NEW PROGRAMS

A crystallographic molecular lattice builder applied to model lipid bilayers

David C. Turner and Bruce Paul Gaber

Center for Biomolecular Science and Engineering, Naval Research Laboratory, Washington, DC, USA

It is often desirable for noncrystallographers to generate graphical models of three-dimensional crystal structures based on published coordinates of the atoms that make up the crystallographic unit cells. This type of visualization is particularly important for small-molecule crystals, such as lipid crystals, where one may be interested in investigating interactions between the individual molecules in addition to their conformations. BILAYER BUILDER is a program that generates a portion of the entire crystal structure from the coordinates of the molecules in a single unit cell. It gives users of small desktop computers, such as the Apple Macintosh, the capability to generate and examine model crystal structures with a molecular graphics display program. BILAYER BUILDER stores the crystal coordinates in a Brookhaven Protein Data Bank file format for possible use in a variety of applications on many different computers. Initially, it was written for use with lipid crystals and bilayers but may be used for building an assortment of molecular crystals.

Keywords: lipid, membrane, bilayer, crystal

INTRODUCTION

Lipid crystal structures are reported in the crystallographic literature as the fractional coordinates of a single lipid molecule, or occasionally several molecules, which represent the minimum data set necessary to reconstruct the crystal. While the coordinates for a single molecule tell us about the configuration of the submolecular groups within the lipid, they are often insufficient to reveal many of the more complex intermolecular interactions among the lipid molecules within the entire lipid crystal, or within a bilayer sheet of that crystal. These interactions can be observed by reconstructing the crystal structure from the given atomic coordinates using the space group symmetries of the crystallographic unit cell. We have written BILAYER BUILDER to allow the noncrystallographer easier access to these crystal structures. The program was written for the Apple Macintosh but also has been ported to UNIX platforms, such as the Silicon Graphics IRIS workstation and the Sun SPARC workstation.

Once constructed, the crystal data set can be used for many purposes, for example: as input to a graphics display program for visualizing the intermolecular interactions among the lipid molecules, such as hydrocarbon chain packing and interdigitation; or as a starting point for a molecular mechanics of dynamics simulation of a lipid bilayer. On the Macintosh, the NRL-developed-program NanoVision[1] is an ideal graphics tool for displaying the crystal structure, allowing rotations, pans, zooms, and a variety of representations of the individual atoms. NanoVision brings graphical visualization of molecular structures to the desktop of the average Macintosh user. The combination of BILAYER BUILDER and NanoVision allows any Macintosh user to investigate the molecular packing in the crystal from many different vantage points. If one is fortunate enough to have access to a more powerful graphics workstation, then BILAYER BUILDER can be combined with more versatile molecular modeling programs for visualization and energy calculations.

PROGRAM DESCRIPTION

BILAYER BUILDER is written in ANSI-compatible C so that it can be ported to any platform whose compiler supports the ANSI standard. Currently, BILAYER BUILDER runs on any Macintosh with 1-Mb RAM and a hard disk drive, on Silicon Graphics IRIS and on Sun SPARC workstations running UNIX. As input, the program requires the magnitudes and angles of the crystallographic unit cell basis vectors (see Figure 1) and gives the user a choice of 11 space group symmetries (Figure 2) covering most of the published space groups for lipid crystals, including sterols and fatty acids. We are continuing to update the program with additional space groups, but the program is designed in a modular way such that the end user may add any space group desired. BILAYER BUILDER can be obtained by contacting the authors.

Atomic coordinates are read in from a standard ASCII file that is compatible with the Brookhaven Protein Data Bank (PDB) style format[2] (Table 1). Since most crystal structures are given in fractional coordinates, the program is capable of converting fractional to Cartesian coordinates when required. Following orthogonalization to Carte-

Color Plates for this article are on page 23.

Address reprint requests to Dr. Gaber at the Center for Biomolecular Science and Engineering, Code 6090, Naval Research Laboratory, Washington, DC 20375, USA.

