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TRAJELIX: A computational tool for the geometric characterization of protein helices during molecular dynamics simulations

Mihaly Mezei[a,*] & Marta Filizola[b]
[a]*Department of Physiology and Biophysics, Mount Sinai School of Medicine, NYU, One Gustave L. Levy Place, Box 1218, New York, NY, 10029, USA;* [b]*Department of Physiology & Biophysics, Weill Medical College of Cornell University, 1200 York Ave, New York, NY 10021, USA*

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Summary

We have developed a computer program with the necessary mathematical formalism for the geometric characterization of distorted conformations of alpha-helices proteins, such as those that can potentially be sampled during typical molecular dynamics simulations. This formalism has been incorporated into TRAJELIX, a new module within the SIMULAID framework (http://inka.mssm.edu/~mezei/simulaid/) that is capable of monitoring distortions of alpha-helices in terms of their displacement, global and local tilting, rotation around their axes, compression/extension, winding/unwinding, and bending. Accurate evaluation of these global and local structural properties of the helix can help study possible intramolecular and intermolecular changes in the helix packing of alpha-helical membrane proteins, as shown here in an application to the interacting helical domains of rhodopsin dimers. Quantification of the dynamic structural behavior of alpha-helical membrane proteins is critical for our understanding of signal transduction, and may enable structure-based design of more specific and efficient drugs.

Introduction

Alpha (α)-helices are prominent structural elements of proteins. In particular, alpha-helical membrane proteins constitute about 20–25% of the proteome, and about half of the potential pharmaceutical targets. Since the most prominent structural characteristic of these proteins is their bundles of transmembrane (TM) α-helices, detailed knowledge of the dynamic structural behavior of each helix in the bundle upon activation is considered vital in structure-based drug design. The usefulness of studying changes in the geometric characteristics of transmembrane helical bundles is illustrated by the observation that helix distortions in membrane proteins may play important functional roles (e.g., see [1]). During typical all-atom molecular dynamics (MD) simulations the protein helices undergo not only changes in their overall appearance in relation to the rest of the bundle, but also continual distortions of their local structural features, as often found in globular proteins [2, 3]. These distortions make the determination of global helix properties a complex task.

For helices with an ideal geometry the atoms defining the helix unequivocally define the axis as well. In contrast, for distorted helices the definition of helical axis is not straightforward, as demonstrated by the number of algorithms available for helix axis determination. A comparative evaluation of these algorithms can be found in [4]. Characterization of changes in the geometric

*To whom correspondence should be addressed.
Phone: +212-241-2186; Fax: +212-860-3369
E-mail: mezei@inka.mssm.edu

characteristics of protein alpha helices occurring during MD simulations is further complicated by the local fluctuation of the helix atoms. In order to average over these local fluctuations, all helix atoms must be taken into account.

We report here the details of the mathematical formalism we developed to calculate and compare the geometric characteristics of helical conformations in a protein during molecular dynamics simulations using all helix atoms. The resulting set of formulae and their implementation were incorporated into TRAJELIX, a new module of the Fortran 77 program SIMULAID [5]. TRAJELIX computes global and local tilts, rotation around the helix axis, displacement of the center of mass (COM) and the helix endpoints, length, number of residues per turn, and extent of bend for each helical conformation sampled during a MD simulation.

The approach presented here is complementary to the helix analysis performed by other programs (e.g., HELANAL by Bansal et al. [6] and GROMACS by van der Spoel et al. [7]). HELANAL uses local helix axes for each four consecutive α-carbon atoms within the helix to calculate local helix properties. Unlike HELANAL, our procedure uses a single helical axis to calculate global and local properties of α-helices during MD simulations. Although HELANAL is expected to provide better representations of local axes in single helices, the axis direction and position would be different for different parts of the helix during a MD simulation. Our approach overcomes this problem by averaging out local distortions in the helix geometry, and allowing more robust results than the ones obtained with methods that rely on representing the helix position by preselected atoms. The g_helix and g_bundle modules of GROMACS [7] use an alternative procedure that avoids the explicit calculation of helix axes to obtain helix descriptors (e.g., radius, twist, rise/per residue). Specifically, this procedure consists of fitting the α-carbon positions of helices identified through consideration of specific values of backbone torsion angles and hydrogen bonds to those of an ideal helix.

An illustration of the type of results of TRAJELIX is provided here in an application to a 5 ns MD simulation trajectory of interacting transmembrane helical bundles within the recently proposed dimeric model of rhodopsin [8] using an implicit membrane model [9].

Methods

The details of the methods used to characterize changes in the global and local geometric characteristics of helices during molecular dynamics simulations are reported below. Specifically, the global structural properties treated are displacement, tilting, and rotation. Local structural properties of the helices are: compression/extension, winding/unwinding, and bending. These local properties characterize the shape of the helix, and are therefore independent of the helix position and/or orientation.

TRAJELIX automatically calculates all of these properties for each conformation of the target helix sampled during MD simulations. Details of its implementation and the procedure used in the program to correct distorted helices are provided below. The integrated suite of algorithms of TRAJELIX were tested and validated on three different 20-residue ideal helices, as well as a 30° bent helix (see the ''Validation'' subsection for details). In the followings the subscript r refers to a reference structure and the subscript s refers to the structure to be compared with the reference structure, e.g., a structure from a simulation trajectory.

When applying TRAJELIX to a simulation trajectory an option is provided to separate the effect of the global molecular motion from the local changes in individual helices. Specifically, each structure s can either be (i) translated by the distance between the center-of-mass of structures s and r; or (ii) overlaid (using the algorithm of Kabsch [10]) on the structure r; or (iii) overlaid on the corresponding molecule of the reference structure r, in case of multimolecular systems like the rhodopsin dimer used as a test-case in this paper.

