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Stereolithographic models of biopolymers

David Yourtee,* Jim Emery,* Robert E. Smith,* and Byrl Hodgson,†

**School of Pharmacy, University of Missouri–Kansas City, Kansas City, Missouri, USA*
†Design Concepts in 3D, Lee's Summit, Missouri, USA

Stereolithography (STL) has been used to make plastic models of the solvent accessible surfaces of biopolymers. Models have been made of proteins and proteins bound to DNA and RNA. The STL process uses a laser to photopolymerize a liquid resin. Using the ACES (accurate, clear, epoxy, solid) building technique, parts are made with minimum postcure shrinkage. Protein Data Bank files are converted to STL files that represent the surface topology of the biopolymer as a series of triangles and an index that describes their orientation. The models are useful in teaching biomolecular structure and the principle of docking. They are especially useful to the visually impaired. © 2000 by Elsevier Science Inc.

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INTRODUCTION

Stereolithography (STL) is a type of rapid prototyping in which a laser stimulates photopolymerization of a liquid resin, converting it into a solid model. Rapid prototyping is a general term applied to several different methods of making a model in a few hours or days (depending on the complexity of the model). In this type of three-dimensional printing, or STL, data in Protein Data Bank (PDB) format can be converted into a model of the solvent accessible surface of a biopolymer. It is the most accurate form of rapid prototyping, capable of making layers as thin as 0.025 mm. It produces models with the strength of engineered plastics. Models derived from x-ray computer tomography data have been reported to be accurate enough to be used in surgical planning, to make custom implants, and to teach surgical anatomy.[1] Research applications for small STL models have been described by others.[2,3]

The first step in STL is to produce an STL file from a drawing produced by a CAD program such as ProEngineer, or from a PDB file, using the proprietary programs used in this report. The STL file then is sliced and the SLI file is sent to a computer in an STL apparatus. The computer guides a laser, which constructs supports and draws the topology of the object, one layer at a time.

In an SLA 3500 STL apparatus, a solid-state, frequency-tripled, 160 mW Nd:YVO_4 laser initiates the cationic polymerization of an epoxy resin in a vat that is 350 mm × 350 mm × 400 mm. Using the ACES (accurate, clear, epoxy, solid) building technique, parts are made with minimum postcure shrinkage (~0.12%).[4] This is accomplished by nearly complete and uniform photopolymerization. Previous building techniques that required UV and heat postcuring could cause some distortion and internal stress.[5] Postcuring is the process of completing the polymerization that is left incomplete after partial photopolymerization.

The STL models offer a potential for molecular visualization. Unlike computer images, they can stimulate the sense of touch, making them especially useful for the visually impaired.[6] They can be used to visualize the docking of several proteins, which is difficult to do with computer visualization. Some proteins, such as glycophorin, viral coat proteins, and the bacterial protein pilin, exist as homopolymers. Many copies of a biopolymer can be made and docked with each other. If the models dock properly, it adds supporting evidence to the accuracy of the individual model along with the data and software used to produce it.

It is widely accepted that structural classifications of proteins are based on the types and arrangement of a protein's secondary structures. This helps establish the evolutionary relationship between proteins. It has made the so-called ribbon model a popular way of depicting protein structure. However, it is easy for students to think that this is what proteins really look like. In fact, the α-helices and β-sheets only represent the backbone, and not the surface of a protein. Although they reflect the evolutionary relationship of proteins, they give no useful information about how small molecules (ligands and

Color plates for this article are on pages 59–60.

Corresponding author: D. Yourtee, School of Pharmacy, University of Missouri–Kansas City, Kansas City, MO 64108, USA. Tel.: 816-235-1998; fax 816-235-1776.

E-mail address: yourteed@umkc.edu (D. Yourtee)

655 Avenue of the Americas, New York, NY 10010

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substrates) dock to a protein or how proteins dock with each other. To teach docking, the surface topology of the ligand and protein should be shown. This can be done to a limited extent on the flat surface of a textbook or computer screen, but this shows only one view at a time and only stimulates the sense of sight.

