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Novel homodimer model of the β-adrenergic receptor in complex with free fatty acids and cholesterol: first-principles calculation studies

Yuka Nakano[1], Yasuo Watanabe[2], Yoshihiko Ito[3], Shizuo Yamada[3] & Hiroaki Tokiwa[1]*

[1]Department of Chemistry, Rikkyo University, Nishi-ikebukuro, Toshimaku, Tokyo 171-8501, Japan; [2]Nihon Pharmaceutical University, 10281 Komuro, Ina-machi, Kitaadachi-gun, Saitama 362-0806, Japan; [3]School of Pharmaceutical Sciences, University of Shizuoka, 52-1, Yada, Suruga-ku, Shizuoka 422-8526, Japan; Hiroaki Tokiwa – Email: tokiwa@rikkyo.ac.jp; *Corresponding author

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Abstract:
We propose a theoretical novel homodimer model of the β- adrenergic receptor (βAR) in complex with a heterogeneous mixture of free fatty acids (FFAs) and cholesterol based on first-principles calculations. We used the density-functional-based tight binding with dispersion (DFTB-D) method, which accurately evaluates van der Waals interactions between FFAs and amino acid residues in the receptor. The calculations suggest that a stable homodimer of βAR can form a complex with FFAs and cholesterol by extensive van der Waals interactions in the cell membrane, and that the heterogeneous composition of the FFAs is important for the stability of the homodimer complex. The stable van der Waals interactions propagate from one of the βAR to the other through the cholesterol and FFAs in the homodimer complex. The energy propagation in the complex has the potential to enhance molecular signaling in adipocytes, because the stability of the complex can influence anti-adiposity effects after oral treatment of the FFA components.

Background:
The G-protein-coupled receptor (GPCR) superfamily is the largest and most diverse protein family in mammals that is involved in signal transduction across membranes. GPCRs share the structural motifs of a seven α-helical transmembrane domain, an extracellular N-terminal domain, and an intracellular C-terminal domain. Approximately 800 functioning receptor proteins are encoded within the human genome. The GPCR superfamily comprises six sub-families that can bind to a diverse range of ligands including photons, ions, lipids, organic compounds, peptides, and proteins **[1].** Once stimulated by these molecules, GPCRs bind to and activate G-proteins. The G-proteins then initiate signal cascades that control numerous important biological processes **[2].** Since GPCRs control major biological processes through signaling, the GPCR superfamily is a prominent therapeutic target for approximately half of all modern drugs **[3].** Despite this fact, relatively few of the GPCR structures have been elucidated because expressing and crystallizing GPCRs as membrane proteins remain challenging. The first GPCR structure to be solved was that of bovine rhodopsin in 2000 **[4].** Seven years later, a high-resolution structure of the human β2-adrenergic receptor (β2AR) was obtained **[5].** Since then, the structures of other class A GPCRs, including the β1-adrenergic receptor, **[6]** have been solved. However, crystal structures do not always reveal the *in vivo* structure, that is, the structure formed in the actual cell membrane. Accordingly, numerous theoretical approaches have been taken to explore the molecular mechanism of GPCR activation. One aspect of the activation mechanism of GPCRs that remains controversial is whether the GPCRs exist and function as monomers, (homo / hetero) dimers, or oligomers in cell membranes **[3].** While there is incontrovertible evidence that class C GPCRs work as dimers **[7],** data on the oligomerization of class A GPCRs are less clear **[8, 9].** Recent studies using single molecule imaging approaches showed the transient formation and decomposition of dimers of a class A GPCR (muscarinic and N-formyl peptide receptor) in living cells **[10, 11].** However, while these imaging studies

suggested that oligomers may form, they did not provide detailed information about how receptors interact with each other at the atomic and molecular levels.

