

# Molecular Modeling of Neurokinin B and Tachykinin NK<sub>3</sub> Receptor Complex

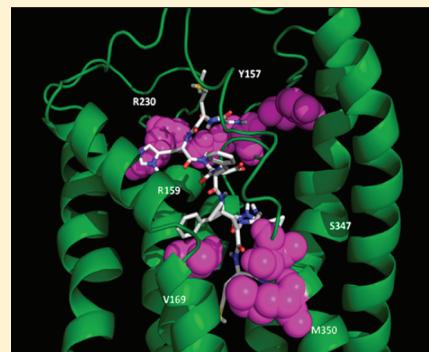
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 Supporting Information

**ABSTRACT:** The tachykinin receptor NK<sub>3</sub> is a member of the rhodopsin family of G-protein coupled receptors. The NK<sub>3</sub> receptor has been regarded as an important drug target due to diverse physiological functions and its possible role in the pathophysiology of psychiatric disorders, including schizophrenia. The NK<sub>3</sub> receptor is primarily activated by the tachykinin peptide hormone neurokinin B (NKB) which is the most potent natural agonist for the NK<sub>3</sub> receptor. NKB has been reported to play a vital role in the normal human reproduction pathway and in potentially life threatening diseases such as pre-eclampsia and as a neuroprotective agent in the case of neurodegenerative diseases. Agonist binding to the receptor is a critical event in initiating signaling, and therefore a characterization of the structural features of the agonists can reveal the molecular basis of receptor activation and help in rational design of novel therapeutics. In this study a molecular model for the interaction of the primary ligand NKB with its G-protein coupled receptor NK<sub>3</sub> has been developed. A three-dimensional model for the NK<sub>3</sub> receptor has been generated by homology modeling using rhodopsin as a template. A knowledge based docking of the NMR derived bioactive conformation of NKB to the receptor has been performed utilizing limited ligand binding data obtained from photoaffinity labeling and site-directed mutagenesis studies. A molecular model for the NKB-NK<sub>3</sub> receptor complex obtained sheds light on the topographical features of the binding pocket of the receptor and provides insight into the biochemical data currently available for the receptor.



## INTRODUCTION

Tachykinin receptors, also known as neurokinin receptors, are considered therapeutically important due to diverse physiological functions and their tractability as drug targets. Tachykinin receptors have been implicated in the pathology of psychiatric diseases such as depression, schizophrenia, and anxiety as well as other conditions, including asthma, pain, emesis, and arthritis.<sup>1</sup> Tachykinin receptors are activated by tachykinin peptide hormones, a family of biologically active peptides, widely distributed in the central and peripheral nervous systems, and are characterized by a common amidated C-terminal sequence, F-X-G-L-M-NH<sub>2</sub> where X represents either an aromatic (F, Y) or a branched aliphatic (V, I) amino acid. The C-terminal region or the message domain is believed to be responsible for activating the receptor.<sup>2</sup> The main mammalian members include substance P (SP), neurokinin A (NKA), and neurokinin B (NKB). The diverse biological effects of the tachykinins are mediated<sup>2–4</sup> via three known human tachykinin receptor subtypes, designated as NK<sub>1</sub>, NK<sub>2</sub>, and NK<sub>3</sub>. The endogenous tachykinin ligands interact with all tachykinin receptors, in spite of a defined agonist rank order of potency such that SP, NKA, and NKB have the highest affinities for the NK<sub>1</sub>, NK<sub>2</sub>, and NK<sub>3</sub> receptors, respectively. NKB (D-M-H-D-F-V-G-L-M-NH<sub>2</sub>), endogenous agonist ligand for NK<sub>3</sub> receptor, has been reported to play a vital role in the potentially life threatening disease pre-eclampsia<sup>5–7</sup> and as a neuroprotective

agent in case of neurodegenerative diseases.<sup>8</sup> A recent study indicates a fundamental role for the NKB pathway in the normal reproductive function.<sup>9</sup>

