# PHENOTYPE

Issue 13 | Michaelmas Term 2012

## The cell reprogramming revolution
*No human embryos required*

## After the Arab Spring
*We investigate the effects of revolution on the scientific community*

## Chagas disease
*A tropical disease uses modern migration to spread*

## Big antibiotics come of age
*Prof Colin Kleanthous introduces colicins, the protein antibiotics of the future*

cover image by
Olivia Berthoumieu

this issue´s winner of the SNAPSHOT scientific image competition
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Interview with a venture capitalist

Wisdom of the crowd

5´ with... Dr Sylvia McLain

Can you crack the cryptic crossword?

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# EDITORIAL

Welcome to the thirteenth issue of *Phenotype* magazine! Lucky number thirteen continues the tradition of wide-ranging content and in-depth articles contributed by PIs, research staff and students from across the University.

Prof Colin Kleanthous introduces us to the world of big antibiotics, proteins capable of killing bacteria, and describes work done in his laboratory to determine how these bacteriocins enter the cell. We also spend five minutes with Dr Sylvia McLain, who shares her unconventional route into academia, near-death experiences included.

The ability to generate artificial tissues and organs in the laboratory for disease treatment and transplantation would revolutionise medicine today. We focus on two lines of current research towards this goal which avoid the controversial use of embryos to generate stem cells. Dr Elizabeth Hartfield describes how stem cells may be generated from a somatic nucleus by using a human oocyte, and the ethical issues surrounding this. OUBS will be hosting Dr Sophie Jarriault later this term. Read more about her work on transdifferentiation in *C. elegans,* the ability to convert a somatic cell to a more plastic state, then into a different somatic state, avoiding the use of embryonic stem cells entirely.

The focus on human biology continues as Daniel Pereda highlights the 'fight or flight' response and the organelle structures that make it possible. Kate Wright introduces Chagas disease, a tropical disease that is hitching a ride on modern migration patterns and may pose a global threat. Looking beyond the research to the researchers, Dr Elwy Okaz takes us to Egypt in the aftermath of the 'Arab Spring' and investigates the effects of revolution on the scientific community.

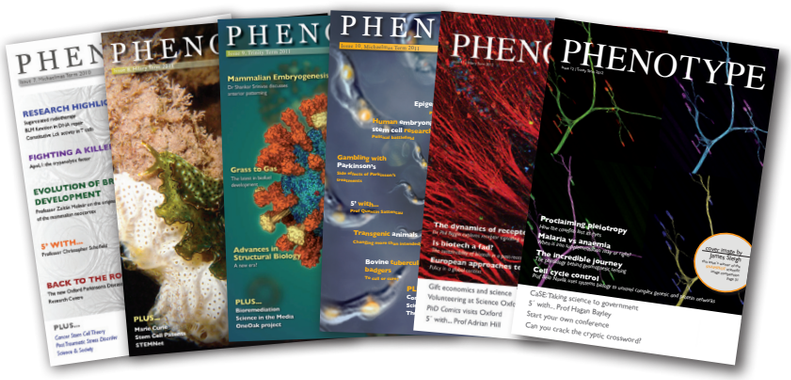
Funding bodies place increasing emphasis on the 'impact' of proposed research programs, but what exactly is impact and how can it be measured? Clara Howcroft Ferreira reports back from the recent Science Communication Conference hosted by the British Science Association, which endeavoured to answer these questions.

Congratulations to alumna Dr Olivia Berthoumieu, the winner of last issue's **SNAPSHOT** competition! Her evocative image of liposome formation graces our cover, and details of her DPhil work investigating receptor proteins using atomic force microscopy can be found on page 31.

Try your hand at beating the cryptographer extraordinaire Homarus. If you can crack the crossword you stand a chance of winning one of the textbooks reviewed in this issue. For those who were stumped by *Issue 12's* crossword, the answers are also available on page 32.

On a personal note, I'd like to say a huge thank you to the team of post-docs and students whose dedication, enthusiasm and hard work result in the magazine in your hands. I've thoroughly enjoyed my tenure as editor for the past year and encourage anyone interested in science communication and publishing to join the *Phenotype* team. Writers, editors and designers all are welcome to get in touch on **oubs@bioch.ox.ac.uk**!

Jennifer de Beyer
Department of Biochemistry

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# CONTENT

## regulars



## science & society



## features



## This issue is supported by:



# TEAM



# *OUBS* SEMINARS

All seminars are held in the Main Meeting Room, New Biochemistry Building from 4 to 5 pm, unless stated otherwise.

**Monday, 22nd October**
Dr Andrew Carter *University of Cambridge* "TBC"

**Monday, 26th November**
Dr Davide Corona *Università degli Studi di Palermo*.
"An RNA-mediated memory mechanism to inherit epigenetic marks"

*Featured Seminar:*

**Monday, 26th November**
Dr Sophie Jarriault, *IGBMC*
"TBC"

**TBC**
Prof Jane Clarke
*University of Cambridge*
"TBC"

**TBC**
Dr Robert Best
*University of Cambridge*
"TBC"

# OUBS Featured Seminar: Dr Sophie Jarriault

by Dr Penelope Mason

On 26 November 2012, OUBS is hosting Dr Sophie Jarriault from the Institute of Genetics and Molecular and Cellular Biology in Strasbourg.

Dr Jarriault has pioneered the use of the nematode worm *Caenorhabditis elegans* in studying transdifferentiation (1). This process allows the transformation of cells of one type into another without going through pluripotency, avoiding the risk of tumour formation.

Multicellular organisms form different specialised cells from a small set of precursors via cellular differentiation. The process is tightly controlled by gene expression, which causes specific changes in metabolism and responsiveness, allowing cells to take on divergent physiological roles. In adults, differentiation is required for normal tissue turnover and replacement, as well as repair.

Totipotent cells, such as those in the germline, can differentiate into all cell types present in the organism, while pluripotent cells, also known as embryonic stem cells (ESCs), can differentiate into all somatic cell types but not germline cells. Adult stem cell pools, in comparison, have limited differentiation capacity and are termed multipotent. The trend throughout development and maturation is hence always towards greater specialisation.

However, there is increasing evidence that cellular plasticity is more fluid than this. Cellular reprogramming can allow terminally-differentiated cells to revert to cell types with wider potency, such as induced pluripotent stem cells (iPSCs), or to convert directly to another type of differentiated cell in a process termed transdifferentiation or lineage reprogramming.

Controlled reprogramming of differentiated cells into a desired cell type would open up new opportunities in stem-cell biology. Direct replacement of diseased or damaged cells *in situ* in a patient from their own cell population is a foreseeable possibility, as it has already been achieved in mice (2). It would also have huge implications for regenerative medicine, potentially allowing transplantation of lab-grown organs from a patient's own cells.

*C. elegans* is a strong model for studying these types of cell conversion, since its cellular lineage is both invariant and known, allowing direct cell type conversions to be tracked with single-cell resolution throughout the process. Transdifferentiation events occur naturally in the organism and Jarriault's lab has recently found that rectal cells are able to transform into motor neurons without exogenous stimulation (3).

They showed that during transdifferentiation, rectal cells dedifferentiate to a state of restricted plasticity, lacking the characteristics of both initial and final cellular identities, and then undergo a stepwise redifferentiation into motor neurons. Dedifferentiation can therefore occur without cell division. It appears that direct transdifferentiation in worms involves cellular transition through tightly controlled discrete stages where cell potential is regulated and restricted, most likely to inhibit unwanted tumourigenic proliferation.

Jarriault's lab is now identifying the molecular networks underlying transdifferentiation *in vivo*. They are also assessing the key conserved aspects in cell reprogramming by comparing different cell plasticity events across tissue types. They recently showed that direct *in vivo* reprogramming of a rectal cell requires the same four-factor molecular cocktail (4) required for maintenance of ESC pluripotency *in vitro* (5), and for laboratory reprogramming of iPSCs. This suggests that the way cellular reprogramming is induced in the lab is similar to that occurring naturally in multicellular organisms.

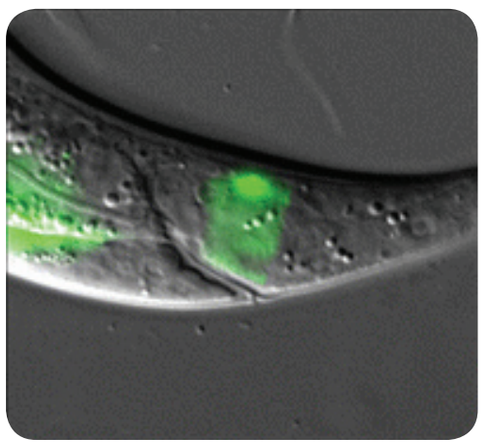

Figure 1: *C. elegans* mutants for one of the four transdifferiation key factors are defective in transdifferentiation. Reproduced with permission (3).

These factors could represent the discovery of a conserved cross-species "plasticity cassette" facilitating cellular dedifferentiation and specialisation. Further investigation of these factors might thus be applied to human cells to facilitate development of future transplant and repair therapies.

### References


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*A selection of recent life sciences research from the University of Oxford*

# RESEARCH HIGHLIGHTS

by Amy Baxter

Metclafe C, Cresswell P & Barclay AN (2012) *Open Biology 2*:110036

## Interleukin-2 signalling is modulated by a labile disulphide bond in the CD132 chain of its receptor

Inflammation is a highly complex process controlled by multiple protein families, in which interleukins (IL) play a key role. CD132 is the common gamma chain for numerous membrane-bound IL receptors, including IL-2 and IL-4. Recent work demonstrated that membrane proteins contain labile disulphide bonds which, when reduced, can cause structural changes affecting downstream signalling. This study aimed to identify and characterise a labile disulphide bond in CD132 and to determine if reducing this bond could alter the signalling properties of the protein.

Metclafe and co-workers screened T cells treated with chemical and enzymatic reduction agents by mass spectroscopy to identify Cys183-Cys232 as a potentially labile disulphide bond. Using the solved structure of the CD132 extracellular domain, they were able to map this bond to the exposed surface, as would be predicted of a labile bond. Furthermore, mutations affecting this bond have been shown to alter the IL-2 signalling properties of CD132, consistent with the idea that the oxidation state of Cys183-Cys232 is functionally important.

The investigators then predicted that if the CD132 disulphide bond was labile, the growth of an IL-2 dependent T cell line would be affected by application of a mild reducing agent. They showed dose-dependent reversible inhibition of proliferation by both chemical (tris(2-carboxyethyl)phosphine) and enzymatic (thioredoxin) reducing agents, which had no effect on the proliferation of a non IL-2 dependent T cell line.

Furthermore, treatment of the IL-2-dependent T cells with a reducing agent decreased phosphorylation of STAT-5, a component of the Jak-STAT pathway through which IL receptors are known to signal. This suggested that reduction of CD132 decreased IL-2 signalling.

This study supports the idea that labile disulphide bonds have a role in signalling *in vivo*, by demonstrating the downstream effects on signalling of bond reduction in a T cell line. These results add weight to the hypothesis that the redox environment modulates IL signalling in inflammation.

Matthews PR, Eastwood SL & Harrison PJ (2012) PLoS One 7(6):e382111

## Reduced myelin basic protein and actin related gene expression in visual cortex in schizophrenia

Schizophrenia is a complex mental disorder with strong genetic associations, characterised by hallucinations and paranoid delusions. Previous studies identified numerous changes in gene expression in the frontal cortex or hippocampus regions in the brain of patients with schizophrenia. Matthews and colleagues utilised a tissue collection to identify alterations in gene expression in the primary visual cortex, a region not previously investigated.

The group employed a two-stage protocol to identify novel gene expression changes. Firstly, they ran DNA microarrays on pooled samples from donors with schizophrenia, bipolar disorder or severe depression, and compared them to healthy controls to identify potential candidates. They uncovered five candidates whose expression was decreased in schizophrenic patients compared to healthy controls: myelin-oligodendrocyte glycoprotein (*MOG*), thymosin b-10 (*TB10*), b-actin (*ACTB*), cervical ganglion-10 (*SCG10*) and myelin basic protein (*MBP*). *SCG10* and *TB10* mRNAs were also decreased in patients with bipolar disorder and *ACTB* transcript was decreased in patients with severe depression.

Next, quantitative reverse-transcription PCR was run on each mRNA to quantify the changes in gene expression. *MBP* expression was significantly reduced in schizophrenic patients compared to those with severe depression, but not reduced in bipolar disorder patients. *ACTB* and *TB10* were decreased in both schizophrenia and bipolar disorder patient groups. Interestingly, further analysis of *TB10* demonstrated that mRNA levels correlated negatively with the age of illness onset and positively with lifetime treatment with antipsychotic drugs.

In conclusion, Matthews and co-workers confirmed that the visual cortex is not 'spared' in schizophrenia, whose neuropathology may involve all areas of the neocortex. Three of the genes identified in the study (*MBP*, *ACTB* and *TB10*) are implicated in cell motility and morphology, hinting at a role for actin polymerisation in this neuropathy. Finally, the finding that two of these transcripts, *ACTB* and *TB10*, were also decreased in bipolar disorder provides the first evidence that the visual cortex may be affected in this pathology.

