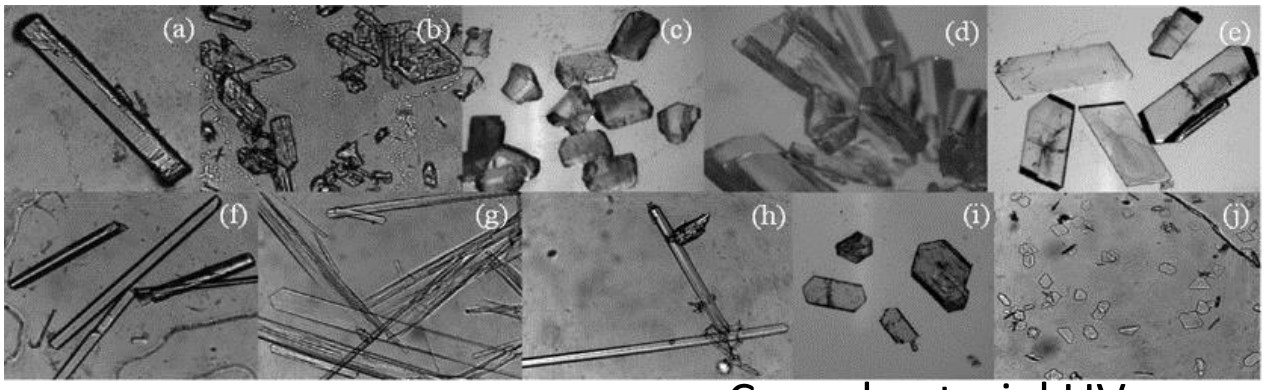
The Institute of Chemistry of Ireland

Irish Chemical News

Feature Articles:

Understanding and Controlling Highly Anisotropic Crystal Growth
Patrick McArdle

From Benzamides to Macrocyclic Imides and beyond.
John F. Gallagher



Microbial cell factories for novel and unique industrial enzymes and Bioactivities.
Catherine Collins and Patrick Murray

Cyanobacterial UV-screening compounds are safe alternative for cosmetics and pharmaceutical applications.
Sushanta Kumar Saha

2014 Issue 2

Editorial

Welcome to the new issue of Irish Chemical News. We have a number of feature articles in this issue that come from the Congress in Limerick this year. As 2014 was the International year of crystallography, Pat McArdle (NUIG) describes his work in crystal growing. Two groups from Limerick have contributed articles on "microbial Cell Factories" and novel applications of Cyanobacteria. Finally, John Gallagher (DCU) discusses some of his recent work on the synthesis of macrocyclic imides.

One of the aims of this issue is that it can be distributed as widely as possible, to members and non-members alike, to highlight the activities of the Institute and hopefully to grow our membership. All our readers are encouraged to visit the website, http://www.chemistryireland.org/index.html, regularly. Details of membership rates can also be found there.

We still need volunteers for articles that can be featured in ICN. There is a bias towards academic articles, so we especially ask our industrial members to participate. If you wish to contribute to the next issue, or have ideas of article types you wish to see, please do not hesitate to contact the editorial team (Robert Baker, Margaret Franklin or Brian Murray).

Dr Robert Baker,

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Introduction from the President

Dear Fellows, Members, Graduate Members,

As my second year as President nears its end in April, I look back on the year since last Christmas and see we can claim some successes. Firstly we had a very successful Congress in Limerick with 75 – 80 delegates attending. The title was "Culturing of Crystals, Chemical and Biochemical Solutions". The event was very well organised by Fergal Barry and his team of LIT with support from UL. We had a good balance of crystallisation and biotechnology talks followed by an excellent poster display from LIT and UL.

We had two significant events in Dublin. Firstly in April The Boyle Higgins Gold Medal & Lecture Award going to Professor Pat Guiry, School of Chemistry and Chemical Biology, UCD. We had a good attendance of approximately 100. This was followed by our annual AGM. The second award was The ICI Annual Award for Chemistry (Eva Philbin Lecture Series). This was awarded to Professor Thorri Gunnlauggson, School of Chemistry at Trinity College. This event was held in conjunction with Inorganic Ireland 2014 at The Royal College of Surgeons in Ireland, on December 11th. It was well attended again with approximately 100 delegates.

Given the range of topics and high standard of all these lectures I urge all chemists to make an effort to attend some of our events, support your profession and enhance to your professional development.

I attended the EuCheMS General Assembly in Torun, Poland where it was announced that the new constitution was formally signed into Belgian Law by the Belgian King and is now a legal European entity.

ICI again we sponsored the 66th Irish Universities Chemistry Research Colloquium held in NUIG this year and many impressive presentations were made. We supported the Regional Rounds of the ISTA Senior Science Quiz and final held at Trinity on Saturday November 22nd with book tokens as prizes. 1035 Leaving Certificate science students participated in the Regionals Rounds in 12 venues nationwide.

The Institute would like to have more events including both academic and industry awards. We can only do this with the support of the Irish chemical community, ICI members, pharmaceutical and chemical companies. On October 1st we announced a new award aimed at the chemical and pharmaceutical industry. This is intended to be a high level award for high achievement in the industry and state bodies supporting the industry. It is called the Institute of Chemistry of Ireland "Industrial Chemistry Award 2015". The prize is €1000 sponsored by Henkel. The closing date is December 31st. It is hoped this will develop into a high profile and much sought after award. With sufficient support it could be presented at a significant event along with additional awards. Reminders are being sent out as the response is very poor. Details of the award are on the Institute's web page.

Complacency seem to be an issue within the chemistry profession with no great desire to join the professional body and enhance the status, standing and public image of the Irish chemist. We get a

small trickle of new members but nowhere near the numbers we should be getting. I said in the last issue that I felt women in particular are underrepresented. The good news is that we now have two new ladies on Council, Dr Patricia Cullen from Henkel our Industrial Award sponsor and Mary Mullaghy from the Irish Science Teachers Association. I have invited more women chemists to join ICI and come on Council and hopefully this will meet with a positive response. We also need more industry chemists to join whatever their gender. Given the contribution of women nationwide to chemistry should the Institute have an award focused on women? If yes it will only be possible with active support from the chemistry community and more people joining ICI. Women are eligible for any current award but your voice is not being heard.

Another concern of the Institute is the position of chemistry in 3rd level institutions and resources allocated to the subject. Sean O'Muircheartaigh from GMIT is coordinating a report on the position of chemistry in our 3rd level institutions and we will review his findings in the New Year and report in due course. Peter Childs of UL has written a research article on "The State of Chemical Education in Ireland" in the last issue of ICN. We have just learned of proposed changes to the Junior Cycle Science curriculum in which chemistry no longer appears to be a core subject and is replaced with "Materials" while physics and biology remain as pillars. I have written to the Minister of Education for clarification and requested copies of the proposals. *The Institute was not consulted*.

Patrick Hobbs MSc. FICI MRSC CChem. CSci.
President, December 2014.

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Letter from David Cole-Hamilton (President of EuCheMS)

Dear EuCheMS Member,

I have just taken over as President of EuCheMS from Ulrich Schubert who did a great job in expanding the Secretariat, moving it to a fine location nearer the premises of the European Institutions, designing a new constitution, which has just been signed into law and developing strong links with the European Parliament and Commission through Nineta H. Majcen, EuCheMS General Secretary.

Ulrich will surely be a very hard act to follow, but I am determined to continue to build the influence of EuCheMS as the independent voice for chemistry in Europe.

I also want to work closely with the **EuCheMS Divisions/Working Parties** to build a strong network that can act proactively and respond nimbly and authoritatively to requests from the Parliament and Commission, and that will continue to strengthen their excellent conferences and other activities.

The **European Chemistry Congresses** have got off to a very good start, but I plan to make them a must for all chemists in Europe and a reference to the world of chemistry. The next ones are in Seville, 2016, and Liverpool, 2018. I very much look forward to welcoming you at these Congresses.

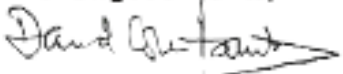
I hope that EuCheMS will be able to celebrate European chemistry with a series of **prestigious European prizes** both across the EuCheMS Divisions/Working Parties and from the EuCheMS as a whole. The European Sustainable Chemistry Award has already become a beacon for environmental friendly European Chemistry research.

With the help of every one of you, I am certain that we can make EuCheMS the voice to be heard for all matters to do with chemistry in Europe.

Please turn the page to see what EuCheMS does for you and what YOU can do for EuCheMS.

Please join me on this very exciting journey by signing up here.

With all good wishes,



David Cole-Hamilton
EuCheMS President

"Europe should be a chemical-free zone"

If you want to change this common unscientific misconception, then EuCheMS is for YOU!

