In the frame of this thesis, different approaches for exploiting proteins and enzymes for non-natural reactions were investigated: generation of novel hybrid catalysts, enzyme promiscuity, non-natural cofactor-regeneration and the use of nicotinamide cofactor mimetics.

In the first project the goal was to generate a hybrid catalyst by introduction of a catalytically active center into a protein host which should primarily confer selectivity in the catalyzed reaction. The ultimate goal of this project was to tune such a bioconjugate by the methods of directed evolution in order to generate enantioselective hybrid catalysts. In a first approach a transition metal catalyst and various organocatalysts were covalently linked to a chosen host protein for which efficient and parallelized expression protocols were established. A flavin-based hybrid catalyst which was generated by this approach proved to be a highly active catalyst for the oxidation of dihydronicotinamides. In the kinetic resolution of various chiral dihydronicotinamides this catalyst and numerous variants produced in a first round of mutagenesis did not exhibit any enantioselectivity. This result could be explained by the fact that in spite of a rather short linker group, the catalytic moiety was probably able to adapt several conformations. The conceptual solution to this problem was to restrict the mobility of the catalytic moiety relative to the protein host. This concept was realized by introducing a metal binding site into the protein host in which the catalytically active metal center is directly bound by the amino acids of the host protein and can only adopt a single conformation. The copper(II)-based model system for this direct-metal approach proved to be an efficient Lewis acid catalyst for an asymmetric Diels-Alder reaction. With the initial hybrid catalyst, a surprisingly high enantioselectivity was observed, as well as a drastically increased endo-selectivity and improved activity. This is the first hybrid catalyst that is based on an artificial metal coordination site. Catalysis experiments with variants of this catalyst suggested that the three putative copper-coordinating amino acids bind the metal center in a cooperative fashion: the replacement of a single one of those amino acids leads to a drop in enantioselectivity, endo-selectivity and activity to the level of plain copper(II) as catalyst.
Characterization of this system by various methods (X-ray crystallography, ENDOR and EXAFS) was initiated in collaboration with other groups specialized in the respective area. The results from those studies might help in understanding the reasons for the observed selectivity and increased activity and will hopefully give detailed structural information of the exact copper-binding mode and thereby provide the necessary information to do efficient directed evolution experiments by a structure based approach such as iterative CASTing. The first objective in this respect would be to tune the enantioselectivity of the model reaction. A more challenging goal would be the generation of an exo-selective variant of the hybrid catalyst for Diels-Alder reactions, a goal that would be extremely difficult to achieve with synthetic catalysts. The use of this catalyst for other copper-catalyzed reactions is another logical possibility as well as the use of other catalytically active metal centers that can be bound in this protein scaffold. The number of catalytically active metals that could be bound in this protein host in combination with the various reactions mediated by the different metals would open up the possibility to tune hybrid catalysts for reactions such as the copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition or chromium- and manganese-catalyzed sulfoxidations. In order to further widen the scope of the concept a protein host which is stable in organic solvents could be found in order to generate hybrid catalysts suitable to catalyze reactions that proceed efficiently in such an environment.

The protein host that was used for the generation of hybrid catalysts was shown to have a catalytically promiscuous hydrolytic activity. In an attempt to localize the active-site responsible for the promiscuous function, an unexpected novel phenomenon was discovered. In contrary to other catalytically promiscuous enzymes, the promiscuous active-site in the present case was not associated to the active-site of the natural reaction. This phenomenon was not reported to date in the literature and was therefore given the name: alternate-site enzyme promiscuity. Since enzyme promiscuity is considered important in understanding the evolution of new enzymatic activities, the phenomenon of alternate-site enzyme promiscuity might also have relevance for this field.

Another field addressed in the frame of this thesis was the direct regeneration of oxidoreductases by unnatural reactions. In this context, a light-driven approach for
the direct regeneration of flavin-dependent enzymes was planned and successfully established for a Baeyer-Villiger monooxygenase and an enoate reductase. Ways to make this concept more efficient and more general in order to apply it to other enzyme classes such as cytochrome P450 were discussed.

As an alternative to the light-driven regeneration of these enzyme classes, initial experiments using NADH mimetics were performed with a flavin-dependent enzyme as well as with cytochrome c as a model system to check for the possibility of directly reducing heme-dependent enzymes that are synthetically useful.

In a side-project, a platform for the directed evolution of the enoate reductase YqjM was established and a preliminary substrate scope was determined and thereby limitations were identified that could potentially be overcome by directed evolution experiments. This would represent the first example for the directed evolution of an enoate reductase. Strategies for such experiments were discussed as well as the use of this enzyme scaffold as catalyst for promiscuous reactions.

In conclusion, several different ways to exploit enzyme- or protein-scaffolds for non-natural reactions were successfully demonstrated in the frame of this thesis. The individual approaches and purposes for each project were different, however they all aimed to some extent at widening the scope and possibilities of biocatalytic processes. Even though the development of practical systems was not the goal of this thesis, further optimization of the systems elaborated here might lead to practical applications in synthetic organic chemistry.