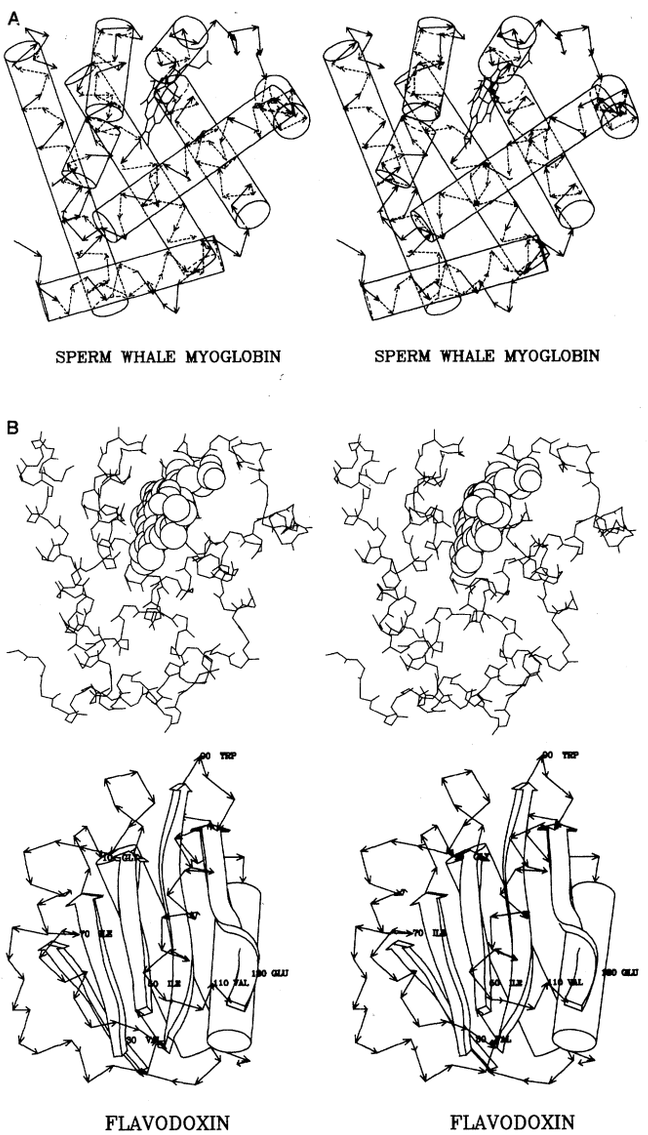
Computer-Generated Schematic Diagrams of Protein Structures

Abstract. *Computer-generated pictures are essential for studying and comparing the structures of proteins that have been solved by x-ray crystallography. Stereoscopic pairs produced by a computer program are particularly useful in providing an intelligible portrayal of the molecular topology.*

One of the most useful representations of protein structures is a schematic diagram in which cylinders represent α-helices and arrows represent β-sheets (*1*). Although other representations—such as pictures simulating wire models or space-filling models—contain more detailed information about atomic positions, the schematic diagrams provide a more perspicuous representation of the topological relationships among elements of secondary structure in proteins. Most diagrams that have appeared in publications were drawn by hand, but computer programs can create similar pictures (*2, 3*).

We extended the state of the art of the automatic generation of schematic diagrams of protein structures by writing a program to produce stereoscopic pairs of diagrams (*4, 5*) (Figs. 1 to 3). Stereo pairs (which are difficult to draw accurately by hand) greatly enhance the viewer's ability to perceive spatial relationships in such complex molecules. "Hidden lines" may be removed entirely, converted to dashed lines, or left unchanged. Strands of β-sheet may be represented by straight arrows or by arrows that curve to follow the twist of the chain (Fig. 2).

These options make the program useful in connection with a variety of computer graphics devices, including color displays (Fig. 3). Removal of a hidden line may not be necessary if the display device simulates real-time rotation.



