

## **Extensive chemoproteomic profiling of polyamine-protein interactions in cancer.**

Humans like to think of themselves as biologically complex organisms. However, when the data obtained from various genome sequencing projects was published in early 2000s, it turned out that the human genome is smaller than originally predicted and not even much bigger than that of a fruit fly, which is a model organism used in biological studies. But if not from the genome itself, where does the human complexity come from?

In fact, the biological complexity arises not on the level of the genes but on the level of the molecular machines of the cell *i.e.* proteins, which are synthesized (translated) according to the information contained in the genome. After the synthesis (post-translationally) structures and functions of proteins are modulated in extraordinarily diverse ways creating biological complexity, and this modulation often involves interactions with small molecules produced in the body.

To date, interactions of proteins with dozens of small molecules have been reported. Some of them are known to be permanent, and others to be dynamic in nature. The permanent interactions always involve formation of a chemical bond whereas the dynamic ones may also involve more transient (non-covalent binding) mechanisms. It is very important to understand various aspects of the small molecule-protein interactions since many of those extensively studied interactions have been proven to be essential for regulation of major cellular processes and importantly, when dysregulated, lead to the development of diseases. However, due to their mostly dynamic nature and often low abundance, these interactions are very difficult to study, and there is only a limited molecular toolset that allows small molecule-protein interactions to be interrogated in living cells or organisms.

For my project, I chose to study interactions of proteins with endogenous polyamines (putrescine, spermidine, spermine), ubiquitous molecules that are essential for cell differentiation, growth, and programmed cell death, whereas dysregulation of cellular levels of these molecules have been implicated in the development and progression of diseases, such as neurodegenerative disorders or cancer. Importantly, polyamine-protein interaction is a molecular phenomenon that on one hand has been shown to control key biological processes and on the other remains mysterious as it has not been investigated extensively enough. Furthermore, the emerging evidence supported dual bond-forming/non-covalent mode of these interactions. In this project, I intend to determine which human proteins interact with polyamines, and with which one of the three endogenous polyamines they do interact preferentially. I will also investigate which polyamine-protein interactions are non-covalent and which are bond-forming, and for the latter which are permanent and which are reversible. I will further perform a detailed study on the significance the interaction with polyamine has on the function of proteins of known importance in health and disease (cancer).

I plan to utilize advanced molecular tools (chemical probes) that I have designed specifically for this project. The probes will closely resemble polyamines naturally occurring in the cell and will be therefore well recognizable by the interacting proteins in living cells. However, unlike the natural polyamines the probes will be equipped with a tracer that will permit their tracking once they engage in interactions with target proteins. For the study of non-covalent interactions, the probes will be additionally equipped with a light-activatable warhead element to permit stabilization of the interaction upon irradiation with UV light, for tracking purposes. As a part of this project, the probes will be utilized to obtain maps of polyamine-protein interactions that occur in cancer cells and specific changes occurring in these interactions in the cells, in which programmed cell death has been activated.

The project is very exciting, as it will enrich our knowledge about proteins modifications in cancer cells, and it will produce new molecular reagents and technologies that could be used even beyond its current scope. For example, they could be applied for high throughput screening of anomalies in polyamine-protein interaction in various other human pathologies with the ultimate goal of identification and validation of novel pharmacological targets and medicines.