Hydrophobicity profiles in G protein-coupled receptor transmembrane helical domains

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Abstract: The lack of a crystallographically derived structure for all but three G (TP [guanosine triphosphate]-binding) protein-coupled receptor (GPCRs) proteins necessitates the use of computationally derived methods to determine their structures. Computational methodologies allow a mechanistic glimpse into GPCR–ligand interactions at a molecular level to better understand the initial steps leading to a protein’s biologic functions, ie, protecting the ligands that activate, deactivate, or inhibit the protein, stabilizing protein structure in the membrane’s lipid bilayer, and ensuring that the hydrophilic environment within the GPCR-binding pocket is maintained. Described here is a formalism that quantifies the amphiphilic nature of a helix, by determining the effective hydrophobicity (or hydrophilicity) at specific positions around it. This formalism will enable computational protein modelers to position helices so that the functional aspects of GPCRs are adequately represented in the model. Hydro-Eff®, an online tool, allows users to calculate effective helical hydrophobicities.

Keywords: GPCR, effective hydrophobicity, amphiphilicity

Introduction

G (TP-binding) protein-coupled receptor (GPCR) proteins are ubiquitous. They transduce cell membranes, span several tissues, and are responsible for myriad functions. GPCRs serve as conduits of material within the cell from outside. Alternatively, their activation following ligand binding serves to initiate intracellular processes (eg, G-protein coupling and signal transduction). The publication of initial and subsequent revised drafts of the human genome brought to light the ubiquity of GPCRs in membrane-related function. Olfactory receptors, which constitute superfamilies in mammalian genomes, are an example of GPCRs.

Structure of GPCRs

GPCRs are believed to possess a tertiary structure comprising an assembly of seven transmembrane helical domains connected by three intracellular and three extracellular loops, and possessing an extracellular N-terminus and an intracellular C-terminus. While this is typical, anomalies do exist; a recent publication has identified a functional olfactory receptor with an anomalous structure of six transmembrane regions and an extracellular C-terminus.

Three GPCRs have been structurally analyzed by X-ray crystallography, ie, rhodopsin, the beta-adrenergic receptor, and the adenosine A2A receptor. All structures have been solved at relatively high resolution, with the resolution for ...
Rationalizing the hydrophilic core of the tertiary protein structure

Individual amino acid residues that point towards the interior of the protein are responsible for ligand binding through a combination of covalent, electrostatic, and van der Waals interactions. A model of a GPCR should preserve these binding features and those that rationalize the protein’s core. Once the transmembrane helical scaffold has been constructed via modeling, helices have to be rotated such that, presumably, the hydrophilic side of the helix is pointed towards the interior of the protein, i.e., where ligand binding is expected to take place. This positions the hydrophobic side of the helix towards the hydrophobic lipid bilayer.

This paper describes a formalism that quantifies helical amphiphilicity by determining the effective hydrophobicities at different positions in a helix. The effective hydrophilicity is calculated at specific angles around the helix, which in this methodology is represented as a helical wheel. The effective hydrophobicity includes not only the hydrophobicity of the amino acid residue at that angle, but also contributions from the hydrophobicities of all other residues in the helix. The effective hydrophobicity thus takes into account how the electronic nature (eg, polarity) of surrounding amino acid residues will affect the hydrophobicity at a specific point (or angle) for a helix.

The first comprehensive review of the structural aspects of GPCRs and membrane proteins was published by Eisenberg. Eisenberg arrived at a theoretic formalism for hydrophobic moments of membrane protein helices. This hydrophobic moment was a single value for the entire helix and quantified as a number per residue. It is a vector sum over all angles of side chains and includes hydrophobicities of the residues involved. The larger this value, the larger the amphiphilicity of the helix. Programs such as the PERSCAN software (currently unavailable) have used Fourier transform methodologies based on differences between substitution frequencies of buried residues in water-soluble proteins and lipid-accessible residues to determine helical amphiphilicities. Recent reviews of research related to membrane protein structures have made inferences based on observations of the crystallographically derived structures of GPCRs, which were not available at the time of the Eisenberg review.

