Dose-discrimination in single cells

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**Short Abstract** — Information about inflammatory cytokines in the extracellular milieu is relayed by the NF-κB transcriptional system to coordinate diverse cellular responses. Although data from cell populations give the impression of a system that can be finely-tuned, widespread heterogeneity between responses when viewed at the level of single cells support a switch-like mechanism for pathway activation with dose information that is severely limited. In this study, we measure dynamic properties of IKK and NF-κB localization in several human cancer cell lines to observe responses of single cells to inflammatory cytokines. We find that the information transmission capacity is heterogeneous between subpopulations of isogenic cells, with some cells carrying significant information about cytokine dose. In all cases, time-course data demonstrates that single cells can discriminate cytokine doses across orders of magnitude in concentration.

**Keywords** — Information theory, NF-κB, IKK, TNF, IL-1, Signal transduction, inflammation.

I. INTRODUCTION

Biological systems rapidly respond to changing environmental conditions in the presence of ‘noise’ that originates from intrinsic and extrinsic sources \cite{1,2}. Intrinsic noise results from fluctuations that are inherent to stochastic collisions between molecules, especially in components that regulate gene transcription and translation in the cell \cite{3,4}. Extrinsic noise arises from sources outside stochastic biochemical processes that lead to cell-to-cell heterogeneity in the abundance and activity of proteins for signal transduction. For instance, states of genetically identical cells can drift over time due to differences in their microenvironment, history or age, cell cycle phase, partitioning during mitosis, among other sources of extrinsic noise that affect rates for essential biological processes. Both sources of noise pose a significant challenge to biological signaling systems because they create uncertainty in how each cell responds to stimuli, thereby limiting the amount of information cells can accurately process about their environment \cite{5}.

II. RESULTS

In this work we set out to look for ‘missing’ information by measuring single-cell responses to cytokines that activate the NF-κB transcriptional system. We compared a panel of human cell lines for sensitivity to TNF or Interleukin-1 (IL-1), using fixed-cell immunofluorescence to quantify variation in nuclear NF-κB abundance. We selected the KYM-1 cell line for expression of a fluorescent reporter, and measured time courses for dynamics of nuclear NF-κB in 1000’s of single cells exposed to various concentrations and durations of TNF. By accounting for digital properties of pathway activation, and dynamic features of NF-κB that regulate transcription, we used an information-theory formalism to quantify the channel capacity of TNF-induced signal transduction. We show that TNF-induced signals have a much larger bit-depth than previously predicted, suggesting that responses to increasing cytokine doses may be graded when viewed in single cells. To test this prediction, we exposed live cells to a series of cytokine pulses. Cells were first exposed to a short-duration pulse of low concentration TNF to provide a point of reference for each cell. The same cells were then imaged again after exposure to a test pulse of the same cytokine but at various concentrations. Despite significant heterogeneity when comparing single cells exposed to the same cytokine conditions, we observed dose-dependent responses when comparing different conditions in the same cell. These observations were confirmed for TNF and IL-1 with two additional cell lines that express a fluorescent fusion protein of IKK from its endogenous locus - an orthogonal reporter for pathway regulation upstream of NF-κB.

III. CONCLUSION

Our results demonstrate that despite heterogeneity, cells that respond to a stimulus are capable of discriminating doses across orders of magnitude in concentration.

**REFERENCES**


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