# PHENOTYPE

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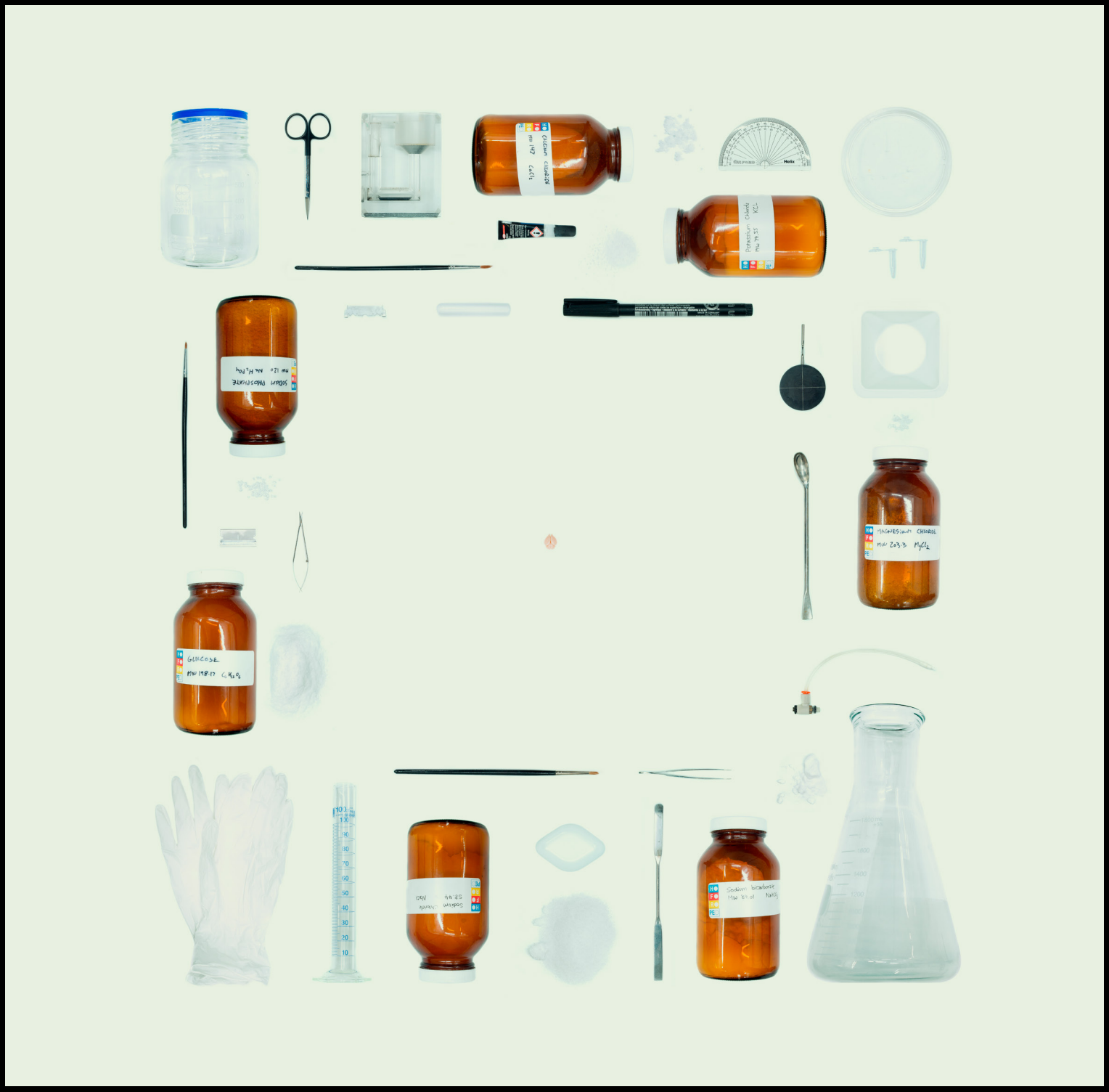

## Anatomy of an Experiment

**Cristiana Vagnoni & Lev Tankelevitch**

*Winners of the SNAPSHOT Scientific Image Competition* ***Page 31***

The Neocortex

Decrypting drug resistance in tuberculosis with CRyPTIC

Special Feature: OUBS Careers Day

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# EDITORIAL

*A very warm welcome to the 24th issue of Phenotype! This term, I have yet again had the privilege of working with many talented writers and reading some truly exciting and inspirational articles.*

*Following on from last term's Neuroscience Supplement, our Features section takes a cerebral turn, with a focus on the Neocortex in our PI article by Professors Zoltan Molnar and Simon Butt & Dr André Marques Smith. Glial cells, the brain's resident defence system, have their moment in the spotlight in two articles by Heather Booth and Kleopatra Andreou, while Rebecca Wallings examines recent research into the fascinating relationship between gut bacteria and the brain.*

*It is always exciting to read about novel technologies, methodologies and treatments. This term, Dr Christakis Constantinides looks at the use of cardiac imaging for phenotyping in cardiovascular disease, while Dr Suvi Honkanen explains the use of activity-based protein profiling to detect active enzymes in plants. James Eaton also takes us on a surprising detour with his piece on how tick spit might be used to cure disease. These articles span clinical and fundamental research, highlighting the amazing diversity of Phenotype's contributors and the fantastic research output from the biological sciences in the University of Oxford. If you need more convincing of this fact, turn to page 5 for Martine Abboud's summaries of some recent high-impact papers from the Department of Chemistry and the Wellcome Trust Centre of Human Genetics.*

*Some of the most important challenges to world health are tackled here in Oxford. Luckily for us, the researchers confronting these issues have taken the time to write for Phenotype and explain the work they do. Drs Ana Gibertoni Cruz and Philip Fowler discuss their approach to identifying drug-resistant tuberculosis with the CRyPTIC consortium, and Dr Anne Raimondo describes the complexity of predicting and treating Type 2 Diabetes and emphasises the need for increased understanding of the biology underlying this disease.*

*I particularly enjoy reading about the incredible opportunities both available to and created by researchers at the University of Oxford. In this issue, Beatrice Tyrell and Tonia Thomas describe the brilliant outreach work they do in primary schools with the student-led Oxford Hands-on-Science, while Dr Burcu Anil Kirmizitas brings us special coverage of the OUBS Careers Day in February. I hope you will enjoy reading about the experiences of the speakers who returned to the Biochemistry Department and find inspiration in the varied careers they pursued after their studies.*

*As ever, our Regulars Section includes a look at some Classic Kit, in Emma Bickford's exploration of the Scanning Electron Microscope, while Lauren Chessum spends 5' with Dr Richard Hopkinson. Congratulations are in order for Cristiana Vagnoni and Lev Tankelevitch, whose stunning artistic representation of an electrophysiology experiment graces our front page. They are the deserved winners of book tokens from our sponsors at Oxford University Press. We also have congratulations for Daniel Yin at Queen's College, who solved Fish's fiendishly cryptic crossword last term to win a book prize from Wiley-Blackwell publishers. For a chance to match Daniel, turn to the back page for another crossword from Fish on the theme of genetics.*

*Our crossword matches the theme of our Supplement this term. We get the low-down on the genetics of C. elegans from Sophie Gilbert, Post-Mendelian genetics from Sara Althari and Meiosis from Dr George Busby.*

*Finally, I must remnd you of opportunities we have here at Phenotype. Whether you would like experience in science writing, editing, designing and advertising, please email rebecca.hancock@linacre.ox.ac.uk for more information.*

*Becky Hancock*
*Editor-in-Chief*

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# EDITORIAL TEAM

# RESEARCH HIGHLIGHTS

by
Martine
Abboud

Makena A *et al.* (2015) Antimicrobial Agents and Chemotherapy (Accepted Manuscript). doi:10.1128/AAC.01768-15

## Comparison of Verona Integron-Borne Metallo-β-lactamase variants reveals differences in stability and inhibition profiles

The increasing problem of antibiotic resistance is a major global public health concern. Today, the use of life-saving β-lactam antibiotics is threatened by the production of bacterial β-lactamases. One major challenge in the antibiotic drug discovery field is to identify broad-spectrum bacterial metallo-β-lactamase (MBL) inhibitors and achieve a breadth of selectivity required for clinical use. In order to meet these criteria, it is important to understand the structural and biochemical properties of MBLs. Verona Integron-borne MBLs (VIM) are amongst the most widespread MBLs, with 46 reported VIM variants. Accordingly, the Schofield group has studied the biochemical and biophysical properties of relevant VIM variants using a combination of different techniques, including kinetic assays, circular dichroism (CD), NMR spectroscopy and X-ray crystallography. VIM variants have been tested against a panel of β-lactam substrates using an absorbance-based assay previously reported by the group. Remarkably, similar kinetic properties were observed for several variants (VIM-1, VIM-2, VIM-4, VIM-5, and VIM-38). The small differences in their catalytic efficiencies support the proposal that changes in substrate selectivity are not the sole evolutionary driving force for MBLs. Similarly, VIM variants have a conserved secondary structure as implied by CD analyses; however, clear differences in thermal stability were observed. This finding suggests that protein stability might be an important factor and a driving force in MBL evolution. Inhibition profiles were substantially different with one isoquinoline derivative selectively inhibiting two variants (VIM-5 and VIM-38) more potently than others (VIM-1, VIM-2 and VIM-4). Even though VIM variants differ by a single-amino acid mutation in most cases, their interactions with inhibitors were clearly different. Accordingly, this work highlights the importance of screening various MBL variants at an early stage during inhibitor development programs.

Davies B *et al.* (2016) Nature. 530:171-176. doi:10.1038/nature16931

## Re-engineering the zinc fingers of PRDM9 reverses hybrid sterility in mice

To date, the molecular bases of speciation are not well understood, with *Prdm9* being the only gene involved in speciation identified in mammals. Importantly, *Prdm9* can contribute to hybrid sterility in particular mouse crosses, which is characterised by the failure of homologous chromosomes to pair and an arrested meiotic prophase due to the lack of repair of recombination intermediates. DNA-binding PRDM9 protein is involved in initiating meiotic recombination by directing the positioning of double-strand breaks (DSBs). The authors report substitution of the murine DNA sequence encoding a zinc finger array with that of the orthologous human sequence in a single allele to produce humanised PRDM9 in C57BL/6 mice. Interestingly, subsequent investigation of its binding to DNA and role in fertility and meiosis showed that this change restored fertility in male hybrids and repositioned the DSB hotspots required for homologous recombination. This finding shows that a change to one of the *Prdm9* alleles affects the behaviour of DSBs at a chromosome level. The humanised PRDM9 is able to bind both chromosomes at the DSB sites and the symmetry of this binding event associates with increased fertility; the symmetric binding might play an important role downstream in the recombination process. Accordingly, subspecies-specific degradation of PRDM9 increases asymmetric binding and supports the proposal of increased hybrid infertility. The data presented in this paper suggest a wider role of PRDM9 in the early stages of mammalian speciation.

# The Neocortex

by Dr André Marques-Smith, Prof Zoltan Molnar and Prof Simon Butt

When trying to picture the brain, it is likely that what you're imagining is the neocortex (Figure 1). The neocortex is the outermost structure in the brain, responsible for advanced cognitive skills in mammals, from mice to humans. In some species the neocortex is smooth, while in others it is lined with grooves and trenches, like a walnut. By examining stained neurons through a microscope, early neuroscientists noticed striking regularities and differences even in its gross anatomy. Each neocortical area could be split into 6 layers, based on neuron density and morphology; however, the relative size of layers and the morphology and density of neurons varies from area to area, offering a structural basis for parcelling the neocortex.

Neocortical neurons come in two main varieties: excitatory *pyramidal* neurons (PNs) and inhibitory GABAergic *interneurons* (INs). When PNs fire an impulse, they release glutamate, a neurotransmitter that excites the cell receiving the connection. INs release GABA, which *inhibits* the cell receiving the connection. *Excitation* and *inhibition* refer, in this context, to the process of bringing a neuron closer to the threshold for firing impulses (excitation), and the process of pushing the neutron away from it (inhibition). The key function of PNs is to relay information from one stage to the next, whereas INs control the flow of PN activity by influencing the timing, likelihood and rate of PN impulse-firing. The coordinated interplay of excitatory and inhibitory connections allows circuits to perform sophisticated logical operations and structure information in complex codes.

