

Molecular Modeling of the Peptide Agonist-Binding Site in a Neurokinin-2 Receptor

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The neurokinin-2 receptor is a member of the rhodopsin family of G-protein coupled receptors, which represents one of the most relevant target families in small-molecule drug design. NK-2 receptors have been implicated in playing a pathophysiological role in asthma. Activation of the NK-2 receptor by its endogenous peptide agonist, tachykinins, is associated with diverse biological responses like bronchoconstriction, vasodepression, and regulation of endocrine functions. Agonist binding to the receptor is a crucial event in initiating signaling, and therefore 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 NKA with its G-protein coupled receptor neurokinin-2 receptor has been developed. A three-dimensional model for the NK-2 receptor has been generated by homology modeling using rhodopsin as a template. A knowledge based docking of the NMR derived bioactive conformation of NKA to the receptor has been performed utilizing the ligand binding data obtained from the photoaffinity labeling and site-directed mutagenesis studies. The molecular model for the NKA/NK-2 receptor complex thus obtained sheds light on the topographical features of the binding pocket of the receptor and provides atomic insight into the biochemical data currently available for the receptor. The results of the receptor modeling studies have been used to discuss the molecular determinants for NK-2 receptor selectivity.

INTRODUCTION

Protein–protein recognition represents a fundamental aspect of biological function. Peptides are the primary means of intracellular communication in many diverse biological systems. This intracellular communication generally consists of interactions between these peptide messengers and receptors embedded in the cell surface. Large subsets of these receptors couple interactions with G-proteins to their interactions with these messengers and hence are called the G-protein coupled receptors (GPCRs). Seven alpha helical transmembrane domains characterize these GPCRs. GPCRs represent one of the most relevant target families in small-molecule drug design, and the molecular basis of peptide hormone recognition by GPCRs has been a topic of interest for a very long time.

The neurokinin-2 (NK-2) receptor is a member of the rhodopsin family of GPCR signaling proteins. The NK-2 receptor is primarily activated by the tachykinin peptide hormone neurokinin A (His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH₂) and its N-terminally extended forms NPK and NP γ . These peptides are characterized by a common C-terminal sequence, Phe-X-Gly-Leu-Met-NH₂, and this C-terminal domain is believed to be responsible for activating the receptor. The NK-2 receptor is found in abundance in the central and peripheral nervous systems. Activation of the

NK-2 receptor by NKA and analogs are associated with diverse biological functions like broncoconstriction, vasodilation, increase in heart rate, regulation of endocrine functions, stimulation of salivary secretion, and antidiagnostic action.^{1,2} In humans, the NK-2 receptors are present on smooth muscle of both large and small airways and mediate tachykinin-induced contraction in isolated large airways.³ In view of their potent effects on the airways, tachykinins have been implicated as possible mediators of asthma, and therefore tachykinin receptor antagonists are a potential new class of antiasthmatic medication. Owing to diverse physiological functions, the NK-2 receptor has been long regarded as an important drug target, and there is considerable interest in NK-2 agonists for drug design, especially in the development of novel antiasthmatics.

Understanding the structure of the ligand-receptor complex and analysis of the topography of the binding pocket of the NK-2 receptor is crucial in rational design of drugs. However, little data are available on ligand receptor interaction and on the NK-2 receptor ligand complex. Lack of detailed structural information and the absence of detailed 3D information regarding the NK-2 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-2 receptor. However the X-ray structure of bovine rhodopsin, a prototype GPCR, has been published (PDB ID 1F88),⁴ and its crystal structure serves as a template reference for homology modeling of all other members of the GPCR family.^{5–9} Though the obtained models exhibit only limited accuracy, it has been demonstrated that ligand binding at

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GPCRs can be rationalized with the aid of homology models based on rhodopsin, and the modeled receptor pocket conformations can be validated or improved via docking of known ligands.^{5–7,10,11} Over the years, several molecular models of the NK-2 receptor have been developed, and the mode of ligand binding of several nonpeptide antagonists has been characterized using these models.^{10,12–15} The development of a similar understanding of the binding of the endogenous peptide ligand NKA to the NK-2 receptor has been much more challenging, and there are few reports on the NKA/NK-2 receptor complex.¹⁶

