Summary and outlook

The protein VASP is important for the regulation of actin dynamics. It prevents the inhibition of filament growth (capping). This process is important for shape changes and migration of cells.
VASP is also involved in infections with the pathogenic bacterium *Listeria monocytogenes* and is recruited to the bacterium's surface. This allows *Listeria* to polymerise actin filaments and propel itself through the cell. The interaction of VASP is mediated by the surface protein ActA, which contains the amino acid sequence motif SFEFPPPPTEDEL.
VASP does not contain a catalytic subunit but rather is involved in several protein protein interactions. They are mediated by the N-terminal EVH1 domain, a central proline rich region and an F-actin binding domain. VASP oligomerises via its C-terminal coiled coil domain.
It was the main goal of this work to gain a detailed understanding of factors influencing the function of VASP and develop means to influence this function. The focus lay on investigating the EVH1 domain, competitive inhibitors containing nonnatural amino acids were developed. These allow for an extension of structural motifs compared to natural amino acids and are less prone to proteolytic digestion *in vivo*.

The interaction of VASP with proline rich motifs is mediated by its EVH1 domain. It binds sequences containing the sequence motif FPxϕP. It is not possible to substitute the two proline residues with any other natural amnio acid. The only substitution leading to a ligand of higher affinity is SFE**W**PPPPTEDEL. In this work new ligands based on peptoidic building blocks were developed for the VASP EVH1 domain. These building blocks are derived from the rare amino acid sarcosin, bearing two hydrogen atoms on Cα and a sidechain on the nitrogen atom. Starting from the peptide SFEFPPPPTEDEL a new ligand was developed by screening and model supported structure optimization, in which the first proline residue as well as the preceeding Phe residue were substituted. The peptomer SFEAXPPPPTEDEL (X: peptoid building block) has a lower affinity compared to the starting ligand, but proves the validity of this method in the development of EVH1 ligands.

For a better understanding of the structure-function-relationship of EVH1 domains the investigation of homologous proteins is very useful. Therefore the structure of the sequence homologous EVH1 domain from the human Spred2 protein was determined. The structure

confirmed the sequence based assumption that this is indeed an EVH1 domain. A comparison of the putative binding site to that of other EVH1 domains shows considerable differences and hints at a different ligand specificity. An interaction partner for the Spred2 EVH1 domain has not been identified in the literature so far.

The affinity of EVH1 domains to their binding partners is low, hence the oligomerization of VASP plays a central role in increasing interactions with target proteins. Hence the C-terminal coiled coil EVH2 domain of VASP was investigated as a modulating factor of VASP function.

Combining analytical ultracentrifugation and NMR spectroscopy it has been possible to show that this domain forms a parallel tetramer over a broad concentartion range (0.0625 - 5.0 mM). Insight into this arrangement is crucial in developing models of VASP fucntion and interaction within protein networks.

Due to the high degree of symmetry of the coiled coil domain is was not possible to determine the complete tetramer structure by NMR so far. Based on NOESY data and residual dipolar couplings a model of a monomer has been calculated. This model is in reasonable agreement with the recently solved crystal structure.