Among the tachykinin receptors, the NK<sub>3</sub> receptor is of particular interest because of its role in the pathophysiology of psychiatric disorders, including schizophrenia.<sup>10,11</sup> It is predominantly expressed in the central nervous system (CNS) and belongs to the rhodopsin family of G-Protein Coupled Receptors (GPCRs). However, the structure of GPCRs at the atomic level is known for a few GPCRs only such as bovine rhodopsin, human  $\beta$ 2-adrenergic receptor, turkey  $\beta$ 1-adrenergic receptor, and human A2A adenosine receptor (AR),<sup>12,13</sup> and, therefore, computer assisted molecular modeling is an alternative means to access potential 3D structures of GPCRs.<sup>14</sup>

The pharmacology of the NK<sub>3</sub> receptor is less well characterized in comparison of NK<sub>1</sub> and NK<sub>2</sub> receptors. Several agonists selected for the NK<sub>3</sub> receptor have been obtained by modification of the primary structure of NKB.<sup>15</sup> Senktide, suc[Asp<sup>6</sup>, MePhe<sup>3</sup>]SP(6–11)), a classical synthetic agonist, has been used extensively to study the role of NK<sub>3</sub> receptors<sup>16</sup> and to facilitate the study of putative NK<sub>3</sub> antagonists.<sup>17</sup> In recent years a number of selective antagonists of diverse chemical classes of the NK<sub>3</sub>

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receptors including osanetant<sup>18,19</sup> and talnetant<sup>20,21</sup> have emerged offering an alternative therapy for psychiatric disorders such as schizophrenia and bipolar syndrome.<sup>22–25</sup> While the residues in the binding site of NK<sub>1</sub> and NK<sub>2</sub> receptors involved in the interaction with endogenous agonist peptides or nonpeptide antagonists have been extensively characterized,<sup>15</sup> little is known about the NK<sub>3</sub> ligand binding pocket except for a few reports showing the involvement of TM2 residues M 134 and A 146 in the species-selectivity of an antagonist, saredutant (SR48968) for human NK<sub>3</sub> receptor.<sup>26,27</sup> Also, studies with chimeric NK1/NK<sub>3</sub> receptors have identified that the carboxyl terminal domain, part of the third extracellular loop and the seventh transmembrane region of the NK<sub>3</sub> receptor, is important for NKB binding.<sup>28</sup>

The determination of the structure of the ligand–receptor complex and an analysis of the topography of the binding pocket of the NK<sub>3</sub> receptor is an important step in the rational design of drugs. Lack of detailed structural information and absence of detailed 3D information regarding the NK<sub>3</sub> receptor has impaired the understanding of ligand–receptor interaction. As is the case for most GPCRs, expression, purification, crystallization, and structure determination remain difficult for the NK<sub>3</sub> receptor. Attempts have been made to build the molecular models of NK<sub>1</sub> and NK<sub>2</sub> receptors characterizing the receptor–ligand interaction.<sup>29–31</sup> A molecular model of the NK<sub>3</sub> receptor has been developed, and the mode of ligand binding of several nonpeptide antagonists has been characterized using this model.<sup>31–33</sup> The development of a similar understanding of the binding of the endogenous peptide ligand NKB to the NK<sub>3</sub> receptor has been much more challenging, and there are no reports to date on the NKB/NK<sub>3</sub> receptor complex.

In the present work, we describe a molecular model for the interaction of the primary endogenous ligand NKB with the NK<sub>3</sub> receptor. A homology model of the NK<sub>3</sub> receptor has been generated using bovine rhodopsin X-ray structure as a template. The membrane bound conformation of a representative potent NK<sub>3</sub> agonist NKB bound to DPC micelles<sup>34</sup> has been used for the docking studies (PDB ID 1P9F). Notably, the degree of amino acid sequence identity within human receptors is 51% between the NK<sub>1</sub> and NK<sub>3</sub> receptors and 41% between the NK<sub>2</sub> and the NK<sub>3</sub> receptors. In the absence of available structural data on the NK<sub>3</sub> receptor, ligand-binding data from the NK<sub>2</sub> and NK<sub>1</sub> receptors has been used for initial docking. The putative binding pocket for NK<sub>3</sub> peptide agonists has been proposed. The model of the NK<sub>3</sub> and NKB receptor complex developed here provides insight into the biochemical data on tachykinin receptors.

