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Gaseous Signalling

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Developed by:
Ghosts, gases and the Mouse’s Petition

by Freddie Theodoulou, Science Editor

It’s hard to watch a kettle boil without thinking about states of matter. Or is that just me, and most folk simply anticipate a hot cup of tea? Either way, I think it’s fair to claim that most school children understand that water forms a solid when cooled and a gas when heated. Thanks to to celebrity chef Heston Blumenthal, we’re also pretty well acquainted with liquid nitrogen and dry ice, if only as novelty ingredients for his culinary creations. But in these enlightened times, it’s easy to forget that for much of human history, gases were mysterious and intangible. Even the etymology is enigmatic, the neologism “gas” being said to derive either from the German “geist” (ghost or spirit) or the Greek “chaos” (roughly translating as “ultra-rarefied water”).

During the eighteenth century, natural philosophers such as Priestley, Lavoisier and Scheele sought to understand and separate gases, eventually establishing the basis for modern chemistry. Each has a different claim to the discovery of oxygen, or “dephlogisticated air”, as Priestley erroneously called it. Priestley also discovered several other gases, often involving the use of animal experiments, which stimulated a discourse on the topic with his literary friend, Anna Laetitia Barbauld, who presented Priestley with “The Mouse’s Petition”1, a mock-serious poem in which the murine subject makes an eloquent case to be spared from experimentation. Writing in her obituary, William Turner elaborated on the story2:

‘The Mouse’s Petition’ had its origin from the following circumstance: Dr Priestley, from the vicinity of his residence to a large brewery had been led to notice the suffocating vapor which is extricated in the process of fermentation (now so well known as the carbonic-acid-gas...). In the course of these investigations, the suffocating nature of various gases required to be determined, and no more easy or unexceptionable way of making such experiments could be devised, than the reserving of these little victims of domestic economy, which were thus at least as easily and as speedily put out of existence, as by any of the more usual modes. It happened that a captive was brought in after supper, too late for any experiment to be made with it that night, and the servant was desired to set it by till the next morning. Next morning it was brought in after breakfast, with its petition twisted among the wires of its cage. It scarcely need be added, that the petition was successful. ”

Not all of Priestley’s experimental animals enjoyed the support of a poetic advocate but the discovery of oxygen is a happier tale in which the mice in question were sustained by plant-derived oxygen, setting the scene for the later understanding of photosynthesis. In honour of Priestley - chemist, theologian, educator, and inventor of soda water (an achievement of which he was most proud), this month’s issue is devoted to gas sensing and signalling, with several examples of biologically important “airs” and their roles in bacteria, plants, humans and yes, even the odd mouse.

1. Anna Laetitia Barbauld. Poems, 1773
2. William Turner. “Mrs Barbauld” The Newcastle Magazine, April 1825
Ethylene: a gaseous signal in plants and bacteria

Ethylene was the first gaseous growth regulator discovered due to its pronounced effects on plant growth and development. Besides plants, many bacteria also have ethylene-binding proteins, indicating that the ability to bind and respond to ethylene is an evolutionarily ancient sensory mechanism. The recent characterization of an ethylene receptor from cyanobacteria and the finding that it plays a role in phototaxis confirms a prokaryotic role for the ethylene receptors and is consistent with the hypothesis that plants acquired ethylene receptors from the endosymbiont that gave rise to the chloroplast. The signalling pathway acting downstream of the plant ethylene receptors is considerably diverged from that found in bacteria, pointing to adaptations that can occur in transitioning from a prokaryotic to a eukaryotic cellular environment. Interestingly, although pathways for ethylene biosynthesis and signalling are conserved in plant lineages extending back to the green algae, there are examples of plants where these pathways have been lost, with ethylene no longer playing a regulatory role.

A brief history of ethylene as a plant hormone

The saying “one bad apple spoils the whole bunch” has a real biological basis. The gaseous hydrocarbon ethylene (C₂H₄) is most commonly known as the fruit-ripening hormone, and a rotting apple will release ethylene and thereby stimulate the ripening and rotting of nearby fruit. Although unaware of ethylene itself, farmers in ancient Egypt and the Middle East exploited its effects through their gashing of unripe sycamore figs, a practice that causes a burst of ethylene which then drives ripening. The modern fruit industry uses ethylene to ripen fruit in transit to stores so that it is shelf-ready when arriving at the market. Ethylene inhibitors, such as the chemical 1-methylcyclopropene (1-MCP) sold under various brand names, are used to preserve fruit. The fact that ethylene affects plant growth and inhibitors exist implies that plants can sense ethylene. Indeed, plants synthesize ethylene and sense it through specific receptors. Physiologically, ethylene has roles in cell elongation, organ abscission, senescence, pathogen resistance, abiotic stress, cell division, metabolism, flowering and fruit development, with many of these roles being of significant agronomic importance. Because of its gaseous nature, ethylene can diffuse rapidly throughout plant cells, cross cell membranes and escape the plant as an environmental signal.

The history of plant research on ethylene extends back over a century to the Industrial Revolution, when observations suggested that illuminating gas was altering plant growth. Illuminating gas, a coal by-product piped throughout cities for street lamps in the 19th and early 20th Centuries, would leak up to 10% of the gas. Plants near illuminating gas pipes were damaged or died due to inhibited root growth, accelerated senescence, leaf and petal abscission, and other alterations to normal growth. In 1901, Dmitriy Neljubow demonstrated that the active component in illuminating gas was ethylene. In the 1930s, Richard Gane showed that plants synthesized their own ethylene by isolating detectable quantities from 27.2 kg (60 lb) of apples, thereby establishing ethylene as an endogenous plant hormone, the first gaseous growth regulator identified in any organism. The biochemical pathway for ethylene biosynthesis was elucidated during the 1960s and 1970s. It was not until the 1990s that the key elements in the ethylene signalling pathway were determined, this major advance taking place as a result of the elegant genetics made possible with the model plant Arabidopsis (Figure 1). For those interested, Bakshi et al. provide an excellent historical overview of ethylene research.

Ancient origin of ethylene receptors

Observations of flowering plants led to the discovery of ethylene as a hormone, but the ability to sense ethylene is evolutionarily ancient, preceding the existence of plants. Sequencing of bacterial genomes has revealed genes that encode proteins with ethylene-binding domains similar to those of plant ethylene receptors. This is perhaps not too surprising in retrospect, because the signal output domains of plant ethylene receptors are related to two-component signalling elements of bacteria. At their simplest, two-component signalling systems involve a membrane-bound receptor that is phosphorylated on a
histidine residue in response to ligand binding (Figure 2). The phosphate is then transferred to an aspartic acid residue in a soluble response regulator, which then elicits downstream responses such as changes in gene expression. Eukaryotic two-component signalling systems are found in fungi, algae and plants. The fact that ethylene-receptor-like proteins, with similar input and output domains, are found in bacteria and plants points to a remarkable conservation of structure across more than a billion years of evolution.

Recent studies with cyanobacteria demonstrate not only that bacteria contain sequences that look like ethylene receptors, but also that these are capable of binding ethylene and eliciting physiological responses, hallmarks for receptor function. Cyanobacteria are blue-green bacteria capable of photosynthesis and responsible for oxygenating Earth's atmosphere two billion years ago. The receptor in question is found in the cyanobacterium *Synechocystis* and is named ETHYLENE RESPONSE1, or SynETR1 for short. SynETR1 contains two input domains: an ethylene-binding domain and a light-sensing domain (Figure 2). Ethylene and light appear to have opposing effects on signal output of the receptor, with UV–Violet light inducing movement away from the light source (negative phototaxis) and ethylene inducing movement towards the light source (positive phototaxis). Ethylene also operates through SynETR1 to promote the formation of bacterial pili involved in bacterial movement. Signalling itself operates through a canonical two-component signalling system such that SynETR1 phosphorylates a response regulator, which in turn controls gene expression. Interestingly, although ethylene-producing bacteria exist, the *Synechocystis* strain with SynETR1 does not synthesize ethylene. This suggests that SynETR1 may be involved in detecting environmental ethylene or perhaps other hydrocarbons, not specifically ethylene. Ethylene is produced from organics exposed to sunlight, so the ability of SynETR1 to sense ethylene and light is likely to play a role in the cyanobacterium finding optimal photosynthesis conditions.

The identification of SynETR1 in cyanobacteria as a genuine ethylene receptor has significant implications for how plants receptor their repertoire of ethylene receptors. A likely hypothesis is that ethylene receptors were co-opted for signalling by higher plants during the endosymbiotic event by which an ancestral SynETR1-like containing cyanobacterium became a chloroplast. As described below, this probably occurred in the green algae that are land plant ancestors. As with many ancestral chloroplast genes, the gene encoding the ethylene receptor of the endosymbiont eventually moved to the nuclear genome of the host cell over evolutionary time. Alternatively, plant ethylene receptors may have their origin via a horizontal gene transfer to algae from a bacterium with an ethylene-receptor-like gene.

![Image](https://example.com/image.png)

**Figure 1.** The morphological response to ethylene of dark-grown *Arabidopsis* seedlings. In response to ethylene, dark-grown *Arabidopsis* seedlings exhibit a shortening and thickening of the hypocotyl stem, an inhibition of root growth and the formation of an exaggerated apical hook. Seedlings are shown grown in the absence (−) or presence (+) of ethylene (scale bar, 5 mm). A close-up of the apical hook is shown on the right.

The functional conservation between the ethylene receptors of cyanobacteria and plants might suggest that plants would also rely on a two-component system to relay the ethylene signal. This, however, is not the case. Although evidence exists that signal output from the receptors can make use of a two-component-like signalling system, this is not the major signalling system operating downstream of the receptors. In fact, the evolutionary trajectory of plant two-component elements frequently seems to move away from the original histidine/aspartic acid phosphorylation framework prevalent in eukaryotes. This is exemplified by the chimaeric nature of the plant ethylene signal transduction pathway (Figure 3). First, although some of the plant ethylene receptors have histidine kinase activity, others have diverged so much that, although they preserve a functional ethylene-binding domain, they lack histidine kinase activity; some in fact appear to have acquired serine/threonine kinase activity. In addition, the primary function of the histidine-kinase-like output domain of the receptors is now as a docking site for a serine/threonine kinase called CTR1, which belongs to the family of Raf-like kinases unique to eukaryotes. Other elements in the plant ethylene signalling pathway include the transmembrane protein EIN2, which also belongs to a eukaryote-specific protein family, and the plant-specific EIN3 family of transcription factors. The ethylene receptors, CTR1 and...

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**Pathways for ethylene biosynthesis and signalling exist in green algae**

The identification of SynETR1 in cyanobacteria as a genuine ethylene receptor has significant implications for how plants receptor their repertoire of ethylene receptors. A likely hypothesis is that ethylene receptors were co-opted for signalling by higher plants during the endosymbiotic event by which an ancestral SynETR1-like containing cyanobacterium became a chloroplast. As described below, this probably occurred in the green algae that are land plant ancestors. As with many ancestral chloroplast genes, the gene encoding the ethylene receptor of the endosymbiont eventually moved to the nuclear genome of the host cell over evolutionary time. Alternatively, plant ethylene receptors may have their origin via a horizontal gene transfer to algae from a bacterium with an ethylene-receptor-like gene.
EIN2 are predominantly localized to the membrane of the endoplasmic reticulum. This might be considered an unusual location for hormone perception, as the plasma membrane is the most logical location for hormone receptors, but internal localization is quite compatible with the cross-membrane diffusion of ethylene.

Given the differences between the ‘modern’ ethylene signalling pathway of plants and the ‘ancient’ two-component ethylene signalling pathway of bacteria, an important question to resolve is when plants acquired their suite of ethylene biosynthesis and signalling genes. The answer to this question has become increasingly tractable through analysis of many species’ genomes and transcriptomes. For instance, the moss *Physcomitrella patens*, which shares a common ancestor with flowering plants about 400 million years ago, has a similar suite of biosynthetic and signalling genes to the angiosperm *Arabidopsis*2. Moreover, ethylene affects *P. patens*’s growth, stimulating growth of peripheral filaments and reducing growth in central area of the moss. Thus ethylene appears to operate similarly throughout land plants separated by 400 million years of evolution. Does the plant ethylene hormone system pre-date the advent of land plants? A recent study demonstrates that it extends back to the charophytes, a division of freshwater green algae, but is lacking in marine green algae7. Furthermore ethylene can stimulate cell elongation in the charophytes. Ethylene also regulates cell elongation in flowering plants, for instance inhibiting cell elongation in dark-grown *Arabidopsis* seedlings (Figure 1), but stimulating cell elongation in deep-water rice, suggesting a remarkable conservation of function for ethylene between green algae and flowering plants. The demonstration that the ethylene hormone system exists in charophytes pushes the conservation of this system back to more than 450 million years, when plant colonization of land occurred. Additionally, the use of ethylene as a hormonal signal in the freshwater green algae may have played a role in land colonization, potentially facilitating adaptive responses to various stresses as ethylene often serves as a plant stress hormone.

Some plants have lost the ability to use ethylene as a signal

On the basis of the variety of ethylene-regulated processes found in plants, it might seem essential for viability. There is evidence, however, that some plant lineages have lost the ability to use ethylene as a signal8. Genome sequencing of eelgrass revealed that the plant lacked genes for ethylene biosynthesis, perception and most of the signalling pathway9. Eelgrass is an aquatic plant found in coastal ecosystems around the northern hemisphere. It seems ironic that a signal with an aquatic origin (i.e. the receptors from cyanobacteria and the signalling system from green algae) has been lost in an ocean-living flowering plant. Ethylene, however, may be under a negative selection force in such submerged environments. A gaseous hormone can be problematic as a signalling molecule under water in a multicellular organism because its diffusion efficiency is lower than in air. As a result, the ethylene concentration can build up and negatively affect growth akin to the terrestrial plants exposed to illuminating gas. Indeed, many non-aquatic plants take advantage of this property of ethylene as a signal indicative of submergence, a stress combated in such ways as the formation of aerenchyma (hollow air cells), elongation to grow out of the submergence zone, or going metabolically quiescent until oxygen levels return to atmospheric levels. Exploiting these ethylene-
There is still much to learn about ethylene and how this simple molecule is integrated into bacterial and plant life. Whether the ancient bacterial ethylene receptors respond mainly to ethylene or other hydrocarbons is still unknown. The precise mechanisms by which ethylene binding to its receptor alters the receptor's activity is not fully understood, nor are some of the downstream regulatory mechanisms. Ethylene signalling and biosynthesis components also interact with other hormone pathways in complex ways that can vary depending on the tissue and plant observed. For such a simple molecule, ethylene's mechanisms are complex and its impact wide. From ancient waters to wounding unripe figs to an urban pollutant affecting plant life to flood-resistant strains of rice, ethylene has had, and continues to have, a profound impact on the biological and human world.

![Image](https://example.com/image.png)

**Figure 3.** The ethylene signal transduction pathway of plants. The pathway incorporates negative and positive regulatory elements of disparate evolutionary origin. In the absence of ethylene (in air), the serine/threonine kinase CTR1 phosphorylates EIN2 to suppress its activity and prevent an ethylene response. Binding of ethylene to the receptors serves to inactivate CTR1, resulting in activation of EIN2 and the induction of the ethylene response. See Shakeel et al. for more details.

dependent submergence responses in rice is facilitating the development of new flood-resistant varieties. In the case of eelgrass, it is still unknown whether another hormone pathway or an alternative input has taken over ethylene's role to regulate its typical downstream responses. The example of eelgrass and a few other aquatic/semi-aquatic plants demonstrates that ethylene is not essential for plant life under all conditions.

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**References**


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**A simple molecule shaping life and culture**

Gaseous Signalling

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**Figure 3.** The ethylene signal transduction pathway of plants. The pathway incorporates negative and positive regulatory elements of disparate evolutionary origin. In the absence of ethylene (in air), the serine/threonine kinase CTR1 phosphorylates EIN2 to suppress its activity and prevent an ethylene response. Binding of ethylene to the receptors serves to inactivate CTR1, resulting in activation of EIN2 and the induction of the ethylene response. See Shakeel et al. for more details.
How bacteria breathe in hydrogen sulfide-rich environments

Hydrogen sulfide (H$_2$S) is now universally recognized as an endogenous signalling molecule playing a central role in human physiology. This gas, although it controls a number of physiological processes at low (submicromolar) concentrations, is toxic at high concentrations as it blocks cell respiration by potently inhibiting cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain. In a recent study on the model micro-organism Escherichia coli, it was shown that the bacterial respiratory oxidase cytochrome $bd$ is resistant to H$_2$S inhibition, thus enabling bacterial O$_2$ respiration and growth in the presence of sulfide. This may be relevant because many microbes are H$_2$S producers and some of them live in sulfide-rich environments, such as the human gut and other natural habitats. The potential impact of this finding in different areas (environment, life evolution and human health) is discussed.

In mammalian cells, H$_2$S is endogenously generated for signalling purposes mainly by three enzymes: cystathionine $\beta$-synthase, cystathionine $\gamma$-lyase, and 3-mercaptopyruvate sulfurtransferase (3-MST)$^4$. Equivalent enzymes are commonly found also in the microbial world, suggesting a fundamental role for H$_2$S from bacteria to humans. As an example, Escherichia coli harbours an orthologue of 3-MST that was shown to contribute to a significant extent to bacterial H$_2$S synthesis$^5$. The regulatory role of H$_2$S in bacterial physiology still needs to be elucidated. Yet, interestingly, in E. coli and other bacteria tested, the ability to produce H$_2$S was shown to enhance antibiotic resistance, thereby providing an adaptive advantage$^5$.

In the human intestinal lumen, H$_2$S is also produced by the resident microflora through bacterial amino acid metabolism, mainly via reduction of thiosulfate and dissimilatory sulfate reduction by sulfate-reducing bacteria (SRB)$^6$. Therefore H$_2$S levels in the gut are high. Direct measurements of the gas in the rat caecum and analysis of human faecal samples proved that the total concentration of sulfide-derived species in the colon is ~1 mM, whereas the concentration of free H$_2$S in the intestinal lumen is ~40–60 $\mu$M. As a result, the gut microbiota is routinely exposed to high levels of H$_2$S, similarly to many other bacteria living in sulfide-rich natural habitats, such as swamps, hot springs, hydrothermal vents and other volcanic areas. As H$_2$S is a potent inhibitor of cellular respiration, the following questions arose: can bacteria accomplish...
Gaseous Signalling

O₂ consumption in sulfide-rich environments? Are there sulfide-insensitive respiratory terminal oxidases in bacteria?