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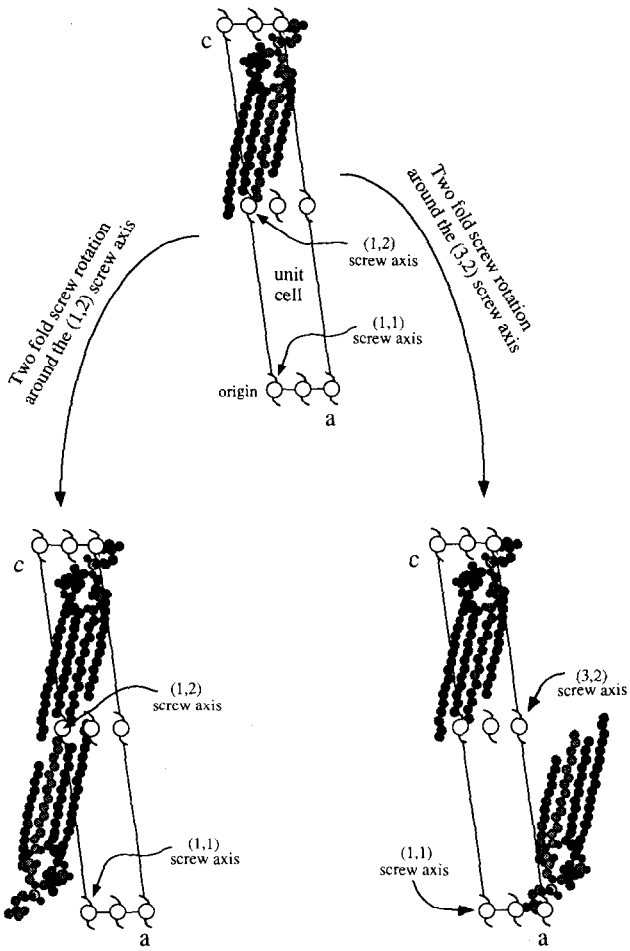

Figure 1. The **a-c** *plane of the $P2_1$ monoclinic unit cell; the* **b***-axis comes directly out of the page. The angles between the three axes are taken to be obtuse by convention and are as follows: α is the angle between* **b** *and* **c**, *β is the angle between* **a** and **c**, *and γ is the angle between* **a** and **b**. *The molecules of DMPC-*A *and -*B *are shown in grey scale. The locations of the twofold screw symmetry axes are noted, with each labeled such that the axis closest to the origin is the (1, 1) axis, and the rest are counted sequentially from that point. This convention is followed for all of the space groups included in BILAYER BUILDER. Consult the* International Tables for Crystallography[3] *for the locations of the rotation and screw axes for each of the different space groups. The figure shows the screw axis process applied to the two DMPC molecules to make the four molecules that can be translated by lattice vectors to fill the true crystallographic unit cell. The twofold screw rotation around the (1, 2) and (3, 2) screw axes is shown for comparison. As noted in the text, all atoms located modulo a lattice translation vector are considered equivalent in the crystal*

```
Enter a, b, c, alpha, beta, gamma: 8.379,8.824,47.683,90,90,90
Choose one of the following space groups:
1 - P1              5 - C2              15 - C2/c
2 - P1(bar)         6 - Pm              19 - P2_1,2_1,2_1
3 - P2              7 - Pc              29 - Pca2_1
4 - P2_1            14 - P2_1/c
19
Enter first screw/rotation coords (S1, S2): -2,1
Enter second screw/rotation axis coords (S3, S4): 1,2
Enter the input file name: ompc.pdb
Enter 'f' for fractional coordinates, default = Cartesian:
Generating the unit cell, please wait...
Enter the output file name: ompc_3x3x3.pdb
Enter the number of replications along the three directions (na,nb,nc):
3,3,3
Replicating.....

Another Crystal (y,n,) ? n
|
```

Figure 2. The input screen for BILAYER BUILDER. The basis vector lengths and angles are given by **a, b, c** *and alpha, beta, and gamma. The space groups are numbered according to the* International Tables for Crystallography *Vol. IV convention.*[3] *The input and output files should be in the current directory or accessed with the proper path name. The user must tell the program whether the atoms are located by fractional or Cartesian coordinates*

sian coordinates, a portion of the crystal structure is generated. There are two parts to this process: completion of the unit cell by operating on the given atomic coordinates with the symmetry operations of the crystal space group; and replication of the completed unit cell. Typical space group symmetry operations include: inversion around a point, mirror reflections, rotations and screw rotations around an axis, and glide plane reflections.[3] These operations are used to transform the minimum data set into a complete unit cell that can be replicated by translations along the basis vectors (Figure 1) to generate the model crystal. Where appropriate, the user is allowed to choose among the different possible rotation or screw axes or inversion centers within the crystal. While each of these axes or centers are equivalent in principle, only one choice will generate the most compact configuration of the molecules. For example, Figure 1 shows the **a-c** plane of a unit cell with $P2_1$ symmetry in relation to the coordinates of the *A* and *B* molecules of the dimyristoyl phosphatidylcholine (DMPC) crystal.[4-6] Since there are four molecules in the proper unit cell, we must do a twofold screw rotation on the molecules to end up with four molecules before replication. The screw axes are labeled such that the (1, 1) axis is located nearest the origin and the others are denoted as shown in the figure, **a** and **c** are the basis vector lengths, **b** is coming out of the page, and β is the angle between the **a**- and **c**-axes in this monoclinic unit cell. This figure shows the process of applying a twofold screw rotation: Each atom is rotated around the axis by π and then translated along the **b**-axis by **b**/2 (out of the page), yielding two additional molecules. Here we should choose to operate around the (1, 2) screw axis because that keeps the four molecules packed tightly together. One can see easily that the choice of screw axis can alter the molecular configuration such that the atoms may be separated by many unit cell basis vectors. For example, doing a screw rotation around the (3, 2) screw axis, the bottom pair of molecules are shifted along the **a** direction by 2**a** (Figure 1). This is not actually a problem, however, because the atomic coordinates are equivalent to those in the unit cell up to a lattice translation

