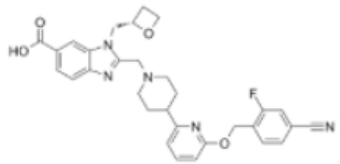
Incretins mimetics, GLP1R agonists : the end of a worldwide metabolic diseases pandemia ?
TUTORIALS

Danuglipron (PF-06882961) is an oral



Molar mass 555.610 g·mol^{-1}

Danuglipron Chemical Structure

CAS No. : 2230198-02-2

Safety, Pharmacokinetics, and Pharmacodynamics of CT-996, an Oral Small-Molecule, Signal-Biased GLP-1 Receptor Agonist Over 4 Weeks in Adults With Obesity

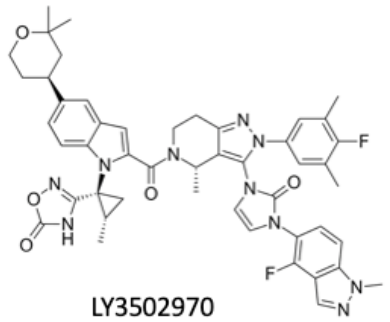
Manu V Chakravarthy,[1] Paul Wabnitz,[2] Jingtao Wu,[1] Stacey Toussaint Touson,[1] Michael A Elliott,[1] Christina C Chang,[3] Jason Lickliter[3]

[1]F. Hoffmann-La Roche Ltd., Basel, Switzerland (previously Carmot Therapeutics Inc.,

File:Orforglipron.svg

File Discussion

File File history File usage on Commons



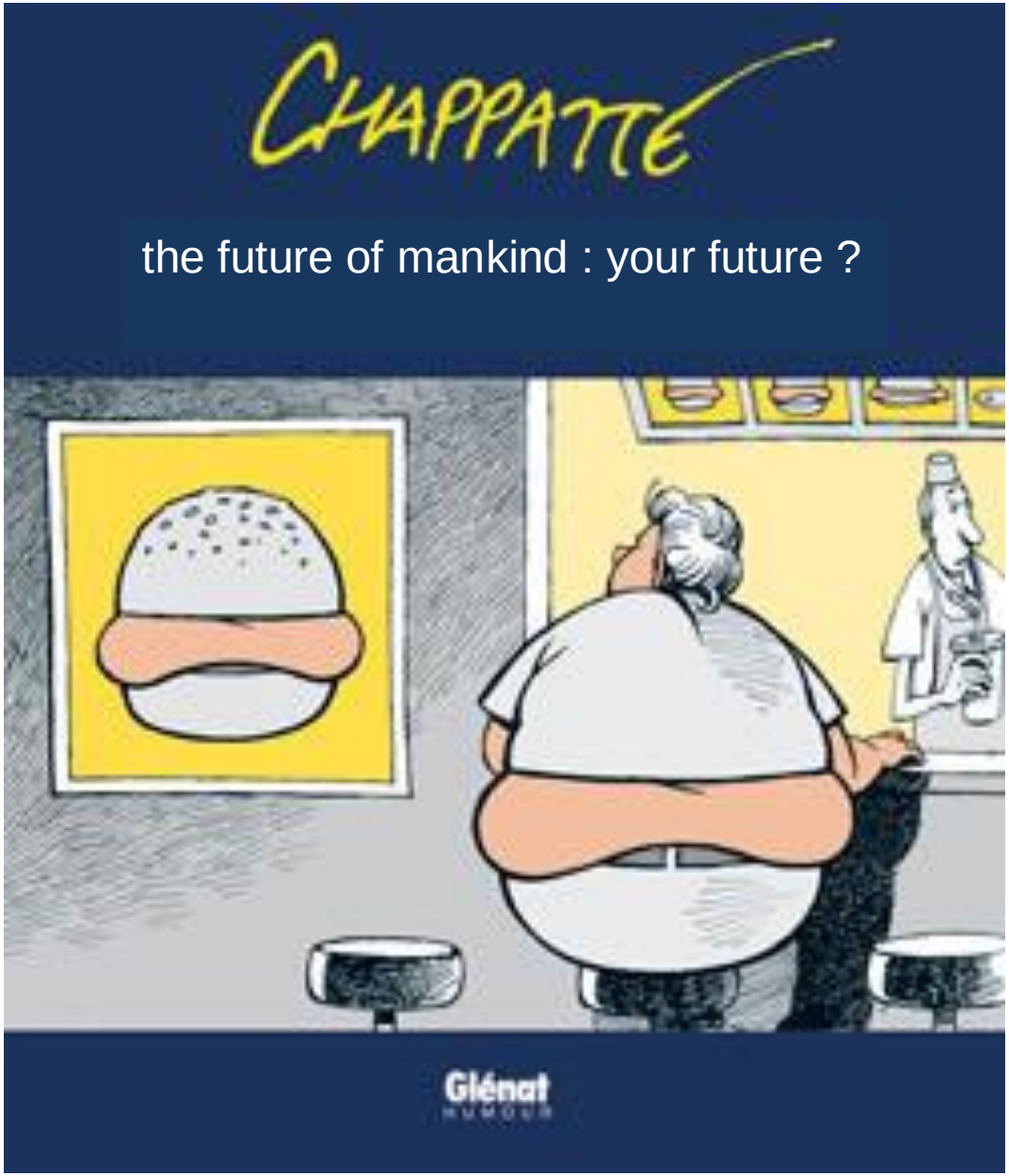
LY3502970
MW 882 Da

EPFL

Sciences de la Vie -SV

Prof Roger G. Clerc

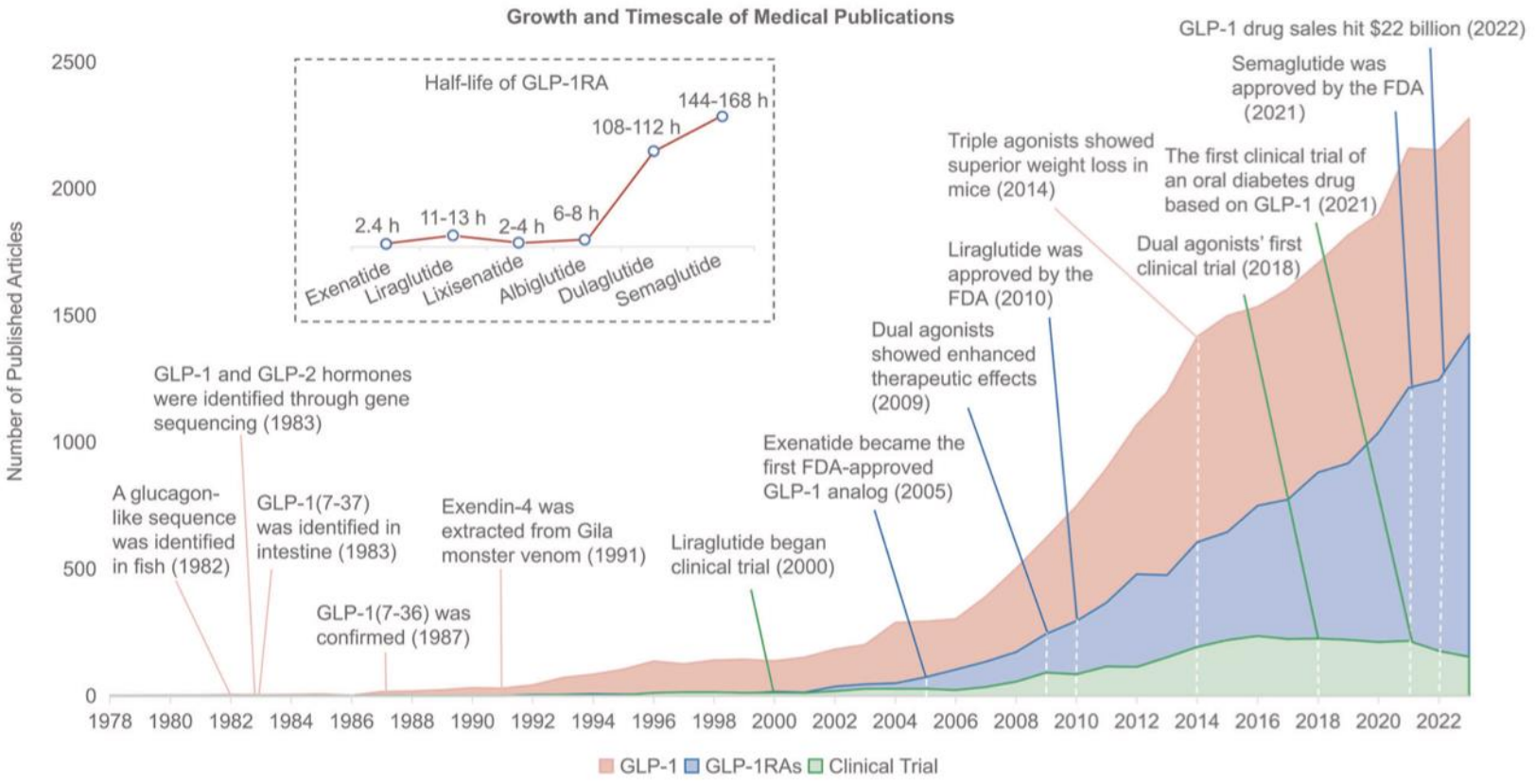
The advent of incretins, GLP1 GLP2 agonists : the end of a worldwide metabolic diseases pandemia ?



