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IMPALA: A Simple Restraint Field to Simulate the Biological Membrane in Molecular Structure Studies

Ph. Ducarme,* M. Rahman, and R. Brasseur
Centre de Biophysique Moléculaire Numérique, Faculté Universitaire des Sciences Agronomiques de Gembloux, Gembloux, Belgium

ABSTRACT **The lipid bilayer is crucial for the folding of integral membrane proteins. This article presents an empirical method to account for water–lipid interfaces in the insertion of molecules interacting with bilayers. The interactions between the molecule and the bilayer are described by restraint functions designed to mimic the membrane effect. These functions are calculated for each atom and are proportional to the accessible surface of the latter. The membrane is described as a continuous medium whose properties are varying along the axis perpendicular to the bilayer plane. The insertion is analyzed by a Monte Carlo procedure applied to the restraint functions. The method was successfully applied to small α peptides of known configurations. It provides insights of the behaviors of the peptide dynamics that cannot be obtained with statistical approaches (e.g., hydropathy analysis). Proteins 30:357–371, 1998.** © 1998 Wiley-Liss, Inc.

Key words: membrane; protein; structure; prediction; hydrophobicity; computer; magainin; melittin; 18A; M2δ; PGLa

INTRODUCTION

IMP represent about half the proteins of cells. They play crucial roles for the cell survey such as the selective passage of solutes or the transmission of information. However, little is known about the IMP structures mainly because classical experimental approaches (NMR and X-rays) can hardly be used with this type of protein. Molecular modeling is therefore of special interest but, compared to the structure prediction of soluble proteins, modeling of IMP is drastically limited because many methods (e.g., homology modeling) lean on the use of known 3D structures. The 3D structures of IMP are too few and thus could be misleading. Fortunately, the structural restraints imposed by the membrane are such that the diversity of secondary and tertiary structures is limited. Thus one often considers that most transmembrane segments of IMP are structured as α helices. This allows the development of very simple and straight algorithms (e.g., hydropathy plots[1]) that can determine the transmembrane segments and provide information about their relative positions. Although these methods afford valuable understanding of the IMP structures, they do not examine the kinks, tilts, extramembrane loops, hindrances, or physical interaction between transmembrane segments, thereby precluding the construction of a precise 3D model. Moreover, the membrane medium seems crucial for IMP folding, and it should be simulated. Since each lipid is described by its atomic coordinates, this approach requires huge calculation times. Our actual calculation capabilities allow very short periods for simulation of the molecule dynamics (e.g., 20 ns in Ref. 2), which is not enough time for determining the structure of a protein from a rough topography. However, one can devise methods that consider the membrane as a continuous medium.[3–5] Milik and Skolnick[6] proposed such a method, predicting with success the orientation and degree of association of several peptides in membranes. However, lateral side chains were modeled as hard spheres and thermodynamic constants were specific for α structure. Thus, this method cannot be used for 3D-model structure refinement.

In this article, we present a method to study the association of peptides with the membrane—named IMPALA for *IMP a*nd *L*ipid *A*ssociation—based on the calculation of restraints, and using a full atomic description of the molecule. The purpose of this approach is not to describe the precise interactions governing the IMP folding[7,8] but rather to obtain more realistic models in accord with the experimentally known features of IMP structures. In this way, we use restraints. These restraints are not energy functions because they do not directly correspond to actual physical interactions, but they are designed so those nativelike structures have lower restraints.

Abbreviations: CD, circular dichroism; IMP, integral membrane protein(s); NMR, nuclear magnetic resonance; PDB, proteins databank reference; SW, SwissProt databank reference; 3D, three-dimensional.

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*Correspondence to: Dr. Ph. Ducarme, Centre de Biophysique Moléculaire Numérique, Faculté Universitaire des Sciences Agronomiques de Gembloux, Passage des Déportés, 2, B5030 Gembloux, Belgium.
E-mail: ducarme.p@fsagx.ac.be
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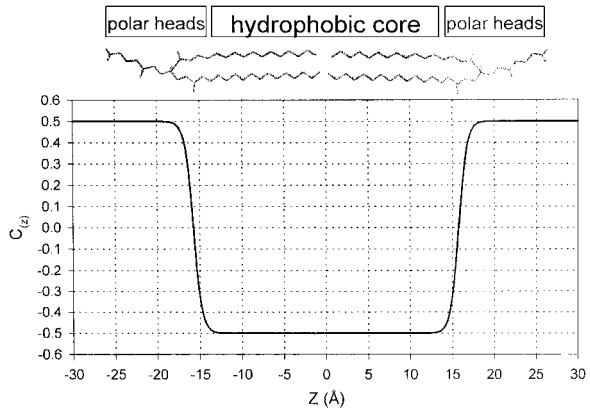


Fig. 1. Function $C(z)$ along the z-axis perpendicular to the membrane; $z = 0$ corresponds to the contact between the two layers of the membrane. Two representative lipids are shown.

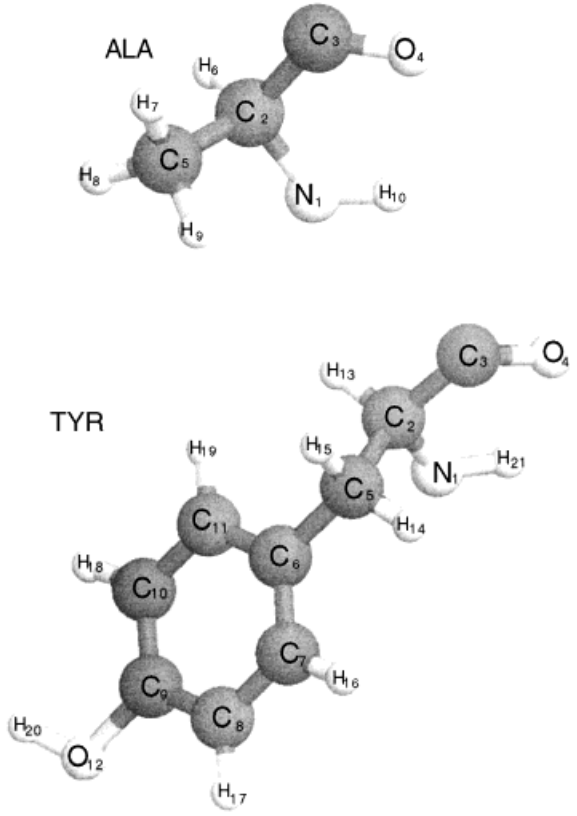


Fig. 2. Structures and atom numbers for Ala and Tyr.

METHOD

Description of the Water Bilayer Interfaces

The assumption is made that the properties of the membrane are constant in the plane of the bilayer (x and y axes). Thus, the lipid/water interfaces are described by a function, $C_{(z)}$, which varies along the z axis only; z (in angstroms) is perpendicular to the plane of the membrane and its origin is at the center of the bilayer. $C_{(z)}$ (Fig. 1) is an empirical function

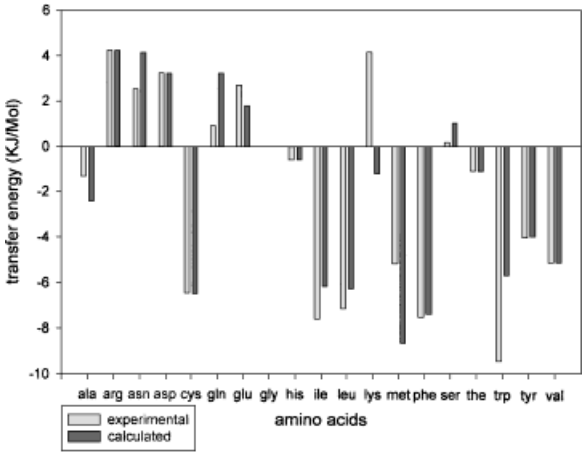


Fig. 3. Experimental[15] and computed (this article) transfer energies for the side chains of 19 of the 20 natural amino acids.