The work described here revisits helical amphiphilicities in GPCRs, in light of published X-ray-determined structures for rhodopsin, the beta-adrenergic receptor and the adenosine A2A receptor.
Methods

Effective hydrophobicity

The effective hydrophobicity, $\Theta_\phi$, for an alpha helix is determined by Equation 1.

$$\Theta_\phi = \sum_{i=\Theta_\phi}^{360-\Theta_\phi} \mu_i \cdot \cos i$$ (1)

This value is calculated at angles $\Theta$, i.e., the angles that residue side chains occupy on a helical wheel; $\mu$ is the hydrophobicity value for each amino acid residue that resides at angle $\Theta$. Although residues occupy 20° intervals on a helical wheel, sequentially the amino acids are positioned at 100° intervals along the helical turns. For example, for a helix FGPTGCNLGFF, the angles on the wheel will be occupied thus: F(0°), G (100°), P (200°), T (300°), G (400° [60° + 300° = 360° = 0° + 40° = 40°]), C (140°), N (240°), L (340°), E (80°), G (180°), F (280°), and F (20°). To illustrate how Equation 1 works, consider a residue at an angle $\Theta = 100°$. This is the angle in question, i.e., the angle at which the effective hydrophobicity will be measured. The first term in Equation 1 is $\mu_{100} \cdot \cos 0$. The vector summation in Equation 1 is then over cosine contributions (projections) from other residues at other angles to the angle being considered. Since residues occupy, in sequence, angles at intervals of 100° in a helical wheel, the next residue in this sequence is at angle 200°. The contribution from this residue to the residue (at the angle in question), i.e., the second term in equation 1, is $\mu_{200} \cdot \cos 100$. The contribution from the third term, 200° away, is $\mu_{300} \cdot \cos 200$, and so forth. Figure 1 illustrates how Equation 1 is used to determine effective hydrophobicities at angles on a helical wheel.

If the sequence is long enough that another residue lies at angle 100° (approximately six turns lower along the helical axis), then this hydrophobicity is added to the one in question times the cosine of the angle 0°. For helices of sequences containing greater than 18 residues, additional residues are superimposed on previously occupied positions on the helical wheel. For example, in Figure 1, the Glu at angle 0° and the Gly at angle 360° are superimposed.

The $\mu$ values used in the equation can be derived from hydrophobicity scales, reviewed extensively by Eisenberg. These hydrophobicities have been variously determined, i.e., through thermodynamic calculations of the free energy changes with an amino acid in an aqueous versus a hydrophobic environment, or, semiempirically, based on a survey of the likelihoods of amino acid residues being buried versus exposed, or a combination of both. The scale most often used (and used in this paper) is the Eisenberg consensus hydrophobicity scale, which were obtained by a simple averaging of hydrophobicities determined, using different conceptual methodologies, by Chothia,32 Janin,33 and Von Heijne and Blomberg.34

Hydro-Eff®: An Internet-accessible tool to determine effective hydrophobicities

An Internet-based tool, Hydro-Eff® (see http://bioinfo.genetics.uab.edu/hydro-eff) allows users to calculate $\Theta_\phi$. Figures 2 and 3 illustrate the web user interface and the results page. The web page contains information about the concepts behind Hydro-Eff. The user can enter a sequence in the following format: Helix1:EPWQFS ..., in the text box. The “Helix1” or user-preferred designation is necessary to identify helices, because Hydro-Eff allows the user to enter unlimited helical sequences in the text box. For example, the input for GPCRs will typically consist of seven helices. Also, important is the use of the “:” that separates the helical designation from the sequence. This format is required by the Hydro-Eff program.

