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Biochimica et Biophysica Acta 1509 (2000) 148–154

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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The optimisation of the helix/helix interaction of a transmembrane dimer is improved by the IMPALA restraint field

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Received 30 November 1999; received in revised form 29 May 2000; accepted 12 July 2000

Abstract

A continuous membrane model (IMPALA) was previously developed to predict how hydrophobic spans of proteins insert in membranes (Mol. Mod. 2 (1996) 27). Using that membrane model, we looked for the interactions between several hydrophobic spans. We used the glycophorin A dimer as an archetype of polytopic protein to validate the approach. We find that the native complex do not dislocate when it is submitted to a 10^5 steps optimisation whereas separated spans converge back to a native-like complex in the same conditions. We also observe that IMPALA restraints are not strictly mandatory but do increase the efficiency of the procedure.

Keywords: Membrane; Protein; Structure; Prediction; Hydrophobicity; Computer; Glycophorin

1. Introduction

Bacteriorhodopsin was the first membrane protein to be completely denatured and refolded in vitro. Refolding has been analysed for four other membrane proteins: bacteria outer membrane β-barrel proteins [1–3] and photosynthetic light-harvesting proteins [4–6]. Numerous recent reviews cover various aspects of the problems of membrane protein structure [7–12]. One of the most difficult steps in modelling integral membrane proteins (IMP) is to assemble membrane spans. In many assays, the investigator has a major action because he forces the structure by choosing the partner spans and the specific membrane properties are not mimicked. The lipids are ignored and the structures are simulated in water or vacuum. In some cases, this can appear reasonable because more realistic approaches are really difficult. Indeed the simulation of pure membranes is a challenge. However, minimising energy in vacuum or in water is clearly misleading as it neglects the strong membrane partitioning effect. Since 1988, we have been calculating three-dimensional models of membrane proteins by predicting spans from sequences and minimising the energy of span interactions using an empirical equation including a term for the solvent hydrophobicity [13]. Several models of cytochrome were calculated. The procedure gave interesting results but is not fully satisfactory because the investigator has a key role in the choice of which

Abbreviations: IMP, Integral membrane protein

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0005-2736/00/$ – see front matter
PII: S0005-2736(00)00290-X

spans interact and some membrane properties, its thickness for instance, are not considered.

A few years ago, we developed the IMPALA approach that uses an implicit model of membrane by adding symmetric restraints to the force field. The IMPALA restraint field [14] has two empirical functions, one for hydrophobicity and another for lipid perturbation. By using a Monte Carlo (MC) procedure on simple α-helical peptides with constant secondary structures, we have shown [14] that the restraint field successfully detects the mode of membrane insertion (i.e., in-plane, transmembrane or fluctuating).

In this paper, we use IMPALA and test the helix/helix interactions in membrane, using the glycophorin A dimer as an archetype. Twenty structures of the transmembrane pair of glycophorin A helices have been obtained by NMR and one of those structures was used [15]. The dimer was dissociated and its capacity to re-associate into a native fold when minimised with IMPALA was investigated.

2. Materials and methods

All calculations were achieved with programs developed in the laboratory and running on a 21 Pentium pro cluster (submitted). Energy of interactions between two glycophorins were calculated as the sum of van der Waals (Lenard Jones's function) and electrostatic (Coulomb's function) terms. The dielectric constant was set to 3 to account for the hydrophobic environment. The atomic charges were Amber's.

In order to consider the membrane environment, the energy of glycophorin interactions was completed by a restraint term, extensively described in a previous paper [1]. This restraint is briefly summarised hereafter.

The lipid/water interfaces are described as a continuum by the function, $C(z)$:

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

where z is the axis perpendicular to the xy plane separating the membrane into two layers, $|z|$ (in Å) the distance of a point of z to the xy plane and where α and z_0 are constants determined so that C is approximately equal to 1 for 18 Å $< |z| < \infty$ (water phase) and equal to 0 for 0 Å $< |z| <$ 13.5 (hydrocarbon core).