The algorithms described below all rely on the user to specify the set of residues forming the helix under investigation. This option to manually insert data regarding the length and location of the helices allows an accurate calculation of local properties. On the other hand, the inflexibility resulting from having to specify the helix location does not allow application of TRAJELIX to the study of folding simulations of helical structures in a fully automatic mode. In this case, the location and stretch of the simulation where a helix is formed need to be detected separately. The SIMULAID framework [5] includes a module that

allows identification of helical segments in simulated trajectories using the DSSP algorithm [10].

Displacement

The position of a helix is characterized by the position vector of the COM of the helix-defining atoms. TRAJELIX allows monitoring of changes in the helix COM position as a function of time. Changes in the positions of the helix end points, which correspond to the projection of the first and last helix atom onto the helix axis, can also be recorded and used as an indication of helix displacement.

Tilting

Global and local tilts define the orientation of each helix in a protein. Specifically, the angle δ_{si} that the helix axis forms with the laboratory frame's coordinate axes i (i stands for one of the three Cartesian axes, and s is a given helix structure) is defined as global tilt. The angle δ_{rs} between the axes of each helix structure s and the reference helix configuration r is used to describe the local tilt, which shows the extent of change in helix direction. TRAJELIX calculates these angles for each snapshot of a MD trajectory, and shows the evolution of these values during time.

Rotation

During MD simulations helices may rotate around their own axes performing a sort of rigid body motion. TRAJELIX calculates angles of rotation for each helix structure sampled during a MD simulation with an algorithm designed to avoid singling out any particular atom in the calculation of the helix orientation. This algorithm includes the following steps:

1. Selection of the reference structure r and calculation of its helix axis $\boldsymbol{a}_r$.
2. For each structure $s \neq r$, calculation of the helix axis $\boldsymbol{a}_s$.
3. Rotation of each structure s around the normal to the plane of $\boldsymbol{a}_s$ and $\boldsymbol{a}_r$ (*i.e.*, around $(\boldsymbol{a}_s \times \boldsymbol{a}_r)$) in such a way that the new axis $\boldsymbol{a}_s$ is parallel to the reference structure's axis $\boldsymbol{a}_r$.
4. For each structure $s \neq r$, calculation of the vectors normal to the axis that goes through the α-carbon atoms $\boldsymbol{n}_{s,i}$ ($i = 1,...,N_{\mathrm{res}}$).
5. For each residue i, calculation of its rotation around the helix axis. Angles between $\boldsymbol{n}_{r,i}$ and $\boldsymbol{n}_{s,i}$, i.e. $\varphi_{rs,i}$ angles, are used to estimate such rotations. Unless the helix is kept rigid at its ideal geometry, these angles will be different for each residue. The overall angle of helix rotation φ_{rs} will then be taken as the average of the $\varphi_{rs,i}$'s over the N_{res} residues.

Compression/Extension

During extensive MD simulations helices can undergo compression or extension – just like a spiral spring. The length of the helix axis can serve to estimate these changes. TRAJELIX calculates helix lengths as the distance between the projections of the first and last α-carbons of the helix axis.

Winding/Unwinding

A transmembrane helix can also become unwound or overwound during a MD simulation. These changes can be monitored using as a guide the number of residues per turn or, equivalently, the turn angle θ per residue, and measuring their deviation from ideal values. TRAJELIX calculates θ using a two-step procedure. Specifically, the two steps are:

1. For a structure s, calculation of the angle $\psi_{i\text{-}1,i}$ between $\boldsymbol{n}_{s,i\text{-}1}$ and $\boldsymbol{n}_{s,i}$ ($i = 2,...,N_{\mathrm{res}}$) and calculation of ψ_{1i} as the sum of the $\psi_{j-1,j}$'s ($j \leq i$), which results in a monotonically increasing sequence.
2. Fitting a straight line of the form $\psi_i = \psi_o + \theta^* i$ to the ψ_{1i} values, which yields the turn angle per residue, θ.

Bending

For relatively long helices, such as membrane-spanning helices (20–30 amino acid residues) in α-helical membrane proteins, the length of the local helix axis may change significantly during MD simulations. This may be the result of bend(s) in the helix (either a single bend or multiple bends following an oscillating pattern), or just random fluctuations. A simple indicator of a single bend is the radius of the circle fitted

to the α-carbons [11]. The smaller the radius the larger the bend. However, if there is more than one bends in the helix, the radius of the fitted circle may be much larger than what would correspond to each bend separately. TRAJELIX uses a novel procedure to detect multiple bends in the helix. Specifically, this procedure consists of the following 5 steps:

1. Fitting a plane to the α-carbons $\{\boldsymbol{C}\alpha_i, i=1,...,N_{\rm res}\}$. This requires the solution of a 3×3 eigenvalue problem [12].
2. Pulling each α-carbon simultaneously toward the helix axis along the normal to the axis that goes through that α-carbon until the one closest to the helix axis reaches it. Thus each α-carbon is moved toward the helix axis a distance that is equivalent to the one between the axis and its closest α-carbon. The new set of coordinates $\{\boldsymbol{C}'\alpha_i,\ i=1,...,N_{\rm res}\}$ will then have the same helical orientation around the axis as the original set, but will be closer to it.
3. Finding the projection of the $\boldsymbol{C}'\alpha_i$'s onto the plane fitted to the $\boldsymbol{C}\alpha_i$'s, $\{\boldsymbol{C}''\alpha_i,\ i=1,...,N_{\rm res}\}$.
4. Fitting a line to the α-carbon projections $\boldsymbol{C}''\alpha_i$ and detecting the places where the lines that connect the projections cross the axis.
5. This final step is to decide if the crossings of the axes determined above represent random fluctuations. Random fluctuations indicate only 'noise' in the data. If, however, the crossings are correlated then the helix backbone is expected to be bent. The standard statistical test for the detection of correlation is the so-called run test [13]. This test requires to count the number of $\boldsymbol{C}''\alpha_i$'s on each side of the line obtained in step 4 and the number of times the line was crossed by the $\boldsymbol{C}''\alpha_I$–$\boldsymbol{C}''\alpha_{i+1}$ line. The run test provides critical numbers for minimal and maximal number of crossings as a function of the number of points on both sides of the axis. If the number of crossings is between the critical values then the data set is considered to be random. When the number of crossings is above its upper threshold, it may be concluded that the data is of the oscillating type, *i.e.*, the deviations from the straight helix are periodic with a short period. With number of crossings below the lower threshold the data is considered to be consisting of a few distinct groups – this is the case when the helix is bent. With a single bend in the helix, one expects just two crossings.

