MICROFLUIDICS-BASED ANALYSIS AND MODELLING OF MAMMALIAN SIGNALING DYANIMCS

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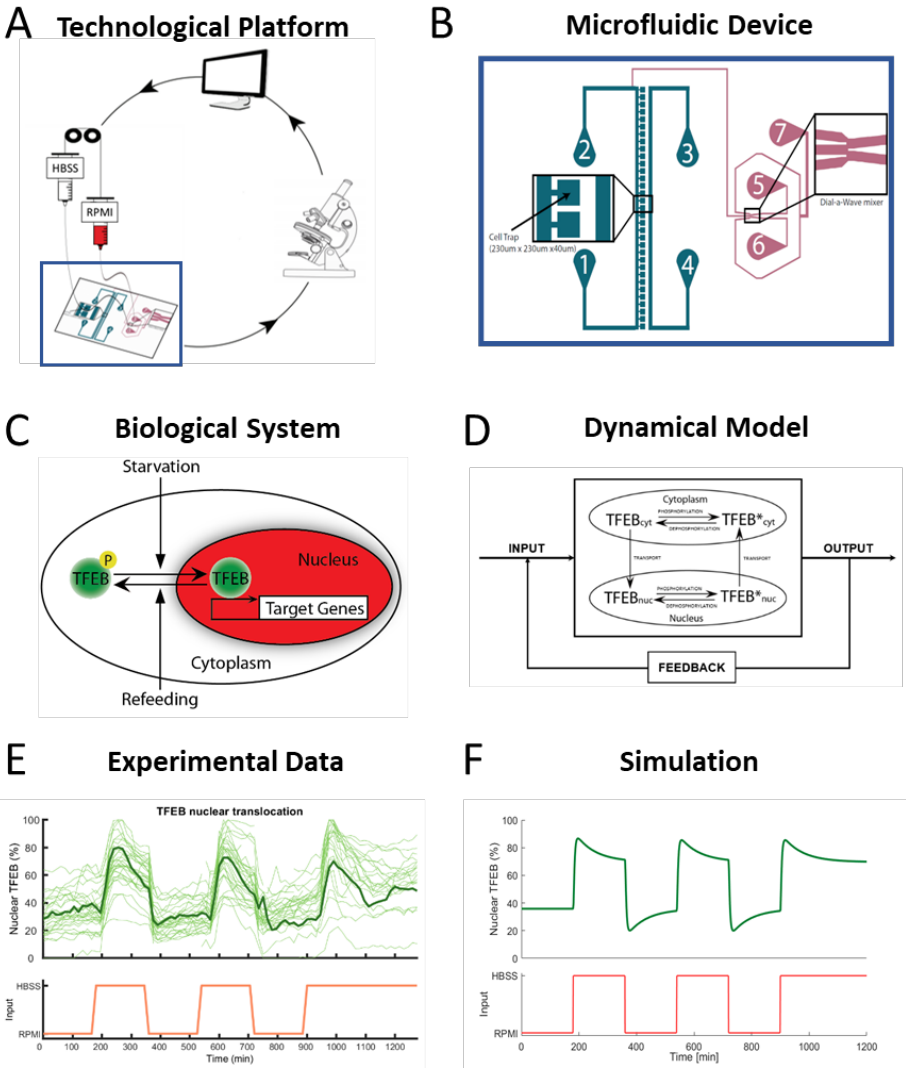
Mammalian cells are dynamical systems, in the sense that they detect, adapt and respond to time-varying inputs such as environmental cues, secreted molecules, and mechanical stimuli. Regulation of these processes is accomplished by signalling pathways that sense extracellular and intracellular environment and relay this information to transcriptional regulatory networks, which ultimately alter thousands of genes and thus the cell state. My PhD project is focused on the quantitative study of signaling dynamics in mammalian cells. Specifically, I focused on the mTORC1 signaling pathway that controls the cellular behaviour (proliferation versus quiescence) depending on cellular nutrients level and has a central role in aging and cancer. Specifically, in response to amino acid starvation, mTORC1 kinase is inhibited and thus it is not able to phosphorylate the transcription factor EB (TFEB), which becomes activated and translocates to the nucleus, where it initiates a transcriptional program leading to restoration of the amino acid balance by a process named autophagy [1-4]. In amino acid rich conditions, mTORC1 is active, and sequesters TFEB in the cytoplasm through phosphorylation [3]. In my project, I will use the TFEB translocation dynamics as a read-out of mTORC1 activity. The aim is to investigate the dynamics observed experimentally and understand what the key players of this behavior are and what is their biological role by integrating mathematical modelling and quantitative single-cell experiments by using an automated microfluidic platform available in the lab, shown in Fig. A, B [5-8].

During my first year of PhD, I learned how to produce microfluidics devices from PDMS, how to grow mammalian cells and how to perform time-lapse microscopy experiments. I also developed a quantitative dynamical model of TFEB translocation system [5] using chemical reactions based on the literature, as schematized in Fig. C, D. The resulting model is a two-compartment model (nucleus and cytoplasm) with two different species (dephosphorylated and phosphorylated TFEB) for each of the two compartments, for a total of four species. The transport and de/phosphorylation kinetics were assumed to be first order kinetics function of the input (the lack of nutrients). Conservation of the total TFEB amount was also assumed. The model parameters were inferred from the literature.

I then performed a series of experiments where I monitored the nuclear localization of a fluorescently tagged TFEB protein in real-time in the microfluidics device following alternating stimulation of cells either with nutrient rich medium or medium depleted of amino acids. I noticed a complex and rich dynamics in the nuclear accumulation of TFEB over time, as depicted in Fig. E, which could not be reproduced by the model derived from the literature. Therefore, I needed to add a feedback to the original model, where nuclear TFEB is able to partly inhibit its own translocation. The final model is a 4D system of differential nonlinear equations in the states and in the input.

The model was able to correctly replicate experimental data and was used to hypothesize the existence of a novel feedback loop driving TFEB localization regulation (in silico analysis). New experiments are being performed by inhibiting key pathways that could be involved in the feedback (autophagy, mTOR signaling, translation, transcription) to validate in vitro this hypothesis.

The next two years of my PhD, I will also search for alternative models (i.e. FeedForward Loop) that can replicate the observed dynamics without requiring the presence of a feedback. I will then design and perform experiments to distinguish among the alternative models and what are the biological mechanisms at the core of the observed dynamics.



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