PHENOTYPE

Issue 21 | Trinity Term 2015

www.phenotype.org.uk

FIrts and the human brain

How flrts drive the development of the most mysterious part of the human body

Hydrophobic gating

Insights into the fundamental behaviour of water on the nanometer scale

The elixer of life

Heterochromatic parabiosis and the study of the ageing process

cover image by

Jonathan Neeve

this issue´s winner of the SNAPSHOT scientific image competition

page 31

Cancer

Optical detection · Metastatic mechanisms · Tumour acidosis

Genetics

Worms, germs and genes · Hijacking transcription · Epigenetics

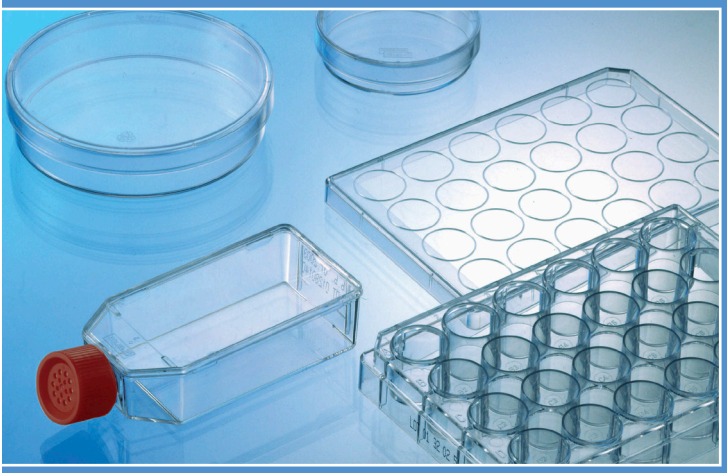
OXFORD UNIVERSITY BIOCHEMICAL SOCIETY

OUBS

We Care - *about our environment . . .*

greiner bio-one

Improved Surface Adhesion



Protein coated cultureware for improved cell adhesion

Tel: **01453 825255**

email: **sales@uk.gbo.com**

. . . **that's why** *our moulding waste is recycled*

www.gbo.com

EDITORIAL

Welcome to a very special 21st instalment of Phenotype! This term we are pleased to be able to include an extraordinary array of thought-provoking articles in a bigger 36 page issue. All of our articles have been produced and edited by undergraduates, postgraduates, postdocs, principle investigators and alumna from a highly diverse range of disciplines throughout the University of Oxford. I truly hope you will find our collaborative science magazine as exciting, interesting and fun to read as it has been for us to produce.

In this issue, our ***Features*** *articles address big questions… How does an organ as mysterious and complex as the human brain develop? Turn to page 8 where Dr Elena Seiradake explains how the neural network that performs such complicated tasks as generating thoughts, emotions, dreams and memories, is formed by interactions between ligands and guidance receptors. Elena highlights how her lab is investigating a particularly versatile class of guidance receptors called Flrt proteins (pronounced 'flirt') that readily interact with other molecules within the same or, indeed, a different family. Then, on page 12, Dr Prafulla Aryal contemplates our understanding of the behavior of the most fundamental molecule of life – water. What properties make water so unique? How does water get in and out of cells? And how do transmembrane pores prevent cells from effectively becoming liquid-filled impermeable plastic bags? As if those questions weren't big enough, Caitlin Clunie-O'Connor addresses the age-old question of the elixir of life. Caitlin's intriguing article focuses on heterochronic parabiosis, a technique in which 'young' and 'old' mice are joined together to create a shared circulatory system. Caitlin proposes on page 14 that such experiments demonstrate that 'young' blood has rejuvenating properties.*

We also have six captivating articles providing an in-depth focus into the fields of cancer and genetics. Edyta Augustyniak, Dr Srinivasa Rao, and Kevin Ray provide interesting explanations of the strategies employed to optically detect premalignant oesophageal cancer (page 16), the molecular mechanisms underpinning bone-metastatic prostate cancer (page 17), and new CEST-MRI-based imaging of tumour acidosis (page 24). Dr Delia O'Rourke, Dr Mónica Martínez Alonzo, and Laura Godfrey also offer insights into the wonderful world of genetics with their articles on C. elegans as a genetic model for bacterial and fungal infections (page 20), how influenza hijacks transcription machinery (page 22), and epigenetic regulation through the methylation of histones by a histone lysine methyltransferase called DOT1 (Page 23).

In our ***Science and Society*** *section, we have two career insights from Dr Kapil Tuladhar, a trainee patent attorney at J A Kemp (page 26), and Dr Cat Kelly, a senior imaging scientist and product manager at Perspectum Diagnostics (page 27). Following our careers section, Anna Senft gives a scientific perspective on travelling to, and conducting research in Madagascar (page 28) and Jason Kaufman explains how increased CO_2 from anthropogenic sources can potentially lead to increased CO_2 release from soil and oceans (page 29). Inés Usandizaga's article on page 30 is a must read for anyone interested in Women's Networks, which enable women to shape their working environment and influence society. Then, on page 31, Dr Michael Fiebig, a Product Development Manager at Absolute Antibody, advocates the use of recombinant antibodies in scientific research.*

As always, our ***Regulars*** *sections brings you Research Highlights, Featured Seminar, Book Reviews, our 5 min with… academic interview and a biography of our SNAPSHOT competition winner. In Research Highlights, Dr Naveed Akbar summarises two key papers that report the development of a real time chemotaxis assay as well as a human CD68 promoter GFP mouse model (page 5). This term's Featured Seminar covers the 2nd Louise Johnson Memorial Lecture, which will be given by Prof John Kuriyan from the University of California, Berkley on Wednesday 20th May (page 6). Dr Anjan Nibber also takes 5 min with… Dr Holger Kramer, who runs the OXION proteomics facility (page 34). Also, we would like to heartedly congratulate Jonathan Neve who has won this term's SNAPSHOT competition, for his heat map of an Ion PI semiconductor sequencing chip from Thermo-Fisher's Ion Proton Sequencer. Further details of Jonathan's research can be found on page 35. As always you can have a go at the crossword on the back cover, which this issue is themed around microorganisms. Be sure to email your answers to christopher.hillyar@jesus.ox.ac.uk for a chance to win one of the excellent Wiley-Blackwell textbooks that have been reviewed by Anna Sigurdsson, Dr Srinivasa Rao, Jason Kaufman, and Rebecca Hancock on pages 32–33.*

Finally, we would like to encourage you to get involved in science communication, writing, and publishing by joining the Phenotype team. We have excellent opportunities for talented people with a passion for science writing, editing, designing or advertising. Please direct enquiries to chrisotpher.hillyar@jesus.ox.ac.uk

Christopher Hillyar
Editor-in-Chief

OXFORD UNIVERSITY BIOCHEMICAL SOCIETY

Phenotype is also available to read online via our website: **www.phenotype.org.uk**

CONTENTS

regulars

science & society

features

EDITORIAL TEAM

Entrepreneurial opportunity

Are you interested in working with a start-up company looking to understand how plant properties can be used in natural skin care solutions with a particular focus on sun protection?
This is an opportunity to be part of an exciting story. A start-up company exploring the use of wholly natural ingredients in cosmetic products is looking for a driven and motivated researcher with an interest in this area. This individual will have the opportunity to help set the direction of the business and will play an integral role in the development and growth of the company. Remuneration will include being paid partly by equity in the company. If interested, please send an email to oadoh47@gmail.com.

This issue is supported by

RESEARCH HIGHLIGHTS

by Dr Naveed Akbar

Iqbal A, *et al.* (2013) *PLoS One* 8(3):e58744.
doi: 10.1371/journal.pone.0058744

A real time chemotaxis assay unveils unique migratory profiles amongst different primary murine macrophages

Chemokines are powerful chemoattractants that are synthesised and secreted at sites of tissue damage. Macrophage activation and infiltration into injured tissues, mediates repair following injury in response to the release of such chemoattractants. Failure to turn off the chemokine synthesis and signalling system causes continual macrophage infiltration, initiating a perpetual cycle of localised inflammation, which can lead to the development of chronic inflammatory diseases. A better understanding of the processes used by macrophages to migrate into tissues may allow drugs to be therapeutically targeted to them, limiting excessive infiltration and in turn preventing chronic inflammatory disease conditions. Cell culture based experiments designed to study the movement of macrophages across membranes and gels have been used previously to assess the ability of macrophages to migrate in response to challenge with chemokines and inflammatory mediators. These types of experiments require researchers to count the number of migrating cells over a time course and to quantify the change in migration due to exposure to a chemoattractant relative to control conditions. Although quantification using this method is very useful, the quantification process can be laborious and time consuming and subject to variability that influences the reproducibility of this approach.

Iqbal *et al.* present a validation of the Real-Time Cell Analyser-Dual Plate (RTCA-DP), an alternative method for the study of macrophage movement (chemotaxis). RTCA-DP applies an electrical current via electrodes located inside a culture chamber containing a membrane through which macrophages can migrate. As macrophages migrate across the chamber membrane towards a chemotactic solution, they create impedance in the electrical current. The impedance that is created by the present of migrating cells offers real-time information on the movement of the cells, unlike traditional cell chamber systems which only offer a single snap shot measurement of migration at a specific time point. In this paper, the authors demonstrate the robust, fast and reproducible readout of the RTCA-DP system using a variety of murine macrophage populations. Whilst the system requires further optimisation for a wider range of chemokines, it provides an operator-friendly and reproducible method for quantifying the movement of macrophages in response to chemoattractant cytokines.

Iqbal A, *et al.* (2014) *Blood* 124 (15):e33-44.
doi: 10.1182/blood-2014-04-568691

Human CD68 promoter GFP transgenic mice allow analysis of monocyte to macrophage differentiation in vivo

Macrophages are specialised cells of the innate immune system with roles in pathogen recognition and elimination, and the regulation of tissue homeostasis and repair after infection or injury. Macrophages also contribute to the inflammatory responses that underpin numerous clinical pathologies including atherosclerosis and rheumatic diseases such as arthritis. Therefore the ability to better understand the biological complexity of these cells and their differentiation may offer avenues for therapeutic intervention. However, the study of macrophages in tissues is often hindered by an inability to discriminate this cell type from other resident cell populations. Previous efforts to visualise macrophages have utilised fluorescence techniques that focus on the detection of proteins such as CX3CR1. Unfortunately, the detection of CX3CR1 has been met with varying success, as reports often show a quenching in the fluorescent signal over the course of the development of the cell that hinders long-term studies.

Recent work by Iqbal *et al.* has harnessed genetic approaches to visualise macrophages and monocytes (macrophage precursors) through fluorescence techniques. Here, Iqbal *et al.* developed a novel transgenic reporter mouse expressing a green fluorescent protein (GFP) under the control of the human CD68 promoter. The CD68 gene encodes a transmembrane glycoprotein that is expressed exclusively in macrophage and monocyte populations. Thus, CD68-GFP mice expressed high levels of GFP only in their macrophages and monocytes. This system allows for the visualisation of macrophages in numerous tissues including the liver, spleen and heart using fluorescence imaging. Importantly, flow cytometry analysis of hematopoietic cells demonstrated that precursor cells that had differentiated into macrophages were still detectable in CD68-GFP mice and no reduction in fluorescence signal was observed over time (up to 72 hours after differentiation).

The CD68-GFP mouse model developed by Iqbal *et al.* is a significant advance that will allow macrophage fate to be mapped more precisely during the study of acute and chronic inflammation or long-term tissue recovery and injury without a loss in fluorescent signal.

OUBS FEATURED SEMINAR

Prof John Kuriyan from the University of California, Berkeley

2nd Louise Johnson Memorial Lecture

Wednesday 20th May 2015

When he was in high school in India, Prof John Kuriyan encountered a series of articles in *Scientific American* on protein structures. He particularly remembers one by Max Perutz on hemoglobin and its structural changes in response to oxygen binding. Like many other budding scientists, Prof Kuriyan was fascinated by the extravagant diversity of animal species, particularly the microscopic creatures that inhabited the streams and drains in his native tropical environment. But the *Scientific American* articles and a very wise early mentor provided him with a new perspective: one hundred years after Darwin, might the molecular world provide an opportunity to experience the thrill that Darwin felt when encountering strange and unexpected species of barnacles through his microscope? This perspective led Prof Kuriyan to switch his field of study from Zoology to Chemistry and, after obtaining an undergraduate degree from Juniata College in Pennsylvania, he completed a PhD and a postdoctoral fellowship in Biophysical Chemistry at MIT and Harvard University, respectively. He then spent fourteen years on the faculty of The Rockefeller University in New York before moving to the University of California, Berkeley, where he is now a Professor of Molecular and Cell Biology and of Chemistry. Prof Kuriyan has been an Investigator of the Howard Hughes Medical Institute since 1990.

Prof Kuriyan's work is focused on understanding the workings of the proteins that regulate intracellular decisions. For much of his early career, he used X-ray crystallography to determine the three-dimensional structures of proteins involved in cell signalling and enabling very high speed DNA replication. More recently his lab has embraced a variety of other techniques to understand the workings of signalling proteins, ranging from computer simulations of protein dynamics to fluorescence microscopy. One class of proteins that Prof Kuriyan considers the most fascinating are the protein kinases, which phosphorylate specific residues on target proteins. Phosphorylation often changes the shape of the protein, affecting the way it interacts with other molecules. The targets of protein kinases regulate most cell functions, and they are especially important in sending messages across the cell membrane. Ultimately, kinases can prompt cells to divide, move, and even die.

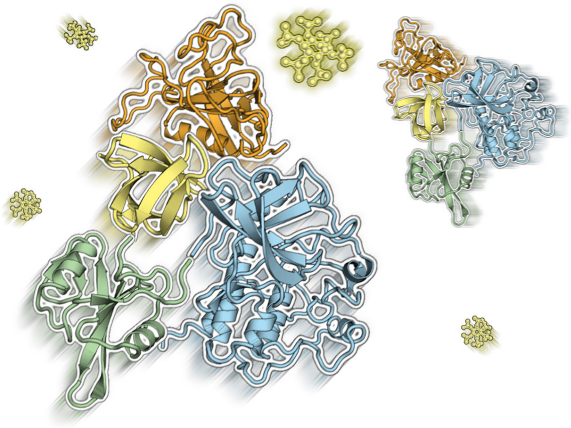


Figure 1: Bruton's tyrosine kinase (BTK) is a multi-domain tyrosine kinase and an important target in cancer and immunodeficiency diseases. Kuriyan and colleagues found that a highly charged small molecule called inositol hexakisphosphate (shown as small yellow molecules) can activate BTK by transiently bringing BTK molecules together to allow them to phosphorylate each other.

In the 1990s, Prof Kuriyan discovered how changes in protein structure affect the regulation of Src kinases, one of which is encoded by the first oncogene ever to be discovered. Concurrently with the laboratory of Stephen Harrison at Harvard, Prof Kuriyan showed how certain parts of Src kinases (regulatory domains called SH2 and SH3 domains) are important in "turning on" the kinase to send messages (1). The way the regulatory parts of the protein worked was not intuitive; it reminded Prof Kuriyan of a contraption designed by Rube Goldberg, an eminent 1904 Berkeley engineering graduate who spent his life designing mechanisms that combined moving parts in the most unexpected ways. A little later, Prof Kuriyan found a Src-like "molecular handshake" mechanism at work in the BCR-Abl kinase, an abnormal protein that is behind most cases of chronic myelogenous leukemia. Prof Kuriyan and colleagues discovered that the anticancer drug Gleevec works by recognising the unique "switched-off" form of BCR-Abl (2). The specificity of Gleevec (it strongly inhibits very few of the 500 or so human protein kinases) makes it a powerful drug, with few side effects. Most recently, Prof Kuriyan's lab, in collaboration with that of Stephen Harrison, discovered that another important cancer target, the tyrosine kinase BTK, is also regulated by a Src-like mechanism. But, in a Rube Goldberg twist, they discovered that a highly charged small molecule, inositol hexakisphosphate, can activate BTK by transiently bringing BTK molecules together to allow them to phosphorylate each other (3).

A key question being addressed by the lab is how cell-surface receptors with only one transmembrane helix, such as the epidermal growth factor receptor

(EGFR), communicate across the cell membrane. Members of the EGFR family have cytoplasmic tyrosine kinase domains, and they are particularly important because two of them, known as HER2 and HER3, are potent in combination even though HER3 is a "crippled" kinase. The interaction between HER2 and HER3 underlies many cancers, including some breast cancers and glioblastomas. Prof Kuriyan and his group discovered that the activation of EGFR involves the formation of an asymmetric dimer between the kinase domains of the receptor, in which one kinase domain activates the other, analogous to the well-known activation of cyclin-dependent kinases by the cell cycle control proteins known as cyclins (4). This new paradigm for the activation of a receptor tyrosine kinase explained why HER2 and HER3 are so potent in combination, as HER3 serves as the activator for the kinase domain of HER2. The results from Prof Kuriyan's group provided the first clear mechanistic insights into how these two receptors form an active signalling complex.

