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The Wild Garden of the Gut Bacteria

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EDITORIAL

Welcome to the 23rd issue of Phenotype, and my very first issue as Editor-in-Chief! As ever, this issue is packed with articles by students, postdocs, principle investigators and alumni from a huge range of discplines across the University and from further afield. We are lucky to have contributors and an editorial team with expertise in an enormous variety of subjects, which is undoubtedly one of the main reasons that the quality of the articles we publish is so high.

This issue is no exception, with our **Features** *section containing articles on research that sits at the cutting edge of biological science. Some of the biggest healthcare challenges we face today come under scrutiny, with Professor Matt Higgins delving into the proteins posing challenges and presenting hope for the production of a malaria vaccine, while Dr Rebecca Perrett explores the role of the endolysosomal pathway in Parkinson's disease. Dr Laura McCulloch asks how bariatric surgery can cure type 2 diabetes and Vivekka Nagendran puts oncoviruses in the spotlight. We also see a particular focus on new technology and methodology in this issue. Dr Tom McAllister explains the use of mRNA display in screening novel cyclic peptide inhibitors of the histone lysine demethylases, while in a similar vein, Josh Bugajski looks at the use of cell-penetrating peptides for drug delivery. Dr Jyothi Menon reviews the mechanisms behind cancer-targeting nanoparticles and Oriol Pavón Arocas describes the enormous value of photo-activatable GFP tracing in mapping neural networks.*

In a slight departure from our usual format, our **Science in Society** *section has been distributed throughout the magazine, reflecting the ever-increasing importance of the translation of research to the wider world. Dr Emily Flashman probes the importance of GM crops in the 21st century, Emma Lawrance looks at the role of neuroscience research in finding hope for mental illness and Dr Gaia Donati champions Open Access Publishing.. Finally, alumnus Dr Fenix Leung provides a career insight into his work as a Business Intelligence Anayst at GlobalData.*

As always, our **Regulars** *section brings you Research Highlights, book reviews, an interview with an academic, the winner of the SNAPSHOT scientific image competition and something new! In Research Highlights (page 5), Dr Cristina Maculescu summarises two key papers from groups in the Department of Chemistry; one probing the role of the O-linked GlcNAcylation post-translational modification on histones, and the other reporting the use of a nanopore sensor to evaluate Pim kinase inhibitors. Then turn to page 15 for our new item, "Classic Kit", in which Evangelia Tzika looks at the history, development and use of the now ubiquitous Mass Spectrometer. On page 30, Lauren Chessum spends 5 min with… Dr Samira Lakhal-Littleton, who is uncovering novel mechanisms of iron homeostasis, whilst page 31 provides details of the winner of this term's SNAPSHOT competition. Congratulations to Dr Nicola Fawcett, whose beautiful image 'The Wild Garden of the Gut Bacteria' showcases her work investigating the effect of antibiotics on the gut microbiome.*

If you fancy a challenge, set your mind to our brain-themed cryptic crossword on the back cover- it's literally a brain-teaser! Be sure to email your answers to me at rebecca.hancock@linacre.ox.ac.uk for a chance to win one of the books from our partners at Wiley-Blackwell, reviewed by Natalie Ng, James Eaton, Emma Bickford and Cassandra Kennedy on pages 28 and 29.

Finally, please do take a look at our second ever themed **Supplement**, *which this issue focusses on Neuroscience, with articles from Professor Vladyslav Vyazovskiy and his team, Ines Barreiros and Vani Rajendran.*

I would also like to highlight the opportunities we have here at Phenotype. Whether you would like experience in science writing, editing, designing and advertising, please email rebecca.hancock@linacre.ox.ac.uk for more information.

I hope that you enjoy reading this issue, which really does showcase the best in both biological science research and science writing that the University has to offer. Please get in touch with any comments, questions or suggestions you may have and have an excellent Hilary term!

Becky Hancock
Editor-in-Chief

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RESEARCH HIGHLIGHTS

by Dr Cristina Marculescu

Lercher. L, *et al.* (2015) Nature Communications 6, 7978. doi: 10.1038/ncomms8978

Generation of a synthetic GlcNAcylated nucleosome reveals regulation of stability by H2A-Thr101 GlcNAcylation

Post-translational modification (PTM) of histones is key for the regulation of gene expression in eukaryotic cells. This paper from the Davis group in the Chemistry department focuses on a recently identified and medically relevant histone PTM, O-linked GlcNAcylation. To date, there are no reports on how this PTM affects transcription. The goal that the Davis group has set out to achieve is a deeper understanding of the role of O-linked GlcNAcylation.

The approach undertaken by the lab to address this question was to generate the post-translationally modified histone *in vitro* and then subject it to various biophysical and interaction analyses. However, currently available conjugation techniques are not selective enough and therefore do not allow the synthesis of a pure modified protein. For example, when used *in vitro*, O-linked *N*-acetylglucosamine transferase (OGT), the enzyme that catalyses GlcNAcylation *in vivo*, produces a mixture of different protein products instead of a single protein.

The authors have overcome this problem by mutating the threonine residue to be O-GlcNAcylated – T101 of *Xenopus laevis* Histone 2A (H2A) – to a cysteine residue. The H2A-T101C variant protein was then subjected to a neat two-step synthesis to give the first homogenous H2A GlcNAcylated mimic at residue 101.

They used the modified histone to reconstruct the nucleosome, by first forming H2A/B dimers and then adding the H3/H4 tetramer and a high-affinity DNA sequence. The properties of the modified nucleosome and its H2A/B dimer component were then compared with corresponding wild type structures. Size exclusion chromatography, circular dichroism spectroscopy and differential scanning fluorimetry data indicate that H2A-T101 GlcNAcylation reduces the tetramer-dimer association, decreasing the overall stability of the nucleosome. Further proof came from nanoelectrospray ionization mass spectrometry analysis, which revealed that the GlcNAcylated nucleosome consists of a mixture of subspecies, as opposed to the wild type nucleosome which is significantly more homogenous.

Finally, having access to the homogeneous O-GlcNAcylated H2A allowed further insights into the role of H2A-T101 O-GlcNAcylation in cells. Pull-down experiments indicated that DNA mismatch proteins are recruited as an effect of nucleosome destabilization, which are thought to further contribute to nucleosome disassembly. Ultimately, this is thought to facilitate transcription by making the DNA more sterically accessible.

Notably, the conjugation strategy described in this paper can be further applied to other proteins of interest and provide further insights into the molecular basis of epigenetic processes.

Harrington, L, *et al.* (2015) Angewandte Chemie International Edition 54, 8154. doi: 10.1002/anie.201503141

Pim kinase inhibitors evaluated with a single-molecule engineered nanopore sensor

Kinases are overexpressed or mutated in different disease states and they are a major target in modern drug discovery. Developing easy to operate, accurate, cost-effective and scalable assays is crucial to screening drugs which may impact kinases.

Screening kinase inhibitors relies heavily on radiometric assays as the gold standard method, followed by fluorescence-based evaluation. However, the most commonly applied method is differential scanning fluorimetry (DSF), which measures the stabilization of the protein fold to thermal denaturation induced by ligand binding. Unfortunately, DSF often leads to false positives and misses out on potent binders.

In this study, the Bayley group proposes a novel method to screen potential kinase inhibitors. Showing its efficiency and superiority over DSF, the group also illustrate that their new method has the potential to become formatted on high throughput chips.

In a recent paper by the group, Harrington *et al.* have applied their expertise in producing nanopores to make a heptameric alpha-hemolysin pore with just a single subunit bearing a peptide sensor element. In the case of their model kinase, Pim-1, which is associated with regulation of cancer progression, the sensor is represented by an analogue of the Pim consensus substrate. The binding affinity of the analyte for the sensor peptide is calculated by monitoring the fluctuations in the ionic current through the nanopore. Kinase binding to the sensor was measured in the presence of MgATP without subsequent phosphorylation. The inhibitory effect of a small library of compounds was determined by comparing the electrical current traces before and after addition of the inhibitor.

The results obtained using the nanopore screen were compared to ones obtained using DSF and a coupled-enzyme assay. In fact, DSF failed to identify a particularly potent compound that was one of the six compounds highlighted by the novel nanopore technology.

Finally, the amenability of this new method to be used for high-throughput screening was addressed by suggesting that this could be achieved by adapting the MinION technology promoted by Oxford Nanopore Technology, a tool currently being developed for DNA sequencing.

The malaria parasite: a master of disguise?

by
Prof Matt Higgins

In July 2015 the BBC reported that the world's first malaria vaccine, Mosquirix, had been approved for use in Africa by the European Medicines Agency (1). The head of vaccine research at GlaxoSmithKline was quoted as describing this as "a dream come true" after working on the vaccine for 30 years. Indeed, as malaria still kills more than half a million people each year, an effective vaccine would be the thing of dreams. Mosquirix is a big step forwards but, at best, it protects just a third of immunized children from developing severe malaria, and only after a complex dosing regimen that requires four doses over the course of 20 months; a logistical nightmare in some malaria-scourged parts of Africa. So why, 36 years after a vaccine led to the eradication of smallpox, are we still struggling to develop a universally effective vaccine to prevent malaria?

The parasites that cause malaria, such as *Plasmodium falciparum*, are complex shape-shifters. Millennia of co-evolution with their human host has led them to develop a variety of strategies to evade detection by the immune system of an infected child, allowing them to survive, replicate and be passed on as a mosquito takes a blood meal. However, to drive many stages of their cell cycle, these parasites need to retain the capacity to make specific interactions with invariant human molecules. This allows them to invade host cells, such as human red blood cells, where they can divide without being detected by the immune system. A major focus of the Higgins lab is to understand the molecular basis for these interactions, determining which features of parasite surface proteins must be retained for their function, and which might be targeted by the therapeutics of the future.

An example is the PfEMP1 protein family, whose members are found on the surfaces of human red blood cells infected with *Plasmodium falciparum*. Damaged red blood cells will normally be cleared from circulation as blood flows through the spleen. However, infected cells evade such fate by employing the PfEMP1 proteins as 'molecular Velcro', allowing them to stick to blood vessel surfaces. This enables parasites to grow and divide safely, increasing their numbers within the patient. It also leads to specific symptoms of severe malaria, with infected red blood cells accumulating in the brain and causing inflammation. PfEMP1 proteins that bind to endothelial protein C receptor (EPCR) are particularly associated with the development of severe childhood malaria, perhaps by preventing the natural role of EPCR in reducing cerebral inflammation (2).

However, the presence of PfEMP1 on the surfaces of infected erythrocytes comes at a cost for the parasite, as these proteins are now exposed to the immune system. Antibodies that recognize PfEMP1 are therefore common in people from countries where malaria is prevalent. To avoid detection by these antibodies, the parasite has amplified and diversified the number of PfEMP1 proteins that it produces into a large protein family. By switching which protein is expressed the parasite can avoid detection. But to what degree are the PfEMP1 proteins diversifiable? How much can one of these proteins vary, driven by the pressure to avoid antibody-mediated detection, while still retaining the ability to bind to a particular human endothelial receptor? This was the question tackled by Clinton Lau, in collaboration with colleagues at the University of Copenhagen (3).

The team analysed ~800 sequences of EPCR-binding PfEMP1 proteins, but this alone did not

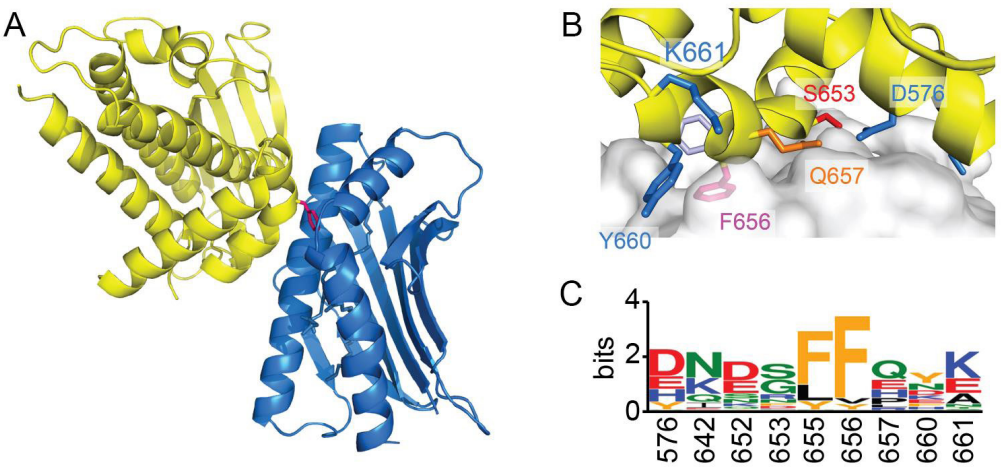

Figure 1. (A) The structure of EPCR (blue) in complex with a domain from a PfEMP1 protein (yellow). (B) A close up of the EPCR-binding site. (C). The residues that make direct contacts with EPCR are not conserved across EPCR-binding domains, but retain conserved chemical properties.

provide the answer. Although conserved residues could be found across this group of proteins, none of these discriminated between EPCR-binders and non-binders. However, a crystal structure of a domain from a PfEMP1 protein in complex with EPCR revealed the molecular details of the interaction, and showed the degree to which features of the binding site remain conserved (Figure 1). While none of the amino acids that directly contact EPCR are kept identical, the overall architecture and chemistry of the EPCR-binding site is maintained throughout the protein family. Therefore, although highly diverse in sequence, all of the EPCR-binding PfEMP1 proteins retain a chemically similar surface to mediate ligand binding. The next challenge is to produce antibodies that can recognize these chemically similar surfaces, generating a reagent that can block EPCR-binding by the entire PfEMP1 family.