The extent of bend detected by this procedure can be tuned by a threshold value $d_{\rm min}$ for the distance of the $\boldsymbol{C}''\alpha_i$'s from the axis. Residues within that threshold could be eliminated from the calculation of crossing, leaving only residues that are farther from the axis. The more residues there are between two crossings of the axis the larger the bend is likely to be.

For helices with a bend, the orientation of the fitting plane represented by the plane's normal can be used to monitor the evolution of the plane orientation. Since the plane of bend is an independent geometric characteristic of the bend, it provides additional information about the helix. While this normal is likely to change direction as a result of a possible rotation of the helix, it can change direction without rotation. This latter case can reveal structural changes of potential functional significance.

Corrections to Distorted Helices

During MD simulations helices can be distorted to varying degrees, making the calculations described above more complicated. The major problem is caused by the incorrect sign of vectors drawn perpendicular to the helix axis, as an extra value of π is added to the values of the angles $\varphi_{rs,i}$ and $\psi_{i-1,i}$. TRAJELIX is able to detect this problem, and it attempts to automatically correct it using the following three-step procedure:

1. It calculates the minimum ($\varphi^{\rm min}$), maximum ($\varphi^{\rm max}$), and average ($\varphi^{\rm av}$) values of the $\varphi_{rs,i}$'s.
2. If $\varphi^{\rm max}-\varphi^{\rm min}<\pi$, it accepts all vector directions.
3. If $\varphi^{\rm max}-\varphi^{\rm min}>\pi$, then some of the perpendicular directions $\boldsymbol{n}_{s,i}$ are reversed and the $\varphi_{rs,i}$'s will be clustered around $\varphi^{\rm max}$ and $\varphi^{\rm min}$. Since it is likely that only a few vectors have incorrect directions, $\varphi^{\rm av}$ is expected to be closer either to $\varphi^{\rm min}$ or to $\varphi^{\rm max}$. By comparing each $\varphi_{rs,i}$ and $\varphi_{rs,i}+\pi$ with $\varphi^{\rm av}$, the one closer to $\varphi^{\rm av}$ will be accepted. Accordingly, the program will reverse the sign of each $\boldsymbol{n}_{s,i}$ vector found to be closer to the incorrect range. This procedure has been found to work in most cases.

Implementation

The set of algorithms that calculate global and local structural properties of helices have been incorporated into TRAJELIX, a new module within the program SIMULAID [5]. SIMULAID is written in Fortran 77, and works with input coordinate files in several different formats, such as PDB [14], Charmm [15], Amber [16], Macromodel [17] InsightII [18], and MMC [19] and trajectories in Charmm (also used by NAMD [20]), Amber, Macromodel, and MMC formats.

Helix axes in TRAJELIX are calculated by an algorithm due to Kahn [12] using subroutines written by Cristopher, Swanson, and Baldwin [4]. Integrity of each helix is checked using the DSSP algorithm of Kabsch and Sander [10]. At the outset of a run of TRAJELIX, the user has to provide the following information:

1. The residue range of the helix to be analyzed in the run.
2. The distance threshold $d_{\min}$ used for the bend analysis.
3. The axis selected for special plotting (x, y or z, *vide infra*).
4. The number of residues to ignore at each end for the turn angle/residue calculations.

Furthermore, the user has to specify if TRAJELIX should:

1. Shift the protein's COM to the reference protein's COM.
2. Overlay the target protein to the reference protein.
3. In case of multimolecular systems, overlay each protein to the corresponding protein in the reference structure.
4. Perform a DSSP check on each structure to check on the integrity of the helix.

For each structure analyzed the program prints the following information to an output file (using Å for distances and degrees for angles):

1. S and E: coordinates of the helix axis initial and final points.
2. RMS: diagnostic of the irregularities in the helix [4].
3. Len: length of the helix
4. D: unit vector in the helix direction
5. D–X, D–Y, D–Z: the tilt angles of the helix with respect to the laboratory frame.
6. Shape: Bent, Random or Oscillating, based on Nup/dn (number of α-carbon projections above and below of the helix axis, resp), and Ncross (the number of axis crossings).
7. $d_{\min}$, the tolerance for being on the axis and Naxis, the number of α-carbons within tolerance.
8. Rc: radius of circle fitted to the alpha carbons.
9. TPR: turn angle/residue.
10. C: coordinates of the center of mass of the helix.
11. N–X, N–Y, N–Z: the tilt angles of the normal to the plane fitting the alpha carbons with respect to the laboratory frame.
12. Rotation: the angle of rotation of helix around its axis from the start.
13 Local tilt: angle between the current and reference helix axes.
14. SD: fluctuation of the rotation angles calculated from each alpha carbon.
15. N/Nr: angle between the normals N in the current and reference state.

