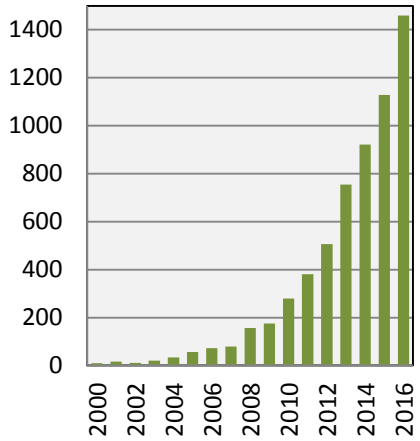
Developing and validating a computational model of the gut microbiota–mucosa interactions to replace and reduce animal experiments

Mini-summary for busy panel members: An explosive growth of research on the gut microbiota, often using rodent models, has amply demonstrated the huge importance of the previously neglected microorganisms for our health. However, the complexity of the system poses a formidable challenge. Our central hypothesis is that mathematical modelling is required to understand and predict such a complex system. Our long-term goal is to replace & reduce animal experiments and improve our understanding by developing and utilizing a general mathematical modelling platform. The specific aim of the studentship is to develop a model of the gut mucosa as part of our eGUT software and to validate the model with an *in vitro* and an insect model as well as training the student in a range of alternatives to rodent models. This is timely as research in this area using animal experiments is growing exponentially. By improving the science, we can best realize the potential of eGUT to ultimately replace & reduce ~75,000 animals per year globally.



Timeliness of the proposal

Research on the gut microbiota using rodent models has been increasing exponentially (FIG. 1). This is driven by the realization of the importance of the microbiota and facilitated by the next-generation sequencing revolution. While it is difficult to predict the future, it is very likely that the number of animal experiments where, e.g., germfree animals will be colonized with various commensal and/or pathogenic microbes, will rise substantially and remain at a high level.

FIG. 1. Number of publications in Web of Knowledge per year using mice or rats to study the gut microbiota.

Impact on the 3Rs

Replacing animal models and improving science go hand in hand as animal models are often poor models so replacing them with *in silico* or *in vitro* models improves science, and only when science is improved will the scientific community overcome inertia and adopt the replacements. Since eGUT, the generic simulation platform we are developing, needs validation for each type of application, limited animal experiments will be initially required before eGUT will be sufficiently validated to fully replace animal experiments in studies similar to previously validated ones. Moreover, data from human plus past animal studies, see REFS (1-5), can be used to parameterize eGUT models, however, this will require more work than can be achieved in three years. The more conservative of two alternative ways of estimating impact suggests the reduction & replacement that eGUT can ultimately achieve is ~75,000 rodents per year worldwide. Please see Pathways to Impact for detailed metrics on the potential impact and our plans to achieve this.

It will be vital to convince researchers of the value of computer simulations. Therefore, the studentship will focus on **validation of the model in the labs of our collaborators Profs Wilmes and Moya**, and finish with **preliminary trials of the software by the UK company Probiotics International and the internationally respected Hardt and Stecher labs in Zurich and Munich** (see letters of support), who could become role models for others to follow.

Scientific impact: computer simulations versus animal experiments

Virtual experiments have several advantages over animal experiments, which will facilitate their adoption (see CV#21 = paper 21 in my CV and publication list). (i) In a simulated experiment, conditions are fully controlled and measurements complete, unbiased and error free. (ii) Computer simulations predict exactly how the system would behave if all assumptions made were true, which can quickly eliminate hypotheses that lead to results contrary to evidence. (iii) Some experiments are difficult to do, e.g., changing the size of a mouse gut, but a computer simulation can easily evaluate how the longer residence time of a larger gut shifts the community composition towards more slowly growing members. (iv) Parameter and structural sensitivity analysis can identify those parameters and modelled processes that have little effect on results and thus do not need to be studied in animal experiments.

