Bacterial respiration keeps amazing us in the 21st century

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The last two decades have seen some remarkable discoveries in bacterial bioenergetics. In this article, we highlight how important societal issues such as antimicrobial resistance and sustainable fuel production have led to a renewed focus on the bioenergetics of bacteria, which is only beginning to show us how much we have left to learn. Advances in technology, particularly through biophysics, have further contributed to a rapid progress in our understanding of the respiratory chain complexes in bacteria. Blue-sky research has seamlessly merged with solutions to societal problems and we stand on the precipice of a number of exciting discoveries in this field.

Using bacterial energetics to combat antimicrobial resistance

Since their introduction in the early 1940s, antibiotics have saved countless lives and cured even more illnesses. However, infections resistant to antibiotics have been affecting us ever since the first antibiotics were discovered, and this issue has come to the forefront in the 21st century when the World Health Organization (WHO) declared antimicrobial resistance (AMR) one of the top 10 biggest threats in the world. The O'Neill Review on Antimicrobial Resistance has projected that AMR, if left unchecked, will cause 10 million deaths per year by 2050, with a total worldwide GDP loss of $100.2tn . Tackling AMR requires action on many fronts and one of the rapidly advancing threads is the discovery of novel antimicrobials.

The majority of antimicrobials developed in the last century target only five major essential molecular processes in prokaryotes, despite the fact that there are approximately 200 conserved essential proteins in most bacterial species. These five molecular processes – cell-wall biogenesis, DNA replication, RNA and protein synthesis and folic acid metabolism – were historically thought to be the only routes for clinically successful compounds. An unprecedented change in this dogma was the development and FDA approval of the drug bedaquiline for the treatment of multidrug-resistant tuberculosis disease caused by *Mycobacterium tuberculosis*. Bedaquiline disrupts ATP synthesis by targeting the membrane enzyme, F_0F_1-ATP synthase (Figure 1), and it significantly shortens the treatment timeframe. Its approval has opened up bacterial bioenergetics, including the respiratory chain complexes, as a new and valid target space. Scientists worldwide are now attempting to develop novel antibiotics targeting various complexes in bacterial respiration (Figure 2) and are extending their search to other bacterial pathogens, such as methicillin-resistant *Staphylococcus aureus* and multidrug-resistant Gram-negative pathogens.

These lines of applied research have also brought scientists back to the fundamental understanding of antibiotics in general. Scientists have long sought, controversially, a universal model for how antibiotics lead to bacterial cell death. It is now beginning to be understood that antibiotics, regardless of their molecular targets, have overlapping effects on bacterial respiration – to the point where it is now understood that bactericidal (bacteria-killing) and bacteriostatic (growth-inhibiting) antibiotics have opposing and competing effects on bacterial respiration. In *Escherichia coli*, bactericidal antibiotics (like ampicillin) increase the rate of aerobic respiration, while bacteriostatic antibiotics (like erythromycin) inhibit the rate of aerobic respiration. Disrupting aerobic respiration, via gene deletions or co-treatment with bacteriostatic antibiotics, prevents the lethal action of normally bactericidal antibiotics. Although much work remains to establish a full causal pathway, it highlights that modulating the activity of the respiratory chain can be used to refine antibiotics and produce highly effective combination therapies.

In addition to true antibiotic resistance, these findings are revolutionizing our understanding of persistence: a non-heritable phenomenon where a subpopulation of bacteria slow or halt growth to resist various environmental stresses, including antibiotic challenge. Such bacteria are incredibly hardy towards antibiotics and require much longer treatment timeframes which can lead to issues with patient compliance. Both these factors promote the acquisition of true AMR in a vicious cycle. The aforementioned *M. tuberculosis* bacterium is one of the most well-studied persisters and it is now understood that the electrochemical gradient (i.e., the very proton-motive force that is central to chemiosmosis) is essential for the survival of persisters. This shows that

