**Alexander Loewer**

"To respond and adapt: dynamics and variability of SMAD signaling in single cells”

**Abstract:** The TGF? pathway is a multi-functional signaling system regulating cellular processes ranging from proliferation and migration to differentiation and cell death. Upon binding of TGF? to its receptors, SMAD proteins translocate to the nucleus and activate numerous genes. While many components of the underlying signaling network have been identified, we are still challenged to understand how its activation is translated into distinct cellular responses of individual cells. We hypothesize that the information is encoded in stimulus- and context-specific dynamics of SMAD translocation. We therefore aimed to quantify the dynamics and variability of SMAD signaling over long time periods and correlate it to the state of individual cells. To this end, we measured the cytoplasmic to nuclear translocation of SMAD proteins with high temporal and spatial resolution in thousands of cells by combining fluorescent reporter cell lines with live-cell microscopy and automated image analysis. We observed that dose-dependent SMAD signaling shows strong and complex cell-to-cell variability and decomposes into distinct classes of dynamic behavior reflecting phenotypic responses. These classes are mainly characterized by the strength of the initial response and the extent of adaptation to a given input. Combining perturbation experiments with mathematical modeling at different levels of complexity, we provide evidence that both receptor internalization / degradation and transcriptional feedbacks contribute to shaping the dynamic response, while variability in the expression levels of regulatory proteins is sufficient to explain the decomposition in signaling classes. By characterizing the response to TGF? at the single cell level, our study provides a deeper understanding of the molecular mechanisms mediating signal processing in this versatile pathway and may open opportunities to modulate it in diseased cells.