Scientific impact: generic modelling platforms versus targeted computational models

There are now a number of very good mathematical models of the gut, e.g., (1–5). They assemble a wide range of empirical details to evaluate the coherence of this information and agreement with observed large-scale patterns (e.g. concentrations of acetate, propionate, butyrate). These custom-built models are not our competition, on the contrary, they are very useful as they already evaluated the choice of parameters and processes that any user of eGUT can benefit from. In contrast to most previous models, our generic

modelling platform eGUT will (i) simulate any number of interlinked compartments, (ii) simulate spatial structure (biofilms), (iii) be a generic platform that can be applied to a range of questions and systems, and (iv) can be used by scientists without the need to write computer code or understand the algorithms. This platform will of course be able to simulate various *in vitro* models, different animal guts and the human gut, forming a proper framework for cross-system comparison. As our tool will enable scientists who are not mathematicians or programmers to run virtual experiments, a much larger number of researchers can use the tool, multiplying the scientific and 3Rs impact.

Scientific potential

Background on gut microbiota and host interactions

The gut is teeming with microbes and they profoundly affect us. There is no space to even list all effects so I will only highlight the two most relevant. (i) Many metabolites in our bloodstream are of microbial origin demonstrating how interlinked the metabolism of microbes in the colon is with our metabolism (6). Hence, metabolic reactions of microbes and the transport of metabolites in the gut can be specified in eGUT and the multi-compartment nature of eGUT enables specification of the metabolism in further compartments, e.g., representing liver, muscle and fat tissues. (ii) Commensal bacteria in the gut provide colonization resistance. Freter in the 1980s used a simple mathematical model to show that dual competition, for resources and for attachment sites, is necessary for the colonization resistance he observed in mice experiments (reviewed in CV#10). The Stecher lab (who agreed to trial eGUT, see letter of support) has recently shown that their synthetic minimal microbiota protect mice against Salmonella infection (7). Objective 1 of this proposal is the addition of the mucosa model to eGUT. This will enable detailed simulation of competition between commensals or probiotics with pathogens that we will use to validate eGUT (Objective 2).

Background on gut models

In vitro models of the gut have been developed since the 1980s, many of these are based on (multiple) reactors of the same volume as human gut sections, e.g., the SHIME model, which is particularly attractive due to the recent addition of a mucin compartment (8). The NC3Rs has shown confidence in the utility of insect models by funding three grants to develop *Drosophila* as a gut model. We have chosen the cockroach model for this project as *Drosophila* does not have an anaerobic gut required for the growth of most human gut commensals. **Cockroaches have been successfully used by our collaborator Prof Moya** to study, e.g., the effect of age (9), diet (10) and antibiotics (unpublished). Importantly, the omnivorous *Blattella germanica* harbours abundant Bacteroidetes and Firmicutes like mammalian guts. Also, Fusobacteria become more abundant in a high protein diet as in carnivorous mammals (kittens and dogs) (10). The NC3Rs has also funded five grants to develop intestinal organoids. These 3D tissue cultures have great potential as alternatives to rodent models and we anticipate that a combined use with our computational model will be mutually beneficial in the future. We have chosen the **HuMiX microfluidic model developed by our collaborator Prof Wilmes** as its membrane-separated microbial and epithelial compartments allow independent control of feeds and continuous sampling of outflow (11, 12). This would be difficult with an organoid. Time series of population dynamics are particularly valuable for testing mathematical models.

Background on individual-based modelling

Mathematical models aim to simplify reality. How to simplify the complexity of the gut microbial community and the animal host is an exciting challenge, especially if the model should generate predictions from experimentally studied mechanisms of interactions on the cellular and molecular scale. This is where Individual-based Models (IbMs) shine because they describe the interactions of the parts of the system–microbes and tissue cells. By simulating parts & interactions, the complexity we observe on the larger scale is generated as an emergent property. Since macroscopic complexity has been shown to emerge from simple microscopic rules (13), IbMs have been successful in modelling complex systems such as forests, the Florida everglades, or agricultural landscapes (13).

