Computational Models for the Helix Tilt Angle

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Received April 27, 2004

The concept of hydrophobic imbalance and that of hydrophobic and hydrophilic centers are used along with side chain models in the computation of helix orientation and tilt angle in or near a membrane. Rotamer statistics are used to infer typical side chain positions and chain length for each amino acid, and the results are used in fast computation of helix orientation. Sliding windows are used to compute local tilt angles on long α-helices that defy idealized modeling and generate tilt angle profiles. Seven different procedures based on different formulas and hydrophobicity scales are used for comparison. These procedures generated very similar tilt angle profiles. These profiles provide insights into helix deformation, membrane destabilization, and similarity and differences between membrane proteins.

1. INTRODUCTION

When a peptide in the form of an α-helix comes into contact with a membrane, its primary structure determines its orientation and in particular the tilt angle at which it attaches or inserts itself into the membrane. This tilt angle is considered as one of the crucial parameters in membrane-protein interplay, which is essential in viral infection, membrane curvature recognition, membrane destabilization, and transmembrane incorporation.

The first tilted peptides were described in 1988.[19] A few experimental results on the tilt angle exist, largely from attenuated total reflection infrared spectroscopy (ATR-FTIR),[1] neutron diffraction,[17] and NMR.[18] Models have been set up to understand the relationship between the primary structure of the peptide and the tilt angle. Hydrophobicity of the amino acids has been considered as the driving force in protein-membrane interaction. Its concentration in part of the peptide has been used in transmembrane prediction.[2] Its distribution or imbalance in a helix has been used as the indication of amphiphilicity.[3] Computational methods have been developed to solve the tilt angle and orientation, ranging from the simplistic[4] to the complicated.[5]

Here, we propose two approaches for computing the orientation and tilt angle of a helix, both are "rigid rotor" approximations based on an idealized helix main chain structure, to achieve the balance between accuracy and efficiency. The idealized main chain backbone is generated using a formula similar to that in ref 4. In the first approach, a rotamer library for α-helices[6] and the transfer energy for individual atoms[5] are used to determine the relative positions of centers for hydrophobicity for the 20 amino acids. The hydrophobicity centers for residues on the idealized helix can then be rapidly computed and so can the orientation and tilt angle.

In the second approach, the three-dimensional freedom of the hydrophobicity centers is reduced to a single value, the distance from the helix axis. The center of a residue is placed on the extension of the line segment from the axis to the α-carbon position of the residue. Different hydrophobicity scales (five scales were used in the examples) can be used to compute a normalized hydrophobicity imbalance (called the alternative definition of the hydrophobic moment in ref 3).

We also provide a scheme to compute local tilt angles along a long peptide, using a sliding window. This generates a "local tilt angle profile", a curve to go along with the sequence. This is used to provide visual comparison between different approaches and different scales as well as different peptides. The sliding window also adds flexibility to the "rigid rotor" model.

The paper is organized as follows. Section 2 is an attempt to unify various models and algorithms for computing orientation and the tilt angle of a peptide. We discuss conditions under which some of these models become equivalent and conditions for coordinate translation invariance. This section establishes the centroid pair and the normalized imbalance as two different modeling strategies that are invariant to coordinate translation. Section 3 deals with the problem of reducing each amino acid into either a pair of points or a single point while maintaining the equivalence of the computation at the atomic level. Parameters for computing the positions of these points are provided. Section 4 discusses different hydrophobicity scales and their use in the computation. Results of computation from different models and scales are compared with each other and with previously published results. Section 5 introduces the local tilt angle profile with some results on its application to the BAR domain and the OTCace protein family. Discussions are in Section 6.

2. THEORETICAL BASIS

The orientation and tilt angle of a chemical complex facing the membrane are often thought as the result of balance and

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10.1021/ci049859g CCC: $27.50
Published on Web 10/15/2004

energy minimization of electrostatic interactions between charged particles. Many simulation tools are based on pairwise additive force fields. These include interactions between particles within the complex, between particles within the membrane, and between particles in the complex and particles in the membrane. The models and algorithms discussed in this section are mostly concerned only with the interactions between the chemical complex (a helical peptide) and the membrane. Both the helix and the membrane are idealized as "rigid rotors", and we are concerned with their position relative to each other, or the orientation of the helix relative to the membrane, idealized as an infinite sheet of lipids.

We will explore several formulations of the tilt angle and the orientation of a chemical complex with respect to a membrane and indicate their similarity and differences. These formulations include the hydrophobic moment of Eisenberg, Weiss, and Terwilliger,[3] the hydrophobic imbalance of Silverman,[7] and the hydrophobic−hydrophilic centroid pair of Chou, Zhang, and Maggiora[4] and Brasseur.[5] We will show the conditions under which some of these become identical and the transformation from one to another.

Let T be a complex of components, attracted or repelled by a membrane, approximated by a plane with a norm $\mathbf{n}$. Let $\mathbf{s}_t$ be the position of the component t in T and h_t the *hydrophobicity* or *transfer energy* in which the membrane attracts t. The *net hydrophobicity*

$$H_T = \sum_{t \in T} h_t \tag{2.1}$$

is a measure of how strong the attraction on T is, with a per-component *mean hydrophobicity*

$$h_t = \frac{1}{|T|} H_T \tag{2.2}$$

We are now interested in the tilting force the membrane has on the complex. If the attraction to one side of the complex is different from that to the other side, then the imbalance of hydrophobicity may force the complex to rotate and approach the membrane at a specific tilt angle.

