A β-peptide agonist of the GLP-1 receptor, a class B GPCR

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SUPPLEMENTARY INFORMATION

I. Materials. β^3-amino acids were purchased (PepTech Corp., Burlington, MA) or prepared from the corresponding commercially available Fmoc-protected α-amino acid (Novabiochem, San Diego, CA) according to established synthetic routes.[1, 2] 1 (7-Azabenzotriazol-1-yloxy)tripyrrolidino-phosphonium hexafluorophosphate (PyAOP) was purchased from Oakwood Products, Inc. (West Columbia, SC). 1-Hydroxy-7-azabenzotriazole (HOAt) was purchased from Chempep, Inc. (Miami, FL). 2-(6-Chloro-H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) was purchased from Peptides International Inc. (Louisville, KY). *N,N*-diisopropylethylamine was purchased from Sigma-Aldrich (St. Louis, MO). Dimethylformamide (DMF), *N*-methyl-2-pyrrolidone (NMP), *N*-methyl morpholine (NMM), trifluoroacetic acid (TFA) and piperidine were purchased from American Bioanalytical (Natick, MA). Glucagon and PACAP were purchased (American Peptide Co., Sunnyvale, CA). Unless mentioned explicitly, all other reagents used for the synthesis of β^3-amino acids were purchased from Sigma-Aldrich (St. Louis, MO).

II. Peptide synthesis. β^3-peptides were synthesized manually on a 25 μmol scale using standard Fmoc chemistry and Rink amide resin (Novabiochem, San Diego, CA). Amide bond formation and deprotection steps were performed using a MARS Microwave Accelerated Reaction System

230/60 (CEM Corp., Matthews, NC) as described.[3] α-peptides were synthesized on a 50 μmol scale using Fmoc-protected α-amino acids (Novabiochem, San Diego, CA) and Rink amide or TGR resin (Novabiochem, San Diego, CA)[4] on a Liberty Model 908505 automated microwave-assisted peptide synthesis apparatus (CEM Corp., Matthews, NC). Synthetic peptides (α- and β-peptides) were cleaved from the solid support and deprotected using a cocktail composed of 92.5% trifluoroacetic acid (TFA), 2.5% tri-isopropylsilane, 2.5% water and 2.5% 3,6-dioxa-1,8-octanedithiol (DODT) for 30 min at 38 ºC utilizing the CEM MARS device. The resin (150 mg for a 50 μmol synthesis) was suspended in 3 mL of the cleavage cocktail, heated to 38 ºC for 2 min, held at this temperature for 30 min, and then cooled for 5 min. The power setting of the CEM MARS device during this procedure was set at 50% of the 400W maximum. All synthetic peptides (α- and β-peptides) carried free amino termini and amidated carboxyl termini.

III. Peptide purification and characterization.

a. **Purification.** Peptides were purified to homogeneity using reverse-phase HPLC and an eluent that ramped between 10% and 70% acetonitrile/0.1% TFA in water/0.1% TFA on a Varian HPLC module (pump model 210, UV/Vis detector model 325, autocollector model 701, Varian, Inc., Palo Alto, CA)[4] using a YMC basic semi-preparative column (150 mm x 10 mm, Model BA99S11-151-WT, 10 μm particle size, YMC Co. Ltd., Kyoto, Japan). The purity of each peptide was assessed using a C8 Grace-Vydac analytical column (300 Å, 5 μm, 4.6 mm x 150 mm, Grace Davison, Deerfield, IL).

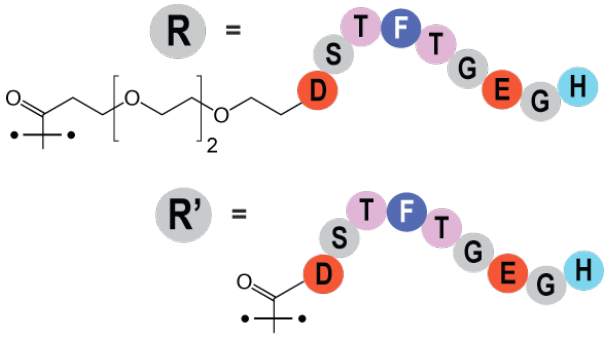
b. **Concentration determination.** Peptides were quantified by UV spectroscopy using a Beckman-Coulter DU 730 UV/Vis Spectrophotometer (Beckman Coulter, Brea, CA)[5] using extinction coefficients predicted by the peptide sequence.[6]

c. **Verification of identity.** The identities of the β^3-peptides evaluated in this work were verified using a Voyager-DE Pro MALDI-TOF mass spectrometer (Applied Biosystems, Foster, CA). The sequences of all synthetic β-peptides are listed in Supplementary Table 1 alongside the expected and observed mass. Sequences are represented as an alternative single letter code in which each letter X corresponds to the β^3-homolog of the corresponding natural amino acid X. Several α-peptides were also synthesized and purified. Their identities were also verified by MALDI-TOF MS (Supplementary Table 2).

