1. Engineering pathways improves prediction of side effects from protein-interaction network models

Mohammadali Alidoost, Jennifer L. Wilson & Department of Bioengineering, University of California Los Angeles (UCLA)

Exploring the prediction of drug side effects remains a challenge for current modeling tools, particularly in the context of protein-protein interaction models. These models, while adept at assessing drug effects based on the proteins around drug targets, often struggle due to their tendency to overpredict drug phenotypes and their dependency on clearly defined pathways. In our research, we utilized PathFX, a protein-protein interaction model, to predict side effects using data derived from active ingredient-side effect pairings on drug labels. Our findings indicate that PathFX, despite its potential, shows constrained efficacy, which led us to innovate new pathways through pathway engineering. This process involved both network-based and gene expression-based methodologies. A key discovery of our study was the inherent balance between sensitivity and specificity in the model's predictions, highlighting a method to reduce excessive false predictions for side effects with sufficient true positive cases. When compared with animal model predictions, our approach showcased comparable effectiveness, suggesting that we may not need perfect evaluation metrics for the practical application of protein-protein interaction models. Our study underscores the significant role of pathway engineering, incorporating true positive examples and omics data, in improving the relevance and accuracy of protein interaction network models for the anticipation of drug effects. This approach presents a forward step in enhancing the predictive accuracy of drug side effects in preclinical research.

2. Astral numbers and the mechanical strength of cells

Shannon McFadden (Huntington Beach High School / UCI); Andrew Rusli (St. Margaret's Episcopal School / UCI); Brady Berg (UCI - MCSB); Jun Allard (UCI - Math & Physics, CCBS)

Cells have the ability to control their rigidity - that is, the structural property of a material that allows it to withstand mechanical force - over orders of magnitude in minutes. This is an ability that material engineers aspire to recreate. It is also an ability that goes wrong in many diseases in which cells are either too stiff (parasitic infections like malaria, some anemias) or too soft (some cancers and heart disease). Recent work has shown that part of the cell's rigid structures (F-actin) into star-shaped patterns called asters that then crosslink into larger networks. In this work, we show that there is an optimal number of filaments per aster -- what we term the "astral number" -- that maximizes rigidity. This work provides a direct connection between a readily-measurable quantity (astral number) to a biomedically-relevant but harder-to-measure one (cell rigidity). This poster is a joint work with Shannon McFadden, Andrew Rusli, and Jun Allard.

3. Chromatin organization is regulated by geometric constraint during cell crowding

Alexandra Bermudez (UCLA), Zoe Latham (UCLA, Jimmy Hu (UCLA), Neil Lin (UCLA)

Cells sense and respond to their surrounding microenvironment, but how physical cues regulate gene expression to control cell behaviors remains an important open question. Cell geometric confinement refers to the spatial constraints imposed on cells due to their physical surroundings, such as during cell crowding, which influences cell homeostasis, morphologies, and movements in several physiological contexts, including epithelial organogenesis, wound healing, and injury repair. However, how this crowding-induced confinement is translated into cell behavioral and shape changes remains underexplored. In this work, we address this knowledge gap by investigating the interplay between geometric confinement, coordination of cell and nucleus sizes, and epigenetic state in Madin-Darby Canine Kidney (MDCK) cells. We found that the geometric constraint of cell packing induces a universal probability distribution of cell size that positively correlates with nucleus size. Furthermore, we found that the nucleus size is correlated with the expression levels of different histone marks, in which cells with a smaller nucleus are associated with a more repressed epigenetic state and cells with a larger nucleus exhibit a more active epigenetic state. This result is thus consistent with previous studies using microprinting pattern 1 and tissue scaffold pores 2 to confine individual cells. Our findings further highlight that crowding-induced confinement has direct impacts on nuclear sizes and the chromatin state.

4. Using the protein interaction network to make drug synergy predictions

Emily Bozich, Jennifer Wilson (Department of Bioengineering, UCLA)

Many have predicted synergy between combined perturbations (i.e., drug combinations), yet sophisticated machine learning models, with ample single perturbation, and cell line information, report model-experiment correlations of ~0.24-0.48. Of note, better performing models leveraged drug target and pathway information. Additionally, protein-protein interaction network methods have successfully identified signaling pathways and have been used to describe drug combination effects. However, few have considered de novo synergy prediction solely from interaction network (interactome) topology.

We applied a network approach to measure the extent to which topological relationships between target pairs can be used to predict their combined perturbation effects. We quantified relative target topological positioning in the interactome by exhaustively testing various distance metrics. In doing so, we ultimately reported model-experiment correlations of ~0.42-0.62 for six cell line models derived from two validation datasets: a pairwise CRISPR knockout screen and small molecule combination cell viability screen.

In comparing model performance using our various distance metrics, we found that network diffusion-based distance measurements outperformed simple, shortest-paths measurements. We also demonstrated the importance of making nuanced assumptions when defining network diffusion features in order to better characterize target distances. Further, we found that highly synergistic combinations are significantly closer than background target pairs (t-test, $P < 0.001$), suggesting that target closeness may sufficiently explain strong experimental effects. In summary, our efficient data-driven, network-based approach used to predict drug synergy can be a viable approach to more effectively design resource-deprived experimental combination screens.

5. PIEZO1 regulates leader cell formation and cellular coordination during collective keratinocyte migration

Jinghao Chen, Jesse Holt, Elizabeth Evans, John Lowengrub, Medha Pathak. (University of California - Irvine)

The collective migration of keratinocytes during wound healing requires both the generation and transmission of mechanical forces for individual cellular locomotion as well as for the coordination of movement across cells. Leader cells initiated along the wound edge transmit mechanical and biochemical cues to ensuing follower cells, ensuring their uniform polarization and coordinated direction of migration across multiple cells. Despite the observed importance of mechanical cues in leader cell formation and controlling coordinated directionality of cell migration, the underlying biophysical mechanisms remain elusive. The mechanically activated ion channel PIEZO1 was recently identified to play an inhibitory role during the reepithelialization of wounds through retraction of keratinocytes located at the wound edge. Here, through an integrative experimental and mathematical modeling approach, we elucidate PIEZO1's contributions to collective migration. Time-lapse microscopy reveals that PIEZO1 activity inhibits leader cell formation along the wound edge. To probe the relationship between PIEZO1 activity, leader cell formation and inhibition of reepithelialization, we developed an integrative 2D continuum model of wound closure that links observations at the single cell and collective cell migration scales. Through numerical simulations and subsequent experimental validation, we found that coordinated directionality plays a key role during wound closure and is inhibited by upregulated PIEZO1 activity. We propose that PIEZO1-mediated retraction suppresses leader cell formation which inhibits the coordinated directionality between cells during collective migration.

6. Integrating mechanistic modeling and quantitative imaging to reveal pancreatic beta cell signaling dynamics

Lynne Cherchia, Falk Schneider, Stacey D. Finley, Scott E. Fraser, Alfred E. Mann (USC)

Signal transduction through the prolactin receptor (PRLR) drives pancreatic cell proliferation; however, the precise mechanisms underlying this proliferative effect remain ill-defined. To harness its potential use as a regenerative diabetes therapy, it is paramount that we establish a quantitative, mechanistic understanding of PRLR signaling. PRLR is a single-pass membrane receptor in the JAK family. Upon PRLR binding to its ligand, prolactin, STAT5 signaling is activated, causing changes in gene expression. Prior studies have demonstrated that PRLR localizes intracellularly; however, the implications of intracellular PRLR pools on signal transduction and cell proliferation have not been investigated. Because they involve interactions between numerous molecular species, spanning many time- and length-scales, signaling networks are challenging to investigate. To bridge these scales, we are implementing a pipeline that uses quantitative imaging studies to inform ordinary differential equation

(ODE) models of the PRLR signaling network. Our integrated approach allows us to investigate the effects of PRLR and STAT5 localization and dynamics on cell proliferation. We have developed a reconstituted minimal expression system in cultured cells, comprising fluorescently labeled PRLR and STAT5, and have applied this system to confirm the presence of intracellular PRLR localization, and to obtain fluorescence correlation spectroscopy (FCS) measurements capturing PRLR and STAT5 dynamics. These imaging and spectroscopy data are used to incorporate the intracellular PRLR pools, the role(s) of which have previously been overlooked. Our fluorescence imaging results and FCS measurements have been used to iteratively extend an ODE model of the PRLR signaling network and to update the model structure. This modeling-imaging pipeline demonstrates the utility and robustness of FCS for capturing the protein dynamics needed for integration into the computational model and provides a powerful, generalizable approach to quantitative biology and cellular signaling networks.

7. Identifying Liver Transplantation Rejection Mechanisms Through Integration of Longitudinal Donor and Recipient Immunological Measurements

Jackson L. Chin, Cyrillus Zhixin Tan, Aaron S. Meyer; UCLA

Introduction: Liver ischemia-reperfusion injuries (LIRI) arise during liver transplantation and are suspected to contribute to the high rate of chronic liver transplant rejection. Current metrics of evaluating LIRI severity—including biopsy pathology scores and liver function tests (LFTs)—are insufficiently explanative of long-term outcome, therefore precluding development of preventative therapies and limiting the effectiveness of donor and recipient screening methods. We hypothesize that this gap in understanding could be attributed to the multitude of LIRI sources that manifest across donor and recipient tissues at varying times across the transplant process, and that understanding the mechanisms driving liver transplant rejection requires a holistic integration of longitudinal donor and recipient measurements. Materials and Methods: To better understand the mechanisms of LIRI and their relationship to transplant rejection, we developed and applied tensor partial least squares (tPLS)—a supervised, tensor-based decomposition method that enables outcome-informed integration of multiple high-dimensional datasets into components—to regress transplant outcomes against longitudinal measurements derived from both transplant donors and recipients. Longitudinal measurements include peripheral blood cytokine and LFT measurements collected from the recipient alongside cytokine measurements taken from the donor liver's portal vein. Results and Discussion: tPLS successfully reduces pre- and post-operation measurements collected from donor and recipient to two components that are strongly associated with transplant outcome, predicting liver transplant rejection with an accuracy over 70%. Interpretation of these components yields novel biological insights into the mechanisms of liver transplant rejection, highlighting the roles of T-helper polarization, granulocyte response, and regenerative factors in liver transplant outcome. Collectively, these findings demonstrate the efficacy of outcome-informed tensor decomposition techniques in integrating longitudinal measurements and highlight determinants of transplant outcome for improving therapeutics and screening methods.