Current research in Prof Kuriyan's group includes investigation of the kinases involved in immunological signalling, the proteins that activate the GTP-binding protein Ras, as well as a particularly intriguing kinase called calcium/calmodulin-dependent protein kinase II (CaMKII), that is involved in learning and memory. Mutation of a specific phosphorylation site in CaMKII leads to forgetful mice, and mutation of two other sites result in mice that have rigid learning, a behavior that Prof Kuriyan calls the "pre-med phenotype". CaMKII is unique among protein kinases for its dodecameric assembly and its ability to respond to the frequency, and not just the amplitude, of stimulating signals. While studying how this protein works, Prof Kuriyan's group discovered that CaMKII has another remarkable property, which is that phosphorylation at the sites that characterise the "pre-med mouse" triggers the exchange of subunits between different CaMKII assemblies, including unactivated ones, enabling the calcium-independent activation of new subunits (5). These findings, which can spread the activating signal beyond those assemblies that are first activated, takes research on CaMKII in the lab in a new and exciting direction for understanding how CaMKII mediates the response to synaptic stimulation.

The idea that activity in one part of a protein can affect the behavior in a totally different part of it is called allostery, and all of the signalling proteins that fascinate Prof Kuriyan exhibit complex allosteric behavior. Allosteric interactions are common in biology; one of the best-known examples is the way in which hemoglobin changes its shape

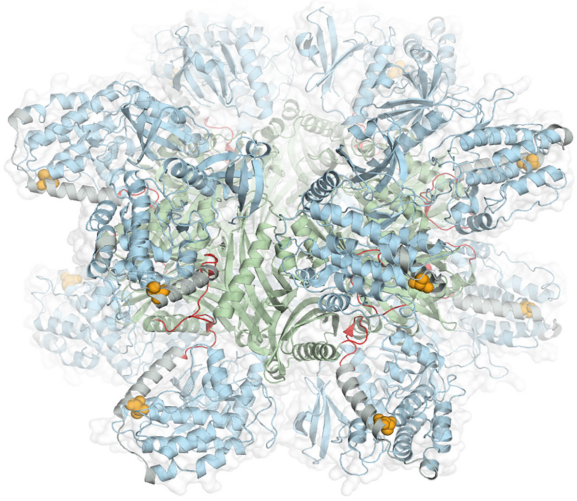


Figure 2: The calcium/calmodulin-dependent protein kinase II (CaMKII) is a serine/threonine-specific protein kinase that is involved in learning and memory, among other key functions. Each CaMKII subunit is composed of a kinase domain (blue), a regulatory segment (grey) and a hub domain (green) that mediates oligomerisation of CaMKII into a dodecameric assembly.

in response to oxygen binding, the topic of Perutz's *Scientific American* article. The diversity of allosteric mechanisms and how they evolve are issues that hark back to Prof Kuriyan's early interest in biological diversity and evolution. Because all kinases carry out the same phosphate-addition reaction, and because many active kinases look similar to one another, Prof Kuriyan originally expected that they would all work the same way. However, his research, and that of others, has revealed that kinase mechanisms are surprisingly diverse, with various kinds of regulatory domains working together with a seemingly haphazard level of interconnectivity that would have delighted Rube Goldberg. The need to understand the evolutionary mechanisms that led to the emergence of these finely tuned and exquisitely sensitive molecular devices continues to keep Prof Kuriyan and his group awake late at night.

References

1. Sicheri F, *et al.* (1997) Crystal Structure of the Src-family tyrosine kinase Hck. *Nature.* 385(6617):602-609.
2. Schindler T, *et al.* (2000) Structural mechanism for STI-571 inhibition of Abelson tyrosine kinase. *Science.* 289(5486):1938-1942.
3. Wang Q, *et al.* (2015) Autoinhibition of Bruton's tyrosine kinase (Btk) and activation by soluble inositol hexakisphosphate. *eLife.* doi: 10.7554/eLife.06074
4. Jura N, *et al.* (2011) Catalytic control in the EGF receptor and its connection to general kinase regulatory mechanisms. *Mol. Cell.* 42:9-22.
5. Stratton M, *et al.* (2013) Activation-triggered subunit exchange between CaMKII holoenzymes facilitates the spread of kinase activity. *eLife.* 3:e01610. doi: 10.7554/eLife.01610.

Flrts and brain development

by
Dr Elena
Seiradake

The brain is perhaps the most mysterious part of the human body. It is the centre of our nervous system, where thoughts, emotions and dreams are generated, as well as where memories are stored. To perform these complicated tasks, the brain contains billions of neurons that form an intricate network of electrical circuits via trillions of contacts: synapses. How such a staggering number of neurons work together is still largely unknown, hampering the design of drugs for neurological disorders such as Alzheimer's disease, schizophrenia, epilepsy and insomnia; to name a few. The good news is that, despite its outstanding complexity, the development and regeneration of neural tissue is steered by a relatively small number of cell-guidance molecules. So how do guidance molecules direct brain development?

The development of complex organs like the brain depends on a huge number of inter-cellular communication events, which result in attraction, repulsion or adhesion between cells. Cells communicate via cell guidance receptors, which are located at the cell surface and act like antennas for chemical cues (ligands) in their environment. Receptor-ligand interactions trigger specific responses in the receptor-bearing cell. Some cell-bound ligands act as receptors themselves and can also trigger a cell response. For example, Eph receptors recognise and bind to ephrin ligands present on neighbouring cells, causing both the Eph-bearing cell and the ephrin-bearing cell to repel one another. Cell repulsion is the dominant mechanism of tissue boundary formation and segregation between cells. In the brain, the Eph/ephrin interaction leads to topographic map formation, used in the visual system to relay spatial relationships of the visual world to the midbrain, for instance (1). Rather than mediating repulsion, other families of receptors provide cell-cell adhesive interactions. These receptors are termed cell adhesion molecules. They provide spatial stability to non-moving cells and traction for migrating cells. Famous examples of homophilic cell adhesion molecules are the cadherins, which bind to their own kind to produce dimers or oligomers. In the brain, cadherins are broadly expressed and important at many stages of development (2).

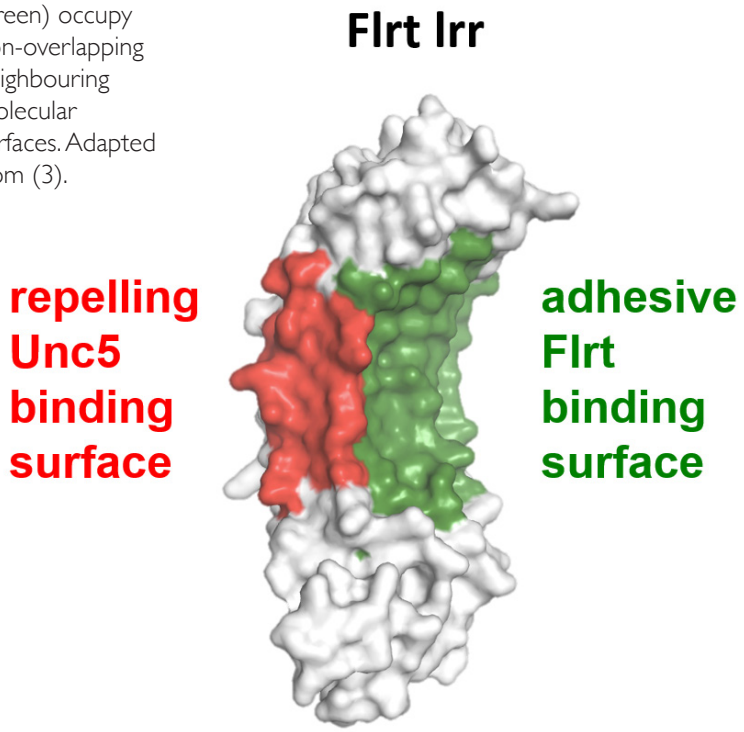


Figure 1: X-ray structure of Flrt2 Irr domain. The Unc5-Flrt (red) and Flrt-Flrt interaction sites (green) occupy non-overlapping neighbouring molecular surfaces. Adapted from (3).

With many of the cell guidance receptors being intensely studied, it is perhaps surprising that we are still far from understanding how the brain develops at the molecular level. A major challenge arises from the overlapping expression patterns of many cell guidance receptors. Instead of acting in isolation from one another, a variety of different receptors are expressed in any given brain cell, known as a neuron, and these may be synergistic in their effects. How a cell responds to its environment depends on the combination of its expressed receptors and the combination of the presented ligands. The number of possible combinations is more than the overall number of guidance receptors and ligands; assuming there were only 100 receptors encoded in the genome, there could be up to 2,100 neurons with a different receptor expression pattern. For reference, 2,100 corresponds to a number with 31 digits, whereas the total number of neuronal cells in the brain only has 10 digits. Thus every neuron could have a different expression profile. Most guidance molecules interact with a multitude of ligands, creating complex interaction networks on the cell surface. Understanding the general mechanisms of how different receptors and ligands interact and synergise to control processes in the brain is the basis of an active field of research.

One of the projects in my lab focuses on a particularly versatile class of cell guidance receptors, the Flrt proteins. Flrt stands for "fibronectin leucine-rich repeat transmembrane" and is pronounced 'flirt'. As the pronunciation suggests,

the three members of the Flrt family, Flrt1-3, readily interact with other molecules, both within the same family and within other classes. By binding to each other, they act as classical homophilic cell adhesion molecules promoting cell-to-cell adhesion. In terms of molecular structure, adhesion involves Flrt-Flrt binding via a concave surface on the leucine-rich repeat (lrr) domain (Figure 1). The affinity between individual Flrt molecules is weak and adhesion only occurs at high local concentrations (3). The weak binding affinity and its concentration-dependency make Flrt an ideal molecule for finely tuning adhesive cell-cell interaction, providing different amounts of traction between cells.

In addition to producing cell adhesion, Flrt repels cells expressing a different type of receptor, Unc5. Unc5-dependent cell repulsion is triggered when an Unc5-expressing cell meets a Flrt-expressing cell, or the free extracellular fragment of Flrt released from cells by proteases (4). In contrast to the low-affinity adhesive binding, repulsive Flrt-Unc5 interaction is of high affinity and mediated by a distinct binding surface on the Flrt lrr domain (Figure 1). Intriguingly, some regions in the brain, such as the rostral thalamus, contain neurons that express both Flrt and Unc5. This raises the question: how do these cells respond to Flrt in their environment? Stripe assay analysis revealed that Flrt, when present on the same cell as Unc5, acts as an attenuator of Unc5 repulsion. Attenuation happens because neurons integrate the two opposing signals elicited by Flrt-Flrt (adhesive) and Flrt-Unc5 (repulsive) interactions (Figure 2). There appears to be no direct physical interaction between Flrt and Unc5 in cis, which is between molecules attached to the same neuron. Because of their dual functions as adhesive and repulsive molecules, Flrts are also termed repelling cell adhesion molecules (3).

Recent work showed that Flrt directs the development of a brain region known as the cortex (3). The human cerebral cortex is the outermost region of the brain where information comes together from the sensory and motor systems, and where information is processed to generate memories, conscious thoughts and decisions. It is tightly organised in horizontal layers and intersecting columns. During development, different types of cortical neurons settle in different layers in an inside-out fashion, i.e. the innermost layers develop first. Flrt-Unc5 repulsive interaction then helps timing the formation of the layers. Flrt2 fragment is released by cells in the outer-most region of the cortex (the cortical plate) and diffuses towards Unc5-expressing cells. This stops the neurons from prematurely migrating to the cortical plate where the layers are formed. Only when the neurons stop expressing Unc5 can they migrate upwards and form a new cortical layer (4).

The intersection of cortical layers by vertical columns is also dependent on Flrt action. Removal of Flrt3 from the mouse cortex results in abnormal dispersion

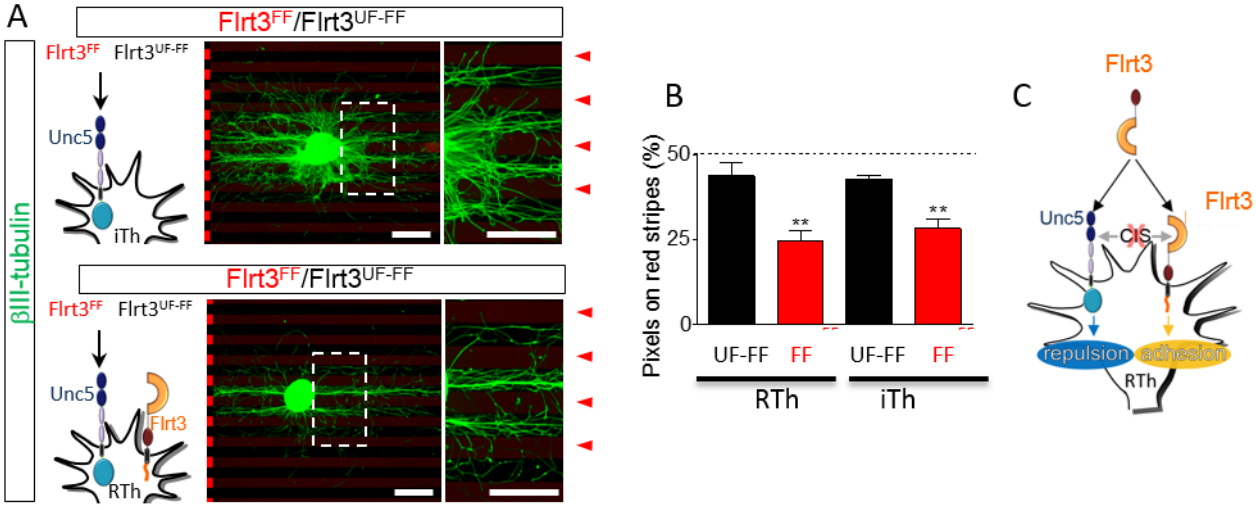


Figure 2: Stripe assays are used to determine whether neurons internally integrate signals received via different receptors. We previously observed that neurons expressing Unc5 are repelled more strongly by wild type Flrt3 compared to neurons expressing both Flrt3 and Unc5 (3). (A) Mutant Flrt proteins (FF and UF-FF) were immobilised on culture surfaces in an alternating horizontal stripe pattern. Red stripes: FF has a mutation in the Flrt-Flrt binding site; thus cannot mediate adhesive binding. Black stripes: UF-FF has the FF mutation plus an additional mutation in the Unc5-Flrt binding surface; thus cannot promote Unc5-mediated repulsive interaction either. Two populations of neurons (green) derived from the mouse thalamus were cultured on the stripe patterns. The neurons express either Unc5 (iTh, top) or Unc5+Flrt3 (RTh, bottom). Red arrows indicate the location of the red FF stripes in the magnified insets. Scale bars represent 300 micrometres. (B) We measured the axonal growth, quantified as green pixels, on FF versus UF-FF stripes in repeated experiments. The results show no significant difference between the two populations of neurons (**$p < 0.01$) (C) The results imply that neurons expressing Flrt3 and Unc5 integrate the adhesive and repulsive Flrt3-mediated signals, rather than exogenous Flrt3 competing with cell-bound Flrt3 for Unc5-binding.

of neurons within their columns. This effect is linked to the adhesive properties of Flrt3, and is independent of Unc5. Lack or over-expression of Flrt3 in neurons disrupts the delicate balance of adhesion/repulsion, which is necessary for correct tangential migration within columns. This function of Flrt3 may be shared by the related Flrt1 that is co-expressed with Flrt3 in the developing cortex and displays similar characteristics (4). A preliminary characterisation of Flrt1/Flrt3 double knockout mice revealed a stronger spatial disruption of tangential neuronal migration than that seen in single Flrt3 knockouts (D. del Toro and R. Klein, unpublished observation).

So far studying the functions of Flrt and Unc5 in the brain has led to exciting conclusions, but these might be just the tip of the iceberg. Both Flrt and Unc5 bind to additional receptors and ligands, which in turn interact with other molecules. For example, Flrt was shown to bind latrophilin, an adhesion-type 'G-protein coupled receptor' expressed in the cortex, with functions in synapse formation and maintenance (5,6). Further interaction partners may exist and may not have been identified yet. How cell surface receptors interact with intracellular proteins is also still largely unknown. The task of understanding the mechanisms and functions of different guidance receptor interactions is immense. Questions on that scale are best tackled in collaboration with several labs, in particular with those having complementary technical expertise. High-resolution structural models of receptors/ligands show how the molecules interact physically, enabling protein engineering to produce mutant molecules. These mutants are very useful tools for functional analysis of cells by using computer modelling, advanced microscopy, cell biology and in vivo methods.

The expected insights from such studies are of medical significance, as many guidance molecules are involved in human disorders. For example, defects in the Flrt interaction partner latrophilin are associated with attention deficit hyperactivity disorder (ADHD) in humans (7). The underlying mechanism appears to be conserved in animals, as decreased activity of latrophilin also elicits ADHD-like behaviour in fish (8). Increased production of an Unc5 receptor in the hippocampus, a brain region important for memory formation, is linked to late-onset Alzheimer's disease (9). Understanding the details of what goes wrong in these terrible and poorly understood conditions will be essential for the rational design of better drugs and treatments.

Beyond the medical interest, brain research is influential in ethical, legal and philosophical aspects. There may be a time when criminal behaviour in humans can be directly linked to physical malfunction, such as too much or too little action of a certain receptor in a specific region of the brain. It is of no doubt that this will blur the distinction between criminals and psychologically-ill individuals. This could lead to more sophisticated and unbiased ways of penalising and preventing crimes. In terms of philosophy, understanding how our brain develops, and how it functions, will lead to a more rigid framework, defined by the abilities and limitations of human reasoning and the way humans perceive the world. After centuries of speculating about who and what we are, our century is the first in which humans have a reasonable chance of experimentally approaching these issues. An interesting conundrum is presented by the fact that our brain's properties enable us to become both the examiner and the research object in this case. Given the broad impact of brain research, and its application to solving medical questions that so far have been almost impossible to address, it is possible that we may see ground-breaking developments over the next decades, which are likely to lead to fundamental changes in our society.