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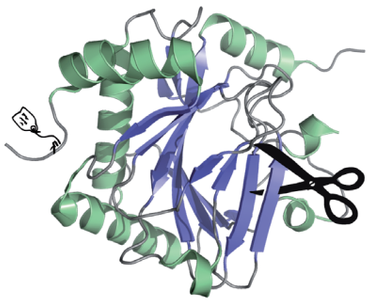

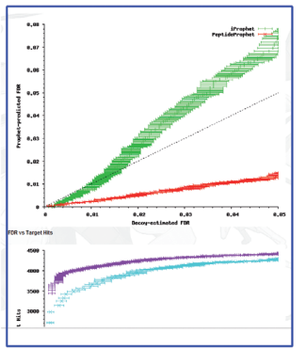

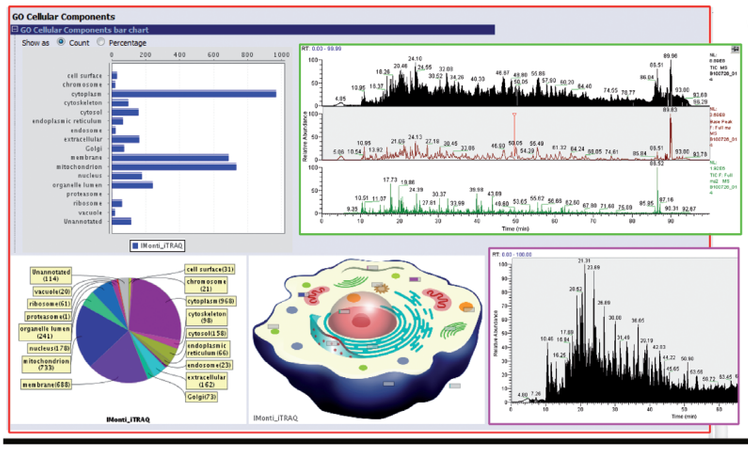

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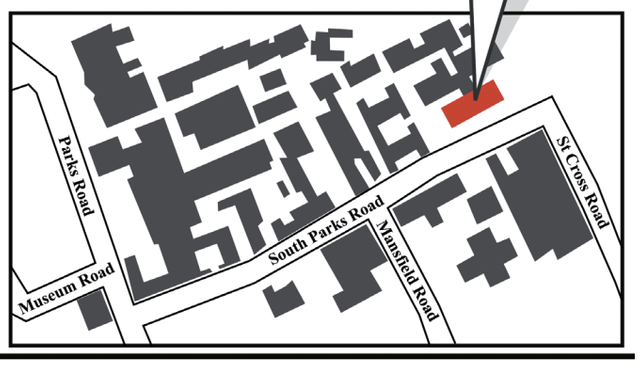


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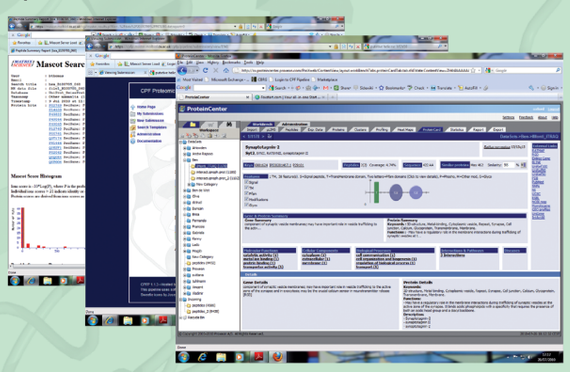

# Big antibiotics come of age

by
Prof Colin
Kleanthous

Discovered before penicillin, protein antibiotics kill bacteria by enzymatic or channel-forming activities. My laboratory studies how such toxins navigate their way into bacteria, as a means of investigating the structure and function of important protein complexes within the cell envelope, as well as developing novel antibacterial strategies.

## The physiology of the cell cycle

When we think of antibiotics we instinctively think of small molecules, such as penicillin, that revolutionised the treatment of bacterial infections in the 20th century. By 'small' I mean molecules containing just a handful of carbon atoms and other heteroatoms, typically with molecular weights <1000 Daltons (Da). However, the hunt for naturally-occurring substances that destroy pathogens was well established when Fleming published his pioneering paper on the antibacterial activity of *Penicillium* in 1929. A largely forgotten pioneer of antibacterial research was the eminent Belgian microbiologist André Gratia (1893-1950) (Figure 1). In 1925, Gratia reported that when two strains of the enteric bacterium *Escherichia coli* were grown next to each other, one of the isolates produced a substance that "antagonised" the other. He christened this substance *colicine* for 'coli killer' (1).

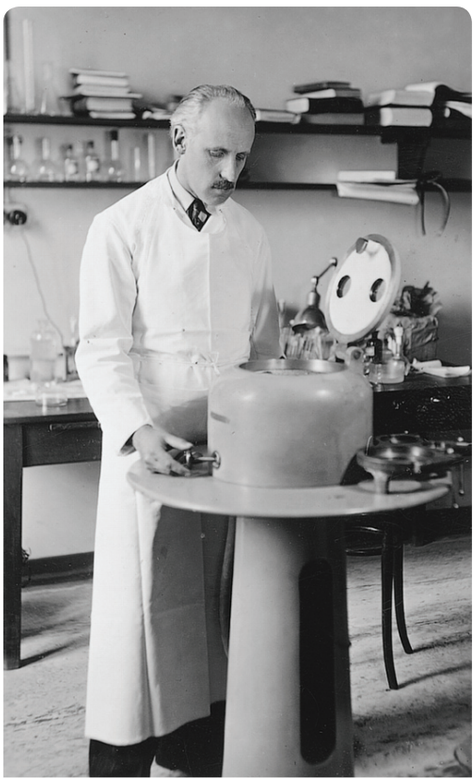

**Figure 1: André Gratia.** Photograph reproduced with permission from (2).

## Bug-killing toxins

Thus was born the field of *bacteriocin* research. Bacteriocins are highly toxic molecules released by bacteria to kill neighbouring organisms of the same species when in competition for resources. Their species selectivity is one feature that distinguishes bacteriocins from broad-spectrum antibiotics such as penicillin. The other is size: bacteriocins tend to be peptides or proteins (molecular weight 2,000-70,000 Da), so are bigger than traditional antibiotics. This combination of size and specificity has limited the use of bacteriocins in medicine. However, bacteriocins have found widespread use as natural preservatives in the food processing industry and are increasingly being evaluated for biomedical applications.

Fast-forward 87 years from Gratia: hundreds of bacteriocins (and dozens of colicins) have been reported. Colicins have a highly modular structure, typically comprising a central receptor-binding (R-) domain, an N-terminal translocation (T-) domain, and a C-terminal cytotoxic domain, either a cytotoxic enzyme or a channel-forming toxin that de-energises the bacterial inner membrane (IM). Importantly, bacteria that make colicins are protected against their own toxin by an immunity protein, which binds and inactivates the cytotoxic domain (3). Our work focuses on nuclease colicins, which elicit cell death by cleaving nucleic acids within the cytoplasm.

## Mission impossible – Colicin import across the bacterial cell envelope

The process by which nuclease colicins (~60 kDa) are imported is complex, involving many protein-protein interactions with endogenous systems throughout the cell envelope. Through a combination of microbiological, biochemical, biophysical and structural approaches, we have been exploring how colicins manage the seemingly impossible feat of translocating their nuclease to the cytoplasm.

The cell envelope serves many essential functions, emphasised by the fact that *E. coli* sacrifices almost a third of its 4,000 proteins to it. Most importantly, it functions as a barrier to keep out unwanted molecules, while allowing essential nutrients into the cell. The organism achieves these mutually exclusive functions by having outer membrane (OM) proteins with pores running through them. These porous proteins, known as porins, only allow molecules below a certain size (<700 Da) to diffuse into the cell (4). This restriction does not pose any problems for molecules such as glucose (or indeed penicillin), but is a significant problem for essential nutrients such as iron or vitamins, which are brought into the cell as large complexes. Specific OM transporter proteins, which have pores big enough to accommodate their specific substrate, are used for these molecules instead. To stop other molecules exploiting OM transporters, the pores are sealed shut by a protein plug. When the ligand of choice binds, the seal is

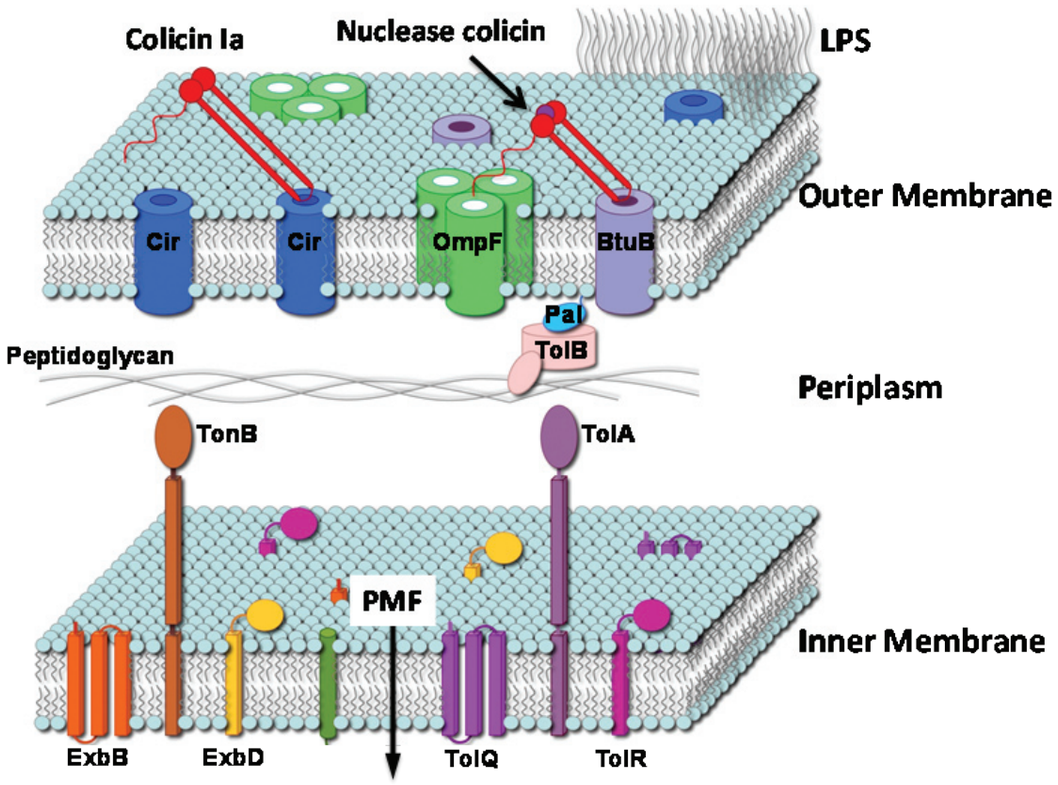


Figure 2: Cartoon depicting systems exploited by colicins to translocate across the *E. coli* cell envelope. Colicins begin their journey into a cell by binding to an OM receptor, typically nutrient transporters. Shown here are colicin Ia bound to the iron transporter Cir, and a nuclease colicin bound to the vitamin B12 transporter BtuB. Receptor-bound colicins then recruit translocator proteins via unstructured regions of the T-domain (wiggly lines). For the Colla-Cir complex the translocator is another copy of Cir, whereas for nuclease colicins it is the trimeric porin OmpF. Translocator proteins deliver peptide epitope signals to the periplasm where they contact either the Ton (Colla) or Tol-Pal (nuclease colicins) systems, both of which are coupled to the IM pmf. For nuclease colicins, this peptide epitope competitively recruits TolB. Contact with TolB induces interaction between TolB and TolA, which in turn triggers toxin entry. LPS, lipopolysaccharide; PMF, proton motive force.

broken, the nutrient allowed into the cell, and the seal re-set. However, breaking the seal requires energy, which poses a significant logistical problem to the bacterium since the OM is not an energised system.

The solution to this problem is provided by the triumvirate complex TonB, ExbB and ExbD, which drives specific ligand entry through OM nutrient transporters by coupling transport to the proton motive force (pmf) across the IM (Figure 2) (5). Such coupling of the pmf to an energy-dependent process at the OM is also the basis for the function of the Tol-Pal system, which although related to Ton, is involved in stabilising the OM of the bacterium. Tol-Pal is composed of three IM proteins (TolA/TolQ/TolR), a soluble periplasmic protein (TolB) and an OM lipoprotein (Pal). Colicins parasitize both the Ton and Tol-Pal systems, presumably because they both span the cell envelope and are linked to the pmf, providing the toxin with an energy source with which to translocate into the cell (6).Two recent stories illustrating how colicins exploit these systems and what we have learnt about the bacterial cell envelope as a result are described below.

## Pore delivery

Following receptor binding, nuclease colicins have to deliver a peptide signal, the TolB binding epitope (TBE) embedded within its intrinsically unstructured T-domain (Figure 2), to the periplasm. We recently discovered that this region houses two OmpF binding sites flanking the TBE. To determine where on OmpF the colicin bound, we solved the crystal structure of a colicin-OmpF complex. This showed the colicin peptide in the lumen (pore) of the porin, with its N-terminus pointing towards the periplasm. This is consistent with sequential binding of the colicin to a single OmpF pore, leaving the intervening TBE dangling in the periplasm to capture TolB, somewhat like bait on a fishing line. Hence, colicins have evolved to pass into the cell a protein ten times larger than the molecular weight cut-off of the pore, by unfolding the polypeptide and threading it through the narrow holes of OmpF pores. Porins are also exploited by some bacteriophages, viruses that infect bacteria, and have been implicated in protein export. Therefore it is possible that this mechanism of transmembrane communication may apply to other biological scenarios.

## Disorder signalling

The central pillars of the Ton and Tol-Pal systems are TonB and TolA, which are long, stalk-like proteins that span the periplasm and are coupled to the pmf through associations with their IM partners ExbB/ExbD and TolQ/TolR (Figure 2), respectively. The pmf is thought to drive rotational changes in TonB and TolA, suggested by the fact that both ExbB/ExbD and TolQ/TolR share sequence identity with stator proteins that are part of the rotational motor of the bacterial flagellum. Notwithstanding their IM similarities, the Ton and Tol-Pal systems have distinct cellular roles, emphasised by different deletion phenotypes and the fact that Tol-Pal has two additional components, TolB and Pal, which form a complex at the OM (Figure 2).