TABLE I. Atomic Surface Transfer Energies for the Seven Atomic Types*

Atomic types	Transfer energies per accessible surface (KJ/mol Å^2)
Csp3	−0.105
Csp2	−0.0134
H(=0)	−0.0397
H(/0)	0.0362
O	0.0403
N	0.112
S	−0.108

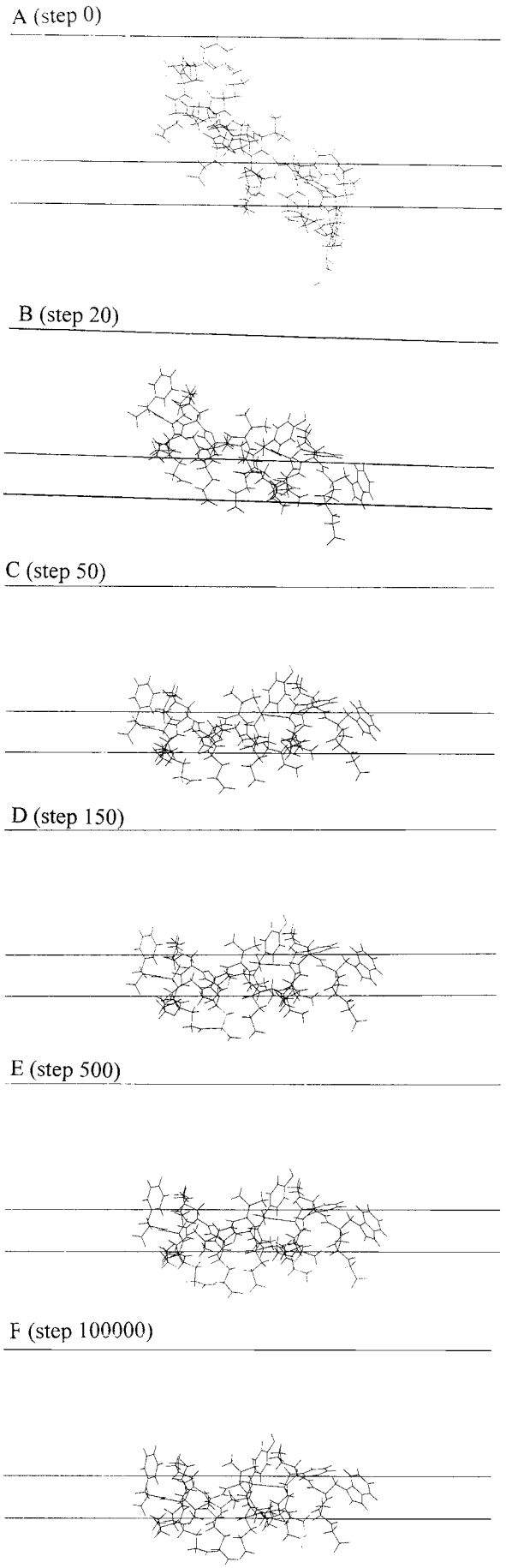
*Csp2, double-bonded and aromatic carbons; Csp3, single-bonded carbons; H(=0), noncharged hydrogen (bound to C); H(/0), charged hydrogen; O, oxygen; N, nitrogen; S, sulfur.

varying from 0.5 (completely hydrophilic) to −0.5 (completely hydrophobic). It is derived from Ref. 6:

$$C_{(z)} = 0.5 - \frac{1}{1 + e^{\alpha(|z|-z_0)}} \qquad (1)$$

where α and z_0 are mathematical parameters calculated so that $C_{(z = 13.5\text{Å})} = 0.49$ and $C_{(z = 18\ \text{Å})} = 0.49$ and that this symmetrical function is approximately constant from $-\infty$ to −18 Å (hydrophilic phase), −13.5 Å to 13.5 Å (hydrocarbon core) and 18 Å to ∞ (hydrophilic phase). $Z = 13.5$ Å is the distance at which the first polar heads appear,[9] and an interface of 4.5 Å gives the best results for the simulation. The same interface width was found in the Monte Carlo technique developed by Milik and Skolnick.[6] This mathematical form of $C_{(z)}$ was chosen because it is continuous and can be rapidly computed.

Because C does not vary along x and y, a molecule is fully described by its internal geometry, two rotations (around x and y) and one translation (along z). The main simplification of this approach is that it totally neglects specific interactions between the molecule and lipids or other molecules buried in the membrane. However, generally these interactions do



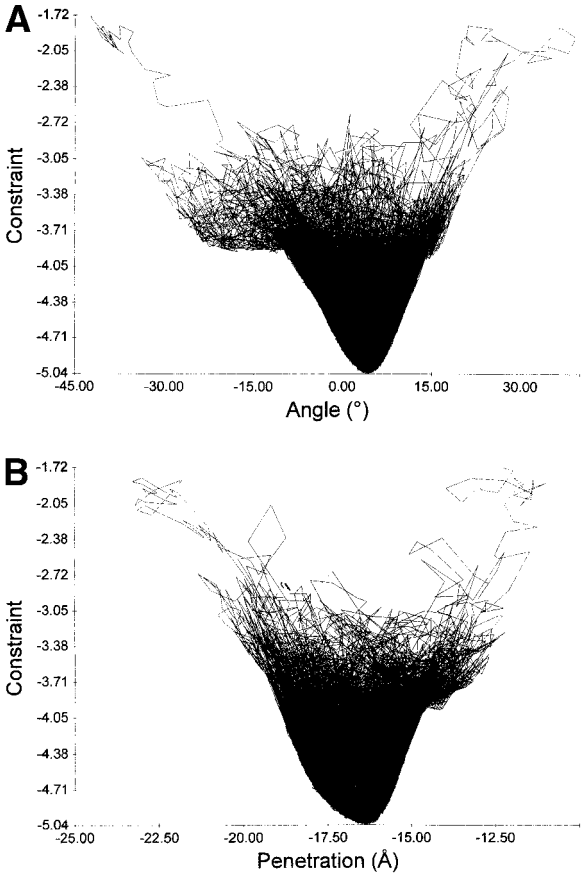


Fig. 5. Profiles of the simulation of the 18A peptide. On each graphic, one point corresponds to a Monte Carlo step. **A:** Sum of restraints [Eqs. (4) and (5)] versus the angle of insertion (i.e., angle between helix axe and the plane of the bilayer). **B:** Restraints versus penetration (i.e., the *z* coordinate of the mass center of the molecule).

not seem crucial for the folding of membrane proteins, as the proteins may refold in vitro under nonbiological conditions.[10] This assumption is also made for the crystallization of IMP where lipids are replaced by detergents. Moreover, the description is so simple that it could be easily adapted to other interfaces (e.g., monolayer, spherical, or discoidal).

Atomic Surface Hydrophobicity

To calculate the restraints we use atomic surface transfer energies. This concept relies on the assumption that the overall transfer energy of a molecule, H, can be calculated as

$$H = \sum_{i=1}^{N} S_{(i)} E_{\mathrm{tr}(i)} \qquad (2)$$

Fig. 4. Steps of the simulation of the 18A peptide. The three planes correspond (from top to bottom) to the center of the membrane, $z = 13.5$ Å and $z = 18$ Å. Step 100,000 is the best configuration found by the Monte Carlo procedure.