Supplementary Table 1. β^3-peptides sequences and their calculated and observed molecular masses as determined by MALDI-TOF MS.

β^3-peptide	**Sequence**	**MW (calculated)**	**MW (observed)**
CC-1	OAVEFWOWVE	1346.4	1346.3
CC-2	OWVEFVOAVE	1346.4	1346.4
CC-3	OVAEVFOVWE	1346.4	1346.4
CC-4	OVWEVFOVAE	1346.4	1346.4
CC-5	OAVEFVOLVE	1273.1	1271.8
CC-6	OLVEFVOAVE	1273.1	1273.2
CC-7	OVAEVFOVLE	1273.1	1273.7
CC-8	OVLEVFOVAE	1273.1	1273.8
CC-9	OVVEFVOWVE	1374.7	1374.8
CC-10	OWVEFVOVVE	1374.7	1373.9

CC-11	OVVEVFOVWE	1374.7	1374.8
CC-12	OVWEVFOVVE	1374.7	1374.7
CC-13	OVVEFVOLVE	1301.4	1301.5
CC-14	OLVEFVOVVE	1301.4	1300.8
CC-15	OVVEVFOVLE	1301.4	1301.0
CC-16	OVLEVFOVVE	1301.4	1301.0
CC-3^{Act}	R-CC3	2481.6	2482.0
CC-11^{Act}	R-CC11	2509.9	2511.0
CC-3.3	OVOEVFOVWE	1389.5	1390.2
CC-3.6	OVAEVAOVWE	1270.3	1270.2
CC-3.9	OVAEVFOVAE	1231.2	1232.4
CC-3.3^{Act}	R-CC-3.3	2524.7	2525.7
CC-3.6^{Act}	R-CC-3.6	2405.5	2404.9
CC-3.9^{Act}	R-CC-3.9	2366.4	2368.0
CC-3.3^{Act-1}	R'-CC3	2278.3	2280.9



Supplementary Table 2. The α-peptides studied herein and their calculated and observed molecular masses as determined by MALDI-TOF MS.

Peptide	Sequence	MW (calc)	MW (obs)
exendin-4	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS	4187.6	4189.9
exendin-$4_{9\text{-}39}$	DLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS	3370.7	3371.2
GLP-1	HAEGTFTSDVSSYLEGQAAKEFIAWLVKGRG	3355.7	3355.4
exendin-$4_{1\text{-}9}$	HGEGTFTSD	949.9	950.9
GIP	YAEGTFISDYSIAMDKIHQQDFVNWLLAQKGKKNDWKHNITQ	4983.5	4985.7

IV. nGLP-1R Expression and Purification

a. Preparation of the nGLP-1R bacterial expression plasmid. The human GLP-1 receptor gene in PCR4-TOPO was obtained from Open Biosystems (Huntsville, AL) (Clone ID: 8327594; Accession #: BC112126; Catalog #: MHS1768-97430513). The sequence encoding the N-terminal extracellular domain (residues 24 through 125)[7, 8] was amplified using the polymerase chain reaction (PCR) and primers NGLP1R-1 and NGLP1R-2. The sequences of these primers, written in the 5' to 3' direction, are as follows: NGLP1R-1: TTTTTTCATATGCGCCCCCAGGGTGCCACTGTGTCCCTCTGGG; NGLP1R-2: TTTTTTGCTGAGCTTAGTAGAGGAACAGGAGCTGCTCCTCCGGGGAGCTTCT. PCR was performed using an MJ Research PTC-200 thermocycler (MJ Research, Inc., Waltham, MA) and Taq-Polymerase (NEB, Ipswich, MA) according to NEB's specification for Taq-Polymerase. The products of the PCR reaction were purified on an