8. Parameter sensitivity analysis of GRNmap, a dynamical systems model of gene regulatory networks

Nikki C. Chun, Kam D. Dahlquist, and Ben G. Fitzpatrick (Loyola Marymount University)

A gene regulatory network (GRN) is a set of transcription factors that regulate the expression of genes encoding other transcription factors. The dynamics of GRNs explain how gene expression changes over time. GRNmap is a complex MATLAB software package that uses ordinary differential equations to model the dynamics of small- to medium-scale GRNs. The program estimates production rates, expression thresholds, and regulatory weights for each transcription factor in the network based on time-course gene expression data, using a penalized least-squares function that minimizes the discrepancy between simulated model outputs and observed data. The optimization problem is constrained by the addition of a penalty term, which consists of the square of the parameter vector, multiplied by α, which is used for weighting. Exploration of the α parameters found that an alpha value of 0.02 is suitable, with a value of 0.002 causing overfitting. Additionally, GRNmap can make parameter estimation from input expression data with missing data points. When using an alpha value of 0.02, there were only slight differences in outputs from workbooks with missing expression data points and no missing data points (which were filled with the average value of the other replicates for that time point). To better understand where these differences are coming from, a sensitivity analysis will be conducted based on a handpicked trial network. Noise will be added to the expression data systematically to see where the sensitivities arise so that we can better interpret the differences in the missing and no missing data outputs.

9. Direct vs indirect activation of ZEB lead to stabilization of alternate states in a tristable model of epithelial to mesenchymal transition

Anupam Dey, Adam MacLean (USC)

Epithelial to mesenchymal transition (EMT) and its reverse, mesenchymal to epithelial transition (MET) are crucial processes known to occur in embryo development, organogenesis, and wound healing. Cancer cells hijack this process to facilitate tumor progression and spread. Epithelial cells are typically adhesive whereas mesenchymal cells lose cell-cell adhesions and behave like individual cells. This process often involves a transition through an intermediate or hybrid E/M state where the cells exhibit properties of both epithelial and mesenchymal types. Evidence suggest this state may be the most metastatic and thus dangerous EMT state. Here, we develop a mathematical model of the core EMT regulatory network, and – via multistability and bifurcation analysis – we discover how the stability of the different EMT phenotypes is governed by various functions of ZEB, a transcription factor known to initiate and promote EMT. We found that the direct activation of ZEB by its upstream regulator SNAIL initiates EMT and stabilizes the hybrid E/M state whereas the indirect activation of ZEB by SNAIL through miR-200 stabilizes EMT by maintaining the mesenchymal state. We also show that varying the activation strength of signaling on ZEB can generate different dynamical responses, thus fundamentally changing the effective potential landscape of the system. Via stochastic simulations of the gene regulatory network dynamics, we demonstrate how this landscape governs the cell state transitions that occur in presence of gene expression noise. Overall, our findings provide direct predictions for how manipulating specific molecular interactions can stabilize or destabilize the hybrid E/M state, of relevance for therapeutics during cancer progression and metastasis. More generally, we provide new insight into a commonly occurring network motif in biology in light of its multistable properties and how these change in the presence of noise.

10. A statistically principled feature selection method for single cell transcriptomics

Emmanuel Dollinger (UCI)

Single cell transcriptomics data (scRNAseq) is inherently high dimensional. Therefore, a standard data processing step is to select a subset of genes (a.k.a. feature selection) for downstream analysis. The impact of feature selection in scRNAseq on downstream dimensionality reduction and clustering is unknown. We show that random feature selection is sufficient for celltype identification for some datasets but not others. We then show that both the number of genes and the feature selection algorithm can greatly affect standard downstream clustering and rare celltype identification. From first principles, we derive an analytical feature selection method that allows for interpretable cutoffs, leading to biologically meaningful rare celltype identification. We describe rules of thumb for how to optimize these cutoffs for a given dataset and find that our method compares favorably to the default methods in scanpy and Seurat along with SCTransform. Feature selection in scRNAseq can strongly affect downstream celltype identification and deserves further exploration.

11. scENCORE: leveraging single-cell epigenetic data to predict chromatin conformation using graph embedding

Ziheng Duan (UCI), Siwei Xu (UCI), Shushrruth Sai Srinivasan (UCI), Ahyeon Hwang (UCI), Che Yu Lee (UCI), Feng Yue (Northwestern U), Mark Gerstein (Yale), Yu Luan (UT San Antonio), Matthew Girgenti (Yale & VA) and Jing Zhang (UCI).

Dynamic compartmentalization of eukaryotic DNA into active and repressed states enables diverse transcriptional programs to arise from a single genetic blueprint, whereas its dysregulation can be strongly linked to a broad spectrum of diseases. While single-cell Hi-C experiments allow for chromosome conformation profiling across many cells, they are still expensive and not widely available for most labs. Here, we propose an alternate approach, scENCORE, to computationally reconstruct chromatin compartments from the more affordable and widely accessible single-cell epigenetic data. First, scENCORE constructs a long-range epigenetic correlation graph to mimic chromatin interaction frequencies, where nodes and edges represent genome bins and their correlations. Then, it learns the node embeddings to cluster genome regions into A/B compartments and aligns different graphs to quantify chromatin conformation changes across conditions. Benchmarking using cell-type-matched Hi-C experiments demonstrates that scENCORE can robustly reconstruct A/B compartments in a cell-type-specific manner. Furthermore, our chromatin confirmation switching studies highlight substantial compartment-switching

events that may introduce substantial regulatory and transcriptional changes in psychiatric disease. In summary, scENCORE allows accurate and cost-effective A/B compartment reconstruction to delineate higher-order chromatin structures heterogeneity in complex tissues.

12. The Role Of Frailty Assessment In Reducing Adverse Liver Transplant Outcomes

Brooke Edwards, Samantha Ramirez, Derek Samson, Aaron Ahearn (UCLA)

Background: Frailty negatively impacts health outcomes before and after liver transplantation (LT). In October 2022, the LT team implemented the use of Liver Frailty Index (LFI), a validated tool for assessing frailty in liver disease patients, at USC. Pre-habilitation services were provided to the identified frail patients to improve clinical outcomes. The frailty assessment was integrated into the LT selection committee criteria aimed to refine the decision-making processes.

Purpose: To analyze the impact of frailty on LT outcomes. To investigate the role of LFI in optimizing patient selection.

Methods: Post transplant outcomes were analyzed for 190 patients that underwent LT between October 2022 – January 2024. Their outcomes were compared to 221 patients who underwent LT prior to LFI implementation (June 2021 - September 2022).

Results: Among the 190 patients, 4 had graft failure within 30 days of LT, 2 died post-transplant during LT admission, 24 had an unplanned return to the OR (RTOR) during LT admission, and 44 were readmitted within 30 days of the LT admission hospital discharge. Both patients that died were frail (LFI scores 4.75 and 5.32). In comparison to the pre-LFI adaption period, 12 had graft failure within 30 days of LT, 7 died post-transplant during LT admission, 42 had an unplanned RTOR during LT admission, and 77 were readmitted within 30 days of the LT admission hospital discharge. The average length of stay (LOS) from transplant to discharge was 17.8 compared to 16.7 days. The median MELD score was 32 compared to 27.

Discussion: The results showed a 71.42% decrease in post-transplant mortality, 66.66% decrease in graft failure, 42.85% decrease in unplanned RTOR, and a 42.85% decrease of readmissions. The median MELD of the transplanted patients was lower. However, the LOS was similar. The adaptation of the LFI into practice enabled the LT team to be more conscientious in determining transplant candidacy among frail patients and resulted in a reduction in poor transplant outcomes.

Conclusion: Frailty significantly impacts post-liver transplant outcomes. The incorporation of the LFI assessment and pre-habilitation at USC has proven instrumental in optimizing patient selection criteria resulting in reductions in post-transplant mortality, graft failure, unplanned return to OR, and readmissions.

13. Targeting Neuroendocrine Vulnerabilities in Recurrent Platinum-Resistant High Grade Serous Ovarian Carcinomas

Favour N. Esedebe, Tanya Singh, Yi Jou (Ruby) Liao,Gabriella DiBernardo, Amir Borujerdpur, Christopher Ochoa,Robert Damoiseaux,Sandra Orsulic,Sanaz Memarzadeh,Thomas Graeber (UCLA)

With a low 5-year survival rate, ovarian cancer ranks among the deadliest gynecologic cancers in the U.S. High-grade serous ovarian carcinoma (HGSOC) is the most commonly diagnosed and lethal subtype of ovarian cancer, accounting for more than 80% of deaths from ovarian cancer. Despite initial response to platinum-based chemotherapy, HGSOC tumors eventually relapse and become resistant to treatment. Patients with platinum-resistant ovarian cancer have few effective treatment options, underscoring the need to discover biomarkers and druggable targets. We analyzed bulk and single cell RNA-sequencing data collected from over 60 longitudinal matched patient and patient-derived-xenografts tumor samples at different treatment timepoints to characterize and map out tumor progression. Our large-scale transcriptomics study, augmented with spatial and histological analyses, revealed substantial pathway shifts and tumor clonal evolution as the cancer advances. Notably, we discovered previously unreported enrichment of neuroendocrine features in platinum-resistant tumors, suggesting a novel resistance mechanism in HGSOC tumors through transdifferentiation. Furthermore, by performing multivariate integration of datasets from drug sensitivity, RNAi knockdown, and CRISPR knockout screens, we detected potential druggable gene and pathway targets associated with resistance and transdifferentiation in

ovarian cancer cell lines. Our findings highlight new and promising avenues for therapeutic intervention in patients with recurrent platinum-resistant HGSOC.

14. Modeling the heterogenous NFκB dynamics of single immune cells

Xiaolu Guo, Adewunmi Adelaja, Supriya Sen, Alexander Hoffmann (UCLA)

Macrophages function as immune sentinel cells, initiating appropriate and specialized immune responses to a great variety of pathogens. The transcription factor NFκB controls macrophage gene expression responses, and its temporal dynamics enable stimulus-specificity of these responses. Using a fluorescent reporter mouse our laboratory recently generated large amounts of single-cell NFB dynamic data and identified dynamic features, termed 'signaling codons', that convey information to the nucleus about stimulus identity and dose. Here, we built and parameterized the mechanistic model of the signaling network to recapitulate the stimulus-specific but highly cell-to-cell heterogeneous NFκB dynamics. The parameters that are subject to biological variation provide the potential to account for the heterogeneity in observed stimulus responses. Visual inspection and quantitative evaluation revealed an excellent concordance between simulations and experimental data. Further, we identified biochemical reactions that may account for the cellular heterogeneity in NFκB dynamics. We verified that the stimulus-specificity of the virtual macrophage NFκB responses was consistent with their live-cell counterparts, as assessed by mutual information and machine learning classification. Furthermore, the virtual NFκB macrophages enabled the exploration of individual cell responses to different ligands. Leveraging this capability, we made predictions regarding combinatorial ligands, that were then experimentally tested. Discrepancies between the experimental results and model predictions led to the identification of a competition mechanism between CpG and PolyIC for endosome trafficking, resulting in non-integrative responses behavior. Our results establish a mathematical modeling tool that may be used to study the molecular determinants of response specificity and dynamical coding in immune sentinel cells at the single cell level.

