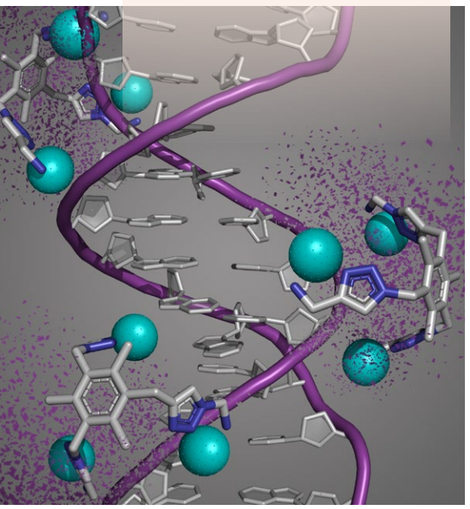
DNA molecular scissors as new cancer chemotherapeutics

The discovery of efficient new metallodrugs with minimal side effects is urgently needed in cancer medicine. Associate Professor Andrew Kellett and Dr Creina Slator, Dublin City University, Ireland, have employed 'click chemistry' to generate new copper DNA molecular scissors with anticancer and gene editing properties. Their molecules have coordinated metal centres and mimic the action of natural products that cause DNA strand cutting, thereby disrupting the DNA-mediated growth of cancer cells. The researchers have successfully synthesised a group of these compounds and described their mode of action, with a view to generate new therapies.



Natural products have historically served as the major source of therapeutic drugs. However, the evolution of preparative chemistry has helped to address limitations associated with the isolation and laborious extraction of bioactive therapeutic agents. Within cancer treatment, some of the most successful therapeutic strategies involve the synthesis of DNA targeted drugs that circumvent drug-resistance pathways. Discoveries of new cancer metallodrugs – compounds whose properties and mode of action are dependent on the chemistry of a particular metal ion – appear promising not only for overcoming resistance factors, but also for treating cancers where limited treatment options exist.

Dr Creina Slator and Associate Professor Andrew Kellett of Dublin City University, Ireland, investigate copper complexes that can initiate cancer cell death by an oxidative mechanism causing DNA damage. The team's work focuses on the design of inorganic, metal-based compounds that act as molecular scissors for DNA excision in cancer therapy and other diagnostic and therapeutic applications.

METALLODRUGS AS ANTICANCER AGENTS

Metallodrugs have been extensively applied due to their ability to cause DNA damage, thereby acting as anticancer agents. Platinum-containing drugs represented by the most well-studied, cisplatin, are essential anticancer agents with proven effects against a variety of tumours. Despite their recognised clinical applications they suffer from several limitations such as poor uptake and cross-resistance, but mainly their mode of action relies on the chemical and physical properties of discrete complexes with non-specific cell recognition or targeting. In practise, this results in adverse side effects, with healthy cells also being targeted. Therefore, there is a need for the discovery of new metallodrugs with improved efficacy and decreased toxicity. In trying to meet this demand, Slator, Kellett and the team have developed new metallodrugs that can act as molecular scissors. Using a process called 'click chemistry', the metallodrugs can be targeted to selectively bind to specific genes of interest and cause DNA damage to tumours.

CLICK CHEMISTRY

Click chemistry is described as the sum of reactions where two groups 'click' together to create a product in the most efficient manner. This translates to high yields, simple reaction conditions, easy purification conditions, and readily available materials. Despite its wide use, Kellett and Slator were among the first to use click chemistry to create new DNA binding metallodrugs. Specifically, the research team, together with group member Dr Natasha McStay, created a library of copper-binding compounds known as the Tri-Click (TC) series. The project is part of the group's ongoing work within the Science Foundation Ireland-funded SSPC centre.