The *hydrophobic imbalance*[7] with respect to a center $\mathbf{c}$ is defined as

$$\mathbf{I}_T = \sum_{t \in T} h_t(\mathrm{s}_t - \mathrm{c}) \tag{2.3}$$

The torque the attraction exerts on component t, with respect to the center $\mathbf{c}$, is $h_t(\mathbf{s}_t - \mathbf{c}) \times \mathbf{n}$, and the total torque is $\sum h_t(\mathbf{s}_t - \mathbf{c}) \times \mathbf{n} = \mathbf{I}_T \times \mathbf{n}$. This total torque vanishes when $\mathbf{I}_T = k\mathbf{n}$ or when the hydrophobic imbalance aligns with the norm of the membrane. When the complex has the freedom to rotate, its insertion angle with respect to the norm of the membrane will be an angle between its hydrophobic imbalance and a direction indicating its geometric axis.

The hydrophobic imbalance $\mathbf{I}_T$ is not invariant with respect to the center $\mathbf{c}$. Indeed, if we can define

$$\mathrm{c} = \frac{\sum_{t \in T} h_t s_t}{\sum_{t \in T} h_t} \tag{2.4}$$

then the imbalance vanishes, $\mathbf{I}_T = 0$.

The imbalance becomes invariant with respect to the center if and only if the net hydrophobicity is zero, $H_T = 0$. This obviously can be realized if we use the *relative hydrophobicity* $h'_t = h_t - h_T$. This scheme was suggested by Eisenberg, Weiss, and Terwilliger,[3] as the "alternative definition" of the hydrophobic moment

$$\mathrm{J}_T = \sum_{t \in T} h'_t s_t \tag{2.5}$$

We also notice that we have $\mathbf{J}_T = \mathbf{I}_T$ when

$$\mathbf{c} = \frac{1}{|T|} \sum_{t \in T} \mathrm{s}_t \tag{2.6}$$

Thus, normalization on hydrophobicity scales is equivalent to selecting the appropriate center $\mathbf{c}$, which in this case is the geometric centroid of the complex.

Notice that the contribution of the component t to the hydrophobic imbalance depends on not only the hydrophobicity h_t but also its distance from the center $\mathbf{c}$ as well. The contribution or influence will be minimal when it is closest to the center.

Most chemical compounds contain some atoms attracted to the membrane (with positive hydrophobicity or being *hydrophobic*) and those repelled by the membrane (with negative hydrophobicity or being *hydrophilic*). In this case, the definition of center (2.4) cannot be used, and separate hydrophobic and hydrophilic centers have been proposed.

Chou, Zhang, and Maggiora[4] and Brasseur[5] adopted this approach in defining

$$\mathbf{c}_T^{+} = \frac{\sum_{t \in T, h_t > 0} \mathbf{s}_t}{H_T^{+}} \quad \mathbf{c}_T^{-} = \frac{\sum_{t \in T, h_t < 0} \mathbf{s}_t}{H_T^{-}} \tag{2.7}$$

where $H_T^{+} = \sum_{t \in T, h_t > 0} h_t$ and $H_T^{-} = \sum_{t \in T, h_t < 0} h_t$. The direction $\mathbf{c}_T^{+} - \mathbf{c}_T^{-}$ has been used as the insertion direction of the complex in the membrane.

This direction is the same as the hydrophobic imbalance if and only if the net hydrophobicity is zero. In general, the hydrophobic imbalance with respect to $\mathbf{c}$ can be computed from the centers as $\mathbf{I}_T = H_T^{+} \mathbf{c}_T^{+} + H_T^{-} \mathbf{c}_T^{-} - H_T \mathbf{c}$.

3. HYDROPHOBIC CENTERS FOR AMINO ACIDS

A chemical compound, like a polypeptide, can be easily divided into subgroups, for example, amino acid residues. Let $T = \cup_{i \in I} \mathrm{T}_i$ and the union be disjoint. The imbalance can be written as

$$\mathbf{I}_T = \sum\nolimits_{i \in I} \mathrm{R}_i \tag{3.1}$$

where

$$\mathbf{R}_i = \sum_{t \in T_i} h_t(\mathbf{s}_t - \mathbf{c}) = \sum_{t \in T_i} h_t \mathbf{s}_t - \sum_{t \in T_i} h_t \mathbf{c} \tag{3.2}$$

When the relative positions of the atoms in a subgroup are fixed, as in the case of relatively rigid amino acid residues, $\mathbf{R}_i$ can be computed as translation and rotation of some precomputed results. The question is whether $\mathbf{R}_i$ can be written as $H_i \mathbf{b}_i$ for some subgroup hydrophobicity H_i and subgroup position $\mathbf{b}_i$.

Table 1. Hydrophobic and Hydrophilic Centers of Amino Acids

	hydrophobic center			hydrophilic center		
amino acid	r_t	θ_t	h_t	r_t	θ_t	h_t
A (Ala)	2.619	0.201	−0.161	1.554	−0.038	0.355
C (Cys)	2.887	0.161	−0.413	1.554	−0.038	0.355
D (Asp)	2.840	0.170	−0.352	2.968	−0.024	−0.656
E (Glu)	3.429	0.307	−0.129	3.571	0.379	−0.228
F (Phe)	4.038	0.365	0.389	1.554	−0.038	0.355
G (Gly)	2.087	0.104	0.271	1.554	−0.038	0.355
H (His)	3.740	0.288	0.180	3.831	0.333	0.772
I (Ile)	3.683	0.212	−0.816	1.554	−0.038	0.355
K (Lys)	4.354	0.378	−0.136	4.585	0.425	−0.088
L (Leu)	4.019	0.298	−0.052	1.554	−0.038	0.355
M (Met)	4.029	0.066	−0.435	1.554	−0.038	0.355
N (Asn)	2.846	0.170	−0.346	3.180	−0.080	−0.767
P (Pro)	2.630	0.123	−1.131	1.581	0.031	0.581
Q (Gln)	3.433	0.304	−0.130	4.112	0.391	−0.176
R (Arg)	4.095	0.367	−0.219	5.986	0.484	−0.107
S (Ser)	2.619	0.201	−0.161	2.472	0.017	−0.348
T (Thr)	3.157	0.278	−0.047	2.448	0.013	−0.370
V (Val)	3.360	0.219	−0.403	1.554	−0.038	0.355
W (Trp)	4.547	0.215	0.612	3.124	0.393	0.720
Y (Tyr)	4.043	0.367	0.390	3.680	0.371	1.166