The relative orientation of the molecule with respect to the interface is due to the hydrophobic effect. To simulate it we calculate the interface restraint for each configuration as follows:

$$E_{\text{int}} = -\sum_{i=1}^{N} S_{(i)} E_{\text{tr}(i)} C_{(z_i)} \quad (2)$$

where N is the total number of atoms, S the accessible surface of atom i to solvent, E_{tr} its transfer energy by unit of the accessible surface area (defined for seven atomic types [16,17]).

E_{lip} accounts for the perturbation of lipids due to the peptide insertion. It is defined as:

$$E_{\text{lip}} = a_{\text{lip}} \sum_{i=1}^{N} S_i C_{(z_i)} \quad (3)$$

where a_{lip} is an empirical factor fixed to 0.018.

As the optimisation is achieved by an MC procedure, a unique structure must be provided to start with. One of the 20 NMR structures of the glycophorin was selected. Selection was at random because all conformers have equal probabilities and the structural dispersion concerns the extra membrane fragments not the transmembrane spans [2]. The position of the dimer in the membrane is not provided in the NMR data. Therefore, the glycophorin A was randomly positioned in the model membrane and subjected to a 10^5 steps MC procedure. During this optimisation, the structure of each monomer, as well as their self-positions, were fixed. The result was hereafter used as starting position and is referenced as GpAr.

In our first validation procedure, the GpAr was subjected to 5 independent MC (5000 steps, maximum 0.5 Å translation, 0.2° rotation per step). Each monomer moved separately while its secondary structure remained constant. The aim of this test was to check whether the constrained MC splits the dimer into isolated helices because, if it does, we may expect that assembling helices together will never converge to a compact structure.

In the second test, three models (GpA1 to GpA3, Fig. 1) were built from GpAr by disrupting the dimer interactions and separating the monomers away. The

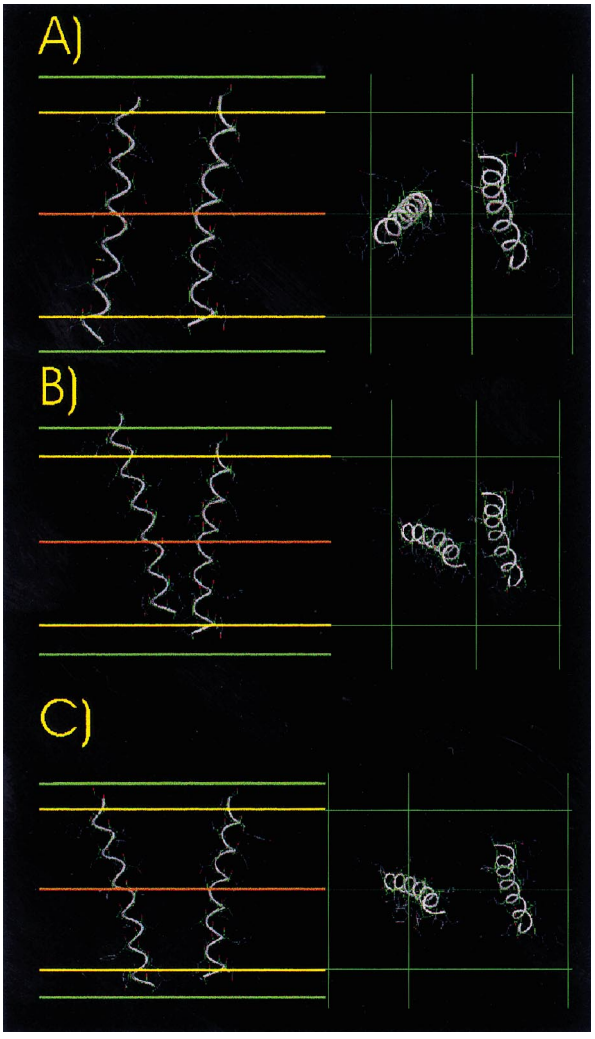

Fig. 1. The three different conformations of the glycophorin A helices used to start the second test of IMPALA. (Left) The view is perpendicular to the membrane section. (Right) The view is from outside the membrane. Green axis is $|z| = 18$ Å, yellow axis is $|z| = 13.5$ Å, orange axis is $z = 0$ Å. Each helix is represented by a grey ribbon.

three models were different structures used as initial structures to start the minimisation in order to simulate various situations (Table 1). Each starting model was optimised by 10^5 steps of MC with and without the restraints described above. Each run was repeated 12 times.