In the present work, we describe a molecular model for the interaction of the primary endogenous ligand NKA (His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH₂) with the NK-2 receptor with the aim of gaining a better understanding of the ligand/receptor interactions and the agonist binding mode. The homology model of the NK-2 receptor has been generated using a bovine rhodopsin X-ray structure as a template, and the NMR structure of NKA has been docked to the receptor based on experimental data from mutational studies and photoaffinity labeling studies. The results of the receptor modeling studies have been used to discuss the molecular determinants for NK-2 receptor selectivity.

METHODS

Homology Modeling. The three-dimensional model for the NK-2 receptor was generated on a Silicon graphics fuel workstation using the Homology module of Insight II (Accelrys). The transmembrane regions of the NK-2 receptor were modeled using the rhodopsin template. The boundaries of TM helices of the NK-2 receptor were set to those of rhodopsin as identified from the crystal structure of rhodopsin (PDB ID 1F88).⁴ The Cα coordinates of rhodopsin were used to construct the seven TM helices for the NK-2 receptor. The backbone atoms of rhodopsin were transferred to those of the corresponding backbone atoms of the NK-2 receptor in accordance to the sequence alignment between them, while the side chains were mutated using the Homology module of Insight II. The flexible loop regions were constructed using the random loop generation and loop search methods implemented in the Homology module. The search loop method used an existing alpha-carbon distance matrix to search for regions of proteins in the PDB whose alpha-carbon distances best fit those of the selected region of the protein being studied, while meeting the additional constraint of having the specified number of residues present between the regions of interest. With the loop generate method a peptide backbone chain was built between two transmembrane segments using randomly generated values for all the Φ and Ψ dihedral angles of the loops. A set of distances was defined about the base of the loop that must meet certain criteria in order for the loop to be considered. Coordinates for Extracellular loop 2 and Extracellular loop 3 were set to the alpha helical structure in correlation to the similar structural feature in the NK-1 receptor as elucidated by NMR.^{17,8} The final 3D model was obtained by optimization through conjugate gradients and molecular dynamics with simulated annealing.

Refinement of the Receptor Model Using Molecular Mechanics and Dynamics. Energy minimization and MD simulation protocols of the receptor were carried out on a Silicon Graphics Fuel workstation (SGI, California, USA).

The *Discover* program (Insight II, Accelrys) was used to refine the homology model generated for the NK-2 receptor. *Discover* provided functionality to minimize the energy of a molecular system to produce a model protein that is chemically and conformationally reasonable. The CVFF force field was used for minimizations of the NK-2 receptor. A distance-dependent dielectric constant was used for summation of nonbonding interactions. For energy minimizations, the steepest descent method was applied first followed by the conjugate gradients method until the final convergence criterion of 0.1 kcal/mol Å was achieved. The *Discover* program was used for extensive molecular dynamics simulation of the homology model generated for the NK-2 receptor. Molecular dynamics simulation was used to refine the homology model generated for the NK-2 receptor and to obtain proper topology of the loop regions. The stereochemical quality of the homology model for NK-2 was validated using Procheck,¹⁸ wherein the accuracy of geometric parameters such as bond length, bond angles, torsion angles, and correctness of the amino acid chiralities was checked.

Characterization of 3D Structure of NKA. The three-dimensional structure of NKA (His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH₂) has been determined by the combined use of 2D ¹H NMR spectroscopy in membrane mimetic solvent, DPC, and the torsion angle dynamics algorithm DYANA.¹⁹ The ensemble of structures has been deposited in the Protein Data Bank (PDB ID code: 1N6T) and published.¹⁹

