# A Molecular Dynamics Approach to Receptor Mapping: Application to the $5HT_3$ and $\beta_2$-Adrenergic Receptors

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A molecular dynamics-based approach to receptor mapping is proposed, based on the method of Rizzi (Rizzi, J. P.; *et al. J. Med. Chem.* **1990**, *33*, 2721). In Rizzi's method, the interaction energy between a series of drug molecules and probe atoms (which mimic functional groups on the receptor, such as hydrogen bond donors) was calculated. These interactions were calculated on a three-dimensional grid within a molecular mechanics framework, and the minima in the grid were associated with the binding site on the receptor. In this extension, dummy atoms, bonded to the drug with appropriate molecular mechanics parameters, were placed at these minima. The distances between the dummy atom sites were monitored during molecular dynamics simulations and plotted as distance distribution functions. Important distances within the receptor became apparent, as drugs with a common mode of binding share similar peaks in the distance distribution functions. In the case of specific $5HT_3$ ligands, the important donor–acceptor distance within the receptor has a range of *ca.* 7.9 – 8.9 Å. In the case of specific $\beta_2$-adrenergic ligands, the important donor–acceptor distances within the receptor lie between *ca.* 7 – 9 Å and between 8 and 10 Å. These distance distribution functions were used to assess three different models of the $\beta_2$-adrenergic G-protein-coupled receptor. The comparison of the distance distribution functions for the simulation with the actual donor–acceptor distances in the receptor models suggested that two of the three receptor models were much more consistent with the receptor-mapping studies. These receptor-mapping studies gave support for the use of rhodopsin, rather than the bacteriorhodopsin template, for modeling G-protein-coupled receptors but also sounded a warning that agreement with binding data from site-directed mutagenesis experiments does not necessarily validate a receptor model.

## Introduction

A common strategy in receptor mapping is to overlay a series of drugs which are believed to bind to the same receptor such that the structural features held in common are superimposed. There are a number of drawbacks with this approach. Firstly, the strategy assumes that all the drugs bind to a common binding site within the same receptor. Secondly, a single geometry for the drug must be assumed, and this is usually the global minimum. Thirdly, it is not always clear how to superimpose diverse chemical structures.

Rizzi and co-workers proposed an elegant approach to the latter problem, by moving the emphasis away from the structure of the drug onto the structure of the receptor.[1] For a series of $5HT_3$ receptor ligands, they proposed that the binding involved (i) a hydrogen bond donor on the receptor, such as a serine OH which was modeled by a serine hydroxy oxygen probe atom, (ii) a hydrogen bond acceptor on the receptor, such as an aspartate which was modeled by a carboxylate oxygen probe atom, and (iii) hydrophobic binding to the aromatic ring system.

Interaction energies between the drug and the probe atoms were calculated within a molecular mechanics framework over a three-dimensional grid surrounding the drug using the GRID software.[2] Assuming that the position of the minimum in the interaction energies could be associated with the key binding residues in the receptor, it was possible to measure the distance between key residues and hence determine aspects of the receptor structure that would be useful in drug design. In practice, they found that the hydrogen bond donor to hydrogen bond acceptor distance was *ca.* 7.7 Å for three of the four compounds studied; the compounds are shown in Chart 1. Compound **4**, however, only complied with this pattern in a conformation about 2 kJ $mol^{-1}$ above the global minimum, thus illustrating the problems of receptor mapping using energy-minimized structures.

Here we describe a modification of this approach in which the drug is modeled using molecular dynamics. The advantages of this approach are 2-fold. Besides giving a more systematic way of choosing the correct conformations of the drug, there may be instances where the additional information on the flexibility of the drug will be useful in mechanistic diagnosis and prediction. Such information may include whether the drug is an agonist or an antagonist.

Recently, the three-dimensional structures of a number of receptors have been determined. These have included the X-ray structures of the human growth hormone receptor complex,[3] the tumor necrosis factor receptor complex,[4] and the cryomicroscopic electron diffraction structure of bacteriorhodopsin.[5] It is therefore an interesting exercise to compare the receptor-mapping studies with *models* of the receptors themselves. In particular, models of G-protein-coupled receptors have been constructed[6–8] by utilizing their homology to bacteriorhodopsin.[5] However, the more recent electron density map for rhodopsin[9] is probably a better starting point for modeling G-protein-coupled

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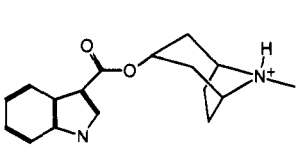
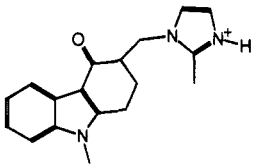
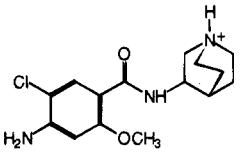
**Chart 1.** Structures of the $5HT_3$ Ligands Studied



