
Publishable JRP Summary for HLT10 BiOrigin Metrology for biomolecular origin of disease

Overview

Diseases originate from molecular changes, as do their cures. Many disorders such as cystic fibrosis or haemophilia can be treated by editing single genes, while antimicrobials able to kill pathogenic bacteria and viruses can eradicate AIDS and tuberculosis. Cures are drugs and drugs are specialist molecules that correct or destroy disease targets. However, our incomplete understanding of what makes effective drugs hampers further progress in drug design and therapy. The BiOrigin project tackled the root of the problem and established critical design principles that can link a molecular structure to the desired therapeutic effect. By way of example, the project has demonstrated the use of these principles for creating next-generation antibiotics, against which bacterial resistance is far less likely. The results of this research have enabled early impacts on commerce and healthcare, while improving precision and specificity with which therapeutically relevant biological functions can be measured and exploited.

Need for the project

Relationships between the molecular origin of disease and therapeutic responses are sparse and fail to keep pace with the attempts to provide solutions to global health issues including antimicrobial resistance. Indeed, over the last 75 years we have relied heavily on antibiotics to cure everything from acne to pneumonia. These pillars of medical treatment are cheap and readily available, and have almost ruled out deaths from minor infections. However, over the last decade antibiotics have lost their effectiveness, in some cases leaving even commonplace infections untreatable.

Research efforts in industry are decreasing too. In the early 90's there were almost 20 active antibiotic R&D programmes, now there are only two by GlaxoSmithKline and AstraZeneca. The tendency is set to continue for several reasons. Firstly, the everyday natural sources of antibiotic discovery, e.g. penicillin mould, have been exhausted. Secondly, many companies are seeing their licenses for antibiotics expire, meaning that the manufacture of these drugs, many of which are no longer effective, ceases to be profitable. Thirdly, there is a lack of commercial incentive for the development of antibiotics compared to other drugs. Antibiotics are not particularly profitable as they are only ever required short term in contrast to long-term courses of diabetes or heart failure medicine. Despite their significant importance for public health, antibiotics are not seen as a lucrative business. This situation demands fundamentally new solutions and such solutions have to be validated by robust and reproducible measurements.

Traditional antibiotics interfere with the construction of bacterial cell walls or inhibit protein biosynthesis. To develop resistance to such treatments requires minor genetic mutations which bacteria can afford and readily develop. In contrast, making resistant cell membranes is a formidable task for bacteria as it requires the rebuilding of their entire genetic apparatus – an extremely high price to pay. Therefore, it is the membrane of a bacterial cell which remains its Achilles' heel. For this reason, this project has focused on membrane-active antibiotics that principally target and destroy bacterial membranes thus making bacteria less likely to become resistant. However, exact mechanisms and rules of engagement with bacterial cells are still full of uncertainties.

Traditional measurement concepts and platforms fall short of providing answers, and so new and innovative principles are required for the development of more effective methods and materials. These are necessary for enabling any sustainable progress in drug design and development, and in particular in the area of antimicrobial resistance given the global impact and urgency of the issue. With this in mind, the project dealt with the pre-validation of predictable and exploitable links between the structure and activity of antimicrobial agents. Consultations with stakeholders from industry, clinic and health research organisations identified two most pressing measurement challenges that this project addressed:

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- Irreproducibility and inconsistency in the detail of what makes a better antibiotic. This was needed in order to provide reliable and predictable links between the structure and activity of last-resort membrane-active antibiotics,
- A lack of associated measurement tools and materials, particularly those that can allow for the monitoring, evaluation and screening of potential antibiotics in their natural cellular environments. This is needed to provide a validated rationale for drug design.

Scientific and technical objectives

The scientific and technical objectives for the BiOrigin project were to:

- *provide a set of validated physical characteristics* that guarantee the desired biological effects thereby enabling purely artificial designs and re-designs of novel and more efficient antimicrobials,
- *establish underpinning methods and materials* for the evaluation and screening of antimicrobial properties of biomolecules (small proteins) in natural and cellular environments (bacterial and human cells) at time and length scales of their therapeutic action,
- *develop experimental and computational methods* to quantitatively determine the mechanistic performance and extent to which antimicrobials engage with their targets with the highest precision and accuracy (molecular to atomistic),
- *innovate imaging methods* for the visual monitoring and imaging of antimicrobial action in real time thus allowing for the mechanistic elucidation of the antimicrobial action,
- *provide a molecular rationale for the prediction of biological activity* by refining and optimising the experimental data with computational analyses.

