[24] Schematic Drawings of Protein Structures

By JANE S. RICHARDSON

An accurate stereo figure is certainly essential to reporting a protein structure. It is aimed at that small but important fraction of readers who have the ability to see the stereo and to interpret what they see, and who are sufficiently interested actually to do so.

However, it is also extremely important to provide accessible, if simplified, three-dimensional information (in mono) for the entire audience. A schematic drawing (such as Fig. 1 or 2) can summarize the overall features of a structure in a quickly graspable and relatively memorable form, and can provide a framework within which to place further details. Such a drawing has inherent dangers, of course: by definition any simplification must omit information, and any representation which aids conceptual understanding must involve interpretation and choice (which is equally true of an automated computer drawing). But even if one is led to miss alternative interpretations something worthwhile is gained, because understanding one conceptualization of a structure is much better than not understanding it at all.

It follows that there are two crucial and nontrivial tasks of a schematic drawing. The first is to portray the overall organization of the structure rather than a collection of details; for example, one should try to draw a β-sheet rather than drawing β-strands. Some degree of interpretation must be done before starting to draw, but feedback from the appearance of the drawing can modify one's interpretation. When the structure is successfully perceived as a unified object, then comparisons and symmetry relationships can also be perceived directly rather than laboriously worked out by matching individual features (e.g., the relation between the two subunits in Fig. 1).

The second major task is to communicate accurate three-dimensional information, by utilizing all available monocular depth cues and, where possible, by mimicking the appearance of a binocular image. For abstract and unfamiliar objects such as these proteins, it is necessary to exaggerate the monocular depth cues in order to achieve a realistic perception of the three-dimensional relationships. Helical ribbons must be made curlier, foreshortening exaggerated, and more than the true amount of the side of an arrow shown as it starts to twist. A direct example of compensating for the lack of the second eye is the case of an arrow viewed edge on as it twists around: the most satisfactory convention shows both faces of the

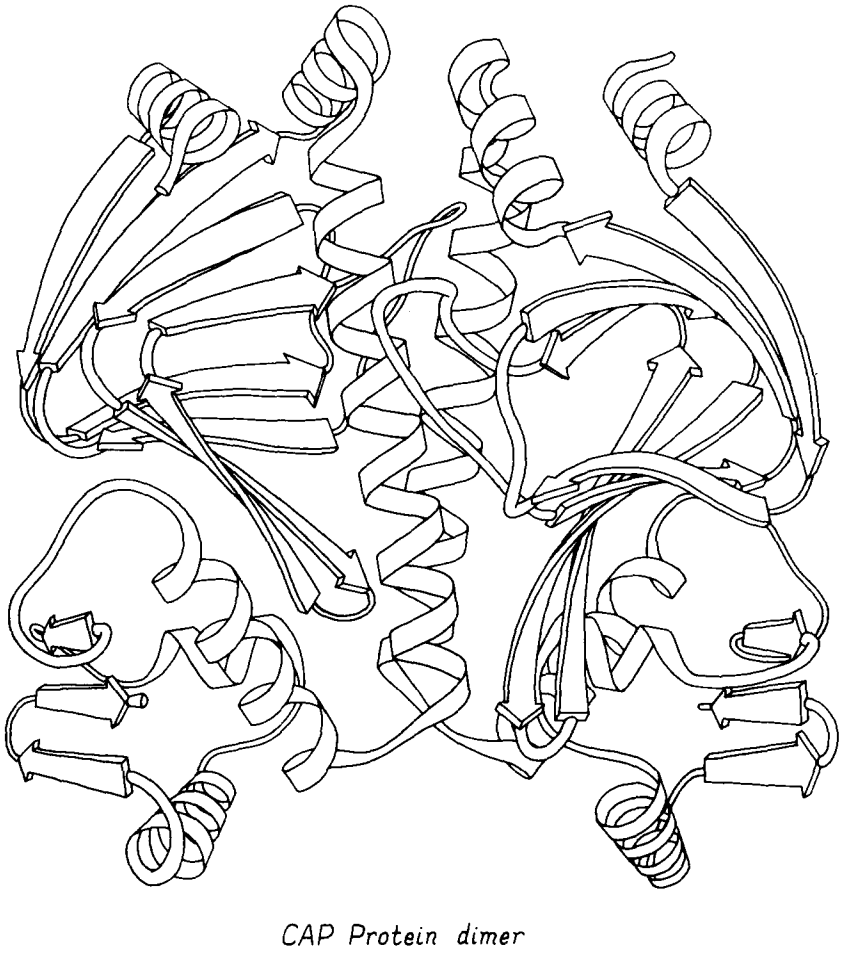

FIG. 1. Schematic drawing of a protein structure, the CAP protein dimer.

arrow as visible around the changeover point (see Fig. 3b), which seems logically peculiar but more nearly matches binocular vision. This can be demonstrated by holding a belt or plastic ruler stretched vertically in front of you with a half twist from top to bottom; look at the crossover point first with just one eye and then with two.

Emphasis on the large-scale features need not compromise accurate positioning. If a fairly favorable view has been chosen, then ambiguities can be resolved and loops smoothed without shifting anything more than 1 or 2 Å. To make a drawing "work" in mono, what needs to be modified is

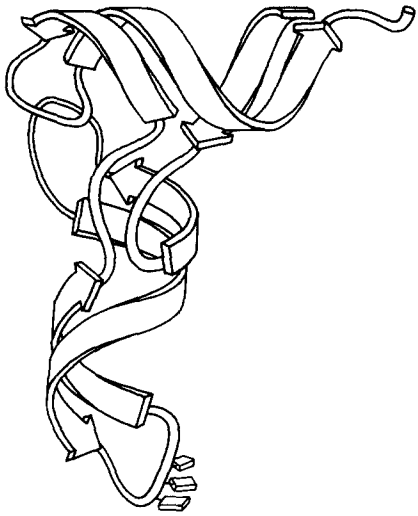

FIG. 2. Yeast $tRNA^{Phe}$: schematic drawing of a nucleic acid structure.

not positions but the local cues to depth and orientation. The overriding criterion for evaluating a schematic drawing is its final overall appearance, judged as a representation of the major patterns and relationships you see in the three-dimensional structure.