Similarly, in the computation of the hydrophobic and hydrophilic centers, the rigidity of the components implies possible simplification of the computation.

Once the base chain of a polypeptide is determined, the positions of the side chains can be predicted based on statistical data of protein structure. One of these sources is the backbone dependent rotamer library.[6] In this paper, we concentrate on the positioning of the residues on an α-helix, whose base chain can also be ideally modeled and has been so in the literature.

We use the method of Chou, Zhang, and Maggiora[4] to compute the position of the α-carbon C_α of the ith residue in an α-helix as

$$x_i = r_{C\alpha}\cos(i\theta_0)$$

$$y_i = r_{C\alpha}\sin(i\theta_0)$$

$$z_i = ih \tag{3.3}$$

where $r_{C\alpha} = 2.3$, $h = 1.5$ (in Å for both), and $\theta_0 = 100°$ form the coordinates in the cylindral system, $(r_{C\alpha}, i\theta_0, ih)$. The α-carbon of the first residue has the position (2.3, 0, 0).

Based on PDB, the average distances of the nitrogen in the amino group of the ith residue, N_i, to $C_\alpha(i)$, $C_\alpha(i+1)$, $C_\alpha(i+2)$, and $C_\alpha(i+3)$ are 1.45, 4.21, 5.30, and 5.59, respectively. From this, we calculate the cylindrical coordinates of N_i as $(r_N, i(\theta_N + \theta_0), ih+h_N)$, where $r_N = 1.55$, $\theta_N = 0.447$, and $h_N = -0.928$. The nitrogen of the first residue has the position (1.398, −0.671, −0.928). Similarly, the carbon in the carboxyl group of the first residue has the position (1.477, 0.800, 0.995).

From Dunbrack's rotamer library[6] for $\phi = -57°$ and $\varphi = -47°$ (α-helix), we determine the most likely positions of each atom in the side chain of each amino acid. This is done with a coordinate system local to each residue. This local coordinate system is defined with the α-carbon C_α at the origin (0, 0, 0), the nitrogen N on the positive z axis, at (0, 0, 1.458), and the carbon C in the carboxyl group in the x−y plane. This determines the y axis and the position of C in the local system (0, 1.436, −0.492). The transform matrix is

Table 2. Hydrophobicity Scores and the $\mathbf{b}_i$ Parameters for Amino Acids

amino acid	H^+	H_i^-	H_i	r_t	θ_t	h_t
A (Ala)	40.156	−28.833	11.32	5.524	0.372	−1.476
C (Cys)	47.350	−28.833	18.51	5.034	0.256	−1.608
D (Asp)	46.480	−52.517	−6.02	6.067	−0.792	−2.992
E (Glu)	63.396	−52.517	10.91	3.051	−0.111	0.351
F (Phe)	78.100	−28.833	49.25	5.577	0.429	0.408
G (Gly)	23.240	−28.833	−5.60	1.360	−2.045	0.707
H (His)	69.499	−62.815	6.89	3.331	−0.214	−5.378
I (Ile)	90.904	−28.833	62.08	4.698	0.251	−1.360
K (Lys)	90.904	−54.434	36.50	4.029	0.298	−0.208
L (Leu)	90.904	−28.833	62.08	5.211	0.344	−0.241
M (Met)	85.487	−28.833	56.64	5.294	0.082	−0.838
N (Asn)	46.480	−61.969	−15.51	4.926	−0.524	−2.030
P (Pro)	73.988	−24.528	49.45	3.155	0.146	−1.981
Q (Gln)	63.396	−61.971	1.42	29.835	−2.287	1.852
R (Arg)	80.312	−88.416	−8.11	25.458	0.672	0.998
S (Ser)	40.156	−44.980	−4.81	4.313	−1.169	−1.904
T (Thr)	57.072	−44.980	12.08	6.560	0.650	1.154
V (Val)	73.988	−28.833	45.13	4.552	0.275	−0.887
W (Trp)	90.748	−45.824	44.94	6.075	0.122	0.503
Y (Tyr)	78.100	−44.980	31.43	4.536	0.362	−0.664

$$R = \begin{pmatrix} -0.036 & -0.785 & -0.619 \\ -0.793 & 0.399 & -0.460 \\ 0.608 & 0.474 & -0.637 \end{pmatrix} \tag{3.4}$$

Given a point $\mathbf{t}$ in the local coordinate system, the position in the helix coordinate system of the first residue is $\mathbf{p} = R\mathbf{t} + (2.3, 0, 0)$. Let $\mathbf{p} = (u, v, w)$. The coordinates of $\mathbf{t}$ in the ith residue can be represented as

$$x_i = r_t\cos(i\theta_0 + \theta_t)$$

$$y_i = r_t\sin(i\theta_0 + \theta_t)$$

$$z_i = ih + h_t \tag{3.5}$$

where the cylindrical coordinate parameters are computed as follows.

$$r_t = \sqrt{u^2 + v^2}$$

$$\theta_t = \arctan\left(\frac{v}{u}\right)$$

$$h_t = w \tag{3.6}$$

The most likely positions of all hydrophobic atoms in an amino acid are summed into a combined position called the *hydrophobic center* for the amino acid and those of all hydrophilic atoms the *hydrophilic center*. The positions of the centers are then translated into the cylindrical coordinate parameters listed in Table 1.