A second story focuses on red blood cell invasion, where complex machinery is used by the malaria parasite to force its way into host cells. This requires a set of proteins that allow the parasite to recognize molecules on the correct cell, forming a junction where the invasion machinery can act. While a set of proteins are present in the parasite to make such interactions, a single protein, RH5, has been shown to be essential for invasion through its interaction with the red blood cell receptor, basigin. If this interaction is blocked through the application of antibodies that interact with either RH5 or basigin, red blood cell invasion is prevented and the parasite remains outside the host cell, unable to replicate and exposed to detection and destruction by the immune system.

To understand the molecular basis for the interaction between RH5 and basigin, Kate Wright, in collaboration with colleagues at the Jenner Institute in Oxford, determined the crystal structure of the RH5:basigin complex and also revealed how monoclonal antibodies can prevent basigin from binding to RH5 (4). These studies revealed a novel 'kite-shaped' architecture for RH5, with a basigin molecule binding at the top, and inhibitory antibodies binding at, or close to, this surface (Figure 2). Remarkably, unlike the PfEMP1 proteins, RH5 is extremely conserved in malaria parasites from around the globe. Mapping the variation observed in this protein onto the structure revealed that the very few observed changes were dispersed over the surface and did not change the basigin-binding surface.

So why is RH5 so invariant when the PfEMP1 proteins are so variable? The secret appears to lie in another of the parasite's favourite tricks. The surface proteins, such as RH5, that play essential roles in the invasion machinery are hidden within a compartment at the tip of the parasite, only to be released when the parasite bumps into a red blood cell. Unlike the PfEMP1 proteins, RH5 is therefore only exposed to the immune system for a brief time during the process of invasion. By hiding away its invasion proteins, the parasite protects them from exposure and reduces the chance that they will be detected and inactivated by the body. Despite this, immunization with RH5 does protect *Aotus* monkeys from developing malaria (5). This means that RH5 is an exciting vaccine candidate, and results from human clinical trials are eagerly awaited.

The parasites that cause malaria therefore use a number of tricks to avoid detection by the immune system. They use sticky proteins to tether infected red blood cells away from destruction by the spleen. They change their surface proteins so that, as the body learns to recognize them, they switch to become unrecognizable again. Finally, they hide away essential machinery in intracellular compartments to be released when needed, reducing its exposure to the immune system. By understanding these complex parasite molecules, and the essential features that they use to interact with the host, we hope to design components that target their conserved regions. These components can join with other molecules, such as those present in the Mosquirix vaccine, to make the next generation of vaccines, aiming towards a universally effective vaccine to prevent this horrific disease.

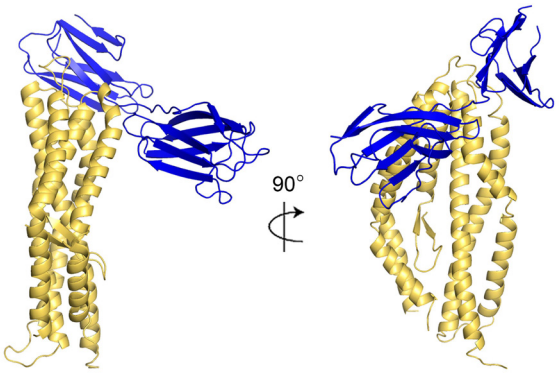

Figure 2. The structure of the complex between RH5 (yellow) and basigin (blue).

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*Matt Higgins is a Professor of Parasitology in the Department of Biochemistry.**The work presented is funded by Arthritis Research UK and the Medical Research Council.*

mRNA display & cyclic peptides: a novel approach to targeting histone demethylases

by
Dr Tom
McAllister

Histone demethylases are enzymes involved in epigenetic regulation with implications for cardiovascular diseases and cancer. The high degree of structural similarity in the active sites of this family of enzymes makes it difficult to target specific members by means of traditional medicinal chemistry approaches. The Kawamura group uses an alternative approach - the identification of cyclic peptides via mRNA display, a highly powerful, broadly applicable technique outlined below.

Nowadays, sequences of DNA strands can be readily identified, whereas an analogous process for sequencing proteins simply does not exist. Thus, in a mixture of proteins, deciphering the sequence of each would be next to impossible using the translated proteins alone. The so-called 'display' technologies provide crucial backwards compatibility and establish a link between the functional protein molecule and the nucleotide strand encoding it. One example of 'display' technologies - mRNA display - directly connects phenotype with genotype and overcomes the difficulty of recovering the sequence of a protein/peptide with a desirable feature.

Starting from a large mixture of 10^7 to 10^{12} different DNA sequences, the corresponding peptides can be produced and those with a desirable characteristic can be isolated and identified by their DNA sequence. Multiple repetitions of the procedure selects for the best binding motifs or most active sequences. In our lab, we start the selection process with a large library of approximately 1012 different DNA sequences, designed to encode for 4 to 12 random amino acid residues flanked by a conserved initial amino acid and a C-terminal cysteine residue (important to make the peptides cyclic – see later).

There are different types of display techniques: phage display, bacterial display, yeast display, mRNA display and ribosome display. Ribosome and mRNA display are *in vitro* techniques, whereas phage, bacterial and yeast display are in vivo techniques that rely on the transformation of cells with the DNA of interest.

The *in vitro* techniques utilise an *in vitro* transcription/translation (IVTT) system. The required components for transcription and translation are produced separately, purified and recombined to yield a solution that produces protein from an input DNA sequence. This mixture includes an RNA polymerase along with nucleotide-triphosphates, ribosomes, tRNAs, amino acids, aminoacyl-tRNA synthetases, initiation factors, release factors and proteins to ensure efficient transcription and translation. The key property of this mixture is its defined composition, lacking DNases, RNases or proteases. Extra elements can be added or other components can be omitted entirely. IVTT systems using the machinery from both eukaryotes and prokaryotes are commercially available.

In ribosome display, release factors are withheld from the system so that ribosomes will stall at a stop codon with both the mRNA and nascent peptide/protein still attached to the same ribosome. mRNA display, as first demonstrated in Nobel laureate Jack Szostak's lab (1), employs a similar approach but uses mRNA modified at the 3' end with a linker bearing a puromycin derivative which can enter the ribosomal A site and be incorporated into nascent peptide chains. This provides a covalent linkage between the peptide and the mRNA encoding its synthesis. In both cases, subsequent reverse transcription can be used to generate cDNA from the mRNA strand.

Each display technique has its own advantages and drawbacks: *in vivo* methods rely on the transformation of cells, but the efficiency is limited and thus require smaller library sizes to ensure full coverage. On the other hand, they do have the advantage of post-translational modifications of the peptides which could confer more beneficial characteristics to them. The in vitro techniques are typically more expensive but allow larger starting library sizes and more opportunities for further development, such as genetic code reprogramming. This is a key element that enables us to produce cyclic peptides and/or peptides containing post-translationally modified residues.

During translation, incoming amino acids are recognised by the complementarity of the anti-codon on the tRNAs to which they are attached, not the amino acids themselves. Hence, a tRNA bearing a different amino acid to the one expected (e.g. a leucyl tRNA with a glycine residue attached) will still be translated in exactly the same fashion. By 'mis-charging' tRNAs with non-cognate amino acids it is possible to reprogram a codon so that it corresponds to a different amino acid. There are several ways to achieve this, but we decided to use tRNA acylating ribozymes termed "flexizymes" and pioneered by Hiroaki Suga's lab (2).

Flexizymes are short single-stranded RNA molecules that attach a chemically synthesised activated ester of the desired amino acid onto tRNAs. Flexizymes do not recognise the amino acids themselves but the activating group on the acid and the conserved 3' end of tRNA molecules. In other words, they can be used to charge any amino

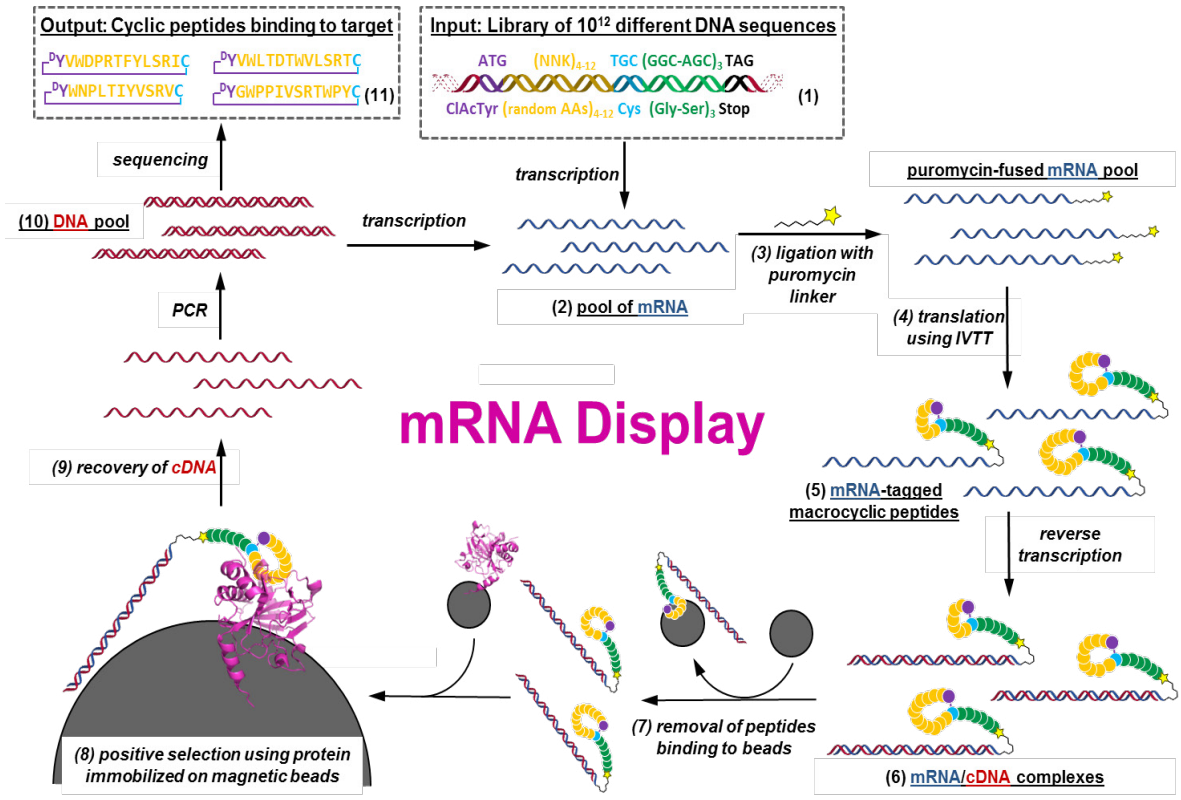

Figure 1.
A typical 'mRNA-display' screening procedure starts with our initial library of 10^{12} different DNA sequences (1) which are transcribed in vitro to generate the corresponding mRNAs (2). A linking oligonucleotide with a 3' puromycin is ligated to the mRNA mixture (3). An IVTT reaction is set up using the puromycin-linked mRNA and a reprogrammed initiator tRNA with a chloroacetyl tyrosine instead of methionine (4). The mRNA is translated by the ribosome to generate a peptide which spontaneously cyclises between the chloroacetyl group and downstream cysteine and at the same time the coding mRNA becomes attached to the nascent peptide through the puromycin (5). A reverse transcription reaction is then performed to generate complementary DNA (cDNA) which forms a stable duplex with the mRNA (6). This serves two purposes: the duplex protects the mRNA from degradation and the duplexation with DNA prevents the mRNA strand from adopting any secondary structure which may itself have affinity for the target. This mixture is then used in the actual selection process for which we use the target protein immobilised on magnetic beads. First, rounds of negative selection using just magnetic beads without the target protein are used (7) to ensure that binding sequences recovered are binding to the target protein and not the beads themselves. A positive selection is then performed using the target protein immobilised onto magnetic beads and the beads are thoroughly washed to remove any non-binding sequences (8). The binders are then recovered (9) by heat-denaturing the immobilised protein, quantified by qPCR and amplified by standard PCR to give sufficient DNA to be used as the input for the next round of selection (10). After several rounds of this procedure to identify the best binders, the output DNA is ligated into a vector, transformed into E. coli and multiple colonies are sequenced to determine both the sequence of the binding peptides and their relative abundance (11).

acid onto a tRNA with any codon. tRNAs acylated using flexizymes are then simply added to the IVTT mixture and are incorporated into the peptides by the ribosome. The only caveat to this is that in order to prevent a background level of incorporation of endogenously charged tRNAs, the amino acid whose codon has been reassigned needs to be excluded from the IVTT reaction. We reassign the start codon from methionine to the unnatural amino acid chloroacetyl tyrosine, which will react with the encoded downstream cysteine residue to generate a cyclic peptide with a thioether linkage.