However, by investigating the exploitation of Tol-Pal by nuclease colicins, we recently discovered that it has more in common with the Ton system than previously suspected (Figure 3) (8). The key to understanding this is to recognise that TonB and TolA each interact with a receptor. In the case of TonB, the receptor is a ligand-bound OM transporter

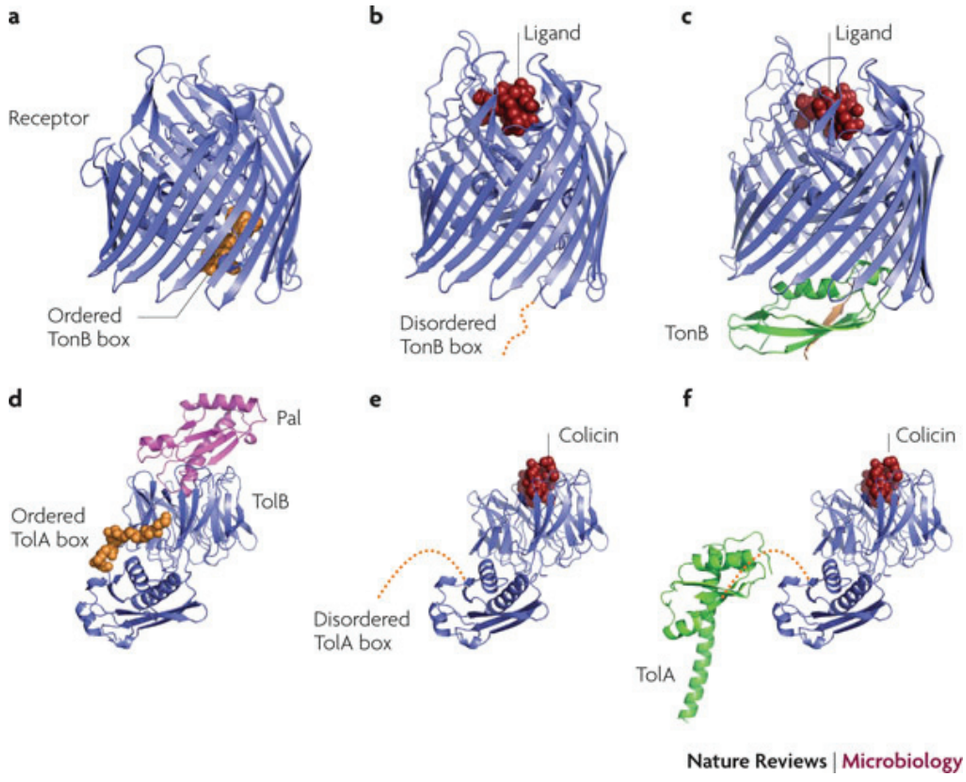


**Figure 3: Similarities in order-disorder signalling of the Ton and Tol-Pal systems and its subversion by translocating colicins.** (a)-(c) Ligand-gated signalling to TonB through an OM transporter, depicted here for the vitamin B12 receptor, BtuB. (a) The TonB box of the receptor is ordered in the unliganded state. (b) Ligand (vitamin $B_{12}$, red atom spheres) binding to the extracellular regions of the receptor promotes disorder of the TonB box in the periplasm, and (c) capture of the C-terminal domain of TonB. (d)-(f) Ligand-gated signalling to TolA through periplasmic TolB. (d) The resting state of the Tol system is thought to be TolB bound to the OM lipoprotein Pal, sequestering the TolA box (orange atom spheres) to the body of TolB. (e) Binding of the TBE (red atom spheres) of a colicin to TolB promotes disorder of the TolA box and (f) capture of TolA. Reproduced with permission from (8).

such as BtuB. The N-terminus of BtuB contains a TonB box within the β-barrel of the receptor. Binding of vitamin $B_{12}$ induces disorder in the TonB box, allowing the ligand-bound receptor to capture TonB. Engagement with TonB induces opening of the transporter plug domain and entry of the vitamin into the bacterial periplasm. We showed that the receptor for TolA is TolB itself (9). The N-terminus of TolB undergoes an order-disorder transition, analogous to that of ligand-bound TonB-dependent receptors, where the disordered N-terminus is the TolA binding site (TolA box in Figure 3). Also similar to the Ton system, we found that contact between TolA and the N-terminus of TolB is dependent on the IM pmf.

Colicins exploit signalling between TolB and TolA to initiate their import across the OM. Although the colicin's TBE has to be unfolded to pass through the OmpF pore, on binding to TolB it folds and mimics many of the interactions made by Pal bound to TolB. Unlike Pal however, colicin is an allosteric activator of TolB, inducing disorder of the TolB N-terminus and hence contact with TolA. Pal on the other hand acts as an allosteric off-switch for the system, sequestering the TolB N-terminus onto the body of TolB, where it shows little or no TolA binding (Figure 3).

In summary, studying colicins has revealed novel functions of porins in the outer membrane of Gram-negative bacteria and uncovered the signalling mechanism of the Tol-Pal assembly. Many questions remain as to how these remarkable molecules enter bacteria. Answering these questions will explain the 'antagonism' between bacteria first described by Gratia as well as revealing facets of the cell envelope, knowledge that may be exploitable for the future development of antibiotics.

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# The Reprogramming Revolution

The way we think about differentiated somatic cells is changing. The advent of reprogramming technologies has revealed that cell fate no longer has to be considered terminal. Cells can be converted from one type to another and even back to a pluripotent state, contradicting the long-held dogma that development occurs in a linear path. Reprogramming technologies now offer the unique opportunity to provide a source of cells for disease modelling and therapeutics, without the need for embryonic stem cells (ESCs). Induced pluripotent stem cells (iPSCs) offer an advantage over ESCs in that they can be produced from adults carrying disease-associated mutations which, once directed to become a terminally differentiated cell of interest, may also recapitulate the disease phenotype *in vitro*. Transplantation of self-derived cells could be on the horizon to replace damaged tissues and avoid immune rejection. Furthermore, iPSCs are produced from fully consenting adults and do not require the destruction of an embryo. Yet the question remains: are iPSCs equivalent to ESCs?

by
Dr Elizabeth
Hartfield

## Induced pluripotent stem cells

Reprogramming has classically been carried out using viral vectors to deliver key pluripotency transcription factors. Mammalian somatic cell reprogramming using exogenous pluripotency factors was first described in 2006 by Takahashi and Yamanaka (1), who coined the term iPSCs. This groundbreaking study revolutionised stem cell research and opened up the field for the generation of patient-specific stem cell lines, while avoiding the ethical dilemmas associated with the use of human embryos. Yamanaka's reprogramming cocktail is now widely used in laboratories around the world and thus far appears to be the most efficient method for generating iPSCs. Somatic cells from many different tissue types have been reprogrammed, including hair, skin, blood, bone and teeth. This illustrates the robustness of the reprogramming system and how easily accessible tissues can be converted back to a pluripotent state. These cells have the capacity to develop into cells representing all three germ layers of the human body (endoderm, ectoderm and mesoderm), and have huge potential applications, including pharmaceutical drug screening, disease modelling or even replacement cell therapies.

Despite their advantages, several reports have suggested that iPSCs are not fully reprogrammed and may contain a genetic 'memory' of the tissue from which they are derived (2). In addition, variations in differentiation potential have been described (3). The use of virally-reprogrammed cells for transplantation studies has also raised concerns over insertional oncogene activation, as the viral vectors used for reprogramming are integrated into the genome during the infection process. Although these vectors are self-inactivating, once they are integrated into the genome and silenced the viral promoter remains and, with it, the possibility of reactivation.

In an attempt to overcome this problem, transposon technologies, where the reprogramming factors are flanked by terminal repeat transposon sequences, have been used to generate iPSCs in which no viral genes remain (4). Following reprogramming, an episomal viral vector expressing a transposase is used to induce seamless excision of the reprogramming transposon. A similar system uses loxP sites and both employ recombination of homologous sequences to excise the reprogramming genes within this region. Removal of viral genes will help reduce safety concerns, making these cells a more attractive option for future transplantation therapies. However, this research is still in its infancy, and with all methods available at present, the efficiency of reprogramming is low.

## Conversion of somatic cells to stem cells via SNCT

The alternative to removing viral gene remnants after reprogramming is to not use a viral vector at all. In 2011, the first report that human oocytes could be used to reprogram somatic cells into a pluripotent state was published (5). Dieter Egli's team, from the New York Stem Cell Foundation, has demonstrated that human oocytes have the ability to reprogram somatic cells into iPSCs through somatic nuclear cell transfer (SNCT), without the requirement of exogenous reprogramming factors.

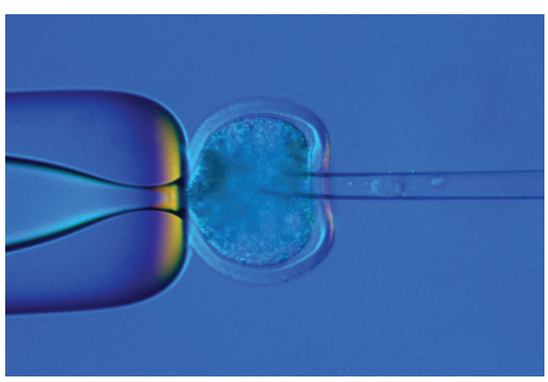
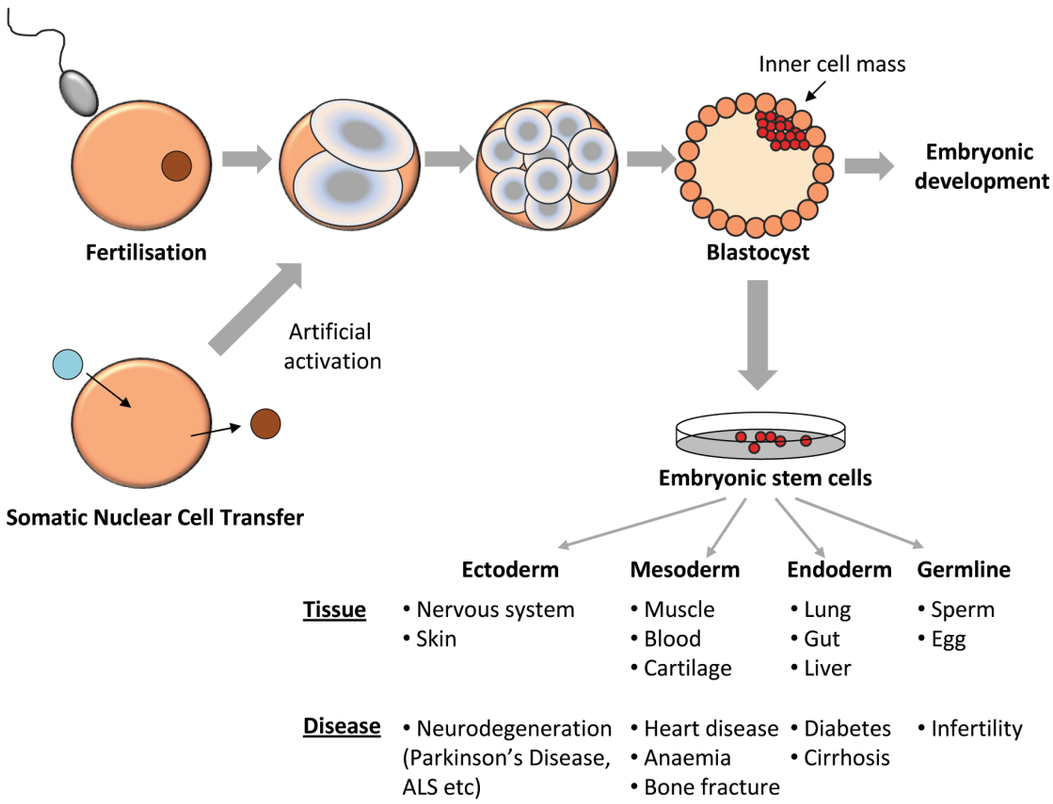


Figure 1: The removal and transfer of a nucleus during SNCT. Reproduced with permission from EuroStemCell.org

Figure 2:
ESC production via traditional and SNCT processes.




This involves removing the nucleus from a somatic cell, injecting it into a human oocyte and then allowing the oocyte to develop to the blastocyst stage before the inner cell mass is harvested. These induced pluripotent stem cells can be continually propagated in culture. Critically, in normal fertilised embryos this cell mass will go on to form the embryo, which is where the controversy surrounding this method arises. The authors found that cells reprogrammed using human oocytes resemble ESCs more closely than iPSCs generated by viral vectors and argue that this makes them a 'cleaner system' with no leftover reprogramming genes or epigenetic 'memory' of cell origin. Such technology could be used to generate patient-specific stem cells for use in the same manner as the iPSCs generated by viral reprogramming, but without the safety concerns associated with viral vectors.

The researchers found that implantation of a somatic nucleus into an enucleated oocyte induced cell division, but that transcription of somatic genes caused blastocyst development to become abnormal and arrested after three days. However, if the somatic genome was simply added to the oocyte then development proceeded normally. This suggests that the oocyte contains unknown factors that, once identified, could be used to produce normal diploid cells from the somatic nucleus – enucleated oocyte system.

When the cells were in triploid form (diploid somatic plus haploid oocyte) they did not arrest in growth, and in fact went on to form the blastocyst from which the inner cell mass could be extracted. Two stem cell lines were successfully established using this approach. Interestingly, the cells expressed pluripotency genes and were able to differentiate into tissues representative of the three germ layers despite the extra set of chromosomes. The blastocysts generated in this experiment were triploid and therefore abnormal, so it is unlikely that they could go on to develop a viable embryo. As current ethical restrictions dictate that these blastocyts are destroyed after 14 days, this cannot be verified.

It is important to weigh up the benefits of this technology with the ethical implication of using human oocytes for research. Although the blastocysts used are not generated from fertilised oocytes, their use is controversial since the formation of a blastocyst is a key stage in human embryonic development. In addition, these stem cells will not be useful as disease models until a normal diploid karyotype can be achieved. However, this technology brings scientists closer to reprogramming true human stem cells from somatic cells.

SNCT has been used to generate stem cells from several mammalian species but research on humans is scarce. This may be due in part to the limited availability of human oocytes for research purposes and the ethical implications surrounding their use. Since Yamanaka's seminal paper in 2006, alternative

methods for the generation of pluripotent stem cells, including the use of proteins, chemicals, plasmids, RNAs and miRNAs, have been developed. However, these are in the region of 100 to 1000-fold less effective than lentiviral-mediated reprogramming (0.01% efficiency). Utilising human oocytes as a reprogramming vehicle resulted in a 21% success rate. More recently, somatic cells have been directly converted from one cell type to another (7-8), even across cellular lineages, without the need to be converted to a pluripotent state. This further emphasises the plasticity of differentiated cells.

## Applications of SNCT technologies

Although these reprogramming technologies are still in their infancy, they are set to reshape the way in which we study disease. Patient-derived stem cells can be differentiated into the cell type of interest to provide a biological model that is more relevant than rodent models or cell lines. In addition, these cells can be employed as a drug-screening platform in which disease-carrying cells can be 'cured' *in vitro* prior to clinical trials being carried out.

Perhaps the most exciting application of this technology is the potential for individually tailored therapies. Somatic cells could be taken from the donor, reprogrammed to replace sick or damaged cells or organs, and then transplanted back into the donor. Given that the DNA sequence from the reprogrammed cells will be identical to that of the recipient, the immunogenic consequences of transplantation from another source will be avoided. Although epigenetic markers may not be erased completely, technologies such as SNCT seek to improve this. Compatibility of reprogrammed cells has not been researched in depth, but one report demonstrated that certain iPSC lines re-introduced to the original donor in mice did induce immunogenicity (9). Once these issues have been resolved, iPSCs have the potential to revolutionise the field of regenerative medicine, offering a source of otherwise unobtainable cells, such as neuronal cells. However, for custom-made cell therapies to be successful, more work needs to be done to determine the precise mechanisms of this immune response.