Unless a single structure is analyzed, the program also prepares a Postscript output file plotting the evolution of the quantities calculated during the simulation analyzed. The program provides three different plots. Specifically, the evolution of global and local tilts, the helix rotation and the turn angle per residue are represented by *dial plots* similar to the ones introduced by the Beveridge laboratory [21]. Conventional *Cartesian plots*, two functions per plot, are used to represent the evolution of the helix length, the radius of the fitted circle, the absolute displacement of the COM of the helix, the displacement of the COM along a selected coordinate direction (e.g., the normal of the bilayer the protein is embedded), the displacement in the other two directions of the COM of the helix, as well as of the two endpoints of the helix. For the displacement of the helix COM, and of the two helix endpoints the program draws a *trace* of their projection on the plane whose normal is the axis selected above. The trace is color-coded by the spectrum going continuously from red through yellow, green, cyan to blue. A similar trace is drawn for the projection of the normal to the plane

Table 1. Calculated values of selected structural properties of ideal helices

Helix type	RMS	TPR/°	Length/Res/Å	R_c/Å
α_R	0.0	99.4	1.459	17.2
α_L	0.0	−99.4	1.459	25.9
3_{10}	0.0	109.9	1.707	22.3
Pi	0.0	86.6	1.265	32.5
Bent α_R	0.82	102.4	1.452	17.6

Legend: RMS: deviation of the axis from the C_α projection; TPR: turn angle per residue; Length/Res: helical rise per residue; R_c: radius of the circle fitted to the C_α atoms.

of bend to the plane of the bend of the reference helix.

When SIMULAID is run on an SGI graphics system, the selected helix, its axis, the radius of the fitted circle, and the normal to the plane of bend are shown in the form of an animation.

Validation

TRAJELIX set of algorithms was validated using 20-residue ideal helices of the type α_R ($\varphi = -65°\psi = -40°$), $\alpha_L(\varphi = 65°\psi = 40°)$, $3_{10}(\varphi = -60°\psi = -30°)$, and Pi($\varphi = -30°\psi = -90°$), as well as a 30° bent α_R helix ($\varphi_i = -65° + 5*\sin(2\pi i/3.6)$ and $\psi_i = -40° + 5*\sin(2\pi i/3.6)$) as test cases. All helices were generated with InsightII [18]. Table 1 shows the values of the helical axis deviation from the C_α projection (RMS), the turn angle per residue (TPR), the helical rise per residue (Length/Res), and the radius of the circle fitted to the C_α atoms (R_c) that TRAJELIX calculated for each test case. The RMS and TPR values shown in Table 1 confirm the ability of TRAJELIX to correctly characterize the axes of ideal helices as well as canonical turn angles per residue, and helical rise per residue. On the other hand, the

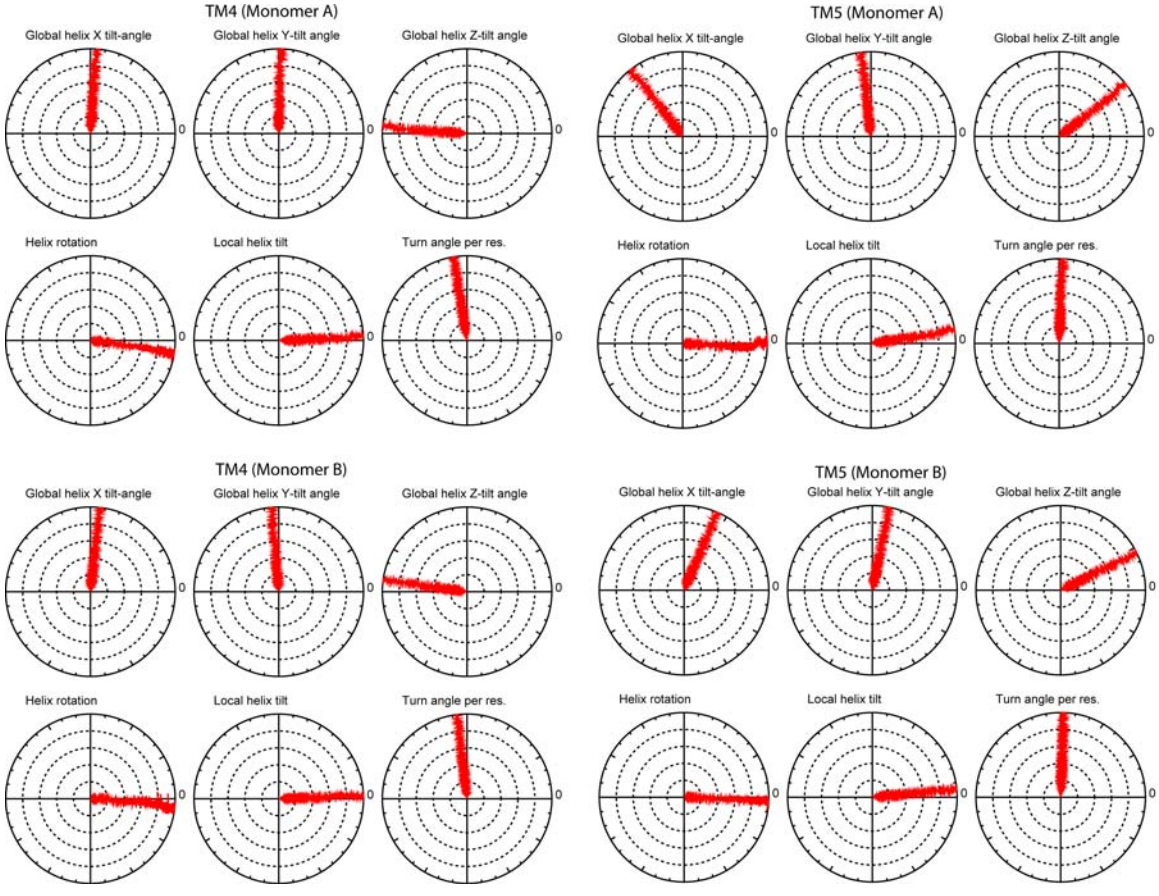

Figure 1. Dial plots of the evolution of global and local tilting angles, rotation angles, and turn-per-residue angles of the interacting TM4 and TM5 helices at the interface between monomers A and B of the rhodopsin dimeric model during MD simulations in an implicit membrane environment.