What EuCheMS does for YOU

- Acts as a single independent voice for Chemistry in Europe
- Advices the European Parliament and Commission on chemistry topics
- Promotes excellence in European research via continuous collaboration with the European Research Council
- Ensures that chemistry is well represented in European projects
- Responds to a variety of consultations from the European institutions on chemistry topics, such as water, phosphorus, mercury
- Runs the biennial European Chemistry Congress
- Has Divisions and Working Parties which run biennial conferences and summer schools in most areas of chemistry as well as other activities
- Has an extremely active and effective Young Chemists Network (EYCN)
- Awards a range of European prizes and lectureships for Chemistry
- Together with the European Chemistry Thematic Network Association (ECTN), promotes high quality teaching of chemistry in European universities

What YOU can do for EuCheMS

- Get involved with a EuCheMS Division or Working Party
- Get involved in the European Young Chemists' Network
- Be prepared to offer expert advice when needed, such as for public consultations
- Promote EuCheMS and the role of chemistry whenever appropriate
- Share with us what is relevant for you

Join us in co-creating The Voice for Chemistry in Europe via on-line request at www.surveymonkey.com/s/euchemsform

David A. Brown

The death has occurred recently (September 27) of David A. Brown, who was Professor of Inorganic Chemistry in UCD for many years, during which time both in the former College of Science in Merrion Street and in the more recent UCD campus in Belfield he played a vital role in the development of inorganic chemistry, both in Ireland and internationally. He served the college in many capacities, including the head of the chemistry department, the dean of the science faculty and a member of the governing body.

He joined UCD in 1959 as a lecturer and was appointed in 1964 as the first Professor of Inorganic Chemistry in UCD at a young age as he was born in 1929 in High Wycombe, England. He was a student at Queen Mary College, now Queen Mary University of London, where he became the president of the student chemical society. Following his studies and research in London he worked as a post-doctoral fellow in Cambridge, where his research focused on theories of chemical bonding, that is on how atoms are held together in molecules. Following these studies in England he worked in Brussels as a European Research Associate, where he became a fluent linguist, and from there he joined the UCD staff.

While an undergraduate in 1949 David Feakins, who joined David in UCD when he became the first Professor of Physical Chemistry, as a first year student remembered David, who was a final year "Olympian" figure, as an outstanding worker. Professor Feakins recalled that David went on to be first in chemistry in the whole university.

Professor Brown was a member of the Royal Irish Academy for 52 years, having been elected in 1962. In 1996 he was awarded the Boyle-Higgins gold medal and lecture awards of the Institute of Chemistry of Ireland, of which he served as President. This is an award for research work carried out in Ireland and is made for an outstanding and internationally recognised research contribution to the advancement of chemistry. He also was a member of the Royal Society of Chemistry for over 60 years.

David, along with Dr Bill Davis of TCD, organized a very successful International Conference on Coordination Chemistry in Dublin in 1974, attended by more than 700 participants. The profit from this conference was used to establish regular inorganic chemistry meetings initially in Greystones and later in Maynooth. These conferences were attended by chemists from both the Republic and Northern Ireland as well as by prominent international speakers. During his career David also was very successful in obtaining funding both from European and other sources.

As well as his work in UCD administration, David was a very active researcher and teacher. He was a lively and inspiring lecturer and in his lectures to undergraduates he introduced developments that were topical and had practical and academic importance, as for example when he described and clarified for students the structure of a new type of compound, with a special type of bonds, ferrocene, that subsequently led on to a whole new field of science. In his research he had interests in many exciting areas, including the theory of bonding, coordination chemistry, the study of organometallic compounds that is those with both organic and inorganic parts, bioinorganic and medicinal chemistry. He published extensively and was internationally well known. His Penguin book on Quantum Chemistry opened up many insights to non-specialists in a clear, concise way and helped readers to appreciate modern developments. David formally retired in 1994 but continued his active research in retirement and his most recent publication was in 2011.

He jointly worked with many leading chemistry groups, including Professor Kevin Nolan of the Royal College of Surgeons in Ireland, and his standing is shown by the many famous chemists who visited UCD over the years. His students appreciated meeting these in David's house where he was a hospitable host.

Recently the very successful inaugural David Brown Lecture of the Institute of Chemistry of Ireland and the Institute of Nanotechnology was given by Professor Annie Powell, who received the first David Brown award. Members of the Brown family attended and this was very much appreciated by the large audience of inorganic chemists in the RCSI where the excellent lecture was given

He was very encouraging to his students, many of whom blossomed exceptionally under his guidance. If a student had personal difficulties David helped, discreetly and effectively. This support has been generously acknowledged and appreciated by former students. He worked very conscientiously himself, and his energy, drive and commitment encouraged his students, to whom he was tremendously loyal. As head of department he was exceptionally fair to all groups in sharing often limited resources in difficult times. This generosity helped to maintain a very positive *esprit de corps* in the chemistry department of UCD,

During his time there he established a flourishing nuclear magnetic resonance centre, with substantial industrial funding and also developed excellent micro analytical facilities, which

were used by both UCD personnel and others from commercial and academic backgrounds.

David was a true humanitarian and set up Unesco courses, where students from developing countries came to UCD; this project was supported by Unesco and was very successful. Through these courses and other opportunities David had many overseas students, whose careers he followed with interest, and his international commitment was shown when he was a visiting professor in Kuwait during 1979. He kept in touch with his former students, and this close contact again illustrated the appreciation that they had for David's interest and support. This acknowledgement is illustrated by the very many sincere condolences received from all around the world.

An indication of his standing in science was illustrated when at an international meeting in Florence a delegate was asked if he seen David and was told, after explaining that as he was so busy with the conference he had not time to visit the Uffizi Gallery, that David Brown was meant.

As well as his tremendous commitment to science and UCD David had other interests, including music, especially opera. Being a student in London he had opportunities to attend operas and he had the pleasure of hearing Maria Callas, and more recently in attending the Verona Opera festival. He enjoyed travel and as well as attending international scientific conferences he travelled widely all his life and as he had a very sharp memory was able to recall and entertain others with his travelling adventures.

He was also a voracious reader and was very knowledgeable about many areas of history, both military and social, being very aware and concerned about political abuses worldwide.

As well as his interests in UCD, science, music, travel and reading he was a great family person. His dedication to his family was most impressive. He was a devoted husband, a wonderful father and an adoring grandfather, as was acknowledged at his funeral service. He is deeply mourned by Rita and children, Geraldine, Paul, Suzanne, Rory, Elizabeth and Catherine, as well as by his eight grandchildren in Ireland, the UK and the USA.

David had exceptional ability as a scientist, administrator and teacher, but especially he will be membered as a person of great loyalty, integrity, energy and friendship. His students will always recall his interest in them and his help in their careers and his family will always cherish his love and dedication.

Peter Allen Start

We were greatly saddened to learn of the death of Peter Start, retired member of the academic staff of the Department of Chemistry, University College Dublin, on October 16th 2014. Peter was a senior lecturer in Physical and Inorganic Chemistry in the Department and served for 14 years as the University Safety Officer.

When the Chemistry Department moved from what is now Government Buildings to the campus at Belfield in 1964, Peter was charged with the enormous task of organizing the chemistry practical classes for first year students, in the vast new laboratories .With the increase in numbers of the student body, practical sessions in first year became a communications challenge, it took time to walk from bench to bench talking with only a handful of the class at any one time. Having identified the problem Peter quickly had a solution "CHEM TV"
CHEM TV was run from a fully-fledged TV studio in the Chemistry Department with TV's installed throughout the laboratories. From the studio Peter and the late Dr J. Gowan would brief and guide all the students through their practical experiments assisted by an army of demonstrators. This method of conducting practical sessions was soon to be used in other Universities. This was a very innovative teaching method at the time, which the students found both entertaining and helpful. The' Gowan-Start' productions along with his memorable lectures are well recalled by many Irish doctors, engineers, agriculturists and vetinarians.

Last January during the 50th BT Young Scientist Exhibition Peter was delighted to recall the announcement of the First Aer Lingus Young Scientist exhibition which took place at the Intercontinental Hotel, Ballsbridge on the 15th April 1964. He was the last remaining member of the trio who were the scientific advisors to the airline's air-education team. The other members were the late Fr Tom Burke (physics) and the late Margaret Duhig (zoology). For some twenty years he continued to assist the airlines acting as a chemistry judge. He was popular with the young fledgling scientists and it was not unusual to see him escorted around the Exhibition by those wishing to understand the many exhibits. Peter was a natural teacher. His opinion when sought was carefully listened to and acted upon. He was a gentleman to his fingertips.

Peter had been a loyal member of The Institute of Chemistry of Ireland for well over fifty years, having first been elected Member of the Institute in 1959 and Fellow in January 1964. He was given the distinction of being made an Honorary Fellow of the Institute earlier in the spring of 2014 and attended the award ceremony accompanied by his family at UCD on the occasion of the annual general meeting of the Institute. Peter commented at the time that for him this was his most cherished award.

Peter was the first Editor of the Institute's journal, 'Orbital' and produced 11 issues in all from 1965 to 1970. He was also for a period of time the Chairman of the Conjoint Chemical Council which produced a very necessary yearly calendar of events run by the chemical societies in Ireland.
He was also a Fellow of the Royal Society of Chemistry and a Chartered chemist.

In the late eighties Peter played a major part in the development of the University's Diploma in Safety, Health and Welfare at Work, collaborating with Dr Caroline Hussey former Registrar and Deputy President and Tom Walsh, former CEO of the Health and Safety Authority. They set about establishing the National Centre for Safety and Health in Ireland building up initially a certificate course and then a full Master's degree programme. He joined the Institution of Occupational Safety and Health (IOSH) and was rapidly elevated to the rank of Fellow. Jointly with RTE he pioneered Ireland's international tele-education course with students as far away as Chile and South America. Recently this satellite version has been converted into an online blended learning format. In 2012 he was awarded the title" OSH Person of the Year" and it was presented to him by the Lord Mayor of Dublin.