The field of cellular reprogramming has completely overturned the way in which we think about cell fate and differentiation and is set to change the course of disease research and medicine. Reprogramming strategies are improving, resulting in a move away from integrating viral vectors and towards safer 'footprint-free' methodologies. The use of human oocytes in research opens up a difficult ethical debate due to their potential to create human life. If regulated properly then we could reap the rewards of using these cells to generate more efficiently reprogrammed iPSCs that more closely resemble ESCs. By improving not only the reprogramming but the differentiation of various cell types, we have the potential to move into an era where for the first time human cellular research can be completed *in vitro* on previously unobtainable cells, or even recapitulate the hallmarks of a disease, thus providing new insight into disease mechanisms and therapies.

Human Fertilisation and Embryology (Research Purposes) Regulations 2001 allows research on human embryos for the following purposes:

- To promote advances in the treatment of infertility
- To increase knowledge about the causes of congenital disease
- To increase knowledge about the causes of miscarriages
- To develop more effective techniques of contraception
- To develop methods for detecting the presence of gene or chromosome abnormalities
- To increase knowledge about the development of embryos
- To increase knowledge about serious disease
- To enable any such knowledge to be applied in developing treatments for serious disease

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# Taming yourself: adrenaline and the chromaffin granules

by Daniel Pereda

"Isabella Swan: Are you going to tell me how you stopped the van?
Edward Cullen: Yeah. Um... I had an adrenaline rush. It's very common. You can Google it."
*Twilight*: the movie

## Fight or flight

Okay, you gloomy vampire, you got it right. According to the Wikipedia definition, "an adrenaline rush is the fight or flight response of the adrenal gland, in which it releases adrenaline. When releasing adrenaline, one's body releases dopamine which can act as a natural pain killer. An adrenaline rush causes the muscles to perform respiration at an increased rate, improving strength. It also works with the nervous system to interpret impulses that trigger selective glands." As definitions go, this one is in need of expansion.

We all know the feeling: breathing and heart rate increase, muscles tense, pupils dilate. At the same time, without us being conscious of it, our immune response is paralysed, our circulatory system redirects blood from the digestive tract to the muscles while glucose levels increase to feed them, and sensorial perception focuses on the source of stress.

This is all part of the fight-or-flight response, a dramatic physiological reaction to danger first described by the American physiologist Walter Bradford Cannon in 1915 (1). It is now recognised as an evolutionary adaptation to face the stress caused by the presence of a predator or any other stimulus considered a threat. But how exactly does this response work and what is the role of adrenaline? Simplifying, we could say it is all a matter of the adrenal glands.

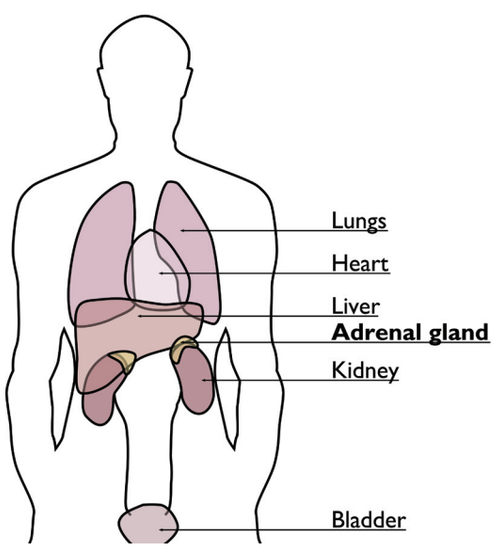


Figure 1:
The human adrenal glands are located above the kidneys and produce a number of hormones, including noradrenaline and adrenaline.

The adrenal glands are located on top of each kidney (Figure 1). They are highly stratified endocrinal glands, formed of an adipose capsule surrounding a trilayered cortex, each layer producing and releasing its own set of peptides and hormones, and a core, innervated by the sympathetic nervous system, in which adrenaline is produced.

## Adrenaline synthesis

The cells responsible for synthesising adrenaline are called chromaffin cells, because they are stained by chromium salts. They are almost exclusively found in the adrenal glands, although they do also appear in other areas of the sympathetic nervous system. Under the microscope, they appear to have a punctuated cytosol, although with higher resolution it is possible to see that the apparent points are actually tiny, round granules, up to 350 nm wide, with a large, electron-dense core. These granules, known as large dense-core vesicles or simply chromaffin vesicles, store adrenaline and release it through exocytosis.

Adrenaline, or epinephrine as it is known in the United States, is a small molecule composed of just 26 atoms. It is the final product of the transformation of the amino acid tyrosine via a highly regulated enzymatic pathway. In chromaffin cells, dopamine is oxidised to the adrenaline precursor, noradrenaline, after entering the granules which are the final storage point for adrenaline. However, the enzyme responsible for the final step of adrenaline synthesis, PNMT, can only be found in the cytosol. Why this apparent waste of time, moving dopamine into the granules, just to ship it back into the cytosol as noradrenaline, then into the granules again as adrenaline? The answer is very elegant: by expressing or not expressing the membrane transporter VMAT1, the protein that carries adrenaline into the granules, the cell can selectively store either just noradrenaline, or both adrenaline and noradrenaline, in the same granules, and release them in different ways.

## Storage and release of catecholamines

Chromaffin granules are fascinating biological structures. Their internal pH is acidic and $Ca^{2+}$ is more abundant here than in the rest of the cell put together. Adrenaline, noradrenaline and dopamine are all catecholamines, organic compounds with a catechol ring and a side chain. Catecholamines are

present in chromaffin granules at a concentration of 0.5-0.6 M, roughly three million molecules per granule, with an osmolality far higher than that of the cell. Yet the granules do not swell with water and explode, as we might expect, but keep a stable diameter. The reason behind this is not yet clear. What we do know is that the effective concentration of catecholamines inside the vesicle is much lower than the theoretical one we can calculate from the number of molecules and the diameter of the granules. This is most likely because catecholamines form weak bonds with the vesicle's protein matrix, which only break when exocytosis occurs and the extracellular medium enters the granule, releasing the neurotransmitters.

Catecholamines are released significantly slower than they should be. We can record and analyse not only the amount of neurotransmitter inside individual vesicles, but also how they are released, using single-cell amperometry (2). This involves placing an electrode able to oxidise catecholamines and generate an electric current, in close contact with the cell while exocytosis is induced. If the catacholamines were free in the vesicle and released by simple diffusion, the kinetics of the amperometric signal should be considerably faster than those that are observed. The logical conclusion is that something holds onto the catecholamines and decelerates the process. This is vital, because adrenaline is such a powerful hormone and neurotransmitter that uncontrolled release of it in the blood could kill us instantly.

## The chromogranine matrix

Our research group, led by Professor Ricardo Borges at the University of La Laguna, Spain, works with transgenic mice to determine what is holding back the catecholamines. We have shown that altering the vesiciular matrix by knocking out the soluble polypeptides chromogranine A and B drastically decreases the amount of catecholamines the vesicle can store (3). However, this modifies the kinetics of the exocytotic events as well. Additionally, the granules swell as expected, with a higher effective concentration of catecholamines which can no longer be buffered.

To make things even more complex, the chromogranines themselves have a wide range of biological activities. Alongside calcium ions and catecholamines, a number of additional products are stored in the vesicles, including less abundant granines, an enzymatic cocktail including proteases and peptidases, opioids, neuropeptides and many other peptides, vitamin C, nucleotides, even Alzheimer's amyloid precursors!

Given this opulence of molecules and the finesse involved in the control mechanisms regulating their exocytosis, it is unsurprising that a number of pathologies appear when the system fails. A common example is phaeochromocytoma, where generally benign tumours of the adrenal medulla result in unregulated release of adrenaline. The result is elevated blood pressure and heart rate, sometimes resulting in multi-organ failure when the blood vessels break and the blood pumps out of them.

Not all effects are as easy to detect as this. Polymorphisms, common genetic variants, in the chromogranin A gene have been linked with cardiovascular disease risk factors such as obesity and hypertension, and several research teams in Asia have correlated polymorphisms in the chromogranin B gene with a higher risk of suffering schizophrenia or Parkinson's disease (4, 5).

Even more interestingly, drugs with a similar structure to the catechol ring of the catecholamines have recently been found to slowly accumulate inside the vesicles (6, 7). This explains why it takes weeks before the therapeutic effects of some drugs appear, and opens new therapeutic opportunities.

Just in case anyone is still wondering though, no, not even chromaffin cells can help you stop a van. So please, don't try this at home.

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# Old disease, new problem: Chagas disease in the 21st century

By Kate Wright

Chagas disease, caused by the parasite *Trypanosoma cruzi* and transmitted by blood-sucking insects, has been a scourge of poor, rural populations in Latin America for centuries. In recent years, human migration patterns have spread Chagas disease to non-endemic parts of the world and new methods of transmission are coming to the fore. This neglected tropical disease is swiftly becoming a global health concern.

Chagas disease, also known as American trypanosomiasis, affects an estimated 10 million people and kills in excess of 15,000 a year. Initial infection with *T. cruzi* generally leads to a mild, short-term illness, which often eludes diagnosis. For one in three infected individuals, however, the disease re-emerges 10-30 years later. Chronic Chagas disease is characterised by cardiac symptoms, such as abnormal heart rhythm or heart failure, and digestive disorders. There are approximately 2-3 million people currently living with, or likely to contract, chagasic heart disease.

## Rude insects transmit *T. cruzi*

*T. cruzi* uses blood-sucking bugs called triatomines as vectors to infect wild and domestic animals, and humans (Figure 1). Species of these bugs inhabit 22 countries in the Americas. Triatomines have been dubbed 'kissing-bugs', or 'barbeiros' (Portuguese for barbers), after their unpleasant tendency to bite sleeping humans around the mouth and face. The insect defecates when it bites, leaving parasite-infested faeces around the wound. The parasite steals into the body through broken skin, often the result of scratching or rubbing of the bite wound, or through the mucous membranes of the eyes and mouth.

## Chagas disease in Latin America

Chagas disease has existed for millions of years in Latin America, shuttling from triatomines to wild animals, and later, to humans. Indeed, *T. cruzi* DNA has been discovered in 9,000 year old mummies of the Chinchorro people, the first settlers in parts of Chile and Peru (2). Some species of triatomines, including *Triatoma infestans*, have adapted to domestic life, burrowing into the mud walls and roofs of huts before creeping out at night to feed on their hosts.

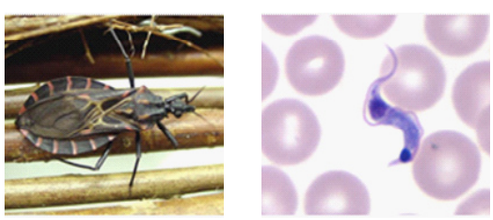

Figure 1: A triatomine insect (left) and a *Trypanosoma cruzi* parasite in a blood smear (right). Reproduced with permission from (1).

During the latter half of the 20th century, rural populations began to move to Latin American cities, bringing *T. cruzi* with them. As there were fewer kissing-bugs in this urban environment, an alternative route for *T. cruzi* transmission was provided by blood transfusion. In 1960, an estimated 10,000 cases of Chagas disease were acquired through contaminated blood in São Paulo hospitals alone (3). It is also possible to contract Chagas disease orally, through food and drink contaminated with triatomines or their faeces. Micro-epidemics of oral transmission are rare but severe, as seen in 2007 when over 100 Venezuelan schoolchildren were infected by contaminated guava juice (4).

## *T. cruzi* abroad

On the night of 25 March 1835, while in Mendoza, Argentina, Charles Darwin was bitten by a triatomine. "It is most disgusting," he grumbled in his diary the next morning, "to feel soft wingless insects…crawling over one's body." For the rest of his life, he suffered from a mysterious illness characterised by a panoply of symptoms. To account for his cardiac and gastric symptoms, some scholars have speculated that he contracted Chagas disease (5). Whether or not Darwin was a victim of Chagas disease, it is clear that Latin American emigrants can unwittingly carry *T. cruzi* with them.

In recent years, large population movements from Latin America to other parts of the world have spread *T. cruzi* (Figure 2). There are an estimated 300,000 infected individuals in the United States and more than 80,000 in Europe (6, 7). Although species of triatomines live in the US and some bugs have been carried to Europe by trade and travel, until now there has been little danger of transmission to humans outside of Latin America. New methods of transmission are now increasing the spread of the disease. In addition to blood transfusions, mothers can pass the parasite on to their children. Laboratory accidents, organ transplants and intravenous needle sharing are truly urban, modern causes of infection.

A recent editorial in *PLoS Neglected Tropical Diseases* branded Chagas disease the "new HIV/AIDS of

the Americas," highlighting similarities between the new epidemiological patterns of Chagas disease and the early years of the HIV/AIDS pandemic (8). Both are chronic and currently incurable conditions, disproportionately affecting the poor. Both have or had an attached stigma: HIV/AIDS that of sexual orientation or intravenous drug use, and Chagas disease that of immigration status. Co-infection with Chagas disease and HIV/AIDS is also emerging as a major health concern.

## A vector-borne epidemic beyond South America?

Currently, human Chagas disease transmitted by triatomines is uncommon outside of Latin America. Although US kissing-bugs range across the southern half of the country and transmit *T. cruzi* to animals, only seven cases of human infection from bug bites have been reported since 1955. Why are there so few cases? Firstly, US houses are less than welcoming for triatomine visitors, with concrete basements and screen doors to keep the bugs out. Secondly, the US species of triatomines have better manners than their southern counterparts, as they tend to feed first and then move on from the host before defecating. By following the (rude) adage that it's best not to 'use the toilet' where you eat, the US species effectively reduce the risk of disease transmission.

On the other hand, there is growing evidence to suggest that a vector-mediated outbreak in the United States is not out of the question. US bugs do harbour *T. cruzi* and certainly bite humans when given the opportunity. A PCR-based approach revealed that the guts of many triatomines in Arizona and California contained human DNA (9). Climate change may also increase the incidence of Chagas disease in the US: as temperatures increase, the range of some triatomine species could extend northward (10). Nevertheless, in the US, there is still a greater chance of experiencing an allergic reaction to the kissing-bug than contracting Chagas disease from it.