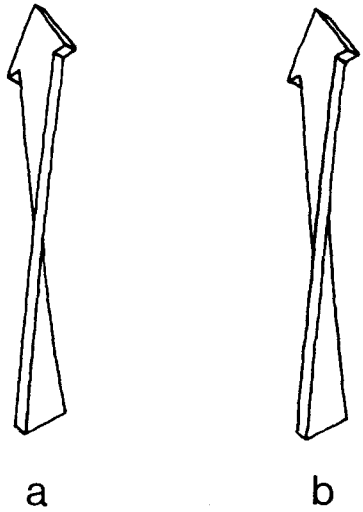

FIG. 3. Comparison of a monocular view (a) of a twisting arrow with a more convincing drawing (b) which imitates a binocular image.

Specific Drawing Methods: Materials and Setup

In this section are provided detailed and explicit instructions for producing schematic drawings of the type shown in Fig. 1 and used for the "mini-atlas" in the previous chapter. There are many different but related types of schematics in the literature, e.g., for myoglobin,[1] carbonic anhydrase,[2] thioredoxin,[3] immunoglobulin,[4] PGM,[5] and tRNA.[6] Along with the specific methods explained here, one could also add or substitute conventions from some of those other representations if they were especially suitable for showing the features of a given structure. For example, in showing large multisubunit structures it is helpful to simplify further to cylindrical helices and entire β-sheets or β-barrels.[7-9]

It is generally desirable to make an original ink drawing two to three times larger than it will be reproduced, so that irregularities in the lines will be less visible. One must then remember to use a wide enough pen for the lines to show well when reduced. The originals for the drawings shown here were made at a scale of approximately 7 Å/inch, and some specific measurements quoted below refer to this scale. The drawings are reproduced at a 3 : 1 reduction in "Advances in Protein Chemistry,"[10] at 2 : 1 in the figures of the previous chapter, and at 1 : 1 in the "Protein Structure Coloring Book."[11] At this scale, a No. 1 size Leroy pen produces a reasonable line width.

Producing the C_α plot to the right scale and from a good viewpoint is a critical step in this process. The best method is to view a backbone model

[1] R. E. Dickerson, *in* "The Proteins" (H. Neurath, ed.), 2nd ed., Vol. 2, p. 634. Academic Press, New York, 1964.

[2] A. Liljas, K. K. Kannan, P.-C. Bergsten, I. Waara, K. Fridborg, B. Strandberg, U. Carlbom, L. Jarup, S. Lovgren, and M. Petef, *Nature (London), New Biol.* **235,** 133 (1972).

[3] A. Holmgren, B.-O. Soderberg, H. Eklund, and C.-I. Branden, *Proc. Natl. Acad. Sci., U.S.A.* **72,** 2307 (1975).

[4] M. Schiffer, R. L. Girling, K. R. Ely, and A. B. Edmundson, *Biochemistry* **12,** 4628 (1973).

[5] J. W. Campbell, H. C. Watson, and G. I. Hodgson, *Nature (London)* **250,** 302 (1974).

[6] S.-H. Kim, F. L. Suddath, G. J. Quigley, A. McPherson, J. L. Sussman, A. Wang, N. C. Seeman, and A. Rich, *Science* **185,** 436 (1974).

[7] G. A. Clegg, R. F. D. Stansfield, P. E. Bourne, and P. M. Harrison, *Nature (London)* **288,** 299 (1980).

[8] R. E. Dickerson and I. Geis, "Structure and Action of Proteins," p. 88. Harper, New York, 1969.

[9] J. S. Richardson, *Nature (London)* **268,** 497 (1977).

[10] J. S. Richardson, *Adv. Protein Chem.* **34,** 181 (1981).

[11] J. S. Richardson, "The Protein Structure Coloring Book." Little River Institute, Bahama, North Carolina, 1979.

on computer graphics: study it well from a variety of directions to decide what features to emphasize, choose a precise viewing direction, and then either obtain plotter output (in stereo) or photograph the screen directly. It is also quite possible to plot C_α coordinates by hand or with a batch computer program, or to photographically enlarge an existing stereo diagram, but this allows less choice, or no choice, of viewpoint. A computer program is now available[12] which produces arrow-and-cylinder drawings in stereo from complete atomic coordinates; it should provide an excellent starting point for making a mono drawing, as well as being very useful in its own right for making stereos.

The following criteria should be considered when choosing an optimal viewing direction:

1. Look through the minimum, or nearly minimum, depth of the structure.
2. Put features of special interest near the front.
3. Do not view either helices or β-strands end on.
4. Minimize the places where features lie behind one another so extensively that their continuity is lost, especially when more than two layers are involved.
5. Favor slight overlaps, which provide valuable hidden-line depth cues.
6. A β-sheet looks best if at least one corner turns over, which helps perception of the shape.
7. If similar structures are to be compared, the viewpoints should be identical, not just similar.
8. For real clarity, it may be worth drawing more than one view (see triose-phosphate isomerase and lactate dehydrogenase in the previous chapter).

If the scale is being adjusted photographically, then either an α-helix or a β-strand lying in the plane of the paper can serve as a standard, using Fig. 4 to adjust the repeat distances. As well as a single C_α plot at standard scale, you need a smaller stereo pair that must be referred to constantly while doing the sketch drawing. This is absolutely essential to producing a mono drawing which accurately represents the three-dimensional relationships.

The first pencil sketch is made on heavy tracing paper taped onto a C_α plot at the correct scale, and the final ink drawing is then traced from the sketch onto translucent Mylar drafting film. Another sheet of tracing paper can be used, but the Mylar does not curl up with heavy ink applica-

[12] A. M. Lesk and K. D. Hardman, *Science* **216**, 539 (1982).