Table 1. PDB format ASCII file giving the Cartesian coordinates with respect to the unit cell origin of the nonhydrogen atoms in OMPC[7]

ATOM	1	C15	OMP	1	1.073	10.205	48.920	1.00	9.10
ATOM	2	N	OMP	1	0.838	9.182	47.823	1.00	5.10
ATOM	3	C14	OMP	1	−0.444	9.490	47.156	1.00	7.10
ATOM	4	C13	OMP	1	1.927	9.287	46.869	1.00	7.80
ATOM	5	C12	OMP	1	0.796	7.832	48.538	1.00	6.40
ATOM	6	C11	OMP	1	0.318	6.677	47.680	1.00	8.10
ATOM	7	O12	OMP	1	1.299	6.297	46.726	1.00	6.50
ATOM	8	P	OMP	1	0.997	4.974	45.868	1.00	6.20
ATOM	9	O13	OMP	1	2.204	4.789	45.010	1.00	8.40
ATOM	10	O14	OMP	1	0.520	3.881	46.726	1.00	7.30
ATOM	11	O11	OMP	1	−0.260	5.398	44.962	1.00	5.00
ATOM	12	C1	OMP	1	−0.008	6.447	43.961	1.00	7.50
ATOM	13	C2	OMP	1	−1.391	6.950	43.532	1.00	7.10
ATOM	14	O21	OMP	1	−1.994	7.612	44.676	1.00	8.20
ATOM	15	C21	OMP	1	−2.941	8.644	44.342	1.00	10.40
ATOM	16	C3	OMP	1	−2.271	5.874	43.007	1.00	6.60
ATOM	17	O31	OMP	1	−3.352	6.500	42.244	1.00	6.90
ATOM	18	C31	OMP	1	−4.332	5.574	41.815	1.00	7.10
ATOM	19	C32	OMP	1	−5.405	6.368	41.148	1.00	7.10
ATOM	20	C33	OMP	1	−6.494	5.486	40.576	1.00	6.50
ATOM	21	C34	OMP	1	−7.626	6.324	39.908	1.00	7.60
ATOM	22	C35	OMP	1	−8.665	5.468	39.288	1.00	7.10
ATOM	23	C36	OMP	1	−9.788	6.297	38.668	1.00	7.50
ATOM	24	C37	OMP	1	−10.886	5.468	38.001	1.00	6.90
ATOM	25	C38	OMP	1	−11.983	6.297	37.333	1.00	7.30
ATOM	26	C39	OMP	1	−13.048	5.468	36.618	1.00	6.30
ATOM	27	C310	OMP	1	−14.145	6.324	36.046	1.00	7.00
ATOM	28	C311	OMP	1	−15.277	5.460	35.426	1.00	6.60
ATOM	29	C312	OMP	1	−16.375	6.297	34.806	1.00	8.80
ATOM	30	C313	OMP	1	−17.380	5.486	34.091	1.00	7.20
ATOM	31	C314	OMP	1	−18.486	6.315	33.471	1.00	8.20
ATOM	32	C315	OMP	1	−19.626	5.460	32.804	1.00	8.20
ATOM	33	C316	OMP	1	−20.749	6.253	32.184	1.00	9.10
ATOM	34	C317	OMP	1	−21.738	5.433	31.564	1.00	8.20
ATOM	35	C318	OMP	1	−22.894	6.253	30.897	1.00	14.00
TER	36								

vector and when replicated enough times wil properly produce the crystal. The optimal choice of screw–rotation axis, therefore, simply reduces the number of replications necessary to produce a recognizable portion of the crystal. Often, referring to the published crystal structure of the molecule of interest will help decide which axis to use.

Figure 2 shows a sample execution of the program, where the user has chosen the $P2_12_12_1$ space group with a screw axis along the **b**-direction (−2, 1) and a screw axis along the **a**-direction (1, 2). As noted above, the axis closest to the origin is described as the (1, 1) axis. The user is asked to choose the number of replications along each unit cell basis vector. The program creates a crystal of these dimensions and writes the structure to an output file in Cartesian coordinates compatible with the PDB file format. One of the significant advantages of BILAYER BUILDER is that it produces a coordinate file in a format compatible with most molecular graphics programs and computers, rather than simply a graphics representation.