References

1. Klein, R. & Kania, A. (2014) Ephrin signalling in the developing nervous system. *Curr. Opin. Neurobiol.* 27:16–24.
2. Hirano, S. & Takeichi, M. (2012) Cadherins in brain morphogenesis and wiring. *Physiol. Rev.* 92(2):597–634.
3. Seiradake, E., *et al.* (2014) FLRT Structure: Balancing Repulsion and Cell Adhesion in Cortical and Vascular Development. *Neuron.* 84(2):370–385.
4. Yamagishi, S., *et al.* (2011) FLRT2 and FLRT3 act as repulsive guidance cues for Unc5-positive neurons. *E.M.B.O. J.* 30(14):2920–2933.
5. O'Sullivan, M.L., *et al.* (2012) FLRT proteins are endogenous latrophilin ligands and regulate excitatory synapse development. *Neuron.* 73(5):903–910.
6. Jackson, V.A., *et al.* (2015) Structural Basis of Latrophilin-FLRT Interaction. *Structure.* In Press.
7. Arcos-Burgos, M., *et al.* (2010) A common variant of the latrophilin 3 gene, LPHN3, confers susceptibility to ADHD and predicts effectiveness of stimulant medication. *Mol. Psychiatr.* 15(11):1053–1066.
8. Lange, M., *et al.* (2012) The ADHD-susceptibility gene lphn3.1 modulates dopaminergic neuron formation and locomotor activity during zebrafish development. *Mol. Psychiatr.* 17(9):946–954.
9. Wetzel-Smith, M.K., *et al.* (2014) A rare mutation in UNC5C predisposes to late-onset Alzheimer's disease and increases neuronal cell death. *Nat. Med.* 20(12):1452–1457.

Dr Elena Seiradake is Group Leader of the Molecular Mechanisms in the Nervous and Vascular Systems group in New Biochemistry

Where's the water?

by
Dr Prafulla
Aryal

Water is fundamental to life. When astrobiologists search for extraterrestrial life, they look for the presence of water or a similar solvent. Yet despite its importance, we still lack a complete grasp on the behaviour of water on a nanometre scale, where most biochemistry occurs. One of the major limitations of studying water at this scale is that we cannot directly observe its dynamic behaviour under physiological conditions. Molecular Dynamics (MD) simulations have been very useful for investigating the behaviour of water, lipids, ions, and proteins in silico under near-physiological conditions. This approach has yielded a number of new insights into the relationship between protein structure, water dynamics, and biological function.

Unique solvent properties of water

So what makes water unique? Open up a biochemistry textbook and in one of the earlier chapters you will find that this molecule, 3 Å (0.3 nm) in diameter, is composed of an oxygen atom that draws electrons away from two hydrogen atoms, creating a dipole. This leads to hydrogen bond formation between adjacent water molecules, making water a liquid at room temperature. In the liquid state, water molecules are free to tumble around, making and breaking hydrogen bonds. This increases the entropy, or randomness, of the system and is energetically favourable. Water's dipoles also make it a good solvent for polar molecules such as sugars, certain amino acids, and salts, moving them away from their crystalline lattices and increasing the entropy of the system. We all know that water and oil don't mix; and this is because the entropy of the system is decreased by interactions between water and non-polar molecules like lipids. Thus water-fearing, or hydrophobic, molecules preferentially self-associate rather than mix with polar molecules.

In an MD simulation box, if we add together atomistic models of water, phospholipids, and salt and let the system equilibrate, we will see within a few tens of nanoseconds that the hydrocarbon tails of phospholipids interact with each other to form a lipid bilayer, while the hydrophilic head groups become oriented to either side of the bilayer, interacting with water and ions. Cell membranes are required to separate our cells from their environment. However, a lipid bilayer alone would effectively turn cells into liquid-filled impermeable plastic bags, incapable of exchanging or interacting with their external environment.

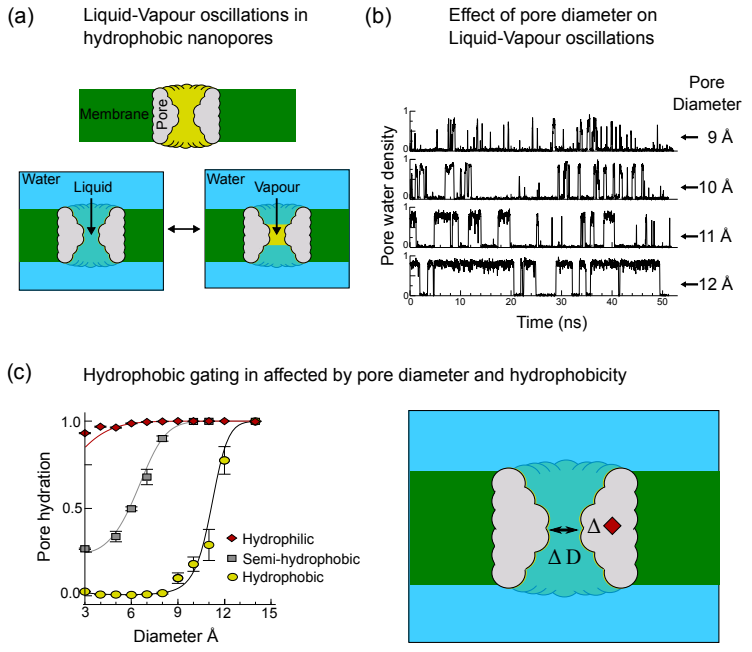


Figure 1: Principles of hydrophobic gating. (a) A cross-section through a model hydrophobic nanopore. Hydrophobic lumen of the pore is shown in yellow, membrane in green, and water in cyan. (b) In MD simulations water density inside the nanopore oscillates between liquid and vapour states. (c) The stability of the liquid (wetted) state is highly dependent on pore diameter and hydrophobicity. Modified from (1).

Transmembrane pores are hydrophobic

Ion channels and transporters are nanoscale pores that allow ions and related polar molecules to permeate across the cell membrane. In most ion channels, the outside surface of the pore is generally hydrophobic where it traverses the membrane, whilst the lumen of the pore is intuitively assumed to be hydrophilic. Most forms of 'gating' subsequently occur through conformational changes which physically close or open the pore. However, there is now a strong body of structural evidence that instead suggests that the lumen of these pores is sometimes rather hydrophobic (1).

Unusual behaviour of water inside hydrophobic pores

What exactly happens when water meets a hydrophobic surface, such as that in an open nanoscopic pore? The biochemistry textbook tells us that water will be forced to adopt the structure of a highly ordered ice-like sheet at this interface, massively increasing the entropy of the system. However, early MD simulations of model hydrophobic nanopores embedded in a bilayer interacting with water revealed unusual behaviour. When the pore was at or below 12 Å in diameter, instead of switching to an ice-like structure, water oscillates between liquid and vapour states, transiently evaporating at the smallest constriction of the pore (Figure 1b). This transition is dependent on the diameter of the hydrophobic pore. If the diameter is below 9 Å, that is three times the size of water and slightly larger than a hydrated sodium or potassium ion, the pore spends most of its time in evaporated (dewetted) state, and thus it is effectively closed. This observation led to the 'hydrophobic

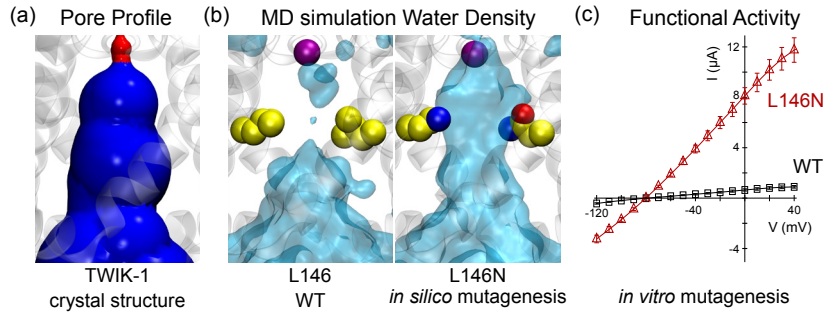


Figure 2: Hydrophobic gating principle applies to TWIK-1 channel pore. (a) 3D surface profile of TWIK-1 pore suggests that the pore is open. (b) However, normalized water density surface map (cyan) inside the pore from MD simulations of TWIK-1 shows that dewetting occurs in wild-type TWIK-1 at its narrowest constriction where L146 residues are located (left). In silico mutagenesis to L146N led to a retention of water inside the pore (right). Carbon atoms at 146 of the dimer are coloured yellow, oxygen red and nitrogen blue. Potassium ion at the selectivity filter in purple. (c) Current measurement of wild type (black) and L146N (maroon) TWIK-1 channels expressed in *Xenopus* oocytes shows that the wild-type channel is effectively silent and that hydrophilic substitutions lead to a large increase in channel activity. Modified from (6).

gating' hypothesis, which states that water and ion conductance through a hydrophobic pore is hindered at dimensions much greater than the size of water and solvated ions. In contrast, water and ions will freely flow through similarly sized hydrophilic pores (Figure 1c).

Hydrophobic gating has been observed not only in the simulation of model nanopores (2,3), but also in biological ion channels, such as the nicotinic acetylcholine receptor (4). Furthermore, long timescale (ms) simulations have revealed that dewetting of the hydrophobic inner pore is one of the first steps that occurs during physical constriction of a potassium channel pore (5).

MD simulation predicts a novel hydrophobic gate

TWIK-1 is a member of the two-pore domain family of potassium channels that control cellular excitability in a diverse range of tissues. Unlike many other family members, which conduct large potassium ion currents through their pores, the activity of the TWIK-1 channel is very low. The reason for this has been unclear for a very long time, but the recent publication of a crystal structure for the TWIK-1 channel (Figure 2a) revealed that the pore was wide open (blue), with the narrowest constriction being 9.5 Å in diameter, at a position 5 Å below the potassium selective filter (red).

In an attempt to understand how these channels function in a bilayer, we ran a self-assembly simulation of the crystal structure with phospholipids, water, and salt, and, as expected, we found that the channel embeds itself across the bilayer. When we monitored the dynamics of water inside the pore, we found that liquid-vapour transitions occurred within the inner pore, leading to a cumulative loss of water (Figure 2b). Examining the amino acids that line the pore revealed that the pore is very hydrophobic, including a ring of leucine residues at the narrowest constriction. Further simulations, in which we dragged a potassium ion through this constriction, revealed that the hydrophobicity of the pore created an energetic barrier not just to water, but also to a solvated ion. As predicted by the hydrophobic gating model, computationally mutating these leucine sidechains to similarly sized but hydrophilic asparagines, led to retention of water and decreased the energetic barrier for ions at the pore.

We then measured the functional activity of these channels using *in vitro* mutagenesis followed by electrophysiological techniques and found that the change from leucine to asparagine dramatically increased the potassium currents flowing through the channel (Figure 2c). Furthermore, by applying the hydrophobic gating principles, we showed an inverse correlation between the hydrophobicity of this barrier and its ability to conduct ions. Similar functional results have now been reported for other members of this ion channel family (1).

Thus, by using MD simulations to ask the question "where's the water?", we were able to both make predictions about and break the functional silence surrounding the TWIK-1 channel. This study demonstrates the importance of the nanoscopic behaviour of water in defining the functional properties of transmembrane pores. Our study also highlights the use of MD in guiding the development of hypotheses that can be tested experimentally. In the future, we hope to use similar approaches to help define whether novel channel structures are open or closed.

References

1. Aryal, P., *et al.* (2015) Hydrophobic gating in ion channels. *J. Mol. Biol.* 427(1):121–130.

2. Beckstein, O. & Sansom, M.S.P. (2003) Liquid-vapor oscillations of water in hydrophobic nanopores. *P. Natl. Acad. Sci. U.S.A.* 100(12):7063–7068.

3. Trick, J.L., *et al.* (2014) Designing a hydrophobic barrier within biomimetic nanopores. *A.C.S. Nano.* 8(11):11268–11279.

4. Beckstein, O. & Sansom, M.S.P. (2006) A hydrophobic gate in an ion channel: the closed state of the nicotinic acetylcholine receptor. *Phys. Biol.* 3(2):147.

5. Jensen, M.O., *et al.* (2012) Mechanism of voltage gating in potassium channels. *Science.* 336(6078):229–233.

6. Aryal, P., *et al.* (2014) A hydrophobic barrier deep within the inner pore of the TWIK-1 K2P potassium channel. *Nat. Commun.* 5.

Dr. Prafulla Aryal is an OXION (Initiative in Ion Channels and Disease) postdoctoral research training fellow, working jointly with Prof Mark Sansom and Assoc Prof Stephen Tucker

Is young blood the elixir of life?

by Caitlin Clunie-O'Connor

Human history is rich in examples showcasing our obsession with unlocking the secret to eternal youth. Alchemists toiled for hundreds of years in search of the philosopher's stone. The first emperor of China, Qin Shi Huang, died from mercury poisoning since the pills he had been prescribed to make him immortal were mainly comprised of mercury. And legend has it that the Countess Elizabeth Báthory de Ecsed bathed in the blood of young virgins in order to retain her youth. However, it now appears that the fabled countess may well have been on to something.

As we age, our bodies lose their ability to replenish and repair damaged tissue and organs. This leads to a steady decline in bodily functions and an increased susceptibility to injury, disease, and death. For years scientists have sought to understand and control the mechanisms of aging. In doing so, several research groups now have evidence to suggest that the blood of young animals is able to reverse some pathological effects of aging and rejuvenate old organs and stem cell populations.

This phenomenon was first observed whilst exploring the 'Frankenstein-esqe' technique of parabiosis: the surgical creation of conjoined twins. Two animals, commonly mice or rats, are joined together in such a way that they develop a shared circulatory system. This allows full exchange of soluble blood factors between the two animals.

By joining together two animals of different ages in a process named 'heterochronic parabiosis', the tissue of the old animal is 'bathed in the blood of a young one'. It is therefore possible to investigate how exposure to young blood affects the function of tissues and organs in older animals and vice versa (Figure 1) (1).

In 1977, Japanese scientists observed physical improvements in liver cells of old mice after nine months of heterochronic parabiosis with a younger partner. They noted that these liver cells consistently contained higher numbers of mitochondria than would be expected in an unpaired mouse of the same age. This was linked with an apparent decline in liver structure and function in the younger mouse. However, it was unclear at this point whether these changes occurred due to the organs of the younger mouse being forced to overcompensate to aid its older companion, or due to some other unknown effect of the shared circulation (2).

It wasn't until almost three decades later, that evidence of a blood-borne 'rejuvenating factor' began to emerge. At the time, a group of scientists at Stanford University were studying how the regenerative ability of 'satellite cells' changes in animals as they age. Satellite cells are stem cells that reside between muscle fibres and contribute to 99% of adult muscle regeneration. In response to injury, satellite cell populations expand and a fraction of them differentiate to form new muscle. This ability to expand and proliferate declines with age. In mice, it was found that satellite cells from young animals increased production of a protein, Delta, after injury whereas satellite cells from older mice did not. However, if correctly stimulated, older satellite cells could be coaxed into producing Delta again, restoring their ability to proliferate and form new muscle. It was well known at the time that cells react and behave according to signals from the tissues and cells around them. The reactivation of aged cells suggested that the decline in response to injury was not due to a loss of intrinsic regenerative ability; rather, the cells no longer existed in an environment that was able to fully stimulate a regenerative injury response (3).

To explore this idea further, the group examined muscle repair in parabiosed mice. In heterochronic pairs, the older mouse consistently demonstrated increased satellite cell activation and muscle regeneration compared to controls. This strongly suggested that the blood of the younger animal was having a rejuvenating effect on satellite cell populations (4).

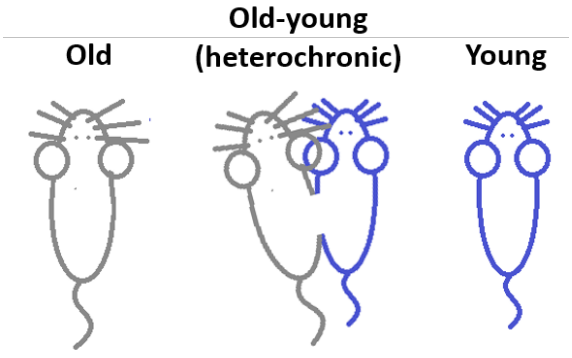


Figure 1: Heterochronic parabiosis. Pairs of mice are surgically joined together to create a shared circulatory system.

Of course this enticing discovery begged the question of whether the effect could be extended to other cell populations. Soon after, it was discovered that exposure to young blood had a similar rejuvenating effect on aged cell populations in the brain. In humans, the number of new brain cells being 'born' (neurogenesis) decreases as we age. This is often accompanied by neuronal inflammation and other detrimental changes that can significantly alter our cognitive ability, affecting both learning and memory. As a similar pathological decline in brain function had been observed in mice, it seemed plausible that these changes may be, in part, reversed if the brain were to be exposed to a younger circulation. Indeed, after five weeks of heterochronic parabiosis, the rate of neurogenesis in older mice significantly increased. Once again the opposite effect was seen in the youngest of the pair.