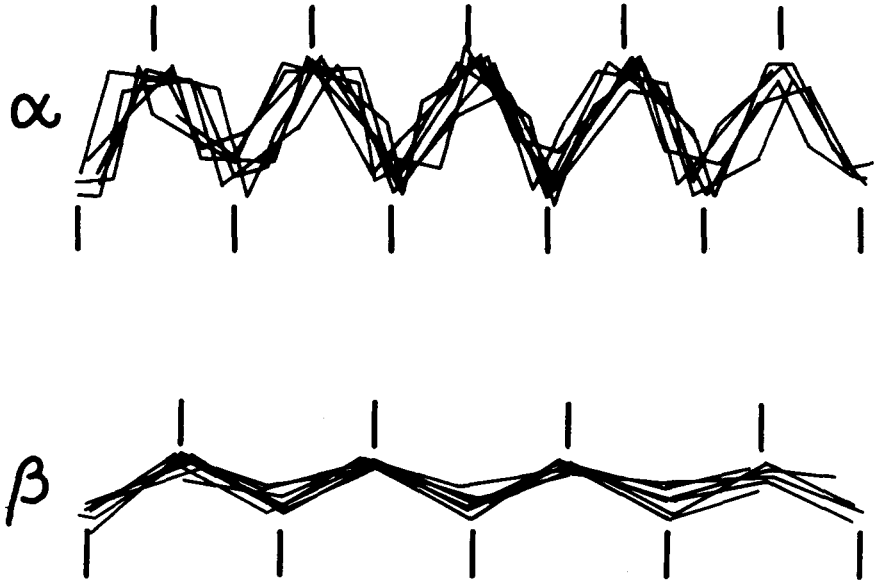

FIG. 4. Standard scale of repeat distances using either (a) an α-helix or (b) a β-strand which lies in the plane of the drawing.

tion, does not become yellow or brittle with age, and takes corrections more easily. Corrections are done by inking in the corrected version and then scraping away the unwanted ink lines with a razor blade or Exacto knife (it is worth changing the blade frequently). Mylar film (usually in large sheets, which can be cut to size), tracing paper, ink, and Leroy or other drafting pens are available at drafting supply stores. It is best to use the heaviest, most expensive tracing paper available, since the lightweight types crumple up. Inks need to be reasonably free flowing in pens, and thus unfortunately none of them are sufficiently opaque black to make high-quality reproduction simple. A fairly acceptable one that is generally available is Higgins "Black Magic."

α-Helices

Helices, in this convention, are shown as spiral ribbons with a cylindrical diameter just a little larger than the C_α positions. Figure 5 lays out the steps in sketching a straight helix lying in the plane of the page. From the stereo, decide where the helix begins and ends. Using a transparent ruler, judge the line of the helix axis and put a tick mark at each end of the axis. Mark a 3/4-in. width at each end perpendicular to the axis, and draw

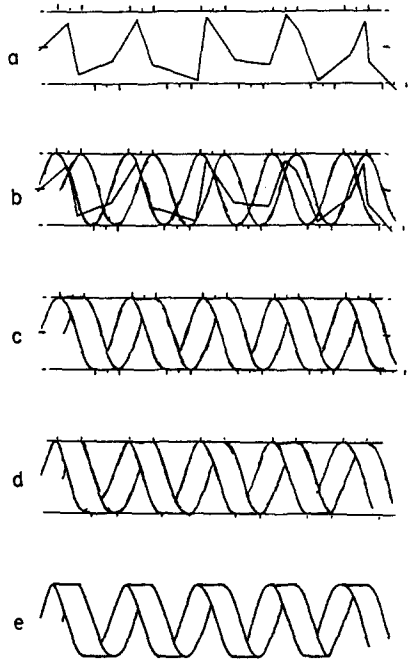

FIG. 5. Successive steps in drawing a spiral ribbon α-helix.

a pair of lines 3/4-in. apart to show the sides of the helix spiral. Along the top line, make a series of equally spaced marks which give the best average locations of the turns in the C_α plot (these marks will be fairly close to 3/4-in. apart). Along the bottom line, put marks centered between the top ones; adjust all of them if necessary. Lay out 1/4 in. surrounding each mark to show where the edges of each turn of the ribbon should fall. You now should have a sketch resembling Fig. 5a. As in Fig. 5b, draw a smooth sine curve joining the left edges of the 1/4-in. marks. Draw a second sine curve through the right edges, keeping the spacing even. Remove hidden lines to produce a right-handed spiral as in Fig. 5c, either by erasing the unwanted lines or by darkening the desired ones. For a helix longer than about three turns, it helps to show that one can see a little farther inside those spiral turns near the ends; this is done by progressively rounding the tops and pointing the bottoms of the sine curves toward the right end and vice versa toward the left, as shown in Fig. 5d. If used, this effect is easiest incorporated between steps b and c. To make the final ink drawing (5e), first use a straightedge to draw the short segments along the sides. Then draw the curves smoothly in freehand, starting at the top and drawing each line in one smooth, slow motion. Tilt the

whole drawing if needed to get a favorable angle for the hand motion (one can draw most smoothly going downward, and from the concave side of a curve). Do the front section of each turn first, and then the back pieces.

In general, I have chosen to show all helices as regular except for bending (see below) and a few cases in which the irregularity is particularly significant (such as the open turn at the active disulfide in glutathione reductase). Varying the size or pitch tends to be confused with depth changes, and apparently irregular helices in initial tracings often become more regular with refinement. However, if you have, for instance, a clear 3_{10} helix, by all means try showing it as such.

Some helices bend significantly and should be shown bent (otherwise the position at an end can easily be off by 5 Å). If several H bonds are missing, a good representation is two separate, straight helices, in which their spiral ribbons meet smoothly (e.g., see the A and B helices of hemoglobin in the "mini-atlas"). If only one H bond is missing (almost always at a proline), the bend can be made by curving the outline for two successive turns, as in the lactate dehydrogenase domain 1 drawing in the previous chapter. Sometimes long helices have a continuous gentle curve, either circular (as in *p*-hydroxybenzoate hydroxylase, see this volume [23]) or spiral (see Figs. 1 and 6). To draw these, simply make a smooth curve along the helix axis with parallel curves 3/8 in. on either side, and proceed as above. If the helix curves in or out of the plane of the paper, then adjust the amount of view inside each turn according to the appropriate local angle (as in the long helices of Fig. 6). The lines forming the top and bottom of each turn should be straight, but tangent to the curve. Curved helices would probably be difficult to show convincingly at highly oblique angles.

To draw straight helices at oblique angles to the line of view, the procedure is very similar to Fig. 5, except that the outer guide lines should taper to show foreshortening and the ribbon edges are smoothly looping curves rather than sine curves. The "curliness" of the curves needs to be shown a bit greater than it would really be in a helix at the desired apparent angle, and the curve is more open toward the back because the visual angle is steeper at the back than the front if the object is not infinitely distant. Figure 7 provides a gallery of helix examples at varying apparent angles. Fairly good results can be obtained by simply tracing the example nearest to the appropriate angle. Directly end-on helices should be avoided if at all possible; the nearest acceptable version I have managed attains an apparent angle of 75–80° by using a "binocular" distortion. In general, the lengths and relationships of helices are perceived best when they are at low angles to the plane of the paper.

