

Coupling Interaction between Thromboxane A2 Receptor and Alpha-13 Subunit of Guanine Nucleotide-Binding Protein

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G protein-coupled receptors (GPCRs) form a large superfamily of membrane proteins that play an essential role in modulating many vital physiological events, such as cell communication, neurotransmission, sensory perception, and chemotaxis. Understanding of the 3D (dimensional) structures of these receptors and their binding interactions with G proteins will help in the design of drugs for the treatment of GPCR-related diseases. By means of the approach of structural bioinformatics, the 3D structures of human alpha-13 subunit of guanine nucleotide-binding protein (G alpha 13) and human thromboxane A2 (TXA2) receptor were developed. The former plays an important role in the control of cell growth that may serve as a prototypical G protein; the latter is a target for nitric oxide-mediated desensitization that may serve as a prototypical GPCR. On the basis of the 3D models, their coupling interactions were investigated via docking studies. It has been found that the two proteins are coupled with each other mainly through the interaction between the minigene of G alpha 13 and the 3rd intracellular loop of the TXA2 receptor, consistent with the existing deduction in the literatures. However, it has also been observed via a close view that some residues of the TXA2 receptor that are sequentially far away but spatially quite close to the loop region are also involved in forming hydrogen bonds with the minigene of G alpha 13. These findings may provide useful information for conducting mutagenesis and reveal the molecular mechanism how the human TXA2 receptor interact with G alpha 13 to activate intracellular signaling. The findings may also provide useful insights for stimulating new therapeutic approaches by manipulating the interaction of the receptor with the relevant G proteins.

Keywords: GPCR • G protein • binding interaction • docking • structural bioinformatics • intracellular signaling • allosteric transition • TXA2 receptor • G alpha 13

I. Introduction

G protein-coupled receptors (GPCRs) form the largest family of cell surface receptors that are involved in the control of every aspect of human behavior and physiology. GPCRs can also be involved in pathological processes and are linked to numerous diseases, including cancer, AIDS, retinal degeneration, as well as cardiovascular and mental disorders.¹ Characterized by their cell-surface localization and tissue-specificity, GPCRs are the targets of 50–60% of all existing medicines including well-known-blockers and anti-histamine therapeutics. Actually, they are among the most frequent targets of therapeutic drugs.²

The functions of many of GPCRs are unknown, and determining their ligands and signaling pathways is both time-consuming and costly. This difficulty has motivated and challenged the development of a computational method which can predict the classification of the families and subfamilies of GPCRs based on their primary sequences so as to help us classify drugs, a technique which might be called “evolutionary pharmacology”.^{3,4}

Meanwhile, many efforts in pharmaceutical research have been aimed at understanding their structure and function.

Unfortunately, because they are difficult to crystallize and that most of them will not dissolve in normal solvents, so far, very few G-protein-coupled receptor structures have been determined. The present study was initiated in an attempt to use the approach of structural bioinformatics⁵ to investigate how GPCRs couple to G proteins. As is known, G proteins are so-called because they bind the guanine nucleotides GDP and GTP. Associated with the inner surface of the plasma membrane and GPCRs, G proteins are heterotrimers constituted by three different subunits: $G\alpha$, $G\beta$, and $G\gamma$, of which $G\alpha$ carries the binding site for the nucleotide. In the inactive state, $G\alpha$ has GDP in its binding site. When a hormone or other ligand binds to the associated GPCR, an allosteric transition^{6,7} takes place from the receptor to $G\alpha$. This will activate $G\alpha$ by replacing GDP with GTP. The activated $G\alpha$ will dissociate from $G\beta\gamma$, and in turn activate an effector molecule. Here, as a prototype, let us consider the interaction of how the human G alpha 13 (alpha-13 subunit of guanine nucleotide-binding protein) binds to the human TXA2 (thromboxane A2) receptor, which is a target for nitric oxide-mediated desensitization. TXA2 receptors are members of GPCR and they couple to several different G protein α subunits including G alpha 13.⁸ Below, we shall first develop the 3D (dimensional) models of human G alpha 13

