# Chemistry in New Zealand

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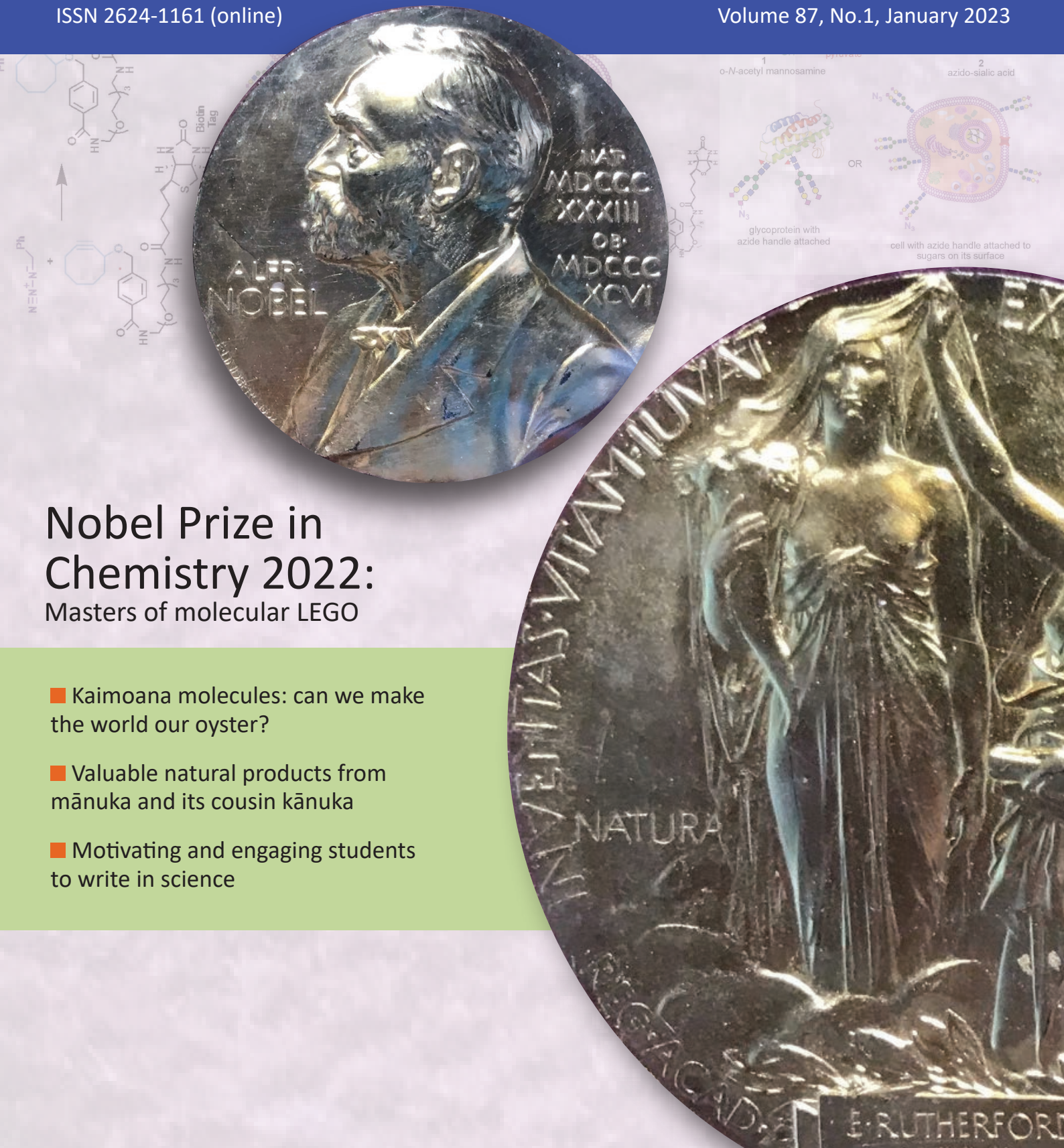


## Nobel Prize in Chemistry 2022:
### Masters of molecular LEGO

- Kaimoana molecules: can we make the world our oyster?
- Valuable natural products from mānuka and its cousin kānuka
- Motivating and engaging students to write in science

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Chemistry in New Zealand

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## Articles and features



## Other columns

# Comment from the President

**Tēnā koutou katoa.**
**Greetings from Wellington at the start of 2023.**

As your new President, let me introduce myself. I'm a Wellingtonian, born and (mostly) bred in Pōneke. I was a student member of the NZIC during my undergraduate and Honours degrees at Victoria University (VUW), and benefitted from the networking and prizes provided by the Branch. After time away doing a PhD at the Australian National University, Canberra, with Professor Martin Banwell and post-doctoral stints at the University of York, UK with Professor Richard J. K. Taylor, I returned to Wellington and took up a lectureship at VUW.

I lead an organic synthesis research group here and teach (mostly organic) chemistry at all levels. I've been involved with the Wellington Branch committee for many years – as secretary, committee member, student liaison rep, chair of the Branch and Branch editor. It's been a pleasure and a privilege to serve as the Vice President in 2022 and I'd like to thank outgoing President Michael Mucalo for his excellent leadership over the past two years, which have been particularly tough ones. I'm grateful to be able to rely on his experience now in his Past-President role. Over the past year, I've learned a huge amount from Michael, as well as from Samantha Eason (our awesome adminstrator), Hamish McDonald (our very wise treasurer) and all the rest of Council. I'm delighted and honoured to take the role of President of NZIC for this 2023–2024 term.

I've been reflecting on the role of NZIC as the key advocate for chemistry and chemists in this motu/country. In doing so, I checked out the aims of the Institute on the website (https://nzic.org.nz/aims) - an enlightening experience. Two phrases particularly stood out to me.

The first was that the Institute was established to "promote the science and practice of chemistry in all its branches...". I find this a helpful reminder of the eminent importance of our Branches as the vehicles by which we operate. For those who aren't aware, we have six regional Branches within NZ (Auckland, Waikato, Manawatu, Wellington, Canterbury, Otago), a Branch for overseas members and two education-related Branches: SCENZ (Secondary Chemistry Educators of NZ) and *tert*-Chem Ed (Tertiary Chemistry Educators of NZ) (https://nzic.org.nz/branches). As we begin a new year, one in which we can hopefully put the disruptions of Covid-19 behind us, each of our Branches can consider how to move forward in this goal to promote chemistry. Perhaps some of the creative ways in which we've operated during Covid times will provide inspiration for complementing our traditional modes of meeting and conducting the business of NZIC, or maybe we will simply be relieved to get back to normality in our Branch activities.

"In this time of shifting attitudes towards truth and fact in our society, this is a timely reminder that we as members of the NZIC need to uphold the veracity and correct practice of chemistry."

Secondly, I was struck by the stated NZIC goal to "promote honourable practice, to repress malpractice, to settle disputed points of practice and to decide all questions of professional usage and etiquette". In this time of shifting attitudes towards truth and fact in our society, this is a timely reminder that we as members of the NZIC need to uphold the veracity and correct practice of chemistry. In addition to ensuring our own actions are honourable, we must encourage our colleagues and students in this regard. Furthermore, each of us is a voice of truth to those around us when it comes to representing chemistry and chemists. There is much misinformation out there that can be gently pointed out and corrected in our day-to-day interactions. One of my pet peeves is the "chemical free" labelling on products that are obviously made of matter and therefore are entirely chemicals! We can look for opportunities to remind our non-scientist contacts of the importance and good of chemistry.

NZIC aims to look after our members through advancing "the interests of the profession of chemistry and of those engaged therein". Given the challenging financial situation of the NZ economy and the education sector at present, advocacy on behalf of our members is becoming more important and necessary. Please do get in touch through your Branches or via the NZIC Office (nzic.office@gmail.com) if there are employment matters concerning you and/or other chemists. It's hard to know how successful we can be but we will aim to strongly represent the cause of chemistry and chemists.

I want to bring attention to the hard work of NZIC/SCENZ folk in the ongoing revision of the NCEA curriculum and assessment framework. The development of excellent educational goals for our secondary school teaching of chemistry in NZ is of utmost importance and we are tremendously grateful for your efforts in providing expertise and vision to the NCEA revisions in partnership with the Ministry of Education.

As we start this year, please consider how NZIC can better represent the chemical sciences in NZ and advocate for our profession. In a more general sense, what is our vision for how chemistry can be used to solve the big problems that we face? We are living in a world facing the impacts of climate change: are there ways in which NZIC should get involved in the science and politics of climate change and in building awareness in the community (beyond the amazing work our members are already doing in this space)?

During the past few years, we have also encountered the effects of being a small and remote market in a global economy, leading to scarcity of many products and resulting in delays in health care and building projects, amongst other issues. Are there opportunities for NZIC (and our members) to facilitate the improvement of our country's resilience by decreasing our reliance on international products? By boosting the chemical industry here in Aotearoa, might we grow job prospects for our members and students of chemistry, while decreasing the cost of transportation and improving the provenance of our products through local manufacturing? If you have ideas for how NZIC can positively impact our society, please get in touch via your Branches or NZIC Office (nzic.office@gmail.com).

As the incoming President of NZIC, I'm keen to visit our regional Branches this year, so I can meet you, see what you're up to, hear about your ideas and visions, and see how we can implement some positive goals. We'll be arranging these visits and I look forward to seeing you in the coming months.

Ka kite ahau i a koutou,
**Joanne Harvey, FNZIC, FRSC**

# NEWS

## AUCKLAND

### UNIVERSITY OF AUCKLAND

EVENTS

#### Inaugural Lecture

Professor Tilo Sohnel gave his inaugural lecture, "Using neutrons and photons to study matter(s) of the world" in October. There was a full house, including several of his former research group. He recounted the interesting start to his University career, beginning under the strict regime of East Germany, then experiencing the changes that occurred with the fall of the Berlin wall and reunification.

He showed us excerpts from some of his early practical classes, making and igniting thermite and analysing the "new" YBCO superconducting materials (they had been discovered in 1986). He showed how some structural features have been a recurring theme through his inorganic materials research. The key roles of the Australian synchrotron and neutron source in Tilo's recent research were evident, as was his continued fascination with structure-property relationships.

#### NZIC Conference

The NZIC Conference was held at the University of Auckland from 21 - 24 November 2022.

#### MOTAT STEM Fair

The School of Chemical Sciences was represented this year at the MOTAT STEM Fair by PhD students Conor Doran, Jen Muhl, Sahil Patel, Claire Webster, Luke Park and Robert Deas along with Cameron Weber. A great time was had by children of all ages including making alginate worms, red cabbage painting and separating felt tip colours using paper chromatography. Thank you to Katrina Graaf for preparing the materials for the day and Joel Rindelaub for helping with the organisation.



MOTAT STEM Fair fun

#### School of Chemical Sciences seminars

**The following seminars were held:**

- Nina Novikova, University of Auckland: "Raman and time resolved spectroscopy: from fundamental excited state dynamics of chromophores to probing complex biological samples in application driven research."

- Professor Ernst-Walter Knapp, Free University of Berlin: "Electrostatics and quantum chemistry for computing protonation equilibria in liquids and proteins."

- Johnathon Muhl Te Pukenga, Southern Institute of Technology: "Microfermentation: an engineer's perspective. Are we stuck in our ways?"

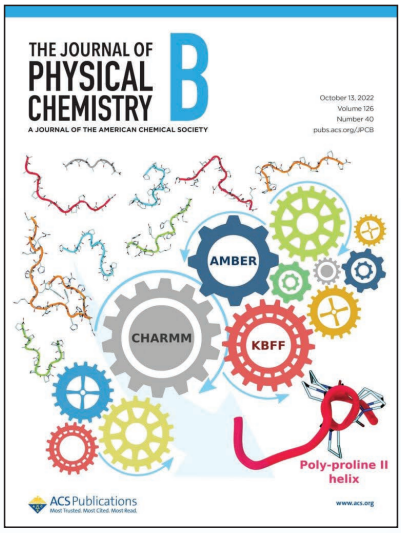
STAFF SUCCESSES

- Geoff Waterhouse has been awarded the Shorland Medal from the New Zealand Association of Scientists. The Shorland Medal is awarded in recognition of major and continued contribution to basic or applied research that has added significantly to scientific understanding or resulted in significant benefits to society.

■ Margaret Brimble won the American Chemical Society Ernest Guenther Award in the Chemistry of Natural Products. The award is to "recognize and encourage outstanding achievements in the analysis, structure elucidation, and chemical synthesis of natural products, with special consideration given to the independence of thought and originality".

The most recent research article from the Mercadante Group was featured on the cover of *Journal of Physical Chemistry B* (McIvor, J.A.P.; Larsen, D.S.; Mercadante, D. Simulating polyproline II-helix-rich peptides with the latest Kirkwood-Buff force field: a direct comparison with AMBER and CHARMM. *J. Phys. Chem. B*. **2022**, *126*, 7833-7846). The published research investigates the sampling of peptide dynamics, using a force field inspired by the Kirkwood-Buff theory of solutions. The work shows that a force field developed beyond a quantum-chemical paradigm and, inspired by means of statistical physics with strong cross-talking between experiments and simulations, is equally capable of sampling peculiar secondary structure elements, such as polyproline II helices, in peptides of biological interest.

The Mercadante Group has had their work featuring the design of large protein inhibitors with enhanced thermal stability published in *Angewandte Chemie* (Angew. Chem. Int. Ed. 2022, e202202711). The research proposes a scalable method for the design of new molecules with higher fitness, based on the collection of information hidden in molecular ancestry and the ability of modelling and simulations to pre-screen viable candidates for experimental testing. This is nested within a pipeline that efficiently selects viable molecular candidates, reducing the costs of experimentation while increasing the probability of finding suitable targets within a sustainable, computing-driven approach to molecular design. Overall, this highlights the ever-increasing role of computations in instructing the rational design of molecules for industrial processes and drug design.




Research from the Mercadante group featured on the cover of Journal of Physical Chemistry B

■ Brent Copp and Geoff Waterhouse have again been named on Clarivate's annual Highly Cited Researcher List. Highly Cited Researchers have demonstrated significant and broad influence reflected in their publication of multiple highly cited papers over the last decade - papers that rank in the top 1% by citations in the Web of Science™. In 2022, fewer than 7,000, or about 0.1%, of the world's researchers, in 21 research fields and across multiple fields, earned this exclusive distinction.

■ Emeritus Professor Ralph Cooney has had an article published in *The Conversation*, this time on recovering space junk: https://bit.ly/3xxZJuU

## Grant Success

Following his recent publication (Heidarsson, Mercadante, *et al.,* Release of linker histone from the nucleosome driven by polyelectrolyte competition with a disordered protein. *Nat. Chem*. **2022**, *14(2)*, 224-231), Davide Mercadante will be an AI in a project successfully funded by a European Research Council Starting Grant. The 1.5M Euro grant will be used to study the activity of pioneer factors in genetic transcription, with the Mercadante group running the computational side of the project and interfacing simulations with NMR experiments performed in Copenhagen (Kragelund group) and single-molecule Förster Resonance Energy Transfer spectroscopy carried out in Reykjavík (Heidarsson group).

Several staff obtained UoA-based Marsden grants:

■ Chris Larsen (Fast Start): "*A molecular machine-based approach to artificial photosynthesis.*"

■ Tristan de Rond (Fast Start): "*Elucidating the biosynthesis of bioactive, chemically-unique terpenoid natural products in a New Zealand marine sponge.*"

■ Jon Sperry (Standard) with Erin Leitao and Tilo Sohnel as AIs: "*A green awakening for radical chemistry*."

■ Dan Furkert as co-PI with Michelle Glass (University of Otago): "*Unlocking the therapeutic potential of the human cannabinoid CB1 receptor: rational design of novel allosteric modulators.*"

■ Cameron Weber as an AI with Patricia Hunt (VUW): "*Unravelling the electronic structure of highly charged hydrogen- and halogen-bonds; rational chemical design and the creation of novel ionic liquid materials.*"

■ Geoff Waterhouse as an AI with Prasanth Gupta (GNS Science) for the Fast Start project: "*Harnessing the power of thermal spikes – a new pathway to fabricate size-controlled transition metal carbide nanoparticles for energy conversion and storage.*"

■ Bruno Fedrizzi as an AI with Maxence Plouviez (Massey University) for the Fast Start project: "*Understanding the mechanisms of microalgal self-aggregation for economic and sustainable harvesting.*"

■ Alan Cameron received an MBIE Smart Ideas grant for his project, "Developing biodegradable quaternary ammonium biocides for sustainable NZ marine biosecurity." Paul Harris received an MBIE Smart Ideas grant for his project, "Boosting crop growth and yield by improving nitrogen uptake and use." Margaret Brimble is an AI on both proposals.

■ Ziyun Wang was awarded a Catalyst: Seeding grant to develop a collaboration with colleagues at the State University of New York at Buffalo and the Chinese University of Hong Kong for the project, "Understanding and designing of bipolar membrane $CO_2$ electrolyser."

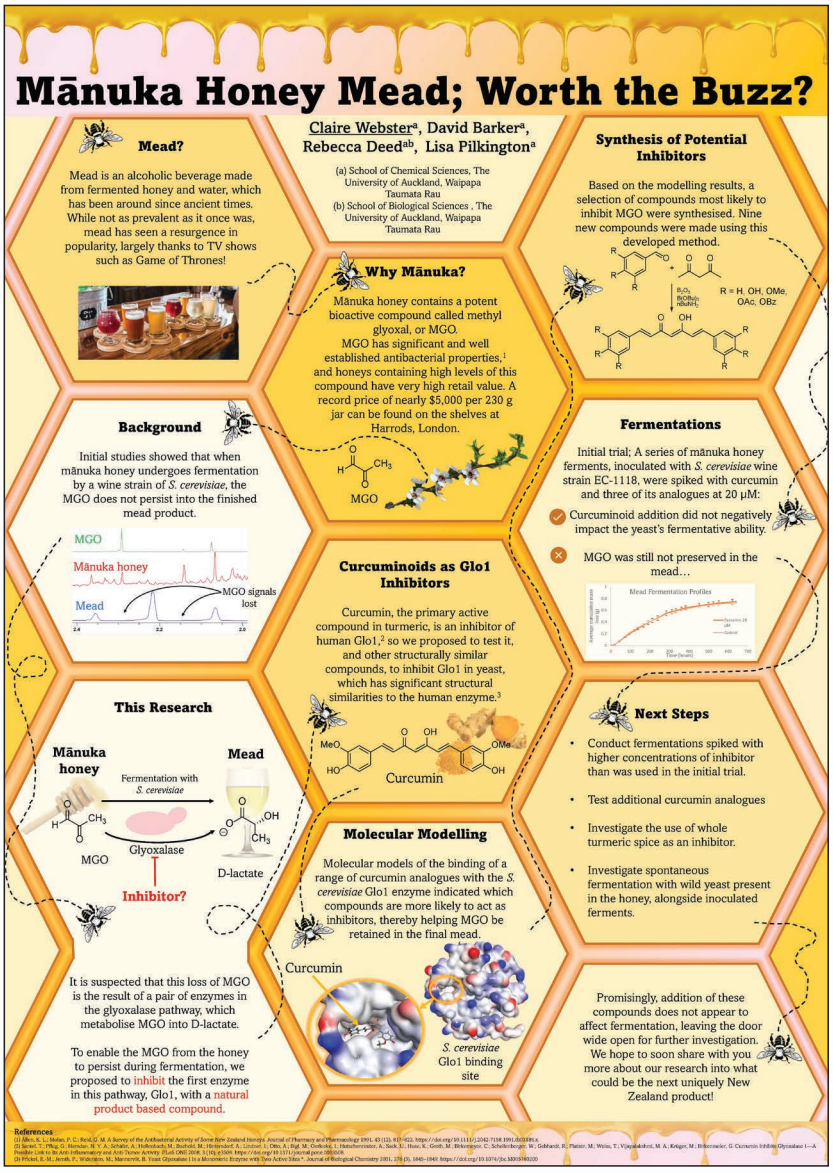
## Farewells

■ Tanya Groutso retired after nearly twenty years' service with the School of Chemical Sciences as our XRD technologist. After gaining a Masters degree in solid state physics from the State University in Belarus and working in the local surface science and metallurgy industry for sixteen years, Tanya moved to New Zealand with her family where she has spent the majority of her time working for the School of Chemical Sciences with stints with Chemical and Materials Engineering and the Light Metals Research Institute.

## Publications

■ McIvor, J.A.P; Larsen, D.S.; Mercadante, D. Simulating polyproline II-helix-rich peptides with the latest Kirkwood-Buff force field: a direct comparison with AMBER and CHARMM. *J. Phys. Chem. B*, **2022**, *126*, 7833-7846.

■ Tian, P.; Lemaire, A.; Sénéchal, F.; Habrylo, O.; Antonietti, V.; Sonnet, P.; Lefebvre, V.; Marin, F.I.; Best, R.B.; Pelloux, J.; Mercadante, D. Design of a protein with improved thermal stability by an evolution-based generative model. *Angew. Chem. Int. Ed*. **2022.** https://doi.org/10.1002/anie.202202711




PhD student Claire Webster was awarded second place in the Faculty of Science Postgraduate Student Poster Competition.

STUDENT SUCCESSES

## PhD student prizes

■ Rebekah Bradley was runner-up (and also winner of the People's Choice Award) in the NZ Inter-University Masters 3 Minute Thesis competition for her presentation on, "Assessment of the MinION as a platform for forensic sequencing of mitochondrial DNA."

■ Ryan England (supervisors: Associate Professor SallyAnn Harbison and Dr Douglas Elliot) was placed on the Dean of Graduate Studies List ("Dean's List") in recognition of excellence achieved with his PhD thesis entitled, "The development and validation of Massively Parallel Sequencing marker panels for use within a New Zealand population in forensic science."

■ Wenyao Zhu (supervisor: Professor Paul Kilmartin) was named on the Dean's List in recognition of his excellent PhD thesis on characterising and differentiating wines based on their volatile composition.

■ PhD student Stefy Gi Peediakal was awarded the best poster prize within the thematic session "Coordination compounds for energy applications, sustainable and environmental chemistry" at the 44th International Conference on Coordination Chemistry (ICCC 2022) held in Rimini, Italy. Stefy's poster was entitled, "Water purification using a green science approach." This award comes on top of another award Stefy received for best poster in the "Future/Green Energy Materials" session at the Royal Australian Chemical Institute (RACI) 2022 National Congress held in Brisbane in July.

### PhD Completions

■ Yimei Wu successfully defended her PhD thesis entitled, "Synthesis of novel photopolymers of additive manufacturing" supervised by Dr Jianyong Jin and Professor Cather Simpson.

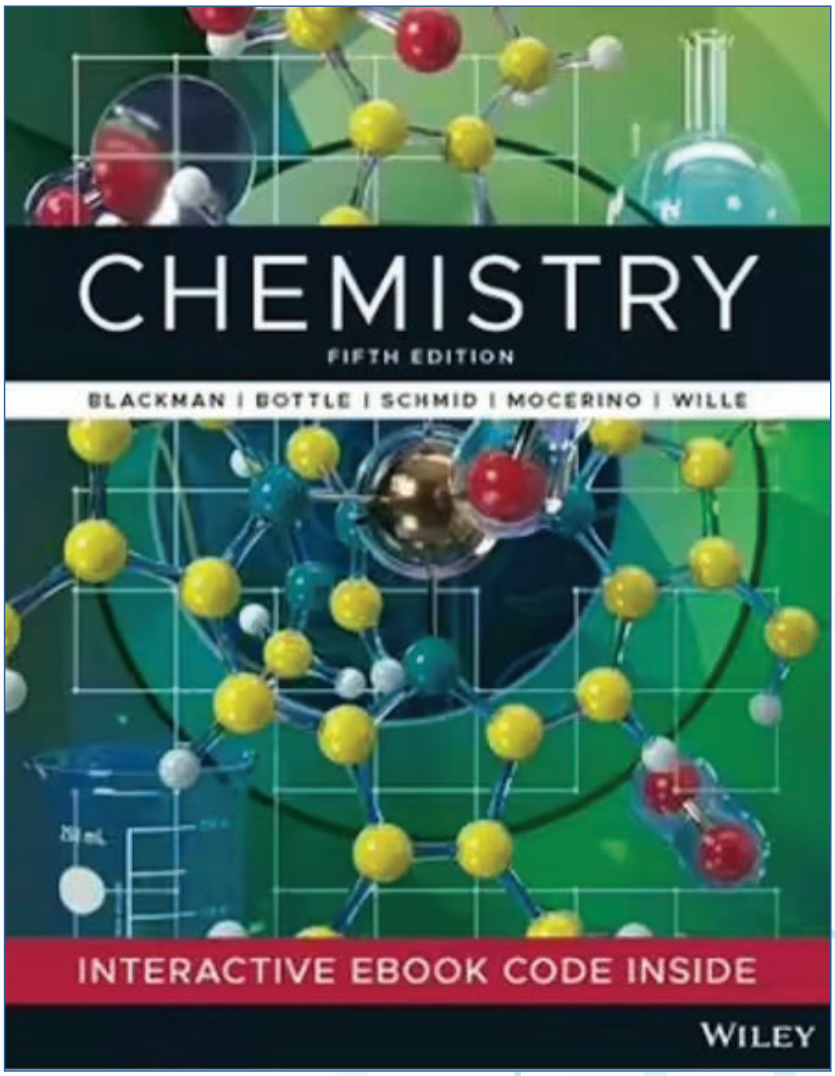
■ Hugh Glossop, a member of Viji Sarojini's group, successfully defended his PhD thesis entitled, *"Studies toward ultrashort peptides as supramolecular biomaterials and fluorinated antimicrobials*."

■ Che Wang defended her PhD thesis entitled, *"Development of spectroscopic non-invasive assessment for quality of dairy powders"* supervised by Dr Marlon Reis and Dr Mariza Gomes Reis (AgResearch), Geoff Waterhouse and Yacine Hemar.

## AUCKLAND UNIVERSITY OF TECHNOLOGY

### NEWS

Thanks go to colleagues around the country and overseas for writing letters in support of keeping chemistry at AUT.




The bestselling first year textbook in Australasia

The 5th Edition of the first-year textbook "Chemistry" by Allan Blackman, Steve Bottle, Siggi Schmid, Mauro Mocerino, and Uta Wille, was published in October by Wiley Australia. This continues to be the largest selling first year textbook in Australasia, having sold close to 100,000 copies since the first edition was published in 2007.

### EVENTS

AUT hosted the ChemEd & BioLive Conference 2022 (https://chemed-biolive.org/) from 16-18 November. This biennial conference was an opportunity for 240 secondary school educators to network, hear about and discuss recent developments in chemistry and biology education. Delegates toured facilities and attended seminars on current leading research in chemistry and biology from AUT and UoA researchers to gain a better understanding of what's on offer for students.

The theme for the conference was "Manaaki whenua, manaaki tangata, haere whakamua" (Care for the land, care for the people, go forward). Professor Allan Blackman gave an invited keynote address and Dr Cassandra Fleming and Associate Professor Don Otter gave invited talks.

Professor Nicola Brasch organised the School of Science Research Showcase, together with colleagues. Anau Lautaha won an award for a lightning (3 minute) talk and Roisin Mooney won an award for her oral presentation.

2022 AUT School of Science Research Showcase

## CONGRATULATIONS

■ Professor Nicola Brasch and her collaborators Dr Brent Seale, Dr Yan Li and Dr Scott Ferguson (University of Otago) have been awarded a Marsden grant of $960,000 to develop vitamin $B_{12}$-antibiotic conjugates to treat resistant gram-negative bacterial infections.

■ Dr Jack Chen and his collaborators, Associate Professor Catherine Whitby (Massey) and Dr Stefan Hill (Scion), have been successful in their application for the 2022 MBIE Endeavour Smart Ideas fund. This project is worth $1 million over three years and will develop a new class of sustainable cellulose-based surfactants that can change properties on-demand, allowing precision control in industrial settings.

■ Ben Stackpole and Olivia Matich were awarded the NZIC most outstanding BSc graduate in chemistry award and Jessica Fredericksen was awarded the School of Science award for the most outstanding BSc(Hons) graduate.