The solvent accessible surface is a more realistic way to depict biopolymer structures. It is formed by rolling a water molecule over the surface of the biopolymer. This monolayer of water (the solvent of life) is tightly bound and has different properties from that of bulk water. The calculation of the solvent accessible surface was described by Connolly.[7] It consists of the parts of the van der Waals surfaces that are accessible to the water probe's surface. A network of concave and saddle-shaped surfaces connects it. The continuous surface contour consists of spheres and tori connected smoothly at circular arcs. There are three kinds of pieces: concave spherically shaped triangles, saddle-shaped rectangles, and convex spherical regions. First, a spherical probe approximately the size of a water molecule is placed tangent to every set of three neighboring atoms. A concave triangle is generated wherever the probe sphere has no collisions with other atoms in the biopolymer. Each concave triangle has three concave arcs as edges. Next, saddle-shaped rectangles are formed by connecting adjacent concave arcs along the inner surfaces of tori. The edges of each rectangle consist of a pair of convex arcs. The arcs on each atom are grouped to form closed cycles. The boundary of each convex face is defined by zero, one or two more cycles.

METHODS

The models were fabricated in an SLA 3500 STL apparatus from 3D Systems (Valencia, CA) with Magics RP software from Materialise (Ann Arbor, MI).

The building process started by making a series of supports. They performed the same function as fixtures in conventional machining: holding the object together while it is being built. They held the model up off the building platform, providing a protective layer to prevent the blade from contacting the model while it was scraping off unused resin. They also secured isolated segments that would otherwise just float away. In the past, supports also served to constrain some topology (like free cantilevers) that might undergo distortion as the photoactive resin is polymerized. This distortion, known as curl, was formerly a significant part of error in STL. Very low shrinkage epoxy resins made by Ciba-Geigy and DuPont, and vinyl ether resins made by AlliedSignal have made curl much less of a problem. Curl distortion occurs when one layer undergoes shrinkage before the next layer can be applied. The distortion can be instantaneous or delayed. The ACES build style uses both X and Y hatch to achieve two levels of cure for each layer. The first irradiation accomplishes most of the solidification, without bonding to the previous layer. This almost eliminates instantaneous cure.

A computer guided the automated laser drawing process. The models were made from STL files that represent the surface topology of the biopolymer as a series of triangles and an index that describes their orientation. It was converted into a sliced file. The commands were sent to a pair of orthogonal mirrors, which reflected the laser downward onto a vat of resin. The resin became locally solidified. The details of the photopolymerization were described in numerous texts on polymer chemistry, but one is directly relevant to the STL process.[8] First, the laser beam traced the boundaries of the slice being drawn. Then the appropriate cross-sections are solidified. This step is called hatching.

After the first layer is completed, the platform holding the object was lowered. Liquid resin then flowed over the polymerized layer, and excess resin was removed with a blade that skimmed the top. The platform was positioned one layer over the previous one, and the laser drew the next slice. With the correct exposure, the border and hatch vectors achieved the proper cure depth to ensure that the layers adhere to each other. This process continued layer by layer, without human intervention, until the model had been completed, from bottom to top. After the final layer was completed, the platform was elevated with the solid model emerging from the vat of liquid resin. Excess resin drained back into the vat and the model, with supports, was taken out. Uncured residual resin was cleaned off and the supports removed.

The ACES process cured a layer of resin so that it was almost entirely solidified before it is bonded to the previous layer. Two consecutive sequences of laser irradiation were used. First, the layer being cured was irradiated by a uniform energy, producing a planar cure depth that almost reached the previous layer. Since there was no bonding, the cured resin was free to shrink without causing distortion to the previous layer. It was irradiated with a second, almost identical beam from an UV laser, but we increased the cure depth to uniformly cure the remaining liquid resin and to assure binding to the previous layer.

The photopolymerization is unique to STL. However, building supports and removing them are common to most rapid prototyping techniques.

The STL files were calculated using a Silicon Graphics O2 computer using proprietary programs. No CAD program was used. Calculations started with the Cartesian coordinates of the center of the atoms in the PDB file. Spheres were drawn around them to construct a molecular surface. The sizes of the spheres depended on the type of atom and can be designated by the user.

