Recent developments in mathematical and computational biomedicine

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The burgeoning field of precision medicine is stimulating special interest in the development of novel mathematical and computational methods and in the integration of omics, clinical and environmental data. Twenty-two researchers met at the BIRS-CMO located in Oaxaca to discuss new developments in Computational Biomedicine. Each day was organized into seminars in the morning and discussion and collaborative activities in the afternoon. Mathematicians and computer scientists were exposed to interesting problems within medicine where modelling and algorithmic input could be transformational. Biologists and medics benefited from a forum with discussion in the context of mathematics and computation.

1 - Overview of the Field

Stochastic modelling and simulation techniques have major advantages over deterministic ones when the goal is to capture variability and heterogeneity. They have the practical disadvantage that current Bayesian techniques for comparing models to data are much easier to use when the model is deterministic. Recent imaging and tracking techniques allow direct observation of cell cycle phases and phenotypes of living cells. Fluorescent ubiquitin-based cell-cycle indicator (FUCCI) is used to measure the times that individual cells, including stem, melanoma, B and T cells, spend in the G1 phase. The long-established Smith-Martin model, of an exponentially-distributed time in the G1 phase, has been superseded by the more sophisticated cyton model developed by the Hodgkin school, validated by comparison to carefully-collected data from single cells and their offspring [6, 8]. Many objects of interest, in vivo, appear to move randomly in a confined domain. Using the basic model of Brownian motion, it is possible to calculate the frequency of encounters and reactions between such objects. The healthy cycle, where theory stimulates new observations which stimulate further theoretical developments, is seen in the study of cell surfaces, of T cells in lymph nodes, and of animal search strategies [11, 12].

Chemical reaction networks are believed to be key to many biological processes. Recent attention has focused on how robust responses may be maintained in the face of variable conditions and intrinsic stochasticity, due to mathematical features of the reaction networks. Stochastic reaction networks describe the time evolution of the numbers of interacting objects, deterministic or stochastic, via a triple (S, C, R) representing species, complexes and reactions. Health and disease are regulated, to a large extent, by our immune system. The immune system not only protects the body from infectious disease, but is involved in a number of conditions of increasing

incidence and morbidity, such as diabetes, rheumatoid arthritis, inflammatory bowel disease and allergies. In cancer, the immune system can be both cause and cure; it contributes to chronic inflammation that promotes tumour development, but it can also provide the ultimate weapon against metastatic disease. Thus, the development of ways to harness, direct or restrain immune responses has great potential for enhancing human health.

In more detail, the ability of the adaptive immune system to respond adequately pathogens encountered is in large part driven by T cells, which are able to recognise pathogens via a cell-surface receptor generated randomly during T cell development, and inherited by daughter cells. Understanding how the repertoire of these receptors, called TCRs, in an individual relates to adequate immune function or disease severity, and how this system can be harnessed to improve human health, are central questions in modern immunology. Research in human immunology is based on *observational studies*. For example, data sets of gene expression profiles are used to identify disease biomarkers. The interaction of T cells with antigen-presenting cells, for example, has been extensively studied in mice using explants and intravital imaging, but such studies are practically impossible in humans. Furthermore, accurate control and manipulation of parameters such as antigen dose and cell ratios is limited. The classical tools in *experimental* human immunology rely heavily on immortalised cell lines, stimulated clonal lines, peripheral blood, and humanised mouse models. Each of these tools has limitations.

Lastly, the complex community of the human microbiota and its specific role in health maintenance and disease has become an intense topic of study over the last years, a topic that is itself intrinsically related to the growing resistance to antimicrobials and their extensive use in human, agricultural and commercial settings. The number of microbial genes inside our bodies is roughly 100 times higher than the number of genes contained in the human genome, impacting human biology in various ways. For instance, the human immune system is in great part composed of and trained by resident microorganisms, and different microbiome compositions associate with the onset and progression of a large variety of human diseases. Causal links, however, remain largely unexplored in many human diseases, including those showing strong associations. The latter is in great part due to sampling limitations, data volume and integration complexity, necessitating novel mathematical and computational methods.

2 - Recent Developments and Open Problems

The extension of the concept of absolute robustness to stochastic systems has potential to reorient the analysis of chemical reactions. It is possible to deduce, directly from the reaction scheme before proceeding to the mathematics proper, which reactants and complexes have steady states that are robust to total concentrations [1].