**Neocortical circuits**

The function of a cortical area depends on its inputs (where it receives information from), its outputs (where it sends information) and how it transforms inputs into outputs using its intrinsic circuitry. Information arrives into and leaves areas or layers *via* PN projections. Though cortical areas differ in the strength and probability of certain connections, there is a remarkable degree of generality in intrinsic excitatory circuitry, at least for primary sensory areas (Figure 1). Sensory information arrives into L4, from where it is relayed into layers (L) 2 and 3 (jointly referred to as L2/3). L2/3 PNs project onto L5, which sends connections to L6. L5 and L6 PNs project to subcortical brain structures involved in movement execution or sensory processing. GABAergic interneurons generally form connections within their own layer, though important translaminar (layer-to-

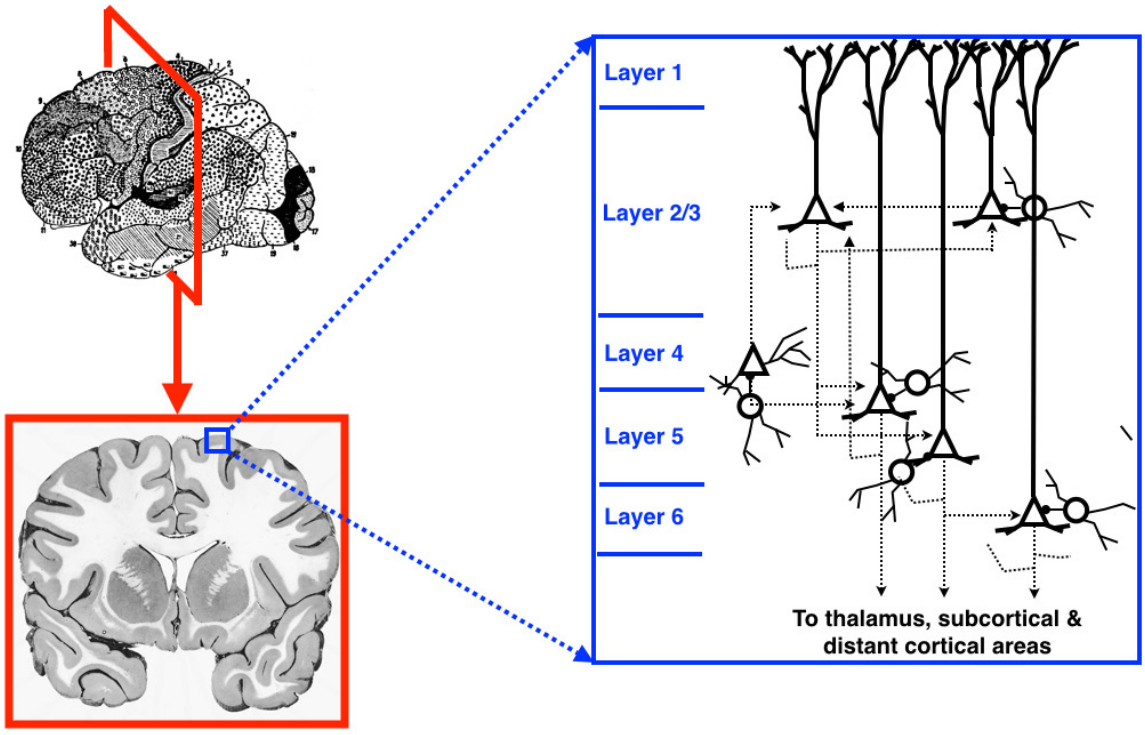


**Figure 1.** The neocortex. Top left: side view of the human brain. Bottom left: a coronal section through the human brain. Inset: the cerebral cortex and its circuits.

layer) connections have also been reported (1, 2). Interestingly, it is in translaminar inhibitory connectivity that primary area circuitry seems to vary the most (1).

### The development of inhibition in L4

The aim of our team at Simon Butt's lab was to investigate the development of inhibitory connections onto L4 (2). PNs in L4 monitor the incoming sensory information and 'decide' whether to relay it for further processing. *But who watches the watchers?* Because INs exert such powerful control over the firing of PNs, they effectively set a threshold to determine whether activity will propagate beyond L4. Set this threshold too low and the natural world would overwhelm our senses. Set it too high and you'd soon become intimate with the belly of a tiger.

We performed experiments on the mouse primary somatosensory cortex, which processes the sense of touch. Prior work showed that in adult mice, L4 excitatory neurons receive practically all their inhibition from INs in L4 (1). The majority of this inhibition comes from a subclass of interneuron that stains for the marker Parvalbumin (PV). Previous studies examined the emergence of the PV to PN connection by recording simultaneously from PV-PN pairs in L4 and checking if they were connected. PV-PN connections are rare before postnatal day (P) 7, but sprout drastically after, coinciding with the engagement of PV INs by sensory (thalamic) input (3, 4). This timing is striking, because sensory input onto L4 PNs matures between P3 and P8 *via* experience-dependent processes (5). Intuitively, it makes sense to delay formation of the PV-PN connection and couple it to the emergence of sensory activity: relieving PNs of inhibition could facilitate activity-dependent maturation processes. Indeed, depriving neonatal mice of tactile input impairs PV to PN connections (3). The wiring of cortical circuits is thus a precisely orchestrated symphony, where every player must come in at the exact time.

### Transient cortical circuits

However, even early circuits rely on precise matching of excitation and inhibition to function. Recordings from living neonatal mice showed that inhibitory activity is present before P7 and required for relaying sensory information correctly (6). Since PV-PN circuits only develop later, a key player was clearly missing. We used a technique called

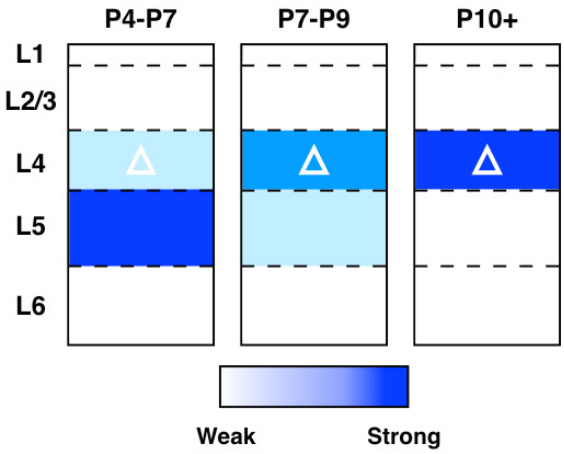


**Figure 2.** Diagram illustrating organisation of inhibitory inputs onto L4 excitatory neurons throughout development.

Laser-Scanning Photostimulation (LSPS) to sequentially activate focal pools of INs across all layers of cortex, whilst recording from L4 PNs (2). This allowed us to *map* which layers inhibit the PNs in L4. Before P7, the majority of inhibition came from the deeper half of L5. Throughout development, L5 inhibitory input declines, concurrent to an increase in L4 input, such that by P10, L4 is the dominant layer. This is in line with previous results (Figure 2) (1, 3, 4).

If not PV, then which IN provides early L5 input? Our lab had a hunch from a project focused on the study of a population of deep L5 INs that stain for the marker Somatostatin (SST). The morphology of these neurons suggested they project to L4. Using LSPS, it was possible to map the excitatory connections L5 SST INs receive. Our hypothesis was that they received excitation from L4, completing a loop between the two layers. Prior to P7, L5 SST interneurons did receive most of their excitation from L4. Throughout development, L4 excitatory input to L5 SST INs declined, concurrent to a rise in L5 input, and by P10 L4 input was gone, with only L5 remaining

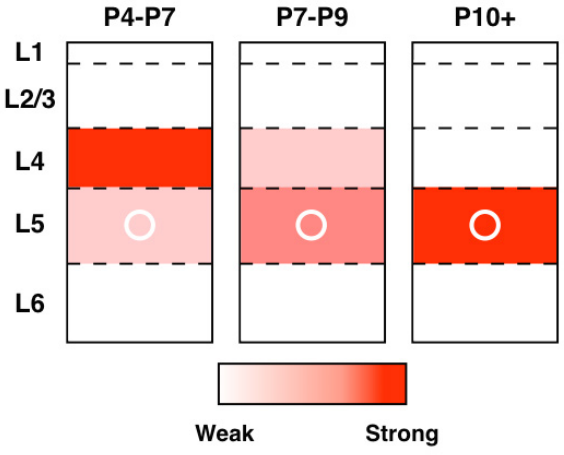


**Figure 3.** Diagram illustrating organisation of excitatory inputs onto L5 SST interneurons throughout development.

(Figure 3). To confirm that these SST INs are responsible for early L5 input onto L4 PNs, we genetically silenced them. This abolished all L5 inhibitory input onto L4, proving early L4 inhibition arises from L5 SST INs.

The overall conclusion from these experiments was that early in development, L4 PNs and L5 SST INs are reciprocally connected, establishing a transient loop between the two layers (Figure 4). But what is it for? In further experiments, we found that L5 SST INs receive thalamic sensory input as early as P3, concurrent to L4 PNs and days earlier than PV INs. Interestingly, normal sensory experience is required for the developmental dismantling of the L4-L5 loop, suggesting a link between it and sensory input maturation. Though a definitive answer will require further experimentation, we found,

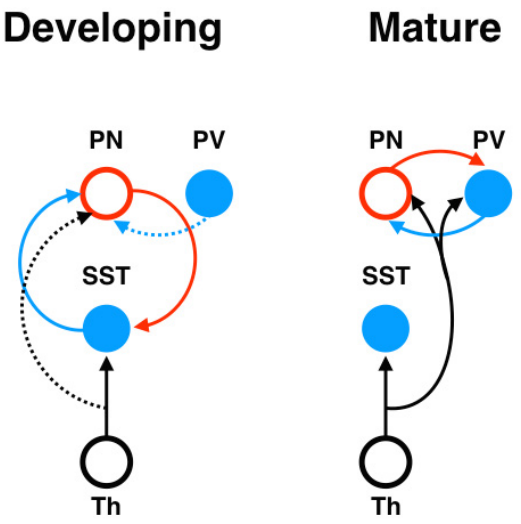


**Figure 4.** Development of thalamic and inhibitory inputs onto L4 PNs

consistent with this hypothesis, that silencing SST INs disrupted the formation of sensory thalamic connections to L4 PNs

**Outlook**

Our results revealed a transient circuit required for normative development of somatosensory cortex. This circuit is like a *scaffold* – important during construction but gone from the finished project. What other transient circuits lurk in the developing brain? Could disruptions in these circuits contribute to neurodevelopmental disorders? And what does that imply for treatment, when several neuropsychiatric disorders are diagnosed in the adult? From a basic science point of view, it is interesting to note that inhibitory connections from deep layers to L4 exist in *adult* primary cortices of other modalities, such as vision. Why is there more area-to-area diversity in inhibitory than excitatory circuits? How does this diversity arise? The results from our group and others show that sensory input reconfigures translaminar inhibitory motifs. This ties into a deeper point about cortical circuits and function: is somatosensory cortex (for instance) somatosensory because it is intrinsically programmed to be so, or is it instructed to become so by sensory experience? This is an old question – but one in which it now seems inhibitory circuits could have a say.

*Zoltan Molnar is a Professor of Developmental Neurobiology and Simon Butt is an Associate Professor of Neuroscience in the Department of Physiology, Anatomy & Genetics, Oxford. Dr André Marques-Smith is a Postdoctoral Research in the Rico Lab, Kings College London.*

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# Microglia: multifaceted brain sentinels?

by Kleopatra Andreou

Microglia, the resident macrophages of the central nervous system (CNS), are haematopoietic cells that originate from the yolk sac and migrate into the central nervous system during early embryogenesis. Evidence suggests that microglia represent a distinct cell population from bone marrow-derived cells, and have the ability to proliferate and expand in the CNS when homeostasis is dysregulated. One key characteristic of ramified (resting) microglia is their processes protruding from the cell body, enabling them to patrol the surrounding microenvironment and communicate with neurons and other glial cells. Activated microglia orchestrate the CNS innate response to injury and infection. However, unresolved microglial activation is often associated with pathological inflammatory conditions that may lead to neurodegeneration and neoplastic diseases. Furthermore, clinical and preclinical studies have shown microglial infiltration of brain lesions in Parkinson's and Alzheimer's disease, multiple sclerosis, and brain tumours (1).

Extensive research has established microglia as versatile cells that have the ability to express distinct functional programs depending on stimuli from the microenvironment in which they reside, similar to macrophages. The classification of activated microglia resembles a well-established T1/T2 dichotomy applied to T helper cells of the immune response. Experimentally, microglia polarise to the classical M1 phenotype upon treatment with Toll-like receptor agonists such as bacterial lipopolysaccharide (LPS) or the pro-inflammatory cytokine interferon-gamma (IFNγ). M1 cells secrete pro-inflammatory cytokines such as tumour necrosis factor-α (TNFα) and interleukin 6 (IL6). The production of oxidative radicals and other cytotoxic effects by M1 cells also potentiates host defences. The alternatively polarised phenotype M2 is induced by interleukins 4 (IL4) and 13 (IL13) and is associated with high levels of the anti-inflammatory cytokine IL10. M2 polarisation promotes angiogenesis, tissue repair, and ultimately the resolution of inflammation (1, 2).