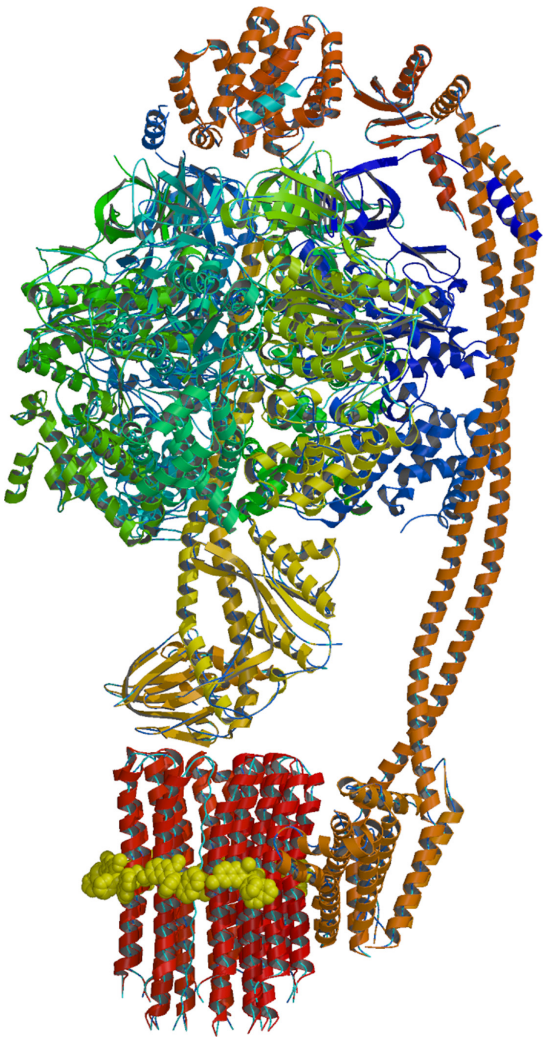

Figure 1. F_oF_1-ATP synthase inhibited by bedaquiline. The structure of the F_oF_1-ATP synthase from *M. tuberculosis* and the FDA-approved drug bedaquiline (in yellow spheres), bound to the c-ring where it disrupts rotation and ATP synthesis. (PDB: 7jg8)

non-growing and slow-growing persistent bacteria still dedicate a large amount of resources to their respiratory chain, opening up new questions and opportunities. For example, can inhibitors of bacterial bioenergetics be used to kill non-multiplying latent bacteria and thus persistent infections? Small-molecule drugs that perturb the lipid membrane have already been proposed to target persistent infections. Together this suggests that inhibitors of respiration are 'two for the price of one', combating both resistance and persistence.

Much of the work on bacterial bioenergetic inhibitors has focused on aerobic bacterial pathogens, but there are a number of important bacterial pathogens that generate energy by anaerobic respiration or fermentation, such as group A streptococci. These bacteria represent an important challenge for scientists as the field moves forward, but also bring new opportunity with it. For example, many facultative anaerobes (bacteria that can adjust their metabolism between aerobic and anaerobic modes) can adapt their metabolism to survive the rapidly changing, and often hostile, environment encountered in the host. These hostile conditions can frequently involve reactive oxygen species (ROS), like superoxide, while simultaneously limiting transition metals, which prevent the synthesis of enzymes that defend against ROS (e.g., superoxide dismutase). Under these conditions, bacteria can deplete the local concentration of oxygen and so prevent the production of ROS by limiting the very oxygen itself. After oxygen is depleted, the facultative anaerobe effortlessly switches to anaerobic respiration with only a minor defect in the pathogen's ability to synthesize ATP. Targeting anaerobic respiration with novel antibiotics could therefore be a strategy to selectively treat infections, instead of indiscriminately killing all bacteria like most broad-spectrum antibiotics.

These exciting new developments in antimicrobial discovery have come at the same time as the aptly named 'resolution revolution' in single-particle cryo electron

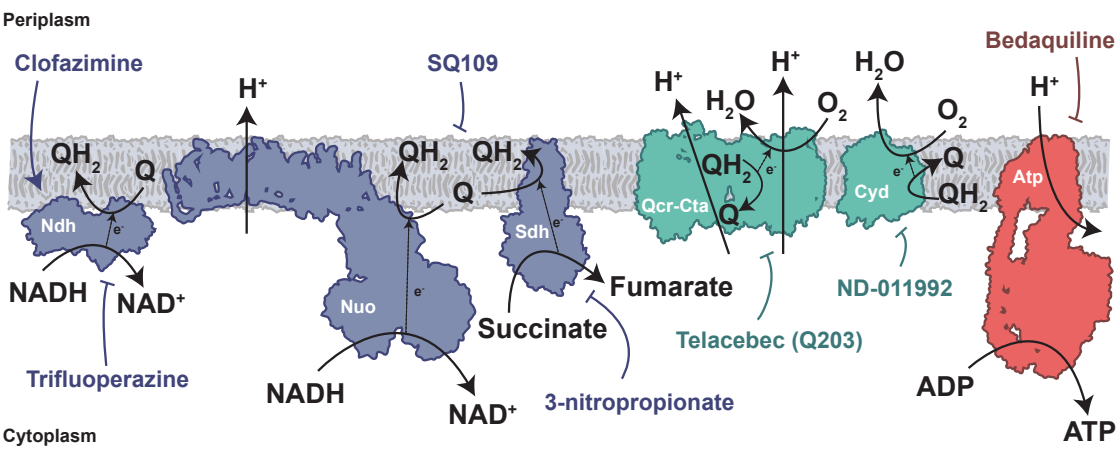

Figure 2. The electron transport chain of *M. tuberculosis* with several novel inhibitors indicated