Patrick Chappatte

40 years on : GLP-1 research reaches global interest only recently

Glucagon-like peptide-1 receptor: mechanisms and advances in therapy
Zheng et al.



Papers : full length human GLP1 apo-receptor structure

Wu et al. Nat Com. 2020 Mar 9;11(1):1272.

nature COMMUNICATIONS

ARTICLE

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https://doi.org/10.1038/s41467-020-14934-5 OPEN

Full-length human GLP-1 receptor structure without orthosteric ligands

Fan Wu[1,2,3], Linlin Yang[4,15], Kaini Hang[1,2,3,15], Mette Laursen[5,15], Lijie Wu[2], Gye Won Han[6], Qiansheng Ren[7], Nikolaj Kulahin Roed[5], Guangyao Lin[2,3], Michael A. Hanson[8], Hualiang Jiang[9,10,11], Ming-Wei Wang[2,9,12,13], Steffen Reedtz-Runge[5], Gaojie Song[14]✉ & Raymond C. Stevens[1,2,6]✉

Glucagon-like peptide-1 receptor (GLP-1R) is a class B G protein-coupled receptor that plays an important role in glucose homeostasis and treatment of type 2 diabetes. Structures of full-length class B receptors were determined in complex with their orthosteric agonist peptides, however, little is known about their extracellular domain (ECD) conformations in the absence of orthosteric ligands, which has limited our understanding of their activation mechanism. Here, we report the 3.2 Å resolution, peptide-free crystal structure of the full-length human GLP-1R in an inactive state, which reveals a unique closed conformation of the ECD. Disulfide cross-linking validates the physiological relevance of the closed conformation, while electron microscopy (EM) and molecular dynamic (MD) simulations suggest a large degree of conformational dynamics of ECD that is necessary for binding GLP-1. Our inactive structure represents a snapshot of the peptide-free GLP-1R and provides insights into the activation pathway of this receptor family.

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Papers : full length human GLP1 apo-receptor structure

Wu et al. Nat Com. 2020 Mar 9;11(1):1272.

How to proceed to get an Xray apo GLP1 receptor structure at Angström level ?

Ectopic expression of GLP1 r in Sf9 Drosophila cells via a baculovirus vector with His tagging for complex, Flag-tag and a TEV tobacco etch site protease site.
As to increase stability of GLP+r rubretoxin fusion protein is inserted into ICL Intracellular loop 2.

Ectopic expression of Fab7F38 in chinese hamster ovary cells CHO with heavy and light chain expression vectors transfected with lipofectamine.
Affinity purification on Sepharose G column elution at low pH with quick neutralization 1M Tris pH8, pooled fractions stored at -80oC.

Purification of GLP1r – Fab7F38 complex by lysing 1L biomass expressing GLP1r low salt buffer and protease free buffer. Membranes were collected, washed. Protein sample extracted from membranes by non ionic detergent DDM,etc, washed,centriguged. Sup was incubated with TALON resin, then washed, and incubated with Fab7F38 ectopically expresed (see above) at 1:1.5 molar ratio. Unbound Fab was washed. Resuspended resin was added TEV protease to remove Nterminal tag. The elution profile containing GLP1r Fab7F38 complex was submitted for crystalization trials.

Cristalization trials was carried out with lipidic cubic phase (LCP) mixture dispense in 96 well plates in 50 nl drops and overlaid with precipitant solution NT8. Crystals appeared in amonium formate, PEG400 and guanidine hydrochloride after 7 days and reached 150µ size in 1 month. Negative stain for Gs nanobody. Xray diffraction data collected at Spring8 beam Hyogo Japan.

Papers : full length human GLP1 apo-receptor structure

Wu et al. Nat Com. 2020 Mar 9;11(1):1272.

