The prevention of cancer, neurodegenerative diseases as well as other developmental disorders is the greatest challenge of modern molecular biology and biophysics. The generation and progression of these diseases is usually mediated by congested molecular highways and crossroads controlled by proteins and nucleic acids. The quantity of these biomolecules is tightly regulated in the fundamental processes that are governed by classical cellular organelles. Over the last decade, however, we have observed that fundamental processes can also be tightly regulated as a result or a consequence of the formation of membrane-less organelles, biomolecular condensates or coacervates. Among the many currently known classes of such organelles, one stands out in particular; P-bodies (processing bodies). Until recently unnoticed, later overlooked. Today they are appreciated and in the spotlight because they contain complex machinery responsible for the supervision of mRNA molecules. Their formation in the cytoplasmic environment is associated with a spontaneous physical process called liquid-liquid phase separation. In this process, interactions between proteins and nucleic acid molecules play a key role, leading to their assembly into liquid droplets, and then to further maturation of these droplets. Each of the biomolecules involved in the formation of P bodies therefore plays its specific and yet not fully understood role. What are these roles? This question remains open. In order to understand the mechanism of P-body regulation, further biophysical studies are needed to characterise the contribution of the individual proteins that are involved in both the formation and maturation process of these droplets.

A key component of P-bodies is a molecular complex composed of a short microRNA molecule, the targeted mRNA to be degraded and a protein called Argonaute (Ago). This structure is called the miRNA-induced silencing complex (miRISC), since it triggers processes leading to silencing the gene encoded in the mRNA molecule. In the complex, the pivotal role is played by Ago that interacts with an intrinsically disordered glycine and tryptophan rich protein of 182 kDa mass (GW182). Their interaction leads to the recruitment of another multiprotein deadenylase complex that starts to digest the 3’ tail of the targeted mRNA. The interactions between the GW182 protein and the deadenylase are mediated by the C-terminal part of GW182 called the silencing domain.

While the involvement of the N-terminal Ago-binding domain of GW182 in liquid phase separation has recently been demonstrated, it remains unclear whether and how the key GW182 silencing domain could be involved in this process. Based on our preliminary results, we propose that the silencing domain of GW182 could also directly contribute to phase separation. We want to verify the hypothesis and explain the molecular mechanism behind the liquid-liquid phase separation involving the silencing domain of GW182. The project aims to advance current knowledge of the biophysical basis of P-body formation and over the post-transcriptional regulation of gene expression in humans, thus shedding new light on the interaction of molecules in the complex mechanisms of gene silencing and RNA metabolism. These results may also provide more detailed data that will enrich the overall study of liquid-liquid phase separation involving proteins, which is another small step towards understanding the mechanism of regulation of membrane-less organelles involved in diseases and disorders that present a modern challenge in molecular biology and biophysics.