The sulfide-insensitivity of the bacterial terminal oxidase cytochrome bd

Bacteria have a highly flexible metabolism that increases their chance to survive and thrive in a changing environment. Typically, they have branched respiratory chains ending with multiple terminal oxidases. The majority of respiratory oxidases identified to date, including mtCcOX, belong to the superfamily of haem–copper oxidases, having a characteristic bimetallic active site comprising a haem and a copper ion (Cu₃). The bd-type terminal oxidases, widely distributed among prokaryotes, but absent from eukaryotes, are phylogenetically unrelated to the haem–copper oxidases. They do not have a copper co-factor, but have a distinctive haem composition consisting of two haems b and one haem d. Characterized by a remarkable affinity for O₂, the cyanide-resistant bd-type oxidases accomplish the complete reduction of O₂ to H₂O using quinols as reducing substrates. The catalysed reaction is electrogenic, but is not associated with proton pumping, thus leading to proton motive force generation with a lower energy yield compared with haem–copper oxidases. Besides its role in bacterial energy metabolism, cytochrome bd was suggested to play other physiological functions, being implicated in the bacterial response to oxidative and nitrosative stress. This respiratory oxidase was identified in a number of pathogens, such as Mycobacterium tuberculosis, Klebsiella pneumoniae, Shigella flexneri, Listeria monocytogenes, Streptococcus, Brucella and Salmonella, and, in many of them, it was intriguingly shown to promote virulence. In this regard, it is worth noting that, compared with haem–copper oxidases, cytochrome bd is more resistant to NO inhibition, owing to a faster dissociation of this gaseous ligand from haem d in the active site. An interesting finding in the light of the information that NO is produced as part of the host immune response to counteract microbial infections.

Since Cu₃ in the active site of mtCcOX has been proposed to take part in the mechanism of sulfide inhibition, the Cu-free cytochrome bd was suggested to be sulfide-insensitive. The hypothesis was tested in E. coli. This model micro-organism is a ubiquitous member of the human gut microbiota that possesses three respiratory terminal oxidases expressed under different O₂ conditions: the haem–copper cytochrome bo₃ preferentially expressed at higher O₂ concentrations, and two bd-type oxidases (bd-I and bd-II), induced under O₂-limiting conditions, such as those found in the human gut. The ability of these three enzymes to sustain bacterial O₂ consumption and growth in the presence of sulfide was tested. Interestingly, by measuring the O₂ reductase activity of the purified terminal oxidases, both bd oxidases were found to be insensitive to sulfide up to 58 μM, whereas under identical experimental conditions the bo₃ oxidase was potently inhibited by sulfide (Figure 1). In E. coli respiratory mutants expressing only a single terminal oxidase, both O₂ consumption and aerobic growth were severely impaired by sulfide when respiration was sustained by the bo₃ oxidase alone, but completely unaffected by less than or equal to 200 μM sulfide when either bd enzyme acted as the only terminal oxidase. Consistently, in the wild-type parental strain, H₂S affected cell growth and respiration only in the early phase of the culture, when O₂ availability was sufficiently high to favour the expression of the bo₃ oxidase, but it caused no effect in the late phase of the culture, when O₂ limitation is expected to stimulate the expression of the bd oxidases. The results demonstrate that, in contrast with the haem–copper bo₃ enzyme,
E. coli bd oxidases can enable respiration and growth in the presence of high levels of sulfide (Figure 2). In this light, it will be important to assess whether the reported H$_2$S insensitivity represents a common feature among bd-type oxidases.

**Life in sulfide-rich environments and evolution**

Thanks to their tremendous metabolic plasticity, prokaryotes are able to colonize very diverse ecological niches on our planet, including sulfide-rich extreme environments that are prohibitive for most living organisms. Naturally produced through geochemical or biological processes, H$_2$S can be found in large amounts in several habitats, such as swamps, hot springs, deep-sea hydrothermal vents and other volcanic areas (Figure 3). In these habitats, microbes have to cope with H$_2$S toxicity and it is therefore conceivable that the expression of bd-type terminal oxidases represents an adaptive advantage, enabling bacterial O$_2$ consumption for bioenergetic or detoxification purposes, despite the presence of H$_2$S. Accordingly, bd type oxidases have been identified in several prokaryotes adapted to these unique environments. For example, in the sulfide-rich waters of hydrothermal vents and hot springs, this enzyme has been found in aerobic bacteria, such as *Salinisphaera hydrothermalis*, *Thiobacillus prosperus* and *Halothiobacillus neapolitanus*, but also in anaerobic bacteria, such as the SRB *Desulfococcus multivorans*, in which cytochrome bd possibly acts as a detoxifier of environmental O$_2$ to protect from oxidative damage.

Interestingly, H$_2$S-rich extreme environments like those found near the deep-sea vents or other volcanic areas are thought to resemble the primordial Earth milieu where life originated. Gases have always played a key role in life evolution on Earth. Before the onset of oxygenic photosynthetic activity of cyanobacteria, due to active volcanism, H$_2$S is thought to have been present at high concentrations in the O$_2$-poor atmosphere of Earth and to be used as an energy source$^{11}$. H$_2$S metabolism probably played a major role in those primordial times, as it still does in many living beings nowadays. When O$_2$ levels started to rise due to the oxygenic photosynthesis, H$_2$S levels decreased dramatically. These notable changes in O$_2$ and H$_2$S availability probably resulted in a strong selective pressure, requiring profound adaptive mechanisms in living beings. In this scenario, it is tempting to speculate that bd-type oxidases with their ability to metabolize O$_2$ in the presence of high H$_2$S levels have played a major role throughout evolution, representing an adaptive advantage for those bacteria living in habitats with coexistent O$_2$ and H$_2$S.

**The human gut ecosystem**

Microbes are an integral part of the human gastrointestinal system. They control many aspects of human physiology and play vital functions such as facilitating digestion, supplying vitamins and providing resistance to invading pathogens. An imbalance in the number or composition of the gut microbial communities can therefore trigger pathological...
consequences. As reported above, H$_2$S is present at high concentration in the intestinal lumen. This gas, derived mainly from bacterial amino acid metabolism and SBR-mediated sulfate reduction, was suggested to play a role in shaping the human gut microbiota, based on bacterial tolerance to sulfide. It is becoming evident that bacteria-derived H$_2$S has a broad impact on human health and disease, and controls several physiological functions, well beyond the intestinal ones. In studies on healthy rats, inhibition of H$_2$S synthesis resulted in inflammation of the small intestine and colon, whereas in a study on germ-free mice the host microbiota inflammation of the small intestine and colon, whereas beneficial versus toxic effects of H$_2$S require further clarification.

The $bd$-type oxidases have been identified in numerous enterobacteria. Expression of these oxidases is probably stimulated in the microaerobic conditions found in the human colon. Not only commensals, but also several pathogenic enterobacteria, such as S. flexneri, Enterococcus faecalis, E. coli or Salmonella enterica, harbour cytochrome $bd$, presumably sustaining growth in the H$_2$S-rich and O$_2$-poor environment of the gut. Several bacterial pathogens are known to make use of cytochrome $bd$ to colonize O$_2$-poor niches of the host and, in some micro-organisms, such as M. tuberculosis, this terminal oxidase was shown to enhance antibiotic resistance. Cytochrome $bd$ has therefore recently emerged as an attractive pharmacological target for the development of new antimicrobial drugs, also because the enzyme is absent from humans. Only a few cytochrome $bd$ inhibitors are known to date, with aurachin D being the only selective example. The identification of novel selective inhibitors of cytochrome $bd$ would certainly represent an important step towards the development of new antibacterials, a research field which has been recently boosted by the resolution of the first crystallographic structure of a $bd$-type oxidase, the enzyme from Geobacillus thermodenitrificans.

In conclusion, the newly discovered insensitivity of E. coli cytochrome $bd$ to H$_2$S has opened new perspectives and will hopefully prompt future studies on these bacterial enzymes with interesting implications in different research areas.

**References**


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Hydrogen sulfide: stench from the past as a mediator of the future

The past decade has witnessed the discovery of hydrogen sulfide (H2S) as a new signalling molecule. Its ability to act as a neurotransmitter, regulator of blood pressure, immunomodulator or anti-apoptotic agent, together with its great pharmacological potential, is now well established. Notwithstanding the growing body of evidence showing the biological roles of H2S, the gap between these roles and the actual mechanism(s) behind these processes is getting larger. We propose a way that protein cysteine residues can be modified to form protein persulfides (P-SSH) and explain how this process is controlled in a physiologically relevant fashion. This article provides an overview of H2S signalling in the human body with particular emphasis on the latest discoveries regarding the mechanisms of protein persulfidation and de-persulfidation, as well as about the biological reactivity of persulfides and their role in health and disease.

Introducing hydrogen sulfide

In order to maintain life, nature uses a limited number of chemical reactions. One of these is sulfur-based chemistry, mainly exploited for the control of intracellular redox homeostasis and redox-based signalling. Hydrogen sulfide has long been known only as a toxic gas that gives the unpleasant smell to rotten eggs. However, the discovery that all our cells produce it in a controllable manner and that it regulates a plethora of physiological functions, changed our view on its role in the human body. This is perhaps not so surprising, as the early life forms in the oceans flourished in an H2S-rich atmosphere. In fact, analysis of recently discovered preserved samples from Stanley Miller’s experiments performed with H2S in 1958 revealed 23 types of amino acids, some of which are the building blocks of proteins and necessary for life, including the sulfur-containing amino acid methionine. This was much more than Miller observed in his initial experiments.

The emergence of H2S as a signalling molecule starts with the seminal discovery by Abe and Kimura that H2S acts as neurotransmitter, which can stimulate long-term potentiation. Over the past decade, H2S was recognized as the third gaseous molecule produced within the body alongside nitric oxide and carbon monoxide.

Mammalian cells produce H2S via enzymatic activity of cystathionine-γ-lyase (CSE) and cystathionine-β-synthase (CBS), which are pyridoxal 5'-phosphate-dependent enzymes and key players of the evolutionary conserved transsulfuration pathway (TSP) (Figure 1). Beside this synthetic pathway, H2S is also produced by the activity of the cysteine aminotransferase (CAT)/3-mercaptopiruvate sulfurtransferase (MST) couple. As substrates for their activity these enzymes use either cysteine and/or homocysteine. CSE and CBS are localized in the cytoplasm in different tissues including the cardiovascular system, nervous system and gastrointestinal system; while MST is expressed in all cell types both in the cytoplasm and mitochondria.

Intracellular concentrations of H2S are still the subject of debate, but the discovery of new detection methods limits these values to submicromolar levels.
Conversely, the flux of H$_2$S production is huge, almost as high as that of glutathione (which reaches millimolar concentrations), suggesting that the removal of H$_2$S is an efficient and tightly regulated process. The sulfide oxidation pathway begins with sulfide:quinone reductase (SQR) and also includes a sulfur dioxygenase, rhodanese and sulfite oxidase (Figure 1). By consuming H$_2$S and its persulfide products SQR and sulfur dioxygenase are important switch-off regulators of sulfide signalling. In the first step, SQR catalyses the oxidation of H$_2$S to sulfane sulfur, which remains covalently attached to the enzyme. In the second step this sulfane sulfur could be transferred to sulfite, to form thiosulfate, or to glutathione making glutathione persulfide, which is further consumed by persulfide dioxygenase (ETHE1) to make sulfite.

Aided by pharmacological modulation and an understanding of the biosynthetic pathway, many physiological functions have been shown to be exclusively or partly regulated by H$_2$S. Knocking out CSE leads to high blood pressure in mice, suggesting that H$_2$S is an endogenous regulator of blood pressure\(^6\). H$_2$S also showed surprising effects on mice, putting them in a suspended animation-like state (hibernation-like state), although mice are not hibernating animals\(^6\). Excitingly, H$_2$S has tremendous pharmacological potential in preventing ischaemia-reperfusion injury in the models of myocardial infarction or stroke\(^6\); several drug-candidate ‘donors of H$_2$S’ have been developed and entered clinical trial with hope of their eventual use in disease treatment\(^8\).

Hydrogen sulfide does not react readily with oxygen, however, solutions of H$_2$S undergo oxidation, just like the solutions of other thiols (such as cysteine and glutathione). Oxidation products of H$_2$S in solution are polysulfides, sulfites, thiosulfates and eventually elemental sulfur.

In biological systems, H$_2$S reactivity can be divided into three groups of reactions: (i) cross-talk with scavenging of reactive oxygen (ROS) and reactive nitrogen species (RNS), (ii) binding to and/or subsequent redox reactions with metal centres and (iii) reaction with proteins, herein called persulfidation (alternatively S-sulfhydration) (Figure 2a). Cross-talk with nitric oxide and its siblings is particularly interesting and could explain some of the vasodilatory and cardio-protective effects of H$_2$S\(^11\). Several biologically active molecules have been shown to be products of this cross-talk, such as thionitrous acid (HSNO)\(^11\) and nitroxyl (HNO)\(^10,12\). Binding of H$_2$S to metal centres in proteins also plays an important role.

For example, nature uses it in the mollusc, *Luciana pectinata*, where H$_2$S binds to haemoglobin I and is
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then transported to the symbiotic chemoautotrophic bacteria living in their gills13.

Hydrogen sulfide signals through protein persulfidation

Probably the most important form of H2S signalling is the modification of protein function by a process called S-sulfhydration, although the more correct term should be persulfidation. Protein persulfides are a novel addition to the list of oxidative post-translational modifications of cysteine (oxPTM), such as S-nitrosation, S-sulfenylation and S-glutathionylation (Figure 2b). Modification of proteins by H2S could explain the plethora of effects that H2S exhibits and several proteins have been identified to be controlled in this way. One such protein is parkin, considered to be an important player in Parkinson’s disease (PD). Parkin is an E3 ubiquitin ligase that catalyses the ubiquitylation of diverse substrates. Mutations in parkin, which lead to the loss of its activity, are one of the causes of PD. Parkin has reactive cysteine residues, which can be subjected to oxidative post-translational modifications. Recently, Solomon Snyder and colleagues demonstrated that several cysteine residues could undergo S-sulfhydration14, leading to an increase in parkin activity which could salvage the neurons from cell death by removing damaged proteins (Figure 2c). Development of H2S donors therefore opens up a possibility of their use in early treatment of PD. However, this field of research is still in its infancy and faces challenges such as the proper choice of detection method, understanding of the mechanism(s) by which persulfidation takes place and the actual impact it has on cellular functions.

To be able to selectively label protein persulfides and study their reactivity we recently developed a tag-switch assay (Figure 3a15,16). The assay is based on a chemical premise that an aromatic thiol blocking reagent would lead to mixed disulfides with protein persulfides making them more reactive towards a proper nucleophile carrying a specific tag, such as biotin. This method allowed us to characterize several protein targets that could be persulfidated and also to localize protein persulfidation into two particular organelles: mitochondria and the endoplasmic reticulum15,16.

How to make a protein persulfide

Although there is growing interest in the persulfidation of proteins, only a few studies have actually addressed the issue of the mechanism(s) behind protein persulfidation. The original misconception was that thiolate on the protein can react directly with H2S
to form protein persulfide. However, that reaction is impossible due to the thermodynamic constraints.

So how are the proteins modified by H₂S? Getting the answer(s) to this question is of the utmost importance for our understanding of H₂S signalling and also for interpreting the vast amount of data accumulated to date. Several mechanisms could be envisioned, but the most probable ones are the reaction of H₂S with disulfides or sulfenic acids.

The reaction towards disulfides could represent a significant consumption pathway for H₂S, mainly in extracellular milieu and plasma, where the diversity and high amounts of disulfides present make them a likely target. However, we recently determined the pH-independent rate constants obtained for HS⁻ reactions with the symmetric disulfides and protein disulfides and showed that they are about one order of magnitude lower than those reported for alkyl thiolates of comparable pKa17. We also estimated the reactivity of H₂S with protein sulfenic acids 17. In contrast to the trend observed for disulfides, the pH-independent rate constant of the HS⁻ reaction with HSA-SOH is higher than those reported for other biological thiols. We confirmed the latter to be an important way to generate protein persulfides as the treatment of the cells with H₂O₂, in order to increase intracellular levels of sulfenic acids, led to an increase of protein persulfidation. This could be completely prevented by inhibiting endogenous H₂S production (Figure 3b)17.

**A protective role for protein persulfidation?**

The pKa of persulfides is lower than that of corresponding thiols, suggesting that at physiological conditions, the majority of persulfide would be in the deprotonated form (R-S-S⁻), making the persulfide “super” nucleophilic. This should dramatically increase the reactivity of persulfides when compared with the corresponding thiols. Indeed, when we assessed the rate constants of protein persulfides with peroxyxinitrite (a powerful oxidant formed in the diffusion-controlled reaction of superoxide with nitric oxide) we found that persulfides react one order of magnitude faster than corresponding thiols17.

This led us to propose that one of the possible roles of protein persulfidation could be the protection of a particular protein from irreversible damage induced by ROS and/or RNS (Figure 3c)17. Thiol oxidation, which initially starts with the formation of sulfenic acids (still reversible modification), could proceed further with the formation of irreversible sulfonic acids. H₂S could react with sulfenic acid preventing this oxidation. In addition, persulfidated protein will react faster with ROS/RNS and form an adduct that could be cleaved by the action of certain enzymes restoring free thiol.

To exert a regulatory function similar to that of phosphorylation/dephosphorylation or S-nitrosation/denitrosation, S-persulfidation levels must be enzymatically regulated18. Intracellular protein disulfide and S-glutathionylation levels are controlled by the thioredoxin (Trx) system19. The enzymatic system, consisting of Trx, thioredoxin reductase (TrxR) and NADPH, represents the main disulfide reductase system in cells. To assess the role of Trx system in de-persulfidation we used low molecular weight persulfides as well as protein persulfide models16. To our surprise, Trx cleaved persulfides one order of magnitude more efficiently than it reduced corresponding disulfides. Inhibition of the Trx system led to an increase of intracellular persulfides confirming that this process occurs in the cells as well (Figure 4). HIV-1 patients with high viral load are known to have increased levels of circulatory Trx19. Significantly lower total sulfane sulfur levels were detected in patients with a high viral load (and therefore high circulatory Trx levels) compared with antiretroviral therapy (ART)-treated patients, confirming the role of Trx as a putative de-persulfidase. This provided the first in vivo evidence that Trx acts as a de-persulfidase16.