Recently, Kubo *et al.* used a radio receptor assay to show that a certain group of four free fatty acids (FFAs), extracted from *Flammulina Velutipes (Curt.:Fr.) Sing,* binds effectively to the β-adrenergic receptor (βAR), but that the binding activity of each FFA independently was poor **[12].** Oral treatment of this grouping of FFAs improved adiponectin and leptin secretion, which are disrupted in some lipid metabolism disorders **[13].**

In addition, a structural biology study showed that cholesterol effectively stabilizes βAR **[5, 14].** Here, we propose a theoretical, novel βAR homodimer model with FFAs and cholesterol that demonstrates enhanced molecular signaling *via* protein–protein interactions in the homodimer. We theoretically analyzed the interactions among βARs, cholesterol, and FFAs by using the density-functional-based tight binding with dispersion (DFTB-D) method **[15],** which accurately estimates van der Waals dispersion energies among protein, FFA, and cholesterol molecules without a large calculation cost. We found that a βAR homodimer complex with cholesterol and FFA molecules is more stable than a βAR homodimer that lacks contact with these molecules. This stability of the homodimer may be caused by additive van der Waals dispersion energies among the cholesterol, FFAs, and βAR. This novel model is the first example of a complex showing explicit interactions among FFAs, cholesterol, and βAR determined by using first-principles calculations. Our novel homodimer complex with FFAs and cholesterol furthers our understanding of the molecular mechanism of activation and dimerization of the GPCR and offers a molecular basis for exploring enhanced signaling and anti-adiposity effects *via* GPCR oligomerization.

Computational Methodology and Discussion:

All first-principles calculations were performed at the level of the density-functional-based tight binding with dispersion (DFTB-D) method, which rapidly and adequately estimates van der Waals (dispersion) interactions among βAR–FFAs, FFA–FFA, and cholesterol–FFA by using the DFTB+ **[15]** and Gaussian09 **[16]** programs. Small molecules and βAR were modeled by using the Gauss View **[17]** and MOE **[18]** programs, respectively. PyMOL (www.pymol.org) was used for the preparation of all structure figures.

FFAs and Cholesterol

We used linoleic acid (**1**), α-linolenic acid (**2**), palmitic acid (**3**) and pentadecanoic acid (**4**) as neutral (not ionized) FFA molecules. Isolated cholesterol (**5**) and FFA molecular geometries were fully optimized by use of the DFTB-D method. This group of FFAs is contained in ethanol extracts of *Flammulina Velutipes (Curt.: Fr.) Sing,* which have anti-adiposity effects on both humans and mice. Oral treatment of this group of FFAs led to the recovery of adiponectin and leptin. The ability to secrete adiponectin and leptin is disrupted in some lipid metabolism disorders **[13].**

βAR

The βAR structure was derived from the recently determined activated form of the human β2AR (PDB ID: 3P0G **[19],** chain A). Hydrogen atoms were added by using the protonate3D wizard in the MOE2010/2011 system **[18]** under pH 7.0 conditions. Then, all of the positions of the hydrogen atoms were optimized by using the AMBER99 force field **[20]** in the MOE system.