microscopy (cryo-EM). Recent progress in cryo-EM, as well as advancements in crystallography of membrane proteins, has generated a record number of structures of membrane proteins at a resolution below 3.5 Å. Structures of bacterial respiratory complexes will benefit the development of compounds inhibiting bacterial respiration. Besides detailed structural data for drug development, cryo-EM has provided us exciting insights into respiratory *super*complexes. Supercomplexes are protein collectives where multiple electron-chain complexes come together to form large respiratory machineries. They are thought to optimize electron chains pathways and some structures of supercomplexes have hinted at mechanisms by which respiration is coupled to the bacteria's defence against oxidative stress. Progress is also made in solving cryo-EM structures of membrane proteins in the lipid membrane rather than in detergent, opening up the possibility to study respiratory chain complexes in the presence of electrochemical gradients. Structural biology of bacterial respiration is entering an exciting era and we eagerly await what comes next.

Bioelectricity and biotechnology

While the development of antibiotics was aimed at inhibiting respiratory chain complexes, other scientists aimed to boost respiration in a class of bacteria known as exoelectrogens. Exoelectrogens are microbes with the remarkable ability to respire on *extra*cellular minerals. For this, electrons need to be transported out of the cell, crossing the cell envelope. Early in the 21st century, research into exoelectrogens received a tremendous push when it was shown that electricity could be harvested from organic matter. This is possible because not only can exoelectrogens respire on minerals outside the cell, but they are equally happy to respire on macroscopic, conducting materials. It was observed that under anaerobic conditions, exoelectrogens like those from the *Geobacter* species can form thick biofilms on electrodes and somehow transfer respiratory electrons through the film and into the electrode. In a microbial fuel cell, the electrode with the biofilm (the anode) is simply connected to another compartment where oxygen is reduced (the cathode) and, hey presto, bioelectricity is generated. Although electrical currents are typically low, microbial fuel cells are easy to make and work is on-going to enhance bioelectricity production. Microbial fuel cells also make for interesting projects. In 2020, we teamed up with an artist to create a light sculpture powered by microbial fuel cells (Figure 3).

Biological tissues, including biofilms, have long been considered electrically isolating materials. Electron transfer through micron thick biofilms was thus very unexpected. Upon further study, proteins and filaments were discovered that conduct electrons on the nano- to micron-scale. Membrane proteins were found in *Shewanella oneidensis* with 20 heme cofactors that

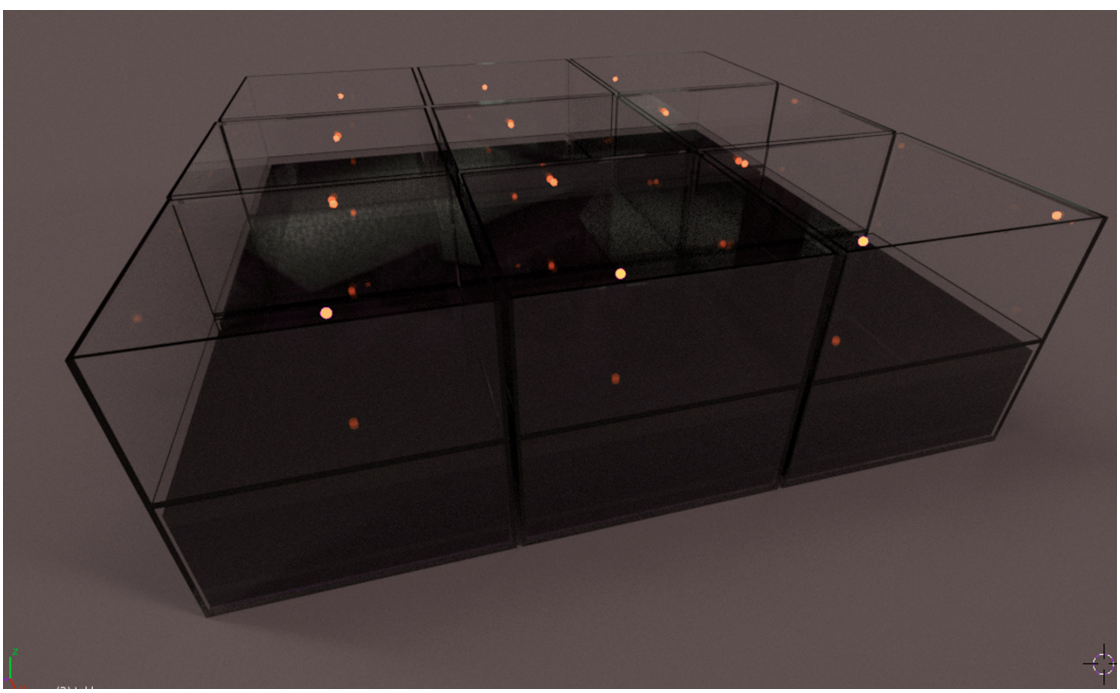

Figure 3. A computer-generated model of a light sculpture, powered by microbial fuel cells. From the artist Jessica Lyodd-Jones (www.jessicalloydjones.com).