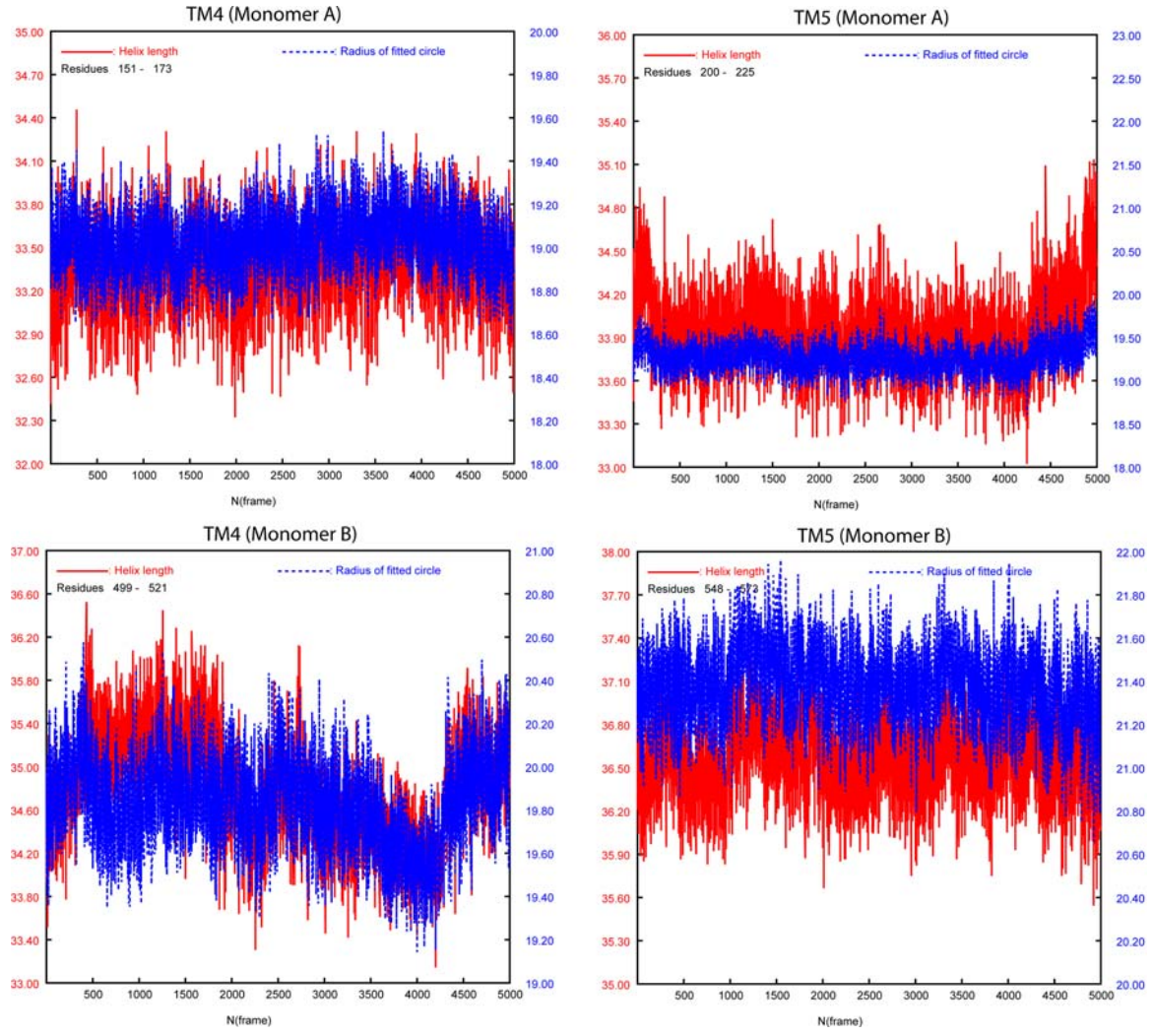

Figure 2. Estimation of helix compression/extension (red line) and helical bending (blue line) of the interacting TM4 and TM5 helices at the interface between monomers A and B of the rhodopsin dimeric model during MD simulations in an implicit membrane environment.

radius of the circle fitted to the C_α atoms does not appear to be very sensitive in detecting helical bends. The reason is that different circles can be fitted for a helix of constant bend depending on the position of the C_α atoms at the ends of the helix. In fact, different values of R_c are obtained by changing the length of the helix by 1 residue. However, since during dynamics the end-effects are expected to cancel each other out, changes in the radius of the circle fitted to the C_α atoms are likely to be good indicators of curvature changes.