Peter and Marie were made for each other. They were delightful hosts in their home 'Shaldon Grange' in Kiltiernan, where many members of the University Chemistry Departments recall the great parties that took place there. On each occasion the development of the grounds was the subject for discussion such as the diversion of an onsite natural stream to flow beside the house and the laying out of six acres of gardens, waterfalls and lakes.' Shaldon Grange' was his life's enjoyment and the party highlight for Peter used to be the opening of the reservoir sluice gates that exhibited a spectacular torrent of water.

Peter was able to handle many trades, besides his design skills he could turn his hand to brick laying, carpentry, plumbing, electrician, slater to mention but a few. At 'Sheldon Grange' he built everything he possibly could himself, he surrounded himself with his family and machinery of all kinds, his pride and joy being the big yellow JCB. Perhaps what was most endearing was his generosity in giving of any of these skills to help his colleagues and friends. His advice was always being sought and given unstintingly.

Peter will be sadly missed by his friends and colleagues in UCD.

He is survived by his wife Marie and his two sons Keith and Nigel

IRISH CHEMICAL NEWS

Research Article

Microbial cell factories for novel and unique industrial enzymes and bioactivities

Catherine Collins and Patrick Murray

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Introduction

Industrial enzymes are widely used to synthesize a wide range of synthetic intermediates and fine chemicals for application in industries such as textile, food, detergent, pharmaceutical, agriculture, biofuel and chemical industry. The global market for industrial enzymes was reported to be worth nearly US$4.5 billion in 2012 and nearly US$4.8 billion in 2013 with the market expected to reach around $7.1 billion by 2018 (BCC research; Global Markets for Enzymes in Industrial Applications; June 2014). Furthermore the requirement for novel and more powerful enzymes is at the forefront of scientific and industrial research such as the EU Horizon 2020 funding call in 2014 entitled "Enhancing the industrial exploitation potential of marine-derived enzymes" or the EU funded project Peroxicats [peroxidases as biocatalysts] which explores novel and more robust fungal peroxidases as industrial biocatalysts (http://www.peroxicats.org/project.php). There is also considerable demand for new bioactives for applications in health and medicine given our rising world population. Huge opportunities and challenges are presented in the search for better industrial enzymes and bioactives with advancement in genomics and biotechnology. This article outlines how such advances are playing a role in search for enzymes and bioactives.

New sources of enzymes/bioactives

In recent years there has been an increased exploration of more extreme environments for enzymes and bioactive discovery made possible by advances in Science. One such environment is that of the marine which has led to a number of EU funded projects listed on Table 1. With approximately 71% of the earth's surface of 361 million square kilometers covered by oceans, and estimated to account for more than 80% of life on earth, marine environments represents an enormous reservoir for enzyme discovery [1]. It is estimated that marine environments, including the subsurface, are said to contain a total of approximately 3•67 × 10^{30} micro-organisms alone [2], while a marine prokaryotic plankton from Sargasso Sea was identified to contain about one million novel open reading frames encoding putative proteins [3]. It is therefore clear that the marine environment contains an enormous pool of as yet largely underexploited biodiversity. New habitats are continually being discovered such as a vertical rock face half a mile below the sea surface off the Irish Coast, which extended upwards for about 150 metres, and was covered in a rich assemblage of bivalves and corals [4]. In the marine environment habitats range from ocean trenches with depths of up to 11 000 m and pressures exceeding 100 MPa [5] to deep-sea hydrothermal vents with temperature as high as approximately 400°C [6]. Thus, the enzymes produced by organisms from such extreme marine environments have the potential capacity to be uniquely suited to many industrial processes. Other examples of environments being explored as sources of enzymes and bioactives, include hot springs, bovine gut microbial community or landfill leachate.

Metagenomics

In recent years the search for enzymes in non-culturable microorganisms has been made possible by the advent of metagenomics which is based on the analysis of DNA from environmental samples while bypassing culturing of the micro-organisms. As only a small percentage microorganism can be cultured (~1%), this means that the type and quantity of enzymes got from traditional cultivation-based method is not enough and cannot meet the industrial demand. It has been estimated that bacteria can achieve densities of up to 10^6 per milliliter of seawater [7] and assuming 3000 genes per single genome, then there could be up to 3 × 10^9 genes mediating up to 1•2 × 10^9 putative reactions in that sample (assuming that 40% of these genes have catalytic activity) [8], making seawater samples a particularly rich source of potential biocatalytic biodiversity.

One way to identify novel enzymes from metagenomic libraries is to apply Functional Based Screening. This approach can be performed by adding chemical dyes and insoluble or chromophore-containing derivatives of enzyme substrates into the growth medium to detect individual clones positively expressing the corresponding enzyme of interest. It has been extensively used to mine novel enzymes and bioactive compounds from metagenomic libraries. For example Beloqui et al., [9] performed an activity-based screen by direct detection of phenotypes in order to identify novel glycosyl hydrolases enzymes. DNA was isolated from the cellulose-depleting microbial community found on fresh manure of Earthworms. This DNA was then inserted into fosmid vectors and transformed into the bacteria Escherichia coli to make a metagenomic library. Clones of E. coli with the metagenomic fosmid DNA were screened for their ability to hydrolyse synthetic substrates p-nitrophenyl-β-d-glucopyranoside and p-nitrophenyl-α-l-arabinopyranoside. Two of the recovered glycosyl hydrolases had no similarity to any known glycosyl hydrolases and represented two novel families of β-galactosidases

Table 1. List of EU Funded Projects

EU-funded Marine Research Projects	Overall Objectives of Existing Projects
MaCuMBA	Marine microorganism biodiscovery from conventional and extreme habitats. (http://mamba.bangor.ac.uk/)
Marex	Exploring marine bioactive compounds through the discovery path.(http://www.marex.fi)
Marine Fungi	Marine fungi for new anti-cancer compounds. (https://www.marinefungi.eu)
Special	Marine sponges metabolites biomaterials. (www.project-special.eu)
BAMMBO	Overcome marine organisms culturing bottle-necks (www.bammbo.eu)
Sunbiopath	Sunlight to biomass conversion in microalgae.(www2.ulg.ac.be/genemic/sunbiopath)
GIAVAP	Genetic Improvement of Algae for Value Added Product. (http://giavap.eu/)
PharmaSea	Commercialisation of new substances from marine organisms (http://www.pham-sea.eu)
Bluegenics	Recombinant novel secondary metabolites from sponges. (www.bluegenics.eu/cms/)
SeaBioTech	Innovative marine discovery pipelines (http://spider.science.strath.ac.uk/seabiotech/)
MicroB3	Access marine data to define new targets for biotechnological applications. (http://www.microb3.eu)
MBT-ERA-NET	ERA-MarineBiotech (www.marinebiotech.eu/)

α-arabinopyranosidases. Similarly, Ferrer et al., (2012) discovered a multifunctional glycosyl hydrolase from the family 43 involved in the breakdown of plant polymers from a calf rumen metagenomic library [10].

Functional screening of metagenomic libraries using solid media based screening assays can be employed to screen large numbers of clones and thus increase the analytical throughput. For example, Tasse et al. [11] screened 200,000 clones against 7 primary and 16 secondary substrates per week using solid media assays to identify activities involved in dietary fibre breakdown

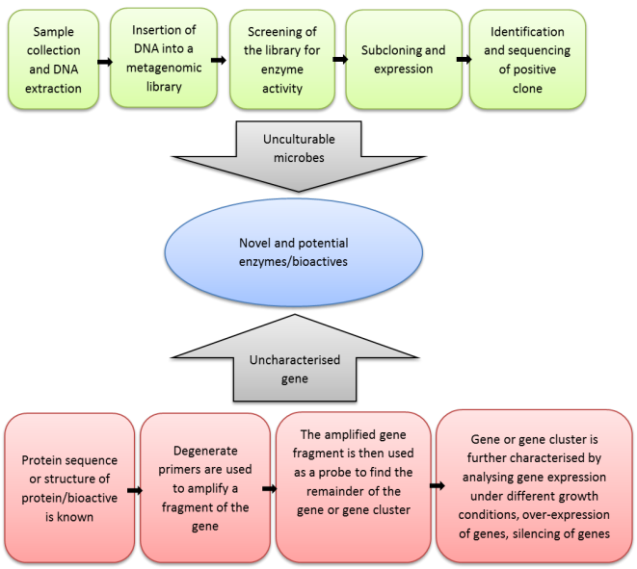

Figure 1. Schematic illustrating Metagenomic technology and genome mining as a means of novel and potential enzyme/bioactive discovery.

in the human gut microbiome. However, solid media based assays exhibit low signal-to-noise ratios in part due to the diffusion of reaction products resulting in decreased detection sensitivity and data with limited quantitative value [12]. Solution-based assays may provide an alternative as they are amenable to automated liquid-handling systems, and coupled with the direct detection of chromogenic or fluorescent substrate transformations, can be used to increase screening throughput, assay reproducibility, and sensitivity [13]. A high-throughput screening assay for detecting cellulase activity in metagenomic libraries has recently been reported [14]. A daily throughput of 38,400 clones by using one labeled substrate, 384 well microplate format, and automated liquid handling was achieved.