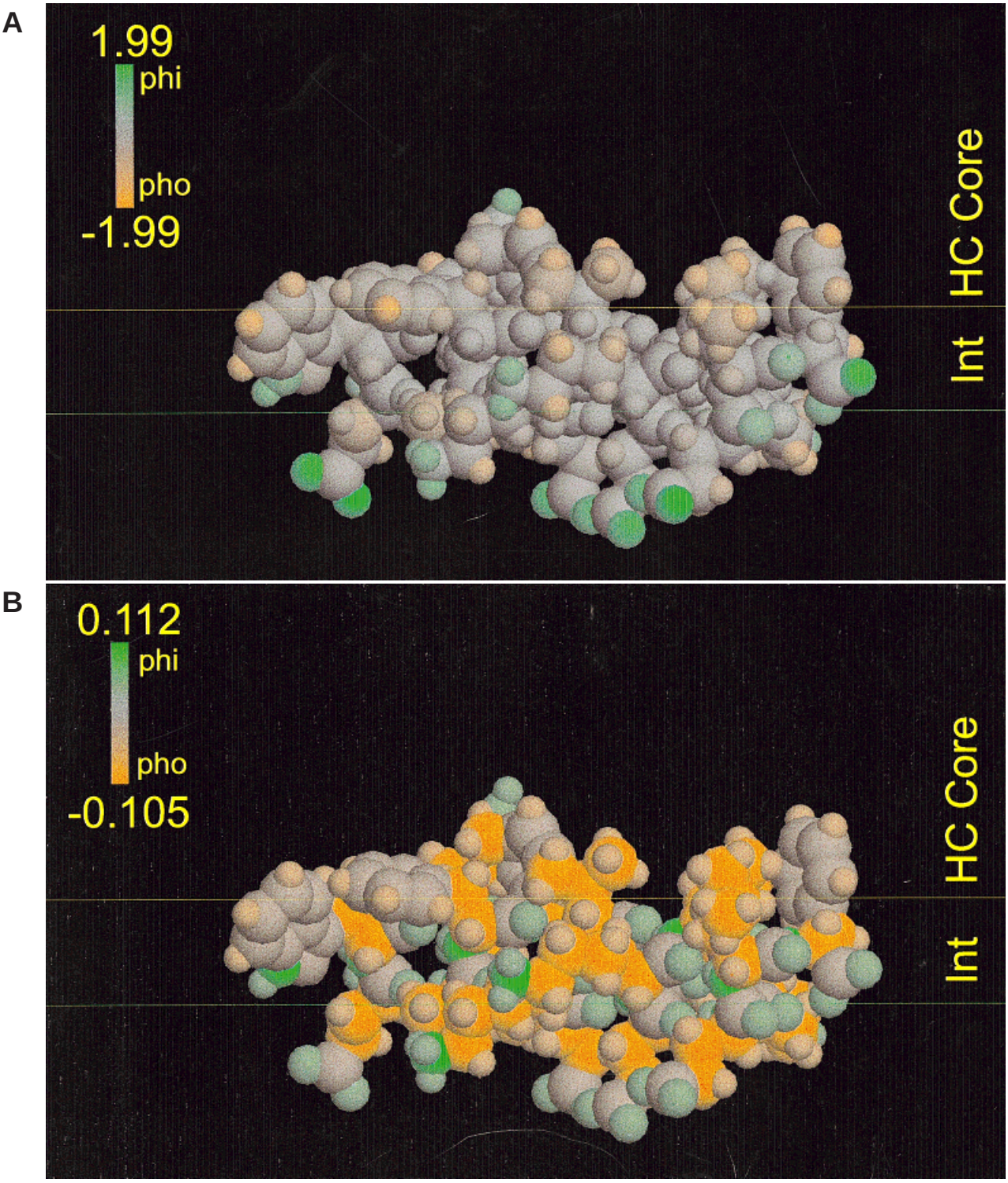


Fig. 6. The best configuration found for the 18A peptide. Atoms are colored according to the factor **A:** S(i)*Etr(i) [see Eq.(2)]. **B:** Etr(i) [see Eq.(3)] *Green,* hydrophilic; *orange,* hydrophobic; *red,* center of membrane; *yellow, z* = 13.5 Å; *green, z* = 18 Å.

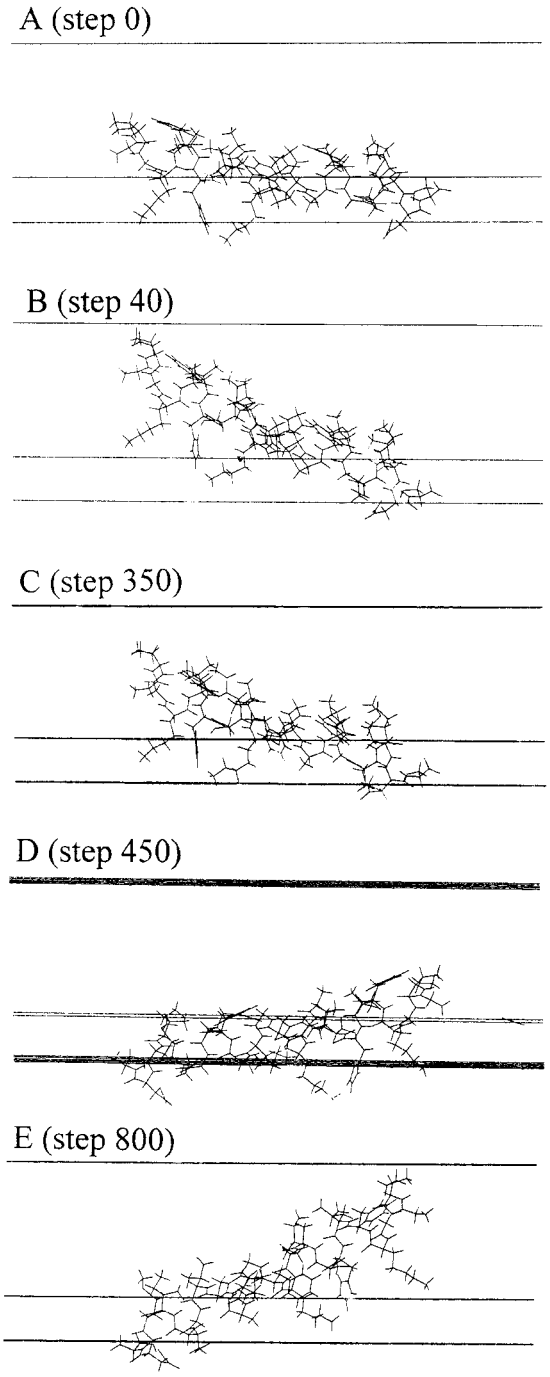


Fig. 7. Steps of the simulation of the magainin. The three planes correspond (from top to bottom) to the center of the membrane, $z = 13.5$ Å and $z = 18$ Å.

where i is an index for the N atoms of the molecule, S (Å^2) the solvent-accessible surface—calculated by using the NSC routine[11]—and E_{tr}(kJ · mol · Å^2) is the transfer energy by surface of individual atoms.

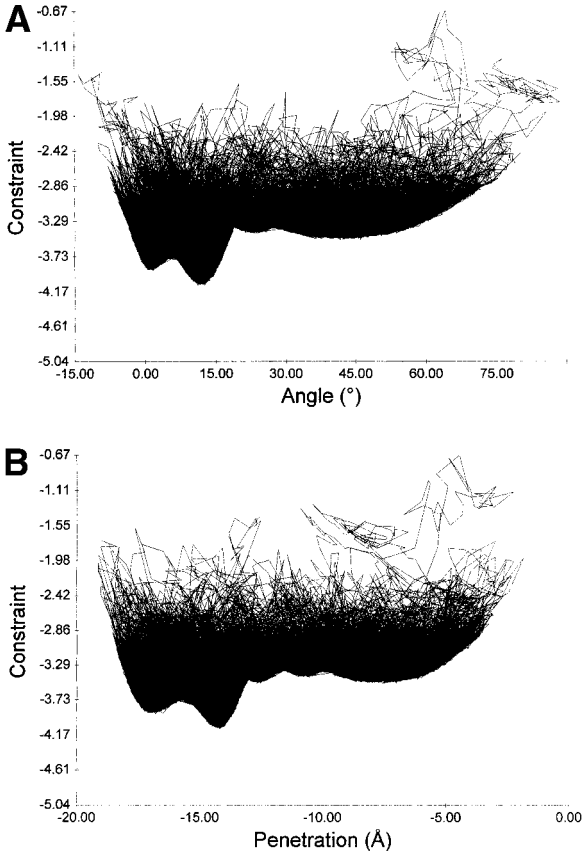


Fig. 8. Profiles of the simulation of the magainin. On each graphic, one point corresponds to a Monte Carlo step. **A:** Sum of restraints [Eqs. (4) and (5)] versus the angle of insertion (i.e., angle between helix axe and the plane of the bilayer). **B:** Restraints versus penetration (i.e., the z coordinate of the mass center of the molecule).

A similar approach was already successfully attempted on small molecules[12] by considering that

$$H = \sum_{i=1}^{N} E_{\mathrm{atr}(i)} \tag{3}$$

where E_{atr} is the atomic transfer energy of one of the N atoms of the molecule considered. In addition we introduce a correcting factor that takes into account the fact that solvent interacts only with accessible atoms. This is known to be essential for a good description of the hydrophobic effect.[13,14] Our formulation is at least in line with two experimental facts: The hydrophobic effect is related to the nature of the solute and to the surface of solute in contact with solvent. To calculate the values of E_{tr} we wrote an equation system by applying Equation (2) to 19 of the 20 natural amino acids (excluding proline for convenience). We used the experimental transfer energies measured by Fauchère and Pliska[15] as H values.

