**Master thesis project:**

## Folic acid derivatives as extracellular signal for aggregation of dictyostelid social amoebae

Dictyostelia are soil-dwelling amoeboid cells that are characterized by a facultative “social” phase, in which single cells cooperate to form a multicellular organism that transforms into fruiting bodies that can spread dormant spores to places with sufficient food supply. The multicellular phase starts with the chemotactic migration of single cells in gradients of secreted signaling molecules known as “acrasins”. If an acrasin mediates aggregation of single amoebae to form multicellular structures, it would be expected that cells are able to (i) secrete this acrasin, (ii) migrate in gradients of the acrasin, and (iii) secrete an enzyme (“acrasinase”) that degrades the acrasin to an inactive metabolite.

Of the four phylogenetically distinct groups of dictyostelid amoebae, only the Dictyosteliaceae are known to use extracellular cyclic AMP as acrasin to regulate aggregation; the acrasin systems of most other species are unknown. All Dictyostelia migrate in chemotactic gradients of folic acid and secrete folic acid and pterin deaminases during early development. Because folic acid derivatives are released by bacteria, it is generally believed that chemotaxis to these compounds is a means to detect food. However, it is unknown whether chemotaxis to folic acid was reconstructed during evolution to serve a second function as an acrasin system. Interestingly, papers from the 1980’s described *Dictyostelium lacteum* and *Dictyostelium minutum* to use a not fully characterized folic acid derivative and an unknown 6-alkylpterin, respectively, as acrasins. The exact structures of these postulated acrasins could not be determined by the time the studies were performed.

In this master project you will aim at identifying folic acid and/or pterin derivatives that are released during aggregation of *Dictyostelium lacteum* and *Dictyostelium minutum* and that are probably used as acrasins. Because these natural compounds are expected to be released in sub-nanomolar amounts, an experimental approach would be required to specifically concentrate these compounds from supernatants of aggregating cells. To accomplish this, you will clone and express a $his_8$-tagged version of human folic acid-binding protein (FOLR1) and establish a transgeneic mammalian cell line to produce the protein. This protein will be purified from media of transfected cells by $Ni^{2+}$/NTA affinity chromatography and used as recombinant protein that is mixed with suspensions of *Dictyostelium lacteum* and *Dictyostelium minutum* to bind folic acid or pterin dervatives secreted by the cells. The FOLR1 protein will then be purified from the cell supernatants and the bound compounds will be extracted and analyzed by HPLC-MS. If successful, you will be the first to report new folic acid and/or perin compounds released from *Dictyostelium lacteum* and *Dictyostelium minutum* amoebae. These compounds are classical candidate acrasins. Their functions can be further analyzed, for example, in chemotaxis assays. Also, these compounds should be substrates for “acrasinase” enzymes that we currently try to identify in both amoeba species.