MIBTP2 PhD Project Proposal

The Midlands Integrative Biosciences Training Partnership 2 (MIBTP) is a BBSRC-funded doctoral training partnership between the University of Warwick, the University of Birmingham and the University of Leicester recruiting students for four-year studentships starting in Oct 2018. These students would do a year of training and start their PhD research in Oct 2019.

MIBTP2 PhD projects should fit within the Strategic Plan of the BBSRC (http://www.bbsrc.ac.uk/publications/planning/strategy/strategy-overview.aspx). In MIBTP2 we are supporting research in the following areas 1) Food security, 2) Bioenergy and industrial biotechnology and 3) world-class bioscience. Exploiting New Ways of Working is a cross-cutting theme throughout MIBTP2; in the taught component, mini-projects and the main research component of the programme. MIBTP2 PhD projects should have two academic supervisors, usually in different disciplines, or at least who approach the biological topic from different directions.

It is expected that labs hosting MIBTP students will be well resourced in terms of current personnel, equipment and consumables and there is a section of the proposal form for supervisors to describe the situation.

If you would like to offer a PhD project, please complete this form and submit it with the file name format "Smith_John_MIBTP_PhD_Proposal.doc" to h.etchell by September 21st.

Please note that while each student is allocated a project budget (max. £4500 pa for consumables, facility use etc.) it is expected that Supervisors will also contribute to the funding of the project if the annual costs are higher than this. Supervisors are also expected to contribute to training of the PhD students by offering master classes and mini-projects.

If you have any questions regarding this form please contact one of the MIBTP directors: Brian Thomas (Brian.thomas@warwick.ac.uk), Chris Thomas (C.M.Thomas@bham.ac.uk), or Jonathan McDearmid (jrm33@leicester.ac.uk).

Principal Supervisor: Jan Kreft, Biosciences

Co-supervisor: Daniele Vigolo, Chemical Engineering

PhD project title: Microfluidics and modelling to map antibiotic resistance of individual cells and populations

University of Registration: Birmingham

Project outline

1. Project outline describing the scientific rationale of the project (max 4,000 characters incl. spaces and returns). There is no need to be too detailed about individual projects – general background, objectives and methods should suffice. One or two key references for potential applicants would be useful.

The rise of antimicrobial and antibiotic resistance threatens our ability to cure infections. If we do not tackle this crisis, we may return to the pre-antibiotic era. Many studies have looked at the evolution of resistance and the effect of inhibitory and sub-inhibitory concentrations of antibiotics. Most of these studies have used populations of many cells assuming that the variation between individuals is not important enough to warrant investigation. Some have studied the response of individual cells to antibiotics, e.g. to see how the response is affected by the age of the cell, but not over a range of different concentrations.

Our central hypothesis is that antibiotic susceptibility and fitness costs of resistance mutations or plasmids are affected by the growth rate and physiology of individual cells. As a consequence, individuals in a population will not be all the same, but differ in important ways. We will construct and use microfluidic devices consisting of two components, a gradient mixer and a microchemostat, to create concentration gradients of growth substrates and/or antibiotics to study the effect of growth rate and antibiotic concentration on individual cells growing under constant and defined conditions in cell-sized channels. We can observe and track many cells with a microscope and then use image analysis to measure e.g. growth rate or time to killing. Using these measurements of many individual cells, we can then use individual-based mathematical models to predict the behaviour of populations and how population responses are affected by the differences between individuals.

For example, generating a gradient of substrate concentrations that will lead to a gradient of growth rates, we can measure how growth rate affects antibiotic susceptibility. As we can have thousands of channels in a microfluidic device, we can record this for hundreds of cells and learn how much the responses differ between cells. Feeding this information into an individual-based model, we can then predict how the antibiotic affects the population. These predictions can be tested in larger culture vessels such as flasks. The model can also make predictions on the effect of antibiotics on biofilms as the growth of a biofilm generates a substrate concentration gradient, and from recording growth and antibiotic inhibition over a range of concentrations, we can predict the growth and inhibition of each cell in the biofilm depending on where in the gradient it is located.

