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Introduction

Cell signaling is a highly complex, yet extremely precise, process that governs many important functions in bacterial and human cells. Each signaling process involves multiple steps, and the major research challenge is to perform appropriate experiments and modeling not only to study the individual steps, but also to understand how these steps are properly coordinated and regulated to produce the end-point response.

Over the course of his career, Los Alamos scientist Byron Goldstein made significant contributions to the area of immune cell signaling induced by receptor-ligand binding. In honor of his retirement, we are holding this symposium and have invited leading experts in this field to discuss the advances they have made by combining experiments and modeling. Many of Byron’s colleagues and collaborators will also participate in the symposium. In addition to invited speakers, we welcome abstracts for additional talks and posters (especially from Ph. D students, post-docs, and junior faculties) to increase the depth and breadth of the symposium.

Key topics are:

- Adaptive immune responses
- Innate immune responses
- Host-pathogen Interactions
- Cell-cell communication in bacteria
- Bacterial virulence and drug resistance

Keynote Speaker: Byron Goldstein, LANL (Honoree)

Dr. Byron Goldstein is a retired Fellow of the Los Alamos National Laboratory. He is a recipient of the 2003 National Institute of General Medical Sciences MERIT award for the study of receptor aggregation and its effects. In the mid 1970's, Dr. Goldstein was recruited to join the newly formed Theoretical Biology and Biophysics Group within Los Alamos’s renowned Theoretical Division. From that point on, he studied various aspects of the immune system, in particular allergic reactions triggered by the high-affinity receptor for IgE antibody. Dr. Goldstein studied IgE receptor signaling for over 35 years, working with many of the leading experimentalists in the field over that time. He developed a great number of models relating antigen properties to antigen-induced signaling events and cellular responses, and he made important contributions to our understanding of how ligand dose, valence, and binding kinetics influence downstream receptor signaling events. He is recognized for his biophysical modeling of ligand-receptor interactions, for modeling intracellular signaling processes in mammalian cell signaling systems that play roles in immunity, and for developing novel approaches for modeling the dynamics of biochemical networks. He became a LANL Fellow in 2004, an AAAS Fellow in 2011.
Scientific Organizing Committee

• Alan Perelson, Chair, Los Alamos
• Barbara Baird, Cornell University
• Thomas Terwilliger, Los Alamos
• Micah Dembo, Boston University
• Srinivas Iyer, Los Alamos
• James Faeder, University of Pittsburgh
• William Hlavacek, Los Alamos
• Goutam Gupta, Los Alamos
• Steve Buelow, New Mexico Consortium
A Symposium on Cell Signaling

**Venue:** Hotel La Fonda at the Plaza, Santa Fe, NM, USA  
**Dates:** August 28-31, 2016

### August 28, 2016

**1:00-6:00 pm**  
**Registration**

**5:30-6:30 pm**  
**Opening Session I at the Lumpkins Ballroom,**  
(Chair: Tom Terwilliger, Los Alamos National Laboratory)

**5:30-5:40 pm**  
Welcome Remarks By LANL, NMC, and the Organizing Committee

**5:40-6:05 pm**  
Michael Weiss, Case Western Reserve University, Abstract #49  
*How insulin binds: structure of a micro-receptor complex and implications for analog design*

**6:05-6:30 pm**  
Geoff Waldo, Los Alamos National Laboratory, Abstract #48  
*A fluorescent protein toolbox for studying host-pathogen interactions*

**6:30-8:00 pm**  
**Reception and Dinner at the La Terraza Hall**

**8:00-8:30 pm**  
An after dinner talk by Byron Goldstein (the honoree), Introduction by Charles De Lisi  
*A few stories*

**8:30-9:00 pm**  
Tribute to Byron (Alan Bishop, Micah Dembo, Alan Perelson. . .)

### August 29, 2016

**8 am-2 pm**  
**Registration**

**7:00-8:30 am**  
**Breakfast at the Lumpkins Ballroom**

**8:30-10:10 am**  
**Session II on “Receptor-Mediated Signaling” at the Lumpkins Ballroom**  
(Chair: Alan Perelson, Los Alamos National Laboratory)

**8:30-8:55 am**  
Leslie Berg, University of Massachusetts Medical School, Abstract #6  
*Regulation of TCR signal strength by the Tec kinase ITK*

**8:55-9:20 am**  
James Faeder, University of Pittsburgh, Abstract #18  
*Determining the role of T cell antigen receptor signaling strength in T cell differentiation*

**9:20-9:45 am**  
Barbara Baird, Cornell University, Abstract #3
How does the plasma membrane participate in IgE receptor-mediated signaling in mast cells?

9:45-10:10 am  
William Hlavacek, LANL (Missing abstract)  
Rule-based modeling of multivalent ligand-receptor interactions

10:10 am-12:00 noon  
Refreshments and Poster Session at the Lumpkins Ballroom

12:00-1:00 pm  
Lunch at La Terraza Hall

1:00-2:50 pm  
Session III on “Cell and Membrane Dynamics” at the Lumpkins Ballroom  
(Chair: Srinivas Iyer, Los Alamos National Laboratory)

1:00-1:25 pm  
Micah Dembo, Boston University, Abstract #13  
Forces Between interacting cells during adhesion, signaling and separation

1:25-1:50 pm  
Marc Herant, Recon Strategy, Abstract #24  
Membrane dynamics and cell mechanics

1:50-2:15 pm  
Dan Hammer, University of Pennsylvania, Abstract #22  
Role of signaling in T-cell homing

2:15-2:40 pm  
S. Gnanakaran, Los Alamos National Laboratory, Abstract #20  
Membrane Transport: Extracellular Signals and Drugs

2:40-3:40 pm  
Refreshments and Poster Session at the Lumpkins Ballroom

3:40-5:20 pm  
Session IV on “Autophagy and Inflammasome” at the Lumpkins Ballroom  
(Chair: Ruy Ribeiro, Los Alamos National Laboratory)

3:40-4:05 pm  
Vojo Deretic, University of New Mexico, Abstract #14  
Autophagy: mechanisms, subsystem organization, selectivity and links to innate immunity

4:05-4:30 pm  
Jeff MacKeigan, VanAndel Research Institute, Michigan, Abstract #34  
Data Driven Model of Autophagy

4:30-4:55 pm  
Anu Chaudhary, University of Washington, Abstract #10  
Human Diversity in a Cell Surface Receptor that Inhibits Autophagy

4:55-5:20 pm  
Mo Lamkanfi, VIB, Belgium, Abstract #31  
Inflammasome signaling in inflammatory disease

5:20-5:35 pm  
Kaitlin Sawatzki, Boston University, Abstract #44  
B cell clones in repeated AVA immunization are first drawn from autoreactive memory, then naïve cells, and evolve continually.

5:35-5:50 pm  
Eugene Douglass, Yale University, Abstract #15  
Modeling Chemotherapy Mechanisms to Improve Cure Rates

6:50-8:20 pm  
Reception and Dinner at the La Terraza Hall
8:20-8:50 pm  An after dinner talk by Alan Perelson, Los Alamos National Laboratory
Approaches to Functional Cure of HIV Infection

August 30, 2016

7:00-8:30 am  Breakfast at the Lumpkins Ballroom

8:30-10:10 am  Session V on “Host-Pathogen Interactions” at the Lumpkins Ballroom
(Chair: Ben McMahon, Los Alamos National Laboratory)

8:30-8:55 am  Joanne N. Engel, UCSF, Abstract #17
*Intracellular pathogens are master cell biologists: understanding how Chlamydia co-opts the host cell to survive intracellularly*

8:55-9:20 am  Richard Sayre, Los Alamos National Laboratory (Abstract missing)
*Molecular modulation of bacterial quorum sensing by the green alga, Chlamydomonas reinhardtii*

9:20-9:45 am  Janelle Ayres, Salk Institute (Abstract missing)
*Microbiota-innate immune interactions protect against cachexia*

9:45-10:10 am  Christopher Basler, Georgia State University, Abstract #4
*Filovirus subversion of innate immune signaling pathways*

10:10-10:35 am  Sun Hur, Harvard University, Abstract #25
*Innate immune mechanism for viral dsRNA Recognition*

10:35-11:00 am  Refreshment break at the Lumpkins Ballroom

11:00-12:30 pm  Session VI on “Technology Development” at the Lumpkins Ballroom
(Chair: Basil Swanson, Los Alamos National Laboratory)

11:00-11:25 am  Andrew Bradbury, Los Alamos National Laboratory, (Abstract missing)
*At the Crossroads: Getting to Recombinant Antibodies That Guarantee Reproducible Research*

11:25-11:50 am  Jennifer Foster-Harris, Los Alamos National Laboratory, Abstract #23
*Mechanical, Chemical, and Cellular Stimuli in Human Organs on a Chip*

11:50-12:15 pm  James Werner, Los Alamos National Laboratory, Abstract #51
*3D Molecular Tracking in Live Cells*

12:15-12:40 pm  Ryan Gutenkunst, University of Arizona, Abstract #21
*Selection on network dynamics constraints protein evolution in signaling and metabolic networks*

12:40-1:05 pm  Anita Kant, Fluidigm Corporation, Abstract #28
*Understanding Biological Heterogeneity and Immune Cell Signaling Through Mass Cytometry*
1:05-2:00 pm Lunch at La Terraza Hall

2:00-4:10 pm Session VI on “Receptor Signaling and Human Diseases” at the Lumpkins Ballroom (Chair: David Holowka, Cornell University)

2:00-2:25 pm Pamela Bjorkman, CalTech, Abstract #7

A molecular arms race: The immune system versus HIV

2:25-2:50 am Arup Chakraborty, MIT (Abstract missing)

