



Systems Biology Symposium

University of Pennsylvania

June 23, 2009

Computational Systems Biology Short Course

June 24, 2009

Levine Hall, Wu and Chen Auditorium

Philadelphia, PA



Day 1, June 23, 2009: Wu and Chen Auditorium

9:30 – 10:00 am	Coffee, Levine Hall Lobby
10:00 – 10:30 am	Stochastic Sampling— A Systems Approach for Discovering Transcriptional Heterogeneities in Tissues Kevin Janes , Ph.D. Biomedical Engineering, University of Virginia
10:30 – 11:00 am	Endocytosis and Signaling: Regulation at Multiple Scales Ravi Radhakrishnan , Ph.D. Department of Bioengineering, University of Pennsylvania
11:00 – 11:30 am	Blood Systems Biology: Patient-Specific Prediction of Thrombosis Scott L. Diamond , Ph.D. Institute for Medicine and Engineering, University of Pennsylvania
11:30 am – 12 noon	Computational Models of the Evolution of Metastasis Carlo C. Maley , Ph.D. Molecular and Cellular Oncogenesis Program, The Wistar Institute, University of Pennsylvania
12:00 – 1:30 pm	Lunch and Poster Session, Levine Hall Reception Area
1:30 – 2:00 pm	Multi-Scale Modeling of Neuronal Adaptation Mediated by Angiotensin II in the Central Regulation of Blood Pressure Rajanikanth Vadigepalli , Ph.D. Pathology, Anatomy and Cell Biology, Thomas Jefferson University
2:00 – 2:30 pm	Minimal Models of Extrinsic Regulation in Cell-Fate Decisions Casim Sarkar , Ph.D. Department of Bioengineering, University of Pennsylvania
2:30 – 3:00 pm	Student Abstract Winners (to be named)
3:00 – 4:00 pm	Cell Signaling in Equations and Embryos Stanislav Shvartsman , Ph.D. Lewis-Sigler Institute for Integrative Genomics, Princeton University
4:00 – 4:30 pm	Reception, Levine Hall Reception Area

Day 2, June 24, 2009: 100B Moore Hall

9:30 – 10:00 am Coffee, Levine Hall Lobby

10:00 – 12:00 noon Bottom-up Simulation: SBML

Jeremy Purvis

12:00 – 1:00 pm Lunch, Levine Hall Reception Area

1:00 – 3:00 pm Top-Down Simulation: Neural Networks

Jeremy Purvis

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Stochastic Sampling— A Systems Approach for Discovering Transcriptional Heterogeneities in Tissues

Cell-to-cell variations and asymmetries in gene and protein expression play an important role in the development and function of many tissues. Studying the mechanisms that give rise to such heterogeneities is an active area of research in systems biology. But, how do we identify the heterogeneities in the first place? In this talk, I will present a new technique (called “stochastic sampling”) that attempts to address this general problem. Stochastic sampling involves the repeated, random selection of very-small cell populations (~10 cells) followed by quantitative gene-expression profiling and simple statistical analysis. Comparing how transcripts are distributed across repeated samplings distinguishes acute molecular heterogeneities from biological noise. We combine laser-capture microdissection, a customized single-cell amplification protocol, quantitative PCR, and oligonucleotide microarrays to implement stochastic sampling in an in vitro model of mammary-gland morphogenesis. The distinct gene-expression patterns predicted by stochastic sampling were verified in situ, identifying coregulated gene clusters that were completely obscured at the population level. Genetic perturbation of several heterogeneities causes profound changes in morphogenesis, suggesting that the endogenous patterns are critical for normal development and maintenance of glandular structures. Stochastic sampling provides an important first step toward identifying the mechanisms of cell-to-cell variation in both normal physiology and disease.