The characteristics of the force field and of the restraint algorithms are important for the efficacy of calculations. Indeed, they effect the level of the acceptance of structures at each step of the MC. If the acceptance is low, movements of the structure will be short and exploration of the structural conformations will be restricted. Conversely, if the acceptance is high, movements of the molecule will be large but the probability to converge toward an energy minima will be decreased. Training shows that, an acceptance between 0.3 and 0.5 is optimal. Previous tests demonstrated that the best way to reach this acceptance is to introduce a balancing factor between the terms representing the protein interactions with the membrane and the term calculating the energy of interactions between the glycophorin monomers. Without restraint, only the Amber force field is used, with restraints, the sum of the Amber force field plus ten times the restraints (Eint+Elip) are used. Introduction of the balancing factor of 10 leads to an acceptance of about 0.4, which is satisfactory. The balance is required because variations of van der Waals energy can be very important when the monomers come into contact. However, when we compared the simulations with and without restraints, we had to multiply the temperature of restrained simulations by 10 (3100 K), otherwise the number of structures rejected during the MC calculations were too different in both simulations.

3. Results and discussion

The backbone RMS of calculated structures was monitored with respect to the GpAr structure during the MC optimisations. Structures are 'native-like' when their RMS is lower than 0.4 Å, that is, the average RMS between the 20 NMR models [2].

Table 1
Parameters of the glycophorin A simulations

Structure	Initial movement	Initial RMS (Å)	Number of simulations	Number of steps by simulation
A	Rotation	3.11	12	5000
B	Translation (perpendicular to the interface)	2.38	12	5000
C	Translation (parallel to the interface)	4.13	12	15000