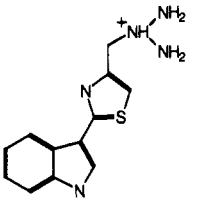
(1) ICS-205-930



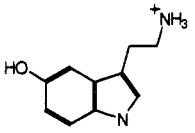
(2) Ondanseteron



(3) Zacopride



(4) 3-[2-guanidinylmethyl-4-thiozoyl] indole



(5) Serotonin

receptors, even though the projection map does not yield atomic information because rhodopsin is a G-protein-coupled receptor. This study suggests that rhodopsin has different dimensions than bacteriorhodopsin.[10] The arrangement of the helices however is very similar in the two structures, even though the orientation of certain helices differs. The assignment of the helices is identical with that used by Baldwin.[10]

For the $\beta_2$-adrenergic receptor, there is sufficient site-directed mutagenesis information on the binding of specific ligands[11,12] to apply this method of receptor mapping to see which template, bacteriorhodopsin or rhodopsin, appears to yield a model receptor structure most consistent with the receptor-mapping studies. (The $5HT_3$ receptor is not a G-protein-coupled receptor, and so a model of the $5HT_3$ receptor cannot be obtained so readily.)

## Methods

**Receptor Mapping.** In order to facilitate a comparison with Rizzi's work, the same four $5HT_3$ ligands were selected for study; see Chart 1.

The structure of each compound was minimized using the AM1 Hamiltonian[13] within the semiempirical molecular orbital program MOPAC 93.[14] The molecular dynamics simulations were performed using the AMBER 4.0 suite of programs.[15] The additional all-atom parameters required were chosen by analogy to the AMBER force field,[16] and the atomic potential-derived charges were computed using the academic version of the *rattler* program,[17] which gives charges compatible with AMBER.[18] Since the interior of the receptor is basically hydrophilic, a distance dependent dielectric constant, $D$ ($D = r$), was used. Over the range of distances sampled during the simulations, this dielectric constanct was significantly more than the gas phase value though it never reached the value for bulk water. The approximation of using a distance dependent dielectric constant is therefore probably ideal for modeling the interior of receptors. All structures were minimized and equilibrated for 20 ps at 298 K.

GRID maps[2] were computed for each drug at the optimized geometry on a 0.5 Å grid spacing. The results were calculated and displayed from within WHATIF[19] and are shown in Figures 1 and 2. The GRID probes selected were the carboxylate oxygen, to represent a hydrogen bond acceptor (*e.g.*, C=O as in Asp), the serine OH oxygen, to represent a hydrogen bond donor (*e.g.*, OH as in Ser), and the phenyl CH probe, to represent hydrophobic interactions. The deepest GRID contours were used to identify the likely interaction sites on the drug. Given the position of these sites, two alternative receptor-mapping strategies arise. (i) The distance between the interaction sites on the drug may be monitored during the molecular dynamics simulations. (ii) Dummy atoms placed at the GRID minimum may be bonded to the interaction sites using molecular mechanics parameters and the distance between the dummy atoms monitored during the molecular dynamics simulation.

This latter approach is preferable since the dummy atoms provide a much better measure of the range of possible receptor structures. This use of dummy atoms is similar to that used to create and annihilate atoms during the course of a free energy perturbation simulation.[20] The bond and angle parameters were identical with those for a hydrogen atom; the barrier height for the dihedral parameters was set to zero. Since the dummy atoms carry no charge and have zero Van der Waals interactions, to all intents and purposes they do not affect the course of the molecular dynamics simulations.

For either approach, distance distribution functions may be plotted. To obtain accurate distance distributions, it is important that phase space is adequately sampled. On the basis of simulations of Ondansetron, 500 ps simulations appear to give reasonable distance distribution functions. Rather than looking for a single distance to represent the distance between key residues in the receptor, as in ref 1, the problem now becomes one of comparing peaks corresponding to the most probable distances between the key residues.

**Models of the $\beta_2$-Adrenergic Receptor.** Three models of the rat $\beta_2$-adrenergic receptors[21] were constructed. The first two used a homology based on multiple-sequence alignment of several hundred G-protein-coupled receptors (*i.e.*, based on homology to bacteriorhodopsin); the homology is essentially the same as that given in ref 8. Side chains were added to the template in their most probable conformation, and the structure was minimized and equilibrated for 20 ps at 298 K using molecular dynamics. The bacteriorhodopsin-based model was then used as a starting point for the rhodopsin-based model. Here the arrangement of the helices was moved using interactive molecular graphics[19] so the overall shape was consistent with the rhodopsin electron density map.[9] The third model used a different homology, derived by Baldwin,[10] and was again based on the rhodopsin template, since Baldwin's studies support the use of this template. Following the molecular dynamics, helix 5 tilted in a manner consistent with the most recent rhodopsin projection map;[22] the map was published after this article was first submitted. The features of the three models essential to this study are shown in Table 1, and the sequences of the transmembrane segments are shown in Table 2.

