Understanding the signaling architecture of calcium-dependent protein kinases in apicomplexan parasites

Awardee: Sebastian Lourido
Award: Early Independence Award
Awardee Institution: Whitehead Institute for Biomedical Research
Co-author: Lucas D. Tilley
Co-author institution: Whitehead Institute for Biomedical Research

Identifying the substrates of different protein kinases remains a major obstacle to understanding the signaling pathways that regulate eukaryotic cells. The complexity and plasticity of these pathways makes it difficult to distinguish the contribution of individual kinases in global studies, and yet *in vitro* assays frequently lack many of the cellular features that dictate kinase-substrate specificity. By engineering kinases that accommodate bio-orthogonal ATP analogues, others have previously demonstrated the feasibility of monitoring the activity of individual kinases in complex lysates. We have improved on these methods using pore-forming toxins to semi-permeabilize cells and quantitative phosphoproteomics to identify the substrates of two related kinases, CDPK1 and CDPK3, in the human parasite *Toxoplasma gondii*. We have previously demonstrated that both calcium-dependent protein kinases (CDPKs) contribute to the regulation of parasite motility and are essential for the infectious cycle. By preserving cellular ultrastructure, we are able to determine the contribution of subcellular localization to kinase specificity. Furthermore, analysis of the substrates has extended our understanding of the cellular processes regulated by these kinases. This includes the previously neglected role of CDPK3 in the regulation of ion homeostasis and the preparation of parasites for extracellular survival prior to exiting the infected host cell. Beyond its relevance to human health, *T. gondii* serves as a model for other apicomplexan parasites, including *Plasmodium spp.*, the causative agents of malaria. We hope that our work will serve as the basis for comparing calcium-regulated signaling within the phylum, and as a model for understanding the role of subcellular localization in the evolution of kinase families.