**Future directions**

Signalling by H₂S is emerging as an important way to control biological functions. Therefore, the development of new H₂S-donating drugs has huge pharmacological potential. To achieve its signalling role H₂S causes modification of protein cysteine...
residues, leading to protein persulfidation. The thioredoxin system represents the first de-persulfidase system in the cells, but other enzymes could have a similar role. Further development of assays, which would allow easy labelling of protein persulfides and their subsequent proteomic analysis would accelerate the progression of the field. In addition, it would help in understanding the spatio-temporal correlation of protein persulfidation with other oxPTM of cysteine in both health and disease. For example, we have recently observed a dramatic decrease of protein persulfidation in spinocerebellar ataxia 3 (SCA 3)20. This is a progressive neurodegenerative disease and could be reversed by overexpression of CSE, an intervention that also protects against tissue loss in the Drosophila SCA3 model20. Future proteomic studies will reveal which proteins are persulfidated in this and many other diseases shown to be linked to the downregulation or overproduction of H2S.

**Glossary**

**Apoptosis** – genetically regulated self-destruction of the cells, also called programmed cell death.

**Protein persulfidation** – post-translational modification of the cysteine residue (R-SH) into R-SSH.

**De-persulfidation** – enzymatic or non-enzymatic process of sulfur removal from protein persulfides (R-SSH) to restore cysteine residues (R-SH).

**Intracellular redox homeostasis** – enzymatic and non-enzymatic systems involved in maintaining reduced environment in the cells.

**Redox-based signalling** – set of intracellular processes, which occur as a response to a disturbance of intracellular redox homeostasis; for example, when cells are exposed to oxidants.

**Long-term potentiation** – persistent strengthening of synapses based on recent patterns of activity; i.e. memory storage.

**Sulfide oxidation pathway** – metabolic pathway for oxidation of sulfide to thiosulfate or sulfate.

**pK_a** – defined as −log_{10} Ka where Ka is acid dissociation constant, a quantitative measure of strength of an acid in solution.

**Vasodilation** – relaxation of blood vessels.

**Ubiquitylation** – addition of ubiquitin to a substrate protein. This process can affect targeted protein in many ways; the best described role for this process is to label targeted protein for degradation.

**Nucleophile** – chemical species that provides a pair of electrons to form a new covalent bond; nucleophilicity refers to a substance’s nucleophilic character.
References


Nitric oxide (NO) is a relatively simple molecule comprising only two atoms. Understanding how this free radical controls an array of complex biological functions provides the platform for much of the research in NO biochemistry and biology. Here, we discuss an updated perspective on how this gas is formed in the body involving a fascinating interplay between the diet, bacteria residing on the tongue, and redox reactions that are regulated by pH and local oxygen tensions. We highlight this as an area primed for novel microbe-targeted therapeutics for controlling NO production and affecting human health and disease.

The functions ascribed to nitric oxide (NO) in humans are many and diverse, including but not limited to regulating blood flow, neuronal signal transduction, innate immunity, inflammation, coagulation, cell survival and death. Like any biological signalling pathway there has to be controlled formation, selectivity in the reactions that follow and activation of a signalling cascade culminating in a cellular response. Finally, there should be mechanisms that counter this activation to allow the pathway to be slowed or turned off. NO signalling checks all these boxes with an extensive scientific literature detailing mechanisms for each of these key aspects. In the standard model of NO signalling, NO is produced by one of three nitric oxide synthase (NOS) enzymes, which catalyse the conversion of L-arginine to NO to amplify local signalling pathways. In this article, we focus on a relatively new or updated perspective on how NO formation occurs in mammals that instead, involves bacteria residing on the tongue. According to this model, these bacteria reduce internally and externally produced nitrate to nitrite, a reaction that bacteria catalyse to support bacterial metabolism. The nitrite that is produced is further metabolized by an array of mammalian (host) proteins to produce NO and mediate NO signalling in all major organs. In this paradigm, NO bioavailability is controlled not by a single host enzyme, but also by symbiotic interactions between the environment, microbes in the mouth and externally produced nitrate to nitrite, a reaction thought of as oxidation products of NO that are inert, and often used as markers for NO formation. That said, there similar reactions that reduce nitrate and nitrite to NO? In mammals, nitrate and nitrite are commonly thought of as oxidation products of NO that are inert, and often used as markers for NO formation. That said, under extreme conditions of low oxygen, and/or very low pH (e.g. in the stomach), nitrite can be converted to NO by one electron to NO and other NO-containing intermediates. The concept that nitrate and nitrite may be sources of NO-signalling equivalents in mammalian systems was largely ignored for a long time because there were no known enzymatic systems equivalent to a plant or bacterial nitrate or nitrite reductase. However, around the time NOS enzymes were discovered, it was also shown that facultative anaerobic bacteria residing on the tongue express functional nitrate-reductase enzymes, and can reduce nitrate to nitrite in saliva. In parallel with these observations were intriguing studies showing that nitrate from fruit and vegetables in the diet was absorbed from the upper gastrointestinal system into the blood and then concentrated into the saliva.
to reach millimolar concentrations. The fact that this is a concerted process suggests biological importance and the idea emerged of an entero-salivary nitrate-reduction cycle that provides a controlled supply of nitrate to oral microbes, which use this as a substrate to generate reducing equivalents for respiration and other microbial processes. While beneficial to the bacteria, the advantage to the mammalian host was less clear and limited to preventing oral infections and dental caries. Systemic effects were overlooked, largely because the product was nitrite, and as indicated above, at the time of these findings, nitrite was not thought to be particularly important for NO bioavailability except under extreme biological conditions.

**Nitrite-reduction mechanisms – the missing piece of the nitrate-reduction puzzle**

Fast forward to today, and we now know that nitrite is not an inert anion in mammals, but in fact tightly controlled and a key player in NO homeostasis. Nitrite may be reduced by a one-electron process to produce NO and other NO-containing products including S-nitrosothiols and nitroproteins. Importantly, mechanisms controlling this biochemistry are operational at physiological nitrite concentrations. The new insight that provided the ‘missing piece’ of the puzzle was the understanding that metallo- and haem proteins (e.g. haemoglobin) are able to facilitate, or increase the rate of nitrite reduction via mechanisms that still involve hypoxia (low oxygen tension) and low pH. The general model is that lowering oxygen tension alters the reactivity of the metal centre with nitrite. Several candidate metalloproteins have been discussed and amongst the first and perhaps most studied is haemoglobin, which also provides a useful model to illustrate the mechanism and function.

Specifically, deoxyferrous haem reduces nitrite to NO. In other words, nitrite reduction to NO only occurs when haemoglobin is deoxygenated. This reaction is also faster at lower pH thereby limiting or compartmentalizing this pathway to metabolically active, high oxygen-consuming and lactate-producing tissues. This pathway has been discussed as a mechanism for how hypoxia increases local blood flow via NO formation, and couples this with oxygen delivery. The comparisons with NOS-dependent NO formation are interesting. Both are regulated by oxygen, but with NOS, oxygen is a substrate, with nitrite-reduction mechanisms, however, oxygen could be considered an inhibitor; for example, oxygen will promote oxyhaemoglobin formation, and oxyhaemoglobin oxidizes and thus depletes nitrite. Several candidate metalloproteins have been described to possess properties similar to haemoglobin that couple oxygen sensing to nitrite reduction, with specific biochemical and functional aspects differing. For example, haemoglobin-dependent nitrite reduction is optimal at ~27 mmHg oxygen and controls blood flow and coagulation, whereas with the related protein myoglobin, oxygen levels have to drop to below ~10 mmHg before nitrite reduction occurs with the function being regulation of mitochondrial respiration and reactive species formation in cardiac muscle cells. This comparison also illustrates how compartmentalized NO formation can occur in this paradigm. So while NOSs are the ‘professional’ NO-generating proteins, whose primary function is to catalyse NO formation, proteins that mediate nitrite reduction could be considered as the ‘back-ups’ that ensure NO signalling is maintained when oxygen levels drop and NOS pathways become inactivated. In this model, metalloproteins that possess nitrite-reduction reactivity are protein catalysts that speed-up nitrite reduction and provide regulation of this process.

**Role of the entero-salivary system: why mouthwash could be bad for you**

Returning to the entero-salivary system discussed earlier; nitrate is concentrated into the saliva where it is reduced to nitrite by tongue bacterial nitrate reductases. Nitrite is then swallowed. In the stomach, some of the
nitrite undergoes acidic disproportionation to form NO and nitroso-species that have local effects in controlling gastric-mucosal functions, but some is also absorbed into the blood where it supports NO signalling in all major organs via reactions with an array of metalloproteins. The functionality of this model has been demonstrated in many physiological and therapeutic contexts especially where low oxygen (i.e. tissue ischaemia) and low pH are biological features. The linkage between nitrate, nitrite and NO may also partly explain the health benefits of consuming diets rich in fruits and vegetables, the latter providing more than 80% of the nitrate exposure to humans. Conversely, lack of nitrate-derived NO may contribute to increased susceptibility to cardiovascular disease in communities with a low intake of fruits and vegetables. The preponderance of evidence for this cycle in humans comes from experiments showing that basal blood pressure, platelet coagulation and exercise performance are modulated by the presence of nitrate in the diet and oral nitrate-reductase activity. The basal effects referred to were gleaned by showing that oral microbe depletion using antiseptic mouthwash, increases basal blood pressure in healthy individuals. This effect is worth pointing out because it demonstrates the potential for the entero-salivary system to play a key role in NO homeostasis in the presence of a functioning NOS-dependent NO-signalling system. There are several excellent reviews and articles which discuss nitrate reductase enzymes that catalyse denitrification i.e. reduce nitrite to NO, N2O and N2. Furthermore, some bacteria may reduce nitrite to ammonia via dissimilatory nitrate-reduction pathways. Conceptually, this sets up an interesting balance where the fate of nitrite generated in the oral cavity is dependent on competing biochemical and physical processes related to saliva volume, swallowing of saliva, nitrite reduction to N2 or ammonia. Can this balance be modulated? Does it change in different pathophysiologic scenarios related to oral health and diseases associated with altered NO bioavailability? Can it be targeted therapeutically to improve NO bioavailability? Moreover, little is known about how nitrate is concentrated into the saliva. These are all questions for which we have little current insight. That said, emerging evidence and ideas suggest that indeed the oral nitrate-reducing microbiota may be regulated.

As proposed recently, diversion from nitrite formation to ammonia formation may exacerbate chronic kidney and heart disease and link oral microbial dysbiosis, loss of NO and chronic inflammation. Moreover, oral microbes are spatially proximal to exposure to reactive and toxic substances present in cigarette smoke and other inhaled environmental pollutants. Such stresses are strongly associated with a host of cardiopulmonary inflammatory diseases characterized by loss of NO bioavailability. Whether inhaled toxicants negatively affect the oral nitrate-reducing microbiome has significant implications for environmental toxicology mechanisms and requires testing. Finally, emerging studies show that oral bactericidal solutions, like chlorohexidine, or the presence of nitrate in the diet shift the oral microbial community. Assuming nitrate reduction to nitrite and then to NO is concomitantly increased or decreased, such data reflect the dynamic nature of this pathway and suggest that indeed, it can be modulated to affect systemic NO bioavailability.

Bacteria expressing nitrate reductase enzymes (NAR) are pivotal for the entero-salivary system. However, our current knowledge regarding the specific bacteria expressing this activity, which classes of NAR are involved, how they are regulated and whether this changes in any pathophysiologic setting remains poor. This is likely to alter with the application of high throughput and sensitive analyses using 16S RNA sequencing and metagenomics, together with the ever-increasing appreciation of the importance of our microbiome in health and disease. Bacterial species that have been identified as containing genes for nitrate reductase include Staphylococcus sp., (S. aureus, S. epidermidis, S. bovis), Streptococcus sp., (S. mitis), Corynebacterium sp., Rothia sp., Actinomyces sp., Prevotella sp., Fusobacterium sp. and Veillonella sp., with the latter considered to be the largest contributor to nitrate reduction. Knowledge is limited regarding their regulation however.

Most bacteria in the oral cavity are facultative anaerobes living in the crypts of the tongue, but why they predominantly occupy the posterior tongue remains unclear. Many of the nitrate-reductase expressing bacteria also express nitrite reductase enzymes that catalyse denitrification i.e. reduce nitrite to NO, N2O and N2. Furthermore, some bacteria may reduce nitrite to ammonia via dissimilatory nitrate-reduction pathways. Conceptually, this sets up an interesting balance where the fate of nitrite generated in the oral cavity is dependent on competing biochemical and physical processes related to saliva volume, swallowing of saliva, nitrite reduction to N2 or ammonia. Can this balance be modulated? Does it change in different pathophysiologic scenarios related to oral health and diseases associated with altered NO bioavailability? Can it be targeted therapeutically to improve NO bioavailability? Moreover, little is known about how nitrate is concentrated into the saliva. These are all questions for which we have little current insight. That said, emerging evidence and ideas suggest that indeed the oral nitrate-reducing microbiota may be regulated.

Prospects

The entero-salivary nitrate-reduction pathway and NOS-dependent NO-formation processes operate, and are regulated by, distinct and complementary mechanisms. Evidence demonstrating that the entero-salivary system is competent to mediate NO-dependent processes underscores the potential for controlling this pathway in the management of diseases associated with decreased NO bioavailability. Changes in lifestyle such as improved oral health and increased dietary nitrate may be relatively simple approaches to treat common but difficult diseases.
including hypertension, atherosclerosis and diabetes. This understanding also raises intriguing possibilities regarding the use of oral probiotics and the notion of being able to repopulate the ‘good’ bacteria or restoring natural conditions from a microbial imbalance. That said, much still needs to be learned about this fascinating interplay of the diet, bacteria in the oral cavity and nitrite-reduction pathways.

Glossary:

**Entero-salivary Nitrate Reduction**: the system of nitrate reduction to nitrite catalysed by nitrate reductases present in bacteria residing on the tongue. Nitrite is swallowed and absorbed into the blood stream, where some is reduced to nitric oxide, and some oxidized to nitrate, which accumulates in the saliva and again provides substrate for oral nitrate reductases.

**Microbial dysbiosis**: development of an imbalance in the microbiota compared to the normal condition.

**Nitric oxide bioavailability**: the ability for NO to stimulate a signal transduction pathway depends on the concentration, or amount of NO. The amount of NO is determined by how much is formed versus how much NO is consumed by other pathways that do not lead to physiologic signalling. The term NO bioavailability reflects this balance and the amount of NO available to stimulate physiologic soluble guanylyl cyclase signalling.

**Further Reading**

- Qin, L., Liu, X., Sun, Q., et al., (2012) (SLC17A5) functions as a nitrate transporter (NO) bioavailability by oral nitrate-reducing bacteria and its impact on acute and chronic lung injury models. Email: kahmed@uabmc.edu
- Rakesh Patel received his PhD from the University of Essex, UK in 1996. He moved to pursue postdoctoral studies at UAB, where he is currently a Professor in the Department of Pathology and Director of the Center for Free Radical Biology. His research interests have centred on understanding the role of nitric oxide and other oxidative/nitrosative intermediates in modulating acute and chronic inflammatory diseases. Email: rakeshpatel@uabmc.edu
- Alexandera L. Nichols is a senior undergraduate majoring in Molecular Biology at the University of Alabama at Birmingham. She belongs to the Science and Technology Honors College at UAB and has been working in the Department of Pathology in Dr Rakesh Patel’s lab on the development of a method for measuring and characterizing oral nitrate reductase activity. Email: aln835@uab.edu
- Khandaker Ahtesham Ahmed received his PhD from Kumamoto University, Japan in 2011. He is currently pursuing his postdoctoral training at the Department of Pathology, UAB. His research interest focuses on the regulation of nitric oxide (NO) bioavailability by oral nitrate-reducing bacteria and its impact on acute and chronic lung injury models.

**References**

Nitric oxide (NO) is a colourless free radical gas, which has diverse and potentially opposing biological effects, depending on the local environment. The identification of NO and discovery of the mechanism of signalling are interesting tales on their own, leading to the Nobel Prize in Physiology or Medicine (1998) awarded to Murad, Furchgott and Ignarro. This article focuses on two very different – and important – consequences of the effects of NO on blood vessels: erectile dysfunction and pre-eclampsia.

The commonality between a male health issue and a potentially devastating pregnancy complication

It may seem strange to begin a discussion about two medical conditions that affect entirely different populations – the first impacting a surprisingly large proportion of men with increasing age, and the second impacting between 5–10% of women during pregnancy. However, there are two important similarities between these two conditions: the underlying pathology is due to dysfunction of the vascular endothelium (the single cell layer that lines blood vessels and they are considered harbingers of the development of future cardiovascular disease. This is particularly critical from a prevention and screening perspective, considering that women who develop pre-eclampsia are in their prime reproductive years, often decades before the cardioprotective effect of oestrogen is lost during menopause. The recognition that these seemingly unrelated events can significantly increase risk for future cardiovascular disease is changing clinical practice and improving screening, prevention and therapeutic guidelines. Now when a man seeks medical counsel for erectile dysfunction, he often leaves with a prudent cardiology workup – a practice change that saves lives. Similarly, this is also occurring with a much younger population of women, who are experiencing or have experienced pre-eclampsia, a more harmful condition.

So what exactly is pre-eclampsia? Why is it important?

Most people are familiar with the term pre-eclampsia, or the older terminology, toxaemia of pregnancy, or know of someone who had it. On occasion, people have told me they heard of pre-eclampsia through entertainment – it was featured on a popular television show or impacted a celebrity. Pre-eclampsia is a serious condition that affects pregnant women, almost exclusive of other animal species. High blood pressure (new onset or worsening) developing after 20 weeks’ pregnancy, accompanied by significantly increased levels of protein in the urine, (proteinuria) are the most common clinical signs. The exact diagnostic criteria for pre-eclampsia have changed over time and depend on which national or international guidelines are followed. This diagnostic difficulty results from signs of pre-eclampsia overlapping with pre-existing conditions or other pregnancy disorders, necessitating a highly sensitive and specific biomarker. The task of identifying a biomarker for accurate diagnosis, or better, prediction, has been hindered by the unclear aetiology of pre-eclampsia. There are many theories to explain the development of pre-eclampsia, but no absolute confirmation of direct cause. The generally
accepted theory is that early in gestation (1st trimester), growth of new blood vessels both from the maternal side and/or presumptive placenta is insufficient. As the placenta matures and requires increasing amounts of nutrients for the fetus, the reduced blood supply from the impaired vasculature results in a stressed placenta and fetus. When an organ is deprived of appropriate blood supply, the organ itself releases factors into the circulation to attempt to mitigate cellular damage from decreased oxygen—these factors also act locally on the vasculature to modify blood flow. The placenta is a unique organ, and is capable of secreting a wider variety of factors to ensure appropriate blood flow towards the fetus. One of the factors released in excessive amounts by the stressed placenta prior to the clinical development of pre-eclampsia is soluble fms-like tyrosine kinase-1 (sFLT1, which is a truncated, non-signalling version of vascular endothelial growth factor receptor-1). sFLT1 is an anti-angiogenic protein (i.e. it inhibits the development of blood vessels) that antagonizes a critical angiogenic protein, vascular endothelial growth factor (VEGF). Both sFLT1 and VEGF are normally secreted during pregnancy—VEGF is required in high amounts for fetal and placental development, while sFLT1 (in low amounts) binds excess VEGF, preventing any negative effects in the mother. In pre-eclampsia, sFLT1 release is overwhelming and prevents VEGF from binding and signalling normally. The excess sFLT1 is largely accepted to be a cause of the signs and symptoms of pre-eclampsia. Despite this knowledge, the only treatment for pre-eclampsia is delivery of the placenta and fetus, which is often pre-term. Even with careful monitoring, pre-eclampsia can become more severe, with complications including kidney and liver damage, stroke, seizures and death. Pre-term delivery is often necessary for both maternal and fetal health.