## METHODS

**Homology Modeling.** The modeling described here utilizes a segmented approach where the C-terminus, the N-terminus, the transmembrane (TM) helices, and the loop regions have been modeled on separate individual templates. The three-dimensional model for the NK<sub>3</sub> receptor was generated on a silicon graphics fuel workstation using homology module of INSIGHT II (Accelrys). Crystal structure of bovine rhodopsin, chain A (PDB ID 1F88)<sup>12</sup> was used as a template for the TM segments of the NK<sub>3</sub> receptor. The ends of the TM segments were defined using TMPred algorithm.<sup>35</sup> The structural features of second and third extracellular loop (EC2 and EC3) of the NK<sub>1</sub> receptor, as determined by high resolution NMR<sup>36,37</sup> were incorporated into EC2 and EC3 loops of NK<sub>3</sub> model. Homology for EC1 and three intracellular loops were identified from Protein data bank using a

random loop generation method and loop search method implemented in homology module. The sequence alignments were undertaken using the ClustalW site (located at [www.align.genome.jp](http://www.align.genome.jp)). The alignments of the NK<sub>3</sub> and bovine rhodopsin TM segments correspond to those identified in the GPCR database<sup>38</sup> located at (<http://www.gpcr.org/7tm>). The Blosum scoring matrix<sup>39</sup> was used for the clustalW alignments. In ClustalW the penalty value for closing or opening a gap was set at 10, and the penalty for extending a gap and separation of a gap was set at 0.1. Using the adjusted alignment, the homology model of the NK<sub>3</sub> receptor was constructed and consequently subjected to splice repair and energy minimizations. The disulfide bridge was added between C 158 and C 233 of EC2 and EC1 loops respectively before further refinement. Molecular dynamics and simulated annealing were carried out using the Discover module of INSIGHT II with specific distance constraints between TM helices of the NK<sub>3</sub> receptor from the GPCR database.<sup>40,41</sup> The modeled structure was validated with PROCHECK<sup>42</sup> and WHATIF.<sup>43</sup>

**Characterization of 3D Structure of NKB.** The three-dimensional structure of NKB (D-M-H-D-F-F-V-G-L-M-NH<sub>2</sub>) has been determined by a combined use of two-dimensional <sup>1</sup>H-NMR spectroscopy in a membrane mimetic solvent, dodecyl phosphocholine (DPC), and the torsion angle dynamics algorithm DYANA. The ensemble of structures has been deposited in Protein Data Bank (PDB ID code: 1P9F) and published.<sup>34</sup>

**Molecular Docking.** Protein flexibility and the absence of robust scoring functions pose a great challenge to docking field.<sup>44</sup> An increasing number of research groups have employed various strategies to include protein flexibility in docking.<sup>45,46</sup> An excellent review dealing with background and principles of existing protein–protein docking methods and algorithms describes challenges of treating flexibility in molecular docking.<sup>47–49</sup> Since currently available docking programs do not work very well for peptides, manual docking was conducted for NKB. The molecular docking of NKB (PDB ID 1P9F) with the NK<sub>3</sub> receptor was carried out on a silicon graphics fuel workstation, using Biopolymer and the docking modules of Sybyl6.9/TRIPOS software package. It was assumed that the same helix packing arrangement is present in all GPCR family a receptors. Therefore, ligand binding and site directed mutagenesis data were collated from a number of different receptors (Supporting Information, Table 1). A manual docking based on the common residues conserved in GPCR<sup>12,50–56</sup> subfamily particularly NK<sub>1</sub>,<sup>28,57–60</sup> NK<sub>2</sub>,<sup>61–64</sup> and NK<sub>3</sub><sup>62,65</sup> receptors was followed by energy minimization of the binding pocket interaction using minimize-dock. The agonist–receptor complex was subjected to global energy minimization by the Powel method using Kollman all atom force field. To achieve meaningful docking modes it was ensured that no steric clashes occurred between any of atoms. Various relative orientations of the peptide within the binding pocket were tried during initial docking, and the final orientation was selected by monitoring docking energy, hydrogen bonds, and van der Waals contacts.