## Results and Discussion

**Receptor Mapping: $5HT_3$ Ligands.** The GRID maps for the $5HT_3$ ligands are shown in Figure 1. The carboxylate probe interaction energies are contoured at *ca.* $-5.5$ kcal mol$^{-1}$; the hydroxy oxygen probe interaction energies are also contoured at *ca.* $-5.5$ kcal mol$^{-1}$, and both are shown in Figure 1. The hydrophobic probe interactions are contoured at *ca.* $-2$ kcal mol$^{-1}$ and are shown in Figure 2.

The normalized distance distributions are shown in Figure 3. A number of features emerge from these plots. The most striking observation is that for most molecules

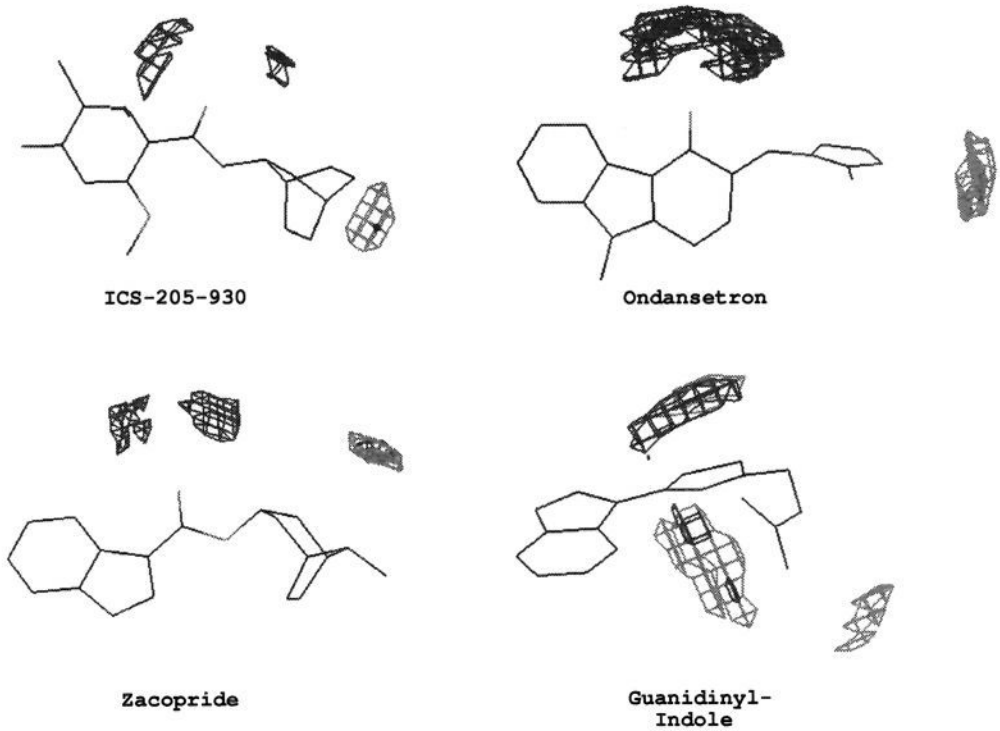



**Figure 1.** GRID maps for molecules **1**–**4**. The interaction energies for the serine hydroxy oxygen atom probe are contoured at −5.0, −4.0, −5.5, and −5.0 kcal mol$^{-1}$, respectively, and are shown in black; the interaction energies for the carboxylate oxygen atom probe are contoured at −6.0, −4.5, −5.5, and −5.5 kJ mol$^{-1}$, respectively, and are shown in gray.