Molecular Docking. Protein flexibility and the absence of robust scoring functions pose a great challenge to docking field.²⁰ An excellent review dealing with background and principles of existing protein protein docking methods and algorithms describes challenges of treating flexibility in molecular docking.²¹ Since currently available docking programs do not work very well for peptides, manual docking was conducted for NKA and molecular docking of NKA to the NK-2 receptor was carried out on a Silicon graphics O2 workstation (SGI, California, USA), using Biopolymer and Docking modules of the Sybyl 6.9/TRIPOS software package (Tripos, St. Louis, MO, USA). The ligand binding data obtained from the photoaffinity labeling and site-directed mutagenesis were used to guide the docking of the NKA to the NK-2 receptor.^{16,22–24} The bioactive conformation of NKA determined by NMR¹⁹ was manually docked in the NK-2 receptor model. 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 the Kollman all atom force field. To achieve meaningful docking modes it was seen that no steric clashes occurred between any atom, and the docked structure well-interpreted the SAR and mutagenesis data for NKA. 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.

RESULTS

Generation and Refinement of Homology Model for the NK-2 Receptor. The structure of GPCRs at the atomic level is known for bovine rhodopsin. The X-ray structure of bovine rhodopsin has advanced our understanding of the

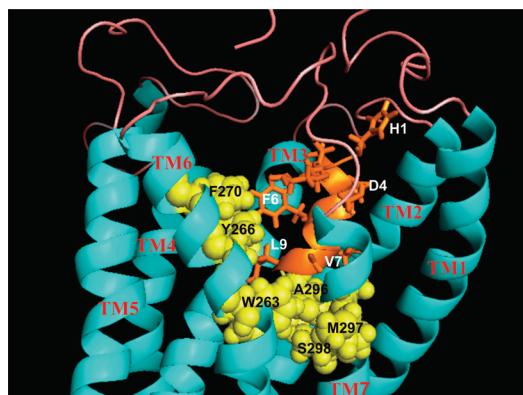


Figure 1. The NKA/NK-2 receptor complex indicating the pharmacophore complementarity. The residues forming the three main hydrophobic binding pockets of the receptor are shown in yellow, and the ligand is colored orange. The TM helices of the receptor are shown in cyan.

structure and activation of GPCRs.⁴ Crystal structure of rhodopsin is widely used as a structural template to model the transmembrane domains of other GPCRs, and it has been demonstrated that such models may be successfully used to identify both agonists and antagonists by virtual screening of compound libraries.²⁵ Homology modeling has been used to generate three-dimensional model for the NK-2 receptor. Rhodopsin serves as a good template for homology modeling of tachykinin receptors as it has been long regarded as a prototype GPCR. Based on the assumption that GPCRs share similar TM boundaries and overall topology, a homology model of the NK-2 receptor has been constructed using the X-ray crystal structure of bovine rhodopsin (PDB ID: 1F88)⁴ as the structural template.

The amino acid sequences of TM helices of the NK-2 receptor were aligned with those of bovine rhodopsin (Figure 2, Supporting Information) guided by the highly conserved amino acid residues.^{26–29} Transmembrane regions for the NK-2 receptor were predicted based on hydrophobicity profiles using the program TMHMM which employs a hidden Markov model to locate transmembrane regions.³⁰ The results were consistent with the boundaries derived from rhodopsin structure. Hence, the same boundaries were applied for the TM helices of the NK-2 receptor as derived from the X-ray crystal structure of bovine rhodopsin.⁴ Extracellular loop 1 and intracellular loops of the NK-2 receptor were constructed by the random loop search and generate loop methods implemented in Insight II, utilizing the Brookhaven protein data bank. Coordinates for extracellular loop 2 and extracellular loop 3 were set to an alpha helical structure in correlation to a similar structural feature in the NK-1 receptor as elucidated by NMR.^{17,8} Based on the finding that Cys 106 and Cys 181 are conserved and form a disulfide bridge, a disulfide link was inserted between the two residues.^{31,32}

The NK-2 receptor model was optimized using a molecular mechanics method employing a distance dependent dielectric constant, a CVFF force field, and conjugate gradient minimization until the energy gradient rms was less than 0.1 kcal/mol. The NK-2 receptor was minimized until the final convergence criterion of 0.1 kcal/mol was achieved. The resulting structure was subjected to a short MD run using *Discover* (Accelrys) in order to remove the initial strain. During this, the backbone topology of the TM helices was preserved by constraining all backbone atoms to initial