Using this protocol, we have identified many novel peptide sequences that bind to their target proteins with high affinity and typically high selectivity over similar proteins. To date, we have found inhibitory sequences, sequences with the ability to bind without inhibiting the native function and even a sequence that increases enzyme activity. In short, this powerful technique is rapidly producing high quality compounds, crucial to further our understanding of the complex world of epigenetic regulation.

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Dr Tom McAllister is a Postdoctoral Research Fellow in the Kawamura Lab, based in the Departments of Chemistry and Cardiovascular Medicine.

Open Research: one expression for many implications

by
Dr Gaia Donati

Open research encompasses a multitude of views on how to make science a more collaborative, transparent and resource-efficient process. Open Access (OA), open data and open education have become widely used expressions, each referring to a specific aspect of the wider debate on good research practice and knowledge transfer. This article focuses on the accessibility of research data in the physical and life sciences, although the paradigm of open research is equally relevant to the humanities and social sciences.

In the traditional model of scientific publishing, authors are not charged to publish their article in journals (although color pages can be very expensive). Publishers instead source their revenue from institutional subscriptions, views and downloads from non-subscribers, and third-party advertisements. In this scheme, commercial publishers report higher profits compared to their counterparts managed by learned societies and academic institutions. The 'figures of the business' can be tricky to obtain: while some publishers refuse to disclose how profitable their journals are, others admit that they lack a clear idea of the publishing cost of an individual article (1).

Despite this uncertainty, some numbers do appear in the publishing literature. A survey by the Association of Research Libraries in the US reported average yearly subscription prices for individual journals well above $1000—for instance, $4215 in chemistry and $2520 in biology (2). These figures raise the question of how lucrative publishing really is. Most publishers stress the added value brought by their services, which ensure a high-quality finished product and successful dissemination of a research team's achievements. However, available data demonstrates an increase in journal subscription costs over time that is not justified by inflation.

If traditional publishers have broken the delicate balance of 'editorial service and economic return', what is the alternative? OA can be regarded as one of the sector's major recent innovations; its growing success suggests that it is a viable alternative to subscription-only publishers. What is clear is that it has already opened a debate into publishing practices involving scientists, librarians, journalists and research funders. Indeed, there is an increasing demand from funding bodies for the results of research that they supported to be made publicly available, which is particularly important for projects funded by the taxpayer.

Broadly, OA sets out to challenge and overcome an issue that is all too familiar to university researchers, teachers, pupils and curious individuals—being unable to read past the abstract of a paper because full access requires an institutional subscription. This situation is described by many OA advocates as 'hitting the paywall'. If you have ever found yourself in this scenario, you may wish to make sure that your own articles will not face the same fate by publishing your results in an OA journal (also called the 'gold route' to OA). The Directory of Open Access Journals (DOAJ) currently lists more than 10,000 OA periodicals. Inclusion in this directory is based on a list of criteria established by the Open Access Scholarly Publishers Association (OASPA) (3) and the overall figure testifies to the success of this alternative publishing model. Authors are usually asked to pay an article processing charge (APC) upon acceptance and prior to publication; from then on, their paper is freely accessible and reusable to the extent dictated by the article license.

An alternative option to OA publishers, already popular among some disciplines, is to self-archive papers, dissertations and theses in one of over 3,000 repositories. This is sometimes referred to as the 'green route' to OA. It is commonly adopted in physics, astronomy, earth sciences and mathematics, but still appears to be poorly acknowledged in the medical and life sciences (4).

Given this picture of the publishing landscape, why does the debate around OA become outright incendiary at times? Some researchers are doubtful of the validity of gold OA due to high APCs; defenders of the OA model reply by recalling that there exist advantageous

membership schemes to cut off such charges, and that partial to full waivers are also available. Some scientists are also persuaded that only subscription periodicals ensure high standards of review and copy-editing for their papers. However, there is no fundamental reason why an OA journal should not be able to provide the same quality of services as its traditional counterparts; furthermore, article retractions can be observed in both publication categories.

Besides matters of personal taste and beliefs, constructive criticism may well benefit the publishing sector as a whole. This already holds true for the OA ecosystem where new ideas, journals and editorial platforms are implemented and reviewed relentlessly — from open peer reviews, which in OA journals such as eLife can be read alongside the main paper, to the possibility of publishing negative results (5) or partial findings followed by updates (6). The DOAJ, OASPA and similar initiatives aim to constantly monitor and improve the quality and reputation of OA publishers: their approach relies on a white list of trustworthy OA periodicals, in a strategy that opposes black lists of 'predatory publishers' such as those compiled by librarian Jeffrey Beall (7).

A closely related initiative to OA is that of open data. Interestingly, stored in the ~3000 web repositories one can find journal articles and theses, but hardly any datasets. The idea of making research code and data available is not always greeted with enthusiasm within the scientific community: the fear of losing the exclusive on a piece of research and the prospect of hours spent reformatting data can discourage even the most open-minded scientists. However, more and more journals require authors to include datasets and analysis algorithms with their papers; willingness to set up new collaborations or accelerate a project may work in favour of the open-data movement. The publishing giant Springer Nature made a significant step in this direction by launching the journal Scientific Data, which revolves primarily around datasets.

OA has generated a greater awareness that publishing should not and cannot remain unchanged over time. If it is to guarantee the dissemination of ideas, it must mirror the evolution of scientific enquiry over the centuries, taking into account the increasing importance of interdisciplinary collaboration, large-scale projects producing terabytes of data, and a growing role for computational approaches. It is possible to read a positive message even amid the arguments: be informed, be aware of your rights and duties as a scientist, and do not take for granted models and practices. After all, open-mindedness may lead to transparent, open research.

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Focus: OA in Oxford

1. Open Access Oxford homepage: http://openaccess.ox.ac.uk.
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Dr Gaia Donati recently completed her DPhil in the Department of Physics

Activating the potential of cell-penetrating peptides

by Joshua Bugajski

Cell-penetrating peptides (CPPs) are short sequences, usually fewer than 30 amino acids in length, which possess the ability to cross cell membranes unimpeded with very little toxicity. Many of the CPPs identified to date are able to transport cargoes up to 120 kDa in size along with them. This finding has spurred scientists to investigate the feasibility of using CPPs to deliver large bioactive molecules to intracellular targets, but they have fallen short with their lack of specificity. Now, however, research is focusing on specific activation of inhibited CPPs to overcome this hurdle.

It was over two decades ago that cell-penetrating peptides (CPPs) were discovered after a series of structural and functional studies exposed the shortest amino acid sequences required for cellular uptake of the Trans-Activator of Transcription (Tat) protein of HIV-1. The ability of hydrophilic molecules to cross the plasma membrane went against all understanding at the time. This discovery propelled further research and discovery of CPPs, many of which retain the ability to cross cell membranes even when covalently or non-covalently attached to cargoes of varying size (1). The potential for this process to revolutionise the delivery of large bioactive and imaging molecules was quickly realised.

The ability for peptides to cross membranes so freely means that any bound cargoes can be internalised into cells. It also means that bioactive molecules delivered by this route would be more likely to exhibit side effects at lower dosages or that they could miss the target cells for treatment completely. If the peptides were to be utilised for imaging in areas such as cancer therapy, these would be hurdles to overcome. To further complicate the issue, various research groups from around the world were struggling to reach a consensus on exactly how CPPs are internalised and transported within cells. Research reviews have pointed at a lack of consistency with experimental design across the different groups, leading to varying and sometimes contradictory results. To date, the strongest evidence points towards both endocytosis and direct translocation across the membrane occurring at the same time (1). However, disputes over this remain, and the effect of various attached cargoes on internalisation is still to be fully understood.

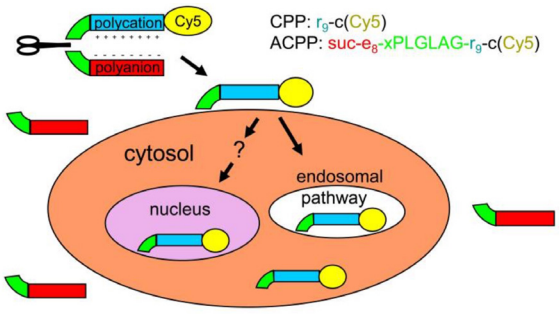

Figure 1. General schematic for ACPP activation. The intact molecule is blocked from cell uptake until cleavage of the linker (green), after which the polycation (blue) becomes unhindered and able to adhere to cells and to become internalised, along with its cargo (in this case a Cy5 fluorophore). Reproduced from (2) with permission of The Royal Society of Chemistry. Available from: http://dx.doi.org/10.1039/b904878b

Despite these obstacles, one notable effort to design an elegant and specific activatable cell penetrating peptide (ACPP) for internalisation into cancer cells came from the research group of Roger Tsien at the University of California. Around a decade ago they began studying the positively charged oligoarginine (r9) CPP, which could be successfully blocked from cell uptake via conjugation with an opposing chain of negatively charged glutamates. The key to this design was the peptide sequence used to link the arginine group to the glutamate sequence, which could be altered so that it could be specifically cleaved by chosen groups of enzymes, in this case by the metalloproteinases MMP2 and MMP9 (2). These appear in many areas of the body where extracellular matrix breakdown and modification is required, but are particularly highly expressed in aggressive cancers where they aid the spread of metastases. In theory the ACPP described above circulates the body in an inert state until cleaved in close proximity to the cancerous cells by the MMPs, allowing internalisation of the oligoarginine portion into the problematic cells. Indeed, studies of this ACPP using a mouse model of high MM2/9 expressing tumours showed promising results (3).

A probe based on this design produced by Avelas Biosciences has recently entered phase I trials for fluorescently guided tumour resection in breast cancer, and further research and optimisation may yield further potential uses in cancer imaging and treatment. There is much scope to change the pharmacokinetics of the ACPP through alterations in its length and to alter the linkers involved to improve their specificity, or to utilise further markers of cancer and other diseases. Watch this space.

Joshua Bugajski is an MRes student in the Vallis/Cornelissen Lab in the Department of Radiation Oncology.

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GM crops: a necessity for the 21st Century?

In the late 1960s, mass famine could have swept across Asia as food production struggled to match population growth. Thankfully, a Green Revolution managed to avert this disaster: new crop strains and farming methods enabled increased crop production and several countries, such as India, went from receiving food aid to becoming food exporters. One of the best known crop developments that contributed to the Green Revolution was a strain of rice called IR8. A fast-maturing, high yielding, semi-dwarf rice variety with strong stems supporting huge seed heads, it was made freely available to farmers by the International Rice Research Institute (IRRI).

by Dr Emily Flashman

IR8, and the subsequent more pest-resistant strain IR36, were developed by crossing multiple rice varieties with different properties. It was not until 2002 that the molecular rationale for the characteristics of IR8 was associated with altered gibberellin signalling. Gibberellins are plant hormones that regulate growth and development and key steps in their biosynthesis are catalysed by the gibberellin oxidases. IR8 carries mutations in the gibberellin oxidase isoform present in leaves and stems, but not in the isoform found in the reproductive organs (1). This results in stunted growth, hence stronger stems, while maintaining fertility.

Today we face another crisis of food security. The world population is due to peak at over 9 billion in the middle of this century and will need to be supported by a 70% increase in food production (2). This agricultural challenge is exacerbated by environmental pressures created by global climate change. Crop production will need to be intensified and strains that can withstand drought, flooding and other stresses will need to be developed.

In part, cross-breeding is helping once again. The sub1a gene, encoding a transcription factor enabling rice plants to survive prolonged submergence by entering into a quiescent metabolic state, is naturally present in a few rare species which grow in flood-prone regions. Excitingly, transferring sub1a into more commonly grown rice strains has resulted in a 45% increase in yield after exposure to flooding. This flood-resistant rice, now used widely by farmers in Asia and again distributed by the IRRI, is being hailed as part of a second Green Revolution (3). Genomics-assisted breeding programmes have also contributed to improved drought tolerance in US maize (though here the improved yields are a less impressive 5-15%) (4).

It is possible to biochemically understand how the cross-bred strains achieve their phenotype. However, now that we have sophisticated gene-editing techniques to hand, we are in a position to take a reverse approach: by specifically modifying proteins of interest in a highly targeted way, we can generate crops with altered phenotypes. Thus, genetically modified crops with better drought, flood or pest resistance could become commonplace.

Despite the potential advantages, public opinion in Europe towards genetically modified (GM) crops is fairly hostile. No GM crops are currently commercially grown in the UK, and since individual EU countries were granted the right to veto GM crop cultivation, several have ruled it out altogether. Nevertheless, GM crops have been successful in countries such as the USA, Brazil and Argentina, with examples including crops engineered with herbicide resistance such that weeds can be effectively targeted with reduced herbicide application. Another GM success story is Golden Rice, which has been modified to synthesise β-carotene, the Vitamin A precursor; this is currently being grown to address nutritional deficiency, predominantly in South-East Asia.

> “We are on the cusp of being able to create ‘designer’ crops suitable for our changing 21st century climate.”

So there is reason for optimism that GM crops could address our global food challenges. We are on the cusp of being able to create ‘designer’ crops suitable for our changing 21st century climate. To mobilise this possibility in Europe, we need to prove the benefits and disprove the fears by pressing ahead with the science.