■ Chloe Ren was awarded the 2022 AUT School of Science Postgraduate Student Award for Excellence in Research and Jessica Fredericksen was awarded the 2022 AUT School of Science Postgraduate Student Award for Excellence in Teaching.

## AUCKLAND CANCER SOCIETY RESEARCH CENTRE

### STAFF NEWS

Dr Daniel Conole has arrived on site and is establishing his group. He recently won a prestigious four-year HRC Sir Charles Hercus Health Research Fellowship to return to the ACSRC from Imperial College. He will establish a next generation drug-screening

Left to right: Brent Seale, Jessica Fredericksen, Nicola Brasch and Yan Li

■ Andres Tiban (left) was awarded the KiwiNet Emerging Innovator award, which provides $10k for him to develop his commercialisation skills and provide market validation for a project on cellulose-based surfactants, on which he works with Dr Jack Chen (right).


Attendees at the ACSRC Research Retreat

research programme centred around DNA-encoded libraries. This new high throughput screening technology is faster, cheaper and more convenient than conventional methods.

Initially, this technique will be deployed to discover new chemical probes for an important class of deubiquitinase enzymes in cancer and inflammation, in collaboration with Professor Mike Waring (Newcastle, UK) and Dr Elton Zeqiraj (Leeds, UK). Further development will explore the ACSRC novel drug collection to increase productivity and success rates for drug discovery screens, in collaboration with Associate Professor Michael Hay.


## ACSRC Research Retreat

At the end of October all staff met at Long Bay for a day to assess where the ACSRC research currently stands and where the centre wants to go. After a panel discussion with major stakeholders in the ACSRC we heard an engaging talk by David Downs about his journey through his cancer. David Downs is well known and has published his blog on Stuff.

The rest of the day we heard from the current major groups and its challenges. The retreat will be followed up with a series of seminars where different focus points are discussed in more detail. The day was ably MCed by Dr Michelle Sullivan.

### Student Review

PhD student Christine Kim (ACSRC Supervisors Jiney Jose and Peter Choi) presented her first PhD year review at an ACSRC seminar titled, "Development of hypoxia-activated prodrug of second generation analogues of bedaquiline for treatment of latent tuberculosis."

### Major Publications

■ Dr Julie Spicer had a major review article on her perforin inhibitor research published in the *Journal of Medicinal Chemistry* that earned high praises from the reviewers:

Spicer, J. A.; Huttunen, K. M.; Jose, J.; Dimitrov, I.; Akhlaghi, H.; Sutton, V. R.; Voskoboinik, I.; Trapani, J. Small Molecule Inhibitors of Lymphocyte Perforin as Focused Immunosuppressants for Infection and Autoimmunity. *J. Med. Chem.* **2022**, *65(21),* 14305-14325.
https://doi.org/10.1021/acs.jmedchem.2c01338

Abstract: New drugs that precisely target the immune mechanisms critical for cytotoxic T lymphocyte (CTL) and natural killer (NK) cell driven pathologies are desperately needed. In this Perspective, we explore the cytolytic protein perforin as a target for therapeutic intervention. Perforin plays an indispensable role in CTL/NK killing and controls a range of immune pathologies, while being encoded by a single copy gene with no redundancy of function. An immunosuppressant targeting this protein would provide the first-ever therapy focused specifically on one of the principal cell death pathways contributing to allotransplant rejection and underpinning multiple autoimmune and post-infectious diseases. No drugs that selectively block perforin-dependent cell death are currently in clinical use, so this Perspective will review published novel small molecule inhibitors, concluding with *in vivo* proof of concept experiments performed in mouse models of perforin-mediated immune pathologies that provide a potential pathway toward a clinically useful therapeutic agent.

■ Dr Jeff Smaill and his group had 3 papers published in the *Journal of Medicinal Chemistry* this year highlighting the work in their field of EGRF and AKT inhibitor research.

■ Fang Yang; Xiaojuan Chen; Xiaojuan Song; Raquel Ortega; Xiaojing Lin; Wuqing Deng; Jing Guo; Zhengchao Tu; Adam V. Patterson; Jeff B. Smaill; Yongheng Chen; Xiaoyun Lu.

Design, Synthesis, and Biological Evaluation of 5-Formylpyrrolo[3,2-b] pyridine-3 carboxamides as New Selective, Potent, and Reversible-Covalent FGFR4 Inhibitors.
https://pubs.acs.org/action/showCitFormats?doi=10.1021/acs.jmedchem.2c01319&ref=pdf

■ Fang Xu; Xin Zhang; Zhipeng Chen; Sheng He; Jing Guo; Lei Yu; Yongjin Wang; Caiyun Hou; Hawaa Al-furas; Zongyao Zheng; Jeff B. Smaill; Adam V. Patterson; Zhi-Min Zhang; Liang Chen; Xiaomei Ren; Ke Ding.

Discovery of Isoform-Selective Akt3 Degraders Overcoming Osimertinib-Induced Resistance in Non-Small Cell Lung Cancer Cells.
https://pubs.acs.org/action/showCitFormats?doi=10.1021/acs.jmedchem.2c01246&ref=pdf

■ Min Shao; Xiaojuan Chen; Fang Yang; Xiaojuan Song; Yang Zhou; Qianmeng Lin; Ying Fu; Raquel Ortega; Xiaojing Lin; Zhengchao Tu; Adam V. Patterson; Jeff B. Smaill; Yongheng Chen; Xiaoyun Lu.

Design, Synthesis, and Biological Evaluation of Aminoindazole Derivatives as Highly Selective Covalent Inhibitors of Wild-Type and Gatekeeper Mutant FGFR4.
https://pubs.acs.org/action/showCitFormats?doi=10.1021/acs.jmedchem.2c00096&ref=pdf

### NZIC Conference

The ACSRC was well represented at the NZIC conference in Auckland with 15 members attending. 7 talks and 6 posters were presented by ACSRC staff and students.

### Cancer Society Auckland Northland Walking Stars

On Saturday 12 November the ACSRC had a team at the start of the fundraising event Walking Stars organised by the Cancer Society Auckland Northland. The ACSRC fundraised over $2,000 for the event. A big thank you to all the team members and the donors.

## ■ CANTERBURY

### NZIC

The Canterbury-Westland Schools' Science and Technology Fair was held on Saturday 24 September in the Ernest Rutherford Building at the University of Canterbury. NZIC prizes were awarded for excellence in chemistry and rigour in the research undertaken for the science fair projects and were awarded to:

**Year 7**
1st ($60): Hansun Xia, St Martins School, for "Hair washing"

2nd ($45): Harrison Croot, Chisnallwood Intermediate, for "Plant power"

3rd ($30): Evelyn Naylor & Katie Stewart, Chisnallwood Intermediate, for "Nails vs rust"

**Year 8**
1st ($60): Page Woodrow & Kate Burgess, Chisnallwood Intermediate, for "Spherification"

**Year 9-10**
1st ($60): Cameron Klempel, John Paul II High School, for "Something sticky"

2nd ($45): India Hood, Liera Yeakley & Lucy Kelly, Cashmere High School, for "Measuring the vitamin C levels in different citrus fruits"

## UNIVERSITY OF CANTERBURY

### PhD successfully defended

Congratulations to Lily Hermanspahn on the successful defence of her PhD thesis on 25 October entitled, "Synthesis of functionalised supramolecular assemblies" and supervised by Paul Kruger.

Well done to Lily on such a polished performance, and good luck to her for the future!

### Seminar

Dr Joel Rindelaub, University of Auckland (https://profiles.auckland.ac.nz/j-rindelaub) gave a well-attended presentation entitled, "Understanding airborne microplastics in Aotearoa New Zealand" on 21 October at the School of Physical and Chemical Sciences. Joel is a Research Fellow at the University of Auckland and uses analytical chemistry to address issues in air quality and environmental science. Originally from Minnesota in the US, Joel moved to New Zealand in 2017 after receiving a PhD from Purdue University. In addition to academic research, Joel is also active in science communication, writing and video production. His presentation abstract follows:

Microplastics have been found across the globe, from the tops of mountain ranges to the snowfall of Antarctica, yet there are currently large uncertainties in the transport mechanisms of these tiny pieces of plastic. My work investigates the presence of microplastics in Aotearoa New Zealand, hoping to better understand how the ocean-atmosphere dynamic contributes to airborne plastics. I am also interested in exposure to plastic-related species, particularly from inhalation, as microplastics have recently been found in both human lungs and the bloodstream. Despite posing a greater toxicological risk compared to ingestion, the inhalation of microplastics has not been studied as extensively, due to issues related to the small sizes relevant to particulate matter inhalation (< 10 µm). Using size-selective air sampling techniques coupled with a pyrolysis GC/MS analysis method, my work aims to uncover the chemical compositions of the small, breathable plastic in the air around us.

### Lyttelton Fire Festival

The University of Canterbury and the Lyttelton Volunteer Fire Brigade held the first Lyttelton Fire Festival on Sunday 14 August 2022. There were science and fire demonstrations, as well as a number of hands-on activities and demonstrations for kids such as kitchen chemistry demonstrations, nitrogen ice-cream, slime making, hover craft rides and a bed of nails. The fire appliances were on display, and there was a sausage sizzle.

A brief video of the event can be seen at: https://www.facebook.com/yttelton VolunteerFireBrigade/videos/786461636021060

The festival was an event organised by the School of Physical and Chemical Sciences at UC and the Lyttelton Volunteer Fire Brigade, aiming to bring science and fun to the local community.

## MANAWATU

The 31st annual Massey-Victoria Chemistry Symposium was held on 11 November at Victoria University of Wellington (VUW). Yiming Zhang (Massey University) and Tehreema Nawaz, Callum Gordon, Brenda Luong, Georgia Richardson, Lara Browne and Emily Mason (all VUW)

Students and staff attending the 31st Massey-Vic symposium

served as organisers. The event featured 15 presentations from students covering a diverse range of research. Emily Stephens of VUW was awarded the best BSc Hons/MSc presentation for her talk entitled, "Towards novel high covalency frameworks". Marryllyn Donaldson, a 2nd year PhD student in the Plieger group, was awarded the best PhD talk for her presentation entitled, "Variable supramolecular assembly with bis-tridentate ligands". The colouring in competition was won by Associate Professor Robin Fulton who steamrolled the other competitors with her black-and-white cross-hatching technique.

Marryllyn Donaldson (left) accepting her award for the best PhD talk from Vyacheslav Filichev (right)

■ Tyson Dais successfully defended his PhD thesis entitled, "From triangles to rings: colourful clusters of substituted naphthalenediols". Tyson was supervised by Professor Paul Plieger and Associate Professor Gareth Rowlands.

■ André Buzás Stowers-Hull has completed his MSc entitled, "Ultra-sensitive SERS detection of organophosphorus compounds via surface modified silver nanostructures". André was supervised by Professor Mark Waterland.

■ Nicole Park has completed her MSc entitled, "Encapsulation of tetrahedral anions in nickel mesocate cages". Nicole was supervised by Professor Paul Plieger.

■ Elnaz Jangodaz has joined the Telfer group as part of a MacDiarmid scholarship with a project entitled, "Carbon dioxide capture using metal-organic frameworks (MOFs)".

■ Sam Staniforth has joined the Rowlands group as part of a summer scholarship and will be working on photoswitchable catalysts.

■ Cara Bosman has joined the Plieger group as part of summer scholarship and will be developing new single molecule magnets.

■ Tomasz Czapik has joined the Filichev group as a post-doctoral fellow.

On 20 October, Professor Steven Bull from the University of Bath visited Massey University to give a presentation entitled, "Biorefineries for sustainable chemical synthesis and fluorescent sensors as tools for investigating biological processes".

On 17 November, Dr Jamie M. Withers from the University of Glasgow gave a presentation entitled, "DNA assembly directed by the fluorous effect, and an investigation of sequence bias in DNA minor-groove binders".

The Nucleic Acids Chemical Biology group at Massey University received several grants near the end of 2022. Dr Elena Harjes as Principal Investigator received a Marsden grant on a project entitled, "Structural basis of viral wars: innate immune system attack on viral genomes and the counterattack by viruses". Emeritus Professor Geoffrey B. Jameson is a co-PI and Associate Professor Vyacheslav V. Filichev is an AI. This project has external links with Professor Kurt Krause from Otago University and Professor Linda Chelico from University of Saskatchewan (Canada).

■ Dr Harikrishnan M. Kurup of the Filichev group has been awarded a prestigious fellowship from the Cancer Society of New Zealand to develop powerful inhibitors of APOBEC3 enzymes. The fellowship covers Harikrishnan's postdoc salary for nearly 3 years.

The $50,000 Massey Innovation Prize was awarded to Associate Professor Vyacheslav V. Filichev and his team from the School of Natural Sciences to begin R&D on a gene manipulation technology with potential to treat aggressive cancers.

A new DNA synthesiser from K&A Labs GmbH (Germany) has been installed in the laboratory of Vyacheslav Filichev. This DNA synthe-

siser allows large-scale production of modified DNA and RNA and will be used in several research programs at Massey University.

## OTAGO

### UNIVERSITY OF OTAGO, DEPARTMENT OF CHEMISTRY

Plant & Food Research (PFR) is running nationwide networking for early career researchers (ECRs). As part of this, Josh Bristowe (PFR Dunedin) and University of Otago chemistry students Kat Handey (BSc Honours), Liam Hewson (MSc) and Ioan Fuller (PhD) visited the PFR research station and labs at Clyde on 10 November. They were treated to lunch over an all-site (Kerikeri to Clyde) video conference with other ECRs, with some great discussion between the sites and a chance to talk to PFR Chief Scientist, Richard Newcomb and PFR People & Culture Leader, Kath Clarke.

The Chemistry Outreach team led by Dave Warren has been visiting schools around Kaitaia as part of their ongoing partnership with Ngāti Kahu. They visited three schools (about 300 kids) in mid-November before heading over to Tāneatua for a Science Wānanga the following week.

## WAIKATO

The branch provided two cash prizes for the NIWA Waikato Science Fair. The prize for the best junior chemistry investigation was won by Naomi Martin and Alex West of Te Awamutu College, whose project "C the difference" looked at the vitamin C content of different fruit juices, while the prize for the best senior chemistry investigation was won by Amisha Sadani of St. Peter's School for her project "Inhibitory tea" which examined the effects of phenolic compounds in black tea on amylase inhibition.

School pupils in Kaitaia participating in chemistry outreach with Otago's Liam Hewson

Early Career Researchers from Plant & Food Research/University of Otago visiting the PFR research station at Clyde, Central Otago. Left to right: Josh Bristowe, Kat Handey, Liam Hewson, Ioan Fuller.

## UNIVERSITY OF WAIKATO

EVENTS

### Chemquest

Nearly 150 students from 18 schools in the greater Waikato and Bay of Plenty region participated in the annual ChemQuest competition, held at the University. This was the 25th anniversary of this popular event which featured music, demonstrations and a "glow show".

ChemQuest combines pop culture with chemistry and students compete for the ChemQuest trophy and cash prizes. It is a fun-filled evening for students studying NCEA Level 2 chemistry and is always a hard-fought contest. Prizes were awarded as follows:

**1st Place:**
**St John's College:** (Matthew Jeremiah, Christian Pearson, Mohnish Singh)

**2nd Place:**
**Hamilton Girls' High School:** (Marina Aleksic, Kennedy Hosking, Kiera Sullivan)

**3rd Place:**
**Hamilton Boys' High School:** (Josef Gillgren, Alex Liu, Alexander Mayo)

**4th Place:**
**Rototuna Senior High School:** (Jaylah Heaton, Zoe Simonson, Alannah Wrigg)

**5th Place:**
**Hamilton Christian School:** (Olivia Bennett, Anika Botha, Joshua Shayak)

The quiz was generously sponsored by the Waikato Branch of NZIC, Hill Laboratories and the School of Science, University of Waikato. Numerous chemistry staff and students contributed to make the event a very successful one.

### University of Waikato Postgraduate Conference

Congratulations to each of the students who participated in the annual

St John's College, first place winners at ChemQuest 2022 with competition sponsors. From left: McGregor Small (Hill Laboratories), Michèle Prinsep (competition organiser), Mohnish Singh, Matthew Jeremiah, Christian Pearson, Peter Allen (Hill Laboratories).


Top, from left: Mohnish Singh, Matthew Jeremiah, Christian Pearson.

Left, St John's College, first place winners at ChemQuest 2022.

Below, **Michèle Prinsep demonstrating the "methanol cannon".**

Above left, Lauren Gris (right) receiving her award from the Dean of Science, Professor Margaret Barbour.

Above right, Edie Thomas (right) with Genevieve Palmer from Tonkin + Taylor

Left, Simon Winship's prizewinning photograph. From left to right the solutions are: spinach extract (chlorophyll), purple highlighter ink, turmeric, honey and tonic water.

University of Waikato Postgraduate Conference.

Matthew Risi, Edie Thomas, Yanan Li, Amber Bell, Lauren Gris, Simon Winship and Jade te Bogt gave oral presentations, whilst Kavitha Harshani, Amber Bell and Simon Winship presented posters.

Lauren Gris won the Waikato Branch sponsored New Zealand Institute of Chemistry Award for the best chemistry-related oral presentation for her talk, "A chemical study of the predator-prey relationship in nudibranchs" and Edie Thomas won the Tonkin + Taylor Award for the best MSc presentation, "Characterising the aroma profile of New Zealand native monofloral honeys".

Simon Winship was the runner up in the scientific photography competition for his photograph of everyday objects that fluoresce under UV light.

Amber Bell has received the Association of Official Analytical Chemists (AOAC) International/Eurofins Foundation "Testing for Life" student award. This is an international award for student researchers advancing science in analytical or molecular testing for food safety, security, defence or authenticity, or health and environmental protection. Amber flew to Arizona, USA for the AOAC annual meeting and gave an oral presentation on her award-winning research. She also presented a poster on her MSc research on the cause of low diastase activity in manuka honey.

■ We welcome Kavitha Harshani Ranaweera and welcome back Mohammad Soleimani Zohr Shiri, both of whom have finally been able to return to PhD studies with the lifting of border restrictions.

## WELLINGTON

NEWS

The NZIC Annual Chemistry Quiz was held on 18 October. It was attended by about 60 members of the Wellington Branch and VUW students. Organised by Lara Browne and Finlay Burke, it was a fun evening.

The Curtis Lecture, held every three years in tribute to the wonderful work of Professor Neil Curtis, took place on 27 October. Professor James Wright from the University of Auckland presented "New coordination compounds with a metallic ring" and provided an elegant display of organometallic chemistry advances by the Wright group.


The 2022 Curtis Lecture given by Professor James Wright (Neil Curtis and his wife Yvonne are in the photo)


Professor Steve Bull with PhD student Goutham Rajendran by the Cuba Mall bucket fountain

### VUW

The School of Chemical and Physical Sciences (SCPS) at VUW hosted a science and technology expo "Dangling Bonds" in September, featuring research and innovation from VUW, Callaghan Innovation, spin-out companies and other technology firms in the Wellington region.

Professor Steven Bull from the University of Bath visited Wellington in late September during his Erskine Fellowship at the University of Canterbury, meeting with staff and student members of the SCPS, and presenting an exciting and enthusiastically received lecture "Biorefineries for sustainable chemical synthesis and fluorescent sensors as tools for investigating biological processes".

Dr Mark Glenny, the Innovation Research Manager at Resene Paints, delivered "Chemistry in a can – perspectives, possibilities and products", which described innovative solutions to many of the application-based challenges encountered in producing high-performing products.

Dr Emma Dangerfield presented the chemistry part of the SCPS student colloquium featuring the Nobel Prizes in Physics and Chemistry in October. Emma's seminar on "Click reactions and bio-orthogonal chemistry" was beautifully pitched and highly informative.

VUW hosted the annual Massey-Vic Student Symposium on 12 November. It was fantastic to join together in person again, and the lecture room was full, with additional attendees online. Fifteen students (Honours, Masters and PhD) spoke about their research. Congratulations to prize winners Marryllyn Donaldson (Massey) for the best PhD talk on "Variable supramolecular assembly with bis-tridentate ligands" and Emily Stephens (VUW) for the best MSc

talk "Towards novel high covalency frameworks". Thanks to the organising committee (Lara Browne, Calum Gordon, Georgia Richardson, Tehreema Nawaz, Brenda Luong, Emily Mason from VUW and Yiming Zhang from Massey) for all the hard work, supported by Kara Eaton, Luke Liu, Robin Fulton and Vyacheslav Filichev.

Dr Mat Anker has secured a Rutherford Discovery Fellowship. Mat has made some very substantial discoveries in his work in the short time of his independent career, with his first paper from his own group being in *Nature Comms*. This award is a very fitting and well-deserved honour.

Professor Tricia Hunt led a successful bid in the 2022 Marsden Fund on "Unravelling the electronic structure of highly charged hydrogen- and halogen- bonds; rational chemical design and the creation of novel ionic liquid materials."

Dr Courtney Davy was awarded the VUWSA Outstanding Lecturer award, as voted by VUW students.

Professor Justin Hodgkiss won the Researcher Entrepreneur Award at the 2022 KiwiNet awards for his leadership in deep-tech commercialisation. This award recognises an experienced entrepreneurial researcher who consistently delivers real world impact from his research. Frank Natali, also from SCPS, was also a finalist in this category.

Congratulations to Tehreema Nawaz (supervised by Grant Williams and Martyn Coles) and Charlotte Page (supervised by Simon Hinkley, Richard Furneaux and Paul Teesdale-Spittle) who have both recently successfully defended their PhD theses.

Justin Hodgkiss and Joanne Harvey have both made recent television appearances, with Justin talking to Newshub about his group's recent work to understand how melanin interacts with UV, and Joanne discussing deck cleaners on TV show Fair Go.

Following the return of international travel, many of our members have recently travelled overseas to attend conferences and visit collaborators. Professor Justin Hodgkiss gave an invited talk "Rapid exciton diffusion in next generation organic semiconductors and its implications for charge photogeneration" at the International Conference on the Science and Technology of Synthetic Metals (ICSM), held in Glasgow in July.

Dr Nathaniel Davis has recently presented "Pushing the limits on renewable energy technology through hybrid organic/inorganic nanomaterials" at the Royal Australian Chemical Institute National Congress, in Brisbane in July, The International Symposium on Singlet Fission and Photon Fusion: Emerging Solar Energy Conversion Technologies, in Milan in October, the Australian Research Council Centre for Excellence in Exciton Science Annual Workshop in Lorne in November, and at the Australasian Community for Advanced Organic Semiconductors Symposium, in Tweed Heads in December. PhD students Matthew Brett, Lara Browne and Calum Gordon also attended and presented at the Royal Australian Chemical Society National Congress in Brisbane.

Associate Professors Rob Keyzers and Joanne Harvey met with research collaborators in Guelph, Ontario in Canada in August and visited the chemistry department at the University of Toronto.

The 1st AUSNZ Natural Products Chemistry and Biology Symposium, held in Melbourne in November, was well attended, with presentations given by Professor Emily Parker (plenary talk on "Reconstructing pathways for indole diterpene production"), Dr Rose McLellan, Dr Alistair Richardson, Dr Luke Stevenson, Dr Daniel Berry, and Dr Rosannah Cameron.

A good contingent from Wellington attended the NZIC Conference in Auckland in November.

Article

# The Nobel Prize in Chemistry 2022: masters of molecular LEGO

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Keywords: Nobel prize, click chemistry, biorthogonal chemistry, azide, alkyne, cycloaddition

## The click chemistry concept

I confess that, after last year's initial slight disappointment with the outcome, this year I did not stay up and watch the Chemistry Nobel prize announcement live on YouTube[1] on the night of 5th October 2022. Of course it transpires that was a mistake, because not only did organic chemistry win again, but two of the three recipients were carbohydrate chemists by background - my own specialist research area.

The Nobel committee awarded the 2022 prize in equal shares to Barry Sharpless, Morten Meldal and Carolyn Bertozzi, for "an ingenious method of building molecules", namely the development of click chemistry and bioorthogonal chemistry. The onomatopoeia of 'click' chemistry refers to the sound that you make when snapping together blocks of LEGO, and indeed one useful analogy is that click chemistry equates to molecular LEGO; complex molecular pieces can be joined together at will to make larger ones (Fig. 1A).



■ Antony Fairbanks undertook both his first degree and DPhil in chemistry at Oxford, the latter with Prof George Fleet. After two postdocs (Prof Pierre Sinaÿ, École Normale Supérieure, Paris and Prof Steve Ley, Cambridge) he became an independent academic at Oxford in 1996. In 2009 he moved to UC and was Head of the Department of Chemistry from 2010-2014 during the Christchurch earthquakes. He is an organic chemist with research interests focussed on carbohydrate chemistry and biology. He has authored/co-authored 170 scientific publications and in 2018 was awarded the New Zealand Institute of Chemistry Maurice Wilkins Centre Award for Chemical Science.

The basic idea of click chemistry, first proposed by Barry Sharpless more than 20 years ago[2], is that using a small selection of 'perfect' 'spring-loaded' reactions, a modular approach could be used to bolt together molecular building blocks into more complex species. Importantly, each building block contains a mutually reactive handle to ensure that bond formation specifically occurs only as desired. Sharpless then set himself, and other chemists, the goal to develop "a set of powerful, selective, and modular blocks that worked reliably in both small and large-scale applications". In theory, click chemistry would not only allow the easy construction of molecular complexity by the assembly of modular fragments (Fig. 1B, here the LEGO analogy is even more obvious), but its use would also allow other larger molecular species to be joined together. Highly useful applications involving linking biomacromolecules or surfaces to fluorescent tags (Fig. 1C), or radiolabelling, immediately spring to mind.

Anyone who has ever heard Sharpless speak knows that his use of the term 'spring-loaded' derives from reactions of epoxides; their super reactivity arising from the strain of the three-membered ring, which is released when the epoxide is opened by attack of a nucleophile. Thus the term 'spring-loaded' refers to a chemical species that is perfectly primed for reaction, but which will only react with the correct partner. So, in this context 'perfect' can be taken to mean that only the desired chemical reaction happens, it irreversibly results in strong covalent bond formation between the two species, and a single predicted

product is formed in 100% yield. Finally, the reaction must always work, i.e. it is completely reliable. Oh, and did I add that it also needs to be compatible with aqueous solvents?

In 2000, when Sharpless wrote his concept paper, click chemistry was therefore a nice concept, but unfortunately there was one major problem: in reality there were no 'perfect' reactions that satisfied all of the required criteria. You may argue that can't be true, saying "there are lots of essentially quantitative yielding chemical processes, take amide bond formation for example". It is indeed true that chemists can make amide bonds in very high yield with excellent reliability. However the problem is that amines also do other reactions, as do the required coupling agents, and you can't routinely do the coupling reactions in water. So amide bond formation just isn't good enough to be a click reaction.

However, only just over a year later Medal and Sharpless independently and almost simultaneously found the first example of a perfect click reaction, namely the Cu-catalysed azide-alkyne cycloaddition. It is for the discovery of the catalysed version of this quite old reaction that they were awarded the prize. Very shortly afterwards, Bertozzi would make a key alteration to this reaction that remarkably allowed it to be performed in biological systems; the invention and application of a bioorthogonal click reaction. Let's now look at the background to this chemical transformation before considering Meldal, Sharpless and then Bertozzi's contributions in turn.