Results and Discussion

The program TRAJELIX presented here aims at a comprehensive screening of changes in the geometric characteristics of protein α-helices during MD simulations. Specifically, helix movements and distortions are analyzed in terms of global and local structural properties of the helix, which include displacement, tilting, rotation, compression/extension, winding/unwinding, and bending. In general, characterization of such structural

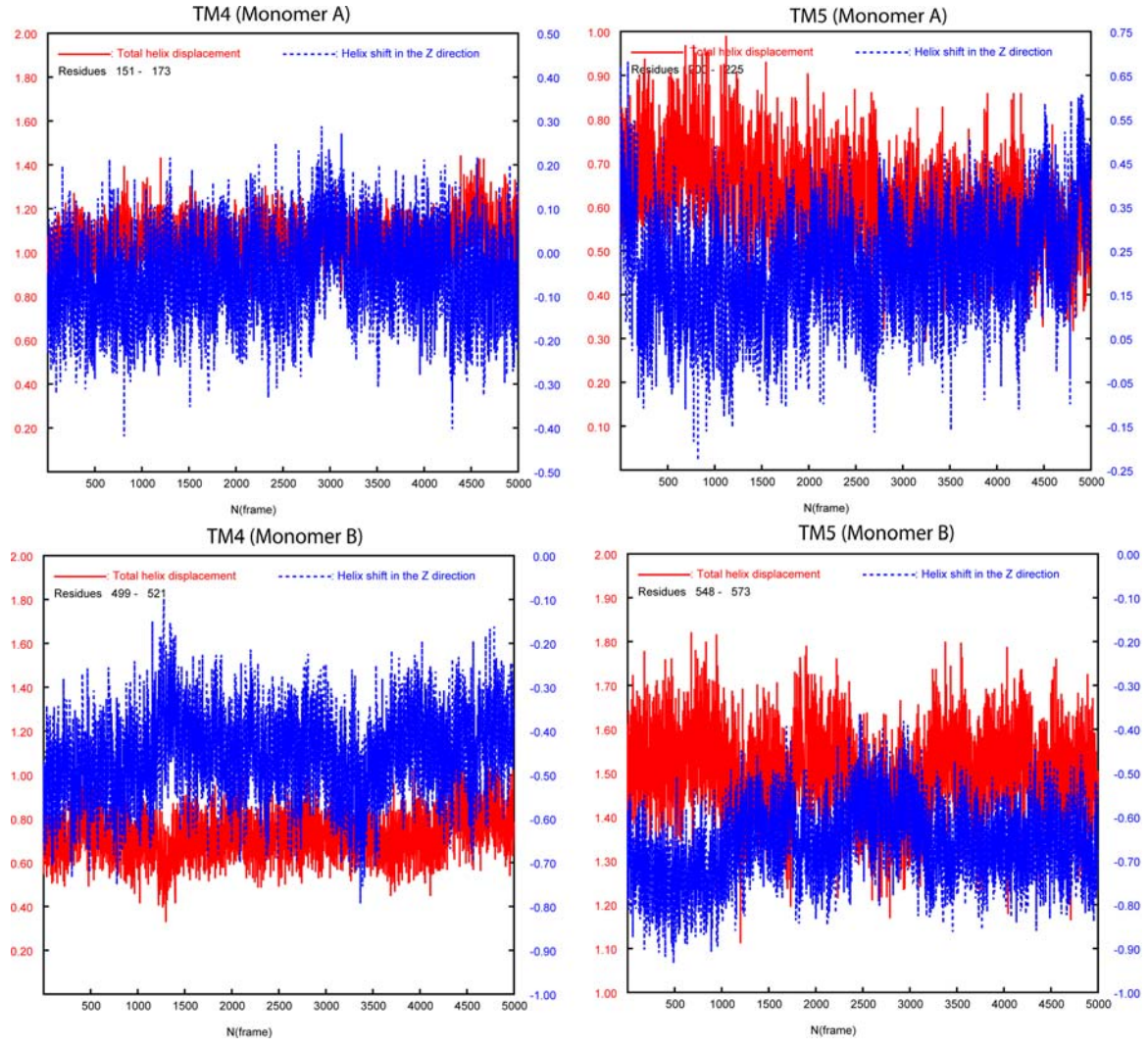

Figure 3. Total displacement (red line) and displacement in the *z* direction (blue line) calculated for each helix at the interface between monomers A and B of the proposed dimeric model of rhodopsin during the 5 ns MD simulation in an implicit membrane environment.

changes can help clarify the contribution of helices to the mechanical properties of proteins. Evaluation of this contribution can be particularly important to understand mechanisms of signal transduction, with the ultimate goal of helping structure-based drug design.

In view of the recent evidence that G-protein coupled receptors (GPCRs), which are the most successful class of drug targets among α-helical membrane proteins, can associate in dimeric/oligomeric complexes (see [22–28] for recent reviews), analysis of changes in the global and local structural properties of transmembrane helices that may occur during MD simulations at the interface of dimerization/oligomerization may help understand the nature of the interaction, and its implication in receptor function. To enable quantitative assessments of possible changes in dynamic molecular models of GPCR dimers we are currently carrying out extensive MD simulations of inactive and activated molecular models of rhodopsin monomer and dimer in explicit lipid-water environments. The results of these calculations, which will be reported elsewhere, are expected to provide new insights into the molecular determinants responsible for GPCR activation. The