Nyyssönen et al. 2013 developed high-throughput screening assays for function-based characterization of activities involved in plant polymer decomposition from environmental metagenomic libraries [13]. They employed multiplexed assays using fluorogenic and chromogenic substrates, combined with automated liquid handling and the use of a genetically modified expression host to enable simultaneous screening of 12,160 clones for 14 activities in a total of 170,240 reactions. Using this platform they identified 374 (0.26%) enzymes involved in the degradation of cellulose, hemicellulose, chitin, starch, phosphate and protein from fosmid libraries prepared from decomposing leaf litter.

DNA Sequencing and screening

The introduction of next-generation sequencing platforms, such as the Roche 454 sequencer, the SOLiD system of Applied Biosystems, and the Genome Analyzer of Illumina, had a big impact on genomic and metagenomic research [15]. The advances in throughput and cost reduction have increased the number and size of metagenomic sequencing projects, such as the Sorcerer II Global Ocean Sampling (GOS) project which studied the genetic potential of surface ocean water bacterioplankton on a global scale [16] or the study by Dinsdale et al., where they compared metagenomes consisting of almost 15 million sequences from 45 distinct microbiomes and 42 viromes [8]. The analysis of the resulting large data sets allows the exploration of the taxonomic and functional biodiversity and of the system biology of diverse ecosystems [17].

We are living in the post-genomic era in which there has been a vast explosion in the amount of genomic data generated. In November 2014, Genbank contained more than 178 million sequence records made up of over 183 billion bases. In 2002, Genbank had nearly 173,000 whole genome sequences whereas in 2014 this figure has risen to over 196 million (http://www.ncbi.nlm.nih.gov/genbank/statistics/). Between 1990 and the publication of a working draft in 2001, more than 200 scientists joined forces in a $3-billion effort to read the roughly 3 billion bases of DNA that comprise our genetic material (International Human Genome Sequencing Consortium Nature 409, 860–921; 2001). The price of sequencing an average human

genome has plummeted from about US$10 million to a few thousand dollars in just six years in 2013 with the cost of genome sequencing expected to be in the region of US$1000 shortly (http://www.genome.gov/sequencingcosts/). Bacterial and fungal genomes are typically much smaller than a human genome and therefore, are being sequenced more routinely in research.

However, a large number of gene sequences from this genomic information are uncharacterized for their definite biological functions. For example, 5,341 nucleotide sequences of nitrilase could be searched in GenBank as of December 2012, and among them, only about 400 sequences have ever been characterized [18]. Despite the vast number of Glycoside Hydrolase enzymes deposited in publicly available databases (eg Genbank contains 96,857 sequences in December 2013), it is estimated that 90% of these enzymes are not characterised. Thus, the mining of this genomic data would be precious resources for discovery of novel enzymes. However, it is clear that we need to match the rate of sequence generation with proving the functional role genes such as those encoding novel enzyme and in confirming functions for environmental homologs of previously characterized genes.

Sequence based screening of genomic data involves the design of DNA probes or primers which are derived from conserved regions of already-known genes or protein families (Figure 2.). For example, the biosynthetic gene cluster of quartromicins was identified by degenerate primer PCR amplification of specific genes involved in the biosynthesis of the tetronate moiety [19]. Quartromicins (QMNs) are antiviral compounds that represent unique members of a family of spirotetronate natural products. The enzyme FkbH-like glyceryl-S-ACP synthase is necessary for biosynthesis of the tetronate moiety of spirotetronate antibiotics. Because a homologous enzyme was predicted to mediate the biosynthesis of QMNs, degenerate primers were designed based on a homolog gene encoding FkbH-like glyceryl-S-ACP synthase. Using these primers which were 20-30bp long and the genomic DNA from the bacterium Amycolatopsis orientalis as a template, a DNA fragment was obtained. This was then used as a probe to screen the A. orientalis genomic library, which resulted in several overlapping fosmids spanning a 50 kb DNA region. A second probe in a different region of the cluster was also used to screen the library to ensure full coverage of the entire QMN gene cluster. Chromosome walking upstream and downstream of the probes led to a 90 kb contiguous DNA region on the chromosome. A bioinformatic analysis of this DNA region revealed 47 open reading frames (ORFs) and according to functional assignment of the deduced products of these, 28 were proposed to constitute the QMN gene cluster. Based on knowledge of the gene cluster, it allowed the authors to reconstruct the tetronate intermediate in vitro and biochemically characterized a PKS module skipping strategy that allows this bacterium to produce two alternative polyketide chains, both of which are essential for QMNs biosynthesis. Once a gene cluster is discovered it can be manipulated by overexpressing genes or switching genes off and so decipher the pathway involved in synthesis of the metabolite.

However, with the approach of using degenerate primers, only

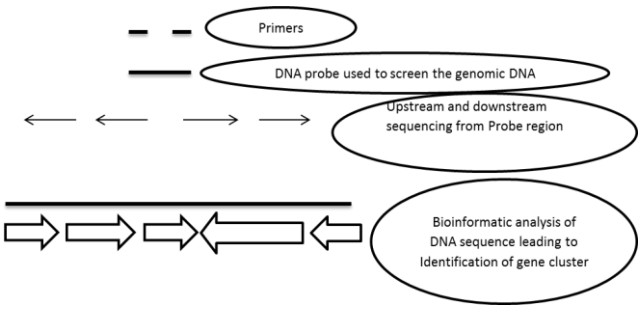

Figure 2. Simple schematic illustrating the process of obtaining several kilobases of DNA sequence information using degenerate primers. Primers which are 20-30bp in length are designed based on known protein/peptide sequence or the enzymes involved in synthesis of the metabolite. These are used to amplify a region of a gene (usually less 1000bp) which then can act as a probe to find the remaining genes in the cluster. A cluster of secondary metabolites can be several thousand bases long.

novel variants of known functional classes of proteins can be identified. Nevertheless, this strategy has led to the successful identification of many genes encoding novel enzymes, such as chitinases [20], dioxygenases [21], dimethylsulfoniopropionate-degrading enzymes [22], [Fe-Fe]-hydrogenases [23], [NiFe] hydrogenases [24], nitrite reductases [25], hydrazine oxidoreductases [26], and glycerol dehydratases [27].

Cell-free protein synthesis

The combination of *in vitro* gene amplification and cell-free protein synthesis followed by *in situ* analysis holds great potential for enzyme screening from the genomic DNA of microbes [28]. Kwon and co-workers successfully adapted the strategy of *in vitro* gene amplification and cell-free protein synthesis followed by *in situ* analysis to discover novel ω-transaminases (ω-TA) enzymes from the putative genes from various microbial species [29]. ω-TA genes were amplified by Polymerase Chain Reaction (PCR) from microbial colonies and directly expressed in a cell-free protein synthesis system i.e. DNA of a gene and everything necessary to make protein were added together in a tube. The expressed enzymes were then screened for their activities towards numerous substrates in a colourimetric assay (Figure 3). By using cell free expression for 11 ω-TA genes and analysing the protein products against 16 different substrates, several enzyme–substrate matches were identified in a matter of hours. The use of a cell free expression is much more amenable to rapid high through put screening and overcomes the limitations and time consumption associated with cell based expression such as misfolded and/or inactive enzymes or non-native enzyme structures.

An elegant application of cell-free protein synthesis is the expression screening of glycoside hydrolases (GH) from a metagenomic library [30]. In their work, putative GH genes cloned from the cow rumen microbiome were expressed by cell-free synthesis reactions in the presence of different substrates of glycan such as amylose, xylan, and cellulose. If the expressed enzyme had functional GH activity, then the glycan substrate in the same pot was hydrolysed by these enzymes resulting in the liberation of monomeric sugars, which were then metabolised in

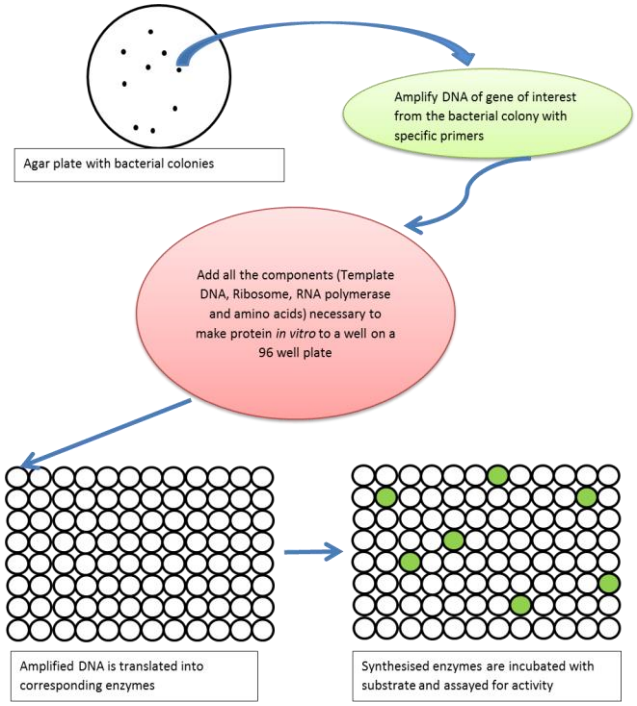

Figure 3. Cell free expression screening of enzymes. DNA encoding enzymes are amplified from bacterial colonies. This DNA then acts as a template when added to a well in a 96 well-microtitre plate which already contains everything necessary to make protein i.e. ribosomes, amino acids and RNA polymerase. After the enzymes have been synthesized they are assayed for activity.

the cell extract to provide additional ATP for continued synthesis of the enzyme. As the cell-free metabolism of the sugars also resulted in the accumulation of acidic by-products, a number of glycan-hydrolysing enzymes were identified by detecting the decrease in pH of the cell-free protein synthesis reaction mixture. This work shows that cell-free protein synthesis reaction can greatly facilitate enzyme discovery from untapped genetic resources when combined with appropriately designed assay schemes. Such an approach was used by Hess *et al*. to characterize biomass-degrading genes and genomes from microbes adherent to plant fibre incubated in cow rumen [31]. They sequenced and analysed 268 gigabases of metagenomic DNA from which they identified 27,755 putative carbohydrate-active genes and expressed 90 candidate proteins, of which 57% were enzymatically active against cellulosic substrates. They also reconstructed 15 uncultured microbial genomes, which were validated by complementary methods including single-cell genome sequencing. This work highlights how a substantially expanded catalogue of genes and genomes participating in the deconstruction of cellulosic biomass can be analysed and used as a strategy for the discovering diverse enzymes encoded in nature.