Further advantages of IbMs are that (i) they do not constrain the number of species or activities considered, (ii) they can use mechanistic knowledge and parameters from pure culture or other laboratory experiments directly as input, (iii) the model structure is organized in the same way as the natural system (containing individual cells, metabolites, suspended particles and transport terms). Many successful applications of IbMs can be found in my CV (CV#3-6, 11, 12, 14-16, 18, 22) and are based on our software development.

Research plans

Preliminary data: My group has been developing open source software for Individual-based Modelling (IbM) since 1998 (CV#3). The second-generation software iDynoMiCS was published in 2011 (CV#14). It has already been used in 20 labs worldwide. The current eGUT development will be the third-generation as we have simplified and modularized the internal organization of the code for individual agents. FIG. 2 shows a demo simulation we produced as an outreach activity to showcase eGUT for the Channel 4 Superfoods programme (unfortunately they dropped this at the end).

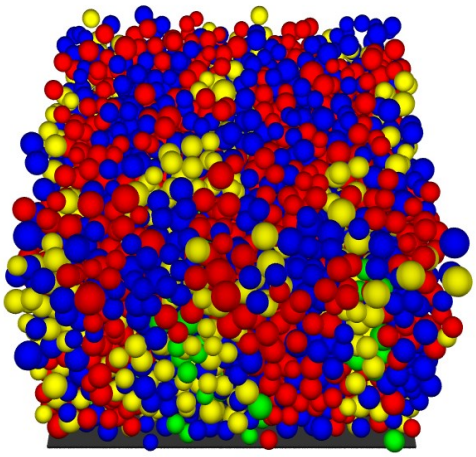


FIG. 2. 3D simulation of four metabolic types common in the colon, following the non-spatial model of REF. 1 but implemented in our spatially-explicit individual-based model where cells can grow as a biofilm. Blue cells hydrolyse polysaccharides into glucose, which they ferment to lactate, Short-Chain Fatty Acids (SCFA) and H_2. Yellow cells are secondary fermenters converting the lactate into SCFA and H_2. Red methanogens and green acetogens compete for H_2. The methanogens win due to higher substrate affinity. (unpublished)

The new, completely modular structure developed for eGUT allows free combination of any property (shape, plasmid content, etc) and activity (set of metabolic reactions, mechanical interactions, production of extracellular matrix, etc) of an agent. Therefore, eGUT is easier to learn to code and improve than iDynoMiCS, enhancing the student's productivity. The main achievements of our work on eGUT, funded by the NC3Rs–for which I am very grateful–are the following: (i) Ability to model multiple compartments and to specify transport of metabolites and agents between compartments. (ii) Introduction of simple compartments without agents where a set of ordinary differential equations (ODEs) describes changes of metabolites (we implemented an ODE solver, which can also be used for intracellular dynamics such as gene regulation), to be used for simple representations of organs such as liver. (iii) Implemented motility leading to attachment of bacteria to the surface. (iv) Rod and filamentous rather than only spherical cell shapes (FIG. 3). (v) Physically correct mechanical interactions between cells based on attractive and repulsive forces and collision detection algorithms (FIG. 3). (vi) Agents are now spatially sorted into a tree for rapid finding of neighbouring agents, important for modelling larger systems. (vii) Improved user interface, guided construction of model specification (protocol maker) and output analysis. However, one very important part of eGUT remains unfinished. This is the mucosa sub-model that the student will implement (Objective 1). Our original aim was to use Voronoi tessellation as the best way to represent the complex 3D structure of the epithelium with crypts and villi accurately and allowing dynamic changes to the epithelial cell population. We had experience with a small-scale 2D model using a generalized Voronoi tessellation (CV#12) and other groups had used simpler Voronoi cells for larger 3D models of animal tissues (14, 15). We had therefore not foreseen that implementing Voronoi tessellation in eGUT would prove very time consuming and lead to a geometrical complexity of the system that could not be handled efficiently for more than a small domain with a single crypt and villus so we had to abandon this in order to complete the other parts. We now know to use a simple geometry for the epithelium and model the effect of villi implicitly as surface enlargement.