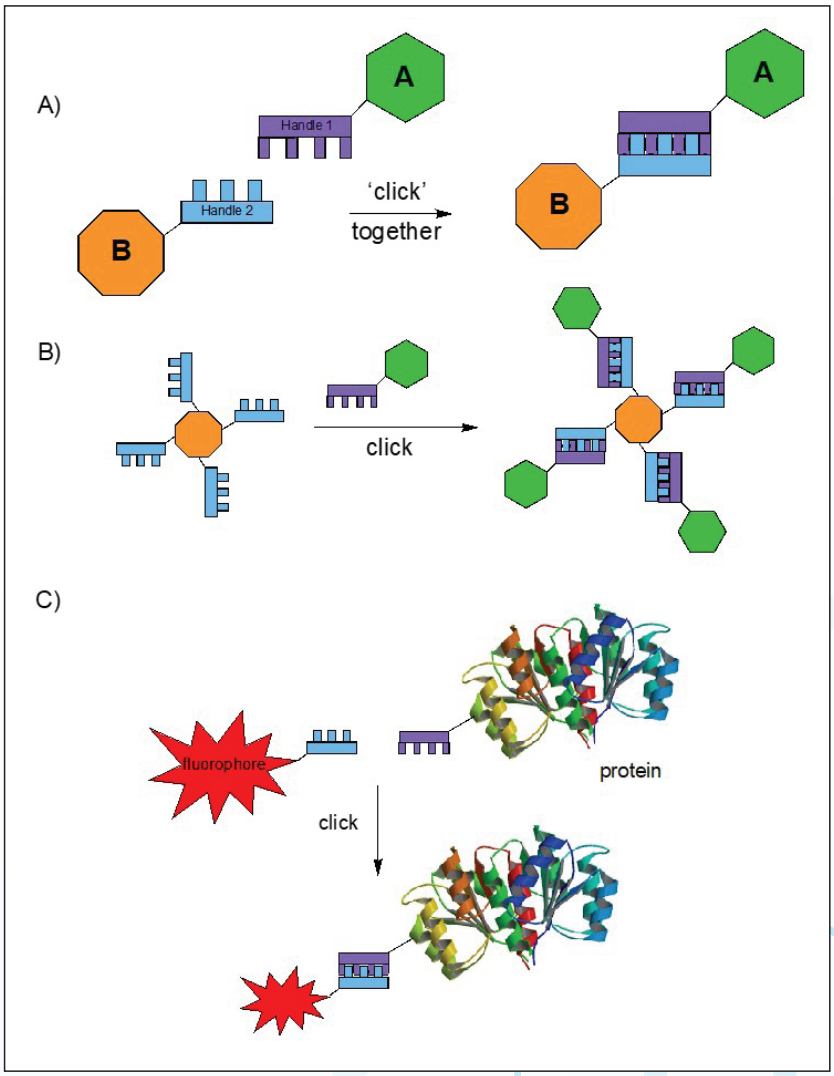



Fig 1. The click chemistry concept. A) Two molecular building blocks A and B, each containing a mutually reactive handle, are covalently linked together with complete selectivity in a high yielding and reliable process. B) Rapid assembly of complex molecular architecture using click chemistry. C) Linking a fluorescent tag to a protein for visualisation studies using click chemistry.

## Background to the Prizes

### 1,3-Dipolar cycloaddition reactions

Cycloadditions are classic examples of pericyclic reactions. The most well-known is probably the Diels-Alder reaction, familiar to all chemistry undergraduates, which occurs between a diene and an alkene that is formally described as a [4+2] cycloaddition. Perhaps slightly less familiar, though certainly on any undergraduate chemistry curriculum, are 1,3-dipolar [3+2] cycloadditions (Fig, 2A). These encompass a wide range of reactions that occur between a '1,3-dipole' as the first component and an unsaturated species, termed a 'dipolarophile', which react together to create a 5-membered ring. A wide range of 1,3-dipoles are known (Fig. 2B), all sharing the common feature that they comprise a conjugated system of three p orbitals containing 4 electrons. While these are neutral species overall, they all contain a separation of charge that is spread over three atoms, hence the term '1,3-dipole'. These 1,3-dipoles all undergo cycloaddition reactions with alkenes, alkynes, and other species that contain carbon-heteroatom multiple bonds to produce different types of 5-membered heterocyclic rings systems that are particularly useful in medicinal chemistry.

A convenient approach[3] to rationalising the rates and regioselectivities of

these processes can be made by considering interaction between the Frontier Molecular Orbitals (FMOs) of the two reacting components (Fig. 2A). Broadly speaking, the lower the energy gap between the highest occupied molecular orbital (HOMO) of one component and the lowest unoccupied molecular orbital (LUMO) of the other component, then the better the overlap between these two and the faster the reaction. Whether the most important FMO interaction is between the HOMO of the 1,3-dipole and the LUMO of the dipolarophile (most common, termed normal electron demand), or alternatively between the HOMO of the dipolarophile and the LUMO of the 1,3-dipole (termed inverse electron demand), depends on the precise reaction in question.

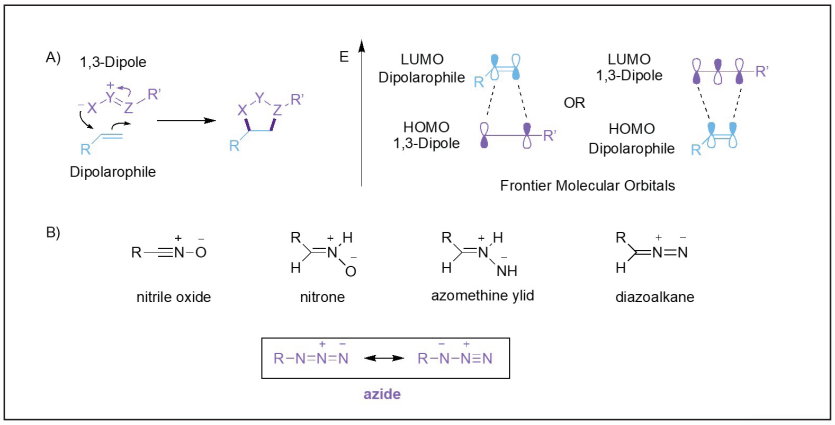



Fig 2. 1,3-dipolar cycloaddition reactions. A) Rection mechanism and Frontier Molecular Orbitals. B) A small selection of examples of 1,3-dipoles.

Regardless, catalysis may be achieved by decreasing the appropriate HOMO-LUMO energy gap. Furthermore, the better matched that the atom coefficients of the FMOs are, then the more regioselective the reaction tends to be. The 2022 Nobel Prize in Chemistry was awarded for the development of superlative versions of just one of these reactions, namely the azide-alkyne cycloaddition; a reaction that bears the name of its greatest exponent as you can see below.

## Rolf Huisgen (1920-2020)[4] - 'creator' of the 1,3-dioploar cycloaddition

The first account of the cycloaddition reaction of an azide and an alkyne was actually made by Arthur Michael in 1893[5], when he reported the first production of 1,2,3-triazoles. However, the reaction was not studied in any significant detail until Rolf Huisgen, a German organic chemist educated at the University of Munich, began his seminal studies, first at Tubingen University and then at the University of Munich.

Professor Rolf Huisgen. Photo credit: Jü, published under Creative Commons License CC0 1.0, https://commons.wikimedia.org/wiki/File:Rolf_Huisgen_and_Franz_Marc%27s_Mandrill.JPG

Rolf Huisgen's research interests centred on 1,3-dipolar cycloaddition reactions (Fig. 2). Indeed, it was Huisgen who actually came up the term '1,3-dipolar cycloaddition' in 1960.[6] By his meticulous research, Huisgen transformed the entire field, providing unifying rationalisation that seems obvious now, but which no one else had previously appreciated. To quote Albert Szent-Györgyi[7], he "saw what everybody else had seen, but thought what nobody else had thought". He even predicted new 1,3-dipoles to create new reactions, and went on to carefully study reaction mechanisms, showing that sometimes stepwise rather than concerted processes occurred. A seminal review published by Huisgen in Angewandte Chemie in 1963 summarises these early studies.[8] Whilst any of the 1,3-dipolar cycloadditions may be sometimes be referred to as a 'Huisgen cycloaddition', it is the azide-alkyne cycloaddition that most commonly bears his name, and our discussion will be limited to this reaction.

Scheme 1 shows a non-catalysed azide-alkyne cycloaddition, often referred to as a Huisgen cycloaddition, that forms a mixture of regioisomeric triazole products.

This cycloaddition occurs when an alkyl azide and an alkyne are heated together, to form a triazole product containing a 5-membered heterocyclic ring (Scheme 1). Typical reaction conditions involve refluxing the two components in toluene, i.e. heating to approximately ~110 °C, with the reaction taking 24 h or longer to reach completion. The precise details of this cycloaddition reaction vary with the identities and electronic properties of the two reacting components. However, the reaction is not usually regioselective, i.e. a mixture of two isomeric products is normally formed, namely the 1,4-triazole and 1,5-triazole (Scheme 1). At first such a process therefore appears to be incompletely incompatible with the click chemistry paradigm created by Sharpless. However closer consideration of this reaction reveals the following important details:

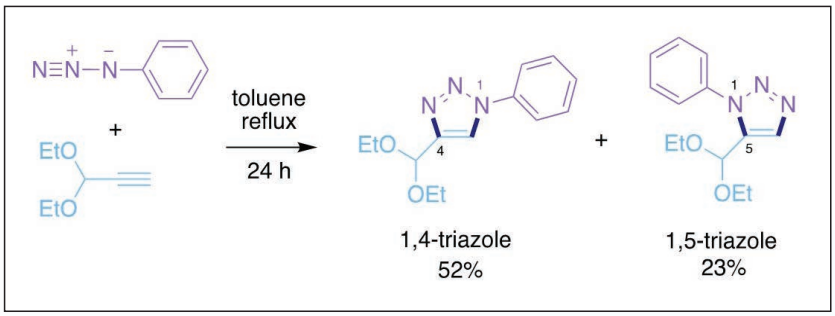
a) Neither azides nor alkynes occur significantly[9] in biological systems.

b) Both of these functional groups are 'stable', and only react with other species under quite specific reaction conditions, none of which occur in biological systems.

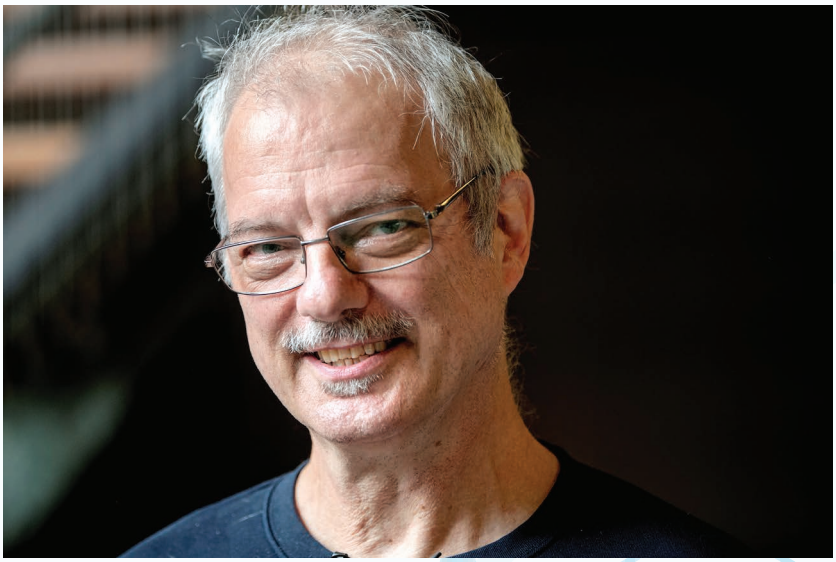
c) The Huisgen cycloaddition reaction is completely compatible with aqueous reaction conditions, and its success is generally insensitive to the local environment.

In respect of point b) above it is worth quoting Sharpless himself: "given their feared explosive nature, azides are remarkably stable to water, oxygen, and most conditions used in organic synthesis, until they are exposed to a dipolarophile."

Thus, while the Huisgen reaction was not a solution, it represented a useful starting point, particularly if methods




Scheme 1. A non-catalysed azide-alkyne cycloaddition.



*Professor Morten Meldal. Photo credit: Nils Meilvang, courtesy of the University of Copenhagen.*

to enhance the rate of reaction and - most importantly - to completely control product regiochemistry could be found. This sets the scene nicely for the development of effective catalysis of this reaction, the impact of which would be so spectacular that it would merit the award of a Nobel Prize.

## THE 2022 PRIZE WINNERS

With regard to precise timing of 'who did it first', it should be noted that Meldal's journal paper received date of December 14th 2001 predates that of Sharpless' work (April 29th 2002) by some 4 months. I shall therefore discuss Meldal's work first.

### Morten Meldal - University of Copenhagen

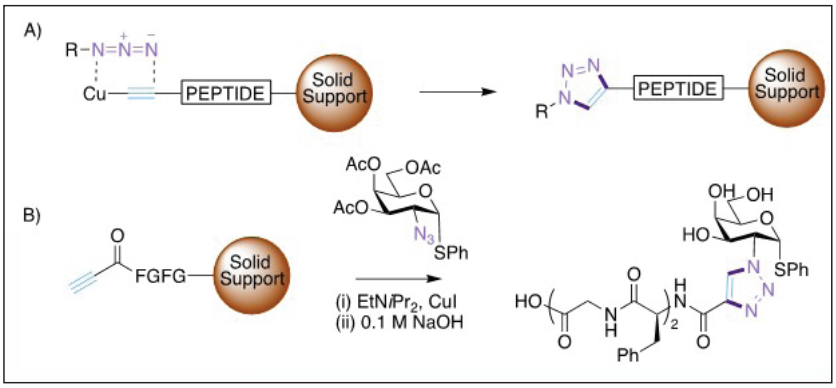
Morten Meldal has a long-standing interest in both carbohydrates, having done his PhD with Klaus Bock at Lyngby, and solid-supported synthesis, the latter particularly when it was applied to combinatorial chemistry. In the key Nobel prize-winning 2002 publication[10], Meldal and his group were seeking to increase the efficiency of the azide-alkyne cycloaddition so that it was compatible with solid-phase combinatorial synthesis of drug molecules. Their goal was to make millions of biologically interesting triazoles available for screening using the split and mix method invented by Furka.[11]

Meldal's key reaction insights, based on the earlier literature, were that some metal salts (Na, Mg, Li) of alkynes underwent the azide-alkyne cycloaddition at much lower temperatures, and that one paper, in which the authors were trying

to synthesise a propargyl azide using copper (I) chloride, reported unexpected triazole formation. Thus, Medal hypothesised that copper acetylides may be formed by reaction of the alkyne with the Cu (I) salt in the presence of a base (e.g. Hünigs base, *N,N*-diisoproplyethylamine), and that these copper acetylides would undergo cycloaddition much more readily.

When this hypothesis was explored experimentally the results were spectacular (Scheme 2). Simply adding catalytic amounts of copper (I) salts (chloride, bromide or iodide) to the reaction mixture led to the formation of triazoles in very high yield (>95% conversion), and crucially with complete regiochemical control at 25°C. Interestingly, no ligands were required for the copper. However, Cu (II) salts were ineffective, and the reaction only worked for terminal alkynes. On the other hand, the reaction worked exceptionally well on the solid-phase, and was subsequently applied to prepare a wide variety of peptide substrates.

Though the precise mechanism was not investigated at the time, given the accepted precedent that Cu insertion into terminal alkynes occurs in the presence of base (e.g. in the Sonogashira coupling), it was presumed that a copper acetylide intermediate was formed which then underwent either a stepwise or concerted cycloaddition with the azide to form only the 1,4-triazole product.




Scheme 2. Meldal's Cu (I)-catalysed Huisgen cycloaddition. A) Formation of a copper acetylide that controls reaction regiochemistry. B) Solid phase synthesis of a glycopeptide.

## Barry Sharpless - Scripps Research Institute La Jolla, California

The name Barry Sharpless is probably known to all chemists. He now joins Fred Sanger as only the second person ever to win the Nobel Prize in Chemistry twice, having won a 50% share in 2001 for his development of asymmetric synthesis - the ability to produce single enantiomers of chiral compounds starting from non-chiral starting materials. More specifically he created both the Sharpless asymmetric epoxidation of allylic alcohols and the Sharpless asymmetric dihydroxylation of alkenes. Both of these transformations have long been established parts of the synthetic organic chemist's repertoire. They are extremely reliable, the outcome is predictable, and which enantiomer of the product is formed can be chosen at will since the chiral catalysts used are readily available.

Professor K. Barry Sharpless. Photo credit: courtesy of Scripps Research.

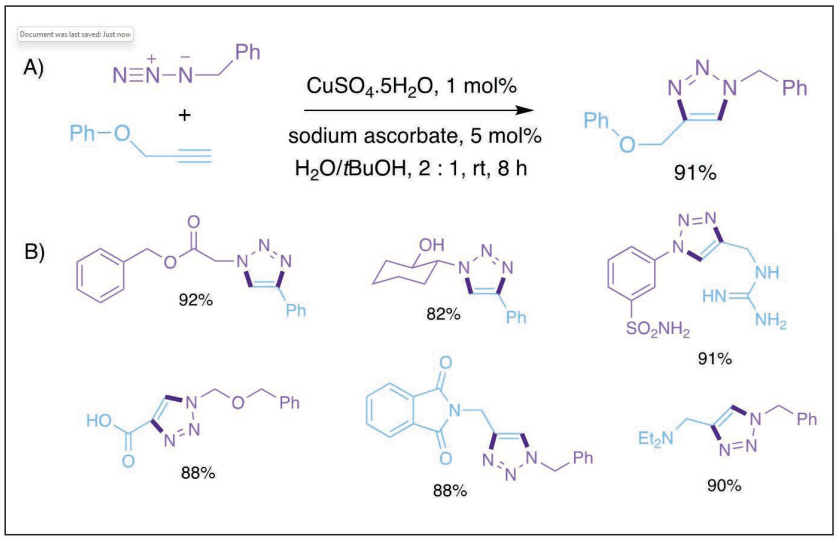
In the organic chemistry world, Sharpless' creativity and imagination are therefore legendary. Indeed, some stories around this topic are probably apocryphal, and certainly libellous. What is certain is that in a stroke of creative genius, Sharpless created the concept and coined the phrase 'click chemistry.' Next, he went on to translate his concept into a working reality by the independent discovery of Cu (I) catalysis of the Huisgen azide-alkyne cycloaddition.

Sharpless's group's[12-13] seminal publication on Cu (I) catalysis of the azide-alkyne cycloaddition appeared in print shortly after Meldal's. In this paper, which has now been cited more than 13,000 times, Sharpless reported that the Cu (I) catalyst was best made by *in situ* reduction of Cu (II) salts, such as $CuSO_4$ hydrate, by ascorbic acid, or sodium ascorbate. As little as 1 mol % of the catalyst was sufficient. The reaction was 'extremely forgiving', did not require any special conditions or precautions, was complete at room temperature after 6-36 h, and worked in a range of solvents, most importantly water without any added organic co-solvent. Thus, the reaction had wide scope and functional group tolerance, and was essentially 'fool-proof.'

In this paper, Sharpless and Fokin went on to propose a catalytic cycle for the Cu(I)-catalysed ligation reaction, which involved an intermediate Cu acetylide. Based on density functional theory (DFT) calculations, a step-wise mechanism

was proposed instead of a concerted cycloaddition process. Later studies by Fokin and Finn would reveal that two Cu(I) ions were involved, and that the mechanism was slightly more complicated than originally proposed.[14]

Despite these minor uncertainties, one thing was sure; the first 'perfect' click reaction had been discovered. Although Meldal's reaction conditions using a Cu (I) additive were reported first, it is the use of Cu (II) and its *in situ* reduction to the active Cu (I) species by sodium ascorbate, that has become the almost exclusively applied procedure. Click chemistry had become a reality, using a simple reaction that could be performed by anyone, anywhere, and at any time.




Scheme 3. Sharpless and Fokin's Cu-catalysed azide-alkyne cycloaddition. A) Generation of the Cu (I) catalyst in situ by reduction of $CuSO_4$ with sodium ascorbate. B) Representative examples.

## Carolyn Bertozzi - Stanford University, California

Like Meldal, Carolyn Bertozzi is a carbohydrate chemist by background, having done her PhD with Mark Bednarski at Berkley on the chemical synthesis of C glycosides. Realising that sugars are of vital importance throughout biological systems, she embarked on her own research programs at the interface of chemistry and biology. The genius of Bertozzi was to recognise the golden opportunities of applying synthetic chemistry techniques directly in biological systems, and click chemistry ultimately provided an excellent means of functionalising biomolecules in living systems.

Bertozzi developed the concept and coined the term 'biorthogonal chemistry' in 2003[15], which is defined as a chemical reaction that can occur within living cells, which neither affects, nor is affected by, the myriad of native biochemical species and metabolic processes that may also be present. It doesn't take much of an intuitive leap to then see that click chemistry, as defined by Sharpless, provides the perfect basis for bioorthogonal chemistry. Indeed the case that Bertozzi made for the using

Professor Carolyn Bertozzi. Photo credit: Armin Kübelbeck, published under Creative Commons License CC-BY-SA, https://commons.wikimedia.org/wiki/File:Carolyn_Bertozzi_IMG_9384.jpg

biorthogonal click chemistry to study biological systems was extremely compelling. For example, would it not be extremely useful if one reacting handle of a click chemistry pair could be incorporated into a cell, and then the other reacting handle used as a probe to find its location? Furthermore, attaching a label such as a fluorophore to a 'clickable' probe would allow techniques such as fluorescence microscopy to be used to visualise the whole process in living cells.

Azide, which is not itself found within biological systems and displays essentially no reactivity towards biological systems or metabolites, appeared to be the perfect reactive handle with which to perform bioorthogonal chemistry. The first key development by Bertozzi was the demonstration that reactive azide handles can be incorporated either into recombinant proteins, and even onto the surface of live cells, simply by feeding cells with a peracetylated azido *N*-acetylmannosamine sugar (Fig. 3A, feedant).

This is possible because the sugar sialic acid is found at the extremities of the sugar chains (glycans, or oligosaccharides) attached to mammalian proteins and to mammalian cell surfaces. Sialic acid is biosynthesised within cells from *N*-acetylmannosamine and pyruvate (shown in red) in an aldol reaction. However, the enzymes that catalyse this process are promiscuous and also accept azido *N*-acetyl mannosamine **1** (formed inside the cell following loss of the acetate groups from the peracetylated feedstock) as a substrate, to produce azido-sialic acid **2**. Thus, when the surrogate azide containing substrate is fed to cells, some of the sialic acids that it makes will then contain an azide. These sialic acids are further metabolically

processed to occupy some of the positions throughout the cell that sialic acid normally occupies, and so provide bioorthogonal reactive handles to potentially carry out click chemistry.

Given this ability to incorporate azide into living systems, the opportunity for selective reaction of azide in order to attach externally administered chemical species became possible. Although Bertozzi had developed other approaches to bioorthogonally react azide, such as the Staudinger ligation[16], the Cu-catalysed variant of the Huisgen cycloaddition developed by Meldal and Sharpless seemed like the perfect reaction to apply.[17] However, there was one big problem; the copper. Copper is toxic, and so cannot be used in cells or living organisms. Furthermore, often the reaction still required heating to a moderate temperature in order to increase its rate, with reactions often taking as long as 36 h at room temperature. So, in order to develop a version of the Huisgen cycloaddition that could be applied directly in biological systems something else was needed. Bertozzi therefore very wisely did something that far too few of us academics ever do; she looked back into the old chemical literature.[18] She then realised that putting an alkyne in a medium sized ring greatly increased its reactivity and so here was a potential solution to the Cu toxicity and rate of reaction problems.

In the first example reported (Scheme 4), Bertozzi used a cyclooctyne as the dipolarophile. An 8-membered ring is the smallest ring into which an alkyne may be incorporated to form a stable product.[19] In this instance the cyclooctyne was attached via a short linker to a biotin 'handle', which was attached as a reporting unit to enable confirmation that subsequent reaction had occurred later on using anti-biotin antibody and Western Blotting techniques. In this way, Bertozzi was able to demonstrate that click reactions had occurred successfully, first using

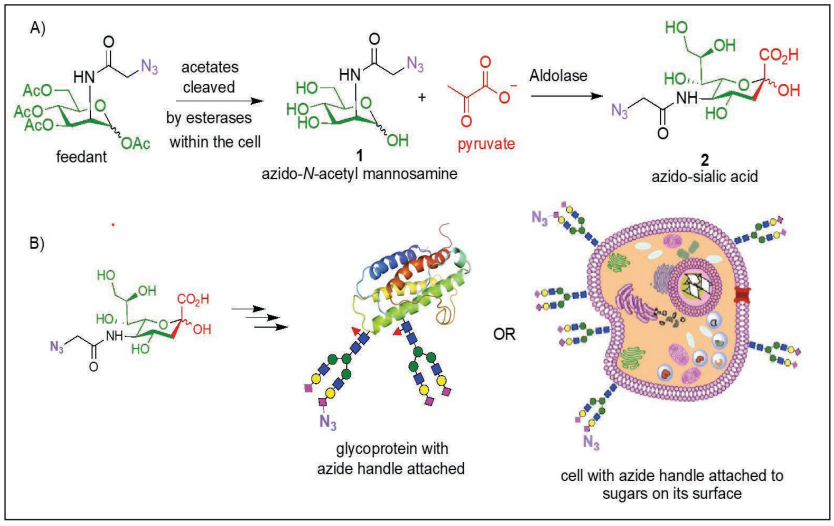
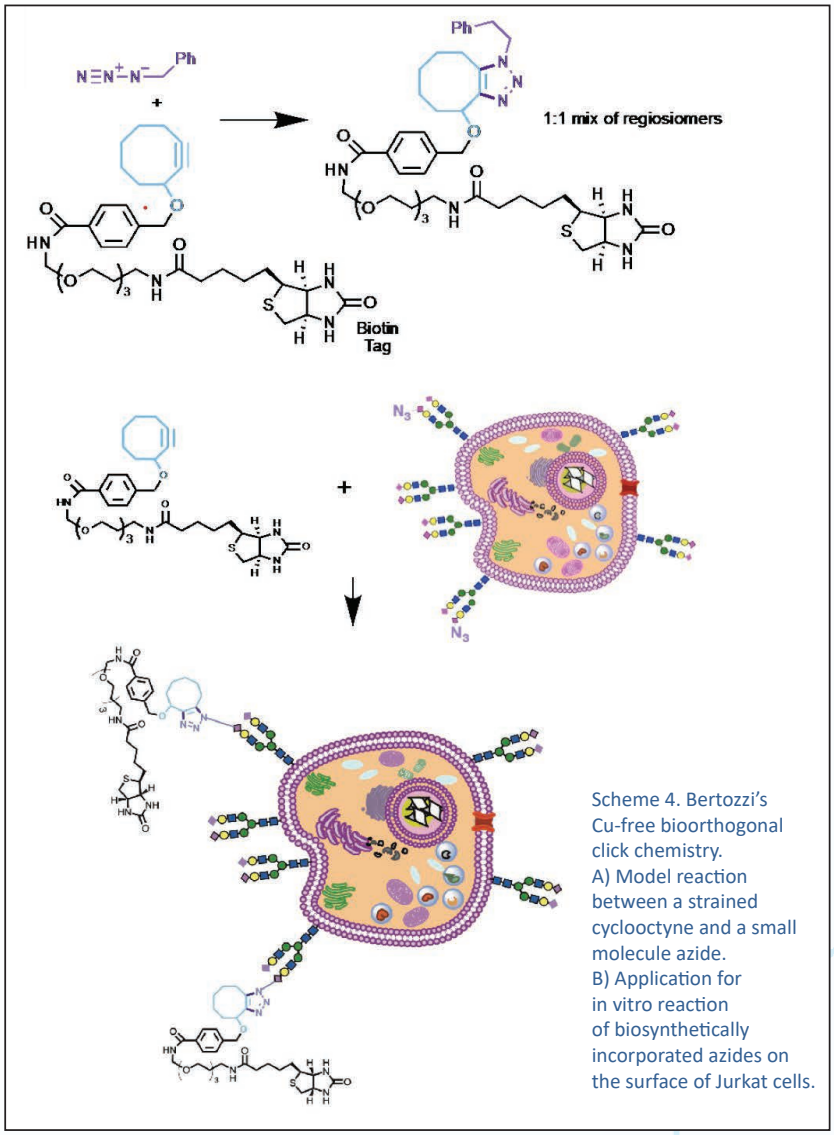



Figure 3. Feeding experiments allow incorporation of azide into biomolecules. A) Biosynthesis of azido-sialic acid **2** by a promiscuous aldolase which accepts azide-containing N-acetyl mannosamine **1** as a substrate. B) Incorporation of azido-sialic acid **2** into sugar chains (glycans) that are attached to proteins and to the surfaces of mammalian cells.