Results

Provide a set of validated physical characteristics

The project applied systematic investigations of antimicrobial activity at the molecular and cellular levels. Model molecules were designed to experimentally obtain and validate physical characteristics that are responsible for antimicrobial activity. Such characteristics relate to the strength and speed of antimicrobial binding to bacteria, the selectivity of binding to bacterial cells as opposed to human cells, minimum concentrations necessary to kill bacteria and the like. The characteristics were then refined computationally and were subsequently used to build generic molecular templates (peptides) comprising different blocks of molecules (amino acids) that can be replaced with other blocks or different combinations of the same blocks to favour a particular biological function. These templates are now used to benchmark the activity of existing and emerging antibiotics and related antimicrobial agents.

Establish underpinning methods and materials

The hallmark of the templates is that they are generic enough to apply to other applications including gene delivery, anticancer treatments and biosensors. The templates are structurally amenable and can accommodate other desired properties in order to support a new biological activity. For example, the antimicrobial activity of the template can be converted to a predominantly cell-penetrating activity that is necessary to deliver drugs into live cells. Because this capability allows to convert one activity into another activity, be it similar or different, core methods that can validate the new acquired properties by comparing them with the original (benchmark) properties are necessary. To realise this, the project integrated physical, biological and imaging methods into one continuous methodology that can trace the desired biological activity down to the elementary structure of the template while providing step-by-step correlations between the mode of action, bacteria killing kinetics and molecular recognition.

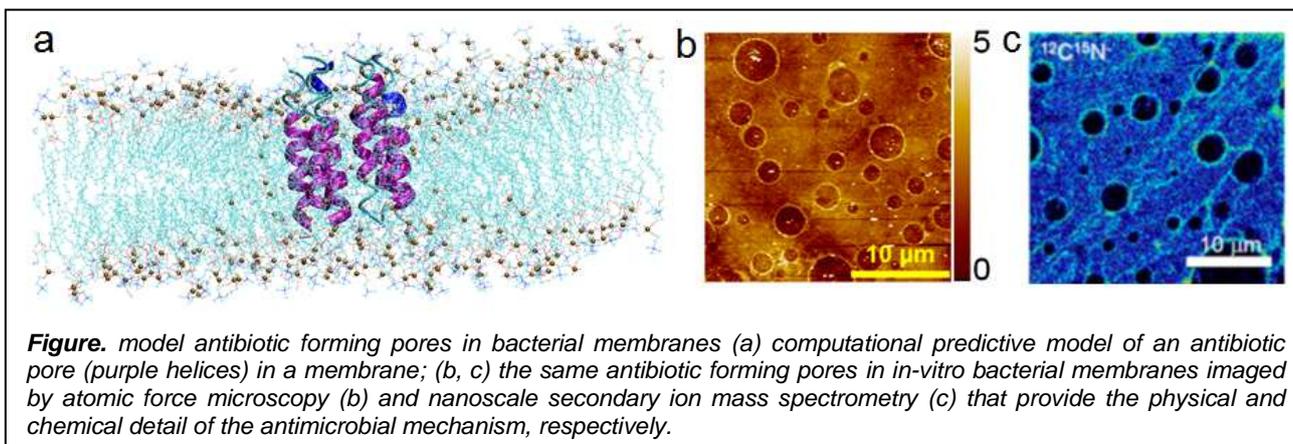
Develop experimental and computational methods

Such correlations were then validated with nearly atomistic detail using high resolution methods including synchrotron radiation and magnetic resonance imaging. With this level of detail, the molecular behaviour of an antibiotic or another drug can be quantified and is the most relevant to drug design as it describes precise

drug-target interactions. Each measurement methodology, which uses a unique physical principle, contributed a critical piece of information that was adapted to develop computational algorithms to predict and model therapeutic mechanisms with atomistic accuracy. This strategy provided the first metrological validation of antibiotic action and underpins a reference methodology for the screening and validation of antibiotics.

Innovate imaging methods

BiOrigin performed measurements in live bacteria and their membranes as well as on live human cells and human cell membranes to demonstrate selectivity to antibiotics. For the first time, it was shown and validated that it is the position and structure of antibiotics in microbial membranes that are responsible for antimicrobial activity, which can thereby be made more selective and stronger. In accordance with our predictions, model antibiotics elicited faster and broad-spectrum antimicrobial responses when compared to commercial antibiotics daptomycin, vancomycin or polymyxins, which kill only particular types of bacteria. Yet, the visual evidence of how antimicrobials interact with bacteria has been elusive before this project, whereas seeing antimicrobial effects in real time has far-reaching benefits for antimicrobial therapy and diagnostics. Therefore, BiOrigin pushed the boundaries of existing capabilities by developing innovative imaging methods to give the first visual evidence of antibiotic action. The imaging capabilities included high-speed atomic force microscopy, magnetic resonance molecular imaging and mass-spectrometry chemical imaging, which provided important insights into antimicrobial mechanisms and the distribution of antibiotics in bacteria (Figure). These findings are already proving crucial for the development of effective antimicrobials and equally for the diagnosis of infectious diseases. The capabilities are beyond the state of the art and hold a considerable promise for use in hospitals while their use for commercial development is being probed in collaboration with industry.