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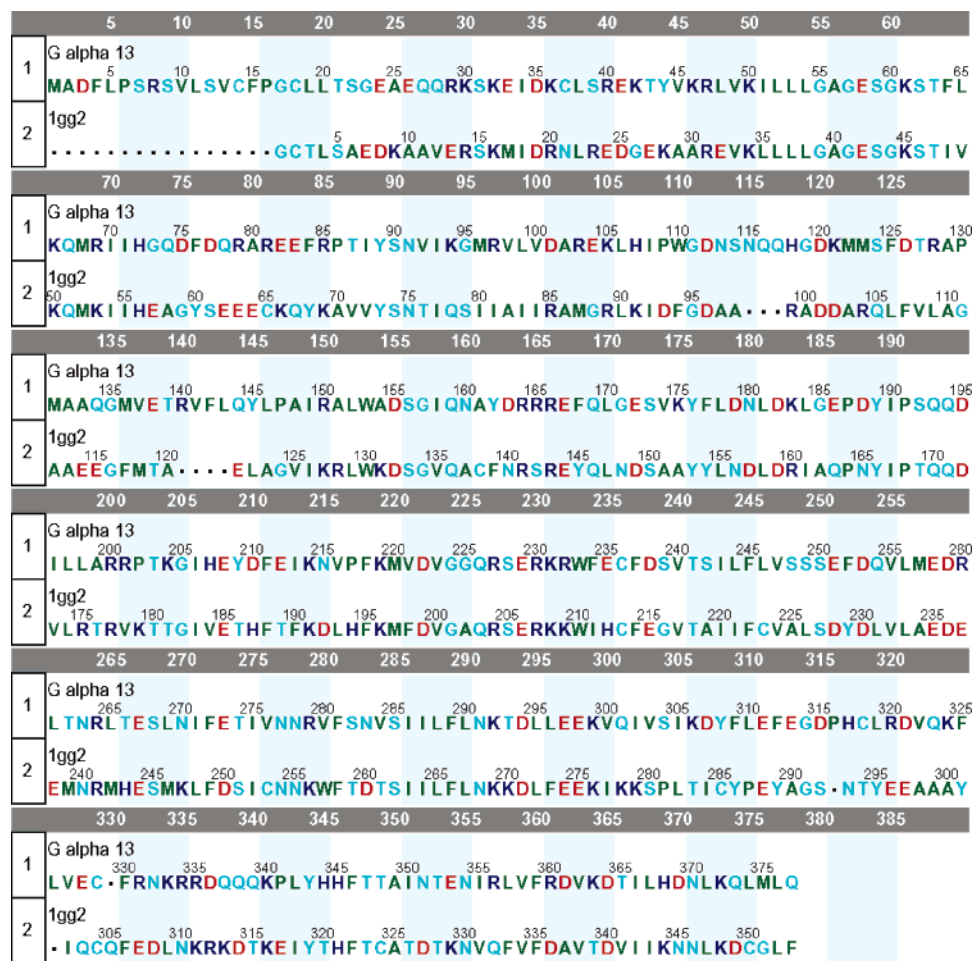


Figure 1. Alignment of the amino acid sequences of G alpha 13 and 1gg2 using PILEUP in the GCG package,¹⁴ where the codes in red represents the acidic amino acids, those in dark blue the basic, green the hydrophobic, and light blue the hydrophilic.

and human TXA2 receptor, followed by docking G alpha 13 to TXA2 receptor.

II. Modeling the 3D Structure of Human G Alpha 13

Constitutively active G alpha 13 causes permissive cell types to proliferate or undergo phenotypic transformation implying its role in the control of cell growth.

The sequence of human G alpha 13 was taken from refs 9 and 10. The accession number for G alpha 13 in Swiss-Prot¹¹ is Q14344. The entire sequence contains 377 amino acid residues. It was found by running a BLAST search^{12,13} that, among the known 3D structure proteins, chain A of 1gg2.pdb (G protein GI alpha 1 chain A) had the highest score bits in sequence similarity with G alpha 13. Actually, it was indicated by running the GAP program of the GCG package¹⁴ that the sequence similarity between the two G proteins was 54.5%. Accordingly, the crystal structure of 1gg2 determined in ref 15 was selected as a template to model the 3D structure of G alpha 13. The sequences of G alpha 13 and 1gg2 were aligned using the PILEUP program in the GCG package.¹⁴ The aligned result is given in Figure 1, where the codes in red represent the acidic amino acids, those in dark blue the basic, green the hydrophobic, and light the hydrophilic.

