Research in our laboratory is focused on the molecular mechanisms of calcium signaling through store-operated Ca2+ channels (SOCs). This class of ion channels is regulated in a unique way, by the depletion of Ca2+ from the lumen of the endoplasmic reticulum (ER) which normally occurs following stimulation of cell surface receptors that generate IP3. They are expressed in practically all cells, where they contribute to diverse functions including secretion, gene expression, and cell differentiation. A SOC called the Ca2+ release-activated Ca2+ channel, or CRAC channel, is particularly important in T cells, where it generates sustained Ca2+ signals.
that are essential for triggering T cells to proliferate and carry out immune functions. Loss of function of the CRAC channel by a single mutation in its structural gene leads to a devastating severe combined immunodeficiency (SCID) syndrome in humans.

A major effort in the lab is to understand how the depletion of Ca2+ from the ER triggers the opening of CRAC channels in the plasma membrane. Since the recent discovery of genes encoding the ER Ca2+ sensor (STIM1) and the CRAC channel (Orai1), we have made rapid progress in revealing the choreographic nature of this process. Following store depletion, STIM1 moves from locations throughout the ER to accumulate in ER subregions positioned within 10-25 nm of the plasma membrane (PM). Simultaneously, the CRAC channel gathers at corresponding sites in the PM directly opposite STIM1, where it is opened through an interaction with STIM1. This is an unprecedented mechanism for channel activation, in which a stimulus acts to bring a channel and its activator/sensor together for interaction across apposed membrane compartments. We are currently studying how changes in ER Ca2+ drive the redistribution of STIM1 and the mechanism by which STIM1 and Orai1 interact across the ER-PM gap. For these studies we combine protein engineering with patch-clamp electrophysiology and live-cell imaging using total internal reflection fluorescence (TIRF), confocal, and single-molecule microscopy.

A second major area of interest is how specific information is encoded in Ca2+ signals. Specificity is an acute problem for pluripotent messengers like Ca2+ that are involved in multiple signaling pathways. We have shown that transcriptional specificity in T cells can be achieved through the amplitude and dynamics of Ca2+ signals generated by CRAC channels. We are now studying how these features contribute to cell fate decisions during T cell development. In the thymus, self-reactive thymocytes are deleted through negative selection, while cells with the appropriate avidity for “self” are allowed to mature into T cells and populate the periphery (positive selection). To study the role of Ca2+ in this choice, we have developed a novel thymic slice preparation in which we use two-photon microscopy to track and record Ca2+ signals in single thymocytes as they migrate through tissue engineered to provide defined selection signals. We have found that positive selection is associated with Ca2+ oscillations, which immobilize the cells at locations of self-antigen recognition to promote gene activation. We are currently comparing signaling during positive and negative selection to determine how the Ca2+ signal “signature” helps a T cell decide whether to live and prosper or die.

### Teaching

#### COURSES

**2019-20**
- Imaging: Biological Light Microscopy: BIO 152, MCP 222 (Aut)

**2018-19**
- How Cells Work: Energetics, Compartments, and Coupling in Cell Biology: MCP 256 (Spr)
- Imaging: Biological Light Microscopy: BIO 152, CSB 222, MCP 222 (Win)

#### STANFORD ADVISEES

**Doctoral Dissertation Reader (AC)**
- Garrison Buss, Eirini Tsekitsidou

**Postdoctoral Faculty Sponsor**
- Michael Kirmiz

**Doctoral Dissertation Advisor (AC)**
- Suzanna Bennett

#### GRADUATE AND FELLOWSHIP PROGRAM AFFILIATIONS

- Biophysics (Phd Program)
- Chemical and Systems Biology (Phd Program)
• Immunology (Phd Program)
• Molecular and Cellular Physiology (Phd Program)
• Neurosciences (Phd Program)

Publications

PUBLICATIONS

• Store-Operated Calcium Channels: From Function to Structure and Back Again. *Cold Spring Harbor perspectives in biology*
  Lewis, R. S.
  2019

• Structural features of STIM and Orai underlying store-operated calcium entry. *Current opinion in cell biology*
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• Physiological CRAC channel activation and pore properties require STIM1 binding to all six Orai1 subunits. *The Journal of general physiology*
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