Provide a molecular rationale for the prediction of biological activity

As per the project concept, all the data generated was rationalised into one generic rationale to predict antimicrobial action and resistance. The rationale was demonstrated in the design of experimental antibiotics, antimicrobial materials and was also probed to reveal unknown antimicrobial resistance mechanisms. Model antimicrobials derived from this rationale performed as predicted. Examples include antibiotics predicted to form pores in bacteria (Figure) and antimicrobial materials designed to resist biofilm formation while supporting the growth of human cells. Matched by experimental validation, computational predictions provide an ever-evolving basis for designing next-generation antimicrobial agents. Thus, the rationale enables limitless prediction-validation cycles leading to constant improvements in drug design. After consultations with key industry stakeholders (IBM, GSK, Malvern Cosmeceutics, Novabiotics, CEM Corp and others) the rationale was tested to design bespoke antibiotics with broad spectrum antimicrobial activities while having no effects on human cells, and was also used for the formulation of bacterial membrane materials that are now developed as high-throughput platforms for screening antibiotics.

Actual and potential impact

The project is a scene setter for the direct and real-time measurements of biomolecular processes of therapeutic relevance. BiOrigin is indeed an exemplary success in delivering high value outcomes within this short period of time. The strong group of industrial stakeholders provided the project with a necessary focus from the start, which proved to be essential for the successful trial of real-world applications.

Dissemination activities

The established capabilities enable the prediction and monitoring of biological processes at the molecular and cellular level that are applicable for the development of novel diagnostics, antibiotics and biofilm-resistant materials. Research was published in co-authorship with industry and clinicians, which demonstrates an early, pre-impact interest from stakeholders. The project has had an exemplary number and quality of outputs including papers in high-impact scientific journals such as *PNAS*, *JACS* and *JBC*, while being featured in industry-oriented and public publications including *Microscopy & Analysis*, *Drug Discovery World* and *The Times*.

Overall and long-term impact

The project has generated a number of know-hows, which have started being taken up by industry (SMEs, corporations) as entry capabilities to new markets and are being developed as screening platforms for antibiotics in collaboration with EU clinicians. The research has created a strong momentum for metrology to respond to the spread of antimicrobial resistance and is progressing towards a global impact in healthcare with a follow-up research program focusing on establishing high-throughput approaches to accelerate antimicrobial discovery and manufacture. The program has attracted important partnerships with academia (Cambridge, Edinburgh, Oxford, UCL), industry (IBM, GSK, CEM, Ingenza), NMIs (NIST, PTB, LNE, LGC, NIBSC) and world-leading research organisations (Turing and Crick Institutes, NIH, Antibiotic Action, EC JRC, EMA).

Immediate impact

Some examples of short-term impacts include the design and production of novel molecular probes for magnetic resonance imaging which are being proposed as highly efficient biosensors. A European company was involved in these activities over the whole project and was thus enabled to generate such biosensors. This new capability is anticipated to increase the company's market share and is already providing it with a competitive edge with know-hows generated by the project.

Another example is a company who collaborated at later stages of the project and who are active in the R&D and sales of membrane-based cosmetics with products sold in Europe, the US and Japan. The project results allowed the company to partially re-direct their R&D programmes to enable entry into other markets thus expanding their product portfolio. Predictive drug delivery and anti-infective technologies are of particular interest to the company.

In addition, a European leader in industrial biotechnology with clients in pharmaceutical and chemical industry is collaborating in the commercialisation of high-throughput methods developed in the project. They place a particular emphasis on *in-situ* metrology of molecular processes inside live cells for the development of high-throughput drug discovery technologies.

These and other examples at different stages of development, from consultations to implementation, manifest the impact the project has had through the introduction of a predictive rationale for drug design and development. The project has initiated a new paradigm in antimicrobial discovery, which is undergoing a next R&D stage of applying the developed approaches to other antimicrobial classes to create a new pipeline of antimicrobial agents to be taken up by early adopters.

List of publications

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