It could have been argued here that physical

changes observed in parabiosed mice were due to the abrupt change in lifestyle that would naturally occur after pairing, rather than a direct result of shared circulation. This theory was disproved when young, unpaired mice were injected with blood plasma from older mice. After ten days of injections, neurogenesis was significantly decreased and the young mice exhibited increased levels of fear and spatial confusion, which are behavioural indicators of age-related cognitive decline (5). On the other hand, older mice injected with young blood plasma demonstrated enhanced learning and memory capacity, along with improved brain function at a cellular level. Collectively, it appeared that changes occurring with age alter the environment to which cells and tissues of the body are exposed, at least in mice. This can then have a knock-on effect on overall heath and behaviour. Furthermore, while younger blood may contain factors that inhibit or even reverse age-related decline, older blood appears to contain factors that can promote it (6).

> “The old animal is bathed in the blood of a young one”

Clearly, it was important at this point to identify the component or components of the blood responsible for these rejuvenating effects. In experiments involving blood plasma donation, no changes were observed in the mice when the plasma had been heated to a high temperature prior to administration. It is known that high temperatures destroy most of the proteins naturally present in the blood, which indicated that the ‘active component’ was likely to be a blood-borne protein.

The identity of one of these mystery components was revealed in 2013 by a research group studying age-related cardiac hypertrophy at the University of Colorado. Cardiac hypertrophy is a pathological condition in which muscle cells of the heart (cardiomyocytes) become enlarged. This causes them to become weakened until they are unable to continue efficiently pumping blood around the body, often leading to heart failure. Study of this phenomenon in a mouse model once again demonstrated the rejuvenating effects of young blood on the progression of this age-related disease. Just four weeks of heterochronic parabiosis significantly reduced heart mass in older mice and down-regulated expression of genes associated with heart failure.

Next, the group compared the protein content of plasma from old and young animals, and discovered that blood from young mice contained much higher levels of growth and differentiation factor 11 (GDF11). Cardiomyocytes were treated with phenylephrine, which normally causes an increase in the cells’ size, but this hypertrophic response was inhibited when the cardiomyocytes were simultaneously exposed to GDF11. Armed with this information, the group then investigated the effect of treating older mice with the GDF11 protein alone. Encouragingly, GDF11 treatment reversed many symptoms of age-related cardiac hypertrophy (7). Subsequent investigations have demonstrated that supplementing levels of GDF11 in aged mice also rejuvenates the satellite cell population, improving skeletal muscle repair, structure and function (8). Increased GDF11 has been shown to improve neurogenesis and also plays an important role in the formation of new bone, which may protect against development of skeletal fragility (9, 10).

In light of these discoveries, it appears that it may be possible to develop therapies to halt or reverse some processes of aging. However, many unanswered questions still remain, not least of which is whether the effects seen in mice could be recapitulated in humans. There may also be other key unknown rejuvenating factors that could be manipulated in order to decelerate the aging process. In spite of this, the discovery of GDF11 is undoubtedly an exciting twist in the tale of humanity’s unwillingness to accept our own mortality.

References

1. McCay, C.M., *et al.* (1957) Parabiosis between old and young rats. *Gerontologia.* 1:7–17.
2. Tauchi, H. & Sato, T. (1980) Changes in hepatic cell mitochondria during parabiosis between old and young rats. *Mech. Ageing. Dev.* 12:7–14.
3. Conboy, I.M., *et al.* (2003) Notch-mediated restoration of regenerative potential to aged muscle. *Science.* 302:1575–1577
4. Conboy, I.M., *et al.* (2005) Rejuvenation of aged progenitor cells by exposure to a young systemic environment. *Nature.* 433:760–764.
5. Villeda, S.A., *et al.* (2014) Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. *Nat. Med.* 20:659–663.
6. Villeda, S.A., *et al.* (2011) The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature.* 477:90–94.
7. Loffredo, F.S., *et al.* (2013) Growth Differentiation Factor 11 Is a Circulating Factor that Reverses Age-Related Cardiac Hypertrophy. *Cell.* 153:828–839.
8. Sinha, M., *et al.* (2014) Restoring Systemic GDF11 Levels Reverses Age-Related Dysfunction in Mouse Skeletal Muscle. *Science.* 344:649–652.
9. Zhang, Y., *et al.* (2015) Growth differentiation factor 11 is a protective factor for osteoblastogenesis by targeting PPARgamma. *Gene.* 557:209–214.
10. Katsimpardi, L., *et al.* (2014) Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. *Science.* 344:630–634.

Caitlin Clunie-O’Connor is a second year DPhil student in Prof Paul Riley’s group in the Department of Anatomy, Physiology and Genetics

Optical detection of premalignant oesophageal cancer

by
Edyta
Augustyniak

The incidence of oesophageal cancer is rising worldwide, yet treatment options are limited. On average, only 15 of every 100 patients will survive another 5 years following diagnosis of oesophageal cancer. The oesophagus, or gullet, is a long tube that carries food from the throat to the stomach. There are two main types of oesophageal cancer: squamous cell carcinoma, found in the upper part of the oesophagus, and adenocarcinoma, which forms in the lower part. Both involve abnormal multiplication of the cells that line the oesophagus or lie inside mucosal glands, respectively.

Poor prognosis in oesophageal cancer is linked to late diagnosis, when the cancer has become advanced and already spread to local lymph nodes or to other parts of the body, in a process called metastasis. Although chemotherapy and radiotherapy are used to control tumour size in patients with metastasising oesophageal cancer, these treatments are not effective enough to provide a cure. Common symptoms of oesophageal cancer include difficulties swallowing, weight loss, throat pain and persistent cough, which is often initially ignored or does not occur until the tumour is of significant size and has become malignant.

Risk factors for oesophageal cancer include tobacco use, poor diet and alcohol consumption. In the case of adenocarcinoma, gastroesophageal reflux disease (GERD) and a condition called Barrett's oesophagus are associated with higher risk of oesophageal cancer (1). Barrett's oesophagus involves transformation of epithelial cells of the lower oesophagus into metaplastic columnar intestinal-like cells. In about 0.1% of patients, metaplastic cells undergo further genetic changes and become dysplastic, where the tissue begins to proliferate and/or differentiate abnormally.

Patients with Barrett's oesophagus undergo regular screening, during which a doctor takes multiple biopsies in the affected area of the oesophagus. These biopsies are used to look for the presence and grade of dysplasia and malignancy. Other screening methods include immune staining for the presence of the progression markers to predict the disease course, to calculate the risk of developing adenocarcinoma. Endoscopy, on the other hand, is an optical screening method that uses a long flexible tube with a camera and a light source to take internal images of the oesophagus. However, dysplastic changes are difficult, if not impossible, to detect under visible light using a conventional endoscope. As a result the early stages of early oesophageal cancer are frequently overlooked (1).

Professors Katherine Vallis and Boris Vojnovic from the Department of Oncology have teamed up to develop a novel optical method of detecting early oesophageal cancer using antibodies against markers of early precancerous changes. The first challenge is to find a novel marker to detect premalignant changes in epithelial cells. Secondly, specially engineered high-affinity antibodies or minibodies (antigen-specific antibody fragments) tagged with a near-infrared dye will be developed. Ideally, these antibodies would be applied atopically to patients, shortly before undergoing endoscopy using a visible light endoscope with a built-in near-infrared detector. This would enable detection of precancerous markers in real time, combining visible light and near infrared images. If successful, it could become a routine method for precision screening of oesophageal cancer in high-risk or Barrett's oesophagus patients. Such patients could then be treated before transformed cells become malignant, thereby improving their chances of survival.

Edyta Augustyniak is a first year DPhil student in the Department of Oncology

References

1. Wild, C.P. & Hardie, L.J. (2003) Reflux, Barrett's oesophagus and adenocarcinoma: burning questions. *Nat. Rev. Cancer*. 3(9):676–84.

Molecular mechanisms in bone-metastatic prostate cancer

by Dr Srinivasa Rao

Prostate cancer is the most common cancer in men in the Western world, with as many as 417,000 men diagnosed in Europe every year (1). The late stages of prostate cancer are characterised by increasing metastases to distant organs. Up to 75% of these occur in the skeletal system and carry a mean 5-year survival of 25%. While distant metastasis are common in many cancers, the predilection of prostate cancer cells to metastasise to bone is shared by only a few other malignancies, including breast cancer and multiple myeloma.

Metastasis to the bone

It was previously thought that bones are robust structures that hardly change throughout adult life. We now know that this is not the case; the skeletal system is in constant flux, with some cells (osteoclasts) breaking old bone down and other cells (osteoblasts) building new bone. This process of bone remodelling is normally in a state of balance, with no net loss or gain of bone unless needed. When prostate cancer cells colonise bone, they upset this balance, increasing both abnormal bone formation and bone destruction (2). The new bone formed near the metastatic tumour is structurally unsound; this, along with the bone destruction, makes the skeletal site susceptible to fractures. Consequently, fractures of the long bones and spine are very common among men with metastatic prostate cancer.

Osteomimicry

Why do prostate cancer cells target bone? Paget proposed the 'seed and soil' hypothesis to explain breast cancer metastasis, whereby cancer cells colonise distant sites with favourable conditions (3). This very likely also applies to prostate cancer cells, which could be drawn to the bone microenvironment by chemoattractant factors that are produced by bone cells during the remodelling process. Another theory proposes that prostate cancer cells can mimic bone cells (osteomimicry), allowing them to better survive in the bone microenvironment (4). In support of this, prostate cancer cells overexpress factors that are well-established markers of bone cells and not normally expressed in prostate cells, like the receptor activator of nuclear factor kappa-B ligand (RANKL) and osteopontin.

Our research group has been studying osteomimetic features that enhance metastatic potential in prostate cancer cells, particularly in the switch from less aggressive (epithelial) to a highly metastatic (mesenchymal) phenotype which is termed as epithelial-mesenchymal transition (EMT) (5). We have identified that an osteoblast-specific factor is a crucial regulator of EMT, controlling mechanisms like cell-cell adhesion, migration and alteration of cell morphology.

Cancer-bone interactions in a petri dish

As mentioned before, the interaction between prostate cancer cells and bone cells is central to the pathology of prostate cancer bone metastasis. Common models to study cancer-bone cell interactions *in vitro* involve co-culturing either osteoblasts or osteoclasts with prostate cancer cells. This ignores the variety of cell-cell and cell-matrix interactions that are essential for the establishment of the bone-tumour niche *in vivo*. We are addressing this by directly co-culturing fresh human bone explants with human prostate cancer cells, and we expect this to be a more accurate model of cancer-bone interactions because the explants represent the bone microenvironment in its entirety: bone, fat, blood, immune cells and even the organic and inorganic bone matrix.

Our research aims to explore the signalling cross-talk between prostate cancer cells and the bone microenvironment. While it is likely that a multitude of factors may be responsible for prostate cancer cells' tendency to metastasise to bone, discovering and characterising as many of these factors as possible would give us a fighting chance to protect against prostate cancer metastasis.

References

1. Cancer Research UK (2014) Cancer statistics: Key facts. Available at http://publications.cancerresearchuk.org/cancerstats/statsprostate/keyfactsprostate.html/ [Accessed 14th April 2015]

2. Mundy, G.R. (2002) Metastasis: Metastasis to bone: causes, consequences and therapeutic opportunities. *Nat. Rev. Cancer.* 2(8):584–593.

3. Paget, S. (1889) The distribution of secondary growths in cancer of the breast. *Lancet.* 133(3421):571-573

4. Rucci, N. & Teti, A. (2010) Osteomimicry: how tumor cells try to deceive the bone. *Front. Biosci.* 2:907–915.

5. Kalluri, R. & Weinberg, R.A. (2009) The basics of epithlial-mesenchymal transition. *J. Clin. Invest.* 119(6):1420–1428.

Srinivasa Rao recently completed his doctorate and is now a post-doctoral researcher in Dr James Edwards' group, Nuffield Dept. of Orthopaedics, Rheumatology and Musculoskeletal Sciences

Tracking and Identifying New

"epidemic over a very large area; affecting a large proportion of the

Natalie Connor-Robson & Óscar Cordero-Llana

The WHO is continua
to monitor global infe
pandemic the organis
tracking its spread an

Centre for Disease Control has a major role in disease tracking using both traditional and new techniques.

#flu
#temperature
#influenza

INFLUENZA

By analysing search terms and tweets around the globe, real time tracking of flu pandemics is being tackled by the likes of Google and Twitter. There are around 55 million tweets per day to sort through, meaning a potential goldmine of data. Initial studies have matched CDC predictions well. These methods cut the 1-2 week lag time required to collect and process surveillance data.

EBOLA

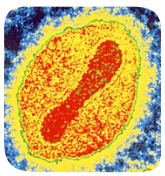
Ebola Google
Searches Hotspots

Monitoring diseases in animals is an important aspect of pandemic tracking. Zoonosis is the ability of infectious agents to move from animals to humans, potentially causing a new pandemic. The most recent example being Ebola whose reservoir host is likely bats. To date the WHO has reported 24,700 cases of Ebola.

Global travel allows for fast a
efficient spread of infectious
In 2014 there were **37.4 mi**

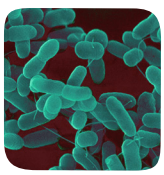
Total Deaths

Causative agent
Emergence



300 million

Variola virus
Small pox (1000 B.C.)



25 million

Yersinia pestis
Black death (1334)

Pandemics

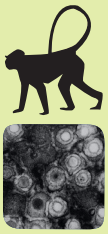
e population"

ally collecting samples and data
ectious disease chatter. During a
sation takes a leading role on
d implementing treatments.

Genetic sequencing is another weapon in our arsenal against pandemics, enabling fast identification of new infections. For example, tracking mutations that make the infection more transmissible. It also allows us to follow genetic evolution of such agents and identify potential changes that make the agent more deadly comparing the genetic sequence with similar strains.

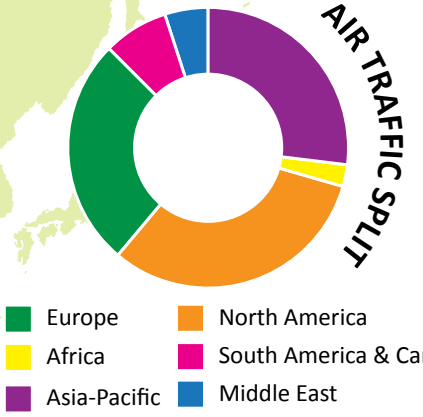
Routes of animal-to-human spread include bush meat hunting and consumption, live markets and farming. New pathogens with pandemic potential can be monitored by taking samples from farms, markets and abattoirs. Taking blood samples from high risk individuals with frequent animal contact is another way to stay ahead.

INFLUENZA

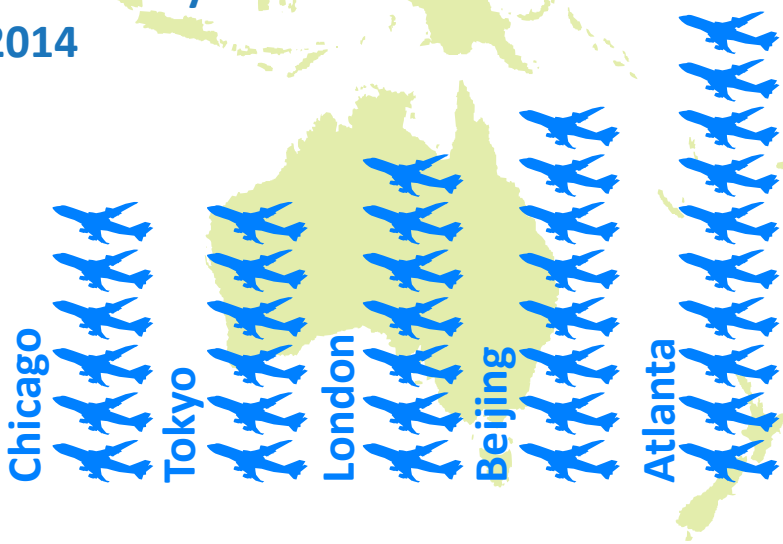


HIV

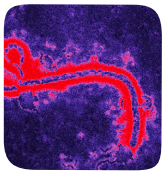
2.97 billion
passengers carried by airlines in 2014



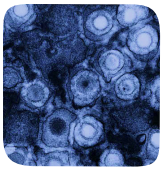
and
diseases.
illion commercial flights!!



50 million
Influenza virus
Spanish flu (1918)



12,000
Ebola virus
Ebola (1976)



39 million
HIV
AIDS (1981)

Worms, germs and genes

by
Dr Delia
O'Rourke

The nematode worm *Caenorhabditis elegans* is an incredibly useful model organism. Its small size, rapid generation time, well-annotated genome sequence, and biological reagents have made it ideal for investigating diverse areas of molecular biology, development, evolution, and infection. *C. elegans* is prey to natural nematode-specific pathogens (1) but can also be infected by human pathogens in the laboratory, including some that cause serious illness or death. Consequently, the worm is a useful model for many human bacterial and fungal infections, such as *Staphylococcus aureus* and *Candida albicans* amongst others (2), and can be used in high-throughput screens for antimicrobials (3). Furthermore, although *C. elegans* itself is harmless, certain nematode species are important parasites of humans, pets, livestock and crops. *C. elegans* is an important model for these parasitic nematode infections, and has led to the discovery of the anthelmintic toxin Cry5b used to treat *Ascaris suum* and *Ascaris lumbriocides*, an infection in pigs and humans (4). Worms lack an adaptive immune system, relying on innate immune responses, avoidance behaviours, and the physical barrier of their cuticle to resist infection. Here, I will describe how our laboratory is using the worm to understand host-pathogen interactions and, in particular, the role of the worm surface in pathogen resistance.