The role can fungi play in the discovery, characterisation and production of novel enzymes and bioactives

Filamentous fungi are dominant producers of a range of primary metabolites such as organic acids including citric, gluconic, fumaric, kojic, itaconic and fatty acids all of which are produced via traditional fermentation technology [32]. In addition they also produce many important secondary metabolites, a number of which have found use as human therapeutics e.g. griseofulvin, lovastatin, taxol , penicillin, cephalosporin, ergot alkaloids and zeranol. They are producers of biosurfactants and polysaccharides as well as being a food source in their own right e.g. mushrooms, single cell protein/biomass. Some fungi are notable enzyme producers which have been exploited for the production of cellulases, pectinases, laccases/ligninases, amylases, amyloglucosidases, phytase, proteases, microbial rennets, lipases and glucose oxidase. Intracellular fungal enzymes are also used as biocatalysts in biotransformations in bioorganic synthesis while other are applied to biodegradative processes such as soil bioremediation.

Filamentous fungi are renowned for high productivity characteristics and many of them are naturally excellent producers of extracellular enzymes which makes them exceptional candidate hosts for the expression of recombinant proteins. For example the fungus *Aspergillus niger* can produce 25-30 g/L of glucoamylase while the fungus *Trichoderma reesei* can produce 100g/L extracellular protein [33]. Fungi have an inherent ability to grow at high rates and to high biomass densities supported by low cost substrates in relatively simple fermenters.

Much research has been done in recent time with regard to the production of antibiotics and secondary metabolites at a DNA level in fungi. The genes involved in the biosynthesis of numerous antibiotics and other secondary metabolites have been cloned and found to be organised in clusters and within these clusters pathway-specific regulatory genes positively or negatively modulate gene expression and secondary metabolite production. The metabolic engineering of *Penicillium chrysogenum* for cephalosporin production illustrates how strategies of introducing recombinant enzymes into strains can result in the development of multipurpose host platforms for the production of non-native secondary metabolites. The production of penicillin G, cephalosporins and cephamycins share a common early pathway to the synthesis of isopenicillin-N intermediate. Relatively low clinical efficacy has been reported from the "natural" cephalosporins produced by *Acremonium chrysogenum.* They are produced semi-synthetically using intermediate building blocks, 7-aminodeacetoxycephalosporanic acid (7-ADCA) and 7-aminocephalosporanic acid (7-ACA). *Penicillium chrysogenum* is an attractive recombinant host for the production of cephalosporins and cephamycins given that it cannot produce these molecules and it has a hyper capacity to produce penicillin via the common precursor isopenicillin N. It has been successfully engineered to produce cephalosporins and 7-ADCA. The transformation of *P. chrysogenum* into a cephalosporin producer involved the introduction of the gene *cefE* from the bacterium *Streptomyces clavuligerus* which encodes the expandase enzyme deacetoxy-cephalosporin-C synthetase [34]. This transformation process resulted in a tremendous improvement of 7-ADCA production in terms of the purity of the end product, more efficient use of energy, reduction in the use of organic solvents and consequently cost reduction. This process highlights the economic and environmental advantages of integrating chemical steps into biological processes. Introducing the genes *cefEF* and *cefG*, which encode the dual expandase/hydroxylase and acetyl-transferase, respectively from *A. chrysogenum* into *P. chrysogenum* resulted in the production

of adipoyl-7-ACA . Approximately two thirds of semi-synthetic cephalosporins in use are derived from 7-ACA. To produce other novel cephem precursors other recombinant proteins have been successfully expressed in *P. chrysogenum* such as the *cmcH* gene from *S. clavuligerus* encoding deacetylcephalosporin O-carbamoyltransferase [35]. This enzyme catalyses carbamoylation of cephalosporins. The resulting products are used for the synthesis of several semi-synthetic cephalosporins. Transcriptomic analysis where by the genes expressed in a fungus during antibiotic production are analysed provides a useful tool to pinpoint the genes involved in metabolite production. Similarly secretome analysis can be used to monitor protein production in metabolite production.

Further advances in proteomics, genomics and improvements in transformation techniques to introduce exogenous DNA into fungi, will result in recombinant expression of multiple proteins and enzymes. Such developments will allow the engineering of metabolic pathways so as to enhance production of primary or secondary metabolites or even facilitate production of novel compounds through introduction of new biosynthetic pathways. Currently known filamentous fungi exhibit a tremendous level of metabolic diversity. Given that only a small number (>5%) of fungi are known from an estimated 1.5 million species [36] which are thought to exist it implies that filamentous fungi will continue to provide the biosynthetic tools for the synthesis of a vast number of novel products for several years to come.

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Dr. Catherine Collins obtained her PhD by investigating the molecular genetics of cellulose degradation in the cellulolytic fungus *Talaromyces emersonii* at The National University of Ireland, Galway. Since then I have worked on a number of fungal projects both in Ireland and the U.K. including a project at The University of Bristol, England, which was in collaboration with GlaxoSmithKline, involving cloning, sequencing and manipulating the genes involved in production of the antibiotic pleuromutilin from the mushroom *Clitopilus passeckerianus*. Currently I am employed as a Post-Doctoral Researcher at Shannon Applied Biotechnology Centre (SABC), Limerick Institute of Technology (LIT) investigating the properties of peat based skin care products. Previously I worked as a researcher on the FP7 EU funded project BAMMBO (Biologically Active Molecules of Marine Based Origin) at SABC, LIT. In this project marine organisms (fungi, yeast, bacteria, macro-algae, micro-algae and sponges) were screened for their potential as sustainable producers of high-added value molecules (HVABs). At Shannon ABC we are applying analytical methods for the extraction, purification and enrichment of bioactive compounds. I have just recently established my own research group which focuses on investigating mushrooms and fungi as functional foods and a source of bioactives. This project is funded by the Department of Agriculture, Food and the Marine and entitled Mushrooms and Fungi, Functional and Life Enhancing Reservoirs (MUFFLER).

Dr. Patrick Murray is a lecturer in Department of Applied Science, Principal Investigator, Shannon Applied Biotechnology Centre and senior researcher of CHIMERA (CHemIcal and Molecular microbial biotEchnology ReseArch) Research group at LIT. As research coordinator at Shannon ABC Centre he has a strong track record of collaboration with industry specifically in identification of innovative ingredients and biological products from natural resources using novel state of the art processes to obtain lead molecules for drug development and value added food, flavour and medicinal products. Dr. Murray was the scientific coordinator and WP4 leader of an FP7 project "BAMMBO" on extraction of high-value bioactive molecules from marine plants and animals with specific interests on environmentally friendly and sustainable extraction processes (using Supercritical Carbon Dioxide). Dr. Murray was the principal investigator of an Enterprise Ireland funded project in partnership with an Irish SME "AlgaeHealth" involving the scale-up of indoor cultivation in photobioreactor systems and extraction of high value bioactive molecules from microalgae. Dr. Murray is currently principal investigator for three Industrial Innovation partnership projects funded by Enterprise Ireland. Dr. Murray has also directed projects involved in bioconversion of target biomass streams to fermentable feedstock's for bioethanol/biodiesel production. Dr. Murray received his Degree in Biochemistry from the National University of Ireland, Galway (NUIG) and subsequently completed his PhD in Fungal Glycobiotechnology, also at NUIG. Dr. Murray developed the molecular biology laboratory as part of the Molecular Glycobiotechnology Group at NUIG, and has previously worked as a visiting scientist at Wallenberg Wood Biotechnology Centre and at VTT research centre in Finland. He is supervising postgraduate students towards their masters and PhD degrees. Dr. Murray is co-owner of 2 patents and the author to a number of peer reviewed scientific articles. Dr. Murray provides scientific leadership to the development of industrially relevant projects as well as technology transfer.