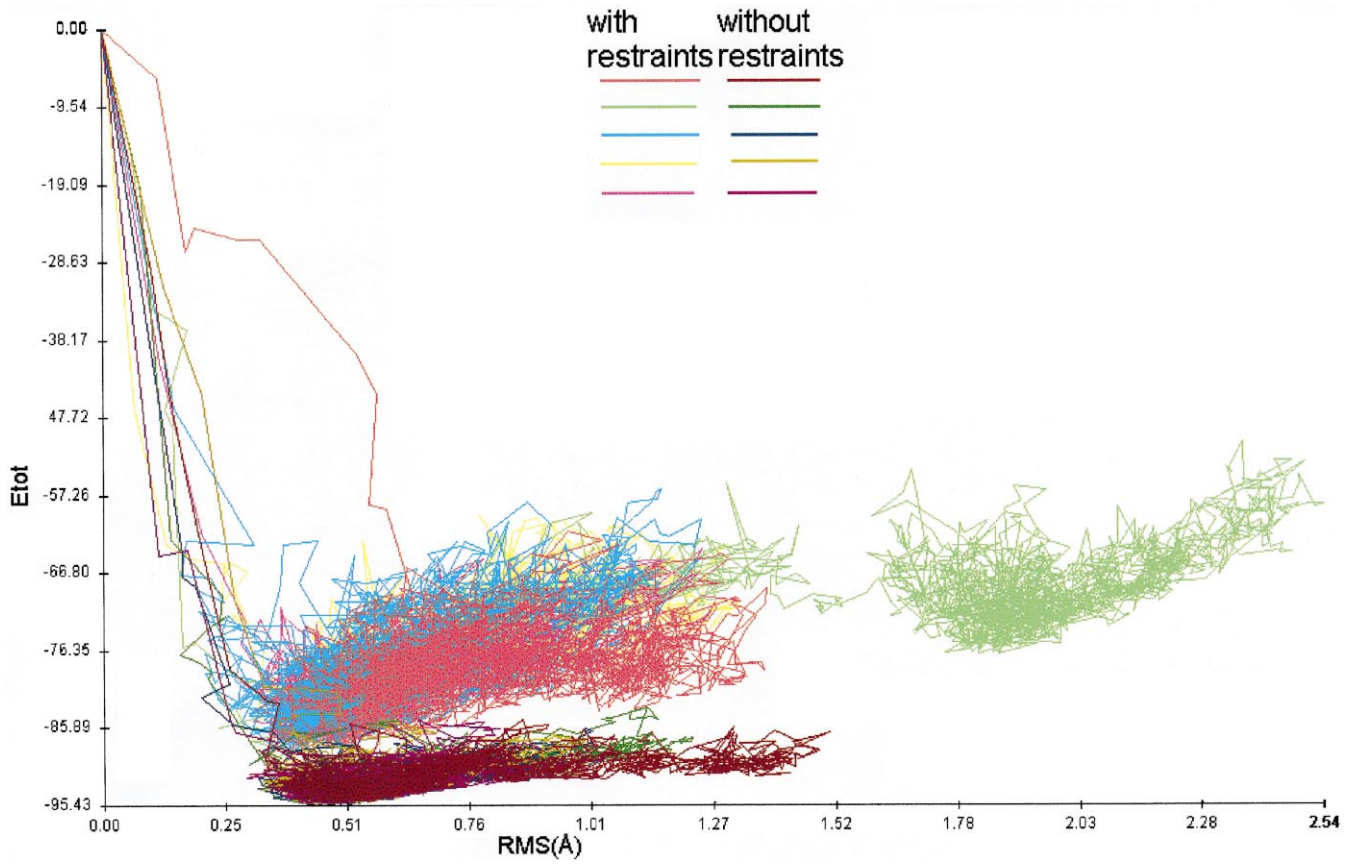

Fig. 2. Variations of restrained and non-restrained energies versus RMSd.

The first set of simulations started from a native conformer and looked for the dissociation of helices (Fig. 2). The following observations are made.

(a) The structure of the GpAtm dimer is almost unchanged after the simulations.

(b) The largest helix movements occur at the very beginning of the simulation (±15 steps). Those movements are due to one or several steric clashes and thus correspond to a decrease in the energy of van der Waals. Since the final RMS is about 0.4 Å, we state that the final structures are not significantly different from the initial one. The small discrepancies between the van der Waals term used to refine the NMR structures and that used here should be responsible for the initial movements.

(c) When the helices of the dimer are separating, the total energy term increases supporting that the starting structure corresponds to a minimum. There is no significant correlation between E_{int} and the RMS, whereas E_{lip} markedly increases with the RMS because separating the helices increases the surface of contact with lipids. The absence of correlation between RMS and E_{int} does not mean that E_{int} is not important in the restraint field. Indeed, we previously showed [14] that, in the absence of E_{int}, a wrong topography of protein insertion in the membrane is found.

At this stage we can conclude that the restraints do not modify the fact that the native-like structure correspond to a minimum of energy.

Whether or not restraints are able to drive a disrupted structure to a native-like structure is investigated in the second sets of simulation (Fig. 3). The RMS variations during the runs show the following.

(a) Simulations with and without restraints can both reach native-like structures.

(b) Some simulations do not reach a native-like structure in the time course of the experiment. When the restraints are applied, successful simulations are more frequent than in their absence. Suc-