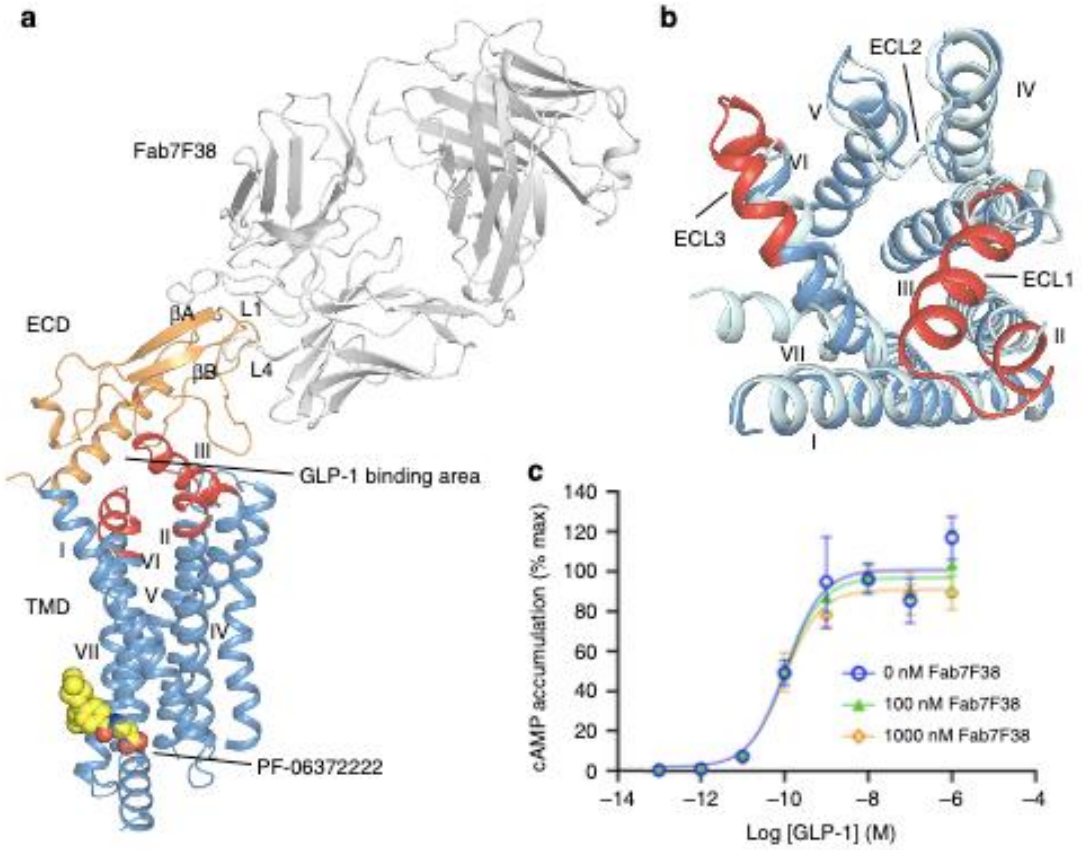


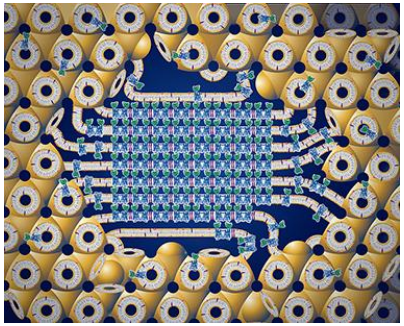
Fig. 1 Overall structure of the GLP-1R-Fab7F38 complex. a Cartoon representation of GLP-1R-Fab7F38 complex. Fab, ECD (residues R24-E127) and TMD

Papers : full length human GLP1 apo-receptor structure

Wu et al. Nat Com. 2020 Mar 9;11(1):1272.

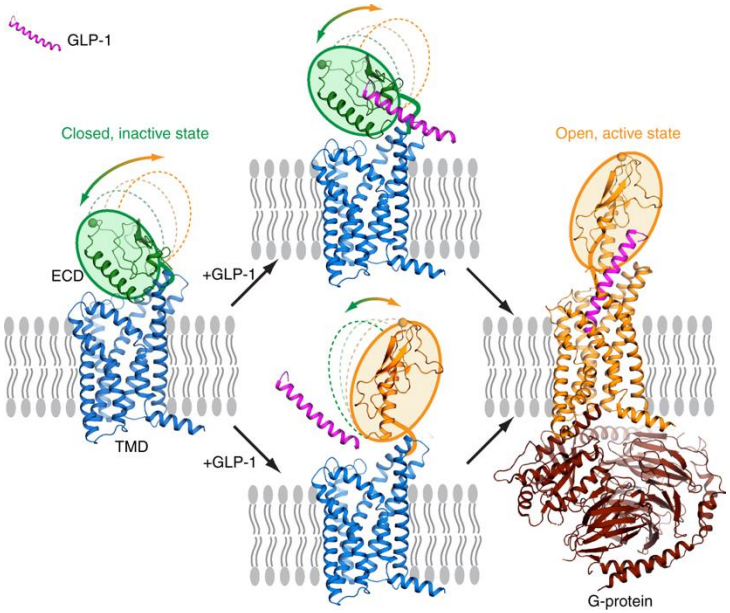
To visualize
You can try **Alphafold** multimer with sequence of GLP1R and GLP1 peptide
Or with **PyMol**
peptide-free GLP-1R–Fab7F38 structure (PDB: **6LN2)**
active-like peptide 5-bound GLP-1R structure (PDB: **5NX2**)
active GLP-1-bound GLP-1R–Gs complex structure (PDB: **5VAI**)

Cherezov lab
https://cherezov.usc.edu/resources.htm



To align two PDB structures in **PyMOL**, follow these steps:
fetch 6LN2, peptidefree
fetch 5NX2, activelike
fetch 5VAI, activebound

Use the align command to perform the alignment:
super peptidefree, activelike
super activebound, activelike
center
color blue, peptidefree
color yellow, activelike
color orange, activebound
select GLP1peptide, activebound and chain P
color pink, GLP1peptide



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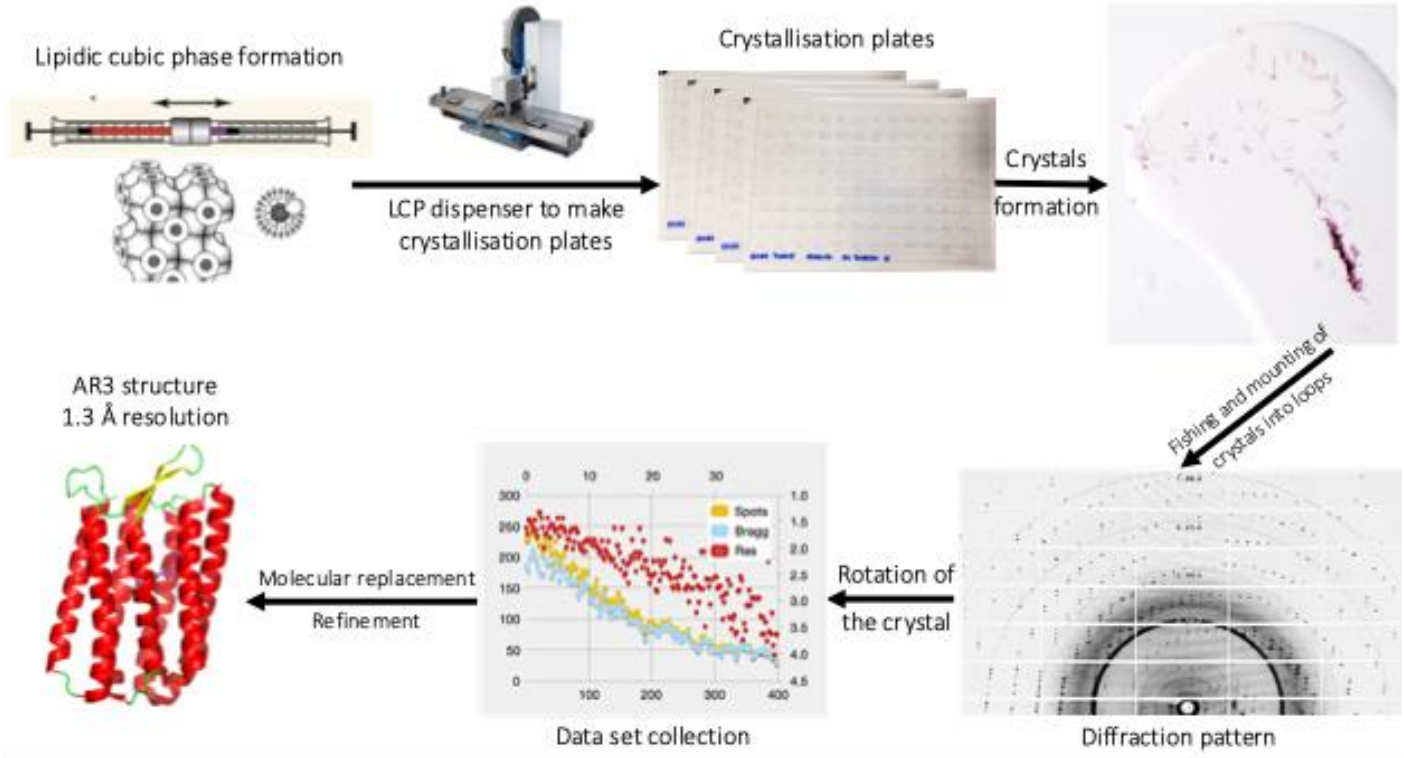

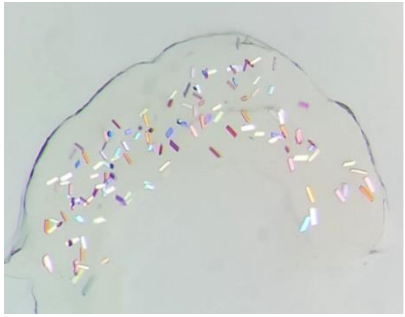

https://phys.org/news/2021-01-high-resolution-archaerhodopsin-protein-receptor-desensitization.html

Figure 4.15: Detergent-free crystallization of AR3. LCP is formed by mixing the claret membrane with the monoolein. Crystallization plates are produced and in some of them some crystals appeared. The crystals are mounted onto crystallization loops for diffraction. Several rounds of optimization were performed until obtaining high-resolution data on different data set. Molecular replacement and refinement were performed to finally obtain the 1.3 Å resolution crystal structure. Picture from syringes and cubic phase adapted from [393, 409].