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Endocytosis and Signaling: Regulation at Multiple Scales

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To gain valuable insights at microscopic and mesoscopic scales we have developed theoretical and computational platforms for quantitatively describing how cell-membrane topologies are actively mediated and manipulated by intracellular protein assemblies. Specific application we focus on is the study of intracellular endocytotic trafficking mechanisms, i.e., active transport mechanisms characterized by vesicle nucleation and budding of the cell membrane orchestrated by protein-interaction networks. The method we employ involves a multiscale integration of lattice-based kinetic Monte Carlo schemes with continuum-based time-dependent Ginzburg Landau methodologies in order to obtain a unified picture of how curvature inducing proteins mediate cellular trafficking mechanisms. The simulation framework helps enable the development of a quantitative link between molecular driving forces and emergent functionality in endocytotic trafficking networks and also provide rigorous foundations for differentiating intracellular trafficking fates on the basis of differences in molecular interactions due to homologous receptors or receptor mutations, which often gain prominence in dysfunctional trafficking pathways.

Blood Systems Biology: Patient-Specific Prediction of Thrombosis

Systems biology seeks to provide a quantitative framework to understand blood as a reactive biological fluid whose function is dictated by prevailing hemodynamics, vessel wall characteristics, platelet metabolism, numerous coagulation factors in plasma, and small molecules released during thrombosis. The hierarchical nature of thrombosis requires analysis of adhesive bond dynamics of activated platelets captured from a flow field to a growing thrombus boundary along with the simultaneous assembly of the coagulation pathway. We have expanded kinetic models of protease cascades to account for platelet activation. These models are tested against 100s of conditions achieved in 384-well plate assay using fluorogenic substrates for thrombin. A full bottom-up model of platelet intracellular metabolism in response to activation of P2Y1 receptor by ADP is now available to simulate the metabolism of resting platelets and platelets exposed to activators. Monte Carlo algorithms can finally accommodate platelet reaction, dispersion, and convection for full simulation of platelet deposition and clotting under flow. Finally, we have developed an experimental/computational technique called "Pairwise Agonist Scanning" to define platelet response to combinations of up to 6 agonists. For clinical applications, the Systems Biology prediction of patient-specific pharmacological response requires the final assembly of platelet intracellular metabolism models with coagulation protease network models.

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Computational Models of the Evolution of Metastasis

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Prior to metastasis, most cancers can be treated surgically, with good prognosis. After metastasis, systemic therapy is required and is rarely curative. Thus, metastasis represents one of the most important transitions in neoplastic progression. The evolution of metastasis is a puzzle because a metastatic clone in the primary tumor is at a competitive disadvantage relative to non-metastatic clones and so should be unable to expand to large numbers. The vast majority of cells that emigrate from the primary tumor fail to establish metastases. So a metastatic clone effectively wastes much of its reproductive potential compared to a non-metastatic clone as they compete for space and resources in the primary tumor. We have developed an agent-based model that shows that heterogeneity of resources in both space and time, caused by poorly regulated angiogenesis and hypoxia, selects for increased cell migration within the primary tumor. Cells that are able to locate unutilized resources are better able to proliferate than their sedentary competitors. We hypothesize that this selection for cell migration leads to increased cell emigration from the primary tumor as a side effect. This would explain why primary tumors with high metastatic potential may be detected early in progression. Expression array studies may be detecting the conditions that select for cell migration or may be detecting increased cell migration within the primary tumor. Our results would also explain why hypoxia, particularly transient periods of hypoxia, are associated with increased metastasis. Our results may lead to better assays to measure the risk of metastasis and to interventions that may reduce that risk.

Multi-Scale Modeling of Neuronal Adaptation Mediated by Angiotensin II in the Central Regulation of Blood Pressure