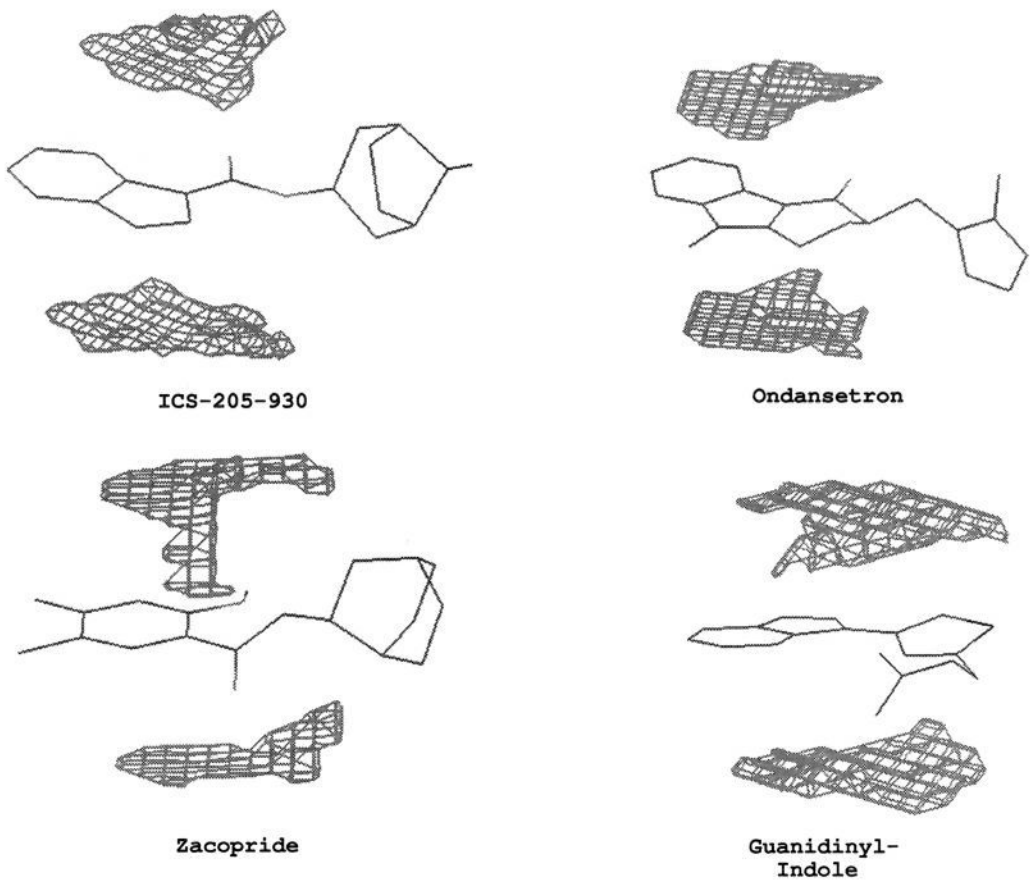



**Figure 2.** GRID maps for molecules **1**–**4**, for the phenyl CH hydrophobic probe atom. The interaction energies are contoured at −1.4, −1.8, −1.7, and −2.2 kJ mol$^{-1}$ for molecules **1**–**4**, respectively.

a peak is observed around 7.4 − 8.9 Å and that all molecules, including Ondansetron, have a reasonable chance of reproducing this distance. Rizzi's characteristic distance of 7.7 Å is well within this range and is

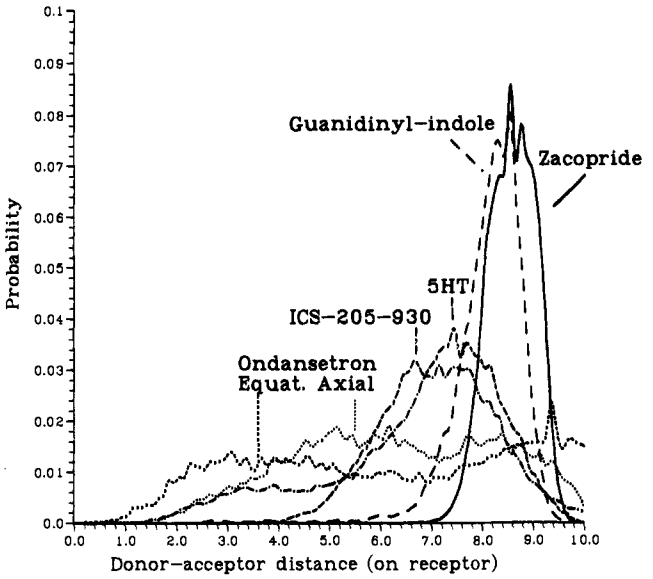



**Figure 3.** Normalized donor–acceptor distance distribution functions for molecules **1**–**5**. The structures of the molecules are shown in Chart 1.