agarose gel, the DNA was excised (Gel Extraction/Purification Kit, Qiagen) and the excised DNA was ligated into pET-15b (Novagen, San Diego, CA) using the NdeI and BlpI restriction sites contained therein. Ligation reactions were incubated overnight with T4 DNA Ligase in T4 DNA Ligase reaction buffer (NEB) on ice with a 1.2 to 1 molar ratio of insert to plasmid. XL-10 gold cells (Stratagene, Agilent Technologies, Inc., Santa Clara, CA) were transformed using freshly ligated DNA and plated on carbenicillin plates; nine colonies were selected and the DNA was amplified and purified using Qiagen's Mini-Preparation kit. DNA sequencing (W.M. Keck Facility at Yale University) verified the sequence of the DNA insert. XL-1 Blue cells (Stratagene, Agilent Technologies, Inc., Santa Clara, CA) were transformed with sequences that contained no mutations in order to amplify the DNA on a Maxi-Preparation scale (Qiagen). This DNA was then transformed into BL21-(DE3) cells as per the manufacturer's recommendations (Stratagene, Agilent Technologies, Inc., Santa Clara, CA) for subsequent expression.

b. Expression. nGLP-1R was expressed and purified according to previously established protocols.[7,8] Briefly, 1 L of LB (supplemented with 50 μg/mL carbenicillin (American Bioanalytical, Manassas, MA)) was inoculated at 37 ºC from a single colony of BL21-(DE3) cells transformed with nGLP-1R in pET-15b. Expression was suppressed using 1 mM glucose until the OD reached 0.6 (2 to 3 h) and subsequently induced with IPTG (1 mM). Under these conditions, nGLP-1R was expressed in inclusion bodies. After 6 h, the cells were lysed and the inclusion bodies isolated,[9] solubilized in 30 mL of 6 M Gdm-HCl (VWR International, Radnor, PA) at 50 mg/mL, and dialyzed against 4 M Gdm-HCl (pH 3.0).

c. **Refolding**. Refolding was performed by adding the dialyzed solution prepared above dropwise into refolding buffer A (RB-A: 100 mM Tris•HCl (pH 8.5), 500 mM L-arginine, 1 mM reduced glutathione (GSH), 5 mM oxidized glutathione (GSSG), 1 mM EDTA). Every six hours a 5 mL aliquot of the solution was added dropwise over the course of 2 to 3 min to 500 mL RB-A. The resulting solution was then added to 1.5 L refolding buffer B (RF-B: 20 mM Tris•HCl, 500 mM NaCl, 10 mM $MgCl_2$, 20 mM imidazole (Sigma-Aldrich, St. Louis, MO) (pH 7.4) and passed through a 0.22 μm filter. The pH of this diluted solution was adjusted to 7.4 using HCl (pH is normally ~9.75 to 10.00 upon dilution) and purified as described below.

d. **Purification and thrombin cleavage.** The refolded sample of nGLP-1R was loaded on a 5 mL Ni-Sepharose His-Trap HP column (GE Healthcare Biosciences, Piscataway, NJ); washed with $MgCl_2$ binding buffer (M-BB: 20 mM Tris•HCl, 20 mM imidazole, 10 mM $MgCl_2$, 500 mM NaCl, (pH 7.4)), washed with binding buffer (BB: 20 mM Tris•HCl, 20 mM imidazole, 500 mM NaCl, (pH 7.4)) and then eluted with elution buffer (EB: 20 mM Tris•HCl, 500m mM NaCl, 500 mM imidazole, (pH7.4)). Fractions containing pure nGLP-1R were combined, dialyzed against thrombin Ni buffer (TNB: 20 mM Tris•HCl, 500 mM NaCl, 20 mM imidazole, 2.5 mM $CaCl_2$ (pH 7.4)) and treated with Biotinylated Thrombin (Stratagene, Agilent Technologies, Inc., Santa Clara, CA) for 16 h at 4 ºC. After thrombin cleavage, to the resulting solution was added 143 μL Streptavidin Agarose (Stratagene) and 1 mL Ni-NTA (Qiagen) beads to remove unreacted nGLP-1R and Biotinylated Thrombin. After filtration, the resulting nGLP-1R was dialyzed against 250 mM L-Arginine, 10 mM NaH_2PO_4, 1 mM EDTA (pH 7.5) for 12 h and again for 24 h at 4 ºC. nGLP-1R was concentrated using Amicon Ultra-4 Centrifugal Concentrators (3k