Why is pre-eclampsia an important condition? This is a fair question—as it only impacts upon a specific section of women of childbearing age. Unfortunately, many hold this point of view, which is reflected in public interest and research funding. An important counterpoint is every one of us was born, and potentially already impacted by in utero effects of a pregnancy complication. Mounting research demonstrates that children born during a pre-eclamptic pregnancy are similarly at elevated risk of developing type 2 diabetes and cardiovascular disease. Any improvement to maternal health not only alleviates immediate maternal and fetal harm, but also has the potential to decrease lifetime cardiovascular risk for mothers and her children.

To understand the pathology of pregnancy, we must understand normal physiology

When we generally consider development, we think of the embryo or fetus; while the role of the mother is known to be crucial, the adaptations that provide required support are often overlooked. Even if you’ve experienced pregnancy, some of these changes are seen as unpleasant, uncomfortable and often, bewildering. Indeed, some of
the most fascinating physiological adaptations occur to the female during pregnancy – and these adaptations are fairly well conserved between placental mammals. This has allowed a great deal of information to be discovered from animal models, keeping in mind key species-specific differences. The cardiovascular changes that occur over pregnancy include an increase in cardiac output, heart rate and blood volume. To balance this, blood pressure and vascular resistance decline – these responses are key in maintaining a healthy pregnancy and also make pregnant physiology so intriguing. It makes logical sense that with a growing fetus, which possesses extraordinary metabolic demand, the maternal heart would compensate by working harder and increasing the total amount of blood circulating. But why a decline in blood pressure? It is important to note that in a normal person, increasing cardiac output and heart rate generally results in increasing blood pressure and an increase in vascular resistance – contributors to endothelial dysfunction, chronic hypertension and atherosclerosis. During pregnancy, blood vessels relax and dilate, all while new vessels are being created within the endometrium. The uterine arteries lengthen, expand and dilate to permit higher blood flow. The increased vascular surface area reduces the overall resistance to blood flow, but importantly, soluble factors are produced that also act to dilate vessels and maintain lower blood pressure. None of these factors are pregnancy specific, however, they play multiple roles in physiology, including embryo and fetal development: prostaglandins, VEGF, bradykinin, the renin-angiotensin-aldosterone system (RAS) and nitric oxide (NO).

The role of nitric oxide in vascular disease

The regulation, mechanism and functions of NO are multiple, extensively explored and critical to many biological systems. The physiological actions of NO on blood vessels include vasodilation via vascular smooth muscle relaxation, local modification of blood flow, inhibition of platelet aggregation and reducing immune cell adhesion. All of these actions are crucial for maintaining healthy blood vessels and perturbations in NO bioavailability or signalling are found in several pathological conditions, including: hypertension, diabetes, atherosclerosis, stroke and coronary artery disease. NO is produced constitutively at relatively low levels in many cell types by endothelial or neuronal nitric oxide synthase (eNOS and nNOS). A third form of NOS is inducible (iNOS), typically in response to immune stimuli. NOS utilizes oxygen and L-arginine to produce NO under normal conditions, but under certain environments, NOS can be switched to produce a damaging free radical that contributes to oxidative stress. NO is released from endothelial cells to act on vasculature smooth muscle by activating guanylyl cyclase to increase cyclic guanosine monophosphate (cGMP), which causes relaxation and vessel dilation. Cyclic GMP is degraded into an inactive form by phosphodiesterase type 5, on which the infamous ‘little blue pill’ (Viagra or sildenafil) pharmacology is based. Sildenafil citrate, an inhibitor of phosphodiesterase type 5, acts to prevent cGMP from being degraded, potentiating the vascular effects of NO and cGMP. The innovative aspect of this drug is that it acts downstream of NOS, avoiding issues related to impaired synthesis, signalling or uncoupling. Sildenafil (and similar medications) have been effective in treating erectile dysfunction, pulmonary hypertension and is under investigation for a number of other conditions.

Can the benefits of sildenafil translate to women?

In a recent study conducted in our lab, we used a mouse model of pre-eclampsia to examine why women with pre-eclampsia have a heightened response to vasoconstrictors (factors that constrict the smooth muscle of blood vessels, restricting flow and increasing pressure), which can exacerbate existing hypertension. Our mouse model used the overexpression of sFLT1, which causes signs similar to that observed in pre-eclamptic women – hypertension, proteinuria, increased resistance in uterine arteries and impaired fetal growth. Vascular sensitivity to vasoconstrictors can be induced by overexpression of sFLT1 in pregnant mice. We were able to demonstrate that sFLT1 acts by decreasing eNOS phosphorylation (activation), thereby reducing the capacity for NO formation. At a cellular level, the altered NO availability combined with increased blood pressure on the vascular endothelium increases oxidative stress, which pushes eNOS towards an ‘uncoupled’ state, where more damaging substances are produced, including superoxide. This resulted in significant oxidative stress within systemic vessels of the pre-eclamptic mice. Our next step was to try
to prevent the complications of pre-eclampsia using sildenafil in pregnant mice. Treatment with sildenafil resulted in normalized blood pressure, reduced uterine artery resistance and increased fetal weights. While these findings may not be translatable to humans, sildenafil treatment had no apparent adverse effects on pregnant mice or pups, which is consistent with the small number of human trials conducted. There have been countless studies upon which we have built our ideas, drawing from numerous disciplines and fields to hopefully provide a cohesive explanation for the vascular dysfunction observed in pre-eclampsia. It remains likely that this vascular dysfunction is related to the long-term risk for cardiovascular disease. Endothelial dysfunction and impaired NO signalling is a common feature of several conditions that would benefit from improvements in therapies.

The idea that NO deficiency may be implicated in pre-eclampsia is not by any means novel – many researchers have tested this idea and suggested the use of sildenafil as therapy. One of the largest hindrances to breakthroughs in maternal–fetal medicine is safety. What is deemed safe for an adult may not be safe for a child, let alone a developing fetus; thus, for excellent reasons, safety profiles of potential treatments must be absolute prior to investigation in pregnant women. Bearing this in mind, several groups have begun clinical trials using sildenafil in pregnancy. One recent double-blind, placebo-controlled trial conducted in Brazil examined early (<34 weeks gestation) pre-eclamptic women randomized to sildenafil or placebo. The results of this 100 patient study found that sildenafil, in combination with standard treatment, reduced blood pressure and uterine artery resistance compared with placebo (and standard treatment). Further, pregnancy was prolonged in the sildenafil group by four days compared with the placebo – this may sound insignificant, but any extra time to allow fetal development has a significant beneficial impact. Potentially of greatest importance, sildenafil was not associated with any adverse maternal or fetal events. While these may seem to be modest benefits, in the world of maternal–fetal medicine, a positive safety record is a great starting point. The fact that clinical trials are beginning to demonstrate benefit for a medication, supported by mechanistic evidence, is absolutely welcome.

**Coming full circle back to cardiovascular disease**

One of the interesting similarities between pre-eclampsia and erectile dysfunction is they are considered to be predictors of cardiovascular disease. While pre-eclampsia is clinically urgent and requires monitoring and treatment, both conditions are associated with endothelial damage. Is it surprising that research into pre-eclampsia is establishing this condition as a warning sign for vascular disease? If it is a surprise, perhaps we need to reconsider our thinking on this complication of pregnancy. Pre-eclampsia is primarily considered an obstetric condition, for obvious reasons, with the placenta being key to disease development. The placenta is comprised of a rich network of blood vessels for the exchange of nutrients and gases between maternal and fetal blood. It is deficiency in these blood vessels that leads to widespread damage to systemic maternal vasculature that in turn, causes hypertension, kidney damage and other complications of pre-eclampsia. While the origins of pre-eclampsia may be the placenta, clear evidence points to a vascular pathology, even prior to clinical presentation. Treating pre-eclampsia as a vascular disease may improve the approach to treatment and similarly explain the elevated risk for future cardiovascular disease.

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**References**


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How decreasing oxygenase activity helps cells cope with hypoxia

The ability of our bodies to adapt to reduced oxygen availability (hypoxia) by increasing red blood cell formation was recognized in the 19th century, but almost 200 years passed before the molecular mechanism underlying this hypoxic response was revealed. In animals, hypoxia tolerance is enabled by turning on a plethora of genes, all under the control of the hypoxia inducible transcription factor protein, HIF. Crucially, levels of HIF are controlled by a set of enzymes, whose activity is exquisitely sensitive to oxygen availability. A low-oxygen response system is arguably more important for plants which, as sessile organisms, cannot employ behavioural responses to hypoxia, and recent findings reveal a distinct yet analogous mechanism. It seems that in plants as well as in animals, oxygen-dependent enzymes are the linchpins connecting oxygen availability with the cellular response to hypoxia.

The need for oxygen regulation

If oxygen supply fails to meet demand, then a hypoxic state arises. In plants this can occur upon submergence in floodwater, an increasing problem for worldwide crop growth and food security in the advent of climate change. In humans, hypoxia is experienced at altitude, but also as a consequence of medical conditions where blood supply is limited, e.g. ischaemia or in the centre of solid tumours. Hypoxic cells have mechanisms to compensate for low oxygen availability and thus help prevent damage and death; this is called the hypoxic response mechanism. In this article, I will summarize current understanding of these mechanisms in both plants and animals.

By definition, all aerobic organisms need oxygen to survive. The primary role of oxygen is in the generation of ATP (adenosine triphosphate), the source of cellular energy. Oxygen is also important as a source of oxygen (O) atoms found in essential molecules of biological systems, for example steroid hormones, signalling molecules and neurotransmitters. Reduced oxygen availability (hypoxia) will not only affect the ability of a cell to sustain these processes, but also results in the increased formation of reactive oxygen species, which damage lipids, proteins and nucleic acids, leading to cell dysfunction and death. It is therefore vital that cells are provided with sufficient oxygen to meet demand. While plants must rely on the diffusion of oxygen through intercellular spaces, in humans our respiratory and circulatory systems have evolved so that each of the 100 trillion cells in adults has an adequate supply of oxygen. Although the hypoxic response mechanism has evolved separately in plants and animals, they appear to have converged on similar strategies and hinge upon enzymes which ‘sense’ oxygen availability (Figure 1).

Mammalian response to low oxygen levels

At the physiological level, if humans experience an oxygen deficit then the body’s immediate response is to invoke chemosensory mechanisms in a group of sensory cells in the carotid artery, called the carotid body, that increase breathing and heart rates. However, prolonged or localized hypoxia (e.g. in the centre of a tumour) results in elevated levels of the transcription factor protein HIF. HIF has two subunits (α and β) and while both subunits are continually produced by the cell, under normal oxygen conditions post-translational modification of HIFα signals for HIFα to be sent to the cell’s degradation machinery. This modification doesn’t take place in hypoxia, so HIFα remains stable in the cell, binds to HIFβ and together they promote the transcription of genes which enable the cell or tissue to address the oxygen deficit, either by increasing supply (e.g. erythropoietin production for blood cell formation) or decreasing demand (e.g. by modulating metabolism). HIFα itself does not detect ambient oxygen availability, but instead the modification of HIFa signals for HIFα to be sent to the cell’s degradation machinery. This modification doesn’t take place in hypoxia, so HIFα remains stable in the cell, binds to HIFβ and together they promote the transcription of genes which enable the cell or tissue to address the oxygen deficit, either by increasing supply (e.g. erythropoietin production for blood cell formation) or decreasing demand (e.g. by modulating metabolism). HIFα itself does not detect ambient oxygen availability, but instead the modification of HIFα is carried out by oxygen-dependent enzymes, the HIF hydroxylases. These enzymes catalyse the addition of a hydroxyl (OH) group to specific proline residues in HIF (Figure 2A), and it is this hydroxylation that targets HIF for degradation. Understanding the hypoxic response and identifying routes for intervention is an important goal for the pharmaceutical industry in treating both cancer (where elevated HIF is typically related to aggressive tumour growth; and ischaemic disease).
Gaseous Signalling

HIF hydroxylase inhibitors may be able to treat ischaemic disease by artificially stabilizing HIF and encouraging vascular growth.

The HIF hydroxylases are therefore the direct link between oxygen availability and HIF stability, and are thus termed oxygen sensors. As well as oxygen, they require iron (Fe(II)) and 2-oxoglutarate (2OG, a cellular metabolite) to catalyse prolyl hydroxylation. There are a number of other Fe(II)/2OG-dependent oxygenases in humans and other organisms, which are capable of performing a wide variety of tricky oxidative reactions. However, the HIF hydroxylases, in particular the prolyl hydroxylase PHD2, seems to be unusually sensitive to oxygen. This means that its activity is tightly coupled to oxygen availability so that when oxygen levels first start to drop, PHD2 activity begins to decrease, HIF hydroxylation is reduced and it starts to become stable in the cell to induce the hypoxic response. The amount by which PHD2 activity is diminished is dependent on the oxygen concentration, such that there is a gradual rather than a steep change in activity. PHD2 therefore acts like a dimmer switch, rather than an on/off switch, to control HIF levels, allowing an

Sub1A rice – a second ‘Green Revolution’?

What do gas sensing, targeted protein breakdown and rice breeding have in common? The answer is Sub-1A rice.

About 20 million hectares of rice in Asia is prone to flooding, often causing catastrophic losses to farmers. Scientists from the International Rice Research Institute responded to this challenge by scouring natural diversity collections to identify genes that could be introduced into elite varieties, leading to the development of submergence-tolerant ‘Sub-1A’ rice. The impact on subsistence farmers has been compared with that of the yield gains achieved by Norman Borlaug’s wheat breeding in the mid-20th century.

The year 2003 saw the identification of the rice Sub1A locus, which encodes a plant-specific transcription factor belonging to the ethylene response factor (ERF) family. Six years later, a subset of ERF transcription factors was implicated as part of an oxygen-sensing mechanism, using the model plant, Arabidopsis.

Under normal oxygen conditions, ERF proteins are turned over owing to oxidation of an N-terminal cysteine residue, which targets them for destruction in the proteasome. When oxygen availability is low, as happens during a flood, cysteine oxidation is prevented and the ERFs switch on the hypoxia response; this type of selective protein breakdown is referred to as the N-end rule. Intriguingly, unlike the Arabidopsis proteins, rice Sub1A somehow evades the N-end rule which might explain how it confers flood tolerance.

A constitutive hypoxia response can be deleterious to some plant species but understanding the molecular mechanism of low oxygen sensing now paves the way to modulating plant metabolism and perhaps to extending the second Green Revolution to many more crops.

Further reading:

appropriate response to the environmental conditions. This remarkable sensitivity appears to be unique to PHD2 amongst the Fe(II)/2OG-dependent oxygenases studied to date, and structural and kinetic studies of this enzyme have shown that the rate at which oxygen either binds or reacts with the enzyme–substrate complex is remarkably slow. Studies to ascertain the particular features of PHD2 responsible for this slow reaction with oxygen are ongoing, and hope to reveal the molecular key to this crucial oxygen-sensing mechanism.

Of course, the HIF hydroxylases are not the only enzymes in human cells that are dependent on oxygen and though hydroxylation of HIF appears to be the primary oxygen-sensing event, the possibility remains that the activity of other cellular processes may also be affected as a result of decreased oxygenase activity in hypoxia. Changes reported in hypoxic cancer cells indicate that one of these processes is histone demethylation. Histone proteins are involved in packaging DNA in our chromosomes. They are post-translationally modified in a variety of ways, affecting access of transcriptional machinery to the DNA and thus turning gene expression on or off. This is one form of epigenetic regulation, and includes histone methylation/demethylation. One example of where this becomes significant is in cancer, as different patterns of gene expression can affect growth and survival. Research is beginning to reveal that certain histone demethylase enzymes, which are similar to the HIF hydroxylases in that they are also Fe(II)/2OG-dependent oxygenases, may also be sensitive to oxygen availability (although whether this occurs to the same extent as for HIF hydroxylases remains to be seen). While we should be cautious in correlating the oxygen-dependent properties of enzymes studied in isolation with their properties in a cellular environment, this does raise the possibility that gene expression could be further modulated by hypoxia in a HIF-independent manner. It also highlights that there is much work to be done to fully understand the ways in which oxygen gradients impact upon global cellular events.

How plants cope with hypoxia

As for animals, plants also require adaptations to help them cope under hypoxic conditions, for example to survive periods of flooding, when oxygen cannot diffuse as efficiently into plant cells as it can in air. Plants deploy a range of morphological responses to low oxygen but a molecular mechanism underpinning hypoxia tolerance has only relatively recently been pieced together, helped by understanding flood responses in rice. Different rice varieties, such as Sub1A (see box), can either undergo a ‘quiescent’ response, going into metabolic shutdown until the flood (hopefully) subsides, or an ‘escape’ response, pushing growth above the level of the water so that photosynthesis can occur efficiently and oxygen can diffuse down tube-like structures called aerenchyma (sometimes called a snorkel response). It was found that a subset of plant-specific transcription factors, the Group VII ethylene response factors (ERF-VII), are responsible for directing these flood survival strategies, and crucially that these transcription factors are hypoxically regulated, similar to HIF. This mechanism is conserved in other plants including barley and the model plant species, Arabidopsis. Like HIF, the ERF-VII are targeted for cellular degradation, however unlike HIF, in plants this is not signalled via prolyl hydroxylation. Instead, ERF-VII transcription factors are degraded by a process which is dependent on the identity of the first amino acid in their protein sequences, called the N-end rule pathway. The ‘N-end rule’ dictates that certain amino acids, if they occur at the beginning of the protein (the N-terminus) following protein cleavage, are destabilizing, and trigger a cascade of reactions that ultimately result in degradation by the cellular proteasome. One of these destabilizing amino acids is cysteine, but only if it is subsequently oxidized. The activity of methionine aminopeptidases reveals a cysteine residue in the N-terminus of many ERF-VII, and crucially it was found in Arabidopsis that ERF-VII N-terminal cysteine oxidation to Cys-sulfenic acid is catalysed by oxygen-dependent enzymes, the Plant Cysteine Oxidases (PCOs, Figure 2B). Therefore, at normal oxygen concentrations ERF-VII N-terminal cysteines are oxidized by the PCOs, targeting them for degradation, but during hypoxia the...
PCOs become less efficient and the ERF-VII N-terminal cysteines are unmodified. In this case, the ERF-VIIs are stable and can induce the expression of genes that promote the flood survival strategies described above.

