Protein phosphorylation is one of the most important processes in cellular signaling. Kinases (PKs), which phosphorylate proteins, and phosphatases (PPs), which catalyze the removal of phosphate groups, have been identified to play important roles in different pathways and networks. The picture however, particularly of network connections between these complementary protein families, is far from being complete. This project will contribute to the understanding of interactions between these proteins and their ligands and substrates, which in return can be PKs or PPs, and thus will help to complete the picture of phosphorylation networks in cellular signaling.

It is envisioned to study protein-ligand interactions of human PKs/PPs using computational tools to map out the most likely ligands, followed by testing using chemical, biochemical and crystallographic approaches. Specific targets of interest include the PRLs (dual specificity phosphatases), PTEN (lipid phosphatase), PTP1B and TCPTP (tyrosine phosphatases).

The envisaged project work flow is as follows:

1. Perform a computational analysis of the proteins, using in house and public tools, including consideration of the sequences, conservation, structures and other biochemical data (J. Thornton). The methods used would include:
   
   a) Protein sequence analysis, including phylogenetic trees. This would exploit our new tools at EBI to combine sequence and small molecule data.
   
   b) Ligand family analysis - using cheminformatics tools to compare and contrast ligands known to bind to the family of proteins
   
   c) Structure analysis, using a whole suite of tools for binding site comparison and ligand prediction developed in the Thornton lab
   
   d) Docking studies - to identify the most likely classes of ligands for a given protein

2. Test the predictions using the most appropriate biochemical methods and assays. This would include two aspects:

   a) (Bio)chemical screening in Heidelberg (M. Köhn): Key interactions between PKs/PPs and potential substrates will be verified using biochemical (de)phosphorylation assays. Peptides that correspond to sequences of proteins in question will be synthesized and tested to enable higher throughput. Phosphoinositides can also be included as potential substrates as they are available in
the Köhn group. For testing particular phosphorylation sites in the protein, in addition to peptides, semi-synthetic proteins can be applied that carry distinct phosphorylation patterns. Non-catalytic interactions between PKs/PPs and ligands (peptides, small molecules) can be investigated using binding assays such as fluorescence polarization applying fluorescently labeled synthesized ligands.

b) Crystallographic screening in Hamburg (M. Wilmanns): Screening for crystallization conditions will be carried out with the HTP crystallization platform in Hamburg (http://www.embl-hamburg.de/services/crystallisation/index.html). Thereby by the chemical ligands mentioned above will be used together with the PP/PK of interest. Structures will be determined using the local expertise and infrastructures (starting 2011, state-of-the-art beamlines at Petra-3 will become available). Training on crystallographic methods ("learning by doing") will be performed in parallel.