Plato's Cave Algorithm: Reverse Engineering Functional Signaling Networks

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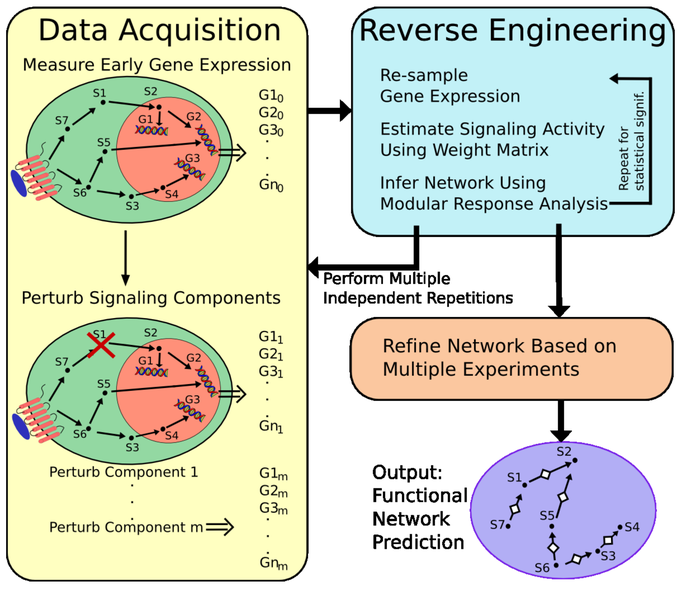
Abstract

The activity levels of components in the signaling network that mediate the changes in gene expression are less accessible to experimental measurement than the genes they regulate. Recently, it was shown that the profile of the early gene program provides a sensitive reflection of the response state of the signaling network. We developed a technique that uses early gene responses to cell stimulation and systematic signaling component perturbation to reverse engineer the functional interactions between the signaling components. This approach is robust to noise and produced novel and verifiable predictions based on experimental datasets.

PLACA Methodology Overview



Modular Response Analysis

A method to reverse engineer interactions between genes or proteins by measuring the steady-state activity of each one both under normal conditions and after sequential perturbations of each one.

[Kholodenko et al., PNAS 99, no. 20 (2002): 12841-12846]

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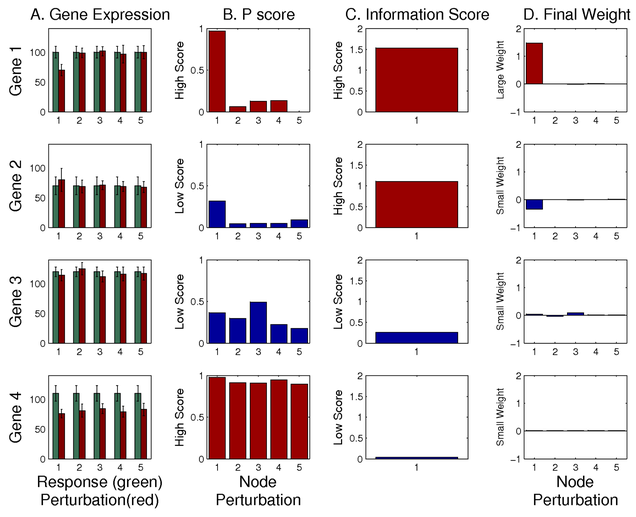
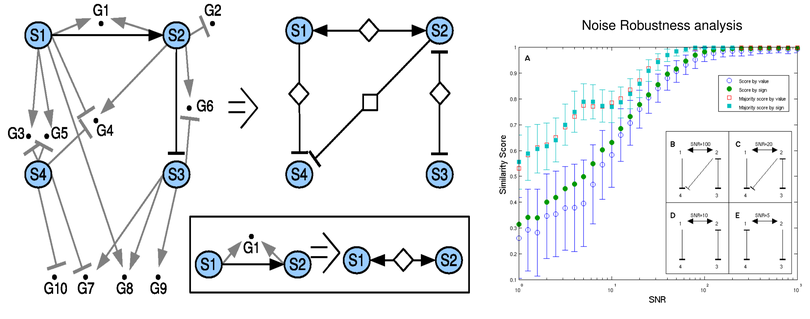
Choosing Good Predictors of Signaling Activity

- Genes that change significantly only for a single perturbation are good predictors
- P-score - the statistical significance of the change
- Information score - based on the Shannon entropy
- The final weight determines how much the change in each gene's activity contributes to the estimated activity of each signaling component



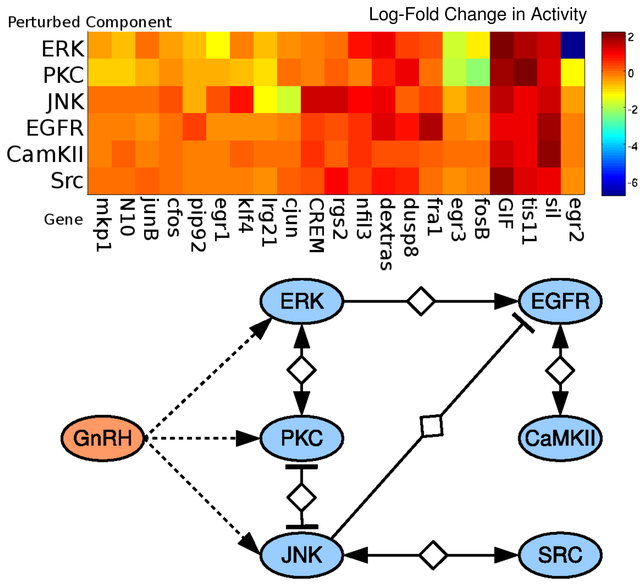
Testing PLACA on Synthetic Data

- A synthetic biochemical interaction network was correctly inferred by PLACA into a functional interactions network.
- Note that S1 and S2 both activate gene g1, therefore they have a positive functional interaction
- PLACA is also robust to noise, especially when multiple experiments are used



Using PLACA on Experimental Data

- LβT2 gonadotropes were treated with 6 chemical inhibitors + GnRH (Y axis)
- The activity of 21 genes was measured (X axis)
- PLACA was applied to data from 5 experiments
- The interactions between ERK and PKC, between JNK and Src, and between EGFR and CaMKII were previously seen experimentally



Validating Predicted Functional Interaction

- JNK is predicted to functionally repress EGFR
- LβT2 cells were treated with a JNK inhibitor and varying levels of EGF, and *klf4* activity measured
- A combination treatment with JNK inhibitor and EGF resulted in a stronger induction of *klf4* as compared to EGF alone or the JNK inhibitor alone