The PCOs therefore appear to be enzymes that link oxygen availability and the plant hypoxic response, performing an equivalent role to the HIF hydroxylases in humans. They could therefore be described as plant oxygen sensors. While it remains to be seen whether the way in which the PCOs respond to oxygen availability is similar to the HIF hydroxylases, including whether they act in a graded or stepwise manner, the PCOs are different to the HIF hydroxylases in that they are not 2OG-dependent (though are Fe(II)-dependent, as many oxygenase enzymes are). Overall then it seems that two different hypoxic response systems have evolved separately to fulfil the same role, both using oxygen-dependent enzymes to control levels of transcription factors whose role is to induce expression of a range of genes that help cells cope with reduced oxygen availability. This quasi-convergent evolution highlights the effectiveness of this system of oxygen sensing.

**Manipulating oxygen sensors**

In both humans and plants, understanding oxygen-sensing strategies may be of practical (as well as academic) interest, as manipulating these systems could help to address health and food security challenges. The oxygen-sensing enzymes are good intervention points for manipulation, both because they act at the interface with oxygen itself but also because enzymes are intrinsically good drug targets: substrate mimics can in principle be designed to bind to their active sites and be intrinsically good drug targets: substrate mimics can in principle be designed to bind to their active sites and inhibit their activity, indeed HIF hydroxylase inhibitors in principle be designed to bind to their active sites and are intrinsically good drug targets: substrate mimics can interface with oxygen itself but also because enzymes points for manipulation, both because they act at the oxygen-sensing enzymes are good intervention points for manipulation, both because they act at the interface with oxygen itself but also because enzymes are intrinsically good drug targets: substrate mimics can in principle be designed to bind to their active sites and are intrinsically good drug targets: substrate mimics can in principle be designed to bind to their active sites and inhibit their activity, indeed HIF hydroxylase inhibitors could achieve the same effect. Gene-editing techniques may also be able to play a role in modifying oxygen sensing at least in plants, with the advantage of avoiding off-target effects (non-specific inhibition by small molecules of other enzymes with similar active site structures is a common problem in drug design).

Although the plant and animal oxygen-sensing systems are apparently analogous, it would be folly to make assumptions about one based upon the other. It is clear then that there is some catching up to do in order to understand the PCOs to the same degree as we understand the HIF hydroxylases, and even there we have work to do to really understand how these fascinating enzymes have evolved to have such unique interactions with oxygen.

**References**


**Glossary**

**Transcription factor** – proteins that bind to specific sequences of DNA (enhancer or promoter sequences) to regulate the transcription of associated genes. Transcription factors allow the selective control of gene expression under different conditions or in different cell types.

**Post-translational modification** – enzyme-catalysed covalent modifications of the chemical structure of amino acids in proteins, which take place after the protein has been synthesized by the ribosome. Post-translational modifications usually alter the chemical properties of the amino acid side chain, extending the chemical repertoire of the proteome.

**Histone methylation** – post-translational addition of up to three methyl (−CH3) groups to amino acid side chains (lysines, arginines) in histone proteins, catalysed by histone methylases. Methyl groups are removed by histone demethylases.

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Interview

Living the high life

Helen Albert speaks to biological anthropologist Professor Cynthia Beall about her research into human evolutionary adaptation to high altitude and the important role of oxygen transport genes in Tibetan highlanders.

Cynthia Beall is a Distinguished University Professor at Case Western Reserve University in Cleveland, Ohio, in the US, where she has worked since the 1970’s following completion of her PhD in anthropology at the Pennsylvania State University. She is a world-renowned expert in human evolutionary adaptation to high altitude hypoxia and has carried out ground-breaking research on the different altitude responses of Andean, Tibetan and East African highlanders over the last four decades. She is a member of the National Academy of Sciences, the American Academy of Arts and Sciences and the American Philosophical Society.

How did you get into researching human adaptation to high altitude? Was it something you were always interested in?

I had my Masters and PhD in biological anthropology and was interested in worldwide variation in human biology. By chance, I studied with someone (Paul Baker) who was already working at altitude and it was kind of natural to follow in that avenue of working on human variation, human evolution and adaptation to the stress of high altitude.

One of the fun things about high altitude from a research standpoint is that at any given altitude everyone has the same stress. You can imagine if you were living in a cold climate or a hot climate, and depending on how much you work, whether you are rich or poor, whether you have heavy or light clothes, a lot of factors influence your exposure to the environmental stress. Altitude is nice because other factors cannot influence your exposure.

I started off working in the Andes where almost all the work had been done at the time. When I first got a job one of the questions someone asked me was ‘have you ever thought of working in the Himalayas?’ He (Mel Goldstein) was already working in the Himalayas and so helped me arrange my first field trip to Nepal to work with indigenous Tibetans living along the Tibetan border in Nepal. I had a very simple research question, which was ‘is everyone responding to altitude in the same way?’ and the answer right away was ‘no’. From then on I have been working largely on that question, by starting off with very simple measurements, because I had absolutely no money. Then as I got a little bit more money and a little bit more evidence it contributed to building up the case that Tibetan and Andean highlanders exposed to the same stress had quantitatively and qualitatively different responses. We expanded that to some Ethiopian East African samples and continued to find variation.

This was all very exciting and one hypothesis was that there was a genetic basis to these differences, but until fairly recently we had no way to ask that question. We were addressing it by migrant studies. For example, when Europeans move to altitude do they or their children achieve the same biological characteristics as the Tibetans or the Andean highlanders? Over the years we tried many different ways to get at this question of genetic variation, because it was starting to look as if we might have a beautiful case, a complete case, of evolution by natural selection on a quantitative trait in humans and I am still working on that.

What effects does living at high altitude have on oxygen intake in different populations?

Virtually everyone is affected. It depends a bit on who you are talking about. For example, take people like us who are born and raised at low altitude. We have a certain set of modifications of gas exchange, gas transport and gas use, that is in terms of oxygen. For instance, if we go to altitude we increase our breathing so that, for example, you may be breathing 8-10 litres per min sitting down in a lowland environment, but if you went to altitude then your breathing would go way up immediately. It would go a bit higher for first few days and would stay high for a few weeks and then start to come down. If you were there for long enough, say you stayed for several years, then your breathing would slowly return toward baseline (from before you went to altitude).

Andean highlanders have, starting from early childhood, what is called blunted ventilation. They have a response similar to the one we would have
if we had been at altitude for a year or so, in that it is not as high as might be expected based on acute exposure. So the proposed physiological explanation for this, was that it would be really costly from an energetic standpoint, to keep on breathing at a high rate. Therefore, it's adaptive to have low ventilation.

Then we studied the same question in Tibetans and Tibetans have a really high ventilation rate, from the age of 8 onwards (the youngest that's been tested) they are very well adapted, they work well, they are adequately nourished and so forth. So we learnt from that that just looking at lowland populations and Andean highlanders can lead to too narrow a perspective. Including Tibetans in that mix was really important to understand that human biology has several ways to respond to long-term altitude.

**What impact does high altitude have on oxygen transport in haemoglobin?**

Andean highlanders and people like us who go to altitude acutely for a few weeks have a very high haemoglobin concentration, it can go up 20% or 25%. Again, when we only knew about us and Andean highlanders we thought that having a very high haemoglobin was a good thing, because the highlanders had it and they are healthy and we have it and we are healthy. Then we started asking the same question of Tibetans and we found that Tibetans have in the order of 2 g/dl less haemoglobin at the same altitude as Andean highlanders. In fact, Tibetans can live at altitudes up to about 13,000 feet or 4200 m with little elevation in haemoglobin concentration. They have a very dampened response and Nayia Petousi and Peter Robins at Oxford have recently measured Tibetans living at sea level in the UK and compared them with Han Chinese living at sea level in the UK and found that even at sea level Tibetans have lower haemoglobin than others. Not pathologically low, but lower within the normal range. So that has led to a lot of questions. We were able to establish that Tibetans were healthy, they were not anaemic, they were not infected with diseases that would cause them to produce less haemoglobin, so we were able to establish that this was consistent with healthy life at altitude.

That raised a very interesting question though, because if Tibetans don't have as much haemoglobin, which carries oxygen, then what's going on? How do they get enough oxygen? Or are they getting enough? Is there some adaptation at the gas use level?

**What impact does nitric oxide have on oxygen transport at high altitude?**

Nitric oxide can widen blood vessels (vasodilation). A colleague (Serpil Erzurum) and I asked how Tibetans offset the lower arterial oxygen content resulting from the lower hemoglobin concentration. We wanted to know how they were getting enough oxygen to their tissues in order to maintain a normal basal metabolic rate. We hypothesised that they were emphasising vasodilation and blood flow. At this time there was a lot of work coming out on nitric oxide and that's how I went down the nitric oxide route.

We went to two places at the same altitude and with the same equipment and asked how much nitric...
oxide was exhaled from the lungs of Andean and Tibetan highlanders. We found that Tibetans exhaled more nitric oxide than Andean highlanders and that both exhaled more than lowlanders acutely exposed to high altitude. When we go to high altitude our exhaled nitric oxide decreases, presumably because of low oxygen, as it requires oxygen as a substrate.

When we measured blood levels, we found that Tibetans had high levels of nitric oxide metabolites and nitrosylated proteins. This has contributed to the idea that some populations are employing vascular responses to hypoxia to a greater extent than other populations such as the Andean highlanders who are relying more on haematological responses.

How does genetic variation influence high altitude adaptation?

After the human genome project and genome analysis techniques became available, I was able with some collaborators, including Hugh Montgomery from University College London, Peter Robbins from Oxford and Changqing Zeng from Beijing Institute of Genomics, to publish a paper in which we linked the relatively low haemoglobin concentration in Tibetans to the high prevalence of a very distinctive variant in a gene called **EPAS1**, which is involved in oxygen homeostasis, so that was thrilling.

The genetics of **EPAS1** first came to light in the 90’s, the late 90s, by the discovery by Greg Semenza, Peter Ratcliffe, William Kaelin, and colleagues, of the oxygen sensing pathway [See ‘Oxygen Sensing and Regulation’ box]. They identified three controllers of oxygen homeostasis called hypoxia inducible factors (HIFs) – HIF 1, 2, and 3. Each of these proteins is made up of two molecules; the beta molecule is the same for all three and the alpha molecule is different among the three. They are active in different tissues and have somewhat different biochemical properties. The HIFs are transcription factors that turn on other genes in the oxygen homeostasis pathway, including erythropoietin.

We didn’t have very much money, so we looked at variants in 3 or 4 genes - HIF1 (HIF1 alpha unit), **EPAS1** (HIF2 alpha unit), and a couple of others. We found that there was a high prevalence of an **EPAS1** variant among the Tibetans that (when homozygous) was associated with a 1.5g lower haemoglobin concentration than if you were homozygous for the ancestral variant.

In terms of living at high altitude, the hypothesis is that increasing haemoglobin concentration is fine as a short term response, but what it does is increase blood viscosity. People at the upper end of the normal curve have very thick blood and they are more vulnerable to a disease called chronic mountain sickness and also more vulnerable to thrombosis, stroke and things like that. The hypothesis is that if you have the relevant variants it makes you less susceptible to disease and more likely to survive as a result.

Interestingly, it turns out that it’s only the Tibetans that have this particular set of variants of **EPAS1**. People have looked for the variants and **EPAS1** associations with haemoglobin in several East African populations and in Andean populations and not found an association. There may be convergent evolution occurring, however, as we found one SNP of unknown function associating with low haemoglobin concentration among Amhara highlanders in East Africa.

Have recent discoveries about the impact of environment on the expression of a person’s genes (epigenetics) influenced your research and/or its interpretation?

Yes, I’ve thought about it a lot, because of the potential for completely confounding what we think we’ve discovered in terms of genetics.

We looked at it in one study with Anna Di Rienzo and Gorka Alkorta-Aranburu of the University of Chicago, and found no altitude differences in CpG methylation signals, a marker of epigenetic modification, at high and at low altitude among Amhara of Ethiopia and among Oromo of Ethiopia. We found that there was a big difference between the two ethnic groups, but not an altitude difference.

One possible explanation is that we were measuring things in saliva or in blood and maybe it’s
really lung epigenetics or muscle epigenetics that’s relevant. So epigenetics is definitely something that we are trying to think about and incorporate into our research.

From the fieldwork point of view, it’s very difficult to get things like lung and muscle samples from people at fieldwork sites. I think we have to really think about good ways to approach collecting this data in a minimally invasive way.

**What is your current research focus?**

The next step is that we want to know what the mechanism is and we want to know if we can link that to survival and reproduction. So what I’m doing now is trying to connect the genetics of oxygen homoeostasis, the biological characteristics that we see in people and fertility and survival of their offspring.

What I think is really important is that we found signals of natural selection at EPAS1 in Tibetans and that has been replicated in half a dozen samples now. It’s very common that one scientist may find one association and another may replicate their methods and not find it, but the EPAS1 association with low haemoglobin levels in Tibetans has withstood a number of replications. Now to me, what I next need to do is to ask the question ‘is this a result of natural selection?’ if so then we should be able to link it with fertility and survival and I’m halfway through a project working with that question among Tibetan women.

Of course when it comes to having babies, a lot of things influence whether or not you have them, such as whether or not you are married, how rich you are and so on, so fertility studies in humans are really complicated. It’s not as easy as looking at that question in mice or in salamanders or other animals!

Looking at all the factors, we have a very interesting situation where we find EPAS1 variants associate with haemoglobin, and haemoglobin associates with fertility in the sense that the higher the haemoglobin the poorer the fertility of Tibetan women living at altitude.

It could be that there is a linked gene acting in a similar pathway to EPAS1, or it could be that EPAS1 is doing something else that we are not measuring and there is a phenotype that we haven’t measured yet. We’ve been arguing that haemoglobin was the trait that selection was acting on, but maybe not, maybe it’s something closely correlated to haemoglobin that we haven’t figured out yet.

### Further reading


### Oxygen Sensing and Regulation – The 2016 Albert Lasker Basic Medical Research Award

The 2016 Albert Lasker Basic Medical Research Award (http://www.laskerfoundation.org/awards/) was awarded to William Kaelin (Dana-Farber Cancer Institute/Harvard Medical School, USA), Peter Ratcliffe (University of Oxford/Francis Crick Institute, UK) and Gregg Semenza (Johns Hopkins University School of Medicine, USA) in September for their discovery of the existence of the oxygen sensing pathway that allows cells to detect and respond to differing levels of oxygen in the environment.

Semenza and Ratcliffe began this work in their respective labs in the early 1990s when they were trying to discover how expression of the erythropoietin gene is triggered by oxygen deprivation. A sequence necessary for activation of genes under conditions of low oxygen, the transcription factor hypoxia inducible factor (HIF)-1, was identified. Later work by Semenza and colleagues showed that HIF-1 contained an alpha and a beta subunit.

Previously it had been assumed that mechanisms influencing production of erythropoietin, which increases under conditions of hypoxia, must also be located in the specialist kidney cells (interstitial fibroblasts) that produce the hormone. Ratcliffe and Semenza showed this was not the case and found that HIF-1 is able to bind and activate genes in a number of different cell types around the body. For example, HIF-1 is able to activate vascular endothelial growth factor (VEGF), a protein that stimulates blood vessel formation.

While HIF-1 levels go up under hypoxic conditions, they also decrease rapidly when oxygen concentration in the atmosphere rises. Kaelin was able to demonstrate how breakdown of the HIF-1 protein occurs through his work on von Hippel-Lindau (VHL) disease, a familial cancer syndrome. Tumours of this type of cancer contain blood vessels and some result in increased levels of red blood cells, which suggested to Kaelin that the VHL protein was linked to hypoxia signalling. Kaelin, Ratcliffe and others later showed that the VHL protein bound to the HIF-1 alpha subunit and targeted it for destruction through the ubiquitin pathway when oxygen levels increased. They later found that the reason this only occurs in conditions of high oxygen is due to the addition of a hydroxyl group to the HIF-1 alpha subunit by an enzyme (prolyl hydroxylase) that allows the VHL protein to recognise it.

The knowledge obtained about the action of HIF-1 and related proteins offers the potential to be exploited for therapeutic purposes. For example, prolyl hydroxylase inhibitors that preserve HIF and activate the production of red blood cells by stimulating erythropoietin could be used to treat conditions such as anaemia. Conversely, blocking HIF activity may be beneficial in treating certain cancers.
Obtaining a PhD is a long and often rocky road. Now in my final year of doctoral study, I am taking a moment to look back at the successes and (far more frequent) failures of PhD life, particularly my first year experience. This was, quite literally, full of ups and downs as I found myself undertaking research as part of the Xtreme Everest 2 (XE2) expedition team.

Background

XE2 was a research expedition undertaken in 2013 by the Centre of Altitude Space and Extreme Environment Medicine, University College London (http://www.xtreme-everest.co.uk/), to follow up Caudwell Xtreme Everest conducted in 2007. The premise of both expeditions was to investigate human physiological responses to low oxygen (O₂) conditions (hypoxia) through high altitude exposure, where the inspired partial pressure of O₂ is decreased. This research goal aligns with those of my own PhD, which broadly involves investigation into metabolic responses to hypoxic conditions.

O₂ is the stuff of life. Mammals are reliant upon a sufficient supply of O₂ to maintain energetic and redox homeostasis. Conditions where O₂ supply is limited, termed hypoxia, present a significant physiological challenge and in severe cases may be fatal. Hypoxia is also a state prevalent in a range of disease conditions where O₂ delivery to the tissues is impaired, making our understanding of hypoxic adaptation highly clinically relevant.

Despite its importance in human physiology, the pathways involved in acclimatisation to hypoxia remain unclear and are particularly difficult to study in pathological states given the plethora of confounding factors and complications present in patients. The Xtreme Everest studies offer an alternative model for exploring hypoxic adaptation through assessment of a large cohort of healthy humans exposed to progressive environmental hypobaric hypoxia during strictly controlled ascent to high altitude. In addition to testing native lowlanders, a fundamental aspect of XE2 was to investigate the responses of the renowned ‘Kings of the Mountains’, the native ancestral high altitude dwellers (Sherpas). Baseline testing of lowland subjects was undertaken in London prior to the start of the expedition and for Sherpa participants at Kathmandu (1,300m). Data were subsequently collected at Namche Bazaar (3,500m) and Everest base camp (5,300m) on the ascent and Kathmandu on the descent. My role was to work as a scientific investigator in the Kathmandu Laboratory.