**Generation and Refinement of Homology Model for NK<sub>3</sub> Receptor.** Chain A from the crystal structure of rhodopsin (PDB ID 1F88) was used as a template for homology modeling of 7TM, IC1, EC1 and C-terminus region of NK<sub>3</sub> receptor. The sequence alignment (Figure 1) of NK<sub>3</sub> plus (conservative substitutions) for each of the seven TM and loop regions are TM1, 5% (46%); TM2, 36% (46%); TM3, 23% (59%); TM4, 22% (44%); TM5, 17% (57%); TM6, 48% (62%); TM7, 32% (59%); EC1, 22% (39%); EC2, 33% (47%); EC3, 26% (30%); IC1, 37% (82%); C-terminus, 37% (45%); N-terminus, 8% (33%). Coordinates

<b>TM1_NK3</b>	ALWSLAYGVVVAVALGNLIVI	<b>EC1_NK3</b>	VNFIFYALHSEWYFGANYCRFQNF
<b>TM1_1F88</b>	SMLAAYMFLILMCLFPINFLTL :: : :: : : *:::	<b>EC1_1F88</b>	TTLYTSIHGYFVFPTGCNLEGF .. : : * . : * * . * . : :
<b>TM2_NK3</b>	VTNYFLVNLAFSDASMAAFNTL	<b>EC2_NK3</b>	--QCLYSKTKVMPGRTLCFVQWPPEGPKQH-----
<b>TM2_1F88</b>	PLNYILLNLAVADLFMVFGGFT **;*:*** . * . *	<b>EC2_NK1</b>	FPQGYYSTETMPSRVVCMIEWPEHPNRTYEKAYHI * **.*.**.*.:.**:::**** * ;:
<b>TM3_NK3</b>	FPIITAVFASIYSSMTAIAVDRYM	<b>EC3_NK3</b>	-----AIYQQLNRWKYIQQV-----
<b>TM3_1F88</b>	FATLGGEIALWSLVLAIERYV *. . ::*::: *::: *:	<b>EC3_NK1</b>	FHVFFLLPYINPDLYLKKFIQQVYLAS * : * * : ****
<b>TM4_NK3</b>	KIVIGSIWILAFLLAFFP-	<b>IC1_NK3</b>	WIILAHKRMRT
<b>TM4_1F88</b>	-MGVAFTWVMALACAAPP : .. *::*: * *	<b>IC1_1F88</b>	YTVQHKKLRT : : * *::*
<b>TM5_NK3</b>	FTYHIIIVILVYCPLLIMGYT	<b>IC2_NK3</b>	AIIDPLKPRLSATAT
<b>TM5_1F88</b>	ESFVYIMFYVHFIIPLIVIFFCY : * :: : : : * *::: : *	<b>IC3_NK3</b>	TIVGITLWGGEIPGDTCDKYHEQLAKRKVVKM
<b>TM6_NK3</b>	MIVVMTFAICWL PYHIYFIL	<b>C_TER_NK3</b>	NKRF RAGFKRA
<b>TM6_1F88</b>	VIIMVIAFLICWL PYAGVAFY : * *::: * ***** :	<b>C_TER_1F88</b>	NKQFRNCMVTT * * : * : :
<b>TM7_NK3</b>	YLASFWLAMSSTMNPPIIYCLL	<b>N_TER_NK3</b>	LTNQFVQPSWR
<b>TM7_1F88</b>	MIIPAFFAKTSAYVNPVIYIMM : . : * : * : * * : * :	<b>N_TER_1F88</b>	APQYYLAEPWQF . : . *::

Random loop generation

**Figure 1.** Sequence alignment of human NK<sub>3</sub> receptor with chain A of bovine rhodopsin. In all sequence alignment figures, an asterisk (\*) indicates an identical amino acid; a ":" indicates a "conserved" residue, which meets the criteria for either highly conservative substitutions or semiconservative substitutions, as defined by ClustalW, using the Gonnet Pam250 matrix.

