Alignment of 3D structures of macromolecular assemblies
Salvatore Lanzavecchia*, Francesca Cantele and Pier Luigi Bellon

Università di Milano, Dipartimento di Chimica Strutturale e Stereoehimica Inorganica
Via G. Venezian 21, 20133 Milano, Italy

Received on June 6, 2000; revised on August 29, 2000; accepted on September 8, 2000

ABSTRACT
Motivation: A number of macromolecular assemblies are being reconstructed in 3D from electron micrographs. The analysis yields a 3D matrix representing the protein density map. In reconstruction processes and in comparing the results of different experiments, it is often necessary to obtain all models oriented the same way in three dimensions. The problem is not trivial since there exist no 3D counterpart of correlation analysis used for 2D images. It is usually solved by time consuming trial and error algorithms.

Results: 3D density distributions can be brought to a ‘canonical’ orientation. The tensor of inertia of the distribution is determined and its eigenvectors are oriented along the coordinate axes. The method is fast and essentially free of reference. It is suitable for structures whose inertial axes do not completely degenerate as they do in icosahedral viruses or if symmetry is cubic. Applications are presented for asymmetric objects and for molecules possessing symmetry axes higher than twofold.

Implementation: The implementation simply requires the accumulation of the inertial tensor and its diagonalisation. Volume data rotation has been already illustrated in this journal by the authors.

Availability: The software is available upon request to the authors.

Contact: salvator@csmtbo.mi.cnr.it

INTRODUCTION

Three-dimensional (3D) electron microscopy (EM) is a sophisticated, powerful tool of structural biology. It aims at reconstructing 3D macromolecular assemblies from projections observed, most often, by cryo-microscopy. The methodologies and related algorithms have been developed in the past years by a relatively small number of dedicated laboratories (see, e.g. Frank, 1996) and, as an outcome, several software packages are now available to the scientific community (see *Journal of Structural Biology*, 116, 1996). The number of proteins reconstructed by this technique is growing steadily and, in the meantime, technological and scientific efforts are pushing the resolution toward 0.5–0.3 nm (De Rosier, 1997). Still, no complete automation has been achieved and several aspects need to be further investigated.

A problem which is often encountered is the alignment of 3D density maps representing the same object in different orientations. This may occur in: (1) merging the reconstruction of molecules obtained by the random conical tilt method (RCT) (Radermacher et al., 1987) if the molecular species exhibits two or more preferred orientations (Frank et al., 1991); (2) averaging preliminary models obtained by reconstruction from sets of projections portrayed in two symmetric exposures (Bellon et al., 1998; Lanzavecchia et al., 1999b); (3) aligning 3D particles reconstructed by single axis automated tomography (Koster et al., 1997); (4) docking high resolution x-ray structures into low resolution quaternary structures obtained by microscopy. Angular correlation (Frank, 1981), by which the mutual rotation of 2D images is determined, has not 3D counterpart. Thus, the problem of 3D alignment of equivalent structures is far from being a trivial one.

The 3D rotation of a model A with respect to an equivalent model B, defined in a reference system (i, j, k), can be described in terms of a rotated Cartesian system (i', j', k'), or of three Euler angles (α, β, γ). The angles α and β (α = longitude, β = latitude) define the orientation of k' with respect to (i, j, k) and γ specifies a rotation around k' in the plane (i', j'). In the absence of symmetry or of other information, bringing A into coincidence with B is usually performed by trial and error. A is rotated systematically by varying a vector (α, β) sampling the 4π solid angle of possible orientations. A measure of how well A and B match each other is obtained by correlation algorithms (Walz et al., 1997). An improvement has been introduced to take into account a relative shift (Öfverstedt et al., 1997).

In this paper we wish to illustrate a quick and direct method to determine how two or more density maps are aligned.

*To whom correspondence should be addressed.
rotated with respect to a ‘canonical’ orientation. Once this has been determined, the volume data can be rotated by accurate algorithms (see, e.g. Tosoni et al., 1996). The result, if necessary, could be refined by the trial and error within a small solid angle. The method makes use of inertia tensors of 3D structures and can be applied in all cases in which at least one tensor eigenvalue does not degenerate. For asymmetric- or twofold symmetric objects the implementation is straightforward and all structures become oriented in a ‘canonical’ way, that is with the directions of their principal axes coinciding with the coordinate axes. Even in the presence of higher symmetry the method works well. Our algorithm has been tested successfully on quite a few structures. Happily enough, even density maps with a missing region in Fourier domain, such as those obtained by RCT or single axis tomography, can be properly aligned.