NMCO, Millipore, Billerica, MA) to a final concentration between 25 and 50 μM. Small (100 μL) samples were flash frozen and stored at -80 ºC. Cloning into pET-15b resulted in a fusion protein in which both a His_6 tag and a thrombin cleavage site are appended to the nGLP-1R N-terminus. The sequence of this fusion protein is as follows: *MGSS*<u>*HHHHHH*</u>*SS****GLVPR****GSHM*RPQGATVSLWETVQKWREYRRQCQRSLTEDPP PATDLFCNRTFDEYACWPDGEPGSFVNVSCPWYLPWASSVPQGHVYRFCTAEG LWLQKDNSSLPWRDLSECEESKRGERSSPEEQLLFLY. In this sequence, the His tag is underlined, the thrombin cleavage site is in bold, and amino acids that are not found in nGLP-1 are shown in italics. After thrombin cleavage, the construct used for all *in vitro* binding assays has the following sequence (Arg_{24} in bold):

GSHM**R**PQGATVSLWETVQKWREYRRQCQRSLTEDPPPATDLFCNRTFDEYACW PDGEPGSFVNVSCPWYLPWASSVPQGHVYRFCTAEGLWLQKDNSSLPWRDLSE CEESKRGERSSPEEQLLFLY

V. Fluorescence polarization assays

a. Preparation of exendin-4^{Flu}. Exendin-4^{Flu} was prepared by modifying the solid phase synthesis procedure used to prepared exendin-4 described above. Following the synthesis of exendin-4, but prior to side chain deprotection and cleavage from the resin, one eighth of the resin (6.25 μmol) was transferred to a separate reaction vessel and two β-alanine residues were added to the N-terminus as described above. After deprotection, the resin was washed and treated with 5-(and-6)-carboxyfluorescein, succinimidyl ester (5(6)-FAM, SE, catalog # C1311, Invitrogen, San Diego, CA) (1.5 equiv, 5 mg/mL in DMF) and 15 μL *N,N*-diisopropylethylamine (Sigma-Aldrich, St. Louis, MO). The reaction vessel was shaken on a rotary shaker at room temperature for 8 h; the resin was

subsequently washed and dried, and the product deprotected, cleaved, purified and identified as described previously. This final product is referred to as exendin-4^{Flu} (calculated MW = 4689.7, observed MW = 4698.0).

b. **Direct fluorescence polarization analysis of binding equilibria.** Samples consisted of between 13.5 μM and 824 pM nGLP-1R in binding buffer (BB, 250 mM L-arginine, 10 mM NaH_2PO_4, 1 mM EDTA, (pH 7.4)) and 5 nM exendin-4^{Flu} or a fluorescently labeled β- or α-peptide. Fluorescence polarization was monitored using an Analyst AD (LJL Biosystems, Inc., Sunnyvale, CA) as previously described.[10-14] Settings for FP were as follows: 3 reading per well, 100 ms between readings, 560 ms integration time, z-height = 1 mm, excitation filter = fluorescein (485 nm), emission filter = fluorescein (530 nm), G-factor = 1, 505 nm dichroic mirror. Time courses established that equilibrium was reached within 30 min. Data were fit to the following equation:

$$F = F_L + \left(\frac{F_{LP} - F_L}{2[L]_T}\right)\left([L]_T + [P]_T + K_d - \sqrt{([L]_T + [P]_T + K_d)^2 - 4[L]_T[P]_T}\right)$$

where *F i*s the measured average fluorescence polarization, F_L is the fluorescence polarization of the free labeled ligand, F_{LP} is the maximum fluorescence polarization of the peptide-protein complex, K_d is the equilibrium dissociation constant, $[L]_T$ and $[P]_T$ are the total concentration of fluorescently labeled peptide and nGLP-1R, respectively.

c. **Competition analysis of binding equilibria.** Samples consisted of 125 nM nGLP-1R in binding buffer (BB, 250 mM L-arginine, 10 mM NaH_2PO_4, 1 mM EDTA, (pH 7.4)), 5 nM exendin-4^{Flu} and between 400 μM and 12.2 nM of the β- or α-peptide acting as the

competitor. Fluorescence polarization was monitored as described above. The IC_{50} value for each competitor was determined by fitting the data to the following equation:[15]

$$F = F_L + \frac{(F_{LP} + F_L)}{1 + (IC_{50}/[I])^{-n}}$$

In this equation, F is the measured fluorescence polarization, F_L is the fluorescence polarization of exendin-4^{Flu}, F_{LP} is the maximum fluorescence polarization of the exendin-4^{Flu}•nGLP-1R complex, [I] is the concentration of the competitor, and n is the Hill coefficient. Data were fit using non-linear least squares analysis and Kaleidagraph v. 3.6 software (Synergy Software, Reading, PA). The value of K_I was computed from the IC_{50} value using the following equation [15, 16]:

$$K_I = \frac{[IC]_{50}}{\left(\frac{[L]_{50}}{K_d} + \frac{[P]_0}{K_d} + 1\right)}$$