How to hit HIV where it hurts

2:50-3:15 pm Bridget Wilson, University of New Mexico, Abstract #52

Transient Homo-Interactions Drive Autonomous Signaling from the Pre-BCR Signaling Complex

3:15-3:40 pm Daniel Coombs, University of British Columbia, Canada, Abstract #11

B cell receptor organization, dynamics and signaling

3:40-3:55 pm Priya Luthra, Georgia State University, Abstract #32

DNA damaging compounds induce activation of innate immune responses and circumvent Ebola virus immune evasion mechanisms

3:55-4:10 pm Youfang Cao, Los Alamos National Laboratory, Abstract #9

Stochastic Bimodal Control of Latency and Reactivation in HIV-1 Infected Cells

4:10-4:30 pm Refreshment Break

4:30-6:10 pm Session VII on “Cell Signaling: modeling and simulation” at the Lumpkins Ballroom (Chair: Micah Dembo, Boston University)

4:30-4:55 pm Ben McMahon, Los Alamos National Laboratory, Abstract #35

Disease specific cytokine profiles in pediatric patients with malaria, HIV, and systemic bacteremia infections

4:55-5:20 pm Michael Blinov, University of Connecticut, Abstract #8

Modeling of multi-molecular ensembles: time-courses, molecular composition at a given time, and accounting for compartments and steric crowding

5:20-5:45 pm Zhiping Weng, University of Massachusetts Medical School, Abstract #50

Computational Identification of Peptide Antigens Bound by T Cell Receptors

5:45-6:10 pm Omer Dushek, University of Oxford, Abstract #16

A minimal signaling architecture explains the T cell response to a 1,000,000-fold variation in antigen affinity and dose

6:40-8:00 pm Reception and Banquet at the La Terraza Hall

8:00-10:00 pm A round table “conversation on changes: impact of immigration on American science in the modern era” chaired by Charles DeLisi, Boston University

August 31, 2016 Departure
Abstracts

1. Libin Abraham, University of British Columbia, Vancouver, V6T 1Z3, Canada

Receptor organization and mobility as the basis for the priming of Marginal Zone B cells

B cells integrate signals from multiple activating and inhibitory receptors in a highly regulated spatiotemporal manner to regulate B cell receptor (BCR) signaling and B cell activation. Marginal Zone (MZ) B cells are a unique subset of B cells that appear to exist in a partially activated or primed state, allowing them to rapidly respond to small amounts of antigens. The molecular basis for this priming is not understood. We propose a novel signal integration mechanism, where altered lateral mobility and nanoscale organization of BCRs serves as the basis for priming in MZ B cells. To this end, we used single molecule tracking (SMT) and drift free super resolution microscope to assess individual receptor mobility and nanoscale organization at high temporal and spatial resolutions, respectively.

Our SMT data revealed higher lateral mobility of BCRs on the surface of MZ B cells, when compared to resting circulating Follicular (FO) B cells. This is consistent with the increased actin dynamics in MZ B cells, which removes actin based barriers to receptor diffusion and reduces BCR confinement. Single molecule localizations of BCRs revealed heterogeneous populations of receptors, and were broadly classified as nanoclusters and oligomers. The oligomeric population was assessed using Hopkins index and we found that BCRs on MZ B cells had large numbers of oligomers and exhibited a lower degree of clustering. The nanocluster population was quantitatively analyzed using Voronoi tessellations. We found that BCRs formed dense nanoclusters in MZ B cells, while their area remained largely unchanged. This suggests that, despite having higher lateral mobility (as seen via SMT), the BCRs do not concatenate into large protein islands, rather they coalesce into denser nanoclusters in MZ B cells. We propose that, the dense BCR nanoclusters act as signaling hubs and contribute to priming in MZ B cells.
2. Eduardo Anaya, University of New Mexico, Albuquerque, NM 87111, USA

Nanoclustering and Calcium Signaling of Dectin-1A and 1B After Activation with Soluble Ï²-Glucans

Dectin-1 is an innate immunoreceptor that recognizes Ï²-glucan that is found in the cell walls of several fungal pathogens. Dectin-1 has eight alternative splice isoforms including, A and B. Dectin-1A has a long stalk region in the ectodomain while Dectin-1B lacks this stalk. Innate immunocytes co-express these isoforms, but their relative responsiveness to Ï²-glucan is little understood. Determining how the different types of soluble Ï²-glucans affect both isoforms will help in unraveling how differences in glucan structure impact the earliest events in Dectin-1 signaling. In this study, we focused on the impact of ligation by soluble Ï²-gluca ns varying in size and quaternary structure on the membrane organization and Ca2+ signaling responses of Dectin-1 isoforms.

To accomplish this, we used super resolved fluorescence imaging (dSTORM) to determine the nanostructure and total amount of Dectin-1A or-1B in stimulated and unstimulated cells. Furthermore, we measured Ca2+ signaling using Fluo-2 leak resistant probe in HEK-293 cell lines expressing Dectin-1A or-1B after stimulation with various glucans.

Our dSTORM results on imaged transfected cells expressing Dectin-1A or -1B have shown that Dectin-1A and 1B exhibit a diffuse, apparently monomeric distribution in resting cells. After stimulation with S. cerevisiae medium molecular weight (ScMMW) and high molecular weight (ScHMW) Ï²-glucan, the density of Dectin-1A increases to about 4 fold while Dectin-1B exhibits a much lower increase density when compared to Dectin-1A. However, there is no evidence of significant receptor clustering after stimulation by either ligand with either isoform.

Stimulation with ScMMW and ScHMW Ï²-glucans resulted in greater amplitude of calcium influx in the Dectin-1A cells. Meanwhile, Dectin-1B cells had decreased signaling compared to Dectin-1A. Further research will look to determine what the interactions between Dectin-1A and -1B have on signaling after stimulation with various Ï²-glucans.
3. Barbara Baird, Cornell University, Ithaca, NY 14850, USA

How does the plasma membrane participate in IgE receptor-mediated signaling in mast cells?

Cells respond to their physical environment and to chemical stimuli in terms of collective molecular interactions that are regulated in time and space. Small molecules may engage specific receptors to initiate a transmembrane signal, and the system amplifies this nanoscale interaction to microscale assemblies within the cell and often to longer length scales involving surrounding tissue and ultimately the whole organism. A striking example of signal integration over multiple length scales is the allergic immune response. This is initiated by antigen binding to IgE receptors (FcRI) on mast cells, which serve as gatekeepers. Indeed, the mast cell system has proven to be a valuable model for investigating receptor-mediated cellular activation more broadly. Spanning the range of cellular responses we use super resolution fluorescence localization microscopy to investigate the earliest signaling events and ligands patterned in micron size features together with confocal microscopy to investigate early and later events. We are characterizing distinctive regulatory roles resulting from the dynamic interplay between plasma membrane organization and the actin cytoskeleton at different stages of IgE-FcRI mediated mast cell responses.
Filovirus subversion of innate immune signaling pathways

The filoviruses Ebola virus (EBOV) and Marburg virus (MARV) cause severe disease characterized by systemic virus replication and suppression of antiviral defenses. We have systematically explored how filoviruses defeat host innate immunity. We have identified mechanisms by which each virus prevents production of the antiviral type I interferon (IFN) family of cytokines and mechanisms by which each virus blocks IFN-induced Jak-STAT signaling. The VP35 proteins of both EBOV and MARV prevent production of IFN by blocking RIG-I-like receptor (RLR) signaling. The inhibition largely correlates with VP35 dsRNA binding activity, sequestration of immune stimulatory RNAs and interaction with PACT, a cellular protein that can facilitate RIG-I activation. Each virus also blocks IFN-induced Jak-STAT signaling, preventing induction of interferon stimulated gene (ISG) expression. The EBOV VP24 protein interacts with select karyopherin alpha nuclear transport factors, preventing activated STAT1 from entering the nucleus. In contrast, MARV uses its VP40 protein to target the kinase function of Jak1, preventing the tyrosine phosphorylation that activates STAT1. Relevance of these functions for pathogenesis is demonstrated by studies using EBOVs with mutated VP35s. Such viruses trigger a robust IFN response and are highly attenuated in vivo. Methods to counteract or bypass these innate immune evasion functions should have therapeutic benefit.
5. Supartim Basu, New Mexico Consortium, Los Alamos, NM 87544 USA

Coordinated Systemic Regulation of Photosynthetic Carbon Metabolism in Rice

With the world’s population expected to surpass 9 billion by 2050, a 26% increase in rice production would be required by 2035 to feed the rising population. Towards this goal, photosynthesis is recognized as the major prospect for improving crop yield on the scale of the past 50 years since the Green Revolution. Evidence that elevated CO2 can increase leaf photosynthesis in crops by as much as 22.6% over the growing season suggests that increasing photosynthesis can increase plant productivity. Several studies revealed good correlations of leaf or canopy photosynthesis, and seed yield but improvement of photosynthetic efficiency was not as successful. We identified an AP2/ERF transcription factor HYR which when overexpressed in rice under the control of the CaMV35S promoter shows increased chlorophyll content and chloroplast number in mesophyll cells. Gas exchange measurements of HYR lines with Li6400XT revealed enhanced photosynthetic capacity, observed as an increase in net CO2 assimilation with higher irradiance (400-1500 Âµmol/m2/s), supported by qRT-PCR data showing up-regulation of a number of photosystem II (PSII) and electron transport genes. Rice transformants expressing affinity tagged (TAP-tagged) HYR protein were used to isolate HYR-bound chromatin, and ChIP-qPCR assays showed in vivo binding to promoters of predicted HYR-regulated genes found up-regulated in microarray studies. Validation of HYR targets were done by trans-activation assays in rice protoplast transformation experiments expressing HYR co-transfected with target promoter-luciferase or HYR fused to Human Estrogen Receptor (HER) constructs confirmed direct up- or down- regulation. HYR bound to the promoters of photosynthesis genes, photosynthetic carbon metabolism (PCM) TFs and other genes, supporting the observed phenotypes. The expression of HYR and its downstream genes regulating PCM were found correlated within a diverse collection of rice genotypes that were screened for higher photosynthesis related processes.
Regulation of TCR signal strength by the Tec kinase ITK

