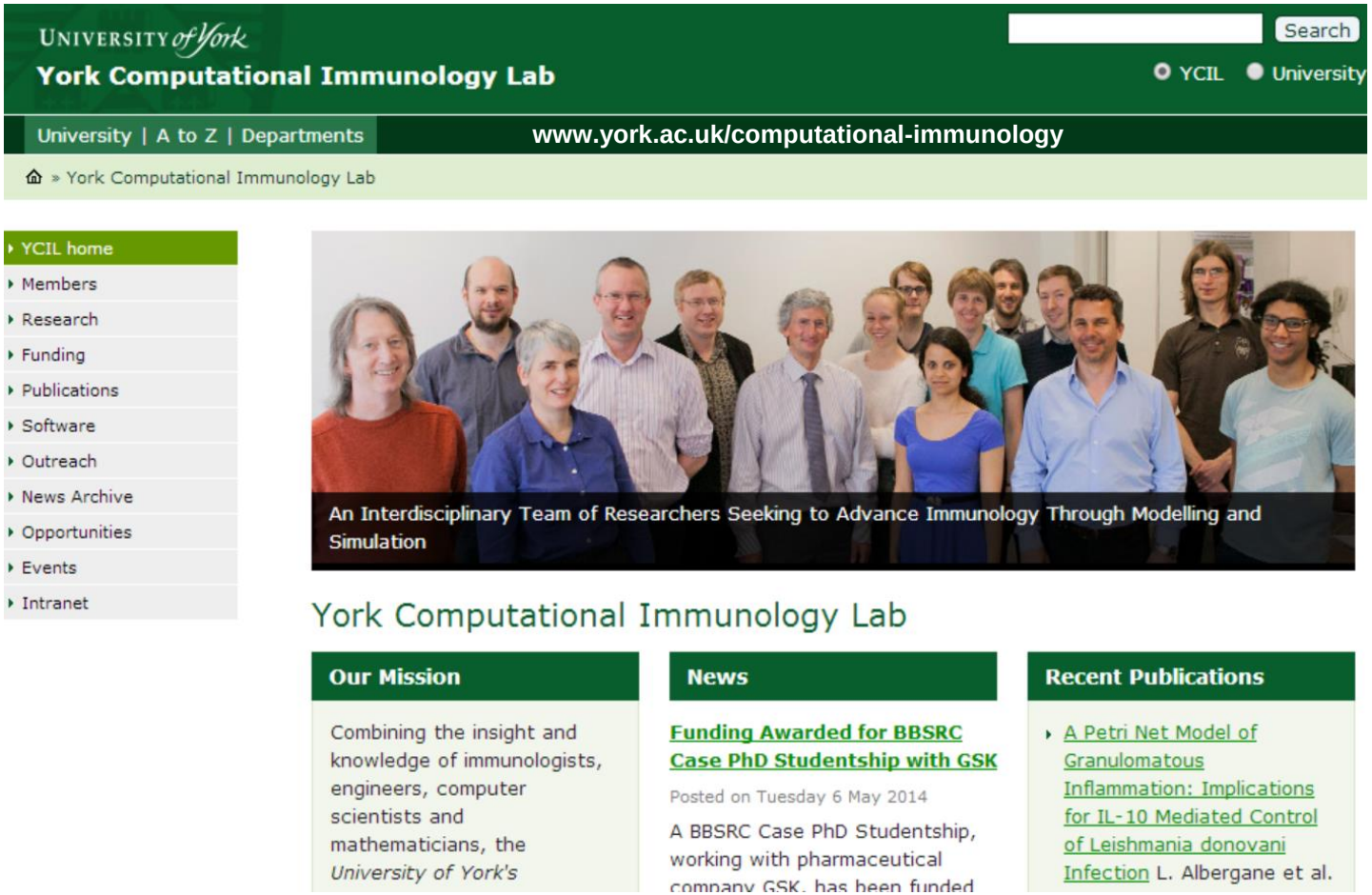
Combining Computational Modelling with Experimentation to Understand Immune System Formation & Function



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Combining Computational Modelling with Experimentation to Understand Immune System Formation & Function

Part 1: Why? Philosophy & Approach

Part 2: How? Modelling Process

Part 3: Example: Multi-scale hybridised Agent Based Model

Increasing Drug Development Success: Understanding Drug-Disease Interactions Through Quantitative Systems Pharmacology

November 2-3, 2015: New York, New York

Description

Roche Pharma Research and Early Development (**pRED**) is sponsoring an exciting interactive forum, from which experts from a variety of specialties – including computational methods, mathematical modeling, clinical investigation, and experimental medicine – will come together to explore how to leverage the interfaces of theoretical and experimental pharmacology for ultimately increasing drug development success rates. The symposium is designed to inspire new research directions as thought leaders with various viewpoints collaboratively analyze the connectivities among different disciplines and disease areas to determine the optimal applications of quantitative systems pharmacology.

Modelling Biology with Mathematics and applying it to drug development: Piet van der Graaf (Leiden), Ben-Fillippo Koppendorf (Roche), Mark Coles (York), Ravi Lyengar (Mount Sinni), Don Mager (Buffalo), Ben Ribbi (Roche),

Investigating disease and drug response heterogeneity: Phillippe Sanseau (GSK), Philip Maini (Oxford), John Issacs (Newcastle)

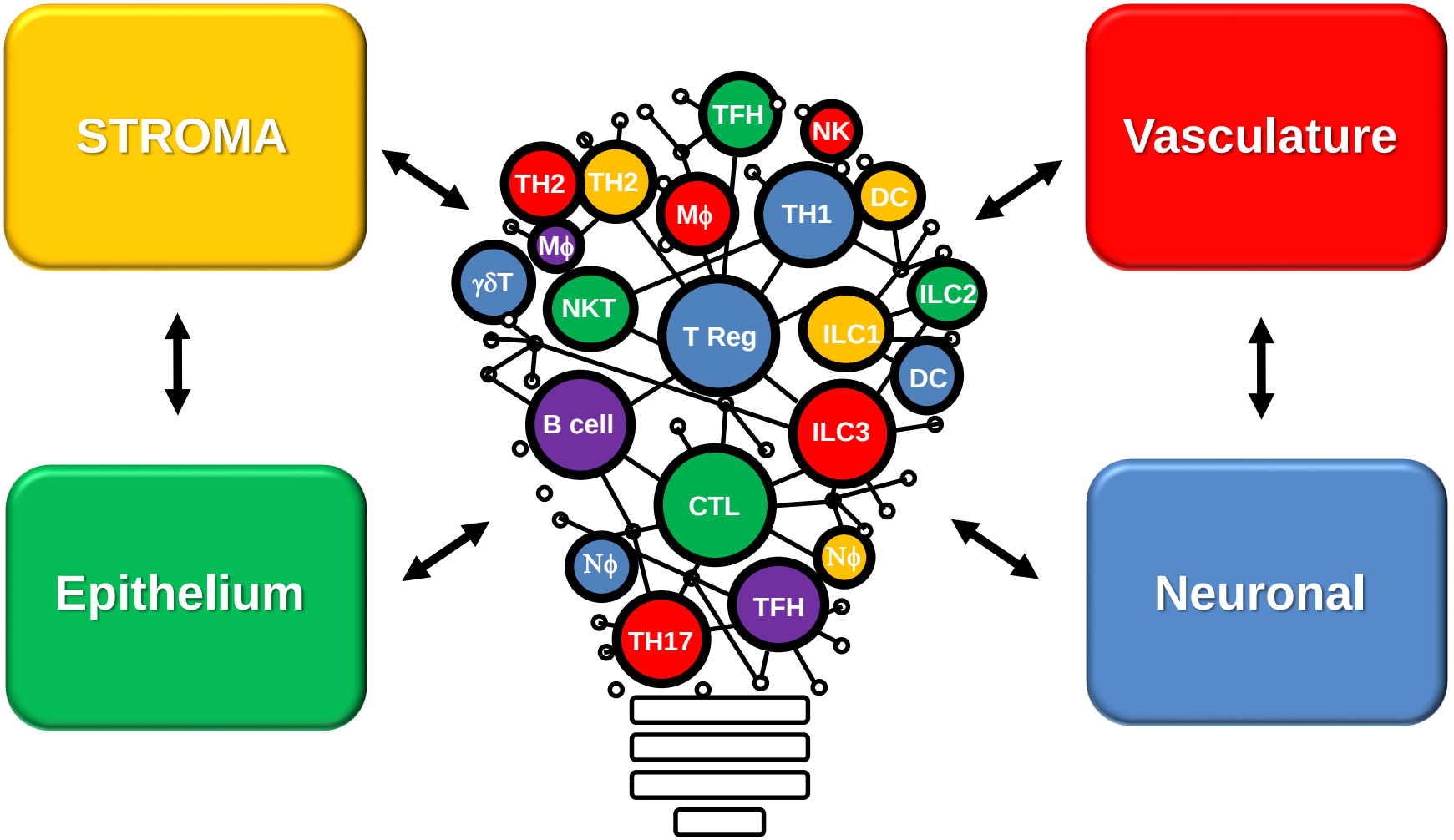
The challenge of treatment failure and the emergence of resistance: Ron Germain (NIH), Karen Bush (Indiana), James Collins (Boston), Elisabet Nielsen (Uppsala)

The challenge of treatment failure and the emergence of drug resistance: Alan Perelson (Los Alamos), William Pao (Roche), Franziska Michor (Harvard)

Information to Register

Attendance will be limited to 100 experts from key disciplines. If you would like to participate, please register your interest at http://tinyurl.com/pREDsymposium. The scientific organizing committee will review all applications and inform you as soon as possible about your application.

Molecular and biophysical mechanisms that regulate immune responses in tissue microenvironments



Understanding and Resolving Pathology:
Cross talk in the context of the microenvironment

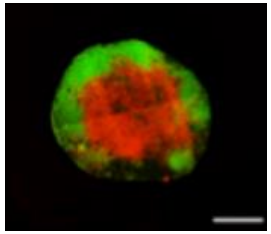
Problems where modelling can address key questions

Why integrate mechanistic modelling with experimental immunology?

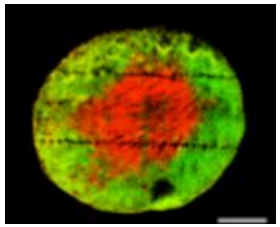
Experimental Driven

- **Analysed time**

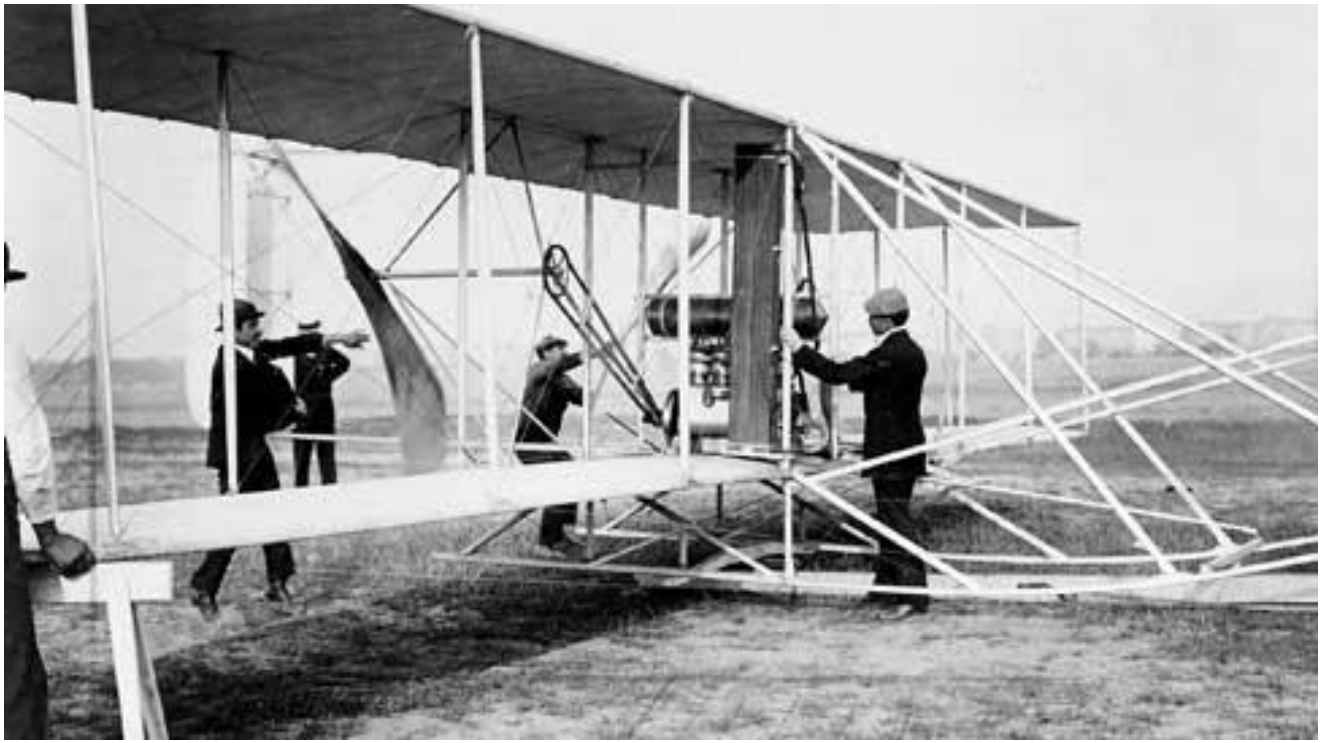
OVA/GLA-SE 24hrs



OVA/GLA-SE 72hrs



- **Expensive**
- **Use of large numbers of animals**
- **Reactionary**

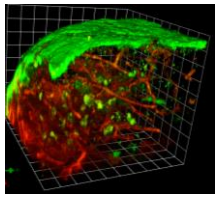


Why integrate mechanistic modelling with experimental immunology?

