Using a RIBBON program to illustrate lipid bilayer packing

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We describe the use of the Priestle RIBBON program suite for the illustration of lipid bilayer packing. The hydrocarbon chains of lecithin-like molecules are representable by β-sheet arrows. The lateral packing, packing across the bilayer midline and any twisting, bowing, or curling of the polymethylene chain are readily detectable.

Keywords: hydrocarbon graphical representations, lipid ribbon models, illustrating polymethylene chains, drawings of bilayers

BACKGROUND

Ball-and-stick illustrations[1] are widely favored for representing the crystal structures of small molecules. Figure 1 shows four ball-and-stick views of the X-ray crystal structure of *racemic* glycerol 1,2-(di-11-bromoundecanoate)-3-(-p-toluene-sulfonate),[2] hereafter DBUTOS. Figure 1a suggests a twist in one fatty acid (Chain 1) and a slight bowing in the other (Chain 2). Because these illustrations are frequently drawn without including the hydrogen atoms, a hydrocarbon chain might be perceived to be a slightly elliptical rod. The observation of a twist, bow, or curl can be easily overlooked or attributed to experimental noise. When viewed from an end and with the hydrogen atoms included, the hydrocarbon chain is more rectangular—a lathlike strip. End-on views of the hydrocarbon chains for several different packing types have been described by Chapman.[3]

Originally, the pseudo-periodic sub-cell packing of long-chain compounds was described by Vand as part of a method for determining the signs of the structure factors[4] that he used for solving crystal structures, such as the β form of trilaurin.[5]

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The sub-cell descriptions have since been used to characterize and classify the lateral packing of long hydrocarbon chains.[6] The sub-cell may be triclinic, monoclinic, or orthorhombic, and the chain-to-chain orientations may be parallel or perpendicular. A few hybrid sub-cell types have been described.[7] Although this sub-cell nomenclature is useful, it seductively suggests a uniform progression of methylene units along a planar all-trans hydrocarbon chain. The sub-cell chain packing in DBUTOS (Figure 1b) can be described as orthorhombic perpendicular; however, the twist along one chain prevents a rigorous periodic repeat of the sub-cell along the chains. The sub-cell terminology is, at best, only qualitative in this case.

Priestle[8] described a suite of FORTRAN programs that are especially good for representing the secondary structure components of proteins and their topological interrelationships by using helical ribbons, twisted arrows, and smooth ropes.[9] Such cartoon drawings provide an uncluttered representation of the folding of α-helices, β-sheets, random coils, and reverse turns, and can provide a clearer visualization of a binding cleft of an enzyme or of a protein–ligand interaction. Molecular cartoons capture the essential features of the tertiary structure of a protein.

LIPID RIBBONS

The β-sheet ribbon is both rectangular and flexible; it is a natural medium for the abstract representation of a hydrocarbon chain. Priestle's program suite, somewhat modified, contains the following features:

(1) Stereo drawings can be produced. The left and right pairs are obtained by either rotating the coordinate system about its axis, or by shifting the viewpoint.[10] The computer calculates the perspective projection of the cartoon to the projection plane from a user-defined viewpoint.
(2) Hidden (overlapped) regions can be removed.
(3) The β-sheet arrow has both width and thickness.
(4) Breaks between residues can be accommodated.