Subject testing: a perspective from the Kathmandu Laboratory

‘Logistics’, a term I was to become all too familiar with during our 3-month testing period. From the outset, XE2 was an incredibly ambitious undertaking: establishing functioning laboratories at each of the test locations was no easy task and required rigorous preparation. At each test centre, a large number of experiments were conducted to gain a detailed characterisation of phenotype. These included: cardiopulmonary exercise testing (CPET), assessment of nitric oxide metabolism, skeletal muscle mitochondrial respirometry and analysis of microvascular blood flow to name a few. To say that each laboratory was
required to be multi-disciplinary is an understatement.

For the Kathmandu lab team, set up began immediately upon arrival to enable testing to commence as soon as possible. This began with testing of Sherpa subjects, which was reliant upon the assistance of Nepali translators, and concluded with the testing of native lowlanders. Each member of the laboratory team had multiple roles in the daily routine. My own included processing of blood, saliva, breath condensate and urine samples, followed by assessment of subjects’ forearm microvascular blood flow.

The very nature of field expeditions requires that experiments are undertaken outside of the comfort of a scrupulously controlled environment. Whilst this was taken to the extremes for our Base Camp team (where certain items were placed in the fridge to keep warm in the face of plummeting night time temperatures!), for us, this included negotiating the temperamental Nepali national grid, hot weather and a general limit in available facilities and consumables. The limited ‘laboratory’ space meant the majority of protocols were performed in the same room. This made for a somewhat unique laboratory environment that was consistently sociable but required highly efficient organisation to avoid falling into chaos. The ability of each team member to adapt and work in a less than optimal environment was tested on a daily basis.

**Trekking the Himalayas**

Having the greatest mountain range on your doorstep is a way of life for most Nepalis. Thankfully, we were able to take advantage of this in between busy testing periods, squeezing in a jaunt up the Khumbu valley to Everest Base Camp, which gave us the chance to visit the other testing laboratories and gain first-hand experience of the trek our subjects were undertaking.

Having survived the runway landing at Lukla airport (527m in length, positioned precariously at a cliff-edge), the trek ascended through luscious greenery and across suspended bridges onto the XE2 laboratory positioned at Namche. This gradually progressed to the barren landscape surrounding the mountain medical centre at Pheriche and eventually into terrain arguably reminiscent of the moon at Everest Base Camp. It was here that I truly began to feel the strain of the drop in atmospheric pressure. With $O_2$ saturation readings in the high 70’s, tasks that would require a low level of cardiovascular strain at sea level became heavily arduous. For instance, ascending 100m to the summit of Kala Pathar (5,643m) to bask in the glorious and quite literally breathing-taking views of the Khumbu icefall left me feeling as though I was winded and suffering from a hangover. Although walking at a snail’s pace, my breathing rate was equivalent to an all-out sprint and I was left with a migraine-like headache. Whilst I am no Mo Farah, I do not consider myself unfit, so this response did leave me unnerved and highly envious of our Sherpa guides whom managed the ascent unruffled, with the extra weight of my backpack. It was at this point I gained a full appreciation of the advantages genetic heritage can provide, in particular the superiority of the Sherpa population and their ability to withstand such low O2 conditions with apparent ease and minimal acclimatisation time.

**The aftermath**

The significance of both Xtreme Everest expeditions in the wider context of hypoxia research is yet to be fully determined as the analysis of the collected data continues. Compared to previous high altitude field studies, they undoubtedly lead the way in their rigorous study design and impressive subject cohort recruited. The ultimate test will be whether findings from this will translate into the significant advancement in our understanding of the pathophysiological effects of hypoxia in patients at sea level.

From the viewpoint of a young scientist about to make a leap into the research world, this expedition was an exceptional experience. As I approach the end of my PhD, I can begin to appreciate the incalculable impact it has had. The success of our laboratory work was entirely dependent upon proficient teamwork, which is something that the solo quest for a PhD does not always entail. Along with projects I have been involved with since, it has confirmed my belief that working as a member of a team trumps laboratory solitude. There are clearly instances when the latter cannot be avoided, but science should not be a lonesome struggle. Beyond the laboratory, the sheer scale of the expedition has inspired me to pursue my research interests and ultimately dream big. If you are intrigued by the extremes of physiology, why not take your experiment to that extreme environment?

At a personal level, gaining first-hand experience of being hypoxic allowed me to put the greater picture of my PhD in context. At later stages, when I have been guilty of becoming lost in the intricacies of research, ‘unlucky to see the wood for the trees’ as my supervisor so eloquently puts it, this experience has helped put it all into context. $O_2$ is indeed the stuff of life.

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**Bibliography**


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Student Focus

I obtained a degree in Physiology from King’s College London in 2012 and began my PhD at the Centre of Human and Aerospace Physiological Sciences (CHAPS) the same year under the supervision of Professor Stephen Harridge (CHAPS) and Dr Lindsay Edwards (GlaxoSmithKline). My research aims to identify the metabolic responses to oxygen insufficiency (hypoxia), which is experienced upon ascent to high altitude and in a number of disease states, and the potential for dietary nitrate supplementation to alleviate hypoxic stress. I am funded by King’s College London on a Graduate Teaching Assistant scheme.
The Science Communication Competition is now in its sixth year. As in previous years, it aims to find young talented science writers and give them the opportunity to have their work published in *The Biochemist*. In 2015, a new branch of the competition was launched to include video entries. Overall this year’s competition attracted 62 entries and these were reviewed by our external panel of expert judges. The second prize in the written category was awarded to Anwen Brown from University College London, whose article is presented here; the second prize in the video category went to Amy (Xinyang) Hong from the University of Oxford. Amy’s video can be viewed on the Society’s YouTube channel: http://bit.ly/2bz3rfz

Ground control to Major Tim

**Anwen Brown**

(University College, London, UK)

By now, at the time of writing, Major Tim Peake is on day 105 of his mission on the International Space Station (ISS). The main reason for his expedition is to perform a number of scientific experiments under the effect of weightlessness, as well as collecting samples from his own body for studies here on earth. He’s blown into lung–function kits, collected blood, stool and urine samples, measured properties of his skin, given himself ultrasounds, conducted eye examinations and completed numerous health questionnaires, just to name a few!

His body has now been feeling the effects of microgravity for over three months, but what kind of consequences do we already know zero gravity is having on his biochemistry? We can all imagine some of the physiological effects he might be experiencing, but what are some of the molecular changes behind these alterations? And how could the experiments he’s performing better aid biochemical research here on earth as well as in space?

**“Take your protein pills and put your helmet on”**

It’s well known that astronauts lose weight in space, in fact, loss is estimated at around 2.4% per 100 days in space. This decrease in weight is due mainly to muscle and bone changes. Our muscles are made up of proteins and the balance between synthesis and breakdown is directly related to the amount of food we eat and the amount of energy we expend. In space, as there is no gravity, the amount of stress on muscles is decreased, which means that less protein is made and more protein is degraded. The likely protein breakdown mechanism involves a protein called ubiquitin and a protein complex called the proteasome. Ubiquitin attaches onto the muscle protein which is then taken to the proteasome for destruction. The proteasome is like a big machine, made up of lots of enzymes which attack the protein and cut it up into its smaller components, amino acids. This then results in loss of muscle mass.

Major Tim’s bone structure will also be affected by the microgravity environment; during the whole mission, he could end up losing up to 20% of his total bone mass! Our bones are made by groups of cells called osteoblasts and are broken down by osteoclasts. Osteoblasts make collagen, a type of dense protein which is the main component of bone, along with skin and hair. Osteoclasts in turn break down and destroy bone by secreting acids and enzymes, reducing it to its molecular components: calcium, magnesium and phosphate; a process known as bone resorption. As Major Tim’s bones do not need to support him in order to stand up and walk around, less collagen is made by osteoblasts and more bone is broken down by osteoclasts, resulting in an overall decrease in bone density.

**“I’m floating in a most peculiar way”**

We take knowing that we are upright and where our arms and legs are for granted; but in space, Major Tim cannot rely on his internal sense of up and down. Our brains consolidate information from passages in our inner ears, our vision and from receptor proteins in our muscles and tendons, to make sense of our motion and orientation. Movement of liquid found in the ear labyrinth stimulates hair cells lining these channels to activate receptors. Similarly, tissue receptors are activated by the stretching of tendons and muscles. On activation, they cause an electrical change involving movement of positive sodium and potassium ions across nerve cell membranes. The change in charge triggers the release of a neurotransmitter, a molecular messenger that allows the electrical signal to pass between nerves to the brain, carrying information about the type of movement made. Of course, these systems are effectively calibrated to upright movement.
against gravity on earth. So in zero gravity space, where there isn't really “up” or “down”, they are thrown off. These systems can actually adapt to microgravity, but it could mean that Major Tim experiences some space motion sickness, or sometimes feels that he is the wrong way round. Problems can also arise with body coordination when performing simple tasks, as fewer forces acting on the body means less stimulation of muscle and tendon receptors. Consciously moving arms and legs can require some re-training of the brain.¹

“The stars look very different today”

The operators of Major Tim’s mission will be monitoring his health during this flight, in particularly his eyes, as up to 20% of astronauts report persistent vision changes. We know that in low gravity situations, body fluids shift upwards causing increased pressure in the head and possibly the nerve which relays information from the eye to the brain. This could be part of the physical explanation, but why does it only affect 20% of astronauts? Previous astronauts’ blood tests revealed that those who experienced vision deterioration had different levels of some molecules compared to those who didn’t. The molecules involved suggested a process called the one-carbon pathway, which can be thought of as an assembly line for making DNA.² Genetic differences between people can sometimes mean that they respond to environmental changes in different ways. For example, people exposed to the same amount of sun can burn, tan or experience very little skin change at all. Similarly, in space the common stress of microgravity affects the one-carbon pathway differently in various individuals. Some astronauts might experience a slowing of this assembly line and consequently a deterioration in eyesight, while others have no adverse effects at all. The way in which this pathway is related to vision is still uncertain, and the experiments that Major Tim is undertaking will provide important insight into making these connections.

“Press your space face close to mine”

It might be obvious from pictures of Major Tim that an astronaut looks rather different in space; and not just because they’re floating. Due to the shift in fluid from the lower body, the face and neck can look full

European Space Agency (ESA) astronaut Timothy Peake prepares to install a space acceleration measurement system sensor inside the European Columbus module aboard the International Space Station. The device is used in an ongoing study of the small forces (vibrations and accelerations) on the International Space Station resulting from the operation of hardware, crew activities, dockings and maneuvering. Results generalize the types of vibrations affecting vibration-sensitive experiments. Photo credit: NASA/Tim Peake
and puffy; indeed, some have even been remarked to regain a youthful appearance during space flight! But this fluid shift can also cause changes to the lungs and cardiovascular system. The increase in pressure around the lungs can temporarily affect their function, and an increase in blood volume in some heart chambers means that the body needs to quickly adapt before the heart is overloaded. It does this by excreting more urine and maintaining a body fluid level 10% lower than on earth. Amount of fluid excretion is controlled by anti-diuretic hormone released from the brain.

The baroreceptor reflex is also affected by microgravity; it is the system responsible for cardiovascular control. It involves receptors, or sensors found in blood vessels which detect changes in blood pressure. At upright gravitational resting blood pressure, the baroreceptors send electrical impulses to the brain carried via the movement of sodium and potassium ions across nerve membranes, at the same rate as the heartbeat. Pressure increases cause receptors to send an increasing rate of electrical impulses. This leads to a decrease in heart output and changes to muscles in the walls of blood vessels, resulting in an overall lowering of blood pressure. However, in space, as the body is no longer in a gravitational upright position, and fewer forces are acting on it, the baroreceptors are not able to sense normal changes in pressure and send moment-to-moment electrical stimuli. This means the baroreceptor reflex needs to adapt and find a new balance appropriate to the microgravity conditions. Indeed, as time goes on during the space flight, blood pressure and heart output seem to return to levels seen on earth.²

“Changes”

So now we know some of the changes that Major Tim’s biochemistry might be undergoing during his mission on the ISS, but his research will without doubt elucidate details of the pathways and mechanisms involved. They will also certainly aid our understanding of diseases affecting people here on earth. For example, experiments on his own bones will help inform research into osteoporosis, a major cause of bone loss in the elderly. His muscle research could elucidate the biochemical mechanisms causing bed-bound patients to lose muscle mass and tone, and how to prevent this. His experience of orientation and spatial awareness could help researchers better understand how damage to the inner ear can affect balance and how electrical signals to the brain can be altered in some disorders. His eye tests could aid research into the causes of migraine and strokes, and investigation into his own heart performance and blood pressure has a clear benefit to people suffering from cardiovascular diseases. Lastly, thinking to the future, his work will also be invaluable as mankind considers longer journeys into space. Even though Major Tim’s physiology and biochemistry has been affected in the short term, the long term benefit of his research could shape the future of biomolecular research and treatments on earth as well as that of space travel itself.

References

AuthorAID – supporting early career researchers in developing countries

Getting your research published is a major milestone in any researcher’s career, but it’s also a journey fraught with challenges and questions from the beginning: How do you analyse and present the data? How do you write up your results into something meaningful and readable? How do you choose the right journal? How do you make sure your paper has impact? And of course, how do you make sure your paper doesn’t end up in the rejection pile?

In many lower and middle-income countries, researchers face the same intense pressures to publish as their counterparts in developed nations, but they often don’t have the access to resources, information, training in research writing, and well-established support networks that we take for granted in the US and Europe. As a result a lot of important research goes unpublished, is rejected, or even worse, ends up in questionable ‘predatory’ journals, which can damage the reputation of the researcher and their work.

In order to address some of these problems, the AuthorAID project was established in 2007 by INASP (www.inasp.info/en/), an Oxford-based international development charity working with a global network of partners to improve access, production and use of research information and knowledge. Over the years, AuthorAID has developed a number of different ways to help early career researchers in developing countries to publish and communicate their work. One of the ways we do this is to help embed research writing skills training in curricula and professional development schemes of universities and research institutes. We are currently working with 10 institutes across four countries, namely Ghana, Tanzania, Sri Lanka and Vietnam.

Support through training, resources, small grants and online discussion

Our other initiatives have a wider reach. We offer small grants twice yearly to researchers in all lower and middle income countries. Travel grants provide early career researchers the chance to present their research at international conferences, network with other researchers and learn from the leaders in their field. Our workshop grants provide opportunities for experienced researchers to run research writing and grant proposal writing training at their own institution or organisation.

Even for researchers who do not win one of our grants, we provide all of our training materials and other resources free of charge to website visitors. Resources are available in English, Arabic, Chinese, French and Spanish: http://www.authoraid.info/en/resources/.

What are the biggest challenges for developing country researchers?

The AuthorAID discussion forum has over 2000 members from around the world. In July this year, we invited an informal straw poll of members to ask what is their biggest challenge in publishing or communicating their research. The top ten answers were as follows:

- Lack of research funding
- Writing in the English language
- Identifying the most suitable, genuine journals
- Delays with peer review
- Getting their papers accepted though peer review
- Publication costs (such as Article Processing Charges)
- Lack of mentors, or people to check and review their manuscripts
- Academic writing style
- Poor or insufficient laboratory equipment
- Difficulty communicating academic research to policy makers and the public

The AuthorAID discussion group is hosted on Dgroups and everybody is welcome to join the conversation – please register at https://dgroups.org/groups/authoraiddiscussion.

Free online courses and MOOCs

In recent years AuthorAID has also developed free online courses in research writing and grant proposal writing. In November 2015, we ran our very first MOOC (Massive Open Online Course) in research writing, attracting
Learning Curve

Guinean researcher Alexandre Delamou (white shirt, fourth from right, front row) won an AuthorAID grant to run a recent medical writing workshop in Conakry, Guinea in July. Alexandre is currently researching the effects of the Ebola virus in rural Guinea, but he has also found the time to register as an AuthorAID mentor.

over 1,200 researchers from 59 countries. The six-week course was hosted on the free open source learning platform Moodle, and covered basics such as literature reviews, publishing ethics, writing your paper and getting published in a journal. The course was run again in April–May 2016 and attracted over 1,600 researchers from 79 countries from as far afield as El Salvador, the Philippines, Somalia as well as refugee researchers from Syria, Iraq and Yemen. Over 900 completed the course and received certificates and a digital badge.

The course benefited greatly from an international team of volunteer ‘guest facilitators’, who were on hand to answer questions in the lively discussion forums, on popular topics such as how to avoid plagiarising, how to spot predatory journals, accessing research behind paywalls, best citation practices, and the meaning of the Impact Factor.

The next Research Writing MOOC runs in October/November 2016. If you would like to be involved as a guest facilitator, email us at authoraid@inasp.info

AuthorAID online mentoring - making a difference for medical researchers

One of the main challenges that early career researchers face is the shortage of mentors – senior researchers and more experienced peers who can provide them with advice on getting their research written up and published. AuthorAID provides an online mentoring system which allows volunteer mentors to use their crucial skills and experience to guide less experienced researchers through the challenges of publishing and communicating their research. The AuthorAID mentoring system was set up in 2008 and over the years we have seen a growing demand for mentoring assistance from developing country researchers. We are continually looking to increase the number of mentors on the system to help meet this growing demand.

15 most common types of support needed by new mentees in 2016:

- Writing
- Article planning
- Proofreading
- Grant proposal development
- Language editing or proofreading support
- Career mentoring
- Thesis and dissertation writing
- Dealing with the publishing process
- Literature reviews
- Study design
- Statistics
- Presentation planning
- Responding to peer review
- Publication ethics
- Technical reports
Our platform is open to researchers from all subject areas, but our key shortage is in medical sciences – over a third of mentees looking for mentoring supporting on the AuthorAID website are researchers in medicine, healthcare or biological sciences.

Who can be a mentor?

Many AuthorAID mentors are senior researchers with years of experience and long lists of publications behind them, but we also have a growing number of postdocs and mid-career researchers who are also keen to put their knowledge and skills into action. We usually ask that mentors have successfully published at least two or three papers in high-quality journals, or have won at least two grant applications. Alternatively, if you have substantial editorial experience we would also like to hear from you.

All mentor applications are reviewed to ensure that we have mentors with the appropriate attitude, skills and experience to support others. A large number of our mentors come from developed countries, but increasingly mentors from developing countries are also signing up. It’s nice to note that many mentors are motivated to volunteer their time and expertise because they themselves have benefited from having such support in the past. You can read the inspiring stories of AuthorAID mentors Joshua Okonya from Uganda1 and Dr. Farooq Rathore from Pakistan2 on the INASP website.