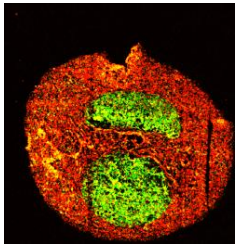
"All models are wrong some are just useful"

Combining Approaches for mechanistic discovery and therapeutic development

Experimental Models



In vivo



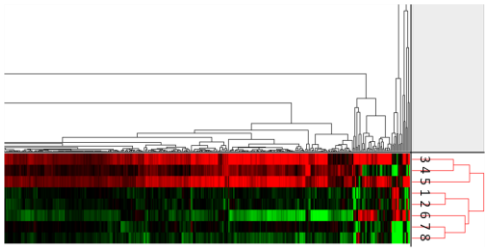
In vitro

- Hypothesis testing
- Use animal models to replicate *in vivo* physiology
- 3D models of human immune microenvironment

Data Driven Modelling

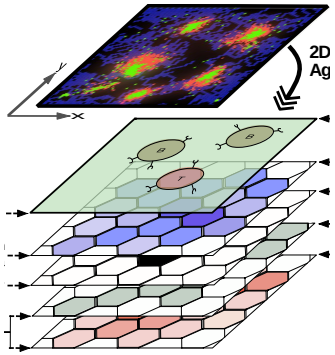
“Polyomics”

Transcriptomics
Epigenomics
Metabolomics
Proteomics
Cytomics



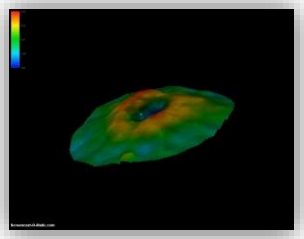
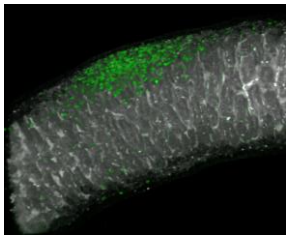
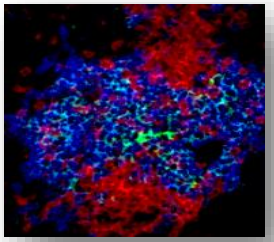
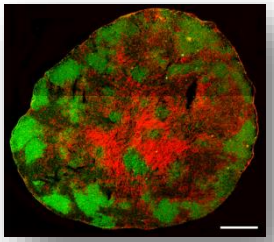
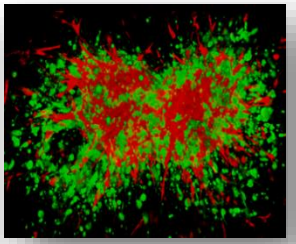
- Data Rich Holistic
- Emergence
- Data Driven rather than hypothesis driven
- Top down approach

Mechanistic Modelling



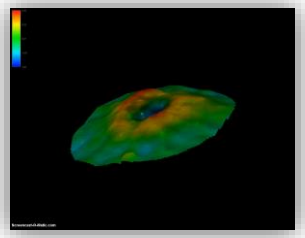
- Holistic
- Emergence
- Mechanism rich
- Hypothesis driven modelling
- Bottom up approach

Understanding Immune Microenvironments: Formation and Function: Pairing an experimental biologist with a modeller

	Wound Healing	Lymphoid Tissue Development	Tertiary Lymphoid Tissue Formation	Lymph Node Remodelling	Inflammatory Tumour Stroma
Experimental Question					
Experimental Biologist	 Gerry Li	 Mark Coles	 Bridget Glaysher	 Anne Thuery	 Katrina Reilly
Computational / Mathematical Modeller	 Liz Gothard	 Kieran Alden	 James Butler	 Jason Cosgrove	 James Butler
Approach	ODE/PDE	ABM	Multiscale	Multiscale	Multiscale

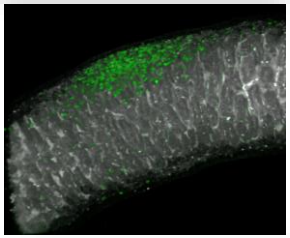
Questions raised by the biology but difficult to address experimentally

Wound Healing



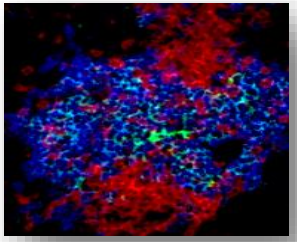
How does the interplay between Innate lymphoid cells (type 3) and macrophages control the wound healing process?

Lymphoid Tissue Development



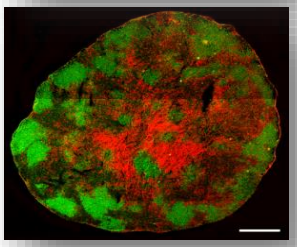
What is the relative role of adhesion and chemokines in the induction and growth of lymphoid tissues during embryonic development?

Tertiary Lymphoid Tissue Formation



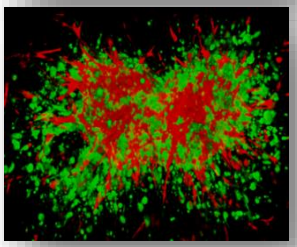
Is a feedback loop between lymphocytes and stroma sufficient to drive the formation of highly organised tertiary lymphoid tissue found in autoimmune pathology?

Lymph Node Remodelling



What are the key factors required for nascent follicle formation in lymph nodes treated with TLR agonists?

Inflammatory Tumour Stroma

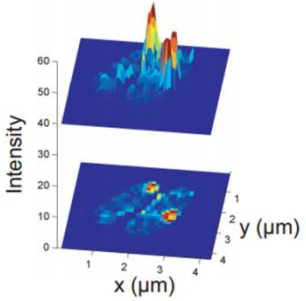


How does competition between VLA4, SPARC and VCAM-1 drive tumour cell migration?

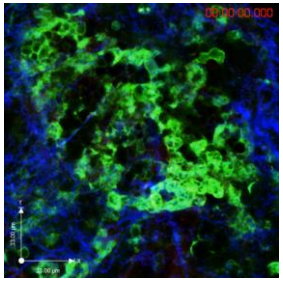
Biophysics of Immune Responses: From Atoms to Organisms

Quantification

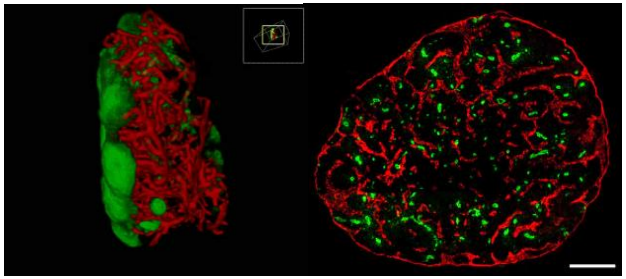
Single Molecule Imaging
(TIRF, STORM, PALM)
Fluorescent Electron Microscopy



Multi-photon imaging
Confocal Microscopy
Flow Cytometry



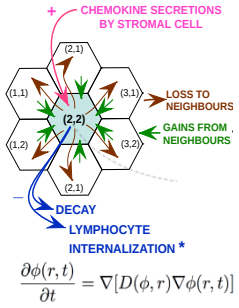
Light Sheet (3D structure)
Immunohistochemistry
Bio-photonic & electrical Devices



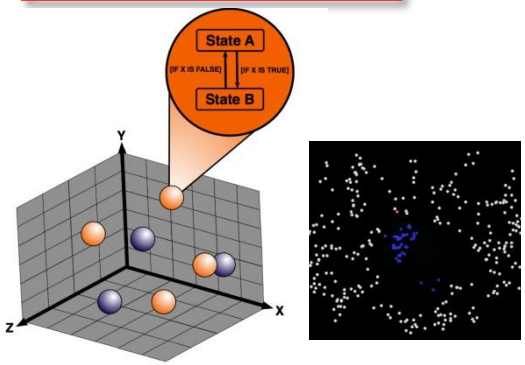
Mechanism

Molecular Diffusion	Cell migration	Physiological Systems
Molecular Interactions	Cellular Interactions	Immune Responses
Subcellular Biophysics	**Cellular Function**	**Tissue Organisation & Function**
Receptor dynamics	Cellular Differentiation	Remodelling
Signalling thresholds	Effector Function	Pathology

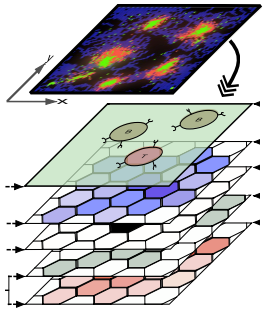
Modelling



Mathematical Models
Monto Carlo Simulations
PDE, ODE models

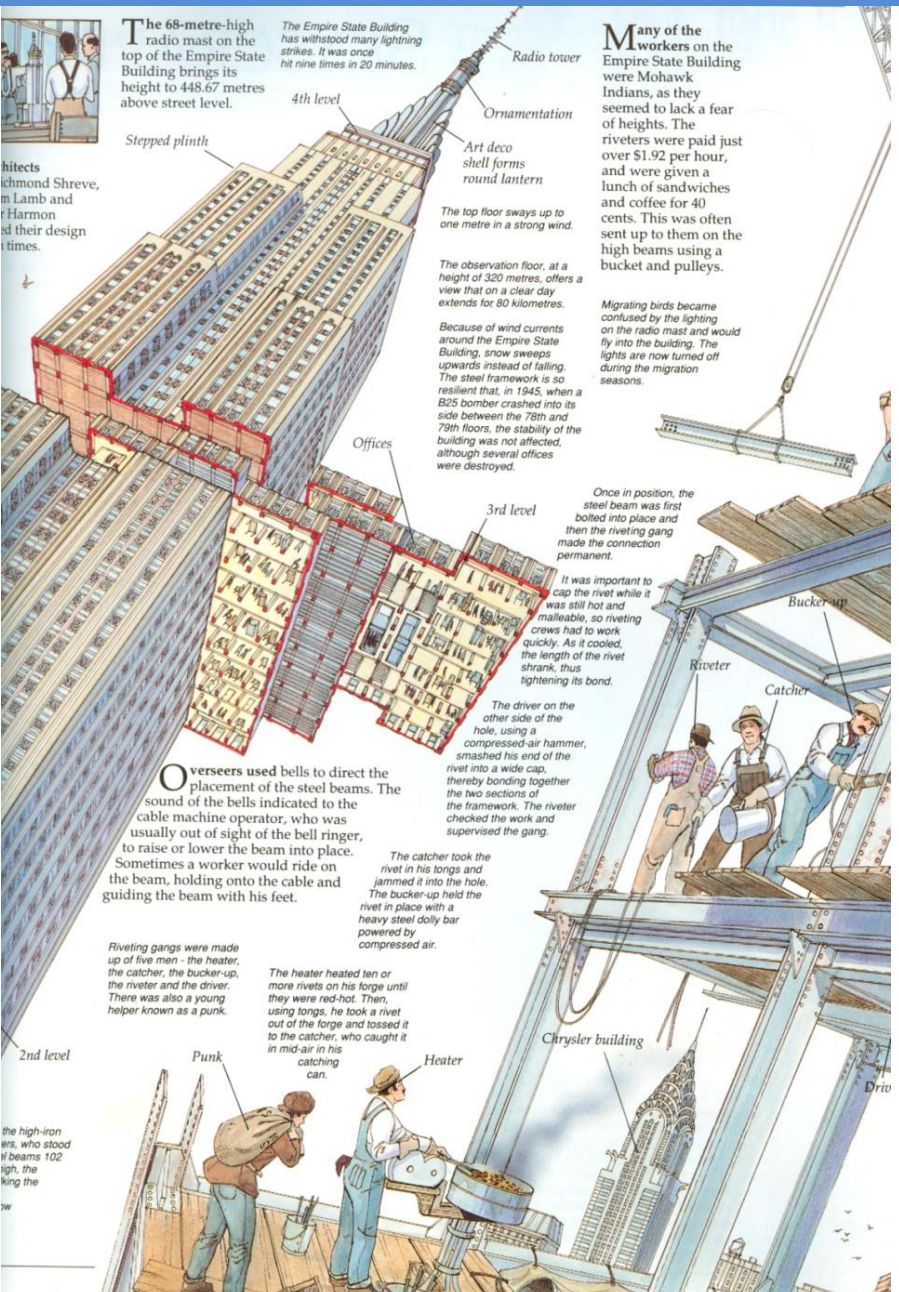


Agent based models
PDEs

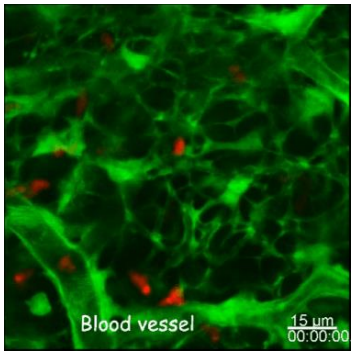


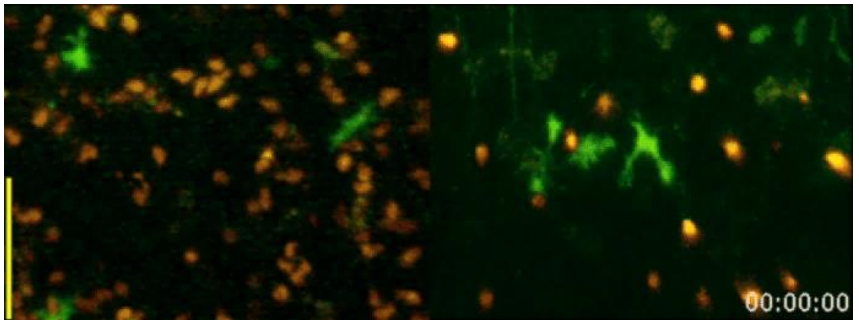
Hybridised Multi-scale Models

Part 2: The biological problem; Immune Function is controlled migration and stochastic cell interactions



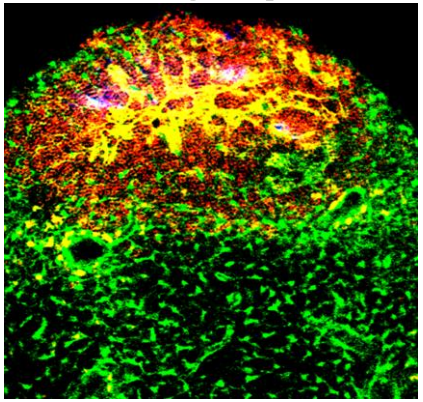
Right Cell, Right Place, Right Time





Structural organisation: Lymph Node Stroma

YFP (MESENCHYME)
CD35
$Wnt1^{cre}Rosa26^{eYFP}$

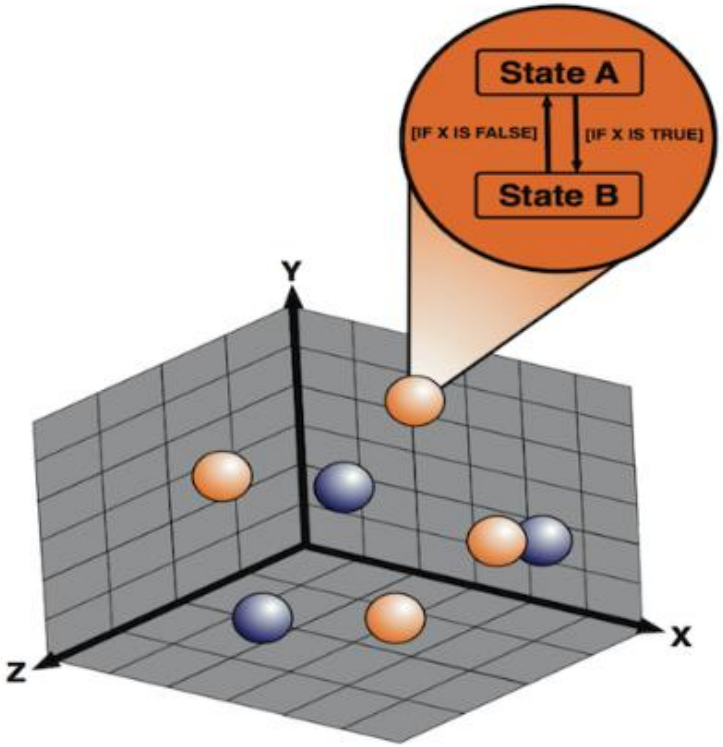


How? Application of multiscale hybrid agent based models to understand immune microenvironments

Agent Based Model (ABM): **A collection of individual entities that interact in and with an environment**

- **Models the components of a complex system**
- **ODE/PDE are used to describe receptor dynamics and cytokine/chemokine diffusion**
- **Behaviours emerge from the models**

