Visualising microorganisms from molecules to cells

Visual methods have been a central part of the study of microbial life since its beginnings. Microbiologists have used increasingly sophisticated microscopes to examine living cells, and fixed, stained and labelled cells, in clever ways to reveal their inner secrets. As part of their work, scientists have documented their findings with photographs, illustrations and models of the cells and their properties. These visual methods have played an important role in presenting microbial research in the FEMS journals. In this second of the four 40th anniversary Commentaries, images from 10 FEMS articles have been chosen to exemplify the diversity of visualisation used in microbiology. Read on to find out how far microbial imaging has come in the last 40 years.

Seeing cells

Microscopy led to the initial discovery and classification of bacteria and, today, advanced methods are revealing the inner molecular structure. The pages of FEMS journals are filled with micrographs from light microscopy and electron microscopy. In some cases, these micrographs stand alone. The freeze-etched SEM image of the surface of a *Pyrodictium* cell (Fig. 1; Rachel *et al.*, 1997) reveals a surface studded with the hexagonal proteins of an S-layer, as well as a bundle of tubules extending from the upper right region, providing interconnections to neighbouring cells. S-layer proteins are found on the surface of some bacteria, archaea and algae. The archaeon *Pyrodictium* was isolated from a deep-sea hydrothermal vent and was the first organism found to grow at temperatures above 100 °C. Its S-layer is assumed to provide mechanical, osmotic and possibly thermal stability.

Fluorescent labelling

In the past two decades, fluorescence microscopy has revolutionised cell biology, including microbial cell biology, by allowing scientists to follow specific cells or specific molecules in cells. Various techniques involving fluorescent molecules have become popular. For instance, green fluorescent protein (GFP) and its variants, such as red fluorescent protein, can be used to tag selected microorganisms and/or to act as reporters for monitoring gene expression in these microorganisms. In other types of experiments, known microorganisms can be stained by specific antibodies conjugated with fluorescent dyes. Furthermore, fluorescently labelled oligonucleotide probes have proved immensely useful for *in situ* hybridisation experiments in which environmental microorganisms may be identified at various taxonomic levels. These techniques have been applied singly or in combination as shown in the following four illustrations (Figs 2–5; Kragelund *et al.*, 2005; Gantner *et al.*, 2006; Elias & Banin, 2012; Schmidt & Eickhorst, 2014).

Authors can interpret more complex experimental images with a descriptive illustration. For instance, in models of mixed biofilms, three different spatial arrangements of two bacterial species used in the mixtures have been observed using confocal microscopy with fluorescent staining (Fig. 2; Elias & Banin, 2012). It appears that bacteria can cooperate with each other as separate microcolonies, as co-aggregates or as layered structures; these arrangements are illustrated by the simple cartoons shown in Fig. 2.

