Mapping neuropeptide-receptor and agonist binding: peptide synthesis strategies to guide the design of novel modulators of appetite regulation

Review Article

With 30–70% of adults in the European Union designated overweight and 10–30% obese (WHO), and more than one third of US adults obese (CDC), the global obesity epidemic has stimulated much research into the fundamentals of appetite regulation. Bo Xu and colleagues, based at Uppsala University in Sweden and collaborating with Novo Nordisk A/S in Denmark, have studied the interaction between the agonist neuropeptide Y (NPY) and the human receptor Y_2 involved in appetite regulation. Using a combination of computational modeling, peptide chemistry and *in vitro* pharmacology analysis the research team has gained insight into interactions that promise to guide the design of novel modulators to fight obesity.

A multidisciplinary approach to refine the Y_2-PYY interaction model

The human neuropeptide Y (NPY) signaling system consists of three peptides (NPY, peptide YY [PYY], and pancreatic polypeptide) and four receptors (Y_1, Y_2, Y_4, and Y_5). Food intake is stimulated by NPY through activation of the Y_1 and Y_5 receptors and is inhibited through activation of receptors Y_4 or Y_2 by PYY-(3-36), an endogenous truncated version of PYY. Many attempts have been made to develop drugs to regulate this system in an effort to treat obesity, including the modification of the common C-terminus of NPY/PYY peptides. The research reported here involved the application of computational modeling, peptide chemistry and *in vitro* pharmacology studies to study binding of the native peptide agonist PYY to the human Y_2 receptor.

Computational modeling is the most common way of investigating receptor structure and receptor-ligand binding and this approach has been greatly assisted by the use of site-directed mutagenesis. The research group had already developed a computational method to estimate the effects of single point mutations on ligand binding affinity and generated a model of the human Y_2 receptor based on crystal structures of other receptors together with mutagenesis and binding studies. The group had also used the model to determine the binding mode of the C-terminal coil, ^{32}TRQRY^{36}–NH_2, of the PYY agonist in the transmembrane (TM) regions of the receptor. In this study, they refined and extended their Y_2-agonist model to the complex with the full PYY peptide.

Peptide synthesis

Peptide analogues were prepared on a Prelude® or Prelude X peptide synthesizer using an Fmoc-chemistry protocol for solid phase peptide synthesis (SPPS) and either Fmoc-Rink Amide or Fmoc-PAL polystyrene resins. The coupling reactions were done for 60 min at 25°C or 30 min at 45–55°C using Fmoc-amino acid-OH and OxymaPure in DMF and activation by DIC and collidine. Deprotection was done with 25% piperidine in NMP or DMF.

C-terminal modified peptides were also synthesized to assess the role of the C-terminal amide. PYY-(3-36) methylamide was synthesized using the linker (3-formylindolyl) acetamidomethyl polystyrene, with coupling of Fmoc-Tyr(OtBu)-OH onto this linker using DIC and OxymaPure in DMF at 55°C. After coupling, the resin was capped using acetic anhydride in DMF. PYY-(3-36)-ol (the C-terminal as primary alcohol) was synthesized by coupling Fmoc-(O-tert-butyl)-tyrosinol in DCM/DPEA to 2-chlorotrityl chloride polystyrene resin. Residues 1–35 for both of the above analogues were coupled using standard SPPS Fmoc-chemistry.

Peptides were cleaved and deprotected with TFA/TIPS/thioanisole or TFA/TIPS/H_2O and purified by preparative RP-HPLC. Purity was determined by analytical RP-UPLC, and molecular weights were determined using MALDI-TOF mass spectroscopy.

N-terminal truncations successively reduce affinity

A model developed by earlier computational modeling showed the interaction between the amidated C-terminal pentapeptide fragment (TRQRY) of PYY and the TM cavity of the Y_2 receptor. The characteristic secondary structure of NPY/PYY/PP peptides contains an α-helix (13–31) that protrudes out in the extracellular loop (EL) region of the receptor, followed by a proline-rich helix (1–12) that interacts through hydrogen bonding within the α-helix domain (Figure 1). This model was used to build complexes between Y_2 and four N-terminal truncated analogues of PYY.