At the core of TC compounds is a bulky carbon structure surrounded by three azides which are clicked to a bank of alkyne-functionalised amine ligands, forming triazole ring systems. The three anchor points in the TC compounds selectively capture copper ions, which

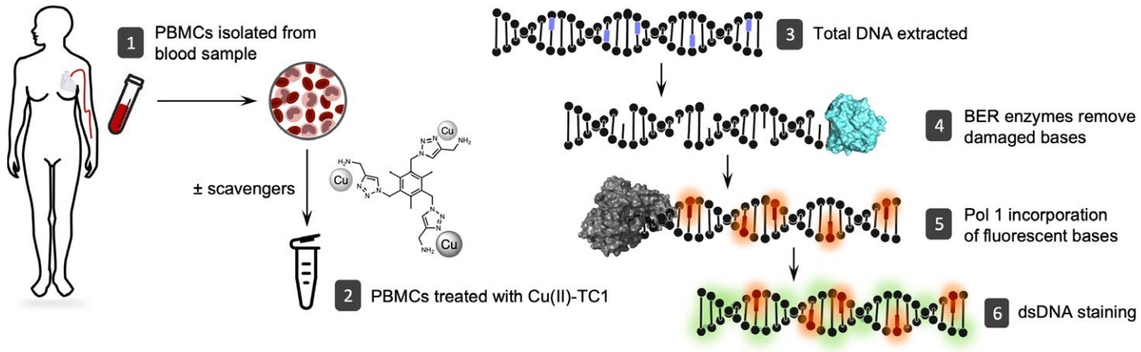
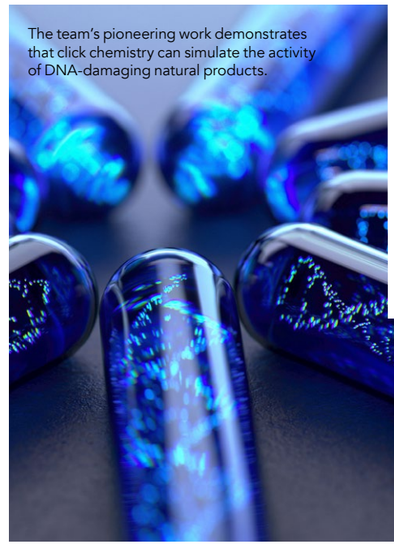
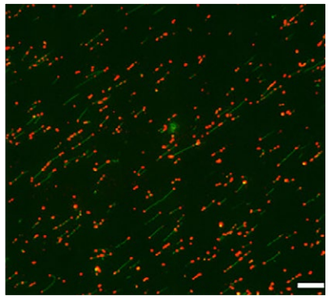


Figure 1. DNA-damaging potential and effect of antioxidants in isolated peripheral mononuclear blood cells. 1–2: Sample collection and treatment; 3–6: DNA labelling process. From: McStay et al, *Nucleic Acids Research* (2021), doi.org/10.1093/nar/gkab817

serve as molecular scissors capable of initiating DNA damage within diseased cells. Fascinatingly, the TC compounds act by causing oxidative DNA damage: a mode of action found in natural products, such as marine alkaloids and metallo-bleomycin. The team's pioneering work demonstrates that click chemistry can simulate the activity of DNA-damaging natural products.





Fluorescence microscope image showing DNA damage in Cu(II)–TC1 treated cells. From: McStay et al, *Nucleic Acids Research* (2021), doi.org/10.1093/nar/gkab817

COPPER-ION MEDIATED DNA DAMAGE BYPASSES CHEMOTHERAPEUTIC RESISTANCE

In 2021, the Kellett group published a paper outlining the Tri-Click ligand development, focusing specifically on the copper ion-mediated DNA damage. Before any drug can exert a therapeutic action, it must first recognise and bind tightly to its target. In the case of anticancer drug development, double-helical DNA binding must occur before the DNA can be damaged. Nucleic acids, the biomolecular building blocks of DNA, are key in mediating faithful cellular replication in all living things. The 2021 paper shows that copper-bound TC compounds recognise nucleic acids and bind to them before initiating the DNA-damaging action. When TC1, one of the most promising TC compounds, is unbound it has a modest binding affinity for DNA. However, it can produce a potent combination of DNA binding and cleaving activity when a copper complex is formed. It is also remarkable that the copper-TC1 combination demonstrates 'self-activating' DNA cleavage, mediating single strand breaks in DNA in the absence of any other external agent.