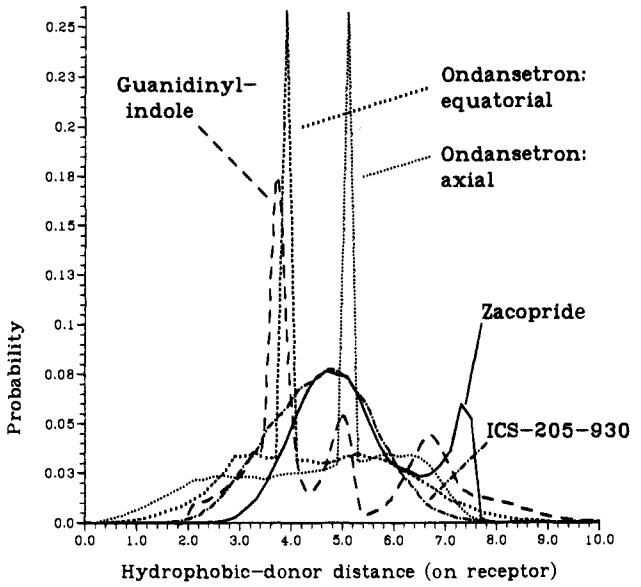



**Figure 4.** Normalized hydrophobic donor distance distribution function for molecules **1**–**5** in Chart 1.

shown to be highly probable. The second striking feature is that for some molecules, the peak is not as sharp as for others. The significance of this is hard to assess on the basis of such a small sample of molecules, but the following comments offer just one possible explanation. Ondansetron has the flattest peak and the highest binding affinity but the lowest antagonist activity.[1] It may be that the flexibility of Ondansetron hinders its function as an antagonist (though it does still function well as an antagonist due to the rigidity conferred by the three fused rings). Here our assumption is that antagonists block the receptor because their rigidity prevents the necessary conformational changes required for receptor activation. The sharper peaks and greater antagonist activity of Zacopride and the guanidinylindole are consistent with this view. These ideas may be most approriately applied to partial agonists,[23] but further work is required to investigate whether there is indeed a relationship between flexibility and

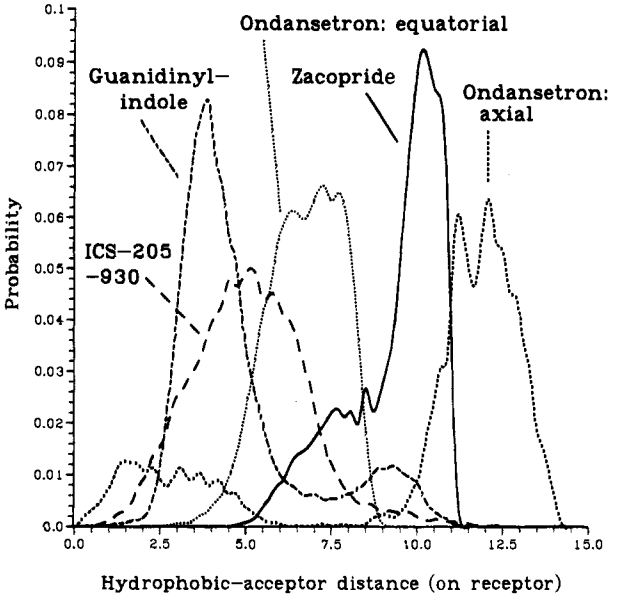



**Figure 5.** Normalized hydrophobic acceptor distance distribution function for molecules **1**–**5** in Chart 1.

**Table 1.** Distances (Å) between the Hydrogen Bond Acceptor (Asp 113) and the Hydrogen Bond Donors (Ser 203, Ser 204, Ser 207) in the Various Receptor Models, Which Are Denoted by Their Template[a]

| | template for model | | |
|---|---|---|---|
| donor–acceptor | bacteriorhodopsin | rhodopsin | Baldwin |
| Ser 203–Asp 113 | 15.67 | 8.61 | 11.26 |
| Ser 204–Asp 113 | 17.33 | 10.24 | 13.45 |
| Ser 207–Asp 113 | 17.93 | 9.52 | 8.47 |

[a] The distances were measured between the hydroxy oxygen of the serine and the nearest carboxylate oxygen of the aspartate. The receptor models were equilibrated for 20 ps in the absence of ligand.

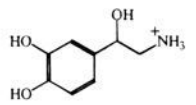
ligand function; the method presented here may be an appropriate tool for such investigations.

In Rizzi's model, the third component of the pharmacophore, hydrophobic binding, was satisfied by aligning the plane of the aromatic rings. In this dynamic model, the hydrophobic binding region is shown in Figure 2 and distance distribution plots are shown in Figures 4 and 5. However, while general conclusions may be drawn as to the requirements of the receptor, the donor–acceptor distance distribution function is the most informative.

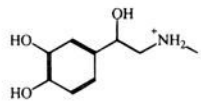
The primary role of the molecular mechanics parameters for the dummy atoms is to reproduce the *position* of the minimum in the GRID interaction energy contours. In future developments of the model, we would expect more elaborate molecular mechanics parameters to allow for the *shape* of the energy minima to be reproduced. This should broaden the distance distribution functions, particularly those involving hydrophobic binding sites, thus improving the overlay between the peaks.

The dynamic nature of the distance distribution function is clearly shown in Figure 3, but different types of distance distribution functions may be expected for agonists and antagonists. For receptor mapping based on a series of agonists, the donor–acceptor distance in the receptor may change during the agonist-induced allosteric change. If so, the range of inter-residue distances should be be encompassed within the observed distance distribution functions.