Adoptive T cell therapies for the treatment of cancer and immunodeficiencies rely on appropriate selection of T cells from the patient, ex vivo clonal expansion through the provision of activating stimuli and, possibly, ex vivo genetic modifications that result in T cells expressing chimeric antigen receptors (CARs) [4, 3]. Immunotherapies elicit, amplify or suppress immune responses in order to treat diseases such as cancer and auto-immune disorders. In the method of Adoptive cell transfer (ACT) [4, 3], T cells are removed from the patient, then possibly genetically modified with chimeric antigen receptors, cultured for expansion in vivo and returned to the same patient. ACT holds great promise as it leverages the targeted precision inherent in T cell adaptive

immunity. Immunomodulators based on naturally occurring immune signalling (interleukins, cytokines and chemokines) have been licensed for medical use. They provide general activation, damping or transport cues to cells of the immune system. Cancer immunotherapies function either by motivating an incipient, but ineffectual, immune response or by creating an entirely new one via receptor engineering.

Successful engineering of T-cells involves the expression of a functional and stable TCR, as well as the preservation of important cellular characteristics. One such characteristic is cell motility which is essential for efficient antigen search. The technique of mRNA electroporation is showing promise, although the induction of expression of an exogenous TCR in CD4 T cells is considerably harder than in CD8 T cells. Dushek, Abu-Shah and coworkers are developing a three-dimensional culture system to study immune cell interactions. In this tissue-like platform, engineered quiescent human T cells are studied and their interactions with dendritic cells observed directly.

In what pertains host-microbiota interactions, several tools and methods have been developed toward integration of multi 'omics' data and multi-scale analysis and predictive (mechanistic) modeling. On the one hand, feature selection and machine learning allows identification of patterns and relationships in large data collections, such as those in human microbiota studies. On the other, mathematical modeling and simulations provide a comprehensive framework to identify connections and key (onset) mechanisms in disease models.

3 - Presentation Highlights

Mike Scheetz (University of Texas Medical Branch, Galveston, USA)

> Professor Scheetz discussed how mechanosensor depletion can drive wound healing and cancer dynamics. It appears that transformed cancer cells lack rigidity sensors and have altered cytoskeletal protein levels. This rigidity sensing is partly due to actin recruitment. Intriguingly, stretching cells can trigger transformed cell apoptosis but protects normal cells. This cell stretching is a key to possible treatment of certain types of cancer such as breast cancer.

John Hancock (University of Texas Health Science Center at Houston, USA)

> Professor Hancock discussed lipid binding specificity of the KRAS membrane anchor. Such anchors mediate plasma membrane binding and spatial organization. Apparently, many cancers arise due to mutations in KRAS. Professor Hancock discussed the role of RAS clustering on the membrane and subsequent initiation of cascading reactions within the cell. Clustering is partly driven by the structure of the actin skeletal mesh.

André Leier (University Alabama at Birmingham School of Medicine, USA)

> Dr Leier discussed neurofibromatosis. Although it is a relatively rare disease (one in approximately 3000 births), life expectancy can be reduced by up to 15 years. Various bioinformatics analyses were discussed based on finding suppressor genes and analysing the RAS pathway. Possible therapies were proposed based on exon skipping.

Pablo Longoria (UNAM, Mexico)

Professor Longoria presented some conceptual links between spatial mathematics and problems in medicine. Two main ideas were proposed. The first was the use of time dependent bifurcation analysis in growth rates as control mechanisms in pattern formation. The second idea explored optimal space filling curves to understand branching structures, as in the lung and the brain. Switching dynamical behaviours can arise due to the effect of spatial limitations.

German Enciso (University of California at Irvine, USA)

Professor Enciso studied the network stabilization of chemical reacting systems through the use of absolutely robust modules. In its simplest form this involves adding reactions $A + B \rightarrow 2B$, $B \rightarrow A$ into a given system. This was analysed both in the deterministic setting and the intrinsic noise setting. The key concept here is the deficiency index associated with a chemical reacting system. If this value is 1 and the system is conservative with positive equilibrium with some additional constraints then it can be shown that the species converges to a Poisson distribution for large time windows and large total protein concentrations.

Linda Kenney (University of Texas Medical Branch, Galveston, USA)

Professor Kenney discussed salmonella lifestyles and how salmonella can form biofilms in gallstones. There are two types of secretion systems and two lifestyle choices for salmonella, through biofilm formation or within vacuole dynamics. Professor Kenney then discussed the potential of salmonella as an anti-tumour agent via the colonization of a tumour. A key idea is that salmonella can activate caspase.