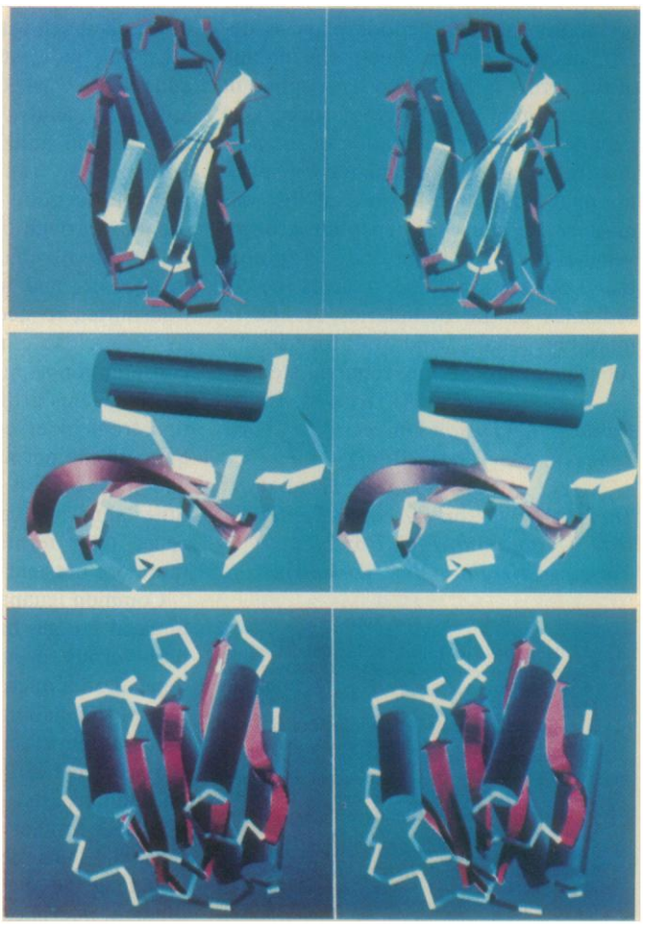


Fig. 1 (top left). Computer-generated drawings of the oxygen-storage protein myoglobin from the sperm whale. The molecule is in an orientation selected by R. E. Dickerson in a much-reproduced illustration (*7*). Although identification or removal of hidden lines informs the eye of the relative depth of occluding segments, stereoscopic rendering is necessary (in a still picture) to define the relative positions of segments that do not lie along the same line of sight. Many combinations of options are available, only some of which are shown. In (A) cylinders represent helices. The entire backbone is traced by linear arrows joining successive α-carbons. Hidden lines are converted to dashed lines. The program generates the labels also. (B) Space-filling representation of the heme group flanked by helices E, F′, and F. Line segments represent chemical bonds between backbone atoms (N—Cα—C—O). Fig. 2 (bottom left). Computer-generated drawing of flavodoxin, an electron-transport protein in certain nitrogen-fixing bacteria. A prosthetic group, flavin mononucleotide, is not shown. The representation of two of the helices are cylinders, and the other two are traced by linear arrows to expose the central β-sheet. The twist in one of the solid arrows that represent strands of sheet occurs at a point where two residues that are not hydrogen-bonded occur in the middle of the strand. The labels, including those of the amino acids, are generated by the program. Fig. 3 (right). The use of color to distinguish different segments of protein molecules. (Top) The double β-sheet structure of the immunoglobulin domain V_L (REI); (middle) bovine pancreatic trypsin inhibitor; and (bottom) flavodoxin (compare Fig. 2).

0036-8075/82/0430-0539$01.00/0

Identification of the regions of secondary structure—and the choice of portions of the protein to be included in the drawing and of the sites to be labeled—are under control of the user through input data.

The program is written entirely in standard FORTRAN IV. The version that produced the line drawings in Figs. 1 and 2 has run on the IBM 370/165 and 168 and on the DEC VAX 11/780. These systems are available to many potential users.

We constructed the program by application of well-known techniques [for example, see (*6*)]. Orientations of α-helices are found by superposition of the backbone coordinates onto a standard helix oriented along the *z*-axis. For ribbon diagrams and strands of β-sheet, the orientation of each peptide plane is determined. For curved sheets, the midline of the arrow is computed by a spline fit to the centers of gravity of successive peptides, and the orientation of the normal to the arrow is calculated by spline fits to the direction cosines of the normals to the successive peptide groups. Whenever the program draws a (potentially) opaque structure, it saves the coordinates of the boundaries of one or more polygonal "windows" for use in a subsequent hidden-line removal step.