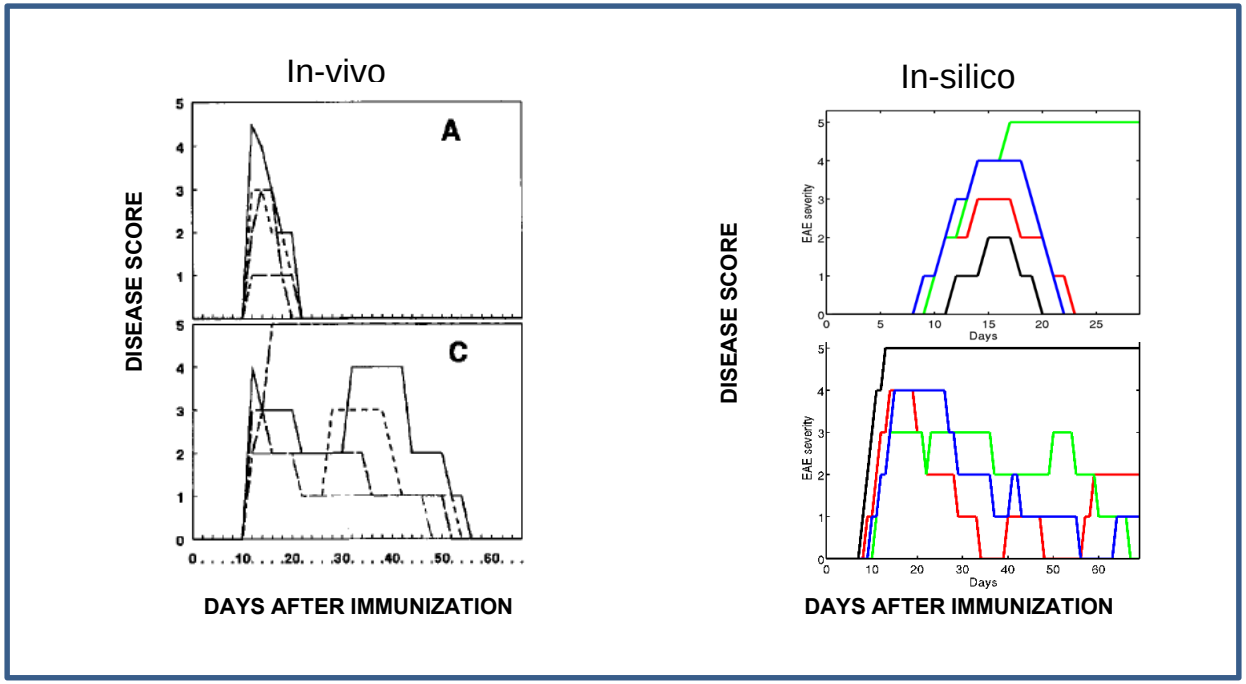
		SPATIALLY RESOLVED	
		NO	YES
CELLULAR HETEREOGENEITY	NO	PBPK ODE	PDE
	YES	State-Based Model	ABM HYBRID-ABM



Independent rule based agents

Agent Based Models (ABM) capture stochastic cell movement and interactions which drive stochastic disease formation

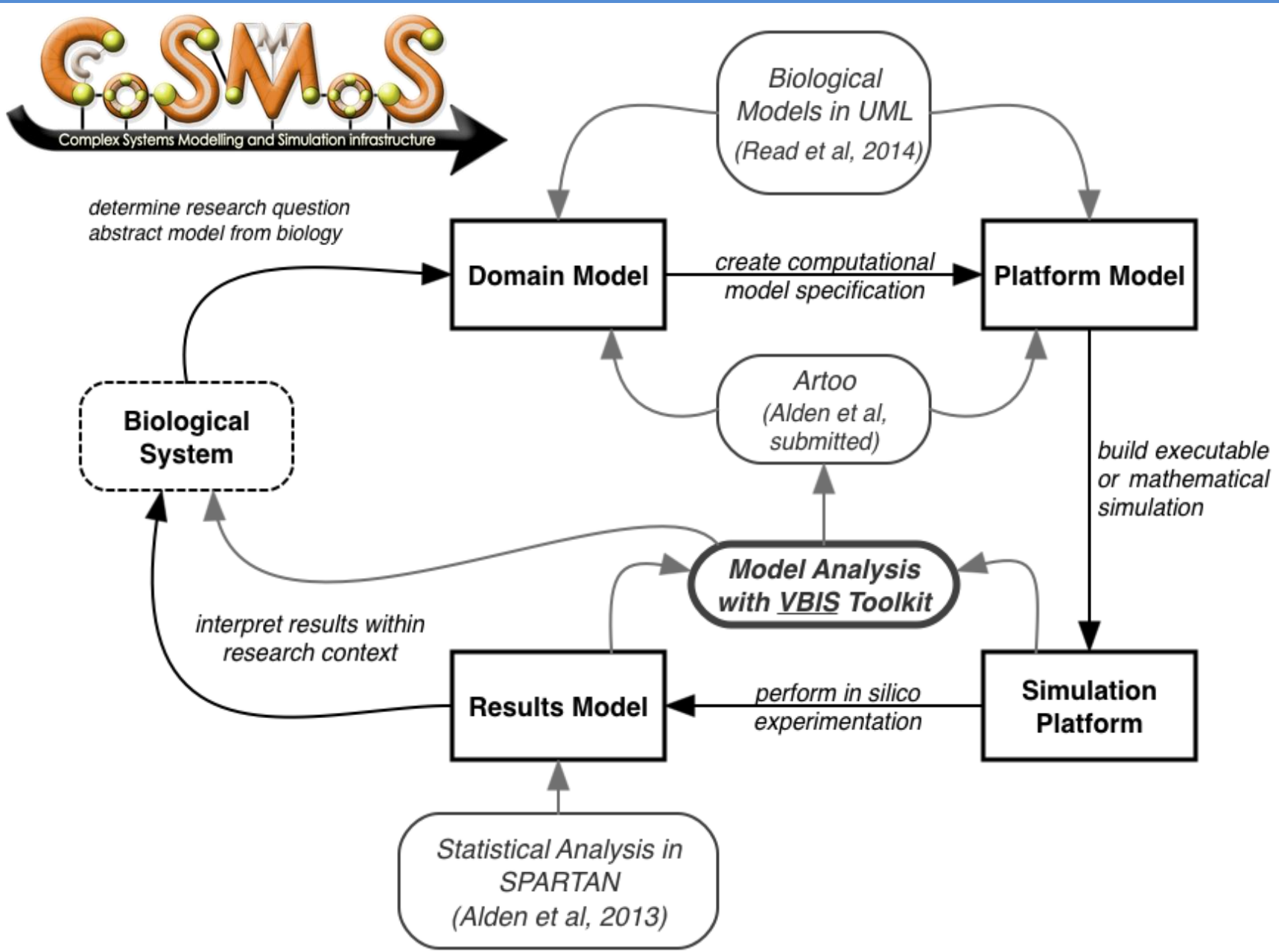
An ABM of Experimental Autoimmune Encephalomyelitis (mouse multiple sclerosis)



- In stochastic computer models different outcomes occur on each simulation run just as observed in mice or humans. This permits analysis of therapeutic intervention. We run thousands of simulations on a cluster.

- However it is important to apply an appropriate technique to an appropriate question, ABMs are useful for certain types of questions, but not others. "Fit for purpose"

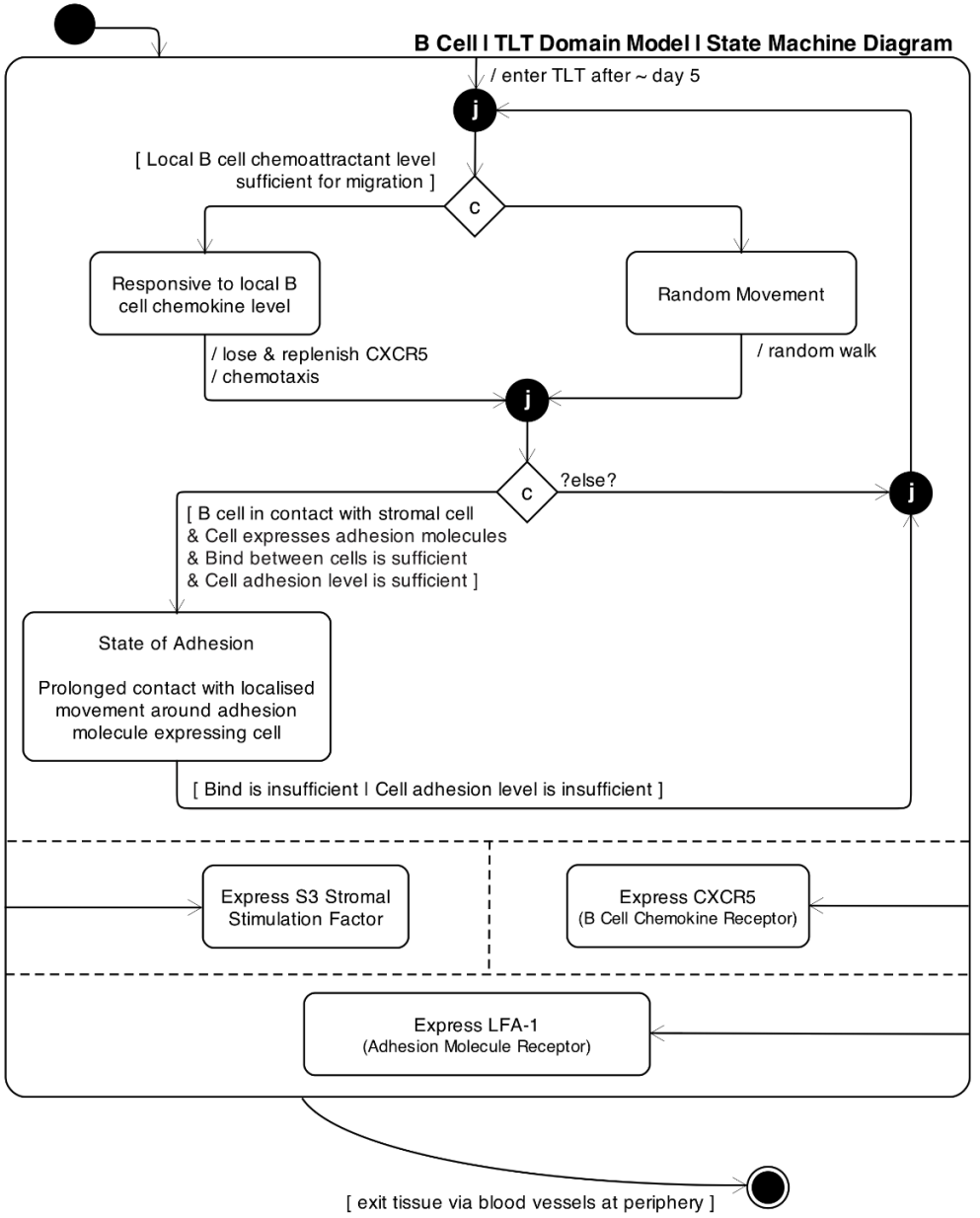
Use a defined process based on engineering principles



UML: Domain Model

Domain State Machines

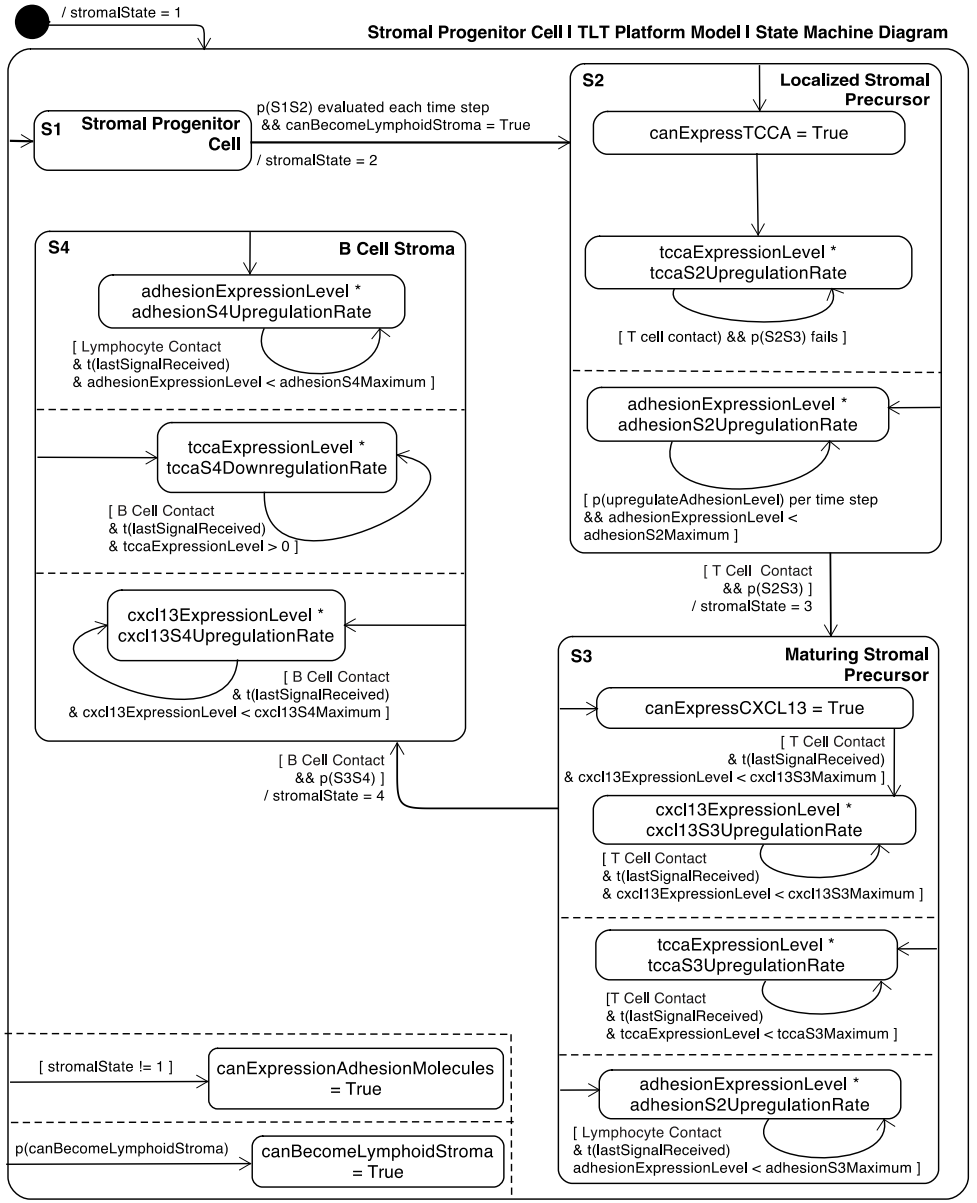
- A ‘domain’ version of each finite state machines defined for each agent in the system
- Defined using the Unified Modelling Language commonly used in software engineering
- Defines what an agent does based on



UML: Platform Model – Defining transition states

Platform State Machines

- A ‘domain’ and ‘platform’ version of each of these finite state machines defined for each agent in the system
- Defined using the Unified Modelling Language commonly used in software engineering
- Defines every possible state and conditions for changing state