## Outlook for the future?

There are currently only two drugs which effectively combat Chagas disease, benznidazole and nifurtimox. Both are long and expensive treatments, accompanied by severe side effects. Crucially, these drugs are not proven to be effective against the chronic stage of Chagas disease, which, for many, is the first time they are diagnosed. Clearly there is an urgent need for new and improved anti-trypanosomal drugs or, better yet, a preventative vaccine. In the meantime, most efforts to combat Chagas disease focus on vector control in endemic areas through use of insecticides. Blood donor screening for *T. cruzi* is now routine in endemic countries, and increasingly in non-endemic countries like the US and France.

The worldwide dissemination of Chagas disease is a powerful warning of what can happen when an obscure and often undiagnosed infection increases its range. Wherever it goes, Chagas disease seeks out disenfranchised, neglected populations, including immigrants, the immunocompromised and those living in acute poverty. It is clear that Chagas is no longer strictly a tropical disease. With heightened awareness, and improved treatment and prevention efforts, we can stop it from remaining a neglected one.

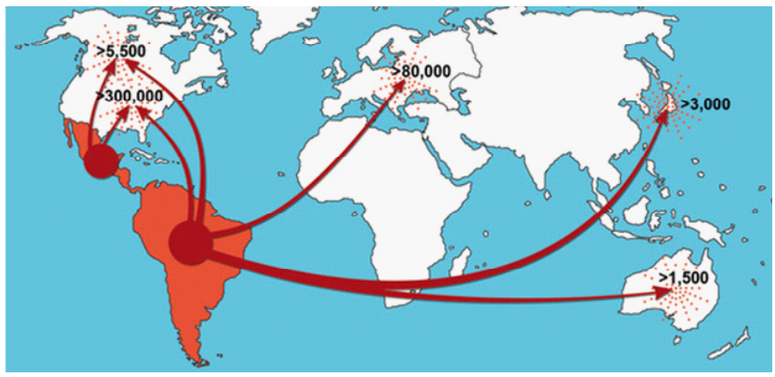


**Figure 2:** Migration patterns from Latin America. Estimates of the number of *T. cruzi*-infected individuals living in non-endemic areas are shown. Sourced from (3).

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# Science after the Arab spring: renaissance or continued disappointment?

by Dr Elwy Okaz

With the dust of last year's Arab spring beginning to settle in Tunisia, Egypt, Libya and Yemen, and with a blood bill still being paid in Syria, there are great expectations in the minds of those who overthrew their dictators. People across the region are waiting for a scientific renaissance. Younger generations believe it is only fair to hope for a better future in a region that held up the torch of knowledge during the European Dark Ages. But are the Arab spring countries taking steps in the right direction towards such a scientific renaissance?

The road to democracy has turned out to be bumpier than initially hoped. In Egypt, for example, despite the ousting of the president Hosni Mubarak last year, power contests, political agendas and behind-the-scene counterattacks from the old regime still shake this pivotal Middle Eastern country. In spite of this, signs of positive change are starting to emerge.

## Optimism in the ranks

The call for science reforms in Arab countries, including Egypt, started several years before the revolution. Egyptian-American Nobel laureate Ahmed Zewail first proposed building a $2 billion 'science and technology city' in Cairo 12 years ago. It was only after revolutions stormed the Middle East that the project was given the green light. This post-revolution 'science city' has attracted a number of Egyptian scientists from abroad, such as Prof Sameh Ali, previously an assistant professor at the University of California, San Diego. How do such leading Egyptian scientists see the prospects for research in the region in light of last year's revolution and in the shadow of the current turbulences?

"Although facing actual difficulties on the ground, I'm very optimistic," said Prof Ali, speaking to *Phenotype*. Ali is now professor and director at the Center for Aging and Associated Diseases, one of the new post-revolution research institutes and part of Zewail City of Science and Technology. "My most profound reason for this optimism is the remarkable energy and devotion that I [have] witnessed in the Egyptian youth," said Ali.

Prof Rania Siam, head of the biology department at the American University in Cairo, is another eminent Egyptian scientist. She already leads a strong research program that she established several years before the revolution. "I am definitely optimistic," she told *Phenotype*. "Scientists should see the revolution as an opportunity to build scientific capacities and revamp the scientific community in Egypt," she said. She also believes a major advantage to undertaking scientific research in post-revolution Egypt is "the enthusiasm and optimism of the young researchers and students".

Many Middle Eastern scientists and scholars, working both in the region and abroad, hail the surge in attention given to education and science after the Arab spring as a major step forward and hope for a return to the past when the Middle East led the world in science. In the Islamic Golden Age, between the eighth and thirteenth centuries, the region was a major contributor to science, with many of the world's greatest minds working in Baghdad, Cairo or Cordoba, in then-Islamic Spain. Great civilizations emerged in the region, whose scholars outlined the rules of the empirical approach, invented algebra, and wrote the most influential medical textbooks of their era. It was these contributions, together with the preservation and propagation of the rich heritage of ancient Greece and Rome, which paved the way for the subsequent Renaissance in Western Europe.

## Physical barriers: funding and infrastructure

Such great expectations though, may be premature. Despite spreading a new optimistic spirit, a small number of post-revolution research institutes cannot single-handedly produce the hoped-for renaissance. The infrastructure of the existing universities and entire science sector needs extensive remodeling. The current poor state of science in the Middle East, especially in Arab countries, is no secret. Only about 0.2% of gross domestic product in the region is spent on scientific research, compared to 1.2% worldwide (1). The lack of research infrastructure, core facilities and training resources is a major obstacle, according to Prof Ali. "This necessitates that we establish everything from scratch," he said. He added that this not only requires great investment, but also determination to overcome the "sterile system in place".

Teaching and research staff across Arab universities, especially those in non oil-rich countries, also have a long list of complaints. These range from low salaries

and limited research funding, to the uncontrolled increase in undergraduate student numbers admitted each year, despite insufficient resources and facilities to train them, and the lack of market demand for new graduates. "We need patience and planning; a short- and long-term comprehensive plan," said Prof Siam. "Basically this is our opportunity to develop a competent scientific system to serve Egypt and the region," she added. "The urge for quick rewards, although understandable, causes a huge pressure on politicians and reformers," said Prof Ali.

In addition, the path towards establishing world-class research labs is not straightforward, even when backed by the better-funded research bodies. Ali believes that one major difficulty stems from the lack of an efficient purchasing system, due to the over-pricing imposed by the agents of foreign vendors, and what he describes as the "bureaucratic rituals" associated with approvals, customs and import inspections. These problems seem reminiscent of the old regime. "The main challenge we face following the revolution is the delay in obtaining consumables and this obviously delays experimentations and project progress," confirms Prof Siam.

Scientific renaissance in these countries will require not only good will, but also patience, a lot of money and radical reforms. The promises from the new governments must be converted into funding and taking strong action to overhaul the bureaucracy. Such changes will also encourage the private sector to take on responsibility and support education and research in the region, which will be essential if it is to become a major scientific power once again.

## Intellectual barriers: science and extremism

In addition to limited funding and a lack of infrastructure, another concern is being discussed behind closed doors: the restriction on freedom of ideas and the lack of openness to several modern research topics. In the year following the Arab spring, Islamist parties have gained parliament majorities in both Tunisia and Egypt. One lesson that is clear from the Islamic Golden age is that the Islamic faith itself has never impeded the freedom of ideas or the flow of imagination when it comes to pursuing scientific questions. Tunisia's ruling Islamist Ennahda party and the ruling Muslim brotherhood in Egypt are considered by many to be moderate, but more hard-line religious parties are gaining popularity.

Science cannot be done without both the freedom to ask questions and the freedom to pursue the answers. Research in fields such as evolutionary biology and stem cell research, for example, might face resistance in these countries. It will be the responsibility of the elected decision makers and those writing post-revolution constitutions to support legislation that promotes the freedom of ideas.

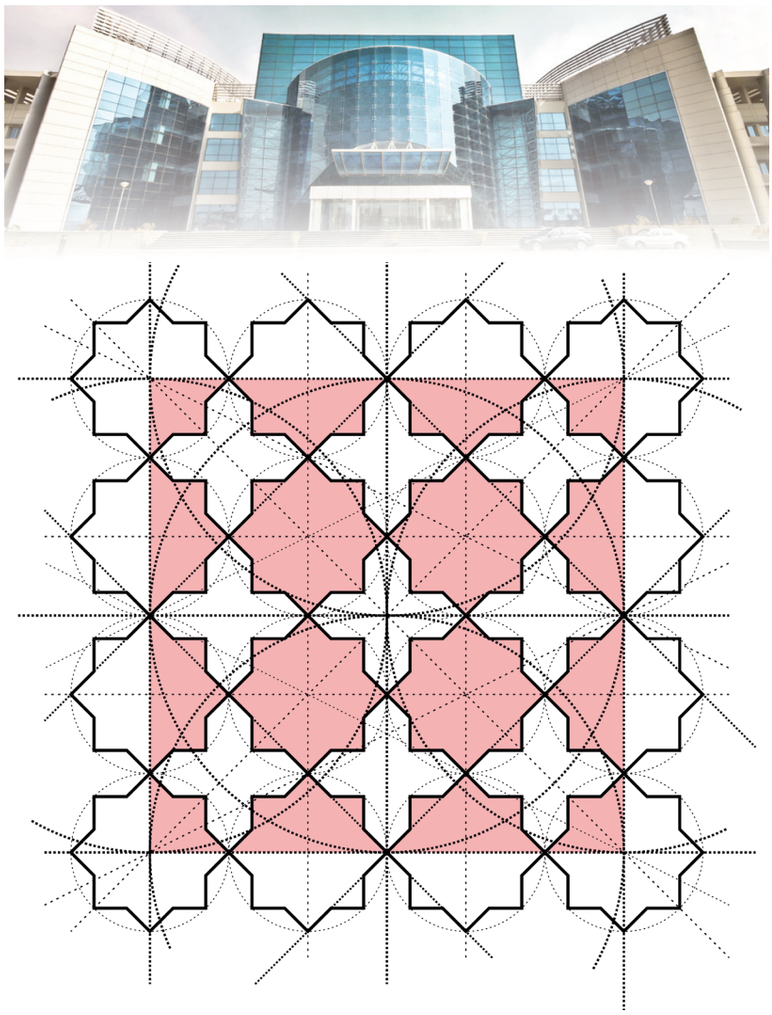

**Figure 1:** The Zewail City of Science and Technology, Egypt.

Will this freedom be secured in the years to come? Will the new leaders and entrepreneurs provide the funds and impose the reforms required for research development? Only time will tell. It is the new 'revolutionary' generation that must build with their hands and minds to make their hoped-for 'scientific revolution' happen, but who also need to monitor the legislative reforms in order to make sure that their sacrifices for a better future are not in vain.

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*Dr Elwy Okaz is a post-doctoral researcher in Prof Nasmyth´s laboratory, Department of Biochemistry.*

# Mass Decision Making

by Dr Carinne Piekema

It is a popularly held belief that two brains are better than one. In fact, this belief plays a major role in everyday life. Brainstorming sessions, board meetings, juries and elections all work on the principle that decisions made by groups are more likely to result in appropriate outcomes than those made by individuals. These ideas have been the research interest of many a scientist.

The first documented investigation into mass decision making dates from the French Revolution. Mathematician and political philosopher Marie-Jean-Antoine-Nicolas de Caritat, later Marquis de Condorcet, proposed in 1785 that large groups of people are capable of making near perfect decisions, even if their members do not possess any particular knowledge of the topic (1).

Over a century later, Sir Francis Galton provided compelling evidence for this 'wisdom of the crowd' effect in an ox meat weight-guessing contest at a meat market in Plymouth. He paraded a bull in front of a crowd of around 800 and asked it to guess the weight of the meat once the bull was slaughtered and dressed. Everyone put in an estimate and Galton calculated the average. It turned out that, while some individual guesses came very close to the actual weight, the average was closer still (2).

But are crowds always smarter than individuals? In some cases crowds can be wrong and importantly, scientists have started to determine the factors at play when the switch from crowd wisdom to foolishness occurs.

Scottish journalist Charles Mackey was among the first to cast doubt on the wisdom of crowds. In 1841 he wrote, "Men, it has been well said, think in herds; it will be seen that they go mad in herds, while they only recover their senses slowly, and one by one." He based these words on his observations that crowds can make terrible mistakes because they forget to think for themselves. In his book, *Extraordinary Delusions and Madness of Crowds*, he chronicled the tulip mania during the Dutch Golden Age. As a result of crowd behaviour, the price of one tulip bulb was driven up to nearly ten times the yearly income of a skilled worker, before it came crashing down with disastrous consequences. He also blamed ill-informed mass decision making for witch hunts and crusades (3).

### To communicate ...

The degree to which members of a group communicate with each other seems to greatly impact their collective wisdom. In Galton's weight-guessing contest, the contestants were not allowed to talk about their estimates, which is potentially why they were so accurate.

A recent evaluation of the impact of communication comes from a study by Andrew King from the Royal Veterinary College of the University of London. He asked 429 people at a campus open day to guess the number of sweets in a jar. Crucially, the guessers were divided into separate groups, which were given different information before guessing. When the guessers were told the most recent guess, or a random previous guess, the median was much less accurate than when no information was supplied (4).

One explanation for this was explored by a research group in Switzerland, led by Jan Lorenz. The group showed that humans are very sensitive to even the mildest social pressure, leading to a narrowing of opinions. Knowledge about the guesses of others narrows the range of estimates, but does not decrease the collective error. However, as a result of consensus, group confidence is boosted even if there is no improvement in their performance (5).

### ... or not to communicate?

Can group decisions only be accurate when communication between group members is forbidden? King and coworkers found that the informed group with the best guess was the one provided with the most accurate answer up until that point (4). In other words, information about the decisions of others can be useful, but only if the information can be trusted as accurate.

Bahador Bahrami and his colleagues at University College London reached a similar conclusion using a visual odd-one-out task. Communication between pairs of participants led to better decisions than those made by either individual on their own, but only if the pair were comparably good at the task. In cases where one participant's visual sensitivity was much worse than the other's, two heads were less accurate than the better of the two (6).

A key factor is that people can communicate both their choice and their confidence in that choice. For instance, in some cricket matches there is now a system that allows players to challenge an umpire's decision on a limited number of occasions. This only works well when the key players, usually the bowler and wicket keeper, indicate how strongly they believe in their appeal and not just how much they would like it to be right.

Bahrami and colleagues observed this too (8). Using the same odd-one-out paradigm as before, they found that when players could speak freely before making their decision, performance was less accurate than when they were only allowed to discuss their confidence level. Free communication, as previously observed by Lorenz, led to a narrowing of opinions and pushed the collective decision away from the correct one.