Angiotensin II type 1 receptor (AT1R) mediated processes in the brainstem play a key role in the homeostatic regulation of autonomic functions, and are critical to the development and maintenance of chronic diseases such as hypertension. While experiments indicated potential components in the AT1R-activated regulatory network, little is known as to which of these play a key role and their dynamical interactions. To this end, we took a computational approach that considers multiple time scales to integrate AT1R signaling dynamics with the short-term electrophysiological behavior, and a longer-term signaling through ERK, PKC, FRK and JNK pathways to affect immediate early transcription factor AP-1 and downstream neuronal gene expression. Analysis of the short-term model for different cases of interactions between signaling kinases and ion channels revealed the contribution of the key regulators to the overall adaptation dynamics. Longer-term model simulations reveal a dynamic balance among distinct dimers of the AP-1 family of transcription factors, resulting in a robust activation of neuronal genes playing a critical role in blood pressure control. This dynamic switch from Fos-Jun to Jun-Jun dimers occurs even at persistently high levels of c-Fos mRNA, indicating the complexity of network interactions with multiple feedbacks and crosstalk. Global sensitivity analysis indicates that the set of key network interactions changes over time, with a shift from FRK-mediated processes to those downstream of JNK. This model-predicted differential dynamic sensitivity of AP-1 activation explains the previously confounding data from kinase inhibition experiments. These experimentally verifiable hypotheses provide insights into the dynamics of interactions between signaling, gene regulation and electrophysiology that underlie neuronal adaptation in the brain.

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Minimal Models of Extrinsic Regulation in Cell-Fate Decisions

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In response to extracellular cues, concentrations of which can be incrementally tuned, a multipotent stem cell can make a discrete, all-or-none fate decision. Although many of the individual biochemical reactions that are critical to such cell-fate decisions are beginning to be elucidated, the network topologies that can convert these 'analog' cues into 'digital' cellular responses remain poorly understood. We are currently examining the role of positive receptor feedback in lineage specification of hematopoietic progenitors and have formulated computational frameworks that integrate this receptor-mediated regulation with classical transcriptional regulation. Analyses of the corresponding network models elucidate novel mechanisms by which positive receptor feedback can generate discrete and stable cell fates in response to cognate extracellular ligands. Our model simulations are in good agreement with experimental findings and may provide new insights into extrinsic regulation during lineage commitment.

Cell Signaling in Equations and Embryos

The Extracellularly Regulated Kinase/Mitogen Activated Protein Kinase (ERK/MAPK) signaling pathway is a critical regulator of cellular processes in adult and developing tissues. Depending on the cellular context, MAPK cascade can act as a rheostat, a switch, or an oscillator. The highly conserved structure of the cascade does not imply a rigid function, as was suggested by the early mathematical models of MAPK signaling, and can instead produce a wide range of input-output maps. Given a large number of pathway components and modes of regulation, it is essential to establish experimental systems that will allow both manipulating the MAPK cascade and monitoring its dynamics. The terminal patterning system in the *Drosophila* embryo appears to be ideally suited for this purpose. Our recent experiments characterized dynamics of the MAPK phosphorylation gradient in the terminal system and proposed that it is regulated by a cascade of diffusion-trapping modules. I will present a biophysical model that can describe the observed dynamics and guide future experiments for exploring the relative importance of multiple layers of MAPK cascade regulation.

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A Mathematical and Computational Approach for Integrating the Major Sources of Cell Population Heterogeneity

During the past decades, several approaches have been used to model heterogeneity in bacterial cell populations, each approach focusing on different source(s) of heterogeneity. However, a holistic approach that integrates all the major sources into a generic framework applicable to cell populations is still lacking.

In this work we present the mathematical formulation of a Master equation that pertains to cell populations and takes into account the major sources of heterogeneity, namely stochasticity in reaction, DNA-duplication, and division, as well as the random partitioning of species contents into the two daughter cells. The formulation also takes into account cell growth and respects the discrete nature of the molecular contents and cell numbers. We further develop a Monte Carlo algorithm for the simulation of the stochastic processes considered here. Using this approach we demonstrate the effect of each source of heterogeneity on the overall phenotypic variability for the two-promoter system used experimentally by Elowitz et al. (2002) to quantify intrinsic versus extrinsic noise.

Elowitz, M. B., A. J. Levine, E. D. Siggia and P. S. Swain (2002). "Stochastic gene expression in a single cell". *Science* 297(5584): 1183-1186.