Stimulation of the T-cell receptor (TCR) leads to activation, a process that includes changes in T cell metabolism, survival, proliferation, cytokine responsiveness, migration behavior, and effector functions. Many of these changes are dependent on TCR signal strength. Our previous studies have demonstrated that the transcription factor IRF4 is upregulated by TCR stimulation in CD8 T cells, and that the maximum level of IRF4 achieved is dependent on the strength of TCR signaling via the Tec kinase ITK. In turn, IRF4 promotes T cell differentiation into massively proliferating antiviral effector cells in a dose-dependent manner. We have investigated the signaling pathways that generate graded expression of IRF4, and find a central role for the calcium response; in contrast, phorbol ester-dependent signals generate only digital changes in IRF4 expression. Further, differences in peak IRF4 levels due to variations in antigen affinity cannot be recapitulated by changing antigen density, whereas analysis of CD69 upregulation, a well-characterized digital response, does not show this behavior. These patterns of graded IRF4 expression are highly dependent on ITK kinase activity. Using a nuclear localization assay, we find rapid and transient activation of NFAT1, whereas NF-B activation is slower and more sustained. Together, these findings demonstrate a central role for ITK in determining the cellular response to variations in TCR signal strength by regulating the magnitude of the calcium response.
A Molecular Arms Race: The Immune System Versus HIV

Over 30 years after the emergence of HIV, there is no effective vaccine, and AIDS remains a threat to global public health. Following HIV infection, the human immune response is unable to clear the virus, partly because the virus rapidly mutates to evade antibodies, one of our most important defenses against pathogens. In the absence of treatment with antiretroviral drugs, unfortunately not readily available in the developing world, an infected person's immune system gradually collapses and cannot fight off normally innocuous pathogens in the environment. Antibodies, which we readily produce against other viruses, do not work well against HIV. We hypothesize this is partly because antibody arms, which can both normally hang on to a virus until it is destroyed, do not have the right dimensions to stay attached to HIV. We seek to alter natural antibodies using molecular engineering so that HIV is powerless to mutate against them. One engineering project involves designing and creating new antibody architectures with arms that remain attached to HIV even as it mutates. We also engineer the antibody combining site by using chemical principles to improve the interface between antibodies bound to HIV proteins, starting with experimentally determined three dimensional structures of antibody complexes with HIV proteins, and using bioinformatics to predict common pathways of HIV escape. The goal is to create potent antibody reagents that can be delivered to prevent or treat HIV.

Few and far between: how HIV may be evading antibody avidity
Klein and Bjorkman, 2010, PLoS Pathogens

For most viruses, two identical Fabs in IgGs permit bivalent binding through inter-spike crosslinking.

Unlike other viruses, HIV has very few spikes, and the spikes are far apart – most antibodies can’t bind with both Fabs.

HIV spikes are relatively immobile in virus membrane
Modeling of multi-molecular ensembles: timecourses, molecular composition at a given time, and accounting for compartments

A significant challenge in modeling cellular biological systems is posed by the fact that interactions among multivalent molecules can lead to formation of large molecular ensembles of various composition and stoichiometry, termed pleomorphic ensembles (Mayer, Blinov & Loew, 2009). Many biological processes are enabled by maintaining of such ensembles, for example aggregation of Nephrin-Nck-NWasp during maintenance of kidney filtration barrier (Li et al., 2012), localized protein expression in RNA granules in neuronal cells (Carson et al., 2008), and transduction of extracellular input by trans-membrane receptor signaling platforms (Goldstein & Perelson, 1984). Pleomorphic ensembles can be described using rule-based approach (Hlavacek et al., 2006), but simulation is challenging because there is potentially an infinite number of individual species that can be generated. The simulations are becoming even trickier when species can be distributed among multiple compartments or anchored to membranes, and when spatial crowding is accounted for. I will describe several algorithms that are being implemented in VCell to deal with these problems: rule-based specification embedded into spatial VCell modeling environment (Schaff et al., 2016); FSGen, a numerical algorithm that generalizes the classical Flory-Stockmayer theory for sol-gel transitions by creating statistical distribution of ensemble composition evolving over time (Falkenberg, Blinov & Loew, 2013); and SpringSaLaD (Michalski & Loew, 2016), a spatial stochastic reaction-diffusion algorithm, implemented as a standalone software tool, which explicitly accounts for the steric crowding of binding partners.
The HIV latent reservoir in resting CD4+ T cells is the major obstacle for complete eradication of HIV infection. The HIV latency and reactivation are stochastically controlled by the HIV Tat circuit - a positive feedback genetic switch. However, detailed mechanisms of the stochastic control of Tat circuit in HIV latency-reactivation are unknown. Here we study the stochastic control of the Tat circuit using the Accurate Chemical Master Equation (ACME) method to directly solve the steady state and time evolution probability landscapes for the Tat circuit in different conditions. We first demonstrate that the Tat circuit is stochastically bimodal. It has a high probability latent state, and a low probability activated state with large Fano factor, implying the high stability in HIV latency and low stability in activation but with high fluctuation in viral production. We further study the effects of reactivating latent cells by targeting key reactions in the Tat circuit. Our model suggests that increasing the binding affinity between P-TEFb and the LTR might be a better target for inducing the latent reservoir than the histone deacetylase inhibitor (HDACi), due to lower viral production and faster rate of activation. Our approach may help to design more effective strategies for HIV treatment.
10. Anu Chaudhary, University of Washington, Seattle, WA 98195 USA

Human Diversity in a Cell Surface Receptor that Inhibits Autophagy

Mutations in genes encoding autophagy proteins have been associated with the susceptibility and severity of human autoimmune diseases. A cellular GWAS screen was performed to explore normal human diversity in responses to rapamycin, a microbial product that induces autophagy. Cells from several human populations demonstrated variability in the expression of cell surface receptor, CD244 (SlamF4, 2B4) that correlated with changes in rapamycin-induced autophagy. High expression of CD244, and receptor activation with its endogenous ligand CD48 inhibited starvation- and rapamycin-induced autophagy due to association of CD244 with the autophagy complex proteins Vps34 and Beclin-1, which reduced Vps34 lipid kinase activity. Lack of CD244 is associated with auto-antibody production in mice. In addition, lower expression of human CD244 has previously been implicated in severity of human rheumatoid arthritis and systemic lupus erythematos. Therefore, increased autophagy as a result of low levels of CD244 may alter disease outcomes.

Human Diversity in a Cell Surface Receptor that Inhibits Autophagy

![Diagram showing cellular GWAS for rapamycin response and the relationship between CD244 expression and autophagy](image-url)
B cell receptor organization, dynamics and signaling

I will describe recent progress in understanding the organization and dynamics of B cell receptors on resting and primed B cells, based on super-resolution light microscopy (primarily, STORM and STED imaging and single particle tracking approaches). Our results underline the importance of spatial effects at the cell surface in modulating immune cell signaling. The attached image shows the heterogeneous distribution of B cell receptors on a resting B cell as obtained by STORM microscopy.
A critical first step in immunological signal transduction is the phosphorylation of an immune receptor - typically by a Src family kinase. Phosphorylation is facilitated by the spatial exclusion of phosphatases from regions of receptor aggregation. By tracking single molecules of CD45 (a common membrane-associated tyrosine phosphatase), we were able to observe this exclusion process in real time in live macrophages presented with patterned, immobilized antigen. We found that integrin molecules form an expanding diffusional barrier that excludes CD45 molecules from sites of receptor aggregation. We analyzed molecular trajectories using a probabilistic model of spatial exclusion. In this model, the strength of the diffusional barrier is measured by an exclusion probability for CD45. I will present key results from this detailed molecular analysis of spatial exclusion, and discuss its consequences on downstream signaling.
Forces Between interacting cells during adhesion, signaling and separation

Using data on the cell shape and on the movements of random markers buried in the substrate, I will describe a method designed to yield an optimal spatial map, of the distribution of cell-substrate traction stresses. By applying a global force balance it then also gives the best possible statistical estimate of the cell-cell forces and torques. We apply our method to characterize the patterns of force and recoil that occur dynamically, when a pair of breast ductal epithelium cells come into close proximity. Surprisingly, we find that as soon as cell-cell contact can be optically observed, the advancing or active cell, has already firmly attached to its more passive partner by a small segment of its leading edge. Further, by some combination of mechano-sensing and adhesive bonding, the advancing cell has deactivated the initial protrusive stress, and, in rapid succession, has activated an inward directed, or “intrusive” stress. This is of unknown mechanism but we propose two simple cartoon models that fit the data. For growing or stable interfaces on different substrates and for different cells, the force/length ranged from a lower bound of about 3 nN/micron to an upper bound about 11 nN/micron. The value of 11 nN/micron.
Autophagy: mechanisms, subsystem organization, selectivity and links to innate immunity

Autophagy is a fundamental biological process that fulfills general and specialized roles in cytoplasmic homeostasis. This presentation will cover the subsystems in autophagy as they apply to mammalian cells and incorporates the recent progress in our understanding of how these modules come together to carry out innate immunity functions.
Modeling Chemotherapy Mechanisms to Improve Cure Rates

Since the 1960s, chemotherapies have been the backbone of late-stage cancer therapies. Unlike most drugs, chemotherapies have very complex mechanisms that involve dozens of genes per drug and hundreds of genes in total (Figure 1). As a result of this complexity, most clinical “standards of care” are poorly understood and have been optimized by trial-and-error. While this strategy has led to dramatic improvements in population-level survival (~70%), it has done little for individuals whose cancer is different from the population-average (~600,000 deaths/year). We attempt to solve this problem with a unique approach that combines our training in organic chemistry, biochemistry, mathematical modeling and data science.

