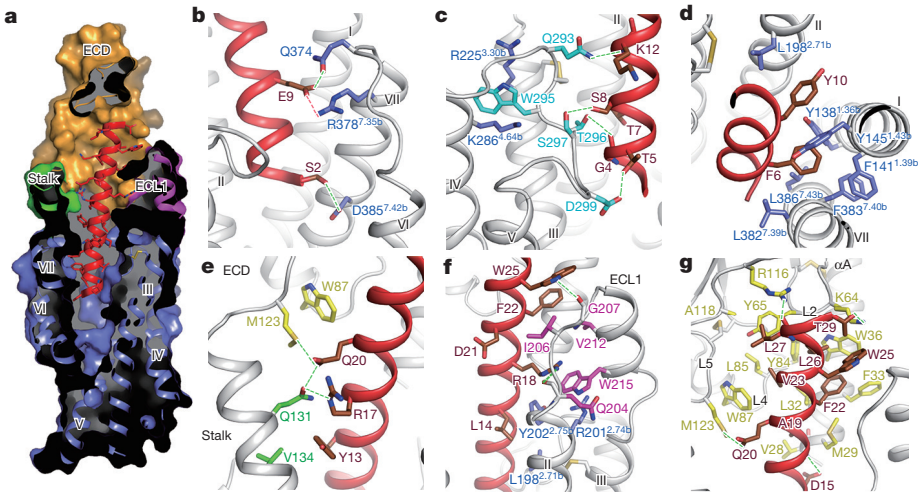

Figure 3 | Binding mode of NNC1702 to GCGR. a, Cutaway view showing NNC1702 binding to the ECD (orange), stalk (green), ECL1 (magenta) and TMD (blue) of GCGR. The receptor is shown in surface and cartoon representations. The peptide ligand is shown as red sticks and a red cartoon. **b**–**d**, Interactions between the NNC1702 N terminus and the GCGR TMD. The receptor and peptide NNC1702 in the GCGR–NNC1702 structure are shown as cartoons and coloured grey and red, respectively. Residues involved in interactions are shown as sticks and coloured brown (NNC1702), blue (GCGR TMD) and cyan (ECL2). Salt bridge and hydrogen bonds are displayed as red and green dashed lines, respectively. **e**, Interactions between NNC1702 and the stalk. Residues of the stalk and ECD are shown as green and yellow sticks, respectively. **f**. Interactions between NNC1702 and ECL1. Residues of ECL1 are shown as magenta sticks. **g**. Interactions between NNC1702 and the GCGR ECD. Residues of the ECD are shown as yellow sticks.

mutant of GLP-1 restores binding of the GLP-1R mutant E387$^{7.42b}$D, whereas the S2A mutant of glucagon rescues binding of the GCGR mutant D385$^{7.42b}$E, which suggests an important role for this hydrogen-bond interaction in recognising the glucagon N terminus. Owing to a spatial hindrance caused by S2 and its contact with D385$^{7.42b}$, the extracellular tip of helix VI shifts away from the central axis of the helical bundle by about 6.5 Å in the GCGR–NNC1702 structure compared to the GCGR–NNC0640–mAb1 structure (Extended Data Fig. 2e). This suggests that the rearrangement of the extracellular half of helix VI may have a role in the peptide ligand recognition of GCGR. We anticipate that further movement of helix VI will occur on G-protein coupling, similar to the conformation observed in the structure of the GLP-1–GLP-1R–Gs complex[13].