Following Brasseur,[12] seven atomic types were considered: double-bonded carbon (Csp2), including

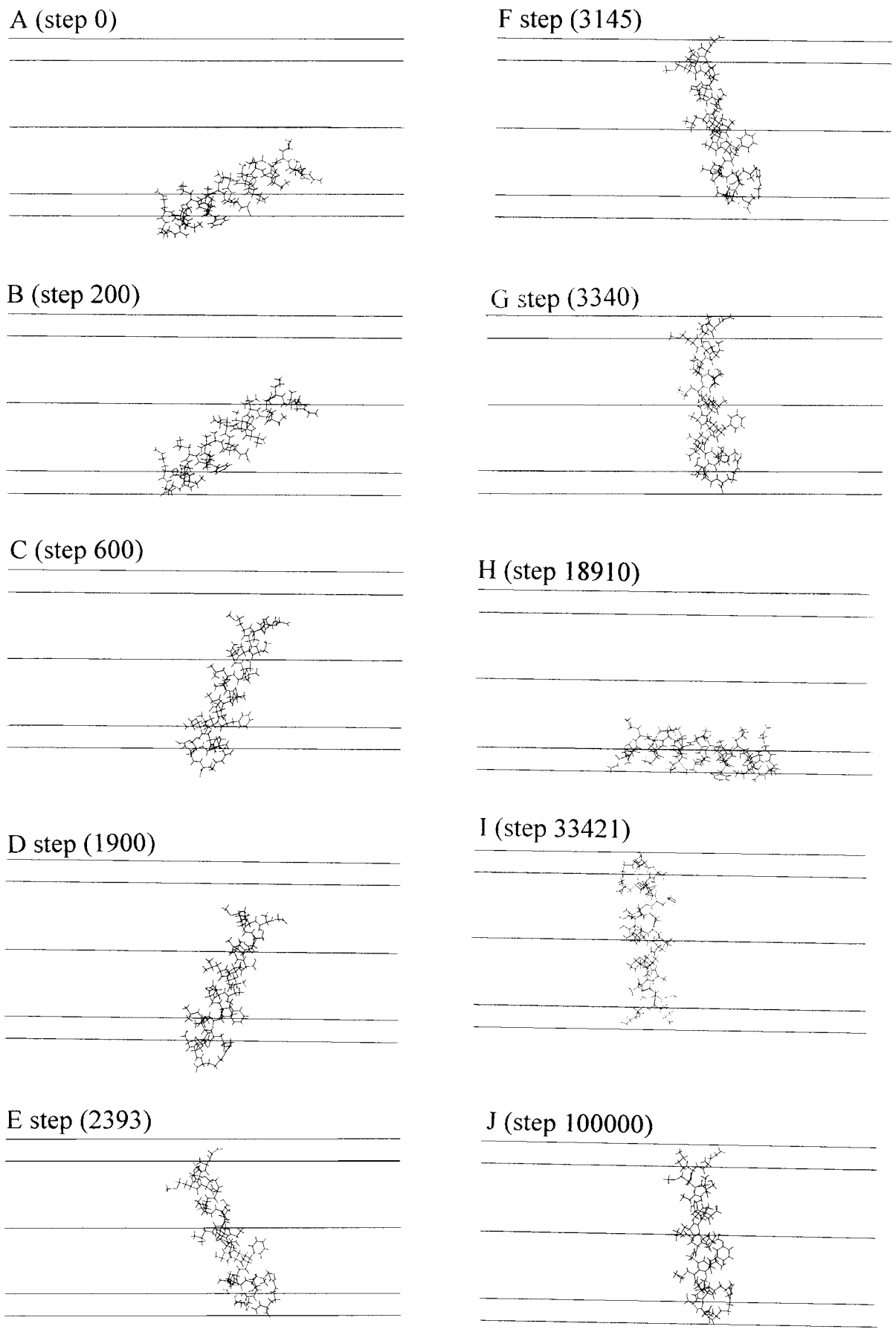

A (step 0)
F step (3145)
B (step 200)
G step (3340)
C (step 600)
H (step 18910)
D step (1900)
I (step 33421)
E step (2393)
J (step 100000)

Figure 9.

aromatic cycles carbons, simple-bonded carbon (Csp3), oxygen (O), sulfur (S), nitrogen (N), non-charged hydrogen, that is, bonded to C (H=0) and charged hydrogen (H/0). Thus, we can, for example, calculate the transfer energy of Ala and Tyr (with the conventions of Fig. 2) as

$$H_{\text{theo(ALA)}} = E_{\text{tr(Csp3)}}(S_2 + S_5) + E_{\text{tr(Csp2)}}S_3 + E_{\text{tr(H=0)}}(S_6 + S_7 + S_8 + S_9) + E_{\text{tr(H/0)}}S_{10} + E_{\text{tr(O)}}S_4 + E_{\text{tr(N)}}S_1$$

$$H_{\text{theo(TYR)}} = E_{\text{tr(Csp3)}}(S_2 + S_5) + E_{\text{tr(Csp2)}} \cdot (S_3 + S_6 + S_7 + S_8 + S_9 + S_{10} + S_{11}) + E_{\text{tr(H=0)}}(S_{13} + S_{14} + S_{15} + S_{16} + S_{17} + S_{18} + S_{19}) + E_{\text{tr(H/0)}}(S_{21} + S_{20}) + E_{\text{tr(O)}}(S_4 + S_{12}) + E_{\text{tr(N)}}S_1.$$

As the equation system written in such a way consists of more equations than variables (19 vs 7), it is likely that a unique solution does not exist and the system must be approximated. In their article, Fauchère and Pliska[15] removed the measured transfer energy of glycine from all the other residues to obtain the transfer energies of the lateral side chains. Thus the error between experimental data and calculated ones for an amino acid x is

$$\delta_{(x)} = |(H_{\text{theo}(x)} - H_{\text{theo(gly)}}) - H_{\text{exp}(x)}|.$$

The total error between the calculated and the experimental H was minimized by a Monte Carlo procedure applied to the atomic transfer energies error.

Restraints

To build restraints, one must define the general structural features of IMP. The observation of bacteriorhodopsin and photosynthetic reaction center[16] shows that IMP are similar to soluble proteins (surface area, interior hydrophobicity and packing, conservation of buried residues, stability). The major difference between IMP and soluble proteins is the nature of surface residues pointing to the lipids, which are much more hydrophobic than the surface residue exposed to water. This feature has been used in many methods aimed to determine the transmembrane segment of IMP. This striking segregation of hydrophobic and hydrophilic parts of the molecule imposed by the interface is due to the hydrophobic effect. To simulate this, for each configuration of the system, we calculate the interface restraint as

$$E_{\text{int}} = -\sum_{i=1}^{N} S_{(i)} E_{\text{tr}(i)} C_{(z_i)}. \quad (4)$$

The general behavior of Equation (4) is that E_{int} increases when accessible hydrophilic atoms (i.e., $E_{\text{tr}} > 0$) penetrate the membrane and decreases when accessible hydrophobic atoms do. The more atoms are accessible, the more the effect is important. E_{int} decreases when two hydrophobic or two hydrophilic atoms come close together in the hydrophilic and hydrophobic phases, respectively.

IMP and water-soluble proteins tend to form compact structures. For water-soluble proteins this is explained, since the protein to minimizes its hydrophobic surface in contact with water. For IMP, Rees and colleagues[16] suggested that a similar effect arises because, when a protein is inserting into the membrane, the interactions between some adjacent lipid are disrupted and replaced by weaker interac-

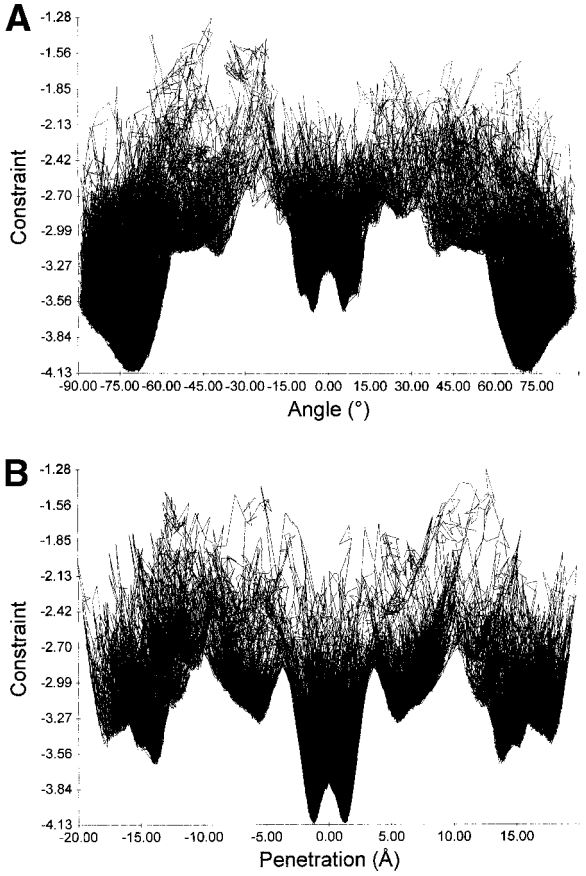


Fig. 10. Profiles of the simulation of M2δ. On each graphic, one point corresponds to a Monte Carlo step. **A:** Sum of restraints [Eqs. (4) and (5)] versus the angle of insertion (i.e., angle between helix axe and the plane of the bilayer). **B:** Restraints versus penetration (i.e., the z coordinate of the mass center of the molecule).