15. Elucidating the stability of scRNA-seq analysis through resampling

Timothy Hamilton, Juan Vergara Najar, Eric J. Deeds (UCLA)

The advent of single cell RNA-seq (scRNA-seq) has ushered in a revolution in a wide array of fields, including systems and developmental biology. By looking at the gene expression of complex tissues at the resolution of single cells, researchers have utilized scRNA-seq to understand the roles that specific cell types play in complex phenotypes and how those phenotypes are altered in various diseases, such as cancer, diabetes, and muscular dystrophy. Yet, while the past decade has heralded an explosion of single cell atlases, each with a supposedly more precise delineation between important cell types, several recent findings point to a lack of robustness in the results of scRNA-seq analysis. For one, recent work has demonstrated that the number of new cell types discovered in scRNA-seq has a strong correlation with the number of cells sequenced in the datasets, regardless of the specific tissue being studied. This suggests that the fine delineation between cell types is a feature of the clustering algorithms themselves rather than a robust reflection of biological differences. Secondly, we recently showed that the highly heterogenous distribution of cells in scRNA-seq datasets may complicate the effective application of current clustering tools to these data. In this work, we explored the effect these factors have on the robustness of clustering results in scRNA-seq. In particular, we formulated a novel resampling-based approach to measure the similarity of clustering analyses if a small percentage of cells are randomly removed from the dataset. Our findings reveal that clustering in scRNA-seq is extremely sensitive to even small variations in the dataset. This suggests that sampling noise and other sources of variation can have a massive impact on clustering results, which likely underlies some of the difficulties the field faces in terms of the reproducibility of results. These findings also call into question the notion that clustering scRNA-seq data generates stable models of cell types.

16. A two-signal model of B-cell activation reveals a tight balance between positive and negative selection in T-dependent B-cell responses

Helen Huang, Haripriya Vaidehi Narayanan, Alexander Hoffmann (UCLA)

In response to vaccination or infection, a successful antibody response must enrich high-affinity antigen-reactive B-cells through positive selection, but eliminate autoreactive B-cells. Reactive B-cells undergo proliferative bursts that are governed by signaling from the B-cell receptor (BCR) which binds the antigen, and the CD40 signal provided by neighboring T-cells that also recognize the antigen. However, how BCR and CD40 signaling are

integrated quantitatively, and jointly determine B-cell selection and proliferation remains unclear. We developed a new differential equations-based model of the BCR and CD40 signaling pathways and linked to models of B-cell fate decision networks. The model correctly recapitulated the population dynamics data of B-cells stimulated in vitro through their BCR and CD40 receptors. However, while the model predicts correctly that CD40 and BCR costimulation induces more NFκB activity, the predicted potentiation at the level of population expansion is not observed experimentally. Model simulations revealed that functional antagonism may be mediated by BCR-induced caspase activity triggering apoptosis in founder cells. We investigated in silico and in vitro the temporal relationship between these antagonistic signals and found that within a limited time window CD40 signaling may effectively rescue cell death triggered by BCR signaling. The window size depends on the strength of the BCR and CD40 signals, but a longer time gap does not allow for B-cell population expansion. We thus propose a form of proofreading that governs the T-cell dependent humoral immune response.

17. DeepAtlas: A Tool to Explore the Manifold Hypothesis

Serena Hughes, Tim Hamilton, Dr. Ivy Xiong, Dr. Eric Deeds - UCLA

The manifold hypothesis states that high-dimensional data sets may lie along low-dimensional latent manifolds, and thus can be described in that low-dimensional space. Current popular manifold learning methods result in a global lower-dimensional embedding, which introduces distortion. The DeepAtlas takes a local approach and yields multiple charts that locally represent the data with less overall average distortion. The DeepAtlas pipeline first generates local neighborhoods, then embeds the neighborhoods in a lower dimension, and finally uses this embedding to train a continuous and invertible neural network. This yields local embeddings with low distortion and a model that maps in both directions between the high and low dimension representations of the data. In order to determine which dimension to embed into, we apply PCA to each lower dimension and plot the resulting embedding's distortion. Since every connected component of a manifold should have a consistent dimension, we expect these plots to show similar behavior for each local neighborhood. However, our findings suggest that most real datasets do not appear to be on a lower dimensional manifold. The DeepAtlas tool thus explores the manifold hypothesis by determining the local dimension of a dataset's latent manifold if one exists and generating an atlas between this dimension and the original high dimensional data.

18. Single cell transcriptomic and epigenomic atlas of >1.5M nuclei from the human PTSD brain

Ahyeon Hwang (UCI), Mario Skarica (Yale), Che Yu Lee (UCI), Jiawei Wang (Yale), Hongyu Li (Yale), Siwei Xu (UCI), Dianne Cruz (Duke), Douglas Williamson (Duke), Hongyu Zhao (Yale), Nenad Sestan (Yale), John Krystal (Yale & VA), Keith A. Young (Texas A&M), Jing Zhang (UCI), Matthew J. Girgenti (Yale & VA)

Post-traumatic stress disorder is a polygenic disorder occurring in the aftermath of extreme trauma exposure. Recent studies have begun to detail the molecular biology of PTSD using bulk-tissue transcriptomic and epigenetic analyses. However, given the array of PTSD-perturbed molecular pathways identified thus far, it is implausible that a single cell type is responsible. Here we profile the molecular responses to traumatic stress in >1.5M nuclei from human postmortem dorsolateral prefrontal cortex of 111 individuals with and without PTSD and major depressive disorder. We identify neuronal and non- neuronal cell type clusters, gene expression changes, transcriptional regulators and map the epigenomic regulome of PTSD in a cell type-specific manner. Our analysis showed PTSD-associated alterations in inhibitory neurons, endothelial cells and microglia and uncovered genes and pathways associated with glucocorticoid signaling, GABAergic transmission and neuroinflammatory processes. Furthermore, we develop methods to integrate genomic levels to detect regulatory mechanisms using peak-to-gene linking and show PTSD-specific gene regulation for ELFN1, MAD1L1, and FKBP5. Taken together, these findings characterize the cell-specific molecular regulatory mechanisms that govern the persisting impact of traumatic stress response on the human prefrontal cortex.

19. Kinase motif analysis and phosphoproteomics reveal conserved HIV-1 virus-host interactions

Prashant Kaushal, Yennifer Delgado, Declan Winters, Sara Makanani, Vivian Yang, Erin Kim, Oliver Fregoso, Mehdi Bouhaddou (UCLA)

The HIV-1 pandemic is an ongoing health crisis, with more than 1.3 million new infections in 2022. A significant barrier to a cure for HIV is the presence of latent HIV-1 genomes in reservoir cells. One way to eliminate these reservoirs is to reactivate HIV transcription to promote viral clearance by the host immune system.

Phosphorylation of HIV-1 proteins is tightly linked to reactivation of viral transcription. Yet, due to their relatively low abundance inside host cells, detecting and quantifying chemically modified HIV-1 proteins through mass spectrometry-based approaches have been historically challenging. As a result, we lack a complete map of HIV-1 phosphorylation sites and their respective kinases, knowledge of which could lead to new strategies to control viral latency. Here, we computationally predicted HIV-1 phosphosites using known substrate specificities of human protein kinases. We computationally aligned HIV-1 subtypes to identify kinase motifs that were highly conserved across subtypes. We then used mass spectrometry (MS) proteomics and phosphoproteomics to quantify changes in viral protein abundance during an HIV reactivation time course in a human T-cell line (J-Lat 10.6) stimulated with phorbol myristate acetate (PMA) to identify HIV-1 phosphorylation sites implicated in the reactivation process. We also characterized the host signaling response to reactivation, including host kinase activity patterns and how they evolved over time. Our computational analysis identified several highly conserved HIV-1 phosphorylation sites that ranked favorably for multiple human kinases, including sites on the viral capsid and reverse transcriptase proteins. Specifically, we found sites 623S, 689S, and 836T on POL and 154S, 285S, and 310T on GAG to be highly conserved, appearing in more than 85% of 21 HIV-1 genomes representative of the major HIV-1 subtypes. Our abundance proteomics analysis also successfully detected lowly abundant viral proteins, including HIV-1 POL, GAG, TAT, and REV. We detected 24 phosphorylation sites (7 in REV and 17 in GAG) 24 hours after HIV-1 latency reactivation through MS-based phosphoproteomics. Excitingly, we detected several previously reported phosphorylation sites on REV and GAG from mutational and biochemical studies. Thus, our MS-based approach led us to the accurate identification of phosphorylation sites with high sensitivity. Interestingly, one of the phosphorylation sites detected in REV is found at S54; modification has been shown to accelerate the formation of an efficient RNA-binding conformation needed for REV transport of HIV-1 mRNA to the cytoplasm for translation. We also found phosphosite GAG S368 in the p2 domain; prior studies have shown its deletion results in reduced production of infectious virions. We hypothesize both sites could contribute to HIV-1 replication and maturation.

20. The language of cytokine repertoire in immune signaling: sentences, not alphabets

Maxim Kuznetsov, Joao Rodrigues Lima Junior, Andrei Rodin, Sergio Branciamore, Peter P. Lee (City of Hope)

Cytokines provide communication among immune cells. Our group developed an experimental approach, based on multiparametric flow cytometry of peripheral blood mononuclear cells, taken from healthy individuals and breast cancer patients. In contrast to traditional studies that focus on individual cytokine applications, we also investigate short-term immune cell response to simultaneous application of pairs of cytokines. Our results reveal that in general cytokine combination effects cannot be predicted in additive manner, with some combinations yielding strong antagonistic outcomes. The most prominent of them is inhibition of IL-10 action, witnessed by decreased phosphorylation levels of STAT3 within 15 minutes of cytokines application. This effect manifests itself for all of studied types of immune cells across combinations of IL-10 with all of studied interleukins, as well as with IFN-α, IFN-γ and TGF-β. In contrast, IL-4 shows opposite behavior, as pSTAT6 levels remain unaffected by concurrent application of other cytokines. Preliminary data suggests that inhibition of IL-10 signal transmission is more pronounced in breast cancer patients, potentially contributing to the suppression of IL-10-mediated immunoregulatory functions in them.

