Cyanobacteria constitute a rich source of biologically active and structurally diverse compounds. The pharmacological potential of these compounds resides among others in their ability to control the proliferation and growth of cancer cell lines and potent disease-causing microbial agents. Despite recent scientific advances the way these compounds interact with the body's molecular structure are still unclear and science still has to discover how the cyanobacterial metabolites interact with cell structures and how cells react to them. In this project we will study yet unexamined cyanobacterial metabolites, especially the compounds which act as chemical ligands for microRNA (miRNA) binding sites, making them promising regulators (inhibitors) of gene networks that are involved in various diseases.

Toward the ambitious objective of our proposal, we will first develop a stable cell line that constitutively expresses a unique miRNA reporter system. Then we will conduct a screen on chemical compounds discovered in Baltic cyanobacteria to identify small molecules with inhibitory activity and specificity to MIR92b-3p. In humans this miRNA has a significant impact on cell behavior. For example, an excess of MIR92b-3p in liver cells leads them to divide uncontrollably. We assume that a successful MIR92b-3p inhibitor will bind to the precursors of MIR92b-3p miRNA, disabling the action of either of the two miRNA processing enzymes involved in the biogenesis of any microRNA in a cell (Drosha or Dicer), thus affecting the MIR92b function.

Our small-molecule screen employs a Huh7 human hepatoma cell line stably transfected with a Renilla luciferase sensor for endogenous MIR92b-3p. It uses a luciferase reporter assay that measures light output from luciferase enzyme, which is incorporated into the cell. The luciferase screen detects the presence of a functional mature MIR92b-3p through repression of the Renilla luciferase signal. In the presence of a small-molecule inhibitor of MIR92b-3p biogenesis (cyanobacterial compound) or a MIR92b-3p antisense agent, luciferase expression is restored. Thus, increased levels of luminescence will indicate a successful inhibitor of MIR92b-3p.

After a suite of chemical and biological tests and bioinformatic analyses, we will learn which chemical structures enable cyanobacterial metabolites to bind directly to and perturb MIR92b functions. With molecular abilities to modulate regulatory elements of cell processes, the bioactive cyanobacterial metabolites that are uncovered would be important tools for clarifying the mechanisms of these cell processes and help in the development of new therapeutic agents. The discoveries made with these chemical molecules could provide insight into the role of the MIR92 pathway in liver diseases and cancer, and possibly, if promising results appear, they may facilitate a strategy for treating some human diseases in the future.