

## On the Comparison of Conformations Using Linear and Quadratic Transformations

BY R. DIAMOND

*Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge, England*

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A means is developed whereby coordinate sets for related molecules may be compared in such a way as to express rigorously the relationship between them in terms of position, orientation, homogeneous strain and curvature. It is thought that such curvatures may be of value in characterizing hinge regions of allosteric enzymes. The method is illustrated with examples taken from haemoglobin and myoglobin.

### 1. Introduction

It is a common circumstance in protein crystallography to find two or more forms of a single molecule or oligomeric cluster for which differences of shape are evident. In this paper we are essentially concerned with the comparison of such shapes.

Given two fully determined structures, a detailed and accurate statement of all the conformational angles in both structures would contain all the information concerning shape differences. In this paper, however, we take a different approach to the comparison for two reasons. Firstly, it would be valuable to have a general means of comparing conformations which could be used before detailed information concerning the conformational angles is available, and secondly, such angular measures, when available, are subject to errors and need to be considered collectively rather than individually in order to appreciate their cumulative effect. For example, the E helix of myoglobin is bent some  $7^\circ$  near its tenth residue (Watson, 1969), yet the fluctuations in  $\varphi$  and  $\psi$  angles from one residue to the next in this helix are not noticeably different from those in other helices, being dominated mainly by the positioning of the carbonyl oxygen atoms. Our aim is therefore to provide a means of comparison at a higher level and in a general way.

Rao & Rossmann (1973) have used superposition techniques very effectively to search for structural similarities between proteins. Cox (Muirhead, Cox, Mazarella & Perutz, 1967) has used superposition techniques to characterize a rotational relationship between the subunits of haemoglobin and Huber, Epp, Steigemann & Formanek (1971) have used similar techniques to compare two related molecules. In all these cases, however, the only transformations considered were linear orthogonal transformations, and in the first instance, although a non-orthogonal transformation was discovered, the information expressed by the non-orthogonality was suppressed.

Linear transformations are limited in that they provide only for a constant orientation of the body throughout the transformed region and cannot therefore describe a region in which bending or twisting occurs. Quadratic transformations provide the possi-

bility of searching for regions in which one molecule is curved in comparison with another, thereby identifying hinge regions associated with conformational differences. The quadratic parts of such transformations introduce an additional 18 degrees of freedom into the transformations which give rise to conceptual difficulties. However, nine of these may be simply summarized in graphical form in terms of three principal curvatures (although this number reduces to eight at any point where the strain is isotropic) and the remaining nine (or ten) do not contribute to changes of orientation.

In the present work transformations with either 12 or 30 degrees of freedom are considered depending on whether linear or quadratic transformations are being used. In either case, the linear part is factorized into the *product* of two transformations, one of which represents pure strain and the other a pure rotation. The pure rotation is then uniquely and rigorously defined as that which rotates the principal axes of strain in one body onto the principal axes of strain in the other, these being, generally, the only three orthogonal directions which remain orthogonal locally under strain. For example, if a square is sheared to a rhombus and rotated, measuring the rotation by reference to its edges will give differing results depending on which edge is used, but if the rotation is measured with reference to its diagonals a uniquely meaningful result is obtained. The rotation remains uniquely defined even if the principal axes of strain are not.

In what follows we suppose that two sets of coordinates exist with a one-to-one correspondence between them. These coordinate sets need not be complete; they might, for example consist of  $C\alpha$  coordinates only. One of the two sets will be regarded as a reference set and the other as an object set. For example, in dealing with the myoglobin E helix, its own coordinates might form the object set, and coordinates for an ideally regular  $\alpha$  helix might be used as a reference set. The analysis then leads to a characterization of the deformations and rotations which must be performed on the reference set in order to make it coincide with the object set. Thus, in the above example we say what must be done to a straight helix to make it look like the myoglobin E helix, rather than saying

what must be done to the E helix to straighten it, but one is, of course, free to make the opposite choice.

Attention is thus focused on the reference set and operations upon it. Transformations are expressed in relation to the coordinate system of the reference set and it is considered that the orientation of the reference molecule is a standard one, that of the object set being arbitrary. If we then wish to compare the superimposed coordinate sets, we may either compare the transformed reference set with the object set, or we may compare the back-transformed object set with the reference set. In order to assist the user to work with a single orientation as standard the latter alternative is chosen. However, this choice requires the evaluation of the inverse of a quadratic transformation which, in principle, is not itself a quadratic transformation. A quadratic approximation to the inverse is given in the Appendix. The program which implements these procedures makes available for display the back-transformed object set and the reference set, and also gives the r.m.s. fitting error for this comparison and for the comparison of the transformed reference set with the object set, the latter normally being the closer fit.

The problem falls naturally into two parts. The first part is concerned with the deformation of a continuum, which involves differentiation, and the second part is concerned with the relationship between this and a deformable assembly of discrete points, in which differentiation has to be replaced by finite difference techniques designed to simulate differentiation. We begin by considering the continuum problem and return to the question of discrete point sets later.

Where no ambiguity arises the mathematics will be written, for clarity, in matrix notation. However, this is not suitable for dealing with the third-rank tensors that occur in the quadratic transformations and it has therefore been necessary to use the more complicated subscript notation. Usually a subscript occurs only once or twice in any term of a tensor expression, summation over that subscript being implied if it occurs twice. In this work, however, there are places where a particular subscript occurs three times, and places where one may occur twice without summation being involved. For this reason capital letter subscripts will be used when summation is not intended, and lower-case subscripts will be used when it is. Furthermore, quantities which relate to the reference axes will be unprimed while those which have been transformed onto the principal axes of strain will be primed with one prime for each subscript on which transformation has been performed. There are several instances in which a third-rank tensor is transformed on the first two subscripts only, and this needs to be distinguished from transformation on all three.

## 2. The continuum problem

We let  $\mathbf{X}$  denote the position vector of a point in the object set, having components  $X_I$  on a cubic Cartesian

set of axes.  $\mathbf{x}$  and  $x_I$  are the corresponding quantities for the corresponding point in the reference set, also on cubic Cartesian axes. Since there is no need in what follows for the object and reference sets to be in the same orientation,  $\mathbf{X}$  and  $\mathbf{x}$  may be considered to be referred to the same set of axes. Then at any point in the reference set we may obtain a matrix of derivatives for which the element on the  $i$ th row and  $j$ th column is

$$D_{IJ} = \frac{\partial X_I}{\partial x_J} \quad (1)$$

and it may readily be verified that  $D$  satisfies the transformation law for second-rank tensors, namely

$$D'_{LM} = a_{Li} a_{Mj} D_{ij}, \quad (2)$$

if the axes of reference are rotated so that  $a_{Li}$  is the cosine of the angle between the  $L$ th axis of the primed set and the  $I$ th axis of the unprimed set, and using the summation convention stated above.