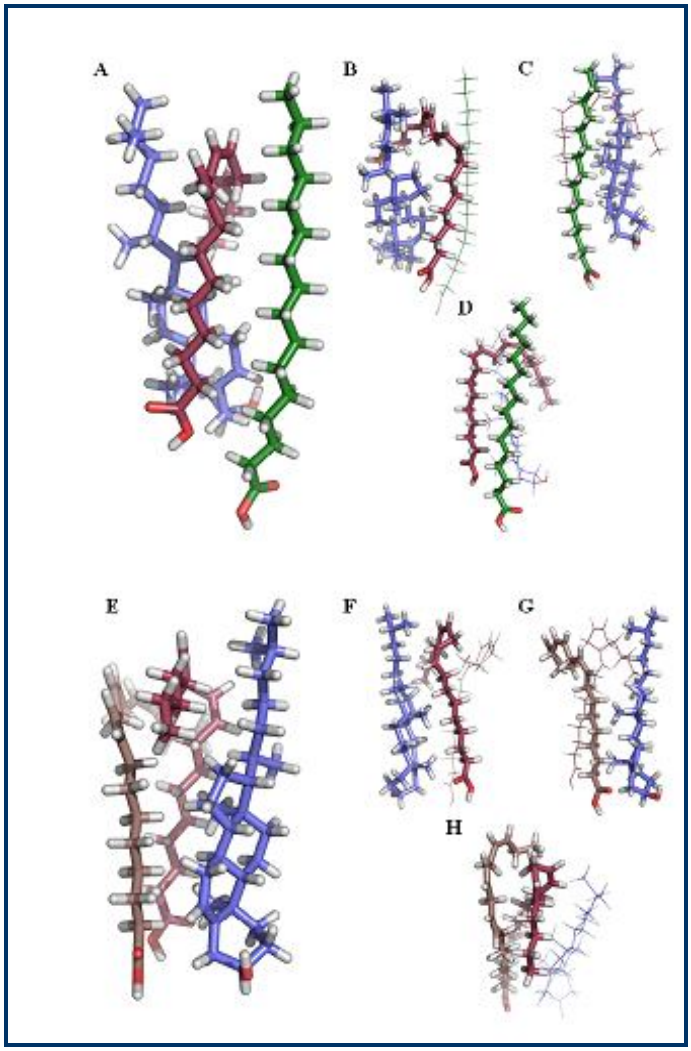

Figure 1: **A)** is the front view of Model IA. The Structures of Model IA constructed with cholesterol (**5**), linoleic acid (**1**) and palmitic acid (**3**) are shown in blue, red, and green as stick models, respectively. These compounds in **B), C),** and **D)** are shown in blue and red as stick models and green as a wireframe model, green and blue as stick models and red as a wireframe model, and green and red as stick models and blue as a wireframe model, respectively**. E)** is the front view of Model IB. The Structures of Model IB constructed with cholesterol (**5**), linoleic acid (**1**) and α-linolenic acid (**2**) are shown in blue, red, and dark salmon, respectively. These compounds in **F), G),** and **H)** are shown in blue and red as stick models and dark salmon as a wireframe model, dark salmon and blue as stick models and red as a wireframe model, and dark salmon and red as stick models and blue as a wireframe model, respectively.

BAR-cholesterol complex

We superimposed a cholesterol molecule, which is contained in the crystal structure of the inactive form of human β2AR (PDB ID: 2RH1 **[5]),** onto the βAR structure. Hydrogen atoms in the cholesterol molecule were added by using the MOE system and were optimized by use of the DFTB-D method. The interaction

energy between βAR and cholesterol was calculated as -14.1kcal/mol, which is more stable than the interaction between βAR and each FFA **[21].** This calculation result is in good agreement with the experimental thermal stability of the structure of the inactive form of human β2AR (PDB ID: 2RH1 **[5])** due to cholesterol binding to the β2AR. Cholesterol effectively interacts with Thr73, Cys77, and Val81 in the helix II of the β2AR **[14].** Similar stable interactions were also observed in our model of the active form of the β2AR-cholesterol complex, that is, the interactions among cholesterol, Leu80, and Ala85 were stable. Thr73 has a van der Waals interaction with the C4 of the sterol ring A of cholesterol. Cys77 forms a hydrogen bond with a hydrogen atom on C9 in the sterol ring B, and a van der Waals interaction with the C11 and C12 of the sterol ring C. The methyl groups of Leu80 and Val81 effectively interact with the hydrophobic group of cholesterol due to van der Waals interaction. Ala85 forms a stable van der Waals interaction with the end of the hydrophobic group of cholesterol.

Model I: Cholesterol (5)-linoleic acid (1) - α-linolenic acid (2)/palmitic acid (3)

We built Model I, as shown in (**Figure 1**). We performed the geometric optimization of Model I, cholesterol (**5**) in complex with linoleic acid (**1**) and palmitic acid (**3**) **(Figure 1A, 1B, 1C, & 1D)** or α-linolenic acid (**2**) **(Figure 1E, 1F, 1G, & 1H),** by using the DFTB-D method. The complex including cholesterol (**5**), linoleic acid (**1**), and palmitic acid (**3**), and the complex including cholesterol (**5**), linoleic acid (**1**), and α-linolenic acid (**2**) are defined as Model IA and Model IB, respectively. Optimized geometries showed that the cholesterol (**5**), linoleic acid (**1**), and palmitic acid (**3**) molecules become more stable as a result of dispersion interactions as the molecules get closer to each other. In Model IA, the double bond in the C12 of linoleic acid (**1**) appears to contribute to the interaction with the hydrophobic group of cholesterol (**5**) through a CH-π interaction. The saturated carbon chain of linoleic acid (**1**) forms effective van der Waals interactions with the sterol rings A, B, C, and D due to the intact spherical nature of those groups. The saturated carbon chain of palmitic acid (**3**) also interacts with the sterol rings A, B, C, and D of cholesterol (**5**). The saturated carbon chain of linoleic acid (**1**) seems to interact with the saturated carbon chain of palmitic acid (**3**) through a van der Waals interaction.