Fig. 9. Steps of the simulation of M2δ. The five planes correspond (from top to bottom) to $z = -18$ Å (transition start), $z = -13.5$ Å, the center of the membrane, $z = 13.5$ Å and $z = 18$ Å. Step 100,000 is the best configuration found by the Monte Carlo procedure.

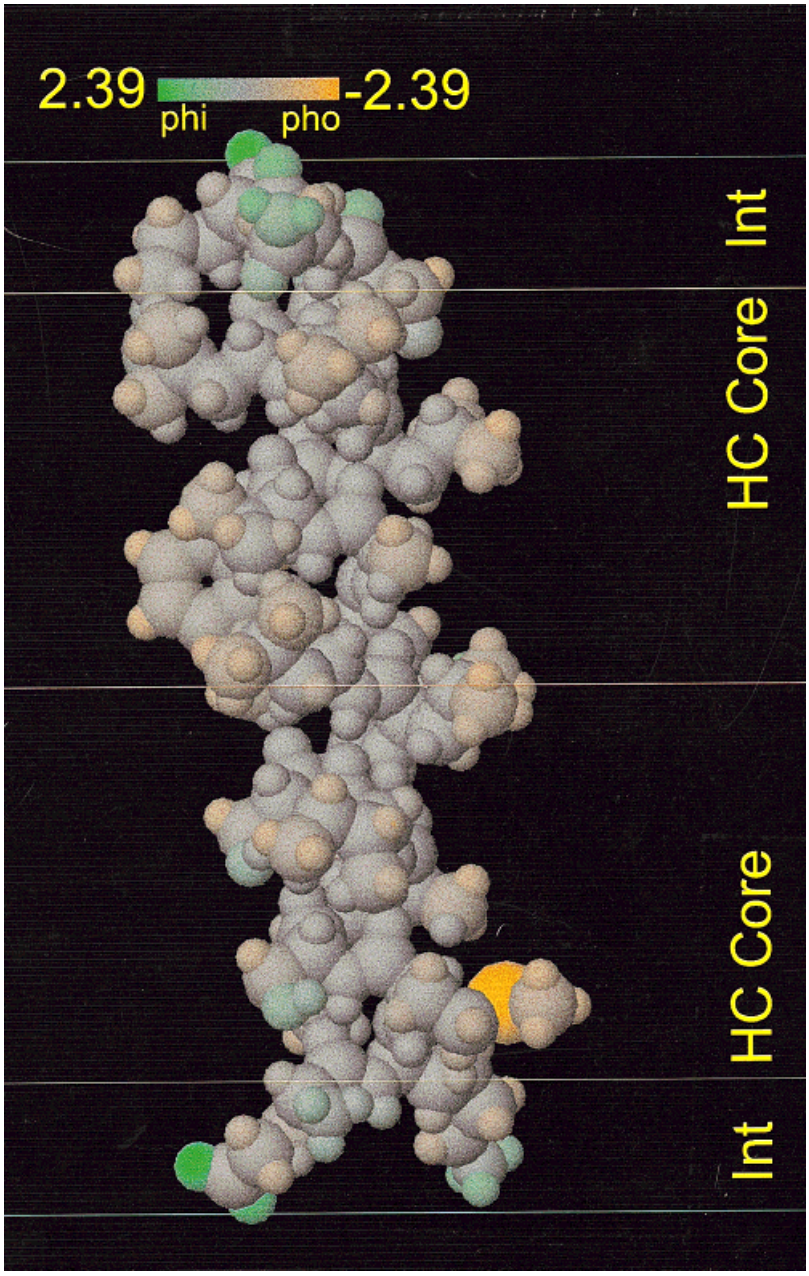


Fig. 11. The best configuration found for M2δ Atoms are colored according to the factor $S(i)*E_{tr}(i)$ [see Eq. (2)]. *Green,* hydrophilic; *orange,* hydrophobic; *red,* center of membrane; *yellow, z* = 13.5 Å; *green, z* = 18 Å.

tions between the protein and the lipids. E_{lip} accounts for the perturbation of the lipid bilayer due to peptide insertion. It is defined as

$$E_{lip} = a_{lip} \sum_{i=1}^{N} S_i C_{(z_i)} \qquad (5)$$

where a_{lip} is an empirical factor fixed to -0.018. The concept of this equation is very simple: E_{lip} increases with the surface of the protein in contact with lipids. The assumption is made that lipids act as a pool of free solvating CH2 groups, although these groups are covalently linked in acyl chains. For calculation performance, CH2 groups have the same radius as water molecule so that the atomic accessible surface is the same for water as for CH2.

Procedure

In order to test these two restraints, we applied them to small α peptides, the configurations of which have been experimentally studied. The structure of

the modelized peptides was fixed to α and no changes of internal structure were allowed. This drastically simplified the problem as follows:

1. Only 3 *df* were considered (two rotations and one translation) so that the Monte Carlo procedure used is far more efficient.
2. We used coulomb, van der Waals, hydrogen bonds, and torsion energies as constants so that the only three variable parameters of the simulation are $z0$, α, and a_{lip}.

This enabled us to rapidly determine a set of parameters leading to predictions in agreement with experimental data (see below).

The peptide structures were constructed by using WinMGM[17] from Ab Initio Technology (OBERNAI, France). The lateral side-chain interactions were not further optimized. The molecules are fully described (H included, no heavy atoms) as this has an obvious effect on the accessible surface used in the calculations. The starting positions of the peptides are determined by an algorithm[12] that predicts the position and the orientation of the hydrophobic/hydrophilic interface of amphipathic peptides. The molecule is translated so that this interface stays at z = 13.5 Å. A standard Monte Carlo procedure is then applied at 310 K for 10^5 steps (i.e., tries of moves) by randomly translating (max. 1 Å) and rotating (max. 5°) the molecule. Each Monte Carlo was run twice.

Material

Calculations were performed on parallel hardware of 21 Tracor Europa Pentium Pro PC cadenced at 180 Mh connected by a 100 Mbytes Network and controlled by an HP Vectra VA Pentium Pro cadenced at 200 MHz. The calculation software has been developed in our laboratory.

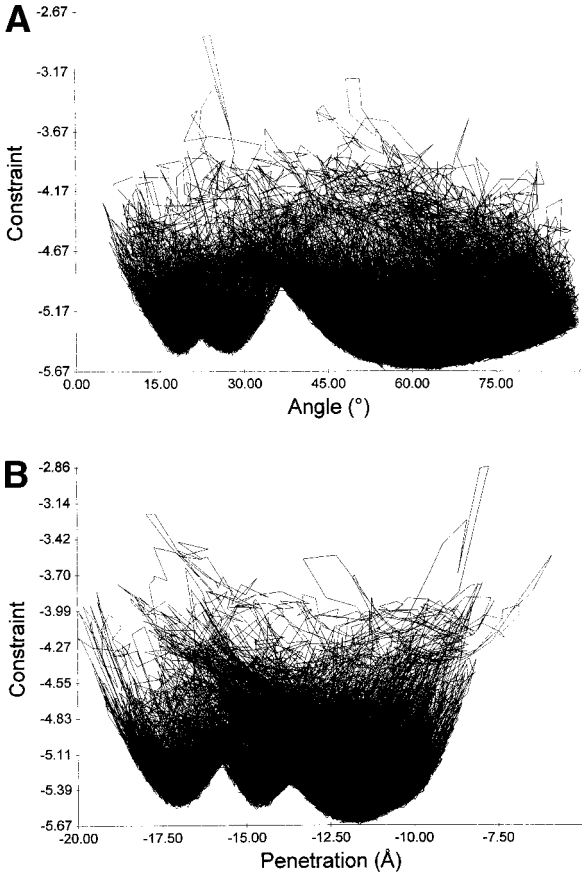


Fig. 12. Profiles of the simulation of melittin. On each graphic, one point corresponds to a Monte Carlo step. **A:** Sum of restraints [eqs. (4) and (5)] versus the angle of insertion (i.e., angle between helix axe and the plane of the bilayer). **B:** Restraints versus penetration (i.e., the z coordinate of the mass center of the molecule).

RESULTS AND DISCUSSION

Atomic Surface Transfer Energies

The choice of an experimental hydrophobicity scale to simulate the membrane is not an easy task. Water/octanol- and water/alkanes partition-derived scales could a priori be used as well. As pointed out by Roseman,[18] it seems that the octanol scales better fit experimental data when the membrane interface is involved in the process studied (e.g., the partition of polar solutes), whereas the alkane scales better reproduced phenomena where the hydrocarbon core is involved (e.g., transport of polar solute through the bilayer). This is probably due to heterogeneity of the chemical properties of the phospholipids along their main axis. Thus the membrane hydrophobicity could be described by a combination of two (or more) hydrophobicity scales. However, we feel that such an approach is not feasible now because of the discrepancies in the available hydrophobicity scales, and, for the present work, we used only one scale. Fauchère's scale was determined by using slightly modified single amino acids, and it provided us a easy way to determine atomic surface transfer energies because we could make the assumption that there are few molecular structure changes when the molecule environment is modified. This is obviously not the case for the Wimley scale[19] where the residue is included in small model peptide. Moreover, Fauchère's scale, determined at pH = 7.1, is more representative of the biological conditions than other hydrophobicity scales.