The N-terminal region of NNC1702 makes multiple interactions with ECL2 of the receptor (Fig. 3c), demonstrating the critical role of ECL2 in peptide ligand binding to GCGR (Supplementary Information). Two aromatic residues (F6 and Y10) within the N-terminal region of NNC1702, together with Y13 and L14 in the middle region of the peptide, form a hydrophobic patch, which has previously been suggested to be important for mediating binding affinity[23]. The side chain of F6 fits in a sub-pocket formed by several hydrophobic residues on helices I and VII (Fig. 3d). The importance of the hydrophobic nature and size of this sub-pocket in peptide ligand recognition is supported by previous mutagenesis data that show that the glucagon binding affinity of GCGR mutants Y145$^{1.43b}$A, Y145$^{1.43b}$N, L382$^{7.39b}$A, L382$^{7.39b}$V, L386$^{7.43b}$A and L386$^{7.43b}$F is completely abolished or reduced by at least fivefold[18]. The other aromatic residue (Y10) in the N-terminal region of NNC1702 wedges into a cleft between helices I and II, and forms hydrophobic interactions with the residues Y138$^{1.36b}$ and L198$^{2.71b}$ of GCGR (Fig. 3d). Similar interactions between GLP-1R and the two corresponding hydrophobic residues (F12 and V16) of the truncated GLP-1 analogue peptide 5 are also observed in the GLP-1R–peptide 5 complex structure[14], which indicates that these two residues have an identical role in binding to GLP-1R to that of F6 and Y10 of glucagon in binding to GCGR.

The only negatively charged residue, E9 (corresponding to D9 in glucagon), within the N-terminal region of NNC1702 forms a salt bridge with residue R378$^{7.35b}$ at the extracellular tip of helix VII and a hydrogen bond with Q374 on the third extracellular loop (ECL3) (Fig. 3b). It was previously reported[24] that the mutation D9E greatly reduced glucagon potency in activating adenylate cyclase, and that the R378$^{7.35b}$Q mutation in GCGR abolished glucagon binding to the receptor[21], both of which support the importance of the interaction with glucagon. The glucagon–GCGR binding pair D9 and R378$^{7.35b}$ is conserved in GLP-1–GLP-1R as D15 and R380$^{7.35b}$. Similarly, mutagenesis studies[25] support a possible ionic interaction between D15 of GLP-1 and R380$^{7.35b}$ in GLP-1R. However, the GLP-1–GLP-1R–Gs electron microscopy structure suggests that this interaction may potentially break up during the transition from an inactive to an active conformation (Supplementary Information). Compared to D9 in glucagon, the longer side chain of E9 in NNC1702 may form a stronger interaction with R378$^{7.35b}$ of GCGR and thus restrict the conformational change of helix VII, consistent with the fact that D9E maintains binding affinity but reduces glucagon potency[24].

The structure of the GCGR–NNC1702 complex reveals molecular details of the essential roles that the stalk and ECL1 of GCGR have in glucagon recognition[26]. The stalk and ECL1 form extensive interactions with the middle portion of NNC1702 (residues Y13–W25) (Fig. 3e, f). The short α-helix (residues D209–S217) within ECL1 not only interacts with the peptide, but also makes contacts with the N-terminal αA helix of the ECD through a hydrophobic core formed by ECL1 residues V212, W215 and L216 and ECD residues M29 and F33 (Fig. 3g). Together with the stalk, the ECL1 α-helix, the ECD αA-helix and the peptide ligand form a four-helical bundle (Extended Data Fig. 3b), which greatly strengthens the interaction between GCGR and the peptide ligand.

Previous efforts[23] to develop potent truncated glucagon antagonists demonstrate that the two arginine resides R17 and R18 of glucagon are sufficient for the peptide to be recognized by the receptor. Besides the hydrogen bond between R17 and residue Q131 on the stalk (Fig. 3e), R18 forms an arginine–π interaction with residue W215 on ECL1 (Fig. 3f) that has a critical role in stabilizing receptor–peptide binding, as the mutation W215L completely abolishes glucagon binding to GCGR[18]. Two bulky residues at the junction between helix II and ECL1, R201$^{2.74b}$ and Y202$^{2.75b}$, have been suggested to be important for glucagon binding[17,18,26]. In the GCGR–NNC1702 structure, Y202$^{2.75b}$ forms hydrophobic interactions with residues L14 and R18 of the peptide (Fig. 3f), in agreement with previous mutagenesis data[18] that show that replacing Y202$^{2.75b}$ with alanine abolishes glucagon binding of the receptor. By contrast, the mutant R201$^{2.74b}$A reduces glucagon binding