The *C. elegans* surface

The surface coat of a nematode constitutes the major interface between the worm and the outside world, and is an important barrier to small molecules and toxins. It is also of critical importance for immune responses to parasitic nematode infections, and influences the design of anthelmintic drugs.

C. elegans is covered by a thick but flexible extracellular collagenous cuticle, composed of multiple layers. The cuticle maintains body shape, enables motility, and protects the worm from the environment. The outermost component, the surface coat or glycocalyx, is a 5 nm glycoprotein-rich layer. It is associated with immune evasion in parasitic nematodes and is important for locomotion in *C. elegans*. Below the glycocalyx lies the epicuticle, an electron-dense lipid-rich layer resembling a thick trilaminate plasma membrane. Beneath the epicuticle are the cortical, medial and basal zones, each comprising an extracellular matrix of collagens and cuticulins (5).

Biochemical analysis of the nematode outer surface has been surprisingly limited. Binding of fungal tectonins to the surface of wild type *C. elegans* suggests that *O*-methylated glycans are exposed (6), and genetic screens identified mutants srf-2, srf-3 and srf-5 (**s**ur**f**ace antigenicity abnormal) with altered surface-exposed glycoproteins (7), suggesting that the worm has a glycoprotein-rich surface. However, specific components remain to be identified.

Using bacterial infection to characterise the surface of *C. elegans*

We are primarily interested in natural and human pathogens that attack the surface coat of *C. elegans*. These include *Yersinia psedotuberculosis*, an important model for plague biofilm formation and two natural *Caenorhabditis* pathogens *Leucobacters* Verde1, which attaches all over the worm, and Verde2, which attaches to the post-anal cuticle (Figure 1).

Verde1 does not kill worms grown on agar plates covered with a bacterial lawn, whereas Verde2 does (8). In liquid culture, Verde1-colonised worms aggregate into lethal 'worm stars', with the bacteria sticking to their tails (8). Our lab has identified over 20 gene knockouts that alter susceptibility to infection by these bacteria. These bus (**b**acterially **u**n**s**wollen) genes were found through a combination of genetic screens and high-throughput sequencing of chemically- and transposon-induced mutants and include alleles of srf-2, srf-3 and srf-5 (9). Many of the encoded proteins are involved in surface synthesis or post-translational protein modifications like *N*-and *O*-glycosylation. Other bus genes encode proteins involved in intracellular transport, lipid metabolism and glycosylphosphatidylinositol-anchor biosynthesis. We also identified nematode-specific membrane proteins of unknown function, as well as secreted proteins that might be surface components. The disorganisation of the surface in these mutants increases drug sensitivity; thus bus mutants are used in high-throughput drug screens (10).

To investigate the biochemistry of the surface changes, our collaborators analysed worm glycomes of three mutants for the glycosyltransferase bus-2, the galactosyltransferase bus-4, and the nucleotide sugar transporter srf-3. In all cases, Ce core-I *O*-glycans were dramatically altered (11), implicating these in bacteria-related pathogenesis. However, there were also large increases in mucin *O*-glycans in bus-2 and bus-4 mutant glycomes, suggesting that saturating the surface with protective mucins may prevent bacterial attachment.

Ether lipids and *agmo-1*

Interestingly, knockouts of many genes that confer resistance to *Leucobacter V*erde2 induce extreme hypersensitivity to 'worm star' pathogen Verde1. Moreover, srf and bus mutants that are lethally susceptible to Verde1 may gain resistance through secondary mutations in suppressor genes, such as

agmo-1 (**a**lkyl**g**lycerol **m**ono**o**xygenase). AGMO-1 is a tetrahydrobiopterin (BH4)-dependent enzyme that degrades ether lipids (alkylglycerols) into aldehyde and glycerol derivatives. In both *C. elegans* and mammals, this is a unique enzymatic activity (12) but its physiological role is unclear.

Analysis of agmo-1 mutants suggests a loss of AGMO-1 activity. The cofactor BH4, required for AGMO-1 function, is synthesised from GTP by GTP cyclohydrolase1 (GCH), PTP synthase (PTPS) and sepiapterin reductase. Mutations in the worm orthologs of GCH and PTPS (cat-4 and ptps-1) were also found in the Verde1 suppressor screens. They act presumably by preventing BH4 synthesis, thus inhibiting AGMO-1 function. The agmo-1, cat-4 and ptps-1 mutations all prevent binding of Verde1 to the worm surface, confirming that they protect Verde1-sensitive strains by preventing bacterial attachment.

Loss of AGMO-1 function might cause accumulation of ether lipids on the nematode surface. We hypothesise that this masks the targets of Verde1 binding. Alternatively, if AGMO-1 affects the biosynthesis of the worm surface, then agmo-1 mutants might lack a Verde1 binding target or cause a collapse in surface structure and loss of targets. We are collaborating with Tony Watts' group to identify surface lipids and proteins in wild-type and agmo-1 worms to establish how loss of AGMO-1 function rescues Verde1 sensitivity.

Summary

Studying 'Worms, germs and genes' has produced new insights into the biosynthesis of the nematode surface, improved the utility of *C. elegans* in high-throughput drug screens, and helped discover a physiological role for agmo-1, which may help to elucidate the role of AGMO-1 and ether lipids in mammals. Further lessons about parasitic nematodes may be learned by applying our methods used to characterise the *C. elegans* surface to other species. Advances in lipidomics, such as lipodisq nanoparticles and lipid characterization by mass-spectrometry, now permit the analysis of nematode surface proteins, lipids, and glycolipids. Understanding nematode host-pathogen interactions may also help in the control of parasitic nematode infections.

References

1. Frézal, L. & Félix, M.A. (2015) *C. elegans* outside the Petri dish. *eLife.* 2015;4:e05849.

2. Marsh, E.K. & May, R.C. (2012) *Caenorhabditis elegans,* a model organism for investigating immunity. *Appl. Environ. Microb.* 78(7):2075–2081.

3. Moy, T.I., *et al.* (2009) High Throughput Screen for Novel Antimicrobials using a Whole Animal Infection Model. *A.C.S. Chem. Biol.* 4(7):527–533.

4. Urban, J.F., *et al.* (2013) Bacillus thuringiensi-derived Cry5B has potent anthelmintic activity against *Ascaris suum. PLoS Negl. Trop. Dis.* 7(6):e2263.

5. Altun, Z.F., *et al.* (ed.s) (2002-2015) WormAtlas. Accessed at http://www.wormatlas.org.

6. Wohlschlager, T., *et al.* (2014) Methylated glycans as conserved targets of animal and fungal innate defense. *P. Natl. Acad. Sci. U.S.A.* 111(27):E2787–E2796.

7. Link, C.D., *et al.* (1992) Genetics Characterization of Caenorhabditis elegans lectin-binding mutants. *Genetics.* 131(4):867–881.

8. Hodgkin, J., *et al.* (2013) Two Leucobacter strains exert complementary virulence on *Caenorhabditis* including death by worm-star formation. *Curr. Biol.* 23(21) 2157–2161.

9. Gravato-Nobre, M.J., *et al.* (2005) Multiple genes affect sensitivity of *Caenorhabditis elegans* to the bacterial pathogen *Microbacterium nemaatophilum. Genetics.* 171(3):1033–45.

10. Partridge, F.A., *et al.* (2008) The *C. elegans* glycosyltransferase BUS-8 has two distinct and essential roles in epidermal morphogenesis. *Dev. Biol.* 317(2):549–559.

11. Parsons, L.M., *et al.* (2014) *Caenorhabditis elegans* bacterial pathogen resistant bus-4 mutants produce altered mucins. *PLoS One.* 9(10):e107250.

12. Watschinger, K., *et al.* (2010) Identification of the gene encoding alkylglycerol monooxygenase defines a third class of tetrahydrobiopterin-dependent enzymes. *P. Natl. Acad. Sci. U.S.A.* 107(31):13672–13677.

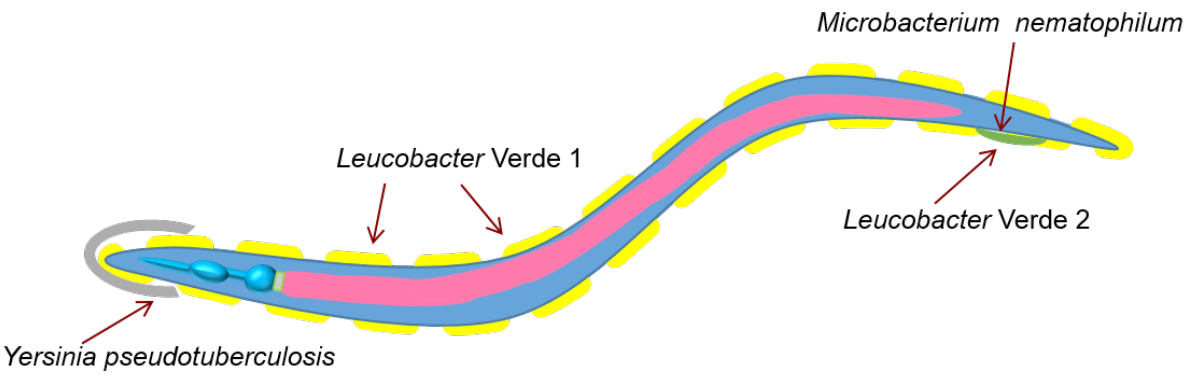


Figure 1: Schematic of an adult worm, showing the locations of bacterial attachment to the worm surface: *Yersinia pseudotuberculosis* (green) to the head, *Microbacterium nematophilum* and *Leucobacter* Verde2 (orange) to the post anal cuticle and *Leucobacter* Verde1 (yellow) to the entire surface.

Dr Delia O'Rourke is a post-doctoral researcher in the Hodgkin Group in the Department of Biochemistry, and a Stipendiary Lecturer in Biochemistry at Hertford College.

How influenza hijacks transcription machinery

by
Dr Mónica Martínez Alonzo

Seasonal influenza results in three to five million cases of severe illness each year and can cause up to half a million deaths. Influenza also causes unpredictable pandemics, which leave people defenceless as available vaccines would not protect against pandemic viruses and a matching vaccine would not be ready in time. In addition, few antivirals are available and viral resistance is a growing issue. It is therefore important to develop new lines of defence by expanding our understanding of the interaction of influenza viruses with their hosts.

Influenza viruses propagate in the nucleus of infected cells, which gives them access to the transcriptional machinery of the host. Viral transcription and replication are carried out by viral ribonucleoprotein complexes (vRNPs), which consist of a heterotrimeric polymerase bound to a viral RNA segment that is wrapped around a nucleoprotein oligomer forming a helical structure. However, initiation of viral transcription within the host nucleus requires the virus to steal capped primers derived from the host cellular mRNA through 'cap-snatching' (1). Hence, influenza requires active transcription by the host RNA polymerase II (Pol II) to provide a supply of cellular transcripts.

Pol II is a large enzymatic complex. Its largest subunit, Rpb1, possesses a unique Carboxy-Terminal Domain (CTD) tail. This tail is flexible, unstructured and serves as a landing platform for cellular factors involved in transcription and mRNA processing. The CTD is composed of a repeating, seven amino acid consensus sequence (YSPTSPS). These amino acids can be subject to post-translational modifications which constitute the 'CTD code'. This code coordinates progression through the transcription cycle and couples transcription with mRNA processing. In particular, phosphorylation of serines correlates with the stages of cellular transcription. Pol II with a hypophosphorylated CTD binds at promoters and engages in transcription initiation, when serine 5 phosphorylation peaks. As Pol II transitions to elongation, levels of phosphorylated serine 5 decrease while serine 2 phosphorylation increases (2).

The influenza virus polymerase interacts with the Pol II form engaged in transcription initiation (3). At this point, transcription is paused and cellular pre-mRNAs are being capped. By targeting Pol II at this stage, the virus might place itself at the right time and in the right place to snatch the capped primers required for its own transcription. However, the molecular details of this interaction remain elusive; it is unclear whether the viral polymerase interacts directly with the CTD of Pol II, or indirectly via mediating cellular factors, and whether the viral polymerase is assembled into vRNPs (Figure 1).

In this regard, a significant advance in this field is the recent crystallisation of the polymerase of two different influenza viruses (4,5). Future research will be directed towards co-crystallising these with their cellular targets; this will shed light on the interaction of influenza virus polymerase with Pol II. Finally, mapping the binding domain(s) in the viral polymerase will provide new targets for rational design of novel antivirals.

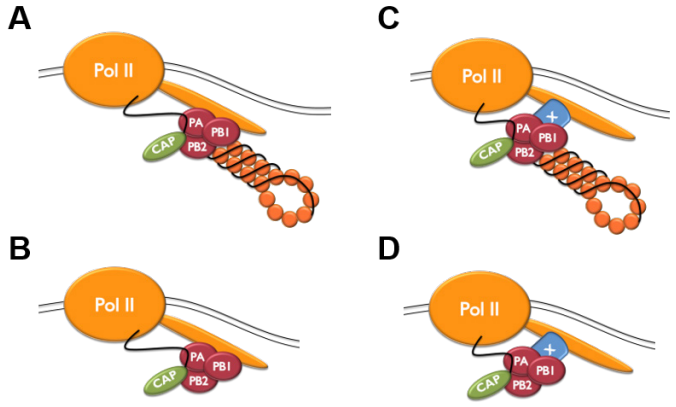


Figure 1: The interaction of influenza virus polymerase with the CTD of Pol II could be direct (A,B) or indirect (C,D) and could involve a fully assembled vRNP (A,C) or a free polymerase (B,D).

Dr Mónica Martínez Alonso is a Marie Skłodowska-Curie Fellow at the Sir William Dunn School of Pathology

References

1. Fodor, E. (2013) The RNA polymerase of influenza A virus: mechanisms of viral transcription and replication. *Acta. Virol.* 57(2):113–122.
2. Eick, D. & Geyer, M. (2013) The RNA polymerase II carboxy-terminal domain (CTD) code. *Chem. Rev.* 113(11):8456–8490.
3. Engelhardt, O.G., *et al.* (2005) Association of the influenza A virus RNA-dependent RNA polymerase with cellular RNA polymerase II. *J. Virol.* 79(9):5812–5818.
4. Pflug, A., *et al.* (2014) Structure of influenza A polymerase bound to the viral RNA promoter. *Nature.* 516(7531):355–360.
5. Reich, S., *et al.* (2014) Structural insight into cap-snatching and RNA synthesis by influenza polymerase. *Nature.* 516(7531):361–366.

Connecting the DOTs

by Laura Godfrey

Epigenetics is a hot topic in gene regulation and disease. There are multiple types of epigenetic changes, one of which is the modification of histone proteins; the proteins around which DNA is wrapped to form nucleosomes. Histones can be modified in a number of ways. One such modification is methylation, which is implicated in both gene activation and repression. Many histone tail residues can be methylated by histone lysine methyltransferases (KMTs). Disruptor of Telomeric Silencing 1 (DOT1) is a KMT that is unique in its ability to mono-, di- or trimethylate lysine 79 of histone 3 (H3K79), a core histone residue.

DOT1 was first identified in a genetic screen with yeast, in which DOT1 overexpression led to telomeric silencing (1). The DOT1 methyltransferase was subsequently found to be conserved in most eukaryotic organisms, ranging from trypanosomes to humans, where it is referred to as DOT1L. DOT1L is distinct from canonical KMTs due to its structurally diverse catalytic domain. It has been suggested that DOT1L is more structurally similar to arginine methyltransferases, indicating that the evolution of DOT1L is divergent from other canonical KMTs.

DOT1L-mediated H3K79 methylation is primarily associated with gene activation, as H3K79 methylation is often found downstream of the transcription start site of actively transcribed genes. Many early studies were carried out in yeast where over 90% of the yeast genome was shown to both have the H3K79 methylation and be actively transcribed (2). In more recently published data, the role of H3K79 methylation in more complex eukaryotic organisms has been explored to demonstrate its association with gene activation. H3K79 methylation has been found to correlate with RNA polymerase II, the enzyme which reads and transcribes DNA, demonstrating a relationship between DOT1L and H3K79 methylation in actively transcribed genes. Little is known about the molecular mechanisms underpinning how methylation functions in transcription, but research linking H3K79 methylation to gene activation does highlight DOT1L as an important protein for further study.

DOT1L-mediated H3K79 methylation is critical in early embryonic development. Murine DOT1L knockout models have shown lethality at embryonic day 10.5 of development. Embryos that survive to this stage display abnormal cardiac development and severe anaemia, highlighting the importance of DOT1L in embryonic blood development or haematopoiesis. As well as being crucial in embryonic development, DOT1L is important in adult haematopoiesis. The phenotype of adult DOT1L knockout mice includes severe anaemia resulting in lethality after 8–12 weeks. This emphasises the pivotal role of DOT1L throughout normal embryonic and postnatal development, and in blood maintenance.