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Dr Emily Flashman is a Royal Society Dorothy Hodgkin Fellow in the Department of Chemistry.

Mapping neural circuits *via* photoactivatable GFP tracing

by Oriol Pavón Arocas

Identifying how neurons interact with one another and are organised into neural networks has been a key challenge since the beginnings of neuroscience research. Notable efforts date as far back as Santiago Ramón y Cajal (1), whose minute drawings of stained subsets of structures within the nervous system inspired the neuron doctrine.

Progress in our understanding of neural circuits has gone hand in hand with the development of more sophisticated techniques to study and manipulate them. Advanced microscopy and labelling techniques are key in establishing the detailed anatomical structure of the nervous system, whereas the ability to target and photoactivate defined subsets of neurons allows us to search for functional connections. Tracing techniques have continued to evolve in parallel with the increasing depth of our understanding of the brain, and now range from tracking anterograde and retrograde processes using biocytin fills or lipophilic dyes to trans-synaptic circuit tracing with rabies virus (2). Continued discovery of new molecules and interesting strategies is inspiring novel applications and techniques that help us to unravel the intricate complexity of neural circuits. One example of such a molecule is a novel variant of the *Aequorea victoria* green fluorescent protein (GFP), named photoactivatable GFP (PA-GFP) (3). The process of photoactivation involves the rapid conversion of a molecule, otherwise quiescent, to its fluorescent state by direct illumination at a specific wavelength. Upon photoactivation, PA-GFP adopts an intensely fluorescent form and is free to diffuse through the intracellular space to achieve a uniformly distributed equilibrium. The fruit fly *Drosophila melanogaster* is an appealing animal model for studying neural circuits, as its nervous system contains a more manageable number of neurons than that of mammals, yet is still capable of generating multiple complex behaviours. The genome of the fly has been sequenced, and there is an extensive and comprehensive genetic toolbox available that allows the neurogenetic dissection of many behaviours (4). Researchers are now able to direct gene expression to multiple specific subsets of neurons by means of independent binary systems such as GAL4-UAS and LexA-LexAop and interfere with specific neural circuits to dissect how the brain works. Putting the pieces together, PA-GFP has been readily combined with the powerful genetic toolbox available in *Drosophila* and the three-dimensional, spatially resolved capabilities of two-photon laser scanning microscopy (5) to develop a combined genetic and optical neural tracing method to study neuronal processes of individual neurons in the fly brain (6). More recently, researchers have managed to generate enhanced versions of PA-GFP, namely C3PA-GFP and SPA-GFP, increasing the sensitivity and usability of these tools.

The main advantage of using this method in *Drosophila* is that we can interchangeably use two independent binary systems of gene expression to label different subsets of neurons simultaneously. In this context, if we wish to know whether two different populations of neurons overlap in regions where they could form synaptic connections, we can use the GAL4-UAS system to express PA-GFP under a specific promoter to restrict expression to one of the subsets (Figure 1, green), and then use the LexA-LexAop system to express mCherry in a second subset of neurons (Figure 1, magenta).

Oriol Pavón Arocas completed an MSc at the Ludwig-Maximilians-Universität, Munich, with a project in the Miesenböck lab, Department of Physiology, Anatomy and Genetics, Oxford.

Once we combine the expression of the respective fluorescent markers in the same fly, the neurons expressing mCherry will be our template to direct and restrain photoactivation to the region we wish to test for co-localisation. In order to do that, we use a two-photon microscope to visualise and define a 3D mask around the region of interest, setting the volume that will be illuminated to achieve photoactivation (Figure 1, red cube).

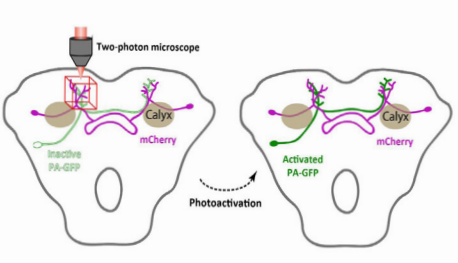

Figure 1. Photoactivatable GFP tracing. A *Drosophila* brain containing a subset of neurons expressing mCherry (magenta) and a different subset expressing PA-GFP (green). A 3D mask has been set comprising the area where we want to check connectivity between the two subsets of neurons (red cube). Photoactivation is achieved by means of a two-photon microscope that repeatedly scans through the 3D mask. If the two sets of neurons overlap in the space, photoactivated PA-GFP molecules diffuse and fill the cells with projections within the 3D mask, allowing visualisation of the anatomy of the labelled neurons (green).

To photoconvert PA-GFP from its inactive to its fluorescent form, the selected volume is repeatedly exposed to brief pulses of 710 nm light, the wavelength that most efficiently converts the fluorophore. The number of repetitions of the photoactivation scan will vary depending on the target volume, the expression levels of PA-GFP and the depth of the photoactivation target. Furthermore, each repetition should be spaced apart by 15 to 30 seconds to allow diffusion of the photoactivated PA-GFP and minimise photodamage of the tissue. After we have completed the photoactivation scan and allowed a few minutes for the photoactivated PA-GFP to diffuse homogenously through the cells, the sample can be fixed and stained with specific antibodies to further characterise the labelled population of neurons and visualise our results with a confocal microscope. Although PA-GFP tracing currently lacks the ability to infer information about functional connections between structures, it is a powerful method to study anatomical overlap between different subsets of neurons and therefore establish potential functional connections. It can be used as an initial screen to narrow down potential candidates for a specific physiological or behavioural effect on our area of interest, or to infer anatomical connectivity between both upstream and downstream structures. But above all, PA-GFP tracing is an asset that opens new roads to navigate the labyrinthine complexity of neural circuits.

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Classic Kit: The Mass Spectrometer

by Evangelia Tzika

Our new section, "Classic Kit", is an introduction to classic laboratory equipment and its development over the years. In this issue, we explore Mass Spectrometers (MS), the instruments that heralded a new era in experimental biology and chemistry. But this isn't a new technique: in fact a century has passed since the concept of characterising particles according to their mass was first developed. Originally, MS was only used by physicists, and then later, chemists. Only in the last fifty years has MS been used for biological experiments, whilst it is only in the last 20 years that the technique has been utilised by biochemists to develop high-throughput protein analysis methods.

Experimentation with MS has produced a phenomenal number of high impact discoveries and Nobel Prizes throughout the 19th and 20th centuries. In 1886, E. Goldstein first observed beams of positively charged ions, or 'canal rays' in gas discharges (1). Later, in 1899, W. Wien constructed a device with parallel electric and magnetic fields to separate these canal rays according to their mass-to-charge ratio (m/Q). In 1912, physicist J. J. Thomson constructed the first MS instrument to measure the mass-to-charge ratios of ions. The fundamental idea behind his instrument was that the ionization of a sample with electrons produces charged fragments, which are then accelerated in a magnetic field and separated according to their mass-to-charge ratio. Thomson noticed that ions of the same mass-to-charge ratio were deflected to the same degree (2). This work led him to discover the electron, for which he was awarded the Nobel Prize in 1906. In 1919, his protégé, F. W. Aston, constructed the first velocity-focusing mass spectrograph. This applies the same amount of energy to all the molecules being studied, but their differing masses, and hence momentum, result in a different angle of detection. This idea improved the resolution of the first MS instruments and Aston was later awarded the Nobel Prize in Chemistry for this astonishing advancement (3).

Now that the seed of scientific motivation had been sewn, MS continued to develop as a discipline in the years that followed. Its uses though, weren't always scientific. Controversially, MS instruments were used for producing the first atomic bomb during World War II. The contribution of MS to nuclear energy began with A. Nier, who used the Calutron mass spectrometer for the separation of uranium and first characterised the plutonium particle. Nier also pioneered 13C enrichment for tracer studies (i.e. to observe the transport of metabolites in plants) (3).

The transition of MS from bespoke instruments to commercialised devices has largely been pioneered by biotechnology firms, but was initially facilitated by F. Field who made MS instruments simpler and easier to use. Later in the 20th century, scientists began tailoring specific instruments according to their needs. Variations of MS instruments that have been developed to date include magnetic deflection instruments, time of flight (TOF)-MS, gas chromatography (GC)-MS, quadrupole instruments, and the Orbitrap MS. The Orbitrap MS was developed in 1999 by A. Makarov, and traps ions in an orbital motion. The current is detected and transformed into a MS spectrum (Figure 1c). Multiple ionisation techniques have also been introduced, with Electrospray Ionization (ESI) and Matrix-Assisted Laser Desorption Ionization (MALDI) being most commonly used for biochemical experiments.

The future of the interdisciplinary field of MS looks very promising, particularly for the molecular characterisation of proteins, DNA and other biological molecules. New applications for MS will no doubt be discovered as a growing international community of scientists collaborate to employ MS to answer increasingly challenging biochemical questions.

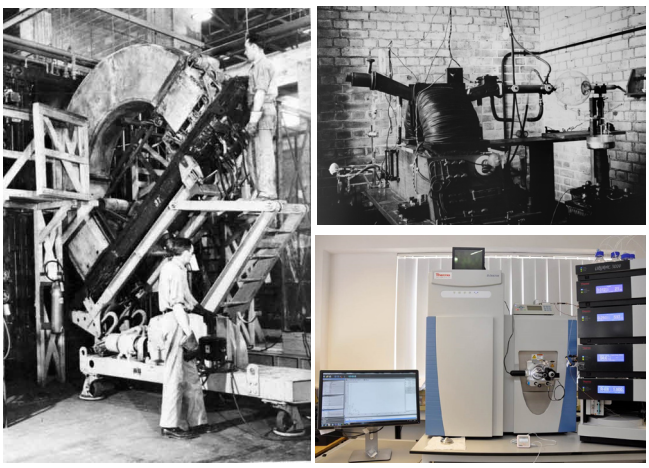

Figure 1. Clockwise from left :Alpha calutron tank, 1930 (4); Aston's mass spectrograph, 1919 (5); A modern day QExactive instrument, Thermo Scientific (2011).

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Evangelia Tzika worked under Prof Nicole Zitzmann in the Department of Biochemistry, and is now at the Max Planck Institute of Psychiatry in Munich.

Career Insights: Business Intelligence Analyst

by Dr Fenix Leung, GlobalData

Do you know how long a drug takes to progress from the lab bench to the market? It often takes more than 10 years for a drug from preclinical studies to become available to patients. For example, the first immune checkpoint modulator, ipilimumab, was initially reported for its in vivo antitumour efficacy in 1996 but only reached the market 15 years later. Many more potential candidates discovered in the lab are never approved. This left me wondering how much I could really contribute to a cancer patient's life if I worked as a research scientist. Despite enjoying the intellectual challenge an academic career could offer, I decided to look for alternative careers in which to make a real impact on pharmaceutical development.

Prior to starting at GlobalData, I undertook in an Internal Research Analyst internship at Oxbridge Roundtable Solutions, where I was involved in market research and business intelligence. My time at ORS provided experience, skills and some essential knowledge required for working in the pharmaceutical business; ultimately, it helped me to secure a position as an Analyst in the Oncology and Hematology team at GlobalData.

The day-to-day responsibility of an analyst here is straightforward: gathering everything required to write a market report on a given disease area. My first report was on the melanoma market. This is currently the most exciting area in oncology due to the launches of the immune checkpoint modulators nivolumab and pembrolizumab. To begin with, I conducted secondary research from documents including clinical literature, clinical trial registries, company announcements, investor presentations and financial statements. Once I had a good understanding of the market dynamics, such as marketed and pipeline products and their competition, primary research was performed from the mid-stage of the authoring process. I interviewed numerous top-ranking physicians and clinical trial investigators ('Key Opinion Leaders') to gain insights into disease management and the future outlook of new therapies. Additionally, a questionnaire surveying high-prescribing physicians was designed, in order to understand their patient referral and drug prescription patterns.

After obtaining all the relevant data, I performed intensive analysis on the competitive landscape, clinical and environmental unmet needs, and pipeline development, which led to a 10-year market forecast based on the knowledge I gained during my research. Finally, all the findings and analyses were written in a 300-page market report. I have written three such reports since I started in July 2014.

I enjoy my job at GlobalData, as I acquire not only expertise in the clinical management of various cancers, but also in other key areas in the pharmaceutical world such as regulatory, pricing and reimbursement landscapes, and drug development strategies. Unlike some larger-scale companies, which split the individual tasks to different teams, I carry out every single task to finish a report, which substantially enhances my skill set. The real job satisfaction comes when I see the daily progress of my work and during discussion with the clients who purchased my reports, where I have real influence in their strategic decisions.

Working in a growing company like GlobalData provides great opportunities for career progression. As my team doubled in size since I joined, I began to mentor junior analysts. After a year and three months I was promoted to Senior Analyst and given new roles, such as managing a project involving a team of analysts and reviewing work from junior- and peer-level analysts. Furthermore, as GlobalData's consulting business matures, I have had the chance to explore the customised side of the business. After assisting the consulting team to win a project worth US$200,000 from a top-three oncology company, I was recommended to take on external management of a six-month project for a Chicago-based big pharmaceutical client.

Overall, I believe GlobalData provides an excellent training ground for young scientists who want to develop a career in market research and business intelligence.

