A fundamental property of the biological proteins and nucleic acid macromolecules is their homochirality. It means that they are composed of only one optically active isomer of amino acids or nucleotides, respectively. Native proteins are composed exclusively of L-amino acids, while natural nucleic acids contain D-sugar residues. However, some D-amino acids and short D-oligopeptides are present in cell walls of some bacteria and the D-oligopeptides are made by non-ribosomal processes. Although origin of the homochirality of biological macromolecules is not fully understood, it is assumed to be essential to ensure the high specificity and fidelity of recognition between them. Mirror-image ribonucleic acids consisting of L-ribose instead of D-ribose are resistant to hydrolysis by cellular nucleases. Also, mirror image RNAs did not induce any immunological responses like that observed upon application of some high molecular weight pharmaceuticals. The great advantage of the application of nucleic acids as therapeutic agents is based on the recognition of relevant nucleic acid targets by complementary Watson-Crick base pairing. Such interactions which include, antisense oligonucleotides, siRNAs and catalytic RNAs (ribozymes), are widely used for controlling gene expression. Interestingly, already 20 years ago, L-DNA has been proposed for use in antisense technologies; however no stable hybrids with D-RNA could convincingly be demonstrated.

We show for the first time that mirror image hammerhead ribozymes and DNAzymes (L-zymes) cleave sequence specifically complementary D-RNA, i.e., target of opposite chirality. These mirror image nucleic acid zymes, have some features, which could make them valuable for future therapeutic and diagnostic applications. Since the precise mechanism of action of A fundamental property of the biological proteins and nucleic acid macromolecules is their homochirality. It means that they are composed of only one optically active isomer of amino acids or nucleotides, respectively. Native proteins are composed exclusively of L-amino acids, while natural nucleic acids contain D-sugar residues. However, some D-amino acids and short D-oligopeptides are present in cell walls of some bacteria and the D-oligopeptides are made by non-ribosomal processes. Although origin of the homochirality of biological macromolecules is not fully understood, it is assumed to be essential to ensure the high specificity and fidelity of recognition between them. Mirror-image ribonucleic acids consisting of L-ribose instead of D-ribose are resistant to hydrolysis by cellular nucleases and do not induce any immunological responses like that observed upon application of some high molecular weight pharmaceuticals.

In this project we will show that mirror image hammerhead ribozymes and DNAzymes (L-zymes) cleave sequence specifically complementary D-RNA, i.e., target of opposite chirality. These mirror image nucleic acid zymes, have some features, which could make them valuable for future therapeutic and diagnostic applications. Since the precise mechanism of action of L-catalytic nucleic acids is not known it will need further investigations. The discovery of catalytic L-hammerhead ribozymes to hydrolyze sequence specifically L-RNAs and D-RNAs in vitro and in vivo, have so far not been described, and very importantly, these new types of nucleic acid enzymes have been entirely designed and synthesized by chemical means. Thus nucleic acid chemistry has been used to develop new types of regulators to be applied in molecular biology and medicine.

The L-hammerhead ribozymes can be considered as an alternative to siRNAs and microRNAs, because they are very stable in human sera and cells, they are not modified D-forms of the common nucleic acids, they are not expected to trigger interferon responses in organisms, they are most likely not toxic, not immunogenic, easily and reproducibly synthesized by a machine, easily made under general manufacture practices (GMP) conditions, not incorporated in the genetic make-up of the cells, they function catalytically at low concentrations and they do not require any co-factors, like the small non-coding RNAs.

The employment of L-hammerhead ribozymes is for example for seen to be of great interest in the areas of astro biology, biochemistry, chemistry, chirality, molecular biology, chemical biology, molecular evolution, molecular medicine and structural biology. With application of the L-catalytic nucleic acids a new technology will be introduced, with great potentials for many disciplines, and it will therefore be of interest to the broad readership. Our project should also appeal to the nonscientific specialists, because new technologies are described, which have the great potentials to revolutionize the medicine of tomorrow.

The expected results imply a large number of potential uses of L-hammerhead ribozymes in the future field of molecular medicine. To the list of potential uses given above, it should also be mentioned that the L-hammerhead ribozymes would not only be restricted as an antidote to L-nucleic acids, but now they could also be used as an antidote to any RNA target in the cells, like antisense, different types of ribozymes, siRNAs and microRNAs, and anyone of the many small and large non-coding RNAs, and not even that, but also of course against other L-hammerhead ribozymes previously introduced into the patient.