Microbiology TODAY

43:3 August 2016

Future Tech

Nanoscale imaging
Two-dimensional infrared spectroscopy
Cryogenic electron microscopy
CRISPR-Cas
Phage therapy

MICROBIOLOGY SOCIETY

CHLORAMPHENICOL

CAPSULES

- Widely distributed throughout the body, including CSF[1]
- Oral levels comparable to i.v. levels[2]
- Rarely implicated with *C.difficile*[3,4]

Effective against serious infections including:

- *H. influenzae*[1,5]
- Typhoid[1,5]
- MRSA[2]
- VRSA[6]
- Neisseria[1,5]
- Legionella[1,5]
- Rickettsia[1,5]
- *C.difficile*[7-10]
- *E. coli*[1]

Abbreviated Prescribing Information
Chloramphenicol Capsules BP 250mg

Presentation: Hard Gelatin Capsules.
Indications: Typhoid fever and life-threatening infections, particularly those caused by *Haemophilus Influenzae*, where other antibiotics will not suffice.
Posology: For oral administration.
Adults and elderly: 50 mg/kg body weight daily in 4 divided doses. For severe infections (meningitis, septicaemia), this dose may be doubled initially, but must be reduced as soon as clinically possible. Children: Not recommended.
Contra-indications: Known hypersensitivity or toxic reaction to chloramphenicol or to any of the excipients. Should not be used for the prophylaxis or treatment of minor infections; during active immunisation; in porphyria patients; in patients taking drugs liable to depress bone marrow function; during pregnancy, labour or by breast-feeding mothers.
Special warnings and precautions for use: Use only if other treatments are ineffective. Use should be carefully monitored. Reduce dose and monitor plasma levels in hepatic or renal impairment; in the elderly; and in patients concurrently treated with interacting drugs.
Interactions: Chloramphenicol prolongs the elimination, increasing the blood levels of drugs including warfarin, phenytoin, sulphonylureas, tolbutamide. Doses of anticonvulsants and anticoagulants may need to be adjusted if given concurrently. Complex effects (increased/decreased plasma levels) requiring monitoring of chloramphenicol plasma levels have been reported with co-administration of penicillins and rifampicin. Paracetamol prolongs chloramphenicol half-life and concurrent administration should be avoided. Chloramphenicol may increase the plasma levels of calcineurin inhibitors e.g. ciclosporin and tacrolimus. Barbiturates such as phenobarbitone increase the metabolism of chloramphenicol, resulting in reduced plasma chloramphenicol concentrations. In addition, there may be a decrease in the metabolism of phenobarbitone with concomitant chloramphenicol use. There is a small risk that chloramphenicol may reduce the contraceptive effect of oestrogens. Chloramphenicol reduces the response to hydroxocobalamin. Chloramphenicol is contra-indicated in patients taking drugs liable to suppress bone marrow function e.g. carbamazepine, sulphonamides, phenylbutazone, penicillamine, cytotoxic agents, some antipsychotics including clozapine and particularly depot antipsychotics, procainamide, nucleoside reverse transcriptase inhibitors, propylthiouracil.
Pregnancy and Lactation: The use of chloramphenicol is contra-indicated as the drug crosses the placenta and is excreted in breast milk.
Effects on ability to drive and use machines: No significant effect on driving ability.
Undesirable Effects: Reversible dose related bone marrow depression, irreversible aplastic anaemia, increased bleeding time, hypersensitivity reactions including allergic skin reactions, optic neuritis leading to blindness, ototoxicity, acidotic cardiovascular collapse, nausea, vomiting, glossitis, stomatitis, diarrhoea, enterocolitis, Gray Baby Syndrome particularly in the newborn, which consists of abdominal distension, pallid cyanosis, vomiting, progressing to vasomotor collapse, irregular respiration and death within a few hours of the onset of symptoms.
Overdose: Stop chloramphenicol immediately if signs of adverse events develop. Treatment is mainly supportive. If an allergy develops, oral antihistamines may be used. In severe overdosage e.g. Gray Baby Syndrome, reduce plasma levels of chloramphenicol rapidly. Resin haemoperfusion (XAD-4) has been reported to substantially increase chloramphenicol clearance.
Pack size and Price: 60 capsules £377.00
Legal Category: POM.
Market Authorisation Number: PL17736/0075.
Market Authorisation Holder: Chemidex Pharma Limited, 7 Egham Business Village, Crabtree Road, Egham, Surrey TW20 8RB, UK.
Date of preparation: January 2016.
See Chloramphenicol Capsules Summary of Product Characteristics for full prescribing information.

Adverse events should be reported. Reporting forms and information can be found at www.mhra.gov.uk/yellowcard. Adverse events should also be reported to Essential Generics on 01784 477167.

References:
1. Martindale: The Complete Drug Reference. Chloramphenicol. [Online]. Available from: http://www.medicinescomplete.com [Accessed 17th September 2015]. **2.** Fluit, A.C., Wielders, C.L.C., Verhoef, J., and Schmitz, F.J. Epidemiology and susceptibility of 3,051 Staphylococcus aureus isolates from 25 university hospitals participating in the European SENTRY Study. Journal of Clinical Microbiology. 2001; 39(10): 3727-3732. **3.** Kelly, C., LaMont. Patient information: Antibiotic-associated diarrhea caused by Clostridium difficile (Beyond the Basics).June 2015. **4.** Bartlett J.G. Antimicrobial agents implicated in Clostridium difficile toxin-associated diarrhea of colitis. Johns Hopkins Med J. 1981; 149(1): 6-9. **5.** Feder, H. Chloramphenicol: What we have learned in the last decade. Southern Medical Journal. 1986; (79)9: 1129-34. **6.** Weigel LM *et al.* High-Level Vancomycin-Resistant Staphylococcus aureus Isolates Associated with a Polymicrobial Biofilm. Antimicrob Agents Chemother. 2007 Jan; 51(1): 231-238. **7.** Ensminger, P., Counter, F., Thomas, L., Lebbehuse, P. Susceptibility, resistance development, and synergy of antimicrobial combinations against Clostridium difficile. Current Microbiology. 1982; 7: 59-62. **8.** Poilane, I., Bert, F., Cruaud, P., Nicolas-Chanoine, MH., Collignon, A. Interest of the disk diffusion method for screening Clostridium difficile isolates with decreased susceptibility to antibiotics. Pathologie Biologie (Paris). 2007; 55(8-9): 429-33. **9.** Cattoir, V., Ould-Hocine, ZF., Legrand, P. Antimicrobial susceptibility of Clostridium difficile clinical isolates collected from 2001 to 2007 in a French university hospital. Pathologie Biologie (Paris). 2008; 56(7-8): 407-11. **10.** Brazier, JS., Levett, PN., Stannard, AJ., Phillips, KD., Willis, AT. Antibiotic susceptibility of clinical isolates of clostridia. Journal of Antimicrobial Chemotherapy. 1985; 15(2): 181-5.

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Editorial

Science is a broad church and the designation of scientific disciplines fascinates me. Four overarching categories map the sciences: formal science, social science, applied science and natural science. The life sciences, considered the study of any living organism, are a strand of the natural sciences, and microbiology, the study of micro-organisms, is one of more than 20 sub-branches found under the 'life sciences' umbrella. As technologies and scientific knowledge grew, the number of branches and sub-branches expanded in tandem and continues to today. The definition of each branch and sub-branch stakes a claim to a unique selling point that gives it an identity and a focus, but, nevertheless, with overlap.

Advances and tools from the study of microbiology underpin several of the branches of life sciences. Restriction enzymes and cloning technologies, cloning vectors and overexpression vectors, heat-stable DNA polymerases and DNA sequencing, and the polymerase chain reaction (PCR) are cornerstone tools of many other branches including biochemistry, molecular biology, genetics, genomics, immunology… The list goes on.

Mining microbes for molecular tools is an on-going process and sea-change technologies continue to emerge. The relative ease with which microbes can be manipulated has also enabled the development of new technologies that can be applied to the study of life sciences and present potential solutions to global problems. This issue celebrates some of these significant tools, techniques and technologies from microbes.

First, S. Andrea Gazze, Lewis Francis and Geertje van Keulen describe how exciting novel microbial properties and behaviours can be revealed by applying nanoscale analytical techniques, such as atomic force microscopy, to pure cultures and *in situ* ecosystems such as the soil.

One of the central themes of biology is that structure is linked to function. Niall Simpson, Neil T. Hunt and Paul A. Hoskisson's article describes how true collaborations between microbiologists and physicists can lead to fascinating insight into biological systems, especially when applying exciting new techniques. They describe how fast, dynamic structure–function relationships can be studied using ultrafast two dimensional infrared spectroscopy (2D IR) that is uniquely sensitive to structural dynamics and is capable of extending the range of information available to microbiologists studying molecular interactions. This is followed by David Bhella's fascinating article on the emerging technology of cryo-electron microscopy.

All organisms are exposed to parasites, and evolved a range of different immune mechanisms to defend themselves. Edze Westra describes how scientists have discovered that bacteria and archaea have a sophisticated adaptive immune system, known as CRISPR-Cas (clustered regularly interspaced short palindromic repeats). The insights gained about this system have led to several applications in industry to protect bacterial species against their viral parasites. In addition, CRISPR-Cas has been turned into a versatile genome editing method that has the potential to treat human genetic diseases.

I have written the last article on the growing global problem of antibiotic resistance. One approach to tackle this issue is to find alternatives to antibiotics used to combat pathogens. In this article I describe how the increasing understanding of bacteriophage and their lifecycles might offer an alternative to new antibiotic discovery.

Finally, Justin O'Grady has written a Comment article that highlights the need for a paradigm shift in diagnostics technology, to allow for the development of a rapid universal diagnostic that can detect any pathogen or resistance for the effective treatment of life-threatening infections. He describes how next-generation sequencing technology, particularly rapid nanopore sequencing and Oxford Nanopore Technologies' MinION device, has the potential to drive this technology gear-shift forward by combining rapidity with comprehensiveness beyond simple microbial culture or PCR.

Microbiology has transformed the life sciences. Microbiologists and microbes continue to be at the forefront of new invention and innovation, and and are pioneering the technology of the future.

Laura Bowater
Editor
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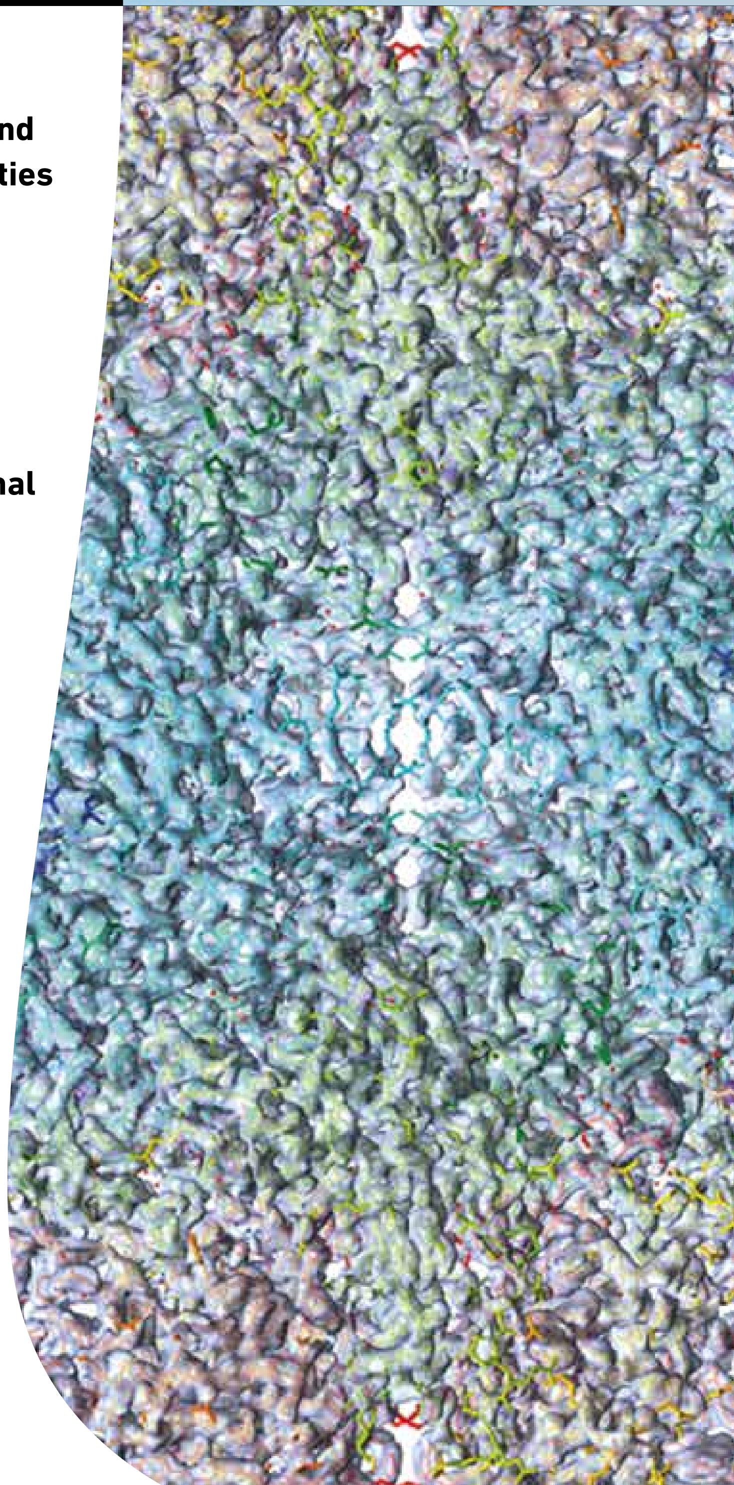

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MICROBIOLOGY SOCIETY

Council 2016

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From the President

As the summer months arrive and the academic terms have been wound up, more time may be available for us to complete manuscripts and grant applications. For Microbiology Society staff, Executive Officers and Committee members this is also a period of intense planning for the future. We are looking forward to seeing our plans from last year transform into action in the coming months. For example the Early Career Microbiologists' Forum will shortly be formed.

Applications for the recently advertised vacancies in our Committees will soon be reviewed and I am pleased to hear that we have had a particularly encouraging number of members applying for the places in our Early Career Microbiologists' Forum. The ECM Forum is our way to capture the voice and insights of active scientists who, for the most part, will still be at the bench, and many will yet to have been appointed to a permanent post. This is the group within our membership who are still generating data and doing experiments, and we are eager to gain input from microbiologists who are on the front line of research. There will be an ECM Forum representative on virtually all of our administrative committees, meaning that we will absorb the perspective of early career researchers across the whole aspect of running the Society. They will be given serious resource and have significant influence to shape the things to come. This will help make us more nimble, and better informed and engaged to enable our organisation to feel confident that its decision-making is truly representative across the entire membership.

The Microbiology Society is also developing an accessible, evidence-based report for policy- and decision-makers exploring human, animal and environmental microbiome research and its relevance to public policy. The Society will be running workshops to inform the development of the report.

Our Society headquarters at Charles Darwin House in London is shared with other professional societies – the Biochemical Society, the British Ecology Society, the Royal Society of Biology – and a great deal of thought is going into ways in which we can achieve new collaborations due to our neighbourly proximities. We will update you of news about how the different societies might help each other while promoting our own agendas in the coming months.

This 'Future Tech' issue of *Microbiology Today* is packed with useful articles exploring current methodologies that are transforming microbiology and other biological disciplines. This is the age when next-generation sequencing is synergising with powerful genome editing methods such as the CRISPR-Cas9 system, making the analysis and manipulation of microbial genes superbly efficient. We are using the CRISPR-Cas9 system in my own laboratory to analyse multigene families in *Candida*, and the acceleration in progress that makes complex genetic constructs possible is extraordinary. In addition, new imaging technologies have opened a Pandora's box of methods to facilitate cell biology and structural biology. Collectively, the sum of these new methods are so potent and broadly applicable that interesting microbes previously recalcitrant to detailed analysis are now becoming accessible to detailed reductionist investigations. Our publications and meetings make an important contribution towards showcasing these advances.

Can I remind you that our operational structures allow our members to promote their own vision of what is important and what defines the horizons of microbiology. For example, if you feel that our portfolio of meetings may be missing a trick in developing an understanding of a particular aspect or area of microbiology, you have the opportunity to present your idea for a session at our Annual Conference or as a Focused Meeting through our Committees. We really do wish to remain responsive to our members providing ideas for meetings and scientific sessions.

I hope that you have enjoyed some summer sun and that you have had an opportunity for a break to help inspire your scientific imaginations.

Neil Gow
President
president@microbiologysociety.org

From the Chief Executive

At this year's Annual Conference in March, we kicked off a major project to find out directly from you, the microbiology community, what more the Microbiology Society can do to support your careers, to connect and empower communities, so that the science of microbiology can make the greatest possible contribution to society. A rewarding scientific career always faces big challenges, and our job is to help you to tackle them. The more you tell Council, me and the rest of the staff about those challenges, the more we can do together to advance microbiology.

We know that many of the activities the Society carries out on your behalf have important impacts because you tell us so. After the Annual Conference this year, you let us know about new collaborations and grants that you will undertake with colleagues you met, about new sponsorship for your research that came directly from contacts you made, and about technical problems you fixed by drawing on the expertise of other delegates you encountered.

We know that some of the Society's other activities are important because of the demand for them. The Scientific Conferences Committee, for example, has to make hard choices about which Focused Meetings we are able to run, because you are submitting so many excellent proposals. Our journals only publish a proportion of the articles that are submitted to them because the Editors and referees work hard to identify the most interesting and scientifically challenging work. And grant schemes like the Harry Smith Vacation Studentships reluctantly have to disappoint some deserving candidates because the demand outstrips our capacity to fund them.

Other activities are judged by the extent to which external audiences rate them. In policy, for example, we can see the impact we have on your behalf every time a politician quotes our briefings, or incorporates one of our recommendations into an official document. The ways of assessing some of what we do use quantitative benchmarks, such as the number of times the papers in our scientific journals are cited by others or the number of hits on the website – *Microbiology Online* received very nearly a million visitors last year.

All of these are valuable indicators that Council, the Committees and Divisions, supported by the staff, are investing in programmes that help you to advance both knowledge about microbiology and also its application into useful processes relevant to the environment, the economy, policymaking or our culture.

But the Society's resources are finite, and we need to be constantly vigilant to the possibility that we could use them even more effectively. So we are using a range of different techniques to consult you about the greatest needs of the microbiology community. At the Annual Conference, we asked you to jot down on Post-it notes what you thought we should be doing. Every time Council or staff members come into contact with any of you, we are listening to what you tell us, and we also use more formal methods like surveys and focus groups.

These methods will all give us extremely valuable information that allows us to build a picture of how we can be most helpful. In the end, there is no substitute for face-to-face conversations. So if you think there are ways in which the Society could do even more for your career, please take the time to tell us. There are many ways you can help to shape the future of your Society. You could think about standing for election to serve on Council or a Committee or Division. You could come to an occasion like one of our conferences or the series of events on the day of the Annual General Meeting, which will be on 8 September. You could invite me to come and visit your laboratory. Or you could just email me and tell me what you think the Microbiology Society does well, what it could do more of, and how we can work together to advance the science of microbiology.

Peter Cotgreave
Chief Executive
p.cotgreave@microbiologysociety.org

News

Focused Meetings 2016

The Society has already hosted two Focused Meetings this year and has a further three taking place in September. These include:

The Dynamic Fungus

5–7 September – Mercure Exeter Rougemont Hotel, Exeter, UK

Molecular Biology and Pathogenesis of Avian Viruses

27–29 September – Charles Darwin House, London, UK

Irish Division: Exploring the Microbe–Immune System Interface

1–2 September – Rochestown Park Hotel, Cork, Ireland

For more information on these events, visit pp. 128–129.

Annual Conference 2017

Next year's Annual Conference will bring us to the historic city of Edinburgh, UK. The date is confirmed as 3–6 April and the event will take place at the Edinburgh International Conference Centre (EICC). More information on planning your trip can be found on pp. 126–127.

Deaths

It is with sadness that the Society announces the death of **Mr Michael Carlile**, who joined the Society in 1956.

New online correction tool for Society journals

Authors publishing in the Microbiology Society's journals can now benefit from an online correction tool available via our new production partner, Exeter Premedia. Authors will be able to view their typeset PDF online, then add corrections directly to the article on the cloud-based production platform. Once they have finished, authors can rebuild the PDF to view their changes in the final PDF before approving the proof.

This will remove the need for an operator to input the author corrections, so we hope that this will reduce the potential for errors to be introduced and speed up the production process.

To find out more about the Microbiology Society's journals and to submit your next article to one of them, visit **www.microbiologyresearch.org**

Parliamentary events link scientists and policy-makers

In May, the Society attended Science and the Assembly 2016 at the National Assembly for Wales in Cardiff. The event, which aims to strengthen ties between the Assembly and the Welsh STEM community, was themed 'Planetary and Space Science'. Speakers included scientists, representatives from the Royal Astronomical Society and the UK Space Agency, and the Chief Scientific Adviser for Wales, Professor Julie Williams CBE. Attendees had the opportunity to hear from and network with Assembly Members.