$D$  is not symmetrical, having nine independent elements. Evidently it transforms a vector  $\delta\mathbf{x}$  in the reference set according to

$$\delta X_I = D_{IJ} \delta x_J. \quad (3)$$

In elementary elasticity theory it is usual to take a matrix similar to  $D$  (involving derivatives of *displacement* with respect to position) and to resolve it into the *sum* of a symmetric and an antisymmetric part and to interpret the symmetric part as strain and the antisymmetric part in terms of a rotation (Nye, 1957). Such a treatment is only valid for small strains and small rotations. In this work we suppose that  $D$  may be factorized according to\*

$$D = RT, \quad (4)$$

in which  $R$  is an orthogonal matrix expressing a pure rotation having three independent elements, and  $T$  is symmetric having six independent elements, thus providing for nine degrees of freedom in  $D$ . Equation (4) states that the transformation (3) is to be considered as the application of a pure strain followed by the application of a pure rotation. [It would, of course, be possible to write the factors in (4) as  $TR$  and to develop the subsequent theory on that alternative and different basis. However, the basis given here is preferred because it provides for the application of strain direct to the unrotated reference set, which the other does not.] Since  $T$  leaves the directions of the principal axes of strain unchanged,  $R$  expresses the orientational relationship exactly in relation to them.

From (4)

$$\tilde{D}D = \tilde{T}\tilde{R}RT = \tilde{T}T \quad (5)$$

(using a tilde to denote a transpose), but since

$$\tilde{T} = T$$

\* Here and elsewhere, when subscripts are omitted matrix notation is intended.

by definition

$$T = (\bar{D}D)^{1/2} \quad (6)$$

and the factorization is completed by writing

$$R = D(\bar{D}D)^{-1/2}. \quad (7)$$

Formation of  $\bar{R}R$  or  $R\bar{R}$  confirms that  $R$  is orthogonal provided  $D$  is of rank 3. The conventional strain tensor,  $S$ , is then given by

$$S = T - I. \quad (8)$$

$\bar{D}D$  is real symmetric positive definite, and therefore  $T$  is also real symmetric.  $(\bar{D}D)^{1/2}$  is a matrix having the same eigenvectors as  $\bar{D}D$  and with eigenvalues equal to the square roots of those of  $\bar{D}D$ . Only the positive square roots need be considered. (Negative roots correspond to tensile strains of the order of  $-2$ , *i.e.* an end-to-end reversal, which would consequentially be associated with an improper rotation  $R$  to reflect it back to its original chirality.)

With this formulation  $R$  can express gross differences of orientation between the object and reference sets and it is of the same form as matrices used by McLachlan (1972) for superimposing one molecule on another. Apart from this function,  $R$  itself is of little interest; it is variations of  $R$  from place to place which express curvature. Likewise a constant  $T$ , other than the identity, corresponds to a homogeneous strain of the entire object set, such as might arise from an error in a crystallographic cell constant. Hence variations of  $T$  from place to place are also of interest, and may be regarded as the causes of changes in orientation.

We consider next the second derivatives of  $\mathbf{X}$

$$\dot{D}_{IJK} = \frac{\partial^2 X_I}{\partial x_K \partial x_J} \quad (9)$$

which may also be shown to satisfy the transformation law for third-rank tensors.  $\dot{D}$  has 18 independent elements because

$$\dot{D}_{IJK} = \dot{D}_{IKJ}, \quad (10)$$

*i.e.* it is unchanged by transposition on the second and third subscripts. In writing a tensor such as  $\dot{D}$  it will always be given with the subscript relating to the numerator in the first position and with subscripts relating to the first and subsequent differentiations in the second and subsequent positions.

We now wish to find  $\dot{T}$  and  $\dot{R}$  in terms of  $D$  and  $\dot{D}$  so that the rates of change of strain and orientation may be determined. Note, first, that the derivative of the  $n$ th power of a matrix  $M$  is not equal to  $nM^{n-1}\dot{M}$  because  $M$  and  $\dot{M}$  do not commute, so we shall differentiate  $T$  implicitly, from first principles.

From (6)

$$T_{IJ}T_{JK} = D_{JI}D_{JK}. \quad (11)$$

Then if  $x$  is a small displacement

$$\begin{aligned} (T_{IJ} + x_I \dot{T}_{IJ}) (T_{JK} + x_m \dot{T}_{JKm}) \\ = (D_{JI} + x_I \dot{D}_{JI}) (D_{JK} + x_m \dot{D}_{JKm}). \end{aligned} \quad (12)$$

Hence, to first order in  $x$

$$x_I \dot{T}_{IJ} T_{JK} + x_m T_{IJ} \dot{T}_{JKm} = x_I \dot{D}_{JI} D_{JK} + x_m D_{JI} \dot{D}_{JKm} \quad (13)$$

and since this holds for any small  $x$

$$\dot{T}_{IJL} T_{JK} + T_{IJ} \dot{T}_{JKL} = \dot{D}_{JIL} D_{JK} + D_{JI} \dot{D}_{JKL}. \quad (14)$$

Now let  $A$  be an orthogonal matrix with elements  $a_{IJ}$  which diagonalizes  $\bar{D}D$  at a point, rows of  $A$  being the eigenvectors of  $\bar{D}D$ . In what follows we use  $A$  without alteration in the vicinity of that point, notwithstanding the fact that neighbouring points may, in general, have differing strains not diagonalized by the same  $A$ . To treat  $A$  as a spatial variable which continuously diagonalizes  $\bar{D}D$  would allow a study of the variation from place to place of the directions of the principal axes of strain, but this is not the same thing as variations from place to place of the orientation of the material, and to study this we find it more convenient to determine  $A$  at a position of interest and to treat it as constant in the vicinity of that point. Thus  $A$  is a diagonal matrix such that

$$A_{IJ}^2 = a_{Ik} a_{Jl} D_{mk} D_{ml} \quad (15)$$

$$A_{II}^2 = \lambda_I^2 \quad (16)$$

$$A_{IJ} = T'_{IJ}. \quad (17)$$

Transforming (14) then gives\*

$$\dot{T}'_{IJL} T'_{JK} + T'_{IJ} \dot{T}'_{JKL} = \dot{D}'_{JIL} D'_{JK} + D'_{JI} \dot{D}'_{JKL}. \quad (18)$$

*i.e.*

$$\dot{T}'_{IKL} \lambda_K + \lambda_I \dot{T}'_{IKL} = \dot{D}'_{JIL} D'_{JK} + D'_{JI} \dot{D}'_{JKL} \quad (19)$$

$$\dot{T}'_{IKL} = \frac{\dot{D}'_{JIL} D'_{JK} + D'_{JI} \dot{D}'_{JKL}}{\lambda_I + \lambda_K} \quad (20)$$

$$\dot{T}'_{IKR} = a_{IR} \dot{T}'_{IKI} = \frac{\dot{D}'_{JIR} D'_{JK} + D'_{JI} \dot{D}'_{JKR}}{\lambda_I + \lambda_K} \quad (21)$$

$$\dot{T}'_{PQR} = a_{IP} a_{kQ} \dot{T}'_{ikR}. \quad (22)$$

Note that the evaluation of  $\dot{T}'$  or of  $\dot{T}$  involves straightforward matrix multiplications to obtain the numerator, but that division by  $\lambda_I + \lambda_K$  is not of this form, being an element operation. For this reason  $\dot{T}$  is not readily obtainable except by way of  $\dot{T}'$  or  $\dot{T}''$ . Note also that

$$\dot{T}'_{IKL} = \dot{T}'_{KIL} \quad (23)$$

and similarly for  $\dot{T}''$  and  $\dot{T}$ . This symmetry differs from that of  $\dot{D}$ .