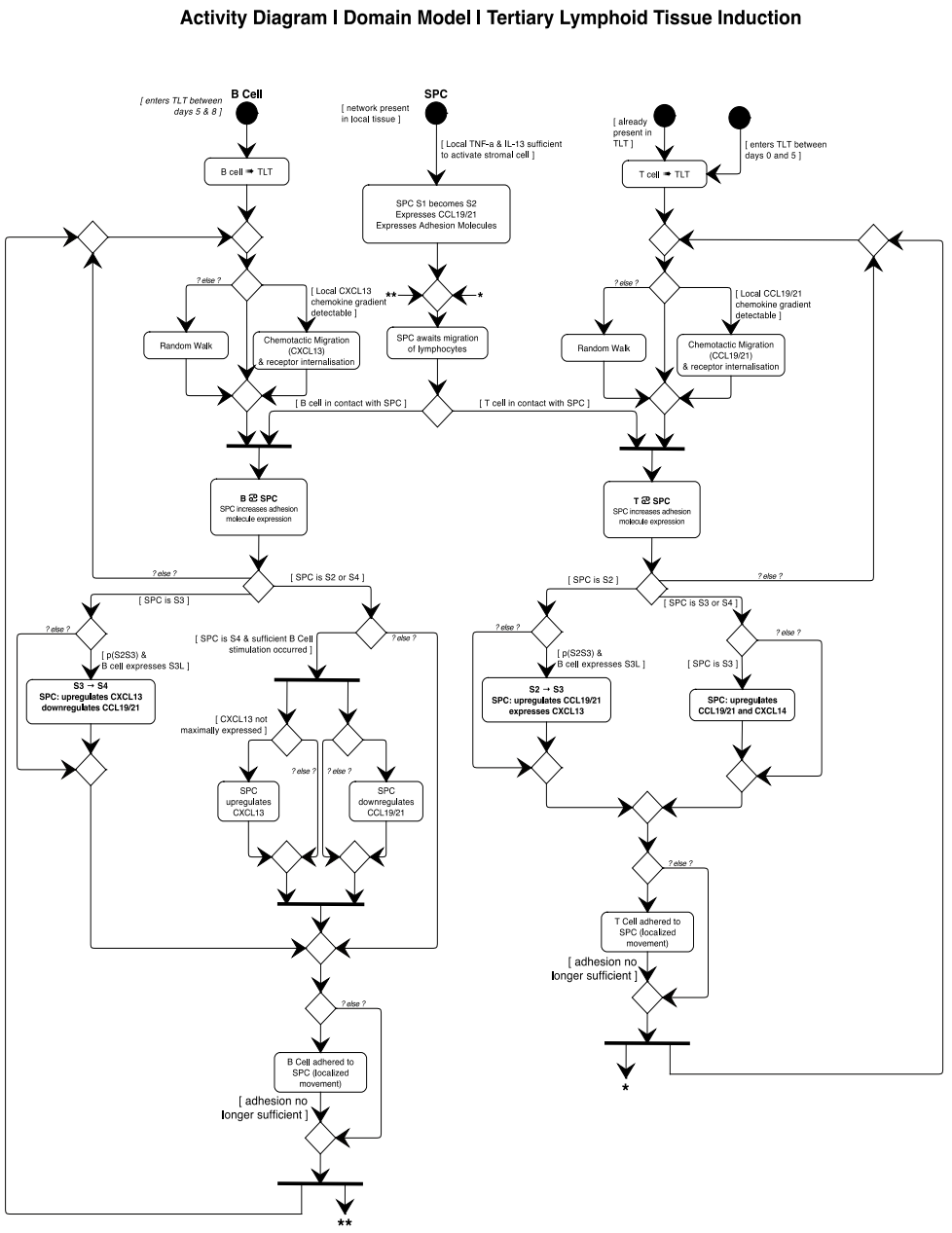


NB: Lymphocyte Cell Contact is true when centre of cell overlaps with any part of the stromal cell

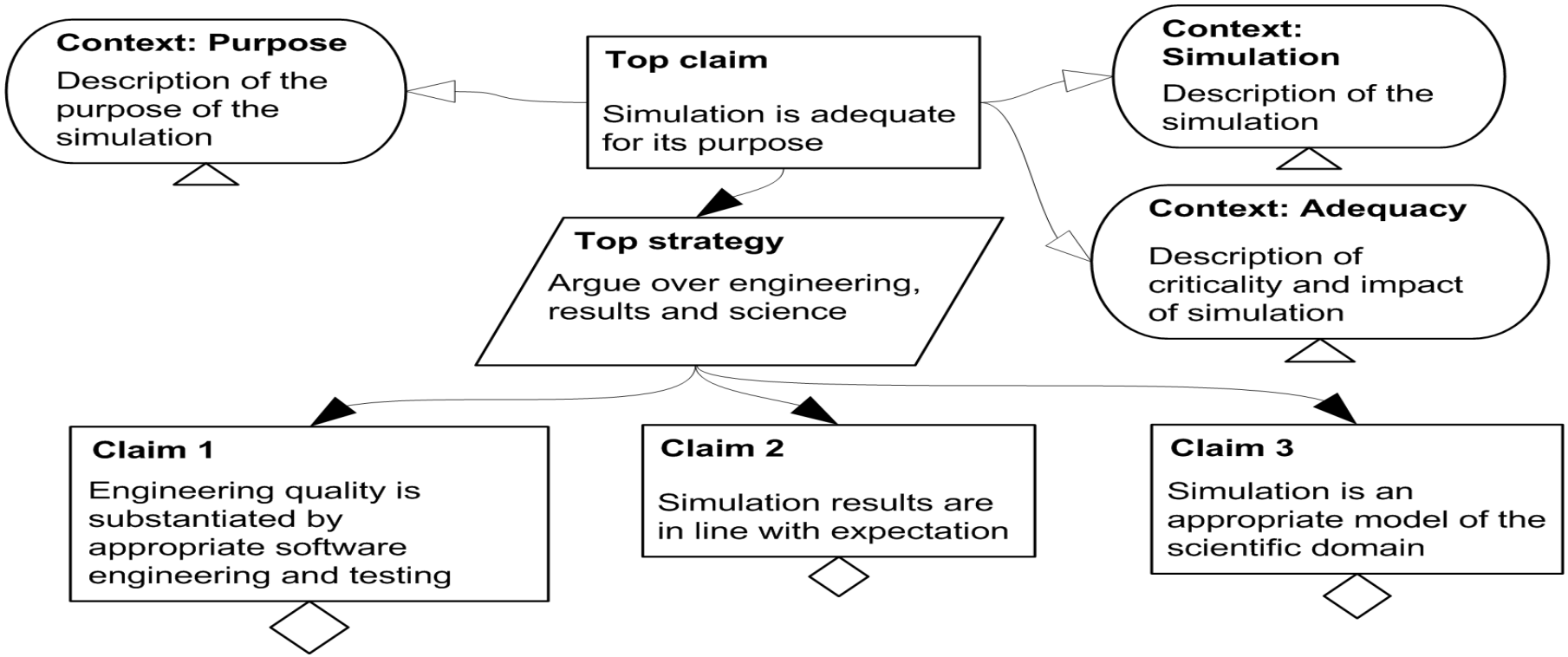
UML: Activity Diagram – Defining the interactions

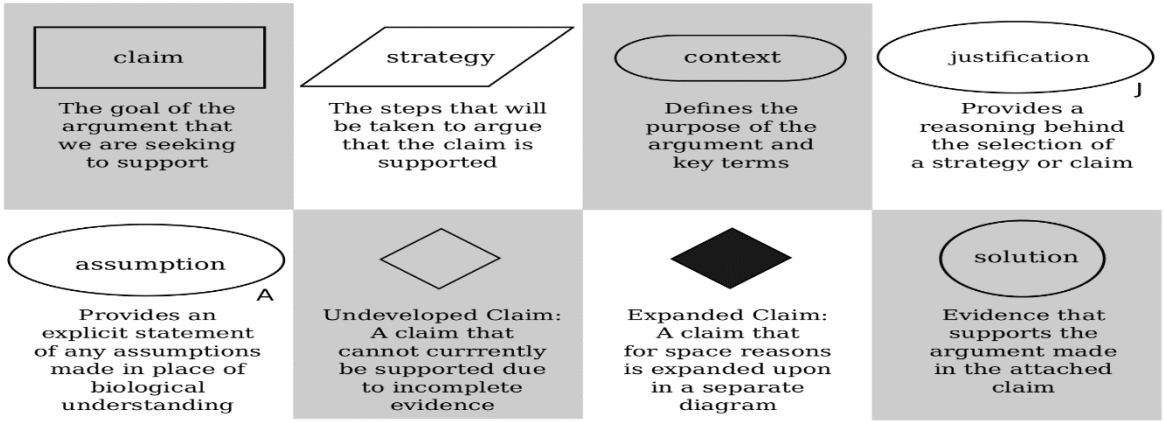
Activity Diagram

- Using the finite state machines, a UML activity diagram is created.
- Defines expected flow of cellular interactions and resultant behaviors.



Confidence and Transparency in Simulations





Defining Arguments

INTERFACE

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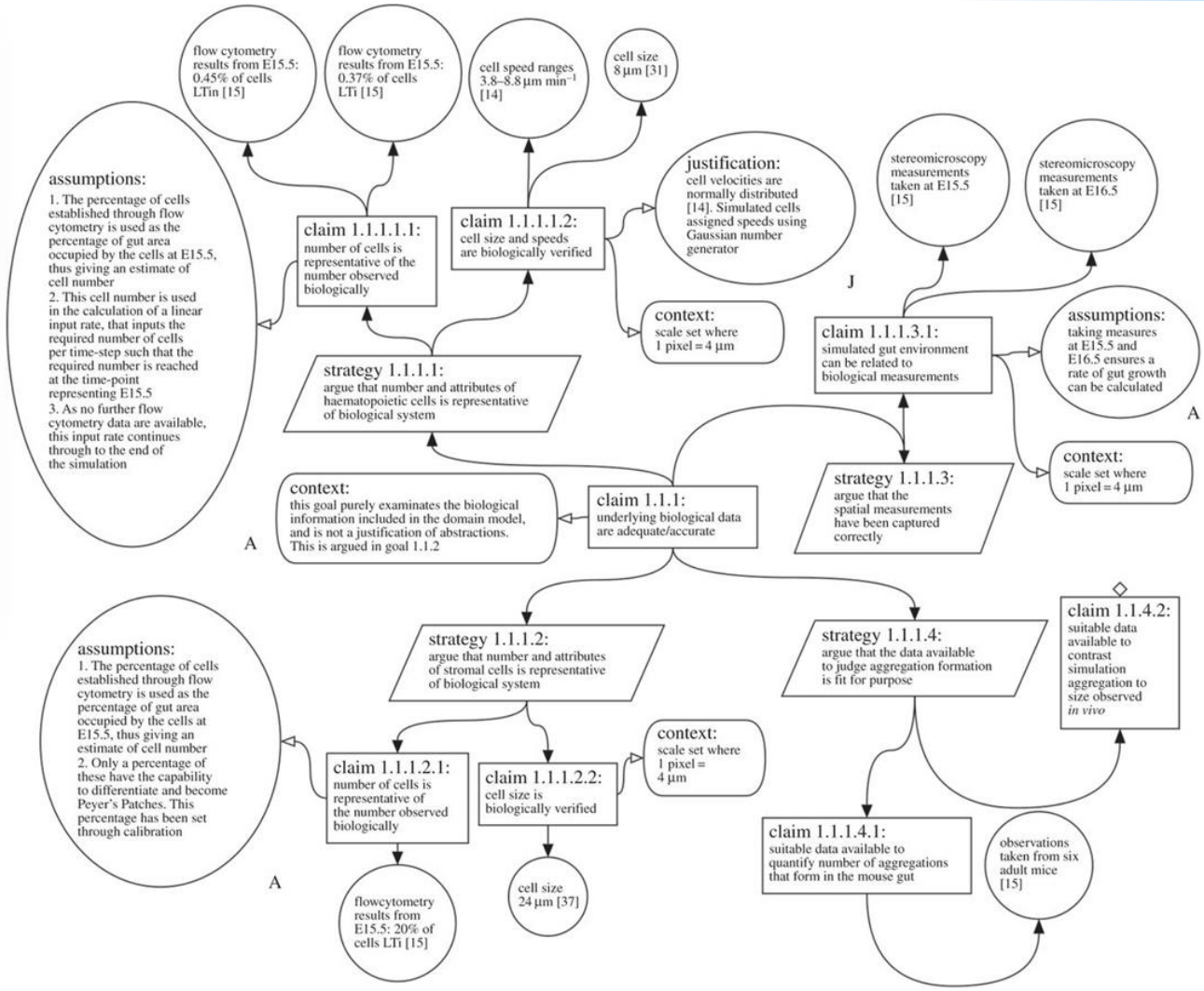
Research

Using argument notation to engineer biological simulations with increased confidence

Kieran Alden[1,2,5], Paul S. Andrews[1,3,4], Fiona A. C. Polack[1,3,4], Henrique Veiga-Fernandes[6], Mark C. Coles[1,2,7] and Jon Timmis[1,5,7]

The *Artoo* Argumentation Tool

File... | Build... | View... | About | Help | Load Example

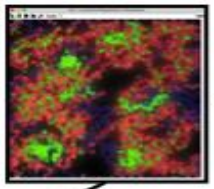


Statistical and Visualization Tools

Virtual Biological Insight into Simulations

Simulation

in silico simulation of biology



transform

VBIS

Generation of novel *in silico* model outputs

Novel Insights

Wet Lab

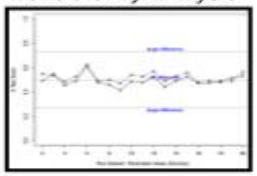
Biological Experimentation

Spartan

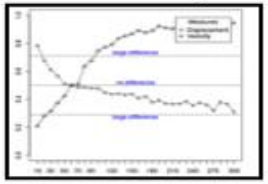
Statistical analysis of *in silico* model outputs