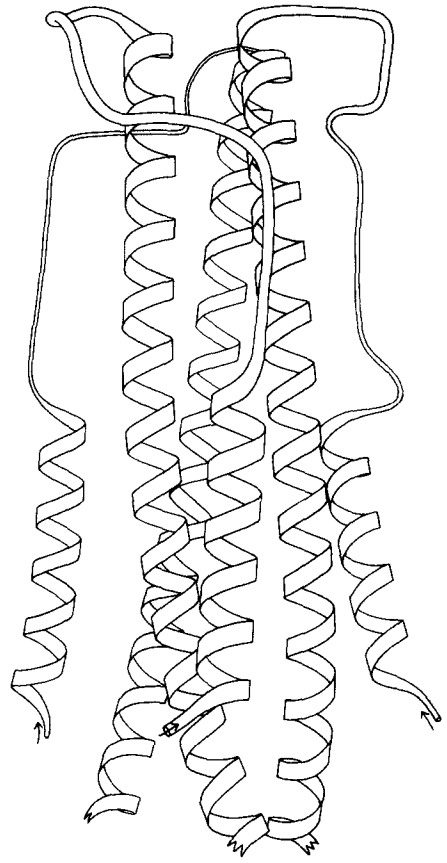

FIG. 6. Long, curved α-helices which spiral around a 3-fold axis (influenza virus hemagglutin HA2).

β-Sheet

In this style of representation, β-strands are shown as arrows with thickness (about one-quarter as thick as they are wide), which gives valuable cues to orientation and twist of the strand. For helices the ribbons are drawn without thickness, since there are already sufficient cues to orientation and the picture would be confusingly complex with those extra lines. The loops, in contrast, are drawn as round "ropes," because none of the simple conventions for showing peptide plane orientation in nonrepetitive structure correspond to meaningful structural features (for helices and β-strands, the ribbon plane really represents the hydrogen bonds

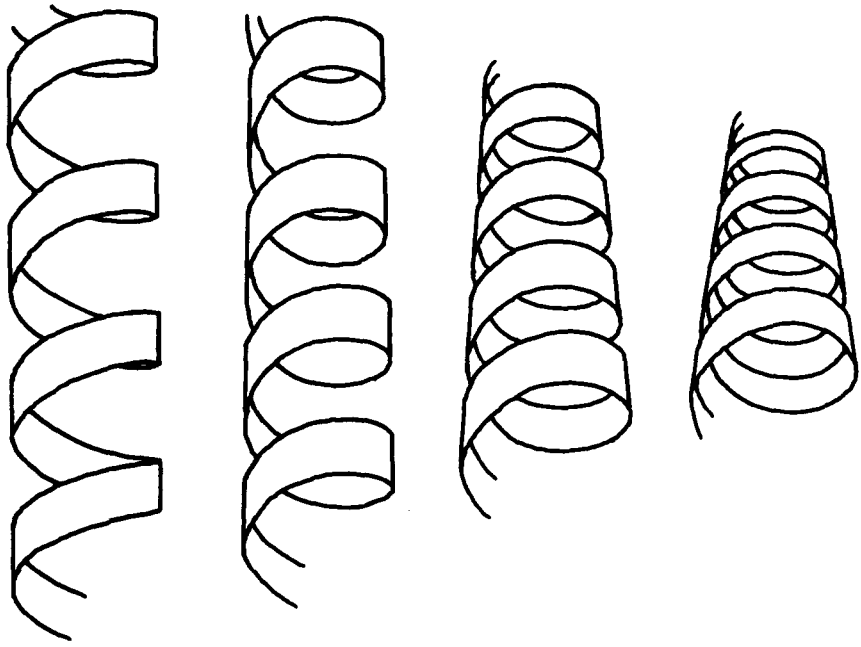

FIG. 7. A collection of α-helices at varying angles to the page.

rather than the peptide direction, since successive peptides flip by 180° in a β-strand but not in a helix). This inconsistent set of conventions surprisingly produces a visually acceptable, unified appearance, and seems to work better than forcing a single convention which is bad for some of the structural features.

The β-strand arrows are about half the width of the strand-to-strand separation. This is a compromise: if the arrows are much wider it is difficult to see features behind them, and if they are narrower there is not enough visual continuity between H-bonded strands. That continuity, which is mainly a matter of making the adjacent strands locally coplanar, is the single most crucial factor in drawing comprehensible β-sheets. The local plane of an arrow (perpendicular to its length) is determined by the average direction to its two H-bonded neighbors or by the direction between it and a single H-bonded neighbor. If it makes no H bonds for a gap of more than two residues, or more than one at an end, then it is no longer shown by an arrow. β-Strands are very seldom completely flat for their entire length, and the correlation of twist and bend from one strand to the next is a powerful signal to perceive them as part of a unified structure.

Since this representation emphasizes the hydrogen-bonding relationships, the extent of a strand (or arrow) can be defined from explicit

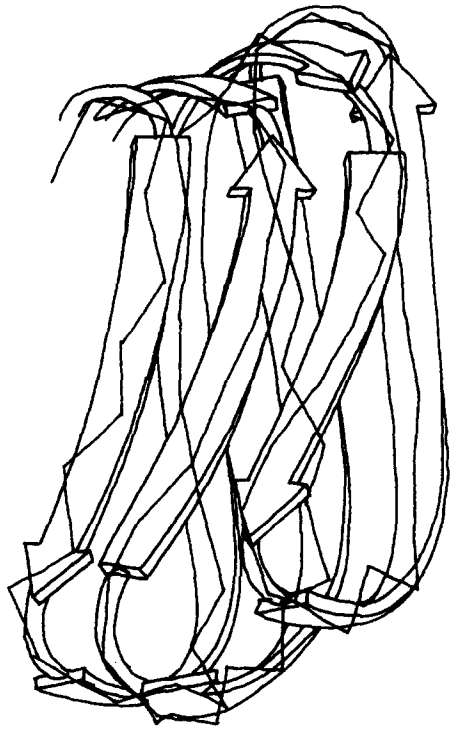

FIG. 8. Sketch of β-strand arrows superimposed on the α-carbon backbone.