Catalysed reporter deposition-fluorescence *in situ* hybridisation (CARD-FISH), a powerful and specific visualisation technique, has been used to detect and
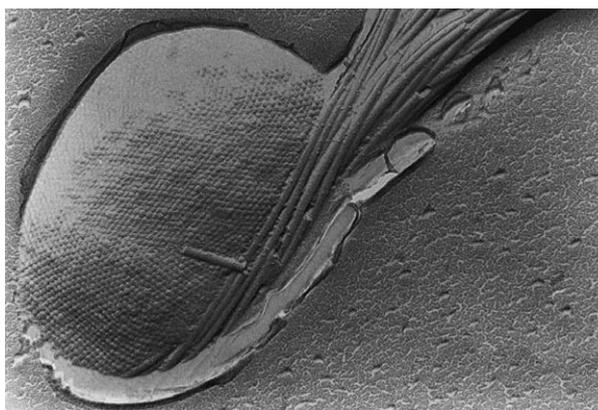
Fig. 1. Freeze-etched scanning electron microscopy image of the surface of a *Pyrodictium* cell (from Rachel *et al.*, 1997).
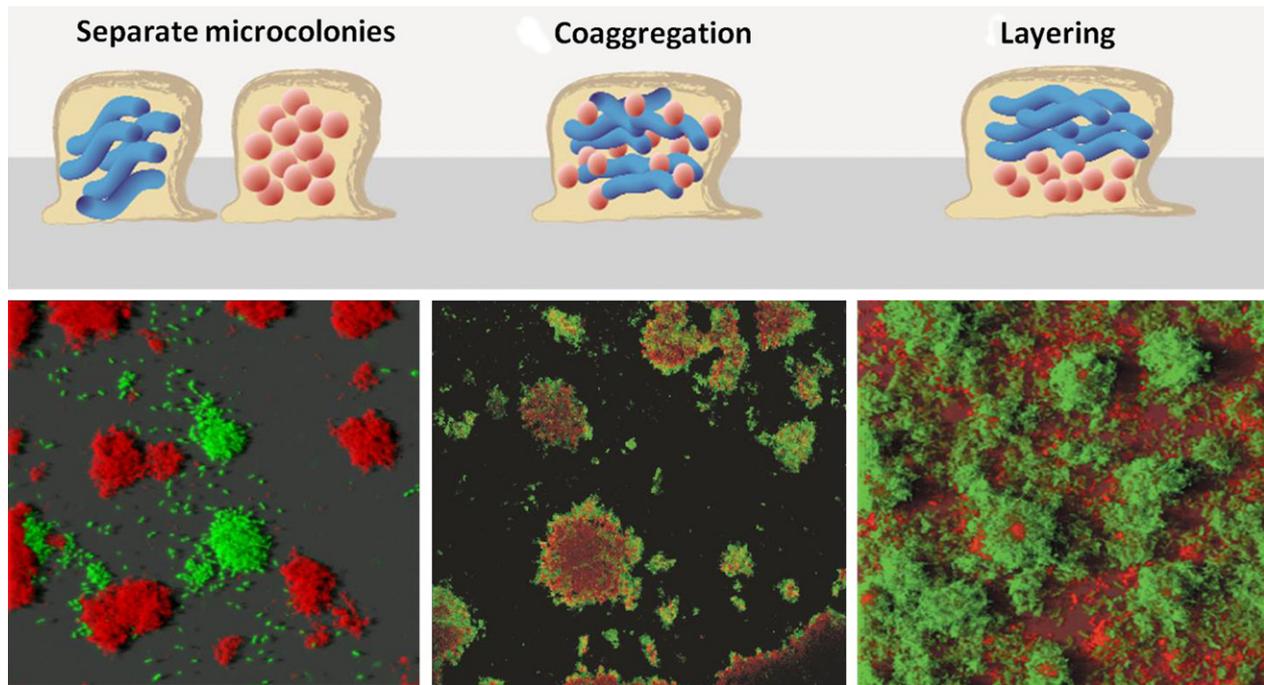
Fig. 2. Different spatial distribution of cells in mixed bacterial biofilms (from Elias & Banin, 2012; confocal images from Nielsen *et al.*, 2000; Rickard *et al.*, 2006 and Hansen *et al.*, 2007).
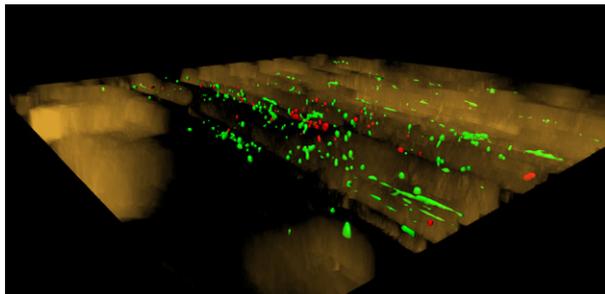
Fig. 3. Sequential hybridization reveals bacteria and archaea on a rice root (from Schmidt & Eickhorst, 2014).

quantitate bacteria (green) and archaea (red) simultaneously on rice roots (Fig. 3; Schmidt & Eickhorst, 2014). *Betaproteobacteria* dominated the bacterial populations on root tips and elongation zones while methanogens were particularly abundant in the rhizoplane.

Combined imaging techniques can show not only which species are present in a natural environment, but are also able to reveal their metabolic activities. In the example given (Fig. 4; Kragelund *et al.*, 2005), FISH-MAR (fluorescence *in situ* hybridisation combined with microautoradiography) simultaneously shows the presence of filamentous eubacteria (green), the alpha-proteobacterium *Meganema perideroedes* (red) and the

uptake of radiolabelled acetate (black dots) in activated sludge.