Papers : GLP1 agonist tomorrow : SMW cpds – work in progress !

developing nonpeptidic small-molecule drugs targeting GLP-1R remains elusive.

LETTER

doi:10.1038/nature22378

Human GLP-1 receptor transmembrane domain structure in complex with allosteric modulators

Gaofie Song[1], Dehua Yang[2]*, Yuxia Wang[1]*, Chris de Graaf[3]*, Qingtong Zhou[1], Shanshan Jiang[4], Kaiwen Liu[1,5,6], Xiaoqing Cai[2], Antao Dai[2], Guangyao Lin[5], Dongsheng Liu[1], Fan Wu[1,5,6], Yiran Wu[1], Suwen Zhao[1,8], Li Ye[4], Gye Won Han[7], Jesper Lau[8], Beili Wu[5,6,9], Michael A. Hanson[10], Zhi Jie Liu[1,8,11], Ming Wei Wang[2,4,5] & Raymond C. Stevens[1,5]

The glucagon-like peptide-1 receptor (GLP-1R) and the glucagon receptor (GCGR) are members of the secretin-like class B family of G-protein-coupled receptors (GPCRs) and have opposing physiological roles in insulin release and glucose homeostasis[1]. The treatment of type 2 diabetes requires positive modulation of GLP-1R to inhibit glucagon secretion and stimulate insulin secretion in a glucose-dependent manner[2]. Here we report crystal structures of the human GLP-1R transmembrane domain in complex with two different negative allosteric modulators, PF-06372222 and NNC0640, at 2.7 and 3.0 Å resolution, respectively. The structures reveal a common binding pocket for negative allosteric modulators, present in both GLP-1R and GCGR[3] and located outside helices V–VII near the intracellular half of the receptor. The receptor is in an inactive conformation with compounds that restrict movement of the intracellular tip of helix VI, a movement that is generally associated with activation mechanisms in class A GPCRs[4–6]. Molecular modelling and mutagenesis studies indicate that agonist positive allosteric modulators target the same general region, but in a distinct sub-pocket at the interface between helices V and VI, which may facilitate the formation of an intracellular binding site that enhances G-protein coupling.

Glucagon-like peptide-1 (GLP-1) is one of the key incretin hormones secreted in response to food intake and gastric motility, and is responsible for glucose homeostasis via the stimulation of insulin secretion through activation of GLP-1R[1]. Peptide analogues of GLP-1 have been successfully developed for the treatment of type 2 diabetes[7], but the development of therapeutically viable non-peptidic GLP-1R agonists has been unsuccessful. Agonist positive allosteric modulators (PAMs) have been identified and used to investigate ligand-directed biased cellular signalling of GLP-1R[7–9]. Previous studies have provided evidence for a two-domain binding mechanism of GLP-1 with its cognate receptor[1,10–13]. Structures of the transmembrane domain (TMD) of the class B GPCRs corticotropin-releasing factor receptor 1 (CRF_1R)[14] and GCGR[3,15] have been reported. Despite these recent advances in structural characterization, little is known about the molecular mechanisms of positive and negative allosteric modulation of GLP-1R and GCGR. To provide a foundation for the discovery of therapeutic agents that allosterically target the GLP-1R and GCGR signalling pathways, we have solved structures of the human GLP-1R TMD in complex with two negative allosteric modulators (NAMs), and complemented these structures with mutagenesis and modelling studies to map the binding site and further our understanding of the activation mechanism for agonist PAMs of GLP-1R.

To facilitate crystallization of the GLP-1R TMD, we generated a thermostabilized construct with 10 mutations, including a disulfide bond ($I317^{5.67b}C$–$G361^{6.50b}C$) (numbers in superscript refer to the Wootten numbering system for class B GPCRs[16]) that links the middle regions of helices V and VI and a GCGR mimicking mutation $C347^{6.36b}F$ in the allosteric modulator binding pocket that stabilizes the interaction interface for NAMs (Methods and Extended Data Fig. 1). These NAMs were previously optimized for GCGR antagonism, but certain analogues were found to antagonize GLP-1R as well. The final modified construct yielded crystals that diffracted to 2.7 Å for PF-06372222 and 3.0 Å for NNC0640 (Fig. 1a, c and Extended Data Table 1).

The TMD architecture of GLP-1R is similar to that of GCGR, consistent with the similarity in their primary sequences (45% identical in the TMDs; Fig. 1b). GLP-1R preserves the conserved and functionally important[17] disulfide bond $C226^{1.29b}$–$C296^{ECL2}$, and contains most of the interhelical hydrogen bonds present in other class B structures[3,14,15] (Extended Data Fig. 2). Helix I of GLP-1R is 2.5 helical-turns shorter than the long stalk region found in the initial structure of the GCGR TMD (PDB code 4L6R)[15] (Fig. 1b). In an accompanying paper[18], the full-length structure of GCGR reveals a rearrangement of the stalk region with the N-terminal helix unwinding to form an extended β-sheet with two strands from the first extracellular loop (ECL1), suggesting a degree of flexibility in this region that may be associated with the functional mechanisms of class B GPCRs.

The NAMs PF-06372222 and NNC0640 bind in a similar pocket outside of helices V–VII as MK-0893 in the crystal structure of the thermostabilized GCGR TMD (PDB code 5EE7)[3] (Fig. 2a–c). The anionic carboxylic acid (PF-06372222) and tetrazole (NNC0640) moieties of the NAMs target a polar cleft between helices VI and VII, and form hydrogen-bond interactions with $S352^{6.41b}$ and $N406^{7.61b}$ in GLP-1R. MK-0893 forms similar interactions with homologous residues in GCGR, and makes an additional hydrogen bond to the side chain of $R346^{6.37b}$ in GCGR. PF-06372222 and NNC0640 each form a hydrogen bond with $T355^{6.44b}$ in GLP-1R, directing their hydrophobic dimethyl cyclobutane (PF-06372222) and cyclohexyl (NNC0640) moieties parallel to helix VI and the lipid bilayer. By contrast, MK-0893 does not hydrogen bond with the homologous $T353^{6.44b}$ in GCGR, and its dichlorophenyl moiety is directed perpendicular to helix VI and the lipid bilayer. The trifluoromethyl-pyrazole group of PF-06372222 binds a hydrophobic surface area of 43 $Å^2$ consisting of $I328^{5.58b}$, $V331^{5.61b}$, $V332^{5.62b}$ and $L335^{5.65b}$ of helix V (Fig. 2a), which is also targeted by the methylsulfone-phenyl group of NNC0640 in GLP-1R (33 $Å^2$ buried surface area; Fig. 2b), but only partially interacts with MK-0893 (9 $Å^2$; Fig. 2c)[3] and NNC0640 (4 $Å^2$)[18] in GCGR (Fig. 2e).