DOT1L and subsequent H3K79 methylation has also been implicated in a form of leukaemia known as MLL-rearranged leukaemia. This disease stems from a chromosomal translocation event in which the Mixed Lineage Leukaemia 1 (MLL) gene is fused to one of over 70 different fusion partners, creating an MLL fusion protein. This type of leukaemia is extremely aggressive and is found in 70% of infant acute lymphoblastic leukaemias, with most patients having a poor prognosis. In these aggressive leukaemias, the mistargeting of DOT1L to the MLL fusion protein leads to hypermethylation of H3K79. This, in turn, contributes to the aberrant up-regulation of genes important in leukaemia pathogenesis (Figure 1). Small molecule inhibitors of DOT1L, which block its methyltransferase activity, are now in stage I clinical trials as a promising potential therapy for MLL-rearranged leukaemias. Recently published data has also shown DOT1L inhibitors to be effective against other types of leukaemia and various types of breast cancer (3). This highlights the benefits of therapeutically targeting DOT1L for the treatment of a range of human cancers and emphasises the importance of further investigating this significant but poorly understood methyltransferase.

References

1. Singer, M.S., *et al.* (1998) Identification of high-copy disruptors of telomeric silencing in Saccharomyces cerevisiae. *Genetics.* 150(2)613–632.

2. Van Welsem, T., *et al.* (2008) Synthetic lethal screens identify gene silencing processes in yeast and implicate the acetylated amino terminus of Sir3 in recognition of the nucleosome core. *Mol. Cell. Biol.* 28(11):3861–3872.

3. Sarkaria, S.M., *et al.* (2014) Primary acute myeloid leukemia cells with IDH1 or IDH2 mutations respond to a DOT1L inhibitor in vitro. *Leukemia.* 28(12):2403–2406.

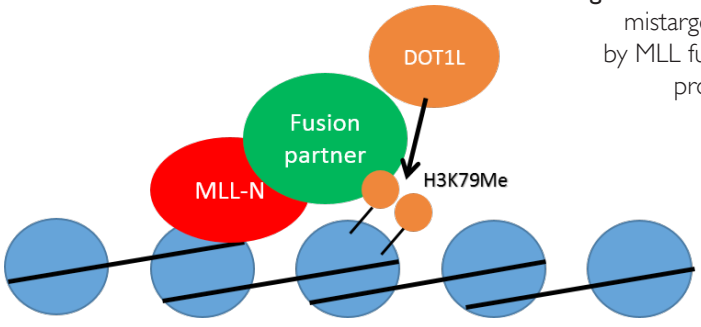


Figure 1: DOT1L mistargeting by MLL fusion protein

Laura Godfrey is a 2nd year DPhil student at the Weatherall Institute of Molecular Medicine (WIMM)

Tumour acidosis: The known unknown

by Kevin Ray

Tumours have long been known to have an altered metabolic status, which drives ATP production via the glycolytic pathway. This is a hallmark of cancerous cells and high levels of glycolysis produces a large abundance of lactate. In a solid tumours, lactate can accumulate in the extracellular space, inducing marked extracellular acidosis and mild intracellular alkalosis.

Studies have shown a correlation between the level of extracellular acidosis and tumour aggression, metastasis, or resistance to chemo- and radiotherapeutic treatments. From a clinical perspective, measurement of the level of acidosis could allow the stratification of patients, which would aid treatment selection and improve patient benefit. And yet, measuring tumour acidosis in the clinic is not commonplace, except in the most accessible of tumours, such as oesophageal and mouth carcinomas.

Non-invasive imaging methods promise to revolutionise the identification of tumour acidosis, because they aim to detect acidosis wherever the site may be located in the human body. Such methods are based on magnetic resonance spectroscopy (MRS). Phosphorous MRS measures the chemical differences between inorganic phosphate and phosphocreatine, which is related to pH by the Henderson-Hasselbalch equation. In addition, standard proton MRS can also be used to measure the resonant frequency of protons, which can be related to pH, on exogenous contrast agents such as the imidazole IEPA (1).

MRS measurements of pH are, however, hindered by a significant lack of sensitivity. The measurements usually obtain average spectra over a 5 mm^3 region, which is sometimes an insufficiently large volume to pick up inhomogeneities in the microenvironment of tumours, and can also make it difficult to exclude non-cancerous tissue. Furthermore, the acquisition times for these measurements are still impractically long for a high throughput clinical environment.

Since 2000, a new technique in magnetic resonance imaging (MRI), known as chemical exchange saturation transfer (CEST), was developed for making non-invasive pH measurements. In a standard MRI scan, the signal observed is from the 'bulk' water protons. In a CEST-MRI scan, the signal from exchangeable (or 'labile') protons on proteins and small molecules (such as those found in amide, amine and hydroxyl groups) can be visualized instead.

Of particular interest is the exchange of amide protons, mainly found on the backbone of proteins and peptides, because the rate at which these protons exchange is pH-dependent. Therefore the rate of exchange of these labile protons, and hence the pH, can be measured by CEST-MRI.

CEST-MRI has been predominantly used in clinical studies of stroke as well as glioblastoma and Alzheimer's disease. Work recently published from Oxford University showed that pH-weighted CEST-MRI of acute stroke patients is predictive for tissue that appears well perfused initially, but later becomes part of the stroke lesion (2). This can help clinicians avoid risky and invasive procedures that will have no benefit for the patient.

However, the application of CEST-MRI to measuring the pH of tumours is complicated by various confounding factors that can contribute to the signal. These include the exchangeable proton concentration, and magnetic properties of the labile protons and bulk water protons. A novel metric to analyse CEST-MRI data developed in Oxford has gone some way to measuring only the contribution of pH to the signal (3), but more work remains to be done to validate this technique. With an interdisciplinary team of engineers, physicists, biologists, chemists and clinicians, we are well placed to make great progress in the field and, hopefully, make the 'known unknown' of tumour acidosis…known.

References

1. Van Sluis, R., *et al.* (1999) *In Vivo* Imaging of Extracellular pH Using 1H MRSI " *Magnetic Resonance in Medicine.* 41(4):743–750.

2. Harston, G.W.J., *et al.* (2015) Identifying the ischaemic penumbra using pH-weighted magnetic resonance imaging. *Brain* 138(1):36–42.

3. Chappell M.A., *et al.* (2013) Quantitative Bayesian model-based analysis of amide proton transfer MRI. *Magnetic Resonance in Medicine.* 70(2):556–7.

4. Tee, Y.K., *et al.* (2014) Comparing different analysis methods for quantifying the MRI amide proton transfer (APT) effect in hyperacute stroke patients. *NMR in Biomedicine.* 27(9):1019–29.

Kevin Ray is a first year DPhil student in the Department of Oncology, jointly supervised by Prof Nicola Sibson and Prof Michael Chappell (IBME)

A CAREER AS A PATENT ATTORNEY . . .

. . . a challenging and rewarding option for Oxford Biochemists

Training as a Patent Attorney is a career path that will enable you to combine your understanding of biochemistry and related disciplines with legal expertise. You will leave the lab environment yet remain at the cutting edge of science and technology, applying your knowledge and skill in a commercial context. Your role will be to help to protect intellectual property assets and grow businesses.

Sound interesting? J A Kemp is a leading firm of Patent and Trade Mark Attorneys with one of the largest concentrations of biotech and pharmaceutical patent expertise in Europe. Three quarters of the firm's Attorneys studied at Oxford or Cambridge, including several Oxford Biochemists. Many have doctorates.

"Commentators praise J A Kemp's attorneys for their 'intelligence, creativity and ingenuity' and are delighted with the consistently high quality of their work."

The Legal 500

"J A Kemp attorneys are extremely sharp, extremely capable and always produce quality work in the life sciences field."

IAM Patents 1000

To find out more visit
www.jakemp.com/careers

J A ♦ KEMP
PATENT ATTORNEYS • TRADE MARK ATTORNEYS

UNIVERSITY OF OXFORD

Isis INNOVATION

TECHNOLOGY TRANSFER AND CONSULTANCY FROM THE UNIVERSITY OF OXFORD

Bringing Isis Innovation closer to Researchers

Isis Innovation now has hot-desks in a number of Oxford University departments.

Isis staff at these locations are available to discuss intellectual property, technology transfer, software commercialisation or academic consultancy services with Oxford researchers, students and support staff.

See **http://tiny.cc/spokes** for a full list of all locations of new and existing hot-desks and when Isis staff will be available.

+44 (0)1865 280830 **www.isis-innovation.com** **innovation@isis.ox.ac.uk**

Careers Insights: Patent Attorney

"Where the business of science comes to life." You may have encountered, ridden on, or been cut up by an Oxford Science Park branded bus or taxi. As the slogan at the top of these vehicles suggests, there is more to science than the pursuit of knowledge. In the words of the Science is Vital campaign, "research enriches society and helps drive the economy". In this regard, the patent attorney can play a key role.

by Dr. Kapil Tuladhar, Trainee Patent Attorney at J A Kemp

What is a patent?

A patent is a legal document that provides a time- and geographically-limited monopoly right.

A patent can be granted for new and inventive subject matters, in exchange for disclosure of the invention.

Patents can be traded in a similar way to physical property. Patents can be deployed as defensive or offensive business tools.

Like many students coming to the end of a science degree - in my case an enjoyable DPhil 'up-the-hill' with Roger Patient - I decided that a career as a professional scientist was not for me. After talking to a patent attorney in the basement of the Sheldonian Theatre, it became clear that training as a patent attorney would allow me to continue to apply the skills that I had developed, in an intellectually stimulating environment.

What we do

On a daily basis, patent attorneys and trainees are exposed to a variety of diverse technologies that are at the leading edge of research. In the life sciences field, you may find yourself working on a new biological molecule such as a new antibody for disease therapy, a new diagnostic test, a new DNA sequencing technique, an invention in the field of stem cells/ regenerative medicine, a transgenic plant or even a new vaccine.

Working in a private practice firm, as I do, you will find yourself acting as an intermediary between clients and patent offices. Your client may be a sole inventor, a University technology transfer office (like ISIS Innovation) or a large corporation. The work ranges from drafting new applications, through arguing about or amending patent applications to convince patent offices that patents should be granted, to defending and attacking patents. You not only apply and develop your scientific knowledge but also your legal knowledge, all in the context of the client's commercial goals.

There is a lot to learn, and difficult exams to pass. While much can be picked up on-the-job, it is inevitable that, as exams loom, you will need to study outside the office. You will also find a supportive network of colleagues and peers willing to provide advice and guidance.

Although the work is heavily deadline-driven, in most cases there are long lead times. Coupled with a degree of freedom to manage your own workload, this means that you can still achieve a healthy work-life balance. As you progress, you will find that your role evolves to include broader and more sophisticated elements. For example, you may find yourself advocating in oral hearings at patent offices or assisting colleagues in courts.

Hence, although pursuing a career as a patent attorney is not an easy option, it can be a rewarding one - I am certainly enjoying the challenge!

Further information

There are a lot of publicly available resources providing further information, such as the "Inside Careers" guide (http://www.insidecareers.co.uk/professions/patent-attorneys/). This also discusses training "in-house" in the patent department of a company.

Patent attorneys form a small profession of which there is increasing awareness, so there is a lot of competition for entry-level positions. If applying, it is important to do your research and take any written exercises ahead of interviews seriously. Should you then find yourself in the great position of working for J A Kemp, you could be riding the Science Park bus to work, while thinking about the latest invention!

Career Insights: Industry

Dr Cat Kelly shares her experiences and advice for building a career in industry after academia

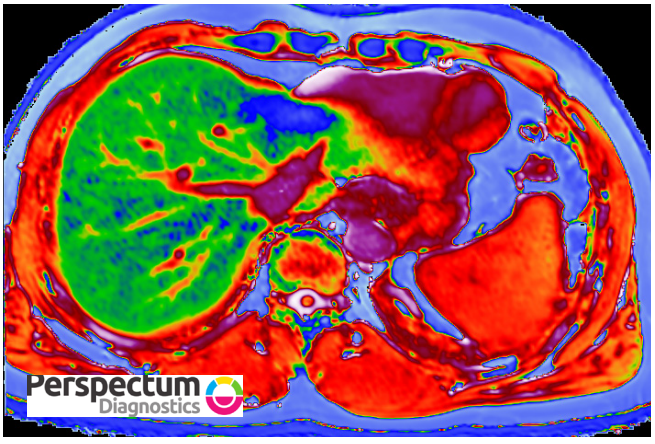
My experience of academia

For my DPhil research, I developed mathematical models describing the kinetics of medical images in hypoxic tumours, namely those that have outgrown their blood supply. By first simulating the distribution of oxygen and then oxygen-dependent imaging tracers on artificial vascular beds of hundreds of different configurations, it is possible to predict the underlying biology from tracer kinetics. After completing my DPhil in 2008, I was keen to spend some time validating this theoretical work with actual data, so I took up a postdoc at the Department of Oncology characterising the development, imaging, and response to therapy of hypoxia in *in vitro* models of tumours (spheroids). As a largely autonomous postdoc, I wrote papers, went to conferences, set up collaborations, supervised students, held a college research fellowship, taught extensively, and spent time abroad working with a different research group. It had its ups and downs, but it was a great experience.

After 10 years at the University, however, I wanted to experience a different kind of scientific development: something closer to the clinic and faster-paced. I must admit that while teaching and analysing data were fun, long repetitive days in the lab were not!

How did I find my job?

I spoke with everyone whose opinion I valued: my DPhil supervisor, the head of my teaching department, and my husband. Through them, I made new contacts and began to understand the different opportunities available to me and, more importantly, what interested me. I found out about my current job during this process. My DPhil supervisor (a 'tech transfer veteran'), was involved in a spin-off company commercialising novel technology, and encouraged me to apply for the position of image analyst.



What does my job entail?

As an imaging scientist, I am responsible for the development of analytical tools to help clinicians interpret images of the liver. As a product manager, I take a more commercial viewpoint, determining what the customer wants and needs, and planning a suitable product. Recently, I have written some code to examine medical images, spoken with the FDA about gaining approval for our product, written a journal article, and discussed the logistics of using our technology in a trial with a pharmaceutical company. I still go to conferences, but primarily as an exhibitor. As the product management needs have expanded, I also needed to recruit new imaging scientists, whom I will line manage.

Running lots of projects can be stressful, but they are often short, meaning that I frequently feel a sense of accomplishment. It's not uncommon to work longer hours when a deadline is approaching. However, the job is predominantly nine-to-five, meaning that I get to spend evenings and weekends with my family, which is a personal priority.

What advice can I offer?

Firstly, do what you enjoy and do it well. When I finally decided that my immediate career trajectory wasn't in academia, I wondered if I'd been too indulgent with my choices to date. I'd always done what interested me – teaching, collaborating with those outside my field, and supervising students. On the contrary, all of these experiences have, in different ways, been useful in my current role. All those hours spent describing concepts to someone outside of my field have proven to be invaluable, i.e. when explaining particular technologies to clinicians and physicists. Supervising the projects of students and research assistants has also provided me with a solid foundation for running a technical team.

Secondly, talk to people in different roles. Ask them what they do on a daily basis, what they like and dislike. Does this fit with what you enjoy doing? How much does the job pay? It may seem an unsavoury question, but be honest with yourself – how much do you need to feel valued? Keep a notebook of interesting people. I still have mine somewhere!

Affiliation:

Cat Kelly is a Senior Imaging Scientist and Product Manager at Perspectum Diagnostics

A scientific perspective of Madagascar

by Anna Senft

The mosquitoes are hungry. With a soft thud, a technician places the crimson container on top of their woven retainer. Instantly, they launch towards the blood. Yellow light emanates from heat lamps over the brooding trays where countless larvae move; ready to hatch.

The mosquito breeding room is one of the core facilities of the Institute Pasteur de Madagascar, Antananarivo. It is the largest such institute in the country, occupied almost entirely by local Malagasy researchers. This situation, however, is quite exceptional. Despite its exotic biodiversity, often featuring in scientific journals, most research in Madagascar is not conducted by the Malagasy people themselves but by researchers from the USA and Europe. With Madagascar being one of the poorest countries in the world, there is almost no possibility to realise Madagascan research.

The Institute Pasteur, subject of a convention between the Malagasy government and the Institut Pasteur in Paris, combines basic research and diagnosis, for example by investigating the aetiology of malaria. "We want research [here] to meet international standards", says Director Christophe Rogier. The Institute's equipment is among the best in the country and the wall in front of Rogier's office is covered with recent publications. Nonetheless, Rogier is aware of the limitations: lack of English proficiency combined with Madagascar's geographical isolation affects exchange with international colleagues. Rogier thus takes every opportunity to send his researchers to conferences abroad.

Scientists from abroad have also helped to improve the situation. American primatologist Patricia Wright came to Madagascar in 1986 to rediscover the great bamboo lemur, believed to have gone extinct. Partially based at Stony Brook University, New York State, she made Madagascar her second home and became involved in establishing the Ranomafana national park in South-Eastern Madagascar.

In 2003, Wright founded Centre ValBio, located within the rain forest in vicinity of the national park, which enabled top tier research in biodiversity and infectious disease. Its own tissue culture lab allows for primary cultures and DNA preparations for genomics projects. Although most scientists at Centre ValBio are visiting from the USA or Europe, Wright's strategy importantly involves Malagasy people. Each international researcher pairs with and funds a Malagasy student and research technician, who both assist in the project. Additionally, Centre ValBio is involved in local outreach programs promoting sustainable economic development to reduce poverty in the area.