for EC2 and EC3 loops were set to an alpha helical structure in correlation to similar structural features in the NK<sub>1</sub> receptor as elucidated by NMR.<sup>36,37</sup> IC2 and IC3 loops were generated by a random loop generation feature of the homology module. The NK<sub>3</sub> receptor model was optimized using a molecular mechanics method employing a distance dependent dielectric constant, CVFF force field, and conjugate gradient minimization until the final convergence criterion of 0.1 kcal/mol was achieved. The resulting structure was then subjected to short a MD run using Discover (Accelrys) in order to remove the initial strain. During this procedure, the backbone topology of the TM helices was preserved by constraining all backbone atoms to initial positions. The model was further refined by extensive simulated annealing, wherein the structure was gradually heated to 300 K over 15 ps followed by a 100 ps MD simulation at a constant temperature of 300 K with the TM backbone conformation held fixed. Snapshots were extracted every 1 ps. The trajectory was analyzed, and the structure with the lowest energy and loops in proper topology (i.e., not interfering with the proposed binding site) was selected as the template for the docking studies.

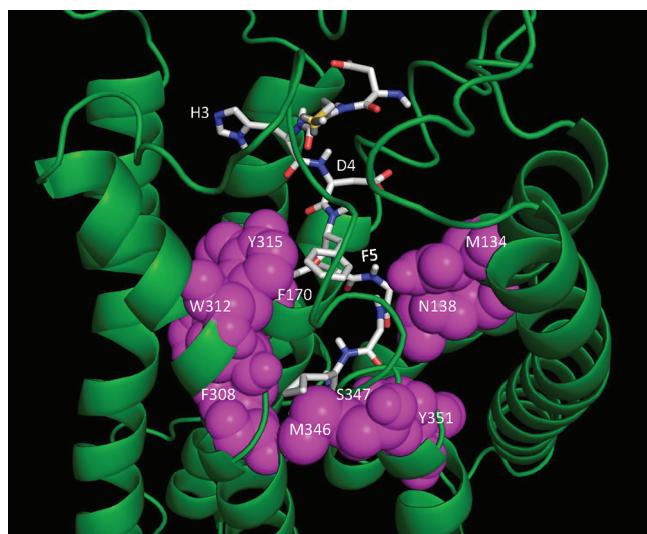
After energy refinement and molecular dynamics, the final selected modeled structure was obtained. The model was further validated with PROCHECK and WHATIF. The PROCHECK statistics showed that 94% of residues in the NK<sub>3</sub> model are in the favored regions of Ramachandran plot. The overall main-chain and side-chain parameters, as evaluated by PROCHECK, are also very favorable. The WHAT IF validation found loose rms-Z scores, which are typical of the model, as opposed to experimental structures.

## RESULTS

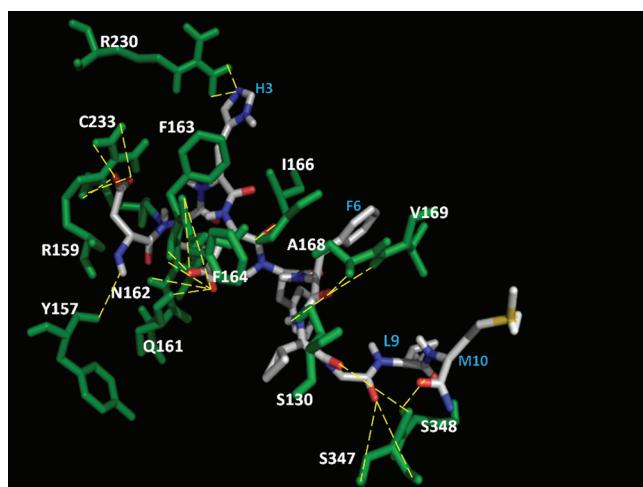
**Molecular Docking Studies.** The three-dimensional structure of NKB derived from NMR studies<sup>34</sup> was utilized in docking