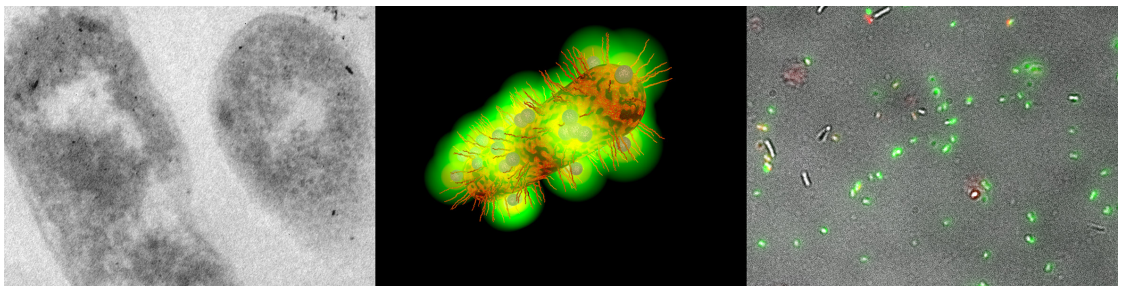

Figure 4. Images of quantum dots bound to bacteria. (Left) A transmission electron micrograph of *S. oneindensis* MR-1 with CdTe quantum dots (small black dots). (Middle) Artistic impression of a bacterium with quantum dots. (Right) An overlay of a bright-field microscope image and a fluorescent microscope image of CdTe quantum dots bound to *S. oneindensis* MR-1.

efficiently transport electrons across the lipid membrane and, together with other heme proteins, possibly in parallel with the membrane. Filaments of *Geobacter sulfurreducens* were shown to be repeated units of heme proteins through which electrons are transferred at high speeds outside the cell. Controversy still exists on which filaments, nanowires or pili can conduct electricity, and how. But the shear fact that bacteria and possible other microbes have evolved proteins and organelles to conduct electrons across microns outside the cell would have been unimaginable only 20 years ago.

Possibly even more unexpected is that scientists have been able to reverse the electron flow. Instead of harvesting respiratory electrons for bioelectricity, electrons can be pumped back into exoelectrogens for electrosynthesis. In this process, bacteria synthesize organics rather than consume them. When Galvani explored bioelectricity in the 18th century, he showed that muscles would contract upon application of electric pulses. This famously gave inspiration for the story of Frankenstein and the idea that electricity could start life. Now, in the 21st century, it has been shown that, although electricity cannot start life, it might be able to 'fuel' it, at least for bacteria.

Taking this one step further, bacteria have been connected to semi-conducting nanoparticles (Figure 4). Semi-conducing materials generate electricity in photovoltaic devices like solar panels. By connecting semi-conducting nanoparticles directly to bacteria, scientists created systems they called 'biohybrids', which one day could synthesize organics, including (bio)fuels, from sunlight. Part inorganic material, part bacteria, these systems are surely inspiring.

Even though we are still early in the 21st century, we have seen a number of fascinating advancements in bacterial bioenergetics that promise to rapidly transform our understanding of this wide-reaching field. Hopefully in this article, it is apparent that these advances range broadly across ecology, medicine and industry, as well as physiology, biochemistry and biophysics. Within this, a number of novel biochemical processes have been resolved, with many new opportunities and challenges for scientists moving forward. We encourage the reader to follow the field with us, as we expect that we are only at the tipping point of what this century has to offer. ■

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Gregory Cook is professor at the Microbiology and Immunology Department of the University of Otago, New Zealand. He did his DPhil at the University of Waikato (1992) and has been a researcher at Cornell University, Kings College London and University of Sheffield before moving back to New Zealand. The Cook laboratory is interested in the metabolism and energetics of bacterial pathogens like Mycobacterium tuberculosis *and multidrug-resistant Gram-negative pathogens.*

Lars J.C. Jeuken is a professor at the Leiden Institute of Chemistry, the Netherlands, where he also finished his PhD in 2001. He has worked at the Universities of Oxford and Leeds before moving back to Leiden University in 2021. The Jeuken group is interested in biological electron transfer and bacterial respiration and has recently started developing novel biotechnological applications of exoelectrogens. Email: L.J.C.Jeuken@lic.leidenuniv.nl