The life of every mammal starts with a single cell – the zygote. Upon cell division and tightly orchestrated stepwise differentiation processes, the zygote transforms into a mature individual. The mechanisms ensuring the remarkable precision and the reproducibility of this process rely on the control of genome activity. Indeed, all the cells in the newly formed organism, even those performing profoundly different tasks, such as brain cells and bone cells, have the same genetic material in their nucleus. The thing that differentiates them from each other is simply the way that they use it. In other words, to forge a different set of properties, cells need to switch some genes on while keeping others silenced. How does it happen? Genomes contain dedicated sequences, which can act to orchestrate gene activity when bound by regulatory proteins. Promoters allow gene expression to start. Enhancers tune it up by positively impacting promoters. They do so by establishing a direct physical interaction with promoters and the precision of gene regulation profoundly depends on the specificity of the interactions between enhancers and promoters. When the enhancer-promoter pairing is lost, gene activity may go awry and escape control, a phenomenon observed in cancer. Remarkably, enhancers can be located extremely far from their cognate promoters. What are the mechanisms regulating the physical interactions between enhancers and promoters? How do cells fine-tune these interactions to keep them robust, dynamic, and specific? These questions are at the heart of this project.

ATP is the energy currency in the living world. The observed high ATP concentration in the cell (3-8mM) might seem appropriate – enzymatic activities underlying cell movement, cargo transport, secretion of hormones, gene activation, and other functions rely on energy. Yet, at any given time, ten times less ATP should be largely sufficient to sustain these activities, raising a question pertaining to the significance of the excess of ATP. Over 50 years ago, it was discovered that in millimolar concentration, ATP acts as a dissolvent that can disrupt aggregates, including protein deposits. However, the functional contribution of these non-energetic, thus non-canonical features of ATP in controlling gene expression, is unknown. Thus, we hypothesize that ATP plays an essential role in regulating the interplay between DNA regulatory elements. Our preliminary results indicate that ATP can disturb contacts between enhancers and promoters. Therefore, by combining state-of-the-art high throughput sequencing and imaging approaches, this project aims to determine the implication of the dissolvent-like properties of ATP in the regulation of gene activity during cell differentiation. We will use mouse embryonic stem (ES) cells and neuronal progenitors (NP) as a model system of cell differentiation during the earliest stages of development. We will take advantage of technologies, that allow to reconstruct genome structure in high resolution, to assess how promoter-enhancer pairs react to varying levels of ATP and how its hydrotrope-like properties contribute to the regulation of contacts between these elements in ES and NP cells. Using transcriptomics, we will gauge the impact of ATP on gene regulation. We will measure how nuclear ATP levels change during the differentiation process. These results will likely change the way of thinking about how gene expression is controlled and identify new players essential to this process.