At Parliamentary Links Day in June, scientists and policy-makers came together at the UK Houses of Parliament for a very topical discussion about what was next for science after the referendum on the UK's membership of the European Union. Society members and staff also attended a luncheon in the House of Lords, where they were joined by Jim Dowd MP. The Society also highlighted its science policy work at an afternoon networking session with Members of Parliament and Peers.

Upcoming grant deadlines

Date	Grant	Notes
1 September 2015	Travel Grants	For conferences and courses from **1 October** onwards*
15 September 2015	Microbiology in Schools Fund	For School Members to receive funding for microbiology teaching initiatives taking place on or after **1 November**
1 October 2015	Research Visit Grants	For visits and events from **1 December** onwards
	International Development Fund	
	Education and Outreach Grants	

Rolling application

Local Microbiology Event Sponsorship

All members can apply for funds to support microbiology-related events, e.g. sponsored talks.

**Please note, you do not need to have received confirmation of abstract acceptance to apply for these grants as conditional offers will be made. In this case, evidence of acceptance is required to claim your grant.*

Annual General Meeting and Showcase of the Society's Achievements

The Microbiology Society Annual General Meeting (AGM) will be held as part of a wider Showcase of the Society's Achievements event on 8 September 2016. This will include the Young Microbiologist of the Year final, a presentation from our Microbiology Outreach Prize winner and a Special Lecture.

The notice of the AGM and all associated papers are now available to members under the AGM and Celebration of the Society's Work page: **www.microbiologysociety.org/agm**

Contributions and feedback

The Society welcomes contributions and feedback from members. Please contact **mtoday@microbiologysociety.org** with your ideas.

Benjamin Thompson

Head of Communications

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Get the latest updates, follow the Society on:

Could you be the next Editor of *Microbiology Today?*

After a successful three-year term of office, Laura Bowater stands down as Editor of *Microbiology Today* at the end of 2016. The Society is looking to appoint a new member who is interested in being the next Editor of our prestigious and highly regarded magazine. The role will begin in January 2017 and is open to any Full, Full Concessionary or Honorary Member of the Society. There will be a handover period from November through to December 2016.

My role as Editor of *Microbiology Today* has provided me with a unique opportunity to develop a worldwide network of contacts and to be introduced to the incredible variety of research being undertaken by committed and dedicated microbiologists. It has been a real privilege and a pleasure to move *Microbiology Today* forward, and it is one of the few roles I have had that garners unsolicited, positive feedback on a regular basis. Laura Bowater

As the Editor you are responsible for the scientific content and integrity of the magazine.
You will be required to:

- attend and chair two Editorial Board meetings per year
- read, assess and edit all submitted feature and Comment articles
- liaise with the Managing Editor, Head of Communications and Chief Executive at the Microbiology Society's main offices with regard to any matters arising that involve the content or development of *Microbiology Today*
- represent and promote the magazine outside the Society

In addition, you will sit on the Communications Committee and will be required to attend two meetings for the Committee each year.

Desirable qualities for the position include:

- an interest in a wide range of subjects across the whole of microbiology
- an ability to assess contributions for their suitability for publication
- excellent communication skills, including the ability to deal with sensitive issues
- enthusiasm about the communication of microbiology to key stakeholders, including other scientists, parliamentarians, policy-makers, educators and the general public

You do not need to have been involved with the Editorial Board or the Society previously to apply, but you do need to be a Full, Full Concessionary or Honorary Member. If you are interested, please read a full description of the role at **www.microbiologysociety.org/MTEditor** and send your CV with the names of two referees to **mtoday@microbiologysociety.org**. The closing date for applications is Monday 12 September 2016. The appointment will be subject to approval by the Communications Committee.

The Microbiology Society is committed to creating an inclusive culture that supports equality and diversity, and fully reflects both its membership and the global microbiology community.

MICROBIOLOGY SOCIETY

The integration of nanoscale imaging and quantitation of nanomechanical properties in microbiology

S. Andrea Gazze, Lewis Francis & Geertje van Keulen

Recent developments in nanotechnology enable the imaging, quantification and manipulation of materials at the near-atomic level. The number of applications of atomic-force microscopy (AFM) in the life sciences is increasing, now allowing the integrated study of topological and quantitative nanoscale mechanical characterization of living cells and their interactions with their environments, which was inconceivable until recently.

AFM is a surface-sensitive technique whereby a sample is scanned with a sharp tip mounted on a micrometre-sized flexible cantilever. Irregularities in surface topography will deflect the cantilever while scanning the surface (Fig. 1a). These deflections are detected, processed and then used to reconstruct a 3D topological image of the sample. Forces between the surface and cantilever tip govern the cantilever deflections differentially when moving towards or away from the sample (Fig. 1b), from which a range of mechanical properties can be quantified at the nanoscale, such

as surface stiffness, adhesion and deformation.

Individual microbial cells, cells within a microbial community, and their subcellular components can readily be imaged by well-known techniques such as confocal laser scanning microscopy (CSLM) and scanning electron microscopy (SEM). These techniques require staining, labelling and/or other sample preparations, which may introduce artefacts. Most excitingly, microbial materials can be analysed quantitatively by AFM under physiological conditions without the need for prior sample preparation.

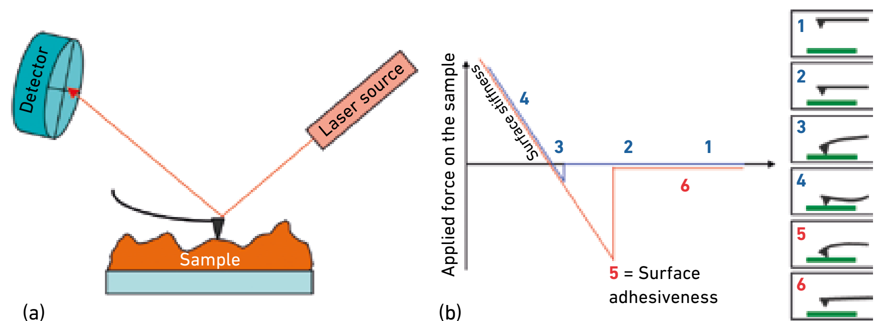

Fig. 1. **Basic working principles of AFM: cantilever deflections leading to determination of (a) surface topography, and (b) force-curve measurements for quantification of nanomechanical properties.** S. Andrea Gazze

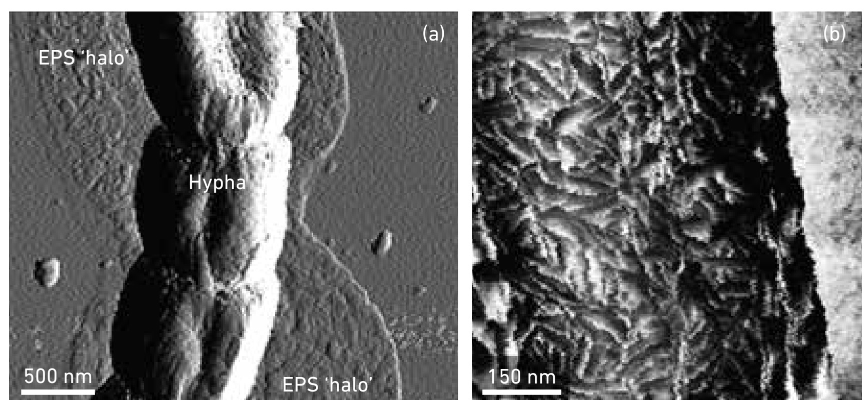

Fig. 2. **Topology of a filamentous bacterial hypha surrounded by a thin film of EPS (a) and rodlet-structured aerial cell surface at higher magnification (b).** S. Andrea Gazze

Nanomechanical properties of microbial cell surfaces and extracellular (polymeric) substances in pure cultures and biofilms

Extracellular polymeric substances (EPS) form a composite biomaterial of water, proteins, nucleic acids and polysaccharides in which microbial cells are embedded to construct a biofilm that favours microbial growth and survival by permitting the local accumulation of nutrients and other metabolites. Biofilms form the main method of microbial colonisation of natural and artificial substrates, with potential to cause significant risk to health and the economy, e.g. by biofouling medical and industrial materials such as implants and membranes. Studying the properties of biofilms and their development is therefore of paramount importance, not only for the advancement of microbiology in general, but also to control detrimental microbial effects on specific applications.

High-resolution topographical imaging and mechanical force analysis of composite biomaterials in their natural state by AFM has already demonstrated its usefulness in studying different stages of biofilm and other types of multicellular development. AFM has revealed that cells are first surrounded by a thin 'halo' of secreted EPS (Fig. 2a), which can be up to a cell's diameter away from the cell surface and a few tens of nanometre in thickness. Importantly, the microbial community can produce overlapping EPS layers with different mechanical properties. Interestingly, both filamentous bacteria (Fig. 2a) and fungi secrete EPS in a similar way, showing that organisms from different kingdoms with similar cellular morphology (growing by hyphal elongation from the tip) can colonise and affect surfaces in a similar way. The next stage of cellular differentiation can also be followed with AFM: chaplin and rodlin proteins are excreted on the aerial cell surface of *Streptomyces* bacteria, which then typically self-assemble into thicker rodlet-shaped amyloidal aggregates, conferring a concomitant change in mechanical properties from wettable to water-repellent (Fig. 2b). This change in surface properties can be visualised and quantified with AFM, providing quantitative nanomechanical insights into morphological differentiation.

AFM can also be co-applied with other analytical techniques such as

CSLM, nanoscale secondary ion mass spectrometry (NanoSIMS), and several others. Such hybrid instrumentation can reveal subcellular localisation of labelled intra- and extracellular molecules, with overlying bionanomechanical properties, leading to deep mechanistic insights such as those demonstrated for the lateral expansion of antimicrobial pores in lipid bilayers.

Nanoscale detection of microbial processes *in situ*: bio-weathering of minerals

Terrestrial microbes play a pivotal role in mineral weathering, soil formation and the generation of essential growth nutrients. This process is especially evident in forested ecosystems dominated by ectomycorrhizal symbioses with tree roots. The role of these fungi in mineral weathering can be assessed *in situ* by evaluating the presence of microbial signatures in rocks and soil particles, such as etch pits, secondary mineral formation and hyphal-shaped channels (Fig. 3a). Inductively-coupled plasma atomic emission spectroscopy (ICP-AES) can be applied to give the overall dissolution rate of minerals, e.g. released mineral cations in solution at parts-per-billion (ppb) levels. AFM can complement ICP-AES data by detecting in *real time* the release of mineral components, the specific geometry of bioweathering features, and the different reactivity of mineral surfaces resulting from exposure to fungal exuded substances such as organic acids (Fig. 3b). Furthermore, the specific nanoscale geometries of such microbial signatures alongside AFM characterisation of mineral components refractive to dissolution have also provided a novel molecular mechanism for mineral bond hydrolysis and dissolution.

Nanoscale detection of microbial processes *in situ*: soil

Microbial activities greatly affect soil properties and soil ecosystem processes such as the formation and breakdown of soil organic matter (SOM). Different techniques exist to evaluate macroscale soil properties such as composition, wettability, bulk density, porosity, soil hardness and deformation. In turn, these properties may affect the severity and impact of runoff, flood risk, soil stability to tillage, and other agricultural and anthropogenic activities. The application of AFM to soils allows nanoscale imaging and quantification of the nanophysical properties of soil building blocks, i.e. single particles and soil aggregates, to understand how these ultimately influence bulk soil properties.

For example, the presence of SOM strongly affects the physical properties of soil aggregates. By applying AFM, high-resolution topological maps can

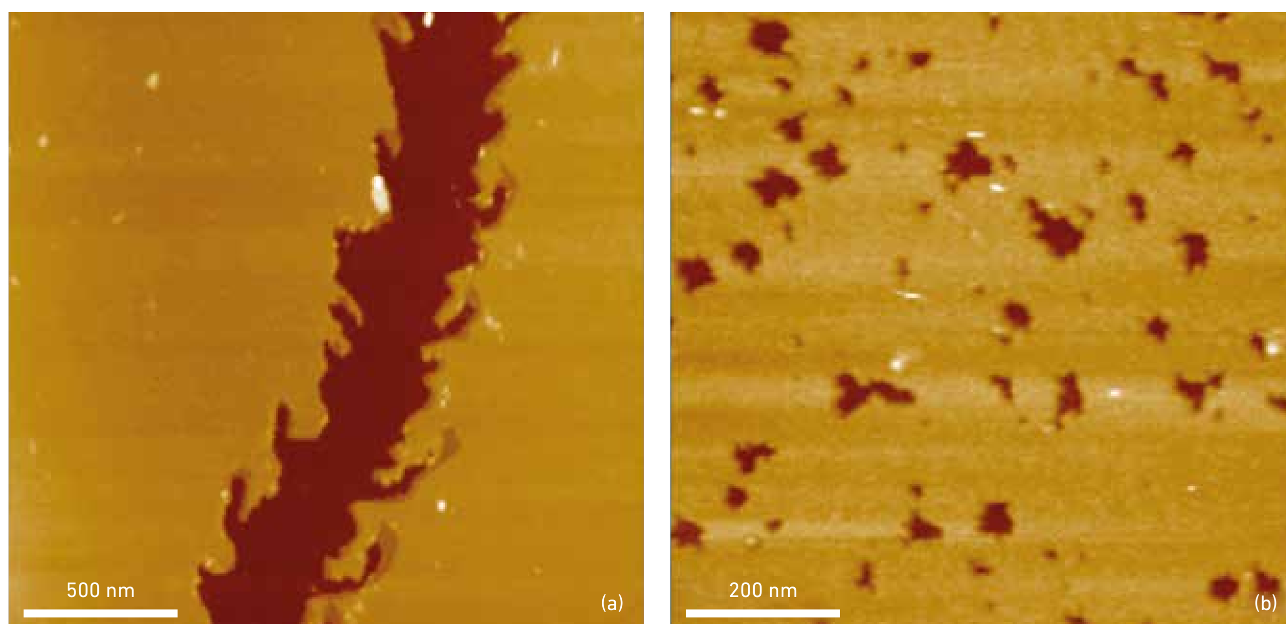

Fig. 3. Bioweathering of silicate minerals by microbial signatures: (a) fungal hypha-created channel and (b) etch pits resulting from fungal organic acid exudates. S. Andrea Gazze

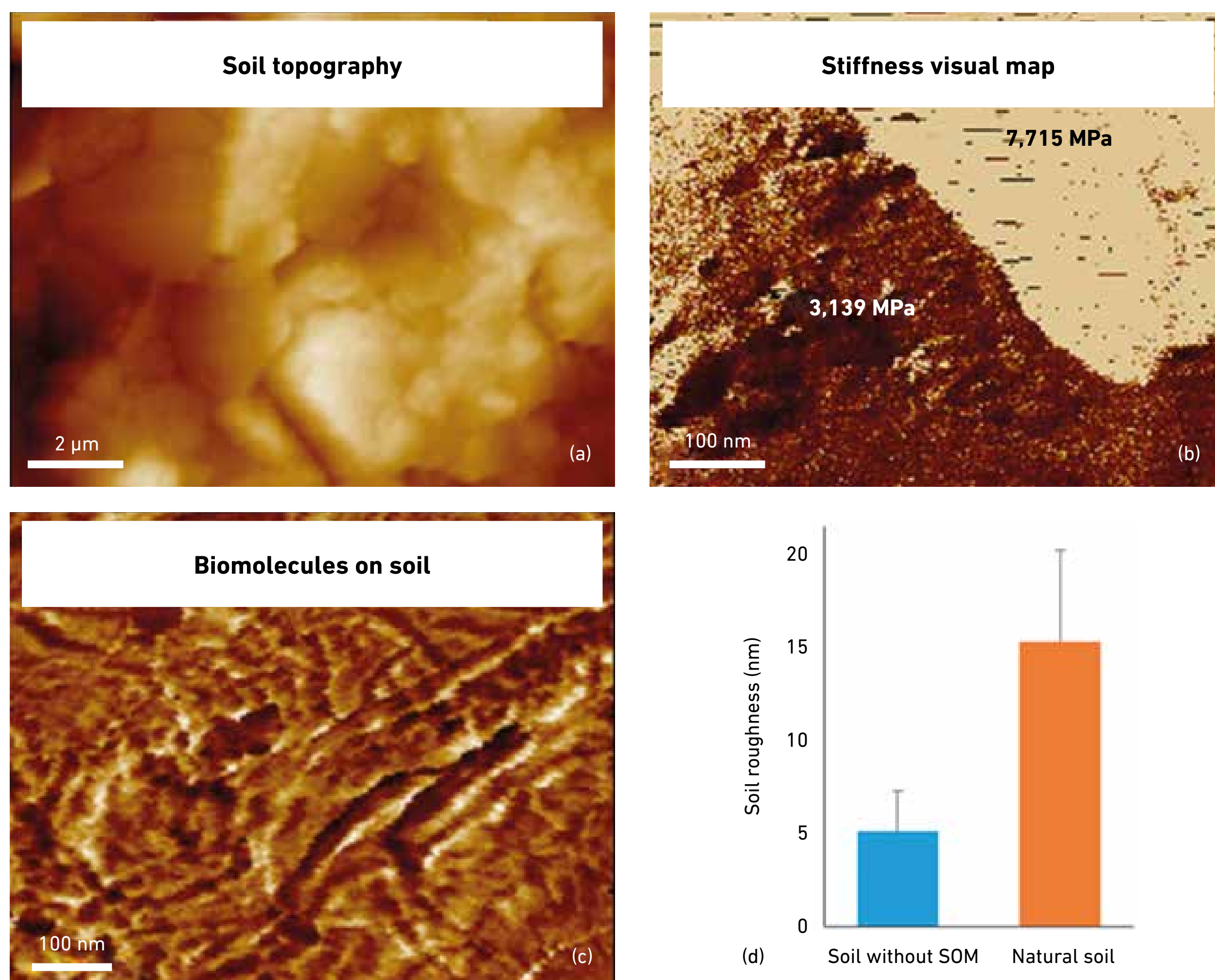

Fig. 4. **Nanoscale natural soil characterisations: (a) topography of soil particle, (b) mineral surface with different stiffness properties (unit MPa), (c) biomolecules on a soil particle, (d) quantitative exemplar of the nano-roughness of natural and organic-matter-deprived soils.** S. Andrea Gazze

be obtained for mineral surfaces of soil particles and the presence, distribution and nature of biological and other organic matter that is covering soil minerals (Fig. 4). How the nanomechanical properties are affected by SOM can be measured, for instance, by the change in elastic modulus (Young's modulus) in the presence and absence of microbial or plant exudates (Fig. 4b), while the surface roughness of single aggregates appears to increase with the presence of bio-organic matter (Fig. 4d). It is therefore very timely to integrate nanoscopic profiling and bulk soil characterisations with functional expression studies, such as metaproteomics, to generate deep mechanistic understanding of the role of microbes in soil ecosystems.

Microbiology: crossing scales

Exciting novel microbial properties and behaviours can be revealed by applying nanoscale analytical techniques *per se* to pure cultures and *in situ* ecosystems. The combined application of biomolecular, nanoscale and macroscopic techniques has the potential to stepwise fill the gaps in understanding microbial(-influenced) processes in the real world across molecular to global scales.