NKB on the NK<sub>3</sub> receptor. NKB was docked manually onto the NK<sub>3</sub> receptor by placing the C-terminal moiety of NKB that composes the pharmacophore of the peptide into the cavity formed by transmembrane segments of the receptor. The positioning was also guided by inspection of the van der Waals contacts and docking energy. At each step of the ligand docking the structure was refined by submitting the receptor–ligand complex to molecular dynamics followed by energy minimization. The manual docking was followed by energy minimization of the binding pocket interaction using minimize–Dock. The agonist–receptor complex was subjected to global energy minimization by the Powel method using Kollman all atom force field. To achieve meaningful docking modes it was seen that no steric clashes occurred between any atom and that the docked structure well-interpreted the structure–activity data for NKB. The final orientation of the agonist within the binding site was selected by monitoring the hydrogen bonds, van der Waals contacts, and docking energy of the agonist. To examine the variations in the ligand and receptor conformation, rmsd with respect to the starting structure was calculated. The final intermolecular docking energy was -85 kcal/mol. The rmsd for both the ligand and receptor backbone on final docking is 0.86 and 1.1 Å respectively.

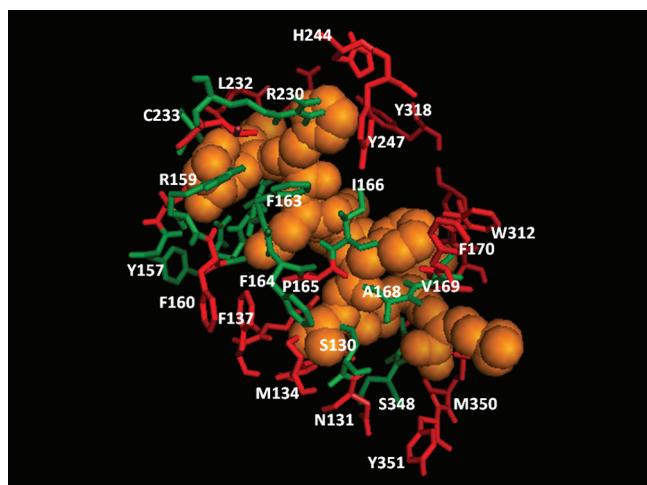
The energy-minimized structure of NKB in its final docked position is shown in Figure 2. In this model the backbone of NKB adopts a helical conformation with the C-terminal NH<sub>2</sub> (amide) group in close proximity to S346/M350 in TM7 of the receptor, and the N-terminus lies in close proximity to the extracellular region, especially the N-terminus of the receptor. After selecting the final docked position of the NKB ligand, the NK<sub>3</sub> receptor–ligand interactions were analyzed. NKB was found to make extensive van der Waals contacts with several of the key residues of the NK<sub>3</sub> receptor (Figure 3). The complete list of these contacts is given in Table 2 in the Supporting Information. Another



**Figure 2.** The NKB-NK<sub>3</sub> receptor complex showing pharmacophore complementarity. The residues forming the three main hydrophobic binding pockets of the receptor are shown in magenta, and the ligand is shown in stick model. The TM helices of the receptor are shown in green.



**Figure 4.** Hydrogen bonds formed between NKB (colored by element CHNO)) and NK<sub>3</sub> receptor (in green). Hydrogen bonds are shown as yellow dashed lines. The residues have been listed in Table 3 of the Supporting Information.



**Figure 3.** The final docked position of NKB (orange) in the binding pocket of NK<sub>3</sub> receptor (regions colored green and red). The residues in red are those, which make van der Waals interactions (3.8 Å) with the ligand. Only those receptor residues that are within 5 Å from the ligand are shown here. All the residues have been listed in Table 2 of the Supporting Information.

important feature of the NKB/NK<sub>3</sub> receptor complex is the occurrence of a tight hydrogen-bonding network (Figure 4). The receptor–ligand complex is stabilized by thirty-two hydrogen bonds, and most of these hydrogen bonds are formed by the N-terminal residues of the NKB receptor. A list of the intermolecular hydrogen bonds formed between NKB and the NK<sub>3</sub> receptor is given in Table 3 in the Supporting Information.