The eq 3.5 can be used to translate these centers into the Cartesian coordinates. These will be the centers in the subgroup (or side chain) T_i

$$\mathbf{c}_i^+ = \frac{\sum_{t\in T_i, h_t>0} h_t s_t}{H_i^+} \quad \mathbf{c}_i^- = \frac{\sum_{t\in T_i, h_t<0} h_t s_t}{H_i^-} \tag{3.7}$$

where

$$H_i^+ = \sum_{t \in T_i, h_t > 0} h_t, H_i^- = \sum_{t \in T_i, h_t < 0} h_t$$

The overall centroid pair can be computed as follows.

$$\mathbf{c}^+ = \frac{\sum_{i \in I} H_i^+ \mathbf{c}_i^+}{\sum_{i \in I} H_i^+}, \mathbf{c}^- = \frac{\sum_{i \in I} H_i^- \mathbf{c}_i^-}{\sum_{i \in I} H_i^-} \quad (3.8)$$

The hydrophobicity scores H_i^+ and H_i^- for individual amino acids are computed based on the transfer energy table in ref 5 and are listed in Table 2. The net hydrophobicity for each amino acid, $H_i = H_i^+ + H_i^-$, or the sum of the two columns is also listed.

Note that the hydrophobic imbalance with respect to **c** can also be written as

$$\mathbf{I}_T = \sum_{i \in I} H_i (\mathbf{b}_i - \mathbf{c}) \quad (3.9)$$

where

$$\mathbf{b}_i = \frac{H_i^+ \mathbf{c}_i^+ + H_i^- \mathbf{c}_i^-}{H_i} \quad (3.10)$$

These centers $\mathbf{b}_i$ for different amino acids can be represented with cylindrical coordinate parameters, as shown in Table 2.

We can replace H_i with $H_i' = H_i - (1/|I|)H_T$ to have an imbalance that is invariant with respect to the center **c**.

$$\mathbf{J}_T = \sum_{i \in I} H_i' \mathbf{b}_i \quad (3.11)$$

The procedures for computing the tilt angles based on the rotamer configuration are listed as Procedure 1 and Procedure 2 below.

Procedure 1. Rotamer-Based Tilt Angle Using the Hydrophobic and Hydrophilic Centroid Pair

INPUT: A sequence of amino acid symbols.

OUTPUT: The angle between the helix axis and the membrane plane.

len = length of the input sequence

Label the sequence elements from 0 to len − 1 and let I be the set of the labels.

Let t be the amino acid label of the ith element of the sequence.

The hydrophobic center for the ith element is computed as $\mathbf{c}_i^+ = (x_i, y_i, z_i)$ as

$$x_i = r_t \cos(i\theta_0 + \theta_t)$$

$$y_i = r_t \sin(i\theta_0 + \theta_t)$$

$$z_i = ih + h_t$$

where $\theta_0 = 100°$, $h = 1.5$, and (r_t, θ_t, h_t) are from Table 1.

The hydrophilic center for the ith element, $\mathbf{c}_i^-$, is similarly computed.

The overall centroid pair $(\mathbf{c}^+, \mathbf{c}^-)$ is computed as (H_i^+ and H_i^- are from Table 2 based on the residue)

$$\mathbf{c}^+ = \frac{\sum_{i \in I} H_i^+ \mathbf{c}_i^+}{\sum_{i \in I} H_i^+}, \mathbf{c}^- = \frac{\sum_{i \in I} H_i^- \mathbf{c}_i^-}{\sum_{i \in I} H_i^-}$$

The angle between the vector $\mathbf{c}^+$-$\mathbf{c}^-$ and the axis of the helix is computed. Its complementary is the predicted tilt angle between the membrane plane and the axis of the helix.

Procedure 2. Rotamer-Based Tilt Angle Using the Imbalance with Relative Hydrophobicity

INPUT: A sequence of amino acid symbols.

OUTPUT: The angle between the helix axis and the membrane plane.

len = length of the input sequence

Label the sequence elements from 0 to len − 1 and let I be the set of the labels.

Let t be the amino acid label of the ith element of the sequence.

The hydrophobic center for the ith element is computed as $\mathbf{b}_i = (x_i, y_i, z_i)$ as

$$x_i = r_t \cos(i\theta_0 + \theta_t)$$

$$y_i = r_t \sin(i\theta_0 + \theta_t)$$

$$z_i = ih + h_t$$

where $\theta_0 = 100°$, $h = 1.5$, and (r_t, θ_t, h_t) are from Table 2.

Compute the relative hydrophobicity $H_i' = H_i - (1/|I|)H_T$ and the imbalance that is invariant to the choice of the center as

$$\mathbf{J}_T = \sum_{i \in I} H_i' \mathbf{b}_i$$

The angle between the vector **J** and the axis of the helix is computed. Its complementary is the predicted tilt angle between the membrane plane and the axis of the helix.