On the basis of the sequence alignment (Figure 1) and the atomic coordinates of the crystal structure 1gg2.pdb,¹⁵ the 3D structure of G alpha 13 was derived by means of the “segment

matching” or “coordinate reconstruction” approach.^{16–19} The rationale of the approach is based on the finding that most hexapeptide segments of protein structure can be clustered into only 100 structurally different classes.²⁰ Thus, comparative models can be built by using a subset of atomic positions from a selected template structure as “guiding” positions, as well as by identifying and assembling short, all-atom segments that fit these guiding positions. The template structure is usually homologous to the targeted one and is preferably with a high structural resolution. The entire operation involves the following steps: (1) breaking the targeted chain into many short sequence segments; (2) searching the database, which contains more than 5200 high-resolution crystal protein structures, for matching the segments according to the sequence alignment of Figure 1 and the “guiding” positions of the template protein chain (1gg2.pdb); (3) fitting the coordinates of the matched segments into the growing target structure under the monitor to avoid any van der Waal overlap until all atomic coordinates of the targeted structure were obtained; (4) repeating the process 10 times and generating an average model, followed by a global energy minimization to create the final 3D structure. The segment matching approach was previously used to model the structure of the protease domain of caspase-8²¹ before the crystal coordinates of caspase-3 were released.²² To deal with the situation of lacking a proper template, the 3D structure of the catalytic domain of caspase-3 was first derived by using

the crystal structure of caspase-1 as a template. Subsequently, the caspase-3 structure thus obtained was used as a template to further model the protease domain of caspase-8. After the crystal coordinates of caspase-3 protease domain were finally released and the crystal structure of the caspase-8 protease domain was determined,²³ it was found that the RMSD (root-mean-square-deviation) for all the backbone atoms of the caspase-3 protease domain between the crystal and computed structures was 2.7 Å, while the corresponding RMSD was 3.1 Å for caspase-8, and only 1.2 Å for its core structure, indicating that the predicted structures of caspase-3 and -8 were quite close to their crystal structures. Shortly afterward, this method was successively applied to model the CARDS (caspase recruitment domains) of Apaf-1, Ced-4, and Ced-3 by using the NMR structure of the RAIDD CARD²⁴ as a template, and to model the Cdk5-Nck5a* complex²⁵ as well as the protease domain of caspase-9.²⁶ Two years after the computed Cdk5-Nck5a* complex structure was published,²⁵ the crystal structure of the complex was determined.²⁷ It was found that the predicted Cdk5 and the crystal Cdk5 are almost the same. Also, it was observed by these authors²⁷ that, upon the binding of Cdk5 and Nck5a* (or p25), the buried surface area was 3400 Å², which was very close to 3461 Å² derived from the computed structure.^{5,25} Meanwhile, stimulated by the computed Cdk5-Nck5a*-ATP structure, the molecular truncation experiments were conducted²⁸ with the conclusion that the experimental results “confirm and extend specific aspects of the original predicted computer model”. The segment match approach was also used to derive the tertiary structure of β -secretase zymogen,²⁹ leading to a compelling elucidation of why the prodomain of β -secretase did not suppress activity like in a strict zymogen, as observed by Shi et al.³⁰ and Benjannet et al.³¹ Recently, the segment match approach was further used to develop the 3D structures of extracellular domains for the subtypes 1, 2, 3, and 5 of GABA-A receptors,³² the extracellular domain of α 7 nicotinic acetylcholine receptor,³³ the protease domain of BACE2,³⁴ the monomer and dimer of GFPT,³⁵ cathepsin-E,³⁶ as well as the cores of human potassium channel and sodium channel.³⁷

The computed 3D structure of G alpha 13 is illustrated in Figure 2, where the minigene segment³⁸ is shown with ball-and-stick drawing and the other part of the protein with ribbon drawing. The minigene segment (res. 367–377) is formed by Leu-367, His-368, Asp-369, Asn-370, Leu-371, Lys-372, Gln-373, Leu-374, Met-375, Leu-376, and Gln-377 (cf. Figure 1).