\* For example, the transform of the first term may be obtained as

$$\begin{aligned} a_{PI} a_{Qk} a_{Rl} \dot{T}'_{Ijl} T_{jk} &= a_{PI} a_{Qk} a_{Rl} \delta_{jm} \dot{T}'_{Ijl} T_{mk} \\ &= a_{PI} a_{Qk} a_{Rl} a_{nm} \dot{T}'_{Ijl} T_{mk} = \dot{T}'_{PnR} T'_{nQ}. \end{aligned}$$

*i.e.* Transformation of  $\dot{T}'_{IJL} T_{JK}$  leads to an expression of the same form with the subscripts renamed and primes appended. The subscript name changes may be reverted.

$\dot{T}$  expresses the rates of change with respect to the untransformed coordinates  $\mathbf{x}$  of the nine elements of strain.  $\dot{T}''$  expresses the rates of change with respect to the transformed coordinates  $\mathbf{x}'$  of the nine elements of strain expressed on the local principal axes of strain. At any particular point, of course, the off-diagonal elements of  $T''$  vanish identically. Nevertheless their spatial derivatives do not vanish because the transformation  $a_{ij}$  is not itself varied, as explained earlier.

To differentiate  $R$  we write

$$R_{ij}T_{jk} = D_{ik}; \quad (24)$$

differentiating and transforming as before gives

$$\dot{R}_{ijl}T'_{jk} + R'_{ij}\dot{T}'_{jkl} = \dot{D}'_{ikl} \quad (25)$$

$$\dot{R}'_{ikl}\lambda_k + \lambda_j^{-1}D'_{ij} \left[ \frac{\dot{D}'_{mjl}D'_{mk} + D'_{mj}\dot{D}'_{mkl}}{\lambda_j + \lambda_k} \right] = \dot{D}'_{ikl} \quad (26)$$

from (17), (20) and the transform of (24). Hence

$$\dot{R}'_{ikl} = \lambda_k^{-1}\dot{D}'_{ikl} - D'_{ij} \left[ \frac{\dot{D}'_{mjl}D'_{mk} + D'_{mj}\dot{D}'_{mkl}}{\lambda_k\lambda_j(\lambda_j + \lambda_k)} \right]. \quad (27)$$

To determine the rate of change of orientation of the object set relative to the reference set in the vicinity of a point, let  $R$  rotate the reference set onto the object set at that point and let  $R(\mathbf{x})$  rotate the reference set onto the object set at a point displaced from it by a small  $\mathbf{x}$  in the reference set, then

$$R_{IK}(\mathbf{x}) = R_{IK} + x_i\dot{R}_{IKi} = R_{Ii}P_{iK}(\mathbf{x}) \quad (28)$$

in which  $P_{LK}(\mathbf{x})$  is an orthogonal matrix representing an incremental rotation associated with the vector increment  $\mathbf{x}$ . In this form  $P$  applies to the reference set of coordinates before the rotation  $R$  is applied. Evidently

$$R_{ij}R_{ik} + x_l R_{ij}\dot{R}_{ikl} = R_{ij}R_{il}P_{iK}(\mathbf{x}) \quad (29)$$

$$P_{JK}(\mathbf{x}) = \delta_{JK} + x_l R_{ij}\dot{R}_{ikl} \quad (30)$$

$$\equiv \delta_{JK} + x_l Q_{JKL} \quad (31)$$

defining  $Q$ . Now

$$Q'_{JKL} = R'_{ij}\dot{R}'_{ikl} \quad (32)$$

$$= \lambda_j^{-1}D'_{ij} \left\{ \lambda_k^{-1}\dot{D}'_{ikl} - D'_{in} \left[ \frac{\dot{D}'_{mnl}D'_{mk} + D'_{mn}\dot{D}'_{mkl}}{\lambda_k\lambda_n(\lambda_k + \lambda_n)} \right] \right\} \quad (33)$$

from (17), (27) and the transform of (24).

Now (15) gives

$$D'_{ij}D'_{in} = A_{jn}^2 \quad (34)$$

so that

$$Q'_{JKL} = \lambda_j^{-1}\lambda_k^{-1}D'_{ij}\dot{D}'_{ikl} - \lambda_j \left[ \frac{\dot{D}'_{mjl}D'_{mk} + D'_{mj}\dot{D}'_{mkl}}{\lambda_k\lambda_j(\lambda_k + \lambda_j)} \right] \quad (35)$$

$$= D'_{ij}\dot{D}'_{ikl} \left\{ \lambda_j^{-1}\lambda_k^{-1} - \frac{1}{\lambda_k(\lambda_k + \lambda_j)} \right\} - \frac{\dot{D}'_{mjl}D'_{mk}}{\lambda_k(\lambda_k + \lambda_j)} \quad (36)$$

$$= \frac{\lambda_j^{-1}D'_{ij}\dot{D}'_{ikl} - \lambda_k^{-1}\dot{D}'_{mjl}D'_{mk}}{\lambda_k + \lambda_j}. \quad (37)$$

Note that

$$Q'_{JKL} = -Q'_{KJL} \quad (38)$$

$$Q'_{JKL} = \frac{\lambda_j^{-1}D'_{ij}\dot{D}'_{ikl} - \lambda_k^{-1}\dot{D}'_{mjl}D'_{mk}}{\lambda_k + \lambda_j} \quad (39)$$

$$Q_{PQL} = a_{jP}a_{kQ}Q'_{JKL} \quad (40)$$

from which the rotation  $P(\mathbf{x})$  may be obtained with (31).

The rotation  $P$ , being differential, may be expressed in terms of a curvature vector  $\mathbf{V}$ , itself a function of  $\mathbf{x}$ . For sufficiently small  $\mathbf{x}$

$$V_H(\mathbf{x}) = \frac{1}{2}\varepsilon_{Hij}P_{ij}(\mathbf{x}) \quad (41)$$

$$= \frac{1}{2}\varepsilon_{Hij}x_k Q_{ijk} \quad (42)$$

in which the direction of  $\mathbf{V}(\mathbf{x})$  is the axis of rotation of the change of orientation associated with a small displacement  $\mathbf{x}$  and its magnitude is the rotation angle in radians for that displacement. If  $\mathbf{x}$  is 1 Å then  $\mathbf{V}$  expresses curvature in radians Å<sup>-1</sup>.  $\mathbf{V}(\mathbf{x})$  parallel to  $\mathbf{x}$  implies twisting and  $\mathbf{V}(\mathbf{x})$  perpendicular to  $\mathbf{x}$  implies bending.

Now

$$\begin{aligned} |\mathbf{V}(\mathbf{x})|^2 &= V_h(\mathbf{x})V_h(\mathbf{x}) = \frac{1}{4}\varepsilon_{hij}\varepsilon_{hlm}x_kx_n Q_{ijk}Q_{lmn} \quad (43) \\ &= \frac{1}{4}(\delta_{il}\delta_{jm} - \delta_{im}\delta_{jl})x_kx_n Q_{ijk}Q_{lmn} \\ &= \frac{1}{4}x_kx_n(Q_{ijk}Q_{ijn} - Q_{ijk}Q_{jin}) \\ &= \frac{1}{2}x_kx_n Q_{ijk}Q_{ijn} \\ &\equiv \frac{1}{2}x_kx_n M_{kn}, \end{aligned} \quad (44)$$

defining  $M$ .

