

**P010** Just an extracellular loop? ECL 2 is critical for GPCR function

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G-protein-coupled receptors (GPCRs) are the targets of over 40% of drugs in clinical use. The extracellular face of peptide-GPCRs provide recognition and interaction sites for ligands. The second extracellular loop (ECL2) of rhodopsin forms a  $\beta$ -hairpin which projects down into the TM bundle to make contacts with the chromophore. ECL2 of other rhodopsin-like (Family A) GPCRs may adopt a similar conformation. A functionally important disulfide bond between ECL2 and the top of TM3 is conserved throughout Family A GPCRs and constrains ECL2.

The  $V_{1a}$  vasopressin receptor ( $V_{1a}R$ ) is a member of a family of related GPCRs activated by neurohypophysial peptide hormones, including vasopressin (AVP).

This study is the first systematic investigation of the role of the ECL2 domain of a peptide-GPCR, all 29 ECL2 residues of the  $V_{1a}R$  were systematically mutated and pharmacologically characterized with respect to (i) binding of four different classes of ligand (ii) inositol phosphates (InsP) signalling and (iii) receptor expression.

Our data establish the functional importance of key residues within ECL2 of the  $V_{1a}R$ , in addition to the disulfide bond conserved in Family A GPCRs. Defining the roles of residues within the ECL domains of GPCRs in this way is fundamental to understanding the molecular mechanisms of ligand-binding and receptor activation and may aid rational drug design.