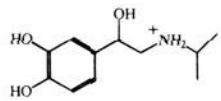
**Chart 2.** Structures of the $\beta_2$-adrenergic Ligands Studied



(1) Norepinephrine



(2) Epinephrine



(3) Isoproteranol

**Evaluating Receptor Models: $\beta_2$-Adrenergic Agonists.** The site-directed mutagenesis studies[11,12] implicate Asp 113, Ser 203, Ser 204, and Ser 207 as key binding residues in the $\beta_2$-adrenergic receptor; thus, the hydrogen bond acceptor referred to above is clearly identified as Asp 113, while the hydrogen bond donor is one of the three serines. The donor−acceptor distances in the two models are shown in Table 1 and are clearly longer in the bacteriorhodopsin template than in the rhodopsin templates. The GRID maps for the $\beta_2$-adrenergic ligands (see Chart 2) are shown in Figure 6. The first set of plots for the donor−acceptor-related distance distribution function plots for the $\beta_2$-adrenergic agonists epinephrine, norepinephrine, and isoproteranol are shown in Figure 7. In this case, the distance is taken between the interaction sites (*i.e.*, the cationic nitrogen and the oxygen of the hydroxyl groups) rather than between dummy atoms bonded to these sites; the key distance falls in a range between 4.0 and 9.0 Å. However, the most this distance can be is *ca.* 11.5 Å, since in each case the distance to the dummy atoms is *ca.* 1.5 Å. Thus, only the rhodopsin and Baldwin templates are consistent with the molecular dynamics receptor-mapping studies. Indeed, attempts to dock the ligands into the bacteriorhodospin template were unsuccessful because the distance between the residues associated with binding was too great. Also, steric clashes between the drug and Phe 289 and Phe 290 at the top of helix 6 were observed, whereas the ligands may be fitted quite readily into both of the $\beta_2$-adrenergic receptor models based on the rhodopsin templates. Thus, the dangers inherent in modeling G-protein-coupled receptors using current techniques, even when site-directed mutagenesis data are available, are clearly illustrated by the fact that two quite different models both fit the data. However, from Figure 7, it is difficult to conclude anything other than that the receptor-mapping studies are consistent with the model of the receptor.

The second set of plots for the donor−acceptor-related distance distribution function plots for the $\beta_2$-adrenergic agonists are shown in Figures 8 and 9. Here, the plots are indeed between the dummy atoms. Even though the peaks do not cluster as closely as for the $5HT_3$ ligands, it now far easier to see how these distances may relate to the precise information in the receptor model structures. Since the common distances for the *m*-hydroxyl group span a range of *ca.* 7−9 Å while the common distances for the *p*-hydroxyl group span a range of *ca.* 8−10 Å, it may appear that the rhodopsin-based template is more appropriate than the Baldwin template and that Ser 203 and Ser 207 are likely to be more important than Ser 204. However, two alternative explanations may apply. Firstly, the results imply that the agonists may not bind to more than one Ser for the full duration of the activation process, and indeed this was observed during molecular dynamics simulations

