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Minimization of Construction Errors in Bent-Wire Protein Models

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Synopsis

Two kinds of errors are found in protein models made with the tool of Rubin and Richardson. Global errors result from the accumulation of many errors too small to localize, while local errors are assignable to particular bends in the model. We here locate the sources of local errors, and show how to minimize both kinds of errors.

INTRODUCTION

The wire-bender of Rubin and Richardson[1] is a convenient tool for constructing α-carbon models of proteins. It has been used in one of our laboratories for building protein backbone models which helped us establish the existence of continuous regions of peptide chain in globular proteins.[2] With this device it is feasible to develop a scale model from a continuous length of wire at a rate of approximately 25 residues per hour.

The original proposal[1] for this means of model building made no claims for accuracy in the resulting models. On the other hand, there is a general need for inexpensive high-fidelity models for various protein structural studies. We therefore undertook an investigation of the sources of error in model construction.

Error can be divided into two categories: local and global. Local errors are those that can be identified with a given bend, while global errors are the cumulative result of many small unidentified local errors. In addition to those mentioned in the original publication[1] including protractor-reading errors, clamp slippage, and the like, we find three additional sources of local error, all of which can be reduced to acceptable levels. These occur because: (1) The bend angle obtained in a piece of wire can differ from the nominal angle read from the bend protractor by as much as 5°. (2) The bending process stretches the wire, the extent of elongation increasing with bend angle. (3) The bending program may call for part of the already bent wire to occupy some of the same space as the bending tool.

The accuracy of backbone models suffers from the accumulation of small errors at each bend. It is important to recognize, however, that the fidelity of a model is degraded not by small local errors *per se*, but rather by their cumulative global effect. Even when the completed model is adjusted by visual comparison with the stereoscopic projections, global misalignment cannot be systematically eliminated. In consequence, we have undertaken the analysis and correction of model errors from both the local and the global point of view.

The global procedure described here provides a simple way to minimize the cumulative effect of local errors. In brief, a set of fiducial points is identified, and the completed model is fastened at these loci. When carefully selected, only a small number of such points is required to stabilize the structure, and the fiducial adjustment can be made with little further effort. In addition, an empirical evaluation was conducted to determine local error contribution from the three sources listed above. That analysis is described together with appropriate local error minimization procedures.

GLOBAL ADJUSTMENT

The accuracy of backbone models produced by the wire-bender suffers from the accumulation of small errors at each bend. One policy for minimizing errors of this kind is to increase the scale, but large models tend to easily deform under their own weight.

The adjustment method considered here consists of attaching the molecule to a set of measured vertical rods that are rigidly positioned in a chosen base plane. The attachment need not be a permanent one. Figure 1 shows a lysozyme model, scaled to 1 cm/Å, permanently attached to its fiducial supports. The model at this scale lacks stability without external support.

Following bending, a set of fiducial points is selected by visual inspection. Likely candidates are chosen from active site and half-cystine residues as well as from the ends of helixes, β-pleated sheet, and other extended stretches. In the event that the model is to be permanently anchored, the desired viewing orientation should be taken into account to avoid unnecessary obstructions.

An acceptable base plane can be determined from any three residues that lie in the same horizontal plane when the model is situated in a desired viewing orientation. From the original coordinate data, a rotation matrix can be found to transform all coordinates to this orientation.[3] A template for cutting the base plane can be generated from these transformed coordinates by projecting the (x,y) positions for fiducial residues onto a scaled plot. Figure 2 shows such a plot for the support rods used in the lysozyme model. The scaled z-coordinate plus a constant (to raise the model above the base plane) determines the length of each rod, as shown in Table I. In some orientations adventitious intersection of the support rods and the model will occur. A projection of the whole skeleton onto the base plane (not shown in Figure 2) proves helpful in avoiding such intersections.

TABLE I
Fiducial Residue Positions for Lysozyme[a]

	C-alpha, in.		
	x	y	z
Residue 41	0	0	2.0000
Residue 49	7.3566	0.0000	2.0000
Residue 85	−0.9300	−2.5310	2.0000
Residue 1	−2.6071	−0.1199	2.3036
Residue 16	−1.8925	−4.5204	8.9856
Residue 23	0.1959	−1.5356	11.4491
Residue 60	5.1831	−2.0735	3.6732
Residue 75	5.1696	−5.3363	5.5521
Residue 90	0.3548	−4.4521	4.6269
Residue 120	−1.4445	2.1377	11.0393
Residue 129	−5.8982	−1.6731	9.6413
Residue 6	−4.9071	0.8477	7.4255
Residue 52	3.7015	0.0160	3.6654
Residue 101	4.8220	−4.0707	9.5961

[a] Centimeters per Angstrom, 1.00; inches per Angstrom, 0.39.