Rafael Peña Miller (UNAM, Mexico)

Professor Peña Miller presented heterogeneity in clonal populations of E. coli. Multicopy plasmid dynamics was described as a stochastic process with division, random replication and random segregation. The resulting stochastic dynamics was then studied through the effects of antibiotics on a population. In such cases, persistors play a role in surviving populations. The effect of fluctuating environment was considered both from experiments and model construction and calibration. The main conclusions are that multicopy plasmids promote genetic heterogeneity and that heterogeneous populations are better adapted in fluctuating environmental conditions. Furthermore, multicopy plasmids can play a role in alleviating evolutionary trade-offs.

Phil Hodgkin (WEHI, Melbourne)

Professor Hodgkin gave a presentation on the cellular calculus in the context of the immune system, highlighting the point that individual cellular paths are heterogeneous but populations of cells behave deterministically. Models of lymphocyte dynamics were presented from a stochastic perspective. The presentation focused on memory and the time between events. This latter component was based on a distributional approach. Other issues that were discussed included the manifestation of correlations through competition and censorship, and the critical role of heterogeneity in an effective immune response.

Grégoire Altan-Bonnet (NIH, USA)

Professor Altan-Bonnet focused on the response of the immune response against tumours with a particular emphasis on leukocytes, T cell immunotherapy and adaptive kinetic proofreading. Experimental data is available through mass cytometry in which machine learning across a very high dimensional space is used to classify cells and identify clusters. Machine learning and neural nets, in particular, are used to establish time kinetics which can then be used either to calibrate adaptive kinetic proofreading or to learn the underlying biochemical pathways.

Tatiana Marquez-Lago (University Alabama at Birmingham School of Medicine, USA)

Professor Marquez-Lago gave an overview of a range of stochastic modelling and simulation techniques that play an important role in Computational Biology. These include the Stochastic Simulation Algorithm, the Chemical Master Equation, and tau-leap methods along with a number of variants. A particular focus was on the role of delays in chemical kinetic modelling. In particular, the presentation illustrated how chains of unimolecular chemical reactions can be reduced to much simpler transitions through the use of time-dependent delays. New work on the role of host-microbiota interactions in complex human diseases and antibiotic resistance was also discussed, and how artificial intelligence and mathematical models can help unravel complex interactions from multi-omics data.

Judy Cannon (University of New Mexico, USA)

Professor Cannon gave an overview of the effect of space on T cell dynamics and movement, with a particular focus on the lymph node. Various issues were discussed including spatial crowding, confinement and motion along networks. Different mathematical models of transport were analysed including Levy walks and lognormal walks. The effects of environmental and chemokine influences were also discussed in terms of the stopping and starting of T cells. Most of the simulations were done in two spatial dimensions with more work needed to upscale to full three-dimensional simulations. This work is likely to offer deep insights into how target distributions and spatial structures affect the search efficiency of T cells and offer new ways of understanding the complexity of the immune system.

Dan Coombs (University of British Columbia, Canada)

Professor Coombs gave an overview on the use of graph-based approaches for analyzing receptor distributions. Various measures of spatial clustering were discussed. These included the Hopkins index, Ripley functions, tessellation-based methods, weighted neighbourhood graphs through community detection, the Louvain method and other hierarchical clustering approaches. Data was obtained through a new and very efficient approach, known as STORM, which is based on stochastic optimal reconstruction microscopy.

Tianhai Tian (Monash University, Australia)

Professor Tian discussed how to inference pseudo-time trajectory and gene network information using single-cell expression data. He first described how single-cell experiments can measure gene expression levels or protein activities in single cells, how this has provided unprecedented opportunities to analyze the heterogeneity in cells, and how the non-time-series data has raised challenges to mathematical modeling, where the necessary first step is to arrange each cell in the pseudo-time trajectory based on the developmental stages of cells. Professor Tian discussed two recent works for the inference of pseudo-time trajectories,

namely the SCOUT algorithm and DTFLOW algorithm. He then discussed a modeling framework for developing both graphic models and dynamic models of gene networks using single-cell expression data.

Enas Abu-Shah (University of Oxford)

Dr. Abu-Shah took us into the world of low-affinity TCR interactions and described a beautiful system for measuring low affinity interactions and the quantitative contributions of defined costimulatory signals.

Matthew Simpson (Queensland University of Technology, Australia)

Professor Simpson discussed some new approaches to spatiotemporal reaction diffusion models of cells in different phases of the cell cycle in terms of scratch assays. Experiments allow cell cycle states to be labelled fluorescently and this provides information about the position of individual cells as well as temporal information. Thus, the total population is composed of 3 (reduced to 2) interacting subpopulations. A continuum approach is used to construct two coupled partial differential equations that can be studied in terms of their wave speed. The models are calibrated against the scratch assays in one spatial dimension.