Dr Fenix Leung is a Senior Analyst in the Oncology and Hematology team at GlobalData.

Cancer-targeting mechanisms of nanoparticles

by
Dr Jyothi
U. Menon

One of the chief limitations of conventional drug administration methods is the possibility of causing systemic toxicity in the patient due to non-specificity of the drug. This also implies that less drug reaches the desired disease site, leading to reduced therapeutic efficacy. Nanoparticles (NPs) are attracting increasing interest as an alternative to current drug administration techniques, especially in cancer research, as they can improve site-specific drug delivery while potentially limiting systemic side effects. This article shall briefly summarize the different NP targeting strategies being studied for cancer treatment.

Passive Targeting

Tumour vasculature is characterized by its 'leakiness': tumour vessels have gaps about 100 - 500 nm wide that allow the extravasation of small molecules, fluids, and NPs into the tumour interstitial space (1). This preferential accumulation of small molecules in the tumour region via the leaky tumour blood vessels was discovered more than 30 years ago and is known as the Enhanced Permeation and Retention (EPR) effect (2). Passive targeting exploits this property to allow accumulation of NPs at the tumour region following administration. The poor lymphatic drainage observed in tumour regions also aids in better retention of the NPs at the site following accumulation. Passive targeting does not require NPs to undergo any surface modification, although coating them with polyethylene glycol (PEG) in a process known as PEGylation has been shown to significantly improve blood half-life of the particles. PEGylation of NP surfaces has been shown to prevent rapid clearance of the particles from the body by protecting them from phagocytosis and complement activation, thus improving their circulation time (3). The longer the time particles spend in circulation, the greater the chances of them reaching the tumour environment instead of being eliminated rapidly from the body. However, this method of NP targeting has a few limitations. Tumour vascularization and angiogenesis can vary between tumours and persons, and this can affect nanoparticle/drug accumulation at the targeted site. In addition, there can be suboptimal interaction between cell surfaces and the NPs resulting in reduced treatment efficacy (4). To overcome these limitations, NPs can be surface-modified for active targeting, as described below.

Active Targeting

Some cancer cells in the body tend to overexpress certain receptors that are either not expressed or are expressed in relatively low quantities in healthy cells. Active targeting involves surface modification of NPs with targeting moieties/ligands such as antibodies, aptamers, peptides, nucleic acids, and other small molecules that tend to show high binding affinity towards these overexpressed receptors on the cancer cells (Figure 1). The bioconjugation of the targeting moieties to the surface of the NPs can be carried out by techniques such as carbodiimide chemistry, thiol-maleimide chemistry, or biotin-streptavidin chemistry. The choice of the specific method depends on the availability of functional groups such as COOH, NH_2, or SH groups on both the NP surface as well as on the ligand of interest (5). Commonly used targeting ligands for active NP targeting include epidermal growth factor (EGF) to target EGF receptors, folic acid to target folate receptors, and transferrin to target transferrin receptors (6). In some cases, the endothelial cells in the tumour vasculature have also been targeted using NPs, as these cells are known to express molecules such as $\alpha_v\beta_3$ integrins, $\alpha_v\beta_5$ integrins, and CD13, which are generally not overexpressed in healthy blood vessels. This method of targeting aids in anti-angiogenic therapy (7). Recently, dual-targeting NPs have also been reported where the particles can be coated with multiple ligands specific for tumour blood vessels as well as for the tumour cells themselves. By this targeting method the NPs can potentially damage the tumour blood vessels, thereby cutting off nutrient and oxygen delivery to the tumour, and also damage the tumour cells themselves by releasing the encapsulated chemotherapeutic agent (8). Although active targeting has the potential to specifically target and treat cancer cells, it is important to note that this method still depends on passive targeting and the EPR effect to facilitate accumulation of the NPs at the tumour site. However, following accumulation these particles would show high affinity towards the targeted cancer cells, enhancing their treatment efficacy.

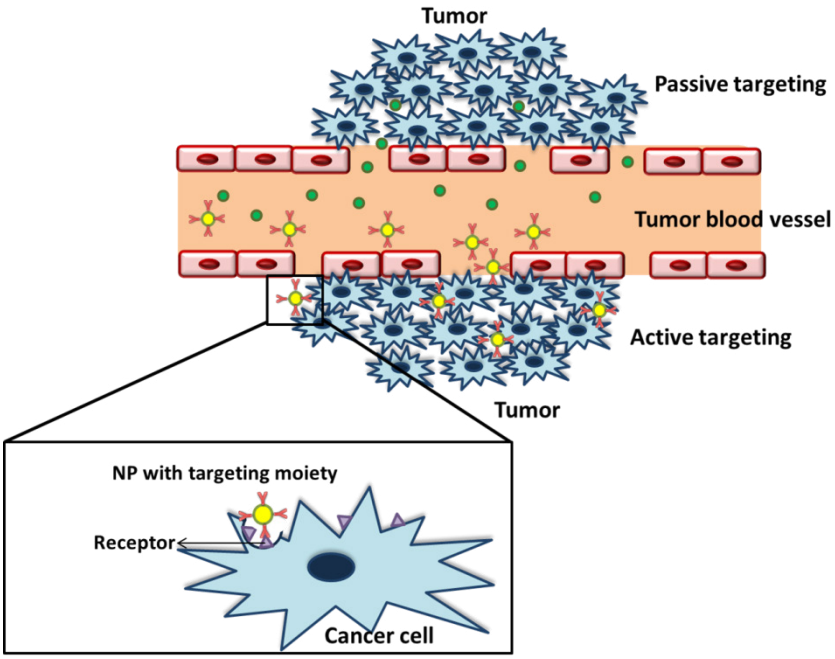

Figure 1.
Representation of passive and active targeting of tumours by NPs for cancer treatment

Targeting by external stimuli

NPs containing superparamagnetic iron oxide (SPIO) have been used for magnetic targeting where an external magnetic field is applied following NP administration to recruit magnetically susceptible NPs to the site of interest. Although this method is promising, magnetic targeting is only effective if the targeted tissue is close to the surface of the body. For example, magnetic targeting has been employed by some groups for the treatment of prostate cancer using iron oxide-containing NPs (9). However, decreasing magnetic strength with increasing distance of the particles from the source of the magnetic field diminishes the effectiveness of this method in targeting tumours deep within the body (10).

Future Outlook

The application of nanotechnology for targeted therapy has great potential in improving health care by providing more effective therapy while potentially limiting toxicity and patient discomfort. This area of research is still in its nascent stages, and thorough investigation into the in vivo NP biodistribution and reproducibility of studies conducted is warranted in order to provide effective treatment to patients. It is encouraging to note that a recent phase II clinical trial employing BIND-014, a docetaxel-containing NP, which is the first nanoformulation to be tested clinically for targeted and controlled cancer therapy, has shown great promise. Clinical data indicates that BIND-014 showed better anti-tumour activity than conventional docetaxel treatment in patients with advanced non-small cell lung cancer (NSCLC) (11). Through the collection of more clinical data, the potential of NPs for targeted delivery will be better understood.

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Dr Jyothi Menon is a Post-Doctoral Researcher in the Vallis lab in the Department of Oncolgy.

The endolysosomal pathway in Parkinson's disease

by Dr Rebecca Perrett

Parkinson's disease (PD) is the second most common late-onset neurodegenerative disease, and is clinically characterized by muscle rigidity, tremor and bradykinesia (slow movement). This is mainly caused by the progressive loss of dopaminergic neurons within the substantia nigra brain region, although other brain areas are affected (for example the basal ganglia and cerebral cortex), leading to cognitive and behavioural impairments.

It is clear from the pathology that PD involves impaired protein degradation and/or excessive protein accumulation, as affected brains are characterized by the presence of Lewy bodies (LBs), intraneuronal inclusions comprised primarily of a small protein called α-synuclein (1). α-synuclein is abundant in the normal human brain, although point mutations and gene amplifications lead to PD (2). α-synuclein has 'prion-like' properties; it can be transmitted to neighbouring cells, initiating a PD-like pathological process (3). Excessive or defective α-synuclein, is often ubiquitinated within LBs to promote its degradation. However, instead of being degraded, it aggregates into fibrils, which are particularly toxic to dopaminergic neurons.

α-synuclein's function may provide some clues regarding the disease aetiology. It regulates membrane fusion events in the endolysosomal pathway, a multi-vesicular pathway involved in protein recycling and degradation (Figure 1). Within this pathway, protein cargo is transported to the early endosome where it can either be recycled back to the plasma membrane or trafficked to the lysosome for degradation. In the latter route, the early endosome matures to become a late endosome, which then fuses with a lysosome, a low pH organelle containing degradative enzymes. In PD, overexpressed or mutated α-synuclein disrupts the endolysosomal pathway by interfering with membrane fusion events. In addition, α-synuclein aggregates at low pH, which may explain its aggregation in late endosomes and lysosomes, causing cellular dysfunction (4).

A number of PD associated genetic mutations or polymorphisms also disrupt protein trafficking and degradation via the endolysosomal

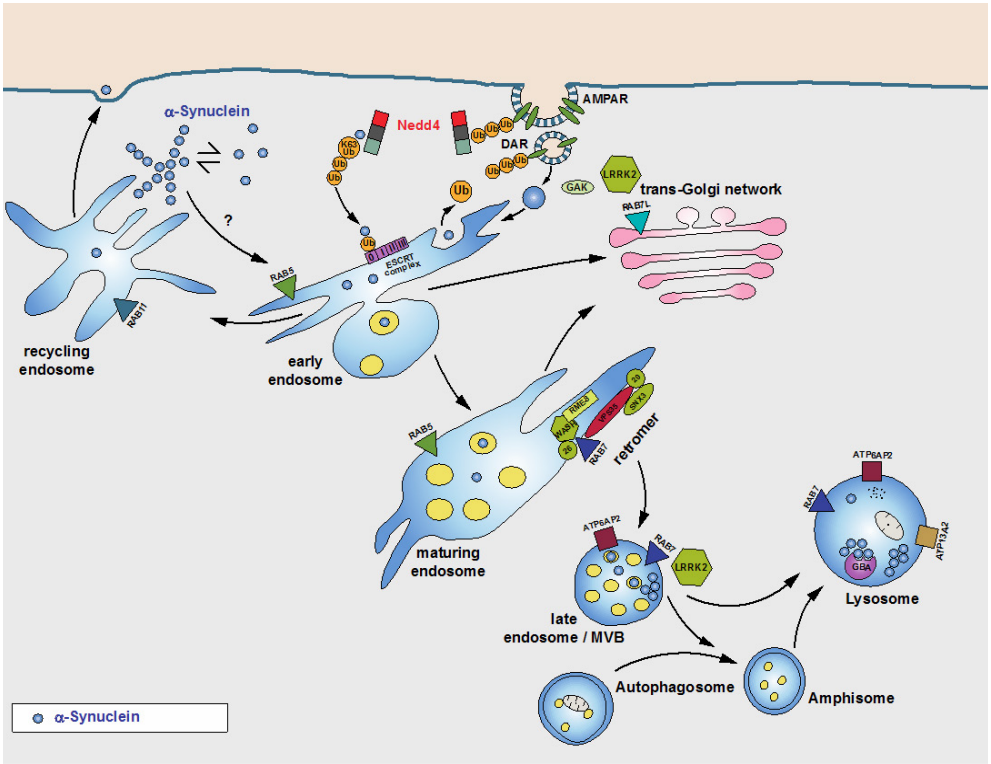

Figure 1. The endolysosomal pathway and PD causing mutations. Protein cargo is transported to the early endosome, where it is either recycled back to the plasma membrane or targeted to the lysosome for degradation. A number of PD causing mutations (LRRK2, VPS35, GBA, ATP13A2, ATP6AP2 and DNAJC13/RME-8) cluster within this pathway. In addition, overexpressed or mutated α-synuclein aggregates at multiple points and causes endolysosomal dysfunction. (10).

pathway. These are highlighted in Figure 1 and include mutations in LRRK2, VPS35, GBA, ATP13A2, ATP6AP2 and DNAJC13/RME-8 proteins. These mutations disrupt various stages of the endolysosomal pathway, the end result being α-synuclein accumulation in late endosomes/lysosomes and neurotoxicity (5).

Mutations in the LRRK2 gene are the most common cause of autosomal dominant PD, as well as 2% of apparently sporadic cases. Mutant LRRK2 interferes with multiple points of the endolysosomal pathway, including endocytosis at the plasma membrane, endosomal sorting and late endosome maturation and fusion (6) It is likely that both α-synuclein and LRRK2 exert synergistic affects to exacerbate toxicity in dopaminergic neurons. Mutations in LRRK2 can also have implications further downstream, directly affecting lysosomal function. Heterozygous mutations in the lysosomal enzyme glucocerebrosidase (GBA) predispose individuals to PD, because of decreased lysosomal α-synuclein degradation, and mutations in the endosomal and lysosomal cation pump ATP13A2 and the lysosomal proton pump ATP6AP2 also trigger PD (5). Improving lysosomal function, via pharmacological stabilisation of GBA partially restored motor function in mice (7, 8), and therefore may be used as a therapeutic target for PD. In fact, Lysosomal Therapeutics are developing a GBA activator for PD, with plans for a clinical trial next year.