III. Modeling the 3D Structure of Human TXA2 Receptor

The sequence of the human TXA2 receptor was taken from ref 39. Its accession number in Swiss-Prot¹¹ is AAC24303. The entire sequence contains 343 amino acid residues. Both the TXA2 receptor and bovine rhodopsin belong to the GPCR family. Accordingly, the crystal structure of bovine rhodopsin, 1f88.pdb, recently determined by Palczewski et al.⁴⁰ was used as a template for modeling the 3D structure of TXA2 receptor. The sequences of TXA2 receptor and 1f88 were aligned with the PILEUP program in the GCG package.¹⁴ The aligned result is given in Figure 3, where the codes in red represents the acidic amino acids, those in dark blue the basic, green the hydrophobic, and light the hydrophilic. On the basis of the sequence alignment (Figure 3) and the X-ray coordinates of 1f88, the 3D structure of the human TXA2 receptor was derived by following the similar procedures as described above for modeling G alpha 13. The computed 3D structure of the human TXA2 receptor



Figure 2. Predicted 3-D structure of human G alpha 13, where the minigene segment (res. 367–377) is shown with ball-and-stick drawing and the other part of the protein with ribbon drawing.

is illustrated in Figure 4, where the segment of res.222–239 (i.e., Leu-222, Cys-223, His-224, Val-225, Tyr-226, His-227, Gly-228, Gln-229, Glu-230, Ala-231, Ala-232, Gln-233, Gln-234, Arg-235, Pro-236, Arg-237, Asp-238, and Ser-239) forms the 3rd intracellular loop.

IV. Binding Interaction between Human TXA2 Receptor and G Alpha 13

On the basis of the computed 3D structures of G alpha 13 and TXA2 receptor, a study of docking G alpha 13 to TXA2 receptor was conducted by the same procedures as described in ref 41. An overview of the binding complex thus obtained is given in Figure 5, where the TXA2 receptor is in yellow, and G alpha 13 in red. Those residues, which are involved in forming hydrogen bonds between G alpha 13 and TXA2 receptor, are shown by the ball-and-stick drawing while the remaining part by the ribbon drawing. As shown from the figure, the two protein molecules are coupled with each other mainly through the interaction between the 3rd intracellular loop of TXA2 receptor^{42,43} and the minigene of G alpha 13.³⁸

A close view of the binding region is provided in Figure 6, from which we can see that seven hydrogen bonds (drawn in green dotted line) are formed between the two protein molecules during the binding interaction. The detailed residues and atoms involved in forming these hydrogen bonds are listed in Table 1, from which we can see: (1) for the TXA2 receptor three of the seven hydrogen bonds are associated with the residues (Gln-234 and Asp-238) of its 3rd intracellular loop, and the remaining four with the residues (Arg-136 and Gly-133) that