Steven Goodman, an American conservation biologist from the Fields Museum in Chicago, uses another strategy. Goodman became interested in Madagascar during an excursion in 1991. Together with the World Wildlife Fund (WWF) Madagascar, he founded the 'Ecological Training Program', enabling field excursions for Malagasy students. The program became independent of the WWF in 2007 and the name changed to Vahatra (Malagasy for 'grass roots'). Today, Vahatra provides motivated biodiversity students with a stipend to cover study and living costs.

The Vahatra House, close to Antananarivo University, offers a scientific library, lab space and representative rooms. The association also initiated the maintenance and expansion of a tremendous array of Madagascan specimens, partially collected by Goodman. Currently, Vahatra is dependent on funds that Goodman raises. He emphasises that "not only money, but also discarded equipment from labs in the USA or Europe is of great value…even the oldest PCR machine is better than no PCR machine".

However, in the long term, Goodman aims to make himself dispensable. Today, Vahatra is headed by three of its former students with Goodman as scientific counsellor. Other former students have gone on to pursue scientific careers all over the country, including the Institute Pasteur. The initiatives of Wright, Goodman and the Institute Pasteur provide a basis for a scientifically successful Madagascar. Together, they promote an environment where Malagasy researchers can go beyond participation to actively leading the country's science.

Anna Dorothea Senft is a DPhil student in Chromosome and Developmental Biology at the Dunn School. She travelled to Madagascar to study how scientific research is performed

Anna travelled to Madagascar with the support of alumni of the Life Science Lab Heidelberg

Carbon sources in the oceans and soil

by Jason Kaufman

SUVs, airoplanes, and factories – we often think of these as the 'bad guys' in global climate change. And we are not wrong to assume this, but they certainly are not alone. The rising levels of carbon dioxide (CO_2) in the atmosphere, and the resulting increase in global temperature, can set off the release of carbon stores elsewhere – in our planet's oceans and soil.

Scientists at Norway's Center for Arctic Gas Hydrate, Climate and Environment examined these phenomena by studying the release of methane gas from permanently frozen soil known as permafrost in Siberia (1). Methane is a powerful greenhouse gas; pound for pound its effect on climate change is 20 times greater than CO_2 since methane traps radiation more efficiently. Methane is released by domestic livestock, during the processing and use of natural gas, and by landfills as waste decomposes.

> "a temperature increase of just two degrees could accelerate the thawing to the extreme"

The scientists studied the Yamal Peninsula in Northern Siberia, which is covered by permafrost. Near this peninsula, permafrost also exists at the bottom of the Kara Sea, under which methane is stored in 'ice like structures' stabilised by the low temperatures in the deep ocean. Permafrost was formed at the bottom of the ocean when, 20,000 years ago, the sea level dropped, exposing the sea floor. The permafrost on the sea floor has been thawing since the climate began warming 12,000 years ago, albeit slowly due to the low water temperature at the bottom of the ocean. While it was thought that the permafrost in the Kara Sea, at a depth of 100 meters, was too deep for gas to penetrate, this study found that there is already gas leaking, suggesting that the permafrost 'seal' is thinner, and thus more fragile, than previously thought.

Permafrost is warmed from both below, where heat from the Earth's interior warms its lower surface, and from above, from warmer-than-freezing ocean water. While there is nothing that can stop geothermal heat from below, an increase in ocean temperatures is cause for concern. According to Portnov, who led these studies, a temperature increase of just two degrees could "accelerate the thawing to the extreme," leading to "an explosive gas release" from areas of the permafrost (1). Another study published in February suggested that the last ice age ended in a similar manner; the release of CO_2 from oceans into the atmosphere ushered in the following warmer period (2).

Like the oceans, soil also stores a massive amount of carbon; twice as much as all of Earth's plants and atmosphere combined. Despite its bad reputation, CO_2 is essential for plant growth. Scientists at Princeton University generated computer models of the interaction between plants, soil, carbon and fluctuations in climatic conditions (3). This study suggested that as plant growth in a CO_2-rich world increases, their roots stimulate microbial activity in the soil that accelerates decomposition of soil carbon and its release into the atmosphere as CO_2. Alternatively, microbial activity stimulated by root growth could actually lock the carbon onto mineral particles, contributing to longer storage of carbon within the soil. While previously it was simply thought that increased plant growth could cancel out some anthropogenic CO_2 as plants do take up the gas, this new model suggests greater complexity in the interactions between plants, bacteria, fungi, minerals and carbon.

Both of these phenomena reveal positive feedback loops in global climate change. That is, increased CO_2 from anthropogenic sources can potentially lead to increased CO_2 release from soil and oceans, continually reinforcing the release of carbon from these sources. Understanding these dynamics of carbon output can hopefully raise appropriate urgency surrounding anthropogenic carbon release.

References

1. Sojtaric, M. (2014) Methane is leaking from permafrost offshore Siberia. Available at http://www.sciencedaily.com/releases/2014/12/141222111559.htm [accessed 14 April 2014].

2. Martinez-Boti, M.A., *et al.* (2015) Boron isotope evidence for oceanic carbon dioxide leakage during the last deglaciation. *Nature.* 518: 219-222.

3. Sulman, B., *et al.* (2014). Microbe-driven turnover offsets mineral-mediated storage of soil carbon under elevated CO_2. *Nature Climate Change.* 4(12):1099-1102.

Jason Kaufman is a MSt student at Jesus College studying American History

Why I support women's networks

by
Inés
Usandizaga

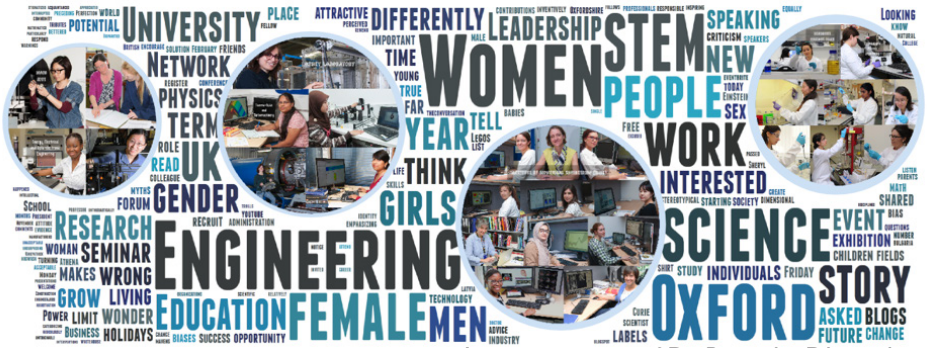


Image courtesy of Dr Priyanka Dhopade

When it comes to women's networks I have something of a track record. At the beginning of my mechanical engineering degree, I came to the realisation that there weren't many women around. I therefore decided to take part in every network that crossed my path. I helped to set up the Women in Engineering group in Oxford, joined Oxford Females in Engineering, Science, and Technology (OxFEST) and Women in Science and Engineering (WISE), and have attended several events organised by Zonta International. Moreover, I am an active member of Femtec, a German network that I joined during my undergraduate degree at the Karlsruhe Institute of Technology. It is thus fair to say that I am very enthusiastic about women's networks and therefore would like to use this platform to share my experiences.

Opportunities

As with any network you become part of, joining a women's network provides you with many new opportunities; both personal and professional. You may meet someone who works in your field and who can offer you the opportunity to join a committee at a conference or who gives you the necessary insight into a company. Or maybe someone is looking for help with an outreach event and you find yourself playing with helicopters at a primary school. Joining a network will give you the chance to be better connected with people within your department, your research field and society in general.

Role models

The importance and regrettable lack of role models for young women in STEM (Science, Technology, Engineering and Mathematics) subjects has been highlighted time and time again. If you work in a male-dominated field, it can be difficult to find inspiring role models. In the women's networks that I have joined so far there have been numerous role models. In my opinion, the key is to meet many different women: maybe you admire one person's communication skills, another person's determination and someone else's ability to juggle work-life responsibilities. It is the combination of these influences that can provide help in figuring out where you want to go and what you want to achieve. Ideally you would even find a mentor who could help steer and support the first steps in your professional career.

Awareness

Joining women's networks has opened my eyes to a great number of issues; together, we have rediscovered the achievements of successful female scientists. We have discussed the image of women in the media. We have thought about biased application processes and how they discriminate against different groups. We have also explored the representation of women in different countries and how this has developed over time. We have discussed the 'leaky pipeline' of women in science, the gender pay gap and wondered how to balance work and family life. Above all, we have cherished the many successes that have come from inspiring women all over the globe!

Join the discussion!

Finally, joining a women's network will provide you with an opportunity to join the discussion and thereby enable you to help shape your working environment and influence society. There are still many important issues left to debate. For instance, what do you think of a quota for women? How can the policy on parental leave be improved? What can universities and companies do to support women? What else can we do to encourage girls to pursue a career in STEM?

Beyond this, there are many global issues that need to be tackled, from global warming to inequality. We cannot afford to waste female potential. At the very least we can support each other and encourage young girls to follow their goals. In short, I support women's networks because there is so much we can change!

Further information

www.facebook.com/WIEOxford

www.ox-fest.org

www.wisecampaign.org.uk

www.zonta.org

www.femtec.org

Inés Usandizaga is a DPhil student in Engineering Science at University College

Antibodies: they are proteins too...

by Dr Michael Fiebig

Johnny stared in disbelief. This was the last Western Blot he needed…one last clear band at the right size…but what was THAT?! The lanes on his blot, even his previously blank negative control, had more bands than Glastonbury. What had happened? Johnny had done everything exactly the same, everything apart from opening a new vial of antibody. It looked the same; same target, species, isotype, supplier, catalogue number and concentration, and yet it dawned on Johnny that he had not the slightest idea what was in that vial.

Usually we biologists want to know. You wouldn't work with a plasmid you haven't sequenced and you want to know the sequence of the protein you're working with. But somehow we don't seem to care about the composition of antibodies we use. Superficially, there's not much to it. There's a constant region, which differs between species and comes in different isotypes, and there are variable domains that recognise the antigen. It's so simple you could get a kid to draw it.

But there is more to it. The sequence that determines binding is extremely important and characteristic of an antibody, but usually it's completely unknown. In a recent article in Nature (co-signed by 110 other leading scientists), Bradbury and Plückthun (1) address the issue of antibodies and reproducibility. They believe "poorly characterized and ill-defined antibodies were in large part to blame for a study (...) being able to replicate the scientific results of only 6 of 53 landmark preclinical studies." Forget poor Johnny and his Western – think of the wasted time and funding (the authors estimate US$350 million per annum in the USA alone) and think about all those 'fantastic' papers you've always been a bit sceptical about.

Of course antibodies are superb reagents, and the contribution readily raised polyclonal antibodies have made to science is undeniable. However, they are inherently only ever available in finite amounts. Hybridoma-based production can also bear problems. Long-term producing hybridomas can 'drift' and the antibody may not be as monoclonal as expected (e.g. multiple light chains (2) or aberrant chains (3)). Bradbury and Plückthun (1) propose that polyclonals should be "phased out of research entirely" and that for hybridoma-produced monoclonal antibodies, sequences should be determined and all antibodies produced recombinantly. This would revolutionize the way antibodies are made, ensuring that antibodies are not just defined by a label on a tube, but by their amino-acid sequence.

This technology exists and has been used extensively by the pharmaceutical sector, but has been inaccessible for most academic scientists – that is, until very recently. The Oxford based start-up, Absolute Antibody, provides antibody sequencing, engineering and entirely recombinant expression services. All production is performed in a serum-free mammalian cell system ensuring ultra-pure, absolutely defined products. Another advantage of producing antibodies starting from their sequence is that they can be reformatted – your favourite mouse IgG1 can now be a rat IgG2B. Likewise, constant domains can be engineered like the Fc-Silent™ format from Absolute Antibody that doesn't bind to complement and Fc-receptors.

Whilst Absolute Antibody are busy generating new antibody formats to suit the needs of our customers, we hope that the next time you pick up a tube of antibody you ask yourself, "do I really know what's in this?" Maybe it's time for a change; maybe it's time for Absolute Antibody.

If any of this interests you and you would like to find out more, email sales@absoluteantibody.com or visit www.absoluteantibody.com.

References

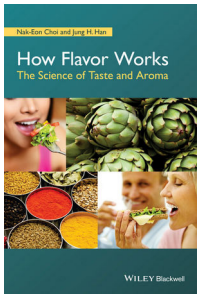
1. Bradbury, A. & Plückthun, A. (2015) Reproducibility: Standardize antibodies used in research. *Nature.* 518(7537):27-29.

2. Yang, J. *et al.* (2013) Comparison of two functional kappa light-chain transcripts amplified from a hybridoma. *Biotechnol. Appl. Biochem.* 60(3):289-297.

3. Ding, G., *et al.*(2010) Identification of two aberrant transcripts derived from a hybridoma with amplification of functional immunoglobulin variable genes. *Cell. Mol. Immunol.* 7(5):349-354.

Dr Michael Fiebig recently completed his DPhil at the Sir William Dunn School of Pathology and now works for Absolute Antibody as Product Development Manager

BOOK REVIEW



How Flavor Works: The Science of Taste and Aroma

David B. Rivers and Gregory A. Dahlem

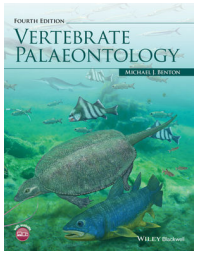
ISBN: 978-1-118-86547-7, Wiley-Blackwell (2015)
Paper, 240 pages, £59.95

Reviewed by Anna Sigurdsson

What is taste? Why do we taste? *How Flavor Works: The Science of Taste and Aroma* is a lovely book that deals with the "how?" and "why?" of flavour. Aimed at a broad audience ranging from food science professionals to anyone with an interest in food, the book is a poetically written introduction to the world of flavour, taste and aroma. The first few chapters examine the mechanisms of taste and smell and explain them in comprehensible and accessible language. The text is interlaced with examples of foods to illustrate the points being made, which makes the book interesting for the layperson as well. The chapter "Taste in general science" focuses on the complexity of taste, showing how different sensory experiences such as vision and sound also contribute to the taste experience, just as cultural and personal factors may contribute to food preferences. The book then continues to describe the art of compounding flavours and odours to create specific aromas.

The book then takes a turn towards the behavioural sciences discusses how flavours influence humans. Grounding the discussion in evolutionary history, Choi and Han discuss the importance of the olfactory system to animals. Although our sense of smell is much less sensitive than in other animals, they argue that it is still a crucial part of human existence. With reference to pheromones and aromatherapy they illustrate how aromas can greatly affect human behaviour. Thereafter, we learn how taste and smell are processed neutrally, and how external factors such as expectation (i.e. due to the price of a food) and experience (i.e. a good product image) can affect the perception of flavour. Finally, the book discusses the future of taste and aroma. Choi and Han predict that more technology will become incorporated into the culinary arts, ponder upon the possibilities and limitations of aroma-releasing movies, and consider how taste modification may be interacting with the issue of obesity.

How Flavour Works: The Science of Taste and Aroma is a delightfully multi-facetted book that serves as an exciting introduction to the world of flavour.



Vertebrate Palaeontology (4th Edition)

Edited by Michael J. Benton

ISBN: 978-1-118-40755-4, Wiley Blackwell (2014)
Hardback, 480 pages, £85

Reviewed by Srinivasa Rao

When I agreed to review *Vertebrate Palaeontology*, I was secretly hoping to learn something about dinosaurs that would allow me to indulge in some petty palaeontological pedantry, like shouting, "Velociraptors have feathers!" at the screening of Jurassic World. Alas, there was not even a mention of the Velociraptor in this textbook; I got that nugget from Wikipedia. An academic textbook cannot hope to compete with online resources in terms of sheer volume of information, but it can present the most important information in a very structured fashion and serve as a solid starting point for further studies. This book does not disappoint.

The book is divided into 11 chapters based on evolutionary age and vertebrate class. Each chapter begins with "key questions" and ends with unanswered questions requiring further research, and a section listing additional reading material (in addition to extensive bibliographies), all of which are invaluable for students seeking a broader perspective. This is a technically complex book aimed at a graduate student level with a background in basic palaeontology. Some familiarity with vertebrate anatomy is assumed, but one chapter does briefly describe the techniques used in vertebrate palaeontology. There is a collection of interesting colour plates in the middle of the book, although the rest of the figures in the book are not in colour.

The book is peppered with boxes covering interesting topics, like the domestication of dogs and horse-eating birds, and references to popular literature, which provide some respite from what often feels like a dry subject. The up-and-coming subfield of palaeogenomics, however, is only covered in passing. A section describing the career choices available to a budding palaeontologist, including case studies, could help students get a rough idea of what they are getting into. Finally, there is a companion website hosting all the figures from the textbook as PowerPoint slides, which could be very useful for teaching purposes. Overall, this is a very well organised textbook for advanced students or teachers of palaeontology, but do not expect to find news of the latest mammoth discovery in here.

Global Food Security and Supply

Wayne Martindale
ISBN: 978-1-118-69932-4, Wiley Blackwell (2014)
Paperback, 323 pages, £50
Reviewed by Jason Kaufman

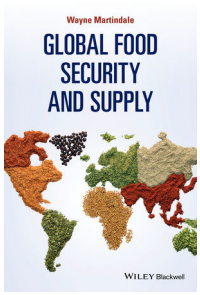
Global Food Security and Supply explores the present state of research and debates in – as the title suggests – global food security, with a particular focus on food supply chains. Its readability and contemporary nature, combined with thorough coverage of important themes in the discipline, make it an appropriate reference for anyone interested in food security or a related subject. The book focuses mainly on two aspects of food supply chains: where products are made, and where they are used. Four main chapters deal with broadly defining food security in terms of genetic diversity, understanding supply chains, and the scientific and sociological basis of food security.