A final question is why are dopaminergic neurons selectively vulnerable in PD? A recent study by Laguna et al. (9) might provide the answer. The authors demonstrated that expression of the transcription factor Lmx1b, which is involved in the early specification of dopaminergic neurons, may prevent dopaminergic nerve degeneration. Critical expression of this transcription factor is lost in PD, directly causing lysosomal dysfunction and dopaminergic nerve degeneration.

In conclusion, both sporadic and familial PD exhibit a shared pathophysiology: endolysosomal dysfunction leading to α-synuclein aggregation and neuronal toxicity, highlighting the importance of endolysosomal transport to dopaminergic neuronal integrity. However, defects in mitochondrial function, lysosome-mediated autophagy and synaptic exo- and endocytosis are all implicated in PD, and some mutations, for example in LRRK2, cause dysfunction in multiple pathways. Until we fully understand the function of mutated gene products in dopaminergic neurons, pharmaceutical intervention at this level is difficult. Most therapies currently in the pipeline are aimed at reducing α-synuclein aggregation into LBs, as despite multiple genetic causes, this is the common pathological endpoint. Roche and Prothena are conducting a clinical trial treating PD patients with a monoclonal antibody (PRX002) against α-synuclein, successful in various animal models, with results to be published this year. As the power of genetic analysis techniques improves, further genes will be implicated in PD, and their physiological functions elucidated. Hopefully, this will lead to novel (potentially patient-specific) therapies targeting the endolysosomal pathway further upstream than α-synuclein aggregation.

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Dr Rebecca Perrett is a Post Doctoral Research Fellow in the Molecular Neurodegeneration Research Group, Nuffield department of Cinical Neurosciences.

Oncoviruses: the ins and outs

by Vivekka Nagendran

It is a common misconception that one can 'catch cancer. Cancer is not contagious, and tumours cannot spread from one individual to the next, however there are certain infections that can increase one's chance of developing certain cancers. In 2006, it was estimated that around 17% of all cancers are caused by viral infections (1); these cancer-causing viruses are known as oncoviruses.

In 1908, Ellerman and Bang demonstrated that the avian sarcoma leukosis virus can infect chickens, leading to the development of leukaemia (2). This was believed to be the first of many experiments revealing the consequences of infection by oncoviruses. In 1964, Epstein, Achong, and Barr identified the first human oncovirus from a sample of Burkitt lymphoma cells (Epstein-Barr virus), where they used electron microscopy and visualised 'several mature virus particles' in the cytoplasm of these cells (3).

Oncoviruses can contain RNA or DNA, and can cause a wide range of cancers. Examples of DNA viruses include Epstein-Barr virus (EBV), hepatitis B virus (HBV) and the human papilloma viruses (HPVs), whilst RNA viruses include hepatitis C virus (HCV) and the human T-cell leukaemia virus type 1 (HTLV-1).

How do oncoviruses increase the risk of getting cancer? There are various ways in which this is achieved, as viruses have evolved many mechanisms to prolong their life in their host. The aim of these mechanisms is to simply promote viral growth inside the host organism; malignant tumour progression is thought to be an occasional, accidental consequence and is of no advantage to the virus or host.

Some oncoviruses, such as HPV, increase the risk of cancer development by interfering with the p53 response, which is involved in regulating apoptosis. An increase in p53 expression acts to increase expression of the p21 gene, which leads to apoptosis of the cell. The p21 protein works by inhibiting cyclin-dependent kinase (CDK) activation. CDK activation usually causes inactivation of the retinoblastoma protein (a tumour suppressor), thereby allowing the cell to enter the cell cycle. Since p21 inhibits the CDK response, its overall effect is to prevent cells from entering the cell cycle. HPV is a papilloma virus, of which many types cause warts (benign tumours of the skin), although 6 serotypes are known to cause cervical carcinoma, which progresses over many years. HPV produces viral oncoproteins called E5, E6 and E7, which alter p53 signalling in different ways and drive unrestrained cell proliferation. E5 allows more survival factor receptors to exist at the cell surface, whilst the E6 and E7 proteins target p53 proteins for proteolysis. E7 inactivates the retinoblastoma protein, thereby relieving its inhibitory effect on cell cycle entry and allowing proliferation.

Another virus that produces these oncoproteins is the Epstein-Barr virus (human herpes virus 4), which is associated with four types of cancer: Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma and non-Hodgkin lymphoma. EBV is well known as the cause of infectious mononucleosis, otherwise referred to as glandular fever, and infection with EBV can occur via oral transfer of saliva and genital secretions. As with HPV, EBV produces proteins that are anti-apoptotic and pro-proliferative – in Burkitt's lymphoma, myc gene overexpression acts to drive proliferation. The myc gene is a proto-oncogene involved in cell cycle progression and cell proliferation, and its dysregulation can drive cancer development. Oncoviruses have helped us identify oncogenes, as oncogenes of retroviruses are thought to be derived from host proto-oncogene mRNAs. Studies have therefore used these viruses to identify a set of genes that drive tumourigenesis when they are overexpressed, and these genes can be used as a target for chemotherapy in humans.

HBV and HCV both increase the likelihood of malignant tumour progression via chronic

inflammation. Although the HBV and HCV viruses have no oncogenes, hepatocellular carcinoma is thought to be attributable to persistent cellular regeneration in the attempt to replace hepatocytes killed by the infection – this persistent regeneration increases the chance of a cancerous mutation occurring in these cells. HIV infection also has an indirect link to cancer progression via the immune system, although this is due to immunodeficiency as opposed to chronic inflammation. The immune system is thought to target and kill many tumourigenic cells (first suggested by Paul Erlich in the early 1900s), and such is the basis of cancer immunotherapy. Severe immunodeficiency in patients suffering from AIDS means a lack of immune response against cancer stem cells, along with an increased susceptibility to oncoviruses and other 'onco-pathogens', leading to an increased risk of cancer development in these individuals.

The significance of oncoviruses was often overlooked due to most preventative measures targeting risk factors such as smoking, sun exposure and diet, and little focus on infection. The link between infection and cancer has been difficult to demonstrate for multiple reasons. Firstly, cancer takes time to develop. It may only arise or be identified years after initial infection and so the link between the virus and malignant tumour formation is not the most obvious.

Additionally, these infections generally do not cause cancer in patients. Only the minority of cases progress to cancer development, most likely due to the interplay of genetics and lifestyle factors. However, oncoviruses are still very important as they represent a significant proportion of the cancer burden world-wide. Studies have shown that if these pathogens were removed, there would be 23.6% fewer cases of cancer in developing countries (1), where the burden of infection is the leading cause of mortality. Therefore, increased education about the prevention of spread of oncoviruses is a central way to tackle the problem, along with appropriate vaccine development.

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Virus	Associated Cancer
Human Papilloma Virus	Cancer of the cervix, anus, vulva, vagina, penis, oropharynx
Kaposi's Sarcoma Virus	Kaposi's sarcoma, multicentri Castleman's disease
Hepatitis B Virus	Hepatocellular carcinoma
Hepatitis C Virus	Hepatocellular carcinoma
Human T-cell Leukaemia Virus Type I	Adult T-cell leukaemia/lymphoma (ATLL)

Table 1. Known oncoviruses and their associated cancers

Vivekka Nagendran is a third year medical student who has recently undertaken a project with Prof Peter Cook, Dunn School of Pathology

Finding hope for mental illness: the effort of the 'It Gets Brighter' campaign

by Emma Lawrance

It is scary to feel something is wrong with us. Having this confirmed by a diagnosis, whether it be diabetes or depression, can simultaneously provide relief and further terror. Relief at knowing what is happening, coupled with the hope that treatment can lead to recovery. Terror for the possible life-changing consequences of such diagnosis. But imagine if on top of trying to deal with the day-to-day reality of living with the condition, you had to worry about people finding out; to worry about telling people in the fear that they might equate your illness with a character weakness, or somehow think it's your fault; to worry that your job prospects, even once you've recovered, could be diminished by the 'tarnish' of a mental illness.

Of course stigma can surround many different illnesses. There was a time when cancer was thought to be contagious, and was stigmatised to the point that patients were unemployable. The resulting shame vastly increased the suffering of cancer patients, and the stigma meant a dearth of research money to develop cures. Such stigma is thankfully now greatly reduced due to much advocacy, and the research to understand cancer physiology and find new cures is in continuous progress.

Now is the era for such a change in our understanding and attitudes towards mental illnesses. Currently, mental health stigma is one of the most ubiquitous barriers to care and has been demonstrated to exacerbate the experience of mental illness. The physiology of mental illnesses is generally poorly understood. Largely diagnosed from a 'symptom clustering' approach, they are treated using methods that range wildly in their effectiveness across individuals for reasons we don't understand. Such a diagnostic approach does not address the key pathological changes that may differ between individuals displaying the same vague symptoms.

Computational psychiatry is working to change that. Its approach starts with the awareness that mental health varies across spectra. By quantifying differences in behaviour and brain function associated with the spectrum of mental illnesses we can find better diagnostic biomarkers and design targeted treatment approaches.

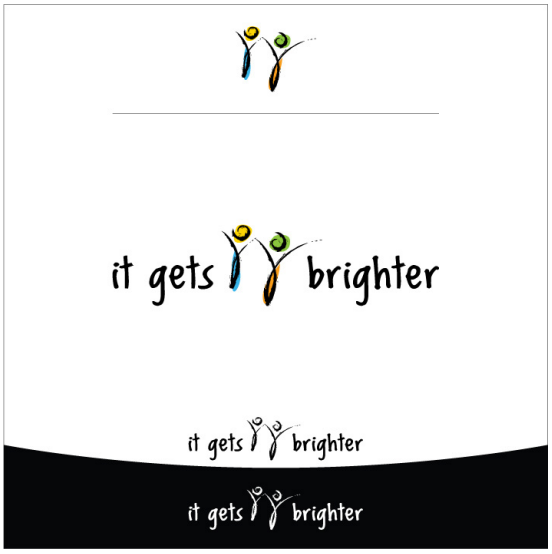
This approach is used by the Bishop Lab (University of Oxford, FMRIB) to examine differences in how people process information from the environment and make decisions, and how these relate to their tendency to experience anxious and depressive symptoms. Examining the differences in such behaviours and their neural underpinnings along the spectrum of 'healthy volunteers,' and in clinical populations, is key to understanding the basis for mental disorders. Previous work has discovered that anxious people have diminished ability to inhibit distractions under low cognitive loads [1]. If you are prone to anxiety, that person walking across your otherwise quiet office is much more likely to pull your focus away from your less-than-fascinating Excel tables. This has been shown to be due to weakened connections in prefrontal cortex, an area of the brain involved in attention and decision making. Remarkably, training to strengthen these pathways and improve focus also reduces anxiety symptoms, in the absence of any traditional symptom-targeted treatment [2].

Our latest line of inquiry is into the changes in decision making associated with anxiety. There is much anecdotal evidence to suggest that anxious people have problems with decision making. But what underlies this? Is this true of all anxious people or only a subset? How could we treat this? In a recently published study, Browning *et al* [3] demonstrated that whilst less anxious people change the significance they attribute to events based upon how much their environment is changing, anxious people don't seem to. This seems to be related to suboptimal functioning of the norepinephrine system, providing more targets for future studies and potential treatments.

Research in this field is providing much needed deeper understanding of the biological basis of

mental illness, which will enable new diagnostic and treatment approaches over the coming decades. With greater understanding, there is the hope also for significant stigma reduction, as we narrow the conceptual divide between physical and mental illnesses.

And hope is something greatly needed for sufferers of mental illness. A recent report from the University of California, Berkeley found that a whopping 47% of its students suffer from depression [4], while a 2005 survey found 10% had contemplated suicide [5]. Many mental health problems surface for the first time when students are in high school or college and it has been estimated that around 53% of academics in UK have experienced mental health issues [6]. Yet despite the ubiquity of these struggles, many people feel unable to speak up for fear of the repercussions. Perhaps this is due to our inability to separate mental health struggles from character, or our (perhaps understandable) inability to conceptualise a pathological imbalance in neurotransmitters, as we would a pathological imbalance in liver enzymes.



This is where the It Gets Brighter (IGB) campaign comes in. Our aim is to bring messages of hope and support to young people struggling with mental health issues. In 2013, I joined a small group from Mind Your Head, an Oxford University based mental health awareness association, to create a video testimonial campaign by and for students. We aim to change conversations around mental illness and show young people they are not alone, and indeed, that a mental illness doesn't have to define them. There are many, many people who have recovered, or learned to manage the symptoms such that it has become much brighter for them. As scientists, we want to make sure we use evidence based approaches, and videos like ours are increasingly recognized as a clinical tool. Additionally, with the changes brought about by social media, video-based campaigns have the potential to be more scalable and cost-effective when compared to social contact interventions.
Our website launched in January 2015, and we now have over 70 videos from people around the world, including several Oxford students, the Vice Chancellor of Oxford, comedian Ruby Wax and the Archbishop of Canterbury. Stephen Fry tweeted his support when we launched, and we have developed partnerships with mental health organisations around the world. We currently work in the UK, Canada, Australia, the USA and Lebanon, with IGB Deutschland to launch soon. Recently, It Gets Brighter China was launched and is spreading with great success.