Radek Erban (University of Oxford, UK)

Professor Erban gave an overview of approaches in the multiscale modelling of stochastic reaction-diffusion problems. These techniques included the combining of Markov Chain models with stochastic partial differential equations and the coarse-graining of molecular dynamics through stochastic models with non-Gaussian force distributions. Professor Erban also discussed the coupling of all-atom molecular dynamics simulations with ions in water through Brownian dynamics. Compartmental methods were also given in terms of a number of molecular dynamics examples.

Abel Palafox (UDG, Mexico)

Dr. Palafox presented analyzes of vaccination effects on epidemiological competition, giving a background on how couples of viruses like Influenza - Respiratory Syncytial Virus (RSV), or Dengue - Zika, are in competition on every epidemiological season. Classical approaches do not consider competition since both viruses are studied separately, and the interaction of both viruses was modelled using Lotka-Volterra equations. One interesting question is to explore the effect of vaccination against one virus, on the second one, for which preliminary results where a real dataset of Dengue and Zika infections were presented, alongside numerical experiments.

Germán Preciat (University of Luxembourg)

German Preciat (PhD candidate) presented an atomically resolved metabolic network and constraint-based model for Parkinson's disease. The elegant iDopaNeuro 1.0 model was generated by integrating omics data from in vitro neuroepithelial stem cell-derived cultures and was supplemented including atom mapping data. Using a mathematical optimization approach and the iDopaNeuro 1.0 model, the flux of isotope-labeled atoms in a dopaminergic neuron was predicted and subsequently validated with in vitro data for healthy and known Parkinson's disease perturbations.

4 - Afternoon discussions and ad hoc talks

The presentations were given in the morning, and after lunch, time was provided for discussions amongst the attendees about new collaborations. An unscheduled chalk talk was volunteered by Kevin Burrage (Queensland University of Technology and Visiting Professor University of Oxford). Professor Burrage gave an overview of modelling and simulation techniques for coping with variability and heterogeneity in both a Biology and Physiology setting. A particular focus was on a new paradigm called populations of models (POMs). In this approach, an ensemble of models are calibrated, with each model having a different set of parameters. Examples were given for the calibration of cardiac cell models in terms of their electrophysiology. A second aspect that was discussed was the role of model reduction through such techniques such as emulation, homogenization and graphs. Finally, an image-based modelling approach was discussed in which an automated computational pipeline was able to generate realisations of different types of fibrotic pattern as a means of both data enrichment and simulation over the images as a way of further elucidating the relationships between structure and function in cardiac electrophysiology.

5 - Scientific Progress Made

Topics for discussion and leading participants included:

- Control of cell fate: how knowledge of signalling pathways in cancer cells can be translated to T cell and B cells. (Phil Hodgkin)

- Machine learning techniques can be applied to measurements on heterogeneous populations of T cells. (Gregoire Altan-Bonnet and Phil Hodgkin)

- The Fucci reporter system. (Phil Hodgkin, Matt Simpson)

- Immune cell signalling and its consequences, especially different roles and forms of the RAS molecule: (Phil Hodgkin and John Hancock)

- Cell-surface receptor motility and cell-surface dynamics. (Dan Coombs, Gregoire Altan-Bonnet)

- New approaches for coupling stochastic simulation techniques across multiple scales. (German Enciso and Radek Erban)

- PDEs in one space dimension
  A model that some participants teach to undergraduate students is the PDE

$$\frac{\partial c}{\partial t} = D\,\frac{\partial^2 c}{\partial t^2} + \lambda c(1-c) \qquad (1)$$

  It is based on the idea that individuals in a population diffuse, and that population growth is density-limited, meaning that each individual's birth rate depends on how many other individuals are present. In current scratch assays, it is possible to observe cells in different

phases of the cell cycle. Thus, the single PDE (1) is replaced by a set of three coupled PDEs, one for each colour. It may also be possible to deduce from the intensity of the red fluorescence, the time since a cell last divided. (Matt Simpson, Grant Lythe, Phil Hodgkin)

- Machine learning and mathematical models for predicting antibiotic resistance and host-microbiota interactions (Tatiana Marquez-Lago, André Leier, Rafael Peña Miller)

- Simulation of growth hormone receptor dynamics, based on super resolution fluorescent microscopy and electron microscopy (André Leier, Tatiana Marquez-Lago, Dan Coombs, John Hancock)

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