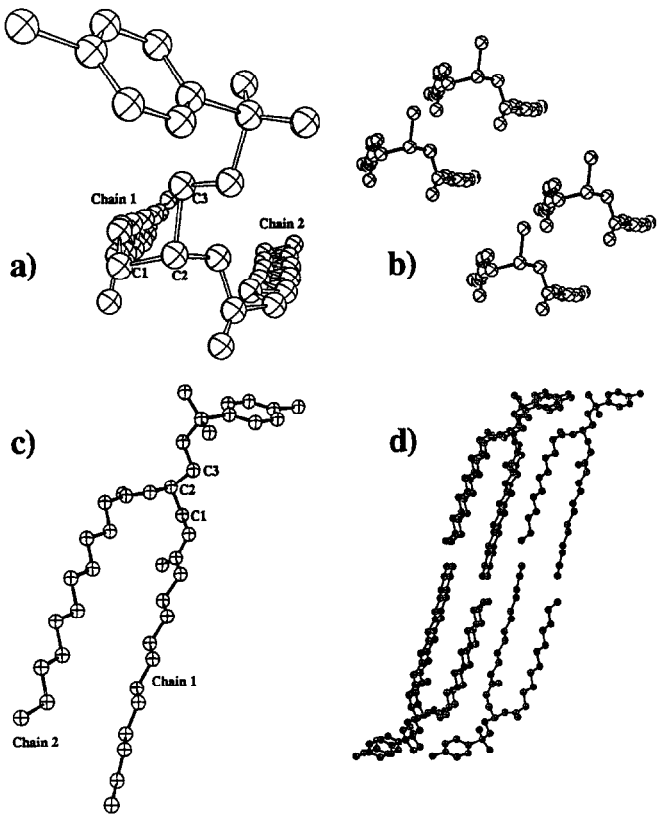

Figure 1. Several ball-and-stick drawings of DBUTOS. a) A single molecule is viewed through the head group toward the tails of the fatty acids; the carbon atoms of the glycerol group are labeled C1, C2 and C3. The twist of Chain 1 and the bowing of Chain 2 are noticeable. b) The orthorhombic sub-cell packing is noticeable in this drawing. The head groups were omitted. c) The long, planar section of the fatty acid of Chain 2 is shown in the plane of the illustration; the carbon atoms of the glycerol group are again labeled C1, C3, and C3. In this view the three-atom carboxyl group of Chain 2, located near C2, is oriented perpendicular to fatty acid plane. The plane of Chain 1 is viewed edge on and appears to be twisted, and the carboxyl group is coplanar with the rest of the Chain. d) This drawing, which attempts to show the bilayer packing of six molecules, should be compared with Figure 2. Note that the packing of the Chain 1 terminal ends form a parallel packed configuration of fatty acid chains. Hydrogen atoms were omitted from all drawings.

(5) HETATM (special atoms) can be input and displayed.
(6) Output can be viewed or plotted on a variety of device types.

The Fortran source code for the suite of RIBBON programs was implemented with some modifications on an AT&T 3B1 computer, and was used to generate plot files for an attached HPLaserJet III printer. Because the RIBBON program was designed for illustrating protein structures, it naturally expects coordinates in Protein Data Base (PDB) format.[11, 12] It uses the orthogonalized coordinates of the α-carbons from each residue; that is, only the *CA* atoms of each amino acid residue are used to define a ribbon. The crystallographic coordinates for small molecules, including lipids, are reported as fractions of the unit cell dimensions; furthermore, the unit cell is often nonorthogonal. The first problem was to generate sets of Cartesian coordinates for input to the RIBBON program.

As an example, consider the case of DBUTOS; it contains two fatty acid chains to be drawn as two β-sheets. All carbon atoms of the fatty acid chains were assigned the label *CA* on individual ATOM cards and given unique "residue" numbers. The numerical ordering of the β-sheet residues is important; a β-sheet fatty acid starts at the tail end, with the residue numbers increasing sequentially from the tail to the carboxyl group of the chain.

We used the GEOM program previously written by Hybl to generate the PDB-formatted coordinates for a bilayer cluster of molecules. GEOM can input the crystallographic coordinates of small molecules in any order. It was used to compute the Cartesian coordinates for DBUTOS relative to the principal axes of the whole molecule. In a preliminary calculation, GEOM was used to compute the distances between the terminal Br atom of Chain 1 and all Chain 1 Br atoms in molecules related by all symmetry operations, and all unit cell translations to a limit of about 10 Å. The list of atoms and distances were output to a dbutos.adc file. This file was edited to select the atom designator codes[1] (ADC) for the molecules to be included in the bilayer cluster. Using the translation and symmetry components of the atom designator codes from the edited dbutos.adc file, and knowing the order of the carbon atoms in each fatty acid, GEOM generates both the dbutos.dat and dbutos.pdb files required by the RIBBON program.

All atoms not in a β-sheet, including the atoms of the glycerol group, the atoms of the para-toluene sulfonate group, and the carboxyl oxygen atoms, as well as the carboxyl carbon atoms, were put into the HETATM group. Each carboxyl carbon atom is entered twice, once on an ATOM card and again on a HETATM card, but with different name and residue number. They provide connectivity between the β-sheets and the HETATM stick structure. A total of thirteen molecules (26 β-sheets) was generated. (The total number of ATOM and HETATM cards generated for this bilayer cluster is larger than some proteins.) The terminal Br on Chain 1 was selected, and duplicate coordinates for two translationally equivalent Br atoms, one at $(x-1, y-1, z)$ having ADC code 44501 and the other at $(x, y-1, z)$ with ADC code 54501, were also added to the HETATM group with atom type codes unknown to the program. This causes the program to draw an asterisk at their positions in the plot, and provides a mechanism for inserting fiduciary points to help identify which molecules belong to which ADC codes; that is, it orients the plot. A line connecting the two asterisks would be parallel to the crystal a-axis.

The second problem was that the CA atoms of a β-sheet in a protein define a plane perpendicular to the face of the β-sheet arrow. We reversed the values defining the width and thickness of the arrow in our work. The principal plane of the lipid is coplanar with the new face (old edge) of the arrow. The face and edge planes of a β-arrow can be shaded by