INDIRECT COMPARISON BETWEEN MODEL AND BIOLOGICAL SYSTEM

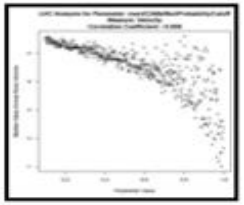
consistency analysis



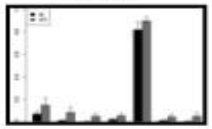
2-output robustness analysis



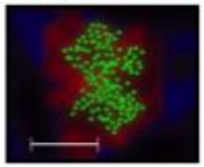
latin hypercube analysis



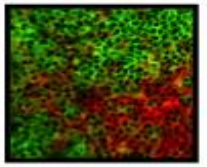
eFAST



v_immunohistochemistry

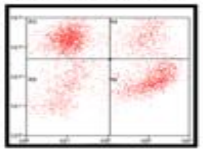


Immunohistochemistry

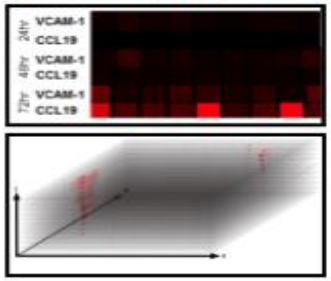


v_flowCytometry

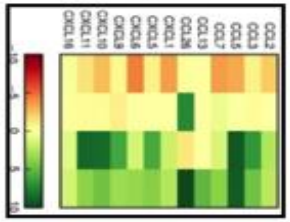
Flow Cytometry



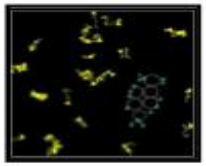
v_sequencing & v_heatmap



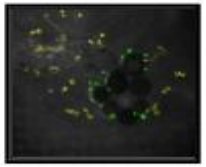
Gene Expression



v_tracking

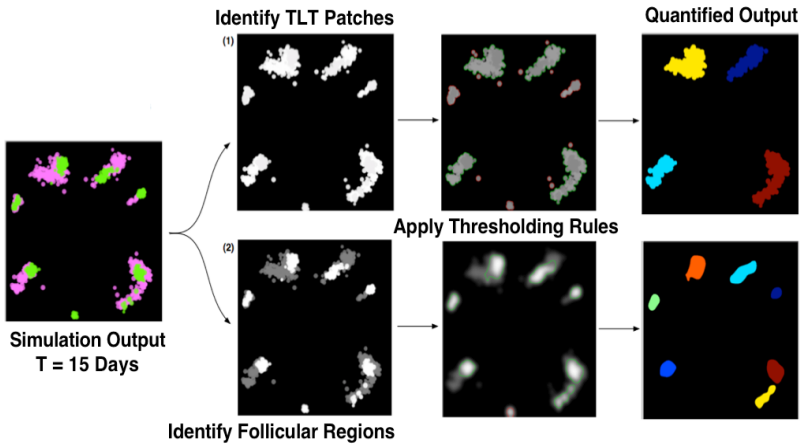


Cell Behaviour/Tracking



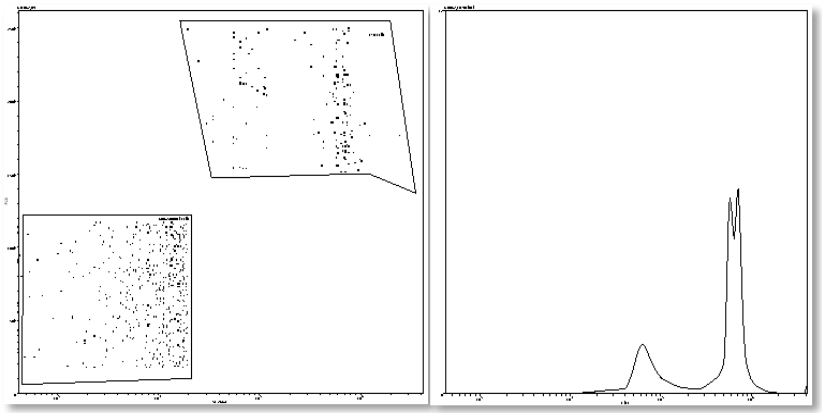
Statistical and Visualization Tools

High Content Analysis of Virtual Histology

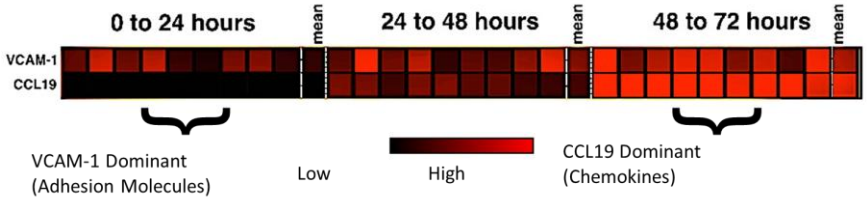


Cell Profiler

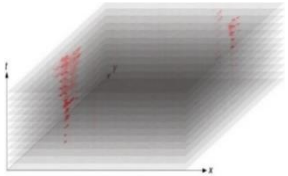
Analysing Models using Flow Cytometry Software



Analysing Models using Heat Maps



3D parameter values over time



Part 3: Applications
Using Agent Based Model to Understand Peyer's Patch Development

Henrique Veiga-Fernandes

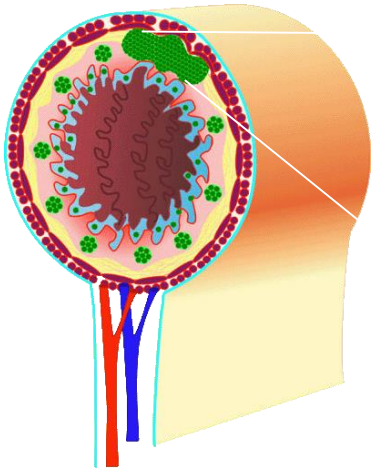
Jon Timmis

Kieran Alden

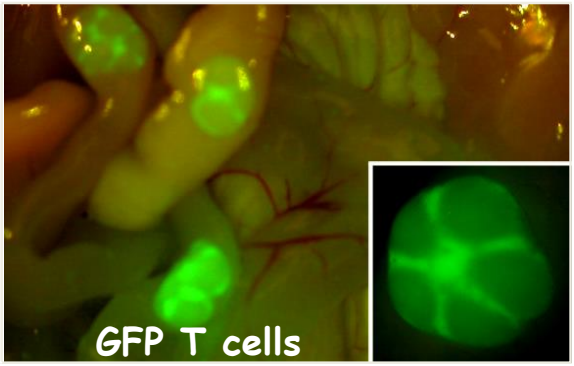
Patel, A, et al. Science Signalling, 2012
Alden, K, et al., Frontiers in Immunology, 2012
Alden, K et a., PLOS Computational, Biology, 2013
Alden, K et al., Natural Computing, 2014

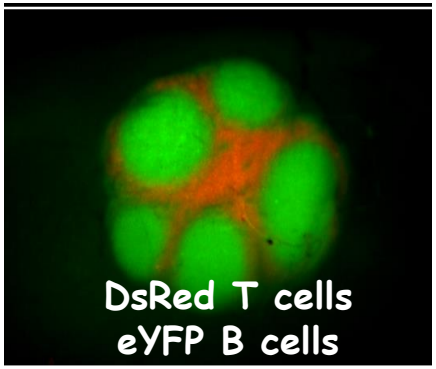
Peyer's Patches Development:

What is the relative role of adhesion and chemokines in the induction and growth of lymphoid tissues during embryonic development?



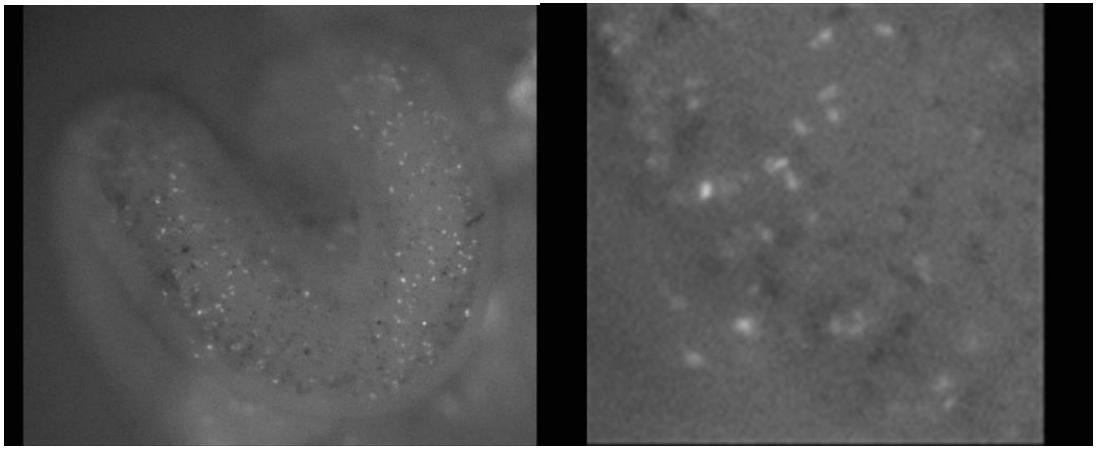
Peyer's Patches



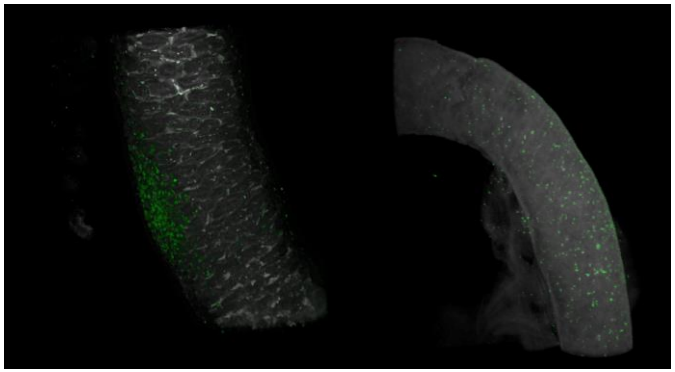


Specialised lymphoid tissue of the intestinal tract

Stochastic Process involving cell movement



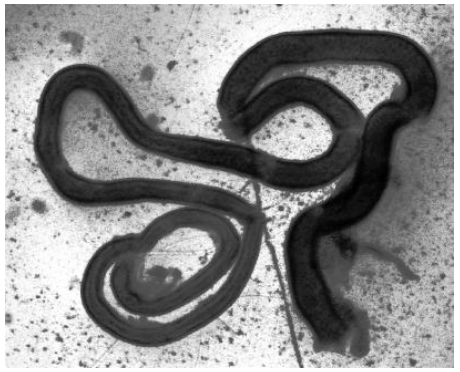
Defined Signalling pathways using genetic mutants



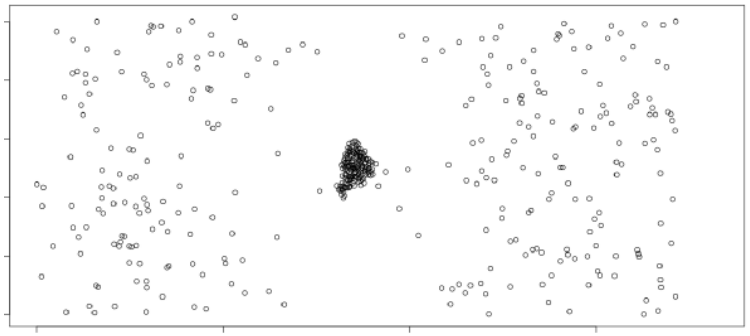
Wild Type Ret Deficient

Coles, et al., PNAS, 2006
Veiga-Fernandes, et al., Nature, 2007

Modelling the Environment



Experimental Measurements Taken:
Measured the length and circumference of the developing mid-gut from 12 embryos using stereomicroscopy (Zeiss) and ImageJ (Fiji)
Averages taken for use in the simulation.
Having both measurements allows for inclusion of growth over the period



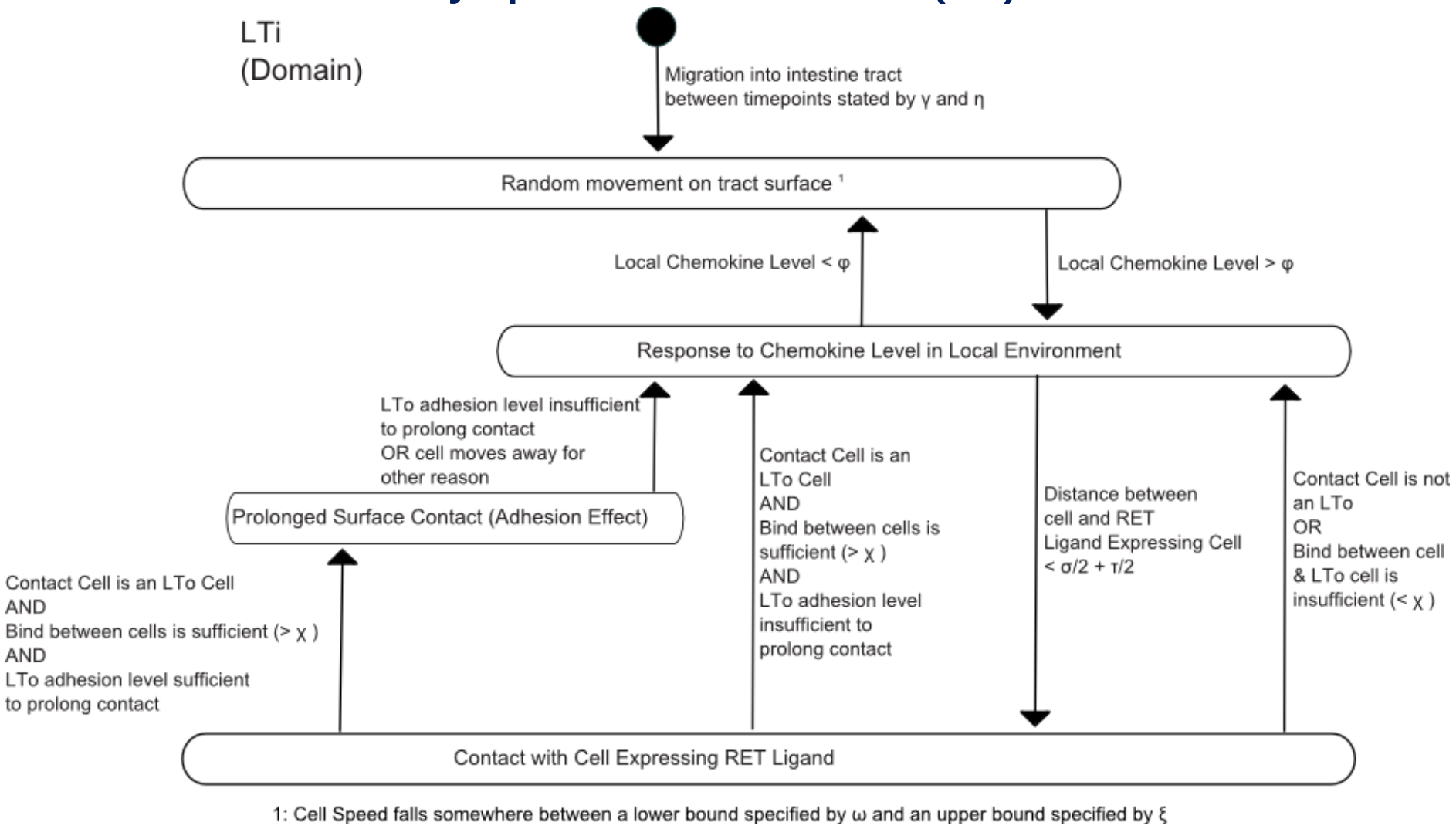
244 pixels -> 0.976mm

7203 pixels -> 28.8mm

Simulation Environment:
1 screen pixel = 4 microns, Length and width represent the gut measurements taken.
Cells that leave top or bottom appear on opposite side. Cells that leave left and right are deemed to take no more part in the simulation