Google’s early success was not due not to its search algorithm but rather to its PageRank algorithm that physically modeled a Random Surfer to analyze the citation network of the World Wide Web. In a similar manner, we have designed “ChemoRank” algorithms that physically model Individual Chemotherapy-Mechanisms to analyze the biochemical-resistance network of cancer cells (Figure 1). For each type of chemotherapy, our models combine gene-expression and mutation data into a single resistance metric. This resistance metric will be used to rank all major types of chemotherapy from most to least effective for a given cancer. In this manner, treatment alternatives can be obtained and ranked when the “standard of care” fails.
A minimal signalling architecture explains the T cell response to a 1,000,000-fold variation in antigen affinity and dose

T cells must respond differently to antigens of varying affinity presented at different doses. Previous attempts to map pMHC affinity onto T cell responses have produced inconsistent patterns of responses preventing formulations of canonical models of T cell signalling. Here, a systematic analysis of T cell responses to 1,000,000-fold variations in both pMHC affinity and dose produced bell-shaped dose-response curves and different optimal pMHC affinities at different pMHC doses. Using sequential model rejection/identification algorithms, we identified a unique, minimal model of cellular signalling incorporating kinetic proofreading with limited signalling coupled to an incoherent feed forward loop, that reproduces these observations. Our work offers a new general approach for studying cellular signalling that does not require full details of biochemical pathways.
Intracellular pathogens are master cell biologists: understanding how Chlamydia co-opts the host to survive intracellularly

Defining host-pathogen interactions is vital to understanding how pathogens cause disease. Chlamydia trachomatis is a leading cause of genital and ocular infections for which no vaccine exists. We subjected putative C. trachomatis inclusion membrane proteins (Incs) to affinity purification-mass spectroscopy (AP-MS). Very few host targets were known for this unique protein family. We identified 354 high confidence Inc-human interactions for 38/58 Incs and found enrichment in host processes consistent with Chlamydia’s intracellular lifecycle. We uncovered significant overlap between targets of Incs and viral proteins, suggesting common pathogenic mechanisms among obligate intracellular microbes. We demonstrate that IncE binds directly to sorting nexins (SNXs) 5/6, components of the retromer, resulting in SNX5/6 relocalization to the inclusion membrane and enhanced inclusion membrane tubulation. Depletion of retromer components enhances progeny production, revealing that retromer restricts Chlamydia infection. Our study demonstrates the broad applicability of AP-MS in unveiling host-pathogen interactions in genetically challenging microbes.
Determining the role of T cell antigen receptor signaling strength in T cell differentiation

Antigen stimulation of the T cell receptor (TCR) in a naïve T cell triggers a complex network of signaling pathways that determine whether the cell becomes a tolerogenic T regulatory (Treg) or immunogenic T helper (Th) cell. We have recently discovered a potential positive feedback loop involving Akt-mediated transcriptional downregulation of PTEN, a lipid phosphatase that opposes activation of Akt. To explore the effects of this feedback we developed a rule-based model of signaling downstream of the TCR, with a focus on the regulation of PTEN and Akt, and calibrated to available experimental data for multiple signaling intermediates. This model recapitulates dose-dependent dynamics of PTEN and Akt activity and predicts a dose- and time-dependent threshold for TCR stimulation to drive the sustained Akt activity required for the differentiation and proliferation of Th cells. The model also shows that sub-threshold signals lead to transient Akt activation, potentially leading to a Treg phenotype. The model identifies several key factors that can influence this threshold in an individual cell, including PTEN expression. Experiments confirm that PTEN expression levels and activity differentially affect the threshold for sustained Akt activation and the outcome of differentiation.
Different Resistance Mechanisms Are Mediated by RND Efflux Pumps in Planktonic vs. Biofilm States of *Pseudomonas aeruginosa*

The resistance-nodulation-cell division (RND) pumps provide multi-drug resistance in *Pseudomonas aeruginosa* and other gram-negative bacteria. The RND pumps are complexes of inner-membrane, periplasm, and outer-membrane protein components. In this work, we focus on the role of the RND pumps plays planktonic and biofilm states of *P. aeruginosa*. For this, we performed gene expression and functional assays for three different strains of *P. aeruginosa*, i.e., the wild type, and knockout and over-expressor of mexA-mexB-oprM both in the presence and absence of the antibiotic, Ciprofloxacin, a DNA gyrase inhibitor. mexA, mexB, and oprM are the genes encoding respectively periplasmic, inner-membrane and outer-membrane protein components.

In the planktonic state of *P. aeruginosa*, two resistance mechanisms are induced in the presence of Ciprofloxacin. One involves the overexpression of efflux pump genes such as mexC-mexD-oprJ and mexE-mexF-oprN whereas the other involves expression of genes encoding pyocins and phage proteins. Both mechanisms are probably due to toxicity to the cell caused by Ciprofloxacin induced DNA damage. The overexpression of efflux pumps directly lowers Ciprofloxacin concentration inside the bacteria. Expression of pyocin and phage proteins is a generic bacterial response to defend against toxic materials produced by neighboring harmful bacteria.

In the biofilm state of *P. aeruginosa*, the resistance mechanism involves the formation of biofilms in the presence of Ciprofloxacin. We observed that the overexpression of an efflux pump facilitates the formation of a biofilm consisting of polysaccharides, DNA, and protein matrix. The biofilm acts as an external barrier that offers resistance to the cells by preventing drug entry. We have also observed another interesting phenomenon associated with efflux pump facilitated biofilm. Biofilm associated Type Two Secretion System (T2SS) enzymes e.g., elastase, ADP-ribosylase, which cause host neutrophil lysis thereby abrogating host innate immune defense.

Thus, bacterial efflux pumps are more than a pump.
Membrane Transport: Extracellular Signals and Drugs

I will briefly describe our recent project with Byron Goldstein that sought answers to a central question on T-cell triggering - How does the extracellular TCR-pMHC engagement get transmitted to the intracellular signalling motifs called immunoreceptor tyrosine-based activation motifs (ITAMs) in the CD3 subunits? The affinity between TCR and pMHC is weak, and the engagement lacks significant conformational changes. Therefore, T-cell triggering may not be a simple process of protein-protein interaction, and, as we show, it is likely that membrane-mediated mechanisms are involved. I will then address the transport of drugs across gram-negative bacteria. Multi-drug resistance efflux pumps are complexes of three membrane proteins that pump antibiotics out of the cell before they can act on targets inside the bacteria. When antibiotics get inside the bacteria, they induce production of additional pumps on the bacterial membrane, leading to enhanced resistance of antibiotics. Efflux pumps can also transport quorum-sensing molecules that lead to the formation of bacterial biofilms that are impenetrable to antibiotics. We will describe our efforts to characterize this multi-layered resistance generated from structural, genetic, and cellular processes and to understand how efflux pumps assemble and function.
Selection on network dynamics constrains protein evolution in signaling and metabolic networks

The long-held principle that functionally important proteins evolve slowly has recently been challenged by studies in mice and yeast showing that the severity of a protein knockout only weakly predicts that protein's rate of evolution. However, the relevance of these studies to evolutionary changes within proteins is unknown, because amino acid substitutions, unlike knockouts, often only slightly perturb protein activity. To quantify the phenotypic effect of small biochemical perturbations, we developed an approach to use computational systems biology models to measure the influence of individual reaction rate constants on network dynamics. We show that this dynamical influence is predictive of protein domain evolutionary rate within signaling and metabolic networks in vertebrates and yeast, even after controlling for expression level and breadth, network topology, and knockout effect. Thus, our results not only demonstrate the importance of protein domain function in determining evolutionary rate, but also the power of systems biology modeling to uncover unanticipated evolutionary forces.
22. Daniel Hammer, University of Pennsylvania, Philadelphia, PA 19104 USA

Role of signaling in T-cell homing

We have combined a model of signal transduction with a mechanically accurate model of cell adhesion under flow to build an algorithm that predicts where T-cells (and other leukocytes) will home to inflammatory sites on endothelial surfaces that present chemokines and adhesive ligands. The model combines Adhesive Dynamics, which uses a force balance on the cell to determine its motion, with a spatio-temporal stochastic model of the signal transduction pathway in the interior of a T-cell, to understand how outside-in signaling of a leukocyte might lead to activation and cell arrest. The key nexus point at the center of the cell response is the conformational change of the integrin adhesion receptors to a stronger, activated state; when sufficiently many of these receptors are activated, the cell will arrest. As a test of the model, we predicted the effect of deletion of a key intracellular enzyme, diacylglycerol kinase (DAGK), would be to increase adhesion, and experiments with T-cells from DAG knock out mice confirm that deletion of DAGK leads to a gain of function mutation. We also present sensitivity analysis that identifies the key pathways that control T-cell adhesion.