SAMPLE APPLICATIONS

BILAYER BUILDER was used to construct two-dimensional slabs of the dimyristoyl phosphatidylcholine (DMPC) crystal structure, and also the 3-octadecyl-2-methyl-D-glycero-1-phosphocholine (OMPC) crystal structure. Figure 1 is the monoclinic unit cell of the DMPC crystal projected onto the **a-c** plane showing four molecules per unit cell, derived from the crystal data of Pearson and Pascher.[4–6] The space group is $P2_1$ with lattice parameters: **a** = 8.72 Å, **b** = 8.92 Å, **c** = 55.40 Å, and β = 97.4°. Here NanoVision is used to display a ball-and-stick model with a grey-scale perspective such that the atoms that appear farthest from the viewer are the darkest. Again note that the unit cell contains two crystallographically distinct molecules, commonly called DMPC-*A* and DMPC-*B*, which yield four molecules per unit cell when the screw axis symmetry is applied. Color Plates 1a and b show some more views of the DMPC crystal created by BILAYER BUILDER and realized with NanoVision. Color Plate 1a shows two monolayers apposed at the headgroups

displayed as balls and sticks, where the balls represent 0.4 of the atoms' van der Waals radii. The magenta balls denote oxygens in the water molecules that are crystallized in the structure. One can use a view such as this to visualize how the lipid head groups hydrogen bond to the water molecules. Finally, Color Plate 1b shows a bilayer containing 25 unit cells, or 100 molecules. This is displayed with a perspective view, in which the atoms become brighter as they appear closer to the viewer. The atoms are represented as balls with their full van der Waals radius. Once again, the magenta balls are the oxygens of the cocrystallized water molecules. A bilayer such as this is an ideal starting data set for a molecular mechanics or dynamics simulation.

The interesting OMPC crystal[7] is shown in Color Plate 2. It crystallizes in an orthorhombic unit cell with space group $P2_12_12_1$ and lattice parameters: **a** = 8.379 Å, **b** = 8.824 Å, and **c** = 47.683 Å. Color Plate 2a shows a ball-and-stick projection of the crystal on the **a-c** plane. The chains are sharply tilted so the crystal shows a herringbone pattern in this plane. Color Plate 2b shows a perspective view in the **b-c** plane, the molecules stitch in and out of the plane. This structure of the crystal is revealed fully only when a sizable portion of the crystal is displayed; it is not evident from the unit cell coordinates alone. The Color Plates cannot convey the true flexibility of the graphical display of the structure: The crystal can be rotated for observation from any perspective the user desires, making it possible to pinpoint the region of the crystal structure of interest.

Finally, one also can generate approximate bilayers (or crystal structures) for molecules that have not yet been crystallized. For example, a chemical-structure drawing program, such as CHEM3D (Cambridge Scientific Computing, Inc.) on the Macintosh, or Quanta (Polygen Corp.) on the IRIS, can be used to create a starting molecular structure, and BILAYER BUILDER then can be used to replicate the structure in some controlled way into a monolayer, bilayer, or an entire three-dimensional pseudocrystal. Color Plate 3 shows a possible bilayer structure for dioleoyl phosphatidylcholine (DOPC), which was created using Quanta. The structure was created by using the crystallographic coordinates of DMPC and altering the hydrocarbons chains to singly unsaturated oleic chains. While the structure is probably not crystallographically correct, one can certainly justify using it as the starting point for a molecular mechanics or dynamics simulation, where the initial phase of the simulation will be relaxation of the hydrocarbon chain conformations.

DISCUSSION

BILAYER BUILDER solves a long-standing problem for noncrystallographers, that is, how a lipid crystal structure, or portion of a crystal structure (such as a bilayer), can be constructed so that it can be observed graphically or used as a starting point for a molecular mechanics or dynamics calculation. By maintaining the information in a generic format, such as PDB, it can be accessed by many different applications. Also, the code itself is easily ported to many different computer systems. In addition, it should be noted that BILAYER BUILDER is not limited to lipid crystals, it can reconstruct any crystal structure whose coordinates are known, as long as the space group has been included in the program. The ability to propose a trial structure and then easily replicate it opens possibilities for simple starting structures for molecular simulations. The docking of small proteins and peptides into a lipid bilayer is an ideal use of this capability. One can make a small patch of bilayer to dock with the peptide and then replicate the proposed structure to an appropriate size for the simulation. In short, BILAYER BUILDER can play a useful role in many different types of research, from simply generating a model bilayer from a known crystal structure, to assisting in the theoretical evaluation of the interactions of small molecules with lipid bilayers.

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