Support rods can be made from the same wire used to bend the model. With the rods in place, alignment is a process of gently deforming the wire until all fiducial residues coincide with their respective support points. This process can be repeated whenever the model becomes misaligned through handling. In the case of permanent attachment, the model can be silver soldered to its supports and then removed together with them for

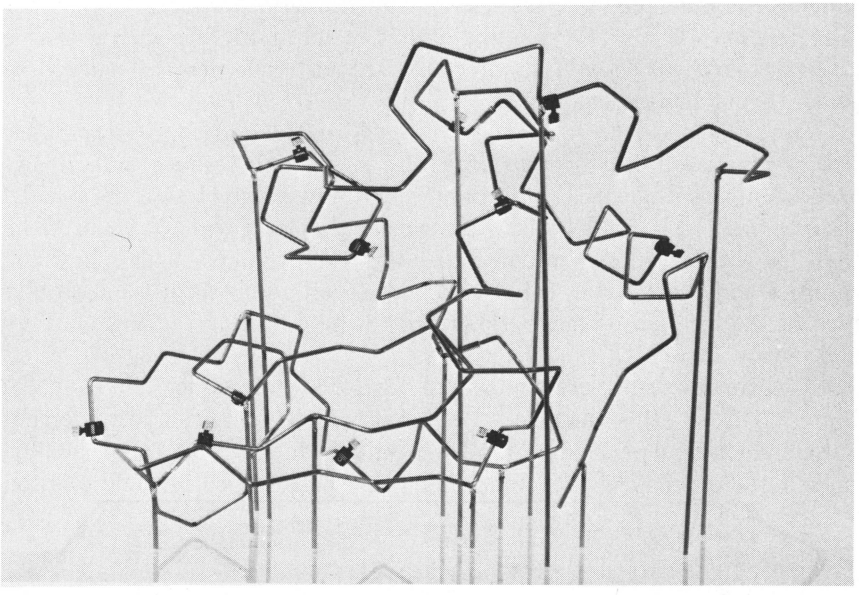

Fig. 1. Bent-wire α-C backbone model of hen egg lysozyme (scale 1 cm/Å), supported on fiducial support standards. Every tenth bend is marked with its sequence number.

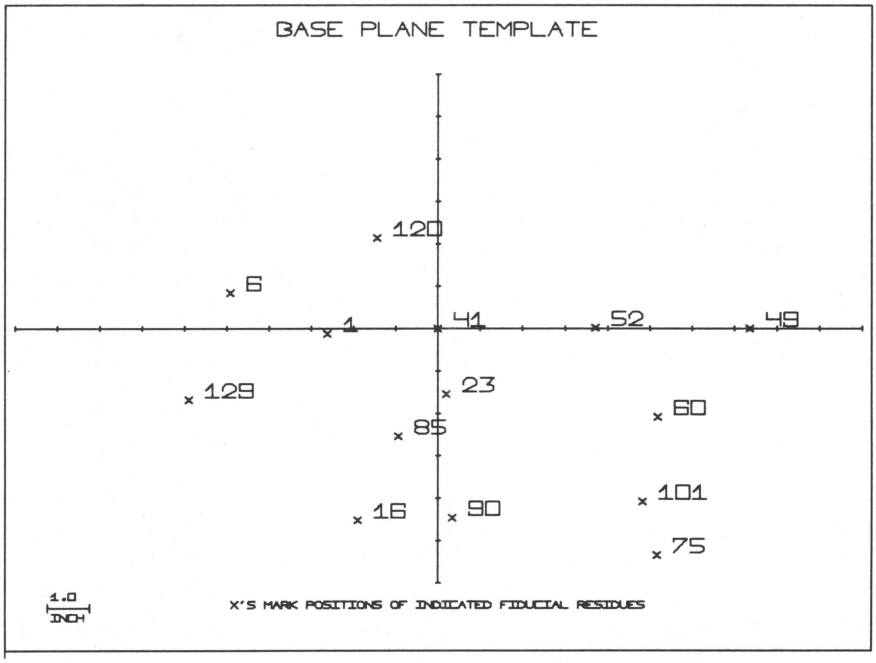

Fig. 2. Scale diagram of base plane template for the fiducial supports of the hen egg lysozyme model shown assembled in Figure 1.

plating. (Note: When soldering, the model can relax and change conformation if the wire is overheated. A low-melting, high tensile strength solder is recommended for this reason, e.g., Eutectic Corporation type 157 Eutecrod, an alloy of silver and tin that melts at 218°C, with a tensile strength of 15,000 psi.) If desired, the support rods may be painted to render them less conspicuous.