The future is looking very bright indeed for our campaigns. Our ultimate goal is to create communities where people are equipped with knowledge and tools to look after their own and others' mental wellbeing, where they are empowered to seek support and treatment, and where people can be open about their struggles without fear of repercussions. Communities where we understand that mental health struggles can strike anyone, regardless of their 'character'. Recovery is possible, even when there isn't yet a cure. For that, we need to look back to the scientists.

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Emma Lawrance is studying for a DPhil in Cllinical Neuroscience in the Bishop Lab, FMRIB.

How does bariatric surgery cure type 2 diabetes?

by
Dr Laura McCulloch

We are currently experiencing a worldwide obesity pandemic. In the UK alone, 62% of adults are currently either overweight (BMI 25-30 kg/m^2) or obese (> 30 kg/m^2) (1). Alarmingly, 55% of adults and a staggering 25% of children are predicted to be obese by the year 2050 (1). The economic burden of dealing with this pandemic is already breaking the backbone of our healthcare system. Obesity does not exist in isolation: it is strongly associated with a number of complications including heart disease, certain forms of cancer and type 2 diabetes (T2D). Annually, the NHS spends £6bn on medical costs associated with obesity and a further £10bn treating diabetes. Cumulatively this equates to over 10% of the total health budget. So how do we stem the tide?

Weight loss surgery, also known as bariatric surgery, is currently the only successful treatment for obesity, and leads to significant weight loss in comparison to dieting or therapeutic interventions alone. Patients are given a choice as to which form of surgical treatment they receive, one of which is a gastric band. This involves placing an inflatable ring around the top of the stomach to restrict the amount of food which can be ingested. Another option is a gastric bypass, in which the digestive system is surgically resected such that a large part of the stomach is bypassed. In the UK, the most common procedure is a form of gastric bypass called Roux-en-Y gastric bypass (RYGB, Figure 1). In this procedure, a small pouch is created at the top of the stomach, then joined directly to the small intestine so that the majority of the stomach and upper intestines are avoided. The duodenum is reattached to the jejunum at a more distal location to allow the efflux of digestive enzymes and stomach acid. This procedure restricts the amount of food that can physically be consumed, as well as limiting the ability of the gut to absorb nutrients.

Although originally developed as a weight loss therapy, it has quickly become evident that RYGB can also exert significant effects on glycaemia. In 80-90% of T2D subjects undergoing RYGB, diabetes enters into full and long-lasting remission (2). Moreover, normalisation of blood glucose occurs within days of surgery and prior to any substantial weight loss, thus, weight loss and nutrient intake cannot be the causal factors responsible for changes in glycaemia (3). This rapid normalisation of the major symptoms of diabetes is astronomical, given that these individuals will have seen a progressive decline in their ability to control their blood glucose over the years. These observations have been so astounding that the National Institute for Health and Care Excellence (NICE) has now amended their guidelines to recommend that bariatric surgery be considered as a treatment strategy for obese individuals newly diagnosed with T2D. The hope is that treating the disease in its infancy will reduce the likelihood of developing diabetes-related complications, such as blindness, cardiovascular disease and amputations, thus reducing the economic burden on the NHS.

So is this it? Have we unwittingly stumbled upon the cure for T2D? Understanding what is happening in these early days post-surgery may give us a clue as to how it is possible that glycaemia, poorly controlled in these subjects for years, has seemingly been restored overnight. Moreover, how can we untangle the molecular mechanisms underlying this phenomenon?

For years, researchers have speculated that the anti-diabetic effects of bariatric surgery are mediated by altered secretion of hormones from the gut, and in particular glucagon-like peptide-1 (GLP-1). GLP-1 is secreted by intestinal L-cells in response to nutrient intake and has two major forms of action: on the brain to signal satiety, and on the pancreatic beta-cells to increase insulin secretion. Numerous studies have reported that systemic GLP-1 levels are elevated following bariatric surgery (4), and many have been quick to assume this correlative relationship to mean causality. However, recent studies using a GLP-1 receptor knockout animal have eloquently shown that the metabolic benefits of bariatric surgery persist in animals lacking any endogenous GLP-1 signalling (5). This suggests that GLP-1 is not the magic bullet

it was once thought to be and we need to cast our exploratory net further.

As pancreatic islet biologists, we can't help but think that the current studies into the effects of bariatric surgery have only addressed half the question. In humans, T2D is characterised by two major physiological perturbations: insulin resistance in peripheral tissues (i.e. liver, muscle and fat), and inappropriate secretion of the glucose-regulating hormones, insulin and glucagon, from the pancreatic islets. The effect of bariatric surgery on systemic glucose homeostasis is well-known. Tests can be performed using glycaemic clamps relatively non-invasive to the subject, but such studies only tell us what is happening at the whole body level. To date, no group has comprehensively studied the direct effects of bariatric surgery on islet function. This is likely in part due to lack of access to human pancreas post-surgery, as well as difficulties in studying islet function *in vivo*. There is also a surprising lack of data from rodent models. Given that the pancreas is a major player in diabetes, we believe that this is a substantial oversight, and studying the effects of surgery on islet function may put us one step closer to understanding how RYGB cures diabetes.

In the Rorsman group (OCDEM) we are taking action to address this fundamental question. With collaborators at the Norwegian University of Science and Technology, Trondheim, we have established a diabetic rat model of RYGB. All of our investigations will take place in the first few days following surgery to mimic the timeframe in which diabetes enters remission in humans. Our primary aim is to determine whether RYGB restores the ability of islets to secrete insulin and glucagon in a glucose-dependent manner, something not seen in diabetic animals. In addition to studying islet function, we will also comprehensively investigate whether RYGB is able to modulate the structure and morphology of the islets. Determining whether islets are able to regenerate or certain cell types are able to proliferate may shed further light on the mechanisms behind the restoration of glycaemia. In parallel, and in light of the recent data ruling out GLP-1, we will chase down gut hormones that may be implicated in the post-RYGB improvements. Indeed, the literature shows that GLP-1 is not the only gut-derived hormone to be elevated after surgery. Therefore, we plan to investigate this aspect further with our model.

It is clear that the process of resecting the digestive tract, as seen in RYGB, has profound effects on the body's metabolic activities. If, as we believe, effects on the pancreas mediate the anti-diabetic effects of surgery, then it is imperative to understand exactly how this is occurring. If we can discover the causal factor and the corresponding signalling pathway, we will be one step closer to developing a novel therapy with the potential to cure diabetes, as well as providing a non-surgical alternative to bariatric surgery.

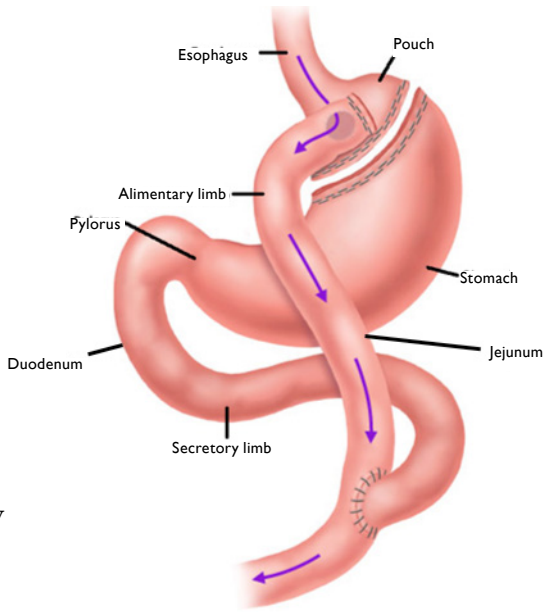

Figure 1. Roux-en-Y gastric bypass (RYGB) (6).

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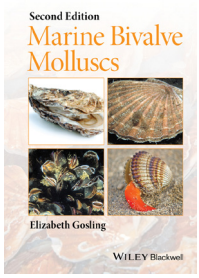
Dr Laura McCulloch is a Post Doctoral Researcher in the Oxford Centre for Diabetes, Endocrinology and Metabolism.

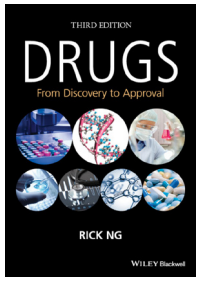
BOOK REVIEWS



Marine Bivalve Molluscs, 2nd Edition

Elizabeth Gosling
IISBN: 978-0-470-67494-9 Wiley-Blackwell (2015)
536 pages: Hardback, £175.00 / eBook, £157.99
Reviewed by Natalie Ng

Marine bivalve molluscs are distributed across aquatic habitats throughout the world and have been ingrained in cultural lifestyles for centuries. *Marine Bivalve Molluscs* is well written for the novice reader in marine biology. It provides a wealth of information covering all aspects of this class of animals with ample detail, and examples, statistics and diagrams make it easy to understand. The structure of the book is commendable, beginning with chapters on bivalve physiology, reproduction, feeding, osmoregulation and excretion, then progressing to management of the population, cultivation, fisheries, bivalve culture, disease, parasites and public health. Each chapter is rounded off with references for more specific further reading. The content is aided by the use of representative species from each group of mussels, oysters, clams and scallops. A particularly impressive quality of this book is its ability to enthuse the reader by drawing on specific examples of bizarre qualities of these seemingly docile animals. For example, the innate defence mechanism of the butter clam, which sequesters diet-derived paralytic algal toxins in its siphons to deter siphon-nipping predators.
Perhaps what makes this book more appealing to a wider audience is the relation of bivalves to the everyday world, as a culinary choice, hobby, or even the provenance of the pearls on your favourite necklace. Dr Elizabeth Gosling highlights the pertinent issue of environmental pollution, a view that resonates with the public audience. Attention is drawn to the use of marine bivalve molluscs within an ecosystem to indicate and measure pollutants in the ocean; although the use of more sophisticated laboratory techniques to quantify pollutants with better sensitivity are also explained.
This book leaves the reader with a greater appreciation of humble marine bivalve molluscs. Overall it is highly informative for those interested in learning about the intricacies of marine bivalves and their global impact. Although out of most students' price range and therefore better suited as a library reference, this would be the perfect textbook to accompany a marine biology course.



Drugs: From Discovery to Approval, 3rd Edition

Rick Ng
ISBN: 978-1-118-90727-6, Wiley Blackwell (2015)
248 pages: Hardback, £66.95 / eBook, £60.99
Reviewed by James Eaton

The drug discovery process lies at the interface of chemistry, biology, and medicine, as well as academia and industry. Consequently, it relies on an understanding of several different disciplines. *Drugs: From Discovery to Approval* is a clear and concise book describing how drugs are discovered and brought to market in the 21st century. It coherently brings together all these different subject areas, providing a clear narrative on how a drug goes from an idea to a physical entity that can be synthesised and sold.
The introduction describes the history of the pharmaceutical industry, giving a historical context for the subsequent chapters. It moves on to describe the pre-clinical phase of drug discovery, providing a good description of the techniques used to identify a molecule that possesses the desired biological activity, including target selection, target validation, and screening approaches. The book gives a well explained molecular description of drug discovery and biological action, covering both in vitro and in vivo aspects. The discussion is helped along by examples and 'Exhibit' boxes explaining concepts that the reader may not be familiar with.
Further sections cover clinical trials and good manufacturing practice, where the design and implementation of a trial, regulatory authorities, and the industrial manufacture of a drug are explained. This phase of the drug discovery process seems to be the most bureaucratic and therefore potentially complicated to an audience with a scientific background, but the supporting figures make it easier to read and understand.
The book concludes with a discussion of future perspectives, giving a frank and honest description of the challenges faced by the pharmaceutical industry in the years to come. It seems that we still have a long way to go but with the current efforts to further our understanding, progress will be made.
This book would suit anyone who wishes to understand the drug discovery process, particularly those who may be familiar with a single aspect, but wish to understand the industry better as a whole. This is a well-written book that is accessible to those even with a limited scientific background.

Bioinformatics and Functional Genomics, 3rd Edition

Jonathan Pevsner
ISBN: 978-1-118-58178-0, Wiley Blackwell (2015)
Hardback, 1160 pages, £80 / eBook, £72.99
Reviewed by Emma Bickford

In recent years, an explosion in the availability of sequence and structural data arising from genomics and proteomics has posed both great opportunities and challenges in terms of data management. *Bioinformatics and Functional Genomics* encompasses the emerging field of bioinformatics; a discipline at the interface between molecular biology and computer technology. It adopts an integrative approach, combining contextual theory with practical applications, whilst illustrating the varied applications of bioinformatics; from identifying the genes associated with disease to understanding evolutionary history.

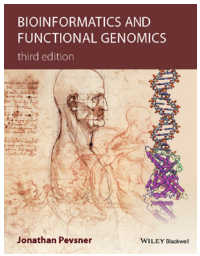
The book's chapters are grouped into three sections. Part I: 'Analyzing DNA, RNA and Protein sequences' focuses on accessing and comparing sequence data, using biological databases, and alignment tools such as BLAST (Basic Local Alignment Search Tool). Part II 'Genome wide Analysis of DNA, RNA and Protein' adopts a functional genomic approach, and introduces next generation sequencing. This section discusses the application of bioinformatics to RNA studies. Part III concentrates on genome-wide analysis to assemble phylogenetic trees, applying the tools discussed previously to genomics. The focus on human genomes leads into the advantages of bioinformatics for studying disease, reflecting the author's background in biomedical research.

