Recent studies have shown that bacterial cell is not a membrane sack filled up chaotically with nucleic acids, proteins and other compounds. Bacterial nucleoids are highly ordered domains, replication and segregation of which proceeds under strictly defined spatiotemporal conditions. Cellular proteins and metabolic pathways may be organized into microcompartments, defined structures. Overlapping in time and space processes of DNA replication, segregation of newly replicated DNA, transcription and translation, determine metabolic activity of the cell, its growth and division cycle. It requires very complex and precisely controlled regulatory networks. This picture is further complicated by the presence of mobile elements, among them plasmids that, on one hand benefit from the host metabolism, on another may modify the host by genetic information brought in and disturbing due to the metabolic burden they cause.

Plasmids according to definition are replicons (self-replicating DNA molecules) independent from host chromosome, however they are liable to the same rules that govern host replication and segregation during cell division. Hence they are perfect research model to study these processes in bacterial cells.

Objects of our studies are plasmids: R751 from IncP-1 and RA3 from IncU groups. These low-copy number, broad -host- range (BHR), conjugative plasmids replicate and are stably maintained in phylogenetically distant bacterial species, common in intensive care hospital units, soil and aquatic environments worldwide. Our interests are concentrated on plasmids-encoded Kfr proteins of almost 100% alpha-helical structure. Proteins of alpha-helical structure are present in all organisms and participate in vital cell processes. In bacteria they are involved in morphogenesis, cytokinesis, nucleoids segregation and motility. Our studies demonstrated that they are also engaged in DNA segregation of BHR plasmids to progeny cells after division. Due to their structure they are challenging to study.

The aim of this project is verification of scientific hypothesis about filamentous structures formed by complexes of Kfr proteins to facilitate directional movements of plasmid molecules (“mitotic spindle”) and possibly other cell microstructures. Identification of cellular partners of Kfr proteins and deciphering the mechanism by which disturbed plasmid segregation may influence host metabolism is another key aspect of the proposed study. This multidisciplinary project links basic research methodology of molecular biology, biophysics and bioinformatics. The electron microscopy will be applied to visualize and analyze the structures formed by alpha-helical Kfr proteins in vitro and fluorescence confocal microscopy will follow Kfr molecules in the cellular context.