Seeing cell–cell communication

Confocal scanning laser microscopy has been combined with reporter gene technology to visualise bacterial signal molecules (N-acyl-homoserine lactones) produced on plant roots and to measure the 'calling distance', that is the distance that such molecules can travel from a producer cell to a recipient cell. In the image shown (Fig. 5; Gantner *et al.*, 2006), the producer *Pseudomonas putida* IsoF (red) emits signal molecules that, when sensed, paint the recipient reporter *Pseudomonas putida* F117 green. This frequently cited paper shows that cell–cell communication mostly occurs at distances of 5–10 µm on plant roots, while a maximal distance of 78 µm has also been measured.

Seeing organelles

In the past decade or so, researchers developed methods of cellular tomography to add the third dimension to electron microscopy. Several approaches may be taken. A tomogram of yeast cells (Fig. 6; Perktold *et al.*, 2007) was created by imaging serial sections of a chemically fixed cell. The sections were then interpreted manually or
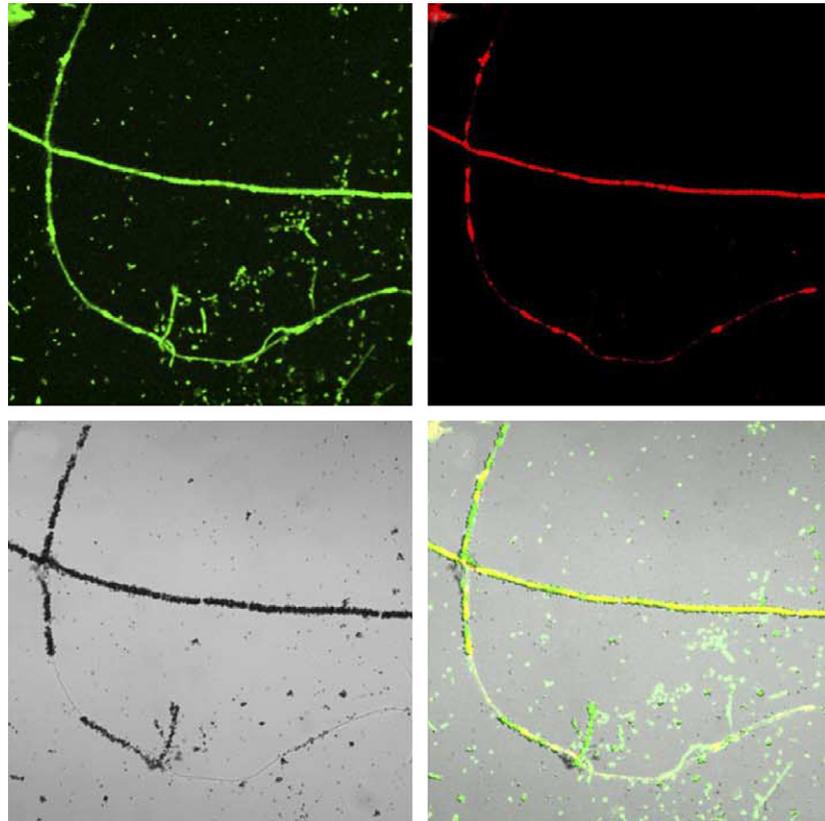
Fig. 4. Filaments of *Meganema perideroedes* reveal metabolic activity (from Kragelund *et al.*, 2005).
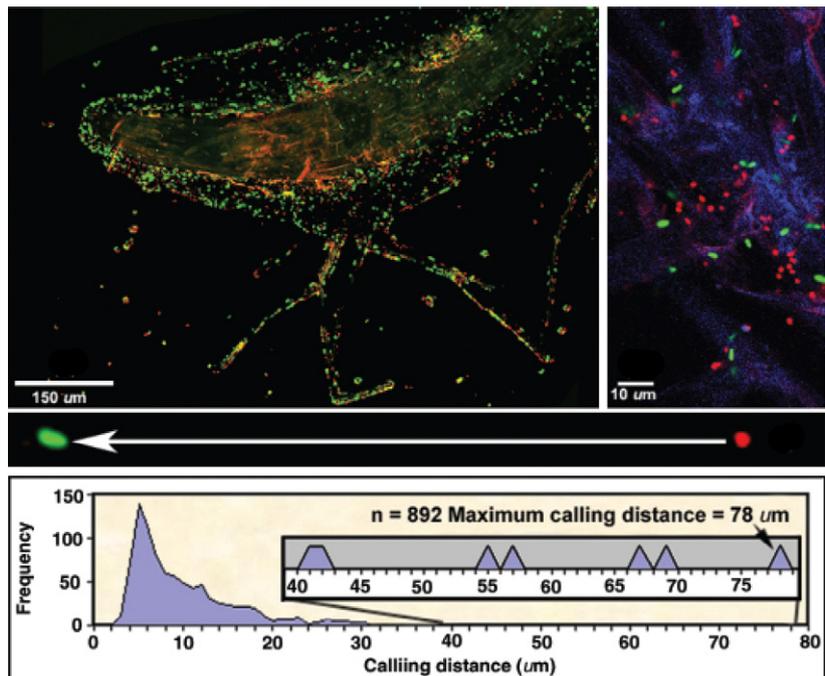