Scheme 4. Bertozzi's Cu-free bioorthogonal click chemistry. A) Model reaction between a strained cyclooctyne and a small molecule azide. B) Application for in vitro reaction of biosynthetically incorporated azides on the surface of Jurkat cells.

small molecule models (Scheme 4A, note the reaction is not regioselective but this is of no consequence), then a recombinant glycoprotein produced in Chinese Hamster Ovary (CHO) cells, and finally demonstrating that click reactions had occurred on the surface of Jurkat cells (Scheme 4B, immortalised human T lymphocyte cells). Later refinements were made to the alkyne component,[20] including the introduction of two election withdrawing fluorines on the cyclooctyne ring (to increase the rate of cycloaddition with azide by lowering the energy of the LUMO of the alkyne), and the development of a water soluble hydrophilic cyclooctyne suitable for reactions in aqueous biological systems.[21]

The 'icing on the cake' was a seminal paper[22] using dynamic imaging which allowed intracellular trafficking of sugar chains (glycans) containing sialic acid to be visualised. These experiments were performed *in vitro* using CHO cells, and even *in vivo* within a living mouse, illustrating the power of bioorthogonal chemistry as a tool to enable biological processes to be interrogated.

## SOME THOUGHTS ON THE PRIZE AWARDS

Morten Meldal's group's seminal publication was quite remarkably published in the *Journal of Organic Chemistry* in 2002, commonly referred to by organic chemists as 'JOC'. Without meaning any disrespect to the editors of, or authors who publish excellent work in JOC, this is in a 'lower tier' of American Chemical Society (ACS) journals and currently has an impact factor of only 4.3. One wonders if this was indeed the journal that Meldal submitted his work to, or perhaps if a journal editor of a higher-ranked ACS journal declined to publish the paper saying it was 'too specialised' or 'not of interest to our broad readership'? This is of course mere speculation on my part, and perhaps JOC was the first and only destination for this particular publication.

However, like many other chemists who fight against perceptions of 'relative importance' during both editorial 'sifting' and then the anonymous peer review process, I am heartened that the Nobel Committee recognised this work for its real value, and that one can be awarded a Nobel prize essentially for a single publication in a middle tier journal such as JOC.

Furthermore, anyone who has studied the history of science in detail, or, perhaps rather like me who has read Bill Bryson's excellent book, *A Short History of Nearly Everything*[23] will be all too aware that throughout history often the real inventors or discoveries of processes have often been forgotten in favour of more 'popular' or 'more powerful' figures who have grabbed all of the limelight. It is particularly pleasing that Morten Meldal was awarded his fair share of this years' prize, despite

## THE RISE OF CLICK CHEMISTRY

Database searches and citation counts give some idea of the impact of a field. A Scopus search (October 2022) for the term 'click' in article titles, keywords or abstracts produced >49,000 hits, with approximately 3,500 hits per year for the years 2019, 2020 and 2021. This massive citation count is unsurprising, since the beauty of click chemistry, as Sharpless originally envisioned, is that it can basically be applied anywhere, anytime, and by anyone. The reaction is by design so robust and insensitive to other reagents or functional groups, that it doesn't matter what the substrates are; the reaction just works. Furthermore, the reaction works well in water and is insensitive to oxygen, so basically anyone can do it (even biologists ☺); prior expertise in synthetic organic chemistry is absolutely not required!

Therefore, it is no surprise that the Cu-catalysed azide-alkyne cycloaddition has now been applied to almost every situation that one can imagine where the objective has been to join two molecules linked together, including (but not limited to) pharmaceutical development and drug discovery, conjugation of tags to biomolecules, DNA sequencing, and throughout materials science.

It is unfortunately not possible in an article such as this to begin to scratch the surface of the myriad applications of this truly remarkably robust reaction, though many excellent reviews are available.[24] Even my own research group jumped on the 'click bandwagon', so to speak, and developed an application of click chemistry to allow the direct conjugation of unprotected sugars to other species in aqueous solvent systems.[25] Other reactions have been promulgated as 'click' chemistry, for example the thiol-ene reaction which comprises the formal addition of a thiol to an alkene, and proceeds either by free radical or Michael-type mechanisms. However, none of these other reactions matches the performance of the Cu-catalysed azide-alkyne cycloaddition. Therefore, to the majority of chemists the Cu-catalysed Huisgen cycloaddition remains the pre-eminent example of click chemistry, and indeed it is widely regarded as synonymous with the term 'click reaction'.

## THE RISE OF BIOORTHOGONAL CHEMISTRY

Whilst the concept of biorthogonality is by definition less widely applicable than click chemistry (a Scopus search produces a mere 2,500 hits), it provides chemists with unique methods to study biomolecule interactions inside cells, and crucially also to decipher disease and disease processes. Indeed, bioorthogonal chemistry has become one of the cornerstones of chemical biology research. For the uninitiated, chemical biology is the application of chemistry, and in particular synthesis, to study and manipulate biological systems. Clearly a chemical reaction that you can perform in water, in the presence of oxygen, that links together two molecules containing mutually reactive handles in essentially quantitative yield is rather useful for the study of biology! Moreover, if you can even carry out click chemistry in living cells, while looking at the cell through a microscope at the same time, then so much the better. Unfortunately, again there is not space in this article to even begin to do justice to the field of bioorthogonal chemistry, and the interested reader is therefore strongly encouraged to consult one of the many excellent specialist reviews on this subject.[26]

the fact that the Nobel laureate Sharpless essentially made the same discovery at the same time.

### AFTERTHOUGHTS

Sharpless is a creative genius, who absolutely deserves his pre-eminence in the chemistry world by the award of a second Nobel Prize. It was he who created the click chemistry concept, but it was Meldal who discovered the first example, pipping Sharpless at the post by a few months. Bertozzi is another creative genius, who furthermore possessed the insight, courage, and drive to tackle the toughest, but perhaps the most rewarding, of problems; how to do synthetic chemistry in living biological systems. All three are truly worthy winners of the 2022 Nobel Prize in Chemistry and I congratulate them wholeheartedly.

Rolf Huisgen's death in 2020, at the amazing age of 99, was ultimately untimely, not least because he fell short of a remarkable century by only three months. Astonishingly Huisgen had continued to publish until 2018.[27] He was a true giant of organic chemistry, and it is fitting that one of his numerous legacies was the fundamental reaction for which the 2022 Nobel Prize was awarded.

Had he still been alive the Nobel Committee may have been faced with a real conundrum as to who to omit from this year's prize, as there can only be three winners. One also wonders whether without the seminal work of Huisgen, would either Sharpless or Meldal would have focussed on that particular cycloaddition reaction as a means of linking two molecules together? On the other hand, Bertozzi had already developed her first bioorthogonal reaction - the Staudinger ligation - so the future development of bioorthogonal chemistry was in some respects already assured.

Without Huisgen one also wonders if alternative solutions would have been found, or in fact whether they even exist? It is notable that, 20 years after the first reports, the Cu-catalysed and strain promoted azide-alkyne cycloaddition reactions are still pre-eminent over all other click reactions that have been developed; nothing else actually comes close. The final question therefore is, "Are there other click reactions out there that are yet to be discovered?" We shall have to wait and see.

Finally I conclude in a similar vein to last year.[28] Katalin Karikó and Drew Weissman surely must win the Nobel Prize in Chemistry for their work on mRNA vaccines at some not too distant point in the future. Maybe 2023 will be their year? If so, then I may be back again to complete a trilogy of articles.

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Article

# Kaimoana molecules: can we make the world our oyster?

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## Introduction

"*Ahakoa iti te pipi o tōku kāinga, he waiū tangata tonu*"[1]

"*The shellfish of my home may be small, but they nourish the people*"

This whakataukī speaks of the value of kaimoana shellfish to the traditional Māori diet: small, yet mighty. Their nutritional value, sought-after taste and ready accessibility to Aotearoa New Zealand's earliest settlers made them an essential part of the traditional coastal Māori diet and many, including pāua, were considered taonga (treasures). Kaimoana was also a valued trading commodity for coastal peoples, exchanged with inland tribes in return for forest products such as birds and rats.

Today, kaimoana shellfish are still highly prized for their nutritional value and are regularly consumed, though more commonly as a product of farming than of foraging. New Zealand's Greenshell™ mussel and Bluff oyster industries are now worth more than 30 million NZD and are claimed to be some of the most sustainable aquaculture practices in the world.[2]

But, as chemists, why should we be interested in these squishy little species? Worldwide, shellfish are renowned as an excellent source of lipids, including omega-3 polyunsaturated fatty acids (PUFAs). Shellfish are also a particularly good source of phospholipids and the combination of omega-3 PUFAs within phospholipid structures makes for a highly diverse class of molecules with potential biological functions. This diversity, along with their biological applications, makes these an interesting and rewarding class of molecules to study.

■ Ioan Fuller obtained his BSc(Hons) in chemistry at the University of Otago in 2018 before spending a year working at Plant & Food Research in Nelson with the Marine Products Team. In 2020 he returned to the University of Otago to begin his PhD in chemistry under the supervision of Prof Nigel Perry, Prof Chris Hepburn and Dr Daniel Killeen, studying the phospholipid composition of kaimoana shellfish. Outside of research, Ioan is a keen climber, runner and musician.

Overseas research has characterised the phospholipid composition of a range of shellfish species: mediterranean mussels, blue mussels, oysters, clams and whelks.[3] Three major phospholipid classes are most commonly reported: phosphocholines, phosphoethanolamines and ceramide aminoethyl phosphonate, alongside many other minor phospholipid classes,[3] some of which are depicted in Fig. 1.

However, New Zealand lipid research on kaimoana shellfish has been confined almost entirely to the commercially relevant Greenshell™ mussel and the lipid profiling techniques have focused primarily on fatty acid analysis by gas chromatography-mass spectrometry (GCMS) or lipid class analysis by thin layer chromatography (TLC) and $^{31}$P nuclear magnetic resonance spectroscopy ($^{31}$P NMR).[4] But beyond Greenshell™ mussels are

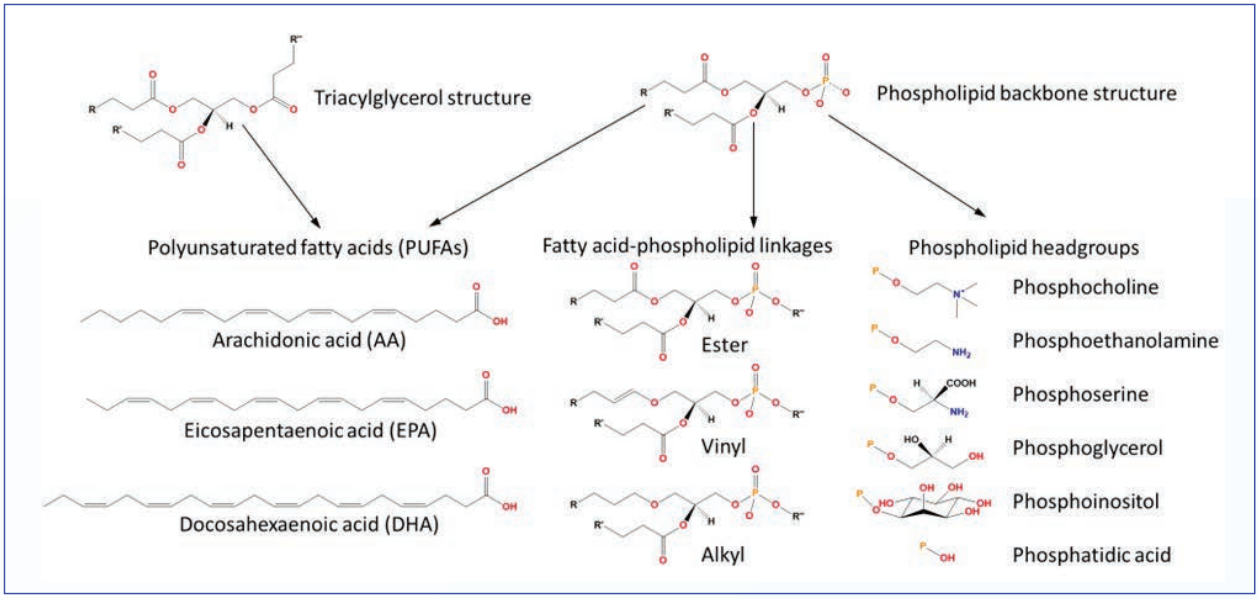


Fig. 1. Sources of variation within triacylglycerol and phospholipid structures, illustrating some common fatty acids, fatty acid-phospholipid linkages and phospholipid headgroups.

many other kaimoana shellfish with unexplored lipid chemistry, some of which are shown in Fig. 2.

Of course, we are not the first to learn about these species. Many of these species have a strong association with Mātauranga Māori, relating to the time of year that they should be eaten, their health benefits and even how to translocate them in an early form of aquaculture.[5] Sustainability was always key to the traditional collection of these kaimoana, and is all the more important today. This research aims primarily to add value to our taonga species by increasing our understanding and appreciation of the world around us and promoting sustainable use to provide benefit to all.

## What is a phospholipid?

Lipids are a class of molecules that include all the things we'd recognise as fats and oils, such as cooking oil, wax or adipose tissue. But they also include a few things that might not jump immediately to mind, for example forming a key part of cell membranes or acting as signalling molecules in our bodies.

Fatty acids form one of the major lipid classes and are incorporated into many more complex lipids. These complex lipids can be broadly grouped into 'neutral' and 'polar' classes. 'Neutral' lipids include tri-, di- and mono-acylglycerols, which consist of fatty acids esterified to a glycerol backbone (Fig. 1). These are primarily used for energy storage in marine organisms and are the easiest lipids to extract and purify, so they have become the lipid class commonly found in supermarket omega-3 supplements. 'Polar' lipids, on the other hand, are much more complex lipid classes, and the most common of these are phospholipids. These consist of one or two fatty acids attached via ester, alkyl, or vinyl bonds at the *sn*-1 and/or *sn*-2 positions of a glycerol backbone, with a phosphate or phosphonate group at the *sn*-3 position. To the phosphate is then attached an organic headgroup, which can vary in size from a small choline group right up to an entire other phosphate-glycerol-fatty acid group. There are five common classes: phosphocholine; phosphoethanolamine; phosphoserine;

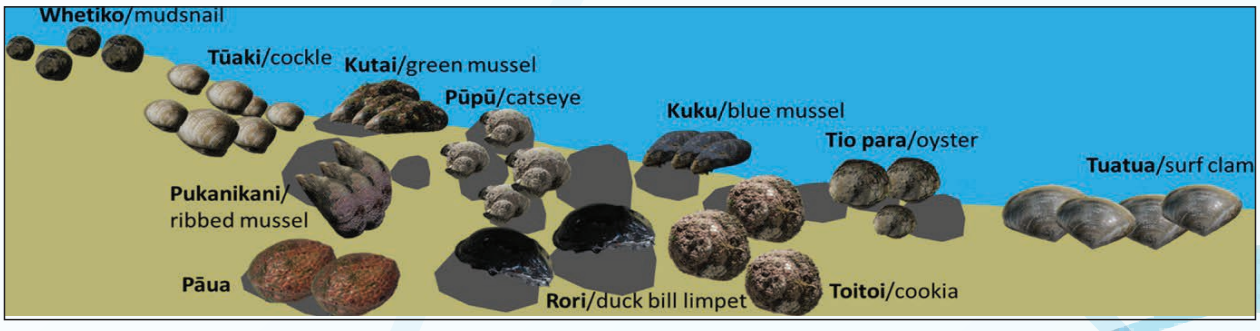


Fig. 2. A selection of some common kaimoana shellfish from the intertidal and subtidal zone.


Fig 3. A workflow for phospholipid identification. Left to right: selection of species for study → oil extraction and fractionation → LCMS analysis → phospholipid identification.

phosphoinositol and phosphoglycerol, their precursor being phosphatidic acid (Fig. 1).[6] Lyso- variants of each class and subclass can also be formed from selective hydrolysis of fatty acids by phospholipases.

The lipid structure can be further modified by the addition of other structures such as ceramides, which form the phosphonolipid class. Phosphonolipids can be formed by the attachment of 2-aminoethylphosphonoic acid (ciliatine) to either a ceramide, diacylglycerol or carbohydrate glycolipid type. The phosphonolipid 'ceramide 2-aminoethylphosphonate' is often found in marine organisms. These lipids are part of the sphingolipid class, which contain a long chain sphingoid base backbone and are characterised by a carbon-phosphorous bond.

In combination, these modifications to the phospholipid structure create a lipid class with thousands of potential molecular structures.[6] Characterising the phospholipid composition of kaimoana shellfish is a satisfying challenge to tackle, and most lipidomics studies identify 150–250 unique phospholipid molecules.[3] This requires a strategic approach. We can use the somewhat regular structure of the molecular class to our advantage and use a raft of analytical techniques to build up a picture of the phospholipid profile of any one species.

The history of lipid analysis started with gas chromatography (GC) to identify different fatty acids, before TLC and high performance liquid chromatography (HPLC) techniques were developed to identify different lipid classes. $^{31}$P NMR also provides insight into the range of phospholipid classes present. More recently, mass spectrometry (MS) techniques have been developed to facilitate the study of both fatty acids and lipid classes simultaneously using fragmentation patterns to identify the molecular structures. Today, liquid chromatography coupled to mass spectrometry (LCMS) is considered the gold standard for phospholipid analyses,[3] and a common workflow is shown in Fig. 3.

"We can use the somewhat regular structure of the molecular class to our advantage and use a raft of analytical techniques to build up a picture of the phospholipid profile of any one species."

These analytical techniques work additively, so carefully combined approaches allow us to understand the lipid profile of kaimoana species.

## Phospholipids for improved omega-3 bioavailability

The potential variation present within the class of phospholipids can give a highly tuneable molecule, with the potential to perform multiple roles in an organism and be affected by many environmental factors. For example, marine creatures in cold environments tend to accumulate more PUFAs, which help maintain better membrane fluidity at low temperatures. In our bodies, it is well established that omega-3 PUFAs are the key lipid structures that confer health benefits and are a common supplement, readily available on supermarket shelves. These are biosynthesised by marine algae and bioaccumulated in filter-feeders and grazers.[7]

Two omega-3 PUFAs are essential: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (structures shown in Fig. 1). EPA is a key prostaglandin precursor, while DHA is the major lipid component of the brain and nervous tissue, and deficiencies in either of these molecules is a sign of multiple neurodegenerative diseases. Our bodies do not do a good job of biosynthesising these long-chain omega-3 polyunsaturated acids, with only a tiny contribution coming from conversion of the primarily plant-sourced α-linolenic acid.[8] Furthermore, the process of moving DHA from the gut to the brain is complex, with many biological hurdles for the molecule to overcome. For example, only free fatty acids and lyso-phospholipids appear able to cross the highly selective blood-brain-barrier.[8] Therefore, it is ideal to include the most direct sources of essential omega-3 PUFAs like EPA and DHA in our diet.

Fish have long provided the major source of our omega-3 PUFAs, and I expect many of us are familiar with the dietary recommendation to include oily fish in our diet at least once a week.[9] However, shellfish also provide an excellent source of omega-3 PUFAs and their production, when done well, can be more sustainable than fishing. Shellfish are also rich in phospholipids, and the combination of phospholipids and omega-3 PUFAs has been the topic of interesting research in recent years. Specific esterification of omega-3 PUFAs to different phospholipid structures has been shown to modify the bioavailability of these omega-3 PUFAs.[8,10] A more bioavailable source of omega-3 PUFAs means that our bodies can more efficiently use what we consume and it may be possible that adequate omega-3 PUFA intake, particularly DHA, can help maintain our brain's health into later years.[8]

When comparing the relative bioavailability of omega-3 PUFAs in different lipid forms, conventional triacylglycerol and ethyl ester omega-3 supplements score the lowest. Free fatty acids are more bioavailable, and phospholipids are the most readily absorbed form. However, bioavailability also varies between phospholipids, and phosphocholines and phosphoethanolamines provide the best omega-3 PUFA bioavailability.[10] These are two of the most common phospholipid classes found in shellfish, which is very promising for the potential efficacy of these species as foods with the potential application to help improve brain health.[3]

"It is important to remember that many of these species are unique to Aotearoa New Zealand and as taonga species, any and all applications should be done in a sustainable manner."

And where might we find these omega-3 PUFA phospholipids, I hear you ask. Do they have to be synthesised via costly, complex organic protocols? Are they difficult to extract, or to prepare? Well, it doesn't seem so to me. Kaimoana shellfish may provide the answer: naturally rich in omega-3 fatty acids, including DHA and EPA, along with a diverse phospholipid composition, they may just be a perfect source of these brain-sustaining molecules. *"Ahakoa iti te pipi o tōku kāinga, he waiū tangata tonu,"* these species are indeed small, yet mighty. It is important to remember that many of these species are unique to Aotearoa New Zealand and as taonga species, any and all applications should be done in a sustainable manner, with appropriate collaboration and engagement with local communities and with Mātauranga Māori.

## Acknowledgements

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Article

# Valuable natural products from mānuka and its cousin kānuka

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Keywords: kānuka, mānuka, natural products, antimicrobial

## Introduction

We are investigating the underexplored chemistry of *Kunzea* (kānuka / tea tree) in Aotearoa New Zealand in search of antimicrobial compounds and returning this knowledge of molecular basis of activity to Māori kaitiaki (guardians) of this taonga (treasured) species. The results of our investigation are being linked into innovative outreach activities that expand traditional offerings, fusing mātauranga (traditional knowledge), chemistry and biology to engage rangitahi (youth) in pūtaiao (science).

This paper reviews natural products from taonga plant species, with a particular focus on the New Zealand Myrtaceae representatives. Within NZ Myrtaceae, the bioactivity of mānuka, the most economically important species, is explored and contrasted with our current knowledge of kānuka bioactive natural products.

■ Liam Hewson recently completed his BSc degree in chemistry and plant biotechnology at the University of Otago in 2021, including undertaking a third-year research project on kānuka.

This year, he is expanding upon his work with kānuka, undertaking an MSc in chemistry with the University of Otago / Plant & Food Research Dunedin, exploring the bioactives chemistry of kānuka under the supervision of Prof Nigel Perry and A/Prof Bill Hawkins. Outside of the lab, Liam loves spending time tramping in the ngahere, and is developing a passion for trail running.

## Novel natural products from taonga species of Aotearoa New Zealand

New Zealand has a unique range of flora species that have differentiated as a result of geological isolation from other landmasses,[1] leading to a high degree of endemism in vascular plants. This distinct evolutionary lineage has allowed these species to develop unique bioactive natural products (with novel structures), which we can today study by isolation and identification.

Knowledge of natural products extends back to the first Māori in New Zealand, who encountered the fruit of the karaka tree – a novel species to Māori – and its highly poisonous seed. Mātauranga records a complex treatment of extended cooking and washing to leach the karakin alkaloid and make this into a nutritious and safe food source. With this decontamination knowledge, karaka was widely planted at Māori settlements around Aotearoa, establishing a reliable propagated food supply. In a similar vein, tutu (*Coriaria spp.*) was made into a refreshing drink, sweetener and jelly by straining the juice of the enfolding petals of the berry through toetoe or raupō flower heads – with multiple uses in healing and antiseptic applications. Failing to do this scrupulously enough caused acute poisoning from the neurotoxic tutin contained in all other plant parts.

A survey of New Zealand indigenous plant extracts showed widespread biological activity across sampled species, especially cytotoxic and antibacterial activities.[2] Chemometric analysis of compounds from native New Zealand medicinal flora found 80% of the bioactive compounds were in known physicochemical drug space, and 10% to be lead-like. Therefore these compounds are well suited to further screening and drug development projects.[3]

Investigation of other taonga species has found numerous compounds with novel structures and properties. Horopito (*Pseudowintera colorata*) was found to have antibacterial, antifungal and insecticidal properties, among other bioactivities. A native mushroom (*Iliodiction cibarium*) used as a food source by Māori, contained uronic acids and an unusual, unresolved polysaccharide. Novel cannabinoids have also been identified in a liverwort (*Radula marginata*).

## NZ Myrtaceae and valuable natural products

Myrtaceae is a large family of flowering plants with leaves containing oil glands that are a rich source of natural products, particularly acylphloroglucinols and phloroglucinol adducts. Familiar examples include *Eucalyptus* (gum trees) and mānuka. It comprises 121 genera and be-

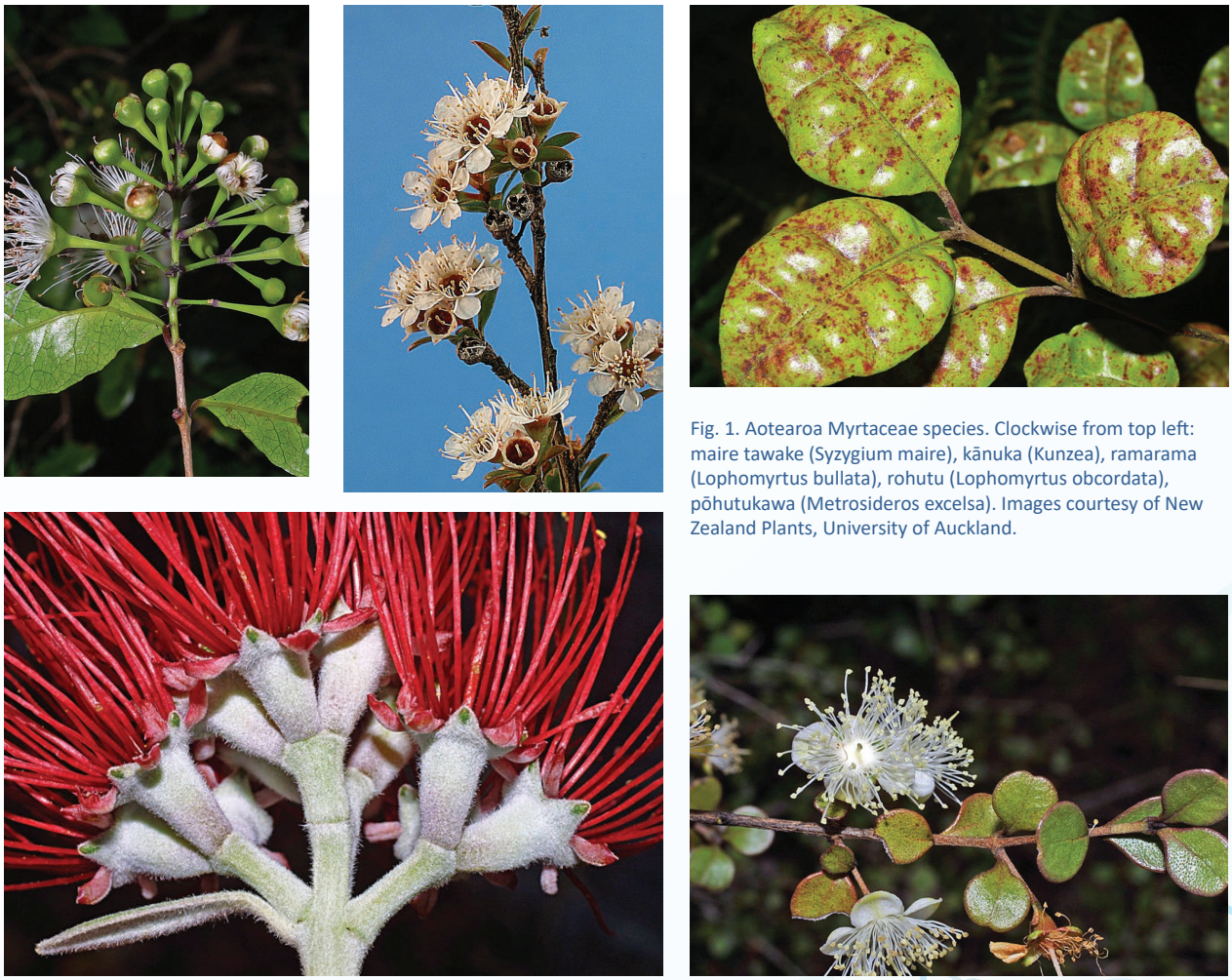

Fig. 1. Aotearoa Myrtaceae species. Clockwise from top left: maire tawake (Syzygium maire), kānuka (Kunzea), ramarama (Lophomyrtus bullata), rohutu (Lophomyrtus obcordata), pōhutukawa (Metrosideros excelsa). Images courtesy of New Zealand Plants, University of Auckland.

tween 3800 and 5800 species, with a tropical-subtropical centre of diversity and reach extending into temperate Australasia.