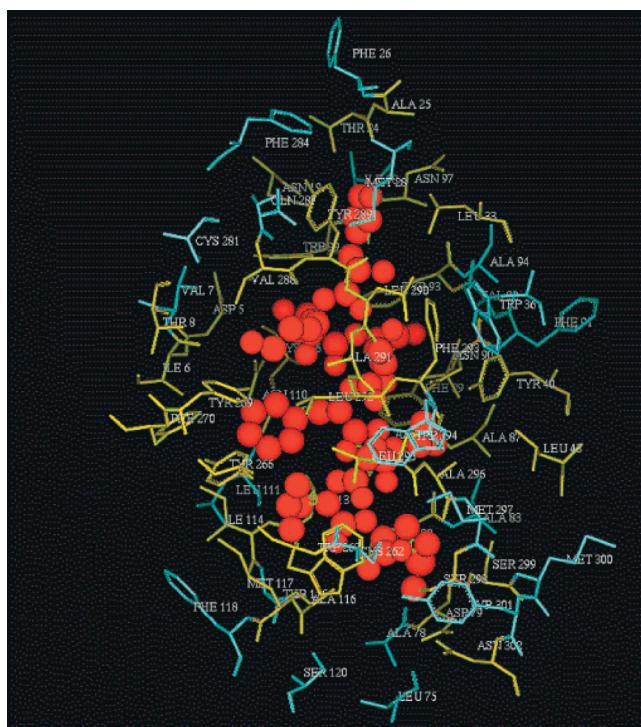
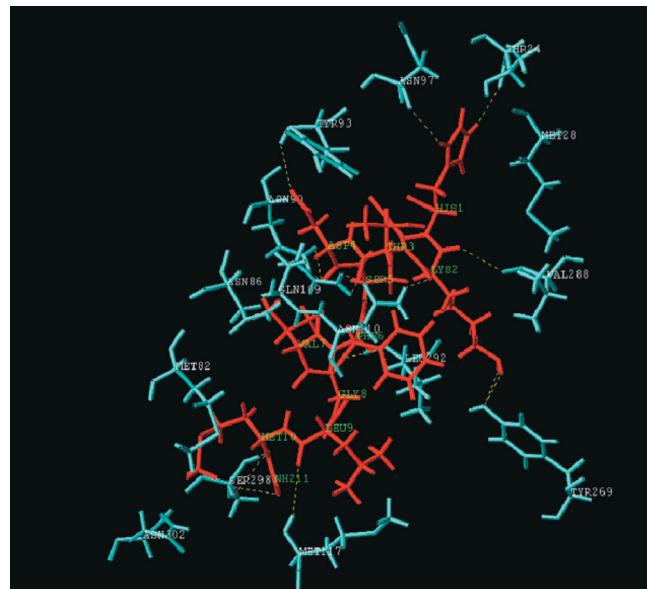


Table 1. Final Energies and RMSD for NKA and the NK-2 Receptor on Final Docking with Respect to Their Respective Starting Conformations

molecule	energy of starting conformation	final energy	rmsd for backbone atoms	rmsd for all atoms
ligand (NKA)	77 kcal	-120 kcal	0.8	1.4
NK-2 receptor	656 kcal	-521 kcal	0.6	1.9

finally, the whole receptor–ligand complex was submitted to energy minimization. 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. The energy-minimized structure of NKA in its final docked position is shown in Figure 1. In this model the backbone of NKA adopts a helical conformation with the C-terminal NH-2 group in close proximity to Ser 298 in TM7 of the receptor, and the N-terminus lies in close proximity to the extracellular region, especially the N-terminus of the receptor. The final intermolecular docking energy was -101.866 kcal. Table 1 shows the final energy of the ligand and receptor. To examine the variations in the ligand and receptor conformation, rmsd with respect to the starting structure was calculated (Table 1).

