In vivo variability of regulatory programs and single cell physiology in central homeostatic control circuits

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The guiding systems biology viewpoint of our research program is that cells are the building blocks of biological function; that function arises from dynamic networks of diverse cells in tissues interacting with the environment to cause functional phenotype. In probing these functional networks we need to be able to assay and manipulate molecular processes occurring in individual cells on a very large scale of sample numbers and types, in their tissue and phenotypical context, to support network reconstruction. In addition, we need to derive functional meaning from the resulting complex datasets, and to develop useful functional hypotheses from the analyses. These requirements/approaches are uncommon in present mammalian systems biology practice and require focused attention on assembling innovative, novel technical approaches into a coherent resource.

Our research focus aims to understand how the *in vivo* adaptive molecular responses within neurons and other cells work together in a local brain region, affecting neurons involved in homeostatic function and disease. Our strategy is to develop innovative approaches to (1) manipulate environmental inputs to drive an in vivo functional response in reproducible and controllable ways using these inputs and genetic methods to perturb, temporal and spatial gene expression patterns across the manipulated in vivo samples (2) query cell networks all the way to individual cells, in order to monitor altered specific molecular processes and network activity in phenotypically homogeneous subsets of cells and individual cells. The ability to manipulate and assay single cells will provide a wealth of data concerning cellular physiology (3) meet analytical method requirements for large sample numbers, yielding 3-dimensional molecular measures in tissue space and in time, producing dense time series, and extensive anatomically and phenotypically specific samples (4) innovate computational approaches to interpret context specific network behaviors (5) identify and model integrative regulatory relationships between molecular processes and cell network responses controlling tissue functional phenotype involving an analysis of systems of systems.

In this context, we will summarize key challenges and limitations in tackling these issues in the context of neuroscience and neurophysiology. We will present our experimental and computational results and perspective on considering variability of transcriptional programs at the single cell level. We will discuss our current and evolving statistical/bioinformatics methods that are combined with mechanistic modeling and simulations. We then interpret the observed range of *in vivo* cellular and system states to derive variability in the regulatory networks driving the expression programs. A key learning from our efforts so far has been that combining pathway-scale views of the regulatory programs with multi-dimensional data-driven analyses is a tractable way to approach the aforementioned challenges.