The growth of our civilization and increasing pollutions, e.g. by various plastics, motivate the search for natural objects, such as proteins, that could remove such polutions. This means that we need to learn how such proteins could function in a non-natural environment. There are two approaches to this aim. One is to design polymer–protein hybrids, which are intriguing materials that can bolster protein stability in non-native environments, thereby enhancing their utility in diverse medicinal, commercial, and industrial applications. One stabilization strategy involves designing synthetic random copolymers with compositions attuned to the protein surface, but rational design is complicated by the vast chemical and composition space. Another approach is to use so called deep eutectic solvents (DES), which enable to stabilize non-native conformations by varying the solvent's inherent properties; this expand the dimensions of protein-based systems that we can access with traditional approach.

We propose a strategy that aims to construct thermally and mechanically robust proteins, which can conduct enzymatic function using a knotting motif, based on active machine learning and facilitated by automated protein expression and characterization platforms. This is a new approach, and moreover can be applied to both polymer–protein hybrids and those with DES. The use of entanglement motifs in proteins with DES allows to stabilize non-native conformations not only by varying the solvent's inherent properties. Thus, this expands the dimensions of protein-based systems beyond what can be accessed with traditional approach. Overall, this work broadens the capabilities to design fit-for-purpose new proteins that promote or otherwise manipulate protein activity, with extensions toward the design of robust polymer–knotted-protein hybrid materials.

This project is possible to conduct due to recent advances in chemistry and structural biology. We will use the AI approach related to this year's (2024) Nobel Prize in chemistry to predict structure and design new proteins. However, our approach goes further, as we will design proteins with non-trivial topology. In parallel to Nobel Prize approach, we already established methods to design knotted proteins, which can be expressed and fold in E. coli organism. We will also used other AI methods developed in my group to detect non-trivial topology based on sequence and geometry. To enhance success rate and understand the basic laws of structural biology, we will also develop two related topics and will apply Ancestral Sequence Reconstruction (ASR) to explore the evolutionary origins of knotted proteins, and advance AI approach to protein folding problem.

The rapid and efficient discovery of new proteins and characterization of their activities is critical for expanding our fundamental knowledge and advancing biotechnological applications. However, current methods for protein expression, purification, and characterization often involve time-consuming, labor-intensive, and error-prone procedures. When testing dozens of protein variants, this presents a significant bottleneck in our laboratory activities.

The proposed project aims to implement a high-throughput automated pipeline for protein discovery, expression, and characterization. Liquid-handling robots dramatically increase the throughput of the screening process by automating repetitive tasks, reducing the need for manual labor, and decreasing the consumption of reagents and consumables. As a result, such a solution would allow us to significantly widen our scope and screen larger libraries in a fraction of the time it would take with manual methods.