SEMS: RESEARCH PROJECT DESCRIPTION

1. Project Background and Description

Introduction: Cells react to their (biomechanical) environment with the regulation of ~7,000 genes, in a timely and spatially coordinated manner. The invention of CRISPR enables to study this dynamical process either by time or spatially induced fluorescence, barcoding or by induced knock out/ knock-in studies.
Performing these studies on a large scale in a parallel way allows one to embrace the above-mentioned complexity. In order to do so, we have created a new high throughput platform which uses reversed transfection to allow eukaryotic cells to uptake guiding RNA in a timely manner. One of the interesting developments is to introduce knock in technology in high throughput platforms. Knock in experiments enable to add fluorescent tags to proteins of interest, add molecular biomarkers (small artificial elements that can be detected by pCR), add inducible elements to provide timed expression of genes. In this application we develop "knock in" technology to test how signaling pathways interact. Although currently unknown why so many signaling pathways are involved, one idea is that signaling pathways act as cascades and timing and frequency of cascades is determined by short and long noncoding RNA.
First, we aim to knock- in GFP for components of a signaling pathway and evaluate the effect of flow on their expression. A signaling pathway is often represented by 50-100 genes which will be spotted in a single lane. Following the GFP expression per spot over time allows to determine the sequential activation of the pathway. The current HT platform allows to test 10 individual signaling pathways in parallel, but the aim is to expand it to ~50 pathways in the current application. The general timing of evaluation of pathways has never been studied in such a way and it enables to time their activity with respect to components in the pathway and among pathways.
Nest, we aim to study whether signaling pathways interact and whether short, fast acting pathways influence slow acting pathways in a sequential manner. To that end entire pathways are blocked by microRNA and the response of the cascading pathways are measured. This then will inform researchers whether cascading pathways exist and whether they can be influenced by other pathways.

Hypothesis. We hypothesise that cascading pathways emerge from an initial fast acting pathway and that this eplxains the comlexity of most processes.

2. Project Scope

Aims. **Our overall objective is to create a new drug-screening platform capable of monitoring the response of cells to complex and specific 3D environments. Specifically, we aim to:**

- Create a new knock in platform for monitoring of the response of entire signaling pathways.
- Modify the platform so that we can study 50 parallel signaling pathways.
- Evaluate the hypothesis by monitoring time dependent processes.

3. Desired Skills from the Student

The student is interested in

- *Working on novel ideas*
- *Molecular interests*
- *Cell culture, gene transfection, plasmid synthesis*
- *Cloning*
- *Microcopic interests.*

4. Supervisory Team

Professor Rob Krams

Dr. Helena Azevedo

Dr. Thomas Iskratsch