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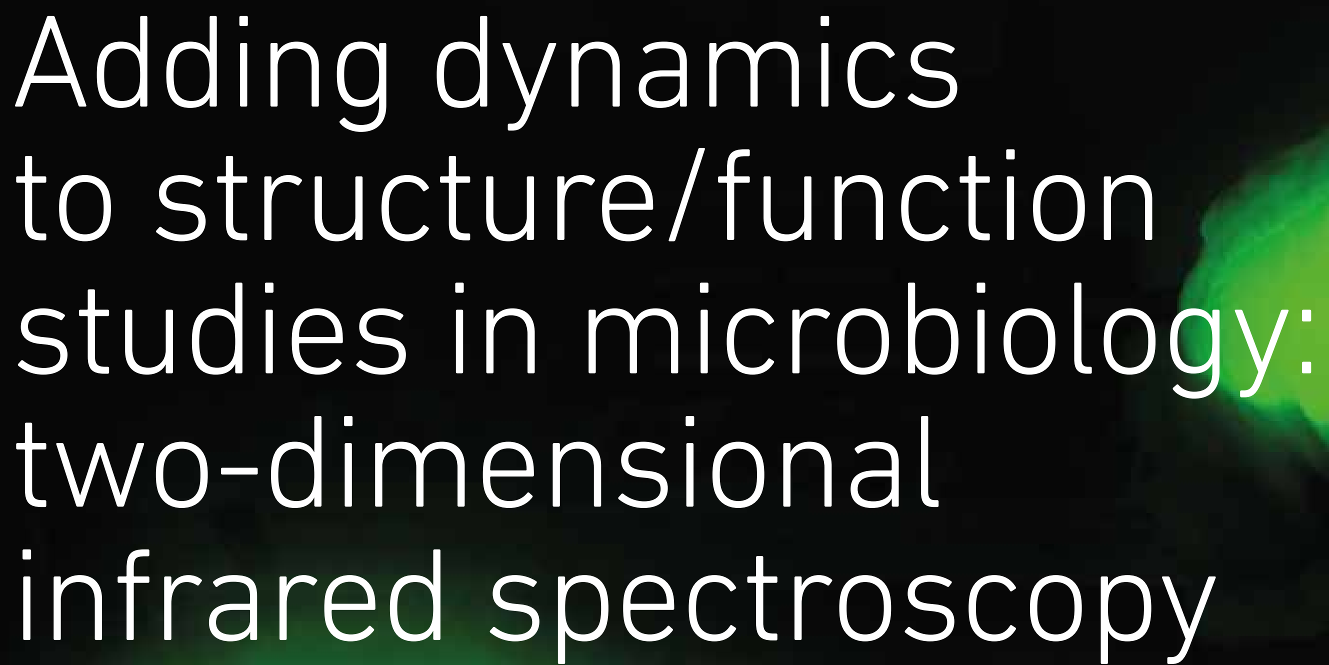
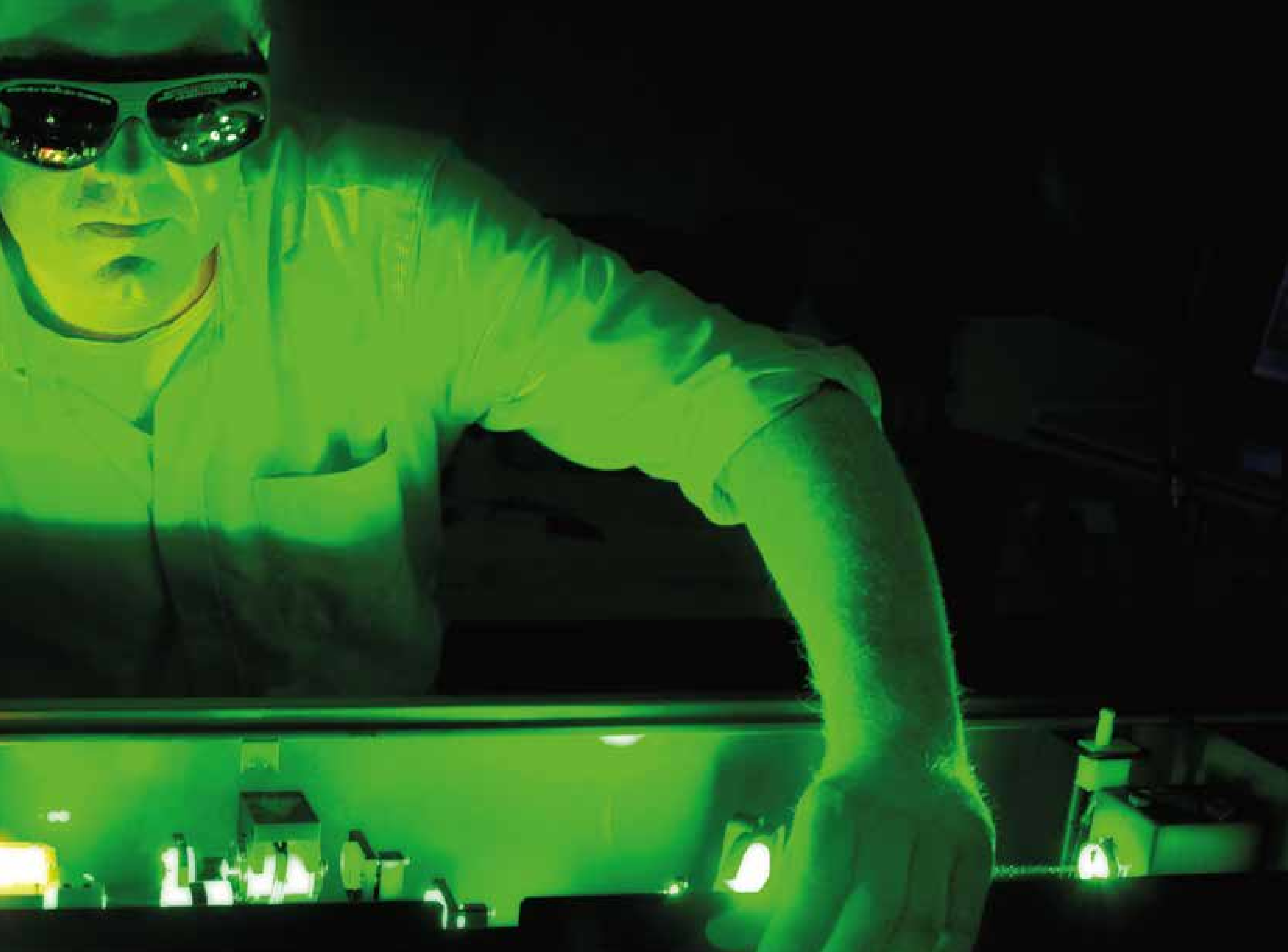


Adding dynamics to structure/function studies in microbiology: two-dimensional infrared spectroscopy

Niall Simpson, Neil T. Hunt & Paul A. Hoskisson

Microbiology is a pioneering discipline. Many of the fundamental processes of biology were first elucidated in microbial systems – deciphering the genetic code, the one gene–one enzyme hypothesis, the mRNA hypothesis, the first genome sequences, and many more key biological breakthroughs were all the result of microbiologists! In an age where interdisciplinary projects are encouraged it is easy to be cynical. However, true collaborations between biologists and physicists can lead to fascinating insight into biological systems, especially when exciting new techniques can be applied to biology.

One of the central themes of biology is that structure is linked to function. At the molecular level, however, biology is dominated by intermolecular interactions, such as those between protein–ligand, protein–protein, protein–nucleic acid and nucleic acid–nucleic acid. As microbiologists, we often look for ways to characterise these kinds of interactions with a view to understanding our favourite (micro)biological system. These chemical interactions are underpinned by hydrogen bonding, and electrostatic or hydrophobic processes, occurring between the molecular partners. As we know, proteins are not static entities, and these intermolecular interactions are generally controlled by rapid structural fluctuations. For example,



Dr Neil Hunt operating a 2D IR spectrometer at the University of Strathclyde. Tim Briggs

the typical lifetime of a hydrogen bond in solution is around 1 picosecond (to put this into context – 1 picosecond is to 1 second as 1 second is to 31,710 years). To fully understand and exploit the biology, therefore, it is necessary to complete the structure–function puzzle by incorporating dynamics. This kind of approach has proved very useful in terms of understanding protein–ligand binding, such as antibiotic molecules binding to their target proteins, or post-translational modifications of proteins.

In order to observe fast processes, you need a 'camera' with a fast shutter speed. One way to approach the understanding of protein dynamics with sub-picosecond temporal resolution is a relatively new experimental technique called ultrafast two-dimensional infrared spectroscopy (2D IR). 2D IR is uniquely sensitive to structural dynamics and it is capable of extending the range of information available to microbiologists studying molecular interactions in a complementary way to established tools, such as macromolecular crystallography, two-dimensional nuclear magnetic resonance (2D NMR), surface plasmon resonance (SPR) and isothermal titration calorimetry (ITC). In terms of microbiological applications, 2D IR is a technique in its infancy and we are only now beginning to discover its potential. We feel 2D IR is capable of making a novel contribution to microbiology research areas, at a time when studies of protein–ligand interactions are fundamental to our understanding of globally relevant microbiological problems such as antimicrobial resistance and climate change.

Infrared (IR) spectroscopy is useful for studying proteins because the C=O bonds found in peptide linkages have stretching vibrational modes (so-called 'amide-I modes') that are intense and sensitive to the inter- and intra-molecular processes (think, school 'mass on a spring' type experiments) that underpin biological function, such as protein folding, ligand binding and enzymatic reactions. These are most commonly studied via IR absorption spectroscopy, which gives rise to a 'one-dimensional' plot of absorbance versus vibrational frequency (See Fig. 1a). However, the

benefits of amide-I mode spectroscopy are restricted because the absorptions from individual amino acid residues in proteins overlap, or couple strongly to form delocalised vibrations that are due to protein secondary structure elements such as α-helices or β-sheets.

Multidimensional spectroscopy methods such as 2D IR measure the same molecular response as an IR absorption experiment, but spread the spectrum out over a 2nd frequency axis in order to uncover features that are not resolved in 1D. Practically, rather than simply measuring absorption, a 2D IR experiment consists of two IR laser pulses that hit the sample with a precisely controlled time delay between them. The first pulse excites the sample at a particular frequency (makes it vibrate) and the second laser pulse measures the effect of this on the molecule via an absorption spectrum. By progressively changing the wavelength of the laser for the first (pump) pulse, one builds up a 2D plot of excitation (pump) versus detection (probe) frequency. The time delay between the laser pulses allows us to examine a protein's structure and its dynamics on the timescale of the pulse duration (~100 fs). This means that the IR absorption spectrum lies along the diagonal of the 2D IR plot (a surface response plot) while new information is found in the off-diagonal region of the spectrum (Fig. 1b). It is these off-diagonal regions that report on interactions between the structural components of our protein molecules – interactions between vibrations on specific amino acid residues or energy transfer processes within the molecule – essentially informing us on the structural interactions within proteins.

An example of a 2D IR spectrum is shown in Fig. 1(b). These can be thought of as a map of the vibrational modes within a molecule and how they interact. This off-diagonal region of the spectrum is richly structured, revealing patterns that are unique to secondary structural elements of the protein, but also reports on interactions between them. Most usefully, in terms of providing complementary information to established microbiological techniques, the anti-diagonal direction of the 2D IR spectrum reports on the flexibility of molecules, as structural motion allows vibrations to undergo small frequency changes during the time delay between laser pulses (Fig. 2). In turn, more flexible molecules give rise to greater anti-diagonal line broadening. These dynamics have been shown to vary sensitively with ligand binding and provide fundamental insight into this fundamental intermolecular contact. Thus, 2D IR provides a large quantity of new information but the difficulty lies in interpreting this in terms of biological function.

An important tool in this is to link 2D IR data to other methods in a complementary fashion. For example, the fast dynamics extracted by 2D IR bridge the gap between the atomic resolution of crystallography and the slower (μs–ms) dynamics accessible by NMR. Importantly, 2D IR measures protein dynamics on the exact timescale that computational simulations like molecular dynamics predict. These methods are used extensively to infer biological

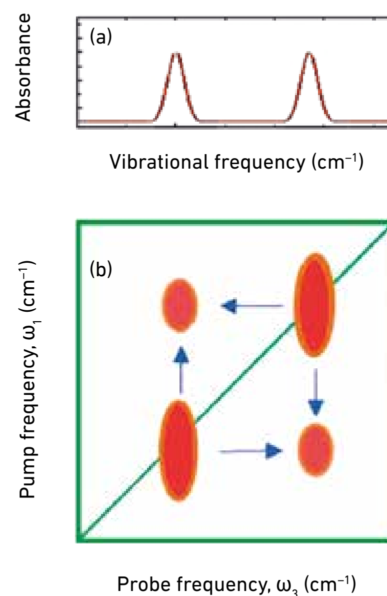


Fig. 1. **(a) Representation of a 'one-dimensional' IR spectrum with two vibrational modes and (b) 2D IR counterpart to the same spectrum, illustrating coupling patterns between the two vibrational modes. These two coupled vibrations will generate off-diagonal patterns, at coordinates indicated by the blue arrows.** Niall Simpson

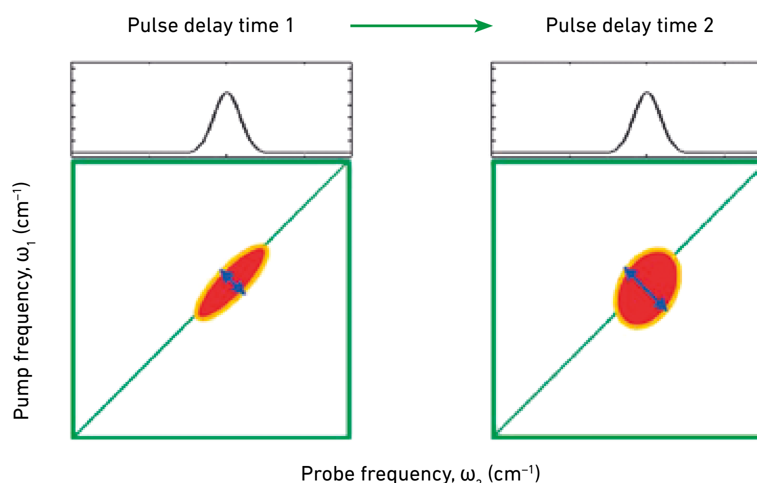
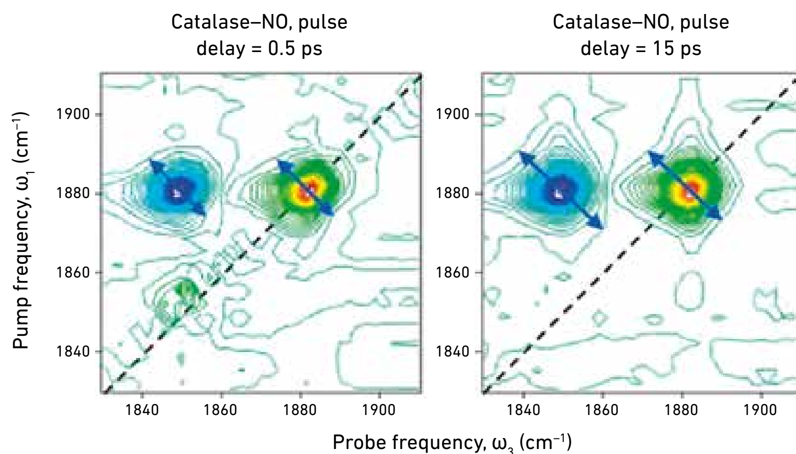


Fig. 2. **2D IR spectra depicting the effect of molecular flexibility on the anti-diagonal width of vibrational modes. With longer pulse delay times, it becomes decreasingly likely to find flexible molecules in the same position or orientation as that at the first pulse. Correspondingly, their excited vibrational frequencies will also evolve with time, resulting in anti-diagonal line-broadening.** Niall Simpson



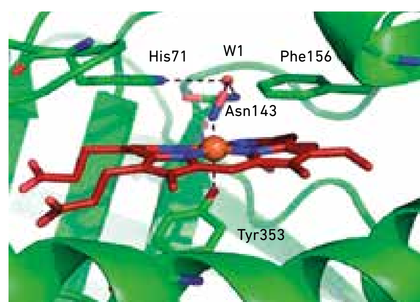


Fig. 3. **2D IR depicting the anti-diagonal line-broadening of a nitric oxide molecule bound at the active site of catalase. Rapid broadening of the NO vibration indicates the presence of a network of mobile water molecules in the distal cavity that are not resolved in the crystal structure, shown below.** Niall Simpson

function, but until now experimental confirmation of the results has not been achievable.

We have used 2D IR to study a range of systems, to shed light on the role dynamics plays in understanding microbiological processes. Recently, we applied 2D IR to an investigation of protein structural dynamics of the active site of catalase from the bacterium *Corynebacterium glutamicum*, and how this enzyme is inhibited by nitric oxide. By recording the dependence of anti-diagonal peak width of the NO group as a function of the time delay between laser pulses (Fig. 3a), we were able to show for the first time that mobile water molecules are present in the active site of the enzyme and that these are responsible for large-scale vibrational modes of the haem pocket that plays a key catalytic role in the enzymatic reaction.

Currently, we are using 2D IR to investigate antimicrobial drug resistance, specifically the inhibition of the essential enoyl reductase (InhA) from *Mycobacterium tuberculosis* by the frontline anti-TB drug isoniazid. We have studied the components of the active isoniazid-NADH complex and are now combining standard molecular biology, such as site-directed mutagenesis and enzymology to augment the 2D IR studies and develop a clear picture of the active site structure–function–dynamic relationship. We have been able to recreate mutations within the InhA protein that lead to clinical resistance and study how these behave when exposed to isoniazid and also natural enzymatic substrates. Understanding the role of molecular dynamics in relation to structure and function can aid the design of novel anti-TB drugs in the future and will hopefully lead to the recognition that 2D IR has a key role to play in microbiology.

Currently, 2D IR is not a bench-top experiment, with each spectrometer being built by the users. However, this was the situation that NMR was in 40 years ago and now it is a standard experimental technique. 2D IR has great potential for microbiology and biochemistry; there are many problems in microbiology that can be assisted with such techniques – so be open to collaboration and befriend a tame physicist!

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Cryogenic electron microscopy – beyond the resolution revolution

David Bhella

Cryo-electron microscopy (cryoEM) has recently emerged as a structural biology technique to rival X-ray crystallography. Technological developments in both instrumentation and software have allowed researchers to determine the structures of macromolecular assemblies at close to atomic resolution. Moreover, assemblies that would be intractable by X-ray methods owing to heterogeneity or disordered regions have become viable targets for structure determination, prompting the wide uptake of a method that, until comparatively recently, was sometimes considered 'niche'. So, what does the future hold for structural electron microscopy and what does it offer the microbiologist?

The long history of a rapidly emerging method in structural biology

Since its invention in the 1930s, the transmission electron microscope (TEM) has been an invaluable tool to investigate the shapes of macromolecular assemblies and cellular ultrastructure. Although the TEM has long been capable of imaging materials at the atomic scale, specimen preparation requirements limited the achievable resolution in biology. Classical biological transmission electron microscopy preserves cells and tissue by chemical fixation and dehydration, followed by resin embedding and ultra-thin sectioning. Purified macromolecules are likewise imaged in a dehydrated state, in negative stain. Drying, fixation and staining severely compromise the structural integrity of biological assemblies, and high-resolution features are lost.

In the 1980s, researchers working in the laboratory of Jacques Dubochet, at the University of Lausanne, pioneered methods for the preparation of biological material in a frozen hydrated state, by freezing suspensions of macromolecules so rapidly that there is no time for ice crystals to form – a process known

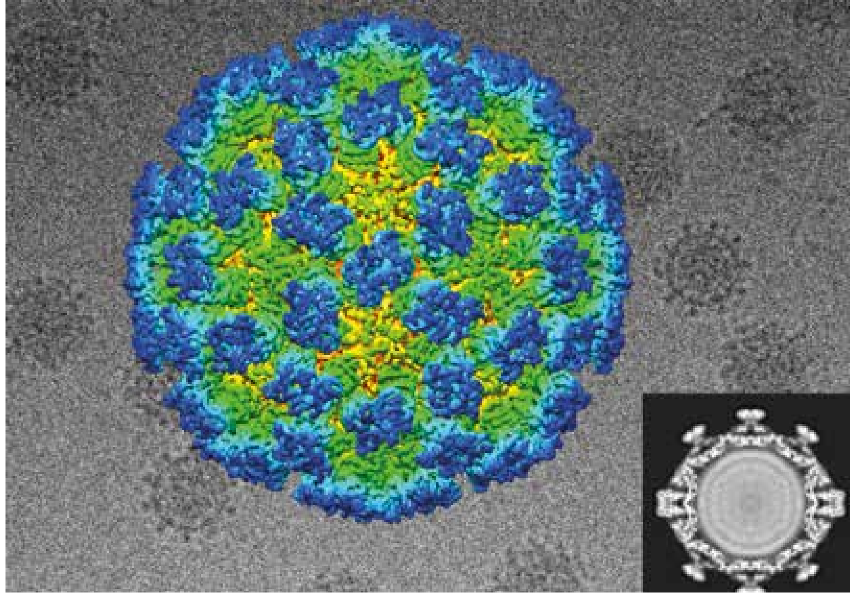

Fig. 1. **Cryomicroscopy of a calicivirus gave 2D projection views of randomly oriented particles (background). These were processed to produce a 3D density map (bottom-right – central slice through the 3D map), which can be represented as a coloured 3D surface.** Michaela Conley and David Bhella, University of Glasgow

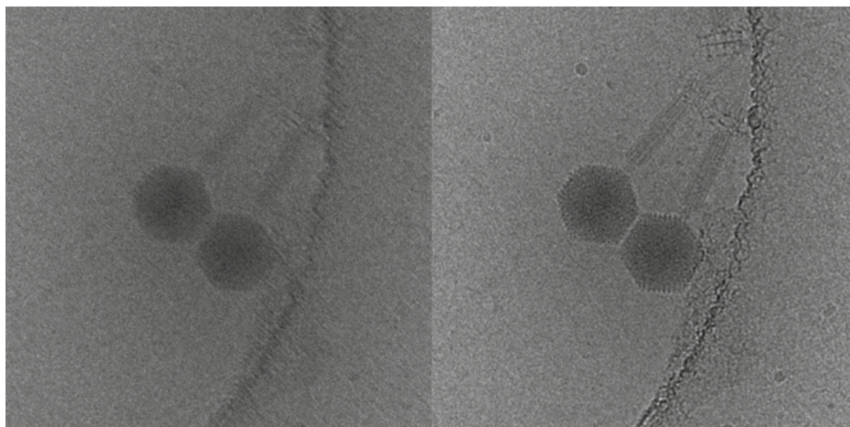

Fig. 2. **Specimen drift can lead to a blurred image that is unsuitable for structure analysis (left). The high frame rate of DDD cameras means that this artefact can be computationally corrected (right), yielding sharp images suitable for 3D reconstruction.** Diana Alves and Toby Jenkins, University of Bath; David Bhella, University of Glasgow

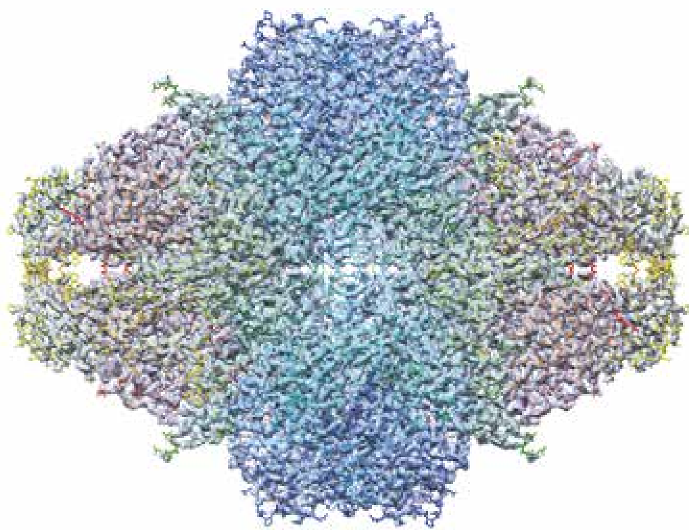

Fig. 3. **The 2.2 Å structure of *β*-galactosidase.** *Science* **348**, 1147–1151 (2015), EMDB 2984 and PDB 5a1a

as vitrification. This led to a landmark publication describing cryogenic TEM imaging of viruses (*Nature* paper in Further reading).