Figure 3 and Table 1 give the results of these calculations. The computed transfer energy of the amino acids fits (regarding the simplicity of the method) the experimental data very well except for Met, Lys, and Trp where the error is >3 kJ/mol. There is no obvious common property to these three residues that may explain such an error (e.g., cycles, or other particular atom groups). Thus the empirical classification of all the atoms into seven types does

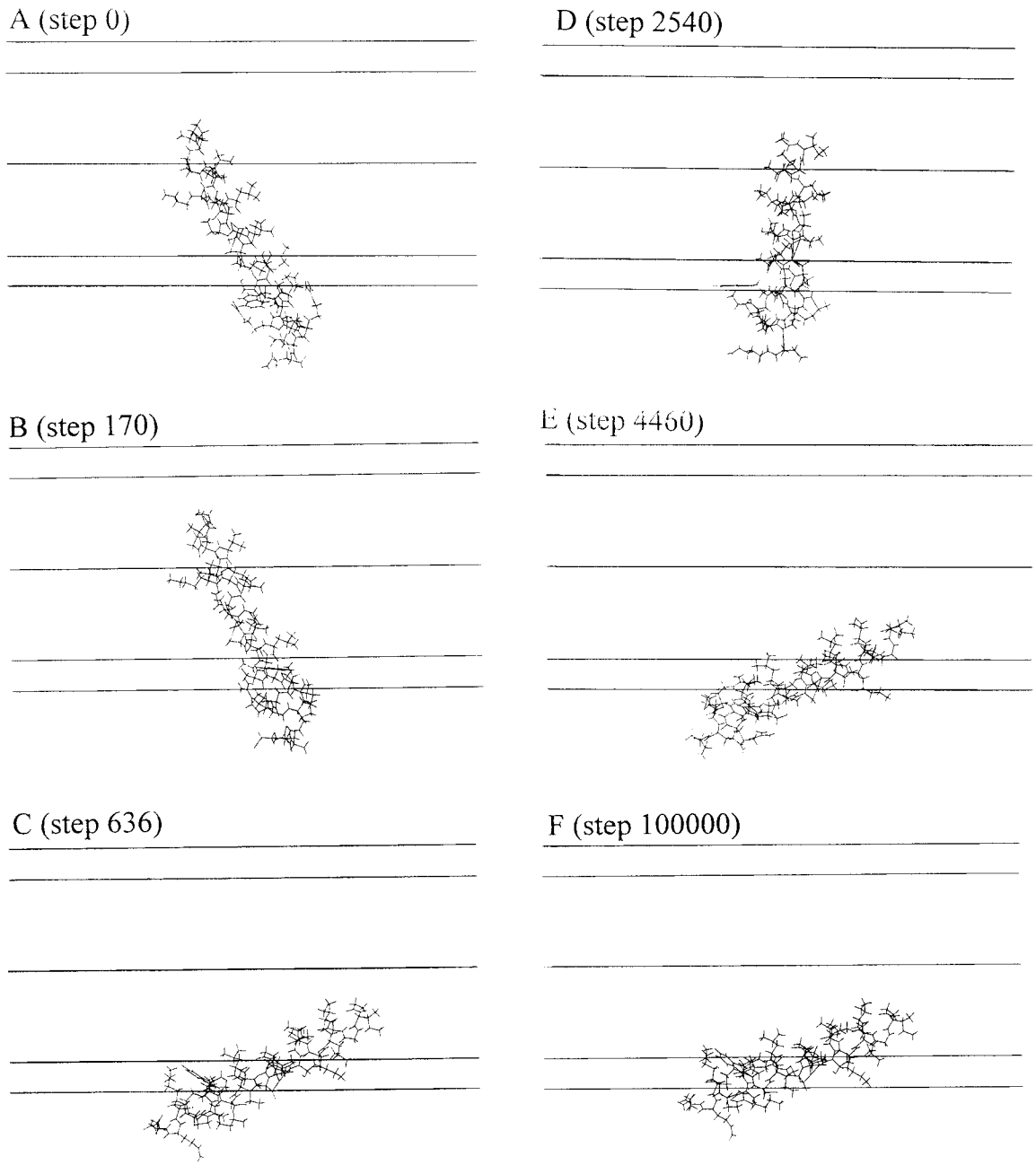


Fig. 13. Steps of the simulation of melittin. The five planes correspond (from top to bottom) to $z = -18$ Å (transition start), $z = -13.5$ Å, the center of the membrane, $z = 13.5$ Å and $z = 18$ Å. Step 100,000 is the best configuration found by the Monte Carlo procedure.

not seem to be the main cause of this error. It is possible that it arises from structure changes during the transfer process and/or specific interactions between the solvent and the molecule, and we intend to take this into account in further refinement studies. Alternatively, the error may be due to specific molecular organization of the solvent (water/octanol in the scale of Fauchère) that we are unable to simulate with this approach.

18A and Lap20 Peptides

18A is a strongly amphipathic synthetic peptide (EWLKAFYEKVLEKLKELF) and is experimentally found adsorbed at the water/lipid interface.[20] In the simulation, the peptide is tightly bound to the membrane surface. As shown by the snapshots of simulation in Figure 4, the peptide never penetrates nor leaves the membrane. Started with an angle of about

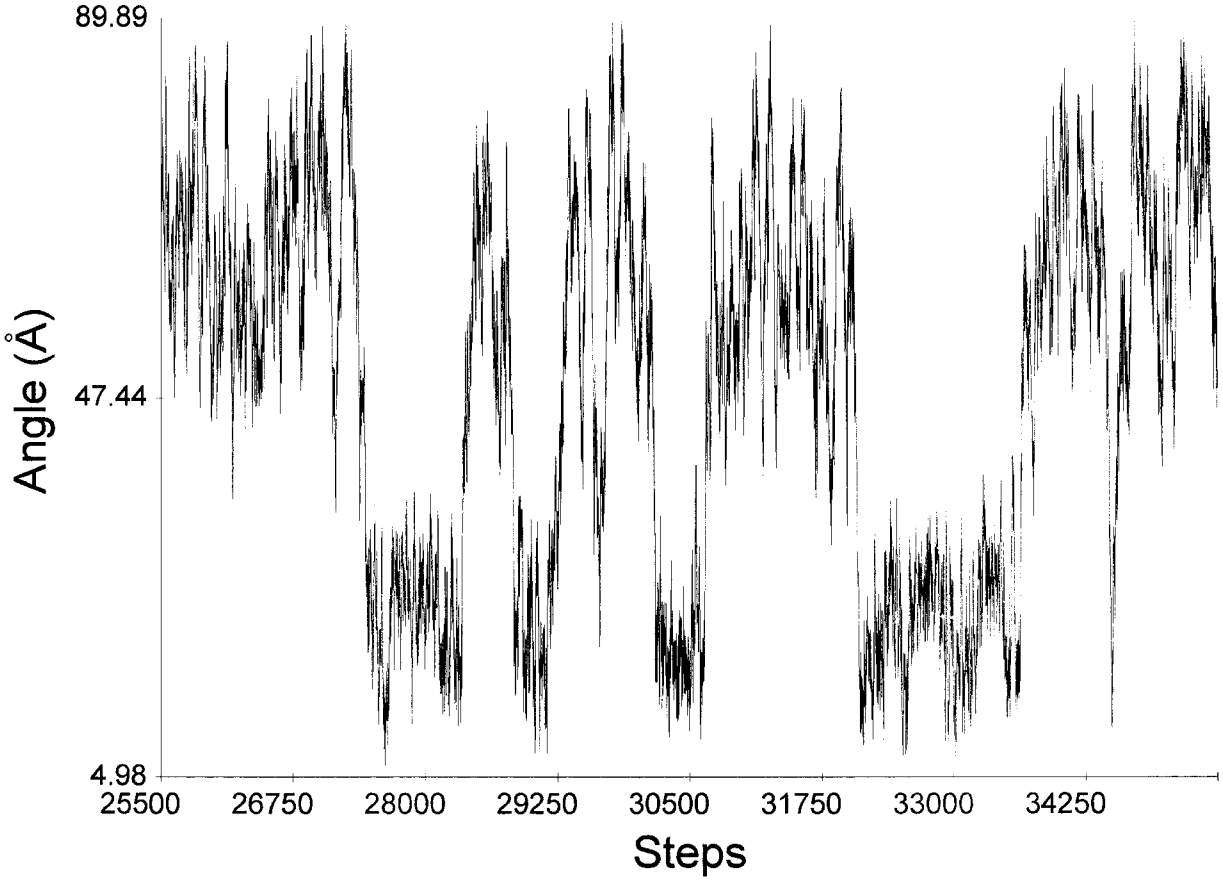


Fig. 14. Angle of insertion (i.e., angle between helix axe and the plane of the bilayer) versus the steps of the Monte Carlo for the melittin simulation.