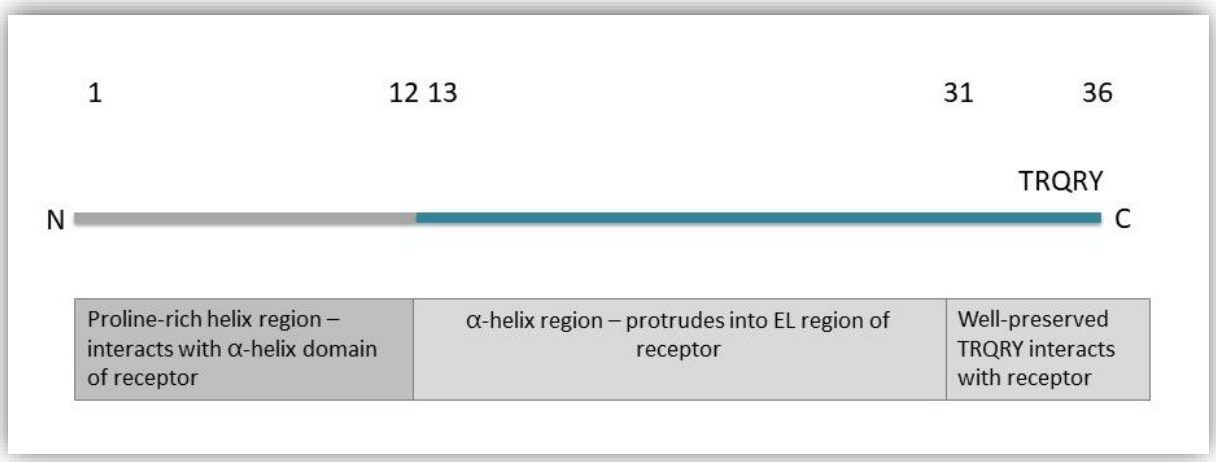

Figure 1. The characteristic secondary structure of intact PYY

Binding studies showed that successively removing residues from the helix-defining N-terminal region reduced the affinity of truncated analogues of PYY, with removal of the first 21 amino acids having a dramatic effect of 25-fold decrease in affinity (Figure 2). The team noted that folding was only stable in full length PYY and the PYY-(3-36) truncated peptide analogue, which retained similar receptor affinity as the parent peptide. Consequently, PYY-(3-36) was used in further studies.

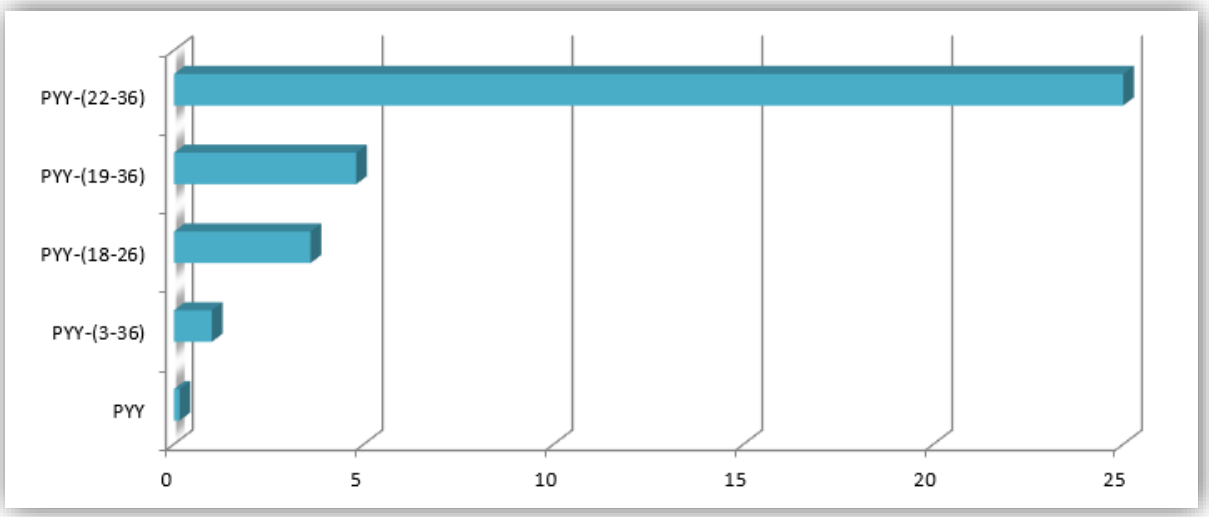

Figure 2. Fold change in binding affinities of PYY and its truncated analogues for WT-hY$_2$. The affinity for PYY-(3-36) was set to 1. Data taken from Table 1, Xu et al, 2018.

The C-terminal TRQRY motif is critical to activation

The team used mutagenesis of Y_2 to examine the interaction between Y_2 and the C-terminal TRQTY motif of PYY. This led them to define a number of receptor-peptide interactions (Figure 3).