NKA was found to make extensive van der Waals contacts with NK-2 receptor residues (Figure 2). The complete list of these residues is given in Table 3 in the Supporting Information. The model was checked for consistency with mutational and known ligand binding data. NKA was found to make extensive contacts with several of the key residues of the NK-2 receptor identified crucial for ligand binding by photoaffinity labeling and mutation data.^{16,22–24,33–35} An important feature of the NKA/NK-2 receptor complex is the occurrence of a tight hydrogen-bonding network, which may play a role in stabilizing the complex (Figure 3). Table 2 lists the intermolecular hydrogen bonds formed between NKA and the NK-2 receptor. The receptor–ligand complex is stabilized by seventeen hydrogen bonds, and most of these hydrogen bonds are formed by the N-terminal residues of NKA.

**Figure 3.** H-bonds formed between NKA (in red) and the NK-2 receptor (in cyan). Hydrogen bonds are shown as dashed yellow lines.**Table 2.** Hydrogen Bonds Formed between NKA and the NK-2 Receptor

ligand atom	receptor atom	distance in Å
His 1:Nε2	Thr ²⁴ :O	2.894
His 1:Nδ1	Asn ⁹⁷ :O	2.457
Lys 2:H	Val ²⁸⁸ :O	2.198
Lys 2:O	Asn ¹¹⁰ :Nδ2	3.521
Lys 2:NH1	Tyr ²⁶⁹ :OH	2.792
Lys 2:NH2	Tyr ²⁶⁹ :OH	2.393
Thr 3:Oγ1	Asn ¹¹⁰ :Nδ2	3.227
Thr 3:O	Gln ¹⁰⁹ :Nε2	2.211
Thr 3:O	Gln ¹⁰⁹ :Nε1	3.440
Asp 4:Oδ2	Tyr ⁹³ :H	3.244
Asp 4:O	Asn ⁹⁰ :Nδ1	3.434
Asp 4:O	Asn ⁹⁰ :Nδ2	1.937
Gly 8:H	Leu ²⁹² :O	2.25
Leu 9:O	Met ¹¹⁷ :H	3.528
Met 10:O	Ser ²⁹⁸ :H	2.60
Met 10:O	Ser ²⁹⁸ :γH	3.06
NH ₂ :HN2	Ser ²⁹⁸ :Oγ	2.896

The key peptide residues Phe6, Leu 9, and Met 10, identified as being important for biological function as well as being identified in our study as pharmacophore points, were found to have significant interactions with the NK2 receptor. Pharmacophore pattern complementarity is observed for the NK2/NKA complex with the three hydrophobic pharmacophores of NKA being bound to three predominantly hydrophobic pockets of the receptor. A hydrophobic binding pocket consisting of Asn 86, Gln 109, Asn 110, Pro 113, Ile 114, Tyr 266, Phe 270, and Leu 292 side chains hosts the aromatic ring of Phe 6 of NKA. The phenyl ring of conserved Phe 6 of NKA appears to be optimally oriented to make aromatic interactions with the phenyl ring systems of Tyr 266 and Phe 270 of the NK-2 receptor. The binding pocket of Leu 9 of NKA is formed by Ile114, Met 117, Ala 116, Trp 263, and Tyr 266, and the binding pocket for Met10 is formed by Ala 296, Met 297, Ser 298, and Met 82.

The C-terminal amide of NKA forms a number of important contacts with the receptor especially with Ala 116, Met 117, Trp 263, and Ser 298. The C-terminal amide also makes a hydrogen bond with Ser 298. This indicates the importance of C-terminal amidation for the agonist binding. Further, structure activity studies have shown that the C-terminal free acid derivatives of NKA are weaker agonists for the NK-2 receptor implicating the importance of C-terminal amidation for agonist activity.³⁶ The conserved C-terminal residues of NKA lie in a well-defined hydrophobic pocket formed by residues mainly from TM2, TM3, TM6, and TM7. Thus hydrophobic interactions are proposed to contribute a major portion of the binding energy of NKA.