Turning Cells into Agents

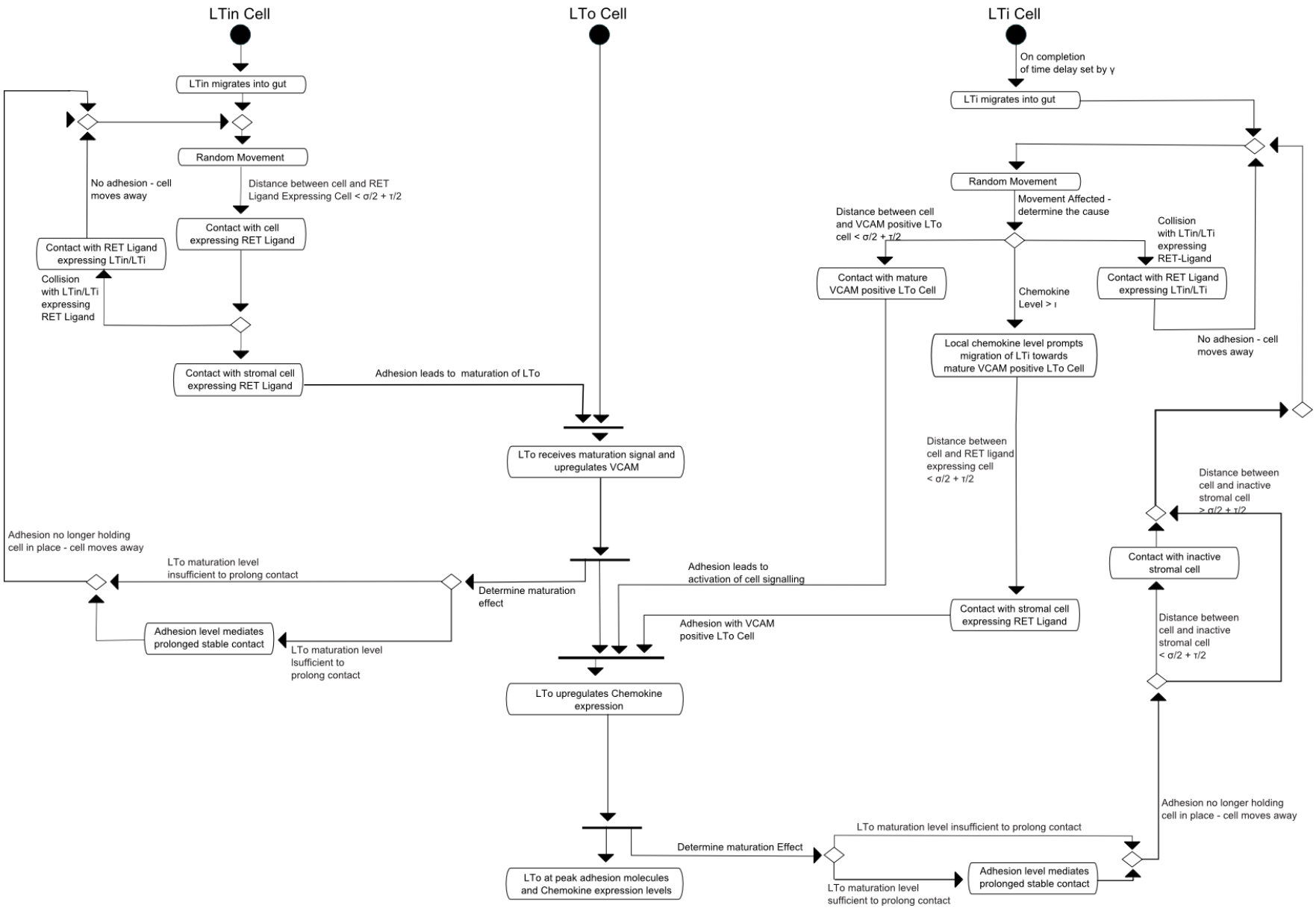
Innate Lymphoid Cell
Lymphoid Tissue inducer (LTi)



1: Cell Speed falls somewhere between a lower bound specified by ω and an upper bound specified by ξ

Alden et al., Frontiers in Immunology, 2012

Simulating Peyer's patch formation

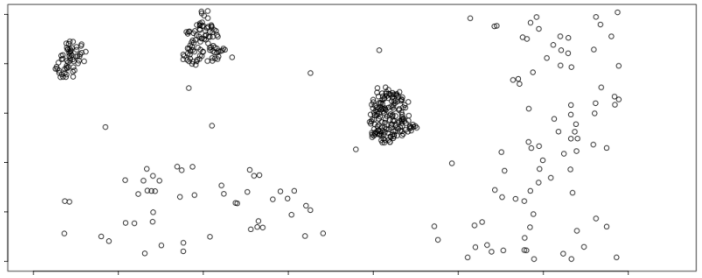


Agent Based Model of Peyer's Patch Development

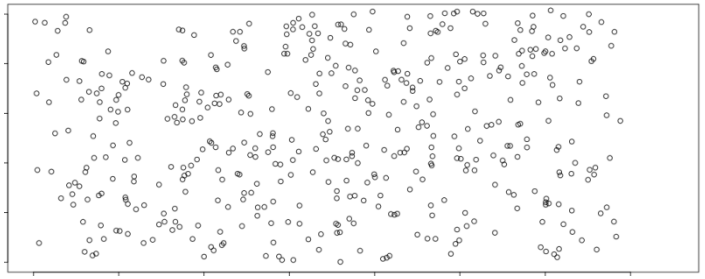
- The ABM had explicit representation of the different cell types involved in Peyer's patch development. The simulation was able to capture the heterogeneous and stochastic cellular behaviours that led to patch formation.
- Why was an ABM the most appropriate approach?
  - Can handle large numbers of individual agents and variables simulating real world like levels of complexity, including incorporating time and space, two key parameters in biology.
  - Importantly we could recreate all the cells in the system, there is a 1:1 correlation between biology and the simulation
  - Peyer's patches are highly stochastic in their development
  - Development occurs in a 2D plane (biologically not always the case)