In this equation, $[IC]_{50}$ is the concentration of the competitor peptide at 50% inhibition (obtained from the competition curve), $[L]_{50}$ is the concentration of free exendin-4^{Flu} at 50% inhibition (determined by calculating the concentration of free and bound exendin-4^{Flu} at 0% inhibition according to the K_d of the exendin-4^{Flu}•nGLP-1R complex and their actual concentrations in the solution, and then assuming that 50% of the *bound* exendin-4^{Flu} would be displaced at 50% inhibition; $[L]_{50}$ being the sum of the initially unbound exendin-4^{Flu} and the bound exendin-4^{Flu} which is displaced by competitor at 50% inhibition), $[P]_0$ is the concentration of free protein at 0% inhibition, and K_d is the independently determined equilibrium dissociation constant of the exendin-4^{Flu}•nGLP-1R

complex (128 nM, as determined by the direct binding of exendin-4^{Flu} to nGLP-1R in the previously described direct FP binding experiment) [16].

VI. GPCR activation assays.

a. Source of expression plasmids. Activation of class B GPCRs was measured by coupling the activation-dependent increase in intracellular cAMP to the expression of a firefly luciferase (*Photinus pyralis)* gene under the control of a cAMP response element binding protein promoter (CREB promoter, pGL4.29, Promega Corp., Madison, WI) [17]. Most of the plasmids used were donated or purchased. Full length GLP-1R (rat) in pCDNA3 was a gift from Professor Dan Donnelly (The University of Leeds, Leeds, UK) and Professor Bernard Thorens (University of Lausanne, Lausanne, Switzerland [18-20]. The plasmids directing the expression of GCGR, VIPR (VPAC2), and PACAPR (PAC1) were purchased from the Missouri S&T cDNA Resource Center (Rolla, MO).

b. Generation of a mammalian expression vector containing the full length GIP receptor. A plasmid encoding the full length GIP receptor in PCR4-TOPO (Clone ID: 8069179; Accession Number: BC101673; Catalog Number: MHS1010-98052657) was obtained from Open Biosystems, (Huntsville, AL). The sequence encoding the receptor was amplified using PCR and the following primers: GIP-1: TTTTTTAAGCTTATGACTACCTCTCCGATCCTGCAGCTGCTGCTGCGGCTCTCACT**;** GIP-2: AAAAAAGCGGCCGCCTAGCAGTAACTTTCCAACTCCCGGCTGGCCTCATT. This resulting sequence was cloned into pcDNA3 using the HindIII and NotI restriction sites.

c. GPCR activation assays. Expression and activation of class B GPCRs was performed in Chinese Hamster Ovary (CHO-K1) cells cultured in F-12K media (Mediatech, Inc., Manassas, VA) supplemented with 100 U penicillin and 100 µg streptomycin (P/S) (Gibco/Invitrogen, Carlsbad, CA), as well as 10% fetal bovine serum (Gibco/Invitrogen, Carlsbad, CA). CHO-K1 cells were grown overnight at a density of 10,000 cells/well in 96-well plates (Corning CoStar #3603, Corning Inc., Corning, NY) and co-transfected with DNA encoding the relevant GPCR and the luciferase reporter, pGL4.29 [17, 21]. Briefly, to each well was added 10 μL of a transfection mixture composed of 100 µL GPCR-encoding plasmid (100 ng/µL in water), 100 µL pGL4.29 (100 ng/µL in water), 1000 µL Optimem (Gibco/Invitrogen) and 80 µL of polyethylenimine (PEI) transfection reagent (catalog number 408727, CAS number 9002-98-6, Sigma-Alrich, St. Louis, MO) {Ehrhardt, 2006 #133}. After 24 h the media was refreshed and after 36 h it was exchanged for F-12K media that was not supplemented with fetal bovine serum (Gibco/Invitrogen) (95 µL per well). After 2 h in serum-free media, 5 µL of a 20X concentration of the appropriate ligand (in 6 mM HEPES, pH 7.4) was added to each well. Cells were incubated for 4 h at 37 ˚C and treated with 100 µL of STEADY-GLO assay reagent (Promega Corp., Madison, WI). Luminescence was detected using an Analyst AD (LJL Biosystems, Sunnyvale, CA) in luminescence mode. A titration of exendin-4 was included in each plate as a control, and the results were normalized to the plateau of exendin-4 activation for that day. EC_{50} values wee calculated using the following equation:[15]

$$L = L_I + \frac{(L_A - L_I)}{1 + ([P]/[EC]_{50})^{-n}}$$

where L is the measured luminescence, L_I is the baseline luminescence at concentrations wherein the peptide does not activate the GPCR, L_A is the maximum luminescence from activation, [P] is the peptide concentration, $[EC]_{50}$ is the half maximal activation and n is the Hill coefficient. Data were fit using Kaleidagraph v 3.6 (Synergy Software, Reading, PA).