Integrating signaling and adhesion (Hammer, Beste and Koretzky)
Human Organs on a Chip (HOCs) have the potential to revolutionize medical and pharmaceutical research because they are designed to recapitulate one or more functions of the parent organ in the human body. The old paradigm of a 2-dimensional culture containing only one cell type is changing rapidly as we are able to recapitulate more and more organ-specific functions in the HOC microenvironment. HOCs generally consist of a fluidic bioreactor platform with pumps, which provide mechanical stimuli, a cell compartment filled with one or more types of primary human cells, or stem cells, and a specialized media, which provides growth factors and other signaling molecules. All three, the mechanical stimuli, the human cells, and the specialized media work together to create an environment where the cells are induced to differentiate or remain differentiated and become functional tissues that behave similar to their counterparts in the human body. In several cases, the signaling events are known or partly known. In many other cases, there is much that still remains to be discovered in order to define what causes the precise differentiation of a tissue. We have utilized known signaling paths to drive differentiation, and also observed new phenomena while building milli-scale hearts, lungs, livers, and neuromuscular junctions on a chip. As we develop more complex organs on a chip, we are discovering more signaling clues behind building these complex cellular systems and recapitulating the human organ environment.
Membrane dynamics and cell mechanics

Traditionally, the cell membrane is thought of as a containment envelope for all the interesting things that happen inside the cell, and as a transduction locus between extracellular and intracellular signals. Here we will illustrate how the dynamics of the membrane play a central role in cell mechanics, ranging from phagocytosis, to cytokinesis, to cell spreading and crawling. In particular, we present a physical model based on a few simple and plausible rules governing adhesion, contractility, polymerization of cytoskeleton, and membrane tension.
Innate immune mechanism for viral dsRNA Recognition

Efficient host defense against viral infection depends on proper functions of pattern recognition receptors. One such family of receptors consists of RIG-I and MDA5, well-conserved cytoplasmic helicases that detect viral RNAs during infection and activate the type I interferon (IFN) signaling pathway. My laboratory has investigated the molecular mechanisms by which these receptors recognize viral dsRNAs and elicit the IFN response against a broad range of viruses. In particular, we have uncovered the filamentous assembly structures of these receptors and how these structures confer the ability to discriminate between self vs. non-self RNA. In this talk, I will also present our recent findings on how certain mutations in RIG-I and MDA5 alter their assembly properties and lead to auto-inflammatory diseases in the absence of infection.
Amyloid toxicity via disruption of cellular membranes

Many neurodegenerative diseases are associated with the deposition of misfolded proteins as amyloid fibrils. It is now widely presumed that these fibrils are largely benign and that the toxic components are actually much smaller oligomeric aggregates. Here, we use atomistic molecular dynamics simulations to evaluate the hypothesis that these misfolded peptide oligomers exert their toxicity by disrupting neuronal membranes. Using enhanced sampling techniques, we show that toxicity of a peptide is related to its ability to penetrate a cell membrane. However, the relationship is complex. Our simulations suggest that amyloidogenic peptides are relatively non-toxic when they are either sufficiently hydrophilic to preclude substantial membrane insertion or when they are sufficiently hydrophobic to permit membrane traversal. Conversely, toxic amyloidogenic sequences have an intermediate hydrophilicity that favors shallow membrane insertion, which may be more disruptive to the membrane’s integrity. Taken together, our results provide a molecular depiction of membrane disruption by β-sheet-rich aggregates of peptides derived from human prion- and Alzheimer’s-related proteins, providing mechanistic insights into the molecular basis of toxicity in this important class of human diseases.
27. Rashmi Joshi, New Mexico State University, Las Cruces, NM 88001 USA

DNA ligase IV modulates the cellular response to DNA replication stress. Rashmi Joshi, Melissa Chavez, John Cavaretta, Neelam

Correctly repairing DNA damage is crucial to the survival and genomic integrity of cells. Double strand breaks (DSBs) are particularly problematic DNA damage, and failure to properly repair DSBs can precede genomic instability leading to cancer. Paradoxically, because of their high proliferative index, replication toxins are successfully used to treat cancer. The two major DNA DSB repair pathways are homologous recombination and nonhomologous end-joining (NHEJ). DNA ligase IV is a central component of NHEJ, performing the final ligation step to reseal the broken DNA ends, in concert with XRCC4. We determined cells lacking another NHEJ protein, DNA-PKcs, restarted DNA replication forks quicker than wild-type following replication stress, though whether other NHEJ proteins are involved is unclear. Our goal was to investigate whether ligase IV, a protein whose cellular functions are exclusive to NHEJ, is involved in the replication stress response. Our hypothesis is ligase IV delays replication restart following exposure to replication toxins. We confirmed our ligase IV knockdown via qPCR and western blotting in multiple cell lines using either pooled or single siRNA constructs. Cellular viability decreased following exposure to siRNA against ligase IV compared to non-target siRNA, and the extent of cell death following ligase IV knockdown was cell-type dependent. A higher percentage of cells treated with non-targeting siRNA incorporated EdU compared to ligase IV knockdown following hydroxyurea exposure, indicating a likely replication phenotype in ligase IV-depleted cells. We are utilizing SCR7, a ligase IV inhibitor, to determine whether similar phenotypes are observed compared to siRNA treatment. Replication stress is a major driver of chromosomal instability and development of drug resistance. Understanding how NHEJ proteins may impact the replication stress response may provide opportunities for developing more effective cancer treatments.
28. Anita Kant, Fluidigm Corporation, Lyons, CO 80540 USA

Understanding Biological Heterogeneity and Immune Cell Signaling Through Mass Cytometry

The immune system provides targeted protection against infectious diseases and emerging tumors.

It is comprised of heterogeneous cell subsets with specialized functions that work together to protect the body from a vast array of diverse threats. Mass cytometry allows simultaneous analysis of more than 40 parameters on per-cell basis to phenotypically and functionally profile cell populations from this complex system. This technology is based on the detection of metal-conjugated antibodies by using cytometry by time-of-flight (CyTOF). It has been instrumental in identifying novel cell populations and analyzing the signaling pathways in immune system to decipher its role in adaptive and innate immunity as well as its interaction with pathogens and tumors.

We will describe the basic principles and workflow of the technology as well as discuss groundbreaking research which was made possible by Fluidigm’s Single Cell Technology Tools.
Approaches for structural analyses of influenza A virus non-structural protein 1 (NS1) complexes with human p85β, CrkL and double

Non-structural protein 1 (NS1) of influenza A virus is a multi-functional virulence factor being involved in a variety of processes during viral infection. NS1 modifies the host-innate immune response and controls virus replication by interference with host mRNA and regulation of viral RNA synthesis and translation interacting with various proteins and RNAs. NS1 has been investigated as a potential target for antiviral drug discovery based on its structure, activities, genetics and importance in virus replication.

In our project, NS1 complexes of two components, NS1 from influenza A virus H7N3 strain and human cellular adapter protein CrkL, of three components, NS1 H7N3, CrkL and phosphoinositide 3-kinase (PI3K) regulatory subunit p85β as well as of four components, NS1 H7N3, CrkL, p85β and double-stranded RNA, are targeted for structural analyses. Since CrkL is known to interact with C-terminal side of NS1, we expect to solve C-terminal structure of NS1 H7N3 that has not been determined yet with its interacting protein besides the entire NS1 H7N3 structure showing its interaction mode with other partners.

To purify complex proteins, we adopted ligation independent cloning and tested expression of varied combinations of complex proteins. Particularly, to generate two different forms of three-proteins Complex I (p85β-NS1-CrkL) and Complex II (NS1-p85β-CrkL), we manipulated NS1 by introducing mutations on NS1 binding site to CrkL. For confirmation of NS1 H7N3 interaction with double-stranded RNA, the technique of dye-ligand affinity chromatography was applied.

We will discuss our approaches to generate complexes of two (NS1 and CrkL), three (NS1, p85β and CrkL) and four (NS1, p85β, CrkL and double-stranded RNA) components along with the progress in crystallization of these complexes.
Detection of Gram-negative vs. Gram-positive Bacterial Biomarkers

The incorrect diagnosis of bacterial pathogens results in the misuse of antibiotics, a major contributor to the evolution of antibiotic resistance. The ability to rapidly distinguish between Gram-negative and Gram-positive bacterial pathogens would facilitate more effective treatment strategies reducing the unnecessary use of antibiotics. Current diagnostics determining the Gram status of a pathogen take days and are therefore unable to inform timely treatment options. Here, we describe the development of a rapid assay to distinguish the presence of Gram-negative or Gram-positive bacteria. Our assay involves the detection of bacterial biomarkers with fluorescently labeled antibodies using a waveguide-based optical biosensor developed at LANL. The bacterial biomarkers that we are targeting are lipid A for Gram-negatives and lipoteichoic acid (LTA) for Gram-positives. We obtained four antibodies targeting either lipid A (1) or LTA (3) from commercial sources and have tested their specificity and sensitivity against various bacterial antigens. The concentrations of antibody at which no cross-reactivity towards antigens in the opposite group was seen ranged from 1 to 230 nM: ~68 nM for the anti-lipid A antibody, ~230 nM for the anti-LTA antibody, ~1 nM for the anti-Gram-positive bacteria antibody, and ~74 nM for the anti-Staph. aureus antibody. Further, the anti-LTA antibody was selected for testing on the waveguide platform using a membrane insertion assay in which LTA was allowed to passively insert itself into a lipid bilayer and was then probed with the anti-LTA antibody. Our waveguide-based biosensor was able to successfully detect the insertion of 100 µg/ml of LTA derived from Streptococcus pyogenes into a lipid bilayer using 10 nM anti-LTA antibody. With this ability to directly detect LTA in a rapid, sensitive and specific manner, we are one step closer to developing a diagnostic tool for point-of-care distinction between Gram-negative and Gram-positive bacterial pathogens.
Inflammasome signaling in inflammatory disease