H-bonding information if it is available. Otherwise it is done by careful evaluation (in stereo) of which regions maintain appropriate strand-to-strand separation and direction, including consideration of whether the backbone "pleats" of neighboring strands are correlated. I prefer to think of β-strands as continuing through β bulges[13] and other local irregularities, and to be rather generous at the ends or edges of large sheets even if the strand may be separated by a bit more than a good H-bond distance (this must especially be considered when working from a list of H bonds, which are sometimes specified by quite strict criteria). When you have decided where the β-strands are, mark the beginning and end of each on the C_α plot. One should also decide in which cases H bonding is lost on one side before the end of the strand.

Figure 8 shows a sketch of β arrows superimposed on the C_α plot. First identify the NH_2 and COOH termini of the protein and follow

[13] J. S. Richardson, E. D. Getzoff, and D. C. Richardson, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2574 (1978).

through the entire chain in the stereo, drawing an arrowhead on the C_α plot at the end of each β-strand. Where the plane of the entire β-sheet is closer than about 45° to the plane of the paper, lightly sketch the two edges of each strand about one-quarter of the way from its midline to its neighbors (so the strand and the gaps are about the same width). Where the plane is more tilted, the controlling factor is determining which strands turn over from this viewpoint, and where. This must be done by following the orientation of the strand and its neighbors in stereo. If a strand begins or ends at right angles to the viewer, then turn the end of it slightly one way or the other (it is usually better to let it turn over if it almost does). This is another case in which avoidance of confusing special positions actually mimics the real binocular image, which is dominated by whichever eye has the more informative view and therefore never appears exactly edge on.

Draw the double line for the front edge of the arrow as it crosses over, and then add the line for the lower edge coming in on either side. Be sure to make both sides visible in a region around the crossover (as in Fig. 3b). Taper the arrow width smoothly in between crossover points and full-width flat regions. In general, arrow width is mainly an orientation cue, and is used for foreshortening only at extreme angles (such as the end-on view of the triose-phosphate isomerase barrel in the previous chapter). Smoothly taper the width of arrow edge visible, always making at least a narrow band show on one side or the other.

Sketch in the angle forming the tip of the arrowhead; it should be almost 90° if the arrow is lying flat, obtuse if you are seeing that arrow more nearly from an end, and acute if from one side. The very tip should lie exactly on the smoothly curving continuation of the arrow midline. Then sketch the line of the arrowhead base and the line across the tail end of the arrow; visualize them as lying in the local H-bond plane of the sheet and perpendicular to the local axis of the arrow. Alter those angles by trial and error until they all look correct and give a consistent idea of the orientation of the sheet. Add the double lines and corner shapes for the edges of the arrowheads and tails. Again, either the top or the bottom of each arrowhead should be made visible. It is good to use a straightedge for drawing the arrowhead and tail (at least on the final ink drawing), but it is better to draw the rest of the arrow freehand, because it almost always has at least a slight curve.

The prealbumin dimer of Fig. 9 includes examples of β-strand arrows with a variety of orientations and twists, and shows how they visually form sheets. Examples of more cylindrical barrels can be seen for triose-phosphate isomerase, catalase, or pyruvate kinase in the previous chap-

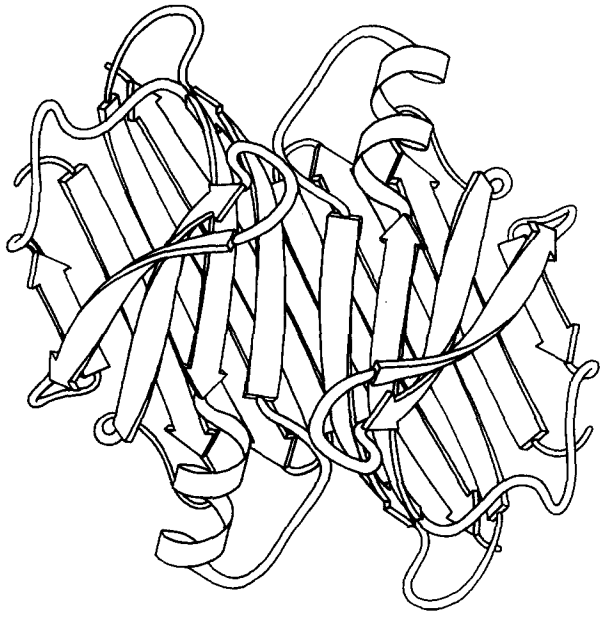

FIG. 9. Prealbumin dimer. β-Strands cross diagonally under other strands; the edges must be offset in order to appear continuous and straight. Front sheet illustrates the feeling of depth produced when a sheet turns over at the corners.

ter, plus examples of twisted sheets viewed edge on in the doubly wound α/β category.

When drawing double sheets that cross at shallow angles as in prealbumin, one should allow for another side effect of binocular vision that produces a perceptual illusion of offset in the back lines. In ordinary perception of such a structure in three dimensions, the two eyes would see slightly around behind the edges of the front arrows, so that in a binocular image the edges of the back arrows would appear offset outward where they emerge from under the top ones. In a mono drawing those back lines must be slightly offset in order to appear straight and continuous; this was done in the prealbumin figure, as can be verified by viewing the page at a glancing angle. Figure 10 shows the appearance of straight and optimally offset lines, and a superposition of the two to show how to achieve the correct offset. Unfortunately, a greater offset in mono merely looks wrong rather than increasing the apparent depth; probably that is because one sees a fused binocular image only for relatively mild

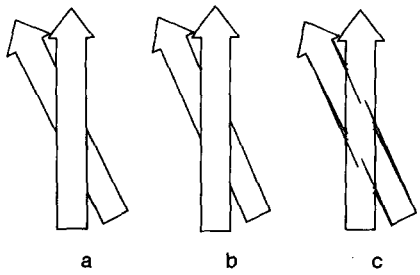

FIG. 10. Offset of rear β-strand necessary to appear straight. (a) Rear arrow edges exactly straight, but appear offset because of optical illusion; (b) edges offset enough to appear straight; (c) superposition of (a) and (b) with offset marked.

offsets and a double image for more extreme ones. Figure 10a is a classic example of the "Poggendorf illusion," which has been known to perceptual psychology since before 1860[14] but has not traditionally been explained in the manner treated here.

