct-dialed transatlantic phone calls were made possible between the US and UK by the combined efforts of AT&T and

the British Post Office. It also marks the 100 years since the birth of *Archer* ***Martin*** who was awarded (with Synge) the Nobel Prize for Chemistry in 1952 for development of paper partition chromatography using two different liquids moving at right angles.

Stanley Lloyd ***Miller***, the American chemist who made a series of famous experiments beginning in 1953 to determine the possible origin of life from inorganic chemicals on the primeval, just-formed earth, is 80 years old today. Mar. 8 is the 35th anniversary of Sir *Robert* ***Robinson***'s death. Sir *Alexander* ***Flemming***, who discovered penicillin, died 11 Mar 1955 and it is the day 50 years ago in 1960 that Pioneer V was launched from Cape Canaveral, Florida. Its launch marked one of the first in-depth attempts to study the solar system.

John Frederic ***Daniell***, the British chemist and meteorologist who invented the Daniell cell, was born 220 years ago on Mar. 12 while the 14th marks 75 years since the death of *Arthur Rudolf* ***Hantzsch***, who at age 25 devised their famed synthesis of pyridines. That day also marks 50 years since the first offshore sulfur mine (off Louisiana) yielded its product.

Frédéric ***Joliot-Curie*** was born on 19 Mar 1900 while Sir *Norman* ***Haworth***, the British chemist and co-winner (with Karrer) of the 1937 Nobel Prize for his work in determining the chemical structures of various carbohydrates and the synthesis of vitamin C, died on his birthday 60 years ago this day. *Torbern Olof* ***Bergman***, the Swedish chemist and naturalist who experimented with carbon dioxide, which he named *aerial acid* and Priestley called *fixed air*, was born 275 years ago on Mar. 20. On this day in 1800, *Alessandro* ***Volta*** dated a letter addressed to Sir Joseph Banks, President of the Royal Society, announcing his invention of the voltaic pile.

25 years ago, on Mar 22 in 1985, the Vienna Convention for the Protection of the Ozone Layer was adopted and opened for signature. The day also marks 50 years since the first laser was patented by *Arthur* ***Schawlow*** and *Charles Hard* ***Townes*** and assigned to the Bell Telephone Laboratories where they worked. On Mar. 25 in 1970, the prototype British-built airplane Concorde 002 made its first supersonic flight and the following day marks 225 years (1885) since the first cremation in England took place at Woking.

Wilhelm Conrad ***Röntgen*** was born 165 years ago on Mar. 27. He received the first Nobel Prize for Physics, in 1901 for his discovery of X-rays. Mar. 29 marks 50 years since British Prime Minister *Harold* ***Macmillan*** reached agreement with US leaders in Washington, DC, on a nuclear test ban treaty to be put to the USSR. Sir *Lawrence* ***Bragg*** was born 120 years ago on Mar. 31; he was the X-ray crystallographer, who at the early age of 25, shared the Nobel Prize for Physics with his father, Sir William Bragg, in 1915. *Isidor* ***Traube***, the father of capillary chemistry, was born on Mar 31, 1860.

On April 1 in 1960, the first weather observation satellite, ***Tiros I***, was launched from Cape Kennedy and provided the first television picture from space. It was the first of several launched in the program, named from its function: *T*elevision *I*nfra*R*ed *O*bservation *S*atellite, and was NASA's first experimental step to determine if satellites could be useful in the study of the Earth. The following day in 1935, Scottish physicist, Sir *Robert* ***Watson-Watt*** was granted a patent for the RADAR (*RA*dio *D*etection *A*nd *R*anging). Apr. 3 marks 100 years since the death of *R. W. H.* ***Abegg***, a German physical chemist who, with Boländer, proposed a theory of valency in 1899.

Edmond H. ***Fischer***, the American biochemist who shared (with Krebs) the 1992 Nobel Prize for Physiology or Medicine for the discovery of reversible protein phosphorylation as a biological regulatory mechanism that governs the activities of proteins in cells, has his 90th birthday on Apr. 6. Apr. 7 marks 150 years since the birth of *W. K.* ***Kellogg***, the American industrialist and philanthropist who founded the W.K. Kellogg Company to manufacture cereal products as breakfast foods in 1906.

Robert Burns ***Woodward***, was born on Apr. 10, 1917, the same day 15 years ago that the world's first national DNA database began operations in the UK. *William* ***Cullen***, the Scottish physician and chemist who held the first independent university lectureship designated for chemistry (founded 1747) in the British Isles at Glasgow University, was born on Apr. 15, 1710. *Charles Fredrick* ***Cross***, the English chemist who, with Bevan and Beadle, discovered that cellulose could be produced (1891) by the dissolution of cellulose xanthate in dilute sodium hydroxide, died this day 75 years ago.

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4. Dr. GNL Jameson, University of Otago.

http://neon.otago.ac.nz/chemistry/contacts/profile/gnlj/

Project title: *Iron's role in the enzyme cysteine dioxygenase: mechanism and biological relevance*

Cysteine dioxygenase (CDO) is a crucially important enzyme that controls levels of the amino acid cysteine in the body by catalyzing the breakdown of cysteine. Failure of this essential process in Parkinson's and other diseases leads to elevated, neurotoxic levels of cysteine. Insight into CDO's regulation and catalytic function are essential to evaluating its role in disease. In the heart of CDO's catalytic site is a single iron atom bound to the protein in a unique way. Although recent X-ray crystal structures define the three-dimensional geometry of the active site, there is no evidence yet as to how the iron supports catalysis within this unusual environment. This proposal will take advantage of our recent results and experience with metal-containing proteins to explore the formation and chemical consequences of the intriguing structure of CDO. Our findings will facilitate understanding of how this enzyme works at a molecular level and eventually show how to prevent its failure in disease.

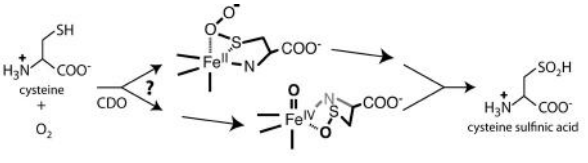

Fig. 4. Key intermediates representing two of several alternative pathways proposed to describe the reaction of CDO. The upper intermediate suggests cysteine activation while the lower suggests oxygen activation.