How does online mentoring work?

The mentoring system helps pair together experienced mentors with researchers who need support at any stage of their writing project. Mentoring relationships can be short or long-term, and mentors and mentees have the option of signing a mentoring agreement to set out clear objectives for both parties.

Mentees can request help with a wide range of tasks, from specific tasks such as planning the structure of an article, language editing and interpreting data; to longer term help such as developing a grant proposal, or career mentoring.

It’s easy to make contact with mentors or mentees – you can use our ‘find a researcher’ search facility, and our mentoring dashboard will automatically suggest suitable ‘matches’ for you based on subject and skills, rather like a dating website for researchers!

The benefits for mentors

So what are the benefits for mentors? Not only are you doing your bit for global development and research, but we believe that mentoring is also a worthwhile personal development process that can widen your perspective and add valuable skills to your CV such as mentoring experience, editing skills, and reviewing manuscripts.

The Researcher Development Framework3 developed by Vitae recognises the importance of not only developing mentoring skills, but also that of global citizenship – engaging and understanding other cultures and international research issue, and developing international contacts and networks.

Please visit the AuthorAID website (http://www.authoraid.info) for more information. Whether you want to be a mentor, a mentee, or just be part of our community, why not sign up as a member today?

References

1. Ugandan entomologist overcame barriers to publication with help from the AuthorAID network http://www.inasp.info/en/publications/details/210/
As I write this article, three weeks post EU referendum, the nation and indeed the world is still reeling from the shock result and the political manoeuvring it has sparked within the UK. I hope that following the appointment of the new Prime Minister we may be about to enter a phase of calm negotiation aimed at defining the country’s new relationship within Europe and indeed the future of the UK science base. It is clear that the true implications of leaving the European political union will be largely unknown for many months, or more likely years. So what can members of the scientific community actually do to influence the debate in a positive manner? To answer this question we need to consider the relationship between scientists and our elected and non-elected policy makers.

Within days of the referendum result I attended Parliamentary Links Day, a very timely event entitled “Science after the Referendum: What Next?”. Although there were many passionate statements on the importance of science to the UK, our economy and the need to secure adequate funding for our sector, it was clear that nobody really knew where to go next. Thankfully, within a few days of this event, the wheels of Government had started to turn and the Biochemical Society’s Policy Committee was busy responding to the first Brexit consultation from the House of Commons Science and Technology Select Committee.

Many MPs are very open about their lack of knowledge regarding science topics and the difficulties in making informed decisions, when voting on important scientific issues. The Speaker of the House of Commons, the Rt Hon John Bercow has freely admitted to his lack of scientific understanding at every Parliamentary Links Day that I have attended. The number of MPs with a science PhD appears to total just one at the present time, however, at least 26 out of the 650 currently elected MPs appear to have a degree in a STEM/medical area¹ and at least 60 more have a declared interest in science². Unfortunately, this represents a decrease on the previous Parliament (2010-15) when over a hundred MPs were linked, in some way, to science and technology. So not a critical mass of science knowledge but it’s a start nonetheless!

The lack of career scientists within the Government has always been an issue but at this crucial time it poses a particular challenge for UK science. MPs need, and do in fact regularly request to be better informed about the decisions they take and the effects their choices may have on the UK science base, the economy and societal impact. However, now more than ever, scientists from across the UK need to make their voices heard within the Westminster corridors of power to facilitate sensible and informed decision making.

Despite the presence of a number of MPs with a science background and the amount of information fed into government by individuals and groups with an interest in science policy. There is every chance that the voice of the science community may still not be loud enough to guarantee the kind of future that we all hope for. There are a number of primary issues we face during the EU exit negotiations, such as availability or replacement of EU funding, free movement of people, ability to maintain and form collaborative networks with other countries, cohesive regulatory frameworks that blend in with current EU rules and continued favourable access to EU science infrastructure. Yet the wider implications for Government, academia and industry and the amount of detail that needs to be negotiated, will only become clear over the next few months. The sheer volume of information that needs to be fed into the process will be vast and keeping up the momentum will be a huge challenge.

At this critical time, it is essential that all scientists do their part to influence the EU negotiation debate and help position UK science at the forefront of the Government’s plans. So what can UK- and EU-based scientists do, especially those that have had little experience in practical science policy work in the past, to inform these discussions? Firstly, it will be important for members of all learned societies to respond to requests for information/evidence that can be fed to policy makers. You may wish to lobby your local MP as an individual, or perhaps take part in a pairing scheme such as that run by the Royal Society, were you work with an individual MP at Westminster and your host institution. There are many ways in which you can...
become involved, and it is important to do so, as it will be by weight of numbers that we make our voice heard above the rest.

To achieve our goals, we need to clearly define what the scientific community wants and enforce the message that putting science at the forefront of the Government’s priority list can benefit the entire population of the UK. This will be a Herculean task, as the lobbying of policy makers by scientists will need to occur at levels never before seen in this country. Yet the price if we fail could be catastrophic for a country that is widely seen as a global superpower of science and technological innovation.

Over the coming months, we will be working along with our sister learned societies and other organizations to urge the UK Government to ensure that both funding and opportunities for scientists to collaborate across the EU are priorities in the forthcoming negotiations.

In order to support these activities, we will continue to gather evidence and case studies to inform our engagement with policy makers. If you have been or expect to be affected by the changes, or have evidence of the challenges or any emerging opportunities for biochemistry, molecular biology and the wider biosciences, please share your experiences by writing to: policy@biochemistry.org.

References
The RSB is currently buzzing in preparation for Biology Week, which takes place from 8–16 October. This will be the fifth annual celebration of the biosciences around the UK and beyond. Biology Week started in 2012 and has been growing year on year; showcasing the important world of the biosciences, and getting everyone from children to professional biologists involved in life science activities.

The week brings together schools, universities, museums, labs, wildlife sanctuaries, charities and everyone in between in an increasingly diverse collection of events.

This year we are excited to once again be holding a debate in partnership with the Biochemical Society at the Royal Institution – we have also partnered with Cancer Research UK and the topic will be: ‘The DNA revolution: Can we predict people's chance of getting cancer? Should we?’

Other Society events during the week will include our annual awards ceremony, where we will announce the winners of our photography, books and science communication awards, and a Parliamentary Reception in the House of Commons for our members, representatives of our Member Organisations, and parliamentarians to share experiences and discuss science policy issues.

Hundreds of schools and universities around the country will take part in biology events and activities, many of which we developed in partnership with the Biochemical Society, including the Biology Week quiz and the 21st Century BioChallenges Activity Kits; tackling issues around antimicrobial resistance and genetic modification.

We want everyone everywhere to have a chance to be involved with the life sciences and celebrate Biology Week with our community. There is a chance for all to get involved online through games, competitions, interactive resources, articles, social media and citizen science.

The RSB is continuing to work closely with all our Member Organisations, individual members, committees and advisory groups to ensure we fully engage policy makers and politicians on the implication of Brexit for bioscience. Working closely with colleagues in the chemical and physical sciences, we are also seeking to ensure common messaging across the sciences, as we have done for the TEF consultation.

For anyone interested in biology teaching at all levels, I would encourage you to take a look at our recently launched free online ‘virtual special issue’ of the Journal of Biological Education to celebrate its 50th birthday and highlight some of the incredible research it has published.

Editor Professor Ian Kinchin CBiol FRSB put together the issue which includes 40 of some of the most cited and downloaded articles including: ‘Learning difficulties in biology’, ‘The effect of eco-schools on children's environmental value and behaviour’, 'The epidemiology of a zombie apocalypse', and ‘Using role play to debate animal testing’. These articles will be free to everyone until the end of 2016.

The JBE is uniquely broad in its reach; it's a really accessible journal for people who teach the biosciences across the spectrum. Looking to the next 50 years of the JBE, I’m excited to see more research into the interface between school and universities.

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Meeting Reports

New Horizons and Emerging Biomedical challenges for Biophysics

Biennal Meeting of the British Biophysical Society 2016, 6-8 July 2016, University of Liverpool, UK

Svetlana Antonyuk and Samar Hasnain (University of Liverpool, UK)

Over 180 researchers, more than half of them young investigators and PhD students, from 14 countries came to Liverpool to participate in the British Biophysical Society (BBS) biennial meeting in July. The meeting brought together scientists in fields ranging from structural biology to drug discovery to synthetic biology, among others, to share their views and promote discussion on ‘New horizons and emerging biomedical challenges for Biophysics’, which helped to create an outstanding agenda.

The three-day conference started with a plenary lecture from Richard Henderson showing recent advances andremaining barriers in single particle Electron Microscopy (EM). Some interesting speakers in the Cryo EM and X-ray Lasers stream included Sriram Subramaniam (NIH, USA), who described the extension of EM applications to tomography and near-atomic resolution structures of macromolecules and how these are being used for drug discovery, and Helen Ginn, a PhD student from Oxford, who gave an overview of some X-ray free electron laser applications as well as some sophisticated data processing approaches. In the parallel Nuclear Magnetic Resonance (NMR) of Proteins and Cells stream, the leading NMR expert Lucia Banci (Florence, Italy) described exciting experiments using in-cell NMR to probe the movement and use of metal ions in living cells.

After the coffee break, delegates were provided with two highly attractive streams on Ion Channels and Transporters and Molecular Recognition. The former was opened by an excellent talk by Sir Munir Pirmohamed (Liverpool, UK) on drug toxicity and the importance of knowing transporters variability in patients in order to prevent adverse drug reactions. The latter was opened with an equally well-received talk by Chris Schofield (Oxford, UK). Both streams gave opportunities for established speakers and young scientists to present their latest work. The scientific session of the day concluded with a flash poster presentation from five poster presenters, each of seven minutes duration, which proved very popular.

The second day opened with a plenary lecture delivered by Toshihide Yamashita (Osaka, Japan) who presented an overview of clinical and biochemical investigations in “Targetting a cure for paralysis”, which examined the recovery of the nervous system from injury and his latest research findings, which focus on the molecular mechanism of neuronal rewiring regulation in neuronal injured and neurodegenerative disorders. His talk had direct implications for a number of neurodegenerative diseases. Later in the morning the delegates could listen to talks on Single Cell Biophysics or Biophysics in Human Diseases. Violaine See (Liverpool, UK) and Mike White (Manchester, UK) provided the latest advances and some beautiful examples of imaging applications to complex systems, Stefan Marklund (Umeå, Sweden) explained how one protein aggregates in different ways in motor neuron disease and Jose Mato (Spain) showed how MAT enzymes were involved in liver disease function.

In the afternoon, Nigel Robinson’s talk was a clear highlight of the Metals in Biology stream where we learnt how many toxic metals are handled by biology, ensuring that all of the essential metals are utilized efficiently.
The second day closed with the highly-enjoyable gala dinner, served in The Crypt Hall of the Metropolitan Cathedral. Sir Tom Blundell welcomed the delegates to the Gala dinner and highlighted some of the excitement of the conference as well as the central importance of these Biennial Biophysics conference which provide a clear focus to the community. The Guest of Honour was Louise Ellman, MP for the local constituency of Liverpool, Riverside and Chair of the Parliamentary Select Committee for Transport. She noted the importance of regional, national and international cooperation in scientific advances and spoke to us about the importance of communicating the importance of our scientific work to the general public. Anthony Watts (Oxford), Chair of the BBS, also spoke to thank the organisers and announced the winner of the BBS 2016 Young Investigator’s Medal Adam Perriman (Bristol), with Sir Tom Blundell presenting the medal.

There were more than 50 excellent posters to choose from and competition for poster prizes was stiff. Some of the well-deserving prize winners included: Ewan Ramsay (Manchester, UK) for the poster “The Structure of the Human Retinal Protein Retinoschisin and Analysis of Disease-causing Mutations”, Florian Stroel (Cambridge, UK) for the poster “dSTORM superresolution imaging to study the role of endogenous alpha-synuclein” and Didi He (Edinburgh, UK) for her poster “How do bacterial nanocompartments store iron?”.

This being Liverpool, the Beatles had to feature somehow, so the dinner was followed by lively dancing to the music of the Mersey Beatles (an accomplished Beatles look-alike and sound-alike band).

The third and final day featured the themes of Drug Discovery and Membrane Proteins and Complexes with three outstanding plenary lectures. Sir Tom Blundell (Cambridge) opened the day with his talk “Fighting Drug Resistance in Cancer and Infectious Diseases: how Biophysics can contribute”. This was followed by two parallel streams. Drug Discovery featured Paul O’Neil and Ben Bax among others while the Membrane Proteins and Complexes stream had highlights from James Naismith and Yvonne Jones. The conference closed with two plenary talks. Adam Perriman, the young scientist BBS award winner gave a talk on “A life less aquatic – structure, function and dynamics in solvent-free liquid proteins”. The final plenary talk was given by Gregory Petsko (Weill Cornell Medical College) in characteristic highly communicative style. He talked on “New Therapeutics for Alzheimer’s and Parkinson’s Diseases Using Structure-Guided Approaches” and kept the audience captivated for the whole hour.

We are very grateful to our sponsors and exhibitors: Astex Therapeutics, the Biochemical Society, Bruker, Dectris, FEI, the International Union of Crystallography, Molecular Dimensions, NanoTemper Technologies, Oxford Cryosystems, Oxford Nanolming and Rigaku, for helping to make the meeting a memorable event.

Ewan Ramsay receives a poster prize from Jonathan Agbenyega of the International Union of Crystallography

Anthony Watts awards a BBS poster prize to Didi He

Florian Stroehl receiving a BBS poster prize from Anthony Watts
Given the success of the event and the large positive feedback received, we hope to meet all the participants again at the next LRRK2 meeting.
Harry Bradford (1938–2016)

Following a fall at home, Harry Bradford passed away after a long hospitalization on the David Marsden ward at King’s College Hospital at Denmark Hill, located across Camberwell Road from the Maudsley Hospital where Harry worked towards his PhD degree.

I met Harry in 1962. I had just finished my neurological training, and came to London for research training in neurochemistry under Professor Henry McIlwain at the Institute of Psychiatry located at the Maudsley Hospital in Denmark Hill. I was told to spend some time with a young graduate student, Harry Bradford, who taught me how to stun a guinea pig and remove its brain and to fractionate the brain material into what are called subcellular fractions. Harry helped me a lot and we became friends. Not only did he teach me some neurochemical techniques, he introduced me to cockney rhyming slang, and when to use the phrase ‘Bob’s your Uncle’. He introduced me to historical scientists such as Charles Darwin and TH Huxley and to the seventeenth century archivist and Naval administrator Samuel Pepys.

After my training I returned to the States, but always kept in contact with Harry and his family, and we often visited one another, and kept in frequent email and telephone touch.

Harry received his PhD from University of London in 1964, followed by an MRC Postdoctoral fellowship to learn neurophysiological techniques in the same laboratory.

In October 1965, Harry joined the biochemistry department at Imperial College as Lecturer under the direction of Professor Sir Ernst Chain, Nobel Laureate for his work on the purification of penicillin. Harry rose to the rank of full Professor and finally Emeritus Professor status upon his retirement in 2003. He became internationally recognized in his chosen field, which was the mechanisms involved in neurotransmitter formation and release. Harry used pinched-off nerve endings called synaptosomes to study transmitters and how they relate to diseases such as epilepsy and Parkinson’s disease. He was first interested in the amino acid glutamate, a major excitatory transmitter that is important in epilepsy. Another neurotransmitter, called dopamine, is critically reduced in Parkinson’s disease. Harry not only studied normal brain tissue, but also studied animal models of Parkinson’s disease and epilepsy and devised ways of turning cells that used one kind of transmitter into ones that used only dopamine. He could put the new dopamine cells into ‘Parkinsonian animals’ and correct their condition.

If he had chosen, he could have become Head of Department, but he preferred to continue his research and trained many important scientists such as Jacqueline De Bellerinche and John Hardy. In 2006 some of his students produced a Festschrift in Harry’s honour. He has earned a number of honours including a Bronze Medal for contributions to neurochemistry from the University of Okinawa, Sandoz Medal Lectures at London University and the Silver Jubilee Lecture and Medal in Calcutta, India.

Harry was a great organizer of scientific meetings on behalf of Biochemical Society in the 1970s and 80s. He had a great interest in the history of science, and as the Society’s Honorary Archivist (1988-1995), Harry developed a programme of 35 in-depth video-interviews with eminent biochemists. I observed one of these interviews in Vancouver and it was very professionally done. Among those interviewed were great scientists such as Fred Sanger, Max Perutz, Dorothy Hodgkin and Joseph Needham. As Features Editor and later as General Editor of The Biochemist (1989-1992) Harry also commissioned several historical articles for the magazine.

After formal retirement, Harry kept very actively involved in science. He has given invited lectures in US, Argentina, Japan, India, Singapore and Canada.

Harry had wide interests and a sense of adventure. He showed me many places in London and Kent, including the church where Samuel Pepys worshipped; Michael Faraday’s laboratory at the Royal Institution; Eltham Palace; and Down House, Darwin’s home, where we were once shooed away by a woman with a broom when we tried to peer in a window when the home was closed. We played Poohsticks on Poohsticks bridge, visited Churchill’s home at Chartwell, antique shops in Brasted, went to plays and operas in London and Seattle and, in recent years, thrift shops in Palm Springs, where Harry would usually find a biography of a Hollywood star.

The Harry I knew was not only an extremely knowledgeable and productive scientist, but also a warm, warm friend. He is profoundly missed by his wife Mary-Therese, his children Sonya and Daniel and his five grandchildren.

By Phillip Swanson
(University of Washington, USA)
Do you know someone who deserves recognition for their outstanding contribution to the molecular biosciences?

**Nominations are now open for the 2018 Biochemical Society Awards.**

Recognizing established and early career researchers, scientists, educators and industry partners for their contribution to the molecular biosciences; we encourage nominations that reflect the diversity of the bioscience community.

**SUBMIT YOUR NOMINATION BY 31 JANUARY 2017**

[www.biochemistry.org/Awards](http://www.biochemistry.org/Awards)

*Nominations can be submitted by both members and non-members of the Biochemical Society. All awards carry an honorarium and recipients are invited to submit an article to a Society-owned publication.*
The 2018 Awards

Announcing three new awards categories for the first time for 2018

Teaching Excellence Award
Recognizes an outstanding individual working in Higher Education who champions the importance of excellence and innovation in biochemistry teaching in order to advance student learning and achievement, both within and beyond their own department and institution.

Industry and Academic Collaboration Award
Awarded to an outstanding individual who has made an inspirational contribution to the biosciences and to industry–academia interactions.