Inflammasomes are multi-protein platforms that are organized in the cytosol to cope with pathogens and cellular stress. The pattern-recognition receptors NLRP1, NLRP3, NLRC4, AIM2 and Pyrin all assemble canonical platforms for caspase-1 activation. Inflammasomes contribute to host defense through their roles in maturation and secretion of the inflammatory cytokines interleukin-(IL)1β and IL18, and they also induce pyroptosis. Contrastingly, inflammasome activation is detrimental in the context of chronic inflammatory diseases. We used a combination of inflammasome knockout mice and purified peripheral blood mononuclear cells (PBMCs) from healthy donors and patients to show that inflammasome activation mechanisms differ in sterile inflammatory disease.
32. Priya Luthra, Georgia State University, Atlanta, GA 30306 USA

DNA damaging compounds induce activation of innate immune responses and circumvent Ebola virus immune evasion mechanisms

DNA damage responses can trigger innate immune responses. Anthracycline antibiotics, a class of compounds widely used as anti-cancer drugs, intercalate DNA, inhibit type II topoisomerase and trigger the DNA damage response. Ebola virus (EBOV) protein VP35 inhibits production of interferon-Î±/Î² (IFN) by blocking RIG-I-like receptor signaling pathways, thereby promoting virus replication and pathogenesis. A high-throughput screening assay was developed to identify compounds that either inhibit or bypass Ebola VP35 interferon-antagonist function. Screening a library of bioactive compounds identified five DNA intercalators as reproducible hits from. Four, including doxorubicin and daunorubicin, are anthracyclines. Treatment with these compounds led to activation of the ATM signaling pathway; furthermore knock down of ATM kinase lead to inhibition of IFN activation, suggesting ATM pathway is important for IFN activation by these compounds. Additionally, these compounds were demonstrated to trigger the DNA-sensing cGAS-STING pathway of IFN induction. Interestingly, VP35 was not able to block IFN activation by these compounds but suppressed Sendai virus (a known activator of RIG-I signaling pathway) mediated inducer of IFN, suggesting that ATM- and cGAS/STING-dependent IFN responses are insensitive to inhibition by VP35. These compounds also suppress EBOV replication in vitro and induce IFN in the presence of IFN-antagonist proteins from multiple negative-sense RNA viruses. This work provides new insight into signaling pathways activated by important chemotherapy drugs and identifies a novel therapeutic approach for IFN induction that may be exploited to inhibit RNA virus replication.
A Tale of Enigmatic Protein Domains: The Grb7 Protein

Our primary research focus is to understand Grb7 signaling in cancer development and progression, specifically with respect to breast and esophageal cancers. In relevance to this, Grb7 gene expression is markedly up-regulated in totipotent human embryos, suggesting Grb7 signaling could be important in cancer development, even of stem cell origin. Defining the functional state and regulation of Grb7 activity is likely crucial for understanding the role of Grb7 in cancer development and/or progression. In prior studies we established an intramolecular binding event between the Grb7 central domains (RA-PH) and the Grb7 C-terminal SH2 domain. We hypothesized this “closed” form of Grb7 could represent an auto-inhibited functional state. Although the Grb7 intramolecular binding event has been established, it has not been characterized in terms of molecular shape, and/or dimerization/oligomerization state of the Grb7 protein. Using dynamic light scattering (DLS), we have begun to address the overall size and shape of the closed Grb7 molecular form. We report results characterizing the closed Grb7 form, and interpret these findings in light of Grb7 regulation and function.
Data Driven Model of Autophagy

Autophagy is an intracellular recycling program engaged by cancer cells to adapt to environmental stressors, such as nutrient starvation, hypoxia, and chemotherapeutic assault. In this regard, autophagy promotes robust cell survival and drug resistance. In contrast, autophagy in excess can contribute to cell death through excessive cellular digestion. The level of autophagic flux is thus an important phenotype of cancer cells; however, our understanding of autophagy in cancer is incomplete. A detailed mechanistic understanding of the core autophagy process, including the identification of sensitive points of intervention, is lacking and even the most fundamental question, in what contexts does autophagy protect cells from environmental stressors and drive survival, is not yet understood. To address the gaps in our quantitative and predictive understanding of autophagy, we have developed an accurate computational model of autophagic flux in single cells. We have begun to integrate predictive computational modeling and high quality cell based measurements to accurately model the autophagic process. We anticipate that our model will help identify the most effective therapeutic strategies for targeting autophagy in cancer.
Disease specific cytokine profiles in pediatric patients with malaria, HIV, and systemic bacteremia infections

We present 680 cytokine profiles of blood drawn from well-characterized pediatric malaria patients in the high-disease-burden environment of Siaya, Kenya. High levels of co-infections were observed in this group, including HIV and bacteremia from non-Typhoidal *Salmonella* (NTS) and *Staphylococcus* sp. Cytokine profiles were placed into one of nine disease categories, including healthy controls, reflecting the bulk of seriously ill suspected malaria patients accepted into our study at the Siaya County Hospital. Distinct cytokine signatures were identified using LASSO, a model selection algorithm. Bootstrapping of the model selection procedure provided robustness to our answers against artifacts arising from the complexity of our data. Linear models using selected cytokines were able to identify comorbidities as the most important complexity impacting the cytokine profile. We were able to distinguish bacteremia from malaria with ROC areas under curve of 0.98, 0.85, and 0.88 for differentiating mono-infection with NTS, co-infection of NTS with malaria, and mono-infection with *Staphylococcus* bacteremia, from mono-infection with malaria, respectively. Uninfected controls could be distinguished from the malaria background with an AUC of 0.91. IL-7, IL-8, TNFa, and MIG were the most informative cytokines for distinguishing NTS bacteremia from malaria, while IL-10 and IL-7 were the most able to distinguish *Staphylococcal* bacteremia from malaria. Progression of malaria to SMA was indicated by high levels of IL-2R and low levels of IP-10, while malaria was distinguished from the healthy controls with IL-2R, IL-10, MIP1b, and RANTES. Additional significant correlations of cytokine profiles with death (IL-8), malnutrition (IL-8, IP-10, TNFa, and IL-15), respiratory distress (Eotaxin and IL-8), high fever (IL-6, IP-10, and IL-10), hemoglobin level (IL-2R, IL-6, and RANTES), age (IL-1b, IL-2R, and Eotaxin), and the reticulocyte production index (RANTES and TNFa), were observed.
Intracellular pathogens co-opt host cell processes as part of their life cycle to maximize infection efficiency. Pathogen manipulation of host factors that play an essential role in infection represents potential intervention points for the development of host-directed novel countermeasures against infectious disease. We performed a RNA interference (RNAi) screen of the human kinome to identify host factors that facilitate intracellular survival of Burkholderia thailandensis, a surrogate for the high-risk intracellular bacterial pathogen B. pseudomallei classified as Category B agent by the CDC. From the RNAi screen, we identified 35 kinases that are required for efficient invasion of B. thailandensis into monocytic THP-1 cells and validated a subset of these kinases in uptake assays using a clinical pathogenic strain of B. thailandensis CDC2721121. We also characterized PKC-η∙/MARCKS signaling as a key event that promotes uptake of unopsonized bacteria by host cells. PKC-η∙ is a non-conventional, Ca2+ independent member of the PKC kinase family and appears to play an indirect role in the activation of autophagosome flux by facilitating phagocytic pathways that promote Burkholderia escape into the cytoplasm. Our study indicates that early upon infection when bacteria are not opsonized with antibodies host receptor usage has a role in the pathogen escape from phagocytic destruction leading to unrestricted bacterial replication into the cytoplasm.
Super-resolution Imaging and Monte Carlo Simulations of Initial Events in Mast Cell Signaling

Our work seeks to understand the molecular basis of signaling by the high affinity IgE receptor, Fc\v\muRI, in mast cells. With one-color super-resolution microscopy (STORM), we observed the formation of IgE clusters upon stimulation (Fig. 1). Structurally defined ligands (Fig. 2) allowed us to control the structure of the cluster formed. To correlate cluster structure with transmembrane signaling, we performed two-color STORM (Fig. 3) in which we imaged IgE and its signaling partner Lyn kinase. Lyn is anchored to the inner leaflet by saturated fatty acyl chains, giving it a preference for the liquid ordered (Lo) membrane phase. We found that Lyn and IgE show the largest stimulated colocalization in cases where IgE clusters have a high density of receptors.