Loops and Miscellaneous

The nonrepetitive loops are shown as round "ropes," made fatter in the foreground and thinner toward the back. Sometimes the taper is locally exaggerated to clarify the orientation of an individual loop. In general these loops are simple to draw, with only two factors needing care: smoothing, and treatment of places where they disappear behind other pieces of structure.

A great deal of simplification is necessary to produce a comprehensible schematic in mono: we have already omitted the side chains and the "pleat" of the β-strands, and smoothing of the loops is another important part of that process. If the detailed zigzags are left in the loops, one loses two essential features of a schematic: the ability to unambiguously follow the backbone from one end to the other, and the perception of the structure as a unified whole. Figure 11 shows a C_α plot and the smoothed loops as sketched on top of it. The position drawn for a point on the loop depends mainly on the nearest C_α, but also to some extent on its neighbors up to two away in each direction along the chain. (This is also true of the midline and the orientation of the β-strand arrows.)

The smoothing is critical for unambiguous perception of the continuity of a loop where it passes behind another piece of chain. The loop should be straight or have a gentle, continuous curve that is readily unified by the

[14] R. L. Gregory, "Eye and Brain," 2nd ed. McGraw Hill, New York, 1973.

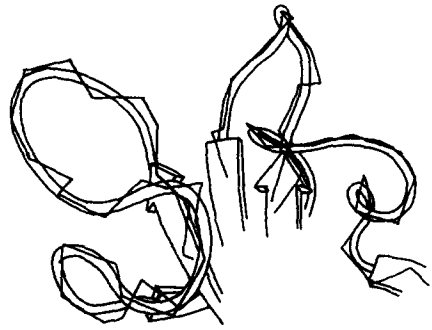

FIG. 11. Sketch of loops superimposed on α-carbon backbone, to illustrate smoothing.

eye. If the angle is oblique, that may require a slight offset (as shown in Fig. 10) in order to *appear* straight. If three chains cross in one spot, a very slight offset of two of them in opposite directions can make the figure unambiguous. Where a loop curls over on itself, the set of representations shown in Fig. 12 all provide a clear visualization of orientation and handedness.

The junctions between loops and helices are simply smooth tapers from one to the other (see Fig. 13 and others); they can be thought of as though the round rope gradually flattened out into the thin helical ribbon. Junctions between loops and arrows are shown best in Figs. 13 and 15. Be sure not to cover very much of the tip of an arrow or the shape becomes confusing.

Other Features

Building on the basic drawing described above, there are a number of possible additions and embellishments. It can be very useful to add selected side chains (e.g., Fig. 14), as long as the total drawing is still kept fairly simple.

The NH_2 and COOH termini must be identifiable. In proteins with a β-strand near one of the termini the directionality of the arrow is sufficient.

FIG. 12. Ways of showing loop ends that curl over by varying amounts.

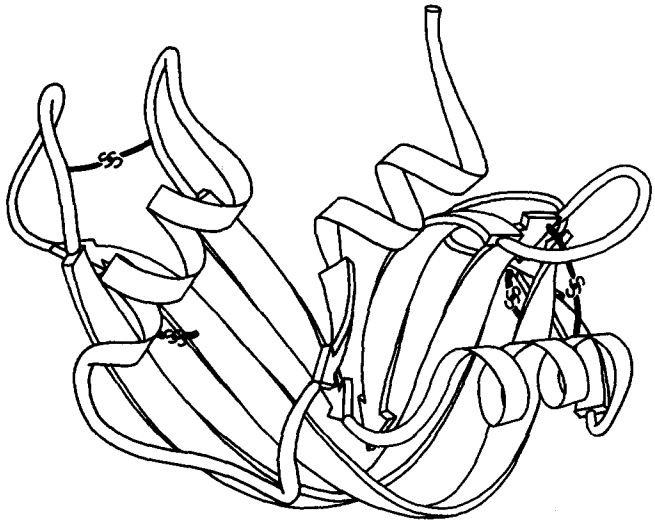

FIG. 13. Ribonuclease A, illustrating a variety of junctions between loops and helices and between loops and β-strand arrows.

In other cases, one or both of the termini can be identified either by small arrows (see Fig. 15) or by letters. For disulfides, I have used either an interlocked SS symbol (Fig. 13) or else a zigzag rather like a conventionalized lightning stroke (see Fig. 14a here and Fig. 16 of previous chapter).

Prosthetic groups or inhibitors can be shown as stick figures (e.g., lactate dehydrogenase domain 1 in the previous chapter) and metals as spheres, either drawn as a single circle or with a couple of partial circles on the lower right to add depth (see Fig. 15). An ordinary drafting compass works well for circles down to about 3/8 in. diameter, but for smaller circles (down to about 1/16 in.) a type of compass called a drop bow pen is extremely useful.

After making a complete sketch of a structure, one should carefully compare again with the stereo to check for errors, especially handedness and whether the correct one is in front where two parts of the chain cross.

If the drawing is to be photographed, it is important to examine it (preferably under a low-power microscope) for line width and blackness. Remember that the eye is very good at compensating for local variations in contrast but the camera is not. Retouch where necessary, using a narrower pen for control, but do not rework the lines too extensively because if the ink is built up thickly or is deeply scratched, that can produce reflections that degrade its blackness when illuminated for photography.