IRISH CHEMICAL NEWS

Research Article

Cyanobacterial UV-screening compounds are safe alternative for cosmetics and pharmaceutical applications

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Introduction

Cyanobacteria (aka blue-green algae) are the pioneer prokaryotic oxygenic phototrophs on earth, which evolved on this planet ca 3.5 billion years ago. They occupy a central position in global nutrient cycling, particularly due to their inherent capacity to fix atmospheric CO_2 and N_2 through Rubisco and nitrogenase enzymes, respectively. They are the inventors of oxygenic photosynthesis and contribute significantly in the biosphere through nitrogen and carbon cycles. Morphologically, they are diverse ranging from unicellular to multicellular; autotrophic to heterotrophic in their mode of nutrition. They survive as psychrophilic to thermophilic, acidophilic to alkylophilic, planktonic to barophilic, and freshwater to marine organisms. They thrive in harsh environments (UV-irradiance, photooxidation, drought and desiccation, nitrogen starvation, heat-cold shocks, anaerobiosis, osmotic and salinity stresses) due to their wide survival strategies (Sinha and Häder, 1996; Saha et al., 2003). UV-irradiance is the major threat to the all living organisms on Earth including human beings. This is because of the fact that the increasing use of anthropogenic environmental hazards such as chlorofluorocarbons, chlorocarbons and organobromides, which are causing the depletion of protective ozone layers. Therefore, all living organisms are exposed to increased amount of harmful UV-A (315-400 nm) and UV-B (280-315 nm) irradiations. Ultraviolet rays are harmful to living systems as these rays produce free radicals that damage cellular components including lipids, proteins and DNA. Cyanobacteria deal with UV exposure by biosynthesising UV-screening compounds, namely, mycosporine-like amino acids (MAAs) and scytonemin pigments. Therefore, cyanobacteria are considered as potentially useful organisms for mankind in various ways including safe natural alternative for bio-cosmetics and bio-pharmaceutical applications.

Mycosporine-like amino acids

Mycosporine-like amino acids (MAAs) are low-molecular-weight molecules with absorption maxima at 310-365 nm. These biomolecules are found in marine, freshwater as well as in terrestrial species. Generally, MAAs are intracellular water-soluble compounds; however, in some cyanobacteria, MAAs are released to their extracellular polysaccharide matrix (Fig. 1), where these compounds are linked to oligosaccharide and are known as OS-MAAs (Ferroni et al., 2010).

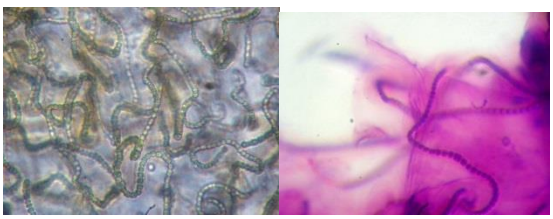


Fig. 1. Micro-morphology of cyanobacterial filaments (*Nostoc* sp.) embedded within polysaccharide matrix, which appeared fluorescent-white under light microscopy (left). Photomicrograph showing binding of crystal violet stain to the extracellular polysaccharide matrix of cyanobacterial filaments (right).

Among the various natural products, MAAs have received considerable interests as sunscreen compounds because of their significant UV-absorption capacity and high photostability (Klisch and Häder, 2008). These UV-screening compounds are also highly resistant to temperature, UV radiation, various organic solvents and pH ranges (Zhang et al., 2005). MAAs absorbs the harmful UV irradiation and dissipates the energy in a harmless form of heat radiation through an uncertain mechanism and thus protects cellular damage from UV-irradiation (Conde et al., 2004). In vitro experiments with MAAs (shinorine, porphyra-334 and mycosporine-glycine) suggested that these bioactive compounds can prevent human fibroblast cells from UV-induced cell death (Oyamada et al., 2008). MAAs have the basic structure of an amino acid or an amino alcohol, which is combined to a cyclohexenone or a cyclohexenimine (Fig. 2). Presently, about 21 MAAs including the pre-dominant forms such as, mycosporine-glycine, palythine, palythinol, asterina-330, porphyra-334, and shinorine have been reported from terrestrial, freshwater and marine organisms (Rastogi et al., 2010). MAAs have been shown to protect skin from UV-A and UV-B-irradiation and hence could be a suitable candidate in the cosmetic industries for the development of bio-sunscreen products. Additionally, MAAs have antioxidant properties through the action of scavenging toxic oxygen radicals, which opens a new avenue for exploration of these bioactive molecules. In fact, "HELIONORI" and "Helioguard 365" are the only two commercial products based on algal extracts (red macroalga Porphyra umbilicalis) containing MAAs (Palythine, Porphyra 334, and Shinorine) are commercialized as safe marine alternative to synthetic UVA filters in cosmetic products for skin protection against UV

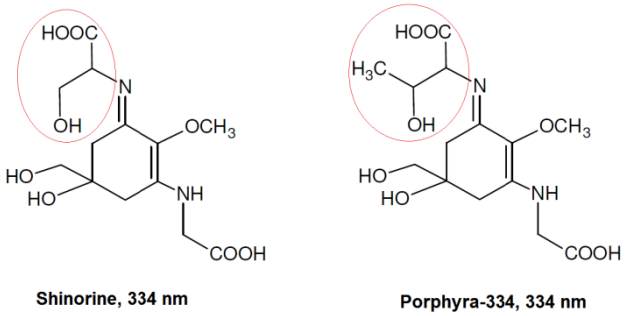


Fig. 2. Basic chemical structures of MAAs (shinorine and porphyra-334) found in cyanobacteria with absorption maxima at 334 nm.

radiation (Ferroni et al., 2010). The source of these natural algae material is mostly wild and depends on local weather conditions. However, cultivable cyanobacteria can be used as natural source for UV-A protective sunscreens. A study demonstrated the effectiveness of methanolic extract of a cyanobacterium Aphanizomenon flos-aquae containing porphyra-334 as significant UV-A protector when compared to two commercial sunscreens (Torres et al., 2006). Another study demonstrated the suitability of MAAs in cosmetics and toiletries as UV protectors and activators of cell proliferation when it was found that the mixture of porphyra-334 and shinorine could suppress UV-induced aging in human skin (Daniel et al., 2004).

Scytonemin pigments

Production of scytonemin pigment in certain cyanobacteria (both unicellular and filamentous) is believed to be the earliest developed mechanism of UV protection, more ancient than the flavonoids or melanins. Scytonemin is a yellowish-brown, lipid soluble, dimeric pigment located in the extracellular polysaccharide sheath of some cyanobacteria (**Fig. 3**) with a molecular weight of 544 Da.

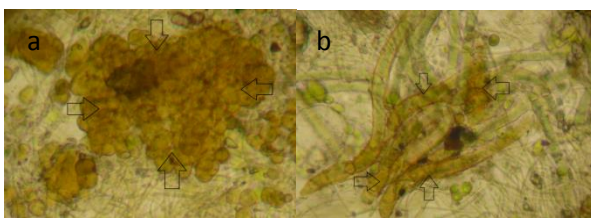
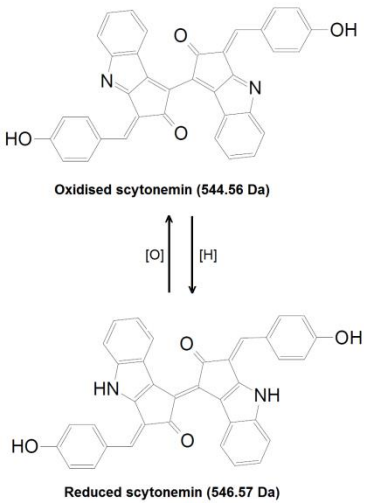


Fig. 3. Micro-morphology of cyanobacteria dominated microbial mat from sun-exposed dry soil surface. Unicellular cyanobacterial colonies of *Gloeocapsa* sp. (arrows) showing thin extracellular sheaths with yellowish-brown pigment scytonemin (a). Heterocyst bearing, filamentous, false-branched cyanobacterium *Tolypothrix* sp. showing thick extracellular sheaths deposited with brown pigment scytonemin (b)

The chemical structure of scytonemin pigment is based on indolic and phenolic subunits. It has an absorption maximum around 384 nm. Scytonemin exist in oxidized (green) and reduced (red) form (**Fig. 4**) depending upon the redox and acid-base conditions during the process of extraction (Garcia-Pichel and Castenholz, 1991). Recently, scytonemin and its derivatives, with absorption maxima in UV-A and UV-B regions, have received much attention for their putative role as UV-screening/absorbing compounds as well as their pharmacological potential. Scytonemin is thought to be synthesized from metabolites of aromatic amino acid biosynthesis and can be induced by high light intensity, deficiency of Fe, Mg or nitrogen, and desiccation and hydration. The scytonemin pigment is highly stable and can perform its screening activity without any further metabolic investment even under prolonged desiccation. Scytonemin pigment possesses anti-inflammatory and anti-proliferative properties apart from its UV screening properties, which makes it a suitable candidate for bio-sunscreens replacing chemical sunscreens and other potential pharmaceutical applications (Singh et al., 2010).