Supplemental Data

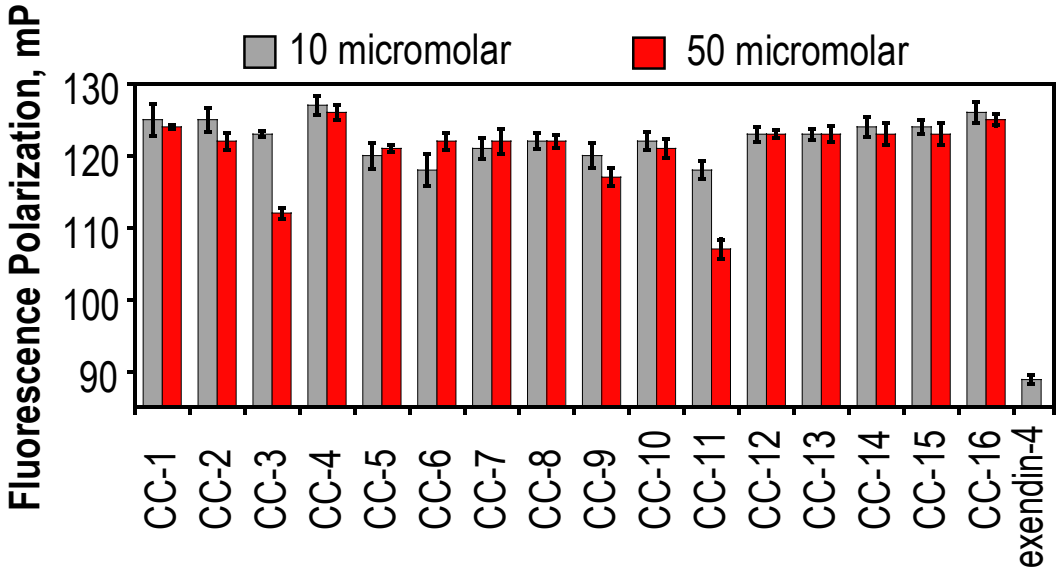


Figure S1. Analysis of *in vitro* nGLP-1R Binding Affinity. Competition between 5 nM Byetta^{Flu} and 10 or 50 μM of the indicated ligand for 125 nM nGLP-1R.