In Model IB, linoleic acid (**1**) and cholesterol (**5**) also have CH-π and van der Waals interactions as seen in Model IA due to the intact spherical structure of those parts of the molecules. The double bond of the C12 in linoleic acid (**1**) appears to contribute an extensive interaction with the double bond of the C12 in α-linolenic acid (**2**) through a π-π stacking interaction. These data indicate that FFA composition is important for the stability of the complex. The heterogeneous complex is more stable than the single composite complex of FFAs and cholesterol.

Model II: Cholesterol-FFAs

Next, we built Model II, as shown (**Figure 2**). A pentadecanoic acid (**4**) was centered in this model, and we analyzed the potential energy by changing the distance between the pentadecanoic acid (**4**) and the palmitic acid **(3)**/ α-linolenic acid **(2) (Figure 2).** The equilibrium distances R_A and R_B between the pentadecanoic acid (**4**) and the palmitic acid **(3)**/ α-linolenic acid (**2**) were 3.92Å and 3.98Å, respectively. The interaction energy of the complex with cholesterol and the FFAs was -86.8kcal/mol. The stable energy is caused by the propagation of van der Waals interactions from one cholesterol molecule to the other through FFA molecules.

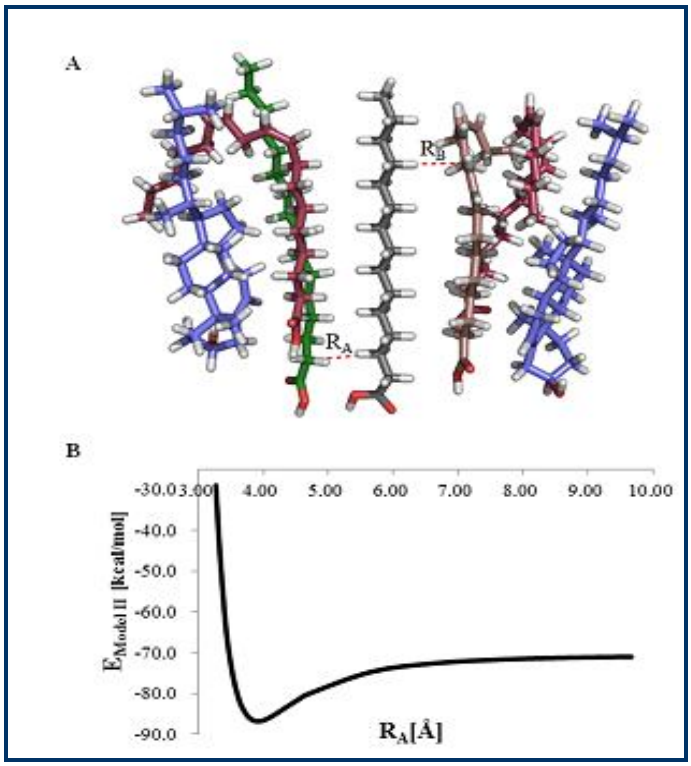

Figure 2: (A) is the front view of Model II. Model II consists of Model IA, Model IB, and a pentadecanoic acid (**4**) in the center. The structure of pentadecanoic acid (**4**) is shown in gray. The distances between the pentadecanoic acid (**4**) and the palmitic acid (**3**)/α-linolenic acid (**2**) are shown as R_A and R_B, respectively; **(B)** is the Potential Energy Curve; the horizontal axis represents R_A, whereas the vertical axis expresses the value of $E_{Model\ II}$. In the Potential Energy Curve, the equilibrium geometry shows that an $E_{Model\ II}$ of -86.8 kcal/mol was gained at R_A = 3.92 Å and R_B = 3.98 Å, respectively.