The Kellett lab, along with collaborators Professor Fredrik Westerlund and Dr Vandana Singh at Chalmers University of Technology, Sweden, then treated DNA isolated from human cells with copper-TC1. Using fluorescence microscopy, the team visualised the location and type of damage in DNA using single molecule detection methods. They demonstrated that copper-TC1 can overcome the innate cellular DNA repair machinery, causing DNA damage that is not possible to achieve with existing metal-based drugs. The study also highlighted that there is good agreement between laboratory observations and a previously observed cellular DNA-damaging mechanism caused by copper complexes found naturally in marine organisms. Thus, TC complexes have applications as high-value anticancer agents. This research paves the way for future investigators to develop enhanced nucleic acid targeting agents through click

The team's pioneering work demonstrates new use for click chemistry to construct DNA-damaging metallodrugs.

chemistry, that circumvent the cellular DNA repair mechanisms at the base of chemotherapeutic resistance.

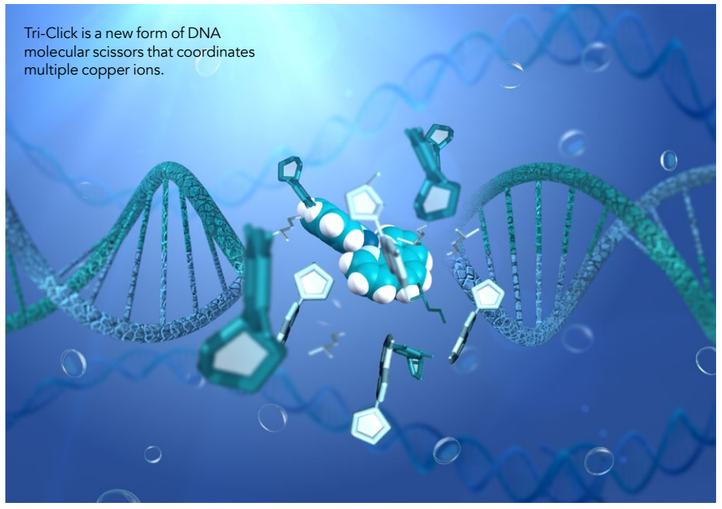
MOLECULAR SCISSORS WITH GENE-EDITING POTENTIAL

Tri-Click is a new form of DNA molecular scissors that coordinates multiple copper ions. The challenge is to now guide these scissors to specific genes of interest using nucleic acid probes. In 2020, Kellett, Slator and collaborators published a new method for directing other classes of copper molecular scissors that mimic nuclease enzymes using nucleic acid click chemistry. This concept was recently extended to direct platinum crossing-linking to specific genes of interest (published 2022 in *Angewandte Chemie*).

The researchers demonstrated that, through click chemistry, the use of triplex-forming oligonucleotides (TFOs) – a short single strand of DNA – can target specific genes. This work is based on the ability of the TFOs to recognise and bind to specific sequences in the helical structure of DNA, forming a DNA triplex. The Kellett laboratory has demonstrated that TFOs hybridised to metal complexes display effective chemical nuclease activity; in other words, in the presence of copper, they can act as molecular scissors with DNA-cutting ability. Studies also show that click chemistry allows scalable and high-throughput generation of hybrid libraries of TFOs that can be tuned to bind and cut specific DNA sequences. These 'molecular scissor' hybrids can be used in artificial gene-editing applications, with gene-of-interest knockout capabilities.

DNA-reactive small molecules which are chemically linked to vectors that recognise and bind to specific genes have recently become the focus of research in the Kellett lab. In collaboration with multi-institutional consortia consisting of academic and industrial partners with a range of expertise in nucleic acid chemistry, including Professor Tom Brown of the University of Oxford and Professor Thomas Carell of LMU Munich, the team aims to develop drugs for future gene editing, immunotherapy, and genetic silencing. By directing metal-based DNA targeting molecules, the long-term goal is to provide effective, personalised treatments for cancer and other genetic diseases. Such individualised therapy offers enhanced efficacy with reduced side effects. The team's current goal is to use click chemistry to develop a new class of compounds that is capable of silencing specific genes to better understand their biological role or to cure disease. Ultimately, the goal is to use a molecular delivery system based on nanoparticles that can transport click chemistry compounds directly into the nucleus of specific target cells. This new approach will constitute a significant step forward towards the achievement of personalised cancer care and personalised medicine.