After the molecular surface file had been written, a solvent accessible surface was calculated. Biopolymers have a tightly bound monolayer of water. Thus, a probe with the radius of water (1.6 Å) was rotated over the molecular surface. To produce a CPK space-filling model, the probe radius was a very low value, such as 0.01 or 0.1 Å. For some protein files, a probe radius of 0.01 sometimes caused errors that disappeared when a radius of 0.1 was used. The spherical probe was rotated around two adjacent spheres, generating a torus. Since a torus is a fourth-degree polynomial, it is called a quartic surface. After the quartic surface was calculated, it was triangulated and a set of vertices and edges of triangles calculated. The final program converted this set into an STL file, in which all the triangles and their normals were properly arranged. The STL files were sliced and made into models.

RESULTS AND DISCUSSION

An STL model of the p53 oncoprotein bound to 21 base pairs of DNA, from PDB file 1tup,[9] is shown in Color Plate 1. The core, DNA-binding region of p53, containing amino acids 94–289, folds into a compact structural domain that contains

the sequence-specific DNA binding activity.[9] The two strands of the DNA double helix are painted red and yellow. The three p53 subunits are painted different shades of blue. A key amino acid, Arg 248, is white. It binds to the minor loop of the DNA and is the most frequently mutated amino acid in human cancers.[10] This model is 30.5 cm in the longest dimension and took about 36 hours to draw in the STL unit. It took about 8 hours to remove the model from the vat, wash it, and remove the supports.

A model of cellulase from PDB file 1cel[11] was made and painted white, as shown in Color Plate 2. This enzyme contains a tunnel into which the cellulose substrate fits. Another model of phenylalanine-tRNA from PDB file 1tra[13] was made and the anticodon region painted blue, as shown in Color Plate 3a. This model, which is 10 cm in the longest dimension, took about 6 hours to draw and 4 hours to wash and remove the supports. In comparison, a space-filling model was generated using the RasMol program *(http://www.umass.edu/microbio/rasmol/)* and is shown in Color Plate 3b.

In a model of acetylcholine esterase from PDB file 1ace,[13] the active site was painted white and the rest of the model blue, as shown in Color Plate 4. The atoms of the acetylcholine substrate were deleted so that the substrate-binding cavity was produced. This model is 12.5 cm in the longest dimension and weighs 306 g. Another useful painting scheme is to paint a protein different colors, based on its structure. As shown in Color Plate 5a, this was done with adenylate kinase (from PDB file 3adk[14]). The α-helical regions are red, the β-sheets are yellow, β-turns are blue, and random coils are white. For comparison, a ribbon model, generated by RasMol, is shown in Color Plate 5b.

All the resin models are solid and inflexible. Flexible models can be made from room temperature vulcanized silicone. Hollow models also have been made.

To see or feel the complete three-dimensional structure, a plastic model has distinct advantages for many people. Computer programs exist to illustrate the docking of a ligand to a protein on a monitor, but this is of little good for the visually impaired. They need a model that they can hold and feel. The difference between a DNA double helix and a protein's α-helix can be discerned through touch. The spatial interrelations between molecules then become evident.

One such STL model was used to make an investment casting and stainless steel model of subtilisin for the "Science in American Life"[15] exhibit in the Smithsonian Museum of American History. It is used to teach the concept of docking. On a computer screen, a visitor can rotate an image of a wire frame model of a hexapeptide substrate and put it into the proper position to dock with an image of the solvent accessible surface of subtilisin. Next to the computer are the stainless steel models of the hexapeptide and subtilisin, so the visitor can hold the wire frame model and fit it into the binding site of the subtilisin.

Computers cannot illustrate the docking of several proteins. Some, like the bacterial protein pilin, assemble into homopolymers. The crystal structure of a single molecule of pilin has been determined. Several copies of CPK space-filling models were made and assembled into models of homopolymers. Models can be made available to anyone. For more information about obtaining a model, contact Byrl Hodgson at *bhodgson@rdtool.com.*

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