The structures demonstrate that these NAMs can accommodate different binding modes, including the variation in hydrophobic

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Article

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Structural Modeling and in Silico Screening of Potential Small-Molecule Allosteric Agonists of a Glucagon-like Peptide 1 Receptor

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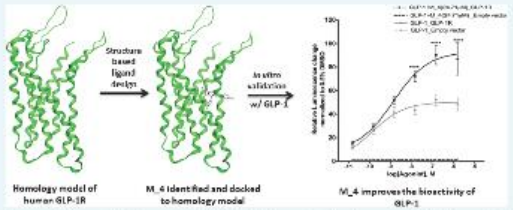
Supporting Information



ABSTRACT: The glucagon-like peptide 1 receptor (GLP-1R) belongs to the pharmaceutically important class B family of G-protein-coupled receptors (GPCRs), and its incretin peptide ligand GLP-1 analogs are adopted drugs for the treatment of type 2 diabetes. Despite remarkable antidiabetic effects, GLP-1 peptide-based drugs are limited by the need of injection. On the other hand, developing nonpeptidic small-molecule drugs targeting GLP-1R remains elusive. Here, we first constructed a three-dimensional structure model of the transmembrane (TM) domain of human GLP-1R using homology modeling and conformational sampling techniques. Next, a potential allosteric binding site on the TM domain was predicted computationally. In silico screening of druglike compounds against this predicted allosteric site has identified nine compounds as potential GLP-1R agonists. The independent agonistic activity of two compounds was subsequently confirmed using a cAMP response element-based luciferase reporting system. One compound was also shown to stimulate insulin secretion through in vitro assay. In addition, this compound synergized with GLP-1 to activate human GLP-1R. These results demonstrated that allosteric regulation potentially exists in GLP-1R and can be exploited for developing small-molecule agonists. The success of this work will help pave the way for small-molecule drug discovery targeting other class B GPCRs through allosteric regulations.

INTRODUCTION

In the past 40 years, the number of overweight people has increased sixfold worldwide with 33.6% of US men and women being obese, and this disturbing trend is projected to continue in the foreseeable future.[1] Obesity can cause a number of health problems including cardiovascular diseases and diabetes, with type 2 diabetes representing 90−95% of diabetes patients. The glucagon-like peptide 1 receptor (GLP-1R), a member of class B family of G-protein coupled receptors (GPCRs), is an effective target for the treatment of type 2 diabetes,[2] and its incretin peptide and varied peptide mimetics are adopted drugs.[3]

Despite remarkable antidiabetic effects, GLP-1 peptide-based agonists have several shortcomings.[4,5] They are available only in a format for injection, lacking effective long-term glucose control capability, and they can cause side effects and result in low quality of life in some patients. Hence, there is significant interest in the development of nonpeptidic small-molecule agonists of GLP-1R with enhanced bioavailability.[6−11] However, such a strategy remains problematic because of the nature of the orthosteric binding site for GLP-1 (Figure 1A), which is large and relatively shallow.[12] Up to now, no small-molecule drugs acting as GLP-1R agonists are available in the market. Therefore, novel approaches in developing small-molecule drugs targeting GLP-1R are very desirable for the treatment of type 2 diabetes.

Given the allosteric nature of GPCRs, targeting the allosteric sites on GPCRs for small-molecule therapeutic intervention represents an alternative and promising approach for drug discovery.[13−16] Cinacalcet, a positive allosteric modulator of

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Papers : GLP1 agonist tomorrow : SMW cpds – work in progress !

developing nonpeptidic small-molecule drugs targeting GLP-1R remains elusive.

Structural Modeling and in Silico Screening of Potential Small-Molecule Allosteric Agonists of a Glucagon-like Peptide 1 Receptor

Tejashree Redij,[†,⊥] Rajan Chaudhari,[‡,⊥,#] Zhiyu Li,[§] Xianxin Hua,[∥] and Zhijun Li*[,†,‡]

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[∥]Department of Cancer Biology, Diabetes Research Center (DRC), University of Pennsylvania, Philadelphia, Pennsylvania 19104, United States

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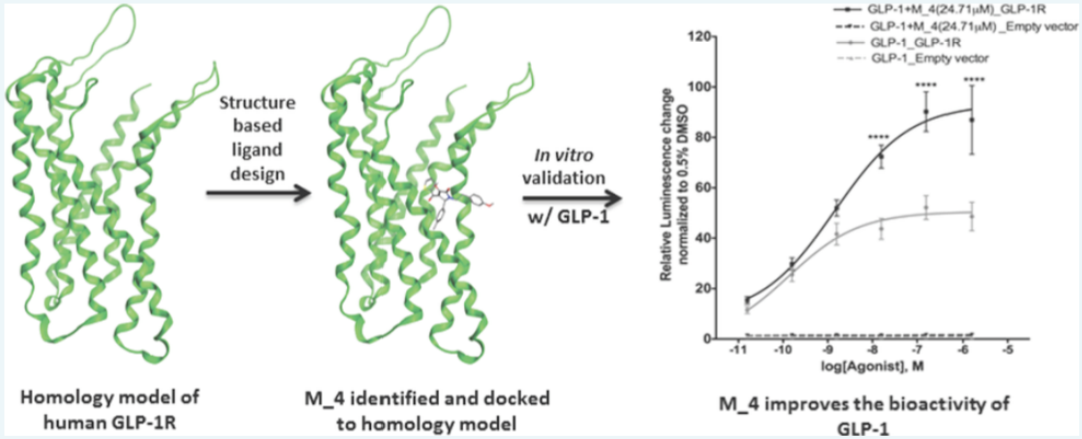


The advent of the glucagon peptide superfamily - 1990s

THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 265, No. 33, Issue of November 25, pp. 20259–20262, 1990
Printed in U.S.A.

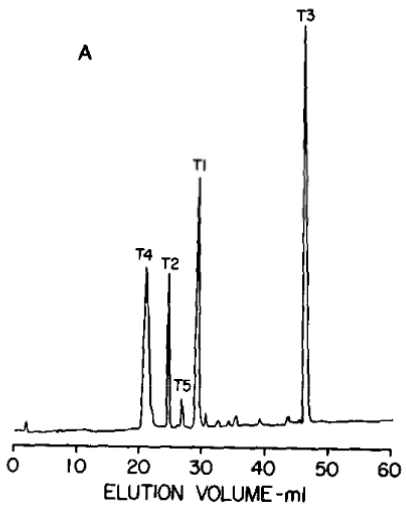
Purification and Structure of Exendin-3, a New Pancreatic Secretagogue Isolated from *Heloderma horridum* Venom*

(Received for publication, April 10, 1990)

John Eng‡§¶, P. C. Andrews‖, Wayne A. Kleinman‡, Latika Singh, and Jean-Pierre Raufman****

*From the ‡Solomon A. Berson Research Laboratory, Veterans Affairs Medical Center, Bronx, New York 10468, the §Department of Medicine, Mount Sinai School of Medicine, New York, New York 10029, the ‖Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan 48109, and the **Department of Medicine, State University of New York-Health Science Center, Brooklyn, New York 11203*

An amino-terminal histidyl structure (His[1]) is characteristic of most peptides in the glucagon superfamily. minal sequencing was used to screen for new His[1] peptides that might be members of the glucagon superfamily. Gila



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100	EXENDIN-3	H S D G T F T S D L S K Q M E E E A V R L F I E W L K N G G P S S G A P P P S #
32	HELOSPECTIN	H S D A T F T A E Y S K L L A K L A L Q K Y L E S I L G S S T S P R P P S S
26	HELODERMIN	H S D A I F T E E Y S K L L A K L A L Q K Y L A S I L G S R T S P P P #
41	SECRETIN	H S D G T F T S E L S R L R D S A R L Q R L L Q G L V #
48	GLUCAGON	H S Q G T F T S D Y S K Y L D S R R A Q D F V Q W L M N T
50	GLP-1	H A E G T F T S D V S S Y L E G Q A A K E F I A W L V K G R #
29	GLP-2	H A D G S F S D E M N T I L D N L A A R D F I N W L I Q T K I T D R
37	PHI	H A D G V F T S D F S R L L G Q L S A K K Y L E S L I #
29	VIP	H S D A V F T D N Y T R L R K Q M A V K K Y L N S I L N #
26	GIP	Y A E G T F I S D Y S I A M D K I R Q Q D F V N W L L A Q K G K K S D W K H N I T Q
13	GRF	Y A D A I F T N S Y R K V L G Q L S A R K L L Q D I M S R Q Q G E S N Q E R G A R A R L #
24	PACAP38	H S D G I F T D S Y S R Y R K Q M A V K K Y L A A V L G K R Y K Q R V K N K #