Simulation predicts Peyer's patch formation

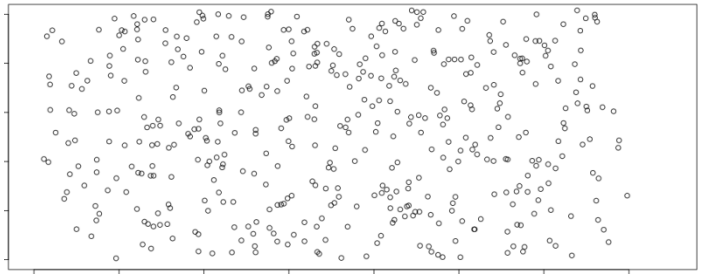
Wild type



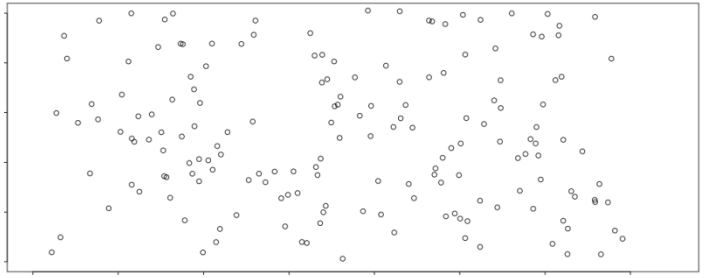
RET$^{-/-}$



CXCL13$^{-/-}$, CCL19/21$^{-/-}$

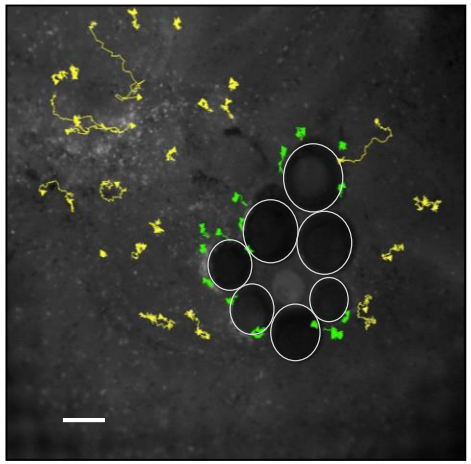


ROR$\gamma^{-/-}$

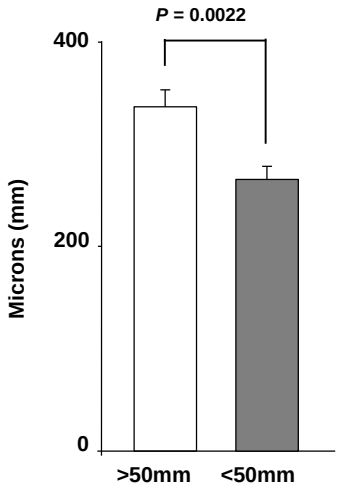


Simulation Predicts Cellular Behaviour

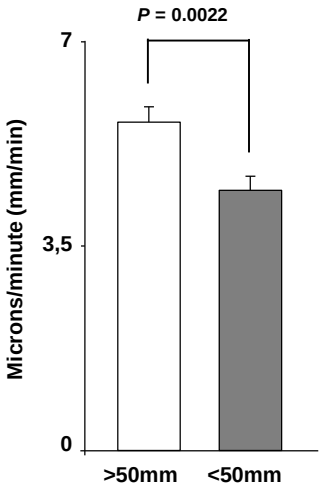
Ex vivo explant culture



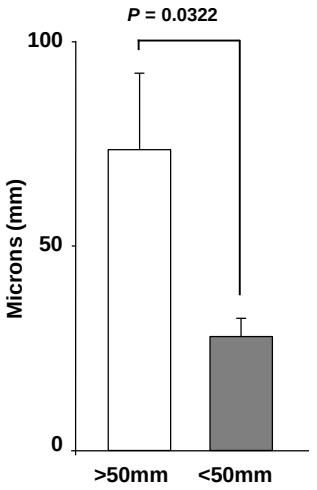
Length



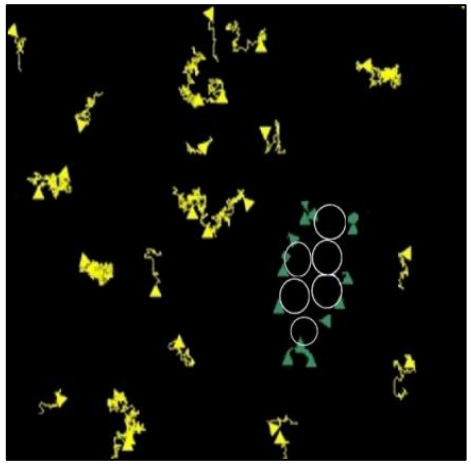
Velocity



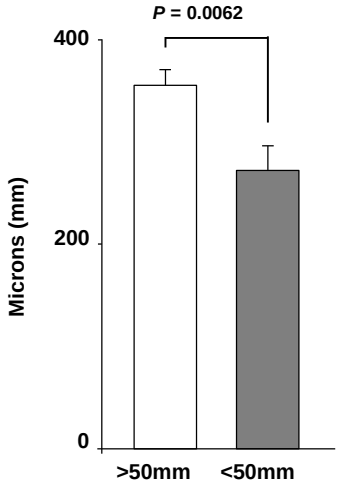
Displacement



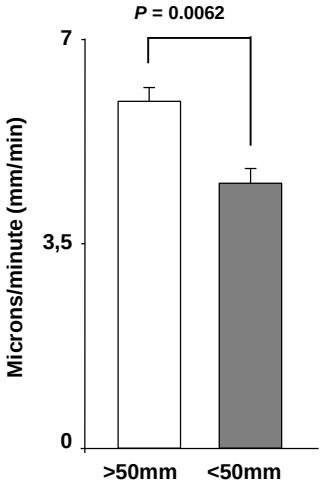
In silico simulation



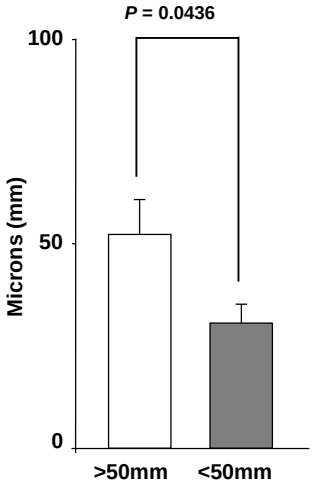
Length



Velocity



Displacement

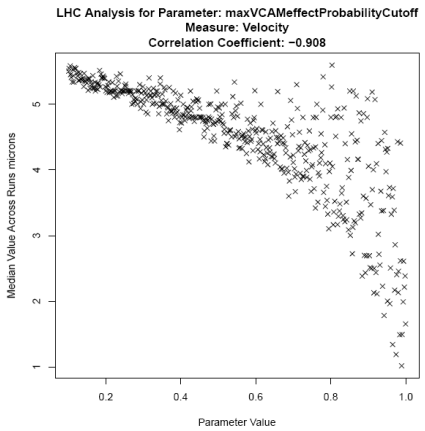


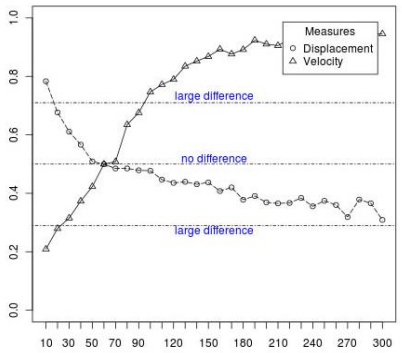
Patel et al., Science Signalling, 2012

Two Step Model of Lymphoid Tissue Induction

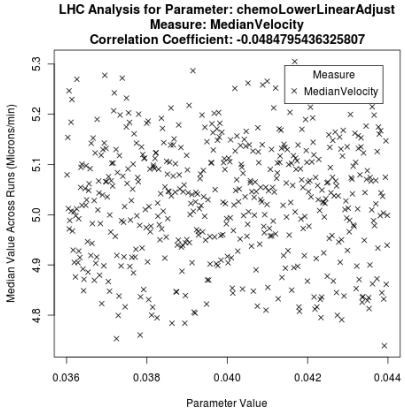
12-13hr time point
Trigger stage

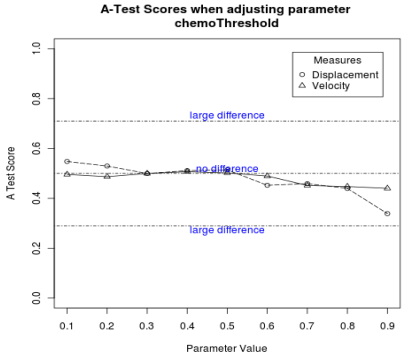
VCAM-1





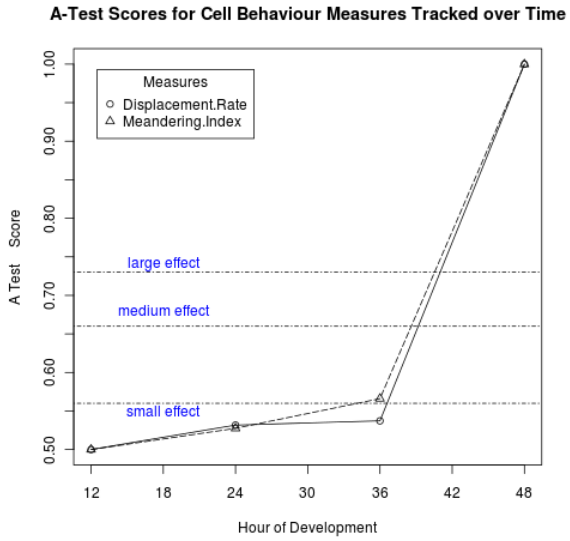
Chemokines





Parameter Value

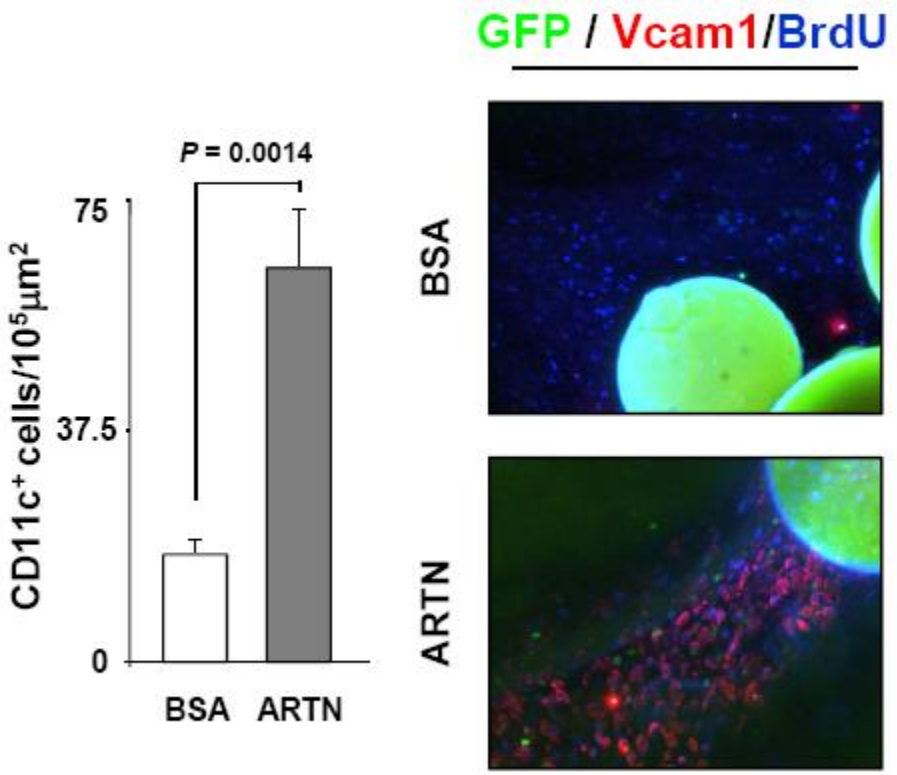
Chemokines are important at later stages in Peyer's patch formation



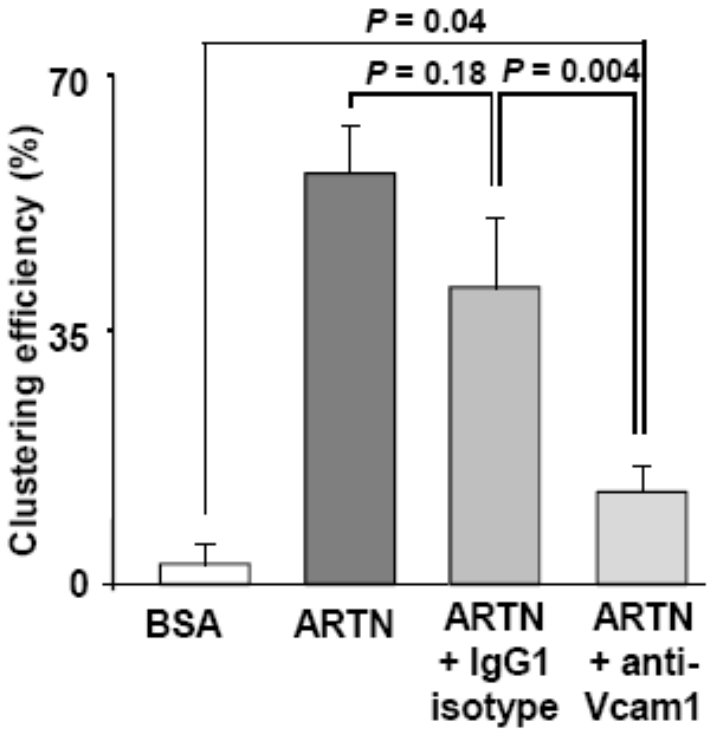
Patel et al., Science Signalling, 2012

Testing Predictions Experimentally

VCAM-1 cells proliferate



Blocking VCAM-1 inhibits LTin cell clustering



Patel et al., Science Signalling, 2012

Using Multi-scale Model to Understand Tissue Pathology

Christopher Buckley

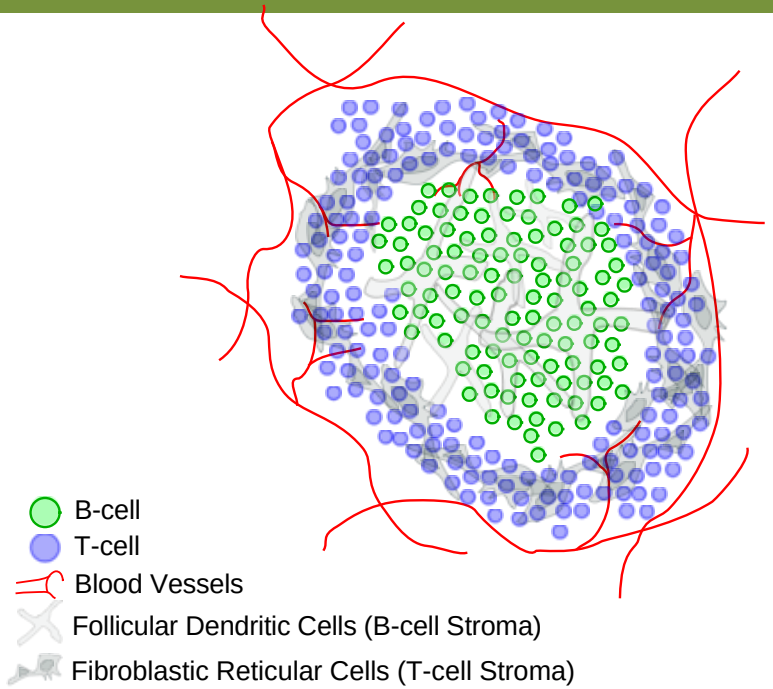
Jon Timmis

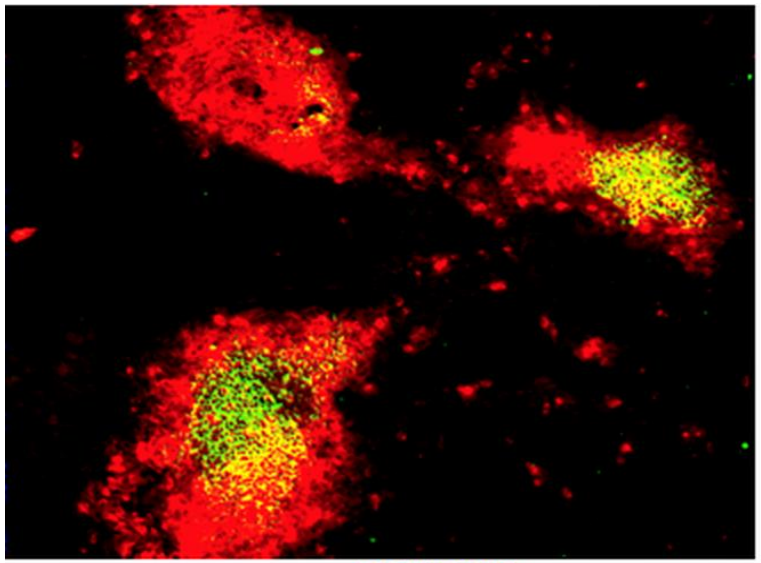
James Butler

Tertiary Lymphoid Tissue formation in Sjögren's syndrome

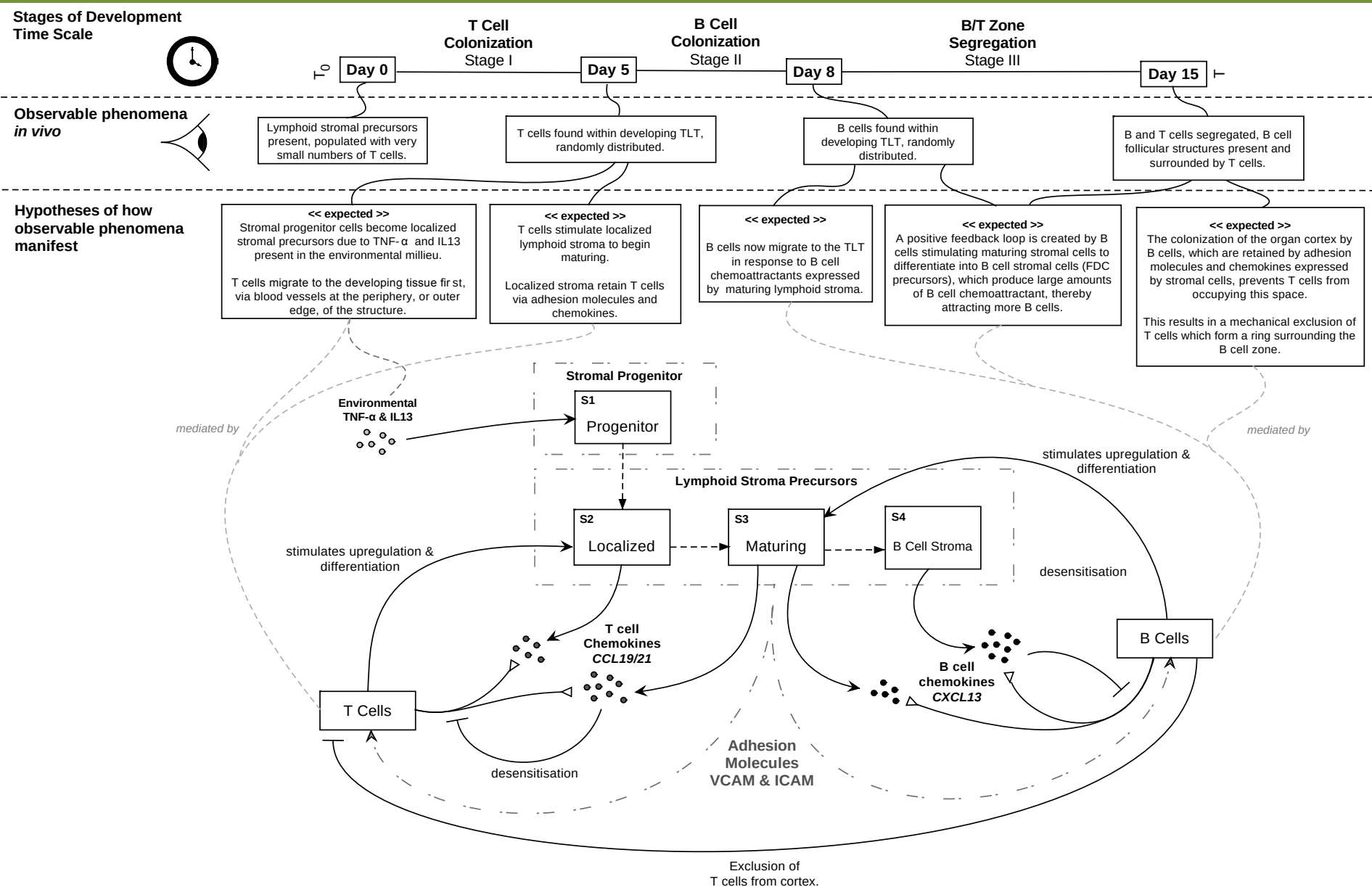
Tertiary Lymphoid Tissues in Autoimmune Pathology

- During autoimmune disease, lymphoid tissues can form where they are not normally found (Tertiary Lymphoid Tissue) they are involved in the pathology.
- Experiments suggest TLTs self-organise through complex interactions involving two inflammatory cytokines and lymphocytic infiltration.
- It is unclear if a simple induction loop model of tissue formation is sufficient to explain the emergence of TLT in autoimmune pathologies. Our model aims to test this.

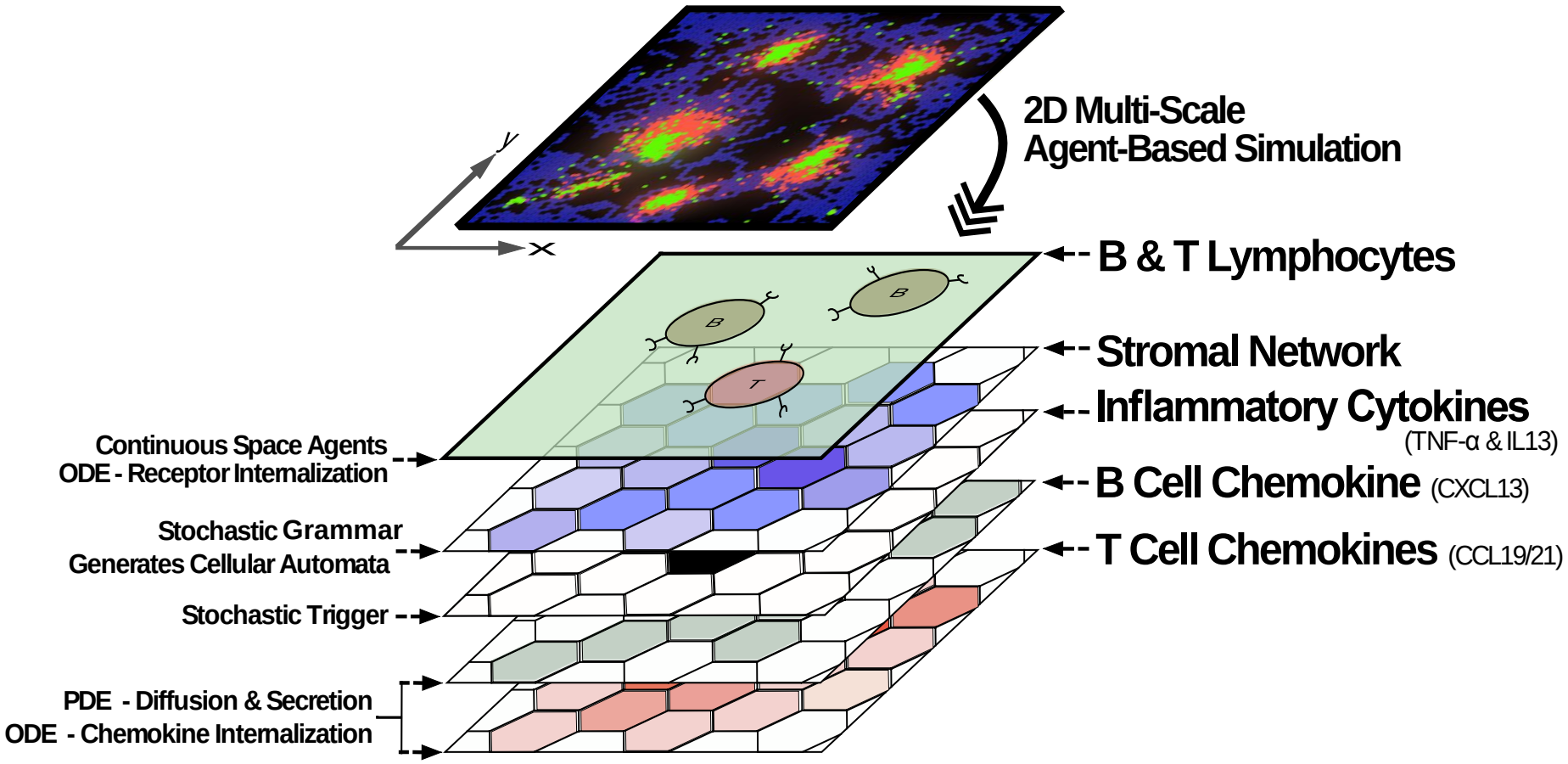




Hypothesis: T cell and B cell amplification loop driven by localised IL13 and TNFα is sufficient to explain highly organised tertiary lymphoid tissue formation and high affinity autoantibody response.



