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Characterization of the binding sites of the Wnt signaling inhibitor Sclerostin using solution NMR

The Wnt signaling pathway is a crucial regulator of bone development in vertebrates. Through its activation, bone mass and mineral density is increased. A negative regulator of this pathway is the protein sclerostin. By binding the LRP5/6 co-receptor of the canonical Wnt pathway, sclerostin suppresses the binding of the Wnt ligand and consequently the bone growth and remodeling. In the presence of glycosaminoglycans (GAGs), it is shown that sclerostin loses its inhibitory effect. Therefore, we are interested in a detailed understanding of the sclerostin-GAG interaction to understand how this suppresses the binding towards LRP5/6 co-receptors, as this is may be a promising target to improve bone healing.

The structure of sclerostin (191 amino acids) contains a rigid cysteine-knot motif as well as extremely flexible termini (with approximately 50 amino acids each). For the investigation of the sclerostin binding mechanism using solution NMR, we established a purification and refolding protocol with a yield of 30 mg per liter fermented culture. In initial experiments, different GAGs were titrated to 15N-labeled sclerostin. In the 1H-15N-HSQC spectrum, shifting peaks were observed that belong to the predicted GAG binding site of sclerostin.

To mimic the binding of sclerostin towards the receptor, a peptide derived from the binding region of the LRP6 co-receptor was synthesized. After titration with this peptide, some peak shifts were observed in the corresponding 1H-15N-HSQC spectrum of sclerostin that belong to some so far unassigned peaks. Upon assignment of these peaks, it will be possible to identify those residues, where the chemical environment was changed during the binding towards GAGs and LRP6.

Prospectively, a successive titration of a GAG and the peptide mimicking the LRP6 co-receptor to 15N-labeled sclerostin will generate a more detailed picture of the interaction of sclerostin, GAGs and LRP5/6.

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