Fig. 5. *In situ* production of *Pseudomonas putida* signal molecules on tomato roots (from Gantner *et al.*, 2006).

semi-automatically and stacked up to create a three-dimensional image showing the cell wall (CW), the endoplasmic reticulum (ER), a lipid particle (LP), mitochondria (M), the nucleus (N) and a vacuole (V).

Alternatively, a tomograph of magnetotactic bacteria (*Magnetospirillum*) was obtained by cryoelectron microscopy (Fig. 7a) and by tilting the specimen and collecting images from many angles (Fig. 7b) (Schüler, 2008). These
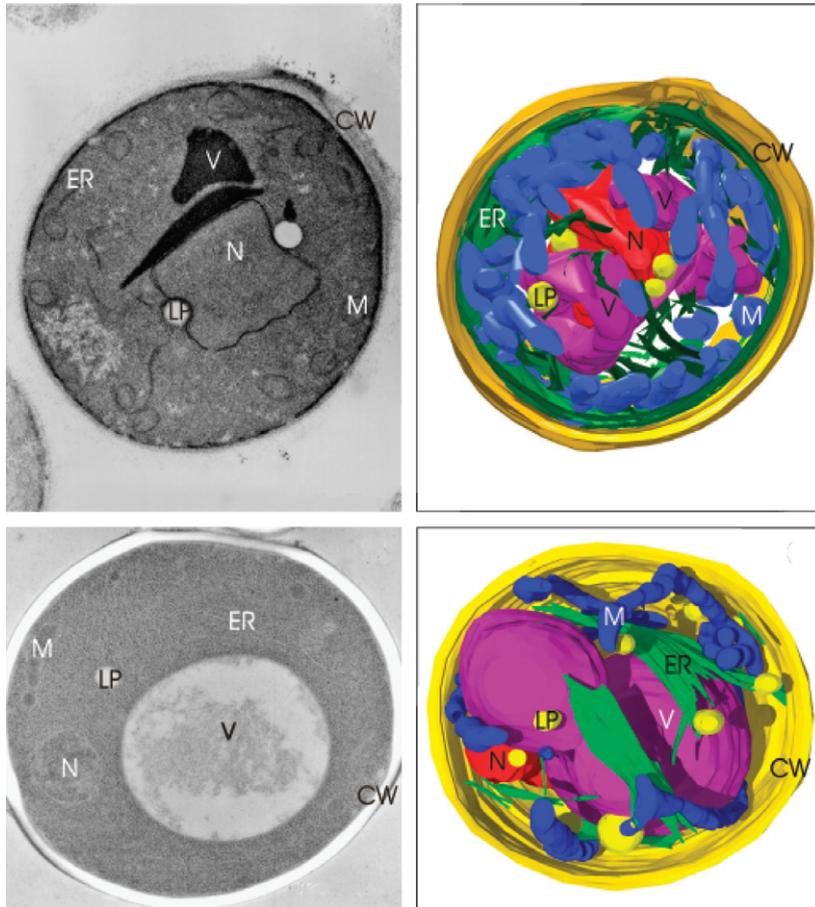
Fig. 6. Transmission electron micrographs and 3D reconstructions of yeast cells (from Perktold *et al.*, 2007).
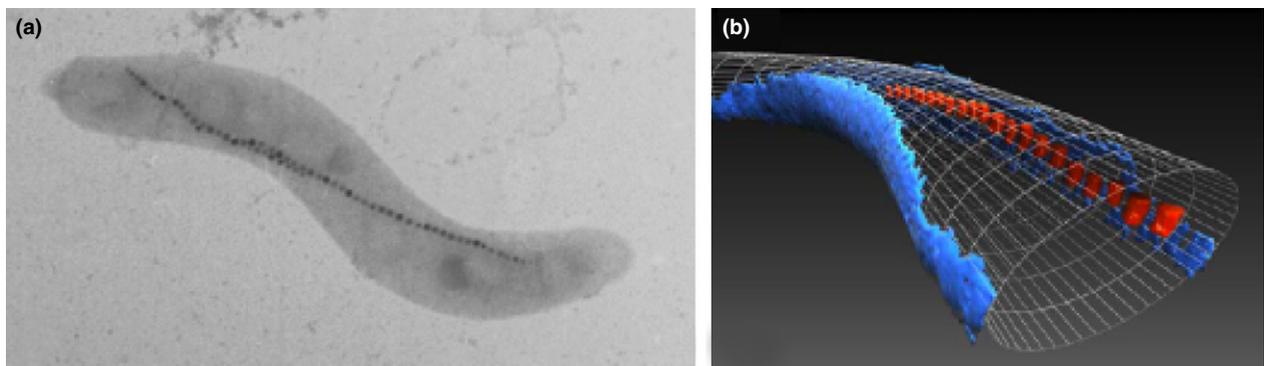
Fig. 7. The magnetosome chain in *Magnetospirillum gryphiswaldense* (from Schüler, 2008).