Hydro-Eff software is written using PERL (Practical Extraction and Report Language). It uses the CGI.pm module to allow access to Hydro-Eff via the Internet. The front page and the results page are annotated with a description of the program with instructions for its use. The author's email address is included, so that users can contact the author for...
Crasto

Helical Hydrophobicity Profiles

Presented here is a methodology to identify effective hydrophobicities of helices. A helix is structurally idealized by a helical wheel. Amino acids in a helical wheel occupy every 20°. The effective hydrophobicity at each 20° interval on the wheel, \( \Theta \), is calculated using the following expression:

\[
\Theta = \sum \mu \cos \theta \quad \text{Equation (1)}
\]

\( \Theta \) is computed by summing the arc contributions to the hydrophobicity moment on that residue from all other points along the helical wheel for a given helix. \( \mu \) is the hydrophobicity for a residue. The above expression allows the hydrophobicity to be defined along an arc of a circle (or a cumulative hydrophobic effect at a specific angle on the helical wheel contributed from hydrophobicities from residues at all other angles) as opposed to the hydrophobicity from a specific residue. Any value of hydrophobicity (\( \mu \)) can be used in the above equation.

Please add the protein sequences for your helix in the text area below. If more than one helix, type sequences one below the other.

The results page shows the effective hydrophobicity \( \Theta \) in a helix using different hydrophobicity scales. These were identified by Eisenberg (the Consensus Scale), Von Heijne, Janin, Chothia, Wolfenden, Kyte and Doolittle, and Argos. The effective hydrophobicity is determined at each angle on the helical wheel. For a helix of residues RTFQNEGSRFT, the angles at which each residue occurs are: R (0°), T (100°), F (200°), G (300°), N (40°), E (140°), G (290°), S (220°), F (120°), T (220°), etc.

Figure 2: A screen capture of the Web page for the Hydro-Eff tool. The user can enter one or more sequences in the text box. The text on the web page explains the rationale for Equation 1 and how it can be used to determine effective hydrophobicities. Hydro-Eff can be accessed at http://bioinfo.genetics.uab.edu/hydro-eff.pl. Accessing this program requires an Internet browser.

help in using Hydro-Eff. To execute Hydro-Eff, only an Internet connection and a browser are needed. All calculations of \( \Theta \) are performed on the server side.

The results are tabulated such that the \( \Theta \) values are obtained at specific angles on the helical wheel. The \( \Theta \) values are calculated using the hydrophobicities (\( \mu \)) determined by Eisenberg (the consensus scale), Von Heijne, Janin, Chothia, Wolfenden, Kyte and Doolittle, and Argos. The values of \( \mu \) are taken from Table 2 of Eisenberg's review. The values for \( \mu \) published by Tanford and Nozaki and Segrest and Feldmann are not included in the calculations for \( \Theta \) because \( \mu \) values for the polar residues Asp, Arg, Lys, and His were not provided by this method. For the same reason, Wolfenden's scales which do not include a \( \mu \) value for Pro, are not included in the calculations.

Results

In Figure 1, the helical wheel was generated using an Internet-based tool (see http://www-nmr.cabm.rutgers.edu/bioinformatics/Proteomic_tools/Helical_wheel/) developed by John K Everett. Figure 1 shows that the amino acid residues are positioned at the 20° angles on a helical wheel for a fictitious helix of sequence: EPWQFSMLAAYFLIMLGFPINFLTLYVTQH. The first residue (Glu) is at angle 0°. The next residue (Pro) is at angle 100°. The third residue (Trp) is at angle 200°, and so on. Consider the summation in equation 1. At angle 0°, the hydrophobicity of Glu (−0.62) contributes to \( \Theta \). Because the angle it makes with itself is 0°, the cos \( \theta \) contribution is 1, and therefore the contribution is from hydrophobicity only. For the next angle at 100°, consider the vector passing through angle 100° on the wheel represented by the Pro side chain. If this vector were resolved, the cos100 projection would fall on the residue at 0°, namely at Glu. This is continued at every 100° interval until \( \Theta \) at all angles and all residues are covered. Figure 1 shows the partial calculation of the effective hydrophobicity, \( \Theta \) at angle 0. At this angle, two residues
The results page shows the effective hydrophobicity $\Theta_0$ in a helix using different hydrophobicity scales. These were identified by Eisenberg (the Consensus Scale), Von Heijne, Janin, Chothia, Woellender, Kyte and Doolittle and Argos. The effective hydrophobicity is determined at each angle on the helical wheel. For a helix of residues RTFQNEGIF, the angles at which each residue occurs are: R (0°), T (100°), F (200°), G (300°), N (40°), E (140°), G (230°), S (30°), F (120°), T (200°), etc.