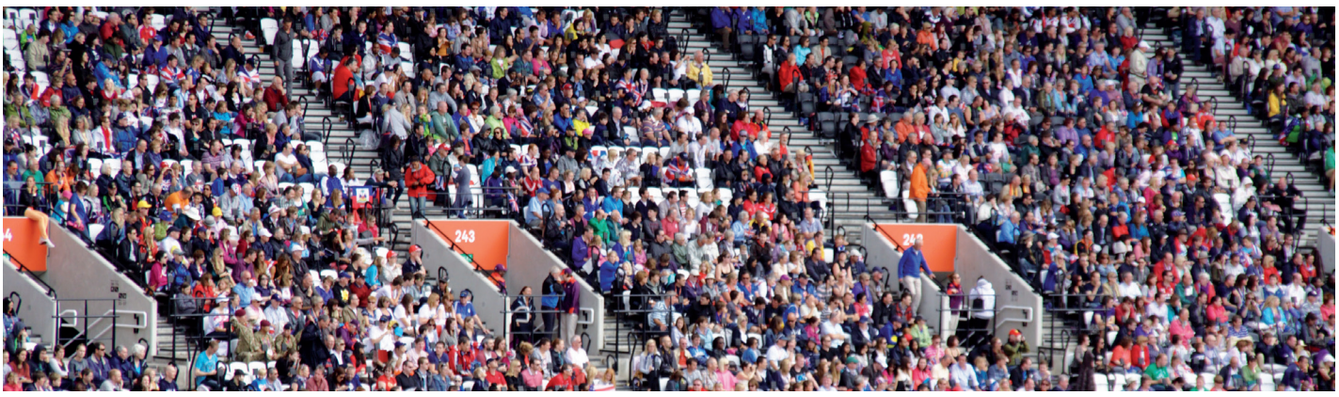
Nonetheless, relying on confidence alone has its own perils. In April of this year, Asher Koriat from the University of Haifa in Israel made an intuitively obvious finding: groups who follow the most confident person make better decisions, but only when most people within the group are making accurate choices. If most people are making mistakes, following a confident person tends to result in poorer choices (7). In other words, don't always trust the overconfident cricketer who believes he has taken a wicket if no-one else agrees.

## Multiple minds

The brain is able to optimally integrate sensory input and past experience. Researchers are now interested in whether the brain can similarly integrate information from multiple minds. They are using computational principles to explore how our brains might represent our trust in the decisions of others.

For example, Tim Behrens and colleagues from the University of Oxford compared brain activity during a task that involved learning from reward or experience, with one involving learning from others (9). They showed that our brains show very similar patterns of activity in these two tasks, but that activity takes place in adjacent regions of the anterior cingulate cortex, a highly important region for decision making. This means that learning from other people occurs in much the same way as learning from our environment through our own senses, suggesting that our brain is capable of judging our confidence in the opinions of others and integrating this information to help us make better decisions.

Paradoxically, it seems the best way to make group decisions is to make the original decision individually and limit communication to an indication of confidence only. Of course, elections, brainstorm sessions and jury verdicts are not going to disappear, and real world situations in which we are only able to communicate our confidence are limited. Therefore, perhaps the most important message to take away from the research discussed above is that it really is important to stand by our own decisions and not be swayed by others trying to convince us that their opinion is more valid than our own.



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# The disappearing male: is the Y chromosome destined for extinction?

The human Y chromosome has attracted much interest due to its small size, degenerate content and specialised role in sex determination and spermatogenesis. Current analysis suggests that the Y chromosome is slowly degrading. This could lead to alternative sex-determining systems in mammals, or to autosomes acting as pseudo-Y chromosomes by acquiring sex-determining genes. However, insights into the Y chromosome show that it might have evolved in such a way that it is able to preserve and protect itself from the degradative forces to which it is exposed.

by Maria Mogni

## The origin of X and Y

Use of the XY system to determine sex is unique to mammals. Birds, reptiles and fish have a range of sex-determining systems, such as environmental cues and XY and ZW systems which bear little resemblance to the mammalian XY system. The most popular theory behind the evolution of the XY system is that the sex chromosome pair evolved from a pair of autosomes (1) (Figure 1). One autosome in the pair acquired a sex-determining gene, becoming the sex-specific partner, Y. Further Y-advantageous alleles accumulated and recombination with X ceased, except at the tips, which share homology with the larger pseudoautosomal X. Multiple mutation and deletion events in the non-recombination region rapidly reduced the sex-specific chromosome to only 60 Mb. This theory explains why only 45 unique genes, many of which are related to genes on the X chromosome, remain on Y. This also provides an explanation for the Y-bearing testis-specific genes involved in sex determination and spermatogenesis.

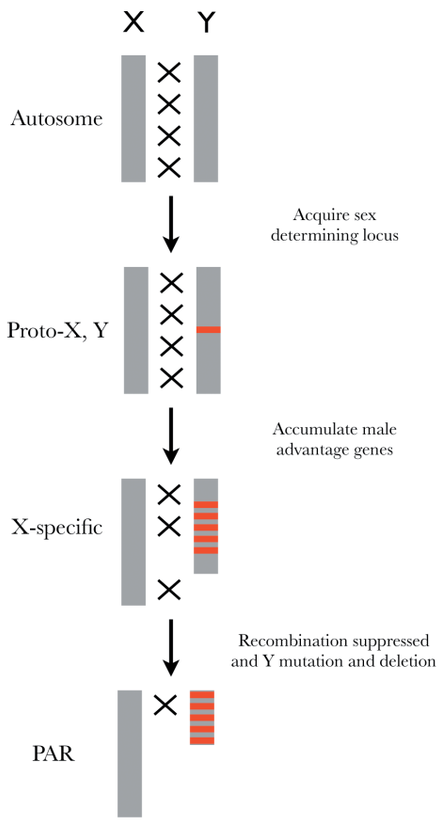


**Figure 1:** Possible formation of the Y chromosome. Adapted from (8).

## Composition of Y

The human Y chromosome can be split into ancient and added regions (Figure 2). The ancient conserved region (YCR) is tiny and is equivalent to the long arm of X and the region above the centromere, whilst most of the Y derives from the added region (YAR). A few genes have also been transposed from other chromosomes. Sequencing of the euchromatic region identified 178 transcribed units, many of which are pseudogenes or amplified copies (1). Several of the 45 unique proteins coded by the Y have a role in sex or fertility, implying Y has some coherence in its function.

Genes on Y were initially subdivided into two classes. Genes of class I are single copy and derived from X, while genes of class II are multicopy and testis-specific with no X equivalent. However, recent analysis showed that most genes with testis-specific expression and function in fertility have X partners from which they evolved. For example, the multicopy testis-specific *RBMY* gene, which has a role in spermatogenesis, evolved from the widely expressed *RBMX* that is involved in brain development. The multicopy testis-specific marsupial *ATRY* evolved from the ubiquitously expressed *ATRX*, mutations in which result in mental retardation and sex reversal in humans (4). Loss and amplification of exons from *RBMX* gave rise to *RBMY*, while the *ATRY* protein bears changes in domains that affect its ability to bind to factors involved in chromatin packaging. Hence, most genes on Y are from an evolutionary continuum and represent degrees of degradation and divergence from the X-borne predecessor. There are a few exceptions, such as *DAZ*, which is a multicopy, testis-specific, spermatogenesis gene closely related to an autosomal gene. This shows that Y can appropriate male-advantage genes from autosomes.

## Y degeneration and degradation

Accepting the idea that Y is a smaller version of X implies that it lost all except 45 of the 1,000 genes with which it started. The unusually rapid degeneration of the Y chromosome can be observed through analysis of its DNA sequence and from the activity and function of the remaining genes. Y is abundant with repetitive sequences of different types. Many multicopy genes are situated in palindromes, where often one or more copies are inactive. Furthermore, half of the heterochromatic long arm is composed of simple repeats with no coding function, which have no phenotypic effect when deleted. Moreover, many genes have been re-tooled for a male-specific function and a few genes with roles in spermatogenesis have been copied onto Y. Hence, alleles that provide a male advantage accumulated in Y, while suppression of recombination with X helped to keep the male sex-specific genes together.

Two major forces are thought to drive Y's degeneration and degradation: a higher mutation rate, and the lack of a partner for recombination. Mammalian Y is prone to far more mutation, deletion and insertion of retrotransposons than the rest of the genome. This bias is thought to be of a factor of 4.8 in humans (2) and explains why many new dominant genetic diseases occur on the father's chromosome. Such bias occurs because Y spends every generation in the testis, which is a hostile environment that requires more cell divisions to produce sperm than required for egg production, thus providing increased opportunity for damage. Furthermore, the sperm is an oxidative environment and is deficient in repair enzymes. Therefore, mutation, deletion and invasion of retrotransposons contribute to Y degradation, leaving it devoid of active genes except for the few that have managed to acquire a vital sex-specific function.

Absence of recombination with a partner is such that the entire Y is inherited as a unit and is susceptible to the vagaries of drift and selection. In addition, the repetitive structure of Y makes it prone to deletion. Recombination between homologous sequences in palindromes can frequently remove 6 or 7 Mb, including fertility genes, as seen in one family of Y chromosomes which are able to survive despite a 1.8 Mb deletion including eight testis-specific gene families (3). Accidental loss of mutant-free Ys from the population can occur if the last possessor of a fit Y bears no sons, because a fit Y cannot be regenerated by recombination.

## What could happen to Y?

A possible outcome for Y's progressive degeneration and degradation is that it will run out of genes and disappear (Figure 3). For example, *Drosophila melanogaster's* Y seems to be non-homologous to X. The initial Y could have been devoid of active genes or even lost and heterochromatin subsequently replaced it as a pairing partner for X. Male-specific genes could then have been acquired from autosomes.

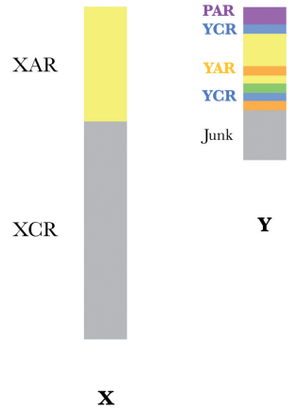


**Figure 2:** Both X and Y chromosomes contain added and ancient regions, while Y also has genes acquired from autosomes (orange) and junk regions (grey). AR, added regions; CR, ancient regions; P, pseudoautosomal. Adapted from(8).

Two rodent groups, the mole vole and the Japanese spinous country rat, have no Y chromosome. DNA analysis in mole voles showed no existence of a new sex-determining system or Y markers (4), while in the country rat it is thought that a region of Y has been transposed to X or an autosome (5).

Alternative sex-determining mechanisms could be put into place should Y disappear. Mutations in genes downstream of *SRY* can cause sex reversal, suggesting that other genes in the pathway could take over as sex-determining master switches. An example of this can be seen in mole voles, where an increased dose of *SOX9*, or an upstream mutation, can produce XX males. The gene that has taken over controlling *SOX9* and testis differentiation genes is yet to be identified.

Alternatively, the Y chromosome may be able to undergo self recombination in order to preserve itself. Palindromes in the Y contain multiple copies

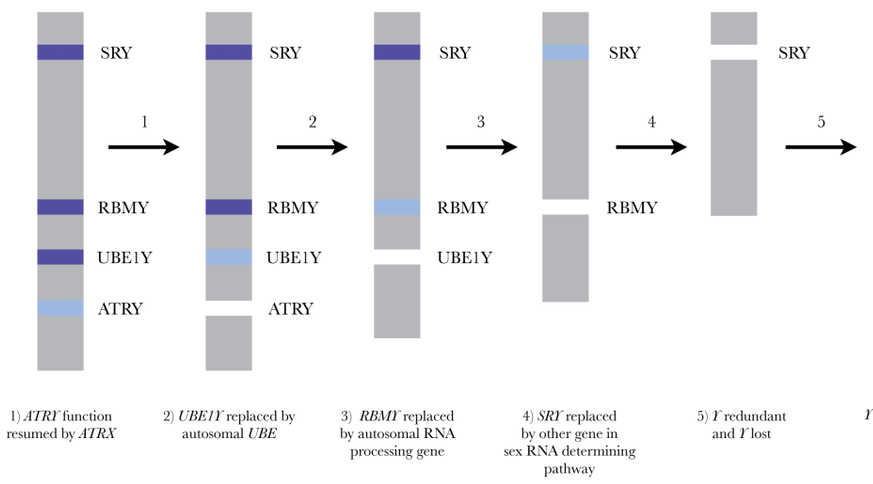


**Figure 3:** Possible fate of the Y chromosome. Adapted from (8).

of genes, with a copy at each end of the palindromic sequence. This provides a back-up mechanism should damaging mutations arise. The mirror-image structure allows the arms to exchange position during DNA division and for mutated genes to be replaced by wild-type copies (6). Furthermore, a study shows that the gene content throughout Y evolution does not experience linear or exponential decay due to degenerative forces (7). Rather, in the later stages of Y evolution, strict conservation of gene content has occurred through purifying selection, which argues against a decline of the Y chromosome.

In conclusion, although the Y chromosome is subject to a cruel evolutionary process that could ultimately lead to its progressive disappearance, there is still the possibility of the perpetuation of sex-determining mechanisms.

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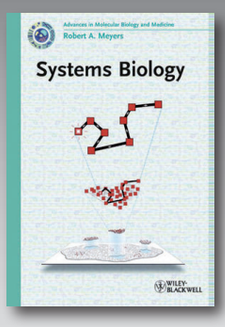
# BOOK REVIEW




## *Systems Biology*

*Edited by Robert A. Meyers*

*IISBN: 978-3-527-32607-5, Wiley Blackwell (May 2012), Hardcover, 726 pages, £ 205.00*

*Reviewed by Stuart Thomas*

Regarding the entire field of biology as an integrated system is one of the hardest challenges facing the scientific community and until now, a holistic understanding of life has proved elusive. However, with today's computer-based technologies allowing realistic and mathematically sound models to be built, and databases to be integrated, it is time for the scientific community to take note of systems biology. Unfortunately, *Systems Biology* is an unlikely candidate for spreading the word.

The book is a struggle to read from a lay point of view. It is not a textbook for students, nor is it a comprehensive review for specialists. The book as a whole lacks direction. The 22 chapters are written by different authors and haphazardly edited by Meyers, who appears to have done little except stitch them together. Many of the sections have tenuous links to systems biology, especially the early chapters which include a concluding paragraph mentioning systems biology as an obligatory link. The first chapter, written by Meyers, is a swamp of terminology and long-winded descriptions. For instance, phrases like "dynamical mesoscopic open spatio-temporally extended non-linear systems" challenge the reader to remain, if not awake, then attentive. While some chapters provide the reader with clear diagrams and case-studies, there is a general lack of explanation of new terminology throughout the book.