methods reveal the inner ultrastructure of cells, in particular the cytoskeletal magnetosome filament (Fig. 7a), but the resolution is currently limited, allowing only the largest molecular complexes, such as ribosomes and the magnetosome filaments, to be seen.

Visualising molecules

Cell biology is increasingly becoming a molecular science and microbiologists rely on a variety of visual tools to explore and present biomolecular structures. The image from Joly *et al.* (2010) is an example of the rich visual language for molecules (Fig. 8). It combines several representations to demonstrate different aspects of the molecular structure of the bacterial phage shock protein (Psp), including a cartoon ribbon to show the folding of the chain, a surface to show the overall shape and location of interaction sites, and a full atomic representation to show the binding of small molecules to the protein. In addition, a schematic diagram of the amino
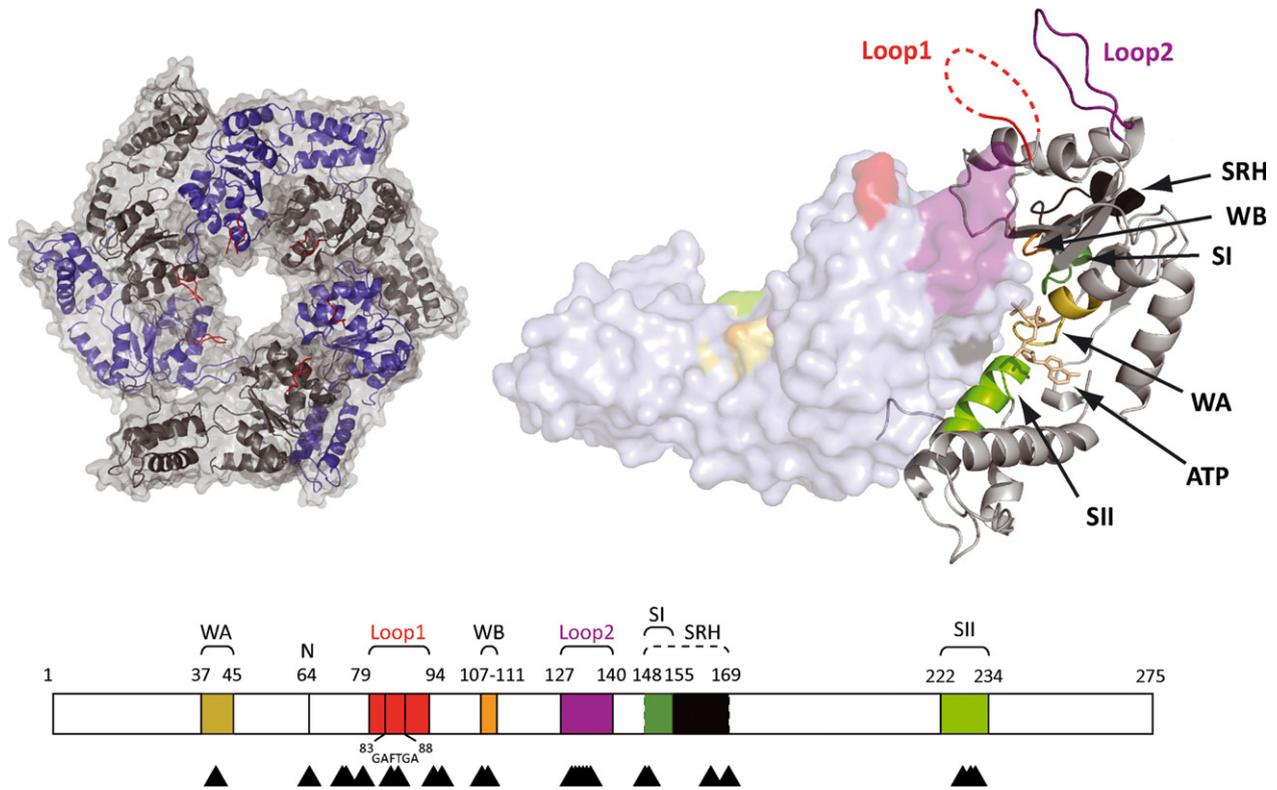
Fig. 8. Structure and motif organization of a phage shock protein (from Joly *et al.*, 2010).
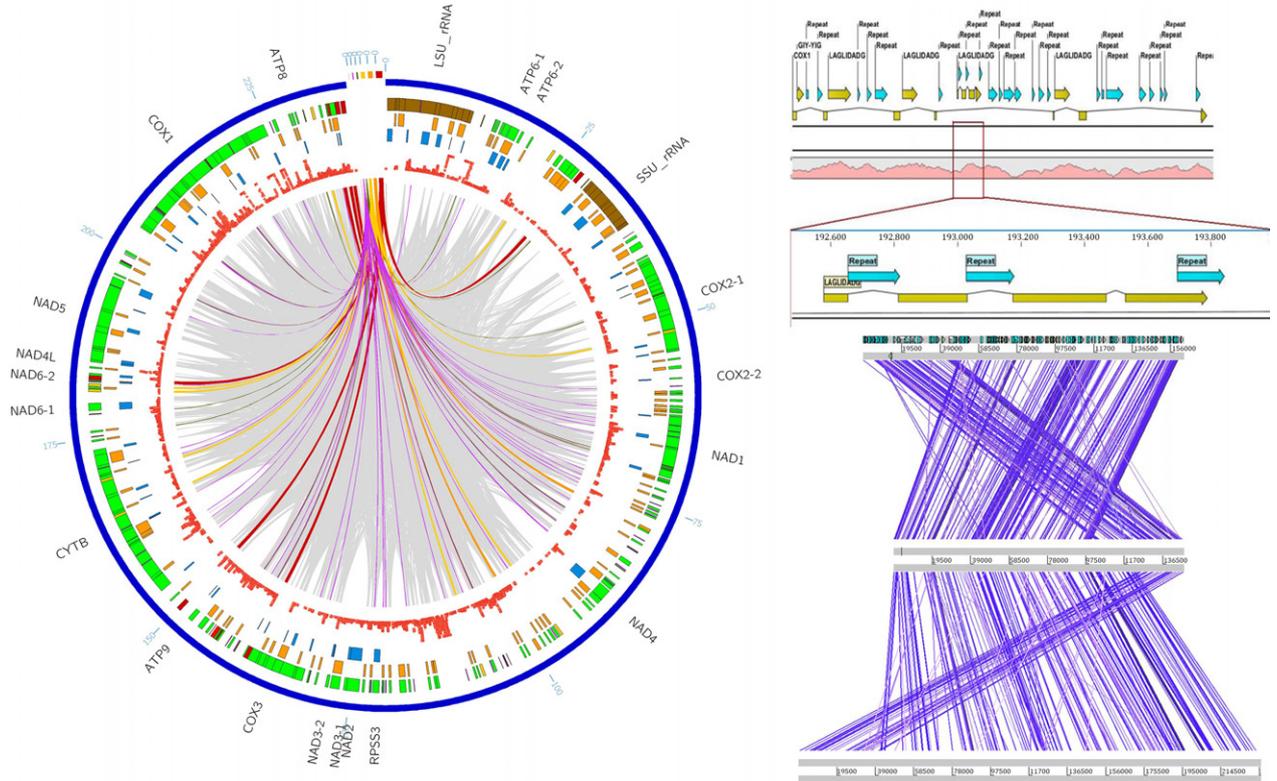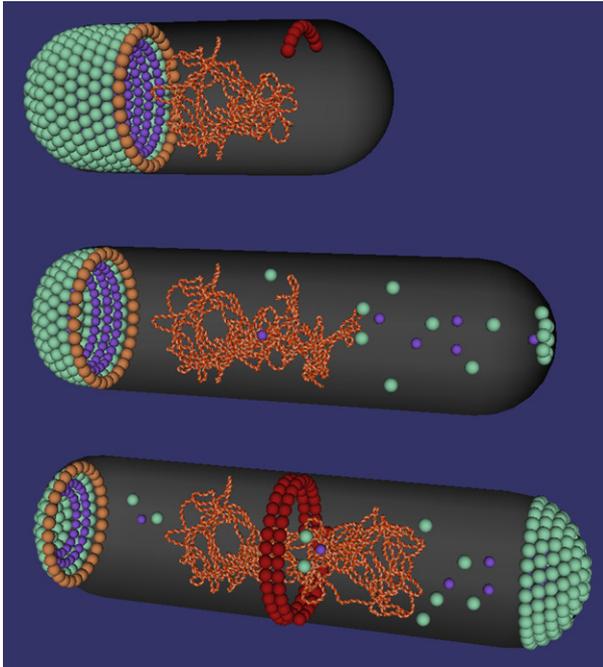