21. Modeling reveals the strength of weak interactions in stacked ring assembly

Leonila Lagunes & Eric J. Deeds (UCLA)

Cells employ many large macromolecular machines for the execution and regulation of processes that are vital for cell and organismal viability. Interestingly, cells cannot synthesize these machines as functioning units. Instead, cells synthesize the molecular parts that must then assemble into the functional complex. Many important machines, including chaperones like GroEL and proteases like the proteasome, are comprised protein rings that are stacked on top of one another. While there is some experimental data regarding how stacked-ring complexes like the proteasome self-assemble, a comprehensive understanding of the dynamics of stacked ring assembly is currently lacking. Here, we developed a mathematical model of stacked trimer assembly, and performed an analysis of the assembly of the stacked homomeric trimer, which is the simplest stacked ring architecture. We found that stacked rings are particularly susceptible to a form of kinetic trapping that we term “deadlock,” in which the system gets stuck in a state where there are many large intermediates that are not the fully-assembled

structure, but that cannot productively react. When interaction affinities are uniformly strong, deadlock severely limits assembly yield. We thus predicted that stacked rings would avoid situations where all interfaces in the structure have high affinity. Analysis of available crystal structures indicated that indeed the majority – if not all – of stacked trimers do not contain uniformly strong interactions. Finally, to better understand the origins of deadlock, we developed a formal pathway analysis and showed that, when all the binding affinities are strong, many of the possible pathways are utilized. In contrast, optimal assembly strategies utilize only a small number of pathways. Our work suggests that deadlock is a critical factor influencing the evolution of macromolecular machines, and provides general principles for not only understanding existing machines but also for the design of novel structures that can self-assemble efficiently.

22. Drug Repurposing to Address Temozolomide Resistance in Glioblastoma

Judith Landau & Nicholas Graham (USC)

Glioblastoma is the most common brain cancer in adults and also the most lethal. A significant barrier to treatment is resistance to temozolomide, the standard chemotherapy for the tumors. Glioblastoma with high levels of MGMT (O6-methylguanine-DNA methyltransferase) are even more resistant because this enzyme can repair DNA alkylation caused by temozolomide. We used a computational tool recently developed in our lab, drug mechanism enrichment analysis (DMEA), to predict drugs to which MGMT-high and temozolomide-resistant glioblastoma would be sensitive. DMEA is a software intended to aid in drug repurposing by identifying drug mechanisms of action that are likely effective for treating diseases in which they have not been explored before. Using correlation coefficients from MGMT protein expression data and the quantified drug response, DMEA predicted that MGMT-high tumors would be sensitive to vitamin D receptor agonists and bromodomain inhibitors. We also employed DMEA's ability to analyze gene expression data from an experiment comparing U251 cells before and after they acquired resistance to temozolomide, and both vitamin D receptor agonists and bromodomain inhibitors were also identified here. These results encourage the evaluation of both drug classes for their ability to reduce temozolomide resistance in glioblastoma and indicates that they may improve treatment outcomes alone or in combination with temozolomide.

23. Deciphering B-cell Evolutionary Dynamics: A Mathematical Modeling Approach

Chengyuan Li, Haripriya Vaidehi Narayanan, Alexander Hoffmann (UCLA)

The antibody response arises from an evolutionary process, where precursor B-cells undergo diversification of their immune receptors through mutation, followed by the selective expansion of clones with high affinity for the target. This evolution relies on the cellular processes of mutation, cell survival/death decision, and cell division, whose probabilities are determined by molecular regulatory networks. Since this complex biological process cannot be reproduced outside living organisms, the evolutionary trajectories of B-cells cannot be directly observed or traced, but must be inferred by sequencing the B-cell receptors and constructing a phylogenetic tree. However, the relationships between phylogenetic tree structure and the cellular parameters of mutation, cell survival/death decision, and cell division are unknown. We developed a mathematical model that connects phylogenetic tree topology with B-cell-intrinsic processes during antibody evolution, by parameterizing the propensity of B-cells to mutate, survive, or proliferate. We performed Monte Carlo simulations of B-cell phylogenetic trees across varying B-cell mutation and selection rates, and analyzed their distributions of graph-theoretic topological metrics, in particular the statistical moments of the root-to-tip depth and the out-degree at internal nodes. Our preliminary results indicate that the combination of mean depth and maximum out-degree is a sensitive and specific metric that identifies changes in selection rate, while the combination of skewness of depth and mean out-degree likewise characterizes variations in mutation rate. This allows us to infer cell decision parameters from the observed topologies of phylogenetic trees. Our model begins to provide a quantitative understanding of multi-scale immunological processes, connecting behaviors at the cellular scale to emergent properties of the immune response at the organism scale.

24. Modeling of CAR T-cell treatment in glioblastoma in PDE and Agent-Based approaches

Runpeng Li, Christine Brown, Russell Rockne, Michael Barish, Heyrim Cho (UCR)

Chimeric Antigen Receptor (CAR) T-cell based immunotherapy has been considered one of the most successful adoptive cell-based immunotherapy in cancer treatment and has been FDA-approved since 2017. While CAR T-

cell therapy has shown its efficacy in leukemia, for solid tumors, the treatment still has a few challenges including (1) trafficking CAR T-cells into solid tumors, (2) a hostile tumor microenvironment that suppresses T-cell activity, and (3) tumor antigen heterogeneity. In my presentation, we mainly investigated the antigen heterogeneity of high-grade glioma and explored the response spatially by agent-based model and PDE model. We used the data provided by City of Hope to simulate and calibrate our model through PhysiCell and were able to optimize the treatment schedule, location of injection, and dosages. More importantly, our model is also capable of capturing the heterogeneity in cancer tissue and allows us to make personalized adjustments on treatments based on the spatially varied antigens.

25. Reliable ligand discrimination in stochastic multistep kinetic proofreading: First passage time vs. product counting strategies

Xiangting Li, Tom Chou (UCLA)

Cellular signaling, crucial for biological processes like immune response and homeostasis, relies on specificity and fidelity in signal transduction to accurately respond to stimuli amidst biological noise. Kinetic proofreading (KPR) is a key mechanism enhancing signaling specificity through time-delayed steps, although its effectiveness is debated due to intrinsic noise potentially reducing signal fidelity. In this study, we reformulate the theory of kinetic proofreading (KPR) by convolving multiple intermediate states into a single state and then define an overall ``processing" time required to traverse these states. This simplification allows us to succinctly describe kinetic proofreading in terms of a single waiting time parameter, facilitating a more direct evaluation and comparison of KPR performance across different biological contexts such as DNA replication and T cell receptor (TCR) signaling. We find that loss of fidelity for longer proofreading steps relies on the specific strategy of information extraction and show that in the first-passage time (FPT) discrimination strategy, longer proofreading steps can exponentially improve the accuracy of KPR at the cost of speed. Thus, KPR can still be an effective discrimination mechanism in the high noise regime. However, in a product concentration-based discrimination strategy, longer proofreading steps do not necessarily lead to an increase in performance. However, by introducing activation thresholds on product concentrations, can we decompose the product-based strategy into a series of FPT-based strategies to better resolve the subtleties of KPR-mediated product discrimination.

Our findings underscore the importance of understanding KPR in the context of how information is extracted and processed in the cell.

26. Flexible strategies for selective delivery of diverse cytokine signals to Tregs

Emily Lin (presenter), Helen Kaidantzis, Brian Orcutt-Jahns, Aaron Meyer (UCLA)

Regulatory T cells (Tregs) are essential in regulating our immune systems, and many immunotherapeutic ligands have been used to target and selectively activate Tregs to treat autoimmune diseases. However, there are still many barriers to selectively targeting Tregs, such as a lack of distinct Treg surface markers, making specifically targeting Tregs without affecting other off-target immune cells difficult. One of the most unique surface markers for Tregs has been identified as the alpha chain of the high-affinity interleukin-2 receptor (IL-2Rα, CD25), and is often targeted in Treg-specific therapeutics using IL-2 and its mutants. However, other ligands have also been shown to promote Treg's suppressive capabilities, such as TGF-β, IL-7, and IL-10, which act through pathways different from IL-2's JAK3/STAT5 pathway, and thus could better potentiate Treg activity.

Here, we developed a flexible computational model to assess the utility of multivalent and multispecific ligands to selectively deliver diverse cytokine signals to Tregs. Previous work has found that tetravalent IL-2, an IL-2 ligand with multiple Fc-fused IL-2 muteins, increases selectivity for Tregs, and we explore that this multivalency approach could be extended to selectively deliver alternative cytokine signals. Further, we investigated whether multispecific targeting (targeting of two or more different surface markers), may enhance Treg targeting by allowing us to more holistically target the heterogeneous Treg population. We first investigated whether receptors in combination could uniquely identify Tregs in a single-cell dataset by calculating 2D distance metrics for receptor pairs. Then, using a multivalent binding model, we found that multispecificity can provide a substantial selectivity gain when targeting Tregs, but this gain is negligible when compared to the selectivity gain that multivalency offers for IL-2 muteins. Having identified multivalency as the most effective strategy for Treg targeting, we profiled the delivery of IL-7 signal to Tregs using a tetravalent IL2Rα-targeting IL-7 mutant, and

found that we could greatly enhance the specificity with which IL-7 was delivered to Tregs. Moving forward, we will perform single-cell RNA-sequencing on Tregs treated with different cytokines to identify additional signals that would provide a unique proliferation or suppression advantage outside of IL-2.

27. Temporal signals from mesenchymal niche fibroblasts regulate hair growth

Yingzi Liu (UCI)

Hair follicles (HFs) are stem cell-rich mini-organs that repetitively renew via cyclical process, known as the hair growth cycle. Dermal papilla (DP) fibroblasts are the key mesenchymal niche cell type of the HF, and they regulate cyclical hair growth by orchestrating paracrine signaling crosstalk with epithelial HF progenitors. However, time-dependent changes that DP niche undergoes during HF growth remain only partially understood. We used single-cell RNA-sequencing (scRNA-seq) and functional experiments to discover new time-dependent features of the DP niche program. Time-series scRNA-seq revealed dynamic heterogeneity of DP cell states, with early states becoming depleted and late states emerging throughout the process of anagen progression. Moreover, signaling network inference analysis using CellChat identified dynamic Scube3 expression by DP cells, that was tightly linked to new hair growth initiation. In situ expression results showed that Scube3 was expressed only in DPs of growing, but not resting HFs. Germline Scube3-/- mice showed distinct anagen initiation delay. Furthermore, intradermal microinjection of SCUBE3 protein was sufficient to activate new hair growth. DP-enriched expression of SCUBE3 and its growth-activating effect were partially conserved in human scalp HFs. Thus, we posit that time-dependent cellular state and signaling heterogeneity in the DP niche are essential for proper hair cycle.