The bipolar M1/M2 paradigm is a useful model for the study of microglial activation *in vitro* but is considered oversimplified for understanding CNS pathologies *in vivo* (3). Clinical studies and preclinical models of CNS diseases provide evidence of a co-existence between M1 and M2 cells in the brain microenvironment as well as intermediate phenotypes (Figure 1). In patients with Alzheimer's disease, microglial activation is reported in conjunction with increased levels of both pro-inflammatory cytokines and angiogenic growth factors. Microglial mediated phagocytosis of amyloid-β is essential for plaque removal and thus neuroprotection. On the other hand, sustained M1 activation upon recognition of the amyloid-β plaques could exert a neurotoxic effect. In the case of primary and secondary brain cancer, balanced coupling of M1 and M2 microglial activation would be beneficial for reducing intracranial tumour burden and maintaining tissue integrity. However, human biopsies and experimental models of gliomas suggest that tumour-associated microglia and macrophages bear an M2 molecular signature and impaired capacity to stimulate T cell anti-tumour immunity - features which sustain neoplastic outgrowth in the brain (1, 4). Is the dynamic equilibrium of microglial polarisation excessively shifted towards either CNS injury or repair by the host in a disease specific context? If so, it could potentially be a promising target for therapeutic intervention in brain pathology.

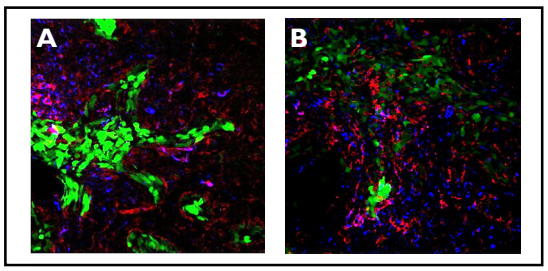


**Figure 1.** M1 (a) and M2 (b) microglia in the metastatic mouse brain. Mammary cancer cells (green) are surrounded by microglia (red, Iba1), some of which are M1 [co-localisation with inducible nitric oxide synthase for M1 phenotype (blue) in a] or M2 [co-localisation with arginase 1 for M2 phenotype (blue) in b]

Although great advances have been made towards defining the association between microglial activation states and brain diseases, the cause-effect relationship remains poorly understood. The identification of molecular determinants of the M1 and M2 inflammatory states in a disease specific setting could yield targets for microglial re-education. The concept of harnessing microglial function has produced promising results experimentally for both neurodegenerative and neoplastic brain disorders. However, the clinical application of this approach is still far from being realised. The lack of detailed knowledge about microglial priming mechanisms and physical limitations for drug delivery into the brain imposed by the blood brain barrier are challenges that need to be overcome in the first instance.

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*Kleopatra Andreou is a DPhil student in the Experimental Neuroimaging Group, supervised by Professor Nicola Sibson, at the Oxford Institute for Radiation Oncology*

# Gut feelings: how gut bacteria influence neurodevelopment and behaviour

by Rebecca Wallings

Microbes within the human body exist as a dynamic super-organism. Much more than a collection of microorganisms thriving off our bodies, the gut microbiota engages in a bidirectional and harmonious relationship with the gut and the central nervous system (CNS), influencing immune function, hormone regulation and neurodevelopment and function. Perturbations in gut microbiota, especially during early life, can have deleterious effects on neurodevelopment that can extend into adulthood. Understanding this delicately balanced relationship between the gut and the brain could open new avenues for developing novel therapies to combat neurodevelopmental and psychiatric disorders.

**The microbiota-gut-brain axis and leaky gut**

The human gastrointestinal (GI) tract is host to nearly 100 trillion microorganisms; 10-fold the number of human cells in the whole body and comprising 150 times as many genes as our own genome. This entire microbial population is collectively known as the gut microbiota. Its key roles are numerous, contributing to the intestinal barrier and protecting against pathogens, promoting epithelial regeneration and intestinal maintenance as well as the metabolism of dietary nutrients and the clearance of toxins and drugs. However, it is becoming increasingly apparent that the influence of the microbiota extends beyond the GI tract.

Encompassing the enteric nervous system, CNS, sympathetic and parasympathetic branches of the autonomic system, and neuroimmune systems, the concept of the gut-brain axis is not a novel one. The reciprocal communication between the GI tract and the brain was first recognised as early as the middle of the nineteenth century. It is *via* this axis that the gut microbiota is believed to influence brain development and behaviour, thus the concept has been extended to the microbiota-gut-brain axis. Such a notion originated from the observation that orally administered antibiotics can reverse encephalopathy in patients suffering from decompensated liver disease (1). Furthermore, the overgrowth of pathobionts within the gut microbiota compromises the epithelial barrier (leaky gut), causing a microbial imbalance and allowing pathobionts to cross the mucosal lining to interact with immune cells and the enteric nervous system (Figure 1). Leaky gut has been associated with various psychiatric disorders such as depression (2), highlighting a potential influence of microbiota on neurophysiology and behaviour.

**Neurodevelopmental disorders**

The pre and postnatal periods in human development are critical windows that are characterised by rapid changes in both microbial and neuronal organisation. Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterised by various social, behavioural and cognitive problems. Laboratory mice that host no microorganisms demonstrated social deficits and increased tendency to engage in repetitive behaviours (3), suggesting that the microbiota is a critical factor for the development of normal social behaviour. Moreover, offspring whose mothers had received an immune insult whilst pregnant display both an imbalance of the gut microbiota and ASD-related behavioural abnormalities (4), highlighting the potential role of microbiota in neurodevelopment. Despite such evidence from animal models, interpretation of data from clinical studies of ASDs is often confounded by marked dietary variations of participants as well as antibiotic use, meaning that definitive conclusions about ASD-related microbiota changes can seldom be drawn.

**Anxiety and Depression**

As the importance of gut microbiota to brain function has gained recognition over the last decade, key findings suggest that stress influences the composition of gut microbiota and that bidirectional communication along the microbiota-gut-brain axis impacts reactivity to stress. The response of the hypothalamic pituitary gland to stress is increased in mice that have no microbiota. This phenotype is diminished by reconstitution with faeces from

healthy mice at 6 weeks, but not 14 weeks, suggesting that establishment of gut microbiota is required during early development of the hypothalamic pituitary gland (5). Although animal studies have consistently demonstrated the benefits of such alteration to microbiota for anxiety and depression-like symptoms, limited clinical work has been carried out in this area. In one pilot study, patients with chronic fatigue syndrome receiving probiotics for two months demonstrated significantly fewer symptoms of anxiety compared to the placebo group (6). Although this study and other pilot studies of a similar nature have been limited to non-psychiatric patients, the evidence suggests that targeting the gut microbiota could help to treat anxiety and other related mood disorders.

## Sex differences

The composition of the gut microbiota, microbiome and its metabolic products change over the course of a human life and can be shaped by various factors, one of which is host sex. Importantly, these variations and periods of instability in microbiota composition appear to correlate with both sex and age-specific disease risk. During the first trimester of pregnancy, the maternal gut microbiota has increased levels of bacteria that metabolise fibre and produce short chain fatty acids (SCFAs), which cross from maternal serum and enter foetal circulation *via* the placenta. These SCFAs are required for normal neurodevelopment of the foetus; however, prolonged and exacerbated exposure during late gestation can have deleterious effects on the foetus later in life in a sex-dependent manner. Male rats, but not females, exposed to SCFA producing bacteria during late gestation exhibited increased anxiety-like behaviour, decreased social interaction and exaggerated stress responses (7). Such sex differences persist into adulthood, resulting in differential effects in neurotransmitter signalling, crucial for normal feedback between the gut and the brain. As neuropsychiatric disorders exhibit a variety of sex differences with regards to severity, presentation and age of onset, a fundamental role of a sexually dimorphic microbiota provides an exciting avenue to explore and identify potential biomarkers and novel therapies.

## Moving forward

As research on the gut microbiota has expanded over the last decade, it has become increasingly apparent that the trillions of microorganisms that reside within our own GI tract are not simply a foreign entity 'along for the ride', but influence our own development as much as our bodies and the environment shape theirs. Although there seem to be critical windows in microbiota and neurodevelopment that coincide and are capable of bidirectional influence, the underlying mechanisms of these effects remain to be elucidated. As developmental changes and sex differences in microbiota parallel those in immune, neural and metabolic systems, bioinformatic approaches capable of integrating complex datasets will be instrumental in providing insight into how these differences and changes are altered in disease. Importantly, this may deliver novel approaches for therapeutic interventions in at-risk populations.

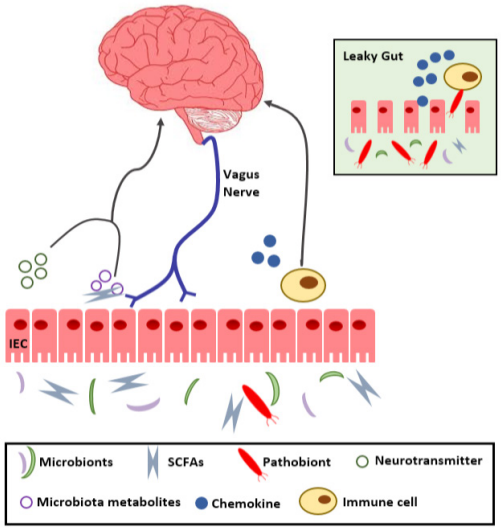


**Figure 1.** The microbiota-gut-brain axis represents a bi-directional communication system whereby both direct and indirect signals can be sent via immune, neural, endocrine and metabolic pathways to influence function. Metabolites produced by microbionts, chemokines produced by immune cells and neurotransmitters produced by enteroendocrine cells are all able to influence the brain. Activation of neural circuits can impact the gut by hormone release as well as altering cytokine release and microbiota composition. Under pathological stress, pathobiont overgrowth can induce inflammation and increased cytokine release, encouraging further translocation of pathobionts across the intestinal mucosa. Such conditions can lead to altered cognition, behaviour and stress responses.

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*Rebecca Wallings is a DPhil student in the Wade-Martins Group, Department of Physiology, Anatomy and Genetics*

# Classic Kit: The Scanning Electron Microscope

by Emma Bickford

The Scanning Electron Microscope (SEM) scans a focussed beam of high-energy electrons over the surface of a sample to produce an image. The electrons interact with the sample, generating signals that reveal a myriad of information about its surface topography and composition.

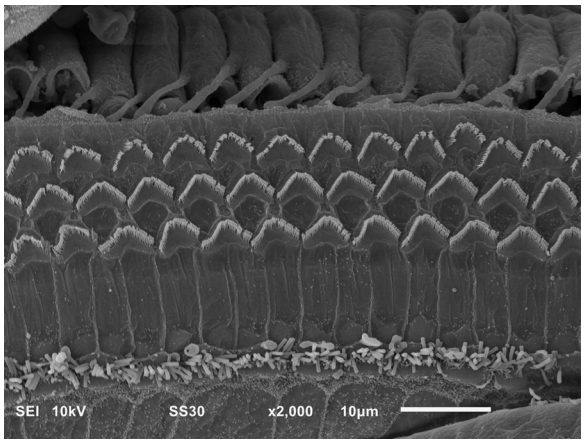
In Berlin in 1937, M. von Ardenne constructed the first true high magnification SEM. The instrument successfully circumvented the problem of chromatic aberration encountered with thick samples using Transmission Electron Microscopy (TEM) and had an impressive resolving power. However, the technological constraints of the era limited the realisation of von Ardenne's ideas. Sadly, his SEM was destroyed in an air raid in the Second World War and he abandoned his research on electron microscopy (1).

Nearly thirty years of intermittent SEM development followed in Germany, the United States, England and Japan. This endeavour was brought to fruition in 1965, when the Cambridge Scientific Instrument Company launched the 'Stereoscan', the world's first commercial SEM device (1).

In a SEM, electrons fired from a 'gun' are accelerated down a column and passed through various lenses and apertures to produce a focussed beam. Scan coils, located above the objective lens, control the position of the electron beam, allowing it to be scanned across the surface of the sample. Interactions between the electron beam and atoms at different depths within the specimen emit signals, which are received by an electron detector and displayed as an image on a computer screen. Prior to analysis, biological samples must be coated with a thin layer of electrically-conductive material such as gold or platinum and are positioned on a stage in the vacuum chamber of the SEM.