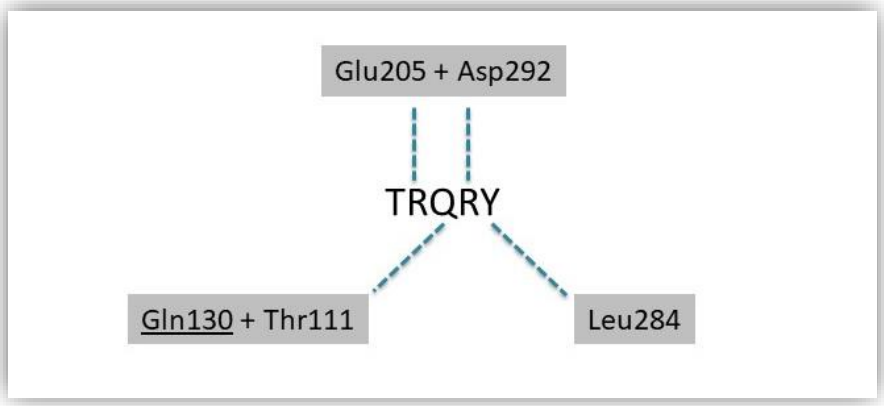

Figure 3. Representation of key interactions between TRQRY motif of PYY and the Y_2 receptor

For example, the mutagenesis results confirmed an earlier prediction that position T^{32} on the peptide should be close to Phe307. To test the binding model further, the team designed three mutants that could be expected to hinder the peptide from entering the binding pocket: Pro127Ala, Pro127Leu, and Cys103Phe. The results confirmed their hypothesis that Pro127 is key to maintaining the integrity of the binding pocket on the upper side of the TM2-TM3 region of the receptor, where Cys103Phe could act as an obstacle at the Y^{36} binding pocket.

A model for the optimization of Y_2 receptor agonists for appetite control

The binding of the common amidated C-terminus was almost identical for PYY and the four truncated analogs, in particular around the TRQRY motif, while truncations of the proline helix mainly affected the folding of the peptides (Figure 4). Simulations indicated that the interactions between the α-helix and the proline-helix region are critical to maintaining the PP-fold, characteristic of all Y-receptor agonist peptides.

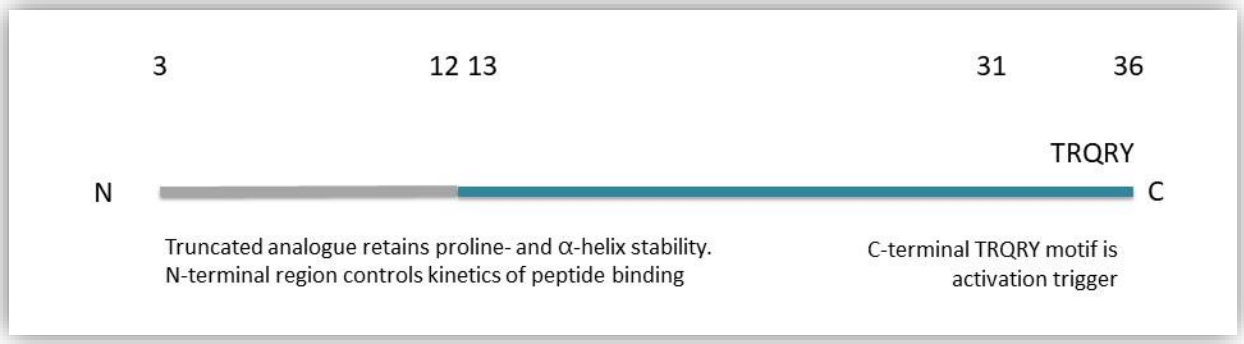

Figure 4. The roles of the N- and C-terminal regions in the PYY-(3-36) truncated analogue

Taken together, the binding affinity data and molecular dynamics analysis supports the current model for Y_2 agonism:

- The activation trigger is located at the amidated C-terminus
- The remaining peptide is involved in the kinetics of peptide binding

Single amino-acid replacements in the peptides had a greater impact than amino-acid changes in the receptor, presumably because the receptor has multiple points of interaction with each residue in the peptide. The researchers concluded that the model agrees with most experimental data available for the Y_2 system and that "the interaction pattern described between ^{32}TRQRY^{36}–NH_2 and hY_2 may guide the optimization of Y_2-selective agonists, with potential applications in the control of appetite."

A synthesizer that increases throughput and enables the synthesis of complex peptides

With 27 years experience of working with solid phase peptide synthesis using various peptide synthesizers, Søren Østergaard, PhD, Principal Scientist at Novo Nordisk A/S greatly appreciated the benefits of the Prelude peptide synthesizers: *"The Prelude X combines the robustness of the former Prelude, which has served as a reliable workhorse for years, with very fast induction heating and the option of pre-activation. This makes the Prelude X more versatile than before, but foremost very fast, which increases the throughput and the complexity of the peptides that can be synthesized."*

References

Elucidation of the binding mode of the carboxyterminal region of peptide YY to the human Y2 receptor**.** Bo Xu et al, Molecular Pharmacology Fast Forward. Published on January 24, 2018 as DOI: 10.1124/mol.117.110627.

http://molpharm.aspetjournals.org/content/molpharm/early/2018/01/24/mol.117.110627.full.pdf

Additional information on obesity:

WHO: http://www.euro.who.int/en/health-topics/noncommunicable-diseases/obesity/data-and-statistics

CDC: https://www.cdc.gov/obesity/data/adult.html