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Author Contributions Ha.Z. optimized the construct, developed the purification procedure and purified the GCGR proteins for crystallization, performed crystallization trials and optimized crystallization conditions. A.Q. helped with construct optimization and crystallization trials. L.Y. performed and analysed molecular dynamics simulations. N.V.E. performed and analysed DEER spectroscopy. K.S.F. performed and analysed binding and potency assays of glucagon and NNC1702. D.Y., A.D. and X.C. designed, performed and analysed the whole-cell glucagon binding assay. Hu.Z. collected the X-ray diffraction data. C.Y. expressed the GCGR proteins. C.C. and L.H. helped to analyse the conformational variety of GCGR. J.L., O.P.E., M.A.H., R.C.S., M.-W.W. and S.R.-R. helped with structure analysis and interpretation, and edited the manuscript. O.P.E. oversaw DEER spectroscopy. M.-W.W. oversaw the whole-cell glucagon binding assay. H.Y. and H.J. oversaw molecular dynamics simulations and commented on the manuscript. S.R.-R. designed the peptide and oversaw ligand characterization of NNC1702. Q.Z. and B.W. initiated the project, planned and analysed experiments, solved the structures, supervised the research and wrote the manuscript with input from all co-authors.

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