40° (A) it rapidly (50 steps) stabilizes at the interface (B, C, D, E). The configuration remains unchanged after the 100,000 steps performed (F). Figure 5 gives the restraints as a function of the angle of insertion (A) and the degree of association (B) and shows a very sharp restraint minimum corresponding to the adsorbed configuration. In Figure 6, the atom colors represent the atomic hydrophobicity, $H(i) = S(i)*E_{tr}(i)$. One can see that, in the stabilized configuration, the larger part of accessible hydrophobic atoms is buried in the membrane, whereas hydrophilic surfaces lay in front of water. Thus, the restraints correctly mimic the segregationing properties of lipid bilayers. Moreover, many atoms (in gray) are not taken into account at all in the calculations because they are not accessible to water. Thus, introducing an accessible surface factor in our formulation has an obvious effect on the results and, as shown by Figure 7 where atoms have been colored according the $E_{tr}(i)$ only, greatly highlights the amphipathic character of the peptide.

Lap20 (VSSLLSSLKEYWSSLKESFS) is another amphipathic synthetic peptide. The results of the simulation, which are obviously similar those of 18A are not shown.

Magainin2

The magainin2 peptide (GIGKFLHSAKKFGKAFVGEIMNS) is found to be structured as an α helix and to lie in the bilayer plane as shown by solid-state NMR spectroscopy.[21] It does not lyse red blood cells but acts as an antibiotic, and this antibacterial activity could be due to the formation of pores into the membrane.[21] Nevertheless, it induces strong perturbations of the membrane (permeability, thickness), and at high concentration, oriented CD spectrometry indicates transmembrane conformations.[22] To this subtle behavior corresponds an amino acid composition less amphipathic than for the 18A peptide. Thus, magainin is a good target to test the restraint field. The results of the simulation (Figs. 7 and 8) are in part similar to those of 18A: in the best configuration found the peptide makes an angle of about 15° with the bilayer plane (Fig. 7a, d). But, conversely to the 18A peptide, magainin can transiently penetrate the membrane (Fig. 7b,c,e) during the dynamics, although this increases the restraints. This may reflect the channel-forming properties of the peptide, and it would be interesting to analyze the behavior of a multimeric assembly. Indeed,

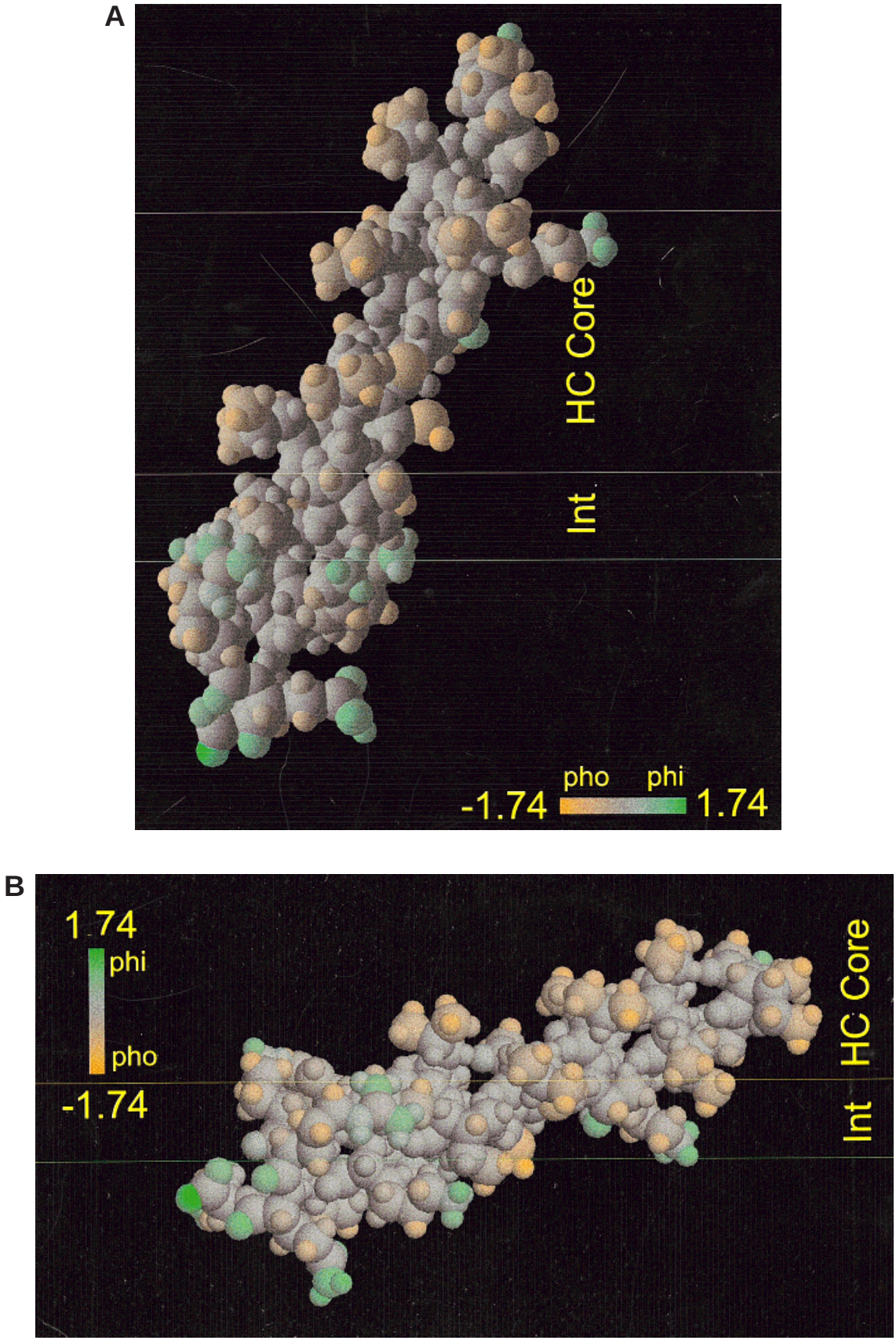


Figure 15.

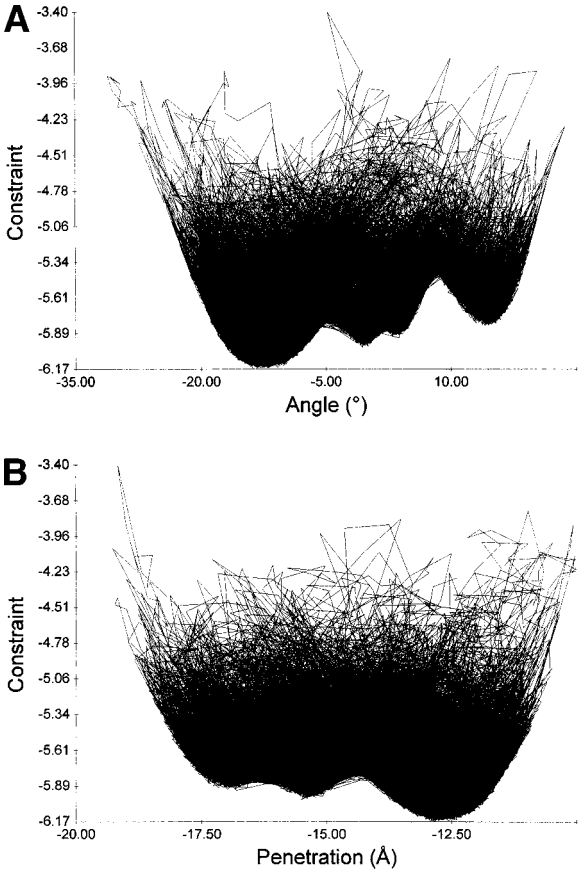


Fig. 16. Profiles of the simulation of LK peptide. On each graphic, one point corresponds to a Monte Carlo step. **A:** Sum of restraints [Eqs. (4) and (5)] versus the angle of insertion (i.e., angle between helix axe and the plane of the bilayer). **B:** Restraints versus penetration (i.e., the *z* coordinate of the mass center of the molecule).