Papers : the advent of the glucagon peptide superfamily - 1990s

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An amino-terminal histidyl structure (His[1]) is characteristic of most peptides in the glucagon superfamily. An assay for His[1] peptides performed by amino-terminal amino acid sequencing was used to screen venom from the Gila monster lizard, *Heloderma horridum*. Two His[1] peptides were identified: helospectin and a new His[1] peptide that has been named exendin-3 to indicate that it is the third peptide to be found in an exocrine secretion of *Heloderma* lizards which has endocrine activity, the first two being helospectin (exendin-1) and helodermin (exendin-2). In the lot of *H. horridum* venom tested, exendin-3 was 5–10-fold more abundant in molar concentration than helospectin. The structure of exendin-3 was analyzed by amino acid sequencing and mass spectrometry. Exendin-3 is a 39-amino acid peptide with a mass of 4200. It contains a carboxyl-terminal amide and has a strong homology with secretin at its amino-terminal 12 amino acids. The complete structure of exendin-3 is His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser-amide. It is 32 and 26% homologous with helospectin and helodermin, respectively. It has greatest homology with glucagon (48%) and human glucagon-like peptide-1 (50%). Exendin-3 (3 μM) stimulated increases in cellular cAMP and amylase release from dispersed guinea pig pancreatic acini.

The glucagon superfamily consists of a diverse group of biologically active peptides that are structurally related by having an amino-terminal histidine residue (His[1]) and a phenylalanine residue at position 6 (Phe[6]) (1) or one of several variant structures such as Tyr[1]-Phe[6] (2, 3), His[1]-Tyr[6] (4), or His[1]-Leu[6] (5). An assay for His[1] can potentially be used to identify peptides belonging to this family. Although special chemical methods have been reported for the detection of His[1] peptides (6, 7), a more general method of assaying for His[1] is amino-terminal amino acid sequencing. This assay was used recently to monitor the purification of glucagon from chinchilla pancreas (8). To test the hypothesis that His[1] is a chemical marker for biologically active peptides, amino-terminal sequencing was used to screen for new His[1] peptides that might be members of the glucagon superfamily. Gila monster venom was chosen for screening because venoms from *Heloderma suspectum* and *Heloderma horridum* had been shown previously to contain two biologically active His[1]-Phe[6] peptides, helospectin (9) and helodermin (10). When *H. horridum* venom was examined with the His[1] assay, a previously unrecognized His[1]-Phe[6] peptide was detected. In this study, we report the purification and structural characterization of this new peptide. It is named exendin-3 to identify it as the third peptide found in an exocrine secretion of *Heloderma* lizards which has endocrine activity. Like the earlier exendins, it is a pancreatic secretagogue.

MATERIALS AND METHODS

H. horridum venom (lot 74F-0240), diphenylcarbamyl chloride-treated trypsin, carboxypeptidase Y, and carboxypeptidase P were purchased from Sigma. Endoproteinase Glu-C (V8 protease) was purchased from Boehringer Mannheim.

His[1] Assay and Amino Acid Sequencing—Amino-terminal amino acid analysis was performed by a single cycle of Edman degradation using an automated gas-phase protein sequenator in combination with an on-line PTH[1]-derivative analyzer (Applied Biosystems). The molarity of the analyzer's weak solvent was adjusted to position PTH-His to elute between PTH-Ala and PTH-dehydroSer. Purified peptides and peptide fragments were sequenced with the gas-phase sequenator.

Isolation of His[1] Peptides from Heloderma Venom—Venom (25 mg) was dissolved in water to a concentration of 10 mg/ml and passed through a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA). The C_{18} cartridge was washed with 5 ml of water and eluted with 2 ml of 0.1% trifluoroacetic acid, 60% acetonitrile. Peptides in the eluate were separated by HPLC on an 8-mm × 10-cm MB C_{18} Radial-Pak column (Waters Associates). The column was eluted with a linear gradient from 20 to 60% acetonitrile in 0.13% heptafluorobutyric acid at a flow rate of 2 ml/min. One-minute fractions were collected. Aliquots from each fraction were assayed for His[1] content.

Enzyme Cleavages—Purified exendin-3 (10 nmol) was dried and digested separately with 0.2 μg of trypsin or 2 μg of V8 protease in 0.1 ml of 0.1 M ammonium bicarbonate overnight at room temperature. Peptide fragments were purified by HPLC on a Nova C_{18} Radial-Pak column (Waters Associates). The elutions were performed with a linear gradient from 0 to 40% acetonitrile in 0.1% trifluoroacetic acid at a flow rate of 1 ml/min.

Exendin-3 (1 nmol) was incubated with carboxypeptidase Y or carboxypeptidase P (1 μg each) in 0.05 ml of 0.05 M sodium acetate, pH 5.5, at room temperature for timed intervals up to 6 h. Aliquots from the mixtures were analyzed for release of free amino acids.

Amino Acid Analysis—Peptides were dried and hydrolyzed with gas-phase 6 M HCl at 150 °C for 60 min. Amino acids were analyzed with an automated amino acid derivatizer (Applied Biosystems) connected to an on-line phenylthiocarbamyl-derivative analyzer.

* This work was supported in part by the Department of Veterans Affairs. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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[1] The abbreviations used are: PTH, phenylthiohydantoin; HPLC, high pressure liquid chromatography; FAB-MS, fast atom bombardment-mass spectrometry; VIP, vasoactive intestinal peptide.

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Isolation and Characterization of Exendin-4, an Exendin-3 Analogue, from *Heloderma suspectum* Venom

FURTHER EVIDENCE FOR AN EXENDIN RECEPTOR ON DISPERSED ACINI FROM GUINEA PIG PANCREAS*

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The recent identification in *Heloderma horridum* venom of exendin-3, a new member of the glucagon superfamily that acts as a pancreatic secretagogue, prompted a search for a similar peptide in *Heloderma suspectum* venom. An amino acid sequencing assay for peptides containing an amino-terminal histidine residue (His[1]) was used to isolate a 39-amino acid peptide, exendin-4, from *H. suspectum* venom. Exendin-4 differs from exendin-3 by two amino acid substitutions, Gly[2]-Glu[5] in place of Ser[2]-Asp[5], but is otherwise identical. The structural differences make exendin-4 distinct from exendin-3 in its bioactivity. In dispersed acini from guinea pig pancreas, natural and synthetic exendin-4 stimulate a monophasic increase in cAMP beginning at 100 pM that plateaus at 10 nM. The exendin-4-induced increase in cAMP is inhibited progressively by increasing concentrations of the exendin receptor antagonist, exendin-(9–39) amide. Unlike exendin-3, exendin-4 does not stimulate a second rise in acinar cAMP at concentrations >100 nM, does not stimulate amylase release, and does not inhibit the binding of radiolabeled vasoactive intestinal peptide to acini. This indicates that in dispersed pancreatic acini, exendin-4 interacts only with the recently described exendin receptor.