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A Network of Stabilizing Hydrogen Bonds is Integral to the Dimer-Mediated Allosteric Activation Mechanism in the ErbB family of Kinases

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Recently, an allosteric activation mechanism was proposed for the receptor tyrosine kinase (RTK) activation in ErbB1 (HER1 or the epidermal growth factor receptor or EGFR) where a novel asymmetric dimer interface activates the kinase. Here, we interpret this allosteric activation mechanism by accounting for the loss of interactions in terms of a contrast in stabilizing interactions surrounding key regions of the kinase in the active versus inactive conformations. Through an analysis of molecular dynamics simulations, we identify a regulatory network of stabilizing interactions surrounding the activation loop and the α C-helix in ErbB1 RTK. We find that the protein residues involved in the RTK dimerization, as well as those associated with mutations in oncogenic cell-lines are poised to preferentially induce network fragility by the disruption of the stabilizing interactions preferentially in the inactive state of the kinase. Based on the network of stabilizing interactions, we further suggest different modes of constitutive kinase activation for the implicated mutations, which qualitatively agree with functional studies of phosphorylation activity reported in the literature. This striking correlation between the disruption of the stabilizing network and changes in the functional activity of the kinase helps to provide a molecular context to how each stimulus such as dimerization or a site-specific mutation or the A-loop tyrosine phosphorylation perturbs the network and hence impacts the kinase activity. We also show that such an analysis is generalizable to the activation of the other members of the ErbB family of kinases.

IntuitCell: An SBML-based Bio-Computing Environment for Large-scale Biological Model Representation, Analysis, and Simulation. Linking Experimental Data to Mathematical Modeling

The development of IntuitCell software has been motivated by the need in our biomedical systems biology research for a coherent approach to relate data and models, and to test the models in the context of various modeling platforms. Our objective is to provide software to automatically convert a list of biochemical reactions to a computer code compatible with a variety of software platforms (SBML, Matlab, etc.). At the same time, IntuitCell might also provide a user-friendly platform that may enable experimental biologists to develop mechanistic computer models without possessing formal training in computer science. Additionally, our approach provides flexibility for users to modify the individual reactions or pathways. IntuitCell uses the Python scripting language to automatically convert user-generated, rule-based Ontology model descriptions to SBML compliant representations and converts these to become Matlab compatible. In our Project, we use IntuitCell as a pipeline to speed up model testing exercises such as (1) investigating the model's regression instability (recently termed "model's sloppiness"), (2) global sensitivity analysis, and (3) bistability bifurcation analysis to provide long-term sustained activation. At present there is no single modeling tool to allow the modeler to perform tests (1) – (3). In our developments, we followed community standards for a simple rule- based and SBML-centered toolset that creates a biochemical model that fulfills all the requirements listed in MIRIAM, Minimum Information Requested in the Annotation of Biochemical Models. IntuitCell will be archived as an open access resource for use and for further developments. For example, in the future it may be desirable for others to extend IntuitCell to also automatically convert SBML representations to be compatible with additional computational platforms such as BioNetGen, little b, Kappa language, matcont, SciPy, etc. and to add algorithms to standardize model calibration, verification and analysis.

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Quantitative Analysis of the Gene Regulatory Network activated by Angiotensin II in the Brainstem

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Angiotensin II type 1 receptor (AT1R) signaling plays an integral role in the synthesis of norepinephrine, a neurotransmitter involved in central cardiovascular regulation. Dysregulation of AT1R signaling within the brain is associated with diseases such as hypertension. AT1R activation affects norepinephrine through a gene regulatory network in which the activated AP-1 family of transcription factors induces expression of tyrosine hydroxylase (TH). However, the dynamics of network components and relative contributions of AP-1 dimers remain unknown. Here, we take a computational modeling approach to investigate the quantitative role of network components on TH gene regulation. Our model simulations predict that AT1R activates a dynamic balance of distinct AP-1 dimers. Model analysis identifies critical mechanisms underlying AP-1 activation, and generates experimentally testable predictions. These results provide quantitative insights into system-wide blood pressure control mechanisms.

