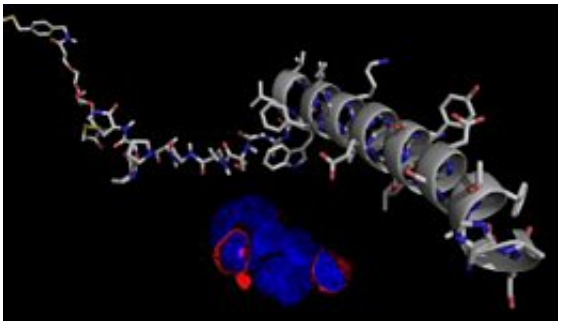
# Ties that bind, and can be untied

February 20 2018



Model of a REductively Cleavable AgONist (RECON) for the reversible control of GPCR-dependent cell signaling pathways. Credit: J. Broichhagen, D. Hodson

LMU researchers have developed a method that enables cell-surface receptors to be switched on and off at will. The technique promises to provide new insights into receptor functions and their effects on intracellular signaling pathways.

G-protein-coupled transmembrane receptors (GPCRs) are intimately involved in the regulation of a wide range of biological processes. They work by binding extracellular messenger molecules – such as hormones – and activating intracellular signaling relays that alter cell functions in specific ways. Not surprisingly, functional defects in these membrane-bound receptors often lead to chronic physiological disorders. This explains why a large proportion of current pharmacological research focuses on finding drugs that target specific GPCRs. A research collaboration involving groups led by Professors Anja Hoffmann-Röder

(Professor of Organic Chemistry) at LMU, Dirk Trauner (formerly LMU, now at New York University) and David Hodson (University of Birmingham) has now designed a suite of molecules with which GPCRs (and potentially other receptor types) can be pharmacologically activated and deactivated at will. These agents promise to uncover new aspects of the molecular mechanisms that underlie the actions of receptors, and in doing so should facilitate the development of novel therapeutic strategies. The new findings appear in the online journal *ACS Central Science*.

The new study is based on earlier work in which the team had developed a light-dependent pharmacological system with the aid of 'SNAP-tags'. A SNAP-tag is a binding protein that can be introduced to a target receptor protein by genetic means. Its purpose is to act as a binding site for the attachment of a synthetic ligand, which then can modulate receptor signaling, i.e. activate or block activation. The new paper describes the application of this technique to a receptor called GLP-1R, which regulates the secretion of insulin. It therefore presents an attractive target for the development of drugs with which to treat diabetes. "We used as the ligand a natural hormone that was equipped with a synthetic extension that acts as an adaptor which is covalently bound by the SNAP-tag. Binding of the hormone activates the receptor, while the direct attachment of the adaptor to the SNAP-tag ensures that the receptor is maintained in the ON state," explains Tom Podewin (Max Planck Institute for Medical Research), joint first author of the new study and until 2017 a doctoral student in Hoffmann-Röder's group. Attachment of the adaptor end to the SNAP-tag effectively tethers the ligand to the receptor. However, the adaptor is flexibly connected to the hormone-binding site of the receptor via a disulfide bond, which can readily be broken by the addition of a reducing agent. This trick allows the hormone to be released from its binding site, thus reversing the interaction and turning the receptor OFF again.

In order to demonstrate the versatility of this 'tethered pharmacology' approach, the team used a different synthetic ligand to activate a receptor that controls the secretion of growth hormone. "Our ligands are actually the largest known tethered molecules that have been shown to act as activators or agonists for membrane-bound receptors. This proves that tethered pharmacology is not restricted to the use of small molecules, but can be extended to peptides and perhaps even to proteins," Hoffmann-Röder points out.

Since binding to the SNAP-tag is covalent, the activating ligand cannot be readily released from the receptor in the absence of a reducing agent. Normally, activated GPCRs are promptly removed from the cell membrane and transported to intracellular vesicles. Once there, their ligands dissociate and they are then recycled to the cell surface. However, the researchers found – to their surprise – that binding of the synthetic ligand inhibits this recycling process, trapping the receptor in the vesicle. "The ability to durably attach any ligand – be it a pharmacological agent or a tag for use in bio-imaging – to a suitably modified receptor affords new opportunities for the manipulation and characterization of complex signaling pathways in cells," adds joint first author Johannes Broichhagen. He and his colleagues believe that the new method will yield a better understanding of receptors and their functions, which will undoubtedly have repercussions for drug development in the future.

**More information:** Tom Podewin et al. Conditional and Reversible Activation of Class A and B G Protein-Coupled Receptors Using Tethered Pharmacology, *ACS Central Science* (2018). DOI: 10.1021/acscentsci.7b00237

Provided by Ludwig Maximilian University of Munich