

Submitter Name: James J. Cai
Submitted email: jcai@tamu.edu

Machine Learning Analysis of Single-cell Gene Regulatory Networks Reveals Signature Transcriptional Responses in Neurons to Acute Morphine Treatment

Daniel Osorio¹, Yan Zhong², Guanxun Li², Jianhua Z. Huang², James J. Cai^{1,3,4}

¹Department of Veterinary Integrative Biosciences; ²Department of Statistics; ³Department of Electrical and Computer Engineering; ⁴Interdisciplinary Program of Genetics; Texas A&M University, College Station, TX 77843, USA.

Single-cell RNA sequencing (scRNA-seq) provides an unparalleled degree of precision to analyze transcriptional regulation, cell history, and cell interactions with rich knowledge. Comparing gene regulatory networks (GRNs) constructed from scRNA-seq data can reveal critical components in the underlying regulatory networks regulating different cellular transcriptional activities. We developed scTenifoldNet—a machine learning workflow built upon principal component regression, low-rank tensor approximation, and manifold alignment—for constructing and comparing single-cell GRNs (scGRNs) to reveal regulatory network changes between samples. We applied scTenifoldNet to a scRNA-seq data set generated by Avey and colleagues in a study on transcriptional responses of mouse neural cells to morphine. The measured cells include 7,972 and 8,912 neurons from morphine- and mock-treated samples, respectively. Using scTenifoldNet, we identified 56 genes showing significant differences in their transcriptional regulation between mock- and morphine-treated neurons. GSEA analysis with the ranked list of all genes showed that differentially regulated genes are enriched for *opioid signaling*, *signaling by G protein-coupled receptors*, *reduction of cytosolic Calcium levels*, and *morphine addiction*. Using the constructed scGRN, we were able to trace significant genes back to their topological positions in the network and examine their interacting genes. In addition to scTenifoldNet, we developed another method, called scTenifoldKnk, to perform virtual knockout, using a similar network science strategy, to predict perturbation caused by gene knockout on a given scGRN. We also developed pseudotime-series network analysis, which can be used to identify top regulators responsible for cell differentiation.