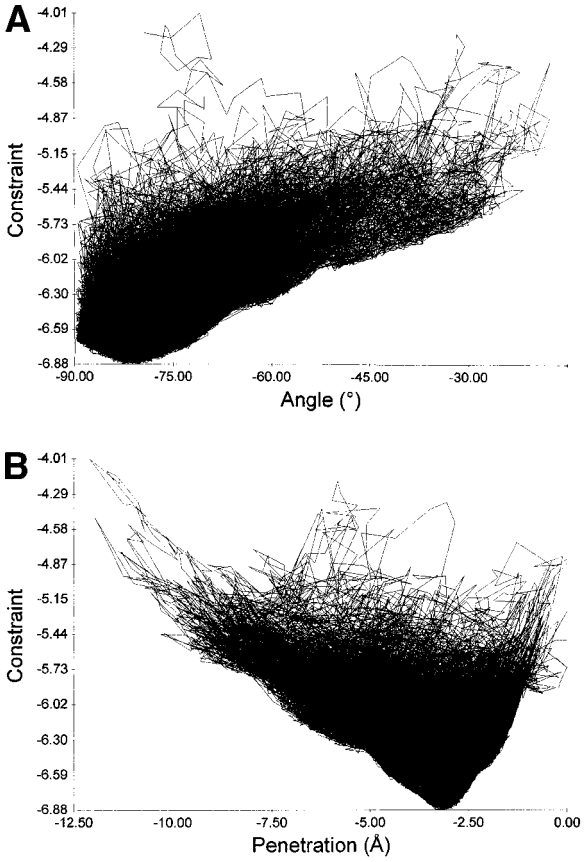


Fig. 17. Profiles of the simulation of LA peptide. On each graphic, one point corresponds to a Monte Carlo step. **A:** Sum of restraints [Eqs. (4) and (5)] versus the angle of insertion (i.e., angle between helix axe and the plane of the bilayer). **B:** Restraints versus penetration (i.e., the *z* coordinate of the mass center of the molecule).

channel-forming peptides must spontaneously penetrate in part, at least, before they can interact. In the simulation magainin is unable to span the membrane alone, and this is in line with the cooperatively observed.[22] Alternatively, this can be a consequence of the error between the computed and experimental transfer energies of lysine of which magainin is rich.

M2δ

M2δ (EKMSTAISVLLAQAVFLLLTSQR) is a segment of the torpedo acetylcholine receptor. It forms an α-helical transmembrane[21] segment and lyses red blood cells.[23] Snapshots of the simulation are given in Figure 9 and profiles in Figure 10. The starting point (Fig. 9a) of the simulation is far from the experimental configuration. This is because the algorithm to compute the initial configuration is designed for simple amphipathic peptide (Pho/Phi), whereas M2δ is bisamphipathic (Phi/Pho/Phi). Anyway, the peptide rapidly sets upright between 40° and 60° and enters the membrane (Fig. 9b,c,d). This type of configuration lasts for about 2,400 steps because the hydrophilic end still lying in the water prevents the peptide from penetrating further in the membrane. In a third phase of the simulation, the peptide crosses a restraint barrier (Fig. 9e,f) and anchors in a transmembrane configuration (around 70°) (Fig. 9g) in which the two hydrophilic ends of the peptide are in contact with water, whereas the hydrophobic core lays in the acyl chains (Fig. 11). This simulation highlights the important stabilizing effect provided by the hydrophilic ends of the transmembrane segments, suggesting that these should always be considered as bisamphipathic and not simply hydrophobic segments, as is the case in most predictive methods. After a longer time (about 16,000 steps), the peptide comes back to a parallel configuration that corresponds to a local restraint minimum

Fig. 15. Near-vertical (**A**) and near-horizontal (**B**) configurations of melittin. Atoms are colored according to the factor $S(i)*E_{tr}(i)$ [see Eq. (2)]. *Green,* hydrophilic; *orange,* hydrophobic; *red,* center of membrane; *yellow, z* = 13.5 Å; *green, z* = 18 Å.

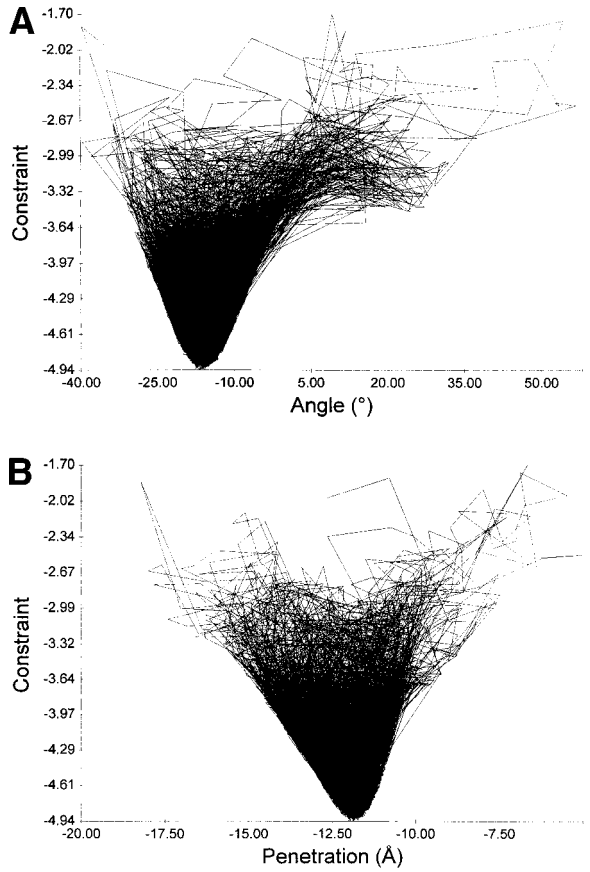


Fig. 18. Profiles of the simulation of PGLa. On each graphic, one point corresponds to a Monte Carlo step. **A:** Sum of restraints [Eqs. (4) and (5)] versus the angle of insertion (i.e., angle between helix axe and the plane of the bilayer). **B:** Restraints versus penetration (i.e., the *z* coordinate of the mass center of the molecule).

(Fig. 9h). From this point, M2δ penetrates the bilayer again (Fig. 9i), but this time the transmembrane configuration is inverted (the N-terminal end is replaced by the C-terminal end). In Figure 10, this phase corresponds to the negative angles. This shows that the hydrophobic properties of the peptide are quite symmetrical.

Melittin

Melittin (GIGAVLKVLTTGLPALISWIKRKRQQ) is a highly hemolytic[24] α helix peptide extracted from the bee venom. In some cases, it has been found to be perpendicular to the bilayer[25] and, in other experimental conditions, parallel.[24] The simulation (Figs. 12 and 13) shows that melittin can access a wide range of angles (from 15° to 90°) having similar restraints. Moreover, during the simulation, the peptide rapidly switches between an almost perpendicular and a parallel configurations (Figs. 14 and 15). This is in line with the experimental *unstability* observed. As the two restraints minima corresponding to these configurations are very similar, one can easily understand that different experimental conditions favor one configuration or the other. Clearly, the ability to analyze the entire conformation space instead of predicting a unique configuration type (e.g., surface or transmembrane in Eisenberg method[26]) gives a more precise description of the peptide capacity.

LK and LA Peptide

The LK peptide (KLLKKLLKLLKKLLKKLKKL-LKKL) is a synthetic that forms an α helix. It is adsorbed at the membrane as shown by solid-state NMR.[27] Here again, the simulation results clearly match the experimental data. In Figure 16 one can see the LK can adopt various conformations around 0° with its best conformation at about −13°. Conversely, the LA peptide (KKKKKALALALALAWALA-LALALAL) was designed as a transmembrane helix. It is composed of five hydrophilic lys followed by hydrophobic amino acids. Solid-state NMR confirms the transmembrane configuration.[27] In the IMPALA simulation, a single restraint minimum is reached for about 80° (Fig. 17).

PGLa

PGLa (GMASKAGAIAGKIAKVALKAL) is another member of the magainin family. As magainin2 it is found parallel to the membrane plane, although it is thought to form pores.[27] Figure 18 shows that a minimum restraint is reached for an angle of about −15°. This suggests that magainin and PGLa have different mode of interaction with the lipid bilayer.

CONCLUSIONS

In this article, we developed a simple restraint field to account for the membrane effect during IMP modeling. This field is efficient to study the mode of insertion of simple α helices and provides more information than a static prediction. Because this approach uses all the atoms and makes no assumption on the molecular structure, it should work with other structure types or even with molecules other than proteins.

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