**Figure 3** A screen capture of results of effective hydrophobicity at different angles (at 20° intervals) on an idealized helical wheel. The user can access results for effective hydrophobicities determined using seven different hydrophobicity scales. The text on the page also provides some information about the different hydrophobicity scales. Hydro-Eff results are accessible via an Internet browser.

<table>
<thead>
<tr>
<th>Angle</th>
<th>Consensus</th>
<th>Von Heijne</th>
<th>Janin</th>
<th>Chothia</th>
<th>Woellender</th>
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<tr>
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<td>1.584</td>
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<tr>
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From a biochemical standpoint, one would expect the hydrophobicity at one angle on the wheel to be perturbed by neighboring and surrounding residues because of the electronic withdrawing and donating effect of their acidic, basic, or neutral natures. As the angle increases from the angle in question, the decreasing value of the cosine of that angle will reduce the perturbing effect of the side chain for that residue.

In Figure 1, the effective hydrophobicity values are on the outside (in blue) and the angles of the helical wheel are on the inside (in red). The other numbers represent the $\mu$ values. The most hydrophilic region illustrated by a red curve is between angles 260° and 360° and is centered between angles 300° and 320°. Figure 1 shows that histidine (320°), glutamic acid (0°), and glutamine (300°) contributed to the hydrophilicity of this side of the helix. The large number of nonpolar residues ensured that the most hydrophobic regions are between angles 80° and 180°, represented by a blue curve. Thus, equation 1 allows the user to quantify the amphiphilicity of a helix.

**Hydro-Eff results**

The results page (Figure 3) shows the tabulated effective hydrophobicities $\Theta_0$ for a helix using six different hydrophobicity scales, ie, Eisenberg, Von Heijne, Janin, Chothia, Kyte and Doolittle, and Argos. The values for $\mu$ are taken from Table 2 by Eisenberg and colleagues. The tabulated results are read, not in terms of the
peptide sequence, but in terms of the effective hydrophobicity at a specific angular location on the helical wheel. Indeed, the contribution from the residues at that specific angle is the highest because the hydrophobicity $\mu$ is multiplied by $\cos 0 = 1$.

**Discussion**

In this paper, a formalism that quantifies the amphiphilic nature of an alpha helix is introduced. This formalism will assist computational biologists and crystallographers in identifying helical amphiphilicities. The availability of crystal structures of three GPCRs allows us to test whether Equation 1 properly represents transmembrane helical positioning for the asymmetric unit within the crystallographic unit cell, and possibly the biologic environment.

Equation 1 provides a mathematic basis that includes the biologic imperative of helical positioning. Hydrophobicity contribution from a side chain at a specific angle will be significantly reduced, enhanced, or otherwise influenced, because a bulky electron density withdrawing or donating side chain is present at neighboring positions (angles on the helical wheel). The cosine vector contribution represents the contribution of a neighboring amino acid residue side chain. As the cosine values decrease with increasing angle, the contribution (biologic and vector) from the cosine component of the angle of the residue side chain is likely to decrease for residue at angles removed from the angle at which the effective hydrophobicity is being calculated.

The structures of rhodopsin (mostly) and the beta-adrenergic receptor have been used to model GPCRs, with a recent publication listing use of the adenosine $A_{2A}$ receptor.

If there is weak homology between the GPCR and rhodopsin or beta-adrenergic receptor (the template used during homology modeling), homology modeling can only be the first step in the modeling protocol. Homology modeling can only be used to establish basic helical positions, much like the low-resolution helical densities from electron diffraction experiments. This affects how the helices are rotated to maintain a hydrophilic core. Alternative protocols have been designed that determine helical rotations in the GPCR transmembrane assembly based purely on lowest system energies. These do not take hydrophobicities into account.