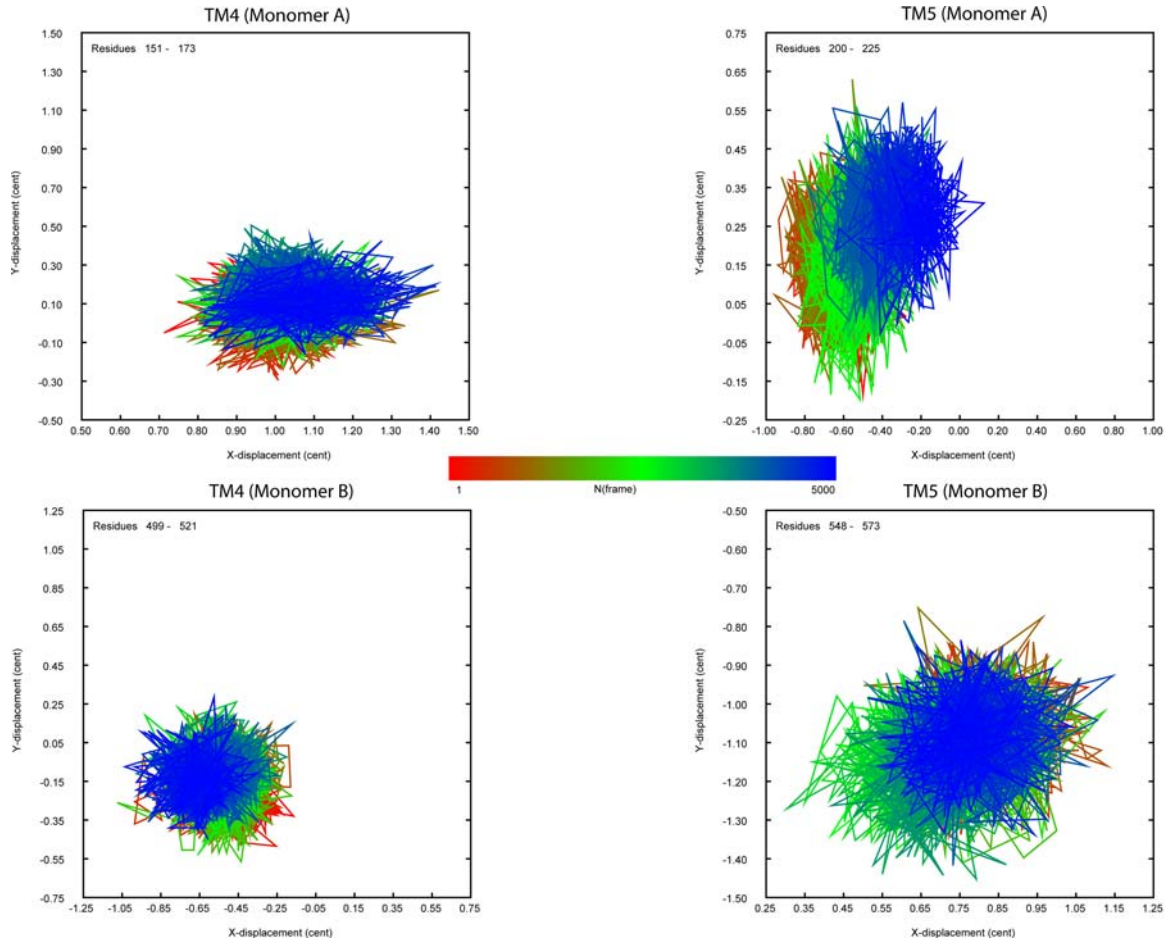

Figure 4. Trace representation, color coded by the spectrum going continuously from red through yellow, green, cyan to blue, of the displacements in the x and y directions for each helix at the interface between monomers A and B of the proposed dimeric model of rhodopsin during the 5 ns MD simulation in an implicit membrane environment.

molecular model of the dimeric interface selected as a starting point for these calculations is the one recently suggested by Liang et al., and available from the PDB database under the accession code 1N3M. Specifically, this model was proposed based on inferences from a recent atomic-force microscopy map of rhodopsin [29], which suggests the organization of rhodopsin protomers into two-dimensional arrays of dimers with TM helices 4 and 5 involved in intradimeric contact.

To test the ability of TRAJELIX to reveal the nature of possible intermolecular changes at the interface of interacting alpha-helical membrane proteins during MD simulations, we carried out a relatively short MD simulation of the proposed TM4,5-TM4,5 dimeric model of rhodopsin using a new energy function for the implicit representation of the membrane-aqueous media (IMM1 [9]), and analyzed the resulting trajectory of 5 ns with our program. It is worth emphasizing here that due to the approximate representation of the environment, and the shortness of the simulation, the results presented here should be considered as a first approximation to the dynamic behavior of the rhodopsin quaternary structure. More accurate assessments of possible changes within the structure of GPCR dimers are expected to result from the more detailed and longer MD simulations of molecular models of inactive and activated forms of rhodopsin monomer and dimer in an explicit lipid-water environment, which are currently ongoing in our laboratory.