28. Systems serology profiling of anti-tumor antibodies in high-grade serous ovarian cancer

Michelle Loui (UCLA), Scott Taylor (Xencor), Crystal Xiao (UCLA), Het Desai (UCLA), Cyrillus Tan (UCLA), Jackson Chin (UCLA), Meera Trisal (UCLA), Allison Brookhart (UCLA), Sophia Ting (UCLA), Gabriella DiBernardo (UCLA), Laura B. James-Allan (UCLA), Sanaz Memarzadeh (UCLA), Aaron S. Meyer (UCLA)

In high-grade serous ovarian cancer (HGSOC) patients, malignant epithelial cells arise from the fallopian tube and ovarian surface epitheliums. Endogenous antibodies (anti- tumor antibodies; ATAbs) target these cells and should promote recognition by the immune system. Patient-derived tumors have been found to be frequently coated in IgG, and ATAbs are present both in the tumor mass and in the fluid that builds up in the peritoneum surrounding the tumor microenvironment. They are derived from B cells that have undergone somatic hypermutation, indicating an active immune response. However, despite their widespread abundance in HGSOC, ATAbs fail to eliminate the tumor cells. We hypothesized that ATAbs are unable to eliminate tumors due to the dysregulation of immune interactions via their Fc region. Therefore, we applied a quantitative, multiplexed assay for profiling the Fc properties and immune receptor interactions of ATAbs. Understanding the mechanisms of humoral immunity evasion will help with the prediction of therapeutic responses in cancer patients and uncover how immunotherapies might reactivate effective humoral immunity.

29. A network model for the growth of a bacterial population in adverse environments

Moitrish Majumdar (UC Merced)

Bacterial populations can consist of several isogenic subpopulations known as phenotypes. Individuals in a bacterial population can switch from one phenotype to another in order to adapt to changing environments. Phenotypic switching can thus confer survival benefits to a bacterial population and may be a mechanism for the development of antibiotic resistance. Kussell and Leibler in "Phenotypic diversity, population growth, and information in fluctuating environments" ("Science", 2005), studied a model of a bacterial population consisting of “n” phenotypes and “n” environments. The population is subjected to these “n” different environments, which occur in a random sequence. In a given environment, a single phenotype is the “fittest", which implies that individuals of that phenotype have the largest growth rate. Kussell and Leibler derived an analytical expression for the Lyapunov exponent, which is a measure of the asymptotic growth rate of the total population. We study a modified version of their model by constructing a network model of the bacterial population, such that each environment can be thought of as a specific antibiotic where the corresponding “fittest” phenotype is resistant and drives the population growth. In our network model, each node represents a single phenotype, and each directed edge represents the ability of one phenotype to switch to some other phenotype. Kussell and Leibler derived an analytical expression for the Lyapunov exponent under the assumption that all the inter-phenotypic switching

pathways are available. We compute the Lyapunov exponent of bacterial populations corresponding to different network models experimentally and analytically, using some approximations. We find that our approaches can be used to compute the Lyapunov exponent when some of these switching pathways are restricted, i.e. some edges are removed from the network. We also derive some results about the growth-maximizing inter-phenotypic switching rates, encoded as the network edge weights, for sparse networks where multiple edges are removed.

30. Gene regulatory network inference with popInfer reveals dynamic regulation of hematopoietic stem cell quiescence upon diet restriction and aging

Megan K. Rommelfanger (USC), Marthe Behrends (FLI), Yulin Chen (FLI), Jonathan Martinez (USC), Martin Bens (FLI), Lingyun Xiong (UC), K. Lenhard Rudolph (FLI) and Adam L. MacLean (USC)

Single-nuclei multiomic data offer new means to learn gene regulatory networks (GRNs). Temporal information can be derived from snapshot data using joint measurements of gene expression (RNA) and chromatin accessibility (ATAC) in the same single cells. We developed popInfer to infer GRNs that characterize lineage-specific dynamic cell state transitions. Benchmarking against alternatives for GRN inference, we showed that popInfer outperforms on hematopoietic data. In application to early cell fate decisions during hematopoiesis, popInfer predicted GRNs regulating the transition from stem to multipotent progenitor cell state. Upon aging or dietary perturbations, we discovered a crucial network controlling entry to/exit from stem cell quiescence that changes dynamically during dietary restriction, and the effects of which are attenuated with aging.

31. Cell type labeling of single-cell RNA sequencing data of hematopoietic stem and progenitor cells enables study of aging effects

David Mastro (UCLA), Apeksha Singh (UCLA), Noa Popko (UCLA), Jennifer J. Chia (UCLA), Alexander Hoffmann (UCLA)

Recognizing established cell types that have been defined by their function, morphology, or cell surface markers, is difficult when only gene expression data is available, such as in single-cell RNA sequencing (scRNA-seq) experiments. In this study, we developed a method to assign cell identities in hematopoietic stem and progenitor cell (HSPC) scRNA-seq datasets to learn how aging affects hematopoiesis. HSPCs have been classified into five subsets: long term hematopoietic stem cells (LT-HSCs), short term hematopoietic stem cells (ST-HSCs), and three multipotent progenitors with varying differentiation potential (megakaryocyte/erythroid-biased MPP2s, myeloid biased MPP3s, and lymphoid biased MPP4s). These cell types are classically defined by the presence or absence of cell surface markers that are not well captured by gene expression data. Thus, we classified these cell types in scRNA-seq datasets by training a multinomial regression model on reference microarray data to identify a marker gene coefficient matrix and then tested its performance on scRNA-seq data derived from surface marker defined and flow cytometry sorted HSPC subsets. We then applied our model to label published scRNAseq datasets of young and aged murine and human HSPCs. Our cell type labeling recapitulated similar cell type compositions as previously reported. Furthermore, differential gene expression analysis between young and aged LT-HSCs recognized increased expression of myeloid and quiescence related gene signatures, consistent with well-established phenotypes of HSC aging.

32. Mathematical Model of Intermediate States in Epithelial-Mesenchymal Transition

MeiLu McDermott & Adam MacLean (USC)

The epithelial-mesenchymal transition (EMT) is a primary biological mechanism of cancer metastasis, involving cells transforming from an adhesive epithelial phenotype to a migratory mesenchymal phenotype. Recent research has identified intermediate EMT states, characterized by hybrid phenotypes experimentally shown to be metastatic. This comparative study investigates these hybrid EMT cells across multiple cancers using single-cell RNA sequencing data. We identified genes upregulated in multiple intermediate EMT states across cancers. Additionally, we developed a mathematical model using ordinary differential equations (ODEs) to describe EMT rates and fitted the model to scRNAseq data. Incorporating data from multiple cancer types, our ODE model provides a discovery tool for identifying genes associated with stabilizing the existence of metastatic, intermediate state EMT cells.

33. Assessing the robustness and reproducibility of RNA-seq quantification tools

Fatemeh Mohebbi, Mohammad Vahed, Fangyun Liu, Serghei Mangul (USC)

One of the fundamental steps in RNA-Seq analysis is to estimate the abundance of genes and transcripts in biological samples. Thus far, numerous quantification tools have been developed to accurately estimate gene and transcript expression levels. Inconsistencies in gene and transcript quantification could have significant implications for the accuracy of diagnostic or therapeutic decisions. It is, however, difficult to achieve accurate and consistent gene expressions due to the presence of experimental variations. Currently, it is unknown which types of experimental variations RNA-Seq quantification tools can mitigate and maintain consistent results and which they cannot account for. Exiting efforts attempting to assess the consistency and reproducibility of quantification tools' results are limited and some of the widely used quantification tools such as Salmon and Kallisto have not undergone a thorough consistency assessment. In this study, we have developed a framework with a scoring metric scheme to evaluate the robustness and reproducibility of RNA-Seq quantification tools.

We studied over ten popular tools and compared the consistency of their gene and transcript expression estimates across both synthetic and real technical replicates. Incorporating both types of replicates allowed us to observe the effect of different experimental variations and the inconsistencies introduced by the tools themselves on the quantification results.

Our analysis revealed a notable disparity in the ability of the quantification tools to maintain consistent estimation of gene and transcript expressions across both technical and synthetic replicates. Importantly, expectation-maximization and mapping-based tools were more effective at maintaining consistency compared to pseudoalignment tools.

34. Role of Notch Dynamics in driving Muscle Stem Cell Fate Specification

Tamas L Nagy & Thomas A Rando (UCLA)

After muscle injury, resident muscle stem cells (MuSCs) exit their dormant state, proliferate, differentiate and fuse into new myotubes to regenerate the tissue. However, a small portion of this population de-differentiates and returns to the dormant state, ready for the next regeneration event. Importantly, the number of cells undergoing this dedifferentiation is tightly controlled at the tissue-level. While we know that highly dynamic signaling pathways like Notch are critical to this decision-making, the importance and role of the actual dynamics is much less clear. We will answer this question by leveraging our in vitro primary mouse MuSC differentiation model that allows us to watch single cell decision-making using time-lapse microscopy. We propose combining live cell biosensors, single cell tracking, and optogenetics to test the necessity and sufficiency of Notch dynamics on muscle stem cell fate specification. This work will improve our understanding of how stochastic and dynamic signaling at the single cell level can lead to robust tissue-scale behavior.

35. A eukaryotic circuit for secretion-coupled cellular autonomy

Lingxia Qiao (UCSD); Pradipta Ghosh (UCSD); Padmini Rangamani (UCSD)

Cancers represent complex autonomous systems, displaying self-sufficiency in growth signaling. Autonomous growth is fueled by a cancer cell's ability to 'secrete-and-sense' growth factors: a poorly understood phenomenon. Using an integrated systems and experimental approach, here we dissect the impact of a feedback-coupled GTPase circuit within the secretory pathway that imparts secretion-coupled autonomy. The GTPase circuit is assembled when the Ras-superfamily monomeric GTPase Arf1, and the heterotrimeric GTPase Gi and their corresponding GAPs and GEFs are coupled by GIV/Girdin, a protein that is known to fuel aggressive traits in cancers. This GTPase circuit ensures the dose information transmission by achieving the dose response alignment behavior of sensing and secretion, leading to self-sustained cell survival by stimulus-proportionate secretion. Findings highlight how enhanced coupling of two biological switches in cancer cells is critical to secretion-coupled autonomy.