Fig. 9. Circular mitochondrial genome of *Rhizoctonia solani* (from Losada *et al.*, 2014).

Fig. 10. Model of the FtsZ ring by nucleoid occlusion and Min systems in *Escherichia coli* (from Vendeville *et al.*, 2011, reproduced with permission from Thanbichler, 2010).

acid sequence is included to present the domain structure of the protein.

Graphical harnessing of data

A vast body of genome, proteome and interactome data is currently available to researchers in microbiology, and methods for visualising and harnessing these data are an area of active research and development. Many of bacterial genomes have been sequenced, providing invaluable insights into the structure and regulation of the cells' genomic information. Most often, published reports include summary illustrations, such as the image of the circular mitochondrial genome from *Rhizoctonia solani* (Fig. 9, Losada *et al.*, 2014). These types of images are perfect for obtaining an overall view; digital tools may then be used to delve into the data in detail.

Modelling the mesoscale

Currently, there is a range of scale that is invisible to experimental methods, between the nanoscale of atomic structures and the microscale of microscopy. Computer simulation is being used to create images of this 'mesoscale', by integrating information from microscopy and molecular biology. This can be quite a treasure hunt to find the necessary information on molecular structure,

cellular ultrastructure, molecular concentrations and locations and, in the best cases, dynamics. The image from Vendeville *et al.* (2011) shows a model from the LifeExplorer visualisation program, which is integrating these types of information to create a model of bacterial cell division (Fig. 10). Quite amazingly, experimental methods are currently narrowing this invisible gap, with continual improvement of micrographic resolution and solution of larger and larger complexes by methods of integrative structural biology.

David S. Goodsell
Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA, USA
E-mail: goodsell@scripps.edu

Dieter Haas
Département de Microbiologie Fondamentale, Université de Lausanne, Lausanne, Switzerland

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