

**Characterizing the  $\beta$ 1 adrenergic receptor and its intact noncovalent complexes with small molecules and a nanobody using native mass spectrometry**A. Gavriilidou<sup>1</sup>, H. Hunziker<sup>1</sup>, D. Mayer<sup>2</sup>, Z. Vuckovic<sup>2</sup>, D. Veprintsev<sup>2\*</sup>, R. Zenobi<sup>1\*</sup><sup>1</sup>ETH Zurich, Department of Chemistry and Applied Biosciences, Switzerland, <sup>2</sup>Laboratory of Biomolecular Research, Paul Scherrer Institut, Villigen, Switzerland

G-protein-coupled receptors (GPCRs) are a prominent class of membrane proteins. GPCRs account for roughly 40 % of all medical targets, for diseases such as cardiac dysfunction, obesity and pain. They are dynamic entities that induce signal transduction pathways upon binding of extracellular ligands. Recently, native mass spectrometry (MS) has been applied to study intact membrane protein complexes. These complexes are released from detergent micelles formed in solution and are transmitted to the gas phase revealing the subunit stoichiometry. High collisional energies need to be applied in order to remove the detergent and therefore preserving drug binding is challenging. Despite the great advances of native MS in the field of membrane proteins, studying G proteins and drug binding to receptors has not yet been investigated.

In this study the turkey  $\beta$ 1 adrenergic receptor (t $\beta$ 1AR) has been investigated with native MS.  $\beta$ -adrenergic receptors activate or deactivate intracellular G proteins upon binding agonists or antagonists respectively. MS experiments were carried out under native-like conditions in 200 mM ammonium acetate, pH 8, supplemented with 1.9 mM n-decyl- $\beta$ -D-maltopyranoside detergent, a concentration above its critical micelle concentration (CMC). Binding experiments of a G protein mimetic nanobody, NB80, against t $\beta$ 1AR in the presence/absence of the agonist isoprenaline (Iso) and the antagonist S32212 hydrochloride (S3) were acquired on a Q-TOF ULTIMA, (Waters/Micromass, Manchester, U.K) in positive ion mode using a commercial nano ESI ion source.

The binding of NB80 to t $\beta$ 1AR was characterized with native MS in the presence/absence of Iso and S3. In the absence of Iso, NB80 bound the receptor with a low affinity. This implied that the active and inactive states of the apo receptor are in equilibrium and therefore binding of the NB80 could occur. However, in the presence of Iso the equilibrium is strongly shifted towards the active state and the affinity of the NB80 to the receptor is greatly increased. The antagonist stabilizes the inactive state of the receptor. Therefore, the disruption of the t $\beta$ 1AR-NB80 complex upon addition of S3 was observed, as expected. The efficiency of S3 to compete Iso was studied by titrating S3 to the t $\beta$ 1AR-NB80-Iso complex, which showed that the affinity of Iso is substantially higher than that of S3. The results suggest that the high collision energy applied to the complex is needed to remove the detergent therefore maintaining the complexes intact and allowing the study of GPCRs with native MS.

Native MS can significantly improve our understanding of the GPCR mechanism, giving an insight into their function. This study shows the potential of native mass spectrometry in characterizing the binding of agonists, antagonists and G proteins to receptors. In this case, it was used to directly determine the degree of NB80 and ligand binding to t $\beta$ 1AR.