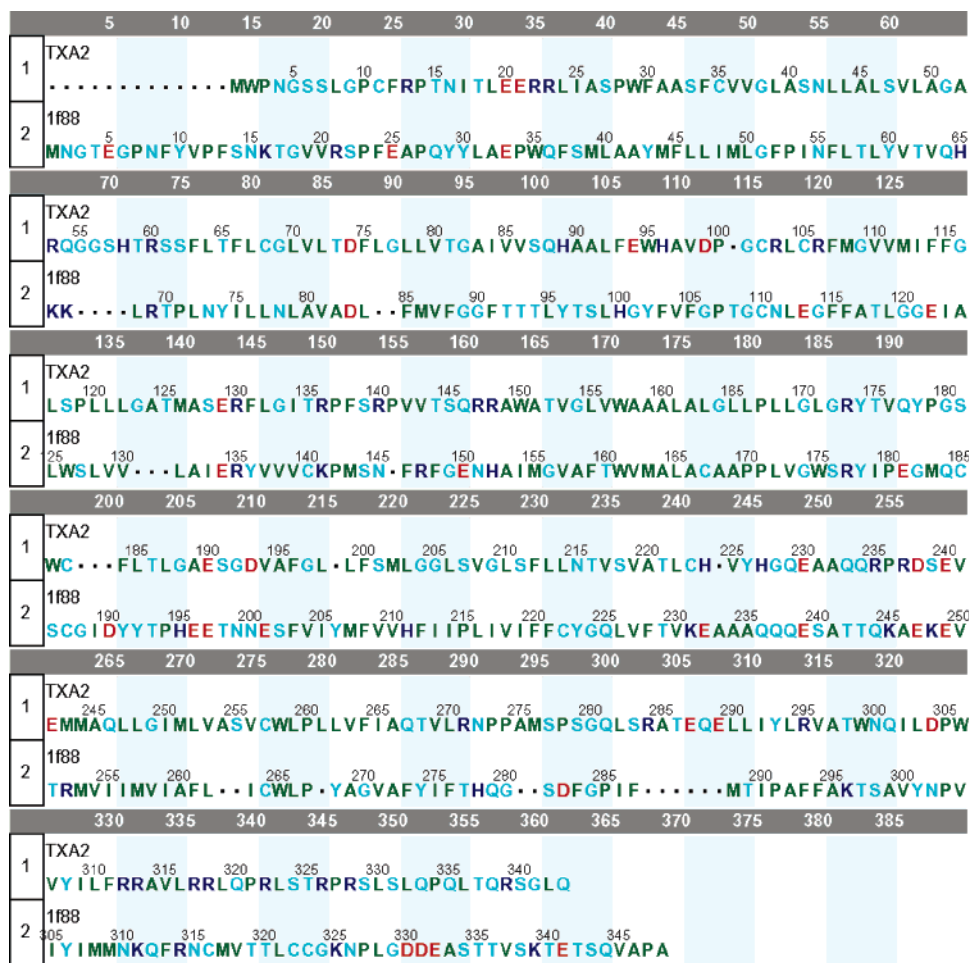


Figure 3. Alignment of the amino acid sequences of the human TXA2 receptor and 1f88 using PILEUP in the GCG package.¹⁴ See the legend of Figure 1 for further explanation.



Figure 4. Computed 3D structure of the human TXA2 receptor, where the segment of res. 222–239 forms the 3rd intracellular loop.

are sequentially far away but spatially quite close to the loop region; (2) for the G alpha 13 all the seven hydrogen bonds are

Table 1. Hydrogen Bond Interactions between Human TXA2 Receptor and G Alpha 13

TXA2 receptor		G alpha 13	
residue	atom	residue	atom
Gln-234 ^a	N ^ε	Asp-364 ^c	O ^{δ1}
Asp-238 ^a	O ^{δ1}	Lys-372 ^c	N ^ζ
Asp-238 ^a	O ^{δ2}	Lys-372 ^c	N ^ζ
Arg-136 ^b	N ^{η1}	Leu-374 ^c	O
Arg-136 ^b	N ^{η2}	Leu-374 ^c	O
Arg-136 ^b	N ^{η1}	Gln-377 ^c	O ^ε
Gly-133 ^b	O	Gln-377 ^c	O ^ε

^a Within the 3rd intracellular loop of TXA2 receptor; the sequence of the loop is: Leu-222, Cys-223, His-224, Val-225, Tyr-226, His-227, Gly-228, Gln-229, Glu-230, Ala-231, Ala-232, Gln-233, Gln-234, Arg-235, Pro-236, Arg-237, Asp-238, and Ser-239. ^b Outside the 3rd intracellular loop of TXA2 receptor. ^c Within the minigene region of G alpha 13; the sequence of the minigene is: Leu-367, His-368, Asp-369, Asn-370, Leu-371, Lys-372, Gln-373, Leu-374, Met-375, Leu-376, and Gln-377.

associated with the residues (Asp-364, Lys-372, Leu-374, and Gln-377) of its minigene region. These findings may provide useful information for conducting mutagenesis and revealing the molecular mechanism of how the human TXA2 receptor interacts with G alpha 13 to activate intracellular signaling. The findings may also provide useful insight for stimulating new therapeutic approaches by manipulating the interaction of the receptor with the relevant G proteins.