The predominant method of detection is secondary electron imaging (SEI). The secondary electrons are emitted in close proximity to the sample's surface, meaning exceptionally high-resolution images of its texture and morphology can be obtained. Scanning electron microscopy unveils a wealth of ultrastructural information that vastly exceeds the capacity of light microscopy. While optical microscopes are limited to a maximum magnification of around 1000x with a resolution approaching 100 nanometers, the SEM is able to magnify in the region of 500,000x with a resolving power exceeding 1 nanometer.

The SEM is crucial for the study of solid specimens and its breadth of applications is unrivalled. Current applications of scanning electron microscopy include inspection of the sensory hair cells of hearing-impaired mouse models (2) and research into the scaled wings of the Lepidoptera (3). Spatial variations in chemical compositions may also be elucidated using scanning electron microscopy, facilitating the elemental and compositional mapping of microstructures.



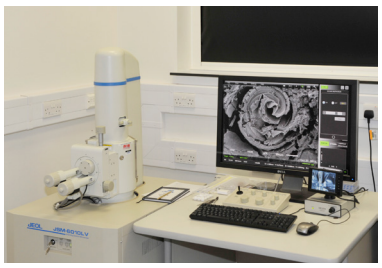



**Figure 1.** Top: SEM image of the sensory epithelium of the mouse inner ear hair cells by Emma Bickford. Left: SEM equipment. Photo courtesy of Jeremy Sanderson, MRC Harwell.

The SEM is easily operated and sample preparation is minimal for many applications. While conventionally limited to samples which are solid, stable in a vacuum and of a size suitable for the specimen chamber, continuing developments in SEM technology may create more specialised instruments. Overall, use of the SEM has precipitated a spectacular array of discoveries. Furthermore, the recent emergence of models designed to operate in a low-vacuum mode or the Environmental SEM (ESEM), which is suitable for analysis of unprocessed non-conductive biological material, only serve to enhance the versatility of this valuable research tool.

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# Inspiration from nature: how tick spit can be used to treat disease

by
James Eaton

Nature has always been an invaluable source of inspiration for drugs. The ongoing battle between different organisms for survival has led to species evolving to produce complex molecules that inhibit vital biological pathways of their competitors. These molecules have proven to be excellent sources of life-saving compounds for use in human medicine. For example, fungi have been locked in a constant battle with bacteria and other organisms for scarce resources and nutrients. As a result of this, they have developed molecules to inhibit the biological pathways of and to destroy their competitors, allowing themselves to flourish instead. The study of these fungi has resulted in the discovery of important compounds such as penicillin, an important antibiotic class used to overcome once-fatal infections, and statins, drugs used to slow the build-up of fatty plaques in human arteries and reduce the number of heart attacks and strokes.

Another battleground in nature that has yielded essential drugs is the one between host and parasite. Ticks are parasites that must live on their hosts for a number of days and are therefore in an evolutionary arms race to develop tools for evading detection by their host's immune system. For the host to initiate an immune response, the immune cells responsible for removing and recognising foreign bodies must first be recruited to the site of infection. One group of proteins responsible for this process is the chemokine family. Chemokines are ***chemo***tactic cyto***kines***, small, secreted proteins responsible for initiating the migration of white blood cells to areas of inflammation (1). There are approximately 40 chemokines and 20 chemokine receptors in total. It is impressive, yet unsurprising, that as chemokines are vital in the initiation of an immune response, ticks have developed proteins in their saliva that are able to bind to them. These proteins are known as Evasins and are able to prevent chemokines from recruiting white blood cells. In the published literature to date, only three Evasins from a single tick species have been characterised (2). Interestingly, each one is able to bind to multiple chemokines. This is important as the chemokine network has been described as 'redundant' due to the ability of multiple chemokines to bind to multiple receptors and *vice versa* (3). What this means is that targeting a single chemokine or receptor with a therapeutic agent will not work as another chemokine or receptor can easily initiate the migration of immune cells in its place. The redundancy in this system may explain the recent failure in the development of drugs to target the chemokine system (4).

**Figure 1.** Do ticks hold the key to treating inflammatory and cardiovascular diseases?

As mediators of the immune response, chemokines play an important role in a number of diseases where pathological inflammation is the main driver of the condition. Examples of these diseases include myocarditis, rheumatoid arthritis and Crohn's disease (5). Each disease has a distinct chemokine expression pattern, which may vary between patients (6-8). By using proprietary screening technology, the Bhattacharya lab has recently identified over thirty novel Evasins from multiple tick genera, each one having a distinct chemokine binding pattern. This means that if the levels of each chemokine involved in a particular disease or patient are known, it might now be possible to select the most suitable Evasin to modulate the activity of the most relevant chemokines. Thus, harnessing tick spit may be able to bring about a targeted and personalised reduction in chemokine-driven deleterious inflammation, improving outcomes for many patients.

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# Co-operation in the brain: the importance of glia in health and disease

by Heather Booth

The human brain contains multiple cell types, the most famous of which is the neuron. When questioning how the brain works, or what is going wrong when it doesn't perform as expected, researchers often turn to neurons for answers. Neurons, however, only make up around half of the cells in our brains, the remainder of which are glia. There are three main sub-classes of glia: oligodendrocytes, microglia and astrocytes (Figure 1).

The main role of oligodendrocytes is to myelinate neuronal processes, which involves wrapping themselves around the axons and dendrites of a neuron to aid in the conduction of the electrical signals that neurons use to communicate. Microglia form part of the brain's immune system and act as the main defence to any insult.

Astrocytes have a wide range of functions, the majority of which revolve around maintaining the wellbeing of neurons. They provide structural and metabolic support, and regulate synaptic transmission, water transport, and blood flow. They also contribute to the blood-brain barrier, separating neurons from meninges and blood vessels. Additionally, upon microglia-mediated initiation of an immune response, astrocytes surround the threatened area and create a barrier to prevent the spread of toxic signals into the surrounding healthy tissue. Neurons in the brain, therefore, cannot easily survive without the support of glial cells (1).

With so many essential functions performed by glia, it is clear that any disruption to the function of these cells could cause major problems: this is the case in Alexander disease. Here, a mutated form of the astrocyte-specific gene Glial Fibrillary Acidic Protein (GFAP) causes the resulting mutated GFAP protein to aggregate within astrocytes. This prevents them from performing their normal role, ultimately leading to neuronal degeneration (2). More common, age-related neurodegenerative diseases such as Alzheimer's and Parkinson's disease have been shown to exhibit increased activation of astrocytes and microglia in regions of neurodegeneration. This is thought to be a response to the toxic environment produced by neuronal cell death; however, it is possible that dysfunctional glia may in fact be initiating these processes (3). Genes that have been implicated in hereditary forms of these diseases are expressed in glia, in some cases at higher levels than in neurons (4). It is therefore plausible that mutations in these genes could be initiating cellular dysfunction in any, or all, of these cell types.

Despite their importance, comparatively little research has been conducted into glial cell function relative to neuronal function. The majority of articles in the field of 'Neurology' focus on neurons. The discovery of stem cells and the establishment of protocols to differentiate them into specialised cell types, has enabled researchers to study disease mechanisms in more relevant cellular models. In the case of neurodegenerative diseases, this approach has generally resulted in the generation and identification of neurons specific to the brain region where the disease pathology is found. Although this can be a very powerful experimental strategy, aspects of the disease may be missed by studying these neurons in isolation. A strong argument is emerging for establishing cell cultures that contain both neurons and glia from the brain region of interest.

With the recent increase in research investment from governments looking to tackle the rising economic costs of mental illness and dementia, such as the "BRAIN Initiative" in the USA and the UK government's "Dementia Challenge", it is important to remember that neurons may not hold all the answers to our questions. Wider investment into the study of other cell types may well prove critically important to our understanding of how the human brain works.

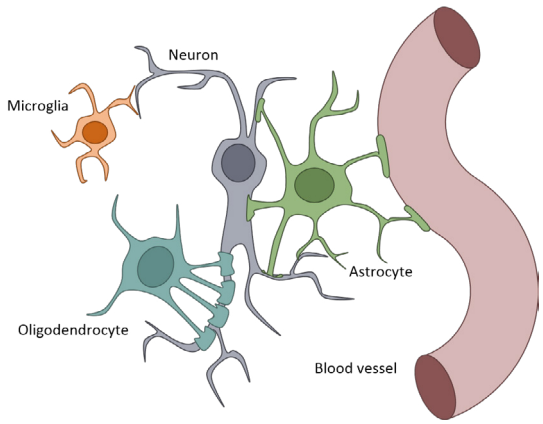


**Figure 1.** Glial subtypes and their interactions

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# Oxford Hands-On Science: a new direction for Oxford public engagement

by Beatrice Tyrrell & Tonia Thomas

Public engagement is a key topic on the scientific community's agenda, with many postdoctoral researchers and principal investigators aware of its importance, yet finding little time to focus on it. Oxford Hands-On Science (OxHOS) is a new student-run society at the University of Oxford, aiming to share research and engage prospective young scientists by involving undergraduate and postgraduate students in outreach. We believe that science is fun and relevant to everyone, and we aim to spread our enthusiasm for science to children and their families. We do this by taking interactive experiments, drawn from a wide range of subject areas, into schools and public venues, and we rely on helpful volunteers from the student body to explain the science behind the experiments.

So where did this idea come from? OxHOS was founded in June 2015, after I had spent the day at a public engagement conference representing Cambridge Hands-On Science (CHaOS), now the sister society of OxHOS. My passion for public engagement was re-ignited, and I thought – if CHaOS has been running successful interactive science Roadshows for over 10 years, why can't I bring the model to Oxford? So, within a few weeks, I had got together a team of excited committee members, and in collaboration with CHaOS, we made a plan to run a pilot Roadshow in Oxfordshire.

**The Autumn 2015 Roadshow**

Our first Roadshow lasted one week. It included three school visits and two public events held in Oxford, during which we were able to involve lots of children and their families in many exciting hands-on activities. It was all made possible by the generosity of the CHaOS team, who loaned us their experiments for the week, and by the sponsorship of the Nuffield Department of Surgical Sciences.

**Figure 1.** Pupils trying out the organ vest at St Andrew's Primary School, Headington.

In schools, we predominantly work with students in years 5-8 and set up around 15 experimental stations covering a range of scientific subjects. We divide classes into small groups, so that pupils can circulate around the stations and have a go with the activities guided by a demonstrator. Providing engaging science activities to this age group is essential: many organizations target A-level and GCSE age students, which is undoubtedly beneficial in promoting access to higher education. However, there is increasing evidence that earlier intervention is key to maintaining students' interest in science. The ASPIRES research report, conducted by the Department of Education at King's College London, investigated the career aspirations of young people aged 10-14. It demonstrated that, by this age, only 15% of students agree that they would like being a scientist (1). It's therefore crucial that we target students earlier to show them how exciting science can be and to combat common misconceptions about "old, grey-haired scientists."

During our Autumn Roadshow, we visited Fitzharrys School in Abingdon, where we held sessions for Year 7 and 8 classes, and also hosted Year 6 students from the local Dunmore Primary School. We also saw Year 4-6 classes at St Andrew's Primary School in Headington and visited the Oxford Academy in Littlemore, where we engaged students from Years 7-9. Feedback from schools was collected through a teacher's questionnaire including comments from students – and it was excellent!

Involving families in public engagement efforts is also very important: ASPIRES reports that "a key factor affecting the likelihood of a student

aspiring to a science-related career by the age of 14, is the amount of 'science capital' a family has" (1). So, alongside visiting schools, we also hold public events or team up with existing events to reach families. During the weekend of the Autumn 2015 Roadshow, we ran two public events at the East Oxford Community Centre and Templars Square Shopping Centre. We were thrilled to see all ages represented at the events and very excited by the number of local families that popped in for a look! It was fantastic to hear a number of parents asking us to visit their children's school or wanting to come to more of our events. Some of our more popular experiments included the mini explosions caused by the interaction between baking soda and lemon juice, the magic of UV light, and the spinning chair, which demonstrates the concept of angular momentum. We also collected some formal questionnaire-based feedback: 100% of our visitors rated the event either fantastic or enjoyable, and the majority of our visitors thought that the content level was about right and had learnt a lot.


## Looking to OxHOS' Future

So what's next for us? The OxHOS committee is busy planning the Summer 2016 Roadshow, when we'll be taking part in the Oxfordshire Science Festival at the end of June, and visiting schools in Oxfordshire before heading off to South Wales for a week. We aim to provide all of our activities free of charge, both to schools and to members of the public, ensuring that financial circumstances aren't a factor in determining audience attendance to the Roadshows. For the same reason, we also visit schools lacking the resources to hold extra-curricular practical activities, and we travel to areas where access to science museums and research-intensive universities is more limited.