DISCUSSION

We have characterized the binding mode of NKA by molecular modeling and docking studies, and our results correlate well with mutation data and the other ligand binding data available for the NK-2 receptor. It is apparent that the C-terminus of NKA binds within the transmembrane bundle of the NK-2 receptor and the hydrophilic N-terminus of NKA interacts with the N-terminus and extracellular loop regions of the receptor. The binding pocket of the NK-2 receptor spans TM1, TM2, TM3, TM7, and TM6, extracellular loop 3, extracellular loop 1 and the N-terminus. This observation

correlates well with the “common binding pocket” hypothesis proposed for the GPCRs wherein a ligand binding pocket located between TM 3, 5, 6, and 7 has been proposed as a common feature throughout the GPCR superfamily.^{27,37}

Our data suggest that the binding pocket of the NK-2 receptor is mainly hydrophobic in nature. The conserved C-terminus of NKA is in close proximity to the core of the transmembrane bundle of the NK-2 receptor formed by mainly TM2, TM3, TM6, and TM7. The N-terminus of NKA especially His 1 and Lys 2 seems to be more solvent accessible than the rest of the peptide and makes several contacts with the extracellular regions of the receptor. Tyr 266 and Phe 270 receptor residues play a ligand-stabilizing role by participating in aromatic–aromatic interactions with the conserved Phe 6 residue of NKA. It was found from site-directed mutagenesis studies that the mutation of Tyr 266 and Phe 270 to Cys and Ala led to a loss of NKA binding, while a mutation of Tyr 266 to Phe and a mutation of Phe 270 to Tyr retained an almost wild-type-like ligand binding.^{16,22,23,38} This suggests the importance of the aromatic nature of these receptor residues in NKA binding, which is also evident from our data. Further, the conserved aromatic microdomain 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.^{37,39,27} The conserved C-terminus of NKA, especially Leu 9 and Met 10, interacts with several hydrophobic residues of the receptor. NKA interacts with several conserved residues of the receptor, especially Ser 298 which makes several van der Waals interactions and hydrogen bonds with the conserved C-terminus of NKA, emphasizing their role in receptor activation. Site-directed mutagenesis studies on the NK-2 receptor have indicated His 198 in TM 5 and His 267 in TM6 to be important for NKA binding to the NK-2 receptor, and a loss of NKA binding was observed when these residues were substituted.^{22,23} However, from our model these residues do not appear to participate in direct binding to NKA, and therefore we propose that these residues may play a role as structural determinants of the local conformation around the binding pocket of the receptor.

The docking data further correlate well with the pharmacophore pattern proposed by us for NK-2 agonists,⁴⁰ and a pharmacophore pattern complementarity has been observed in the ligand–receptor complex, wherein the aromatic side chain of Phe 6 is found to closely interact with an aromatic microdomain in the NK-2 receptor, and hydrophobic Leu 9 and Met 10 are found to interact with hydrophobic residues of the receptor (Figure 1). 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.⁴¹ The docking studies reported here suggest that the NKA/NK-2 complex is stabilized mainly by hydrophobic interactions and van der Waals interactions, while no stabilizing electrostatic interactions were observed. Moreover hydrogen bonds also play a role in stabilizing the complex.

A previous study on the NKA-NK-2 complex model¹⁶ proposed that NKA bound partly within the transmembrane region and NKA adopted an extended looplike conformation wherein Phe6, Leu9, and Met10 appeared to cluster together. In this model NKA binding is suggestive of a mode of NKA-NK-2 receptor interaction where Phe 6 and Val7 of NKA