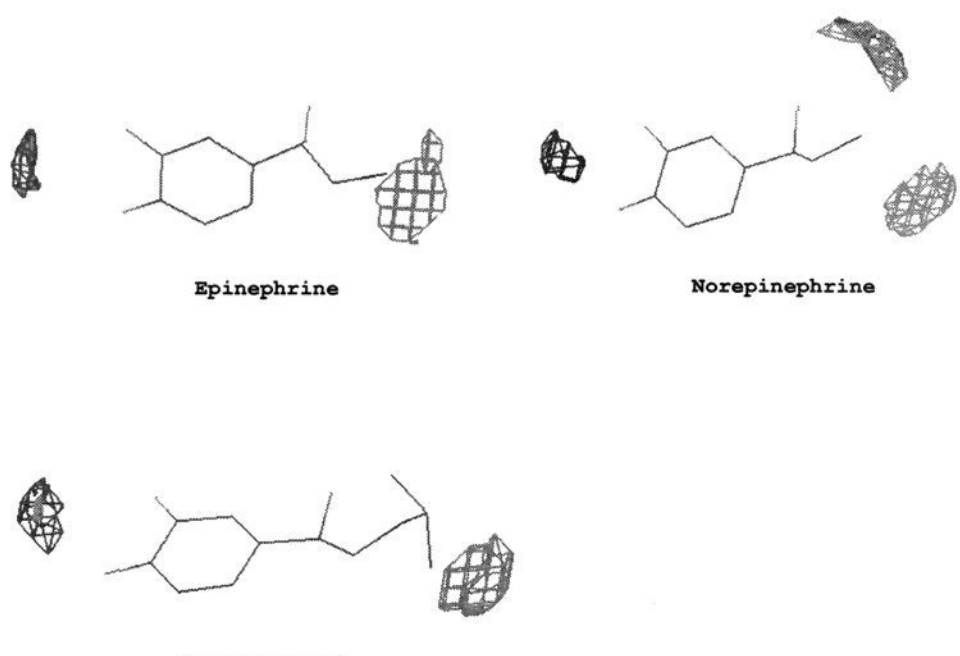



**Figure 6.** Donor and acceptor GRID maps for the $\beta_2$-adrenergic ligands. The interaction energies for the serine hydroxy oxygen atom probe are contoured at −7.5, −7.0, and −7.2 kJ mol$^{-1}$ for norepinephrine, epinephrine, and isoproteranol, respectively, and are shown in black; the interaction energies for the carboxylate oxygen atom probe are contoured at −5.3, −5.0, and −5.1 kJ mol$^{-1}$, respectively, and are shown in gray.

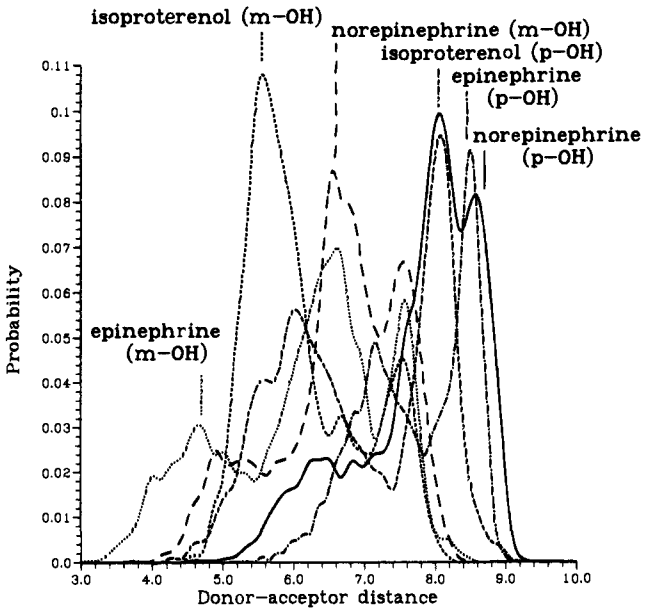



**Figure 7.** Normalized donor–acceptor distance distribution functions between the interaction sites for molecules 1–3. The structures of the molecules are shown in Chart 2.

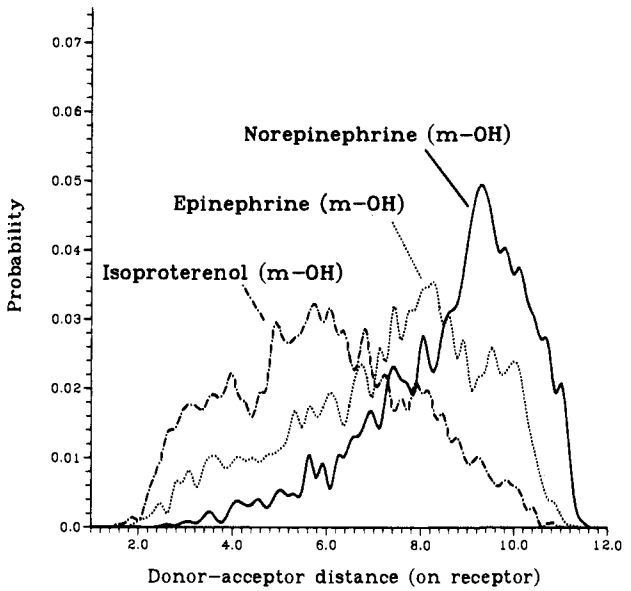



**Figure 8.** Normalized donor–acceptor distance distribution functions between the dummy atoms on the amino group and the *m*-hydroxyl group for molecules 1–3. The structures of the molecules are shown in Chart 2.

on both the rhodopsin model (with epinephrine and norepeinephrine) and the Baldwin model (containing norepinephrine). Secondly, during simulations on the rhodospin model with agonist present, the donors and acceptors on the receptor move. The new distance ranges are 5.9–7.9, 9.2–10.5, and 8.2–9.9 Å for Ser 203, Ser 204, and Ser 207, respectively. These results clearly show that the distance distribution functions derived from the ligand may give a clearer idea of the distances within the activated receptor than models of the receptor alone. Thus, a role for Ser 204 in binding the agonist is no longer ruled out on the basis of these simulations. Similarly, the distance information presented here is insufficient to distinguish between the rhodopsin and Baldwin models.