Principal curvatures may thus be found from (42) by setting  $\mathbf{x}$  equal to each of the eigenvectors of  $M$  since these give stationary values of  $|\mathbf{V}(\mathbf{x})|/|\mathbf{x}|$ .

A further result of interest may be obtained as follows. Suppose that, in (42), we allow  $x_k$  to be replaced by  $x_{kL}$  which is an orthogonal matrix,  $k$  running over the elements of each vector and  $L$  varying from vector to vector, these vectors being mutually perpendicular; then the curvature vectors  $\mathbf{V}$  themselves form a matrix

$$V_{HL}(\mathbf{x}) = \frac{1}{2}\varepsilon_{Hij}x_{kL}Q_{ijk} \quad (45)$$

$$\begin{aligned} W_{HM} &= V_{Hl}(\mathbf{x})x_{Ml} = \frac{1}{2}\varepsilon_{Hij}x_{kl}x_{Ml}Q_{ijk} \\ &= \frac{1}{2}\varepsilon_{Hij}Q_{ijM} \end{aligned} \quad (46)$$

and the trace of  $W$  is

$$Y = W_{hh} = \frac{1}{2}\varepsilon_{hij}Q_{ijh} = V_{hi}(\mathbf{x})x_{hi} \quad (47)$$

and the significance of this quantity is that it is the sum over the three vectors  $\mathbf{x}$  of the scalar products of these vectors with their associated curvatures  $\mathbf{V}$ , and it is independent of the choice of vectors  $\mathbf{x}$  except that they shall be mutually orthogonal unit vectors. Fur-

thermore,  $Y$  being a scalar, is unchanged on transformation\* so that

$$Y = \frac{1}{2} \varepsilon_{pqr} Q''''_{pqr} \quad (48)$$

$$= \frac{1}{2} \varepsilon_{pqr} \frac{\lambda_p^{-1} D''_{ip} \dot{D}''''_{iqr} - \lambda_q^{-1} \dot{D}''''_{ipr} D''_{ia}}{\lambda_q + \lambda_p} \quad (49)$$

$$= \varepsilon_{pqr} \frac{\lambda_p^{-1} D''_{ip} \dot{D}''''_{iqr}}{\lambda_q + \lambda_p}$$

which may be simplified to

$$Y = \frac{1}{K} \varepsilon_{pqr} \lambda_p^{-1} D''_{ip} \dot{D}''''_{iqr} \lambda_r^2 \quad (50)$$

in which

$$K = (\lambda_1 + \lambda_2) (\lambda_2 + \lambda_3) (\lambda_3 + \lambda_1). \quad (51)$$

$Y$  vanishes if the eigenvalues are equal (isotropic strain) because, for a given  $p$ ,  $\lambda_R^2 - \lambda_Q^2$  is involved as a factor since  $\dot{D}''''_{iQR} = \dot{D}''''_{iRQ}$ . For small strains  $Y$  remains small. As an illustration of the meaning of this, consider a cylinder twisted about its length. For  $\mathbf{x}$  along its length  $\mathbf{V}$  is parallel to  $\mathbf{x}$ ; for two other directions normal to each other and to the axis of the cylinder,  $\mathbf{V}$  must be anti-parallel to these radii and of half the magnitude of the axial twist in order that  $Y$  should vanish. Consideration of a twisted cylinder shows that this is so.

As an illustration of some of the foregoing ideas consider a thick plank, initially a rectangular parallelepiped, having  $x_1$  along its length,  $x_2$  in the direction of its thickness and  $x_3$  in the direction of its width and let it be bent to an arc of a circle of radius  $p^{-1}$  measured to the arc of unchanged length, the straight plank being reference and the curved one the object. Then, writing

$$s = \sin px_1, \quad c = \cos px_1, \quad a = 1 - px_2 \quad (52)$$

and

$$\begin{aligned} X_1 &= (p^{-1} - x_2)s \\ X_2 &= p^{-1}(1 - c) + x_2c \\ X_3 &= x_3 \end{aligned} \quad (53)$$

gives

$$D = \begin{pmatrix} ac & -s & 0 \\ as & c & 0 \\ 0 & 0 & 1 \end{pmatrix}, \quad T = \text{diag}(a, 1, 1), \quad A = I,$$

$$R = \begin{pmatrix} c & -s & 0 \\ s & c & 0 \\ 0 & 0 & 1 \end{pmatrix} \quad (54)$$

\*  $\varepsilon_{hij} Q_{ijh} = \varepsilon_{hij} \delta_{hk} \delta_{il} \delta_{jm} Q_{klm} = \varepsilon_{hij} a_{ph} a_{pk} a_{ql} a_{rj} a_{rm} Q_{klm}$   
 $= a_{ph} a_{ql} a_{rj} \varepsilon_{hij} a_{pk} a_{ql} a_{rm} Q_{klm} = \varepsilon_{pqr} Q''''_{pqr}.$

$$\dot{D}_{IJK} = \begin{bmatrix} -aps & -pc & 0 \\ apc & -ps & 0 \\ 0 & 0 & 0 \end{bmatrix} \quad K=1$$

$$\begin{bmatrix} -pc & 0 & 0 \\ -ps & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \quad K=2$$

$$\begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \quad K=3$$

$$\dot{D}_{IJK} D_{IJ} = \begin{bmatrix} 0 & pa & 0 \\ -pa & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \quad K=1$$

$$\begin{bmatrix} -pa & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \quad K=2$$

$$\begin{bmatrix} 0 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \quad K=3 \quad (55)$$

$\dot{T}_{112} = -p$ , all other elements zero. And for the  $K=1$  layer

$$\dot{R} = \begin{pmatrix} -ps & -pc & 0 \\ pc & -ps & 0 \\ 0 & 0 & 0 \end{pmatrix}, \quad Q = \begin{pmatrix} 0 & -p & 0 \\ p & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}$$

$$V = \begin{pmatrix} 0 \\ 0 \\ -p \end{pmatrix}, \quad (56)$$

$\dot{R}$ ,  $Q$  and  $V$  being null for  $K=2$  or  $3$ . Note that the curvature  $V$  expresses the fact that the orientation of the plank alters by  $p$  radians about  $X_3$  per unit displacement in the direction of  $x_1$  (because  $K=1$ ) and that it does not express the fact that the concave side of the plank is bent to a greater curvature than the convex side because it expresses change of orientation per unit displacement in the reference body, and the curvature is the same everywhere on this basis.

### 3. On the relationship between curvature and the spatial derivatives of strain

The distinction between  $\dot{T}$  and  $\dot{T}''''$  is one which depends only on the change of axes to which these tensors are referred and this change is itself a function only of  $D$  and not of  $\dot{D}$ . It follows that we may consider  $\dot{T}''''$  as having the same number of degrees of freedom as does  $\dot{T}$  for the purpose of relating  $\dot{T}$  and  $Q$ , and we shall base the following discussion on the primed quantities.

