Brain tumor cellular architectures are predicted through phosphoprotein signaling measurements in two-cell system.

Nataly Kravchenko-Balasha[a], Young Shik-Shin[b], Jun Wang[b], R.D. Levine[c,d] and James R. Heath[b]

[a]Bio-Medical Sciences department, The Faculty of Dental Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel, e-mail:natalyk@ekmd.huji.ac.il,+972-2-6757583; [b]NanoSystems Biology Cancer Center, Division of Chemistry and Chemical Engineering, Caltech, Pasadena, United States; [c]The Institute of Chemistry, The Hebrew University of Jerusalem, Jerusalem, Israel; [d]Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, UCLA, Los Angeles, United States.

Understanding of tumor architectures and the forces that drive their formation is of high importance in cancer research. To understand cell-to-cell spatial organization, we developed a methodology that combines single cell functional proteomics and theoretical analysis. We have found that signal transduction in two interacting glioblastoma (GBM) cancer cells depends on the cell-cell separation distance. Using thermodynamic-based analysis of protein concentrations as a function of cell-cell distance in two interacting cells we were able to identify the cell-cell separation distance that corresponded to the steady state of the cell-cell protein signaling. That length scale was found to be the dominant cell separation distance in bulk tissue culture [1]. Thereby we predicted that aggressive GBM cells would exhibit a scattered distribution, whereas less aggressive GBM cells would closely pack, consistent with the experimental observations of others *in vivo*. Furthermore we recently demonstrated that proteins secreted by 2 communicating GBM cells generate a free energy gradient that induces a directed cell-cell motion towards the most stable cell-cell separation distance. Neutralizing the secreted proteins most involved in establishing the free energy gradient canceles the directed motion, such that cell pairs show a random Brownian motion, similar to the case of isolated single cells.

[1] Kravchenko-Balasha, N., et al., *Glioblastoma cellular architectures are predicted through the characterization of two-cell interactions.* Proc Natl Acad Sci U S A, 2014. **111**(17): p. 6521-6.