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4. The pictures of sperm whale myoglobin in the figures were computed from coordinates supplied by S. E. V. Phillips; pictures of other proteins were computed from coordinates in the Brookhaven National Laboratories protein data bank (*5*).
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8. We thank Douglas Richardson of University College, London, for advice in the early stages of this project. This work was supported in part by grant PCM80-12007 from the National Science Foundation and by the IBM Thomas J. Watson Research Center.
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27 July 1981; revised 28 December 1981

Transfer of Fermentative Microbes Between Generations in a Herbivorous Lizard

Abstract. Iguana iguana *is herbivorous throughout life and utilizes a microbial fermentation system in the elaborated hindgut to break down plant cell walls. Iguanas reared in captivity grew more slowly than wild hatchlings and failed to develop the same complex populations of fermentative microbes. Captive hatchlings fed fresh fecal material from an adult iguana acquired the complex microflora and grew as rapidly as wild hatchlings. In the field, hatchlings actively associated with adults during the first weeks of life and obtained the complex microflora during this time. Acquisition of the fermentative microflora by neonatal iguanas apparently requires direct contact with older conspecifics.*

Most herbivorous mammals satisfy part of their energy requirements through the fermentative activity of specialized bacteria and protozoans in some portion of the digestive tract. These complex populations of microbes break down plant cell wall materials, which are otherwise indigestible to the herbivore, and release wastes, including certain volatile fatty acids, which the herbivore absorbs and utilizes for energy via the Krebs cycle (*1*). Young herbivorous mammals acquire their fermentative microflora through contact with the mother, usually by consuming fecal material. Protozoans and some bacteria can be transmitted to neonatal ruminants only by very close contact, such as by the consumption of feces or rumen eructate (*2*). Neonates artificially reared in isolation from older conspecifics fail to acquire all the normal species of microbes and may exhibit impaired growth and other abnormalities (*2*), presumably because their fermentative capacity and therefore nutrient intake are impaired.

Common iguanas (*Iguana iguana*) are unusual among lizards in that they are herbivorous throughout life (*3*). Iguanas depend on a microbial fermentation system in the elaborated hindgut (*4*) for as much as 30 percent of their daily energy requirements (*5*). Unlike their mammalian counterparts, neonatal iguanas have no direct contact with their mothers and thus lack this obvious mechanism for obtaining the specialized populations of hindgut microbes. In the Gatun Lake area of Panama, female iguanas travel to nesting sites in February, excavate nests, deposit their eggs, and then return to their home ranges, which may be as far as several kilometers from the nesting site (*6*). The hatchlings emerge in May, leave the nest area, and often settle in habitats quite distant from any adult iguanas (*7, 8*). Even in those areas where both adults and young are found, such as the outer edge of the forest around Gatun Lake, iguanas of different ages typically occupy different habitats; adults dwell in the forest canopy (15 to 30 m high) and hatchlings in low vegetation (1 to 4 m). I investigated how iguanas—reptiles that lack parental care—obtain the fermentative microflora, and how important the common microbe populations are for normal growth and utilization of plant food.

During 1979 and 1980, I hatched and reared iguanas in captivity (*9*) and found that they always grew more slowly than hatchlings in the wild. In addition, they failed to develop the same hindgut microflora (*10*). Although it is generally impossible to identify species of bacteria or to infer their biochemical function on the basis of appearance, many protozoans and some bacterial forms are morphologically unique and can be identified by simple microscopic examination. I found two such morphologically distinct microbes only in the hindguts of wild hatchlings. The first, a bacterial complex or sarcina (*Lampropedia merismopedioides*), also occurs in the hindguts of a variety of herbivorous reptiles, as well as in the rumen of cattle and sheep (*2, 11*). The second, a large ciliate protozoan, is probably an undescribed species (*12*), but resembles the holotrichs of the rumen (*2*). The occurrence of the *Lampropedia* and the protozoan exclusively in the hindguts of wild hatchlings suggested that these animals were exposed to additional sources of fermentative microbes, possibly by direct contact with other iguanas.

In 1981, I conducted experiments to locate sources of the morphologically distinctive microbes and to determine, under controlled conditions, whether possession of the more complex microflora was associated with improved growth rate and digestive efficiency. I used the ciliate protozoan as a marker, without assuming any useful function of the microbe itself. Because protozoans have the most stringent requirements for transmission (*2*), conditions that allow passage of the recognizable protozoan between iguanas should also permit transfer of the important, but visually unrecognizable, bacterial species.

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