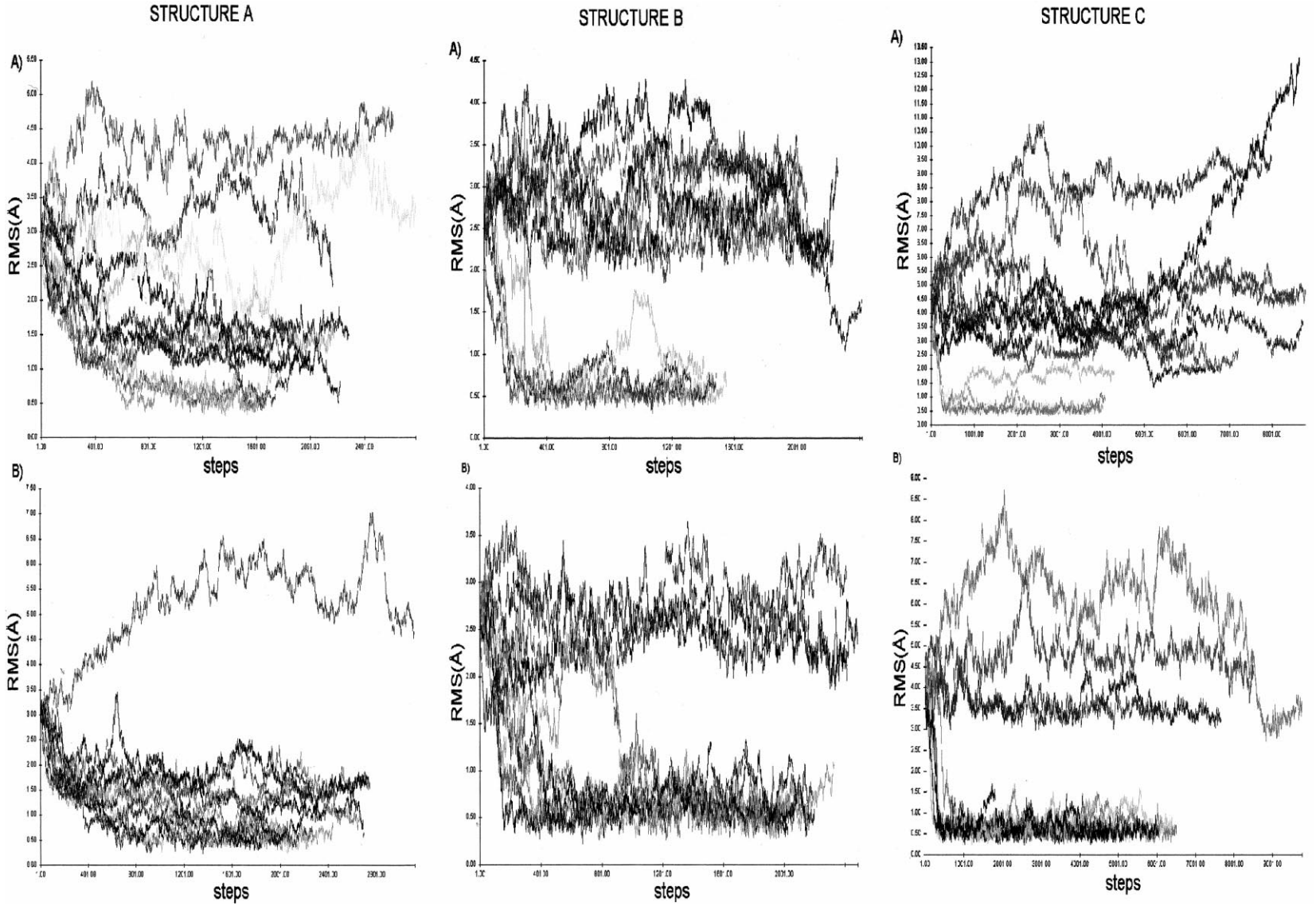

Fig. 3. Variations of the RMS during the simulation. For the RMS calculation, the reference is the NMR model. Above: simulation without restraint; below: simulation with restraints. In each case, ten runs were made.