To test this method, the distance was measured from residue one at every fifth residue position, together with any supported residues not already included in this sequence. This data is shown in Table II with the fiducial residues marked by an asterisk. It is clear that at a scale of 1 cm/Å the model is within the resolution of the X-ray data. The model shown in Figure 1 and the data in Table II are based on global adjustments only; none of the local adjustments described in the following paragraphs were incorporated here.

It is clear that bent-wire models can be made to the same scale as space-filling (CPK, or other) models. While the fiducial mounting arrangement of Figure 1 might provide a useful hanger on which to mount spacefilling models, the details of how compatibility can be achieved in such a liaison are not yet clear.

LOCAL ADJUSTMENT

Local adjustment procedures are developed to minimize the effect or errors at a given bend. In addition to those mentioned in the original

TABLE II
Typical Interresidue Distances in Centimeters

Residue	Computed	Measured	Difference
1-6[a]	14.5	15.3	0.8
1-11	12.8	13.4	0.6
1-16[a]	20.4	20.5	0.1
1-21	26.8	26.9	0.1
1-23[a]	24.6	24.6	0.0
1-26	20.1	20.8	0.7
1-31	16.9	16.6	0.3
1-36	11.1	11.0	0.1
1-41[a]	6.7	6.3	0.4
1-46	22.8	22.5	0.3
1-49[a]	25.3	25.5	0.2
1-51	19.1	19.6	0.5
1-52[a]	16.4	16.4	0.0
1-56	13.1	12.9	0.2
1-60[a]	20.7	20.2	0.5
1-61	24.4	24.0	0.4
1-66	20.0	20.5	0.5
1-71	29.3	29.7	0.4
1-75[a]	25.2	25.7	0.5
1-76	21.8	22.3	0.5
1-81	13.5	13.9	0.4
1-85[a]	7.5	7.5	0.0
1-86	5.1	5.7	0.6
1-90[a]	14.6	15.3	0.7
1-91	13.6	13.8	0.2
1-96	21.1	21.5	0.4
1-101[a]	28.3	29.0	0.7
1-106	25.6	25.8	0.2
1-111	21.3	21.3	0.0
1-116	25.9	25.7	0.2
1-120[a]	23.1	23.5	0.4
1-121	23.6	23.9	0.3
1-126	21.7	22.2	0.5
1-129[a]	20.8	21.0	0.8

[a] Fiducial residue.

publication (protractor-reading errors, clamp slippage, and the like), we find three additional sources of error.

First, the bend angle obtained in a piece of wire can differ from the nominal angle read from the bend protractor by as much as 5°. This is shown in Figure 3A. The "true angle" was measured with a Nikon Profile Projector, Model 6C, and was reproducible to ±0.2° on repetitive measurements of the same bend. The nominal bend angle when read on the bend protractor, which has a small radius, is uncertain by as much as ±1°. The ordinate of each point of Figure 3A is, therefore, obtained as the difference: "true angle" (averaged over seven measurements) − nominal angle (the same for each of seven bends). It appears that occasional recalibration would be advisable, due to wear on the bearing surfaces of the pin about which the bending arm rotates.