$\dot{T}''''$  has 18 distinct elements and  $Q''''$  has nine, yet all 27 are functions of the 18 independent elements of  $\dot{D}$  so that the elements of  $Q''''$  and of  $\dot{T}''''$  cannot be independent of each other. If, therefore, we try to summarize the quadratic character of the transformation in terms of the principal curvatures (which derive directly from  $Q''''$  alone) we must ask what types of quadratic

features remain unexpressed by such a summary. Both  $V(\mathbf{x})$  and  $\mathbf{x}$  of the principal curvatures are functions of  $Q$  so that the principal curvatures would appear to possess nine degrees of freedom. However, if the eigenvalues,  $\lambda$ , of  $T$  are approximated by unity,  $Y$  vanishes by (50), and (48) becomes a linking equation among elements of  $Q$  and its freedoms are thereby reduced from nine to eight. It follows that there must (within this approximation) be ten degrees of freedom available to  $\hat{T}$  which describe curvature-free quadratic deformations which may be present to any degree in a deformed body and which will not be detected by calculation of the principal curvatures.

Equations (10), (20) and (37) may be rearranged to give

$$(\lambda_I + \lambda_J) (\hat{T}''''_{IJK} - \hat{T}''''_{JKI}) = \lambda_I(\lambda_I + \lambda_K) Q''''_{IKJ} + \lambda_J(\lambda_I - \lambda_K) Q''''_{KJI} \quad (57)$$

and if the eigenvalues are approximated to unity this becomes

$$\hat{T}''''_{IJK} - \hat{T}''''_{JKI} = Q''''_{IKJ}, \quad (58)$$

which makes up in clarity what it lacks in precision. This shows that if some increment is added to  $\hat{T}''''_{IJK}$  and the same increment is added to those elements of  $\hat{T}''''$  related to it by cyclic permutation of the subscripts then  $Q''''$  is unchanged by this operation, and conversely, the principal curvatures cannot reveal strain fields contributing equally to cyclicly related elements of  $\hat{T}''''$ . There are, in all, ten such fields, three of the form  $\hat{T}''''_{III}$ , six of the form  $[\hat{T}''''_{IIJ}$  with  $\hat{T}''''_{JII}$  and  $\hat{T}''''_{JJI}$  ( $J \neq I$ ) and one of the form  $[\hat{T}''''_{IJK}$  with  $\hat{T}''''_{JKI}$  and  $\hat{T}''''_{KJI}$ ] ( $I \neq J \neq K \neq I$ ). [If the distinction between the eigenvalues  $\lambda$  is retained, equation (57) leads to conclusions which differ only in detail.] An example of the first kind is provided by a heavy elastic rope suspended from one end and stretched by its own weight. The tensile strain varies linearly with height ( $\hat{T}''''_{111} \neq 0$ ) but there is no curvature.

These three types of curvature-free strain fields are typified by the transformations

$$\left. \begin{aligned} X_1 &= x_1 + \frac{1}{2} p x_1^2 \\ X_2 &= x_2 \\ X_3 &= x_3 \end{aligned} \right\} \quad (59)$$

$$\left. \begin{aligned} X_1 &= x_1 - p x_1 x_2 \\ X_2 &= -\frac{1}{2} p x_1^2 + x_2 \\ X_3 &= x_3 \end{aligned} \right\} \quad (60)$$

and

$$\left. \begin{aligned} X_1 &= x_1 + p x_2 x_3 \\ X_2 &= x_2 + p x_3 x_1 \\ X_3 &= x_3 + p x_1 x_2 \end{aligned} \right\} \quad (61)$$

and these are illustrated in Fig. 1(a), (b) and (c) respectively. For all these three transformations the deformation is curvature-free in the sense that the principal axes of strain everywhere in the deformed object bear a constant orientational relationship (in these

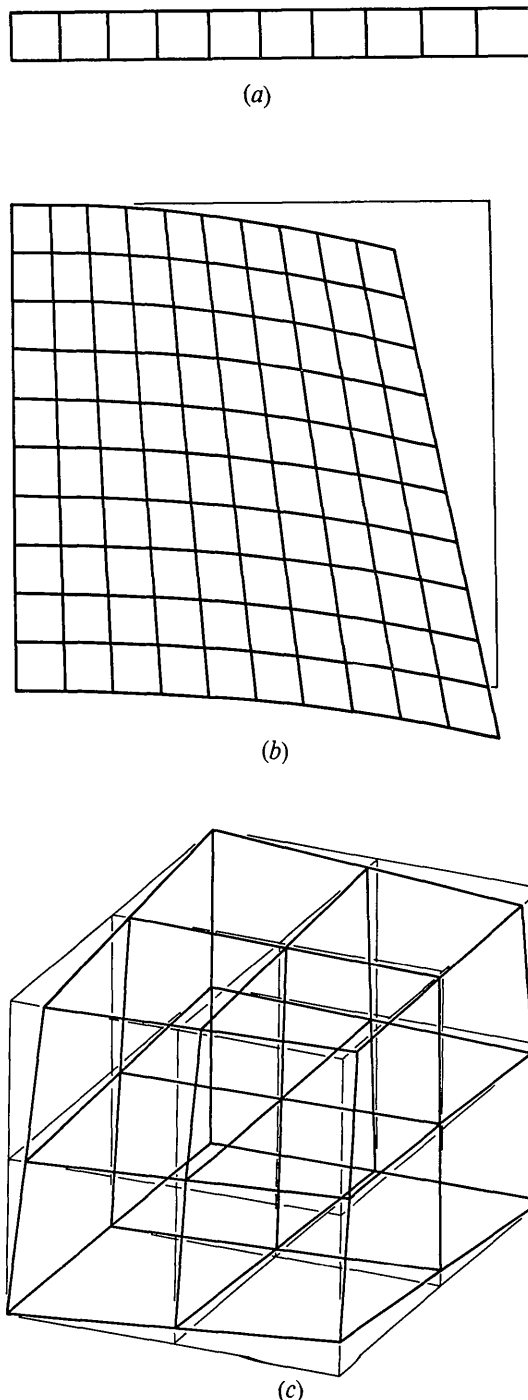


Fig. 1. The three types of curvature-free quadratic deformations, (a), (b) and (c) corresponding to equations (59), (60) and (61) respectively. All of these are curvature-free in the sense that the principal axes of strain in the deformed body are everywhere parallel to the corresponding lines in the reference body. (a), (b) and (c) are essentially one-, two- and three-dimensional in character. The ten curvature-free degrees of freedom are made up of three like (a), six like (b) and one like (c). (a) and (b) show the deformation of arrays of elementary squares, while (c) shows the deformation (in orthographic projection) of eight contiguous elementary cubes.

cases parallel) to the corresponding lines in the reference body. This does not mean that all straight lines in the reference body map into straight lines in the object body, nor does it mean that the orientation of the principal axes of strain does not vary from place to place. In (60) for example, the slopes of the principal axes of strain in the 1,2 plane are given by

$$\frac{\delta X_1}{\delta X_2} = \frac{\delta x_1}{\delta x_2} = \frac{x_2}{2x_1} [1 \pm \sqrt{1 + 4x_1^2/x_2^2}] \quad (62)$$

and the left-hand equality is preserved everywhere, even though the function on the right varies from place to place.

$\hat{T}'''$  may always be resolved into the sum of two strain fields  $B'''$  and  $C'''$  such that  $B'''$  is curvature-free and  $C'''$  is pure curvature, according to

$$B'''_{IJK} = \frac{1}{3}(\hat{T}'''_{IJK} + \hat{T}'''_{JKI} + \hat{T}'''_{KIJ}) \quad (63)$$

$$C'''_{IJK} = \hat{T}'''_{IJK} - B'''_{IJK} \quad (64)$$

so that, if the deformation is to be summarized by the principal curvatures, then the residual deformation which is not contained in the summary may be obtained from (63), the resulting numbers being the coefficients of the ten curvature-free quadratic strain fields already described.