An assay for His[1] peptides was recently used to identify the presence of helospectin and a new, previously unrecognized His[1]-Phe[6] peptide in *Heloderma horridum* venom (1). This new peptide, designated exendin-3, is a pancreatic secretagogue. At concentrations greater than 100 nM, exendin-3 interacts with VIP[1] receptors on guinea pig pancreatic acini to stimulate an increase in cellular cAMP and amylase release (2). At lower concentrations (0.1–10 nM), however, exendin-3 interacts with a putative exendin receptor that causes an increase in acinar cAMP but not amylase release. This conclusion is based on the observation that increasing concentrations of a specific antagonist, exendin-3-(9–39) amide, progressively inhibit exendin-3-induced increases in cAMP (2). Because venom from a closely related lizard, *Heloderma suspectum*, has been shown to contain helodermin (3), a peptide closely related in structure to helospectin (4), a search was undertaken for a His[1] peptide in *H. suspectum* venom that might be analogous to exendin-3. We report the isolation from *H. suspectum* venom of such an analogue that has been named exendin-4. Exendin-4, unlike exendin-3, is not a pancreatic secretagogue. Instead, it interacts exclusively with the newly described exendin receptor (2) to increase pancreatic acinar cAMP.

MATERIALS AND METHODS

H. suspectum venom (lots HS198Z and HS208Z) was purchased from Miami Serpentarium Laboratories (Salt Lake City, UT). Diphenylcarbamyl chloride-treated trypsin was purchased from Sigma. Endoproteinase Asp-N was purchased from Boehringer Mannheim.

His[1] Assay and Amino Acid Sequencing—Amino-terminal amino acid analysis was performed by a single cycle of Edman degradation using an automated gas phase protein sequenator in combination with an on-line PTH-amino acid analyzer (Applied Biosystems, Foster City, CA). PTH-His was positioned to elute between PTH-Ala and PTH-dehydro-Ser. Purified peptides and peptide fragments were sequenced with the gas phase sequenator.

Isolation of His[1] Peptides from Heloderma Venom—Venom (25 mg) was dissolved in distilled water (10 mg/ml) and passed through a C_{18} Sep-Pak cartridge (Waters Associates, Milford, MA). The C_{18} cartridge was washed with 5 ml of water and eluted with 2 ml of 0.1% trifluoroacetic acid, 60% acetonitrile. Peptides in the eluate were separated by HPLC on an 8 mm × 10-cm μBondapak C_{18} Radial-Pak column (Waters Associates). The column was eluted with a linear gradient (20–60%) of acetonitrile in 0.13% heptafluorobutyric acid at a flow rate of 1 ml/min. One-minute fractions were collected, and aliquots were assayed for His[1] content.

Enzyme Cleavages—Purified exendin-4 (5–20 nmol) was digested with 0.2 μg of trypsin or with 0.2 μg of endoproteinase Asp-N. The peptide fragment exendin-(9–39) amide was prepared as described previously (2). Although this fragment was previously referred to as exendin-3-(9–39) amide (2), the name has been shortened to exendin-(9–39) amide to indicate that the carboxyl-terminal 31 amino acids of exendin-3 and exendin-4 are identical. Peptide fragments were purified by HPLC on a Nova C_{18} Radial-Pak column (Waters Associates).

Amino Acid Analysis—Peptides were dried and hydrolyzed with gas phase 6 M HCl at 150 °C for 60 min. Amino acids were analyzed with an automated amino acid derivatizer (Applied Biosystems) connected to an on-line phenylthiocarbamyl-derivative amino acid analyzer.

Mass Spectrometry—The mass of the COOH-terminal fragment generated by trypsin digestion of exendin-4 was determined by fast atom bombardment-mass spectrometry. Mass accuracy of greater than ±0.1 unit was achieved by peak matching to appropriate cesium chloride cluster ions. Fast atom bombardment-mass spectrometry was performed by the Laboratory for Macromolecular Analysis at the