36. Predicting postmortem neuropathology of Alzheimer's disease and related dementias using widely accessible clinical data

Yueqi Ren & Craig Stark (UCI)

Objectives: Faced with a rapidly aging population and the rising prevalence of Alzheimer's disease (AD) and related dementias, the field needs to urgently consider screening tools that utilize widely accessible data modalities. We have previously shown that lower-cost data, operationalized as data modalities accessible at primary care visits, can indeed accurately predict AD clinical diagnosis and that clustering these data can provide useful information. Here, we apply a similar approach to predicting histopathological status.

Methods: We first applied our previously developed feature extraction method based on a supervised encoder (SE) to transform potentially noisy input features while maintaining or amplifying relevant information. We next performed classification and clustering to stratify subjects by their neuropathology. Data for this study come from the National Alzheimer's Coordinating Center, funded by NIA/NIH Grant U24 AG072122 and contributed to by NIA-funded ADRCs.

Result: We found that relatively high classification accuracy of neuropathologic lesions was possible using widely accessible, lower cost clinical data. We identified distinct trajectories of subjects based upon changes in cluster assignment over time. These trajectory subgroups have significantly different risk of showcasing each type of neuropathologic lesion obtained from postmortem neuropathology.

Conclusions: Our framework benefits from the combined strengths of clustering and classification methods while avoiding drawbacks of unsupervised methods. By using lower cost features, this work is broadly generalizable and has direct implications for screening of neuropathologic lesions of AD and related dementias for the public.

37. Selection and Exploration of Models of Malignant Myelopoiesis and Tyrosine Kinase Therapy

Jonathan Rodriguez, Abdon Iniguez, Nilamani Jena, Prasanthi Tata, Zhong-Ying Liu, Arthur D Lander, John Lowengrub, Richard A Van Etten (UCI)

Chronic myeloid leukemia (CML) is a blood cancer characterized by dysregulated production of maturing myeloid cells driven by the product of the Philadelphia chromosome, the BCR-ABL1 tyrosine kinase. Tyrosine kinase inhibitors (TKI) have proved effective in treating CML but there is still a cohort of patients who do not respond to TKI therapy even in the absence of mutations in the BCR-ABL1 kinase domain that mediate drug resistance. To discover novel strategies to improve TKI therapy in CML, we developed a nonlinear mathematical model of CML hematopoiesis that incorporates feedback control and lineage branching. Cell-cell interactions were constrained using an automated model selection method together with previous observations and new in vivo data from a chimeric BCR-ABL1 transgenic mouse model of CML. The resulting quantitative model captures the dynamics of normal and CML cells at various stages of the disease, exhibits variable responses to TKI treatment, predicts key factors of refractory response to TKI treatment, and predicts potential combination therapy efficacy. Recent experiments reveal that interactions and competition between different cellular compartments and between normal and BCR-ABL1-expressing cells form a threshold that determines whether the malignant cells can expand and cause leukemia. To capture these experimental dynamics, we found it necessary to incorporate additional biological factors through the introduction of new cell types and interactions. We applied an adapted model selection scheme to explore the unknown cell-cell interaction space and find subsets of models consistent with experimental dynamics. We analyzed common motifs across experimentally consistent models and identified interactions as targets for experimental design to further narrow the valid models.

38. A mathematical model reveals the regulatory logic of interferon-β expression

Allison Schiffman, Zhang Cheng, Diana Ourthiague, and Alexander Hoffmann (UCLA)

The expression of type I interferon (IFNβ), a critical determinant of the innate immune response, is controlled by a regulatory region that contains one binding site for NFκB (κB) and two binding sites (IRE and gIRE) for members of the IRF family. Classic molecular biology studies of IFNβ expression suggest that NFκB and IRF function synergistically by forming an 'enhanceosome' complex. However, this synergy is not a simple AND gate, as knockout mouse studies have shown that NFκB is only required for transcription of IFNβ induced by specific stimuli. We developed a quantitative thermodynamic state model that captures the stimulus-specific requirement for NFκB. Examining the parameters revealed that differential affinities of the two IRF binding sites are critical for this regulatory logic and that IFNβ expression may be mediated by synergistic pairing of either the two adjacent IRFs (IRE+gIRE) or adjacent IRF-NFκB (gIRE+κB) complexes. Through these pairings, the three binding sites form a double AND gate with each being dominant for a subset of IFNβ-inducing stimuli. We then asked whether

the model could also account for the reported role of NFκB p50:p50 in enforcing stimulus-response specificity by blocking basal IRF binding to the second IRF binding site. We found that this modeling framework is insufficient to capture the data from p50 knockouts, suggesting that p50:p50 alters the actual binding kinetics of IRF to its cognate site. We will explore this possibility with a kinetic formulation of the IFNb regulatory logic.

39. Insights into Bud Morphogenesis Dynamics in Aging Yeast

Navaira Sherwani (UCR)

Understanding cellular aging is crucial for extending organismal lifespan and studying age-related degenerative diseases. In budding yeast recent experiments revealed two aging modes—nucleolar and mitochondrial decline. Bud dimensions measured over a cell cycle show linear growth in both modes. In one, cells maintain bud size and spherical shape, whereas bud size increases and shape becomes tubular in the other. We introduce a chemical-mechanical coupled model predicting that linear bud growth results from delivering new cell surface materials to Cdc42 polarization at a constant rate. Simulations confirm the generation of elongated buds by locally inserting materials at the bud tip. These findings suggest cellular aging may impact the maintenance of chemical signaling polarization that directs the delivery of new materials. Our aim now is to simulate aging under varying environmental conditions with cellular signaling and determine the role played by the septin neck ring and actin cables.

40. Leveraging gene correlations in single cell transcriptomic data

Kai Silkwood, Emmanuel Dollinger, Josh Gervin, Scott Atwood, Qing Nie, Arthur D Lander (UCI)

Many approaches have been developed to overcome technical noise in single cell RNA-sequencing (scRNAseq). As researchers dig deeper into data—looking for rare cell types, subtleties of cell states, and details of gene regulatory networks—there is a growing need for algorithms with controllable accuracy and fewer ad hoc parameters and thresholds. Impeding this goal is the fact that an appropriate null distribution for scRNAseq cannot simply be extracted from data when ground truth about biological variation is unknown (i.e., usually). We approach this problem analytically, assuming that scRNAseq data reflect only cell heterogeneity (what we seek to characterize), transcriptional noise (temporal fluctuations randomly distributed across cells), and sampling error (i.e., Poisson noise). We analyze scRNAseq data without normalization—a step that skews distributions, particularly for sparse data—and calculate p-values associated with key statistics. We develop an improved method for selecting features for cell clustering and identifying gene-gene correlations, both positive and negative. Using simulated data, we show that this method, which we call BigSur (Basic Informatics and Gene Statistics from Unnormalized Reads), captures even weak yet significant correlation structures in scRNAseq data. Applying BigSur to data from a clonal human melanoma cell line, we identify thousands of correlations that, when clustered without supervision into gene communities, align with known cellular components and biological processes, and highlight potentially novel cell biological relationships. New insights into functionally relevant gene regulatory networks can be obtained using a statistically grounded approach to the identification of gene-gene correlations.

41. Stimulus-Response signaling dynamics characterize macrophage polarization states

Apeksha Singh, Supriya Sen, Michael Iter, Adewunmi Adelaja, Alexander Hoffmann (UCLA)

The functional states of many cells are dependent on microenvironmental context. Prior studies described how polarizing cytokines alter macrophage transcriptomes and epigenomes. However, functional cell states may involve kinetic information that is not captured by single cell measurements of mRNA abundances or epigenetic modifications at a single steady-state timepoint. Here we characterized the functional responses of 6 differentially polarized macrophages by measuring the dynamics of transcription factor NFκB in response to 8 stimuli. The resulting dataset of single-cell NFκB trajectories were analyzed by three approaches: 1) Machine learning on time-series data revealed losses of stimulus distinguishability with polarization, reflecting canalized effector functions. 2) Informative trajectory features driving stimulus distinguishability ('signaling codons') were identified and used for mapping a cell state landscape, that could then locate macrophages conditioned by an unrelated condition. 3) Kinetic parameters, inferred using a mechanistic NFκB network model, provided an alternative mapping of cell states and correctly predicted biochemical findings. Together, this work demonstrates that a single analyte's dynamic trajectories may distinguish functional states of single cells and furthermore, reveal the molecular network states underlying them that may not be readily discerned from other "omics" profiling.

42. A systems biology approach to deciphering the health impacts of the most commonly used cooking oil in the U.S.

Frances M. Sladek, Poonamjot Deol, Jose Martinez-Lomeli, Margarita Curras-Collazo, James Borneman, John Leano, Gary Chen, Patrick H. Degnan (UCR)

Soybean oil (SO) is the most commonly used cooking oil in the U.S. and its use is increasing worldwide. As a plant-derived oil with low saturated fat, it was originally assumed that it would be much healthier than other fats, especially saturated ones. However, our results over the past several years have shown that a high fat diet (HFD) based on SO, similar to the current American diet, is actually more obesogenic and diabetogenic than other HFDs, and increases susceptibility to colitis in mice. To investigate underlying molecular mechanisms of the adverse effects of excess SO in the diet, we have performed transcriptomic, proteomic, cistromic, metabolomic and microbiome analysis using a variety of diets in multiple tissues – liver, blood, intestines and hypothalamus. This presentation will provide an overview of these systems biology approaches and our results thus far including those from a mouse model with the nuclear receptor HNF4a that is resistant to SO-induced obesity and colitis.

43. Identifying Critical Immunological Features of Tumor Control and Escape Using Mathematical Modeling

Rachel Sousa, John Lowengrub, Francesco Marangoni (UCI)

The immune system can eradicate cancer, but various immunosuppressive mechanisms active within a tumor curb this beneficial response. Cytotoxic T cells (CD8s), regulatory T cells (Tregs), and antigen-presenting dendritic cells (DCs) play an important role in the immune response; however, it is very cumbersome to unravel the effects of multimodal interactions between tumor and immune cells and their contributions to tumor control using an experimental approach alone. Thus, to better understand the mechanisms that govern the interactions between immune cells and tumor cells and to identify the critical immunological features associated with tumor control and tumor escape, we built a mechanistic mathematical model of CD8s, Tregs, DCs, and tumor cells.

We used an automated model selection procedure known as Design Space Analysis to identify regulatory feedback mechanisms sufficient to reproduce experimentally observed behaviors that regulate the immune system and determined stable regions of parameter space. The model accounts for tumor immunogenicity, the effects of IL-2 prolonging T cell lifespan, Treg suppression of antitumor immune response through CTLA-4, recruitment of immune cells into the tumor environment, and interferon-gamma upregulation of PD-L1 on DCs and tumor cells to deactivate T cells.

