Adaptive immunity, a major evolutionary innovation of vertebrates, relies on the diverse lymphocyte receptors and antibodies generated anew during the individual's life. In contrast to innate immunity, adaptive immunity is pathogen-specific and thus precise, has low maintenance costs, carries a low risk of collateral damage once employed, and results in immunological memory. However, an efficient adaptive immune response requires a large pool of diverse lymphocytes and takes time to mount. Both these requirements may pose a serious challenge for many fish and amphibians that hatch at a very small size and continue their development in the pathogen-rich aquatic environment. The full-fledged adaptive immune response in such small animals will be constrained by a low number of the available lymphocytes and the time needed to develop the immune repertoire. It has been hypothesized that the use of the so-called unconventional lymphocytes that carry receptors of limited diversity would be an efficient solution in the face of such constraints. Unconventional lymphocytes would confer some specificity towards pathogens, providing a quick and efficient defence mechanism when the number of lymphocytes is limited. The benefits of such a system may be particularly pronounced in amphibians that undergo metamorphosis, a dramatic event that profoundly remodels their immune system. Indeed, research on the frog *Xenopus* demonstrated that the larval immunity against some viruses and bacteria depends crucially on unconventional T lymphocytes (T cells) that recognise as yet unidentified pathogen molecules presented on the surface of other cells by the so-called nonclassical Major Histocompatibility Complex (MHC) class I proteins. The MHC is an essential component of adaptive immunity in jawed vertebrates. In addition to classical MHC, that "show" pathogen antigens to the T lymphocytes with diverse receptors, nonclassical MHC have been detected in all species investigated to date. An important function of nonclassical MHC-I (MHC-Ib) molecules is antigen presentation to unconventional T cells.

Salamanders are a major amphibian group that is characterised by diverse developmental modes. Some salamanders deposit in water eggs that develop into a free-living aquatic larva that later metamorphoses into a terrestrial adult, some species give birth to the larvae or even fully metamorphosed individuals, there are also species that deposit on land eggs that undergo direct development, i.e. already metamorphosed individuals hatch from the eggs. In this project, we take advantage of diverse developmental modes and considerable phylogenetic diversity of salamanders to test some key hypotheses about the evolution of nonclassical MHC-I genes and T-cell receptor (TCR) repertoires. We will identify and characterize MHC-Ib genes in salamanders, estimate the number of independent origins and losses of MHC-Ib genes through the salamander evolution, and date these events. We will also investigate the expression of MHC-Ib and the diversity of TCR repertoires throughout the individual's life. We postulate that the developmental program for unconventional T cells remains conserved across salamanders, but the MHC-Ib molecules these cells recognise originate repeatedly from the classical MHC-I. We also hypothesise that the mode of development determines the way the expression of classical and nonclassical MHC-I, as well as the diversity of TCR repertoire, develop during the individual's life. Overall, the project will provide information crucial for the understanding of the evolution of nonclassical MHC-I genes and the interface between innate and adaptive immunity. Amphibians, including salamanders, are undergoing a global biodiversity crisis, caused in no small part by emerging infectious diseases. Information about evolutionary mechanisms shaping MHC-I evolution and TCR repertoires in salamanders is likely to stimulate research to establish more direct links between immunity and diseases. The project thus also has implications for amphibian conservation.