Prediction and Analysis of Calcium Signaling in Human Platelets

Because of its small size, rudimentary genome, and well-studied biochemistry, the platelet is an ideal test system for developing quantitative models of blood function for prognostic purposes or designing therapeutic strategies for patients. We assembled the first computational model of platelet signaling that accurately captures the platelet phosphoinositide (PI) and calcium (Ca^{2+}) release response to adenosine diphosphate (ADP), an important agonist for platelet aggregation. Using a novel strategy to construct the model, we found that the resting structure of platelets places strong restrictions on several biophysical quantities, such as the resting concentration of free inositol 1,4,5-trisphosphate (IP3) (~1000 molecules per cell), the ratio of Ca^{2+} ATPase pumps to IP3 receptor channels (>1000), and the size of the dense tubular system (2-4% of the cytosolic volume). We also performed stochastic simulations of a single platelet that reproduced the characteristic asynchronous Ca^{2+} spiking behavior observed for ADP-stimulated platelets. Artificially increasing the volume of the cellular compartments 100-fold eliminated spiking, suggesting that this stochastic behavior is a consequence of the platelet's small size. To capture the platelet calcium response to multiple agonists acting simultaneously, we tested all pair-wise combinations of 6 platelet agonists (convulxin, ADP, thromboxane analog [U46619], PAR1 agonist peptide, PAR4 agonist peptide, and PGE2) at 3 doses (0.1, 1, 10 X IC_{50}). These 154 calcium traces trained a 2-layer (12 processing nodes) neural network (NN) to successfully predict sequential agonist responses and all ternary combinations of convulxin, TRAP and ADP. The NN model also identified 45 conditions of 4, 5, and 6 agonists predicted to display a range of synergistic signaling, which were confirmed experimentally. Taken together, these "bottom-up" and "top-down" modeling approaches provide complementing analytical tools for understanding signal transduction in human platelets.

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Pairwise Agonist Scanning of Human Platelets Reveals the High-Dimensional Calcium Response to Combinatorial Mediators of Thrombosis

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During thrombosis, platelets respond simultaneously to collagen activation of GPVI and $\alpha_2\beta_1$, ADP activation of P_2Y_{11} , P_2Y_{12} , P_2X_1 , thromboxane activation of TP, and thrombin activation of PAR1 and PAR4, while NO and PGI_2 dampen responsiveness. Using intracellular calcium mobilization to quantify platelet activation, we developed a high throughput assay to rapidly phenotype an individual's platelets. Dose responses (EC50) of convulxin (GPVI activator), ADP, U46619 (thromboxane analog), SFLLRN (PAR1 agonist), AYPGKF (PAR4 agonist) and the IP agonist PGE_2 were first determined. Then, platelets were challenged with all 154 pairwise combinations (2 replicates) of these 6 agonists at concentrations of 0, 0.1, 1 and 10x EC50 to yield the level of mutual synergy or antagonism. A neural network (NN) was trained on pairwise interaction data (34,000 data points) to predict the experimentally observed pairwise synergies/antagonisms. Once trained, the NN predicted the measured 64 ternary combinations of the agonists ADP, SFLLRN and convulxin, as well as various sequential tests of these agonists where striking desensitization and cross down-regulation were noted. NN predictions of 45 conditions (ranging from extreme synergy to extreme antagonism) out of 4077 possibilities in the 6 dimensional agonist space were experimentally verified ($r = 0.88$). "Super-synergy" conditions at high U46619/ PGE_2 ratio in the 6-agonist space were consistent with the known cardiovascular risk of COX-2 therapy. Additionally, a novel thromboxane-PAR1 synergy was detected in all donors. Finally, Pairwise Agonist Scanning (PAS) for 4 healthy individuals demonstrated unique inter-donor traits, thus allowing a patient-specific Systems Biology prediction of global platelet-dependent thrombotic response.

Microfluidic Focal Thrombosis Model for Measuring Murine Platelet Deposition and Stability: PAR4 Signaling Enhances Shear-Resistance of Platelet Aggregates

Background: Flow chambers allow the ex vivo study of platelet response to defined surfaces at controlled wall shear stresses. However, most assays require 1 to 10 mL of blood and are poorly suited for murine whole blood experiments.

