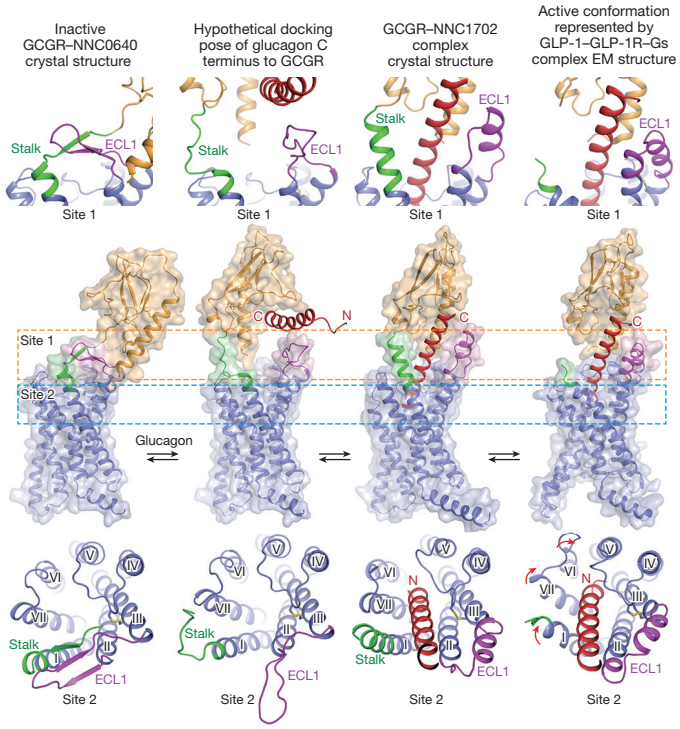

Figure 4 | Dual-binding-site trigger model for GCGR activation. The inactive GCGR–NNC0640–mAb1 crystal structure (PDB ID: 5XEZ, mAb1 removed), a hypothetical docking pose of glucagon C terminus to GCGR, the GCGR–NNC1702 crystal structure and the active GCGR conformation represented by the GLP-1–GLP-1R–Gs electron microscopy (EM) structure (PDB ID: 5VAI) are shown in cartoon and surface representations in the middle panel. The ECD, stalk, ECL1 and TMD of the receptor and the peptide ligands are coloured orange, green, magenta, blue and red, respectively. The two sites of the receptor–peptide interactions that trigger receptor activation, site 1 and site 2, are shown in the top panel and bottom panel, respectively, highlighting the conformational changes of the receptor from the inactive to the active conformation. Top panel, side view; bottom panel, extracellular view. The red arrows in the bottom right panel indicate the shifts of helices I, VI and VII in the active conformation compared to the inactive conformation.

by only about sixfold[18]; this could be explained by a lack of direct contact between this residue and the peptide in the GCGR–NNC1702 structure. Notably, an R201$^{2.74b}$D mutation considerably decreases the binding of GCGR to glucagon[18,26]. Further analysis of the peptide-bound GCGR structure revealed a 'sandwich' stacking interaction formed by R201$^{2.74b}$ and W215 on ECL1 and the peptide residue R18 (Fig. 3f) that stabilizes the conformation of ECL1 and its interaction with the peptide. This structural feature is supported by the fact that the R201$^{2.74b}$D mutant loses glucagon-binding ability, probably as a result of disturbance to the arginine–π–arginine stacking interaction caused by this negatively charged residue.

Together, the GCGR–NNC1702 crystal structure and the structure of the inactive GCGR–NNC0640–mAb1 complex expand the previously established[9–11] two-domain peptide-binding model of class B GPCRs by incorporating another agonist trigger associated with an inter-domain conformational shift coupled with a change of secondary structure in the stalk region and ECL1 (Fig. 4). Binding of the glucagon C terminus to the ECD may disrupt the β-sheet structure of the stalk and ECL1 and result in dissociation between these two regions, which potentially triggers a conformational change of the ECD relative to the TMD and initiates receptor activation. Using double electron–electron resonance (DEER) spectroscopy, we have demonstrated the conformational change of the ECD on peptide binding, which shows that the peptide NNC1702 induces a conformational rearrangement of the receptor ECD to accommodate peptide binding (Extended Data Fig. 5). The second set of interactions between the peptide N terminus and the TMD may enable further conformational changes of the stalk and ECL1 in secondary structures. The conformational change of the stalk may not only mediate the receptor–peptide interaction, but also potentially facilitate conformational movements of the TMD helical bundle through its effect on the conformation of helix I, which shifts towards helix VII on the extracellular side in the active structures of calcitonin receptor and GLP-1R compared to the inactive class B GPCR structures[12–14]. Together with the movement of helix I, the rearrangements of helices VI and VII at the extracellular ends, which may be partially induced by the interaction between H1 of glucagon and the receptor (as suggested by our molecular dynamics simulation studies; Extended Data Fig. 6 and Supplementary Information), are further relayed into conformational changes in the cytoplasmic domain, which lead to G-protein coupling and full receptor activation. In contrast to the two-domain binding model, the interactions in the middle region of the peptide (site 1) are critical not only for driving affinity of the peptide but also for triggering the necessary conformational changes of the stalk and ECL1 that are associated with full receptor activation. The peptide N terminus (site 2) induces further conformational rearrangement of the transmembrane helical bundle that is also essential for full receptor activation (Fig. 4). This dual-binding-site trigger model for GCGR activation updates the long-standing paradigm that N-terminal peptide interactions are solely responsible for triggering agonist-associated conformational changes, and is consistent with the idea that truncated peptides for class B GPCRs can act as partial agonists[27], potentially by triggering conformational changes in the ECD, stalk and/or ECL1.

In summary, the GCGR–NNC1702 crystal structure sheds light on both the complexity and the molecular details that govern the peptide binding and receptor activation of GCGR, and thereby greatly expand our understanding of signal transduction by class B GPCRs.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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