The Multi-scale Computational Model: An Overview



A multi-scale hybridised agent-based model incorporating cellular automata, generative grammar, PDEs, ODEs and Monte Carlo methods.

Implementing the Simulation

- **Programmed in Java using MASON Toolkit.**
- **Integrates Generative Grammar, Agent-Based Model, ODEs, PDEs, Monte Carlo methods & Cellular Automata into one hybrid multi-scale model.**
- **Simulation has independent visualisation layer, developed to generate images comparable to histology or confocal microscopy images for validation & experimentation.**

Calibrating Simulation vs. Observed Phenomena

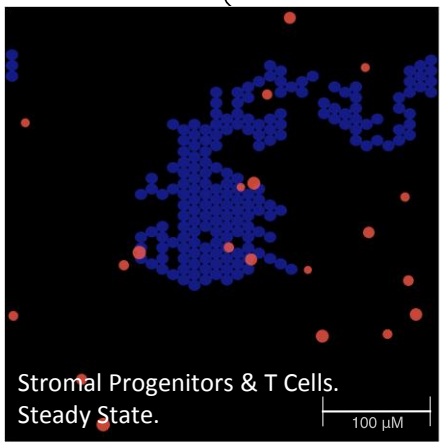
Parameter Set 002

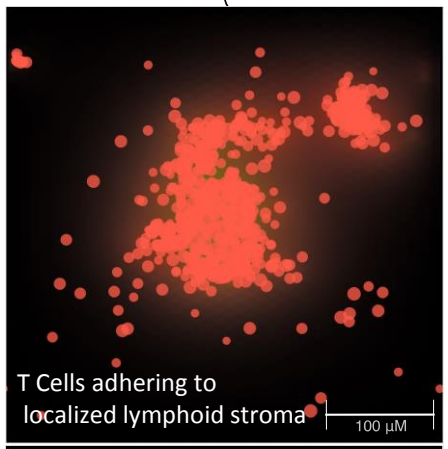
Stages of Development
Time Scale

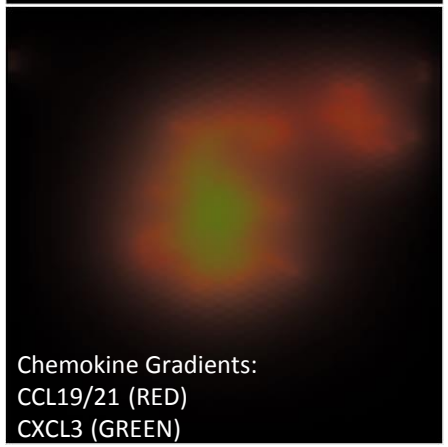
T_0 — **Day 0** **0 TS** — **T Cell Colonization** Stage I — **Day 5** **720 TS** — **B Cell Colonization** Stage II — **Day 8** **1152 TS** — **B/T Zone Segregation** Stage III — **Day 15** **2160 TS**

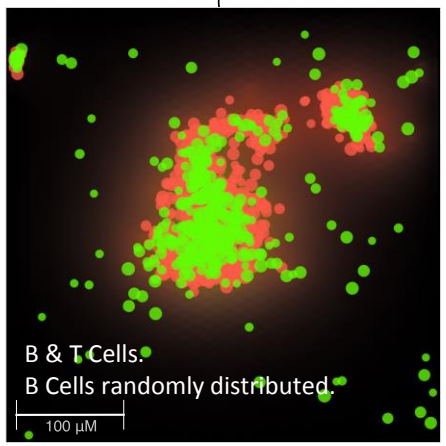
Observable phenomena ***in vivo***

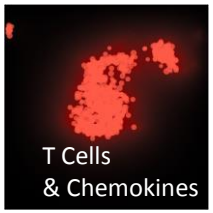
- Lymphoid stromal precursors present, populated with very small numbers of T cells.
- T cells found within developing TLT, randomly distributed.
- B cells found within developing TLT, randomly distributed.
- B and T Cells segregated, with tendency for B cells to form clusters surrounded by T Cells.

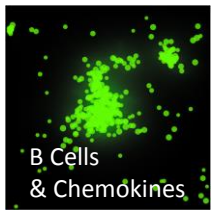


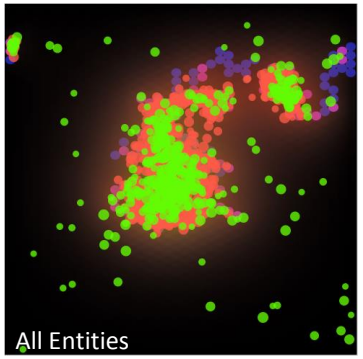


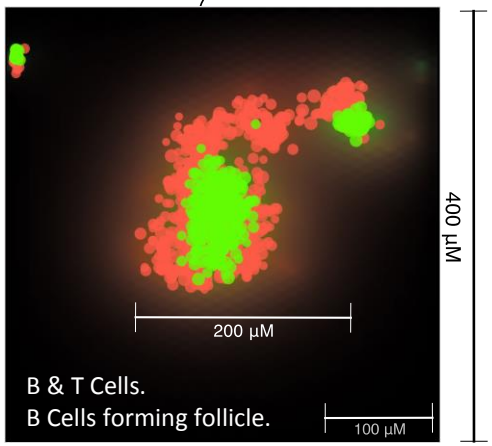


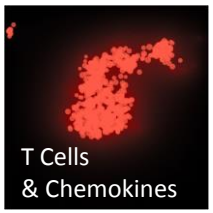


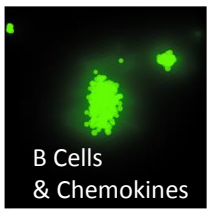


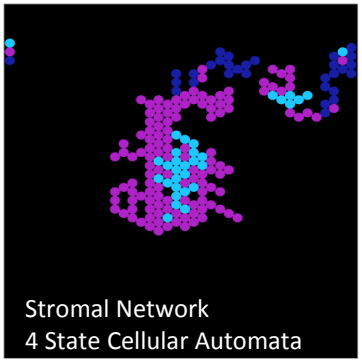








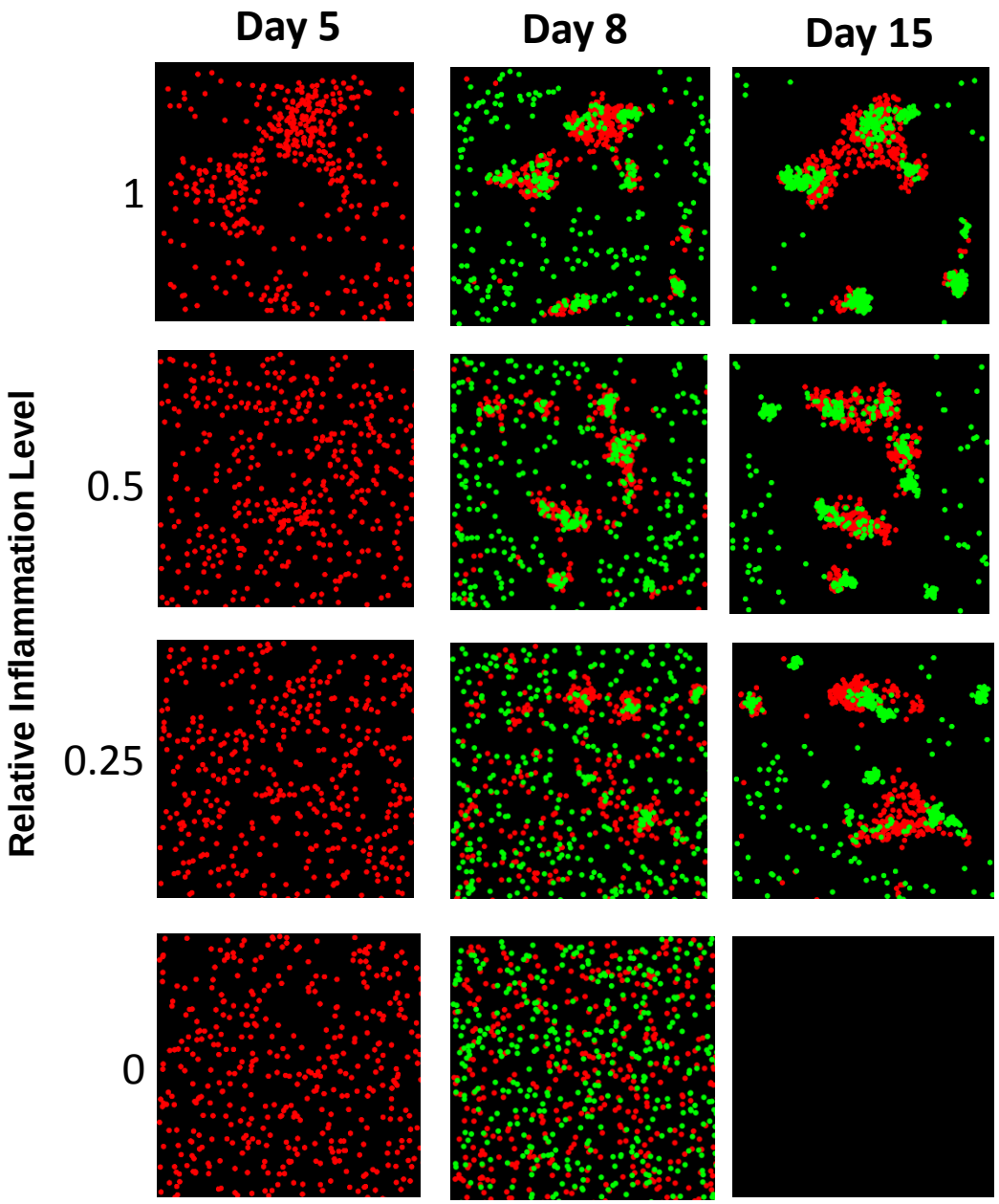




Key

- Stroma
- FRC
- FDC
- B cell
- T cell

Effect of TNFα/IL13 Reduction on TLT Formation



- Demonstrates *critical* timing of dose
- Reducing levels of inflammatory cytokines TNFα/IL13 seeded into environment over first 5 days has significant effect on TLT induction.
- Less inflammation results in less organized B Cell follicular zone.
- This could be considered an abstraction for administering anti-TNFα mAbs or looking at KO mice.
- **This brings us one step closer to designing or testing drug regimes *in silico* – esp. biologics.**

Multiscale Model of Tertiary Lymphoid Tissue Formation

- **The multi-scale model was sufficient . It supported two key findings of Peyer's patch development:**

1) ***Adhesion is important in triggering but not important for the growth, in contrast chemokines are key for the growth but not triggering of structure***

2) ***A two step mechanism provided by two distinct cell types is required for triggering vs. growth (PP: LTin & LTi)(TLT: Epithelium, Lymphocytes)***

- **Why was a multi-scale model approach:**
  - **Although an ABM can handle large numbers of individual agents and variables simulating real world like levels of complexity, ODE/PDEs are key to including key signalling events that occur within and between individual agents. This is key to replicating biology.**
  - **TLT formation is highly variable in size and composition.**

Leishmania Simulation:

Virtual Infectious Disease Laboratory

Exemplar Project: LeishSim

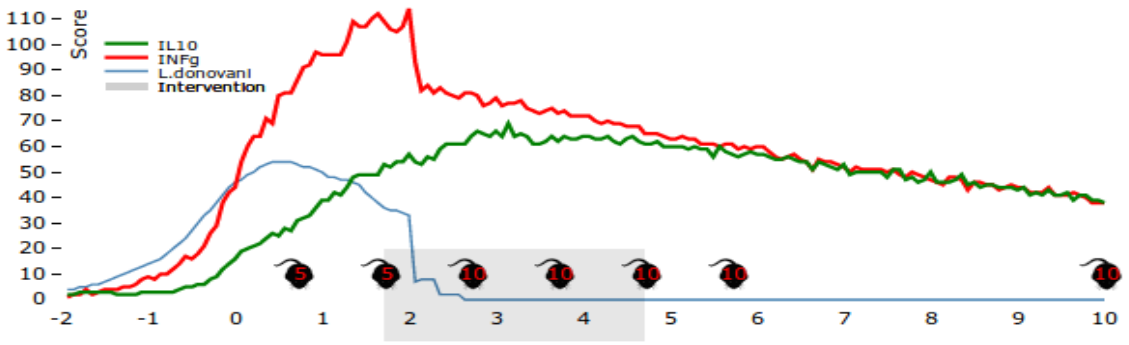
Sim Omics Evidence

LeishSim 1.1

Virtual Lab powered by SimOmics

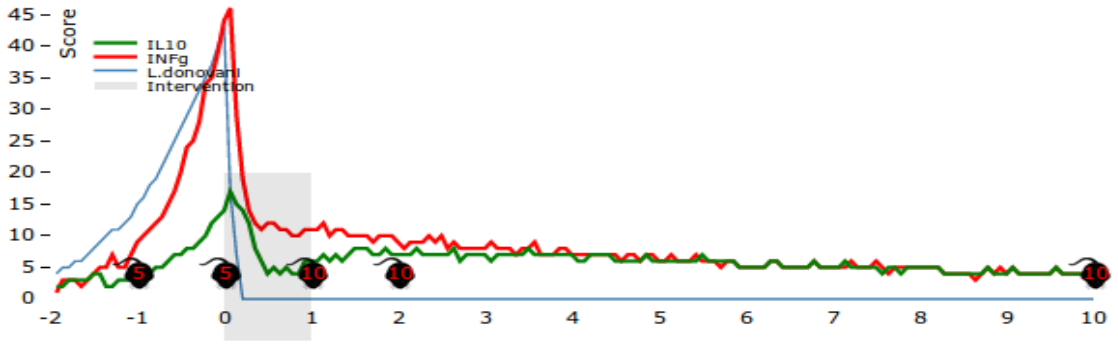
Home | Intervention | Simulation | Stats | Biomarkers

Host:
Species: Mus musculus
Strain: wild type
Compartment: Liver
Parasite:
Species: L.donovani
Strain: Lab adapted
Intervention:
immune null: 75 mg/kg, every 48 hours, from day 12 to day 33



Intervention 1

Host:
Species: Mus musculus
Strain: wild type
Compartment: Liver
Parasite:
Species: L.donovani
Strain: Lab adapted
Intervention:
immune null: 60 mg/kg, every 8 hours, from day 0 to day 7



Submit intervention to Virtual Lab

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CRACK IT

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

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