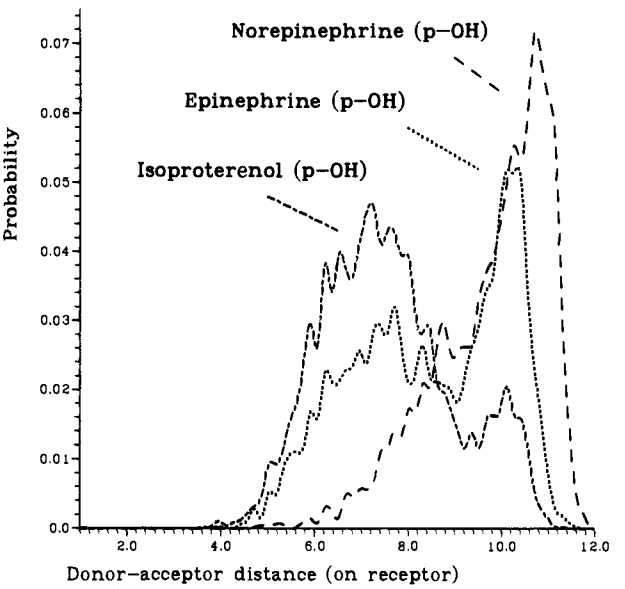



**Figure 9.** Normalized donor–acceptor distance distribution functions between the dummy atoms on the amino group and the *p*-hydroxyl group for molecules 1–3. The structures of the molecules are shown in Chart 2.

**Table 2.** Sequences of Transmembrane Regions in the Three Models of the $\beta_2$-Adrenergic Receptor[a]

| helix | start residue no. | model sequence | end residue no. |
|---|---|---|---|
| 1 | 32 | PVVGMAILSVIVLAIVFGNVLVIT | 56 |
| 1 | 34 | VGMAILSVIVLAIVFGNVLVITAIA | 59 |
| 2 | 71 | FITSLACADLVMGLAVVPFGASHIL | 95 |
| 2 | 66 | TVTNYFITSLACADLVMGLAVVPFGA | 88 |
| 3 | 107 | EFWTSIDVLCVTASIETLCVIAVDRY | 132 |
| 3 | 105 | WCEFWTSIDVLCVTASIETLCVIAVDRY | 132 |
| 4 | 148 | KARVVILMVWIVSGLTSFLPIQ | 170 |
| 4 | 147 | NKARVVILMVWIVSGLTSFLPIQMHW | 173 |
| 5 | 199 | YAIASSIVSFYVPLVVMVGUY | 219 |
| 5 | 198 | AYAIASSIVSFYVPLVVMVGUYSRVF | 223 |
| 6 | 270 | KALKTLGIIMGTFTLCWLPFFIVNIV | 295 |
| 6 | 271 | ALKTLGIIMGTFTLCWLPFFIVNIVNIVH | 299 |
| 7 | 302 | LIPKEVYILLNWLGYVMSAFNLPLI | 325 |
| 7 | 306 | EVYILLNWLGYVMSAFNLPLIYCRSPD | 331 |

[a] The bacteriorhodopsin and rhodopsin models (top) have the same sequence, the Baldwin model is shown below.

## Conclusions

An approach to receptor mapping is proposed, based on a molecular dynamics extension to the method of Rizzi.[1] In this method, pseudoreceptor sites were bonded to a series of drug molecules at a position corresponding to the minimum in the GRID interaction energies. The distances between these dummy atom sites were monitored during molecular dynamics simulations and plotted as distance distribution functions. Important distances within the receptor then become apparent, as drugs with a common mode of binding show similar peaks in the distance distribution functions. In the case of specific $5HT_3$ ligands, the important donor–acceptor distance within the receptor has a range of *ca.* 7.9–8.9 Å. The corresponding distance within the drug (results not shown) is *ca.* 5.9–6.9 Å. The method may have applications beyond suggesting whether a new drug will bind or not, since the shape of the distance distribution function may give an indication of activity.

In the case of specific ligands for the $\beta_2$-adrenergic

receptor, similar distance distribution functions may be obtained. Here, these have been used to assess three models of the $\beta_2$-adrenergic G-protein-coupled receptor, based on the bacteriorhodopsin, rhodopsin, and Baldwin templates. The comparison of the donor–acceptor distance distribution functions for the simulation with the actual donor–acceptor distances in the receptor models suggests the rhodopsin and Baldwin models are much more consistent with the receptor–mapping studies, thus enhancing the support for the use of the rhodopsin template for modeling G-protein-coupled receptors. The fact that two different models of the receptor both agree with the receptor-mapping studies implies the agreement with binding data does not necessarily validate a receptor model. Moreover, the distance distribution functions derived from the ligand give a better indication of the corresponding distance in the activated receptor than can be derived from simulations on the receptor model alone.

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**Supporting Information Available:** MOPAC-optimized structures, atomic charges, AMBER atom types, and additional AMBER parameters for all compounds (4 pages). Ordering information is given on any current masthead page.

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