The Kellett group have identified a new targeting strategy for metallodrugs, overcoming the challenges these compounds face in the field of cancer.



Tri-Click is a new form of DNA molecular scissors that coordinates multiple copper ions.

'IMAGE AND DESTROY': THE ULTIMATE RADIO-THERAPEUTIC STRATEGY

Slator and Kellett are now collaborating with leading Danish scientists, Professor Christine McKenzie, Professor Helge Thisgaard, and Professor Vickie McKee, to bring together two crucial aspects of cancer treatment: accurate and timely diagnosis, and advanced therapeutics that halt the spead of malignant tissue. The ambitious venture, funded by the Novo Nordisk Foundation and led by Professor McKenzie, will incorporate aspects of cellular imaging together with the 'cell-destroying' potential of radioactive compounds. This 'Image and Destroy' project aims to design targeted radiopharmaceuticals which will act both as diagnostics and therapeutics tools. These metallo-hybrids are composed of novel coordination ligands that are bound to underexploited radioactive elements known as Auger electron emitters (AEEs). These promising molecules will also include a TFO vector that recognises specific sections of DNA. The team will optimise these AEE-hybrids for the selective targeting of breast and brain cancers that have a poor prognosis, with the aim of increasing therapeutic effectiveness and survival rates.

CHANGING CANCER THERAPY AND DIAGNOSIS

The Kellett group has opened a new potential for metallodrugs, which aim to overcome the challenges these compounds face in the field of cancer. This pioneering work provides the opportunity for future research to develop enhanced DNA damaging agents with unique chemotherapeutic and diagnostic properties that can circumvent innate cellular DNA repair machinery and offer new opportunities for cancer therapy and diagnosis.

Behind the Research

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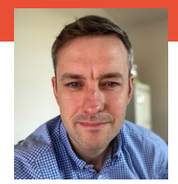

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Research Objectives

Targeted metallodrug–nucleic acid interactions for biochemical and therapeutic applications.

References

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Personal Response

Are there any medical conditions, aside from cancer, that would benefit from your click chemistry approach?

"The application of this innovative hybrid strategy extends beyond the treatment of cancer. With genomic DNA serving as the therapeutic destination of novel molecular scissors, several genetic diseases could avail of this potential, including discrete defects such as naturally occurring somatic genetic diseases, metabolic disorders, or single nucleotide polymorphisms causing deleterious point mutations. Furthermore, by tuning the nucleic acid targeting vector, this technology could be applied within antisense therapies targeting RNA which may offer new potential in treating viruses such as SARS-CoV-2 which causes COVID-19."

Detail

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Bio

Creina Slator obtained her BSc in chemical and pharmaceutical sciences at Dublin City University (DCU) where she completed her PhD in medicinal bioinorganic chemistry in the Kellett Research Group in 2017, and continued as a postdoctoral researcher and research fellow. She has authored 14 research papers and review articles. Creina was awarded the Faculty of Science and Health Distinguished Scholar Studentship (2012), an Irish Research Council Scholarship (2014), and a Technology Innovation Development Award funded by Science Foundation Ireland (2019).

Andrew Kellett is Associate Professor of Inorganic and Medicinal Chemistry in the School of Chemical Sciences at DCU. He completed his BSc in chemistry from Maynooth University and his PhD at the Technological University Dublin (TUD). He started his independent career at DCU in 2011 with research interests focusing on the discovery of metallodrug–nucleic acid interactions for biochemical and therapeutic applications. He has authored over 60 publications, is a PI within Biodesign Europe, the coordinator of back-to-back Horizon 2020 Marie Skłodowska-Curie Innovative Training Networks (ITNs), NATURE-ETN and ClickGene, and his lab is supported by Science Foundation Ireland, the Irish Research Council, and the SFI centres: Synthesis and Solid-State Pharmaceutical Centre, and CÚRAM.

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Collaborators

- **Click and Cut:** Professor Fredrik Westerlund and Dr Vandana Singh, Department of Biology and Biological Engineering, Chalmers University of Technology (CUT), Gothenburg, Sweden.
- **Image and Destroy:** Professor Christine McKenzie, University of Southern Denmark (SDU); Professor Helge Thisgaard, University Hospital Odense (OUH); and Professor Vickie McKee (DCU).