In multicellular organisms like the human body, trillions of cells, of multiple different cell types communicate with each other by releasing a thousand types of molecules such as hormones, growth factors, cytokines, or chemokines to coordinate an organism in all aspects of its function. The functioning of biochemical signaling, however, does simply not comply with the principles of communication engineering. For instance, an engineer designing a communication system would use few distinct signaling components while ensuring that the output of each component is highly reproducible. Natural evolution came up with a different solution: cells have many interconnected, cross-wired pathways that produce highly variable output signals. How can cells function reliably with highly variable signaling outputs? What is the explanation for cross-wired architecture? What are the implications of variable cross-wired signaling for health and disease? These questions reflect tangible gaps in our understanding of how cellular signaling functions.

In the prevalent view, the highly variable signaling outputs of single-cells result from stochasticity (molecular noise)—and not deterministic factors—and therefore diminish signaling fidelity. In our recent work, using bi-nuclear syncytia, we showed that less than 10% of the cell-to-cell heterogeneity of primary signaling outcomes can be attributed to noise along the signaling pathway inside the cell. The remaining 90% of cell-to-cell heterogeneity arises from phenotypic variability. Further, in our earlier theoretical work, we suggested that expansion of signaling components, be it receptors or signaling effectors, via duplication and enlistment of promiscuously acting cues is virtually the only accessible evolutionary strategy to achieve overall high-signaling capacity despite overlapping specificities and molecular noise. This mode of expansion explains the highly cross-wired architecture of signaling pathways. In addition, we have recently used probabilistic modeling and information-theory, to introduce fractional response analysis (FRA), which enables a systematic investigation of cell-to-cell heterogeneity structure for multivariate and high-throughput data in a wide range of situations, in which response analysis in single-cells is of relevance.

The main goal of this proposal is to follow the research avenues opened by our recent work, in order to address the specific long-lasting problems in signaling:

Aim 1: Characterise determinants of cell-to-cell heterogeneity of cytokine responses;

Aim 2: Uncover evolutionary forces that might have shaped cross-wired signaling;

Aim 3: Examine the impact of aging on cell-to-cell heterogeneity cytokine response;

Aim 4: Examine the impact of cell-to-cell heterogeneity on the efficacy of single-cell inhibition in EGF signaling dependent cancer cells.

The exact implications of cell-to-cell variability and cross-wired architecture of signaling pathways for understanding of cells' functioning as well as for etiology and treatment of human disease are only beginning to emerge. For instance, recent evidence, revealed that cell-to-cell variability of gene expression increases during aging. Besides, in pharmacology, the cell-to-cell variability acts as a catalyst for a paradigm shift in preclinical studies, by increasingly incorporating single-cell measurements. In cancer, for instance, fractional killing or incomplete growth inhibition of clonal tumor cells is a significant problem, which cannot be inspected with population measurements. The proposed research agenda is aimed to fill certain gaps in our understanding how cell-to-cell heterogeneity and cross-wired architecture should be accounted for when aiming to understand functioning of cellular signaling systems. In particular, we expect to explain what factors determine cytokine responses of individual cells, as well as provide insights into what forces might have cross-wired architecture of signaling. In addition, our results are intended to demonstrate how aging changes cell-to-cell heterogeneity structure of cytokine responses in human immune cells so that in the future, cell-to-cell heterogeneity could be developed into a quantitative trait comparable across individuals, and a biomarker for aging, or immune vulnerability. Furthermore, our experiments may provide valuable insight that can further inform the preclinical development of effective therapeutic interventions in signaling.