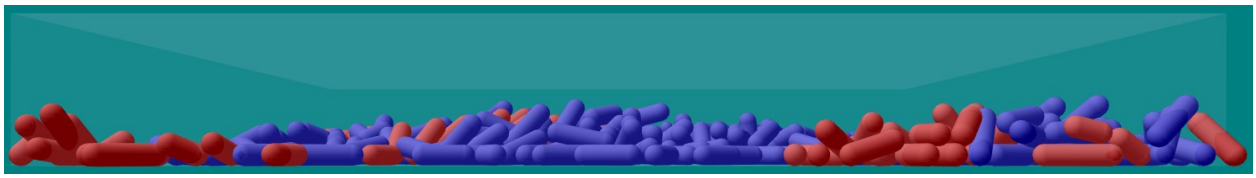


FIG. 3. 3D simulation showing how mechanical forces that push elongating, rod-shaped bacteria out of each other's way lead to partial alignment and buckling. (unpublished)

Objective 1: *Develop a computational model of the mucosa compartment (12 months, 1 publication)*

eGUT is already capable of simulating multiple interconnected compartments. The mucosa sub-model will be integrated as one such compartment. We will use a simple, grid-based representation of epithelial cells that form a simple, flat epithelial layer. On top of this layer will be a mucin layer to which bacteria can attach from a connected gut lumen compartment. Underneath the epithelium will be a connective tissue layer (connected to a bloodstream compartment).

The activities of each individual epithelial cell will be specified as a list of rules and a list of reactions that are described by kinetic equations. Reactions will include transport from and to the lumen and bloodstream sides, e.g. uptake of SCFAs, transport of nutrients from lumen to blood, secretion of enzymes, antibodies, antimicrobials and mucin as these mediate interactions between bacteria and host cells. Cells will be allowed to differ; not all cells have to carry out all reactions, e.g. a 'goblet' cell may only produce mucin and consume SCFAs. Time permitting, we may add sensing of bacteria and immune responses using simple rules.

Objective 2: *Validate the mucosa sub-model–and the complete eGUT model–with* in vitro *and insect models (18 months, 2 publications)*

We will use Paul Wilmes' new HuMiX *in vitro* model to **validate the mucosa sub-model**, as it has the same layers: an epithelial cell layer with a mucin layer and bacterial compartment on top (with medium and aerobic

or anaerobic gas supply), and a collagen layer with separate medium and gas supply underneath. The design of this microfluidic device allows inoculation of each compartment with any cells at any time and continuous sampling of the outflowing metabolites from each compartment and measurement of the integrity of the epithelial barrier (11, 12). (6 mo stay in Luxemburg)

Then, we will use the omnivorous cockroach *Blattella germanica* as a model to **validate the whole of eGUT** as roach guts are large enough for oxygen to be completely consumed inside, resulting in an anaerobic compartment that harbours a microbiota similar to mammals that have similar diets. Our collaborator Prof Andrés Moya has developed and successfully used this model to study microbiota succession with age (9), changes with diet (10) or effects of antibiotics (unpublished). (6 mo stay in València)

The 'same' experiments in HuMIX and cockroach will study colonization resistance and competition between commensal or probiotic bacteria. eGUT will make predictions on which commensals or probiotics are more competitive than the pathogen *Salmonella*, so we can use at least one species predicted to be more and one predicted to be less competitive than *Salmonella*. Time permitting, we will test eGUT predictions of which components of the diet (e.g. prebiotics) will make probiotics more or less competitive to prevent infections.

Objective 3: *Disseminate, teach and support trials of eGUT (6 months spread over last year)*

The student will produce online learning material, documentation and YouTube tutorials and hold training workshops as satellites of relevant conferences as we did with great success in Vienna. Moreover, the student will support the eGUT trials of our **early adopters at Probiotics International and the Hardt and Stecher labs** (see letters of support).

Risk mitigation: Essentially, we will reduce risk by rapid application development, i.e., by getting a basic prototype model working first and then improve it as much as time permits. This emphasises completion over sophistication. Unforeseen difficulties will reduce sophistication rather than risk not completing.