Another example of the use of the microfluidic device and mathematical modelling is to study the cell-to-cell variation of fitness costs of resistance genes or plasmids. Fitness costs are typically measured for populations growing under optimal conditions, that is, at high growth rates. We do not know whether fitness costs will be different when cells grow more slowly but this could have huge consequences. What we do know is that bacteria grow much more slowly in their natural environment than under optimal conditions: *E. coli* has a doubling time of about 24 hours in the gut. It may also be that individual cells with higher fitness costs (more retarded growth) have lower antibiotic sensitivity. There is a lot we do not know and the methodology in this project will be able to address many fundamentally important and clinically relevant questions. There is a host of opportunities to go beyond the state of the art. We have developed some prototype microfluidic devices with the help of two MSc project students and optimized some procedures, but it will need some more testing and optimization before we can use them routinely in high throughput mode. We have explained how the integration of single cell measurements and individual-based modelling will advance microbial sciences in the opinion article below.

References

Hellweger FL, Clegg RJ, Clark JR, Plugge CM, Kreft JU (2016). Advancing microbial sciences by individual-based modelling. *Nature Reviews Microbiology* **14**: 461–471.

Hol FJH, Dekker C (2014). Zooming in to see the bigger picture: Microfluidic and nanofabrication tools to study bacteria. *Science* **346**: 1251821.

Relevance to BBSRC and Approvals

1. How does this project fit within the remit of the BBSRC? (3-4 lines)

The project contributes to the following strategic priorities of BBSRC: combating antimicrobial resistance; food, nutrition and health; systems approaches to the biosciences.

2. Select the relevant BBSRC Strategic Research Priority: Food Security/Industrial Biotechnology and Bioenergy/World Class Bioscience

Food Security (including Crop science and Farm animal health) is addressed as the project aims to understand the conditions that lead to antibiotic resistance or its loss in animal agriculture.

3. How does the project comply with BBSRC's requirement for multidisciplinarity and new ways of working? (5 lines)

The project is truly multidisciplinary combining engineering of microfluidic devices with mathematical modelling, image analysis, microbiology and molecular biology.

New ways of working that underpin the project are quantitative biology, systems biology as well as microfluidics and advanced optical microscopy detection techniques.

4. Please list the techniques that will be undertaken during the project.

Design, manufacture (i.e., soft-lithography) and operation of microfluidic devices.

Automated microscopic imaging (including fluorescence, phase contrast and DIC) and image analysis.

All optical velocimetry technique to evaluate the flow field within the microfluidic device (Ghost Particle Velocimetry).

General laboratory and microbiological methods.

Molecular microbiology and strain construction.

Individual-based modelling.

Statistics.

5. Please describe how your lab can properly support a MIBTP student if appointed (personnel, resources, grants from BBSRC and elsewhere)

The Kreft lab will support the modelling and microbiology and the Vigolo lab the microfluidics and microscopy. Both supervisors have experience in image analysis.

The Vigolo lab is well equipped for the manufacture of PDMS microfluidic devices, including the fabrication of silicon moulds having access to a clean room facility equipped with a mask aligner for soft-lithography. The Vigolo lab is fitted with syringe pumps to precisely control the flow within the microfluidic devices, a spin coater for the realisation of thin layers on glass slides and silicon wafers, and a motorised optical microscope for fluorescence, phase contrast and DIC microscopy. Finally, the Vigolo lab is capable of the quantitative analysis of the flow field within microchannels by all optical techniques, such as Ghost Particle Velocimetry, exploiting the available fast cameras (capable of more than 50,000 fps).

There will be 2 other PhD students in the Kreft lab carrying out combined computational and wet lab projects and 1 research fellow using mathematical modelling and Bayesian inference.

Currently there are 5 PhD students in the Vigolo lab working on microfluidic-based projects (development of biocompatible materials, study of deep veins thrombosis, encapsulation of bacteria in droplets, study of foams and surfactant behaviour during single droplet formation), and a PDRA will be soon appointed on a project based on the controlled generation of biocompatible materials with local gradient of mechanical properties ,studying the behaviour of cells (i.e., fibroblasts, cancer cells, etc.) grown on such a substrate.

Jan Kreft currently has one grant from NERC (Co-I) and one from MRC (PI of Birmingham partner) and is Birmingham coordinator as part of the BBSRC and Innovate UK funded Biofilms Innovation Knowledge Centre (IKC) that just been awarded (confidential before official launch in Nov).

Daniele Vigolo currently has an EPSRC First Grant (PI) on the generation of biocompatible material with intrinsic concentration gradient by thermophoresis and has submitted a number of other grant applications as PI or Co-I (Royal Society, EPSRC).