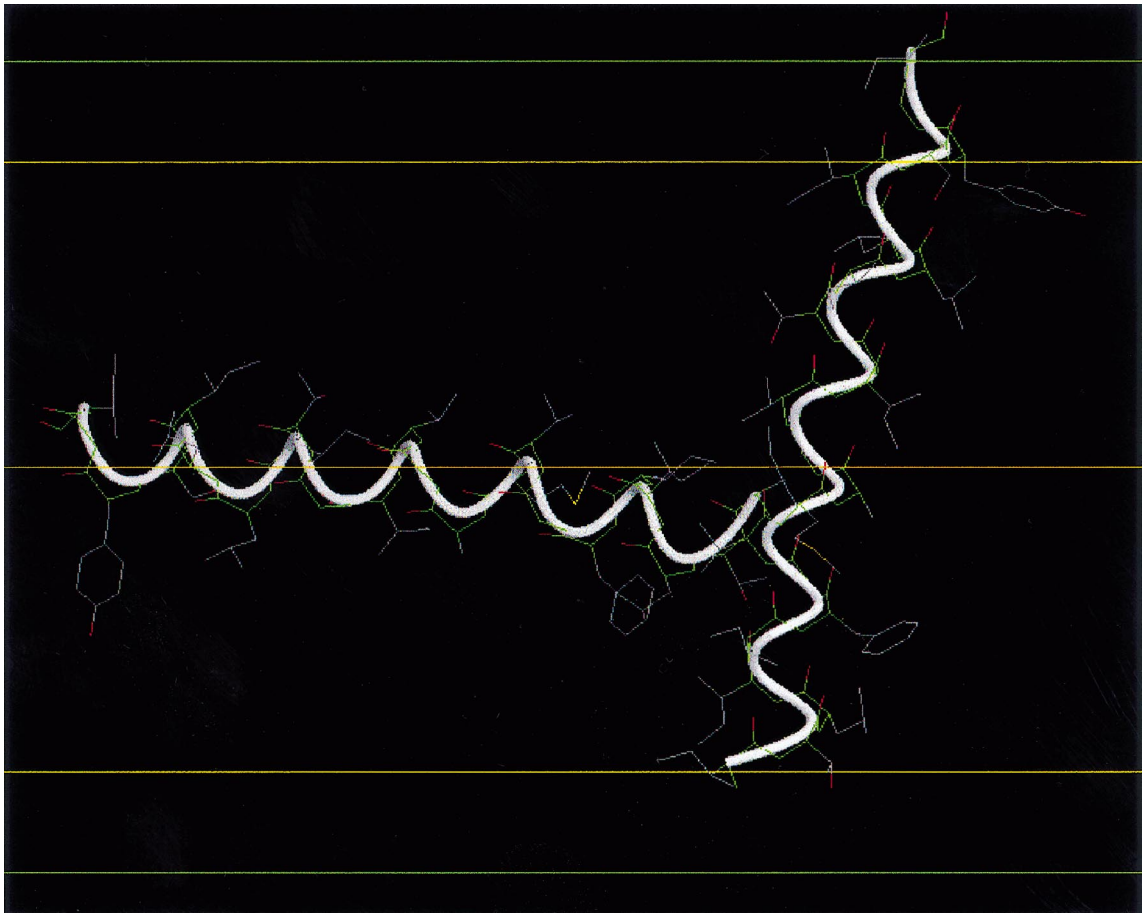

Fig. 4. One of the unrealistic configurations reached when no restraint is applied. Green: $|z| = 18$ Å; yellow: $|z| = 13.5$ Å; orange: $z = 0$ Å. Each helix is represented by a grey ribbon.

cess ratios are 7/5, 9/3 and 8/4 (with/without restraints) over the 12 runs using models A, B and C (Fig. 1) respectively.

Conclusions are that restraints are not mandatory to reach native-like structures; however, they shorten the search since fewer steps are needed to reach a native-like structure. Therefore in reasonable conditions, the probability for correct structures increases. Restraints decrease the probability for unrealistic conformations: Fig. 4 shows one of those unrealistic structure obtained in one of the non-restrained simulations.

Since, even without restraints, conformations of minimal energy correspond to the native like structure, we cannot support that the membrane has crucial effect on dimerisation, as generally admitted. However, since two glycophorin A monomers present extensive structural fitting leading to very favourable van der Waals interactions, there is not yet evidence that this conclusion will be applicable to all kinds of hydrophobic polymers, especially those involving electrostatic interactions.

4. Conclusions

We show that the IMPALA restraint field increases the MC algorithm ability to assemble a dimer structure. Enhancing the MC efficiency should be valuable to study polytopic membrane proteins because the number of different conformations drastically increases with the number of membrane spans.

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