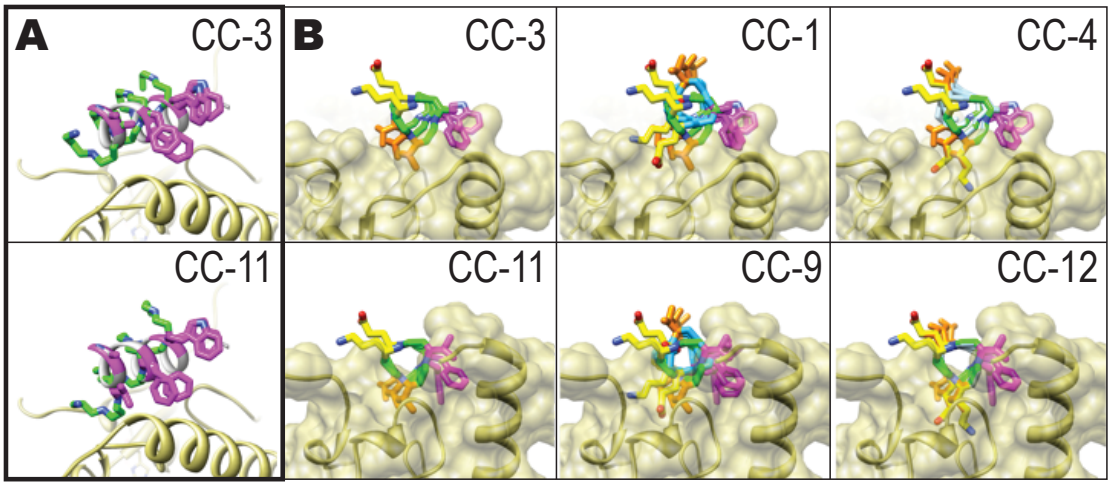


Figure S2. *Post hoc* modeling of potential β-hormones (A) Alignment of CC-3 and CC-11 with Byetta™ (gray ribbon) in the Byetta-nGLP-1R complex. Epitope face side chains are shown in magenta. (B) Modeled complexes of β-peptides that differ from CC-3 or CC-11 in terms of the N-to-C arrangement of the epitope face or stereochemistry (see Figure 1). In the case of CC-3 (AFW) and CC-11 (VFW), this alignment orients the valine face (orange) towards the GLP-1R hydrophobic surface and orients the salt bridge face (yellow) towards solvent. Reversing the C-to-N directional orientation of the AFW/VFW epitope in CC-1 and CC-9 or reversing the relative orientation of the salt bridge and valine faces in CC-4 and CC-12 directs the salt bridge to the hydrophobic groove and the valine face towards the solvent.

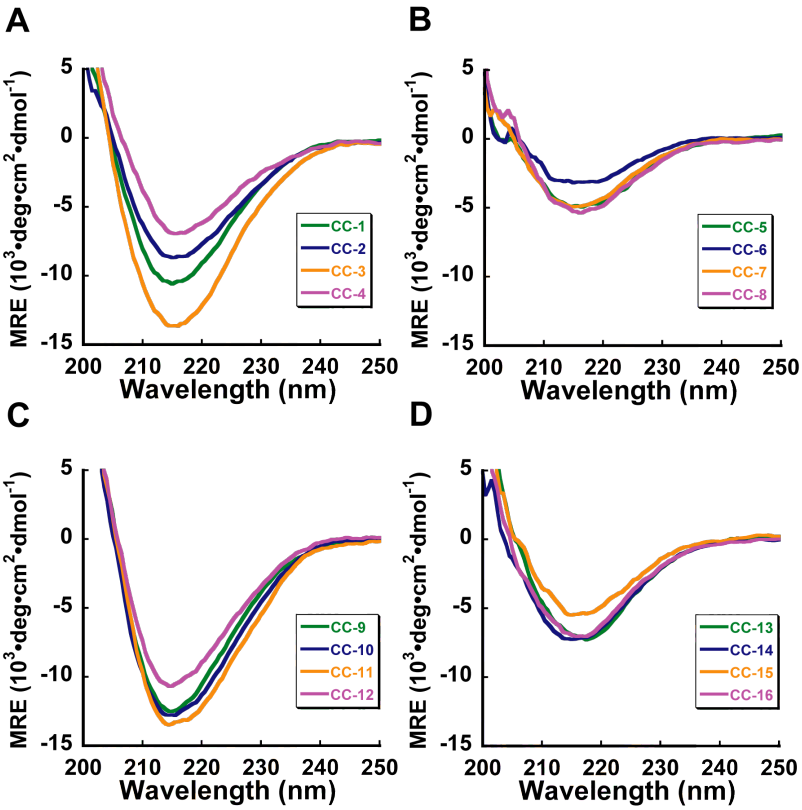


Figure S3. Circular dichroism (CD) spectroscopy was performed using a Jasco J-810 Spectropolarimeter (Jasco, Tokyo, Japan). Circular dichroism spectra of each β-peptide (RT, 25 μM) in 6 mM HEPES buffer (pH 7.4) were collected between 190 and 300 nm as described.[3, 4, 22-27]

Luminescence Reporter Assay Forskolin Control

CHO-K1 cells were transfected as described below with pcDNA3 lacking the gene for GLP-1R or any other class B GPCR. Forskolin (Acros Organics/Fisher Scientific Company, LLC,

Pittsburgh, PA, Cat No. 328240050, CAS No. 66575-29-9) at concentrations between 1 nM and 10 μM was used to stimulate adenylyl cyclase and induce expression of the luciferase reporter (Figure S5A). To evaluate the effect of CC-3^{Act} on this activation pathway, transfected CHO-K1 cells were incubated with 10 μM forskolin in the presence of between 1 nM and 100 µM CC-3$^{Act.}$ The luciferase activity in each sample was normalized to the output of a sample treated only with 10 μM forskolin (Figure S5B).