The key peptide residues F6, L9, and M10, identified as being important for pharmacological function as well as being identified in our study as pharmacophore points,<sup>66</sup> were found to have significant interactions with the NK<sub>3</sub> receptor. The pharmacophore pattern complementarity is observed for the NK<sub>3</sub>/NKB

complex with the three hydrophobic pharmacophores of NKB being bound to three predominantly hydrophobic pockets of the receptor. A hydrophobic binding pocket consisting of S130, P165, I166, A168, V169, F170, and W312 side chains encloses the aromatic ring of F6 of NKB. The phenyl ring of conserved F6 of NKB appears to be optimally oriented to interact with the phenyl ring systems of W312 and F170 of the NK<sub>3</sub> receptor. The binding pocket of L9 of NKB is formed by C311, W312, P314, L344, A345, M346, and S347 and the binding pocket for M10 is formed by F123, V169, S172, M176, V304, F308, C311, S347, S348, and M350. The C-terminal amide of NKB forms a number of important contacts with the receptor especially with S347, S348, M350, and Y351. Further, structure activity studies have shown that the C-terminal free acid derivatives of NKB are weaker agonists for the NK<sub>3</sub> receptor and thus implicating the importance of C-terminal amidation for agonist activity and agonist binding. The conserved C-terminal residues of NKB lie in a well-defined hydrophobic pocket formed by residues mainly from TM7.

## ■ DISCUSSION

This manuscript describes the binding mode of NKB to the NK<sub>3</sub> receptor by molecular modeling and docking studies. We have used a segmented approach for modeling NK<sub>3</sub>, a GPCR receptor. Such a procedure is only justified if there is evidence that the individual segments in GPCRs can fold independently. For rhodopsin, it has been shown that the C-terminal region and the intracellular loops form compact structures that retain functional activity when examined as isolated polypeptides.<sup>67–69</sup> Furthermore, it has been recently shown that various TM segments of the  $\alpha$ -factor receptor can fold and assemble into functional forms independent of the loop segments.<sup>70</sup> Another key feature of the NK<sub>3</sub> model presented here is the inclusion of the coordinates for EC2 and EC3 loops in correlation to similar structural features in NK<sub>1</sub> receptor.

It is apparent from our modeling studies that the C-terminus of NKB binds within the transmembrane bundle of the NK<sub>3</sub> receptor and the hydrophilic N-terminus of NKB interacts with extracellular loop regions of the receptor. The putative binding

pocket of the NK<sub>3</sub> receptor spans TM2, TM3, TM6, and TM7 along with EC1 and EC2 loops (Figure 2). In the absence of extensive structure–activity studies on the NK<sub>3</sub> receptor, our docking studies offer a theoretical basis for new experimental investigations.

Our results show that the N-terminus of NKB specifically D1, M2, and H3 seem to be more solvent accessible than the rest of the peptide and make several contacts with the extracellular regions of the receptor. The conserved C-terminus of NKB, especially L9 and M10 interacts with several hydrophobic residues of the receptor. We propose that Y315, W312, and F170 receptor residues play a ligand-stabilizing role through van der Waals interactions with the conserved F6 residue of NKB. It is interesting to note that Y315 is conserved in the NK<sub>2</sub> receptor and is shown to be important for binding of NKA by site directed mutagenesis studies.<sup>61–64</sup> Further, the conserved aromatic micro domain formed by a cluster of aromatic residues in TM5 and TM6 has been suggested by several studies to play an important role in ligand binding in several GPCRs.<sup>50,55</sup>

Our data also identify residues of the NK<sub>3</sub> receptor that have the potential to form hydrogen bonds with NKB and therefore may be involved in its correct localization and receptor selectivity. D1 residue shows salt bridge interaction with R159 residue of the receptor. We propose that the N-terminal residues of NKB, D1, H3, and D4 act as anchoring points and might be important for the receptor selectivity. Hydrophobic interactions have been proposed as contributing a major portion of the binding energy in case of NK<sub>1</sub> and NK<sub>2</sub> receptors.<sup>30,33,36</sup> In contrast, we propose that both hydrophobic and electrostatic interactions as observed in this study might play an important role and contribute toward binding energy in the case of NKB and the NK<sub>3</sub> receptor complex. These observations are corroborated by concepts expressed in recent modeling studies on peptide–protein interactions.<sup>71–73</sup>

The docking data, further, correlate well with the pharmacophore pattern proposed by us for NK<sub>3</sub> agonists,<sup>66</sup> and a pharmacophore pattern complementarity is observed in the ligand–receptor complex (Figure 2). This is further in accordance with the hypothesis that the binding site will be complementary to the distribution of polar/nonpolar atoms of the ligands in their bioactive conformations.