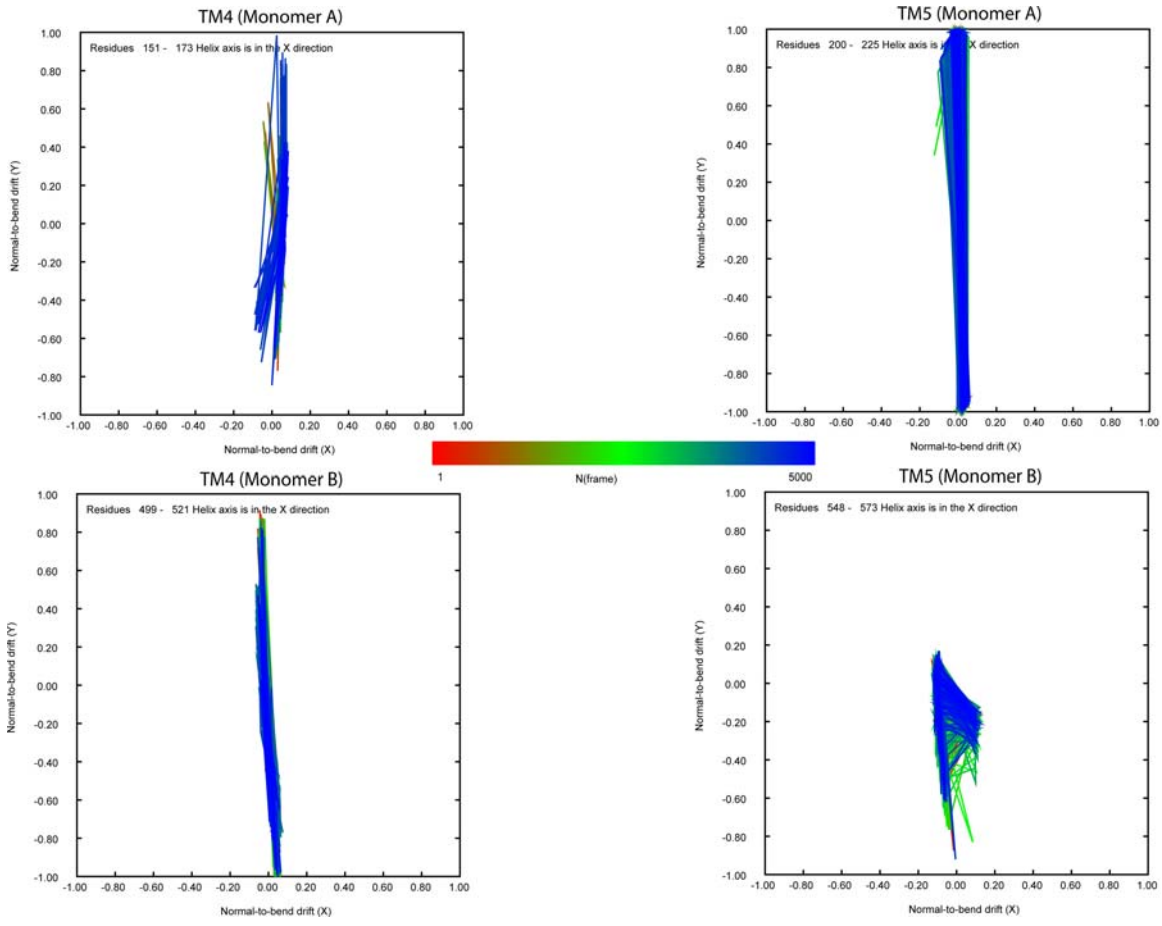

Figure 5. Trace representation, color coded by the spectrum going continuously from red through yellow, green, cyan to blue, of the projection of the normal to the plane fitted to the helix (the plane of the bend) to the plane of the bend of the reference helix for each helix at the interface between monomers A and B of the proposed dimeric model of rhodopsin during the 5 ns MD simulation in an implicit membrane environment.

Root mean square deviation (rmsd) values of each sampled conformation from a reference structure (usually the initial minimized structure) are generally used to assess the stability of the simulation as well as to evaluate changes that may occur in the simulated protein structure. The α-carbon rmsd values calculated for each TM helix of the proposed TM4,5-TM4,5 rhodopsin dimer with respect to initial minimized structures are kept below 1 Å during the 5 ns simulation, suggesting the overall stability of the helix conformations within the dimer. The aim of our program TRAJELIX is to reveal the details of even slight changes in the global and local geometric characteristics of protein helices during MD simulations. For instance, Figure 1 shows in the form of dial plots the evolution of global and local tilting angles, rotation angles, and turn-per-residue angles calculated by TRAJELIX for the interacting TM4 and TM5 helices at the interface of the rhodopsin dimeric model during the calculated 5 ns trajectory. Rotations of up to 20 degrees are observed for the interacting TM4 helices around their axes, whereas TM5 helices do not seem to undergo any appreciable rotation. In addition, the global tilt of both interacting TM5 helices appears more pronounced than the tilt calculated for TM4 helices. An evaluation of helix compression/extension during the MD simulation is provided in Figure 2 (red line), also showing an estimate of helical bending (blue line). Specifically, Figure 2 shows that TMs 4 and 5 remain pretty straight during the MD simulation, and they do not

seem to undergo any significant compression or extension (values within 1 angstrom). Figures 3 and 4 show the total displacement, and the displacement in the x, y, and z directions of the COM of each helix at the interface of the proposed dimeric model of rhodopsin. These movements are minimal for these helices during the 5 ns simulation, as also seen in case of the helix endpoint displacements (data not shown). Finally, Figure 5 shows the time-evolution of the normals to the plane of the bend for TM4 and TM5 helices of monomers A and B within the rhodopsin dimer. In each case the normals show large oscillation in the Y direction and very little change in the X direction. The large oscillations are indicative of bends that change randomly in the direction of the oscillation (Y direction in this specific case) whereas the small changes in the X direction indicate a stable bend in that direction. Taken together, data shown in Figures 1–5 confirm the stability of the quaternary structure of an inactive rhodopsin dimer during the first 5 ns of MD simulation in an implicit membrane model, but at the same time allow quantification of the helix global and local structural properties that were subjected to mild changes. Specifically, a slight clockwise rotation around the helical axes of the interacting TM4 helices, as well as a 30–40 degrees change in the global tilt of interacting TM5 helices were identified as the major helical structural rearrangements at the interface between inactive rhodopsin monomers during molecular dynamics using an implicit membrane environment. However, these suggested changes in the global and local structural properties of helices at the interface of a GPCR dimer will need to be confirmed by the more detailed and longer MD simulations of molecular models of rhodopsin dimers that are currently ongoing in our laboratory.

Application of TRAJELIX to the 5 ns trajectory calculated for the proposed inactive TM4,5-TM4,5 rhodopsin dimer has served to the scope of providing the details of possible local and global structural changes of interacting helical domains of transmembrane bundles. It is worth emphasizing, however, that the proposed algorithm is a fundamental tool that can be applied to any protein structure containing helical domains. Moreover, its addition to the SIMULAID package, which includes a large number of conversion and analysis features, makes TRAJELIX a more complete piece of software than the ones currently available for geometric characterization of protein α-helices. TRAJELIX is available on-line at the URL http://inka.mssm.edu/~mezei/simulaid.

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