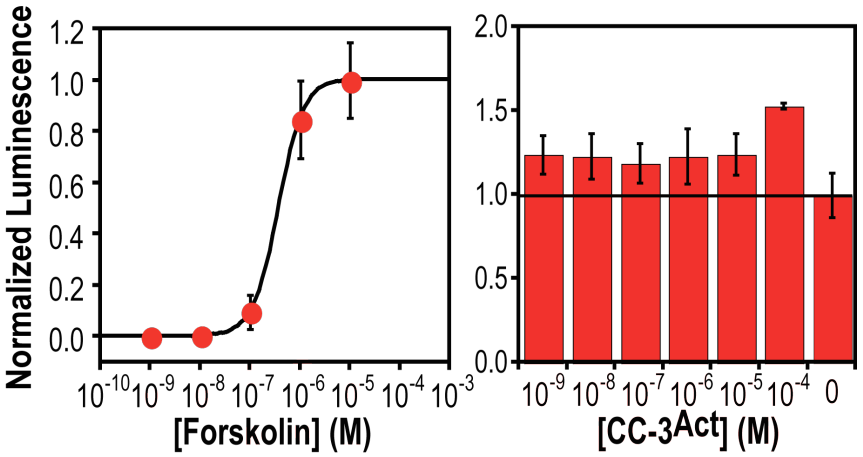


Figure S4. Effect of CC-3^{Act} on forskolin-dependent cAMP activation. (A) Upregulation of cAMP-dependent luciferase activity as a function of [forskolin]. (B) Upregulation of cAMP-dependent luciferase activity in the presence of 10 µM forksolin and the indicated concentration of CC-3^{Act}. The luciferase activity in the absence of CC-3^{Act} is identified by the blue line.

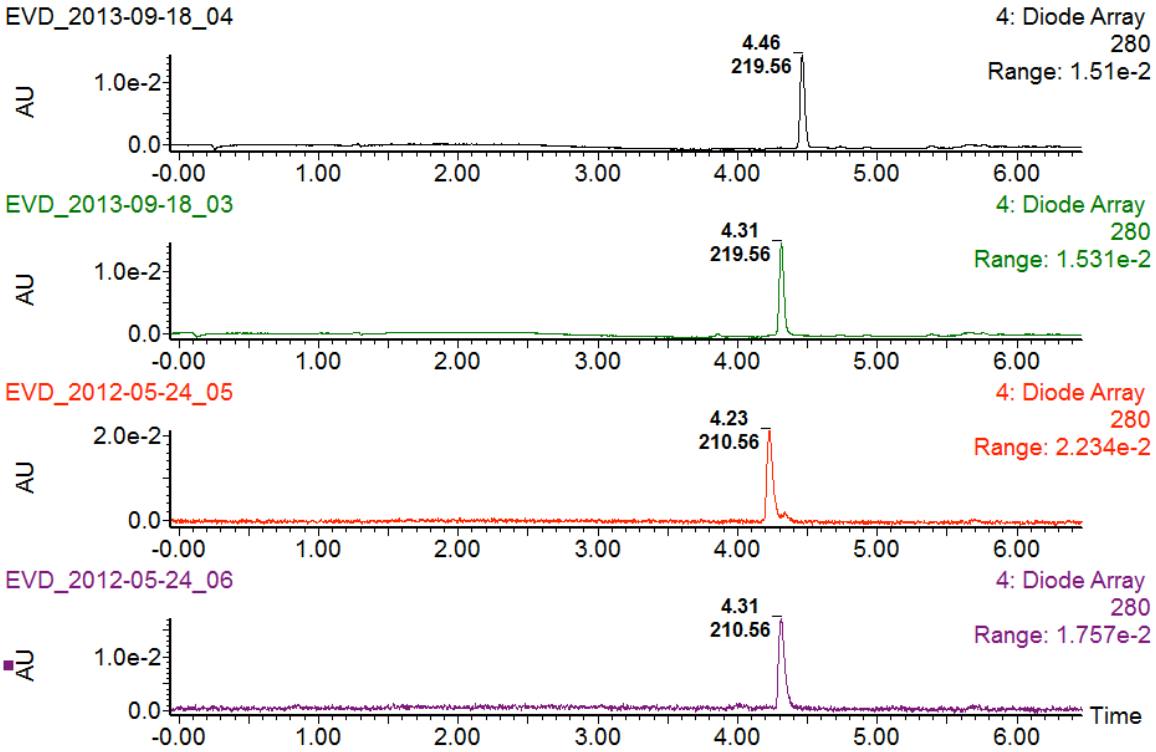


Figure S5: Representative RP-UPLC traces monitored by absorbance at 280 nm. Purity was confirmed using an Acquity BEH300 C18 analytical column (2.1 mm x 100 mm, Part 186003686, 1.7 µm particle size) and a gradient of 5% - 85% acetonitrile/0.1% TFA in water/0.1% TFA over 4.25 min. Peptides shown are (A) CC-11; (B) CC-3; (C) CC-3^{Act}; and (D) CC-11^{Act}.

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