Despite these many drawbacks, there is gold to be found through persistence. The meat of the book is comprehensive and extremely interesting, containing diverse chapters from the philosophy of systems biology to the practical computational aspects. The section on the systems biology of evolution is particularly fascinating, providing a comprehensive review of bioinformatics and the modelling involved in evolutionary predictions. The hot-button topic of personalised medicine, which aims to model individual patients to determine best treatments and outcomes, is included. A chapter on E-cell technology is equally notable, although it reads more as an advertisement and the limitations of the programme are only briefly mentioned.

On the whole, *Systems Biology* is an interesting but flawed book, which serves as an acceptable introduction to the subject and as a go-to guide. Future editions would benefit significantly from a tighter edit to make it more accessible to a wider audience.

## *Epigenetic Regulation and Epigenomics*

*Edited by Robert A. Meyers*

*ISBN: 978-3-527-32682-2, Wiley Blackwell (April 2012), Hardback, 2 Volumes, 1254 pages, £285.00*

*Reviewed by Ben Trigg*

*Epigenetic Regulation and Epigenomics* is one of the latest textbooks to be released into the *Encyclopedia of Molecular Cell Biology and Molecular Medicine*. Aimed at everyone from undergraduate students to researchers, the textbook manages the impressive feat of covering the details while remaining accessible. Each article begins with a glossary of terms followed by a concise summary of the subject to be covered, ensuring that only basic preliminary knowledge is required to understand the key parts of the subsequent text.

The textbook is divided into five main subject areas. *Analytical Methods* focuses on relevant methodologies and what can be learnt from each of them. For example, RNA-based techniques are emphasised as these are key to the study of gene expression. *Basic Molecular Mechanisms* contains the bulk of the theory and covers topics from nuclear and chromatin organisation and stem cell epigenetics, to imprinting and DNA modification. Interesting niche topics are also presented, such as the epigenetics of prion proteins in yeasts, which I had not previously come across.

The rest of the text deals with the practical applications of epigenetics. The human epigenome and computational epigenomics are covered in *The Epigenome*, while *Medical Applications* introduces a wide range of topics from the pharmaco-epigenomics of cancer and epigenetic medicine, to the involvement of epigenetics in the immune system and ageing. Finally, *Model Organisms* ends with an overview of epigenetic systems in a range of extensively studied species.

Articles, averaging 35 pages and containing extensive reference lists, were contributed by over 90 selected authors and subsequently peer-reviewed. The editor should take pride in the work's quality and in-depth, up-to-date coverage of the field. My one criticism of the textbook is that diagrams are used too sparingly, as there are many long passages that could benefit from a diagram, both to aid in the text's explanation and to break up the sometimes overwhelming mass of text.

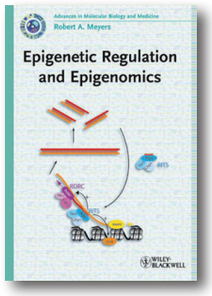
Although priced outside of most students' budgets, this textbook is a great reference that I would definitely recommend to any library or department looking to keep their coverage of this fast moving field up-to-date. It would also be of value to researchers as a reference guide or source of ideas for alternative experimental techniques.

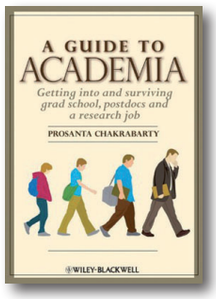
## *A Guide to Academia: Getting into and Surviving Grad School, Postdocs and a Research Job*

*Prosanta Chakrabarty*

*ISBN: 978-0-470-96041-7, Wiley-Blackwell (February 2012), Paperback, 192 pages, £20.50*

*Reviewed by Isobel Steer*




*A Guide to Academia* is based on the American education system, so will undoubtedly be invaluable for those wishing to continue their studies in the USA. Despite detailed information on US specific issues, such as health insurance and the Graduate Record Exam (GRE), this book is still useful for students wanting to progress in the global scientific community in general. The slim volume is filled with universally applicable titbits of advice that range from the obvious (not having a compromising 'frat party' Facebook profile picture; bringing a notebook and pencil to tutorials) to the insightful (if you have the academic background to skip a Master's degree, skip it). The narrative avoids being too anecdotal, although occasional stories liven the text.

The text follows the chronology of an academic career, from undergraduate to research professor. At each stage incredibly detailed advice is given, including how to build a CV, write an application letter, charm at an interview, give a presentation, and secure grant funding. Pages are dedicated not only to the interview or presentation itself, but also how to make yourself feel comfortable beforehand, making the book feel a lot more human than the instruction manual it could otherwise have been. Slightly off-putting is the fact that every chapter starts with "The Hard Truth about [PhDs, postdocs, etc.]". Despite these ominous introductions the style of writing is light and colloquial, interspersed with pithy asides and metaphors. The writer is clearly a fan of gangster movies, frequently referring to the department as a 'mafia boss' and a postdoc as a 'hired gun'.

The book advocates for an ethical career. Readers are encouraged to stand up for themselves; for example, if you have done all the work for a paper, your name should be first author, not your supervisor's. Respect for employees is also emphasised, as "graduate students are not free labour; they are trainees and future colleagues".

For students in a hurry, this book gives useful hints and checklists to ease the postgraduate program application process. In the library of self-improvement books, this work undoubtedly has a place on the shelf – that is, until you are able to replace it with your own academic publications.

# Thoughts from a biotechnology venture capitalist

Anna French

3rd year DPhil student in Prof Suzanne Watt's laboratory, Oxford Stem Cell Institute.

Kate Bingham is one of the pre-eminent life sciences venture capitalists in the UK. In addition to her role as a managing partner at SV Life Sciences, she sits on numerous advisory boards such as the Wellcome Trust Technology Transfer Strategy Panel. Kate read for a biochemistry degree at Oxford University before undertaking an MBA at Harvard as a Kennedy scholar. Prior to joining SV Life Sciences, she spent time as a strategy consultant for Monitor and has worked in business development at Vertex Pharmaceuticals.

**Can you tell me about your early career decisions? Why did you move into the venture capital sector?**

After my biochemistry degree I worked as a strategy consultant at Monitor Company. This was a great learning tool and provided me with solid experience which I have subsequently been able to draw on. The plan was always to use my consulting experience as a training period, so I went on to do an MBA at Harvard before starting at Vertex Pharmaceuticals in business development. Whilst at Vertex I got headhunted into venture capital and entered the profession quite out of the blue – not particularly useful for readers with similar aspirations, I'm afraid!

**What kind of tasks is a venture capitalist involved in on a regular basis?**

Well, there are a number of tasks and responsibilities that I might be engaged in on an average day. Every three to four years we are involved in generating new funds. In between those times, we need to ensure that we are keeping investors interested and expectation management is an essential part of what we do.

Anna French with Kate Bingham (left).

Besides generating new funds, we also search for new investment opportunities and therefore need to keep on top of what is happening in the life sciences sector. We spend a considerable amount of time working on the investments that we make and then, of course, the final step is to make a successful sale. With all of the different elements of the job we have close contact with a number of individuals, such as the investor assessment manager (affiliated professionals who manage additional private funds), the people who buy our companies and of course, with entrepreneurs. All of the partners and investors at SV Life Sciences have had a career in discovery and development; it is clearly easier to teach someone how to do a deal rather than the background of biotech innovations!

**What is it about your job that excites you most?**

Excitement arises when we move into a new area. There might be a need for a new approach and yet we have a limited amount of data to use. Will we be able to make it work? The whole sector is extremely fast moving. I am also interested in the science behind the business and the effect that a product might have on patients' lives. For example, our portfolio includes a company focusing on potassium channel inhibitors for hearing loss. Until now, there has been no treatment for such a condition; hearing aids have limited capabilities and can overload the user with background noise.

**How do you go about differentiating promising start-ups from those that are less likely to succeed?**

There are a number of things that we consider: novelty of the science, whether the market opportunity is present, and the competitor profile. Additionally, we take into account whether there is scope for a strong intellectual property application and if there is a sensible timeframe in which we can receive feedback on the clinical efficacy of a product. We also carefully consider whether the company has the right people to enable it to do the job and be

successful. Negatives such as ill-defined products or too many people make us think twice about the opportunity on the table.

**Have you encountered any major challenges during your career and if so how did you approach such hurdles?**
Yes, there have been a number of challenges, especially when companies fail. For example, there is a difference between the UK and the US with regards to trading insolvency. In the US you can trade until your very last cent, irrespective of debt, whilst in the UK you have to stop trading once your capital has reached your level of debt. In such a situation, the importance of managing expectations from the offset is paramount.

**In a recent letter to *The Guardian* you wrote about a shift in the pharmaceutical industry which has led to a much higher rate of biotech acquisition and a decrease in in-house R&D. In your opinion does this shift in the sector have any negative connotations?**
There are no real negatives with this relationship. Biotech companies, which are comparably smaller, have greater capabilities with regard to decision making. This increases efficiency as well as the potential for creativity. A direct analogy with how the film industry operates can be made; large corporations, such as Warner Bros., get ideas from smaller producers who pitch to the corporations. They in turn provide the required capabilities, marketing and distribution.

**What advice would you have for biochemistry grad students and finalists who are unsure as to their future career path?**
If you want to get involved in the development of new drugs, then there is no substitute for experience. When selecting and applying for positions, you obviously need to ensure that you will be well funded and that you have done a lot of research on the company and the employer. Make sure that it is possible to make a meaningful contribution within the available timescale of the project or role that you are applying for. It is always a good idea to do a post-doc with an entrepreneurial professor. Prof Steve Jackson at Cambridge University, for example, is known to allow interested scientists to get involved in his spin-out companies.

**What does the future have in store for the biotech sector? What are the major challenges that it will face?**
I believe that the practice of small biotech companies feeding into big pharma companies will continue and that this symbiotic relationship will keep getting stronger. Individual pharma companies will need to find a happy balance between internal and external R&D. Pharma now buys companies in a different way, in that they are paying less upfront but have a structure for longer term returns. This influences the interaction between the different people involved. In general, I think that academics with greater flexibility will prosper most through the resulting spin-outs.

This article is co-featured on the OBR Roundtable Review website. OBR is a rapidly growing network of students, academics and professionals committed to innovation in the life sciences. A running conversation of science, business and everything OBR can be found at the Roundtable Review (www.obrreview.com).

## Write for *Phenotype*?

- The deadline for article submissions is Friday of 8th week, 30 November 2012
- We accept articles on any aspect of biological sciences research, books or science education
- Articles can be either 650 or 1300 words

If interested, please get in touch: oubs@bioch.ox.ac.uk.

## Work for *Phenotype*?

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

# Towards an effective 'public involvement' in science

Clara Howcroft Ferreira

2nd year DPhil student in Prof Gero Miesenböck's laboratory, Department of Physiology, Anatomy and Genetics.

The theme of this year's Science Communication Conference from the British Science Association was impact. Assessing impact in science communication activities is difficult, particularly when initiatives are aimed at public understanding of science and often constitute non-formal educational activities. While questionnaires can be used to evaluate the activity itself, a direct correlation between the activity and its impact, for example if there is an increase in young people's uptake of science at school or an increase in public engagement in science, is difficult to assess.

An emergent topic at this conference was that scientific research institutions, funding bodies, policy makers and the public are increasingly interested in establishing an effective role for society in influencing how scientific research is funded, guided, scrutinized and used to inform policy making. A number of ideas were discussed on how to achieve this, as well as the associated concerns and potential pitfalls for all stakeholders.

Here, we explore these ideas and their possible limitations through two examples of successful public involvement in science. Both were presented during this conference at the panel discussion session *International Collaborations in Science Communication*. These successful international projects move far beyond traditional science communication activities aimed at public understanding of science and consist of on-going international networks that deal with societal issues at the local level.

## PLACES: developing partnerships

Antonio Gomes da Costa presented the project PLACES (1). This project has been running since June 2010 and involves 69 science communication institutions, coupled with their local authorities. It encompasses 10 European regions covering over 26 countries and is coordinated by the European Network of Science Centres and Museums (ECSITE) (2). As the 'impact assessor' of the project, the Universitat Pompeu Fabra in Barcelona is involved in developing tools to assess the impact of science communication projects and policies. The key features of this project are:

1. Developing city partnerships between science communication institutions and local policy-makers, research institutes, universities, non-governmental organisations and citizens
2. Developing action plans, with clear objectives and timings, to tackle local science-related issues
3. Long-term perspective
4. Local and European perspective, which is meaningful for the citizens and has an impact at the European level
5. Clear output in the form of recommendations
6. Development of an Impact Assessment toolkit

Gomes da Costa explains that, in practical terms, these features translate into "developing science policies driven by the citizens, with science communication institutions as mediators", and enable issues such as climate change to be addressed locally. The key strategies of this project focus on the science communication policies, with recurring dialogue with adult citizens, and in moving science communication from 'nice to necessary'. Gomes da Costa feels that this project is "changing the way policy makers and citizens look at science communication institutions by turning them into forums for active citizenship."

## Living Knowledge: focus on science shops

Another project, the international science shop network Living Knowledge (3), was presented by Norbert Steinhaus. This project "focuses on building partnerships for public access to research, where science shops are mediators and the scientific issues tackled are community based". The concept of science shops was first developed in the late 1970s in the Netherlands, and is spreading fast throughout the world. Science shops provide independent participatory research support in response to concerns raised by society, offering free or low cost access to scientific or technical knowledge. They mediate the science and society interface by creating bridges between the issues that arise in civil society organisations and the research that is done in order to address these issues. This translates into demand-driven research with no commercial interest, public availability of results and feedback to science institutions.

Examples of research work undertaken through this model are wide spanning and include studies such as those into the auditory pollution caused by turbines in Bonn. Steinhaus explains that "the issues tackled are not always necessarily big research questions, but practical problem solving", such as how to deal with fasting Muslim communities being prescribed medicines that have to be taken following food intake.

Steinhaus also explains that "this type of approach benefits all involved". It benefits society, by providing access to research and increasing both science visibility and citizen empowerment through participation. It also aids education, by providing students with problem-based learning embedded in the curriculum and an awareness of science and society issues. Scientific research also benefits, through the presentation of potential new research topics and by scientists becoming more aware of societal demands and needs.