The OxHOS committee is also working on a whole new repertoire of interactive experiments, drawn from across the sciences. Our current committee consists primarily of DPhil students, so we're all working on translating elements of our research into activities suitable for school students – with topics ranging from neuroscience to immunology, and from angular momentum to electrolysis!

## How do I get involved?

At the heart of OxHOS is the enthusiasm of our volunteers – and soon we'll be recruiting for the Summer 2016 Roadshow: keep your eyes peeled if you're interested! In the run-up to the Roadshow we'll be running sessions to introduce you to our experiments and pass on tips for how to communicate science in a fun and accessible way: no experience is necessary to get involved!

In addition, we're always on the lookout for anyone who can support us with resources for experiments, contacts in schools or pointers for fundraising.

You can visit our website at https://oxfordhandsonscience.wordpress.com/ and email us at contactoxhos@gmail.com!

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*Tonia Thomas is studying for a DPhil in Cardiovascular Regenerative Medicine in the Department of Physiology, Anatomy and Genetics, Oxford*

# Cardiac image-based phenotyping: a scientific fairytale or an emerging reality?

by Dr Christakis Constantinides

*"What would you do if your physician offered you a free screen of your entire genome that would estimate your risk factors for developing certain diseases throughout your entire life?"*, the attending physician asked the child of a thrombocytopenia patient, who was seated at the front of the lecture theatre packed with first year medical and doctoral students at the Johns Hopkins Hospital. It was the weekly clinical correlation session on a gloomy afternoon in the autumn of 1995. While major ethical and confidentiality concerns exist in addressing this simple question (beyond plain personal choice), there are also important political, financial, and scientific implications and consequences.

**Scientific advancements**

Twenty-one years later, the question remains controversial. Nonetheless, tremendous advancements in the field of genomic sequencing and manipulation have been achieved. Both the human and mouse genomes have been mapped (in 2001 and 2003, respectively) due to pioneering efforts initiated by the National Institutes of Health, USA. In addition, targeted mutagenesis, knockout (loss of function), conditional knockout and knock-in (gain of function) studies have now become routine. Correspondingly, tremendous technological advances have been documented, providing a plethora of tools for rapid and high-throughput screening. Clinical screens for single nucleotide polymorphisms (SNPs) that may contribute to disease susceptibility are now being routinely conducted for detection of transcriptional variability, while mutation identification studies routinely embark on the hunt for candidate disease genes, or for manifestations of the observable clinical characteristics, collectively described as the **phenotype**. In the pre-clinical cardiovascular field, scientists have targeted six important areas of cardiac function for further investigation: (a) the excitation-contraction cascade, (b) the beta-adrenergic system, (c) the cytosolic/structural system and the cytoskeleton, (d) the extracellular matrix and its coupling to important cytosolic elements that assist in the generation or propagation of mechanical force, (e) identification of molecules that influence mechanical changes (for example, due to differential gene expression, protein modification, or activation of foetal development gene programmes), and (f) the energetic-metabolic status of the muscle. Capitalising on the accumulated knowledge and reinforcing findings from these branches, international scientific efforts have now turned to phenotyping, in an effort to understand how ultrastructural molecular and cellular changes are manifested at the organ/system level.

**Phenotyping- the role of imaging modalities**

Collectively, the phenotype encompasses the observable characteristics that are the cumulative effect of an underlying genetic variant or variants and/or environmental influences. A phenotype can be morphological, developmental, physiological, or behavioral. Given the fact that numerous diseases are influenced by multiple genetic variants, recent efforts have aimed to investigate these variants and map genotype to phenotype. For example, genome-wide association studies (GWAS) have recently become popular in medicine, and involve methodical screening for genetic markers that are identifiable in individuals with a particular disease but not in a matched control group. Concerted and internationally collaborative efforts have also focused on molecular and genetic mechanisms of disease or on high-throughput phenotyping (including the Jackson Labs [https://www.jax.org/], the European Mouse Phenotyping Resource of Standardised Screens (EMPReSS) [http://empress.har.mrc.ac.uk/], and the International Knockout Consortium (IKMC) [http://www.knockoutmouse.org]).

From an imaging standpoint, phenotyping has traditionally been based on anatomical, structural and functional screening. Magnetic resonance imaging (MRI) has pioneered cardiac translational work (spanning the human-mouse-embryo scales – Fig. 1), but Positron Emission Tomography (PET), Computer Tomography (CT), Single Photon Emission Computer Tomography (SPECT), and optical imaging techniques have also been used extensively for either pre-clinical or clinical screening. Despite all these efforts, the need for more accurate,

sensitive, and sophisticated image-based phenotyping of biomarkers remains. For example, novel MRI techniques routinely allow non-invasive estimation of changes in tissue displacement, and provide regional and highly sensitive indices of circumferential, radial, and longitudinal mechanical strain, as cardiac functional biomarkers for contraction and relaxation. However, complete characterization of the mechanical response of biological tissue necessitates knowledge of both strain and stress (the innate ability of tissue to develop force) that is currently not possible to quantify using MRI. Hence, a technique that provides information on stress-related biomarkers would be more appropriate and relevant to cardiomyofibre and sarcomeric changes. However, the development of techniques relevant to stress quantification in the heart are in general highly complex and difficult to achieve.

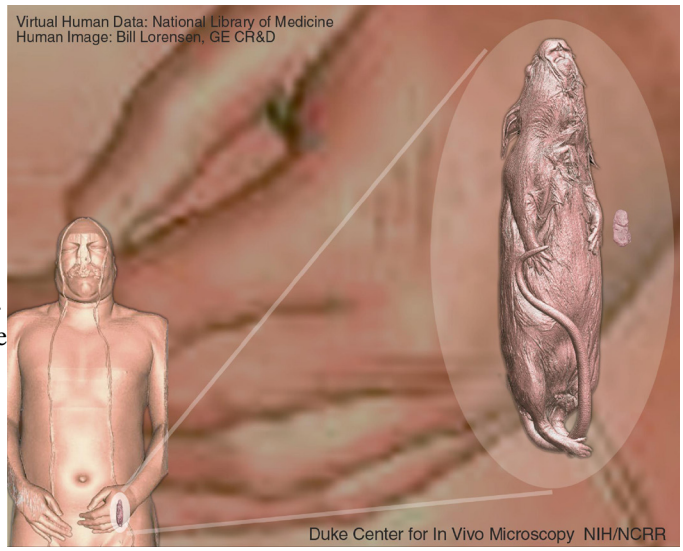


**Figure 1:** Translational phenotyping efforts are now streamlined using MRI-based protocols spanning the spatial anatomical scales of the human, mouse, and mouse embryo.Figure reproduced with permission from Professor G. Allan Johnson, Center for In Vivo Microscopy, Duke University Medical Center, USA and InTech Publications,

## The future

Despite the complexities and technical difficulties inherent to identifying new and more sensitive image-based biomarkers for cardiac function, tremendous amounts of data have become available. Beyond the issues relevant to 'big-data' handling and management, additional concerns relate to the appropriate scientific classification of these biomarkers, either as population-averaged indices, or in the form of registered anatomical atlases (capitalising on ongoing work, such as the Cardiac Physiome Project). While the former approach is, and has been, the convention, anatomically based atlases are becoming popular and may establish morphometric and quantitative phenotypic norms in the near future.

## Conclusion

More than two decades have elapsed since the initial, ground-breaking question of personalised genomic screening. Despite our current and understandable reluctance to take a stand on such fundamentally important dilemmas on health and quality of human life, scientific communities have expended tremendous efforts in streamlining processes and technologies to enable us to do this. Ultimately, it appears that phenotyping and image-based screening is becoming a reality of modern medicine, paving the way for personalised therapies.


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# Decrypting drug resistance in tuberculosis around the world with CRyPTIC

by Dr Ana Gibertoni Cruz & Dr Philip Fowler

As I write for *Phenotype*, I think about phenotypes: more specifically, about the drug susceptibility profiles of *Mycobacterium tuberculosis*, the causal agent of tuberculosis (TB). Globally, tuberculosis is now the leading cause of death due to a single infectious agent: in 2015, TB caused 9 million people to fall ill and 1.5 million to die (1). The phenotype of each infection is a very important determinant of the clinical response to treatment; in many circumstances, it determines whether you will be cured or whether you will remain confined to an existence of physical and emotional suffering, unable to study or work and with a significantly shortened life expectancy.

Thanks to decades of work to reduce TB occurrence here in the UK, it is unlikely that you will know someone who has had it, although London ranks as the city with the second highest TB rate in Europe (the highest rate is found in Lisbon) (2). Yet the past effects of TB still linger in our collective cultural consciousness. Think of the tragedy of Mimí in the opera La Bohème, Nicole Kidman's character in the film Moulin Rouge, or James Herriott traipsing around the Yorkshire Dales testing herds of dairy cattle for TB. Even the BCG (Bacillus Calmette-Guérin) vaccine commonly used to confer immunity to TB appears different: the characteristic dimpled pattern on your arm, a permanent scar that represents rather variable degrees of protection against the disease.

The World Health Organisation (WHO) has chosen 24 March to mark World TB Day. This initiative aims to build, year-by-year, public awareness that even today tuberculosis remains an epidemic in much of the world. The 2016 theme, 'Unite to End TB', highlights the need for a concerted effort by the TB research community to effectively address gaps in health care delivery, treatment and diagnosis. Oxford is leading CRyPTIC (Comprehensive Resistance Prediction in Tuberculosis: an International Consortium) as a part of this effort.

The tuberculosis bacterium has a tough, thick cell wall that is both its strength and its weakness. It slows the passage of both nutrients and drugs, resulting in a bacterium that is slow-growing and difficult to kill. The best current regimen is a six-month combination of antibiotics, which poses a challenge in terms of drug interactions and toxicity, especially in the context of HIV-TB co-infection. Unfortunately, the proportion of TB cases that are resistant to this treatment is rising – it is estimated that 3.5% of new cases in 2015 (480,000) were multi-drug resistant (MDR)-TB but that fewer than 45% of the estimated numbers were actually diagnosed as such. To make matters worse, of all the MDR-TB diagnoses made, only 70% received appropriate therapy (1), resulting in a contingent of people who are not only likely to fail treatment but also to spread MDR bacteria. In line with this, the WHO has declared the rising prevalence of MDR-TB a public health crisis.

There are two ways in which drug-resistant TB can be identified. The first is by culture-based drug susceptibility testing; this technique is expensive and laborious, requiring specialised laboratory facilities with trained technicians. It can also take several weeks to return a result to the clinician, due to TB's slow growth rate. Molecular tests, such as line-probe assays or real time PCR, are faster, but the main challenge for these technologies is that they can only identify a small number of specific resistance-conferring mutations; they inevitably, therefore, focus on the most common drug-resistant genetic variants.

CRyPTIC is a global collaboration that aims to develop better, faster and more targeted treatments of drug-resistant tuberculosis. The focus is on the development and application of genetic methods for diagnosing drug-resistant TB and, more importantly, rapidly predicting the most appropriate treatment from a panel of the 15 most clinically used drugs. Unlike *Enterobacteraceae*, such as *E. coli*, drug resistance in TB arises only through chromosomal mutations, which makes it well-suited to genetic approaches.

Inferring the phenotype from the genotype is a significant departure from traditional culture-

based methods and is predicated by huge advances in genetic sequencing, which have seen the cost to sequence a single human genome fall from $1 million in 2008 to around $1,000 last year. This technology now allows the whole genome of a bacterial infection to be routinely sequenced for around $100 in a few days. As before, a clinician collects a sample from the patient but rather than it being cultured, the genome of the bacterium is sequenced and examined for mutations. Many mutations that confer resistance to the front-line drugs are already known and therefore a list of effective drugs, tailored to that patient, can be rapidly compiled. One of the aims of the CRyPTIC project is to produce a complete catalogue of mutations associated with one or more drugs, building a large dataset to detect all genomic variation accountable for drug resistance.