Images recorded in the TEM are two-dimensional (2D) projections of the three-dimensional (3D) object. For structure analysis, images of identical objects viewed in different orientations may be computationally processed to determine a 3D density map (3D reconstruction – Fig. 1). Methods to recover 3D structure data from negative-stain TEM images were first developed by David DeRosier and Aaron Klug in the late 1960s, working at the Medical Research Council Laboratory of Molecular Biology in Cambridge. In the late 1980s, the application of computational 3D reconstruction methods to cryoEM images led to the publication of the first low-resolution structures for unstained icosahedral viruses and the birth of a new discipline in the field of structural biology.

From blob-ology to atoms

These early reconstructions provided insights into the quaternary arrangements of larger assemblies and allowed the visualisation of domains in protein structures. Technological innovations in the intervening years led to a steady increase in attainable resolution. Improvements in microscope design and the development of algorithms to correct an imaging artefact known as the contrast-transfer function allowed researchers to determine structures at better than 10 Å (1 nm) resolution in the late 1990s. For the first time this produced reconstructions of sufficient detail to permit the modelling of protein folds under certain optimal conditions. At resolutions around 8–9 Å, α-helices are seen as tubes of density.

At 5 Å, β-strands start to resolve and the larger amino acid side-chains become visible.

A step change in the capacity of cryoEM to solve protein structures came about as a consequence of several technological developments over the past five years. First and foremost, the development of second-generation digital cameras for TEM that directly detect electrons as they pass through the sensor. Direct detection devices (DDD) record high-resolution information far more efficiently than the previous CCD-based cameras or photographic film. DDDs also operate at very high frame rates, between 20 and 400 fps. Thus, rather than recording a single frame, micrographs are recorded as movies of many tens of images. At the highest frame rates, DDD cameras can count individual electrons passing through the camera, greatly reducing noise.

DDDs have not only vastly improved the quality of images recorded in the TEM, they have also informed our understanding of specimen behaviour under the electron beam. Specimen drift had been a major problem in cryoEM, which would severely degrade image quality. Images recorded as movies can be aligned and averaged to compensate for specimen drift during the exposure (Fig. 2). DDD movies have, however, revealed that specimen movement during imaging is much more complex than previously thought. In addition to lateral drift of the specimen, particles also rotate and move relative to each other as the ice layer 'domes' under the electron beam, an effect that can also now be corrected in the reconstruction process.

Recent developments in electron microscope design, including more stable lenses and automation, have improved data quality and throughput. Finally, modern software algorithms allow researchers to perform *in silico* purification: classification of heterogeneous datasets to solve multiple structures or exclude damaged particles from the 3D reconstruction. Taken together, these technological advances have extended attainable resolution to 2–4 Å, a point at which protein models can be built with a high degree of confidence.

The first *de novo* structure deposited in the protein data bank (PDB) derived from images recorded in the cryoEM was solved by analysis of 2D crystals of bacteriorhodopsin as early as 1990. It was nearly 20 years before comparable resolutions were achieved for particulate assemblies – helical and icosahedral objects were solved in 2007 and 2008, respectively. The lower symmetry 20S proteasome (a 14-mer) and asymmetric ribosome were solved in 2013 using DDD image data. More recently, astonishing resolutions have been achieved such as the 2.2 Å structure of *β*-galactosidase (Fig. 3). The improved contrast of DDD cameras operated in electron counting

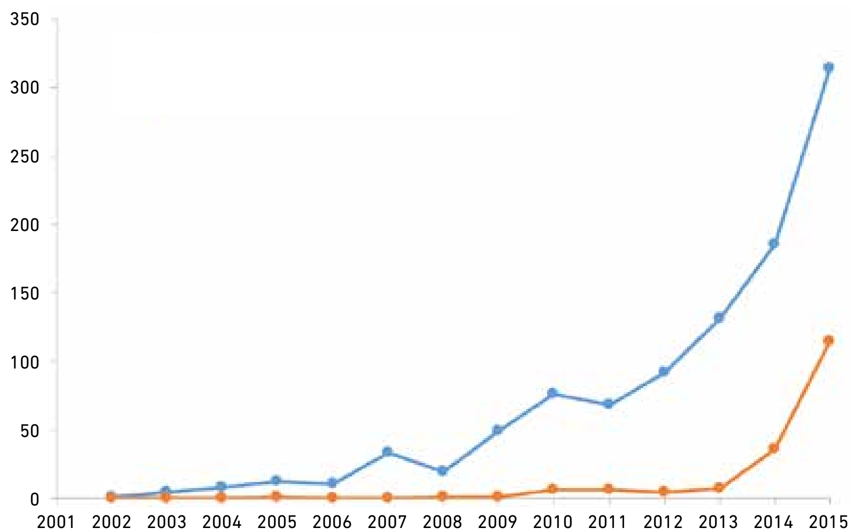

Fig. 4. **A plot to show the number of structures deposited in the EM databank each year that are better than 10 (blue) and 4 (orange) Å resolution. This illustrates the recent explosion of high-resolution structures determined by cryoEM.**

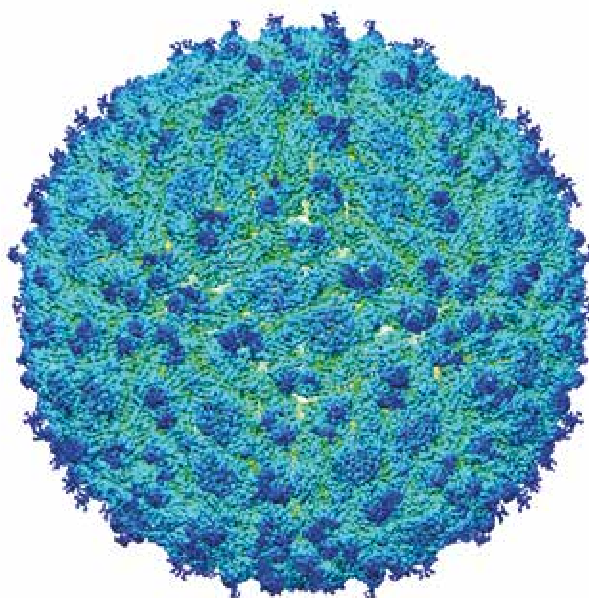

Fig. 5. **The 3.8 Å structure of Zika virus.** *Science* **352**, 467–470 (2016), EMDB 8116

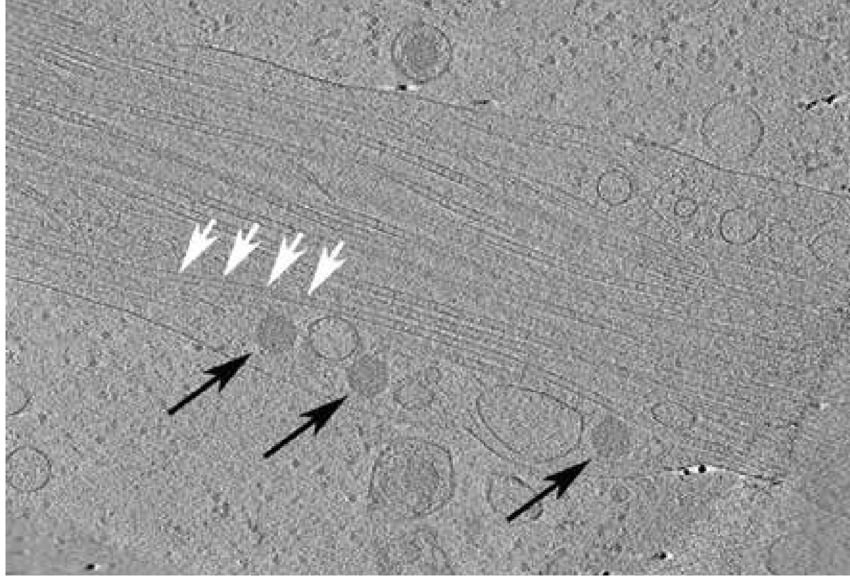

Fig. 6. **A central slice through a tomogram of a neuronal cell that is infected with herpes simplex virus type 1, capsids (black arrows) are seen travelling along microtubules (white arrows).** Rebecca Lauder and Frazer Rixon, University of Glasgow.

modes means that smaller assemblies are also becoming viable targets for analysis, as illustrated by the recent structure of the ~170 kDa human γ-secretase.

Technological advances in cryoEM have led to a seismic shift in the field of structural biology, bringing about a rapid expansion in the number of close to atomic-resolution structures in the EM data bank (Fig. 4). Such structures can be determined swiftly, often only requiring one or two days of microscope time followed by several weeks of computation. This is illustrated by the speed with which structures of Zika virus have been produced (Fig. 5).

Taking structural biology into the cell

Electron microscopy as a tool in structural biology might be said to have come of age in recent years, finally realising its potential as a high-resolution method. In my view, the cryomicroscopy revolution has just begun. Single particle reconstruction still has considerable potential for improvement; minimising specimen movement should lead to improved resolution. Larger, faster DDD cameras with improved imaging performance will increase throughput and data quality, while software methods to deal with domain movement within assemblies are beginning to allow researchers to characterise dynamic processes.

The TEM is a versatile instrument, however. In addition to high-resolution analysis of purified assemblies, cryo-electron tomography (cryoET) allows us to investigate the structure of unique entities. This is achieved by rotating the specimen in the electron beam and recording an image every 1–3 degrees. The resulting tilt-series of images is then processed to compute a 3D reconstruction or tomogram. Repeated features within the tomogram may be extracted, brought into common register and averaged – subtomogram averaging.

As well as providing insights into the structure of pleomorphic structures such as enveloped viruses, cryoET raises the tantalising prospect of *in situ* structure determination: the capacity to solve the structure of an object functioning in the cellular environment. Assemblies such as flagellar motors or bacteriophages can be investigated *in situ* through tomography of frozen-hydrated intact bacteria, albeit presently at lower resolution. *In situ* structure analysis of macromolecular assemblies within eukaryotic cells is also becoming a possibility. In virology, many aspects of the replication cycle, for example viral attachment, entry, trafficking through cellular organelles and morphogenesis, are becoming open to investigation. This remains challenging, as the size of objects that may be imaged in the TEM is limited by the penetrating power of the electron beam. Several approaches have been taken to overcome this; firstly, it is possible to propagate viruses in cells that have thin regions and may be imaged directly (Fig. 6). Many of the most interesting processes occur at the heart of the cell and in large organelles. This requires the cell to be sectioned in a frozen-hydrated state. While this is far from trivial, tantalising first steps are being taken to achieve this.

CryoEM has opened new horizons in structural biology research that will have broad application to address fundamental questions in microbiology. Rapid calculation of high-resolution structures for purified macromolecular assemblies, as well as the developing capacity to determine structures *in situ*, are tremendously powerful additions to the microbiologist's toolbox that will increasingly inform our understanding of molecular structure and function in the coming years.

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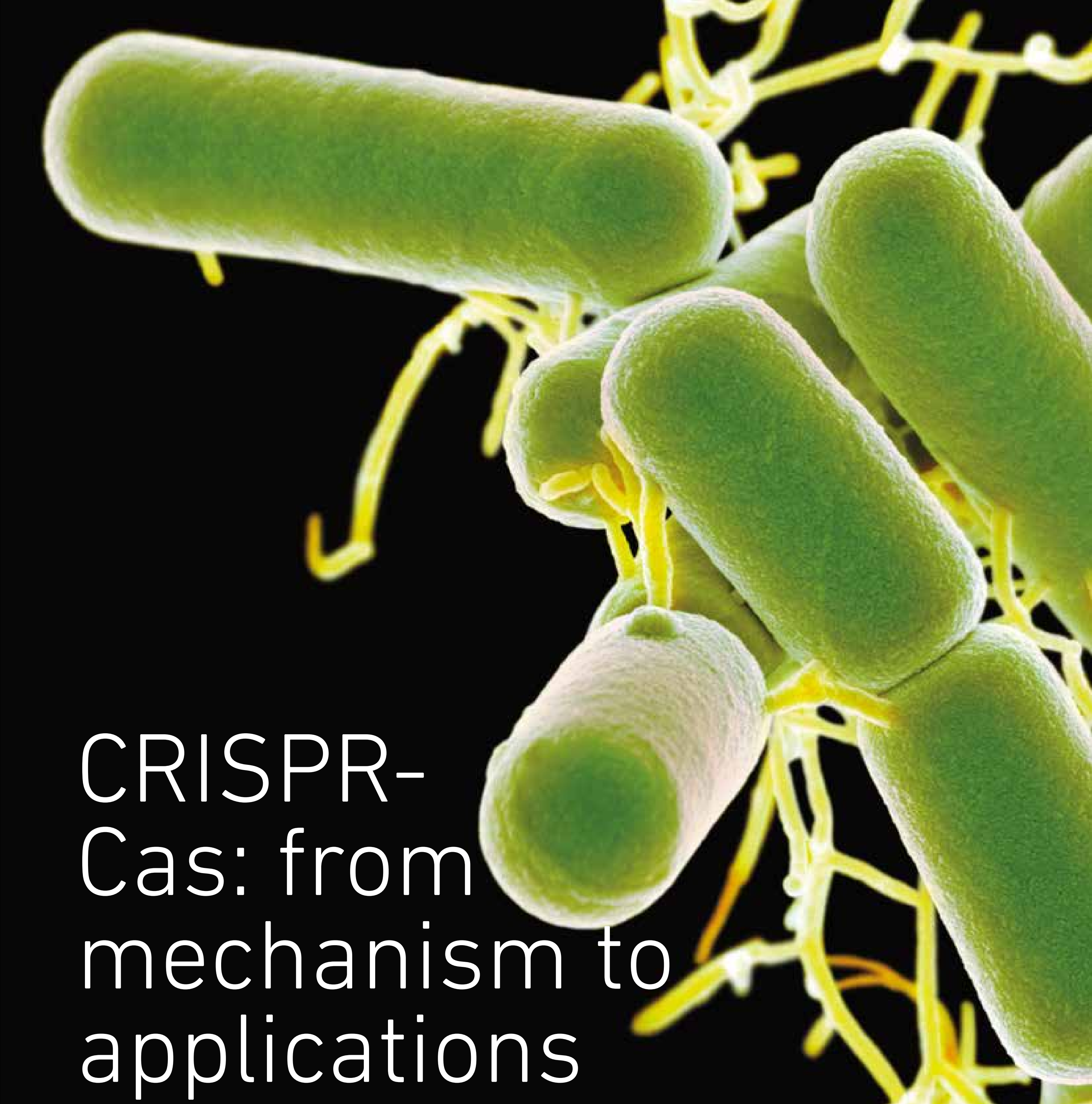


CRISPR-Cas: from mechanism to applications

Edze Westra

Coloured scanning electron micrograph of *Lactobacillus* bacteria. Science Photo Library

All organisms are exposed to parasites, so they evolved a range of different immune mechanisms to defend themselves. About 10 years ago, scientists discovered that bacteria and archaea, generally regarded as primitive organisms, have a sophisticated adaptive immune system, known as CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats). The insights gained about this system led to applications in industry to protect bacterial species against their viral parasites. In addition, CRISPR-Cas has been turned into a versatile genome editing method that has the potential to treat human genetic diseases.

What is adaptive immunity?

There are many ways to categorise immune systems. One common distinction is whether immune systems are pre-programmed (innate) or re-programmable (adaptive). The best known example of adaptive immunity is the antibody response in vertebrates. Unlike innate immunity, adaptive immunity is acquired during the lifetime of the host and always results in a response that is highly specific towards the parasite that the host interacts with. For a long time it was presumed that adaptive immunity was restricted to higher organisms. Adaptive immunity in prokaryotes has been one of the most bewildering discoveries in microbiology.

The architecture of a CRISPR-Cas adaptive immune system

CRISPR-Cas systems consist of two key components. The first is the so-called CRISPR locus, which is located on the bacterial (or archaeal) genome. CRISPR loci function as a genetic memory of previous infections. They are composed of repeating sequences that are typically around 30 nucleotides in length. These repeating sequences (repeats) are separated by unique sequences of similar length. It is these unique sequences that matter most. These are the memory sequences, derived from parasite genomes, and they are referred to as 'spacers'. Spacers in the CRISPR locus confer immunity against any parasites that carry a matching sequence. The sequence information stored in the CRISPR locus is used by the second key element of CRISPR-Cas systems, the Cas proteins. Cas (for CRISPR-associated) proteins are often encoded next to a CRISPR locus.

The three stages of CRISPR-Cas

How do CRISPR-Cas systems work? In a nutshell, CRISPR-Cas systems capture pieces of DNA from a parasite (for example, a virus), integrate this into the CRISPR locus, and use this information to destroy the parasite when it infects again. This process is generally described as having three (more or less) independent stages. The first is the 'Adaptation' stage, which occurs immediately upon infection with a novel parasite. During 'Adaptation', dedicated Cas proteins capture a piece of parasite DNA and integrate this into the CRISPR locus, where it forms a novel spacer. Next, the 'Expression' stage takes place, during which the entire CRISPR locus is copied into an RNA molecule. This long RNA transcript is cleaved by dedicated ribonucleases to generate small crRNA molecules that contain only a single

The interaction between CRISPR-Cas and antibiotic resistance is unclear; some studies support the hypothesis that CRISPR-Cas blocks the spread of resistance genes, whereas other studies found no evidence for this. Understanding the short- and long-term implications of CRISPR-Cas is key if we are to predict and manipulate the spread of antibiotic resistance.

spacer sequence. A single crRNA forms a complex with one or multiple Cas proteins. This complex is one of the key functional modules of the CRISPR-Cas immune system, and functions analogous to an antibody in parasite detection. In the event of re-infection with the same parasite, the 'Interference' stage is triggered. During this stage, Cas-crRNA complexes bind and cleave the genome of the parasite, which results in host immunity.

Two classes of CRISPR-Cas systems

CRISPR-Cas systems are highly diverse, and have been classified into two main classes, and several types and subtypes. One of the most obvious differences is that Class 1 systems encode many more Cas proteins compared with Class 2 systems. Unlike Class 1 systems, Class 2 Cas-crRNA complexes contain only a single Cas protein – the most famous example being Cas9 – and because of their relatively low complexity, Class 2 systems are uniquely suited for genome engineering applications.