4. HYDROPHOBICITY SCALES

Hydrophobicity is arguably the most important property of amino acid side chains in the determination of protein structure and interaction. It is often measured or computed as the transfer energy of the amino acid from nonaqueous phases to water. The scales differ on different nonaqueous phases and different computation methods. The H_i listed in Table 2 can be treated as a hydrophoicity scale, even though Brasseur[5] uses the ratio H_i^+/H_i^- as the indicator of hydrophobicity.

For the purpose of this study, we chose the Fauchere-Pliska scale reported in ref 8, the Eisenberg scale reported in ref 7, the Kyte-Doolittle hydropathy reported in ref 9, the Wimley-White scale reported in ref 10, and H_i listed in Table 2. These scales are listed in Table 3.

Kyte and Doolittle, in the program TGREASE,[2] use the net hydrophobicity over a window on the protein sequence to predict transmembrane regions. Eisenberg, Weiss, and Terwilliger[3] applied hydrophobicity to the two-dimensional helical wheel, which, in our coordinate system, is a projection on the x−y plane. Residues are represented by vectors fanning out at 100° angle intervals. The vector length

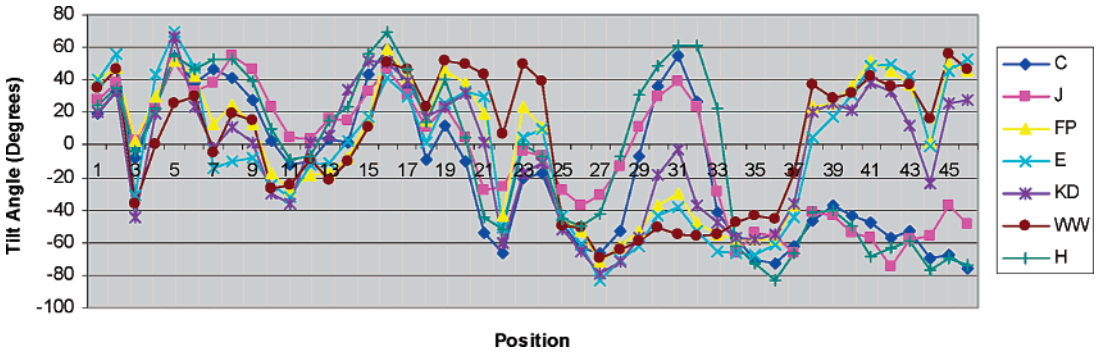

Figure 1. Local tilt angle profile of helix 2 of *Drosophila* amphiphysin (dAmph), based on the seven different computation methods. C (center pairs) and J (imbalance) are based on rotamer data. FP (Fauchere-Pliska), E (Eisenberg), KD (Kyte-Doolittle), WW (Wimley-White), and H (H_i) are hydrophobicity scales with varying radius. The window size is 15. The peptide sequence is YDALQ-AQTGASESLWADFAHKLGDQVLIPLNTYTGQFPEMKKKVEKRNRKLIDYDGQRHSF.

Table 3. Hydrophobicity Scales and Side Chain Length

amino acid	hydrophobicity scales: Fauchere-Pliska[8]	Eisenberg[7]	Kyte-Doolittle[9]	Wimley-White[10]	H_i/10	side chain length r_t in Å[11]
A (Ala)	1.8	0.25	1.8	−0.50	1.13	2.20
C (Cys)	8.7	0.04	2.5	0.02	1.85	3.34
D (Asp)	−4.4	−0.72	−3.5	−3.64	−0.60	3.81
E (Glu)	−3.6	−0.62	−3.5	−3.63	1.09	5.33
F (Phe)	10.2	0.61	2.8	1.71	4.93	6.21
G (Gly)	0.0	0.16	−0.4	−1.15	−0.56	1.11
H (His)	0.8	−0.40	−3.2	−0.11	0.69	5.38
I (Ile)	10.3	0.73	4.5	1.12	6.21	4.83
K (Lys)	−5.6	−1.10	−3.9	−2.80	3.65	7.14
L (Leu)	9.7	0.53	3.8	1.25	6.21	4.84
M (Met)	7.0	0.26	1.9	0.67	5.66	6.35
N (Asn)	−3.4	−0.64	−3.5	−0.85	−1.55	4.13
P (Pro)	4.1	−0.07	1.6	−0.14	4.95	3.38
Q (Gln)	−1.3	−0.69	−3.5	−0.77	0.14	5.51
R (Arg)	−5.7	−1.76	−4.5	−1.81	−0.81	8.22
S (Ser)	−0.2	−0.26	−0.8	−0.46	−0.48	2.66
T (Thr)	1.5	−0.18	−0.7	−0.25	1.21	3.60
V (Val)	6.9	0.54	4.2	0.46	4.51	3.62
W (Trp)	12.8	0.37	−0.9	2.09	4.49	6.97
Y (Tyr)	5.5	0.02	−1.3	0.71	3.14	6.92

represents the hydrophobicity of the residue (including negative ones). They claimed that a large sum of the vectors indicates that the helix is amphiphilic perpendicular to its axis. Chou, Zhang, and Maggiora[4] adopt a three-dimensional view and thus a three-dimensional amphiphilic direction for the helix.

While Kyte and Doolittle[2] represented residue hydrophobicity as points in a one-dimensional space, Eisenberg, Weiss, and Terwilliger[3] extended this to a two-dimensional space, and Chou, Zhang, and Maggiora[4] to a three-dimensional space. Still, the positions of these points are fixed in all cases regardless of the differences between individual amino acids. For example, the location the hydrophobicity of a residue takes place in ref 4 is the location of the α-carbon of the residue in an idealized helix model.