A particular strength of the CRyPTIC project is its global coverage: isolates will be sequenced from patients in areas of the world which have been under-represented in previous sequencing efforts, namely China, India, South America, Pakistan, Africa and Central and Southeast Asia. Inevitably, novel genetic variants will be encountered during this process and in any future clinical diagnostic pipeline. Whilst the majority are not likely to confer resistance, a small and possibly increasingly number will be novel resistance-causing mutations. These will be functionally validated *via* the generation of unmarked *M. tuberculosis* mutants by allelic exchange experiments. Another approach, currently being developed by Philip Fowler as part of the CRyPTIC project, uses sophisticated computer simulation methods, to ***predict*** whether a previously unseen mutation causes resistance. At the heart of this method is the assumption that many chromosomal mutations causing resistance are simply reducing how well the antibiotic binds to a specific protein, whilst not affecting the binding of any natural substrates. If this assumption is true, then the change in binding free energy of an antibiotic upon making the mutation will indicate the degree of resistance conferred. Philip has preliminary data showing that the approach can successfully predict which mutations cause resistance to trimethoprim in S*taphylococcus aureus.* Currently, he is also investigating a small subset of mutations in *pncA*, a gene encoding for an *M. tuberculosis* protein that binds pyrazinamide, one of the most commonly used TB drugs.

Calculating binding free energies is notoriously difficult and a plethora of methods have been developed over the years. One class of methods, dubbed alchemical free energy methods, is theoretically exact, having been derived from classical statistical mechanics. Since each calculation requires hundreds or thousands of computer hours, binding free energies have, until now, remained an academic curiosity. However, the continued increase in computer speeds combined with the recent advent of distributed computing, including cloud computing, now make it feasible to apply these techniques in a high-throughput manner.

Supported by multiple funders, CRyPTIC is a truly global strategy to tackle a global disease. In coordinating this unprecedented collaborative effort to generate vast amounts of data and to apply cutting-edge analytical methods to the findings, Oxford embodies the 2016 World TB Day spirit by uniting the most important TB research institutions worldwide in an effort to end TB.

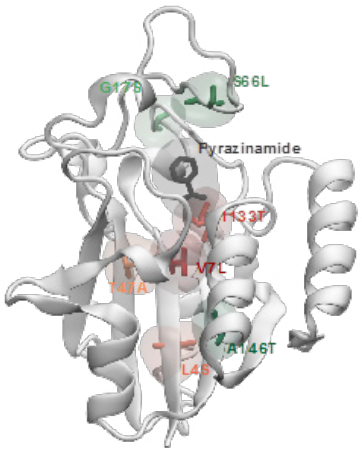


**Figure 1.** The tertiary structure of M. tuberculosis pncA, showing the different mutations under study. The mutations thought to confer resistance are coloured red; the mutations not thought to confer resistance are coloured green.

**Further information**

The University of Oxford press release, 23 March 2016: 'Global team aim for faster, more effective TB diagnosis'. http://www.ox.ac.uk/news/2016-03-23-global-team-aim-faster-more-effective-tb-diagnosis [Accessed 31 March 2016].

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*Dr Ana Gibertoni Cruz is a clinical research fellow, and Dr Philip Fowler is a senior researcher at Modernising Medical Microbiology, Nuffield Department of Medicine, Oxford*

# Diabetes: complex solutions for a complex disease

by
Dr Anne Raimondo

*Am I at risk of developing diabetes?* It's a topical question, but not always an easy one to answer. Statistics alone suggest that the answer may well be "Yes, eventually". In England, the number of people diagnosed with diabetes has more than doubled from 1.4 million to 3.5 million in less than ten years, and current estimates indicate that one adult in ten worldwide will be affected by diabetes by 2040 (1). The UK National Health Service spent approximately 10% of its budget on diabetes in 2010-2011, which equates to about one million pounds every hour (1, 2). In fact, the World Health Organisation is so concerned about the rising global 'diabetes epidemic' that it has made diabetes prevention, management, and surveillance the main focus of World Health Day 2016.

Diabetes is of course not a single disease, and the current global increase in its prevalence is overwhelmingly due to increased incidence of type 2 diabetes (T2D). Unlike type 1 diabetes – an early-onset autoimmune disease that completely destroys the insulin-producing beta-cells of the pancreas – T2D is normally diagnosed in adulthood as a consequence of decreased insulin production (insulin deficiency) or action (insulin resistance) (Figure 1). Most of us know that lifestyle factors such as diet and exercise heavily influence T2D risk, and it has indeed been estimated that you are seven times more likely to develop T2D if you are obese compared to someone of healthy weight (3). Is this then the answer? Can you abolish your risk of developing T2D simply by eating well, exercising regularly, and maintaining a healthy weight?

Actually, as a means of improving your overall health, that's a pretty effective strategy. However, there are several interesting features of T2D that tell us it's more than simply a lifestyle disease. First of all, it clearly runs in families. You are two to six times more likely to develop T2D if you have a positive family history compared to someone who doesn't (4). T2D prevalence is also greater in Black, Asian and other minority ethnic groups compared to Caucasians (1). These facts could simply be attributed to families and ethnic communities sharing similar diet and lifestyle habits as well as genes; however, studies examining the extent to which twins share a particular trait despite having been raised in different environments show that concordance rates for T2D are significantly higher for genetically identical monozygotic twins compared to non-identical dizygotic twins (5). There is also the interesting observation that first-line treatments for T2D, such as the drug metformin, can induce unpredictable side-effects or simply show no therapeutic effect at all in some people. Surely if all T2D individuals have the same disease, they would all respond to treatment in the same way?

The evidence suggests that T2D itself is not a single disease, but a heterogeneous mix of diseases with a shared set of symptoms. The bottleneck seems to lie in our inability to understand and predict differences in T2D pathogenesis between individuals, which could be improved by a better understanding of the underlying biology. Even though the genetic component to T2D has been recognised for decades, the first genetic drivers of the disease were only identified in the early 1990s. These studies relied on familial linkage analysis, which looks for co-segregation between a particular genetic mutation and disease penetrance in family pedigrees, to identify diabetes susceptibility genes. These were followed by somewhat larger 'candidate gene' studies, which

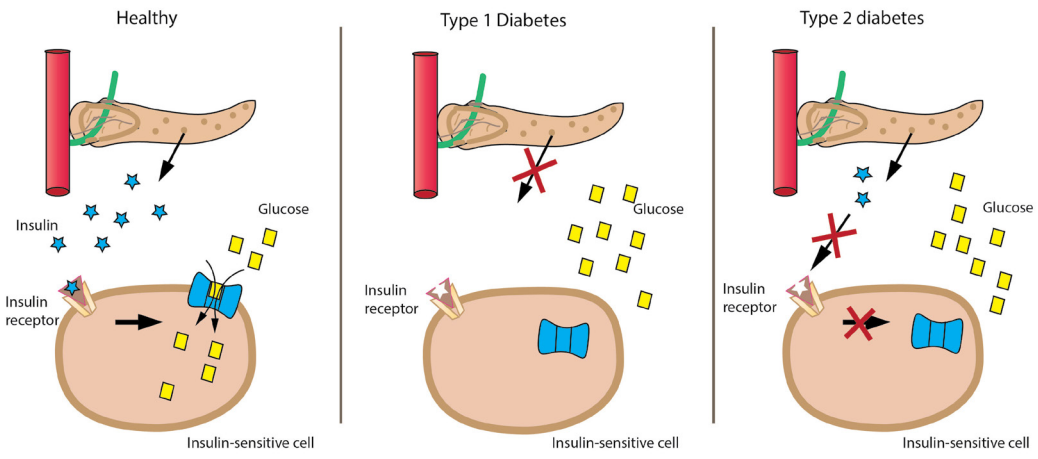


**Figure 1.** Schematic of the physiological alterations that characterise type 1 and type 2 diabetes. Decreased production of insulin by the pancreas results in insulin deficiency, whereas decreased insulin signalling in peripheral tissues results in reduced glucose uptake and insulin resistance. Type 1 diabetes occurs as the result of complete insulin deficiency, whereas type 2 diabetes can be the consequence of one or both of these mechanisms.

essentially involved the selection of biologically plausible genes for further investigation in families and unrelated human populations, usually on the basis of a known role in beta-cell function, insulin uptake, or other metabolic conditions thought to increase T2D risk. Unfortunately, many of the findings from these early studies were not independently reproducible. The candidate gene approach also restricted discovery efforts to genes of known function. What was needed was an experimental methodology that enabled hypothesis-free identification of novel T2D susceptibility genes in sample sets large enough to distinguish pathogenic from benign genetic variation.

The advancement of high-throughput sequencing technologies in the early 21st century has provided scientists with the necessary tools to sequence the genomes of increasingly large and more diverse sample sets, enabling the discovery of previously unappreciated genetic contributors to T2D risk. As the technology has become cheaper and faster to use, more and more genetic variants associated with T2D have been identified. There are now more than 100 regions of the genome known to influence T2D risk, and we are only just beginning to understand the biological, cellular and molecular pathways through which they act. In some cases the results have already paid dividends. For example, mutations in the *KCNJ11* and *ABCC8* genes can cause a type of diabetes known as Maturity-Onset Diabetes of the Young (MODY), thus named because it presents with some of the hallmarks of regular T2D but in much younger patients. *KCNJ11* and *ABCC8* encode two subunits of an ATP-sensitive potassium channel expressed on beta-cells that normally closes in response to glucose signalling, generating an ion gradient that stimulates insulin secretion. Once it was discovered that some MODY individuals possess pathogenic mutations in *KCNJ11* or *ABCC8* that prevent closure of this potassium channel, expensive and problematic insulin regimes could be substituted for cheaper and more effective oral sulphonylureas, which bind and inhibit the potassium channel and restore insulin secretion.

So what is the future of T2D research? The implications for associated health complications (such as cardiovascular disease, blindness, and nerve damage) and premature mortality alone justify our continued investment in T2D research. There is still a substantial gap between our knowledge of the genetic variants statistically associated with T2D risk and the ways in which they perturb normal cellular behaviour.

> “The evidence suggests that T2D itself is not a single disease, but a heterogenous mix of diseases with a shared set of symptoms.”

Interestingly, the majority of these variants appear to influence insulin secretion and beta-cell function, as opposed to insulin resistance in peripheral tissues, putting pancreas development and function at the forefront of our efforts to identify causal biological pathways for drug development. Recent breakthroughs in our ability to generate patient-derived beta-cell models from induced pluripotent stem cells (iPSCs) offer an exciting opportunity to study beta-cell function and patient-specific responses to new drugs in the lab in a relatively high-throughput manner. Worldwide multi-centre research initiatives such as the European Union-funded DIabetes REsearCh on patient straTification (DIRECT) project also hope to discover more effective, personalised treatments for T2D subtypes through a combination of genetic and phenotypic profiling, identification of novel biomarkers, and preliminary clinical trials (6). One day, perhaps, human genetics may be of equal predictive value to lifestyle factors in our attempts to predict, diagnose, treat, and maybe even prevent T2D.

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# Special Feature:
# The OUBS Careers Day

by
Dr
Burcu Anil
Kirmizitas

It was the morning of February 23rd: the coffee and cookies were laid out on the table in the Biochemistry department's main seminar room, ready for the Oxford University Biochemistry Society (OUBS) Career Day. The organisers were excited to welcome the speakers and the programme for the day looked like we were in for a treat, finding out about all kinds of industries Oxford biochemistry alumni work in.

After everybody had taken their seats and Professor Jane Mellor made her opening remarks, the first speaker of the day took to the stage. Christine Whyte, is a Senior Technology Transfer Manager from ISIS Innovation, and she immediately tried to get an idea about the audience. It turned out about half of the attendees were undergraduates, about 40% DPhil students and the rest were postdocs. Christine then started telling us about her journey. She obtained a first class honours degree in Microbiology from the University of Bristol, then undertook her DPhil in gene expression in bacteria at the University of Oxford. During her DPhil studies she realised she did not like focusing on a subject only a small group of people were interested in. She also knew that she liked talking to people and organising things. After attending courses offered by the Career Development Organisation (CRAC) and Procter & Gamble, she decided she wanted to pursue a career in industry. Over the years, she has worked in different roles, including as an R&D Manager and Product Evaluation Manager in different companies like Unilever, Oxoid and Cancer Research Technology. She always enjoyed collaborating with people, including academics, while working in different companies. Her qualifications and the knowledge she gained on technology transfer, patents and licensing in her job in Cancer Research Technology were what got her into ISIS Innovation. She has been there since 2007, putting together business proposals from academics and different types of companies. Christine loves her job and she finds it very exciting to hear about novel ideas from many different areas of science. Moreover, she can manage her family life well, being able to work flexible hours. When I asked her about job openings, she told me that the technology transfer area has been expanding all over the UK. Needing to know about the research areas entrepreneurs want to start businesses in, a PhD in science proves to be very beneficial to her job. Her advice to job seekers was to build on experience and to network.