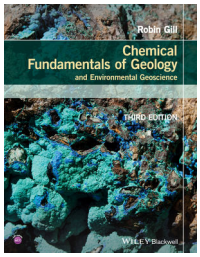
The main strength of this book is its incorporation of current trends, research, debates, and concerns into the broad framework of the field. While paying appropriate homage to important happenings and findings of the 20th century, the book also strives to find new ways of thinking about food security in the 21st century. The second chapter aims to make readers aware of the different writers and critics of the food system, placing them within a wider context. Most significantly, the chapter explores how big data can be used effectively to better understand and make informed decisions about food systems. This chapter also contemplates food security following the first green revolution, discussing how the "second green revolution" should focus on optimising nutrition across supply chains.

A contemporary focus weaves itself in interesting ways into the final chapter on the sociological basis for food security. The chapter discusses how now, alongside new technologies to change food production, environmental and social consequences of changing food supply are becoming just as important to measure and consider. The chapter proposes that big data could be used to perform carbon footprinting for food manufacturers, offering greater visibility in sustainability reporting. This chapter also deals with issues of affordability and accessibility. Food security, as a constantly evolving problem alongside a constantly changing global climatic and anthropogenic landscape, requires a textbook that considers contemporary challenges and opportunities. *Global Food Security and Supply* answers this need admirably.

Chemical Fundamentals of Geology and Environmental Geoscience

Robin Gill
ISBN: 978-0-470-65665-5, Wiley Blackwell (2015)
Paperback, 288 pages, £32.50
Reviewed by Rebekka Hancock



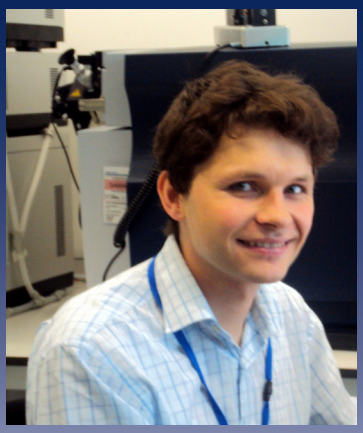


As all chemists will tell you, chemistry is the science behind most other sciences. As a chemist now masquerading as a biologist, my acceptance of this doctrine is somewhat wavering. Nevertheless, I was intrigued by this idea, which forms the basis of *Chemical Fundamentals of Geology and Environmental Geoscience*. Could a book of a mere 267 pages really impart all of the precious chemical knowledge needed by geoscientists? I would suggest so. This textbook provides an excellent grounding in the basic principles of physical and inorganic chemistry, and would, in fact, be of use to any scientist lacking chemical knowledge.

The book is well organised, beginning with an introduction to many elementary principles of physical chemistry. The first three chapters explore thermodynamics, equilibria and kinetics. Further chapters expand to cover aqueous solutions, electrons and atoms and the basics of inorganic chemistry. The key points pertaining to these subjects are neatly summarised in boxes throughout the text, making them very accessible. The language used in explaining these concepts is generally clear, although may prove frustrating for anyone other than a beginner. The author has been careful to keep in mind his intended audience, illustrating his points with many geological examples. This culminates in the final four chapters of this textbook, which discuss crystals, geologically important elements, and the use of isotopes in geology. It is also here that the more biochemical aspects of this book, such as a discussion of the evolution of the atmosphere and establishment of marine biogenic oxygen, can be found, providing interesting reading for both physical and biological scientists.

Along with the content, several other features of this book deserve praise. Each chapter ends with a review of the main points, followed by exercises designed to reinforce the reader's knowledge. Appendices covering some basic mathematical principles and chemical information are also of value. *Chemical Fundamentals of Geology and Environmental Geoscience* triumphs as a reference text for geoscientists in need of a chemical initiation. Whilst the subject matter is too dry to make it a feature of anyone's coffee table, it would be a welcome addition to many a bookshelf.

5' with ... Dr Holger Kramer



Holger Kramer completed his undergraduate and MSci in Chemistry in Marburg, Germany and at the University of Birmingham in 2002. After completing his doctoral studies in Oxford at the Department of Organic Chemistry, Holger started postdoctoral research at the Nuffield Department of Medicine. It was here that Holger was introduced to the use of mass spectrometry and proteomics to study infectious diseases. As of 2010, Holger manages and runs the OXION Proteomics facility. He also held a retained lectureship in Biochemistry at Pembroke College and currently teaches for Trinity and St Anne's colleges.

What early influences led you to pursue a career in science?
I had a very inspirational teacher in Chemistry and Biology. I was fortunate to have undertaken two projects with him, one on 'forests' and another on 'mineralogy and geology'. These were chosen from a range of projects offered by my school to be taken in the last week before the summer vacation. Both projects were really good opportunities to learn many things that are not touched upon in the curriculum and, more importantly, to experience and begin to understand my teacher's fascination with science.

If you were not a scientist, you would be…
A psychologist. I believe that as scientists we like to think of ourselves as sensible and logical beings. However, when you look beneath the surface you find that our motivations and desires are often driven by forces quite remote from rationality, which is a study that greatly fascinates me.

What do you like most about being a research scientist?
The freedom to be creative and to have the opportunity to follow and realise your own ideas. Also, the fact that you keep on learning constantly throughout your research work and career appeals to me.

What are you most proud of in your career so far?
To have managed the successful transition between quite different scientific fields. The first half of my DPhil project was spent on synthetic organic chemistry, focusing on utilising transition metal catalysis. However, the second half involved site-directed mutagenesis, protein expression and unnatural amino acid incorporation using *E.coli*. I initiated this work when I found in the literature that unnatural amino acids represented a more direct and cleaner way to introduce reactive groups into proteins, which we required for conjugation of carbohydrates. I guess the lesson is that an hour spent reading can save months in the lab!

What was your worst disaster in the lab?
Thankfully I have had no serious lab accidents! But during my postdoc I ended up causing a spill of 2.5 litres of diethyl ether just next to one of the mass spectrometers in the lab. Any source of ignition could have turned this into a rather expensive insurance claim, but luckily we were spared a flaming inferno…

Do you have a favorite classical experiment?
Otto von Guericke's demonstration of atmospheric pressure. He showed that 30 horses could not separate two hemispheres once the inside had been evacuated by his previously developed vacuum pump (1654).

What advice would you give to students looking to pursue a career in research?
I would strongly suggest working on interdisciplinary projects and at the interface of different fields. Also, seek out those areas that are less popular, but which have the ability or potential to significantly advance our understanding. Always do what you enjoy doing, but be creative!

What is the biggest contribution chemistry can make towards our understanding of biology?
Chemistry allows us to elucidate the processes that define living cells and organisms at the molecular level. It also gives us the tools to design interventions in disease or to alter normal physiological function.

Write for *Phenotype*?

- The deadline for article submissions is Friday of 8th week, 19th June 2015
- We accept articles on any aspect of biological sciences research, books or science education
- For next issue we particularly welcome articles related to plant science or the environment
- Articles can be either 650 or 1300 words

If interested, please get in touch: christopher.hillyar@jesus.ox.ac.uk

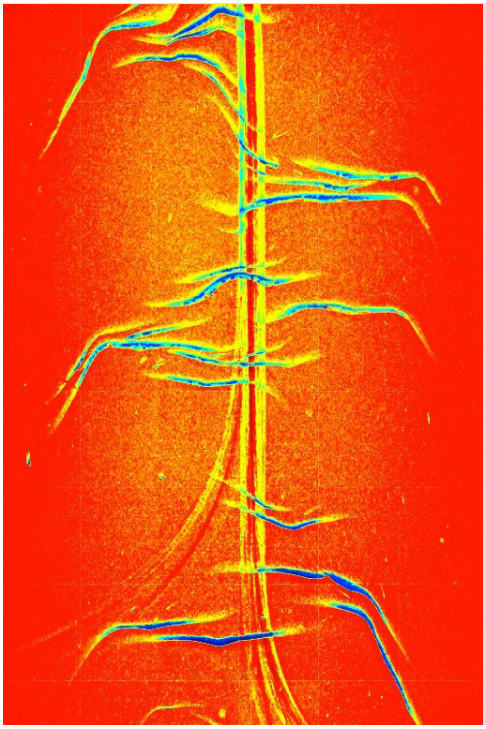
Work for *Phenotype*?

If you'd like to get involved in editing, production or management of *Phenotype*, please get in touch: christopher.hillyar@jesus.ox.ac.uk

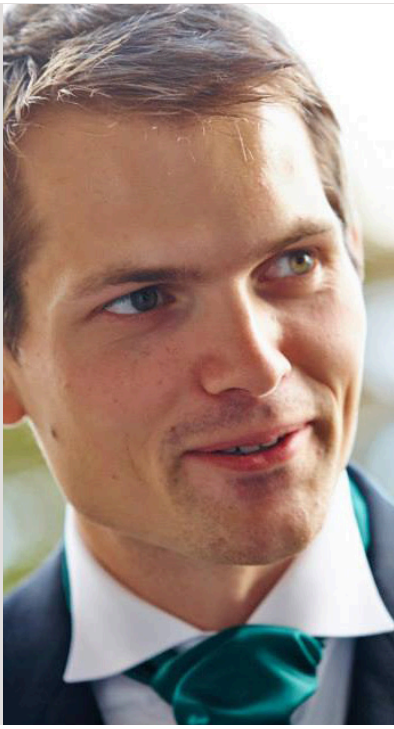
SNAPSHOT Research Image Competition

This issue's winner is...

Jonathan Neve



Jonathan is a third year DPhil student in Professor Andre Furger's group in the Department of Biochemistry. His research focuses on how switching polyadenylation sites within a single gene affects RNA metabolism and ultimately protein expression. The winning image shows a heat map of an Ion PI semiconductor sequencing chip from Thermo-Fisher's Ion Proton Sequencer. The 2cm x 2cm chip contains 165 million wells, each containing an individual sequencing reaction, in which DNA polymerase joins dNTPs to form a DNA strand complementary to a template strand specific to each well. Protons released with each nucleotide addition alter the pH level of each well. By sequentially flowing different nucleotides over the chip, a pH change is registered in the wells, thereby identifying the next base in that template strand. In the case of homopolymers, multiple nucleotides will be incorporated, releasing a number of protons corresponding to the number of bases in that homopolymeric stretch.



In 2011, Jonathan obtained an MSc in Molecular and Cellular Biochemistry at the University of Oxford and completed his part II project in Professor Mahadevan's group. From there Jonathan made the rather unconventional move of accepting a position with British Sugar, as Laboratory Services Manager for a factory in Newark-on-Trent. Although life in Nottinghamshire was sweet, Jonathan realised that a long-term career in the sugar industry was not for him and returned to the Department of Biochemistry as a DPhil student a year later in Professor Furger's group.

During his DPhil, Jonathan is investigating the implications of alternative cleavage and polyadenylation of messenger RNA. To do this, Jonathan uses a modified version of RNA-sequencing that extracts 50–100 nucleotides directly upstream of cleavage and polyadenylation sites and uses this RNA for deep-sequencing. This approach can globally map all the polyadenylation sites within a given RNA sample, and measure the relative frequency at which these sites are used. To identify the genes regulated by alternative polyadenylation, Jonathan uses a subcellular fractionation approach. Differences in the relative abundance of alternative polyadenylation RNA isoforms between the cytoplasm and the nucleus are indicative of pathways that destabilise post-transcriptionally processed RNA, such as microRNA-targeted destabilisation pathways. Jonathan uses a genome-wide approach to identify genes that give rise to alternative polyadenylation RNA isoforms that are differentially post-transcriptionally regulated by such pathways. This therefore provides a platform to identify alternative polyadenylation events that are likely to be of physiological significance.

The Ion Proton Sequencer is housed on the second-floor in New Biochemistry but can be utilised by all departments. During the 12 months since it was installed, the sequencer has completed nearly 200 runs and generated more than 10,000,000,000 sequence reads. The workflow is very simple and the sequencing data can be obtained within 24 hours. Very little hands-on time is required thanks to the robotic Ion Chef System, which prepares a fully loaded chip from the sequencing library that is then transferred onto the Ion Proton Sequencer. Currently, it is possible to get around 70–80 million reads per run, which is set to increase to 200+ million reads with the upcoming release of the new Ion PII chip.

Outside of the lab, most of Jonathan's time is taken up by swimming, cycling and running, and he has recently competed in his first iron distance triathlon at Ironman Wales. When asked about his plans for after his DPhil Jonathan replied, "I am leaving my options open".

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 Michaelmas 2015 issue of *Phenotype*.

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

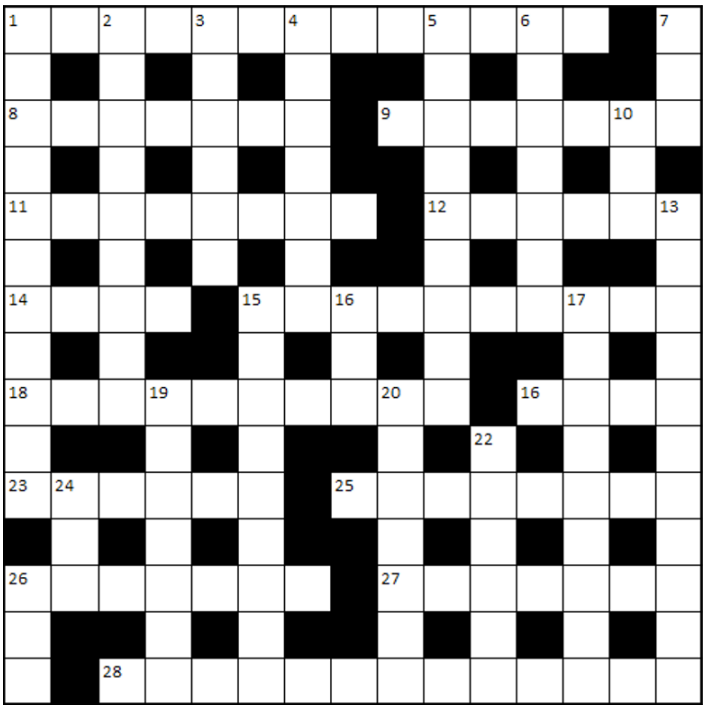
PHENOTYPE crossword

Fish challenges you to this latest cryptic crossword on the theme of microorganisms. Can you crack it? You can get the answers to last issue's crossword at the bottom of the page.

Enter this term's competition by sending your answers to christopher.hillyar@jesus.ox.ac.uk. Entries received before the 22nd June 2015 will be entered into a prize draw to win one of the four books reviewed in this issue.

The winner can choose one of the four books reviewed in this issue, generously provided by Wiley-Blackwell.

WILEY-BLACKWELL



ACROSS

1. *"Psychotic cola!" is made from bunches of grapes (13)*
8. *Where ginger or brandy might be found quickly? (2,1,4)*
9. *Nature documentary covering 26 or 1 across, perhaps? (7)*
11. *Mysteriously selects leaders of Oxford Cricket Club, but in the end loses a friend (8)*
12. *Began work, but need compound (6)*
14. *see 16*
15. *That which nuns share runs from the Olympic to the Titanic, for example (6,4)*
18. *Being grounded is equated with vulgarity . . . (10)*
21. *. . . Until demonstrated by interruptor (2,2)*
23. *Doctor interjected: "heart proteolipids can produce clots" (6)*
25. *Got it between the teeth on second attempt (and split it in half?) (3,2,3)*
26. *'Invoice takes into account one's rods (7)*
27. *Anticonvulsant started for twitch can't be used as a proton donor (7)*
28. *Way for salesman to head company: ring 200 of us in a chain (13)*

DOWN

1. Commanding officer tears up as he drinks hot tea - he's in a spiral (11)
2. Without vessels, Naval centre's using car to get around University of London (9)
3. Trade name (6)
4. Ali is sick so swallows a tablet made of a type of volcanic rock (7)
5. Olly Murs declared in turn he'll use shorter strings (9)
6. Some say they have 13 or 28 in their chests? (7)
7. see 26
10. Shelter for Stan and Bruce (3)
13. Submerge the crazy Spanish copper in caesium so he'll grow (as) a pair (11)
15. I'll aspire to intertwine helices (9)
16,14. Superheroine who can fuse helium and hydrogen when in a bad mood? (3-4)
17. Stupidly I ate pitch, causing liver inflammation (9)
19. Left-back is leading saint (who takes a figurative view of the Scripture) (7)
20. Greek stew is best if adoringly trimmed (7)
22. Teardrop falls as six on the moon drink bromine (6)
24. Cry endlessly: Big Bird is dead! (3)
26,7. A hospital in chaos (6)

Answers to the crossword from Issue 20 - Hilary '15

Across 1. Thymine, 5. Discuss, 8. Standards, 10. Stent, 13. Crick, 14. Guanosine, 16. Ice, 17. Filleting, 21. Backchats, 22. Dry, 24. Sentinels, 25. Taint, 27. Sepal, 29. Extensile, 30. Amoebae, 31. Adenine

Down 2. Hattrick, 3. Monaco, 4. Neat, 6. Cytosine, 11. Theca, 12. Instate, 15. Wilkins, 18. Castrate, 19. Franklin, 20. Bases, 23. Watson, 26. Geld, 28. Elm