What we have done in the previous section is to place the location of hydrophobicity of each residue differently. We used different cylindrical coordinate parameters (r_t, θ_t, h_t) for different amino acids (Table 2). A coarser way to do this is to vary the radius from the axis of the helix only,

Table 4. Helix Tilt Angles in Degrees Reported in the Literature and Those Computed Using Procedures 1−3

sequence	lit.	rotamer-based: Procedure 1	rotamer-based: Procedure 2	Procedure 3 with different scales: H_i	F.-P.	Eisenberg	K.-D.	W.-W.
GVFVLGFLGFLA	50	−14	10	15	11	−6	2	16
AVGIGALFLGFL	45	25	62	56	55	**41**	**37**	55
FAGVVLAGAALG	77	**−69**	−46	−62	−60	−60	**−75**	−64
FIGAIIGSVALGVATAAG	73	**−68**	**−70**	**−66**	**−69**	**−64**	−54	**−70**
FLGFLLGVGSAIASGVA	63	**−57**	**−63**	**−57**	**−61**	−51	−35	**−65**
FFGAVIGTIALGVATSA	72	**−66**	**−63**	**−65**	**−68**	**−68**	−54	**−65**
SPVAALTLGLAL	60	31	34	**51**	**66**	78	**64**	**69**
GPVSLTLALLLGGLTMG	63	**−57**	−50	−21	3	35	−42	−4
GAAIGLAWIPYFGPAAE	45	−80	−6	**43**	**−40**	−61	−76	**−46**
MLLQAFLFLLAGFAAKISA	55	**−61**	−75	**−61**	**−62**	**−50**	**−46**	−65
RPALLALLALPA	45	24	14	**38**	**50**	57	56	**48**
VTVVLWSAYPVVWLIG	65	−41	15	35	50	33	−9	16
GAGIVPLNIETLLFMVLD	31	**33**	72	71	63	**−29**	8	**33**
AGAVVGGLGGYMLGSAMS	25	**−31**	**22**	**31**	16	−70	−56	55
GAIIGLMVGGVV	50	−33	−15	15	9	12	34	9
IKKAGTELVNFLSYFVEL	30	13	**29**	**23**	45	**39**	**31**	45
ASLLSFMQGYMKHAT	45	−34	**−42**	−20	**−39**	**−47**	−55	**−39**
FGFPEHLLVDFLQSLS	30	**−29**	**−29**	**−24**	**−22**	**−34**	−11	−3
DFFTIWLDLNMFL	40	**40**	50	**49**	23	26	50	30
FLELYRHIAQHGF	47	**−42**	**−41**	**−51**	−27	−7	−23	−7
IGEAIRVIAERGL	55	−35	−25	−17	−15	−25	−18	−3
CGTLHCHSGSITPI	60	38	**66**	**57**	21	47	**51**	35
VIGTNAVSIETNIE	50	−39	−20	−24	**−50**	−60	−62	**−51**
GLFGAIAGFIENGWEGMIDG	47	−36	−29	−25	−36	**−52**	**−54**	**−46**
DTRCGRLICGLSTTAQYP	50	**43**	35	**50**	**46**	**50**	31	73
MENITSGFLGPLLVLQ	57	34	52	45	**51**	**49**	**61**	27
GAIIGLMVGGVVIA	50	−40	−6	29	26	**54**	61	27
GVIIILMVGGAVGA	85	**−86**	**−80**	**−82**	**−85**	**−77**	−75	**−84**

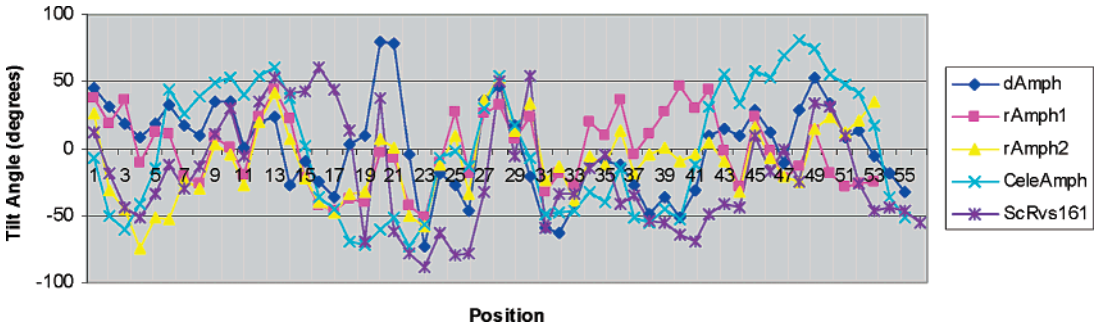

Figure 2. Local tilt angle profiles of helix 3 of five members of the BAR family, computed with Procedure 1 and window size 15. See ref 15 for the alignment and sequences.

which is equivalent to fixing $\theta_\tau = 0$ and $h_t = 0$. We decided to use the "side chain lengths" shown in Table 3 for the radius r_t. (We actually used half of the side chain length plus r_{C_α} as r_t.) The procedure for this coarse method is listed as follows.

Procedure 3. Tilt Angle Computation Using Variable Side Chain Lengths

INPUT: A sequence of amino acid symbols and a hycrophobicity scale.

OUTPUT: The angle between the helix axis and the membrane plane.

len = length of the input sequence

Label the sequence elements from 0 to len − 1 and let I be the set of the labels.

Let t be the amino acid label of the ith element of the sequence.

The hydrophobic center for the ith element is computed as $\mathbf{b}_i = (x_i, y_i, z_i)$ as

$$x_i = r_t\cos(i\theta_0 + \theta_t)$$

$$y_i = r_t\sin(i\theta_0 + \theta_t)$$

$$z_i = ih + h_t$$

where $\theta_0 = 100°$, $h = 1.5$, $\theta_t = 0$, $h_t = 0$, and r_t are from the last column of Table 3.