People and track record (see SI for research environment and student training)

Dr Jan-Ulrich Kreft is a Lecturer in Computational Biology at the University of Birmingham. He is a microbiologist who has pioneered the use of **Individual-based Models (IbMs)** to simulate interactions between microorganisms and their environment. He has extensively applied IbMs in his research, making important contributions to science such as predicting the existence of a bacterium able to completely oxidise ammonia in 2006 (CV#7), which was discovered in 2015 by two groups (16, 17). He has concurrently been developing open source software for IbMs, first **BacSim** and then in collaboration with Prof Smets (DTU) the more advanced **iDynoMiCS**, which is now used by 20 groups worldwide. **eGUT** is the third generation of much extended and improved IbM software that his lab has been developing, thanks to an NC3Rs grant. Three of his PhD students have graduated, one is in year 1 and one is to start in Oct. 2017.

Prof Tariq Iqbal is Honorary Professor at the University of Birmingham and a Consultant Gastroenterologist at the University Hospital Birmingham where he runs a busy Inflammatory Bowel Disease (IBD) practice. He has been **leading many clinical trials**. Currently, he is PI for two interventional trials (colonic biomarkers for stratifying colon cancer risk and faecal transplantation (FMT) in Ulcerative Colitis (UC)). He is regional lead for GI research and member or chair of a number of guideline committees and expert panels.

Conclusion: *We are the right team to develop the mucosa model as part of eGUT and to validate eGUT with our expert collaborators. eGUT fills an important gap as a generic modelling platform, its development is timely, offers scientific advantages and will substantially reduce animal experiments in the long term.*

References: **1.** Munoz-Tamayo et al. (2010) Mathematical modelling of carbohydrate degradation by human colonic microbiota. **2.** Munoz-Tamayo et al. (2011) Kinetic modelling of lactate utilization and butyrate production by key human colonic bacterial species. **3.** Lawson et al. (2011) Bayesian analysis of non-linear differential equation models with application to a gut microbial ecosystem. **4.** Kettle et al. (2014) Modelling the emergent dynamics and major metabolites of the human colonic microbiota. **5.** Cremer et al. (2017) Effect of water flow and chemical environment on microbiota growth and composition in the human colon. **6.** Wikoff et al. (2009) Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. **7.** Brugiroux et al. (2016) Genome-guided design of a defined mouse microbiota that confers colonization resistance against *Salmonella*. **8.** Van den Abbeele et al. (2013) Butyrate-producing *Clostridium* cluster XIVa species specifically colonize mucins in an *in vitro* gut model. **9.** Carrasco (2014) Succession of the gut microbiota in the cockroach *Blattella germanica*. **10.** Pérez-Cobas et al. (2015) Diet shapes the gut microbiota of the omnivorous cockroach *Blattella germanica*. **11.** Shah et al. (2016) A microfluidics-based *in vitro* model of the gastrointestinal human-microbe interface. **12.** Eain et al. (2017) Engineering solutions for representative models of the gastrointestinal human-microbe interface. **13.** Grimm et al. (2005) Individual-based modeling and ecology. **14.** Schaller et al. (2005) Multicellular tumor spheroid in an off-lattice Voronoi-Delaunay cell model. **15.** Van Liedekerke et al. (2015) Simulating tissue mechanics with agent-based models: concepts, perspectives and some novel results. **16.** Daims et al. (2015) Complete nitrification by *Nitrospira* bacteria. **17.** van Kessel et al. (2015) Complete nitrification by a single microorganism.

