**Development of new purification methods for proteins with key roles in cancer**

Structural and biochemical studies require the production of proteins by bacterial or insect cell expression systems. One of the easiest ways to purify the expressed recombinant proteins is metal chelate affinity chromatography, where the protein is produced fused to a His-tag. However, in many cases the efficiency of the commercially available Ni-NTA columns is insufficient. Purifying disordered proteins by Ni-NTA affinity chromatography is hindered by the fact that the His-tag is easily masked by the flexible amino acid chain.

The aim of our cooperation is the production and testing of new chelators bound magnetic nanoparticles (MNP) that are able to bind His-tagged proteins with higher affinity than the widely used Ni-NTA agarose. Our special focus is on the affinity of the new MNPs towards disordered proteins.

To this aim we plan to study two series of MNP affinity carriers. One series consists of MNPs that carry the trifunctional metal binder produced from EDTA anhydride on a short hydrophobic arm close to the surface, while the arm is longer and hydrophilic in the other series and thus the metal binding unit is kept more distant from the surface. The two series contain 14 different lanthanides and other heavy metals that are less toxic than the nickel and cobalt ions and may possess better ligand-binding characteristics.

As a continuation of our studies we plan to carry out structural and biochemical experiments with the proteins purified with the best affinity materials. We will study proteins that are connected with different types of cancer, like matrix metalloproteinase 9, B-cell CLL/lymphoma 9 protein and DNA mismatch repair protein.