Compute the relative hydrophobicity $H_i' = H_i - (1/|I|)H_T$ and the imbalance that is invariant to the choice of the center as

$$\mathbf{J}_T = \sum_{i\in I} H_i'\mathbf{b}_i$$

The angle between the vector **J** and the axis of the helix is computed. Its complementary is the predicted tilt angle between the membrane plane and the axis of the helix.

Now we have several ways to compute the tilt angle of a helix at a membrane. Table 4 shows seven different but efficient ways to compute the angle and a comparison to published angle data. The first 21 sequences are from ref 12, and the next five are from ref 13. The last two are from ref 14. The column with heading "lit." contains angles reported in these publications. Column 3 contains angles computed using rotamer-based hydrophobic and hydrophilic centers for individual residues (Procedure 1). Column 4 contains angles computed using a single-point center for each residue, also based on the rotamer data (Procedure 2). The last five columns contain computation using the varying radius for different residues and different hydrophobicity scales (Procedure 3). Computation results within 10° are marked with **bold face** and the ones closest to the literature are underlined. Angles are measured in degrees, between the helix axis (from the N-terminal to the C-terminal) and the membrane plane and have a range of (−90, 90].

The results in Table 4 show that no method was an obvious winner. The varying radius with the Eisenberg hydrophobicity scale and the rotamer with center pairs perform a little better than the others, if we use the number of close predictions plus the number of best predictions as the score for ranking. On the other hand, the method based on the varying radius with H_i as the hydrophobicity scale seems to have the least number of outliers in its predictions.

5. TILT ANGLE PROFILES

When the helical section of a protein is long, or when the nature of the secondary structure is less certain, the idealized base chain structure may be questionable and the computation of a tilt angle for the complete helix may be error prone. Instead, we devised a strategy to compute the tilt angles of local segments of a helix, along its length, with a fixed window size. This results in a tilt angle profile for a primary peptide structure and can be used to compare the peptides, the procedures, or the hydrophobicity scales. It may also provide insights into the nature of helix distortion in membrane and membrane destabilization due to the presence of peptides. We will use proteins with the BAR (Bin/amphiphysin/Rvs) domain[15] for demonstration purposes.

The procedure for computing the local tilt angle profile is given as follows.

Procedure 4. Local Tilt Angle Profile Computation

INPUT: A sequence of amino acid symbols, a window size d, and a tilt angle procedure.

OUTPUT: A sequence of tilt angles representing the local tilting tendencies along the peptide.

len = length of the input sequence

Label the sequence elements from 0 to len − 1.

For i = 0 to len − d − 1 do

Apply the tilt angle procedure to the subsequence from i to i + d − 1.

The len − d tilt angles form the local tilt angle profile.

Figure 1 shows the local tilt angle profile of the second helix in the *Drosophila* amphiphysin (dAmph). It can be seen that the seven different tilt angle formulas generally provide similar readings, especially the directions of change. Notice at the right-hand side of the graph, the methods branch into two groups, largely based on whether the atomic transfer energy parameters[5] were used.