Another area we got a chance to hear about was publishing and editing from Ann Le Good, a senior editor at *Nature Communications*. Ann graduated from Oxford in 1990 and subsequently completed her PhD at the Imperial Cancer Research Fund. After two postdoctoral stints at the University of Alberta and the Swiss Institute for Experimental Research, she decided to pursue a career as an editor. She started as Assistant Editor at Biomed Central where she worked on content for various journals. She then became a deputy editor for *BMC Biology* and continued working there for 4 years. She has been at *Nature Communications* for a year and she truly enjoys having the opportunity to read about cutting edge science in her job. As an editor, she is involved in all the stages a paper goes through for publication, starting from the initial acceptance, to analysis of reviewers' comments, while making sure everything is done in a timely manner. "Although you have to be able to understand material written about many different areas very quickly in my job, you are in a team of people assigned to a specific area," she told us. Her areas are molecular cell and developmental biology. She and her team also need to be knowledgeable in ethical considerations and editorial policies since they make decisions on articles dealing with sensitive subjects such as human gene

editing. When asked about the hiring process, job security and salary, she told me that *Nature Communications* and other journals are expanding their teams at the moment and there are a lot of job adverts out there. When someone applies for an assistant editor job, they will be given a test to complete. The test will be a manuscript to edit and to deduce key points from. Once you get the job, you will be trained during your probationary period, after which it is possible to work from home most of the week. "You don't really worry about the economy in my line of work, because journals like *Nature* will always be there," Ann remarked. One of the perks about her job is travelling. She gets to attend conferences all over the world a few times a year, which she loves, because of the chance to hear about the techniques and advances in science she always reads about. This way she can gain insight into the areas she is responsible for and can judge the manuscripts she receives more objectively.

The day then took a surprising turn. Who knew you could end up in a helicopter after obtaining a degree in biochemistry? Well, Edward Norris-Cervetto did just that, and made all of us in the audience proud when he told us all about how he is out there saving lives as an A&E doctor. Ed completed his undergraduate and PhD degrees in biochemistry in Oxford. Thinking about a career as a doctor led him to start doing voluntary work at the John Radcliffe hospital, which made his aspirations stronger. He then became a student once again, in order to get his medical degree and he is now a registrar in emergency and pre-hospital medicine at the JR and with the Thames Valley Air Ambulance. His job is inherently stressful; making it to the scene of a very serious accident as quickly as possible, rescuing a patient and performing the critical interventions are just some of the challenges. His job also requires intensive training, during which scenes such as car crashes and collapsed buildings are put together for the doctors. "The training is essential for my job," Ed said, "because we have to be able to do it with our eyes closed." Ed put up a graph he had made, projecting the changes in income of a medical doctor and an academic in biochemistry versus the working years of an individual. The point he raised was that in the worst-case scenario as a medic, one would make more money than in the best case as a biochemistry academic, which is becoming a professor. His concluding remark was "When you get a degree in biochemistry from Oxford, I hate to break it to you, but you are not a biochemist, you are an Oxford graduate. This means there is nothing you cannot do!" Well, that is good news to receive, I would say.

Bridget Harris, the Head of Finance at the Academy of Medical Sciences (AMS), was there to tell us about her journey from "Science to Finance and Back Again". AMS is an independent body in the UK that promotes medical science and its translational benefits for society. Bridget's journey has been full of adventure. She got her degree from Oxford in 1982 and started her DPhil afterwards, but never enjoyed being a DPhil student. Advice from the Careers Service steered her towards becoming an accountant: her first job was at Buzzacott. An accountant has to be "a bit of a detective, very good with numbers and learn about law and finances," she found out at her first job at Buzzacott. It takes 3 years to become qualified as an accountant, after which job security increases substantially. Bridget is now responsible for securing money from funding bodies at AMS and is quite glad to combine her knowledge in science and accounting. At the moment, AMS is hiring policy staff and they are interested in people

at the postdoctoral level as well as DPhil students.

And now off to law. David Lancaster and Sarah Faircliffe took us into this interesting field and told us how their science degrees helped them in their jobs. David was an undergraduate in biochemistry at Oxford in the late 90's and got his PhD in biological chemistry under the supervision of Prof. Chris Schofield in 2006. He did not want to have an academic career and thought going into patent law could be interesting. He got a diploma in intellectual property, law and practice in Oxford as a member of Pembroke College. He is now a senior associate and barrister at Powell Gilbert in London. Sarah obtained an honours degree in biochemistry from Oxford and went straight into the College of Law, qualifying as a solicitor in 1992. She was a Legal Adviser with the European Medicines Agency (EMA) for 10 years and is now a legal director in The International Life Sciences Group at Bird & Bird's. At EMA, Sarah and her team were responsible for all legal questions relating to assessment and granting of EU marketing authorisations for human and animal medicines. They also dealt with issues relating to special medicines such as orphan and pediatric ones. In private practice, she now gives legal advice to companies on regulatory issues and builds client relationships. She finds her personal skills are very important for this aspect of her job. She also thinks her scientific background helps her to think technically. Her advice to undergraduates interested in law was, "Try to organise work experience, attend law fairs and apply for training jobs in your last term." It takes around 4 years for an individual to become qualified in law and a university degree in any subject is the starting point. Sarah's job accommodates her family life very well and she can work from home. David, as a patent attorney, is very much involved with the scientific side of things. He works directly with scientists and writes patents for them. David thinks a PhD is not necessary to do what he does, but having a science degree is an advantage. Looking at the title of the patent application he distributed to us in the audience, I agreed that a science degree is indeed advantageous, as I don't think everybody knows about "compositions and methods for stabilising biological molecules upon lyophilisation!"

> "When you get a degree in biochemistry from Oxford... there is nothing you cannot do!"

The inspiring talk by Katy Gearing, the Director of the Biological Sciences Department at GlaxoSmithKline in Stevenage, centered on the pharmaceutical industry. Katy obtained her biochemistry degree from Oxford in 1982 and her DPhil in biochemistry and molecular biology in 1986. She really wanted to work abroad, so after working at British Biotech, she went to Sweden to work at the Karolinska Institute as a postdoc. She started working at GSK in 1993. Climbing up the ladder at the company, she worked in different subject areas such as virology, target validation, and the generation, scaling-up and screening of biological reagents. Katy was really insightful about the industry and gave very useful tips to job seekers. When a company like GSK advertises a position, the advert stays on their website for at least 5 days. The average time for advertisement is 2 weeks, however, if they receive enough applications in the first 5 days, they can close the opening at that point. "It's very important to tailor your CV for a particular job and the most important thing when doing that is to put the most relevant qualifications and skills at the beginning of your CV," Katy stressed. She said she spends only seconds scanning through a CV for the first time and if she doesn't see what she's looking for, she will immediately send a rejection message to the applicant. She also told us that GSK works with a lot of platform companies and encouraged applicants to apply to different jobs advertised by different companies. "Working at GSK, you won't become rich," Katy pointed out, "but you will live very, very comfortably." The starting salary for a new graduate is in the £20-30,000 bracket and for PhDs it's £30-40,000. After about 20 years at the company you may expect to earn between £45,000 and £65,000. The employees also benefit from performance

related bonuses, share and pension schemes, private healthcare and allowances of 10-50% of their base salary. One aspect of a project at a pharmaceutical company that is very different from an academic one is the fast turnover rate. When a project doesn't look like it's going to work within the targeted time frame, it will be aborted. For employees this may translate to a shift in roles, reassignment to another project, and sometimes redundancy. GSK helps employees by providing training when the change is a shift within the company. Redundancies are not as bad as they sound for jobs like these because there are so many jobs available for researchers in industry.


A degree in biochemistry is a special gift when you want to brew beer and introduce novel techniques into this age-old tradition. The last speaker of the day, Robert Wicks, expanded our horizons when he told us how his degree paved the way for his success in beer brewing. Robert graduated in 1983 and after working in stock exchange, sales and the pharmaceutical industry for many years, he started his own brewery with his wife in 2004. Westerham Brewery Company is based in Kent and the location is one of the key factors for making the good quality pale ale they produce, as the water alkalinity there is perfect for this type of beer. In addition to producing delicious pale ales at the brewery, Robert and his colleagues came up with a brilliant technique for making gluten-free beer. They use proline-specific endoprotease to chop up barley hordein, a gluten protein that individuals with celiac disease are sensitive to. Robert is also promoting the benefits of moderate beer consumption. Most of us enjoy beer without thinking what it contains and for me, it was very interesting to find out that beer is high in vitamins and silicon, an essential for healthy bones. The hops in beer have anti-inflammatory, anti-allergic and vasculoprotective properties. Needless to say, at the end of the day, when we were sampling the ales Robert brought for the Careers Day, we were enjoying ourselves a bit more guilt-freely!

T he Careers Day was without a doubt a big success for OUBS. We in the audience learned so much about the variety of roles and jobs biochemistry graduates obtain after Oxford. It was also a great opportunity to be able to talk to the speakers, who were all very approachable, not only for me as an interviewer, but also everybody else who had questions to ask. After talking to some of the audience members, I can conclude that a career day of small to medium size is ideal to gain a lot from, and I would advise all Oxford students and employees to be on the lookout for these events. All in all, networking is the key to landing a good job these days, and where better to start than at a Career Day?

*Dr Burcu Anil Kirmizitas is a Post Doctoral Research Associate working with Prof Neil Brockdorff in the Department of Biochemistry*

# Detecting active glycosidases using activity-based protein profiling

Activity-based protein profiling (ABPP) is a powerful functional proteomics method that detects active enzymes in biological systems. ABPP utilises small molecular probes that label active enzymes by binding their catalytic site in an activity-dependent manner (Figure 1). It can be performed using living cells or whole cell extracts without pre-purification of the enzymes, and does not require knowledge of the substrate specificity of the enzymes. Therefore, ABPP is a useful method for detecting enzymes that are active in a given context, for example in response to pathogen infection, or in a cell type of interest.

by Dr Suvi Honkanen

Carbohydrates carry out important functions in all living cells. Simple carbohydrates, such as the monosaccharide glucose, exist as intermediates of energy metabolism. Monosaccharide units become linked together through glycosidic bonds to produce complex carbohydrates; long branched or unbranched carbohydrate chains. Some of these complex carbohydrates have a structural role (e.g. cellulose and chitin) or act as energy storage compounds (e.g. starch and glycogen), while others become covalently linked to proteins, hormones and lipids, in many cases modifying their activity (1).

Glycosidases (glycoside hydrolases) are enzymes that hydrolyse the glycosidic linkage between two carbohydrate moieties, thereby catalysing the conversion between different forms of carbohydrates. Compared to animals, plants have a particularly broad range of complex carbohydrates, many of which form structural components of the plant cell wall. Consistently, plants also produce a large number of glycosidases with different substrate specificities. For example, the model plant *Arabidopsis* has approximately 400 glycosidase-encoding genes, the majority of which remain uncharacterised. Most glycosidases are members of large subfamilies and have redundant functions. In addition, the substrates of many glycosidases are difficult to synthesise, while some glycosidases only act on their natural substrates and not on synthetic analogues. Furthermore, the activity of many glycosidases depends on post-translational modifications. These features make glycosidases challenging to study.

Renier van der Hoorn's group at the Oxford University Department of Plant Sciences has developed and validated activity-based probes for a range of enzymes, including proteases, lipases, esterases, ATP-binding proteins and glycosidases. Balakumaran Chandrasekar, a PhD student in the van der Hoorn group, has validated activity-based probes for retaining glycosidases in plants. His work recently identified that cyclophellitol aziridine-based small molecular probes, which have previously been used in animal systems (2) can be used to label plant glycosidases in an activity-dependent manner (3). A biotinylated cyclophellitol-aziridine probe named JJB111 was used to label active glycosidases in *Arabidopsis* leaf extracts and secreted proteomes of tobacco. The labelled enzymes were then purified using streptavidin beads, separated using gel electrophoresis, and identified using mass spectrometry. The major signal in *Arabidopsis* leaf extracts was attributed to two myrosinases; TGG1 and TGG2. In addition, a further 18 active glycosidases were detected, including an enzyme encoded by the *TGG3* gene, which was previously reported as a pseudogene (4). In tobacco, 19 active glycosidases were identified in the apoplast; the fluid that fills the space between the plant cell wall and the plasma membrane. Therefore, in total, the active state of nearly 40 plant glycosidases was reported, the majority

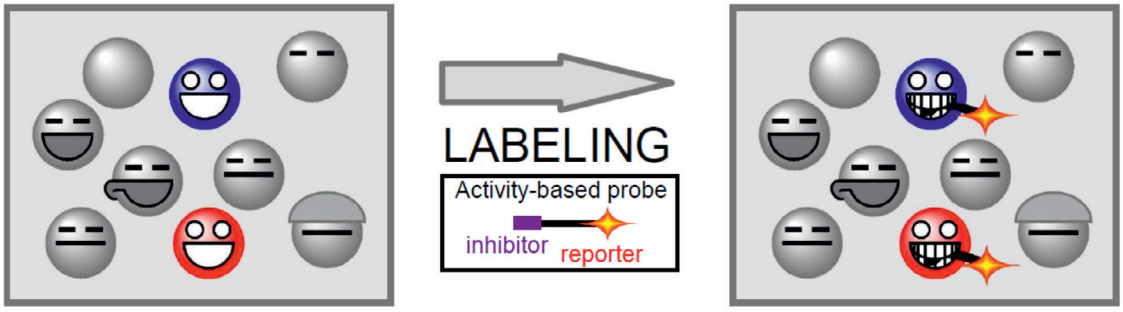


**Figure 1.** Activity-based probes react with the active site of enzymes in an activity-dependent manner, thereby allowing labelling of active enzymes in cell extracts or living cells. Happy smileys represent active enzymes. Copied from (5).

of which were previously uncharacterised. The glycosidases labelled belonged to seven families and included glucosidases, xylosidases, galactosidases, glucuronidases and myrosinases, showing an unexpectedly broad range of substrate specificities. The labelling with activity-based probes was successful in extracts from seeds, seedlings, flowers, leaves and senescing leaves, indicating that ABPP can be carried out in a wide range of tissue types. Furthermore, these probes were shown to label glycosidases *in vivo* in *Arabidopsis* cell culture. Currently, Balakumaran is applying these probes to study the differential activities of these glycosidases under biotic stress conditions.