Myrtaceae is a family known for producing secondary metabolites (essential oils) in oil glands, and has a rich history of ethnomedicinal use worldwide.[4] This family has been fruitful in natural products chemistry worldwide, but is less investigated in New Zealand.

New Zealand is home to six indigenous genera of Myrtaceae (Fig. 1): *Kunzea* (approx. 10 species), *Leptospermum* (*L. scoparium* – two varieties), *Lophomyrtus* (*L. bullata and L. obcordata*), *Metrosideros* (12 endemic species), *Neomyrtus* (monotypic endemic genus - *N. pedunculata*), and *Syzygium* (one endemic species, *S. maire*).[5] These indigenous species have high ecological, cultural and social value, and are iconic, such as pōhutukawa (*Metrosideros excelsa*), rātā (*Metrosideros spp.*), and mānuka (*Leptospermum scoparium*). Others, including *Lophomyrtus bullata*, contain unprecedented molecular frameworks.

"The reason for the DHA content in mānuka honey remains a mystery, which is still actively under investigation."

Locally and internationally, mānuka honey is perhaps the most recognised and economically important example of a product from NZ Myrtaceae species. A 2020 MPI report estimated that the industry generated $374 million NZD in export revenue,[6] representing a significant high-value sector and of particular note given the minimal manufacturing investment required.

Mānuka honey contains MGO (methylglyoxal), derived from nectar, DHA (dihydroxyacetone) and other bioactives that provide antimicrobial activity beyond that of other floral honey. The reason for the DHA content in mānuka honey remains a mystery, which is still actively under investigation.

Alongside this sits mānuka leaf oil, which is known to also be highly

antimicrobial, but due to different compounds compared with honey. The bioactivity of mānuka oil varies extensively by regional chemotypes,[7] with the best oil being sourced from the East Cape of the North Island. Currently demand is outstripping supply for this oil chemotype.[8]

This particular chemotype is enriched with the beta-triketones (Fig. 2), the class of antibacterial (and herbicidal) compounds in mānuka oil – especially leptospermone which is the major bioactive component.[9] The β-triketones are considered to derive from polyketide biosynthetic pathways and are mainly found in plants within Myrtaceae. These bioactive structural moieties are commonly found as building blocks in more complex bioactive molecules across New Zealand and overseas Myrtaceae – often as adducts with other products of secondary metabolism (flavanones, monoterpenes and sesquiterpenes, for example).

While mānuka (oil and honey) has a clearly established chemotypic pattern and understanding of bioactivity, particularly antimicrobial activities, kānuka (*Kunzea sp.*) is underexplored in terms of bioactives chemistry. While there is evidence of some variation present in *Kunzea*, this has not been quantified to the same extent as mānuka owing to the lesser commercial interest in kānuka.

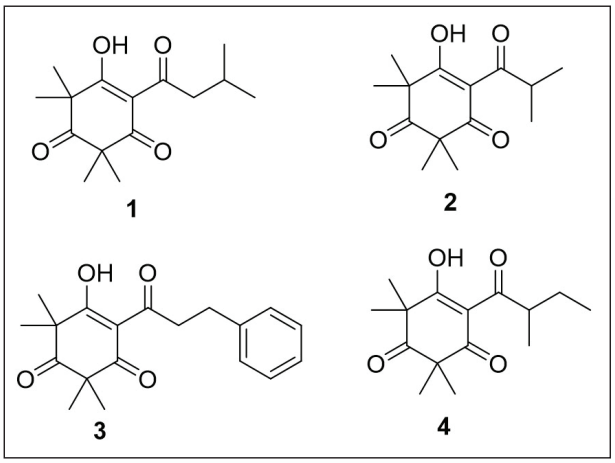


Fig. 2. Beta-triketones of Leptospermum scoparium (mānuka). 1: Leptospermone, 2: flavesone, 3: grandiflorone, 4: isoleptospermone.

"It is important to signal that while we as scientists may use binomial names and classifications, Māori do not necessarily distinguish these species in the same way."

## Mānuka vs kānuka

Kānuka (the *Kunzea* species complex in NZ) is similar in application and appearance[10] to the related mānuka (*Leptospermum scoparium* – a botanically distinct species, like a plant cousin to kānuka), which contains several rare and bioactive compounds effective against bacteria, viruses, other plants, and some autoimmune conditions.[10] While mānuka is relatively well characterised, kānuka remains much more of a mystery in terms of chemical composition, variation and even the number of species.

It is important to signal that while we as scientists may use binomial names and classifications, Māori do not necessarily distinguish these species in the same way: other names used include the generic 'tea-tree', kahikatea, red (*L. scoparium*) and white (*Kunzea*) mānuka;[10] pronunciation also varies by dialect.

Mānuka and kānuka can be distinguished by their flowers/seeds and leaf morphology; mānuka have large, singular flowers/seeds, while kānuka have clusters of small flowers/seeds (Fig. 3).

Fig. 3. Left: Kānuka (Kunzea sp. – flower diameter 4-5 mm) and right: mānuka (Leptospermum scoparium – flower diameter 1-2cm) flowers – note the smaller and clustered flowers in kānuka. Images courtesy of New Zealand Plants, University of Auckland.

**Fig. 4. Left: Kānuka (*Kunzea* sp.) and right: mānuka (*Leptospermum scoparium*) leaves - note the pronounced and sharp leaf tip in mānuka. Leaf length approx. 1.2 cm. Images courtesy of New Zealand Plants, University of Auckland.**

The leaves of kānuka are also much softer and have a rounder tip compared to the sharply pointed leaf tips of mānuka (Fig. 4).

Kānuka (*Kunzea* sp.) are trees or shrubs in the Myrtaceae family endemic to New Zealand. Currently the *Kunzea* complex refers to a group of 10 recognised species defined on a genetic basis due to an extremely variable phenotype.[11] Kānuka (*Kunzea* spp.) is botanically distinct from mānuka (*Leptospermum scoparium*); however, both species are Myrtaceae and valued by Māori, with these generally interchangeable (referred to as kahikatea or white and red mānuka) in rongoā (Māori traditional medicinal practice).[9]

**Indigenous knowledge**

A notable aspect of natural products chemistry is the utilisation of traditional knowledge to inform the choice of species for investigation. Combined with bioassay-directed isolation methods, using indigenous knowledge and traditional plants accelerates structural elucidation and understanding of the chemical ecology underlying macro-scale biological effects.

Indigenous knowledge in New Zealand is held within Mātauranga Māori, including rongoā (Māori medicinal practises), as a taonga of Māori. Engaging with the guardians of this knowledge is a privilege, representing a knowledge system developed from generations of lived experience across the motu. I am extraordinarily grateful to have this opportunity to work with Māori kaitiaki within my project, and I thank and acknowledge the significant role of iwi - as tangata whenua and kaitiaki of the taonga species we are privileged to work with - in my research as integral partners and research directors.

Kānuka is widespread but little studied by natural product chemists, making it an exciting plant to study – particularly in the current climate of demand for novel antiviral compounds.

An example of this approach is presented in Lawrence *et al*.,[12] who interwove Mātauranga Māori around kānuka to discover inhibitory activity against *Phytophthora agathidicida* (causative pathogen of Kauri dieback disease), highlighting the value gained from this intersection of bodies of knowledge.

The authors emphasise that the findings are part of academic research, but the mātauranga and rongoā are taonga (treasures) belonging to tangata whenua, the indigenous people of Aotearoa.

**Natural products of kānuka**

Why kānuka? Kānuka has been used by Māori to treat a range of ailments and illness including viral infections – captured in the written record of traditional uses by Riley *et al*.[10] Kānuka is widespread but little studied by natural product chemists, making it an exciting plant to study – particularly in the current climate of demand for novel antiviral compounds.

A chemotaxonomic analysis of *Kunzea* was undertaken in 1997, which found the essential oils were dominated by α-pinene (up to 67% of the oil).[13] Further chemotaxonomic studies of kānuka identified a number of less volatile compounds that were expected to have bioactive properties.[14] These included an array of compound classes covering various carbon and oxygen-containing frameworks, and conjugated derivatives of these.

Four antiviral acyl phloroglucinol derivatives structurally related to leptospermone have been identified from solvent extracts of *K. sinclairii*

and *K. ericoides*.[15] Recent work using Mātauranga Māori-guided screening found three new flavanones active against *Phytophthora agathidicida*, an oomycete pathogen that is the causative agent of kauri dieback.[12] Hence we see that there is evidence of a diverse bioactive chemistry present in *Kunzea* species.

Using another widespread species, mānuka, outreach has been undertaken across NZ schools with herbicidal triketones and lettuce seeds as an interactive medium to explore Mātauranga Māori and this taonga species in terms of chemical ecology, nationwide variation and economic potential.[16] The combination of community-based sampling and Plant & Food Research chemical analysis created complementary datasets that created scope for use across age and ability ranges up to year 13 students – with potential for subsequent investigations by interested students. This approach proved successful, especially for engaging Māori and rural students across NZ, with the assay, chemotype variation and visualisation proving key highlights for students and teaching staff.[17] Building upon this, I aim to develop a complementary antimicrobial assay for use with kānuka extracts collected with schools.

## Acknowledgements

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Article

# Metal assemblies on cavity platforms

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Keywords: metal complexes, supramolecular chemistry, host-guest chemistry, cages

## Introduction

Nature provides the inspiration to design synthetic receptor mimics for molecular recognition of guests of complementary size, shape and dimensions. The development of such synthetic receptors facilitates a better understanding of the molecular mechanism of receptor site and enzyme activity through an *in vitro* approach. In the 1980s, Nobel laureate Donald J. Cram reached a milestone with the synthesis of a carcerand derived from covalent bond formation. Since then, more such synthetic receptors have come into existence. To mimic metalloenzymes, the development of a synthetic receptor bearing a metal centre is indispensable, where synthetic ligands can duplicate the *in vivo* scenario for a working metal. The molecules possess a predefined cavity that can act as an optimum building block for the synthesis of these biomimics as metal complexes and coordination cages. In this review article, aspects of cavity-based metal assembly and propagation into subspecies are described.

Ramkrishna Mandal is a doctoral candidate at the Department of Chemistry, University of Otago. He is pursuing his doctoral research under the supervision of A/Prof Nigel Lucas. Ramkrishna obtained his MS in medicinal chemistry from NIPER-Kolkata, India. After that, he worked as a research assistant at the Indian Institute of Chemical Biology. Ramkrishna's PhD research primarily encompasses the synthesis of nanoscopic covalent cage architectures from chiral precursors.

## Metal complexes on cavity platforms

A functionalisable, well-defined molecular cavity architecture could be considered the optimum candidate for designing metal complexes. The control of metal ion nuclearity and the relative position of the metal complex on the cavity platform are generally achieved with a rationally functionalised cavity molecule. Thus, calixarene, cyclodextrin (CD), cyclotriveratrylene (CTV), and resorcin[4]arene-derived cavitands are commonly used as cavity molecules to develop potential ligands for metal complexation. In the case of water solubility, cyclodextrins stand out with high water solubility, which favours the formation of metal complexes as molecular receptors in aqueous solution. On the other hand, parent calixarenes, cyclotriveratrylenes, and resorcin[4]arene-derived cavitands are mostly soluble in organic solvents.

### Calixarene-based metal complexes

Among all calixarenes, calix[4]arene has gained attention for use as a molecular platform for metal binding due to the well-explored functionalisation chemistry of calix[4]arene. Reinhoudt *et al*. exploited calix[4]arene-based Zn(II) complexes for ester hydrolysis.[1] They studied bimetallic catalysis with two calix[4]arene-based metal complexes, where Zn(II) is coordinated by 2,6-bis(dimethylaminomethyl)pyridine positioned 1,2-vicinal **1**, 1,3-distal **2**, and 1,2,3- units **3** on the upper rim (Fig. 1). They concluded that the distance between two metal ions is critical for catalysis. With an extension of this study, three Zn(II) complexes at the 1, 2, and 3 positions evidenced catalysis in ester cleavage. After one year, Reinhoudt and colleagues reported calix[4]arene-derived ligands functionalised with [12]aneN$_3$ on 1,2-vicinal **4**, 1,3-distal **5**, and 1,2,3-positions **6** of the upper rim, which could coordinate with zinc(II) and copper(II) (Fig. 1). The synthesised metal complexes were investigated for catalytic activity for phosphodiester cleavage.[2]

Besides calix[4]arene, calix[6]arene can be functionalized at alternate 1-, 3-, and 5- positions of the upper rim,

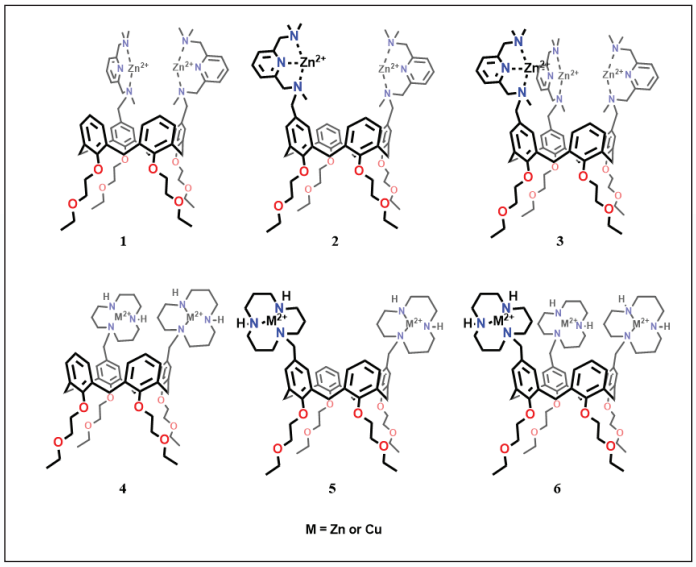



Fig. 1. (above) The metal complexes synthesised by Reinhoudt et al. with calix[4] arene decorated with 2,6-bis(dimethylaminomethyl)pyridine or [12]aneN3[3]

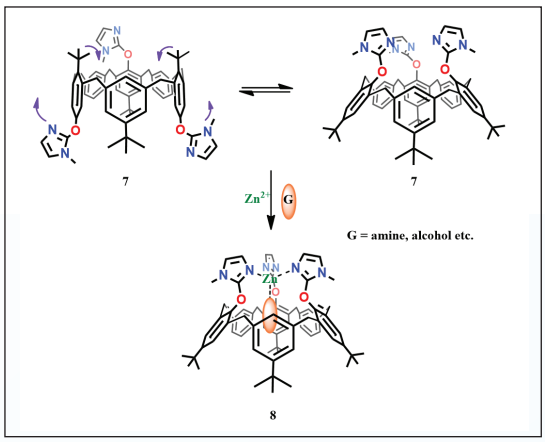



Fig. 2. (above, right) Calix[6]arene derived flexible ligand and zinc(II)

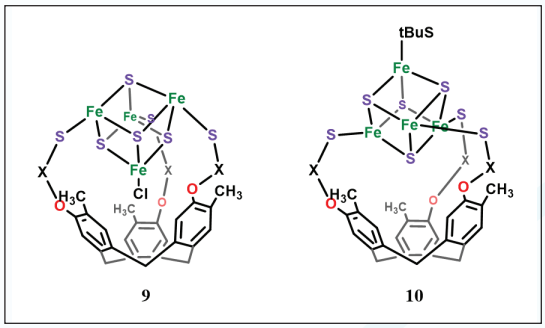



Fig. 3. (right) Nolte et al. reported cyclotriveratrylenes (CTV)-based metal complexes coordinated funnel complex

which facilitate the incorporation of electron donor moieties (like imidazole) to coordinate with the metal centre. The conformational flexibility of calix[6]arene **7** helps to adopt several relative conformations. In a complexation reaction with zinc(II) or copper(II), calix[6]arene adopts a funnel-like conformation. For complexation with a zinc(II) metal centre **8**, calix[4]arene adopts a pseudo tetrahedral geometry consisting of an electron donating group and guest ligand inside the cavity (Fig. 2).

The guest ligand could be an exchangeable primary amine, nitrile, amide, or alcohol; the affinity not only depends on coordination with the metal centre but also on the affinity toward hydrogen bond formation and CH–π interaction inside the calix[6]arene cavity. The selectivity of guest-binding was achieved with consideration of critical factors like donor ability of the guest (e.g. an amine is a considerably better guest compared to an alcohol), steric hindrance and shape of guest (e.g. a primary amine has less steric hindrance than secondary amines and linear molecules are accommodated suitably inside the cavity).[3]

"In some cases, the metal embedded in the cavity influences the guest recognition property of the cavitands while in other cases the metal itself changes the property by being embedded. "

### Cyclotriveratrylenes (CTV)-based metal complexes

Collet *et al.* reported two novel metal complexes with an *n*-propyl thiol and a *m*-substituted benzene thiol derivative of a $C_3$-symmetric cyclotriveratrylene (CTV). The equimolar reaction between a $[Fe_4S_4]$ cluster and one tridentate CTV ligand furnishes the desired metal complex in quantitative yield.[4] In one study, Nolte *et al.* disclosed that a metal complex obtained in a reaction between $[Fe_4S_4Cl_4]^{2-}$ and tri-thiol-CTV derivative, with a chloride ligand placed inside the cavity (**9**). On the other hand, when $[Fe_4S_4(S^tBu)_4]^{2-}$ is used as a metal precursor, a bulky *t*-butyl group is coordinated from outside (**10**) (Fig. 3).[5]

### Resorcin[4]arene-derived cavitand-based metal complexes

A distinct library of cavitand-based mono- and polymetallic complexes has been prepared. To obtain a cavitand-based metal complex, the initial step is the rational modification of cavitand to act as anchor(s) for coordinating a metal centre. In some cases, the metal embedded in the cavity influences the guest recognition property of the cavitands while in other cases the metal itself changes the property by being embedded. Some early examples were reported by Puddephatt and his research group[6] by developing phosphonito-cavitand and phosphinito-resorcin[4]arene multidentate ligands.

Phosphonito-cavitand and phosphinito-resorcin[4]arene assembled with Au(I) and Pt(II), respectively, to

give metal complexes. The X-ray crystal structure of the phosphonito-cavitand tetragold complex clearly shows three out of four AuCl units placed on the upper rim of a phosphonito-cavitand and the fourth AuCl embedded inside the cavity (Fig. 4a). Following this line of research, Puddephatt and colleagues[7] exploited phosphonito-cavitand derived tetracopper(I) (Fig. 4b) and tetrasilver(I) (Fig. 4c) complexes and their halide scavenging properties as well as reactivity towards halide replacement. In 1997, Puddephatt *et al*.[8] described the cation recognition property of the previously reported halide inclusion metal complex. This study revealed cations like Hg and Pb (Fig. 4d) formed the dimeric metal complex. With the development of various derivatives of phosphonic-cavitand, more similar types of metal complexes were prepared by coordination with various metal centres, e.g. gold,[9] silver[10-11] and copper.[12]

### Cyclodextrin (CD)-based metal complexes

In comparison with other cavity molecules, cyclodextrin's water solubility allows it to act as a host in aqueous media (Fig. 5a). In 2000, Kim *et al*.[13] reported a carboxypeptidase mimic by connecting triazacyclododecane and β-cyclodextrin, following Zn(II) complexation. This metalloprotease prototype exibits hydrolysis of *p*-nitrophenyl acetate (PNPA) and influence the hydrolysis rate by around 300-fold. From this study, it was concluded that PNPA only bounded with CD, without coordinating with Zn(II) metal, where Zn(II) coordinated water molecule involved in hydrolysis process (Figure 5b).

## Resorcin[4]arene-derived cavitand-based coordination cages

Through their seminal work, Fujita and co-workers have opened new horizons in the application of coordination chemistry in multidisciplinary areas.[14-15] In a search for size-persistent host molecules, this group reported a *cis*-protected Pd(II) coordination macrocycle with the 4,4'-bi-pyridyl repeating unit (Fig. 6a).[14] The reported authentic framework adopted a square framework by coordinating linear ligands at a 90° coordination angle. The thermodynamically stable macrocyclic could recognise guests in an aqueous system by accommodating them between the π electron-rich eight pyridine motifs. Based upon this knowledge, Dalcanale and Jacopozzi[16] successfully accomplished the first-of-its-kind co-facial cage by coordinating cavitand-based tetradentate ligands and four square planer Pd metals. The cavitand was derivatised with four cyano groups on the upper rim to anchor to Pd. Single-crystal X-ray diffraction analysis and $^{19}F$ NMR revealed one out of eight trifluoromethanesulfonate counteranions are incarcerated inside the cavity, while seven stayed outside the cage.

In an extension of this research, Dalcanale *et al.* thoroughly investigated the factors that influence self-assembly in coordination cage synthesis (Figure 6b).[17] The investigation disclosed aspects of consideration for cage synthesis: (i) the angle between the chelating ligand and the metal precursor is close to 90°, (ii) a transition metal for complexation (like Pd,

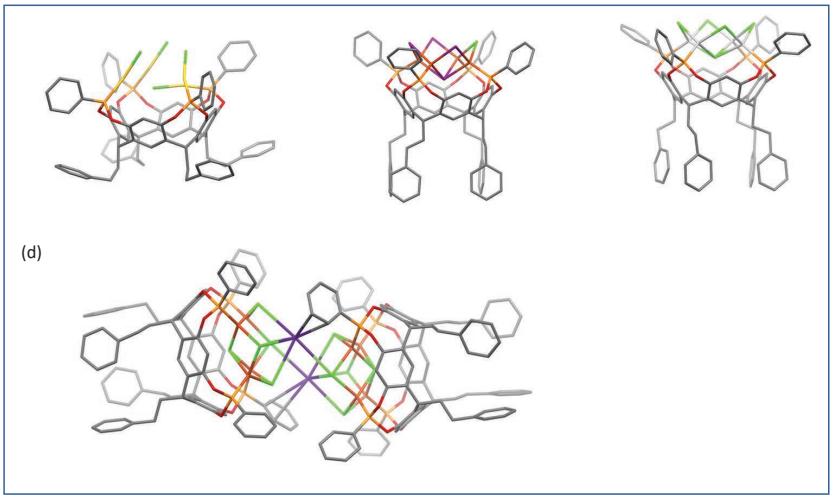



Fig. 5. (a) Chemical structure of cyclodextrin (CD), α-CD (n = 1), β-CD (n = 2); (b) metalloprotease prototype from β- cyclodextrin reported by Kim et al.

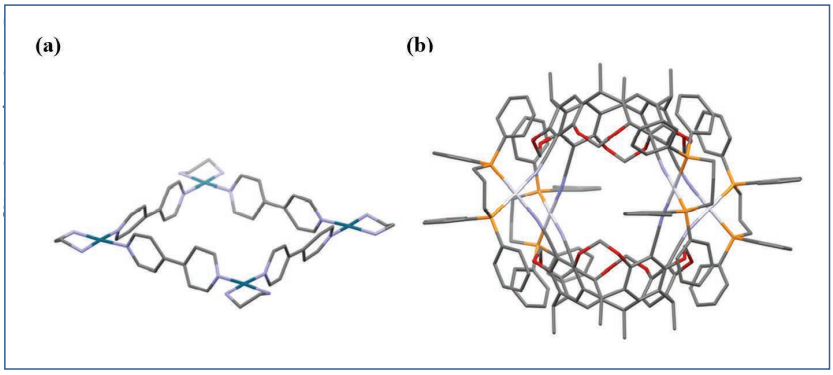
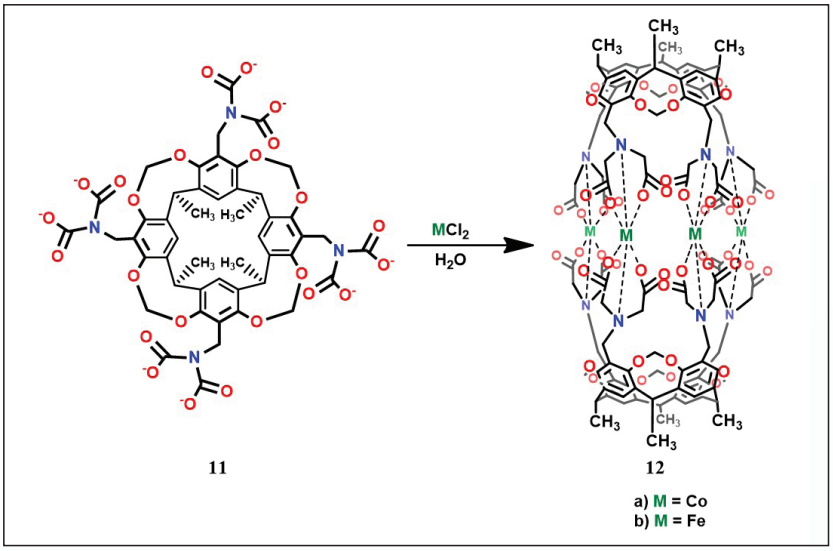


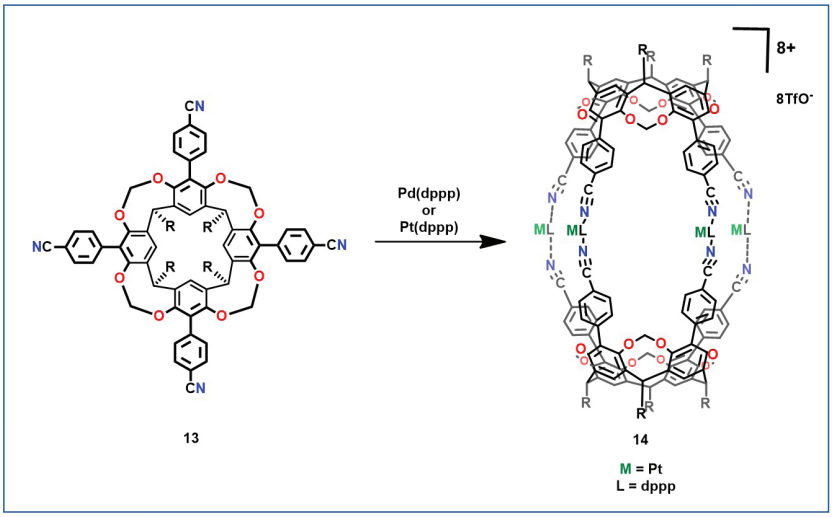

Fig. 6. (a) X-ray crystal structure of Fujita's[72] Pd(II) coordinated square assemblies. Hydrogen atoms, counteranions and solvents are omitted for clarity. (b) X-ray crystal structure of Dalcanale et al.[17] who reported the first cavity-based coordination cage with Pt. Hydrogen atoms, counteranions and solvents are omitted for clarity. Undecyl alkyl chain on feet depicted as methyl for clarity.

Pt), (iii) preorganisation of the tetradentate cavitand and (iv) various counterions.

Following the Dalcanale legacy in building cavity-based coordination cages, $CoCl_2$[18] and $FeCl_2$[19] mediated water-soluble coordination cages (**12a** and **12b**) were reported by Harrison *et al*. The decoration of the cavitand with apically-situated tetra-iminodiacetate moieties as a ligand was the first step of the reaction (Scheme 1). While $FeCl_2$ is used as a metal source, employing a reducing agent or oxygen-free environment was essential to minimise iron oxidation. The research team disclosed that the self-assembly of the cages in aqueous media was favoured at pH > 5. In an extension of the study, encapsulation of a small guest inside the $CoCl_2$-mediated coordination cage was investigated.[20-21] The hydrophobicity as well as cavity compatibility to small organic guests act as a driving force to entrap them inside the hydrophobic cavity of the cages. It was realised that small organic guests were incarcerated during cage formation due to insolubility in aqueous media.




Scheme 1. Synthesis of Co- and Fe-mediated water-soluble coordination cages from 11




Scheme 2. Synthesis of Pd- and Pt-mediated extended cavity coordination cages from tetrakis(4-cyanophenyl) cavitand 13

**Extended cavity co-facial coordination cages**

To accommodate larger guests inside the cavity, a cage consisting of an extended cavity is obligatory. There are two distinct approaches to obtaining an oversized coordination cage: 1) pre-coordination modification of cavitand to achieve an elongated ligand for co-facial coordination cage, or 2) synthesis by multi cavitand assembly (i.e. trimeric, tetrameric coordination cage).