Our tumor-immune model exhibits bistability in which both a tumor-free and a tumor state exist and are stable. We use the model to explore how the initial immunological conditions dictate the final tumor state and ultimately impinge the success of immunotherapy. We also use the model to make inferences on the mechanisms of resistance to various immunotherapies and identify immunotherapy scheduling to stimulate CD8 but not Treg activation. We are currently expanding our model to include spatial dynamics of cellular interactions, guided by intravital imaging data, to understand how the spatial distribution of T cells contributes to tumor control and how that can be exploited to develop more efficacious immunotherapies.

44. Graph homogeneity analysis of single-cell epigenetic states

Breanne Sparta, Timothy Hamilton and Eric J. Deeds (UCLA)

The prevailing interpretation of Waddington's landscape is that attractors in gene expression space produce and stabilize distinct cell types. This hypothesis motivates the standard practice of clustering cells in single-cell omics data, prior to the analysis of differential gene expression. Yet in practice, the analysis of single-cell data has revealed incredible heterogeneity in cell state, regardless of the particular measurement technology employed. Indeed, the ubiquitous fractal-like density distributions of single cells in epigenetic space are inconsistent with the expected densities that would be produced near a cell-type attractor. As such, the extreme heterogeneity of single-cell data poses a challenge for the robust identification of cell types in epigenetic space. For example, when the standard analytical pipeline applies nonlinear transformations and dimensionality reduction steps to fractally distributed data, the produced cell-type clusters are often very parameter sensitive and difficult to reproduce. In this work, we propose an alternative method for asking questions of single-cell data that is directly informed by

the underlying structure of the data. We develop a graph-homogeneity approach to more finely characterize how tissue composition changes in health and disease. Without clustering the data, we characterize differences in gene expression across local regions of interest, and report reproducible findings across experimental replicates.

45. Mathematical Modeling Predicts Treatment Efficacy in Transthyretin Cardiac Amyloidosis

Ashley F Stein-Merlob, Lihua Jin, Alan Garfinkel (UCLA)

Introduction: Increased recognition of transthyretin cardiac amyloidosis (ATTR-CA) led to identification of multiple treatment targets. Optimal patient selection, treatment choice, timing and duration remain unclear. We evaluated the efficacy and timing of common and emerging ATTR-CA treatments with a mathematical model based on therapeutic mechanisms.

Methods: We developed a system of ordinary differential equations describing ATTR deposition and myocardial clearance by clusterin (Figure 1A,B). Published data and equilibrium analyses determined parameters for initial maximal ATTR density. Mechanisms of four therapies were modeled and simulated varying efficacy. Complete inhibition defines 100% efficacy, i.e. full stabilization of tetramer. Expected clinical efficacy (ECE) is based on studies of clinical doses. Minimum effective clearance (MEC) is the theoretical efficacy required to reduce ATTR fibrils below the threshold for complete clearance.

Results: All treatments reduced ATTR fibril density, correlating with efficacy (Fig 1C). At ECE, only NI006 demonstrated complete clearance of ATTR fibrils. With other current treatment strategies, amyloid density was lowered but never cleared. The secondary mechanism of diflunisal did not change equilibrium behavior compared to tafamidis. MEC and time to clearance at MEC were lowest for NI006 (14.7%; 65 days) and doxycycline (16.1%; 36 months). Tetramer stabilization required almost complete efficacy (99.3%) and about a decade (119 months) for clearance.

Conclusion: This mathematical model predicts ATTR clearance by four distinct mechanisms. The novel anti-ATTR antibody NI006 is a promising treatment with predicted faster onset and higher effectiveness for myocardial clearance of ATTR fibrils. This model can evaluate future treatment strategies and therapeutic targets.

46. The structure is the message: preserving experimental context through tensor decomposition

Cyrillus Tan and Aaron S. Meyer (UCLA)

Recent biological studies have been revolutionized in scale and granularity by multiplex and high-throughput assays. Profiling cell responses across several experimental parameters, such as perturbations, time, and genetic contexts, leads to richer and more generalizable findings. However, these multidimensional datasets necessitate a reevaluation of the conventional methods for their representation and analysis. Traditionally, experimental parameters are merged to flatten the data into a two-dimensional matrix, sacrificing crucial experiment context reflected by the structure. As Marshall McLuhan famously stated, “The medium is the message.” In this work, we propose that the experiment structure is the medium in which subsequent analysis is performed, and the optimal choice of data representation must reflect the experiment structure. We introduce tensor-structured analyses and decompositions to preserve this information. We contend that tensor methods are poised to become integral to the biomedical data sciences toolkit.

47. Characterizing nascent strand DNA methylation within long-read sequencing data

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Multiple layers of epigenetic modifications are thought to act coordinately to direct chromatin architecture and establish the global epigenetic landscape. For example, CpG methylation is generally associated with repressive histone modifications and condensation of genomic DNA. Multiple bidirectional modes of crosstalk between these epigenetic marks and/or their associated writers/readers have been proposed to mutually reinforce silencing of genes. However, largely missing from the current picture of multi-layered cross-talk is the dynamics of how these marks propagate and influence each other in time and space. Given that the epigenome is dismantled and reestablished with every cycle of DNA replication, cross-talk between epigenetic modifiers/modifications can be hypothesized to play a role in reshaping chromatin architecture on replication-associated (i.e., sub-cell-cycle)

timescales. We posit that the dynamics of these modifications on sub-cell-cycle timescales could provide insights into these mechanisms of epigenetic cross-talk, yet they remain poorly characterized largely due to technological limitations. Our labs have previously demonstrated that global CpG methylation is reestablished with a pronounced temporal delay following genome replication and that neighboring CpG sites exhibit correlated remethylation kinetics. To further elucidate the replication-associated temporal dynamics of epigenetic modifications (and epigenetic cross-talk), this project aims to identify potential correlations between DNA methylation patterns and DNA strand maturity. We combined BrdU labeling with long-read sequencing to track DNA methylation patterns on nascent chromatin over time. Using long-read methylation measurements and correlation metrics, we demonstrate that nascent and mature chromatin can be separated through computational analysis. Thus, our data suggest that DNA methylation patterns can be used to predict post-replication DNA maturity and (when coupled with other protein-mapping DNA modification strategies) could support a platform technology to better elucidate epigenetic cross-talk at sub-cell-cycle timescales.

48. Multivariate Antibody-Dependent Effector Response Profiling

Meera Trisal, Manmeet Bains, Scott Taylor, Victoria Gong, Kalyan Pande, Aaron S. Meyer (UCLA, Merck)

Antibody-dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP) are key protective functions of antibodies. They are regulated by a variety of factors, such as the affinity of the Fc region of the antibody to the Fc receptors of immune cells, the abundance of cell surface antigens, the cell types present within a tissue, and the expression of each Fc receptor. This project aims to quantitatively profile ADCC, ADCP, and cytokine responses across multiple dimensions. Four variants of the anti-EGFR monoclonal antibody Cetuximab, each with a different alteration in the Fc region, are used in this study. PC-9 and A549 are two lung cancer cell lines used as a model of the target antigens. Monocytes and NK cells isolated from PBMCs are used as the immune effector cells. Previous work has shown that, compared to A549 cells, PC-9 cells have higher total expression of EGFR. Flow cytometry results, however, indicated that Cetuximab bound to A549 cells roughly three-fold more than to PC-9 cells. Cytokine release from co-culture experiments elucidated a lack of IL-6 and TNF-α response from NK cells and monocytes when using PC9, whereas both effector cells showed distinct dose response profiles with A549. The results demonstrate that several factors regulate ADCC and cytokine release. Future experiments will aim to determine the mechanisms behind Cetuximab binding, as well as the role of individual receptors between ADCC and ADCP. Ultimately, the goal is to get a multivariate understanding of antibody-directed effector responses and then compare these measurements to a mechanistic binding model.

49. Application of State-transition theory and treatment modeling for predicting response to chemotherapy in a mouse model of acute myeloid leukemia

Lisa Uechi (City of Hope)

AML is an aggressive cancer that initiates in the bone marrow (BM) and circulates in the body via peripheral blood (PB). Time-series experiments using the Cbfb-MYH11 (CM) knock-in mouse model, along with pharmacokinetic dynamics modeling, were performed to approach AML treatment processes from the state-transition theory perspective. We applied the state-transition theory to characterize Acute Myeloid Leukemia (AML) disease progression, modeling AML as a stochastic dynamical system that undergoes evolution in AML potential. We investigated AML treatment processes through chemotherapy, examining the hypothesis that the treatment dynamically alters the AML potential.

The CM mice were treated with a combination of Ara-C and Daunorubicin after the detection of overt leukemia to model the 7+3 standard of care treatment for newly diagnosed AML. Samples of PB (n=174) and BM (n=11) were collected weekly before, during, and following chemotherapy and subjected to RNA sequencing. The modeling of the pharmacokinetic dynamics of chemotherapy to the AML potential was performed from the state-transition theory. The effects of chemotherapy were modeled by drug dose and the half-life of Ara-C and Daunorubicin with a Heaviside step function. Several treatment protocols, by changing doses and timing, were examined using the state-transition treatment modeling.

All samples were mapped to the state space of the state-transition model, and the 10 AML mice achieved a partial response to chemotherapy with a mean time to relapse of 5 weeks. The state-transition treatment model predicted the longest survival probability when the second dose was applied at the transition point of the Fokker-Planck

solution. The state transition treatment model has implications for improving therapeutic strategies by targeting transcriptome state transition in human AML.

50. Comprehensive Benchmarking of TCR-Seq Data Analysis Methods: Assessing Performance and Accuracy

Mohammad Vahed, Yu Ning Huang, Fatemeh Mohebbi, Jiaqi Fu, Serghei Mangul (USC)

The remarkable diversity of T cell receptor (TCR) repertoires poses challenges in accurately cataloging alleles, exacerbated by underrepresentation of non-European populations in existing databases. To address this, computational methods for inferring novel TCR and BCR alleles from sequencing data have emerged. However, the absence of robust benchmarking methodologies raises concerns about their precision and sensitivity.

In this study, we propose comprehensive benchmarking strategies for six existing tools capable of inferring V(D)J alleles, three dedicated to both TCR-Seq and BCR-Seq data and three specific to BCR-Seq. Leveraging simulation data from ImmuneSIM and real samples from diverse populations, we aim to evaluate the specificity and sensitivity of these methods.

