Bone morphogenic proteins (BMPs) belong to the transforming growth factor-β (TGF-β) superfamily of multifunctional cytokines. Signaling of these growth factors is achieved by binding to two types of transmembrane serine/threonine kinase receptor chains classified as type I and type II. We have analyzed the recognition and binding mechanism of three different BMP proteins (BMP-2, BMP-6 and GDF-5) to their high-affinity type I receptors (BMPR-IA, BMPR-IB and ActR-I) [1-3]. Although all three BMP ligands can bind to either one of the receptor proteins with nanomolar affinity range, there are subtle but significant differences in the individual affinities to the different receptor ectodomains. BMP-2 binds to BMPR-IA with 5-fold higher affinity as to BMPR-IB, on the other hand GDF-5 and BMP-6 bind preferentially to BMPR-IB and interact with BMPR-IB with 10-fold higher affinity than with BMPR-IA. Although these differences might look small, they probably play an important role for the proper preparation and maintenance of the morphogenetic gradients that are required for patterning and organ development. Since transcription factors have to read out the concentration gradient of several different factors and modulator proteins, binding affinity differences have to be small.

In order to understand the promiscuity in the ligand-receptor interactions of the BMP family one has to investigate the structural and functional basis of this protein-protein interaction. In an in vitro situation BMP-2 can bind to different type I receptor with low nanomolar affinities and the biological functions of these different type I receptors seem very similar. However if we consider a more physiological situation in which several factors are present at slightly different local concentrations as well as different cell types with different receptors expressed at their cell surface, these small affinity differences become extremely important whether and which factor will bind and activate a particular cell.

So far mutagenesis studies did not reveal residues of BMP-2 that are clearly responsible for the discrimination between these two receptor subtypes. We have determined a high-resolution structure (1.86Å) of the complex of BMP-2 and the BMPR-IA receptor at the Swiss Light Source (SLS). One BMP-2 dimer is bound to two BMPR-IA receptor ectodomains forming a symmetrical heterotetramer arrangement (fig. 1). Main binding determinants, i.e. residue pairs which are absolutely crucial for the interaction, have been identified by mutagenesis using the high resolution structure data. Interestingly a main chain-side chain hydrogen bond in the center of the interface seems to be most important for high affinity binding. This element is highly conserved throughout the BMP protein family suggesting it might be a common anchor point. Structure determination of the complex BMP-2 bound to the ectodomain of BMPR-IB will enable us to compare the structural epitope with that of the complex of BMP-2 bound to BMPR-IA and propose possible candidates for a BMP receptor subclass specific recognition. Together with functional studies we want to generate BMP-2 variants that will only bind to one type I receptor, and might be used as receptor-specific BMP antagonists.

Crystals for the complex of BMP-2 and BMPR-IB have been obtained from several different conditions (fig. 2). Gelectrophoresis confirmed that these contained all components of the complex. However, diffraction experiments of these crystals showed a large degree of anisotropy, having areas of the crystal yielding no diffraction and others showing diffraction up to 5 Å resolution (ESRF beamline ID-13). Crystals of different crystallization conditions have to be tested to obtain data for the BMP-2 BMPR-IB complex.
Figure 1: The structure of the complex of BMP-2 dimer (blue and yellow) and two BMPR-IA monomers (green).

Figure 2: Crystals of the complex of BMP-2:BMPR-IB obtained from PEG conditions, average crystal size about 60x20x20µm.

References