**Speaker:** Aaron Streets

**Affiliations:** University of California, Berkeley

**Title:** Single-cell multimodal analysis of thymocyte development reveals drivers of CD4/CD8 lineage commitment

**Abstract:** CD4 and CD8 T cells play critical roles in the mammalian immune system. While their development within the thymus from the CD4+CD8+ stage has been widely studied as a model of lineage commitment, the underlying mechanism remains unclear. To deconstruct this process, we applied CITE-seq, measuring the transcriptome and over 100 surface proteins in thymocytes from wild-type and lineage-restricted mice. We developed totalVI, a probabilistic model for joint analysis of transcript and protein abundance measurements to build a comprehensive timeline of cell state in each lineage. This integrated analysis supported a sequential model of lineage determination in which both lineages go through an initial phase of CD4 lineage audition, which is followed by divergence and specification of CD8 lineage cells. We identified early differences implicating T cell receptor signaling via calcineurin-NFAT in driving CD4 lineage commitment. Pharmacological inhibition of TCR signaling validated the requirement of calcineurin- NFAT for CD4, but not CD8, lineage development, providing insight into the CD4/CD8 commitment mechanism.