Genome engineering

Introducing specific mutations into a genome has long been extremely challenging. Our detailed understanding of CRISPR-Cas systems has facilitated the development of a CRISPR-Cas9-based genome editing technology that makes it much more straightforward to introduce any mutation of interest into the genome of our favourite study organism. Some first studies indicate that this technology can be used to cure human diseases that are caused by specific genetic mutations. What makes CRISPR-Cas9 systems so suitable for genome editing? Firstly, a key feature of crRNA-Cas9 complexes is that they will specifically interact with DNA sequences that are complementary to the crRNA. Secondly, this specificity can be readily adjusted by changing the crRNA sequence, which provides extreme control over the crRNA-Cas9/DNA interaction. Thirdly, Cas9 will cleave the corresponding sequence, resulting in a DNA break, which is an essential step in the genome editing process. The double-stranded break will be repaired by a DNA repair pathway. Depending

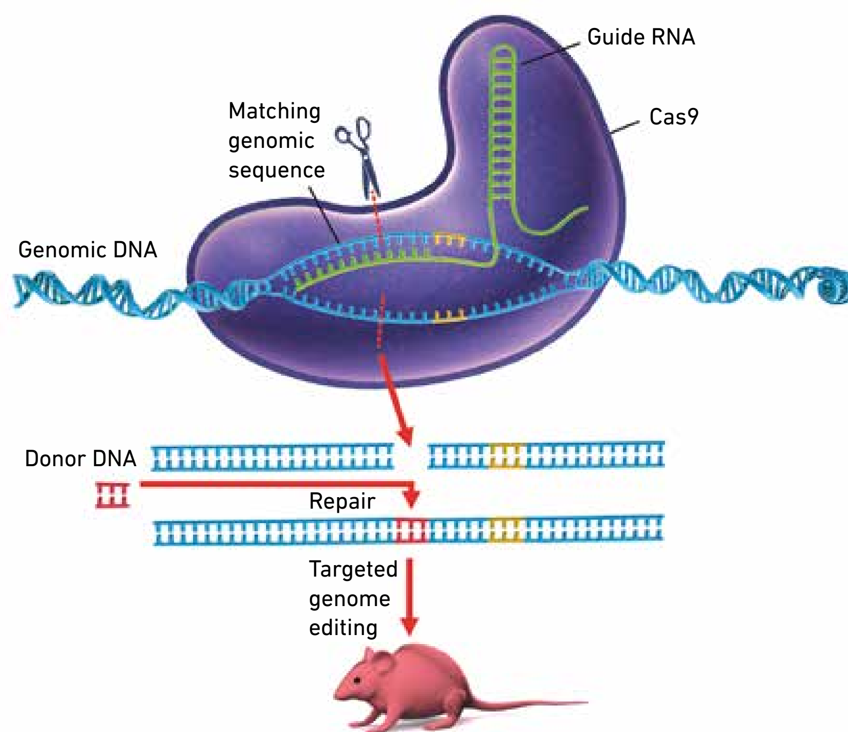
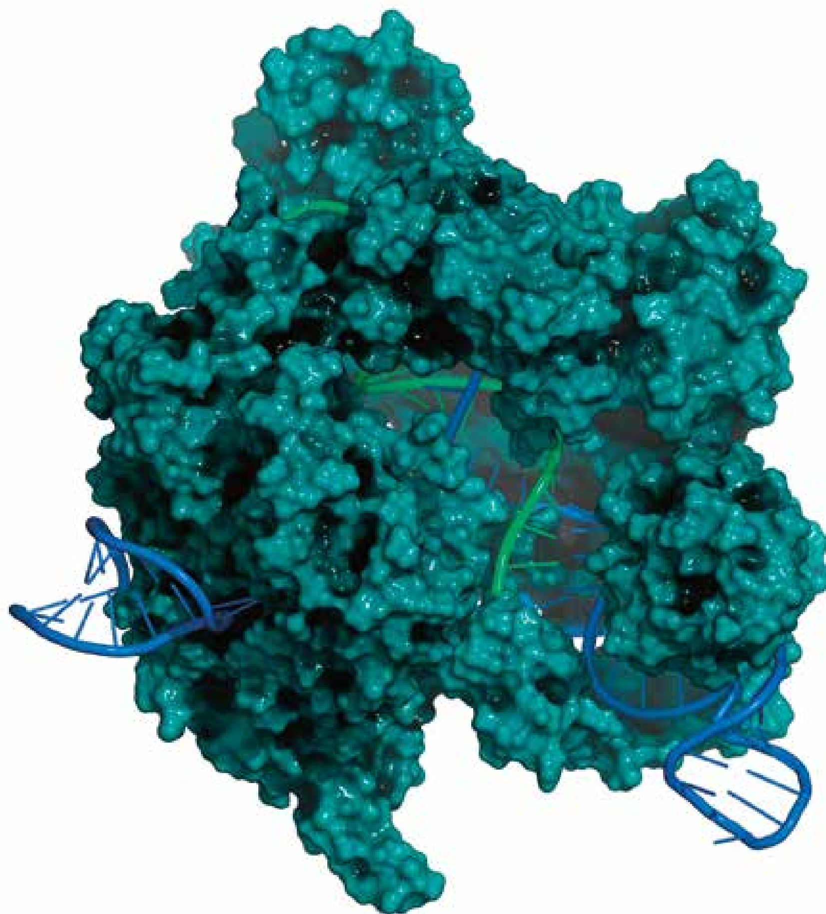


Diagram of the process that occurs within the CRISPR-Cas9 gene editing complex. This protein (purple) is used in genome engineering to cut DNA. It uses a guide RNA sequence (green) to cut DNA (blue) at a complementary site. The cut area of DNA is shown in yellow. The process of inserting a fragment of donor DNA (red) is shown at lower centre, with a genetically modified laboratory mouse. Gunilla Elam / Science Photo Library



CRISPR-Cas9 gene editing complex from *Streptococcus pyogenes.* Molekuul / Science Photo Library

on the method that is used, DNA will be repaired through the non-homologous end joining (NHEJ) pathway, or the homology-directed repair (HDR) pathway. NHEJ will result in higher efficiency of mutation incorporation, but with little control over the mutations that are introduced, while HDR provides full control over the mutations but with lower efficiency.

CRISPR-Cas to protect bacterial species in industry

Much of the initial interest in CRISPR-Cas came from the industry working with lactic acid bacteria. For example, yoghurt production can fail due to virus infections during the fermentation, which leads to product downgrading or loss. In the food industry, there is great interest in bacterial strains with naturally evolved resistance, and CRISPR provides an ideal system to generate strains that are more robust. However, these applied aspects of bacterial culture protection naturally lead to questions concerning the evolution and ecology of CRISPR-Cas systems. It was recently demonstrated that mounting a CRISPR-Cas immune response is associated with a fitness cost for the host. This may help to explain why only half of all bacteria have these sophisticated adaptive immune systems. Understanding the costs and benefits of CRISPR-Cas across different environments will help to design optimal defence strategies for bacterial species.

CRISPR-Cas and antibiotic resistance

Antibiotic resistance genes often move horizontally between bacterial species, for example through plasmid conjugation. Bacterial immune systems may limit such spread, since they can form a barrier for incoming DNA. The interaction between CRISPR-Cas and antibiotic resistance is unclear; some studies support the hypothesis that CRISPR-Cas blocks the spread of resistance genes, whereas other studies found no evidence for this. Understanding the short- and long-term implications of CRISPR-Cas is key if we are to predict and manipulate the spread of antibiotic resistance.

The discovery of the CRISPR-Cas system led to a sea of change in the microbiological sciences. Adjusting to the concept that 'primitive organisms', such as bacteria and archaea, had sophisticated adaptive immune systems was just the start. Since then, scientific endeavour has seen the number of potential uses for this primitive immune system, including genetic engineering with higher organisms, increase rapidly. In turn, the potential applications for this primitive microbial system are providing ethical questions that need to be addressed by society as the implications for the CRISPR-Cas system continue to emerge, especially within clinical settings.

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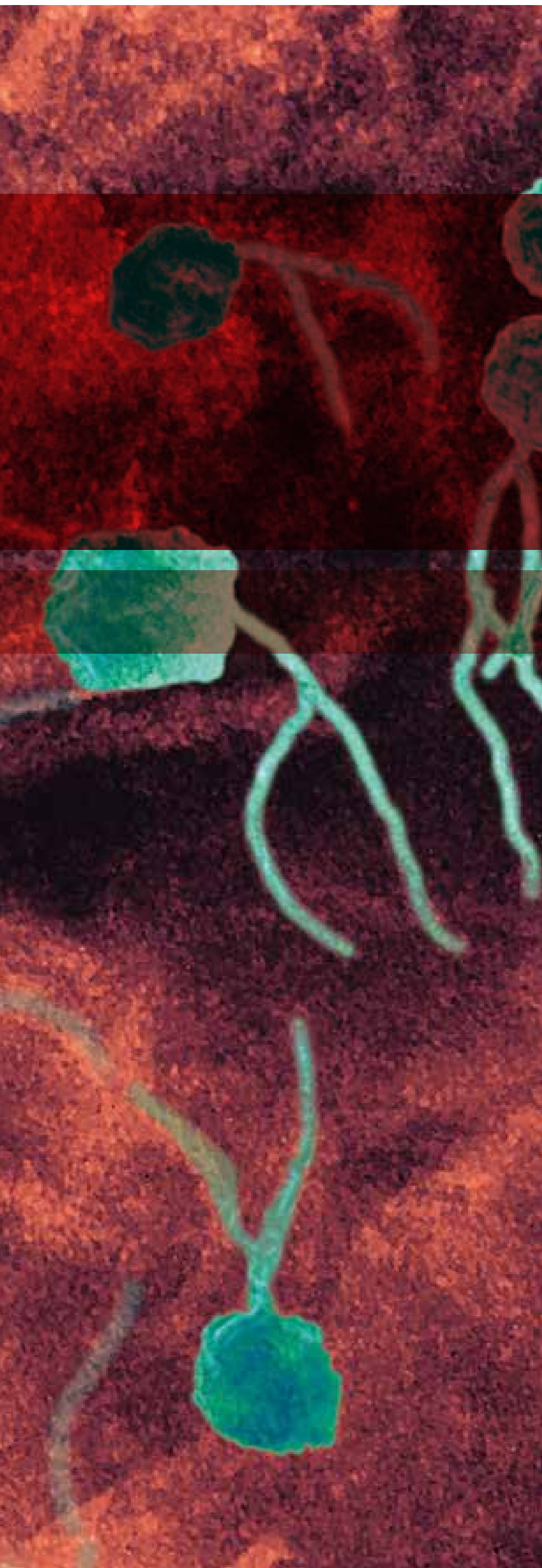
Phage therapy

Laura Bowater

Coloured transmission electron micrograph of bacteriophage particles (turquoise) attacking a bacterial cell (red). AMI Images / Science Photo Library



As early as 1945, in his Nobel Prize Lecture titled 'Penicillin' Alexander Fleming declared, "There is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant".

Fleming's speech was prescient. In May of this year (2016) headlines around the world reported the case of the first known US patient to be infected with *Escherichia coli* bacteria that possess resistance to a 'last resort' antibiotic, colistin. This was not the first time that ominous stories about colistin-resistant bacteria hit the headlines; colistin-resistant bacteria have been found in China, Canada and Europe, including the UK. The implications are clear: we are running out of effective antibiotics as the number of antibiotic-resistant pathogens continues to increase and even the antibiotics of last resort are losing their efficacy.

Tackling this growing crisis is an urgent priority and attention is focusing on protecting and preserving the antibiotics that we have at our disposal, as well as hunting for new antimicrobial products. Antibiotics work by targeting and destroying bacteria. However, they are not unique in that respect. Bacteriophages, or phages for short, can also target and destroy bacterial cells. Phages are ubiquitous; they are found in seawater, freshwater, soil and sewage slurries. It is estimated that there are 10^{30}–10^{32} phages in the biosphere and every 48 h, phages infect and destroy about half the bacteria in the world. The remarkable thing about phages is their specificity to target particular bacterial species.

Phage therapies from the past

In 1915, more than 10 years before Fleming's article on penicillin, an English doctor called Frederick Twort (1877–1950) published an article entitled 'The Infectious Disease of the Micrococcus' in *The Lancet*. It described how a phage infection of colonies of *Staphylococcus* gave rise to clear zones of lysis as the phages appeared to destroy the bacteria. In 1917, the French Canadian microbiologist, Félix d'Herelle (1873–1949) described these infectious particles as bacteriophage (as they 'eat' bacteria). The name has stuck to this day. In 1919, shortly after the end of the First World War, d'Herelle used phages to treat dysentery for the first time in the Hôpital des Enfants-Malades in Paris. The phage preparation was administered to a 12-year-old boy suffering with severe dysentery. The patient's symptoms improved immediately and the boy recovered fully within a few days. Encouraged by the success of his original solution, new preparations of bacteriophages were prepared to target other bacteria, including *Vibrio cholera* and *Yersinia pestis*. Soon, reports began to emerge that these new phage solutions were successfully treating

thousands of people in China, Laos, India, Vietnam and Africa who were suffering from cholera and bubonic plague.

Pharmaceutical companies began to sell phage solutions commercially. D'Herelle's laboratory in Paris produced phage preparations against various bacterial infections that were sold by what became the large French company L'Oréal. In the 1940s, Eli Lilly and Company (Indianapolis, Indiana, USA) also produced several phage products for human use that targeted pathogens such as staphylococci, streptococci and *E. coli*. These pathogens are still causing concern today as antibiotic-resistant species continue to increase.

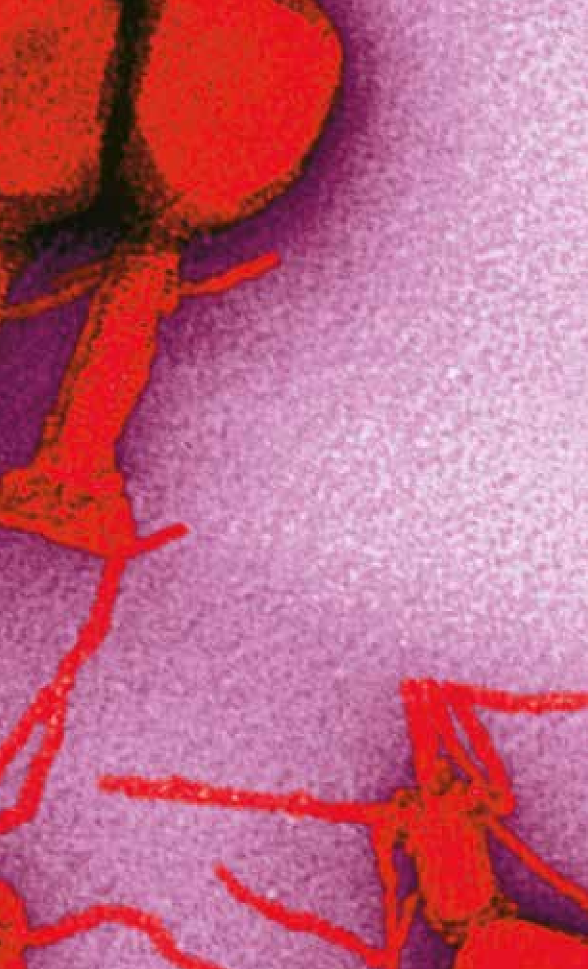
D'Herelle's collaborations with scientists in Tbilisi, Georgia, ensured that phage therapy was adopted as a clinical treatment for bacterial infection in the former Soviet Union and Eastern Europe. Unfortunately, the results of these studies did not use the rigorous, double-blinded clinical trials that are the current gold standard used to test new therapeutic drugs and interventions today. These studies were extensively published in non-English (primarily Russian, Georgian and Polish) journals. As a result, they were not readily accessible to the western scientific and medical community, and phage therapy rapidly fell out of favour in the west, especially after antibiotics became commercially available.

Colour-enhanced transmission electron micrograph of enterobacteria phage T4, a phage that infects *Escherichia coli* bacteria. Omikron / Science Photo Library

Phage therapy in the future

However, as there is a dearth of new, clinically relevant antibiotics emerging through the antibiotic discovery pipelines, there is renewed interest in revisiting phage therapy. Currently, there are clinical trials, which are examining the safety and efficacy of phages as topical therapies for treating skin and ear infections in humans, as well as food additives designed to reduce bacterial contamination of ready-to-eat meat, fruit, vegetables and dairy products.

Scientists are exploiting phages in other ways. After phages enter the bacterial cell they force the bacteria to synthesise new phage particles that accumulate inside the cell. At the end of their replication cycle the phages use suicidal enzymes, called endolysins (phage lysins) to degrade the peptidoglycan found in the bacterial cell walls and the progeny phages are released into the environment. The lysis of the bacterial cell is also enabled by another group of phage enzymes, the holins, which are produced during the late stages of phage infection. Holins and endolysins are encoded by the bacteriophage genomes and synthesised by the bacterial cellular machinery. As the holins oligomerise, they punch holes through the inner bacterial membrane. This allows the lysins to move through the cellular membrane to attack and degrade the bacterial cell wall. Phage endolysins differ depending on whether they target the cell wall of Gram-positive or Gram-negative cells. What makes endolysins so interesting is that they can be applied exogenously as recombinant proteins to destroy bacterial cells. In addition, compared with many antibiotics still in clinical use, the endolysins have an added advantage; they exhibit a genus or species specificity. These characteristics offer exciting therapeutic possibilities. Lysins encoded by phages that infect Gram-positive hosts usually contain two distinct domains. They have an N-terminal catalytic domain (CD) and a C-terminal cell-wall binding domain (CBD). Engineering chimeric lysins, or 'chimeolysins', by mixing and matching the two domains from various natural lysins offers opportunities for finding novel enzymes with improved properties such as increased solubility and an extended lytic spectrum. Currently,



> Lysins destroy the peptidoglycan directly, killing both growing and non-growing cells. And, unlike antibiotics that often kill bacteria indiscriminately, lysins display a high specificity that ensures that the normal commensal microflora is left undisturbed.

methods for developing chimeolysins, such as domain shuffling, depend largely on a trial-and-error approach, and this limits the efficiency for discovering effective chimeolysins.

Scientific research is currently underway to examine how lysins can be used to treat and destroy pathogens associated with food production such as *Clostridium perfringens*, which causes necrotic enteritis in poultry and is one of the leading causes of food poisoning in humans. In addition, Staphefekt is the first endolysin to be registered for human use against common skin conditions such as eczema, acne and rosacea, which are caused or exacerbated by *Staphylococcus aureus*.

Despite the promise of viable antibiotic alternatives and a previous medical history, phage therapy and phage derivatives still provoke serious concerns among the scientific establishment. Issues include the fact that bacteria are just as capable of developing resistance to phages and phage lysins as they are to develop resistance to antibiotics. But phages can also mutate their endolysins and holins, enabling them to re-infect phage-resistant bacteria, and this process happens rapidly and naturally; it does not rely on human intervention. Although humans and animals are constantly exposed to phages, much more research is required to ensure that phages and phage-derived therapy do not cause adverse reactions. As lysins are proteins, they are capable of stimulating an immune response when administered mucosally or systemically. This immune response can potentially decrease the lysis activity. It is absolutely essential that the phages or phage derivatives, such as lysins, are screened to make sure that they are safe to use for human and animal consumption.

Lysins offer enormous potential as effective antibacterials in the fight against infectious disease caused by pathogens with multidrug resistance. Antibiotics such as penicillin and cephalosporin inhibit peptidoglycan synthesis, and only dividing cells are lysed. In contrast, lysins destroy the peptidoglycan directly, killing both growing and non-growing cells. And, unlike antibiotics that often kill bacteria indiscriminately, lysins display a high specificity that ensures that the normal commensal microflora is left undisturbed. As bacteriophages are considered the most abundant biological entities on Earth, they are a rich natural source of these enzymes. It is hoped that bioinformatic and proteomic studies will lead to new opportunities for domain swapping, and the production of specifically engineered designer chimeolysins with diverse applications that will be part of the solution to the growing problem of antibiotic resistance.

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Further reading

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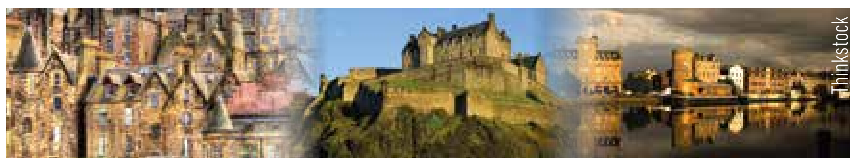
Annual Conference 2017 #Microbio17

Work continues with the preparations for our Annual Conference 2017, which takes place at the Edinburgh International Conference Centre (EICC) between 3 and 6 April. Abstracts submission will be opening soon and closing just before Christmas, so start working on your submissions today to be in with a chance of presenting your work through an offered paper or poster! You can now register online and take advantage of our early bird rate, and don't forget to apply for your grants in time to ensure you meet the deadlines.

Visit the Society website to view all of the information about the Annual Conference and follow the Twitter hashtag #Microbio17 for regular updates.



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Destination Edinburgh

Scotland's capital city is globally recognised as a world-leading authority in the sciences, technology and education. Home to more than 3,000 researchers and 100 market-leading companies, it is a major part of Scotland's science and technology sector – a sector that continues to lead the world.

Did you know that it was in Edinburgh that chloroform was first used as an anaesthetic? Dolly the sheep was cloned at the University of Edinburgh's The Roslin Institute, and the first 'bionic' hand – a powered prosthetic with articulating fingers – was developed by a spin-out company from the city's Princess Margaret Rose Hospital.

So along with Edinburgh's status as a thriving scientific hub; plus plenty of attractions, lots of history, superb travel systems and fantastic accommodation, it was an obvious choice as the location for our 2017 Annual Conference.

Must-sees in Edinburgh

There's so much to see and do in Edinburgh, why not plan some time before or after the conference to visit some of the must-see attractions as recommended by Convention Edinburgh:

- Make some time to explore the delights of Edinburgh Castle, which is visible from almost every main street in the city centre. It's a medieval fortress built upon the site of an extinct volcano and provides breath-taking panoramic views of Edinburgh.
- Scotland's Parliament Building is located at the bottom of the Royal Mile and is open to visitors all year round, except when Parliament is in recess. Guided tours are free of charge.
- The Royal Yacht Britannia is one of the UK's top tourist attractions. Berthed at Leith Docks, it's available for guided group tours.
- The National Museum of Scotland provides a diverse collection that will take you on a journey of discovery through the history of Scotland, the wonders of nature and world cultures – all under one roof and with free admission.