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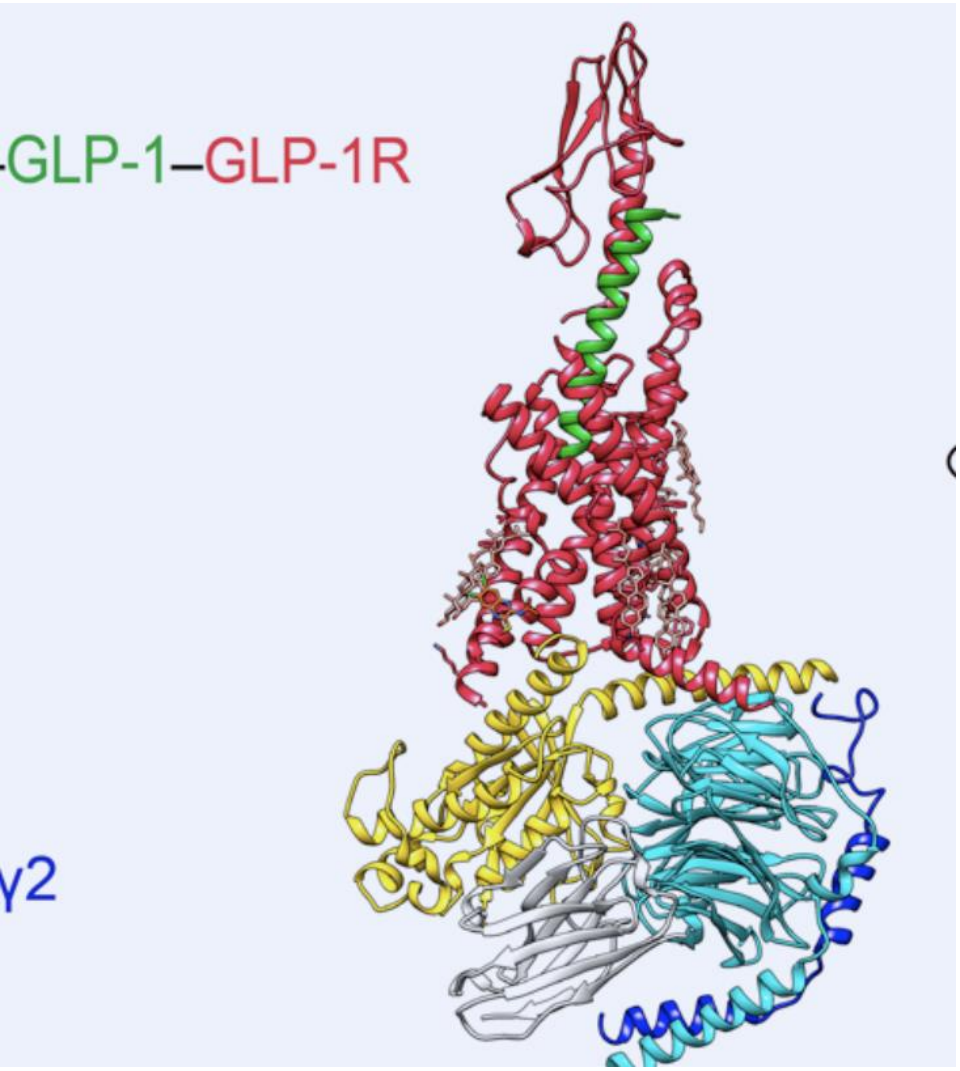
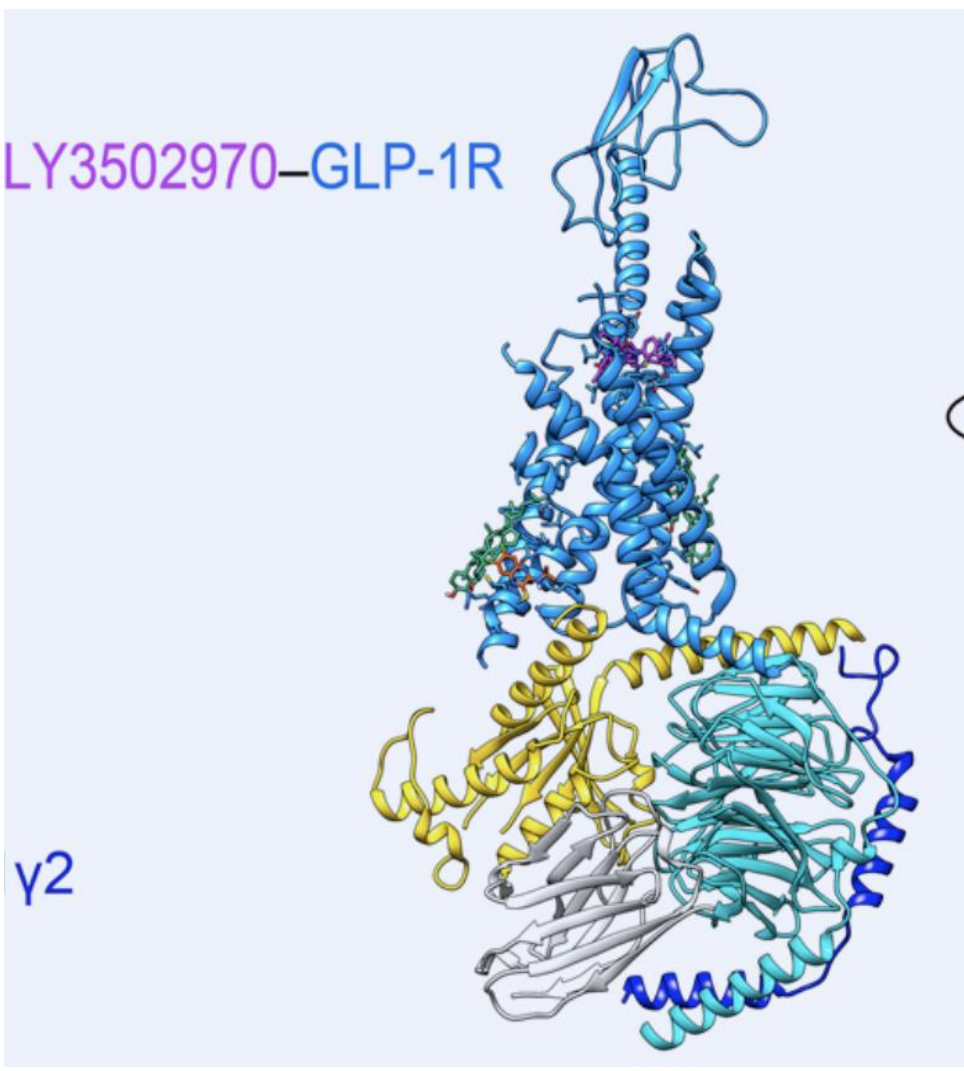
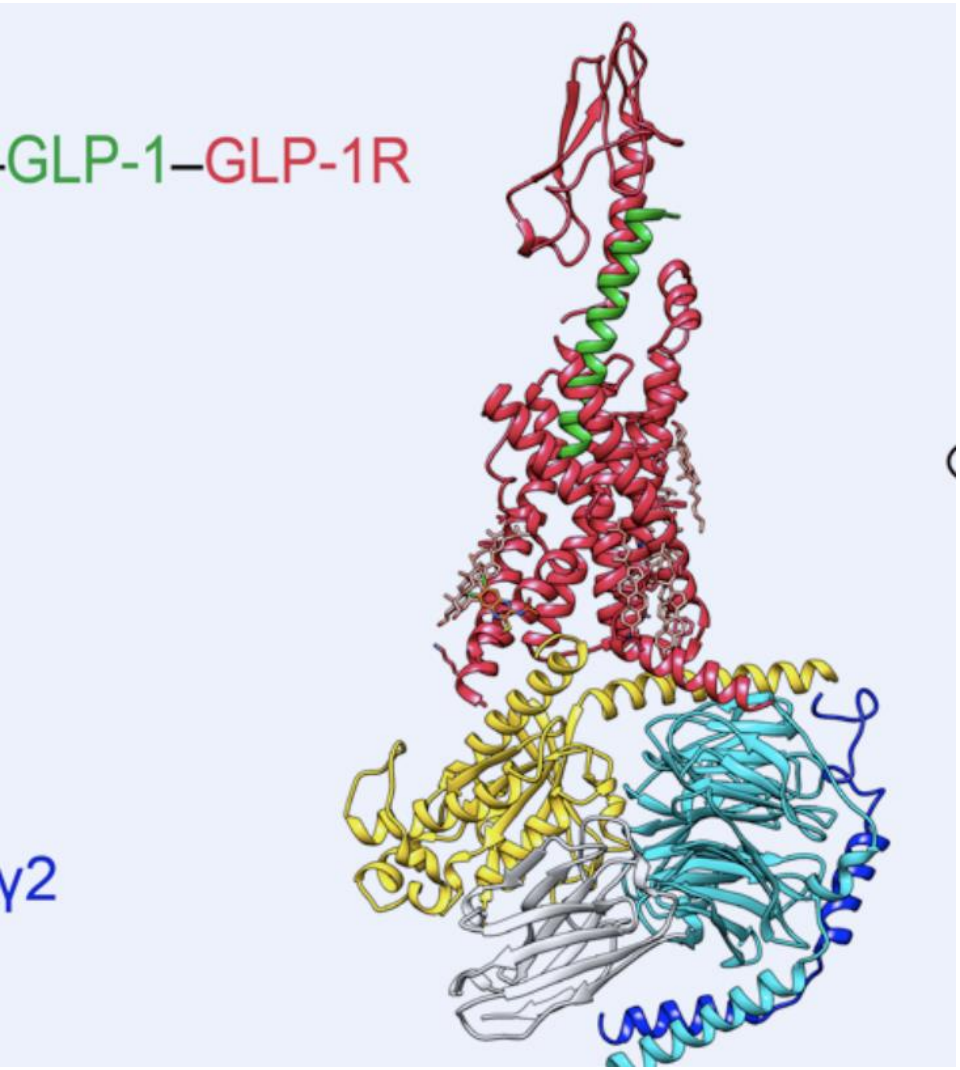
Cryo EM : GLP-1 peptide vs Orforglipron (LY3502970)





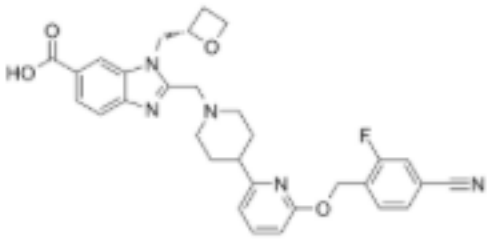
Cryo EM : GLP-1 peptide vs Danuglipron Pfizer



Danuglipron (Synony

Cat. No.: HY-125824 Purete

Danuglipron (PF-06882961) is an orall



Molar mass	555.610 g·mol^{-1}

Danuglipron Chemical Structure

CAS No. : 2230198-02-2

Danuglipron is a small-molecule GLP-1 agonist developed by Pfizer[1] that, in an oral formulation, is under investigation as a therapy for diabetes mellitus. Initial results from

J Biol Chem. 2012 Nov 7;287(53):44121–44129. doi: 10.1074/jbc.M112.361386

Metformin Regulates Glucose Transporter 4 (GLUT4) Translocation through AMP-activated Protein Kinase (AMPK)-mediated Cbl/CAP Signaling in 3T3-L1 Preadipocyte Cells*

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Diabetes. 2001 Oct;50(10):2296-300. doi: 10.2337/diabetes.50.10.2296.

Troglitazone not only increases GLUT4 but also induces its translocation in rat adipocytes

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Affiliations + expand

PMID: 11574411 DOI: 10.2337/diabetes.50.10.2296

Abstract