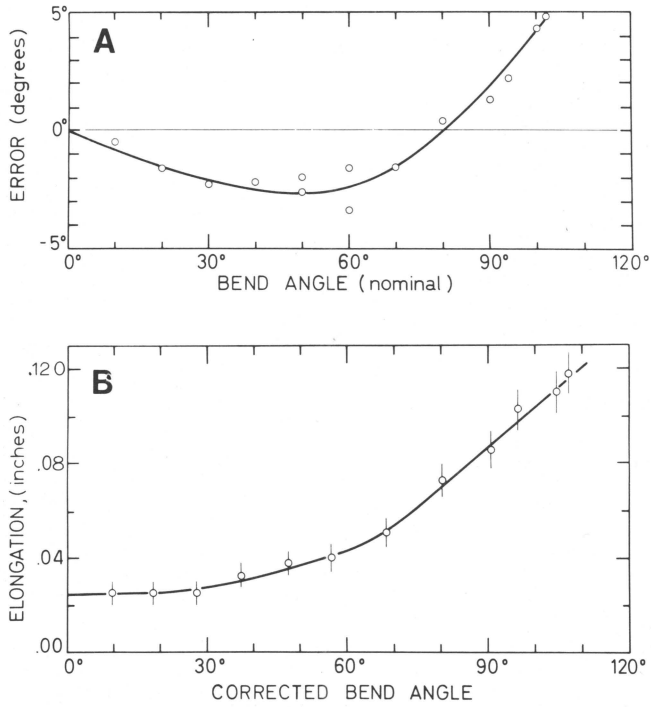

Fig. 3. (a) Bend angle error: (angle read on bend protractor) − ("true angle"). (b) Error in distance between bends: ordinate: (measured length/segment) − (computed length/segment). Abscissa: corrected bend angle. Mild steel rod 0.125-in. diameter and a prototype bending tool similar to that described by Rubin and Richardson.[1]

Second, the bending process irreversibly stretches the wire, the extent of elongation increasing with bend angle. For a bend of 100° in $^1/_8$-in. diameter mild steel, this elongation is 0.080 in. (0.105 in. less 0.025 in. micrometer displacement, see below) or 11% on a scale where the average distance between α-C atoms is 0.70 in. Elongation of wire in bending is well known and can be described mathematically for idealized cases.[4,5] Since the necessary parameters for computing the so-called bend allowance are not easily determinable, we carried out an empirical evaluation of the elongation. A series of test pieces were constructed, each containing seven identical bends (eight wire segments) each nominally 0.700 in. long. In bending the test pieces, the dihedral angle was increased by 180° after each bend, so that all the test pieces were planar zigzag structures. Test pieces were built with "true" bend angles varying from 9.5° to 106.8°. The linear distance from the beginning of the first wire segment to the end of the eighth segment was measured directly with the Nikon Profile Projector, and the length expected from this series of bends (with no wire elongation) was computed trigonometrically. In all cases the measured lengths were greater than those computed, as shown by Figure 3B. A 0,0 intersection was expected, but the plot shows an error of 0.025 inches at zero bend angle.

This was found to result from a fixed displacement of the micrometer mounting on the bender. The elongation due to bending becomes perceptible at bend angles >30°, and increases curvilinearly with increasing bend angle. Surprisingly, application of the elongation corrections (setting a corrected transfer distance for each bend) does not greatly increase the model-assembly time.

Third, when the bending program calls for part of the already bent wire to occupy some of the same space as the bending tool, we cut the wire halfway between that bend and the one immediately preceding. A new bending sequence is begun, and to construct the final model, separate pieces of peptide chain are assembled by inserting the cut ends into a short length of $^1/_8$ in. (i.d.) brass sleeve and silver soldering. The dihedral angle for this joint is set by separately bending an "overlap peptide" of a few residues, which can be clamped to one of the wire segments to be joined, providing a template for setting the dihedral angle error to an estimated ±2° with negligible (±1°) introduction of error in bend angle at such a joint.

CROSSLINKS

When a connected model has been assembled, crosslinks can be introduced by silver soldering appropriate lengths of wire to backbone bends representing half-cystines. We have also found it useful to introduce crosslinks *ad libitum* in models to increase their resistance to deformation by ordinary handling. The latter crosslinks can be conveniently made from $^1/_4$-in. hollow tubing of transparent methacrylate, cut to scaled lengths, V-notched at each end to reduce slippage. They are fastened to an appropriate pair of bends by a piece of nylon monofilament making a double traverse through the tube, looping around the wire at each end of the tube, drawn tight and tied. For example, a model of human carbonic anhydrase C (257 bends),[8] at a scale of 0.47 cm/Å was well stabilized with six such crosslinks. These stiffening crosslinks connected residue pairs 6–241, 29–194, 56–174, 81–255, 115–145, and 134–209. Since stabilizing crosslinks are placed between residues distant in sequence, some global correction of the model results from their incorporation.

To obtain a bent-wire model of maximum fidelity to the three-dimensional coordinates provided by a crystallographic study, it appears necessary to apply corrections to the nominal bend angles and to the nominal α-C to α-C distances, and finally to perform a global adjustment. The local corrections can be expected to vary in detail from one bending instrument to another, and from one kind of wire to another (brazing rod bends quite differently from mild steel). We have been informed (J. Richardson, personal communication) that design modifications not present in the prototype bending instrument used in this work have been incorporated into the instrument produced commercially by the Supper Co.[6] These modifications are believed to reduce significantly both the distance and bend

errors. For some model-building purposes, it may be possible to safely neglect distance and bend errors. For more precise work, the investigator should determine the magnitude of these errors with his particular instrument, in order to make an informed decision about employing these corrections.

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