The method

A density distribution \( D(x, y, z) \) can be regarded as a rigid body whose dynamics is conveniently studied by using the inertia tensor (see, e.g. Goldstein, 1980). This entity has the same representation of a 3 × 3 matrix. If \( D \) is discrete and confined in a cube with edges of length \( n \), \( x \), \( y \), \( z \) range from 1 to \( n \), and in the hypothesis that the centre of mass coincides with the origin, the tensor is given by:

\[
\begin{bmatrix}
\sum_{s,y,z} D_{s,y,z}(y^2 + z^2) & - \sum_{s,y,z} D_{s,y,z} x y & - \sum_{s,y,z} D_{s,y,z} x z \\
- \sum_{s,y,z} D_{s,y,z} x y & \sum_{s,y,z} D_{s,y,z} (x^2 + z^2) & - \sum_{s,y,z} D_{s,y,z} y z \\
- \sum_{s,y,z} D_{s,y,z} x z & - \sum_{s,y,z} D_{s,y,z} y z & \sum_{s,y,z} D_{s,y,z} (x^2 + y^2)
\end{bmatrix}
\]

If the mass centre, easy to determine, is not at the origin an appropriate shift should be applied first. The tensor can also be regarded as a quadratic form representing the inertia ellipsoid of the distribution. The directions of the three semi-axes coincide with the tensor eigenvectors and the squared lengths of the semi-axes are the eigenvalues. As is true for other functions (e.g. a spectrum in reciprocal space or a correlation in real space) there exists an infinity of density distributions, symmetric and asymmetric, possessing the same inertia tensor. Bearing this limitation in mind, the ellipsoid changes its orientation as the distribution rotates in space. Clearly, the inertia tensor is useless if the three eigenvalues degenerate or, in other words, if the ellipsoid is actually a sphere. This would happen, for instance, withicosahedral viruses and with structures possessing cubic or tetrahedral symmetry. Even an asymmetric structure may exhibit complete degeneracy because of its density distribution. Further, the presence of noise could prevent the differences among the eigenvalues from being significant.

Based on the inertia tensor, preliminary steps in orienting a reconstruction are: (i) the 3D density distribution is accurately centred; (ii) the tensor is accumulated; and (iii) the eigenvectors and eigenvalues are computed with some standard routine.

In the general case of a structure in which the three principal axes of the representative ellipsoid have different lengths (three different eigenvalues are found), the knowledge of the eigenvectors allows the 3D map to be rotated and the principal axes brought into coincidence with the co-ordinate axes unambiguously. With axial symmetry higher than twofold, as is the case of several oligomeric complexes, two eigenvalues degenerate and a revolution ellipsoid is obtained; still the method can be used. The structures are aligned so that the symmetry axis coincides with one of the system axes, say \( k \). The rotation along \( k \) can be determined by correlation algorithms.

IMPLEMENTATION

Consider first the general case of a fully asymmetric structure, like the random knot of Figure 1a, which we normally use to test reconstruction algorithms (Bellon et al., 1998; Lanzavecchia et al., 1999a). This test function can be computed analytically in a number of different orientations (Figure 1b–c) and, for each orientation, the tensor of inertia furnishes the directions of the three axes (Figure 1d–f). Since the three eigenvalues differ quite a bit one from the other, a model can be rotated to bring each eigenvector into coincidence with the \( i, j, k \) directions. The largest eigenvalue can, for instance, be associated to \( k \), the middle to \( j \) and the smallest to \( i \). It is important to notice that the indication furnished by the eigenvectors holds true for both the correct- and

![Fig. 1. The analytic function in the volume rendering (a) can be obtained in different orientations as in (b) and (c). The corresponding inertial ellipsoids (d), (e), (f) are equal, though oriented in different ways. A ‘canonical’ orientation corresponds to ellipsoids whose semi-axes point along the three coordinate axes.](image-url)
the reverse direction. There exist four different ways to align the model with respect to the system (Figure 2). If a number knots like those of Figure 1a–c are to be aligned, a suitable strategy is as follows: (i) align the first model at any of the four possible ways; (ii) align all other models of the set in the four different ways and check by correlation in which way the best match with the first model is obtained. Notice that once the eigenvectors coincide with the reference axes and a model has been rotated accordingly, three more orientations are simply obtained by appropriate sign changes of the indices. With reference to Figure 2, three more maps are obtained if $D(x, y, z)$ becomes $D(-x, -y, z)$, $D(-x, y, -z)$ and $D(x, -y, -z)$ respectively.

In the presence of a symmetry axis with order higher than twofold two eigenvalues, associated with eigenvectors orthogonal to the symmetry axis, degenerate (revolution ellipsoid). In this case the alignment is accomplished by rotating all models so that the unique eigenvector coincides with $k$. An up–down indeterminacy still remains, to be removed later on. Once the structures have been oriented in this way, their projections along $k$ are mutually aligned by 2D angular correlation. In this way $y$ is determined and the volume data can be rotated around $k$. This is the end of the story if the structure possesses twofold axes orthogonal to the main symmetry axis. Otherwise, a further check by correlation is required to discriminate upward from downward orientation.

In accumulating the inertia tensor, disturbing effects besides noise can arise from non-positive values in the map or from a density shift with respect to zero. These cause significant changes of the ellipsoid shape. As a remedy, we subtract from the 3D map the mean values of the region surrounding the molecule and accumulate the tensor by considering positive density only. A spherical mask enclosing the molecule is used to limit noise effects. As for correlation, since each reconstruction is centred before accumulating the tensor, there is no need to determine a shift. In this case, the amount of correlation is evaluated by computing the overlap integral extended all over two structures. In the discrete version the integral becomes $\sum_{x,y,z} D(x, y, z) \times D'(x, y, z)$. This is faster than using algorithms based on FFT. The overlap integral is recommended for aligning structures determined from RCT or single axis tomography. A cone or a wedge of zeroes, present in the Fourier transform of these structures, would create artefacts in a correlation computed via Fourier transform.