The polar core of a transmembrane assembly is typically centered on one or more highly polar amino acid residues, such as arginine, lysine, histidine, aspartic acid, and glutamic acid. Deploying Equation 1 precludes the need to identify residues that might contribute to helices individually. These are centered on a specific angle on the helical wheel, as illustrated by the red and blue curves in Figure 1, and not on a specific amino acid residue.

**Effective hydrophobicities for transmembrane domains in rhodopsin and beta-adrenergic receptors**

Mutagenesis experiments and functional analysis experiments identify agonists and antagonists that bind to these receptors and activate or inhibit their function. Binding ligands may form covalent or electrostatic bonds with specific amino acids. Because ligand binding is followed by GPCR activation, it is likely that other nonpolar interactions also facilitate the activation. The evidence for the role of specific residues in binding is that function diminishes or is enhanced by mutating these residues using residues of varying polarity and size and length of side chains.

Hydro-Eff was used to determine the effective hydrophobicities of rhodopsin and the beta-adrenergic and adenosine receptors. The helical sequences were identified by observing the structures of asymmetric units of the three protein structures: PDB code 1U19 for rhodopsin; PDB code 2R4R for the beta-adrenergic receptor; and PDB code 3EML for the adenosine $A_{2A}$ receptor. Here, the structure of the beta-adrenergic receptor with the PDB identifier 2R4R is used. Another structure of the beta-adrenergic receptor with a tobacco etch virus cleavage site has also been published (PDB identifier 2R4S). Both structures were inspected, and the peptide sequences that constitute helices in both structures are not different. Because the Hydro-Eff methodology relies on sequences that make up a helix, any results stemming from the calculation of effective hydrophobicities will be identical for both structures.

The hydrophilic regions predicted by Hydro-Eff are in green and the polar residues are in red, with side chains shown. Figures 4–6 illustrate how polar residues significantly impact the amphiphilicities (through their $\mu$ values), although these residues do not often exactly coincide with the effective hydrophobicity for that angle. In Figures 4 and 5, where the effective hydrophobicities coincide with polar residues, the side chains are shown and the residue is colored green.

For rhodopsin, the hydrophilic transmembrane regions I, VI, and II, as determined using Hydro-Eff, are pointed towards the lipid membrane. The polar sides of the transmembrane regions III and VII are pointed towards the interior. The polar transmembrane regions IV and V are pointed towards each other (Figure 4). For the beta-adrenergic receptor, the polar side of the transmembrane helices II, III, IV, and V are pointed...
towards the interior of the protein. Residues on transmembrane regions III and IV are implicated in ligand binding. Transmembrane regions I, VI, and VII are pointed away from the interior of the protein and mostly towards other transmembrane helices (Figure 5).

The blue, space-filled side chains in Figures 4 and 5 represent the primary binding interaction between the ligand and receptor. For rhodopsin, the ligand is retinal (aldehyde) and forms a covalent Schiff base interaction with Lys296.55 Figure 4 shows that this lysine is positioned in the hydrophilic interior, making it amenable to ligand binding; the hydrophilicity is centered on Ser298, pointed towards the interior of the protein, away from the bilayer, allowing lysine to be accessible to ligand binding. For the beta-adrenergic receptor, the binding ligands are from the alcohol family, ie, antagonists (alprenolol and propranolol) and agonists (isoproterenol and ephedrine). Asp79, Asp113, and Asn318 are implicated in binding.56,57 These residues mostly coincide with the position of effective hydrophobicities.

Figure 4 Amino acids and side chains for the residues for the transmembrane helices on which the effective hydrophobicity residues for rhodopsin. The green regions (without side chains) in the helices show the direction of the effective hydrophobicity for that helix. The amino acid residue and side chains, highlighted in red, show charged residues in the helices of the protein (Arg, Lys, Asp, Glu, His). This is to illustrate that although the effective hydrophobicities do not necessarily reside on the most charged residue, equation 1 takes into account the charged residues in its determination of $\Theta$ which is pointed in roughly the same direction as the polar side chains. Amino acids in green with side chains showing are those where the effective hydrophobicity $\Theta$ resides specifically on a polar amino acid residue. The space-filled blue residue is Lys296 implicated in a covalent bonding with retinal. This residue is pointed into the interior of the protein, where ligand binding is likely to take place.