The first approach was exemplified by Dalcanale and colleagues,[22] when Pt metal centre derived coordination cage **14** was accomplished with extended cavitand (Scheme 2). Cage synthesis was achieved with the development of a tetrakis(4-cyanophenyl) cavitand **13** as a hemispheric ligand to coordinate. With the development of extended benzyl-connected cavitand, Dalcanale and coworkers also synthesised a cavitand decorated acetal position with a tripyridyl electron-donor group. The reaction between the tetra-pyridyl cavitand with Pd or Pt metal centre furnished to the coordination cage, endowing an ellipsoid cavity with measured volume of 840 $Å^3$.[23]

Extension of the study by Dalcanale *et al*. revealed that the capsule has enough space to accommodate methano[60]fullerene derivatives as a 1:1 complex in $CD_2Cl_2$.[24] Formation of the complex is driven by dispersion forces and π-stacking between the fullerene derivative and cavity wall. Later, a less-symmetric coordination cage was reported by Dalcanale *et al.* while reacting tris(4-cyanophenyl)-(4-pyridylethynyl)-cavitand, $Pd(dppp)OTf_2$ and $Pt(dppp)OTf_2$ in 2:3:1 ratio.[25]

Based upon this knowledge, Kobayashi and colleagues[26] demonstrated the development of homogenous and heterogenous cages with consideration of steric demand and coordination potential of the cavi-

tand. The study found that cavitands **13** and **15** could produce homocavitand cages **17** and **18**, respectively. However, equimolar self-assembly of **13** and **15** with 4 equivalent metal precursor resulted in aggregates. In contrast, cavitands **13** and **16** in equimolar co-facial coordination afforded a heterogenous coordination cage **19**. The unsuccessful attempt of heterogeneous cage synthesis with **13** and **15** explained that the longer arms in **15** skewed the coordination angle to form a square planer metal complex (Fig. 7).

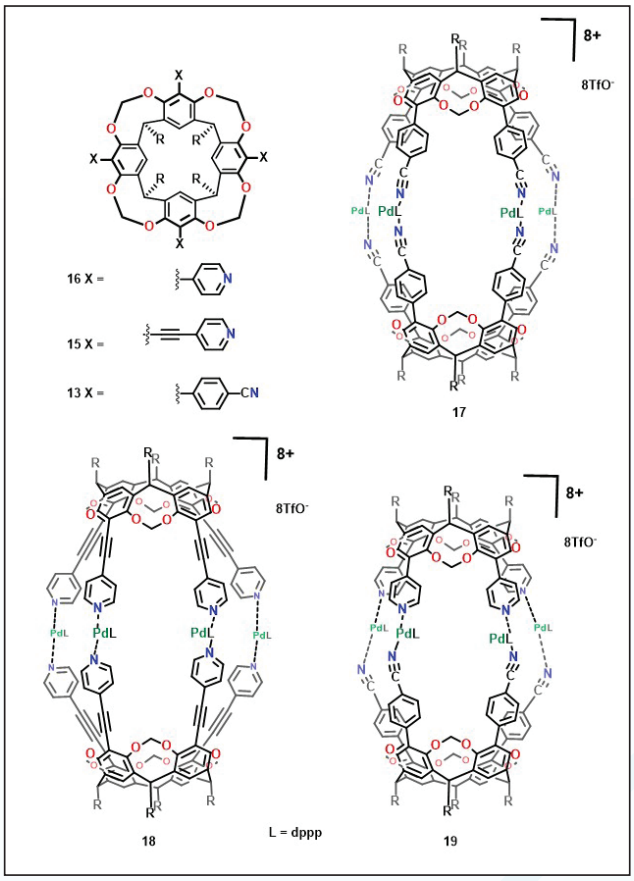
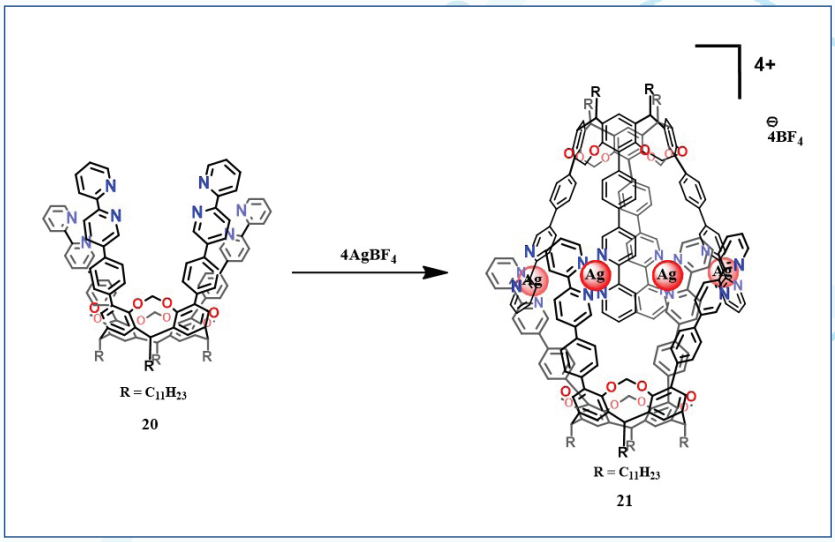



Fig. 7. Homogeneous (17 and 18) and heterogenous (19) cages

In this vein, Haino and co-workers developed more extended octadentate cavitand **20** by linking bipyridyl (bipy) moieties with phenyl spacer. Their work demonstrated the synthesis of the dimeric coordination cage **21** by coordinating two of tetra(bipyridyl) cavitand **20** with four $Ag^{+}$ metal centres (Scheme 3).[27] The guest inclusion ability for such an extended cavity containing cage **21** was investigated. A large aromatic compound as a guest could be incarcerated by the cage and form a thermodynamically, as well as kinetically, stable complex in $CDCl_3$.




Scheme 3. Synthesis of Ag-mediated extended cavity coordination cage from 20

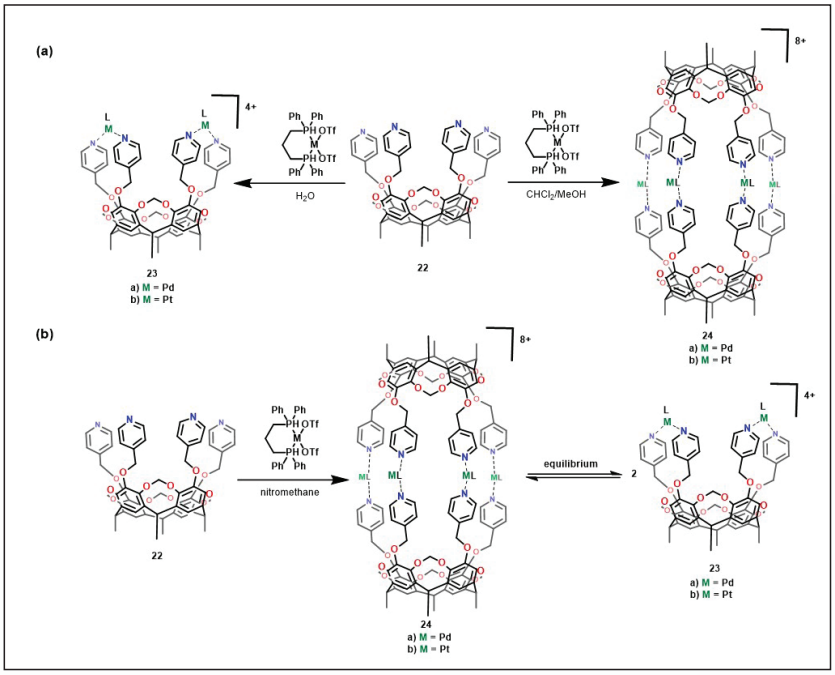
Tadokoro and colleagues[28] developed a cavitand bearing four pyrimidyl groups on the upper rim. The nitrogen atoms on each pyrimidyl group directed to the cavity could complex with a metal by an inner convergent approach, and alternative nitrogen atoms on each pyrimidyl group could form metal complexes individually by an outer divergent method. The coordination with metals for such cavitands could lead to three possible outcomes: 1) a co-facial coordination cage, 2) a coordinated square array and 3) a polymeric chain.

Hong and co-worker developed a new tetradentate cavitand **22** by attaching flexible pyridine moiety to the apical position.[29] Their research reported the synthesis of intramolecularly connected coordination bowls **23** with Pd or Pt metal-mediated coordination cages **23**, simultaneously. Later, continuation of this study revealed the influence of the solvent system on the formation of exclusively intermolecularly coordinated cages or intramolecularly coordinated bowl molecules (Scheme 4).[30]

A variation of phosphonate cavitands was developed by Dalcanale *et al.*[31] and their corresponding ditopic Re metal complex was synthesised. Both cavitands and ditopic architectures were investigated for molecular recognition and complexation properties with N-methylpyridinium salt. Their

study concluded that the number of P=O groups in apical positions on cavitands was pivotal for molecular recognition and host-guest complexation.

Some hybrid coordination cages were reported with $C_{2v}$-symmetrical cavitand, where cage formation was derived by coordination bond formation by connecting metal centre as well as hydrogen bond formation.[32-34] In these cages, encapsulation of guests was carried out by dissociation of the weaker bond (hydrogen bond), while the stronger bond maintained the topology of the cage. Precursor cavitands of these hybrid coordination cages were designed in such a way that an electron-donating coordinating moiety and hydrogen donor-acceptor groups were placed alternatively on the apical macrocycle.

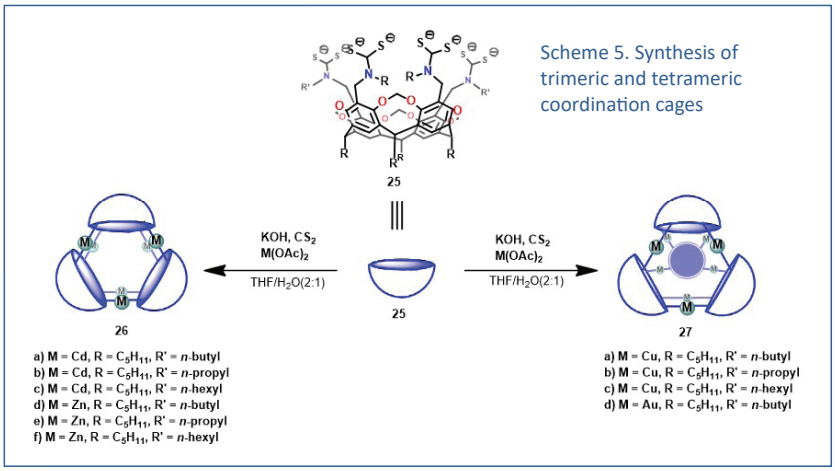



Scheme 4. Metal-mediated self-assembly from 22. (a) Solvent dependent exclusive formation of 23 and 24; (b) intralinked and interlinked self-assembly in dynamic equilibrium.

### Extended cavity multi-cavitands coordination cages

In a search for larger cavity coordination cages, Beer and co-workers[35] pioneeringly disclosed the synthesis of trimeric and tetrameric coordination cages. Where cavitands were ligated through coordination bonds with various *d*-block metal centres, their investigation revealed the metal influence in conformational arrangement of dithiocarbamate cavitands to cage synthesis. Metals like Cu(II) and Au(I) directed coordination assembly to exclusively give tetrameric cages **27**. On the other hand, trimeric architecture **26** was solely obtained when Cd(II) and Zn(II) were used as metal centres (Scheme 5). Later study revealed that Ni(II), Pd(II), and Pt(II) also favoured tetrameric coordination cage synthesis.[36] A guest inclusion study showed Cd(II) and Zn(II) mediated cages could accommodate fullerenes $C_{60}$ and $C_{70}$ in their complementary cavity and formed a 1:1 inclusion complex.[37] Cu(II) based coordination cages also showed encapsulation properties with $C_{60}$ and $C_{70}$.

A hexameric coordination cage was reported by Holman and co-workers,[38] where assembly consists of six




Scheme 5. Synthesis of trimeric and tetrameric coordination cages

cavitands decorated with four carboxylic acids on the upper rim and sixteen Zn(II) ions. Atwood and co-workers reported nearly the same hexameric cage.[39] In contrast to other hexameric cages, pyrogallol[4]arenes was used in place of resorcin[4]arene derived cavitand and Cu(II) was employed in place of Zn(II) as the metal precursor.

## Conclusions

To date, a plethora of discrete cavity-based metal assemblies has been reported. With the booming host-guest chemistry, cavity-based coordination cages have transformed into more complex multi-component coordination cages from the co-facial cage. Meanwhile, cavity-based metal complexes have become predominant in biomimetics. Even after considerable exploration, the future of this field is bright and prosperous. The latent potential of cavity molecules as precursors to metal assemblies has now been understood. This is only the beginning of investigation into the rational synthesis of cavity-based metal complexes and coordination cages with different properties.

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Survey

# CLN5 neuronal ceroid lipofuscinosis (NCL): from analytical chemistry to clinical neuroscience

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## Introduction

You may have heard the exciting news that work on sheep in New Zealand has led to US Federal Drug agency (FDA) sanctioned clinical trials to treat the fatal inherited neurodegenerative disease in children, CLN5 Batten disease (CLN5 neuronal ceroid lipofuscinosis, NCL). For me this has been an exciting journey from analytical chemistry to clinical neuroscience.

It began in 1980 when I accepted a position as a Research Officer working with Professor Bob Jolly in the Department of Veterinary Pathology and Public Health at Massey University, Palmerton North. Bob had established a considerable international reputation from his diagnosis of α-mannosidosis in cattle in collaboration with Dennis Hocking via biomolecular analysis of α-mannose rich oligosaccharides in blood and urine and subsequent assays of α-mannosidase activities. He had realised the importance of lysosomal storage diseases in domestic animals as models of human disease.

Encouraged by people including Nobel laureate Christian de Duve, he applied for NIH funding to pursue these studies highlighting their relevance to the emerging cognisance of human lysosomal storage diseases. While visiting a farm to collect some cow urine, his attention was drawn to a couple of blind South Hampshire sheep that also behaved like pet lambs. After initially dismissing them, he decided that a professional veterinary pathologist was obliged to investigate this thoroughly, had a *post-mortem* done, immediately noted the atrophied brain and diagnosed a ceroid lipofuscinosis, the diagnosis alerted from the similar brain atrophy in a pup he had diagnosed earlier.[1] He procured some of the parental stock and established a research flock of these sheep to study as a model of the human disease.

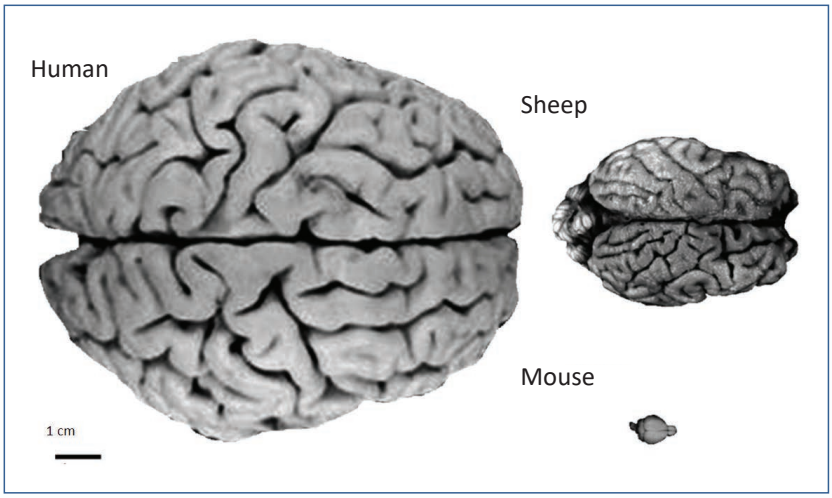



Fig. 1. A human, sheep and mouse brain. Note the gyri and sulci (ridges and wrinkles) in the human and sheep brains and how small the mouse brain is in comparison.

## My induction

The NIH provided the funds and I was employed to find out what was going on. I had done a degree in chemistry at Canterbury but was always attracted to biological chemistry and "doing something useful." Biomedical research had been my career choice since my appetite was whetted by a short stint with Professor Robin Carrell in Christchurch Hospital in 1970, after which I went to the University of Toronto and then to University College London to develop my biological chemistry and biochemistry knowledge for this. By this time, 1980, and back in New Zealand, I was looking for something other than murdering healthy rats in the interests of human health and physiology, so the opportunity to study unhealthy animals to help human medicine was highly attractive.

Sheep are ideal models. They are independent easy-care animals, particularly in our pastoral conditions, their gyrencephalic ovine brain is similar in size and physical organisation to non-human primates (unlike the tiny sulci and gyri-lacking smooth rat or mouse brain) and overall they are similar in size and organisation to human brains (Fig. 1). These facts have stimulated this longitudinal investigation into the ovine disease progression, and therapeutic efficacy and translatability of viral-mediated gene transfer in established CLN5 and CLN6 NCL sheep models.

## The neuronal ceroid lipofuscinoses and lipid peroxidation

These are a group of inherited neurodegenerative lysosomal storage diseases of humans and other animals. Affected children start life normally but develop dementia, blindness

and seizures, culminating in premature death. Characteristic pathological features are brain atrophy, retinal degeneration and the accumulation of fluorescent storage bodies in neurons and most other cells. Cases are relatively common and an estimate of 1 in 12,500 live births worldwide is broadly in line with the incidence in New Zealand. With intensive supportive therapy patients live for a number of years at great human and economic cost.

The name ceroid lipofuscinosis derives from the similar histochemical and fluorescent properties of the disease characteristic storage bodies and those of the intracellular dense body inclusions, ceroid and lipofuscin, still commonly thought to result from abnormal lipid peroxidation (Fig. 2). At that time the dogma was that these diseases arose from a failure of antioxidant protection causing a surfeit of free radical damage leading to peroxidation of lipids; the consequent aldehydes and ketones then cross linking to amines (in a pseudo aldol condensation) to form fluorescent Schiff base polymers resulting in the accumulation of "autofluorescent" lipofuscin storage bodies.

These characteristic intracellular inclusions that give the diseases their name are highly fluorescent *in situ* and were likened to the so called normal "age pigment" lipofuscin. Based on experiments by Chio and Tappel who had stewed up a variety of biological molecules with oxygen and got fluorescent polymers with spectra similar to lipofuscin,[2] the same cause was postulated - abnormal lipid peroxidation products linking with amines. There were a number of publications attesting to a disease association with the abnormal peroxidation of polyunsaturated fatty acids, enhanced peroxidase activities, lack of antioxidant activity and so on, however the methodology in them was poor and the evidence only circumstantial at best. I thought these arguments of structural determination by analogy from similar fluorescence rather tenuous and it was not even clear where this abnormal peroxidation was meant to occur - in lysosomes, in the cell lumen or extracellularly.

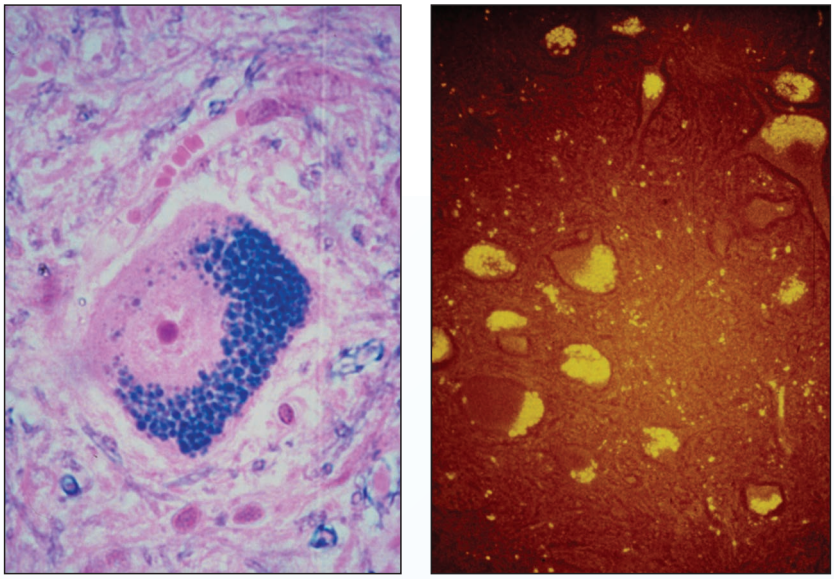


Fig. 2. Storage bodies in situ, stained with Luxol fast blue which stains for hydrophobic surfaces (left) and under a fluorescent microscope (right).

Because of the rumen reduction of polyunsaturated fatty acids, ruminants have a very limited effective essential fatty acid supply and have special mechanisms to conserve them, thus a drain on them would be expected to lead to a net loss of polyunsaturated fatty acids. However, a careful analysis of the fatty acids of affected sheep phospholipids did not reveal any loss of peroxidation susceptible polyunsaturated fatty acids, which would be expected if the peroxidation mechanism was true.[3] There is an adage in lysosomal storage diseases that if you figure out the structure of the stored compound you can guess the enzyme that should be next in the catabolic pathway, e.g. and re previously, the storage of oligosaccharides ending in an α-linked mannose indicated an α-mannosidosis, thus our next set of investigations centred on isolating the storage bodies and analysing their molecular composition. I'd done time in London isolating functional microsomes by gradient centrifugation and started off isolating storage bodies this way, but experience showed they behaved more like viral particles than microsomes and were refractory to sonication, which made it easy to obtain clean preparations.

## Analytical chemistry

Given the predisposition to a lipidosis arising from the peroxidation hypothesis, the apparent histological lipoid nature of the storage bodies and an alternative hypothesis then fashionable in the literature - that the disease was caused by underlying defects in isoprenoid metabolism resulting in the accumulation of dolichols and/or derivatives - we started with a Folch extraction of storage body lipids and analysed them. Nearly all of the storage bodies dissolved in chloroform/methanol with ammonium acetate, and later we found in 100% formic acid with mercaptoethanol and also in lithium dodecyl sulphate or sodium dodecyl sulfate with heaps of mercaptoethanol.

This exercise in analytical chemistry established that the lipid content of the storage bodies was that expect-

ed of lysosome derived organelles and there was no sign of a loss of polyunsaturated fats to peroxidation. There was no fast migrating fluorescent material on silica gel thin layer chromatographs (TLC) that could equate to the postulated "polymalonaldehyde" either.[4]

On the other hand, leaving lipid samples around in air, or leaving TLC plates out in air overnight resulted in the creation of lots of fluorophores, so I have no doubt that it is easy to make fluorescent artefacts. In fact, preparative TLC showed that the lysosomal phospholipid, bis(monoacylglycero)phosphate, present in large amounts, had very high proportions of the essential polyunsaturated fatty acids linolenic and linoleic acids. No fluorophor was found, nor evidence for the highly fluorescent peroxidation formed polymer postulated to be the consequence of the peroxidation.[5]

An efficient normal-phase HPLC method of analysing dolichols, cholesterol and ubiquinones was developed.[6] These compounds were present in storage bodies only in amounts expected in lysosome derived organelles. However, their concentrations were lower than expected if the storage bodies were merely the aggregation of lysosomal membranes which indicated the accumulation something else. Freeze fracture and x-ray diffraction experiments showed that the apparently membranous nature of storage bodies disguised their solid nature (Fig. 3).[7-8] Their isopycnic density (1.18-1.25) was far higher than expected of a lipid conglomerate. When in suspension or *in situ* the storage bodies were fluorescent, but when in solution the fluorescence disappeared and there was no significant fluorescent component, nor even one with a suitable uv/vis absorption spectrum.[9]

Analyses revealed that most of the storage body mass precipitated from the aqueous methanol/chloroform interface in Folch extractions. This insoluble residue was not fluorescent when directly irradiated with 366 nm light. Elemental analysis was consistent with it being proteinaceous; 53.19% C, 7.81% H, 12.71% N and 2.59% S by weight. Gravimetric analysis showed it to be about 70% of the total lipopigment mass, and Lowry protein estimations of sonicated suspensions of storage bodies gave the same proportion as protein.[5]

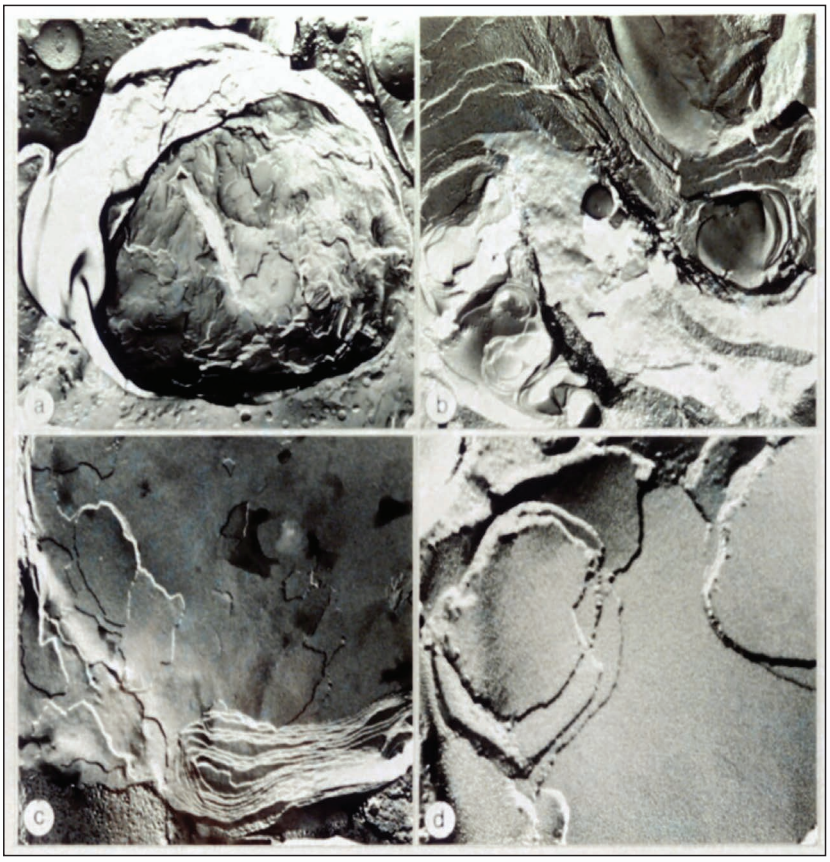



Fig. 3 Freeze fracture electron micrographs of storage bodies in situ, at approx. 150,000x magnification. Note the 'rock like' appearance.

When this residue was suspended as a dry powder in glycerol it was highly fluorescent in the fluorescence microscope, but so are glycerol suspensions of casein and bovine serum albumin, indicating that this apparent fluorescence did not arise from a classical fluorophore. The metal content of the isolated storage bodies was consistent with a lysosomal origin of them and did not support a metal catalysed peroxidative mechanism of lipopigment formation.[10]

We thought it likely the fluorescence arose from the environmentally dependent fluorescence of protein, in a similar way to the observed fluorescence of the globules of protein that accumulate in the livers of people with alpha-1-antitrypsin (alpha-1-protease inhibitor) deficiency.[11] However, it was a rather unusual protein, being highly insoluble in most protein solvents and soluble in lipid solvents. With wonderful help from Gill Ellis (then Barns) we found that if you treated it really gently and ran it in LDS gels at low loading you could detect two Coomassie insensitive bands with a specially developed silver stain technique; a dominant band with an apparent molecular mass of 3,500 kDa and a lesser component with an apparent mass of 14,800 kDa, and some

smears in between.[12] I was excited and went to a number of conferences about these inclusions where I realised how entrenched the "dangerous free radical damage" dogma is. I also found that the evidence these people presented for it was largely cooked up, rather à la Chio and Tappel. The dogma around free radical damage continues to persist.

The similarities between lipofuscin and the storge bodies in ceroid-lipofuscinosis suggest a similar composition, albeit for different reasons to those usually advanced. Dense bodies have also been observed in neurons of rat brains into which protease inhibitor leupeptin had been perfused.[13] They formed quickly, their formation was reversible, and they had structural and fluorescent properties that drew attention to their similarity to lipofuscin and the storage bodies in the ceroid-lipofuscinoses. Lipofuscin and other similar intracellular inclusion bodies are also likely to be largely proteinaceous, and the consequence of general disruptions in lysosomal function particularly affecting lysosomal proteolysis.