**RESULTS**

The random knot of Figure 1 has been obtained in a number of different orientations and the volume data have been corrupted with Gaussian noise down to $S/N = 1$. Thanks to the large differences among the three eigenvectors, not seriously affected by this type of noise, the method above is able to bring the knots to the ‘canonical’ orientation with negligible errors.

In two simulations closer to reality, we started from an already determined structure possessing D6 symmetry, namely the hematic pigment Chlorocruorior of *Sabella spallanzanii*. In one experiment, we assumed the molecule to lay in three preferred orientations on the EM grid. Three sets of noise corrupted projections were computed, in RCT geometry and 50° of tilt. The structures were reconstructed via weighted back projection (WBP Radermacher et al., 1987; Harauz and van Heel, 1986) and via the Radon transform (Lanzavecchia et al., 1999a). Due to the different orientations of the missing cones in the structure transforms, the results were appreciably different. In real experiments it is necessary to assign consistent orientations to the three sets of projections in order to obtain a reconstruction based on all available data. This can be done once the mutual orientations of the three structures has been determined. We have aligned the density maps and compared the angles thus determined with the theoretical ones. For models obtained with use of WBP, we found an average rotation error of 2°, whereas using the Radon transform approach (which partly fills the missing FT cone; Lanzavecchia et al., 1999a) the average...
error was 1.5°. The alignment of each map, 64³ pixel wide, required about 30 s on a PC with Pentium II processor at 450 MHz (C language, LINUX operating system).

A second experiment is based on micrographs of the same molecule embedded in ice. No preferred orientation is observed. A two exposure technique at symmetric tilt angles (Bellon et al., 1998; Lanzavecchia et al., 1999b) has been devised to manage the problem of projections with completely random orientations. With our reconstruction software, a number of preliminary 3D models can be obtained, characterised by different orientation. A substantially improved model is obtained by averaging the models properly aligned. Four models with different orientations are presented in Figure 3a–d. Since the molecule possesses D6 symmetry, two eigenvalues degenerate. All models have been aligned to bring the unique axis along \( k \) and then rotated in \( i, j \) plane to obtain best overlap. The final average, with improved quality, is shown in Figure 4a, whereas Figure 4b is obtained with sixfold symmetry imposed. The latter structure, still a rough representation of the molecule, is a sound starting model for subsequent refinement by projection matching (Frank, 1996).

**DISCUSSION AND CONCLUDING REMARKS**

The problem of aligning 3D density distributions may find an exact or approximated solution, depending upon the equivalence of the distributions. If the objects are identical, an exact solution exists; if the objects are not strictly congruent, the alignment is based on a matching criterion. In the case of 3D models obtained from electron micrographs in the early stage of a structure determination, strict equivalence cannot be observed. The reason may be ascribed only in part to noise; more serious discrepancies are originated by missing regions in Fourier space. This is the case of reconstructions coming from RCT or single axis tomography. In the case of the Chlorocruorin molecules shown in Figure 3, density mismatches mostly arise from improper determination of the projection angles. The average reconstruction represents an improvement in much the same way as the signal to noise ratio does in an averaging process.

It is worth noticing that the alignment based on inertia tensors is basically a ‘reference free’ approach. For fully asymmetric objects the alignment is automatic and all models are independently aligned in the ‘canonical’ way. Checking the correct direction of the eigenvector, by correlation with a model already aligned is again an automatic process, which cannot be influenced by the order by which the maps are processed. In the cases where two eigenvectors degenerate, part of the alignment is based on correlation and requires a reference to be fixed, as in 2D orientation processes. Different strategies could be devised to minimise the effect of the choice. Anyhow, the basic rotation to align the unique eigenvector along \( k \), is reference independent.

Once an average model has been obtained, one may wish to improve the result by refining the orientations of preliminary models with respect to it. This can be done by using a trial and error correlation process (see Section Introduction). The method is slow since, to evaluate a
figure of merit of matching, the maps need to be rotated by every set of Euler angles to be tested. The method described here offers a good starting point, the region of Euler space to be explored being small. We have used a trial and error technique to improve the alignment of Chlorocruorin models with respect to the symmetric average of Figure 4b. However, the refined average of Figure 4c, obtained in this way, does not appreciably differ. This shows that the first alignment was already good. In most cases, a time consuming refinement of 3D orientation is worthless. It is best to use a preliminary model of sound quality to refine the orientation angles of projections.

As a concluding remark, the method above is characterised by an extremely simple implementation. No new software is required, apart from two routines to accumulate the inertia tensor and to compute overlap integrals. An efficient library for volume data rotation is finally required. A version of the latter has been recently proposed by the authors (Tosoni et al., 1996).

REFERENCES