The Van Der Hoorn group has performed pioneering work in the development and validation of activity-based probes and protocols for their use in plants. As a result, the group now has a collection of hundreds of probes that can be used for identifying the active states of thousands of enzymes, providing a powerful tool for addressing a wide range of biological problems.

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*Dr Suvi Honkanen recently completed a DPhil in the Dolan Group, Department of Plant Sciences, University of Oxford*


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# 5' with ... Dr Richard Hopkinson

Dr Richard Hopkinson completed his DPhil at the Department of Chemistry, Oxford in 2012 under the supervision of Professor Christopher J Schofield. Upon completion of his postgraduate studies, Rich was awarded a William R Miller Junior Research Fellowship in Molecular Aspects of Biology to continue his work in this lab. His research focuses on the cellular reactions of formaldehyde in biological systems, including its production and metabolism in both bacterial and human cells. Through characterising the cellular pathways involving formaldehyde, Rich aims to further understanding of its mutagenic and carcinogenic properties, as well as links to disease-onset and potential therapies.

**How did you first become interested in the biological importance of formaldehyde?**
My interest in formaldehyde began during my DPhil studies. I was working on a family of human enzymes called histone demethylases which are involved in regulating gene expression. These enzymes catalyse demethylation on histone proteins and DNA by converting the methyl groups into formaldehyde. While the toxic and carcinogenic properties of formaldehyde are well known (particularly in exposed industrial workers), there is remarkably little information on how these effects are induced at the molecular level. As a chemist, I was particularly interested to see whether formaldehyde's high reactivity with biological molecules could be the cause, and so my work ever since has been aimed at answering that question.

**If you were not a scientist, you would be…?**
I think working in science was always likely for me. I definitely caught the research bug during my Part II year and haven't really looked back since. I guess if I were not in research, I would enjoy doing something sporty.

**What do you like most about your field of research?**
I feel very fortunate to be working in such a new and exciting area of research. Formaldehyde production inside cell nuclei is a relatively recent discovery and consequently there have been very few studies on its biological roles. There is clear potential for this cellular formaldehyde to trigger disease in our cells but it may also play regulatory roles that have yet to be appreciated. Given its high reactivity, chemical as well as biochemical and cellular studies are essential to determining its roles in cells. Therefore I feel it is the perfect research question for my interests and skills.

**What are you most proud of in your career so far?**
My most significant work to date has been with demethylases. These enzymes are key epigenetic regulators of gene expression and as such are of intense biomedical interest. My work in this area has included inhibitor discovery and biochemical studies that have pioneered the ongoing pharmaceutical efforts in this field. One important aspect of my work was to determine whether demethylases could have other substrates. This work has led to many interesting discoveries which we are beginning to rationalise in cells.

**What has been the biggest challenge you have faced during your career?**
From a scientific aspect, the biggest challenge I face is in deciding what to focus my time and effort on. If, like me, you are interested in most aspects of science, it can very difficult to identify what to prioritise.

**What advice would you give to students looking to follow in your footsteps?**
I think the most important thing is to be excited by your research and to keep developing new ideas. Getting experience of other disciplines and practical techniques is also very beneficial, particularly if you wish to pursue interdisciplinary research.

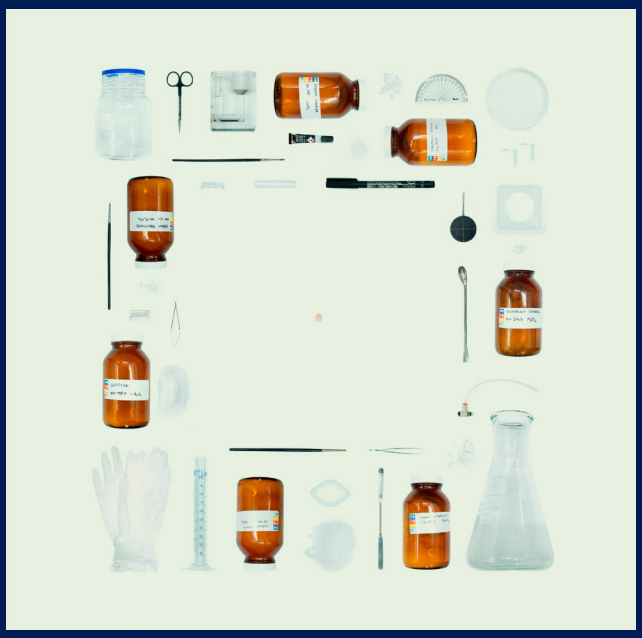
**Who has inspired you during your career?**
My biggest research inspiration has to be Chris Schofield in the Department of Chemistry in Oxford. Chris supervised me during my DPhil work and has been a mentor to me throughout my research career. His passion for science, his willingness to challenge accepted theories and his collaborative approach are inspiring.

**Where do you see research on formaldehyde going in the next 5 years?**
Formaldehyde biological research is in its early stages but I expect to see some pioneering work in the next few years. A key development will be to identify chemical modifications in cells that are derived from endogenously-produced formaldehyde. Once we know what these modifications are, we can then begin to determine how they affect cell functions. Hopefully in 5 years we will be in a much stronger position to probe what formaldehyde does in cells, and therefore to identify how to prevent and/or treat formaldehyde-related disease.

*As told to Lauren Chessum*

# SNAPSHOT Research Image Competition

## This issue's winners are...



**Cristiana Vagnoni & Lev Tankelevitch**

This term's winners of the SNAPSHOT competition are Cristiana Vagnoni and Lev Tankelevitch with their image titled "Anatomy of an Experiment".

Cristiana is currently a DPhil student in Neuroscience working under the supervision of Dr. Simon Butt and Prof. Zoltán Molnár where she studies sensory neural circuit formation in the developing mouse brain.

Lev is also a DPhil student in Neuroscience, working under Dr. Mark Stokes and Prof. Matthew Rushworth, where he studies the human visual attention system using non-invasive imaging techniques such as functional magnetic resonance imaging.

Their image is a photographic representation of the preparation of patch clamp electrophysiology technique, which allows the electrical activity of single neurons in the brain to be observed. The chemicals shown in the image are used to replicate the cerebrospinal fluid, keeping neurons healthy and functional for the experiment and the tools shown are used in extracting and preparing the brain, which in this image is that of a mouse (centre, roughly 1 cm width in actual size).

Even though neuroscience has been more widely publicized within recent years, with many members of the public aware of major results, the public's knowledge of how these results are obtained is lacking. With this image and their artistic collaboration, Lev and Cristiana hope to provide a way to conceptualize these methods, making research more accessible to the general public and inspire other researchers to do the same.

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**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 2016 issue of *Phenotype*.

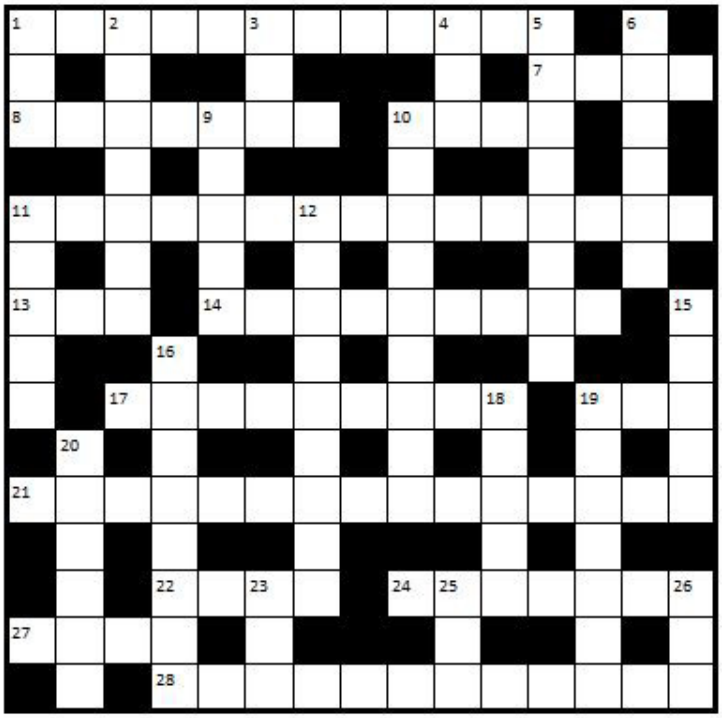
To enter, send images to rebecca.hancock@linacre.ox.ac.uk with a brief description (maximum 100 words). Please get permission from your supervisor before sending any images.

# PHENOTYPE crossword

Fish challenges you to this latest cryptic crossword on the theme of genetics. Can you crack it? Answers to last issue's crossword are given at the bottom of the page. Enter this term's competition by sending your answers to rebecca.hancock@linacre.ox.ac.uk. Entries received before the 1st August 2016 will be entered into a prize draw to win a book from Wiley-Blackwell.

The winner of the crossword competition will receive a book prize kindly provided by

WILEY-BLACKWELL



## ACROSS

1.,24. Sections flanking **7,28** are left in the original language by Signore Cook? (12,7)
7, 28. Start studying structure of a protein-coding sequence (4,7,5)
8. Try endless love hotels without interruptions for 15? (7)
10. Ring changes and smile (4)
11, 20. At the end of the message it's confused, ailing, pasty and lonely - in need of love! (15,6)
13. See **23**
14. "Mathematical mnemonic gives order to chaos" - a hot mess (9)
17. Designed for comfort, and therefore acoustic? (9)
19. Bother about regular cardio (3)
21. Airborne missile destroyed organisms that make rocks (15)
22. Flip coin to see seven heads (4)
24. See **1**
27. What an Australian calls their friend's partner? (4)
28. See **7**

## DOWN

1. In short, where one goes to study French one on one (3)
2. To add up completely (7)
3. Sister says nothing (3)
4. Rodent goes back to black? (3)
5 Gift for party people? (8)
6. Run around the Spanish academic (6)
9. Rounds of **25**s and eels' tails (5)
10. Blood glucose level controls brain cells' absorbtion of yttrium, calcium and 50% of haem (9)
11. Prisoner of the queen has influence (5)
12. Solo appears in screen adaptation of "**16** modifiers" (9)
15. 10 working without directions - together they assemble an **7,28** (5)
16. Publicist who directs transcription (8)
18. Stick to anoxic cooling (5)
19. Promotion raises one to join heads of medicine, anatomy and cancer (7)
20. See **11**
23, 13. Spooner says that all of us speak of this shipping passage (6)
25. For example, before midnight it can be poached (3)
26. Observe odd Swedes (3)

Answers to the crossword from Issue 23 Hilary 2016:
**Across:** **1.**Cerebellum **5.**Dura **9.**Retrains **10.**Botany **12.**Extinct **13.**Tie-Dyed **14.**Hippocampus **19.**Pineal Gland **24.**Protein **25.**Centaur **27.**Runway **28.**Ganglion **29.**Lobe **30.**Substantia
**Down:** **1.**Cortex **2.**Rats Tail **3.**Brain **4.**Lunatic **6.**Uranyl **7.**Amygdala **8.**Nonequal **11.**Stem **15.**Primeval **16.**Aga **17.**Temporal **18.**Annalist **20.**Etna **21.**Lactams **22.**Hobnob **23.**Crania **26.**Nigra