One of the most toxic proteins known in nature are the Ribosome Inactivating Proteins (RIPs), which are found in plants, bacteria, and other organisms; they are present in human food and are also used in ethnomedicine. Moreover, RIP proteins pose a high social risk due to the fact that they can be used as biological weapons. These toxins are not only a threat to human life and health but also attract attention due to the wide possibilities of biotechnological application in medicine; however, from the point of view of their so-called biology, the molecular mechanism of action of these toxins remains unclear. Most RIPs are classified as single-chain (type 1) or double-chain (type 2) proteins in which the enzymatically active A chain is linked by a disulfide bond to the B chain (lectin-like), which ensures their highly efficient ability to penetrate into mammalian cells. Therefore, type 2 RIP proteins are substantially more toxic than type 1 RIPs. Within the RIP family, a third class has also been identified, referred to as type 3, which exists as a proenzyme consisting of a single polypeptide that is active only after proteolytic processing. The currently adopted model of the operation of RIP proteins assumes that these proteins depurinate the 60S ribosomal subunit (they remove one adenine base in the Sarcin Ricin Loop, SRL), which results in inhibition of protein biosynthesis. The blockade of protein synthesis is believed to lie at the core of RIP toxicity and is regarded as the main trigger of apoptotic cell death. However, it should be stressed that there is no clear functional link between depurination and cell death, showing the gap in the current knowledge of the molecular aspects of RIP toxicity. The research work carried out in the Department of Molecular Biology suggests that depurination may have a different biological effect, i.e. it may induce the so-called ribotoxic stress response (RSR), which results in the induction of signaling pathways leading to apoptosis. Moreover, our observations are also consistent with the recent discoveries of the so-called collided ribosomes, which act as a metabolic sensor that transmits information to various signaling pathways, independent of translation blockage, and the response is dependent on the severity of ribosome collisions.

Based on our preliminary results and published original data, the primary objective of the project is to unravel, at the molecular level, the consequences of RIP action that leads to their toxicity in the eukaryotic cell. Our scientific hypothesis postulates that RIPs were evolutionary adapted to highjack the eukaryotic translational machinery by interacting with eukaryotic-specific ribosomal P-proteins and trigger the disome-dependent ISR/RSR pathways. Therefore, we are questioning the generally accepted concept that protein biosynthesis inhibition lies at the heart of RIP toxicity.

Thus, to achieve the project objectives, two analytical platforms are proposed: 1) structural to cast light on the interaction between RIPs and the ribosome as the primary target for the toxins using structural and biophysics approaches (cryo-EM and protein-protein interactions); 2) cell biology to address the mechanism of RIPs action at the cellular level. We postulate that RIP proteins, despite the high level of their structural similarity and enzymatic activity, show differentiation in terms of their biological effects, which may contribute to a new application of the RIPs proposed in the project. The second part of the project is dedicated to define the physiological impact of RIP activity on the performance of the ribosome *in vivo* and on the re-programming of cellular signaling. Within the task, we developed tightly regulated RIP expression systems in the ON/OFF mode to have the toxin in the cell at the right time and in the right amount for time course-analysis of metabolic changes in mammalian cells, i.e. the system is based on splitting of RIPs into two innocuous subunits on their own that can be reunited through the intein re-joining phenomenon into active protein. Such a regulation will allow a time-controlled RIP synthesis, especially the regulation at the post-translational level will enable for the first time studying the metabolic fluctuations caused by RIPs in minute details. It should be underlined that the new tools will not only reveal the biology of RIPs but also open a way for drug discovery. Our approach will provide unique opportunities to study the biology of RIPs; additionally, by controlling toxic proteins inside the cell, it will pave the way for development of safe RIP-based anti-cancer-targeted technologies.