Search online for more must-sees and further information on tickets and opening times.

Impact of Annual Conference attendance

As part of our evaluation following this year's event we discovered that the impact of attending our Conference is far greater than we could have imagined. Here are some of the outcomes delegates shared with us, relating to professional development and developing connections:

Professional development

- I gained experience of presenting my work
- I updated my knowledge
- I have been invited to present at other meetings
- I identified funding opportunities
- I identified the latest research to support with my dissertation topics
- I arranged an external examiner for my PhD student
- I met the expert in my field
- I found the opportunity to recruit young talent
- I was able to identify job opportunities and new job leads

Developing connections

- I was able to meet face-to-face with others
- I developed contacts and research groups
- I met with collaborators
- I developed joint projects
- I developed long-term friendships and networks
- I networked with researchers at a similar stage in their career to me
- I met and discussed with people who were able to advise on current difficulties in my research
- Some of the companies at the stalls have agreed to sponsor our research/send us free samples.
- I discovered new products from the commercial stands
- I found a PhD student working on the same topic as me

Keep up-to-date with events, follow the Society on Twitter: **@MicrobioSoc**

Accommodation and Travel

Because Edinburgh provides so much to see, do and explore, the destination attracts many visitors all year round and therefore hotels are continuously in use. So if you're planning on joining us for Conference next year we would highly recommend you secure your accommodation and make your travel plans as early as possible.
To aid you with securing your accommodation you can visit our website where we provide a link to our booking service via Reservation Highways. We have instructed them to secure negotiated rates at hotels to suit a range of budgets and you can make your reservation today, or you may wish to make your own enquiries by searching online travel agents or visiting the local tourist board. Either way, avoid delay so you can get the best value for money!

One last thought – if you are planning on travelling as a group, why not try and make your travel plans together? Perhaps you can share a twin room or even hire an apartment; plus,- when travelling together you may be able to save on fares with some train companies who offer discounts for multiple bookings. And why not share taxi fees when you arrive?

Getting to Edinburgh is easy and below is a guide to assist your plans:

Air Edinburgh Airport is served by more than 40 airlines, connecting 130 worldwide destinations. Only 12 km from the city centre, it's served by excellent bus, tram and taxi services that link directly to the town. More information available from **www.edinburghairport.com**

Rail Edinburgh station is linked to all the UK's major cities and airports. A high-speed link to London takes just over four hours. The Eurostar service connects to Paris in around eight hours. There are trains to major cities across the UK, as well as to Scotland's favourite tourist destinations. More information available from **www.nationalrail.co.uk**

Road Edinburgh is easily accessed by a network of motorways and trunk roads, chiefly the M74, A1 and A68 from the south and the M8 from the west. The M9 and M90 head north. Edinburgh's bus station on St Andrew Square connects to all the major cities in Britain. More information on bus services available from **www.nationalexpress.com**

Visit the website to book your place at the Annual Conference: **www.microbiologysociety.org/events**

We look forward to seeing you there.

Conference Programme 2017

You can view the current 2017 programme online, including a list of over 150 invited speakers and their talk titles and abstracts. Here is a list of the scientific sessions for 2017:

Main symposia:

- Anaerobes in infection
- Aquatic microbiology
- Cell biology of pathogen entry into host cells
- Circadian rhythms
- EndCritical health challenges in medical mycology?
- Epigenetic and non-coding RNAs in eukaryotes
- Geomicrobiology
- Heterogeneity and polymicrobial interactions in biofilms
- Just passing through – virus infections of the gastrointestinal tract
- Macromolecular machines
- Microbial cell surfaces
- Microbial genomics: whole population to single cell
- Microbial mechanisms of plant pathology
- Annual General Meeting of Protistology-UK Society: Intracellular infection and endosymbiosis within protists
- Regulation of RNA expression during virus infection
- Synthetic and systems biology approaches to microbiology

Prokaryotic forums:

- Prokaryotic Genetics and Genomics Forum
- Prokaryotic Microbial Infection Forum
- Environmental and Applied Microbiology Forum
- Microbial Physiology, Metabolism and Molecular Mechanisms Forum

Virus workshops:

- Antivirals and vaccines
- Clinical virology
- Evolution and virus populations
- Gene expression and replication
- Innate immunity
- Pathogenesis
- Plant virology
- Viral assembly

**Titles are subject to change*

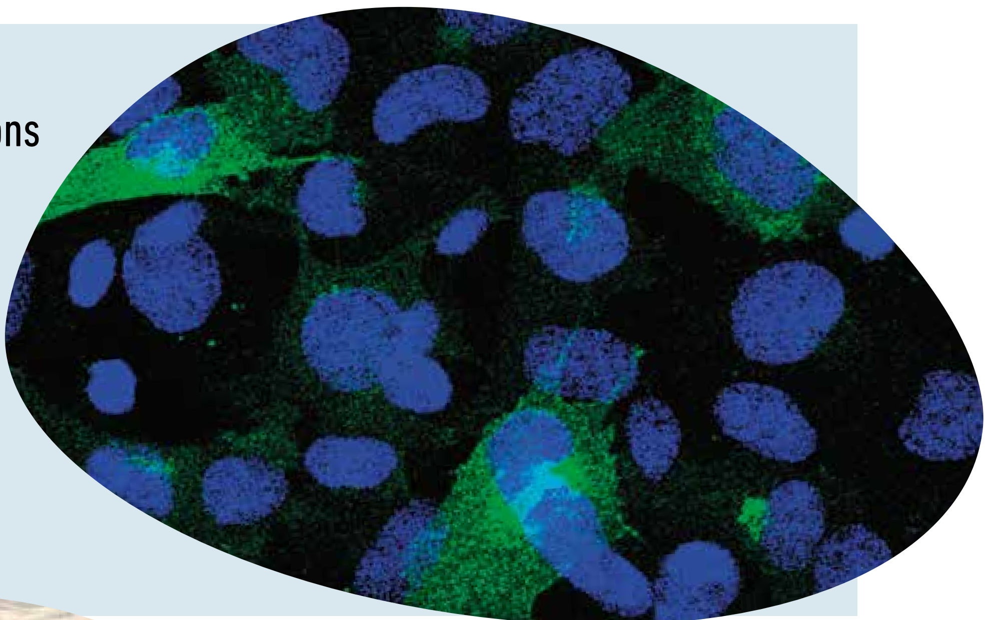
Focused Meetings

Each year the Society runs a series of Focused Meetings in the UK and Ireland that have been submitted by our members. These meetings are a fantastic way to learn from, network with and present to the leaders working in each specific field of microbiology.

Irish Division
Host–Pathogen Interactions

The first in our series of Focused Meetings took place at the end of June in Trinity College, Dublin. Invited speakers from local and international institutes presented their research on host–pathogen interactions.

The meeting took place over two half-days and included plenty of networking opportunities and poster presentations, along with offered papers during the programmed talks.



Gerald Barry / Massimo Palmarini

Thorsten Allers

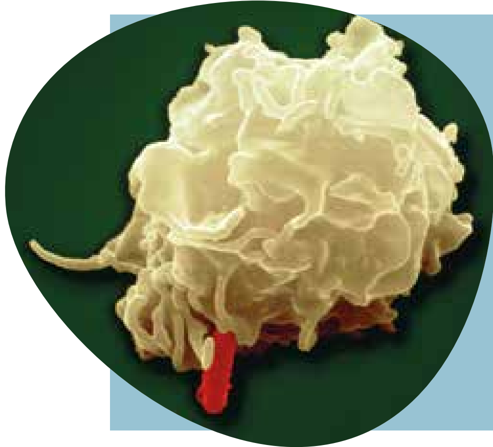
Molecular Biology of Archaea 5

The second in our series has just taken place at the London School of Hygiene and Tropical Medicine. It attracted a large number of international delegates and over 65 abstract submissions were added to the programme as posters and offered papers. The event also included an evening at the Charles Darwin Centre in the Natural History Museum, where the delegates enjoyed a supper, to round off an excellent meeting.

Thanks to all of those who helped make the meetings a success, and please continue to submit ideas for future meetings.

Keep up-to-date with events, follow the Society on Twitter: **@MicrobioSoc**

Still to come in the series...



Juergen Berger / Science Photo Library

#MISinterface

Irish Division
Exploring the Microbe–Immune System Interface

1–2 September 2016 – Rochestown Park Hotel, Cork, Ireland

In this meeting, we will explore our current understanding of how commensal and pathogenic bacteria interact with the host immune system during infection, and the consequences of these interactions in health and disease.

Topics will include:

- Host colonisation
- Host–microbiome interactions
- Infection and immunity
- Immune regulation

Speakers will include:

Jose Bengoechea (Queen's University Belfast, UK)
Lydia Lynch (Harvard Medical School, USA)
John O'Shea (National Institutes of Health, USA)
Vincent Young (University of Michigan, USA)

The Dynamic Fungus

#DynamicFungus

5–7 September 2016 – Mercure Exeter Rougemont Hotel, Exeter, UK

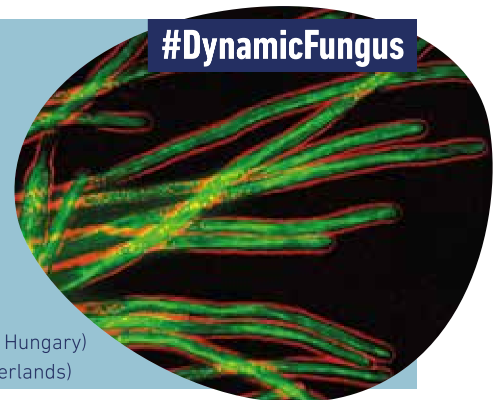
This meeting aims to celebrate the dynamic nature of fungi and provide an overview of some of the most cutting-edge current research in fungal biology.

Topics will include:

- Dynamics of the fungal cell
- Mathematical modelling in fungal science
- Dynamics of cellular differentiation
- Dynamics in fungal pathogenicity
- Dynamic evolution and adaptation of fungi

Speakers will include:

Geoff Gadd (University of Dundee, UK)
Jeff Gore (Massachusetts Institute of Technology, USA)
Balázs Papp (Biological Research Centre, Hungary)
Han Woesten (University of Utrecht, Netherlands)



Gero Steinberg

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#Avian16

Molecular Biology and Pathogenesis of Avian Viruses

27–29 September 2016 – Charles Darwin House, London, UK

This timely meeting focusing on avian viruses will bring together the international scientific community to assess the extent of the problem and help find solutions.

Topics will include:

- Molecular biology and genetics of avian virus replication
- Tropism and host range restriction
- Pathogenesis of avian viruses
- Host antiviral responses and virus immunomodulation
- New and improved approaches to the control of avian viruses

Speakers will include:

Wendy Barclay (Imperial College London, UK)
Paul Britton (The Pirbright Institute, UK)
Laura Delgui (National University of Cuyo, Argentina)
Roy Duncan (Dalhousie University, Canada)

You can view each of the programmes, along with a list of invited speakers, on our website **www.microbiologysociety.org/events** where registration remains open for each meeting. Visit the website today to secure your spot.

Legionella turns 40

Delegates attending the *Legionella* symposium at Colindale. Mark Greenley

2016 marks the 40th year since the now infamous outbreak at the 58th American Legion's convention, Bellevue-Stratford Hotel, Philadelphia, USA, in 1976 resulting in many unexplained deaths. It was Joseph McDade, a specialist in rickettsia at the Centers for Disease Control and Prevention (CDC), Atlanta, USA, who along with his team finally discovered the causative agent, the Legionnaires' disease (LD) bacterium, by inoculating guinea pigs with clinical material from patients. This bacterium was later formally named as *Legionella pneumophila*.

To mark this milestone, Public Health England (PHE) arranged a one-day symposium at Colindale, London, on 31 March 2016 called '*Legionella pneumophila* (1976 to 2016) – from whole guinea pigs to whole genome sequencing'. The aim was to bring together *Legionella* experts and those with an interest in *Legionella* to gain new insights, particularly concerning the application of the new disruptive technology of whole genome sequencing (WGS) to public health.

The opening presentation was by Professor Jacob Moran-Gilad on 'Legionellosis in Israel - it's not the heat, it's the humidity', which covered an infant death due to LD caused by a domestic humidifier. Next, Dr Valeria Gaia presented '*Legionella*: the Swiss experience' and described the link between increased incidence of LD and climate conditions. Dr Sophie Jarraud reported on an investigation into one of the largest LD outbreaks in France in 2004, illustrating new insights gained from the application of WGS analyses. Dr Natalia Kozak-Muiznieks, from the CDC, illustrated how both the current consensus typing method, sequence-based typing (SBT), and WGS analysis of a US healthcare-associated outbreak, led to a revisiting of the concept of *L. pneumophila* subspecies. Sophia David, a final-year PhD student (Sanger Institute/PHE), described the evaluation of WGS for the epidemiological typing of *L. pneumophila* and the rationale for a new core genome WGS scheme. The next speaker was Sharon Carney, a first-year PhD student (Imperial College London/PHE), who presented the application of metagenomics for improved detection of *Legionella* in low-abundance specimens. In May–July 2012, a large LD outbreak occurred in Edinburgh, Scotland. Professor J. Ross Fitzgerald presented data on WGS analysis of the clinical isolates, which revealed subtypes within a single sequence type, raising the possibilities of diversification within a single environmental source or multiple point sources. The title of Professor Julian Parkhill's talk was 'Recent emergence of *L. pneumophila* disease-associated clones: what does it mean?', with the somewhat controversial hypothesis that the only possible explanation is that human infection is involved in the transmission cycle. Finally, the day also marked the retirement of Dr Tim Harrison who gave his insightful 'Reflections from the Legionella Reference Laboratory'.

Acknowledgments

Special thanks to Professor Maria Zambon, PHE Directorate and Microbiology Society for financial support; Professor Nick Phin and Dr Tim Harrison (Chairs), Mark Greenley (audio visual support), Sheila Culkin (administrative support) and Kastro Doda (catering).

Norman Fry

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This event was supported by the Society via a Society-Supported Conference Grant. Find out about other Society-Supported conferences taking place this year and how you could apply for a grant for your event at **www.microbiologysociety.org/ssconferences**

MICROBIOLOGY SOCIETY

Microbiology invites submissions of research articles and reviews that focus on established and emerging topics in biotechnology.

There are a wide range of applications for microbiology in the field of biotechnology, from industrial and environmental processes to developments in biopharmaceuticals. Articles are welcome that utilise microbial systems to develop technologies and products that could improve our lives or our environment.

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- Metabolic engineering
- Metabolomics
- Genetic engineering
- Synthetic biology
- Directed evolution
- Bioremediation
- Bioengineering
- Protein engineering
- Microbes as cell factories
- Bioenergy

Editor-in-Chief
Dr Tanya Parish
Infectious Disease Research Institute, USA

The biotechnology section of the journal is overseen by:

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University of York, UK

Editor
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MICROBIOLOGY

Microbial Genomics: One-Year Anniversary!

July marked the one-year anniversary of the Society's newest open access journal, *Microbial Genomics* (MGen). We recap on the journal's extraordinary first year and meet the journal's Senior Editors to find out what achievement they are most proud of and what papers they hope to see published in the journal in the future.

The past year has seen a wave of change across the Society's journal publishing department, and paving the way in innovation is the Society's open access and open data journal *Microbial Genomics* (MGen), which aims to champion the discoverability and accessibility of open research data. In support of this mission, four MGen Editors joined a panel discussion on Open Data (**https://microbepost.org/tag/exploring-open-data**) at the Society's Annual Conference to discuss why it's important to share datasets in real-time and connect on a wider scale to target outbreaks; the differences in open data needs for academics and public health clinicians; and being open about science and the importance of metadata.

Another exciting achievement for the journal is its highest Altmetric score in the Society. The journal's published paper 'Applying phylogenomics to understand the emergence of Shiga-toxin-producing *Escherichia coli* O157:H7 strains causing severe human disease in the UK' (**http://microb.io/1TPZYtf**) peaked at an Altmetric score of 84. This is an incredible achievement for a journal that is barely a year old, and lead author Timothy Dallman acknowledged, "I think this shows the impact that social media, and particularly Twitter, can have in rapidly disseminating scientific knowledge to our peers, the press and the public."

At six months, Senior Editor Kat Holt reviewed the variety of papers published, from cutting-edge genomic epidemiology, antibiotic resistance, to several articles focusing on functional genomics to investigate microbial gene regulation. Also key to developing the area of microbial genomics are methods papers, which are critical to driving research forward.

Six months on and submissions are on the increase, with the launch of the *Microbial Genomics* poster prizes, focused interviews with the Senior Editors of each subject category and a wealth of inspiring scientists drawing interest for the journal's feature 'Standing on the Shoulders of Giants' (**http://microb.io/1OcuHOl**).

As we continue to build a strong community of *Microbial Genomics* researchers, we interviewed each of our Senior Editors to get a better insight into their subject categories.

Meet the Senior Editors

Find out about their subject category, what they are most proud of achieving so far and what subjects they would like to see more of in MGen in the next 12 months.

Jennifer Gardy
BCCDC Canada

Microbial Evolution and Epidemiology is the place for papers that shine a light on microbial population genomics, and we're proud to say it's becoming a go-to spot for papers in the emerging field of genomic epidemiology.

I am so proud of the remarkable calibre of authors we've attracted for MGen! Looking through the list of authors who have published in the journal it really is a who's who of the microbial genomics community – there are so many people doing truly leading-edge work!

I'd love to see more genomic epidemiology outbreak reports in the journal – we can really carve out a niche for ourselves here. I'd also love to see our community start taking a look at combining genomic datasets published by multiple groups in order to do some really large-scale comparative genomics.

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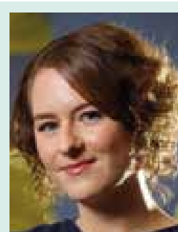
Alan Walker University of Aberdeen, UK
Microbial Communities publishes research employing marker gene, full metagenomic or metatranscriptomic sequencing to characterise and understand the structure, function and dynamics of microbial communities present in a range of host-associated and environmental niches. One of the key strengths of the journal is the commitment to open data and open access, ensuring that both results and raw data are freely available to the wider scientific community immediately upon publication. The hope is that this will benefit submitting authors by increasing audience exposure to their research, and also benefit the scientific process by allowing others to validate or further utilise data. We would like to encourage further submissions in the area of host–microbiota interactions, with a particular emphasis on studies where the sequence data provides real biological insight into the role that microbial communities play in host health.

Jukka Corander University of Oslo, Norway
Genomic Methodologies highlights the new depths of microbial sequencing that necessitates new tools to make the most out of the data and the Genomic Methodologies focus of MGen aims to promote methods which make a difference for turning bases into biological insights. This new journal has very rapidly attracted the attention of both method developers and practitioners, publishing biological highlights with a steady pace from the start. Novel methods for pangenomic analysis are particularly welcome as subjects as the need for such tools is increasing as the data sets get more and more complex.

Christos Ouzounis CPERI, CERTH, Greece
Systems Microbiology covers a combination of systems biology and microbial genomics, addressing big-data issues, omics approaches, perturbation experiments, networks and pathways, microbial diversity and population dynamics under a systems perspective. MGen has already published some very interesting studies on the subject, including the comparative genomics of bacterial strains, genome-wide analyses of environmental responses, clinical proteomics, and others. I would love to see more of those efforts flourish, and invite work on single-cell genomics, comparative-omics, including transciptomics, pangenome analyses and perhaps emerging application domains, such as metabolic engineering work for biofuels and bioenergy.

Carmen Buchrieser
Pasteur Institute, France
Microbe-Niche Interactions should improve our understanding of the interactions that pathogenic, commensal or environmental microbes have with their niches. The journal welcomes articles that use new genomic, proteomic, transcriptomic, metabolomic or imaging techniques to analyse these interactions. Having an excellent, gender-balanced, motivated and innovative Editorial Board that loves working together and promotes the journal is a great achievement. We would like more high-throughput imaging and metabolomics analysis of the interactions and influences of the microbe on the host and the host on the microbe in future papers.



Kathryn Holt
University of Melbourne, Australia
Responses to Human Interventions covers genomic studies of microbial responses to human interventions including clinical interventions such as antibiotic therapy, vaccines or infection control measures; as well as agriculture and environmental changes such as climate change or pollution. I am proud of the variety and quality of articles published so far in MGen and also the journal's innovative feature 'Standing on the Shoulders on Giants' which highlights pioneers in the field of genomics. In future we would like to engage the experimental evolution community to publish their ground-breaking studies on evolutionary responses to antimicrobial stresses, as well as those with longitudinal genomic data sets examining evolution in response to treatment and host-associated stresses.