Our theoretical work with the Ising model further addresses the relationship between receptor clustering and lipid redistribution. The model considers a membrane near a critical phase transition, and examines the consequences of immobilizing proteins with Lo phase preference (e.g. IgE receptors) in close proximity to each other. Fig. 4 shows a snapshot and average of a Monte Carlo simulation of this model. We found that the cluster stabilizes a local region of increased order, which gives an energetic preference for recruiting other Lo-preferring molecules (e.g. Lyn kinase). This preference is larger when the receptors are more closely packed, consistent with our experimental findings using structurally defined ligands.
Lipid regulation of G protein-coupled receptor signaling

Intercellular communication is essential for many facets of multicellular life. An integral component of this signaling involves G protein-coupled receptors (GPCRs), eukaryotic membrane proteins that have evolved to bind thousands of different chemicals and evoke varied cellular responses. However, it is unclear how receptors exhibit robust signaling in vivo when active states are adopted only transiently in vitro. To resolve this apparent contradiction, I will show how phospholipids from the membrane's native environment can regulate receptor activity via specific lipid-protein binding interactions that sterically block collapse of the receptor's cytosolic binding pocket. Furthermore, I will show how negatively charged lipids stabilize active states more readily than neutral lipids, providing a molecular mechanism for the experimental observation that anionic lipids can dramatically enhance receptor activity in vitro. The dependence of this specific protein-lipid interaction on lipid composition may permit biologically relevant modulation of receptor activity based on cell type, cell-cycle stage, diseases such as leukemia, and receptor localization in membrane microdomains.
Mannan Molecular Sub-structures control nanoscale glucan exposure in Candida

N-linked mannans in the cell wall of Candida albicans are thought to mask \( \beta^2-(1,3) \)-glucan from recognition by the transmembrane C-type lectin (CTL) Dectin-1, which contributes to innate immune evasion. Cell wall surface exposures of glucan on C. albicans are predominantly single receptor-ligand interaction sites and are restricted to nanoscale geometries (Fig. 1). It is thought that bulky N-linked mannan polysaccharides of the outer cell wall provide steric masking of glucan to restrict its exposure. Significant Candida species pathogens exhibit a wide range of basal glucan exposures (C. albicans < C. parapsilosis < C. glabrata), and the mannans produced by these species vary in size and complexity at the molecular level. Using super resolution fluorescence imaging and a series of mannosyltransferase mutants in C. albicans and C. glabrata, we investigated the role of specific mannan structural features in regulating the nanoscale geometry of glucan exposure. Mutant strains with decreased size and molecular complexity of N-mannans exhibited significant increases in surface density and size of glucan exposures on the nanoscale (Fig. 2). Furthermore, a recent C. albicans clinical isolate with high glucan exposure had much smaller and less complex N-linked mannan and nanoscale glucan exposure characteristics similar to the mannosyltransferase mutants examined above. Our studies demonstrate that specific sub-molecular features of N-linked mannan, especially the acid-labile fraction and \( \beta^2-(1,6) \)-backbone length, are important determinants of the immunogenic glucan pattern perceived by Dectin-1 at molecular dimensions.
T cells are capable of distinguishing between multiple interfaces

As essential components of the adaptive immune system, T cells continuously scan multiple antigen presenting cells (APCs) for foreign or cancer derived peptide-loaded major histocompatibility complexes (pMHCs) using their hypervariable T cell receptor (TCR). Despite extensive work, it is presently unclear how T cells integrate pMHC signals across multiple APCs.

Here, we stimulated T cells expressing an affinity-enhanced TCR with different numbers of beads coated with pMHCs at different concentrations as surrogate APCs. While increasing the pMHC concentration per bead led to both increases and decreases in T cell activation, increasing the number of beads only resulted in improved T cell activation. We formulated a minimal model able to reproduce these observations.
Developing diagnostic assays for Salmonella bacteremia infections

Salmonella, and especially non-Typhi Salmonella (NTS) are a major cause of infections in sub-Saharan Africa. Invasive NTS has a case fatality rate of between 4.4-27% in children and 22-47% in adults. This is compounded with the lack of proper resources in some of these communities. We are working towards developing fieldable, rapid diagnostic assays for the detection of Salmonella using two methods: pathogen biomarker detection using a LANL-developed waveguide-based platform, and real-time PCR. The former requires minimal handling of the samples, and allows rapid detection of the antigen using a fluorescently-labeled probe. Two different types of assay strategies were used for the waveguide-based assays: membrane insertion assay and lipoprotein capture assay, both developed by our team. Both assays utilize a lipid bilayer for functionalization of the waveguide. Using lipid lysates prepared from ATCC control strains of Salmonella and clinical strains from rural Kenya, we are able to detect pathogen-specific signatures using these assays. Further work includes identification and characterization of the antigen target, and testing patient samples from Kenya to validate the assay. Real-time PCR primers for Salmonella detection have been developed and tested to further characterize the infectious agent. Testing is currently being performed with a Gram detection assay to help differentiate between Gram positive and negative bacteria. Further validations will be done on clinical samples for these assays as well. We hope to deploy these technologies in our clinical site in rural Kenya within a year’s time, and train local personnel to run them, thereby improving health care infrastructure in country.
Hidden influence of membrane composition on cellular level movement of oxygen and key signaling molecules

Oxygen is widely assumed to cross membranes so readily that the membrane’s influence on cellular oxygenation can be neglected. Similarly, small and relatively nonpolar signaling molecules such as nitric oxide, carbon monoxide, hydrogen sulfide, and hydrogen peroxide are thought to diffuse quite readily across cellular membranes. We show evidence from well-validated computer simulations, suggesting that the research community has overestimated the general permeability of biological membranes. In particular, POPC phospholipid bilayers are similar in permeability to pure water, and inclusion of membrane cholesterol reduces the permeability below that level. Cholesterol’s impact is strongest when its solubility threshold is exceeded and some cholesterol molecules phase separate from the phospholipid. The resulting cholesterol-only regions have relatively low permeability, and they seem to reduce the bulk permeability of the membrane in proportion to the area they occupy. Experimental evidence indicates that membrane proteins also have low oxygen permeability and should, likewise, tend to reduce the bulk permeability of the membrane. Therefore, we suggest that biological membranes present a non-negligible barrier to the diffusion of oxygen and key signaling molecules. Variation in the magnitude of the barrier, due to normal or pathological variation in membrane composition, may have broad biological significance.
Promoter analysis of drought-induced genes using BY-2 cell culture

Drought is one of the major environmental factors affecting crop production worldwide. In order to understand the potential mechanisms involved in plants responses to drought, characterization of promoters of target genes need to be conducted. In this study, we employed tobacco cv Bright Yellow-2 (BY-2) cell culture to characterize the promoters of drought-induced transcription factors and downstream genes. A total of three WRKY genes and three downstream genes (ubiquitin protein ligase) like 1, galactinol synthase and raffinose synthase) were selected from tobacco and two WRKY genes (GmWRKY) were selected from soybeans. These candidate genes were selected based from our transcriptome profile dataset for drought-stressed tobacco and soybean plants. GFP and GUS quantification of the WRKY and enzyme-coding genes showed varying response of these genes to PEG, NaCl, jasmonic acid or mannitol treatments in BY-2 cells. These showed that drought-induced genes could also be induced by other stresses.
B cell clones in repeated AVA immunization are first drawn from autoreactive memory, then naïve cells, and evolve continually. . .

Anthrax Vaccine Adsorbed (AVA) immunization protects against anthrax by eliciting a specific, humoral response, however, protection is not conferred in humans until three immunizations have been administered over six months. Even then, AVA does not provide long-term immunity without two more booster doses and an annual booster. We followed six healthy volunteers over the standard five-dose, 18-month AVA schedule to characterize the plasmablast repertoire during the immunization series. After primary immunization, the mutation frequencies of the observed plasmablast clones indicated that they were derived from previously affinity matured memory cells. Further, 9.2% of sequences had very high mutation frequencies (>10%), which prior studies have shown to be associated with multiple antigen exposures. After secondary immunization, naïve-derived plasmablast clones were observed and mutation patterns consistent with affinity maturation occurred following subsequent immunizations. We synthesized and tested a subset of naïve- and preexisting memory-derived antibodies for binding specificity. While memory-derived antibodies exhibited no affinity to the immunodominant AVA antigen, they displayed significant affinity to a variety of autoantigens. This is in contrast to naïve-derived antibodies, which had increasing affinity to AVA antigen, and little to no autoantigen binding. Thus, while primary AVA immunization elicits autoreactive, pre-existing memory plasmablasts, these clones are highly transient and do not contribute to the protective response which is not initiated until the second immunization.
StormGraph: A graph-based clustering algorithm for the analysis of super-resolution microscopy data

With super-resolution microscopy techniques such as Stochastic Optical Reconstruction Microscopy (STORM), it is possible to image fluorescently labeled proteins on a cell membrane with high precision. Often, the extent to which such proteins cluster is biologically meaningful; for example, in B-cells, clustering of the B-cell receptor (BCR) is associated with increased intracellular signaling and B-cell activation, and spontaneous BCR clustering can cause chronic active BCR signaling that results in an aggressive B-cell malignancy. Computational methods are therefore needed to make quantifiable comparisons between the observed clustering in different data sets, such as for different cell types or different experimental conditions.

Inspired by the success of graph-based clustering algorithms such as PhenoGraph in other research areas, we developed StormGraph, a graph-based clustering algorithm for analyzing Single Molecule Localization Microscopy (SMLM) data such as would be obtained by STORM. StormGraph reliably distinguishes clusters from random background and assigns individual localizations to specific clusters, allowing for a detailed analysis of statistics such as cluster area and density.