International Award
Recognizes distinguished and independent interdisciplinary research conducted outside of the UK and Ireland that illustrates the importance of molecular biosciences in the advancement of life science research.

The Centenary Award—Awarded to an international biochemist of distinction

The Colworth Medal—Recognizes outstanding research by a biochemist in the early stage of their career

Early Career Research Awards
In 2018 the awards recognize the breadth of science across the following areas:
- Biological Systems
- Cells
- Computational Biology
- Molecular Structure and Function

GlaxoSmithKline Award—Recognizes research in the field of biochemistry related to medicine

Keilin Memorial Lectureship—Awarded to a research scientist working in the fields of bioenergetics, electron transfer and mitochondrial biology

Morton Lectureship—Recognizes outstanding contributions to lipid biochemistry

The Novartis Medal and Prize—in recognition of contributions to the development of any branch of biochemistry


‘Holding Hands with Bacteria: The Life and Work of Marjory Stephenson’

Soňa Štrbáňová (2016) DOI 10.1007/978-3-662-49736-4

The Czech scholar, Soňa Štrbáňová, has written a delightful biography of the pioneering biochemist and microbiologist Marjory Stephenson (1885-1948), a founding figure in her subject and one of the first two women elected to be a fellow of the Royal Society. Although quite short, this is the first full biography of Stephenson and it catches effectively her work and her personality and puts them in the context of the times she lived in.

For a girl in the late 19th century, Stephenson was fortunate to be encouraged to study and to take the opportunities then available for women’s education. By 1903 she was studying chemistry and biology at Newnham College Cambridge and soon after was earning her living as a Lecturer at a College of Domestic Science and then at ‘King’s College for Women’ (later Queen Elizabeth College of London University). In 1911, she was invited to UCL by Robert Plimmer (a founder of the Biochemical Society) to join him in his research and to teach nutritional biochemistry. Soon after that, she was awarded a prestigious Beit Research Fellowship. But World War I intervened, and Stephenson volunteered to run kitchens and nutritional services supporting troops in France and later in Salonika; she was clearly a good manager, improviser and organizer and was awarded the MBE for this war work. At the war’s end she reactivated her Beit Fellowship and moved to Cambridge to join the growing focus of biochemical research there, stimulated by F. Gowland Hopkins. Although she initially continued in nutritional research, by 1921 she switched to study bacterial metabolism and it was her work in this field over the next quarter-century that made her name and established bacteria and other microorganisms as ideal model organisms for biochemical – and later genetic – research.

Štrbáňová traces the development of Stephenson’s research from the early introduction of techniques such as working on washed suspensions (‘resting cells’) and the development of nutrient ‘balance sheets’ to analysis of intracellular enzymes, some – such as lactate dehydrogenase – already known in higher organisms, and others entirely novel – such as the enzymes involved in reducing formate to methane. A development from this work was the realization (though not the terminology) that some enzymes are constitutive while others are only found after exposure to the appropriate nutritional conditions; this latter group were defined as ‘adaptive’. During the 1930s, Stephenson and her students and colleagues popularised E.coli as a model organism and characterised extensively the phenomenon of enzyme ‘adaptation’, laying the foundations for the work of Monod and colleagues (from 1940 onwards) that underlie our current understanding of the regulation of gene expression.

Stephenson regularly reviewed the field of bacterial metabolism for Annual Reviews of Biochemistry and produced in 1930, 1939 and (posthumously) in 1949, three successive editions of her major textbook ‘Bacterial Metabolism’. These volumes consolidated her position as an international authority but did not consolidate her professional position or her funding. The account of Stephenson’s relationships with the MRC and with Cambridge University is among the most interesting aspects of Štrbáňová’s biography. From the early 1920s, Stephenson had received personal salary funding from the MRC and from 1929 was appointed a member of MRC ‘External Staff’. The MRC then, as now, ran much of its research through ‘Units’ embedded in universities and clearly regarded their funding of Stephenson as their major investment in bacterial metabolism – but they could not bring themselves to provide consistent technical support or to appoint her as a Unit Director. So they did not formally establish her operation as a Unit and Stephenson conducted a polite but pointed war with the MRC over the lack of formal recognition for her operation; Štrbáňová gives a wry account of this, based on MRC archives and Stephenson’s personal correspondence. (Immediately after her death, the group was formally established as a Unit and Stephenson’s former student, Ernest Gale – almost 30 years her junior – was appointed as its Director!). Similarly, although Stephenson taught bacterial metabolism and supervised Cambridge PhD students throughout the 1920s and ’30s, the University only recognised the subject and appointed her to a Readership in 1947!

It was only after World War 2, during which Stephenson had redirected the research of her group to topics of immediate national importance, that formal honours came her way. She had been active in discussions with a wide range of medical, industrial, academic and agricultural microbiologists that led to the formation in 1945 of the Society of General Microbiology (renamed last year as the Microbiology Society) and she became its second President in succession to Sir Alexander Fleming. And in 1945, when the Royal Society finally acknowledged that its exclusion of women had been illegal for some years, Stephenson was one of the first two women elected FRS.

Throughout the biography, Štrbáňová uses primary sources (correspondence, MRC archives, Newnham College Archives) not only to trace Stephenson’s career but also to point up her striking personality – modest and unassuming but self-confident in her abilities, unpretentious and a derider of pretension in others, an able organizer, a caring and supportive research supervisor and someone who engaged with wider world affairs without wearing her political heart on her sleeve. During the 1930s, she was active in ensuring that biochemists from Germany and Central Europe could find positions in Britain, including the Czech scholars Waelsch and Kleinzeller, and most notably Hans Krebs. As early as April 1933, when Jewish academics were beginning to be dismissed from academic posts in Nazi Germany, Stephenson wrote to Otto Warburg in Berlin enquiring about Krebs’ situation and offering her support. As a result, Krebs was able to come to work in Cambridge in June 1933 (bringing with him Warburg manometers, then novel instruments for metabolic research), and this began a lifelong working relationship between Krebs and Stephenson based on good-humoured mutual respect.

Stephenson clearly possessed a self-deprecating wit and made many contributions to the Cambridge Department of Biochemistry’s house magazine ‘Brighter Biochemistry’ (1923 – 1931) including a droll mock book review of her own ‘Bacterial Metabolism’ and an
Soňa Štrbáňová’s achievement in this outstanding biography is that she stimulates thought on all these issues while giving a full and well-illustrated account of the life, work and times of her subject.

Robert Freedman (University of Warwick, UK)

Manual of Clinical Microbiology Volumes I & II


Manuals of Clinical Microbiology I & II is unequivocally the A to Z of clinical microbiology as every subject imaginable from “Abiotrophia to Zygomycetes” is covered. As books, they are behemoths. That the manuals take up so much space is not surprising; every conceivable classical microbiological technique is here, neatly dovetailing with contemporary techniques in molecular microbiology which together, characterise, classify and identify the most obscure microorganisms and their metabolites. Some of these techniques are state of the art: matrix–assisted laser desorption ionisation (MALDI-TOF) for the analysis of proteins, polymerase chain reaction (PCR) amplicons and microbial metabolites. Other important methods such as 16S sequencing and microbiome analysis have revolutionized the identification of lineages within species and can precisely identify base pair differences between genes. In these manuals, there is a comfortable acknowledgement of classical microbiological methods at work and homage is paid to classical microbiological methods which are the backbone of contemporary microbiology practices.

Not being intended to be read from cover to cover, these books should be used for referencing and fact finding. However, a chapter on Bioterrorism is edifying from a historical perspective; apparently this form of warfare was prevalent centuries ago. In the 1300s, Tartars catapulted Yersinia pestis riddled corpses into besieged cities to start epidemics. In the 1760s, British soldiers gave native Indians smallpox-riddled blankets to reduce their numbers in order to illegally seize their lands. Indeed, as recently as 20–30 years ago, the USA legislated for the acquisition, transfer and use of bioterrorism agents after a white supremacist fraudulently obtained Yersinia pestis from a Biotechnology company. Thankfully, these threats are not prevalent in today’s society.

The gut microbiome is cited as the forgotten organ and is currently a hot topic as perturbations in microbiome consortia are believed to contribute to several debilitating diseases/disorders such as Crohn’s Disease, ulcerative colitis, type 2 diabetes and obesity. With as many as 10^{12} bacteria per gram, the main bacteria in the gut comprise the phyla Bacteroidetes and Firmicutes and depending on diet and weight, bacterial levels fluctuate, leading to perturbations in gut function. Metagenomics has revolutionised this aspect of molecular microbiology and provides insights into hitherto unknown interactions between these bacteria and adjacent human cells.

Volume II contains extensive sections on mycology, how to grow and characterise yeast isolated from clinical samples. One section that will resonate with those of us allergic to work on Mondays is the notion of “Sick Building Syndrome” (SBS). Cited as indoor environments suspected of causing health problems for occupants, mycotoxins are proposed as one possible source of this malady. Supposedly emanating from Aspergillus, Penicillium and Alternaria spp to name but a few, there is no concrete proof these mycotoxin producing fungi are responsible. Indeed, the evidence suggests otherwise, mycotoxins are not volatile, some conditions are not conducive for mould growth, and others differ in their abilities to produce mycotoxins. So feeling glum on a Monday morning is likely to be purely psychosomatic, but not something clinical microbiology can fix.

In some parts of the manuals, there is a dearth of images and figures which can be slightly numbing to the brain. However, where there are images, they are striking. For instance who knew Yersinia enterocolitica and Yersinia pseudotuberculosis could be so spectacular on sheep blood agar. Similarly, the geometric uniformity of gastroenteritis viruses is almost artistic: images include the norovirus which afflicts hospitals and emergency departments. This review does not do justice to these manuals. The accumulated knowledge is exhaustive which is why these books take four years to update and complete. Suffice it to say, every clinical microbiology laboratory ought to have these manuals in its clinical armoury.

John Phelan (University College Cork, Ireland)
Unsurprisingly, the result of the EU referendum has been a major subject of discussion within scientific circles since June. It will undoubtedly have a considerable impact upon the molecular bioscience community and the wider science sector. The Biochemical Society is keen to ensure that the UK molecular bioscience community maintains its high international profile and that its open approach to trans-national collaborative opportunities remains clear. Therefore, we are working together with the Royal Society of Biology (RSB), Campaign for Science and Engineering (CaSE) and other learned societies to ensure that the science agenda is represented in the forthcoming EU exit negotiations. Over the next few months, we will be gathering evidence to inform our policy activities. If you have any case studies of ways in which your institution or colleagues have been affected by this issue, please do email us on at policy@biochemistry.org.

You may have read in previous issues about the Society’s Governance Review that Society members were asked to vote on at the Annual General Meeting in July. The changes proposed by the Governance Review were accepted by the membership and we hope will:

• Create clear, transparent and responsive governance structures, policies and procedures
• Readjust the Trustee body to ensure more proportional representation of the breadth of our membership, and enable enhanced focus on strategic matters
• Create an agile Executive Management Team to ensure continued momentum on the implementation of our strategy
• Encourage inter-functional and inter-Society collaboration, in particular with the Biochemical Society Strategy for the Molecular Biosciences (Three Year Strategy)\(^1\)

With these, and other approved changes, we hope to deliver a Society with governance systems to enable us to move more seamlessly into the future. The changes related to the review, including the changes to our constitution will be implemented over a period of six to eight months. Full details will shortly be available on the Society website ‘About Us’ page.

We have now appointed a new Head of Conferences and Events, Lorraine Reese. Lorraine joined us at the beginning of October from the International Society of Ultrasound in Obstetrics and Gynaecology where she worked as an Events Manager. She will work closely with Honorary Meetings Secretary, Sheila Graham, the Theme Panels, the Conferences Committee and myself to ensure that the Society continues to deliver high quality scientific meetings. She will also to work closely with other departments to ensure that opportunities for collaboration are identified with our journals and training activities.

I am delighted to welcome the Editors-in-Chief for our two new journals. Colin Kleanthous, Professor of Biochemistry at the University of Oxford, UK, (and the first Chair of the Biochemical Society I worked with when I became CEO) is now Editor-in-Chief of Emerging Topics in Life Sciences and Aideen Sullivan, Professor of Neuroscience at University College Cork (UCC), Ireland, is the Editor-in-Chief of Neuronal Signaling.

Next month we will be attending Neuroscience 2016 where we will showcase Neuronal Signaling ahead of its launch in early 2017. The conference will be the 46th Society for Neuroscience annual meeting and will take place November 12–16 at the San Diego Convention Center.

October also sees the fifth UK and international Biology Week (8–16 October), an annual celebration of the biosciences organized by the Royal Society of Biology (RSB). One of the highlights of the week promises to be a Biochemical Society panel discussion at the Royal Institution (RI) in London in partnership with the RSB and Cancer Research UK. The event, chaired by the science writer and broadcaster Vivienne Parry OBE, will explore the efficacy and impact of DNA testing for cancer susceptibility. Last year’s debate at the RI was a sell-out, attracting over 440 attendees, so we are hoping for another successful event. Other Biochemical Society events taking place in Biology Week include the Bioscience Careers Festival (in partnership with RSB), Big Biology Day (in partnership with the British Pharmacological Society) and a Policy Lates event on antimicrobial resistance in partnership with other UK members of the European Federation of Biotechnology and Learned Society Partnership on Antimicrobial Resistance (LeSPAR).

Coming up next month, we have the 2nd Synthetic Biology UK conference. SynBio UK will take place from 14–16 November 2016 in Edinburgh and aims to showcase UK Synthetic Biology research and to create a focal point for the community. Further information on the conference is available here: http://bit.ly/2csOU2r. Following last year’s success, topics covered at this meeting will be published in Biochemical Society Transactions. You can read review articles showcasing the exciting synthetic biology research presented at SynBio UK 2015 in Issues 44(3) and 44(4) of the journal. In addition, make sure you do not miss the next issue of Essays in Biochemistry on Synthetic Biology guest edited by Vitor Pinheiro, University College London (UCL), which will be published in late November.

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Since I wrote my last piece for *The Biochemist*, the big news has been the UK electorate’s vote to exit from the European Union, and the installation of a new government that will oversee this. For science in general, and life sciences in particular, this is not good news, but as this week’s *Nature* proclaims, UK science is sufficiently resilient to survive and I am sure it will. Meanwhile many column inches are being filled, and words being spilled, discussing the amount of damage that UK Science will suffer, with predictions ranging from ‘none’ to ‘a lot’, but with nobody foreseeing any benefits. To my mind this just underscores the importance of explaining our science and arguing our case. In my opinion, it also shows that science really wasn’t very high on the agenda of the folk who voted ‘leave’. Of course, the other matter I mentioned in my last ‘From the Chair’ piece was the forthcoming AGM and the need to take a decision on the proposed new governance arrangements for the Society. In the event, the AGM was a happy affair and the proposals were approved, so now I believe that we will be ‘fit for purpose’ for the future, as we enter the world of Brexit and beyond. During his welcome speech to guests at a small reception after the AGM, our President, David Baulcombe, pointed out that Learned Societies are needed now more than ever before. I thought that was well worth underscoring and I also think that our molecular bioscience focus gives us a clear and distinct role, as we push our message. Talking of the distinct nature of our Society, I write this from the ninetieth Harden conference. Hopefully most of you will know that these conferences have a distinct brand, running right back to the first one, organised by David Phillips in 1969. When I was a student the Hardens were mostly held at Wye College just outside Canterbury in Kent. These days the venues are scattered all over the UK and this time we are in a village on the edge of the Peak District, in a rather grand hotel, with over 100 participants, enjoying top notch science around the theme ‘machines on genes’. I do think that this formula works, and not only for the early career participants, simply because everyone is together and stays together. Successive Honorary Meetings Secretaries have grappled with the issue of what makes the perfect meeting. I suspect there is no one answer, but I do think that the Harden formula has a lot to offer.

Finally, the theme of this issue, gaseous signalling, is going to provide us with some interesting insights that I suspect might have fascinated the Society’s founding fathers, who put the word ‘Biochemical’ into our name. So let me finish with a little challenge. How much of the cell’s present biochemistry derives from the time when there was no free oxygen gas, but high carbon dioxide, hydrogen and lots of soluble iron? Then came the key cyanobacterial moment when water could be converted to oxygen. Which parts of our present biochemistry does this account for? Enjoy!

Reference

### Crossword Competition

**Win**

This month’s crossword prize is a Weather Station With Outdoor Sensor / Transmitter.

Simply email the missing word, made up from letters in the highlighted boxes to biochemist@biochemistry.org, by Monday 31st October 2016. Please include the words ‘October crossword competition’ in the email subject line.

**Congratulations to the winner of the August competition:**

The missing word from last issue’s competition was EXTRACELLULAR. Orla Coleman from Dublin received a nerve cells mug as the prize.

**Terms and conditions:** only one entry per person, entrant must be a current Biochemical Society member; closing date Monday 31 October 2016. The winner will be drawn independently at random from the correct entries received. The winner will receive a Weather Station With Outdoor Sensor / Transmitter. No cash alternative available. No employee, agent, affiliate, officer or director of Portland Press Limited or the Biochemical Society is eligible to enter. The winner will be notified by email within 7 days of the draw. The name of the winner will be announced in the next issue of *The Biochemist*. The promoter accepts no responsibility for lost or delayed entries. Promoter: Biochemical Society, Charles Darwin House, 12 Roger Street, London WC1N 2JU; do not send entries to this address.
Summer Vacation Studentships

Vacation lab placements for undergraduate students. Summer 2017

Grants are available for stipends of £200 per week for 6 - 8 weeks, and up to £1,600 in total, to support an undergraduate student to carry out a summer lab placement.

This scheme not only benefits the student as they get valuable research experience, but the supervisor also gains an extra pair of hands in the lab.

THE DEADLINE FOR APPLICATIONS IS 24TH FEBRUARY 2017

For full details on the criteria and more information on how to apply, please visit www.biochemistry.org/Grants/EducationalGrants/SummerVacationStudentships or contact education@biochemistry.org
Apply now for a Biochemical Society Diversity in Science Grant 2016

The Biochemical Society is offering grants of £500 to individuals, groups and charities to support and address issues relating to diversity in science.

Whether you are planning an event or activity to encourage diversity in science, or conducting research into the lack of representative diversity within the science sector, apply for one of our grants now!

Funding will be available for projects completed by 30 November 2017. This grant scheme is also open to applicants from outside of the UK and non-members.

Please note: in exceptional circumstances, at its discretion, the Society may award up to £1,000 for individual projects.

To find out more or apply, visit www.biochemistry.org/Grants/DiversityinScienceGrants

Deadline for applications: 31 October 2016.

Image: Dundee Women in Science Festival 2016