Homodimer in complex with cholesterol and FFAs

The complex of the homodimer with cholesterol and FFAs was formed by superimposing Model II on cholesterol (**5**) in the βAR-cholesterol complex. We obtained the stable homodimer with cholesterol and FFAs by using optimizing geometry with the DFTB-D method. The structure of this complex is shown in (**Figure 3)**. The distance between the βARs is defined as the distance between Phe104 and Phe101 from helix III, R_{AR-AR}. The interaction energy among the βARs, cholesterol, and FFAs from each isolated system, that is from the βAR homodimer, cholesterol, and each FFA molecule, was -108.9kcal/mol. On the other hand, the interaction energy between each βAR in isolation was only -0.18kcal/mol at the same distance between each βAR. Note that the stability of the homodimer complex with the cholesterol and FFAs is caused by the propagation of effective interactions from one βAR to the other βAR through cholesterol, linoleic acid, and the FFA molecules. The stable homodimer complex with cholesterol and FFAs has the potential to enhance molecular signaling and consequently mediate anti-adiposity effects.

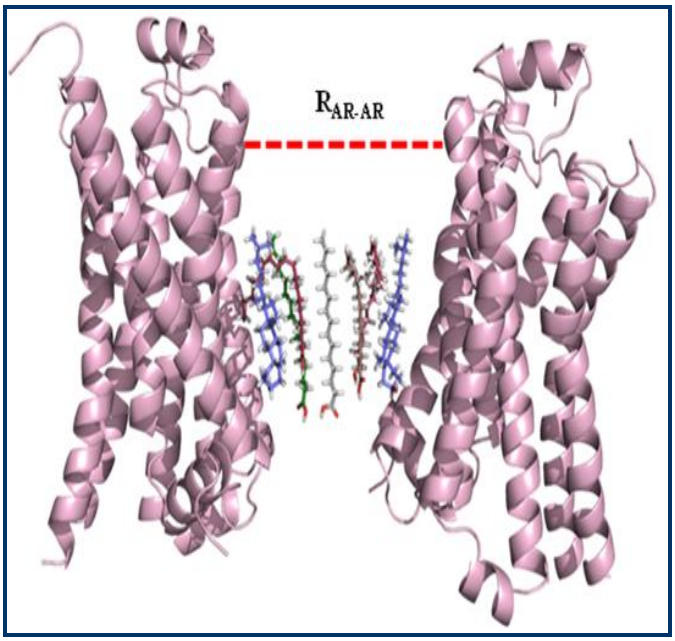

Figure 3: Shown is the novel βAR homodimer model with cholesterol and FFAs. This model is derived from the βAR homodimer and the Model II-centered homodimer. The distance between each βAR, $R_{AR\text{-}AR}$, is 25.3 Å.

Conclusion:

On the basis of first-principles calculations using the DFTB-D method, we determined that a novel homodimer model of the βAR could be formed in which FFA and cholesterol molecules are inserted between the βAR homodimer as a result of interactions between the βARs and FFAs via cholesterol. This stable energy may be caused by the propagation of van der Waals dispersion interactions from one βAR to the other via FFA–FFA and cholesterol–FFA interactions. The main interaction between the βARs and the FFAs via cholesterol is probably the hydrophobic interaction between the carbon chain in the hetero-complex of the FFA and cholesterol molecules, because the total interaction energy between the cholesterol and FFA was -87.5kcal/mol. On the other hand, the interaction energy between the two βARs in direct contact was only -0.18kcal/mol. Our calculation results show that this specific group of four FFAs extracted from *Flammulina Velutipes (Curt.: Fr.) Sing* is an important heterogeneous composition for the key interactions that form the stable homodimer. From these observations, we believe that this stable homodimer complexed with cholesterol and these FFAs could mediate and enhance molecular signals, resulting in anti-adiposity effects. Our model offers the molecular basis for the activation mechanism of the βAR based on FFA composition.

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