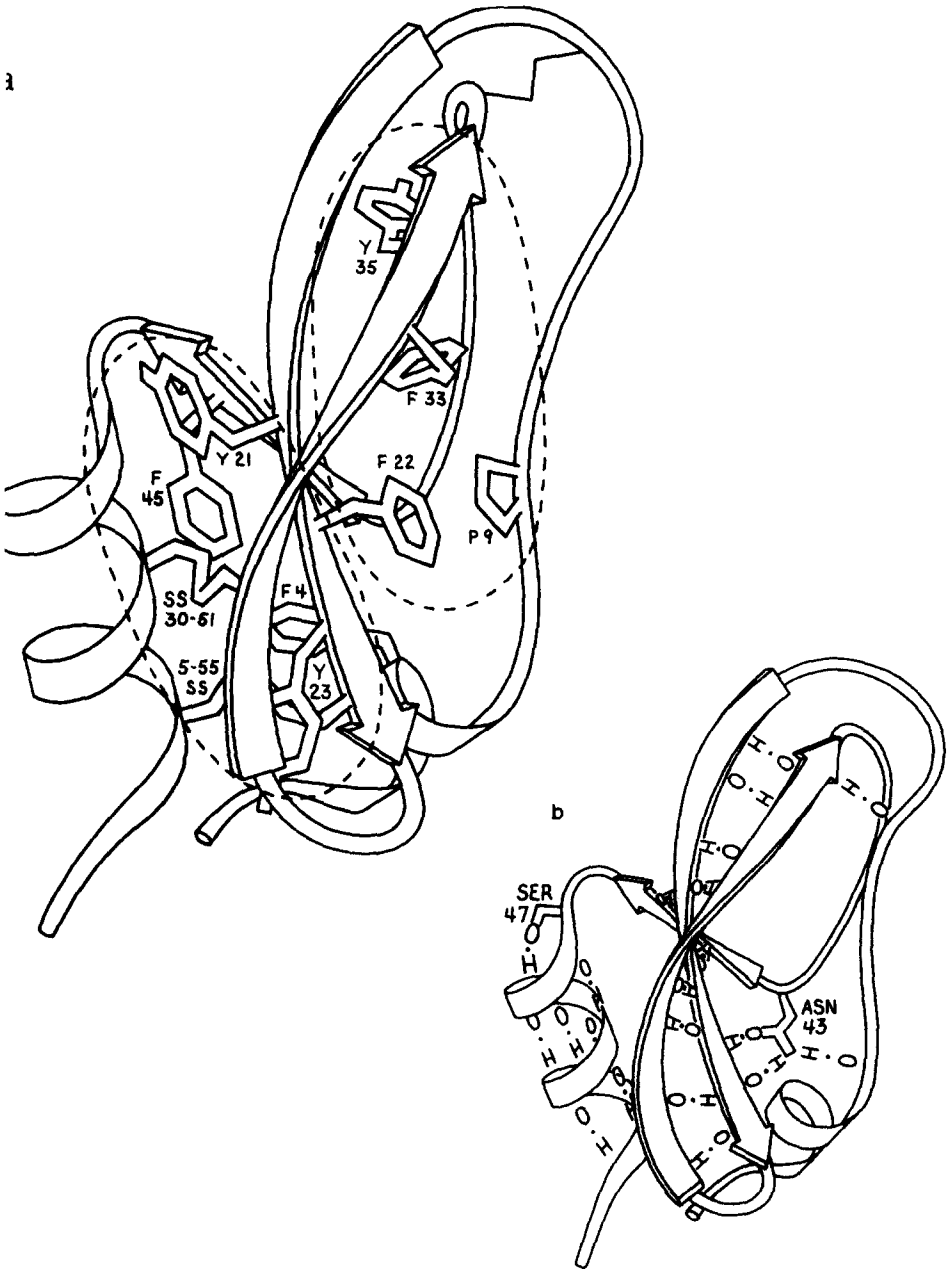

FIG. 14. (a) One way of adding selected side chains to a schematic drawing: the hydrophobic clusters in pancreatic trypsin inhibitor. (b) Pancreatic trypsin inhibitor with its major hydrogen bonds illustrated. The view point had to be slightly different from (a).

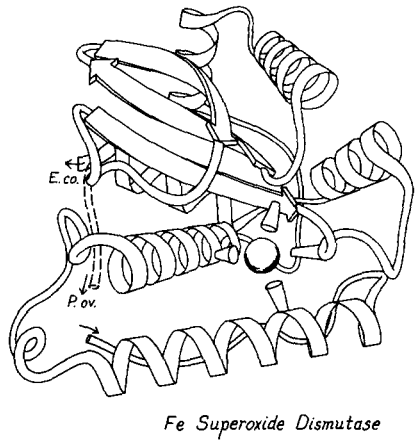

FIG. 15. Ways of shading a sphere, indicating unspecified metal ligands, chain termini, and alternate conformations (Fe superoxide dismutase).

Shading and Color

Shading helps greatly in conveying a feeling of three-dimensionality; the only problem is that it often does not reproduce well. For ordinary line reproduction (as opposed to half-tone), the shading must consist of fine black lines or dots whose size and spacing determine the shade. Small areas can be done by hand, such as the edge of the metal sphere in Fig. 15, but if an entire schematic is to be shaded one should use stick-on plastic film with preprinted dot patterns, which is a standard commercial art material. The suitable number of dots per inch depends on how much the original is to be reduced; for the 7 Å/inch scale it is reasonable to use film with 30 dots/inch (as in Fig. 16). The darkness of the shade is determined by the size of the dots, expressed in percentage area of black, usually available in 10% increments from 10 to about 70% (darker ones would have such tiny clear areas that they would inevitably be lost in reproduction). Unfortunately, most available brands have dark gray dots on a light gray background rather than black on white, and often a "50%" may actually be lighter than a "40%." The only acceptable brand we have discovered so far is Formatt (see notes at end for mail-order sources if you cannot find a good brand locally).

These films are used by cutting out an oversized area with an Exacto knife or Olfa cutter, peeling it off its backing, sticking it over the part of the drawing to be shaded, carefully cutting along the outline desired, and

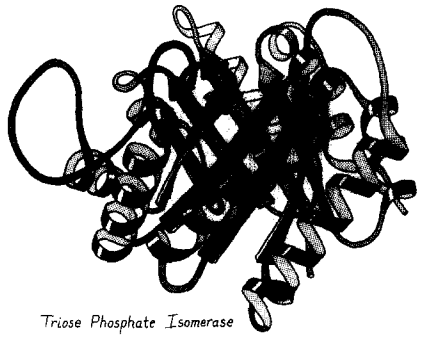

FIG. 16. Triose-phosphate isomerase, with black-and-white shading to show depth, and highlights on the α-helices.

peeling away the excess from around the edges. Light shadings should be cut along the inside of the ink line (since if left across it they may make it a lighter gray), while dark shades should be cut near the outside edge, both to avoid leaving a thin line of white and also because some of the ink may peel off with the film.