View the journal online:
http://mgen.microbiologyresearch.org

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Diagnosing and sequencing Ebola in Sierra Leone

Professor Ian Goodfellow in the Public Health England diagnostic laboratory in Makeni, Sierra Leone. I. Goodfellow

In November 2014, I was deployed with Public Health England (PHE) to establish one of the first diagnostic laboratories in Sierra Leone. Our team of 10 scientists was composed primarily of biomedical or clinical scientists from various UK hospitals or diagnostic laboratories. We were sent to Makeni, to work in an Ebola treatment centre (ETC) operated by International Medical Corps (IMC).

When we arrived the ETC was still under construction so the first weeks were spent moving boxes and equipment around a bustling building site. The early days were rather chaotic, logistics were unpredictable so the establishment of the laboratory required a lot of creative approaches including the repurposing of household items purchased from the local market for use in the laboratory.

The diagnostic lab opened on 13 December 2014 and this significantly reduced the time taken between samples being collected to the results being available from days to as short as four hours.

During my first deployment to Sierra Leone, and in discussions with Dr Tim Brookes (PHE) and Professor Jeremy Farrar (Wellcome Trust), we had identified an urgent need to be able to sequence viruses in real-time. The ability to sequence patient samples within a 24–48 h time period would make the data of use for epidemiological tracing and the identification of transmission networks. By making the data freely available, it would also enable scientists to gain a better insight into how the virus was evolving in real-time rather than being reliant on the release of data following publication, which often occurred many months after samples had been isolated. The ability to sequence viruses in Sierra Leone rapidly has been critical in the recent emergence of new cases from persistently infected survivors.

I returned home on 23 December 2015 and what followed was a frantic period of contacting suppliers, obtaining quotations and putting together a grant to perform next-generation sequencing in a tent next to the diagnostic lab. In April 2015, my third deployment to

Professor Ian Goodfellow discussing the interpretation of Ebola virus sequencing data with a member of the World Health Organization Ebola response team. I. Goodfellow

Makeni Team #1 within the PHE diagnostic laboratory. The team were sent to establish the diagnostic laboratory at the Mateneh Ebola treatment centre in Makeni. From left to right: Dr Stan Ko, University of Central Lancashire; Prof. Ian Goodfellow, University of Cambridge; Cristina Leggio, PHE Porton; Stephanie Leung, PHE Porton; Lisa Berry, University Hospitals Coventry and Warwickshire (UHCW) NHS Trust; Steve Diggle, PHE Manchester; Laura Grice, PHE Manchester; Catherine Moore, PHW Cardiff; Kate Baldwin, North Bristol NHS; Angela Short, PHE Bristol. I. Goodfellow

Makeni Team #1 preparing to establish the diagnostic laboratory at the Mateneh Ebola treatment centre run by International Medical Corps. From left to right: Kate Baldwin, North Bristol NHS; Lisa Berry, University Hospitals Coventry and Warwickshire (UHCW) NHS Trust; Catherine Moore, PHW Cardiff; Steve Diggle, PHE Manchester; Dr Stan Ko, University of Central Lancashire; Stephanie Leung, PHE Porton; Cristina Leggio, PHE Porton; Angela Short, PHE Bristol; Laura Grice, PHE Manchester; Prof. Ian Goodfellow, University of Cambridge. I. Goodfellow

Sierra Leone, I returned to Makeni to install an Ion Torrent sequencer in the ETC using support from the Wellcome Trust. Dr Armando Arias, a postdoctoral fellow in my lab, and I set off to install all the equipment and establish the sequencing workflow. Neither of us had operated a sequencer before but we had 5 days of training beforehand to enable us to install, run and, if necessary, repair the equipment. The sequencer and the other equipment arrived on 13 April 2015 and within a period of 3 days we were sending our first Ebola virus sequences back to our collaborators at the Wellcome Sanger Centre and University of Edinburgh for analysis. We have since sequenced >1,200 samples, providing >600 viral genomes, ~1/3 of all the viral sequences from the outbreak. By sharing our data with other scientists, including a team run by Dr Nick Loman, also a Microbiology Society member who was performing real-time sequencing in Guinea, we were able to provide invaluable information on the movement of cases across the borders in real-time.

In September 2015, we subsequently relocated to the University of Makeni (UniMak) and established the UniMak Infectious Disease Research Laboratory to provide both local and international scientists with access to in-country sequencing technology. The laboratory has provided support to a number of ongoing clinical trials and is providing longer-term capacity building of scientists in state-of-the-art research methods. The sequencing facility continues to provide essential support to the outbreak, including the sequencing of the last cases in Sierra Leone and the new cases from Guinea.

I would encourage all members of the Society to get involved in work in low- and middle-income countries, as it has provided me with an invaluable insight into the impact of infectious diseases on vulnerable populations. Although challenging, the six months I spent in Sierra Leone working as part of the response was without a doubt one of the most rewarding experiences of my scientific career.

I have just recently returned from a two-week trip to Makeni to run microbiology practicals for the undergraduate students at the local university (UniMak) and to begin a public engagement project in collaboration with EducAid, a UK-based charity involved in education in Sierra Leone. The Wellcome Trust-funded engagement project aims to improve the awareness of infectious diseases in secondary school students within Sierra Leone and will run until September 2017.

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Professor Ian Goodfellow and Sunday Kilara (International Medical Corps) preparing to unpack the equipment required to establish the Ebola virus sequencing facility. I. Goodfellow

Developing microbiology around the world

The Society's International Development Fund supports members' development activities in low-income economy countries. We spoke to two previous recipients to find out more about their projects.

Professor Iruka Okeke, University of Ibadan, Nigeria

"It is important to me to teach at the highest level – the International Development Fund allowed us to make a high-impact curricular change and start a collaborative research project that engages students as well as faculty and staff members."

In 2015, Iruka received Society support to transform the Masters course in Pharmaceutical Microbiology at the University of Ibadan, Nigeria. Iruka relocated to Ibadan from Haverford College, USA, after receiving an African Research Leader's Award under the Medical Research Council/Department for International Development concordat agreement. She noticed that, due to resource limitations, graduates joining the Pharmaceutical Microbiology programme often had little microbiology wet-lab experience.

Society funding helped develop a discovery-based laboratory course, where students with limited previous experience of bacteriology learned practical skills in microbiology, molecular biology and bioinformatics via investigation of leaf microbiomes of plants used in Nigerian ethnomedicine. The project has brought the expertise of individuals in the department – bacteriologists, molecular biologists and natural product researchers – together around a new research endeavour. According to Iruka, the wet-lab and research training has been invaluable both for the development of the individuals, and future generations of microbiologists that they will go on to train.

Discovery-based learning course: student Pelumi Adewole loading his agarose gel. Ayandiran Tunmise, PHM712 class

Dr Dorina Timofte, University of Liverpool, United Kingdom

"Having worked in the UK for several years, I see major gaps that occur in antimicrobial resistance detection, surveillance and antimicrobial stewardship in countries like Romania. The grants were the perfect vehicles to enable significant impact in this area."

Dorina received Society support in 2012 and 2014 for antimicrobial resistance (AMR)-focused projects in Romania. During previous collaborations in Romania, she noticed that one contributing factor for AMR was the lack of national guidance regarding routine testing and reporting methodology for detection of antimicrobial-resistant bacteria. As an Antibiotic Action champion, Dorina addressed this by implementing rapid diagnostic methods in order to improve patient care and prevent the spread of resistance in hospitals.

The first grant supported a workshop to exchange knowledge of detection methods for resistant bacteria in Romanian diagnostic laboratories. This was the first time such a meeting of human and veterinary microbiologists was held in Romania, and participants received protocols for detection of resistant bacteria to support implementation of routine testing. Dorina continued this work in 2014 by implementing a pilot scheme for detection of carbapenamase-producing Gram-negative bacteria (CPGNB) in Romanian hospitals. She established wide collaborations and generated evidence of the need for a national surveillance system to rapidly identify CPGNB-infected or colonised patients to allow implementation of appropriate infection control measures. Participating laboratories continue to use these protocols with the aim of them being implemented as part of a wider screening policy throughout Romania.

Advice for prospective applicants

Iruka: "Apply! The awards have truly transformative potential. The opportunities are far greater than we had predicted and we're working on sustaining our discovery-based course."

Dorina: "The Fund is a great way to help microbiology in less economically fortunate parts of the world. If you have an idea that fits with the remit of the scheme, do not hesitate to apply – you never know what opportunities it may lead to."

The next closing date for the International Development Fund is 1 October 2016. Contact **grants@microbiologysociety.org** for details.

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Membership

"I had a brilliant day!"

I mentioned in the last issue that we are undertaking some research to help us identify what we may need to do differently in future, to ensure Society membership remains relevant and attractive.

I talked to members and delegates at our Annual Conference in Liverpool in March, to find out just what it is that continues to draw participants in ever increasing numbers to our annual showcase event. After all, it's a serious commitment both in terms of time and money. The quote above is not from me (although I did have a brilliant day too, getting to meet and talk to many from across the organisation). The quote is actually from Mark Jones, an undergraduate student at John Moores University in Liverpool. Mark had come along to the Annual Conference for the first time and summed up his experience in these words. He'd arrived not really knowing what to expect, listened to a variety of presentations, met new people, discussed his work, and went home very happy.

It was a sentiment that was echoed by many. Increasingly, members seem to be enjoying the opportunity to meet first-hand, hear world-class presentations on a very wide range of topics and enjoy the interactions that naturally follow both within and outside the formal programme.

So maybe we shouldn't try to over-complicate the member research that's currently underway, looking for all the answers in an in-depth analysis of questionnaires and spreadsheets. Maybe some of the answers are closer and more obvious than we think. From my (admittedly not very scientific) observations, opportunities to meet other members, talk to them, discuss ideas and compare notes all seem to be high up our members' wish list. So too does having the opportunity to listen to some great science and get closer to the originators of it. Wrap this all up in a supportive and friendly environment (along with regular opportunities to socialise) and we seem to be on the right track in delivering, at least in part, what many members are looking for. In a world increasingly dominated by online interactions, these opportunities to engage face-to-face are ones that seem to be striking a real chord with members.

But these are just my observations from Conference. What about the members who don't go to Conference? What about those who live overseas? What about those members who are less than satisfied and feel we fall short? What about members who want a more enhanced online experience? And what about those who have made a conscious decision not to join us? We have members – and prospective members – with a wide range of views and opinions and we need to ensure these are captured too. So inevitably there has to be some form of information collection and analysis to get a representative cross-section of views that will help us better understand why a member feels a sense of place or home in one organisation and not in another. And this is why we need your help.

We need you to tell us what you think about your membership of the Society – what we do well, what we don't do well and what we could do differently in future. You don't have to be a current member. You can be a former member or not even have been a member at all. If you have thoughts, ideas and opinions around the subject, please let us have them. We'd love to hear from you. There are a number of ways to join the conversation. You can:

- Take part in our Member Survey. It's a short online questionnaire for current, former and 'never been' members. You should have already received an email invitation to take part, but if you haven't, please contact me and I will send one to you.
- Take part in a focus group meeting. There will be face-to-face group meetings taking place across September 2016 to share insight and discuss membership-related issues in more detail. If you would like to register your interest, please email me. Final selections, timings and locations will be advised closer to the time.
- Email me with your thoughts and suggestions on how we can make membership even more attractive in the future.

With your help, we want to create a membership offering that is attractive and relevant, valued and recognised. In short we want many more of you to be able to say 'I had a brilliant day'.

Paul Easton
Head of Membership Services
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Old School, New School:

determining phylogenetic relationships and evolutionary history of micro-organisms using bioinformatics

Bioinformatics is the method of using computer programming to interpret biological data. The teaching of bioinformatics in schools is becoming an integral part of the curriculum and understanding the evolutionary relationships between species at the genomic level is an important skill. However, the basic process of how these relationships are determined is fundamental in understanding all of these relationships.

The activities below demonstrate how the evolutionary history of different strains of a virus or other micro-organism can be determined by using both modern (computer) and historical (observation) methods.

Phylogenetic comparisons of genetic sequence data to determine the origins of HIV

HIV (human immunodeficiency virus) is a type of virus that attacks and weakens the immune system and most notably can lead to AIDS (acquired immune deficiency syndrome). Since the discovery of HIV in 1981, the origin of the virus has been widely debated. The most widely accepted hypothesis, which is backed up by the scientific evidence, is that HIV came from cross-species transmission of related viruses. Closely related non-human primates (monkeys and apes) carry viruses that are very similar to HIV, called SIVs (simian immunodeficiency viruses).

By comparing the genetic sequences of these closely related viruses it is possible to make assumptions about evolutionary relationships and origins of different strains. The following procedure is taken from a case study on the DNA to Darwin website (**www.dnadarwin.org**). The activity makes use of Geneious software, which aligns protein sequences following nucleotide translation and builds phylogenetic trees that allow the user to visualise how closely related sequences are to one another.

Student procedure

1. Download the free software, *Geneious*, from **www.geneious.com** and the Geneious document from **www.dnadarwin.org/casestudies/7** containing the 'DNA-HIV1andSIV.geneious' file. This data file contains 16 nucleotide sequences of the *pol* gene from the HIV and SIV viruses from chimpanzees, gorillas and humans.
2. Open the *Geneious* program and the DNA-HIV1andSIV.geneious file. The 16 sequences will be visible on the central window. Use the zoom function (magnifying glass button) to view the sequences at the nucleotide level. You should be able to see differences between the sequences at the base level. How can you tell the difference between an RNA and DNA virus at the nucleotide level?
3. Make sure all 16 sequences are selected by clicking and highlighting the file name in the top window of the program. Click on the 'Translate' button to convert the nucleotide sequence to a protein sequence. (A box will appear, make sure the genetic code selected is 'Standard' and the translation frame is '1').
4. A new file will be created and as you did in Step 2, you can zoom in on this sequence and view the sequences at the amino acid level. Each letter represents a different amino acid. Can you guess which amino acid is represented by the letters?
5. Now to ensure the sequences are aligned (they should be already, however). Make sure the protein sequence file is selected in the top window and select the 'Alignment' button. A box will appear and the 'Geneious Alignment' method needs to be selected (it is the only option with the basic software). The alignment can take a few minutes to process.
6. A new alignment file will appear in the top window, select this and click on the 'Tree' button to create a phylogenetic tree. Another box will appear and the following options need to be selected; Genetic Distance Model: Jukes-Cantor, Tree build method: Neighbour-Joining, and Outgroup: No Outgroup. Make sure the 'Resample Tree' box is unchecked and select 'OK'.
7. Your phylogenetic tree should appear! You can zoom in and out and re-size the tree to properly study it. The tree should show you the different subgroups of the HIV-1 family and how these relate to the SIV sequences found in gorillas and chimpanzees. An easy way to visualise the tree is to print it out and highlight different relationships you can see.

Questions to consider

- Can you visualise the branches of the tree that are from human, chimpanzee or gorilla origin?
- How closely related are the different HIV subgroups to each other and to the SIV sequences. What can you conclude from this about the origins of HIV?

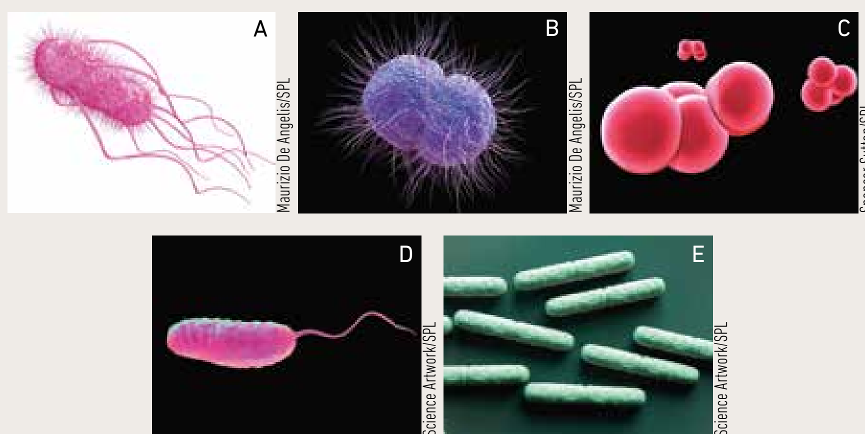
For further information, see our factfile on HIV and AIDS on our education website, Microbiology Online: **www.microbiologyonline.org.uk/teachers/resources**

Building a phylogenetic tree based on morphological characteristics

A simple way to make assumptions about the evolutionary history of a group of organisms is to observe morphological similarities between them and build a picture of how recently they shared a common ancestor based on the number of different features. The activity below is a basic introduction to how phylogenetic trees can be created using morphological characteristics of different micro-organisms. The trees will show common origins and how species can be related to one another based on morphology.

Student procedure

1. Below are five illustrations (A–E) of well-known bacteria. They each have differences and similarities to one another.



Maurizio De Angelis/SPL
Maurizio De Angelis/SPL
Spencer Sutton/SPL
Science Artwork/SPL
Science Artwork/SPL

2. Using the table below, record the features for each of the micro-organisms. The features listed are purely suggestions, include your own.

	Shape (rod or cocci)	Colour	Flagella present?	Texture (smooth or spiked)
A				
B				
C				
D				
E				

3. In the following table, record the number of differences between each micro-organism. The numbers in each of the boxes indicate how the different micro-organisms relate to one another based on their morphology.

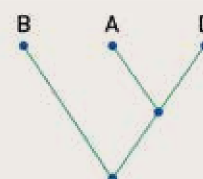
	B	C	D	E
A				
B				
C				
D				

4. To draw the phylogenetic tree, the five micro-organism letters will be placed at the top of the tree as these are the currently living micro-organisms. Firstly, you need to identify which of the micro-organisms are the closest relatives; these will have the least number of differences between them. Place this pair next to each other at the top of the tree. These two will share a recent common ancestor; place a dot below the two letters and draw lines leading to the letters showing their evolutionary lineages.

5. To find the pairs of organisms that have a common ancestor that lived before the most recent pair, look for the micro-organisms that have two differences. Draw a dot below these organisms and link these too.

6. Do the same for both three differences and when the organisms differ by four: this means they share the oldest common ancestor and the tree is complete.

An example of a smaller phylogenetic tree developed using this technique is shown below:



Things to consider:

- Are there other ways to classify the morphology of these micro-organisms? Think about possible lab techniques that you could use.
- Is it possible any of the morphological features have evolved independently of the other species (convergent evolution); are there ways you could determine this?
- Look at the world around you; think of other everyday items you could determine relatedness of with this technique. A tasty suggestion is a biscuit sampler tray; you could look at features such as chocolate coating and shape.

For further information about bioinformatics, see our open access journal, *Microbial Genomics*: **http://mgen.microbiologyresearch.org**

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Outreach

Light at the bottom of the sea

Recipient of the Education and Outreach Grant, Society member Dr Jenny Search shares her work on engaging primary school children using bacterial luminescence.

Large anglerfish at the school's Lumiere festival. J. Search

I have worked in the field of science communication for over 10 years now and spend most of my time working with school children, promoting all the science, technology, engineering and research (STEM) subjects and encouraging youngsters to think of themselves as potential scientists and engineers of the future. It is always exciting when I get to do something linked to immunology and microbiology – the topics of my own undergraduate and postgraduate studies.

Sadly, there is little microbiology in the current English national curriculum at primary school level. I was approached by Denise Armstrong from Vane Road Primary School in County Durham, as she wanted to run a microbiology event in school but, like many primary schools, they couldn't afford the time to run activities not directly linked to the national curriculum. Denise also wanted the children to develop something that could be used

Primary school children making anglerfish. J. Search

as part of the school's activities for their Lumiere festival. We came up with the topic of 'Light at the bottom of the sea' where we linked bacterial luminescence with the circuit-making requirements of the national curriculum.

I developed a workshop to introduce the children (aged 10–11) to bacteria, symbiosis and light production, particularly bioluminescence under the sea. The children then used craft materials and electrical components to create a model anglerfish with a light that can be turned on and off with a basic switch. As the light in the anglerfish lure is provided by bioluminescent bacteria, this provides a good link to exploring the marine microbial environment.

The children investigated several marine creatures that have light-producing symbiotic bacteria. They were quite taken with the wide range of strange, glowing creatures that live under the sea. They came up with their own ideas for creating light-up creatures that could be used as their part of the school's annual Lumiere festival. They worked with an artist to create larger model anglerfish and other bioluminescent sea creatures and then I helped them add circuits and glow-sticks to their models so they lit up.

As well as producing a fantastic display of light at the bottom of the sea, the children found out a lot more about general microbiology, the scale of micro-organisms and the wide range of environments in which they live. Evaluation showed the workshop countered some common misconceptions, such as viruses and bacteria being the same, and bacteria being only known as causing disease. The children found making the light-up anglerfish tricky and they needed perseverance to get it to work; however, most of the children stated this was their favourite part of the workshop. They also enjoyed finding out about marine life and building circuits.

Jenny Search
http://drresearch.co.uk
@DocReSearch

The next deadline for the Education and Outreach Grant is **1 October**. For more information, please see **www.microbiologysociety.org/grants** or contact **grants@microbiologysociety.org**

Making anglerfish. J. Search

Jenny Search demonsrating luminol (above) and jellyfish at the school's Lumiere festival (below). J. Search

Science policy update

The Society's policy team work to ensure appropriate scientific information and expert opinion is made available to policy-makers. We also engage in science policy to ensure that microbiology is supported as a discipline. Here is an overview of just some of our activities over a busy first half of 2016.