In this poster, we present StormGraph and show that it outperforms two leading methods for clustering SMLM data when applied to simulated STORM data. Furthermore, we apply StormGraph to analyze clustering of BCRs on B-cells (see attached figure).
Drug Discovery for Chagas Disease

Nearly 20 million people worldwide are infected with Trypanosoma cruzi, the etiologic agent of Chagas disease. The suboptimal effectiveness and significant toxicities of existing antiparasitic drugs drives the search for new therapeutic strategies in the treatment of this disease. T. cruzi are most commonly transmitted through a vector and have four well-defined developmental stages that include distinct morphological and functional characteristics, each of which affect the parasites’ ability to infect human hosts. The search for antiparasitic compounds began by establishing an infection in mammalian cells. The different stages of T. cruzi were confirmed by confocal microscopy and the appropriate parasitic stage was used for infection. African green monkey kidney cells (Vero) were cultured and infected with T. cruzi for 24 hours before being treated with the novel compound AKS7. Post-treatment with sub-toxic concentrations of AKS7 revealed a dramatic reduction in the presence of both extracellular and intracellular parasites. To determine if infection could be prevented, Vero cells were infected with T. cruzi and co-treated with AKS7. After an incubation of 4 days, confocal microscopy revealed that co-treatment with AKS7 had prevented infection in Vero cells and had inhibited replication of extracellular parasites. Furthermore, a natural tea extract from leaves of Arctostaphylos uva ursi (Uva ursi) offered surprising and promising results. Uva ursi prevented an infection from being established in Vero cells at 0.25% v/v, a dose that was ten times lower than the IC50 of 2.5% v/v in Vero cells. This data provides a promising new lead on the search for a better understanding of the different T. cruzi developmental stages as well as for the search for a less toxic, more effective treatment of T. cruzi infection.
Developing Phosphorylation Specific Antibodies to Study Cell Surface Receptor Activation in Allergy Pathway

The high affinity IgE receptor, FceR1, is expressed on mast cells, basophils, and antigen presenting cells. Phosphorylation of this receptor and the ensuing signal cascade play a critical role in the development of allergic inflammation. The receptor has three subunits: a cell surface alpha-domain that binds to IgE, the beta-chain that functions to enhance receptor maturation, and a gamma-homodimer that enhances signal transduction. The immune-receptor tyrosine-based activation motif (ITAM) regions of the beta and gamma subunits have multiple tyrosines that can be phosphorylated.

Here, we describe the successful selection and characterization of phosphorylation specific antibodies to the beta and gamma subunits of the FceR1 receptor. We utilized phage display and yeast display technologies to select and affinity mature phosphorylation specific antibodies from a highly diverse single chain antibody library. Phosphorylated peptides corresponding to the ITAM region of the high affinity FceR1 receptor were used as antigens in the selection process (Figure 1). We selected three beta-subunit antibodies recognizing: phosphorylation of the N-terminal tyrosine (p1), C-terminal tyrosine (p3), and phosphorylation of all three tyrosines (p123) (figure 2). We utilized these antibodies to study the receptor activation timeline in rat basophilic leukemia cells (RBL) upon stimulation with DNP-BSA (figure 3). We have also selected an antibody able to recognize the N-terminal phosphorylation site of the gamma-subunit of the receptor and utilized this antibody to evaluate the receptor activation (figure 4). Recognition patterns of these antibodies show different timelines for phosphorylation of tyrosines in the ITAM region, indicating various stages of receptor activation/maturation and the corresponding signal cascade.

The methodology we have developed provides a strategy to select antibodies specific to post-translational modifications. These antibodies will be able to facilitate greater understanding of immune cell surface receptor activation and their role in cell signaling.
A fluorescent protein toolbox for studying host-pathogen interactions

We describe the split fluorescent protein toolbox we have developed for studying protein trafficking and protein-protein interactions. The system differs from others by the use of small peptide tags rather than large protein fragments. We describe case studies of its application in our lab and other labs to host-pathogen protein-protein interactions, trafficking of effectors, and protein-RNA interactions.
How insulin binds: structure of a micro-receptor complex and implications for analog design

The discovery of insulin in 1921 represented a landmark in molecular medicine and led to extensive investigation of the structure and function of this globular protein hormone with application to therapeutic analog design. This presentation provides a summary of the current structural understanding of the active conformation of insulin in relation to its mechanism of receptor binding. Implications of recent crystal structures and NMR studies will be discussed as a foundation for the engineering of novel ultra-stable single-chain analogs, intended as basal (long-acting) insulin formulations for use in regions of the developing world lacking access to refrigeration. Prospects will be envisaged for the extension of such protein technology to implantable intra-peritoneal insulin pumps whose present use is complicated by degradation of the hormone at body temperature on a time scale of 1–3 months.

A key constraint in the design of therapeutic insulin analogs is posed by their physical degradation to form amyloid. Spectroscopic studies of insulin fibrils has provided structural constraints regarding the molecular structure of a protofilament and distorted conformations of insulin proposed as intermediates in the process of fibrillation. Consideration of such constraints has highlighted the potential utility of single-chain insulin analogs containing foreshortened connection domains. Such foreshortened tethers must accommodate inducted fit of the hormone on receptor binding, a binding mechanism that entails splaying of the C-terminal segment of the B chain. Insight into the hormone-binding surfaces of the ectodomain of the insulin receptor and “micro-receptor” models of the hormone-receptor complex have enabled visualization at low resolution of how the splayed B chain inserts between domains of the receptor. These recent structures provide a new and promising foundation for analysis of structure-activity relationships with direct application to the design of novel insulin analogs. Efforts are underway toward the optimization of insulin analogs to address unmet needs of patients with diabetes mellitus in affluent societies and in the developing world.
Computational Identification of Peptide Antigens Bound by T Cell Receptors

The binding of T cell receptors (TCRs) to their target peptide MHC (pMHC) ligands initializes the cell-mediated immune response. In autoimmune diseases, T cells erroneously recognize self peptides as foreign and activate an immune response against healthy cells. Evidence suggests such responses can be triggered by cross-recognition of the autoreactive TCR with other foreign peptides; however, the large sequence space of relevant candidate peptides presents an obstacle in the identification of the causative foreign antigens. Here, we present an in silico scoring and design method which utilizes the structural properties of TCR-pMHC complexes to predict binding specificity. We analyzed three mouse TCRs and one human TCR isolated from a patient with the autoimmune disorder multiple sclerosis. Cross-reactive peptides for these TCRs were previously identified via yeast display coupled with deep sequencing, providing a rich dataset for validating results. With a design and scoring protocol, our method accurately selects the top binding peptides from sets containing greater than 100,000 unique peptides.
3D Molecular Tracking in Live Cells

We have been developing methods for following 3D motion of selected biomolecular species throughout mammalian cells. Our approach exploits a custom designed confocal microscope that uses a unique spatial filter geometry and active feedback 200 times/second to follow fast 3D motion. We have used this instrument to follow individual quantum dot labeled allergy receptors (IgE-Fc(epsilon)RI) during cell stimulation with antigen and during down-regulation by endocytosis. By exploiting new non-blinking quantum dots, individual molecular trajectories can be observed for several minutes. We also will discuss recent instrument upgrades, including the ability to perform fluorescence resonance energy transfer (FRET) measurements while tracking and the ability to observe 3D cell structure by spinning disk microscopy performed simultaneously with 3D molecular tracking experiments.
Transient Homo-Interactions Drive Autonomous Signaling from the Pre-BCR Signaling Complex

Autonomous signaling mediated by the pre-B Cell Receptor (pre-BCR) is essential for survival of B cell progenitors during normal B cell development and is potential therapeutic target in precursor B acute lymphoblastic leukemia (BCP-ALL). To enable two-color single particle tracking of the pre-BCR and measurements of receptor homo-dimerization in real time, we have developed probes based on anti-Igβ Fab fragments conjugated to Quantum Dots (QDs). We report the first studies of the dynamics of pre-BCR diffusion on the surfaces of cultured pre-B cells, as well as blasts isolated from BCP-ALL patients. Results show that pre-BCR engage in transient but frequent homotypic interactions. Motion is correlated only at short separation distances, consistent with formation of dimers and potentially larger order oligomers. Homo-interactions are blocked by anti-VpreB1 Fabs that bind with nano-molar affinity, demonstrating that binding is mediated by surrogate light chain components specific to the pre-BCR. Mathematical modeling based upon these quantitative data sets provides insight into the steady-state dimer events that drive autonomous signaling from pre-BCR. By contrast, galectin-mediated crosslinking of pre-BCR leads to interactions with extended lifetimes, markedly slowed diffusion and differential signaling. Consistent with pre-BCR signals providing pro-survival signals, treatment of BCP-ALL cells with inhibitors targeting downstream signaling partners (Syk, Lyn, SHIP) induce cell death. The pre-BCR and its signaling pathway represent potential therapeutic targets in BCP-ALL, the most common malignancy in children and an aggressive disease in adults.
Understanding Host Innate Immune Defense Provides Strategies for Developing Pre-symptomatic Diagnosis and Therapy of Infectious Diseases

Host innate immunity is induced early as the first line of defense against invading pathogens. Innate immune defense breaks down during successful pathogenic infection. This breakdown manifests as the up- and down-regulation of a set of host innate immune genes during the early stages of infection. Therefore, we developed a strategy to use these genes as biomarkers for pre-symptomatic diagnosis of disease caused by a given pathogen. Similarly, some host antimicrobial peptides (an important arm of innate immune defense) are also underexpressed or inactivated during the early stages of infection. We have developed a strategy to design antimicrobial proteins that are expressed at a high level and show enhanced activity against the invading pathogen.

We successfully applied our strategies against a citrus disease called Huanglongbing (HLB), the most devastating disease of citrus worldwide. Gram-negative bacteria called Liberibacter, transmitted by psyllid vectors, cause HLB. Both a cure and pre-symptomatic diagnosis of HLB are urgently needed for the citrus industries.

We performed transcriptome studies to discover and validate citrus gene biomarkers that are differentially altered during the pre-symptomatic stages of Liberibacter infection and are also present throughout the tree. Hence, samples collected from any part of the tree will contain these biomarkers. These biomarkers belong to two classes of innate immune pathways: one involving pattern-recognition receptors on the cell surface and the other involving intracellular nucleotide-binding oligomerization domain (NOD)-like receptors.