Each chapter opens with learning objectives, with boxes throughout highlighting key principles. Concepts are introduced in a biological context, using examples such as breast cancer and retinal-binding protein. The book gradually introduces the programming language R using clear scripts, and demonstrates the strengths and limitations of the software. There is an emphasis on the versatility afforded by command-line software, complemented by simple web-based approaches.

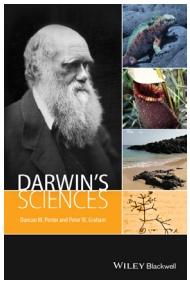
At the end of each chapter there is a 'Perspective' section that describes when the techniques were established and how rapidly they are developing, concluding with the 'Pitfalls'of the tools, advice for students and a quiz enabling self-evaluation.

The growing importance of computer technology and its integration with biological sciences renders *Bioinformatics and Functional Genomics* valuable reading. I would recommend the book for students and professionals at all levels; it remains accessible to readers without a background in computer science and coherently conveys the foundations of the emerging field of bioinformatics.

Darwin's Sciences



Duncan Porter & Peter Graham
ISBN: 978-1-4443-3035-9 Wiley Blackwell (2015)
Paperback, 264 pages, £24.99 / eBook, £15.65
Reviewed by Cassandra Kennedy



Upon receiving this book, I was excited to get started – the glossy front cover promises an interesting read, and I felt like it was time to broaden my knowledge in different fields of science. Where better to start than the life and science of the world's most iconic evolutionary biologist, Charles Darwin? It turns out he did a lot more than just pave the way for the theory of evolution…

Darwin's Sciences is a thorough biography, looking in depth at Charles Darwin's life and the impact of his discoveries. The layout of this book makes it particularly novel – instead of following a chronological order, the authors look separately at the different disciplines of science that interested Charles Darwin throughout his life. With chapters dealing with Geology, Zoology, Botany and Social Sciences, the authors are able to draw connections between key events in the fields and letters and communications that Darwin had at the time. This has enabled the writers to communicate a great deal information about each discipline, and create an easily navigable resource.

The extensive references to letters written by Charles Darwin to his friends, family and fellow scientists make this book an excellent starting point for students who want to delve into these primary resources. In addition, the reader gains a fascinating insight into the character of this iconic scientist, and by the end of the book a tangible sense of his personality is developed.

As a chemist with limited experience of evolutionary biology beyond the A level Biology syllabus, this book gave me a fascinating insight into both the personal and professional lives of Charles Darwin. The lesser-known implications of his research were also highlighted well. The highly detailed descriptions of specific ecological systems would have been more useful to a specialist in the field than they were to me, and might have benefited from diagrams and illustrations (but I'm just a chemist, so what do I know?). Overall, the book serves as an interesting introduction to the life and scientific achievements of Charles Darwin, well suited to evolutionary biologists and interested non-experts alike.

5' with ... Dr Samira Lakhal-Littleton

Dr Samira Lakhal-Littleton completed her DPhil at the Weatherall Institute of Molecular Medicine, Oxford in 2007 under the supervision of Professor Vincenzo Cerundolo. She then remained in Oxford for two post-doctoral positions, first working with Professor Sir Peter Ratcliffe at the Wellcome Trust Centre for Human Genetics and then with Professor Matthew Wood in the Department of Physiology, Anatomy and Genetics (DPAG), during which time she also started a family. In 2013, Samira was awarded a British Heart Foundation (BHF) Intermediate Basic Science Research Fellowship and set up her own group in DPAG. Her research focuses on the molecular pathways that regulate iron and oxygen levels in the heart, as well as the effects of altered iron homeostasis on cardiac physiology and disease.

When did you first decide you wanted to be a scientist?
I decided to become a scientist when considering the courses I wanted to study at University. I wanted to follow a path that combined the excitement of discovery with the satisfaction of a career devoted to the greater good. Science fulfilled both criteria.

How did you become interested in studying iron homeostasis?
During my DPhil studies, I focused on an immune-modulatory enzyme that was iron-dependent. Those studies made me consider the wider consequences of physiological iron deficiency, beyond just anaemia. I realised that iron homeostasis was important for a range of physiological and signalling functions, and that modulation of iron homeostasis presented therapeutic opportunities that were yet to be explored.

If you weren't a scientist, you would be…?
A teacher...I love teaching. The opportunity to teach is another attraction of a career in science.

What are you most proud of in your career so far?
My current BHF-funded research has uncovered novel mechanisms of iron homeostasis, and is therefore challenging the conventional wisdom around how our tissues regulate their iron levels. This project was born from a chance and unexpected experimental finding during my first post-doctoral project. So, I am proud to have had the imagination and determination to build on that finding and to obtain the necessary funding to set up my own research program.

What has been the biggest challenge you have faced during your career?
The transition from post-doctoral researcher to an independently-funded research fellow has been the most challenging stage of my career. It involved a great deal of trial and error, and can, at times, test one's resolve.

What advice would you give to junior researchers in your field?
My advice to junior researchers wishing to progress to an independent investigator position is to identify a scientifically important and original question, to believe in it, to seek the right supportive environment to test it, and not to be discouraged by failure.

Who has inspired you during your career?
I have taken inspiration from many people that I have worked with over the years. Each has inspired me in their own way. Some have taught me persistence, others patience, tolerance and leadership.

Where do you see the field of iron homeostasis going in the next five years?
There is a growing realisation that iron deficiency does not just manifest in anaemia, but also results in adaptive changes in a myriad of signalling and metabolic pathways. In some cases, those changes are maladaptive. The focus now is to identify those iron-responsive maladaptive pathways and to target them for disease therapy. This is already happening for chronic heart failure.

As told to Lauren Chessum

Write for Phenotype?

- The deadline for article submissions is Friday of 8th week, 11th March 2016
- We accept articles on any aspect of biological research, science impact & science education
- Articles can be either 650 or 1300 words

If interested, please get in touch: rebecca.hancock@linacre.ox.ac.uk

Work for Phenotype?

If you'd like to get involved in editing, production or management of Phenotype, please get in touch: rebecca.hancock@linacre.ox.ac.uk

SNAPSHOT Research Image Competition

This issue's winner is...

Dr Nicola Fawcett, NDM

This winning image by Dr Nicola Fawcett is entitled 'The Wild Garden of the Gut Bacteria' and was created by growing normal gut bacteria on nutrient agar with antibiotic-containing paper disks as an entry for the American Society of Microbiology's Agar Art Competition.

This issue's winner, Dr Nicola Fawcett, is an MRC Clinical Research Fellow in the Modernising Medical Microbiology Group based in the Nuffield Department of Medicine and is chief investigator of the Antibiotic Resistance in the Microbiome Oxford Study (ARMORD), which is examining the effects of antibiotics on gut bacteria through sequencing bacterial DNA. In collaboration with Artist Anna Dumitriu, Nicola has produced these images to communicate the importance of the gut microbiome in maintaining health and preventing disease.

The importance of the microbes that live on us and with us (our 'microbiome') is becoming better understood. It is now known the gut bacteria play important roles in metabolism, and provide protection against infection. The diversity and balance of the gut bacteria plays a role in keeping people healthy, and antibiotic use can disrupt this balance, effectively changing the microbiome within our bodies.

Nicola's research aims to understand how the diverse population of bacteria in the gut are affected by antibiotics. It studies which antibiotics cause the most, and least, change in diversity. It also looks at how antibiotic use encourages the emergence of antibiotic-resistant bacteria in the gut, and resistant infections in the future. Ultimately it aims to identify which antibiotics we should be using more, and less, to keep patients and their guts healthy.

Nicola's microbial artwork will also be on display at the John Radcliffe Hospital in the Corridor Gallery from February 13 to May 14, 2016 through the Artlink initiative.

More information on Nicola's microbial artwork can be found at https://livinginamicrobialworld.wordpress.com/2015/09/01/the-wild-garden-of-the-gut-bacteria/.

OXFORD UNIVERSITY PRESS

SNAPSHOT

Research Image Competition

Win a £50 book voucher kindly provided by Oxford University Press!

Do you have an image from, or inspired by your research? Why not enter it in **SNAPSHOT**?
We are now accepting entries for pictures to be featured on the cover of the Trinity 2016 issue of *Phenotype*.

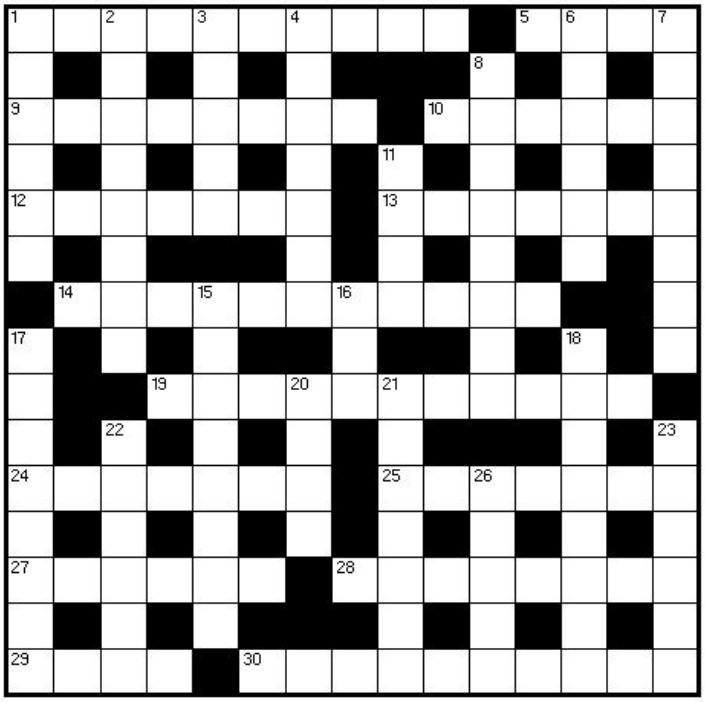
To enter, send images to rebecca.hancock@linacre.ox.ac.uk with a brief description (maximum 100 words). Please get permission from your supervisor before sending any images.

PHENOTYPE crossword

Fish challenges you to this latest cryptic crossword on the theme of the brain. Can you crack it? Answers to last issue's crossword are given at the bottom of the page. Enter this term's competition by sending your answers to rebecca.hancock@linacre.ox.ac.uk. Entries received before the 13th March 2016 will be entered into a prize draw to win one of the four books reviewed in this issue.

The winner of the cross-word competition will recieve their choice of one of the books reviewed on pages 36 to 37, kindly provided by

WILEY-BLACKWELL



Answers to the crossword from Issue 22, Michaelmas 2015: **Across: 1.** Hodgkin, **4.** Noether, **9.** Sinoussi, **10.** Yonath, **11.** Blackburn, **13.** Barre, **16.** Marie Curie, **18.** Lace, **20.** Love, **22.** McClintock, **24.** Moser, **25.** Linda Buck, **29.** Doudna, **31.** Nusslein, **32.** Volhard, **33.** Greider. **Down: 2.** Oriel, **3.** Krunk, **5.** Onyx, **6.** Ten, **7.** Enteric, **8.** Mirror, **12.** Became, **14.** Allot, **15.** Behind, **16.** Mol, **17.** Irene, **19.** Elk, **21.** Olorosso, **23.** Client, **26.** Aisle, **27.** Chime, **28.** Fair, **30.** Doh.

ACROSS

1. Confusing clue about revolutionary headmaster in the **3** (10)
5. Half the duration of a **3** cover (4).
9. Learns anew top recipe: trim **3** **11** ends and scramble (8)
10. Young man absorbs leading textbook and article on the study of plants (6)
12. Incorporated into former pair of 2s that died out (7)
13. Hippy-dippy coloured? (3-4)
14. **3** region where one may study African mammals? (11)
19. Formulate plan dealing with part of the **3** (6,5)
24. Polypeptide supporting a measure of tellurium absorption (7)
25. Half-man, half-horse, third-centipede, half-minotaur (7)
27. A fleeing fugitive at the airstrip (6)
28. **3** cells cluster around Team Cat (8)
29. See **17**
30, 26. Roman reports dark matter in the **3** (10,5)

DOWN

1. Heartless tribunal imposes on former part of the **3** (6)
2. Start to be dubious and feel bad about hairstyle (4,4)
3, 11. Tribesman mixes up the medulla, pons and midbrain (9)
4. The one in France with a twitch is mad (7)
6. Web address through which some acquire $[UO2]^{2+}$ (6)
7. A boy exercises on a revolving piece of the **3** (8)
8. It's different when one qualifies for full immersion (8)
11. See **3**
15. First five and first fifty are pristine (8)
16. Khan uses a gallium stove (3)
17, 29. Pacing Research Assistant is 50:50 to receive honour from **3** region (8,4)
18. One who records events - and who examines them, reportedly? (8)
20. Raise stake in volcano (4)
21. Cyclic amides (like penicillin) are leaders in limited, acute/chronic therapy against multiple sclerosis (7)
22. Mix with a biscuit (6)
23. IRA can make cases for **3**s (6)
26. see **30**