The Living Knowledge network, along with

other science communication projects, such as Public Engagement with Research and Research Engagement with Society (PERARES) (4), constitutes a means of providing international expertise and open exchange. This will increase the accessibility and visibility of community-led research, as well as research information and results dissemination.

## Challenges ahead

These two projects provide the network for a new type of society-driven research that takes into account public needs and issues, which are translated into science policies. Furthermore, the international network that supports these projects allows knowledge and good practice exchange, ultimately making local initiatives more effective. However, these types of approaches are not problem-free.

There are a number of controversial issues which arise, such as how to ensure everybody participates in public dialogues and how to interact with different groups with different agendas. In both cases presented, ensuring an open dialogue with all parties involved proved difficult in debates surrounding 'hot' topics such as nuclear energy or climate change. Examples were given where radical anti-nuclear energy groups boycotted scheduled dialogues, and climate change sceptics impaired on-going dialogues with closed arguments, preventing free discussion. Since it is pivotal to these dialogues not to exclude any of society's voices, alternative platforms rather than physical presence debates might have to take place in cases like these using, for example, web-based dialogue platforms with impartial moderators. The converse could also prove problematic, with stakeholders of companies that profit from a particular course of action manipulating and coercing public opinion. These examples show the importance of an unbiased and impartial moderator, a role which the speakers and the public attending the conference believe should be taken on by science communication institutions.

Nevertheless, these projects are important steps into bridging the gap between society and research institutions, by creating spaces for 'public involvement' in science. This will make scientific research more transparent and open to public scrutiny, and enable society-informed, science-related policy making.

## References


1. PLACES: http://www.openplaces.eu
2. ECSITE: http://www.ecsite.eu/
3. Living Knowledge: http://www.livingknowledge.org/livingknowledge/
4. PERARES: http://www.livingknowledge.org/livingknowledge/perares


# 5´ with... Dr Sylvia McLain

Dr Sylvia McLain moved to the Department of Biochemistry in October 2011 from King's College London. Her group focuses on understanding the atomic-level interactions between biological molecules in physiologically relevant environments. Let's meet the person who rocks the best pair of boots in New Biochemistry.

*Interviewed by Andrea Szöllösi*

**From Physics to Biology, rock climbing to kayaking, bicycle mechanic to teaching, you've done it all! Could you tell us how it all fits into your life?**

Not all at the same time! I have an undergraduate degree in Zoology, which took me a while to finish. I went to university in the US and, under that system, you could start and stop, which was helpful to me as I worked in many different jobs during my undergraduate studies. For instance, I spent a year as a Fisheries Field Technician in the Great Smoky Mountains National Park, delivered pizzas, lived in China for a year teaching English as a Second Language, and was a raft guide. After being a lab technician for four years, I returned to university to get my teaching certificate. I taught secondary school for a year – physical sciences and biology – then returned to university again to get my PhD (in Chemistry) as a mature student.

# 5´ with... Dr Sylvia McLain

**If you were not a scientist you would be…**
A philosopher or an historian, or both even – I am fascinated by the history and philosophy of science.

**If you are not in the lab you are…**
Reading a book, working in my garden, refinishing some random piece of furniture or eating/cooking (drinking) with my friends.

**What was your worst disaster in the lab?**
For part of my PhD work, I synthesized silver fluoride compounds using fluorine gas dissolved in anhydrous hydrogen fluoride (HF). Both of these are highly dangerous chemicals, especially when combined. On one occasion, I hadn't properly sealed a metal ampoule containing about 200 ml of HF. When the mixture warmed up, it reacted highly explosively to water in the air, which caused the ampoule to shoot like a bullet backwards out of the hood. Fortunately, my reflexes were fast that day. I was able to slam the hood sash shut before the ampoule hit me or I was sprayed in the face by HF, which would have killed me. I am very lucky!

**What has been the most memorable finding of your career so far?**
I study the structure and dynamics of molecules in solution (mostly water) on an atomic scale. A few years ago, I investigated the structure of a series of peptides in water. These peptides have increasing hydrophobic character. We found that electrostatic interactions between the C-termini and N-termini were the 'driving force' for association between these peptides, not the hydrophobic-hydrophobic contacts. On top of this, the peptides that aggregated the most through their charged groups also showed the greatest hydrophobic interaction, leading to the hypothesis that it is actually electrostatic charges (on an atomic scale) that drive peptides and perhaps proteins to associate.

**Do you have a favourite classical experiment?**
I have two. One is not really a classical experiment: the Sanger method of sequencing DNA. I remember first using this method when I was a lab technician almost 20 years ago – it is just very elegant.

The second one would be the experiments of Ignaz Semmelweis, the nineteenth century Austro-Hungarian physician. He was the first person to demonstrate that hand washing by physicians could greatly reduce mortality rates in hospitals and clinics. He did this by plotting mortality rates before and after hand washing was introduced. Amazingly, his work was very badly received by the medical establishment who, at the time, didn't think hand washing was an issue. Semmelweis was later vindicated by Louis Pasteur, but sadly this happened after his death.

**In your opinion, what makes a good scientist?**
Being able to hang in there, even when everything is going wrong! Practising science, especially if it is new science, can be very frustrating. There are lots of failures, whether it is getting a certain new experiment to work or persevering when trying to get research funding. I also think a good scientist is someone who can graciously accept when their hypothesis or favourite theory is proved wrong.

**What is the best advice you have ever received?**
I always quadruple check everything I publish, but sometimes I still worry I have missed something. Once, I found a mistake I had made in a paper, which of course I found very upsetting. I went and talked to my post-doc supervisor who very calmly told me: "Don't worry so much about finding mistakes in things; everyone makes them and all you can do is hold your hands up and admit it. And sometimes when you look back at what you did, you think that was actually pretty good."

**What advice would you give to a young scientist in your field?**
It's your career and your life – remember that. Of course you always will have to do things you don't particularly want to do, but when deciding on what job/career path you take, remember it is you that has to do it and take ownership of it, no one else. If you ever decide to change or head down a different career path try not to think of your past as 'wasted time'. There is no such thing as wasted time; what you have learnt at every stage will help you in the long run.

**How do you imagine biological research will change over the next twenty years?**
Discoveries come from the most amazing places and often in ways that you never expect or can predict. It will be interesting to see what happens to Big Pharma, as the patents for many drugs expire and new drugs become more difficult to discover. I imagine that drug design will change drastically in the next 20 years, but I think it is much too soon to make a prediction as to exactly how!

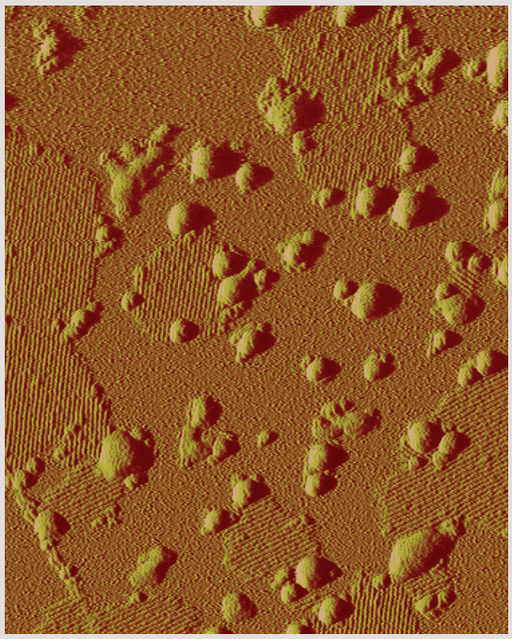
# SNAPSHOT Research Image Competition

## This issue´s winner is...

### Dr Olivia Berthoumieu

Olivia completed her DPhil in June 2012 in Professor Anthony Watts' group in the Oxford University Biomembrane Structure Unit (OUBSU) within the Department of Biochemistry.

Her high resolution image of liposomes on a mineral substrate was obtained using atomic force microscopy. The liposomes were produced by an extrusion process and formed a 'moon-like' surface as they fused into lipid bilayers on the surface of the substrate. The total scan size is 1 μm.



The Watts lab uses biophysical techniques within the OUBSU but also has access to solid state nuclear magnetic resonance performed at the Biological Solid State NMR Facility, at the Rutherford Appleton Laboratory near Didcot. Their research focuses on the structural resolution of small molecules at their native sites of action in cell membranes. Their main research interest is G protein-coupled receptors (GPCRs), the largest group of membrane receptors, involved in a variety of biological and pathological processes and one of the largest classes of drug targets. By studying the dynamics and structural details of membrane proteins and lipids at high resolution they hope to elucidate the mechanism of action of hormones, ions and drugs at their membrane-bound receptors.

While the majority of Olivia's DPhil focused on bacteriorhodopsin, her cover image resulted from an initial side project on neurotensin. Neurotensin has significant involvement in the regulation of the dopaminergic system and therefore may be involved in Parkinson's disease and colon cancer. Olivia purified the neurotensin receptor 1 (NTS1), a class A GPCR that mediates most of the effects of the neurotensin ligand. She then reconstituted it into liposomes, which are small 'bubbles' of lipid membrane. Olivia created 100 μm-wide liposomes and inserted the receptor into the membranes by an extrusion process, where a solution of polar lipids is passed through filters of decreasing size until the desired diameter of vesicle is obtained. Atomic force microscopy (AFM) was then used to confirm the size and integrity of the resulting liposomes. The receptor can in this way be studied at high resolution and in its native conformation within the simplified lipid membrane system.

During AFM, the sample is scanned with an ultra fine probe (2 nm) located at the end of a flexible cantilever, rather than obtaining images from optical or magnetic lenses. Local attractive or repulsive forces established between the probe and the sample result in subtle movements of the cantilever about the scanning surface. This movement is detected by a laser and reflected onto a photodiode detector. This technique can be used to image living cells, proteins, DNA and molecular interactions and, importantly, under native conditions including in solution.

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**SNAPSHOT**
Research Image Competition

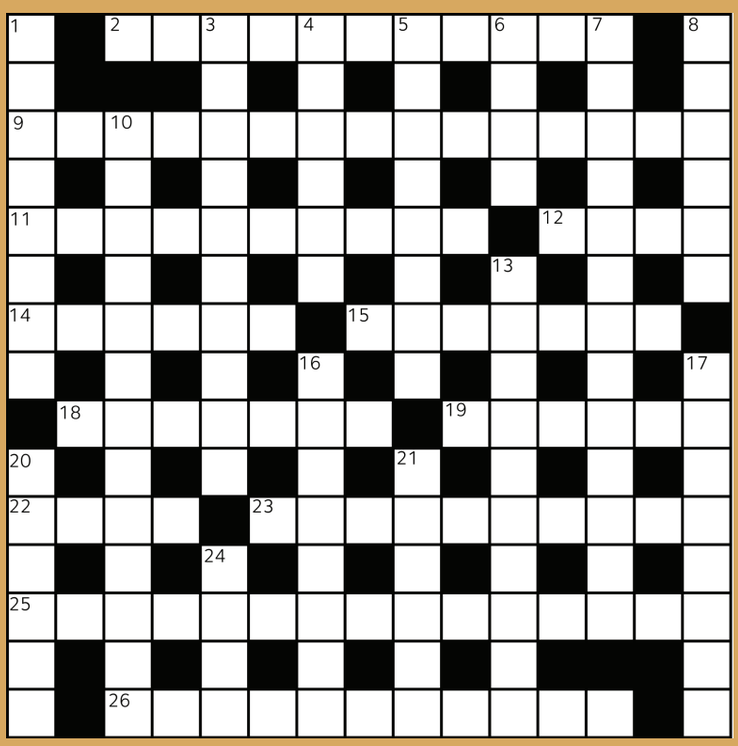
### Win a £50 book voucher kindly provided by Oxford University Press!

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 *Phenotype* HT 2013. To enter, send images to oubs@bioch.ox.ac.uk with a brief description (maximum 100 words). Please get permission from your supervisor before sending any images. There is no limit to the number of entries per person.
The deadline for the competition is 30 November 2012.

# PHENOTYPE crossword

Enter the competition by sending your answers to oubs@bioch.ox.ac.uk or leave a paper copy in a sealed envelope in the OUBS pigeonhole at the New Biochemistry reception. Entries received by 31 December 2012 will be entered into the prize draw.

Our resident cryptographer, Homarus (www.homaruscryptic.com), challenges *Phenotype* readers to crack this cryptic crossword on the theme of molecular biology.



The winner will receive their choice of three books reviewed in this issue, generously provided by Wiley-Blackwell.

WILEY-BLACKWELL

Across

2 WMD is unfeeling, enclosing an evident smell (7,4)
9 Revolting actor in mix-up in somewhere like Guildford (15)
11 Dig expressed region holding bubbly and titanium (10)
12 Spots broken stick (4)
14 About the drugs: let's go over it again (6)
15 Yummy mummy to grease yarrow (7)
18 Operon rearranged in a woody plant that produces syrup (7)
19 Hirer of the French diocese (6)
22 Cogan's battle (4)
23 Haemoprotein dissolved my ochre cot (10)
25 Snackfood containing chicken offal, German pig and herbs (8,7)
26 Missey and Donny mixed together; their names are the same now (11)

Down

1 Something from the chemist to work at the start of every attempt (8)
3 see 10
4 Waste products seen in college's tank (6)
5 Raised a castle in one step with new technology (8)
6 Blower to ring in an honour (4)
7 Spineless model orders: Obama's not rich (13)
8 Yogi stops in bulls' element (6)
10,3 Confused, Mac raises a screechy voice to brewer (13,10)
13 Scorsese film when PCR has finished? (5,5)
16 Balkan sick of your little lan (8)
17 Swiss protein-coding locus against splicing enhancer (8)
20 Quarry coastal Cumbria for mineral (6)
21 Back up pole before adding ... (6)
24 ... shock at rising seeds (4)

Congratulations to Daniel Scott from the Dunn School of Pathology who won the Trinity '12 crossword competition.

*Answers to Trinity '12:*
*Across* 1. cytogeneticist 9. elegans 10. xenopus 11. ophiuroidea 14. arm 15. hid 16. oxfordshire 17. biodynamics 19. sir 20. ing 21. endotrophic 22. ionised 24. spignel 25. cagney and lacey
*Down* 1. caenorhabditis 2. teethed 3. granulocyte 4. nus 5. taxidermist 6. can 7. soprani 8. asymmetrically 12. offhandedly 13. assessorial 18. oogonia 19. sphenic 23. sin 24. son