Since, in comparing protein conformations, changes of orientation associated with curvature are of primary interest the computer program which has been written for this purpose gives the principal curvatures as a summary. The ten coefficients of the curvature-free fields are usually small and are commonly ignored, but may be regained if required.

#### 4. Discrete coordinate sets

In cases in which the object and reference sets consist of discrete points rather than a differentiable continuum it is necessary to replace  $D$  and  $\hat{D}$  by finite difference approximations to the derivatives. To obtain  $D$  at a point it is only necessary to expand (3) to include three column vectors  $\delta X_I$  and  $\delta x_J$  for three corresponding atoms (expressed relative to that point and the transform of that point) in square matrices  $\delta X_{IL}$  and  $\delta x_{JL}$  so that

$$\delta X_{IL} = D_{IJ} \delta x_{JL} \quad (65)$$

$$D_{IJ} = \delta X_{Ik} \delta x_{kJ}^{-1}, \quad (66)$$

provided that the vectors  $\delta \mathbf{x}$  are not coplanar. In practice, however, it is preferable that  $D$  should depend on more than three corresponding atoms in such a way that  $D$  becomes the least-squares solution to the fitting of  $\delta \mathbf{X}$  to  $\delta \mathbf{x}$ . If this is done, (65) implies that whatever point is taken as origin in the reference set must transform *exactly* to the origin in the object set, so that, if one atom is chosen as a reference point, (the  $\delta \mathbf{x}$  and  $\delta \mathbf{X}$  values being then coordinates of other atoms relative to the chosen one) then the chosen atom is

given infinite weight in the fitting. An alternative would seem to be to relate the atoms in both object and reference sets to their corresponding centroids. This leads to the best possible conditioning of the least-squares determination of  $D$  (and of  $\hat{D}$ ), but the centroid of the reference set only maps onto the centroid of the object set in the absence of quadratic terms. It is therefore necessary to allow for a floating origin and to write

$$X_{IL} = d_I + D_{IJ} x_{JL} + \frac{1}{2} \hat{D}_{IJK} x_{JL} x_{kL} \quad (67)$$

in which  $X_{IL}$  and  $x_{JL}$  ( $L=1 \dots N$ ) represent the coordinates of the  $N$  corresponding points in each set expressed relative to their own centroids.  $D$  and  $\hat{D}$  then provide the leading terms in a Taylor expansion about the centroid and  $d$  allows the origin to float.

$d$ ,  $D$  and  $\hat{D}$  together have 30 independent elements, so that a minimum of ten atoms are required to determine them. The results are less sensitive to error if more than ten atoms are used and (67) is interpreted in a least-squares sense by writing

$$X = (d|D|E) \begin{pmatrix} 1 \\ x \\ \varphi \end{pmatrix} + \varepsilon \quad (68)$$

in which  $(d|D|E)$  is a partitioned matrix having three rows and ten columns, one for  $d$ , three for  $D$  and six for  $E$ .  $E$  contains the 18 independent elements of  $\hat{D}$  and  $\varphi$  contains the products  $x_{IL} x_{JL}$  with the factor  $\frac{1}{2}$  when  $I=J$ , and  $\varepsilon$  contains residual errors.

The least-squares solution is then

$$(d|D|E) = X(\tilde{1}|\tilde{x}|\tilde{\varphi}) \left[ \begin{pmatrix} 1 \\ x \\ \varphi \end{pmatrix} (\tilde{1}|\tilde{x}|\tilde{\varphi}) \right]^{-1}. \quad (69)$$

#### 5. Implementation

A program has been written which determines  $d$ ,  $D$  and  $\hat{D}$  from (69) and displays graphically the reference molecule in its own orientation with the object molecule back-transformed to superimpose upon it. The program distinguishes three regions in all. The primary region consists of atoms selected from the reference molecule, together with corresponding atoms found in the object molecule by corresponding names or positions in a list. The primary region is used to determine the transformation (67) by (69). The secondary region consists, optionally, of further atoms selected in the same way, which play no part in the determination of the transformation, but to which the transformation may be applied. This allows an extrapolation of the superposition into a neighbouring region to be observed. For example, if the relative movement in the  $\alpha_1\beta_2$  interface of haemoglobin is to be examined then choosing a primary region in  $\alpha_1$  and a secondary region in  $\beta_2$  shows up the relative displacement when oxy- and deoxy-haemoglobin are compared. The third region consists of all the rest of the molecule. This

plays no part in the current calculation, except that it is held in core and is available for the selection of subsequent primary and secondary regions.

The program provides the option of using either linear or quadratic transformations, so that rigid-body superpositions may be done if preferred. This is done by omitting portions containing  $E$  or  $\varphi$  from (69). It should be noted, however, that the linear part of a quadratic transformation determined by least squares is not the same as a linear transformation determined by least squares with the quadratic terms constrained to zero. (This is analogous to the distinction between the best straight line fitted to a section of a parabola, and a tangent to it at  $x=0$ .)

The back-transformation required to transform the object molecule onto the reference molecule for display purposes may be written

$$x_{iL} = d_i^* + D_{iJ}^* X_{jL} + \frac{1}{2} \dot{D}_{ijk}^* X_{jL} X_{kL} \quad (70)$$

and it is shown in the Appendix that

$$\dot{D}_{ijk}^* = -D_{iP}^{-1} D_{qJ}^{-1} D_{rK}^{-1} \dot{D}_{pqr} \quad (71)$$

$$D_{iJ}^* = D_{iJ}^{-1} + D_{iP}^{-1} D_{qJ}^{-1} D_{rs}^{-1} \dot{D}_{pqr} d_s \quad (72)$$

$$d_i^* = -D_{iJ}^{-1} d_j - \frac{1}{2} D_{iP}^{-1} D_{qs}^{-1} D_{rt}^{-1} \dot{D}_{pqr} d_s d_t \quad (73)$$

which may be substituted in (70). A simpler alternative is to write

$$x_{iL} = D_{iJ}^{-1} (X_{jL} - d_j) - \frac{1}{2} D_{iP}^{-1} D_{qJ}^{-1} D_{rk}^{-1} \dot{D}_{pqr} (X_{jL} - d_j) (X_{kL} - d_k) \quad (74)$$

but the choice between (70-73) and (74) may depend on whether or not one wishes to preserve the  $X$  values unmodified by subtraction of  $d$ .

Three back-transformations may be requested for display. The first is

$$x_{iL} = R_{jI} (X_{jL} - d_j) \quad (75)$$

In this case the axis of rotation is also shown as a line with a length scaled to represent the rotation angle. Similarly a rotation axis associated with the matrix product

$$\tilde{R}_{old} R_{new}$$

is shown in which  $R_{new}$  is the orthogonal matrix associated with the current primary region, and  $R_{old}$  is that associated with the immediately previous one. The axis and angle generated in this way therefore express the total bend of the object molecule relative to the reference molecule between the present and previous primary regions.

The second back-transformation is

$$x_{iL} = D_{iJ}^{-1} (X_{jL} - d_j) \quad (76)$$

If this is requested, the principal strains are shown as arrows pointing towards or away from the centroid, marked in percentage of compression or extension respectively.