Figure 5 Amino acids and side chains for residues of transmembrane helices on which are located the effective hydrophobicity residues for the beta adrenergic receptor. The green regions on the helices show the direction of the effective hydrophobicity for that helix. The amino acid residue and side chains, highlighted in red, show charged residues in the helices of the protein (Arg, Lys, Asp, Glu, His). This is to illustrate that although the effective hydrophobicities do not necessarily reside on the most charged residue, equation 1 takes into account the charged residues in its determination of $\Theta$ which is pointed in roughly the same direction as the polar side chains. Amino acids in green with side chains showing are those where the effective hydrophobicity $\Theta$ resides specifically on a polar amino acid residue. The residues represented by blue space-filled side chains are implicated in ligand binding.

One reason for the slight shift of the effective hydrophobicities from polar residues implicated in binding for both structures is probably that these residues are found at the junctions of the helices and extracellular loops, and are therefore subject to larger displacements at the alpha-carbon backbone than other residues in the helices. To test this, in the case of the adenosine A2A receptor,58 prior to using Hydro-Eff, the helical sequences were truncated by one turn at the N- and C-ends of the helix. Figure 6 shows that for all transmembrane helices, the green regions depicting the angles of highest effective hydrophilicity were pointed towards the center of the transmembrane assembly, which confirms that Equation 1 can be used to identify helical amphiphilicity and properly position helices in the GPCR transmembrane assembly. In most cases, the effective hydrophobicity was in the general direction of most polar residues on the helix, the side chains being identified in red. This indicates that the dynamic behavior of the protein at the helix-loop junctions does not identify side chain positioning with certainty, and should not be considered while using Hydro-Eff.

Figures 4–6 illustrate that while it is not necessary for polar residues to be the sole determinant of a helix’s amphiphilicity. The side chains of these polar residues when
Amino acids and side chains for residues of transmembrane helices on which are located the effective hydrophobicity residues for the adenosine A2A receptor. The green regions in the helices show the direction of the effective hydrophobicity for that helix. The amino acid residue and side chains, highlighted in red, show charged residues in the helices of the protein (Arg, Lys, Asp, Glu, His). This is to illustrate that although the effective hydrophobicities do not necessarily reside on the most charged residue, Equation 1 takes into account the charged residues in its determination of $\Theta \theta$, and the effective hydrophobicities are pointed in roughly the same direction as the polar side chains. Amino acids in green with side chains showing are those where the $\Theta \theta$ resides specifically on a charged residue. The blue space-filled side chains are for residues that are implicated in ligand binding. The figure shows that these residues are pointed into the interior of the protein where ligand binding is likely to take place.

Positioned into the interior of the protein, by virtue of their hydrophobicity values, are the highest value-contributors to the effective hydrophobicities of the helix. If polar residues are on directly opposite sides, then the amphiphilicity determined through calculations from Equation 1 help in positioning the helices. It is possible that, in addition to ligand binding, some polar residues also contribute toward stabilizing the helical bundle, thus protecting the ligand. It is also likely that, in addition to the polar residues, ligand binding is also stabilized by van der Waals interactions from the side chains of nonpolar amino acid residues. Mustafia and Palczewski have extensively assessed the binding in rhodopsin, the beta-adrenergic receptor, and the adenosine A2A receptor, based on the receptor structures. These are considerations that go beyond sequence and structure, ie, to specific biologic functions associated with specific GPCRs.

Using effective hydrophobicities as an aid to modeling GPCRs

As has been seen from our calculations of effective hydrophobicity for rhodopsin, the beta-adrenergic receptor, and the adenosine A2A receptor, as well as the discussions in the foregoing paragraphs, the hydrophilic side does not always face the binding region in the interior of the pocket, although the residues implicated in binding are positioned where they would be amenable to the interacting ligand.