Antimicrobial resistance

Antimicrobial resistance (AMR) remains a key priority for our policy work. As a founding member of the Learned Society Partnership on AMR (LeSPAR) we aim to support research and present a unified scientific voice on AMR (**http://microb.io/1Ontlzi**). Meeting with research funders and policy-makers, the Societies have been highlighting issues and ideas raised by our collective memberships at a series of interdisciplinary LeSPAR workshops held last year.

In February, the Microbiology Society was invited to a stakeholder workshop in Brussels about the European Union's AMR Action Plan; there was interest in the report from our LeSPAR workshops and we were able to highlight the importance of research and the environmental dimension of AMR.

Get involved

Our policy work depends on the expertise and input of our members. It is a great way to promote microbiology and increase its impact. Contact the team at **policy@microbiologysociety.org** to find out how to get involved or flag an issue. Keep an eye on the monthly newsletter and our website (**www.microbiologysociety.org/policy**) for policy news, opportunities and resources.

In May, the Society endorsed recommendations made by Lord O'Neill's independent review on AMR, including building AMR research capacity and raising global awareness.

Policy consultation responses

Our consultation response to the House of Commons Environmental Audit Committee *UK Soil Health* inquiry in January highlighted environmental microbiology, while our joint response with the Society for Applied Microbiology to the House of Commons Science and Technology Committee inquiry on *Science in emergencies: UK Lessons from Ebola* saw several issues our members raised, such as better drawing on and supporting microbiology volunteers in disease emergencies, cited in the Committee's report. We also highlighted our forward-thinking Antibiotics Unearthed outreach project in our response to the Committee's *Science Communication* inquiry.

View our consultation responses on our website: **www.microbiologysociety.org/consultationresponses**

Linking microbiologists and policy-makers

Early-career members questioned leading UK politicians on science policy at Voice of the Future 2016. Members Professor Mike Skinner and Professor Wendy Barclay attended a Parliamentary and Scientific Committee meeting on the safety of pathogen research. Society staff and members also attended Parliamentary Links Day at the UK Parliament and Science and the Assembly in Wales.

The Society is also represented on several external policy committees and groups. Professor Dawn Arnold was elected to the advisory committee of the UK Plant Sciences Federation, while Dr John McGrath replaced Dr Nigel Ternan as the Society's representative on the Northern Ireland Learned Societies and Professional Bodies Forum.

Fungal diseases and microbiomes

We continue to proactively highlight important microbiology issues and research to policy-makers through our series of policy briefings, including our one on *Human Fungal Diseases*, which was sent to UK and Irish policy-makers.

All briefings are accessible on our website: **www.microbiologysociety.org/briefings**

The Society has also established a Microbiome Expert Working Group, chaired by Professor Julian Marchesi, to produce a major evidence-based document for policy-makers, which will explore the research on human, animal and environmental microbiomes.

Paul Richards
Policy Officer
p.richards@microbiologysociety.org

Timber rattlesnake (*Crotalus horridus*). Frupus / Flickr

www.microbepost.org

Best of the blog

It's been a busy few months for us here on the blog, and we've covered research from across the world (and out of it).

Back in April, we learnt about *Ophidiomyces ophidiicola*, the causative agent of Snake Fungal Disease, an emerging pathogen that is spreading rapidly across North America. Anand Jagatia interviewed Dr Jeffrey Lorch from the National Wildlife Health Center in America about his work on the fungus. First reported on the East Coast in 2006, this often-fatal disease has now been detected in 16 American states and in Canada (**http://microb.io/1rffeTQ**).

Members of the UK Parliament often have to debate issues outside of their area of expertise. How do they get the background information they need to stay up-to-date on the latest research? The answer is the Commons Library, staffed by 70 specialist staff working across eight subject areas. Our Policy Officer, Paul Richards, reviewed a 'Policy Lunchbox' event that saw Ed Potton, Head of Science and Environment at the Library, explain the service (**http://microb.io/1TiqViJ**).

For the podcast in June (**http://microb.io/28IFjar**), I was lucky enough to interview Dr Kate Rubins, a virologist who at this moment is orbiting the Earth at 17,000 miles an hour aboard the International Space Station. At the time of our interview, Kate was in the final few weeks of training ahead of the mission's launch. We talked about her transition from one career to another, having to learn chemistry and physics, and whether a pipette works upside down!

Parasitic worms affect millions of people and represent a significant health burden worldwide. However, might there be an upside to these infections? On the April podcast, Anand interviewed Dr Ken Cadwell from New York University, who has been investigating whether worms can cure inflammatory conditions of the digestive tract (**http://microb.io/1VEBgfp**).

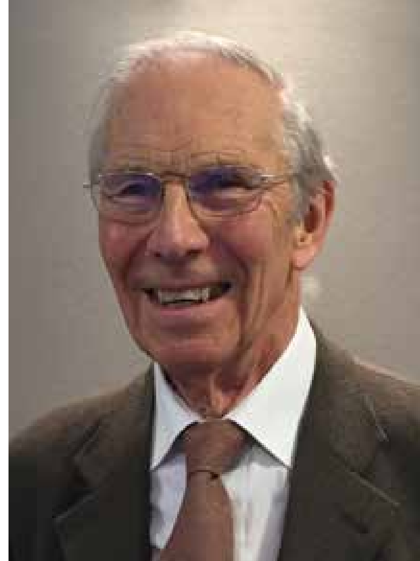
Finally in this round up, we looked at the history of vaccines, from their beginnings in the 15th century through to the modern methods used to create them today (**http://microb.io/1VYrZgQ**).

I'll be back again in the next issue to tell about some more of the content you might have missed. In the meantime, keep an eye on the blog at **www.microbepost.org** and search for the podcast on iTunes or on SoundCloud.

Benjamin Thompson
Head of Communications
b.thompson@microbiologysociety.org

Membership Q&A

This is a regular column to introduce our members. In this issue, we're pleased to introduce Dr Martin Cole.


M. Cole

What is your current position?

I retired some years ago after a career at Beecham Pharmaceuticals and SmithKline Beecham. I joined George Rolinson's Department of Microbiology at Brockham Park in 1958, later becoming head of the Department of Biochemistry and a Project Manager. When I retired I was Director of Research Programmes at Brockham Park.

What was your area of specialisation?

Microbial biochemistry, particularly searching for potentially useful substances. Initially I worked on 6-aminopenicillanic acid (6-APA), the nucleus of all penicillins, which is made in small amounts by *Penicillium chrysogenum* and from which a great variety of semi-synthetic penicillins could be made. One of my early achievements was to find an enzyme, made by a strain of *Escherichia coli,* which would readily deacylate benzylpenicillin to give 6-APA and hence allow bulk production of the new penicillins such as amoxicillin.

You worked in the field of antibiotic resistance, what did this involve?

The very useful broad-spectrum penicillin, amoxicillin, is destroyed by beta-lactamases made by many clinically important bacteria. A big effort was mounted to see if we could find an inhibitor of these enzymes. My colleagues and I found such an agent, a novel substance made by the soil microbe *Streptomyces clavuligerus,* which we named clavulanic acid. Developing its use for treating amoxicillin-resistant infections was a bit of an uphill struggle as there was no precedent for such an approach. A product containing amoxicillin with potassium clavulanate was eventually launched under the trade name Augmentin (co-amoxiclav) and is widely used around the world.

What was the worst moment of your career?

Having my briefcase stolen from my car while I was in a local pub with colleagues after work. It contained a draft of a multi-author paper and my draft for the patent application on clavulanic acid. With the help of our local police chief and two young boys from the nearby village, all the pages were retrieved, sodden, from roadside ditches, having been chucked away by the criminals when they found no money or drugs. I was very relieved!

What happened when you retired?

Initially, I did consultancy work for my previous employer. I also helped the United Nations Industrial Development Organisation in China and the Royal Botanic Gardens at Kew. As more time became available I joined local organisations, becoming Chairman of Dorking Museum, Convenor of the Local History Group and President of the Rotary Club in Dorking. I was also Vice-Chairman of NESCOT, a college of further and higher education in Epsom. I still enjoy reading *Microbiology Today*, *The Biologist*, *The Biochemist* and *Chemistry & Industry*.

What might your colleagues not know about you?

That I have flown over the City of London and up the Thames in the helium-filled Skyship 500, taking photographs over my old college – Imperial College. Also, I successfully took a course on driving the famous steam locomotive Flying Scotsman but did not exceed 25 mph.

What do you do to relax?

Together with my wife, Maureen, son and daughter we restored our 2.5 acre Victorian garden and now enjoy looking after it. With the help of our local museum and history group, and also the Surrey Gardens Trust, we researched the history of where we live and have just published a book, *The Capel Leyse Estate, South Holmwood, Surrey*. I also enjoy visiting other peoples' gardens, supporting the Royal Botanic Gardens, Kew and going on fungal forays. Oh, I nearly forgot, I collect things but there is not much space left in my study or my Victorian summer house!

If you would like to be featured in this section or know someone who may, contact Paul Easton, Head of Membership Services, at **p.easton@ microbiologysociety.org**

Focused Meeting 2016 – Irish Division:

Exploring the Microbe–Immune System Interface

1–2 SEPTEMBER
ROCHESTOWN PARK HOTEL
CORK, IRELAND

Topics will include:

- Host colonisation
- Host–microbiome interactions
- Infection and immunity
- Immune regulation

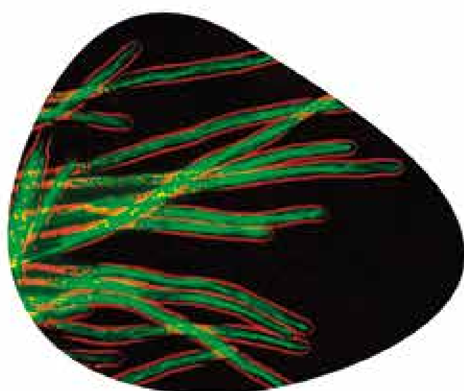
Organising committee:

Beth Brint (APC Microbiome Institute, University College Cork, Ireland)
David Clarke (APC Microbiome Institute, University College Cork, Ireland)

Focused Meeting 2016:

The Dynamic Fungus

5–7 SEPTEMBER
MERCURE EXETER ROUGEMONT HOTEL
EXETER, UK



Topics will include:

- Dynamics of the fungal cell
- Mathematical modelling in fungal science
- Dynamics of cellular differentiation
- Dynamics in fungal pathogenicity
- Dynamic evolution and adaptation of fungi

Organising committee:

Steven Bates, Gero Steinberg, Sarah Gurr, Ken Haynes, Ivana Gudelj, Tom Richards, David Studholme, Nick Talbot (University of Exeter, UK)

British Mycological Society promoting fungal science

Focused Meeting 2016:

Molecular Biology and Pathogenesis of Avian Viruses

27–29 SEPTEMBER
CHARLES DARWIN HOUSE
LONDON, UK

Topics will include:

- Molecular biology and genetics of avian virus replication
- Tropism and host range restriction
- Pathogenesis of avian viruses
- Host antiviral responses and virus immunomodulation
- New and improved approaches to the control of avian viruses

Organising committee:

Mike Skinner (Imperial College London, UK)
Venugopal Nair OBE (The Pirbright Institute, UK)

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Reviews

Biofilms in Bioremediation: Current Research and Emerging Technologies

Edited by G. Lear
Caister Academic Press (2016)
£159.00 ISBN 978-1910190296

The book has three sections, with each chapter written by one or more specialists in the area. This makes for easy navigation and chapters of specific interest can be read as stand-alone items. However, there is considerable repetition of basic biofilm information if read as a whole.

Ch. 1 is a useful introduction, if only for microbiologists to understand the importance of mass transfer! This should help to inform their research so that it is of more direct use to engineers. From my point of view, Table 1.2 is missing 'landfill leachate'.

Ch. 2 is a comprehensive account of biofilm biology and its advantages for bioremediation. However, 19th century trickling filter technology, one of the earliest uses of biofilms for used water bioremediation, is not mentioned. I'm unconvinced that shear forces act on microbial cells at surfaces other than in the lab, but I'm glad this boundary layer is mentioned in relation to substrate transport.

In 'Methods and Monitoring', Fig. 4.11 shows a more comprehensive range of biofilm reactors than in Ch. 1 and I wonder why it was not included there. Ch. 4 also considers the role of chemotaxis – anomalous in a book about biofilms.

The brief Ch. 6, on microscopy, lacks images illustrating the applications of each type. In Ch. 8, immobilisation of cells in gels or foam cubes should not be considered as 'biofilm', nor 'floating' aggregations (flocs) and rhizosphere communities in Ch. 10.

Ch. 11 provides an excellent introduction to biofilm permeable reactive barriers, with many useful illustrations. Ch. 12 is not particularly convincing about biofilm cells having higher rates of biodegradation than planktonic ones.

Ch. 13a presents interesting growth-promoting effects of phenol-degrading bacteria on *Lemna* roots, while Ch. 13b presents convincing data for the use of specific strains of *Sphingobium*.

A useful book for those new to the area, but probably as a library copy. A second edition would benefit from closer collaboration between the chapter authors, to avoid unnecessary repetition.

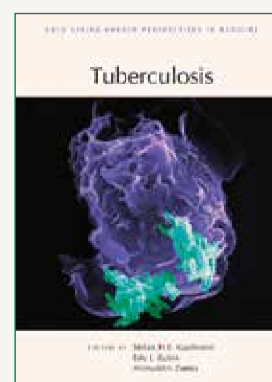
Mike Dempsey
Manchester Metropolitan University

Tuberculosis

Edited by S. H. E. Kaufmann, E. J. Rubin & A. Zumla
Cold Spring Harbor Laboratory Press (2015)
US$135.00 ISBN 978-1621820734

Tuberculosis is a comprehensive and updated amalgamation of contemporary knowledge of fundamental and clinical research on tuberculosis (TB) and its aetiological agent, *Mycobacterium tuberculosis*. The first section, the most diverse, covers topics including basic research on TB-related cell biology and immunology, advances in vaccine development, and the development of laboratory models of infection, ranging from zebrafish to primates. Diverse animal models have accelerated our understanding of host–pathogen interactions and vaccine design and I felt that this topic (four chapters) perhaps deserved a section of its own. The second section starts with a focus on the biology of the tubercle bacillus. I particularly liked the interlinking of this fundamental knowledge to subsequent chapters on drug development, discovery and use. The final section is dedicated to clinical aspects of TB, including updated information on epidemiology, diagnostics, TB in the context of HIV co-infection, management of latent TB, the worrying trend of drug resistance, and advanced imaging methods for diagnostics.

Tuberculosis also offers alternatives or challenges to conventional views on TB, such as a newer look at the granuloma as a structure that is beneficial to mycobacterial expansion, and a critical analysis of comparative genomics in defining 'virulence' factors, and of the protective role of humoral immune response. The book offers updated information and views to a



wide range of readers including, but not limited to, PhD students, early career postdoctoral fellows and clinicians pursuing research on TB.

Apoorva Bhatt
University of Birmingham

More reviews can be found at: **www.microbiologysociety.org/MicrobiologyToday**

Comment

Moving microbial diagnostics into the 21st century

Justin O'Grady

Thinkstock

Rapid and accurate diagnosis is critical for the effective treatment of life-threatening infections, such as bloodstream, respiratory tract and complicated urinary tract infections. These clinical syndromes have complex aetiology and require the recognition of pathogens within a variety of challenging sample types.

The 'gold standard' culture techniques are labour intensive, have long turnaround times and often offer poor clinical sensitivity. Even with the addition of mass spectrometry for rapid identification of bacterial isolates, and automated culture systems for isolation, the turnaround time is at least two days: one to grow the bacteria and, at best, one to identify pathogens and test their antimicrobial susceptibility. Meanwhile, in the absence of pathogen or resistance information, the patient is treated empirically with broad-spectrum therapy, which has evolved over time to include previously reserved potent antibiotics as a result of increasing antimicrobial resistance. This empirical therapy is often unnecessarily potent and, conversely, sometimes ineffective due to inherent or acquired resistance. Nucleic acid amplification-based diagnostic tests are a step in the right direction, providing results within hours, but are limited by the range of pathogens and resistances they can detect.

MinION. Oxford Nanopore Technologies

A paradigm shift in diagnostics technology is required, to allow the development of a universal diagnostic that can detect any pathogen or resistance. Next-generation sequencing (NGS) technology, particularly rapid nanopore sequencing using Oxford Nanopore Technologies' MinION device, has the potential to drive this shift by combining rapidity with comprehensiveness beyond that of culture or PCR. All pathogens contain nucleic acids and so identification in clinical samples by NGS, for example, metagenomics sequencing, is potentially universal (i.e. capable of detecting bacteria, viruses and fungi). Nothing has to be known about the pathogen in advance of testing, unlike PCR which only targets predetermined pathogens or culture, which requires different approaches for bacteria, fungi and viruses. Recent advances in nanopore sequencing technology include: reduced capital and running costs (US$1,000 returnable deposit for the MinION nanopore sequencer and as little as US$45/sample when barcoding 12 samples per flow cell); portability (MinION devices were recently used to track Ebola in rural West African communities) and rapid real-time analysis (pathogen ID within 10 mins of starting the device) using freely available analysis tools in Metrichor. It has now become realistic to investigate the utility of metagenomics sequencing for rapid diagnosis and antimicrobial susceptibility testing in clinical microbiology.

So why are researchers and clinical microbiologists across the world not implementing this technology in their laboratories as we speak? You've guessed it – sample preparation is the remaining bottleneck, a commonly neglected research area as it produces few 'glamour journal' articles. Working directly with clinical samples, without any culture enrichment steps, is challenging as competing nucleic acid, particularly that of the human host, make metagenomics sequencing-based pathogen diagnosis insensitive and costly (spending time and money sequencing unwanted host nucleic acid and struggling to find all or any of the pathogen genome with the sequencing depth available). Some sample types are particularly challenging, e.g. blood, where the ratio of human:bacterial DNA in a septic blood sample can be as high as 10 billion:1. Enrichment is necessary, but, for metagenomics sequencing, rather than time-consuming culture enrichment we need pathogen nucleic acid enrichment and/or host nucleic acid depletion. For metagenomics sequencing-based clinical microbiology to succeed, effective enrichment is vital.

We are currently developing novel pathogen DNA enrichment and human DNA depletion strategies and applying them to blood, sputum and urine for the metagenomics sequencing-based diagnosis of sepsis, hospital-acquired pneumonia and complicated urinary tract infections (UTIs). The different sample types come with different challenges, such as large numbers of leukocytes and low pathogen numbers in blood vs large numbers of leukocytes and pathogens in urine; therefore, we modify our enrichment/depletion strategies depending on the sample type being tested. This ensures that the cost–benefit is appropriate for the various sample types. Our current metagenomics sequencing pipeline (including host DNA depletion) for UTI and hospital-acquired pneumonia samples takes approximately 4 hours from sample to answer (pathogen identification and antimicrobial resistance gene identification) using MinION nanopore sequencing. The sepsis pipeline takes approximately 7 hours.

By providing comprehensive, rapid pathogen and antimicrobial resistance identification, metagenomics sequencing will reduce the time that patients remain on empirical broad-spectrum antibiotics, enabling clinicians to move away from a 'one-size-fits-all' approach to antibiotic treatment to a stratified medicine approach. This will lead to a reduction in the use of inappropriate antibiotics, reduced risk of antibiotic resistance and reduced patient morbidity and mortality. In the final O'Neill report, Jim O'Neill called for governments to "mandate now that by 2020, all antibiotic prescriptions will need to be informed by up-to-date surveillance information and a rapid diagnostic test wherever one exists". MinION metagenomics sequencing will ensure that a rapid diagnostic test exists for every disease.

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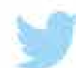

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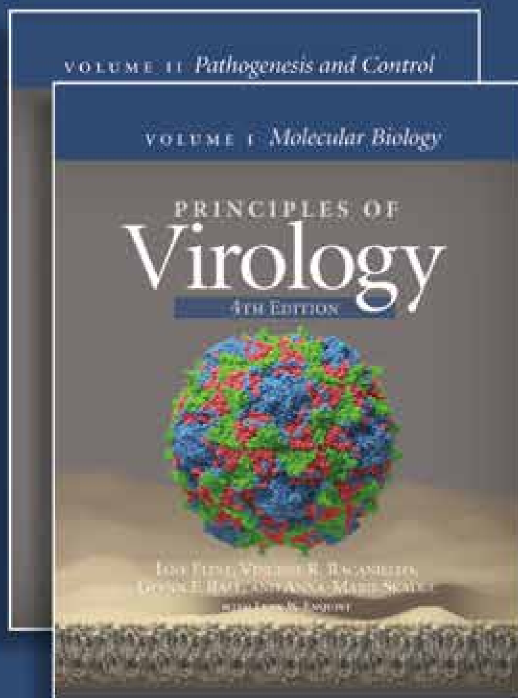
Glenn F. Rall and **Anna-Marie Skalka,** both at Fox Chase Cancer Center, USA



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