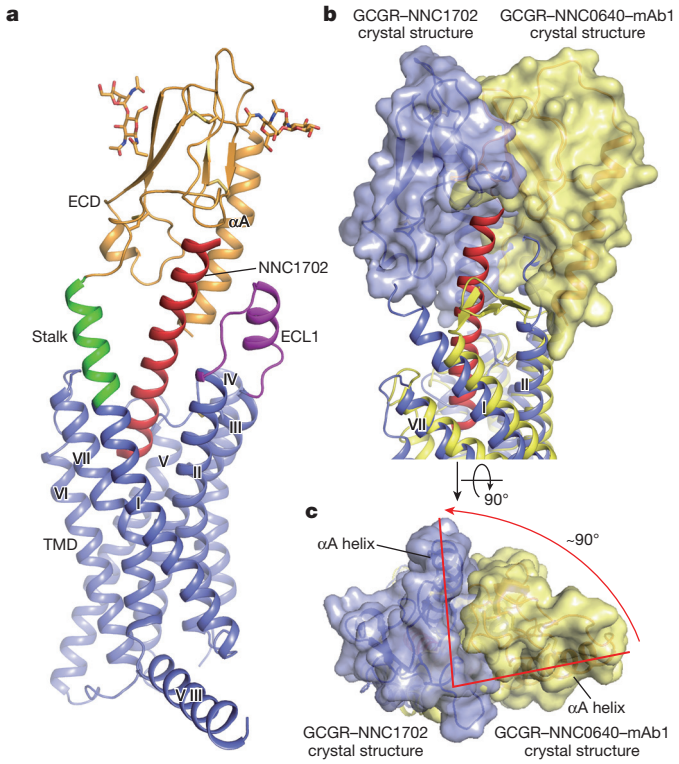

Figure 1 | Overall structure of GCGR–NNC1702 complex. a, Crystal structure of GCGR–NNC1702 complex. GCGR and NNC1702 are shown in cartoon representation. The ECD (residues Q27–D124), stalk (residues G125–K136), TMD (residues M137–Y202 and V221–E426) and ECL1 (residues S203–A220) of the receptor and the peptide ligand NNC1702 are coloured orange, green, blue, magenta and red, respectively. Glycan modifications in the ECD and disulfide bonds are displayed as orange and yellow sticks, respectively. **b**, **c**, Structural comparison between the GCGR–NNC1702 structure and the GCGR–NNC0640–mAb1 structure, shown in side (**b**) and extracellular (**c**) views. The GCGR–NNC1702 structure and the receptor in the GCGR–NNC0640–mAb1 structure (PDB ID: 5XEZ) are shown in cartoon representation and coloured blue and yellow, respectively. The peptide NNC1702 is in red. The ECD of the receptor in both structures is also shown in surface representation. The red arrow in **c** indicates a rotation of the ECD in the GCGR–NNC1702 structure compared to the GCGR–NNC0640–mAb1 structure.