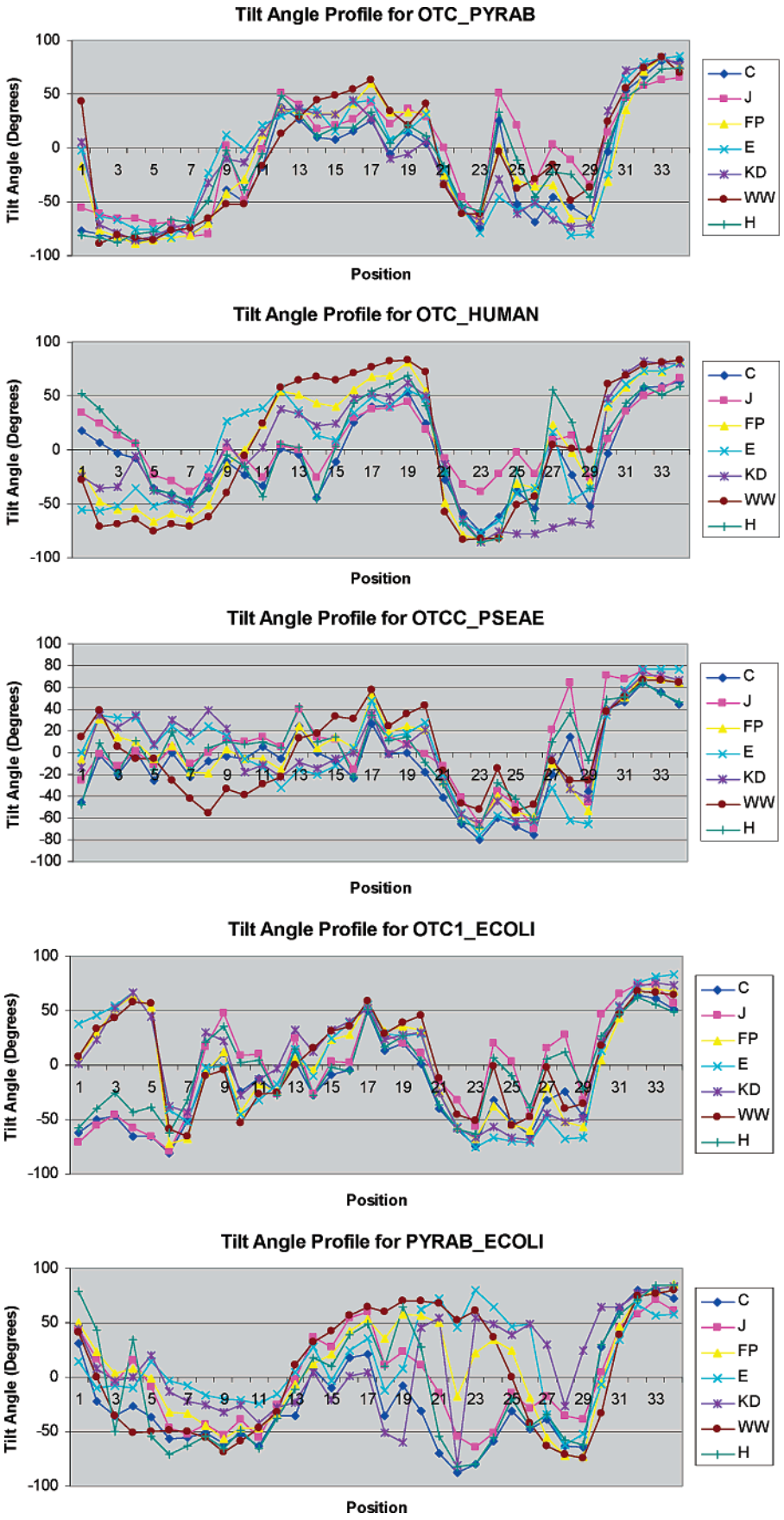

Figure 3. Tilt angle profiles for members of the OTCace family. These are (top-down) OTC_PYRAB/262−310, OTC_HUMAN/294−342, OTCC_PSEAE/284−332, OTC1_ECOLI/283−331, and PYRB_ECOLI/256−304.[16] Seven tilt angle procedures were used and see Figure 1 for procedure abbreviations. The sequences are listed in Table 5.

Table 5. Sequences in the Protein Family OTCace

protein	position	sequence
OTC_PYRAB	262-310	FMHCLPAHRGEEVTDDVIDSPNSVVWDEAENRLHAQKAVLALLL
OTC_HUMAN	294-342	WTFLHCLPRKPEEVDDEVFYSPRSLVFPEAENRKWTIMAVMVSLL
OTCC_PSEAE	284-332	KQIAEqYPNLANgIEVTEDVFESPYNIAFEQAENRMHTIKAILVSTL
OTC1_ECOLI	283-331	MAEeFGLHGGMEVTDEVFESAASIVFDQAENRMHTIKAVMVATL
PYRB_ECOLI	256-304	MKVLHPLPRVDEIATDVDKTPHAWYFQQAGNGIFARQALLALVL

Table 6. Sequences with Close Angle Predictions from All Seven Models[a]

sequence	C	J	H	FP	E	KD	WW
FVCLKIGACPSAHKP	−78	−75	−84	−83	−81	−84	−81
YLPVILDIIKGEMSR	−65	−59	−57	−62	−64	−64	−63
IYTDVWASMGQEAEA	−52	−49	−52	−57	−49	−50	−59
KEICALVGFCDEVKE	−43	−44	−33	−36	−34	−37	−40
TKLAEQYAKENGTKL	−10	−5	−9	−4	0	−3	−1
KVFEEFNVDLQEELP	1	4	2	9	2	6	2
EVTDEVFESAASIVF	50	54	52	58	57	53	59
TNDPLEAAHGGNVLI	62	69	68	70	71	66	61

[a] See Figure 1 for abbreviations.

The local tilt angle profiles on different peptides in a protein family can shed light on their similarities and differences. Figure 2 contains the tilt angle profiles on the helix 3 of five members of the BAR family, all computed using Procedure 1.

The tilt angle profile can be used on a family of proteins to find out some of their common features and also the differences. Figure 3 shows the tilt angle profiles at the ends of the proteins in the family OTCace.[16] There is a helix at the end of each of these proteins, and they share very similar tilt angle profiles. The sequences involved are quite different, and they are in Table 5.

The seven computational methods give different tilt angle readings to many sequences. But they give very close readings for some sequences. Table 6 shows some of these sequences.

6. DISCUSSION

We have proposed two computational models for rapid calculation of helix orientation and tilt angle in or near the membrane. These models are based on the idealized α-helix structure and idealized side chain positions. The first model uses different three-dimensional parameters for side chain positioning of each amino acid, based on the statistically most likely positions published in rotamer libraries. The orientation of the helix is determined with either the centroid pair or the imbalance method. The second model uses side chain length as a one-dimensional parameter for each amino acid and different hydrophobicity scales to compute hydrophobic imbalance. We applied these models to helices as a whole and to subsequences in helices, and the latter approach resulted in tilt angle profiles.

We tested these models (seven procedures altogether) on protein helices with published tilt angle measurement or calculation and found out that even though all provided credible approximation in most cases, none was the obvious winner. There were sequence segments (Table 6) on which all formulas agree quite well. From many tilt angle profile computation results (Figures 1 and 3), we can see that these models and procedures gave similar overall predictions.

Compared with other available formulations, our models and methods provide a middle ground between the extremes in the spectrum of tilt angle computation. Our methods allows computation speed to be identical or similar to the most oversimplified models at one end and accuracy to approach the most complex energy minimization models at the other end. This allows us to perform large and genomic scale computation and find signature features in protein families. The tilt angle profile of a peptide shows the local interactions between the peptide and the membrane. This may be used in prediction of helix distortion and membrane destabilization. The consensus within tilt angle profiles on members of a protein family may indicate necessary configurations common to the family members for a certain biological function.

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CI049859G