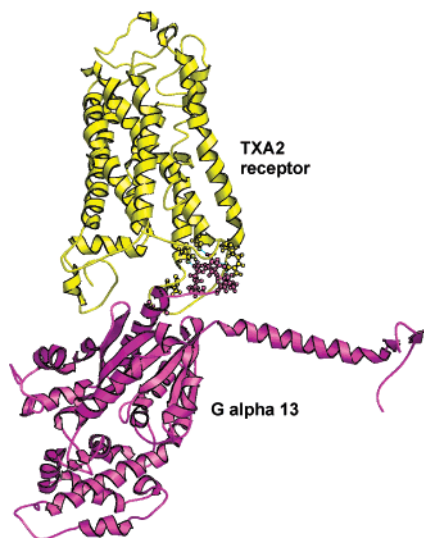


Figure 5. Overview of the binding interaction of the human TXA2 receptor (yellow) with G alpha 13 (purple). The residues involved in forming hydrogen bonds between the two protein molecules are shown by the ball-and-stick drawing, while the remaining part by the ribbon drawing. As expected, the two protein molecules are coupled with each other mainly through the interaction between the 3rd intracellular loop of TXA2 receptor and the minigene of G alpha 13.

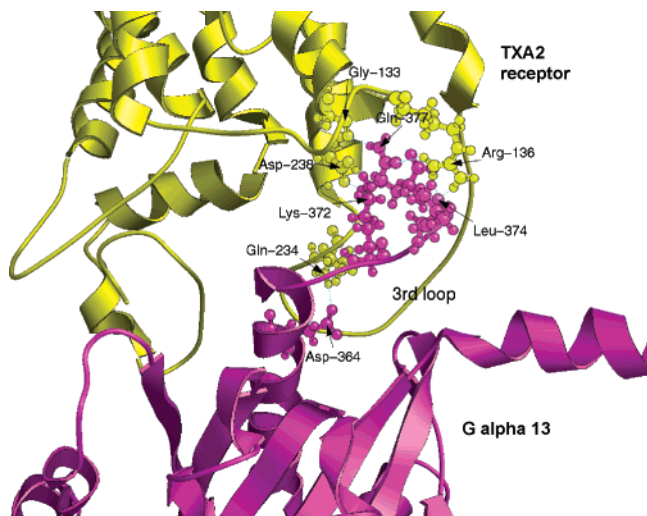


Figure 6. Close view of the binding region of the human TXA2 receptor and G alpha 13. The hydrogen bonds between the two molecules are shown by the green dotted lines. See Table 1 for the detailed information about the atoms involved in forming the seven hydrogen bonds.

V. Conclusion

The coupling of human TXA2 with G alpha 13 is mainly through the interaction between the minigene of G alpha 13 and the 3rd intracellular loop of the TXA2 receptor, which is consistent with the existing deduction in the literatures.^{38,42,43} Therefore, to conduct mutagenesis study, the residues in the 3rd intracellular loop (res. 222–239) of TXA2 receptor and those (res. 367–377) in the minigene of G alpha 13 might be the good candidates for mutation. Furthermore, although Arg-136 and Gly-133 of the TXA2 receptor do not belong to the segment of its 3rd intracellular loop, they are also involved in forming hydrogen bonds with the minigene of G alpha 13, implying that

they might play some role during the coupling interaction, thereby being good targets for mutation as well.

Abbreviations: 3D, 3-dimensional; GPCR, G protein-coupled receptors; TXA2 receptor, thromboxane A2 receptor; G alpha 13, alpha-13 subunit of guanine nucleotide-binding protein; Igg2, crystal structure of G protein GI alpha 1 chain A; 1f88, crystal structure of bovine rhodopsin.

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