The third back-transformation is given by (74) and in this case the principal curvatures obtained from (42) are shown as vectors attached to the extremes of the eigenvectors of  $M$  [equation (44)].

Fig. 2 shows an example of the first two types of plot. In this case the reference molecule is a regular  $\alpha$  helix of polyalanine, calculated by the method of Diamond (1966) with all residues having  $\varphi = -57.37^\circ$ ,  $\psi = -47.52^\circ$ ,  $\omega = 180^\circ$ ,  $\tau = 109.66^\circ$ . The object molecule is the E helix of myoglobin. Reference atoms are plotted as concentric circles, with a single diameter drawn

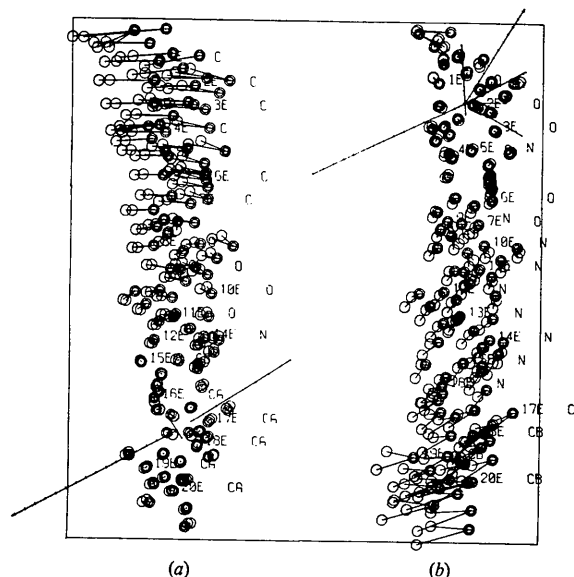


Fig. 2. The application of linear transformations to fit the myoglobin E helix to a regular helix, the fitting being accomplished at the lower part of the figure in (a) and in the upper part in (b). About two thirds of the helix in each case is in the secondary region and is not fitted but shows the effect of extrapolating the fit into these regions. In stepping in this way from one end of the helix to the other, the program reports a change in orientation of some  $12.4^\circ$ . For further details see text.

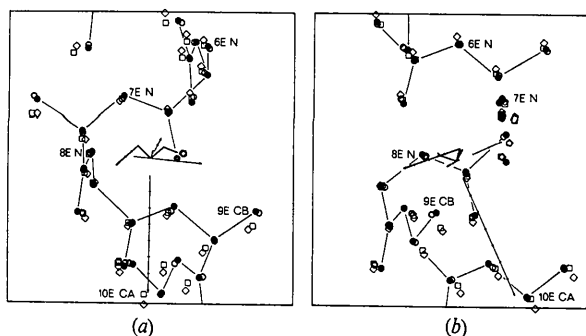


Fig. 3. (a) and (b). Two orthogonal views of the application of the quadratic transformation to the most curved portion of the myoglobin E helix. Residue 8 is glycine. For details see text.



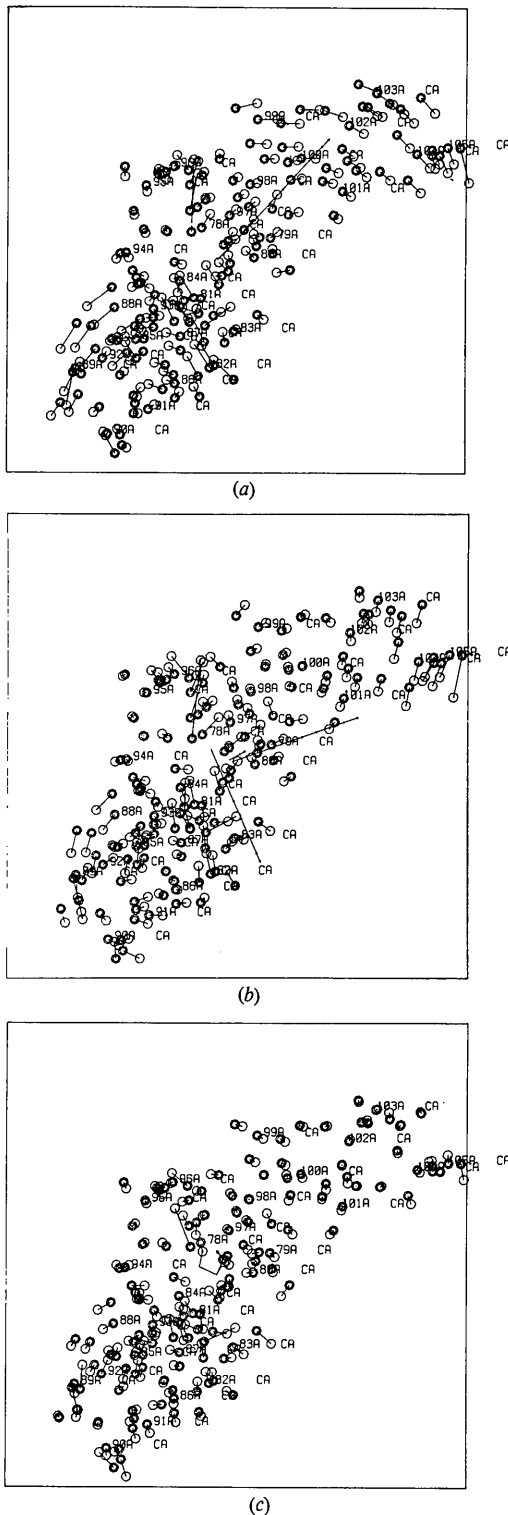


Fig. 4. (a), (b) and (c) Application of orthogonal, linear and quadratic transformations respectively to the comparison of horse oxy- with deoxy-haemoglobin. The substantial discrepancy vectors in the upper right and lower left of (a) are largely removed in (c), although some differences between the two structures are visible in the central region which are of a local nature. For further details see text.

through any which is in the secondary region only. The single circles mark the myoglobin E helix transformed back onto the reference helix both orthogonally and by the general linear transformation. No quadratic parts were calculated for this figure so that they represent the 'best' linear transformations rather than the linear part of the best quadratic transformation. The primary region in Fig. 2(a) is in the lower part of the figure and contains 31 atoms from E13 O to E20 C. The arrow pointing to the upper right in Fig. 2(a) indicates that within the primary region the myoglobin helix is stretched 7.6% in this direction and the small arrow shows it to be compressed 1.6% in this second direction relative to the regular helix. The arrow pointing to the lower left shows that the object and reference molecules are rotated  $162^\circ$  relative to one another about the line of this arrow. Within the primary region the orthogonal transformation provides superposition to  $0.284 \text{ \AA}$  r.m.s. and the linear one to  $0.232 \text{ \AA}$ . Throughout the primary and secondary regions together the corresponding figures are  $1.783$  and  $2.130 \text{ \AA}$ , indicating that the strains detected in the primary region are not characteristic of the helix as a whole. Each object atom is connected to its reference atom and these lines clearly show the bend of the helix.

In Fig. 2(b) a similar calculation is shown (in the same orientation) in which the primary region consists of 27 atoms from E1 N to E6 C. Strains of  $+4.6\%$ ,  $-5.4\%$  and  $-6.3\%$  are found, and again these are local rather than general, and may be influenced by the fact that E1 N and E1 C are within the primary region but are not truly part of the E helix. The uppermost arrow on the right of the figure shows that the overall difference of orientation of the two ends of the myoglobin helix is  $12.4^\circ$  about this arrow as axis, which corresponds approximately to a  $6^\circ$  twist about the helix axis and a  $10.8^\circ$  bend.