Objective: To measure murine platelet deposition and stability in response to focal zones of prothrombotic stimuli using 100 μ L of whole blood and controlled flow exposure.

Methods: Microfluidic methods were used for patterning acid-soluble collagen in 100 μ m x 100 μ m patches and creating flow channels with a volume of 150 nL. Within 1 min of collection into PPACK and fluorescent anti-mouse CD41 mAb, whole blood from normal mice or from mice deficient in the integrin α 2 subunit was perfused for 5 min over the patterned collagen. Platelet accumulation was imaged at venous and arterial wall shear rates. After 5 min, thrombus stability was measured with a "shear step-up" to 8000 sec⁻¹.

Results: Wild type murine platelets adhered and aggregated on collagen in a biphasic shear dependent manner with increased deposition from 100 to 400 sec⁻¹, but decreased deposition at 1000 sec⁻¹. Adhesion to patterned collagen was severely diminished for platelets lacking a functional α 2 β 1 integrin. Those integrin α 2-deficient platelets that did adhere were removed from the surface when challenged to shear step-up. PAR4 agonist (AYPGKF) treatment of thrombus at 5 min enhanced aggregate stability during the shear step-up.

Conclusions: PAR4 signaling enhances aggregate stability by mechanisms independent of other thrombin-dependent pathways such as fibrin formation.

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Lattice Kinetic Monte Carlo Simulations of Platelet Aggregation and Deposition

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Platelet aggregation is a key step in clot formation during the body's hemostatic and thrombotic responses. Modeling large scale platelet aggregation is difficult due to the complex platelet response to various agonists in combination, the changing chemical environment at the clot surface, and the perturbation of the fluid flow. Detailed models, e.g. Mann model for coagulation, exist for many individual processes in clot formation, but a unified large scale model would elucidate the interplay between processes. New tools and methods such as multiscale models are required for coupling information from the disparate time and length scales present in complex biological systems. We present a new approach based on the lattice kinetic Monte Carlo (LKMC) method to address platelet aggregation. Recently, we reported an LKMC algorithm which allows the incorporation of both convective and diffusive particle transport, and we extend this algorithm for deposition of platelets onto an injured vessel wall. Platelet activation and interactions within LKMC will be modeled by simple models and detailed ODE models, and perturbation of fluid flow will be determined by lattice Boltzmann. Coupling information between models will be discussed. The growth rate and morphology of the clot from LKMC with different levels of coarse-graining will be compared to experimental results.

Architectural Algorithmic Abstraction to Understand 4-Dimensional Matrix-Driven Cell Motility

Cell motility represents an abstract endpoint of the convergence between numerous intrinsic and extrinsic effectors, including cell adhesion to the extracellular matrix (ECM). The means to quantify and visualize how the ECM controls cell motility, however, remain highly subjective, especially within 4-D systems. To redress this, we have developed novel computational tools using approaches used in architectural digital design.

As a model biological system, normal human pulmonary artery (PA) smooth muscle cells (SMCs) were cultivated either on 3-D fibrillar or non-fibrillar type I collagen thin films that mimic the micro-environment of a normal or hypertensive PA respectively.

Using real-time image acquisition, dynamic PA SMC edge morphologies were manually traced and passed through a series of scripted algorithms that yield an unbiased “signature package” of visual and numerical information describing cell motility.

In developing the digital tools, our process of continuously abstracting 3-D geometric representations of cellular edges gave rise to an array of systematic methodologies that inherently provide equivalent numerical data used to quantitatively describe the differences in cell motility observed on native and denatured collagen. Metrics derived through this process include the furthest reach of a cell from its center of mass, velocity of cellular edge points, edge fragmentation and cell-cell edge proximities.

This computational approach provides ways of describing and representing morphological change in a hierarchical manner such as filopodial wavering, fibrillar alignment, cell repulsion and attraction as well as and central cohesion, at the scale of the local cell edge, the entire cell, and cell-to-cell interactions.

The discovery of such behaviors was prompted during the interpretation of visual abstractions, as they reveal relationships between the cell and its micro-environment.

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