As observations from Figures 4 and 5 indicate, the residues that have polar side chains pointed outwards are at the junction of the helices and loops either on the extracellular side or in the cytoplasm. Therefore, around the helix–loop junctions, a polar amino acid residue, the side chain of which is pointed away from the protein’s core will not necessarily violate polar-nonpolar interactions by being buried in the lipid bilayer. These are the transmembrane amino acid residues that are responsible for ligand intake prior to binding, or on the cytoplasmic side, are those responsible for G-protein coupling or residues involved in catalyzing signal transduction or other intracellular processes. One example is the MAYDRY peptide sequence motif, which is found at the junction of the third transmembrane helix and the second intracellular loop. This sequence (or a sequentially similar) motif is ubiquitous for all GPCRs. The polar residues, Asp and Arg at this junction will not be in the lipid bilayer.

Figure 7 shows the transmembrane helices for rhodopsin truncated to regions that are possibly embedded in the plasma membrane. The transmembrane amphiphilicities were then re-determined using Hydro-Eff. Figure 7 shows the transmembrane helices for rhodopsin truncated to regions that are possibly embedded in the plasma membrane. The transmembrane amphiphilicities were then re-determined using Hydro-Eff. Figure 7 was created for two reasons, ie, to assess how the helical amphiphilicity

![Figure 6](https://www.dovepress.com/)

**Figure 6** Amino acids and side chains for residues of transmembrane helices on which are located the effective hydrophobicity residues for the adenosine A2A receptor. The green regions in the helices show the direction of the effective hydrophobicity for that helix. The amino acid residue and side chains, highlighted in red, show charged residues in the helices of the protein (Arg, Lys, Asp, Glu, His). This is to illustrate that although the effective hydrophobicities do not necessarily reside on the most charged residue, Equation 1 takes into account the charged residues in its determination of $\Theta \theta$, and the effective hydrophobicities are pointed in roughly the same direction as the polar side chains. Amino acids in green with side chains showing are those where the $\Theta \theta$ resides specifically on a charged residue. The blue space-filled side chains are for residues that are implicated in ligand binding. The figure shows that these residues are pointed into the interior of the protein where ligand binding is likely to take place.

![Figure 7](https://www.dovepress.com/)

**Figure 7** Structure of rhodopsin with helices truncated to regions that will be embedded in the plasma membrane. The effective hydrophobicities were then determined again using all the scales determined in Hydro-Eff. The different colors indicate $\Theta \theta$ for different hydrophobicity scales, ie, Eisenberg consensus scales (red), Von Heijne (blue), Janin (yellow), Chothia (green), Wallenfend (pink), Kyte (orange), and Argos (purple). Despite some outliers, the figures suggest that during computational protein modeling, the helices should be rotated based on hydrophobicities of helices determined on truncated sequences that are likely to be embedded in the plasma membrane. Effective hydrophobicities are not necessarily pointed towards the interior of the protein, but might be pointed towards other helices, which might sustain the helical system.
determined by Hydro-Eff would allow the observation of helical position if residues at the helix–loop boundaries were not considered in the calculations using Equation 1 and to test how the Hydro-Eff system works for scales other than the Eisenberg consensus scales.

For each of the rhodopsin helices, $\Theta_\theta$ was determined based on the hydrophobicity scales of, in addition to the Eisenberg consensus scales (red), Von Heijne (blue), Janin (yellow), Chothia (green), Wolfenden (pink), Kyte (orange), and Argos (purple). The colors represent the residues on which the effective hydrophobicity is centered.

For rhodopsin, the effective hydrophobicities using different scales do not always exactly agree. However, they correctly represent the amphiphilic nature of helices, and are pointed in the expected directions, ie, towards the hydrophilic interior. In cases where all the colors representing the different hydrophobicity scales are not observed, this is where the effective hydrophobicity $\Theta_\theta$ from different scales coincide.