Preliminary results demonstrate discrepancies between tools, with varying detection rates of undocumented alleles. Notably, IMPre revealed more potential novel alleles compared to IgDiscover, underscoring the need for a gold standard dataset for validation. To address this, we plan to introduce PCR-based molecular validation methods, bridging the gap between computational inference and empirical validation.

Our approach not only enhances the precision of allele identification but also aims to enrich databases with population-specific alleles, especially from underrepresented non-European cohorts. By utilizing state-of-the-art computational methods for genetic ancestry inference, we anticipate discovering a wealth of novel alleles in diverse populations, paving the way for a more inclusive and comprehensive understanding of TCR repertoires.

51. Modeling Signaling and Differentiation in Radiotherapy Resistance of Glioblastoma

Alice Vo (UCI) and John Lowengrub (UCI)

Glioblastoma is the most lethal and prevalent form of cancer to the central nervous system. Median life expectancy for patients is five years after standard treatment of care. Experiments have indicated that radiotherapy can result in the growth of cancer stem cells (CSCs) as the tumor growth recurrence takes place. More recent evidence suggests that radiotherapy-induced differentiation in the forward and reverse directions contributes to that recurrence. To address this, a mathematical model incorporating proliferation, (de-)differentiation, and radiotherapy is analyzed to identify conditions consistent with an increase in CSC fraction and tumor size after radiotherapy. Reverse differentiation, radiotherapy, and feedback on non-CSC growth rates provide the ingredients to create a cocktail of tumor regrowth and increase in CSC fraction. Under these conditions, the model also predicts plausible improvements over conventional radiotherapy through sub-toxic, hypofractionated radiotherapy schedules.

52. Comparison of hyphal growth in fusing and non-fusing mycelia using a multiscale model of fungal growth

Khoi Vo (UCR)

Bacterial-fungal interaction is important in crop biofuel development. In this work, we focus on the interactions between the fungus, Laccaria bicolor, and the bacterium, Psuedomonas fluorescens, and their integral role in the fitness of the roots of Populus species. Laccria bicolor synthesizes malate which stimulates growth and chemotaxis of P. flourescens while P. flourescens provides L. bicolor with thiamine thereby increasing fungal mass. We developed a multiscale computational model to investigate these interdependent interactions. The growth and branching of the fungal mycelia are modeled using an off-lattice spatial discrete submodel which is dependent on both diffusive and active translocation of internal nutrients and uptake of external nutrients. Malate secretion acts as a source of diffusive chemoattractant for P. fluorescens. The bacteria colony are represented by point sources of diffusing thiamine.

53. Synergistic antimicrobial effects of Histones and Antimicrobial Peptides using Super-Resolution Microscopy and modeling

Babu Reddy Janakaloti Narayanareddy, Albert Siryaporn, Steven Gross (UCI)

Histones, conventionally associated with chromosome condensation, exhibit increased extracellular concentrations in human blood during bacterial infections and tissue damage. However, the antimicrobial mechanism of histones remains elusive. Utilizing super-resolution microscopy, we examined histone and antimicrobial peptides (AMPs) cooperation against bacterial infections. Histones facilitate AMP localization on cell membranes, while AMPs enhance histone cytoplasmic uptake, indicating a synergistic bactericidal effect. Quantitative analysis reveals synergistic histone and AMP molecule localization on cells. A predictive model suggests synergy arises from histone and AMPs targeting distinct membranes. Our findings illuminate the multifunctional roles of histones and offer a framework to combat antibiotic resistance.

54. Sexual dimorphism in renal metabolism, hemodynamics and diseases

Lingyun (Ivy) Xiong, Alan Garfinkel, Eric J. Deeds (UCLA)

Age-related decline of renal function is faster in males than in age-matched females, manifesting in increased susceptibility to both chronic and acute kidney diseases among males. In the mouse kidney, sexually dimorphic gene activity maps predominantly to proximal tubule (PT) segments, where most reabsorption of water and salt happens, with a high energetic demand. Recent work suggests that male PTs undergo excessive oxidative stress to meet the high energetic demand, while female PTs exhibit an anti-oxidation state. However, it remains elusive how the observed molecular differences relate to sex disparities in renal physiology and pathophysiology. Glomerular filtration rate (GFR) is tightly regulated by tubuloglomerular feedback (TGF) within renal tubules to optimize ultrafiltration of plasma and reabsorption of essential molecules. Importantly, GFR shows a sustained oscillatory pattern over time in rodents, and loss of GFR oscillations is associated with cessation of reabsorption activities and with the occurrence

of ischemia-reperfusion injuries in the kidney, as well as with systemic hypertension. To study the relationship between intracellular metabolic events and renal physiology, we developed a mathematical model of TGF linking metabolic regulation within PT cells to fluid handling and salt reabsorption in the nephron, using renal blood flow and intra-vital imaging data. Analysis of this model revealed that dynamical properties of the system differ between male and female kidneys, resulting in male kidneys being more prone to stress-induced damage, thus building towards an explanation for sexual dimorphism in renal aging and diseases. With this mechanistic model, our work also provides insight into how pharmacological interventions can be employed to confer renoprotection.

55. Approximation of Intractable Likelihood Functions in Systems Biology via Normalizing Flows

Vincent D. Zaballa & Elliot E. Hui (UCI)

Systems biology relies on mathematical models that often involve complex and intractable likelihood functions, posing challenges for efficient inference and model selection. Generative models, such as normalizing flows, have shown remarkable ability in approximating complex distributions in various domains. However, their application in systems biology for approximating intractable likelihood functions remains unexplored. Here, we elucidate a framework for leveraging normalizing flows to approximate complex likelihood functions inherent to systems biology models. By using normalizing flows in the Simulation-based inference setting, we demonstrate a method that not only approximates a likelihood function but also allows for model inference in the model selection setting. We showcase the effectiveness of this approach on real-world systems biology problems, providing practical guidance for implementation and highlighting its advantages over traditional computational methods.

56. The Role of PHLDA2 in Autophagy Regulation and Breast Cancer Progression via Hyperspectral Imaging

Songning Zhu (University of California, Irvine)

Pleckstrin homology like domain family A member 2 (PHLDA2) has emerged as a significant player in the intricate landscape of cancer biology, particularly in the context of breast cancer. Situated within a crucial tumor suppressor gene region, alterations in PHLDA2 have been linked to the onset and progression of breast cancer, notably the aggressive triple-negative subtype (TNBC). While initially identified for its role in promoting cell growth and inhibiting apoptosis through the PI3K/AKT pathway, recent investigations have unveiled its involvement in the regulation of autophagy.

Autophagy, a cellular process crucial for maintaining homeostasis by degrading and recycling damaged organelles and proteins, has garnered attention for its dual role in cancer progression. PHLDA2 appears to intricately modulate this process, exerting inhibitory effects on autophagy. Through its interactions with key signaling pathways such as PI3K/AKT, PHLDA2 exerts control over the delicate balance between cell survival and death, tipping the scales towards tumor progression.

Studies at the cellular level have demonstrated that heightened expression of PHLDA2 promotes cell proliferation, further highlighting its potential contribution to tumorigenesis. Moreover, its correlation with metastatic triple-negative breast cancer (TNBC) underscores the urgent need to decipher the mechanistic connections between PHLDA2 and autophagy regulation. To address this, we propose an organelle-omic approach, aiming to elucidate the roles of various organelles downstream of PHLDA2 gene alterations. By employing multiplexing techniques targeting endosomes, lysosomes, mitochondria, microtubules, and the nucleus, we aim to unravel the intricate interplay between PHLDA2 and autophagy. This approach holds promise for the development of targeted therapeutic interventions tailored to combat aggressive breast cancer subtypes.

57. Parameter sensitivity analysis of GRNmap, a dynamical systems model of gene regulatory networks

Nikki C. Chun, Kam D. Dahlquist, and Ben G. Fitzpatrick (Loyola Marymount University)

A gene regulatory network (GRN) is a set of transcription factors that regulate the expression of genes encoding other transcription factors. The dynamics of GRNs explain how gene expression changes over time. GRNmap is a complex MATLAB software package that uses ordinary differential equations to model the dynamics of small- to medium-scale GRNs. The program estimates production rates, expression thresholds, and regulatory weights for each transcription factor in the network based on time-course gene expression data, using a penalized least-squares function that minimizes the discrepancy between simulated model outputs and observed data. The optimization problem is constrained by the addition of a penalty term, which consists of the square of the parameter vector, multiplied by α, which is used for weighting. Exploration of the α parameters found that an alpha value of 0.02 is suitable, with a value of 0.002 causing overfitting. Additionally GRNmap can make parameter estimation from input expression data with missing data points. When using an alpha value of 0.02, there were only slight differences in outputs from workbooks with missing expression data points and no missing data points (which were filled with the average value of the other replicates for that time point). To better understand where these differences are coming from, a sensitivity analysis will be conducted based on a handpicked trial network. Noise will be added to the expression data systematically to see where the sensitivities arise so that we can better interpret the differences in the missing and no missing data outputs.

58. Epithelial cell competition is directed by signaling from macrophages

Yilun Zhu (UCI), Zeba Wunderlich (BU) and Arthur D. Lander (UCI)

In developing epithelia, when cell neighbors proliferate at even slightly different rates, an active process directs slower cells (“losers”) to arrest and die, allowing “winner” cells to fill the remaining territory. Termed “cell competition”, the process is thought to serve a quality control function, eliminating unfit or abnormal cells, even nascent tumor cells. Although cell competition happens in many species, it is best understood in Drosophila, where methods exist for creating inducible juxtapositions of distinct cell types. In Drosophila imaginal discs, clones of Myc-over-expressing cells eliminate wildtype neighbors, whereas wildtype neighbors eliminate clones with ribosomal mutations. It has been shown that elimination of dead loser cells is required to sustain cell competition, suggesting winner cells require space to move into. Consequently, when hemocytes—phagocytic cells homologous to mammalian macrophages—were shown to be required for cell competition, it was assumed their role was related to removal of cell corpses. Here we challenge this assumption. Working in wing and eye imaginal discs, we show that recruitment of hemocytes to sites of cell competition occurs early during cell competition, before cell death, and occurs even if death is genetically blocked (a condition under which winner clones do not overgrow). As with mammalian macrophages, a key signaling molecules produced by hemocytes is tumor necrosis factor alpha (TNFa), the Drosophila gene for which is called eiger. We find that depleting hemocytes of eiger using hemocyte-specific RNAi also blocks cell competition. These results indicate that cell competition is a three-way, tissue-scale interaction among winner cells, loser cells, and hemocytes, in which hemocytes play an early

signaling role. What remains to be determined is how hemocytes identify sites of cell competition before any cells have died, and how their release of TNFa leads to the preferential loss of loser cells.