When using any sort of shading or color, one has the opportunity to show highlights, which add considerably to the three-dimensionality and appeal of the schematic. The simplest highlights to make, and probably the most effective, are those on the outside of the helices (see Fig. 16). The highlight is a narrow strip of brightness slightly high and/or to the left of the helix midline. For dot or color overlay films, use a straightedge to guide a pair of parallel cuts, and peel off the highlight strips. Exact distance in from the edge is not very critical, but it is important that it be accurately parallel to the edge of the helix (or at the correct intermediate angle if the helix tapers) and that it line up correctly from one turn to the next. If coloring with markers, make the highlight edges accurate in average position and direction, but irregular.

Figure 16 shows a dot-shaded schematic carefully produced with high-quality dot film, retouched, and reproduced from a 1 : 1 Kodalith 4 × 5 negative so that only a single reproduction step is involved. Of course, I do not know in advance whether it is an example of successful or unsuccessful reproduction; however, in the best cases the shading can be extremely effective, especially for the more complicated multilayer structures. For journal reproduction it is probably safer to stay with unshaded, simple-line schematics, but for making slides, which is an inherently simpler process over which you can have direct control (see below), shading is definitely worthwhile. The most effective simple system of shading involves making features in front the darkest, gradually becoming lighter

(and lower contrast) toward the back. This presumably works because it emphasizes what you would see best and it suggests the fade-out into the misty distance that one sees, for example, in mountain landscapes. Since there are not enough different shading levels available to differentiate everything, a reasonable compromise is to use 10% for the back (inner) side of all helices.

Even more information can be conveyed with the use of color, while the corresponding difficulties and expense of reproduction are even greater. The simplest method is to photocopy your original, then use markers or colored pencils to color the copy, and take a slide of it. Colored pencils are very easy to control and shade but tend to give pale results. Markers provide striking colors, but they fade after several months. They also should be tested to find types and colors that blend fairly smoothly between new and dry ink.

Stick-on plastic film is also made in colors (called color overlay film), and produces very uniform, brilliant colors. It is best used directly on the ink original, since it lifts off the black lines on copies. Some companies (e.g., Pantone) make varying percentage shades of a single color (produced with an extremely fine dot screen, usually in 20% increments) with which one can make shaded color drawings.

If a drawing is to be done in color for publication, the best system is to use what are called "color separations." Using a special dark orange plastic film on a clear plastic background (or black ink on paper for pure line work), make a separate positive for each shade of each color. A set of three registration marks (available as stick-on cross hair symbols) is exactly lined up on the original and each separation. Tape the orange film (on its backing) over the original, cut around the relevant edges, and peel off the orange film everywhere except where that particular color shade is to be. The printer will then make one master negative for each color, by combining the separations for all shades of that color. This process is time-consuming and expensive, but it does produce excellent results as long as the registration of the different colors is done carefully in the final printing step.

Reproduction

Many of the steps described here involve photography, and in a very real sense such a drawing has not been finished until it is translated into a reproducible medium. Some of this can be done by handing it to a professional photographer, but it is faster and can produce much more reliably high-quality results if you do it yourself.

The main criteria for both color and black-and-white photography are sharp line reproduction and very high contrast (so that the background is completely clear and the lines are really black or the colors really saturated). This cannot be achieved with normal snapshot films and ordinary development. If you are in a hurry (as one often is!) you can photograph a color original in *bright* sunlight with Kodachrome or illuminate it on a copy stand and use tungsten-light Ektachrome film. Best exposures will be about two stops more open than the meter reading (because of the large white spaces), but be sure to bracket them by at least a stop on each side, in half-stop increments. The results will be worth showing, but the colors will be a bit pale and the background usually slightly tinted or, at best, light grey. Slide films are usually balanced to come out very slightly yellowish, so one can use color-compensating filters to obtain a neutral background. The exact balance varies with the batch of film, but for Ektachrome 50 we have used something between 5C 5M and at most 20C 20M.

The only system I know of for achieving really high-contrast color slides was worked out by David Richardson. It uses Kodak photomicrography film (type 2483), which requires a strong green filter (about 90C 50Y) to produce true colors but is inherently highly saturated and has a clear base. It is developed in E-4 chemistry with doubled times for both the first and color developers, and fresh chemicals for each run. Unfortunately, he has not found an E-6 process film that will give a really clear background even with push-processing.

For any copy photography, but especially for color, evenness of illumination is very critical. It cannot be judged by eye, but should be checked out with a light meter to be within one-half stop over the entire area the original will occupy. For large originals, this usually requires four lights. They must be set so that no reflective highlights are visible through the camera, since they will locally wash out the image. The backing behind the original should be a bright white, and if the drawing has any tendency to curl it should be taped down.

For good black-and-white slides or prints, probably the simplest system to handle is Kodak high-contrast copy film (type 5069) developed in D-19. It seems to be identically replaceable with the 35-mm movie film type 5369. Both are very high contrast, fine grain, and have a completely clear base. Correct exposure for the initial negative will be two to three stops more open than the meter reading. When choosing the optimum exposure, examine the negatives under magnification to look for narrowing or unevenness of the (white) ink lines or a brownness of narrow black areas if exposure is too long, and for spreading of the white areas or a gray

background if exposure is too short. A low-power microscope is also useful for judging color slides or black-and-white positives, but they should also always be projected.

To make a positive slide from a 35-mm negative it is useful to have a slide copier with bellows, to adjust magnification; a light box makes a reasonable light source. Exposure latitude is wide enough with high-contrast copy film so that once you have worked out the correct *f*-stop and exposure for your setup, you should not need to bracket exposures each time.

Another useful black-and-white film is Kodalith, either for 35-mm negatives or positives, or for 4 × 5 high-quality negatives for publication. The exposure latitude for producing a good negative is much narrower than for copy film, but once achieved it will produce good prints over a very broad range since the blacks are totally opaque. They may, however, show tiny clear pinhole defects which need to be blacked out on the negative.

Sources of Materials

For graphics supplies, including Formatt dot screen overlays, Pantone color overlay films both solids and % shades, Amberlith masking film (for color separations), stick-on register marks, Olfa or HT cutters with snap-off blades, etc.:

Graphic Supplies and Services
2166 Faulkner Road, N.E.
Atlanta, Georgia 30324

Charrette Corporation
31 Olympia Avenue
Woburn, Massachusetts 01801

A. I. Friedman
25 West 45th Street
New York, New York 10036

For Leroy lettering pens, high-quality tracing paper, drop bow pens, etc.:

Keuffel and Esser Co.
Morristown, New Jersey 07960