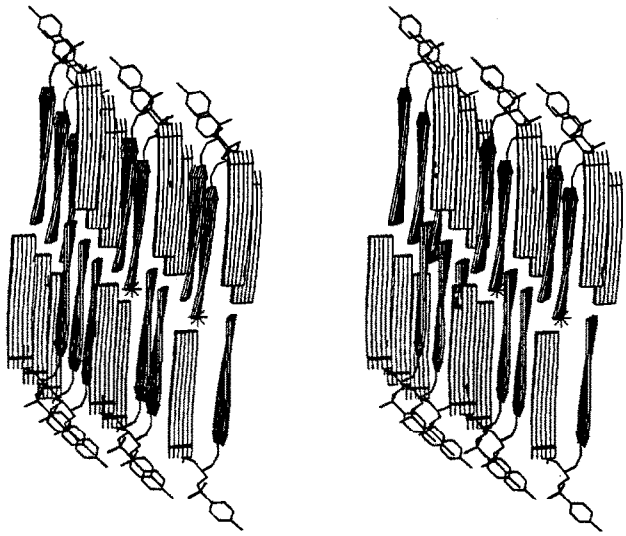

Figure 2. A stereo RIBBON drawing for a 13-molecule bilayer cluster of DBUTOS molecules. A line connecting the two asterisks would be parallel to the crystal a-axis and is parallel to the central plane of the bilayer. (This is not a symmetry plane—it is just a plane that divides the two parts of the bilayer.) The 30° twist in Chain 1 is clearly illustrated, as is the bowing of Chain 2. The intercalated parallel packing of the Chain 1 terminal ends across the bilayer midline is in stark contrast to the perpendicular packing of the Chain 1 and Chain 2 stacks on each side of the bilayer. This drawing and the ball-and-stick drawings (Figures 1c and 1d) are complementary in that each helps to provide details that the other does not or cannot illustrate.

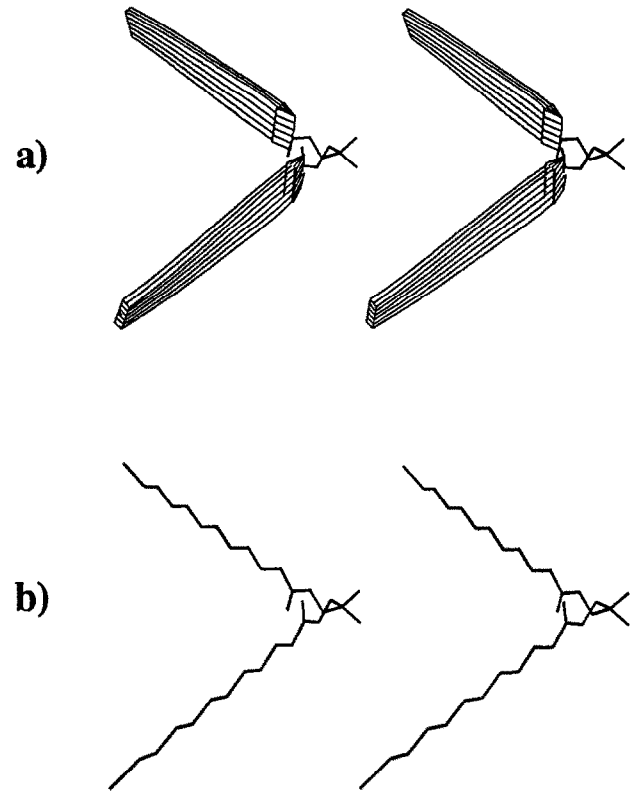

Figure 3. Two stereographic drawings of DBU contrast a RIBBON plot (a) with a pure stick plot (b). The two hydrocarbon chains in DBU are related by a twofold axis of symmetry, and are twisted by 34° along their long axes.

drawing additional lines parallel to the edges, the number being specified by the user. We added one shading line along an edge and four shading lines on each face.

Our result for the DBUTOS molecule is shown in the stereo illustration in Figure 2. The 30° twist in Chain 1 is clearly illustrated, as is the bowing of Chain 2. The intercalated parallel packing of the Chain 1 terminal ends across the bilayer midline is in stark contrast to the perpendicular packing of the Chain 1 and Chain 2 stacks on each side of the bilayer. Figures 1c and 1d help to visualize the region of intercalated packing.

Two less complex stereo drawings, shown in Figure 3, of the 1,3-diglyceride of 11-bromoundecanoic acid (DBU)[13] contrasts a ribbon plot (Figure 3a) with a pure stick representation (Figure 3b). Again, there is a 34° twist in the hydrocarbon chain that is more clearly illustrated by the ribbon plot.

BUGS AND ENHANCEMENTS

The section of code that eliminates overlapped regions misses some lines, as evidenced in one of the arrow heads in Figure 3a and by the short spurious streaks in several arrow faces shown in Figure 2. It also incorrectly removed the leading edge of the arrow heads in Figure 2. Such problems can be touched up by hand on a final drawing. More serious is the slowness of the hidden-line removal code—replacement is strongly indicated. Additional shading modalities should be added; for example, one might desire to illustrate the twist induced strain along the hydrocarbon chain by changes in shading density. Furthermore, many other standard clusterings of atoms can be represented by various cartoon objects.

CONCLUSIONS

We have shown that the fatty acid chains of a lecithin-like molecule are representable by β-sheet arrows. The lateral packing, the packing across the bilayer midline, the twisting, and bowing of polymethylene chains are more clearly revealed by ribbon drawings than by ball-and-stick illustrations.

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