lie in a horizontal plane with Leu9 and Met10.¹⁶ This is not supported by the model presented here and our NMR data wherein NKA adopts a helical conformation and side chains of Phe 6, Leu 9, and Met 10 clusters on the same side of the helix. Contrary to the model proposed by Labrou et al.¹⁶ wherein it was suggested that Gln 109 of the NK-2 receptor may not participate in direct NKA interaction but rather may influence the local conformation around the binding site, our results show that Gln 109 interacts with several residues of the ligand, thereby it is proposed to play an important role in receptor activation. It was found from our model that the Gln 109 side chain makes two hydrogen bonds with Thr 3 of NKA. Moreover, site-directed mutagenesis studies have indicated that a mutation of Gln 109 to His and Ala led to a loss and a decrease in ligand binding, respectively; this could be attributed to the loss of H-bond formation which might be crucial for ligand stabilization.^{22,23} However, our data do confirm some of the interactions proposed by Labrou et al.,¹⁶ and we agree with their findings on the proposed aromatic–aromatic interactions of Tyr 266 and Phe 270 receptor residues with the conserved Phe 6 residue of NKA and the interaction of the receptor residue Met 297 with Leu9 and Met10 of NKA.

Comparison between the NKA binding site in the NK-2 receptor as described here and that of the nonpeptide NK2 receptor antagonists SR48968^{10,15} and ibudantant⁴² provides interesting information. The data indicate that TM6 and TM7 are critical for binding both the peptide agonist, NKA, and the nonpeptide antagonists, SR48968 and ibudantant, and these ligands share common points of anchorage in the NK-2 receptor like Tyr 266, Phe 270, and Tyr 289. NKA, SR48968, and ibudantant share similar interactions with the aromatic ring of the receptor residue Tyr 266.^{10,42} However other significant interactions of SR48968 with receptor residues Gln 166 and His 198 are not shared by NKA. Similarly, interactions of ibudantant with receptor residues Ile 202 and Tyr 206 are not shared by NKA. Therefore, the agonist and antagonist binding sites are distinct but partially overlap, sharing strong anchoring points in the receptor such as Tyr 266. This would clearly account for the competitive antagonist behavior observed for SR48968 and ibudantant.^{10,42}

The procedure of combining information about the structure–activity relationship of a known bioactive ligand, the spatial structure of a homologous receptor protein (rhodopsin), and mutation data, applied in this study, provides new perspectives to rational drug design for neurokinin receptors. However, it must 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-2 receptor. Moreover the micelle bound NMR structure of NKA utilized in docking is an average structure from an ensemble of structures and may not necessarily reflect the 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, considering 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 receptor. Moreover, a recent study demonstrated that GPCRs maintain their general

folding characteristics by means of structural mimicry, despite low homology between these receptors.²⁷ Ligand-binding data available for NKA 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. A strong rationale for utilizing the lipid-induced NMR structure of NKA in docking studies of the NK-2 receptor comes from a recent study wherein a comparison of the receptor-bound and micelle-bound states of a peptide hormone indicated only small differences in the conformation.⁴³

The model of the NKA/NK-2 receptor complex developed here provides molecular insight into the biochemical data currently available and would help guide targeting of future experiments to probe specific ligand/receptor interactions. Examination of the current model allows the identification of the broad features of the binding site. This information can be used to suggest the type of molecules to be made or selected from a compound bank or database as a choice of scaffold for lead generation. In the absence of crystal structure, a homology model such as the one described here can be used in drug discovery programs for high throughput docking and in silico screening of compound libraries. This model can also be used to select an initial set of receptor mutants to be generated through molecular biology techniques. Analyzing the binding site can also guide the selection of those properties to be included in a subset of lead compounds, such as size, charge, relative lipophilicity, or other specific pharmacophoric pattern complementarities.

Abbreviations. GPCR, G-protein coupled receptor; NMR, nuclear magnetic resonance; TM, transmembrane; MD, molecular dynamics; CVFF, consistent valence force field; DPC, dodecylphosphocholine; NK, neurokinin; SP, substance P; NKA, neurokinin A; NKB, neurokinin B; NPK, neuropeptide K; NP γ , neuropeptide gamma.

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Supporting Information Available: Ensemble of NKA structure¹⁹ and data on the NKA-NK2 receptor complex. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Note Added after ASAP Publication. This paper was released ASAP on June 17, 2009, with errors in the Supporting Information. The correct version was posted on June 19, 2009.

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