A strong support for our modeling results is provided by the recently reported site directed mutagenesis studies to identify the binding pocket of NK<sub>3</sub> receptor ligands Me-talnetant and osanetant.<sup>33</sup> Comparison between the NKB binding site in the NK<sub>3</sub> receptor as described here and that of the nonpeptide NK<sub>3</sub> receptor antagonists Me-talnetant and osanetant<sup>33,18</sup> provides interesting information. The data indicate that TM6 and TM7 are critical for the binding of both the peptide agonist, NKB and the nonpeptide antagonists Me-talnetant and osanetant,<sup>33</sup> and these ligands share common points of anchorage in the NK<sub>3</sub> receptor. The agonist and antagonist binding sites are distinct but partially overlap. The residues N138, N142, L232, Y315, F342, and M346 have been reported to be critical for the NKB binding site.<sup>33</sup> However, from our model, N142 residues do not appear to participate in direct binding to NKB, and therefore we propose that this residue may play a role as structural determinants of the local conformation around the binding pocket of the receptor. Moreover, the prediction of the correct binding mode of an antagonist seems to be a more difficult goal than the modeling of the agonist bound receptor. Small molecule agonists of a given Family A GPCR are perhaps more likely than other antagonists

to occupy a conserved region of the receptor, because of a commonality in the receptor activation mechanism. In theory, in order to block agonist action, antagonists need to overlap only partially with the docked position of agonists. Thus, the hypothesis that antagonists display a more diverse range of docking modes than agonists is reasonable.<sup>74</sup>

In conclusion, the study reported here provides an experimentally testable hypothesis for a putative NK<sub>3</sub> agonist-binding pocket. As more potent and selective antagonists emerge it should be possible to model more precisely binding epitopes for the NK<sub>3</sub> receptor. It should be noted that there are several limitations and uncertainties in modeling GPCR pockets on the basis of the bovine rhodopsin structure and the homology model described here is of low resolution and approximate, thus may not correctly reflect the native configuration of the NK<sub>3</sub> receptor. Moreover the micelles bound NMR structure of NKB utilized in docking is an average structure from an ensemble of structures and may not necessarily reflect that active receptor bound conformation of the peptide as there could be structural deviations in the ligand upon binding to the receptor. Hence, the results presented in this study should be treated with caution. However, recently a comparison of the receptor-bound and micelle-bound states of a peptide hormone indicated only a small difference in the conformation.<sup>75</sup> This offers a strong rationale for utilizing the lipid-induced structure of NKB in docking studies of NK<sub>3</sub> receptor. Moreover, a recent study demonstrated that GPCRs maintain their general folding characteristics by means of structural mimicry, despite low homology between these receptors.<sup>49</sup> Therefore, given the conservation of key residues in the family of GPCRs, there are good reasons to assume that the geometry of the transmembrane region will be conserved among the receptors. The limited ligand-binding data available for NKB indicate that most of the crucial ligand–receptor interactions occur within the transmembrane region of the receptor, and, therefore based on these observations, the model described here appears sufficiently relevant.

## ASSOCIATED CONTENT

**S Supporting Information.** Data on NKB-NK3 receptor complex (conserved residues, van der Waals, and hydrogen bond contacts). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## ■ ABBREVIATIONS:

GPCR, G-protein coupled receptor; NKB, neuropeptide NKB; tachykinin receptor subtypes, NK<sub>1</sub>, NK<sub>2</sub>, NK<sub>3</sub>; SP, substance P; DPC, dodecyl phosphocholine

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