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IRISH CHEMICAL NEWS

Research Article

Understanding and Controlling Highly Anisotropic Crystal Growth

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Introduction

Cyanobacteria (aka blue-green algae) are the pioneer prokaryotic oxygenic phototrophs on earth, which evolved on this planet ca 3.5 billion years ago. They occupy a central position in global nutrient cycling, particularly due to their inherent capacity to fix atmospheric CO_2 and N_2 through Rubisco and nitrogenase enzymes, respectively. They are the inventors of oxygenic photosynthesis and contribute significantly in the biosphere through nitrogen and carbon cycles. Morphologically, they are diverse ranging from unicellular to multicellular; autotrophic to heterotrophic in their mode of nutrition. They survive as psychrophilic to thermophilic, acidophilic to alkylophilic, planktonic to barophilic, and freshwater to marine organisms. They thrive in harsh environments (UV-irradiance, photooxidation, drought and desiccation, nitrogen starvation, heat-cold shocks, anaerobiosis, osmotic and salinity stresses) due to their wide survival strategies (Sinha and Häder, 1996; Saha et al., 2003). UV-irradiance is the major threat to the all living organisms on Earth including human beings. This is because of the fact that the increasing use of anthropogenic environmental hazards such as chlorofluorocarbons, chlorocarbons and organobromides, which are causing the depletion of protective ozone layers. Therefore, all living organisms are exposed to increased amount of harmful UV-A (315-400 nm) and UV-B (280-315 nm) irradiations. Ultraviolet rays are harmful to living systems as these rays produce free radicals that damage cellular components including lipids, proteins and DNA. Cyanobacteria deal with UV exposure by biosynthesising UV-screening compounds, namely, mycosporine-like amino acids (MAAs) and scytonemin pigments. Therefore, cyanobacteria are considered as potentially useful organisms for mankind in various ways including safe natural alternative for bio-cosmetics and bio-pharmaceutical applications.

Mycosporine-like amino acids

Mycosporine-like amino acids (MAAs) are low-molecular-weight molecules with absorption maxima at 310-365 nm. These biomolecules are found in marine, freshwater as well as in terrestrial species. Generally, MAAs are intracellular water-soluble compounds; however, in some cyanobacteria, MAAs are released to their extracellular polysaccharide matrix (Fig. 1), where these compounds are linked to oligosaccharide and are known as OS-MAAs (Ferroni et al., 2010).

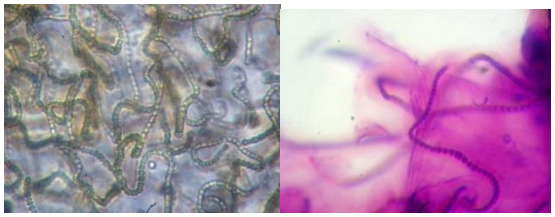

Fig. 1. Micro-morphology of cyanobacterial filaments (*Nostoc* sp.) embedded within polysaccharide matrix, which appeared fluorescent-white under light microscopy (left). Photomicrograph showing binding of crystal violet stain to the extracellular polysaccharide matrix of cyanobacterial filaments (right).

Among the various natural products, MAAs have received considerable interests as sunscreen compounds because of their significant UV-absorption capacity and high photostability (Klisch and Häder, 2008). These UV-screening compounds are also highly resistant to temperature, UV radiation, various organic solvents and pH ranges (Zhang et al., 2005). MAAs absorbs the harmful UV irradiation and dissipates the energy in a harmless form of heat radiation through an uncertain mechanism and thus protects cellular damage from UV-irradiation (Conde et al., 2004). In vitro experiments with MAAs (shinorine, porphyra-334 and mycosporine-glycine) suggested that these bioactive compounds can prevent human fibroblast cells from UV-induced cell death (Oyamada et al., 2008). MAAs have the basic structure of an amino acid or an amino alcohol, which is combined to a cyclohexenone or a cyclohexenimine (Fig. 2). Presently, about 21 MAAs including the pre-dominant forms such as, mycosporine-glycine, palythine, palythinol, asterina-330, porphyra-334, and shinorine have been reported from terrestrial, freshwater and marine organisms (Rastogi et al., 2010). MAAs have been shown to protect skin from UV-A and UV-B-irradiation and hence could be a suitable candidate in the cosmetic industries for the development of bio-sunscreen products. Additionally, MAAs have antioxidant properties through the action of scavenging toxic oxygen radicals, which opens a new avenue for exploration of these bioactive molecules. In fact, "HELIONORI" and "Helioguard 365" are the only two commercial products based on algal extracts (red macroalga Porphyra umbilicalis) containing MAAs (Palythine, Porphyra 334, and Shinorine) are commercialized as safe marine alternative to synthetic UVA filters in cosmetic products for skin protection against UV

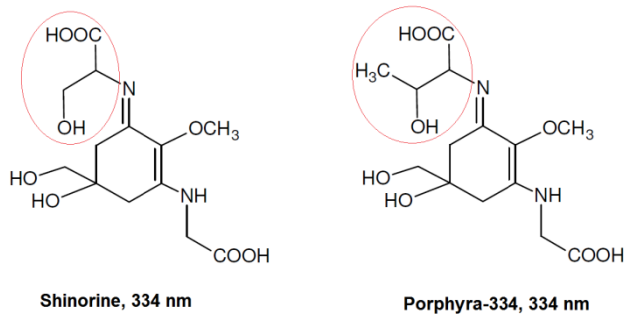

Fig. 2. Basic chemical structures of MAAs (shinorine and porphyra-334) found in cyanobacteria with absorption maxima at 334 nm.

radiation (Ferroni et al., 2010). The source of these natural algae material is mostly wild and depends on local weather conditions. However, cultivable cyanobacteria can be used as natural source for UV-A protective sunscreens. A study demonstrated the effectiveness of methanolic extract of a cyanobacterium Aphanizomenon flos-aquae containing porphyra-334 as significant UV-A protector when compared to two commercial sunscreens (Torres et al., 2006). Another study demonstrated the suitability of MAAs in cosmetics and toiletries as UV protectors and activators of cell proliferation when it was found that the mixture of porphyra-334 and shinorine could suppress UV-induced aging in human skin (Daniel et al., 2004).

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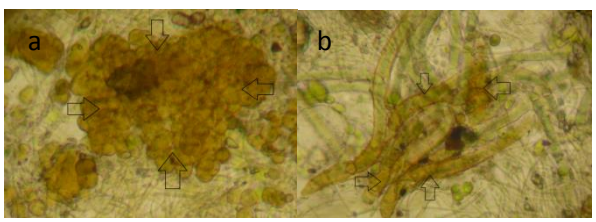

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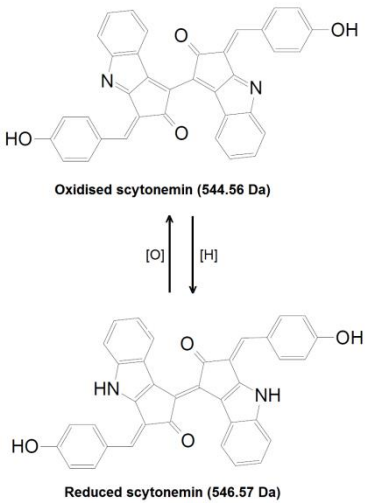

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From Benzamides to Macrocyclic Imides and *beyond*.

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Introduction

Our interest in benzamide chemistry arises from on-going structural and physicochemical research studies of $n \times m$ isomer grids of benzamides, carboxamides ($n, m \geq 3$) *e.g.* **NxxF** (**Figure 1**) where **x** = *ortho*/*meta*/*para*. The objective is to examine relationships by interchanging H/F/Cl/Br/I/CH_3 atoms/groups in a structural systematic fashion about (hetero)aromatic rings, as well as probing atoms and groups *e.g.* F, CH_3 in unusual structural environments and participating in atypical interactions.[1-4]

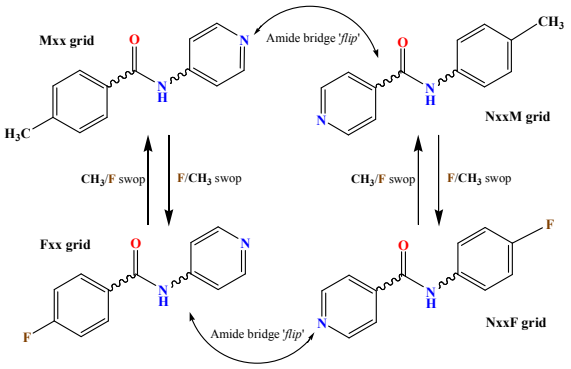

Figure 1 Series of 3 × 3 benzamide/carboxamide isomer grids (with $n, m = 3$).[1-4]

The isomer grids[1-4] have produced a plethora of single crystal structures of which the hydrogen bonded dimer (with a short C-H...π[arene] contact using H23) as **Moo**[1] and the unusual **NmpF**[2] tetrameric assembly as a molecular St. Brigid's cross are two of the more interesting crystal structures that have been studied thus far (**Figure 2**).

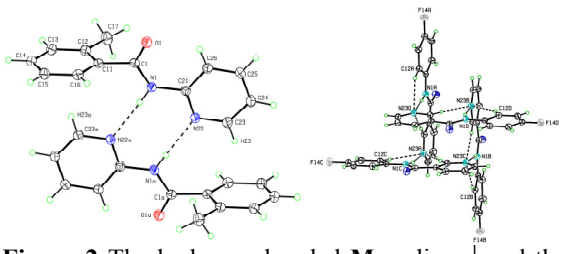

Figure 2 The hydrogen bonded **Moo** dimer[1] and the **NmpF** tetrameric assembly.[2]

A typical synthetic reaction route uses benzoyl chlorides that readily react with 2-aminopyridine (2-AP) in dichloromethane (CH_2Cl_2) at ambient temperatures in the presence of triethylamine (Et_3N) yielding *N*-benzoyl-*N*-pyridin-2-yl-benzamides (2:1 imides); this is often in preference to the expected (1:1) benzamide products.[5-6] This dibenzoylation reaction is well known (**Figure 3**), having first been reported by Marckwald in 1894.[1] Subsequent research has expanded on this condensation reaction in the 20th/21st centuries by examining the reaction mechanism, kinetics and structures.[6] However, given the plethora of benzamides reported as scaffolds in many drugs and materials, the *open-chain* (2:1) imides (**Figure 3**) have not been exploited in any particular synthetic route or application, thus far.