The transmembrane regions 1, 2, 3, 5, 6, and 7 in Figure 7 point to the interior. The effective hydrophobicities for TM4 variably point towards TM2 and TM5, depending on the scales used. While there is general consensus among the methods used to represent the amphiphilic side of the helix, there are some outliers when using different scales. In Figure 7, the effective hydrophobicities for TM1 using the Janin scales, TM4 using Argos, scales and TM7 using Wolfenden’s scales, are pointed away from the protein’s interior.

Consequences for protein–ligand binding

Determining amphiphilicities is crucial to membrane protein modeling, because properly representing the interior of the protein is necessary if accurate predictions are to be made with regard to ligand binding. This is also consequential to postbinding activities, such as signal transduction. Consider the computationally-driven interaction between an odorant molecule and an olfactory receptor (which are Class A GPCRs).19,43,47 Erroneous predictions of binding can result if the helices are not rotated correctly to maintain the hydrophilicity of the binding pocket. Equation 1 thus represents a reassessment of the paradigm that allows a protein computational modeler to position the helices.

Making predictions and inferences from computational studies should necessarily go hand in hand with experimental (mutagenesis) studies. Using computational methodologies to identify and predict putative agonists and antagonists among binding ligands have been attempted. These inferences, following static ligand docking, implicate amino acid residues that are at hydrogen bonding distances from the ligand key to binding and activation. Several computational studies, with support from experimental functional analyses, have been reported of the use of binding affinities to predict agonists versus antagonists.25,44–46 Lai et al published the first study of ligand–olfactory interactions using molecular dynamic simulation studies which better mimic the dynamic biologic environment.20 These studies have identified residues, in addition to those identified through static docking, as necessary for GPCR binding and activation.

Jaakola et al, who used the antagonist ZM241385 bound to the adenosine A2 receptor to crystallize the complex, make the point that the dynamic nature of the binding pocket has to be considered before making inferences about the nature of ligand-binding.58

Computational docking seeks to place a ligand in a region in the protein that is least hindered and can accommodate the binding ligand. Mutagenesis studies identify residues that result in activation or inhibition, and computational studies can identify the regions that house these residues. Studies (currently submitted) led by the author point to two specific binding regions in two olfactory receptors, ie, one which preferably binds ligands that serve as activators of olfactory receptors and one which binds ligands that inhibit the receptor. These results stem from experimental functional data for these olfactory receptors.50

It is critical then that for any computational modeling protocol to be successful, specifically in the case of GPCRs, that the transmembrane helices be properly identified and modeled, and the biochemical nature of the protein’s interior and exterior (its hydrophilicity) be properly maintained. Systems such as the Hydro-Eff are then valuable in this endeavor.

Conclusion

A formalism that quantifies the amphiphilicity of a helix is developed in this paper. For a specific angle on the helical wheel, Hydro-Eff considers hydrophobicity contributions from all other residues in the helix at the angle in question. The web page for the Hydro-Eff tool allows users to input peptide sequences for several helices and obtain tabulated results using differing hydrophobicity scales. Because each hydrophobicity scale was determined based on differing concepts and methodologies, a modeler will have to take into account the function of the protein before deciding which scale to consider. Indeed, results differ, as seen in Figure 7.

Another issue that a prospective modeler would have to consider is how much of the helix would contribute to amphiphilicity. Figures 4 and 5, when contrasted with Figures 6 and 7 in terms of amphiphilicity, indicate that Hydro-Eff predicts the hydrophilic side more effectively.
if residues that border the boundary between the helices and the interhelical loops are not used in determination of $\Theta_0$. This would be important when positioning helices following homology modeling, especially when there is weak homology between GPCRs to be modeled and GPCRs for which structures have already been determined.

Acknowledgments
This work is partially supported by 1R21DC011068-01 (National Institute on Deafness and Other Communication Disorders, National Institutes of Health), a Faculty Development Grant awarded to the author at University of Alabama at Birmingham, University of Alabama at Birmingham Comprehensive Cancer Center, Howell and Elizabeth Heffin Center for Genome Sciences, and the CTSA University of Alabama at Birmingham Center of Clinical and Translational Science.

Disclosure
The author reports no conflict of interest in this work.

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