After gazing at these two bands on gels and lots of unsuccessful efforts to separate them, it occurred to me when calculating mole % of each protein rather than the masses that the 3,500 kDa band was about 90% of the moles, so if it was one protein it should be sequencable without further purification. Without further ado and with the help of the able Julian Reid we loaded the protein into an ABI automatic N-terminal Edman degradation sequencer. The result was astonishing, a clear read of the 40 amino acids from one dominant protein, identical to the c-subunit of mitochondrial ATP synthase, and of the first 10 amino acids of another minor component, the c-subunit of vacuolar ATPase.[14] Later work in collaboration with Sir John Walker and Ian Fearnley, then at the MRC Laboratory of Molecular Biology in Cambridge, established that the c-subunit of ATP synthase stored is the complete and normal 75 amino acid peptide, i.e. not truncated or covalently modified in any way except for the usual and completely normal trimethylation of a lysine.[15] It was originally called the mitochondrial proteolipid as it is soluble in lipid solvents, having been discovered by precipitation from Foch extractions, is insoluble aqueous solutions and stains poorly with Coomassie blue.

"The similarities between lipofuscin and the storge bodies in ceroid-lipofuscinosis suggest a similar composition, albeit for different reasons to those usually advanced."

Quantitative analyses, comparing the sequencer yield to the total amino acids loaded, indicated that this protein alone accounts for the bulk of the storage bodies' mass. We subsequently found, via sequencing from gel bands that the 14,800 kDa band is an oligomer of subunit c. Whether the vacuolar subunit is a genuinely stored component or arises from the lysosomal membrane is not resolved but the variability of yield between different storage body preparations suggests the latter. We reconstituted these bodies from normal phospholipids and Folch extracted c-subunit and they were fluorescent. This led us to conclude that the fluorescence is an aggregate property, not an intrinsic molecular one.[9]

**People and NCLs**

Meanwhile progress was being made on NCL genetics. Originally the human diseases were classified into four forms; infantile, late infantile, juvenile and adult, based on the age of presentation of clinical symptoms and the clinical progress. An infantile form, INCL (CLN1), is most common in Finland. Two late infantile forms, classical and variant LINCL (CLN2 and CLN6) and a juvenile form, JNCL (CLN3), occur worldwide as do several other variants. A confusing number of eponyms have been used to describe these diseases. The best known, Batten disease, is usually used as a generic term for the group as a whole. Over time, 13 different genetically distinct forms have been recognised, now designated CLN 1-8 and 10-14, each gene having a range of disease causative mutations (www.ucl.ac.uk/ncl).

I was very interested in determining the relevance of our sheep findings to NCLs in humans and other animals. In a series of visits to New York, Montreal, Helsinki and particularly Cambridge, we established that the complete and normal ATP synthase c-subunit is also stored in the juvenile and late infantile forms.[16] This was exciting. Subsequent studies showed that it is stored in all forms of Batten disease in humans and a large number in animals, with the exception of the infantile form, where we discovered specific storage of the sphingolipid activator proteins (SAPs or saposins), A and D.[17] General protein turnover is not disturbed. This led to led to the concept that a number of gene products are necessary and unique components of a subunit c turnover pathway. However, there is nothing obvious in the gene products that interlink them. Some are soluble lysosomal proteins, some are not and there is increasing evidence of interactions between some of them in the autophagic pathway to lysosomal digestion. Along the way we found and established another flock, of Borderdale sheep with a CLN5 form, being a dysfunction of a soluble lysosomal protein.[18]

The nature of the international conferences was the other exciting

thing. Initially they were very medically focused but progressed through to a considerable parent and support group involvement by 1990, led by the late Lance Johnston and Elaine, his wife. They built up the Batten Disease Support and Research Association (BDSRA) to provide educational material, beds, wheelchairs and furniture suitable for the patients and conferences brought all the parties together, including healthy siblings of affected children who have their own demons. Meeting parents brought me face-to-face with the human realities of the NCLs and gave me a sense of vocation. I had not fully appreciated the halo of psychological stress that affects a patient's close family, siblings' guilty anxiety at being normal but in fear of their fertility, and all this in addition to the patient's need for 24 hour care with demands at any hour of the day or night that drains parents and caregivers. This is after the stress and loneliness of years of having no credible diagnosis for their children's obvious decline.

Parents became friends and I became interested in joining them in communicating the full costs of the disease, both financial and emotional. In New Zealand we fall under the Australian branch of the BDSRA. They can be contacted at https://bdsraaustralia.org/contact. I have attended a number of their conferences found them informative and humbling. They were very interested in and supportive of our research and what it might mean for them. We had some meetings in New Zealand bringing together researchers, parents, carers, research funders and some patients too (Fig. 4).

Batten disease cases have been reported in Māori, Pacifica, Pakeha and New Zealanders of Indian descent.

Back Row. Dr Craig Bunt (Lincoln), Martin Wellby (Tech Lincoln), Dr Nigel Anderson, (U of O, Christchurch), Dr Tracy Melzer, (U of O, Christchurch), Hollie Wicky (Tech, U of O, Dunedin), Steve Bennett (Parent, Whangarei).

3rd Row. Brett Archer (Parent, Auckland), Janet Xu (Student, Lincoln), Dr Imke Tammen (Sydney), Dr Bruce Scoggins (CureKids), Hannah Best (Student, U of O, Dunedin), Mark Timms (Parent, Timaru).

2nd Row. Nadia Mitchell (Student, Lincoln), Amy Smaill (Tech, Lincoln), Jarol Chen (Student, Lincoln), Erin McCutcheon (Parent, Dannevirke), Lisa Archer (Parent, Auckland). Nicole Neverman (U of O, Dunedin), Glen McCutcheon (Parent, Dannevirke), Sharon Noble (Parent, Auckland). Ra Timms (Parent and Family Co-ordinator, Batten Disease, New Zealand, Timaru)

Front Row. Tim Edmonds (CureKids), John Forman (Lysosomal Diseases New Zealand), Katharina Russell (Student, Lincoln), Professor David Palmer (Lincoln), Noble (Caregiver, Auckland), Dr Stephanie Hughes (U of O, Dunedin), Margie Frazier (Batten Disease Support and research Association, USA), Antonya Noble (Patient, Auckland), Brad Timms (Patient, Timaru). No photo: Lynda Dougan (Parent, Taranaki), Dr Graham Barrell, (Lincoln)

Fig. 4. A group of the New Zealand Batten disease community at a day-long meeting at Lincoln. Sadly Antonia and Brad are no long with us, but don't you love Brad's thumbs up? Batten disease cases have been reported in Māori, Pacifica, Pakeha and New Zealanders of Indian descent.

## Towards treatments

We continued with the pathology, particularly the neuropathology, and noticed early neuroimmune response and glial activation that might drive the disease.[19-20] However, an experiment to treat sheep with chronic oral administration of anti-inflammatory minocycline did not prevent neuroinflammation or disease progression.[21] We are still working on this, but experiments with gene therapy have taken over. These began at the instigation of Stephanie Hughes who had returned to New Zealand after a postdoc with Dr Beverley Davidson who encouraged this. This was the time of the anti-science anti-GM/GE furore which made progress difficult. The practical and administrative impediments invented were extremely onerous, not helped by a fretful approach of university administrators who were not all supportive. Fortunately Otago stepped in and gave Stephanie a position and a brief to make vectors.

The concept is really neat. To make a vector one starts with a virus known to be able to penetrate into cells and discharge genetic cargo that will use the host cell machinery to make more virus. All the genetic information coding for the replication of this virus is removed from the vector and is replaced by the code for the protein wanted, in this case a good copy of the dysfunctional gene. The result is a vector particle capable of entering a cell and expressing a healthy protein, but not of replicating vector or any viral proteins. Trillions of copies can be cloned, free of the risk of any of the native virus.

## Vectors into sheep

Meanwhile the highly proficient and dedicated Nadia Mitchell had joined us and was doing an excellent job. In short order the lab was humming,

we cloned the normal ovine CLN5 and CLN6 genes in collaboration with Tony Frugier and Imke Tammen in Sydney and determined the mutations in the sheep.[18,22] Stephanie cloned these sequences into lentiviral and AAV9 vectors and we injected these into the brain parenchyma of affected sheep.[23]

CLN5-affected Borderdale sheep received lentiviral- or AAV9-mediated gene therapy between 2.1 - 3.5 months of age, prior to the onset of neurological disease signs. Viral vectors were delivered bilaterally into the cerebral lateral ventricles (intracerebroventricular; ICV) and to two intraparenchymal (IP) (cortical) sites which have been shown to degenerate significantly in the affected sheep. Clinical, cognitive and behavioural tests were begun, as were quantitative central nervous system imaging techniques, to assess disease progression and monitor therapeutic correction longitudinally.

The study endpoint was initially defined as 18 months of age, equivalent to an advanced disease state in untreated affected sheep, at which age the treated sheep were to be euthanised and collected tissues evaluated for any pathological amelioration. Once it became apparent that the treatment was functionally efficacious, the study was continued until five of the six treated sheep were euthanised at 27 months of age, exceeding the typical humane endpoint for untreated CLN5 deficient animals of 22 months of age. Their only symptoms were related to retinal degeneration and they became blind and clumsy, but post mortem examination revealed none of the parieto-occipital cortical atrophy typical of untreated affected sheep. The clinical outcomes were similar for both of the viral vector platforms tested.

Results from the CLN6 trials were not as spectacular, with only one of the six treated sheep responding positively. This was actually a pleasant surprise. Aside from genetic classification, the

Fig. 5. A chimeric lamb (with its surrogate mother) made by fusing half each of an affected black faced and footed affected sheep 16 cell embryo with half of a normal plain white embryo. The "footy socks" testify that it is indeed a chimera.

NCL gene products fall into two general subclasses – *bona fide* soluble glycosylated lysosomal proteins (CLN1, CLN2, CLN5 CLN10, CLN13) or predicted transmembrane proteins of unknown function. The lysosomal glycoproteins are taken up into lysosomes via a mannose 6 phosphate marker receptor, both intracellularly and extracellularly via the plasma membrane, and some of the protein produced is excreted from cells. This means that treated cells can cross-correct affected cells.

On the other hand, the dogma is that the insoluble membrane bound lysosomal proteins, of which CLN6 is one, are cell intrinsic, ruling out cross correction. However, an analysis of CLN6/normal chimeric sheep we created by embryo fusion (Fig. 5) indicated that normalised cells do correct affected cells, so long as their location and connectivity are appropriate.[24]

A number of improvements enhanced subsequent trials. We settled on recombinant adeno-associated viral vector serotype 9 (AAV9) as the vector backbone as this had become widely used worldwide and commercially available, allowing the testing of much higher titres and did not scare the horses as much as using lentiviral constructs. The vector does not integrate into the host genome, instead persisting in an extrachromosomal state as episomal concatemers in the host cell nucleus. In dividing cells the episomal DNA is not replicated with the host cell DNA and the AAV genome is lost at mitosis. However, in post-mitotic cells, such as differentiated neurons, AAV episomes remain intact providing sustained transgene expression and therapeutic protein production for the life of the host cell. Thus one set of injections into the right parts of the central nervous system should last indefinitely.

A construct expressing enhanced green fluorescent protein was purchased from the vector core at the University of Pennsylvania. Nadia used this to map the consequences of different routes of administration. We settled on the intracerebroventricular injections alone (Fig. 6) as they bypassed the blood-brain barrier and led to widespread transduction of the central nervous system, notably parts of the brain susceptible to disease atrophy (Fig. 7). Higher doses were more efficient. The hysteria around GM/GE issues had abated and some of the more inane and onerous housing restrictions were relaxed so the sheep could now graze in the open, a massive improvement for them and for us. Intravitreal injections of vector added to the protocol prevented the remaining vision pathology subsequent to ICV injections alone.[25]

We also realised the benefits of long-term *in vivo* monitoring of treated sheep and the need to justify that in applications for translation of the gene therapy to human medicine. For this we developed tests that included

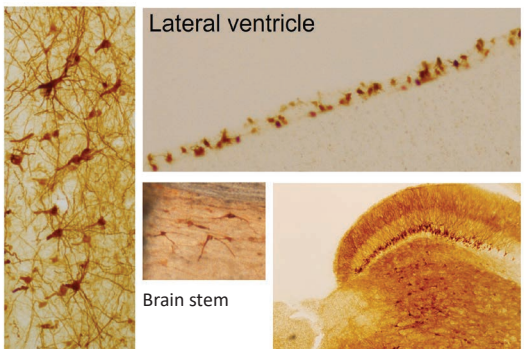
## GENE THERAPY IN SHEEP

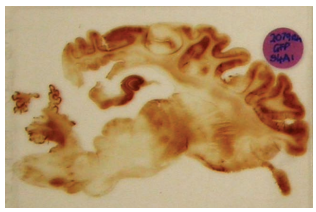

Fig. 6. Nadia performing the gene injection with Martin Wellby and Graham Barrell assisting. It takes about an hour from the beginning anaesthesia to the end of the surgery. An hour later the sheep are awake and starting to show an interest in food.

ITR = inverted terminal repeat; MND = a synthetic promoter

## AAV MEDIATED GFP

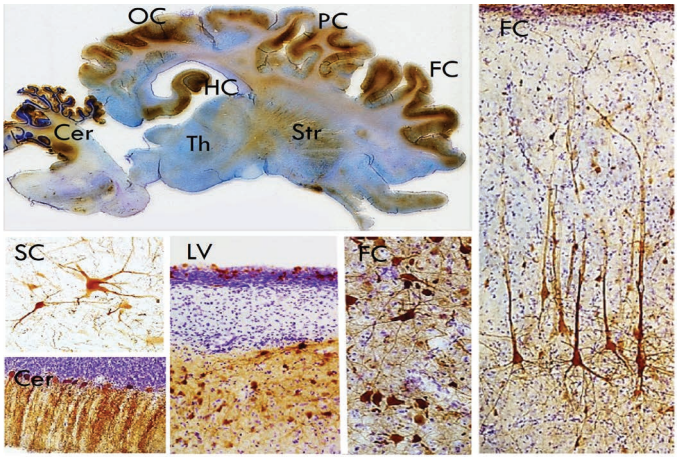
Commercial AAV9-GFP

Intraventricular Bilateral injection

Lateral ventricle




## AAV9 DELIVERY IN SHEEP

Intraventricular delivery results in good penetrance through the brain and spinal cord

Fig. 7. Immunostaining of virally delivered green fluorescent protein. Note the beautiful staining of the length of neurons in the frontal cortex (FC).

scAAV9 = self-complementary adeno-associated virus-9; CBA = chicken β-actin promoter; GFP = green fluorescent protein

OC = occipital cortex; PC = parietal cortex; FC = frontal cortex; HC = hippocampus Cer = Cerebellum; Th = thalamus; Str = striatum; SC = somatosensory cortex; LV = lateral ventricle

a neuro-opthalmic examination assessing ocular function, the central visual pathways and the visual cortex.[26] Auricular (acoustic startle) reflexes - the movement of the pinna in response to a loud noise - were also tested. Mentation, gait, head carriage and postural traits, as well as manifest tremor or seizure onset, were assessed while sheep were herded up a set of stairs and a graded slope to the testing facility. Functionality of the optic cranial nerve II, visual cortex and all cerebral areas associated with motor function were assessed by behavioural vision testing through negotiation of a maze in the field. Sheep were assigned a clinical score after examination by two independent assessors.

Computed tomography (CT) scans were performed every 2 months on animals who received intracranial gene therapy and intracranial volumes from the experimental animals were then compared with historical cohorts of untreated affected and heterozygous control sheep of both genotypes. Magnetic resonance imaging (MRI) was instigated after visiting some French colleagues and a longitudinal study of both CLN5 and CLN6 affected brains performed in collaboration with Dr Tracy Melzer, at the NZ Brain Research Institute.[27-28] Ophthalmic examinations were performed on the treated sheep by an independent veterinary ophthalmologist. Electroretingraphic (ERG) and funduscopic techniques to monitor disease-associated loss of vision longitudinally were developed by Katharina Russell.[29]

The treated sheep were disease-free. They benefitted from a profound improvement in quality of life, preservation of cognitive and neurological function and normalisation of intracranial volume and brain structure compared to untreated animals. Longitudinal studies of non-invasive biomarkers were used to assess the efficacy of lentiviral and AAV-based therapeutics in sheep *in vivo*. Monthly neurological examination and clinical scoring using a sheep-specific rating scale revealed the long-lasting functional efficacy of treatment, with treated animals being clinically indistinguishable from untreated heterozygous controls until 20.5 ± 0.6 months and 24.1 ± 1.5 months for the lentiviral and AAV9 cohorts respectively, when they begin to show effects of their visual deficits. Aside from this loss of vision, at 27 months of age treated sheep were still highly interactive with their environment. Longitudinal monitoring showed that the CLN5 sheep did really well, likewise the CLN6 treated ones.

They found these data compelling and human clinical trials have begun. So far so good. We have also established sheep as an ideal model species and others follow.

## To clinical trials

While this was happening, we were trying to find an entity willing to give human trials a go, or at least work towards that, and had been to lots of conferences and meetings with parents to encourage that. The BDSRA was very supportive. Drug companies, wanting millions of sales, calculated that there was not enough money in it. Pharmac thought the disease was not serious enough as only a small number of people were afflicted, to my mind a very strange world view. Two American parents contacted us who were very interested in the CLN6 treatment. Their agent visited us, and we provided all the information we could and offered to help. However, they decided to do it all in America and our involvement ceased, somewhat to my chagrin and to the relief of our administration.

By now Nadia was in charge. The good news is that we were contacted by an American firm, Neurogene, who were really keen on giving human gene therapy of CLN5 a go. Their principal visited us, Nadia provided a really large and comprehensive summary of our work including results of our trials in sheep in the demanding format for US FDA approval.[30] They found these data compelling and human clinical trials have begun. So far so good. We have also established sheep as an ideal model species and others follow.[31,32]

## Unfinished business

This is not the end though. Nadia and co are setting up for long term studies of the treated sheep to see if any problems arise later, so we can preview any need that treated people may have. This includes the issue of non-central nervous system pathology that may become a problem later, and we still have no really satisfactory explanation of the fluorescence. A paper appeared in *J. Phys. Chem*. that Sir John Walker and I are interested in,[33] and we are now in contact with the authors. BUT this still leaves the big unanswered question from the beginning. Why the specific storage of subunit c?

## Acknowledgements

Firstly, thank you to all my colleagues and co-workers at Massey, Lincoln, Otago and overseas who have played integral roles in this adventure. Sorry I could not name you all, but you know who you are. Also thank you to the parents for their staunch support, good will and encouragement. It's been a privilege to meet you all. None of this would have been possible without the financial support and encouragement from various charity funding bodies who have been there for us. We realise it is not intuitive that funding research on sheep is a way to help children's health needs so thank you to the Neurological Foundation of New Zealand, CureKids New Zealand, The Canterbury Medical Research Foundation, Lysosomal Diseases New Zealand (LDNZ) and especially the Batten Disease Support and Research Association (BDSRA) and and US NIH.

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# Biosensors: tools for the future with a spectrum of applications

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Keywords: biosensors, microfluidics, electrochemical sensors, 3D printing, diagnostics

## Introduction

During the COVID-19 pandemic, many phrases like "test and trace" or "test and release" were very common not only in healthcare facilities but also in almost all publicly accessible areas (Fig. 1). While these were new to many, it actually underpinned a decades-old technology that enabled rapid identification and/or quantification of analytes that are biologically relevant. Such technology was key to allow the infection rate to be curbed by following self-isolation and border control procedures.

During the COVID-19 outbreak, the ability to analyse samples quickly was critical to reduce turnaround time and prevent further spread of infection. Initially, samples were only analysed in central testing facilities using the well-established nucleic acid assay called known as polymerase-chain reaction, or PCR, with a turnaround time between a few hours to a few days along with the need for highly trained operators and expensive equipment. Later, other convenient alternatives to this strategy started to emerge where untrained users could simply analyse their nasopharyngeal swabs at home in a few minutes using a lateral flow device (LFD) or rapid antigen test (RAT).

This transition from laboratory-based testing to rapid testing was not possible without the accumulating knowledge and research that supported the development of biosensors. A biosensor is a device that can report on a pathophysiological condition using a simple and easy to use device that allows untrained users (or at least users with basic training) to test their own samples at the point-of-need, either at home or in a healthcare facility. These devices reduce the need for sample transport to central laboratories and allow swift management of health conditions by providing quick and reliable information enabling accurate clinical assessments.[1]

One of the first commercially available (and most successful) biosensor

Mohamed Sharafeldin is a lecturer in the Department of Chemistry, University of Otago. After finishing his PhD in 2019 at the University of Connecticut, USA (with Prof James Rusling), Mohamed moved to University of Oxford, UK (with Prof Jason Davis) where he worked as a postdoctoral research associate between 2019 and 2022. Mohamed is interested in the development of biosensors for different applications. He specialises in integrating modern microfluidic fabrication techniques into electrochemical and optical platforms for production of highly sensitive, scalable, low-cost sensors.

Mohamed has extensive experience using 3D printing along with interfacial/surface chemistry to develop highly specific, semi-automated assays for cancer and cardiovascular disease diagnostics.

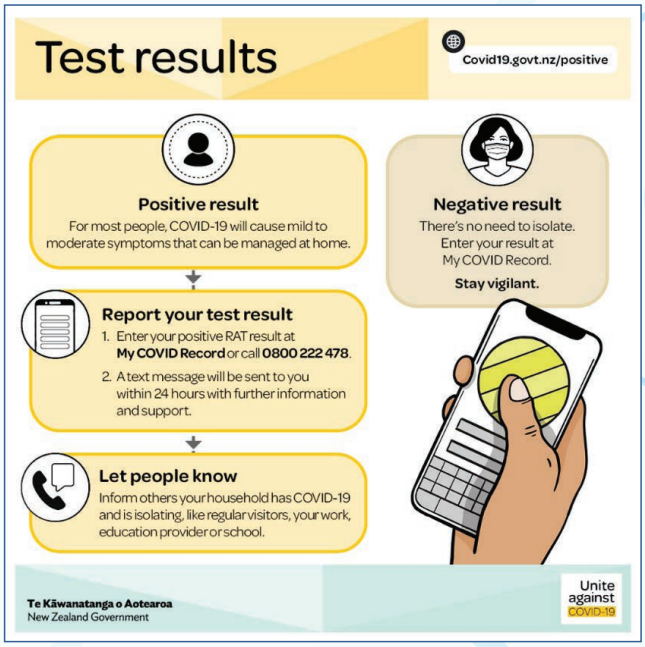


Fig. 1. Typical signage showing instructions for testing at home using a rapid antigen test (RAT) and reporting results online. RATs were central to establishing an efficient COVID-19 testing system to allow contact tracing. Image courtesy of Greater Wellington COVID-19 Hub.

was the glucose sensor or glucometer, widely used to measure the levels of glucose in blood collected from a finger prick. While insulin discovery was one of the most significant, life-changing events for millions of diabetic patients, its significance was not fully realised until these glucometers were invented.[2]

Initially, glucose levels used to be measured in urine samples using Benedict's reagent invented in 1908. Benedict's reagent is a copper-based mixture to detect reducing sugars that requires a cumbersome heating procedure and is susceptible to interferences from many reducing chemicals.[3] The introduction of glucose home testing kits in the 1970s was a landmark change where the concept of self-monitoring of blood glucose (SMBG) became the standard for diabetic care. These glucose sensors, built on electrochemical platforms, enabled a much more accurate glucose analysis with a quick response and high sensitivity. Glucose sensors did not only provide better management of diabetes, but also initiated a new field of research focusing on biosensor development and investigation of their use in biomedical applications, the pharmaceutical industry, food quality control and drug and border control.

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## Biosensors for biomedical applications

The development of biosensors for biomedical applications spans a range of disease diagnostics and point-of-care (POC) health monitoring. With the huge leap in drug development targeting specific biochemical pathways underlying different diseases, there was a pressing need for more precise and accurate diagnostics at the POC - also known as personalised or precision medicine. The genetic and physiological variations between patients are known to be decisive in determining the best course of treatment for a specific condition.

Biosensors targeting specific biomarkers (protein, nucleic acid or metabolites) abnormally expressed during disease progression, can accurately identify disease stage and report on response to therapeutic regimes. Do to their sensitivity, ease of use and fast response, biosensors can support early detection of diseases, personalised treatment and monitoring of disease progression as well as guide personalised therapeutic interventions. To highlight the biomedical applications of biosensors we herein demonstrate the significance of using biosensors in the detection of two leading causes of death; cardiovascular diseases (CVDs) and cancer.

Cardiovascular diseases are the leading cause of death worldwide accounting for more than 31% of all deaths in 2016.[4] Patients admitted to emergency rooms with chest pain are usually examined using diagnostic electrocardiogram (ECG). Half of CVD patients show normal or no diagnostic electrocardiograms complicating the accurate and fast diagnosis of the condition.[5] Analysis of biomarkers in serum is a more accurate and sensitive alternative but requires central laboratory testing which, in some cases (especially in areas with limited resources), can delay life-saving treatments. Biosensors can bridge this gap by enabling a fast, sensitive analysis of CVD biomarkers at the POC where analysis in an ambulance or emergency room support early-stage diagnosis which allows successful treatment and recovery of CVD patients.

An array of biomarkers has been identified and validated for different CVD conditions including cardiac troponin I (cTnI) and creatine kinase MB subform (CK-MB) for acute myocardial infarction; B-type natriuretic peptide (BNP) for acute coronary syndromes or heart failure; C-reactive protein (CRP) and tumour necrosis factor alpha (TNF-α) as markers for inflammation and cardiac risk factors. Several commercial biosensors are now available for such applications; for example the Minicare© system (Philips) for the quantitation of cTnI and BNP in serum, TriageTrue© (Quidel) for detection of cTnI and i-STAT© (Abbott) to determine BNP and cTnI.[6] Although these systems offer life-saving tools that assist clinicians in the emergency room, there is a need for better sensing performance in terms of cost, time and sensitivity. For example, most commercial systems can detect cTnI down to 1 ng/mL while its normal range in blood is between 0-0.04 ng/mL (well below the range detectable by commercial sensors), underpinning the need for more sensitive techniques to detect concentrations down to a few pg/mL.[7]

On the other hand, cancer as the second leading cause of death worldwide is heterogenous in nature and its diagnosis is as challenging as its treatment. The ability to diagnose cancer at an early stage before presentation of symptoms is associated with effective and successful treatment. Current diagnostic strategies rely mainly on the development of

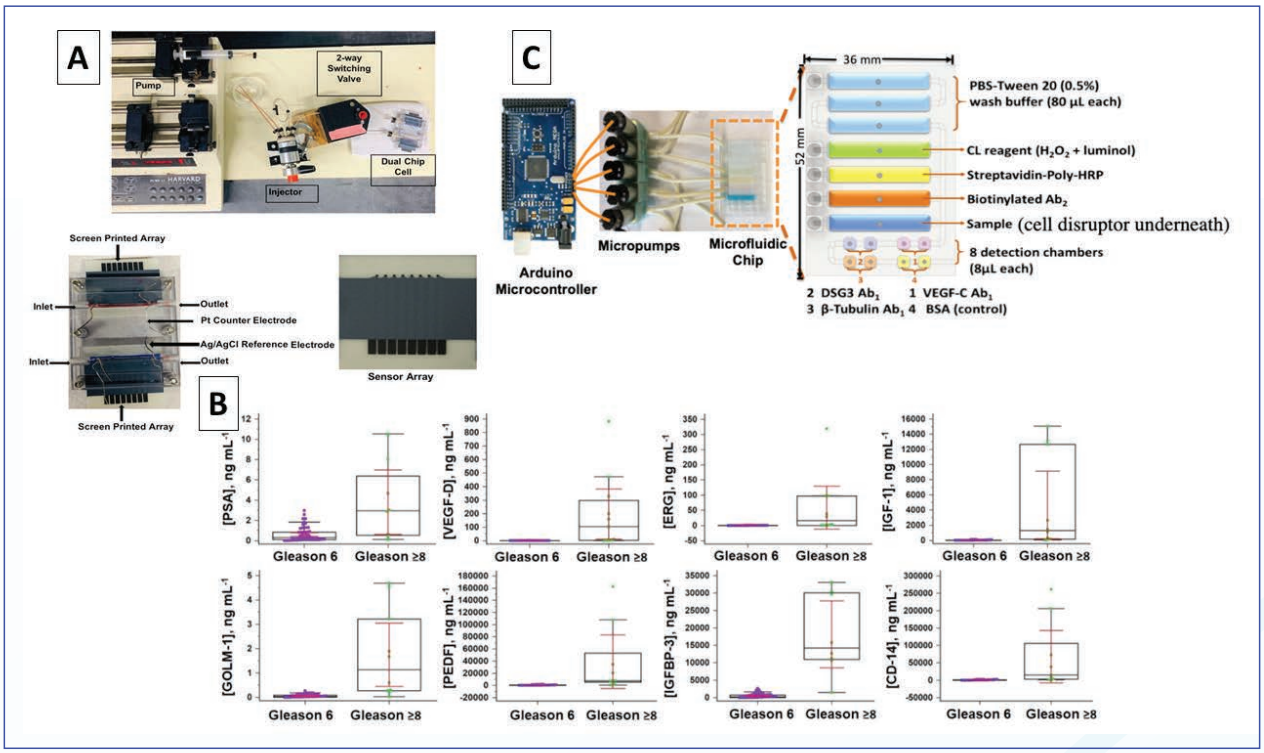


Fig. 2. Examples of biosensors developed for diagnosis of cancer. (A) A microfluidic chip housing a 16-electrode array to analyse 8-protein biomarkers as a tool to identify indolent and aggressive forms of prostate cancer. (B) Box plot analysis of results obtained from 130 male patients samples showing the expression levels of the selected biomarker panel of indolent (Gleason score 6) vs aggressive (Gleason score >8) prostate cancer (outliers excluded). Reproduced with permission from reference 9. (C) A 3D-printed microfluidic chip for analysis of cell-membrane bound protein, desmoglein 3 (DSG3), a cancer metastasis biomarker. The system integrates an automated on-chip cell lysis compartment for DSG3 extraction and automated sample and reagent delivery to biomarker detection compartment. Reproduced with permission from reference 10.

symptoms and imaging techniques that usually identify cancer after development of significant cancerous lesions (tissue), limiting the efficacy of treatment and increasing the risk of spreading cancer to other parts of the body, known as metastasis.