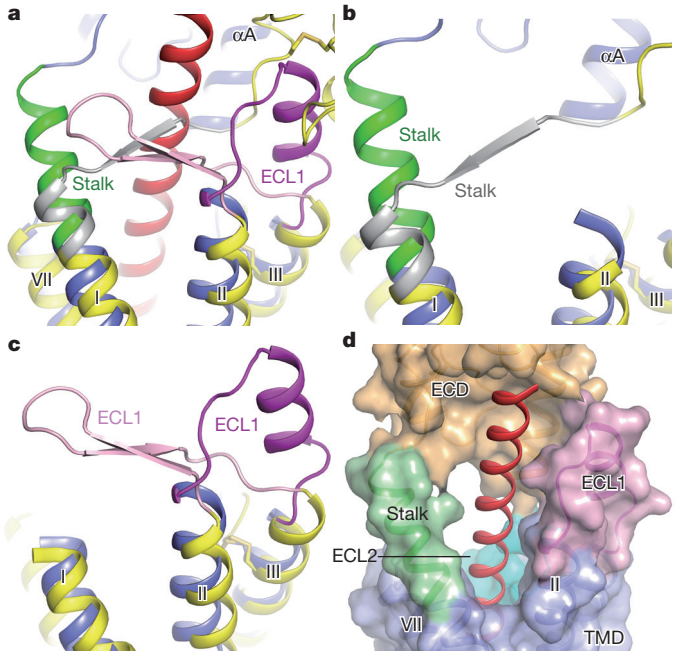

Figure 2 | Conformations of the stalk and ECL1. a, Comparison of the stalk and ECL1 between the GCGR–NNC1702 structure and the GCGR–NNC0640–mAb1 structure. The GCGR–NNC1702 structure and the receptor in the GCGR–NNC0640–mAb1 structure (PDB ID: 5XEZ) are shown in cartoon representation and coloured blue and yellow, respectively. The peptide NNC1702 is in red. The stalk and ECL1 in the GCGR–NNC1702 structure are coloured green and magenta, respectively. The stalk and ECL1 in the GCGR–NNC0640–mAb1 structure are coloured grey and pink, respectively. **b**, Highlight of the conformational difference between the stalks in GCGR–NNC1702 (green) and GCGR–NNC0640–mAb1 (grey) structures. **c**, Highlight of the conformational difference between ECL1 in GCGR–NNC1702 (magenta) and GCGR–NNC0640–mAb1 (pink) structures. **d**, Entrance to the orthosteric ligand-binding pocket within the TMD. The receptor is shown in surface and cartoon representations. The ECD, stalk, ECL1, ECL2 (residues E290–G302) and TMD of GCGR are coloured orange, green, magenta, cyan and blue, respectively. The peptide NNC1702 is shown in cartoon representation and coloured red.

helix I (Fig. 2b), a conformation similar to that observed in the previously solved structure of the GCGR TMD (RCSB Protein Data Bank (PDB) ID: 4L6R)[18]. The stalk has not been modelled in the GLP-1–GLP-1R–Gs electron microscopy structure[13]; in the GLP-1R–peptide 5 structure, the corresponding linker region forms an unstructured loop rather than a helix[14], which may be explained by the absence of interaction between this linker region and the truncated peptide ligand that is thought to stabilize the helical conformation of the stalk[17].

In the peptide-bound GCGR structure, ECL1 of the receptor no longer forms a β-hairpin conformation. Instead, it is dissociated from the stalk region, and stands upwards in line with helices II and III (Fig. 2c). The N-terminal segment of ECL1 (residues S203–I206) lacks secondary structure; the C-terminal residues (D209–S217) form a 2.5-turn α-helix that is connected with helix III by a short linker (residues D218–A220). A similar conformation of ECL1 is observed for GLP-1R in the structures of the GLP-1–GLP-1R–Gs and GLP-1R–peptide 5 complexes[13,14]. However, further structural details of ECL1 in inactive non-peptide-bound GLP-1R are required to determine whether the ECL1 of GLP-1R can undergo a similar conformational change to that observed in the GCGR structures.

Previous mutagenesis and hydrogen–deuterium exchange (HDX) studies[16–18] suggest that the stalk and ECL1 of GCGR are involved in peptide ligand binding. Indeed, both regions form extensive interactions with the peptide ligand in the GCGR–NNC1702 structure. The stalk and ECL1 act as two 'arms' that hold the peptide tightly and greatly strengthen the binding between the receptor and the middle portion of the peptide (Fig. 2d). It has been proposed[17] that the relative movement and interaction dynamics of the ECD and TMD via the stalk pivot point may be a common feature of class B GPCRs. Both the GCGR–NNC1702 structure and the inactive GCGR–NNC0640–mAb1 structure support this concept and demonstrate that a large conformational rearrangement of the stalk and ECL1, which includes the dissociation of these two regions and their changes in secondary structure, is required for peptide ligand binding. These data further support the importance of the stalk and ECL1 in GCGR signal transduction.

The GCGR–NNC1702 crystal structure supports the two-domain model of hormone recognition by class B GPCRs[9,10] (Fig. 3, Extended Data Fig. 3 and Extended Data Table 2). In the structure, NNC1702 forms a continuous α-helix throughout the whole length of the peptide (Extended Data Fig. 4). The N-terminal half of the peptide ligand (residues S2–L14; residue numbering is consistent with that in glucagon) binds to the TMD ligand-binding pocket bordered by helices I, II and VII and the second extracellular loop (ECL2) (Extended Data Fig. 3a). The side chain of the N-terminal residue S2 of NNC1702, which is an alanine (A8) in GLP-1, forms a hydrogen bond with residue D385$^{7.42b}$ (numbers in superscript refer to the modified Ballesteros–Weinstein numbering system for class B GPCRs[19,20]) on helix VII (Fig. 3b). This agrees with previous data[21,22] showing that the A8S