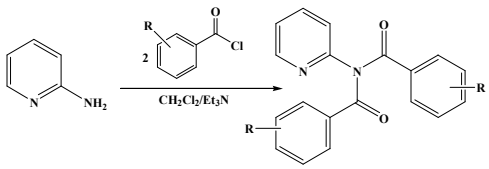

Figure 3 *N*-benzoyl-*N*-pyridin-2-yl-benzamide (2:1 imides) synthesis from 2-aminopyridine (2-AP)

Macrocyclic Imides

Aromatic imides are ubiquitous in materials science. The research focus on imides has largely grown from their potential usage in a wide variety of applications based on their spectroscopic and electronic properties.[7-9] Macrocyclic imides have been developed and typically derive from pyromellitic[7] and perylene bisimides;[8] together with naphthalene-diimides, these almost exclusively incorporate rigid aromatic imide components.[7-9] Such planar diimides contrast with the relatively unexplored *open-chain* and more flexible macrocyclic tetrameric imides as first reported on by Evans and Gale in 2004.[10,11] They discovered a new class of macrocyclic imides by simply condensing isophthaloyl dichloride with penta-/tetra-fluorobenzenes in a similar condensation reaction to that depicted in **Figure 3**.[10]

The [4+4] macrocycle synthesis provides the products in low yields though this is not unexpected

from both steric and electronic considerations and also taking into account the trade-off between oligomer/polymer formation at each step of the potential macrocycle cyclisation stages. The macrocycles as R1 (C_6F_5) and R2 (C_6HF_4) are shown in **Figure 4**,[10] with the crystal structure of a molecule of the R1 derivative depicted in **Figure 5** (left).

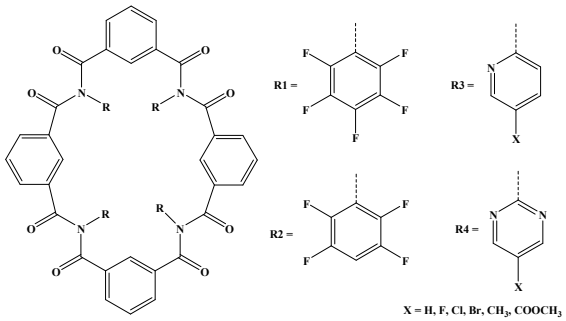

Figure 5 Macrocyclic imides from fluorobenzenes R1, R2,[10] and substituted pyridines/pyrimidines R3, R4.[12]

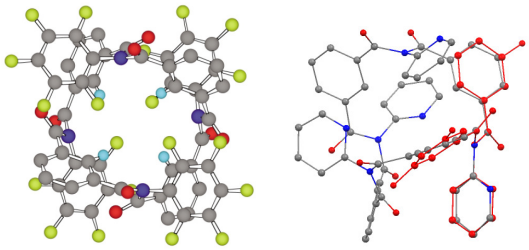

Figure 6 View of the tetrameric macrocycle R1 (left) and superposition of a 2:1 imide (**red**) on R3 (right).

Trezimides and **Tennimides**[12-14]

Using basic reaction chemistry as encountered during the synthesis of the benzamide isomer grid (for the 2-AP derivatives),[1-4] it was decided to attempt the synthesis of analogous pyridine derived macrocycles similar to R1, R2. This was successful using substituted 2-aminopyridines and provided a range of pyridine R3 and pyrimidine macrocycles R4 though these were isolated and purified in modest yields.[12-14]

In addition, the previously unreported and novel trimeric macrocycle named as **trezimides** (**Figure 7**) was isolated and additionally given that the number of tetrameric systems (**Figures 5,6**) has been expanded, these have been named as **tennimides** (as the scaffold backbone has the shape of a tennis ball).[12-14]

The **trezimides** are of interest for several reasons. They can adopt two conformations (**P**) and (**R**) that can be isolated in the solid state and these can readily interconvert though slowly in solution as monitored by ^{1}H NMR. Both conformations are unsymmetrical (**Figure 7**). Molecular modelling studies using potential energy surface scans (PES) demonstrates that the flexibility in the **trezimide** and **tennimide** molecular structure is primarily due to the flexible nature of the isophthalic moieties and with a smaller influence from the imide hinge that is usually seen at angles of 90±15°.[12]

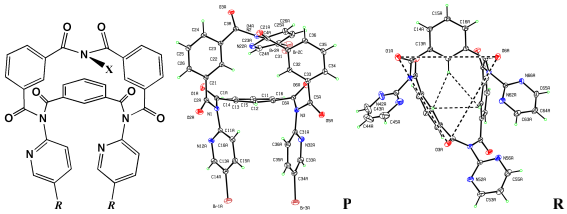

Figure 7 Schematic and ORTEP diagrams of two **trezimides** showing the **P** and **R** conformations.[12]

Several single crystal X-ray diffraction studies on a range of **trezimide** and **tennimide** derivatives show interesting and unusual results. One structural result of a **tennimide** pyrimidyl derivative provides three distinct conformations in the solid-state as two 1-D columns comprising of (*a*) alternating *closed-closed* (*cc*) and *open-open* (*oo*) macrocycles and (*b*) *open-closed* macrocycles (**Figure 8**). The latter conformation is intermediate in structure between the two former states. In the structure, columns of disordered halogenated solvent align between the two distinct 1-D macrocycle columns and the three conformations directly show the semi-flexible nature of the **tennimide** macrocyclic structure.[12]

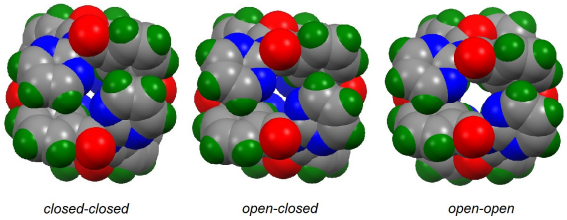

Figure 8 Three solid-state conformations of a **tennimide** with a view of the molecular cavity.[12]

Halogen bonding[15]

Halogen bonding studies have evolved in the past couple of decades into many areas of scientific research from materials, through chemistry and biology.[15] The challenge remains to utilize halogen components (usually incorporated as M–X/C–X) in the design of and synthesis of new materials. A key desire is to exploit their unique functionality where possible, and often in a complementary role, to augment the primary role of the material.[15]

For the brominated **trezimides** and **tennimides**, halogen bonding effects[15] were analysed and typically manifesting as C-Br...O=C and C-Br...$N_{pyrimidyl}$ halogen bonds with $N_c \sim 0.90$ (**Figure 9**).[14]

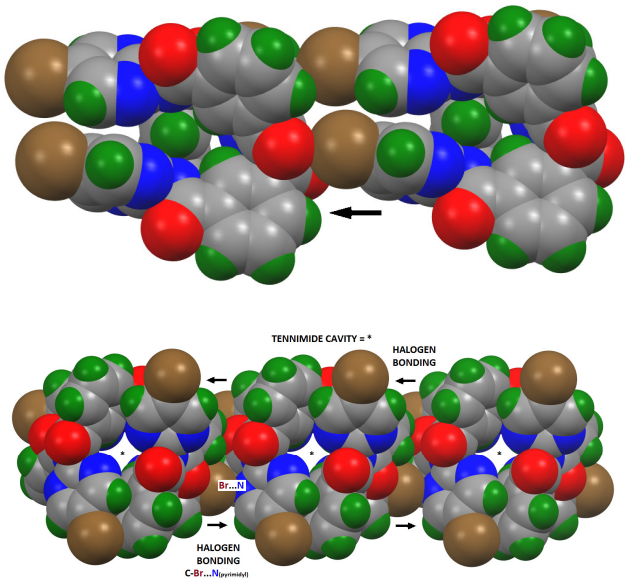

Figure 9 1-D directed C-Br...O=C/$N_{pyrimidyl}$ halogen bonding in a brominated **trezimide** (top) and **tennimide** (bottom) structure.

Future research studies

Further work is directed towards elucidating the structure of larger ring imide-based macrocycles and the chemistry of the oligomeric species generated with the **trezimide** and **tennimide** macrocycles.

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Several molecules are available for viewing at
http://www.youtube.com/channel/UC9FOUhx_uqnj9uyObrcnmUQ
http://www.youtube.com/user/tennimidandtrezimid

Conference posters and a related PhD thesis are available at
http://doras.dcu.ie/view/people/Gallagher,_John_F=2E.html
http://doras.dcu.ie/17256/

Biographical Sketch

Dr. John F. Gallagher has undertaken PhD research in University College Galway (1986-1990) on rhenium-rhenium quadruple bonds between metal atoms, postdoctoral research at the University of Guelph, Canada (1990-1993) in chemical crystallography and the University of Cambridge (1993-1995) on metal atom clusters. Since 1995, research has developed on weak interactions, structural systematics ($n \times m$ isomer grids) and bioorganometallic chemistry. As a co-Editor of *Acta Crystallographica* Sections C (2000-2011) and Section E (2011-2014) of the International Union of Crystallography with currently >190 research papers have been published to date and with ~3000 citations.


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