Recent genetic, proteomic and metabolomic analyses have correlated the abnormal expression of several biomarkers to different types of cancer. These biomarkers, advantageously, can identify cancer at a very early stage well before the development of any symptoms. Assessing the expression of these biomarkers supports cancer screening programmes that enable efficient and successful therapeutic interventions.[8]

The most prominent examples of these programmes are breast and prostate cancer screening adopted in many healthcare systems. Testing serum levels of prostate specific antigen (PSA) and examining BRCA1 and BRCA2 gene variations as predisposition signs of prostate and breast cancer respectively allow identification of more susceptible individuals initiating, personalised health-monitoring programmes. Alas, current screening techniques suffer from low specificity, resulting in false positive diagnoses which puts many patients under psychological stress while adding more cost and pressure on healthcare systems. While there are no commercially available cancer biosensors, research in this field can allow highly sensitive cancer diagnosis with good sensitivity. In addition, biosensors can offer an accessible tool for physicians to tailor treatment regimes and dosing according to individual responses to administered drugs.

Interestingly, recent evidence supports the use of biomarker panels for cancer identification rather than less-reliable single biomarker assays. For example, a panel of 8 biomarkers (including PSA, vascular endothelial growth factor-D (VEGF-D), gene fusion proteins ETS related gene (ERG), Golgi membrane protein 1 (GOLM-1), pigment epithelial derived factor (PEDF), insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFPB-3) and serum monocyte differentiation antigen CD-14 (CD-14)) can accurately predict aggressive vs indolent forms of prostate cancer. This test was performed in an electrochemical microfluidic system that can be easily integrated into a commercial platform (Fig. 2).[9]

Other endeavours investigating system automation and integration of

multiple steps demonstrated biosensors' ability to detect cell-bound biomarkers for cancer metastasis. The system was built on a 3D-printed microfluidic chip that can extract cell-membrane bound protein, desmoglein 3 (DSG3), a metastatic biomarker for oral cancer. The extracted DSG3 was then quantified within the same microfluidic chip using a well-established chemiluminescence assay with very high sensitivity down to fg/mL (Fig. 2). Such a system allows clinicians to decide on a specific course of treatment since a high level of DSG3 indicates the presence of metastatic cancerous tissue that require dissection.[10] These examples highlight the potential capabilities of using biosensors in CVD and cancer diagnosis and demonstrate the advantages of integrating them into the healthcare system workflow where it can allow better, more efficient treatment and improve quality of care while reducing costs and time spent in hospitals.

## Biosensor applications in the pharmaceutical industry

Biosensors, by virtue of their ability to monitor events in real-time, offer an excellent tool to evaluate different processes within pharmaceutical industries, helping companies meet production and compliance goals. Biosensor tasks can vary from simple functions like monitoring oxygen and pH to quantifying drugs for quality control, assessing sterility, detecting common impurities or contaminants and running bioavailability and pharmacokinetic studies. These functions can be easily achieved by integrating optical or electrochemical sensors within a production line to guide scale up or scale down activities. A well-established example of integrating biosensors within production lines is the use of multiple infrared sensors to monitor the homogeneity of active ingredients within a powder blend during tablet manufacturing. This enables semi-automated control on the blend end-point to ensure tablet content uniformity.[11]

"Biosensors, by virtue of their ability to monitor events in real-time, offer an excellent tool to evaluate different processes within pharmaceutical industries, helping companies meet production and compliance goals. "

The application of biosensors in the pharmaceutical field has been further highlighted by the rapid evolution of immunotherapeutic agents which usually require expensive, lengthy and laborious analysis. The increased interest in using immunomodulators or therapeutic vaccines for treatment of cancer or other conditions requires efficient tools for their quantitation and evaluation of their functionality. The quality control process of immunotherapies is not limited to just quantitative analysis but should also report on their pharmacological and therapeutic effect including multi-parametric analysis of cell function, real-time detection of secreted signals and facile retrieval of cells of interest (Fig. 3).[12]

Currently, polychromatic flow cytometry is the main technique applied for monitoring immunotherapeutic agents, both in preclinical tumour immunology and in cancer immunotherapy trials. While polychromic flow cytometry offers the ability to detect multiple target analytes simultaneously, it is expensive and requires very sophisticated equipment, highly trained operators and complex data for *in vitro* analysis to study cells during manufacturing and testing processes. This is reflected in the high cost of immunotherapy development that requires prolonged testing and evaluation procedures.[13]

The use of biosensors in such process can drastically reduce the associated cost of immunotherapy development and evaluation, especially with the development of cell-based biosensors that can provide real-time quantitative response to immunotherapeutic agents. This has been extensively studied using label-free optical biosensors, particularly those using surface plasmon resonance where an evanescent wave generated at the plasmonic active interface (usually gold) interacts with a specially engineered incident beam of light and responds sensitively to changes close to the interface. These sensors have been applied to the detection and quantitation of secreted proteins, intracellular changes (known as cytosolic dynamic mass redistribution (DMR)) and tumour and anti-tumour cells in analysed samples (Fig. 3).[14]

## Food quality control

The application of biosensors in the food processing industry is gaining a lot of momentum, especially with the immense need for energy saving and minimising the environmental impact of food production and waste management (Fig. 4). As previously mentioned, biosensors offer rapid and sensitive real-time monitoring of specific target analytes enabling fast detection of the presence of hazardous byproducts or viable cells during food processing. This information is critical in automating food processing and can reduce cost and time as such tighter quality control measures reduce waste, improve food production quality and sustainability.

Biosensors ranging from pH probes to sensing tags integrated within food packaging allow monitoring of storage and transport conditions. In addition, biosensors have been

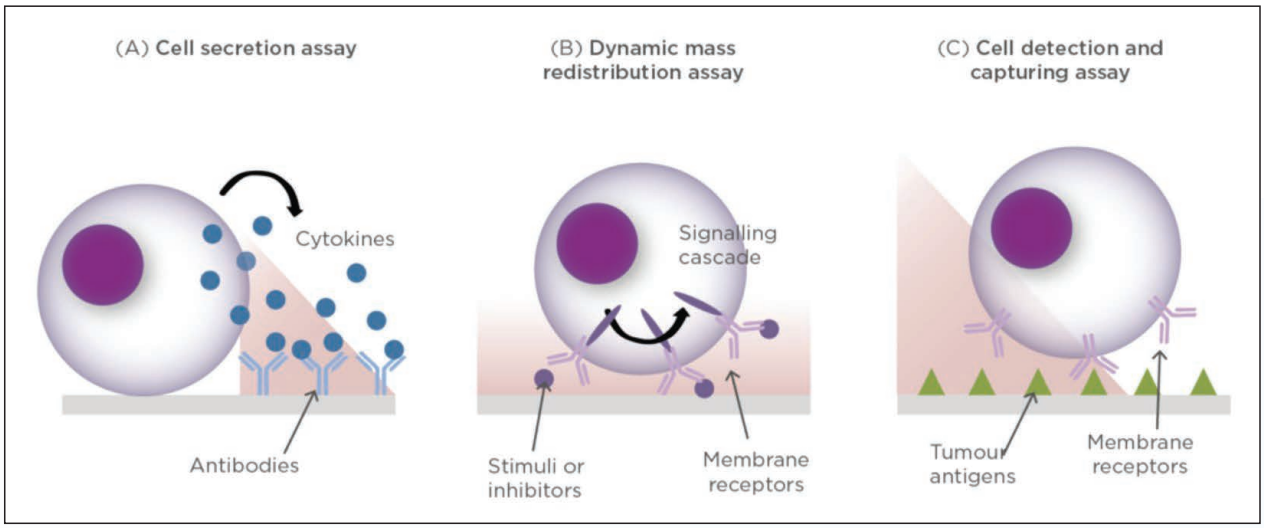


Fig. 3. Schematic depiction of different types of cell-based label-free optical immunosensors that can identify (A) specific secreted proteins; (B) cytosolic dynamic mass redistribution (DMR); and (C) detect and capture specific anti-tumour/tumour cells. These sensors employ the interaction between an evanescent wave (depicted as pink shading) and molecules at dielectric/plasmonic interface to probe changes happening at the biosensor surface. Reproduced from reference 14 under a Creative Commons 4.0 License.

widely applied in the detection of food contaminants like pesticides, toxins or foodborne pathogens. A plethora of commercial food biosensors have been introduced into the food industry over the past two decades; for example RIDACUBE SCAN by r-biopharm© for onsite monitoring of lactic and acetic acid, sugars, ascorbic acid and alcohol; VitaFast© systems for vitamins; BIOFISH 300 from BIOLAN for sulfite detection and PremiTest from r-biopharm for the detection of antibiotic residues.[15] Unfortunately, there is still a huge gap between market need and available biosensors due to the increasing range of food contaminants, high rate of food counterfeiting and adulteration and the uncontrolled use of antibiotics and pesticides.

## Future scope

The examples discussed in this article are by no means an exhaustive list of biosensors' applications. Rather they highlight the benefits the biosensor industry can bring to many fields ranging from healthcare affordability to food safety. Biosensors can provide critical information quickly and specifically to allow swift decision-making based on real-time analyses. While current technology has achieved many milestones required for efficient biosensing applications, there are still many areas where further development is required.

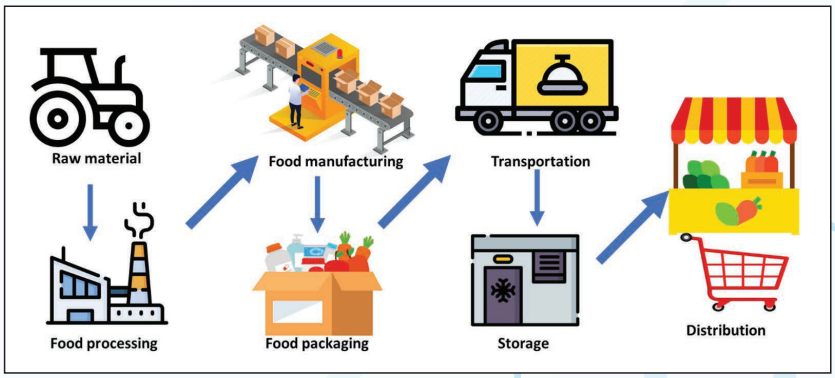


Fig. 4. Biosensors are used across the entire food processing chain from examining raw material to testing final products.

For example, biosensor fabrication has emerged from high-cost sophisticated machinery to using paper-based and 3D printed chips for both prototyping or mass-production. Using 3D printing to manufacture biosensors can enable new levels of scalability and affordability to be reached, capitalising on the availability of low-cost desktop 3D printers. This can enable production of previously difficult-to-manufacture architectures that can further improve sensors' performance and capabilities. Additionally, the advances in engineered micro-components that can be integrated in electrochemical, optical or spectro-electrochemical sensors can allow further miniaturisation without compromising sensor sensitivity.

The use of portable hand-held potentiostats, complementary metal oxide semiconductor (CMOS) sensors, LEDs and fiber optics can allow the production of next-generation biosensors that can achieve bench-

mark performance of laboratory equipment within a small, low-cost device.

The development of cell-based responsive biosensors, tissue-on-chip and organ-on-chip is another area of development where biosensors can pave the way for fast drug development and validation. The introduction of new sensing platforms that improve sensor sensitivity and reduce the number of steps typically required to run an immunoassay is an equally important area of development. Biosensors with high sensitivity that can detect ultra-low concentrations of target species allow discovery of new biomarkers that are usually missed with current analytical tools. Additionally, biosensors can support studies required to validate panels of biomarkers to evaluate different diseases or physiological conditions. These can afford accurate non-invasive tools for disease diagnostics, improve the quality of healthcare systems, ensure tighter quality control measures in food and pharmaceutical industries and strengthen the current inventory of tools required for preparedness for future healthcare crises or pandemics.

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# Motivating and engaging students to write in science

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Keywords: science education, scientific writing, literacy

## Writing in science

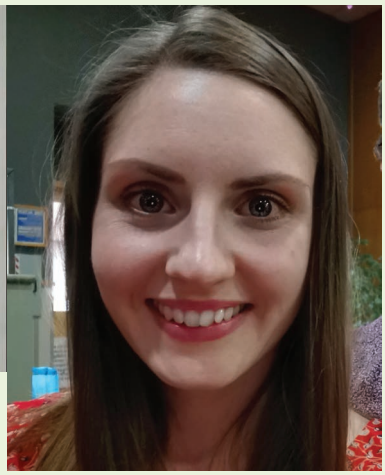
*"When students have developed the positive attitudes that lead them to become fluent and independent readers and writers, they gain life-long benefits."*[1]

Writing is a form of communication which students are required to master in the course of their learning in order to succeed with written assessments needed to be completed for NCEA. It is especially important in science, certainly at the higher levels of NCEA, and therefore it is essential to begin a focus on teaching writing in junior school.

Glynn & Muth[2] state that, "learning to read prepares a student for reading to learn' (p. 1060) and that "learning to write prepares students for writing to learn" (p. 1064). Thus, learning to read and write influences students' abilities to use those skills to learn science (cited in Nixon & Akerson[3], p. 198). It is acknowledged that reading skills support writing and writing improves one's reading skills. When you read and then write, the text presented teaches one how to do the writing. It is through writing, that you can then understand the learning content. By supporting students to become confident readers and writers in science, we can positively impact their literacy skills but also deepen their science understanding.

Snow and Moje[4] stated that "deep learning in subject areas requires complex literacy skills" (p. 66). In the article they talked about adolescent learners often struggling to achieve the level required without explicit instruction in areas such as science and integrating literacy skills into the teaching so that they can achieve successfully (p.67). Therefore, when teachers focus on explicit instruction to develop reading and writing skills in their classroom science learning programme, it is possible to foster deeper learning through inquiry and problem-solving. Students can learn the science content, building on prior learning and making connections to the real world constructively. The motivation to learn is greater when students master a range of skills needed to achieve in science and their engagement grows with their success.

This article seeks to share the perspectives of a teacher, Kayla Robertson (the co-author), about a group of Year 9 students in a secondary school setting in 2021. This group of



Dr Mal Thompson (above, left) was a literacy consultant for many years at the University of Otago College of Education and completed her PhD on literacy challenges in reading science texts in 2012. In the last 3 years she has been tutoring students in NCEA subjects and working as a relief teacher.

Kayla Robertson (above, right) is a 4th year teacher in chemistry and science at Logan Park High School, Dunedin. She wants to raise the achievement of her students and help them in their understanding of science concepts and knowledge using a metacognitive approach in her teaching and learning programme. Having a focus on building students' understanding of science language and texts was really beneficial for her development of classroom resources (that involved chunks of texts and instructions) and teaching strategies to provide deeper learning experiences. It was rewarding to see the students making progress with their written explanations of experiments by linking observations to reasons (scientific concepts).

students were studying science and the initiative was designed to engage students and improve their achievement over the year through a range of activities, particularly online, to help them build their literacy skills in writing. It was also designed to boost their self-management skills and motivate them to achieve in science.

## Background to the science literacy study

Following lockdowns in 2020-21, discussion between the co-authors led to a decision to try to improve motivation and engagement of a group of young students in reading and writing in science. We wanted to help the students with reading science text and then teach them how to write in science, which we hoped would improve their NCEA achievement in science over the year. By integrating the reading and writing and making the learning purposeful, we believed this could lead to the students being more engaged in what they were learning and bring them greater success. We also believed that "motivation is an orientation to learning" as stated in an Education Hub article[5] because it helps those students who struggle to read and write at the level expected. Could an additional literacy focus address our students' struggle to motivate themselves and build a deeper understanding in science?

In addition, a 2021 Education Review Office (ERO) report[6] outlined the impact of COVID-19 stating that most teachers were concerned about student learning in practical subjects and writing. Whilst schools mobilised to support students online from the first lockdown in March 2020, the impact on students' writing was not initially considered but could be viewed as one of the challenges of the pandemic.

Recognising that writing can play a powerful role in the learning of science, "students with competent writing skills are well on their way to achieving scientific literacy; however, the attainment of literacy also presupposes effective writing activities and strategies".[2] Developing those writing skills would have been challenging during lockdown as it required some explicit teaching of science writing.

| Stanine | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Listening | | | | | | | | | |
| (24 students) | | | 2 | 1 | 9 | 8 | 2 | 2 | |
| Reading | | | | | | | | | |
| (27 students) | | | | 3 | 5 | 9 | 5 | 4 | 1 |
| Vocabulary (25 students) | | | | 1 | 6 | 11 | 4 | 1 | 2 |

Table 1. Year 9 test score baseline data.

Kayla stated that the students found writing particularly difficult and because of this they were not motivated in writing in science at all. It was acknowledged that these students may have faced challenges in writing with lockdown in 2020 and this could impact on their achievement in 2021.

According to an Education Hub report in 2022,[7] studies have shown that teachers across a range of secondary school subjects tend to ask closed rather than open questions during literacy-based activities, rarely provide students with texts to read that are sufficiently challenging, and do not provide explicit literacy instruction on key aspects of written texts such as structure, purpose, audience and so on. Kayla agreed with this and was enthusiastic about her learning and making changes to support the students.

Furthermore, the Education Hub report[7] stated that assessment data to support the instruction was seen as not always being used effectively: "there is evidence that literacy assessment is not always being used to identify children who have particular literacy needs or to design effective interventions to support them" (p.30). It was clear that we needed to use assessment data to plan interventions to support our students fully through the science teaching programme.

## Assessment data gathering

Kayla and I brainstormed what assessment data we could use for our study and decided to use data already gathered in school such as Progressive Achievement Test (PAT) scores, a writing assessment (from the English department) and then carry out a diagnostic literacy assessment previously used in postgraduate work and subsequently in a number of secondary schools, particularly in the science area.

Early in Term 1, the students were asked to sit a diagnostic literacy test in science (on laboratory safety) to give the teacher some data on their literacy skills in reading a science text. This produced some valuable information to target their learning: three areas of interest were using text features, reading for deeper meaning and vocabulary strategies. The test gave us information on areas where their knowledge was weak and a range of teaching and learning strategies were then developed through the classroom programme over the year for the class. Table 1 shows the baseline data and includes the range of PAT stanines which scales test scores on a nine-point scale with a mean of five and a standard deviation of two.

While incomplete for all 29 students in the class, the PAT data did give us an indication of the range of abilities in this group of students. However, it should be noted that often the only data available for science teachers are the assessments carried out mostly by English teachers and is difficult to apply this to what happens in the science classroom without some assistance.

There was a large group of students in the middle range of 5-6 stanines with smaller numbers below average and also above average. Observations of the students supported some of the findings, especially the listening skills of the students needing to be developed further.

The diagnostic literacy test also linked to the PAT data, indicating the range in the class in their literacy skills. Both pieces of data gave us insight into the students' needs so that we could make a difference over the year. Mostly, it was interesting to see how much students wrote and commonly they would give one-word answers. Word knowledge was weak and their writing indicated they needed to expand their vocabulary and sentence structures in science to help them demonstrate greater understanding.

The only writing data, from an assessment carried out in English in creative writing, did not accurately reflect the abilities of students who prefer science and do not enjoy writing in English. However, we were able to gauge roughly from the writing students' potential achievement in science, placing it alongside the other data.

## The teaching plan

Using this data, we ensured that the students were given opportunity to use text features so they could find the information to help them understand the science more clearly. Text features such as headings, bullet points, captions and font (e.g. bold, italics etc.), as well as skimming and scanning, references, illustrations and diagrams etc. were focused on to help the students read the text. The students found this helpful and understood that these tools made it easier to read the text. Kayla pointed out to the students in any text they were reading what to look for and notes were often written on the board to remind them. The students appeared to find this supportive and were quick to use these features to help them find the information they needed. This repetitive process helped students to develop their skills in reading science text. Instructional information was used to ensure that deeper learning occurred.

It was also important to direct the students' attention to text structure to help develop their writing skills which would be needed at a higher level. A range of activities were constructed on specific topics. In a unit on optics, waves and energy, a booklet was put together which helped students explore the sense of sight. Students had to read material and engage in some activities such as optical illusions and eye facts before writing down what they could see. The science concepts were then introduced and students were given a diagram of the human eye with words and descriptions to match up and talk about labelling a diagram.

There were many other activities related to the topic for students, mostly online, but they supported the teaching in the classroom. Completion of the activities was encouraged but not compulsory. Kayla also worked with the class as a whole and then summarised what they were saying on the board. It was observed that they began to write more and contribute more in class sessions. A booklet with many optical illusions gave students much enjoyment in understanding how the eye works and increased their engagement in learning further about the concept of sight.

| Characteristics | Gases | Liquids | Solids |
|---|---|---|---|
| Volume | | | |
| Shape | | | |
| Free space between particles | | | |
| Flow or movement of particles | | | |
| Illustration of particles | | | |

Table 2. Student recording sheet for an activity related to states of matter.

Another unit of work on states of matter asked students to write a summary of the characteristics of gases, liquids and solids in terms of shape, volume, free space between particles and flow or movement of particles (Table 2).

The second step of the activity was to identify one specific example of each state of matter and explain why it is a solid, liquid, or gas in terms of its shape, volume, free space between particles and flow of particles. They were required to write at least five complete sentences.

Explanations of how to fill the table and then use the information to write their sentences were very helpful for students who could then complete the task easily. Tasks were engaging, such as using graphic organisers and playing games like dominoes where concepts and definitions could be linked to the topic of solids, gases and liquids.

Another theme of 'Adaptations for survival' inspired students to relate this to their own lives. An initial ac-

tivity of finding key words in the text led to filling in a sheet with the features of birds. The students had to sort images into categories and then write a summary statement. These activities supported them in their writing. The use of open questions also encouraged students to inquire, think more deeply and find solutions to what they were learning.

Other learning activities included regular quizzes which recapped their learning and was a fun activity for the students who competed well to show their learning. Vocabulary was often a focus, especially new words in the unit of work. Repetitive activities strengthened their working vocabulary and also gave them opportunities to use the words in their written work. Reading strategies, such as working out meaning from context and also linking new words to word families was helpful for the students. These strategies also allowed students to develop their literacy skills. Most of the activities were designed to strengthen their ability to working independently online and increase their self-management skills.

Activities in any units of work were designed with online learning in mind as this enabled us to better prepare them for working online at home, when the need arose. However, so that no student was excluded if they did not have access to a device while at home, the work was available as a booklet. Yates and Starkey[8] pointed out the need for practical strategies and collaborative learning in the classroom to be extended for learning at home. The activities were also designed to increase their writing time and set them up for continuing study in science.

## The findings

Initial observations showed that the students did not focus fully on what they were being taught and tended to talk while the teacher was talking or be distracted by other happenings in the classroom. As the year progressed it was evident that a number of students were more engaged in the classroom teaching and learning and interacted a lot more with the teacher. This reflected in the time spent by the teacher in explaining the topic and then scaffolding the writing required which helped students to develop their comprehension skills as well.

| Grade | Mixing & Separating | Botany | Optics | Matter | Food & Digestion |
|---|---|---|---|---|---|
| E | **4** | **5** | **2** | **6** | **11** |
| M | 9 | 10 | 12 | 10 | 8 |
| A | 10 | 11 | 11 | 10 | 7 |
| N | 3 | 0 | 1 | 0 | 0 |

Table 3. Year 9 results for 26 students who completed all assessments.
E = Excellence, M = Merit, A = Achieved, N = Not achieved

| Grade | Chemistry | Biology | Physics | Practical |
|---|---|---|---|---|
| E | 8 (31%) | 8 (31%) | 3(12%) | 8 (31%) |
| M | 11 (42%) | 11 (42%) | 9 (35%) | 15 (58%) |
| A | 6 (23%) | 7 (27%) | 10 (38%) | 3 (11%) |
| N | 1 (4%) | 0 | 4 (15%) | 0 |

Table 4. Year 9 results for science subjects and practical.
E = Excellence, M = Merit, A = Achieved, N = Not achieved

Often working in groups, the students enjoyed carrying out the tasks together. Feedback from students was positive: students felt they were learning lots in science and found it easier than at the beginning of the year. Kayla's strategy of mixing up groups encouraged students to work with different people and learn from them with the emphasis on the tasks rather than 'friend' groups which could lead them lose focus at times.

End-of-year data showed that students had made great progress. Table 3 (different science topics) and Table 4 (different science disciplines) show the results for all testing over the year, including the end of year assessments. The school (like many others) uses the same assessment grades as NCEA. Kayla and I were looking at the results to see the patterns in achievement over the year.

Table 3 shows all the units of work where students were assessed with an end of unit test. The results indicate that most students were achieving in all the units of work and that about 70% of the students achieved highly. It also showed that their writing improved over the course of the year with the answers they gave; students were observed to write more than just a word, gave more detail in their answers and began to write at least a paragraph when required. Their vocabulary also increased and supported their ability to write more.

Table 4 indicates that the top group of students are performing well with 8 students (31%) attaining Excellence in chemistry, biology and practicals. Again, those achieving Merit and Excellence comprised 70% of the students in the class. There was only 1 student (4%) in chemistry and 4 students (15%) in physics not achieving. These were pleasing results overall. The quality and quantity of writing increased with written work was designed to scaffold their learning.

## Conclusions

The in-depth planning and teaching work carried out impacted on student achievement. All students showed an improvement in their science learning. The teaching and learning programme designed specific activities to build students' literacy skills and helped them to become more motivated learners. Targeting their learning literacy needs showed they could also deepen their scientific knowledge and achieve well in science.

Overall, the pleasing results reinforced our belief that the students needed to be set up better for learning by scaffolding the reading and writing so they could complete tasks more successfully online and at home. Students were more motivated to succeed and this demonstrates that deeper learning does result from focused and teaching specifically what is needed.

The importance of writing skills was shown by the results; students who struggled with reading found the writing a great challenge. The students who scored highly in reading tended to achieve well in the writing tasks, but most students improved over the year in writing. Students are now able to recognise different text structures and language and transfer this knowledge to their writing. This indicated that we could improve student achievement with the teaching of reading and writing together.

Feedback from the students also supported the improved achievement as they could see that they could achieve in science. It also showed that students were more motivated to learn science. This is important as they often perceive science to be too hard, especially as they progress through school. This sometimes can lead to making bad choices which can impact on their desired pathway on leaving school.

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