Experimental design and methodology appendix

Methodology

The methodology of developing computer code to simulate individual cells and their interactions, as well as transport of nutrients and cells in the environment, is a cycle of coding and testing the correct implementation of the code. Unit testing is routinely used and will test whether the new code breaks any functionality of the code, but this is not enough for scientific simulations as we also need to check that the output is numerically correct. This requires setting up simple simulation test cases where the correct outcome can be compared with simulations of an ordinary differential equation (ODE) model or with analytical solutions of the model that may be available for simple geometries and cases. For example, to test an individual-based model of plasmid transfer, we have compared it with a simple mass action model of plasmid transfer, which is described by ODEs. To test a reaction diffusion solver simulating the transport of solutes in the environment from a source to a sink, we have compared the simulation output with analytical solutions of Fick's laws. These are known for simple cases, such as starting with a step function (concentration of solute = 1 on the left half of the domain, and zero concentration on the right). In this case, the analytical solution has a sigmoidal shape (described by the error function) that is getting flatter over time. See the book "Accuracy and Reliability in Scientific Computing" edited by Bo Einarsson (SIAM: Philadelphia, 2005) for more details.

Experimental design of computer simulations

Simulations of stochastic models, such as our individual-based models, in a computer are virtual experiments and are therefore statistically analysed in the same way as physical experiments. They also need to be replicated. We usually do three replicates as a pilot and analyse the output. If e.g. trajectories of population sizes diverge, we add more replicates. Running 3 or 30 replicates requires the same human effort as we write scripts to analyse and start 'n' simulations so we only need to change 'n' from 3 to 30. The cpu time is of course tenfold higher. If there are positive feedbacks in the system, it can happen that initially small differences between replicates grow over time (diverge) so it will be necessary to run many replicates. Also, initial cell positions can have strong effects if there are only few cells colonizing a surface (FIG. 1). Power calculations are easy as we can estimate SD and effect size from the three replicates we do by default and run as many replicates as power calculations suggest.

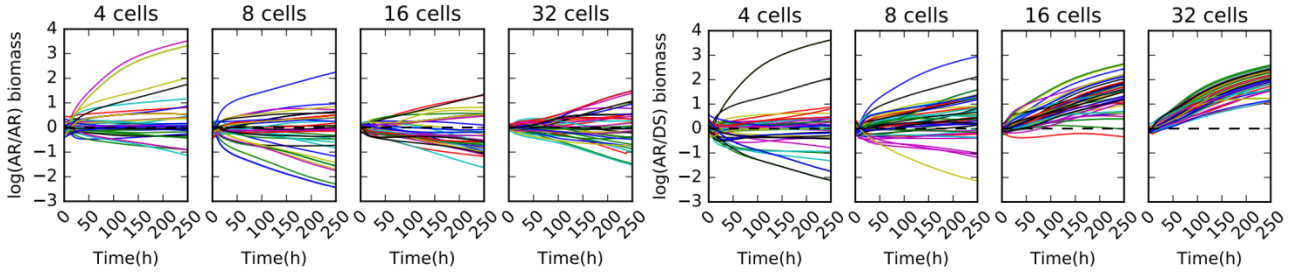


FIG. 1: Biomass ratios (log scale) of two competing species forming mixed biofilms, showing the effect of random initial positions of cells on fitness, which is much stronger if there are only a few cells at the start (n=50). Set on the left are controls where both species are identical. Set on the right shows that species AR is more competitive than DS when results are less influenced by random initial positions. (unpublished)

Randomization is automatically taken care of by initializing the simulation with randomly varied initial values and parameters. Placebos and blinding are not needed as bias is avoided by automatic, programmed analysis guaranteeing objectivity.

Experimental design of in vitro *and* in vivo *experiments*

The HuMiX and cockroach experiments will be designed with the NC3Rs Experimental Design Assistant (EDA) online tool to optimize the experiments and for the student to learn the process and tool.

Comparison of computer simulations with in vitro *and insect model results*

The software is written to produce output at user chosen time intervals. This output is detailed, providing most of the state of each individual cell (and concentration fields for the spatially structured environment). If even more information is needed, the code can be easily changed to output any extra data. Simulation output can be compared with experiments in the same way as different experiments can be compared using statistical tests. However, the simulation output is often higher resolution, provides more information and contains hardly any measurement noise (noise due to numerical approximations and error tolerances of solvers is usually much lower). Therefore, simulation data may have to be filtered and coarsened.