In Fig. 3(a) and (b) two orthogonal views are given of 25 atoms from E4 O to E10 C, all of them in the primary region. The reference body is again the regular  $\alpha$  helix and its atoms are marked with solid circles. The squares with vertical diagonals show the object body orthogonally back-transformed, the squares with vertical sides show the general linear back-transformation and the open circles are the quadratic back-transformation of (74). These are generally closer to the solid circles than are the squares. The light arrows show tensile strains of  $7.1\%$  and  $1.6\%$  and a compressive strain of  $4\%$ . The heavy arrows show the principal curvatures attached to the extremities of the eigenvectors of  $M$  [equation (44)], the largest of these being some  $3.6^\circ \text{ \AA}^{-1}$  about an axis which is about  $72^\circ$  to the helix axis, per  $\text{\AA}$  of displacement in a direction at  $46^\circ$  to the helix axis. The accumulated change in orientation between one end of the helix and the other of  $12.4^\circ$  [Fig. 2(b)] is about an axis which is within  $45^\circ$  of the axis of largest curvature found in this central region. The fact that the squares generally fall to the left of their circles in both upper

and lower parts of Fig. 3(a) is a visible indication of curvature.

Fig. 4(a), (b) and (c) shows respectively the orthogonal, linear and quadratic transformations applied to the superposition of main chain and C $\beta$  atoms from residues 77 to 105 of horse oxy- and deoxy-haemoglobin  $\alpha$  chains. The oxy form is treated as reference, and there is no secondary region. The discrepancy vectors are substantial in Fig. 4(a) and clearly may not be eliminated by a further rotation since improvements made in this way in part of the figure would be deleterious elsewhere. The linear transformation in Fig. 4(b) changes the directions of the discrepancy vectors, but does little to improve the fit, the r.m.s. discrepancy having fallen only from 1.03 to 0.97 Å. These figures are very similar and both of them well outside the estimated accuracy of the coordinates. In Fig. 4(c) the discrepancies are clearly much reduced by the quadratic transformation, the r.m.s. discrepancy being now 0.66 Å. Clearly, several atoms still do not superimpose well, indicating conformational changes of a more local character, but the overall curvatures are as little as 1.26, 0.86 and 0.34° Å<sup>-1</sup>

I am indebted to Drs MacLachlan and Perutz for the haemoglobin coordinates, and to several of my colleagues for criticism of the manuscript.

## APPENDIX

### Derivation of the reverse quadratic transformation

Given the forward transformation

$$X_I = d_I + D_{IJ}x_J + \frac{1}{2}\dot{D}_{Ijk}x_jx_k, \quad (A1)$$

substitution of  $X$  into the reverse transformation

$$x_I = d_I^* + D_{IJ}^*X_J + \frac{1}{2}\dot{D}_{Ijk}^*X_jX_k \quad (A2)$$

gives

$$\begin{aligned} x_I = & d_I^* + D_{IJ}^*d_J + \frac{1}{2}\dot{D}_{Ijk}^*d_jd_k \\ & + [D_{IJ}^*D_{jk} + \frac{1}{2}\dot{D}_{Ijl}^*(D_{jk}d_l + D_{lk}d_j)]x_k \\ & + \frac{1}{2}[D_{IJ}^*\dot{D}_{jkl} + \dot{D}_{Ijp}^*(\frac{1}{2}\dot{D}_{jkl}d_p + \frac{1}{2}\dot{D}_{pkl}d_j + D_{jk}D_{pl})]x_kx_l \\ & + \frac{1}{4}\dot{D}_{Ijp}^*[D_{jm}\dot{D}_{pkl} + \dot{D}_{jml}D_{pk}]x_kx_lx_m \\ & + \frac{1}{8}\dot{D}_{Ijp}^*\dot{D}_{jml}\dot{D}_{pnk}x_kx_lx_mx_n. \end{aligned} \quad (A3)$$

Comparing coefficients of powers of  $x$ , ignoring the cubic and quartic terms, gives, from the linear term

$$D_{IJ}^*D_{jk} + \frac{1}{2}\dot{D}_{Ijl}^*(D_{jk}d_l + D_{lk}d_j) = \delta_{IK} \quad (A4)$$

which, on multiplying by  $D_{kM}^{-1}$ , rearranging and re-naming subscripts gives

$$D_{IJ}^* = D_{IJ}^{-1} - \frac{1}{2}\dot{D}_{Irs}^*(D_{rt}d_s + D_{st}d_r)D_{tJ}^{-1} \quad (A5)$$

and from the quadratic term [substituting (A5)]

$$\begin{aligned} 0 = & \dot{D}_{jKL}[D_{IJ}^{-1} - \frac{1}{2}\dot{D}_{Irs}^*(D_{rt}d_s + D_{st}d_r)D_{tJ}^{-1}] \\ & + \dot{D}_{Irs}^*(\frac{1}{2}\dot{D}_{rKL}d_s + \frac{1}{2}\dot{D}_{sKL}d_r + D_{rK}D_{sL}) \\ = & \dot{D}_{jKL}D_{IJ}^{-1} + \dot{D}_{Irs}^*[\frac{1}{2}(\dot{D}_{rKL} - \dot{D}_{jKL}D_{rt}D_{tJ}^{-1})d_s \\ & + \frac{1}{2}(\dot{D}_{sKL} - \dot{D}_{jKL}D_{st}D_{tJ}^{-1})d_r + D_{rK}D_{sL}] \end{aligned} \quad (A6)$$

and the round brackets vanish, so that multiplying by  $D_{kP}^{-1}D_{IQ}^{-1}$  gives

$$\dot{D}_{IPQ}^* = -D_{IJ}^{-1}D_{kP}^{-1}D_{IQ}^{-1}\dot{D}_{jkl} \quad (A7)$$

and substitution in (A5) gives

$$D_{IJ}^* = D_{IJ}^{-1} + D_{Ip}^{-1}D_{qJ}^{-1}D_{rI}^{-1}\dot{D}_{pqr}d_l. \quad (A8)$$

Finally, the zero-order term gives

$$0 = d_I^* + D_{IJ}^*d_J + \frac{1}{2}\dot{D}_{Ijk}^*d_jd_k,$$

whence

$$d_I^* = -D_{IJ}^{-1}d_J - \frac{1}{2}D_{Ip}^{-1}D_{qk}^{-1}D_{rI}^{-1}\dot{D}_{pqr}d_kd_l. \quad (A9)$$

Equation (74) follows from (A7), (A8) and (A9) with  $d=0$ .

If the left-hand side of (A3) is denoted  $y_I$ , substitution of the foregoing results gives

$$\begin{aligned} y_I = & x_I - F_{Iqr}(F_{qst}x_r + F_{rst}x_q)x_sx_t \\ & - F_{Iqr}F_{qtm}F_{rst}x_tx_mx_sx_n, \end{aligned} \quad (A10)$$

in which

$$F_{LJK} = \frac{1}{2}D_{Li}^{-1}\dot{D}_{IJK} \quad (A11)$$

so that the absolute error in the back transformation involves the second and third powers of  $\dot{D}$  and the third and fourth powers of  $x$ .

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